例21で得た3'-N-(Boc-Gly-Phe-Leu-Gly)-NH-A (A-NH_2 =DX-8951) (97 mg)をトリフルオロ酢酸 (3 ml) に溶かし、1時間放置した。溶媒を留去し、メタノール(30 ml) で共沸を2回、エタノール(30 ml) で共沸を2回行った後、残渣をエーテルで洗浄して、標記化合物(95 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ : 8.57 (d, 1H, J=8.3Hz), 8.47 (d, 1H, J=8.3Hz), 8.32 (d, 1H, J=7.8Hz), 8.17 (t, 1H, J=5.5Hz), 7.81-7.91 (br, 3H), 7.79 (d, 1H, J=10.7Hz), 7.32 (s, 1H), 7.21-7.23 (m, 5H), 7.12-7.17 (m, 1H), 6.45-6.55 (br, 1H), 5.57 (q, 1H, J=4.4Hz), 5.43 (d, 1H, J=16.1Hz), 5.34 (d, 1H, J=16.1Hz), 5.23 (s, 2H), 4.67 (dt, 1H, J=4.0Hz, J=9.0Hz), 4.31 (dd, 1H, J=8.5Hz, J=15.0Hz), 4.0-4.4 (br, 1H), 3.74-3.76 (m, 2H), 3.56 (dd, 1H, J=6.0Hz, J=16.0Hz), 3.41 (dd, 1H, J=6.0Hz, J=16.0Hz), 3.17-3.19 (br, 2H), 3.02 (dd, 1H, J=4.0Hz, J=14.0Hz), 2.70 (dd, 1H, J=10.0Hz, J=14.0Hz), 2.40 (s, 3H), 2.05-2.15 (m, 1H), 1.85 (dt, 2H, J=7.0Hz, J=14.0Hz), 1.50-1.55 (m, 1H), 1.45 (t, 2H, J=6.0Hz), 1.35 (s, 9H), 0.88 (t, 3H, J=7.4), 0.85 (d, 3H, J=6.4Hz), 0.80 (d, 3H, J=6.4Hz).

例23：カルボキシメチルデキストランポリアルコール-Gly-Phe-Leu-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例13で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (690 mg) をN,N-ジメチルホルムアミド(50 ml) に溶解させた。この溶液に、例22で得た3'-N-(Gly-Phe-Leu-Gly)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (95 mg)のN,N-ジメチルホルムアミド(10 ml) 溶液、トリエチルアミン(0.03 ml) 、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン (690 mg) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 に調整した後、透析膜 (スペクトルポア1、カットオフ分子量 6000-8000) を用いて、純水に対して透析した。透析内液をミリポアフィルター (0.22μm)で濾過した後、濾液を凍結乾燥して標記化合物 (600 mg) を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づい

て定量したところ、 4.8% (W/W) であった。

例24：カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成

デキストランT500 (50 g, ファルマシア社製, 分子量 500K) を0.1 M 酢酸緩衝液 (pH5.5, 5000 ml) に溶解し、過ヨウ素酸ナトリウム(165.0 g) の水溶液(5000 ml) を加えた。遮光しながら4 ℃で10日間撹拌した後、エチレングリコール(35.0 ml) を加え、一晩撹拌した。反応液を8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。水素化ホウ素ナトリウム (70 g) を加えて溶解した後、一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して4 ℃で1時間撹拌した後、8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。得られた水溶液をバイオマックス-50 膜を用いて限外濾過法による低分子画分の除去を行なった。高分子画分を凍結乾燥して、デキストランポリアルコール (27.1 g) を得た。この物質の分子量 (ゲル濾過、プルラン標準) は、140Kであった。

このデキストランポリアルコール(5 g) を、水酸化ナトリウム (21 g) を水 (150 ml) に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸 (30 g) を加えて溶解させた後、室温で一晩反応させた。この反応液を酢酸でpHを8に調整した後、バイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥してカルボキシメチルデキストランポリアルコールのナトリウム塩(5.6 g) を得た。この物質の分子量 (ゲル濾過、プルラン標準) は 263K であり、カルボキシメチル化度は0.4 であった。

このカルボキシメチルデキストランポリアルコールのナトリウム塩(2.0 g) を水に溶解し、Bio -Rad AG 50W-X2 (200-400 メッシュ、 H^+ 型) カラム (直径 44 mm、長さ 210 mm)にのせ、水で溶出した。この溶出液にトリエチルアミン (4 ml) を加えた後、凍結乾燥して標記化合物(2.2 g) を得た。

例25：カルボキシメチルデキストランポリアルコールのトリメチルアンモニウム塩の合成

例24で得たカルボキシメチルデキストランポリアルコールのナトリウム塩(1.0 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、$Me_3N\ H^+$ 型) カラムにのせ、水で溶出した。この溶出液を凍結乾燥して標記化合物 (950 mg) を得た。

例26：3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951)塩酸塩の合成

例14と同様の方法で3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) (400 mg) から得た3'-N-(Gly-Gly-Phe-Gly)-NH-A トリフルオロ酢酸塩を水-MeOH (1:4) に溶解して、Bio-Rad AG 1-X8 (200-400 メッシュ、Cl^- 型) カラム (1.5 cm × 8.6 cm) にのせ、上記溶媒で溶出した。この溶出液を濃縮した後、凍結乾燥して標記化合物 (310 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ : 8.53 (d, 1H, J=8.5Hz), 8.46-8.48 (m, 1H), 8.37-8.39 (m , 1H), 7.95 (d, 1H, J=8.0Hz), 7.80 (s, 3H), 7.78 (d, 1H, J=11.1Hz), 7.34 (s, 1H), 7.14-7.24 (m, 5H), 6.50(s, 1H), 5.56-5.60 (m, 1H), 5.35-5.40 (m, 2H), 5.24 (s, 2H), 4.51-4.56 (m, 1H), 3.86 (dd, J=4.8, 13.5Hz, 1H), 3.68-3.79 (m, 3H), 3.54 (s, 2H), 3.15-3.22 (m, 2H), 3.01 (dd, J=5.6, 13.5Hz, 1H), 2.78 (dd, J=9.6, 3.5Hz, 1H), 2.41 (s, 3H), 2.12-2.23 (m, 2H), 1.81-1.89 (m, 2H), 0.88 (t, 3H, J=7.2Hz).

Mass (FAB); m/e 753 (M+1)

例27：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例25で得たカルボキシメチルデキストランポリアルコールのトリメチルアンモニウム塩(0.1 g) をN,N-ジメチルホルムアミド (6 ml) に溶解させた。この溶液に、例26で得た 3'-N-(Gly-Gly-Phe-Gly)NH-A (A-NH_2 =DX-8951) の塩酸塩 (24 mg) のN,N-ジメチルホルムアミド(10 ml) 溶液、トリエチルアミン(5μl)、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(0.1 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-30 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(90 mg) を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ 11% (W/

W)であった。

例28：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例25で得たカルボキシメチルデキストランポリアルコールのトリメチルアンモニウム塩(0.1 g) をN,N-ジメチルホルムアミド (6 ml) に溶解させた。この溶液に、例26で得た3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) の塩酸塩 (36 mg) のN,N-ジメチルホルムアミド(10 ml) 溶液、トリエチルアミン(8μl)、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(0.1 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3M塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 12とした。得られた水溶液をバイオマックス-30 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(80 mg) を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC (カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速：0.8 ml/min) で分析した結果、及び本化合物の紫外線吸収スペクトル (0.1 M トリス緩衝液、pH 9.0、 36 μg/ml) をそれぞれ図８及び図９に示す。本化合物の医薬化合物基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ15% (W/W) であった。

例29：カルボキシメチルデキストランポリアルコール-Gly-Gly-Gly-Phe-NH-A'(A-NH_2 =DX-8951)の合成

デキストランT250(20 g, EXTRASYNTHESE製, 平均分子量 250K)を0.1 M 酢酸緩衝液 (pH5.5, 2000 ml) に溶解し、過ヨウ素酸ナトリウム (66.0 g) の水溶液(2000 ml) を加えた。遮光しながら４℃で10日間撹拌した後、エチレングリコール(14.0 ml) を加え、一晩撹拌した。反応液を氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。水素化ホウ素ナトリウム (28 g) を加えて溶解した後、室温で一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して４℃で１時間撹拌した後、氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。得

られた水溶液をバイオマックス-30 膜を用いた限外濾過法により低分子画分を除去して、膜を通過しない残留溶液１を得た。別に、デキストランT250(50 g, EXTRASYNTHESE製, 平均分子量 250K)を0.1 M 酢酸緩衝液 (pH5.5, 5000 ml) に溶解し、過ヨウ素酸ナトリウム(165 g) の水溶液(5000 ml) を加えた。遮光しながら４℃で10日間撹拌した後、エチレングリコール(35.0 ml) を加え、一晩撹拌した。反応液を氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。水素化ホウ素ナトリウム (70 g) を加えて溶解した後、室温で一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して４℃で１時間撹拌した後、氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。得られた水溶液をバイオマックス-30 膜を用いた限外濾過法により低分子画分を除去して、膜を通過しない残留溶液２を得た。残留溶液１と残留溶液２を合して、限外濾過法によりバイオマックス-50 膜を通過する画分をバイオマックス-30 膜を用いて低分子画分を除去し、凍結乾燥してデキストランポリアルコール (25.7 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は、47K であった。

このデキストランポリアルコール(5 g) を、水酸化ナトリウム (35 g) を水 (150 ml) に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸 (50 g) を加えて溶解させた後、室温で18時間反応させた。この反応液を酢酸でpHを８に調整した後、バイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(7.2 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は 127K であり、カルボキシメチル化度は0.8 であった。このカルボキシメチルデキストランポリアルコールのナトリウム塩(2.2 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、 H^+ 型) カラム（直径 44 mm、長さ 210 mm)にのせ、水で溶出した。この溶出液にトリエチルアミン (4 ml) を加えた後、凍結乾燥してカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (2.69 g) を得た。

このカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (2.67 g) をN,N-ジメチルホルムアミド (200 ml) に溶解した。この溶液に、例２と同様に合成した3'-N-(Boc-Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951) (350

mg）から例16と同様の方法で脱Boc 化して得られる3'-N-(Gly-Gly-Gly-Phe)-NH-A のトリフルオロ酢酸塩とトリエチルアミン（0.116 ml）をN,N-ジメチルホルムアミド（10 ml)に溶かして得られる溶液、1-エトキシカルボニル-2- エトキシ-1,2 - ジヒドロキシキノリン（2.67 g）をN,N-ジメチルホルムアミド(10 ml）に溶かして得られる溶液を順次加え、室温で一晩撹拌しながら反応させた。この反応液に3 M 塩化ナトリウム水溶液（100 ml）を加え、8 mlずつを各30 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液（1 ml）、ジエチルエーテル(5 ml）を加えて、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱をアセトンで洗浄した後、水に溶解し、3 M 塩化ナトリウム水溶液(10 ml) を加えた後、0.1 M 水酸化ナトリウム水溶液で pH 9 に調整し、37℃で1時間処理した。この処理液をバイオマックス-10 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物（2.30 g）を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC （カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速：0.8 ml/min）で分析した結果、及び本化合物の紫外線吸収スペクトル（0.1 M トリス緩衝液, pH 9.0, 0.20 mg/ml）をそれぞれ図10及び図11に示す。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ、 5.8% (W/W) であった。

例30：<u>カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成</u>

デキストランT10 （20 g, ファルマシア社製、平均分子量10K ）の0.1 M 酢酸緩衝液（pH 5.5）溶液（2000 ml)に、過ヨウ素酸ナトリウム（66.0 g）の水溶液(2000 ml）を加えた。遮光しながら4℃で10日間撹拌した後、エチレングリコール(14.0 ml）を加え、一晩撹拌した。反応液を氷冷下で8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。水素化ホウ素ナトリウム（28 g）を加えて溶解した後、室温で一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して4℃で1時間撹拌した後、氷冷下で8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。得られた水溶液をバイオマックス-5膜（ミリポア社製）を用いた限外濾過法により低分子画分を除去して、膜を通過しない残留溶液をバイオマックス-30 膜を通過させ

た。得られた通過液を凍結乾燥して、デキストランポリアルコール(8.0 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は、13K であった。

このデキストランポリアルコール(3.7 g) を、水酸化ナトリウム（25.9 g) を水（111 ml) に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸（37 g) を加えて溶解させた後、室温で20時間反応させた。この反応液を酢酸で pH 8 に調整した後、バイオマックス-5膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(6.2 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は、37K であり、カルボキシメチル化度は0.9 であった。

このカルボキシメチルデキストランポリアルコールのナトリウム塩(6.0 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、H^+ 型）カラムにのせ、水で溶出した。この溶出液にトリエチルアミン（9.3 ml) を加えた後、凍結乾燥して標記化合物(7.2 g) を得た。

例31：カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成

例30で得たデキストランポリアルコール(3.9 g) を、水酸化ナトリウム（16.3 g) を水（117 ml) に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸（23.4 g) を加えて溶解させた後、室温で18時間反応させた。この反応液を酢酸で pH 8 に調整した後、バイオマックス-5膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(5.0 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は、28K であり、カルボキシメチル化度は0.5 であった。このカルボキシメチルデキストランポリアルコールのナトリウム塩(4.8 g) を例30と同様にトリエチルアンモニウム塩に変換して、標記化合物(5.6 g) を得た。

例32：カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成

デキストラン4（20 g 、フナコシ社製、平均分子量 4K-6K) の0.1 M 酢酸緩衝液（pH 5.5) 溶液(2000 ml) に、過ヨウ素酸ナトリウム（66.0 g) の水溶液(2000

ml）を加えた。遮光しながら4℃で10日間撹拌した後、エチレングリコール(14.0 ml）を加え、一晩撹拌した。反応液を氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。水素化ホウ素ナトリウム（28 g）を加えて溶解した後、室温で一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して4℃で1時間撹拌した後、氷冷下で8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。得られた水溶液をバイオマックス-3膜（ミリポア社製）を用いた限外濾過法により低分子画分を除去した。得られた通過液を凍結乾燥してデキストランポリアルコール(6.0 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、9Kであった。このデキストランポリアルコール(2.7 g）を、水酸化ナトリウム（18.9 g）を水(81 ml）に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸（27 g）を加えて溶解させた後、室温で20時間反応させた。この反応液を酢酸で pH 8 に調整した後、バイオマックス-5膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(4.2 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、20K であり、カルボキシメチル化度は0.9 であった。

このカルボキシメチルデキストランポリアルコールのナトリウム塩(4.0 g）を例30と同様にトリエチルアンモニウム塩に変換して、標記化合物(4.8 g）を得た。

例33：<u>カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成</u>

例32で得たデキストランポリアルコール(2.7 g）を、水酸化ナトリウム（11.3 g）を水(81 ml）に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸（16.2 g）を加えて溶解させた後、室温で18時間反応させた。この反応液を酢酸で pH 8 に調整した後、バイオマックス-5膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(2.7 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、16K であり、カルボキシメチル化度は0.5であった。このカルボキシメチルデキストランポリアルコールのナトリウム塩(2.7 g）を例30と同様にトリエチルアンモニウム塩に変換して、標記化合物(3.1 g）を得た。

例34：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例30で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(1.5 g) N,N-ジメチルホルムアミド(90 ml) 溶解させた。この溶液に、トリエチルアミン(0.07 ml) と3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951)のトリフルオロ酢酸塩 (210 mg) のN,N-ジメチルホルムアミド(40 ml) 溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(1.5 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-3膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(1.3 g) を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC (カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速： 0.8 ml/min) で分析した結果、及び本化合物の紫外線吸収スペクトル (0.1 M トリス緩衝液、pH 9.0、 65 μg/ml) をそれぞれ図12及び図13に示す。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ 6.4% (W/W) であった。

例35：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例31で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(1.2 g) をN,N-ジメチルホルムアミド(90 ml) 溶解させた。この溶液に、トリエチルアミン (0.056 ml) と3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (168 mg) のN,N-ジメチルホルムアミド(30 ml) 溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(1.2 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集

めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-3膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(1.0 g) を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ、 4.8% (W/W) であった。

例36：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例32で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(1.2 g) をN,N-ジメチルホルムアミド(90 ml) 溶解させた。この溶液に、トリエチルアミン (0.056 ml) と3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (168 mg) のN,N-ジメチルホルムアミド(30 ml) 溶液、 1- エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(1.2 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-3膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(1.0 g) を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ、 5.9% (W/W) であった。

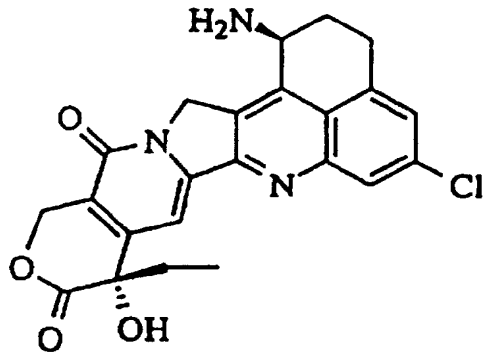
例37：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例33で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(1.5 g) をN,N-ジメチルホルムアミド(90 ml) 溶解させた。この溶液に、トリエチルアミン(0.07 ml) と3'-N-(Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (210 mg) のN,N-ジメチルホルムアミド(40 ml) 溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(1.5 g) を順次加え、

室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液（2.5 ml）、ジエチルエーテル(20 ml）を加えて、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-3膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(1.3 g）を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ、4.6%（W/W）であった。

例38：Boc-Gly-Gly-Phe-Gly-NH-A（A-NH_2 =DW-8286）の合成

Boc-Gly-Gly-Phe-Gly（42 mg）およびN-ヒドロキシスクシンイミド(12 mg）をN,N-ジメチルホルムアミド（2 ml）に溶解し、4℃に冷却した後、N,N'- ジシクロヘキシルカルボジイミド(22 mg）を添加した。この溶液に下記式：



で表される化合物［(1s,9s)-1-アミノ-5- クロロ-9- エチル-2,3- ジヒドロ-9-ハイドロキシ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオン：DW-8286]の塩酸塩(50 mg）とトリエチルアミン(0.01 ml）を溶解したN,N-ジメチルホルムアミド（6 ml）溶液を加えて、遮光して、室温で16時間撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー（溶出液： 0.5% 酢酸を含むジクロロメタン：メタノール＝10：1 溶液）で精製して標記化合物(27 mg）を得た。

^{1}H-NMR($CDCl_3$) δ： 8.10-8.20 (br,1H), 7.95-8.05 (br,1H), 7.70-7.80 (br,2H), 7.50-7.60 (br,1H), 7.40-7.50 (br,1H), 7.10-7.25 (m,5H), 7.05-7.15 (br,1H),

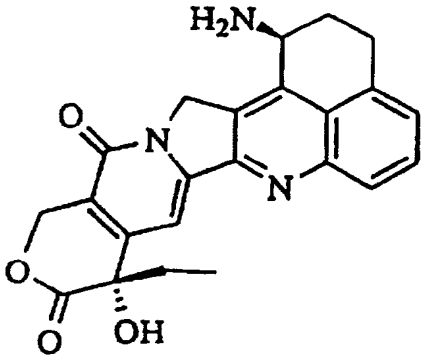
5.85-5.95 (br, 1H), 5.50-5.60 (br, 1H), 5.40-5.50 (m, 1H), 5.25-5.35 (m, 1H), 5.05-5.15 (m, 1H), 4.90-5.00 (m, 1H), 4.70-4.80 (br, 1H), 4.10-4.25 (br, 2H), 3.60-3.90 (m, 4H), 3.10-3.40 (m, 3H), 2.95-3.05 (br, 1H), 2.15-2.30 (br, 1H), 1.75-1.90 (br, 2H), 1.39 (s, 9H), 0.80-1.00 (m, 3H).

例39：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DW-8286)の合成

例24で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩（175 mg）をN,N-ジメチルホルムアミド(20 ml）に溶解させた。この溶液に、例38で得られた3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DW-8286) (27 mg)から例4と同様の方法で脱Boc 化して得られる 3'-N-(Gly-Gly-Phe-Gly)-NH-Aのトリフルオロ酢酸塩(29 mg) とトリエチルアミン(9μl)のN,N-ジメチルホルムアミド（5 ml）溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン（175 mg）を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液（2.5 ml）、ジエチルエーテル(20 ml）を加えて、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-30 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物（135 mg）を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC （カラム：東ソー TSK Gel PW-4000XL、溶媒：0.1M NaCl、流速： 0.8 ml/min ）で分析した結果、及び本化合物の紫外線吸収スペクトル（0.1 M トリス緩衝液、Ph 9.0、 99 μg/ml）をそれぞれ図14及び図15に示す。本化合物の医薬化合物残基の含量は、0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ、 6.1% (W/W) であった。

例40：3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DW-8089)の合成

Boc-Gly-Gly-Phe-Gly (163 mg)およびN-ヒドロキシスクシンイミド(45 mg) をN,N-ジメチルホルムアミド(10 ml) に溶解し、4℃に冷却した後、N, N'-ジシクロヘキシルカルボジイミド(79 mg) を添加した。この溶液に下記の式：



で表される化合物 [(1s,9s)-1-アミノ-9- エチル-2,3- ジヒドロ-9- ハイドロキシ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオン: DW-8089]のトシル酸塩 (170 mg) とトリエチルアミン (0.054 ml) を溶解したN,N-ジメチルホルムアミド(30 ml) 溶液を加えて、遮光下に室温で一晩撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー (溶出液：0.5%酢酸を含むジクロロメタン：メタノール＝94：6溶液) で精製して標記化合物 (100 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.51 (d,1H,J=8.5Hz), 8.41 (t,1H,J=5.6Hz), 8.29 (s,1H), 8.17 (d,1H,J=8.0Hz), 8.03 (d,1H,J=8.0Hz), 7.90 (dd,1H,J=4.8,5.6Hz), 7.79 (t,1H,J=5.6Hz), 7.53 (d,1H,J=7.2Hz), 7.36 (s,1H), 7.13-7.25 (m,5H), 6.94-6.95 (m,1H), 5.60-5.63 (m,1H), 5.36-5.47 (m,2H), 5.21-5.30 (m,2H), 4.42-4.47 (m,1H), 3.63-3.96 (m,3H), 3.51-3.59 (m,3H), 3.31-3.40 (m,1H), 3.09-3.21 (m,1H), 3.02 (dd,1H,J=4.8,13.5Hz), 2.76-2.81 (m,1H), 2.13-2.17 (m,2H), 1.85-1.90 (m,2H), 1.37 (s,9H), 0.89 (t,3H,J=8.0Hz).

Mass (FAB); m/e 822 (M+1)

例41：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-NH-A'(A-NH_2 =DW-8089)の合成

例24で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(1.6 g) をN,N-ジメチルホルムアミド(60 ml) に溶解させた。この溶液に、例40で得られた3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DW-8089) (200 mg) から例４と同様の方法で脱Boc 化して得られる 3'-N-(Gly-Gly-Phe-Gly)-NH-Aのトリフルオロ酢酸塩とトリエチルアミン(0.07 ml) をN,N-ジメチルホルムアミド(20

ml）に溶かして得られる溶液、1-エトキシカルボニル-2- エトキシ-1, 2-ジヒドロキシキノリン(1.6 g) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(25 ml) を加えて、析出した沈澱を遠心分離 (2500 rpm, 8分) により集めた。この沈澱をエタノールで洗浄した後、水に溶解し、3 M 塩化ナトリウム水溶液(20 ml) を加え、0.1 M 水酸化ナトリウム水溶液で pH 9 に調整した。この溶液をバイオマックス-10 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物 (1.20 g) を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC (カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速：0.8 ml/min) で分析した結果、及び本化合物の紫外線吸収スペクトル (0.1 M トリス緩衝液, pH 9.0, 0.26 mg/ml) をそれぞれ図16及び図17に示す。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ、 5.0% (W/W) であった。

例42：Trt-Gly-Gly-Phe-Gly-OHの合成

Trt-Gly-Gly-Phe-Gly-OBzl (670 mg) 、10% Pd-C (100mg)およびギ酸アンモニウム (200 mg) をDMF (5 ml)中に加え、3時間撹拌した。反応液を濾過し、濾液を減圧乾固した後、残渣をシリカゲルカラムクロマトグラフィー (溶出液：ジクロロメタン：メタノール＝８：１溶液) で精製して標記化合物 (300 mg) を得た。

^{1}H-NMR(CD_3OD) δ： 7.16-7.45 (m, 20H), 4.66 (dd, 1H, J=9.8, 5.4Hz), 3.93 (d, 1H, J=16.6Hz), 3.80 (d, 1H, J=17.6Hz), 3.78 (d, 1H, J=16.6Hz), 3.68 (d, 1H, J=17.1Hz), 3.23 (dd, 1H, J=14.2, 5.4Hz), 2.90 (d, 1H, J=13.7Hz), 2.90 (s, 1H).

例43：3'-N-(Gly-Gly-Phe-Gly)-DXR塩酸塩の合成

Trt-Gly-Gly-Phe-Gly-OH (100 mg) およびN-ヒドロキシスクシンイミド(22 mg) をDMF (4 ml)に溶かし、氷冷下N, N'- ジシクロヘキシルカルボジイミド(40 mg) を加え４℃で２時間撹拌した。この溶液にN-メチルモルホリン (0.019 ml) とドキソルビシン(DXR) 塩酸塩(92 mg) を溶解したDMF (20 ml) 溶液を加えて、４℃で16時間攪拌した。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー (溶出液：ジクロロメタン：メタノール＝２０：１溶液) で精製した。得

られた化合物を 75%酢酸 1 ml に溶解し、1時間撹拌した。水 (20 ml)を加えて析出した固体を濾過して除き、濾液を凍結乾燥して、得られた粉末を水 (5 ml) に溶かした。この溶液をAG-1X8 (Cl^- 型) カラムに通し、水で溶出した後、ジクロロメタンで洗い、水層を凍結乾燥して、標記化合物(40 mg) を得た。

^{1}H-NMR(CD_3OD) δ : 7.95 (d, 1H, J=7.3Hz), 7.82 (t, 1H, J=7.8Hz), 7.54 (d, 1H, J=8.3Hz), 7.16-7.26 (m, 5H), 5.43 (d, 1H, J=3.4Hz), 5.14 (br, 1H), 4.72 (s, 2H), 4.42 (dd, 1H, J=8.3, 6.8Hz), 4.30 (q, 1H, J=6.8Hz), 4.14-4.18 (m, 1H), 4.03 (d, 1H, J=16.6Hz), 4.02 (s, 3H), 3.86 (d, 1H, J=18.5Hz), 3.83 (d, 1H, J=17.1Hz), 3.75 (d, 1H, J=16.1Hz), 3.73 (d, 1H, J=16.1Hz), 3.62 (br, 1H), 3.58 (d, 1H, J=16.6Hz), 3.10-3.15 (m, 2H), 3.00 (d, 1H, J=18.6Hz), 2.94 (dd, 1H, J=14.2, 8.8Hz), 2.38 (d, 1H, J=14.2Hz), 2.18 (dd, 1H, J=14.2, 4.4Hz), 1.94-2.00 (m, 1H), 1.71 (dd, 1H, J=12.7, 4.4Hz), 1.28 (d, 3H, J=6.3Hz).

例44：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-DXRの合成

例24で得たカルボキシメチルデキストランポリアルコールのナトリウム塩(1.5 g) を例25と同様の方法でトリメチルアンモニウム塩(1.2 g) に変換した後、この 400 mg をN,N-ジメチルホルムアミド(24 ml) に溶解させた。この溶液に、3'-N-(Gly-Gly-Phe-Gly)-DXRの塩酸塩(76 mg) のN,N-ジメチルホルムアミド(24 ml) 溶液、トリエチルアミン(24 μl)、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン (400 mg) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液 (2.5 ml) 、ジエチルエーテル(20 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、バイオマックス-30 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(40 mg) を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC (カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速： 0.8 ml/min) で分析した結果、及び本化合物の紫外線吸収スペクトル (0.01 Mトリス緩衝液、pH 7.4、 36 μg ml) をそれぞれ図18及び図19に示す。本化合物の医薬化合物残基の含量は、PBS (pH 7.4)中での 480 nm における吸光度に基づいて定量したところ、 6.0% (W/W)

であった。

例45：カルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩の合成

デキストランT150（20 g、ファルマシア社製、平均分子量 150K ）を0.1 M 酢酸緩衝液（pH 5.5、2000 ml)に溶解し、過ヨウ素酸ナトリウム（66.0 g）の水溶液（2000 ml）を加えた。遮光しながら4℃で10日間撹拌した後、エチレングリコール（14.0 ml）を加え、一晩撹拌した。反応液を氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。水素化ホウ素ナトリウム（28 g）を加えて溶解した後、室温で一晩撹拌した。反応液を氷冷して、酢酸で pH 5.5 に調整し、4℃で1時間撹拌した。氷冷下で8 M 水酸化ナトリウム水溶液を用いてpHを7.5 に調整した。得られた水溶液をバイオマックス-5膜（ミリポア社製）を用いた限外濾過法により、500 mlまで濃縮して溶液1を得た。別に、デキストランT110（20 g）について上記の一連の操作を行い、溶液2を得た。溶液1と溶液2を混合し、混合溶液のpHを3.0 とし、40℃で4時間インキュベートした後、pHを7に調整して低分子化されたデキストランポリアルコールを含む溶液を得た。バイオマックス-30 膜を通過させ、次いでバイオマックス-5膜を用いて脱塩した後、凍結乾燥してデキストランポリアルコール(4.6 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、17K であった。

このデキストランポリアルコール(2.5 g）を、水酸化ナトリウム（17.5 g）を水（75 ml）に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸（25 g）を加えて溶解させた後、室温で20時間反応させた。この反応液を酢酸でpHを9に調整した後、バイオマックス-5膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、カルボキシメチルデキストランポリアルコールのナトリウム塩(4.0 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は 45Kであり、CM化度は0.9 であった。

このカルボキシメチルデキストランポリアルコールのナトリウム塩(3.7 g）を水に溶解し、Bio-Rad AG 50W-X2 （200-400 メッシュ、H^+ 型）カラムにのせ、水で溶出した。この溶出液にトリエチルアミン（5.8 ml）を加えた後、凍結乾燥して標記化合物(4.4 g）を得た。

例46：カルボキシメチルデキストランポリアルコール-Gly-Gly-Gly-Phe-NH-A'(A-NH_2 =DX-8951)の合成

例45で得られたカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(4.4 g)をN,N-ジメチルホルムアミド（300 ml）に溶解させた。この溶液に、トリエチルアミン(0.19 ml）と3'-N-(Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951)のトリフルオロ酢酸塩（580 mg）を含むN,N-ジメチルホルムアミド(45 ml)溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(4.4 g)を順次加え、遮光して室温で一晩撹拌しながら反応させた。この反応液を1 M 水酸化ナトリウム水溶液で pH 10に調整した後、5 mlずつを各25 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液（1 ml）、ジエチルエーテル（5 ml）を加えて、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱を水に溶解し、透析膜（スペクトルポア１、カットオフ分子量 6000-8000）を用いて、純水に対して透析し、透析内液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物(3.4 g）を得た。本化合物の医薬化合物残基の含量を0.1 トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ、 4.6% (W/W) であった。

例47：α－メチルカルボキシメチルデキストランポリアルコール-Gly-Gly-Gly-Phe-NH-A'(A-NH_2 =DX-8951)の合成

例45で得られたデキストランポリアルコール(2 g）を、水酸化ナトリウム（14 g）を水(60 ml）に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でα－ブロモプロピオン酸(19 ml）を加えて溶解させた後、室温で18時間反応させた。この反応液を酢酸で pH 8 に調整した後、バイオマックス-50膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥して、α－メチルカルボキシメチルデキストランポリアルコールのナトリウム塩（2.95 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、 45Kであった。糖残基あたりのα－メチルカルボキシメチル化度は、カルボキシメチルデキストランポリアルコールの場合に準じて以下の様に求めた。α－メチルカルボキシメチルデキストランポリアルコールのナトリウム塩の水溶液を Bio-Rad AG 50W-X 2 (H^+ 型）カラムに付し、通過液を凍結乾燥して試料として用いた。この試料を所

定過剰量の0.1 N 水酸化ナトリウム水溶液に溶解し、フェノールフタレインを指示薬として0.1 N 塩酸で滴定した。試料の採取量を s mg 、0.1 N 水酸化ナトリウム水溶液の所定過剰量を a (ml) 、0.1 N 塩酸の滴定量を b (ml) とし、α－メチルカルボキシメチル化度を 13.4(a-b)/[s-7.2(a-b)] の式により求めた。その結果α－メチルカルボキシメチル化度は0.8 であった。

このα－メチルカルボキシメチルデキストランポリアルコールのナトリウム塩 (2.2 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、H^+ 型) カラム (直径 44 mm、長さ 210 mm)にのせ、水で溶出した。この溶出液にトリエチルアミン (4 ml) を加えた後、凍結乾燥してα－メチルカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (2.69 g) を得た。

このα－メチルカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (2.68 g) をN,N-ジメチルホルムアミド(60 ml) に溶解した。この溶液に、例２と同様に合成した3'-N-(Boc-Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951) (350 mg) から例16と同様の方法で脱Boc 化して得られる3'-N-(Gly-Gly-Gly-Phe)-NH-A のトリフルオロ酢酸塩とトリエチルアミン (0.116 ml) をN,N-ジメチルホルムアミド (10 ml) に溶かして得られる溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン (2.68 g) をN,N-ジメチルホルムアミド(10 ml) に溶かして得られる溶液を順次加え、室温で一晩撹拌しながら反応させた。この反応液に3 M 塩化ナトリウム水溶液(40 ml) を加え、6 mlずつを各30 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (1 ml) 、ジエチルエーテル (5 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, ８分) により集めた。この沈澱をアセトンで洗浄した後、水に溶解し、3 M 塩化ナトリウム水溶液(10 ml) を加え、0.1 M 水酸化ナトリウム水溶液で pH 9 に調整し、37℃で１時間処理した。この処理液をバイオマックス-10 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物 (2.15 g) を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC (カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速： 0.8 ml min) で分析した結果、及び本化合物の紫外線吸収スペクトル (0.1 M トリス緩衝液, pH 9.0, 0.21 mg/ ml)をそれぞれ図20および図21に示す。本化合物の医薬化合

物残基の含量を0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ、 5.9%（W/W）であった。

例48：3'-N-(Gly-Phe-Gly)-NH-A（A-NH_2 =DX-8951）トリフルオロ酢酸塩の合成

Phe-Gly-OBzlのp-トルエンスルホン酸塩（3.06 g）、Boc-Gly-OH（1.10 g）、N-ヒドロキシスクシンイミド（941 mg）、N-メチルモルフォリン（0.725 ml）、N,N-ジメチルホルムアミド(40 ml）の混合物を４℃に冷却した後、N,N'- ジシクロヘキシルカルボジイミド（1.56 g）を加えた。室温で一晩撹拌しながら反応させた後、反応液を減圧乾固した。残渣をシリカゲルカラムクロマトグラフィー（溶出液：ジクロロメタン：メタノール＝98：２溶液）で精製してBoc-Gly-Phe-Gly-OBzl（1.93 g）を得た。

^{1}H-NMR（DMSO-d_6）δ：8.52（dd, 1H, J=5.6, 6.4Hz), 7.97（d, 1H, J=8.8Hz), 7.30-7.39（m, 5H), 7.15-7.26（m, 5H), 6.83（t, 1H, J=5.6Hz), 5.14（s, 1H), 4.52-4.57（m, 1H), 3.87-3.96（m, 2H), 3.57（dd, 1H, J=5.6, 16.7Hz), 3.43（dd, 1H, J=5.6, 16.7Hz), 3.01（dd, 1H, J=4.8, 14.3Hz), 2.77（dd, 1H, J=5.6, 14.3Hz), 1.37（s, 9H).

得られたBoc-Gly-Phe-Gly-OBzl（1.78 g）を酢酸エチル(60 ml）に溶解し、5%-Pd-C（1.8 g）存在下、24時間接触還元した。触媒を濾去し、濾液を減圧濃縮し、Boc-Gly-Phe-Gly-OH（1.41 g）を得た。

^{1}H-NMR（DMSO-d_6）δ：8.35（t, 1H, J=5.6Hz), 7.94（d, 1H, J=8.8Hz), 7.15-7.26（m, 5H), 6.85（dd, 1H, J=5.6, 6.4Hz), 4.52-4.58（m, 1H), 3.76（d, 2H, J=5.6Hz), 3.56（dd, 1H, J=6.4, 16.7Hz), 3.43（dd, 1H, J=5.6, 16.7Hz), 3.03（dd, 1H, J=5.0, 13.5Hz), 2.79（dd, 1H, J=9.5, 13.5Hz), 1.37（s, 9H).

上で得られたBoc-Gly-Phe-Gly-OH（500 mg）およびN-ヒドロキシスクシンイミド（161 mg）をN,N-ジメチルホルムアミド(10 ml）に溶解した。この溶液に DX-8951のメタンスルホン酸塩（530 mg）とトリエチルアミン（0.146 ml）を溶解したN,N-ジメチルホルムアミド(50 ml）溶液を加えて、４℃に冷却した後、N,N'- ジシクロヘキシルカルボジイミド（268 mg）を加えて、遮光下に室温で一晩撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー（溶出液：ジクロロメタン：メタノール＝96：４溶液）で精製して3'-N-(Boc

-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) (100 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.39 (d, 1H, J=8.0Hz), 8.34 (t, 1H, J=5.6Hz), 7.98 (d, 1H, J=7.2Hz), 7.78 (d, 1H, J=10.3Hz), 7.33 (s, 1H), 7.13-7.24 (m, 5H), 6.80 (dd, 1H, J=5.6, 6.4Hz), 5.55-5.61 (m, 1H), 5.44 (d, 1H, J=16.0Hz), 5.41 (d, 1H, J=16.0Hz), 5.25 (s, 2H), 4.43-4.46 (m, 1H), 3.69-3.79 (m, 2H), 3.50 (dd, 1H, J=5.6, 16.7Hz), 3.41 (dd, 1H, J=5.6, 16.7Hz), 3.16-3.19 (m, 2H), 2.98 (dd, 1H, J=4.8, 14.3Hz), 2.79 (dd, 1H, J=9.5, 14.3Hz), 2.41 (s, 3H), 2.19-2.25 (m, 1H), 2.10-2.15 (m, 1H), 1.82-1.90 (m, 2H), 1.35 (s, 9H), 0.88 (t, 3H, J=8.0Hz).

Mass (FAB); m/e 797 (M+1)

得られた3'-N-(Boc-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) (100 mg) をトリフルオロ酢酸 (3 ml) に溶かし、1時間放置した。溶媒を留去し、メタノール(30 ml) で共沸を2回、エタノール(30 ml) で共沸を2回行った後、残渣をエーテルで洗浄して、標記化合物(80 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.52-8.62 (m, 1H), 7.94 (s, 3H), 7.79 (t, 1H, J=11.1Hz), 7.34 (s, 1H), 7.15-7.27 (m, 5H), 6.52 (s, 1H), 5.57-5.61 (m, 1H), 5.36-5.46 (m, 2H), 5.24 (s, 2H), 4.66-4.70 (m, 1H), 3.69-3.81 (m, 2H), 3.61-3.68(m, 1H), 3.40-3.47 (m, 1H), 3.15-3.23 (m, 1H), 3.01 (dd, 1H, J=4.0, 13.5Hz), 2.77 (dd, 1H, J=9.5, 13.5Hz), 2.12-2.23 (m, 2H), 1.81-1.91 (m, 2H), 0.89 (t, 3H, J=7.2Hz).

Mass (FAB); m/e 697 (M+1)

例49：3'-N-(Phe-Gly)-NH-A (A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成

Boc-Phe-Gly(771 mg) およびN-ヒドロキシスクシンイミド (300 mg) をN,N-ジメチルホルムアミド(10 ml) に溶解した。この溶液に DX-8951 のメタンスルホン酸塩(1058 mg) とトリエチルアミン (0.293 ml) を溶解したN,N-ジメチルホルムアミド(50 ml) 溶液を加えて、4℃に冷却した後、N,N'- ジシクロヘキシルカルボジイミド (494 mg) を加えて、遮光下に室温で一晩撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー (溶出液：ジクロロメタン：メタノール＝98：2溶液) で精製して3'-N-(Boc-Phe-Gly)-NH-A (A-NH_2 =DX-8951)(1.20 g)を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.29 (d, 1H, J=8.0Hz), 8.21 (t, 1H, J=4.8Hz), 7.76 (d, 1H, J=10.3Hz), 7.32 (s, 1H), 7.13-7.25 (m, 5H), 6.92 (d, 1H, J=7.2Hz), 6.49 (s, 1H), 5.56-5.61 (m, 1H), 5.44 (d, 1H, J=15.9Hz), 5.38 (d, 1H, J=15.9Hz), 5.25 (s, 2H), 4.08-4.12 (m, 1H), 3.78 (d, 1H, J=4.8Hz), 3.16-3.25 (m, 2H), 2.99 (dd, 1H, J=4.0, 13.5Hz), 2.72 (dd, 1H, J=10.3, 13.5Hz), 2.40 (s, 3H), 2.09-2.35 (m, 2H), 1.80-1.91 (m, 2H), 1.16 (s, 9H), 0.88 (t, 3H, J=8.0Hz).

Mass (FAB); m/e 741 (M+1)

上で得られた3'-N-(Boc-Phe-Gly)-NH-A (170 mg)をトリフルオロ酢酸 (4 ml) に溶かし、1時間放置した。溶媒を留去し、メタノール(10 ml) で共沸を2回、エタノール(10 ml) で共沸を2回行った後、残渣をエーテルで洗浄して、標記化合物 (100 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.88 (t, 1H, J=4.8Hz), 8.68 (d, 1H, J=8.7Hz), 8.05-8.15 (m, 3H), 7.79 (d, 1H, J=11.1Hz), 7.26-7.36 (m, 5H), 6.52 (d, 1H, J=7.2Hz), 5.57-5.62 (m, 1H), 5.43 (d, 1H, J=15.9Hz), 5.38 (d, 1H, J=15.9Hz), 5.19-5.28 (m, 1H), 4.10-4.18 (m, 1H), 3.93 (dd, 1H, J=4.8, 16.7Hz), 3.82 (dd, 1H, J=4.8, 16.7Hz), 3.17-3.24 (m, 2H), 3.14 (dd, 1H, J=4.8, 13.5Hz), 2.95 (dd, 1H, J=8.0, 13.5Hz), 2.42 (s, 3H), 2.14-2.25 (m, 2H), 1.83-1.91 (m, 2H), 0.89 (t, 3H, J=8.0Hz).

Mass (FAB); m/e 640 (M+1)

例50：3'-N-Gly-NH-A (A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成

DX-5981 のメタンスルホン酸塩 (530 mg) とトリエチルアミン(0.28 ml) をN,N-ジメチルホルムアミド(10 ml) に溶解し、4℃に冷却した後、Boc-Gly のN-ヒドロキシスクシンイミドエステル (327 mg) を加えた。遮光下に室温で一晩撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー (溶出液：ジクロロメタン：メタノール＝98：2溶液) で精製して3'-N-(Boc-Gly)-NH-A (A-NH_2 =DX-8951) (500 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.38 (d, 1H, J=8.3Hz), 7.77 (d, 1H, J=10.7Hz), 7.31 (s, 1H), 6.89-6.91 (m, 1H), 6.49 (s, 1H), 5.55-5.59 (m, 1H), 5.45 (d, 1H, J=16.1Hz), 5.38 (d, 1H, J=16.1Hz), 5.27 (d, 1H, J=19.0Hz), 5.18 (d, 1H, J=19.0Hz), 3.50-3.62 (m, 2H), 3.15-3.19 (m, 2H), 2.41 (s, 3H), 2.18-2.24 (m, 1H), 2.08-2.12

(m, 1H), 1.81-1.91 (m, 2H), 1.31 (s, 9H), 0.87 (t, 3H, J=8.0Hz).

Mass (FAB); m/e 593 (M+1)

上で得られた3'-N-(Boc-Gly)-NH-A (100 mg)をトリフルオロ酢酸 (2 ml) に溶かし、1時間放置した。溶媒を留去し、メタノール(10 ml) で共沸を2回、エタノール(10 ml) で共沸を2回行った後、残渣をエーテルで洗浄して、標記化合物(70 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.88 (d, 1H, J=8.8Hz), 8.08 (s, 3H), 7.81 (d, 1H, J=11.2Hz), 7.34 (s, 1H), 6.52 (s, 1H), 5.63-5.67 (m, 1H), 5.45 (d, 1H, J=16.7Hz), 5.40 (d, 1H, J=16.7Hz), 5.36 (d, 1H, J=19.1Hz), 5.25 (d, 1H, J=19.1Hz), 3.56 (s, 2H), 3.11-3.19 (m, 2H), 2.43 (s, 3H), 2.23-2.28 (m, 1H), 2.11-2.19 (m, 1H), 1.81-1.91 (m, 2H), 0.88 (t, 3H, J=8.0Hz).

Mass (FAB); m/e 493 (M+1)

例51：<u>カルボキシメチルデキストランポリアルコールのトリメチルアンモニウム塩の合成</u>

デキストランT500 (50 g, ファルマシア社製, 分子量 500K) を0.1 M 酢酸緩衝液 (pH5.5, 5000 ml) に溶解し、過ヨウ素酸ナトリウム(165.0 g) の水溶液(5000 ml) を加えた。遮光しながら4℃で10日間撹拌した後、エチレングリコール(35.0 ml) を加え、一晩撹拌した。反応液を8 M 水酸化ナトリウム水溶液を用いて pH 7 に調整した。水素化ホウ素ナトリウム (70 g) を加えて溶解した後、一晩撹拌した。反応液を氷冷し、酢酸で pH 5.5 に調整して4℃で1時間撹拌した後、8 M 水酸化ナトリウム水溶液を用いて pH 7.5 に調整した。得られた水溶液をバイオマックス-50 膜を用いて限外濾過法による脱塩を行なった。膜を通過しない残留溶液を凍結乾燥して、デキストランポリアルコール (20.2 g) を得た。この物質の分子量 (ゲル濾過、プルラン標準) は、159Kであった。

このデキストランポリアルコール(7.5 g) を、水酸化ナトリウム (31.5 g) を水 (225 ml) に溶かして得られる水溶液に加え、室温で溶解させた。この溶液に氷冷下でモノクロル酢酸 (45 g) を加えて溶解させた後、室温で一晩反応させた。この反応液を酢酸でpHを8に調整した後、バイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液を凍結乾燥してカルボキシメチルデキ

ストランポリアルコールのナトリウム塩(8.5 g) を得た。この物質の分子量（ゲル濾過、プルラン標準）は 274K であり、カルボキシメチル化度は0.4 であった。このカルボキシメチルデキストランポリアルコールのナトリウム塩(2.0 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、H^+ 型) カラム（直径 44 mm、長さ 210 mm)にのせ、水で溶出した。この溶出液にトリエチルアミン (4 ml) を加えた後、凍結乾燥して標記化合物(2.2 g) を得た。

例52：カルボキシメチルデキストランポリアルコール-Gly-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例51で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (200 mg) をN,N-ジメチルホルムアミド (7 ml) に溶解させた。この溶液に、例48で得た3'-N-(Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩(41 mg) のN,N-ジメチルホルムアミド (5 ml) 溶液、トリエチルアミン (0.014 ml) 、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン (100 mg) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液 (2.0 ml) 、ジエチルエーテル(25 ml) を加えて、析出した沈澱を遠心分離 (3500 rpm, 8分) により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物 (190 mg) を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液 (pH 9.0) 中での 362 nm における吸光度に基づいて定量したところ 4.5% (W/W) であった。

例53：カルボキシメチルデキストランポリアルコール-Phe-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例24で得たカルボキシメチルデキストランポリアルコールのナトリウム塩(2.5 g) を水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、Et_3N H^+ 型) カラムにのせ、水で溶出した。この溶出液を凍結乾燥してカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(2.5 g) を得た。

このカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム

塩（200 mg）をN,N-ジメチルホルムアミド(12 ml）に溶解させた。この溶液に、例49で得た3'-N-(Phe-Gly)-NH-A（A-NH_2 =DX-8951） のトリフルオロ酢酸塩(42 mg) とトリエチルアミン（0.016 ml）のN,N-ジメチルホルムアミド（5 ml）溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン（200 mg）を順次加え、遮光して室温で一晩撹拌しながら反応させた。この反応液に水（300 ml）を加え、限外濾過膜10K （フィルトロン社製）を用いて限外濾過した。膜を通過しない残留溶液を0.1 N 水酸化ナトリウム水溶液で pH 10とし、濾過膜（0.16μm 、フィルトロン社製）を通過させた。通過した溶液をバイオマックス-50 膜を用いた限外濾過法により脱塩し、次いでミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物（180 mg）を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ 6.1%（W/W）であった。

例54：カルボキシメチルデキストランポリアルコール-Gly-NH-A'(A-NH_2 =DX-8951)の合成

例51で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩（370 mg）をN,N-ジメチルホルムアミド(10 ml）に溶解させた。この溶液に、例50で得た3'-N-Gly-NH-A（A-NH_2 =DX-8951） のトリフルオロ酢酸塩(57 mg) のN,N-ジメチルホルムアミド（3 ml）溶液、トリエチルアミン（0.027 ml）、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン（185 mg）を順次加え、室温で一晩撹拌しながら反応させた。この反応液の5 mlずつを各10 ml のエタノール中に滴下した。これに3 M 塩化ナトリウム水溶液（2.0 ml）、ジエチルエーテル(25 ml）を加えて、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱を0.5 M 食塩水に溶解し、氷冷下0.1 M 水酸化ナトリウム水溶液で pH 9 とした。得られた水溶液をバイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物（290 mg）を得た。本化合物の医薬化合物残基の含量を0.1 M トリス緩衝液（pH 9.0）中での 362 nm における吸光度に基づいて定量したところ 0.5%（W/W）であった。

例55：カルボキシメチルデキストランポリアルコール-Gly-Gly-Phe-Gly-D51-7059

の合成

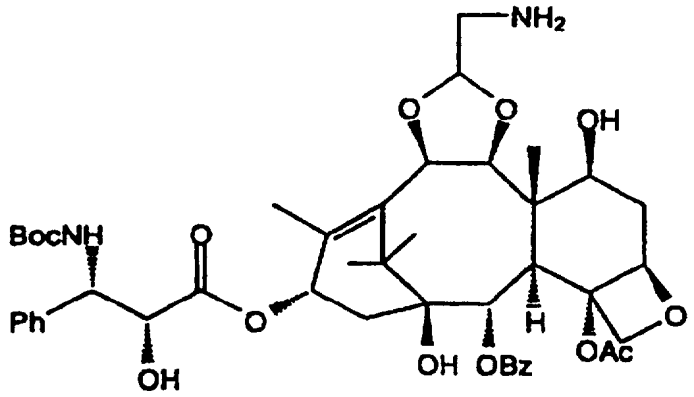
Boc-Gly-Gly-Phe-Gly-OH (200 mg) をトリフルオロ酢酸 (4 ml) に溶かし、1.5 時間撹拌した。溶媒を留去し、メタノール(10 ml) で共沸を2回、エタノール(10 ml) で共沸を2回行った後、残渣をエーテルで洗浄して、Gly-Gly-Phe-Gly-OHのトリフルオロ酢酸塩 (225 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.48 (dd, 1H, J=5.6, 5.6Hz), 8.59 (dd, 1H, J=5.6, 6.4Hz), 8.29 (d, 1H, J=4.8Hz), 7.23-7.26 (m, 4H), 7.16-7.20 (m, 1H), 4.58 (ddd, 1H, J=4.8, 4.8, 10.4Hz), 3.89 (dd, 1H, J=5.6, 16.7Hz), 3.76-3.79 (m, 2H), 3.67 (dd, 1H, J=5.6, 16.7Hz), 3.56 (s, 2H).

上で得られたGly-Gly-Phe-Gly-OHのトリフルオロ酢酸塩 (200 mg) を、水(10 ml) に溶解し、トリエチルアミンを加えて pH 9.0 に調整した後、9-フレオレニルメチルN-ヒドロキシスクシンイミジルカルボネート (200 mg) のアセトニトリル (5 ml) 溶液を加え、次いでトリエチルアミンを用いて pH 8.0-8.5 に維持しながら室温で4時間撹拌した。反応液に1.5 N 塩酸 (50 ml)を加え、析出した沈澱を濾取し、水で洗浄した後、シリカゲルカラムクロマトグラフィー (溶出液：ジクロロメタン：メタノール＝4 ： 1 溶液) で精製してFmoc-Gly-Gly-Phe-Gly-OH (151 mg)を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.28-8.32 (m, 1H), 8.08-8.12 (m, 1H), 7.85-7.89 (m, 2H), 7.68-7.72 (m, 2H), 7.57-7.65 (m, 1H), 7.38-7.43 (m, 2H), 7.29-7.34 (m, 2H), 7.20-7.25 (m, 4H), 7.14-7.17 (m, 1H), 4.45-4.52 (m, 1H), 4.26-4.30 (m, 2H), 4.19-4.24 (m, 1H), 3.77 (dd, 1H, J=5.6, 16.7Hz), 3.58-3.69 (m, 4H), 3.42-3.52 (m, 1H), 3.06 (dd, 1H, J=4.0, 13.5Hz), 2.78 (dd, 1H, J=4.0, 13.5Hz).

上で得られたFmoc-Gly-Gly-Phe-Gly-OH (24 mg) 、下記の式：



で表されるタキソール誘導体（D51-7059: 9,10-O-(2-アミノエチリデン)-13-O-[3-(tert-ブトキシカルボニルアミノ)-2-ヒドロキシ-3- フェニル]-プロパノイル-10-デアセチル-9- ジヒドロバカチン III）（20 mg）およびN-ヒドロキシスクシンイミド（7 mg）をN,N-ジメチルホルムアミド（1 ml）に溶解した。この溶液を4℃に冷却した後、N, N'-ジシクロヘキシルカルボジイミド（9 mg）を添加し、室温で一晩撹拌しながら反応させた。この反応液を減圧乾固し、残渣をシリカゲルカラムクロマトグラフィー（溶出液：ジクロロメタン：メタノール＝96：4溶液）で精製してFmoc-Gly-Gly-Phe-Gly-D51-7059（21 mg）を得た。

^{1}H-NMR ($CDCl_3$) δ: 8.06 (d, 2H, J=8.1Hz), 7.75 (d, 2H, J=8.1Hz), 7.18-7.61 (m, 23H), 7.62 (dd, 1H, J=7.2, 8.0Hz), 6.07 (dd, 1H, J=7.9, 8.8Hz), 5.98 (d, 1H, J=4.8Hz), 5.63 (d, 1H, J=8.8Hz), 5.00-5.40 (m, 4H), 4.92 (s, 1H), 4.60-4.69 (m, 2H), 4.41 (d, 2H, J=6.4Hz), 4.35 (d, 1H, J=8.0Hz), 4.29 (d, 1H, J=8.0Hz), 4.21 (t, 1H, J=7.5Hz), 3.96-4.07 (m, 3H), 3.73-3.86 (m, 4H), 3.37-3.41 (m, 1H), 3.19-3.23 (m, 1H), 3.00 (dd, 1H, J=8.0, 13.5Hz), 2.85-2.89 (m, 3H), 2.29 (s, 3H), 2.05-2.40 (m, 4H), 1.57 (s, 3H), 1.56 (s, 3H), 1.53 (s, 3H), 1.40 (s, 9H), 1.22 (s, 3H).

Mass (FAB); m/e 1413 (M+Na)

上記で得られたFmoc-Gly-Gly-Phe-Gly-D51-7059（21 mg）をジクロロメタン（1.8 ml）に溶解させ、ピペラジン（0.2 ml）を加えた後、室温で1時間反応させた。この反応液をシリカゲルカラムクロマトグラフィー（溶出液：ジクロロメタン：メタノール＝94：6溶液）で精製してGly-Gly-Phe-Gly-D51-7059（16 mg)を得た。

^{1}H-NMR ($CDCl_3$) δ: 8.10 (d, 2H, J=8.1Hz), 7.89-7.94 (m, 1H), 7.62 (dd, 1H, J=7.2, 8.0Hz), 7.45-7.50 (m, 2H), 7.17-7.42 (m, 12H), 7.10-7.16 (m, 1H), 6.97 (dd, 1H, J=5.6, 6.4Hz), 6.08 (dd, 1H, J=8.0, 8.7Hz), 6.02 (d, 1H, J=4.8Hz), 5.62 (d, 1H, J=11.1Hz), 5.23-5.30 (m, 1H), 5.23 (d, 1H, J=7.2Hz), 5.10 (s, 1H), 4.98-5.00 (m, 1H), 4.60-4.63 (m, 1H), 4.38 (d, 1H, J=8.8Hz), 4.33 (d, 1H, J=8.8Hz), 4.13 (s, 1H), 4.04 (dd, 1H, J=5.6, 16.7Hz), 3.93 (dd, 1H, J=5.6, 16.7Hz), 3.82 (d, 1H, J=7.2Hz), 3.73-3.82 (m, 2H), 3.43-3.49 (m, 1H), 3.30-3.38 (m, 2H), 3.24 (dd, 1H, J=6.4, 14.3Hz), 3.04 (dd, 1H, J=8.0, 14.3Hz), 2.89-3.07 (m, 3H), 2.30 (s, 3H), 2.01-2.50 (m, 4H), 1.70 (s, 3H), 1.62 (s, 3H), 1.61 (s, 3H), 1.40 (s, 9H), 1.26 (s, 3H).

Mass (FAB); m/e 1169 (M+1)

以上の方法で合成したGly-Gly-Phe-Gly-D51-7059(33 mg) をN,N-ジメチルホルムアミド (0.5 ml) に溶解させた。この溶液に、例24で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩 (180 mg) のN,N-ジメチルホルムアミド (7 ml) 溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン (180 mg) を順次加え、室温で一晩撹拌しながら反応させた。この反応液の4 mlずつを各10 ml のエタノール中に滴下した。それぞれに3 M 塩化ナトリウム水溶液 (2.0 ml) 、ジエチルエーテル(25 ml) を加えて、析出した沈澱を遠心分離 (2500 rpm, 8分) により集めた。この沈澱をエタノールで洗浄した後、水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシュ、Na^+ 型) カラム (直径 15 mm、長さ 85 mm) にのせ、水で溶出し、溶出液1を得た。別に、Gly-Gly-Phe-Gly-D51-7059 (10 mg) をN,N-ジメチルホルムアミド (0.5 ml) に溶解させた後、例24で得たカルボキシメチルデキストランポリアルコールのトリエチルアンモニウム塩(60 mg) のN,N-ジメチルホルムアミド (5 ml) 溶液、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(60 mg) のN,N-ジメチルホルムアミド(0.25 ml) 溶液を順次加え、室温で一晩撹拌しながら反応させた。この反応液を10 ml のエタノール中に滴下した後、3 M 塩化ナトリウム水溶液 (2.0 ml) 、ジエチルエーテル(25 ml) を加え、析出した沈澱を遠心分離 (2500 rpm, 8分) により集めた。この沈澱をエタノールで洗浄した後、水に溶解し、Bio-Rad AG 50W-X2 (200-400 メッシ

ュ、Na^+ 型）カラム（直径 15 mm、長さ 85 mm）にのせ、水で溶出し、溶出液２を得た。溶出液１と溶出液２を合した後、バイオマックス-50 膜を用いた限外濾過法により脱塩した。膜を通過しない残留溶液をミリポアフィルター(0.22 μm)で濾過した後、凍結乾燥して標記化合物（208 mg）を得た。本化合物を0.1 M 塩化ナトリウム水溶液に溶解後、GPC （カラム：東ソー TSK Gel PW-4000XL、溶媒： 0.1M NaCl、流速： 0.8 ml/min ）で分析した結果、及び本化合物の紫外線吸収スペクトル（メタノール：水＝10：１溶液、1.69 mg/ml）をそれぞれ図22および図23に示す。本化合物の医薬化合物残基の含量をメタノール：水＝10：１溶液中での 240 nm における吸光度に基づいて定量したところ、 5.3% (W/W) であった。

例56：本発明の薬物複合体の抗腫瘍作用

例11と同様の方法により Meth A 担癌マウスを作成し（１群６匹）、例15の薬物複合体について、例12と同様の方法で単回投与した場合の抗腫瘍作用を調べた。その結果、例15の薬物複合体は例12の医薬化合物自体に比べて、顕著な抗腫瘍効果の増強と有効用量域の拡大を示した。

被検化合物	投与量(mg/kg)[1)]	抑制率(%)
例15の化合物	10	100
	5	99
	2.5	95
	1.25	83

1)医薬化合物換算量

例57：本発明の薬物複合体の抗腫瘍作用

ヒト胃癌 SC-6 の腫瘍塊をヌードマウス（BALB/c-nu/nu, 雄）の右鼠径部皮下に移植して SC-6 担癌ヌードマウスを作成した（１群５匹）。移植後24日目に注射用蒸留水に溶解した例15の薬物複合体を静脈内に単回投与し、抗腫瘍作用を医薬化合物自体と比較した。その結果、例15の薬物複合体は医薬化合物自体に比べて、毒性死を示すことなく、高い抗腫瘍効果を発揮した。

被検化合物	投与量(mg/kg)	抑制率(%)	死亡マウス数／使用マウス数
医薬化合物自体	60	98	2/5
	15	61	0/5
例15の化合物	8[1)]	100	0/5
	2[1)]	71	0/5

1)医薬化合物換算量

例58：本発明の薬物複合体の抗腫瘍作用

例57と同様の方法によりヒト肺癌 QG-90担癌ヌードマウスを作成した（１群５匹）。移植後16日目に注射用蒸留水に溶解した例15の薬物複合体を静脈内に単回投与し、抗腫瘍作用を医薬化合物自体と比較した。その結果、例15の薬物複合体は医薬化合物自体に比べて、顕著な抗腫瘍効果の増強と有効用量域の拡大を示した。

被検化合物	投与量(mg/kg)	抑制率(%)	死亡マウス数／使用マウス数
医薬化合物自体	50	60	0/5
	12.5	51	0/5
例15の化合物	7[1)]	98	0/5
	1.75[1)]	97	0/5

1)医薬化合物換算量

例59：本発明の薬物複合体の抗腫瘍作用

例11と同様の方法により Meth A 担癌マウスを作成し（１群６匹）、例12と同様の方法で例41の薬物複合体を単回投与した場合の抗腫瘍作用を医薬化合物自体と比較した。その結果、例41の薬物複合体は医薬化合物自体に比べて、顕著な抗腫瘍効果の増強と有効用量域の拡大を示した。

被検化合物	投与量(mg/kg)	抑制率(%)
医薬化合物自体	100	64
	50	56
	25	34
例41の化合物	25[1)]	99
	12.5[1)]	95
	6.25[1)]	81
	3.125[1)]	61

[1)]医薬化合物換算量

例60：本発明の薬物複合体の抗腫瘍作用

例11と同様の方法により Meth A 担癌マウスを作成し（１群６匹）、例12と同様の方法で例29、例46および例47の各薬物複合体をそれぞれ単回投与した場合の抗腫瘍作用を調べた。

その結果、何れの薬物複合体も高い抗腫瘍効果と広い有効用量域を示した。

被検化合物	投与量(mg/kg)[1)]	抑制率(%)
例29の化合物	30	99
	20	99
	10	89
	5	79
例46の化合物	100	94
	80	92
	40	82
	20	75
例47の化合物	100	96

80	94
40	97
20	75

1)医薬化合物換算量

例61：本発明の薬物複合体の抗腫瘍作用

例11と同様の方法により Meth A 担癌マウスを作成し（１群６匹）、例44の薬物複合体について、例12と同様の方法で単回投与して抗腫瘍作用を医薬化合物自体（ドキソルビシン）と比較した。その結果、例44の薬物複合体は医薬化合物自体に比べて、顕著な抗腫瘍効果の増強と有効用量域の拡大を示した。

被検化合物	投与量(mg/kg)	抑制率(%)	死亡マウス数／使用マウス数
医薬化合物自体	20		6/6
	10	64	0/6
	5	39	0/6
例44の化合物	40[1)]	96	0/6
	20[1)]	96	0/6
	10[1)]	87	0/6
	5[1)]	76	0/6

1)医薬化合物換算量

例62：本発明の薬物複合体の体内動態

例11と同様の方法により Meth A 担癌マウスを作成し、例15の薬物複合体について、例12と同様の方法で単回投与（10 mg/kg：医薬化合物換算量）して、薬物複合体の各組織内における濃度推移を調べた。その結果、例15の薬物複合体は著しく高い血中滞留性、腫瘍組織への高い移行性および肝臓と小腸に対する高い腫瘍選択性を示した。結果を図24に示す。

産業上の利用可能性

例えば抗腫瘍剤である医薬化合物の残基を導入した本発明の薬物複合体は、腫瘍部位選択性に優れており、高い抗腫瘍作用を発揮できるとともに、毒性の発現も軽減されているという特徴を有している。

請　求　の　範　囲

1．1個のアミノ酸からなるスペーサー又はペプチド結合した 2～8 個のアミノ酸からなるスペーサーを介してカルボキシC_{1-4}アルキルデキストランポリアルコールと医薬化合物の残基とが結合していることを特徴とする薬物複合体。

2．カルボキシC_{1-4}アルキルデキストランポリアルコールを構成するデキストランポリアルコールが、実質的に完全にポリアルコール化可能な条件下でデキストランを処理して得られたデキストランポリアルコールであることを特徴とする、請求の範囲第1項に記載の薬物複合体。

3．カルボキシC_{1-4}アルキルデキストランポリアルコールがカルボキシメチルデキストランポリアルコールである請求の範囲第1項または第2項に記載の薬物複合体。

4．医薬化合物が抗腫瘍剤又は抗炎症剤である請求の範囲第1項ないし第3項のいずれか1項に記載の薬物複合体。

5．医薬化合物が濃度依存型の抗腫瘍作用を発現する抗腫瘍剤である請求の範囲第4項に記載の薬物複合体。

6．医薬化合物が時間に依存した抗腫瘍作用を発現する抗腫瘍剤である請求の範囲第4項に記載の薬物複合体。

7．抗腫瘍剤がドキソルビシン又は（1S,9S)-1- アミノ-9- エチル-5- フルオロ-2,3- ジヒドロ-9- ハイドロキシ-4- メチル-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオンである請求の範囲第4項に記載の薬物複合体。

8．スペーサーが -X-Z-で表されるジペプチド（-X-Z- は疎水性アミノ酸(X) と親水性アミノ酸(Z) とがそれぞれN末端側及びC末端側となってペプチド結合して形成されるジペプチドのN末端のアミノ基及びC末端のカルボキシル基から、それぞれ1個の水素原子及び1個の水酸基を除いた残基を示す）であるか、又は該ジペプチドを部分ペプチド配列として含むスペーサーである請求の範囲第5項ないし7項のいずれか1項に記載の薬物複合体。

9．疎水性アミノ酸がフェニルアラニンであり、親水性アミノ酸がグリシンであ

る請求の範囲第８項に記載の薬物複合体。

１０．スペーサーが（N 末端)-Gly-Gly-Phe-Gly-である請求の範囲第９項に記載の薬物複合体。

１１．抗腫瘍剤の残基の導入量が 1～15重量% の範囲である請求の範囲第５項ないし第１０項のいずれか１項に記載の薬物複合体。

１２．抗腫瘍剤の残基の導入量が 3～10重量% の範囲である請求の範囲第５項ないし第１０項のいずれか１項に記載の薬物複合体。

１３．抗腫瘍剤の残基の導入量が 5～6 重量% の範囲である請求の範囲第５項ないし第１０項のいずれか１項に記載の薬物複合体。

１４．H_2N-Gly-Gly-Phe-Gly-COOHで示されるペプチドのN末端がカルボキシメチルデキストランポリアルコールのカルボキシル基に酸アミド結合しており、該ペプチドのC末端が（1S,9S)-1- アミノ-9- エチル-5- フルオロ-2,3- ジヒドロ-9- ハイドロキシ-4- メチル-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオンの1-アミノ基と酸アミド結合した請求の範囲第１項に記載の薬物複合体。

１５．（1S,9S)-1- アミノ-9- エチル-5- フルオロ-2,3- ジヒドロ-9- ハイドロキシ-4- メチル-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオン残基の導入量が 2～10重量% の範囲である請求の範囲第14項に記載の薬物複合体。

１６．カルボキシC_{1-4}アルキルデキストランポリアルコールが分子量 5,000ないし 500,000の範囲のカルボキシメチルデキストランポリアルコールであり、カルボキシメチル化度が 0.01 ～2.0 の範囲である請求の範囲第14項又は第15項に記載の薬物複合体。

１７．カルボキシC_{1-4}アルキルデキストランポリアルコールが分子量50,000～450,000 の範囲のカルボキシメチルデキストランポリアルコールであり、カルボキシメチル化度が 0.1～1.0 の範囲である請求の範囲第14項又は第15項に記載の薬物複合体。

１８．カルボキシC_{1-4}アルキルデキストランポリアルコールが分子量 200,000～400,000 の範囲のカルボキシメチルデキストランポリアルコールであり、カルボキシメ

チル化度が 0.3～0.5 の範囲である請求の範囲第14項又は第15項に記載の薬物複合体。

１９．(1S,9S)-1- アミノ-9- エチル-5- フルオロ-2,3- ジヒドロ-9- ハイドロキシ-4- メチル-1H,12H-ベンゾ[de]ピラノ[3',4':6,7] インドリジノ[1,2-b] キノリン-10,13(9H,15H)- ジオン残基の導入量が 5～6 重量% の範囲であり、カルボキシC_{1-4}アルキルデキストランポリアルコールの分子量が約 228,000であり、カルボキシメチル化度が約 0.4である請求の範囲第14項に記載の薬物複合体。

２０．医薬化合物を結合するための薬物送達のキャリアーであって、カルボキシC_{1-4}アルキルデキストランポリアルコールからなる薬物送達のキャリアー。

２１．カルボキシC_{1-4}アルキルデキストランポリアルコールの分子量が 5,000～500,000 の範囲であり、カルボキシC_{1-4}アルキル化度が0.01～2.0 の範囲である請求の範囲第20項に記載の薬物送達のキャリアー。

２２．カルボキシC_{1-4}アルキルデキストランポリアルコールの分子量が50,000～450,000 の範囲であり、カルボキシC_{1-4}アルキル化度が 0.1～1.0 の範囲である請求の範囲第20項に記載の薬物送達のキャリアー。

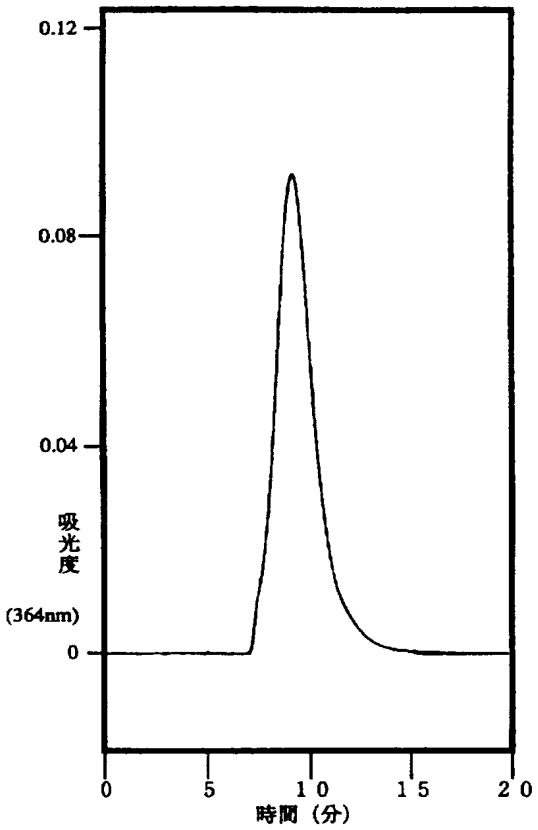
２３．カルボキシC_{1-4}アルキルデキストランポリアルコールの分子量が 200,000～400,000 の範囲であり、カルボキシC_{1-4}アルキル化度が 0.3～0.5 の範囲である請求の範囲第20項に記載の薬物送達のキャリアー。

２４．カルボキシC_{1-4}アルキルデキストランポリアルコールがカルボキシメチルデキストランポリアルコールである請求の範囲第20項ないし第23項のいずれか１項に記載の薬物送達のキャリアー。

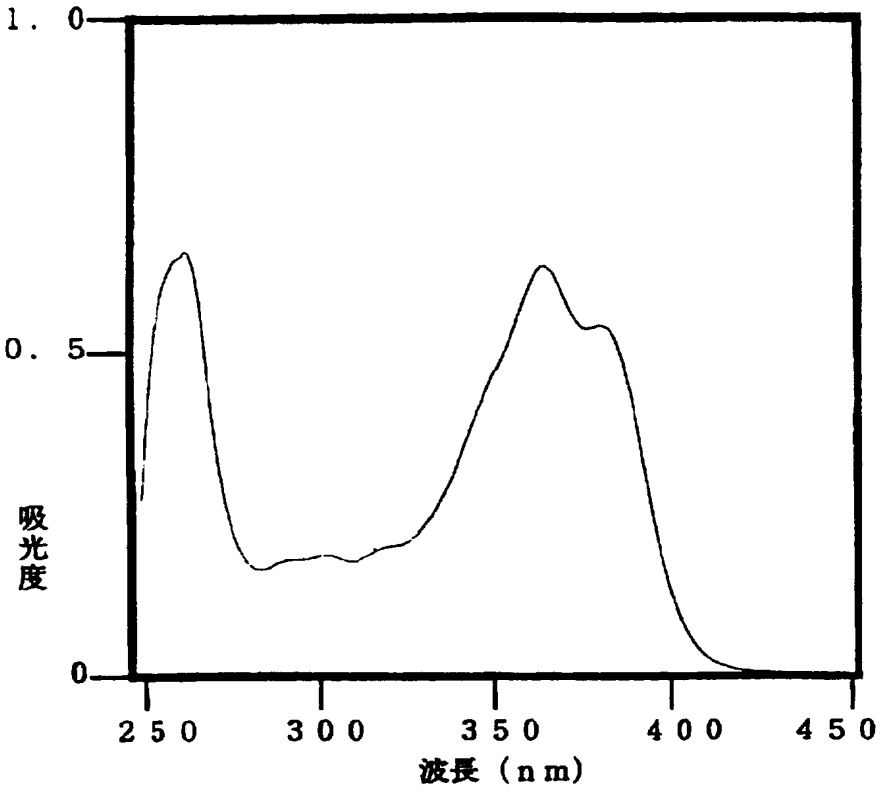
２５．医薬化合物の残基と結合したカルボキシC_{1-4}アルキルデキストランポリアルコールを含む薬物複合体の製造のためのカルボキシC_{1-4}アルキルデキストランポリアルコールの使用。

２６．医薬化合物の残基とカルボキシC_{1-4}アルキルデキストランポリアルコールとがスペーサーを介して、又はスペーサーを介さずに結合した薬物複合体の製造のためのカルボキシC_{1-4}アルキルデキストランポリアルコールの使用。

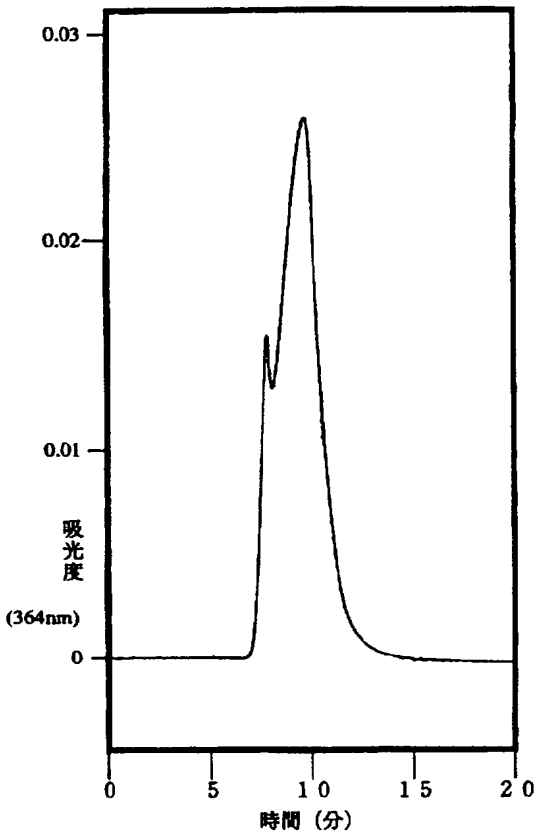
第１図


0.12
0.08
0.04
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

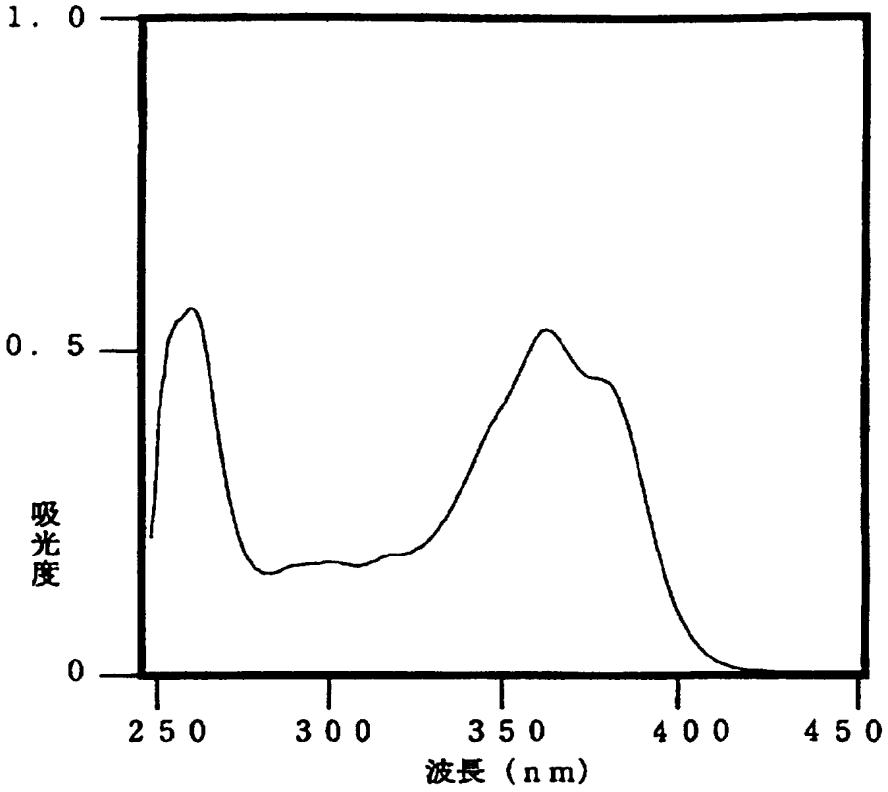
第２図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

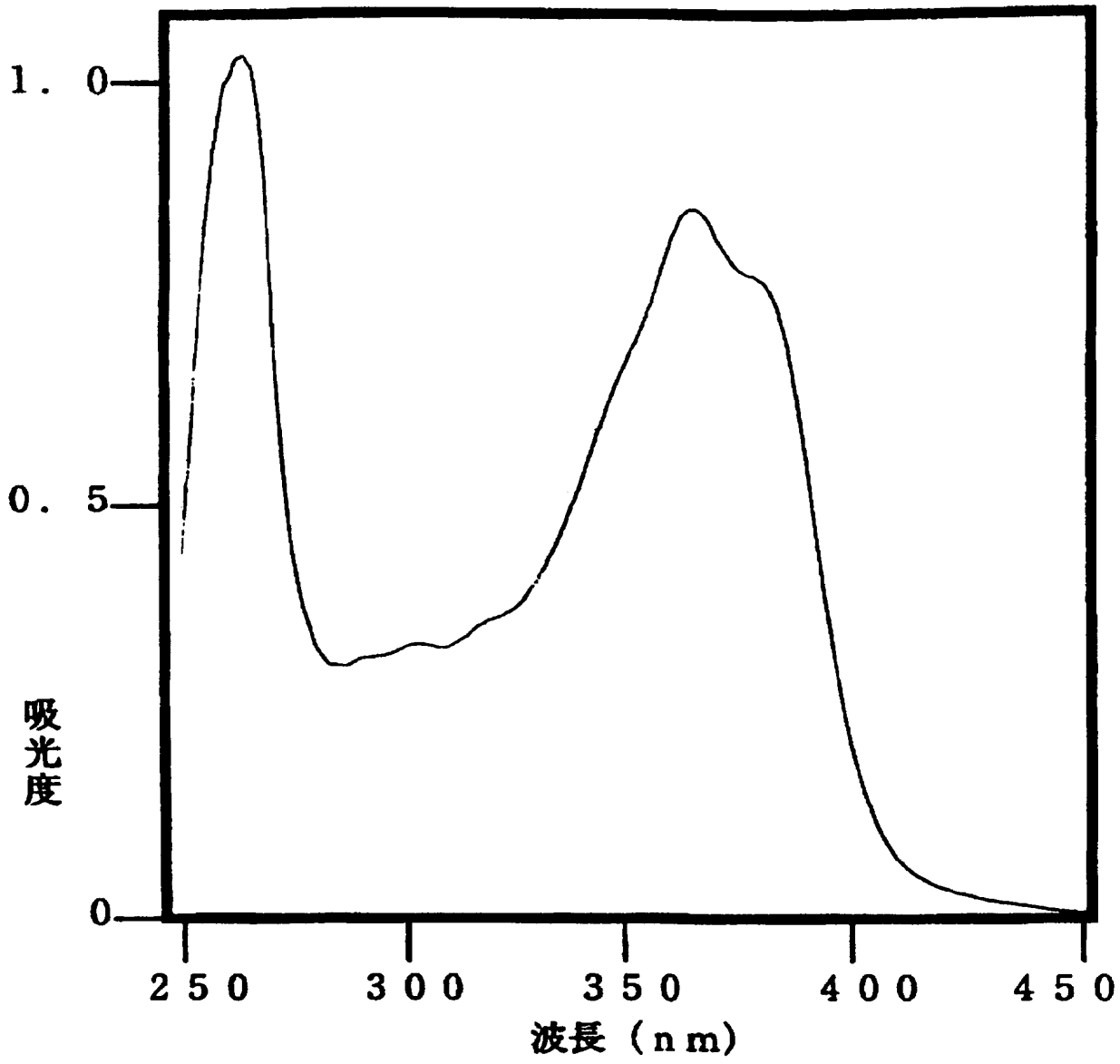
第３図


0.03
0.02
0.01
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

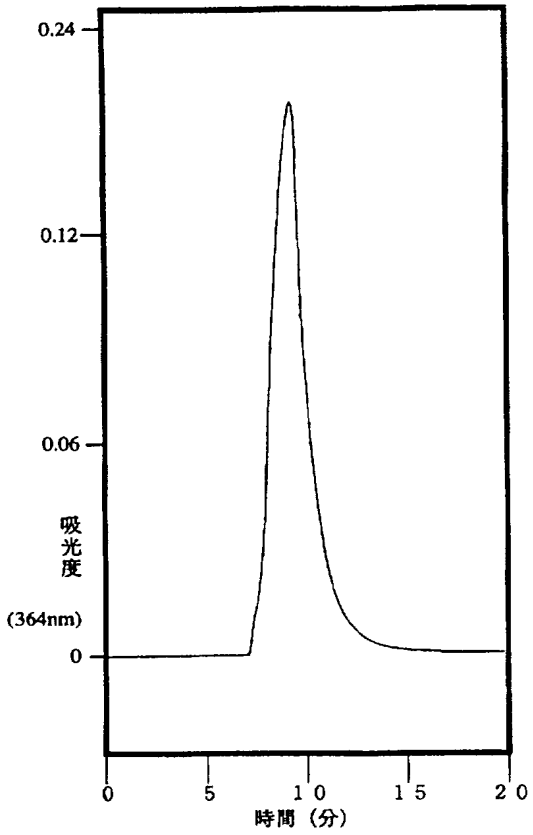
第４図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

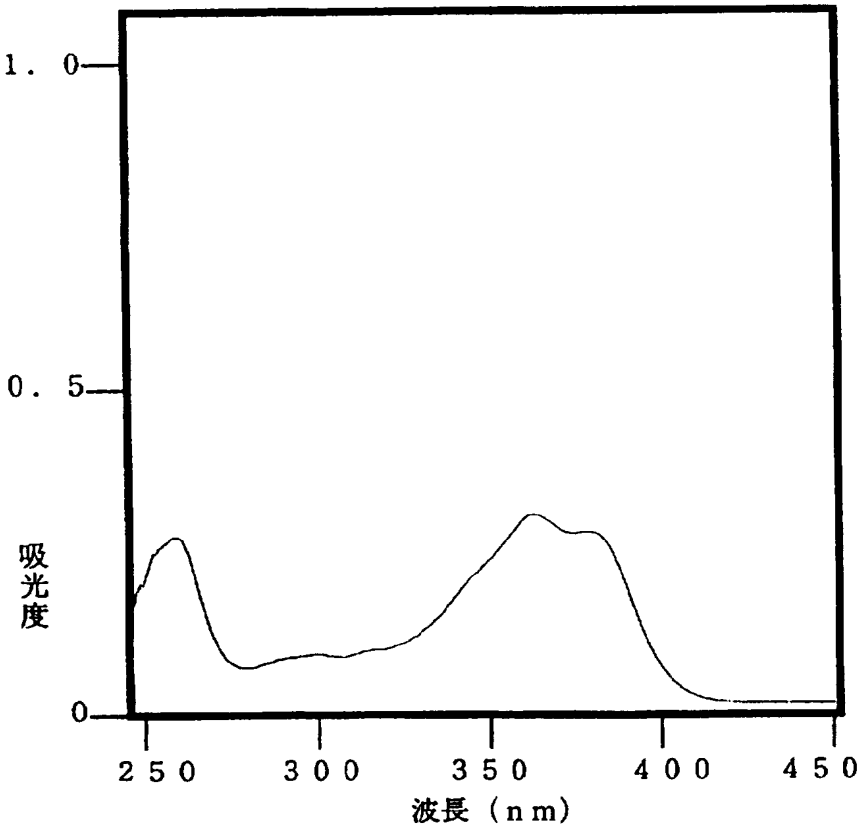
第５図



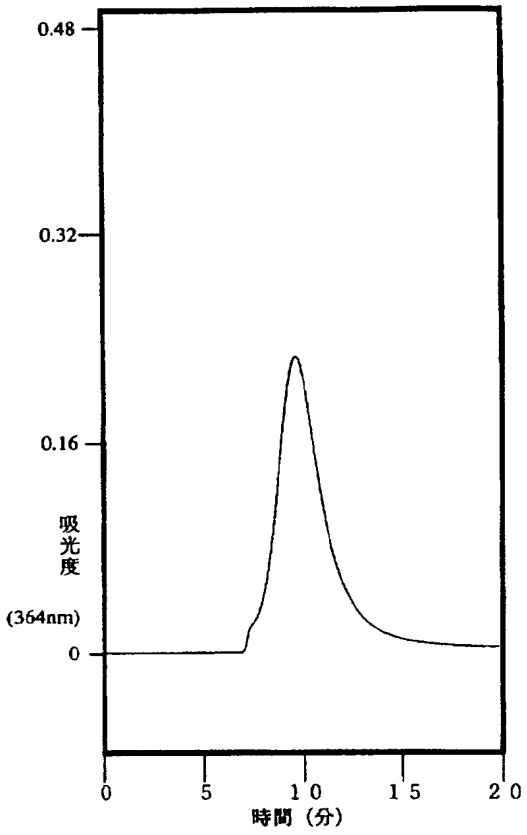
第６図


0.24
0.12
0.06
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

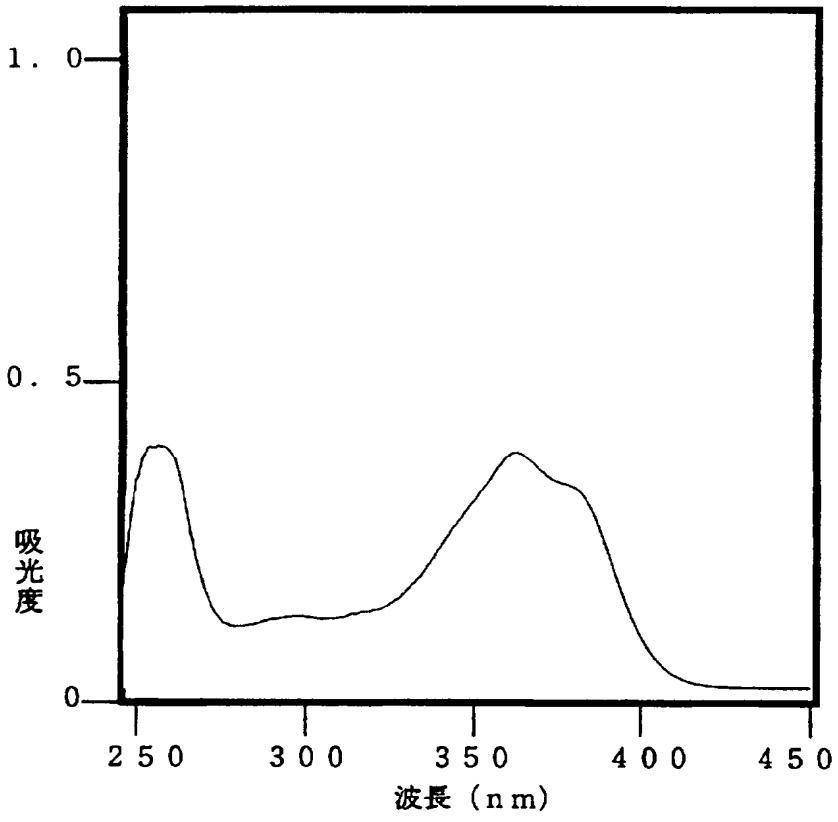
第７図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

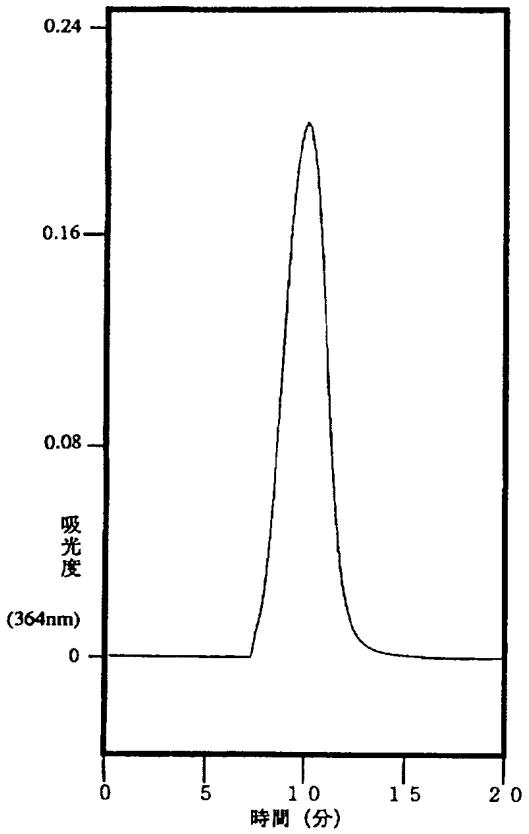
第８図


0.48
0.32
0.16
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

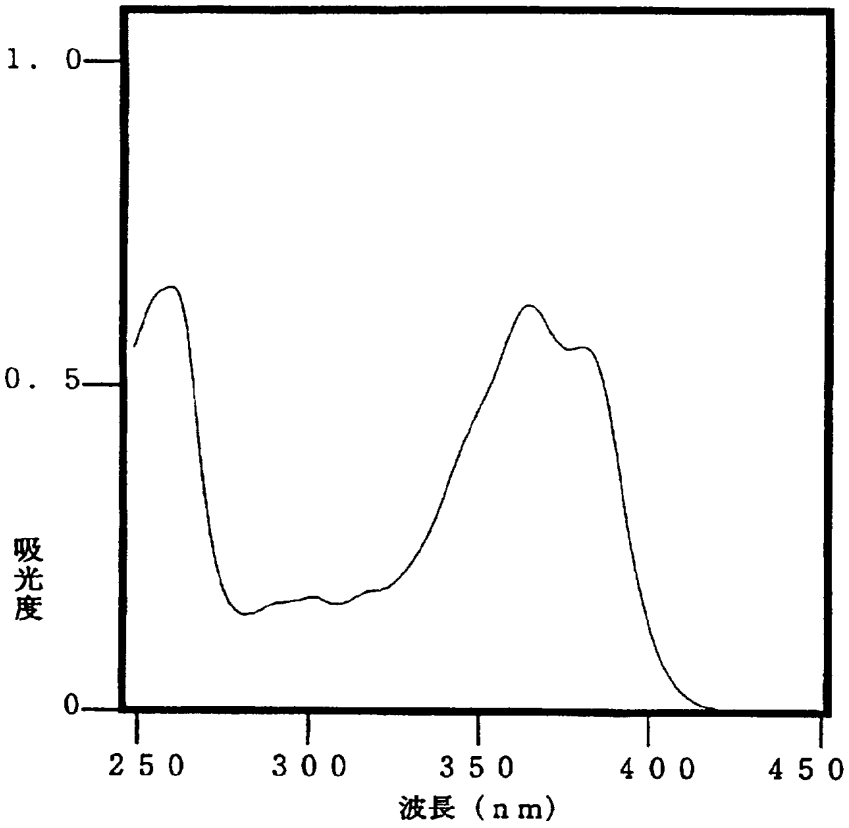
第９図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（ｎｍ）

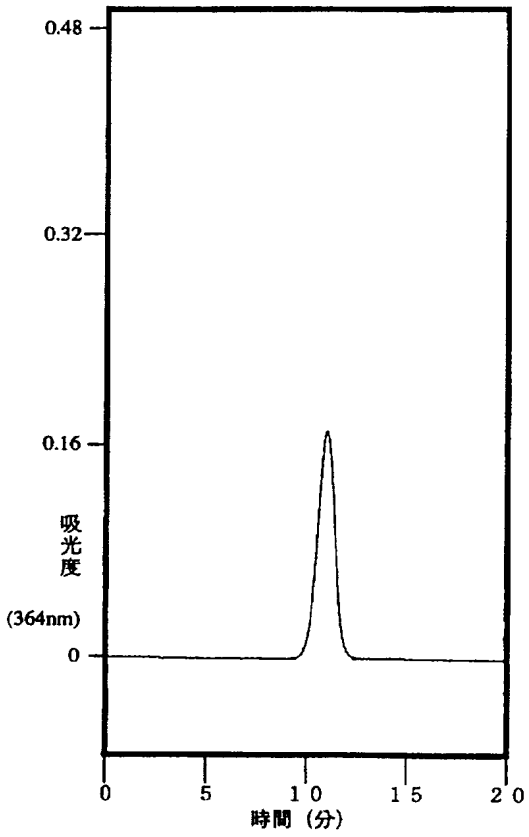
第10図


0.24
0.16
0.08
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

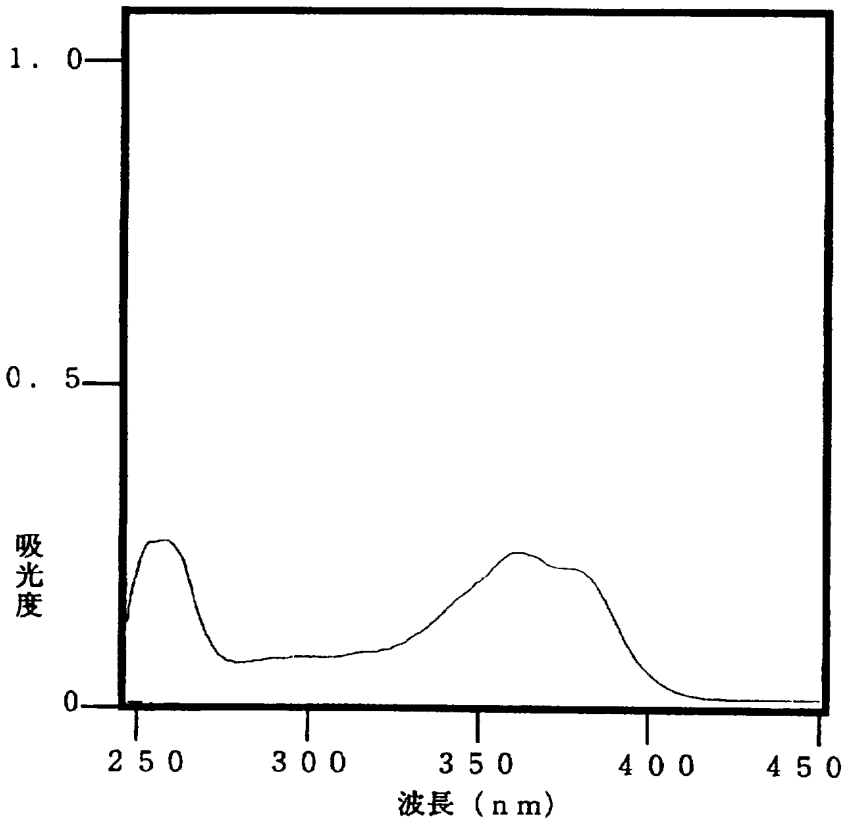
第11図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

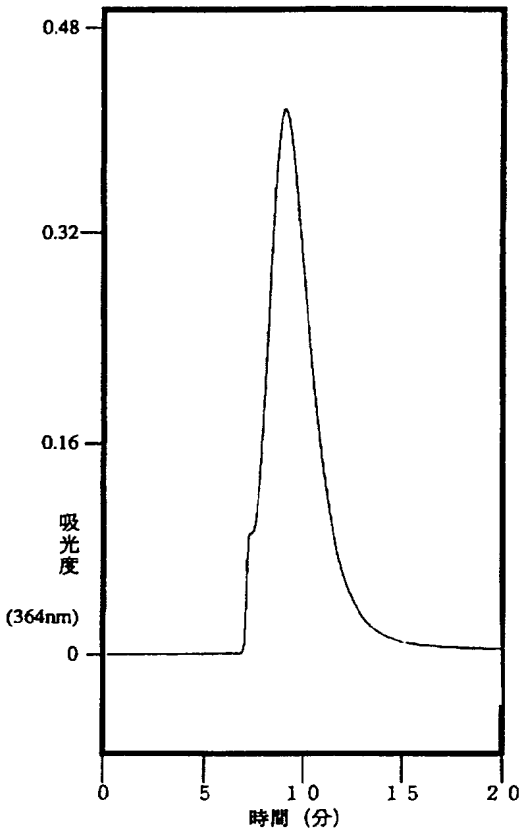
第12図


0.48
0.32
0.16
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

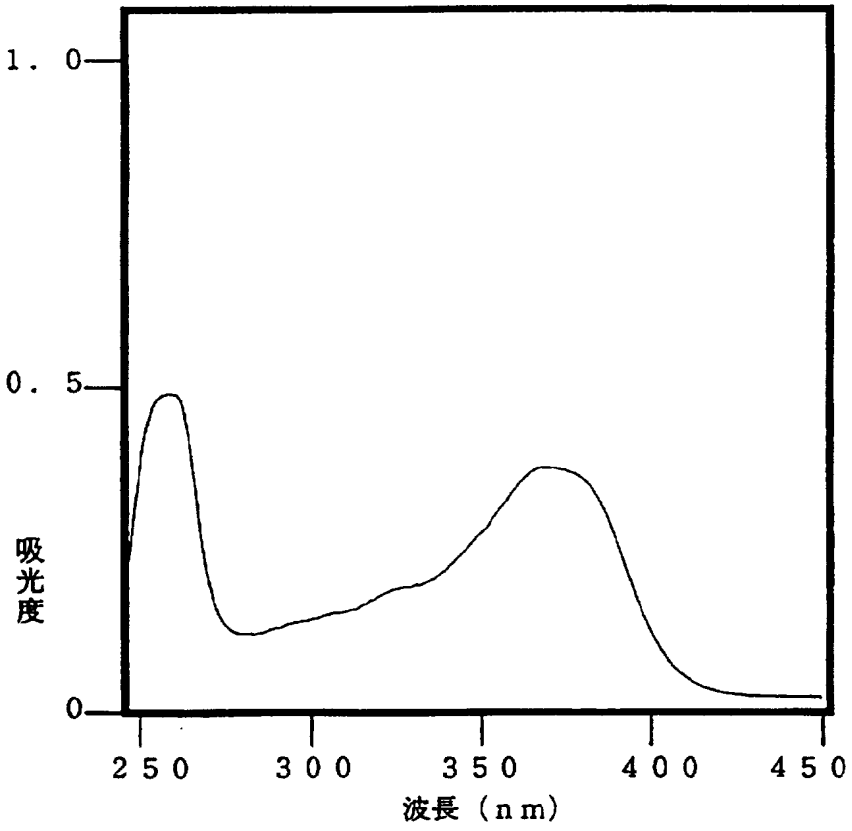
第13図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

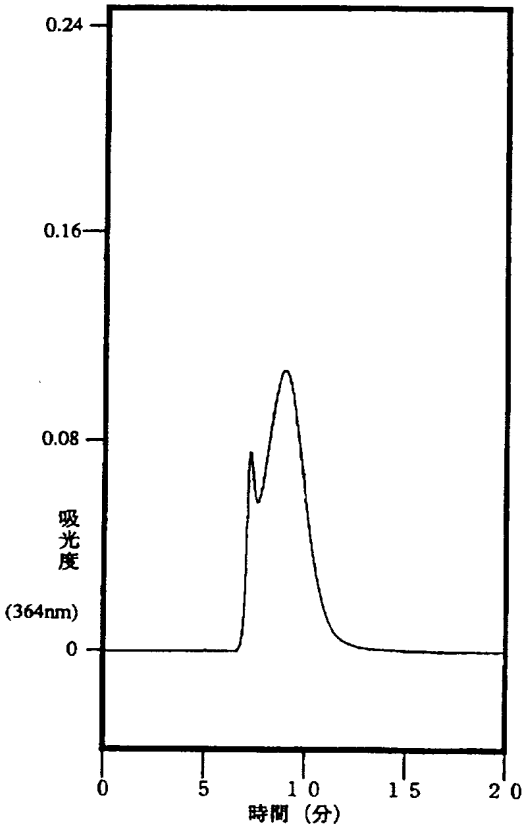
第14図


0.48
0.32
0.16
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

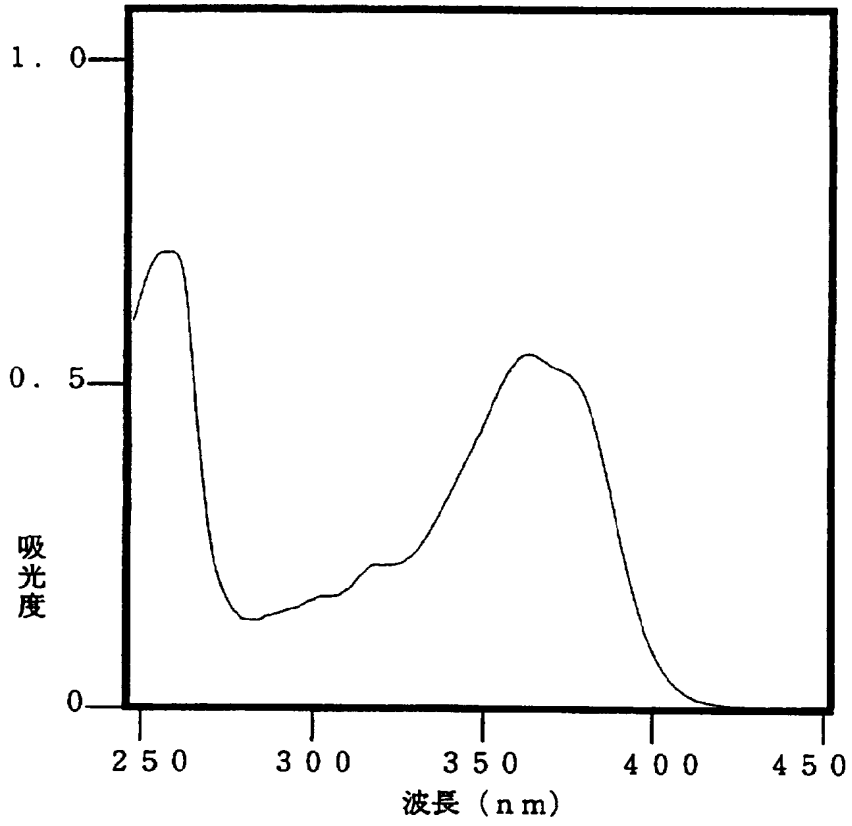
第15図


1．0
0．5
吸光度
0
250
300
350
400
450
波長（nm）

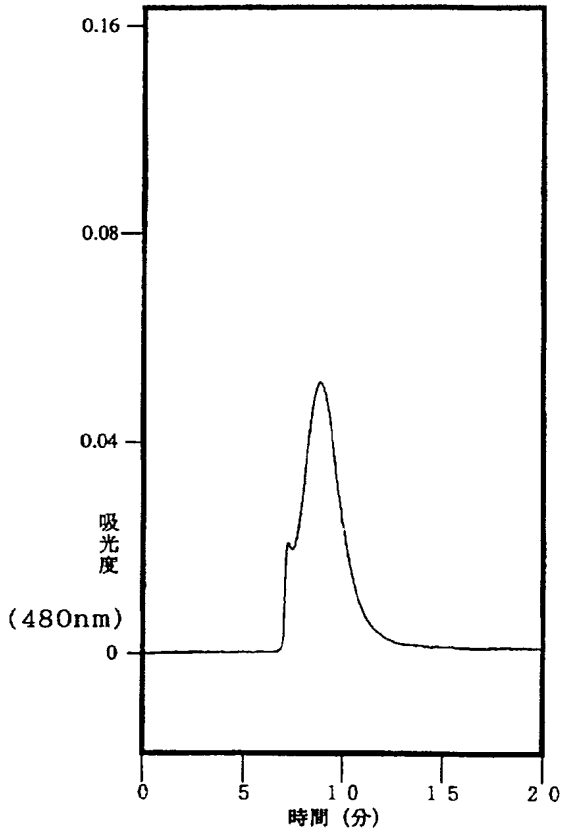
第16図


0.24
0.16
0.08
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

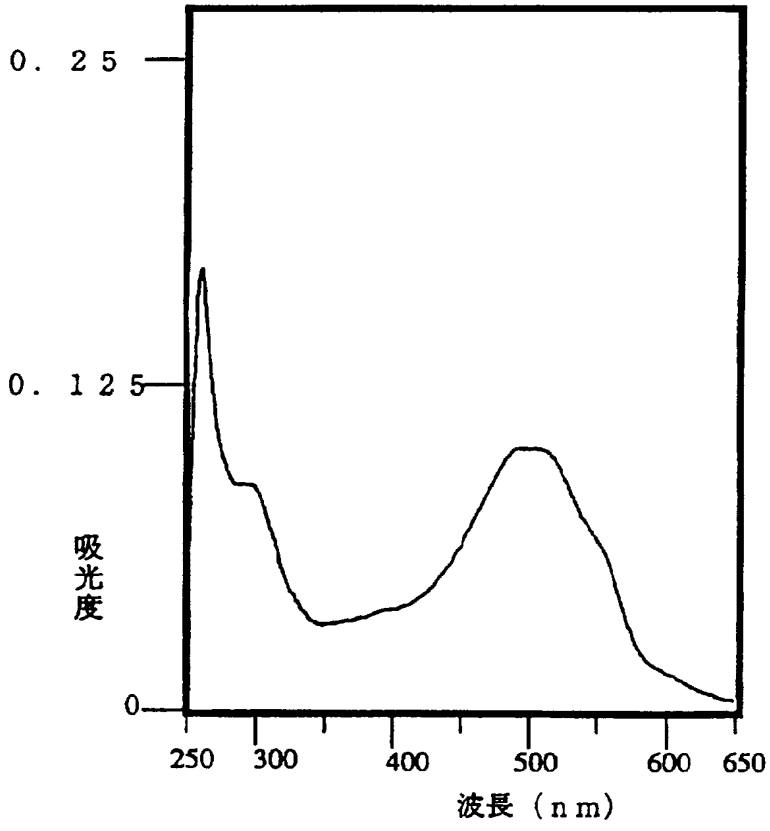
第17図


1．0
0．5
0
吸光度
250
300
350
400
450
波長（nm）

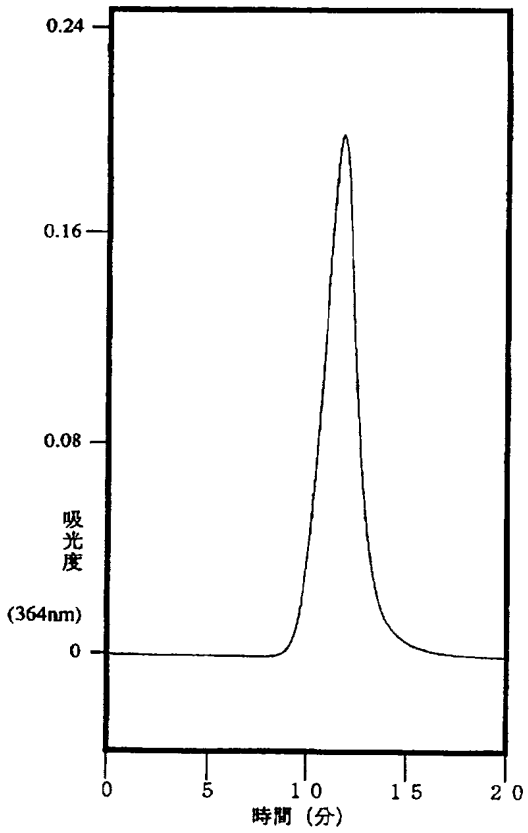
第18図


0.16
0.08
0.04
吸光度
(480nm)
0
0
5
10
15
20
時間（分）

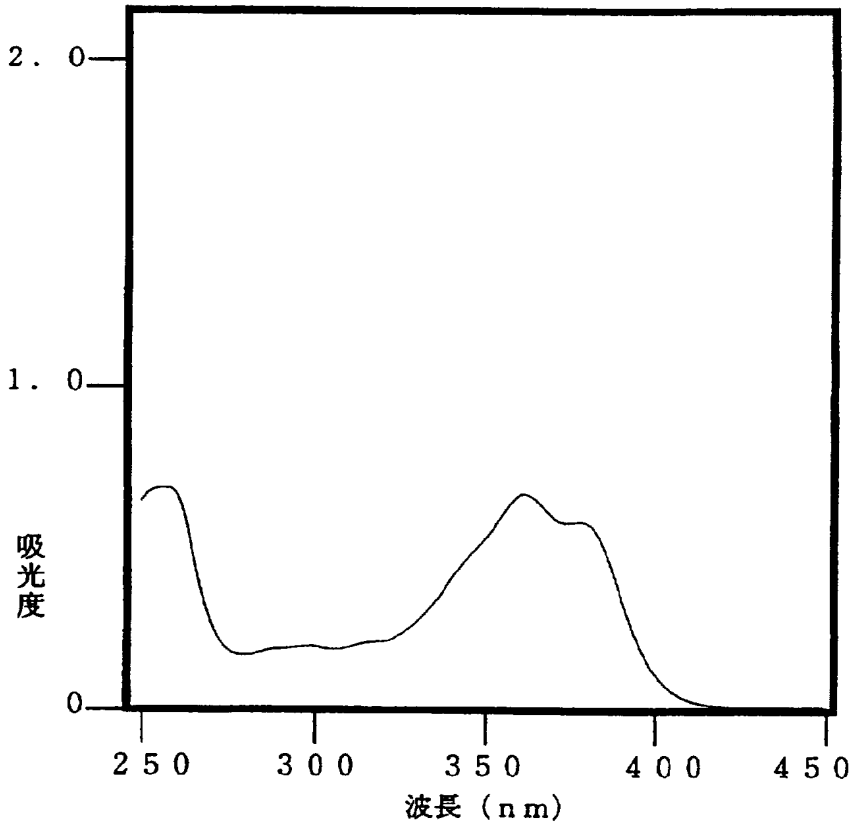
第19図


0．25
0．125
吸光度
0
250
300
400
500
600
650
波長（nm）

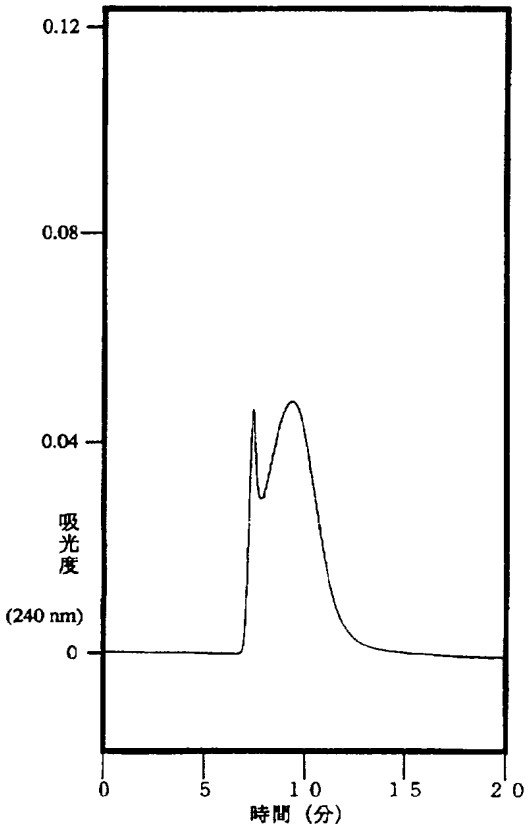
第20図


0.24
0.16
0.08
吸光度
(364nm)
0
0
5
10
15
20
時間（分）

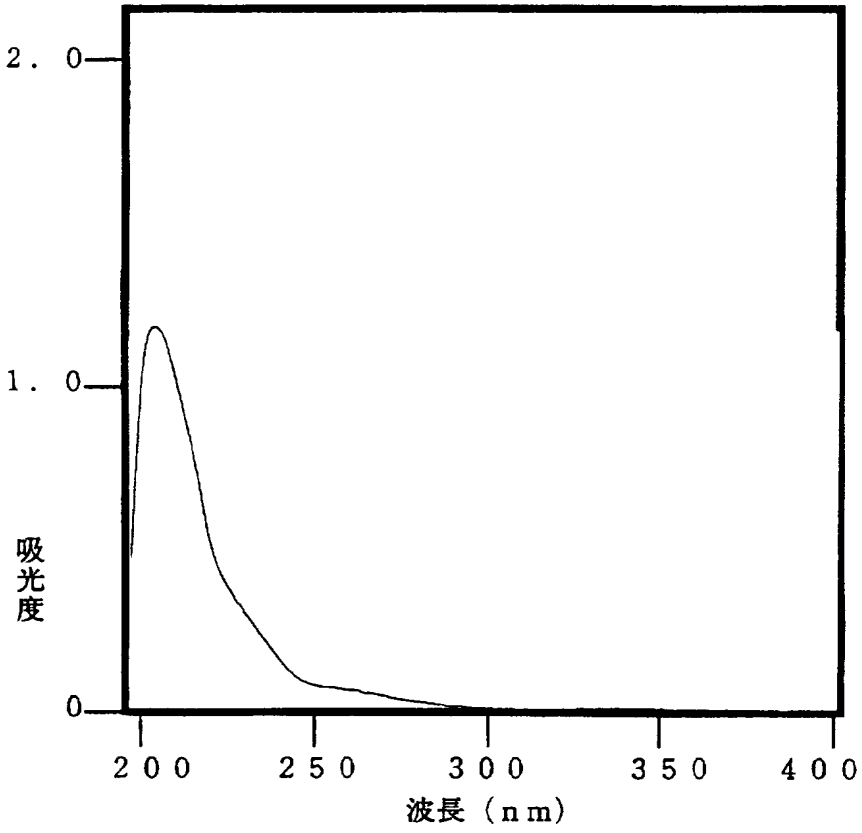
第21図


2．0
1．0
0
吸光度
250
300
350
400
450
波長（nm）

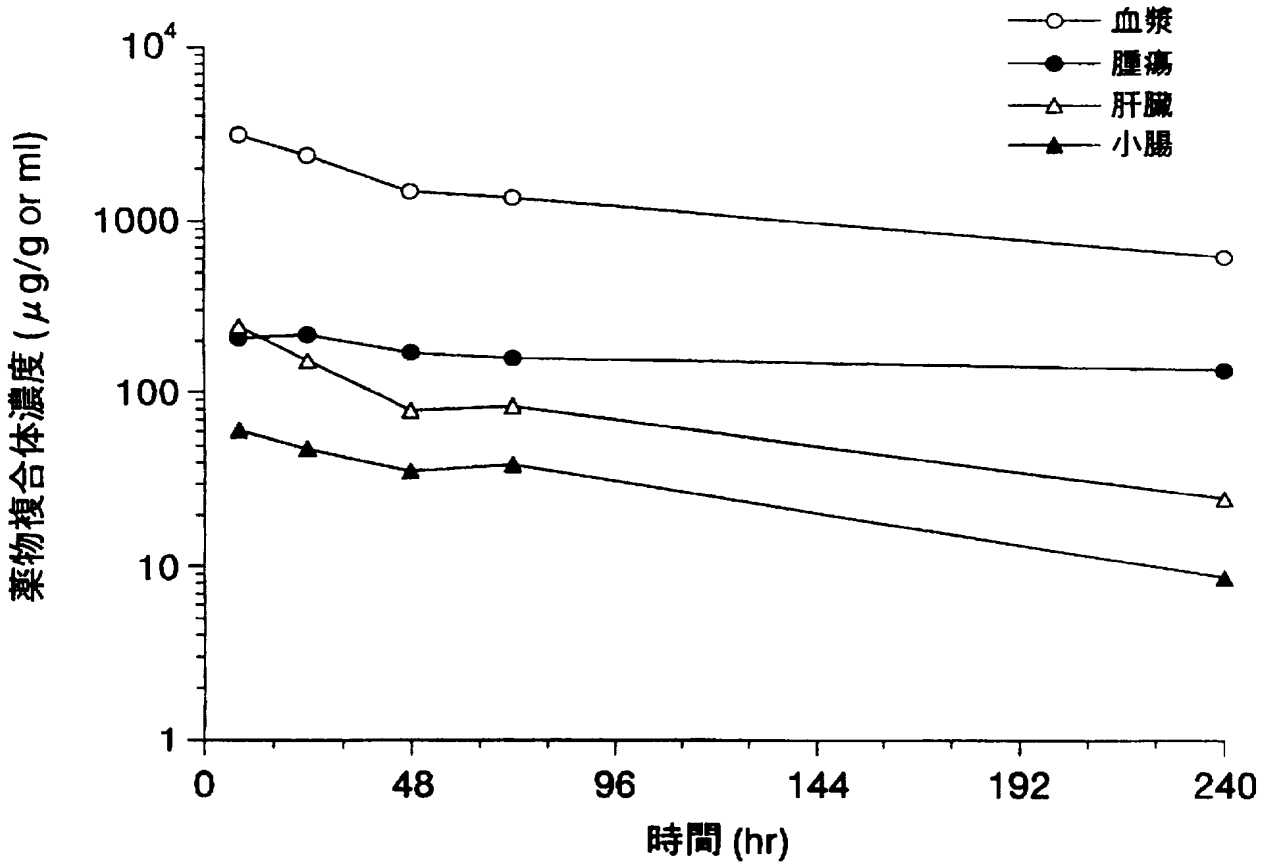
第22図


0.12
0.08
0.04
吸光度
(240 nm)
0
0
5
10
15
20
時間（分）

第23図


2．0
1．0
吸光度
0
200
250
300
350
400
波長（nm）

第24図


血漿
腫瘍
肝臓
小腸
薬物複合体濃度 (μg/g or ml)
10^4
1000
100
10
1
0
48
96
144
192
240
時間 (hr)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/01914

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl^6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl^6 A61K47/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 5-39306, A (Drug Delivery System Institute, Ltd.), February 19, 1993 (19. 02. 93)(Family: none)	1 - 26
A	JP, 59-220197, A (Snow Brand Milk Products Co., Ltd.), December 11, 1984 (11. 12. 84)(Family: none)	1 - 26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general atate of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an nral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
August 11, 1997 (11. 08. 97)	August 19, 1997 (19. 08. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

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国際調査報告

国際出願番号　ＰＣＴ／ＪＰ９７／０１９１４

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Ｉｎｔ．Ｃｌ[6]　Ａ６１Ｋ４７／４８

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Ｉｎｔ．Ｃｌ[6]　Ａ６１Ｋ４７／４８

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
Ａ	ＪＰ，５－３９３０６，Ａ（株式会社デイ・デイ・エス研究所），19.2月.1993 (19.02.93)，（ファミリーなし）	１－２６
Ａ	ＪＰ，５９－２２０１９７，Ａ（雪印乳業株式会社），11.12月.1984(11.12.84)，（ファミリーなし）	１－２６

☐ Ｃ欄の続きにも文献が列挙されている。

☐ パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」先行文献ではあるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
「Ｐ」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「Ｔ」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「Ｘ」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Ｙ」特に関連のある文献であって、当該文献と他の１以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「＆」同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
１１．０８．９７	19.08.97

国際調査機関の名称及びあて先	特許庁審査官（権限のある職員）		
日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００ 東京都千代田区霞が関三丁目４番３号	弓ム 真　謙二　印	４Ｃ	７４３３
	電話番号　０３－３５８１－１１０１　内線　３４５２		

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（１９９２年７月）

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Ex. 1038

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
PD20-9009WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/JP2015/002020	10 April 2015 (10-04-2015)	10 April 2014 (10-04-2014)
Applicant		
DAIICHI SANKYO COMPANY, LIMITED		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

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Ex. 1038

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2015/002020

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

OnCusp

International application No Ex. 1038
PCT/JP2015/002020

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/019024 A2 (IMMUNOGEN INC [US]; SETIADY JULIANTO [US]; SKALETSKAYA ANNA [US]; RUI) 9 February 2012 (2012-02-09) cited in the application Claims 1, 40	1-38
A	WO 2012/064733 A2 (MEDIMMUNE LLC [US]; DIMASI NAZZARENO [US]) 18 May 2012 (2012-05-18) Figure 11; page 120, example 8	1-38
X,P	WO 2014/061277 A1 (DAIICHI SANKYO CO LTD [JP]) 24 April 2014 (2014-04-24) claims 1, 50-54	1-38

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 2015	20/07/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bettio, Andrea

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No Ex. 1038
PCT/JP2015/002020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012019024 A2	09-02-2012	NONE	
WO 2012064733 A2	18-05-2012	EP 2638066 A2	18-09-2013
		JP 2014505463 A	06-03-2014
		US 2013330350 A1	12-12-2013
		WO 2012064733 A2	18-05-2012
WO 2014061277 A1	24-04-2014	TW 201420118 A	01-06-2014
		WO 2014061277 A1	24-04-2014

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Electronic Acknowledgement Receipt

EFS ID:	40723681
Application Number:	17010162
International Application Number:	
Confirmation Number:	6661
Title of Invention:	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE
First Named Inventor/Applicant Name:	Atsuko SAITO
Customer Number:	22428
Filer:	Michael D. Kaminski/Tuan Tran
Filer Authorized By:	Michael D. Kaminski
Attorney Docket Number:	098065-0278
Receipt Date:	01-OCT-2020
Filing Date:	02-SEP-2020
Time Stamp:	11:02:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	B123--WO_2008_144891A1.pdf	6357316 2ac7ce6d4c98b0a32d6ee1f72b3d403deec6cc0f	no	115

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2	Foreign Reference	B124--WO_2011_011474A1.pdf	3261970 31b6c698d3b65d4d640bf41f772e06daecac5abd	no	64
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3	Foreign Reference	B125--WO_2011_021397A1.pdf	10820544 1659887212e500128f786d16eabc8ac4b5686ac5	no	334
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4	Foreign Reference	B126--WO_2011_068845A1.pdf	4852233 7f77c222c7188ae2a5e1f482d66643b99727f3e4	no	89
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5	Foreign Reference	B127--WO_2011_145744A1.pdf	5677878 2b16d9cf3298308a582e4f75e3fe300c091b89c1	no	138
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6	Foreign Reference	B128--WO_2011_155579A1.pdf	5696673 401e6c300f565d7bbb4db9bb540cdc1283532d65	no	144
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7	Foreign Reference	B129--WO_2012_019024A2.pdf	7370009 d0bcdc35b8b645a598655f421eb1b5343ac3e19c	no	150
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8	Foreign Reference	B130--WO_2012_064733A2.pdf	8342969 08a9e834cd56f24236dc22c7a14eb665f95c cea5	no	169
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9	Foreign Reference	B131--WO_2013_068946A2.pdf	7301504 f7e10c75aed2d93c9a2952d688acab10d73 31f5a	no	149
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10	Foreign Reference	B132--WO_2013_077458A1.pdf	8328448 19aecd06284a17559e8bc84977e218f6005 8b6d6	no	207
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11	Foreign Reference	B133--WO_2013_163229A1.pdf	5733884 a0a9c7289c2bd46949d1695a6df63842b51 45d0c	no	108
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12	Foreign Reference	B134--WO_2013_188740A1.pdf	15797848 b15d011d4c02203eddd149078415d92eb1 831c1a	no	296
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13	Foreign Reference	B135--WO_2014_061277A1.pdf	10782710 a33f8bd4978868d2168dbc5a621bf7484fe4 83c8	no	327
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14	Foreign Reference	B136--WO_2014_107024A1.pdf	6520188 50b924dd4fb83f0dfed6a068e2f650286c52 7d27	no	182

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15	Foreign Reference	B137--WO_2015_155998A1.pdf	20203990 6ce9b4903ba62b81dba7564b15308e3b6c03a341	no	264
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16	Foreign Reference	B138--WO_97_46260A1.pdf	2159793 c9370fbca763ff78c985bfabf9f26dfe6d8cba85	no	82
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17	Non Patent Literature	B139--ACCHIONE_ET_AL__Impact_of_linker_and_con.pdf	1931701 f5669016ce7e053d211f1d2ce6933281c8a0cba7	no	12
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18	Non Patent Literature	B140--ADAMS_ET_AL____Humanization_of_a_recombi.pdf	2180370 51bbed73bae6a87ecf816e56be6059551f9d1e6b	no	11
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19	Non Patent Literature	B141--ALIMANDI_ET_AL____Cooperative_signaling_.pdf	1531965 abef4d913e4f58c857cb78c60562835bbd17480f	no	9
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20	Non Patent Literature	B142--ALLEY_ET_AL____Antibody_drug_conjugates_.pdf	1004531 e8deb9dab17482070a6cbe3c8a9c3245210c2151	no	9
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21	Non Patent Literature	B143--Allowance_dated_July_4__2017__in_Japanes.pdf	74250 a28e9e2e2edc56f0486af82998d173950d45e7ba	no	3
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22	Non Patent Literature	B144--Allowance_issued_in_connection_with_Taiw.pdf	123466 06971e23b167becbacebb14fb927ffebb26ceb3c	no	3
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23	Non Patent Literature	B145--Australian_Intellectual_Property_Office_.pdf	191942 8d3719abc487ba5f556bf27d9e31978387e321af	no	5
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24	Non Patent Literature	B146--BARGINEAR_ET_AL____Trastuzumab_DM1__A_Re.pdf	772322 1306c455eb48756418c650433980e5dc81d5407d	no	6
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25	Non Patent Literature	B147--BAROK_ET_AL__Trastuzumab_DM1_is_highly_e.pdf	946458 488365751f5440ea6ed965068bde3baaf1c6bd3f	no	9
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26	Non Patent Literature	B148--BASELGA_ET_AL____Phase_II_Study_of_Weekl.pdf	1456508 6a9debe3fd84725a5212ee97744b9c7e5bb47673	no	8
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27	Non Patent Literature	B149--BASU_ET_AL____The_Epithelial_Carcinoma_A.pdf	1921265 bf8126987fe2f8fd12a3e67c7cf8576d98df3140	no	8
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28	Non Patent Literature	B150--BECK__Alain___The_Next_Generation_of_Ant.pdf	1019967 46b385aa0226ba9f8d9f62ef597cc22176dde4ec	no	8
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29	Non Patent Literature	B151--Behrens_et_al__Methods_for_site_specific.pdf	1390145 2f2b85ba89f24107ed23950cbd643156f8979eb5	no	9
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30	Non Patent Literature	B152--Blok_et_al____Cytoplasmic_Overexpression.pdf	1459243 0d44f2b5f1fbc667c88d216d5112e11787e1ec42	no	11
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31	Non Patent Literature	B153--BURKE_PATRICK_J_et_al__Design__Synthesis.pdf	1170005 280af34b5fa8f859d632f1f6b23c990e7284d2dc	no	9
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32	Non Patent Literature	B154--CALABRESE_ET_AL____Assignment_of_TACSTD1.pdf	217818 05b41c89d0ea0658c5be8ea112017c5cc1da69ef	no	2
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33	Non Patent Literature	B155--Canadian_Examiner_s_Interview_Summary_is.pdf	691127 5f4525a0d2a0a387a995057d1954953b5ac502e5	no	2
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34	Non Patent Literature	B156--Canadian_Office_Action_dated_April_13__2.pdf	468140 6de4c163c6dba6b9dd564db1283545c80df1a704	no	9
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35	Non Patent Literature	B157--Chinese_Office_Action_dated_November_1__.pdf	1405904 d77213bccbb8ca555af73727bc7a18aff73fd328	no	24
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36	Non Patent Literature	B158--Chinese_Office_Action_dated_November_8__.pdf	150427 8e2eb3376edd342ab2455b624270ada60c444e2e	no	4
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37	Non Patent Literature	B159--Chinese_Office_Action_issued_to_correspo.pdf	348703 cf3513e647e682102306006c9aa18b36f361e566	no	5
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38	Non Patent Literature	B160--Chinese_Search_Report_dated_June_24__202.pdf	34690 4ab0a4f11f61e72f6a36037d53ec8ec2125fe8e3	no	1
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39	Non Patent Literature	B161--Colombian_Reconsideration_Petition_dated.pdf	1657633 40be2b98cb3e0fdee908825cb95c058b86be3366	no	36
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40	Non Patent Literature	B162--COUSSENS_ET_AL____Tyrosine_Kinase_Recept.pdf	1810309 c7b1c75302215f58e1cd6e516ab3eb357df6f085	no	8
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41	Non Patent Literature	B163--D__LOO_ET_AL___Development_of_an_Fc_Enha.PDF	1705108 9902523d1d89c4828870a4a974874aa97929b544	no	13
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42	Non Patent Literature	B164--DAMLE__Nitin_K____Tumour_targeted_chemot.pdf	936951 eb3952adcffec15a45b995902e55b97284346401	no	8
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43	Non Patent Literature	B165--DE_JAGER_ET_AL____DX_8951f__Summary_of_P.pdf	836484 86ee84730add60404b077f07bb9da96079aeffd3	no	16
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44	Non Patent Literature	B166--DEFAZIO_ET_AL____Expression_of_c_erbB_Re.pdf	2639451 449cedeca4ce0ccb25d454275e2696e83a3d0a7b	no	12
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45	Non Patent Literature	B167--DI_FIORE_ET_AL____erbB_2_Is_a_Potent_Onc.pdf	1223534 a870c5bb1969efed67f739f5154b19b8e9fb3f90	no	5
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46	Non Patent Literature	B168--DOSIO_et_al____Antibody_targeted_leucino.pdf	516873 f6afced4bd7cc03786bfcb5a0de444de11e78e88	no	8
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47	Non Patent Literature	B169--DUCRY_ET_AL____Antibody___Drug_Conjugate.pdf	903017 1e206de991840621ff38abf0a9df20c7120ea28b	no	9
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48	Non Patent Literature	B170--EL_SEWEDY_ET_AL____Cloning_of_the_Murine.pdf	1367736 fb42e1de22c73e2a5b627cff02147c439a6be380	no	7
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49	Non Patent Literature	B171--English_language_translation_of_Internat.pdf	117613 22863844ee09e4f0cd0b9e52cb2b2e5613039f1a	no	4
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50	Non Patent Literature	B172--Esteva_et_al___A_Phase_II_Study_of_Intra.pdf	858225 121eaa02013e0e5f87f799ed06f834407ed62259	no	8
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51	Non Patent Literature	B173--European_Search_Report_issued_in_corresp.pdf	190710 330b896fd861dc0765657b5b901c5023d94b8d56	no	7
Warnings:					
Information:					
52	Non Patent Literature	B174--Extended_European_Search_Report_dated_Fe.PDF	184683 5201330b1297696e164df654d340b8df567e2fa5	no	7
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53	Non Patent Literature	B175--Extended_European_Search_Report_dated_Ma.pdf	391414 cc76af77e93a8be49cea141060045daa67e63ff6	no	11
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54	Non Patent Literature	B176--Extended_European_Search_Report_dated_Ma.pdf	276660 d5a8ca7f53dde432192ef0b664e1ecd27def0b4a	no	7
Warnings:					
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55	Non Patent Literature	B177--Extended_European_Search_Report_dated_Ma.pdf	235020 39bfec29a6831dd549e4773b8eeca97709530082	no	7
Warnings:					
Information:					
56	Non Patent Literature	B178--Extended_European_Search_Report_issued_i.pdf	285091 73a8dd7ef48bd75c6ae858dc254c40a4959b5cf6	no	10
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57	Non Patent Literature	B179--FAULK_ET_AL____Antigens_of_human_trophob.pdf	1280438 44be37d2f595e24b7420c0849f4c3366516aa1cf	no	5
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58	Non Patent Literature	B180--FENDLY_ET_AL____Characterization_of_Muri.pdf	1241822 8388b0296ca2b879b118ab207a47c22e6c6f7465	no	9
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59	Non Patent Literature	B181--Final_Office_Action_issued_in_U_S__Paten.pdf	245329 156836c6aa6bfa33d7ef476147f2bebe0617ee63	no	8
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60	Non Patent Literature	B182--FONG_ET_AL____High_expression_of_TROP2_c.pdf	664860 7af5e5481d8e664b018bca11526f0d72fff57826	no	6
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		Total Files Size (in bytes):	169267865		

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New Applications Under 35 U.S.C. 111

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National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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Electronic Acknowledgement Receipt

EFS ID:	40731570
Application Number:	17010162
International Application Number:	
Confirmation Number:	6661
Title of Invention:	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE
First Named Inventor/Applicant Name:	Atsuko SAITO
Customer Number:	22428
Filer:	Michael D. Kaminski/Tuan Tran
Filer Authorized By:	Michael D. Kaminski
Attorney Docket Number:	098065-0278
Receipt Date:	01-OCT-2020
Filing Date:	02-SEP-2020
Time Stamp:	11:05:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	B243--OGUMA_ET_AL__Validation_study_of_a_metho.pdf	864657 25f2921fa7436fc9d6f5a2839d767a92de88121c	no	8

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2	Non Patent Literature	B244--OHMACHI_ET_AL____Clinical_Significance_o.pdf	1351321 e6eca63f4cdf5eb513d3528289bc7dacd0938364	no	7
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3	Non Patent Literature	B245--Opposition_dated_May_3__2017__against_co.pdf	756705 52c2cf504273ad6edbaeace0538a517b96e1d585	no	16
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4	Non Patent Literature	B246-OTTO_SOEPENBERG___chromatographic_assays.pdf	1184726 58c6e660d76e606c611768a759c3a24db1f1922a	no	8
Warnings:					
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5	Non Patent Literature	B247--P__Janne__et_al____Phase_1_study_of_the_.pdf	486122 b168196c5a2dad69e86bfc5cad8d4e96749f2de4	no	3
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6	Non Patent Literature	B248--PLOWMAN_ET_AL____Molecular_cloning_and_e.pdf	1052683 11e552615e97d3ddfc904c4473748ad3c26d5080	no	5
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7	Non Patent Literature	B249--RIPANI_ET_AL____Human_Trop_2_is_a_Tumor_.pdf	1167735 c226f744575aa62ad18e28c9814d203515351da0	no	6
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8	Non Patent Literature	B250--ROWINSKY__Preclinical_and_Clinical_Devel.pdf	1166003 3497946cdcc82d3accfee381639e9e0f7999177c	no	25
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9	Non Patent Literature	B251--Russian_Office_Action_dated_March_5__201.pdf	1294734 296bf01b1e4e835a1aed8dc6082fc64a24ccbced	no	16
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10	Non Patent Literature	B252--Russian_Office_Action_dated_October_11__.pdf	1009933 0315c85c4be9abd3de070071c922e2d47c9f3ed7	no	13
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11	Non Patent Literature	B253--SENTER_ET_AL____The_discovery_and_develo.pdf	1184288 3247b18cc3e76814eca67271a4ae3ac566f564dd	no	7
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12	Non Patent Literature	B254--Shen_et_al__Conjugation_site_modulates_t.pdf	1954413 e09c70dff1424dba2a0c4cde692fcda6a606a746	no	7
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13	Non Patent Literature	B255--SHIOSE_et_al___Systematic_Research_of_Pe.pdf	2054761 5d44f7a1565781204 5f99a7aa6ca4221e4eb4d11	no	11
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15	Non Patent Literature	B257--SIEVERS_ET_AL__Antibody_Drug_Conjugates_.pdf	1965884 6b023ef80dc3ceacf7a4696620491f1e39bc8cf5	no	18
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16	Non Patent Literature	B258--SLAMON_ET_AL____Human_Breast_Cancer__Cor.pdf	1619900 67608eeff73a8b3dff6ae41988f72dce213580cd	no	6
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17	Non Patent Literature	B259--SLAMON_ET_AL____Studies_of_the_HER_2_neu.pdf	1548140 b2d537cb6a1bdd3508670365d913445825651180	no	6
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18	Non Patent Literature	B260--SLIWKOWSKI_ET_AL____Coexpression_of_erbB.pdf	780679 343d42e63f2b3704339ff2707bb6110edfab02a9	no	5
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19	Non Patent Literature	B261--SLIWKOWSKI_ET_AL____Nonclinical_Studies_.pdf	1726585 e4afa76b4ea675c4adbb83dd66cef0899613f624	no	11
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20	Non Patent Literature	B262--SOEPENBERG_ET_AL____Phase_I_and_Pharmaco.pdf	1495989 dcacec72c5b789a2907aec90969c1dec93d013bd	no	9
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21	Non Patent Literature	B263--STEPAN_et_al____Expression_of_Trop2_Cell.pdf	1748003 2d209829d0252d45c1123343951050c8c2f4d0df	no	10
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22	Non Patent Literature	B264--Taiwanese_Office_Action_dated_July_30__2.pdf	513028 13959b73fe8a4fe1a2fe3800596782c1c6ceab88	no	5
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23	Non Patent Literature	B265--Taiwanese_Office_Action_issued_in_Taiwan.pdf	1371496 6c637164dece8432f33a3eee42aeafe5086fb089	no	5
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24	Non Patent Literature	B266--TAKIGUCHI_ET_AL____Antitumor_Effect_of_D.pdf	1793669 7e460d896ad3a47e862bc86702919ea7e4d26420	no	10
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25	Non Patent Literature	B267--THOMAS_M__CARDILLO___Humanized_Anti_Trop.pdf	4472480 3f46b5ba62eaed414be592515edc3066c18ab9d5	no	14
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26	Non Patent Literature	B268--US_Notice_of_Allowance_on_US_15_187179_D.pdf	349608 326bc8699b7396614a4963afcedf129dee567ecd	no	5
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27	Non Patent Literature	B269--US_Notice_of_Allowance_on_US_15_187179_D.pdf	409576 1a5b150b4df678f856e36eec1b05851618c2a2e2	no	7
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28	Non Patent Literature	B270--VOGEL_ET_AL____Efficacy_and_Safety_of_Tr.pdf	1344212 167bd8c4866cf986a3e5e38414c1c82f1399925d	no	8
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29	Non Patent Literature	B271--WANG_ET_AL____Identification_of_Trop_2_a.pdf	1155754 7aea2fc92a4d8decbee368d0e731e6ea7e29c908	no	6
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30	Non Patent Literature	B272--WENTE_ET_AL____DE_310__a_macromolecular_.pdf	1893205 035ebaa266ec60ec97f577f2206be0054dbc801e	no	9
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31	Non Patent Literature	B273--YANO_ET_AL____Comparison_of_HER2_gene_am.pdf	858516 6c68b02fea7ebff36ad370f5e69df3cda514bf1b	no	7
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		Total Files Size (in bytes):	41197552		

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PTO/SB/08 (modified)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	17/010162
Date Submitted: September 30, 2020				**Filing Date**	9/2/2020
				First Named Inventor	Atsuko SAITO
(use as many sheets as necessary)				Art Unit	1600
				Examiner Name	LUCAS, ZACHARIAH
Sheet	1	of	12	Attorney Docket Number	098065-0278

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	B1	5,480,968	01-02-1996	KRAUS ET AL	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	17/010162
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Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
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(54) Titre : UTILISATION D'AGENTS DE LIAISON HER3 DANS LE TRAITEMENT DE LA PROSTATE
(54) Title: USE OF HER3 BINDING AGENTS IN PROSTATE TREATMENT

(57) Abrégé/Abstract:
Described herein are materials and methods for using a HER3 binding agent for prostate treatment. The HER3 binding agent can be, for example, an antibody, and can be used to treat conditions such as benign prostate hyperplasia (BPH) and prostate cancer.

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(54) Title: USE OF HER3 BINDING AGENTS IN PROSTATE TREATMENT

(57) Abstract: Described herein are materials and methods for using a HER3 binding agent for prostate treatment. The HER3 binding agent can be, for example, an antibody, and can be used to treat conditions such as benign prostate hyperplasia (BPH) and prostate cancer.

TITLE OF THE INVENTION

USE OF HER3 BINDING AGENTS IN PROSTATE TREATMENT

REFERENCE TO SEQUENCE LISTING, A TABLE, OR A COMPUTER PROGRAM LISTING COMPACT DISC APPENDIX

[0001] A Sequence Listing is being submitted in ASCII character and file format in compliance with 37 CFR § 1.52(e) on a properly labeled CD in compliance with 37 CFR § 1.96. A copy of the Sequence Listing is also being submitted in computer readable form (CRF) in accordance with the requirements of §1.824. The sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing.

TECHNICAL FIELD

[0002] This document relates to materials and methods for treating prostate conditions, and more particularly to materials and methods for reducing HER3 activity in prostate tissue to treat conditions such as benign prostate hyperplasia (BPH) and prostate cancer.

BACKGROUND OF THE INVENTION

[0003] Prostate cancer patients in the United States undergo radical prostatectomy or radiation therapy for treatment of localized prostate cancer. It is estimated that >15-20% of these patients experience tumor recurrence due to metastasis (*See:* Melamed et al., "Prostate Cancer Pathologic Parameters and Clinical Outcome: Results from the Cooperative Prostate Cancer Tissue Resource," National Cancer Institute, Bethesda, MD (2004)). Recurrent prostate cancer typically is treated with androgen withdrawal therapy, which often is initially effective but ultimately fails, indicating development of androgen independent prostate cancer (AIPC) (*See:* Petrylak, *Brit. J. Urol. Int.* 96 (Suppl 2):41-46 (2005)). Treatment options for patients who fail AWT are limited (*See:* Javidan et al., *Cancer Invest.* 23(6):520-528 (2005)).

BRIEF SUMMARY OF THE INVENTION

[0004] This document is based in part on the discovery that androgen ablation is accompanied by an increase in the levels of the receptor tyrosine kinase HER3, which stimulates Akt phosphorylation and induces the ability of cells to propagate in medium without androgens. The materials and methods provided herein relate, in part, to using a HER3 binding agent (*e.g.*, a fully human anti-HER3 monoclonal antibody such as U1-59) during androgen withdrawal to inhibit HER3, thus preventing androgen independence.

[0005] This document also is based in part on the discovery that intravenous (i.v.) treatment of rats with a HER3 binding agent, such as a HER3 binding antibody, resulted in reduced prostate weight. Thus, this document provides materials and methods for decreasing prostate weight (*e.g.*, to treat conditions such as BPH) with a HER3 binding agent.

[0006] The present document describes methods for treating benign prostate hyperplasia (BPH) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising a HER3 binding agent. The HER3 binding agent may a small molecule compound or an antigen-binding protein that binds to HER3.

[0007] In one embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of, for example, SEQ ID NOs: 236, 251, 252, and 256; a CDRH2 selected from the group consisting of, for example, SEQ ID NOs:258, 278, 280, and 282; and a CDRH3 selected from the group consisting of, for example, SEQ ID NOs:283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of, for example, SEQ ID NOs: 320, 334, 337, and 340; a CDRL2 selected from the group consisting of, for example, SEQ ID NOs: 343, 356, 351, and 344; and a CDRL3 selected from the group consisting of, for example, SEQ ID NOs:360, 381, 385, and 387.

[0008] In another embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of (a) CDRH1's as shown in, for example, SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in, for example, SEQ ID NOs: 258, 278, 280, and 282; and (c) CDRH3's as shown in, for example, SEQ ID NOs: 283, 285, 309, 313, and 315.

[0009] In a further embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a light chain amino acid sequence that comprises at least one of the CDR´s selected from the group consisting of: (d) CDRL1's as shown in, for example, SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in, for example, SEQ ID NOs: 343, 356, 351, and 344; and (f) CDRL3's as shown in, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0010] In yet another embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of, for example, (a) CDRH1's as shown in SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs: 258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs: 283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs: 343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs:360, 381, 385, and 387.

[0011] In yet another embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of, for example, SEQ ID NOs: 236, 251, 252, and 256, a CDRH2 selected from the group consisting of, for example, SEQ ID NOs: 258, 278, 280, and 282, and a CDRH3 selected from the group consisting of, for example, SEQ ID NOs: 283, 285, 309, 313, and 315, or a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of, for example, SEQ ID NOs: 320, 334, 337, and 340, a CDRL2 selected from the group consisting of, for example, SEQ ID NOs: 343, 356, 351, and 344, and a CDRL3 selected from the group consisting of, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0012] In another embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 42, 54, 70, 92, and 96. Alternatively, the antigen-binding protein comprises a light chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 44, 56, 72, 94, and 98. In some embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 42, 54, 70, 92, and 96; and a light chain amino

acid sequence selected from the group consisting of, for example, SEQ ID NOs: 44, 56, 72, 94, and 98. The HER3 binding agent may comprise the heavy chain amino acid sequence of SEQ ID NO: 42 and the light chain amino acid sequence of SEQ ID NO: 44, or the heavy chain amino acid sequence of SEQ ID NO: 54 and the light chain amino acid sequence of SEQ ID NO:56, or the heavy chain amino acid sequence of SEQ ID NO: 70 and the light chain amino acid sequence of SEQ ID NO: 72.

[0013] In some embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDRH3 selected from the group consisting of, for example, SEQ ID NOs: 283, 285, 309, 313, and 315. In other embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDHL3 selected from the group consisting of, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0014] In certain examples, the antigen-binding protein is directed against the extracellular domain of HER3. The binding of the antigen-binding protein to HER3 may have one or more effects selected from the group consisting of reduction of HER3-mediated signal transduction, reduction of HER3 phosphorylation, reduction of cell proliferation, reduction of cell migration, and increasing downregulation of HER3.

[0015] In certain embodiments, the antigen-binding protein that binds to HER3 is an antibody. The antibody may be a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof. The antibody fragment may be a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule. For example, the antibody is of the IgG1-, IgG2-, IgG3- or IgG4-type.]

[0016] In some embodiments, the antigen-binding protein is coupled to an effector group. The effector group may be a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group. The therapeutic or chemotherapeutic group may be selected from the group consisting of calicheamicin, auristatin-PE, geldanamycin, maytansine and derivatives thereof.

[0017] The method of the invention may include identifying the subject as having BPH, and/or monitoring prostate size in the subject after administering the composition.

[0018] The present document also relates to methods for reducing prostate weight in a subject, comprising administering to the subject a therapeutically effective amount of a

composition comprising a HER3 binding agent. The HER3 binding agent may be a small molecule compound or an antigen-binding protein that binds to HER3.

[0019] In one embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of, for example, SEQ ID NOs: 236, 251, 252, and 256; a CDRH2 selected from the group consisting of, for example, SEQ ID NOs: 258, 278, 280, and 282; and a CDRH3 selected from the group consisting of, for example, SEQ ID NOs: 283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of, for example, SEQ ID NOs: 320, 334, 337, and 340; a CDRL2 selected from the group consisting of, for example, SEQ ID NOs: 343, 356, 351, and 344; and a CDRL3 selected from the group consisting of, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0020] In other embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of, for example, (a) CDRH1's as shown in SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs: 258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs: 283, 285, 309, 313, and 315.

[0021] In yet other embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a light chain amino acid sequence that comprises at least one of the CDR´s selected from the group consisting of, for example, (d) CDRL1's as shown in SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs: 343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs: 360, 381, 385, and 387.

[0022] In yet other embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of, for example, (a) CDRH1's as shown in SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs: 258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs: 283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs: 343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs: 360, 381, 385, and 387.

[0023] In again other embodiment, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of, for example, SEQ ID NOs: 236, 251, 252, and 256, a CDRH2 selected from the group consisting of, for example, SEQ ID NOs: 258, 278, 280, and 282, and a CDRH3 selected from the group consisting of, for example, SEQ ID NOs: 283, 285, 309, 313, and 315, or a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of, for example, SEQ ID NOs: 320, 334, 337, and 340, a CDRL2 selected from the group consisting of, for example, SEQ ID NOs: 343, 356, 351, and 344, and a CDRL3 selected from the group consisting of, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0024] In certain embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 42, 54, 70, 92, and 96. Alternatively, the antigen-binding protein comprises a light chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 44, 56, 72, 94, and 98.

[0025] The HER3 binding agent may comprise a heavy chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 42, 54, 70, 92, and 96; and a light chain amino acid sequence selected from the group consisting of, for example, SEQ ID NOs: 44, 56, 72, 94, and 98.

[0026] The HER3 binding agent may be an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO: 42 and the light chain amino acid sequence of SEQ ID NO: 44. Alternatively, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO: 54 and the light chain amino acid sequence of SEQ ID NO:56. Again, alternatively, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO: 70 and the light chain amino acid sequence of SEQ ID NO: 72.

[0027] In certain embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDRH3 selected from the group consisting of, for example, SEQ ID NOs: 283, 285, 309, 313, and 315. In yet other embodiments, the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDHL3 selected from the group consisting of, for example, SEQ ID NOs: 360, 381, 385, and 387.

[0028] In certain embodiments, the antigen-binding protein is directed against the extracellular domain of HER3. The binding of the antigen-binding protein to HER3 may have one or more effects selected from the group consisting of reduction of HER3-mediated signal transduction, reduction of HER3 phosphorylation, reduction of cell proliferation, reduction of cell migration, and increasing downregulation of HER3.

[0029] In certain embodiments, the antigen-binding protein that binds to HER3 is an antibody. The antibody may be a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof. The antibody fragment may be a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule. For example, the antibody is of the IgG1-, IgG2-, IgG3- or IgG4-type.

[0030] In some embodiments, the antigen-binding protein is coupled to an effector group. The effector group may be a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group. The therapeutic or chemotherapeutic group may be, for example, a calicheamicin, a auristatin-PE, a geldanamycin, a maytansine or derivatives thereof.

[0031] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. Publications, patent applications, patents, and other references mentioned herein are hereby ncorporated herein by reference in their entireties. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0032] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

[0033] FIGS. 1A and 1B are pictures of blots showing ErbB levels in various cell lines. FIG. 1C is a graph plotting luciferase activity in LNCaP cells transfected with a luciferase construct tagged to a PSA promoter, indicating the effects of culture in medium

containing charcoal stripped serum (CSS) on androgen receptor (AR) transcriptional activity. FIG. 1D is a picture of a blot showing expression levels of various proteins after culture of LNCaP cells in medium containing CSS.

[0034] FIG. 2A is a picture of a blot showing levels of several proteins after over-expression of HER2 and HER3 in LNCaP cells. Overexpression of HER3 increased HER2 expression. FIG. 2B is a graph plotting LNCaP growth rates in CSS-containing medium for control cells, cells overexpressing HER2 or HER3, and an androgen independent (AI) subline. Both HER2 and HER3 caused LNCaP cells to grow in medium containing CSS. FIG. 2C is a graph plotting growth rates for the LNCaP AI subline cultured with fetal bovine serum (FBS) or CSS, with control or HER3 siRNA. Inhibition of HER3 in the AI subline prevented this cell growth in CSS-containing medium. FIG. 2D is a graph plotting growth of LNCaP cells with or without HER3 overexpression, with or without an Akt inhibitor. The HER3-induced increase in cell growth was prevented by Akt inhibition.

[0035] FIG. 3A is a graph plotting the percentage of cells in S-phase for mock transfected LNCaP and C4-2 cells (control cells were treated with LIPOFECATMINE® 2000 alone), or cells transfected with scrambled or HER3-specific siRNA duplex. Bars represent mean ± S.E. from three individual experiments. *$p<0.01$ in siRNA transfected vs. mock transfected cells. FIG. 3B is a picture of a Western blot and a graph plotting band intensity to indicate HER3 protein levels in LNCaP and C4-2 cells incubated with scrambled or HER3 siRNA. HER2 (HER2) was used as loading control. Bars represent mean ± S.E. of band intensity quantization of HER3 bands normalized to HER2 bands from three individual experiments. FIG. 3C is a graph plotting the percentage of LNCaP cells in S-phase after transfection with empty vector (pCDNA3) or pCDNA3-HER3-myc cDNA and treatment with bicalutamide (an AR antagonist marketed as CASODEX® by AstraZeneca, Wilmington, DE) or DMSO (control). Bars represent mean ± S.E. of S-phase percentages from three individual experiments. *$p<0.01$ in bicalutamide treated vs. untreated cells. FIG. 3D is a picture of a Western blot and a graph plotting protein levels of HER3 in LNCaP or C4-2 cells transfected with control (empty pCNDA3 vector) or pCDNA3-HER3-myc cDNA, confirming overexpression of HER3 in the appropriate cells.

[0036] FIGS. 4A and 4B are pictures of western blots using lysates from DU-145 prostate cancer cells treated with IgG, U1-59, cetuximab, panitumumab, c2C4, trastuzumab, or combinations thereof, as indicated. Blots were probed with antibodies

against HER3 phosphorylated at Tyrosine 1289 or 1197. Actin was used as a control. FIGS. 4A and 4B represent separate experiments.

[0037] FIG. 5 is a graph plotting colony numbers for PC-3 prostate cancer cells incubated with IgG, U1-59, or either of two other anti-HER3 antibodies (U1-53 and U1-49), as indicated.

[0038] FIGS. 6A and 6B are pictures of western blots showing phospho-HER3 levels in rat RG2 glioma cells (FIG. 6A) and cynomolgus monkey JTC-1.P3 cells (FIG. 6B) incubated with heregulin, U1-59, IgG control, or combinations thereof, as indicated. □-actin was used as a control for loading.

DETAILED DESCRIPTION OF THE INVENTION

1. General Overview

[0039] This document provides materials and methods related to treating prostate conditions such as BPH and prostate cancer. Unless otherwise defined herein, scientific and technical terms used in connection with this document shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0040] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques described herein generally are performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See*: Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001); Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992); and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which publications are hereby incorporated herein by reference in their entireties. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic

chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0041] It should be understood that this invention is not limited to the particular methodology, protocols, reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the disclosed, which is defined solely by the claims.

[0042] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages may mean ±1%.

2. The Prostate and HER Family Members

[0043] Androgen withdrawal therapy (AWT) for treatment of prostate cancer does not induce cell death, but rather results in cell cycle arrest. The development of androgen independence triggers a release from that arrest, leading to cell cycle progression even in the absence of androgens (*See:* Agus et al., *J. Natl. Cancer Inst.* 91(21):1869-1876 (1999)). Some of the studies described herein were conducted to determine whether induction of apoptosis during AWT can prevent androgen independent cell cycle progression.

[0044] Androgen withdrawal is accompanied by an increase in phosphorylation (activation) of Akt (*See:* Mikhailova et al. in: Hormonal Carcinogenesis V, Springer Science and Business Media, New York, NY, pp.397-405 (2008); and Murillo et al. *Endocrinol.* 142(11):4795-4805 (2001)), a serine threonine kinase that promotes cell survival (*See:* Graff et al., *J. Biol. Chem.* 275(32):24500-24505 (2000); and Xin et al., *Proc. Natl. Acad. Sci. USA* 103(20):7789-7794 (2006)). Direct inhibition of Akt phosphorylation may cause some toxicity since Akt is an essential kinase (*See:* Posadas et al., *Cancer Biol. Ther.* 4(10):1133-1137 (2005); and Chee *et* al., *Clin. Genitourin Cancer* 5(7):433-437 (2007)). The inventors hypothesized that inhibition of pathways stimulating Akt phosphorylation during androgen withdrawal would prevent cell survival and eventually prevent the emergence of androgen independent cells.

[0045] The HER family of proteins, which activate Akt in prostate epithelial cells, includes the epidermal growth factor receptor (EGFR, also known as HER1 and ErbB1), HER2 (also known as ErbB2 and neu), HER3 (also known as ErbB3), and HER4 (also known as ErbB4) (*See:* Olayioye et al., *EMBO J.* 19:3159-3167 (2000)). EGFR and HER2 regulate cell proliferation, differentiation, angiogenesis, and survival (*See:* Yarden and Sliwkowski, *Nat. Rev. Mol. Cell. Biol.* 2(2):127-137 (2001)). Less is known about HER3 and HER4, but microarray analysis revealed increased expression of the HER3 gene in prostate cancer vs. normal prostate (*See:* Chaib et al. *Neoplasia* 3(1):43-52 (2001)), and immunohistochemistry analysis of prostate cancer tissues revealed that >90% of displayed cytoplasmic HER3 staining (*See:* Koumakpayi et al., *Clin. Cancer Res.* 12(9):2730-2737 (2006)). Further, activation of HER3 in an animal model induced activation of the AR and promoted prostate cancer recurrence (*See:* Gregory et al., *Clin. Cancer Res.* 11(5):1704-1712 (2005)). Thus, increased HER3 levels may promote prostate cancer progression.

[0046] The HER family receptors are activated by ligand binding, dimerization and phosphorylation. Each receptor except HER2 has a specific ligand (*See:* Olayioye et al., *EMBO J.* 19(13):3159-3167 (2000)). As such, HER2 requires heterodimerization with other ErbB receptors for phosphorylation and activation. HER3 also requires heterodimerization with other ErbB receptors for kinase activity. Despite this, HER3 has multiple phosphorylation sites that specifically bind and activate downstream targets. In particular, HER3 has six binding sites for phosphatidylinositol 3-kinase (PI3K), an upstream activator required for the phosphorylation and activation of Akt. EGFR and HER2 can also activate Akt independent of HER3, but this activation is indirect, *via* transactivation of PI3K by Src kinases.

[0047] BPH is an enlargement of the prostate gland, and is very common in older men due to continued growth of the prostate throughout life. Although BPH is noncancerous, it can lead to urinary problems such as urinary tract infection, bladder stones, blood in the urine and, in some extreme cases, kidney failure. Treatment for BPH can range from changes in diet and exercise or use of medication in mild cases, to destruction of overgrown tissue (*e.g.*, using microwave thermal therapy, laser therapy, transurethral needle ablation, or other minimally invasive therapies) in moderate cases, to transuretheral prostate resection, or to open surgery in severe cases.

3. HER3 Binding Agents

[0048] As described herein, an agent that binds to HER3 can be a biological compound, including, but not limited to, an antigen binding protein (*e.g.*, an antibody) or a small molecular tyrosine kinase inhibitor. As used herein, an "antigen binding protein" or "binding protein" means a protein that specifically binds a particular target antigen, such as member of the HER family, *e.g.*, HER3. An antigen binding protein is understood to "specifically bind" its target antigen when the dissociation constant (K_D) is $\leq 10^{-8}$ M. The antibody specifically binds antigen with "high affinity" when the K_D is $\leq 5x10^{-9}$ M, and with "very high affinity" when the K_D is $\leq 5x\ 10^{-10}$ M. In one embodiment, the antibody has a K_D of $\leq 10^{-9}$ M and an off-rate of about $1x\ 10^{-4}$/sec. In one embodiment, the off-rate is about $1x10^{5}$/sec. In other embodiments, the antibodies will bind to a specified member of the HER family with a K_D of between about 10^{-8} M and 10^{-10} M, and in yet another embodiment it will bind with a $K_D \leq 2x10^{-10}$. Further, as used herein, a small molecule compound is a low molecular weight compound that has been chemically synthesized to inhibit the enzymatic activity of one or more protein kinases, including serine, threonine, or tyrosine kinases.

[0049] Where the HER3 binding agent is a biological compound, the agent can be an antigen binding protein (*e.g.*, an anti-HER3 antibody). Thus, provided herein for use in compositions and methods of treating HER3 associated diseases are HER binding proteins, including anti-HER3 antibodies. In some embodiments, an antibody targeted to HER3 can be directed against the extracellular domain (ECD) of HER3. For example, an anti-HER3 antibody as described herein can interact with at least one epitope in the extracellular part of HER3. The epitopes can be located in the amino terminal L1 domain (aa 19-184), in the S1 (aa 185-327) and S2 (aa 500-632) cysteine-rich domains, in the L2 domain (328-499), which is flanked by the two cysteine-rich domains, or in a combination of HER3 domains. The epitopes also may be located in combinations of domains such as, without limitation, an epitope comprised by parts of L1 and S1.

[0050] A HER3 binding protein can be further characterized in that its binding to HER3 reduces HER3-mediated signal transduction. A reduction of HER3-mediated signal transduction may, *e.g.*, be caused by a downregulation of HER3 resulting in an at least partial disappearance of HER3 molecules from the cell surface or by a stabilization of HER3 on the cell surface in a substantially inactive form, *i.e.*, a form that exhibits a lower signal transduction compared to the non-stabilized form. Alternatively, a reduction of HER3-mediated signal transduction also may be caused by influencing, *e.g.*, decreasing or inhibiting, the binding of a ligand or another member of the HER family to HER3. For example, a reduction of HER3

mediated signal transduction also can be caused by, decreasing the formation of HER3 containing dimers with other HER family members (*e.g.*, EGF-R).

[0051] A HER3 binding agent can be a scaffold protein having an antibody-like binding activity (*e.g.*, having activity similar to an anti-HER3 antibody) or an antibody, *i.e.*, an anti-HER3 antibody. As used herein, the term "scaffold protein" means a polypeptide or protein with exposed surface areas in which amino acid insertions, substitutions or deletions are highly tolerable. Examples of scaffold proteins that can be used in accordance with the present methods include protein A from *Staphylococcus aureus*, the bilin binding protein from *Pieris brassicae* or other lipocalins, ankyrin repeat proteins, and human fibronectin (reviewed in Binz and Plückthun, *Curr. Opin. Biotechnol.* 16:459-69 (2005)). Engineering of a scaffold protein can be regarded as grafting or integrating an affinity function onto or into the structural framework of a stably folded protein. Affinity function means a protein binding affinity according to the present document. A scaffold can be structurally separable from the amino acid sequences conferring binding specificity. In general, proteins appearing suitable for the development of such artificial affinity reagents may be obtained by rational, or most commonly, combinatorial protein engineering techniques such as panning against HER3, either purified protein or protein displayed on the cell surface, for binding agents in an artificial scaffold library displayed *in vitro*, skills which are known in the art (*See:* Skerra, *J. Mol. Recog.* 13:167-87 (2000); and Binz and Plückthun, *supra*). In addition, a scaffold protein having an antibody like binding activity can be derived from an acceptor polypeptide containing the scaffold domain, which can be grafted with binding domains of a donor polypeptide to confer the binding specificity of the donor polypeptide onto the scaffold domain containing the acceptor polypeptide. The inserted binding domains may be, for example, the complementarity determining region (CDR) of an antibody, in particular an anti-HER3 antibody. Insertion can be accomplished by various methods known to those skilled in the art including, for example, polypeptide synthesis, nucleic acid synthesis of an encoding amino acid as well by various forms of recombinant methods well known to those skilled in the art.

[0052] The term "antibody" includes monoclonal antibodies, polyclonal antibodies, recombinant antibodies, human antibodies, humanized antibodies (*See:* Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992)), chimeric antibodies (*See:* Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)), multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two antibodies, or antibody fragments thereof. The term "antibody fragment"

comprises any portion of the afore-mentioned antibodies, such as their antigen binding or variable regions. Examples of antibody fragments include Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, diabodies (*See:* Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993)), single chain antibody molecules (*See:* Plückthun: The Pharmacology of Monoclonal Antibodies 113, Rosenburg and Moore, eds., Springer Verlag, N.Y. 269-315, (1994)), and other fragments as long as they exhibit the desired capability of binding to HER3.

[0053] In addition, the term "antibody," as used herein, also includes antibody-like molecules that contain engineered sub-domains of antibodies or naturally occurring antibody variants. These antibody-like molecules may be single-domain antibodies such as V_H-only or V_L-only domains derived either from natural sources such as camelids (*See:* Muyldermans et al., *Rev. Mol. Biotechnol.* 74:277-302 (2001)) or through *in vitro* display of libraries from humans, camelids or other species (*See:* Holt et al., *Trends Biotechnol.* 21:484-90 (2003)). In certain embodiments, the polypeptide structure of the antigen binding proteins can be based on antibodies, including, but not limited to, minibodies, synthetic antibodies (sometimes referred to as "antibody mimetics"), human antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments thereof, respectively.

[0054] An "Fv fragment" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain variable domain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three CDR's of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDR's confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR's specific for an antigen) has the ability to recognize and bind the antigen, although usually at a lower affinity than the entire binding site. The "Fab fragment" also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. The "Fab fragment" differs from the "Fab' fragment" by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain, including one or more cysteines from the antibody hinge region. The "$F(ab')_2$ fragment" originally is produced as a pair of "Fab' fragments" which have hinge cysteines between them. Methods of preparing such antibody fragments, such as papain or pepsin digestion, are known to those skilled in the art.

[0055] An antibody can be of the IgA-, IgD-, IgE, IgG- or IgM-type, including IgG- or IgM-types such as, without limitation, IgG1-, IgG2-, IgG3-, IgG4-, IgM1- and IgM2-types. For example, in some cases, the antibody is of the IgG1-, IgG2- or IgG4- type.

[0056] In certain respects, *e.g.*, in connection with the generation of antibodies as therapeutic candidates against HER3, it may be desirable that the antibody is capable of fixing complement and participating in complement-dependent cytotoxicity (CDC). There are a number of isotypes of antibodies that are capable of the same including: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, human IgG3, and human IgA, for example. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and the antibody can be isotype switched by appending the molecularly cloned V region genes or cDNA to molecularly cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art. The isotype-switched antibody may also possess an Fc region that has been molecularly engineered to possess superior CDC over naturally occurring variants (*See:* Idusogie et al., *J. Immunol.* 166:2571-2575 (2001)) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (*See:* US Patent No. 4816397), cell-cell fusion techniques (*See:* US Patent Nos. 5916771 and 6207418), among others. In the cell-cell fusion technique, a myeloma or other cell line such as CHO is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line such as CHO is prepared that possesses the light chain. Such cells can thereafter be fused, and a cell line expressing an intact antibody can be isolated. By way of example, a human anti-HER3 IgG4 antibody that possesses the desired binding to the HER3 antigen can be readily isotype switched to generate a human IgM, human IgG1 or human IgG3 isotype, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity). Such a molecule might then be capable of fixing complement and participating in CDC.

[0057] Moreover, an antibody also may be capable of binding to Fc receptors on effector cells such as monocytes and natural killer (NK) cells, and participating in antibody-dependent cellular cytotoxicity (ADCC). There are a number of antibody isotypes that are capable of the same, including, without limitation, the following: murine IgG2a, murine IgG2b, murine IgG3, human IgG1 and human IgG3. It will be appreciated that the antibodies that are generated need not initially possess such an isotype but, rather the

antibody as generated can possess any isotype and the antibody can be isotype switched by appending the molecularly cloned V region genes or cDNA to molecularly cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art. The isotype-switched antibody may also possess an Fc region that has been molecularly engineered to possess superior ADCC over naturally occurring variants (*See:* Shields et al., *J. Biol. Chem.* 276:6591-6604 (2001)) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (*See:* US Patent No. 4816397), cell-cell fusion techniques (*See:* US Patent Nos. 5916771 and 6207418), among others. In the cell-cell fusion technique, a myeloma or other cell line such as CHO is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line such as CHO is prepared that possesses the light chain. Such cells can thereafter be fused, and a cell line expressing an intact antibody can be isolated. By way of example, a human anti-HER3 IgG4 antibody that possesses the desired binding to the HER3 antigen could be readily isotype switched to generate a human IgG1 or human IgG3 isotype, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity). Such molecule might then be capable of binding to FcR on effectors cells and participating in ADCC.

[0058] TABLE 1 herein provides amino acid sequences for a number of CDR's that can be included in antibodies against HER3. In some embodiments, an isolated binding protein targeted to HER3 can include a heavy chain amino acid sequence containing at least one CDR selected from the group consisting of: (a) CDRH1's as shown in SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, (b) CDRH2's as shown in SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and (c) CDRH3's as shown in SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and/or a light chain amino acid sequence comprising at least one of the

CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, (e) CDRL2's as shown in SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, and (f) CDRL3's as shown in SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232. All sequences referred to herein are shown in the sequence listing in US Patent No. 7705130. These sequences are hereby incorporated herein by reference in their entireties.

TABLE 1

CDR SEQUENCES

Ab chain	Pat ID	SEQ ID NO	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3
heavy	U1-1	235	GGSINSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-1	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-2	236	GGSISSGDYYWS	259	YIYYSGSTYYNPSLRS	283	ADYDFWSGYFDY
light	U1-2	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-3	237	GGSISSGGYYWS	258	YIYYSGSTYYNPSLKS	284	DGYDSSGYYHGYFDY
light	U1-3	319	KSSQSVLYSSNNKNYLA	344	WASTRES	362	QQYYSTPLT
heavy	U1-4	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-4	318	RASQGIRNDLG	343	AASSLQS	363	LQHNNYPWT
heavy	U1-5	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-5	318	RASQGIRNDLG	343	AASSLQS	364	LQHNTYPWT
heavy	U1-6	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	285	ADYDFWNGYFDY
light	U1-6	318	RASQGIRNDLG	343	AASSLQS	364	LQHNTYPWT
heavy	U1-7	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-7	320	RASQDIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-8	238	GYTLTELSMY	260	GFDPEDGETIYAQKFQG	286	GWNYVFDY
light	U1-8	321	RSSQSLLHSNGYNYLD	345	LDSHRAS	365	MQALQTPLT
heavy	U1-9	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	285	ADYDFWNGYFDY
light	U1-9	320	RASQDIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-10	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-10	318	RASQGIRNDLG	343	AASSLQS	363	LQHNNYPWT
heavy	U1-11	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-11	318	RASQGIRNDLG	343	AASSLQS	364	LQHNTYPWT
heavy	U1-12	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-12	318	RASQGIRNDLG	343	AASSLQS	363	LQHNNYPWT

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Ab chain	Pat ID	SEQ ID NO	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3
heavy	U1-13	237	GGSISSGGYYWS	258	YIYYSGSTYYNPSLKS	287	EDDGMDV
light	U1-13	322	RSSQSLLHSNGYNYLE	346	LGSNRAS	366	MQALQTPIT
heavy	U1-14	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-14	318	RASQGIRNDLG	343	AASSLQS	364	LQHNTYPWT
heavy	U1-15	239	GGSVSSGGYYWS	261	YIYYSGSTNYNPSLKS	288	DGDVDTAMVDAFDI
light	U1-15	323	RASQSLSGNYLA	347	GASSRAT	367	QQYDRSPLT
heavy	U1-16	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-16	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-17	236	GGSISSGDYYWS	262	YIYYSGSTYYNSSLKS	283	ADYDFWSGYFDY
light	U1-17	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-18	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-18	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-19	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	289	GDYDFWSGEFDY
light	U1-19	sequence not available					
heavy	U1-20	237	GGSISSGGYYWS	263	YIYDSGSTYYNPSLKS	290	DQGQDGYSYGYGYYYGMDV
light	U1-20	324	QASQDISNYLN	348	VASNLET	368	QQCDNLPLT
heavy	U1-21	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-21	320	RASQDIRNDLG	349	AASRLQS	360	LQHNSYPWT
heavy	U1-22	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-22	318	RASQGIRNDLG	350	AASSLQN	360	LQHNSYPWT
heavy	U1-23	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-23	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-24	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	285	ADYDFWNGYFDY
light	U1-24	318	RASQGIRNDLG	343	AASSLQS	363	LQHNNYPWT
heavy	U1-25	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-25	318	RASQGIRNDLG	350	AASSLQN	360	LQHNSYPWT
heavy	U1-26	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	291	ADYDFWSGYFDF
light	U1-26	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-27	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	291	ADYDFWSGYFDF
light	U1-27	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-28	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	292	ADYDFWSGYFDS
light	U1-28	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-29	240	GFTFNSYDMH	264	VIWYDGSNKYYADSVKG	293	DRLCTNGVCYEDYGMDV
light	U1-29	324	QASQDISNYLN	351	DASNLET	369	QHYDTLPLT
heavy	U1-30	236	GGSISSGDYYWS	265	YIYYSGTTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-30	325	RAGQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-31	241	GYTFTNYGIS	266	WISAYDGYRNYAQKLQG	294	DVQDYGDYDYFDY
light	U1-31	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-32	236	GGSISSGDYYWS	265	YIYYSGTTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-32	325	RAGQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-33	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	295	ADYDFWSGHFDC
light	U1-33	327	RASQGIRDDLG	352	AESSLQS	371	LQHHSYPWT
heavy	U1-34	241	GYTFTNYGIS	266	WISAYDGYRNYAQKLQG	294	DVQDYGDYDYFDY
light	U1-34	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-35	242	GFTFSDYYMS	267	YISSSGNNIYHADSVKG	296	ERYSGYDDPDGFDI
light	U1-35	328	QASQDISNYLS	351	DASNLET	372	QQYDNPPCS

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Ab chain	Pat ID	SEQ ID NO	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3
heavy	U1-36	243	GGSISSGYYYWS	268	YIYYSGTTYYNPSFKS	297	ADYDFWSGHFDY
light	U1-36	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-37	244	GYTFTSYGIS	269	WISAYDGHTNYAQKLQG	298	DPHDYSNYEAFDF
light	U1-37	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-38	245	GFSLSTSGVGVG	270	LIYWNDDKRYSPSLKS	299	RDEVRGFDY
light	U1-38	329	RSSQSLVYSDGYTYLH	353	KVSNWDS	373	MQGAHWPIT
heavy	U1-39	246	GFTVSSNYMS	271	VIYSGGSTYYADSVKG	300	GQWLDV
light	U1-39	321	RSSQSLLHSNGYNYLD	354	LGFHRAS	374	RQALQTPLT
heavy	U1-40	237	GGSISSGGYYWS	272	YIYSSGSTYYNPSLKS	301	DRELELYYYYYGMDV
light	U1-40	330	RSSQSLLYSNGYNYLD	346	LGSNRAS	365	MQALQTPLT
heavy	U1-41	237	GGSISSGGYYWS	258	YIYYSGSTYYNPSLKS	302	DRELEGYSNYYGVDV
light	U1-41	331	RASQAISNYLN	343	AASSLQS	375	QQNNSLPIT
heavy	U1-42	247	GYSFTSYWIG	273	IIYPGDSDTRYSPSFQG	303	HENYGDYNY
light	U1-42	332	RASQSIRSYLN	343	AASSLQS	376	QQSNGSPLT
heavy	U1-43	237	GGSISSGGYYWS	259	YIYYSGSTYYNPSLRS	304	DREREWDDYGDPQGMDV
light	U1-43	333	RASQSISSYLH	343	AASSLQS	377	QQSYSNPLT
heavy	U1-44	247	GYSFTSYWIG	274	IIWPGDSDTIYSPSFQG	303	HENYGDYNY
light	U1-44	332	RASQSIRSYLN	343	AASSLQS	378	QQSISSPLT
heavy	U1-45	248	GYTFTSYDIN	275	WMNPNSGDTGYAQVFQG	305	FGDLPYDYSYYEWFDP
light	U1-45	326	RASQSISSYLN	343	AASSLQS	379	QQSYSTPLT
heavy	U1-46	249	GDSVSSNSAAWN	276	RTYYRSKWYNDYAVSVKS	306	DLYDFWSGYPYYYGMDV
light	U1-46	sequence not available					
heavy	U1-47	249	GDSVSSNSAAWN	276	RTYYRSKWYNDYAVSVKS	307	DYYGSGSFYYYYGMDV
light	U1-47	326	RASQSISSYLN	355	AASNLQS	380	QQSYSTPRT
heavy	U1-48	250	GGSISSYYWS	277	HIYTSGSTNYNPSLKS	308	EAIFGVGPYYYYGMDV
light	U1-48	sequence not available					
heavy	U1-49	251	GYTFTGYYMH	278	WINPNIGGTNCAQKFQG	309	GGRYSSSWSYYYYGMDV
light	U1-49	334	KSSQSLLLSDGGTYLY	356	EVSNRFS	381	MQSMQLPIT
heavy	U1-50	239	GGSVSSGGYYWS	261	YIYYSGSTNYNPSLKS	310	GGDSNYEDYYYYYGMDV
light	U1-50	335	RASQSISIYLH	343	AASSLQS	382	QQSYTSPIT
heavy	U1-51	250	GGSISSYYWS	261	YIYYSGSTNYNPSLKS	311	DSSYYDSSGYYLYYYAMDV
light	U1-51	319	KSSQSVLYSSNNKNYLA	344	WASTRES	383	QQYYTTPLT
heavy	U1-52	237	GGSISSGGYYWS	279	NIYYSGSTYYNPSLKS	312	GGTGTNYYYYYGMDV
light	U1-52	336	RASQSVSSSYLA	357	GASSWAT	384	QQYGSSPLT
heavy	U1-53	252	GFTFSIYSMN	280	YISSSSSTIYYADSVKG	313	DRGDFDAFDI
light	U1-53	337	QASQDITNYLN	351	DASNLET	385	QQCENFPIT
heavy	U1-55.1	253	GGSVSSGGYYWN	281	YINYSGSTNYNPSLKS	301	DRELELYYYYYGMDV
light	U1-55.1	same as U1-55					
heavy	U1-55	same as U1-55.1					
light	U1-55	338	RSSQSLLYSNGYKYLD	346	LGSNRAS	366	MQALQTPIT
heavy	U1-57.1	same as U1-57					
light	U1-57.1	338	RSSQSLLYSNGYKYLD	346	LGSNRAS	366	MQALQTPIT
heavy	U1-57	254	GGSVSSGGYYWN	281	YINYSGSTNYNPSLKS	301	DRELELYYYYYGMDV
light	U1-57	same as U1-57.1					
heavy	U1-58	255	GFTFSSYGMH	264	VIWYDGSNKYYADSVKG	314	AARLDYYYGMDV
light	U1-58	339	RASQSINSYLN	358	GASGLQS	386	QQSYSSPLT

Ab chain	Pat ID	SEQ ID NO	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3
heavy	U1-59	256	GGSFSGYYWS	282	EINHSGSTNYNPSLKS	315	DKWTWYFDL
light	U1-59	340	RSSQSVLYSSSNRNYLA	344	WASTRES	387	QQYYSTPRT
heavy	U1-61.1	257	GVSISSGGYYWS	258	YIYYSGSTYYNPSLKS	316	DSESEYSSSSNYGMDV
light	U1-61.1	same as U1-61.1					
heavy	U1-61	257	GVSISSGGYYWS	258	YIYYSGSTYYNPSLKS	316	DSESEYSSSSNYGMDV
light	U1-61	341	RASQTISSYLN	359	AASSLQG	377	QQSYSNPLT
heavy	U1-62	247	GYSFTSYWIG	273	IIYPGDSDTRYSPSFQG	317	QMAGNYYYGMDV
light	U1-62	342	RASQSVISIYLA	347	GASSRAT	388	QQYGSSPCS

[0059] In some embodiments, an isolated binding protein targeted to HER3 can include a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and/or a light chain amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232. The sequences referred to are shown in the sequence listing in US Patent No. 7705130, and are hereby incorporated herein by reference in their entireties.

[0060] In some embodiments, an anti-HER3 antibody can include a heavy chain amino acid sequence and a light chain amino acid sequence as shown in SEQ ID NOS: 2 and 4, 6 and 8, 10 and 12, 14 and 16, 18 and 20, 22 and 24, 26 and 28, 30 and 32, 36 and 38, 42 and 44, 46 and 48, 50 and 52, 54 and 56, 60 and 58, 62 and 64, 66 and 68, 70 and 72, 74 and 76, 78 and 82, 80 and 82, 84 and 86, 88 and 90, 92 and 94, 96 and 98, 100 and 102, 104 and 106, 108 and 110, 112 and 114, 116 and 118, 122 and 124, 126 and 128, 130 and 132, 134 and 136, 138 and 140, 142 and 144, 146 and 148, 150 and 152, 154 and 156, 158 and 160, 162 and 164, 166 and 168, 170 and 172, 174 and 176, 178 and 180, 182 and 184, 186 and 188, 190 and 192, 194 and 196, 198 and 200, 202 and 204, 206 and 208, 210 and 212, 214 and 216, 218 and 220, 222 and 224, 226 and 228, 230 and 232, or a heavy chain amino acid sequence as shown in any one of SEQ ID NOS: 34, 40, 60, 62, and 120, or a light chain amino acid sequence as shown in either of SEQ ID NOS: 58 and 64. The sequences referred to appear in the disclosure and the sequence listing of US Patent No. 7705130, and are hereby incorporated herein by reference in their entireties.

[0061] In some embodiments, a protein targeted to HER3 can be a scaffold protein having an antibody-like binding activity (*e.g.*, having activity similar to an anti-HER3 antibody), or an antibody, *e.g.*, an anti-HER3 antibody. The anti-HER3 antibody can be selected from the group consisting of antibodies designated U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, and U1-62, or an antibody having at least one heavy or light chain of one of the aforeto antibodies. The antibodies designated as U1-49 (SEQ ID NO: 42/44), U1-53 (SEQ ID NO: 54/56), and U1-59 (SEQ ID NO: 70/72), or an antibody having at least one heavy or light chain of one of these antibodies, may be particularly useful. All sequences of the antibodies and sequences thereof referred to are shown in the sequence listing and disclosure of US Patent No. 7705130, and are hereby incorporated herein by reference in their entireties.

[0062] It is to be understood that the amino acid sequence of the HER3 binding proteins provided herein is not limited to the twenty conventional amino acids (*See:* Immunology - A Synthesis, 2nd Edition, Golub and Gren, eds., Sinauer Associates, Sunderland, MA (1991)), which publication is hereby incorporated herein by reference in its entirety. For example, the amino acids may include stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as I-,I-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids. Examples of unconventional amino acids, which may also be suitable components for the binding proteins provided herein, include: 4-hydroxyproline, K-carboxyglutamate, M-N,N,N-trimethyllysine, M-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids, *e.g.*, 4-hydroxyproline.

[0063] Furthermore, minor variations in the amino acid sequences shown in SEQ ID NOS: 1-390 (as set forth in the appendix filed herewith) are contemplated as being encompassed by the present disclosure, provided that the variations in the amino acid sequence maintain at least 75% (*e.g.*, at least 80%, 90%, 95%, or 99%) of the sequences shown in SEQ ID NOS: 1-390. Variations can occur within the framework regions (*i.e.*, outside the CDRs), within the CDRs, or within the framework regions and the CDRs. In

some embodiments, variations in the amino acid sequences shown in SEQ ID NOS: 1-390, *i.e.*, deletions, insertions and/or substitutions of at least one amino acid, can occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other binding proteins of known structure and/or function. Methods for identifying protein sequences that fold into a known three-dimensional structure are known in the art. *(See:* Bowie et al., *Science* 253:164 (1991); *Proteins, Structures and Molecular Principles*, Creighton, Ed., W. H. Freeman and Company, New York (1984); Introduction to Protein Structure, Branden and Tooze, eds., Garland Publishing, New York, N.Y. (1991); and Thornton et al. *Nature* 354:105 (1991), which publications are hereby incorporated herein by reference in their entireties. Thus, those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the proteins described herein.

[0064] Variations in the amino acid sequences shown in SEQ ID NOS:1-390 can include those that lead to a reduced susceptibility to proteolysis or oxidation, alter glycosylation patterns or alter binding affinities or confer or modify other physicochemical or functional properties of the binding protein. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Amino acid families include the following: acidic family = aspartate, glutamate; basic family = lysine, arginine, histidine; non-polar family = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and uncharged polar family = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Alternative families include: aliphatic-hydroxy family = serine and threonine; amide-containing family = asparagine and glutamine; aliphatic family = alanine, valine, leucine and isoleucine; and aromatic family = phenylalanine, tryptophan, and tyrosine. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting binding protein, especially if the replacement does not involve an amino acid within a framework site. However, all other possible amino acid replacements also are encompassed herein. Whether an amino acid change results in a functional HER3 binding protein that reduces

signal transduction of HER3 can readily be determined by assaying the specific HER3 binding activity of the resulting binding protein by ELISA or FACS, or *in vitro* or *in vivo* functional assays.

[0065] In some embodiments, a HER3 binding protein can be coupled to an effector group. Such a binding protein can be especially useful for therapeutic applications. As used herein, the term "effector group" refers to a cytotoxic group such as a radioisotope or radionuclide, a toxin, a therapeutic group or other effector group known in the art. Examples of suitable effector groups are radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I) or non-radio isotopes (*e.g.*, 2D), calicheamicin, dolastatin analogs such as auristatins, and chemotherapeutic agents such as geldanamycin and maytansine derivates, including DM1. Thus, in some cases, a group can be both a labeling group and an effector group. For example, this includesToxins, RNA Polymerase Inhibitors or other compounds suitable for use as ADC (Antibody Drug conjugate). Various methods of attaching effector groups to polypeptides or glycopolypeptides (such as antibodies) are known in the art, and may be used in making and carrying out the compositions and methods described herein. In some embodiments, it may be useful to have effector groups attached to a binding protein by spacer arms of various lengths to, for example, reduce potential steric hindrance.

[0066] This document also relates to processes for preparing an isolated HER3 binding protein, comprising the step of preparing the protein from a host cell that expresses the protein. Host cells that can be used include, without limitation, hybridomas, eukaryotic cells (*e.g.*, mammalian cells such as hamster, rabbit, rat, pig, or mouse cells), plant cells, fungal cells, yeast cells (*e.g.*, *Saccharomyces cerevisiae* or *Pichia pastoris* cells), prokaryotic cells (*e.g.*, *E. coli* cells), and other cells used for production of binding proteins. Various methods for preparing and isolating binding proteins, such as scaffold proteins or antibodies, from host cells are known in the art and may be used in performing the methods described herein. Moreover, methods for preparing binding protein fragments, *e.g.*, scaffold protein fragments or antibody fragments, such as papain or pepsin digestion, modern cloning techniques, techniques for preparing single chain antibody molecules (Plückthun, *supra*) and diabodies (Hollinger *et al.*, *supra*), also are known to those skilled in the art and may be used in performing the presently described methods.

[0067] In some embodiments, a HER3 binding protein can be prepared from a hybridoma that secretes the protein. See: Köhler et al. *Nature* 256:495 (1975).

[0068] In some embodiments, a HER3 binding protein can be prepared recombinantly by optimizing and/or amplifying expression of the binding protein in host cells, and isolating the binding protein from the host cells. To this end, host cells can be transformed or transfected with DNA (*e.g.*, a vector) encoding a HER3 binding protein, and cultured under conditions appropriate to produce the binding protein. *See:* US Patent No. 4816567. Useful host cells include, for example, CHO cells, NS/0 myeloma cells, human embryonic kidney 293 cells, *E. coli* cells, and *Saccharomyces cerevisiae* cells.

[0069] HER3 binding proteins that are antibodies can be prepared from animals genetically engineered to make fully human antibodies, or from an antibody display library made in bacteriophage, yeast, ribosome or *E. coli*. *See:* Clackson et al., *Nature* 352:624-628 (1991); Marks et al., *J. Mol. Biol.* 222:581-597 (1991); Feldhaus and Siegel, *J. Immunol. Methods* 290:69-80 (2004); Groves and Osbourn, *Expert Opin. Biol. Ther.* 5:125-135 (2005); and Jostock and Dubel, *Comb. Chem. High Throughput Screen* 8:127-133 (2005), which publications are hereby incorporated herein by reference in their entireties.

[0070] In some embodiments, antibodies as provided herein can be fully human or humanized antibodies. Human antibodies avoid certain problems associated with xenogeneic antibodies, such as antibodies that possess murine or rat variable and/or constant regions. The presence of xenogeneic-derived proteins can lead to an immune response against the antibody by a patient, subsequently leading to the rapid clearance of the antibody, loss of therapeutic utility through neutralization of the antibody, and/or severe, even life-threatening, allergic reactions. To avoid using murine or rat-derived antibodies, fully human antibodies can be generated through the introduction of functional human antibody loci into a rodent or another mammal or animal so that the rodent, other mammal or animal produces fully human antibodies.

[0071] One method for generating fully human antibodies is to utilize XENOMOUSE® strains of mice that have been engineered to contain 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus. Other XENOMOUSE® strains of mice contain 980 kb and 800 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus. Still other XENOMOUSE® strains of mice contain 980 kb and 800 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus plus a 740 kb-sized germline configured complete human lambda light chain locus. *See:* Mendez et al., *Nature Genetics* 15:146-156 (1997); and Green and Jakobovits, *J. Exp. Med.*

188:483-495 (1998). XENOMOUSE® strains are available from Amgen, Thousand Oaks, CA.

[0072] The production of XENOMOUSE® mice is further discussed and delineated in: US Patent Publication No. 20030217373, filed November 20, 2002; US Patent Nos. 5939598, 6075181, 6114598, 6150584, 6162963, 6673986, 6833268, and 7435871; and Japanese Patent Nos. 3068180B2, 3068506B2, and 3068507B2. Also *See*: Patent No. EP 0463151; and PCT Publication Nos. WO94/02602, WO96/34096, WO98/24893, and WO00/76310. Each of the disclosures of the above publications and patents is hereby incorporated herein by reference in it's entirety.

[0073] Alternatively, a "minilocus" approach can be used. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (*e.g.*, a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in US Patent Nos. 5545806, 5545807, 5569825, 5591669, 5612205, 5625126, 5625825, 5633425, 5643763, 5661016, 5721367, 5770429, 5789215, 5789650, 5814318, 5874299, 5877397, 5981175, 6023010, 6255458, the disclosures of which are hereby incorporated herein by reference in their entirety. Also *See:* Patent No. EP0546073; PCT Publication Nos. WO92/03918, WO92/22645, WO92/22647, WO92/22670, WO93/12227, WO94/00569, WO94/25585, WO96/14436, WO97/13852, and WO98/24884, the disclosures of which are hereby incorporated herein by reference in their entireties.

[0074] Human antibodies also can be generated from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. *See:* Patent Application Nos. EP773288 and EP843961, the disclosures of which are hereby incorporated herein by reference in their entireties. Additionally, KM™ mice, which are the result of cross-breeding of Kirin's Tc mice with Medarex's minilocus (Humab) mice have been generated. These mice possess the HC transchromosome of the Kirin mice and the kappa chain transgene of the Medarex mice (*See:* Ishida et al., *Cloning Stem Cells* 4:91-102 (2002)).

[0075] Human antibodies also can be derived by *in vitro* methods. Suitable examples include, but are not limited to, phage display (as commercialized by Cambridge Antibody Technology, Morphosys, Dyax, Biosite/Medarex, Xoma, Symphogen, Alexion

(formerly Proliferon), and Affimed), ribosome display (as commercialized by Cambridge Antibody Technology), yeast display, and the like.

[0076] As described herein, antibodies were prepared using XENOMOUSE® technology, as described below. Such mice are capable of producing human immunoglobulin molecules and antibodies, and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein. For example, transgenic production of mice and antibodies therefrom is disclosed in US Patent Application No. 08759620, filed December 3, 1996, now abandoned, PCT Publication Nos. WO98/24893, published June 11, 1998 and WO00/76310, published December 21, 2000, the disclosures of which are hereby incorporated herein by reference in their entirety. Also *See:* Mendez et al., Nature Genetics, 15:146-156 (1997), the disclosure of which is hereby incorporated herein by reference in its entirety.

[0077] Using technology as described herein, fully human monoclonal antibodies to a variety of antigens can be produced. For example, XENOMOUSE® lines of mice can be immunized with a HER3 antigen of interest (*e.g.*, HER3 or a fragment thereof), lymphatic cells (such as B-cells) can be recovered from mice that express antibodies, and the recovered cell lines can be fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. These hybridoma cell lines can be screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. Provided herein are methods for the production of multiple hybridoma cell lines that produce antibodies specific to HER3. Further provided herein are methods for characterizing antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

[0078] In general, antibodies produced by fused hybridomas as described below are human IgG1 heavy chains with fully human kappa light chains, although some antibodies described herein possess human IgG4 heavy chains as well as IgG1 heavy chains. Antibodies also can be of other human isotypes, including IgG2 and IgG3. The antibodies generally have high affinities, with a K_D typically from about 10^{-6} to about 10^{-13} M or below, when measured by solid phase and cell-based techniques.

[0079] This document also provides isolated nucleic acid molecules that encode HER3 binding proteins as described herein. The term "isolated nucleic acid molecule," as used herein, refers to a polynucleotide of genomic, cDNA, or synthetic origin, or some

combination thereof, which (1) is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Further, the term "nucleic acid molecule," as used herein, means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, such as nucleotides with modified or substituted sugar groups and the like. The term also includes single and double stranded forms of DNA.

[0080] In some embodiments, a nucleic acid molecule can be operably linked to a control sequence. The term "control sequence," as used herein, refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoters, ribosomal binding sites, and transcription termination sequences. In eukaryotes, generally, such control sequences include promoters and transcription termination sequences. The term "control sequence" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Furthermore, the term "operably linked", as used herein, refers to positions of components so described which are in a relationship permitting them to function in their intended manner. Moreover, an expression control sequence operably linked to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the expression control sequence.

[0081] Also provided herein are vectors comprising a nucleic acid molecule encoding a binding protein as disclosed herein. The nucleic acid molecule can be operably linked to a control sequence. Furthermore, the vector may additionally contain a replication origin or a selection marker gene. Examples of vectors that may be used include, *e.g.*, plasmids, cosmids, phages, and viruses.

[0082] This document also provides host cells transformed with a nucleic acid molecule or vector as described herein. Transformation can be accomplished by any known method for introducing polynucleotides into a host cell, including, for example, packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector), or by transfection procedures known in the art, as exemplified by

US Patent Nos. 4399216, 4912040, 4740461, and 4959455. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art, and include, without limitation, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. Examples of host cells that may be used include hybridomas, eukaryotic cells (*e.g.*, mammalian cells such as hamster, rabbit, rat, pig, mouse, or other animal cells), plant cells (*e.g.*, corn and tobacco cells), fungal cells (*e.g.*, *S. cerevisiae* and *P. pastoris* cells), prokaryotic cells such as *E. coli*, and other cells used in the art for production of antibodies. Mammalian cell lines available as hosts for expression are well known in the art and include, for example, many immortalized cell lines available from the American Type Culture Collection (ATCC; Manassas, VA). These include, without limitation, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2 cells), and a number of other cell lines.

[0083] In other embodiments, the agent binding to HER3 is a small molecule compound. Such compounds can be identified using, for example, physical or virtual libraries of small molecules. In some embodiments, for example, useful small molecule compounds can be identified using consensus virtual screening methods based on known HER3 inhibitors and models of HER3 active and inactive state structures. Compounds that appear to be of interest can be further analyzed for structural novelty and desirable physicochemical properties. Candidate compounds identified by virtual screening can be tested *in vitro* for, *e.g.*, the ability to inhibit growth of cells that overexpress HER3. In other embodiments, useful small molecule compounds can be identified from a library of small molecule compounds, using high throughput methods to screen large numbers of compounds for the ability to bind to and/or inhibit activity of HER3 (*e.g.*, in cells that overexpress HER3). Small molecule HER3 inhibitors can be synthesized using standard chemical synthesis methods, for example.

[0084] In yet anther embodiment, the agent that binds to HER3 may be a siRNA that interferes with the expression of HER3. An example of siRNA is EZN-3920 (antisense targeting HER3 mRNA) (Santaris Pharma, Hoersholm, Denmark).

[0085] In yet other embodiments, the agent that binds HER3 may be a natural substance. For example, Kahalalide F, a marine-derived agent, has been suggested to inhibit HER3 oncogenic signaling (*See:* Jimeno et al., *J. Translational Med.* 4:3 (2006)) by

down-regulating HER3 protein expression and AKT signaling (*See:* Janmaat et al., *Mol. Pharmacol.* 68:502-510 (2005)).

4. Additional Agents

[0086] In some cases, the compositions and methods provided herein for prostate treatment can include a first agent that binds to HER3 in combination with a second agent. In some cases, the second agent can bind and/or inhibit at least one other member of the HER family, such as EGF-R, HER2, or HER4. Such a second agent can be, without limitation, a biological drug (*e.g.*, a binding protein, such as an antibody that specifically binds to a member of the HER family, a small molecular compound that binds to and/or alters (*e.g.*, inhibits) the activity of at least one member of the HER family other than (or in addition to) HER3, an siRNA, or a natural substance. As used herein, the terms "other HER family members" and "another HER family member" refer to HER family members that are not HER3. Examples include EGF-R, HER2, and HER4, but "HER family member" also includes family members that have not yet been identified.

[0087] The second agent can alter the activity (*e.g.*, increase or decrease the activity) of the other HER family member, either through a direct effect or an indirect effect on the HER family member. It is noted, however, that all second agents as provided herein will have an effect on HER family function and activity. In some cases, for example, the second agent can be an antibody that can bind to another HER family member (*e.g.*, EGF-R, HER2, or HER4), or to another molecule that in turn can affect the activity of the other HER family member. Such an antibody can be targeted, for example, to the extracellular domain of the other HER family member, or to any other suitable domain thereof (*e.g.*, a kinase domain or a dimerization domain).

[0088] A second agent can be further characterized in that its effect on another HER family member reduces HER-mediated signal transduction. A reduction of HER-mediated signal transduction may, *e.g.*, be caused by downregulation of the targeted HER family member, resulting in an at least partial disappearance of the HER molecule from the cell, or by a stabilization of the HER family member in a substantially inactive form. Alternatively, a reduction of HER-mediated signal transduction may be caused by influencing, *e.g.*, decreasing or inhibiting, the binding of a ligand to the HER family member, the binding of the HER family member to HER3, or the binding of GRB2 to HER2 or GRB2 to SHC, or, by inhibiting receptor tyrosine phosphorylation, AKT phosphorylation, PYK2 tyrosine phosphorylation, or ERK2 phosphorylation, or any other cellular component

affecting the HER-family mediated signal transduction pathway. For example, a reduction of HER mediated signal transduction can be caused by decreasing the formation of dimers containing HER3 and another HER family member (*e.g.*, EGF-R, HER2, or HER4). Regardless of the mechanism behind the function, it is noted that the second agent can serve to amplify the effect of the first agent that is targeted to HER3.

[0089] In some embodiments, an agent that binds to another HER family member or another protein that in turn affects activity of another HER family member can be a scaffold protein having an antibody like binding activity (*e.g.*, having activity similar to an anti-HER3 antibody) or an antibody (*e.g.*, an anti-EGF-R, anti-HER2, or anti-HER4 antibody). Scaffold proteins and antibodies in this context are as defined and described above for agents targeted to HER3.

[0090] It is noted that in some embodiments, the first agent that binds to HER3, and the second agent that binds to and/or inhibits another HER family member are combined within one compound, such as a bispecific antibody.

[0091] Also as described above, the amino acid sequences of proteins that bind to other HER family members, or to other proteins that in turn affect the activity of another HER family member, are not limited to the twenty conventional amino acids. Further, as for the HER3 binding proteins described herein, an agent that binds to or otherwise affects the activity of another HER family member can be coupled to an effector group.

[0092] This document also relates to processes for preparing isolated proteins (*e.g.*, antibodies) that can bind to other HER family members, for example. Such processes include those described above in the context of HER3 binding proteins. In some embodiments, antibodies (*e.g.*, anti-HER, anti-HER2, or anti-HER4 antibodies, respectively) can be prepared from animals engineered to make fully human antibodies, or from an antibody display library made in bacteriophage, yeast, ribosomes, or *E. coli*. Further, an antibody targeted directly or indirectly to another HER family member can be fully human or humanized, as described above.

[0093] Also provided herein are isolated nucleic acid molecules (*e.g.*, vectors) expressing proteins that can bind to other HER family members and other proteins that can affect the activity of other HER family members. Protein coding sequences within such nucleic acid molecules can be operably linked to one or more control sequences, as described above. Further, nucleic acid molecules can be transformed or transfected into a host cell as described above.

[0094] In some embodiments, the second agent is a small molecular tyrosine kinase inhibitor provided that the agent can affect (either directly or indirectly) the activity of a HER family member other than (or in addition to) HER3. Such inhibitors can be identified using, for example, physical or virtual libraries of small molecules. In some embodiments, for example, useful small molecule compounds can be identified using consensus virtual screening methods based on known tyrosine kinase inhibitors and models of HER family member structures in active and inactive states. Compounds that are initially identified as being of potential interest can be further analyzed for structural novelty and desirable physicochemical properties. Candidate compounds identified by virtual screening can be tested *in vitro* for, *e.g.*, the ability to inhibit growth of cells that overexpress a HER family member other than HER3. In other embodiments, useful small molecule tyrosine kinase inhibitors can be identified from a library of small molecule compounds and using high throughput methods to screen large numbers of the compounds for the ability to bind to and/or inhibit activity of one or more HER family members other than HER3 (*e.g.*, in cells that overexpress the HER protein). Small molecular tyrosine kinase inhibitors can be synthesized using, for example, standard chemical synthesis methods.

[0095] Agents that can affect an activity of EGF-R (HER) include AEE-788 (Novartis, Basel, Switzerland), BIBW-2992(N-[4-(3-chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (Boehringer Ingelheim, Ingelheim, Germany), BMS-599626 (Bristol-Myers Squibb, New York, NY), BMS-690514 (Bristol-Myers Squibb, New York, NY), carnetinib dihydrochloride (N-[4-[N-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazolin-6-yl]acrylamide dihydrochloride (Pfizer, New York, NY), CNX-222 (Avila Therapeutics, Waltham, MA), CUDC-101 (Curis, US Patent No. 7547781), Dimercept (Receptor Biologix, Palo Alto, CA), lapatinib (ditosilate hydrate (N-[3-chloro-4-[(3-fluorobenzyl)oxy]phenyl]-6-[5-[[[2-(methylsulfonyl)ethyl]amino]methyl]furan-2-yl]quinazolin-4-amine bis(4-methylbenzenesulfonate) monohydrate (GlaxoSmithKline, London, England), MP-412 (Mitsubishi Tanabe Pharma Co., Osaka, Japan), neratinib ((2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide)(Wyeth, Madison, NJ), S-222611 (Shionogi, Osaka, Japan), varlitinib (4-N-[3-chloro-4-(thiazol-2-ylmethoxy)phenyl]-6-N-[(4R)-4-methyl-4,5-dihydrooxazol-2-yl]quinazoline-4,6-diamine bis(4-methylbenzenesulfonate) (Array BioPharma, Boulder, CO), AGT-2000 (ArmeGen Technologies, Santa Monica, CA), AZD-4769 (AstraZeneca, London, England), BIBX-1382 (Boehringer Ingelheim, Ingelheim, Germany), CGP-52411 (4,5-

bis(phenylamino)-1H-isoindole-1,3(2H)-dione) (Novartis, Basel, Switzerland), CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide) (Wyeth, Madison, NJ), CP-292597 (Pfizer, New York, NY), DAB-1059 (Mitsubishi Tanabe Pharma Co., Osaka, Japan), erlotinib (hydrochloride(4-(3-ethynylphenylamino)-6,7-bis(2-methoxyethoxy)-quinazoline hydrochloride (OSI Pharmeceuticals, Long Island, NY, US Patent No. 5747498), gefitinib(4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl)propoxy]quinazoline) (AstraZeneca, London, England, US Patent No. 5821246), HMPL-813 (Hutchison China MediTech, Hong Kong), MDP-01, (Med Discovery, Plan-Les-Ouates, Switzerland), MT-062 (Medisyn Technologies, Minneapolis, MN), ONC-101 (Oncalis, Schlieren, Switzerland), PD-153035,(4-(3-bromophenylamino)-6,7-dimethoxyquinazoline) (AstraZeneca, London, England), PD-169540 (Pfizer, New York, NY), pelitinib (Wyeth Pharmaceuticals, Madison, NJ), PF-299804 (Pfizer, New York, NY), PKI-166 (4-(R)-phenethylamino-6-(hydroxyl)phenyl-7H-pyrrolo[2.3-d]-pyrimidine) (Novartis, Basel, Switzerland), vandetanib (N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine) (AstraZeneca, London, England), VGA-1102 (Taisho Pharmaceuticals, Tokyo, Japan), WHI-P154 (4-(3'-bromo-4'-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline), ZD-1838 (AstraZeneca, London, England), cetuximab (ImClone Systems, New York, NY), panitumumab (Amgen, Thousand Oaks, CA).

[0096] Agents that can affect an activity of HER2 include AEE-788 (Novartis, Basel, Switzerland), ARRY-333786 (Array BioPharma, Boulder, CO), ARRY-380 (Array BioPharma, Boulder, CO), BIBW-2992 (N-[4-(3-chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (Boehringer Ingelheim, Ingelheim, Germany), BMS-599626 (Bristol-Myers Squibb, New York, NY), BMS-690514 (Bristol-Myers Squibb, New York, NY), carnetinib dihydrochloride (N-[4-[N-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazolin-6-yl]acrylamide dihydrochloride) (Pfizer, New York, NY), CNF-201 (Biogen Idec, San Diego, CA), CNX-222 (Avila Therapeutics, Waltham, MA), CP-654577 (OSI Pharmaceuticals, Long Island, NY), CP-724714 (2-methoxy-N-[3-[4-[3-methyl-4-(6-methyl-pyridin-3-yloxy)phenylamino]quinazolin-6-yl]-E-allyl]acetamide) (OSI Pharmaceuticals, Long Island, NY), CUDC-101 (Curis, Cambridge, MA, US Patent No. 7547781), D-69491 (Baxter International, Deerfield, IL), Dimercept (Receptor Biologix, Palo Alto, CA), EHT-102 (ExonHit Therapeutics, Paris, France), HER2 antagonist (Centgent Therapeutics, San Diego, CA), HER/neu vaccine (Corixa, Seattle, WA), Herzyme (Sima Therapeutics, San Francisco, CA), HuMax-Her2 (Genmab, Copenhagen, Denmark), INSM-18 (Insmed,

Richmond, VA), lapatinib (ditosilate hydrate(N-[3-chloro-4-[(3-fluorobenzyl)oxy]phenyl]-6-[5-[[[2-(methylsulfonyl)ethyl]amino]methyl]furan-2-yl]quinazolin-4-amine bis(4-methylbenzenesulfonate) monohydrate) (GlaxoSmithKline, London, England), MP-412 (Mitsubishi Tanabe Pharma Co., Osaka, Japan), mu-4-D-5 (Genentech, South San Francisco, CA), mubritinib (1-[4-[4-[[2-[(E)-2-[4-(trifluoromethyl)phenyl]ethenyl]oxazol-4-yl]methoxy]phenyl]butyl]-1H-1,2,3-triazole) (Takeda Pharmaceuticals, Deerfield, IL), neratinib ((2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide): (Wyeth, Madison, NJ), pertuzumab (Genentech, South San Francisco, CA), PX-103.1 (Pharmexa, Copenhagen, Denmark), PX-103.2 (Pharmexa, Copenhagen, Denmark), PX-104.1 (Pharmexa, Copenhagen, Denmark), S-222611 (Shionogi, Osaka, Japan), TAK-285 (Takeda Pharmaceuticals, Deerfield, IL), trastuzumab (Genentech, South San Francisco, CA), Trastuzumab-DM1 (ImmunoGen, Waltham, MA), varlitinib (4-N-[3-chloro-4-(thiazol-2-ylmethoxy)phenyl]-6-N-[(4R)-4-methyl-4,5-dihydrooxazol-2-yl]quinazoline-4,6-diamine bis(4-methylbenzenesulfonate)) (Array BioPharma, Boulder, CO), VM-206 (ViroMed, Minneapolis, MN).

[0097] Agents that can affect an activity of HER4 include Dimercept (Receptor Biologix, Palo Alto, CA), neratinib ((2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide) (Wyeth, Madison, NJ).

[0098] Particular non-limiting examples of agents that can bind to and/or alter activity of other HER family members and can be used in the compositions and methods provided herein are described below.

[0100] Panitumumab, marketed as VECTIBIX® (Amgen, Thousand Oaks, CA), is a fully human monoclonal antibody specific to EGF-R.

[0101] Erlotinib, marketed as TARCEVA™ (Genentech, South San Francisco, CA; OSI Pharmaceuticals, Long Island, NY; Roche, Basel, Switzerland), is a drug used to treat NSCLC, pancreatic cancer, and several other types of cancer. Erlotinib specifically targets the EGF-R tyrosine kinase, binding reversibly to the ATP binding site of the receptor.

[0102] Lapatinib (GlaxoSmithKline, London) is an orally active small molecule for the treatment of solid tumors such as breast cancer. Lapatinib is a dual tyrosine kinase inhibitor that inhibits tyrosine kinase activity associated with EGF-R and HER2/neu (human EGF-R type 2).

[0103] Trastuzumab, also known as HERCEPTIN® (Genentech, South San Francisco, CA), is a humanized monoclonal antibody that interferes with the HER2/neu receptor.

[0104] T-DM1 (Genentech, South San Francisco, CA; Roche) is an antibody-drug conjugate that includes trastuzumab chemically linked to a potent antimicrotubule drug (DM1) derived from maytansine. Maytansine has been used as a free drug, and has shown effectiveness in, *e.g.*, breast and lung cancer patients. The non-reducible thioether MCC linker is used in T-DM1, providing a stable bond between trastuzumab and DM1, prolonging exposure, and reducing the toxicity of T-DM1 while maintaining activity.

[0105] Pertuzumab, marketed as OMNITARG™ (Genentech, South San Francisco, CA) and also known as c2C4, is a monoclonal antibody that inhibits the dimerization of HER2 with other HER receptors.

[0106] Cetuximab, marketed as PERTUZUMAB® (ImClone, New York, NY; and Bristol Myers Squibb, New York, NY), is a chimeric (mouse/human) monoclonal antibody that binds to and inhibits EGF-R.

[0107] Gefitinib, marketed as IRESSA® (AstraZeneca, London; and Teva, Petah Tikva, Israel), is a drug that acts in a similar manner to erlotinib. Gefitinib selectively inhibits EGF-R's tyrosine kinase domain.

[0108] Neratinib (Pfizer Inc., New York, NY) is an inhibitor of the HER2 receptor tyrosine kinase. Neratinib binds irreversibly to the HER2 receptor and thereby reduces autophosphorylation in cells, apparently by targeting a cysteine residue in the ATP-binding pocket of the receptor. Treatment of cells with neratinib results in inhibition of downstream signal transduction events and cell cycle regulatory pathways, arrest at the G1-S-phase transition of the cell cycle, and ultimately decreased cellular proliferation. In addition, neratinib inhibits the EGF-R kinase and proliferation of EGF-R-dependent cells.

[0109] In some embodiments, a method for treatment of HER3-associated disease can include administration of U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, or U1-62, U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-

9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, or U1-62,U1-49, U1-53 or U1-59, in combination with any of the above agents (*e.g.*, simultaneously or separately), or U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, or U1-62, U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, or U1-62,U1-49, U1-53 or U1-59, in combination with any of the above agents and any other agent(s), for treatment of prostate conditions, including, *e.g.*, BPH and prostate cancer. For sequence identification of the CDR's antibodies listed above, *see*, TABLE 1 and the sequence listing and disclosure of US Patent No. 7705130, and which is hereby incorporated herein by reference in its entirety.

[0110] In some embodiments, the second agent can be a chemotherapeutic drug. For example, agents that act as microtubule stimulants include NK-105(paclitaxel) [(-)-(1S,2R,3S,4S,5R,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate] (NanoCarrier, Chiba, Japan), milataxel (1,10β-dihydroxy-9-oxo-5β,20-epoxy-3zeta-tax-11-ene-2α,4,7β13α-tetrayl 4-acetate 2-benzoate 13-[(2R,3R)-3-(tert-butoxycarbonylamino)-3-(furan-2-yl)-2-hydroxypropanoate] 7-propanoate) (Taxolog, Fairfield, NJ), laulimalide (Kosan Biosciences, Hayward, CA (B-M Squibb)), sarcodictyin A (3-(1-methylimidazol-4-yl)-2(E)-propenoic acid (1R,4aR,6S,7S,10R,12aR)-11-methoxycarbonyl-7,10-epoxy-10-hydroxy-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,7,10,12a-octahydrobenzocyclododecen-6-yl ester) (Pfizer, New York, NY), simotaxel ((2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxete-6,9,12,12b-tetrayl 12b-acetate 12-benzoate 6-

cyclopentanecarboxylate 9-[(2R,3R)-2-hydroxy-3-[[(1-methylethoxy)carbonyl]amino]-3-(thiophen-2-yl)propanoate]) (Taxolog, Fairfield, NJ), SYN-2001 (CLL Pharma, Nice, France), TL-310 (Taxolog, Fairfield, NJ), TL1836 (Taxolog, Fairfield, NJ), tesetaxel (2'-[(dimethyla-mino)methyl]-1-hydroxy-5β,20-epoxy-9α,10α-dihydro[1,3]dioxolo[4',5':9,10]tax-11-ene-2α,4,13α-triyl4-acetate 2-benzoate 13-[(2R,3S)-3-[(tert-butoxycarbonyl)amino]-3-(3-fluoropyridin-2-yl)-2-hydroxypropanoate) (Daiichi Sankyo, Tokyo, Japan), TL-1892 (Taxolog, Fairfield, NJ), TPI-287((2'R,3'S)-2'-hydroxy-N-carboxy-3'-amino-5'-methyl-hexanoic, N-tert-butyl ester, 13 ester 5β-20-epoxy-1,2α,4,7β,9α,10α,13α-heptahydroxy-4,10-diacetate-2-benzoate-7,9-acrolein acetal-tax-11-ene (Tapestry Pharmaceuticals, Boulder, CO), ortataxel (2aR-[2aα,4β,4aβ,6β,9α(2R,3S),10β,11β,12α,12aα,12bα]-3-(tert-butoxycarbonyl-amino)-2-hydroxy-5-methyl-hexanoic acid 6,12b-diacetoxy-12-benzoyloxy-10,11-carbonyldioxy-4-hydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benz[1,2-b]oxet-9-yl ester) (Indena, Milan, Italy), paclitaxel poliglumex (L-pyroglutamylpoly-L-glutamyl-L-glutamic acid partially γ-esterified with (1R,2S)-2-(benzoylamino)-1-[[[(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-6,12b-bis(acetyloxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-7,11-methano-1H-cyclodeca[3,4]benzo[1,2-b]oxet-9-yl]oxy]carbonyl]-2-phenylethyl) (Cell Therapeutics, Seattle, WA), paclitaxel protein-bound particles (paclitaxel : (-)-(1S,2R,3S,4S,5R, 7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (Abraxis BioScience, Los Angeles, CA), paclitaxel(NCI)((-)-(1S,2R,3S,4S,5R,7S,8S,10R, 13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (NCI(NIH)), paclitaxel (NeoPharm, Lake Bluff, IL)((-)-(1S,2R,3S,4S,5R,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (NeoPharm, Lake Bluff, IL), patupilone((1S,3S,7S,10R,11S,12S, 16R)-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1E)-1-(2-methyl-1,3-thiazol-4-yl)prop-1-en-2-yl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione) (US Publication No. 20030104625, Novartis, Basel, Switzerland), PEG-paclitaxel (Enzo Pharmaceuticals, Long Island, NY), docetaxel hydrate((-)-(1S,2S,3R,4S,5R,7S,8S,10R,13S)-4-acetoxy-2-benzoyloxy-5,20-epoxy-1,7,10-trihydroxy-9-oxotax-11-ene-13-yl(2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate trihydrate) (Sanofi-Aventis, Bridgewater, NJ), eleutherobin (3-(1-methylimidazol-4-yl)-2(E)-propenoic acid (1R,4aR,6S,7S,10R,12aR)-11-(2-O-acetyl-β-D-arabinopyranosyloxymethyl)-

7,10-epoxy-1-isopropyl-10-methoxy-4,7-dimethyl-1,2,4a,5,6, 7,10,12a-octahydrobenzocyclododecen-6-yl ester) (Bristol-Myers Squibb, New York, NY), IDN-5390 (Indena, Milan, Italy), ixabepilone ((1S,3S,7S,10R,11S,12S,16R)-7,11-dihydroxy-8,8,10,12, 16-pentamethyl-3-[(1E)-1-methyl-2-(2-methylthiazol-4-yl)ethenyl]-17-oxa-4-azabicyclo[14.1.0] heptadecane-5,9-dione) (Bristol-Myers Squibb, New York, NY), KOS-1584 (Kosan Biosciences, Hayward, CA (B-M Squibb)), KOS-1803 (17-iso-oxazole 26-trifluoro-9,10-dehydro-12,13-desoxy-epothilone B) (Kosan Biosciences, Hayward, CA (B-M Squibb)), KOS-862 (Kosan Biosciences, Hayward, CA (B-M Squibb); US Patent Nos. 6204388 and 6303342), larotaxel (1-hydroxy-9-oxo-5β,20-epoxy-7β,19-cyclotax-11-ene-2α,4,10β,13α-tetrayl 4,10-diacetate 2-benzoate 13-[(2R,3S)-3-[(tert-butoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoate] dehydrate) (Sanofi-Aventis, Bridgewater, NJ, PCT Publication Nos. WO 95/26961 and WO 96/1259), ANG-1005 (Angiopep-2/paclitaxel conjugate) (AngioChem, Montreal, Canada, US Patent No. 7557182), BMS-184476 (*See:* Bristol-Myers Squibb, New York, NY, EP Publication No. 639577), BMS-188797 (Bristol-Myers Squibb, New York, NY), BMS-275183 (3'-tert-butyl-3'-N-tert-butyloxycarbonyl-4-deacetyl-3'-dephenyl-3'-N-debenzoyl-4-O-methyoxycarbonyl-paclitaxel) (Bristol-Myers Squibb, New York, NY), BMS-310705 (Bristol-Myers Squibb, New York, NY), BMS-753493 (Bristol-Myers Squibb, New York, NY), cabazitaxel (1-hydroxy-7β,10β-dimethoxy-9-oxo-5β,20-epoxytax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2R,3S)-3-[[(tertbutoxy)carbonyl]amino]-2-hydroxy-3-phenyl-propanoate]) (Sanofi-Aventis, Bridgewater, NJ), DHA-paclitaxel (Protarga, King of Prussia, PA, TAXOPREXIN®), disermolide ([3S-[3α,4β,5β,6α(2R*,3Z,5R*,6R*,7S*,8Z,11R*,12S*, 13S*,14S*,15R*,16E)]]-6-[14[(amino-carbonyl)oxy]-2,6,12-trihydroxy-5,7,9,11,13,15-hexamethyl-3,8,16,18-nonadecate-traenyl]tetrahydro-4-hydroxy-3,5-dimethyl-2H-pyran-2-one) (*See:* Novartis, Basel, Switzerland, US Patent Nos. 4939168 and 5681847). Some of these microtubule stimulants have a taxane ring in their chemical structures; such compounds having a taxane ring are referred as "taxanes" herein.

[0111] Anthracyclins include actinomycins such as actinomycin D (Dactinomycin: 2-amino-N,N'- bis[(6S,9R,10S,13R,18aS)- 6,13-diisopropyl- 2,5,9-trimethyl- 1,4,7,11,14-penta-oxohexadecahydro- 1H-pyrrolo[2,1-i] [1,4,7,10,13] oxatetraazacyclohexadecin- 10-yl]- 4,6-dimethyl- 3-oxo- 3H-phenoxazine- 1,9-dicarboxamide), bleomycin (bleomycin hydrochloride: (3-{[(2'-{(5S,8S,9S,10R,13S)-15-{6-amino-2- [(1S)-3-amino-1-{[(2S)-2,3-diamino-3-oxopropyl] amino}-3-oxopropyl] -5-methylpyrimidin-4-yl}-13-[{[(2R,3S,4S,5S,6S)-3- {[(2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6- (hydroxymethyl)tetrahydro-2H-

pyran-2-yl]oxy} -4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]oxy} (1H-imidazol-5-yl)methyl]-9-hydroxy-5-[(1R)-1-hydroxyethyl]-8,10-dimethyl-4,7,12,15- tetraoxo-3,6,11,14-tetraaza-pentadec-1-yl}-2,4'-bi-1,3- thiazol-4-yl)carbonyl]amino}propyl)(dimethyl)sulfonium), daunorubicin hydrochloride (daunorubicin: 8S-cis)-8-Acetyl-10-((3-amino-2,3,6-trideoxy-alpha-L-lyxo-hexopyranosyl)oxy)-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride), doxorubicin hydrochloride (doxorubicin: (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride) (Alza, Mountain View, CA), idarubicin hydrochloride ((7S,9S)-9-acetyl-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-7-O-(2,3,6-trideoxy-3-amino-α-L-lyxo-hexopyranosyl)-5,12-naphthacenedione hydrochloride) (*See:* Pfizer, New York, NY, US Patent Nos. 4046878 and 4471052), and mitomycin ((1aS,8S,8aR, 8bR)-6-Amino-4,7-dioxo-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methylazirino[2,3:3,4] pyrrolo[1,2-α]indol-8-ylmethylcarbamate) (Kyowa-Hakko-Kirin, Tokyo, Japan).

[0112] Cisplatin and gemcitabine are additional examples of chemotherapeutic agents. Cisplatin or cis-diamminedichloroplatinum(II) is a platinum-based drug used to treat various types of cancers. The cisplatin platinum complex reacts *in vivo*, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis. Gemcitabine is a nucleoside analog in which the hydrogen atoms on the 2' carbons of deoxycytidine are replaced by fluorine atoms. Like fluorouracil and other pyrimidine analogues, gemcitabine replaces cytidine during DNA replication, which arrests tumor growth since further nucleosides cannot be attached to the "faulty" nucleoside, resulting in apoptosis. Gemcitabine is marketed as GEMZAR® by Eli Lilly and Company (Indianapolis, IN). In some embodiments, a combination for treatment of HER3-associated disease can be: U1-49, U1-53 or U1-59 in combination with a second agent as described herein and cisplatin or gemcitabine and other agent(s), for treatment of cancer which is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer.

[0113] Capecitabine (pentyl[1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H-pyrimidin-4-yl]aminomethanoate, Xeloda, Roche) is an orally-administered chemotherapeutic agent. Capecitabine is a prodrug that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue.

In some embodiments, a combination for treatment of HER3-associated disease can be: U1-49, U1-53 or U1-59 in combination with a second agent as described herein (*e.g.*, lapatanib) and capecitabine for treatment of cancer, wherein the cancer is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer. In some cases, such a combination can be administered after failure of prior treatment with an anthracyclin or taxane, for example.

[0114] Docetaxel((2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5, 20-epoxy-1, 2, 4, 7, 10, 13-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate) and paclitaxel((2α,4α,5β,7β,10β,13α)-4,10-bis(acetyloxy)-13-{[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl be) are chemotherapeutic agents. Docetaxel is marketed as Taxotere by Sanofi Aventis. Paclitaxel is marketed as Taxol by Bristol-Myers Squibb. In the formulation of Taxol, paclitaxel is dissolved in Cremophor EL and ethanol, as a delivery agent. A formulation in which paclitaxel is bound to albumin is marketed as Abraxane. In some embodiments, a combination for treatment of HER3-associated disease can be: U1-49, U1-53 or U1-59 in combination with a second agent as described herein (*e.g.*, trastuzumab) and docetaxel or paclitaxel and other agent(s) such as trastuzumab, for treatment of cancer, wherein the cancer is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer.

[0115] Doxorubicin hydrochloride liposome injection is marketed as Doxil, a liposome formulation comprising doxorubicin chloride. In some embodiments, a combination treatment for HER3-associated disease can include administering U1-49, U1-53 or U1-59 in combination with a second agent as described herein and doxorubicin hydrochloride liposome injection, with or without one or more other agents such as paclitaxel or platinum-based chemotherapeutic agents, for treatment of cancer such as breast cancer, gastro-intestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, renal cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma,

metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma.

[0116] Irinotecan hydrochloride hydrate (irinotecan: (+)-(4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino)carbonyloxy]-1H-pyrano[3',4': 6,7]indolizino[1-2-b]quinoline-3,14(4H, 12H)-dione hydrochloride trihydrate) (*See:* Yakult, EP Publication Nos. 137145 and 56692) is marketed as Campto, Camptosar and Ircan. In some embodiments, a combination treatment for HER3-associated disease can include administering U1-49, U1-53 or U1-59 in combination with a second agent as described herein and irinotecan hydrochloride hydrate, or U1-49, U1-53 or U1-59 in combination with a second agent as described herein, irinotecan hydrochloride hydrate, and one or more other agent(s) such as 5-FU(5'-deoxy-5-fluorouridine or 5-fluoro-2,4(1H,3H)-pyrimidinedione), calcium folinate (N-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methylamino]benzoyl]-L-glutamic acid calcium salt (1:1)) or calcium levofolinate ((-)-calcium N-[4-[[[(6S)-2-amino-5-formyl-1,4,5,6,7, 8-hexahydro-4-oxo-6-pteridinyl]methyl]amino] benzoyl]-L-glutamate), and combinations thereof, for treatment of cancer such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, renal cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma.

[0117] As further described below, these and other agents can be contained within the compositions provided herein, and can be administered in a variety of different forms, combinations and dosages.

5. *Compositions*

[0118] HER3 binding agents as described herein can be incorporated into compositions for treatment of a prostate condition such as BPH or prostate cancer. Thus, this document also provides the use of a HER3 binding agent in the manufacture of a medicament for treating BPH or prostate cancer, for example. The compositions can further include, for example, one or more pharmaceutically acceptable carriers, diluents and/or adjuvants, as well as a second agent (*e.g.*, an agent that binds to another HER family member, or a chemotherapeutic agent). The term "pharmaceutical composition," as used herein, refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient (*See:* The McGraw-Hill

Dictionary of Chemical Terms, Parker, Ed., McGraw-Hill, San Francisco (1985)). The potency of the pharmaceutical compositions provided herein typically is based on the binding of the at least one binding protein to HER3. In some embodiments, this binding can lead to a reduction of the HER3-mediated signal transduction.

[0119] A "pharmaceutically acceptable carrier" (also referred to herein as an "excipient" or a "carrier") is a pharmaceutically acceptable solvent, suspending agent, stabilizing agent, or any other pharmacologically inert vehicle for delivering one or more therapeutic compounds (*e.g.*, HER binding proteins) to a subject, which is nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Pharmaceutically acceptable carriers can be liquid or solid, and can be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties, when combined with one or more of therapeutic compounds and any other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers that do not deleteriously react with amino acids include, by way of example and not limitation: water, saline solution, binding agents (*e.g.*, polyvinylpyrrolidone or hydroxypropyl methylcellulose), fillers (*e.g.*, lactose and other sugars, gelatin, or calcium sulfate), lubricants (*e.g.*, starch, polyethylene glycol, or sodium acetate), disintegrates (*e.g.*, starch or sodium starch glycolate), and wetting agents (*e.g.*, sodium lauryl sulfate). Pharmaceutically acceptable carriers also include aqueous pH buffered solutions or liposomes (small vesicles composed of various types of lipids, phospholipids and/or surfactants which are useful for delivery of a drug to a mammal). Further examples of pharmaceutically acceptable carriers include buffers such as phosphate, citrate, and other organic acids, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins such as serum albumin, gelatin, or immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose or dextrins, chelating agents such as EDTA, sugar alcohols such as mannitol or sorbitol, salt-forming counterions such as sodium, and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0120] Liposomes are vesicles that have a membrane formed from a lipophilic material and an aqueous interior that can contain the composition to be delivered. Liposomes can be particularly useful due to their specificity and the duration of action they offer from the standpoint of drug delivery. Liposome compositions can be formed, for

example, from phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylgly-cerol, or dioleoyl phosphatidylethanolamine. Numerous lipophilic agents are commercially available, including LIPOFECTIN® (Invitrogen/Life Technologies, Carlsbad, CA) and EFFECTENE™ (Qiagen, Valencia, CA).

[0121] In some embodiments, at least one of the agents contained in a pharmaceutical composition (*e.g.*, a HER3 binding agent or an agent that binds and/or inhibits another HER family member) can be coupled to an effector such as calicheamicin, duocarmycins, auristatins, maytansinoids, a radioisotope, or a toxic chemotherapeutic agent such as geldanamycin and maytansine. Such conjugates can be particularly useful for targeting cells (*e.g.*, cancer cells) expressing HER3.

[0122] Linking binding proteins to radioisotopes can provide advantages to tumor treatments. Unlike chemotherapy and other forms of cancer treatment, radioimmunotherapy or the administration of a radioisotope-binding protein combination can directly target cancer cells with minimal damage to surrounding normal, healthy tissue. With this "magic bullet," patients can be treated with much smaller quantities of radioisotopes than other forms of treatment available today. Suitable radioisotopes include, for example, yttrium^{90} (^{90}Y), indium^{111} (^{111}In), ^{131}I, ^{99}mTc, radiosilver-111, radiosilver-199, and Bismuth^{213}. The linkage of radioisotopes to binding proteins may be performed with, for example, conventional bifunctional chelates. Since silver is monovalent, for radiosilver-111 and radiosilver-199 linkage, sulphur-based linkers may be used (*See:* Hazra et al., *Cell Biophys.* 24-25:1-7 (1994)). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. Furthermore, tiuxetan is an MX-DTPA linker chelator attached to ibritumomab to form ibritumomab tiuxetan (Zevalin) (*See:* Witzig, *Cancer Chemother. Pharmacol.* 48 (Suppl 1):91-95 (2001)). Ibritumomab tiuxetan can react with radioisotypes such as indium^{111} (^{111}In) or ^{90}Y to form ^{111}In-ibritumomab tiuxetan and ^{90}Y-ibritumomab tiuxetan, respectively.

[0123] The binding proteins described herein, particularly when used to treat cancer, can be conjugated with toxic chemotherapeutic drugs such as maytansinoids, (*See:* Hamann et al., *Bioconjug. Chem.* 13:40-46, (2002)), geldanamycinoids (*See:* Mandler et al., *J. Natl. Cancer Inst.* 92:1549-1551 (2000)) and maytansinoids, for example, the maytansinoid drug, DM1 (*See:* Liu et al., *Proc. Natl. Acad. Sci. USA*, 93:8618-8623, (1996)). Linkers that release the drugs under acidic or reducing conditions or upon

exposure to specific proteases may be employed with this technology. A binding protein may be conjugated as described in the art.

[0124] In some embodiments, a binding protein can be conjugated to auristatin-PE. Auristatin-PE, *e.g.*, is an antimicrotubule agent that is a structural modification of the marine, shell-less mollusk peptide constituent dolastatin 10. Auristatin-PE has both anti-tumor activity and anti-tumor vascular activity (*See:* Otani et al., *Jpn. J. Cancer Res.* 91:837-44 (2000)). For example, auristatin-PE inhibits cell growth and induces cell cycle arrest and apoptosis in pancreatic cancer cell lines (*See:* Li et al., *Int. J. Mol. Med.* 3:647-53 (1999)). Accordingly, to specifically target the anti-tumor activity and anti-tumor vascular activities of auristatin-PE to particular tumors, auristatin-PE may be conjugated to a binding protein as provided herein.

[0125] The pharmaceutical compositions provided herein also can contain at least one further active agent. Examples of further active agents include antibodies or low molecular weight inhibitors of other receptor protein kinases, such as IGFR-1 and c-met, receptor ligands such as vascular endothelial factor (VEGF), cytotoxic agents such as doxorubicin, cisplatin or carboplatin, cytokines, or anti-neoplastic agents. Many anti-neoplastic agents are known in the art. In some embodiments, an anti-neoplastic agent can be selected from the group of therapeutic proteins including, but not limited to, antibodies and immunomodulatory proteins. In some embodiments, an anti-neoplastic agent can be selected from the group of small molecule inhibitors and chemotherapeutic agents consisting of mitotic inhibitors, kinase inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, histone deacetylase inhibitors, anti-survival agents, biological response modifiers, anti-hormones (*e.g.*, anti-androgens), microtubule stimulants, anthracyclins, and anti-angiogenesis agents. When the anti-neoplastic agent is radiation, treatment can be achieved either with an internal source (*e.g.*, brachytherapy) or an external source (*e.g.*, external beam radiation therapy). The one or more further active agent(s) can be administered with the HER3-binding agent and the second agent either simultaneously or separately, in a single formulation or in individual (separate) formulations for each active agent.

[0126] The pharmaceutical compositions provided herein can be especially useful for diagnosis, prevention, or treatment of a prostate condition (*e.g.*, a prostate condition associated with increased HER family signal transduction). The condition can be, for example, associated with increased HER3 phosphorylation, increased complex formation between

HER3 and other members of the HER family, increased PI_3 kinase activity, increased c-jun terminal kinase activity and/or AKT activity, increased ERK2 and/or PYK2 activity, or any combination thereof. The prostate condition can be, *e.g.*, BPH or prostate cancer.

[0127] Pharmaceutical compositions can be formulated by mixing one or more active agents with one or more physiologically acceptable carriers, diluents, and/or adjuvants, and optionally other agents that are usually incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A pharmaceutical composition can be formulated, *e.g.*, in lyophilized formulations, aqueous solutions, dispersions, or solid preparations, such as tablets, dragees or capsules. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (18th ed, Mack Publishing Company, Easton, PA (1990), particularly Chapter 87 by Block, Lawrence, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies as described herein, provided that the active agent in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. Also *See:* Baldrick, *Regul. Toxicol. Pharmacol.* 32:210-218 (2000); Wang, *Int. J. Pharm.* 203:1-60 (2000); Charman, *J. Pharm. Sci.* 89:967-978 (2000); and Powell et al., *PDA J. Pharm. Sci. Technol.* 52:238-311 (1998), and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0128] Methods for formulating and subsequently administering therapeutic compositions are well known to those skilled in the art. Dosing generally is dependent on the severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Persons of ordinary skill in the art routinely determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages can vary depending on the relative potency of individual polypeptides, and can generally be estimated based on EC_{50} found to be effective in *in vitro* and *in vivo* animal models. Typically, dosage is from about 0.01 μg to about 100 μg per kg of body weight, and may be given once or more daily, biweekly, weekly, monthly, or even less often. Following

successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state.

[0129] In various embodiments dosage may be from about 0.01 to about 0.1 μg per kg of body weight, from about 0.1 to about 1 μg per kg of body weight, from about 1 to about 10μg per kg of body weight, from about 10 to about 100 μg per kg of body weight or greater than about 100 μg per kg of body weight.

[0130] Pharmaceutical compositions can be administered by a number of methods, depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be, for example, topical (*e.g.*, transdermal, sublingual, ophthalmic, or intranasal); pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols); oral; or parenteral (*e.g.*, by subcutaneous, intrathecal, intraventricular, intramuscular, or intraperitoneal injection, or by intravenous drip). Administration can be rapid (*e.g.*, by injection) or can occur over a period of time (*e.g.*, by slow infusion or administration of slow release formulations). For treating tissues in the central nervous system, HER3 binding proteins can be administered by injection or infusion into the cerebrospinal fluid, typically with one or more agents capable of promoting penetration of the polypeptides across the blood-brain barrier.

[0131] Compositions and formulations for parenteral, intrathecal or intraventricular administration can include sterile aqueous solutions, which also can contain buffers, diluents and other suitable additives (*e.g.*, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers).

[0132] Pharmaceutical compositions include, without limitation, solutions, emulsions, aqueous suspensions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, for example, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other; in general, emulsions are either of the water-in-oil (w/o) or oil-in-water (o/w) variety. Emulsion formulations have been widely used for oral delivery of therapeutics due to their ease of formulation and efficacy of solubilization, absorption, and bioavailability.

[0133] HER binding agents can further encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly)

the biologically active metabolite or residue thereof. Accordingly, for example, this document provides pharmaceutically acceptable salts of small molecules and polypeptides, prodrugs and pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form and is converted to an active form (*i.e.*, drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the polypeptides provided herein (*i.e.*, salts that retain the desired biological activity of the parent polypeptide without imparting undesired toxicological effects). Examples of pharmaceutically acceptable salts include, but are not limited to, salts formed with cations (*e.g.*, sodium, potassium, calcium, or polyamines such as spermine); acid addition salts formed with inorganic acids (*e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, or nitric acid); and salts formed with organic acids (*e.g.*, acetic acid, citric acid, oxalic acid, palmitic acid, or fumaric acid).

[0134] Some embodiments provided herein include pharmaceutical compositions containing one or more HER3 binding agents, with or without one or more second agents (*e.g.*, one or more that bind to another HER family member, or one or more chemotherapeutic agents), and one or more other agents that function by a different mechanism. For example, anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, can be included in compositions. Other non-polypeptide agents (*e.g.*, chemotherapeutic agents) also are within the scope of this document. Such combined compounds can be used together or sequentially.

[0135] Compositions additionally can contain other adjunct components conventionally found in pharmaceutical compositions. Thus, the compositions also can include compatible, pharmaceutically active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or additional materials useful in physically formulating various dosage forms of the compositions provided herein, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. Furthermore, the composition can be mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, and aromatic substances. When added, however, such materials should not unduly interfere with the biological activities of the

polypeptide components within the compositions provided herein. The formulations can be sterilized if desired.

[0136] The pharmaceutical formulations, which can be presented conveniently in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients (*e.g.*, the HER family binding agents provided herein) with the desired pharmaceutical carrier(s) or excipient(s). Typically, the formulations can be prepared by uniformly and bringing the active ingredients into intimate association with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Formulations can be sterilized if desired, provided that the method of sterilization does not interfere with the effectiveness of the polypeptide contained in the formulation.

[0137] The compositions described herein can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions also can be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions further can contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, and/or dextran. Suspensions also can contain stabilizers.

[0138] HER binding agents can be combined with packaging material and sold as kits for treating prostate conditions. Components and methods for producing articles of manufacture are well known. The articles of manufacture may combine one or more of the polypeptides and compounds set out in the above sections. In addition, the article of manufacture further may include, for example, buffers or other control reagents for reducing or monitoring reduced immune complex formation. Instructions describing how the polypeptides are effective for treating prostate conditions can be included in such kits.

6. Methods

[0139] This document also provides methods for treating or preventing a prostate condition (*e.g.*, BPH or prostate cancer) in a subject. For example, a method can include administering to a subject (*e.g.*, a mammal such as a human) a HER3 binding agent or a composition containing a HER3 binding agent, as described herein. The methods can be used to, for example, treat or prevent BPH in a subject in need thereof, reduce prostate weight in a subject, treat or prevent prostate cancer; inhibit or reduce growth of prostate cancer cells, or prevent, reduce, or reverse androgen independent growth and/or proliferation of prostate cancer cells.

[0140] The term "treatment or prevention," when used herein, refers to both therapeutic treatment and prophylactic or preventative measures, which can be used to prevent, slow, or lessen the effects of the targeted condition or disorder. Those in need of prevention or treatment can include those already having the disorder, as well as those who may be likely to develop the disorder, or those in whom the disorder is to be prevented. The subject in need of prevention or treatment can be a mammalian patient (*i.e.*, any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, and rabbits). In some embodiments, the subject in need of treatment is a human patient.

[0141] Methods for preventing or treating prostate conditions in subject in need thereof can include administering to the subject an effective amount of at least one HER3 binding agent as described herein. In some cases, the methods also can include administering at least one other agent (*e.g.*, an agent against another HER family member, or a chemotherapeutic compound, as described above). Such treatment can, for example, inhibit abnormal cell growth, migration or invasion. When a combination of agents is administered, they can be administered simultaneously (*e.g.*, when they are contained in the same composition, or by admixture into a common i.v. bag), or separately (*e.g.*, sequentially).

[0142] As used herein, the term "effective amount" is an amount of an agent that results in a decrease or stabilization in one or more symptoms or clinical characteristics of the prostate condition being treated. For example, administration of an effective amount of a composition as described herein can result in decreased prostate size (*e.g.*, as determined by weight or diameter), slowing of tumor growth, decreased tumor size, or decreased activation of HER3 or HER3-responsive biomarkers (*e.g.*, Akt, HER2, ERK, or EGF-R). The decrease or slowing can be any reduction as compared to a previous value (*e.g.*, a 5%, 10%, 20%, 25%, or more than 25% reduction in symptom or characteristic). In some embodiments, an "effective amount" can result in stable disease.

[0143] In addition to classical modes of administration of potential binding protein therapeutics, *e.g.*, *via* the above mentioned formulations, newly developed modalities of administration may also be useful. For example, local administration of ^{131}I-labeled monoclonal antibody for treatment of primary brain tumors after surgical resection has been reported. Additionally, direct stereotactic intracerebral injection of monoclonal antibodies and their fragments is also being studied clinically and pre-clinically. Intracarotid

hyperosmolar perfusion is an experimental strategy to target primary brain malignancy with drug conjugated human monoclonal antibodies.

[0144] As described above, the dose of the agents administered can depend on a variety of factors. These include, for example, the nature of the agents, the tumor type, and the route of administration. It should be emphasized that the present methods are not limited to any particular doses. Methods for determining suitable doses are known in the art.

[0145] Depending on the type and severity of the condition to be treated, up to about 20 mg/kg of HER binding agent can be administered to a patient in need thereof, *e.g.*, by one or more separate administrations or by continuous infusion. A typical daily dosage might range from about 1 µg/day to about 100 mg/day or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition to be treated, the treatment can be sustained until a desired suppression of disease symptoms occurs.

[0146] In some cases, a method as provided herein can include one or more steps for monitoring the therapeutic outcome of the treatment. For example, a subject can be monitored for symptoms of their disease, to determine whether a reduction in symptoms has occurred. The subject also can be monitored, for example, for potential side effects of the treatment. The monitoring can be done after the administration step, and, in some embodiments, can be done multiple times (*e.g.*, between administrations, if dosages are given more than once). Such methods can be used to assess efficacy and safety of the treatment methods described herein, for example.

[0147] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – HER3 expression is increased during androgen withdrawal

[0148] U1-59 is a fully human anti-HER3 monoclonal antibody that has *in vivo* anti-tumor activities in various types of human cancers (*See:* Treder et al., "Fully human Anti-HER3 monoclonal antibodies (mAbs) inhibit oncogenic signaling and tumor cell growth *in vitro* and *in vivo*," AACR Annual Meeting, San Diego, CA (2008); and Freeman et al., "Fully human Anti-HER3 monoclonal antibodies (mAbs) have unique *in vitro* and *in vivo* functional and antitumor activities versus other HER family inhibitors," AACR Annual Meeting, San

Diego, CA (2008)). This antibody may be useful to inhibit proliferation and xenograft tumor growth, as well as to restore androgen dependence, in various prostate cancer cell lines. Androgen dependent LNCaP cells become androgen independent (AI) when cultured over extended periods of time in medium containing low androgen levels (*See:* Murillo et al., (*supra*) (2008); and Hsieh et al., *Cancer Res.* 53(12):2852-2857 (1993)). Comparison of LNCaP cells with their AI sublines LNCaP-AI and C4-2 showed that the AI sublines overexpressed HER2 and HER3 but not EGFR (FIG. 1A), while HER4 levels were extremely low (FIG. 1B). The increase in HER2 and HER3 was associated with androgen withdrawal. LNCaP cells were plated in medium containing FBS and then switched to low-androgen medium containing charcoal stripped serum. As shown in FIG. 1C, this treatment caused a sharp decrease in AR transcriptional activity from a PSA promoter construct, and also in AR expression (FIG. 1D, upper panel). There was a significant increase in the expression of HER2 (3rd panel), HER3 (4th panel), but not EGF-R. In addition, despite the lack of functional PTEN (a negative regulator of PI3K activation), LNCaP cells still experienced an increase in Akt phosphorylation (5th panel).

Example 2 – HER3 overexpression induces LNCaP propagation in the absence of androgens

[0149] LNCaP cells were stably transfected with cDNA encoding HER2 or HER3 (pCDNA3-HER2 and pCDNA3-HER3) to generate the stable cell lines LNCaP-HER2 and LNCaP-HER3 (FIG. 2A). Multiple clones were screened to select those that expressed HER2 and HER3 at levels comparable to the levels in LNCaP-AI cells. Significantly, overexpression of HER3 also induced the overexpression of HER2. Overexpression of both HER2 and HER3 resulted in an increase in proliferation in low-androgen media versus the untransfected control cell line (FIG. 2B). On the other hand, transfection of HER3 siRNA inhibited proliferation in LNCaP-AI cells in both FBS and charcoal stripped serum (FIG. 2C). Inhibition of Akt prevented proliferation caused by HER3 overexpression (FIG. 2D). Thus, HER3 regulates cell growth in an Akt-dependent manner.

Example 3 – HER3 regulates proliferation and androgen dependence in prostate cancer

[0150] Flow cytometric analyses to evaluate the percentage of cells in S-phase revealed that transfection with HER3-specific siRNA, but not a scrambled siRNA, reduced proliferation rates in both LNCaP and C4-2 cells (FIG. 3A), indicating that HER3 is necessary for proliferation of these cells. As before, C4-2 cells expressed higher levels of HER3 compared to LNCaP cells (approximately 2X), and HER3-specific siRNA resulted in

the downregulation of HER3 expression in both cell lines (FIG. 3B). Cells were transfected with 10 □M siRNA because it appeared that this amount of HER3-specifc siRNA duplex downregulated HER3 levels in C4-2 cells back to the levels seen in LNCaP cells. Co-transfection with □-galactosidase followed by □-gal assay showed similar levels of transfection efficiency in all cases. Similarly, overexpression of HER3 using a pCDNA3-HER3 plasmid increased proliferation rates in LNCaP cells (FIG. 3C) as well as C4-2 cells (data not shown). Importantly, flow cytometric analysis revealed that LNCaP cells transfected with an empty vector were growth-inhibited by treatment for 48 hours with the AR antagonist bicalutamide (5 □M), indicating androgen dependence. In contrast, bicalutamide did not significantly reduce proliferation in cells that overexpress HER3, indicating androgen-independence (FIG. 3C). HER3 overexpression was confirmed by western blotting, and showed that LNCaP cells transfected with 2 □g pCDNA3-HER3 increased HER3 levels to that of C4-2 cells (FIG. 3D). As before, transfection efficiency was determined by co-transfection of □-gal, and □-gal assays confirmed similar transfection efficiencies in all cases.

Example 4 – Does inhibition of HER3 inhibit proliferation rates and confer androgen dependence to androgen-independent prostate cancer cells?

[0151] HER3 is overexpressed in prostate cancer relative to normal prostate (*See:* Chaib et al., *supra* (2001)). The Examples above show that HER3 is overexpressed in androgen independent clones of LNCaP cells compared to the androgen dependent parental cell line. Further, overexpression of HER3 in LNCaP cells conferred androgen independence, while inhibition of HER3 reduced proliferation. Culture in the absence of androgens (in medium containing charcoal stripped serum) and treatment with the anti-androgen bicalutamide inhibits cell proliferation in LNCaP cells but not in C4-2 cells (*See:* Mikhailova et al., *Adv. Exp. Med. Biol.* 617:397-405 (2008); and Wang et al., *Oncogene* 26(41):6061-6070 (2007); and Wang et al., *Oncogene* 27(56):7106-7117 (2008)). Transfection of HER3 cDNA into LNCaP cells prevented this inhibition, and also induced LNCaP cells to proliferate in the absence of androgens, HER3-specific siRNA prevented androgen independent growth in androgen-independent sublines of LNCaP cells.

[0152] Further studies are conducted to determine whether inhibition of HER3 using the fully human anti-HER3 monoclonal antibody, U1-59, inhibits proliferation in androgen-independent cells and reverts the cells back to an androgen-dependent phenotype. LNCaP cells or the androgen independent sublines C4-2 and LNCaP-AI, as

well as "normal-like cells" RWPE1 and pRNS-1-1 (Shi *et al.* (2007) *Prostate* 67(6):591-602) and androgen independent cells such as MDA-PCa-2b and CWR22Rv1, are treated with U1-59 or vehicle as per manufacturer's recommended protocol at a range of concentrations to determine the optimum concentration needed to inhibit HER3 oncogenic signaling. The rate of cell growth is determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and the rate of proliferation vs. apoptosis is determined by flow cytometry followed by Western blotting to determine the effect of the drug on cell cycle proteins (*e.g.*, phosphorylation of cyclins D1, E, A, cdk2, and cdk4/6, and expression of p27 and p21), as well as on proteins involved in apoptosis (*e.g.*, Bcl2, BclXL, BAD, and BAX).

[0153] To determine the effect of U1-59 on AR transcriptional activity, levels of AR and PSA expression are determined by Western blotting in cells that express PSA. AR transcriptional activity also is evaluated by transfecting cells with hPSA-luc, a luciferase-tagged PSA promoter region expressing two AR binding regions (ARE), and treating transfected cells with vehicle or antibody and then carrying out luciferase assays to determine AR transcriptional activity. Assays also are conducted to evaluate effects of the antibody on cell motility and invasiveness, growth in soft agar, and effects on downstream effectors of HER3 (*i.e.*, the Akt and ERK signaling pathways).

[0154] To determine whether U1-59 retards androgen independence, the growth of cells in FBS vs. charcoal stripped serum is assessed in the presence or absence of bicalutamide, together with the antibody or vehicle. HER3 inhibition is detected in the cells by Western blotting in plasma membrane fractions obtained by Triton X-114 fractionation as previously described (*See:* Ghosh et al., *Oncogene* 18(28):4120-4130 (1999); Ghosh et al., *Biochim. Biophys. Acta* 1359(1):13-24 (1997); and Ghosh et al., *J. Cell. Biochem.* 74(4):532-543 (1999)). Freshly synthesized active HER3 localizes to the membrane and lack of HER3 in the membrane fraction is indicative of effective HER3 silencing (*See:* Walters et al., *Oncogene* 22(23):3598-3607 (2003)). The other half of the cells is fixed in 70% ethanol, stained with PI and the % cells in S-phase detected by flow cytometry (*See:* Ghosh et al., *Cancer Res.* 62(9):2630-2636 (2002)). It is expected that inhibition of HER3 will make the cells responsive to bicalutamide.

Example 5 – Does inhibition of HER3 during androgen withdrawal prevent development of androgen independent tumors in animal models of prostate cancer?

[0155] Increased HER3 expression is associated with androgen independence *in vitro*, and HER3 levels are increased during androgen withdrawal. In androgen dependent cells, HER3 levels are negatively regulated by the androgen receptor. On the other hand, increased HER3 levels prevent androgen regulation of HER3. Studies are conducted to determine whether inhibition of HER3 *in vivo* during androgen withdrawal will prevent androgen independent growth of prostate cancer cells. An appropriate model of prostate cancer recurrence, the CWR22 model, is available for studies *in vivo*. CWR22 tumors are easily established in nude mice, are androgen dependent in the primary tumor state, and undergo rapid regression upon castration of the host (*See:* Nagabhushan et al., *Cancer Res.* 56(13):3042-3046 (1996)). Within 3-4 months, however, androgen independent tumors arise in the host animals, thus replicating the effect seen in human patients. The CWR22 recurrence model therefore is used to determine the effect of U1-59 on tumor development and tumor regression.

[0156] To determine whether inhibition of HER3 prevents tumor incidence, 4- to 5-week old nude/nude athymic male mice (Harlan, Indianapolis) are implanted with testosterone pellets prior to subcutaneous injections in both flanks of 20 million cells each extracted from CWR22 tumors in a 1:1 mixture with 50% Matrigel (n=40). Three weeks after implantation (or when palpable tumors are observed), the mice are castrated. These animals are followed for up to 12 months following surgery. It is expected that tumors will regress initially but will recur within 8-9 months in 50% of the animals. Growth of tumors in castrated mice indicates hormone-refractory growth. Mice bearing recurrent tumors are divided into two groups (n=20 tumors/group) that receive vehicle or U1-59, respectively. Treatment is initiated when the size of the recurrent tumor reaches 150 mm^3. Tumor size is measured every other day with digital calipers, and tumor volume is calculated. Growth inhibition is calculated by tumor volume of treated mice divided by tumor volume of control mice (T/C). The effect of antibody treatment on tumor volume is followed for up to 12 weeks following the start of treatment. At the end of the study, all remaining tumors are collected, and recurrent tumors are weighed and bisected. One part was fixed in 10% formalin for immunohistochemistry, and the rest are quick frozen (isopropanol in liquid nitrogen).

[0157] Experiments also are conducted to determine whether inhibition of HER3 at the time of androgen withdrawal prevents recurrence, by determining whether progression of prostate cancer cells to androgen independence in castrated nude mice can be prevented by prior treatment with U1-59. Mice (n=40) are subcutaneously and bilaterally injected with CWR22 cells as described above, and the animals are castrated when

palpable tumors are observed (approximately three weeks after injection). In these experiments, however, the castrated animals are treated with (i) vehicle or (ii) U1-59 (n=20/group) in the days following castration, well before the onset of recurrent tumors. This protocol is maintained for up to 9 months, or whenever the control animals begin to develop recurrent tumors. It is expected that the control animals will ultimately develop recurrent tumors, whereas treated animals either will not develop recurrent tumors or will do so at a reduced rate as compared to controls. At the end of the study (*i.e.*, about 9-10 months after the start of treatment), the animals are sacrificed and any tumors formed are collected as described above.\

[0158] Immunohistochemistry is used to evaluate the effect of the antibody on HER3 expression and phosphorylation, as well as on downstream effectors of HER3 (*e.g.*, Akt and ERK). Paraffin-embedded tissues are sectioned and immunostained for total and phospho-HER3 (Tyr1189), phospho-HER3 (Tyr1222), phospho- HER3 (Tyr1289), phospho-Akt (Ser 473), and phospho-ERK (Thr202/Tyr204). Staining intensity in treated vs. untreated tumors is analyzed to determine whether the antibody has the desired effects. Scores are reported as percent of tumor staining positively multiplied with the intensity of staining (on a 1 to 3 scale, with 1 being weak staining intensity, 2 being moderate staining, and 3 being strong staining), resulting in a scale of 0 to 300.

[0159] The time to tumor incidence after implantation and time to tumor recurrence after castration are explored with Kaplan-Meier survival and hazard function curves (n=20 per group), and the effect of treatment on survival evaluated by log rank tests (*See:* Dawson-Saunders and Bat, "Methods for analyzing survival data," *Basic and Clinical Biostatistics*, Appelton & Lange, Norfolk, CT, p.188 (1994)). Further analyses use proportional hazards models to determine the extent to which this relationship is mediated through tumor weight. The mean differences in tumor volume across the four comparison groups are assessed by analysis of variance (ANOVA). Equality of variance across the four groups is assessed using Levene's homogeneity of variance test (*See:* Bonham et al., *J. Natl. Cancer Inst.* 94(21):1641-1647 (2002)). Contrasts (each with one degree of freedom) are used to compare all treatment groups with the control group. Dunnett's multiple comparisons procedure also is used to compare all treatment groups with the control group (Bonhan *et al.*, *supra*). Dunnett's procedure is used to create simultaneous (adjusted for multiple comparisons) 95% confidence intervals (CIs). P values are reported for all significance tests, and all statistical tests are two-sided. Main effects, interactions, and multiple comparisons are done, after assuring assumptions are met for a valid analysis.

Standard transformations and/or the bootstrap procedure are considered if needed. Contrasts of the appropriate means can be done for specific comparisons following the analysis of variance.

Example 6 – *In vitro* effects of U1-59 on prostate cancer cell lines

[0160] DU-145 prostate cancer cells were obtained from DSMZ (Braunschweig, Germany) and were incubated for 24 hours with IgG control, U1-59, the anti-EGF-R antibodies cetuximab and panitumumab (VECTIBIX®), the anti-HER2 antibody trastuzumab (HERCEPTIN®), the HER2 inhibitor c2C4, or combinations thereof, and effects on HER3 phosphorylation were evaluated by Western blot analysis. As shown in FIGS. 4A and 4B, western blotting of cell lysates with antibodies against particular phosphorylated tyrosine residues on HER3 (pHER3 Tyr1289 and pHER3 Tyr1197; Cell Signaling Technology, Beverly, MA)) demonstrated that U1-59 alone completely inhibit phosphorylation of HER3, while cetuximab and panitumumab increased phosphorylation, c2C4 partially inhibited phosphorylation, and trastuzumab had little effect. U1-59 in combination with cetuximab, c2C4, and trastuzumab resulted in complete or nearly complete inhibition of HER3 phosphorylation.

[0161] In separate experiments, the effect of U1-59 on anchorage independent growth of PC-3 prostate cancer cells, obtained from American Type Culture Collection (Manassas, VA), was evaluated in a colony formation assay (CFA). PC-3 cells were pre-incubated with 10 □g/ml each of IgG control, U1-59, or two anti-HER3 antibodies (U1-53 and U1-49) and effects on colony formation were determined in a three-layer soft agar system. The bottom agar layer contained 0.75% agar and 20% FBS in IMDM without phenol red. The top agar layer contained antibody-pre-incubated cells in 0.4 % agar. The top layer was covered with a liquid feeding layer of 50 µl IMDM. Cells were cultivated at 37°C for 12 days and colonies were counted following staining with MTT (0.21 mg/ml). As shown in FIG. 5, anchorage-independent growth of PC-3 cells was significantly inhibited by all anti-HER3 antibodies. A 78% inhibition in colony formation was obtained in the presence of U1-59 and the anti-HER3 antibody U1-53, whereas the anti-HER3 antibody U1-49 inhibited colony formation by 52%.

Example 7 – U1-59 inhibits ligand-induced HER3 phosphorylation

[0162] Rat RG2 glioma cells and cynomolgus monkey JTC-12.P3 kidney cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were incubated in serum free medium with 10 □g/ml IgG control or U1-59 for 1 hour prior to

a 15 minute incubation with 100 ng/ml heregulin or protein control. After treatment the cells were lysed in RIPA buffer and the levels of phosphorylated HER3 were examined by western blotting (pHER3 Tyr1289, Cell Signaling Technology, Beverly, MA). As shown in FIGS. 6A and 6B, the HER3 phosphorylation that was stimulated by heregulin was blocked by U1-59, while the IgG control had no effect.

Example 8 – Toxicity study of U1-59 in rats and monkeys

[0163] Rats (n=10/gender/dose) and cynomolgus monkeys (n=5/gender/dose) were treated once weekly for 4 weeks (five doses) with intravenous (i.v.) injection of U1-59, at doses of 20, 60, or 200 mg/kg/day. TABLE 2 shows key study findings noted after five doses and exposure levels during the fourth week of treatment. In the rats (sexually mature), the only significant finding was a reduction in prostate weight. No adverse effects were observed in either rats or monkeys. Further, there were no changes in testis weight and no microscopic changes. Statistically significant differences in prostate weights were not noted at the end of a 3-month treatment-free phase, indicating that the effects in rats were reversible.

TABLE 2

Toxicity Study Key Findings

Species	Dose (mg/kg/day)	AUC0-tau a (ug-hr/mL)	Key Findings
Rat	20 (NOEL)	2070	–
	60	4800	↓ mean prostate weight (16% mean decrease)
	200 (NOAEL)	10300	↓ mean prostate weight (22% mean decrease)
Cyno	20	2180[b]	–
	60	5900	–
	200 (NOEL; NOAEL)	13600	–

[a]$AUC_{0\text{-}tau}$ = Area under the serum concentration time curve during the final dosing interval beginning on Day 22
[b] Two animals that tested positive for anti-U1-59 antibodies were excluded from group mean calculation.
NOEL, no observed effect level; NOAEL, no observed adverse effect level.

OTHER EMBODIMENTS

[0164] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use of a HER3 binding agent for treating benign prostate hyperplasia (BPH)

2. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is a small molecule compound or an antigen-binding protein that binds to HER3.

3. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises:

a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of SEQ ID NOs:236, 251, 252, and 256; a CDRH2 selected from the group consisting of SEQ ID NOs:258, 278, 280, and 282; and a CDRH3 selected from the group consisting of SEQ ID NOs:283, 285, 309, 313, and 315; and

a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of SEQ ID NOs:320, 334, 337, and 340; a CDRL2 selected from the group consisting of SEQ ID NOs: 343, 356, 351, and 344; and a CDRL3 selected from the group consisting of SEQ ID NOs:360, 381, 385, and 387.

4. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of (a) CDRH1's as shown in SEQ ID NOs:236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs:258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs:283, 285, 309, 313, and 315.

5. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a light chain amino acid sequence that comprises at least one of the CDR´s selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs:343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs:360, 381, 385, and 387.

6. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of (a) CDRH1's as shown in SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs:258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID

NOs:283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs:320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs:343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs:360, 381, 385, and 387.

7. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of SEQ ID NOs: 236, 251, 252, and 256, a CDRH2 selected from the group consisting of SEQ ID NOs: 258, 278, 280, and 282, and a CDRH3 selected from the group consisting of SEQ ID NOs: 283, 285, 309, 313, and 315, or a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of SEQ ID NOs: 320, 334, 337, and 340, a CDRL2 selected from the group consisting of SEQ ID NOs: 343, 356, 351, and 344, and a CDRL3 selected from the group consisting of SEQ ID NOs: 360, 381, 385, and 387.

8. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 42, 54, 70, 92, and 96.

9. The use of a HER3 binding agent of claim 8, wherein the antigen-binding protein comprises a light chain amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 56, 72, 94, and 98.

10. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 42, 54, 70, 92, and 96; and a light chain amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 56, 72, 94, and 98.

11. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO:42 and the light chain amino acid sequence of SEQ ID NO:44.

12. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain

amino acid sequence of SEQ ID NO:54 and the light chain amino acid sequence of SEQ ID NO:56.

13. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO:70 and the light chain amino acid sequence of SEQ ID NO:72.

14. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDRH3 selected from the group consisting of SEQ ID NOs: 283, 285, 309, 313, and 315.

15. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDHL3 selected from the group consisting of SEQ ID NOs: 360, 381, 385, and 387.

16. The use of a HER3 binding agent of any one of claims 3 to 15, wherein the antigen-binding protein is directed against the extracellular domain of HER3.

17. The use of a HER3 binding agent any one of claims 3 to 15, wherein binding of the antigen-binding protein to HER3 has one or more effects selected from the group consisting of reduction of HER3-mediated signal transduction, reduction of HER3 phosphorylation, reduction of cell proliferation, reduction of cell migration, and increasing downregulation of HER3.

18. The use of a HER3 binding agent of any one of claims 3 to 17, wherein the antigen-binding protein that binds to HER3 is an antibody.

19. The use of a HER3 binding agent of claim 18, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

20. The use of a HER3 binding agent of claim 19, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

21. The use of a HER3 binding agent of claim 18, wherein the antibody is of the IgG1-, IgG2-, IgG3- or IgG4-type.

22. The use of a HER3 binding agent of claim 1, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and wherein the antigen-binding protein is coupled to an effector group.

23. The use of a HER3 binding agent of claim 22, wherein the effector group is a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group.

24. The use of a HER3 binding agent of claim 23, wherein the therapeutic or chemotherapeutic group is selected from the group consisting of calicheamicin, auristatin-PE, geldanamycin, maytansine and derivatives thereof.

25. The use of a HER3 binding agent of claim 1, further comprising identifying the subject as having BPH.

26. The use of a HER3 binding agent of claim 1, further comprising monitoring prostate size in the subject after administering the composition.

27. Use of a HER3 binding agent for reducing prostate weight.

28. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is a small molecule compound or an antigen-binding protein that binds to HER3.

29. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises:

a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of SEQ ID NOs:236, 251, 252, and 256; a CDRH2 selected from the group consisting of SEQ ID NOs:258, 278, 280, and 282; and a CDRH3 selected from the group consisting of SEQ ID NOs:283, 285, 309, 313, and 315; and

a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of SEQ ID NOs:320, 334, 337, and 340; a CDRL2 selected from the group consisting of SEQ ID NOs: 343, 356, 351, and 344; and a CDRL3 selected from the group consisting of SEQ ID NOs:360, 381, 385, and 387.

30. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain

amino acid sequence that comprises at least one of the CDR's selected from the group consisting of (a) CDRH1's as shown in SEQ ID NOs:236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs:258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs:283, 285, 309, 313, and 315.

31. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a light chain amino acid sequence that comprises at least one of the CDR´s selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs:343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs:360, 381, 385, and 387.

32. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of (a) CDRH1's as shown in SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2's as shown in SEQ ID NOs:258, 278, 280, and 282; and (c) CDRH3's as shown in SEQ ID NOs:283, 285, 309, 313, and 315; and a light chain amino acid sequence that comprises at least one of the CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOs:320, 334, 337, and 340; (e) CDRL2's as shown in SEQ ID NOs:343, 356, 351, and 344; and (f) CDRL3's as shown in SEQ ID NOs:360, 381, 385, and 387.

33. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence that comprises a CDRH1 selected from the group consisting of SEQ ID NOs: 236, 251, 252, and 256, a CDRH2 selected from the group consisting of SEQ ID NOs: 258, 278, 280, and 282, and a CDRH3 selected from the group consisting of SEQ ID NOs: 283, 285, 309, 313, and 315, or a light chain amino acid sequence that comprises a CDRL1 selected from the group consisting of SEQ ID NOs: 320, 334, 337, and 340, a CDRL2 selected from the group consisting of SEQ ID NOs: 343, 356, 351, and 344, and a CDRL3 selected from the group consisting of SEQ ID NOs: 360, 381, 385, and 387.

34. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 42, 54, 70, 92, and 96.

35. The use of a HER3 binding agent of claim 38, wherein the antigen-binding protein comprises a light chain amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 56, 72, 94, and 98.

36. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 42, 54, 70, 92, and 96; and a light chain amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 56, 72, 94, and 98.

37. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO:42 and the light chain amino acid sequence of SEQ ID NO:44.

38. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO:54 and the light chain amino acid sequence of SEQ ID NO:56.

39. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises the heavy chain amino acid sequence of SEQ ID NO:70 and the light chain amino acid sequence of SEQ ID NO:72.

40. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDRH3 selected from the group consisting of SEQ ID NOs: 283, 285, 309, 313, and 315.

41. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and comprises a CDHL3 selected from the group consisting of SEQ ID NOs: 360, 381, 385, and 387.

42. The use of a HER3 binding agent of any one of claims 29 to 41, wherein the antigen-binding protein is directed against the extracellular domain of HER3.

43. The use of a HER3 binding agent of any one of claims 29 to 41, wherein binding of the antigen-binding protein to HER3 has one or more effects selected from the group consisting of reduction of HER3-mediated signal transduction, reduction of HER3 phosphorylation, reduction of cell proliferation, reduction of cell migration, and increasing downregulation of HER3.

44. The use of a HER3 binding agent of any one of claims 29 to 43, wherein the antigen-binding protein that binds to HER3 is an antibody.

45. The use of a HER3 binding agent of claim 44, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

46. The use of a HER3 binding agent of claim 45, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

47. The use of a HER3 binding agent of claim 44, wherein the antibody is of the IgG1-, IgG2-, IgG3- or IgG4-type.

48. The use of a HER3 binding agent of claim 27, wherein the HER3 binding agent is an antigen-binding protein that binds to HER3, and wherein the antigen-binding protein is coupled to an effector group.

49. The use of a HER3 binding agent of claim 48, wherein the effector group is a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group.

50. The use of a HER3 binding agent of claim 49, wherein the therapeutic or chemotherapeutic group is selected from the group consisting of calicheamicin, auristatin-PE, geldanamycin, maytansine and derivatives thereof.

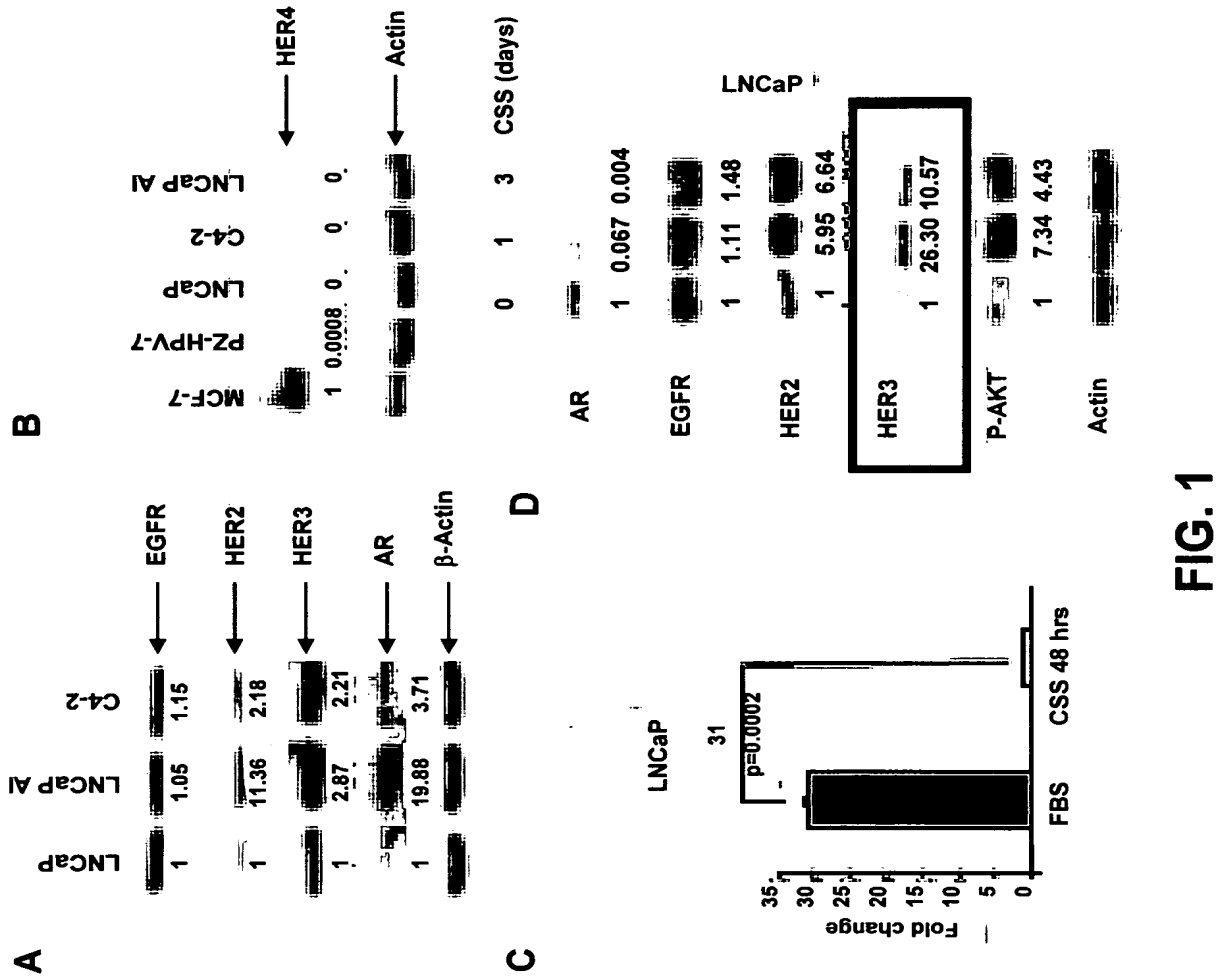

A
LNCaP
LNCaP AI
C4-2
EGFR
HER2
HER3
AR
β-Actin
1
1.05
1.15
1
11.36
2.18
1
2.87
2.21
1
19.88
3.71
B
MCF-7
PZ-HPV-7
LNCaP
C4-2
LNCaP AI
HER4
Actin
1 0.0008 0 0 0
C
LNCaP
31
p=0.0002
FBS
CSS 48 hrs
Fold change
35
30
25
20
15
10
5
0
D
CSS (days)
0 1 3
AR
1 0.067 0.004
EGFR
1 1.11 1.48
HER2
1 5.95 6.64
HER3
1 26.30 10.57
P-AKT
1 7.34 4.43
Actin
LNCaP

FIG. 1

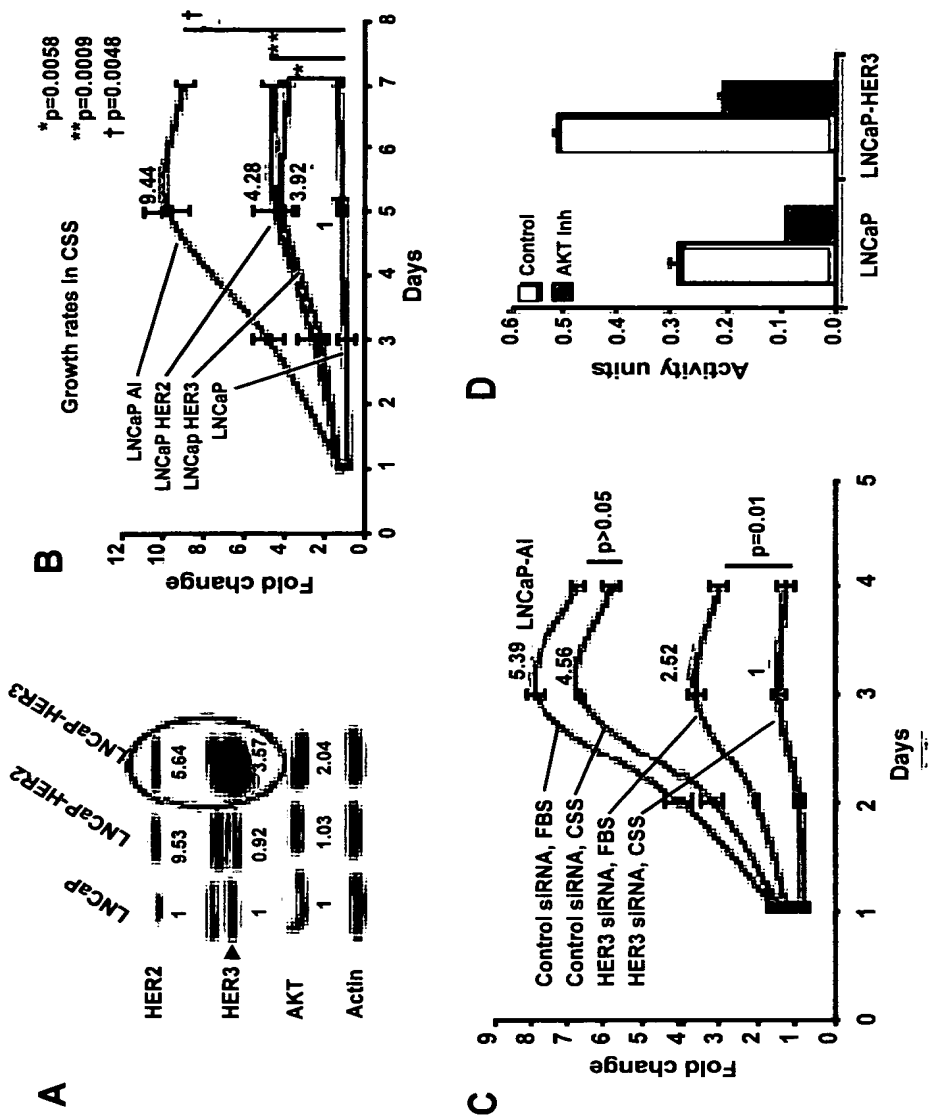

A
LNCaP
LNCaP-HER2
LNCaP-HER3
HER2
HER3
AKT
Actin
9.53
5.64
3.57
1
0.92
1
1.03
2.04
B
Growth rates in CSS
*p=0.0058
**p=0.0009
† p=0.0048
LNCaP AI
LNCaP HER2
LNCap HER3
LNCaP
9.44
4.28
3.92
1
Fold change
Days
C
Control siRNA, FBS
Control siRNA, CSS
HER3 siRNA, FBS
HER3 siRNA, CSS
5.39
4.56
2.52
1
LNCaP-AI
p>0.05
p=0.01
Fold change
Days
D
Control
AKT Inh
Activity units
LNCaP
LNCaP-HER3

FIG. 2

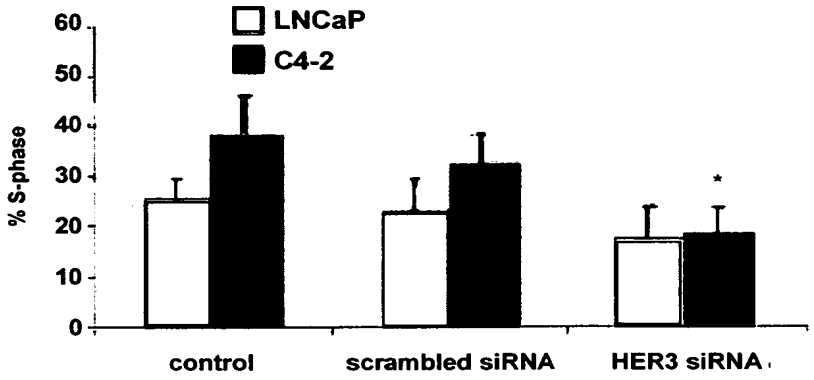

LNCaP
C4-2
% S-phase
60
50
40
30
20
10
0
*
control
scrambled siRNA
HER3 siRNA

FIG. 3A

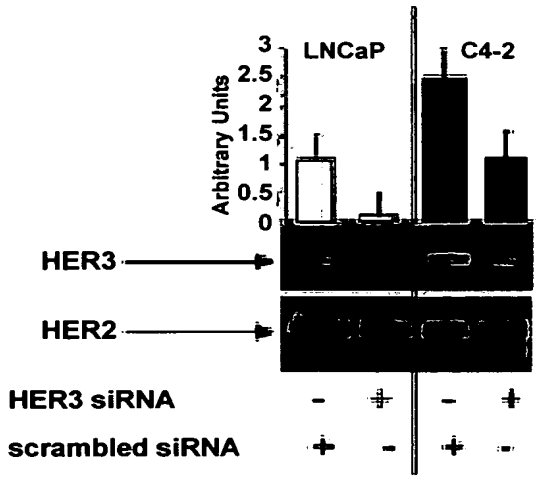

LNCaP
C4-2
Arbitrary Units
3
2.5
2
1.5
1
0.5
0
HER3
HER2
HER3 siRNA
scrambled siRNA

FIG. 3B

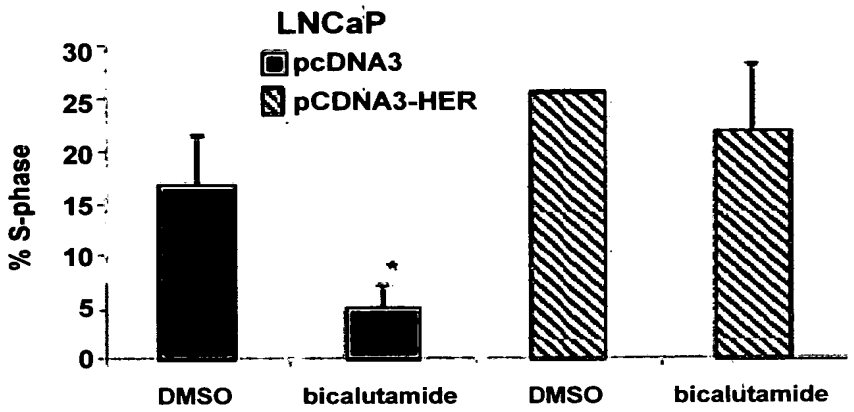

LNCaP
pcDNA3
pCDNA3-HER
% S-phase
30
25
20
15
10
5
0
DMSO
bicalutamide
DMSO
bicalutamide

FIG. 3C

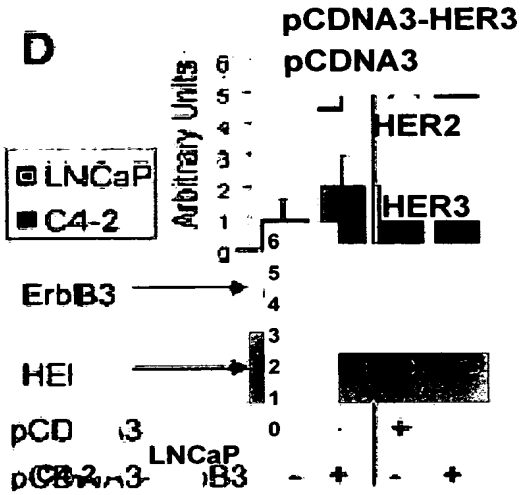

D
pCDNA3-HER3
pCDNA3
Arbitrary Units
LNCaP
C4-2
HER2
HER3
ErbB3
HEI
pCD 3
LNCaP
B3

FIG. 3D

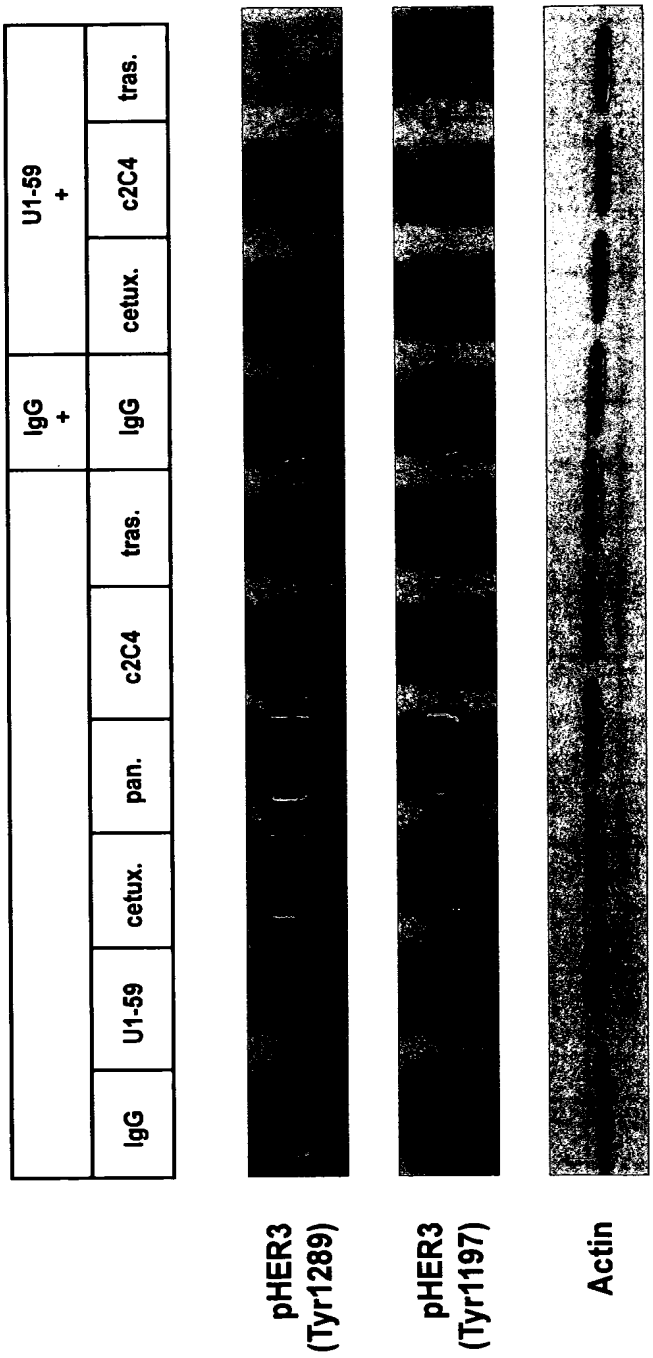

IgG
U1-59
cetux.
pan.
c2C4
tras.
IgG +
U1-59 +
IgG
cetux.
c2C4
tras.
pHER3 (Tyr1289)
pHER3 (Tyr1197)
Actin

FIG. 4A

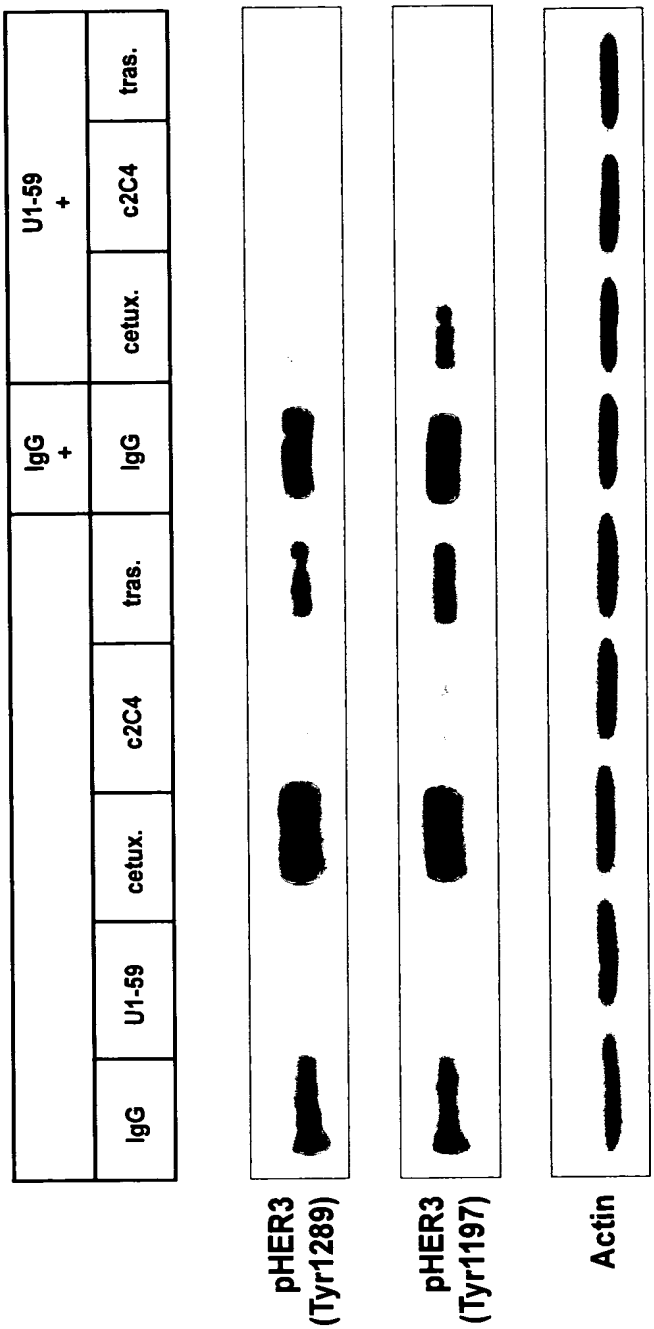

IgG
U1-59
cetux.
c2C4
tras.
IgG +
U1-59 +
IgG
cetux.
c2C4
tras.
pHER3 (Tyr1289)
pHER3 (Tyr1197)
Actin

FIG. 4B

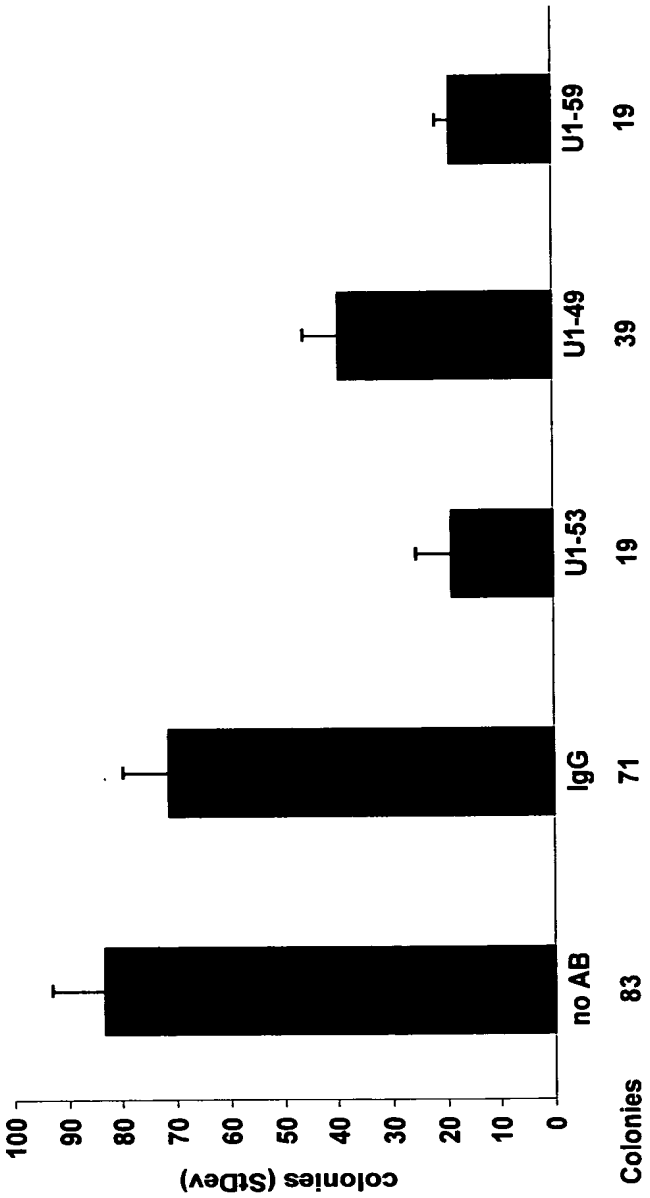

colonies (StDev)
100
90
80
70
60
50
40
30
20
10
0
Colonies
no AB
83
IgG
71
U1-53
19
U1-49
39
U1-59
19

FIG. 5

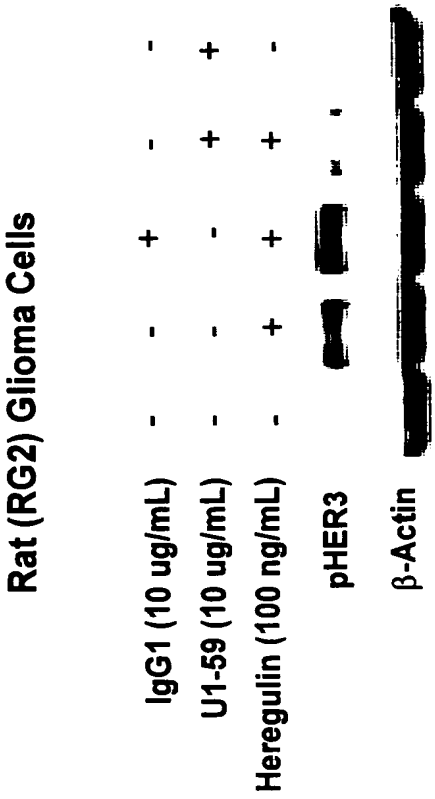

Rat (RG2) Glioma Cells
IgG1 (10 ug/mL)
U1-59 (10 ug/mL)
Heregulin (100 ng/mL)
pHER3
β-Actin
- - + -
- - - +
+ - + +
- + + -

FIG. 6A

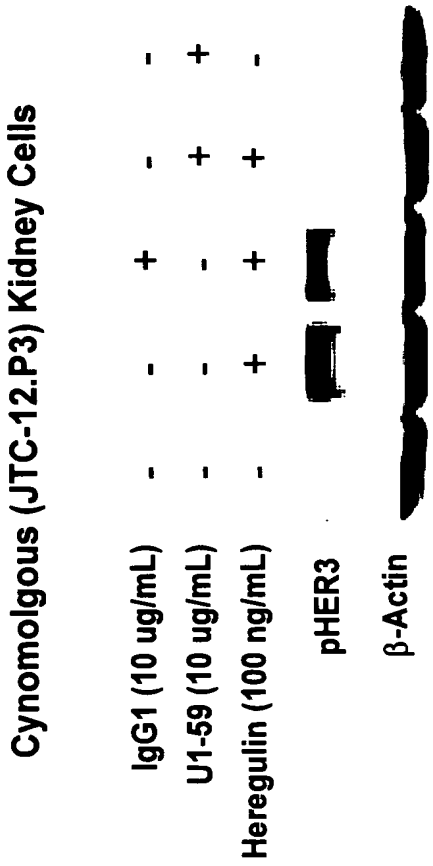

Cynomolgous (JTC-12.P3) Kidney Cells
IgG1 (10 ug/mL)
U1-59 (10 ug/mL)
Heregulin (100 ng/mL)
pHER3
β-Actin
- - + -
+ - + +
- + - +
- - + +

FIG. 6B

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(51) Cl.Int./Int.Cl. *A61K 47/48* (2006.01)

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(54) Titre : NOUVEAUX CONJUGUES LIANT-PRINCIPE ACTIF (ADC) ET LEUR UTILISATION
(54) Title: CONJUGATE (ADCS) AND THE USE THEREOF

(57) Abrégé/Abstract:
The present application relates to new antibody drug conjugates (ADCs) of N,N dialkylauristatins directed against the target FGFR2, drug metabolites of said ADCs, a method for producing said ADCs, the use of said ADCs for the treatment and/or prevention of illnesses as well as the use of said ADCs for producing pharmaceuticals for the treatment and/or prevention of illnesses, particularly of hyperproliferative and/or angiogenic diseases such as carcinosis. Such treatments can be carried out as monotherapy or in combination with other pharmaceuticals or additional therapeutic measures.

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(13) A1

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Abstract

The present application relates to new antibody drug conjugates (ADCs) of N,N dialkylauristatins directed against the target FGFR2, drug metabolites of said ADCs, a method for producing said ADCs, the use of said ADCs for the treatment and/or prevention of illnesses as well as the use of said ADCs for producing pharmaceuticals for the treatment and/or prevention of illnesses, particularly of hyperproliferative and/or angiogenic diseases such as carcinosis. Such treatments can be carried out as monotherapy or in combination with other pharmaceuticals or additional therapeutic measures.

diseases has seen a considerable general increase in recent years, based on the activity of such agents as trastuzumab (Herceptin®), rituximab (Rituxan®), cetuximab (Erbitux®) and bevacizumab (Avastin®), which have since been approved for the therapy of individual, specific tumour diseases [see e.g. G. P. Adams and L. M. Weiner, *Nat. Biotechnol.* 23, 1147-1157 (2005)]. Consequently there has also been a marked increase in interest in so-called immunoconjugates such as, for example, the aforementioned ADCs, in which an internalizing antibody directed against a tumour-associated antigen is joined covalently via a linking unit ("linker") to a cytotoxic agent. Following introduction of the ADC into the tumour cell and subsequent cleavage of the conjugate, either the cytotoxic agent itself or another metabolite with cytotoxic activity, formed from the cytotoxic agent, is released within the tumour cell, where it is able to develop its effect directly and selectively. In this way it would be possible to keep the damage to normal tissue within significantly closer limits in comparison to a conventional chemotherapy of the cancer disease [see e.g. J. M. Lambert, *Curr. Opin. Pharmacol.* 5, 543-549 (2005); A. M. Wu and P. D. Senter, *Nat. Biotechnol.* 23, 1137-1146 (2005); P. D. Senter, *Curr. Opin. Chem. Biol.* 13, 235-244 (2009); L. Ducry and B. Stump, *Bioconjugate Chem.* 21, 5-13 (2010)].

Instead of antibodies, it is also possible for binders from the small-molecule drug sphere to be used as binders which bind selectively to a specific target location ("target"), such as to a receptor, for example [see e.g. E. Ruoslahti *et al.*, *Science* 279, 377-380 (1998); D. Karkan *et al.*, PLoS ONE 3 (6), e2469 (June 25, 2008)]. Also known are conjugates of cytotoxic drug and addressing ligand that exhibit a defined cleavage point between ligand and drug for the release of the drug. A "predetermined break point" of this kind may exist, for example, within a peptide chain which can be cleaved selectively at a particular site by a specific enzyme at the location of action [see e.g. R. A. Firestone and L. A. Telan, US Patent Application US 2002/0147138].

The activity of monoclonal antibodies is demonstrated in various types of cancer. Thus HERCEPTIN® and Erbitux® are used successfully in the treatment of HER2-positive breast cancer and EGFR-positive colorectal cancer, respectively.

The coupling of cytotoxic compounds to antibodies forms an extended possibility for additionally improving cancer therapy, since these conjugates allow tumour-specific toxophore accumulation and at the same time reduce the systemic toxicity. With respect to activity and tolerance, clinical studies with brentuximab vedotin in Hodgkin's lymphoma and with trastuzumab-DM1 in breast cancer have yielded highly promising results, which support the development of new antibodies and new ADCs against other tumour antigens.

Antibody-based therapy proves to be very powerful in the treatment of various carcinomas, including solid tumours. Thus, for example Herceptin® has been deployed successfully in the

treatment of breast cancer, and Rituxan® is powerful in forms of carcinoma associated with the B cells. At the focal point of the development of a successful antibody-based therapy is the isolation of antibodies against cell surface proteins which are expressed preferably on tumour cells.

The fibroblast growth factor receptors are tyrosine receptor kinases (RTK), of which four are known in mammals (FGFR1, FGFR2, FGFR3, FGFR4). As ligands, 22 human fibroblast growth factors (FGF) have been identified (Eswarakumar and Schlessinger, Cytokine & Growth Factor Reviews 2005, 16:139-149; Shimada et al., Proc. Natl. Acad. Sci. USA 2001, 98:6500-6505). The FGFRs consist of three extracellular immunoglobulin (Ig)-like domains, namely D1-D3, with domains 2 and 3 being necessary for ligand binding, and also of an individual transmembrane domain and a cytoplasmic domain, which contains the catalytic centre of the protein tyrosine kinase (a schematic illustration is given in Figure 1).
Schematic illustration of the structure of FGFR2. Alpha (SEQ ID NO: 1) and beta (SEQ ID NO: 2) splice variants are compared with one another. The illustration shows the three Ig-like domains (D1, D2 and D3), the transmembrane domain (TM) and the intracellular kinase domain. The heparin binding site (HBS), the acidic box (AB), and the alternative IIIb/IIIc domains are likewise labelled. The amino terminus is marked by N, the carboxy terminus by C.

The extracellular component additionally harbours the acidic box (AB) and the heparin binding site (HBS) (see Figure 1). An important feature of the FGFR family of RTKs is that there are different, alternatively spliced variants in existence. The full-length FGFR is identified as FGFR alpha (SEQ ID NO: 1), while the isoform, which is missing D1, is identified as FGFR beta (SEQ ID NO: 2) (Figure 1). An alternative splicing in domain 3 leads to two different variants, namely FGFR2 IIIb, which is encoded by the exons 7 and 8, and FGFR2 IIIc, which is encoded by the exons 7 and 9 (Figure 1). The latter splicing influences the ligand binding, leading to the specificity pattern. FGFR2 IIIc is expressed primarily by mesenchymal cells, and FGFR2 IIIb essentially by epithelial cells. FGF7 is also known as keratinocyte growth factor (KGF), and binds only to FGFR2 IIIb, which is therefore also called KGFR. When the FGFs bind to their receptors, there is subsequent dimerization and phosphorylation of the FGFRs, and a downstream signalling via the FRS-GRB2 docking protein complex to the RAS-MAPK signal transduction cascade and the PI3K-AKT signal transduction cascade. The former signal transduction cascade is involved in cell growth and in cell differentiation, while the latter is involved in the survival of the cells and in the determination of cell fate (Katoh und Katoh, Int. J. Oncol. 2006, 29:163-168).

For correct organogenesis during embryogenesis, an orchestrated signal transduction of all four receptors (FGFR1 to FGFR4) and of their splicing variants via the different FGFs is necessary (Ornitz et al., Genome Biol 2001, 2:3005). In the case of FGFR2, the absence of all FGFR2 variants leads to defects in placental and limb bud formation, and therefore leads to fatality at the

E10.5 stage. Specific knock-out of FGFR2 IIIb in the mouse leads likewise to fatality (at P0) in association with agenesis of the lungs, of the anterior lobe, of the thyroid gland, of the teeth and of the limbs, whereas disruption of the FGFR2 IIIc variant is survivable, with incidence of retarded ossification, proportional dwarfism and synostosis of the base of the skull (Eswarakumar and Schlessinger, 2005). Activating mutations of FGFR2 in the germ track lead to severe malformations during embryogenesis, such as coronal synostosis and cranial synostosis in the case of Apert's syndrome or in the case of Pfeiffer's syndrome in humans (Robin et al., in Gene Reviews, NCBI Bookshelf Washington, edited by Pagon et al., 1993). In adults, FGFR2 signal transduction is involved in wound healing, in epithelial repair and in the protection of cells of the skin and mucous membrane (Braun et al., Phil. Trans. R. Soc. Lond. B 2004, 359:753-757) and in regeneration in the event of liver damage (Steiling et al., Oncogene 2003, 22:4380-4388; Böhm, dissertation, Eidgenössische Technische Hochschule Zürich, 2009). A role of FGFR2 signal transduction in the migration of EPDC (epicardial derived cells) into the heart following infarction is therefore under discussion because during embryogenesis, FGF10-FGFR2 signal transduction is necessary for the migration of EPDC into the compact myocardium, a process which is necessary for the development of the heart (Vega-Hernández et al., Development 2011:3331-3340; Winter and De Groot, Cell Mol. Life Sci. 2007, 64:692-703). All of these roles played by FGFR2 are regenerative in nature, and apparently of essential importance only under non-physiological conditions, as a result of a disruption in tissue homeostasis. Increased somatic signal transduction via FGFR2 is involved in various pathological conditions such as acne (Katoh, J. of Invest. Dermatol. 2009, 129:1861-1867), psoriasis (Finch et al., Am. J. Pathol. 1997, 151:1619-1628; Xu et al., J. Invest. Dermatol. 2011:131:1521-1529) and cancer (see below).

A number of studies have been published which show a strong association of FGFR2 expression with an adverse outcome in cancer patients:

The overexpression of FGFR2 and/or KGF is accompanied by expansive growth of stomach carcinoma and shorter survival of the patients (Matsunobu et al., Int. J. Cancer 2006, 28:307-314; Toyokawa et al., Oncol. Reports 2009, 21:875-880). The overexpression of FGFR2 was detected in 31-36.5% of all stomach carcinoma samples examined (Matsunobu et al., Int. J. Cancer 2006, 28:307-314; Toyokawa et al., Oncol. Reports 2009, 21:875-880). Adenocarcinoma (70% of all stomach carcinomas) is subdivided, additionally, into two different pathological types, namely stomach cancer of the intestinal type of and of the diffuse type. Interestingly, the first, less aggressive type is associated with an activated ErbB2 signal pathway, whereas, in the case of the latter, more aggressive type, aberrations occur in the FGFR2/PI3K signal pathway (Yamashita et al., Surg. Today 2011, 41:24-38). FGFR2 overexpression occurred in 53% of all samples of stomach cancer of the diffuse kind (Yamashita et al., Surg. Today 2011, 41:24-38). Drawing

together all of the data, HER2 expression and FGFR2 expression appear to occur in two different patient populations. To some extent possibly, the expression of FGFR2 is the result of a gene amplification, since amplifications of FGFR2 are found in approximately 7-10% of all primary stomach carcinomas (Kunii et al., Cancer Res. 2008, 68:23-40-2348). Moreover, FGFR2 expression has not only been found in metastases, but was in fact even greater in metastases than in primary tumours (Yamashita et al., Surg. Today 2011, 41:24-38).

In the case of breast cancer, FGFR2 IIIb expression was found in 57% of tumour samples, but hardly at all in healthy tissue (Tamaru et al. 2004, 84:1460-1471). KGF (FGF7) occurred in 45% of random samples, generally together with FGFR2 IIIb. The co-expression of FGF7 and its single receptor FGFR2 IIIb was associated with a significantly reduced number of apoptotic cells within the primary tumour by comparison with primary breast carcinomas, where neither FGF7 nor FGFR2 IIIb was expressed (Tamaru et al. 2004, 84:1460-1471). As in the case of stomach cancer, a gene amplification was found for breast cancer as well, and also in 4% of triply negative breast carcinomas (TNBC) (Turner et al., Oncogene 2010, 29:2013-2023). In breast cancer, a number of changes in individual nucleotides (Single Nucleotide Polymorphism, SNP) have been identified that are associated with an increased risk of breast cancer (Hunter et al., Nature Genetics 2007, 6:870-874). If the SNPs are localized within Intron 2, this leads to a transcriptional upregulation of FGFR2 (Katoh, Expert Reviews 2010, 10:1375-1379). Interestingly, FGFR1 is upregulated preferentially in the case of oestrogen receptor (ER) positive breast carcinomas, while FGFR2 is upregulated in the case of ER-negative breast carcinomas (Katoh, Expert Reviews 2010, 10:1375-1379).

In the case of pancreatic carcinoma, the overexpression of FGFR2 IIIb and/or FGF7 is heavily correlated with venal invasion (Cho et al., Am. J. Pathol. 170:1964-1974), with co-expression of FGFR2 and FGF7 having been found in tumour cells, but occurring even more frequently in the stroma cells which are adjacent to the tumour cells (Ishiwata et al., Am. J. Pathol. 1998, 153:213-222).

In the case of epithelial ovary cancer, 80% of cases showed upregulation of FGRF2 as compared with normal tissue, and in 70% there was FGF7 in the ascitic fluid (Steele et al., Oncogene 20:5878-5887).

The FGFR2 protein was found in all invasive cervical carcinomas tested, with strong expression at the invasive front of the tumours (Kawase et al., Int. J. Oncol. 2010, 36:331-340).

In the case of pulmonary adenocarcinoma, co-expression of FGF7 and FGFR2 occurred in 51.6% of cases, and is correlated with lower degrees of differentiation, higher rate of proliferation, lymph node metastasis, and shorter 5-year survival (Yamayoshi et al., J. Pathol. 2004, 204:110-118).

In the case of endometrial carcinoma, activating FGFR2 mutations occur in approximately 16% of cases (Pollock et al., Oncogene 2007, 26:7158-7162).

In the case of oesophageal cancer (OC), co-expression of FGF7 and FGFR2 in cancer cells was found in 26% of patients, and was associated with a trend towards a shorter survival time (Yoshino et al., Int. J. Oncol. 2007, 31:721-728).

In the case of liver cell carcinoma, the expression of FGFR2 was upregulated 4.7 times more strongly in poorly differentiated tumours. This expression is associated with the incidence of portal vein invasion and lower disease-free survival times (Harimoto et al., Oncology 2010, 78:361-368).

A number of publications, with *in vitro* and *in vivo* experimental data, show a causal connection between altered FGFR2 signal transduction and tumour growth.

Knock-down or inhibition of FGFR2 in cells of stomach cancer (Takeda et al., Clin. Cancer Res. 2007; 13:3051-3057; Kunii et al., Cancer Res. 2008; 68:2340-2348), breast cancer (Turner et al., Oncogene 2010, 29:2013-2023), ovarian cancer (Cole et al., Cancer Biol. Ther. 2010, 10:495-504) and squamous carcinoma of the head and neck (Marshall et al., Clin. Cancer Res. 2011, 17:5016-5025) led to reduced proliferation or increased apoptosis of the tumour cells. In tumour xenotransplants as well, knock-down of FGFR2 and inhibition of FGFR2 in tumour cell lines which overexpress FGFR2 was observed to cause growth inhibition in stomach cancer cell lines (Takeda et al., Clin. Cancer Res. 2007; 13:3051-3057) and ovarian cancer cell lines (Cole et al., Cancer Biol. Ther. 2010, 10:495-504). Moreover, FGF7, which exclusively activates FGFR2, increases the proliferation of stomach cancer cell lines (Shin et al., J. Cancer Res. Clin. Oncol. 2002, 128:596–602), breast cancer cell lines (Zhang et al., Anticancer Res. 1998, 18:2541-2546) and ovarian cancer cell lines (Cole et al., Cancer Biol. Ther. 2010, 10:495-504) *in vitro* and *in vivo*. Furthermore, knock-down of FGFR2 in endometrial cancer cell lines which contain FGFR2 with activating mutations likewise led to the standstill of the cell cycle and to the induction of cell death (Byron et al., Cancer Res. 2008, 68:6902-6907).

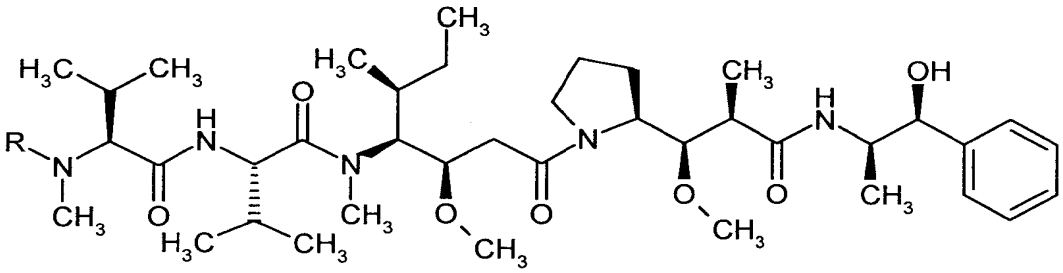
FGFR2 signal transduction promotes the migration and invasion of stomach cancer cell lines (Shin et al., J. Cancer Res. Clin. Oncol. 2002, 128:596–602), breast cancer cell lines (Zhang et al., Anticancer Res. 1998, 18:2541-2546) and pancreatic cancer cell lines *in vitro* (Nomura et al., Br. J. Cancer 2008, 99:305-313; Niu et al., J. Biol. Chem. 2007, 282:6601-6011).

In the case of oesophageal cancer, FGFR2 is the most highly upregulated gene in tumour-associated fibroblasts. Isolated tumour-associated fibroblasts released a soluble factor which promotes the proliferation of oesophageal cancer cells (Zhang et al., hum. Cancer Biol. 2009,

15:4017-4022), thereby demonstrating that FGFR2 expressed by stroma cells is also able to promote tumour progression.

There are only a few reports on anti-FGFR2 antibodies. Fortin et al. (J. Neurosci. 2005, 25: 7470-7479) describe a blocking anti-FGFR2 antibody. Wei et al. (Hybridoma 2006, 25: 115-124) have shown antibodies with exclusive specificity for FGFR2 IIIb, which inhibit the KGF-induced cell proliferation. WO2007/144893 describes inhibiting antibodies which bind FGFR2 and FGFR3. In WO2010/054265 and in Zhao et al. (Clin. Cancer Res. 2010,16:5750-5758), antibodies which inhibit FGF binding are described, including for example GAL-FR21 and GAL-FR22. Bai et al. (Cancer Res. 2010, 70:7630-7639) describe antibodies having specificity for FGFR2 IIIb. R&D Systems market anti-FGFR2 antibodies which have an activity-neutralizing effect in the manufacturer's assays. WO2005/066211 describes antibodies which are directed against various cell-surface FGFRs, including FGFR2. WO2009/100105 describes isoform-specific anti-FGFR2 antibodies which can be linked covalently to effector molecules. WO2007/134210 describes methods for treating colorectal cancer using anti-FGFR2 antibodies or immunoconjugates. WO2007/144893 describes FGFR2 antibodies with binding affinity for further FGFRs, which block the ligand-dependent and the constitutive ligand-independent FGFR2 receptor activation.

Auristatin E (AE) and monomethylauristatin E (MMAE) are synthetic analogues of the dolastatins, a specific group of linear pseudopeptides which were originally isolated from marine sources and which have in some cases very potent cytotoxic activity with respect to tumour cells [for a review see e.g. G. R. Pettit, *Prog. Chem. Org. Nat. Prod.* 70, 1-79 (1997); G. R. Pettit *et al.*, *Anti-Cancer Drug Design* 10, 529-544 (1995); G. R. Pettit *et al.*, *Anti-Cancer Drug Design* 13, 243-277 (1998)].



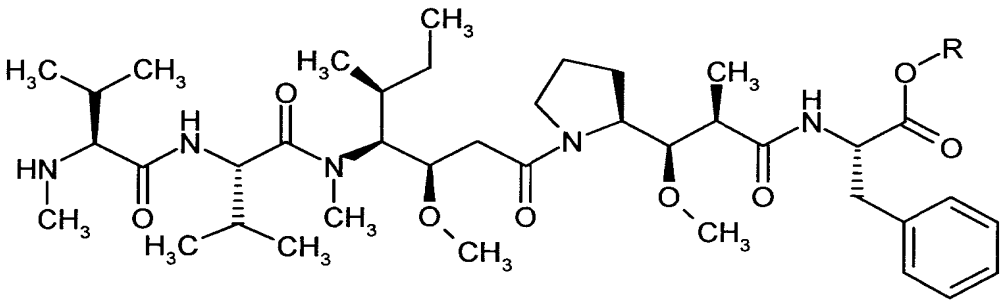
Auristatin E (AE): R = CH_3

Monomethylauristatin E (MMAE): R = H

MMAE, however, possesses the disadvantage of a comparatively high systemic toxicity. For improving tumour selectivity, MMAE is used more particularly in conjunction with enzymatically cleavable valine-citrulline linkers in the ADC setting for more targeted tumour therapy

[WO 2005/081711-A2; S. O. Doronina *et al.*, *Bioconjugate Chem.* 17, 114-124 (2006)]. Following proteolytic cleavage, MMAE is released preferably intracellularly from corresponding ADCs.

Monomethylauristatin F (MMAF) is an auristatin derivative having a C-terminal phenylalanine unit which exhibits only moderate antiproliferative activity in comparison to MMAE. This fact is very probably attributable to the free carboxyl group, whose polarity and charge adversely affect the capacity of this compound to access cells. In this connection, the methyl ester of MMAF (MMAF-OMe) has been described, as a neutral-charged prodrug derivative with cell access capability, which, in comparison to MMAF, has an *in vitro* cytotoxicity for various carcinoma cell lines that is increased by a number of orders of magnitude [S. O. Doronina *et al.*, *Bioconjugate Chem.* 17, 114-124 (2006)]. It can be assumed that this effect is brought about by MMAF itself, which, following uptake of the prodrug into the cells, is rapidly released by intracellular ester hydrolysis.



Monomethylauristatin F (MMAF): R = H

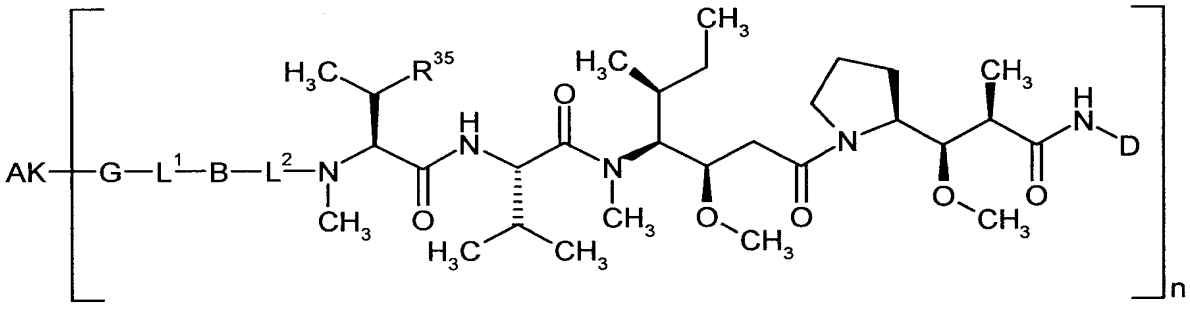
Monomethylauristatin F methyl ester (MMAF-OMe): R = CH_3

However, drug compounds based on simple ester derivatives are generally subject to the risk of chemical instability on account of non-specific ester hydrolysis, independent of the intended site of action, by means, for example, of esterases that are present in the blood plasma; this non-specific hydrolysis may significantly restrict the usefulness of such compounds in therapy.

Monomethylauristatin F (MMAF) and also various ester derivatives and amide derivatives thereof have been disclosed in WO 2005/081711-A2. Further auristatin analogues with a C-terminal, amidically substituted phenylalanine unit are described in WO 01/18032-A2. WO 02/088172-A2 and WO 2007/008603-A1 claim MMAF analogues which relate to side-chain modifications of the phenylalanine, while WO 2007/008848-A2 claims those in which the carboxyl group of the phenylalanine has been modified. Auristatin conjugates linked via the C-terminus have been recently described in WO 2009/117531-A1 [see also S. O. Doronina *et al.*, *Bioconjugate Chem.* 19, 1960-1963 (2008)].

The problem addressed with the present invention was that of providing new binder-drug conjugates (ADCs) which, through combination of new N,N-dialkylauristatin derivatives with innovative, suitable linkers and binder, exhibit a very attractive activity profile, such as, for example, in terms of their specific tumour effect and/or the reduced potential of the metabolites formed intracellularly to be a substrate with respect to transporter proteins, and which are therefore suitable for the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases, such as cancer diseases, for example.

The present invention provides binder-drug conjugates of the general formula (Ia)



(Ia),

in which

n is a number from 1 to 50,

AK is a binder which binds FGFR2,

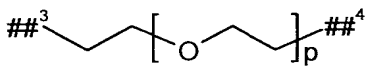
the group §-G-L^1-B-§§ is a linker,

where

§ marks the linkage site with the group AK and

§§ marks the linkage site with the nitrogen atom,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

[3] marks the linkage site with the group B,

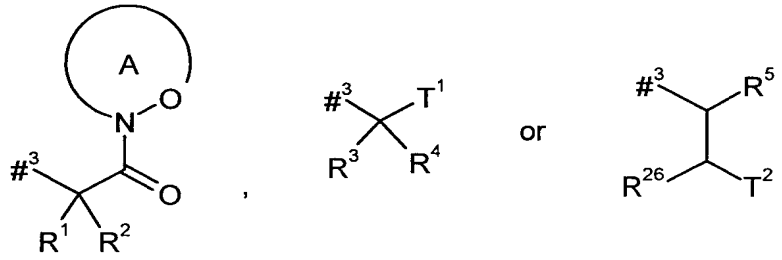
[4] marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



where

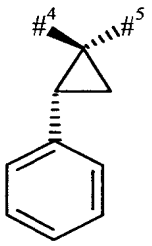
#[3] marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

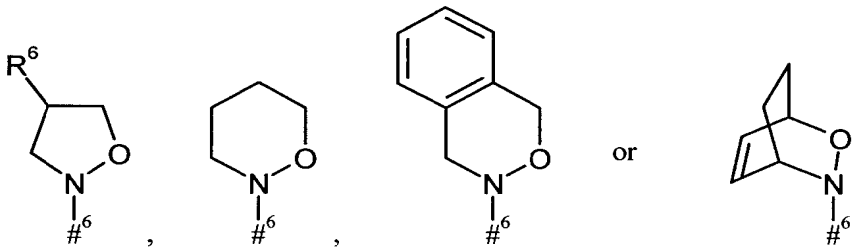


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

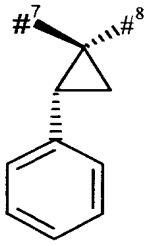
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

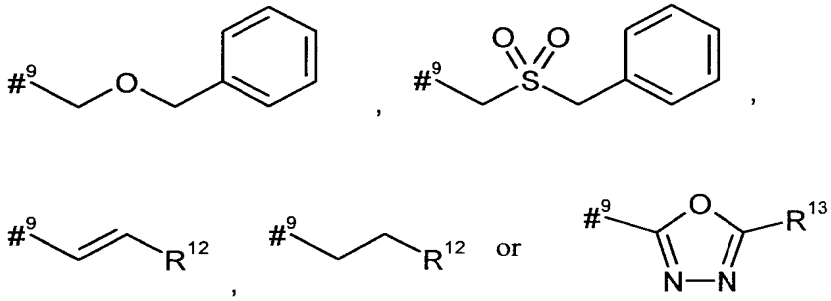
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Compounds of the invention are the compounds of the formula (I) and their salts, solvates and solvates of the salts, the compounds of the formulae identified below and encompassed by formula (I), and their salts, solvates and solvates of the salts, and also the compounds identified below as working examples and encompassed by formula (I), and their salts, solvates and solvates of the salts, to the extent that the compounds identified below and encompassed by formula (I) are not already salts, solvates and solvates of the salts.

Depending on their structure, the compounds of the invention may exist in different stereoisomeric forms, i.e. in the form of configurational isomers or else where appropriate as conformational isomers (enantiomers and/or diastereoisomers, including those in the case of atropisomers). The present invention therefore encompasses the enantiomers and diastereomers and their respective mixtures. The stereoisomerically homogeneous constituents can be isolated from such mixtures of enantiomers and/or diastereomers in a known way; for this purpose it is preferred to use chromatographic processes, more particularly HPLC chromatography on an achiral or chiral phase.

Where the compounds of the invention can occur in tautomeric forms, the present invention encompasses all of the tautomeric forms.

The present invention also encompasses all suitable isotopic variants of the compounds of the invention. An isotopic variant of a compound of the invention is understood here to mean a compound in which at least one atom within the compound of the invention has been exchanged for another atom of the same atomic number but with a different atomic mass from the atomic mass which occurs commonly or predominantly in nature. Examples of isotopes which can be incorporated into an inventive compound are those of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine, chlorine, bromine and iodine such as ^{2}H (deuterium), ^{3}H (tritium), ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{33}S, ^{34}S, ^{35}S, ^{36}S, ^{18}F, ^{36}Cl, ^{82}Br, ^{123}I, ^{124}I, ^{129}I and ^{131}I. Particular isotope variants of a compound of the invention, such as more particularly those in which one or more radioactive isotopes are incorporated, may be of benefit, for example, for investigating the mechanism of action or the distribution of drug in the body; owing to the comparative ease of preparation and detectability, compounds labelled with ^{3}H or ^{14}C isotopes are especially suitable for these purposes. Furthermore, the incorporation of isotopes, such as of deuterium, for example, may lead to certain therapeutic advantages as a consequence of greater metabolic stability of the compound, such as an extension to the half-life in the body or a reduction in the active dose required, for example; such modifications of the compounds of the invention may therefore, where appropriate, also constitute a preferred embodiment of the present invention. Isotopic variants of the compounds of the invention can be prepared by the processes known to the skilled person, as for example in accordance with the methods described later on below and the procedures reproduced in the working examples, by using corresponding isotopic modifications of the respective reagents and/or starting compounds.

Preferred salts in the context of the present invention are physiologically acceptable salts of the compounds of the invention. Also encompassed are salts which although themselves not suitable for pharmaceutical applications may nevertheless be used, for example, for isolating or purifying the compounds of the invention.

Physiologically acceptable salts of the compounds of the invention encompass acid addition salts of mineral acids, carboxylic acids and sulphonic acids, examples being salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid, ethanesulphonic acid, benzenesulphonic acid, toluenesulphonic acid, naphthalenedisulphonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

Physiologically acceptable salts of the compounds of the invention also encompass salts of customary bases, such as, by way of example and preferably, alkali metal salts (e.g. sodium and potassium salts), alkaline earth metal salts (e.g. calcium and magnesium salts) and ammonium salts, derived from ammonia or organic amines having 1 to 16 C atoms, such as, by way of example and preferably, ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylamine, *N*-methylpiperidine, *N*-methylmorpholine, arginine, lysine and 1,2-ethylenediamine.

Solvates in the context of the invention are those forms of the compounds of the invention that form a complex in the solid or liquid state through coordination with solvent molecules. Hydrates are one specific form of solvates, in which the coordination takes place with water. Preferred solvates in the context of the present invention are hydrates.

Furthermore, the present invention also encompasses prodrugs of the compounds of the invention. The term "prodrugs" here identifies compounds which may themselves be biologically active or inactive but are converted during their residence in the body into compounds of the invention (by metabolism or hydrolysis, for example).

In the context of the present invention the definitions of the substituents, unless otherwise specified, are as follows:

$(C_1\text{-}C_4)$-Alkyl in the context of the invention is a linear or branched alkyl radical having 1 to 4 carbon atoms. By way of example and with preference, the following may be mentioned: methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, 1-methylpropyl and *tert*-butyl.

Alkanediyl in the context of the invention is a linear, α,ω-divalent alkyl radical having the particular number of carbon atoms indicated. By way of example and of preference, the following may be mentioned: methylene, ethane-1,2-diyl (1,2-ethylene), propane-1,3-diyl (1,3-propylene), butane-1,4-diyl (1,4-butylene), pentane-1,5-diyl (1,5-pentylene), hexane-1,6-diyl (1,6-hexylene), heptane-1,7-diyl (1,7-hexylene), octane-1,8-diyl (1,8-octylene), nonane-1,9-diyl (1,9-nonylene), decane-1,10-diyl (1,10-decylene).

$(C_3\text{-}C_7)$-Cycloalkyl and 3- to 7-membered carbocycle respectively in the context of the invention is a monocyclic, saturated cycloalkyl group having 3 to 7 carbon atoms. By way of example and of preference, the following may be mentioned: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

The side group of an α-amino acid in the definition of R^{19} encompasses not only the side groups of the naturally occurring α-amino acids but also the side groups of homologues and isomers of these α-amino acids. The α-amino acid here may be in the L or D configuration or else may be present as a mixture of the L and D forms. Examples that may be given of side groups are as follows: methyl (alanine), propan-2-yl (valine), propan-1-yl (norvaline), 2-methylpropan-1-yl (leucine), 1-methylpropan-1-yl (isoleucine), butan-1-yl (norleucine), *tert*-butyl (2-*tert*-butylglycine), phenyl (2-phenylglycine), benzyl (phenylalanine), p-hydroxybenzyl (tyrosine), indol-3-ylmethyl (tryptophan), imidazol-4-ylmethyl (histidine), hydroxymethyl (serine), 2-hydroxyethyl (homoserine), 1-hydroxyethyl (threonine), mercaptomethyl (cysteine), methylthiomethyl (*S*-methylcysteine), 2-mercaptoethyl (homocysteine), 2-methylthioethyl (methionine), carbamoylmethyl (asparagine), 2-carbamoylethyl (glutamine), carboxymethyl (aspartic acid), 2-carboxyethyl (glutamic acid), 4-aminobutan-1-yl (lysine), 4-amino-3-hydroxybutan-1-yl (hydroxylysine), 3-aminopropan-1-yl (ornithine), 2-aminoethyl (2,4-diaminobutyric acid), aminomethyl (2,3-diaminopropionic acid), 3-guanidinopropan-1-yl (arginine), 3-ureidopropan-1-yl (citrulline). Preferred α-amino acid side groups in the definition of R^{19} are methyl (alanine), propan-2-yl (valine), 2-methylpropan-1-yl (leucine), benzyl (phenylalanine), imidazol-4-ylmethyl (histidine), hydroxymethyl (serine), 1-hydroxyethyl (threonine), 4-aminobutan-1-yl (lysine), 3-aminopropan-1-yl (ornithine), 2-aminoethyl (2,4-diaminobutyric acid), aminomethyl (2,3-diaminopropionic acid), 3-guanidinopropan-1-yl (arginine). The L configuration is preferred in each case.

A 4- to 7-membered heterocycle in the context of the invention is a monocyclic, saturated heterocycle having a total of 4 to 7 ring atoms, which contains one or two ring heteroatoms from the series N, O, S, SO and/or SO_2 and is linked via a ring carbon atom or optionally a ring nitrogen atom. Preference is given to a 5- to 7-membered heterocycle having one or two ring heteroatoms from the series N, O and/or S, more preferably a 5- or 6-membered heterocycle having one or two ring heteroatoms from the series N and/or O. By way of example, the following may be mentioned: azetidinyl, oxetanyl, pyrrolidinyl, pyrazolidinyl, tetrahydrofuranyl, thiolanyl, piperidinyl, piperazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, morpholinyl, thiomorpholinyl, hexahydroazepinyl and hexahydro-1,4-diazepinyl. Preference is given to pyrrolidinyl, tetrahydrofuranyl, piperidinyl, piperazinyl, tetrahydropyranyl and morpholinyl.

In the formula of the group which may be represented by A, B, D, G, L^1, L^2, L^4, R^1, R^2, R^3, R^4 and R^5, respectively, the end point of the line at which the symbol $\#^6$, *, **, $\#^3$, $\#^1$, $\#^2$, $\#\#^1$, $\#\#^2$, $\#\#^3$, $\#\#^4$, ***, ****, $\#^4$, $\#^5$, $\#^6$, $\#^7$, $\#^8$ or $\#^9$ is located is not a carbon atom or a CH_2 group, but instead is part of the bond to the atom designated in each case, to which the A, B, D, G, L^1, L^2, L^4, R^1, R^2, R^3, R^4 or R^5 is bonded.

In the context of the present invention, all radicals which occur multiply have their definition independently of one another. If radicals in the compounds of the invention are substituted, the radicals, unless otherwise specified, may be substituted one or more times. Substitution by one or by two identical or different substituent(s) is preferred. Particularly preferred is substitution by one substituent.

In the context of the present invention the terms used, unless otherwise specified, have the following definitions:

The term "linker" is understood in the broadest sense as a chemical unit which comprises a covalent bond or a series of atoms that links a binder covalently to a drug. The term "linker" is understood preferably as a series of atoms in the sense of the present invention that links a binder covalently to a drug. Furthermore, linkers may be represented, for example, by divalent chemical units, such as alkyldiyls, aryldiyls, heteroaryldiyls, heterocyclyldiyls, dicarbonyl acid esters, dicarbonyl acid amides.

The term "binder" is understood in the broadest sense as a molecule which binds to a target molecule which is present on a particular target cell population to be addressed with the binder-drug conjugate. The term "binder" should be understood in its broadest interpretation and encompasses, for example, lectins, proteins which are able to bind particular sugar chains, or phospholipid-binding proteins. Such binders comprise, for example, high molecular mass proteins (binding proteins), polypeptides or peptides (binding peptides), non-peptidic (e.g. aptamers (US5,270,163) review article by Keefe AD., et al., Nat. Rev. Drug Discov. 2010; 9:537-550), or vitamins) and all other cell-binding molecules or substances. Binding proteins are, for example, antibodies and antibody fragments or antibody mimetics such as, for example, affibodies, adnectins, anticalins, DARPins, avimers, nanobodies (review articles by Gebauer M. et al., Curr. Opinion in Chem. Biol. 2009; 13:245-255; Nuttall S.D. et al., Curr. Opinion in Pharmacology 2008; 8:608-617). Binding peptides are, for example, ligands of a ligand-receptor pair, such as VEGF in the ligand-receptor pair VEGF/KDR, such as transferrin of the ligand-receptor pair transferrin/transferrin receptor, or cytokines/cytokine receptor, such as TNFalpha in the ligand receptor pair TNFalpha/TNFalpha receptor.

The term "epitope" as used herein encompasses any determinants of a protein that are able to bind specifically to an immunoglobulin or T-cell receptor. Such determinants commonly consist of chemically active surface arrangements of molecules, such as amino acids, carbohydrates or a combination thereof, for example, which commonly have a specific three-dimensional structure and also defined charge properties. Two antibodies bind to the same epitope if it is shown in a

competitive binding assay format that the first antibody competes with the second antibody. Binding assays of this kind are known to the skilled person.

A "target molecule" is understood in the broadest sense to be a molecule which is present in the target cell population, and may be a protein (e.g. a receptor of a growth factor) or a non-peptidic molecule (e.g. a sugar or phospholipid). Preferably it is a receptor or an antigen.

The term "extracellular" target molecule describes a target molecule which is attached to the cell and which is located on the outside of a cell or the part of a target molecule which is located on the outside of a cell, i.e. a binder may bind to an intact cell at its extracellular target molecule. An extracellular target molecule may be anchored in the cell membrane or may be part of the cell membrane. The skilled person knows of methods for identifying extracellular target molecules. For proteins this may be done via determination of the transmembrane domain(s) and the orientation of the protein in the membrane. This data is generally recorded in protein databases (e.g. SwissProt).

The term "cancer target molecule" describes a target molecule which is multiply present on one or more cancer cell types in comparison to non-cancer cells of the same tissue type. The cancer target molecule is preferably present selectively on one or more cancer cell types in comparison to non-cancer cells of the same tissue type, with "selectively" describing an at least twofold accumulation on cancer cells in comparison to non-cancer cells of the same tissue type (a "selective cancer target molecule"). The use of cancer target molecules allows selective therapy of cancer cells with the conjugates of the invention.

The binder may be linked via a bond to the linker. The linking of the binder may take place by means of a heteroatom of the binder. Inventive heteroatoms of the binder that may be used for linking are sulphur (in one embodiment via a sulphhydryl group of the binder), oxygen (in accordance with the invention by means of a carboxyl or hydroxy group of the binder) and nitrogen (in one embodiment via a primary or secondary amine group or amide group of the binder). These heteroatoms may be present in the natural binder or may be introduced by means of methods of chemistry or molecular biology. In accordance with the invention, the linking of the binder to the toxophore has little influence over the binding activity of the binder to the target molecule. In a preferred embodiment the linking has no influence on the binding activity of the binder to the target molecule.

The term "antibody" is understood in accordance with the present invention in its broadest sense and encompasses immunoglobulin molecules, examples being intact or modified monoclonal antibodies, polyclonal antibodies or multispecific antibodies (e.g. bispecific antibodies). An

immunoglobulin molecule preferably comprises a molecule having four polypeptide chains, two heavy chains (H chains) and two light chains (L chains), which are linked typically by disulphide bridges. Each heavy chain comprises a variable domain of the heavy chain (abbreviated to VH) and a constant domain of the heavy chain. The constant domain of the heavy chain may encompass, for example, three domains CH1, CH2 and CH3. Each light chain comprises a variable domain (abbreviated to VL) and a constant domain. The constant domain of the light chain comprises one domain (abbreviated to CL). The VH and VL domains may be further subdivided into regions having hypervariability, also called complementarity-determining regions (abbreviated to CDR), and regions having a low sequence variability ("framework region", abbreviated to FR). Each VH and VL region is typically composed of three CDRs and up to four FRs. For example, in the following order from the amino terminus to the carboxy terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody may be obtained from any species suitable for the antibody, such as, for example, rabbit, llama, camel, mouse or rat. In one embodiment the antibody is of human or murine origin. An antibody may for example be human, humanized or chimeric.

The term "monoclonal" antibody identifies antibodies which have been obtained from a population of substantially homogeneous antibodies, i.e. individual antibodies of the population are identical except for naturally occurring mutations which may occur in small numbers. Monoclonal antibodies recognize a single antigenic binding site with a high specificity. The term "monoclonal antibody" does not refer to a particular production method.

The term "intact" antibody refers to antibodies which comprise not only an antigen-binding domain but also the constant domain of the light and heavy chain. The constant domain may be a naturally occurring domain, or a variant thereof in which a plurality of amino acid positions have been altered.

The term "modified intact" antibody refers to intact antibodies which have been fused with another polypeptide or protein, not originating from an antibody, via the amino terminus or carboxyl terminus thereof, by means of a covalent bond (e.g. a peptide linkage). Furthermore, antibodies may be modified by introducing reactive cysteines at defined locations, in order to facilitate coupling to a toxophore (see Junutula et al. Nat Biotechnol. 2008 Aug;26(8):925-32).

The term "human" antibody identifies antibodies which can be obtained from a human being or are synthetic human antibodies. A "synthetic" human antibody is an antibody which in parts or as a whole is obtainable from synthetic sequences *in silico* which are based on the analysis of human antibody sequences. A human antibody may be encoded, for example, by a nucleic acid which has been isolated from a library of antibody sequences which are of human origin. One example of such antibodies can be found in Söderlind et al., Nature Biotech. 2000, 18:853-856.

The term "humanized" or "chimeric" antibody describes antibodies which consist of a non-human and of a human sequence component. In these antibodies, part of the sequences of the human immunoglobulin (recipient) is replaced by sequence components of a non-human immunoglobulin (donor). In many cases the donor is a murine immunoglobulin. With humanized antibodies, amino acids of the CDR in the recipient are replaced by amino acids of the donor. In some cases, amino acids of the framework as well are replaced by corresponding amino acids of the donor. In some cases the humanized antibody contains amino acids which were present neither in the recipient nor in the donor and which were inserted during the optimization of the antibody. In the case of chimeric antibodies, the variable domains of the donor immunoglobulin are fused with the constant regions of a human antibody.

The term complementarity-determining region (CDR) as used here refers to those amino acids in a variable antibody domain that are necessary for binding to the antigen. Every variable region typically has three CDR regions, identified as CDR1, CDR2 and CDR3. Each CDR region may comprise amino acids according to the definition of Kabat and/or amino acids of a hypervariable loop, defined according to Chotia. The definition according to Kabat encompasses, for example, the region of approximately amino acid position 24 – 34 (CDR1), 50 – 56 (CDR2) and 89 – 97 (CDR3) of the variable light chain and 31 – 35 (CDR1), 50 – 65 (CDR2) and 95 – 102 (CDR3) of the variable heavy chain (Kabat et al., Sequences of Proteins of Immulological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The definition according to Chotia encompasses, for example, the region of approximately amino acid position 26 – 32 (CDR1), 50 – 52 (CDR2) and 91 – 96 (CDR3) of the variable light chain and 26 – 32 (CDR1), 53 – 55 (CDR2) and 96 – 101 (CDR3) of the variable heavy chain Chothia and Lesk; J Mol Biol 196: 901-917 (1987)). In some cases a CDR may comprise amino acids from one CDR region as defined by Kabat and Chotia.

Depending on the amino acid sequence of the constant domain of the heavy chain, antibodies may be divided into different classes. There are five main classes of intact antibodies: IgA, IgD, IgE, IgG and IgM, and a number of them may be broken down into further subclasses (isotypes), e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The constant domains of the heavy chain that correspond to the different classes are identified as [alpha/α], [delta/δ], [epsilon/ε], [gamma/γ] and [mu/μ]. Both the three-dimensional structure and the subunit structure of antibodies are known.

The term "functional fragment" or "antigen-binding antibody fragments" of a antibody/immunoglobulin is defined as a fragment of an antibody/immunoglobulin (e.g. the variable domains of an IgG) which further encompasses the antigen binding domains of the antibody/immunoglobulin. The "antigen binding domain" of an antibody typically encompasses

one or more hypervariable regions of an antibody, e.g. the CDR, CDR2 and/or CDR3 region. However, the "framework" or "scaffold" region of an antibody may also play a part with regard to the binding of the antibody to the antigen. The framework region forms the scaffold for the CDRs. The antigen-binding domain preferably encompasses at least amino acids 4 to 103 of the variable light chain and amino acid 5 to 109 of the variable heavy chain, more preferably amino acid 3 to 107 of the variable light chain and 4 to 111 of the variable heavy chain, particular preference being given to the complete variable light and heavy chains, i.e. amino acid 1 – 109 of the VL and 1 to 113 of the VH (numbering according to WO97/08320).

"Functional fragments" or "antigen-binding antibody fragments" of the invention encompass, non-conclusively, Fab, Fab', $F(ab')_2$ and Fv fragments, diabodies, Single Domain Antibodies (DAbs), linear antibodies, individual chains of antibodies (single-chain Fv, abbreviated to ScFv); and multispecific antibodies, such as bi and tri-specific antibodies, for example, formed from antibody fragments C. A. K Borrebaeck, editor (1995) Antibody Engineering (Breakthroughs in Molecular Biology), Oxford University Press; R. Kontermann & S. Duebel, editors (2001) Antibody Engineering (Springer Laboratory Manual), Springer Verlag). Antibodies other than "multispecific" or "multifunctional" antibodies are those having identical binding sites. Multispecific antibodies may be specific for different epitopes of an antigen or may be specific for epitopes of more than one antigen (see, for example WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., 1991, J. Immunol. 147:60 69; U. S. Pat. Nos. 4,474,893; 4,7 14,68 1; 4,925,648; 5,573,920; 5,601,8 19; or Kostelny et al., 1992, J. Immunol. 148: 1547 1553). An $F(ab')_2$ or Fab molecule may be constructed such that the number of intermolecular disulphide interactions occurring between the Ch1 and the CL domains can be reduced or else completely prevented.

"Functional fragments" or "antigen-binding antibody fragments" may be fused with another polypeptide or protein, not originating from an antibody, via the amino terminus or carboxyl terminus thereof, by means of a covalent bond (e.g. a peptide linkage). Furthermore, antibodies and antigen-binding fragments may be modified by introducing reactive cysteines at defined locations, in order to facilitate coupling to a toxophore (see Junutula et al. Nat Biotechnol. 2008 Aug;26(8):925-32).

Polyclonal antibodies can be prepared by methods known to a person of ordinary skill in the art. Monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Köhler and Milstein, Nature, 256, 495-497, 1975). Human and humanized monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Olsson et al., Meth Enzymol. 92, 3-16 or Cabilly et al US 4,816,567 or Boss et al US 4,816,397).

A person of ordinary skill in the art is aware of diverse methods for preparing human antibodies and fragments thereof, such as, for example, by means of transgenic mice (N Lonberg and D Huszar, Int Rev Immunol. 1995;13(1):65-93) or Phage Display Technologies (Clackson et al., Nature. 1991 Aug 15;352(6336):624-8). Antibodies of the invention may be obtained from recombinant antibody libraries consisting for example of the amino acid sequences of a multiplicity of antibodies compiled from a large number of healthy volunteers. Antibodies may also be produced by means of known recombinant DNA technologies. The nucleic acid sequence of an antibody can be obtained by routine sequencing or is available from publically accessible databases.

An "isolated" antibody or binder has been purified to remove other constituents of the cell. Contaminating constituents of a cell which may interfere with a diagnostic or therapeutic use are, for example, enzymes, hormones, or other peptidic or non-peptidic constituents of the cell. A preferred antibody or binder is one which has been purified to an extent of more than 95%, relative to the antibody or binder (determined for example by Lowry method, UV-Vis spectroscopy or by SDS capillary gel electrophoresis). Furthermore an antibody which has been purified to such an extent that it is possible to determine at least 15 amino acids of the amino terminus or of an internal amino acid sequence, or which has been purified to homogeneity, the homogeneity being determined by SDS-PAGE under reducing or non-reducing conditions (detection may be determined by means of Coomassie Blau staining or preferably by silver coloration). However, an antibody is normally prepared by one or more purification steps.

The term "specific binding" or "binds specifically" refers to an antibody or binder which binds to a predetermined antigen/target molecule. Specific binding of an antibody or binder typically describes an antibody or binder having an affinity of at least 10^{-7} M, with the antibody or binder having an at least two times higher affinity for the predetermined antigen/target molecule than for a non-specific antigen/target molecule (e.g. bovine serum albumin, or casein) which is not the predetermined antigen/target molecule or a closely related antigen/target molecule.

Antibodies which are specific against a cancer cell antigen can be prepared by a person of ordinary skill in the art by means of methods with which he or she is familiar (such as recombinant expression, for example) or may be acquired commercially (as for example from Merck KGaA, Germany). Examples of known commercially available antibodies in cancer therapy are Erbitux® (cetuximab, Merck KGaA), Avastin® (bevacizumab, Roche) and Herceptin® (trastuzumab, Genentech). Trastuzumab is a recombinant humanized monoclonal antibody of the IgG1kappa type which in a cell-based assay (Kd = 5 nM) binds the extracellular domains of the human epidermal growth receptor with high affinity. The antibody is produced recombinantly in CHO cells.

A preferred subject of the invention are binder-drug conjugates of the general formula (Ia) in which

n is a number from 1 to 50,

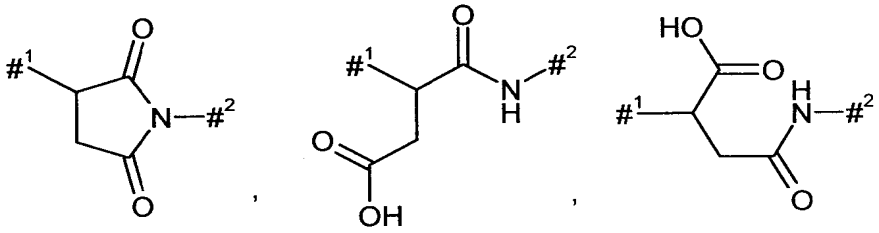
AK is AK_1 or AK_2

where

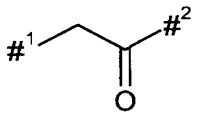
AK_1 is a binder which binds FGFR2 and is bonded via a sulphur atom of the binder to the group G,

AK_2 is a binder which binds FGFR2 and is bonded via a nitrogen atom of the binder to the group G,

G when AK = AK_1, is a group of the formula



or



where

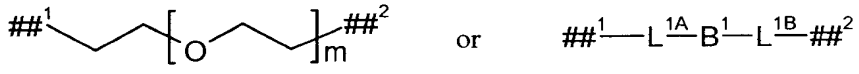
$\#^1$ marks the linkage site with the sulphur atom of the binder,

$\#^2$ marks the linkage site with the group L^1,

or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula



where

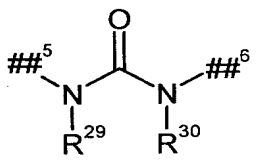
m is a number from 2 to 6,

[1] marks the linkage site with the group G,

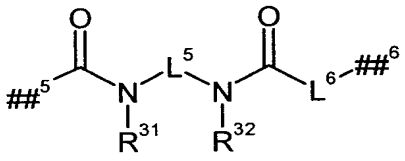
[2] marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

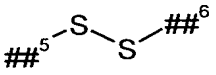
B^1 is a group of the formula



,



or



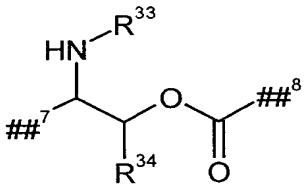
in which

[5] marks the linkage site with the group L^{1A},

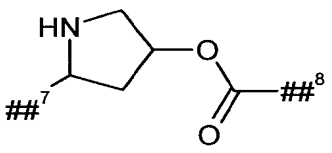
[6] marks the linkage site with the group L^{1B},

L^5 is a bond or (C_2-C_4)-alkanediyl,

L^6 is a bond or a group of the formula



or



in which

[7] marks the linkage site with the carbonyl group,

[8] marks the linkage site with L^{1B},

R^{33} is hydrogen, (C_1-C_4)-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

R^{34} is hydrogen or methyl,

R^{29} is hydrogen or (C_1-C_4)-alkyl,

R^{30} is hydrogen or (C_1-C_4)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or (C_1-C_4)-alkyl,

R^{32} is hydrogen or (C_1-C_4)-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear (C_2-C_{10})-alkanediyl,

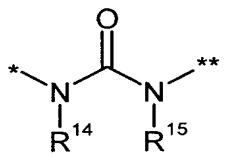
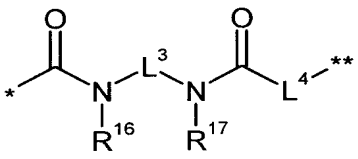
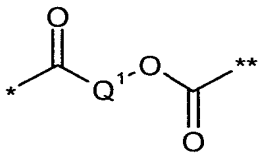
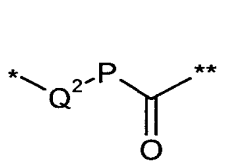
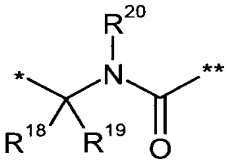
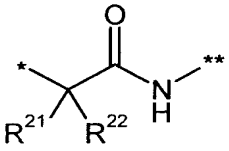
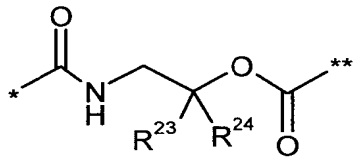
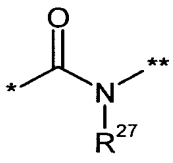
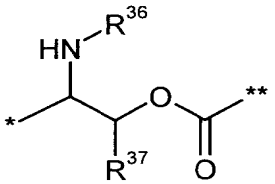
and

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula

where

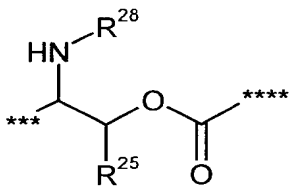
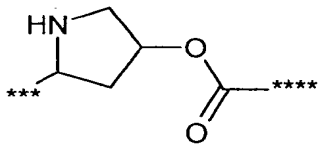
* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

L^3 is a bond or $(C_2\text{-}C_4)$-alkanediyl,

L^4 is a bond or a group of the formula


or


in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, (C_1-C_4)-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{14} is hydrogen or (C_1-C_4)-alkyl,

R^{15} is hydrogen or (C_1-C_4)-alkyl,

or

R^{14} and R^{15} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{16} is hydrogen or (C_1-C_4)-alkyl,

R^{17} is hydrogen or (C_1-C_4)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{18} is hydrogen or (C_1-C_4)-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or (C_1-C_4)-alkyl,

R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{23} is (C_1-C_4)-alkyl,

R^{24} is hydrogen or (C_1-C_4)-alkyl,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

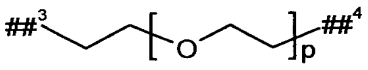
R^{36} is hydrogen, (C_1-C_4)-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

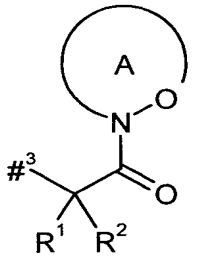
##4 marks the linkage site with the nitrogen atom,

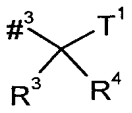
where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

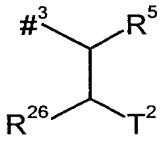
and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



,


or


in which

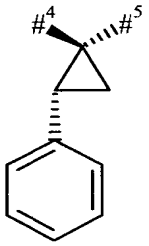
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

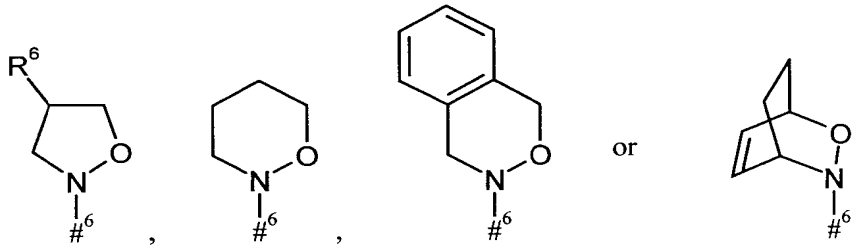


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

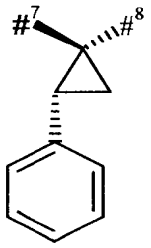
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

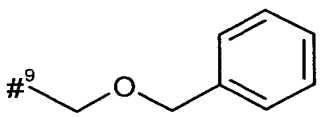
or

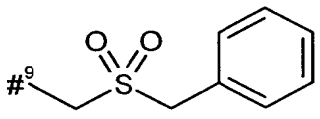
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

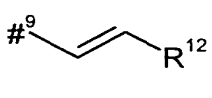
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

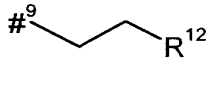
R^5 is hydrogen, methyl or a group of the formula

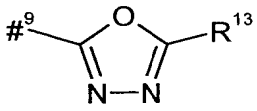


,


,



,


or


in which

#9 marks the linkage site with –CHC(R^{26})-T^2,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula –$S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Preferred subject of the invention are binder-drug conjugates of the general formula (Ia) in which

n is a number from 1 to 20,

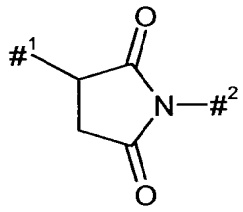
AK is AK_1 or AK_2

where

AK_1 is a binder which binds FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

AK_2 is a binder which binds FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G when AK = AK_1, is a group of the formula



in which

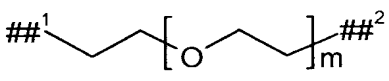
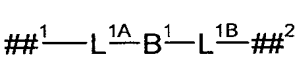
$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula

 or 

where

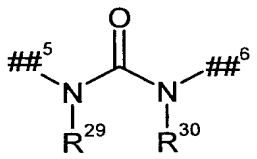
m is a number from 2 to 6,

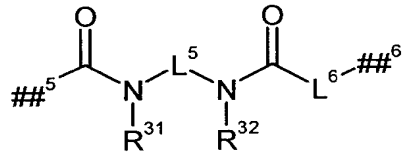
[1] marks the linkage site with the group G,

##[2] marks the linkage site with the group B,

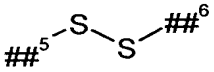
L^{1A} is linear (C_2-C_6)-alkanediyl,

B^1 is a group of the formula



,


or



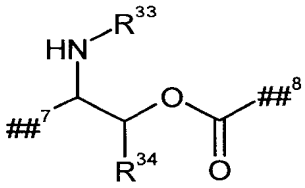
in which

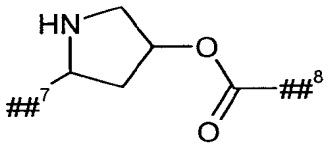
[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond,

L^6 is a bond or a group of the formula



or


in which

[7] marks the linkage site with the carbonyl group,

[8] marks the linkage site with L^{1B},

R^{33} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{34} is hydrogen or methyl,

R^{29} is hydrogen,

R^{30} is hydrogen,

R^{31} is hydrogen or methyl,

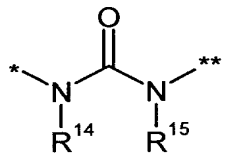
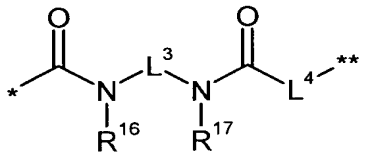
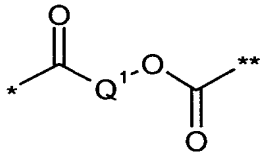
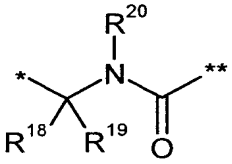
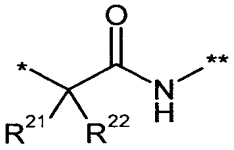
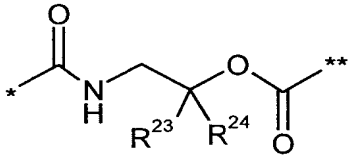
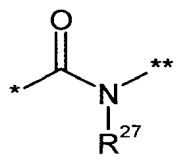
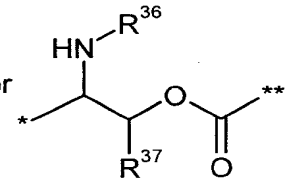
R^{32} is hydrogen or methyl,

L^{1B} is linear (C_2-C_6)-alkanediyl,

and

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula








or


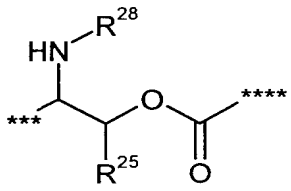
where

* marks the linkage site with L^1,

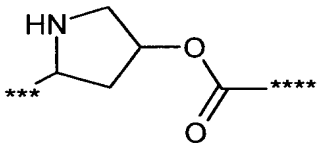
** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



or



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

Q^1 is a 4- to 7-membered heterocycle,

R^{14} is hydrogen,

R^{15} is hydrogen,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

R^{18} is hydrogen,

R^{19} is hydrogen, methyl, propan-2-yl , 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} is hydrogen or methyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or methyl,

R^{22} is hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{23} is methyl,

R^{24} is hydrogen or methyl,

R^{27} is hydrogen,

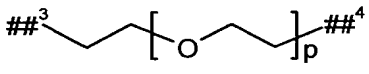
R^{36} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



where

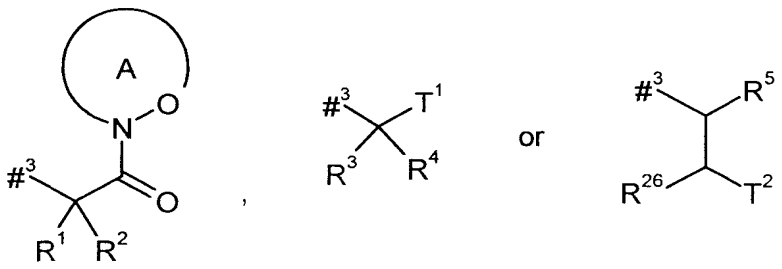
p is a number from 2 to 6,

3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 or 2 methyl substituents,

D is a group of the formula



where

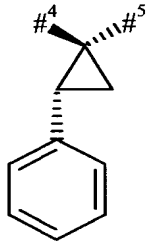
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-yl-methyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

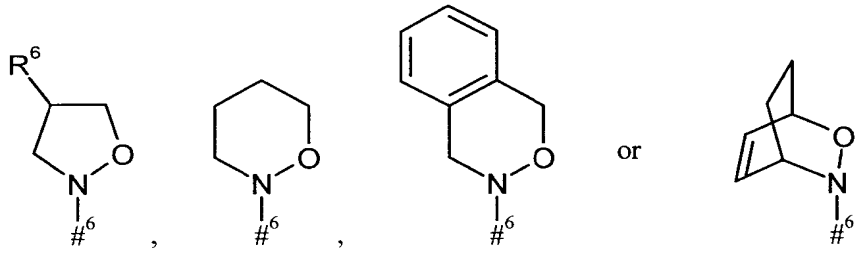


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

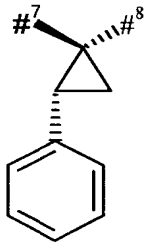
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-yl-methyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula -C(=O)-OR^7, -C(=O)-NR^8R^9, -C(=O)-NH-NH-R^{10} or -CH_2-O-R^{11},

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

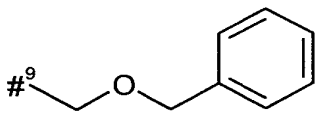
or

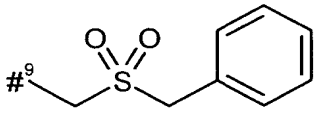
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

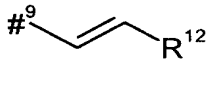
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

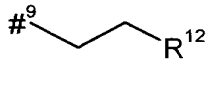
R^5 is hydrogen, methyl or a group of the formula

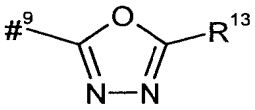


,


,



,


or


in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Preferred subject matter of the invention are binder-drug conjugates of the general formula (Ia), in which

n is a number from 1 to 10,

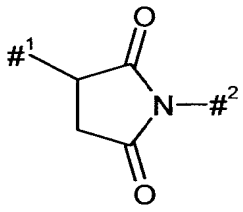
AK is AK_1 or AK_2

where

AK_1 is a binder which binds FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

AK_2 is a binder which binds FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G when AK = AK_1, is a group of the formula



in which

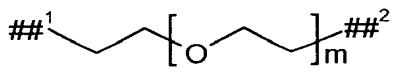
$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula



where

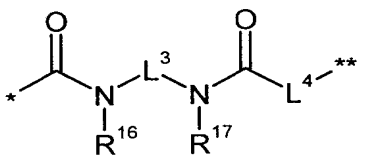
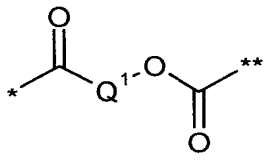
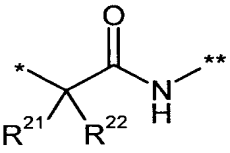
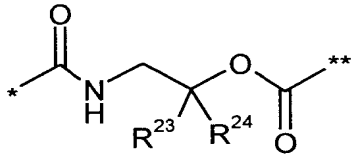
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

$\#\#^2$ marks the linkage site with the group B,

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula




or


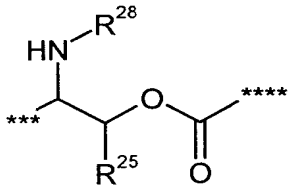
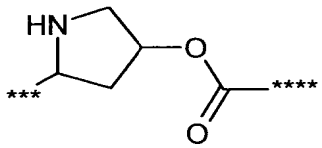
where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula


or


in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

Q^1 is piperidine-1,4-diyl,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

R^{21} is hydrogen or methyl,

R^{22} is hydrogen or methyl,

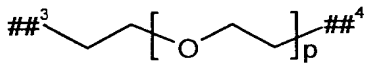
or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{23} is methyl,

R^{24} is hydrogen,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



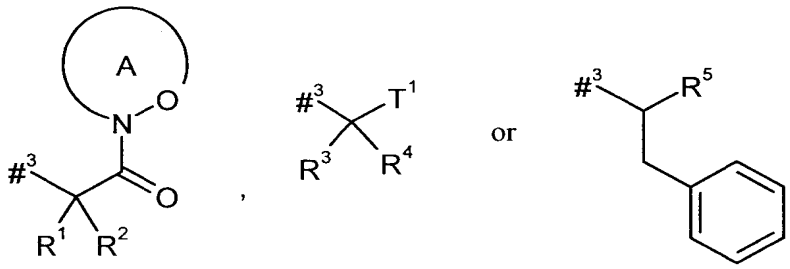
where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

D is a group of the formula



where

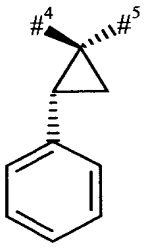
#3 marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

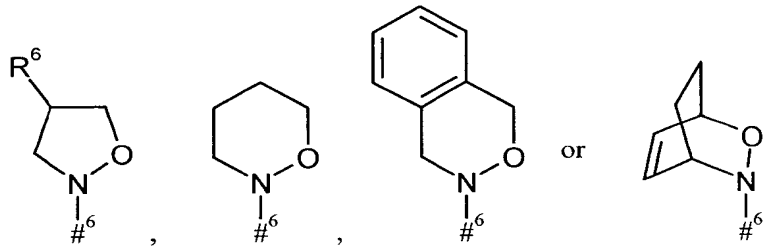


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

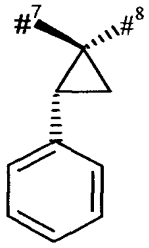
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$ or $-CH_2-O-R^{11}$,

in which

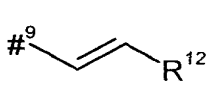
R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

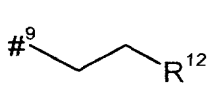
R^8 is hydrogen or methyl,

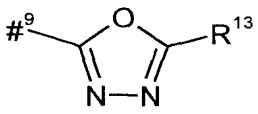
R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



,


or


in which

$\#^9$ marks the linkage site with $-CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Preferred subject matter of the present invention are binder-drug conjugates of the general formula (Ia), in which

n is a number from 1 to 10,

AK is AK_2,

where

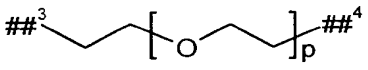
AK_2 is a binder which binds FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G is carbonyl,

L^1 is a bond,

B is a bond,

L^2 is linear $(C_3$-$C_6)$-alkanediyl or is a group of the formula



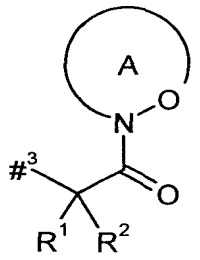
where

p is a number 2 or 3,

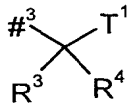
3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

D is a group of the formula



or



where

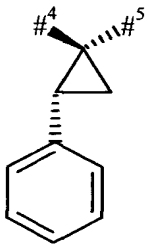
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

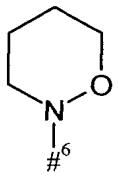


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

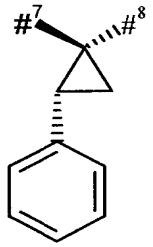
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen,

R^9 is hydrogen or benzyl,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

Preferred subject matter of the invention are binder-drug conjugates of the general formula (Ia), in which

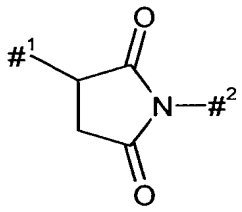
n is a number from 1 to 10,

AK is AK_1,

where

AK_1 is a binder which binds FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

G is a group of the formula

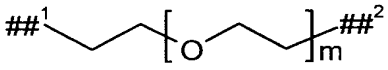


where

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

L^1 is a bond, linear $(C_3\text{-}C_5)$-alkanediyl or a group of the formula



where

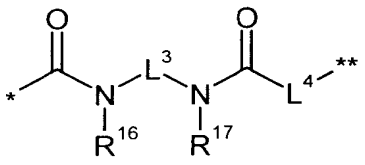
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

$\#\#^2$ marks the linkage site with the group B,

where $(C_3\text{-}C_5)$-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



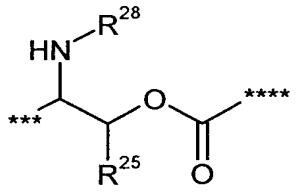
where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

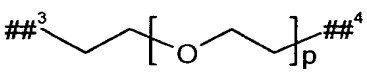
R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

L^2 is linear (C_3-C_5)-alkanediyl or is a group of the formula



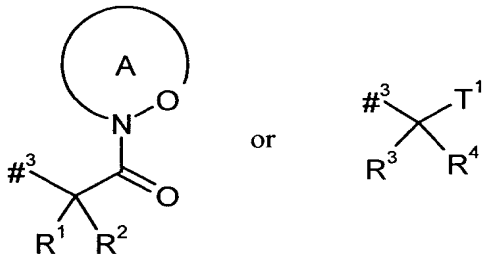
where

p is a number 2 or 3,

[3] marks the linkage site with the group B,

[4] marks the linkage site with the nitrogen atom,

D is a group of the formula



where

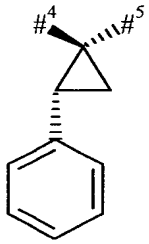
#3 marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

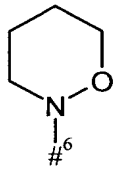


in which

#4 marks the linkage site with the adjacent nitrogen atom,

#5 marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

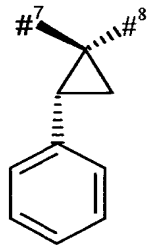
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen,

R^9 is hydrogen or benzyl,

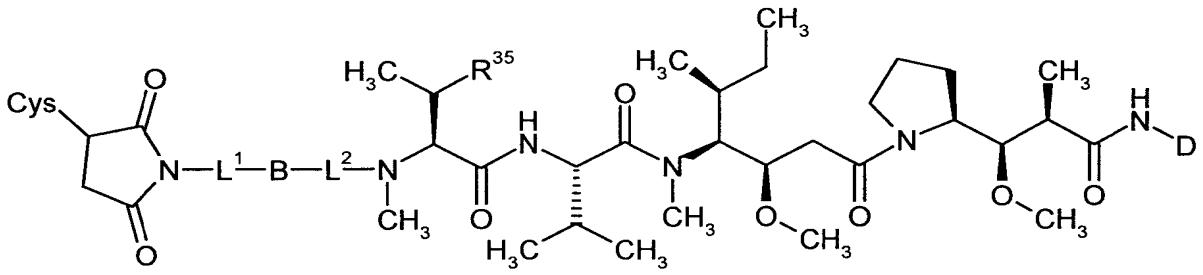
R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

Additionally preferably provided by the present invention is a binder-drug conjugate as described above, where the binder comprises the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1-b, reproduced in SEQ ID NO: 14 (Vl) and SEQ ID NO: 13 (Vh),

the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1-b reproduced in SEQ ID NO: 9 (light chain) and SEQ ID NO: 10 (heavy chain).

Additionally provided by the present invention are compounds of the formula (XXXa)

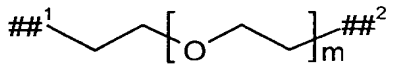


(XXXa),

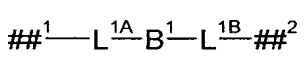
in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain to a carbon atom of the succinimide,

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula



or



where

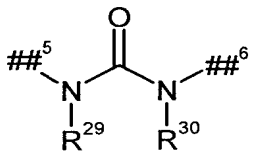
m is a number from 2 to 6,

$##^1$ marks the linkage site with the group G,

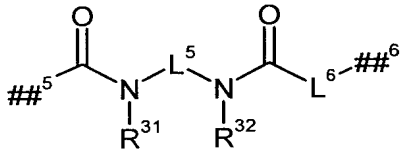
$##^2$ marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

B^1 is a group of the formula



or



in which

[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond or $(C_2$-$C_4)$-alkanediyl,

L^6 is a bond,

R^{29} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{30} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{32} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear $(C_2$-$C_{10})$-alkanediyl,

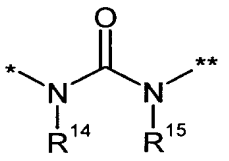
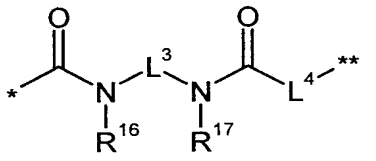
and

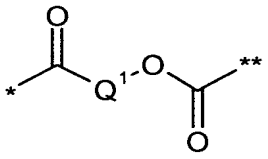
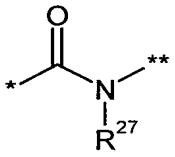
where $(C_1$-$C_{10})$-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula


,

,


or


where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

L^3 is a bond or (C_2-C_4)-alkanediyl,

L^4 is a bond,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{14} is hydrogen or (C_1-C_4)-alkyl,

R^{15} is hydrogen or (C_1-C_4)-alkyl,

or

R^{14} and R^{15} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{16} is hydrogen or (C_1-C_4)-alkyl,

R^{17} is hydrogen or (C_1-C_4)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{18} is hydrogen or (C_1-C_4)-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or (C_1-C_4)-alkyl,

R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

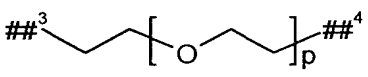
R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{23} is (C_1-C_4)-alkyl,

R^{24} is hydrogen or (C_1-C_4)-alkyl,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

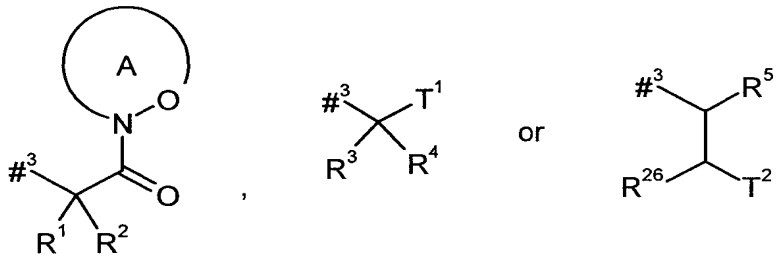
$\#\#^4$ marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



where

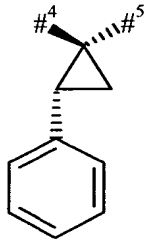
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

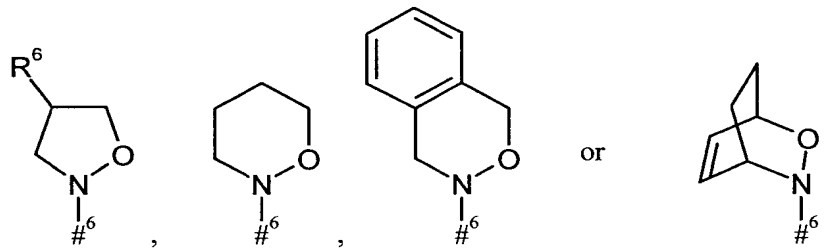


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

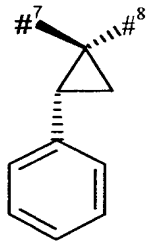
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

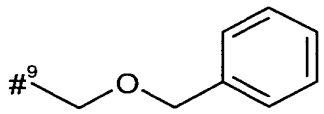
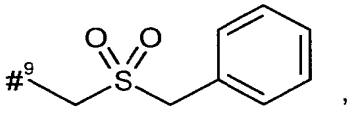
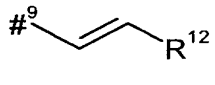
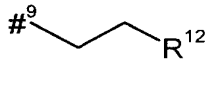
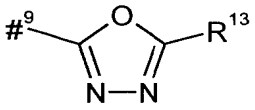
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula





or


in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

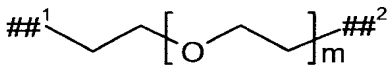
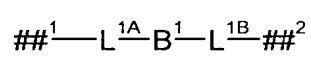
R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are additionally also compounds of the formula (XXXa) in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain via a carbon atom of the succinimide,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula


or


where

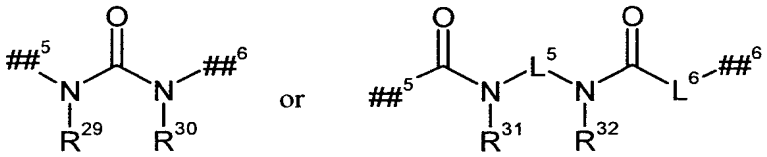
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

$##^2$ marks the linkage site with the group B,

L^{1A} is linear (C_2-C_6)-alkanediyl,

B^1 is a group of the formula



in which

$##^5$ marks the linkage site with the group L^{1A},

$##^6$ marks the linkage site with the group L^{1B},

L^5 is a bond,

L^6 is a bond,

R^{29} is hydrogen,

R^{30} is hydrogen,

R^{31} is hydrogen or methyl,

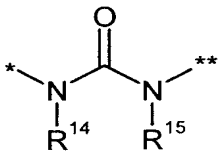
R^{32} is hydrogen or methyl,

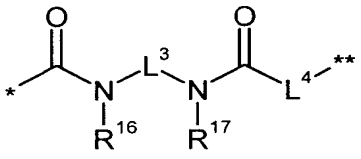
L^{1B} is linear (C_2-C_6)-alkanediyl,

and

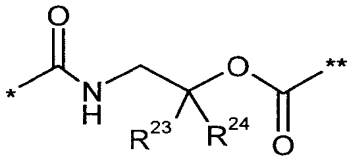
where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula





or



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond,

R^{14} is hydrogen,

R^{15} is hydrogen,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

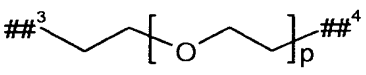
or

R^{16} and R^{17} together with the atoms to which they are bonded form piperazinyl ring,

R^{23} is methyl,

R^{24} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



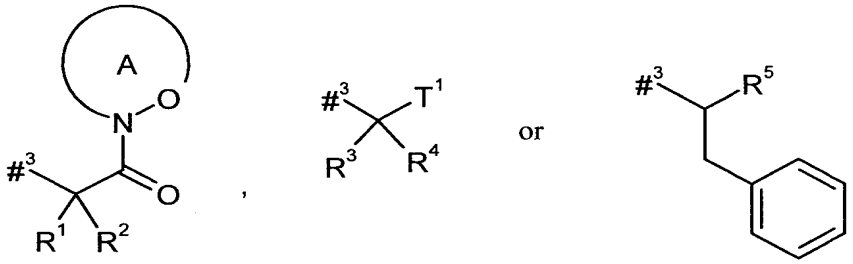
where

p is a number 2 or 3,

[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

D is a group of the formula



where

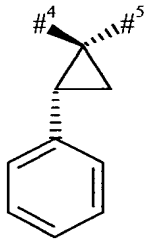
#[3] marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-yl-methyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

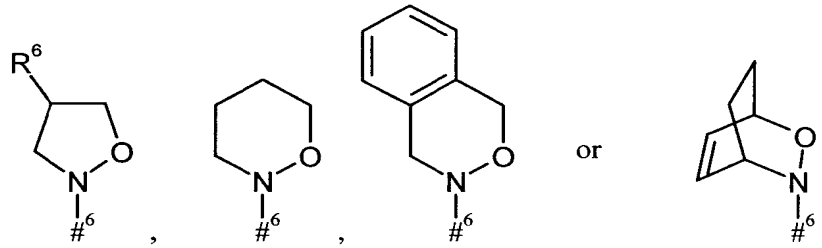


in which

#[4] marks the linkage site with the adjacent nitrogen atom,

#[5] marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

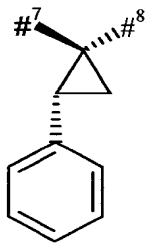
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-yl-methyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

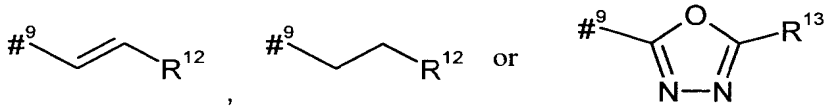
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with –$CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula –$S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

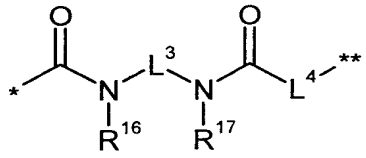
and also their salts, solvates and solvates of the salts.

Particularly preferred in the context of the present invention are additionally also compounds of the formula (XXXa) in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain via a carbon atom of the succinimide,

L^1 is a bond or linear (C_2-C_6)-alkanediyl,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

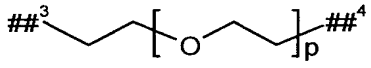
L^3 is a bond,

L^4 is a bond,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



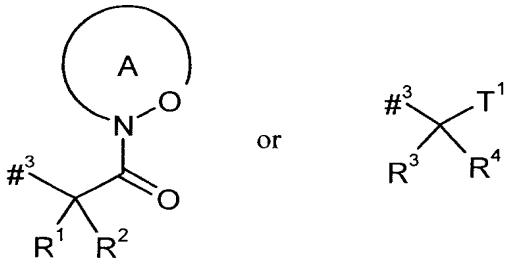
where

p is a number 2 or 3,

^{3} marks the linkage site with the group B,

$##^4$ marks the linkage site with the nitrogen atom,

D is a group of the formula



or

where

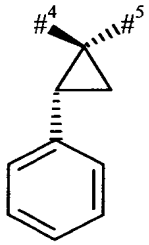
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

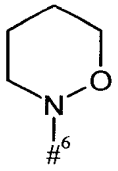


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

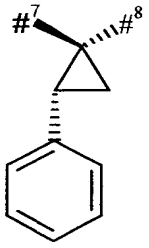
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

in which

R^7 is hydrogen,

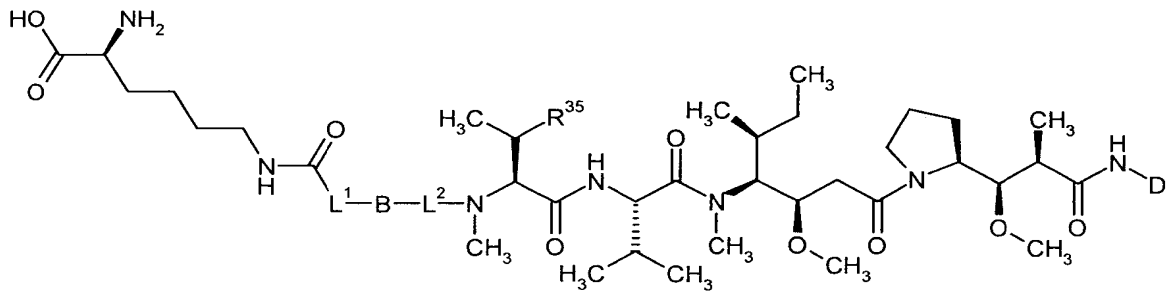
R^8 is hydrogen,

R^9 is hydrogen,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

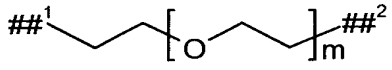
The present invention additionally provides compounds of the formula (XXXI)



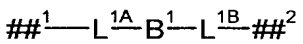
(XXXI),

in which

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula



or



where

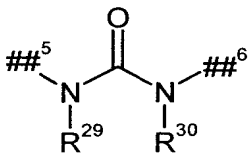
m is a number from 2 to 6,

[1] marks the linkage site with the group G,

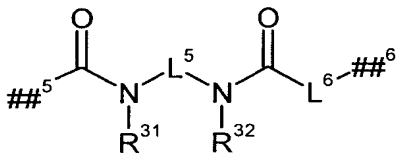
$##^2$ marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

B^1 is a group of the formula



or



in which

[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond or (C_2-C_4)-alkanediyl,

L^6 is a bond,

R^{29} is hydrogen or (C_1-C_4)-alkyl,

R^{30} is hydrogen or (C_1-C_4)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or (C_1-C_4)-alkyl,

R^{32} is hydrogen or (C_1-C_4)-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear (C_2-C_{10})-alkanediyl,

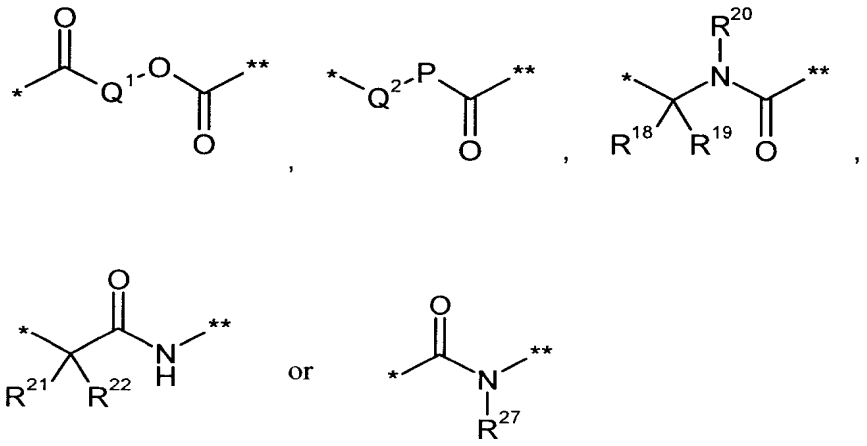
and

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{18} is hydrogen or (C_1-C_4)-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or (C_1-C_4)-alkyl,

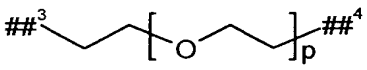
R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

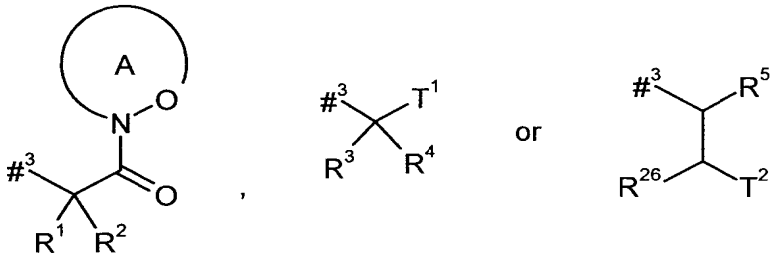
##4 marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



in which

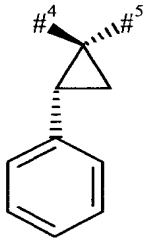
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

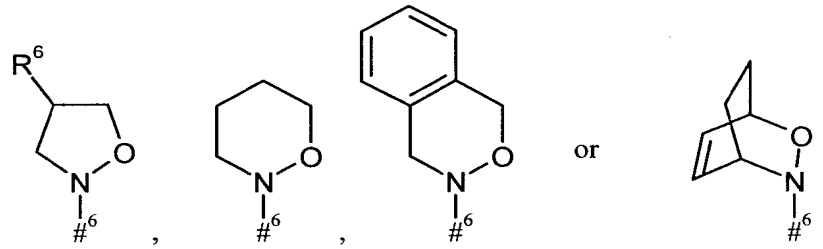


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

#6 marks the linkage site with the carbonyl group,

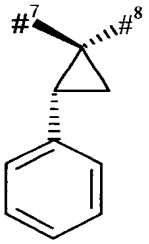
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

#7 marks the linkage site with the adjacent nitrogen atom,

#8 marks the linkage site with the group T^1,

T^1 is a group of the formula -C(=O)-OR^7, -C(=O)-NR^8R^9, -C(=O)-NH-NH-R^{10} or -CH_2-O-R^{11},

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

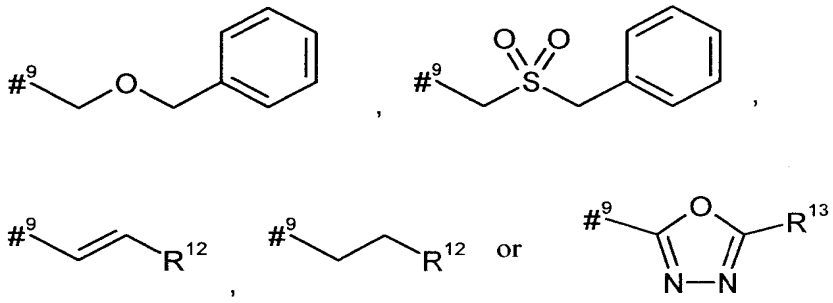
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

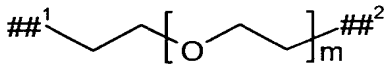
T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (XXXI) in which

L^1 is a bond, linear (C_2-C_6)-alkanediyl or a group of the formula



where

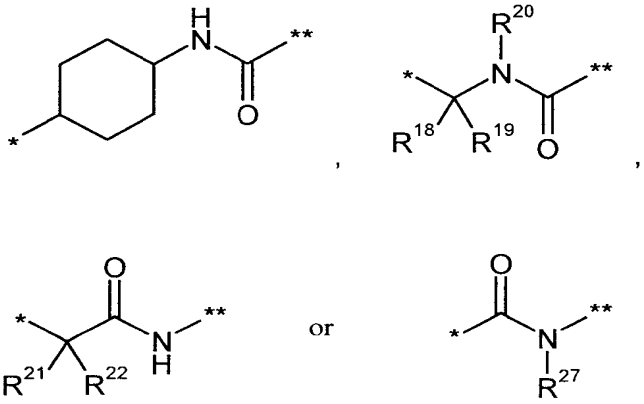
m is a number 2 or 3,

[1] marks the linkage site with the group G,

##[2] marks the linkage site with the group B,

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

R^{18} is hydrogen,

R^{19} is methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or methyl,

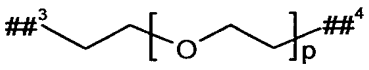
R^{22} is hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{27} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

3 marks the linkage site with the group B,

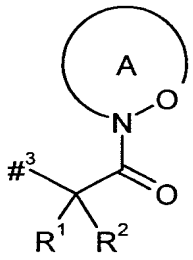
##4 marks the linkage site with the nitrogen atom,

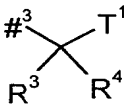
where (C_2-C_{10})-alkanediyl may be substituted by 1 or 2 methyl substituents,

and

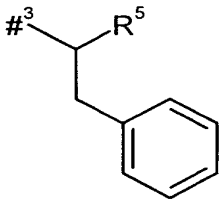
where two carbon atoms of the alkanediyl chain in 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a phenyl ring,

D is a group of the formula





or



in which

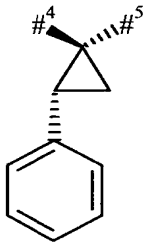
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

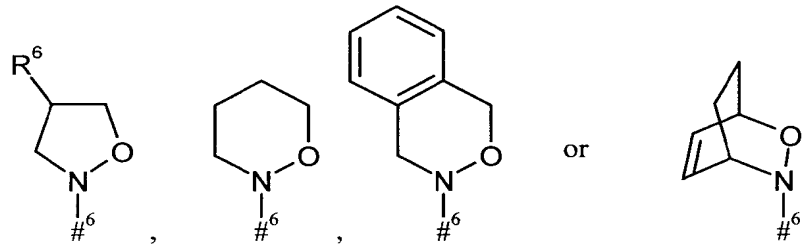


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

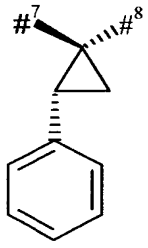
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-yl-methyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropan-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

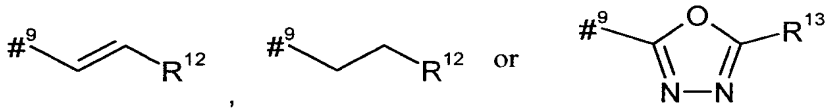
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with $-CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

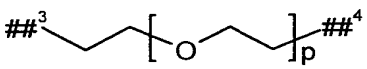
and also their salts, solvates and solvates of the salts.

Particularly preferred in the context of the present invention are also compounds of the formula (XXXI) in which

L^1 is a bond,

B is a bond,

L^2 is linear $(C_2\text{-}C_6)$-alkanediyl or is a group of the formula



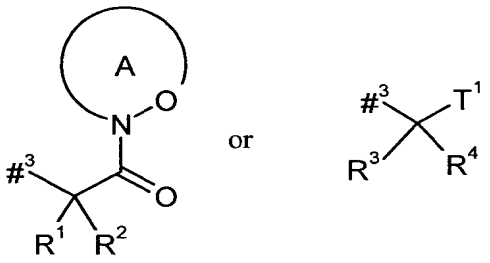
where

p is a number 2 or 3,

[3] marks the linkage site with the group B,

[4] marks the linkage site with the nitrogen atom,

D is a group of the formula



where

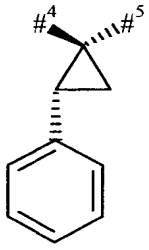
#[3] marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

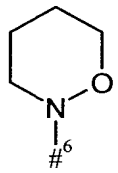


in which

#[4] marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

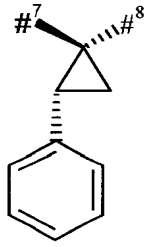
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula -C(=O)-OR^7 or -C(=O)-NR^8R^9,

in which

R^7 is hydrogen,

R^8 is hydrogen,

R^9 is hydrogen,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

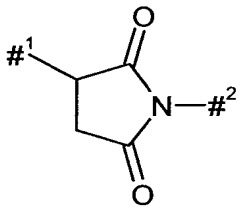
Preferred in the context of the present invention are also compounds of the formula (Ia), in which

AK is AK_1

where

AK_1 is an antibody or an antigen-binding antibody fragment which binds to FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

G is a group of the formula



where

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

and

n, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), in which

AK is AK_2

where

AK_2 is an antibody or an antigen-binding antibody fragment which binds to FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G is carbonyl,

and

n, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

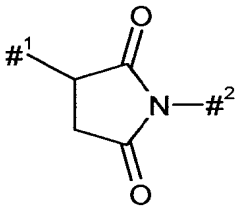
Preference in the context of the present invention is also given to compounds of the formula (Ia), in which

AK is AK_1

where

AK_1 is a binder which binds FGFR2, and which is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

G is a group of the formula



where

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

and

n, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preference in the context of the present invention is also given to compounds of the formula (Ia), in which

AK is AK_2

where

AK_2 is an a binder which binds FGFR2 and which is bonded via the NH side group of a lysine residue of the binder to the group G,

G is carbonyl,

and

n, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preference in the context of the present invention is also given to compounds of the general formula (Ia), in which

AK is AK_2

where

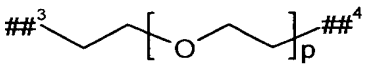
AK_2 is a binder which binds FGFR2 and which is bonded via the NH side group of a lysine residue of the binder to the group G,

G is carbonyl,

L^1 is a bond,

B is a bond,

L^2 is linear (C_3-C_6)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

##3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

n, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

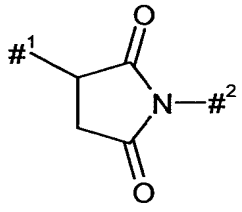
Preference in the context of the present invention is also given to compounds of the general formula (Ia), in which

AK is AK_1

where

AK_1 is a binder which binds FGFR2 and which is attached via the sulphur atom of a cysteine residue of the binder to the group G,

G is a group of the formula

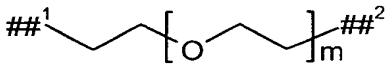


where

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

L^1 is a bond, linear (C_3-C_5)-alkanediyl or a group of the formula



where

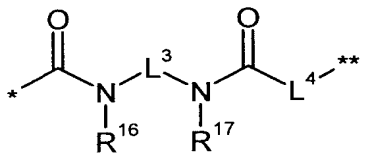
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

$\#\#^2$ marks the linkage site with the group B,

where (C_3-C_5)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



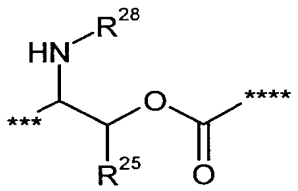
where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

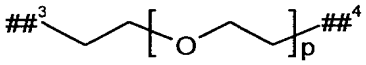
R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

L^2 is linear (C_3-C_5)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

##[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

and

n, D and R^{35} have the definitions indicated above,

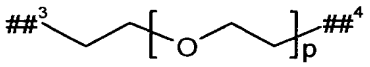
and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

L^1 is a bond,

B is a bond,

L^2 is linear (C_3-C_6)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

##[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

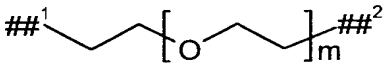
and

n, AK, Cys, G, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), in which

L^1 is linear (C_1-C_{10})-alkanediyl or a group of the formula



where

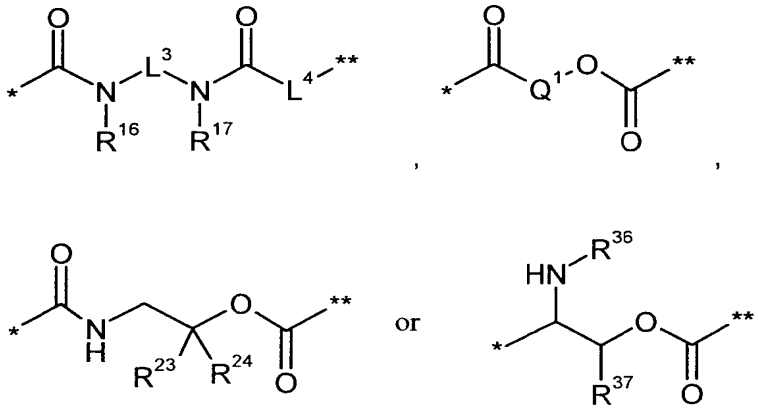
m is a number from 2 to 6,

$##^1$ marks the linkage site with the group G,

$##^2$ marks the linkage site with the group B,

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxyl and benzyl,

B is a bond or a group of the formula



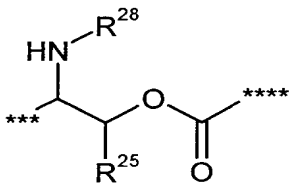
where

* marks the linkage site with L^1,

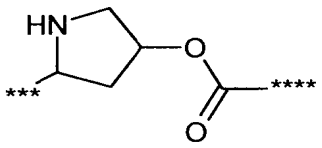
** marks the linkage site with L^2,

L^3 is a bond or (C_2-C_4)-alkanediyl,

L^4 is a group of the formula



or



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

Q^1 is a 4- to 7-membered heterocycle,

R^{16} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{17} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{23} is $(C_1$-$C_4)$-alkyl,

R^{24} is hydrogen or $(C_1$-$C_4)$-alkyl,

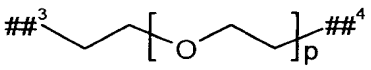
R^{36} is hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear $(C_2$-$C_{10})$-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

##[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxyl and benzyl,

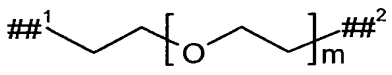
and

n, AK, G, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), in which

L^1 is linear (C_2-C_6)-alkanediyl or a group of the formula



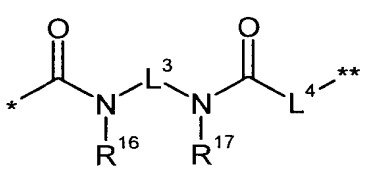
where

m is a number 2 or 3,

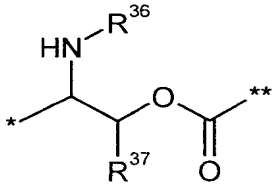
##[1] marks the linkage site with the group G,

##[2] marks the linkage site with the group B,

B is a bond or a group of the formula



or



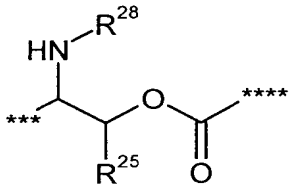
where

* marks the linkage site with L^1,

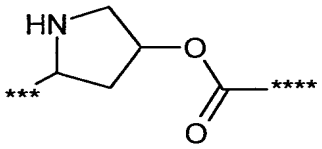
** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a group of the formula



or



where

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

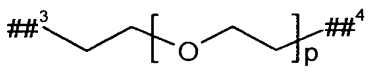
R^{36} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

$\#\#^3$ marks the linkage site with the group B,

$\#\#^4$ marks the linkage site with the nitrogen atom,

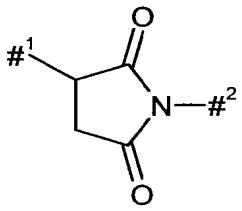
and

n, AK, G, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia) and (XXXa), in which

G is a group of the formula

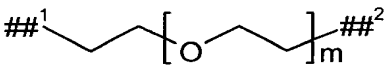


in which

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

L^1 is linear (C_3-C_5)-alkanediyl or a group of the formula



in which

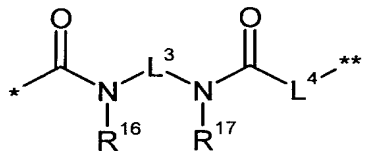
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

$\#\#^2$ marks the linkage site with the group B,

where (C_3-C_5)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



where

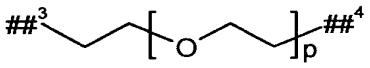
* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond,

L^2 is linear (C_3-C_5)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

$\#\#^3$ marks the linkage site with the group B,

$\#\#^4$ marks the linkage site with the nitrogen atom,

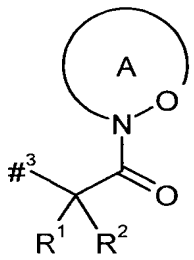
and

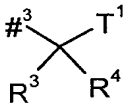
n, AK_1, Cys, D, R^{16} and R^{17} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

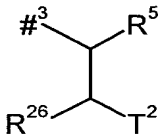
Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

D is a group of the formula





or



where

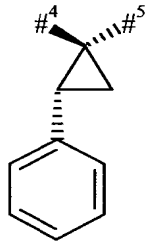
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

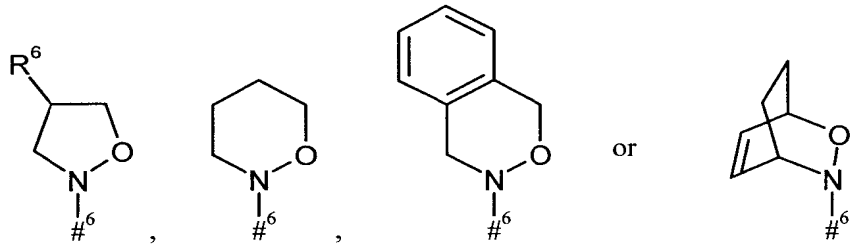


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

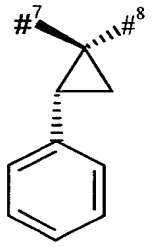
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

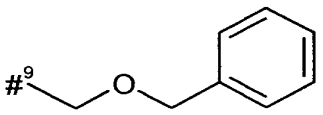
or

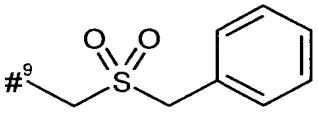
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

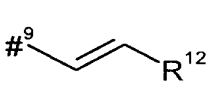
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl

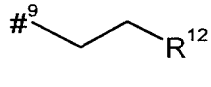
R^5 is hydrogen, methyl or a group of the formula

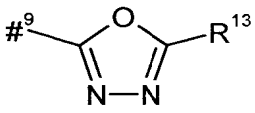


,


,



,


or


in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

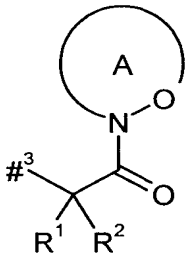
and

n, AK, Cys, G, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

D is a group of the formula



where

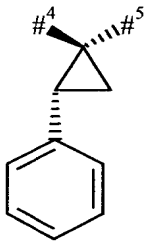
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

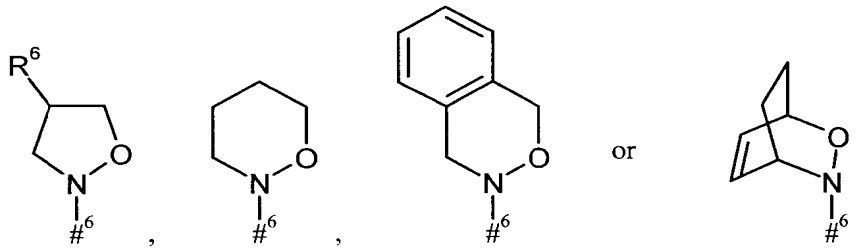


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

R^6 is hydrogen, hydroxy or benzyloxy,

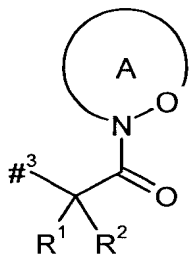
and

n, AK, Cys, G, L^1, B, L^2 and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

D is a group of the formula



where

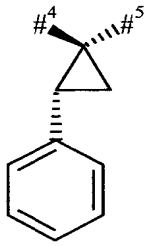
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

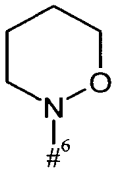


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

and

n, AK, Cys, G, L^1, B, L^2 and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

R^{35} is hydroxyl,

and

n, AK, Cys, G, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

Preferred in the context of the present invention are also compounds of the formula (Ia), (XXXa) and (XXXI), in which

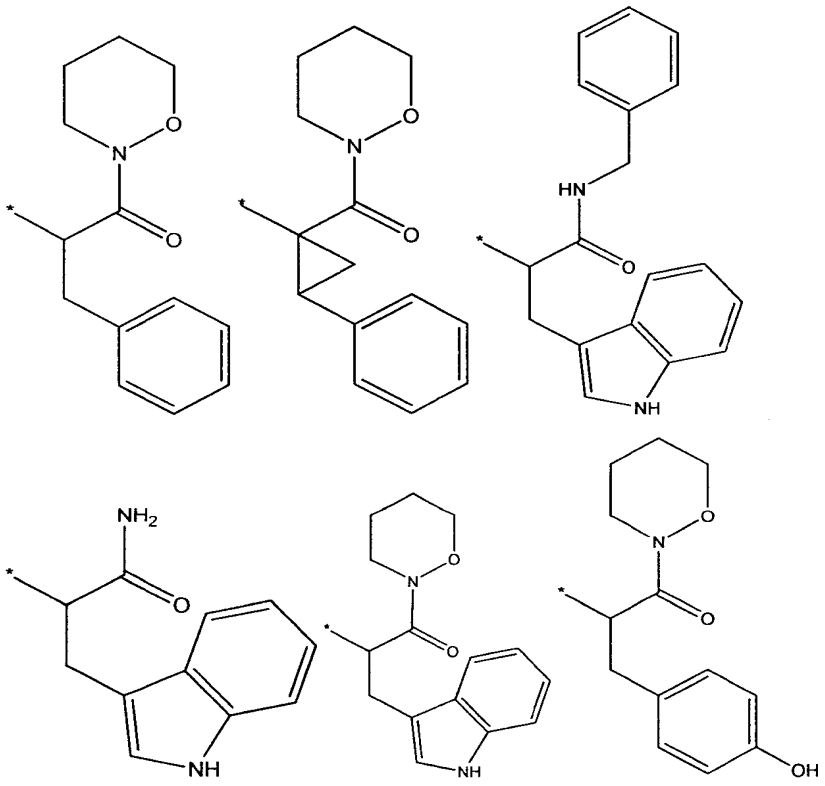
R^{35} is methyl,

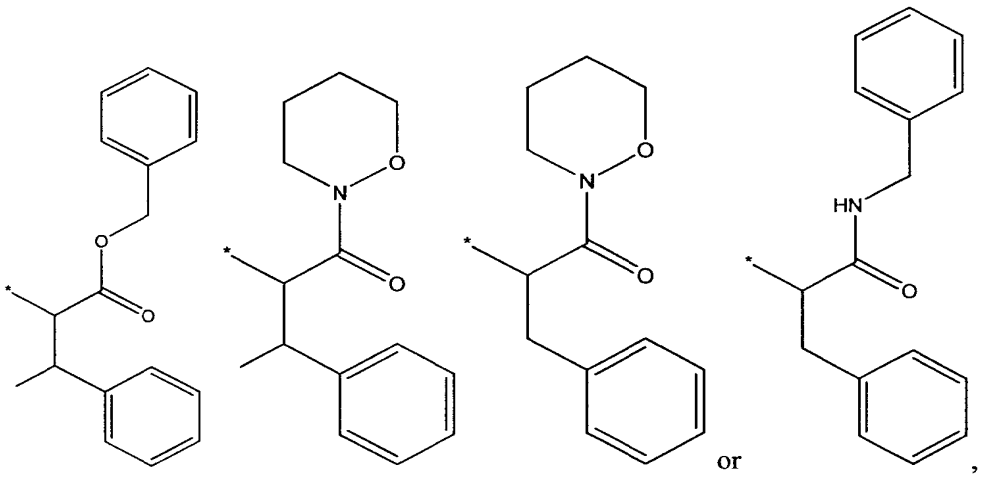
and

n, AK, Cys, G, L^1, B, L^2, D and R^{35} have the definitions indicated above,

and also their salts, solvates and solvates of the salts.

A preferred subject of the present invention are binder-drug conjugates of the general formula (Ia) in which D can have the following structures and * stands for the linkage site with the nitrogen atom:





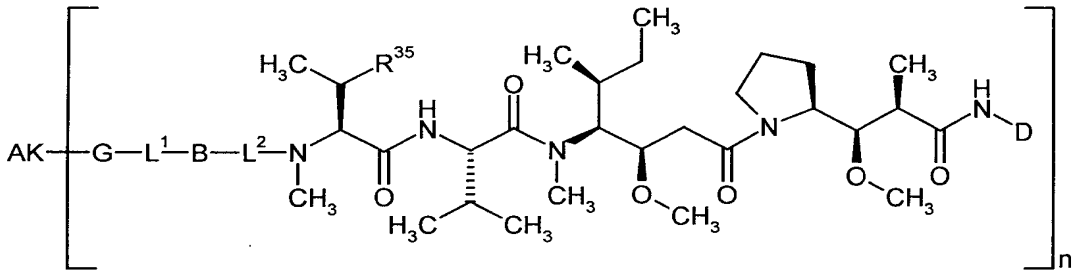
and also their salts, solvates and solvates of the salts.

A preferred subject of the present invention are binder-drug conjugates of the general formula (Ia) in which D has a structure which is disclosed by one of the intermediates of the present invention; and the linker unit §-G-L^1-B-L^2-§§ and also all other variables are defined in accordance with the present invention; and their salts, solvates and solvates of the salts. AK is preferably an anti-FGFR2 antibody or antigen-binding fragment thereof.

A preferred subject of the present invention are binder-drug conjugates of the general formula (Ia) in which the linker-drug unit has a structure which is disclosed by one of the intermediates or examples of the present invention; and their salts, solvates and solvates of the salts. AK is preferably an anti-FGFR2 antibody or antigen-binding fragment thereof.

A preferred subject of the present invention are binder-drug conjugates of the general formula (Ia) in which the linker-drug unit has a structure which is disclosed by one of the examples of the present invention; and their salts, solvates and solvates of the salts. AK is preferably an anti-FGFR2 antibody or antigen-binding fragment thereof.

Particularly preferred subject of the present invention are binder-drug conjugates of the general formula (Ia)



(Ia),

in which

n is a number from 1 to 50,

AK is a binder which binds to FGFR2,

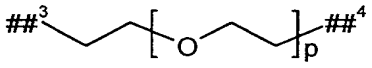
the group §-G-L^1-B-§§ is a linker,

where

§ marks the linkage site with the group AK and

§§ marks the linkage site with the nitrogen atom,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

^{3} marks the linkage site with the group B,

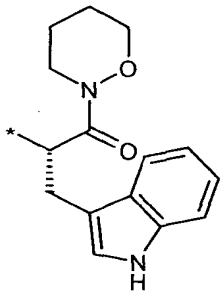
^{4} marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 methyl substituents selected independently of one another from the group consisting of methyl, hydroxyl and benzyl

and

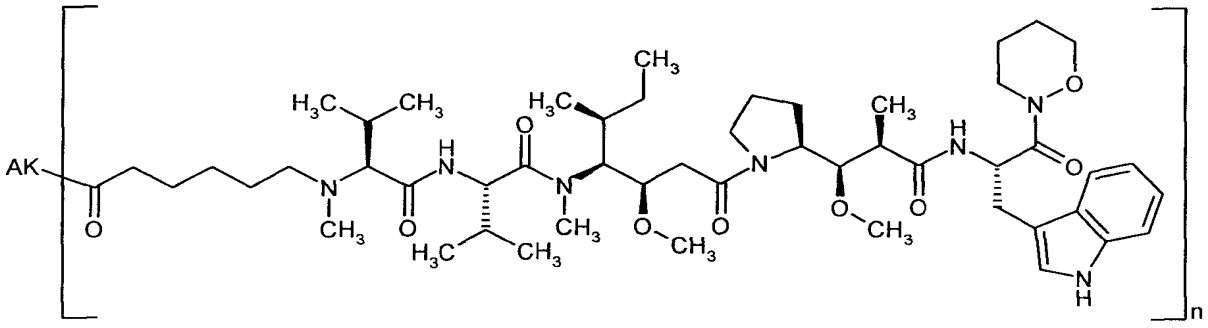
where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the following formula, where * is the linkage site to the nitrogen atom



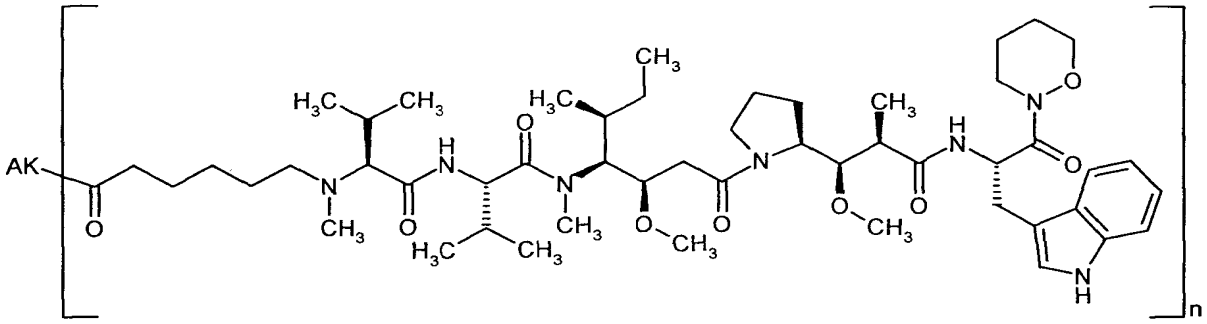
and also their salts, solvates and solvates of the salts.

Particularly preferred subject of the present invention are compounds of the following formula



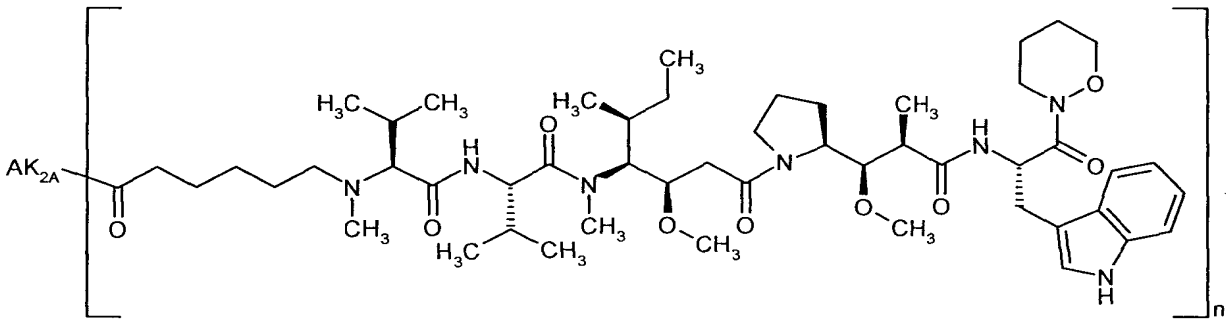
where AK is a binder which binds FGFR2, and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts. It is preferred if the binder is bonded via a NH side group of a lysine residue to the linker-toxophore unit.

Particularly preferred subject of the present invention are compounds of the following formula



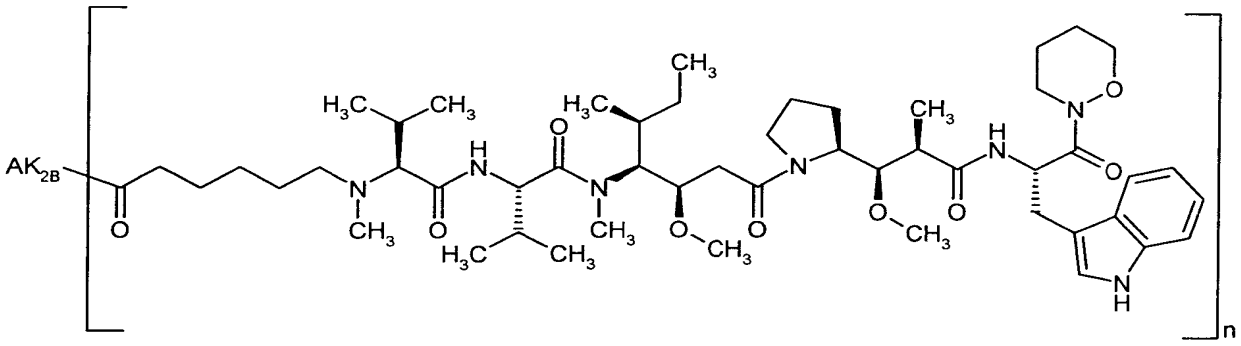
where AK is an antibody or an antibody fragment which binds FGFR2, and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts. It is preferred if the antibody or antibody fragment is bonded via an NH side group of a lysine residue of the antibody or antibody fragment to the linker-toxophore unit.

Particularly preferred subject of the present invention is the compound of the following formula



where AK2A is M048-D01-hIgG1 and n is a number from 1 to 10, and also the salts, solvates and solvates of the salts thereof.

A further particularly preferred subject of the present invention is the compound of the following formula



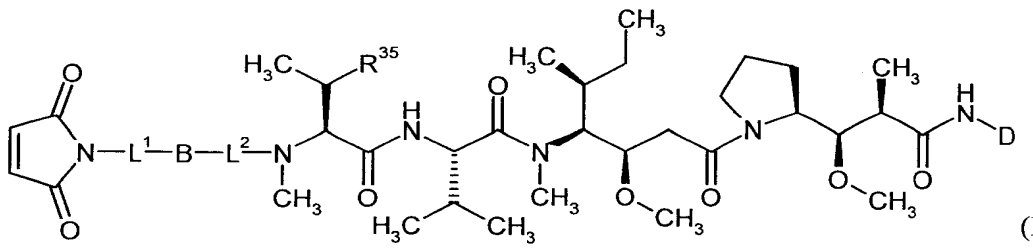
where AK2B is M048-D01-hIgG1-b and n is a number from 1 to 10, and also the salts, solvates and solvates of the salts thereof.

The definitions of radicals that are indicated individually in the respective combinations and preferred combinations of radicals are also replaced arbitrarily by radical definitions of other combinations, independently of the respective combinations of radicals that are indicated.

Especially preferred are combinations of two or more of the abovementioned preference ranges.

Further provided by the invention is a process for preparing the compounds of the invention of the formula (Ia), characterized in that a solution of the binder in PBS buffer

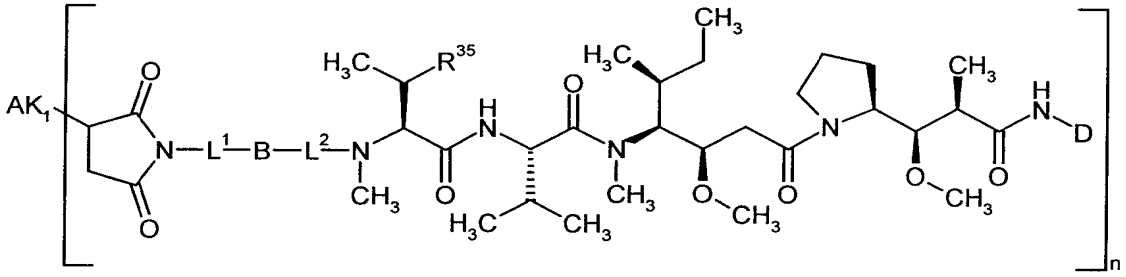
[A] is admixed with a suitable reducing agent, such as, for example, dithiothreitol or tris(2-carboxyethyl)phosphine hydrochloride, and is subsequently reacted with a compound of the formula (IIa)



(IIa),

in which D, L^1, B, L^2 and R^{35} each have the definitions indicated above,

to give a compound of the formula (I-A)

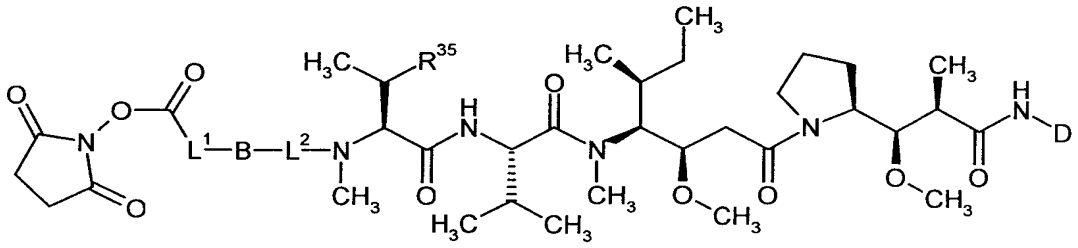


(Ia-A),

in which n, AK_1, D, L^1, B, L^2 and R^{35} each have the definitions indicated above,

or

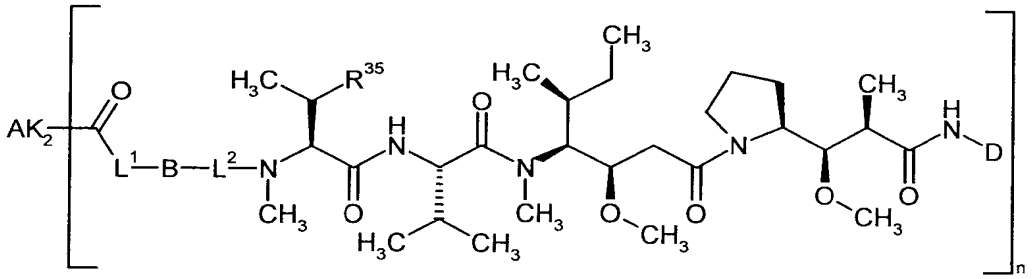
[B] is reacted with a compound of formula (IIIa)



(IIIa),

in which D, L^1, B, L^2 and R^{35} each have the definitions indicated above,

to give a compound of the formula (Ia-B)



(Ia-B),

in which n, AK_2, D, L^1, B, L^2 and R^{35} each have the definitions indicated above.

Cysteine coupling:

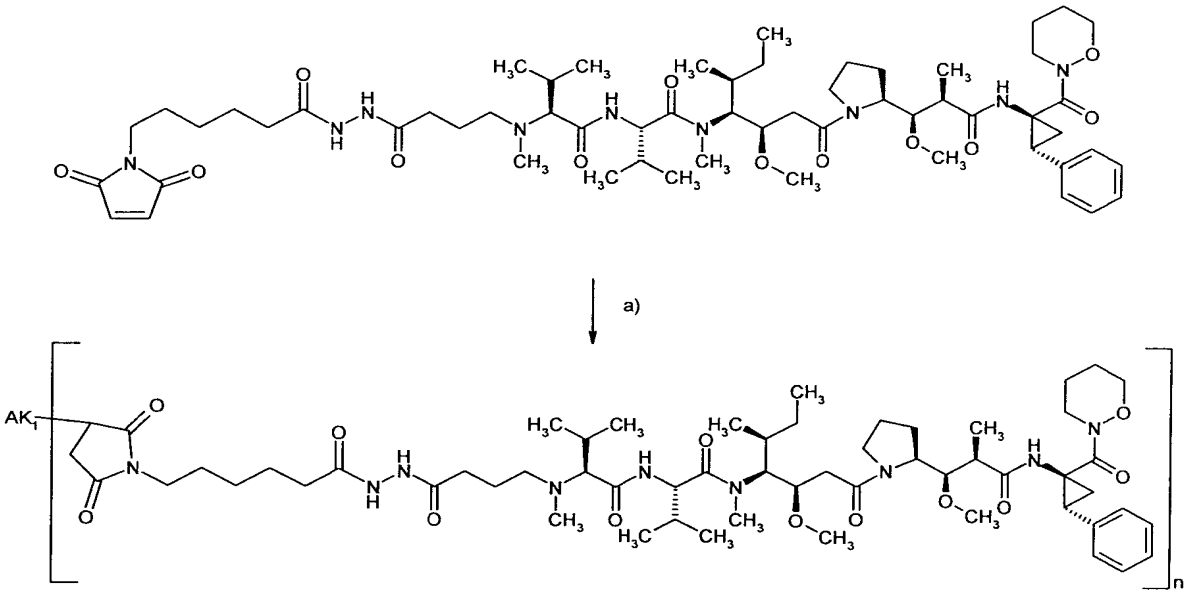
The partial reduction of the antibody and also the subsequent conjugation of the (partially) reduced antibody with a compound of the formula (IIa) takes place in accordance with the methods known to the skilled person, see e.g. Ducry et. al., Bioconj. Chem. 2010, 21, 5 and references herein, Klussman et. al., Bioconj. Chem. 2004, 15(4), 765-773. The mild reduction of the antibody is accomplished preferably by addition of 2-6 equivalents of TCEP to the antibody, which is present in a suitable buffer solution, preferably phosphate buffer, and by stirring for 30-180 minutes at temperatures between 15 and 40°C, preferably at RT. This is followed by the conjugation, by addition of a solution of a compound of the formula (IIa) in DMSO, acetonitrile or DMF to the solution of the (partially) reduced antibody in PBS buffer, and subsequent reaction at a temperature of 0°C to +40°C, more particularly of +10°C to +30°C, for a period of 30 minutes to 6 hours, more particularly 1 to 2 hours.

Lysine coupling:

First of all the compounds of the formula (IIIa) or comparable activated carboxyl components are prepared by conventional methods of peptide chemistry. They are then taken up in inert solvents such as DMSO or DMF, for example, and added to the antibody, which is preferably present in phosphate buffer at a neutral pH. The solution is stirred for 1-16 h at a temperature between 15 and 40°C, preferably RT.

The preparation processes described above are elucidated by way of example using the schemes below (Scheme 1 and 2):

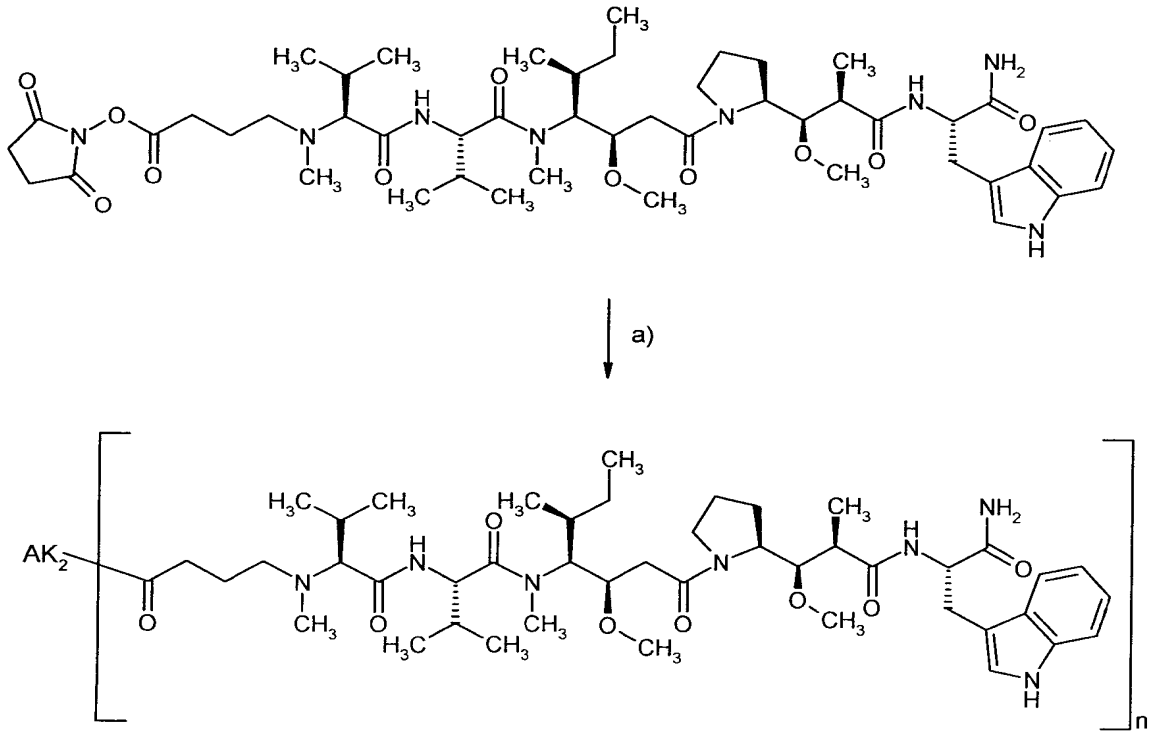
Scheme 1



[a): 1. AK (antibody), TCEP, PBS buffer, RT; 2. Addition of the maleimide derivative in DMSO, RT].

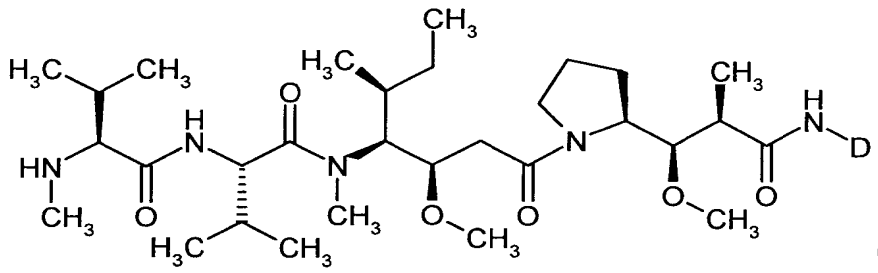
72

Scheme 2



[a): AK (antibody), PBS buffer, RT admix with activated carboxyl derivative of the linker-drug components].

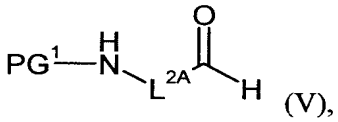
The compounds of the formula (II) in which L^1 and B are a bond can be prepared by subjecting a compound of the formula (IV)



(IV),

in which D has the definition indicated above,

to reductive amination in an inert solvent with a compound of the formula (V)



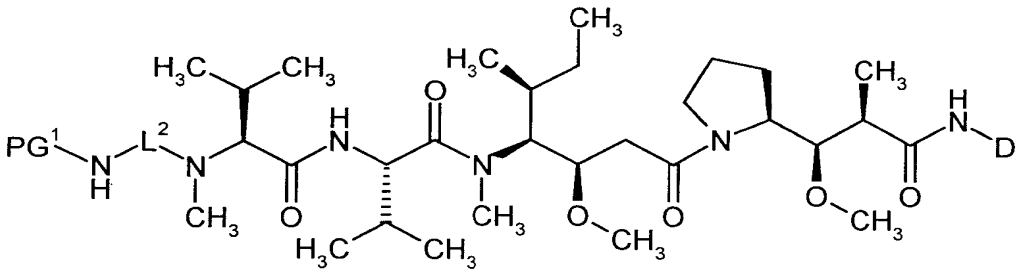
(V),

in which

L^{2A} has the above-defined definition of L^2, but is shortened by one carbon atom in the alkyl chain length,

PG^1 is an amino-protective group such as, for example, (9*H*-fluoren-9-ylmethoxy)carbonyl, *tert*-butoxycarbonyl or benzyloxycarbonyl,

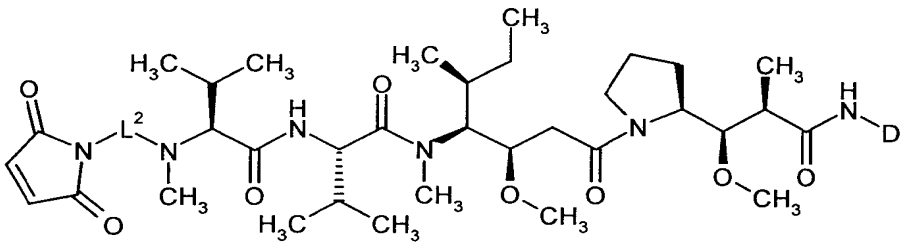
to give a compound of the formula (VI)



(VI),

in which D, L^2 and PG^1 have the definition indicated above,

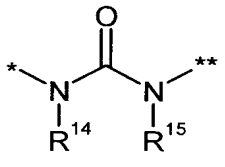
eliminating the protective group PG^1 from this compound by methods known to the skilled person, and reacting the deprotected compound in an inert solvent in the presence of a suitable base with methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate to give a compound of the formula (II-A)



(II-A),

in which D and L^2 each have the definitions indicated above.

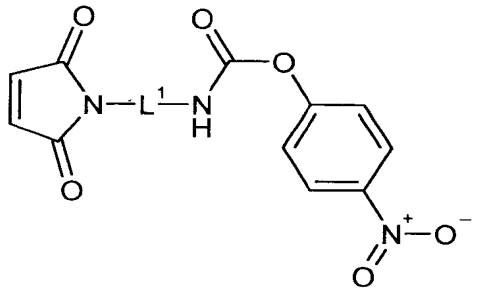
The compounds of the formula (II) in which B is a group of the formula (B^1)



(B^1),

in which *, **, R^{14} and R^{15} each have the conditions indicated above,

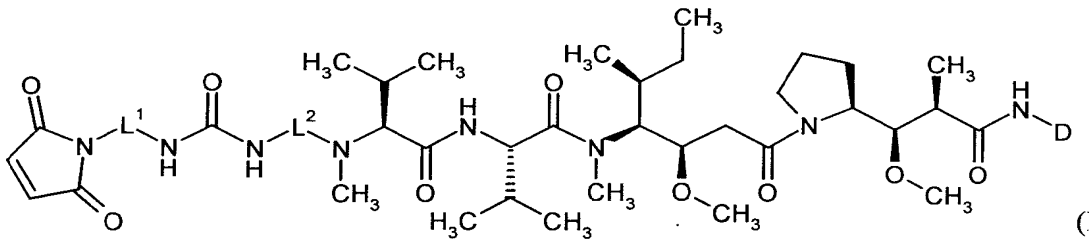
can be prepared by eliminating the protective group PG^1 from a compound of the formula (VI) by methods known to the skilled person, and reacting the deprotected compound in an inert solvent in the presence of a suitable base with a compound of the formula (VII)



(VII),

in which L^1 has the definition indicated above,

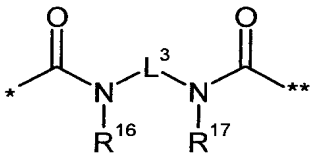
to give a compound of the formula (II-B)



(II-B),

in which D, L^1 and L^2 each have the definitions indicated above.

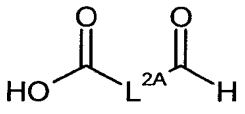
The compounds of the formula (II) in which B is a group of the formula (B^2)



(B^2),

in which *, **, L^3, R^{16} and R^{17} each have the conditions indicated above can be prepared by subjecting a compound of the formula (IV)

to reductive amination in an inert solvent with a compound of the formula (VIII)

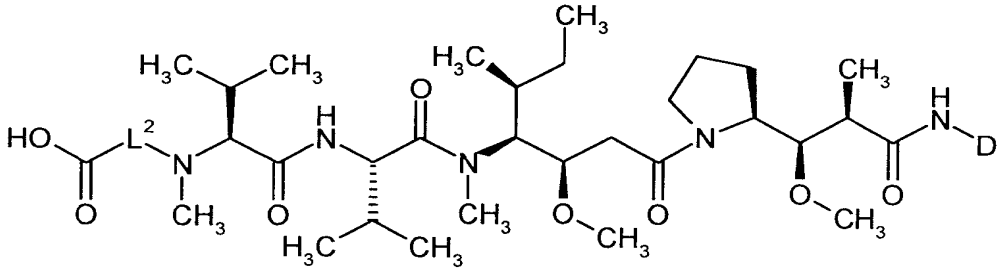


(VIII),

in which

L^{2A} has the above-defined definition of L^2, but is shortened by one carbon atom in the alkyl chain length,

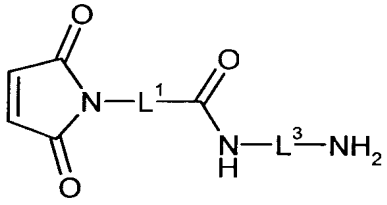
to give a compound of the formula (IX)



(IX),

in which D and L^2 have the definitions indicated above,

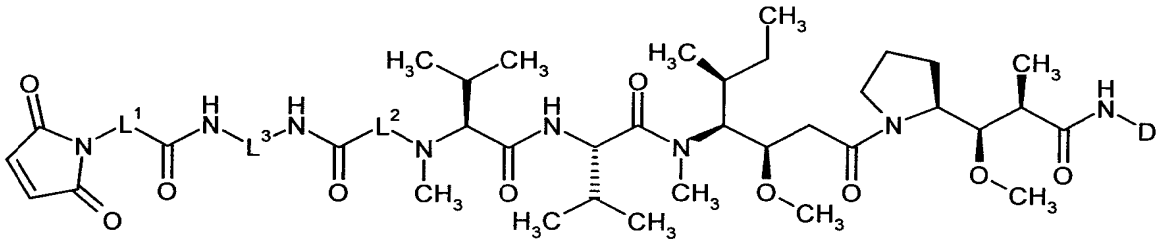
and reacting this compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (X)



(X),

in which L^1 and L^3 each have the definitions indicated above,

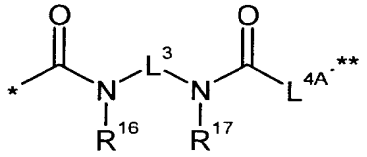
to give a compound of the formula (II-C)



(II-C),

in which D, L^1, L^2 and L^3 each have the definitions indicated above.

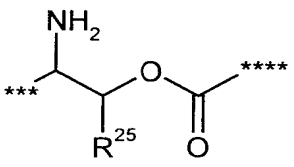
Compound of the formula (II), in which B is a group of the formula (B^3)



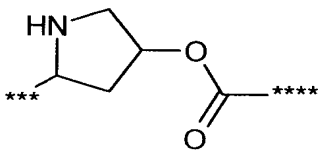
(B^3),

in which *, **, L^3, R^{16} and R^{17} each have the conditions indicated above and

L^{4A} is a group of the formula



or



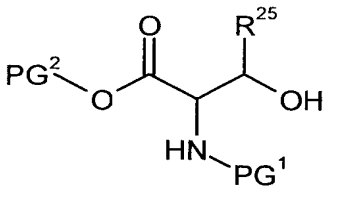
in which

*** marks the linkage site with the carbonyl group,

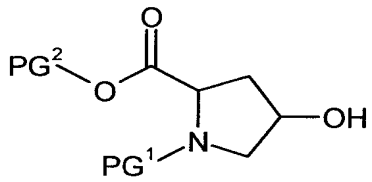
**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of the formula (XI-A) or (XI-B)



(XI-A) or

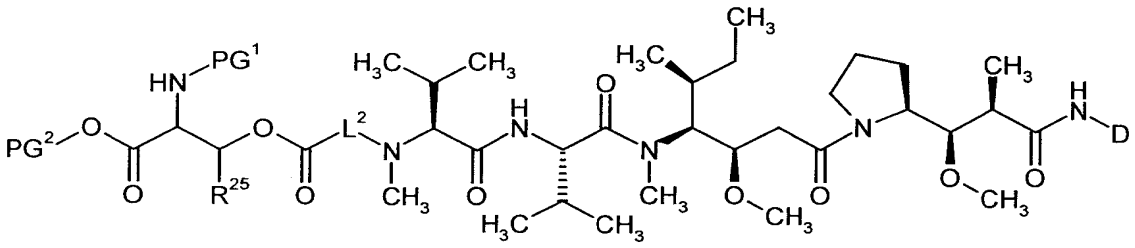


(XI-B),

in which R^{25} and PG^1 each have the definitions indicated above and

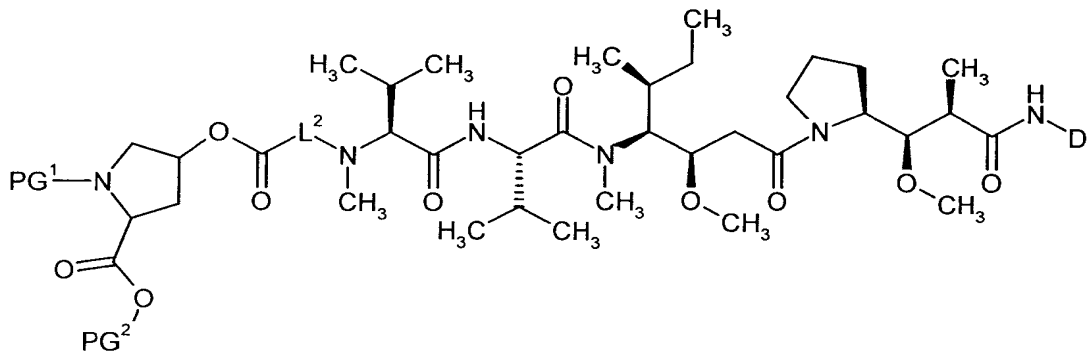
PG^2 is a suitable carboxyl-protective group, more particularly benzyl,

to give a compound (XII-A) or (XII-B)



(XII-A)

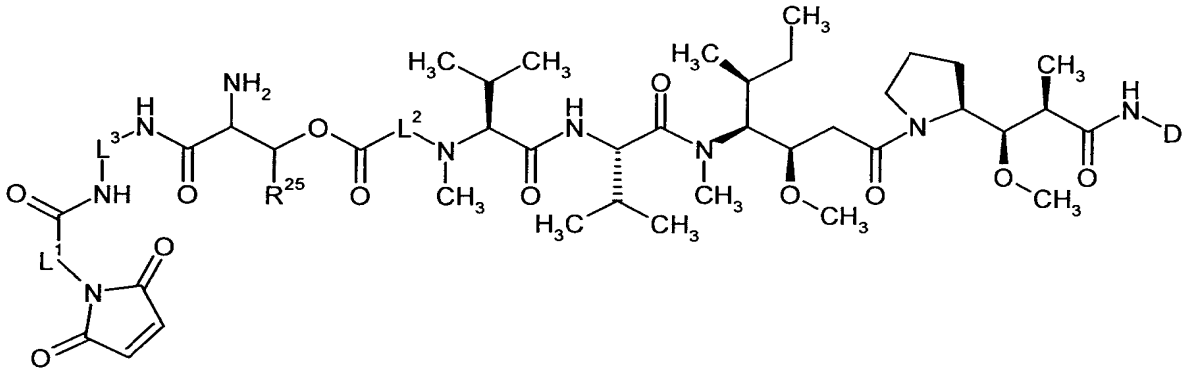
or



(XII-B),

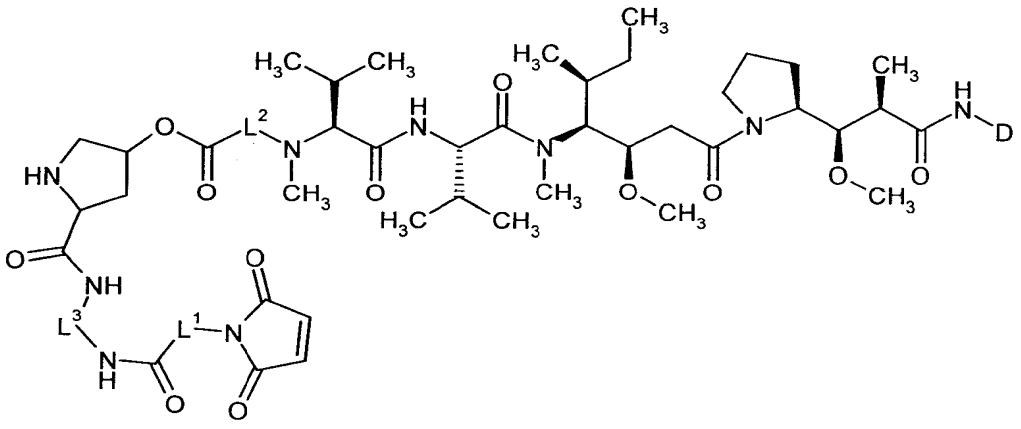
in which D, PG^1, PG^2 and L^2 have the definitions indicated above,

eliminating the protective group PG^2 from this compound subsequently, by methods known to the skilled person, and reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (X), and finally, eliminating the protective group PG1 from this compound, by methods known to the skilled person, to give a compound of the formula (II-D-A) or (II-D-B)



(II-D-A),

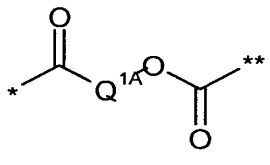
or



(II-D-B),

in which D, L^1, L^2 and L^3 have the definitions indicated above.

Compound of the formula (II), in which B is a group of the formula (B^4)

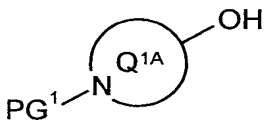


(B^4),

in which *, ** each have the conditions indicated above and

Q^{1A} is an N-linked 4- to 7-membered heterocycle,

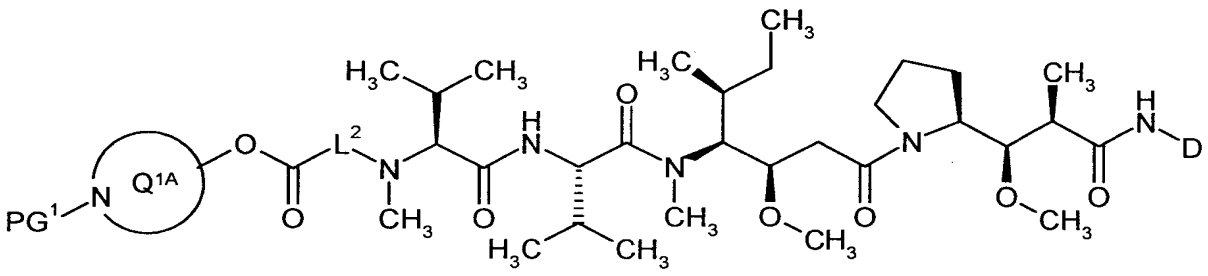
can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of the formula (XXI)



(XXI),

in which PG^1 and Q^{1A} each have the definitions indicated above,

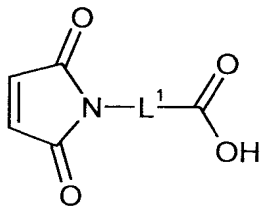
to give a compound of the formula (XXII)



(XXII),

in which PG^1, Q^{1A}, D and L^2 have the definitions indicated above,

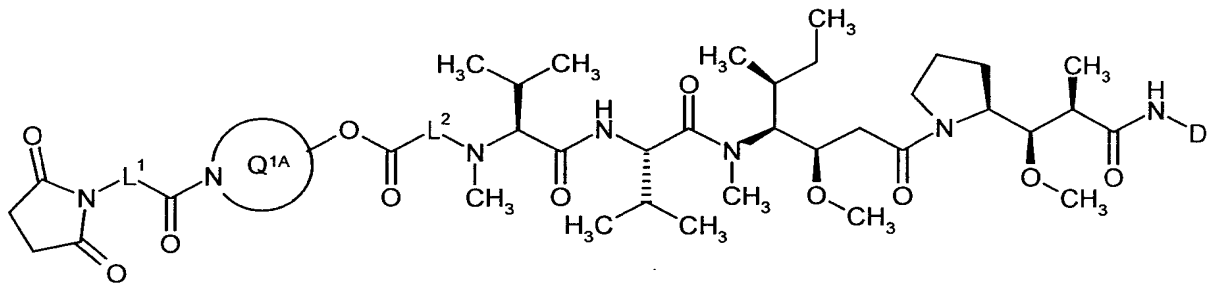
eliminating the protective group PG^1 from this compound, by methods known to the skilled person, and subsequently reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (XXIII)



(XXIII),

in which L^1 has the definition indicated above,

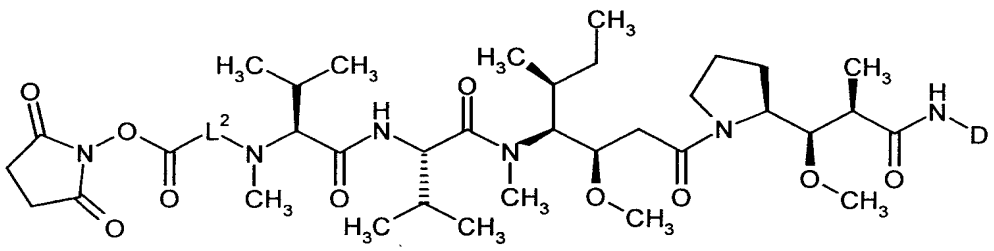
to give a compound of the formula (II-D)



(II-D),

in which Q^{1A}, D, L^1 and L^2 have the definitions indicated above.

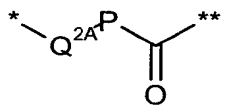
The compounds of the formula (III), in which L^1 and B are a bond can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with N-hydroxysuccinimide to give a compound of the formula (III-A)



(III-A),

in which D and L^2 each have the definitions indicated above.

The compounds of the formula (III), in which L^1 is a bond and B is a group of the formula (B^{5A})

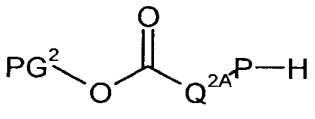


(B^{5A}),

in which *, ** and P each have the definitions indicated above and

Q^{2A} is a 3- to 7-membered carbocycle,

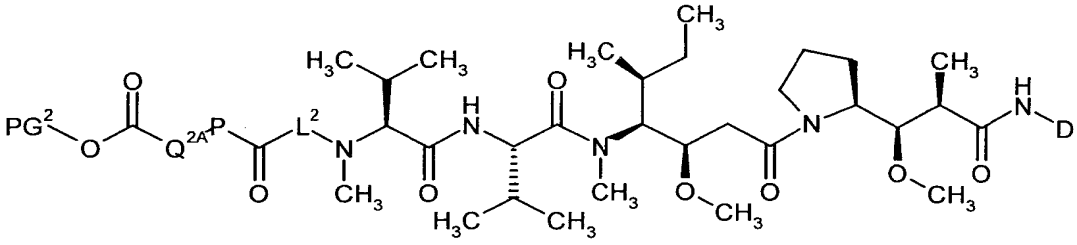
can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (XIII)



(XIII),

in which P, Q^{2A} and PG^2 each have the definitions indicated above,

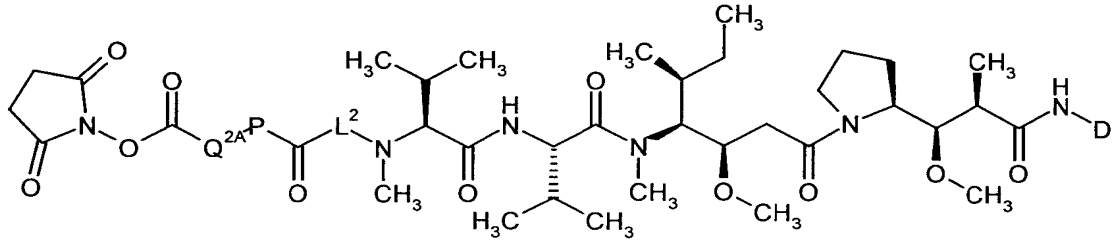
to give a compound of the formula (XIV)



(XIV),

in which D, P, Q^{2A}, L^2 and PG^2 each have the definitions indicated above,

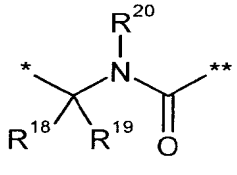
eliminating the protective group PG^2 from this compound by methods known to the skilled person, and subsequently reacting the deprotected compound in an inert solvent in the presence of a suitable base with N-hydroxysuccinimide to give a compound of the formula (III-B)



(III-B),

in which D, P, Q^{2A} and L^2 each have the definitions indicated above.

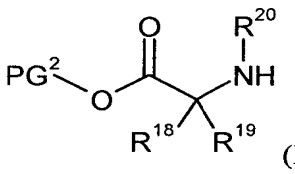
The compounds of the formula (III), in which L^1 is a bond and B is a group of the formula (B^6)



(B^6),

in which *, **, R^{18}, R^{19} and R^{20} each have the definitions indicated above,

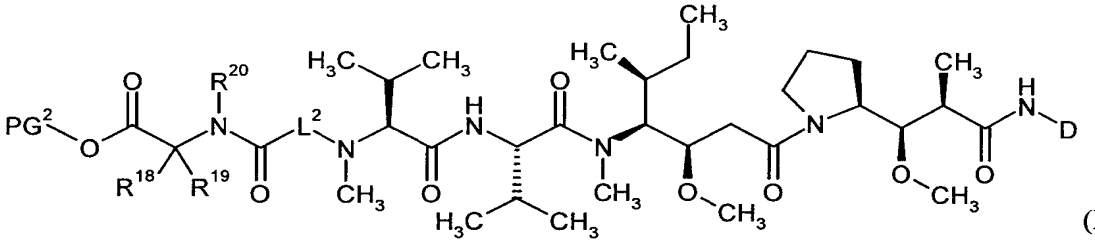
can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (XV)



(XV),

in which R^{18}, R^{19}, R^{20} and PG^2 each have the definitions indicated above,

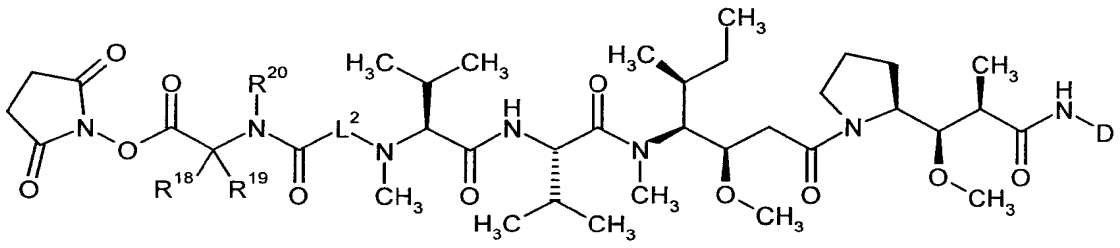
to give a compound of the formula (XVI)



(XVI),

in which D, R^{18}, R^{19}, R^{20}, L^2 and PG^2 each have the definitions indicated above,

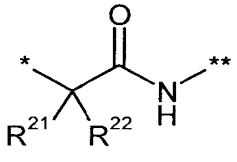
eliminating the protective group PG^2 from this compound by methods known to the skilled person, and subsequently reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with N-hydroxysuccinimide to give a compound of the formula (III-C)



(III-C),

in which D, R^{18}, R^{19}, R^{20} and L^2 each have the definitions indicated above.

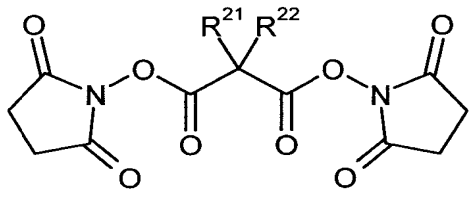
The compounds of the formula (III), in which L^1 is a bond and B is a group of the formula (B^7)



(B^7),

in which *, **, R^{21} and R^{22} each have the definitions indicated above,

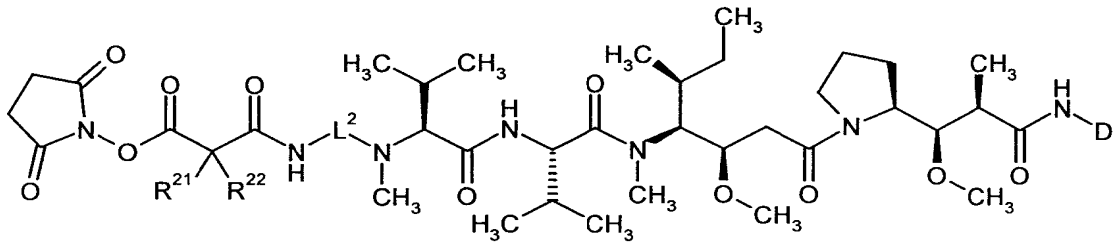
can be prepared by eliminating the protective group PG^1 from a compound of the formula (VI) by methods known to the skilled person, and reacting the resultant deprotected compound in an inert solvent in the presence of a suitable base with a compound of the formula (XVII)



(XVII),

in which R^{21} and R^{22} each have the definitions indicated above,

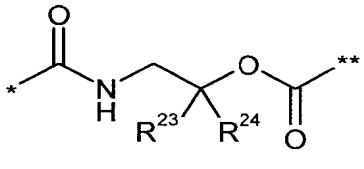
to give a compound of the formula (III-D)



(III-D),

in which D, R^{21}, R^{22} and L^2 each have the definitions indicated above.

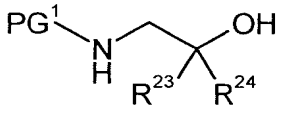
The compounds of the formula (III), in which B is a group of the formula (B^8)



(B^8),

in which *, **, R^{23} and R^{24} each have the definitions indicated above,

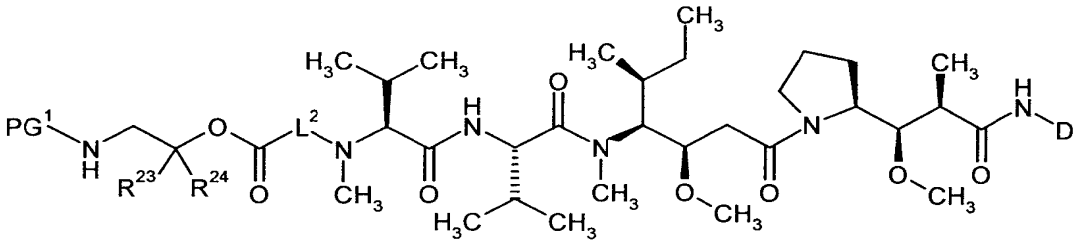
can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (XVIII)



(XVIII),

in which R^{23}, R^{24} and PG^1 each have the definitions indicated above,

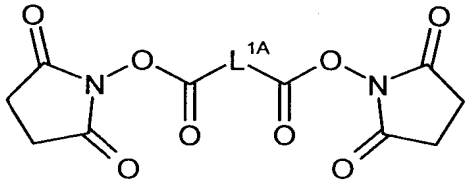
to give a compound of the formula (XIX)



(XIX),

in which D, R^{23}, R^{24}, L^2 and PG^1 each have the definitions indicated above,

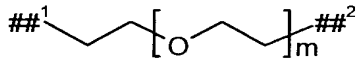
eliminating the protective group PG^1 from this compound by methods known to the skilled person, and subsequently reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of the formula (XX)



(XX),

in which

L^{1A} is linear (C_1-C_{10})-alkanediyl or is a group of the formula



where

m is a number from 2 to 6,

$##^1$ marks the linkage site with the group G,

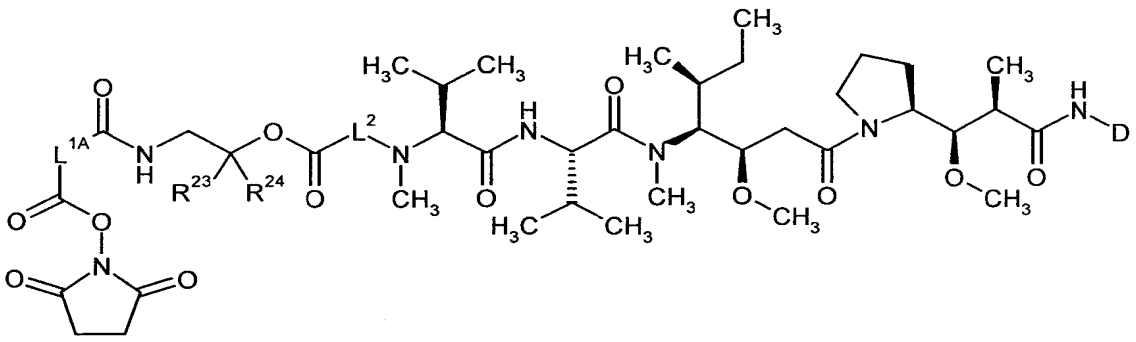
$##^2$ marks the linkage site with the group B,

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 methyl substituents,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

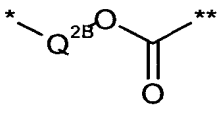
to give a compound of the formula (III-E)



(III-E),

in which D, R^{23}, R^{24}, L^{1A} and L^2 each have the definitions indicated above.

The compounds of the formula (III), in which B is a group of the formula (B^{5B})

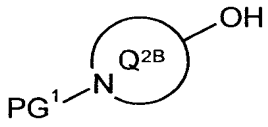


(B^{5B}),

in which * and ** each have the definitions indicated above and

Q^{2B} is an N-linked 4- to 7-membered heterocycle,

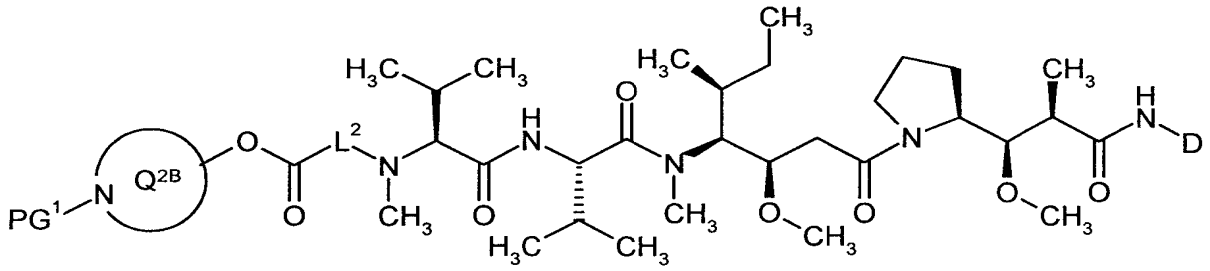
can be prepared by reacting a compound of the formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of the formula (XXIV)



(XXIV),

in which PG^1 and Q^{2B} each have the definitions indicated above,

to give a compound of the formula (XXV)

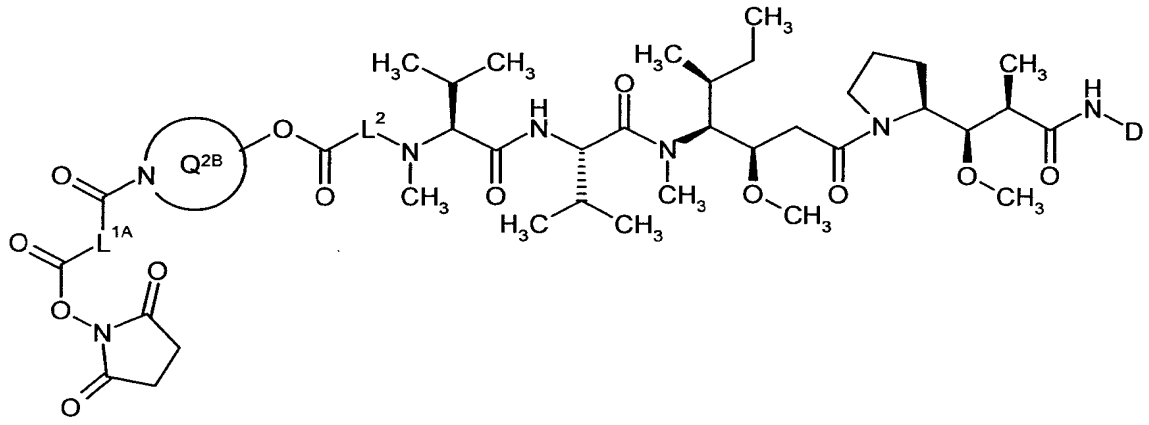


(XXV),

in which PG^1, Q^{2B}, D and L^2 have the definitions indicated above,

eliminating the protective group PG^1 from this compound by methods known to the skilled person,

and subsequently converting the deprotected compound in an inert solvent in the presence of a suitable base with a compound of the formula (XX) into a compound of the formula (III-F)



(III-F),

in which Q^{2B}, D, L^{1A} and L^2 have the definitions indicated above.

The reactions (IV) + (V) → (VI) and (IV) + (VIII) → (IX) take place in the solvents which are customary for a reductive amination and are inert under the reaction conditions, optionally in the presence of an acid and/or of a water-removing agent as catalyst. Such solvents include, for example, alcohols such as methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol or *tert*-butanol, ethers such as tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis(2-methoxyethyl) ether, or other solvents such as dichloromethane, 1,2-dichloroethane, *N,N*-dimethylformamide or else water. It is also possible to use mixtures of these solvents. As solvent it is preferred to use a 1,4-dioxane/water mixture, with addition of acetic acid or dilute hydrochloric acid as catalyst.

Reducing agents suitable for this reaction are, in particular, complex borohydrides, such as, for example, sodium borohydride, sodium cyanoborohydride, sodium triacetoxyborohydride, tetra-

n-butylammonium borohydride or borane-pyridine complex. It is preferred to use sodium cyanoborohydride or borane-pyridine complex.

The reactions (IV) + (V) → (VI) and (IV) + (VIII) → (IX) take place in general in a temperature range from 0°C to +120°C, preferably at +50°C to +100°C. The reactions may be carried out under atmospheric, increased or reduced pressure (e.g. from 0.5 to 5 bar); it is usual to operate at atmospheric pressure.

The above-described coupling reactions (IX) + (X) → (II-C), (XII-A) or (XII-B) + (X) → (II-D-A) or (II-D-B), (IX) + (XIII) → (XIV), (IX) + (XV) → (XVI) and (XXII) + (XXIII) → (II-D) (amide formation from amine component and carboxylic acid component respectively) are carried out by standard methods of peptide chemistry [see e.g. M. Bodanszky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin, 1993; M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1984; H.-D. Jakubke and H. Jeschkeit, *Aminosäuren, Peptide, Proteine*, Verlag Chemie, Weinheim, 1982].

Examples of inert solvents for these coupling reactions are ethers such as diethyl ether, diisopropyl ether, *tert*-butyl methyl ether, tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis(2-methoxyethyl) ether, hydrocarbons such as benzene, toluene, xylene, pentane, hexane, heptane, cyclohexane or petroleum fractions, halogenated hydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, 1,2-dichloroethane, trichloroethylene or chlorobenzene, or dipolar-aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulphoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropyleneurea (DMPU) or *N*-methylpyrrolidinone (NMP). It is also possible to use mixtures of such solvents. Preference is given to using *N,N*-dimethylformamide.

Examples of suitable activating/condensing agents for these couplings include carbodiimides such as *N,N'*-diethyl-, *N,N'*-dipropyl-, *N,N'*-diisopropyl-, *N,N'*-dicyclohexylcarbodiimide (DCC) or *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), phosgene derivatives such as *N,N'*-carbonyldiimidazole (CDI) or isobutyl chloroformate, 1,2-oxazolium compounds such as 2-ethyl-5-phenyl-1,2-oxazolium 3-sulphate or 2-*tert*-butyl-5-methylisoxazolium perchlorate, acylamino compounds such as 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, phosphorus compounds such as propanephosphonic anhydride, diethyl cyanophosphonate, bis(2-oxo-3-oxazolidinyl)phosphoryl chloride, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate or benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), or uronium compounds such as *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 2-(2-oxo-1-(2*H*)-pyridyl)-1,1,3,3-tetramethyluronium

tetrafluoroborate (TPTU), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) or *O*-(1*H*-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium-tetrafluoroborate (TCTU), optionally in combination with further auxiliaries such as 1-hydroxybenzotriazole (HOBt) or *N*-hydroxysuccinimide (HOSu), and also, as bases, alkali metal carbonates, e.g. sodium or potassium carbonate, or tertiary amine bases such as triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine.

In the context of the present invention, as activating/condensing agents for such coupling reactions, it is preferred to use *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) in combination with 1-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine, or *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) likewise in conjunction with *N,N*-diisopropylethylamine.

The coupling reactions (IX) + (X) → (II-C), (XII-A) or (XII-B) + (X) → (II-D-A) or (II-D-B), (IX) + (XIII) → (XIV) , (IX) + (XV) → (XVI) and (XXII) + (XXIII) → (II-D) are carried out in general in a temperature range from -20°C to +60°C, preferably at 0°C to +40°C. The reactions may take place under atmospheric, at increased or at reduced pressure (e.g. from 0.5 to 5 bar); it is usual to operate under atmospheric pressure.

The esterifications (IX) + (XVIII) → (XII) and (IX) + (XI-A) or (XI-B) → (XII-A) or (XII-B), (IX) + (XXIV) → (XXV) and also (IX) + (XXI) → (XXII) take place in analogy to the above-described amide coupling reactions. These reactions take place preferably in dichloromethane, using *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylamino-pyridine at a temperature of +50°C to 100°C under atmospheric pressure.

The functional groups optionally present in the compounds – such as amino, hydroxyl and carboxyl groups in particular – may also be present in a temporarily protected form during the above-described process steps, if useful or necessary. In these cases, such protective groups are introduced and removed in accordance with customary methods known from peptide chemistry [see, for example, T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, Wiley, New York, 1999; M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1984]. Where two or more protected groups are present, they can be liberated again optionally simultaneously in a one-pot reaction, or else liberated again in separate reaction steps.

As an amino-protective group PG^1 it is preferred to use *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (*Z*) or (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc); for a hydroxyl or carboxyl function it is preferred to use *tert*-butyl or benzyl as protective group PG^2. The elimination of a

tert-butyl or *tert*-butoxycarbonyl group is typically accomplished by treatment with a strong acid, such as hydrogen chloride, hydrogen bromide or trifluoroacetic acid, in an inert solvent such as diethyl ether, 1,4-dioxane, dichloromethane or acetic acid; this reaction may optionally also be carried out without addition of an inert solvent. In the case of benzyl or benzyloxycarbonyl as protective group, this group is removed preferably by hydrogenolysis in the presence of a suitable palladium catalyst, such as palladium on activated carbon, for example. The (9*H*-fluoren-9-ylmethoxy)carbonyl group is generally eliminated using a secondary amine base such as diethylamine or piperidine.

The reaction (VI) → (II-A) takes place in a solvent which is inert under the reaction conditions, such as, for example, ethers such as tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis(2-methoxyethyl) ether, alcohols such as methanol, ethanol, isopropanol, n-butanol or *tert*-butanol, or dipolar-aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulphoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropyleneurea (DMPU) or *N*-methylpyrrolidinone (NMP) or water. It is also possible to use mixtures of such solvents. Preference is given to using a mixture of 1,4-dioxane and water.

Suitable bases for the reaction (VI) → (II-A) are, for example, alkali metal carbonates such as potassium carbonate, sodium carbonate or lithium carbonate, alkali metal hydrogencarbonates such as sodium or potassium hydrogencarbonate or alkali metal alkoxides such as sodium methoxide, sodium ethoxide or potassium tert-butoxide. It is preferred to use sodium hydrogencarbonate.

The reaction (VI) → (II-A) takes place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may take place under atmospheric, under elevated or under reduced pressure (e.g. from 0.5 to 5 bar); it is usual to operate under atmospheric pressure.

The reaction (VI) + (VII) → (II-B) takes place in a solvent which is inert under the reaction conditions, such as, for example, ethers such as tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis(2-methoxyethyl) ether, alcohols such as methanol, ethanol, isopropanol, n-butanol or *tert*-butanol, or dipolar-aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulphoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropyleneurea (DMPU) or *N*-methylpyrrolidinone (NMP) or water. It is also possible to use mixtures of such solvents. Preference is given to using DMF.

Suitable bases for the reaction (VI) + (VII) → (II-B) are, for example, tertiary amine bases such as triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine. Preference is given to using *N,N*-diisopropylethylamine.

The reaction (VI) + (VII) → (II-B) takes place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may take place under atmospheric, under elevated or under reduced pressure (e.g. from 0.5 to 5 bar); it is usual to operate under atmospheric pressure.

The reactions (IX) → (III-A), (XIV) → (III-B) and (XVI) → (III-C) and also (VI) + (XVII) → (III-D), (XIX) + (XX) → (III-E) and (XXV) + (XX) → (III-F) take place in a solvent which is inert under the reaction conditions. Examples of suitable solvents are ethers such as diethyl ether, diisopropyl ether, *tert*-butyl methyl ether, tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis(2-methoxyethyl) ether, hydrocarbons such as benzene, toluene, xylene, pentane, hexane, heptane, cyclohexane or petroleum fractions, halogenated hydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, 1,2-dichloroethane, trichloroethylene or chlorobenzene, or dipolar-aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulphoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropyleneurea (DMPU) or *N*-methylpyrrolidinone (NMP). It is also possible to use mixtures of such solvents. Preference is given to using *N,N*-dimethylformamide.

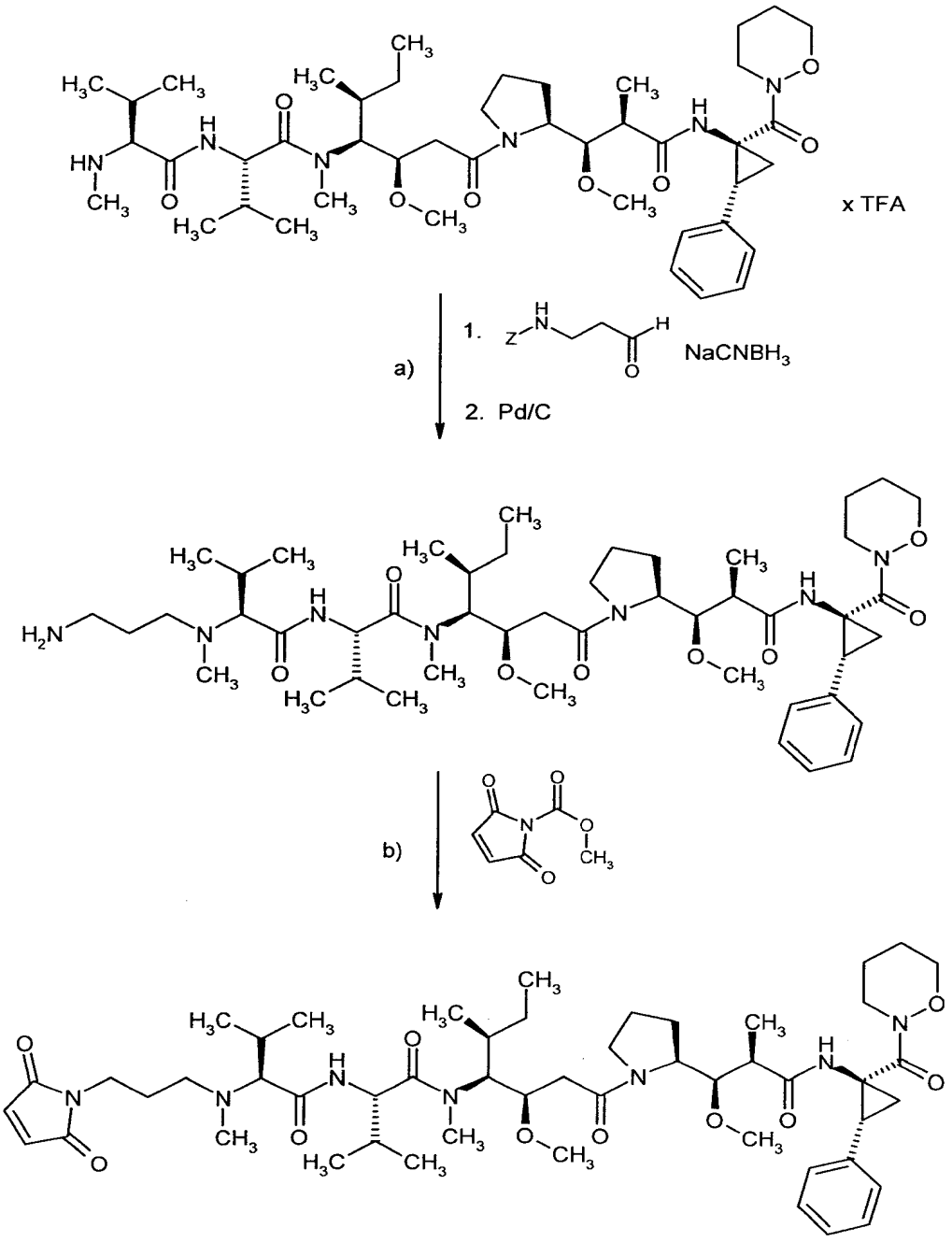
Suitable bases for these reactions are, for example, tertiary amines such as triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine. Preference is given to using *N,N*-diisopropylethylamine, optionally with addition of 4-*N,N*-dimethylaminopyridine.

The reactions (IX) → (III-A), (XIV) → (III-B) and (XVI) → (III-C) and also (VI) + (XVII) → (III-D) and (XIX) + (XX) → (III-E) take place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may take place under atmospheric, under elevated or under reduced pressure (e.g. from 0.5 to 5 bar); it is usual to operate under atmospheric pressure.

The compounds of the formulae (II) and (III) are sub-quantities of the compounds of the formulae (IIa) and (IIIa) respectively, where R^{35} is methyl. The preparation of the compounds (IIa) and (IIIa) takes place in analogy to the preparation of the compound of the formulae (II) and (III) as described above.

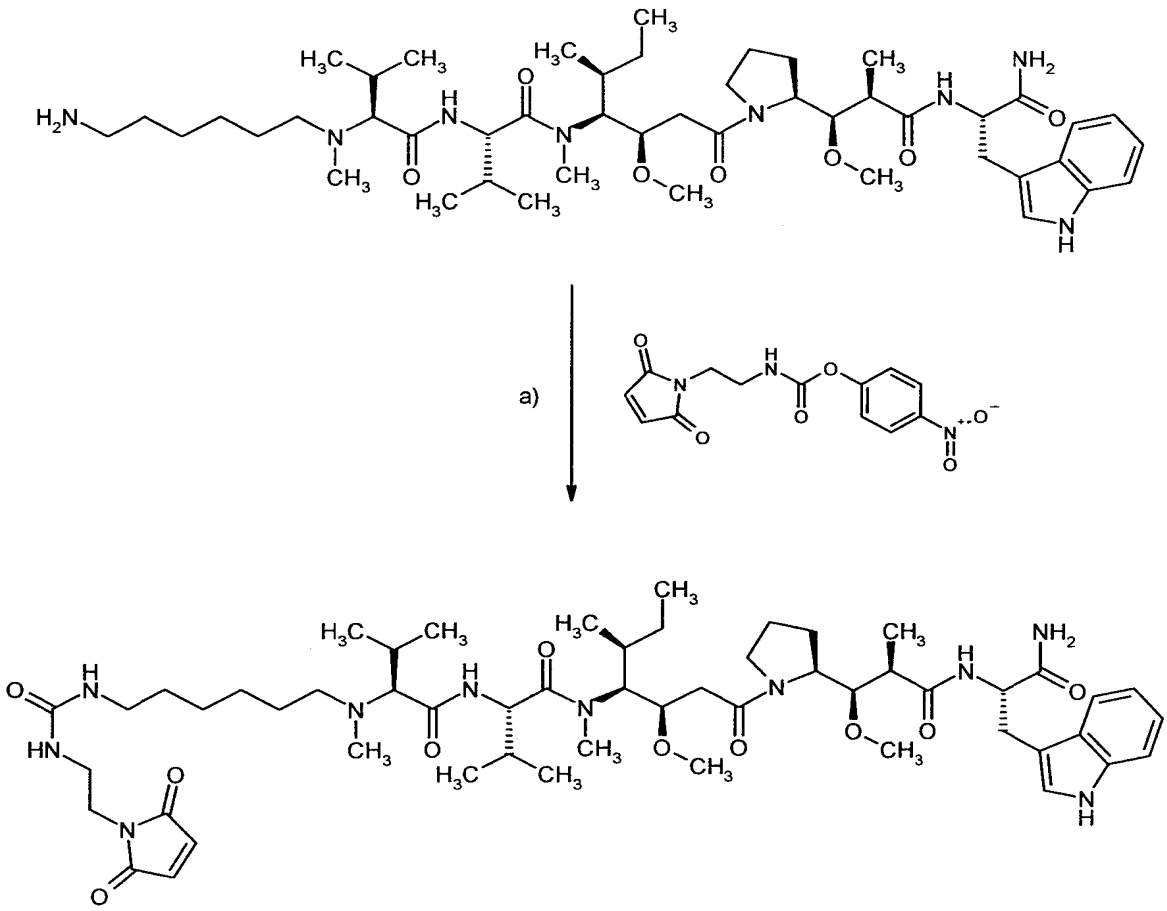
The above-described processes are illustrated by way of example by the following synthesis schemes (Scheme 3 to 13, 18):

Scheme 3



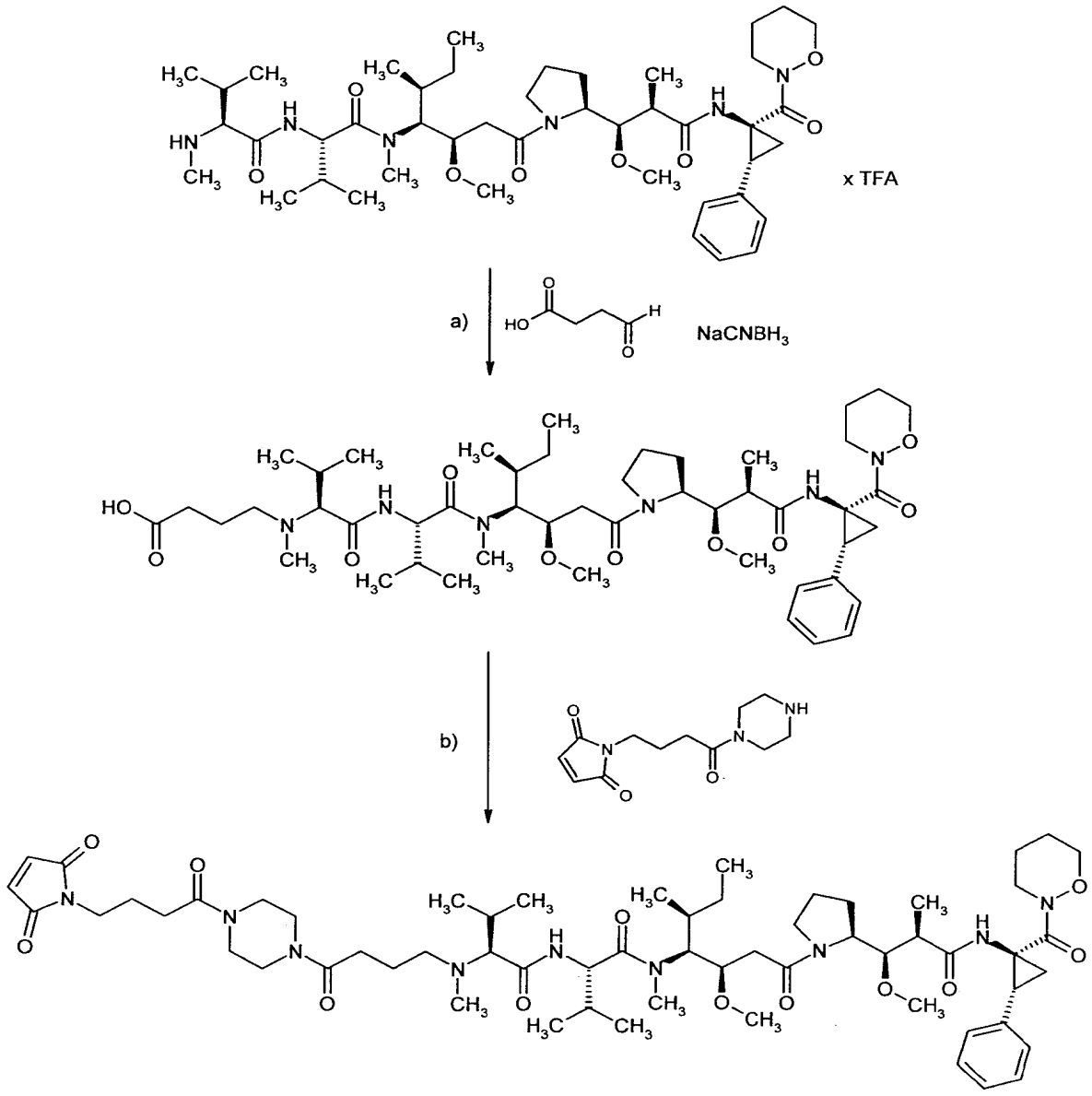
[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H_2, Pd/C, methanol, RT; b): $NaHCO_3$, H_2O, dioxane, RT].

Scheme 4



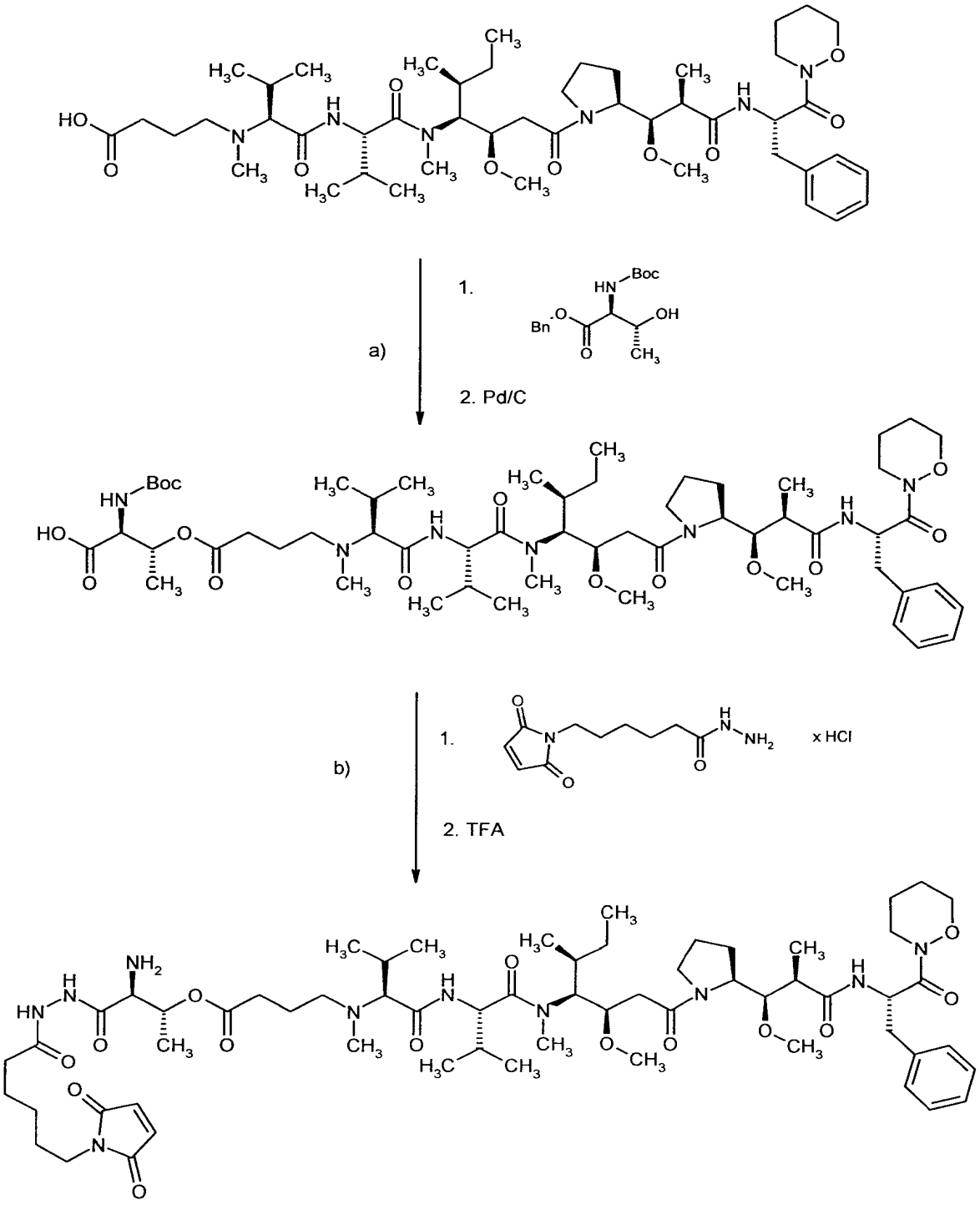
[a): Diisopropylethylamine, DMF, RT].

Scheme 5



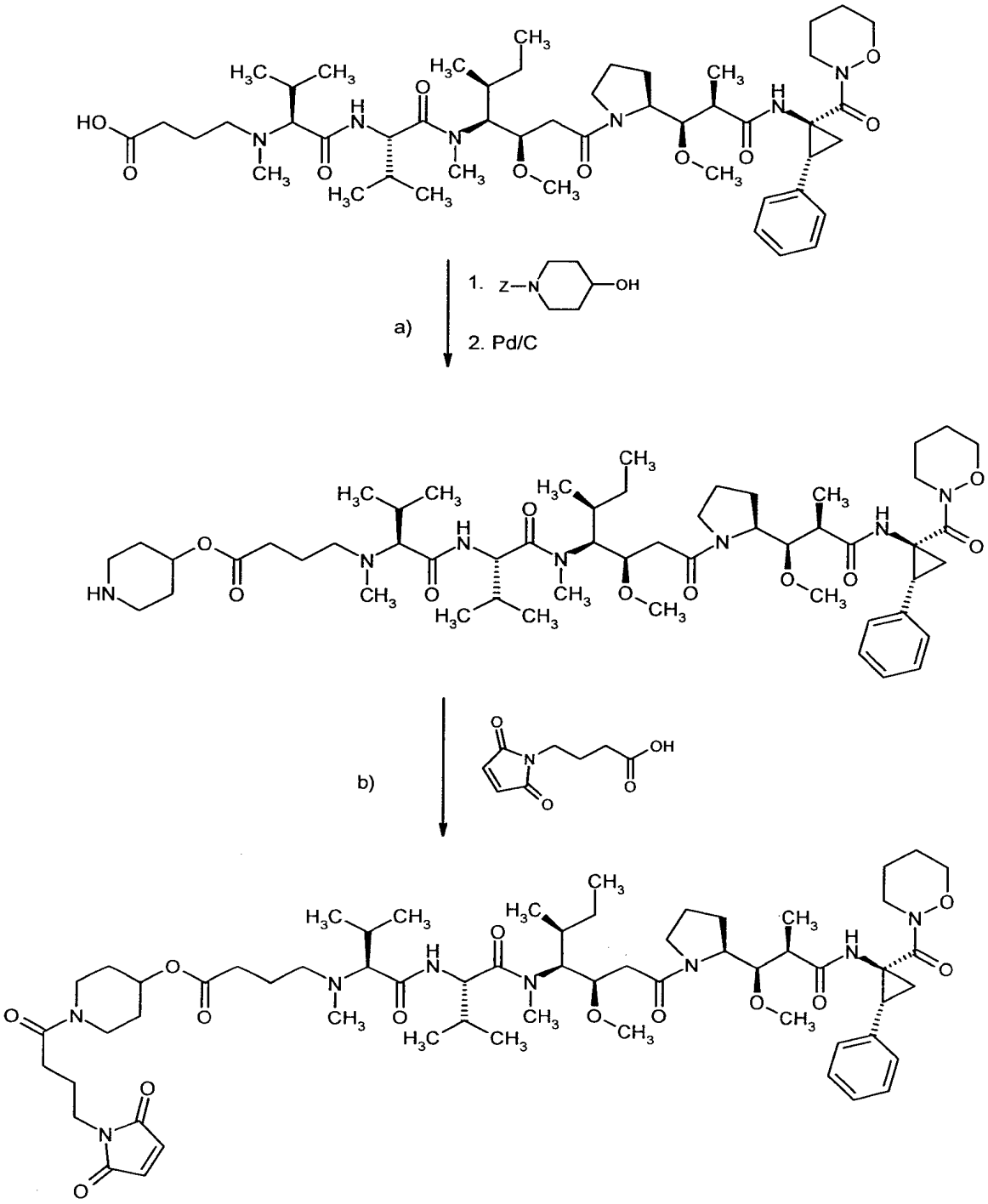
[a): Water/dioxane, 1N HCl, 100°C; b): HATU, diisopropylamine, DMF, RT].

Scheme 6



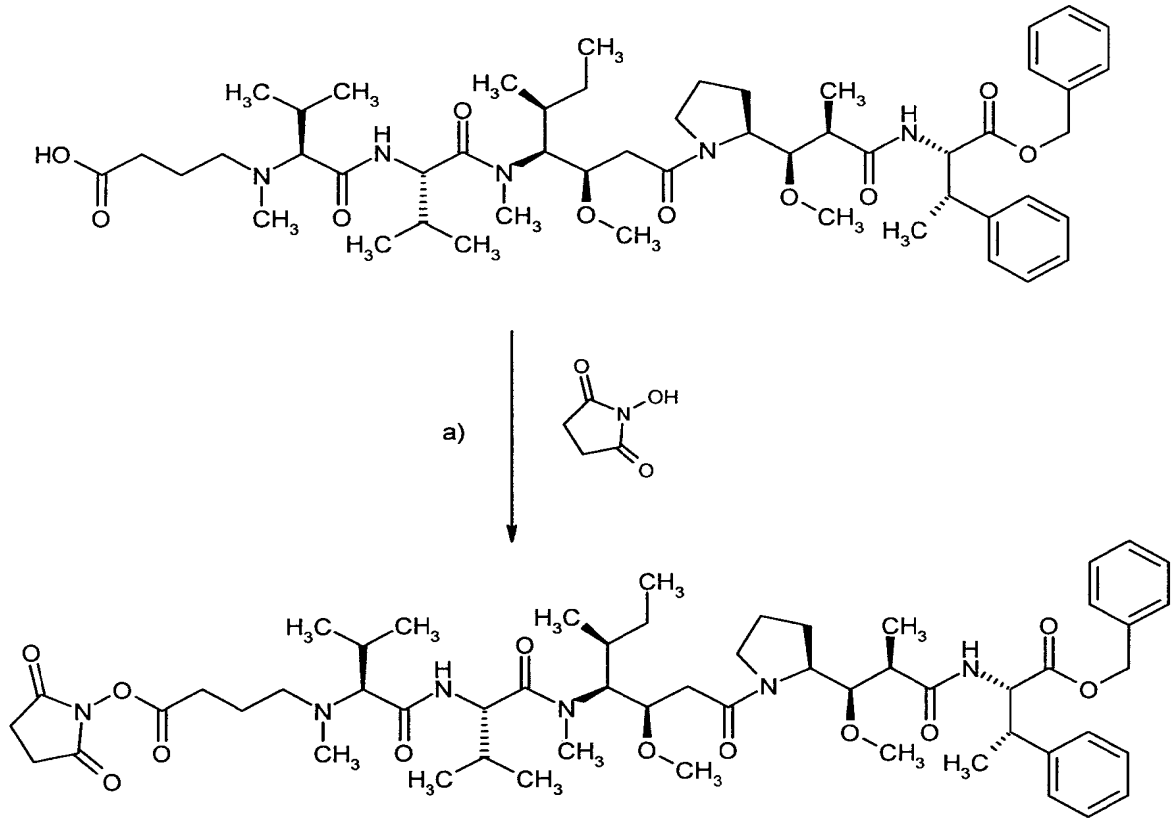
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H_2, methanol, RT ; b): 1. EDCI, HOBt, diisopropylamine, DMF, RT; 2. dichloromethane, RT].

Scheme 7



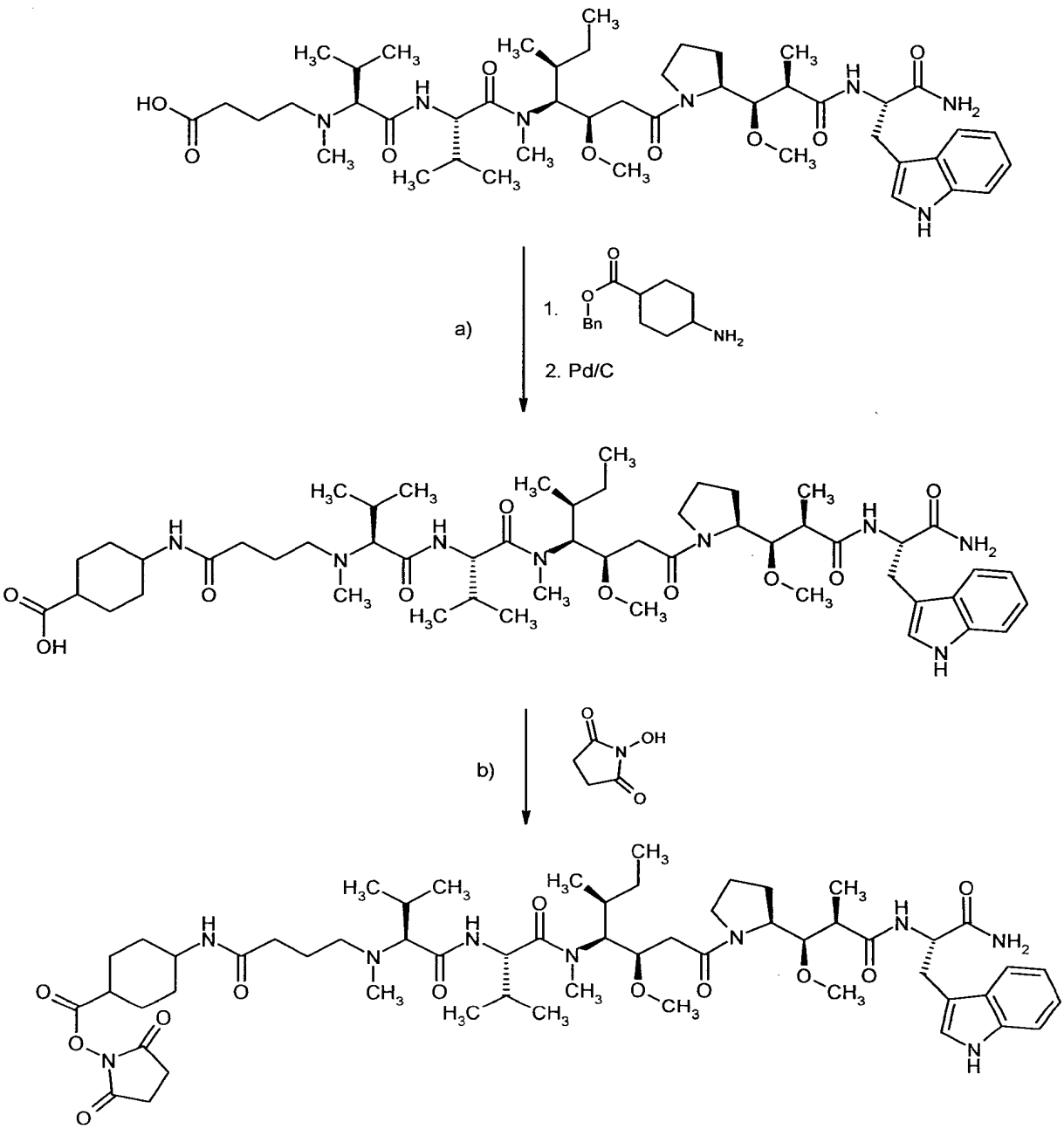
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H_2, methanol, RT ; b): HATU, diisopropylamine, DMF, RT].

Scheme 8



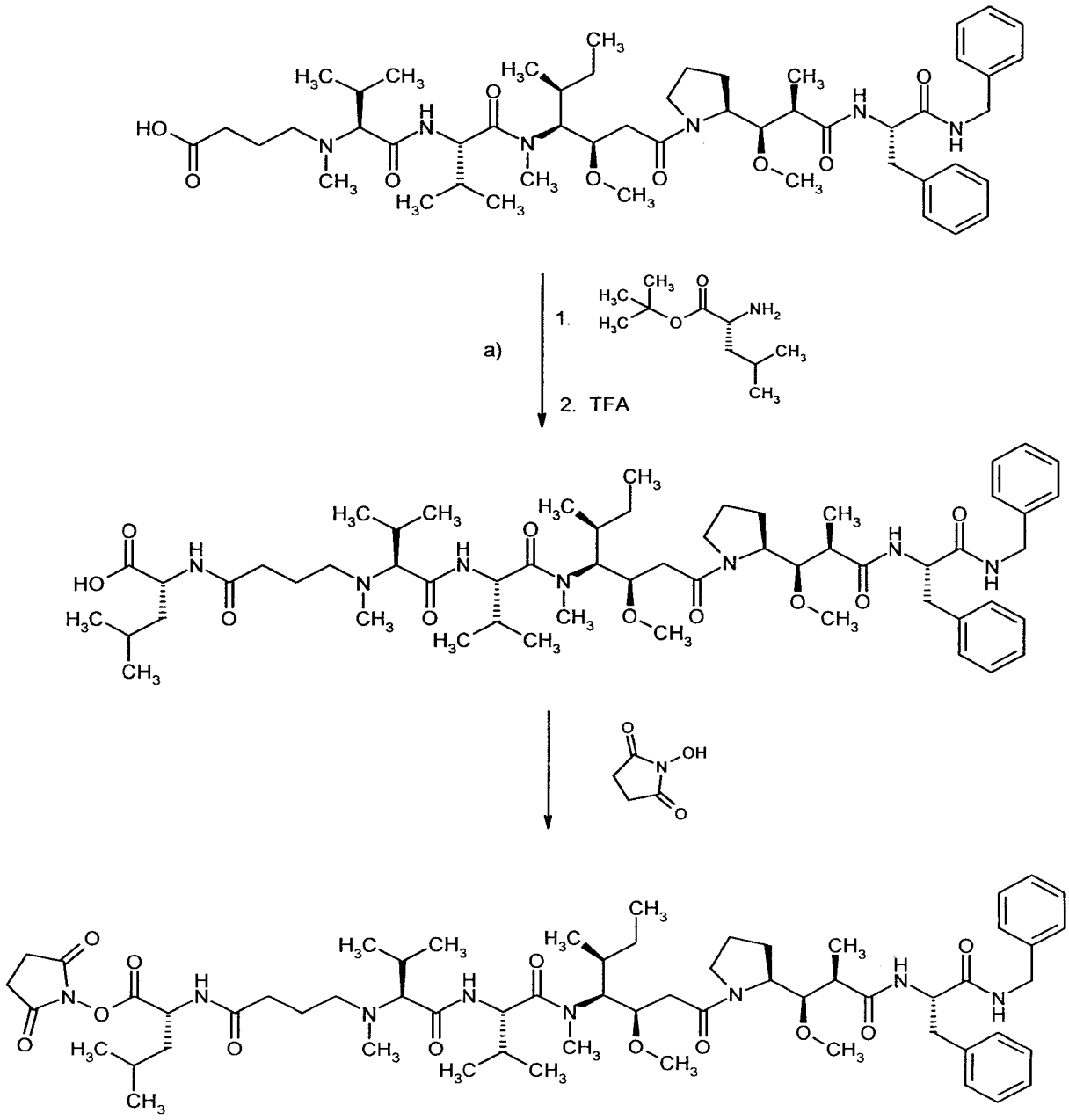
[a): EDCI, dichloromethane, RT].

Scheme 9



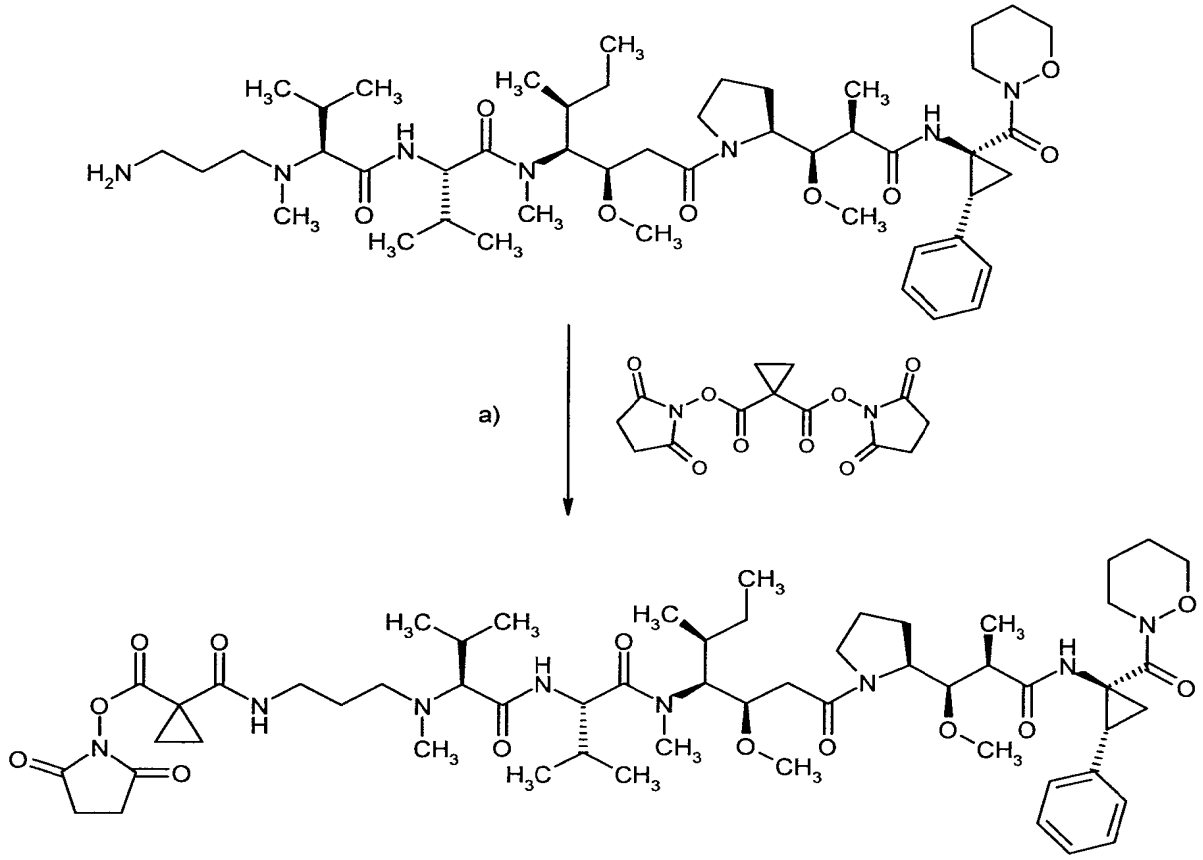
[a): HATU, diisopropylethylamine, DMF, RT; 2. H_2, methanol, RT; b): EDCI, DMAP, dichloromethane, RT].

Scheme 10



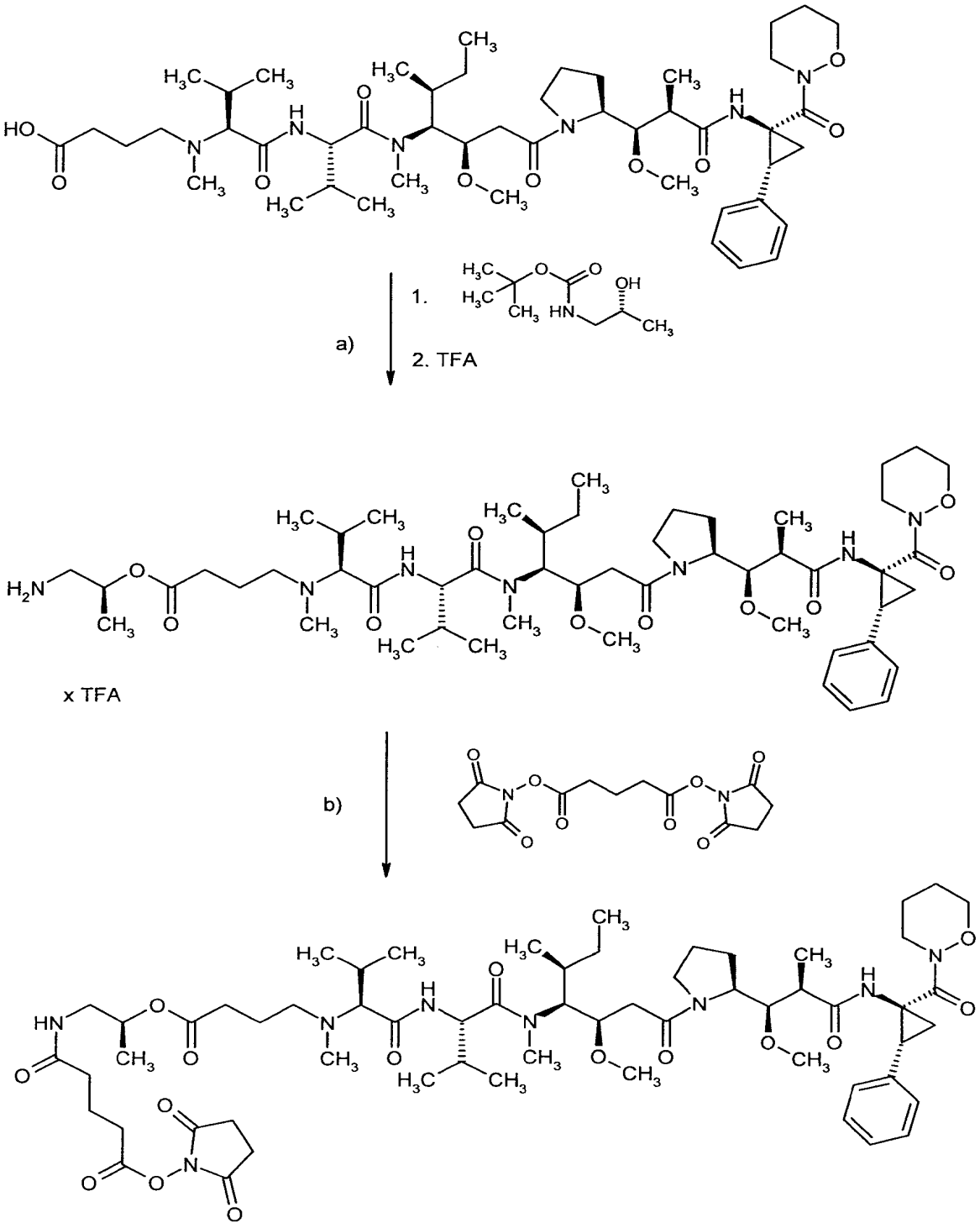
[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. dichloromethane, RT; b): EDCI, DMAP, dichloromethane, RT].

Scheme 11



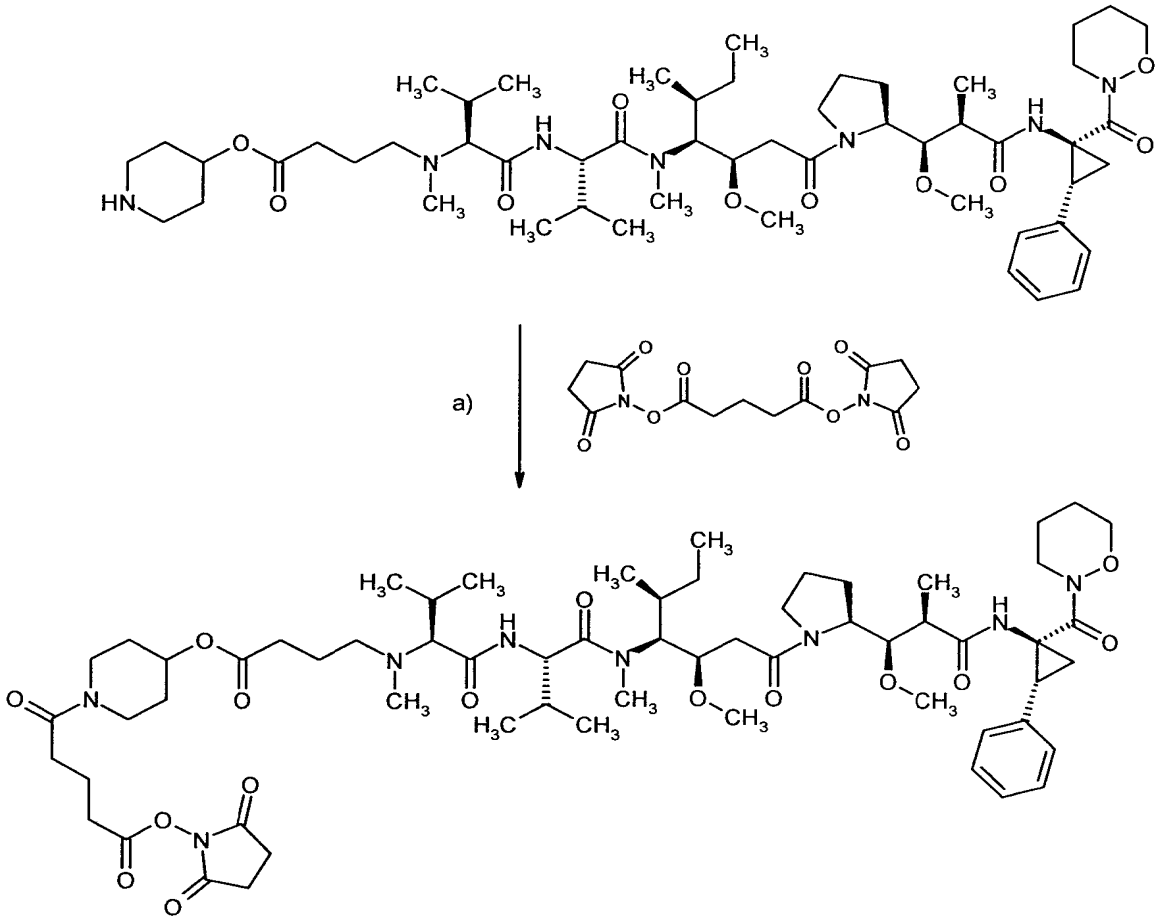
[a): Diisopropylethylamine, DMF, RT].

Scheme 12



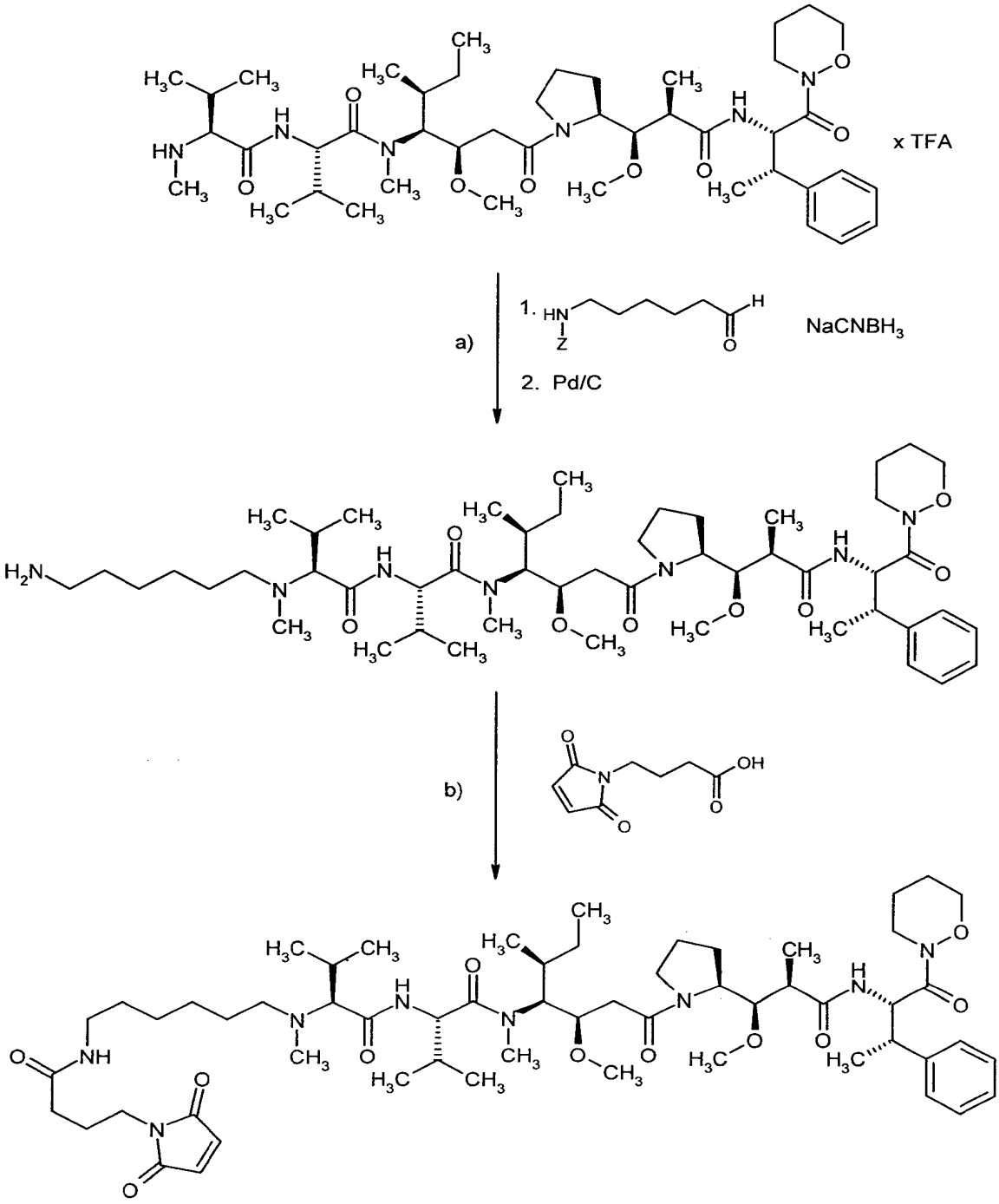
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. dichloromethane, RT; b): diisopropylamine, DMAP, dichloromethane, RT].

Scheme 13



[a): DMAP, diisopropylamine, dichloromethane, RT].

Scheme 18

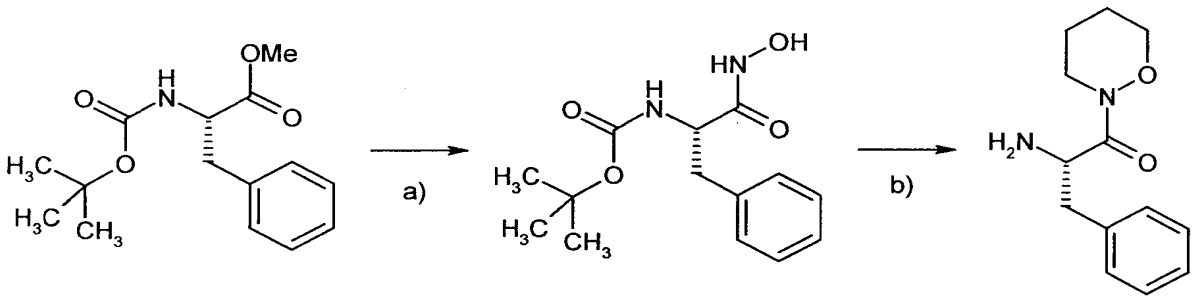


[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H_2, Pd/C, methanol, RT; b): HATU, diisopropylethylamine, RT].

The compounds of the formula (IV) can be prepared from commercially available amino acid building blocks or those known from the literature (see, for example, Pettit *et al.*, *Synthesis* 1996, 719; Shioiri *et al.*, *Tetrahedron Lett.* 1991, *32*, 931; Shioiri *et al.*, *Tetrahedron* 1993, *49*, 1913;

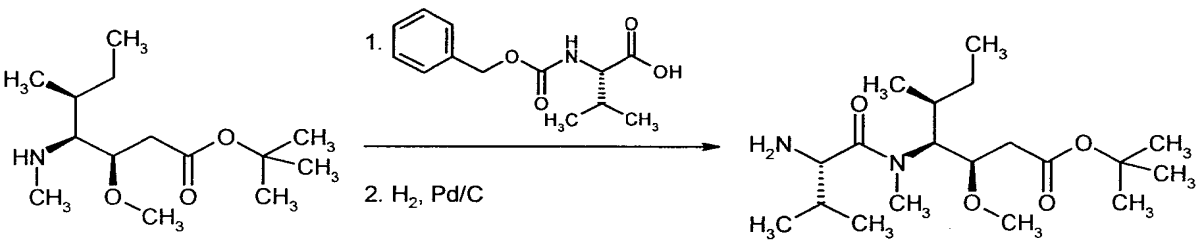
Koga *et al.*, *Tetrahedron Lett.* 1991, *32*, 2395; Vidal *et al.*, *Tetrahedron* 2004, *60*, 9715; Poncet *et al.*, *Tetrahedron* 1994, *50*, 5345. Pettit *et al.*, *J. Org. Chem.* 1994, *59*, 1796) in analogy to processes known from the literature, in accordance with customary methods of peptide chemistry, and as described in the present experimental section. The synthesis schemes below (Scheme 14 to 16) illustrate the preparation by way of example.

Scheme 14



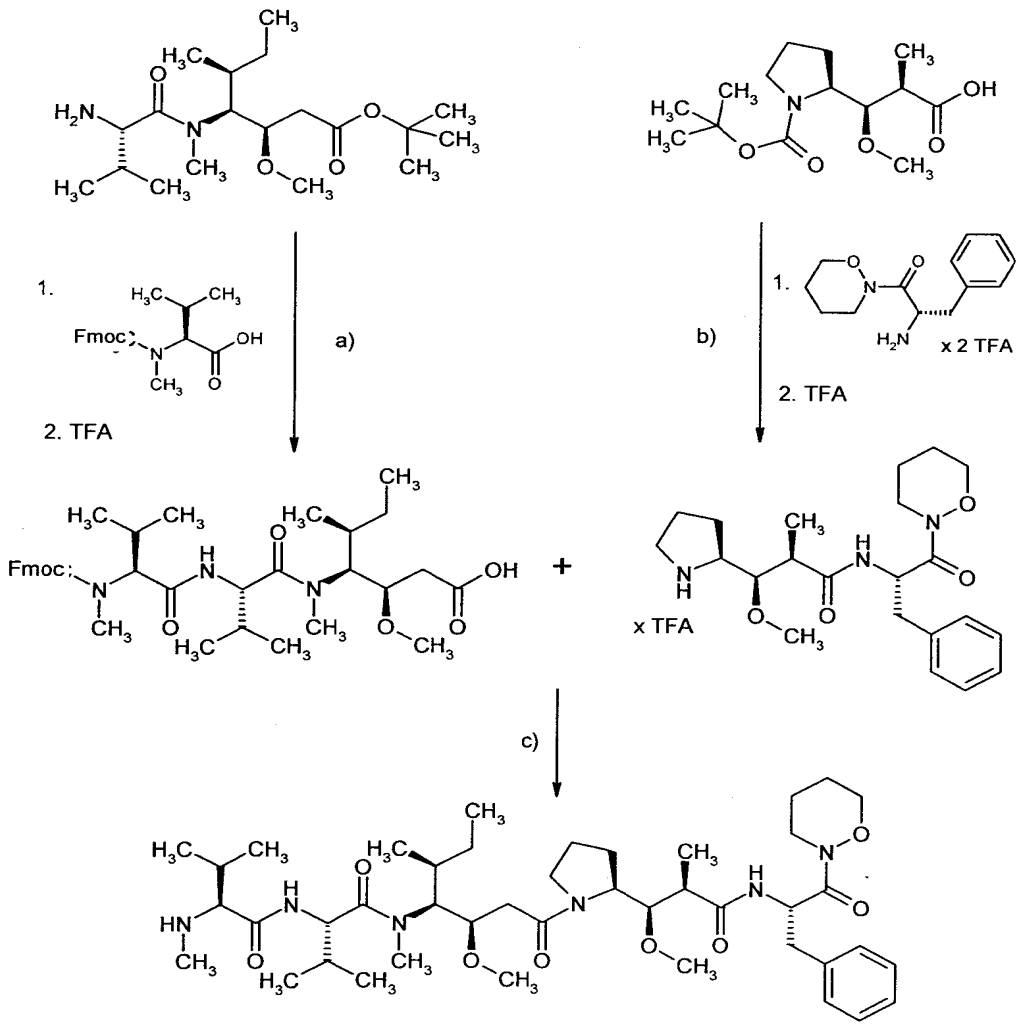
[a): Hydroxylamine hydrochloride, KOH, MeOH, 0°C → RT; b): $BrCH_2(CH_2)_2CH_2Br$, K_2CO_3, acetone, reflux].

Scheme 15



[a): 1. Diisopropylethylamine, BEP, dichloromethane, -10°C → RT; 2. MeOH].

Scheme 16



[a): 1. Diisopropylethylamine, BEP, DMF, RT; 2. dichloromethane; b): 1. HATU, diisopropylethylamine, DMF, RT; 2. dichloromethane, RT; c): 1. HATU, diisopropylethylamine, DMF, RT; 2. piperidine, DMF, RT].

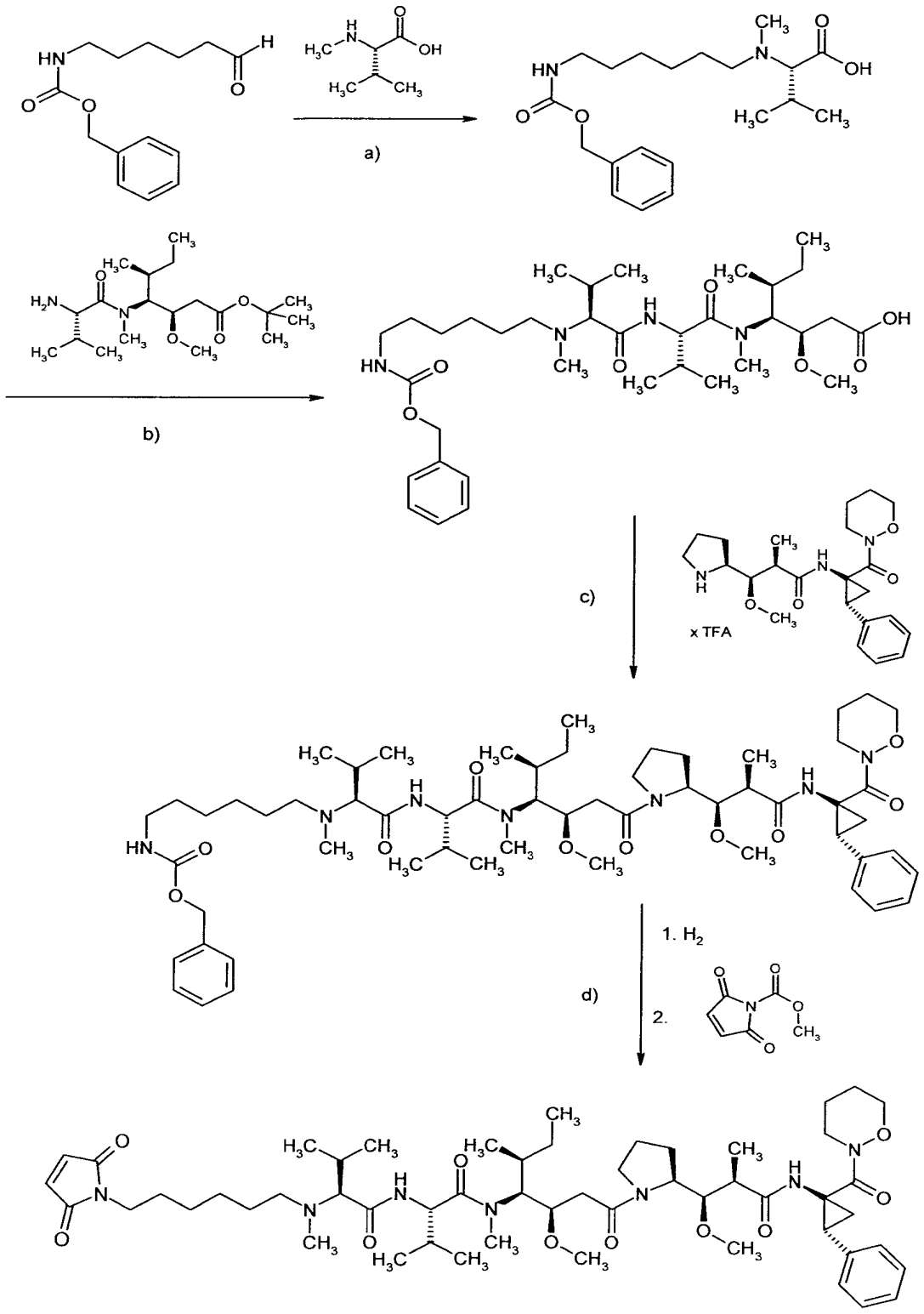
The compounds of the formulae (XI), (XIII), (XV), (XVII) and (XXI), including, where appropriate, chiral or diastereomeric forms thereof, are available commercially or are described as such in the literature, or they can be prepared by routes that are obvious to the skilled person, in analogy to methods published in the literature. Numerous comprehensive instructions and also literature information on the preparation of the starting materials are also given in the experimental section, in the section relating to the preparation of the starting compounds and intermediates.

The compounds of the formulae (V), (VII), (VIII), (X), (XVIII), (XX) and (XXIII), including, where appropriate, chiral or diastereomeric forms thereof, are known from the literature, or can be prepared by routes which are obvious to the skilled person, in analogy to methods published in the

literature. Numerous comprehensive instructions and also literature information on the preparation of the starting materials are also given in the experimental section, in the section relating to the preparation of the starting compounds and intermediates.

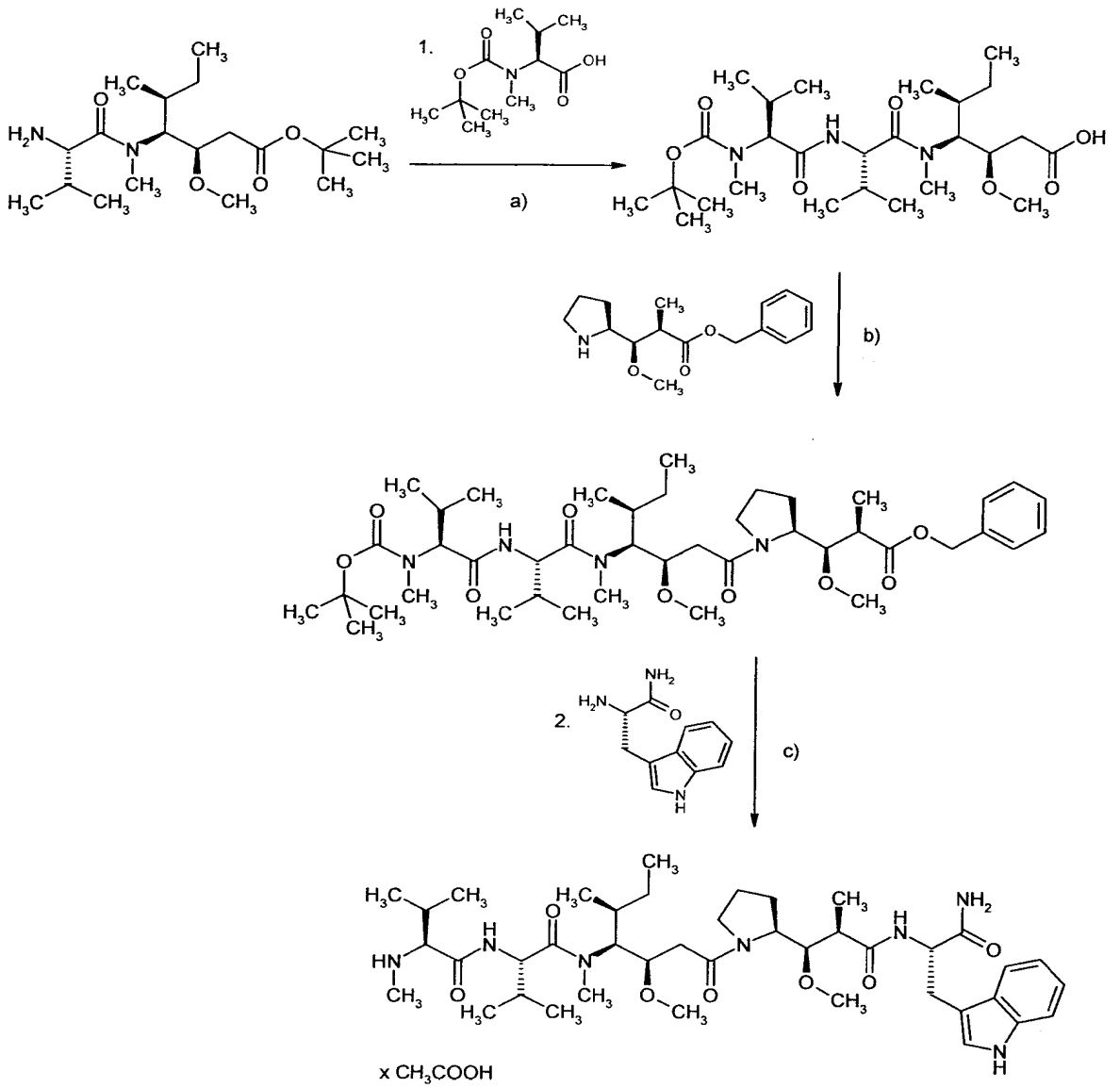
Alternatively, individual steps of the preparation sequence may be carried out in a different order or with other protective group combinations. This approach is illustrated by way of example in the synthesis schemes below (Scheme 17, 19, 20 and 21).

Scheme 17



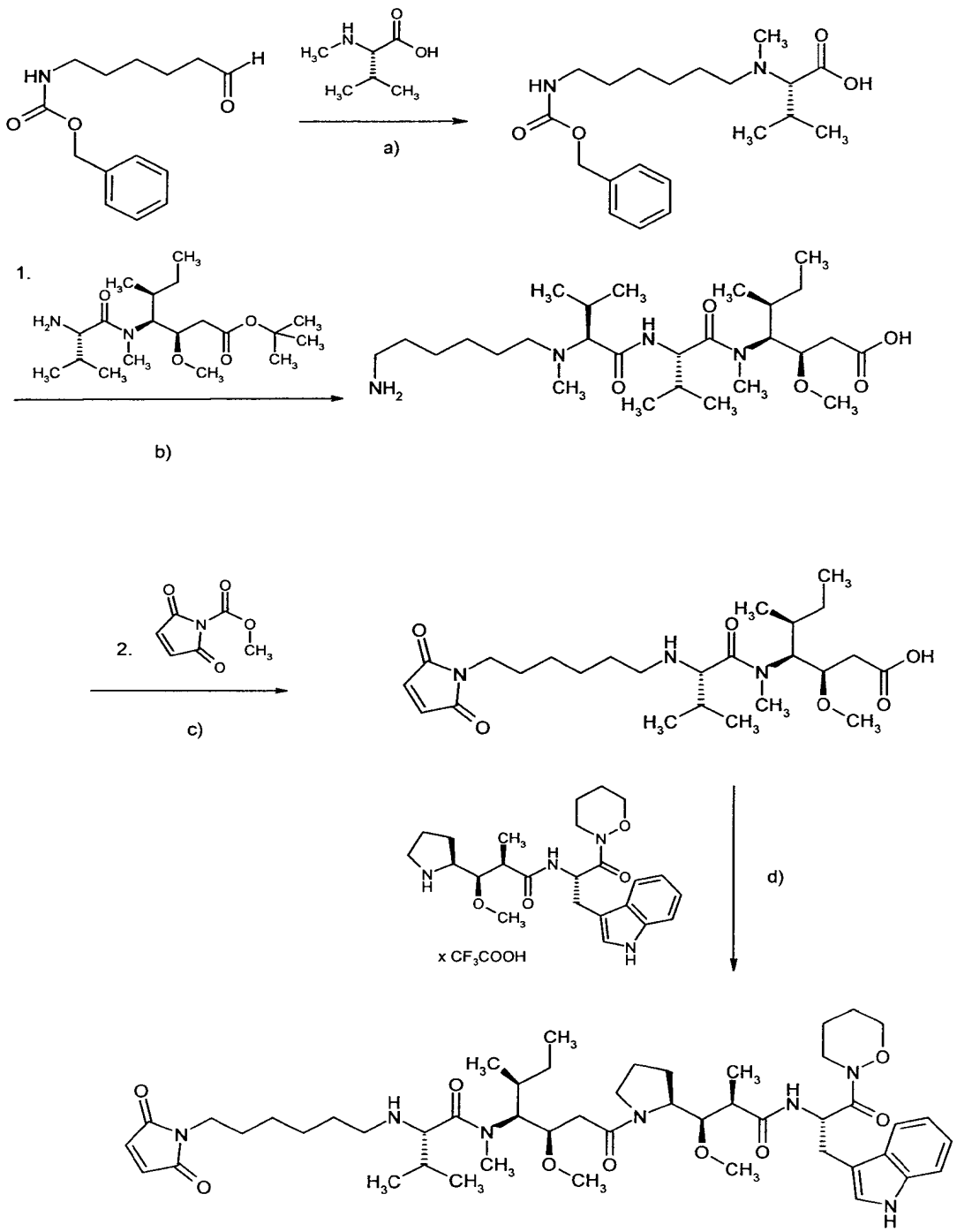
[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; c): HATU, diisopropylethylamine, DMF, RT; d): 1. Pd/C, MeOH, RT; 2. $NaHCO_3$, dioxane, water].

Scheme 19



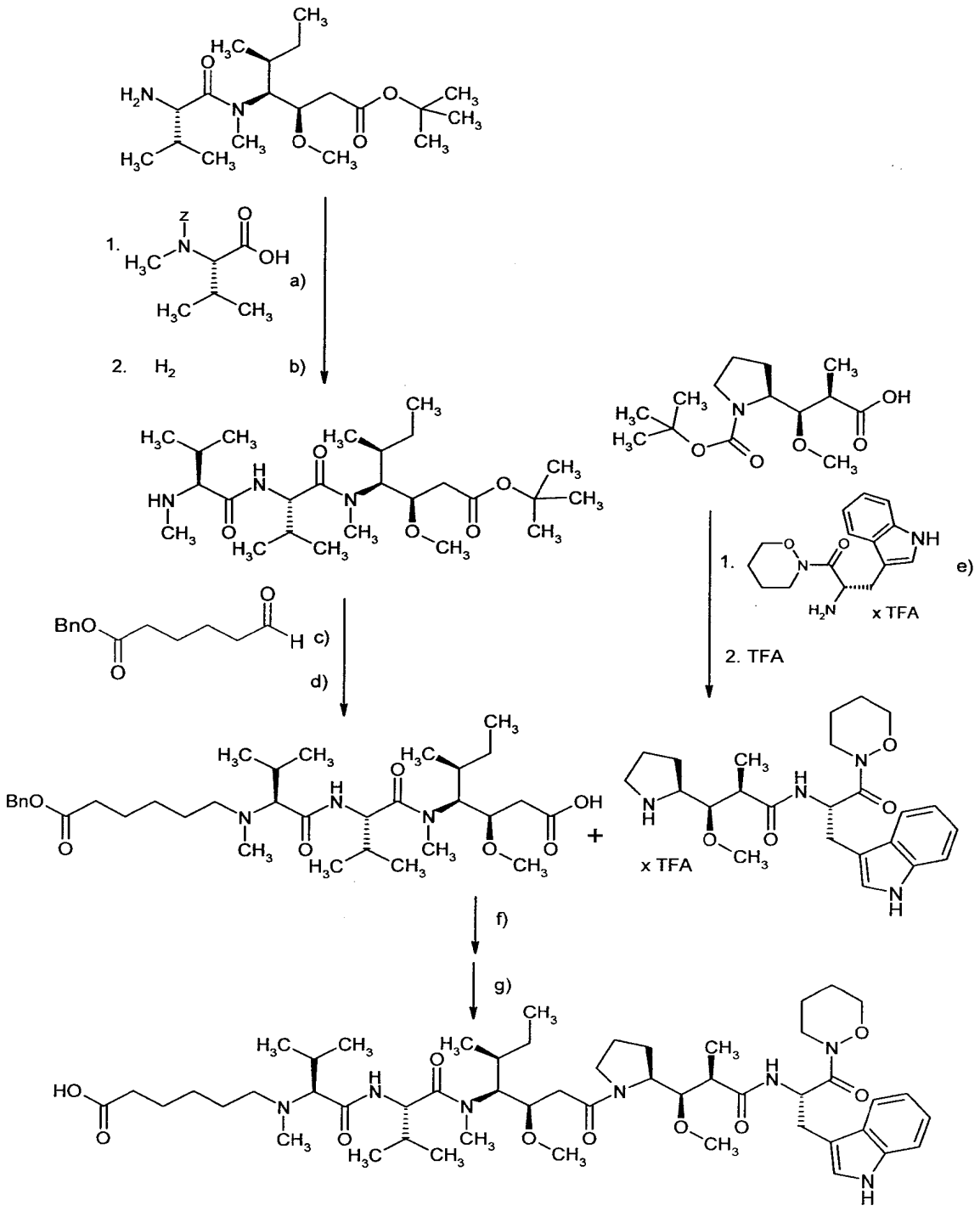
[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; 3. $((H_3C)_3C(C=O))_2O$, DMF, diisopropylethylamine; b): diisopropylethylamine, BEP, DMF, RT; c): 1. H_2, Pd/C (10%), methanol, RT; 2. HATU, diisopropylethylamine, DMF, RT; 3. TFA, dichloromethane, RT].

Scheme 20



[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; 3. H_2, Pd/C, MeOH, RT c): 1. $NaHCO_3$, dioxane, water; d): HATU, diisopropylethylamine, DMF, RT].

Scheme 21



[a): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; b) H_2, Pd/C, MeOH, RT; c) borane-pyridine complex, acetic acid, MeOH; d) TFA, dichloromethane, RT; e): HATU, diisopropylethylamine, DMF, RT; f): HATU, diisopropylethylamine, DMF, RT; g) H_2, Pd/C, MeOH, RT].

The FGFR2 cancer target molecule of the binder of the present invention is known to the skilled person. The full-length FGFR2 is identified as FGFR2 alpha (SEQ ID NO: 1), while the isoform lacking the D1 domain is identified as FGFR2 beta (SEQ ID NO: 2) (see Figure 1). Alternative splicing in domain 3 leads to two different variants, namely FGFR2 IIIb, which is encoded by the exons 7 and 8, and FGFR2 IIIc, which is encoded by the exons 7 and 9 (see Figure 1).

In one embodiment of the invention the binder binds – preferably specifically – to FGFR2. In a further subject of the invention, the binder binds – preferably specifically – to the extracellular domain of the target molecule FGFR2 (see Figure 1).

In a further embodiment of the invention, the binder binds – preferably specifically – to one or more forms of the human FGFR2 polypeptide. In a further subject of the invention, the binder binds – preferably specifically – to all isoforms and splice variants of FGFR2. In the text below, the concept of different "forms" of FGFR2 includes, though is not limited to, different isoforms, different splice variants, different glycoforms or FGFR2 polypeptides which undergo different translational and post-translational modifications.

In a further embodiment of the invention, the binder binds - preferably specifically – to the N-terminal domains of the cancer target molecule FGFR2. In a further subject of the invention, the binder binds – preferably specifically – to the extracellular N-terminal epitope (^{1}RPSFSLVEDTTLEPE^{15}) of FGFR2 (SEQ ID NO: 23).

In a further embodiment of the invention, the binder also binds – preferably specifically – to the FGFR2 of different species. The effect of this is that the conjugates of the invention can more easily, pharmacologically, be investigated in these species. Preferred species are rodents, more particularly mice or rats, but also dogs, pigs and non-human primates.

In one preferred embodiment the binder, after binding to FGFR2 on the target cell, is internalized by the target cell as a result of the binding. The effect of this is that the binder-drug conjugate, which may be an immunoconjugate or an ADC, is taken up by the target cell.

In one embodiment the binder is a binding protein. In one preferred embodiment the binder is an antibody, an antigen-binding antibody fragment, a multispecific antibody or an antibody mimetic.

Preferred antibody mimetics are affibodies, adnectins, anticalins, DARPins, avimers, or nanobodies. Preferred multispecific antibodies are bispecific and trispecific antibodies.

In one preferred embodiment the binder is an antibody or an antigen-binding antibody fragment, more preferably an isolated antibody or an isolated antigen-binding antibody fragment.

Preferred antigen-binding antibody fragments are Fab, Fab', $F(ab')_2$ and Fv fragments, diabodies, DAbs, linear antibodies and scFv. Particularly preferred are Fab, diabodies and scFv.

In one particularly preferred embodiment the binder is an antibody. Particularly preferred are monoclonal antibodies or antigen-binding antibody fragments thereof. Further particularly preferred are human, humanized or chimeric antibodies or antigen-binding antibody fragments thereof.

In one preferred embodiment, the antibody or the antigen-binding fragment comprises the amino acid sequence of the CDR sequences of the variable light and heavy chain of the antibody M048-D01-hIgG1.

In a further preferred embodiment, the antibody or the antigen-binding fragment comprises the amino acid sequence of the CDR sequences of the variable light and heavy chain of the antibody M048-D01-hIgG1 represented in SEQ ID NO: 15 (H-CDR1), SEQ ID NO: 16 (H-CDR2), SEQ ID NO: 17 (H-CDR3), SEQ ID NO: 18 (L-CDR1), SEQ ID NO: 19 (L-CDR2) and SEQ ID NO: 20 (L-CDR3).

In a further preferred embodiment, the antibody or the antigen-binding fragment comprises the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1 or M048-D01-hIgG1-b.

In a further preferred embodiment, the antibody or the antigen-binding fragment comprises the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1, represented in SEQ ID NO: 12 (Vl) and SEQ ID NO: 11 (Vh), or of the variable light and heavy chains of the antibody M048-D01-hIgG1-b, represented in SEQ ID NO: 14 (Vl) and SEQ ID NO: 13 (Vh).

In a further particularly preferred embodiment, the antibody or the antigen-binding fragment comprises the amino acid sequence of the variable light and heavy chain of the antibody M048-D01- hIgG1-b represented in SEQ ID NO: 14 (Vl) und SEQ ID NO: 13 (Vh).

In a further particularly preferred embodiment, the antibody comprises the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1-b represented in SEQ ID NO: 9 (light chain) and SEQ ID NO: 10 (heavy chain).

In a further particularly preferred embodiment, the antibody comprises the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1 represented in SEQ ID NO: 7 (light chain) and SEQ ID NO: 8 (heavy chain).

Examples of further FGFR2 antibodies are the GAL-FR21-mIgG1 (SEQ ID NO: 3 and SEQ ID NO: 4) and GAL-FR22-mIgG2a (SEQ ID NO: 5 and SEQ ID NO: 6) antibodies that are described in this invention. The two last-mentioned antibodies were constructed on the basis of WO2010/054265 from the variable regions, described therein, of the light (Vl) and heavy (Vh) chains of the antibodies GAL-FR21 (SEQ ID NO: 1 and SEQ ID NO: 4 from WO2010/054265) and GAL-FR22 (SEQ ID NO: 7 and SEQ ID NO: 8 from WO2010/054265), with the variable regions of GAL-FR21 having been reformatted into an mIgG1 format, while the variable regions of GAL-FR22 were reformatted into an mIgG2a format.

Antibodies or antigen-binding antibody fragments which bind cancer target molecules may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Binders for cancer target molecules may be acquired commercially or may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Further processes for preparing antibodies or antigen-binding antibody fragments are described in WO2007070538 (see page 22 "Antibodies"). The skilled person knows how processes such as phage display libraries (e.g. Morphosys HuCAL Gold) can be compiled and used for discovering antibodies or antigen-binding antibody fragments (see WO2007070538, page 24 ff and Example 1 on page 70, Example 2 on page 72). Further processes for preparing antibodies that use DNA libraries from B-cells are described for example on page 26 (WO2007070538). Processes for humanizing antibodies are described on page 30-32 of WO2007070538 and in detail in Queen, et al., Pros. Natl. Acad. Sci. USA 86:10029-10033, 1989 or in WO90/0786. Furthermore, processes for the recombinant expression of proteins in general and of antibodies in particular are known to the skilled person (see, for example, in Berger and Kimrnel (Guide to Molecular Cloning Techniques, Methods in Enzymology, Vo1. 152, Academic Press, Inc.); Sambrook, et al., (Molecular Cloning: A Laboratory Manual, (Second Edition, Cold Spring Harbor Laboratory Press; Cold Spring Harbor, N.Y.; 1989) Vol. 1-3); Current Protocols in Molecular Biolony, (F.M. Ausabel et al. [Eds.], Current Protocols, Green Publishing Associates, Inc./John Wiley & Sons, Inc.); Harlow et al., (Monocl0nal Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (19881, Paul [Ed.]); Fundamental Immunology, (Lippincott Williams & Wilkins (1998)); and Harlow, et al., (Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1998)). The skilled person knows the corresponding vectors, promoters and signal peptides which are necessary for the expression of a protein/antibody. Commonplace processes are also described in WO2007070538 on pages 41–45. Processes for preparing an IgG1 antibody are described for example in WO2007070538 in Example 6 on page 74 ff. Processes which allow the determination of the internalization of an antibody after binding to its antigen are known to the skilled person and are described for example in WO2007070538 on page 80. The skilled person is able to use the

processes described in WO2007070538 that have been used for preparing carboanhydrase IX (Mn) antibodies in analogy for the preparation of antibodies with different target molecule specificity.

Further examples of FGFR2 binders are the single chain Fv antibody fragments PRO-007 (binds FGFR2 with high affinity) and PRO-001 (binds FGFR3 with high affinity and FGFR2 with low affinity) described in WO2007144893.

The compounds of the invention possess valuable pharmacological properties and can be used for the prevention and treatment of diseases in humans and animals.

The binder-drug conjugates (ADCs) of the invention, of the formula (Ia), exhibit a high and specific cytotoxic activity with regard to tumour cells, as may be shown on the basis of the assays set out in the present experimental section (Section C). This high and specific cytotoxic activity on the part of the binder-drug conjugates (ADCs) of the invention, of the formula (Ia), is achieved through the appropriate combination of the new N,N-dialkylauristatin derivative and binder with linkers which exhibit not only an enzymatically, hydrolytically or reductively cleavable predetermined break point, for the release of the toxophores, but also no such predetermined break point. More particularly, through the use of stable linkers which have no enzymatically, hydrolytically or reductively cleavable predetermined break point for the release of the toxophores, and which, following uptake of the ADCs into the tumour cell and following complete intracellular, enzymatic breakdown of the antibody, still remain wholly or partly intact, the activity is confined very specifically to the tumour cell. Compatibility between ADCs and stable linkers presupposes, among other things, that the metabolites formed intracellularly can be formed with sufficient efficacy, are able to reach their target and are able there to develop their anti-proliferative activity on the target with sufficient potency, without being carried out of the tumour cell again beforehand by transporter proteins. Such a compatibility of the ADCs with a stabile linker chemistry and with the target in question offers an enlarged therapeutic window (see, e.g., L. Ducry, Bionconjugate Chem. 2010, 21-5; A.G. Polson, Cancer Res. 2009, 69, 2358).

More particularly, the binder-drug conjugates of the invention, of the formula (Ia), exhibit a high and specific cytotoxic activity with respect to tumour cells which express FGFR2. The activity with respect to tumour cells which do not express FGFR2 is significantly weaker at the same time.

On the basis of this profile of properties, the compounds of the invention are therefore suitable to a particular degree for the treatment of hyperproliferative diseases in humans and in mammals generally. The compounds are able on the one hand to inhibit, block, reduce or lower cell proliferation and cell division, and on the other hand to increase apoptosis.

The hyperproliferative diseases for the treatment of which the compounds of the invention can be employed include in particular the group of cancer and tumour diseases. In the context of the present invention, these are understood as meaning, in particular, the following diseases, but without being limited to them: mammary carcinomas and mammary tumours (ductal and lobular forms, also *in situ*, triple-negative, HER2-negative), tumours of the respiratory tract (parvicellular and non-parvicellular carcinoma, bronchial carcinoma), cerebral tumours (e.g. of the brain stem and of the hypothalamus, astrocytoma, medulloblastoma, ependymoma and neuro-ectodermal and pineal tumours), tumours of the digestive organs (oesophagus, carcinomas of the oesophagogastric junction (=EGJ), stomach (diffuse and intestinal forms), gall bladder, small intestine, large intestine, rectum), liver tumours (including hepatocellular carcinoma, cholangiocellular carcinoma and mixed hepatocellular and cholangiocellular carcinoma), tumours of the head and neck region (larynx, hypopharynx, nasopharynx, oropharynx, lips and oral cavity), skin tumours (squamous epithelial carcinoma, Kaposi sarcoma, malignant melanoma, Merkel cell skin cancer and non-melanomatous skin cancer), tumours of soft tissue (including soft tissue sarcomas, osteosarcomas, malignant fibrous histiocytomas, lymphosarcomas and rhabdomyosarcomas), tumours of the eyes (including intraocular melanoma and retinoblastoma), tumours of the endocrine and exocrine glands (e.g. thyroid and parathyroid glands, pancreas and salivary gland), tumours of the urinary tract (tumours of the bladder, penis, kidney, renal pelvis and ureter) and tumours of the reproductive organs (carcinomas of the endometrium, cervix, ovary, vagina, vulva and uterus in women and carcinomas of the prostate and testicles in men). These also include proliferative blood diseases in solid form and as circulating blood cells, such as lymphomas, leukaemias and myeloproliferative diseases, e.g. acute myeloid, acute lymphoblastic, chronic lymphocytic, chronic myelogenic and hair cell leukaemia, and also AIDS-correlated lymphomas, Hodgkin's lymphomas, non-Hodgkin's lymphomas, cutaneous T-cell lymphomas, Burkitt's lymphomas and lymphomas in the central nervous system.

Preferred hyperproliferative diseases for anti-FGFR2 binder-drug conjugates

Hyperproliferative diseases for the treatment of which the compounds of the invention can be preferably employed are FGFR2-expressing tumours, such as, for example, stomach carcinoma (intestinal and diffuse types), signet ring carcinoma, especially of diffuse type, oesophageal cancer, cancer of the oesophagogastric junction (EGJ), breast cancer, cancer of the large intenstine, colorectal carcinoma, rectal carcinoma, prostate cancer, kidney cancer, carcinomas of the head and neck region, pancreatic cancer, liver cancer, cervical carcinoma, ovarian carcinoma, endometrial carcinoma, more particularly of endometrioid type, of papillary serous type, or of clear cell subtype, lung cancer, more particularly non-small-cell lung carcinoma (NSCLC), adenocarcinoma, squamous carcinoma and pancreatic carcinoma.

These well-described diseases in humans can also occur with a comparable aetiology in other mammals and can be treated there with the compounds of the present invention.

In the context of this invention, the term "treatment" or "treat" is used conventionally and means the care, management and support of a patient with the aim of combatting, diminishing, attenuating or relieving a disease or health defect and of improving the living conditions which are adversely affected by this disease, such as in the case of a cancer disease.

The present invention thus further provides the use of the compounds of the invention for the treatment and/or prevention of diseases, in particular the abovementioned diseases.

The present invention furthermore provides the use of the compounds of the invention for the preparation of a medicament for the treatment and/or prevention of diseases, in particular the abovementioned diseases.

The present invention furthermore provides the use of the compounds of the invention in a method for the treatment and/or prevention of diseases, in particular the abovementioned diseases.

The present invention furthermore provides a method for the treatment and/or prevention of diseases, in particular the abovementioned diseases, using an effective amount of at least one of the compounds of the invention.

The compounds according to the invention can be employed by themselves or, if required, in combination with one or more other pharmacologically active substances, as long as this combination does not lead to undesirable and unacceptable side effects. The present invention furthermore therefore provides medicaments comprising at least one of the compounds of the invention and one or more further drugs, in particular for the treatment and/or prevention of the abovementioned diseases.

For example, the compounds of the present invention can be combined with known antihyperproliferative, cytostatic or cytotoxic substances for the treatment of cancer diseases. Suitable drugs in the combination which may be mentioned by way of example are as follows:

aldesleukin, alendronic acid, alfaferone, alitretinoin, allopurinol, aloprim, aloxi, altretamine, aminoglutethimide, amifostine, amrubicin, amsacrine, anastrozole, anzmet, aranesp, arglabin, arsenic trioxide, aromasin, 5-azacytidine, azathioprine, BCG or tice-BCG, bestatin, betamethasone acetate, betamethasone sodium phosphate, bexarotene, bleomycin sulphate, broxuridine, bortezomib, busulfan, calcitonin, campath, capecitabine, carboplatin, casodex, cefesone, celmoleukin, cerubidin, chlorambucil, cisplatin, cladribin, clodronic acid, cyclophosphamide,

cytarabine, dacarbazine, dactinomycin, daunoxome, decadron, decadron phosphate, delestrogen, denileukin diftitox, depomedrol, deslorelin, dexrazoxane, diethylstilbestrol, diflucan, docetaxel, doxifluridine, doxorubicin, dronabinol, DW-166HC, eligard, elitek, ellence, emend, epirubicin, epoetin-alfa, epogen, eptaplatin, ergamisol, estrace, estradiol, estramustine sodium phosphate, ethinylestradiol, ethyol, etidronic acid, etopophos, etoposide, fadrozole, farstone, filgrastim, finasteride, fligrastim, floxuridine, fluconazole, fludarabin, 5-fluorodeoxyuridine monophosphate, 5-fluorouracil (5-FU), fluoxymesterone, flutamide, formestane, fosteabine, fotemustine, fulvestrant, gammagard, gemcitabine, gemtuzumab, gleevec, gliadel, goserelin, granisetron hydrochloride, histrelin, hycamtin, hydrocortone, erythro-hydroxynonyladenine, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, interferon-alpha, interferon-alpha-2, interferon-alpha-2α, interferon-alpha-2β, interferon-alpha-n1, interferon-alpha-n3, interferon-beta, interferon-gamma-1α, interleukin-2, intron A, iressa, irinotecan, kytril, lentinan sulphate, letrozole, leucovorin, leuprolide, leuprolide acetate, levamisole, levofolic acid calcium salt, levothroid, levoxyl, lomustine, lonidamine, marinol, mechlorethamine, mecobalamin, medroxyprogesterone acetate, megestrol acetate, melphalan, menest, 6-mercaptopurine, mesna, methotrexate, metvix, miltefosine, minocycline, mitomycin C, mitotane, mitoxantrone, modrenal, myocet, nedaplatin, neulasta, neumega, neupogen, nilutamide, nolvadex, NSC-631570, OCT-43, octreotide, ondansetron hydrochloride, orapred, oxaliplatin, paclitaxel, pediapred, pegaspargase, pegasys, pentostatin, picibanil, pilocarpine hydrochloride, pirarubicin, plicamycin, porfimer sodium, prednimustine, prednisolone, prednisone, premarin, procarbazine, procrit, raltitrexed, rebif, rhenium-186 etidronate, rituximab, roferon-A, romurtide, salagen, sandostatin, sargramostim, semustine, sizofiran, sobuzoxane, solu-medrol, streptozocin, strontium-89 chloride, synthroid, tamoxifen, tamsulosin, tasonermin, tastolactone, taxoter, teceleukin, temozolomide, teniposide, testosterone propionate, testred, thioguanine, thiotepa, thyrotropin, tiludronic acid, topotecan, toremifen, tositumomab, tastuzumab, teosulfan, tretinoin, trexall, trimethylmelamine, trimetrexate, triptorelin acetate, triptorelin pamoate, UFT, uridine, valrubicin, vesnarinone, vinblastine, vincristine, vindesine, vinorelbine, virulizin, zinecard, zinostatin-stimalamer, zofran; ABI-007, acolbifen, actimmune, affinitak, aminopterin, arzoxifen, asoprisnil, atamestane, atrasentan, avastin, BAY 43-9006 (sorafenib), CCI-779, CDC-501, celebrex, cetuximab, crisnatol, cyproterone acetate, decitabine, DN-101, doxorubicin-MTC, dSLIM, dutasteride, edotecarin, eflornithine, exatecan, fenretinide, histamine dihydrochloride, histrelin hydrogel implant, holmium-166 DOTMP, ibandronic acid, interferon-gamma, intron-PEG, ixabepilone, keyhole limpet hemocyanine, L-651582, lanreotide, lasofoxifen, libra, lonafarnib, miproxifen, minodronate, MS-209, liposomal MTP-PE, MX-6, nafarelin, nemorubicin, neovastat, nolatrexed, oblimersen, onko-TCS, osidem, paclitaxel polyglutamate, pamidronate disodium, PN-401, QS-21, quazepam, R-1549, raloxifen, ranpirnas, 13-*cis*-retic acid, satraplatin, seocalcitol, T-138067, tarceva, taxoprexin, thymosin-

alpha-1, tiazofurin, tipifarnib, tirapazamine, TLK-286, toremifen, transMID-107R, valspodar, vapreotide, vatalanib, verteporfin, vinflunin, Z-100, zoledronic acid and combinations of these.

In a preferred embodiment, the compounds of the present invention can be combined with antihyperproliferative agents, which can be, by way of example – without this list being conclusive as follows:

aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine, bleomycin, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, diethylstilbestrol, 2',2'-difluorodeoxycytidine, docetaxel, doxorubicin (adriamycin), epirubicin, epothilone and its derivatives, erythro-hydroxynonyladenin, ethinylestradiol, etoposide, fludarabin phosphate, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, 5-fluorouracil, fluoxymesterone, flutamide, hexamethylmelamine, hydroxyurea, hydroxyprogesterone caproate, idarubicin, ifosfamide, interferon, irinotecan, leucovorin, lomustine, mechlorethamine, medroxyprogesterone acetate, megestrol acetate, melphalan, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitotane, mitoxantrone, paclitaxel, pentostatin, *N*-phosphonoacetyl L-aspartate (PALA), plicamycin, prednisolone, prednisone, procarbazine, raloxifen, semustine, streptozocin, tamoxifen, teniposide, testosterone propionate, thioguanine, thiotepa, topotecan, trimethylmelamine, uridine, vinblastine, vincristine, vindesine and vinorelbine.

The compounds of the invention can also be combined in a very promising manner with biological therapeutics such as antibodies (e.g. avastin, rituxan, erbitux, herceptin). The compounds of the invention can also achieve positive effects in combination with therapies directed against angiogenesis, such as, for example, with avastin, axitinib, recentin, regorafenib, sorafenib or sunitinib. Combinations with inhibitors of the proteasome and of mTOR and also with antihormones and steroidal metabolic enzyme inhibitors are likewise particularly suitable because of their favourable profile of side effects.

Generally, the following aims can be pursued with the combination of compounds of the present invention with other agents having a cytostatic or cytotoxic action:

- an improved activity in slowing down the growth of a tumour, in reducing its size or even in its complete elimination compared with treatment with an individual drug;
- the possibility of employing the chemotherapeutics used in a lower dosage than in monotherapy;

- the possibility of a more tolerable therapy with few side effects compared with individual administration;
- the possibility of treatment of a broader spectrum of tumour diseases;
- the achievement of a higher rate of response to the therapy;
- a longer survival time of the patient compared with present-day standard therapy.

The compounds according to the invention can moreover also be employed in combination with radiotherapy and/or surgical intervention.

The present invention furthermore provides medicaments which comprise at least one compound of the invention, conventionally together with one or more inert, non-toxic, pharmaceutically suitable excipients, and the use thereof for the abovementioned purposes.

The compounds of the invention can act systemically and/or locally. They can be administered in a suitable manner for this purpose, such as for example parenterally, possibly by means of inhalation, or as an implant or stent.

The compounds of the invention can be administered in suitable administration forms for these administration routes.

Parenteral administration can be effected with bypassing of an absorption step (e.g. intravenously, intraarterially, intracardially, intraspinally or intralumbally) or with inclusion of an absorption (e.g. intramuscularly, subcutaneously, intracutaneously, percutaneously or intraperitoneally). Administration forms which are suitable for parenteral administration include injection and infusion formulations in the form of solutions, suspensions, emulsions or lyophilizates. Parenteral administration is preferred, in particular intravenous administration.

In general, it has proved advantageous in the case of parenteral administration to administer amounts of from about 0.001 to 1 mg/kg, preferably about 0.01 to 0.5 mg/kg of body weight to achieve effective results.

Nevertheless it may be necessary to deviate from the amounts mentioned, and in particular depending on the body weight, administration route, individual behaviour towards the active compound, nature of the formulation and point of time or interval at which administration takes place. Thus in some cases it may be sufficient to manage with less than the abovementioned minimum amount, while in other cases the upper limit mentioned must be exceeded. In the case

where relatively large amounts are administered, it may be advisable to distribute these into several individual doses over the day.

The following examples illustrate the invention. The invention is not limited to the examples.

The percentage figures in the following tests and examples are percentages by weight, unless stated otherwise; parts are parts by weight. Solvent ratios, dilution ratios and concentration data of liquid/liquid solutions in each case relate to the volume.

A. Examples

Abbreviations and acronyms:

ABCB1	ATP-binding cassette sub-family B member 1 (synonym for P-gp and MDR1)
abs.	absolute
ADC	antibody-drug-conjugate
Ac	acetyl
aq.	aqueous, aqueous solution
ATP	adenosine triphosphate
BCRP	breast cancer resistance protein, an efflux transporter
Boc	*tert*-butoxycarbonyl
br.	broad (in NMR)
Ex.	example
ca.	*circa*, approximately
CAIX	carboanhydrase IX
CI	chemical ionization (in MS)
d	doublet (in NMR)
d	day(s)
TLC	thin-layer chromatography
DCI	direct chemical ionization (in MS)
dd	doublet of a doublet (in NMR)
DMAP	4-*N,N*-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMEM	Dulbecco's modified eagle medium (standardized nutrient medium for cell culture)
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulphoxide
DPBS, D-PBS, PBS	Dulbecco's phosphate-buffered saline solution PBS = DPBS = D-PBS, pH 7.4, from Sigma, No. D8537 Composition: 0.2 g KCl 0.2 g KH_2PO_4 (anhydrous) 8.0 g NaCl 1.15 g Na_2HPO_4 (anhydrous) make up to 1 l with H_2O

dt	doublet of a triplet (in NMR)
DTT	DL-dithiothreitol
EDC	*N'*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
EI	electron impact ionization (in MS)
ELISA	enzyme-linked immunosorbent assay
eq.	equivalent(s)
ESI	electrospray ionization (in MS)
ESI-MicroTofq	ESI- MicroTofq (name of the mass spectrometer, with Tof = time of flight and q = quadrupole)
FCS	foetal calf serum
FGFR2	fibroblast growth factor receptor 2
Fmoc	(9*H*-fluoren-9-ylmethoxy)carbonyl
sat.	saturated
GTP	guanosine 5'-triphosphate
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOAc	acetic acid
HOBt	1-hydroxy-1*H*-benzotriazole hydrate
HOSu	*N*-hydroxysuccinimide
HPLC	high-pressure, high-performance liquid chromatography
IC_{50}	half-maximum inhibitory concentration
i.m.	intramuscular, administration into the muscle
i.v.	intravenous, administration into the vein
Kato III	human tumour cell line
conc.	concentrated
LC-MS	liquid chromatography-coupled mass spectrometry
LLC-PK1 cells	Lewis lung carcinoma pork kidney cell line
L-MDR	human MDR1 transfected LLC-PK1 cells
m	multiplet (in NMR)
MDA-MB-231	human tumour cell line
MDR1	multidrug resistence protein 1

MFM-223	human tumour cell line
min	minute(s)
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
NCI-H716	human tumour cell line
NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance spectrometry
NMRI	mouse strain, originating from Naval Medical Research Institute (NMRI)
Nude mice	experimental animals
NSCLC	non-small cell lung cancer (non-parvicellular bronchial carcinoma)
PBS	phosphate-buffered saline solution
Pd/C	palladium on activated carbon
P-gp	P-glycoprotein, a transporter protein
PNGaseF	enzyme for sugar elimination
quant.	quantitative (for yield)
quart	quartet (in NMR)
quint	quintet (in NMR)
R_f	retention index (for TLC)
RT	room temperature
R_t	retention time (for HPLC)
s	singlet (in NMR)
s.c.	subcutaneous, administration beneath the skin
SCID mice	experimental mice with a severe combined immunodeficiency
SNU-16	human tumour cell line
SUM52-PE	human tumour cell line
t	triplet (in NMR)
tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UV	ultraviolet spectrometry
v/v	volume to volume ratio (of a solution)
Z	benzyloxycarbonyl

HPLC and LC-MS methods:

Method 1 (LC-MS):

Instrument: Waters Acquity SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8µ 50 mm x 1 mm; eluent A: 1 l water + 0.25 ml 99% strength formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% strength formic acid; gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A; flow rate: 0.40 ml/min; oven: 50°C; UV detection: 210-400 nm.

Method 2 (LC-MS):

Instrument: Micromass QuattroPremier with Waters UPLC Acquity; column: Thermo Hypersil GOLD 1.9µ 50 mm x 1 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 90% A → 0.1 min 90% A → 1.5 min 10% A → 2.2 min 10% A; flow rate: 0.33 ml/min; oven: 50°C; UV detection: 210 nm.

Method 3 (LC-MS):

Instrument: Micromass Quattro Micro MS with HPLC Agilent Series 1100; column: Thermo Hypersil GOLD 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 100% A → 3.0 min 10% A → 4.0 min 10% A → 4.01 min 100% A (flow rate 2.5 ml/min) → 5.00 min 100% A; oven: 50°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 4 (LC-MS):

MS instrument: Micromass ZQ; HPLC instrument: HP 1100 Series; UV DAD; column: Phenomenex Gemini 3µ 30 mm x 3.00 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 90% A → 2.5 min 30% A → 3.0 min 5% A → 4.5 min 5% A; flow rate: 0.0 min 1 ml/min → 2.5 min/3.0 min/4.5 min 2 ml/min; oven: 50°C; UV detection: 210 nm.

Method 5 (HPLC):

Instrument: HP 1090 Series II; column: Merck Chromolith SpeedROD RP-18e, 50 mm x 4.6 mm; preliminary column: Merck Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; injection volume: 5 µl; eluent A: 70% $HClO_4$ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 20% B → 0.50 min 20% B → 3.00 min 90% B → 3.50 min 90% B → 3.51 min 20% B → 4.00 min 20% B; flow rate: 5 ml/min; column temperature: 40°C.

Method 6 (HPLC):

Instrument: Waters 2695 with DAD 996; column: Merck Chromolith SpeedROD RP-18e, 50 mm x 4.6 mm; Ord. No.: 1.51450.0001, preliminary column: Merck Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; Ord. No.: 1.51470.0001, eluent A: 70% $HClO_4$ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 5% B → 0.50 min 5% B → 3.00 min 95% B → 4.00 min 95% B; flow rate: 5 ml/min.

Method 7 (LC-MS):

MS instrument: Waters ZQ; HPLC instrument: Agilent 1100 Series; UV DAD; column: Thermo Hypersil GOLD 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 100% A → 3.0 min 10% A → 4.0 min 10% A → 4.1 min 100% A (flow rate 2.5 ml/min); oven: 55°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 8 (LC-MS):

MS instrument: Waters ZQ; HPLC instrument: Agilent 1100 Series; UV DAD; column: Thermo Hypersil GOLD 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 100% A → 2.0 min 60% A → 2.3 min 40% A → 3.0 min 20% A → 4.0 min 10% A → 4.2 min 100% A (flow rate 2.5 ml/min); oven: 55°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 9 (LC-MS):

Instrument: Waters Acquity SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8µ 50 mm x 1 mm; eluent A: 1 l water + 0.25 ml 99% strength formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% strength formic acid; gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A; oven: 50°C; flow rate: 0.35 ml/min; UV detection: 210-400 nm.

Method 10 (HPLC):

Instrument: Agilent 1200 Series; column: Agilent Eclipse XDB-C18 5µ 4.6 mm x 150 mm; preliminary column: Phenomenex KrudKatcher Disposable Pre-Column; injection volume: 5 µl; eluent A: 1 l water + 0.01% trifluoroacetic acid; eluent B: 1 l acetonitrile + 0.01% trifluoroacetic acid; gradient: 0.00 min 10% B → 1.00 min 10% B → 1.50 min 90% B → 5.5 min 10% B; flow rate: 2 ml/min; column temperature: 30°C.

For all reactants or reagents whose preparation is not explicitly described below, they were obtained commercially from generally available sources. For all other reactants or reacents whose preparation is likewise not described below, and which were not available commercially or were obtained from sources which are not generally available, a reference is given to the published literature in which their preparation is described.

Method 11 (LC-MS):

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8 µ 30 x 2 mm; eluent A: 1 l water + 0.25 ml 99% strength formic acid , eluent B: 1 l acetonitrile + 0.25 ml 99% strength formic acid; gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A oven: 50°C; flow rate: 0.60 ml/min; UV detection: 208 – 400 nm.

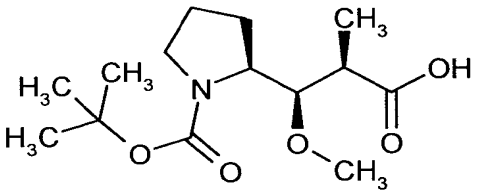
Method 12 (HPLC):

Instrument: Agilent 1200 Series with column oven and DAD; column: Merck Chromolith SpeedROD RP-18e, 50 mm x 4.6 mm; Ord. No.: 1.51450.0001; preliminary column: Merck Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; Ord. No.: 1.51470.0001; eluent A: 70% $HClO_4$ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 5% B → 0.50 min 5% B → 3.00 min 95% B → 4.00 min 95% B; flow rate: 5 ml/min; column temperature: 30°C.

Starting Compounds and Intermediates:

Starting Compound 1

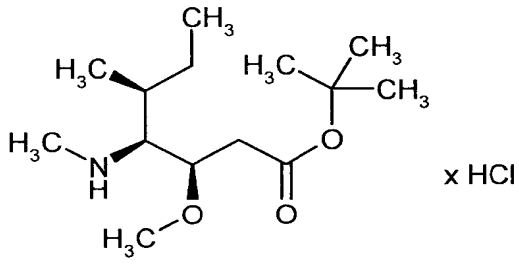
(2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Boc-dolaproine)



The title compound can be prepared in various ways according to literature methods; see, for example, Pettit *et al.*, *Synthesis* 1996, 719; Shioiri *et al.*, *Tetrahedron Lett.* 1991, *32*, 931; Shioiri *et al.*, *Tetrahedron* 1993, *49*, 1913; Koga *et al.*, *Tetrahedron Lett.* 1991, *32*, 2395; Vidal *et al.*, *Tetrahedron* 2004, *60*, 9715; Poncet *et al.*, *Tetrahedron* 1994, *50*, 5345. It was prepared either as the free acid or as a 1:1 salt with dicyclohexylamine.

Starting Compound 2a

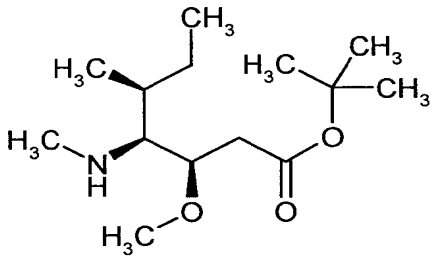
tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate hydrochloride (dolaisoleucine-OtBu x HCl)



The title compound can be prepared in various ways according to literature methods; see, for example, Pettit *et al.*, *J. Org. Chem.* 1994, *59*, 1796; Koga *et al.*, *Tetrahedron Lett.* 1991, *32*, 2395; Shioiri *et al.*, *Tetrahedron Lett.* 1991, *32*, 931; Shioiri *et al.*, *Tetrahedron* 1993, *49*, 1913.

Starting Compound 2b

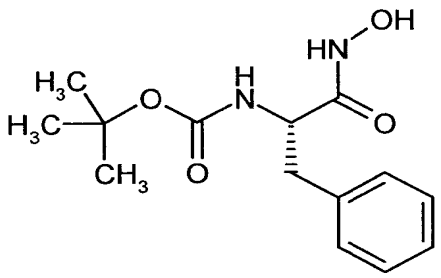
tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate (dolaisoleucine-O^{t}Bu)



The compound was prepared in analogy to starting compound 2a, except that the hydrogenation was performed without addition of 1N hydrochloric acid.

Starting Compound 3

*N*α-(*tert*-butoxycarbonyl)-*N*-hydroxy-L-phenylalaninamide



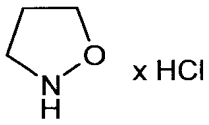
The title compound was prepared by the literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, *59*, 4602).

Yield: 750 mg (75% of theory)

LC-MS (Method 3): R_t = 1.67 min; MS (ESIpos): m/z = 281 $(M+H)^+$.

Starting Compound 4

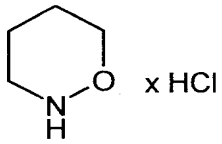
1,2-oxazolidine hydrochloride



The title compound can be prepared by literature methods (see, for example, H. King, *J. Chem. Soc.* 1942, 432); it is also commercially available.

Starting Compound 5

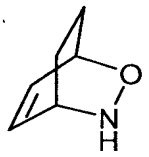
1,2-oxazinane hydrochloride



The title compound can be prepared by literature methods (see, for example, H. King, *J. Chem. Soc.* 1942, 432).

Starting Compound 6

2-oxa-3-azabicyclo[2.2.2]oct-5-ene

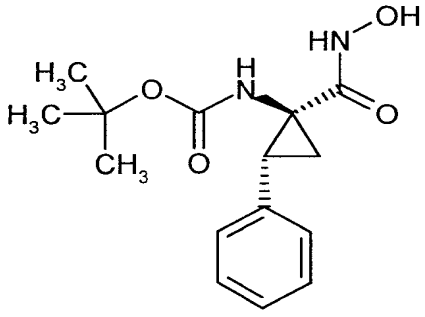


The title compound can be prepared in Boc-protected form by the literature method (see, for example, C. Johnson *et al.*, *Tetrahedron Lett.* 1998, *39*, 2059); the deprotection was effected in a customary manner by treatment with trifluoroacetic acid and subsequent neutralization.

Yield: 149 mg (89% of theory)

Starting Compound 7

tert-butyl (1*S*,2*R*)-1-(hydroxycarbamoyl)-2-phenylcyclopropyl carbamate



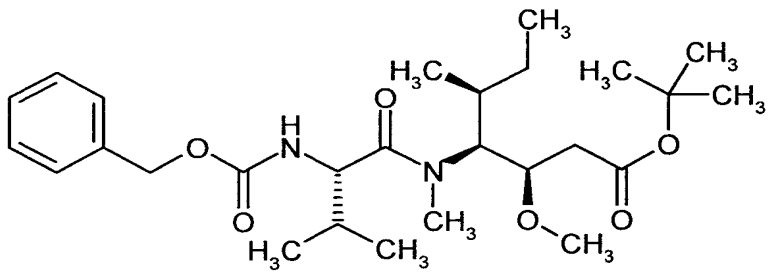
The title compound was prepared by a literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, *59*, 4602) proceeding from commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid (C. Cativiela *et al.*, *Chirality* 1999, 11, 583).

Yield: 339 mg (59% of theory)

LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 293 $(M+H)^+$.

Intermediate 1

tert-butyl (3*R*,4*S*,5*S*)-4-[{*N*-[(benzyloxy)carbonyl]-L-valyl}(methyl)amino]-3-methoxy-5-methylheptanoate



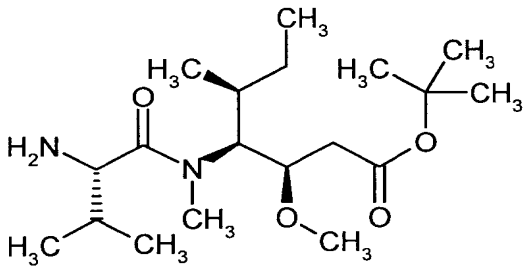
10.65 g (41.058 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate (starting compound 2b) were taken up in 250 ml of dichloromethane and the solution was cooled to -10°C. Then, while stirring, 10.317 g (41.058 mmol) of N-[(benzyloxy)carbonyl]-L-valine, 16.866 g (61.586 mmol) of 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) and 28.6 ml of *N,N*-diisopropylethylamine were added, and the mixture was subsequently stirred at RT for 20 h. The reaction mixture was then diluted with dichloromethane and shaken twice with saturated sodium chloride solution, dried over sodium sulphate, filtered and concentrated. The residue was purified by flash chromatography on silica gel with 4:1 petroleum ether/ethyl acetate as the eluent. The corresponding fractions were concentrated and the residue was dried under high vacuum overnight. 10.22 g (51% of theory) of the title compound were obtained as a yellowish oil.

HPLC (Method 5): R_t = 2.3 min;

LC-MS (Method 2): R_t = 1.59 min; MS (ESIpos): m/z = 493 $(M+H)^+$.

Intermediate 2

tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoate



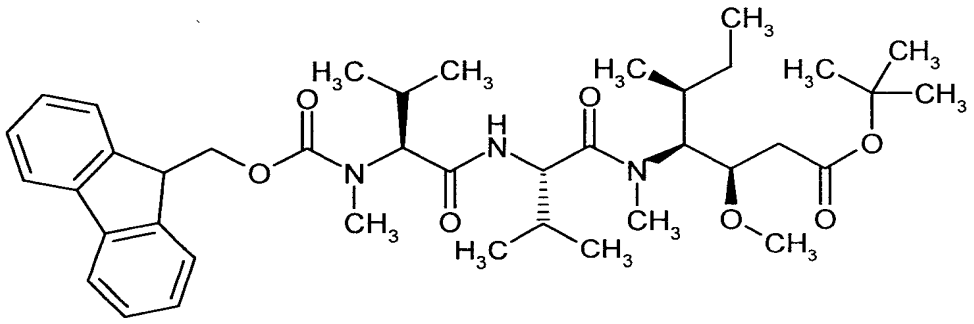
500 mg (1 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-4-[{*N*-[(benzyloxy)carbonyl]-L-valyl}(methyl)amino]-3-methoxy-5-methylheptanoate (intermediate 1) were dissolved in 50 ml of methanol and, after addition of 100 mg of 10% palladium on activated carbon, hydrogenated under standard hydrogen pressure at RT for 1 h. The catalyst was then filtered off and the solvent was removed under reduced pressure. This gave 370 mg (quant.) of the title compound as a virtually colourless oil.

HPLC (Method 5): R_t = 1.59 min;

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 359 $(M+H)^+$.

Intermediate 3

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



4.64 g (13.13 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valine were dissolved in 20 ml of DMF and admixed successively with 4.28 g (11.94 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoate (Intermediate 2), 2.75 g (14.33 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 2.2 g (14.33 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium

sulphate, filtered and concentrated. The residue was used directly in the next stage, without further purification.

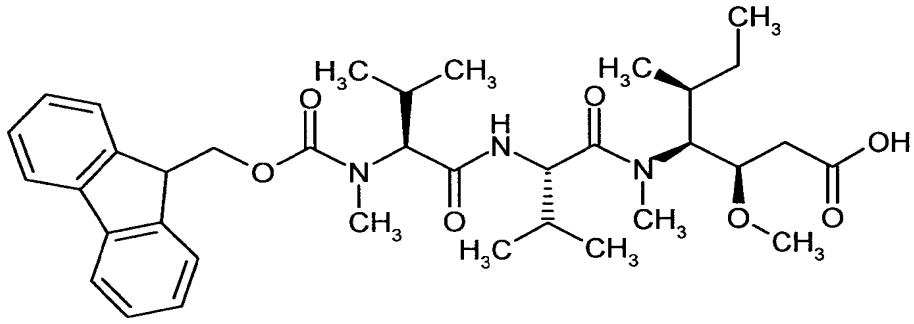
Yield: 9.1 g (quant., 60% purity)

HPLC (Method 5): R_t = 2.7 min;

LC-MS (Method 2): R_t = 1.99 min; MS (ESIpos): m/z = 694 $(M+H)^+$.

Intermediate 4

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



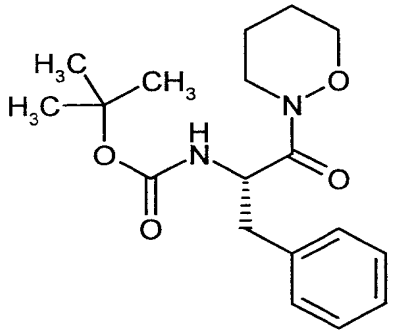
9.1 g of the crude product *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 3) were taken up in 56.6 ml of dichloromethane, 56.6 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was purified by flash chromatography, using dichloromethane, 3:1 dichloromethane/ethyl acetate and 15:5:0.5 dichloromethane/ethyl acetate/methanol as eluent. After purification of the corresponding fractions and concentration, 5.8 g (86% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.2 min;

LC-MS (Method 1): R_t = 1.3 min; MS (ESIpos): m/z = 638 $(M+H)^+$.

Intermediate 5

tert-butyl (2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl carbamate



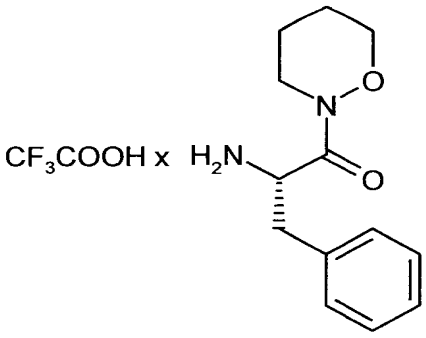
500 mg (1.9 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were dissolved in 10 ml of DMF and admixed successively with 466 mg (3.8 mmol) of 1,2-oxazinane hydrochloride (Starting Compound 5), 433 mg (2.3 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 382 mg (2.8 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate and 731 mg (5.7 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. 620 mg (98% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 2): R_t = 1.62 min; MS (ESIpos): m/z = 235 $(M-C_4H_8-CO_2+H)^+$.

Intermediate 6

(2*S*)-2-amino-1-(1,2-oxazinan-2-yl)-3-phenylpropan-1-one trifluoroacetate



620 mg (1.85 mmol) of *tert*-butyl (2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl carbamate (Intermediate 5) were taken up in 5 ml of dichloromethane, 10 ml of trifluoroacetic acid were added and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was lyophilized from

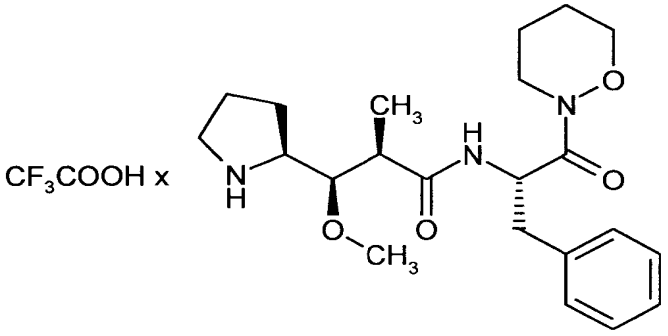
water/acetonitrile. In this way, 779 mg (91% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 0.45 min;

LC-MS (Method 3): R_t = 1.09 min; MS (ESIpos): m/z = 235 $(M+H)^+$.

Intermediate 7

(2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate



360 mg (1.25 mmol) of (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) were taken up in 10 ml of DMF and admixed successively with 579.2 mg (1.25 mmol) of (2*S*)-2-amino-1-(1,2-oxazinan-2-yl)-3-phenylpropan-1-one trifluoroacetate (Intermediate 6), 714.5 mg (1.88 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and 655 µl of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 16 h. The reaction mixture was then concentrated, and the residue was taken up in ethyl acetate and extracted by shaking first with 5% aqueous citric acid solution, then with 5% aqueous sodium hydrogencarbonate solution and subsequently with saturated sodium chloride solution. The organic phase was concentrated and the residue was purified by flash chromatography on silica gel with 16:4 dichloromethane/methanol as the eluent. The corresponding fractions were combined and the solvent was removed under reduced pressure. After the residue had been dried under high vacuum, 503.5 mg (74% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.12 min; MS (ESIpos): m/z = 504 $(M+H)^+$.

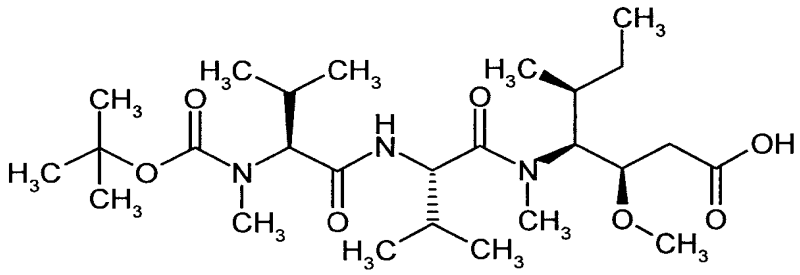
503 mg (1 mmol) of this intermediate were taken up in 20 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and redistilled with dichloromethane. The remaining residue was precipitated from ethyl acetate with n-pentane, and the solvent was decanted off. The residue thus obtained was dissolved in water and extracted by shaking with ethyl acetate, and the aqueous phase was subsequently lyophilized. In this way, 462 mg (89% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): R_t = 1.53 min;

LC-MS (Method 11): R_t = 0.57 min; MS (ESIpos): m/z = 404 $(M+H)^+$.

Intermediate 8

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



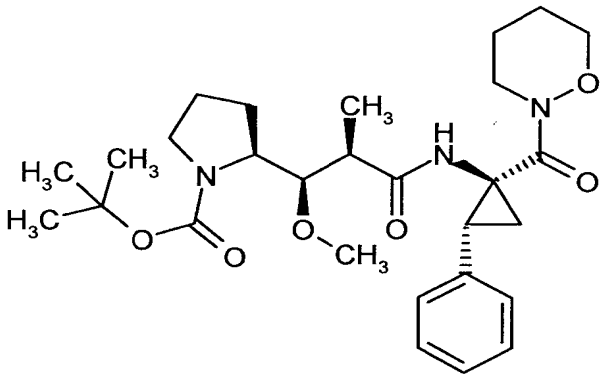
51 mg (0.08 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were dissolved in 10 ml of DMF, and 0.5 ml of piperidine was added. After stirring at RT for 10 min, the reaction mixture was concentrated under reduced pressure and the residue was stirred with diethyl ether. The insoluble constituents were filtered off and washed repeatedly with diethyl ether. Then the filter residue was taken up in 5 ml of dioxane/water and the solution was adjusted to pH 11 with 1 N sodium hydroxide solution. Under ultrasound treatment, a total of 349 mg (1.6 mmol) of di-*tert*-butyl dicarbonate were added in several portions, in the course of which the pH of the solution was kept at 11. After the reaction had ended, the dioxane was evaporated off and the aqueous solution was adjusted to a pH of 2-3 with citric acid. The mixture was extracted twice with 50 ml each time of ethyl acetate. The organic phases were combined, dried over magnesium sulphate and concentrated under reduced pressure. The residue was taken up in diethyl ether and the of the title compound was precipitated with pentane. The solvent was removed by decantation. The residue was digested several times more with pentane and finally dried under high vacuum. 40 mg (97% of theory) of the title compound were thus obtained.

HPLC (Method 6): R_t = 2.2 min;

LC-MS (Method 2): R_t = 1.32 min; MS (ESIpos): m/z = 516 $(M+H)^+$.

Intermediate 9

tert-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate



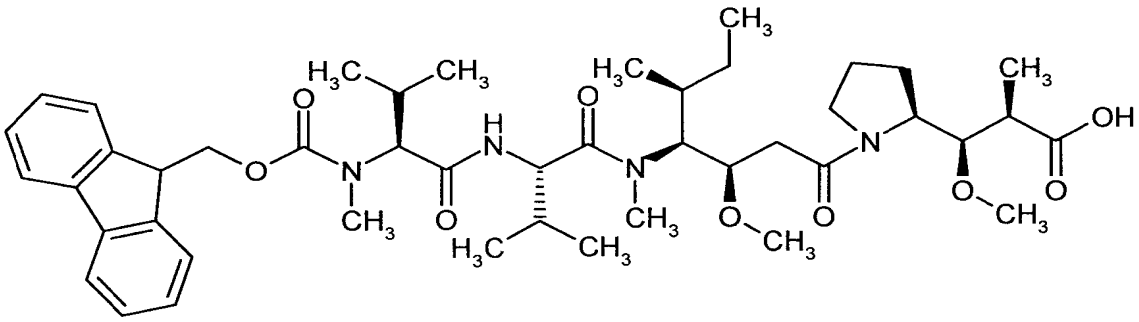
The title compound was prepared in analogy to the synthesis of Intermediates 5, 6 and 7 over three stages, by coupling of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with 1,2-oxazinane hydrochloride (Starting Compound 5), subsequent deprotection with trifluoroacetic acid and coupling with Starting Compound 1. The end product was purified by preparative HPLC.

HPLC (Method 5): R_t = 2.12 min;

LC-MS (Method 2): R_t = 1.25 min; MS (ESIpos): m/z = 516 $(M+H)^+$.

Intermediate 10

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



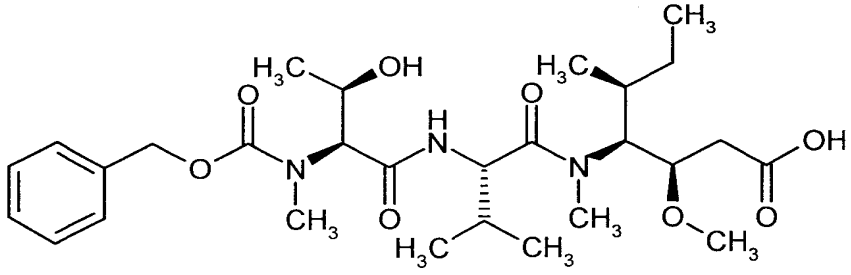
315 mg (0.494 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were dissolved in 12 ml of DMF, and admixed with 104 mg (0.543 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 83 mg (0.543 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate, and the mixture was stirred at RT for 90 min. Subsequently, 112 µl of *N*,*N*-diisopropylethylamine and 149 mg (0.494 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoic acid trifluoroacetate, which had been prepared beforehand from Starting Compound 1 by elimination of the Boc protecting group by means of trifluoroacetic acid, were added. The mixture was stirred at RT for 2 h and then concentrated under high vacuum. The remaining residue was purified twice by preparative HPLC. 140 mg (35% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 5): R_t = 2.40 min;

LC-MS (Method 1): R_t = 1.38 min; MS (ESIpos): m/z = 807 $(M+H)^+$.

Intermediate 11

N-[(benzyloxy)carbonyl]-*N*-methyl-L-threonyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



First, *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine was released from 237 mg (0.887 mmol) of its dicyclohexylamine salt thereof by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and

concentrated. The residue was taken up in 16 ml of DMF and admixed successively with 365 mg (1 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoate (Intermediate 2), 185 mg (0.967 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 148 mg (0.967 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was stirred at RT for 2 h. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. The residue was purified by preparative HPLC. 283 mg (53% of theory) of the *tert*-butyl ester intermediate *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were thus obtained.

HPLC (Method 5): R_t = 2.17 min.

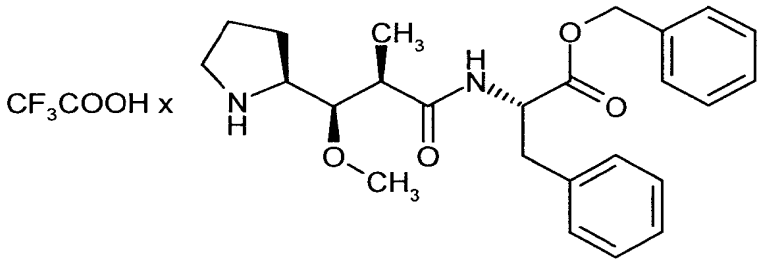
283 mg (0.466 mmol) of this intermediate were taken up in 5 ml of dichloromethane, 5 ml of anhydrous trifluoroacetic acid were added, and the mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated under high vacuum and the remaining residue was purified by means of preparative HPLC. This gave 156 mg (61% of theory) of the title compound as a colourless foam.

HPLC (Method 5): R_t = 1.50 min;

LC-MS (Method 2): R_t = 1.09 min; MS (ESIpos): m/z = 552 $(M+H)^+$.

Intermediate 12

benzyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate



In the first step, Starting Compound 1 was released from 600 mg (1.28 mmol) of the corresponding dicyclohexylammonium salt by dissolving the salt in 100 ml of ethyl acetate and extractive shaking, first with 50 ml of 0.5% sulphuric acid and then with saturated sodium chloride solution.

Then the organic phase was dried over magnesium sulphate, filtered, concentrated and reacted immediately with benzyl L-phenylalaninate in analogy to the synthesis of Intermediate 7, and then deprotected.

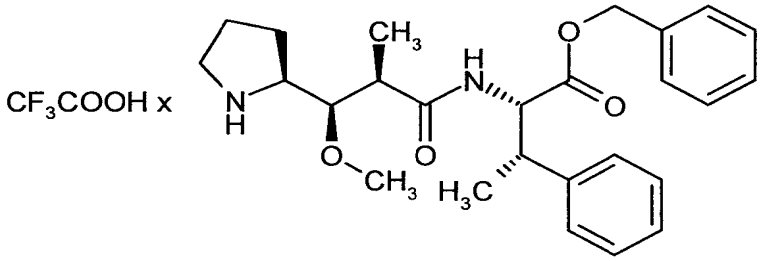
Yield: 650 mg (94% over 2 stages)

HPLC (Method 6): R_t = 1.76 min;

LC-MS (Method 2): R_t = 1.68 min; MS (ESIpos): m/z = 425 $(M+H)^+$.

Intermediate 13

benzyl (β*S*)-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-β-methyl-L-phenylalaninate trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid was released from 351 mg (0.75 mmol) of the dicyclohexylamine salt (Starting Compound 1) by taking it up in ethyl acetate and extractive shaking with aqueous 5% potassium hydrogensulphate solution. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and admixed successively with 373 mg (0.75 mmol) of benzyl (β*S*)-β-methyl-L-phenylalaninate trifluoroacetate [prepared from commercially available (β*S*)-*N*-(*tert*-butoxycarbonyl)-β-methyl-L-phenylalanine by EDC/DMAP-mediated esterification with benzyl alcohol and subsequent detachment of the Boc protecting group with trifluoroacetic acid], 428 mg (1.125 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 392 µl of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 20 h. The reaction mixture was then poured onto a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, and subsequently concentrated. The residue was purified by means of preparative HPLC. This gave 230 mg (57% of theory) of the Boc-protected intermediate benzyl (β*S*)-*N*-{(2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl}-β-methyl-L-phenylalaninate.

HPLC (Method 6): R_t = 2.3 min;

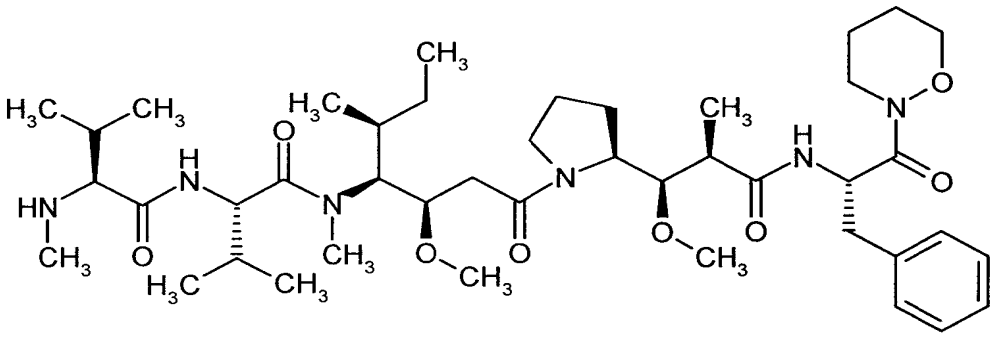
LC-MS (Method 1): R_t = 1.36 min; MS (ESIpos): m/z = 539 $(M+H)^+$.

230 mg (0.42 mmol) of this intermediate were taken up in 5 ml of dichloromethane, 5 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure. The remaining residue was the reaction mixture dried further under reduced pressure and then lyophilized from acetonitrile/water. In this way, 230 mg (quant.) of the title compound were obtained.

HPLC (Method 6): R_t = 1.6 min.

Intermediate 14

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



x CF_3COOH

143 mg (0.223 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 15 ml of DMF and admixed successively with 141 mg (0.22 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 7), 102 mg (0.27 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and 128 µl (0.74 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 3 h. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. This gave 275 mg (quant.) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-

ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 5): R_t = 2.73 min;

LC-MS (Method 4): R_t = 3.19 min; MS (ESIpos): m/z = 1023 $(M+H)^+$.

46 mg (0.045 mmol) of this intermediate were dissolved in 4 ml of DMF. After 1 ml of piperidine had been added, the reaction mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by means of preparative HPLC (eluent: acetonitrile + 0.01% TFA / water + 0.01% TFA). 22 mg (54% of theory) of the title compound were obtained as a colourless foam.

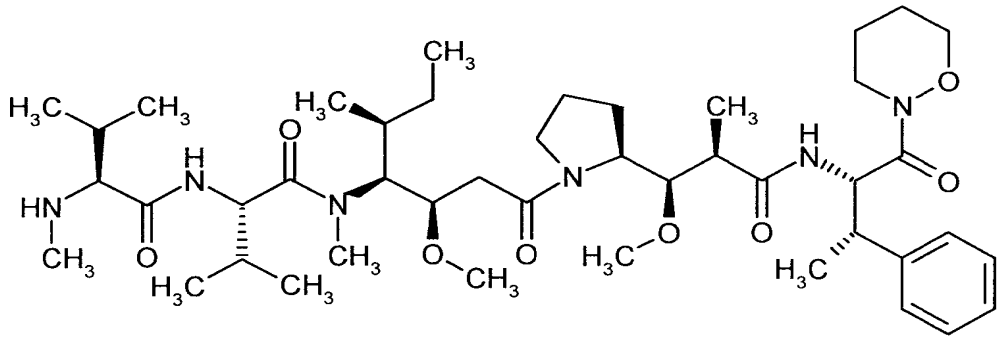
HPLC (Method 5): R_t = 1.68 min;

LC-MS (Method 2): R_t = 1.03 min; MS (ESIpos): m/z = 801 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.8 (m, 2H), 8.7 (m, 1H), 8.42 and 8.15 (2d, 1H), 7.3-7.1 (m, 5H), 5.12 and 4.95 (2m, 1H), 4.70 and 4.62 (2m, 1H), 4.62 and 4.50 (2t, 1H), 4.1-3.9 (m, 3H), 3.85 (m, 1H), 3.75-3.6 (m, 2H), 3.23, 3.18, 3.17, 3.14, 3.02 and 2.96 (6s, 9H), 3.1-2.9 and 2.75 (2m, 2H), 2.46 (m, 3H), 2.4-2.1 (m, 2H), 2.05 (br. m, 2H), 1.85-1.55 (br. m, 6H), 1.5-1.2 (br. m, 3H), 1.1-0.8 (m, 18H), 0.75 (t, 3H) [further signals hidden under H_2O peak].

Intermediate 15

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



x CF_3COOH

126 mg (0.198 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 10 ml of DMF and admixed successively with 105 mg (0.198 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 17), 41.6 mg (0.217 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 33 mg (0.217 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate and 79 µl (0.454 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. This gave 220 mg (quant.) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 5): R_t = 2.77 min;

LC-MS (Method 1): R_t = 1.5 min; MS (ESIpos): m/z = 1037 $(M+H)^+$.

220 mg (0.212 mmol) of this intermediate were dissolved in 5 ml of DMF. After 1 ml of piperidine had been added, the reaction mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by means of preparative HPLC (eluent: acetonitrile + 0.01% TFA / water + 0.01% TFA). 91 mg (46% of theory) of the title compound were obtained as a colourless foam.

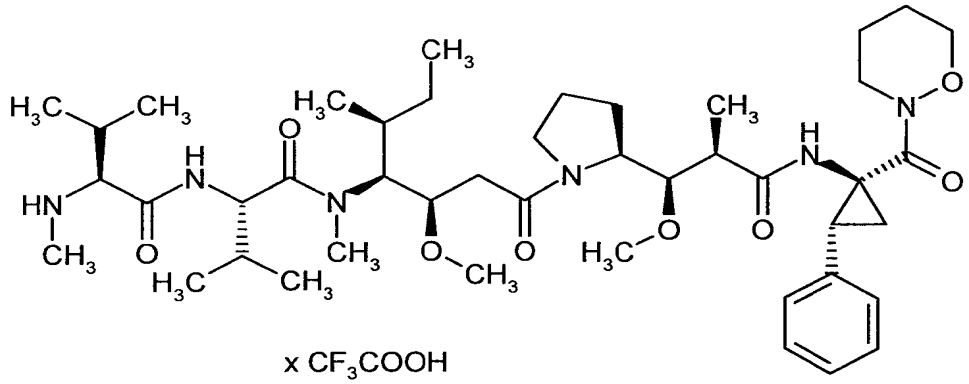
HPLC (Method 5): R_t = 1.71 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 815 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.87 and 8.80 (2d, 2H), 8.75 (m, 1H), 8.40 and 7.98 (2d, 1H), 7.3-7.1 (m, 5H), 5.45 and 5.2 (2t, 1H), 4.78 and 4.62 (2m, 1H), 4.73 and 4.58 (2t, 1H), 4.2-4.0 (m, 3H), 3.7-3.6 (m, 1H), 3.35, 3.20, 3.18, 3.14, 3.12 and 3.00 (6s, 9H), 3.1 and 2.95 (2m, 2H), 2.46 (m, 3H), 2.4-2.0 (m, 4H), 1.9-1.6 (m, 4H), 1.6-1.2 (m, 5H), 1.1-0.75 (m, 21H), 0.80 (t, 3H) [further signals hidden under H_2O peak].

Intermediate 16

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



617 mg (1.2 mmol) of *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate (Intermediate 24) were taken up in 44 ml of dichloromethane, 4.4 ml of trifluoroacetic acid were added and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was lyophilized from dioxane/water. 702 mg (quant.) of the deprotected compound (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate were obtained as a crude product, which was used in the following stage without further purification.

470 mg (0.74 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 57 ml of DMF and admixed successively with 390 mg (approx. 0.74 mmol) of the above-obtained (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate, 336 mg (0.88 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and 423 µl (2.4 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 2 h. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over sodium sulphate, filtered and concentrated. The residue was purified by preparative HPLC. This gave 453 mg (59% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-

[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 5): R_t = 2.58 min;

LC-MS (Method 1): R_t = 3.10 min; MS (ESIpos): m/z = 1035 $(M+H)^+$.

453 mg (0.438 mmol) of this intermediate were dissolved in 24 ml of DMF. After 2.4 ml of piperidine had been added, the reaction mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (eluent: acetonitrile / 0.1% TFA in water). 260 mg (64% of theory) of the title compound were obtained as a colourless foam.

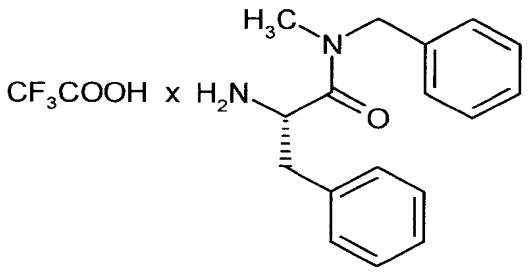
HPLC (Method 5): R_t = 1.64 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 813 $(M+H)^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.8 (m, 2H), 8.65 (m, 2H), 7.3-7.1 (m, 5H), 4.8-4.05 (m, 2H), 4.0 and 3.82 (2m, 2H), 3.8-3.5 (m, 8H), 3.32, 3.29, 3.20, 3.19, 3.12 and 3.00 (6s, 9H), 2.65 (t, 1H), 2.5-2.45 (m, 3H), 2.4-1.3 (m, 15H), 1.15-0.85 (m, 18H), 0.8 and 0.75 (2d, 3H) [further signals hidden under H_2O peak].

Intermediate 17

N-benzyl-*N*-methyl-L-phenylalaninamide trifluoroacetate



1000 mg (3.77 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were dissolved in 10 ml of DMF and admixed with 457 mg (3.77 mmol) of *N*-methylbenzylamine, 2150 mg (5.65 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 657 µl of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 30 min and then concentrated under reduced pressure. The residue was taken up in dichloromethane and extracted by shaking three times with water. The organic phase was dried over magnesium sulphate and concentrated.

The residue was purified by flash chromatography on silica gel with 3:1 petroleum ether/ethyl acetate as the eluent. The product fractions were concentrated and the residue was dried under high vacuum. This gave 1110 mg (75% of theory) of the Boc-protected intermediate *N*-benzyl-N^{α}-(*tert*-butoxycarbonyl)-*N*-methyl-L-phenylalaninamide.

HPLC (Method 6): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.14 min; MS (ESIpos): m/z = 369 $(M+H)^+$.

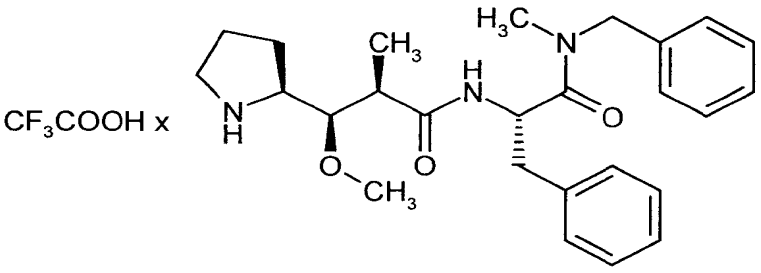
1108 mg (3,007 mmol) of this intermediate were taken up in 30 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure, the remaining residue was stirred with dichloromethane and the solvent was distilled off. The residue was stirred twice more with pentane, the solvent was decanted off again each time and the of the title compound was finally dried under high vacuum. 1075 mg (93% of theory) of the title compound were thus obtained as a resin.

HPLC (Method 6): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.6 min; MS (ESIpos): m/z = 269 $(M+H)^+$.

Intermediate 18

N-benzyl-N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-*N*-methyl-L-phenylalaninamide trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) was released from 141 mg (0.491 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and 187.6 mg (0.49 mmol) of *N*-benzyl-*N*-methyl-L-phenylalaninamide trifluoroacetate (Intermediate 9), 190.3 mg (1.47 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 256 µl of *N,N*-diisopropylethylamine were

added. The mixture was stirred at RT for 1 h. The reaction mixture was then concentrated, the residue was taken up in ethyl acetate and the solution was subsequently extracted by shaking successively with saturated ammonium chloride solution, saturated sodium hydrogencarbonate solution and water. The organic phase was dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography on silica gel with 30:1 acetonitrile/water as the eluent. The product fractions were concentrated and the residue was dried under high vacuum. This gave 168 mg (64% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidine-1-carboxylate.

HPLC (Method 6): R_t = 2.2 min;

LC-MS (Method 2): R_t = 1.22 min; MS (ESIpos): m/z = 538 $(M+H)^+$.

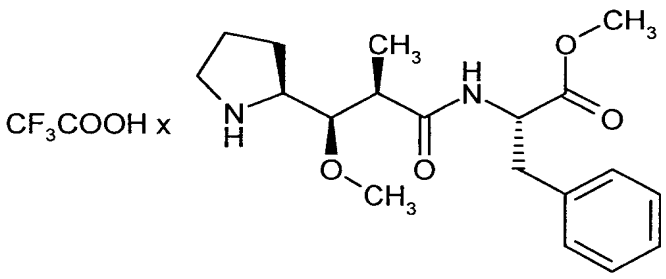
168 mg (0.312 mmol) of this intermediate were taken up in 15 ml of dichloromethane, 3 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure. The remaining residue was stirred first with dichloromethane, then with diethyl ether, and the solvent was distilled off again each time. After drying under high vacuum, 170 mg (99% of theory) of the title compound were obtained as a resin.

HPLC (Method 6): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.73 min; MS (ESIpos): m/z = 438 $(M+H)^+$.

Intermediate 19

methyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate



The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid

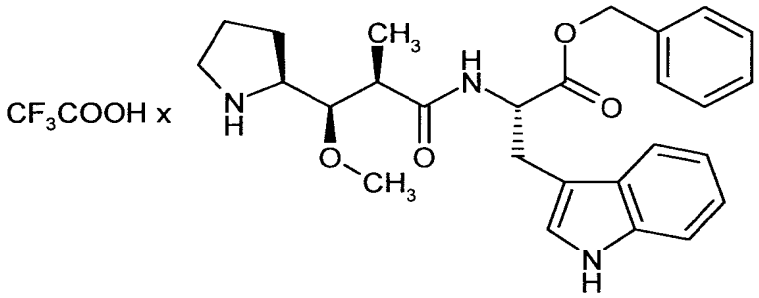
(Starting Compound 1), which was released from the dicyclohexylamine salt, and methyl L-phenylalaninate hydrochloride.

HPLC (Method 5): R_t = 0.6 min;

LC-MS (Method 3): R_t = 1.17 min; MS (ESIpos): m/z = 349 $(M+H)^+$.

Intermediate 20

benzyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanate trifluoroacetate



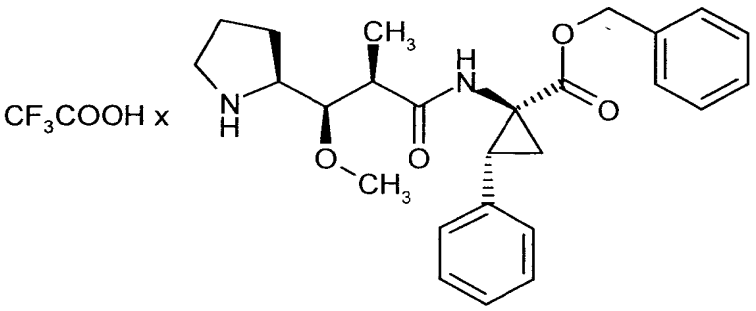
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl L-tryptophanate.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 464 $(M+H)^+$.

Intermediate 21

benzyl (1*S*,2*R*)-1-({(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}amino)-2-phenylcyclopropanecarboxylate trifluoroacetate



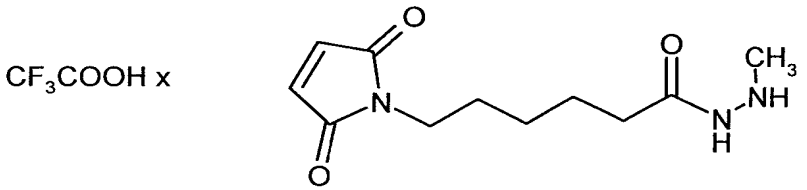
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate. Benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate had been prepared beforehand by standard methods, by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with benzyl alcohol and subsequent Boc detachment with trifluoroacetic acid.

HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 2): R_t = 0.93 min; MS (ESIpos): m/z = 437 $(M+H)^+$.

Intermediate 22

6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-N'-methylhexanehydrazide trifluoroacetate



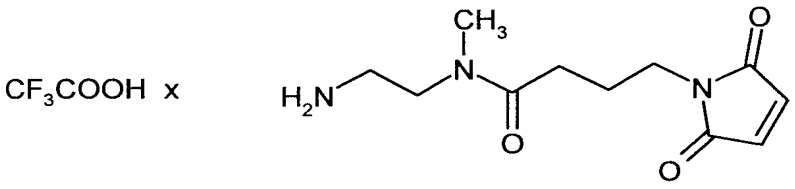
100 mg (473 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid were dissolved in 71 µl of DMF and then admixed with 139 mg (947 µmol) of tert-butyl 1-methylhydrazinecarboxylate, 182 mg (947 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 145 mg (947 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was stirred at RT overnight and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 129 mg (80% of theory) of the protected intermediate were obtained as a colourless foam.

Subsequently, the 129 mg (380 µmol) were deblocked with 2 ml of trifluoroacetic acid in 8 ml of dichloromethane. After stirring at RT for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was lyophilized from acetonitrile/water, which left 125 mg (83% of theory) of the title compound as a colourless foam.

LC-MS (Method 1): R_t = 0.38 min; MS (ESIpos): m/z = 240 $(M+H)^+$

Intermediate 23

N-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-N-methylbutanamide trifluoroacetate



First, 35 mg (164 µmol) of tert-butyl 2-(methylamino)ethyl carbamate hydrochloride trifluoroacetate, 30 mg (164 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 75 mg (197 µmol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 57 µl of *N,N*-diisopropylethylamine were combined in 5 ml of DMF and stirred at RT overnight. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane/water, 35 mg (63% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 1.6 min;

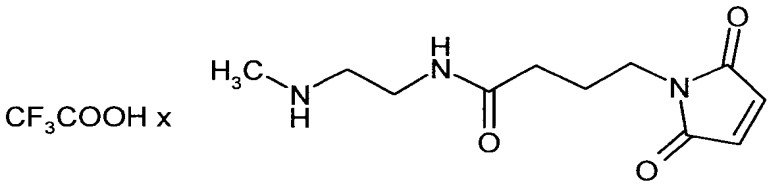
LC-MS (Method 1): R_t = 0.71 min; MS (ESIpos): m/z = 340 $(M+H)^+$.

Subsequently, the entire amount of the protected intermediate was deblocked with 1 ml of trifluoroacetic acid in 5 ml of dichloromethane to obtain 28 mg (77% of theory) of the title compound.

LC-MS (Method 3): R_t = 0.75 min; MS (ESIpos): m/z = 240 $(M+H)^+$.

Intermediate 24

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-N-[2-(methylamino)ethyl]butanamide trifluoroacetate



First, 35 mg (164 µmol) of tert-butyl (2-aminoethyl)methyl carbamate hydrochloride trifluoroacetate, 30 mg (164 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 75 mg (197 µmol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 57 µl of *N,N*-diisopropylethylamine were combined in 5 ml of DMF and stirred at RT for 30 min. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and,

by lyophilization from dioxane/water, 51 mg (91% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 1.6 min;

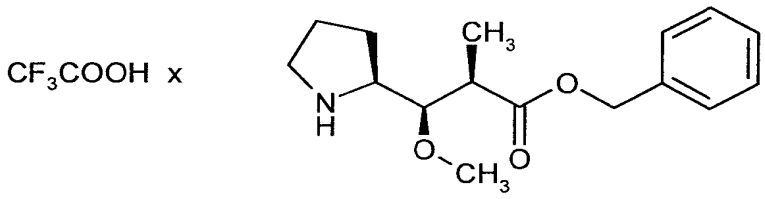
LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 340 $(M+H)^+$.

Subsequently, the entire amount was deprotected with 1 ml of trifluoroacetic acid in 5 ml of dichloromethane to obtain 45 mg (69% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.19 min; MS (ESIpos): m/z = 240 $(M+H)^+$.

Intermediate 25

benzyl (2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoate trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid was released from 1.82 g (388 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 100 ml of 0.5% sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of dioxane and 10 ml of water, 1517 mg (4.66 mmol) of caesium carbonate were added, and the mixture was treated in an ultrasound bath for 5 min and concentrated under reduced pressure and redistilled once with DMF. The residue was then taken up in 15 ml of dichloromethane, and 1990 mg (11.64 mmol) of benzyl bromide were added. The mixture was treated in an ultrasound bath for 15 min and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water, and the organic phase was removed and extracted by shaking with saturated sodium chloride solution and then concentrated. The residue was then purified by preparative HPLC. This gave 1170 mg (80% of theory) of the Boc-protected intermediate.

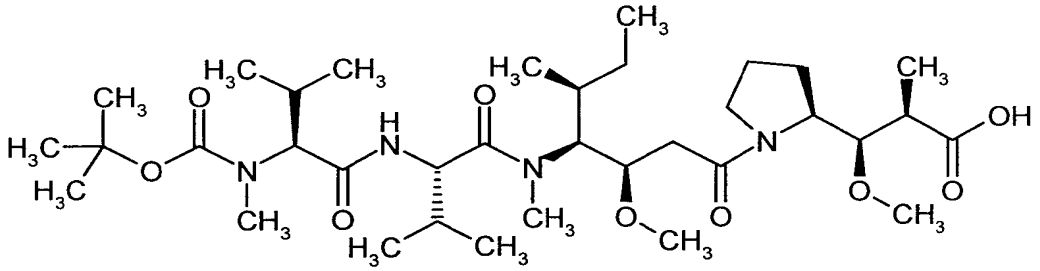
Subsequently, these 1170 mg were deprotected immediately with 5 ml of trifluoroacetic acid in 15 ml of dichloromethane. After stirring at RT for 15 min, the reaction mixture was concentrated under reduced pressure. The residue was lyophilized from dioxane. After drying under high vacuum, there remained 1333 mg (84% of theory) of the title compound as a yellow oil.

HPLC (Method 6): R_t = 1.5 min;

LC-MS (Method 1): R_t = 0.59 min; MS (ESIpos): m/z = 278 $(M+H)^+$.

Intermediate 26

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



1200 mg (2.33 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 5) were combined with 910.8 mg (2.33 mmol) of benzyl (2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoate trifluoroacetate (Intermediate 14), 1327 mg (3.49 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 2027 µl of *N*,*N*-diisopropylethylamine in 50 ml of DMF, and the mixture was stirred at RT for 5 min. Thereafter, the solvent was removed under reduced pressure. The remaining residue was taken up in ethyl acetate and extracted by shaking successively with 5% aqueous citric acid solution and saturated sodium hydrogencarbonate solution. The organic phase was removed and concentrated. The residue was purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 1000 mg (55% of theory) of the benzyl ester intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-(benzyloxy)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide as a resin.

LC-MS (Method 1): R_t = 1.56 min; MS (ESIpos): m/z = 775 $(M+H)^+$.

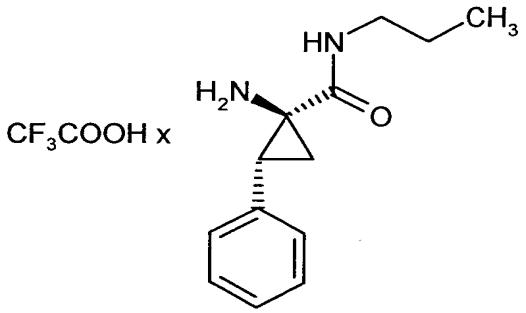
The entire amount of this intermediate obtained was taken up in 25 ml of a mixture of methanol and dichloromethane (20:1), and the benzyl ester group was removed by hydrogenation under standard hydrogen pressure with 10% palladium on activated carbon as a catalyst. After stirring at RT for 30 min, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. This gave 803 mg (91% of theory) of the title compound as a white solid.

HPLC (Method 6): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.24 min; MS (ESIpos): m/z = 685 $(M+H)^+$.

Intermediate 27

(1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide trifluoroacetate



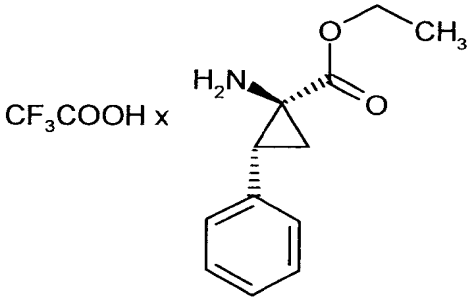
The title compound was prepared by coupling commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with *n*-propylamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and subsequent Boc detachment with trifluoroacetic acid (yield: 85% of theory over both stages).

HPLC (Method 6): R_t = 1.2 min;

LC-MS (Method 1): R_t = 0.52 min; MS (ESIpos): m/z = 219 $(M+H)^+$.

Intermediate 28

ethyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate

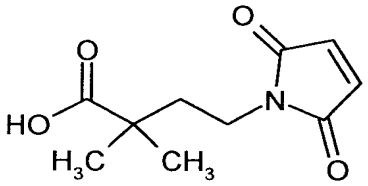


The title compound was prepared by standard methods, by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with ethanol and subsequent Boc detachment with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.50 min; MS (ESIpos): m/z = 206 $(M+H)^+$.

Intermediate 29

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoic acid

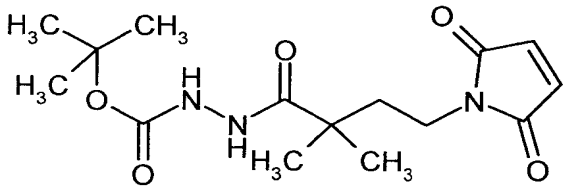


To a solution of 1.39 g (8.95 mmol) of N-methoxycarbonylmaleimide in 44 ml of saturated sodium hydrogencarbonate solution were added, at 0°C, 1.5 g (8.95 mmol) of 4-amino-2,2-dimethylbutyric acid, and the mixture was stirred for 40 min. Subsequently, the cooling bath was removed and the reaction mixture was stirred for a further 1 h. While cooling with ice, the reaction mixture was then adjusted to pH 3 by adding sulphuric acid, and extracted with ethyl acetate. The combined organic phases were dried over magnesium sulphate and concentrated. 1.17 g (79% purity, 49% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.64 min; m/z = 212 $(M+H)^+$.

Intermediate 30

tert-butyl 2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazinecarboxylate

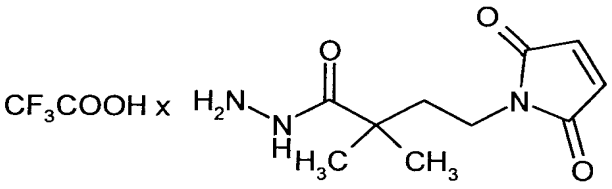


To a solution of 50 mg (237 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoic acid in 2 ml of THF were added, at 0°C, first 26 µl (237 µmol) of 4-methylmorpholine and then 31 µl (237 µmol) of isobutyl chloroformate. After removing the cooling bath and stirring at RT for a further 15 min, 31.3 mg (237 µmol) of tert-butyloxycarbonyl hydrazide were added. The reaction mixture was stirred overnight and then concentrated. The residue was purified by preparative HPLC. 50.8 mg (66% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.71 min; m/z = 324 $(M-H)^-$.

Intermediate 31

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate

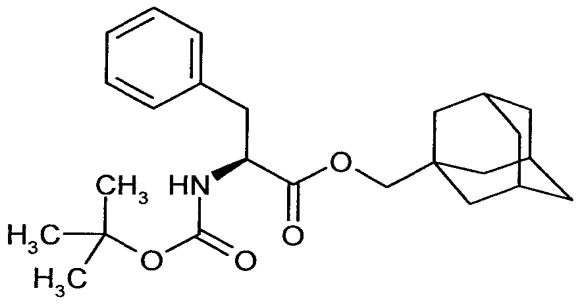


50 mg (154 mmol) of *tert*-butyl 2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazinecarboxylate were dissolved in 2 ml of dichloromethane, and 0.4 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated. 55.2 mg (93% purity, 99% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.36 min; m/z = 226 $(M+H)^+$.

Intermediate 32

adamantan-1-ylmethyl *N*-(*tert*-butoxycarbonyl)-L-phenylalaninate

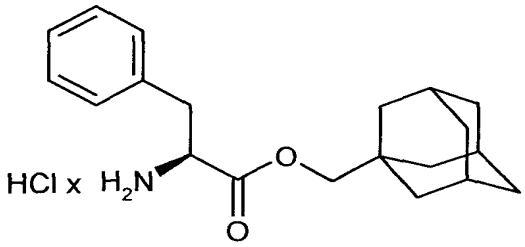


To a solution of 500 mg (1.89 mmol) of *N*-Boc-L-phenylalanine in 25 ml of dichloromethane were added, at RT, 1192 mg (6.2 mmol) of EDC, 578 µl (4.1 mmol) of triethylamine, 345 mg (2.8 mmol) of DMAP and 345 mg (2.1 mmol) of 1-adamantylmethanol. The reaction mixture was stirred overnight, then diluted with 50 ml of dichloromethane, and was successively washed with 10% aqueous citric acid solution, water and saturated sodium chloride solution. The organic phase was dried over magnesium sulphate, then concentrated, and the residue was purified by preparative HPLC. 769 mg (90% of theory) of the title compound were obtained.

LC-MS (Method 2): R_t = 1.84 min; m/z = 414 $(M+H)^+$.

Intermediate 33

adamantan-1-ylmethyl *L*-phenylalaninate hydrochloride

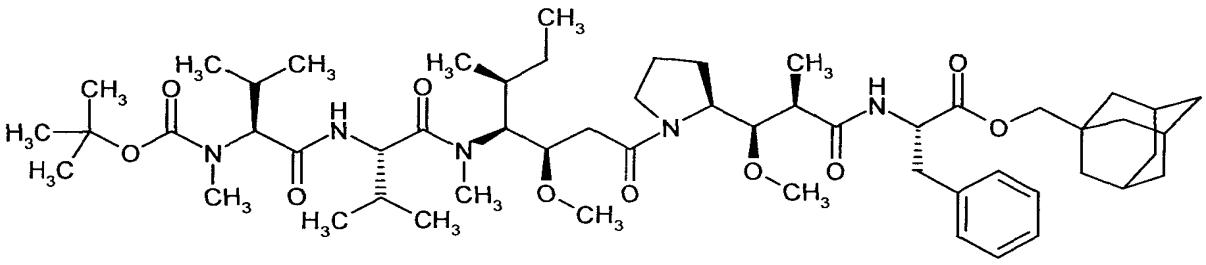


769 mg (1.86 mmol) of adamantan-1-ylmethyl N-(*tert*-butoxycarbonyl)-*L*-phenylalaninate (Intermediate 13) were dissolved in 25 ml of a 4 N solution of hydrogen chloride in dioxane and stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated and the residue was dried under reduced pressure. 619 mg (95% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.82 min; m/z = 314 $(M+H)^+$.

Intermediate 34

N-(*tert*-butoxycarbonyl)-N-methyl-*L*-valyl-N-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide

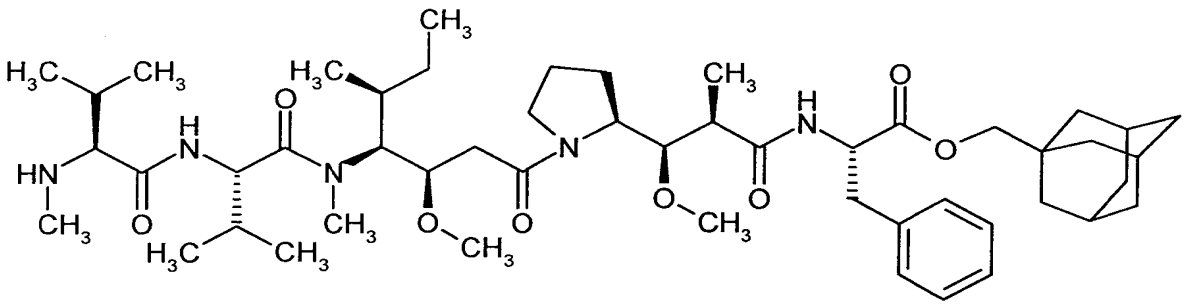


To a solution of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added, at RT, 15.3 µl (88 µmol) of *N,N*-diisopropylethylamine, 6.7 mg (44 µmol) of HOBt and 6.7 mg (35 µmol) of EDC, and the mixture was stirred for 30 min. Subsequently, 10.1 mg (32 µmol) of adamantan-1-yl *L*-phenylalaninate hydrochloride were added. After stirring overnight, the reaction mixture was separated directly into its components via preparative HPLC. 27.5 mg (93% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 1.70 min; m/z = 980 $(M+H)^+$.

Intermediate 35

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



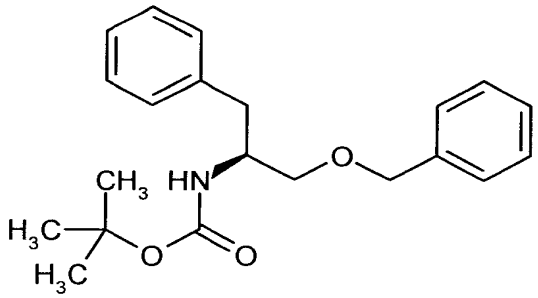
x CF_3COOH

27.5 mg (28 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1.8 ml of dichloromethane, and 361 µl of TFA were added. The reaction mixture was stirred for 30 min and then concentrated. The residue was taken up in water and lyophilized. 22.7 mg (81% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 1.14 min; m/z = 880 $(M+H)^+$.

Intermediate 36

tert-butyl (2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl carbamate



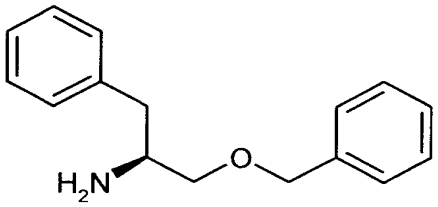
Under an argon atmosphere, 500 mg (1.99 mmol) of *N*-Boc-*L*-phenylalaninol were dissolved in 5 ml of DMF and cooled to 0°C. Subsequently, 159 mg (3.98 mmol) of a 60% suspension of sodium hydride in paraffin oil were added. The reaction mixture was stirred until the evolution of gas had ended and then 260 µl (2.19 mmol) of benzyl bromide were added. The cooling bath was removed

and the reaction mixture was stirred at RT for 2 h. Thereafter, the reaction mixture was concentrated, the residue was taken up in ice-water and the mixture was extracted with dichloromethane. The organic phase was washed with saturated sodium chloride solution, dried over magnesium sulphate and concentrated. The residue was purified by means of preparative HPLC. 226 mg (33% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 1.28 min; m/z = 342 $(M+H)^+$.

Intermediate 37

(2*S*)-1-(benzyloxy)-3-phenylpropan-2-amine hydrochloride

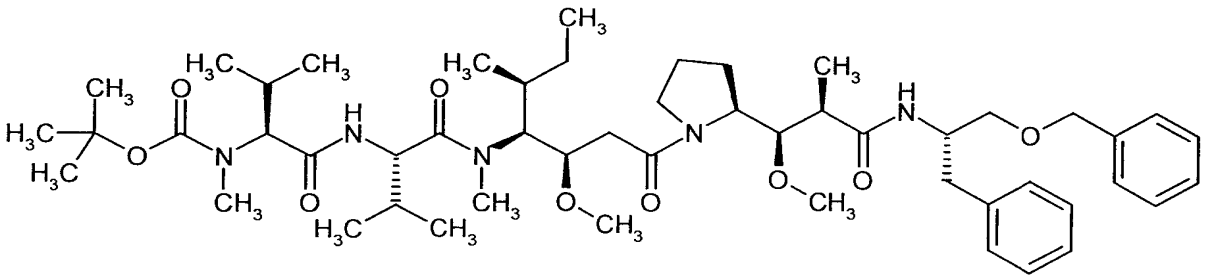


220 mg (644 µmol) of *tert*-butyl (2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl carbamate were dissolved in 11 ml of a 4 N solution of hydrogen chloride in dioxane and stirred at RT for 1 h. Then the reaction mixture was concentrated and the residue was dried under reduced pressure. 138 mg (77% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.65 min; m/z = 242 $(M+H)^+$.

Intermediate 38

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

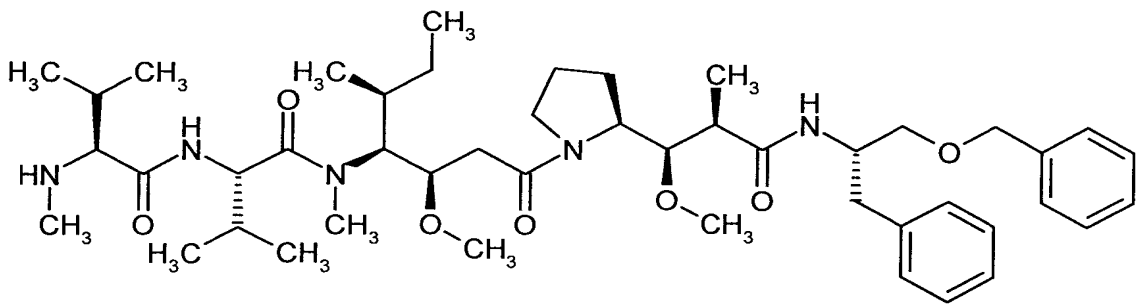


To a solution of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added, at RT, 15.3 µl (88 µmol) of *N*,*N*-diisopropylethylamine, 6.7 mg (44 µmol) of HOBt and 6.7 mg (35 µmol) of EDC, and the mixture was stirred for 30 min. Subsequently, 7.8 mg (32 µmol) of (2*S*)-1-(benzyloxy)-3-phenylpropan-2-amine hydrochloride were added. After stirring overnight, the reaction mixture was separated directly into its components via preparative HPLC. 26 mg (98% of theory) of the title compound were obtained.

LC-MS (Method 1): Rt = 1.51 min; m/z = 909 (M+H)+.

Intermediate 39

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



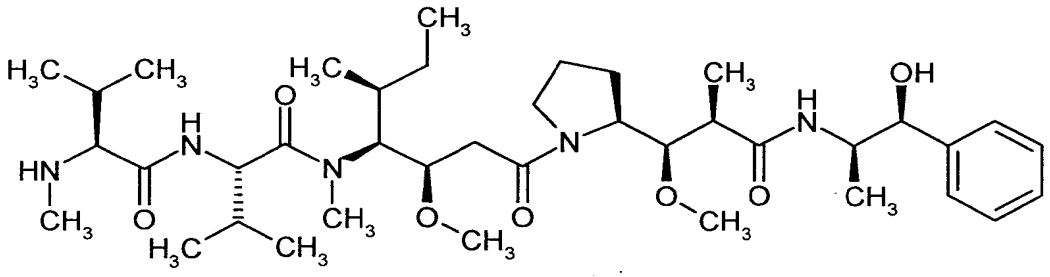
x CF_3COOH

26 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1.8 ml of dichloromethane, and 370 µl of TFA were added. The reaction mixture was stirred at RT for 30 min and then concentrated. The residue was taken up in water and lyophilized. 26.4 mg (quant.) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.97 min; m/z = 809 $(M+H)^+$.

Intermediate 40

N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



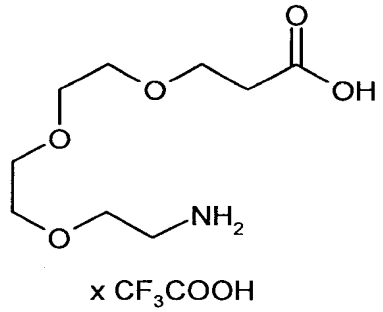
50 mg (70 µmol) of Intermediate 26 and 11 mg (70 µmol) of (1S, 2R)-2-amino-1-phenylpropan-1-ol in 10 ml of DMF were admixed with 42 mg (0.11 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate and 25 µl of *N,N*-diisopropylethylamine, and the reaction mixture was stirred at RT for 5 min. This was followed by concentration and purification of the residue by means of preparative HPLC. After combining the corresponding fractions, concentrating and drying under high vacuum, 49 mg (81%) of the protected intermediate were obtained. Subsequently, the Boc group was detached by known conditions with trifluoroacetic acid in dichloromethane. Concentration was followed by the purification of the title compound by preparative HPLC, and 26 mg (52%) of the title compound were obtained.

HPLC (Method 12): R_t = 1.65 min;

LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 718 $(M+H)^+$.

Intermediate 41

3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate



x CF_3COOH

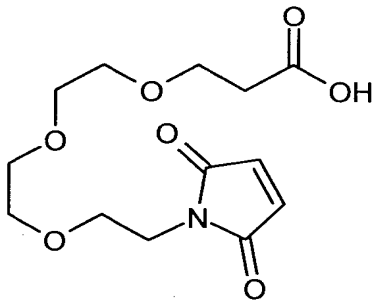
150 mg (541 µmol) of *tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate were dissolved in 3 ml of dichloromethane, 1.5 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 1 h, then concentrated.

181 mg (100% of theory) of the title compound were obtained.

MS (EI): m/z 222 $(M+H)^+$

Intermediate 42

3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanoic acid



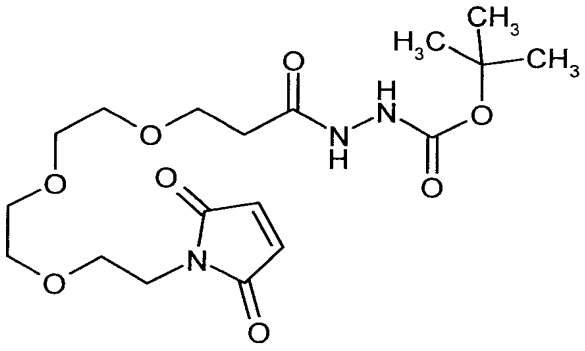
186 mg (555 µmol) of 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate were dissolved in 2.6 ml of saturated sodium hydrogencarbonate solution and admixed at 0°C with 86 mg (555 µmol) of N-methoxycarbonylmaleimide. The reaction mixture was stirred at 0°C for 40 min and at RT for 1 h, then cooled again to 0°C, adjusted to pH 3 with sulphuric acid and extracted 3x with 25 ml of ethyl acetate. The combined organic phases were dried over magnesium sulphate and concentrated.

126 mg (75% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.53 min; m/z = 302 $(M+H)^+$.

Intermediate 43

tert-butyl 15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4-oxo-7,10,13-trioxa-2,3-diazapentadecan-1-oate



125 mg (417 µmol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy) propanoic acid were dissolved at 0°C in 2.1 ml of THF and admixed with 46 µl (417 mmol) of 4-methylmorpholine and 54.5 µl (417 µmol) of isobutyl chloroformate. The ice bath was removed and the reaction mixture was stirred at RT for 30 min. Subsequently, at 0°C, 55 mg (417 µmol) of

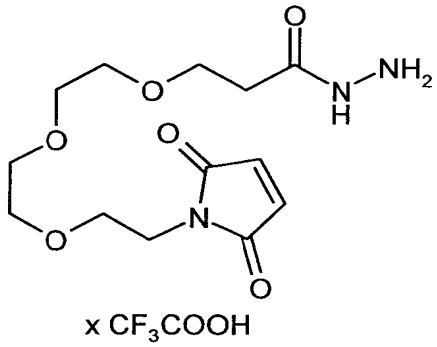
tert-butyloxycarbonyl hydrazide were added. The reaction mixture was warmed to RT overnight, concentrated and purified by preparative HPLC.

60 mg (33% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.66 min; m/z = 416 $(M+H)^+$.

Intermediate 44

3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide trifluoroacetate



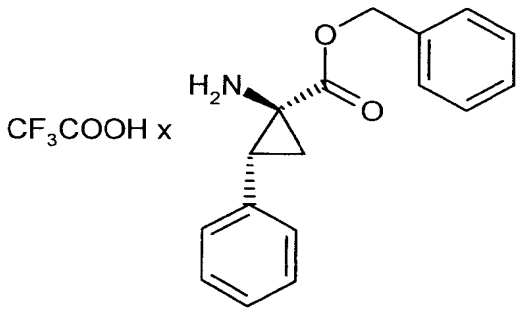
60 mg (145 µmol) of *tert*-butyl 15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4-oxo-7,10,13-trioxa-2,3-diazapentadecan-1-oate were dissolved in 1 ml of dichloromethane, and 0.2 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated.

62 mg (100% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.35 min; m/z = 316 $(M+H)^+$.

Intermediate 45

benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate

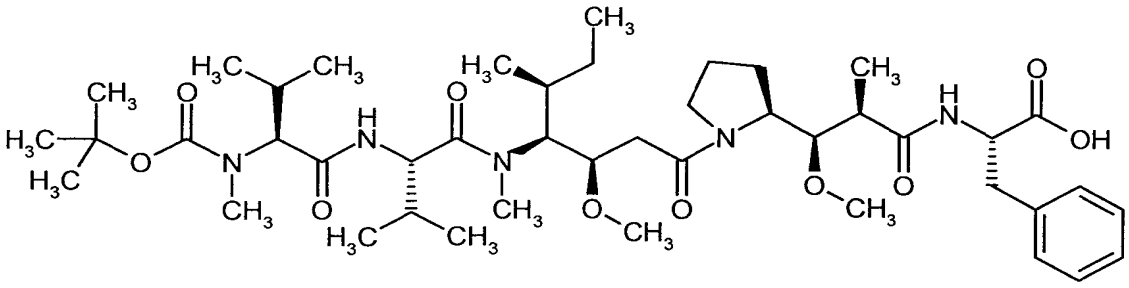


The title compound was prepared by standard methods, by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with benzyl alcohol and subsequent Boc detachment with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.72 min; MS (ESIpos): m/z = 268 $(M+H)^+$.

Intermediate 46

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



383 mg (0.743 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 8) were combined with 485 mg (0.743 mmol) of benzyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate (Intermediate 12), 424 mg (1.114 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 388 µl of *N*,*N*-diisopropylethylamine in 15 ml of DMF, and the mixture was stirred at RT for 10 min. Subsequently, the solvent was removed under reduced pressure. The remaining residue was taken up in ethyl acetate and extracted by shaking successively with 5% aqueous citric acid solution and saturated sodium hydrogencarbonate solution. The organic phase was removed and concentrated, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. 335 mg (48% of theory) of the benzyl ester intermediate were obtained as a foam.

LC-MS (Method 1): R_t = 1.49 min; MS (ESIpos): m/z = 922 $(M+H)^+$.

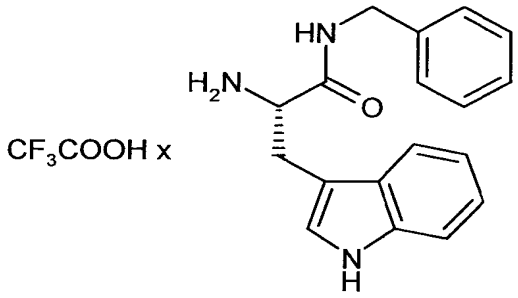
100 mg (0.11 mmol) of this intermediate were taken up in 15 ml of methanol and the benzyl ester group was removed by hydrogenation under standard hydrogen pressure with 10% palladium on activated carbon as a catalyst. After stirring at RT for 1 h, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. After lyophilization from dioxane, 85 mg (94% of theory) of the title compound were obtained as a solid.

HPLC (Method 12): R_t = 2.4 min;

LC-MS (Method 1): R_t = 1.24 min; MS (ESIpos): m/z = 832 $(M+H)^+$.

Intermediate 47

N-benzyl-L-tryptophanamide trifluoroacetate



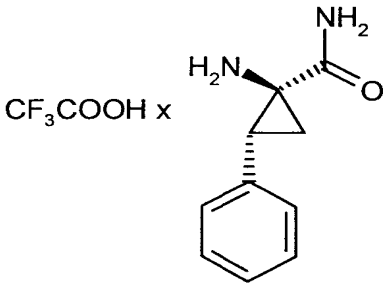
202 mg (0.5 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(tert-butoxycarbonyl)-L-tryptophanate and 45 mg (0.42 mmol) of benzylamine were dissolved in 10 ml of DMF, and 110 µl (630 µmol) of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 3 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (eluent: 20:0.5:0.05 dichloromethane/methanol/17% aq. ammonia). The corresponding fractions were combined and concentrated. The resulting residue was digested with diethyl ether and then dried under high vacuum. Subsequently, this residue was taken up in 10 ml of dichloromethane, and 3 ml of anhydrous trifluoroacetic acid were added. After stirring at RT for 45 minutes, the mixture was concentrated and the residue was purified by preparative HPLC. After drying under high vacuum, 117 mg (57% of theory over both stages) of the title compound were obtained.

HPLC (Method 12): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.66 min; MS (ESIpos): m/z = 294 $(M+H)^+$.

Intermediate 48

(1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxamide trifluoroacetate



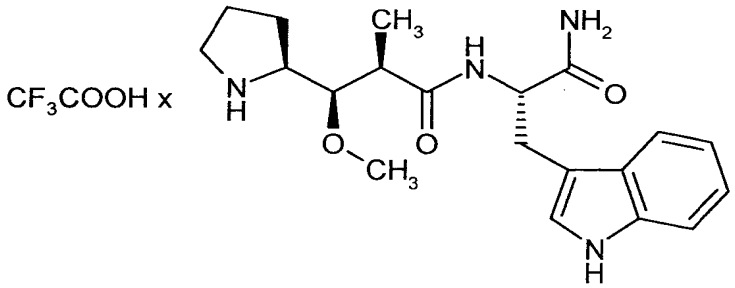
50 mg (180 µmol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid were dissolved in 5 ml of DMF, 94 µl (541 µmol) of *N*,*N*-diisopropylethylamine, 31 mg (270 µmol) of *N*-hydroxysuccinimide and 41.5 mg (216 µmol) of EDC were added, and then the mixture was stirred at RT overnight. The reaction mixture was then concentrated, the residue was taken up in dioxane, 71 mg (901 µmol) of ammonium hydrogencarbonate were added, and the reaction mixture was then left to stand at RT for 3 days. The reaction mixture was then diluted with a 1:1 mixture of ethyl acetate and water. The organic phase was removed, dried over magnesium sulphate and concentrated. The resulting residue was subsequently taken up in 3 ml of dichloromethane, and 3 ml of anhydrous trifluoroacetic acid were added. Stirring at RT for 1 h was followed by concentration. The residue was stirred with pentane, filtered off with suction and lyophilized from dioxane. In this way, 32 mg (62% of theory over both stages) of the title compound were obtained.

HPLC (Method 6): R_t = 0.38 min;

LC-MS (Method 1): R_t = 0.20 min; MS (ESIpos): m/z = 177 $(M+H)^+$.

Intermediate 49

N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate



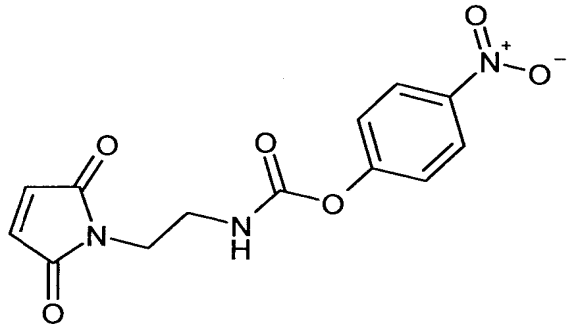
The title compound was prepared in analogy to the synthesis of Intermediate 13 from Starting Compound 1 and L-tryptophanamide hydrochloride.

HPLC (Method 5): R_t = 1.4 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 473 $(M+H)^+$.

Intermediate 50

4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate



813 mg (3.1 mmol) of triphenylphosphine were dissolved in 25 ml of THF and cooled to -70°C under argon. After dropwise addition of 627 mg (3.1 mmol) of diisopropyl azodicarboxylate, the mixture was stirred for 5 min. Subsequently, 500 mg (3.1 mmol) of tert-butyl 2-aminoethyl carbamate dissolved in 5 ml of THF were added dropwise, and the reaction mixture was stirred at -70°C for a further 5 min. Then 136.6 mg (1.55 mmol) of 2,2-dimethyl-1-propanol dissolved in 1 ml of THF and 301 mg (3.1 mmol) of maleimide were added, the reaction mixture was stirred at -70°C for a further 10 min and then the mixture was warmed to RT. After stirring at RT for a further 16 h, the solvent was removed under reduced pressure and the residue was purified by means of preparative HPLC. This gave 463 mg (62%) of the protected intermediate.

After removing the Boc protecting group under standard conditions, 652 mg of 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione were obtained as the trifluoroacetate.

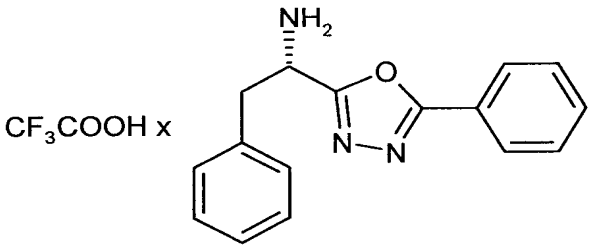
112.9 mg (543 µmol) of nitrophenyl chloroformate were dissolved in 30 ml of THF and, after addition of 100 mg (271.6 µmol) of 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione trifluoroacetate, the mixture was stirred at RT for 30 min. The mixture was filtered and the filtrate was concentrated to dryness and then slurried with diethyl ether. After filtration with suction and drying, 60 mg (95% of theory) of the title compound were obtained.

HPLC (Method 5): Rt = 0.65 min;

LC-MS (Method 1): Rt = 0.74 min; MS (ESIpos): m/z = 306 (M+H)+.

Intermediate 51

(*1S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate



200 mg (0.75 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were initially charged at 0°C in 5.5 ml of dichloromethane, and 128 mg (0.79 mmol) of 1,1'-carbonyldiimidazole were added. After 30 min, 103 mg (0.75 mmol) of benzoyl hydrazide were added. After a further 45 min at 0°C, 500 mg (1.5 mmol) of carbon tetrabromide and 395 mg (1.5 mmol) of triphenylphosphine were finally added. The reaction mixture was stirred first at 0°C for 2 h and then at RT overnight. The mixture was subsequently concentrated on a rotary evaporator, and the residue was dried under high vacuum. The crude product thus obtained was purified by means of preparative HPLC. 217 mg (78% of theory) of the Boc-protected intermediate *tert*-butyl (1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl carbamate were obtained.

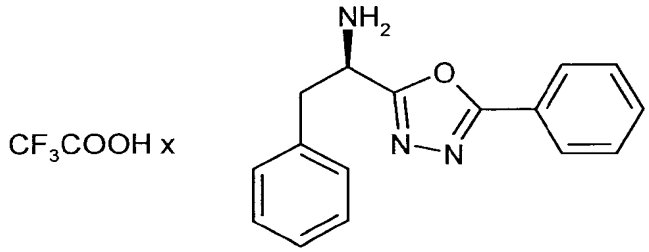
LC-MS (Method 12): R_t = 1.15 min; MS (ESIpos): m/z = 366 $(M+H)^+$

217 mg (0.59 mmol) of this intermediate were taken up in 3 ml of dichloromethane, 0.6 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure. The remaining residue was the reaction mixture dried further under reduced pressure and then lyophilized from dioxane. In this way, 214 mg (90% of theory) of the title compound were obtained.

LC-MS (Method 11): R_t = 0.62 min; MS (ESIpos): m/z = 266 $(M+H)^+$

Intermediate 52

(*1R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate



200 mg (0.75 mmol) of *N*-(*tert*-butoxycarbonyl)-D-phenylalanine were initially charged at 0°C in 5.5 ml of dichloromethane, and 128.3 mg (0.79 mmol) of 1,1'-carbonyldiimidazole were added. After 30 min, 103 mg (0.75 mmol) of benzoyl hydrazide were added. After a further 45 min at 0°C, 500 mg (1.5 mmol) of carbon tetrabromide and 395 mg (1.5 mmol) of triphenylphosphine were finally added. The reaction mixture was stirred first at 0°C for 2 h and then at RT overnight. The mixture was subsequently concentrated on a rotary evaporator, and the residue was dried under high vacuum. The crude product thus obtained was purified by means of preparative HPLC. 219 mg (80% of theory) of the Boc-protected intermediate *tert*-butyl (1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl carbamate were obtained.

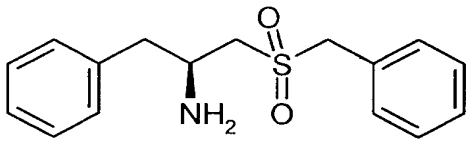
LC-MS (Method 2): R_t = 1.36 min; MS (ESIpos): m/z = 366 $(M+H)^+$

219 mg (0.6 mmol) of this intermediate were taken up in 3 ml of dichloromethane, 0.6 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure. The remaining residue was the reaction mixture dried further under reduced pressure and then lyophilized from dioxane. In this way, 196 mg (86% of theory) of the title compound were obtained as a solid.

HPLC (Method 10): R_t = 2.41 min

Intermediate 53

(*2S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine



200 mg (1.13 mmol) of (4*S*)-4-benzyl-1,3-oxazolidin-2-one were initially charged in 3 ml of *tert*-butanol, and 280 mg (2.26 mmol) of benzyl mercaptan were added. The mixture was subsequently heated under reflux for 2 days. Thereafter, the reaction mixture was concentrated on a rotary evaporator and the resulting (2*S*)-1-(benzylsulphanyl)-3-phenylpropan-2-amine intermediate was converted further directly, without workup.

HPLC (Method 10): R_t = 2.63 min

LC-MS (Method 1): R_t = 0.67 min; MS (ESIpos): m/z = 258 $(M+H)^+$

The crude intermediate obtained above was dissolved in a solution of 2 ml of 30% hydrogen peroxide and 5 ml of formic acid, and the mixture was stirred at RT for 12 h. Then the reaction

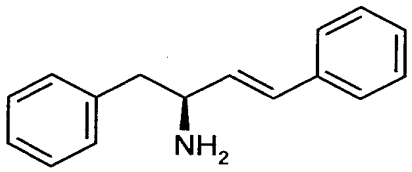
mixture was added to saturated sodium sulphate solution and extracted three times with ethyl acetate. The organic phase was dried over magnesium sulphate and concentrated under reduced pressure. The crude product obtained was purified by means of preparative HPLC. 343 mg (61% of theory) of the title compound were thus obtained.

HPLC (Method 10): R_t = 2.40 min;

LC-MS (Method 12): R_t = 0.65 min; MS (ESIpos): m/z = 290 $(M+H)^+$

Intermediate 54

(*2S,3E*)-1,4-diphenylbut-3-en-2-amine



552.7 mg (9.85 mmol) of potassium hydroxide were dissolved in methanol, adsorbed onto 1.1 g of neutral aluminium oxide and then dried under high vacuum. To a solution of 240 mg (0.82 mmol) of (*2S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine and 1.56 g of the potassium hydroxide on aluminium oxide thus prepared in 6.2 ml of *n*-butanol were added dropwise, at 5-10°C, 307 µl (3.3 mmol) of dibromodifluoromethane. The reaction mixture was stirred at RT for 2 h, then filtered through Celite, and the residue was washed thoroughly with dichloromethane. The filtrate was concentrated and the resulting residue was dried under reduced pressure. The crude product thus obtained was purified by means of preparative HPLC. 98 mg (35% of theory) of the title compound were obtained with an *E/Z* diastereomer ratio of 4:1.

HPLC (Method 10): R_t = 2.46 min;

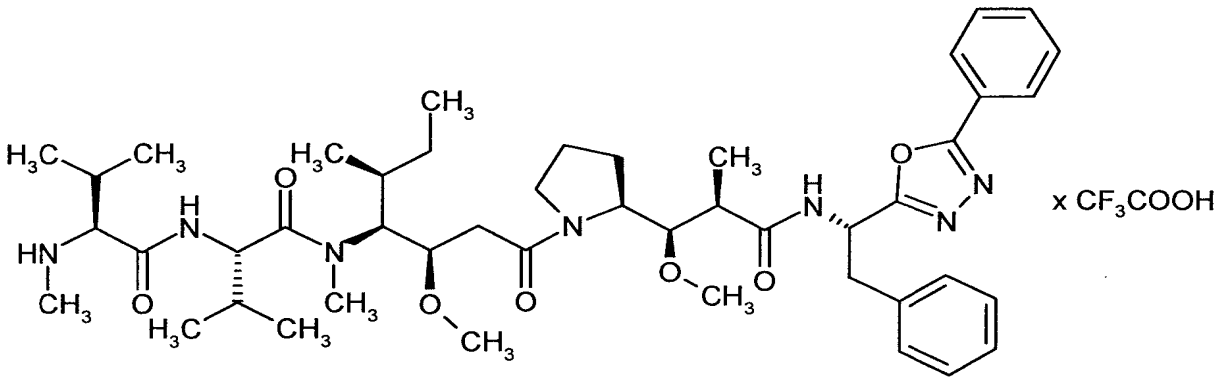
LC-MS (Method 12): R_t = 0.75 min; MS (ESIpos): m/z = 224 $(M+H)^+$

The *E/Z* diastereomer mixture obtained above was dissolved in 2 ml of ethanol and 0.2 ml of *N,N*-diisopropylethylamine, and separated by means of HPLC on chiral phase [column: Daicel Chiralpak AD-H, 5 µm 250 mm x 20 mm, eluent: hexane/(ethanol + 0.2% diethylamine) 50:50 v/v; UV detection: 220 nm; temperature: 30°C]. The appropriate fractions were concentrated on a rotary evaporator, and the residue was dried under reduced pressure. 45 mg of the title compound were obtained.

^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] = 2.62 - 2.83 (m, 2 H) 3.52 - 3.71 (m, 1 H) 6.18 - 6.30 (m, 1 H) 6.34 - 6.46 (m, 1 H) 6.98 - 7.57 (m, 10 H) [further signals hidden under solvent peaks].

Intermediate 55

N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide trifluoroacetate



20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1 ml of DMF, 13.3 mg (35 µmol) of HATU and 15.3 µl (88 µmol) of *N,N*-diisopropylethylamine were added, and the mixture was stirred at RT for 30 min. Subsequently, 12.2 mg (32 µmol) of (1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate were added. The reaction mixture was stirred at RT overnight and then separated by preparative HPLC. This gave 22 mg (81% of theory) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

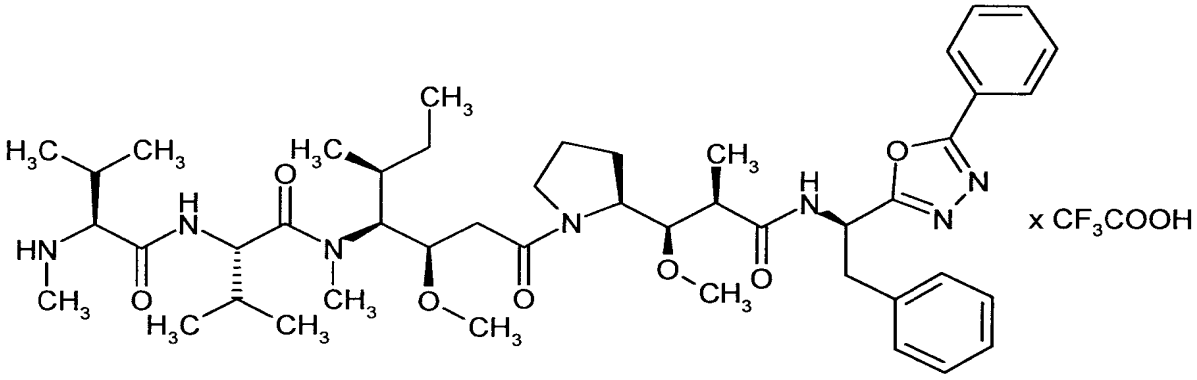
LC-MS (Method 12): R_t = 1.45 min; MS (ESIpos): m/z = 933 $(M+H)^+$

By subsequently detaching the BOC protecting group with trifluoroacetic acid, 22.4 mg (98% of theory) of the title compound were then obtained.

LC-MS (Method 11): R_t = 0.85 min; MS (ESIpos): m/z = 833 $(M+H)^+$

Intermediate 56

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



N-(*tert*-Butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reaction of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide with 12.2 mg (32 µmol) of (1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate.

Yield: 17 mg (64% of theory)

HPLC (Method 10): R_t = 3.74 min;

LC-MS (Method 1): R_t = 1.45 min; MS (ESIpos): m/z = 933 $(M+H)^+$

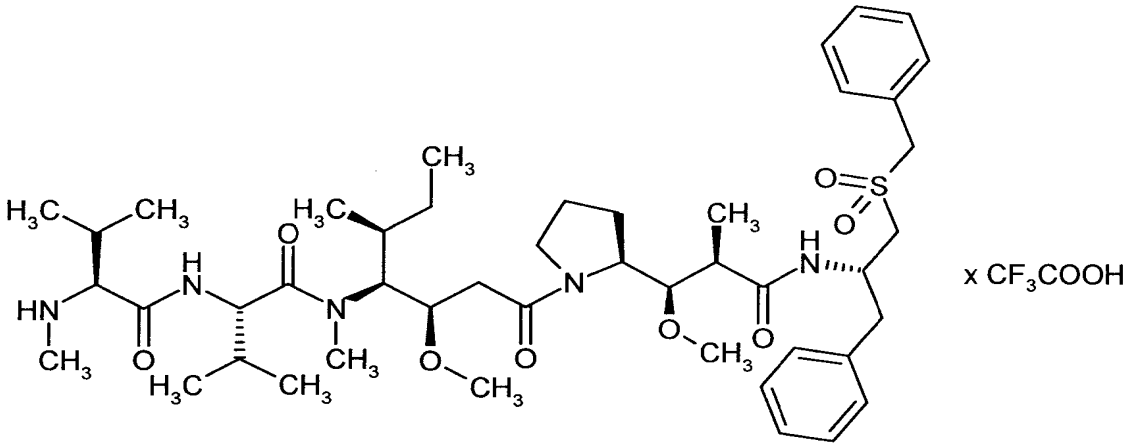
By subsequently detaching the BOC protecting group with trifluoroacetic acid, 17.1 mg (99% of theory) of the title compound were then obtained.

HPLC (Method 10): R_t = 2.55 min;

LC-MS (Method 11): R_t = 0.85 min; MS (ESIpos): m/z = 833 $(M+H)^+$

Intermediate 57

N-methyl-*L*-valyl-N-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide trifluoroacetate



N-(*tert*-Butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reaction of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide with 9.3 mg (20 µmol) of (2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine.

Yield: 19.2 mg (68 % of theory)

HPLC (Method 10): R_t = 3.5 min;

LC-MS (Method 12): R_t = 1.41 min; MS (ESIpos): m/z = 957 $(M+H)^+$

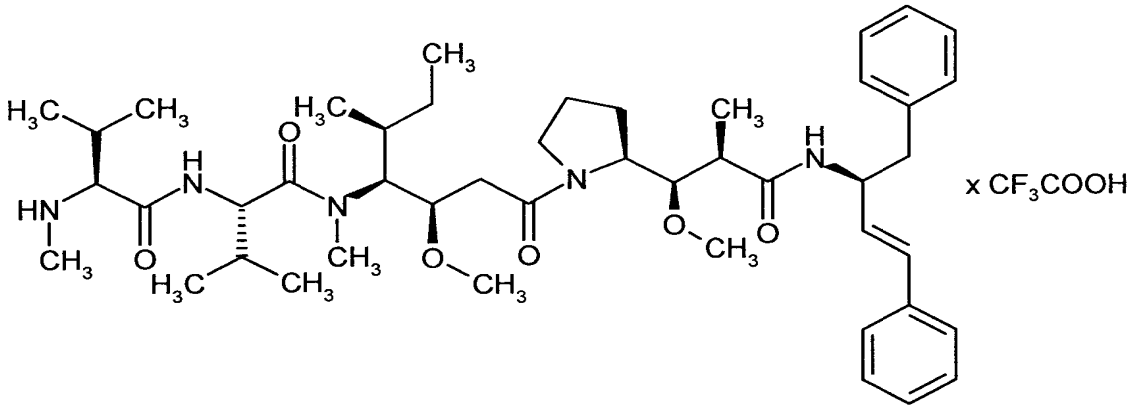
By subsequently detaching the BOC protecting group with trifluoroacetic acid, 19.3 mg (99% of theory) of the title compound were then obtained.

HPLC (Method 10): R_t = 2.52 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 857 $(M+H)^+$

Intermediate 58

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



N-(*tert*-Butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reaction of 20 mg (29 µmol) *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(*3R,4S,5S*)-1-{(*2S*)-2-[(*1R,2R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide with 7.1 mg (32 µmol) of (2*S*,3*E*)-1,4-diphenylbut-3-en-2-amine.

Yield: 15.1 mg (58 % of theory)

HPLC (Method 10): R_t = 4.2 min;

LC-MS (Method 12): R_t = 1.51 min; MS (ESIpos): m/z = 891 $(M+H)^+$

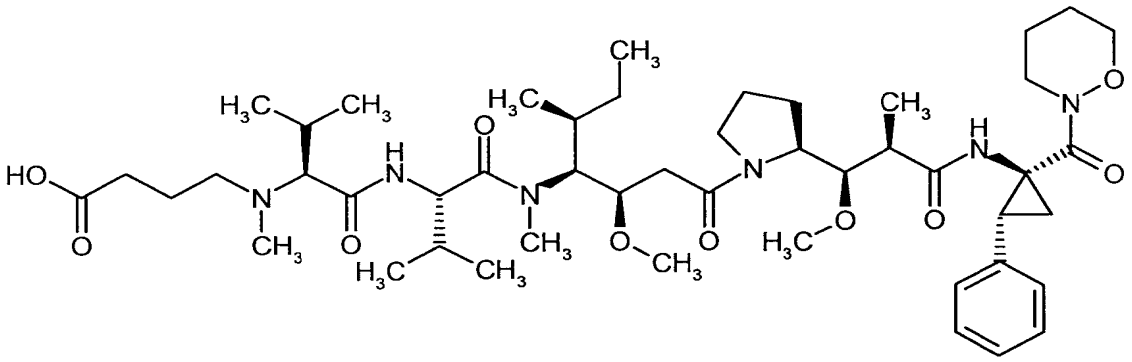
By subsequently detaching the BOC protecting group with trifluoroacetic acid, 15.7 mg (99% of theory) of the title compound were then obtained.

HPLC (Method 10): R_t = 2.62 min;

LC-MS (Method 12): R_t = 0.97 min; MS (ESIpos): m/z = 791 $(M+H)^+$

Intermediate 61

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



50 mg (0.054 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) were dissolved in 8 ml of dioxane/water, and 70 ml (0.108 mmol) of a 15% solution of 4-oxobutanoic acid in water were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 3.7 mg (0.059 mmol) of sodium cyanoborohydride were added and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for a further 2 h. After cooling, another 70 ml (0.108 mmol) of the 15% 4-oxobutanoic acid solution were added and the reaction mixture was again stirred at 100°C for 1 h. Then a further 3.7 mg (0.059 mmol) of sodium cyanoborohydride were added and about 300 µl of 0.1 N hydrochloric acid were subsequently used to adjust the pH back to 3. The reaction mixture was then stirred at 100°C for another 2 h. In the event of conversion still being incomplete, this procedure was repeated for a third time. The reaction mixture was finally concentrated and the residue was purified by means of preparative HPLC. In this way, 32 mg (65% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 5): R_t = 1.64 min;

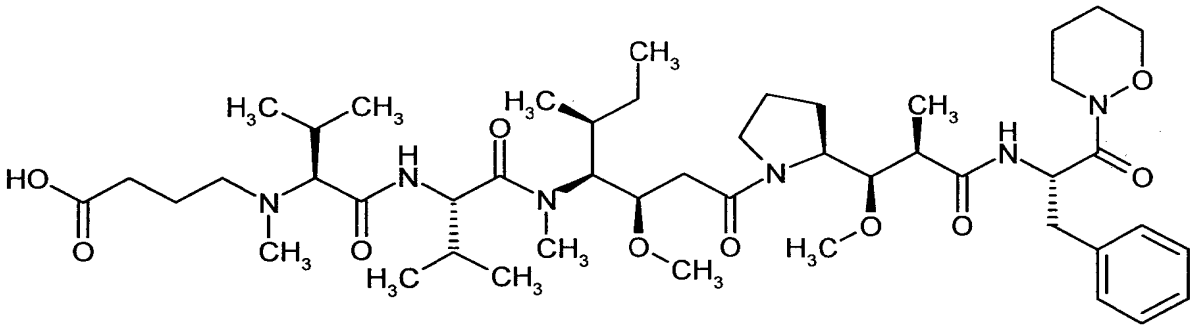
LC-MS (Method 9): R_t = 4.76 min; MS (ESIpos): m/z = 899 $(M+H)^+$

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.95 and 8.8 (2m, 1H), 8.88 and 8.65 (2s, 1H), 7.4-7.1 (m, 5H), 5.0, 4.78, 4.65 and 4.55 (4m, 2H), 4.1-3.7 (m, 5H), 3.32, 3.29, 3.20, 3.12, 3.1 and 3.0 (6s,

9H), 2.75 (m, 2H), 2.63 (t, 1H), 2.4-2.2 (m, 4H), 2.1-1.2 (m, 12H), 1.2-0.8 (m, 16H), 0.75 (m, 3H) [further signals hidden under H_2O and DMSO peaks].

Intermediate 62

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 61, by reaction of 50 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 14) with 4-oxobutanoic acid.

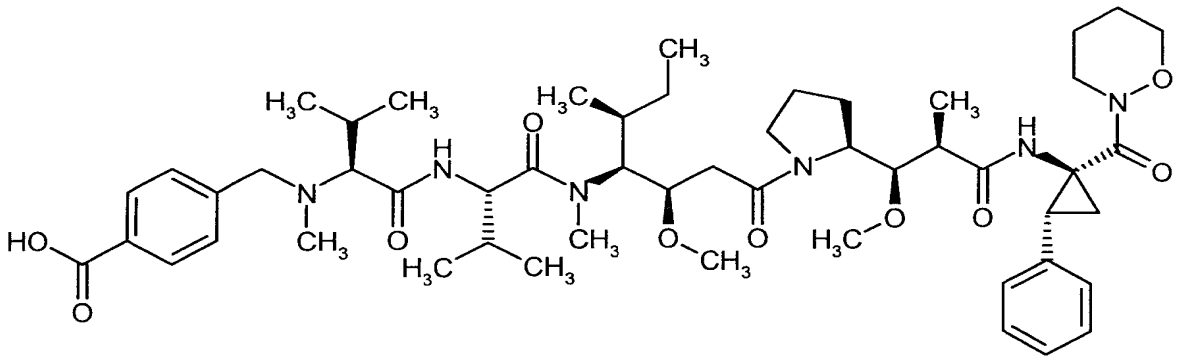
Yield: 34 mg (70% of theory)

HPLC (Method 5): R_t = 1.64 min;

LC-MS (Method 9): R_t = 4.77 min; MS (ESIpos): m/z = 887 $(M+H)^+$.

Intermediate 63

N-(4-carboxybenzyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 61, by reaction of 15 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with 4-formylbenzoic acid.

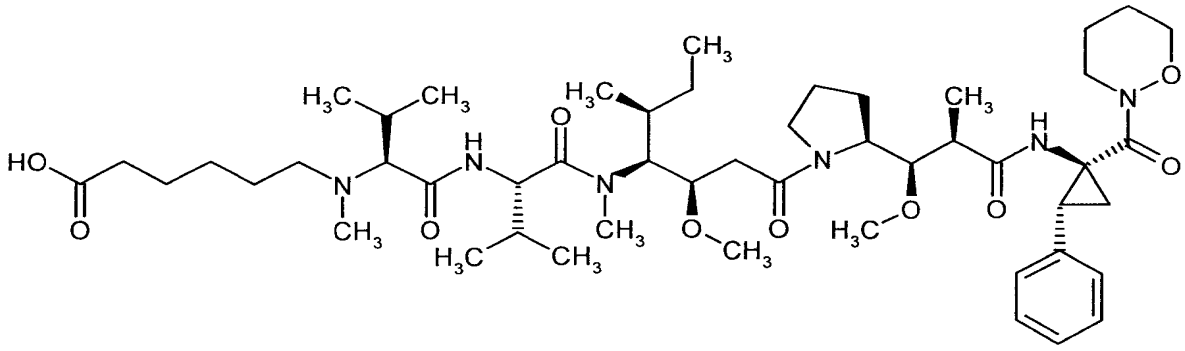
Yield: 7.5 mg (48% of theory)

HPLC (Method 5): R_t = 1.75 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 947 $(M+H)^+$.

Intermediate 64

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10 mg (0.011 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) were dissolved in 2 ml of dioxane/water, and 2.8 mg (0.022 mmol) of 6-

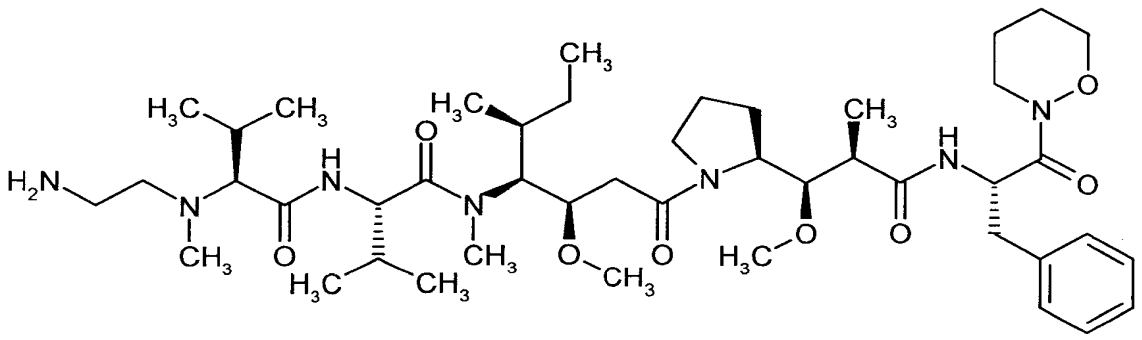
oxohexanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 0.75 mg (0.012 mmol) of sodium cyanoborohydride was added and the mixture was adjusted to a pH of 3 by adding 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for a further hour. After cooling, another 2.8 mg (0.022 mmol) of 6-oxohexanoic acid were added and the reaction mixture was again stirred at 100°C for 1 h. A further 0.75 mg (0.012 mmol) of sodium cyanoborohydride was added and 0.1 N hydrochloric acid was subsequently used to adjust the pH back to 3. The reaction mixture was then stirred at 100°C for another 1 h. This procedure was then repeated for a third time. The reaction mixture was finally concentrated and the crude product was purified by means of preparative HPLC. This gave 6.4 mg (64% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): R_t = 1.68 min;

LC-MS (Method 9): R_t = 4.86 min; MS (ESIpos): m/z = 927 $(M+H)^+$.

Intermediate 65

N-(2-aminoethyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide bistrifluoroacetate



x 2 CF_3COOH

The title compound was prepared by reaction of 68 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 14) with *tert*-butyl 2-oxoethyl carbamate and subsequent detachment of the Boc protecting group with trifluoroacetic acid.

Yield: 49 mg (62% of theory over two stages)

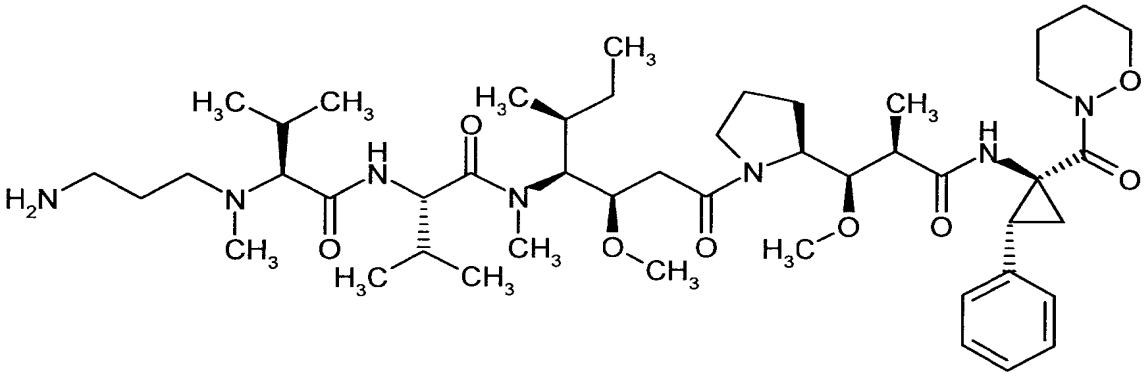
HPLC (Method 5): R_t = 1.58 min;

LC-MS (Method 2): R_t = 1.05 min; MS (ESIpos): m/z = 844 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.25 (m, 1H), 8.45 and 8.15 (2d, 1H), 7.65-7.55 (m, 3H), 7.23-7.1 (m, 5H), 5.12 and 4.95 (2m, 1H), 4.72 and 4.62 (2m, 1H), 4.6 and 4.52 (2t, 1H), 4.2-3.8 (m, 4H), 3.7 (d, 1H), 3.23, 3.20, 3.19, 3.18, 3.03 and 2.98 (6s, 9H), 3.0-2.7 (m, 6H), 2.4-1.2 (m, 15H), 1.05, 1.0, 0.88 and 0.82 (4d, 6H), 0.92 (m, 6H), 0.73 (m, 6H) [further signals hidden under H_2O peak].

Intermediate 66

N-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 65, by reaction of 25 mg (0.027 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with benzyl 3-oxopropyl carbamate and subsequent hydrogenolytic detachment of the Z protecting group (with 10% palladium on charcoal as a catalyst, in ethanol as a solvent).

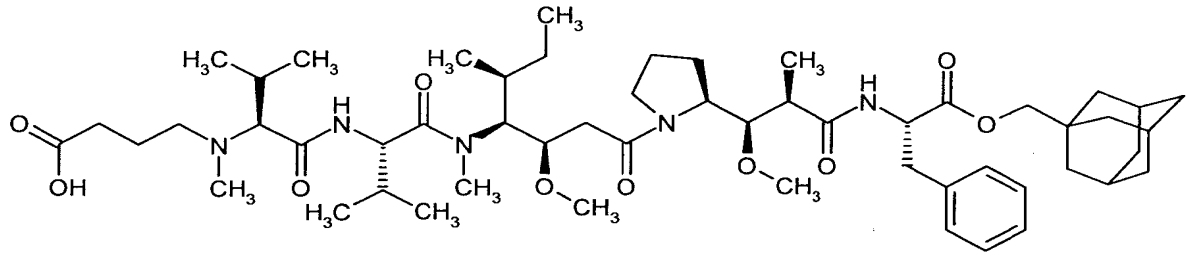
Yield: 11 mg (41% of theory over two stages)

HPLC (Method 5): R_t = 1.53 min;

LC-MS (Method 1): R_t = 0.72 min; MS (ESIpos): m/z = 870 $(M+H)^+$.

Intermediate 67

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

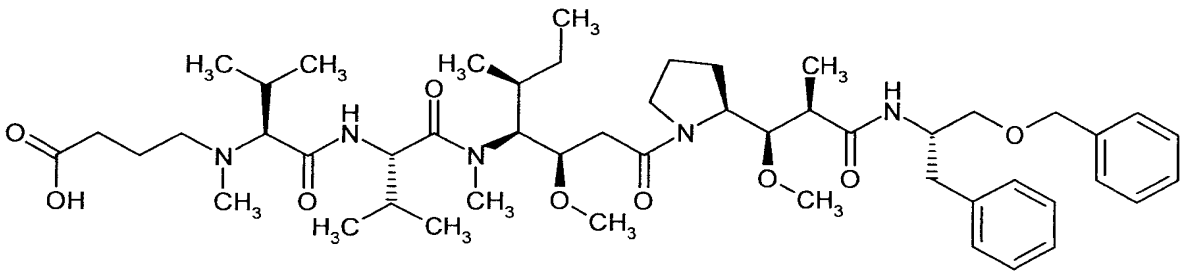


26 mg (26 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and 33.9 µl of a 15% aqueous succinaldehydic acid solution (53 µmol) were dissolved in 957 µl of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.81 mg (29 µmol) of sodium cyanoborohydride were added. The reaction mixture was adjusted to pH 3 by adding 0.1 N hydrochloric acid and the mixture was heated to 100°C for a further 2 h. After again adding the same amounts of succinaldehydic acid solution, sodium cyanoborohydride and hydrochloric acid, the mixture was heated once again to 100°C for 2 h. The reaction mixture was then separated directly into its components by means of preparative HPLC. 18.5 mg (73% of theory) of the title compound were thus obtained.

LC-MS (Method 1): R_t = 1.17 min; m/z = 967 $(M+H)^+$.

Intermediate 68

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

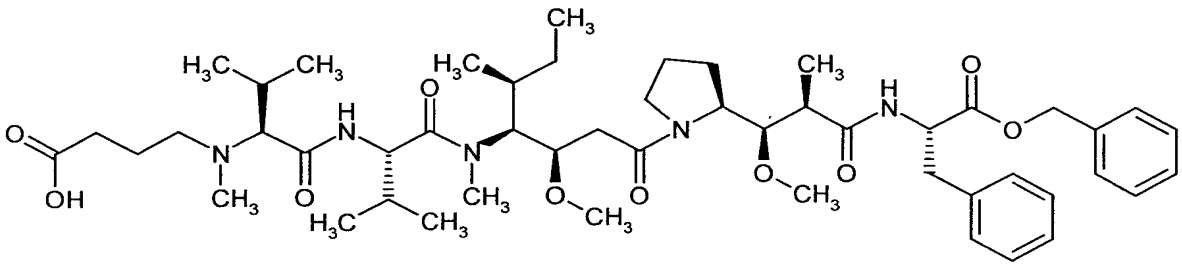


24 mg (26 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and 33.7 µl of a 15% aqueous succinaldehydic acid solution (52 µmol) were dissolved in 953 µl of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.80 mg (29 µmol) of sodium cyanoborohydride were added. The reaction mixture was adjusted to pH 3 by adding 0.1 N hydrochloric acid and the mixture was heated to 100°C for a further 2 h. After again adding the same amounts of succinaldehydic acid solution, sodium cyanoborohydride and hydrochloric acid, the mixture was heated once again to 100°C for 2 h. The reaction mixture was then separated directly into its components by means of preparative HPLC. 15.2 mg (65% of theory) of the title compound were thus obtained.

LC-MS (Method 1): R_t = 1.01 min; m/z = 895 $(M+H)^+$

Intermediate 69

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



53 mg (84 µmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and 45 mg (84 µmol) of benzyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate (Intermediate 12) were taken up in 2 ml of DMF, 19 µl of *N*,*N*-diisopropylethylamine, 14 mg (92 µmol) of HOBt and 17.6 mg (92 µmol) of EDC were added and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. This gave 59 mg (68% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

LC-MS (Method 1): R_t = 1.55 min; m/z = 1044 $(M+H)^+$.

57 mg (0.055 mmol) of this intermediate were treated with 1.2 ml of piperidine in 5 ml of DMF to detach the Fmoc protecting group. After concentration and purification by means of preparative HPLC, 39 mg (76% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 1.01 min; m/z = 822 $(M+H)^+$.

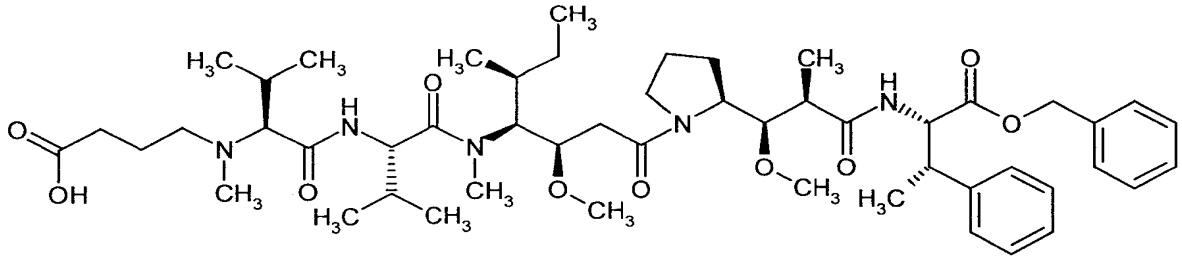
37 mg (0.045 mmol) of this intermediate were dissolved in 5 ml of dioxane/water and, analogously to the preparation of the compound in Intermediate 66, reacted with a 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. 16 mg (39% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 6): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 908 $(M+H)^+$.

Intermediate 70

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 26, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared.

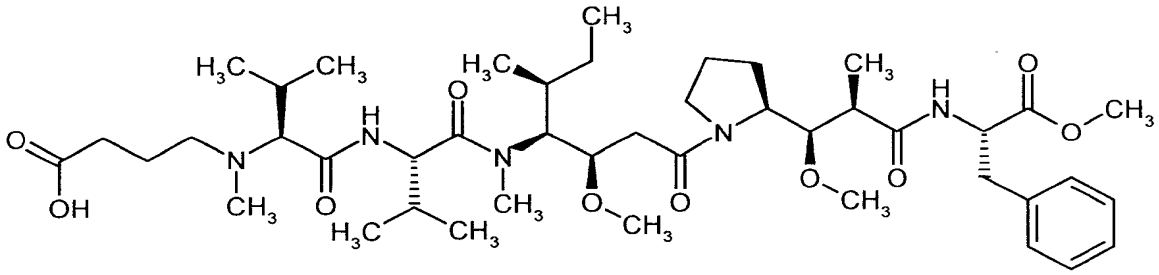
30 mg (0.032 mmol) of this compound were dissolved in 6 ml of dioxane/water, and 41 µl (0.063 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2.2 mg (0.035 mmol) of sodium cyanoborohydride were added and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for a further 2 h. After cooling, another 41 µl (0.063 mmol) of the 15% 4-oxobutanoic acid solution were added and the reaction mixture was again stirred at 100°C for 1 h. Then a further 2.2 mg (0.035 mmol) of sodium cyanoborohydride were added and about 300 µl of 0.1 N hydrochloric acid were subsequently used to adjust the pH back to 3. The reaction mixture was then stirred at 100°C for another 2 h. In the event of conversion still being incomplete, this procedure was repeated for a third time. The reaction mixture was finally concentrated and the crude product was purified by means of preparative HPLC. This gave 24 mg (82% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 9): R_t = 5.15 min; MS (ESIpos): m/z = 922 $(M+H)^+$.

Intermediate 71

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-{[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



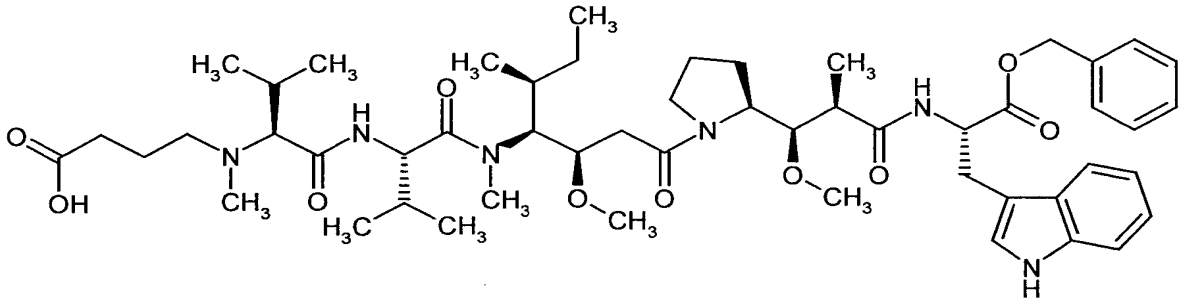
First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 39, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-{[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared. 7 mg (0.009 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2 mg (22% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 6): R_t = 1.9 min;

LC-MS (Method 2): R_t = 1.06 min; MS (ESIpos): m/z = 832 $(M+H)^+$.

Intermediate 72

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



212 mg (411 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 8) and 237 mg (411 µmol) of benzyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanate trifluoroacetate (Intermediate 20) were taken up in 30 ml of DMF, and 188 mg (493 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 215 µl *N*,*N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 20 h, then concentrated under reduced pressure, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 315 mg (80% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide as a colourless foam.

LC-MS (Method 1): R_t = 1.45 min; m/z = 961 $(M+H)^+$.

50 mg (52 µmol) of this intermediate were treated with 1 ml of trifluoroacetic acid in 9 ml of dichloromethane to detach the Boc protecting group. After concentration and purification by means of preparative HPLC, 29 mg (57% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

LC-MS (Method 1): R_t = 0.99 min; m/z = 861 $(M+H)^+$.

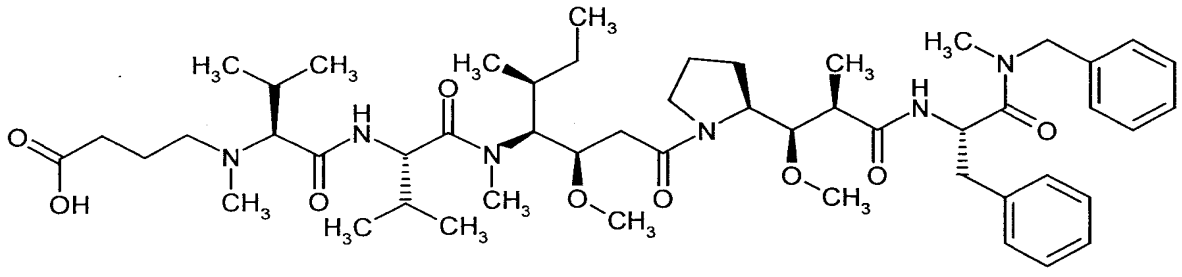
29 mg (0.03 mmol) of this intermediate were dissolved in 6 ml of dioxane/water, and 39 µl (0.059 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2 mg (0.033 mmol) of sodium cyanoborohydride were added and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for a further 2 h. After cooling, another 39 µl (0.059 mmol) of the 15% 4-oxobutanoic acid solution were added and the reaction mixture was again stirred at 100°C for 1 h. Then a further 2 mg (0.033 mmol) of sodium cyanoborohydride were added and about 300 µl of 0.1 N hydrochloric acid were subsequently used to adjust the pH back to 3. The mixture was then stirred at 100°C for another 2 h. Thereafter, the reaction mixture was poured onto a 1:1 mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed with saturated sodium chloride solution, dried over sodium sulphate and concentrated. The residue was freeze-dried from water/ acetonitrile. This gave 27 mg (94% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): R_t = 2.2 min;

LC-MS (Method 9): R_t = 5.04 min; MS (ESIpos): m/z = 947 $(M+H)^+$.

Intermediate 73

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 38, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared. 25 mg (0.026 mmol) of this compound were then used, in analogy to the preparation of

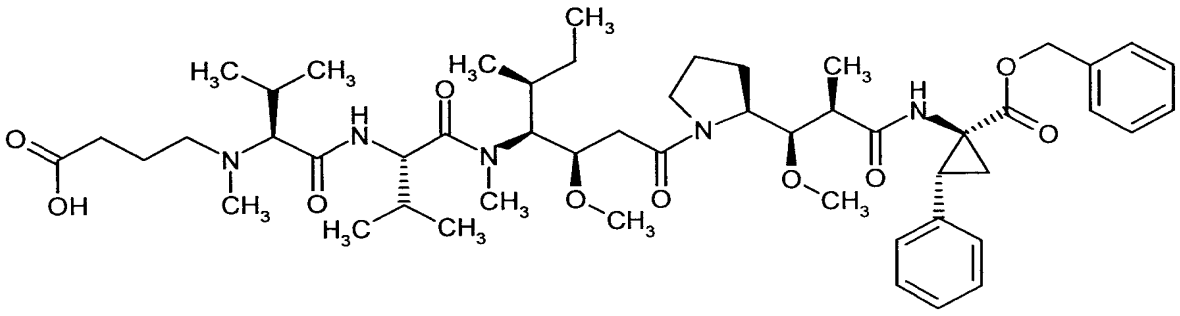
Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 13 mg (54% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 9): R_t = 5.01 min; MS (ESIpos): m/z = 921 $(M+H)^+$.

Intermediate 74

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



50 mg (73 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 28 mg (73 µmol) of benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate (Intermediate 45) were taken up in 5 ml of DMF, and 42 mg (110 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 38 µl of *N*,*N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 5 h, then concentrated under reduced pressure, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated. After lyophilization from dioxane/water, 35 mg (51% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as a colourless foam.

LC-MS (Method 1): R_t = 1.52 min; m/z = 934 $(M+H)^+$.

35 mg of this intermediate were treated with 1 ml of trifluoroacetic acid in 5 ml of dichloromethane to detach the Boc protecting group. After concentration and lyophilization from dioxane/water, 34 mg (97% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-

[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

LC-MS (Method 1): R_t = 0.91 min; m/z = 834 $(M+H)^+$.

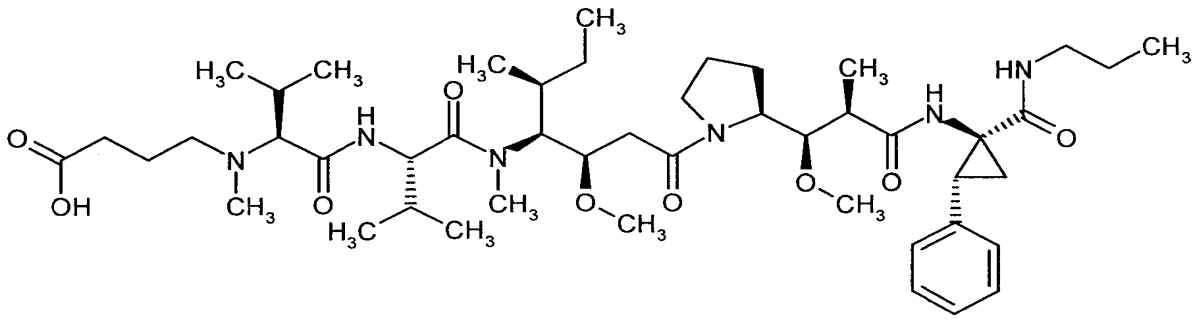
11 mg (0.011 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 66, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2.5 mg (24% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 9): R_t = 5.1 min; MS (ESIpos): m/z = 920 $(M+H)^+$.

Intermediate 75

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and (1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide trifluoroacetate (Intermediate 27) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 14 mg (0.016 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic

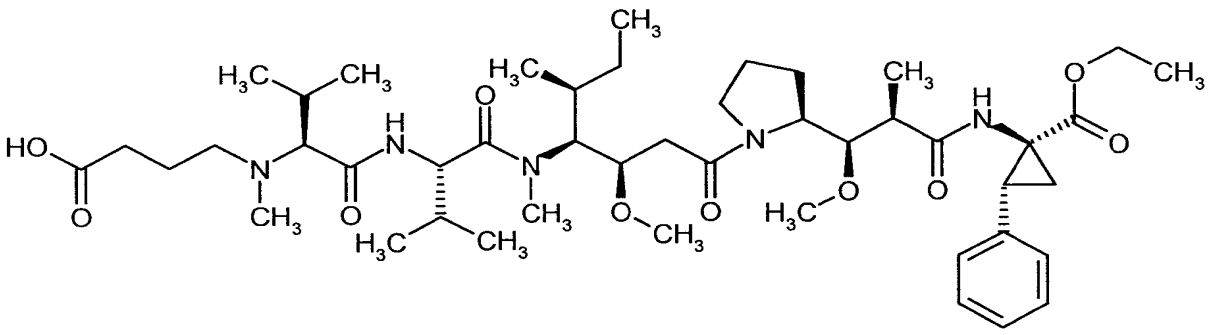
acid in the presence of sodium cyanoborohydride, to obtain 11.3 mg (83% of theory) of the title compound.

HPLC (Method 6): R_t = 1.9 min;

LC-MS (Method 2): R_t = 1.27 min; MS (ESIpos): m/z = 871 $(M+H)^+$.

Intermediate 76

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



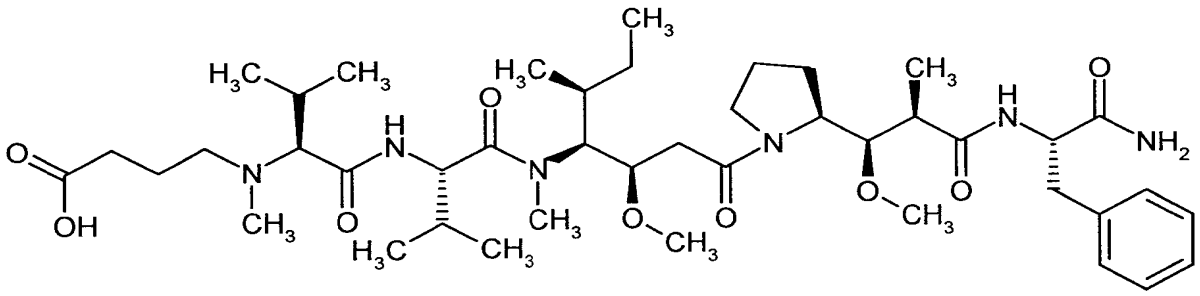
First, by coupling of Intermediate 46 (*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide) with Intermediate 48 (ethyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate) in the presence of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent Boc detachment, the starting compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate was prepared. 70 mg (0.079 mmol) of this starting material were then used, by reaction with 4-oxobutanoic acid, in analogy to Intermediate 61, to obtain 46 mg (68% of theory) of the title compound.

HPLC (Method 6): R_t = 1.9 min;

LC-MS (Method 2): R_t = 1.28 min; MS (ESIpos): m/z = 858 $(M+H)^+$

Intermediate 77

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 75, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and L-phenylalaninamide hydrochloride in the presence of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 47 mg (0.049 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 39 mg (96% of theory) of the title compound.

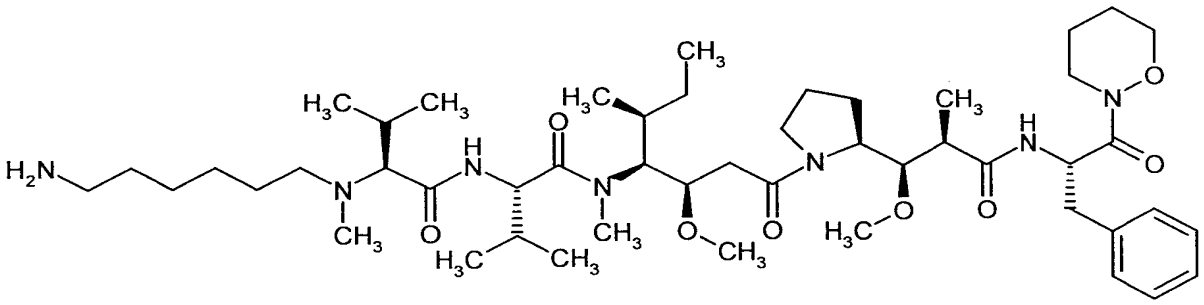
HPLC (Method 6): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.44 min; MS (ESIpos): m/z = 817 $(M+H)^+$

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.95 and 8.8 (2m, 1H), 8.25 and 8.0 (2d, 1H), 7.45, 7.35 and 7.0 (3s, broad, 2H), 7.3-7.1 (m, 5H), 4.8-4.4 (2m, 3H), 3.95 (m, 1H), 3.82 (m, 1H), 3.72 (d, 1H), 3.22, 3.18, 3.15, 3.05 and 3.00 (5s, 9H), 2.85-2.7 (m, 4H), 2.45-1.6 (m, 12H), 1.5-1.2 (m, 3H), 1.1-0.7 (m, 21H) [further signals hidden under solvent peaks].

Intermediate 78

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5S)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 66 over 2 stages, proceeding from 20 mg (16 µmol) of the compound from Intermediate 14 and benzyl 6-oxohexyl carbamate, and the hydrogenation was performed in methanol as the solvent.

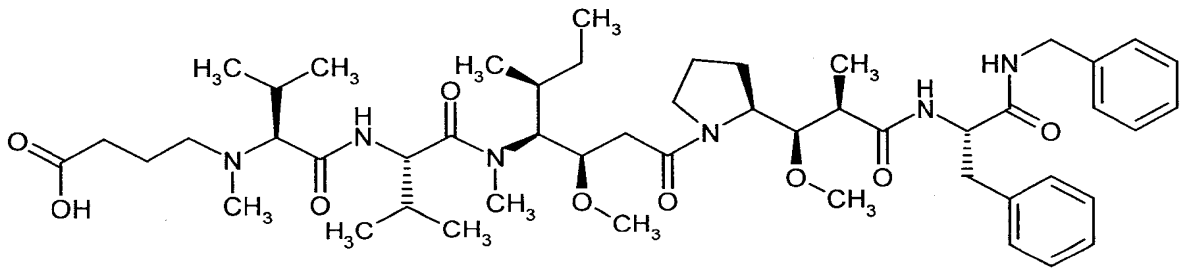
Yield: 7.6 mg (55% of theory over 2 stages)

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.7 min; MS (ESIpos): m/z = 901 $(M+H)^+$.

Intermediate 79

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



36 mg (43 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and 4.6 mg (43 µmol) of benzylamine were taken up in 5 ml of DMF, 7.5 µl (88 µmol) of *N,N*-diisopropylethylamine, 10 mg (65 µmol) of HOBt and 10 mg (52 µmol) of EDC were added, and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. 29 mg (73% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-

oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained.

LC-MS (Method 1): R_t = 1.43 min; m/z = 921 $(M+H)^+$.

29 mg of this intermediate were treated with 1 ml of trifluoroacetic acid in 6 ml of dichloromethane to detach the Boc protecting group. After concentration and lyophilization from dioxane/water, 30 mg (quant.) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

LC-MS (Method 1): R_t = 0.95 min; m/z = 821 $(M+H)^+$.

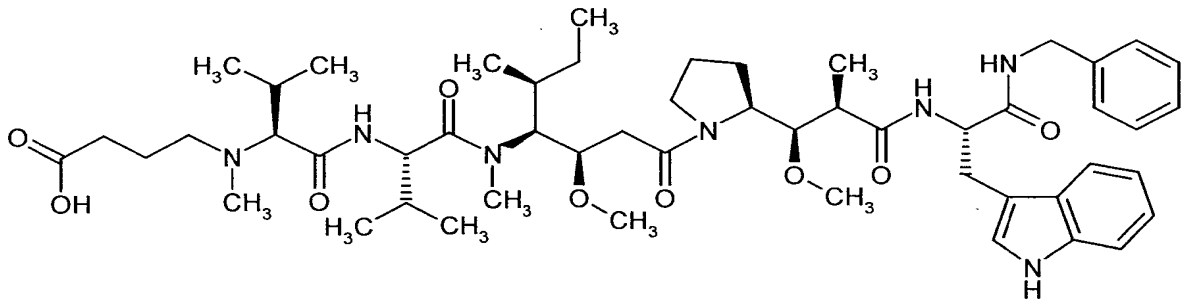
17 mg (0.018 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 13 mg (80% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.97 min; MS (ESIpos): m/z = 907 $(M+H)^+$.

Intermediate 80

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *N*-benzyl-L-tryptophanamide trifluoroacetate (Intermediate 47) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate and

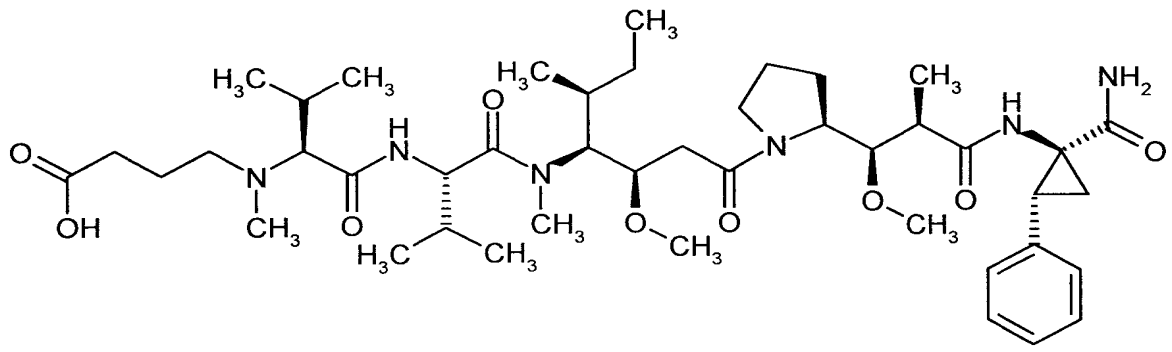
subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 10 mg (0.01 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2.5 mg (26% of theory) of the title compound.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.13 min; MS (ESIpos): m/z = 946 $(M+H)^+$.

Intermediate 81

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



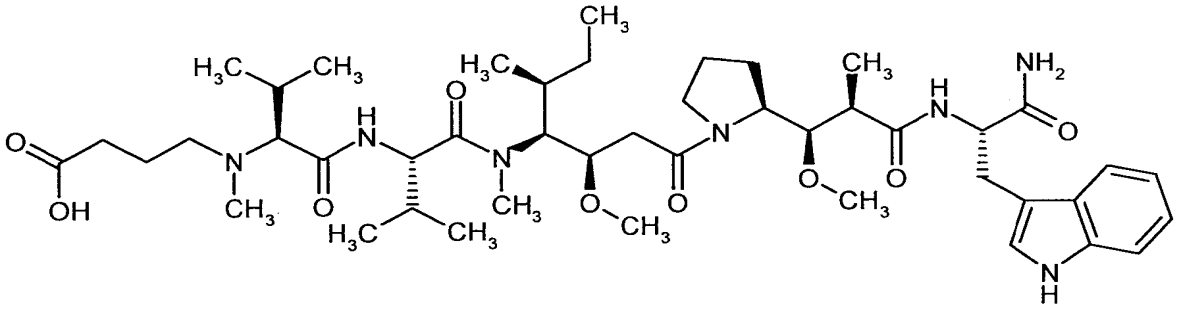
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxamide trifluoroacetate (Intermediate 48) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 14 mg (0.0163 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 8 mg (57% of theory) of the title compound.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 9): R_t = 4.64 min; MS (ESIpos): m/z = 829 $(M+H)^+$.

Intermediate 82

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



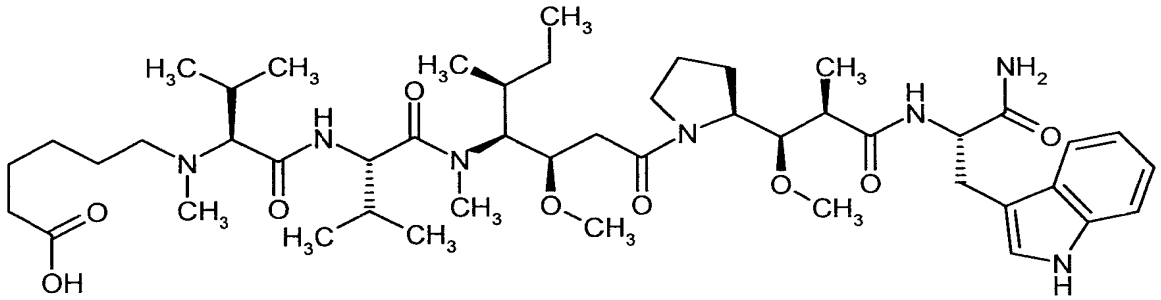
First, in analogy to the synthesis described in Intermediate 69, by coupling of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Fmoc protecting group by means of piperidine, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 78 mg (0.088 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 68 mg (90% of theory) of the title compound.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 9): R_t = 4.49 min; MS (ESIpos): m/z = 856 $(M+H)^+$.

Intermediate 83

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the compound in Intermediate 82, proceeding from 20 mg (26 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride prepared.

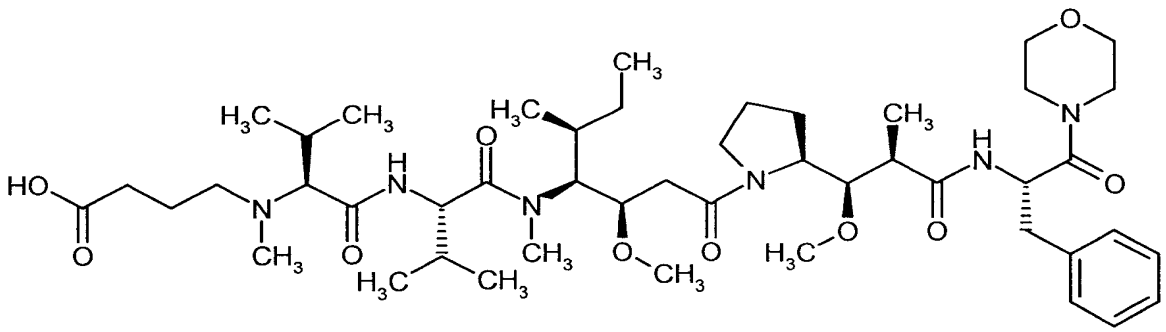
Yield: 5 mg (25% of theory)

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.72 min; MS (ESIpos): m/z = 884 $(M+H)^+$.

Intermediate 84

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 79, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and morpholine in the presence of EDC and HOBT and subsequent detachment of the Boc protecting group by means of trifluoroacetic

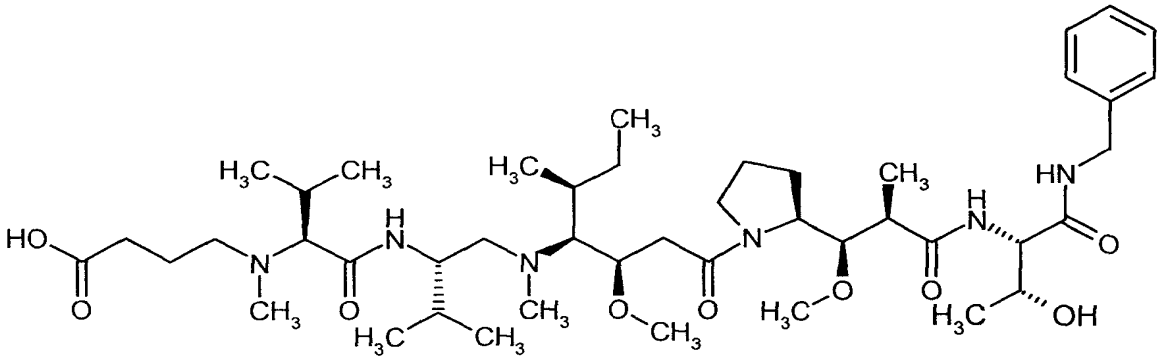
acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 30 mg (0.033 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 22 mg (76% of theory) of the title compound.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 9): R_t = 4.58 min; MS (ESIpos): m/z = 887 $(M+H)^+$.

Intermediate 85

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



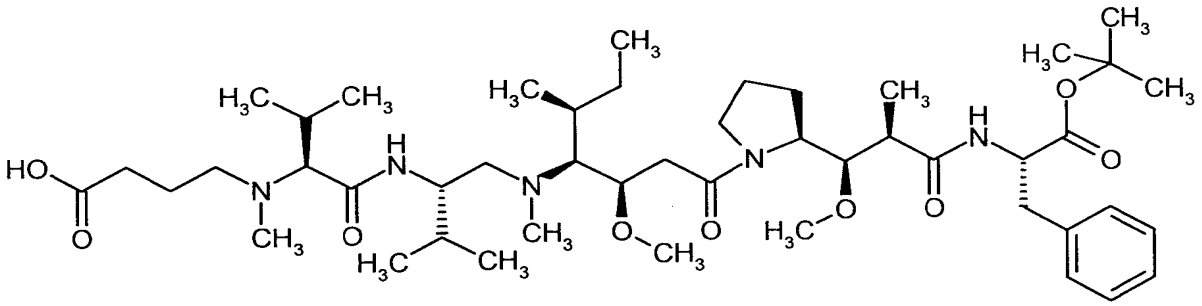
First, in analogy to the synthesis described in Intermediate 79, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and *N*-benzyl-L-threoninamide trifluoroacetate in the presence of HATU and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 21 mg (0.024 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 20 mg (97% of theory) of the title compound.

HPLC (Method 5): R_t = 1.54 min;

LC-MS (Method 9): R_t = 4.49 min; MS (ESIpos): m/z = 861 $(M+H)^+$.

Intermediate 86

4-{[(2*S*)-1-{[(2*S*)-1-{[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*.-Butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-phenylalaninate hydrochloride in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid to obtain the *tert*-butyl ester (stirring with trifluoroacetic acid in dichloromethane for 40 minutes), the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 22 mg (0.02 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 16 mg (94% of theory) of the title compound.

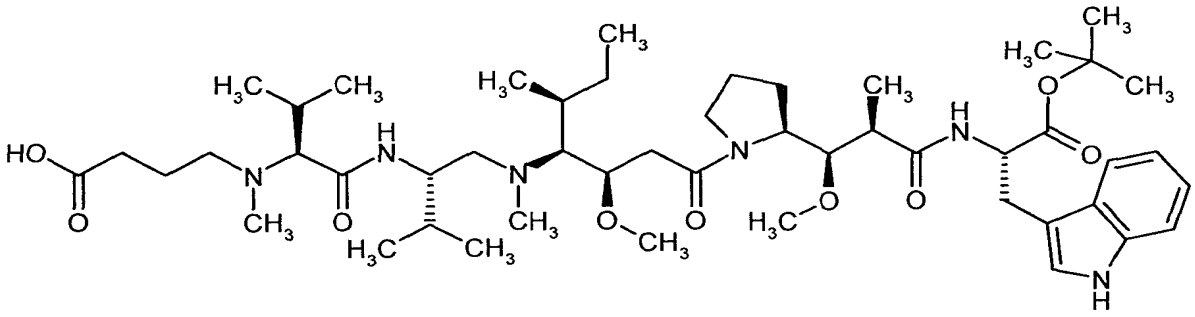
HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 9): R_t = 5.05 min; MS (ESIpos): m/z = 874 $(M+H)^+$.

Intermediate 87

4-{[(2*S*)-1-{[(2*S*)-1-{[(3*R*,4*S*,5S)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-Butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-

oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid



This compound was prepared in analogy to the synthesis described in Intermediate 86, proceeding from 230 mg (336 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-tryptophanate hydrochloride over 3 stages.

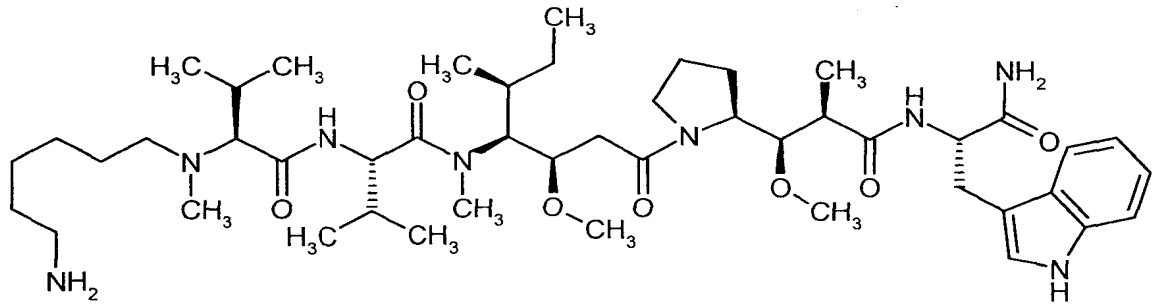
Yield: 95 mg (31% of theory over 3 stages)

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 9): R_t = 5.05 min; MS (ESIpos): m/z = 913 $(M+H)^+$.

Intermediate 88

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the syntheses described in Intermediate 69, by coupling of *N*-[(9H-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-

2-yl]propanoyl}-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent detachment of the Fmoc protecting group by means of piperidine, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 30 mg (0.03 mmol) of this compound were then used, in analogy to the preparation of the compound of Intermediate 61, by reaction with benzyl 6-oxohexyl carbamate, which had been obtained beforehand by oxidation of benzyl 6-hydroxyhexyl carbamate, in the presence of sodium cyanoborohydride, to obtain 17 mg (45% of theory) of the Z-protected compound. Subsequently, hydrogenolysis in methanol over 10% palladium/activated carbon afforded the title compound.

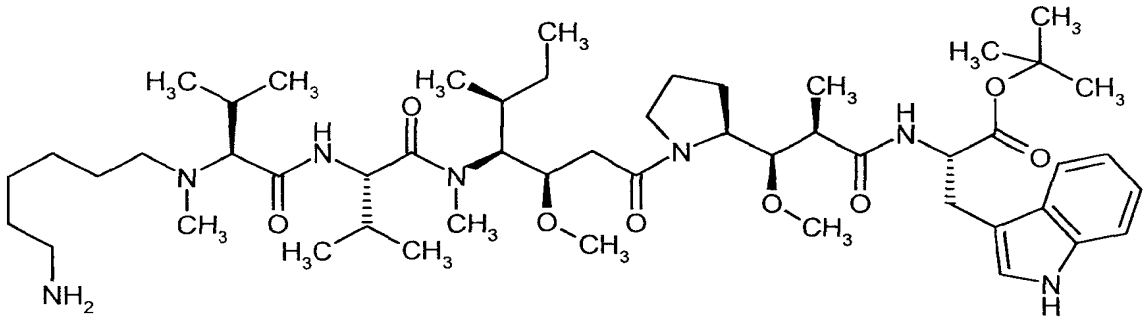
Yield: 14 mg (95% of theory)

HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 1): R_t = 0.73 min; MS (ESIpos): m/z = 869 $(M+H)^+$.

Intermediate 89

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert-but*oxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 86, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-tryptophanate hydrochloride in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid to obtain the *tert*-butyl ester (stirring with 1:10 trifluoroacetic acid/dichloromethane for 30 min), the amine compound *N*-

methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 71 mg (0.075 mmol) of this compound were then used, in analogy to the preparation of the compound of Intermediate 61, by reaction with benzyl 6-oxohexyl carbamate, which had been obtained beforehand by oxidation of benzyl 6-hydroxyhexyl carbamate, in the presence of sodium cyanoborohydride, to obtain 35 mg (44% of theory) of the Z-protected compound. Subsequently, hydrogenolysis in methanol over 10% palladium/activated carbon afforded the title compound.

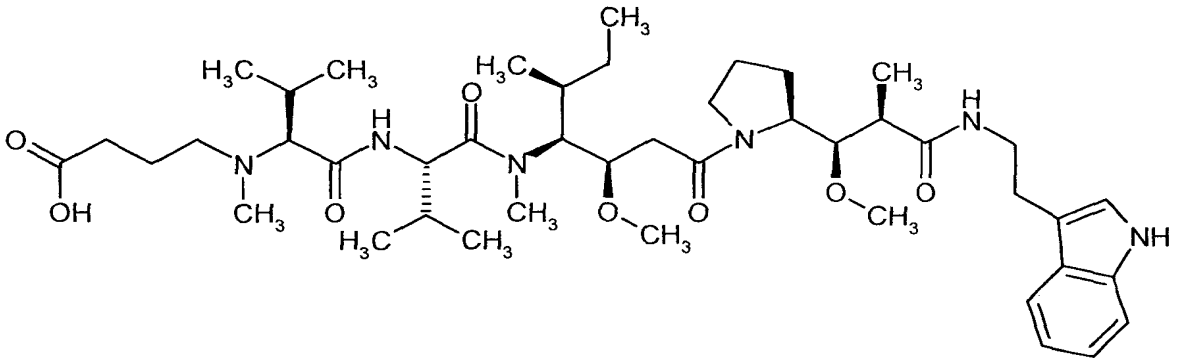
Yield: 30 mg (98% of theory)

HPLC (Method 5): Rt = 1.9 min;

LC-MS (Method 1): Rt = 0.77 min; MS (ESIpos): m/z = 926 (M+H)+.

Intermediate 90

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 2-(1*H*-indol-3-yl)ethanamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 100 mg (0.119 mmol) of this compound were then used, in

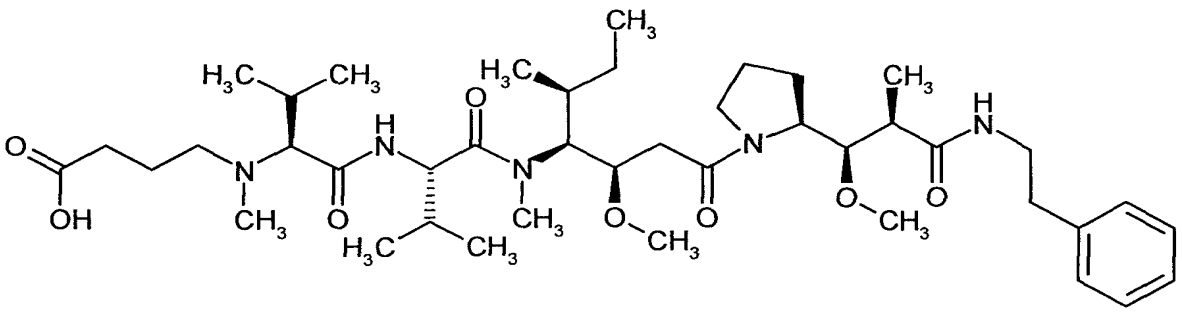
analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 50 mg (49% of theory) of the title compound. The title compound was purified here by flash chromatography on silica gel with dichloromethane/methanol/17% ammonia as the eluent, in the course of which the mixing ratio was switched from initially 15/2/02 to 15/4/0.5.

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 813 $(M+H)^+$.

Intermediate 91

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-{(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-{(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl}pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl}-*N*-methyl-L-valinamide



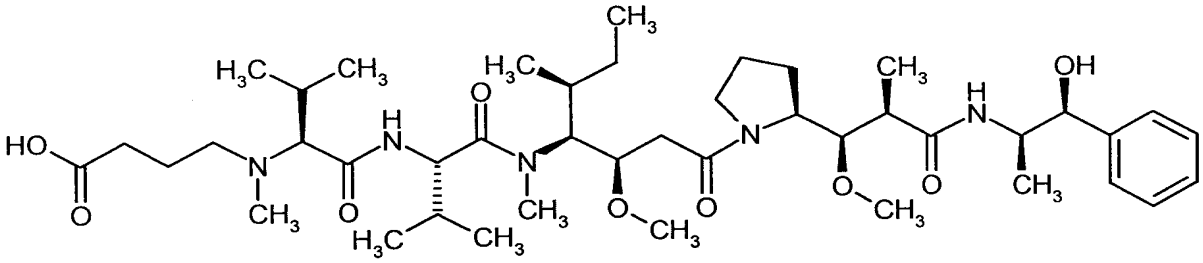
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and phenylethylamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-{(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-{(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl}pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl}-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 57 mg (0.071 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 44 mg (80% of theory) of the title compound. The title compound can also be purified here by flash chromatography on silica gel with dichloromethane/methanol/17% ammonia as the eluent (15/2/02 -> 15/4/0.5).

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.64 min; MS (ESIpos): m/z = 774 $(M+H)^+$.

Intermediate 92

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



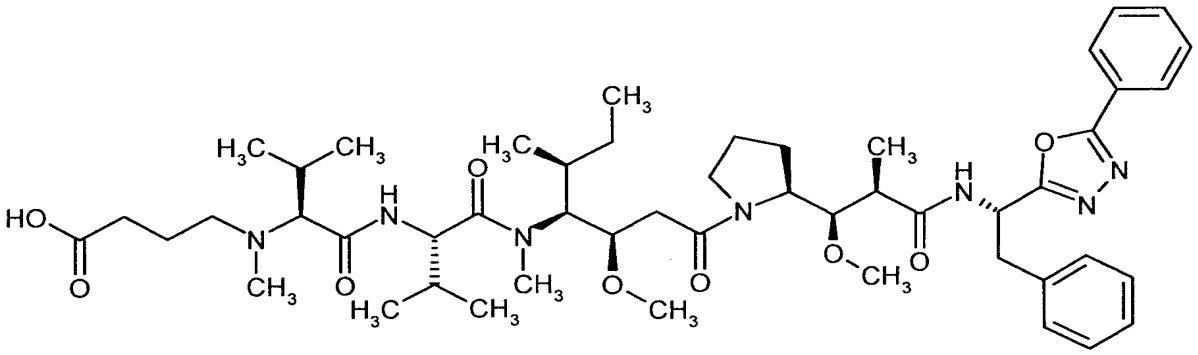
100 mg (0.139 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(*1R*,2*R*)-3-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 40) were used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 94 mg (84% of theory) of the title compound. The title compound was purified by preparative HPLC.

HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 9): R_t = 4.46 min; MS (ESIpos): m/z = 804 $(M+H)^+$.

Intermediate 93

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



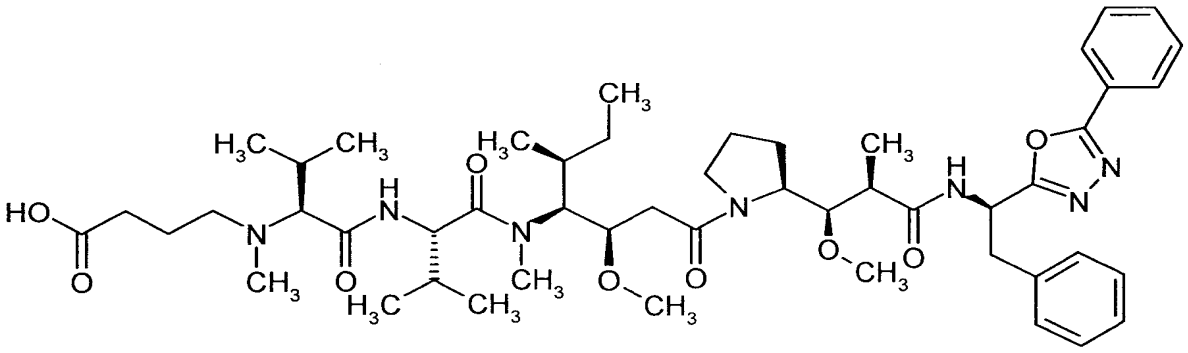
22.4 mg (24 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.4 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 8.2 mg (38% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): R_t = 2.54 min

LC-MS (Method 12): R_t = 0.94 min; MS (ESIpos): m/z = 919 $(M+H)^+$

Intermediate 94

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



17.1 mg (18 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

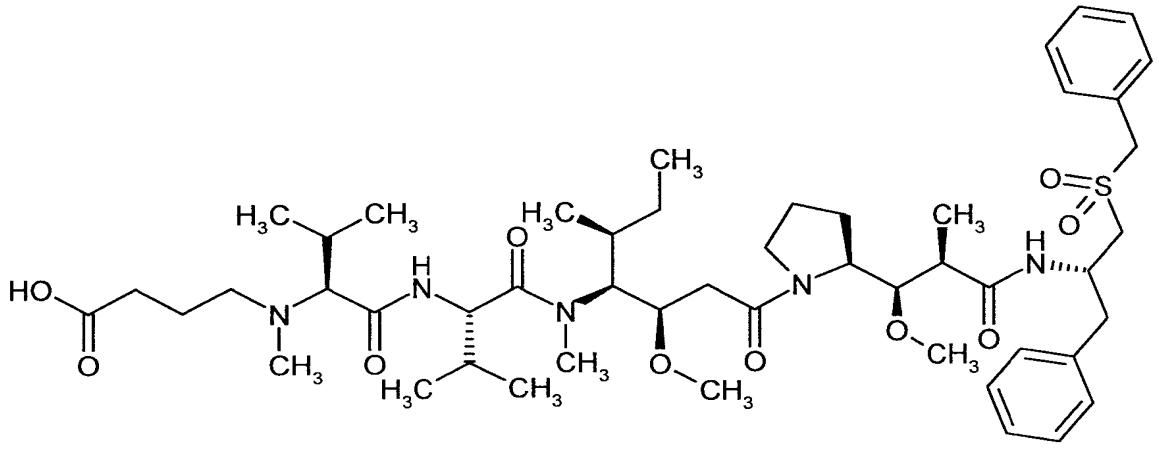
trifluoroacetate were dissolved in 1.1 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 14.8 mg (89% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): R_t = 2.54 min;

LC-MS (Method 12): R_t = 0.92 min; MS (ESIpos): m/z = 919 $(M+H)^+$

Intermediate 95

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

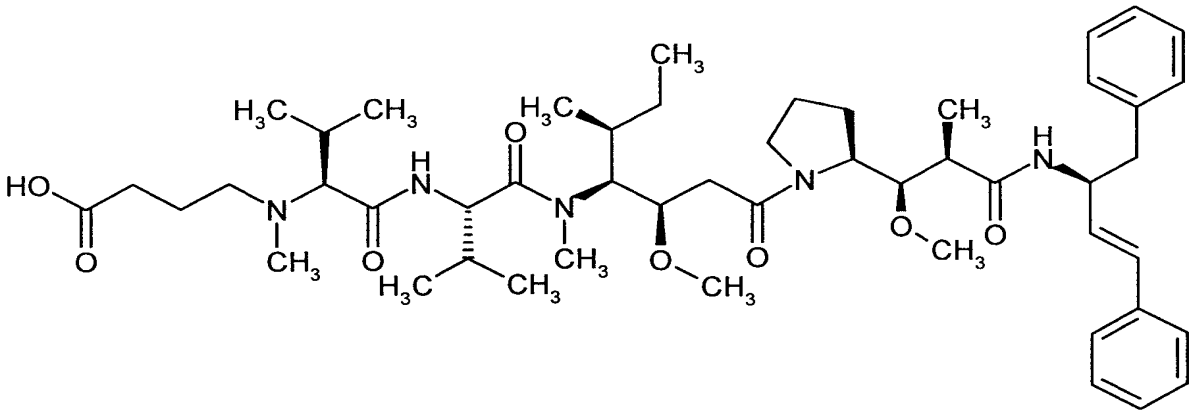


19.3 mg (20 µmol) *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.2 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 8.6 mg (45% of theory) of the title compound were obtained in the form of a solid.

LC-MS (Method 11): R_t = 0.85 min; MS (ESIpos): m/z = 943 $(M+H)^+$

Intermediate 96

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



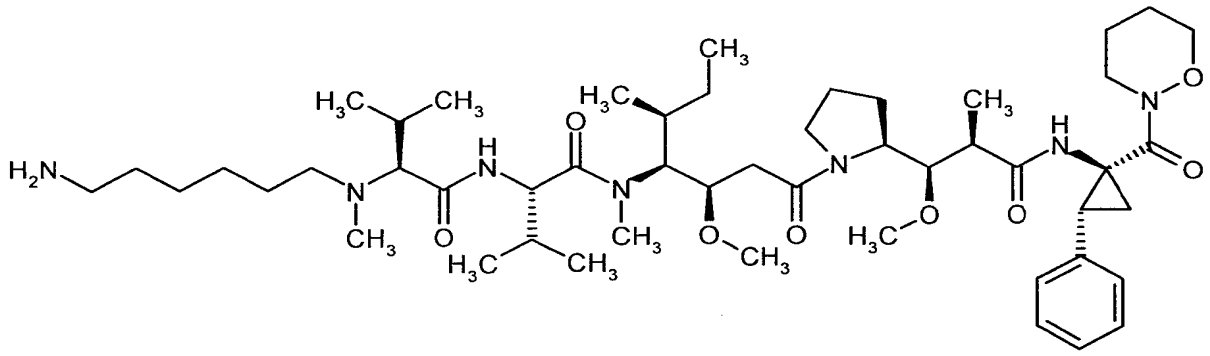
15.5 mg (10 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.0 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 10.3 mg (68% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): R_t = 2.59 min;

LC-MS (Method 11): R_t = 0.94 min; MS (ESIpos): m/z = 877 $(M+H)^+$

Intermediate 97

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 66, by reaction of 200 mg (0.108 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with benzyl 3-oxohexyl carbamate and subsequent hydrogenolytic detachment of the Z protecting group (with 5% palladium on charcoal as a catalyst, in methanol as a solvent).

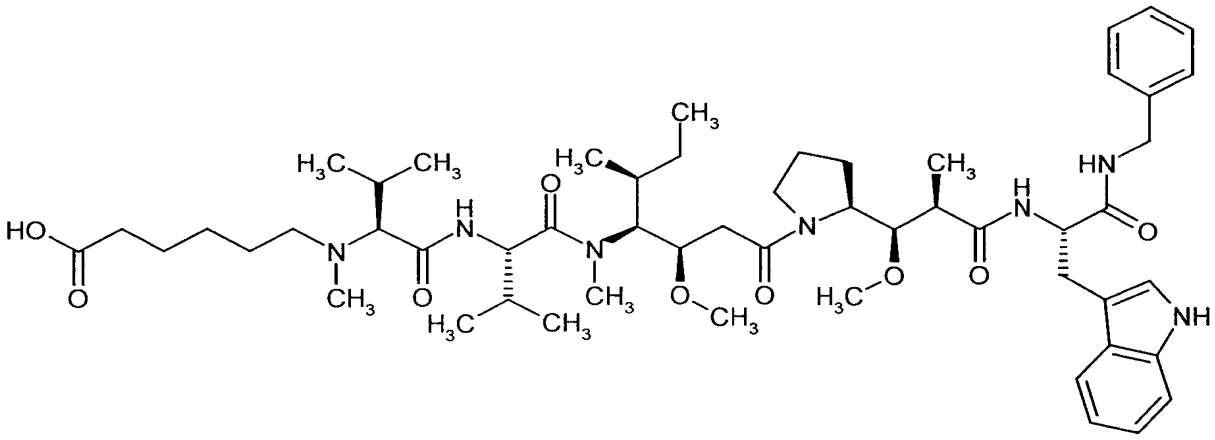
Yield: 69 mg (65% of theory over two stages)

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.76 min; MS (ESIpos): m/z = 912 $(M+H)^+$.

Intermediate 98

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 80. The purification was effected by preparative HPLC.

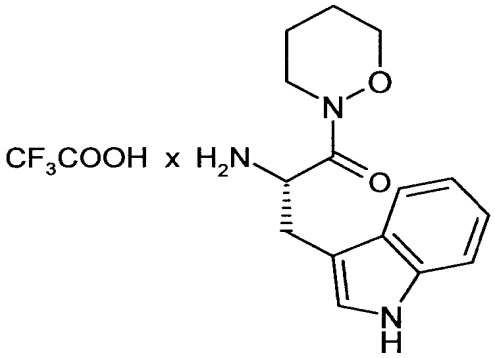
Yield: 40 mg (29% of theory over 3 stages)

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 974 $(M+H)^+$.

Intermediate 99

(2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate



324 mg (0.81 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(*tert*-butoxycarbonyl)-L-tryptophanate were dissolved in 20 ml of DMF, and 200 mg (1.62 mmol) of 1,2-oxazinane hydrochloride (Starting Compound 5) and 850 µl of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at 50°C overnight and then concentrated under reduced pressure. The residue was taken up in dichloromethane and extracted with water. The organic phase was dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography on silica gel with 4:1 dichloromethane/ethyl acetate as the eluent. The product fractions were concentrated and the residue was dried under high vacuum. This gave 147.5 mg (48% of theory) of the Boc-protected intermediate.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 374 $(M+H)^+$.

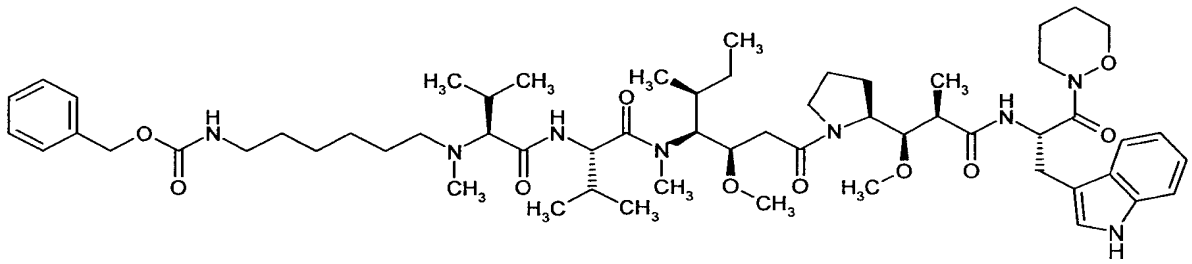
Using 166 mg (444.5 µmol) of this intermediate, under standard conditions with 3 ml of trifluoroacetic acid in 20 ml of dichloromethane, the Boc protecting group was detached and, after HPLC purification, 155 mg (86% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.43 min;

LC-MS (Method 11): R_t = 0.56 min; MS (ESIpos): m/z = 274 $(M+H)^+$.

Intermediate 100

N-(6-{[(benzyloxy)carbonyl]amino}hexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



177 mg (260 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 100 mg (260 µmol) of (2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate (Intermediate 99) were taken up in 15 ml of DMF, and 118 mg (310 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 140 µl of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 30 min, then concentrated under reduced pressure, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated. After lyophilization from dioxane, 170 mg (68% of theory) of the Boc-protected intermediate were obtained.

LC-MS (Method 1): R_t = 1.36 min; m/z = 940 $(M+H)^+$.

170 mg of this intermediate were treated with 3 ml of trifluoroacetic acid in 30 ml of dichloromethane for 30 min to detach the Boc protecting group. Then the reaction mixture was concentrated under reduced pressure and the residue was purified by means of preparative HPLC to obtain 155 mg (86% of theory) of the deprotected N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl] pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide intermediate.

HPLC (Method 12): R_t = 1.85 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 840 $(M+H)^+$.

50 mg (0.052 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 97, with benzyl 3-oxohexyl carbamate in the presence of sodium cyanoborohydride

and subsequent hydrogenolytic detachment of the Z protecting group (with 5% palladium on charcoal as a catalyst, in methanol as a solvent), prepared to prepare the title compound.

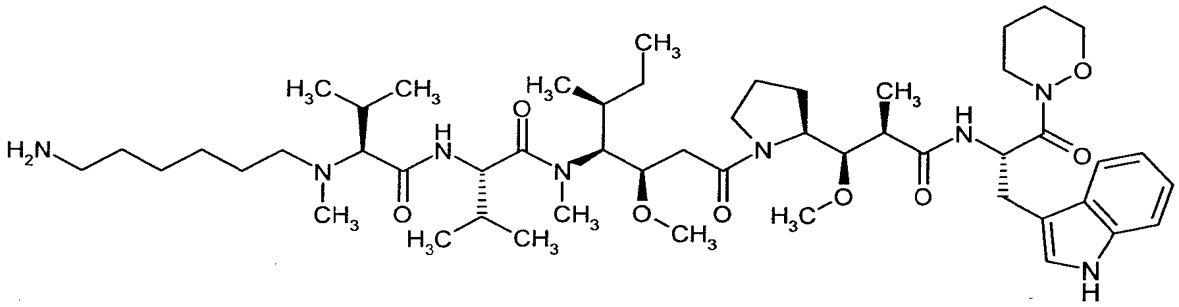
Yield: 21 mg (37% of theory)

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 1073 $(M+H)^+$.

Intermediate 101

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



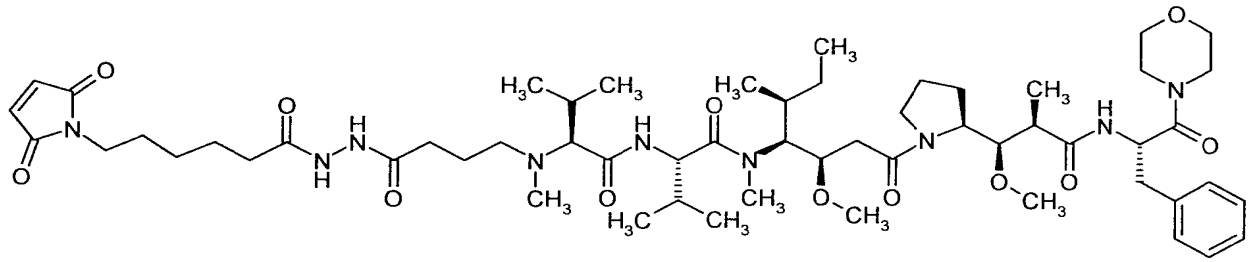
26.7 mg (24.87 µmol) of Intermediate 100 were dissolved in 10 ml of methanol and hydrogenated over palladium/activated carbon (5%) under standard hydrogen pressure for 30 min. The catalyst was filtered off and the solvent was evaporated off under reduced pressure. After the residue had been dried under high vacuum, 22.5 mg (96% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.76 min; MS (ESIpos): m/z = 939 $(M+H)^+$.

Intermediate 102

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

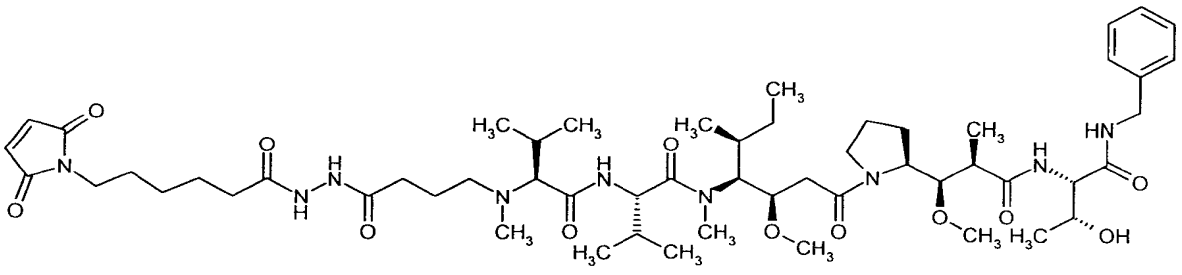
Yield: 8 mg (71% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1094 $(M+H)^+$.

Intermediate 103

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

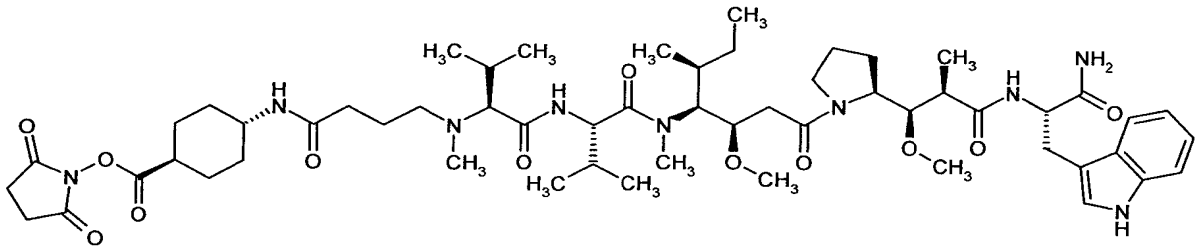
Yield: 3 mg (22% of theory)

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.78 min; MS (ESIpos): m/z = 1069 $(M+H)^+$.

Intermediate 104

N-{4-[(trans-4-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}cyclohexyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, benzyl trans-4-aminocyclohexanecarboxylate trifluoroacetate was prepared from *trans*-4-aminocyclohexanecarboxylic acid by introducing the Boc protecting group, then introducing the benzyl ester protecting group and subsequently detaching the Boc protecting group by conventional peptide chemistry methods.

15 mg (18 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were then dissolved in 5 ml of dimethylformamide and subsequently admixed with 13 mg (35 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 9 µl of *N*,*N*-diisopropylethylamine and with 15 mg (44 µmol) of benzyl *trans*-4-aminocyclohexanecarboxylate trifluoroacetate. The mixture was stirred at RT for 1 h and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. The corresponding fractions were combined and the solvent was evaporated off under reduced pressure. After the residue had been dried under high vacuum, 14.7 mg (78% of theory) of the protected intermediate were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1072 $(M+H)^+$.

From this protected intermediate, the benzyl ester was first removed by hydrogenolytic means and the free carboxyl component was obtained in quantitative yield. 14 mg (14 µmol; 1 equiv.) of the

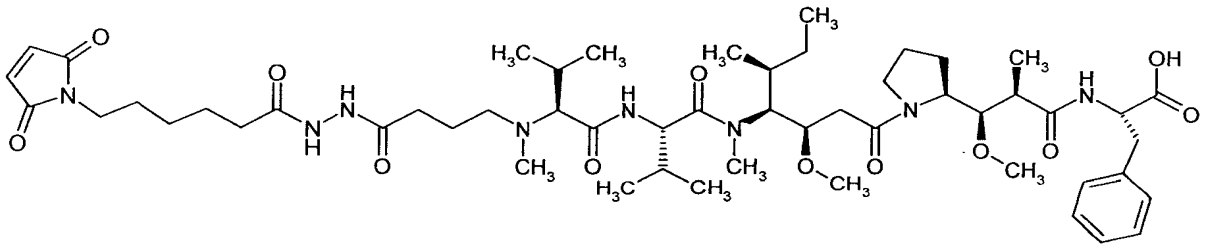
deprotected compound were taken up in 5 ml of DMF and admixed with 3.3 mg (29 µmol; 2.1 equiv.) of *N*-hydroxysuccinimide in the presence of 4.1 mg (21 µmol; 1.5 equiv.) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 7.5 µl (44 µmol; 3.1 equiv.) of *N,N*-diisopropylethylamine and 0.9 mg (7 µmol; 0.5 equiv.) of 4-dimethylaminopyridine, and the mixture was stirred at RT overnight. Then another 10 equiv. of *N*-hydroxysuccinimide, 5 equiv. of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5 equiv. of *N,N*-diisopropylethylamine and 0.5 equiv. of 4-dimethylaminopyridine were added and the reaction mixture was treated in an ultrasound bath for 5 h. Subsequently, the solvent was evaporated off, the residue was purified by means of preparative HPLC and the corresponding fractions were combined and concentrated. After lyophilization of the residue from dioxane, 9.7 mg (62% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 11): R_t = 0.77 min; MS (ESIpos): m/z = 1078 $(M+H)^+$.

Intermediate 105

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



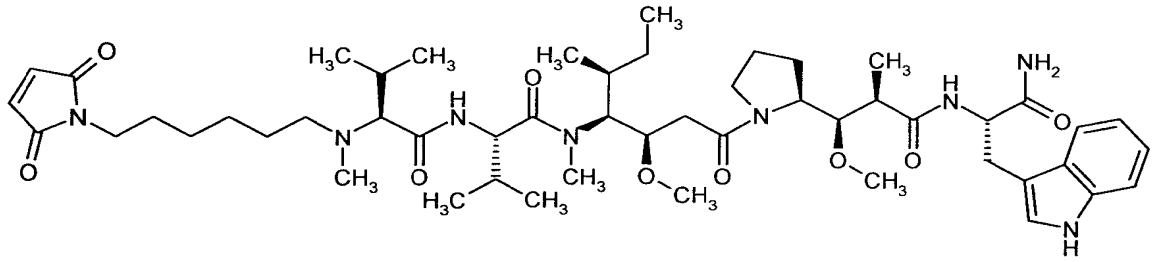
This compound was prepared in analogy to the synthesis described in Intermediate 157, proceeding from 4-{[(2*S*)-1-{[(2*S*)-1-{[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The ester intermediate was obtained in 42% yield. In a second step, 6 mg (6 µmol) of this intermediate were cleaved with trifluoroacetic acid the tert-butyl ester. After HPLC purification, 3.4 mg (48% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.66 min;

LC-MS (Method 2): R_t = 1.04 min; MS (ESIpos): m/z = 1025 $(M+H)^+$.

Intermediate 106

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



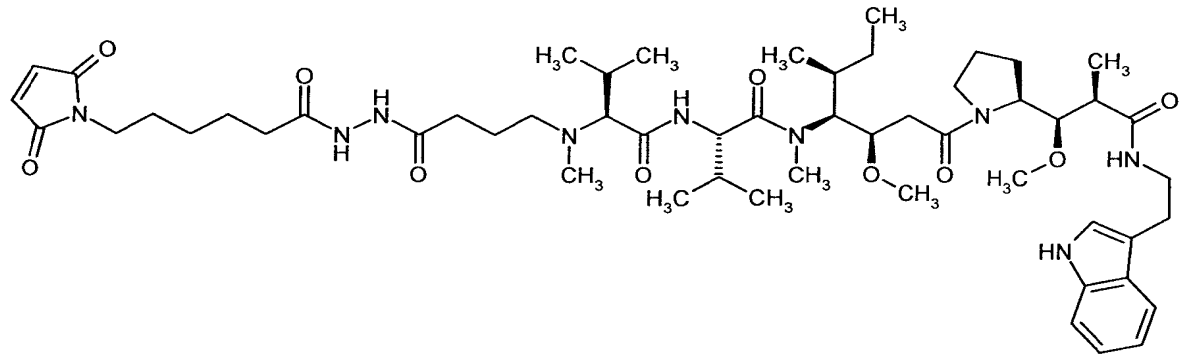
14 mg (16 µmol) of *N*-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 87) were taken up in 750 µl of dioxane and admixed with 1.5 ml of saturated sodium hydrogencarbonate solution and then with 3.2 mg (21 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 5.5 mg (36% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 949 $(M+H)^+$.

Intermediate 107

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



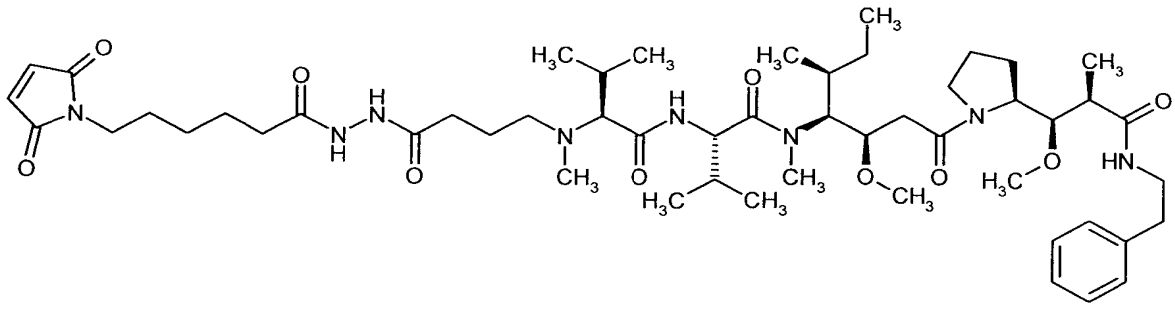
38 mg (47 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 37 ml of DMF and then admixed with 71 mg (187 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 33 µl of *N,N*-diisopropylethylamine and with 37 mg (140 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 12.2 mg (26% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1020 $(M+H)^+$.

Intermediate 108

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-{(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-{(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl}pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl}-*N*-methyl-L-valinamide



The compound was prepared in analogy to Intermediate 107.

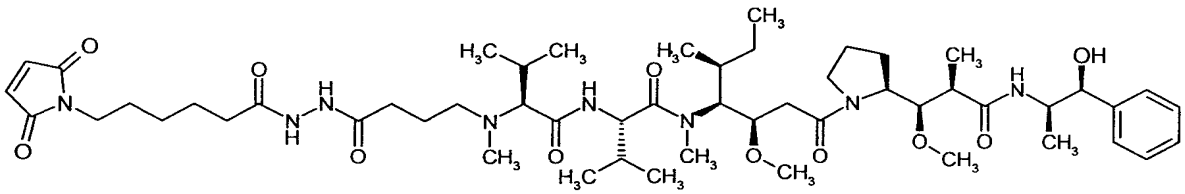
Yield: 2.5 mg (30% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 981 $(M+H)^+$.

Intermediate 109

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The compound was prepared in analogy to Intermediate 107 from the compound in Intermediate 92.

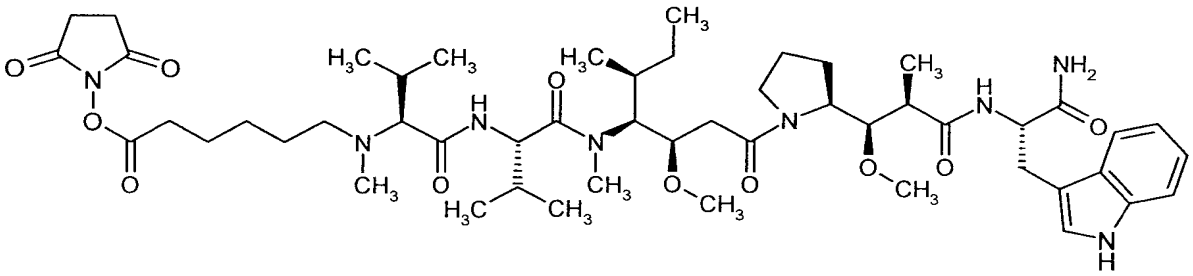
Yield: 35 mg (65% of theory)

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 11): R_t = 0.76 min; MS (ESIpos): m/z = 1011 $(M+H)^+$.

Intermediate 110

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 147 from the compound in Intermediate 83.

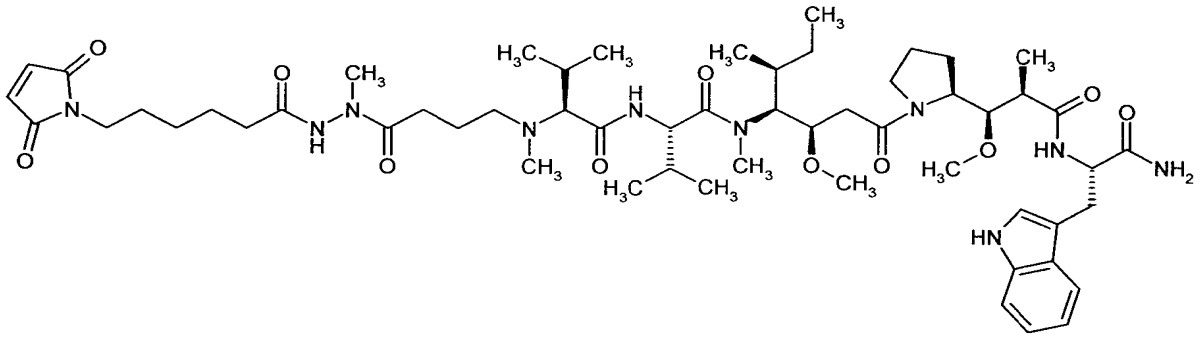
Yield: 2.4 mg (24% of theory)

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 981 $(M+H)^+$.

Intermediate 111

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 140 from Intermediate 82 and Intermediate 22.

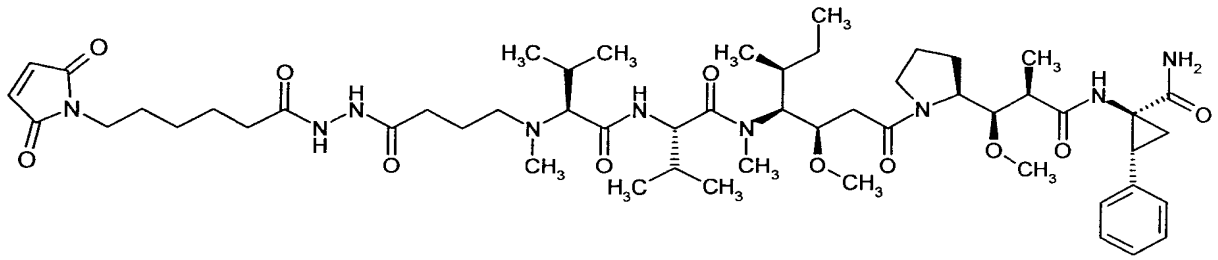
Yield: 6.5 mg (51% of theory)

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 1): R_t = 4.71 min; MS (ESIpos): m/z = 1077 $(M+H)^+$.

Intermediate 112

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 157 from the compound in Intermediate 81.

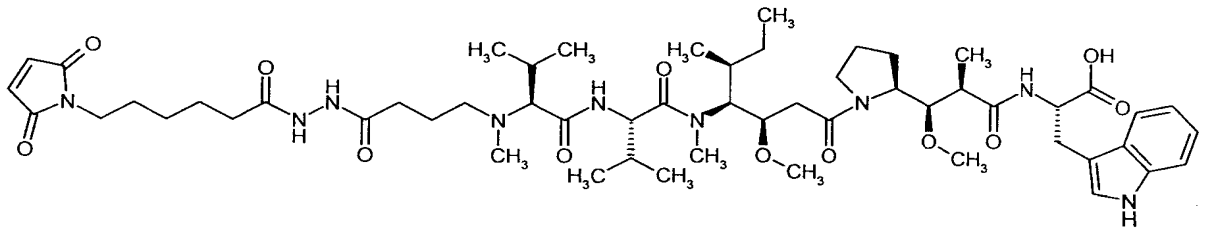
Yield: 5.7 mg (57 % of theory)

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1036 $(M+H)^+$.

Intermediate 113

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



95 mg (104 µmol) of 4-{[(2*S*)-1-{[(2*S*)-1-{[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid were dissolved in DMF and then admixed with 79.5 mg (209 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 73 µl of *N*,*N*-diisopropylethylamine and with 68 mg (261 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 2 h. This was followed by concentration under high vacuum and purification of the remaining residue by

means of preparative HPLC. Thus, 104 mg (89% of theory) of the tert-butyl ester of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.93 min; MS (ESIpos): m/z = 1121 $(M+H)^+$.

The intermediate was taken up in 33.4 ml of dichloromethane, 17 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC.

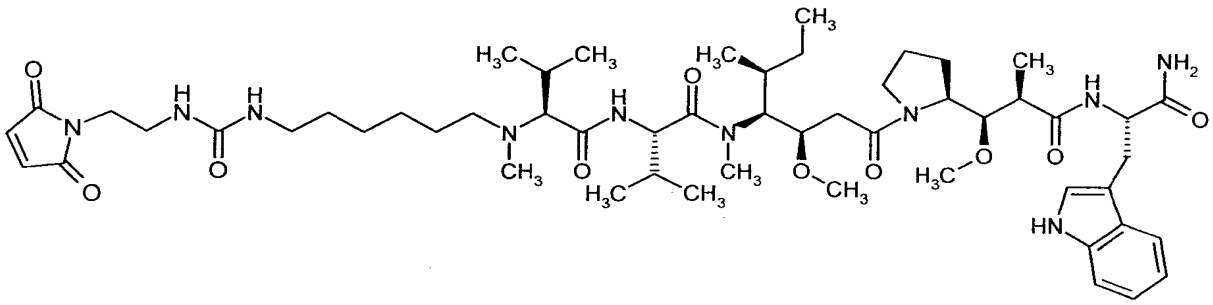
Thus, 61 mg (62 % of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1064 $(M+H)^+$.

Intermediate 114

N-[6-({[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamoyl}amino)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5 mg (5 µmol) of *N*-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were taken up in 885 µl of DMF and admixed with 5.3 mg (8 µmol) of 4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate and 2.8 µl of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 2 h and then concentrated to dryness. The residue was purified by means of preparative HPLC.

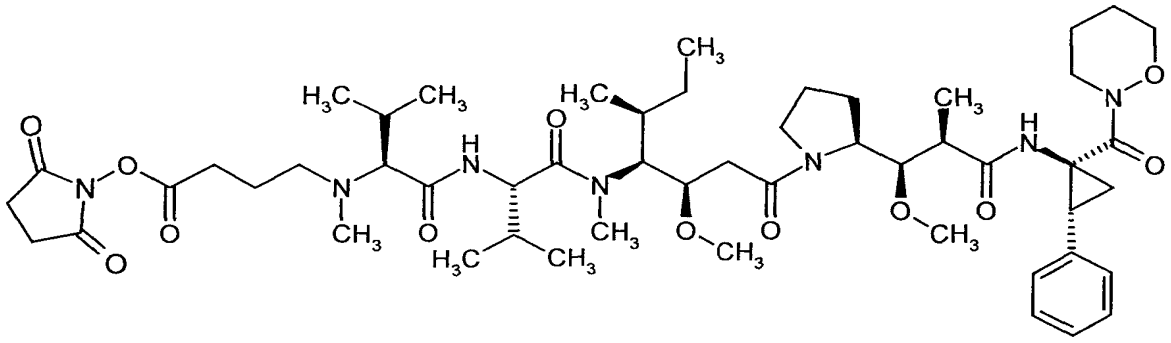
Yield: 0.58 mg (11% of theory) of a colourless foam

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 1035 $(M+H)^+$.

Intermediate 115

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the compound in Intermediate 147, proceeding from 8 mg (9 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide. After concentration, the activated ester was purified by means of preparative HPLC and, after removal of the solvent under reduced pressure, reacted immediately with the antibody.

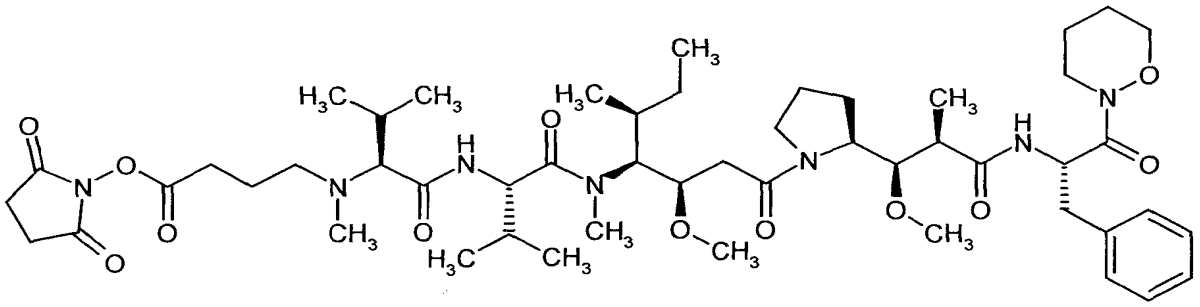
Yield: 3 mg (27% of theory) (hydrolysis-sensitive)

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 996 $(M+H)^+$.

Intermediate 116

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the compound in Intermediate 147, proceeding from 5 mg (6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide. After concentration, the activated ester was purified by means of preparative HPLC and, after removal of the solvent under reduced pressure, reacted immediately with the antibody.

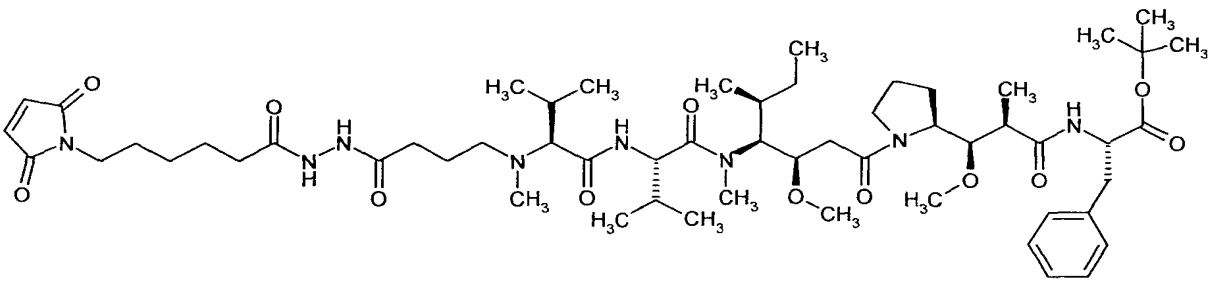
Yield: 3.2 mg (43% of theory) (hydrolysis-sensitive)

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 984 $(M+H)^+$.

Intermediate 117

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-tert-butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 157 from the compound in Intermediate 86.

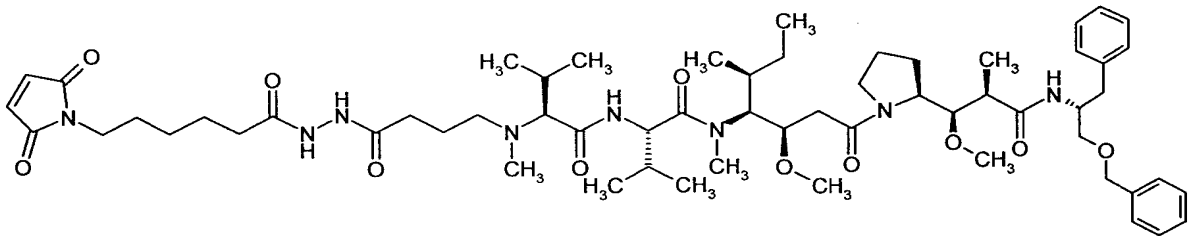
Yield: 7 mg (42% of theory)

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1081 $(M+H)^+$.

Intermediate 118

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*R*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

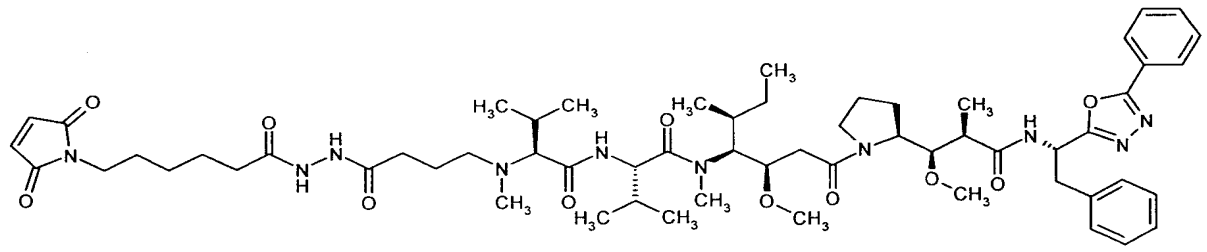


The target compound was prepared analogously to Intermediate 157 from 7 mg (7.8 µmol) of the compound in Intermediate 68. Yield: 6.3 mg (53% of theory)

LC-MS (Method 1): R_t = 1.00 min; MS (ESIpos): m/z = 1102 $(M+H)^+$.

Intermediate 119

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



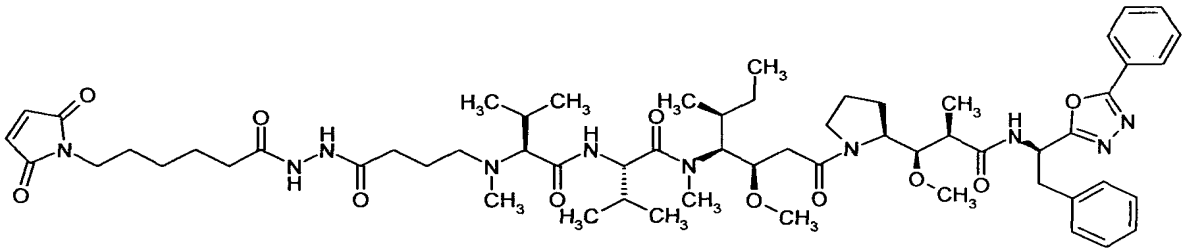
7.4 mg (8.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.3 mg (24.2 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride

were coupled and worked up in analogy to Intermediate 157. 1.6 mg (13% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): R_t = 0.89 min; MS (ESIpos): m/z = 1126 $(M+H)^+$

Intermediate 120

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

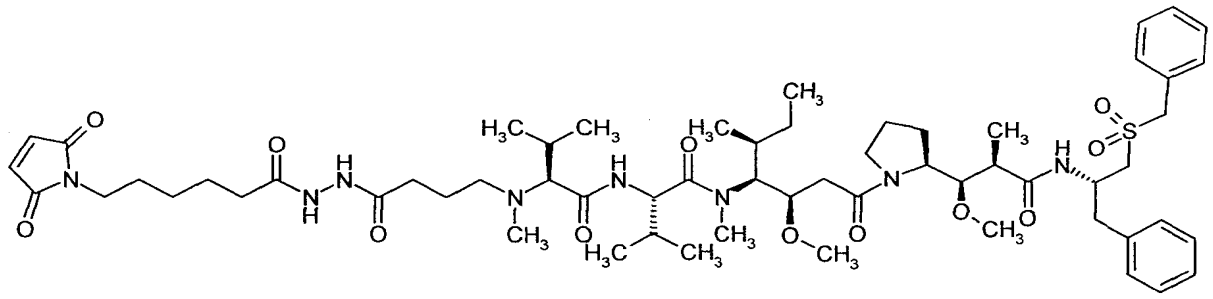


12.8 mg (13.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 10.9 mg (41.8 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 10.8 mg (59% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): R_t = 0.90 min; MS (ESIpos): m/z = 1126 $(M+H)^+$

Intermediate 121

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

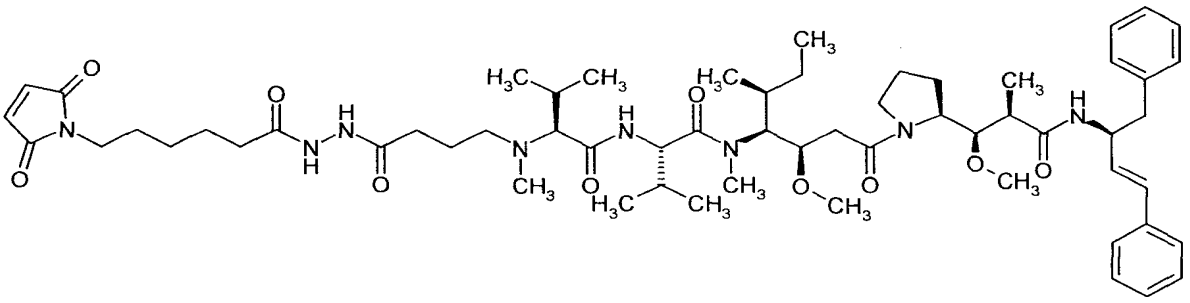


7.4 mg (7.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.2 mg (23.5 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 6.9 mg (74% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): R_t = 0.87 min; MS (ESIpos): m/z = 1150 $(M+H)^+$

Intermediate 122

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

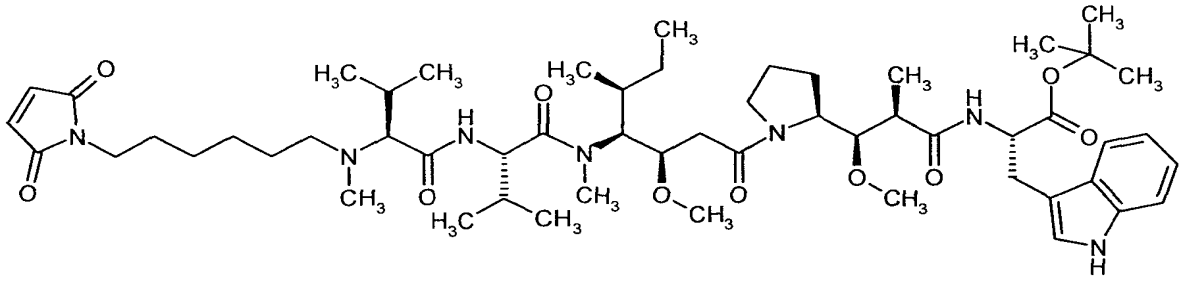


8 mg (9.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 7.2 mg (27.4 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 8.2 mg (82% of theory) of the title compound were obtained as a white solid.

LC-MS (Method 11): R_t = 0.95 min; MS (ESIpos): m/z = 1083 $(M+H)^+$

Intermediate 123

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



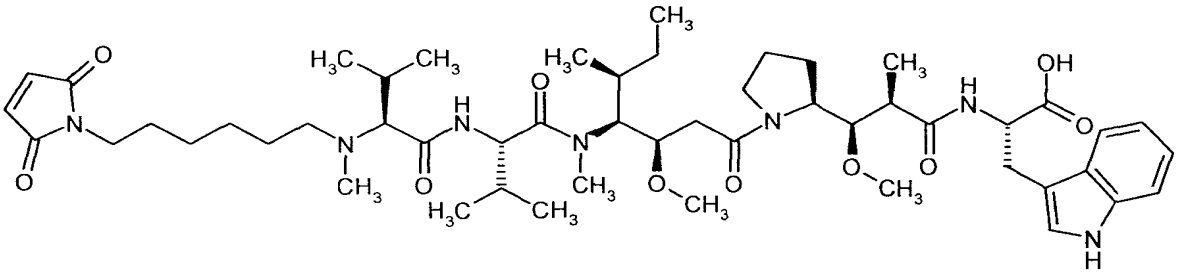
30 mg (30 µmol) of Intermediate 89 were taken up in 2 ml of 1,4-dioxane and admixed with 4 ml of saturated sodium hydrogencarbonate solution and then with 7.5 mg (50 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 24 mg (74% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.2 min;

LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 1006 $(M+H)^+$.

Intermediate 124

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



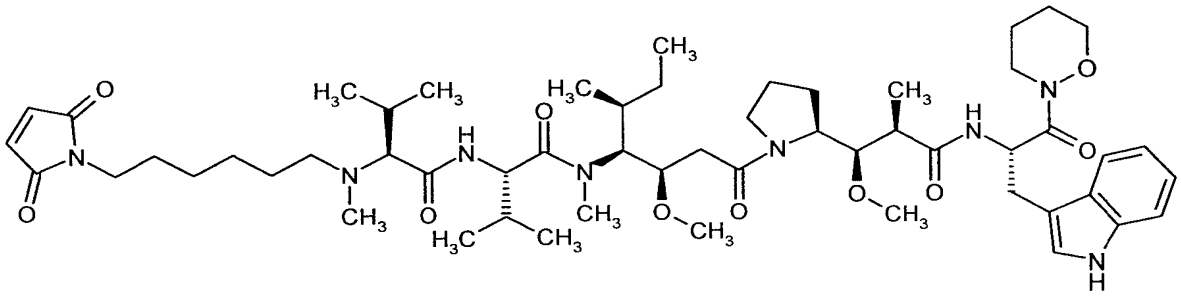
22 mg (20 µmol) of Intermediate 123 were reacted with 4 ml of trifluoroacetic acid in 8 ml of dichloromethane at RT for 1 h. Thereafter, the reaction mixture was concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 11 mg (54% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 11): R_t = 0.85 min; MS (ESIpos): m/z = 950 $(M+H)^+$.

Intermediate 125

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



22.5 mg (20 µmol) of Intermediate 101 were taken up in 2 ml of 1:1 dioxane/water and then admixed with 5.6 mg (40 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate and with 0.25 ml of saturated sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 30 min. Then another 0.25 ml of the saturated sodium hydrogencarbonate solution was added and the reaction mixture was stirred at RT for a further 15 min and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 12.8 mg (50% of theory) of the title compound were obtained as a colourless foam.

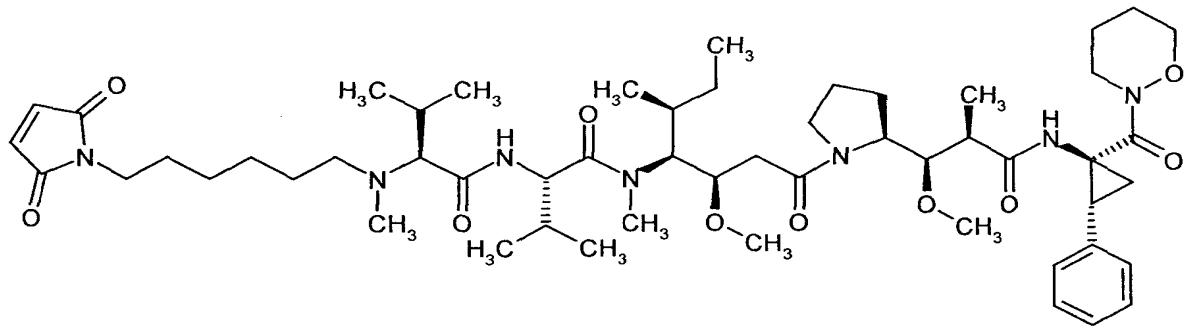
HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1019 $(M+H)^+$.

Intermediate 126

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-

phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



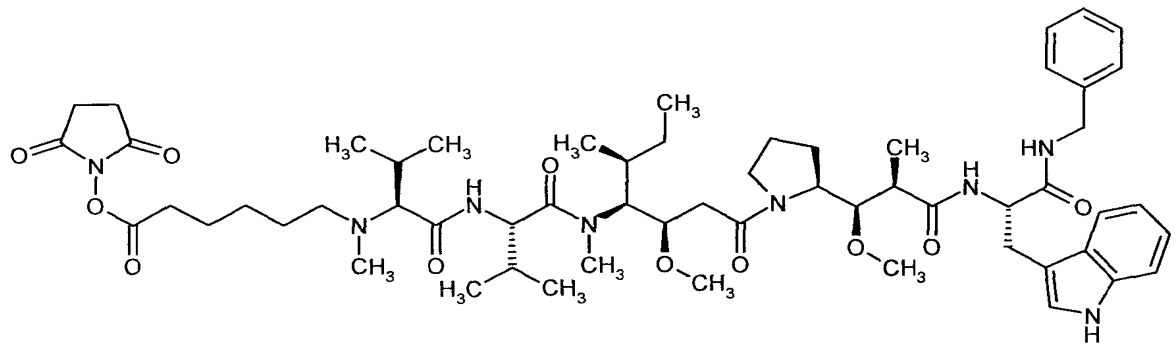
64 mg (70 µmol) of *N*-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 97) were taken up in 3 ml of 1:1 dioxane/water, then adjusted to pH 9 with 4 ml of saturated sodium hydrogencarbonate solution and subsequently admixed with 16.3 mg (110 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated under reduced pressure. Then another 8 mg (55 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate were added, and the reaction mixture was adjusted again to pH 9 and stirred at RT for a further hour. This was followed by concentration and purification of the remaining residue by means of preparative HPLC. At first, 31 mg of an as yet uncyclized intermediate were obtained. 27 mg of this intermediate were taken up again in 2 ml of 1:1 dioxane/water and then admixed with 250 µl of saturated sodium hydrogencarbonate solution. After stirring at RT for 2 hours, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. After lyophilization, 20 mg (29% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.96 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 992 $(M+H)^+$.

Intermediate 127

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



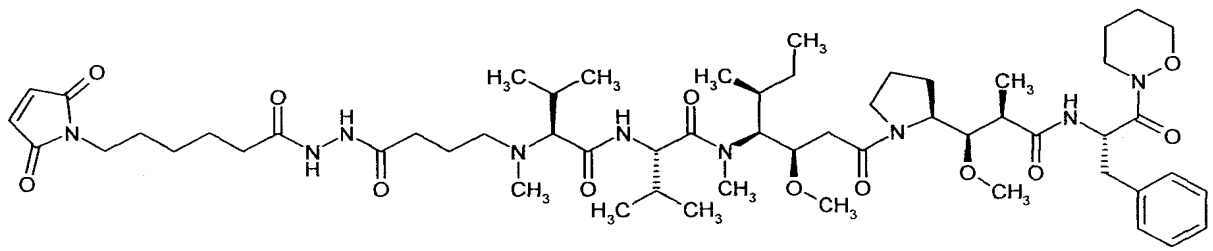
17 mg (18 µmol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 98) were dissolved in 2.8 ml of dichloromethane and admixed with 20 mg (174 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 10 mg (52 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.21 mg (0.17 µmol) of DMAP. After stirring at RT for 4 h, the reaction mixture was concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 8.2 mg (43% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 1071 $(M+H)^+$.

Intermediate 128

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-

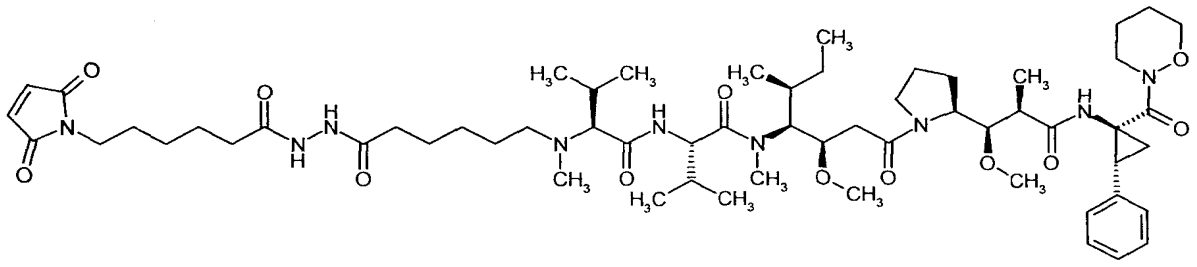
3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 845 µl of DMF and then admixed with 3.2 mg (17 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.6 mg (17 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 1.96 µl of *N,N*-diisopropylethylamine and with 5.9 mg (22.5 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 2.2 mg (36% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1094 $(M+H)^+$.

Intermediate 129

N-(6-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



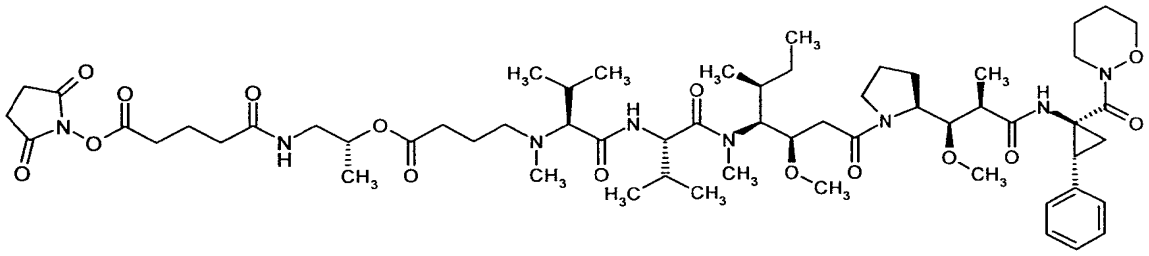
4 mg (4.3 µmol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 646 µl of DMF and then admixed with 2.5 mg (13 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.0 mg (13 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.25 µl of *N,N*-diisopropylethylamine and with 4.5 mg (17 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 3 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 1.9 mg (39% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.9 min; MS (ESIpos): m/z = 1134 $(M+H)^+$.

Intermediate 130

N-(4-{[(2*R*)-1-({5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}amino)propan-2-yl]oxy}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10.5 mg (11.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3.7 ml of dichloromethane and then admixed with 6.7 mg (35 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.7 mg (5.8 µmol) of 4-dimethylaminopyridine and with 8.2 mg (47 µmol) of commercially available *tert*-butyl (2*R*)-2-hydroxypropyl carbamate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 7.5 mg (61% of theory) of the Boc-protected intermediate were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 1056 $(M+H)^+$.

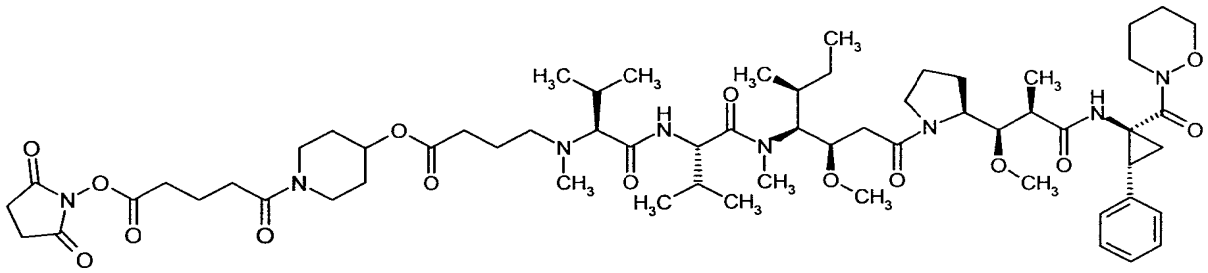
Subsequently, the Boc protecting group was detached with trifluoroacetic acid. 4.9 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.7 mg (0.011 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 2.4 µl (0.014 mmol) of *N,N*-diisopropylethylamine and 0.6 mg (5 µmol) of 4-dimethylaminopyridine. The mixture was stirred at RT for 2 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 0.77 mg (15% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.93 min; MS (ESIpos): m/z = 1167 $(M+H)^+$.

Intermediate 131

N-{4-[(1-{5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}piperidin-4-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10 mg (11 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2 ml of dichloromethane and then admixed with 4.3 mg (22 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.88 mg (6 µmol) of 4-dimethylaminopyridine and with 5.2 mg (22 µmol) of commercially available benzyl 4-hydroxypiperidine-1-carboxylate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 5 mg (40% of theory) of the Z-protected intermediate were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.04 min; MS (ESIpos): m/z = 1116 $(M+H)^+$.

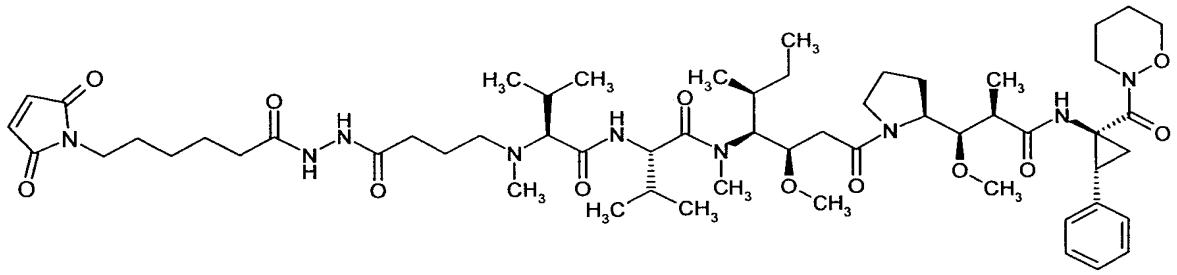
Subsequently, the Z protecting group was detached by hydrogenolytic means in ethanol over palladium/activated carbon. 4.6 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.8 mg (0.012 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 0.8 µl (0.005 mmol) of *N,N*-diisopropylethylamine and 0.6 mg (5 µmol) of 4-dimethylaminopyridine. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 0.96 mg (16% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1193 $(M+H)^+$.

Intermediate 132

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazinyl}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



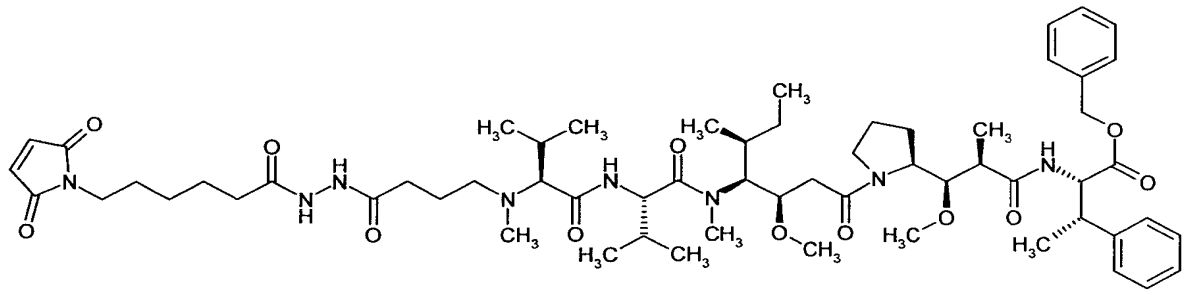
15 mg (16.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2500 µl of DMF and then admixed with 9.6 mg (50 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 7.6 mg (50 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 5.8 µl of *N,N*-diisopropylethylamine and with 17.4 mg (67 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 11.2 mg (52% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.09 min; MS (ESIpos): m/z = 1106 $(M+H)^+$.

Intermediate 133

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazinyl}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



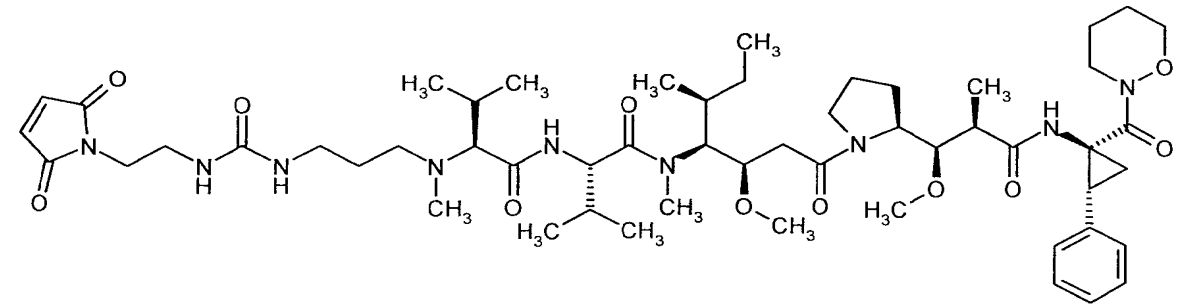
5.8 mg (6.3 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 943 µl of DMF and then admixed with 3.6 mg (19 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.9 mg (19 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.2 µl of *N,N*-diisopropylethylamine and with 6.6 mg (25 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 4.5 mg (64% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 1129 $(M+H)^+$.

Intermediate 134

N-[3-({[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamoyl}amino)propyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, 4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate was prepared under standard conditions, proceeding from commercially available 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione trifluoroacetate and 4-nitrophenyl chlorocarbonate.

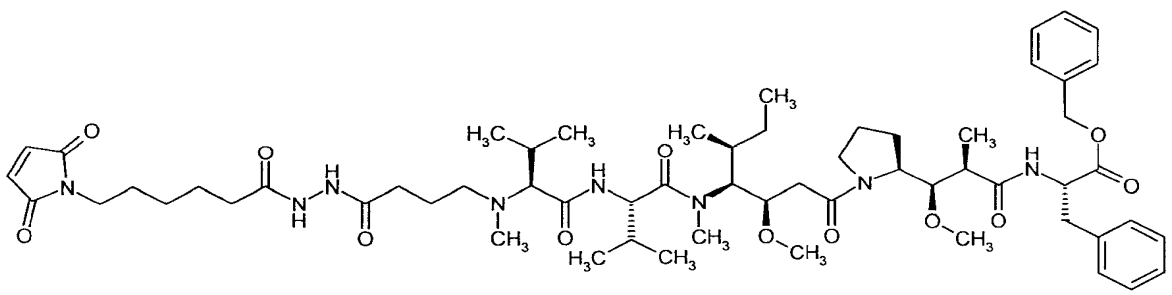
5 mg (6 µmol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1000 µl of DMF and then admixed with 2 µl of *N,N*-diisopropylethylamine and with 2.2 mg (9 µmol) of 4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate. The mixture was stirred at RT for 1 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 1.6 mg (23% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.09 min; MS (ESIpos): m/z = 1036 $(M+H)^+$.

Intermediate 135

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10 mg (11 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 4000 µl of DMF and then admixed with 6.3 mg (33 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4.5 mg (33 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 5.7 µl of *N,N*-diisopropylethylamine and with 11.5 mg (44 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture

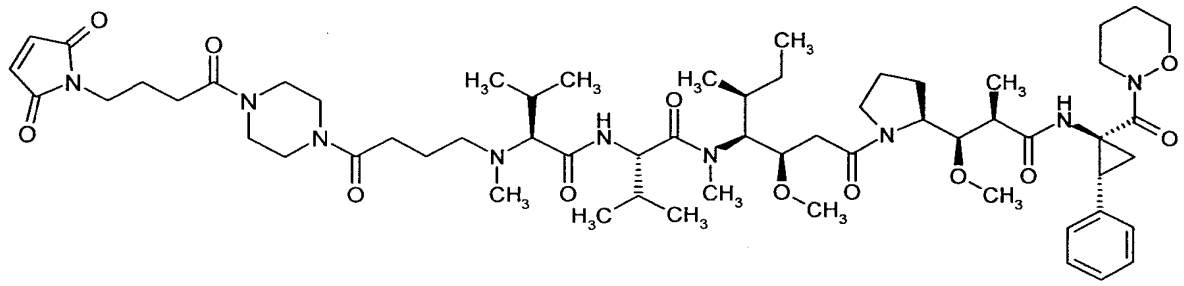
was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 2.6 mg (14% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 1115 $(M+H)^+$.

Intermediate 136

N-(4-{4-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]piperazin-1-yl}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, 1-[4-oxo-4-(piperazin-1-yl)butyl]-1*H*-pyrrole-2,5-dione trifluoroacetate was prepared under standard conditions, proceeding from tert-butyl piperazine-1-carboxylate and 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid over 2 stages.

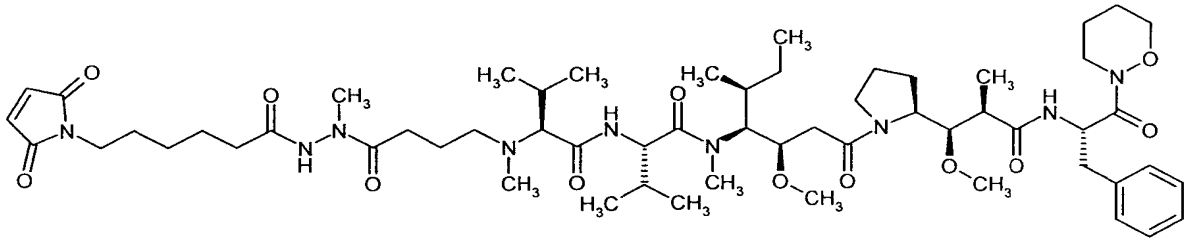
5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1000 µl of DMF and then admixed with 2.1 mg (11 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.7 mg (11 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2 µl of *N*,*N*-diisopropylethylamine and with 3.5 mg (5.6 µmol) of 1-[4-oxo-4-(piperazin-1-yl)butyl]-1*H*-pyrrole-2,5-dione trifluoroacetate. The mixture was stirred at RT overnight. Then 2.1 mg (5.6 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate were added and the reaction mixture was stirred at RT for a further 3 h. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from water, 0.6 mg (10% of theory) of the title compound was obtained as a colourless foam.

HPLC (Method 6): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 1132 $(M+H)^+$.

Intermediate 137

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*'-methylhexanehydrazide trifluoroacetate was prepared under standard conditions, proceeding from commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid and *tert*-butyl 1-methylhydrazinecarboxylate over 2 stages.

6.9 mg (8 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2540 µl of DMF and then admixed with 3.6 mg (9 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 3 µl of *N*,*N*-diisopropylethylamine and with 4.1 mg (12 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*'-methylhexanehydrazide trifluoroacetate. The mixture was stirred at RT overnight. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. Thus, 3.9 mg (45% of theory) of the title compound were obtained as a colourless foam.

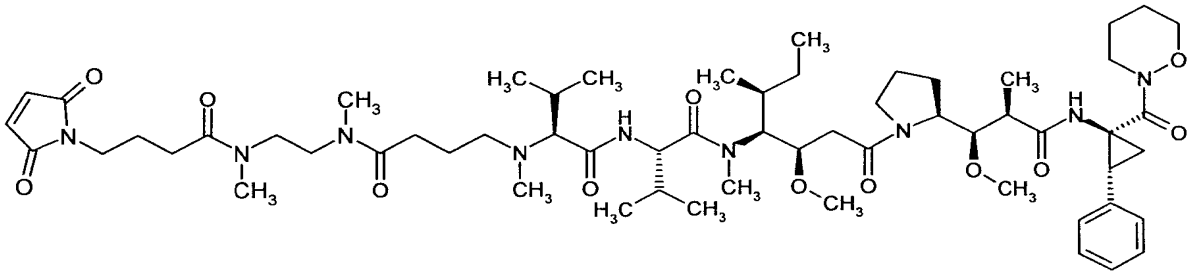
HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.93 min; MS (ESIpos): m/z = 1108 $(M+H)^+$.

Intermediate 138

N-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl](methyl)amino}ethyl)(methyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-

methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



Proceeding from *tert*-butylmethyl 2-(methylamino)ethyl carbamate and 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, over 2 stages, 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methyl-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate was first prepared by.

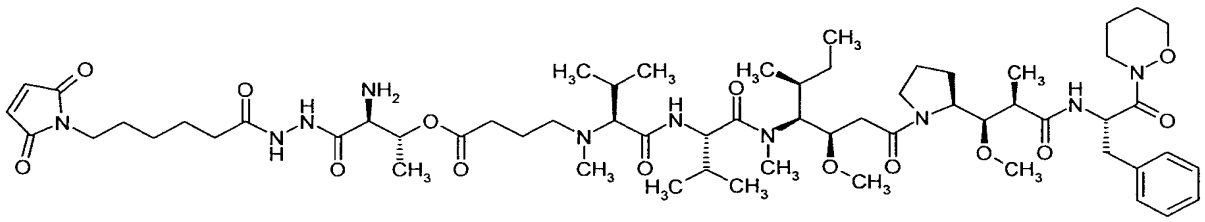
6.6 mg (7.3 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2000 µl of DMF and then admixed with 5.6 mg (14.7 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 2.6 µl of *N*,*N*-diisopropylethylamine and with 4.1 mg (9 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methyl-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate. After stirring at RT for 3 h, the same amounts of HATU and *N*,*N*-diisopropylethylamine were added once again, and the reaction mixture was then stirred at RT overnight. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. Thus, 4 mg (44% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 1134 $(M+H)^+$.

Intermediate 139

(2*R*,3*S*)-3-amino-4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3*R*,4*S*,7*S*,10*S*)-4-[(2*S*)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



13 mg (14.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 10 ml of dichloromethane and then admixed with 8.4 mg (44 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5.4 mg (44 µmol) of 4-dimethylaminopyridine and with 9 mg (29.3 µmol) of commercially available benzyl *N*-(*tert*-butoxycarbonyl)-L-threoninate. The mixture was stirred at RT for 5 h. Subsequently, the reaction mixture was twice extracted by shaking with water and the organic phase was dried over sodium sulphate and concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 14 mg (81% of theory) of the protected intermediate were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.3 min;

LC-MS (Method 1): R_t = 1.13 min; MS (ESIpos): m/z = 1178 $(M+H)^+$.

Subsequently, the Z protecting group was detached by hydrogenolytic means in methanol over 10% palladium/activated carbon. 9.5 mg (0.0087 mmol) of the deprotected crude product were then, without further purification, taken up in 5 ml of DMF, and admixed 5 mg (26.2 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4 mg (26.2 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 54.6 µl of *N,N*-diisopropylethylamine and with 9.1 mg (34.9 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 9.5 mg (84% of theory) of the Boc-protected intermediate were obtained.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1295 $(M+H)^+$.

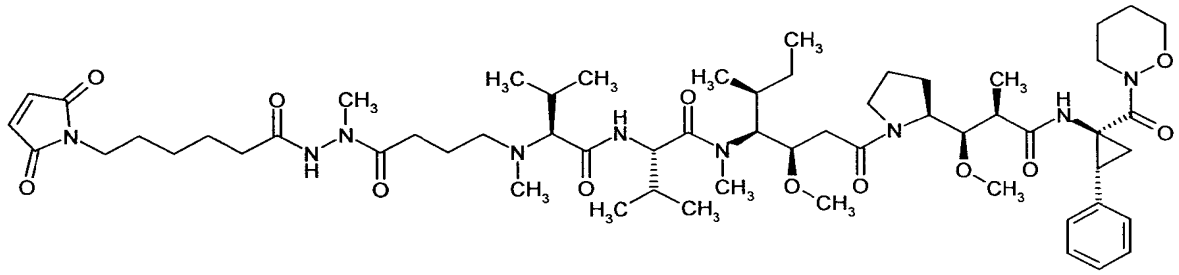
Subsequently, 9.5 mg (7.3 µmol) were deprotected with 0.5 ml of trifluoroacetic acid in 2 ml of dichloromethane of the Boc-protected intermediate and, after lyophilization from dioxane, 9 mg (82% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 1195 $(M+H)^+$.

Intermediate 140

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



4.1 mg (12 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N'*-methylhexanehydrazide trifluoroacetate (Intermediate 22) were dissolved together with 6.9 mg (8 µmol) of the compound from Intermediate 61 in 2.5 ml of DMF and then admixed with 3.5 mg (9 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 µl of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 2.6 mg (30% of theory) of the title compound were obtained.

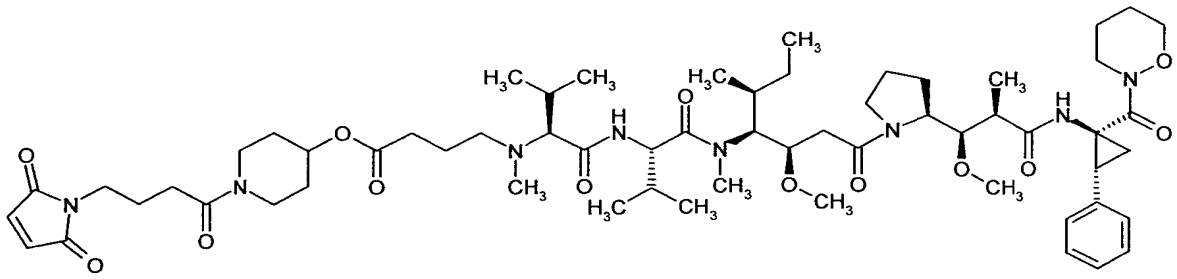
HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.90 and 0.91 min; MS (ESIpos): m/z = 1120 $(M+H)^+$.

Intermediate 141

N-[4-({1-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]piperidin-4-yl}oxy)-4-oxobutyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-

(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



44 mg (49 µmol) of N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2 ml of dichloromethane and then admixed with 18.8 mg (98 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 3.8 mg (24 µmol) of 4-dimethylaminopyridine and with 23 mg (98 µmol) of commercially available benzyl 4-hydroxypiperidine-1-carboxylate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 22 mg (40% of theory) of the Z-protected intermediate were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.04 min; MS (ESIpos): m/z = 1116 $(M+H)^+$.

Subsequently, the Z protecting group was detached by hydrogenolytic means in ethanol over palladium/activated carbon.

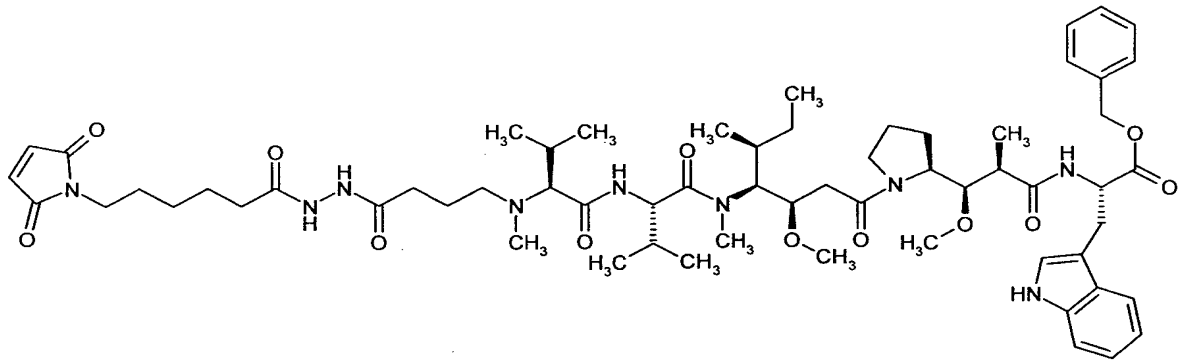
19 mg (19 µmol) of the deprotected crude product were then, without further purification, taken up in 4 ml of DMF and admixed with 7 mg (39 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 11 mg (29 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 5 µl of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 1 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 7.5 mg (34% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1147 $(M+H)^+$.

Intermediate 142

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



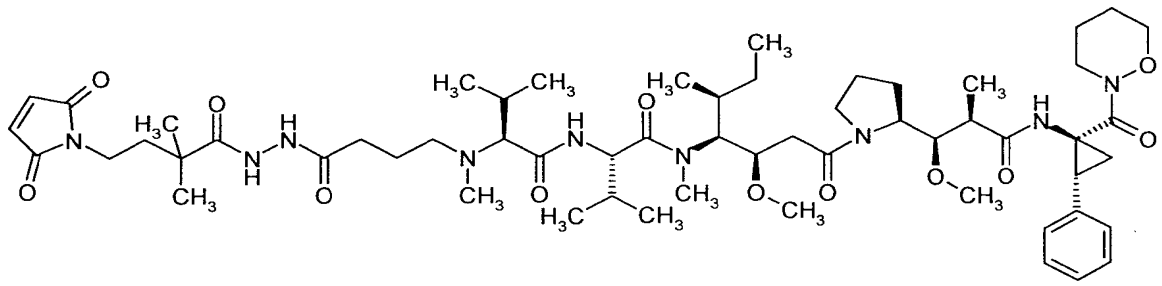
9 mg (9.5 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 72) were dissolved in 1000 µl of DMF and then admixed with 10 mg (38 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide, 7.2 mg (19 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 8 µl of *N*,*N*-diisopropylethylamine, and the reaction mixture was stirred at RT for 1 h. Subsequently, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization, 6.4 mg (58% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 1154 $(M+H)^+$.

Intermediate 143

N-(4-{2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



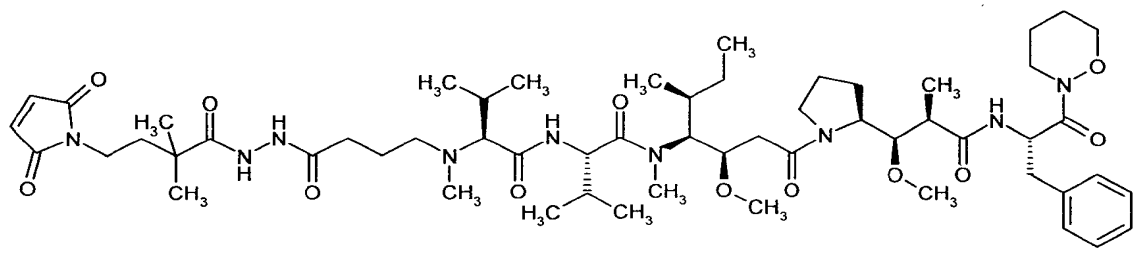
6 mg (6.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were reacted with 3 mg (8.7 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate in analogy to Intermediate 142 to give 2 mg (27% of theory) of the title compound.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 3): R_t = 1.92 min; MS (ESIpos): m/z = 1106 $(M+H)^+$.

Intermediate 144

N-(4-{2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



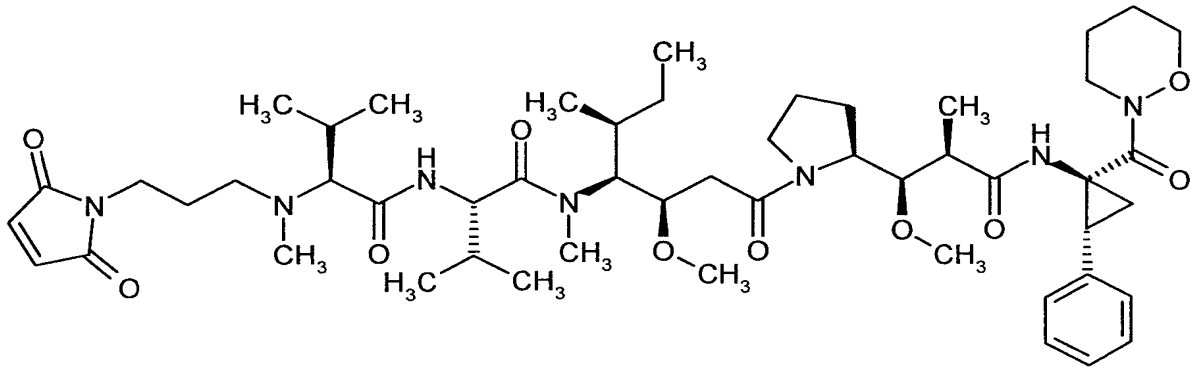
To a solution of 5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added 7.65 mg (22.5 µmol) of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate, 3.2 mg (16.9 µmol) of EDC, 1.96 µl (11.3 µmol) of diisopropylethylamine and 2.6 mg (16.9 µmol) of HOBT. The reaction mixture was stirred at RT for 3 h. Subsequently, a further 0.95 mg (2.8 µmol) of 4-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate was added. After stirring overnight, the reaction mixture was concentrated and purified by preparative HPLC. 3.5 mg (85% purity, 48% of theory) of the title compound were obtained.

LC-MS (Method 3): R_t = 1.86 min; m/z = 1094 $(M+H)^+$.

Intermediate 145

N-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



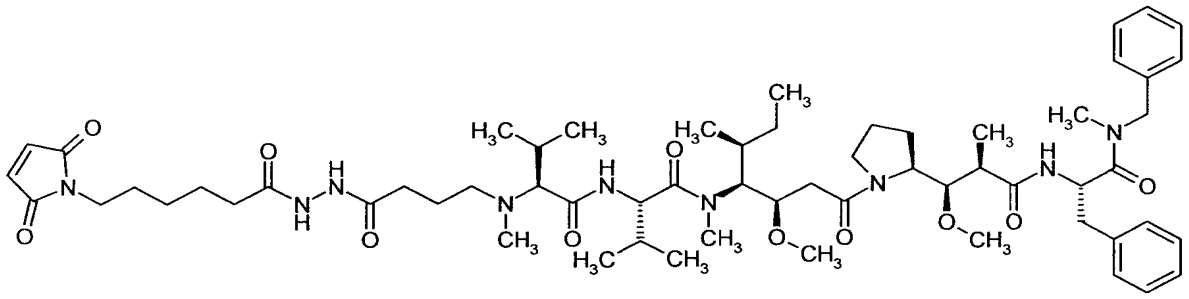
12 mg (14 µmol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 66) were taken up in 750 µl of dioxane and admixed with 1.5 ml of saturated sodium hydrogencarbonate solution and then with 3.2 mg (21 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 4.2 mg (32% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 950 $(M+H)^+$.

Intermediate 146

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



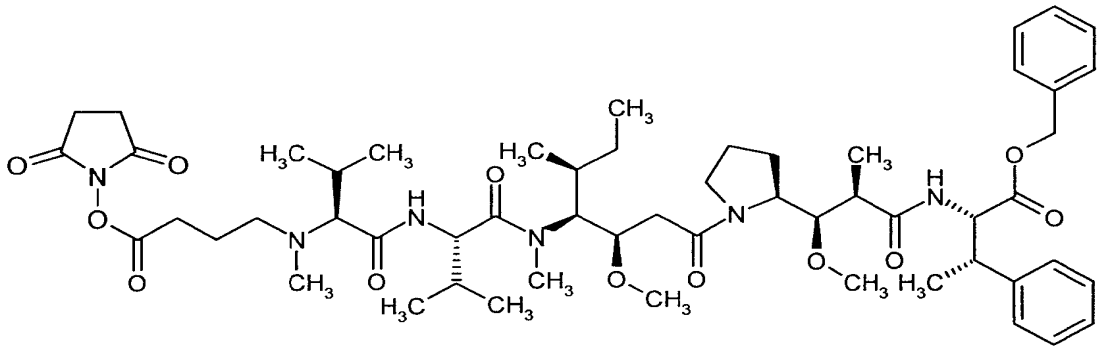
9 mg (9.8 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 73) were reacted in analogy to Intermediate 133 with 10 mg (39 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide to give 1.8 mg (15% of theory) of the title compound.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 9): R_t = 5.11 min; MS (ESIpos): m/z = 1128 $(M+H)^+$.

Intermediate 147

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

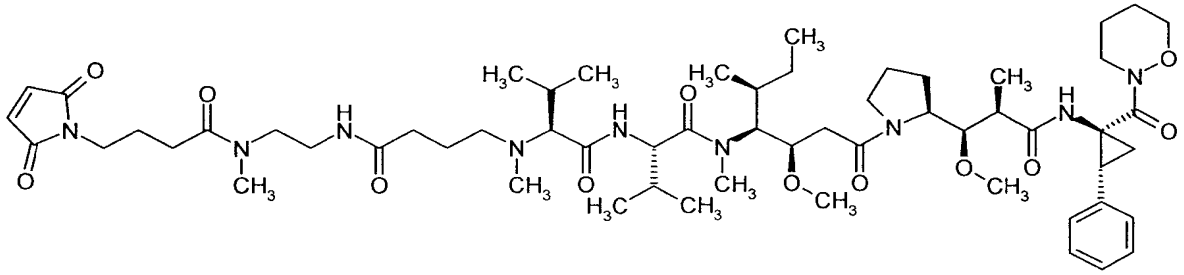


16 mg (17 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 70) were dissolved in 2 ml of dichloromethane and admixed with 2.6 mg (23 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 4 mg (21 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. After stirring at RT for 2 h, the same amounts of 1-hydroxypyrrolidine-2,5-dione and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added once again. Then stirring at RT overnight, the reaction mixture was concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 10 mg (56% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.0 min;

Intermediate 148

N-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl](methyl)amino}ethyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



6 mg (7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]

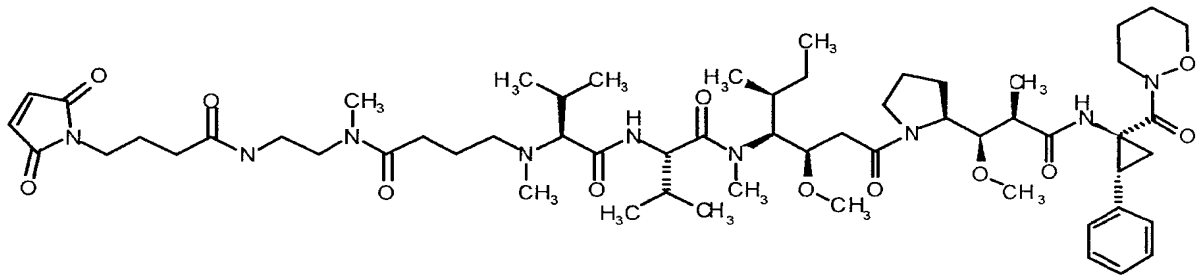
amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 2.8 mg (8 µmol) of *N*-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methylbutanamide trifluoroacetate, 10.1 mg (27 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 µl of *N,N*-diisopropylethylamine in 2 ml of DMF and stirred at RT overnight. Then another 5 mg (23.5 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 µl of *N,N*-diisopropylethylamine were added. After stirring at RT for a further 5 h, the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane, 1.3 mg (15% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 2): R_t = 1.21 min; MS (ESIpos): m/z = 1120 $(M+H)^+$.

Intermediate 149

N-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]amino}ethyl)(methyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



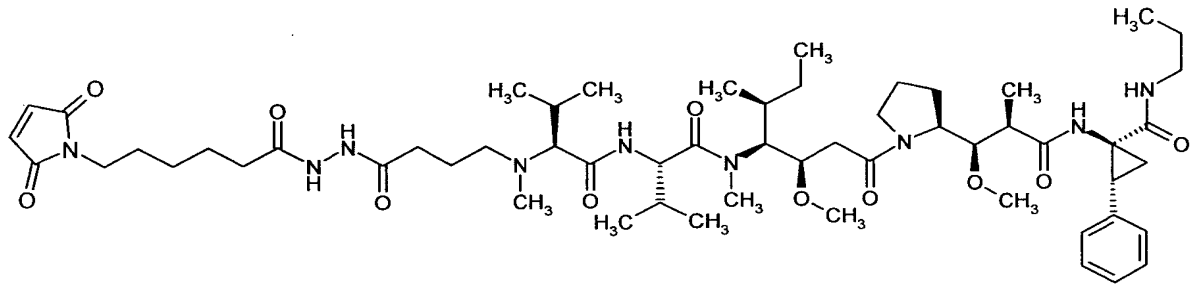
6 mg (7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 3.1 mg (9 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate, 10.1 mg (27 µmol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 µl of *N,N*-diisopropylethylamine in 2 ml of DMF, and the mixture was stirred at RT for 4 h. Then the solvent was removed under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane, 1 mg (13.4% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1121 $(M+H)^+$.

Intermediate 150

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



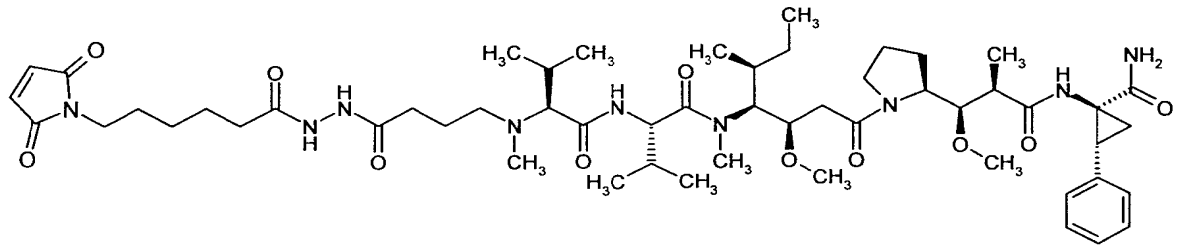
7.9 mg (9 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3 ml of DMF and then admixed with 10.4 mg (54 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 8.3 mg (54 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 9 µl of *N,N*-diisopropylethylamine and with 9.5 mg (36 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 4.3 mg (22% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 1.9 min;

LC-MS (Method 9): R_t = 4.93 min; MS (ESIpos): m/z = 1078 $(M+H)^+$.

Intermediate 151

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



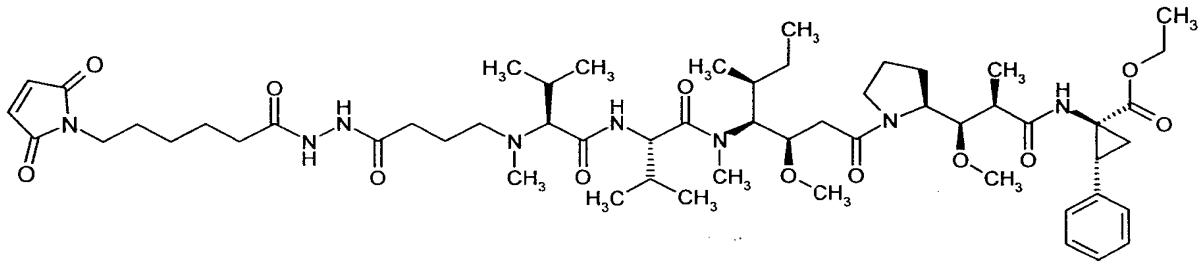
The compound was prepared analogously to Intermediate 150, proceeding from the compound in Intermediate 81.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1036 $(M+H)^+$.

Intermediate 152

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



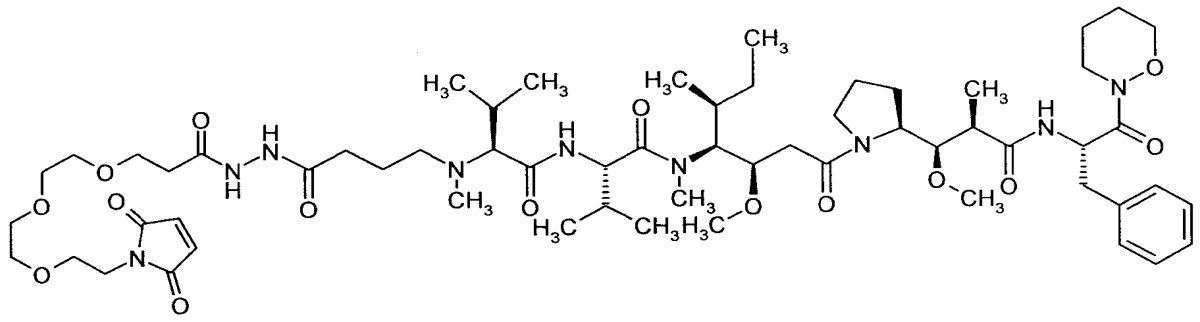
10 mg (12 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3 ml of DMF and then admixed with 8.9 mg (23 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 10 µl of *N*,*N*-diisopropylethylamine and with 12 mg (47 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 5.8 mg (37 % of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 9): R_t = 4.99 min; MS (ESIpos): m/z = 1066 $(M+H)^+$.

Intermediate 153

N-[1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-12,15-dioxo-3,6,9-trioxa-13,14-diazaoctadecan-18-yl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



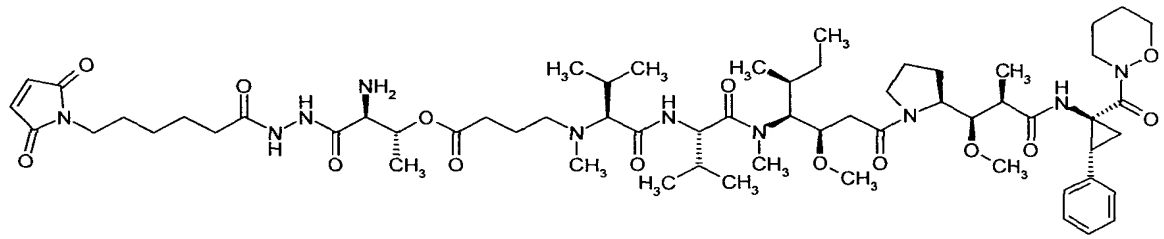
To a solution of 5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added 9.7 mg (22.5 µmol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide trifluoroacetate, 3.2 mg (16.9 µmol) of EDC, 1.96 µl (11.3 µmol) of N,N-diisopropylethylamine and 2.6 mg (16.9 µmol) of HOBT. The reaction mixture was stirred at RT for 3 h. Subsequently, a further 1.2 mg (2.8 µmol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide trifluoroacetate were added. The reaction mixture was stirred at RT overnight and then purified by preparative HPLC.

3.6 mg (51% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.90 min; m/z = 1185 $(M+H)^+$.

Intermediate 154

(2*R*,3*S*)-3-amino-4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3*R*,4*S*,7*S*,10*S*)-4-[(2*S*)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



15 mg (17 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*)-1-(1,2-oxazinan-2-yl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 10 ml of dichloromethane and then admixed with 12.8 mg (67 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 10 mg (83 µmol) of 4-dimethylaminopyridine and with 10.3 mg (33 µmol) of commercially available benzyl *N*-(*tert*-butoxycarbonyl)-L-threoninate. The mixture was heated to reflux for 4 h. Then the same amounts of coupling reagent and 4-dimethylaminopyridine were added again and the reaction mixture was heated under reflux overnight. Subsequently, the reaction mixture was diluted with dichloromethane and extracted by shaking once with water, and the organic phase was removed and concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 7.7 mg (37% of theory) of the protected intermediate were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.5 min;

LC-MS (Method 1): R_t = 1.13 min; MS (ESIpos): m/z = 1190 $(M+H)^+$.

Subsequently, the benzyl ester protecting group was removed by hydrogenation under standard hydrogen pressure in methanol over 10% palladium/activated carbon, and the acid thus obtained, as described in Intermediate 151, was joined to 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. In a last step, the Boc protecting group was detached with trifluoroacetic acid. The remaining residue was purified by means of preparative HPLC. Thus, 0.22 mg (2.5% of theory over 3 stages) of the title compound was obtained as a colourless foam.

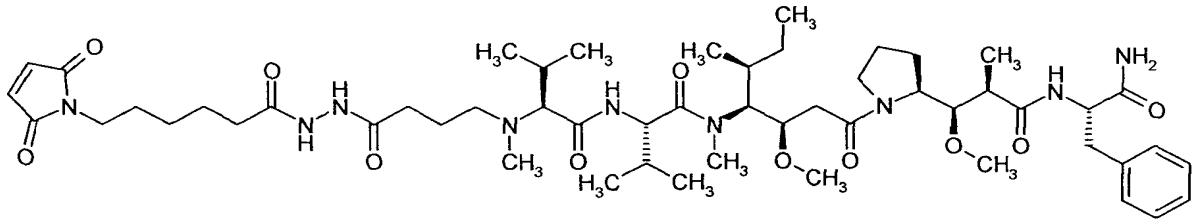
HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 1207 $(M+H)^+$.

Intermediate 155

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-

methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



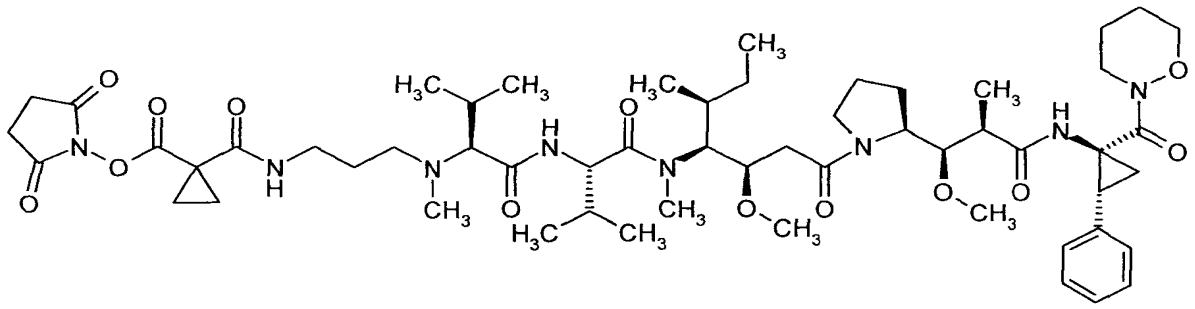
This compound was prepared in analogy to the synthesis described in Intermediate 152, from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 1024 $(M+H)^+$.

Intermediate 156

N-(3-{[(1-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}cyclopropyl)carbonyl]amino}propyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in the last stage of Intermediate 131, from *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 1,1'-

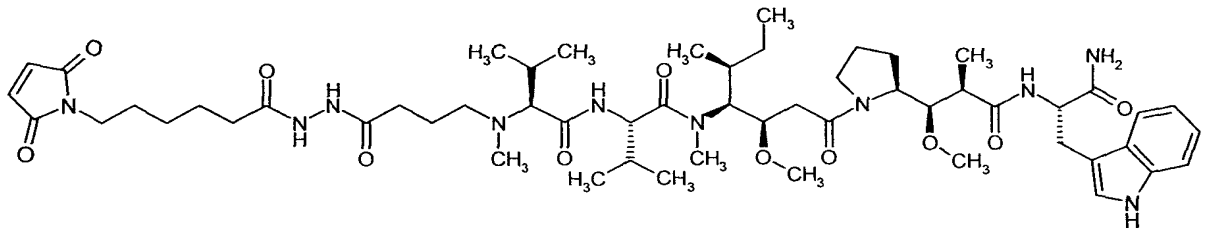
[cyclopropane-1,1-diylbis(carbonyloxy)]dipyrrolidine-2,5-dione, which had been obtained from the corresponding dicarboxylic acid beforehand.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1080 $(M+H)^+$.

Intermediate 157

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15 mg (18 µmol) of (*N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3.8 ml of DMF and then admixed with 27 mg (70 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 12 µl of *N*,*N*-diisopropylethylamine and with 14 mg (53 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The reaction mixture was stirred at RT for 1 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 6.2 mg (33% of theory) of the title compound were obtained as a colourless foam.

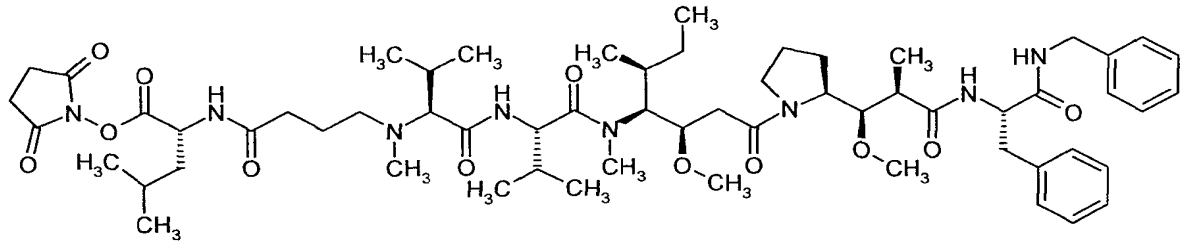
HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1063 $(M+H)^+$.

^{1}H-NMR (500 MHz, DMSO-d_6, characteristic signals): δ = 10.8 (d, 1H), 9.8-9.7 (m, 2H), 9.6 and 9.4 (2m, 1H), 8.9, 8.88, 8.78 and 8.75 (4d, 1H), 8.08 and 7.85 (2d, 1H), 7.6-6.9 (m, 9H), 4.7-4.4 (m, 3H), 3.4 (t, 2H), 3.23, 3.2, 3.18, 3.0, and 2.99 (5s, 9H), 2.8 (m, 3H), 2.1 (t, 2H), 1.06 and 1.01 (2d, 3H), 0.95-0.8 (m, 15H), 0.8-0.75 (dd, 3H).

Intermediate 158

N-[4-({(2*R*)-1-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-methyl-1-oxopentan-2-yl}amino)-4-oxobutyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



13 mg (14.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 4 ml of dimethylformamide and then admixed with 9.4 mg (25 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 6 µl of *N*,*N*-diisopropylethylamine and with 7 mg (31 µmol) of commercially available *tert*-butyl D-leucinate hydrochloride trifluoroacetate. The mixture was stirred at RT for 5 h and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 6.5 mg (49% of theory) of the protected intermediate were obtained as a colourless foam.

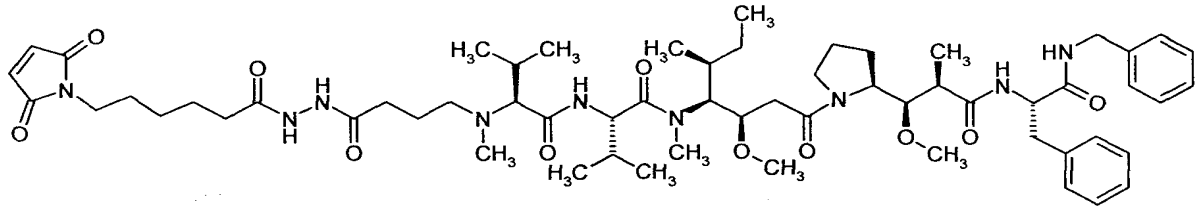
HPLC (Method 5): R_t = 2.2 min;

LC-MS (Method 1): R_t = 1.21 min; MS (ESIpos): m/z = 1076 $(M+H)^+$.

Trifluoroacetic acid in dichloromethane was first used to detach the Boc protecting group from this protected intermediate, giving 6.2 mg (99% of theory) of the deprotected compound. 5.2 mg (5 µmol) of this intermediate were taken up in 1.5 ml of dichloromethane and reacted with 0.8 mg (7 µmol) of *N*-hydroxysuccinimide in the presence of 1.2 mg (6 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.16 mg (1 µmol) of 4-dimethylaminopyridine. After stirring at RT for 2 h, the reaction mixture was concentrated and purified by means of preparative HPLC. 1.3 mg of the title compound were obtained, some of which was hydrolysed to the reactant.

Intermediate 159

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157, from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

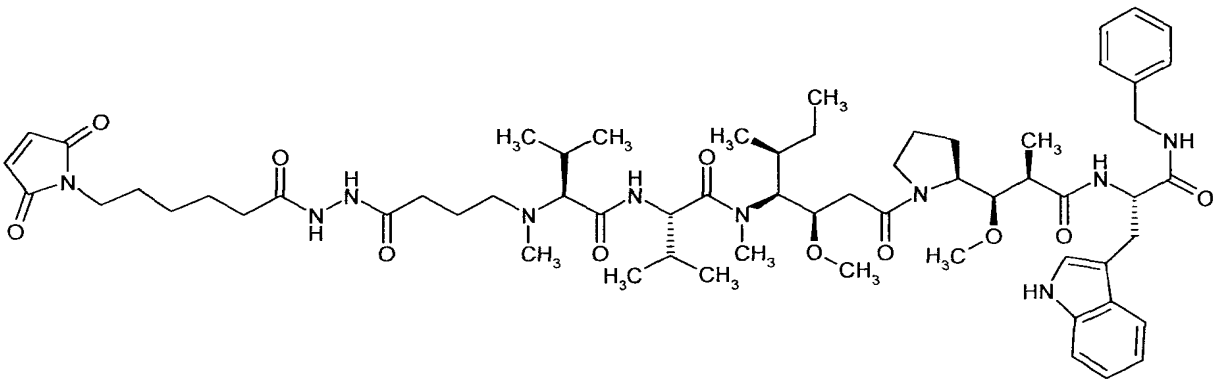
Yield: 6 mg (53% of theory)

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1114 $(M+H)^+$.

Intermediate 160

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157, from 20

mg (21 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

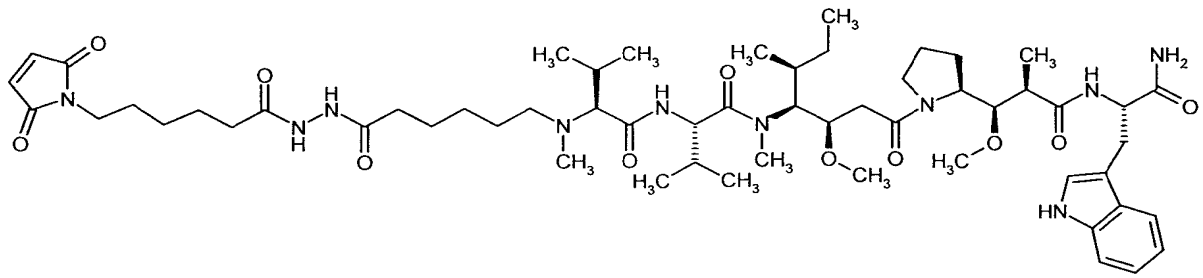
Yield: 13 mg (52% of theory)

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1153 $(M+H)^+$.

Intermediate 161

N-(6-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157, from *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

Yield: 0.8 mg (16% of theory)

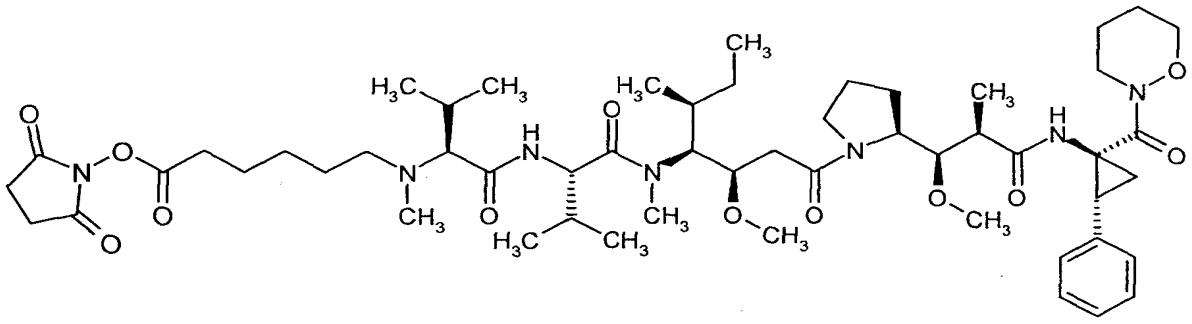
HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.78 min; MS (ESIpos): m/z = 1092 $(M+H)^+$.

Intermediate 162

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-

phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



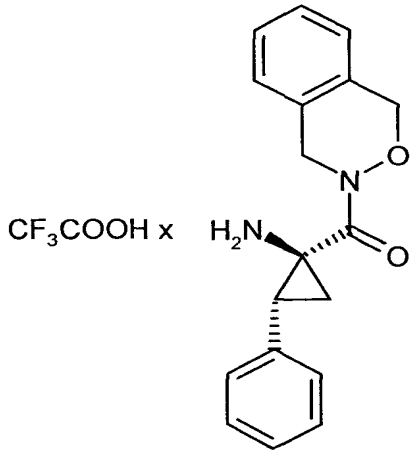
18 mg (20 µmol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 64) were dissolved in 3.2 ml of dichloromethane and admixed with 22 mg (190 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 11 mg (60 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 µmol) of DMAP. After stirring at RT for 2 h, another 22 mg (190 mmol) of 1-hydroxypyrrolidine-2,5-dione, 11 mg (60 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 µmol) of DMAP were added and the reaction mixture was stirred at RT for a further hour. This was followed by concentration under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 8.2 mg (41% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 11): R_t = 0.9 min; MS (ESIpos): m/z = 1024 $(M+H)^+$.

Intermediate 163

[(1*S*,2*R*)-1-amino-2-phenylcyclopropyl](1,4-dihydro-3*H*-2,3-benzoxazin-3-yl)methanone trifluoroacetate



First, proceeding from 265 mg (0.82 mmol) of *tert*-butyl (1*S*,2*R*)-1-(hydroxycarbamoyl)-2-phenylcyclopropyl carbamate (Starting Compound 7), by reaction with 1,2-bis(bromomethyl)benzene, analogously to a literature method (see H. King, *J. Chem. Soc.* 1942, 432), the Boc-protected tert-butyl (1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl carbamate intermediate was prepared.

Yield: 108 mg (34% of theory)

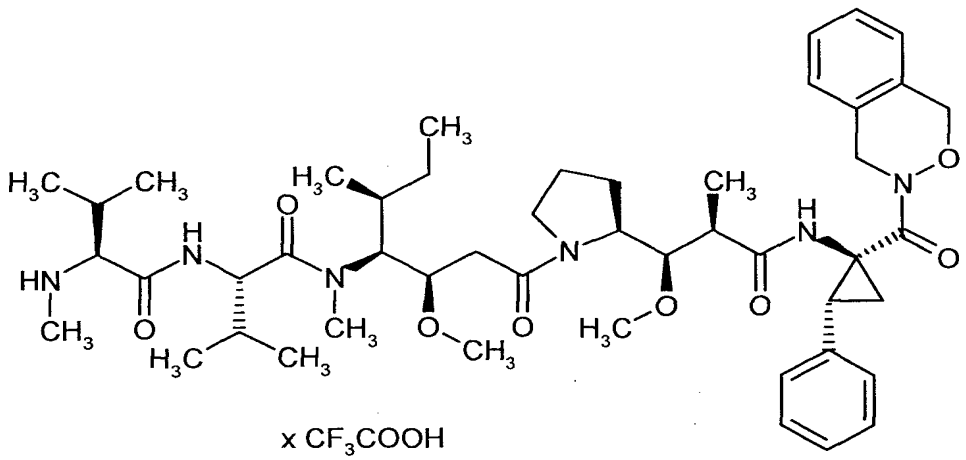
LC-MS (Method 2): R_t = 1.3 min; MS (ESIpos): m/z = 395 $(M+H)^+$.

108 mg (0.27 mmol) of this intermediate were taken up in 3.7 ml of dichloromethane, 1.8 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 15 min. This was followed by concentration under reduced pressure and lyophilization of the remaining residue from dioxane. 112 mg of the title compound were obtained in quantitative yield as a colourless foam.

LC-MS (Method 1): R_t = 0.7 min; MS (ESIpos): m/z = 295 $(M+H)^+$.

Intermediate 164

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(1,4-dihydro-3*H*-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



166 mg (0.196 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 10) were taken up in 40 ml of DMF and admixed successively with 80 mg (0.196 mmol) of [(1*S*,2*R*)-1-amino-2-phenylcyclopropyl](1,4-dihydro-3*H*-2,3-benzoxazin-3-yl)methanone trifluoroacetate (Intermediate 163), 112 mg (0.294 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and 682 µl (3.9 mmol) of *N*,*N*-diisopropylethylamine. The mixture was subsequently stirred at RT overnight. The reaction mixture was then concentrated under reduced pressure, the residue was taken up in ethyl acetate and the solution was washed with saturated aqueous sodium chloride solution. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was finally purified by preparative HPLC. In this way, 19 mg (9% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(1,4-dihydro-3*H*-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained.

HPLC (Method 5): R_t = 1.68 min;

LC-MS (Method 1): R_t = 1.51 min; MS (ESIpos): m/z = 1083 $(M+H)^+$.

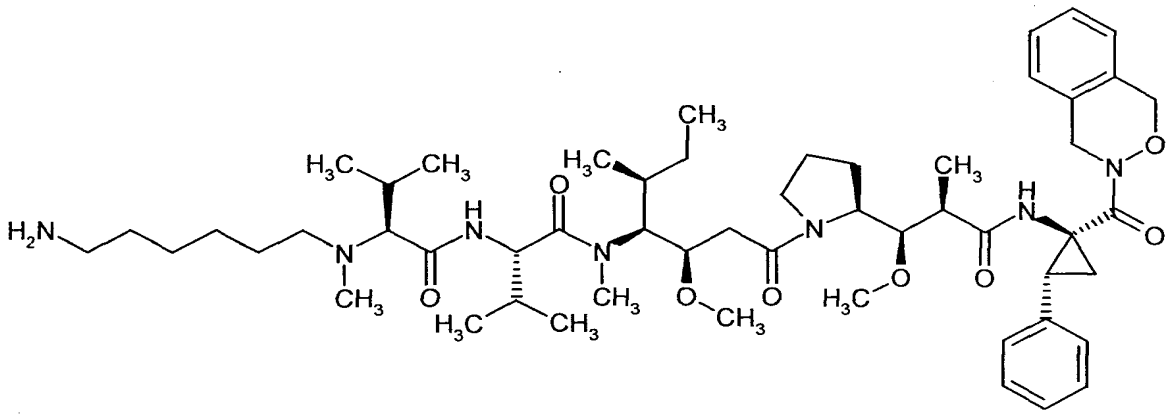
19 mg (0.015 mmol) of this intermediate were dissolved in 4 ml of DMF. After 817 µl of piperidine had been added, the reaction mixture was stirred at RT for 5 min. This was followed by concentration under reduced pressure, and the residue was first digested with diethyl ether and then purified by means of preparative HPLC (eluent: acetonitrile + 0.1% TFA / 0.1% aq. TFA). The corresponding fractions were combined, the solvent was removed under reduced pressure and then the residue was lyophilized from dioxane/water. 12 mg (92% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 861 $(M+H)^+$.

Intermediate 165

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



20 mg (0.021 mmol) of Intermediate 164 were used, in analogy to the preparation of Intermediate 97, with benzyl 3-oxohexyl carbamate in the presence of sodium cyanoborohydride and subsequent hydrogenolytic detachment of the Z protecting group (with 5% palladium on charcoal as a catalyst, in methanol as a solvent), to prepare the title compound.

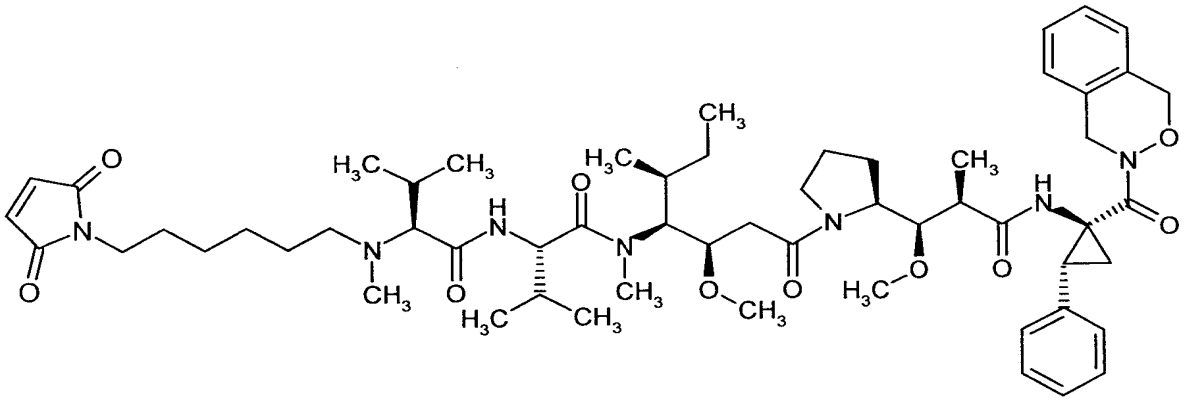
Yield: 4.5 mg (23% of theory over 2 stages)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 960 $(M+H)^+$.

Intermediate 166

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



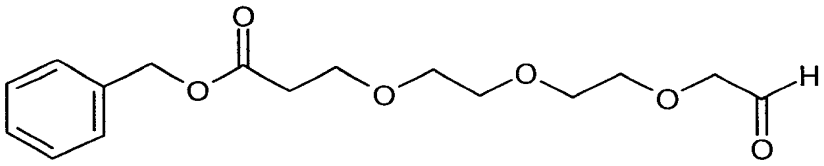
4.4 mg (4.5 µmol) of Intermediate 165 were taken up in 1 ml of 1:1 dioxane/water and then admixed with 1 mg (6.8 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 50 µl of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 30 min. Then another 50 µl of the saturated aqueous sodium hydrogencarbonate solution were added and the reaction mixture was stirred at RT for a further 15 min and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization, 1 mg (21% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.08 min; MS (ESIpos): m/z = 1040 $(M+H)^+$.

Intermediate 167

benzyl 3-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}propanoate



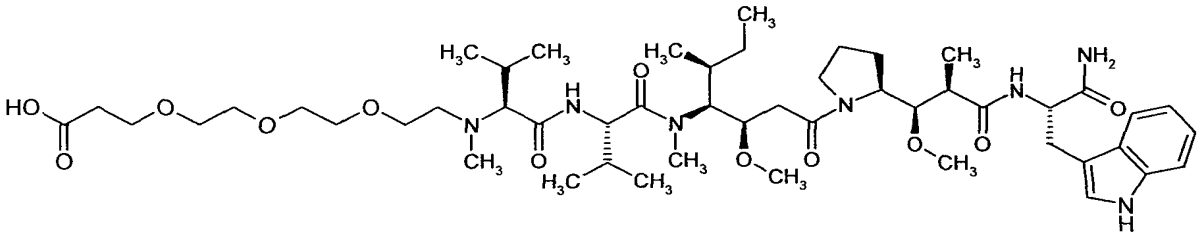
The title compound was prepared from 6 g (21.55 mmol) of commercially available 3-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}propanoic acid under standard conditions, first by esterification with benzyl chloride and caesium carbonate and subsequent oxidation with sulphur trioxide-pyridine complex.

Yield: 611 mg (10% of theory over 2 stages)

LC-MS (Method 2): R_t = 1.69 min; MS (ESIpos): m/z = 311 $(M+H)^+$.

Intermediate 168

N-(2-{2-[2-(2-carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 69, by coupling of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Fmoc protecting group by means of piperidine, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate.

25 mg (0.028 mmol) of this compound and 17.5 mg (0.06 mmol) of Intermediate 167 were combined in 2 ml of methanol and admixed with 12.6 mg (0.14 mmol) of borane-pyridine complex and 2.5 ml of acetic acid. The reaction mixture was stirred at RT overnight. Then the same amounts of borane-pyridine complex and acetic acid were added once again and the reaction mixture was stirred at RT for a further 24 h. This was followed by concentration under reduced pressure, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 26.5 mg (88% of theory) of the Z-protected title compound were obtained.

HPLC (Method 12): R_t = 2.04 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1064 $(M+H)^+$.

25 mg (0.024 mmol) of this intermediate were taken up in 10 ml of methanol and hydrogenated over 10% palladium on activated carbon under standard hydrogen pressure at RT for 45 min. The

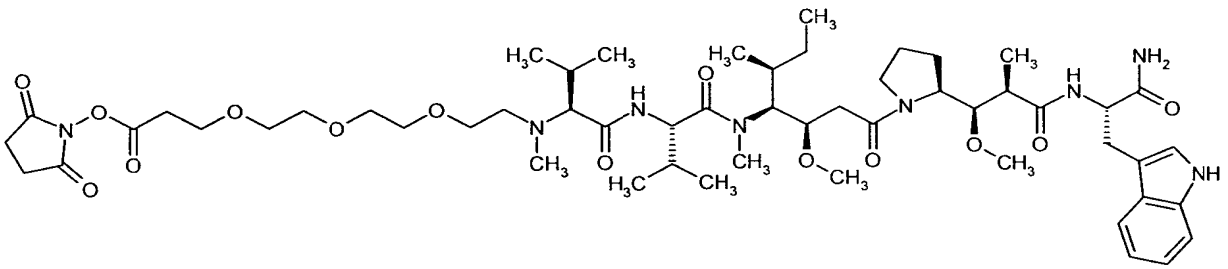
catalyst was then filtered off and the solvent was removed under reduced pressure. After lyophilization from dioxane, 19.7 mg (85% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 974 $(M+H)^+$.

Intermediate 169

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



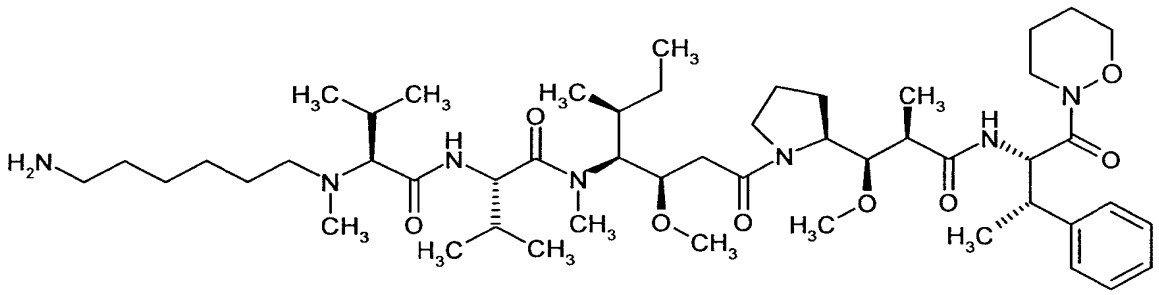
10 mg (10 µmol) of Intermediate 168 were dissolved in 3 ml of DMF and admixed with 3.5 mg (30 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 5 µl of N,N-diisopropylethylamine. After stirring at RT for 20 h, 8 mg (0.02 mmol) of HATU were added and the reaction mixture was stirred once again at RT overnight and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 8.6 mg (64% of theory) of the title compound were obtained.

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 11): Rt = 0.81 min; MS (ESIpos): m/z = 1071 (M+H)+.

Intermediate 170

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 101 over 2 stages, proceeding from 26 mg (0.028 mmol) of Intermediate 15.

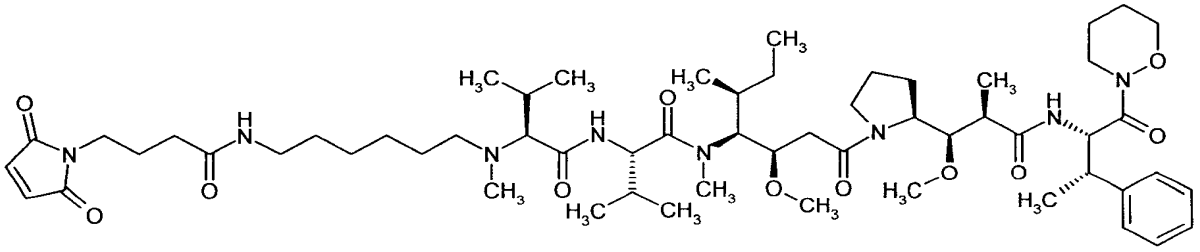
Yield: 16.7 mg (63% of theory over 2 stages)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 914 $(M+H)^+$.

Intermediate 171

N-(6-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



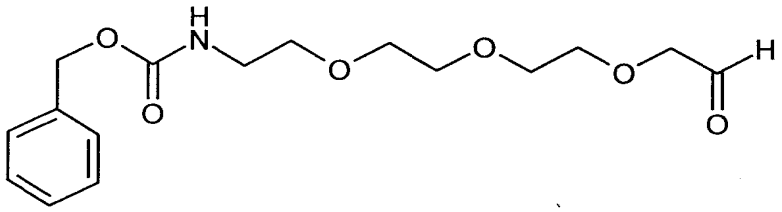
6.7 mg (7.3 µmol) of the compound formed from Intermediate 170 and 3 mg (14.7 µmol) of commercially available 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid were taken up in 2 ml of DMF and admixed with 5.6 mg (14.7 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 2 µl of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 30 min. The reaction mixture was concentrated and the residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was removed under reduced pressure and then the residue was lyophilized from dioxane. Thus, 4.5 mg (56% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.12 min; MS (ESIpos): m/z = 1079 $(M+H)^+$.

Intermediate 172

benzyl 2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl carbamate



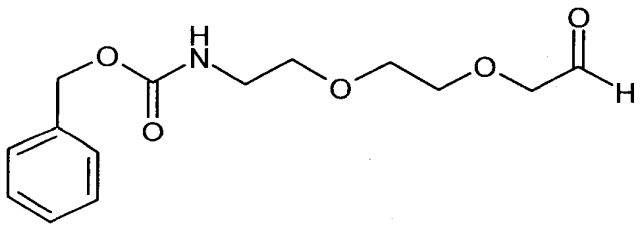
The title compound was prepared from commercially available 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol under standard conditions, by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

HPLC (Method 12): R_t = 1.4 min;

LC-MS (Method 11): R_t = 0.65 min; MS (ESIpos): m/z = 326 $(M+H)^+$.

Intermediate 173

benzyl {2-[2-(2-oxoethoxy)ethoxy]ethyl carbamate



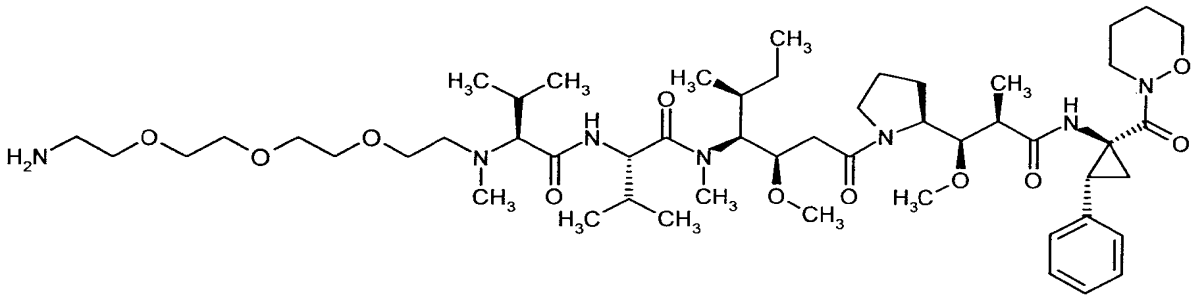
The title compound was prepared analogously to Intermediate 172 from commercially available 2-[2-(2-aminoethoxy)ethoxy]ethanol under standard conditions, by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

HPLC (Method 12): R_t = 1.3 min;

LC-MS (Method 11): R_t = 0.68 min; MS (ESIpos): m/z = 282 $(M+H)^+$.

Intermediate 174

N-(2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



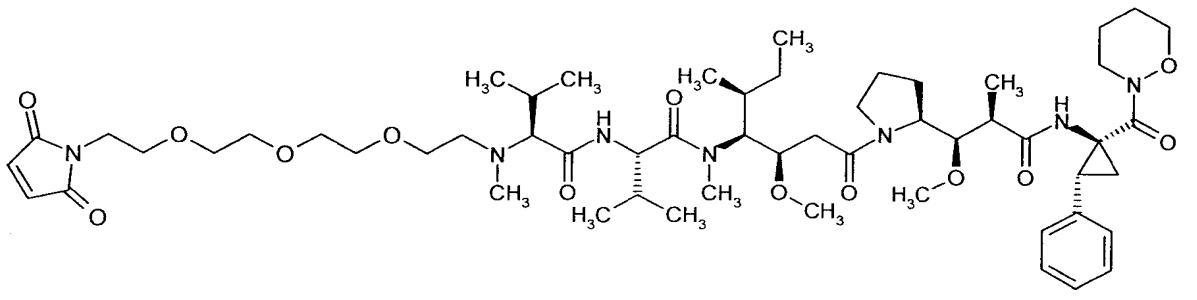
47 mg (0.05 mmol) of Intermediate 16 were reductively aminated in analogy to the preparation of Intermediate 167 with benzyl 2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl carbamate in the presence of borane-pyridine complex. Subsequently, the Z protecting group was removed by hydrogenolytic means with 5% palladium on charcoal as a catalyst and in methanol as a solvent, and 38 mg (66% of theory over 2 stages) of the title compound were prepared.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 988 $(M+H)^+$.

Intermediate 175

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was effected in analogy zu Intermediate 166, proceeding from 34 mg (0.03 mmol) of Intermediate 174.

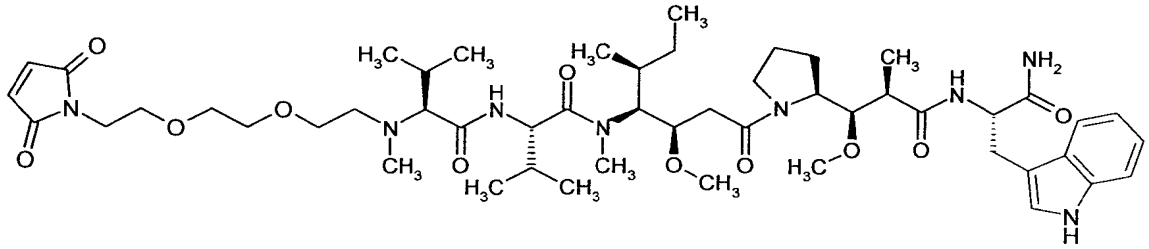
Yield: 8.3 mg (23% of theory)

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1068 $(M+H)^+$.

Intermediate 176

N-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



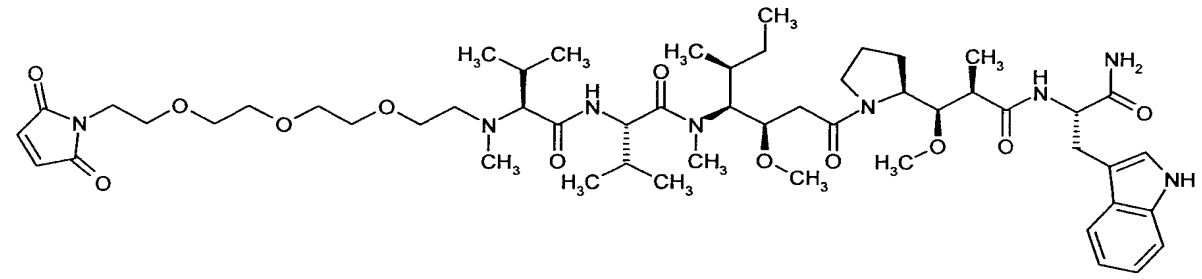
The preparation was effected in analogy to Intermediates 174 and 175, commencing with the reductive amination of Intermediate 16 with Intermediate 173, subsequent deprotection and formation of the maleimide.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 11): R_t = 0.8 min; MS (ESIpos): m/z = 981 $(M+H)^+$.

Intermediate 177

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



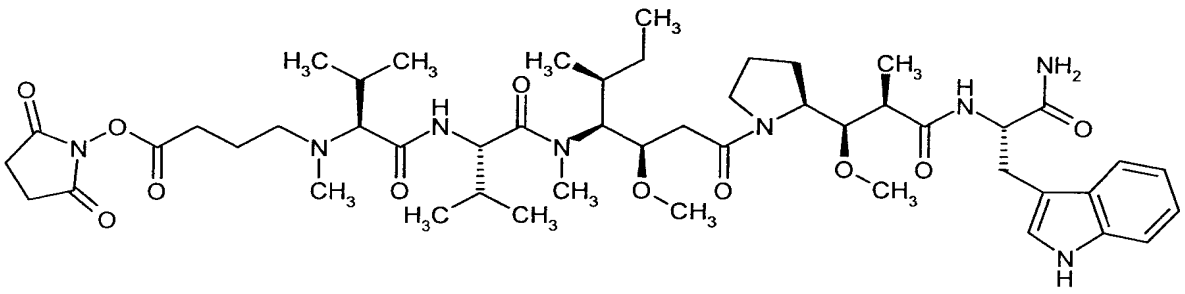
The preparation was effected in analogy to Intermediates 174 and 175, commencing with the reductive amination of Intermediate 16 with Intermediate 172, subsequent deprotection and formation of the maleimide.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1025 $(M+H)^+$.

Intermediate 178

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

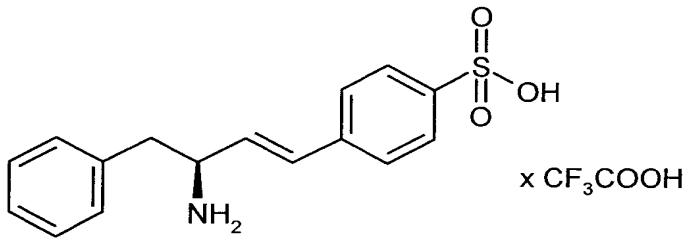


The preparation was effected in analogy to Intermediates 162, proceeding from 6 mg of Intermediate 82.

LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 953 $(M+H)^+$.

Intermediate 179

4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate



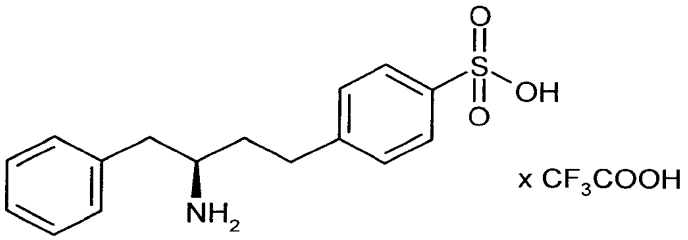
A mixture of 13.6 mg (0.06 mmol) of palladium(II) acetate, 469 mg (1.46 mmol) of potassium 4-iodobenzenesulphonate, 300 mg (1.21 mmol) of (S)-tert-butyl 1-phenylbut-3-en-2-yl carbamate, 16.5 mg (0.12 mmol) of phenylurea and 167.6 mg (1.21 mmol) of potassium carbonate in 7.5 ml of DMF was heated to 160°C in a microwave for 15 min. The crude product was subsequently purified directly by preparative HPLC. This gave 312 mg of a mixture of 31% of the BOC-protected compound and 69% of the free amine.

This mixture was subsequently taken up in 30 ml of dichloromethane, admixed with 1 ml of trifluoroacetic acid and stirred at RT for 20 h. After concentrating under reduced pressure, the residue was stirred with diethyl ether, and the precipitate formed was filtered off with suction and washed with diethyl ether. This gave 200 mg (62% of theory) of the title compound.

LC-MS (Method 11): R_t = 0.44 min; MS (ESIpos): m/z = 304 $(M+H)^+$.

Intermediate 180

4-[(3R)-3-amino-4-phenylbutyl]benzenesulphonic acid



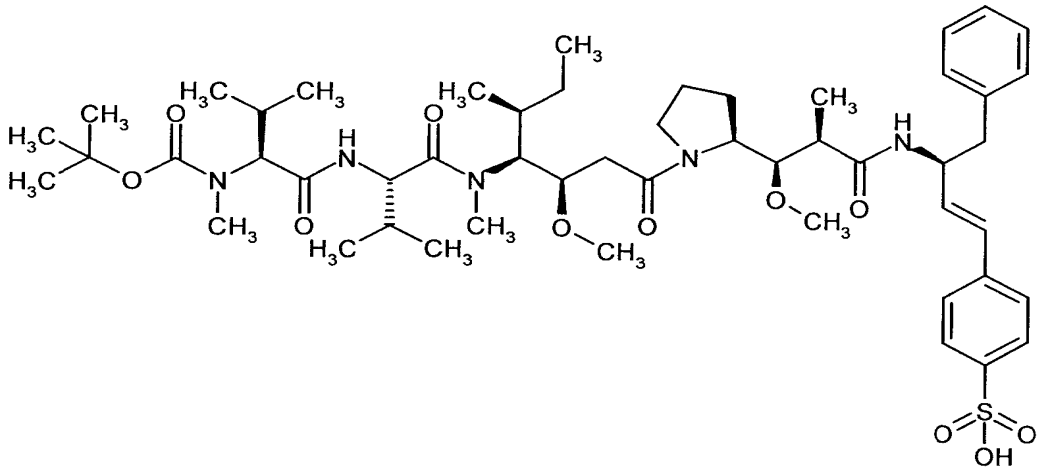
100 mg (0.25 mmol) of 4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate were suspended in 10 ml of acetic acid and a few drops of DMF and water, admixed with 70 mg (0.07 mmol) of palladium on charcoal (10%) and hydrogenated at hydrogen pressure 2.2 bar for 24 h. The solution was filtered and the filtrate purified by prep. HPLC.

29 mg (76% purity, 21% of theory) of product were obtained.

LC-MS (Method 1): R_t = 0.46 min; MS (ESIpos): m/z = 306 $(M+H)^+$.

Intermediate 181

N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

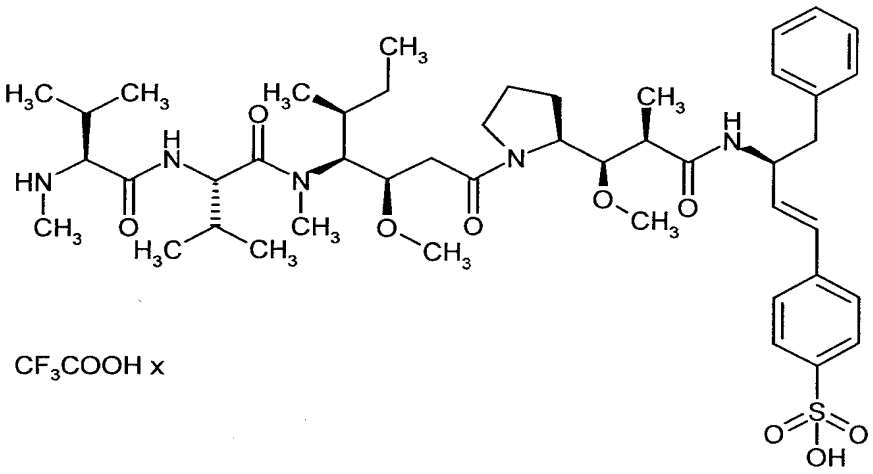


To a solution of 90 mg (0.13 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 4 ml of DMF were added 60 mg (0.16 mmol) of HATU and 69 µl of (0.39 mmol) Hünig's base. The reaction mixture was stirred at RT for 30 min and then admixed with 60 mg (0.15 mmol) 60.3 mg (0.13 mmol) of 4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate. After stirring overnight, the reaction mixture was purified by prep. HPLC. This gave 127 mg of a 44:56 mixture of the title compound and of the already deprotected amine.

LC-MS (Method 1): R_t = 1.21 min; MS (ESIpos): m/z = 971 $(M+H)^+$; R_t = 0.84 min; MS (ESIpos): m/z = 871 $(M+H)^+$ for the deprotected compound.

Intermediate 182

N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



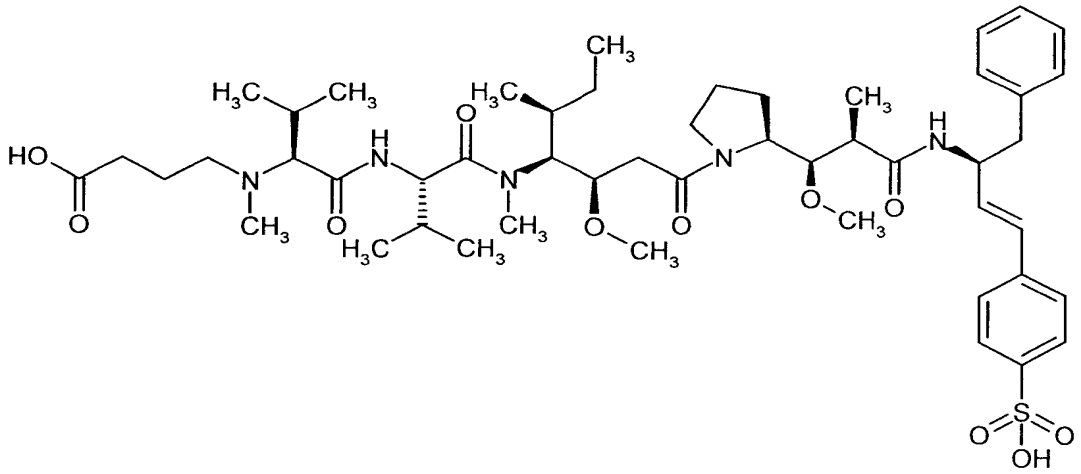
90 mg of Intermediate 180 were dissolved in 4.6 ml of dichloromethane, and 0.92 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated. The crude product obtained was purified by prep. HPLC.

91 mg (98% of theory) of the target compound were obtained.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 871 $(M+H)^+$

Intermediate 183

N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



16.7 µl (0.03 mmol) of a 15% aqueous succinaldehyde solution were initially charged in 943 µl of methanol and admixed with 17 mg (0.02 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-

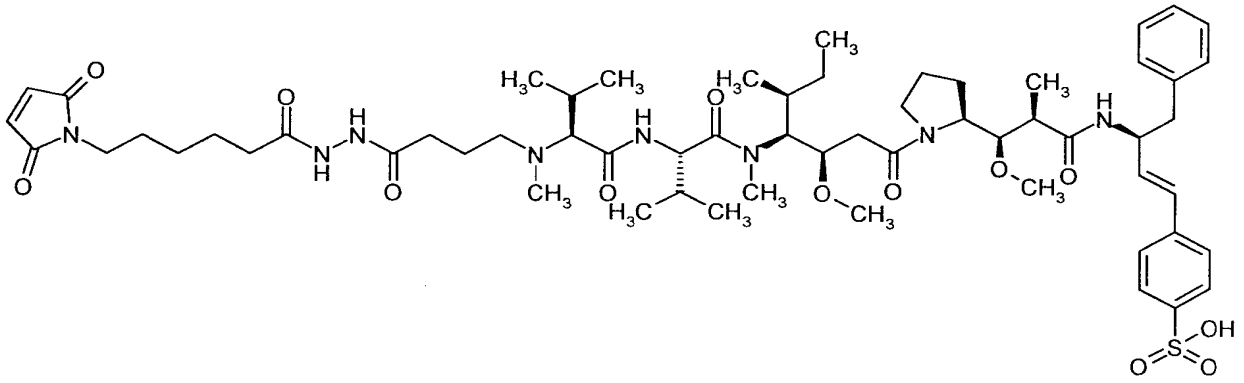
yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 181) and 1.1 µl (0.02 mmol) of acetic acid. The reaction mixture was stirred for 5 min at RT and then 2.9 µl (0.02 mmol) of borane-pyridine complex were added. After 1 h, a further 2 equivalents each of succinaldehyde, acetic acid and borane-pyridine complex were added and the mixture was stirred at RT for 20 h. The reaction mixture was then purified by prep. HPLC.

This gave 20 mg (83% purity, 80% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 957 $(M+H)^+$

Intermediate 184

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



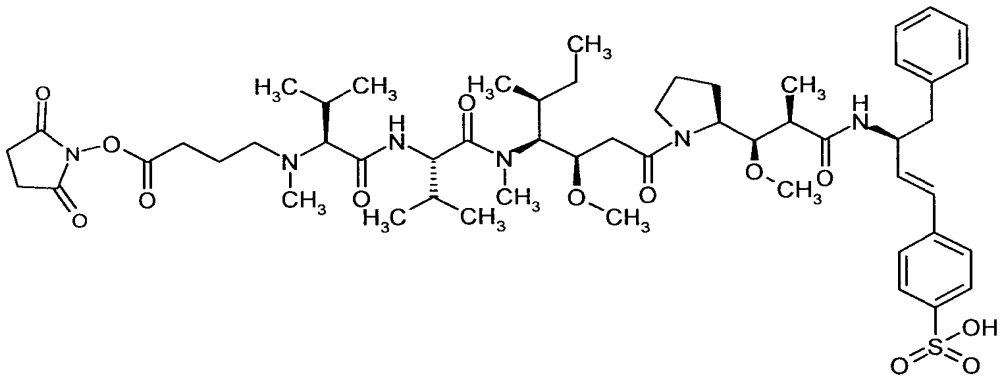
8 mg (7.5 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 2.8 mg (8.2 µmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide trifluoroacetate, 3.4 mg (9 µmol) of HATU and 3.9 µl of Hünig's base were stirred in 0.77 ml of DMF at RT for 20 h. Subsequently, the reaction mixture was purified by prep. HPLC.

3 mg (31% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 1164 $(M+H)^+$

Intermediate 185

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

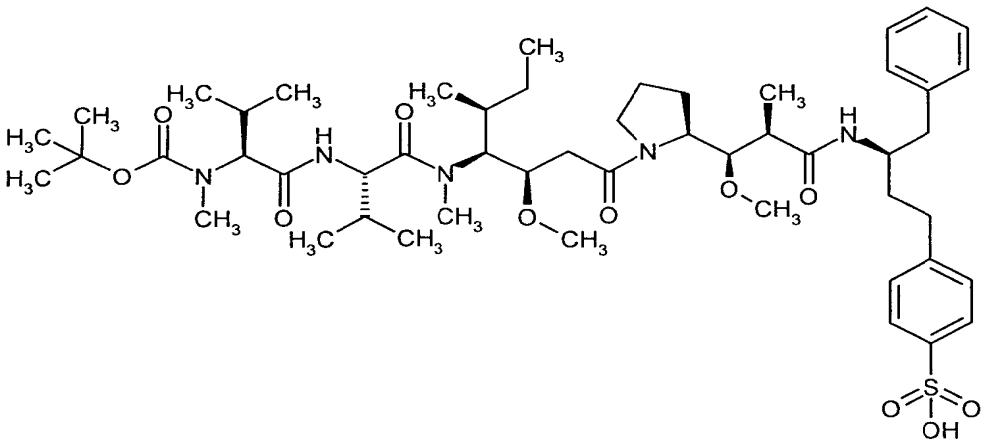


To a solution of 8 mg (7.5 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2 ml of DMF were added 8.6 mg (74.8 µmol) of N-hydroxysuccinimide, 8.5 mg (22.4 µmol) of EDCI and 0.1 mg (0.75 µmol) of DMAP. The reaction mixture was stirred at RT for 20 h. Subsequently, 1.3 µl (7.5 µmol) of Hünig's base were added and the mixture was stirred for 1 h. The reaction mixture was then purified by prep. HPLC. 2.6 mg (72% purity, 21% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1054 $(M+H)^+$

Intermediate 186

N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

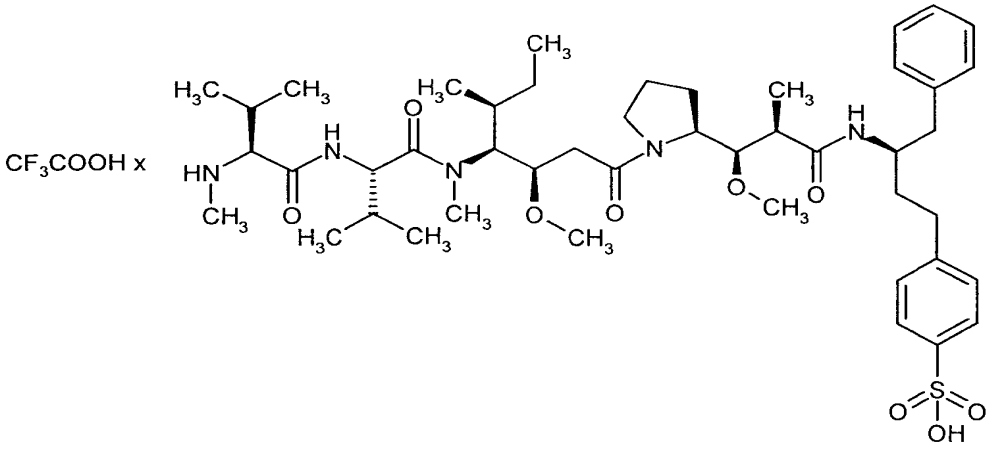


To a solution of 43 mg (0.06 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 1.9 ml of DMF were added 29 mg (0.07 mmol) of HATU and 33 µl (0.19 mmol) of Hünig's base. The reaction mixture was stirred at RT for 30 min and then admixed with 29 mg (0.07 mmol) of 4-[(3R)-3-amino-4-phenylbutyl]benzenesulphonic acid trifluoroacetate. After stirring overnight, the reaction mixture was purified by prep. HPLC. This gave 58 mg of a 45:55 mixture of the title compound and of the already deprotected amine.

LC-MS (Method 1): R_t = 1.09 min; MS (ESIpos): m/z = 973 $(M+H)^+$; R_t = 0.87 min; MS (ESIpos): m/z = 873 $(M+H)^+$ for the deprotected compound.

Intermediate 187

N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



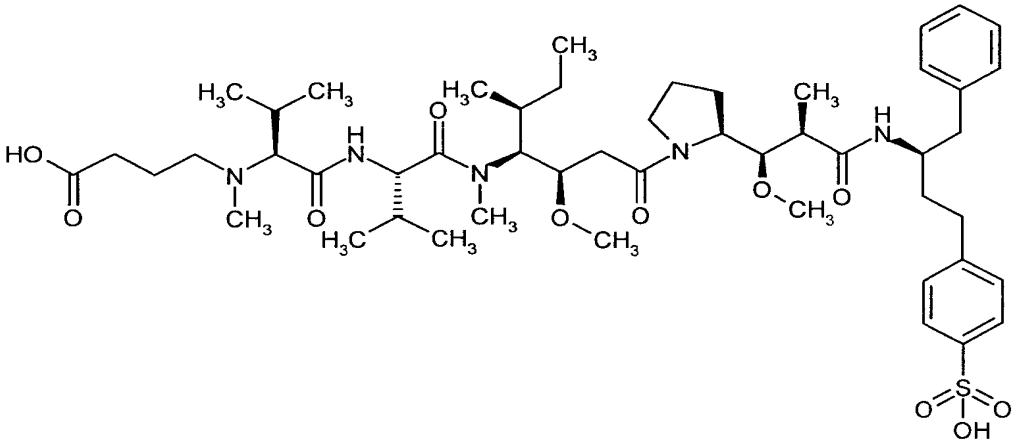
58 mg of Intermediate 186 were dissolved in 4.1 ml of dichloromethane, 0.41 ml of trifluoroacetic acid was added and the mixture was stirred at RT for 30 min. After concentration under reduced pressure, the crude product was purified by prep. HPLC.

50 mg (90% purity, 85% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 873 $(M+H)^+$

Intermediate 188

N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



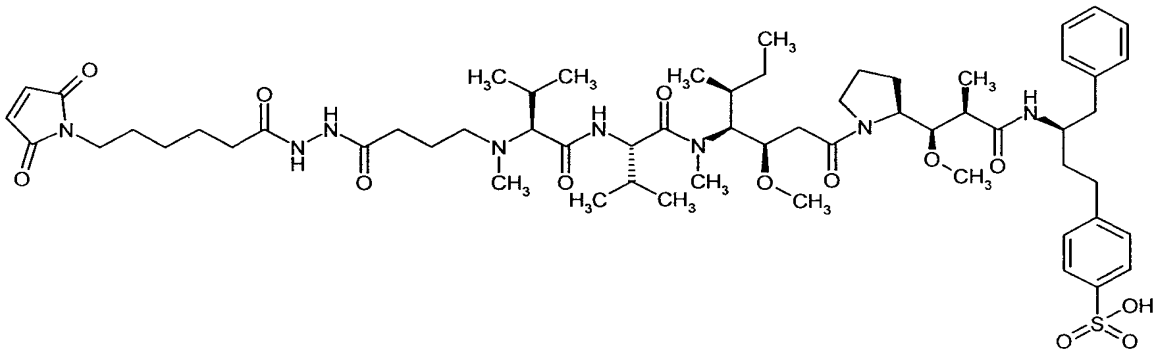
171 µl (0.26 mmol) of a 15% aqueous succinaldehyde solution were initially charged in 2.5 ml of methanol and admixed with 50 mg (0.05 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate and 11.6 µl (0.2 mmol) of acetic acid. The reaction mixture was stirred for 5 min at RT and then 30 µl (0.24 mmol) of borane-pyridine complex were added. After stirring for 24 hours, a further equivalent of borane-pyridine complex was added and the mixture was stirred for a further 2 h. The reaction mixture was then purified by prep. HPLC.

40 mg (90% purity, 66% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 959 $(M+H)^+$

Intermediate 189

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



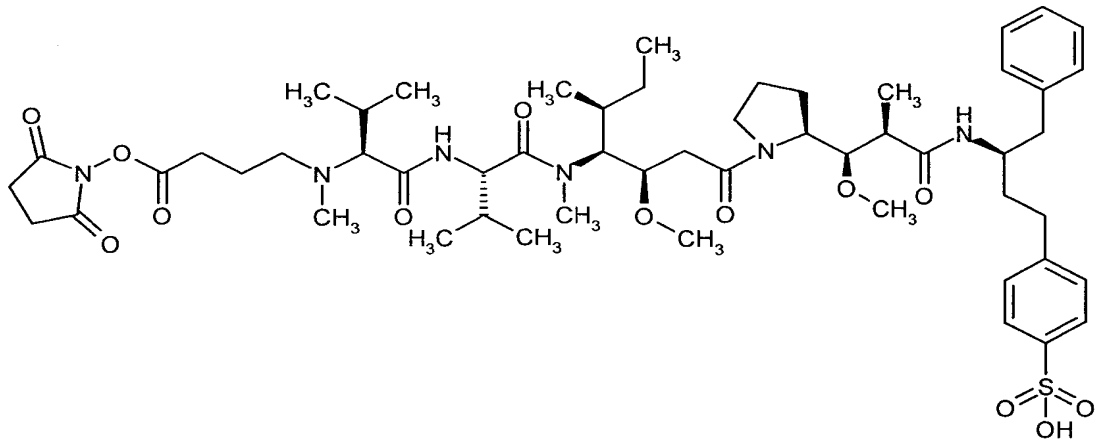
10 mg (9.3 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 3.5 mg (10.3 µmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide trifluoroacetate, 4.3 mg (11.2 µmol) of HATU and 4.9 µl (28 µmol) of Hünig's base were stirred in 1 ml of DMF at RT for 20 h. Subsequently, the reaction mixture was purified by prep. HPLC.

4.2 mg (92% purity, 33% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 1166 $(M+H)^+$

Intermediate 190

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



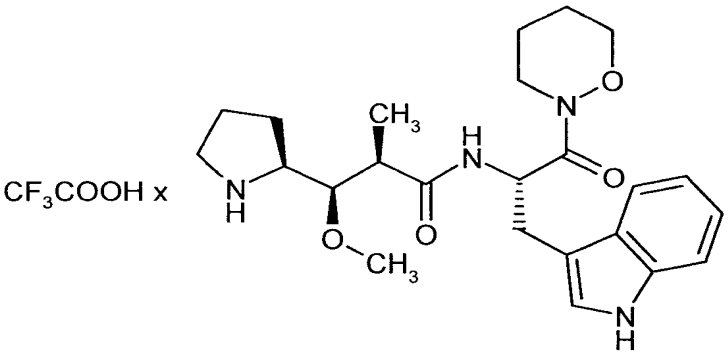
To a solution of 10 mg (9.3 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2.5 ml of DMF were added 10.7 mg (93 µmol) of N-hydroxysuccinimide, 10.6 mg (28 µmol) of EDCI and 0.12 mg (0.9 µmol) of DMAP. The reaction mixture was stirred at RT for 20 h and then purified by prep. HPLC.

3.8 mg (72% purity, 25% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 1055 $(M+H)^+$

Intermediate 191

(2*R*,3*R*)-*N*-[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate



The title compound was prepared in analogy to the synthesis of Intermediate 7 over two stages from Starting Compound 1 and (2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate (Intermediate 99).

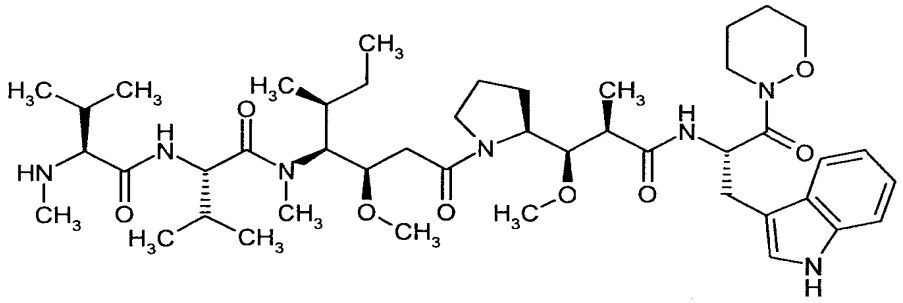
Yield over 2 stages: 62 mg (67% of theory)

HPLC (Method 6): R_t = 1.65 min;

LC-MS (Method 1): R_t = 0.7 min; MS (ESIpos): m/z = 443 $(M+H)^+$.

Intermediate 192

N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



1015 mg (1.59 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 50 ml of DMF, admixed with 654 mg (2.39 mmol) of 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) and 2.8 ml of *N,N*-diisopropylethylamine, and stirred at RT for 10 min. Then 1083 mg (1.75 mmol) of (2*R*,3*R*)-*N*-[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 191) were added and then the mixture was treated in an ultrasound bath at RT for 30 min. The reaction mixture was then concentrated under reduced pressure and the residue was taken up in 300 ml of ethyl acetate. The organic phase was washed successively with 5% aqueous citric acid solution and 5% aqueous sodium hydrogencarbonate solution, dried over magnesium sulphate, filtered and concentrated. The crude product thus obtained (1684 mg), without further purification, was taken up in 20 ml of acetonitrile, 2 ml of piperidine were added and the reaction mixture was then stirred at RT for 10 min. Then the mixture was concentrated under reduced pressure and the residue was admixed with diethyl ether. The solvent was concentrated by evaporation again and the residue was purified by flash chromatography on silica gel (eluent: 15:1:0.1 -> 15:2:0.2 dichloromethane/methanol/17% aqueous ammonia solution). The corresponding fractions were combined, the solvent was removed under reduced pressure and the residue was lyophilized from acetonitrile/water. Thus, 895 mg (67% over 2 stages) of the title compound were obtained.

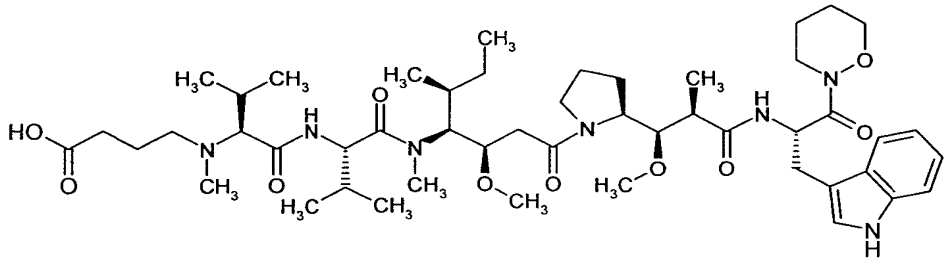
HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 840 $(M+H)^+$.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.8 (d, 1H), 8.3 and 8.05 (2d, 1H), 8.0 (d, 1H), 7.5 (m, 1H), 7.3 (m, 1H), 7.15 and 7.08 (2s, 1H) 7.05-6.9 (m, 2H), 5.12 and 4.95 (2m, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 4.1-3.8 (m, 4H), 3.75 (d, 1H), 3.23, 3.18, 3.17, 3.12, 2.95 and 2.88 (6s, 9H), 3.1-3.0 and 2.85 (2m, 2H), 2.65 (d, 1H), 2.4-2.2 (m, 3H), 2.15 (m, 3H), 1.95 (br. m, 2H), 1.85-0.8 (br. m, 11H), 1.08 and 1.04 (2d, 3H), 0.9-0.75 (m, 15H), 0.75-0.65 (dd, 3H) [further signals hidden under H_2O peak].

Intermediate 193

N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



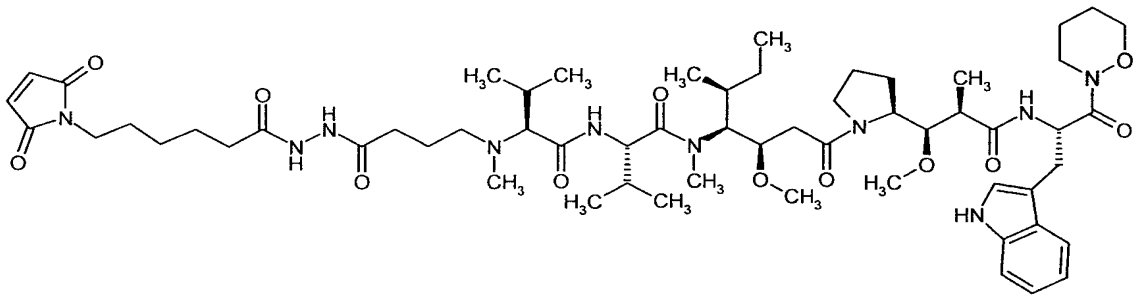
50 mg (0.052 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 192) and 204 µl einer of a 15% aqueous solution of 4-oxobutanoic acid were combined in 2 ml of methanol and admixed with 23.4 mg (0.252 mmol) of borane-pyridine complex and 6 µl of acetic acid. The reaction mixture was stirred at RT overnight. This was followed by concentration under reduced pressure, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions, 38 mg (78% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.7 min; MS (ESIpos): m/z = 926 $(M+H)^+$.

Intermediate 194

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from 10 mg (11 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide.

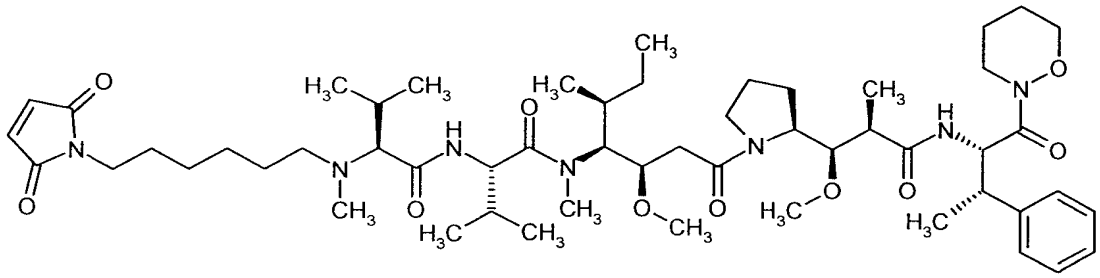
Yield: 4.4 mg (35% of theory)

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 1133 $(M+H)^+$.

Intermediate 195

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 166, proceeding from 9 mg (0.010 mmol) of Intermediate 170.

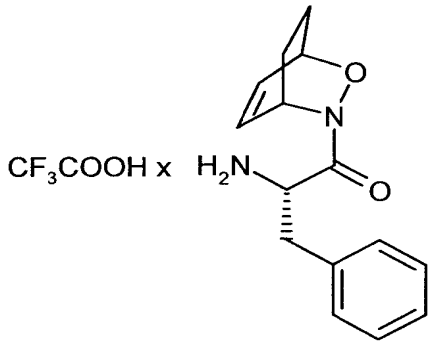
Yield: 1.1 mg (10% of theory)

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 994 $(M+H)^+$.

Intermediate 196

(2*S*)-2-amino-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-3-phenylpropan-1-one trifluoroacetate



41 mg (0.37 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(*tert*-butoxycarbonyl)-L-phenylalaninate were taken up in 10 ml of DMF and admixed with 149 mg (0.41 mmol) of 2-oxa-3-azabicyclo[2.2.2]oct-5-ene (Starting Compound 6) and 72 µl (0.41 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 1 h. The solvent was removed under reduced pressure, and the residue was taken up in ethyl acetate and extracted by shaking with 5% aqueous citric acid solution and then with 5% aqueous sodium hydrogencarbonate solution. The organic phase was concentrated and the residue was purified by flash chromatography on silica gel with 10:1 toluene/ethanol as the eluent. The corresponding fractions were combined and the solvent was removed under reduced pressure. After the residue had been dried under high vacuum, 69 mg (47% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl carbamate were thus obtained as a diastereomer mixture.

LC-MS (Method 1): R_t = 1.1 min; MS (ESIpos): m/z = 359 $(M+H)^+$.

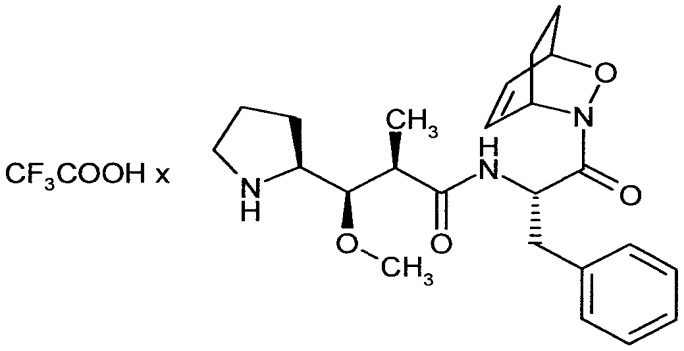
64 mg (0.18 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 1 ml of trifluoroacetic acid was added, and the mixture was stirred at RT for 30 min. This was followed by concentration under reduced pressure and lyophilization of the remaining residue from water/dioxane. In this way, 66 mg (quant.) of the title compound were obtained as a foam.

HPLC (Method 6): R_t = 1.45 min;

LC-MS (Method 3): R_t = 1.12 min; MS (ESIpos): m/z = 259 $(M+H)^+$.

Intermediate 197

(2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) was released from 83 mg (0.18 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous potassium hydrogensulphate solution. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and admixed successively with 66 mg (0.18 mmol) of (2*S*)-2-amino-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-3-phenylpropan-1-one trifluoroacetate (Intermediate 196), 101 mg (0.266 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) and 93 µl (0.53 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 30 min. The reaction mixture was then concentrated and the residue was purified by preparative HPLC. This gave 52 mg (56% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate.

HPLC (Method 6): R_t = 2.13 min;

LC-MS (Method 1): R_t = 1.13 min; MS (ESIpos): m/z = 528 $(M+H)^+$.

52 mg (0.1 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 1 ml of trifluoroacetic acid was added, and the mixture was stirred at RT for 20 min. This was followed by concentration under reduced pressure and stirring of the remaining residue with 20 ml of diethyl

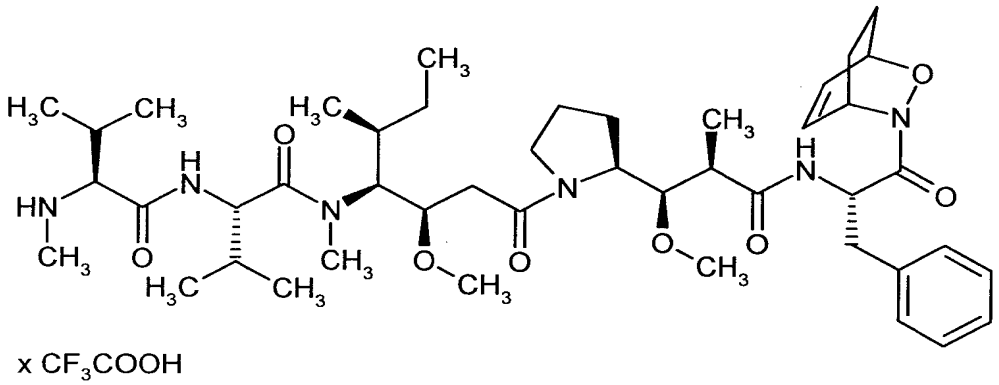
ether. After 10 min, the mixture was filtered and the filter residue was dried under high vacuum. In this way, 39 mg (72% of theory) of the title compound were obtained.

HPLC (Method 6): R_t = 1.62 min;

LC-MS (Method 1): R_t = 0.68 min; MS (ESIpos): m/z = 428 $(M+H)^+$.

Intermediate 198

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



x CF_3COOH

44.5 mg (0.071 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 10 ml of DMF and admixed successively with 38.6 mg (0.071 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 197), 32.5 mg (0.086 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and 41 µl (0.235 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at RT for 1 h. The reaction mixture was then concentrated under reduced pressure and the residue was taken up in ethyl acetate. The organic phase was washed successively with 5% aqueous citric acid solution and 5% aqueous sodium hydrogencarbonate solution, dried over magnesium sulphate, filtered and concentrated. This gave 73 mg (98% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 6): R_t = 2.78 min;

LC-MS (Method 3): R_t = 2.96 min; MS (ESIpos): m/z = 1047 $(M+H)^+$.

73 mg (0.071 mmol) of this intermediate were dissolved in 5 ml of DMF. After 0.5 ml of piperidine had been added, the reaction mixture was stirred at RT for 10 min. This was followed by concentration under reduced pressure, and the residue was digested repeatedly with diethyl ether. After the diethyl ether had been decanted off, the residue was purified by preparative HPLC (eluent: acetonitrile / 0.1% aq. TFA). 16 mg (26% of theory) of the title compound were obtained as a foam.

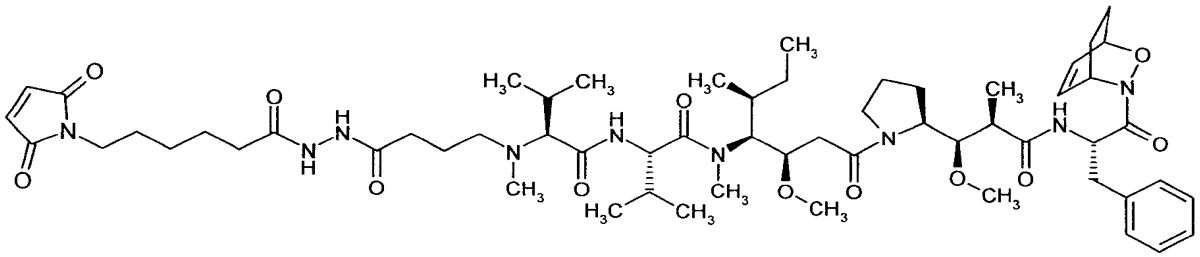
HPLC (Method 6): R_t = 1.94 min;

LC-MS (Method 3): R_t = 1.71 min; MS (ESIpos): m/z = 825 $(M+H)^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.9-8.6 (m, 3H), 8.4, 8.3, 8.1 and 8.0 (4d, 1H), 7.3-7.1 (m, 5H), 6.7-6.5 (m, 2H), 5.2-4.8 (m, 3H), 4.75-4.55 (m, 3H), 4.05-3.95 (m, 1H), 3.7-3.4 (m, 4H), 3.22, 3.17, 3.15, 3.05, 3.02 and 2.95 (6s, 9H), 3.0 and 2.7 (2 br. m, 2H), 2.46 (m, 3H), 2.4-1.2 (br. m, 13H), 1.1-0.85 (m, 18H), 0.75 (m, 3H) [further signals hidden under H_2O peak].

Intermediate 199

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



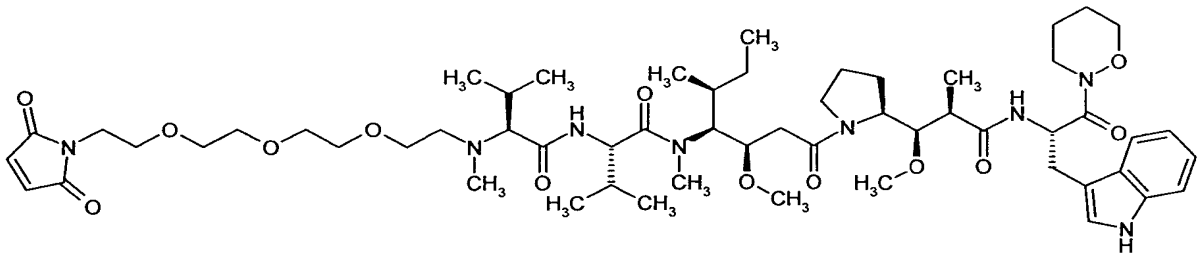
The title compound was prepared in analogy to Intermediates 193 and 194, proceeding from 23 mg (24 µmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 198).

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 2): Rt = 2.1 min; MS (ESIpos): m/z = 1118 (M+H)+.

Intermediate 200

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



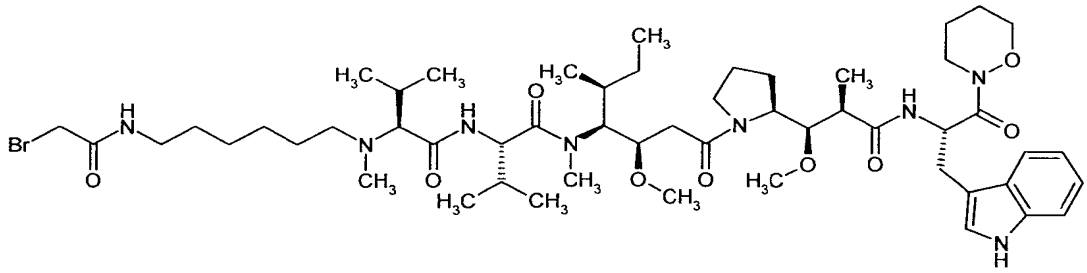
The preparation was effected in analogy to Intermediates 174 and 175, commencing with the reductive alkylation of Intermediate 192 with Intermediate 172, subsequent deprotection and formation of the maleimide.

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 1): Rt = 0.86 min; MS (ESIpos): m/z = 1025 (M+H)+.

Intermediate 201

N-{6-[(bromoacetyl)amino]hexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



22 mg (0.023 mmol) of N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 101) were dissolved in 9.5 ml of THF and admixed at 0°C with 4.2 µl of

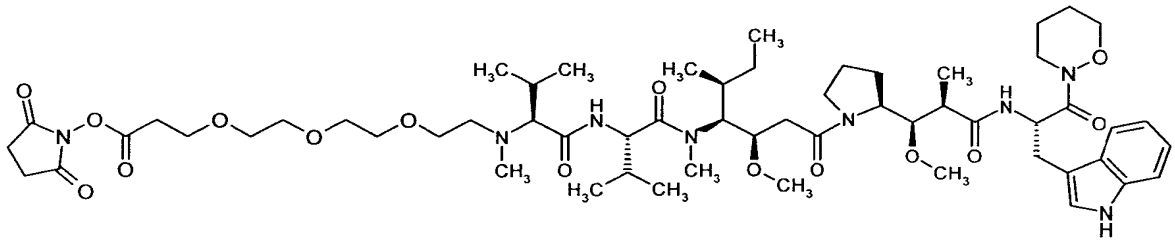
triethylamine. A solution of bromoacetyl chloride in THF was added dropwise and the reaction mixture was stirred at 0°C for 30 min. The reaction mixture was concentrated and the residue was purified by preparative HPLC. Thus, 6.9 mg (26% of theory) of the title compound were obtained as a foam.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 11): R_t = 0.9 min; MS (ESIpos): m/z = 1059 and 1061 $(M+H)^+$.

Intermediate 202

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was at first effected in analogy to Intermediate 168, commencing with the reductive alkylation of Intermediate 192 with Intermediate 167 and subsequent hydrogenolytic cleavage of the benzyl ester of N-(2-{2-[2-(2-carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide.

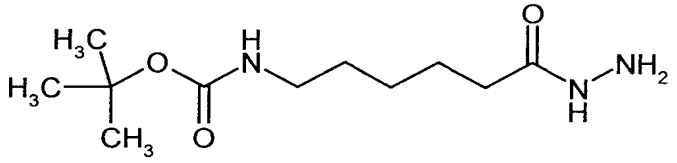
13 mg (10 µmol) of this intermediate were dissolved in 5 ml of DMF and admixed with 2.1 mg (20 mmol) of 1-hydroxypyrrolidine-2,5-dione, 6.5 µl of *N,N*-diisopropylethylamine and 7.1 mg (0.02 mmol) of HATU. The reaction mixture was stirred at RT overnight and then concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 9.2 mg (62% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 2): R_t = 2.1 min; MS (ESIpos): m/z = 1141 $(M+H)^+$.

Intermediate 203

tert-butyl 6-hydrazino-6-oxohexyl carbamate

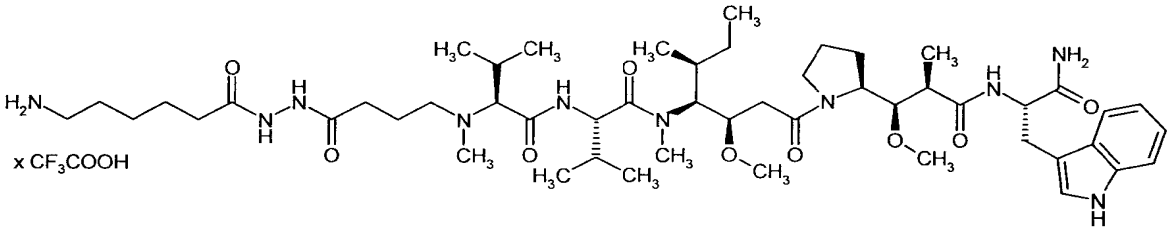


This compound was prepared by standard peptide chemistry methods, by coupling of 6-[(*tert*-butoxycarbonyl)amino]hexanoic acid with benzyl hydrazinecarboxylate in the presence of EDCI and HOBT, and subsequent hydrogenolytic cleavage of the benzyloxycarbonyl protecting group.

LC-MS (Method 11): R_t = 0.59 min; MS (ESIpos): m/z = 246 $(M+H)^+$.

Intermediate 204

N-{4-[2-(6-aminohexanoyl)hydrazino]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



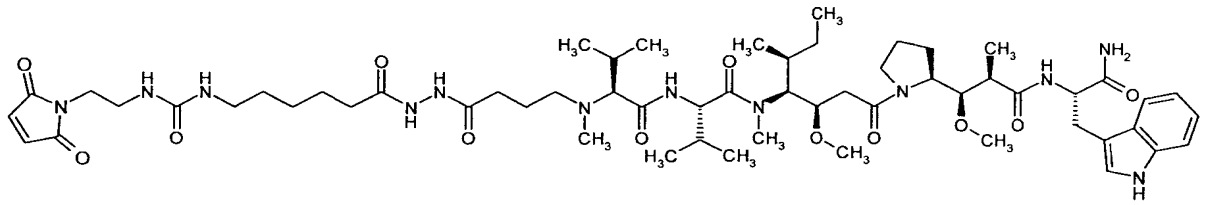
146 mg (50 µmol) of (N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide were dissolved in 5 ml of DMF and then admixed with 30.6 mg (80 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 19 µl of N,N-diisopropylethylamine and with 22.4 mg (60 µmol) of *tert*-butyl 6-hydrazino-6-oxohexyl carbamate. The reaction mixture was stirred at RT for 1.5 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 43 mg (68% of theory) of the protected intermediate were obtained, which were then taken up in 10 ml of dichloromethane and deprotected with 1 ml of trifluoroacetic acid. The reaction mixture was concentrated and the residue was stirred with dichloromethane, and the solvent was removed again under reduced pressure. Thus, 45 mg (68% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.66 min; MS (ESIpos): m/z = 983 $(M+H)^+$.

Intermediate 205

N-(4-{2-[6-({[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl}amino)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 114, proceeding from Intermediates 50 and 204.

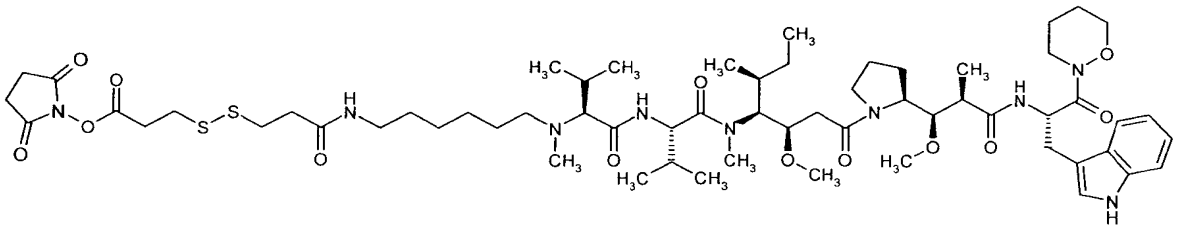
Yield: 4 mg (78% of theory)

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 11): R_t = 0.73 min; MS (ESIpos): m/z = 1149 $(M+H)^+$.

Intermediate 206

N-(6-{[3-({3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropyl}disulphanyl)propanoyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



8 mg (10 µmol) of Intermediate 101 were dissolved in 2 ml of DMF and admixed with 8.6 mg (20 µmol) of 1,1'-{disulphanediylbis[(1-oxopropane-3,1-diyl)oxy]}dipyrrolidine-2,5-dione and 3.7 µl

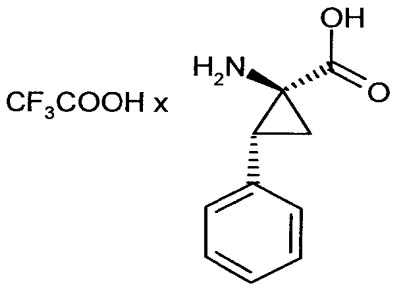
of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 2 h and then the solvent was evaporated off under reduced pressure and the residue was purified by preparative HPLC. 7.2 mg (68% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 11): R_t = 0.94 min; MS (ESIpos): m/z = 615 [½ ($M+2H^+$]

Intermediate 207

(1S,2R)-1-amino-2-phenylcyclopropanecarboxylic acid trifluoroacetate

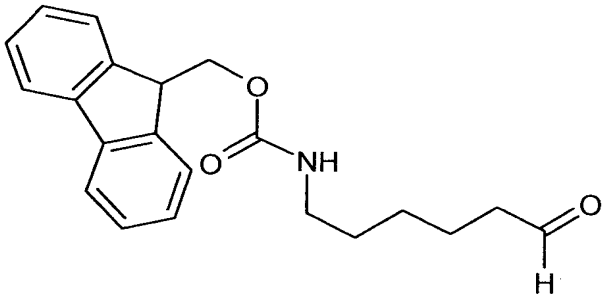


The title compound was obtained in quantitative yield by deprotecting 210 mg (0.76 mmol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.23 min; MS (ESIpos): m/z = 178 $(M+H)^+$.

Intermediate 208

9H-fluoren-9-ylmethyl 6-oxohexyl carbamate



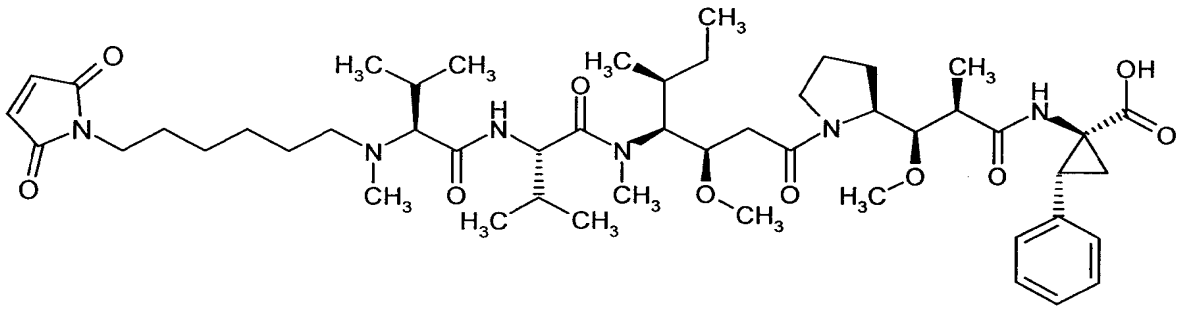
The title compound was prepared from 1 g (2.95 mmol) of commercially available 9H-fluoren-9-ylmethyl 6-hydroxyhexyl carbamate under standard conditions, by oxidation with sulphur trioxide-pyridine complex. 840 mg (85% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.1 min; MS (ESIpos): m/z = 338 $(M+H)^+$.

Intermediate 209

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-carboxy-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 75, by coupling of N-(*tert*-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 26) and (1S,2R)-1-amino-2-phenylcyclopropanecarboxylic acid trifluoroacetate (Intermediate 207) in the presence of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, the amine compound N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-carboxy-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide was prepared as the trifluoroacetate.

To 22 mg (0.026 mmol) of this compound in 10 ml of methanol were then added 17 mg (0.05 mmol) of 9H-fluoren-9-ylmethyl 6-oxohexyl carbamate (Intermediate 208) and 2.3 mg of acetic acid, and also 11.4 mg (0.12 mmol) of borane-pyridine complex. The reaction mixture was stirred at RT overnight. Then the same amounts of borane-pyridine complex and acetic acid, and also 8 mg of fluoren-9-ylmethyl 6-oxohexyl carbamate, were added once again and the reaction mixture was stirred at RT for a further 24 h. This was followed by concentration under reduced pressure, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions, the product was used immediately in the next stage.

33 mg of the still contaminated intermediate were taken up in 5 ml of DMF, and 1 ml of piperidine was added. After stirring at RT for 15 min, the reaction mixture was concentrated and the resulting residue was purified by preparative HPLC. Thus, 11 mg (55% of theory over 2 stages) of the aminocarboxylic acid intermediate were obtained.

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 11): R_t = 0.7 min; MS (ESIpos): m/z = 843 $(M+H)^+$.

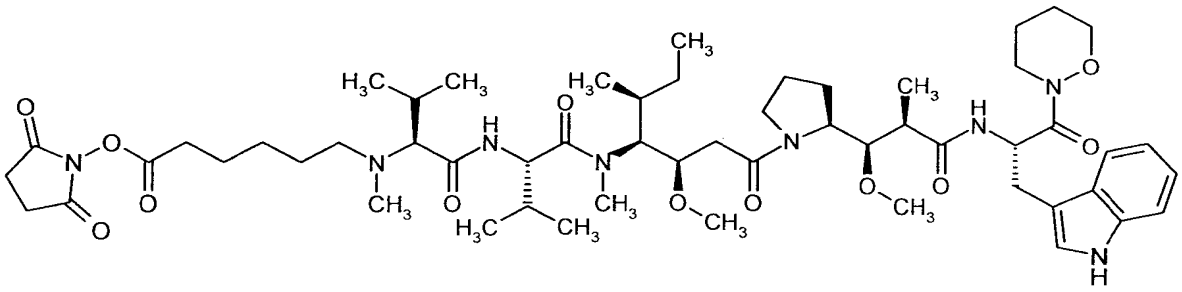
6 mg (7.12 µmol) of this intermediate were taken up in 1 ml of dioxane and then admixed with 6.6 mg (42.7 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 5 µl of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 1 h. Then another 3 portions each of 50 µl of the saturated aqueous sodium hydrogencarbonate solution were added and the reaction mixture was stirred at RT for a further 30 min. Then the reaction mixture was acidified to pH 2 with trifluoroacetic acid and subsequently concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 4 mg (60% of theory) of the title compound were obtained as a foam.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 11): R_t = 0.88 min; MS (ESIpos): m/z = 923 $(M+H)^+$.

Intermediate 210

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First, 6-oxohexanoic acid was prepared by a literature method (J. Org. Chem. 58, 1993, 2196-2200).

80 mg (0.08 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 192) and 65.4 mg (0.5 mmol) of 6-oxohexanoic acid were combined in 9 ml of methanol and admixed with 10 µl of acetic acid and 37.4 mg (0.4 mmol) of borane-pyridine complex. The reaction mixture was stirred at RT overnight. This was followed by concentration under reduced pressure, and the residue was taken up in 1:1 acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. The reaction mixture was concentrated again and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions, 70 mg (86% of theory) of N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide were obtained as the trifluoroacetate.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 955 $(M+H)^+$.

^{1}H NMR (500 MHz, DMSO-d_6, characteristic signals): δ = 12.0 (br. M, 1H), 10.8 (s, 1H), 9.4 (m, 1H), 8.9 and 8.8 (2d, 1H), 8.3 and 8.02 (2d, 1H), 7.5 (m, 1H), 7.3 (m, 1H), 7.15 and 7.1 (2s, 1H) 7.05-6.9 (m, 2H), 5.12 and 4.95 (2m, 1H), 4.7-4.5 (m, 2H), 4.1-3.8 (m, 4H), 3.75 (d, 1H), 3.25, 3.2, 3.18, 3.13, 2.98 and 2.88 (6s, 9H), 2.8 (m, 3H), 1.08 and 1.04 (2d, 3H), 0.95-0.8 (m, 15H), 0.8-0.65 (dd, 3H).

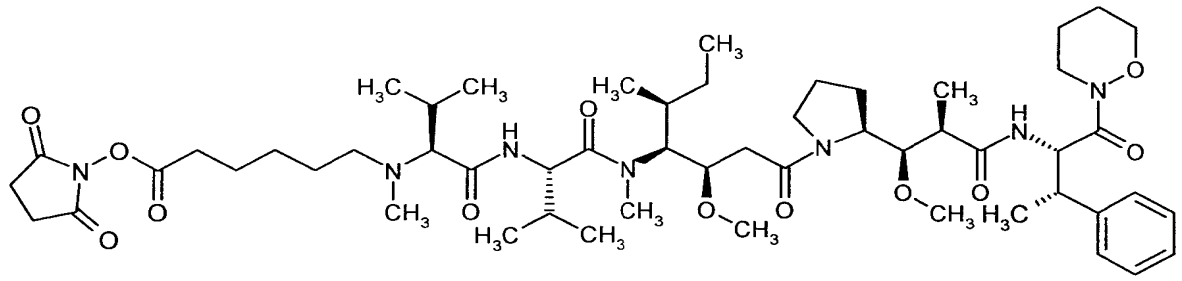
22 mg (23 µmol) of this intermediate were dissolved in 1.8 ml of dichloromethane and admixed with 13.2 mg (70 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 26.5 mg (230 µmol) of 1-hydroxypyrrolidine-2,5-dione and 0.28 mg (2 µmol) of dimethylaminopyridine, and the reaction mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 21.3 mg (88% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1052 $(M+H)^+$.

Intermediate 211

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



15 mg (20 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 15) were reductively alkylated with 6-oxohexanoic acid, in analogy to Intermediate 210.

Yield: 9.2 mg (61% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 929 $(M+H)^+$.

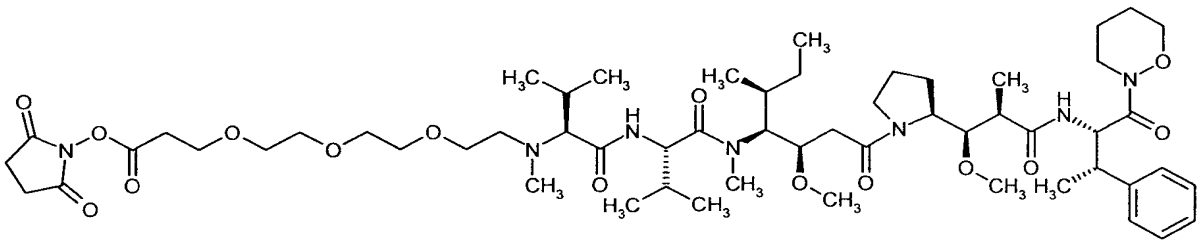
9 mg (10 µmol) of this intermediate were dissolved in 3 ml of DMF and admixed with 5.6 mg (48 µmol) of 1-hydroxypyrrolidine-2,5-dione, 5 µl of *N,N*-diisopropylethylamine and 5.5 mg (0.015 mmol) of HATU, and the reaction mixture was treated in an ultrasound bath for 6 h. In the course of this, 5.5 mg of HATU were added every hour. Subsequently, the reaction mixture was concentrated under reduced pressure, and the residue was taken up in acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. After concentrating again under reduced pressure, the remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 5.8 mg (57% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1027 $(M+H)^+$.

Intermediate 212

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was at first effected in analogy to Intermediate 168, commencing with the reductive alkylation of Intermediate 15 with Intermediate 167 and subsequent hydrogenolytic cleavage of the benzyl ester of N-(2-{2-[2-(2-carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide.

8.4 mg (8 µmol) of this intermediate were dissolved in 3 ml of DMF and admixed with 9.5 mg (80 µmol) of 1-hydroxypyrrolidine-2,5-dione, 10 µl of *N,N*-diisopropylethylamine and 9.4 mg (25 µmol) of HATU, and the reaction mixture was stirred at RT overnight and then concentrated under reduced pressure. Subsequently, the reaction mixture was concentrated under reduced pressure, and the residue was taken up in acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. After concentrating again under reduced pressure, the remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 4 mg (32% of theory) of the title compound were obtained.

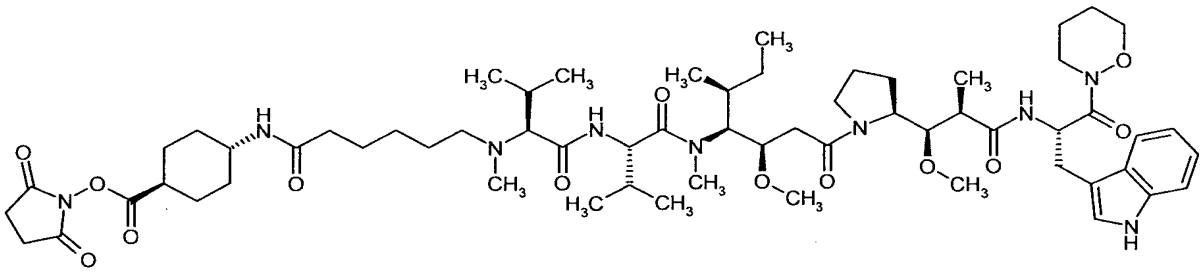
HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.96 min; MS (ESIpos): m/z = 1117 $(M+H)^+$.

Intermediate 213

N-{6-[(trans-4-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}cyclohexyl)amino]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-

yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



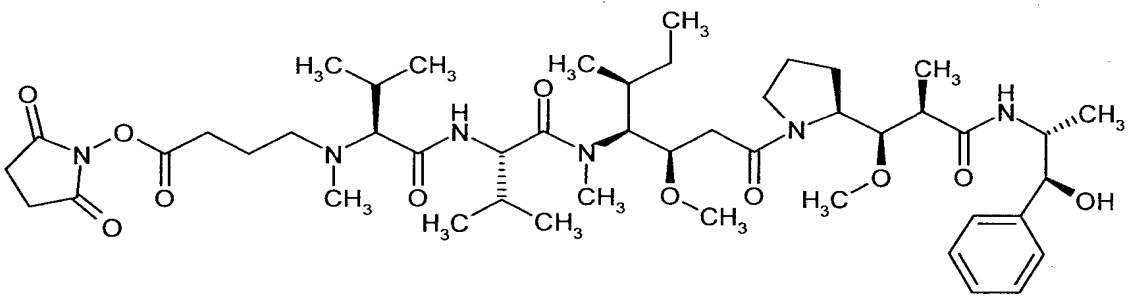
This compound was prepared in analogy to Intermediate 104, proceeding from N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, the synthesis of which was described under Intermediate 210. 9.3 mg of the title compound (37% of theory over 3 stages) were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 1177 $(M+H)^+$.

Intermediate 214

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



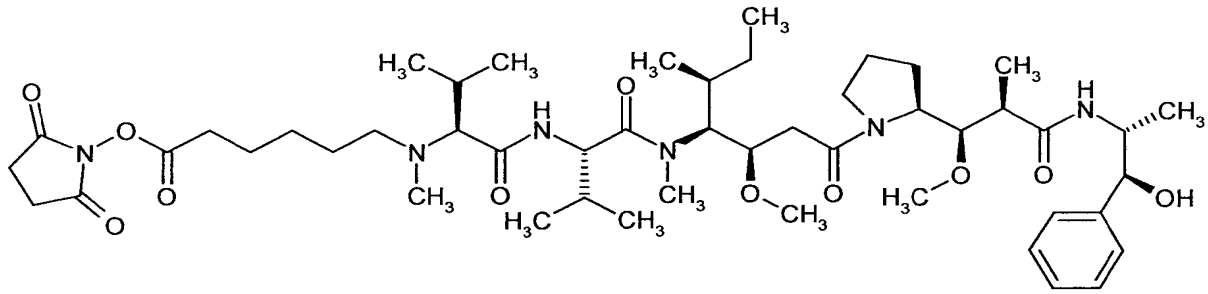
This compound was prepared in analogy to Intermediate 210, by conversion of Intermediate 92 to the active ester.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.82 min; MS (ESIpos): m/z = 901 $(M+H)^+$.

Intermediate 215

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



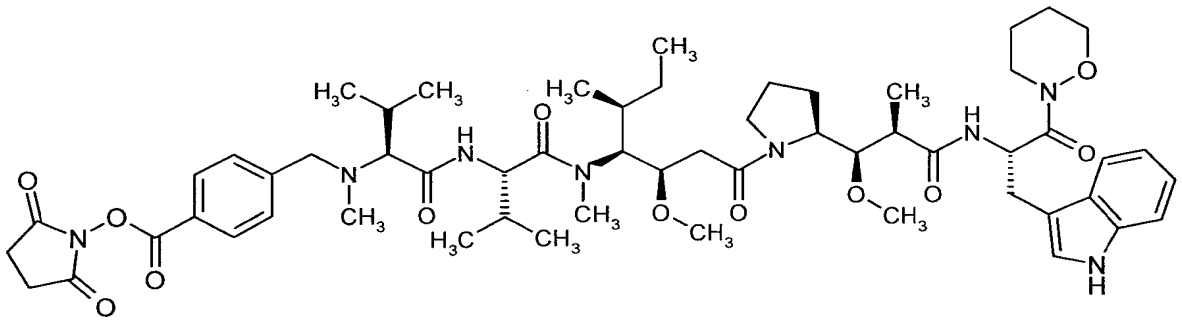
First, Intermediate 40, in analogy to Intermediate 183, was used with borane-pyridine complex to prepare N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide. From this compound, in analogy to Intermediate 210, the active ester was then generated. 34 mg (36% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 930 $(M+H)^+$.

Intermediate 216

N-(4-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}benzyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



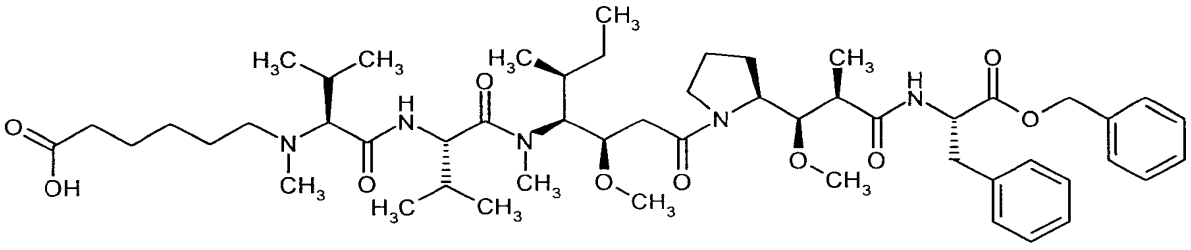
First, in analogy to the preparation of Intermediate 183, Intermediate 192 was reacted with 4-formylbenzoic acid with borane-pyridine complex to give N-(4-carboxybenzyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide. This compound was then used, in analogy to Intermediate 210, to generate 11 mg (68% of theory) of the title compound.

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 1.13 min; MS (ESIpos): m/z = 1072 $(M+H)^+$.

Intermediate 217

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



53 mg (84 µmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and 45 mg (84 µmol) of benzyl *N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate (Intermediate 12) were taken up in 2 ml of DMF, 19 µl of *N,N*-diisopropylethylamine, 14 mg (92 µmol) of HOBt and 17.6 mg (92 µmol) of EDC were added and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. This gave 59 mg (68% of theory) of

the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

LC-MS (Method 1): R_t = 1.55 min; m/z = 1044 $(M+H)^+$.

57 mg (0.055 mmol) of this intermediate were treated with 1.2 ml of piperidine in 5 ml of DMF to detach the Fmoc protecting group. After concentration and purification by means of preparative HPLC, 39 mg (76% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 1.01 min; m/z = 822 $(M+H)^+$.

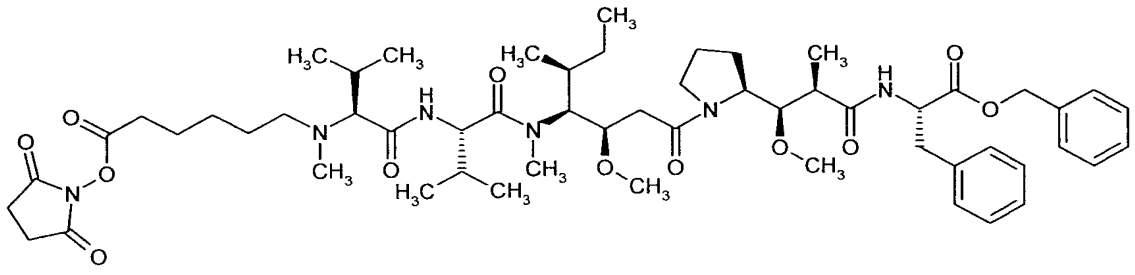
60 mg (0.06 mmol) of this intermediate were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. 45 mg (75% of theory) of the title compound were obtained as a foam.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 9936 $(M+H)^+$.

Intermediate 218

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared by conversion of 42 mg (0.05 mmol) of Intermediate 217 to the active ester.

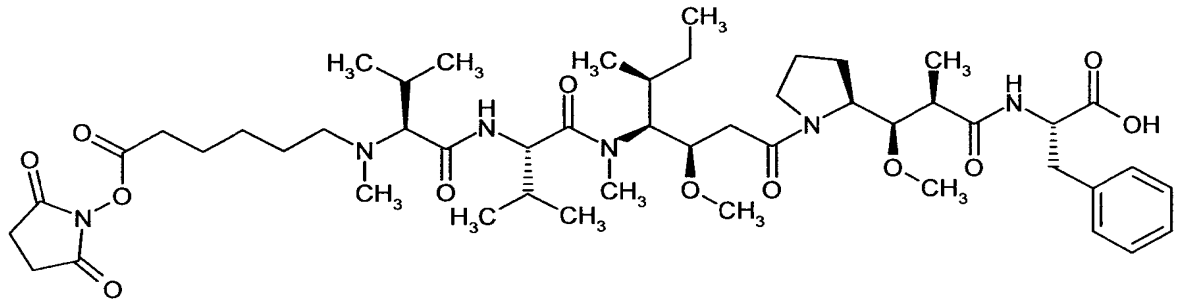
Yield: 26 mg (54%)

HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 1034 $(M+H)^+$.

Intermediate 219

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



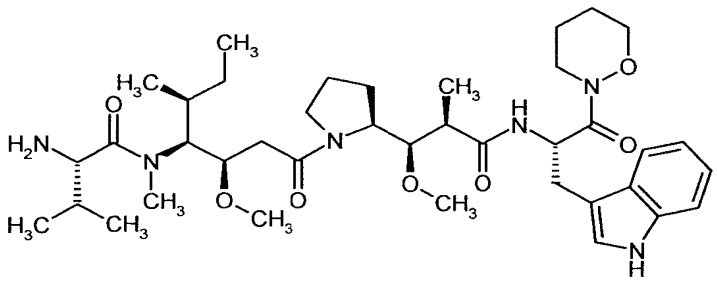
20 mg (0.02 mol) of the compound from Intermediate 218 were taken up in 2.4 ml of methanol and hydrogenated over 5% palladium on activated carbon under standard hydrogen pressure at RT for 30 min. The catalyst was then filtered off and the solvent was removed under reduced pressure. The residue was lyophilized from 1:1 acetonitrile/water. This gave 14 mg (92% of theory) of the title compound as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 944 $(M+H)^+$.

Intermediate 220

N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



500 mg of this intermediate were dissolved in 20 ml of DMF and admixed with 466 mg (3.8 mmol) of Intermediate 191, 382 mg (1.01 mmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 440 µl (2.5 mmol) of N,N-diisopropylethylamine. The mixture was stirred at RT for 1 h and then concentrated. The residue was taken up in dichloromethane and extracted by shaking first twice with 5% aqueous citric acid solution and then with saturated aqueous sodium hydrogencarbonate solution. The organic phase was concentrated and the residue was purified by flash chromatography on silica gel with 95:5 dichloromethane/methanol as the eluent. The corresponding fractions were combined and the solvent was removed under reduced pressure. After the residue had been dried under high vacuum, 562 mg (65% of theory over both stages) of the Z-protected intermediate were obtained.

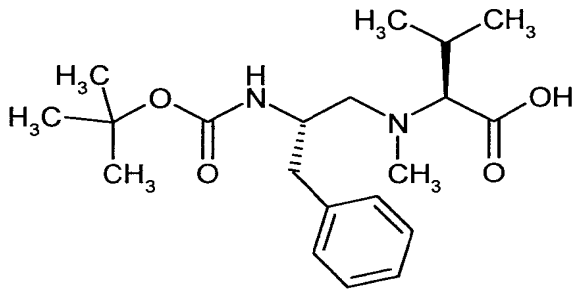
562 mg (0.57 mmol) of this intermediate were taken up in 50 ml of methanol and hydrogenated with 155 mg of 10% palladium on activated carbon under standard hydrogen pressure at RT for 20 min. The catalyst was then filtered off and the solvent was removed under reduced pressure. The residue was purified by preparative HPLC. The corresponding fractions were combined, the solvent was evaporated off under reduced pressure and the residue was lyophilized from dioxane. This gave 361 mg (87% of theory) of the title compound as a foam.

HPLC (Method 5): double peak with Rt = 1.75 and 1.86 min;

LC-MS (Method 1): double peak at Rt = 0.84 min and 0.91 min with the same mass; MS (ESIpos): m/z = 944 $(M+H)^+$.

Intermediate 221

N-{(2S)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropyl}-N-methyl-L-valine



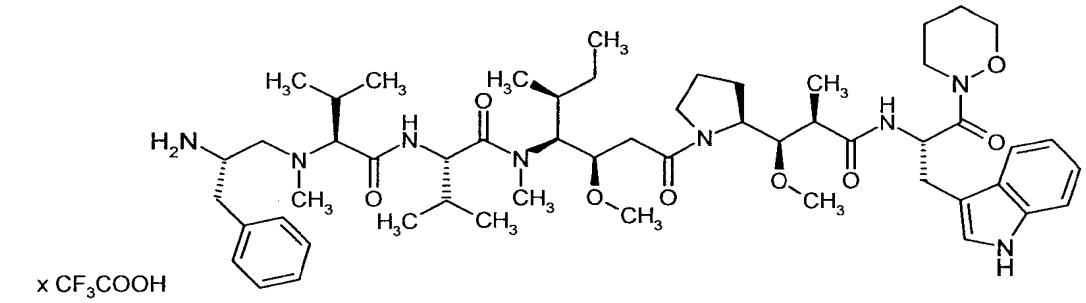
100 mg (0.76 mmol) of commercially available N-methyl-L-valine and 285 mg (1.14 mmol) of commercially available *tert*-butyl (2S)-1-oxo-3-phenylpropan-2-yl carbamate were combined in 22 ml of methanol and admixed with 340 mg (3.66 mmol) of borane-pyridine complex and 70 µl of acetic acid. The reaction mixture was stirred at RT overnight. This was followed by concentration under reduced pressure, and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol/17% aqueous ammonia solution as the eluent. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 259 mg (93% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.76 min; MS (ESIpos): m/z = 365 $(M+H)^+$.

Intermediate 222

N-[(2S)-2-amino-3-phenylpropyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



x CF_3COOH

40 mg (0.11 mmol) of N-{(2S)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropyl}-N-methyl-L-valine (Intermediate 221) were dissolved in 5 ml of DMF and admixed with 80 mg (0.11 mmol) of N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 220), 50 mg (0.13 mmol) of *O*-(7-azabenzotriazol-1-

yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 57 µl (2.5 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 1 h and then concentrated. The residue was taken up in ethyl acetate and washed first with 5% aqueous citric acid solution and then with water. The organic phase was concentrated and the residue was purified by means of preparative HPLC. The corresponding fractions were combined and the solvent was removed under reduced pressure. After lyophilization from dioxane, 60 mg (50% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 1): R_t = 1.17 min; MS (ESIpos): m/z = 1073 $(M+H)^+$.

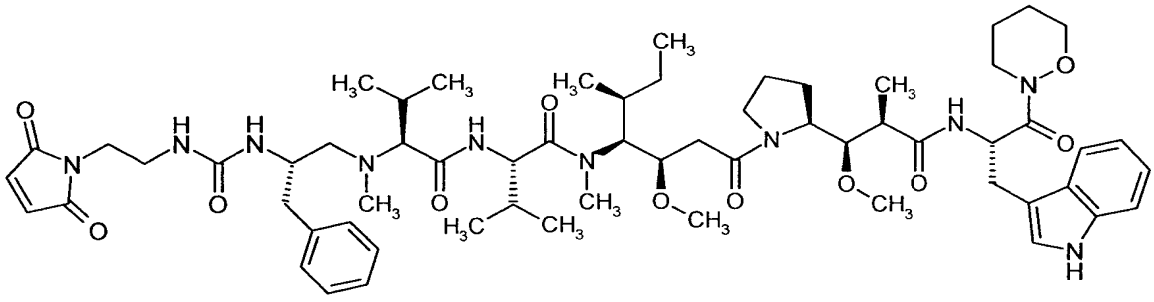
60 mg (0.05 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 2 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 1.5 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was removed under reduced pressure and the residue was lyophilized from dioxane/water. In this way, 25 mg (42% of theory) of the title compound were obtained as a foam.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 974 $(M+H)^+$.

Intermediate 223

N-[(2S)-2-({[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl}amino)-3-phenylpropyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



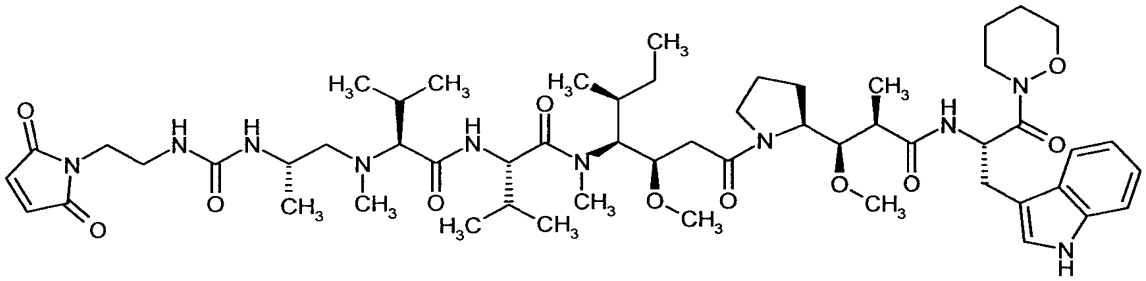
The preparation was effected in analogy to Intermediate 134, proceeding from 5 mg (4.6 µmol) of Intermediate 222. 3.4 mg (65% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 1140 $(M+H)^+$.

Intermediate 224

N-[(2S)-2-({[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl}amino)propyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



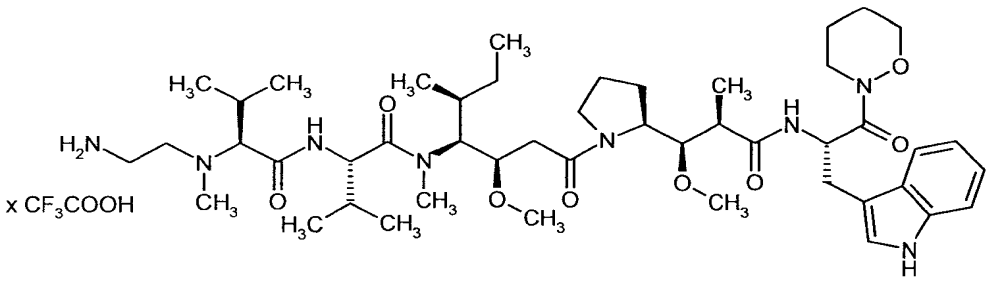
The preparation was effected in analogy to the synthesis of Intermediate 223.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1064 $(M+H)^+$.

Intermediate 225

N-(2-aminoethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



100 mg (0.76 mmol) of commercially available N-methyl-L-valine and 182 mg (1.14 mmol) of commercially available *tert*-butyl 2-oxoethyl carbamate were combined in 20 ml of methanol and admixed with 340 mg (3.66 mmol) of borane-pyridine complex and 65 µl of acetic acid. The reaction mixture was stirred at RT overnight. This was followed by concentration under reduced pressure, and the residue was purified by flash chromatography on silica gel with

dichloromethane/methanol/17% aqueous ammonia solution (15/4/0.5) as the eluent. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 190 mg in 39% purity (35% of theory) of the intermediate were obtained, which were converted further without further purification.

50 mg (0.07 mmol) of this intermediate were dissolved in 10 ml of DMF and admixed with 52 mg (0.07 mmol) of N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 220), 32 mg (0.09 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 37 µl (0.2 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight and then concentrated. The residue was taken up in ethyl acetate and extracted by shaking first with 5% aqueous citric acid solution and then with water. The organic phase was concentrated and the residue was purified by means of preparative HPLC. The corresponding fractions were combined and the solvent was removed under reduced pressure. After lyophilization from dioxane, 53 mg (76% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 984 $(M+H)^+$.

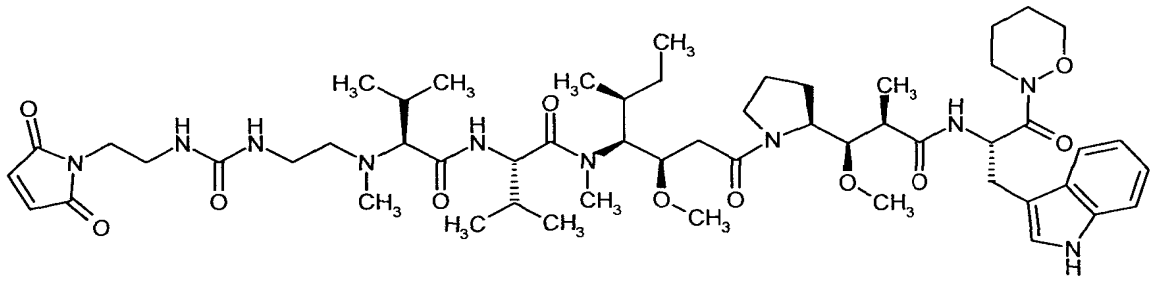
53 mg (0.05 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 2 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was removed under reduced pressure and the residue was lyophilized from dioxane/water. In this way, 21 mg (40% of theory) of the title compound were obtained in 65% purity.

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 884 $(M+H)^+$.

Intermediate 226

N-[2-({[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl}amino)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



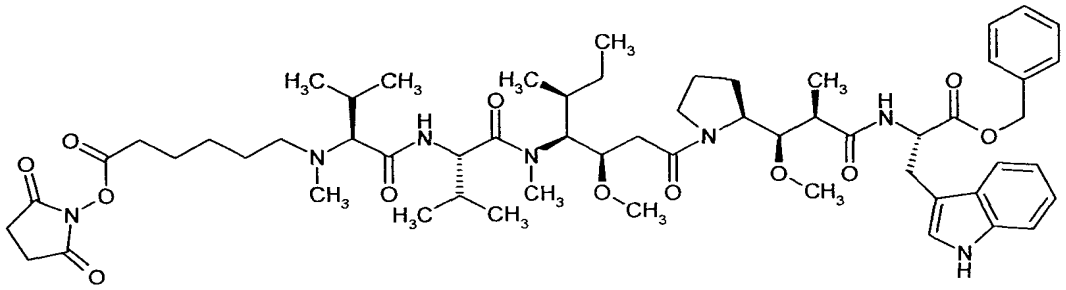
The preparation was effected proceeding from Intermediate 225, in analogy to the synthesis of Intermediate 134. 11.6 mg (59% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 1050 $(M+H)^+$.

Intermediate 227

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzyloxy)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared analogously to Intermediate 218, by conversion to the active ester.

Yield: 18 mg (51% of theory)

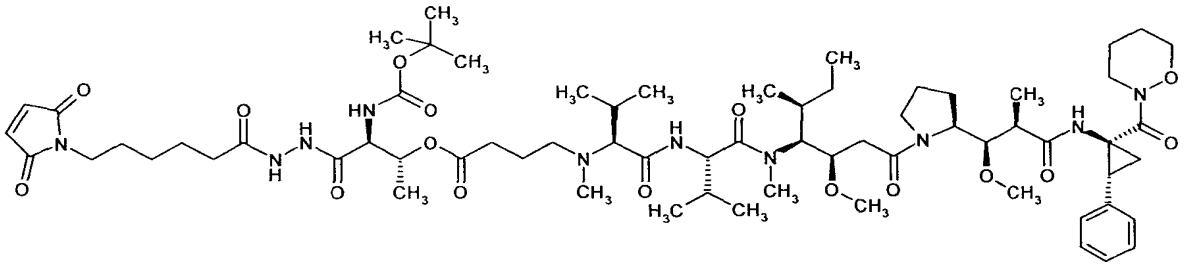
HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 1073 $(M+H)^+$.

Intermediate 228

(2R,3S)-3-[(*tert*-butoxycarbonyl)amino]-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl(3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-

phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



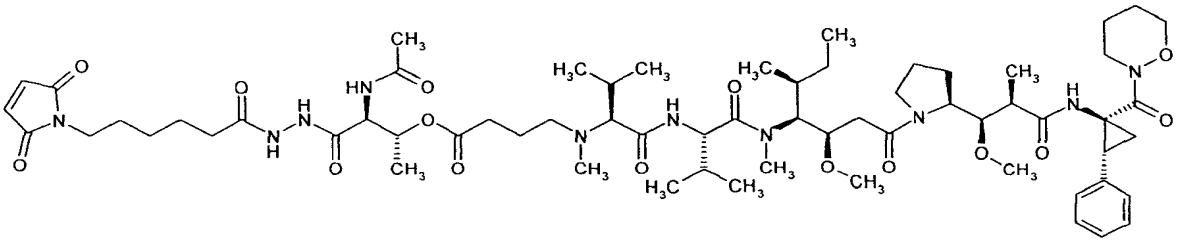
The title compound was obtained as intermediate in the synthesis of Intermediate 154.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1308 $(M+H)^+$.

Intermediate 229

(2R,3S)-3-acetamido-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



The title compound was prepared from 7.5 mg (2.5 µmol) of Intermediate 154 by acetylation with 2.3 µl of acetic anhydride in 1 ml of DMF in the presence of 0.4 µl of *N,N*-diisopropylethylamine.

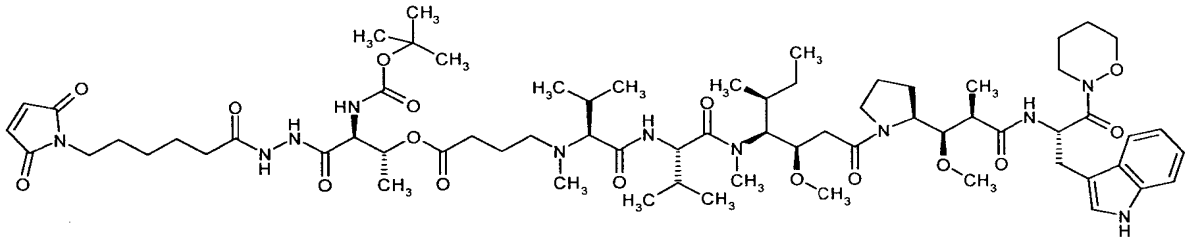
Yield: 1.4 mg (40% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1250 $(M+H)^+$.

Intermediate 230

(2R,3S)-3-[(*tert*-butoxycarbonyl)amino]-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl] hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl] pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



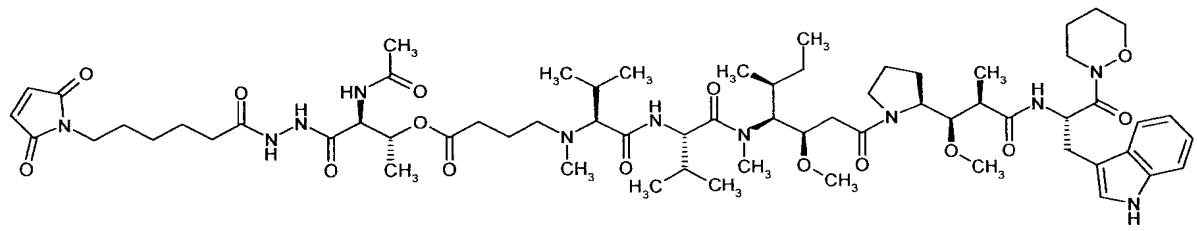
This compound was prepared in analogy to Intermediate 229, proceeding from Intermediate 193. 16 mg (30% of theory over 3 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 1335 $(M+H)^+$.

Intermediate 231

(2R,3S)-3-acetamido-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



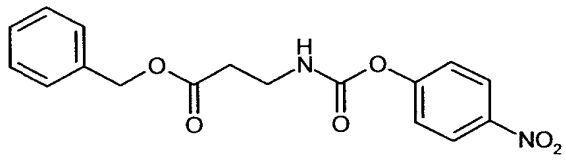
This compound was prepared from 8 mg (6 µmol) of Intermediate 230, first by deprotection with trifluoroacetic acid and subsequent acetylation with acetic anhydride in DMF in the presence of *N,N*-diisopropylethylamine. 2 mg (37% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1277 $(M+H)^+$.

Intermediate 232

benzyl N-[(4-nitrophenoxy)carbonyl]-beta-alaninate



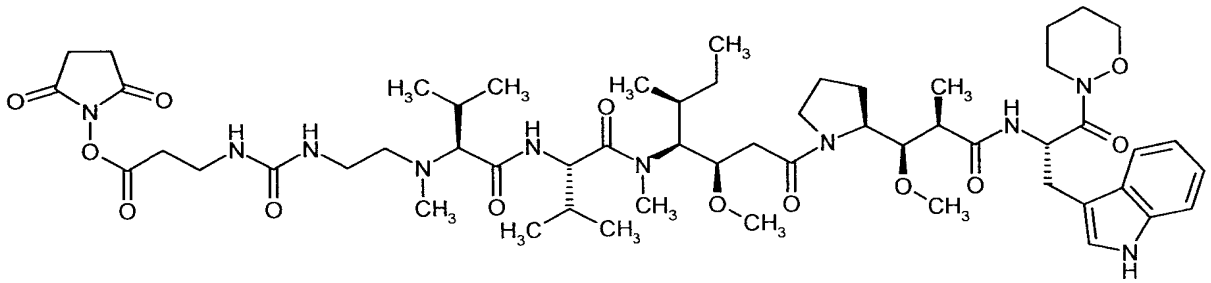
200 mg (0.57 mmol) of commercially available 4-methylbenzenesulphonic acid-benzyl beta-alaninate and 229 mg (1.14 mmol) of 4-nitrophenyl chlorocarbonate were taken up in 15 ml of tetrahydrofuran and the reaction mixture was then heated to reflux for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions and drying of the residue under high vacuum, 86 mg (44% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 1.07 min; MS (ESIpos): m/z = 345 $(M+H)^+$.

Intermediate 233

N-{2-[({3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropyl}carbamoyl)amino]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



13 mg (10 µmol) of Intermediate 225 and 6.7 mg (20 µmol) of Intermediate 232 were dissolved in 3 ml of DMF, and then 7 µl of N,N-diisopropylethylamine were added. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by

means of preparative HPLC. After concentration of the corresponding fractions and drying of the residue under high vacuum, 5.4 mg (38% of theory) of the protected intermediate were obtained.

HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0. 6in; MS (ESIpos): m/z = 1089 $(M+H)^+$.

5.4 mg (5 µmol) of this intermediate were dissolved in 5 ml of methanol and, after addition of 2 mg of 10% palladium on activated carbon, hydrogenated under standard hydrogen pressure at RT for 20 min. The catalyst was then filtered off and the solvent was removed under reduced pressure. After the residue had been dried under high vacuum, 5 mg (quant.) of the acid intermediate were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 999 $(M+H)^+$.

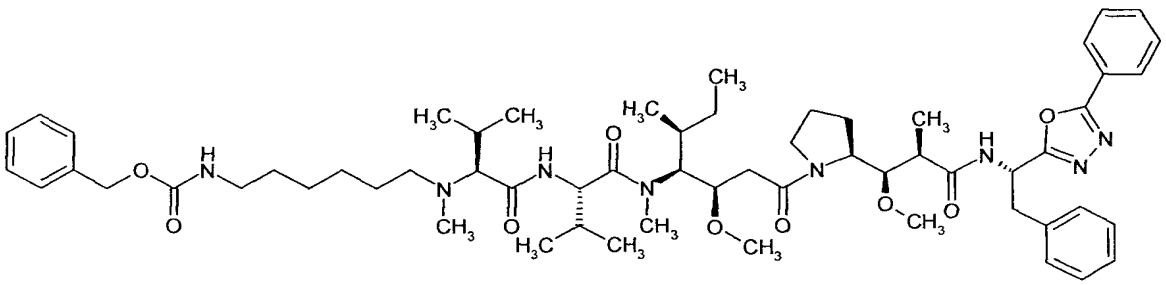
5 mg (10 µmol) of this intermediate were dissolved in 1 ml of DMF and admixed with 5.8 mg (50 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.6 µl of *N,N*-diisopropylethylamine and 3.8 mg (10 µmol) of HATU. After stirring at RT for 20 h, the reaction mixture was concentrated under reduced pressure. The remaining residue was purified by means of preparative HPLC. After lyophilization from 1:1 dioxane/water, 1.1 mg (20% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1096 $(M+H)^+$.

Intermediate 234

N-(6-{[(benzyloxy)carbonyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

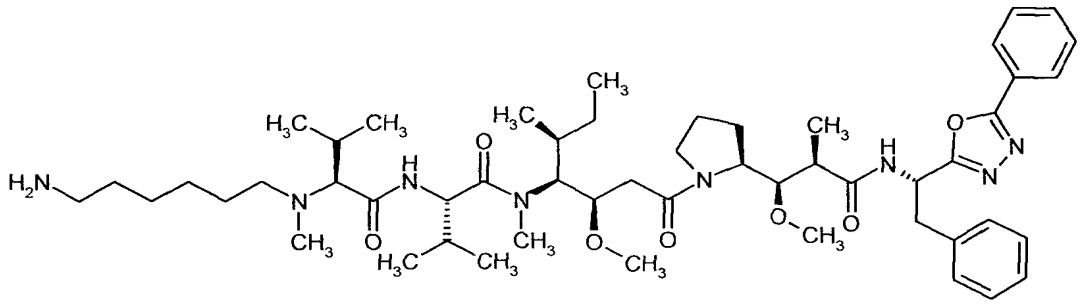


25 mg (30 µmol) of N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide (Intermediate 55) and 45 mg (180 µmol) of benzyl 6-oxohexyl carbamate were taken up in 3 ml of methanol and acidified with acetic acid. At room temperature, 15 µl (144 µmol; 9.4M) of borane-pyridine complex were subsequently added. The mixture was subsequently stirred at RT for 24 h, and acetic acid and 15 µl (144 µmol; 9.4M) of borane-pyridine complex were added again after 8 h. The reaction mixture was subsequently adjusted to pH 2 with TFA and purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 15 mg (46% of theory) of the title compound as a foam.

LC-MS (Method 1): R_t = 1.03 min; m/z = 1066 $(M+H)^+$.

Intermediate 235

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

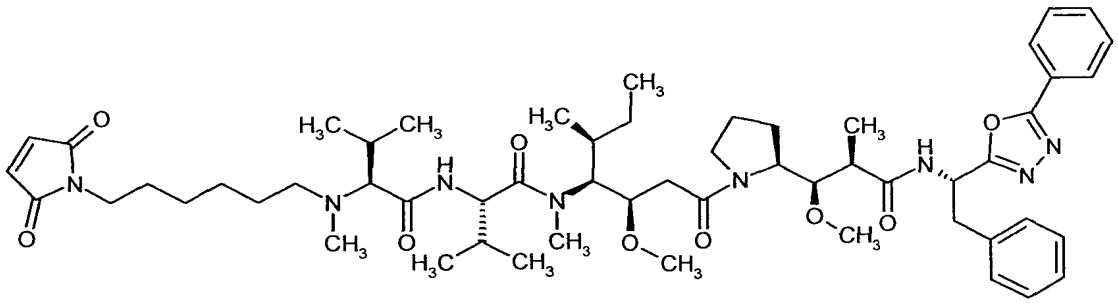


15 mg (14 µmol) of N-(6-{[(benzyloxy)carbonyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 234) were taken up in 3 ml of methanol, and 1.8 mg of palladium on charcoal (5%) were added. The reaction mixture was subsequently hydrogenated under standard hydrogen pressure at RT for 2 h. The catalyst was then filtered off and the solvent was removed under reduced pressure. The residue was lyophilized from 1:1 acetonitrile/water. 11 mg (86% of theory) of the title compound were obtained as a foam.

LC-MS (Method 1): R_t = 0.81 min; m/z = 932 $(M+H)^+$.

Intermediate 236

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

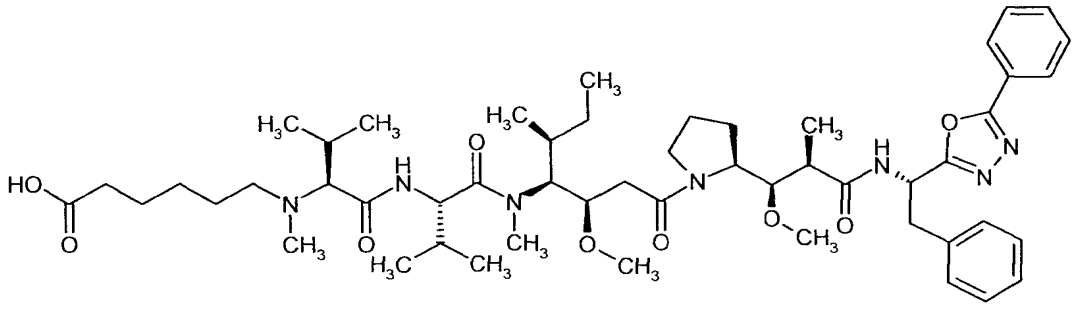


11 mg (12 µmol) of N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 235) were taken up in 500 µl of 1:1 dioxane/water and admixed with 253 µl of 1M aqueous sodium hydrogencarbonate solution and then with 2.8 mg (18 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 30 min and then acidified with trifluoroacetic acid. The reaction mixture was purified by means of preparative HPLC. After lyophilization, 0.8 mg (7% of theory) of the title compound was obtained.

LC-MS (Method 1): R_t = 1.01 min; m/z = 1012 $(M+H)^+$.

Intermediate 237

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

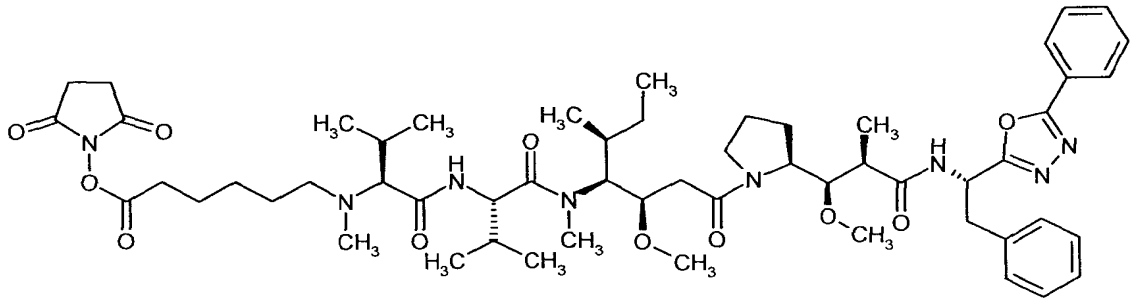


25 mg (30 µmol) of N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide (Intermediate 55) and 23 mg (180 µmol) of 6-oxohexanoic acid were taken up in 3 ml of methanol and acidified with acetic acid. At room temperature, 15 µl (144 µmol; 9.4M) of borane-pyridine complex were subsequently added. The reaction mixture was subsequently stirred at RT for 20 h, and acetic acid and 15 µl (144 µmol; 9.4M) of borane-pyridine complex were added again after 8 h. The reaction mixture was subsequently adjusted to pH 2 with trifluoroacetic acid and purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was lyophilized. 21 mg (74% of theory) of the title compound were thus obtained as a foam.

LC-MS (Method 1): R_t = 0.91 min; m/z = 947 $(M+H)^+$.

Intermediate 238

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

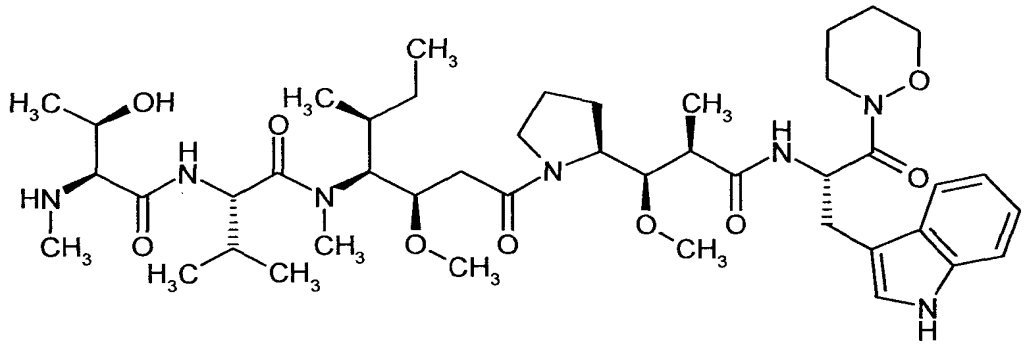


21 mg (22 µmol) of Intermediate 237 were dissolved in 1 ml of DMF and admixed with 38 mg (333 µmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 19 µl of N,N-diisopropylethylamine. After stirring at RT for 2 h, the reaction mixture was purified by means of preparative HPLC. After lyophilization from dioxane, 22 mg (96% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.95 min; m/z = 1044 $(M+H)^+$.

Intermediate 239

N-methyl-L-threonyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl] pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



First, *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine was released from 237 mg (0.887 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and concentrated. 14.7 mg (0.055 mmol) of *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine were taken up in 3 ml of DMF and admixed successively with 40 mg (0.055 mmol) of Intermediate 220, 12.7 mg (0.066 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 10 mg (0.066 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was subsequently stirred at RT for 2 h. The solvent was then removed under reduced pressure and the residue purified by preparative HPLC. 29 mg (54% of theory) of the Z-protected intermediate were thus obtained.

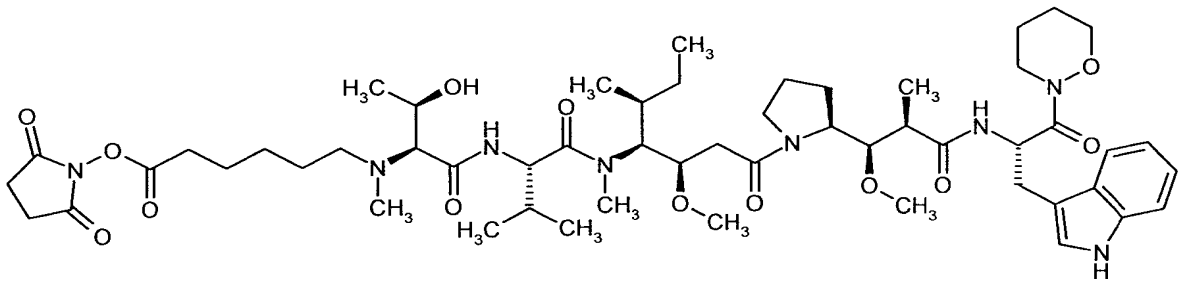
LC-MS (Method 1): R_t = 1.15 min; MS (ESIpos): m/z = 976 $(M+H)^+$.

29 mg (0.003 mmol) of this intermediate were dissolved in 5 ml of methanol and hydrogenated over 5 mg of 5% palladium/charcoal at RT and standard pressure for 1 h. The catalyst was subsequently filtered off and the solvent was evaporated off. The remaining residue was purified by preparative HPLC. 17 mg (54% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 842 $(M+H)^+$.

Intermediate 240

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-threonyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



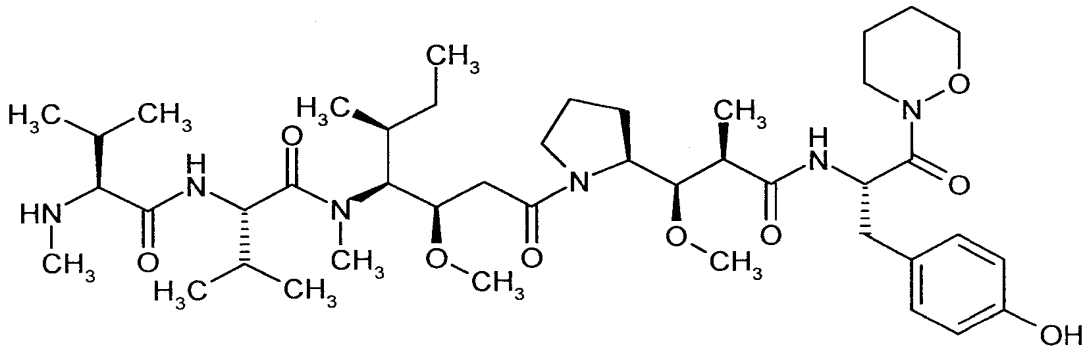
This compound was prepared in analogy to Intermediate 210 from 15.6 mg (0.016 mmol) of Intermediate 239. 10.8 mg (67% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1053 $(M+H)^+$.

Intermediate 241

N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



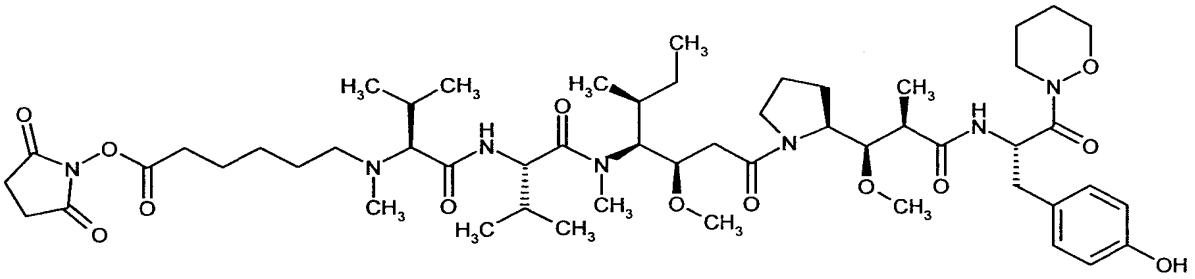
First, in analogy to Intermediate 5, trifluoroacetic acid-(2S)-2-amino-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)propan-1-one (1:1) was prepared. This reagent was then used, in analogy to the synthesis described in Intermediate 75, by coupling with *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) in the presence of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid, to prepare the title compound.

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 817 $(M+H)^+$.

Intermediate 242

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



50 mg (0.05 mmol) of Intermediate 241 were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. Subsequently, 22.5 mg (0.02 mmol) of the resulting acid were converted to the activated ester. 13.5 mg (36% of theory over 2 stages) of the title compound were obtained.

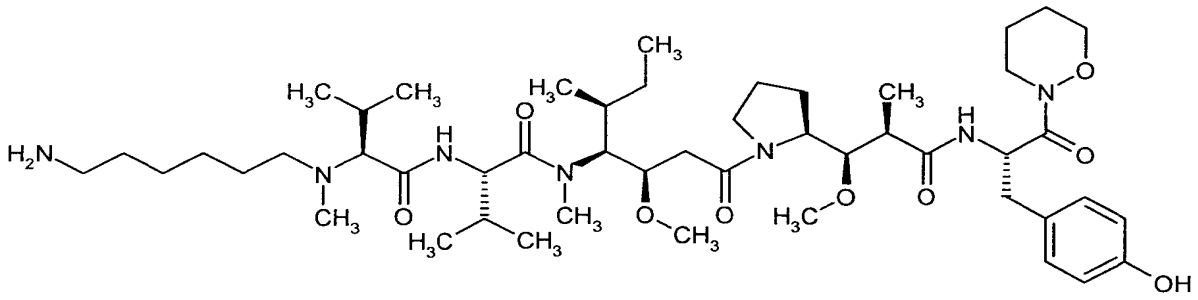
HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1028 $(M+H)^+$.

Alternatively, the title compound can also be obtained by one-hour catalytic hydrogenation of Intermediate 250 at room temperature in methanol over 10% palladium on active carbon under hydrogen standard pressure.

Intermediate 243

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was effected in analogy to Intermediate 78, by reductive alkylation of Intermediate 241 with benzyl 6-oxohexyl carbamate and borane-pyridine complex and subsequent hydrogenation in methanol as the solvent.

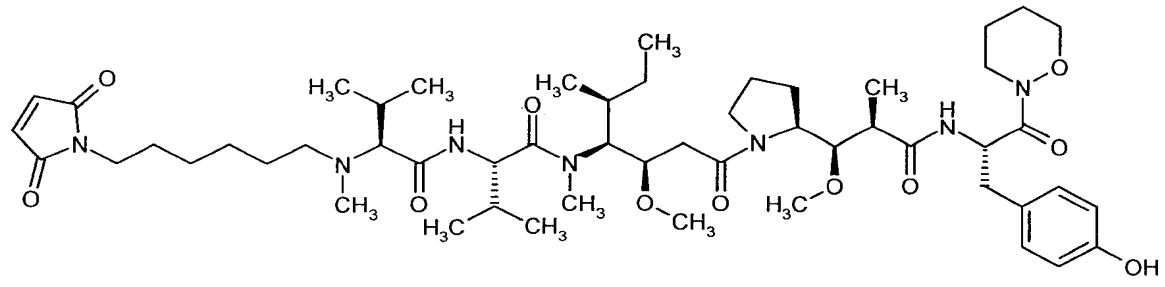
Yield: 17.5 mg (34% of theory over 2 stages)

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.63 min; MS (ESIpos): m/z = 916 $(M+H)^+$.

Intermediate 244

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was effected in analogy to Intermediate 166, proceeding from Intermediate 243.

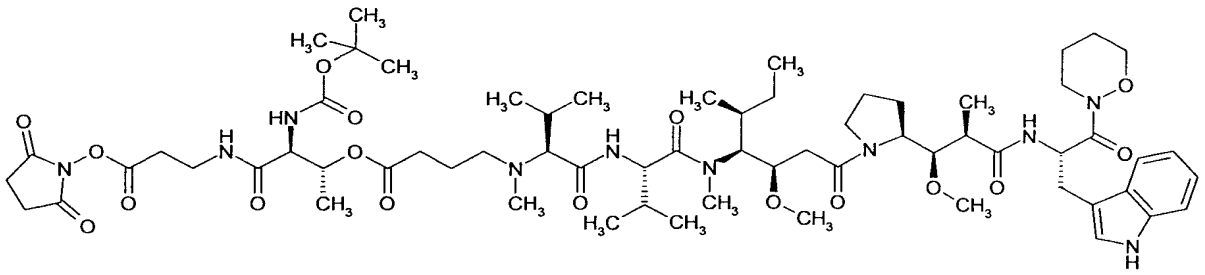
Yield: 1.3 mg (12% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 996 $(M+H)^+$.

Intermediate 245

2,5-dioxopyrrolidin-1-yl O-[(3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9,15-trioxo-2-oxa-5,8,11-triazapentadecan-15-yl]-N-(tert-butoxycarbonyl)-L-threonyl-beta-alaninate



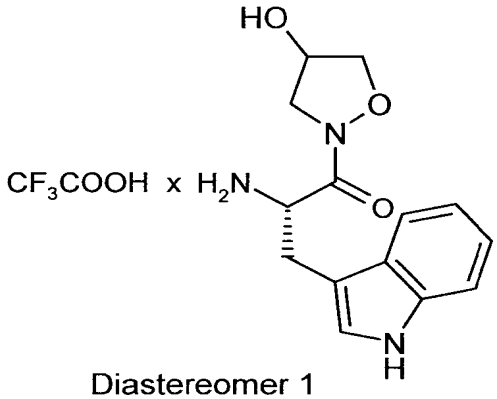
First, Intermediate 193, as described under Intermediate 154, was reacted with benzyl N-(tert-butoxycarbonyl)-L-threoninate and then the benzyl ester was removed by hydrogenolysis. 30 mg (0.027 mmol) of the N-[4-({(1S,2R)-1-[(tert-butoxycarbonyl)amino]-1-carboxypropan-2-yl}oxy)-4-oxobutyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide thus obtained were then coupled with 4-methylbenzenesulphonic acid-benzyl beta-alaninate (1:1) in the presence of HATU and the benzyl ester was removed again by hydrogenolysis (yield: 24 mg (71% of theory over 2 stages)). Finally, 10 mg (0.008 mmol) of the resulting acid were converted to the activated ester. After HPLC purification, 2.7 mg (23% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.9 min;

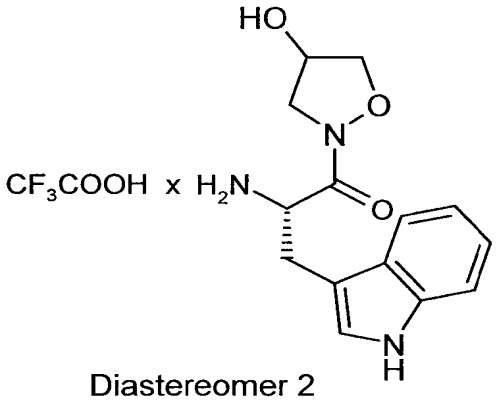
LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 1295 $(M+H)^+$

Intermediate 246a

(2S)-2-amino-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)propan-1-one trifluoroacetic acid (1:1) Diastereomer 1



Diastereomer 1



Diastereomer 2

1.6 g (3.982 mmol) of 2,5-dioxopyrrolidin-1-yl N-(tert-butoxycarbonyl)-L-tryptophanate were dissolved in 15 ml of DMF and admixed with 500 mg (3.982 mmol) of 1,2-oxazolidin-4-ol and 100 µl of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight. Then another 100 µl of *N,N*-diisopropylethylamine were added, and the mixture was first treated in an ultrasound bath for 5 h, then stirred at RT overnight, and subsequently concentrated under reduced pressure. The remaining residue was taken up in ethyl acetate and shaken first twice with 5% citric acid solution, then with saturated sodium hydrogencarbonate solution and finally with water. The organic phase was concentrated and the residue was separated into the diastereomers by flash chromatography on silica gel with 95:5 dichloromethane/methanol as the eluent. The corresponding fractions of both diastereomers were combined and the solvent was removed under reduced pressure. After the residues had been dried under high vacuum, 272 mg (18% of theory) of Diastereomer 1 (R_f = 0.18 (95:5 dichloromethane/methanol) and 236 mg (16% of theory) of Diastereomer 2 (R_f = 0.13 (95:5 dichloromethane/methanol), and also 333 mg (22% of theory) of a mixed fraction of the Boc-protected intermediates were obtained.

Under standard conditions, 5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used to detach the Boc protecting group from 272 mg (725 µmol) of Diastereomer 1 of this intermediate

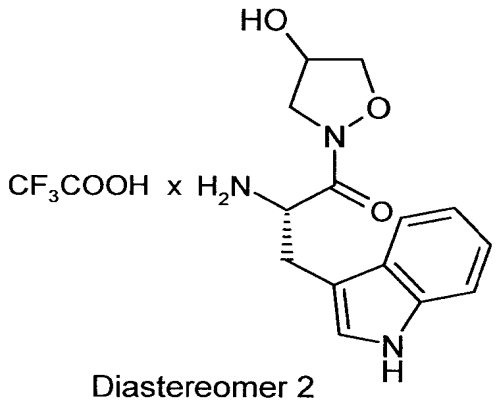
and, after lyophilization from dioxane/water, 290 mg (quant) of the title compound were obtained in 75% purity and used in the next stage without further purification.

HPLC (Method 12): R_t = 1.1 min;

LC-MS (Method 13): R_t = 1.80 min; MS (ESIpos): m/z = 276 $(M+H)^+$

Intermediate 246b

(2S)-2-amino-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)propan-1-one trifluoroacetic acid (1:1) Diastereomer 2



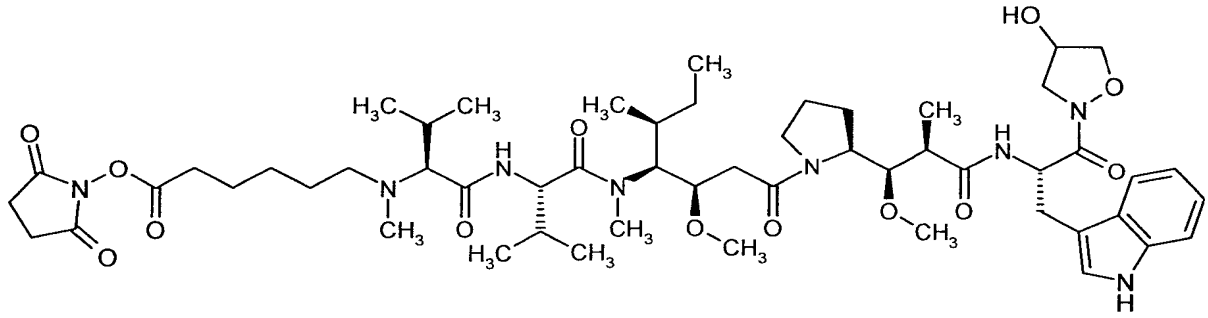
Diastereomer 2

Under standard conditions, 5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used to detach the Boc protecting group from 236 mg (630 µmol) of Diastereomer 2 of the intermediate described in 246a and, after concentration, stirring with diethyl ether and drying of the residue under high vacuum, 214 mg (76%) of the title compound were obtained.

LC-MS (Method 13): R_t = 1.84 min; MS (ESIpos): m/z = 276 $(M+H)^+$

Intermediate 247a

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



Diastereomer 1

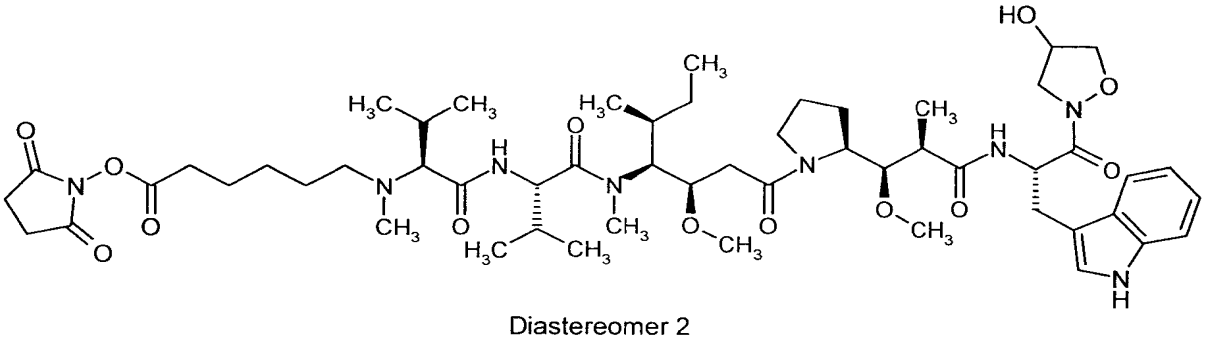
To synthesize this compound, the coupling of Intermediates 26 and 246a with subsequent detachment of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of the acid to the active ester were performed as described in Intermediate 210. The title compound was purified by preparative HPLC.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1053 $(M+H)^+$

Intermediate 247b

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



Diastereomer 2

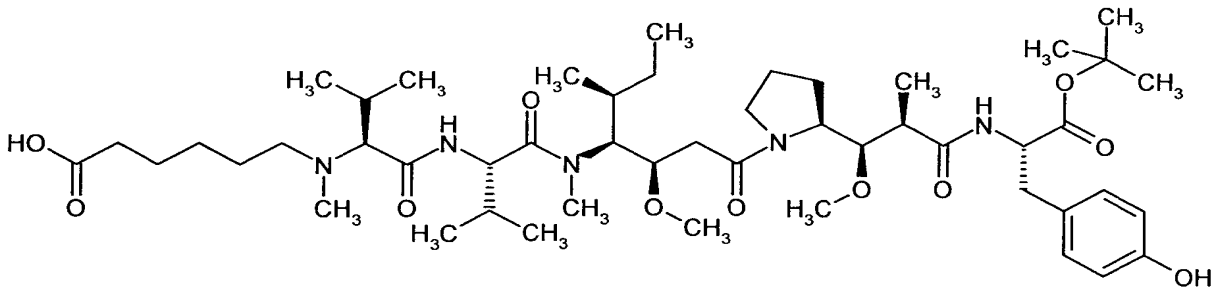
To synthesize this compound, the coupling of Intermediates 26 and 246b with subsequent detachment of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of the acid to the active ester were performed as described in Intermediate 210. The title compound was purified by preparative HPLC.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1053 $(M+H)^+$

Intermediate 248

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-tert-butoxy-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



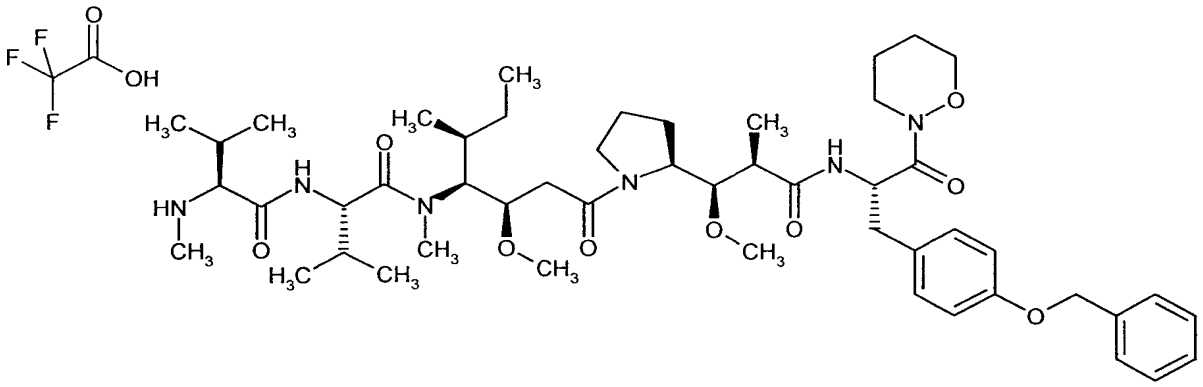
First, in analogy to the synthesis described in Intermediate 86, by coupling *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and tert-butyl L-tyrosinate in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent detachment of the Boc protecting group by means of trifluoroacetic acid to obtain the tert-butyl ester (stirring with trifluoroacetic acid in dichloromethane for 40 min), the amine compound tert-butyl N-[(2R,3R)-3-methoxy-3-{(2S)-1-[(3R,4S,5S)-3-methoxy-5-methyl-4-(methyl{(2S)-3-methyl-2-[(N-methyl-L-valyl)amino]butyl}amino)heptanoyl] pyrrolidin-2-yl}-2-methylpropanoyl]-L-tyrosinate was prepared as the trifluoroacetate. 38 mg (0.04 mmol) of this compound were then used, in analogy to the preparation of Intermediate 210, by reaction with 4-oxohexanoic acid in the presence of borane-pyridine complex, to obtain 31 mg (99% of theory) of the title compound.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 918 $(M+H)^+$.

Intermediate 249

Trifluoroacetic acid-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-[4-(benzyloxy)phenyl]-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide(1:1)



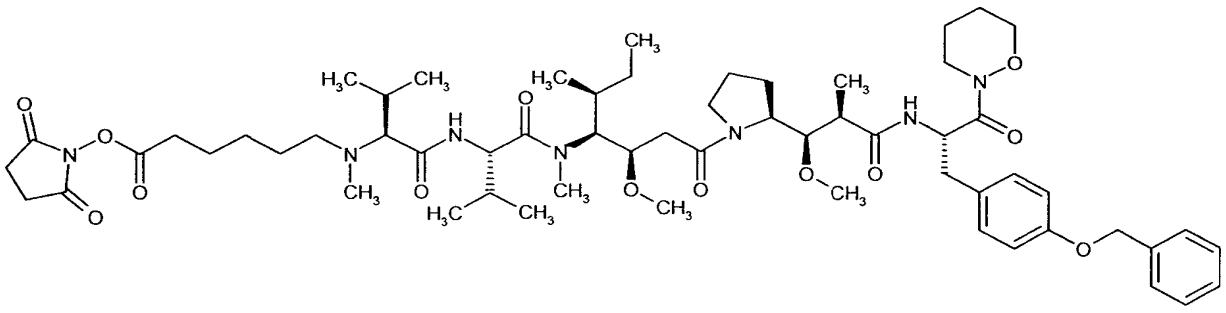
First, in analogy to Intermediates 5 and 6, starting from O-benzyl-N-(tert-butoxycarbonyl)-L-tyrosine, trifluoroacetic acid-(2S)-2-amino-3-[4-(benzyloxy)phenyl]-1-(1,2-oxazinan-2-yl)propan-1-one(1:1) was prepared. Then, from this building block, in analogy to the synthesis described in Intermediate 75, coupling with *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) in the presence of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and subsequent elimination of the Boc protecting group by means of trifluoroacetic acid gave the title compound.

HPLC (Method 12): R_t = 2.15 min;

LC-MS (Method 1): R_t = 0. 99 min; MS (ESIpos): m/z = 908 $(M+H)^+$.

Intermediate 250

N-{6-[(2,5-Dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-[4-(benzyloxy)phenyl]-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



100 mg (0.088 mmol) of Intermediate 249 were reacted in analogy to Intermediate 210 with 6-oxanoic acid in the presence of borane-pyridine complex. Then 30 mg (0.029 mmol) of the

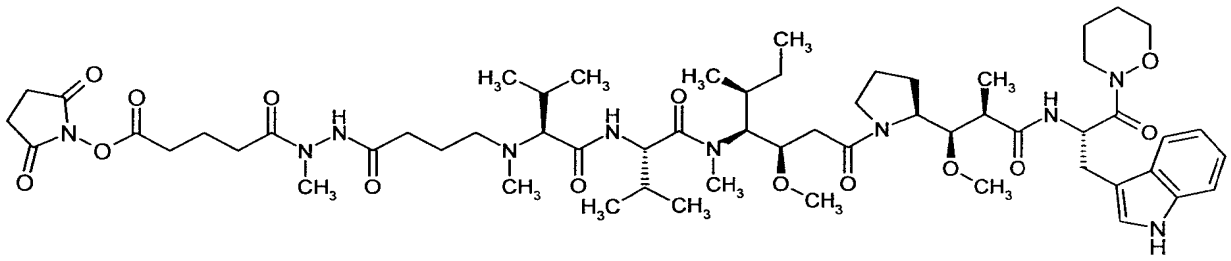
resulting acid were converted into the activated ester. This gave 15 mg (40% of theory over 2 stages) of the title compound.

HPLC (Method 12): R_t = 2.26 min;

LC-MS (Method 1): R_t = 1.05 min; MS (ESIpos): m/z = 1119 $(M+H)^+$.

Intermediate 251

N-[4-(2-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-2-methylhydrazino)-4-oxobutyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First of all, 30 mg (0.032 mmol) of Intermediate 193 were converted into the activated N-hydroxysuccinimide ester. 10.3 mg (0.009 mmol) of this active ester were dissolved in 2 ml of DMF, admixed with 2.7 mg (0.018 mmol) of tert-butyl 1-methylhydrazinecarboxylate and 8 µL *N,N*-diisopropylethylamine, and stirred at RT for 16 h. This operation was repeated, and then the batch was concentrated and the residue which remained was purified by preparative HPLC. Concentration of the corresponding fractions and drying under a high vacuum gave 5.4 mg (43%) of the intermediate.

LC-MS (Method 1): R_t = 0. 99 min; MS (ESIpos): m/z = 1054 $(M+H)^+$.

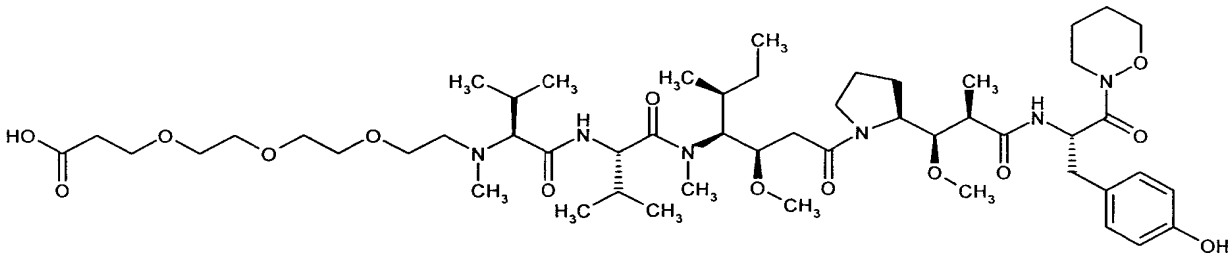
The Boc protecting group was removed from 3.5 mg (0.002 mmol) of this intermediate, using trifluoroacetic acid in dichloromethane. Following concentration and drying under a high vacuum, the residue was taken up in 4 ml of dichloromethane and admixed with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione and 2 µl of *N,N*-diisopropylethylamine. After stirring at RT for 1 h, the batch was concentrated and the residue which remained was purified by preparative HPLC. Concentration of the corresponding fractions and drying under a high vacuum gave 1.4 mg (44%) of the title compound.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1166 $(M+H)^+$.

Intermediate 252

N-(2-{2-[2-(2-Carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



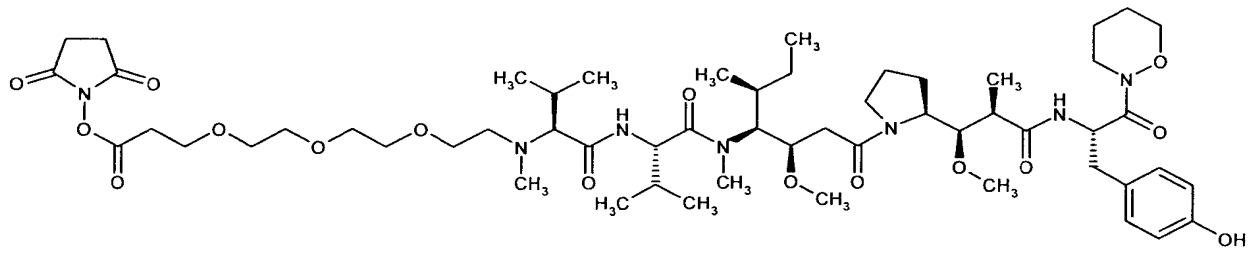
100 mg (0.088 mmol) of Intermediate 249 and 109 mg (0.350 mmol) of Intermediate 167 were combined in 10 ml of methanol and admixed with 39 mg (0.42 mmol) of borane-pyridine complex and 15 µl of acetic acid. The batch was stirred at RT overnight. Then the same amounts of borane-pyridine complex and of acetic acid were added once again, and the batch was stirred for a further 24 h at RT. It was then concentrated under reduced pressure, and the residue was purified by means of preparative HPLC. Concentration of the corresponding fractions and lyophilization from dioxane/water 1:1 gave 98 mg (93% of theory) of the bis-benzyl intermediate. This intermediate was taken up in 18.5 ml of methanol and subjected to catalytic hydrogenation over 5% palladium on activated carbon under standard hydrogen pressure at room temperature for 1 h. Filtration, concentration, and lyophilization of the residue from dioxane gave 73 mg (87% of theory) of the title compound.

HPLC (Method 12): R_t = 1.85 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 1021 $(M+H)^+$.

Intermediate 253

N-{2-[2-(2-{3-[(2,5-Dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



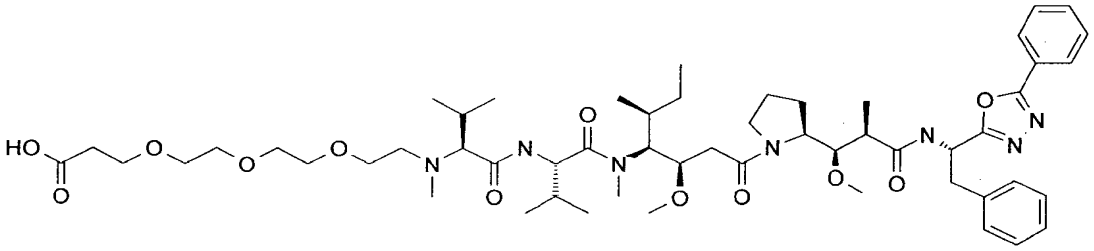
22 mg (22 µmol) of Intermediate 252 were dissolved in 8.5 ml of DMF and admixed with 25 mg (215 µmol) of 1-hydroxypyrrolidine-2,5-dione and subsequently with 12.3 mg (32 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 37 µl of N,N-diisopropylethylamine. After stirring at RT for 2 h, the reaction mixture was purified by means of preparative HPLC. Lyophilization from dioxane gave 16 mg (62% of theory) of the title compound.

HPLC (Method 5): R_t = 1.57 min;

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 1118 $(M+H)^+$.

Intermediate 254

N-(2-{2-[2-(2-Carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



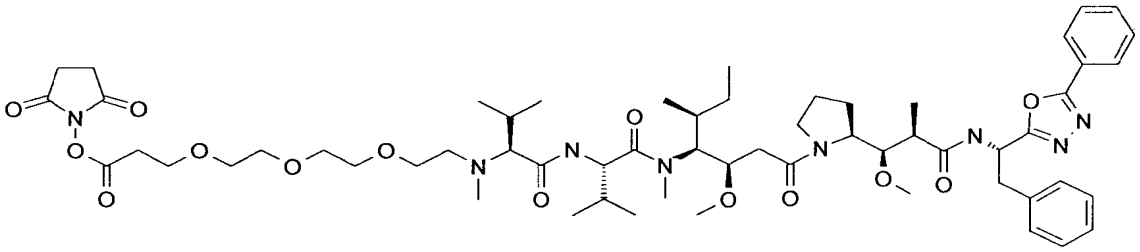
10 mg (0.012 mmol) of Intermediate 55 and 11 mg (0.036 mmol) of Intermediate 167 were combined in 1 ml of methanol and admixed with 5.4 mg (0.058 mmol) of borane-pyridine complex and 1 µl of acetic acid. The batch was stirred at RT overnight. Then the same amounts of borane-pyridine complex and of acetic acid were added once again, and the batch was stirred at RT for a further 20 h. It was then concentrated under reduced pressure and the residue was purified by means of preparative HPLC. Concentration of the corresponding fractions and lyophilization from dioxane/water 1:1 gave 8 mg (58% of theory) of bis-benzyl intermediate. This intermediate was taken up in 2 ml of methanol and subjected to catalytic hydrogenation over 5% palladium on activated carbon under standard hydrogen pressure at room temperature for 1 h. Filtration,

concentration, and lyophilization of the residue from dioxane gave 7 mg (95% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 1036 $(M+H)^+$.

Intermediate 255

N-{2-[2-(2-{3-[(2,5-Dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

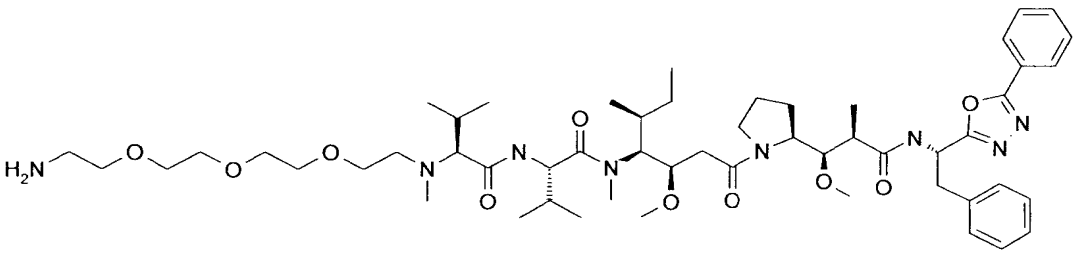


7.3 mg (7 µmol) of Intermediate 254 were dissolved in 0.3 ml of DMF and admixed with 12 mg (106 µmol) of 1-hydroxypyrrolidine-2,5-dione and subsequently with 13.5 mg (35 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 6 µl of N,N-diisopropylethylamine. After stirring at RT for 2 h, the reaction mixture was purified by means of preparative HPLC. Lyophilization from dioxane gave 7.7 mg (79% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1134 $(M+H)^+$.

Intermediate 256

N-(2-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

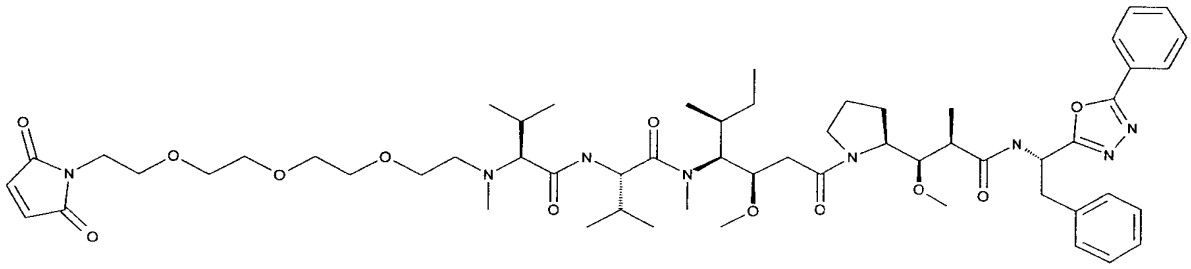


20 mg (0.02 mmol) of Intermediate 55 were subjected to reductive amination in analogy to the preparation of Intermediate 254, with benzyl (2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl)-carbamate (Intermediate 172) in the presence of borane-pyridine complex. The Z protecting group was subsequently removed by hydrogenolysis, using 5% palladium on carbon as catalyst and in methanol as solvent, and 21 mg (85% of theory over 2 stages) of the title compound were prepared.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1008 $(M+H)^+$.

Intermediate 257

N-[2-(2-{2-[2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

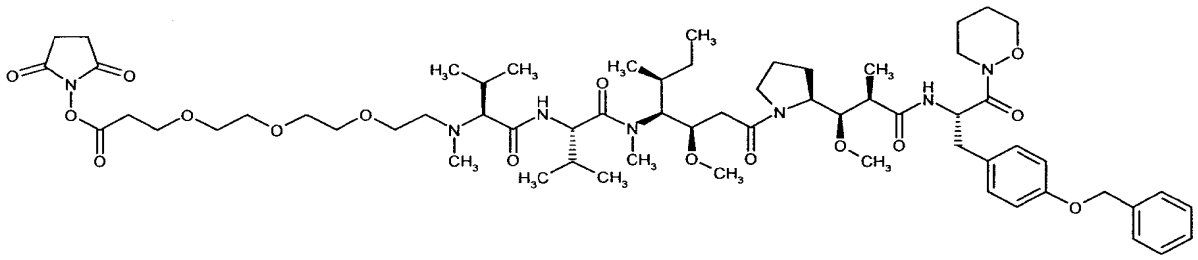


21 mg (20.8 µmol) of Intermediate 256 were taken up in 1 ml of dioxane/water 1:1 and then admixed with 4.9 mg (31.2 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and also wtih 42 µl of 1M aqueous sodium hydrogen carbonate solution. The reaction mixture was stirred at RT for 30 min. Then a further 374 µl of the 1M aqueous sodium hydrogen carbonate solution were added, and the reaction mixture was stirred at RT for a further 30 min and then concentrated under reduced pressure. The residue which remained was purified by means of preparative HPLC. Lyophilization gave 4.5 mg (20% of theory) of the title compound, as a colourless foam.

LC-MS (Method 1): R_t = 1.04 min; MS (ESIpos): m/z = 1088 $(M+H)^+$.

Intermediate 258

N-{2-[2-(2-{3-[(2,5-Dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-[4-(benzyloxy)phenyl]-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



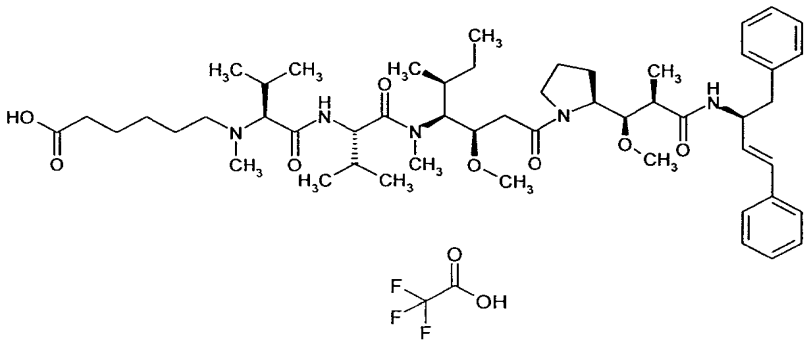
This compound was prepared starting from Intermediate 249 by reductive alkylation using tert-butyl 3-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}propanoate, followed by t-butyl ester cleavage and conversion into the N-hydroxysuccinimide ester.

HPLC (Method 5): R_t = 1.96 min;

LC-MS (Method 1): R_t = 1.11 min; MS (ESIpos): m/z = 1208 $(M+H)^+$.

Intermediate 259

N-(5-Carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S,3E)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

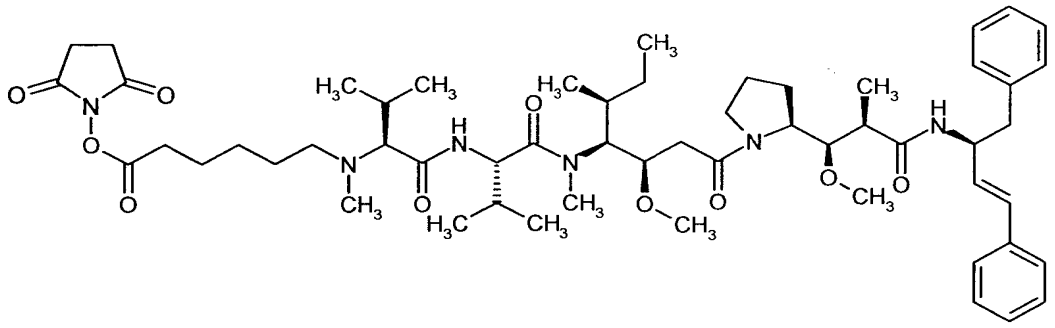


9.6 mg (8.4 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 58) and 6.5 mg (51 µmol) of 6-oxohexanoic acid were taken up in 769 µl of methanol and acidified with acetic acid. Then, at room temperature, 4 µl (40 µmol) of borane-pyridine complex were added. The reaction mixture was subsequently stirred at RT for 20 h. The reaction mixture was then adjusted to a pH of 2 with trifluoroacetic acid, and purified by means of preparative HPLC. The production fractions were combined and concentrated and the residue was lyophilized. This gave 8 mg (93% of theory) of the title compound, as a foam.

LC-MS (Method 1): R_t = 1.06 min; m/z = 1019 $(M+H)^+$.

Intermediate 260

N-{6-[(2,5-Dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S,3E)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

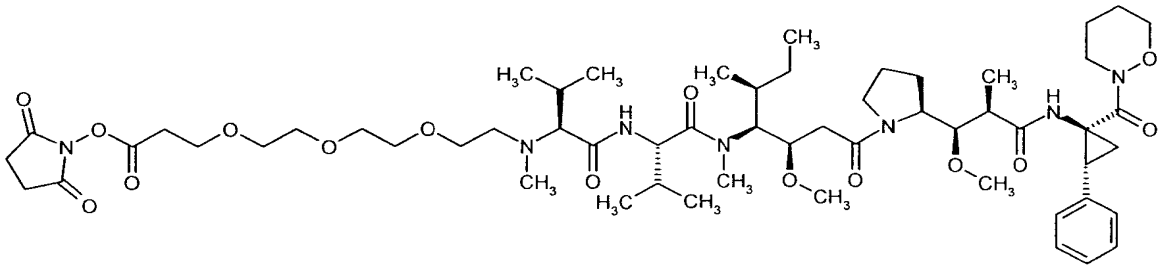


7.5 mg (7.4 µmol) of Intermediate 259 were dissolved in 332 µl of DMF and admixed with 12.7 mg (110 µmol) of 1-hydroxypyrrolidine-2,5-dione and subsequently with 14 mg (37 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 6 µl of N,N-diisopropylethylamine. After stirring at RT for 2 h, the reaction mixture was purified by means of preparative HPLC. Lyophilization from dioxane gave 4 mg (55% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.19 min; m/z = 1002 $(M+H)^+$.

Intermediate 261

N-{2-[2-(2-{3-[(2,5-Dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared starting from Intermediate 16 by reductive alkylation with benzyl 3-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}propanoate, subsequent benzyl ester cleavage by hydrogenolysis, and conversion into the N-hydroxysuccinimide ester.

HPLC (Method 5): R_t = 1.83 min;

LC-MS (Method 1): R_t = 0.93 min; MS (ESIpos): m/z = 1114 $(M+H)^+$.

B: Preparation of antibody-drug conjugates (ADCs)

B-1. Generation of anti-FGFR2 antibodies

The human anti-FGFR2 antibodies M048-D01-hIgG1 and M048-D01-hIgG1-b of the present invention were isolated by phage display technology, using the naïve Fab antibody library n-CoDeR from Bioinvent International AB (Lund, Sweden; described in Söderling et al., Nat. Biotech. 2000, 18:853-856). One screening hit was the parental Fab fragment M048-D01. The variable region of the heavy chain Vh of this Fab fragment is given by SEQ ID NO: 21; the variable region of the light chain Vl is given by SEQ ID NO: 22. Following identification of the M048-D01 Fab fragment, it was expressed under the name M048-D01-hIgG1-b (SEQ ID NO: 10 for the heavy chain, and SEQ ID NO: 9 for the light chain) in the form of human IgG. For efficient cloning, the first three amino acids of the N-terminus of the heavy chain of M048-D01-hIgG1-b [EVQ] were also expressed, alternatively, as QVE. This variation gave the antibody M048-D01-hIgG1 (SEQ ID NO: 8 for the heavy chain and SEQ ID NO: 7 for the light chain). The variable regions Vh and Vl of M048-D01-hIgG1 are given by SEQ ID NO: 11 and SEQ ID NO: 12; the variable regions Vh and Vl of M048-D01-hIgG1-b are given by SEQ ID NO: 13 and SEQ ID NO: 14. Both antibodies have the same CDR sequences, which are given by SEQ ID NOs: 15 (H-CDR1), 16 (H-CDR2), 17 (H-CDR3), 18 (L-CDR1), 19 (L-CDR2) and 20 (L-CDR3).

The antibodies GAL-FR21-mIgG1 and GAL-FR22-mIgG2a are described in WO2010/054265 as mIgG1 (GAL-FR21) and mIgG2b (GAL-FR22). GAL-FR21 is defined therein via Vh (SEQ ID NO: 4 in WO2010/054265) and via Vl (SEQ ID NO: 1 in WO2010/054265), and GAL-22 is defined via Vh (SEQ ID NO: 8 in WO2010/054265) and via Vl (SEQ ID NO: 7 in WO2010/054265). For the studies described in the present application, Vl and Vh of GAL-FR21 were reformatted into the murine IgG1 format, resulting in the antibody GAL-FR21-mIgG1 (SEQ ID NO: 3 and SEQ ID NO: 4 of the present application). Vl and Vh of GAL-FR22 were reformatted into the murine IgG2a format, resulting in the antibody GAL-FR22-mIgG2a (SEQ ID NO: 5 and SEQ ID NO: 6 of the present application).

B-2. Expression of anti-FGFR2 antibodies

The antibodies M048-D01-hIgG1, M048-D01-hIgG1-b, GAL-FR21-mIgG1 and GAL-FR22-mIgG2a were produced transiently in mammalian cell culture.

The expression constructs were produced as described below:

In order to transfer the Vh and Vl regions of the M048-D01 Fab originating from the phage display into the IgG format, and in order to change the expression system from *E. coli* to mammalian cells, the Vh and Vl sequences from the phage display *E. coli* clone were amplified using PCR primers. The flanking restriction enzyme cleavage sites were introduced at the 5' and 3' ends both of Vh and of Vl. These restriction enzyme cleavage sites were used for the cloning of Vh and Vl into an expression vector which contained the IgG backbone.

The *E. coli* cells were incubated in a test tube with 100 µl of water at 95°C for 10 minutes and then placed on ice for 5 minutes. Following brief vortexing, the solution was clarified by centrifugation. The supernatant separated off was used for DNA amplification. The PCR reactions were carried out separately for Vh and Vl. This was done using specific primer pairs with BamHI and NotI cleavage sites for Vl and MfeI, and MfeI and BlpI cleavage sites for Vh. The PCR reactions were carried out using the AccuPrime Pfx polymerase (Invitrogen, # 12344-024) according to manufacturer instructions. The PCR products were analysed on 1% agarose gels. To obtain compatible ends, the expression vectors and PCR products were digested according to manufacturer instructions with the corresponding restriction endonucleases at 37°C for 2 hours (see Table 1). Digestion was halted by incubation at 70°C for 15 minutes. The resulting fragments were ligated into the expression vector, and *E. coli* or mammalian cells were transformed with the constructs by standard methods.

The DNA sequences of the Vh and Vl domains or of complete light chains of certain antibodies (GAL-FR21-mIgG1, GAL-FR22-mIgG2a and M048-D01-hIgG1-b) were synthesized by Geneart gene synthesis and gene optimizer technology for mammalian gene expression (life technologies, Grand Island, NY, USA). During the gene synthesis, the V domains on sequences coding for a mammalian signal peptide were fused with upstream Kozaki sequence. Flanking restriction cleavage sites were inserted on 5' and 3' ends of the synthesized DNA constructs. These restriction cleavage sites were used for the cloning of the Vh and Vl or the light chains into an expression vector which contained the coding constant regions of the IgG.

The expression vectors and the Geneart constructs were digested according to manufacturer instructions with the respective restriction endonucleases at 37°C for 2 hours, to give compatible ends (see Table 1). Digestion was halted by incubation at 70°C for 15 minutes. The resulting

fragments were ligated, and *E. coli* cells were transformed with the ligates. Plasmids obtained from these transformants were used to transform mammalian cells according to standard methods.

Table1: Restriction cleavage sites for the cloning of Vh and Vl or of the light chain into IgG expression vectors

Antibody	Restriction cleavage site for the cloning of:	
	VH	VL or light chain*
GAL-FR21-mIgG1	HindIII – BlpI	XbaI – BsiWI
GAL-FR22-mIgG2a	HindIII – BlpI	XbaI – BsiWI
M048-D01-hIgG1#	MfeI-BlpI	BamHI – NotI*
M048-D01-hIgG1-b	HindIII-BlpI	XbaI – NotI*

* Cloning of the complete light chain

Cloning of the PCR product into EcoRI-BlpI and BamHI-NotI-digested expression vectors.

The antibodies were expressed transiently in mammalian cell culture, as described in Tom et al. (Tom et al., Chapter 12 in Methods Express: Expression Systems, edited by Michael R. Dyson and Yves Durocher, Scion Publishing Ltd, 2007): for the expression of the anti-FGFR2 antibodies, for example M048-D01-hIgG1, GAL-FR21-mIgG1 and GAL-FR22-mIgG2a, HEK293 6E cells were transfected transiently with a suitable CMV promoter-based expression plasmid. The cell culture scale was either up to 1.5 l in a shake flask or 10 l in a "wave-bag". Expression took place at 37°C for 5-6 days in F17 medium (Invitrogen) supplemented with tryptone TN1 (Organotechnie) with 1% "FCS ultra low IgG" (Invitrogen) and 0.5 mM valproic acid.

Alternatively, the anti-FGFR2 antibodies, for example M048-D01-hIgG1-b, were expressed in a stably transfected Chinese hamster ovary (CHO) cell line. This was done using a one-vector system. Fermentation took place in bioreactors on a different scale in a fed-batch process.

The parental Fab fragment M048-D01 (Vh: SEQ ID NO: 21, Vl: SEQ ID NO: 22) belonging to the M048-D01-based antibodies from the phase display was expressed as follows: 20-50 ml of LB medium (admixed with 0.1 mg/ml ampicillin and 0.1% glucose) were inoculated with a preliminary culture of a corresponding *E. coli* clone, which contained the initial pBif vector, which was lacking the geneIII sequence, but which had had the M048-D01 Fab sequence cloned into it. Production of the sFab was started by addition of 0.5 mM IPTG (final concentration). Incubation took place at 30°C overnight at 250 rpm.

B-3. Purification of the FGFR-2 antibodies

The antibodies, for example M048-D01-hIgG1, M048-D01-hIgG1-b, GAL-FR21-mIgG1 and GAL-FR22-mIgG2a, were obtained from the cell culture supernatants. The cell supernatants were clarified by centrifugation from cells. The cell supernatant was subsequently purified by affinity chromatography on a MabSelect Sure (GE Healthcare) chromatography column. For this purpose, the column was equilibrated in DPBS pH 7.4 (Sigma/Aldrich), the cell supernatant was applied and the column was washed with around 10 column volumes of DPBS pH 7.4 + 500 mM NaCl. The antibodies were eluted in 50 mM Na acetate pH 3.5 + 500 mM NaCl, and subsequently purified further by gel filtration chromatography on a Superdex 200 column (GE Heathlcare) in DPBS pH 7.4.

The parental M048-D01 Fab fragment expressed in *E. coli* was purified as follows: the *E. coli* cells were harvested by centrifugation and lysed by incubation at 4°C for 1 hour in lysis buffer (20% sucrose (w/v), 30 mM TRIS, 1 mM EDTA, pH 8.0, 1 mg/ml lysozyme (Sigma L-6876) and 2.5 U/ml Benzonase (Sigma E1014)). Thereafter the same volume of PBS was added. After that, the clarified supernatant was applied to Dynabeads for his-tag isolation (Invitrogen, 101-03D) and the mixture was swirled at 4°C for 2 hours. Thereafter the matrix was washed three times with buffer 1 (50 mM Na phosphate buffer, pH 7.4, 300 mM NaCl, 5 mM imidazole, 0.01% Tween 20). Subsequently a single washing step in buffer 2 was carried out (PBS admixed with 0.005% of Tween 20). Lastly, the Fabs were eluted with buffer E (10 mM Na phosphate buffer, pH 7.4, 300 mM NaCl, 300 mM imidazole) and concentrated using PBS buffer in Vivaspin 500 concentrators (cut-off 10 000, from GE, 28-9322-25).

B-4. Construction of the cross-reactivity profile of the M048-D01-based antibodies

The cross-reactivity of the human antibodies M048-D01-hIgG1 and M048-D01-hIgG1-b of the present application was determined using the parental Fab fragment M048-D01 (comprising Vh: SEQ ID NO: 21 and Vl: SEQ ID NO: 22).

The M048-D01 Fab fragment was tested in an ELISA for binding to the various FGF receptor variants listed in Table 2.

Table 2: List of recombinant proteins used for establishing the cross-reactivity profile of the FGFR2 binders

Protein	Origin	Cat. No. (RnD Systems)
hFGFR2β-Fc (IIIb)	Human	665-FR
mFGFR2β-Fc (IIIb)	Mouse	708-MF
hFGFR2α-Fc (IIIb)	Human	663-FR
hFGFR2β-Fc (IIIc)	Human	684-FR
hFGFR1β-Fc (IIIc)	Human	661-FR
hFGFR1β-Fc (IIIb)	Human	765-FR
hFGFR3-Fc (IIIc)	Human	766-FR
hFGFR3-Fc (IIIb)	Human	1264-FR
hFGFR4-Fc	Human	685-MF
mFGFR2β-Fc (IIIc)	Mouse	716-MF
mFGFR3-Fc (IIIc)	Mouse	710-MF
hTRAIL-Fc	Human	630-TR

All of the variants took the form of Fc fusion proteins in carrier-free preparations. The proteins were biotinylated according to manufacturer instructions, using a 2-fold molar excess of biotin-LC-NHS (Pierce; Cat. No. 21347) and desalted using Zeba desalting columns (Pierce; Cat. No. 89889).

For the ELISA, 96-well plates pretreated with streptavidin (Pierce, 15500) were loaded overnight at 4°C with 1 µg/ml biotinylated protein. Wells which had been loaded with biotinylated TRAIL-Fc served as a reference. The next day the plates were washed 3× with PBST (1 × PBS admixed with 0.05% Tween 20 (Sigma, P7949)), treated with blocking buffer (PBST admixed with 3% BSA (Sigma A4503)), and again washed three times with PBST. 100 µl of the purified Fab (1 µg/ml) were added, and incubation was carried out at room temperature for 1 hour. After threefold washing with PBST, an HRP-coupled anti-hIgG (Fab-specific) (diluted 1:2500, Sigma, A0293) was added, and incubation took place at room temperature for 1 hour. The colour reaction was activated by addition of 50 µl of TMB (Invitrogen, 2023) and halted after 5-15 minutes by addition of 50 µl of H_2SO_4 (Merck, 1120801000). The colour reaction was monitored at 450 nm in a plate reader (Tecan). The signal strengths of the wells containing TRAIL-Fc were used as background values, and the signal-to-background ratios were calculated as summarized in Table 3.

Table 3: Summary of the ELISA data on the cross-reactivity of M048-D01

		hFGFR2β-Fc (IIIb)	hFGFR2β-Fc (IIIc)	hFGFR2α-Fc (IIIb)	mFGFR2β-Fc (IIIb)	mFGFR2β-Fc (IIIc)	hFGFR1β-Fc (IIIb)	hFGFR1β-Fc (IIIc)	hFGFR3-Fc (IIIb)	hFGFR3-Fc (IIIc)	mFGFR3-Fc (IIIc)	hFGFR4-Fc
M048-D01		+++	+++	+++	+++	+++	0	0	0	0	0	0

Signal-to-background ratios: 0: <2; +: 2-3; ++: 3-5; +++: >5

As is evident from Table 2, the M048-D01-based antibodies M048-D01-hIgG1 and M048-D01-hIgG1-b bind to human and murine FGFR2, and do so independently of whether the forms in question are alpha or beta isoforms, or are IIIb and IIIc splice forms. As is likewise apparent from the table, the M048-D01-based antibodies of the invention do not bind to FGFR1, FGFR3 and FGFR4.

B-5. Epitope mapping by means of CLIPS technology

In order to test the binding characteristics of the M048-D01-based antibodies, an intensive epitope mapping operation was carried out on the basis of Pepscan's proprietary "Chemically Linked peptides on Scaffolds" (CLIPS) Technologie (Timmerman et al., J. Mol. Recognit. 2007, 20:283-99). In all, 8653 different CLIPS peptides were designed, from 15 amino acids to 30 amino acids in length, which cover linear, conformational and discontinuous epitopes on the human FGFR2. The peptides were synthesized on peptide arrays. The human antibody M048-D01-hIgG1 was tested on the peptide arrays in an ELISA-based procedure. The peptides which gave the highest ELISA values were analysed to isolate common, similar amino acid sequences.

In order to reconstruct discontinuous epitopes of the target molecule, a library of structured peptides was synthesized. The CLIPS technology allows peptides to be structured in individual loops, double loops, triple loops, sheet-like loops, helix-like loops, and combinations of these structural elements: for this purpose, CLIPS templates are coupled to cysteine residues of the peptide arrays. For example, a 0.5 mM solution of the T2 CLIPS template 1,3-bis(bromomethyl)benzene was dissolved in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1 (v/v)). This solution was added to the peptide arrays. The CLIPS template bound to the side chains of 2 cysteines of the peptides present in the peptide arrays (455 well plate with 3 µl wells). The peptide arrays were cautiously swirled in the solution for 30 to 60 minutes, with complete coverage by the solution. At the conclusion, the arrays were washed thoroughly with water in

excess and were treated in disrupt buffer (1% SDS, 0.1% beta-mercaptoethanol in PBS (pH 7.2)) at 70°C for 30 minutes in an ultrasound bath. The treatment in the ultrasound bath was subsequently repeated in water for a further 45 minutes. T3 CLIPS-bearing peptides were prepared in a similar way.

The binding of the antibodies to each peptide was tested in a PEPSCAN-based ELISA (Sloostra et al., Molecular Diversity 1996, 1: 87-96). The peptide arrays were preincubated (1 h, 20°C) with 5% to 100% of binding buffer. The binding buffer consisted of 1% Tween 80, 4% horse serum and 5% ovalbumin (w/v) in solution in PBS. After a washing step, the peptide arrays were incubated at 4°C overnight with primary antibody solution (1 to 5 µg/ml) in 1% Tween 80 in PBS. After a further washing step, the peptide arrays were incubated at 25°C for an hour in a 1/1000 dilution of an antibody peroxidase conjugate (anti-human-IgG) in 100% binder buffer. After a further washing step, the peroxidase substrate 2,2'-azino-di-3-ethylbenzothiazolinesulphonate (ABTS) and 2 µl/ml 3% strength H_2O_2 were added. After an hour, the development of colour was measured and was quantified using a CCD camera and an image processing system.

The crude data obtained in this method are optical values which range from 0-3000 mAU (milli-absorption-units).

The result is that all the M048-D01-based antibodies bind on an epitope which consists of the 15 N-terminal residues of FGFR2 (^{1}RPSFSLVEDTTLEPE^{15}). Analysis of 1257 CLIPS and linear peptides gave consistently high ELISA values for N-terminal peptides.

The N-terminal residues (^{1}RPSFSLVEDTTLEPE^{15}) are present in all splice variants of human FGFR2, independently of alternative splicing in domain D3, which results in the IIIb and IIIc isoforms. The epitope is also present if the domain D1 is removed from the full-length FGFR2 (FGFR2 alpha, SEQ ID NO: 1) by splicing, resulting in the shorter beta-form of FGFR2 (SEQ ID NO: 2). In this case the epitope is situated directly before the domain D2.

A matter of particular interest is the fact that the N-terminal sequence is conserved in human, mouse, rat and rhesus monkey. This allows the broad inter-species cross-reactivity of the M048-D01 antibodies. The binding epitope of M048-D01-hIgG1 and M048-D01-hIgG1-b, two example antibodies of the present invention, is marked as the striped box in Figure 1.

B-6. General process for coupling to cysteine side chains

The antibodies used in the coupling reactions were as follows:

M048-D01-hIgG1

M048-D01-hIgG1-b

Added to a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/ml and 10 mg/ml were 3 equivalents of tris(2-carboxyethyl)phosphine hydro-

chloride (TCEP), in solution in PBS buffer, and the mixture was stirred at RT for 1 hour. Subsequently, depending on the desired loading, between 2 and 10 equivalents of the maleimide precursor compound or halide precursor compound for coupling from the Intermediates 102, 103, 105-109, 111-114, 117-126, 128, 129, 132-146, 148-155, 157, 159-161, 166, 171, 175-177, 184, 189, 194-195, 199-201, 205, 209, 223-224, 226, 228-231, 236, 244 and 257 were added as a solution in DMSO. The amount of DMSO here ought not to exceed 10% of the overall volume. The batch was stirred at RT for 60-120 minutes and then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) equilibrated with PBS, and eluted with PBS buffer. Optionally a concentration procedure was carried out additionally by means of ultracentrifugation.

Normally, unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer were used for the reduction and the subsequent coupling. Following purification via the PD10 column, this gave, in each case, solutions of the corresponding ADC in 3.5 ml of PBS buffer. The particular protein concentration indicated was then determined for these solutions. Furthermore, the loading of the antibody (drug/mAb ratio) was determined in accordance with the methods described below.

This process was used to prepare the immunoconjugates represented in Examples 1, 3, 5-6, 8, 10-12, 14, 15, 27 and 32.

In the structural formulae illustrated, the definition of AK_{1A} and AK_{1B} is as follows

AK_{1A} = anti-FGFR2 antibody M048-D01-hIgG1 (partially reduced)- $S^{\S 1}$

AK_{1B} = anti-FGFR2 antibody M048-D01-hIgG1-b (partially reduced)- $S^{\S 1}$

where

$\S^1$ denotes the link with the succinimide group,

and

S stands for the sulphur atom of a cysteine residue of the partially reduced antibody.

B-7. General process for coupling to lysine side chains:

The following antibodies were used in the coupling reactions:

M048-D01-hIgG1

M048-D01-hIgG1-b

GAL-FR21-mIgG1

GAL-FR22-mIgG2a

Added to a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/ml and 10 mg/ml were, depending on the desired loading, between 2 and 5 equivalents of the precursor compound for coupling from the Intermediates 104, 110, 115, 116, 127, 130, 131, 147, 156, 158, 162, 169, 178, 185, 190, 202, 206, 210-216, 218, 219, 227, 233, 238,

240, 242, 245, 247a, 247b, 250, 251, 253, 255, 258 and 260-261 as a solution in DMSO. After 30 minutes of stirring at RT, the same amount of precursor compound in DMSO was added again. The amount of DMSO here ought not to exceed 10% of the overall volume. After a further 30 minutes of stirring at RT, the batch was applied to PD 10 columns (Sephadex® G-25, and eluted with PBS buffer. Optionally a concentration procedure was carried out additionally by means of ultrafiltration. If necessary, for more effective removal of low molecular mass constituents, the concentration by ultrafiltration was repeated after re-dilution with PBS buffer.

Normally, unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer were used for the coupling. Following purification via the PD10 column, this gave, in each case, solutions of the corresponding ADC in 3.5 ml of PBS buffer. The particular protein concentration indicated was then determined for these solutions and the loading of the antibody (drug/mAb ratio) was determined in accordance with the methods described below.

This process was used to prepare the immunoconjugates represented in Examples 2, 4, 7, 9, 13, 16-17, 25, 26, 28-31 and 33-35.

In the structural formulae illustrated, the definition of AK_{2A}, AK_{2B}, AK_{2D} and AK_{2E} is as follows

AK_{2A} = anti-FGFR2 antibody M048-D01-hIgG1 - NH§^{2}

AK_{2B} = anti-FGFR2 antibody M048-D01-hIgG1-b - NH§^{2}

AK_{2D} = anti-FGFR2 antibody GAL-FR21-mIgG1 - NH§^{2}

AK_{2E} = anti-FGFR2 antibody GAL-FR22-mIgG2a - NH§^{2}

where

§^{2} denotes the link with the carbonyl group

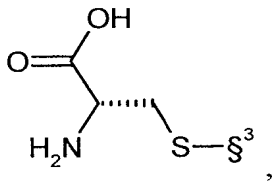
and

NH stands for the side chain amino group of a lysine residue of the antibody.

B-8. General process for preparing cysteine adducts:

10 µmol of the above-described maleimide precursor compounds were taken up in 3 ml of DMF and admixed with 2.1 mg (20 µmol) of L-cysteine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC.

In the structural formulae illustrated, the definition of Cys is as follows



where

$§^3$ denotes the link with the linker-toxophore unit.

B-9. General process for preparing lysine adducts:

10 µmol of the above-described active ester precursor compounds were taken up in 5 ml of DMF and admixed, in the presence of 30 µmol of N,N-diisopropylethylamine, with α-amino-protected L-lysine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC. The protective group was then removed by known methods.

Further purification and characterization of the conjugates of the invention

After reaction had taken place, in certain cases the reaction mixture was concentrated, by ultrafiltration, for example, and then desalted and purified by means of chromatography, for example using a Sephadex® G-25. Elution took place with, for example, phosphate-buffered saline (PBS). The solution was subsequently subjected to sterile filtration and freezing. An alternative option is to lyophilize the conjugate.

B-10. Determination of the toxophore loading

The toxophore loading of the resultant solutions of the conjugates described in the working examples, in PBS buffer, was determined as follows:

The toxophore loading of lysine-linked ADCs was determined by mass-spectrometric determination of the molecular weights of the individual conjugate species. In this case, to start with, the antibody conjugates were deglycosylated by means of PNGaseF, and the sample was acidified and, following HPLC separation, was analysed by mass spectrometry using an ESI-MicroTofQ (Bruker Daltonik). All of the spectra were added via the signal in the TIC (Total Ion Chromatogram), and the molecular weight of the various conjugate species was calculated on the basis of MaxEnt Deconvolution. Following signal integration of the different species, the DAR (Drug/Antibody Ratio) was then calculated.

For protein identification, in addition to the molecular weight determination, a tryptic digestion was carried out after deglycosylation and/or denaturing, and this digestion, after denaturing, reduction and derivatization, confirmed the identity of the protein on the basis of the tryptic peptides detected.

The toxophore loading of cysteine-linked conjugates was determined via reversed-phase chromatography of the reduced and denatured ADC. The ADC solution (1 mg/mL, 50 µL) was admixed with guanidinium hydrochloride (GuHCl) (28.6 mg) and with a solution of DL-dithiothreitol (DTT) (500 mM, 3 µL). The mixture was incubated at 55°C for an hour and analysed by HPLC.

The HPLC analysis was carried out on an Agilent 1260 HPLC System with detection at 220 nm. The column used was a Polymer Laboratories PLRP-S Polymeric Reversed Phase column (catalogue number PL1912-3802) (2.1 × 150 mm, 8 µm particle size, 1000 Å) with a flow rate of 1 mL/min, using the following gradient: 0 min, 25% B; 3 min, 25 % B; 28 min, 50% B. Eluent A consisted of 0.05% trifluoroacetic acid (TFA) in water, eluent B of 0.05% trifluoroacetic acid in acetonitrile.

The peaks detected were assigned by retention time comparison with the light chain (L0) and the heavy chain (H0) of the unconjugated antibody. Peaks which were detected exclusively in the conjugated sample were assigned to the light chain, with a toxophore (L1), and to the heavy chains, with one, two and three toxophores (H1, H2, H3).

The average loading of the antibody with toxophores was determined from the peak areas determined by integration as follows: the light-chain loading was calculated as the sum of the toxophore number weighted integration results of the peaks belonging to the light chains divided by the sum of the singularly weighted integration results of the peaks belonging to the light chains. The heavy-chain loading was calculated as the sum of the toxophore number weighted integration results of the peaks belonging to the heavy chains divided by the sum of the singularly weighted integration results of the peaks belonging to the heavy chains. The average drug load results therefrom as the twofold sum of light-chain loading and heavy-chain loading. In certain individual cases it may be impossible exactly to determine the toxophore loading, owing to co-elutions of certain peaks.

B-11. Testing of the antigen binding of the ADC

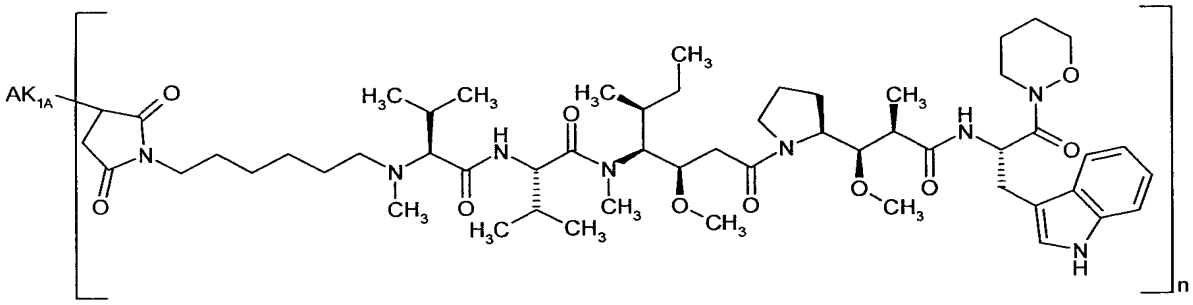
The binding capacity of the binder to the target molecule was tested after coupling had taken place. The skilled worker knows of diverse methods for achieving this – for example, the affinity of the conjugate can be tested by means of ELISA technology or surface plasmon resonance analysis

(BIAcore™ measurements). The conjugate concentration can be measured by the skilled person using common methods – for example, for antibody conjugates, by means of protein determination (see also Doronina et al.; Nature Biotechnol. 2003; 21:778-784 and Polson et al., Blood 2007; 1102:616-623).

5

Working examples - immunoconjugates

Example 1

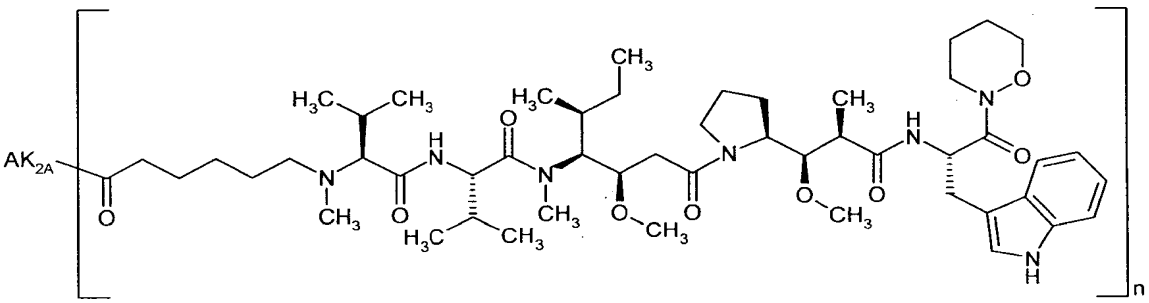


Coupling here was carried out using 30 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation, re-diluted with PBS and concentrated again.

Protein concentration: 15.5 mg/ml

Drug/mAb Ratio: 3.7

Example 2

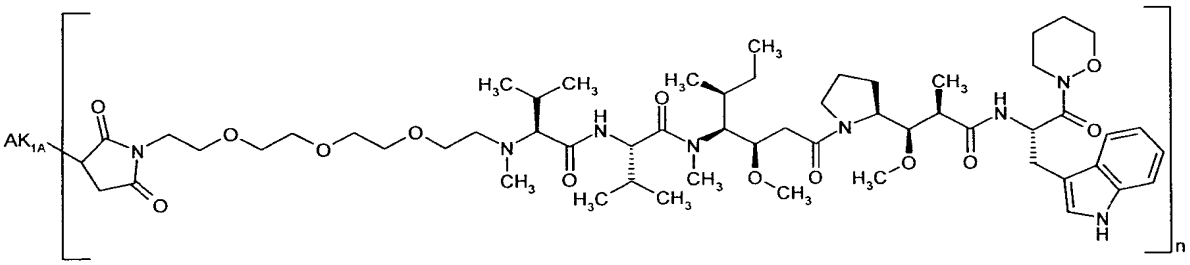


Coupling here was carried out using 32 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentifugation, re-diluted with PBS and concentrated again.

Protein concentration: 11.7 mg/ml

Drug/mAb Ratio: 3.9

Example 3

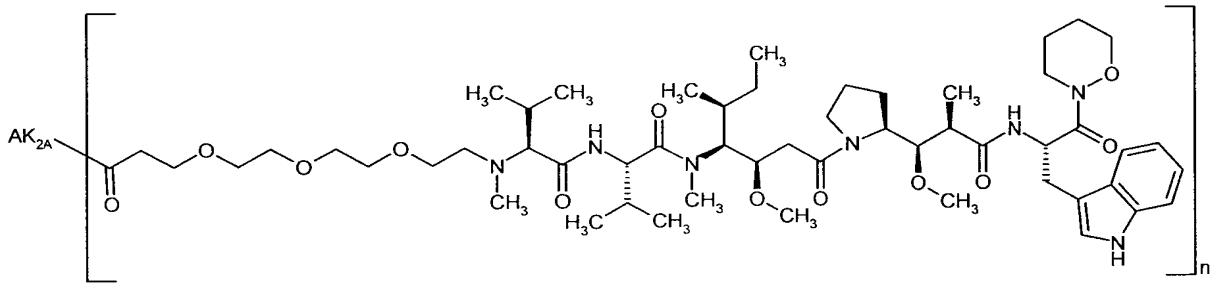


Coupling here was carried out using 30 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation, re-diluted with PBS and concentrated again.

Protein concentration: 12.5 mg/ml

Drug/mAb Ratio: 3.7

Example 4

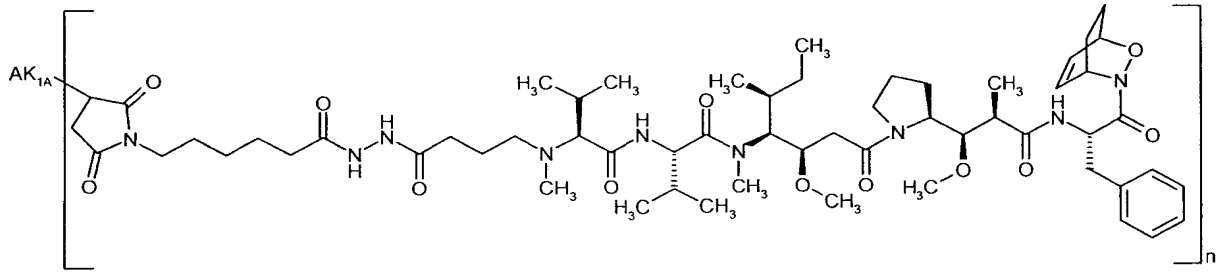


Coupling here was carried out using 2 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentifugation and re-diluted with PBS.

Protein concentration: 1.92 mg/ml

Drug/mAb Ratio: 3.7

Example 5

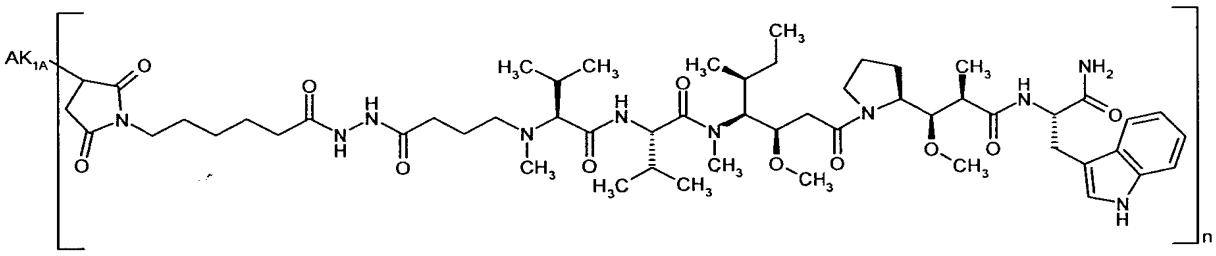


Coupling here was carried out using 3 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 2.54 mg/ml

Drug/mAb Ratio: 3.1

Example 6

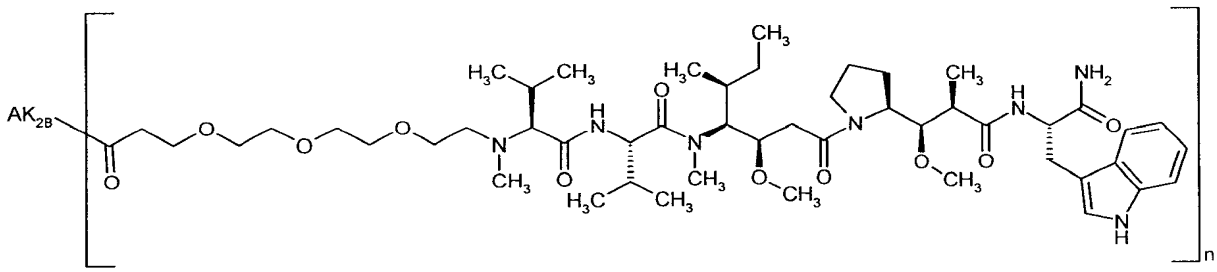


Coupling here was carried out using 4 mg of M048-D01-hIgG1 in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.53 mg/ml

Drug/mAb Ratio: 2.7

Example 7

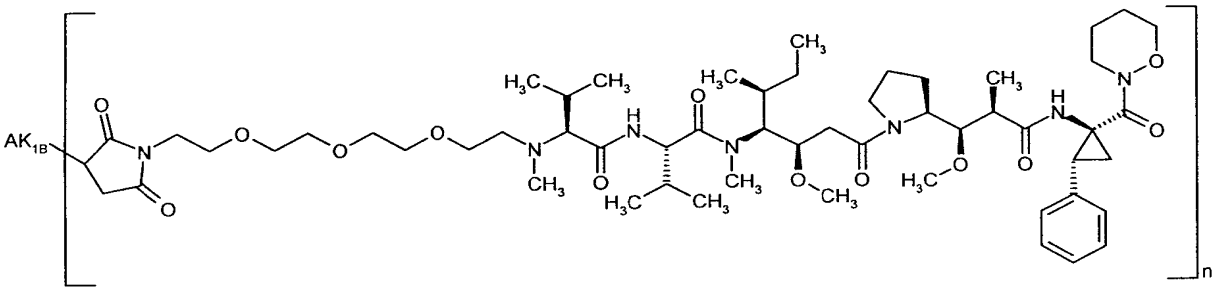


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.28 mg/ml

Drug/mAb Ratio: 6.1

Example 8

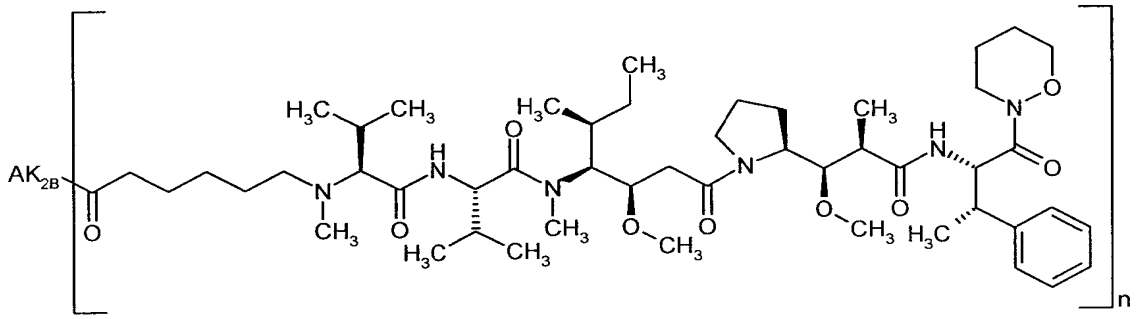


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentifugation and re-diluted.

Protein concentration: 1.26 mg/ml

Drug/mAb Ratio: 3.5

Example 9

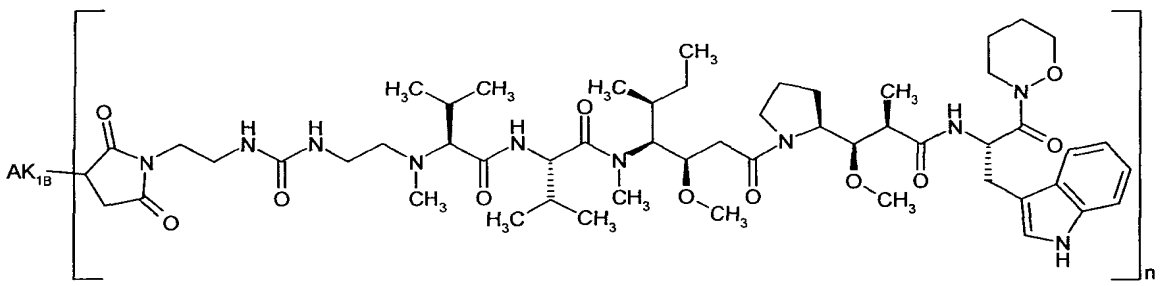


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentifugation and re-diluted.

Protein concentration: 1.28 mg/ml

Drug/mAb Ratio: 6.1

Example 10

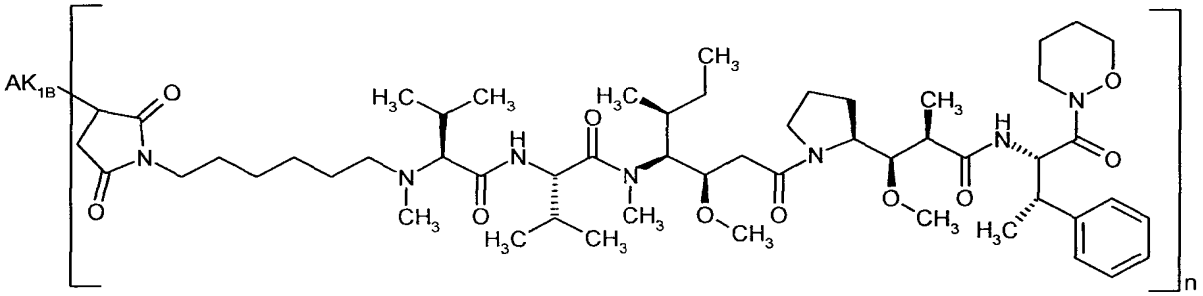


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.36 mg/ml

Drug/mAb Ratio: 4.4

Example 11

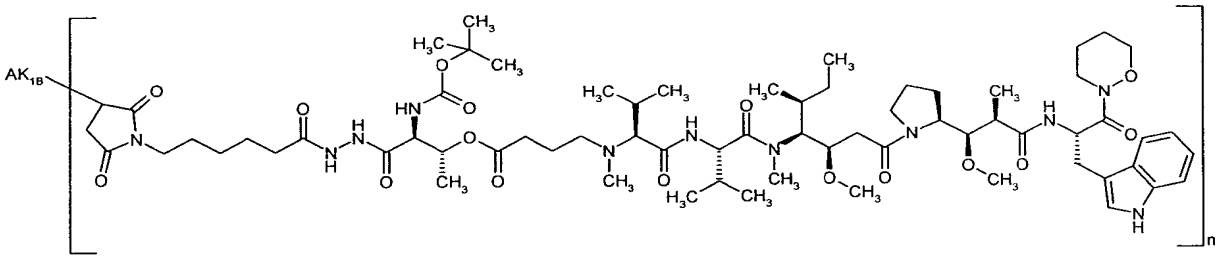


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.27 mg/ml

Drug/mAb Ratio: 4.8

Example 12

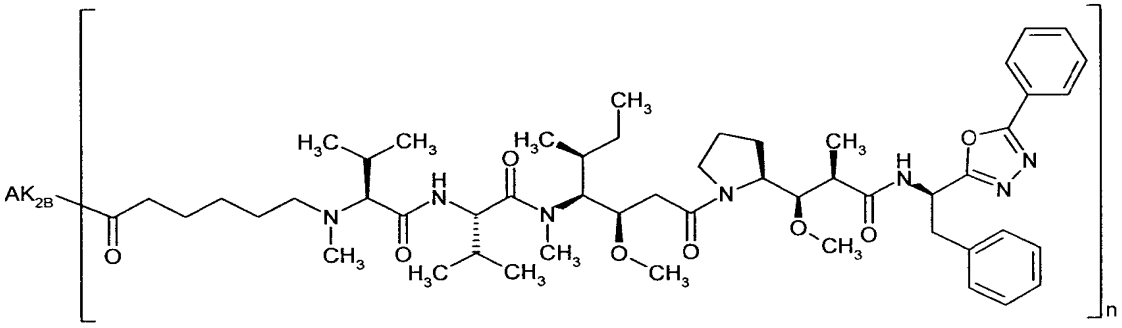


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.48 mg/ml

Drug/mAb Ratio: 4.0

Example 13

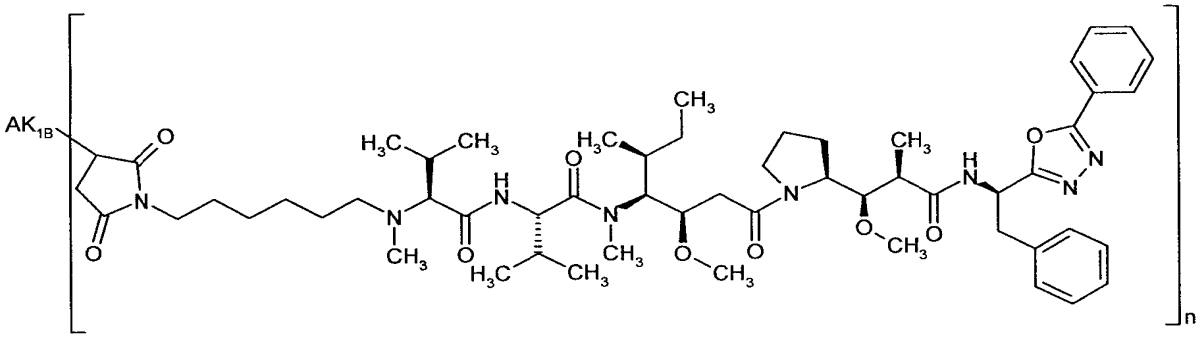


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and following the Sephadex purification the batch was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.21 mg/ml

Drug/mAb Ratio: 1.5

Example 14

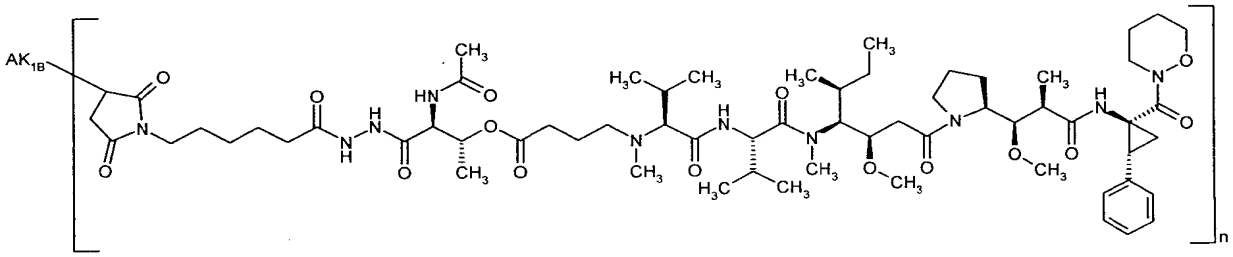


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.27 mg/ml

Drug/mAb Ratio: 2.7

Example 15

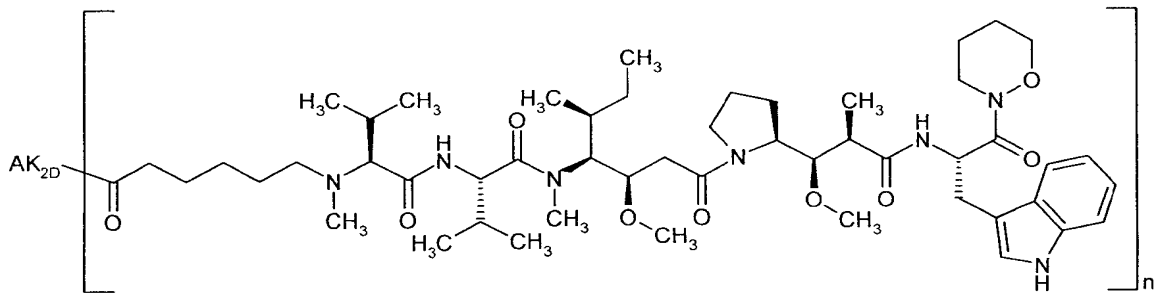


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted with PBS.

Protein concentration: 1.37 mg/ml

Drug/mAb Ratio: 3.9

Example 16

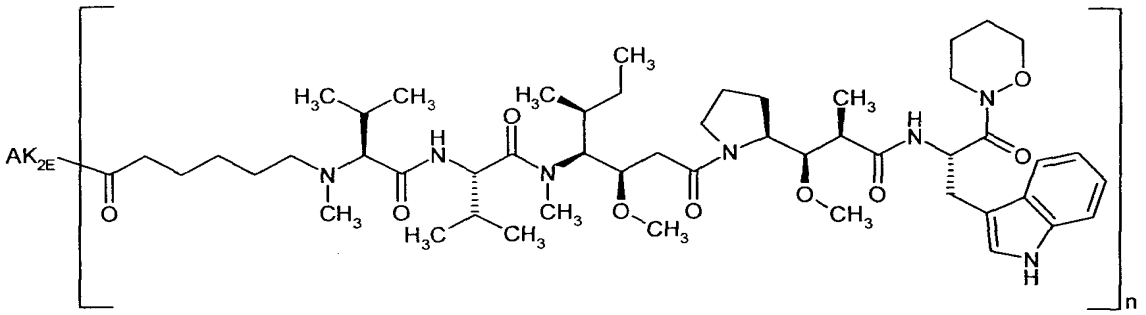


Coupling here was carried out using 4 mg of GAL-FR21-mIgG1 in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted with PBS.

Protein concentration: 1.63 mg/ml

Drug/mAb Ratio: 8.7

Example 17



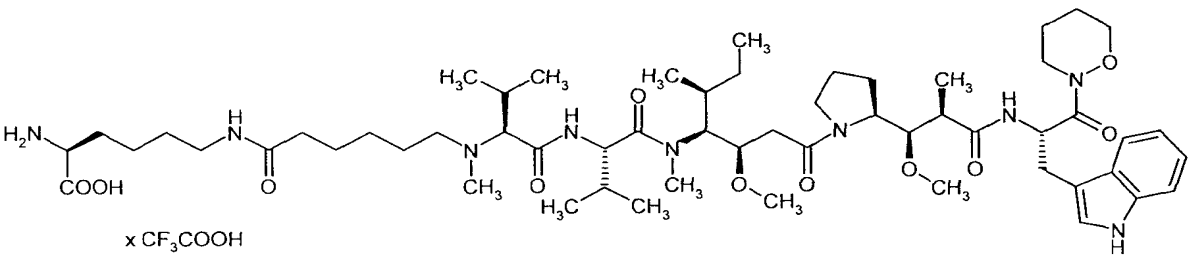
Coupling here was carried out using 4 mg of GAL-FR22-mIgG2a in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted with PBS.

Protein concentration: 1.60 mg/ml

Drug/mAb Ratio: 8.1

Example 18

N-(6-{[(5S)-5-Amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



15.5 mg (15 µmol) of Intermediate 210 were taken up in 5 ml of DMF and admixed with 4.4 mg (18 µmol) of N^2-(tert-butoxycarbonyl)-L-lysine and also 7.7 µL (44 µmol) of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight and then concentrated

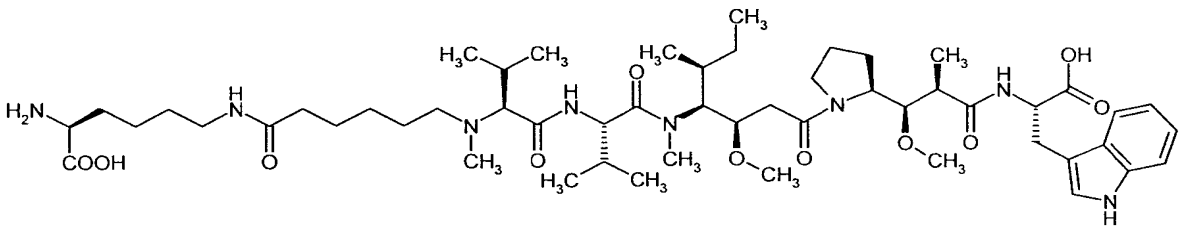
under reduced pressure. The residue was subsequently purified by preparative HPLC. This gave 14 mg (81% of theory) of the protected intermediate of the title compound, which was subsequently taken up in 1 ml of dichloromethane and deprotected with 1 ml of trifluoroacetic acid. The batch was concentrated and, following lyophilization of the residue from acetonitrile/water (1:1), 15 mg (97% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.79 min; MS (ESIpos): m/z = 1083 $(M+H)^+$.

Example 19

N-(6-{[(5S)-5-Amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



40 mg (40 µmol) of Intermediate 227 were taken up in 5 ml of DMF and admixed with 11.5 mg (40 µmol) of N^2-[(benzyloxy)carbonyl]-L-lysine and also 13 µL (80 µmol) of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight, then concentrated under reduced pressure and subsequently purified by preparative HPLC. This gave 32.5 mg (70% of theory) of the protected intermediate of the title compound.

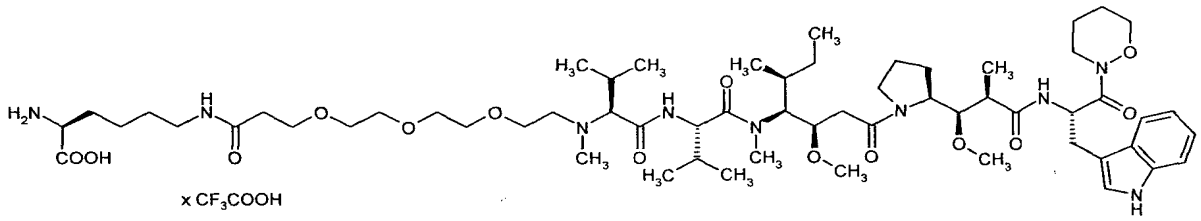
This 32.5 mg of the intermediate were dissolved in 10 ml of methanol and, following addition of 2 mg of 10% palladium on activated carbon, were hydrogenated under standard hydrogen pressure at RT for 30 minutes. The catalyst was then removed by filtration and the solvent was removed under reduced pressure. Lyophilization of the residue from dioxane/water 1:1 gave 26 mg (99% of theory) of the title compound.

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.76 min; MS (ESIpos): m/z = 1014 $(M+H)^+$.

Example 20

N-[(18S)-18-Amino-18-carboxy-12-oxo-3,6,9-trioxa-13-azaoctadec-1-yl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



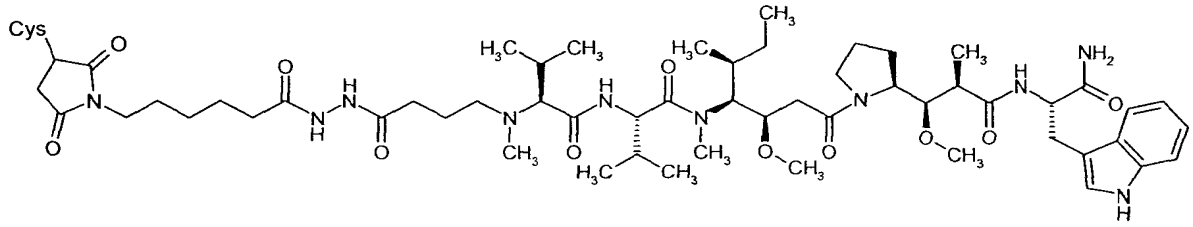
3.5 mg (3 µmol) of Intermediate 202 were taken up in 2 ml of DMF and admixed with 0.8 mg (3 µmol) of N^2-(tert-butoxycarbonyl)-L-lysine and also 1.6 µL (10 µmol) of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight and then concentrated under reduced pressure. The residue was taken up in acetonitrile/water (1:1), brought to a pH of 2 with trifluoroacetic acid and then purified by preparative HPLC. This gave 1 mg (25% of theory) of the protected intermediate of the title compound, which was subsequently taken up in 500 µl of dichloromethane and deprotected with 500 µl of trifluoroacetic acid. The batch was concentrated and, following lypophilization of the residue from acetonitrile/water (1:1), 1 mg (89% of theory) of the title compound was obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 1173 $(M+H)^+$.

Example 21

N-(4-{2-[6-(3-{[(2R)-2-Amino-2-carboxyethyl]sulphanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



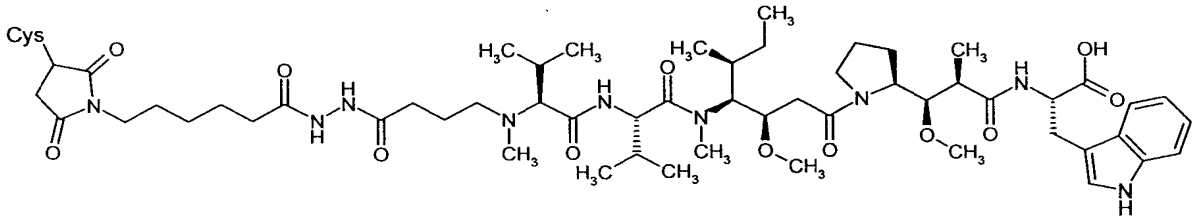
10 mg (10 µmol) of Intermediate 157 were taken up in 5.2 ml of DMF and admixed with 2.28 mg (20 µmol) of L-cysteine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC. This gave 5.8 mg (48% of theory) of the title compound.

HPLC (Method 5): R_t = 1.45 min;

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 1184 $(M+H)^+$.

Example 22

N-(4-{2-[6-(3-{[(2R)-2-Amino-2-carboxyethyl]sulphanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



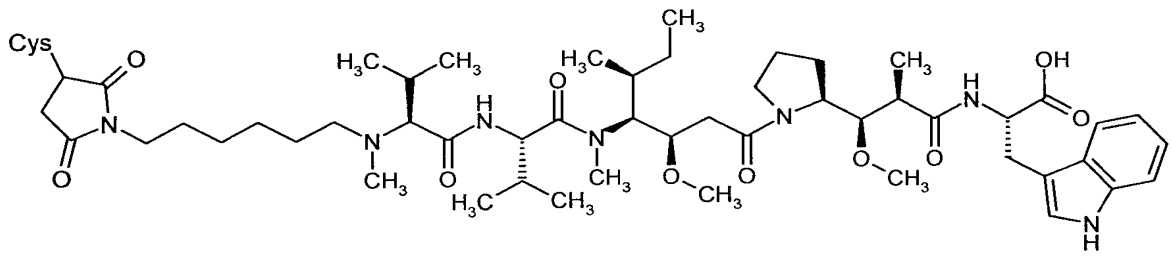
10 mg (10 µmol) of Intermediate 113 were taken up in 5.2 ml of DMF and admixed with 2.28 mg (20 µmol) of L-cysteine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC. This gave 6 mg (54% of theory) of the title compound.

HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 1185 $(M+H)^+$.

Example 23

N-[6-(3-{[(2R)-2-Amino-2-carboxyethyl]sulphanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



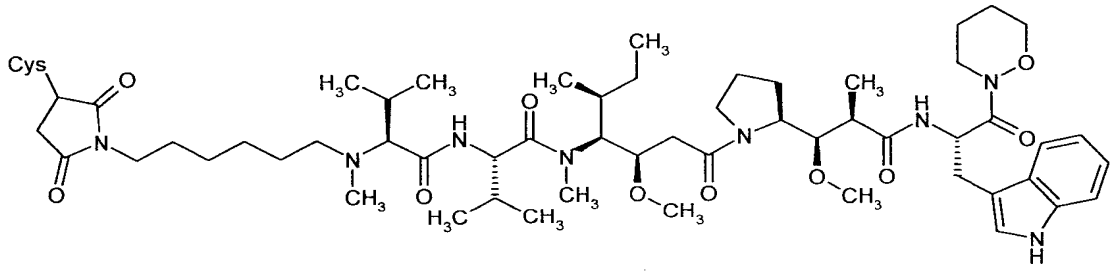
10 mg (10 µmol) of Intermediate 124 were taken up in 4 ml of DMF and admixed with 2.5 mg (20 µmol) of L-cysteine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC. This gave 7.2 mg (64% of theory of the title compound.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 1071 $(M+H)^+$.

Example 24

N-[6-(3-{[(2R)-2-Amino-2-carboxyethyl]sulphanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

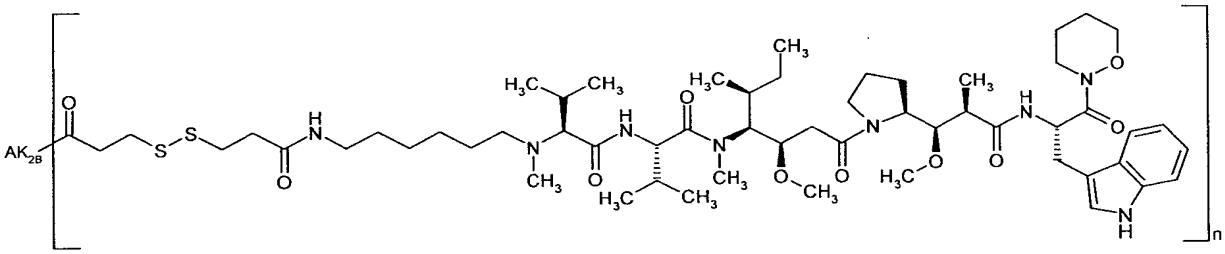


10 mg (10 µmol) of Intermediate 125 were taken up in 4 ml of DMF and admixed with 2.4 mg (20 µmol) of L-cysteine. The reaction mixture was stirred at RT for 2 hours, then concentrated under reduced pressure and subsequently purified by preparative HPLC. This gave 7.7 mg (69% of theory of the title compound.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.91 min; MS (ESIpos): m/z = 1140 $(M+H)^+$.

Example 25

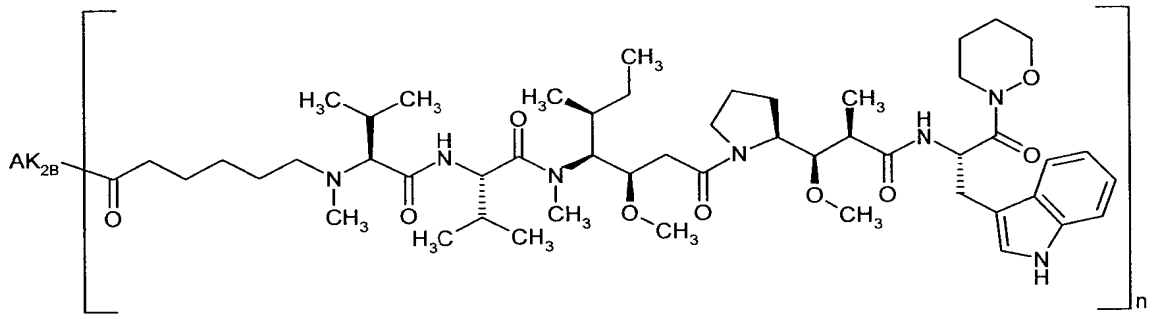


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.27 mg/ml

Drug/mAb Ratio: 1.0

Example 26

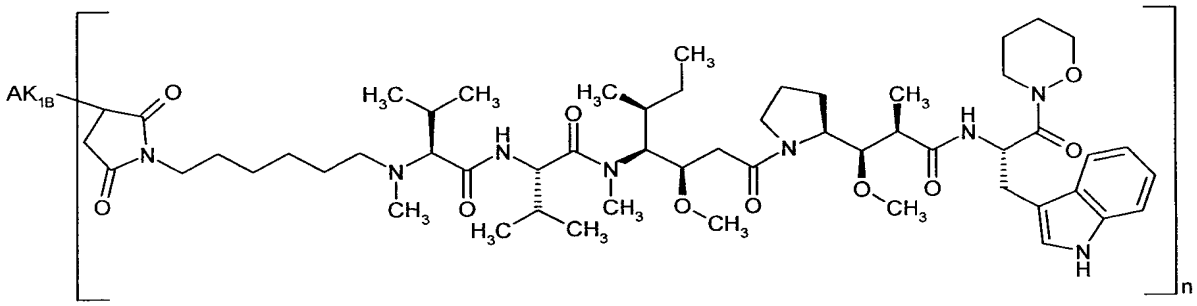


Coupling here was carried out using 35 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation, re-diluted with PBS and concentrated again.

Protein concentration: 11.60 mg/ml

Drug/mAb Ratio: 3.7

Example 27

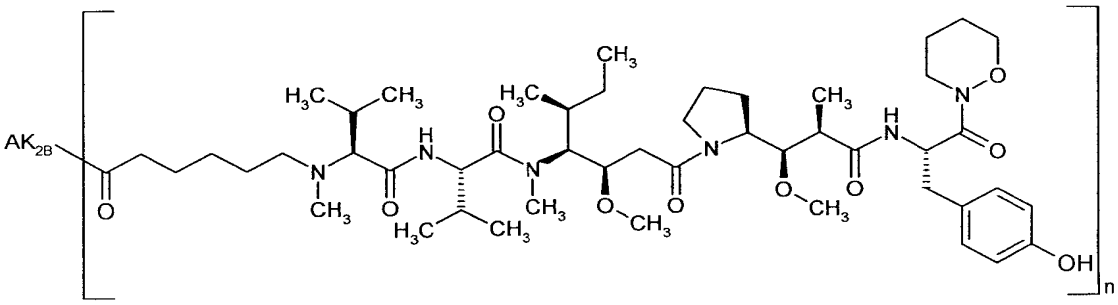


Coupling here was carried out using 35 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation, re-diluted with PBS and concentrated again.

Protein concentration: 11.7 mg/ml

Drug/mAb Ratio: 4.2

Example 28

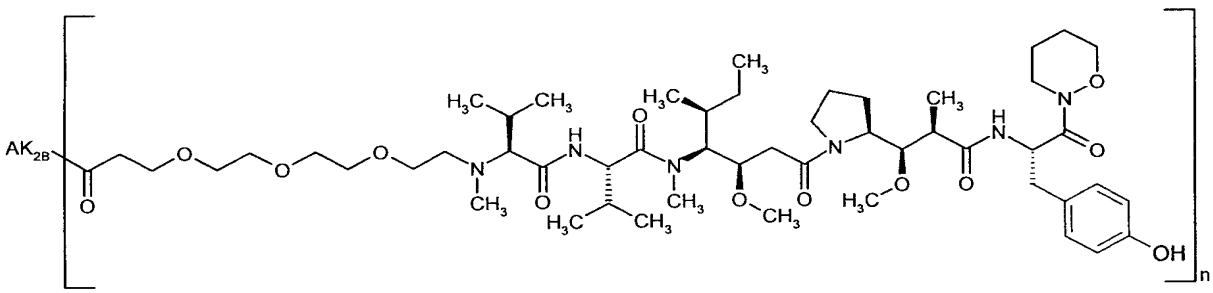


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted with PBS.

Protein concentration: 1.31 mg/ml

Drug/mAb Ratio: 3.7

Example 29

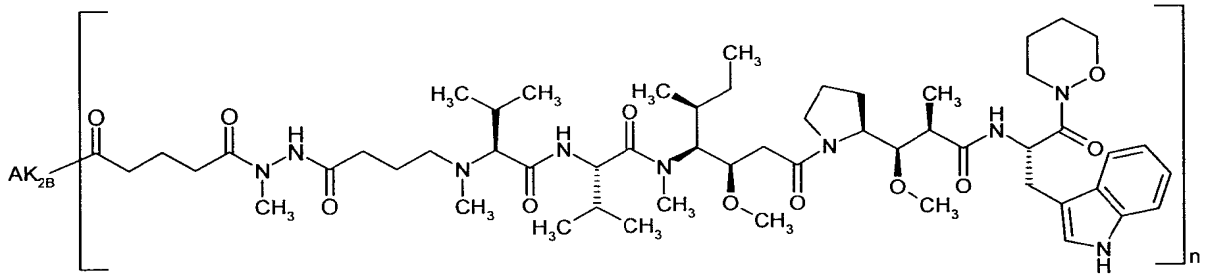


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted with PBS.

Protein concentration: 1.53 mg/ml

Drug/mAb Ratio: 1.3

Example 30

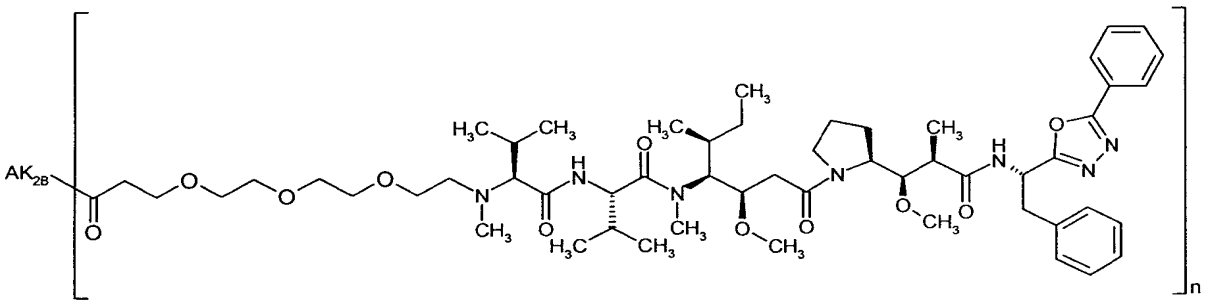


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the batch, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.61 mg/ml

Drug/mAb Ratio: 3.9

Example 31

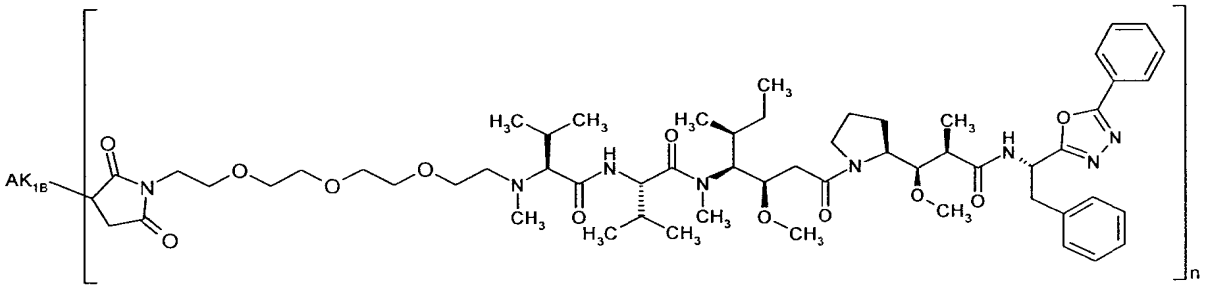


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.32 mg/ml

Drug/mAb Ratio: 1.7

Example 32

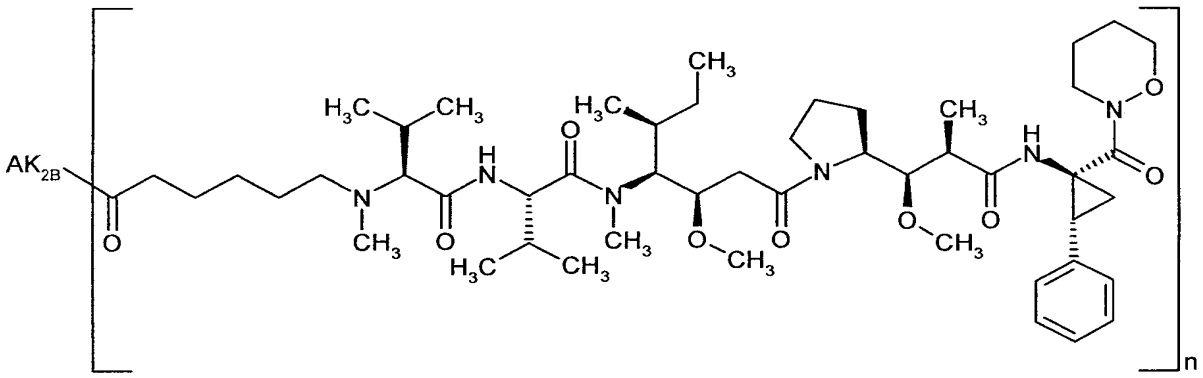


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.12 mg/ml

Drug/mAb Ratio: 0.2

Example 33

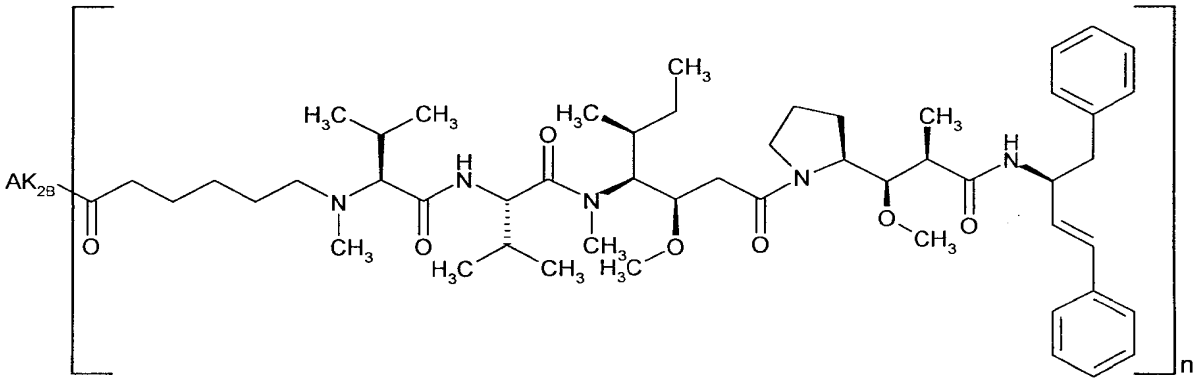


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.29 mg/ml

Drug/mAb Ratio: 5.5

Example 34

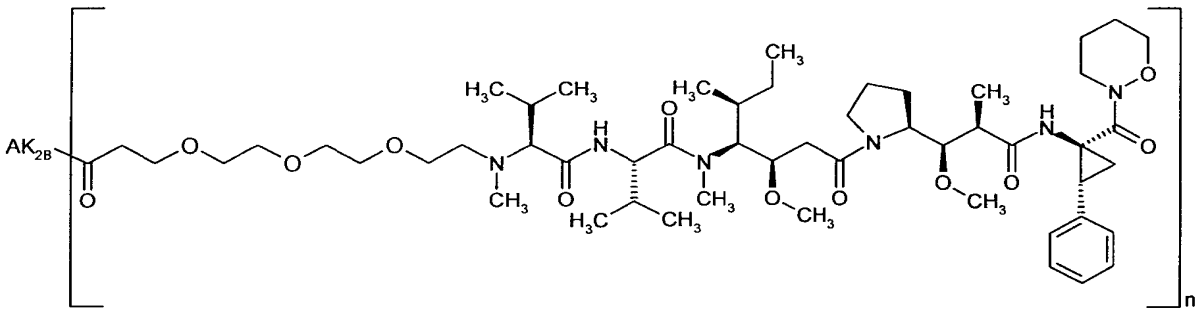


Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.04 mg/ml

Drug/mAb Ratio: 0.5

Example 35



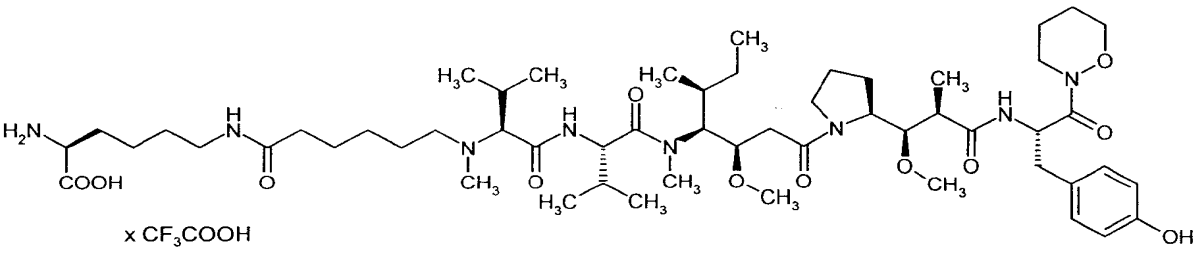
Coupling here was carried out using 5 mg of M048-D01-hIgG1-b in PBS and the reaction mixture, following Sephadex purification, was concentrated by ultracentrifugation and re-diluted.

Protein concentration: 1.66 mg/ml

Drug/mAb-Ratio: 3.4

Example 36

N-(6-{[(5S)-5-Amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



8 mg (8 µmol) of Intermediate 242 were taken up in 3 ml of DMF and admixed with 2.9 mg (12 µmol) of N^2-(tert-butoxycarbonyl)-L-lysine and also 2.7 µL (16 µmol) of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight, then admixed again with the same amounts of N^2-(tert-butoxycarbonyl)-L-lysine and *N,N*-diisopropylethylamine, and stirred at RT for a further 4 hours. The batch was subsequently concentrated under reduced pressure. The residue was then purified by preparative HPLC. Lyophilization from acetonitrile/water gave 6.5 mg (72% of theory) of the protected intermediate of the title compound, which was

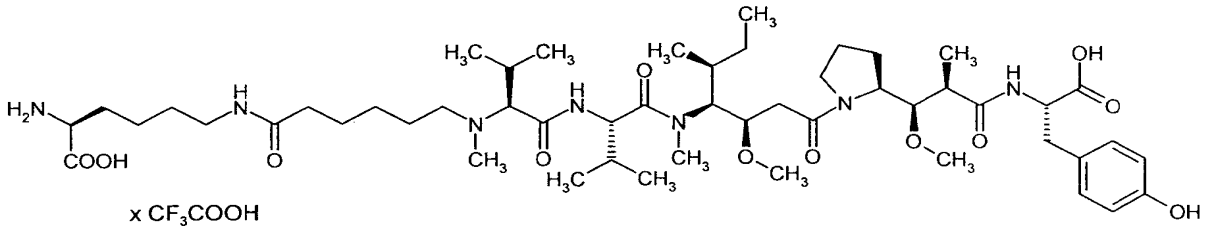
subsequently taken up in 5 ml of dichloromethane and deprotected with 0.75 ml of trifluoroacetic acid. The batch was concentrated, and lyophilization of the residue from dioxane/water gave 5 mg (76% of theory) of the title compound.

HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.69 min; MS (ESIpos): m/z = 1059 $(M+H)^+$.

Example 37

N-(6-{[(5S)-5-Amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



38 mg (41 µmol) of Intermediate 248 were first converted into the N-hydroxysuccinimide ester. 72 mg of the crude product obtained were taken up in 5 ml of DMF and admixed with 24 mg (100 µmol) of N^2-(tert-butoxycarbonyl)-L-lysine and 23 µL of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight, and then admixed again with 16 mg of N^2-(tert-butoxycarbonyl)-L-lysine and 12 µL of *N,N*-diisopropylethylamine, and subsequently treated in an ultrasound bath for a further 2 hours. The batch was then concentrated under reduced pressure and the residue was purified by preparative HPLC. Lyophilization from acetonitrile/water gave 20 mg (50% of theory) of the protected intermediate of the title compound.

15 mg (12 µmol) of this intermediate were subsequently taken up in 3 ml of dichloromethane and admixed with 1 ml of trifluoroacetic acid. After 40 minutes of stirring at RT, a further 1.5 ml of trifluoroacetic acid were added and the batch was treated in an ultrasound bath for 1 hour. Thereafter the batch was concentrated, and lyophilization of the residue from dioxane/water gave 13 mg (90% of theory) of the title compound.

HPLC (Method 12): R_t = 1.5 min;

LC-MS (Method 1): R_t = 0.68 min; MS (ESIpos): m/z = 990 $(M+H)^+$.

C: Evaluation of biological activity:

The biological effect of the compounds of the invention was demonstrated by the assays described below:

C-1. Identification of tumour cells with different FGFR2 levels on the cell surface

To determine the amounts of FGFR2 available for the antibody on the cell surface, the cell binding of the FGFR2 antibody on different tumour cell lines was analysed in flow cytometry. The cell lines below were used for the experiments. The information on mutations and copy number of the FGFR2 comes from the Sanger Center Genome Project:

- **SNU-16** human stomach carcinoma cells, FGFR2 gene amplification (copy number 14; ATCC-CRL-5974, RPMI 1640 (Biochrom FG1215) + 10% FCS

- **KatoIII** human stomach carcinoma cells, FGFR2 gene amplification (copy number 14 ATCC-TCP-1008; Iscove's (Biochrom FG0465) + 20% FCS

- **SUM52-PE** human breast cancer cells, FGFR2 gene amplification (copy number 14; Asterand Lot No.: 28062A1-6004; Ham's F12 (Biochrom FG0815) + 5% FCS + 10 mM Hepes buffer + 1 µg/ml hydrocortisone + 5 µg/ml insulin

- **MFM-223** human breast cancer cells, FGFR2 gene amplification (copy number 14; ECACC-98050130, MEM Earle (Biochrom F0315) + 10% FCS + 2mM glutamine

- **NCI-H716** human colorectal carcinoma cells, FGFR2 gene amplification (copy number: 8; ATCC-CCL-251; RPMI 1640 (Biochrom FG1215) + 10% FCS)

- **MDA-MB-231** human breast cancer cells (no FGFR2 gene amplification, copy number 3; ATCC-HTB-26, DMEM/HAM´s F12 (Biochrom FG4815) + 10% FCS

For the experiments, adherent cells were washed twice with PBS (without calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions and detached using enzyme-free, PBS-based cell dissociation buffer (Invitrogen). Approximately 1×10^5 cells per well were suspended in FACS buffer (PBS without Ca^{2+} and Mg^{2+} with 3% FCS (Biochrom)), followed by centrifugation (250 g, 5 min, 4°C), and the supernatant was discarded. The cells were resuspended in antibody dilutions (5 µg/ml in 80 µl) in FACS buffer, and incubated on ice for 1 hour. Next, the cells were washed once with 100 µl of cold FACS buffer and 80 µl of 1:150 diluted secondary antibodies (PE-coupled goat anti-mouse IgG, Jackson Immuno Research #115-115-164 for GAL-FR21-mIgG1 and for GAL-FR22-mIgG2a, and PE-coupled goat anti-human IgG, Dianova #109-115-098 for M048-D01-hIgG1) were added.

Following incubation on ice for 1 hour, the cells were again washed with cold FACS buffer, resuspended in 100 µl of FACS buffer, and analysed in an FACS array flow cytometer (BD Biosciences). The results were calculated as the geometric average value of the cell population detected with FGFR2 antibody, minus the background fluorescence, which was measured by incubating the cell population only with the secondary antibody. The values were analysed by the following system: geometric average value FGFR2 antibody minus geometric average value only secondary antibody > 10 +, > 100 ++, > 1000 +++, > 10000 ++++, -: no signal. Values in the vicinity of the category limits are marked with ().

The binding of FGFR2 antibodies to tumour cells is indicated in Table 4:

Table 4

	M048-D01-hIgG1	GAL-FR21-mIgG1	GAL-FR22-mIgG2a
MFM-223	+++	+++	+++
SNU-16	++(+)	++(+)	++
MDA-MB-231	-	nd[1)]	nd[1)]

[1)] nd: not determined

The FGFR2 antibodies detect FGFR2 on the cell surface of MFM-223 and SNU-16 cancer cells.

C-2. Determination of the cytotoxic effect of the ADCs directed against FGFR2

The cytotoxic effect of the FGFR2 ADCs was determined on various cell lines with different expression quantities of FGFR2, as follows:

The cells were cultured according to a standard method, using the growth media indicated under C-1. For the implementation, the cells were detached with a solution of trypsin (0.05%) and EDTA (0.02%) in PBS (Biochrom AG #L2143), pelletized, resuspended in culture medium, counted, and seeded into a 96-well culture plate with a white base (Costar #3610) (at 75 µl/well, following cell counts per well: SNU-16: 3000; MFM-223: 7000; MDA-MB-231: 4000; SUM52-PE: 3000; NCI-H716: 3000; KatoIII: 3000) and incubated in an incubator at 37°C under 5% carbon dioxide. After 24 hours, the antibody-drug conjugates in 25 µl of culture medium (four-fold concentration) were applied to the cells, giving final antibody-drug conjugate concentrations of 3×10^{-7} M to 3×10^{-11} M on the cells (triplicates). The cells were then incubated in an incubator at 37°C and 5% carbon dioxide. In a parallel plate, the cell vitality at the beginning of drug treatment (day 0) was determined using the Cell Titer Glow Luminescent Cell Viability Assay (Promega #G7573 and #G7571). For this, 100 µl of the substrate were added per cell batch, and the plates were then

covered with aluminium foil, shaken at 180 rpm with a plate shaker for 2 minutes, left to stand on the laboratory bench for 8 minutes, and then measured using a luminometer (Victor X2, Perkin Elmer). The substrate detects the ATP content in the living cells, producing a luminescence signal whose level is directly proportional to the vitality of the cells. After 72 hours of incubation with the antibody-drug conjugates, the vitality was determined in these cells as well, using the Cell Titer Glow Luminescent Cell Viability Assay as described above. From the data measured, the IC_{50} of growth inhibition was calculated in comparison to that on day 0, using the laboratory software MTS (developed by Schering AG and Bayer Business Services 1999-2009) on the basis of a 4-parameter adaptation.

Table 5 below lists the IC_{50} values[1)] of representative working examples from this assay:

Table 5

Example	SNU-16, IC50, 72 hr [nM]	MFM-223, IC50, 72 hr [nM]	MDA-MB-231, IC50, 72 hr [nM]	SUM52-PE, IC50, 72 hr [nM]	NCI-H716, IC50, 72 hr [nM]	KatoIII, IC50, 72 hr [nM]
1	0.214	0.0907	> 300			
2	1.19	0.86	> 300			
3	0.15	0.195	> 300			
4	0.567	1.47	> 300			
5	0.496	4.00	> 300			
6	0.497	6.85	> 300			
7	0.209	0.628	> 300			
8	2.06	8.67	256			
9	2.05	4.78	230			
10	0.519	10.3	> 300			
11	0.614	0.581	80.9			
12	0.389	0.145	> 300			
13	0.254	0.431	6.61			
14	0.914	1.91	67.5			
15	16.6	16.9	> 300			
16	< 0.03	0.0788	> 300			
17	0.0616	0.278	> 300			
26	0.455	na	> 248	< 0.097	4.69	na

27	0.159	0.109	> 248	< 0.053	0.517	0.198
28	0.277	na	na	0.101	na	na
29	1.70	na	na	0.106	na	na
30	1.84	9.16	> 248	0.244	na	na
31	5.88	na	na	na	na	na
32	> 300	na	na	na	na	na
33	8.50	na	128	0.104	na	0.920
34	7.41	na	> 248	3.37	na	na
35	na	na	> 248	0.105	na	0.523

[1] The activity data reported relate to the working examples described in the present experimental section, with the drug/mAB ratios indicated. The values may possibly deviate for different drug/mAB ratios.

Working Example 1 inhibited the proliferation of the SNU-16 and MFM-223 cancer cell lines which expresses FGFR2 at the cell surface, with an IC50 in the subnanomolar concentration range. Working Example 1 inhibited the proliferation of the MDA-MB-231 cancer cell line which do not express FGFR2 at the cell surface, with an IC50 of 300 nM. As is apparent from the data, all tested antibody-drug conjugates (Working Examples 1-31, 33-35) selectively inhibit the proliferation of FGFR2-expressing cancer cell lines (SNU-16, MFM-223, SUM52-PE, KatoIII or NCI-H716).

C-3. Determination of the effect on tubulin polymerization

Cancer cells are denatured cells which frequently lead to the formation of tumours also as a result of increased cell division. Microtubuli form the spindle fibres of the spindle apparatus and are an essential constituent of the cell cycle. The regulated construction and breakdown of microtubuli allows the precise division of the chromosomes among the daughter cells, and constitutes a continuously dynamic process. Disruption to this dynamic process results in incorrect cell division and ultimately in cell death. The increased cell division of cancer cells, however, also makes them particularly sensitive towards spindle fibre poisons, which constitute a fixed constituent of chemotherapy. Spindle fibre poisons such as paclitaxel or epothilone lead to a sharply increased polymerization rate of the microtubuli, while vinca alkaloids or else monomethylauristatin E (MMAE) lead to a sharply reduced polymerization rate of the microtubuli. In both cases, the necessary dynamism of the cell cycle is critically disrupted.

Tubulin polymerization was investigated using the "Fluorescence-based Microtubule Polymerisation Assay Kit" from Cytoskeleton (Denver, Colorado, USA; order number: BK011). With this assay, GTP is added to unpolymerized tubulin, allowing polymerization to take place

spontaneously. The assay is based on the binding of the fluorophore 4',6-diamidino-2-phenylindole (DAPI) to tubulin. Free and bound DAPI can be differentiated on the basis of different emission spectra. Since DAPI exhibits a significantly high affinity for polymerized tubulin in comparison to non-polymerized tubulin, the tubulin polymerization can be followed via the increase in the fluorescence of bound DAPI fluorophores.

For the implementation of this assay, the compounds of the invention, in solution in DMSO, were diluted from their initial concentration of 10 mM to 1 µM in water. In addition to the buffer control, paclitaxel, with a polymerization-increasing effect, and vinblastin, with a polymerization inhibiting effect, were run additionally as assay controls. Measurement was carried out using 96-well plates with a half base area. The kinetics of the tubulin polymerization were monitored in a Fluorimeter at 37°C for 1 hour. The excitation wavelength was 355 nm, and emission was monitored at 460 nm. For the region of linear increase within the first 10 minutes, a calculation was made of the change in fluorescence per minute (ΔF/min), which represents the polymerization rate of the microtubuli. The potency of the test substances was quantified on the basis of their respective reduction of the polymerization rate. Table 6 below gives data for the influence of representative working examples on tubulin polymerization.

Table 6: Blockade of tubulin polymerization by selected examples of toxophore variants.

Working example	Concentration of toxophore [µM]	Tubulin polymerization in the presence of toxophore in [%]. Tubulin polymerization rate at 1 µM MMAF set at 100%
MMAF	1	100
MMAF	10	34
MMAF	100	0
18	1	45
18	10	1
19	1	80
19	10	14
20	1	60

Working example	Concentration of toxophore [µM]	Tubulin polymerization in the presence of toxophore in [%]. Tubulin polymerization rate at 1 µM MMAF set at 100%
20	10	0
21	1	88
21	10	25
22	1	109
22	10	27
24	1	121
24	10	35
36	1	88
36	10	21
37	1	90
37	10	17

The MMAF toxophore and the working examples inhibit tubulin polymerization as a function of their concentration. At 100 µM MMAF, the tubulin polymerization is inhibited completely. The compounds investigated in the context of the present invention result in a reduced polymerization rate of the microtubuli. Working Examples 18-21 inhibit the tubulin polymerization at 1 µM to 45-88% of the value measured for 1 µM MMAF.

C-4. *In vitro* tests for determining cell permeability

The cell permeability of a substance can be investigated by means of *in vitro* testing in a flux assay using Caco-2 cells [M.D. Troutman and D.R. Thakker, *Pharm. Res.* 20 *(8)*, 1210-1224 (2003)]. For this purpose, the cells were cultured for 15-16 days on 24-well filter plates. For the determination of permeation, the respective working example was applied in a HEPES buffer to the cells either apically (A) or basally (B) and incubated for 2 hours. After 0 hours and after 2 hours, samples

were taken from the cis and trans compartments. The samples were separated by HPLC (Agilent 1200, Böblingen, Germany) using reverse phase columns. The HPLC system was coupled via a Turbo Ion Spray Interface to a Triple Quadropol mass spectrometer API 4000 (Applied Biosystems Applera, Darmstadt, Germany). The permeability was evaluated on the basis of a P_{app} value, which was calculated using the formula published by Schwab *et al.* [D. Schwab *et al.*, *J. Med. Chem.* 46, 1716-1725 (2003)].

Of critical importance for toxophores which are released intracellularly is the permeability from B to A [P_{app} (B-A)]: the lower this permeability, the longer the residence time of the working example in the cell following intracellular release, and hence also the longer the time available for interaction with the biochemical target (in this case: tubulin).

Table 7 below sets out permeability data for representative working examples from this assay:

Table 7

Working example	**P_{app} (B-A) [nm/s]**
18	2
19	1
21	2
22	2
23	1
36	1.5
37	0.9

The working examples exhibit a low permeability from B to A [P_{app} (B-A) and therefore have a long residence time in the CaCo-2 cells. In comparison, monomethylauristatin E (MMAE) and monomethylauristatin F (MMAF) in this test exhibit a P_{app} (B-A) value of 73 nm/s, and therefore have a significantly shorter residence time in the Caco-2 cells.

C-5. *In vitro* tests for determining the substrate properties for P-glycoprotein (P-gp)

Many tumour cells express transporter proteins for drugs, and this frequently accompanies the development of resistance towards cytostatics. Substances which are not substrates of such

transporter proteins, such as P-glycoprotein (P-gp) or BCRP, for example, could therefore exhibit an improved activity profile.

The substrate properties of a substance for P-gp (ABCB1) were determined by means of a flux assay using LLC-PK1 cells which overexpress P-gp (L-MDR1 cells) [A.H. Schinkel *et al.*, *J. Clin. Invest.* 96, 1698-1705 (1995)]. For this purpose, the LLC-PK1 cells or L-MDR1 cells were cultured on 96-well filter plates for 3-4 days. For determination of the permeation, the respective test substance, alone or in the presence of an inhibitor (such as Ivermectin or Verapamil, for example), was applied in a HEPES buffer to the cells either apically (A) or basally (B) and incubated for 2 hours. After 0 hours and after 2 hours, samples were taken from the cis and trans compartments. The samples were separated by HPLC using reverse phase columns. The HPLC system was coupled via a Turbo Ion Spray Interface to a Triple Quadropole mass spectrometer API 3000 (Applied Biosystems Applera, Darmstadt, Germany). The permeability was evaluated on the basis of a P_{app} value which was calculated using the formula published by Schwab *et al.* [D. Schwab *et al.*, *J. Med. Chem.* 46, 1716-1725 (2003)].

Of critical importance for toxophores which are released intracellularly is the permeability from B to A [P_{app} (B-A)]: the lower this permeability, the longer the residence time of the working example in the cell following intracellular release, and hence also the longer the time available for interaction with the biochemical target (in this case: tubulin).

Table 8 below lists permeability data for representative working examples from this assay, which was carried out in L-MDR1 cells:

Table 8

Working example	**P_{app} (B-A) [nm/s]**
18	6
19	4
21	3
22	4
23	4

The working examples exhibit a low permeability from B to A [P_{app} (B-A) and therefore have a long residence time in the L-MDR1 cells.

C-6. Pharmacokinetics in the SNU-16 tumour model

Following intravenous administration of various ADCs, the plasma concentrations and tumour concentrations of ADC and also of potential metabolites are measured and the pharmacokinetic parameters such as clearance (CL), area under the curve (AUC) and half-life ($t_{1/2}$) are calculated.

Analysis for quantifying the potentially occurring metabolites

The measurement of the compounds in plasma and tumour takes place following precipitation of the proteins with methanol, by means of high-pressure liquid chromatography (HPLC) coupled to a tandem mass spectrometer (MS).

For the processing of 100 µL of plasma, it is admixed with 400 µL of methanol and 10 µL of internal standard (ISTD, 50 ng/mL in methanol) and shaken for 10 seconds. After centrifuging for 5 minutes at 16 000 g, 250 µL of supernatant are transferred to an autosampler vial, which was made up with 250 µL of ammonium acetate buffer (AAC, 10 mM, pH 6.8) and shaken again.

For the processing of a tumour, it is admixed with 4 times the amount of methanol. In a Tissuelyser II (Quiagen), the sample is comminuted at 30 impacts per minute for 6 minutes and then centrifuged off at 16 000 g for 5 minutes. 50 µL of the supernatant are transferred to an autosampler vial and made up with 50 µL of ammonium acetate buffer (10 mM, pH 6.8) and with 5 µL of ISTD. After again being shaken, the tumour sample is ready for measurement.

The measurement of both matrix samples takes place, lastly, with the aid of an HPLC-coupled, atmospheric pressure ionization/tandem mass spectrometer by means of a Turbo Ion Spray Interface (TISP) on an API4000 instrument from SCIEX.

HPLC/LC-MSMS (TISP) analysis runs on an HP1100 pump (Agilent) with a Gemini column (5 µm C18 110 A, 50 × 3 mm, Phenomenex).

C-7. Activity test in vivo

The activity of the conjugates of the invention was tested in vivo by means for example of xenograft models. The skilled person knows of methods in the prior art for testing the activity of a conjugate of the invention (see, for example, WO 2005/081711; Polson et al., Cancer Res. 2009 Mar 15;69(6):2358-64). For this purpose, for example, rodents (e.g. mice) were implanted with a tumour cell line which expresses the target molecule of the binder. These tumour-carrying rodents were subsequently administered either a conjugate of the invention or a control antibody

conjugate, or isotonic salt solution. Administration took place singly or more often. The tumour growth was determined twice a week by means of a sliding caliper. After a tumour growth of several weeks, the tumour size of conjugate-treated animals and the control group was compared. The conjugate-treated animals showed a significantly lower tumour size.

C-7a. Testing of ADCs in experimental tumours in the mouse

Human tumour cells which express FGFR2 were inoculated subcutaneously into the flank of immunosuppressed mice, such as nude mice or SCID mice. 1-10 million cells are detached from the cell culture, centrifuged and resuspended with 100 µl of medium, 50% medium/50% Matrigel or 100% Matrigel. The cell suspension was injected beneath the skin of the mouse.

Within a few days, a tumour grew. Treatment began no earlier than at a tumour size of 20-25 mm^2 after establishment of a tumour.

Treatment with conjugate took place via the intravenous route into the caudal vein of the mouse. The conjugate was dissolved in PBS and is administered with a volume of 5-10 ml/kg.

The treatment scheme was governed by the pharmacokinetics of the antibody. As a standard, treatment took place three times following every fourth day or every seventh day. Treatment, however, may also be continued further, or a second cycle with three days of treatment may follow at a later point in time.

As a standard basis, 8 animals were used per treatment group. This number may be higher if particularly strong fluctuations in tumour growth or after treatment are anticipated. As well as the groups which receive the active substances, one group, as a control group, was treated only with the buffer, in accordance with the same scheme.

In the course of the experiment, the area of the tumour was measured regularly using a sliding caliper in two dimensions (length/width). The area of the tumour was calculated by means of the formula length × width.

At the end of the experiment, the tumours were removed and weighed. The ratio of the average tumour weights for the therapy group (T) to the control group (C) was expressed as T/C. If control and treatment groups finished at different times, then the T/C value was calculated using the tumour areas of the last common measurement of all treatment and control groups.

C-7b. Testing of FGFR2-ADC in the SNU-16 xenograft model in the mouse

Two million SNU-16 stomach carcinoma cells were inoculated subcutaneously into the flank of female NODscid mice.

Intravenous treatment with the conjugates was commenced at an average tumour size of 20-30 mm^2. When the control groups had reached the maximum permissible size, these groups were ended. The experimental groups treated with FGFR2 conjugates were ended when the tumours began to grow again. The activity of the conjugates was determined on day 31, the last point in time at which the vehicle control was still in the experiment. All of the FGFR2 conjugates tested inhibited tumour growth in a dose-dependent manner. At a dose of 5 mg/kg, Example 1 achieved a T/C of 0.08, Example 3 a T/C of 0.06 and Example 26 a T/C of 0.10. For all of the animals treated, the tumour at this point in time was smaller than at the beginning of treatment (partial regression of the tumour). At a dose of 2.5 mg/kg, Example 26 achieved a T/C of 0.14. At this dose, Example 26 led to partial regression in 40% of the animals. At the dose of 1 mg/kg, Example 1 achieved a T/C of 0.15 and Example 3 of 0.36. At this dose as well, Example 1 leads to partial regression in all of the animals treated, whereas in the case of Example 3 no partial regressions were obtained. Significant anti-tumour effect in comparison to the control was achieved for all of the conjugates tested, up to a dose of 1 mg/kg. The corresponding control antibody-conjugates showed no activity at all in this model at the same doses.

C7c Testing of FGFR2-ADC in the MFM-223 xenograft model in the mouse

10 million MFM-223 breast carcinoma cells were inoculated subcutaneously into the flank of female NMRI nu/nu mice. These mice had been supplemented beforehand with estradiol pellets.

Intravenous treatment with the conjugates was commenced at an average tumour size of 30-35 mm^2. When the control groups had reached the maximum permissible size on day 40, all of the treatment groups were ended and the tumour weights were ascertained. Example 26, with a dose of 10 mg/kg, achieved a T/C of 0.09, a T/C of 0.13 at a dose of 5 mg/kg and a T/C of 0.26 at a dose of 1 mg/kg. Significant anti-tumour effect in comparison to the control was achieved for all three doses tested. The corresponding control antibody conjugate showed a significant non-specific effect in this model only at the dose of 10 mg/kg.

C7d Testing of FGFR2-2 ADC in the NCI-H716 xenograft model in the mouse

1.5 million NCI-H716 intestinal carcinoma cells were inoculated subcutaneously into the flank of female NMRI nu/nu mice.

Intravenous treatment with the conjugates was commenced at an average tumour size of 25-30 mm^2. When the control groups had reached the maximum permissible size on day 36, all of the treatment groups were ended and the tumour weights were ascertained. Treatment with Example 26 resulted in a significant reduction in tumour weight, achieving a T/C of 0.24 at 5 mg/kg. At this dose, the corresponding control conjugate had no activity at all in this model.

D. Working examples for pharmaceutical compositions

The compounds of the invention can be converted as follows into pharmaceutical preparations:

i.v. Solution:

The compound of the invention is dissolved at a concentration below the saturation solubility in a physiologically tolerated solvent (e.g. isotonic saline solution, D-PBS, or a formulation with glycine and sodium chloride in citrate buffer with addition of polysorbate 80). The solution is subjected to sterile filtration and dispensed into sterile and pyrogen-free injection containers.

i.v. Solution:

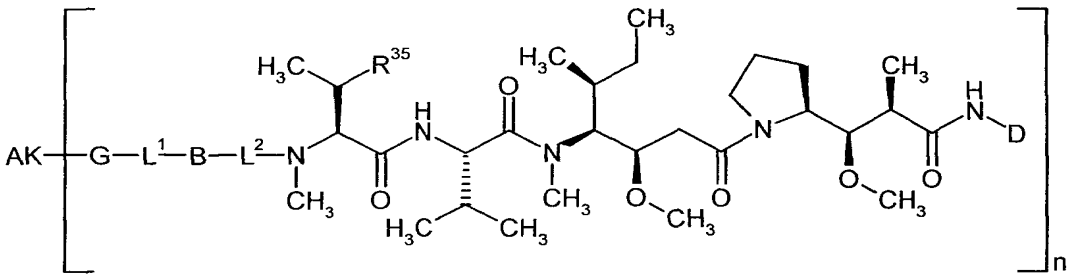
The compounds of the invention can be converted into the administration forms cited. This can be accomplished in a known way by "mixing with" or "dissolving in" inert, non-toxic, pharmaceutically suitable excipients (e.g. buffer substances, stabilizers, solubilizers, preservatives). The following, for example, may be present: amino acids (glycine, histidine, methionine, arginine, lysine, leucine, isoleucine, threonine, glutamic acid, phenylalanine and others), sugars and related compounds (glucose, saccharose, mannitol, trehalose, sucrose, mannose, lactose, sorbitol), glycerol, sodium salts, potassium, ammonium salts and calcium salts (e.g. sodium chloride, potassium chloride or disodiumhydrogenphosphate and many others), acetate/acetic acid buffer systems, phosphate buffer systems, citric acid and citrate buffer systems, trometamol (TRIS and TRIS salts), Polysorbates (e.g. Polysorbate 80 and Polysorbate 20), Poloxamers (e.g. Poloxamer 188 and Poloxamer 171), Macrogols (PEG derivatives, e.g. 3350), Triton X-100, EDTA salts, glutathione, albumins (e.g. human), urea, benzyl alcohol, phenol, chlorocresol, metacresol, benzalkonium chloride and many others.

Lyophilizate for subsequent conversion into an i.v., s.c. or i.m. solution:

Alternatively the compounds of the invention may be converted into a stable lyophilizate (possibly with the aid of abovementioned excipients) and, before being administered, reconstituted with a suitable solvent (e.g. injection-grade water, isotonic saline solution) and administered.

Claims

1. Binder-drug conjugates of the general formula (Ia)



(Ia),

in which

n is a number from 1 to 50,

AK is a binder which binds to FGFR2,

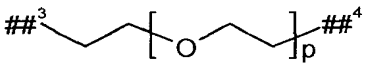
the group §-G-L^1-B-§§ is a linker,

where

§ marks the linkage site with the group AK and

§§ marks the linkage site with the nitrogen atom,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

##^{3} marks the linkage site with the group B,

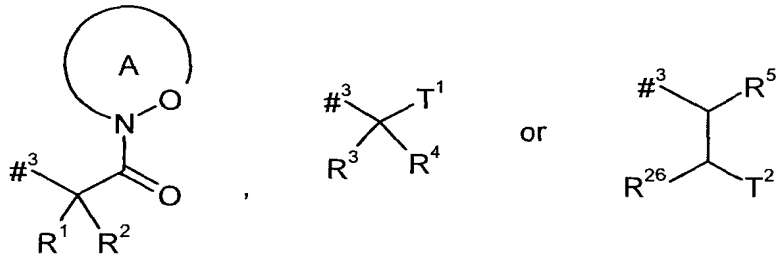
##^{4} marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



where

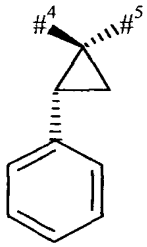
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

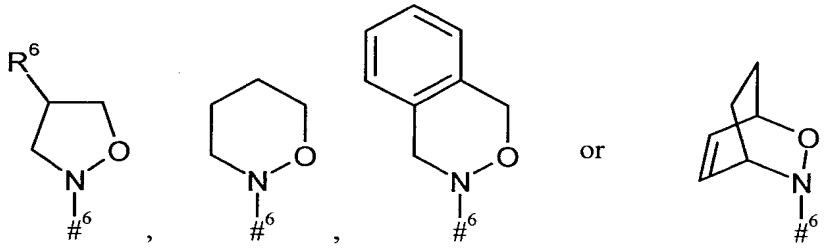


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

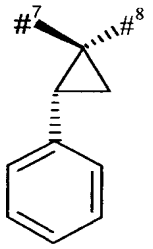
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

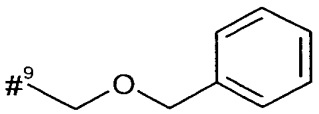
or

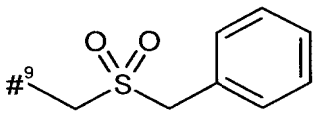
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

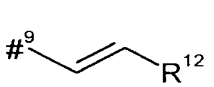
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

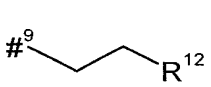
R^5 is hydrogen, methyl or a group of the formula

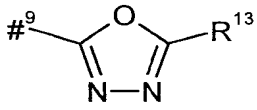


,


,



,


or


in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

2. Binder-drug conjugates of the general formula (Ia) according to Claim 1, in which

n is a number from 1 to 50,

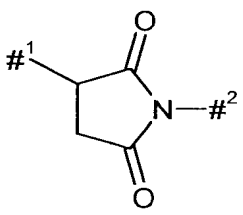
AK is AK_1 or AK_2

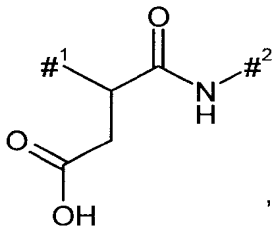
where

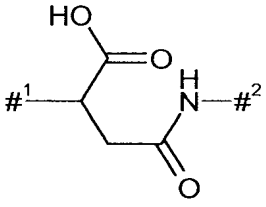
AK_1 is a binder which binds to FGFR2 and is bonded via a sulphur atom of the binder to the group G,

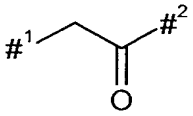
AK_2 is a binder which binds to FGFR2 and is bonded via a nitrogen atom of the binder to the group G,

G when AK = AK_1, is a group of the formula



,


,


or


where

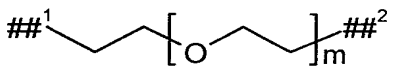
$\#^1$ marks the linkage site with the sulphur atom of the binder,

$\#^2$ marks the linkage site with the group L^1,

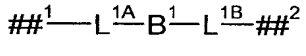
or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula



or



where

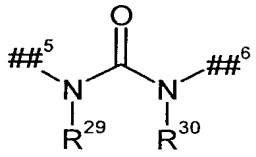
m is a number from 2 to 6,

1 marks the linkage site with the group G,

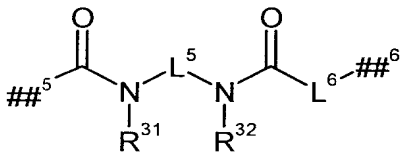
$\#\#^2$ marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

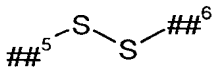
B^1 is a group of the formula



,



or



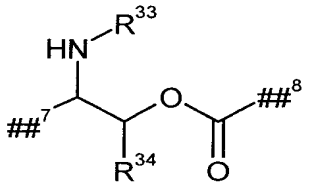
in which

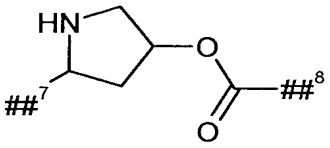
5 marks the linkage site with the group L^{1A},

6 marks the linkage site with the group L^{1B},

L^5 is a bond or (C_2-C_4)-alkanediyl,

L^6 is a bond or a group of the formula



or


in which

[7] marks the linkage site with the carbonyl group,

[8] marks the linkage site with L^{1B},

R^{33} is hydrogen, (C_1-C_4)-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

R^{34} is hydrogen or methyl,

R^{29} is hydrogen or (C_1-C_4)-alkyl,

R^{30} is hydrogen or (C_1-C_4)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or (C_1-C_4)-alkyl,

R^{32} is hydrogen or (C_1-C_4)-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear (C_2-C_{10})-alkanediyl,

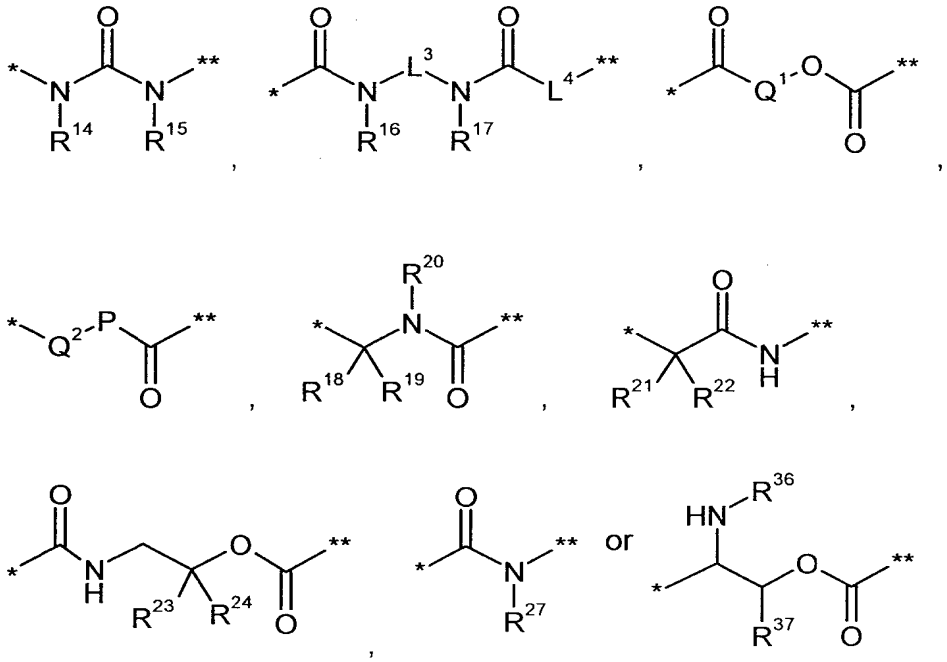
and

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula



where

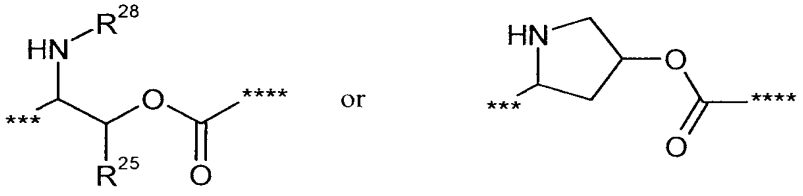
* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

L^3 is a bond or (C_2-C_4)-alkanediyl,

L^4 is a bond or a group of the formula



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, tert-butyloxycarbonyl or benzyloxycarbonyl,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{14} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{15} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{14} and R^{15} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{16} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{17} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{18} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{23} is (C_1-C_4)-alkyl,

R^{24} is hydrogen or (C_1-C_4)-alkyl,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

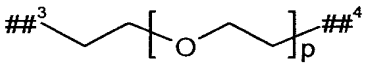
R^{36} is hydrogen, (C_1-C_4)-alkylcarbonyl, tert-butyloxycarbonyl or benzyl-oxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

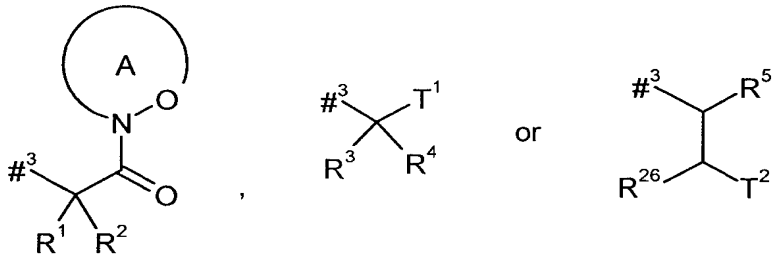
##4 marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



in which

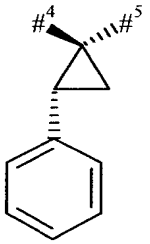
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

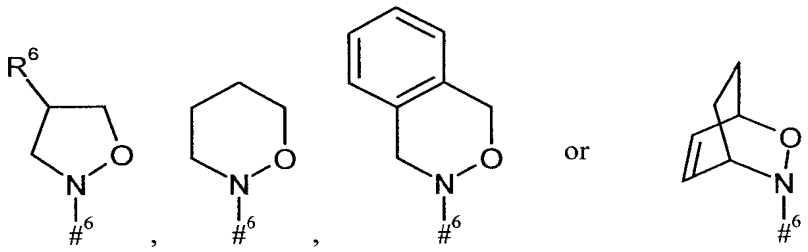


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

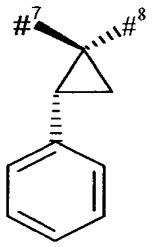
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula -C(=O)-OR^7, -C(=O)-NR^8R^9, -C(=O)-NH-NH-R^{10} or -CH_2-O-R^{11},

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

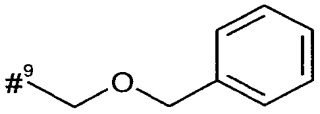
or

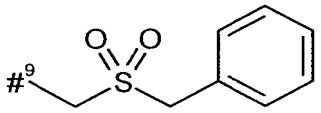
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

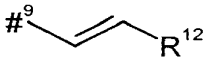
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

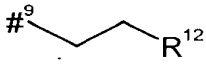
R^5 is hydrogen, methyl or a group of the formula

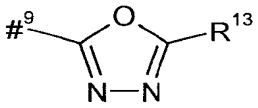


,


,



,


or


in which

$\#^9$ marks the linkage site with –CHC(R^{26})-T^2,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula –$S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

3. Binder-drug conjugates of the general formula (Ia) according to Claim 1 or 2, in which

n is a number from 1 to 20,

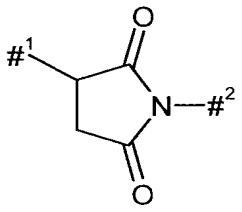
AK is AK_1 or AK_2

where

AK_1 is a binder which binds to FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

AK_2 is a binder which binds to FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G when AK = AK_1, is a group of the formula



in which

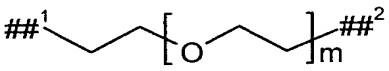
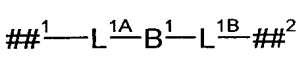
$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula

 or 

where

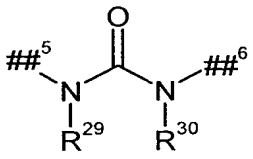
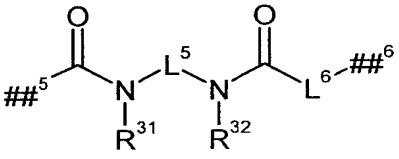
m is a number from 2 to 6,

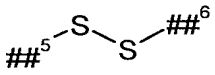
[1] marks the linkage site with the group G,

[2] marks the linkage site with the group B,

L^{1A} is linear (C_2-C_6)-alkanediyl,

B^1 is a group of the formula

 , 

or 

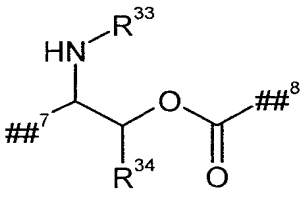
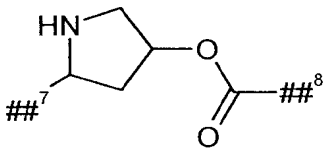
in which

[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond,

L^6 is a bond or a group of the formula

 or 

in which

[7] marks the linkage site with the carbonyl group,

[8] marks the linkage site with L^{1B},

R^{33} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{34} is hydrogen or methyl,

R^{29} is hydrogen,

R^{30} is hydrogen,

R^{31} is hydrogen or methyl,

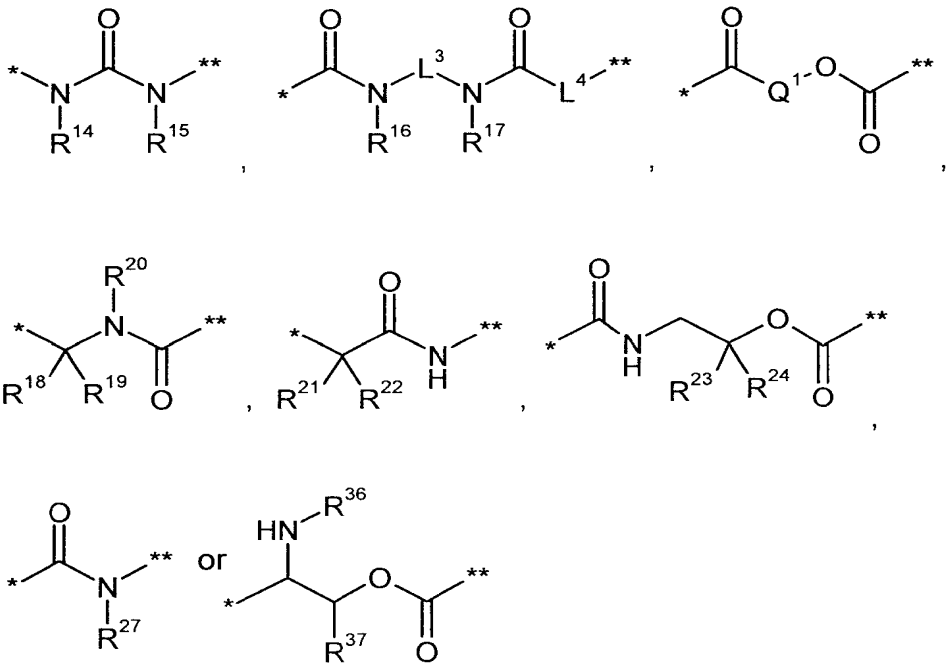
R^{32} is hydrogen or methyl,

L^{1B} is linear (C_2-C_6)-alkanediyl,

and

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



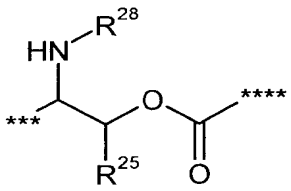
where

* marks the linkage site with L^1,

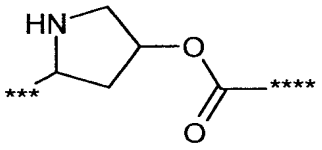
** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



or



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is hydrogen or methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

Q^1 is a 4- to 7-membered heterocycle,

R^{14} is hydrogen,

R^{15} is hydrogen,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

R^{18} is hydrogen,

R^{19} is hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} is hydrogen or methyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or methyl,

R^{22} is hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{23} is methyl,

R^{24} is hydrogen or methyl,

R^{27} is hydrogen,

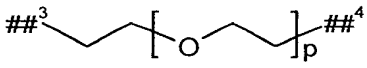
R^{36} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

R^{37} is hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bonded form a pyrrolidine ring,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



where

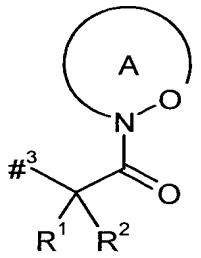
p is a number from 2 to 6,

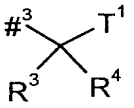
3 marks the linkage site with the group B,

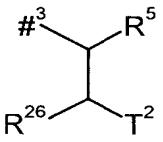
##4 marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 or 2 methyl substituents,

D is a group of the formula



,


or


where

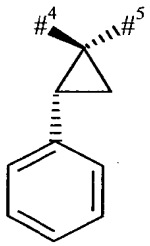
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

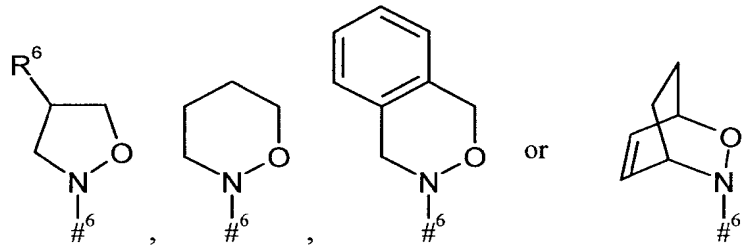


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

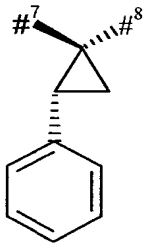
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

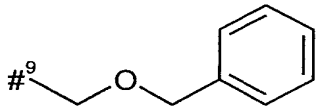
or

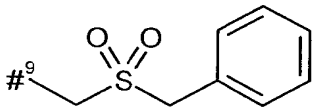
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

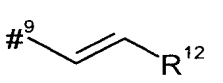
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

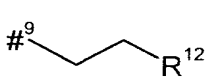
R^5 is hydrogen, methyl or a group of the formula

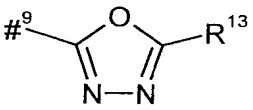


,


,



,


or


in which

#9 marks the linkage site with –CHC(R^{26})-T^2,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula –$S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

4. Binder-drug conjugates of the general formula (Ia) according to any of Claims 1 to 3, in which

n is a number from 1 to 10,

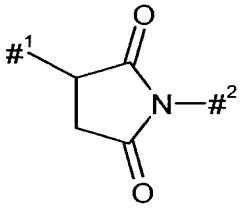
AK is AK_1 or AK_2

where

AK_1 is a binder which binds FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

AK_2 is a binder which binds FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G when AK = AK_1, is a group of the formula



in which

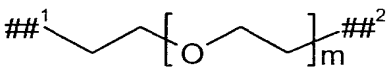
$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

or

when AK = AK_2, is carbonyl,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula



where

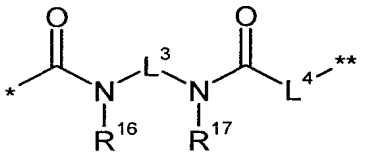
m is a number 2 or 3,

[1] marks the linkage site with the group G,

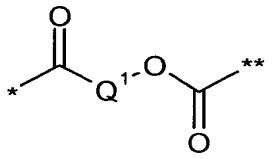
##[2] marks the linkage site with the group B,

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

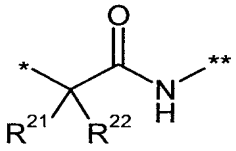
B is a bond or a group of the formula



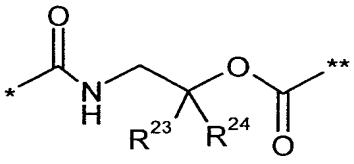
,



,



or



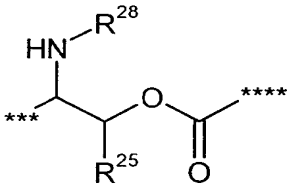
where

* marks the linkage site with L^1,

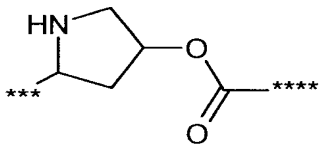
** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



or



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

Q^1 is piperidine-1,4-diyl,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

R^{21} is hydrogen or methyl,

R^{22} is hydrogen or methyl,

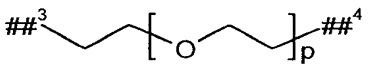
or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{23} is methyl,

R^{24} is hydrogen,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



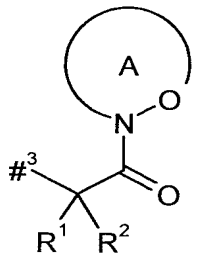
where

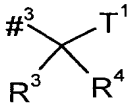
p is a number from 2 to 6,

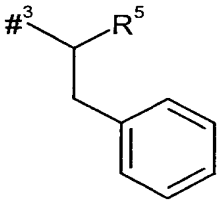
3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

D is a group of the formula



,


or


where

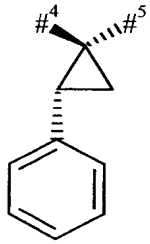
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula