부분적으로 환원된 유도체를 모두 포함한다. 상기 아로메틱기는 5 내지 15각형으로 이루어진 단순 또는 융합 고리형이며, 헤테로아로메틱기는 산소, 황 또는 질소를 하나 이상 포함하는 아로메틱기를 의미한다. 대표적인 아릴기의 예로는 페닐, 나프틸, 피리디닐(pyridinyl), 푸라닐(furanyl), 티오페닐(thiophenyl), 인돌릴(indolyl), 퀴놀리닐(quinolinyl), 이미다졸리닐(imidazolinyl), 옥사졸릴(oxazolyl), 티아졸릴(thiazolyl), 테트라히드로나프틸 등이 있으나 이에 한정되는 것은 아니다.

[76] 상기 C_1-C_8의 알킬기, C_3-C_6의 시클로알킬기, C_1-C_4의 알콕시기, C_1-C_4의 알킬기, 및 아릴기는 한 개 또는 그 이상의 수소가 C_1-C_4의 알킬기, C_2-C_4의 알케닐기, C_2-C_4의 알키닐기, C_3-C_{10}의 시클로알킬기, C_1-C_4의 할로알킬기, C_1-C_4의 알콕시기, C_1-C_4의 티오알콕시기, 아릴기, 아실기, 히드록시, 티오(thio), 할로겐, 아미노, 알콕시카르보닐, 카복시, 카바모일, 시아노, 니트로 등으로 치환될 수 있다.

[77] 본 명세서에서 항체는 최대한 넓은 의미로 사용되며, 구체적으로 무손상(intact) 모노클로날 항체, 폴리클로날 항체, 2종 이상의 무손상 항체로부터 형성된 다중특이성 항체(예를 들어, 이중특이성 항체), 및 목적하는 생물학적 활성을 나타내는 항체 단편을 포함한다. 항체는 특이적인 항원을 인식하고 결합할 수 있는 면역계에 의하여 생성되는 단백질이다. 그 구조적인 면에서, 항체는 통상적으로 4개의 아미노산 쇄(2개의 중쇄 및 2개의 경쇄)로 이루어진 Y-형상의 단백질을 가진다. 각각의 항체는 주로 가변 영역 및 불변 영역의 2개의 영역을 가진다. Y의 팔의 말단 부분에 위치한 가변 영역은 표적 항원에 결합하고 상호작용한다. 상기 가변 영역은 특정 항원 상의 특이적 결합 부위를 인식하고 결합하는 상보성 결정 영역(CDR)을 포함한다. Y의 꼬리 부분에 위치한 불변 영역은 면역계에 의하여 인식되고 상호작용한다. 표적 항원은 일반적으로 다수의 항체 상의 CDR에 의하여 인식되는, 에피토프라고도 하는 다수의 결합 부위를 가지고 있다. 상이한 에피토프에 특이적으로 결합하는 각각의 항체는 상이한 구조를 가진다. 그러므로, 한 항원은 하나 이상의 상응하는 항체를 가질 수 있다.

[78] 본 발명의 한 실시형태로서, 항체는 암세포 항원에 면역특이적으로 결합하는 항체일 수 있다. 암세포 항원에 면역특이적인 항체는 상업적으로 입수하거나, 재조합 발현 기술과 같이 당업계에 공지된 방법에 의해 제조될 수 있다. 암세포 항원에 면역특이적인 항체를 코딩하는 뉴클레오티드 서열은 예를 들어 진뱅크 데이터베이스 또는 문헌으로부터 얻거나, 통상의 클로닝 및 시퀀싱에 의해 얻을 수 있다. 암 치료에 이용가능한 항체의 예는 전이성 유방암의 치료를 위한 인간화된 항-HER2 항체인 허셉틴®(제넨테크사의 트라스투주맙), 비-호지킨 림프종의 치료를 위한 키메라 항-CD20 항체인 리툭산®(제넨테크사의 리툭시맙), 두부 및 목의 암과 같은 상피성장인자 양성 암의 치료를 위한 키메라 항-EGFR 항체인 어비툭스®(임클론 시스템즈사의 세툭시맙) 및 육종의 치료를 위한 인간화된 항-인테그린 αvβ3 항체인 비탁신®(메드이뮨사)를 포함하나, 이에

제한되지 않는다.

[79] 본 발명의 항체-링커-약물 결합체에서 링커-약물은 항체의 라이신 잔기에 직접 결합된다. 따라서, 항체의 분자내 디설파이드 결합의 환원, 티올기 생성 시약으로 인한 잔기의 잔류 등 항체 단백질 구조의 불필요한 변형이 발생하지 않고, 항체의 비정상적인 파편이나 응집의 발생이 최소화되어 90% 이상, 바람직하게는 92% 이상, 더욱 바람직하게는 97% 이상, 더욱 바람직하게는 98%이상의 정상 항체 구조 비율을 가진다.

[80]

[81] 본 발명의 항체-링커-약물 결합체는 바람직하게는,

[82] i) 상기 화학식 I-1에서,

[83] Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

[84] X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

[85] R_1은 수소, 히드록시 또는 C_1-C_4의 알콕시기이고,

[86] R_2는 수소 또는 C_1-C_4의 알킬기이며,

[87] R_3는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

[88] R_4는 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐인 항체-링커-약물 결합체 이거나,

[89] ii) 상기 화학식 I-2에서,

[90] Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

[91] X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

[92] R_1, R_2 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

[93] R_3는 C_1-C_4의 알킬기이며,

[94] Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐이고,

[95] Z는 질소이며,

[96] R_5는 수소 또는 C_1-C_4의 알킬기이고,

[97] R_6는 수소, 히드록시 또는 C_1-C_4의 알콕시기인 항체-링커-약물 결합체 이거나,

[98] iii) 상기 화학식 I-3에서,

[99] Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

[100] X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

[101] R_1, R_2 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

[102] R_3는 C_1-C_4의 알킬기이며,

[103] R_5는 수소, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,

[104] Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,

[105] Y는 질소 또는 산소이고,

[106] R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지

않는 항체-링커-약물 결합체 이거나,

[107] iv) 상기 화학식 I-4에서

[108] Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

[109] X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

[110] R_1, R_2, R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

[111] R_3는 C_1-C_4의 알킬기이며,

[112] R_5는 수소, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,

[113] Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,

[114] Y는 질소 또는 산소이고,

[115] R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않는 항체-링커-약물 결합체 이다.

[116]

[117] 보다 바람직하게는, 본 발명의 항체-링커-약물 결합체는

[118] i) 상기 화학식 I-1에서,

[119] Ab가 트라스투주맙(trastuzumab)이고,

[120] X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

[121] R_1은 수소, 히드록시 또는 메톡시이고,

[122] R_2는 수소 또는 메틸이며,

[123] R_3는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

[124] R_4는 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐인 항체-링커-약물 결합체 이거나,

[125] ii) 상기 화학식 I-2에서,

[126] Ab가 트라스투주맙(trastuzumab)이고,

[127] X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

[128] R_1, R_2 및 R_3는 메틸이고,

[129] R_4는 수소 또는 메틸이며,

[130] Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐이고,

[131] Z는 질소이며,

[132] R_5는 수소 또는 메틸이고,

[133] R_6는 수소, 히드록시 또는 메톡시인 항체-링커-약물 결합체 이거나,

[134] iii) 상기 화학식 I-3에서,

[135] Ab가 트라스투주맙(trastuzumab)이고,

[136] X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

[137] R_1, R_2 및 R_3는 메틸이고,

[138] R_4는 수소 또는 메틸이며,

[139] R_5는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,

[140] Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,

[141] Y는 질소 또는 산소이고,

[142] R_6는 Y가 질소일 때 수소 또는 메틸이고, Y가 산소일 때 존재하지 않는 항체-링커-약물 결합체 이거나,

[143] iv) 상기 화학식 I-4에서

[144] Ab가 트라스투주맙(trastuzumab)이고,

[145] X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

[146] R_1, R_2 및 R_3는 메틸이고,

[147] R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 메틸이며,

[148] R_5는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,

[149] Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,

[150] Y는 질소 또는 산소이고,

[151] R_6는 Y가 질소일 때 수소 또는 메틸이고, Y가 산소일 때 존재하지 않는 항체-링커-약물 결합체 이다.

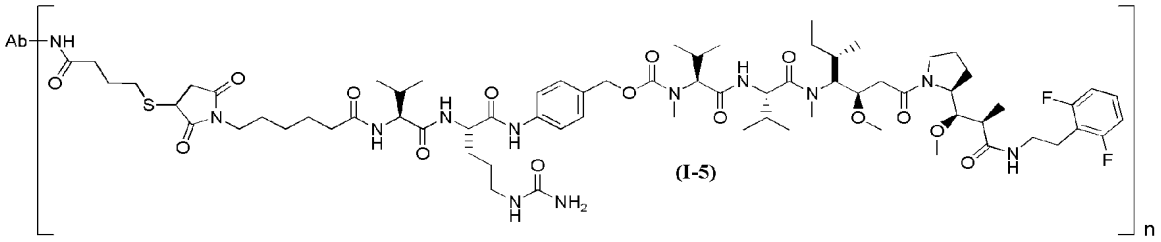
[152]

[153] 본 명세서에서 약제학적으로 허용되는 염은 무독성 무기산염 및 유기산염 모두를 포함하며, 예를 들어 염산염, 황산염, 질산염, 인산염, 아세테이트산염, 벤젠설포네이트산염, 시트레이트산염 등을 포함하나 이에 한정되는 것은 아니다.

[154]

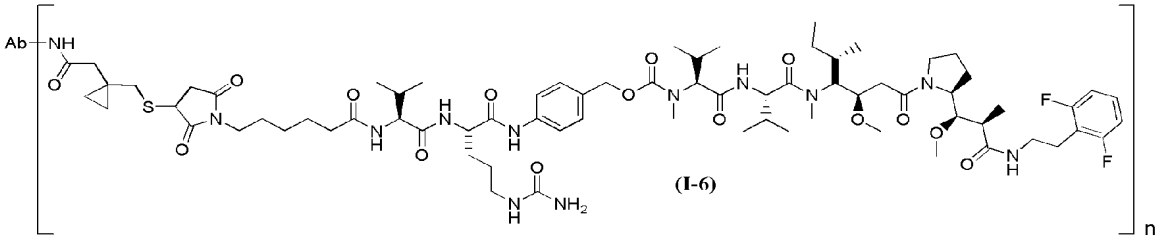
[155] 본 발명의 항체-링커-약물 결합체 중 대표적인 물질은 하기 그룹에서 선택될 수 있다.

[156]



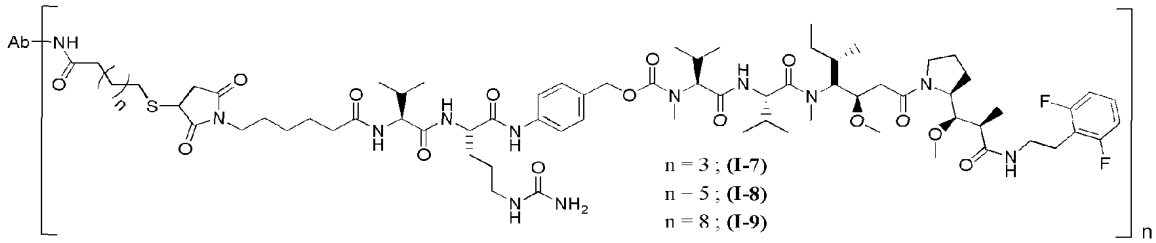
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[157]

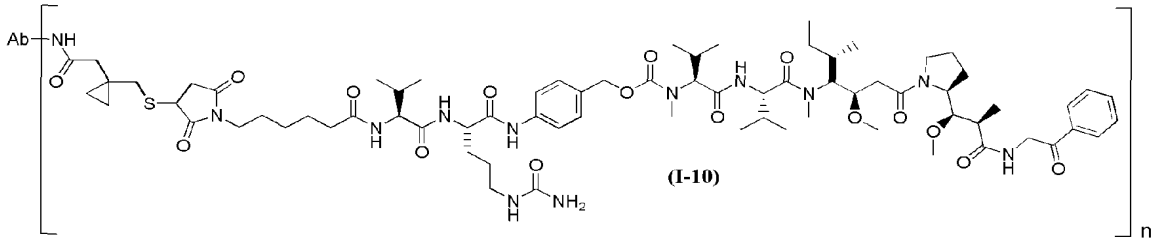


(I-6)

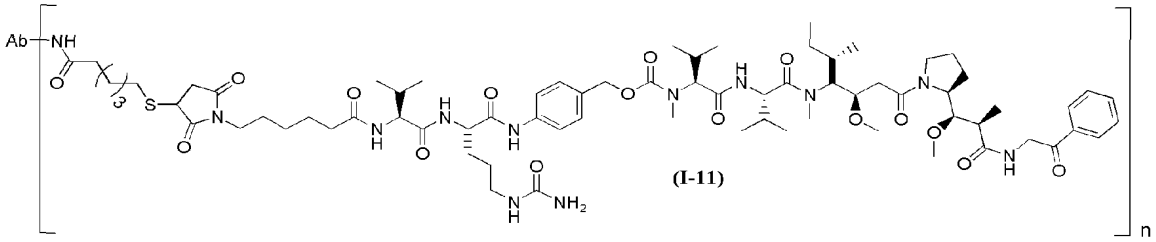
[158]



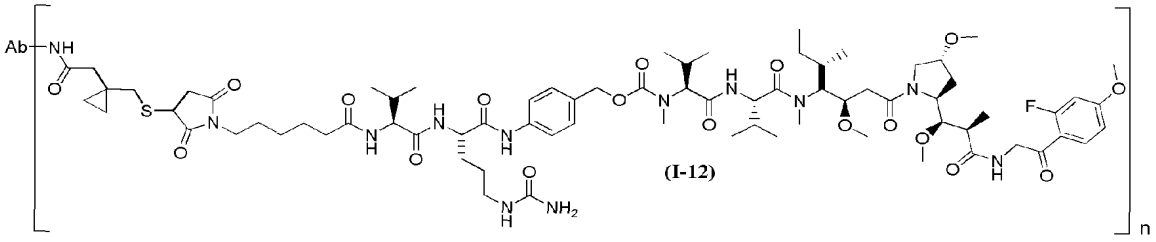
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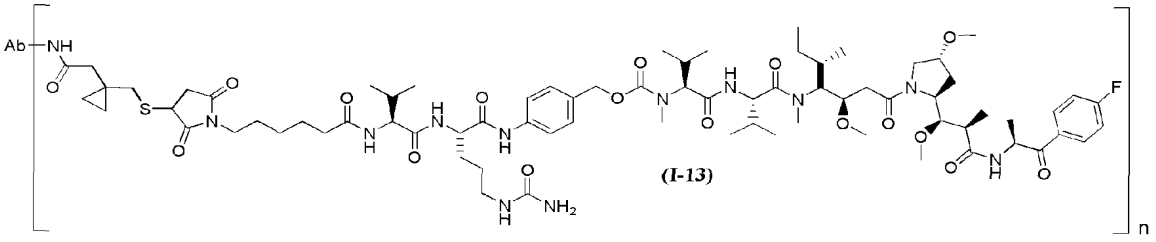
[160]



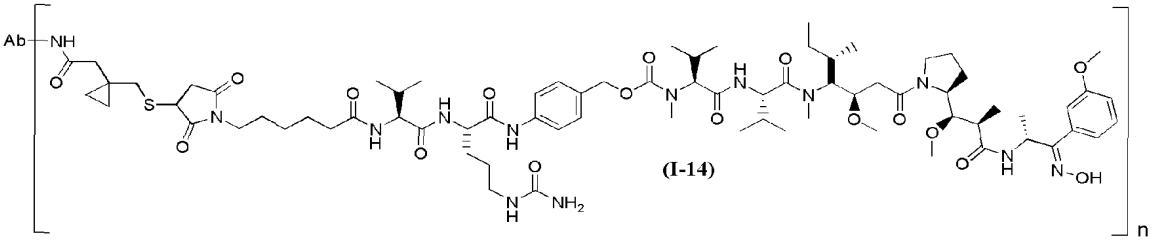
[161]



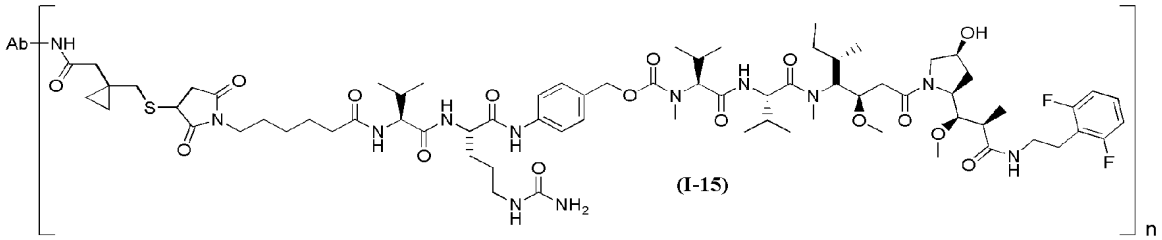
[162]



[163]

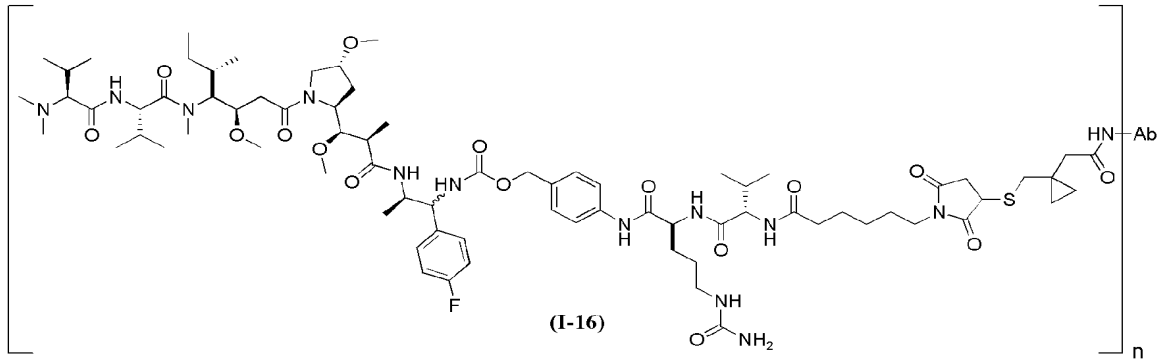


[164]



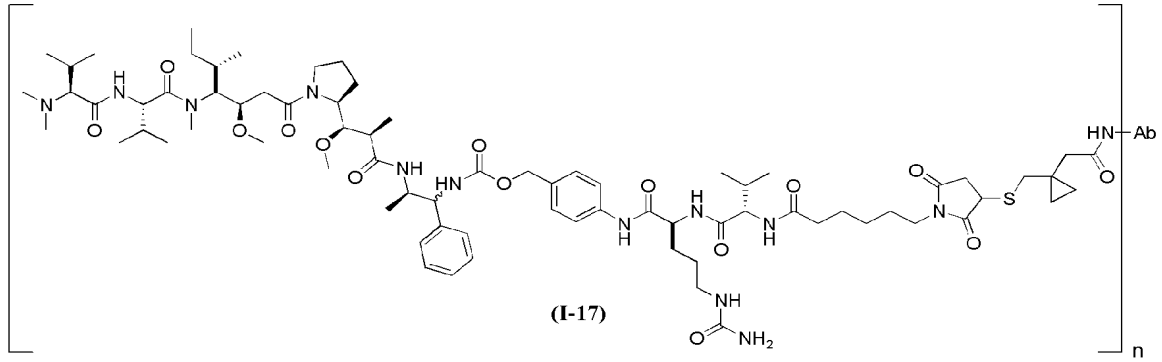
(I-15)

[165]



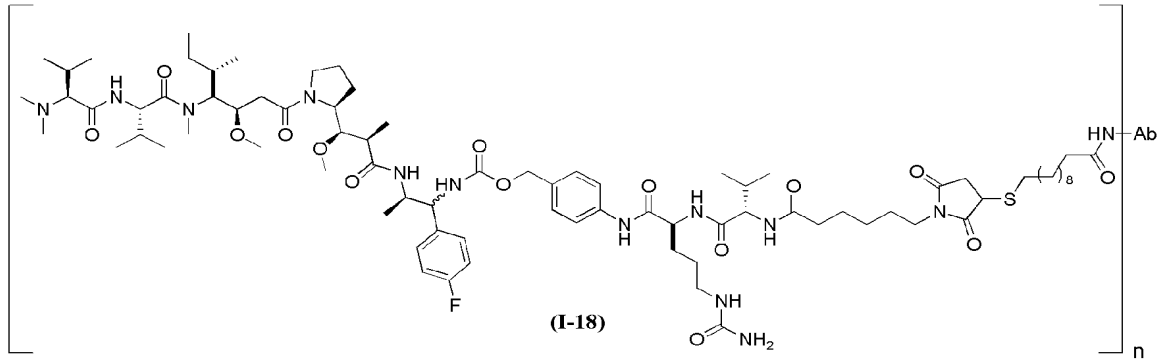
(I-16)

[166]



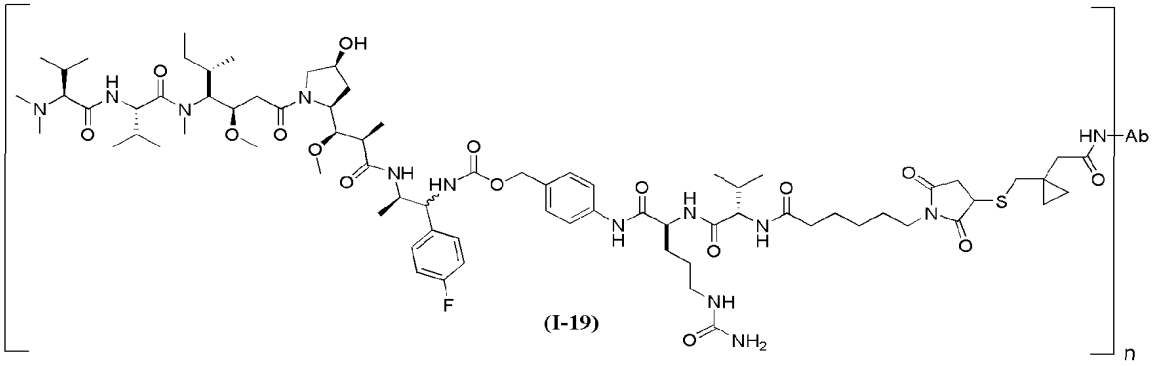
(I-17)

[167]



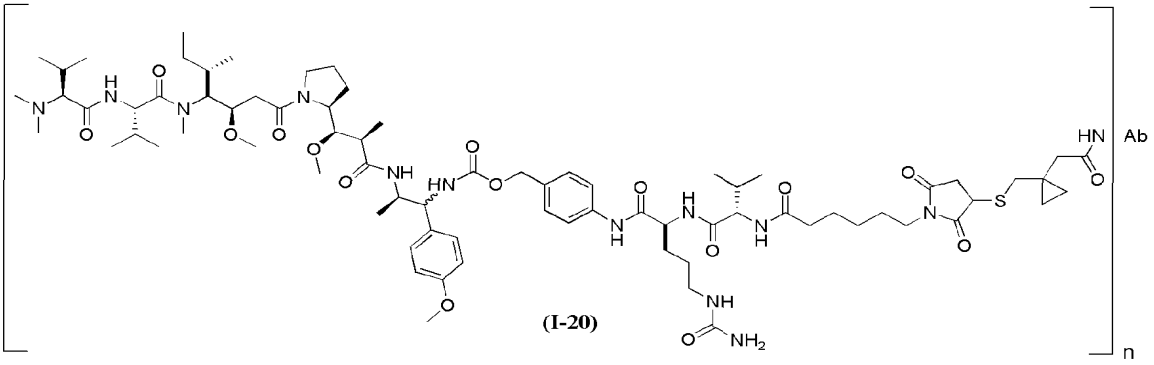
(I-18)

[168]



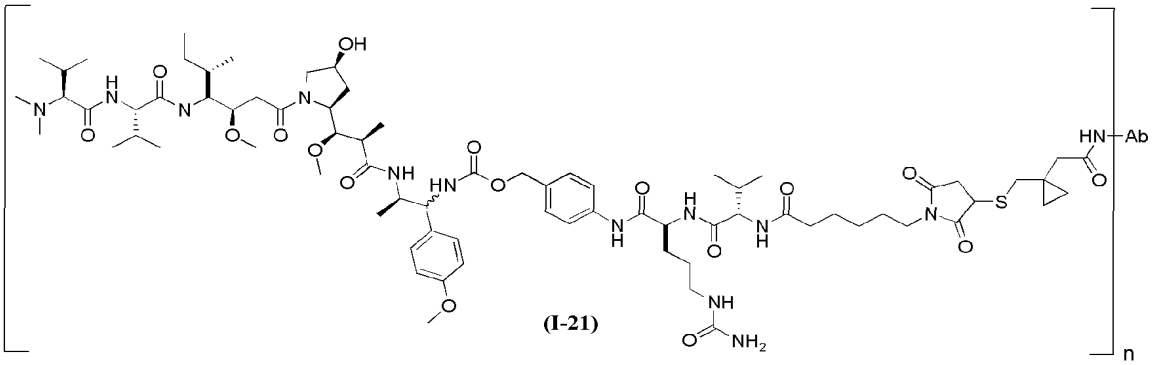
(I-19)

[169]



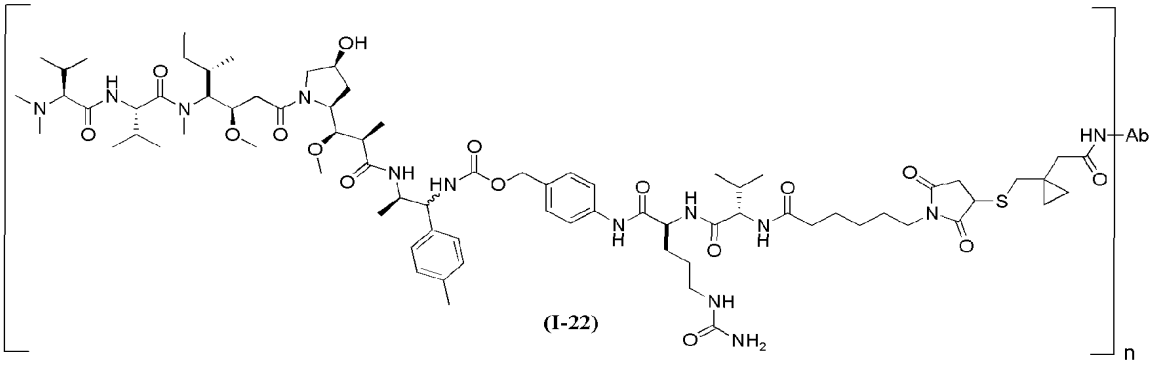
(I-20)

[170]



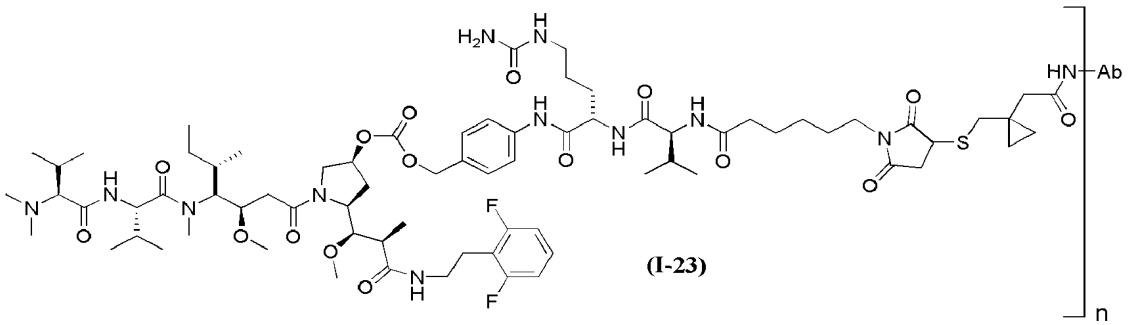
(I-21)

[171]

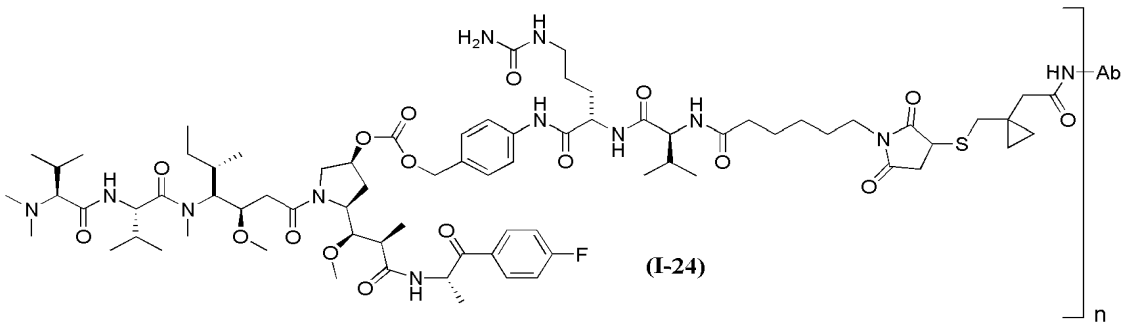


(I-22)

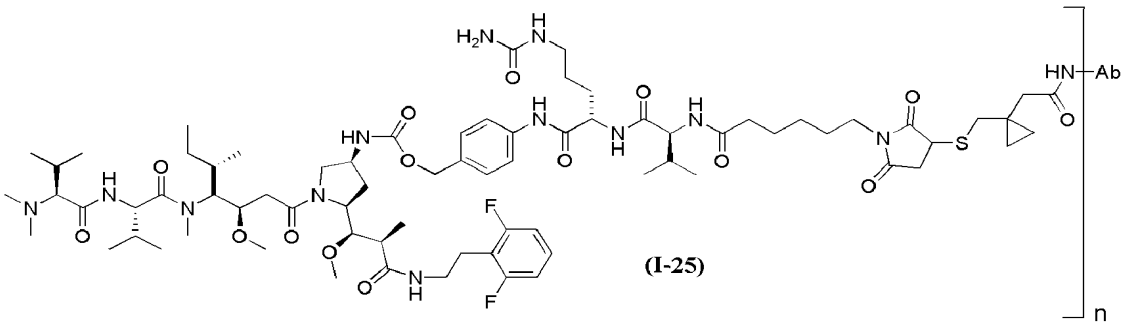
[172]



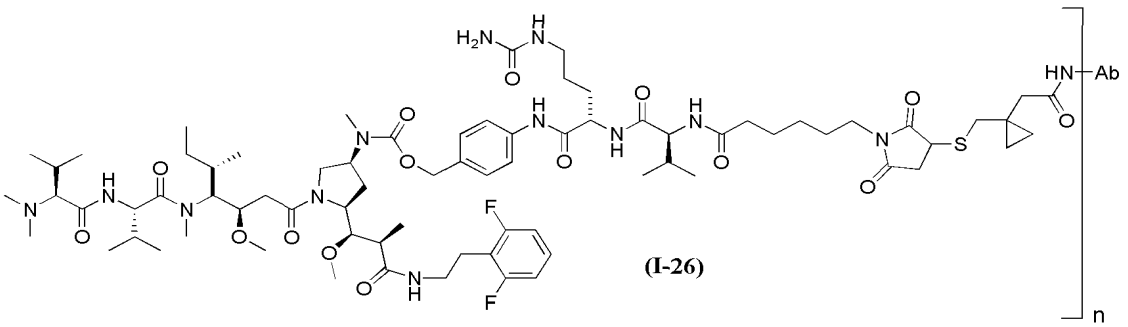
[173]



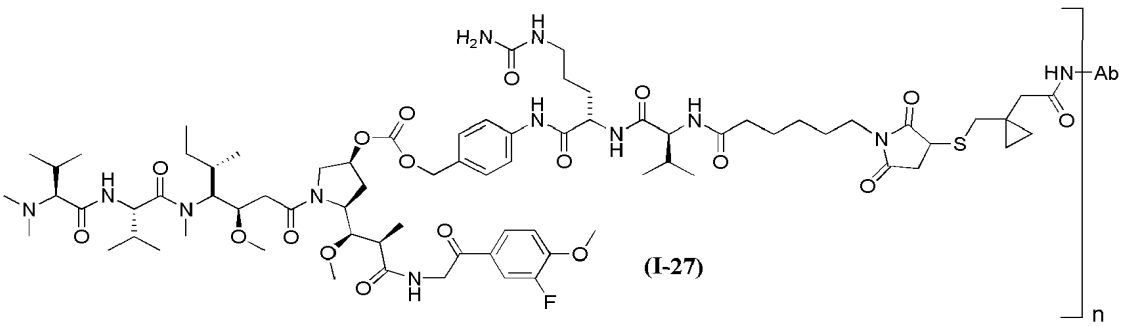
[174]



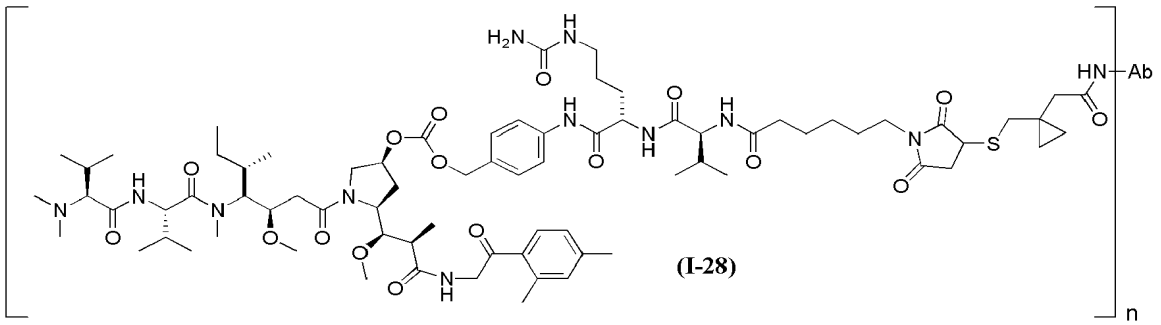
[175]



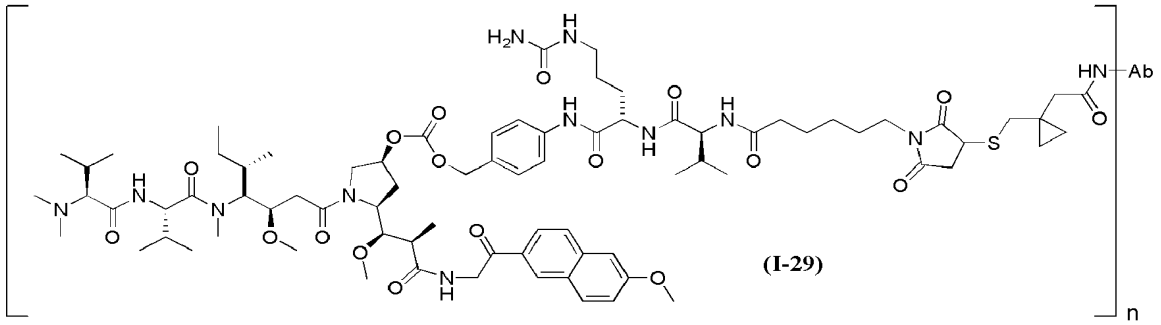
[176]



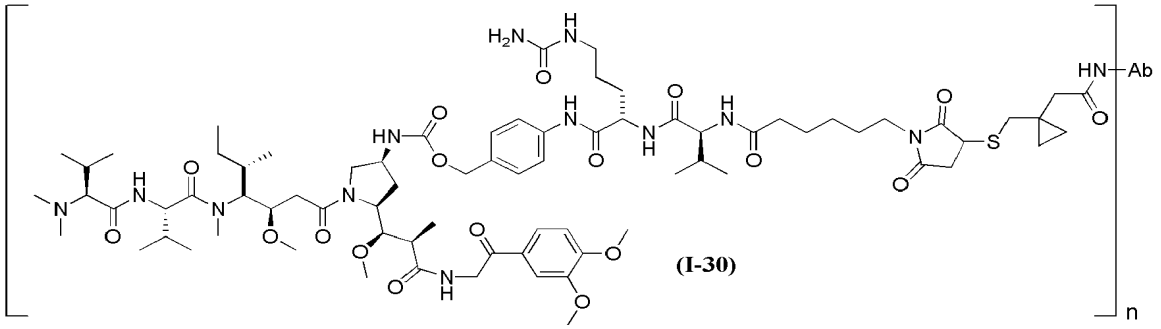
[177]



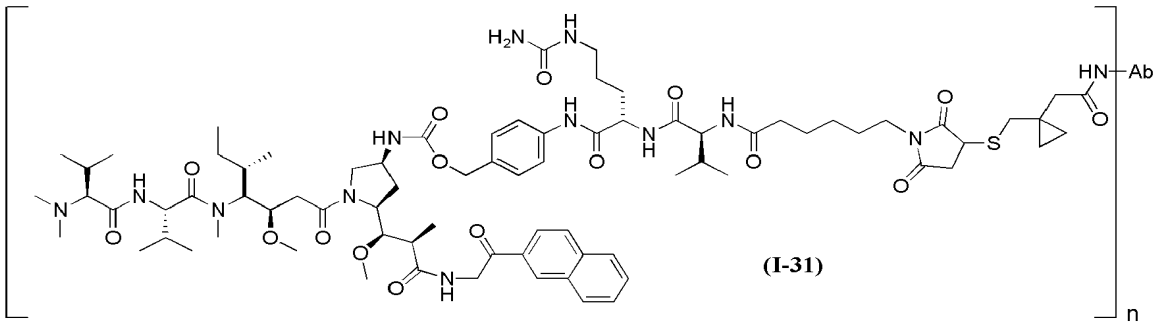
[178]



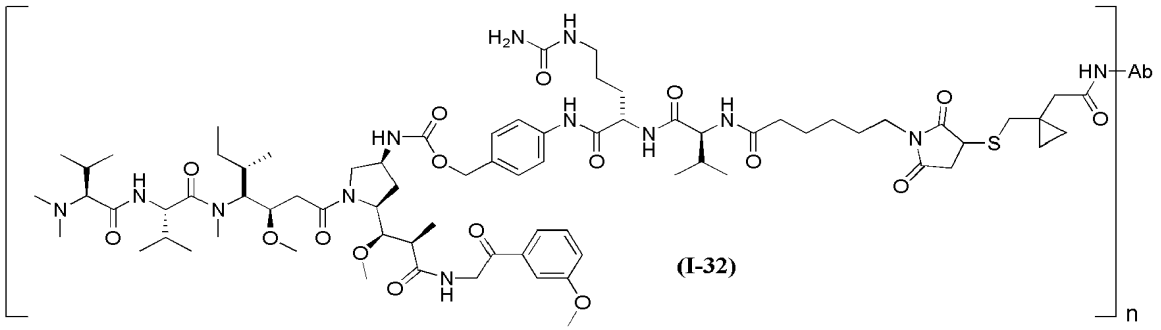
[179]



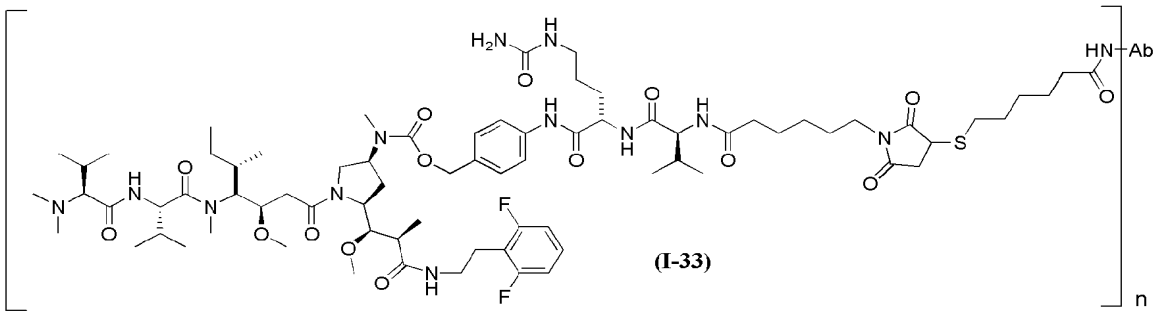
[180]



[181]

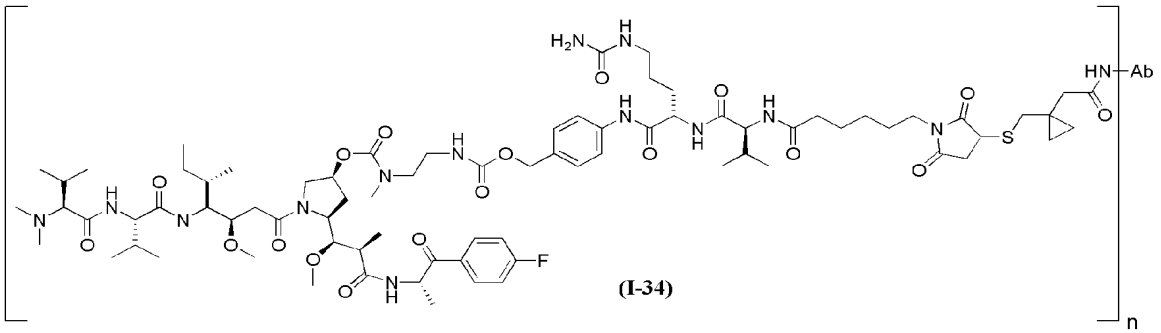


[182]



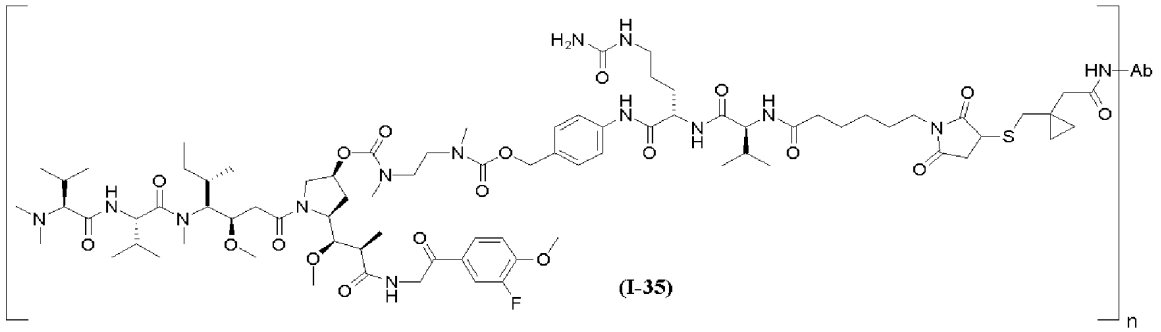
(I-33)

[183]



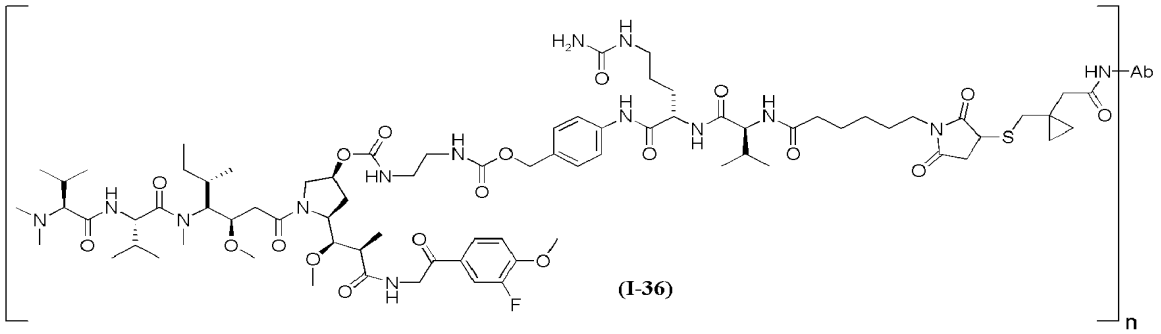
(I-34)

[184]



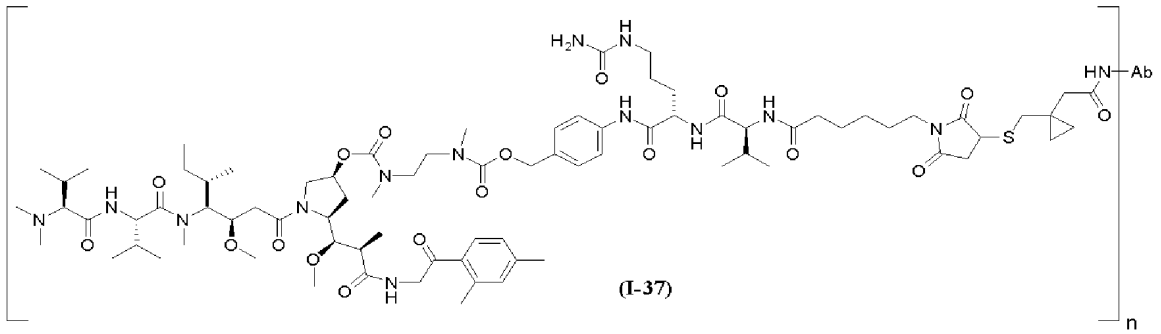
(I-35)

[185]



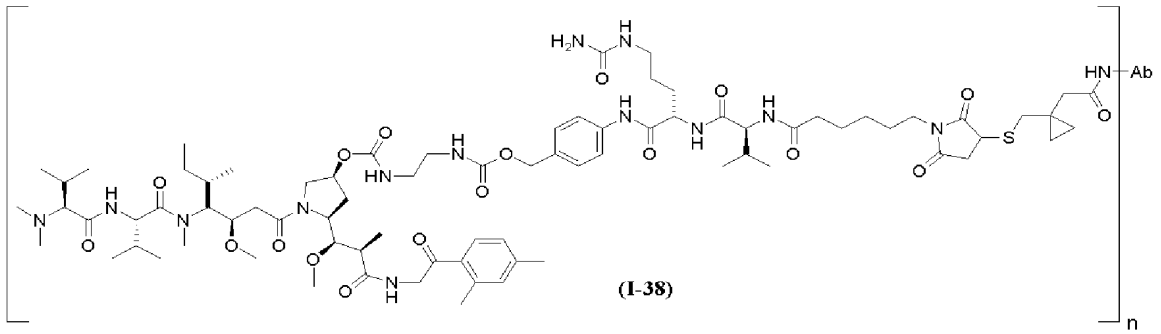
(I-36)

[186]

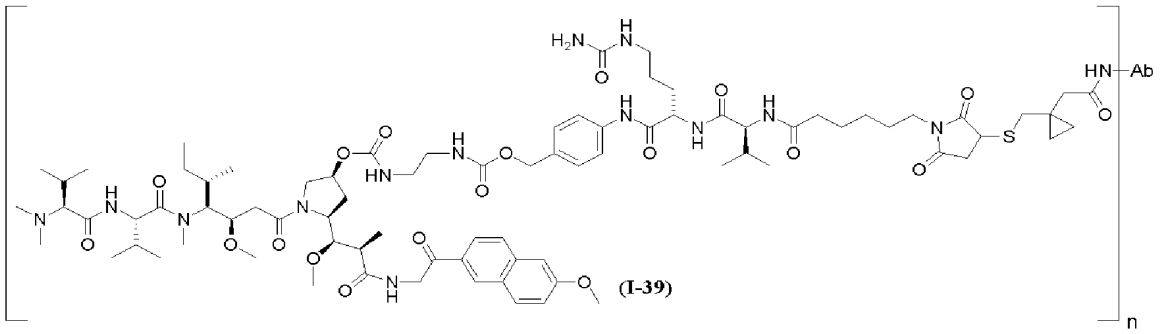


(I-37)

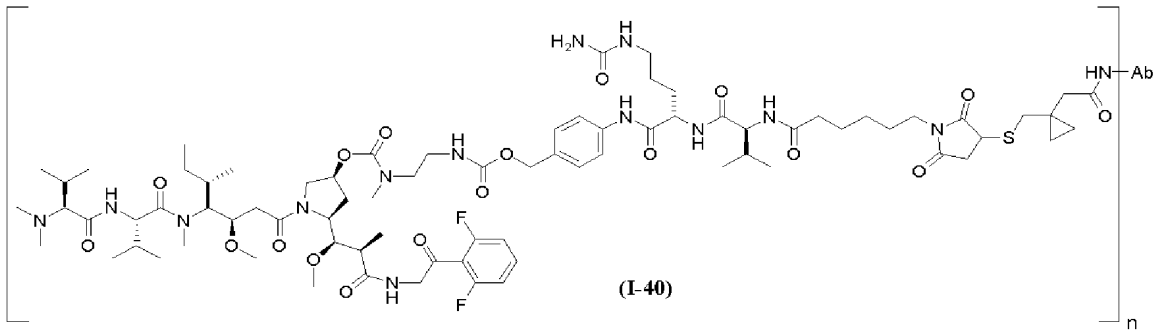
[187]



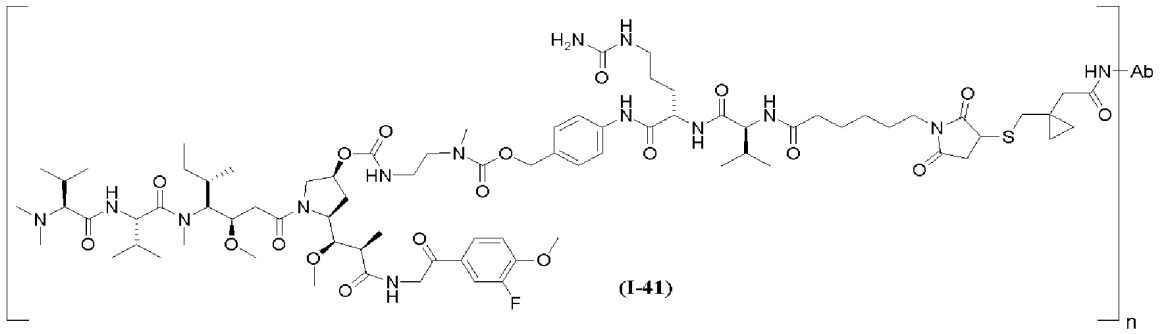
[188]



[189]



[190]



[191] 상기 화학식 I-5 내지 I-41 에서, n은 1 내지 5이다.

[192]

[193] 한편으로, 본 발명은 상기 화학식 I-1, I-2, I-3 또는 I-4의 항체-링커-약물 결합체의 제조방법에 관한 것으로, 본 발명의 제조방법은

[194] (i) 하기 화학식 II-1의 화합물과 하기 화학식 III-1, III-2 또는 III-3의 돌라스타틴 10 유도체를 축합반응시켜 각각 하기 화학식 IV-1, IV-2, IV-3의 화합물을 수득하거나, 하기 화학식 II-2의 화합물과 하기 화학식 III-4의 돌라스타틴 10

유도체를 축합반응시켜 하기 화학식 IV-4의 화합물을 수득하는 단계;

[195] (ii) 하기 화학식 IV-1, IV-2, IV-3 또는 IV-4의 화합물에 하기 화학식 V의 화합물을 첨가반응시켜 각각 하기 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물을 수득하는 단계;

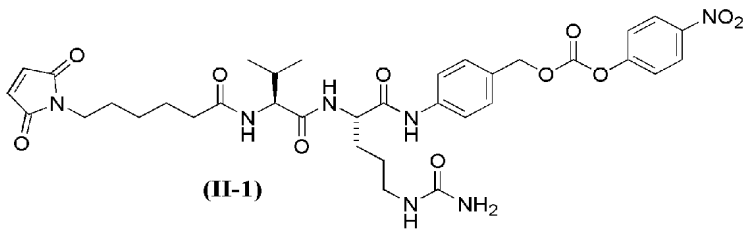
[196] (iii) 하기 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물과 N-히드록시숙신이미드를 축합반응시켜 각각 하기 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물을 수득하는 단계; 및

[197] (iv) 하기 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물과 항체를 반응시키는 단계를 포함한다.

[198]

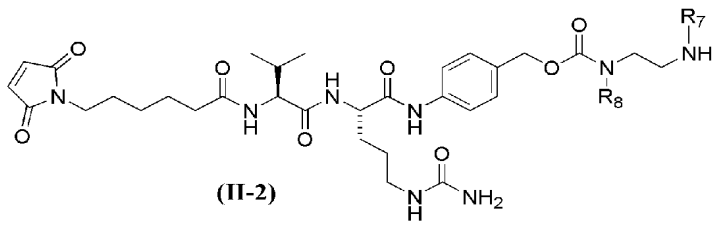
[199] [화학식 II-1]

[200]



[201] [화학식 II-2]

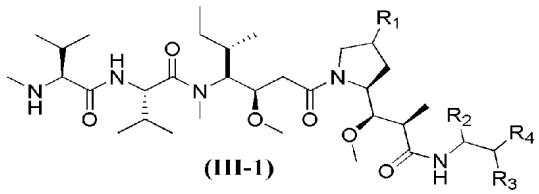
[202]



[203] 상기 식에서 R7 과 R8은 상기 화학식 I-4에서 정의한 바와 같다.

[204] [화학식 III-1]

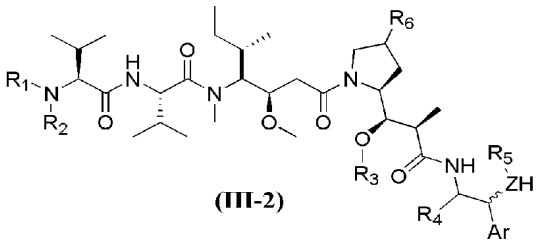
[205]



[206] 상기 식에서 R1, R2, R3 및 R4는 상기 화학식 I-1에서 정의한 바와 같다.

[207] [화학식 III-2]

[208]

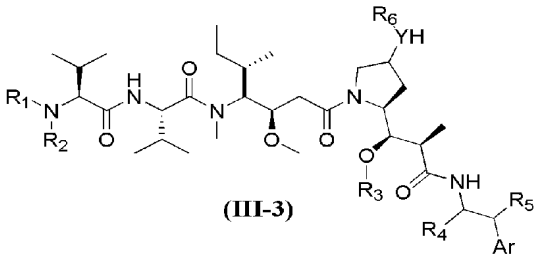


[209] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar 및 Z는 상기 화학식 I-2에서 정의한 바와

같다.

[210] [화학식 III-3]

[211]

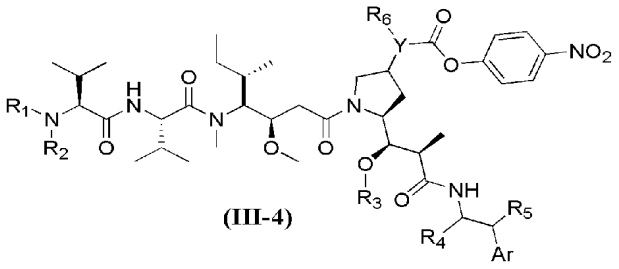


(III-3)

[212] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar 및 Y 는 상기 화학식 I-3에서 정의한 바와 같다.

[213] [화학식 III-4]

[214]

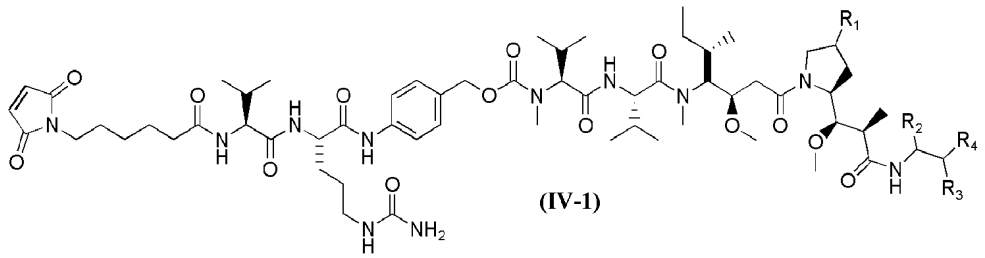


(III-4)

[215] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar 및 Y는 상기 화학식 I-4에서 정의한 바와 같다.

[216] [화학식 IV-1]

[217]

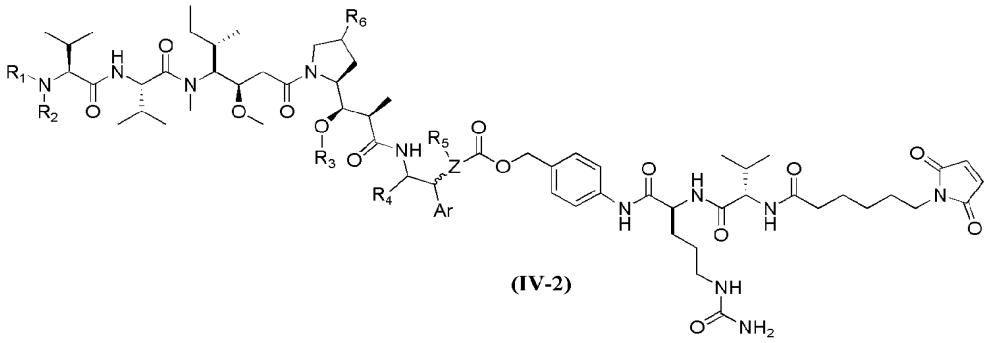


(IV-1)

[218] 상기 식에서 R1, R2, R3 및 R4는 상기 화학식 I-1에서 정의한 바와 같다.

[219] [화학식 IV-2]

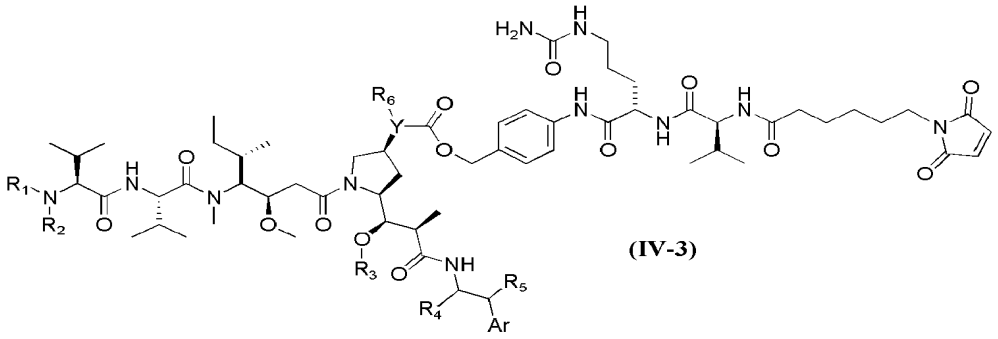
[220]



(IV-2)

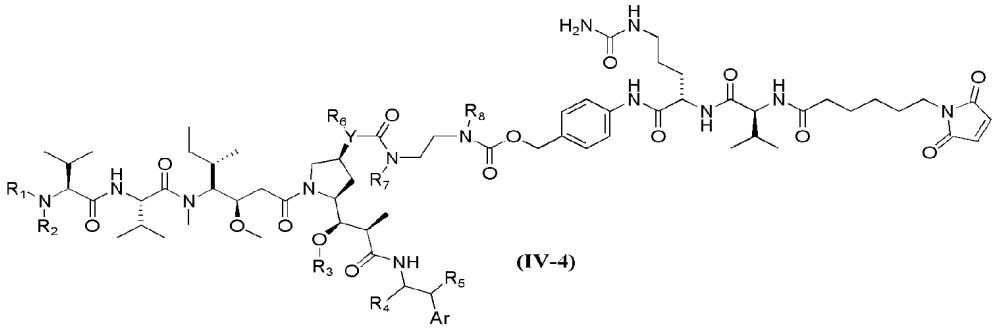
[221] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar 및 Z는 상기 화학식 I-2에서 정의한 바와 같다.

[222] [화학식 IV-3]

[223] 

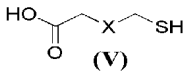
[224] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar 및 Y는 상기 화학식 I-3에서 정의한 바와 같다.

[225] [화학식 IV-4]

[226] 

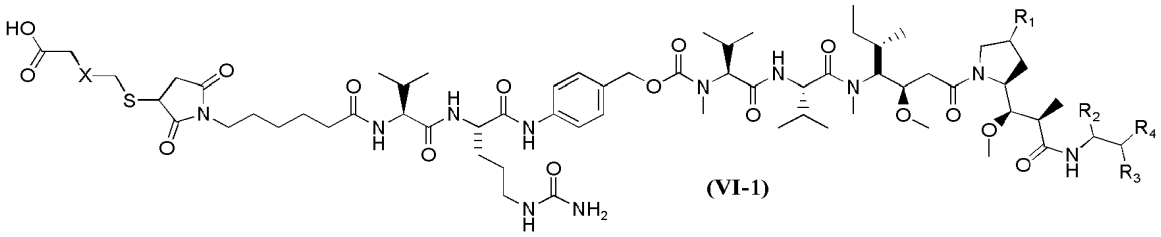
[227] 상기 식에서 R1, R2, R3, R4, R5, R6, R7, R8, Ar 및 Y는 상기 화학식 I-4에서 정의한 바와 같다.

[228] [화학식 V]

[229] 

[230] 상기 식에서 X는 상기 화학식 I-1, I-2, I-3 또는 I-4에서 정의한 바와 같다.

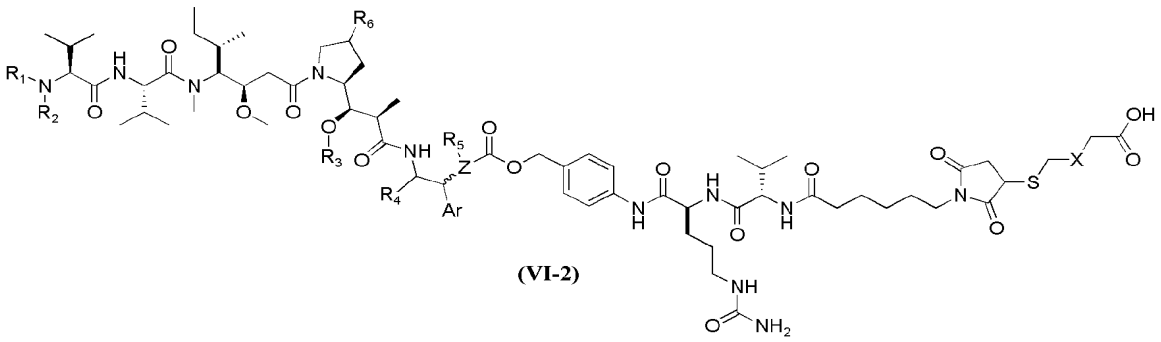
[231] [화학식 VI-1]

[232] 

[233] 상기 식에서 R1, R2, R3, R4 및 X는 상기 화학식 I-1에서 정의한 바와 같다.

[234] [화학식 VI-2]

[235]

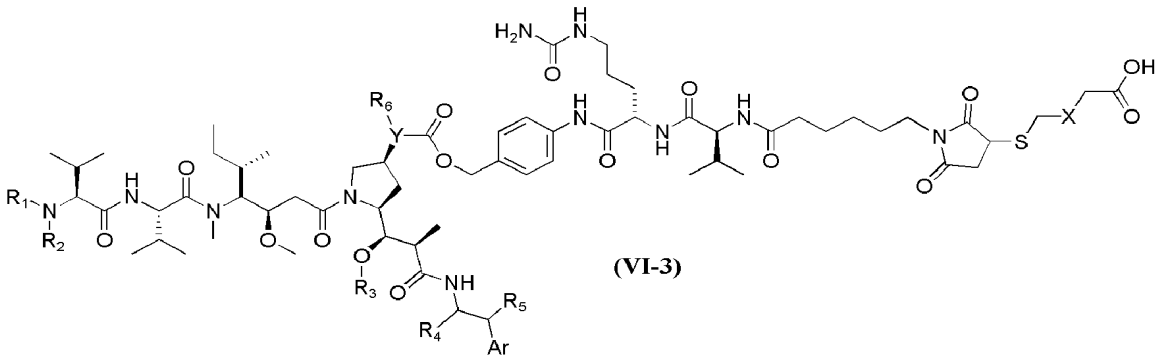


(VI-2)

[236] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar, X 및 Z는 상기 화학식 I-2에서 정의한 바와 같다.

[237] [화학식 VI-3]

[238]

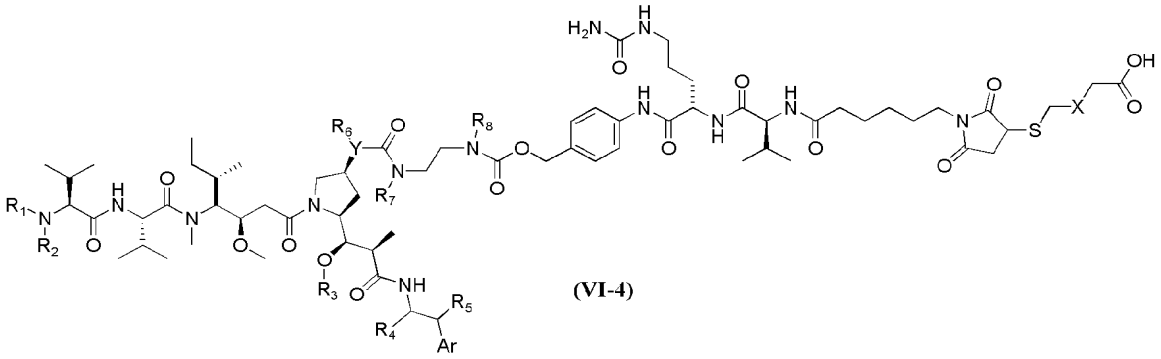


(VI-3)

[239] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar, X 및 Y는 상기 화학식 I-3에서 정의한 바와 같다.

[240] [화학식 VI-4]

[241]

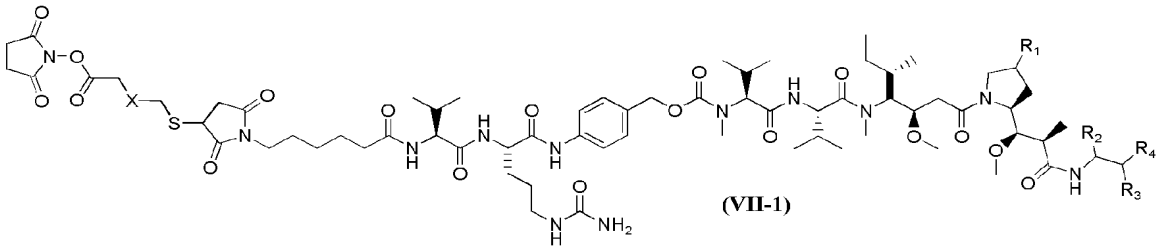


(VI-4)

[242] 상기 식에서 R1, R2, R3, R4, R5, R6, R7, R8, Ar, X 및 Y는 상기 화학식 I-4에서 정의한 바와 같다.

[243] [화학식 VII-1]

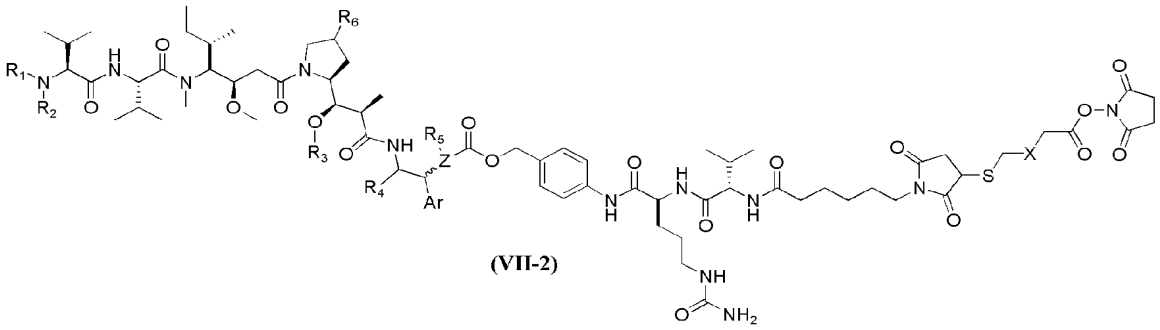
[244]



(VII-1)

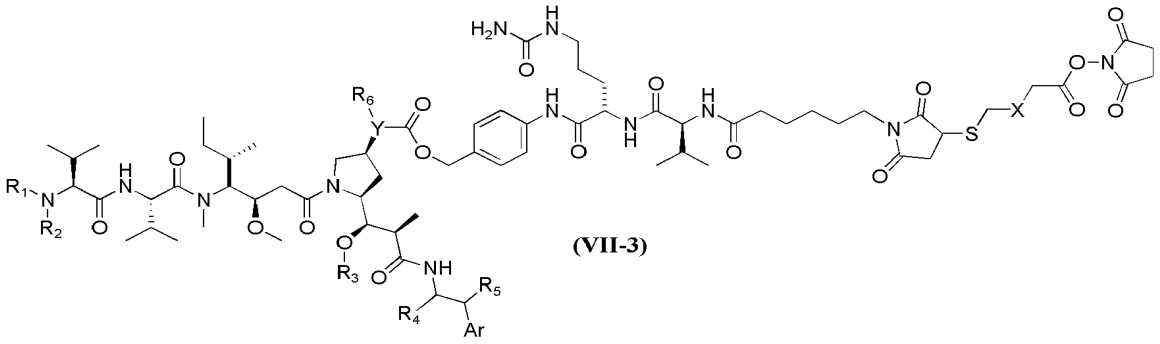
[245] 상기 식에서 R1, R2, R3, R4 및 X는 상기 화학식 I-1에서 정의한 바와 같다.

[246] [화학식 VII-2]

[247] 
(VII-2)

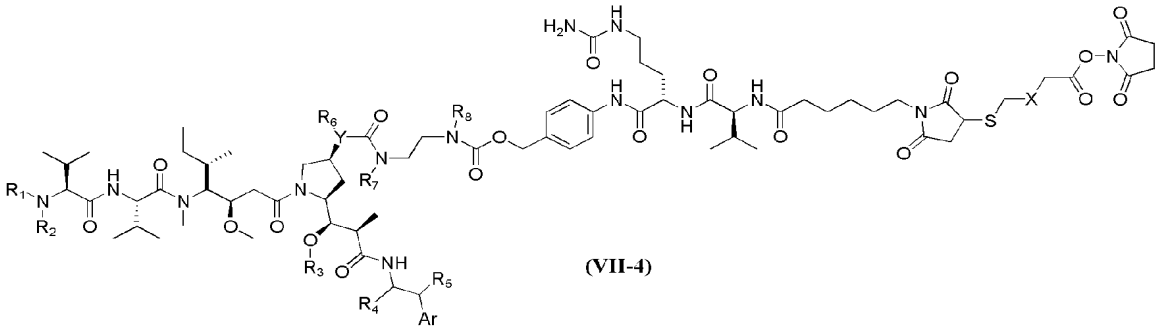
[248] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar, X 및 Z는 상기 화학식 I-2에서 정의한 바와 같다.

[249] [화학식 VII-3]

[250] 
(VII-3)

[251] 상기 식에서 R1, R2, R3, R4, R5, R6, Ar, X 및 Y는 상기 화학식 I-3에서 정의한 바와 같다.

[252] [화학식 VII-4]

[253] 
(VII-4)

[254] 상기 식에서 R1, R2, R3, R4, R5, R6, R7, R8, Ar, X 및 Y는 상기 화학식 I-4에서 정의한 바와 같다.

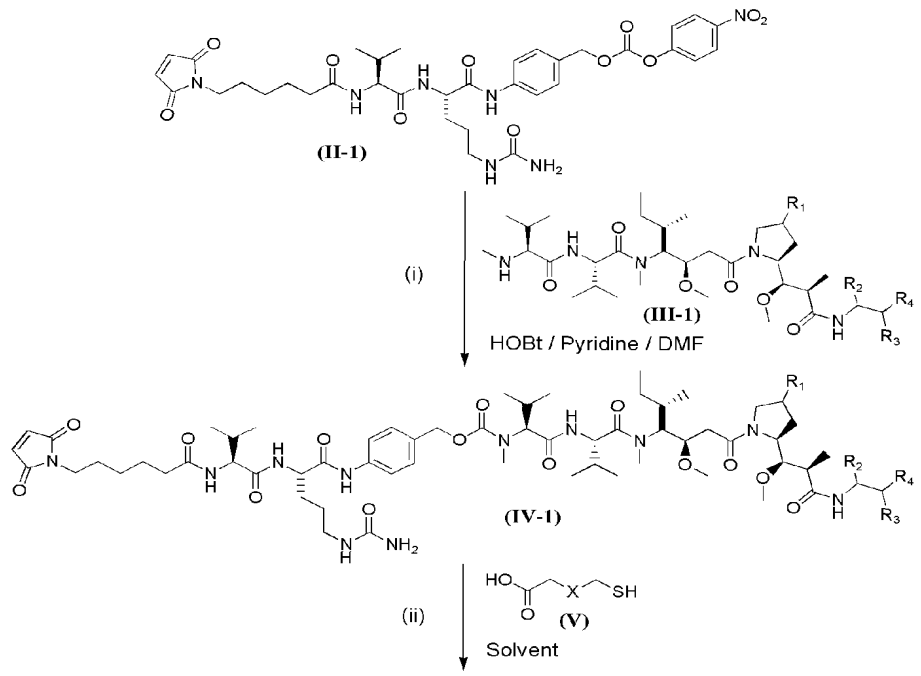
[255]

[256] 이하, 본 발명에 따른 상기 화학식 I-1 내지 I-4의 항체-링커-약물 결합체의 제조방법을 각각 하기 반응식 I-1 내지 I-4를 참조로 상세히 설명한다. 하기 반응식 I-1 내지 I-4에 기재된 방법은 대표적으로 사용된 방법을 예시한 것일 뿐 단위조작의 순서, 반응시약, 반응조건 등은 경우에 따라 얼마든지 변경될 수

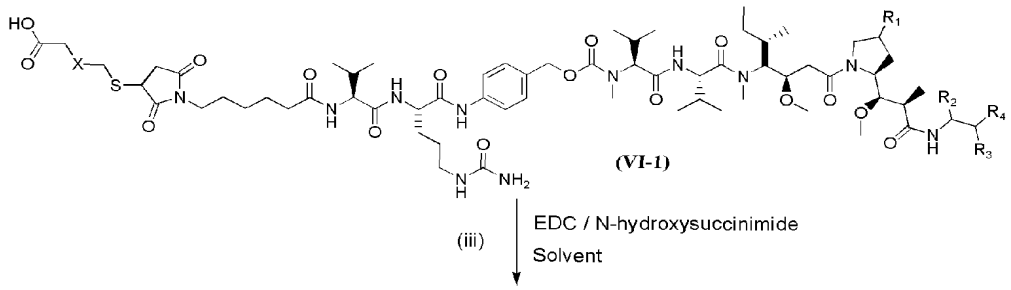
있다.

[257] [반응식 I-1]

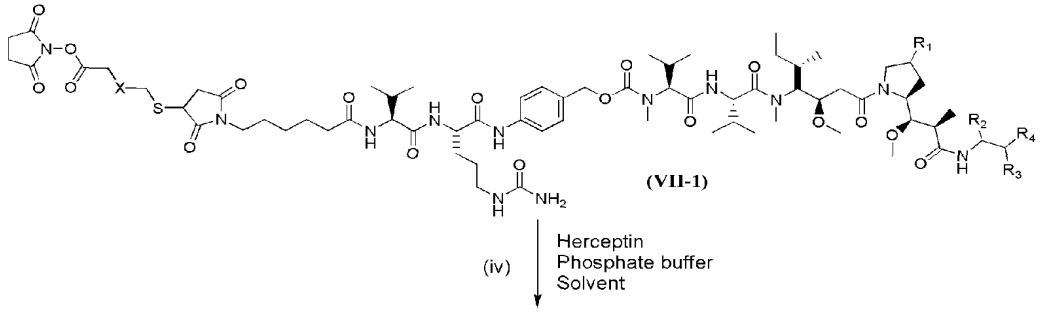
[258]



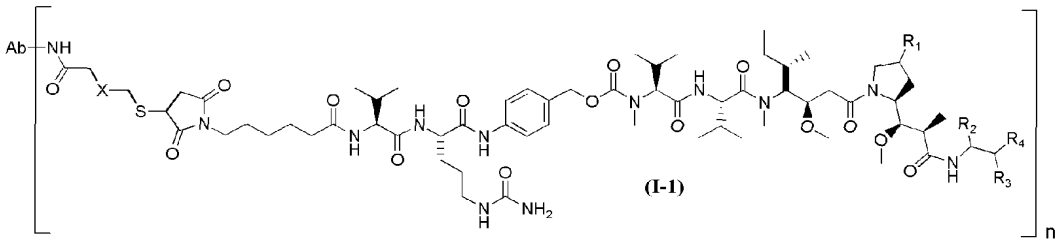
[259]



[260]



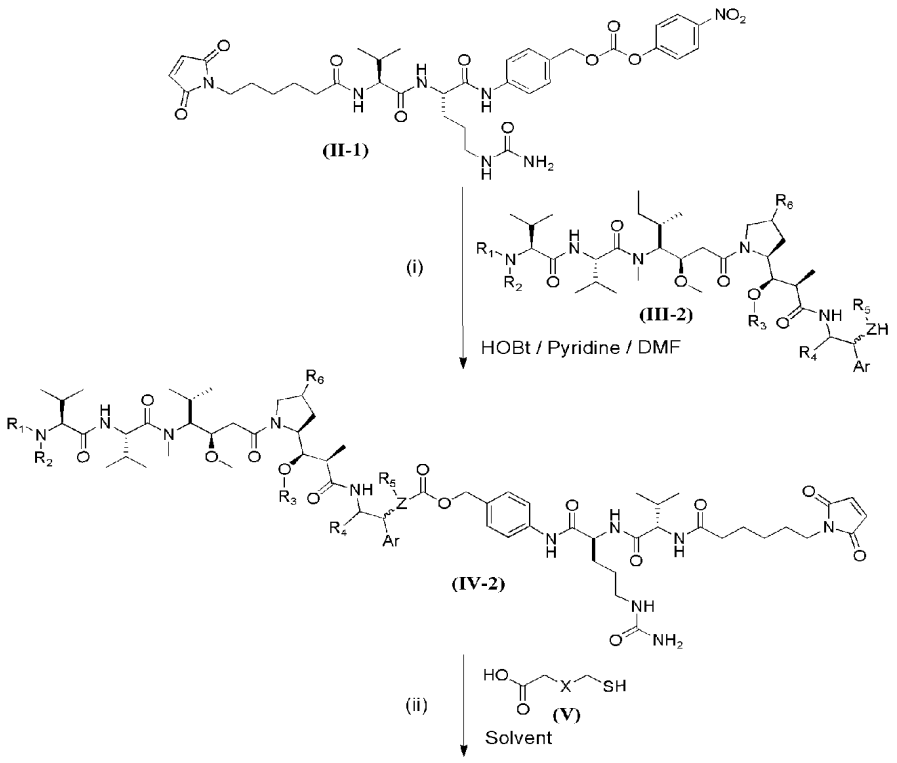
[261]



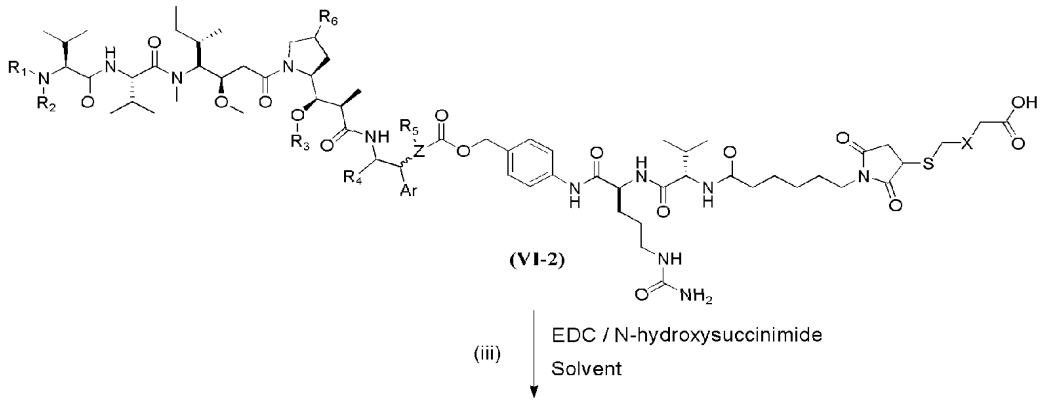
[262]

[263] [반응식 I-2]

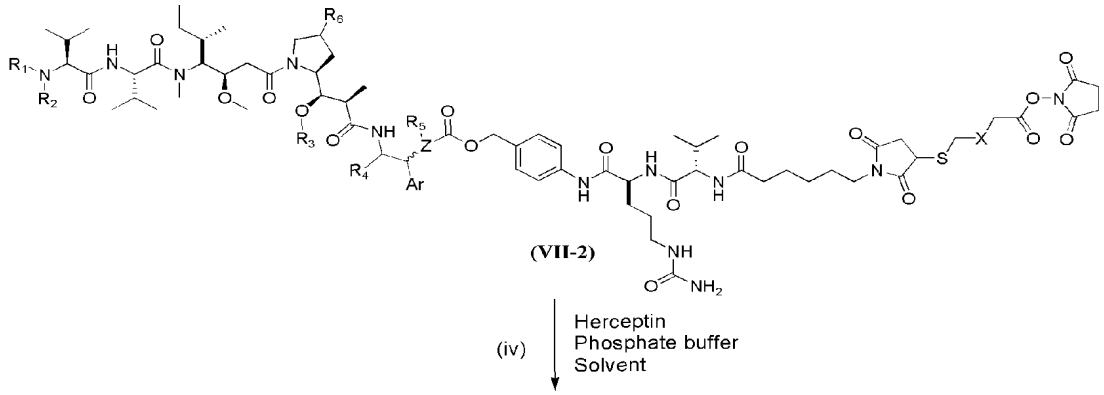
[264]



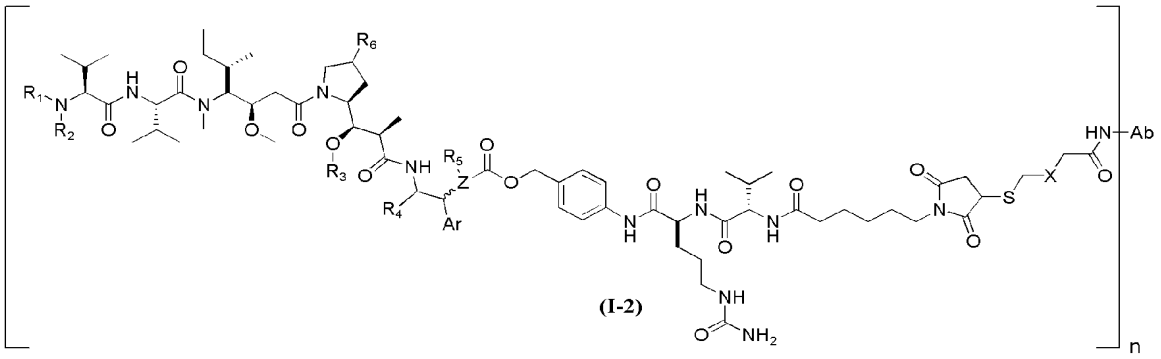
[265]



[266]



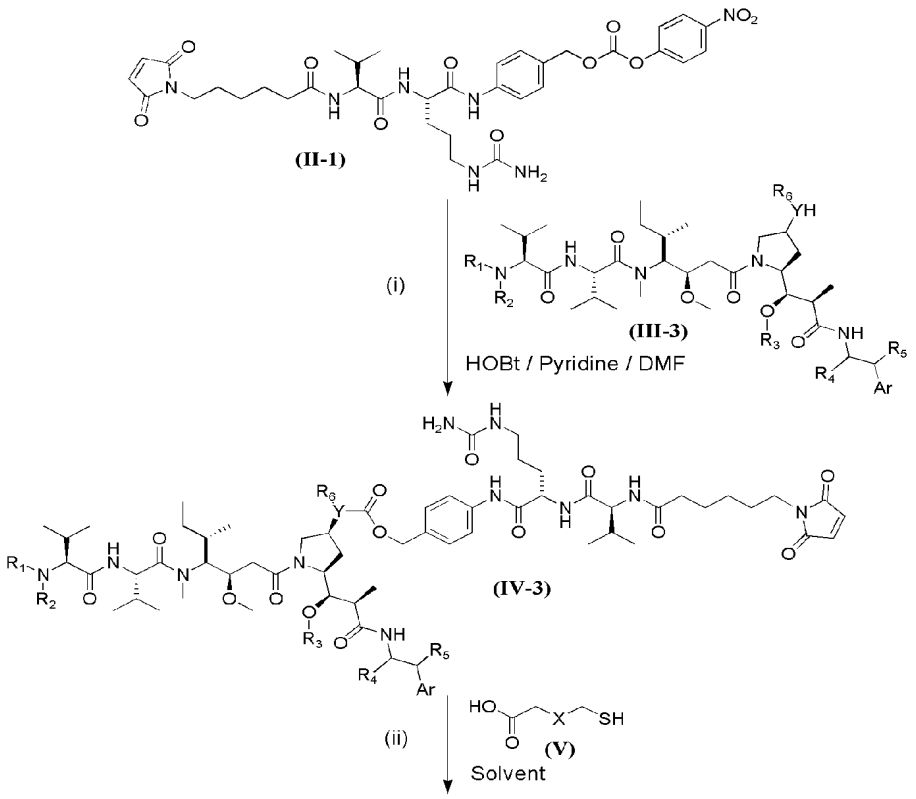
[267]



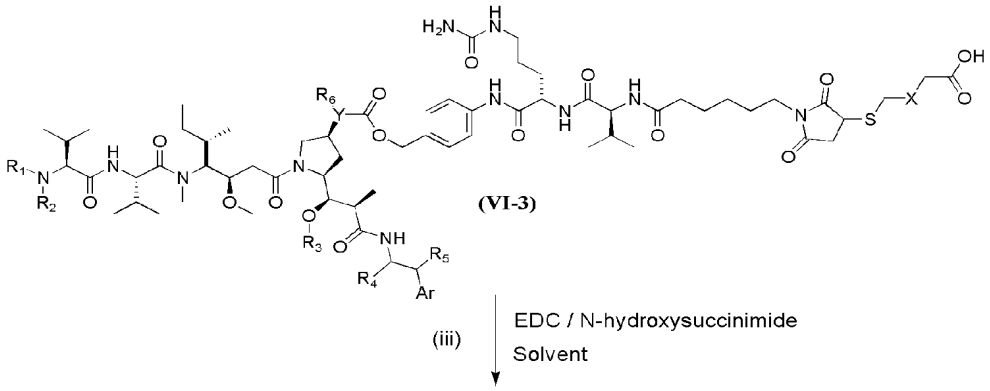
[268]

[269] [반응식 I-3]

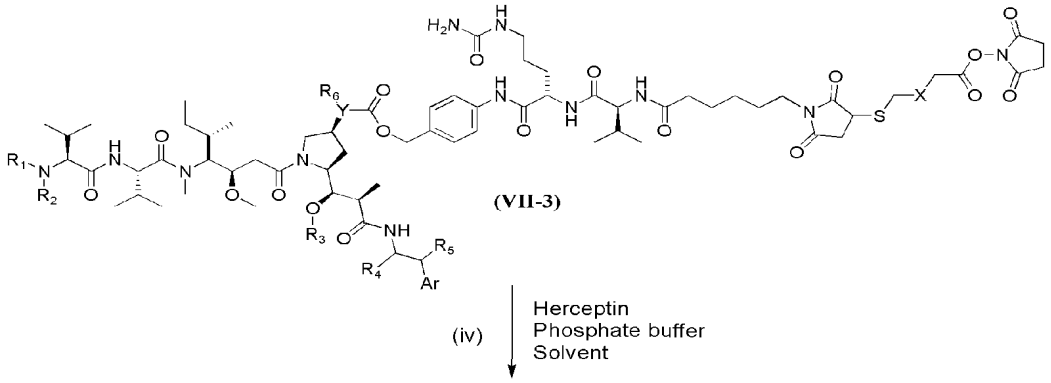
[270]



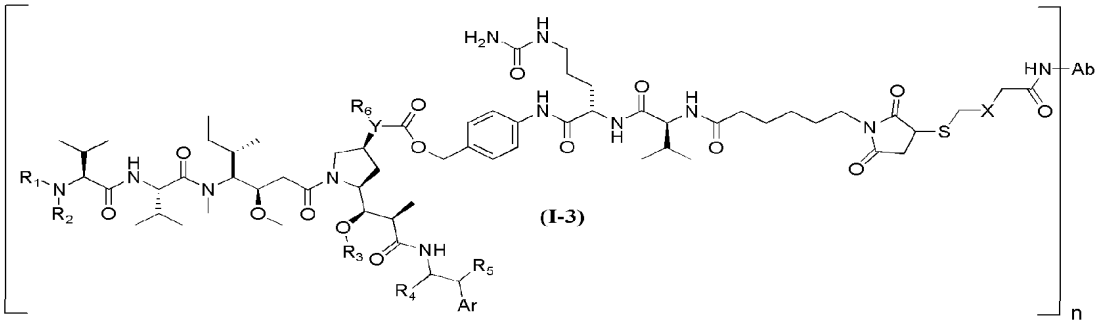
[271]



[272]



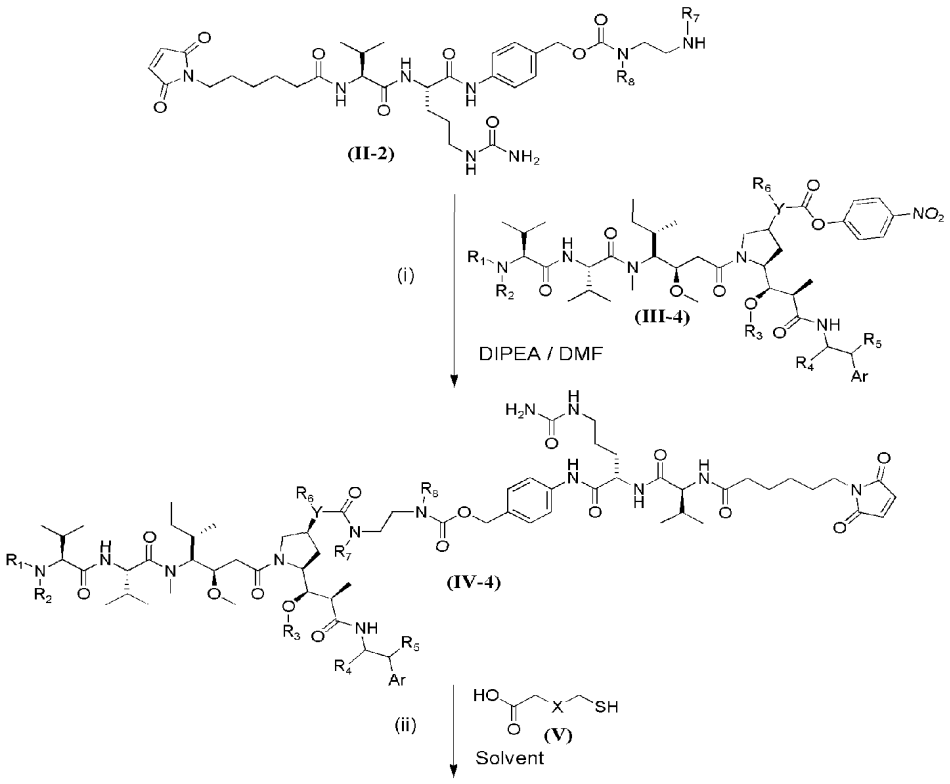
[273]



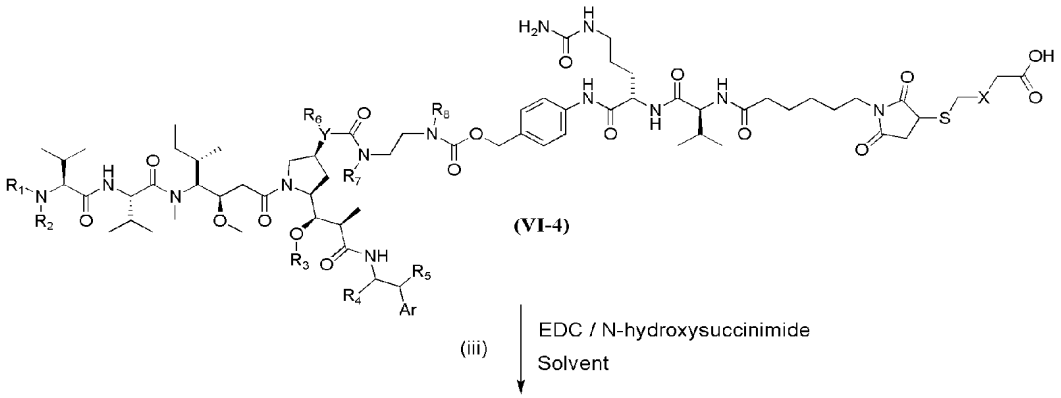
[274]

[275] [반응식 I-4]

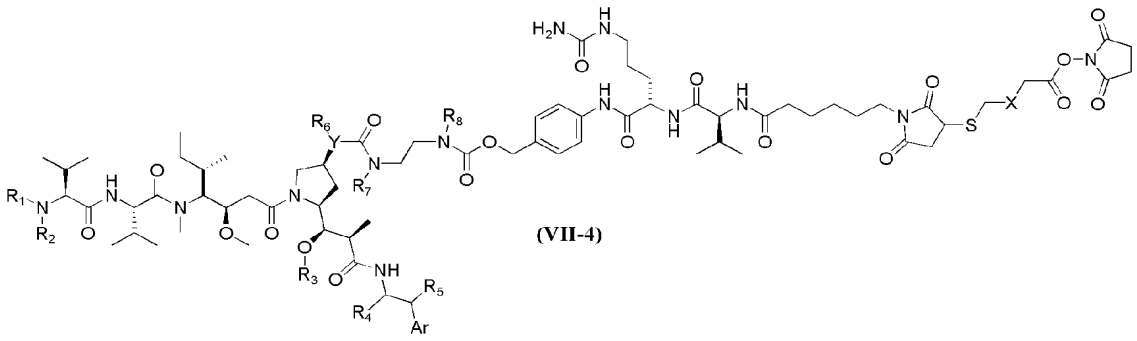
[276]



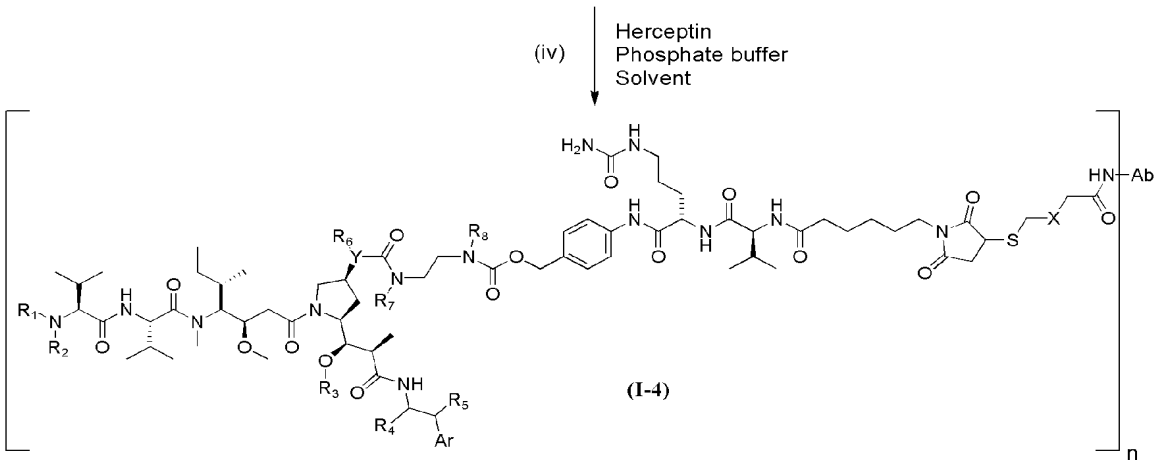
[277]



[278]



[279]



[280]

[281] 상기 단계 (i)에서는 화학식 II-1의 화합물과 화학식 III-1, III-2 또는 III-3의 돌라스타틴 10 유도체를 축합반응시켜 각각 화학식 IV-1, IV-2 또는 IV-3의 화합물을 수득하거나, 화학식 II-2의 화합물과 화학식 III-4의 돌라스타틴 10 유도체를 축합반응시켜 각각 화학식 IV-4의 화합물을 수득한다

[282] 상기 축합반응은 축합제의 존재 하에 수행될 수 있으며, 축합제로는 히드록시벤조트리아졸(HOBt), 히드록시아자벤조트리아졸(HOAt), 히드록시숙신이미드(HOSu) 등을 사용할 수 있으나 이에 한정되는 것은 아니다.

[283] 축합반응시 필요하다면, 피리딘, 디이소프로필에틸아민과 같은 유기 염기를 축합제와 함께 사용할 수 있다.

[284] 반응용매로는 디메틸포름아미드(DMF), 디메틸아세트아미드(DMA),

디메틸술폭시드(DMSO), N-메틸-2-피롤리돈(NMP) 등을 사용할 수 있으며, 반응온도는 20 내지 25 ℃가 바람직하다.

[285]

[286] 상기 단계 (ii)에서는 화학식 IV-1, IV-2, IV-3 또는 IV-4의 화합물에 화학식 V의 화합물을 첨가반응시켜 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물을 수득한다.

[287] 반응용매로는 디메틸술폭시드(DMSO), 아세토니트릴(MeCN), 디메틸포름아미드(DMF) 등을 사용할 수 있으며, 반응온도는 20 내지 25 ℃가 바람직하다.

[288]

[289] 상기 단계 (iii)에서는 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물과 N-히드록시숙신이미드를 축합반응시켜 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물의 카르복시산기를 활성화한다.

[290] 상기 축합반응은 축합제의 존재 하에 수행될 수 있으며, 축합제로는 디시클로헥실카르보디이미드(DCC), (3-디메틸아미노프로필)카르보디이미드(EDC) 등을 사용할 수 있으나 이에 한정되는 것은 아니다.

[291] 반응용매로는 디메틸포름아미드(DMF), 디메틸아세트아미드(DMA), 디클로로메탄(DCM) 등을 사용할 수 있으며, 반응온도는 20 내지 25 ℃가 바람직하다.

[292]

[293] 상기 단계 (iv)에서는 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물과 항체를 반응시켜 최종 생성물인 화학식 I-1, I-2, I-3 또는 I-4의 항체-링커-약물 결합체를 수득한다.

[294] 반응용매로는 pH 6.0 내지 8.0의 포스페이트 버퍼를 사용할 수 있으며, 세포독성 약물의 종류에 따라 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물인 링커-세포독성 약물의 포스페이트 버퍼에서의 용해도가 달라지므로, 필요에 따라 디메틸포름아미드(DMF), 디메틸술폭시드(DMSO), 디메틸아세트아미드(DMA), 아세토니트릴(MeCN), 1,4-디옥산 등의 유기용매를 혼합하여 사용할 수 있다. 이때 혼합용매 중 유기용매의 비율은 50% 이하인 것이 바람직하다.

[295] 상기 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물은 항체를 기준으로 3 내지 25 당량 사용하는 것이 바람직하다. 이때 제조된 항체-링커-약물 결합체에서 1 분자의 항체에 결합된 약물의 수인 약물-항체 비(drug-antibody ratio: DAR)은 약 1 내지 5가 된다.

[296]

[297] 상기 화학식 II-1 또는 II-2의 화합물은 효소-분해형(enzyme cleavable) 펩타이드 링커로 공지된 물질로, 미국특허 제6,214,345호에 기재된 방법에 따라 용이하게 제조할 수 있다.

[298]

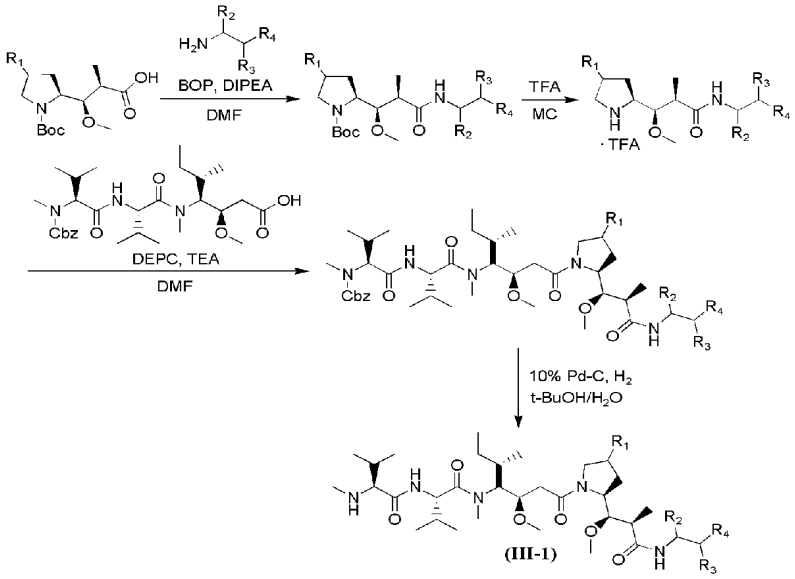
[299] 또한 상기 화학식 III-1, III-2, III-3 또는 III-4의 돌라스타틴 10 유도체는 세포독성 약물로서, 본 출원인에 의해 2012년 9월 20일자 출원되어 계류중인 대한민국 특허출원 제10-2012-0104710호 또는 미국특허 제5,599,902호에 기재된 방법에 따라 제조할 수 있다. 이에 상기 특허문헌을 전부 본 명세서에 참조로 포함시킨다.

[300] 예를 들어, 상기 화학식 III-1, III-2, III-3 또는 III-4의 돌라스타틴 10 유도체는 각각 하기 반응식 II-1 내지 II-4에 나타낸 방법에 따라 제조될 수 있다.

[301]

[302] [반응식 II-1]

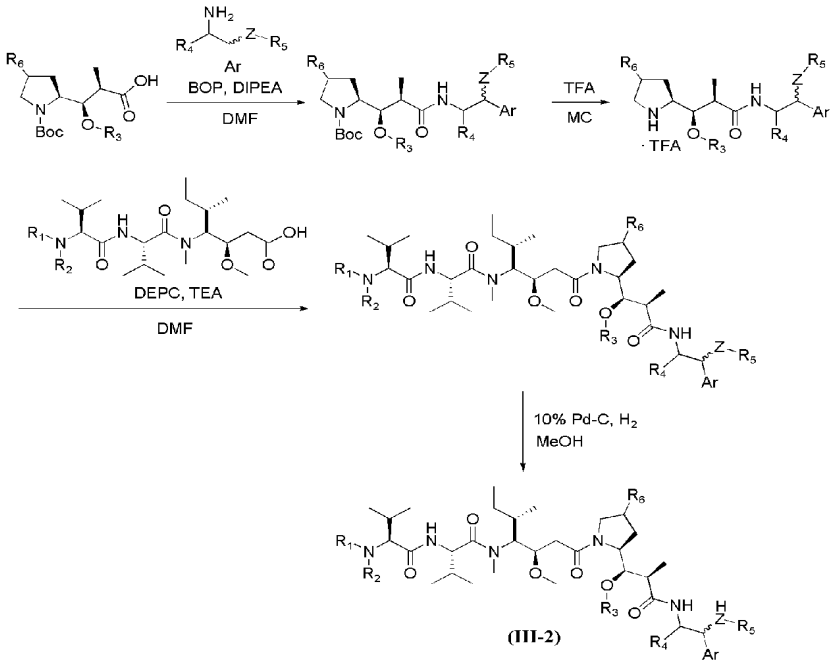
[303]



[304]

[305] [반응식 II-2]

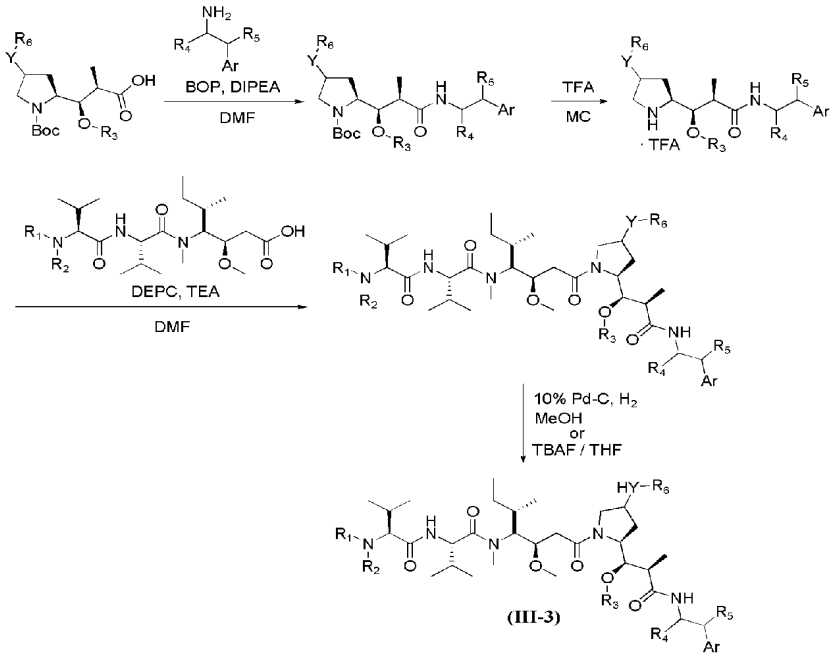
[306]



[307]

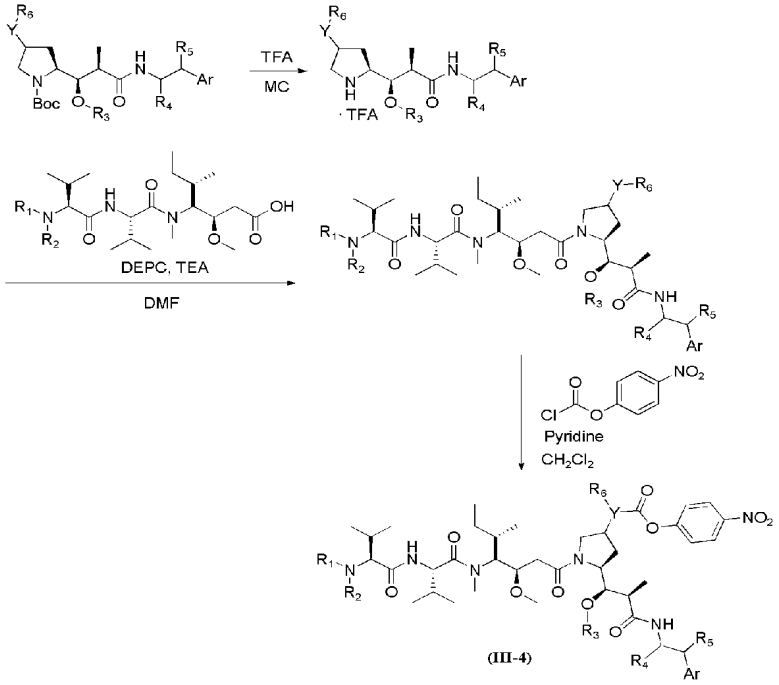
[308] [반응식 II-3]

[309]



[310]

[311] [반응식 II-4]

[312] 

[313]

[314] 본 발명의 항체-링커-약물 결합체는 우수한 항종양 활성을 나타낸다(시험예 4 및 시험예 6 참조).

[315] 따라서, 본 발명은 상기 화학식 I-1 내지 I-4의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염을 약제학적으로 허용가능한 담체와 함께 포함하는 항암제 조성물, 특히 유방암 치료용 조성물에 관한 것이다.

[316] 본 발명에 따른 항암제 조성물은 경구적으로(예를 들면, 복용 또는 흡입) 또는 비경구적으로(예를 들면, 주사, 침착, 이식, 좌약) 투여될 수 있으며, 주사는 예를 들면, 정맥주사, 피하주사, 근육내주사 또는 복강내주사일 수 있다. 본 발명에 따른 항암제는 투여 경로에 따라, 정제, 캡슐제, 과립제, 파인 서브틸래(fine subtilae), 분제, 설하 정제, 좌약, 연고, 주사제, 유탁액제, 현탁액제, 시럽제, 분무제 등으로 제형화될 수 있다. 상기 여러 가지 형태의 본 발명에 따른 항암제 조성물은 각 제형에 통상적으로 사용되는 약제학적으로 허용되는 담체(carrier)를 사용하여 공지기술에 의해 제조될 수 있다. 약제학적으로 허용되는 담체의 예는 부형제, 결합제, 붕해제(disintegrating agent), 윤활제, 방부제, 항산화제, 등장제(isotonic agent), 완충제, 피막제, 감미제, 용해제, 기제(base), 분산제, 습윤제, 현탁화제, 안정제, 착색제 등을 포함한다.

[317] 본 발명에 따른 항암제 조성물은 약제의 형태에 따라 다르지만, 본 발명의 화합물 또는 그의 약제학적으로 허용되는 염을 약 0.01 내지 95 중량%로 포함한다.

[318] 본 발명의 항암제 조성물의 구체적인 투여량은 치료되는 사람을 포함한 포유동물의 종류, 체중, 성별, 질환의 정도, 의사의 판단 등에 따라 다를 수 있다.

바람직하게는, 경구투여의 경우에는 하루에 체중 1 kg당 활성성분 0.01 내지 50 mg이 투여되고, 비경구투여의 경우에는 하루에 체중 1 kg당 활성성분 0.01 내지 10 mg이 투여된다. 상기 총 일일 투여량은 질환의 정도, 의사의 판단 등에 따라 한번에 또는 수회로 나누어 투여될 수 있다.

[319]

발명의 효과

[320] 본 발명에 따른 항체-링커-약물 결합체는 링커-약물이 항체의 라이신 잔기에 직접 결합되어 항체 단백질 구조의 불필요한 변형이 발생하지 않으며, 생체내 안정성을 확보하면서 세포독성 약물의 효과적이고 선택적인 전달을 가능케 함으로써 1회 투여로 암, 특히 유방암을 효과적으로 치료할 수 있다. 아울러, 본 발명의 제조방법에 따르면, 항체-링커-약물 결합체를 고수율로 제조할 수 있다.

[321]

도면의 간단한 설명

[322] 도 1은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-6)과 (I-8)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[323] 도 2는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-10)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[324] 도 3은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-11)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[325] 도 4는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-16)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[326] 도 5는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-18)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[327] 도 6은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-19)와 (I-21)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[328] 도 7은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-20)과 (I-22)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[329] 도 8은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-23)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[330] 도 9는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-24)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[331] 도 10은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-25)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[332] 도 11은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-26)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[333] 도 12는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-32)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[334] 도 13은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-33)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[335] 도 14는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-34)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[336] 도 15는 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-35)와 (I-36)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[337] 도 16은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-37)과 (I-38)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[338] 도 17은 BT-474 세포에 대한 본 발명에 따른 항체-링커-약물 결합체 (I-39)와 (I-40)의 시험관 내 세포생장 저해능을 나타낸 그래프이다.

[339] 도 18은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-6), (I-10) 및 (I-11)을 각각 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[340] 도 19는 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-6), (I-10) 및 (I-11)을 각각 투여하고 56일 경과 후의 종양 무게를 나타낸 그래프이다.

[341] 도 20은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-16)을 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[342] 도 21은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-16)을 각각 투여하고 28일 경과 후의 종양 무게를 나타낸 그래프이다.

[343] 도 22는 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-23)을 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[344] 도 23은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-23)을 투여하고 28일 경과 후의 종양 무게를 나타낸 그래프이다.

[345] 도 24는 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-25)를 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[346] 도 25는 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-25)를 투여하고 28일 경과 후의 종양 무게를 나타낸 그래프이다.

[347] 도 26은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-26)를 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[348] 도 27은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-26)를 투여하고 28일 경과 후의 종양 무게를 나타낸 그래프이다.

[349] 도 28은 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-34)를 투여한 후 시간에 따른 종양 부피 변화를 나타낸 그래프이다.

[350] 도 29는 암 유발 동물모델에 본 발명에 따른 항체-링커-약물 결합체 (I-34)를 투여하고 28일 경과 후의 종양 무게를 나타낸 그래프이다.

[351]

발명의 실시를 위한 형태

[352] 이하, 실시예에 의해 본 발명을 보다 구체적으로 설명하고자 한다. 이들

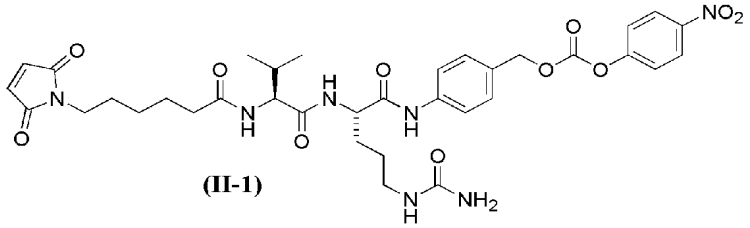
실시예는 오직 본 발명을 설명하기 위한 것으로, 본 발명의 범위가 이들 실시예에 국한되지 않는다는 것은 당업자에게 있어서 자명하다.

[353]

[354] **제조예 1: 화학식 II-1, II-2의 화합물의 제조**

[355] **제조예 1-1: 화합물 (II-1)의 제조**

[356]



(II-1)

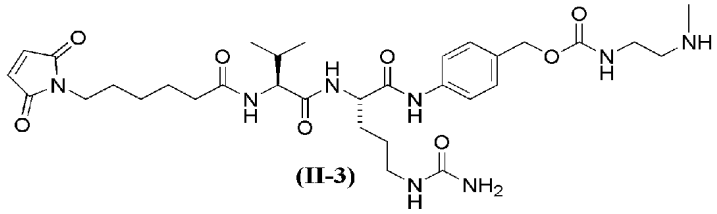
[357] 6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)-N-((S)-1-(((S)-1-((4-(히드록시메틸)페닐)아미노)-1-옥소-5-우레이도펜탄-2-일)아미노)-3-메틸-1-옥소부탄-2-일)헥산아미드 (5.0 g, 8.7 mmol, Dubowchik et al., Bioconjugate Chem., 2002, 13 (4), pp 855-869)를 아르곤 기류 하에서 무수 디메틸포름아미드 60 mL에 녹인 후, N,N-디이소프로필에틸아민 (3.0 mL, 17.4 mmol)을 가하고 0 ℃로 냉각하였다. 반응혼합물에 비스(4-니트로페닐) 카르보네이트 (7.94 g, 26.1 mmol)를 한번에 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물 (3.65 g, 57%)을 미색 고체로 얻었다.

[358] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 1.09 (t, J = 7.2 Hz, 1 H), 1.19 (m, 2 H), 1.34 - 1.76 (m, 7 H), 1.96 (m, 1 H), 2.15 (m, 2 H), 2.99 (m, 2 H), 3.37 (m, 2 H), 4.19 (t, J = 7.8 Hz, 1 H), 4.39 (m, 1 H), 5.24 (s, 2 H), 5.41 (s, 2 H), 5.97 (brt, J = 5.6 Hz, 1 H), 7.00 (s, 2H), 7.41 (d, J = 8.4 Hz, 2 H), 7.57 (d, J = 7.2 Hz, 2 H), 7.65 (d, J = 8.4 Hz, 2 H), 7.80 (d, J = 8.4 Hz, 1 H), 8.09 (d, J = 7.2 Hz, 1 H), 8.31 (d, J = 7.2 Hz, 2 H), 10.05 (brs, 1 H)

[359]

[360] **제조예 1-2: 화합물 (II-3)의 제조**

[361]



(II-3)

[362] 6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)-N-((S)-1-(((S)-1-((4-(히드록시메틸)페닐)아미노)-1-옥소-5-우레이도펜탄-2-일)아미노)-3-메틸-1-옥소부탄-2-일)헥산아미드(5.0 g, 8.7 mmol, Dubowchik et al., Bioconjugate Chem., 2002, 13 (4), pp 855-869)를 아르곤 기류 하에서 무수 디메틸포름아미드 60 mL에 녹인 후, N,N-디이소프로필에틸아민(3.0 mL, 17.4 mmol)을 가하고 0 ℃로 냉각하였다. 반응혼합물에 비스(4-니트로페닐) 카르보네이트(7.94 g, 26.1 mmol)를 한번에

가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 4-((S)-2-((S)-2-(6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)헥산아미도)-3-메틸부탄아미도)-5-우레이도펜탄아미도)벤질 (4-니트로페닐) 카르보네이트(3.65 g, 57 %)를 미색 고체로 얻었다.

[363] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 1.09 (t, J = 7.2 Hz, 1 H), 1.19 (m, 2 H), 1.34 - 1.76 (m, 7 H), 1.96 (m, 1 H), 2.15 (m, 2 H), 2.99 (m, 2 H), 3.37 (m, 2 H), 4.19 (t, J = 7.8 Hz, 1 H), 4.39 (m, 1 H), 5.24 (s, 2 H), 5.41 (s, 2 H), 5.97 (brt, J = 5.6 Hz, 1 H), 7.00 (s, 2H), 7.41 (d, J = 8.4 Hz, 2 H), 7.57 (d, J = 7.2 Hz, 2 H), 7.65 (d, J = 8.4 Hz, 2 H), 7.80 (d, J = 8.4 Hz, 1 H), 8.09 (d, J = 7.2 Hz, 1 H), 8.31 (d, J = 7.2 Hz, 2 H), 10.05 (brs, 1 H)

[364]

[365] 4-((S)-2-((S)-2-(6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)헥산아미도)-3-메틸부탄아미도)-5-우레이도펜탄아미도)벤질 (4-니트로페닐) 카르보네이트(3.65 g, 4.95 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 90 mL에 용해한 후, t-부틸 (2-아미노에틸)(메틸)카르바메이트(0.86 g, 4.95 mmol)를 가하고 20 - 25 ℃에서 2시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (II-3)의 아미노기가 보호화된 화합물(3.8 g, 99%)을 수득하였다.

[366] LC-MS m/z : 773.5 $[M+H]^+$

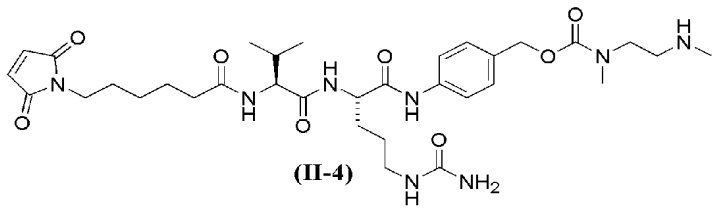
[367]

[368] 화합물 (II-3)의 아미노기가 보호화된 화합물(146 mg, 0.186 mmol)을 디클로로메탄 5 mL에 녹이고 트리플루오로아세트산 2 mL를 적가한 후 20 - 25 ℃에서 2시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 mL를 2회 첨가하여 트리플루오로아세트산을 완전히 제거하여 표제화합물의 TFA염 농축액을 수득하였다.

[369]

[370] **제조예 1-3: 화합물 (II-4)의 제조**

[371]



[372] 4-((S)-2-((S)-2-(6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)헥산아미도)-3-메틸부탄아미도)-5-우레이도펜탄아미도)벤질 (4-니트로페닐) 카르보네이트(6.8 g, 9.22 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 150 mL에 용해한 후, t-부틸 메틸(2-(메틸아미노)에틸)카르바메이트(1.82 g, 9.68 mmol)를 가하고 20 - 25 ℃에서 19 시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전

농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (II-4)의 아미노기가 보호화된 화합물(5.14 g, 71%)을 수득하였다.

[373] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78-0.8 (d, 3 H), 0.8-0.83 (d, 3 H), 1.09 -1.99 (m, 10 H), 1.33 (s, 9 H), 1.92 (m, 1 H), 2.07-2.19 (m, 2H), 2.66-2.73 (d, 3H), 2.8-2.81 (m, 3H), 2.9-3.0 (m, 2H), 3.37 (m, 2 H), 4.15 (t, 1 H), 4.34 (m, 1 H), 4.94 (s, 2 H), 5.38 (s, 2 H), 5.94 (brt, 1 H), 6.96 (s, 2H), 7.23 (d, 2 H), 7.55 (d, 2 H), 7.77 (d, 1 H), 8.04 (d, 1 H), 9.95 (brs, 1 H)

[374] LC-MS *m/z* : 787.5 $[M+H]^+$

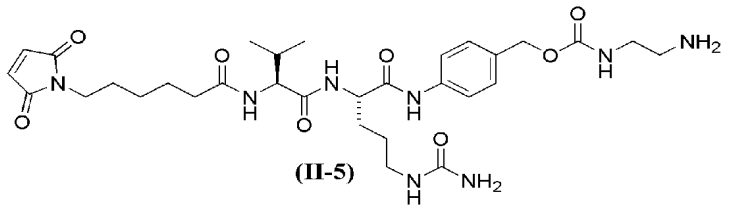
[375]

[376] 화합물 (II-4)의 아미노기가 보호화된 화합물을 사용하여 제조예 1-2와 동일한 방법으로 표제화합물의 TFA염 농축액을 수득하였다.

[377]

[378] **제조예 1-4: 화합물 (II-5)의 제조**

[379]



(II-5)

[380] 4-((S)-2-((S)-2-(6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)헥산아미도)-3-메틸부탄아미도)-5-우레이도펜탄아미도)벤질 (4-니트로페닐) 카르보네이트(6.8 g, 9.22 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 80 mL에 용해한 후, t-부틸 (2-아미노에틸)카르바메이트(1.63 g, 10.14 mmol)를 가하고 20 - 25 ℃에서 15 시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (II-5)의 아미노기가 보호화된 화합물(4.13 g, 59%)을 수득하였다.

[381] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78-0.8 (d, 3 H), 0.8-0.83 (d, 3 H), 1.09 -1.99 (m, 10 H), 1.33 (s, 9 H), 1.92 (m, 1 H), 2.07-2.19 (m, 2H), 2.9-3.0 (m, 2H), 3.37 (m, 2 H), 4.15 (t, 1 H), 4.34 (m, 1 H), 4.94 (s, 2 H), 5.38 (s, 2 H), 5.94 (brt, 1 H), 6.76 (m, 1 H), 6.96 (s, 2H), 7.15 (m, 1H), 7.23 (d, 2 H), 7.55 (d, 2 H), 7.77 (d, 1 H), 8.04 (d, 1 H), 9.95 (brs, 1 H)

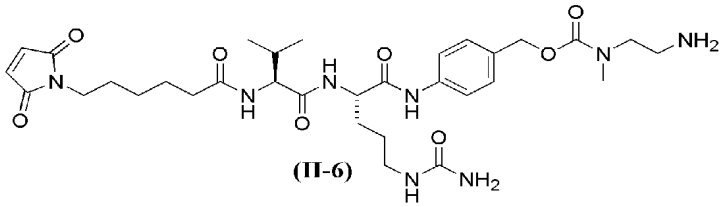
[382] LC-MS *m/z* : 759.5 $[M+H]^+$

[383]

[384] 화합물 (II-5)의 아미노기가 보호화된 화합물을 이용하여 제조예 1-2와 동일한 방법으로 표제화합물의 TFA염 농축액을 수득하였다.

[385]

[386] **제조예 1-5: 화합물 (II-6)의 제조**

[387] 
(II-6)

[388] 4-((S)-2-((S)-2-(6-(2,5-디옥소-2,5-디히드로-1H-피롤-1-일)헥산아미도)-3-메틸부탄아미도)-5-우레이도펜탄아미도)벤질 (4-니트로페닐) 카르보네이트(0.74 g, 1 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 30 mL에 용해한 후, t-부틸 (2-(메틸아미노)에틸)카르바메이트(0.25 g, 1.4 mmol)를 가하고 20 - 25 ℃에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (II-6)의 아미노기가 보호화된 화합물(0.36 g, 48%)을 수득하였다.

[389] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (d, 3 H), 0.82 (d, 3 H), 1.13-1.7 (m, 10 H), 1.32-1.39 (d, 9 H), 1.89-1.93 (m, 1 H), 2.07-2.17 (m, 2 H), 2.79-2.82 (d, 2 H), 2.89-3.02(m, 5H), 3.14 (d, 1 H), 3.2 (m, 1H), 4.15 (t, 1 H), 4.34 (m, 1 H), 4.9-4.94 (d, 2 H), 5.37 (s, 2 H), 5.941 (brt, 1 H), 6.81-6.85 (m, 1 H), 7.24 (d, 2H), 7.54 (d, 2 H), 7.76 (d, 1 H), 8.04 (d, 1 H), 9.95 (brs, 1 H)

[390] LC-MS *m/z* : 773.5 $[M+H]^+$

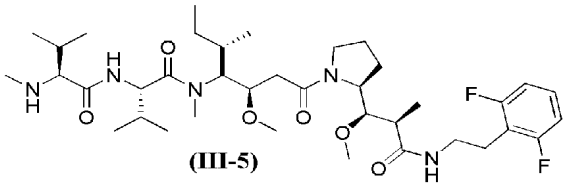
[391]

[392] 화합물 (II-6)의 아미노기가 보호화된 화합물을 이용하여 제조예 1-2와 동일한 방법으로 표제화합물의 TFA염 농축액을 수득하였다.

[393]

[394] **제조예 2: 화학식 III-1, III-2, III-3 또는 III-4의 화합물의 제조**

[395] **제조예 2-1: 화합물 (III-5)의 제조**

[396] 
(III-5)

[397] (2R,3R)-3-((S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 (0.63 g, 2.2 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 녹인 후, 2-(2,6-디플루오로페닐)에탄아민 (0.38 g, 2.4 mmol)을 가하고 반응혼합물을 0 ℃로 냉각한 다음,
벤조트리아졸-1-일-옥시-트리스(디메틸아미노)포스포늄헥사플루오로포스페이트 (0.97 g, 2.2 mmol)와 디이소프로필에틸아민 (1.0 mL, 6.6 mmol)을 순차적으로 가하고, 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 (R)-t-부틸 2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트 (0.60 g, 64%)을 얻었다.

[398] 상기 화합물 (0.6 g, 1.4 mmol)을 디클로로메탄 10 mL에 녹이고 트리플루오로아세트산 1 mL를 가한 후 상온에서 3시간 동안 교반하고, 반응이 종결되면 반응용매를 감압 농축한 후 디클로로메탄 10 mL를 2회 첨가하여 반복 농축하여 잔류 트리플루오로아세트산을 완전히 제거하여 (2R,3R)-N-(2,6-디플루오로페네틸)-3-메톡시-2-메틸-3-((R)-피롤리딘-2-일)프로판아미드의 트리플루오로아세트산염을 얻었다.

[399] 상기 농축액(TFA염)과 (5S,8S,11S,12R)-11-((S)-sec-부틸)-5,8-디이소프로필-12-메톡시-4,10-디메틸-3,6,9-트리옥소-1-메틸-2-옥사-4,7,10-트리아자테트라데칸-14-산 (0.77 g, 1.4 mmol)를 디메틸포름아미드 10 mL에 용해하고, 0 ℃에서 디에틸 시아노포스포네이트(DEPC) (0.23 mL, 1.54 mmol)와 트리에틸아민 (1.17 mL, 8.4 mmol)을 순차적으로 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 용매를 제거하고 얻어진 잔사를 에틸아세테이트 50 mL에 용해시켜 증류수 30 mL로 씻은 후, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (III-5)의 N-말단 아미노기가 보호화된 화합물 (0.96 g, 80%)를 얻었다.

[400] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 - 1.06 (m, 17 H), 1.18 - 1.38 (m, 5 H), 1.71 - 2.15 (m, 7 H), 2.21 - 2.52 (m, 4 H), 2.87 - 2.91 (m, 4 H), 2.99 (s, 3 H), 3.10 (s, 3 H), 3.38 (s, 3 H), 3.43 - 3.51 (m, 3 H), 3.82 (m, 1 H), 4.09 - 4.15 (m, 4 H), 4.69 (m, 1 H), 5.12 - 5.23 (m, 2 H), 6.50 - 6.60 (m, 2 H), 6.82 - 6.87 (m, 2 H), 7.13 - 7.17 (m, 1 H), 7.30 - 7.34 (m, 5 H)

[401]

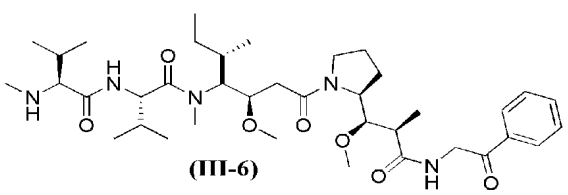
[402] 화합물 (III-5)의 N-말단 아미노기가 보호화된 화합물 (0.96 g, 1.1 mmol)를 t-부틸알코올 18 mL와 물 2 mL에 녹이고 10% 팔라듐 카본 (0.1g)을 가한 후 수소 대기 하에서 3시간 동안 교반하였다. 반응이 종결되면 반응 혼합물을 셀라이트로 여과하고 메탄올로 수회 세척한 후, 감압 하에서 용매를 제거하여 표제화합물 (0.79 g, 99%)을 얻었다.

[403] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 - 0.98 (m, 16 H), 1.06 (m, 1 H), 1.16 - 1.27 (m, 4 H), 1.38 (m, 1 H), 1.65 - 1.76 (m, 6 H), 1.89 - 2.02 (m, 4 H), 2.31 - 2.38 (m, 5 H), 2.71 - 2.75 (m, 1 H), 2.88 - 2.89 (m, 2 H), 3.00 (s, 3 H), 3.29 (s, 3 H), 3.41 - 3.48 (m, 3 H), 3.79 - 3.82 (m, 1 H), 4.08 - 4.15 (m, 2 H), 4.72 - 4.86 (m, 2 H), 6.56 (m, 1 H), 6.79 - 6.85 (m, 2 H), 7.10 - 7.15 (m, 1 H), 7.16 - 7.22 (m, 1 H), 7.55 (d, 1 H)

[404]

[405] **제조예 2-2: 화합물 (III-6)의 제조**

[406]



(III-6)

[407] 2-(2,6-디플루오로페닐)에탄아민 대신에 2-아미노아세토페논을 사용하여 제조예 2-1과 동일한 방법으로 화합물 (III-6)의 N-말단 아미노기가 보호화된 화합물 2.36 g (81%)을 얻었다.

[408] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 - 1.04 (m, 17 H), 1.31 - 1.32 (m, 4 H), 1.77 - 1.81 (m, 2 H), 1.92 - 2.04 (m, 4 H), 2.17 - 2.61 (m, 4 H), 2.89 - 2.95 (m, 3 H), 2.99 (s, 3 H), 3.30 (s, 3 H), 3.46 (m, 3 H), 3.43 - 3.52 (m, 1 H), 3.99 - 4.01 (m, 1 H), 4.09 - 4.15 (m, 1 H), 4.24 (m, 1 H), 4.69 - 4.76 (m, 6 H), 5.09 - 5.23 (m, 2 H), 6.46 - 6.49 (m, 1 H), 7.12 (m, 1 H), 7.26 - 7.34 (m, 5H), 7.50 (t, *J* = 7.6 Hz, 2 H), 7.62 (t, *J* = 7.2 Hz, 1 H), 7.97 (d, *J* = 7.6 Hz, 2 H)

[409]

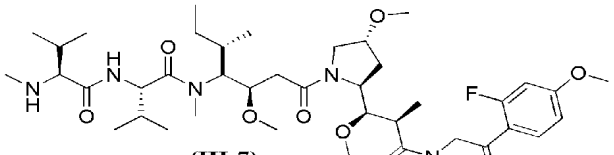
[410] 화합물 (III-6)의 N-말단 아미노기가 보호화된 화합물을 사용하여 제조예 2-1과 동일한 방법으로 표제화합물 1.89 g (96%)을 얻었다.

[411] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 - 0.85 (m, 3 H), 0.92 - 1.14 (m, 13 H), 1.25 - 1.43 (m, 5 H), 1.46 - 1.89 (m, 6 H), 1.92 - 2.22 (m, 5 H), 2.33 (s, 3 H), 2.45 - 2.54 (m, 2 H), 2.74 (d, *J* = 4.8 Hz, 1 H), 3.02 (s, 3 H), 3.32 (s, 3 H), 3.43 (d, *J* = 9.2 Hz, 3 H), 3.52 - 3.84 (m, 2 H), 3.92 - 4.06 (m, 2 H), 4.75 - 4.80 (m, 2 H), 4.94 - 5.23 (m, 1 H), 6.76 - 6.94 (m, 1 H), 7.34 - 7.43 (m, 4H), 7.60 (m, 1 H)

[412]

[413] **제조예 2-3: 화합물 (III-7)의 제조**

[414]



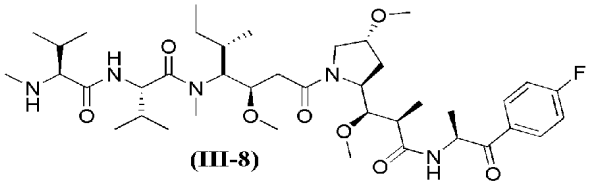
(III-7)

[415] (2R,3R)-3-((S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산을 사용하고, 2-(2,6-디플루오로페닐)에탄아민 대신에
2-아미노-1-(2-플루오로-4-메톡시페닐)에타논을 사용하여 제조예 2-1과 동일한 방법으로 표제화합물 0.18 g (89%)을 수득하였다.

[416] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 - 0.84 (m, 3 H), 0.88 - 1.03 (m, 17 H), 1.32 (d, *J* = 7.2 Hz, 3 H), 1.35 - 1.39 (m, 2 H), 1.98 - 2.10 (m, 4 H), 2.35 (s, 3 H), 2.36 - 2.51 (m, 3 H), 2.74 (m, 1 H), 3.02 (m, 3 H), 3.29 (s, 3 H), 3.34 (s, 3 H), 3.45 (s, 3 H), 3.46 - 3.56 (m, 1 H), 3.87 (s, 3 H), 3.99 (dd, *J* = 7.6 Hz, 2.8 Hz, 1 H), 4.10 - 4.15 (m, 1 H), 4.25 - 4.28 (m, 1 H), 4.58 - 4.64 (m, 2 H), 4.73 - 4.78 (m, 1 H), 6.67 (m, 1 H), 6.78 (qd, *J* = 10.4 Hz, 2.8 Hz, 1 H), 7.95 (m, 1 H)

[417]

[418] **제조예 2-4: 화합물 (III-8)의 제조**

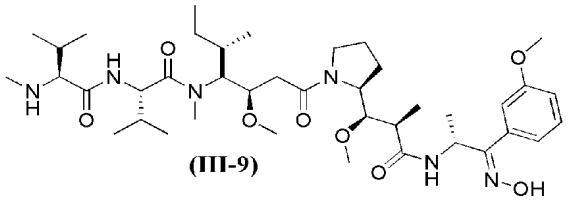
[419] 
(III-8)

[420] (2R,3R)-3-((S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산을 사용하고, 2-(2,6-디플루오로페닐)에탄아민 대신에
(S)-2-아미노-1-(4-플루오로페닐)프로판-1-온을 사용하여 제조예 2-1과 동일한 방법으로 표제화합물 0.45 g (100%)을 수득하였다.

[421] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 - 1.03 (m, 20 H), 1.22 (d, 3 H),1.33 - 1.37 (m, 4 H), 1.40 - 1.44 (m, 3 H), 1.60 - 1.81 (m, 4 H), 1.98 (m, 2 H), 2.26 (m, 2 H), 2.90 (s, 3 H), 3.21 (s, 3 H), 3.32 (s, 3 H), 3.43 (s, 3 H), 4.06 - 4.15 (m, 2 H), 4.22 - 4.27 (m, 2 H), 4.64 - 4.69 (m, 2 H), 5.09 - 5.13 (m, 2 H), 5.23 - 5.30 (m, 2 H), 5.50 (m, 1 H), 7.18 (t, 2 H), 8.01 - 8.05 (m, 2 H)

[422]

[423] **제조예 2-5: 화합물 (III-9)의 제조**

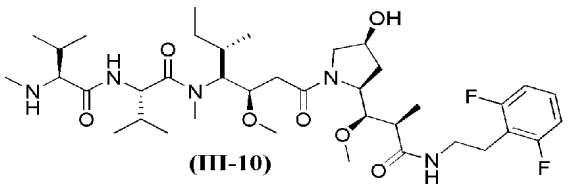
[424] 
(III-9)

[425] 2-(2,6-디플루오로페닐)에탄아민 대신에 (R,
E)-2-아미노-1-(3-메톡시페닐)프로판-1-온 옥심을 사용하여 제조예 2-1과 동일한 방법으로 표제화합물 0.17 g (94%)을 수득하였다.

[426] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 0.98 (m, 20 H), 1.01 (m, 4 H), 1.19 - 1.27 (m, 4 H), 1.79 - 1.83 (m, 2 H), 2.21 - 2.43 (m, 4 H), 2.77 (m, 2 H), 3.00 (m, 2 H), 3.29 (s, 6 H), 3.42 (s, 3 H), 3.99 - 4.14 (m, 3 H), 4.15 - 4.37 (m, 3 H), 4.70 (m, 1 H), 5.21 - 5.23 (m, 2 H), 5.36 (m, 1 H), 7.03 (m, 1 H), 7.15 (m, 2 H), 7.49 (m, 1 H)

[427]

[428] **제조예 2-6: 화합물 (III-10)의 제조**

[429] 
(III-10)

[430] (2R,3R)-3-((S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4S)-1-(t-부톡시카르보닐)-4-((t-부틸디메틸실릴)옥시)피롤리딘-2-

일)-3-메톡시-2-메틸프로판산을 사용하여 제조예 2-1과 동일한 방법으로 화합물 (III-10)의 N-말단 아미노기와 히드록시기가 보호화된 정제하지 않은 화합물 1.0 g (100%)을 얻었다.

[431] 상기 화합물 (1.0 g, 1.02 mmol)을 디메틸포름아미드 10 mL에 녹이고, 1.0 M 테트라부틸암모늄 플루오라이드 (3.1 mL, 3.1 mmol)를 첨가한 후 5시간 동안 교반하였다. 반응 완결 후 용매를 제거하고 얻어진 잔사를 에틸아세테이트 50 mL에 용해시켜 포화 탄산수소나트륨 용액 및 소금물로 씻은 후, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (III-10)의 N-말단 아미노기가 보호화된 화합물 (0.75 g, 84%)를 얻었다.

[432] ES-MS *m/z* : 874 $[M+H]^+$

[433]

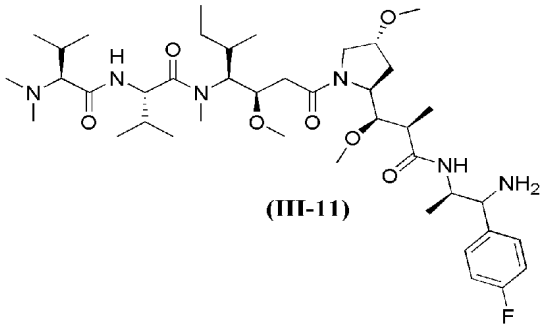
[434] 화합물 (III-10)의 N-말단 아미노기가 보호화된 화합물을 사용하여 제조예 2-1과 동일한 방법으로 표제화합물 0.64 g (100%)을 얻었다.

[435] ES-MS *m/z* : 740 $[M+H]^+$

[436]

[437] **제조예 2-7: 화합물 (III-11)의 제조**

[438]



(III-11)

[439] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산(0.70 g, 2.2 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 녹인 후, (2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염(0.47 g, 2.4 mmol)을 가하고 반응혼합물을 0 ℃로 냉각한 다음, 벤조트리아졸-1-일-옥시-트리스(디메틸아미노)포스포늄헥사플루오로포스페이트(0.97 g, 2.2 mmol)와 디이소프로필에틸아민(1.0 mL, 6.6 mmol)을 순차적으로 가하고, 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 (2S,4R)-t-부틸 2-((1R,2R)-3-(((2R)-1-아지도-1-(4-플루오로페닐)프로판-2-일)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-메톡시피롤리딘-1-카르복실레이트(0.61 g, 56%)를 얻었다.

[440] LC-MS *m/z* : 494.2 $[M^+]^+$, 516.3 $[M+Na]^+$

[441]

[442] 상기 화합물(0.61 g, 1.23 mmol)을 디클로로메탄 10 mL에 녹이고 트리플루오로아세트산 7 mL 적가한 후 20 - 25℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 mL를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[443] 반응 농축액(TFA염)과 (3R,4S,5S)-4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵탄산(0.53 g, 1.23 mmol)를 디메틸포름아미드 5 mL에 용해하고, 0 ℃에서 디에틸 시아노포스포네이트(DEPC) (0.20 mL, 1.35 mmol)와 트리에틸아민(1.56 mL, 11.09 mmol)을 가하고 상온에서 16시간 동안 교반하였다. 반응이 종결되면 반응용매를 제거하고 에틸아세테이트 20 mL에 용해시킨 후, 1 M 포타슘하이드로젠설파이트, 물, 포화 탄산수소나트륨 용액 및 소금물로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 화합물 (III-11)의 아미노기가 보호화된 화합물(0.25 g, 25%)을 수득하였다.

[444] LC-MS *m/z* : 779.6$[M+H]^+$

[445]

[446] 화합물 (III-11)의 아미노기가 보호화된 화합물(0.25 g, 0.31 mmol)을 메틸알코올 10 mL에 녹이고 10% 팔라듐 카본(15 mg)을 가한 후 수소 대기 하에서 16시간 동안 교반하였다. 반응이 종결되면 반응 혼합물을 셀라이트로 여과하고 메탄올로 수회 세척한 후, 감압 하에서 용매를 제거하여 백색 고체의 표제화합물(97 mg, 41%)을 수득하였다.

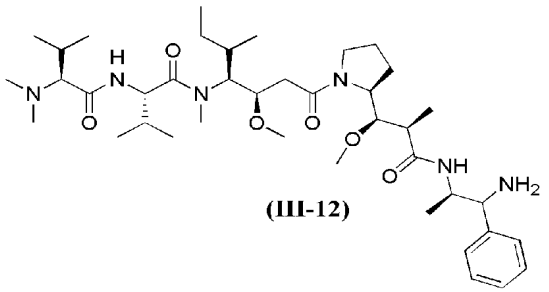
[447] LC-MS *m/z* : 753$[M^+]^+$, 775$[M+Na]^+$

[448] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (t, 3 H), 0.77-1.02 (m, 17 H), 1.01 (d, 3 H), 1.15 (d, 3 H), 1.32 (m, 1 H), 1.78 (brd 2H), 1.85-2.12 (m, 4 H), 2.18 (S, 6 H), 2.32-2.50 (m, 4H), 3.30 (s, 3H), 3.38 (s, 6H), 3.48(m, 1H), 4.00-4.15 (m, 4H), 4.32(m, 2H), 4.85 (m, 2H), 6.60 (s, 1H), 6.88 (s, 1H), 6.99-7.06 (m, 2H), 7.22-7.32 (m, 2H)

[449]

[450] **제조예 2-8: 화합물 (III-12)의 제조**

[451]



(III-12)

[452] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에

(2R,3R)-3-((2S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 (92 mg, 0.32 mmol)을 사용하고, (2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염 대신에 (2R)-1-아지도-1-페닐프로판-2-아민 염산염(68 mg, 0.32 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 표제화합물(44 mg, 50%)을 수득하였다.

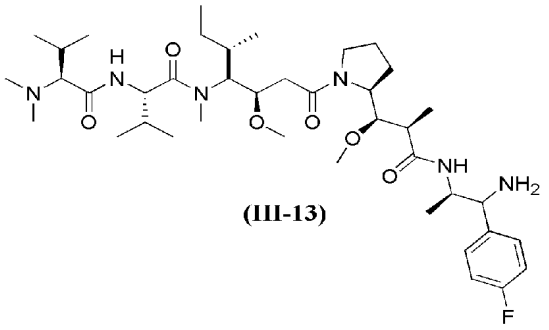
[453] MALDI-TOF MS *m/z* : 731.6 $[M+H]^+$, 753.5 $[M+Na]^+$

[454] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (t, 3 H), 0.88-1.07 (m, 17 H), 1.18 (d, 3 H), 1.21 (d, 3 H), 1.47 (m, 1 H), 1.74 (brd, 2H), 1.87- 2.17 (m, 3 H), 2.24 (S, 6 H), 2.25-2.47 (m, 4H), 3.04 (s, 3H), 3.28-3.34 (m,1H), 3.31 (s, 3H), 3.38 (s, 3H), 3.45(m, 1H), 3.79 (d, 1H), 3.82 (m, 1H), 4.05-4.12 (m, 4H), 4.24(m, 2H), 4.75 (m, 2H), 6.73 (s, 1H), 6.95 (d, 1H), 7.24-7.35 (m, 5H)

[455]

[456] **제조예 2-9: 화합물 (III-13)의 제조**

[457]



(III-13)

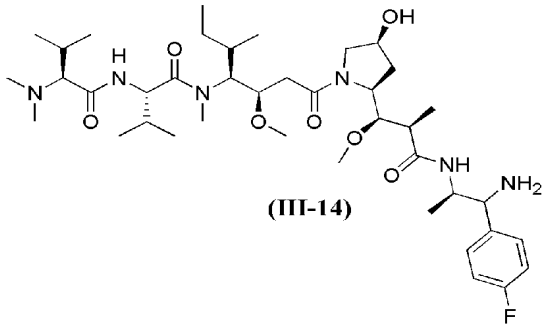
[458] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산(0.80 g, 2.8 mmol)을 사용하고, (2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염 (0.64 g, 2.8 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 표제화합물(0.5 g, 79%)을 수득하였다.

[459] LC-MS *m/z* : 749.47 $[M+H]^+$, 771.44 $[M+Na]^+$

[460] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (t, 3 H), 0.91-1.1 (m, 17 H), 1.16 (d, 3 H), 1.20 (d, 3 H), 1.35 (m, 1 H), 1.78 (brd, 2H), 1.87- 2.10 (m, 4H), 2.24 (S, 6 H), 2.28-2.48 (m, 4H), 3.03 (s, 3H), 3.28-3.34 (m,1H), 3.31 (s, 3H), 3.38 (s, 3H), 3.48(m, 1H), 3.82 (d, 1H), 4.03-4.10 (m, 4H), 4.22(m, 2H), 4.77 (m, 2H), 6.56 (d, 1H), 6.86 (s, 1H), 6.99-7.07 (m, 2H), 7.23-7.35 (m, 2H)

[461]

[462] **제조예 2-10: 화합물 (III-14)의 제조**

[463] 
(III-14)

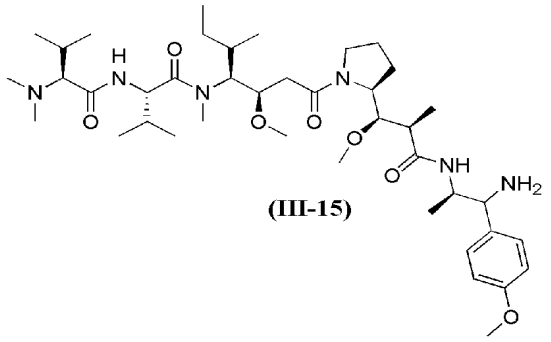
[464] ((2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-히드록시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 (340 mg, 1.12 mmol)을 사용하고,
(2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염(258 mg, 1.12 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 표제화합물(350 mg, 71%)을 수득하였다.

[465] LC-MS *m/z* : 765.46$[M^+]^+$

[466] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, 3 H), 0.90-1.09 (m, 17 H), 1.11 (d, 3 H), 1.25 (d, 3 H), 1.32 (m, 1 H), 1.78 (brd, 2H), 2.04- 2.09 (m, 3 H), 2.23 (S, 6 H), 2.35-2.50 (m, 4H), 3.03 (s, 3H), 3.32 (s, 3H), 3.38(m, 1H), 3.47 (s, 3H), 3.54-3.56 (m, 2H), 3.90 (m, 1H), 4.0-4.32 (m, 6H), 4.75 (m, 2H), 6.53 (s, 1H), 6.76 (d, 1H), 6.99-7.04 (m, 2H), 7.32-7.35 (m, 2H)

[467]

[468] **제조예 2-11: 화합물 (III-15)의 제조**

[469] 
(III-15)

[470] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S)-1-(t-부톡시카르보닐)피롤리딘-2-일)-3-메톡시-2-메틸프로판산 (0.17 g, 0.58 mmol)을 사용하고, (2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염 대신에 (2R)-1-프탈이미도-1-(4-메톡시페닐)프로판-2-아민 염산염(0.2 g, 0.58 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 화합물 (III-15)의 아미노기가 보호화된 화합물(0.34 g, 94%)을 수득하였다.

[471] LC-MS *m/z* : 891.62[M]+

[472] 화합물 (III-15)의 아미노기가 보호화된 화합물(0.34 g, 0.38 mmol)을 에탄올 10mL에 용해시키고, 히드라진 수화물 1mL를 가한 후 16시간 동안 상온에서 교반하였다. 반응이 종결되면 감압 하에서 용매를 제거하여 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 백색 고체의 표제화합물(0.11 g, 38%)을 수득하였다.

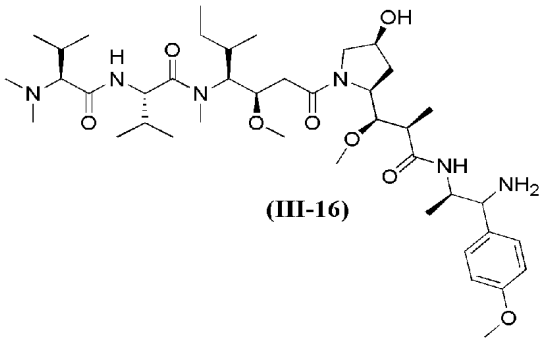
[473] LC-MS *m/z* : 761.56[M+]+, 783.60[M+Na]+

[474] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (m, 3H), 0.88-1.07 (m, 18H), 1.19 (d, 3 H), 1.15(m, 1H), 1.22 (m, 1 H), 1.25(d, 3H), 1.70- 1.90 (m, 4H), 2.07(S, 6 H), 2.26(s, 3H), 2.28-2.48 (m, 4H), 3.06 (s, 3H), 3.32 (s, 3H), 3.38 (m, 1H), 3.43(s, 3H), 3.50(m, 1H), 3.76 (m, 1H), 3.80(s, 3H), 3.78(d, 1H), 3.90-4.25 (m, 4H), 4.54 (m, 2H), 6.89 (d, 2H), 7.35 (d, 2H)

[475]

[476] **제조예 2-12: 화합물 (III-16)의 제조**

[477]



(III-16)

[478] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-히드록시피롤리딘-2-일)-3-메톡시-2-메틸프로판산(0.36 g, 1.17 mmol)을 사용하고,
(2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염 대신에
(2R)-1-프탈이미도-1-(4-메톡시페닐)프로판-2-아민 염산염(0.41 g, 1.17 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 화합물 (III-16)의 아미노기가 보화화된 화합물(0.59 g, 74%)을 수득하였다.

[479] LC-MS *m/z* : 907.54[M]+

[480]

[481] 화합물 (III-16)의 아미노기가 보호화된 화합물(0.59 g, 0.65 mmol)을 이용하여 제조예 2-11과 동일한 방법으로 백색 고체의 표제화합물(0.19 g, 38%)을 수득하였다.

[482] LC-MS *m/z* : 777.42[M+]+, 799.39[M+Na]+

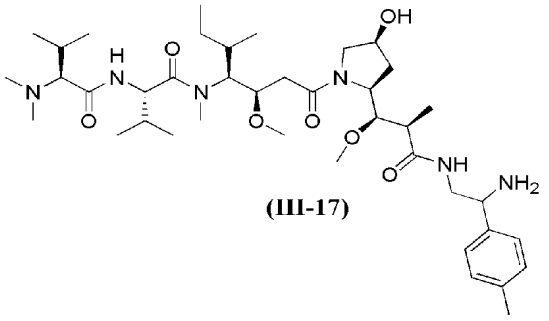
[483] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.859(m, 3H), 0.86-1.06(m, 17H), 1.13(d, 3H), 1.24-1.40(m, 6H), 1.62-1.84(m, 2H), 1.97-2.41(m, 3H), 2.20-2.32(m, 7H),

2.36-2.54(m, 4H), 3.06(s, 3H), 3.31(s, 3H), 3.46(t, 1H)3.50(s, 3H), 3.56(brs, 1H), 3.62(m, 1H), 3.67(m, 1H),3.72-3.77(m, 1H), 3.80(s, 3H), 3.97-4.28(m, 4H), 4.30(m, 1H), 4.68(m, 1H), 4.72-4.83(m, 1H), 6.89(d, 2H), 7.33(d, 2H)

[484]

[485] **제조예 2-13: 화합물 (III-17)의 제조**

[486]



(III-17)

[487] (2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-메톡시피롤리딘-2-일)-3-메톡시-2-메틸프로판산 대신에
(2R,3R)-3-((2S,4R)-1-(t-부톡시카르보닐)-4-하이드록시피롤리딘-2-일)-3-메톡시-2-메틸프로판산(1.43 g, 4.7 mmol)을 사용하고,
(2R)-1-아지도-1-(4-플루오로페닐)프로판-2-아민 염산염 대신에
2-프탈이미도-2-(p-톨릴)에틸아민 염산염(1.65 g, 5.2 mmol)을 사용하여 제조예 2-7과 동일한 방법으로 화합물 (III-17)의 아미노기가 보호화된 화합물(2.35 g, 78%)을 수득하였다.

[488] LC-MS *m/z* : 878$[M+1]^+$

[489]

[490] 화합물 (III-7)의 아미노기가 보호화된 화합물(2.08 g, 2.37 mmol)을 이용하여 제조예 2-11과 동일한 방법으로 백색 고체의 표제화합물(1.7 g, 94%)을 수득하였다.

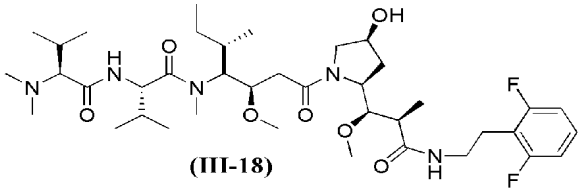
[491] LC-MS *m/z* : 748$[M+1]^+$

[492] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.84(m, 3H), 0.91-1.03(m, 19H), 1.24-1.29(m, 4H), 1.29-1.40(m, 1H), 1.90-2.14(m, 4H), 2.25(s, 6H), 2.32(s, 3H), 2.38-2.48(m, 3H), 3.03(s, 3H), 3.14(brd, 1H), 3.24-3.30(m, 1H), 3.32(s, 3H), 3.38-3.43(m, 1H), 3.48(s, 3H), 3.54-3.62(m, 3H), 4.04-4.18(m, 3H), 4.24-4.34(m, 2H), 4.77-4.90(m, 2H), 6.37(m, 1H), 7.14-7.28(m, 4H)

[493]

[494] **제조예 2-14: 화합물 (III-18)의 제조**

[495]



(III-18)

[496] (2R,3R)-3-((2S,4S)-1-(t-부톡시카르보닐)-4-((t-부틸디메틸실릴)옥시)피롤리딘-2-일)-3-메톡시-2-메틸프로판산(0.92 g, 2.2 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 녹인 후, 2-(2,6-디플루오로페닐)에탄아민(0.38 g, 2.4 mmol)을 가하고 반응혼합물을 0 ℃로 냉각한 다음, 벤조트리아졸-1-일-옥시-트리스(디메틸아미노)포스포늄헥사플루오로포스페이트(0.97 g, 2.2 mmol)와 디이소프로필에틸아민(1.0 mL, 6.6 mmol)을 순차적으로 가하고, 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.87 g, 70.9%)를 얻었다.

[497] 상기 화합물(0.87 g, 1.56 mmol)을 디클로로메탄 10 mL에 녹이고 트리플루오로아세트산 7 mL를 적가한 후 20 - 25℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 mL를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[498] 반응 농축액(TFA염)과 (3R,4S,5S)-4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵탄산(0.67 g, 1.56 mmol)을 디메틸포름아미드 5 mL에 용해하고, 0 ℃에서 디에틸 시아노포스포네이트(DEPC) (0.26 mL, 1.56 mmol)와 트리에틸아민(1.09 mL, 7.82 mmol)을 가하고 상온에서 16시간 동안 교반하였다. 반응이 종결되면 반응용매를 제거하고 에틸아세테이트 20 mL에 용해시킨 후, 1 M 포타슘하이드로젠설파이트, 물, 포화 탄산수소나트륨 용액 및 소금물로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피(에틸아세테이트:헥산 = 2:1 → 에틸아세테이트)로 정제하여 화합물 (III-18)의 히드록시가 보호화된 화합물(1.14 g, 84%)을 수득하였다.

[499] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.81 - 1.06 (m, 19 H), 1.19 (m, 3 H), 1.46 (m, 2 H), 1.65 (m, 2 H), 1.97 - 2.17 (m, 3 H), 2.44 - 2.46 (m, 3 H), 2.71 - 2.80 (m, 3 H), 2.81 - 2.89 (m, 1 H), 2.91 - 2.94 (m, 2 H), 2.71 - 2.80 (m, 3 H), 3.04 (s, 3 H), 3.26 - 3.33 (m, 2 H), 3.37 (s, 3 H), 3.74 (m, 2 H), 4.28 - 4.31 (m, 2 H), 4.75 - 4.82 (m, 2 H), 6.18 (m, 1 H), 6.84 - 6.89 (m, 2 H), 7.15 - 7.19 (m, 1 H), 7.58 (d, 1 H)

[500]

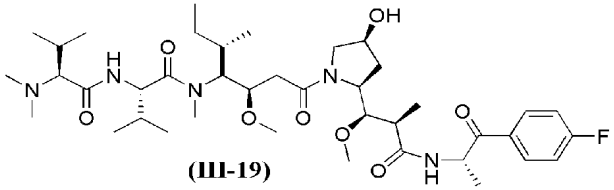
[501] 화합물 (III-18)의 히드록시기가 보호화된 화합물(1.14 g, 1.31 mmol)을 테트라히드로퓨란 95 mL에 녹이고, 1.0 M 테트라부틸암모늄 플루오라이드 (4.9 mL, 4.09 mmol)를 첨가한 후 5시간 동안 교반하였다. 포화된 암모늄클로라이드 수용액으로 반응을 종결시키고 에틸아세테이트 450 mL와 물 300 mL로 추출한 후 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피 (디클로로메탄:메탄올 = 9:1)로 정제하여 표제화합물(0.45 g, 45%)을 수득하였다.

[502] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 1.05 (m, 19 H), 1.26 (m, 3 H), 1.45 (m, 2 H), 1.66 (m, 2 H), 1.97 - 2.17 (m, 3 H), 2.44 - 2.46 (m, 3 H), 2.71 - 2.80 (m, 3 H), 2.81 - 2.89 (m, 1 H), 2.91 - 2.94 (m, 2 H), 2.71 - 2.80 (m, 3 H), 3.04 (s, 3 H), 3.26 - 3.33 (m, 2 H), 3.37 (s, 3 H), 3.74 (m, 2 H), 4.28 - 4.31 (m, 2 H), 4.75 - 4.82 (m, 2 H), 6.18 (m, 1 H), 6.84 - 6.89 (m, 2 H), 7.15 - 7.19 (m, 1 H), 7.58 (d, 1 H)

[503] LC-MS *m/z* : 754[M$^+$]$^+$

[504]

[505] **제조예 2-15: 화합물 (III-19)의 제조**

[506] 

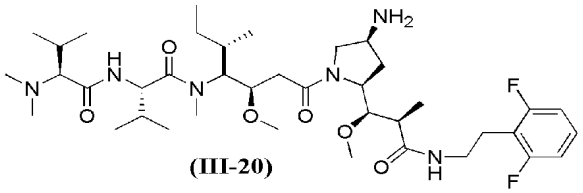
(III-19)

[507] 2-(2,6-디플루오로페닐)에탄아민 대신에 (S)-2-아미노-1-(4-플루오로페닐)프로판-1-온을 사용하여 (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-(((S)-1-(4-플루오로페닐)-1-옥소프로판-2-일)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.44 g, 0.78 mmol)를 얻고, 이를 사용하여 제조예 2-14와 동일한 방법으로 표제화합물 0.34 g (58%)를 수득하였다.

[508] LC-MS *m/z* : 764[M$^+$]$^+$

[509]

[510] **제조예 2-16: 화합물 (III-20)의 제조**

[511] 

(III-20)

[512] (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 4-아지도-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.33 g, 0.71 mmol)를 사용하여 제조예 2-14와 동일한 방법으로 화합물 (III-20)의 아미노기가 보호화된 화합물(0.42 g, 76%)을 수득하였다.

[513] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 1.07 (m, 15 H), 1.20 (d, 2 H), 1.99 -2.11 (m, 4 H), 2.18 - 2.28 (m, 5 H), 2.33 - 2.45 (m, 4 H), 2.90 - 3.27 (m, 6 H), 3.32 - 3.42 (m, 6 H), 3.47 - 3.54 (m, 2 H), 3.75 - 3.78 (m, 1 H), 3.85 - 3.98 (m, 2 H), 4.19 - 4.21 (m, 1 H), 4.39 - 4.44 (m, 1 H), 4.46 - 4.80 (m, 1 H), 4.87 - 4.90 (m, 1 H), 6.17 (m, 1 H), 6.84 - 6.95 (m, 2 H), 7.14 - 7.26 (m, 1 H)

[514]

[515] 화합물 (III-20)의 아미노기가 보호화된 화합물 (0.24 g, 0.31 mmol)을 메틸알코올 10 mL에 녹이고 10% 팔라듐 카본 (15 mg)을 가한 후 수소 대기 하에서 14시간 동안 교반하였다. 반응이 종결되면 반응 혼합물을 셀라이트로 여과하고 메탄올로 수회 세척한 후, 감압 하에서 용매를 제거하여 백색 고체의 표제화합물(0.23 g, 99%)를 수득하였다.

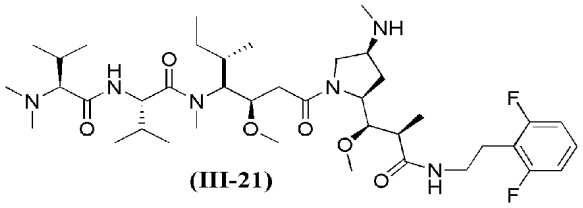
[516] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 1.03 (m, 14 H), 1.21 - 1.22 (m, 5 H), 2.06 - 2.45 (m, 6 H), 2.89 - 3.02 (m, 4 H), 3.30 - 3.44 (m, 5 H), 3.47 - 3.52 (m, 2 H), 3.67 - 3.69 (m, 1 H), 3.71 - 4.39 (m, 3 H), 4.78 - 4.80 (m, 1 H), 6.17 (m, 1 H), 6.84 - 6.95 (m, 2 H), 7.14 - 7.26 (m, 1 H)

[517] LC-MS *m/z* : 753[M$^+$]$^+$, 775[M+Na]$^+$

[518]

[519] **제조예 2-17: 화합물 (III-21)의 제조**

[520]



(III-21)

[521] (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 4-(벤질(메틸)아미노)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.13 g, 0.24 mmol)를 사용하여 제조예 2-14와 동일한 방법으로 화합물 (III-21)의 아미노기가 보호화된 화합물(0.13 g, 64%)을 수득하였다.

[522] LC-MS *m/z* : 857.5[M$^+$]$^+$

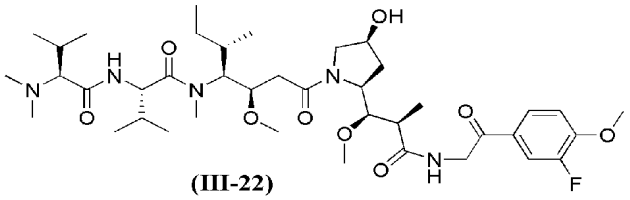
[523]

[524] 화합물 (III-21)의 아미노기가 보호화된 화합물(0.13 g, 0.15 mmol)을 메틸알코올 10 mL에 녹이고 20% 수산화팔라듐(66 mg)을 가한 후 수소 압력(55 psi) 하에서 12시간 동안 교반하였다. 반응이 종결되면 반응 혼합물을 셀라이트로 여과하고 메탄올로 수회 세척한 후, 감압 하에서 용매를 제거하여 백색 고체의 표제화합물(115 mg, 100%)을 수득하였다.

[525] LC-MS *m/z* : 767.6[M$^+$]$^+$, 789.4[M+Na]$^+$

[526]

[527] **제조예 2-18: 화합물 (III-22)의 제조**

[528] 

(III-22)

[529] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트(1.28 g, 2.73 mmol)를 디클로로메탄 30 mL에 녹이고 트리플루오로아세트산 10 mL를 적가한 후 20 - 25 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 ㎖를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[530] (3R,4S,5S)-t-부틸 4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵탄노에이트(1.33 g, 2.73 mmol)를 디클로로메탄 30 mL에 녹이고 트리플루오로아세트산 10 mL를 적가한 후 20 - 25 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 mL를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

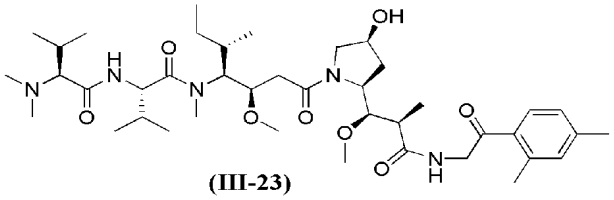
[531] 두 반응 농축액(TFA염)을 디클로로메탄 60 mL에 용해하고, 0 ℃에서 디에틸시아노포스포네이트(DEPC) (0.52 mL, 3.28 mmol)와 트리에틸아민(3.8 mL, 27.32 mmol)을 가하고 상온에서 16시간 동안 교반하였다. 반응이 종결되면 반응용매를 제거하고 에틸아세테이트 50 mL에 용해시킨 후, 1 M 포타슘하이드로젠설파이트, 물, 포화 탄산수소나트륨 용액 및 소금물로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피(에틸아세테이트:헥산 = 2:1 → 에틸아세테이트)로 정제하여 표제화합물(1.3 g, 60%)을 수득하였다.

[532] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 0.88 (m, 3 H), 0.91-1.07 (m, 17 H), 1.31-1.37 (m, 5 H), 1.99 - 2.11 (m, 3 H), 2.28 (s, 6 H), 2.43 - 2.62 (m, 6 H), 3.06 (s, 3 H), 3.11 - 3.16 (m, 2 H), 3.34 (s, 3 H), 3.56 (s, 3H), 3.57 -3.65 (m, 2 H), 3.97 (s, 3H), 4.08-4.09 (brs, 1H), 4.18-4.2 (d, 1H), 4.3 (brs, 1H), 4.39-4.42 (d, 2 H), 4.7 -4.72 (t, 2 H), 4.76-4.8 (m, 2H), 4.85-4.91 (brs, 1H), 7.03-7.1 (m, 1H), 7.69-7.78 (m, 2H)

[533] LC-MS *m/z* : 780.8 $[M+H]^+$, 802.8 $[M+Na]^+$

[534]

[535] **제조예 2-19: 화합물 (III-23)의 제조**

[536] 

(III-23)

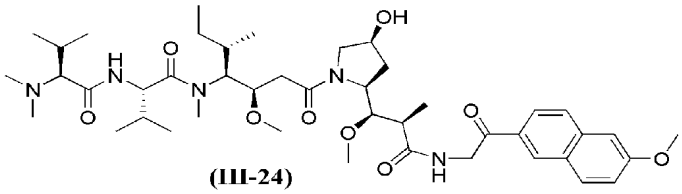
[537] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(2,4-디메틸페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트를 사용하여 제조예 2-18과 동일한 방법으로 표제화합물(3.2 g, 89%)을 수득하였다.

[538] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.85 (m, 3 H), 0.91-1.13 (m, 14 H), 1.15-1.19 (m, 3H), 1.35-1.38 (m, 5 H), 2.00 - 2.10 (m, 3 H), 2.18-2.24 (m, 3H), 2.37 (s, 3 H), 2.39-2.48 (m, 1H), 2.53 (s, 3H), 2.55-2.62 (m, 1H), 2.87 (s, 6H), 2.89 (s, 1H), 3.02 (s, 3H), 3.07-3.17 (m, 2H), 3.32 (s, 3H), 3.52 (s, 3H), 3.51-3.61 (m, 2H), 4.11-4.12 (brs, 1H), 4.16-4.18 (d, 1H), 4.30 (m, 1H), 4.37-4.4 (d, 1H), 4.64-4.66 (t, 2H), 4.69-4.74 (m, 2H), 6.90 (t, 1H), 7.09-7.11 (m, 2H), 7.66-7.68 (d, 2H)

[539] LC-MS *m/z* : 760.6 $[M+H]^+$, 782.6 $[M+Na]^+$

[540]

[541] **제조예 2-20: 화합물 (III-24)의 제조**

[542] 

(III-24)

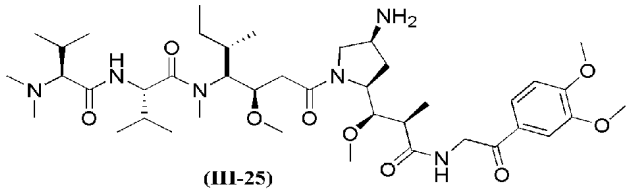
[543] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 4-히드록시-2-((1R,2R)-1-메톡시-3-((2-(6-메톡시나프탈렌-2-일)-2-옥소에틸)아미노)-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트를 사용하여 제조예 2-18과 동일한 방법으로 표제화합물(0.17 g, 67.5%)을 수득하였다.

[544] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.82 (m, 3H), 0.85-1.07 (m, 17H), 1.26 -1.40 (m, 5H), 1.98-2.10 (m, 6H), 2.39-2.48 (m, 3H), 2.58-2.64 (m, 1H), 3.01 (s, 3H), 3.34 (s, 3H), 3.52-3.73 (m, 2H), 3.57 (s, 3H), 4.12 (brs, 1H), 4.19 -4.21 (d, 1H), 4.25-4.31 (brs, 1H), 4.41-4.44 (d, 1H), 4.75-4.83 (m, 2H), 4.87-4.94 (m, 3H), 6.91-6.94 (t, 2H), 7.17 (s, 1H), 7.22-7.24 (d, 1H), 7.78-7.92 (m, 2H), 7.96-7.98 (d, 1H), 8.42 (s, 1H)

[545] LC-MS *m/z* : 812.7 $[M+H]^+$, 834.7 $[M+Na]^+$

[546]

[547] **제조예 2-21: 화합물 (III-25)의 제조**

[548] 
(III-25)

[549] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에(2S, 4S)-t-부틸-4-아지도-2((1R, 2R)-3-((2-(3, 4-디메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.39 g, 0.77 mmol)를 사용하여 제조예 2-18과 동일한 방법으로 화합물 (III-25)의 아미노기가 보호화된 화합물 (0.56 g, 89%)을 수득하였다.

[550] LC-MS *m/z* : 817.5 $[M+H]^+$, 839.5 $[M+Na]^+$

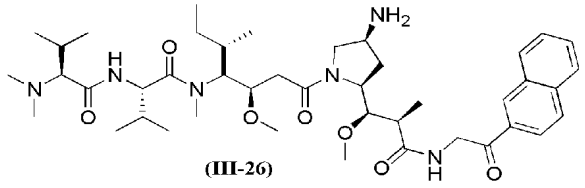
[551]

[552] 화합물 (III-25)의 아미노기가 보호화된 화합물을 사용하여 제조예 2-16과 동일한 방법으로 표제화합물 (0.38 g, 69%)을 수득하였다.

[553] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.82 (t, 3 H), 0.88-1.02 (m, 17 H), 1.23-1.28 (m, 1 H), 1.31-1.34 (m, 3 H), 1.96-2.12 (m, 2 H), 2.25-2.28 (m, 6 H), 2.36-2.52 (m, 2 H), 2.56-2.65 (m, 1 H), 2.98-3.03 (m, 3 H), 3.25-3.33 (m, 5 H), 3.47-3.49 (m, 1 H), 3.51-3.57 (m, 3 H), 3.72-3.73 (m, 1 H), 3.83-3.96 (d, 6 H), 4.09-4.16 (m, 2 H), 4.18-4.24 (m, 2 H), 4.25-4.36 (m, 1 H), 4.68-4.69 (m, 1 H), 4.74-4.88 m, 2 H), 4.82-4.88 (m, 1 H), 6.91-6.93 (d, 1 H), 7.50 (s, 1 H), 7.60-7.63 (d, 1 H)

[554]

[555] **제조예 2-22: 화합물 (III-26)의 제조**

[556] 
(III-26)

[557] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에(2S, 4S)-t-부틸-4-아지도-2-((1R, 2R)-1-메톡시-2-메틸-3-((2-(나프탈렌-2-일)-2-옥소에틸)아미노-3-옥소프로필)피롤리딘-1-카르복실레이트(0.40 g, 0.81 mmol)를 사용하여 제조예 2-18과 동일한 방법으로 화합물 (III-26)의 아미노기가 보호화된 화합물(0.60 g, 92%)을 수득하였다.

[558] LC-MS *m/z* : 807.5 $[M+H]^+$, 829.5 $[M+Na]^+$

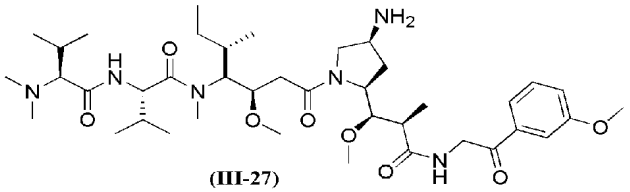
[559]

[560] 화합물 (III-26)의 아미노기가 보호화된 화합물을 사용하여 제조예 2-16과 동일한 방법으로 표제화합물(0.28 g, 48%)을 수득하였다.

[561] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78-0.82 (t, 3 H), 0.87-1.01 (m, 17 H), 1.22-1.28 (m, 1 H), 1.33-1.34 (m, 3 H), 1.80-2.12 (m, 2 H), 2.24-2.28 (m, 6 H), 2.38-2.50 (m, 2 H), 2.58-2.68 (m, 1 H), 2.95-3.05 (m, 3 H), 3.06-3.13 (m, 1 H), 3.20-3.23 (m, 1 H), 3.28-3.40 (m, 3 H), 3.43-3.45 (m, 1 H), 3.48-3.59 (m, 3 H), 3.60-3.67 (m, 1 H), 3.72-3.75 (m, 1 H), 4.04-4.17 (m, 2 H), 2.20-2.30 (m, 1 H), 4.32-4.36 (m, 1 H), 4.73-4.81 (m, 2 H), 4.85-4.86 (m, 1 H), 4.96-5.02 (m, 1 H), 7.57-7.65 (m, 2 H), 7.88-8.01 (m, 4 H), 8.51 (s, 1 H)

[562]

[563] **제조예 2-23: 화합물 (III-27)의 제조**

[564] 
(III-27)

[565] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸-4-아지도-2-((1R,2R)-1-메톡시-3-((2-(3-메톡시페닐)-2-옥소에틸)아미노)-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.30 g, 0.63 mmol)를 사용하여 제조예 2-18과 동일한 방법으로 화합물 (III-27)의 아미노기가 보호화된 화합물(0.50 g, 100%)을 수득하였다.

[566] LC-MS *m/z* : 787.4 $[M+H]^+$, 809.5 $[M+Na]^+$

[567]

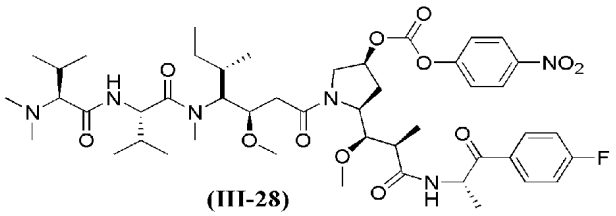
[568] 화합물 (III-27)의 아미노기가 보호화된 화합물을 사용하여 제조예 2-16과 동일한 방법으로 표제화합물(0.23 g, 48%)을 수득하였다.

[569] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78-0.82 (t, 3 H), 0.88-1.02 (m, 17 H), 1.25-1.29 (m, 3 H), 2.07-2.09 (m, 2 H), 2.18-2.31 (m, 6 H), 2.33-2.40 (m, 2 H), 2.52-2.64 (m, 1 H), 2.92-3.08 (m, 3 H), 3.20-3.25 (m, 1 H), 3.28-3.33 (m, 3 H), 3.38-3.40 (m, 1 H), 3.48-3.76 (m, 1 H), 3.78-3.88 (m, 3 H), 4.06-4.16 (m, 2 H), 4.19-4.28 (m, 1 H), 4.29-4.39 (m, 1 H), 4.70-4.71 (m, 1 H), 4.74-4.81 (m, 2 H), 4.88-4.95 (m, 1 H), 6.96-6.99 (d, 1 H), 7.38-7.42 (t, 1 H), 7.47 (s, 1 H), 7.53-7.55 (d, 1 H)

[570]

[571] **제조예 2-24: 화합물 (III-28)의 제조**

[572]



(III-28)

[573] (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-(((S)-1-(4-플루오로페닐)-1-옥소프로판-2-일)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.87 g, 1.56 mmol)를 디클로로메탄 10 mL에 녹이고 트리플루오로아세트산 7 mL를 적가한 후 20 - 25℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 ㎖를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[574] 반응 농축액(TFA염)과 (3R,4S,5S)-4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵탄산(0.67 g, 1.56 mmol)를 디메틸포름아미드 5 mL에 용해하고, 0 ℃에서 디에틸 시아노포스포네이트(DEPC) (0.26 mL, 1.56 mmol)와 트리에틸아민(1.09 mL, 7.82 mmol)을 가하고 상온에서 16시간 동안 교반하였다. 반응이 종결되면 반응용매를 제거하고 에틸아세테이트 20 mL에 용해시킨 후, 1 M 포타슘하이드로젠설파이트, 물, 포화 탄산수소나트륨 용액 및 소금물로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피(에틸아세테이트:헥산 = 2:1 → 에틸아세테이트)로 정제하여 히드록시가 보호화된 화합물(1.14g, 84%)을 수득하였다.

[575] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.81 - 1.06 (m, 19 H), 1.19 (m, 3 H), 1.46 (m, 2 H), 1.65 (m, 2 H), 1.97 - 2.17 (m, 3 H), 2.44 - 2.46 (m, 3 H), 2.71 - 2.80 (m, 3 H), 2.81 - 2.89 (m, 1 H), 2.91 - 2.94 (m, 2 H), 2.71 - 2.80 (m, 3 H) 3.04 (s, 3 H), 3.26 - 3.33 (m, 2 H), 3.37 (s, 3 H), 3.74 (m, 2 H), 4.28 - 4.31 (m, 2 H), 4.75 - 4.82 (m, 2 H), 6.18 (m, 1 H), 6.84 - 6.89 (m, 2 H), 7.15 - 7.19 (m, 1 H), 7.58 (d, 1 H)

[576]

[577] 히드록시기가 보호화된 화합물(1.14 g, 1.31 mmol)을 테트라히드로퓨란 95 ㎖에 녹이고, 1.0 M 테트라부틸암모늄 플루오라이드(4.9 mL, 4.09 mmol)를 첨가한 후 5시간 동안 교반하였다. 포화된 암모늄클로라이드 수용액으로 반응을 종결시키고 에틸아세테이트 450 mL와 물 300 mL로 추출한 후 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피 (디클로로메탄:메탄올 = 9:1)로 정제하여 (S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N-((3R,4S,5S)-1-((2S,4S)-2-((1R,2R)-3-(((S)-1-(4-플루오로페닐)-1-옥소프로판-2-일)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-일)-3-메톡시-5-메틸-1-옥소헵탄-4-일)-N,3-디

메틸부탄아미드(0.45g, 45%)을 수득하였다.

[578] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 1.05 (m, 19 H), 1.26 (m, 3 H), 1.45 (m, 2 H), 1.66 (m, 2 H), 1.97 - 2.17 (m, 3 H), 2.44 - 2.46 (m, 3H), 2.71 - 2.80 (m, 3 H), 2.81 - 2.89 (m, 1 H), 2.91 - 2.94 (m, 2 H), 2.71 - 2.80 (m, 3 H), 3.04 (s, 3 H), 3.26 - 3.33 (m, 2 H), 3.37 (s, 3 H), 3.74 (m, 2 H), 4.28 - 4.31 (m, 2 H), 4.75 - 4.82 (m, 2 H), 6.18 (m, 1 H), 6.84 - 6.89 (m, 2 H), 7.15 - 7.19 (m, 1 H), 7.58 (d, 1 H)

[579] LC-MS *m/z* : 754 $[M+H]^+$

[580]

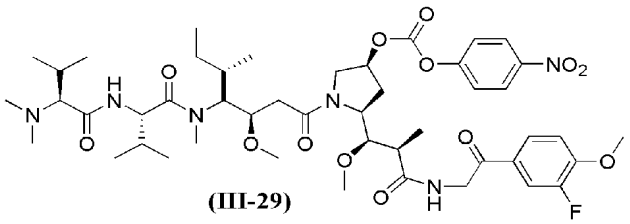
[581] 상기 화합물(0.45 g, 0.59 mmol)을 디클로로메탄 20 ml에 용해하고 -50 내지 -60 ℃로 냉각한 후 피리딘 (0.29 ml, 3.54 mmol)을 가하였다. 생성된 반응용액에 p-니트로페닐 클로로포르메이트 (0.71 g, 3.54 mmol)을 디클로로메탄 10 ml에 용해하여 20분 동안 서서히 적가한 후 -50 내지 -60 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용액에 디클로로메탄 20 ㎖를 더 가하고, 0.5 M 포타슘하이드로젠설파이트 용액으로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(0.4 g, 74%)을 수득하였다.

[582] LC-MS *m/z* : 929.6 $[M+H]^+$

[583]

[584] **제조예 2-25: 화합물 (III-29)의 제조**

[585]



(III-29)

[586] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트(1.28 g, 2.73 mmol)를 디클로로메탄 30 mL에 녹이고 트리플루오로아세트산 10 mL를 적가한 후 20 - 25℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 10 ㎖를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[587] (3R,4S,5S)-t-부틸 4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵탄노에이트(1.33 g, 2.73 mmol)를 디클로로메탄 30 mL에 녹이고 트리플루오로아세트산 10 mL를 적가한 후 20 - 25 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 10 mL를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[588] 두 반응 농축액(TFA염)을 디클로로메탄 60 mL에 용해하고, 0 ℃에서 디에틸 시아노포스포네이트(DEPC) (0.52 mL, 3.28 mmol)와 트리에틸아민(3.8 mL, 27.32 mmol)을 가하고 상온에서 16시간 동안 교반하였다. 반응이 종결되면 반응용매를 제거하고 에틸아세테이트 50 mL에 용해시킨 후, 1 M 포타슘하이드로젠설파이트, 물, 포화 탄산수소나트륨 용액 및 소금물로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피(에틸아세테이트:헥산 = 2:1 → 에틸아세테이트)로 정제하여 (S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N-((3R,4S,5S)-1-((2S,4S)-2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-일)-3-메톡시-5-메틸-1-옥소헵탄-4-일)-N,3-디메틸부탄아미드(1.3 g, 60%)을 수득하였다.

[589] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 -0.88 (m, 3 H), 0.91-1.07 (m, 17 H), 1.31 -1.37 (m, 5 H), 1.99 - 2.11 (m, 3 H), 2.28 (s, 6 H), 2.43- 2.62 (m, 6 H), 3.06 (s, 3 H), 3.11 - 3.16 (m, 2 H), 3.34 (s, 3 H), 3.56 (s, 3H), 3.57-3.65 (m, 2 H), 3.97 (s, 3H), 4.08-4.09 (brs, 1H), 4.18-4.2 (d, 1H), 4.3 (brs, 1H), 4.39 - 4.42 (d, 2 H), 4.7 - 4.72 (t, 2 H), 4.76-4.8 (m, 2H), 4.85 -4.91 (brs, 1H), 7.03-7.1 (m, 1H), 7.69 -7.78 (m, 2H)

[590] LC-MS *m/z* : 780.8 $[M+H]^+$, 802.8 $[M+Na]^+$

[591]

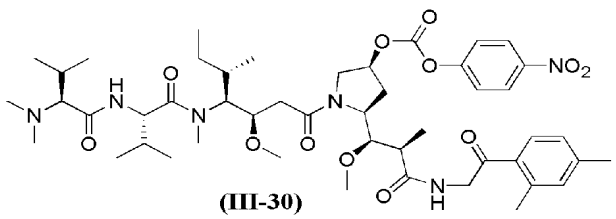
[592] 상기화합물(1.27 g, 1.63 mmol)을 디클로로메탄 20 ml에 용해하고 -50 내지 -60 ℃로 냉각한 후 피리딘(1.3 ml, 16.28 mmol)을 가하였다. 생성된 반응용액에 p-니트로페닐 클로로포르메이트(16.28 g, 3.3 mmol)을 디클로로메탄 10 ml에 용해하여 20분 동안 서서히 적가한 후 -50 내지 -60 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용액에 디클로로메탄 20 ㎖를 더 가하고, 0.5 M 포타슘하이드로젠설파이트 용액으로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(0.8 g, 54%)을 수득하였다.

[593] LC-MS *m/z* : 945.7 $[M+H]^+$, 967.7 $[M+Na]^+$

[594]

[595] **제조예 2-26: 화합물 (III-30)의 제조**

[596]



(III-30)

[597] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸

2-((1R,2R)-3-((2-(2,4-디메틸페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트를 사용하여 제조예 2-25와 동일한 방법으로

(S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N-((3R,4S,5S)-1-((2S,4S)-2-((1R,2R)-3-((2-(2,4-디메틸페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-일)-3-메톡시-5-메틸-1-옥소헵탄-4-일)-N,3-디메틸부탄아미드(3.2 g, 89%)을 얻었다.

[598] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 0.85 (m, 3 H), 0.91-1.13 (m, 14 H), 1.15-1.19 (m, 3H), 1.35-1.38 (m, 5 H), 2.00 - 2.10 (m, 3 H), 2.18-2.24 (m, 3H), 2.37 (s, 3 H), 2.39-2.48 (m, 1H), 2.53 (s, 3H), 2.55-2.62 (m, 1H), 2.87 (s, 6H), 2.89 (s, 1H), 3.02 (s, 3H), 3.07-3.17 (m, 2H), 3.32 (s, 3H), 3.52 (s, 3H), 3.51-3.61 (m, 2H), 4.11-4.12 (brs, 1H), 4.16-4.18 (d, 1H), 4.30 (m, 1H), 4.37-4.4 (d, 1H), 4.64-4.66 (t, 2H), 4.69-4.74 (m, 2H), 6.90 (t, 1H), 7.09 - 7.11 (m, 2H), 7.66 - 7.68 (d, 2H)

[599] LC-MS *m/z* : 760.6 $[M+H]^+$, 782.6 $[M+Na]^+$

[600]

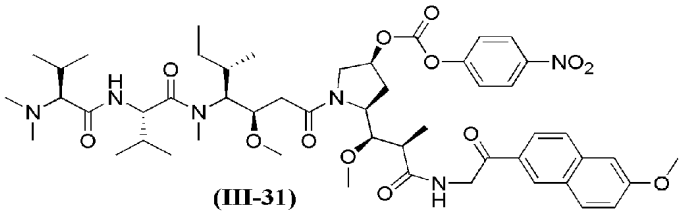
[601] 상기 화합물을 사용하여 제조예 2-25와 동일한 방법으로 표제화합물(1.6 g, 43%)를 얻었다.

[602] LC-MS *m/z* : 925.6 $[M+H]^+$, 947.6 $[M+Na]^+$

[603]

[604] **제조예 2-27: 화합물 (III-31)의 제조**

[605]



(III-31)

[606] (2S,4S)-t-부틸

2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 4-히드록시-2-((1R,2R)-1-메톡시-3-((2-(6-메톡시나프탈렌-2-일)-2-옥소에틸)아미노)-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트를 사용하여 제조예 2-25와 동일한 방법으로

(S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N-((3R,4S,5S)-1-((2S,4S)-4-히드록시-2-((1R,2R)-1-메톡시-3-((2-(6-메톡시나프탈렌-2-일)-2-옥소에틸)아미노)-2-메틸-3-옥소프로필)피롤리딘-1-일)-3-메톡시-5-메틸-1-옥소헵탄-4-일)-N,3-디메틸부탄아미드(0.17 g, 67.5%)를 얻었다.

[607] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.82 (m, 3H), 0.85-1.07 (m, 17H), 1.26 -1.40 (m, 5H), 1.98-2.10 (m, 6H), 2.39-2.48 (m, 3H), 2.58-2.64 (m, 1H), 3.01 (s, 3H), 3.34 (s, 3H), 3.52-3.73 (m, 2H), 3.57 (s, 3H), 4.12 (brs, 1H), 4.19 -4.21 (d, 1H), 4.25-4.31 (brs,

1H), 4.41-4.44 (d, 1H), 4.75-4.83 (m, 2H), 4.87-4.94 (m, 3H), 6.91-6.94 (t, 2H), 7.17 (s, 1H), 7.22 -7.24 (d, 1H), 7.78 - 7.92 (m, 2H), 7.96 - 7.98 (d, 1H), 8.42 (s, 1H)

[608] LC-MS *m/z* : 812.7 $[M+H]^+$, 834.7 $[M+Na]^+$

[609]

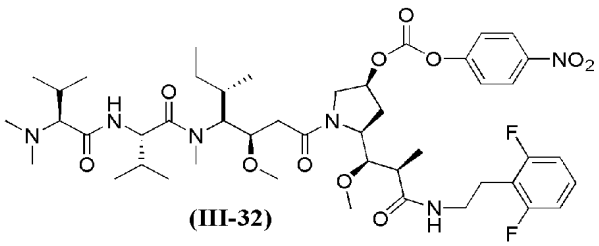
[610] 상기 화합물을 사용하여 제조예 2-25와 동일한 방법으로 표제화합물(0.15 g, 87%)를 얻었다.

[611] LC-MS *m/z* : 977.9 $[M+H]^+$, 999.9 $[M+Na]^+$

[612]

[613] **제조예 2-28: 화합물 (III-32)의 제조**

[614]



(III-32)

[615] (2S,4S)-t-부틸 2-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-카르복실레이트 대신에 (2S,4S)-t-부틸 4-((t-부틸디메틸실릴)옥시)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-1-카르복실레이트(0.87 g, 70.9%)를 사용하여 제조예 2-24와 동일한 방법으로 (2S)-N-((3R,4S)-1-((2S,4S)-2-((1R,2R)-3-((2,6-디플루오로페네틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)-4-히드록시피롤리딘-1-일)-3-메톡시-5-메틸-1-옥소헵탄-4-일)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미드(0.45 g, 45%)을 수득하였다.

[616] ^1H NMR (400 MHz, $CDCl_3$) δ 0.79 - 1.05 (m, 19 H), 1.26 (m, 3 H), 1.45 (m, 2 H), 1.66 (m, 2 H), 1.97 - 2.17 (m, 3 H), 2.44 - 2.46 (m, 3 H), 2.71 - 2.80 (m, 3 H), 2.81 - 2.89 (m, 1 H), 2.91 - 2.94 (m, 2 H), 2.71 - 2.80 (m, 3 H), 3.04 (s, 3 H), 3.26 - 3.33 (m, 2 H), 3.37 (s, 3 H), 3.74 (m, 2 H), 4.28 - 4.31 (m, 2 H), 4.75 - 4.82 (m, 2 H), 6.18 (m, 1 H), 6.84 - 6.89 (m, 2 H), 7.15 - 7.19 (m, 1 H), 7.58 (d, 1 H)

[617] LC-MS *m/z* : 754$[M^+]^+$

[618]

[619] 상기 화합물을 사용하여 제조예 2-24와 동일한 방법으로 표제화합물(0.46 g, 83%)를 얻었다.

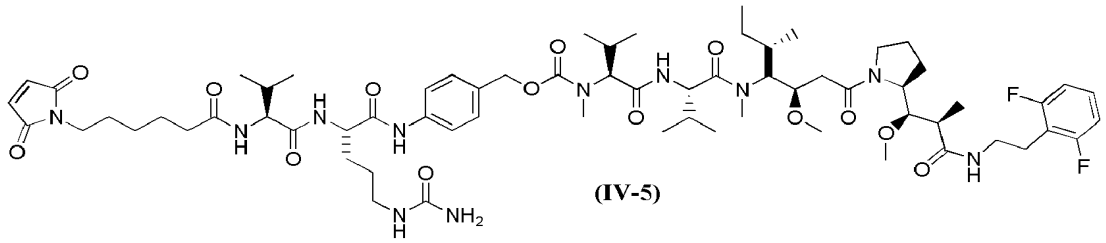
[620] LC-MS *m/z* : 920.1 $[M+H]^+$

[621]

[622] **제조예 3: 화학식 IV-1, IV-2, IV-3 또는 IV-4의 화합물의 제조**

[623] **제조예 3-1: 화합물 (IV-5)의 제조**

[624]



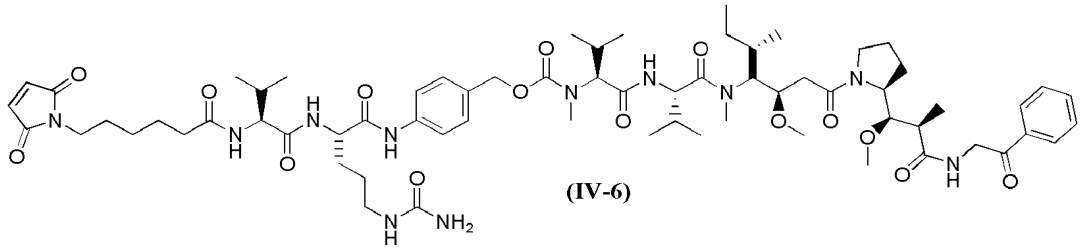
[625] 제조예 2-1에서 수득한 화합물 (III-5) (0.36 g, 0.50 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 14 mL에 녹인 후, 제조예 1-1에서 수득한 화합물 (II-1) (0.37 g, 0.50 mmol)을 가하였다. 반응혼합물을 0 ℃로 냉각한 후 HOBt (81 mg, 0.60 mmol)와 무수 피리딘 3.5 mL를 순차적으로 가하고, 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물 (0.49 g, 74%)을 미백색 고체로 얻었다.

[626] MALDI-TOF MS *m/z* : 1345.6 $[M+Na]^+$, 1361.6 $[M+K]^+$

[627]

[628] **제조예 3-2: 화합물 (IV-6)의 제조**

[629]



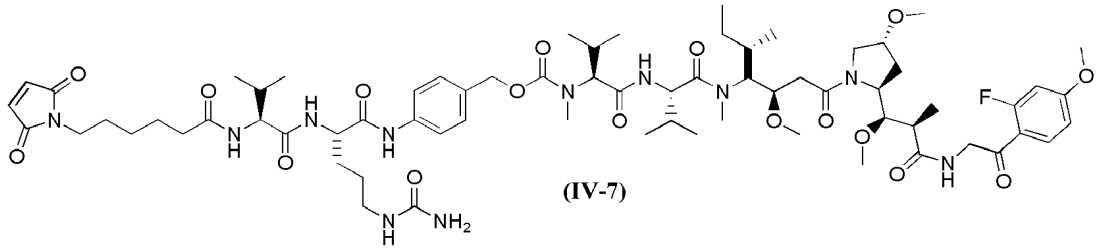
[630] 제조예 2-1에서 수득한 화합물 (III-5) 대신에 제조예 2-2에서 수득한 화합물 (III-6) (0.35 g, 0.50 mmol)를 사용하여 제조예 3-1과 동일한 방법으로 표제화합물 (0.42 g, 72%)를 얻었다.

[631] MALDI-TOF MS *m/z* : 1322.8 $[M+Na]^+$, 1339.0 $[M+K]^+$

[632]

[633] **제조예 3-3: 화합물 (IV-7)의 제조**

[634]



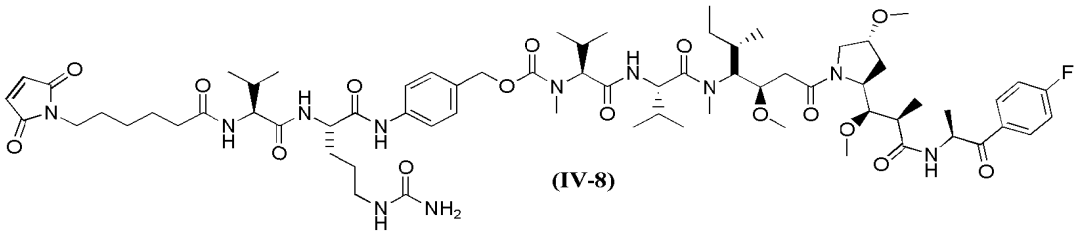
[635] 제조예 2-1에서 수득한 화합물 (III-5) 대신에 제조예 2-3에서 수득한 화합물 (III-7) (79 mg, 0.10 mmol)를 사용하여 제조예 3-1과 동일한 방법으로 표제화합물 (97 mg, 78%)를 얻었다.

[636] MALDI-TOF MS *m/z* : 1400.3 $[M+Na]^+$

[637]

[638] **제조예 3-4**: **화합물 (IV-8)의 제조**

[639]



(IV-8)

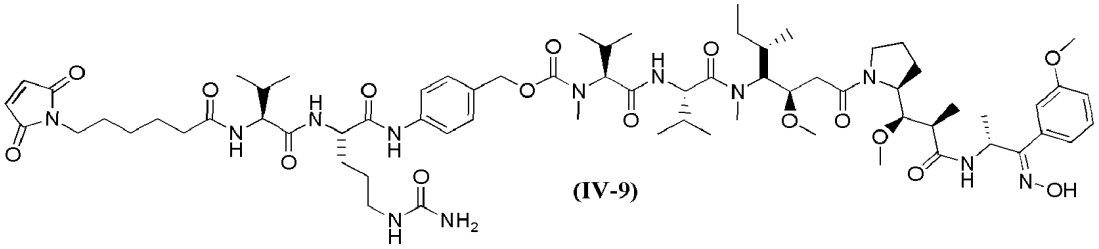
[640] 제조예 2-1에서 수득한 화합물 (III-5) 대신에 제조예 2-4에서 수득한 화합물 (III-8) (104 mg, 0.14 mmol)를 사용하여 제조예 3-1과 동일한 방법으로 표제화합물 (86 mg, 48%)를 얻었다.

[641] MALDI-TOF MS *m/z* : 1386.9 $[M+Na]^+$, 1402.9 $[M+K]^+$

[642]

[643] **제조예 3-5: 화합물 (IV-9)의 제조**

[644]



(IV-9)

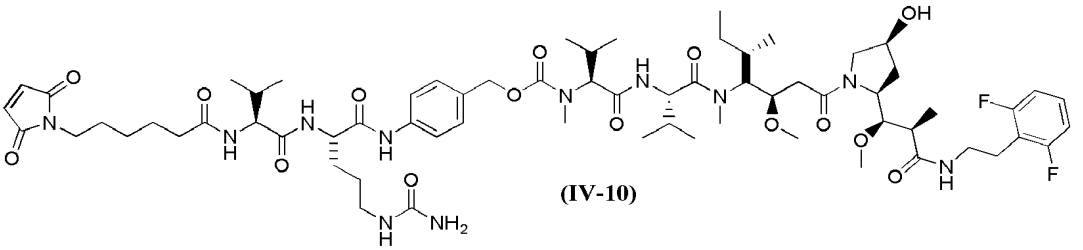
[645] 제조예 2-1에서 수득한 화합물 (III-5) 대신에 제조예 2-5에서 수득한 화합물 (III-9) (65 mg, 0.085 mmol)를 사용하여 제조예 3-1과 동일한 방법으로 표제화합물 (43 mg, 41%)를 얻었다.

[646] MALDI-TOF MS *m/z* : 1381.6 $[M+Na]^+$

[647]

[648] **제조예 3-6: 화합물 (IV-10)의 제조**

[649]



(IV-10)

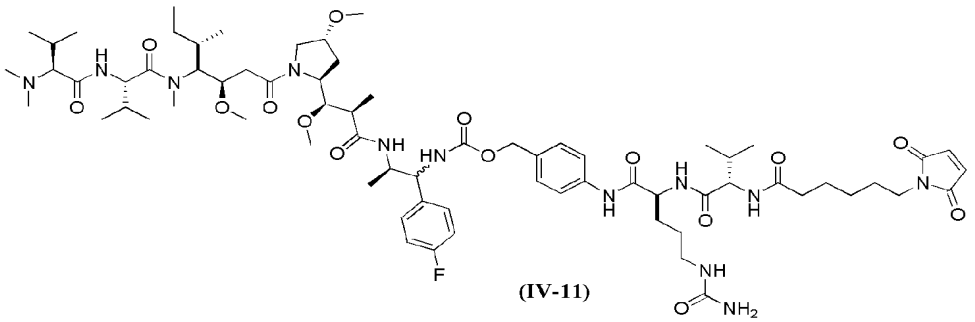
[650] 제조예 2-1에서 수득한 화합물 (III-5) 대신에 제조예 2-6에서 수득한 화합물 (III-10) (82 mg, 0.11 mmol)을 사용하여 제조예 3-1과 동일한 방법으로 표제화합물 (89 mg, 66%)를 얻었다.

[651] MALDI-TOF MS *m/z* : 1360.0 $[M+Na]^+$, 1377.2 $[M+K]^+$

[652]

[653] **제조예 3-7: 화합물 (IV-11)의 제조**

[654]



(IV-11)

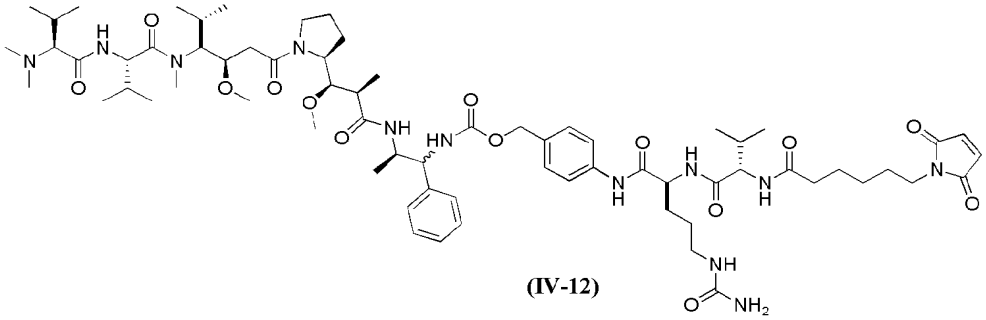
[655] 제조예 2-7에서 수득한 화합물 (III-11)(89 mg, 0.116 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 8 mL에 녹인 후, 제조예 1-1에서 수득한 화합물 (II-1)(78 mg, 0.106 mmol)을 가하였다. 반응혼합물을 0 ℃로 냉각한 후 HOBt (17 mg, 0.128 mmol)와 무수 피리딘 2.0 mL를 순차적으로 가하고, 상온에서 17시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(106 mg, 73%)을 얻었다.

[656] LC-MS *m/z* : 1377.8 $[M^+]^+$, 1400.8 $[M+Na]^+$

[657]

[658] **제조예 3-8: 화합물 (IV-12)의 제조**

[659]



(IV-12)

[660] 제조예 2-7에서 수득한 화합물 (III-11) 대신에 제조예 2-8에서 수득한 화합물 (III-12)(17 mg, 0.023 mmol)를 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(26 mg, 93%)을 얻었다.

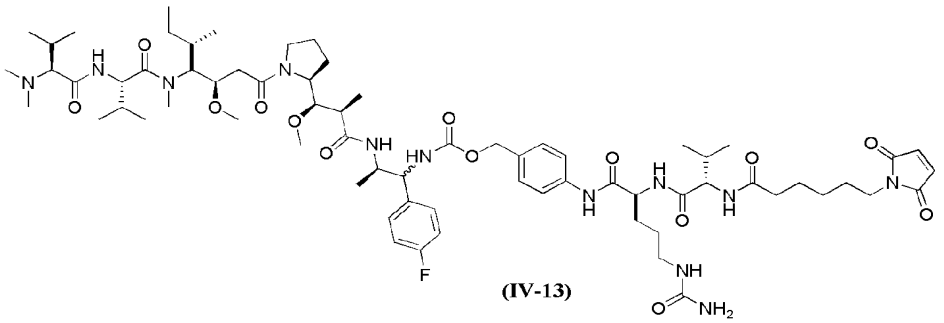
[661] MALDI-TOF MS m/z : 1351.3 $[M+Na]^+$, 1367.7 $[M+K]^+$

[662] ^{1}H NMR (400 MHz, CD_3OD) δ 0.81-0.91(m, 6H), 0.91-1.03(m, 21H), 1.63(d, 2H), 1.22(d, 1H), 1.28(m, 2H), 1.32(m, 1H), 1.50-1.63(m, 6H), 1.68(m, 2H), 1.82-1.90(m, 2H), 1.95(s, 3H), 1.98(m, 4H), 2.23-2.28(m, 3H), 2.34(brd, 1H), 2.40(s, 6H), 2.58-2.70(m, 1H), 2.80-2.85(m, 1H), 2.91-2.98(m, 1H), 3.12(s, 3H), 3.15-3.27(m, 2H), 3.26-3.33(m, 8H, OCH_3), 3.34(d, 2H), 3.42-3.48(m, 5H, OCH_3), 3.52(m, 1H), 3.7-3.96(m, 2H), 3.96-4.08(m, 2H), 4.15(d, 1H), 4.18(m, 1H), 4.44-4.54(m, 2H), 4.66(d, 1H), 4.54-5.10(m, 5H), 6.76(m, 1H), 7.02-7.12(m, 2H), 7.23-7.28(m, 2H), 7.30-7.38(m, 2H), 7.51-7.58(m, 2H)

[663]

[664] **제조예 3-9: 화합물 (IV-13)의 제조**

[665]



(IV-13)

[666] 제조예 2-7에서 수득한 화합물 (III-11)대신에 제조예 2-9에서 수득한 화합물 (III-13) (478 mg, 0.638 mmol)을 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(347 mg, 40%)을 얻었다.

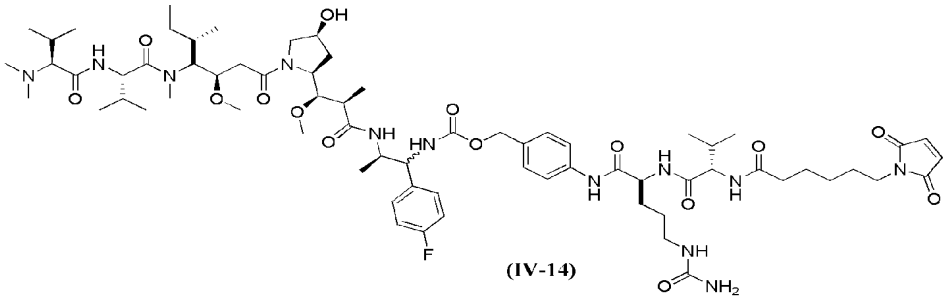
[667] LC-MS *m/z* : 1347 $[M^+]^+$

[668] ^{1}H NMR (400 MHz, CD_3OD) δ 0.76-0.90(m, 6H), 0.90-1.12(m, 18H), 1.16(m, 4H), 1.19(m, 3H), 1.28(m, 3H), 1.28(m, 3H), 1.40(m, 2H), 1.50-1.66(m, 9H), 1.66-1.80(m, 2H), 1.93(s, 3H), 2.0-2.14(m, 4H), 2.26(m, 3H), 2.38(d, 6H), 2.44(m, 1H), 2.50-2.65(m, 1H), 2.77(m, 2H), 3.10-3.24(m, 6H), 3.25-3.36(m, 7H, OCH_3), 3.45(t, 3H), 3.58(m, 1H), 3.66(m, 1H), 3.82(m, 1H), 4.06(m, 1H), 4.14(m, 2H), 4.26(m, 1H), 4.36(m, 1H), 4.48(m, 2H), 4.63(m, 1H), 4.69(m, 3H), 6.78(s, 2H), 6.97(m, 1H), 7.08(m, 1H), 7.25(m, 2H), 7.34(m, 2H), 7.54(m, 2H)

[669]

[670] **제조예 3-10: 화합물 (IV-14)의 제조**

[671]



(IV-14)

[672] 제조예 2-7에서 수득한 화합물 (III-11) 대신에 제조예 2-10에서 수득한 화합물 (III-14)(0.39 g, 0.51 mmol)을 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(453 mg, 65%)을 얻었다.

[673] LC-MS *m/z* : 1364$[M+1]^+$

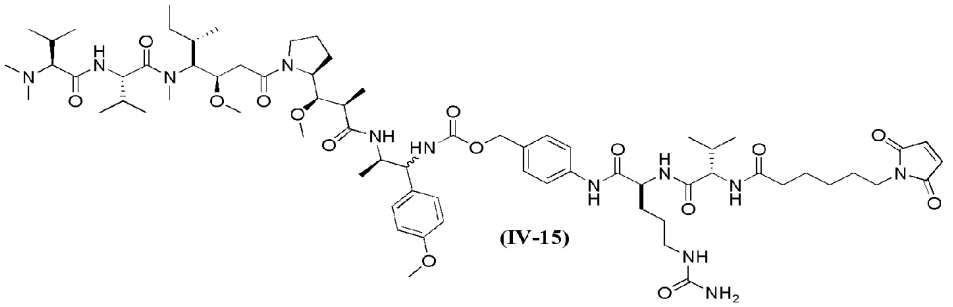
[674] ^{1}H NMR (400 MHz, CD_3OD) δ 0.81-0.91(m, 6H), 0.91-1.03(m, 21H), 1.13(d, 2H), 1.22(d, 1H), 1.28(m, 2H), 1.32(m, 1H), 1.50-1.63(m, 6H), 1.68(m, 2H), 1.82-1.90(m, 2H), 1.95(s, 3H), 1.98(m, 4H), 2.23-2.28(m, 3H), 2.34(brd, 1H), 2.40(s, 6H), 2.58-2.70(m, 1H),2.80-2.85(m, 1H), 2.91-2.98(m, 1H), 3.12(s, 3H), 3.15-3.27(m, 2H), 3.27-3.33(m, 8H, OCH_3), 3.34(d, 2H), 3.42-3.48(m, 5H, OCH_3),3.52(m, 1H), 3.7-3.96(m, 2H), 3.96-4.08(m, 2H), 4.15(d, 1H), 4.18(m, 1H), 4.44-4.54(m, 2H), 4.66(d, 1H), 4.74-5.10(m, 5H), 6.76(m, 1H), 7.02-7.12(m, 2H), 7.23-7.28(m, 2H),

7.30-7.38(m, 2H), 7.51-7.58(m, 2H)

[675]

[676] **제조예 3-11: 화합물 (IV-15)의 제조**

[677]



[678] 제조예 2-7에서 수득한 화합물 (III-11) 대신에 제조예 2-11에서 수득한 화합물 (III-15)(110 mg, 0.144 mmol)를 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(82 mg, 42%)을 얻었다.

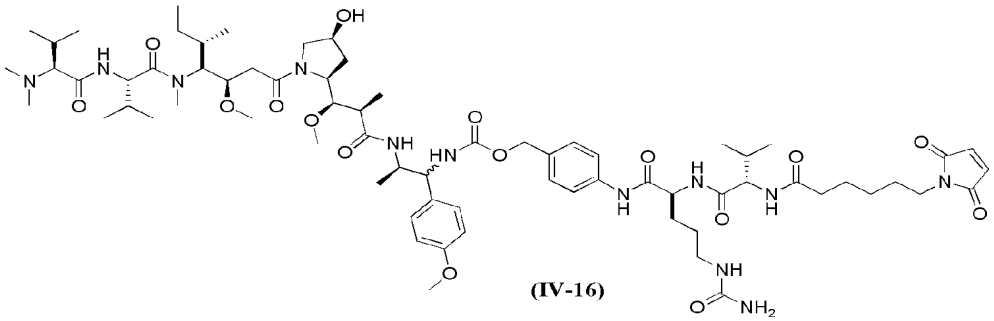
[679] LC-MS *m/z*: 1360 $[M+1]^+$

[680] ^{1}H NMR (400 MHz, CD_3OD) δ 0.81-0.91(m, 6H), 0.92-1.05(m, 19H), 1.3-1.2(m,3H), 1.22(d, 1H), 1.28(m, 2H), 1.32(m, 1H), 1.50-1.63(m, 6H), 1.68(m, 2H), 1.82-1.90(m, 2H), 1.95(s, 3H), 1.98(m, 3H), 2.23-2.28(m, 3H), 2.37(d, 6H), 2.61(m, 1H), 2.78(m, 1H), 3.12(s, 3H), 3.16-3.24(m, 2H), 3.26-3.32(m, 15H, OCH_3), 3.32-3.28(m, 5H, OCH_3), 3.42-3.50(m, 1H), 3.74(d, 3H), 3.86(m, 1H), 3.96-4.08(m, 2H), 4.14(d, 1H), 4.18-4.10(m, 1H), 4.42-4.52(m, 2H), 4.64-5.10(m, 5H), 6.76(d, 2H), 6.87(m, 2H), 7.24(m, 4H), 7.55(m, 2H)

[681]

[682] **제조예 3-12: 화합물 (IV-16)의 제조**

[683]



[684] 제조예 2-7에서 수득한 화합물 (III-11) 대신에 제조예 2-12에서 수득한 화합물 (III-16)(179 mg, 0.23 mmol)를 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(268 mg, 85%)을 얻었다.

[685] LC-MS m/z : 1375 $[M+1]^+$

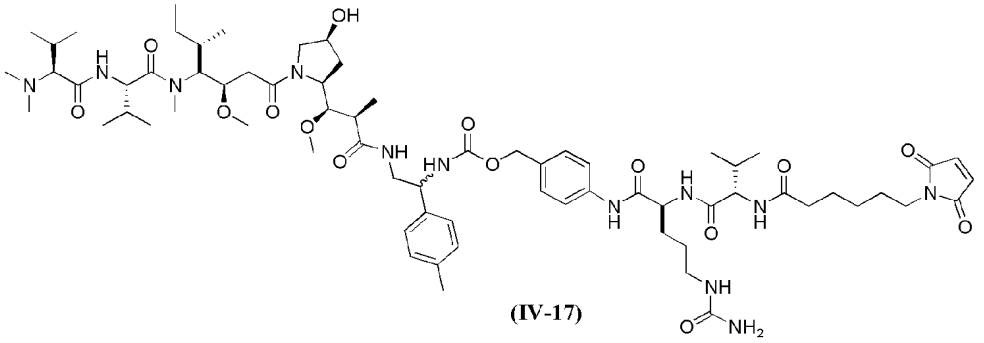
[686] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71-0.80(m, 5H), 0.80-0.98(m, 18H), 0.98-1.10(m, 4H), 1.18(m, 2H), 1.50(m, 6H), 1.55-1.75(m, 4H), 1.81-2.00(m, 8H), 2.05-2.30(m, 5H), 2.26(d, 6H), 2.40-2.61(m, 1H), 2.65-2.80(m, 2H), 2.90-3.08(m, 6H),

3.15(d, 3H), 3.30(d, 3H), 3.42(s, 2H), 3.67(s, 3H), 3.70(s, 3H), 3.15-3.43(m, 3H), 3.85-4.05(m, 4H), 4.18(brt, 2H), 4.38(m, 2H), 4.52(t, 1H), 4.56-4.80(m, 3H), 4.88(d, 1H), 5.01(m, 2H), 5.10(d, 1H), 6.00(m, 1H), 6.84-8.20(m, 10H), 10.0(d, 1H)

[687]

[688] **제조예 3-13: 화합물 (IV-17)의 제조**

[689]



(IV-17)

[690] 제조예 2-7에서 수득한 화합물 (III-11) 대신에 제조예 2-13에서 수득한 화합물 (III-17)(327 mg, 0.431 mmol)를 사용하여 제조예 3-7과 동일한 방법으로 표제화합물(440 mg, 75%)을 얻었다.

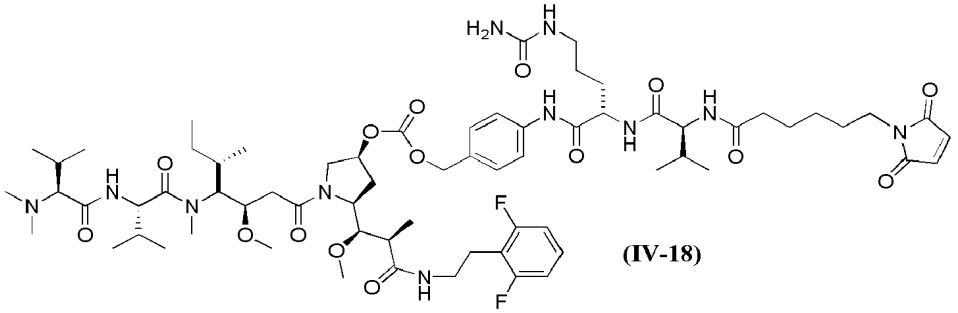
[691] LC-MS m/z : 1346 $[M+1]^+$

[692] ^{1}H NMR (400 MHz, CD_3OD) δ 0.81-1.02(m, 23H), 1.13-1.20(m, 3H), 1.25-1.32(m, 2H), 1.40(m, 1H), 1.50-1.63(m, 6H),1.72-1.78(m, 2H), 1.82-1.98(m, 3H), 1.96(s, 3H), 2.00-2.09(m, 4H), 2.15-2.30(m, 5H), 2.34-2.42(d, 6H),2.40-2.60(m, 3H), 2.75(m, 1H), 2.98(m, 1H), 3.05(m, 3H), 3.16-3.24(m, 2H), 3.26-3.35(m, 8H, OCH_3), 3.38-3.48(m, 6H, OCH_3), 3.52-3.60(m, 1H), 3.65-3.80(m, 1H), 3.90-4.16(m, 5H), 4.48(m, 1H), 4.63-5.10(m, 8H), 6.76(s, 2H), 7.09-7.28(m, 6H), 7.53(m, 2H)

[693]

[694] **제조예 3-14: 화합물 (IV-18)의 제조**

[695]



(IV-18)

[696] 제조예 2-14에서 수득한 화합물 (III-18) (0.36 g, 0.48 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 녹인 후, 제조예 1-1에서 수득한 화합물 (II-1) (0.32 g, 0.43 mmol)을 가하였다. 반응혼합물을 0 ℃로 냉각한 후 HOBt (70 mg, 0.52 mmol)와 무수 피리딘 2.5 mL를 순차적으로 가하고, 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(0.29 g, 51%)을 미백색

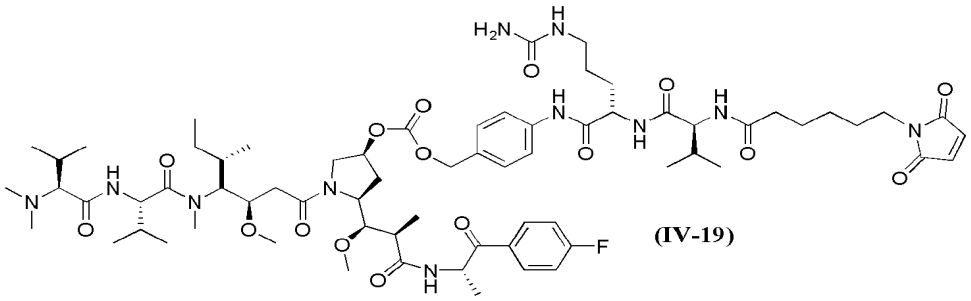
고체로 얻었다.

[697] LC-MS *m/z* : 1353 $[M^+]^+$, 1375 $[M+Na]^+$

[698]

[699] **제조예 3-15: 화합물 (IV-19)의 제조**

[700]



(IV-19)

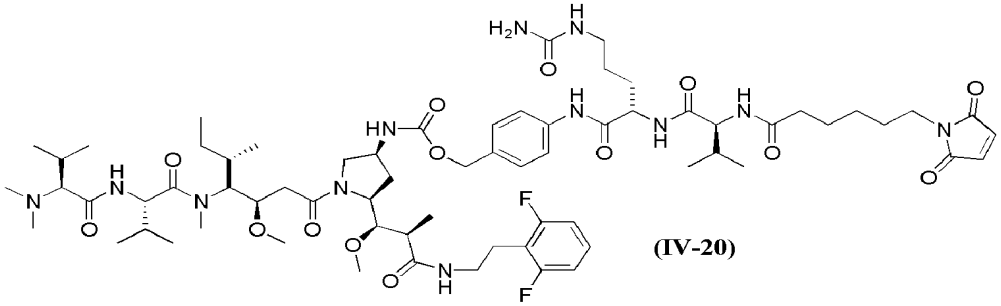
[701] 제조예 2-14에서 수득한 화합물 (III-18) 대신에 제조예 2-15에서 수득한 화합물 (III-19) (0.34 g, 0.45 mmol)를 사용하여 제조예 3-14와 동일한 방법으로 표제화합물(0.28 g, 45%)을 얻었다.

[702] LC-MS *m/z* : 1362.8 $[M^+]^+$, 1384.8 $[M+Na]^+$

[703]

[704] **제조예 3-16: 화합물 (IV-20)의 제조**

[705]



(IV-20)

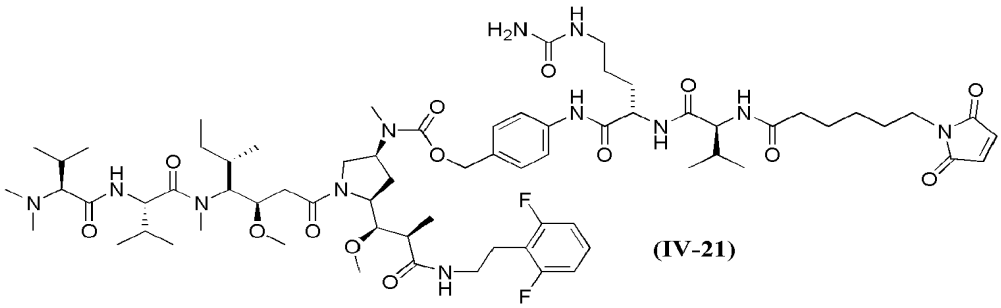
[706] 제조예 2-14에서 수득한 화합물 (III-18) 대신에 제조예 2-16에서 수득한 화합물 (III-20) (91 mg, 0.12 mmol)를 사용하여 제조예 3-14과 동일한 방법으로 표제화합물(122 mg, 82%)을 얻었다.

[707] LC-MS *m/z* : 1352 $[M^+]^+$

[708]

[709] **제조예 3-17: 화합물 (IV-21)의 제조**

[710]



(IV-21)

[711] 제조예 2-14에서 수득한 화합물 (III-18) 대신에 제조예 2-17에서 수득한 화합물

(III-21) (110 mg, 0.14 mmol)를 사용하여 제조예 3-14와 동일한 방법으로 표제화합물(153 mg, 86%)을 얻었다.

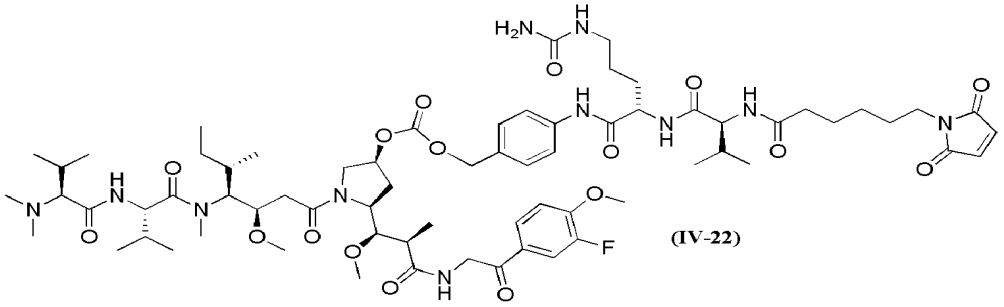
[712] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70-0.77 (m, 5 H), 0.81-0.97 (m, 17 H), 1.02-1.07 (m, 3 H), 1.16-1.29 (m, 8 H), 1.34-1.40 (m, 1 H), 1.48-1.51 (m, 6 H), 1.54-1.63 (m, 1 H), 1.63-1.79 (m, 2 H), 1.91-1.97 (m, 5 H), 2.03-2.30 (m, 7 H), 2.35-2.44 (m, 1 H), 2.59-2.61 (m, 1 H), 2.61-2.69 (m, 1 H), 2.70-2.84 (m, 4 H), 3.07-3.12 (m, 1 H), 3.12-3.24 (m, 3 H), 3.26-3.40 (m, 3 H), 3.40-3.48 (m, 1 H), 3.62-4.03 (m, 4 H), 4.03-4.25 (m, 3 H), 4.25-4.68 (m, 4 H), 4.88-5.09 (s, 2 H), 5.35-5.47 (s, 2 H), 5.85-6.03 (t, 1 H), 6.85-7.10 (s, 2 H), 7.14-7.40 (m, 2 H), 7.56-7.66 (m, 1 H), 7.74-7.83 (d, 1 H), 7.90-8.22 (m, 3 H), 10.00 (brs, 1 H)

[713] LC-MS *m/z* : 1366 $[M^+]^+$

[714]

[715] **제조예 3-18: 화합물 (IV-22)의 제조**

[716]



[717] 제조예 2-18에서 수득한 화합물 (III-22) (1.27 g, 1.63 mmol)을 디클로로메탄 20 ml에 용해하고 -50 내지 -60 ℃로 냉각한 후 피리딘(1.3 ml, 16.28 mmol)을 가하였다. 생성된 반응용액에 p-니트로페닐 클로로포르메이트(16.28 g, 3.3 mmol)을 디클로로메탄 10 ml에 용해하여 20분 동안 서서히 적가한 후 -50 내지 -60 ℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용액에 디클로로메탄 20 ㎖를 더 가하고, 0.5 M 포타슘하이드로젠설파이트 용액으로 추출한 다음, 유기층을 무수 황산나트륨으로 건조시키고 감압 농축하였다. 농축 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 (3S,5S)-1-((3R,4S,5S)-4-((S)-2-((S)-2-(디메틸아미노)-3-메틸부탄아미도)-N,3-디메틸부탄아미도)-3-메톡시-5-메틸헵타노일)-5-((1R,2R)-3-((2-(3-플루오로-4-메톡시페닐)-2-옥소에틸)아미노)-1-메톡시-2-메틸-3-옥소프로필)피롤리딘-3-일 (4-니트로페닐) 카르보네이트(0.8 g, 54%)을 수득하였다.

[718] LC-MS *m/z* : 945.7 $[M+H]^+$, 967.7 $[M+Na]^+$

[719]

[720] 상기 화합물(0.3 g, 0.32 mmol)과 t-부틸 ((S)-1-(((S)-1-((4-(히드록시메틸)페닐)아미노)-1-옥소-5-우레이도펜탄-2-일)아미노)-3-메틸-1-옥소부탄-2-일)카바메이트(0.3 g, 0.64mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 용해한 후, 4-메틸아미노피리딘(0.039 g,

0.32mmol)을 가하고 20 - 25 ℃에서 19시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 아미노기가 보호화된 화합물(0.1 g, 25%)을 수득하였다.

[721] LC-MS *m/z* : 1285.5 $[M+H]^+$

[722]

[723] 아미노기가 보호된 화합물(0.18 g, 0.14 mmol)를 디클로로메탄 8 mL에 녹이고 트리플루오로아세트산 2 mL를 적가한 후 20 - 25℃에서 3시간 동안 교반하였다. 반응이 종결되면 반응용매를 감압 농축하여 제거하고, 톨루엔 5 ㎖를 2회 첨가하여 트리플루오로아세트산을 완전히 제거한 후 반응을 진행하였다.

[724] 반응 농축액(TFA염)과 2,5-디옥소피롤리딘-1-일 6-(2,5-디옥소-2,5-디히드로-1-피롤-1-일)헥사노에이트를 디메틸포름아미드 10 mL에 용해하고, 트리에틸아민(0.1 mL, 0.7 mmol)을 가하고 상온에서 18시간 동안 교반하였다. 반응 완결 후 고진공 하에서 반응용액을 완전 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(0.14 g, 71%)을 수득하였다.

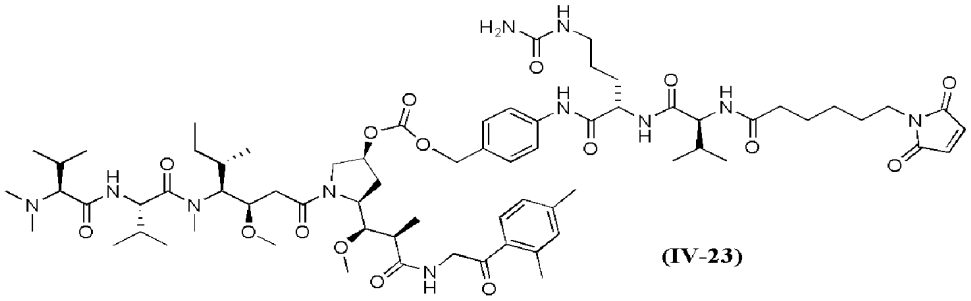
[725] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70-0.78 (m, 6H), 0.81-0.92 (m, 13H), 1.16-1.24 (23H), 1.46-1.51 (m, 4H), 1.84-1.97 (m, 3H), 3.06-3.17 (m, 12H), 3.30-3.38 (m, 15H), 3.84-3.93 (m, 3H), 4.08-4.85 (m, 8H), 4.86-5.04 (m, 1H), 5.10 (s, 2H), 5.40 (s, 2H), 6.03 (t, 1H), 7.00 (s, 1H), 7.15-7.35 (m, 2H), 7.53-7.55 (d, 1H), 7.77-7.98 (m, 2H), 8.10-8.12 (d, 1H), 8.19-8.48 (m, 1H), 9.44-9.49 (brs, 1H), 10.03 (s, 1H)

[726] LC-MS *m/z* : 1378.7 $[M+H]^+$

[727]

[728] **제조예 3-19: 화합물 (IV-23)의 제조**

[729]



(IV-23)

[730] 제조예 2-18에서 수득한 화합물 (III-22) 대신에 제조예 2-19에서 수득한 화합물 (III-23) (0.8 g, 0.865 mmol)을 사용하여 제조예 3-18과 동일한 방법으로 표제화합물(0.22 g, 69%)을 수득하였다.

[731] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.73-0.75 (m, 4H), 0.81-0.92 (m, 11H), 1.16-1.19 (23H), 1.46-1.50 (m, 4H), 1.54-1.73 (m, 2H), 1.90-1.97 (m, 2H), 2.08-2.25 (m, 4H), 2.28-2.38 (m, 4H), 2.94-2.99 (m, 4H), 3.07-3.17 (m, 11H), 3.33-3.38 (m, 12H), 3.95-4.05 (m, 1H), 4.12-4.27 (m, 3H), 4.51-4.67 (m, 1H), 4.87-4.95 (m, 1H), 5.11(s,

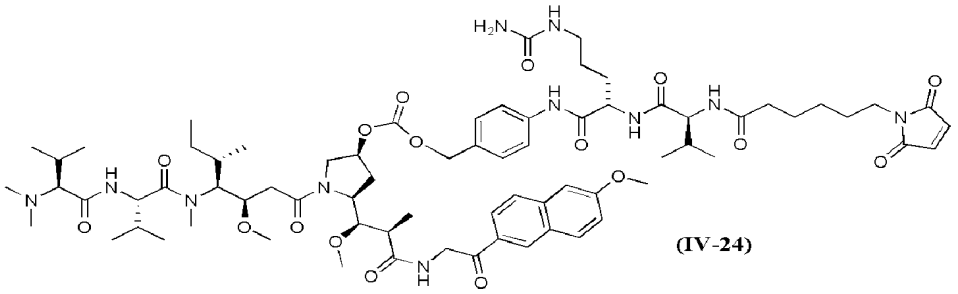
2H), 5.43 (s, 2H), 6.00 (t, 1H), 7.00 (s, 1H), 7.07-7.15 (m, 1H), 7.22-7.24 (d, 1H), 7.32-7.35 (m, H), 7.53-7.56 (d, 1H), 7.61-7.62 (d, 1H), 7.81-7.84(d, 1H), 8.07-8.13 (m, 1H), 8.28-8.50 (m, 1H), 9.26 (brs, 1H), 9.91 (s, 1H), 10.06 (s, 1H)

[732] LC-MS *m/z* : 1380.4 $[M+H]^+$

[733]

[734] **제조예 3-20: 화합물 (IV-24)의 제조**

[735]



(IV-24)

[736] 제조예 2-18에서 수득한 화합물 (III-22) 대신에 제조예 2-20에서 수득한 화합물 (III-24) (0.44 g, 0.45 mmol)를 사용하여 제조예 3-18과 동일한 방법으로 표제화합물(0.16 g, 64%)을 수득하였다.

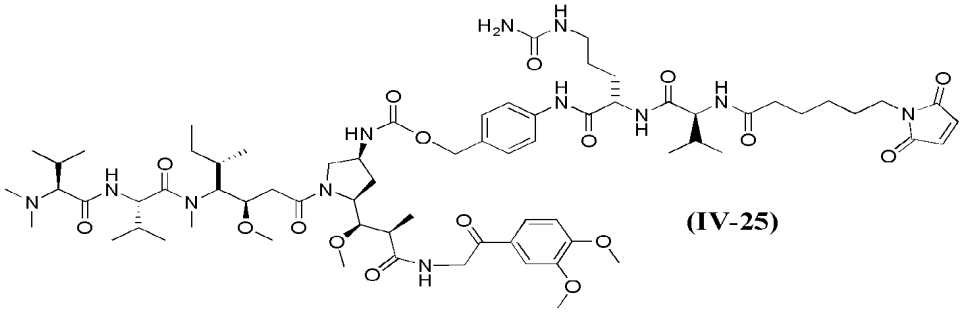
[737] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48-0.50 (d, 1H), 0.58-0.59 (d, 1H), 0.68 -0.77 (m, 4H), 0.81-0.92 (m, 14H), 1.16-1.23 (20H), 1.46-1.51 (m, 4H), 1.81-1.88 (m, 3H), 3.06-3.13 (m, 12H), 3.35-3.38 (m, 15H), 3.84-3.92 (m, 4H), 4.08 -5.00 (m, 8H), 5.13 (s, 2H), 5.44 (s, 2H), 6.01-6.03 (t, 1H), 7.00 (s, 1H), 7.22-7.28 (m, 1H), 7.33-7.36 (m, 2H), 7.39-7.42 (d, 1H), 7.60-7.65 (d, 2H), 7.82-7.84 (d, 1H), 7.89-7.93 (m, 2H), 8.00-8.03 (d, 1H), 8.11-8.13 (d, 1H), 8.27-8.53 (m, 1H), 8.64 (s, 1H), 9.38 (brs, 1H), 10.06 (s, 1H)

[738] LC-MS *m/z* : 1410.5 $[M+H]^+$

[739]

[740] **제조예 3-21: 화합물 (IV-25)의 제조**

[741]



(IV-25)

[742] 제조예 2-18에서 수득한 화합물 (III-22) 대신에 제조예 2-21에서 수득한 화합물(III-25) (0.37 g, 0.47 mmol)를 사용하여 제조예 3-14와 동일한 방법으로 표제화합물(0.47 g, 72%)을 얻었다.

[743] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.57-0.78 (m, 8 H), 0.78-0.98 (m, 15 H), 1.04-1.28 (m, 6 H), 1.28-1.39 (m, 1 H), 1.39-1.54 (m, 5 H), 1.54-1.63 (m, 1 H), 1.63-1.84 (m, 3

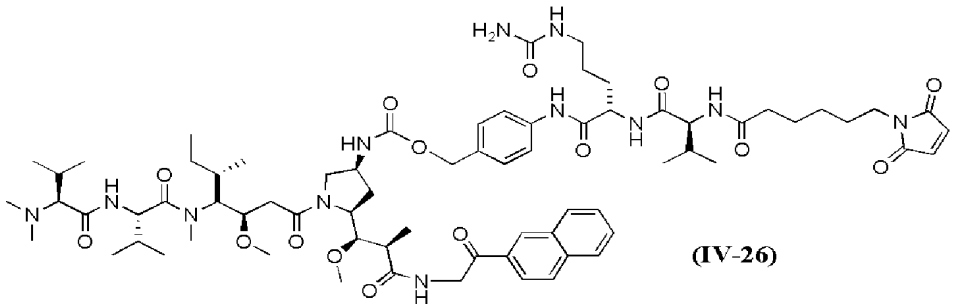
H), 1.84-2.03 (m, 3 H), 2.03-2.25 (m, 7 H), 2.25-2.40 (m, 2 H), 2.53-2.69 (m, 3 H), 2.69-2.83 (m, 1 H), 2.85-3.06 (m, 5 H), 3.09-3.21 (m, 3 H), 3.26-3.42 (m, 3 H), 3.44-3.52 (m, 1 H), 3.74-3.88 (m, 6 H), 3.88-4.05 (m, 3 H), 4.07-4.16 (m, 1 H), 4.16-4.23 (m, 1 H), 4.23-4.34 (m, 1 H), 4.34-4.48 (m, 2 H), 4.48-4.57 (m, 1 H), 4.58-4.73 (m, 2 H), 4.97 (s, 2 H), 5.42 (s, 2 H), 5.93-6.04 (t, 1 H), 6.98-7.04 (s, 1 H), 7.04-7.12 (d, 1 H), 7.25-7.36 (m, 1 H), 7.42-7.48 (s, 1 H), 7.48-7.57 (m, 1 H), 7.57-7.64 (d, 1 H), 7.64-7.72 (m, 1 H), 7.77-7.86 (m, 1 H), 8.01-8.16 (m, 1 H), 10.01 (brs, 1 H), 10.56 (brs, 1 H)

[744] LC-MS *m/z* : 1389.6 $[M^+]^+$, 1412.6 $[M+Na]^+$

[745]

[746] **제조예 3-22: 화합물 (IV-26)의 제조**

[747]



(IV-26)

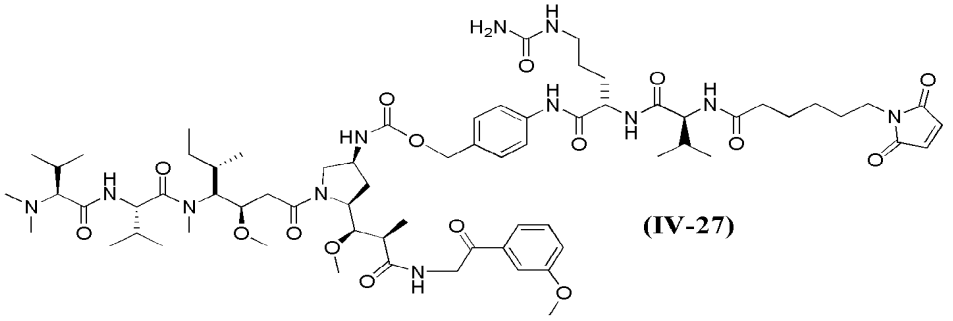
[748] 제조예 2-18에서 수득한 화합물 (III-22) 대신에 제조예 2-22에서 수득한 화합물 (III-26)(0.28 g, 0.36 mmol)을 사용하여 제조예 3-14와 동일한 방법으로 표제화합물(0.24 g, 48%)을 얻었다.

[749] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.66-0.78 (m, 5 H), 0.78-1.06 (m, 19 H), 1.07-1.29 (m, 6 H), 1.29-1.38 (m, 1 H), 1.38-1.53 (m, 5 H), 1.53-1.63 (m, 1 H), 1.63-1.85 (m, 2 H), 1.85-2.02 (m, 3 H), 2.02-2.14 (m, 3 H), 2.14-2.27 (m, 6 H), 2.27-2.41 (m, 2 H), 2.56- 2.60 (m, 3 H), 2.61-2.83 (m, 1 H), 2.86-3.08 (m, 5 H), 2.08-3.20 (m, 3 H), 3.20-3.27 (m, 1 H), 3.27-3.40 (m, 3 H), 3.45-3.63 (m, 1 H), 3.64-3.88 (m, 1 H), 3.88-4.06 (m, 2 H), 4.06-4.26 (m, 2 H), 4.28-4.46 (m, 1 H), 4.46-4.74 (m, 2 H), 4.74-4.91 (m, 1 H), 4.97 (s, 2 H), 5.43 (s, 2 H), 5.88-6.10 (t, 1 H), 7.01 (s, 1 H), 7.12-7.38 (m, 2 H), 7.38-7.72 (m, 4 H), 7.72-7.92 (m, 2 H), 7.92-8.22 (m, 3 H), 8.25-8.63 (m, 2 H), 8.73 (s, 1 H), 10.06 (brs, 1 H), 10.59 (brs, 1H)

[750] LC-MS *m/z* : 1379.6 $[M^+]^+$, 1402.5 $[M+Na]^+$

[751]

[752] **제조예 3-23: 화합물 (IV-27)의 제조**

[753] 
(IV-27)

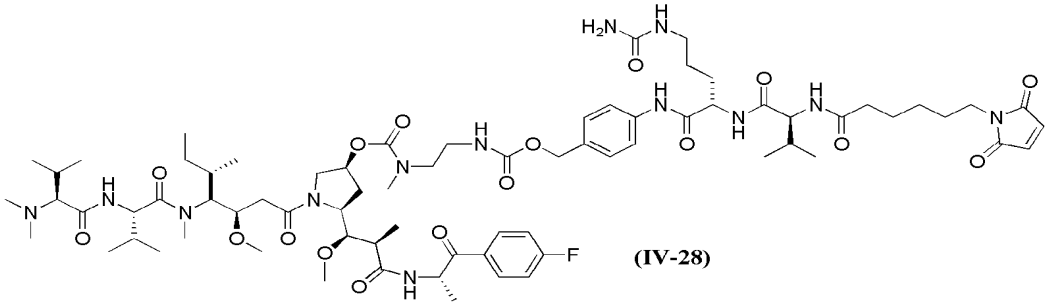
[754] 제조예 2-18에서 수득한 화합물 (III-22) 대신에 제조예 2-23에서 수득한 화합물 (III-27) (0.24 g, 0.32 mmol)을 사용하여 제조예 3-14와 동일한 방법으로 표제화합물(0.28 g, 64%)을 얻었다.

[755] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.59-0.78 (m, 6 H), 0.78-0.97 (m, 15 H), 0.97-1.06 (m, 2 H), 1.06-1.28 (m, 6 H), 1.28-1.39 (m, 1 H), 1.39-1.54 (m, 5 H), 1.54-1.64 (m, 1 H), 1.64-1.84 (m, 3 H), 1.84-2.02 (m, 3 H), 2.05-2.29 (m, 8 H), 2.30 - 2.42 (m, 2 H), 2.55-2.60 (m, 3 H), 2.60-2.83 (m, 1 H), 2.86-3.06 (m, 5 H), 3.06-3.21 (m, 6 H), 3.21-3.32 (m, 3 H), 3.36-3.44 (m, 1 H), 3.44-3.52 (m, 1 H), 3.65-3.74 (m, 1 H), 3.74-3.87 (m, 2 H), 3.89-4.05 (m, 2 H), 4.15-4.25 (m, 1 H), 4.31-4.73 (m, 4 H), 4.96 (s, 2H), 5.43 (s, 2 H), 5.92 -6.06 (t, 1 H), 6.71-6.83 (m, 1 H), 6.83-6.94 (m, 1 H), 6.97-7.04 (m, 1 H), 7.16-7.35 (m, 2 H), 7.38-7.67 (m, 3 H), 7.75-7.88 (d, 1 H), 8.00-8.17 (m, 2 H), 10.01 (brs, 1 H), 10.55 (brs, 1 H)

[756] LC-MS *m/z* : 1361.6 $[M^+]^+$, 1384.5 $[M+Na]^+$

[757]

[758] **제조예 3-24: 화합물 (IV-28)의 제조**

[759] 
(IV-28)

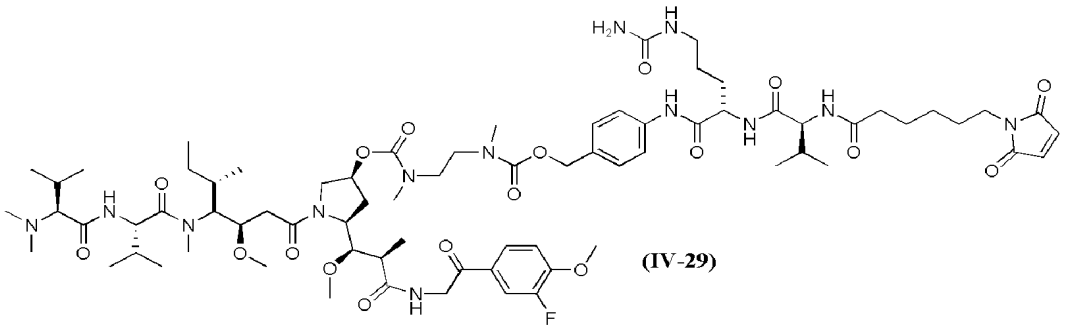
[760] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염(0.88 mg, 0.11 mmol)에 무수 디메틸포름아미드 5 mL와 디이소프로필에틸아민 (0.18 mL, 1.03 mmol)을 첨가하여 얻어진 용액을, 제조예 2-24에서 수득한 화합물 (III-28) (75 mg, 0.08 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 5 mL에 녹인 용액에 적가한 후 20 - 25℃에서 17시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(135 mg, 100%)을 얻었다.

[761] LC-MS *m/z* : 1462.9 $[M+H]^+$, 1485.9 $[M+Na]^+$

[762]

[763] **제조예 3-25: 화합물 (IV-29)의 제조**

[764]



(IV-29)

[765] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-3에서 수득한 화합물 (II-4)의 TFA염(0.17 g, 0.212mmol)을 사용하고, 제조예 2-24에서 수득한 화합물 (III-28) 대신에 제조예 2-25에서 수득한 화합물 (III-29) (0.2 g, 0.212 mmol)를 사용하여 제조예 3-24와 동일한 방법으로 표제화합물(0.14 g, 44%)를 얻었다.

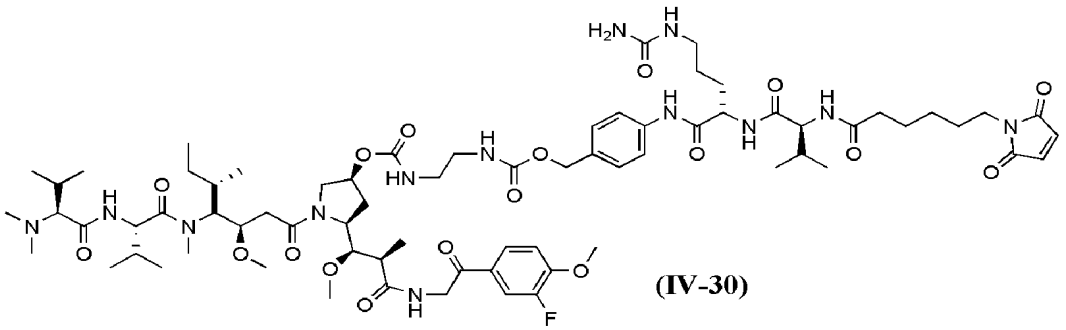
[766] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.69-0.78 (m, 6H), 0.81-0.90 (m, 14H), 1.11-1.64 (m, 3H), 1.18-1.26 (m, 10H), 1.38-1.51 (m, 6H), 1.58-1.62 (m, 1H), 1.63-1.83 (m, 3H), 1.91-1.99 (m, 3H), 2.11-2.26 (m, 7H), 2.35-2.42 (m, 2H), 2.87 (s, 6H), 2.92-3.05 (m, 3H), 3.13-3.18 (m, 4H), 3.32-3.38 (m, 17H), 3.62 (brs, 1H), 3.92-3.99 (m, 4H), 4.07-4.09 (m, 1H), 4.15-4.84 (m, 9H), 4.98 (s, 2H), 5.4 (s, 2H), 5.96-5.99 (t, 1H), 6.99 (s, 1H), 7.22-7.3 (m, 2H), 7.53-7.65 (m, 2H), 7.77-7.80 (m, 2H), 7.84-7.89 (t, 1H), 8.05-8.07 (d, 1H), 8.37- 8.43 (m, 1H), 9.97 (s, 1H)

[767] LC-MS *m/z* : 1492.8 $[M+H]^+$, 1514.7 $[M+Na]^+$

[768]

[769] **제조예 3-26: 화합물 (IV-30)의 제조**

[770]



(IV-30)

[771] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-4에서 수득한 화합물 (II-5)의 TFA염(0.16 g, 0.212 mmol)을 사용하고, 제조예 2-24에서 수득한 화합물 III-28 대신에 제조예 2-25에서 수득한 화합물 (III-29) (0.2 g, 0.212mmol)를 사용하여 제조예 3-24와 동일한 방법으로 표제화합물(0.13 g, 40%)를 얻었다.

[772] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.69-0.76 (m, 6H), 0.81-0.92 (m, 14H), 1.10-1.20 (m, 4H), 1.25-1.26 (m, 8H), 1.44-1.51 (m, 5H), 1.55-1.65 (m, 1H), 1.66-1.85 (3H),

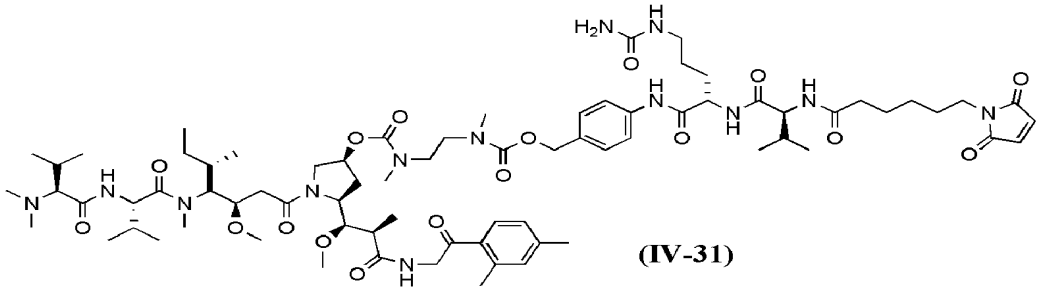
1.86-2.05 (m, 3H), 2.06-2.27 (m, 6H), 2.28-2.46 m, 2H), 2.84 (s, 1H), 2.87-2.96 (m, 1H), 2.99-3.04 (m, 5H), 3.14-3.18 (m, 4H), 3.32-3.47 (m, 11H), 3.62 (brs, 1H), 3.92-3.93 (d, 3H), 4.00-4.91 (m, 8H), 4.94 (s, 2H), 5.41 (s, 2H), 5.97-6.00 (t, 1H), 7.00 (s, 1H), 7.24-7.32 (m, 3H), 7.58-7.60 (d, 2H), 7.77-7.80 (m, 2H), 7.85-7.89 (t, 1H), 8.05-8.07 (d, 1H), 8.19 (t, 1H), 8.44 (brs, 1H), 9.97 (s, 1H)

[773] LC-MS *m/z* : 1464.8 $[M+H]^+$, 1487.9 $[M+Na]^+$

[774]

[775] **제조예 3-27: 화합물 (IV-31)의 제조**

[776]



(IV-31)

[777] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-3에서 수득한 화합물 (II-4)의 TFA염(0.26 g, 0.324 mmol)을 사용하고, 제조예 2-24에서 수득한 화합물 (III-28) 대신에 제조예 2-26에서 수득한 화합물 (III-30) (0.2 g, 0.216mmol)을 사용하여 제조예 3-24와 동일한 방법으로 표제화합물(0.2 g, 64%)를 얻었다.

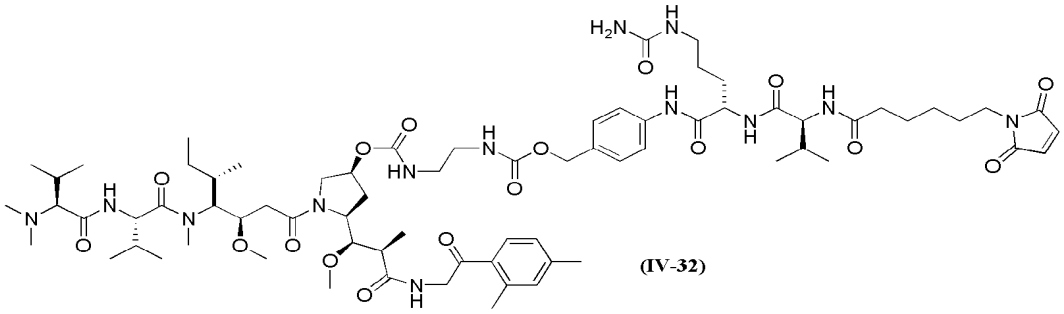
[778] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71-0.75 (m, 6H), 0.80-0.91 (m, 15H), 1.18-1.19 (m, 3H), 1.22-1.27 (m, 9H), 1.43-1.49 (m, 6H), 1.90-1.98 (m, 3H), 2.09-2.23 (m, 3H), 2.25-2.40 (m, 7H), 2.83-2.86 (m, 4H), 2.96-3.01 (m, 4H), 3.15-3.19 (m, 3H), 3.26 (s, 3H), 3.35-3.38 (m, 16H), 3.85-3.96 (m, 3H), 4.11 -4.88 (m, 8H), 4.97 (s, 2H), 5.42 (s, 2H), 5.97-5.99 (t, 1H), 6.99-7.01 (m, 1H), 7.06-7.16 (m, 2H), 7.28 (brs, 2H), 7.60-7.69 (m, 2H), 7.81-7.83 (d, 1H), 8.09-8.12 (d, 1H), 8.29 (brs, 1H), 10.09 (s, 1H)

[779] LC-MS *m/z* : 1472.9 $[M+H]^+$, 1494.9 $[M+Na]^+$

[780]

[781] **제조예 3-28: 화합물 (IV-32)의 제조**

[782]



(IV-32)

[783] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-4에서 수득한 화합물 (II-5)의 TFA염 (0.24 g, 0.324 mmol)을 사용하고, 제조예 2-24에서 수득한

화합물 (III-28) 대신에 제조예 2-26에서 수득한 화합물 (III-30) (0.2 g, 0.216 mmol)을 사용하여 제조예 3-24와 동일한 방법으로 표제화합물 (0.2 g, 67%)를 얻었다.

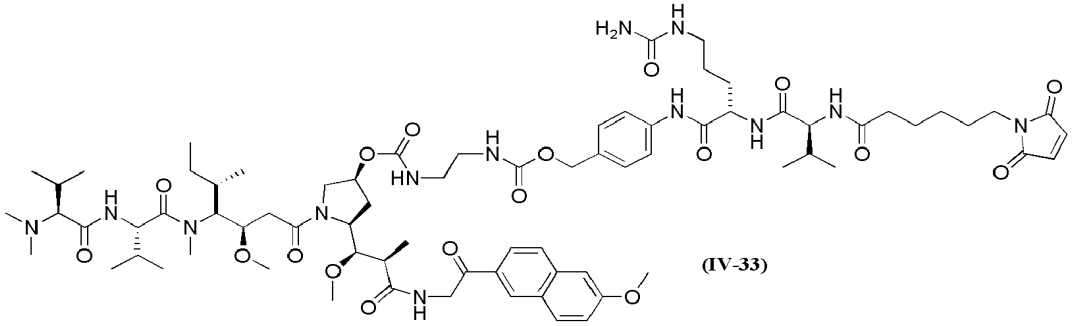
[784] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71-0.75 (m, 7H), 0.81-0.92 (m, 16H), 1.03-1.04 (m, 2H), 1.11-1.12 (m, 1H), 1.18-1.27 (m, 7H), 1.46-1.49 (m, 5H), 1.90-1.98 (m, 3H), 2.09-2.23 (m, 6H), 2.28-2.38 (m, 7H), 2.96-3.03 (m, 9H), 3.15-3.17 (m, 4H), 3.28 (s, 3H), 3.35-3.38 (m, 14H), 3.62-3.65 (m, 2H), 3.85 -4.00 (m, 3H), 4.17-4.88 (m, 8H), 4.94 (s, 2H), 5.43 (s, 2H), 5.88-6.00 (t, 1H), 7.00 (s, 1H), 7.06-7.16 (m, 2H), 7.27-7.29 (m, 3H), 7.58-7.69 (m, 2H), 7.82-7.84 (d, 1H), 8.09-8.12 (d, 1H), 8.29 (brs, 1H), 10.00 (s, 1H)

[785] LC-MS *m/z* : 1444.9 $[M+H]^+$, 1466.9 $[M+Na]^+$

[786]

[787] **제조예 3-29: 화합물 (IV-33)의 제조**

[788]



(IV-33)

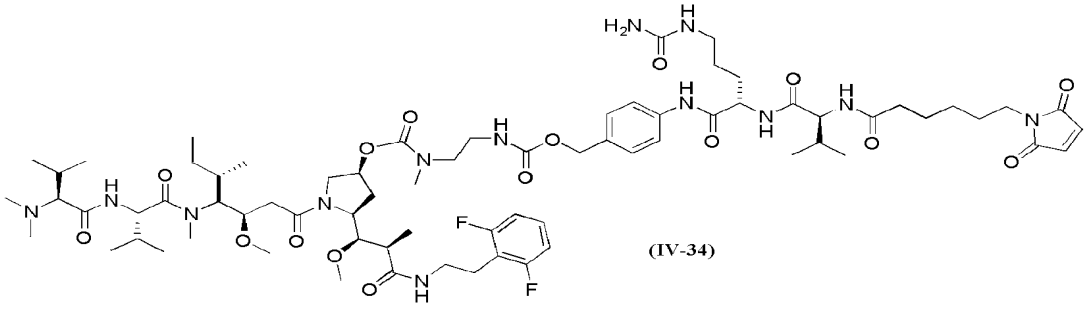
[789] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-4에서 수득한 II-5 화합물의 TFA염(0.23 g, 0.307 mmol)을 사용하고, 제조예 2-24에서 수득한 화합물 (III-28) 대신에 제조예 2-27에서 수득한 화합물 (III-31) (0.2 g, 0.205 mmol)를 사용하여 제조예 3-24와 동일한 방법으로 표제화합물 (0.16 g, 51%)를 얻었다.

[790] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71-0.75 (m, 7H), 0.81-0.92 (m, 16H), 1.03-1.04 (m, 2H), 1.11-1.12 (m, 1H), 1.18-1.27 (m, 7H), 1.46-1.49 (m, 5H), 1.90-1.98 (m, 3H), 2.09-2.23 (m, 6H), 2.28-2.38 (m, 7H), 2.96-3.03 (m, 9H), 3.15-3.17 (m, 4H), 3.28 (s, 3H), 3.35-3.38 (m, 14H), 3.62-3.65 (m, 2H), 3.85 -4.00 (m, 3H), 4.17-4.88 (m, 8H), 4.94 (s, 2H), 5.43 (s, 2H), 5.88-6.00 (t, 1H), 7.00 (s, 1H), 7.06-7.16 (m, 2H), 7.27-7.29 (m, 3H), 7.58-7.69 (m, 2H), 7.82-7.84 (d, 1H), 8.09-8.12 (d, 1H), 8.29 (brs, 1H), 10.00 (s, 1H)

[791] LC-MS *m/z* : 1497.6 $[M+H]^+$, 1519.6 $[M+Na]^+$

[792]

[793] **제조예 3-30: 화합물 (IV-34)의 제조**

[794] 
(IV-34)

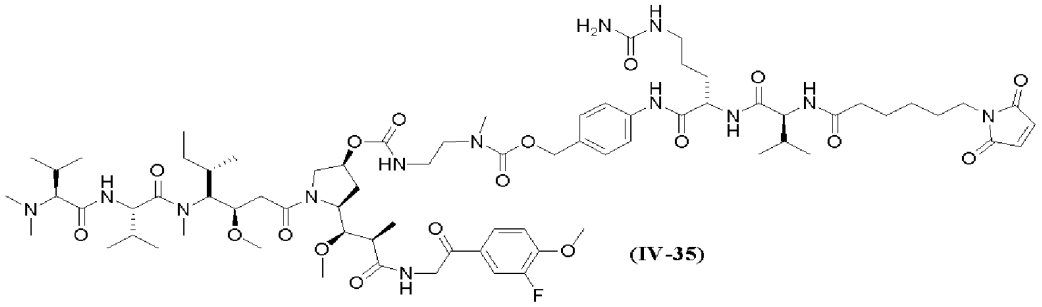
[795] 제조예 1-2에서 수득한 II-3 화합물의 TFA염 (1 g, 1.294 mmol)을 사용하고, 제조예 2-24에서 수득한 화합물 (III-28) 대신에 제조예 2-28에서 수득한 화합물 (III-32) (1.2 g, 1.294mmol)를 사용하여 제조예 3-24와 동일한 방법으로 표제화합물 (1.3 g, 72%)를 얻었다.

[796] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48-0.49 (m, 1H), 0.58-0.59 (m, 2H), 0.64 -0.65 (m, 2H), 0.70-0.76 (m, 5H), 0.81-0.92 (m, 16H), 1.11-1.13 (m, 2H), 1.16 -1.20 (m, 3H), 1.22-1.28 (m, 9H), 1.44-1.49 (m, 5H), 1.90-1.98 (m, 3H), 2.09 -2.23 (m, 7H), 2.96-3.04 (m, 9H), 3.13-3.18 (m, 4H), 3.33-3.38 (m, 14H), 3.60 -3.64 (m, 1H), 3.90-3.94 (m, 4H), 3.99-4.88 (m, 8H), 4.94 (s, 2H), 5.42 (s, 2H), 5.86-5.99 (t, 1H), 7.22-7.29 (m, 4H), 7.38-7.42 (m, 1H), 7.58-7.60 (d, 2H), 7.80-7.82 (d, 1H), 7.89-7.96 (m, 2H), 8.00-8.10 (m, 2H), 8.29 (brs, 1H), 8.70 (s, 1H), 10.00 (s, 1H)

[797] LC-MS *m/z* : 1452.8 $[M+H]^+$, 1474.7 $[M+Na]^+$

[798]

[799] **제조예 3-31: 화합물 (IV-35)의 제조**

[800] 
(IV-35)

[801] 제조예 1-2에서 수득한 화합물 (II-3)의 TFA염 대신에 제조예 1-5에서 수득한 II-6화합물의 TFA염(0.18 g, 0.233 mmol)과 제조예 2-25에서 수득한 화합물 (III-29) (0.2 g, 0.212 mmol)를 사용하여 제조예 3-24와 동일한 방법으로 표제화합물 (0.16 g, 51%)를 얻었다.

[802] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68-0.76 (m, 6H), 0.81-0.90 (m, 14H), 1.08-1.20 (m, 4H), 1.23-1.27 (m, 9H), 1.44-1.49 (m, 6H), 1.58-1.62 (m, 1H), 1.63-1.83 (m, 3H), 1.91-1.99 (m, 3H), 2.13-2.20 (m, 6H), 2.35-2.42 (m, 2H), 2.85-2.87 (m, 4H), 2.99-3.01 (m, 4H), 3.14-3.17 (m,4H), 3.28-3.38 (m, 19H), 3.62 (brs, 1H), 3.92-3.94 (m, 3H), 4.07-4.09 (m, 1H), 4.15-4.84 (m, 8H), 4.98 (s, 2H), 5.43 (s, 2H), 5.98-6.00 (t, 1H), 7.01 (s, 1H), 7.22-7.36 (m, 3H), 7.54-7.64 (m, 2H), 7.77-7.97 (m, 2H), 8.08-8.11 (d,

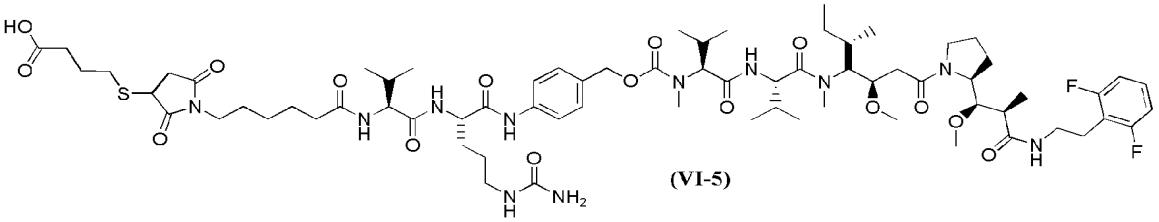
1H), 8.22-8.45 (m, 2H), 10.01 (s, 1H)

[803] LC-MS *m/z* : 1478.5 $[M+H]^+$, 1500.5 $[M+Na]^+$

[804]

[805] **제조예 4: 화학식 VI-1, VI-2, VI-3 또는 VI-4의 화합물의 제조**

[806] **제조예 4-1: 화합물 (VI-5)의 제조**

[807] 

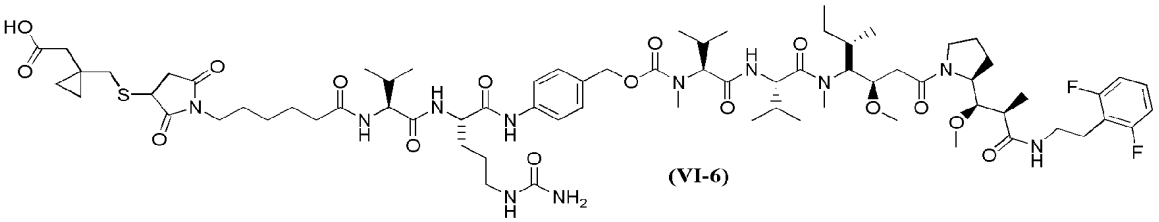
(VI-5)

[808] 제조예 3-1에서 수득한 화합물 (IV-5) (274 mg, 0.21 mmol)에 아세토니트릴 5.0 mL를 가하여 용해시키고, 4-머캅토부티르산 (V-1) (126 mg, 1.05 mmol)과 DMSO 0.5 mL를 가하여 실온에서 4시간 동안 교반하였다. 반응혼합물을 감압 농축하여 표제화합물 (290 mg, 98%)을 얻었다.

[809] MALDI-TOF MS *m/z* : 1465.3 $[M+Na]^+$

[810]

[811] **제조예 4-2: 화합물 (VI-6)의 제조**

[812] 

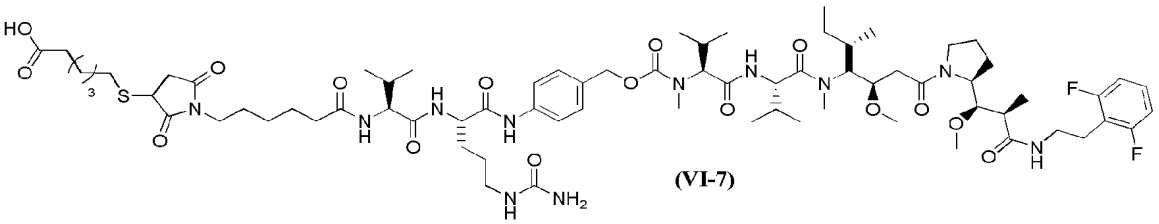
(VI-6)

[813] 제조예 3-1에서 수득한 화합물 (IV-5) (70 mg, 0.053 mmol)에 아세토니트릴 2.5 mL를 가하여 용해시키고, 2-(1-(머캅토메틸)시클로프로판)아세트산 (V-2) (44 mg, 0.3 mmol)과 DMF 0.7 mL를 가하여 실온에서 4시간 동안 교반하였다. 반응혼합물을 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물 (60 mg, 77%)을 고체로 얻었다.

[814] MALDI-TOF MS *m/z* : 1492.0 $[M+Na]^+$

[815]

[816] **제조예 4-3: 화합물 (VI-7)의 제조**

[817] 

(VI-7)

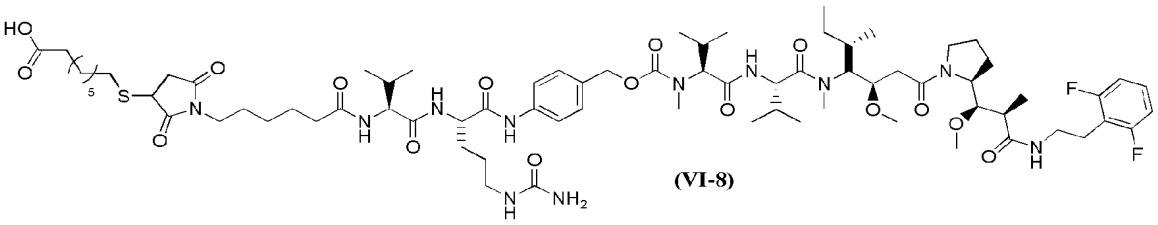
[818] 2-(1-(머캅토메틸)시클로프로판)아세트산 (V-2) 대신에 6-머캅토헥사노익산 (V-3)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (54 mg, 69%)을

고체로 얻었다.

[819] MALDI-TOF MS *m/z* : 1493.2 $[M+Na]^+$

[820]

[821] **제조예 4-4: 화합물 (VI-8)의 제조**

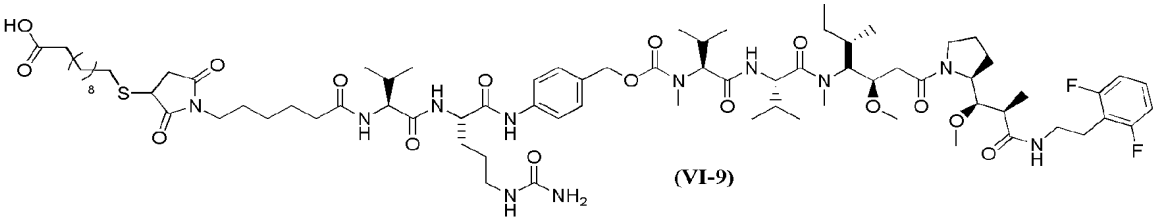
[822] 

[823] 2-(1-(머캅토메틸)시클로프로판)아세트산 (V-2) 대신에 8-머캅토옥타노익산 (V-4)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (66 mg, 84%)을 고체로 얻었다.

[824] MALDI-TOF MS *m/z* : 1522.0 $[M+Na]^+$

[825]

[826] **제조예 4-5: 화합물 (VI-9)의 제조**

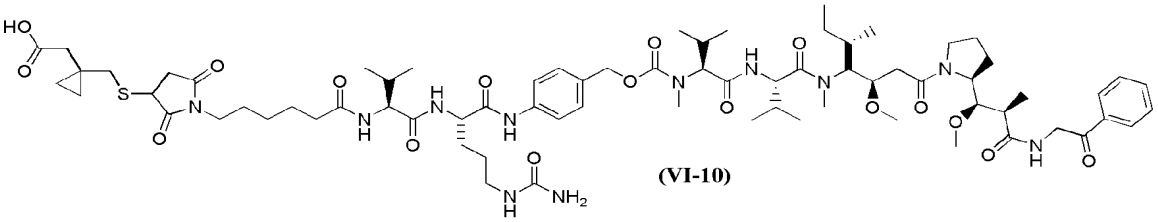
[827] 

[828] 2-(1-(머캅토메틸)시클로프로판)아세트산 (V-2) 대신에 11-머캅토운데카노익산 (V-5)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (71 mg, 86%)을 고체로 얻었다.

[829] MALDI-TOF MS *m/z* : 1563.3 $[M+Na]^+$

[830]

[831] **제조예 4-6: 화합물 (VI-10)의 제조**

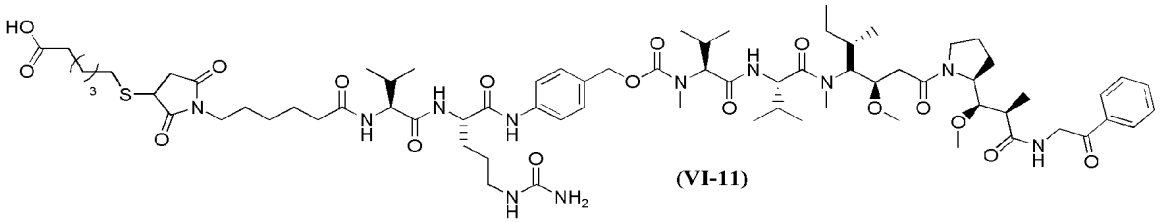
[832] 

[833] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-2에서 수득한 화합물 (IV-6) (70 mg, 0.054 mmol)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (53 mg, 68%)을 고체로 얻었다.

[834] MALDI-TOF MS *m/z* : 1469.5 $[M+Na]^+$

[835]

[836] **제조예 4-7: 화합물 (VI-11)의 제조**

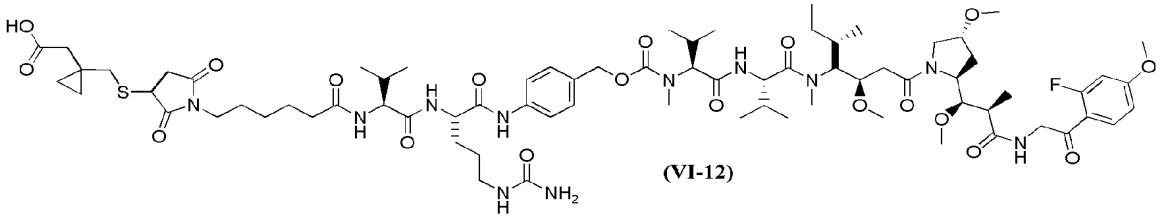
[837] 
(VI-11)

[838] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-2에서 수득한 화합물 (IV-6) (70 mg, 0.054 mmol)을 사용하고,
2-(1-(머캅토메틸)시클로프로판)아세트산 (V-2) 대신에 6-머캅토헥사노익산 (V-3)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (46 mg, 59%)을 고체로 얻었다.

[839] MALDI-TOF MS *m/z* : 1471.6 $[M+Na]^+$

[840]

[841] **제조예 4-8: 화합물 (VI-12)의 제조**

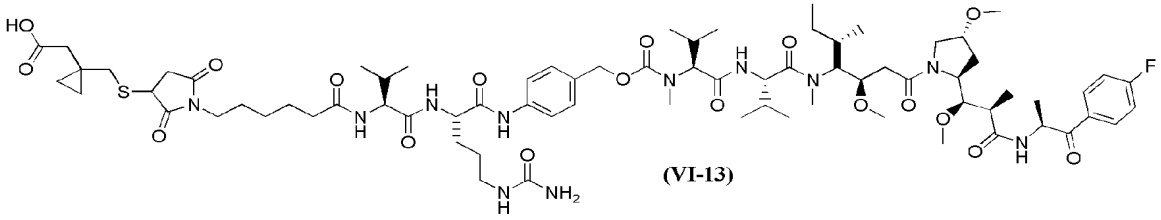
[842] 
(VI-12)

[843] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-3에서 수득한 화합물 (IV-7) (88 mg, 0.064 mmol)을 사용하여 제조예 4-2와 동일한 방법으로
표제화합물 (48 mg, 49%)을 고체로 얻었다.

[844] MALDI-TOF MS *m/z* : 1548.1 $[M+Na]^+$

[845]

[846] **제조예 4-9: 화합물 (VI-13)의 제조**

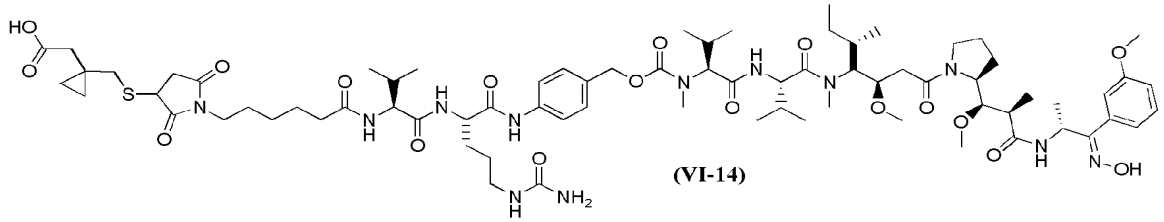
[847] 
(VI-13)

[848] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-4에서 수득한 화합물 (IV-8) (71 mg, 0.052 mmol)을 사용하여 제조예 4-2와 동일한 방법으로
표제화합물 (46 mg, 59%)을 고체로 얻었다.

[849] MALDI-TOF MS *m/z* : 1529.4 $[M+Na]^+$

[850]

[851] **제조예 4-10: 화합물 (VI-14)의 제조**

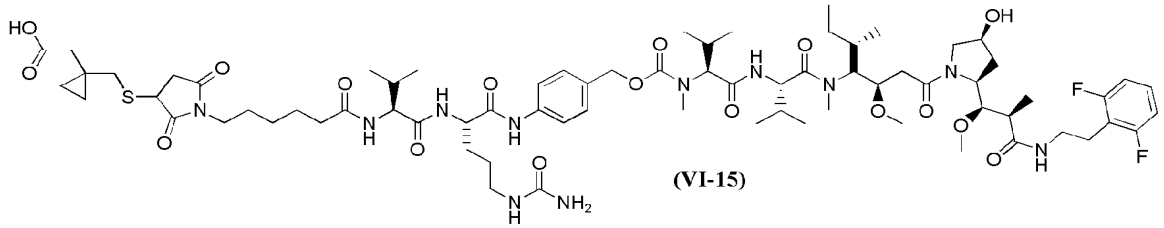
[852] 
(VI-14)

[853] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-5에서 수득한 화합물 (IV-9) (35 mg, 0.026 mmol)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (29 mg, 74%)을 고체로 얻었다.

[854] MALDI-TOF MS *m/z* : 1528.2 $[M+Na]^+$

[855]

[856] **제조예 4-11: 화합물 (VI-15)의 제조**

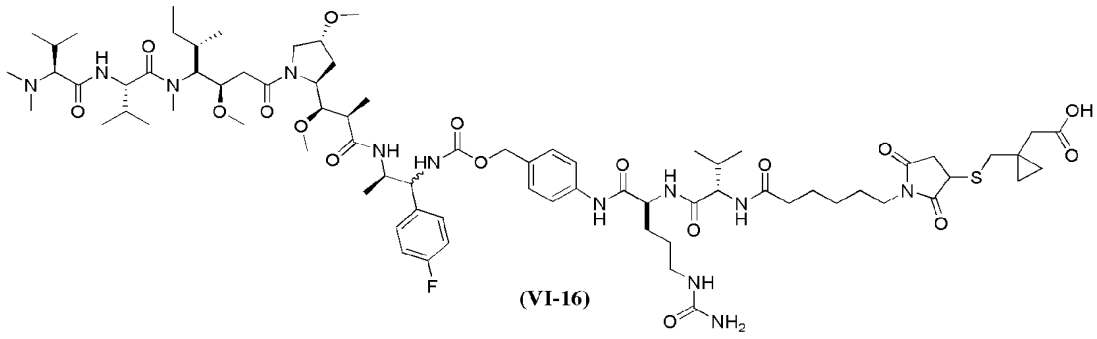
[857] 
(VI-15)

[858] 제조예 3-1에서 수득한 화합물 (IV-5) 대신에 제조예 3-6에서 수득한 화합물 (IV-10) (78 mg, 0.058 mmol)을 사용하여 제조예 4-2와 동일한 방법으로 표제화합물 (77 mg, 90%)을 고체로 얻었다.

[859] MALDI-TOF MS *m/z* : 1508.1 $[M+Na]^+$, 1524.8 $[M+K]^+$

[860]

[861] **제조예 4-12: 화합물 (VI-16)의 제조**

[862] 
(VI-16)

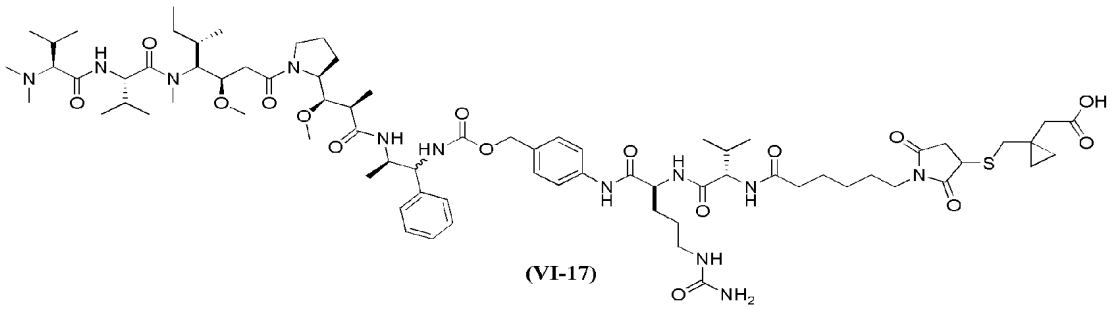
[863] 제조예 3-7에서 수득한 화합물 (IV-11)(58 mg, 0.042 mmol)에 메틸알코올 5 mL를 넣고 무수 디메틸포름아미드 1.5 mL를 가하여 용해시키고, 2-(1-(머캅토메틸)시클로프로판)아세트산(19 mg, 0.126 mmol)를 가하여 실온에서 4시간 동안 교반하였다. 반응혼합물을 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(60 mg, 93%)을 얻었다.

[864] LC-MS *m/z* : 1523.8 $[M^+]^+$, 1545.8 $[M+Na]^+$

[865]

[866] **제조예 4-13: 화합물 (VI-17)의 제조**

[867]



(VI-17)

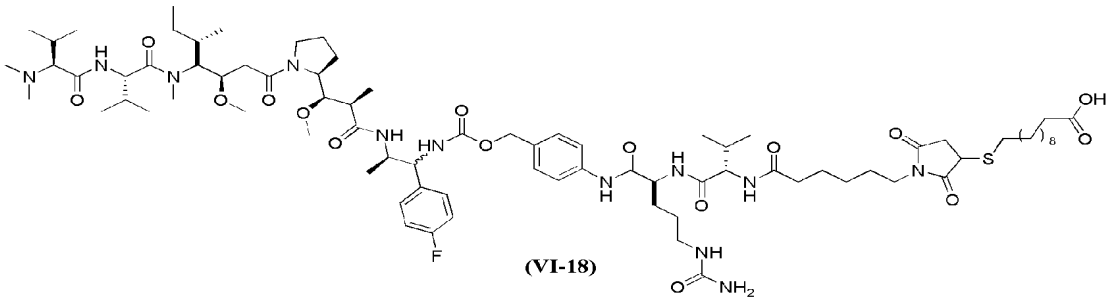
[868] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-8에서 수득한 화합물 (IV-12)(41 mg, 0.031 mmol)을 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(39 mg, 85%)을 고체로 얻었다.

[869] MALDI-TOF MS *m/z* : 1496.8 $[M+Na]^+$

[870]

[871] **제조예 4-14: 화합물 (VI-18)의 제조**

[872]



(VI-18)

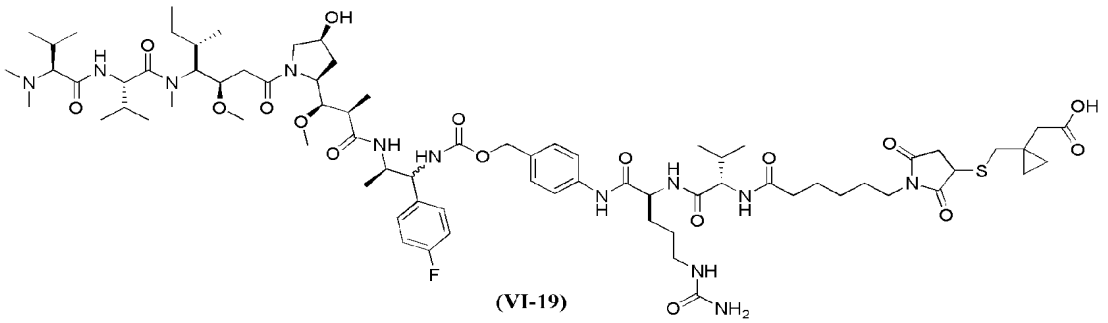
[873] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-9에서 수득한 화합물 (IV-13) (347 mg, 0.257 mmol)을 사용하고,
2-(1-(머캅토메틸)사이클로프로판)아세트산 대신에 11-머캅토운데칸산(112 mg, 0.514 mmol)을 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(266 mg, 66%)을 얻었다.

[874] LC-MS *m/z* : 1567 $[M+1]^+$

[875] ^{1}H NMR (400 MHz, CD_3OD) δ 0.76-0.90(m, 10H), 0.90-1.14(m, 20H), 1.17(m, 3H), 1.26(m, 4H), 1.29(brs, 20H), 1.37(m, 4H), 1.50-1.64(m, 14H), 1.68-1.78(m, 3H), 1.88(m, 2H), 1.94(s, 3H), 2.21-2.26(m, 6H), 2.99-2.48(m, 10H, N-CH_3*2), 2.70(m, 1H), 2.84(m, 1H), 3.07-3.19(m, 6H), 3.26-3.45(m, 6H), 3.45(m, 4H), 3.82(m, 1H), 4.15(m, 1H), 4.47(m, 2H), 4.64(m, 1H), 4.81(m, 1H), 4.98(m, 3H),6.96(t, 1H), 7.08(t, 1H), 7.25(d, 2H), 7.28-7.40(m, 2H), 7.53(d, 2H)

[876]

[877] **제조예 4-15: 화합물 (VI-19)의 제조**

[878] 

(VI-19)

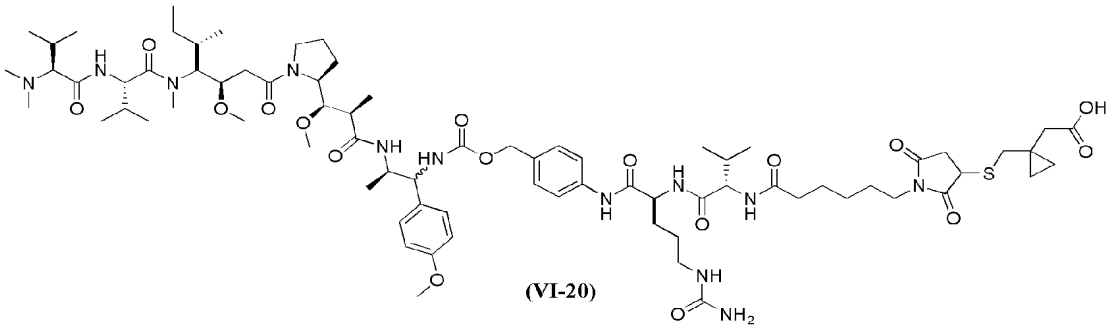
[879] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-10에서 수득한 화합물 (IV-14)(453 mg, 0.332 mmol)를 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(60 mg, 93%)을 얻었다.

[880] LC-MS *m/z* : 1510 $[M+1]^+$,

[881] ^{1}H NMR (400 MHz, CD_3OD) δ 0.46-0.68(m, 5H), 0.82-1.10(m, 27H), 1.17(d, 2H), 1.22(d, 1H), 1.30(m, 2H), 1.40(m, 1H), 1.50-1.63(m, 6H), 1.68-1.80(m, 2H), 1.82-1.90(m, 2H), 1.94(s, 3H), 2.00-2.15(m, 4H), 2.20-2.35(m, 5H), 2.42(s, 6H), 2.38-2.50(m, 2H), 2.81-2.89(m, 1H), 2.91-2.98(m, 2H), 3.08-3.40(m, 6H), 3.26-3.33(m, 10H, OCH_3), 3.43(d, 2H), 3.45-3.48(m, 5H, OCH_3), 3.52-3.64(m, 1H), 3.74-3.86(m, 1H), 3.906-4.10(m, 3H), 4.15(d, 1H), 4.24(m, 1H), 4.44-4.54(m, 2H), 4.66(d, 1H), 4.74-5.10(m, 5H), 7.09-7.12(m, 2H), 7.23-7.27(m, 2H), 7.33-7.37(m, 2H), 7.52-7.59(m, 2H)

[882]

[883] **제조예 4-16: 화합물 (VI-20)의 제조**

[884] 

(VI-20)

[885] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-11에서 수득한 화합물 (IV-15)(82 mg, 0.063 mmol)을 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(38 mg, 42%)을 얻었다.

[886] LC-MS *m/z* : 1506 $[M+1]^+$

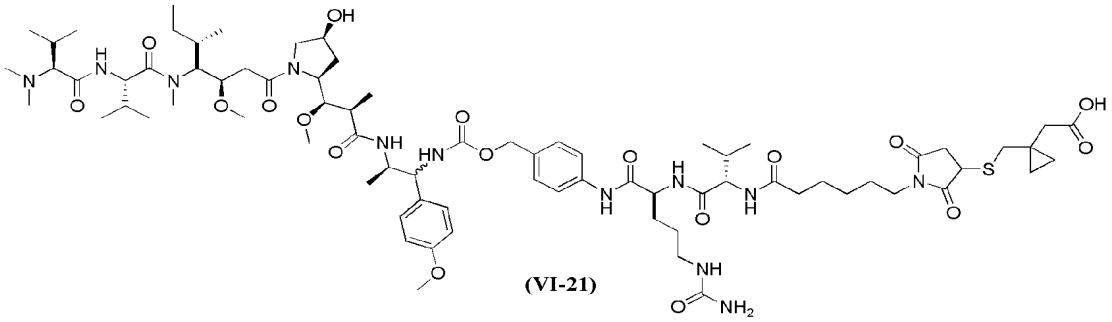
[887] ^{1}H NMR (400 MHz, CD_3OD) δ 0.42-0.67(m, 4H), 0.81-1.08(m, 27H), 1.13-1.20(m, 4H), 1.29(m, 2H), 1.40(m, 1H), 1.50-1.68(m, 6H), 1.75(m, 2H), 1.82-1.90(m, 2H), 1.94(s, 3H), 2.01-2.18(m, 4H), 2.23-2.32(m, 3H), 2.42(s, 6H), 2.35-2.50(m, 1H), 2.61(m, 1H), 2.79-3.00(m, 3H), 3.10-3.20(m, 5H), 3.28-3.40(m, 19H, 2*OCH_3), 3.43-3.48(m, 3H), 3.60(m, 1H), 3.74(d, 3H), 3.85-4.00(m, 3H), 4.14(d, 1H),

4.21-4.30(m, 1H), 4.48(m, 2H), 4.64-5.10(m, 5H), 6.86-7.60(m, 8H)

[888]

[889] **제조예 4-17: 화합물 (VI-21)의 제조**

[890]



(VI-21)

[891] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-12에서 수득한 화합물 (IV-16)(268 mg, 0.195 mmol)을 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(132 mg, 46%)을 고체로 얻었다.

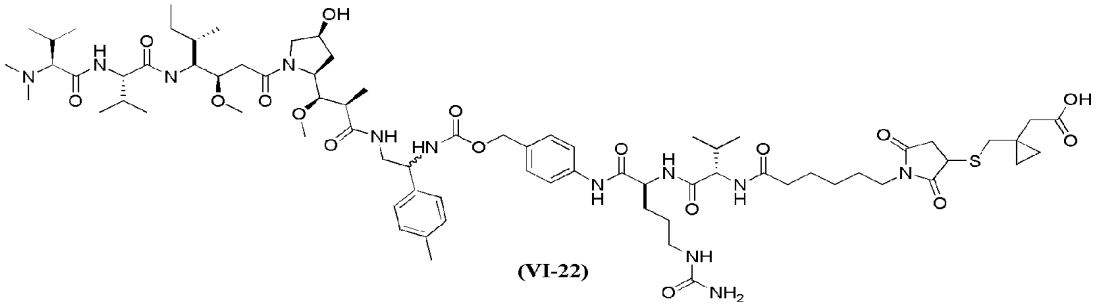
[892] LC-MS *m/z* : 1521[M]$^+$

[893] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48-0.50(m, 6H), 0.71-1.08(m, 27H), 1.16-1.22(m, 4H), 1.42-1.54(m, 9H), 1.60(m, 1H), 1.70(m, 1H), 1.94(s, 3H), 2.10-2.32(m, 8H), 2.32-2.46(m, 6H), 2.50(s, 6H), 2.78-3.10(m, 8H), 3.16(m, 2H), 3.21(m, 1H), 3.28-3.40(m, 6H, 메톡시기 6H), 3.62(s, 1H), 3.70(s, 2H), 3.86- 4.00(m, 5H), 4.19(t, 2H), 4.38(m, 2H), 4.52(t, 1H), 4.60(m, 1H), 4.76(m, 1H), 4.88(m, 1H), 5.00(m, 2H), 5.08(m, 1H), 5.42(brs, 2H), 5.98(m, 1H), 6.81(d, 1H), 7.17(d, 1H), 7.28(dd, 1H), 7.50-7.70(m, 3H), 7.82(d, 1H), 8.10(d, 1H), 10.0(d, 1H)

[894]

[895] **제조예 4-18: 화합물 (VI-22)의 제조**

[896]



(VI-22)

[897] 제조예 3-7에서 수득한 화합물 (IV-11) 대신에 제조예 3-13에서 수득한 화합물 (IV-17)(440 mg, 0.33 mmol)을 사용하여 제조예 4-12와 동일한 방법으로 표제화합물(296 mg, 60%)을 얻었다.

[898] LC-MS m/z : 1492 [M+1]$^+$

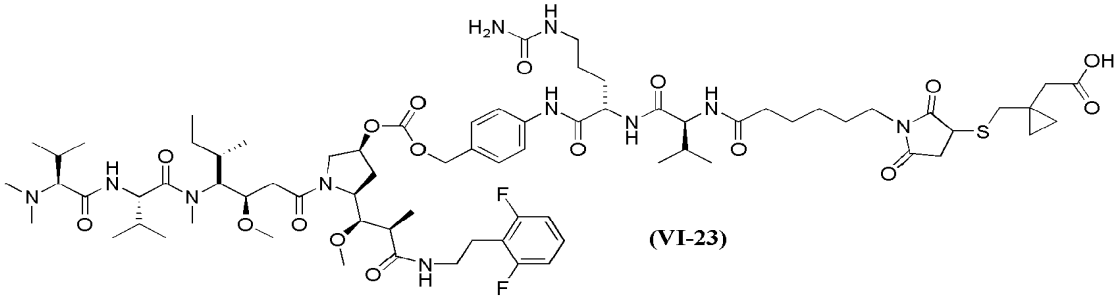
[899] ^{1}H NMR (400 MHz, CD_3OD) δ 0.47-0.62(m, 5H), 0.81-1.02(m, 26H), 1.13-1.20(m, 3H), 1.25-1.32(m, 2H), 1.40(m, 1H), 1.50-1.68(m, 6H), 1.72-1.78(m, 2H), 1.82-1.98(m, 3H), 1.96(s, 3H), 2.00-2.09(m, 4H), 2.25-2.35(m, 5H), 2.38-2.48(m, 6H),

2.850-2.90(m, 3H), 3.05-3.20(m, 6H), 3.26-3.35(m, 9H, OCH_3), 3.40-3.48(m, 6H, OCH_3), 3.50-3.60(m, 2H), 3.65-3.80(m, 3H), 3.90-4.18(m, 5H), 4.48(m, 1H), 4.64(m, 1H), 4.70-5.10(m, 8H), 7.09-7.58(m, 8H)

[900]

[901] **제조예 4-19: 화합물 (VI-23)의 제조**

[902]



(VI-23)

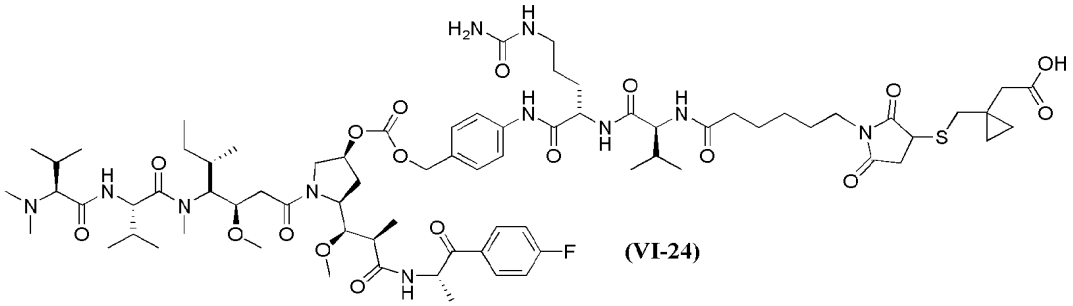
[903] 제조예 3-14에서 수득한 화합물 (IV-18) (37 mg, 0.027 mmol)에 메틸알코올 5 mL를 넣고 무수 디메틸포름아미드 1.5 mL를 가하여 용해시키고, 2-(1-(머캅토메틸)시클로프로판)아세트산(12 mg, 0.083 mmol)를 가하여 실온에서 4시간 동안 교반하였다. 반응혼합물을 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(33 mg, 81%)을 고체로 얻었다

[904] LC-MS *m/z* : 1499 $[M^+]^+$, 1451 $[M+Na]^+$

[905]

[906] **제조예 4-20: 화합물 (VI-24)의 제조**

[907]



(VI-24)

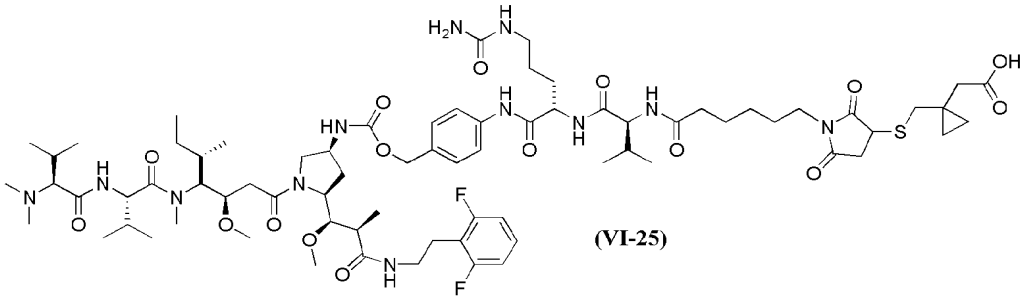
[908] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-15에서 수득한 화합물 (IV-19) (69 mg, 0.051 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(55 mg, 72%)을 고체로 얻었다.

[909] LC-MS *m/z* : 1508.9 $[M^+]^+$

[910]

[911] **제조예 4-21: 화합물 (VI-25)의 제조**

[912]



(VI-25)

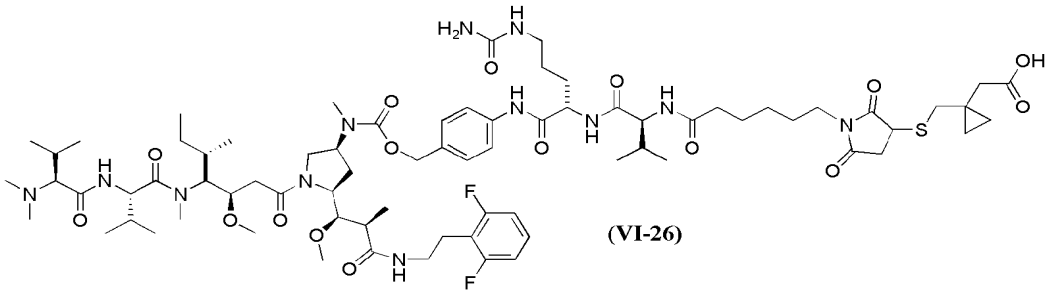
[913] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-16에서 수득한 화합물 (IV-20) (94 mg, 0.07 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(99 mg, 94%)을 고체로 얻었다.

[914] LC-MS *m/z* : 1498 $[M^+]^+$

[915]

[916] **제조예 4-22: 화합물 (VI-26)의 제조**

[917]



(VI-26)

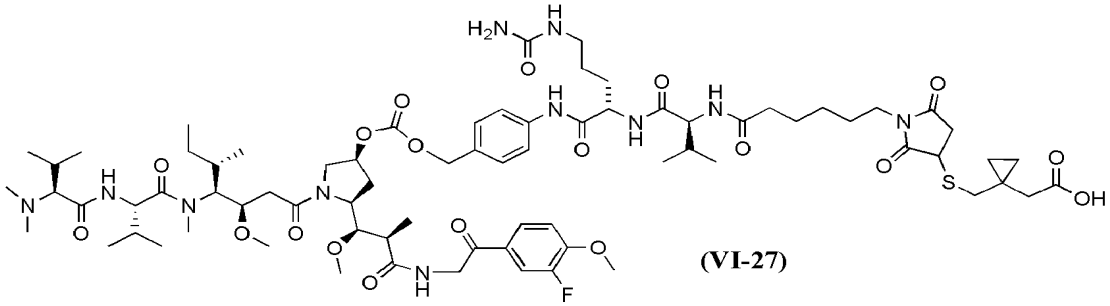
[918] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-17에서 수득한 화합물 (IV-21) (144 mg, 0.105 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(116 mg, 73%)을 고체로 얻었다.

[919] LC-MS *m/z* : 1512 $[M^+]^+$

[920]

[921] **제조예 4-23: 화합물 (VI-27)의 제조**

[922]



(VI-27)

[923] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-18에서 수득한 화합물 (IV-22) (0.14 g, 0.099 mmol)를 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(0.11 g, 75%)을 고체로 얻었다.

[924] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.50-0.52 (m, 4H), 0.65-0.76 (m, 6H), 0.82 -0.92 (m, 13H), 1.08-1.24 (18H), 1.43-1.49 (m, 6H), 1.54-1.82 (m, 3H), 1.84-1.98 (m, 3H),

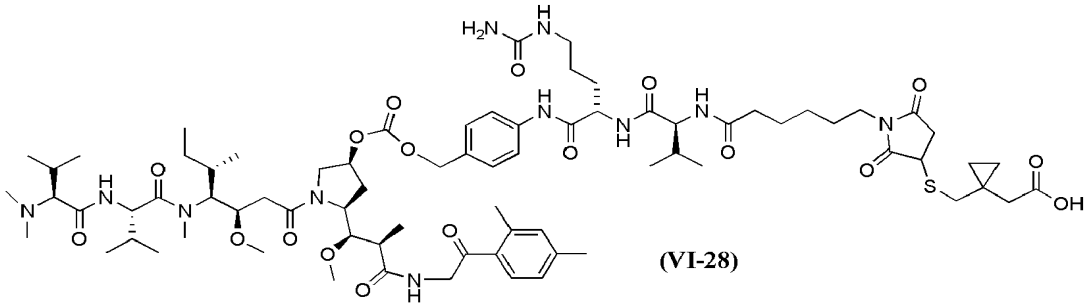
2.10-2.19 (m, 6H), 2.79-3.04 (m, 12H), 3.15-3.24 (m, 3H), 3.30 -3.44 (m, 15H), 3.87-3.89 (m, 3H), 3.97-4.00 (m, 1H), 4.08-4.86 (m, 8H), 4.86 -5.04 (m, 1H), 5.10 (s, 2H), 5.46 (s, 2H), 6.03 (t, 1H), 7.22-7.36 (m, 2H), 7.62-7.65 (d, 1H), 7.77-7.80 (d, 1H), 7.87-7.89 (m, 2H), 8.17-8.23 (m, 1H), 8.48 (brs, 1H), 10.11 (s, 1H)

[925] LC-MS *m/z* : 1524.4 $[M^+]^+$

[926]

[927] **제조예 4-24: 화합물 (VI-28)의 제조**

[928]



(VI-28)

[929] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-19에서 수득한 화합물 (IV-23) (0.2 g, 0.15 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(0.06 g, 27%)을 고체로 얻었다.

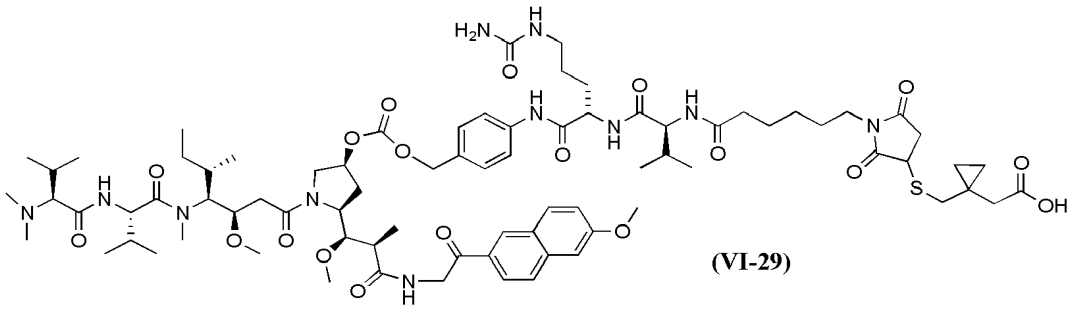
[930] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43-0.52 (m, 6H), 0.69-0.76 (m, 7H), 0.82 -0.92 (m, 15H), 1.02-1.06 (m, 7H), 1.11-1.13 (m, 2H), 1.18-1.23 (m, 4H), 1.43 -1.47 (m, 6H), 1.54-1.65 (m, 1H), 1.66-1.84 (m, 2H), 1.89-1.98 (m, 3H), 2.09 -2.20 (m, 6H), 2.91-3.03 (m, 10H), 3.15-3.22 (m, 5H), 3.33-3.46 (m, 15H), 3.87-4.02 (m, 3H), 4.15-4.95 (m, 8H),5.08 (s, 2H), 5.44 (s, 2H), 6.03 (t, 1H), 7.07-7.16 (m, 2H), 7.33-7.35 (m, 2H), 7.62-7.70 (d, 2H), 7.85-7.91 (m, 1H), 7.98-8.07 (m, 1H), 8.20 (brs, 1H), 8.28-8.49 (m, 1H), 10.10 (s, 1H)

[931] LC-MS *m/z* : 1504.4 $[M^+]^+$

[932]

[933] **제조예 4-25: 화합물 (VI-29)의 제조**

[934]



(VI-29)

[935] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-20에서 수득한 화합물 (IV-24) (0.1 g, 0.1 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물(0.08 g, 53%)을 고체로 얻었다.

[936] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43-0.47 (m, 5H), 0.48-0.50 (d, 1H), 0.58 -0.59

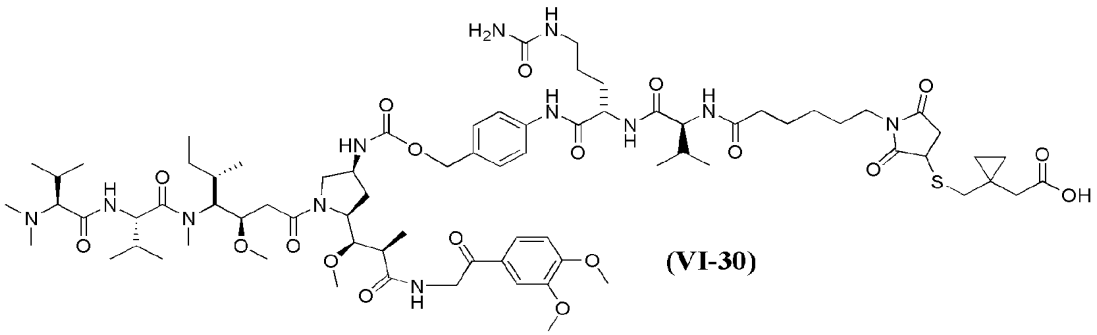
(d, 1H) 0.69-0.76 (m, 3H), 0.82-0.92 (m, 16H), 1.05-1.09 (m, 6H), 1.11 -1.13 (m, 2H), 1.16-1.23 (m, 4H), 1.43-1.47 (m, 6H), 1.54-1.65 (m, 1H), 1.66 -1.84 (m, 2H), 1.89-1.98 (m, 3H), 2.10-2.20 (m, 8H), 2.31-2.43 (m, 2H), 2.58 -2.68 (m, 1H), 2.70-2.82 (m, 3H), 2.87-2.88 (m, 2H), 2.95-3.00 (m, 4H), 3.13 -3.22 (m, 4H), 3.31-3.46 (m, 16H), 3.90-3.92 (m, 3H), 3.97-4.00 (m, 1H), 4.06 -5.00 (m, 8H), 5.10 (s, 2H), 5.47 (s, 2H), 6.10 (t, 1H), 7.22-7.28 (m, 1H), 7.33-7.43 (m, 2H), 7.55-7.58 (d, 1H), 7.88-7.99 (m, 2H), 8.01-8.03 (d, 1H), 8.22-8.54 (m, 2H), 8.64 (s, 1H), 10.15 (s, 1H)

[937] LC-MS *m/z* : 1556.4 $[M^+]^+$

[938]

[939] **제조예 4-26: 화합물 (VI-30)의 제조**

[940]



(VI-30)

[941] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-21에서 수득한 화합물 (IV-25) (0.46 g, 0.33 mmol)를 사용하여 제조예 4-19와 동일한 방법으로 표제화합물 (0.49 g, 96%)을 고체로 얻었다.

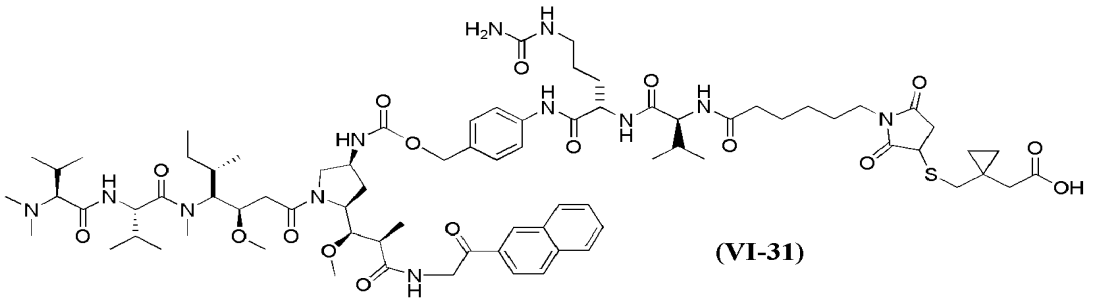
[942] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.30-0.58 (m, 6 H), 0.59-0.78 (m, 8 H), 0.78-1.00 (m, 16 H), 1.05-1.32 (m, 6 H), 1.32-1.54 (m, 6 H), 1.54-1.64 (m, 1 H), 1.64-1.84 (m, 3 H), 1.84-2.05 (m, 3 H), 2.06-2.27 (m, 10 H), 2.27-2.43 (m, 4 H), 2.55-2.71 (m, 1 H), 2.71-2.85 (m, 2 H), 2.85-3.07 (m, 7 H), 3.09-3.18 (m, 3 H), 3.18-3.30 (m, 3 H), 3.74-3.88 (m, 6 H), 3.88-4.05 (m, 4 H), 4.06-4.22 (m, 2 H), 4.22-4.43 (m, 2 H), 4.43-4.53 (m, 1 H), 4.59-4.75 (m, 2 H), 4.96 (s, 2 H), 5.43 (s, 2 H), 5.78 (s, 1 H), 5.96-6.10 (d, 1 H), 7.01-7.13 (d, 1 H), 7.21-7.37 (m, 2 H), 7.41-7.48 (s, 1 H), 7.49-7.57 (m, 1 H), 7.57-7.75 (m, 3 H), 8.00-8.11 (m, 1 H), 10.00-10.20 (brs, 1 H)

[943] LC-MS *m/z* : 1535.5 $[M^+]^+$

[944]

[945] **제조예 4-27: 화합물 (VI-31)의 제조**

[946]



(VI-31)

[947] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-22에서 수득한 화합물 (IV-26) (0.24 g, 0.17 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물 (0.19 g, 73%)을 고체로 얻었다.

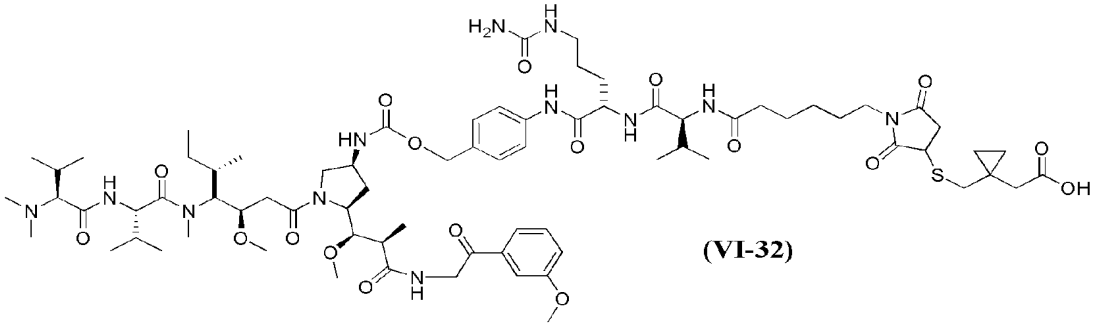
[948] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.37-0.58 (m, 9 H), 0.60-0.78 (m, 6 H), 0.78-0.97 (m, 16 H), 1.06-1.37 (m, 7 H), 1.37-1.53 (m, 6 H), 1.54-1.85 (m, 5 H), 1.86-2.03 (m, 3 H), 2.05-2.26 (m, 10 H), 2.26-2.43 (m, 3 H), 2.58-2.67 (m, 1 H), 2.68-2.84 (m, 2 H), 2.85-3.09 (m, 8 H), 3.10-3.24 (m, 6 H), 3.24-3.33 (m, 3 H), 3.62-3.78 (m, 1 H), 3.78-4.05 (m, 3 H), 4.05-4.24 (m, 2 H), 4.27-4.40 (m, 1 H), 4.46-4.61 (m, 1 H), 4.61-4.72 (m, 1 H), 4.72-4.89 (m, 1 H), 5.01 (s, 2 H), 5.44 (s, 2 H), 5.96-6.14 (t, 1 H), 7.21-7.37 (m, 2 H), 7.45-7.56 (m, 1 H), 7.56-7.74 (m, 4 H), 7.93-8.08 (m, 3 H), 8.08-8.19 (m, 1 H), 8.28-8.39 (m, 1 H), 8.49-8.61 (m, 1 H), 8.73 (s, 1 H), 10.14 (brs, 1 H)

[949] LC-MS *m/z* : 1535.5 $[M^+]^+$

[950]

[951] **제조예 4-28: 화합물 (VI-32)의 제조**

[952]



(VI-32)

[953] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-23에서 수득한 화합물 (IV-27) (0.21 g, 0.15 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물 (0.21 g, 91%)을 고체로 얻었다.

[954] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.34-0.56 (m, 5 H), 0.64-0.78 (m, 6 H), 0.78-0.96 (m, 15 H), 0.96-1.07 (m, 2 H), 1.07-1.27 (m, 5 H), 1.29-1.39 (m, 1 H), 1.39-1.54 (m, 5 H), 1.54-1.65 (m, 1 H), 1.65-1.84 (m, 3 H), 1.84-2.03 (m, 3 H), 2.05-2.31 (m, 10 H), 2.31-2.44 (m, 2 H), 2.44-2.48 (m, 1 H), 2.58-2.61 (m, 2 H), 2.75-2.85 (m, 1 H), 2.86-3.08 (m, 7 H), 3.09-3.15 (m, 1 H), 3.24-3.55 (m, 14 H), 3.64-3.76 (m, 2 H),

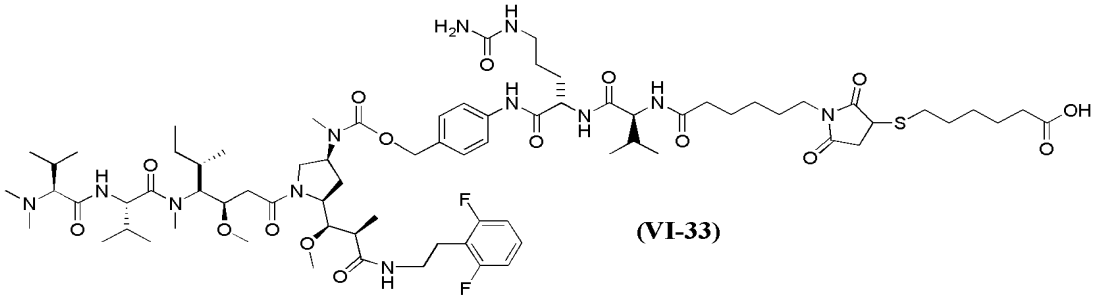
3.77-3.86 (m, 2 H), 3.87-4.04 (m, 3 H), 4.04-4.14 (m, 2 H), 4.14-4.25 (m, 2 H), 4.33-4.40 (m, 1 H), 4.40-4.61 (m, 2 H), 4.61-4.76 (m, 1 H), 4.97 (s, 2 H), 5.44 (s, 2 H), 5.98-6.11 (t, 1 H), 6.82-6.94 (m, 1 H), 7.16-7.25 (m, 1 H), 7.25-7.37 (m, 2 H), 7.40-7.49 (m, 1 H), 7.49-7.68 (m, 3 H), 7.81-7.99 (m, 1 H), 7.99-8.12 (m, 1 H), 8.24-8.45 (m, 1 H), 10.12 (brs, 1 H)

[955] LC-MS *m/z* : 1535.5 $[M^+]^+$

[956]

[957] **제조예 4-29: 화합물 (VI-33)의 제조**

[958]



(VI-33)

[959] 제조예 3-14에서 수득한 화합물 (IV-18) 대신에 제조예 3-17에서 수득한 화합물 (IV-21) (0.28 g, 0.21 mmol)을 사용하고,
2-(1-(머캅토메틸)사이클로프로판)아세트산 대신에 6-머캅토헥사노익산 (0.07 g, 0.41 mmol)을 사용하여 제조예 4-19와 동일한 방법으로 표제화합물 (0.26 g, 81%)을 고체로 얻었다.

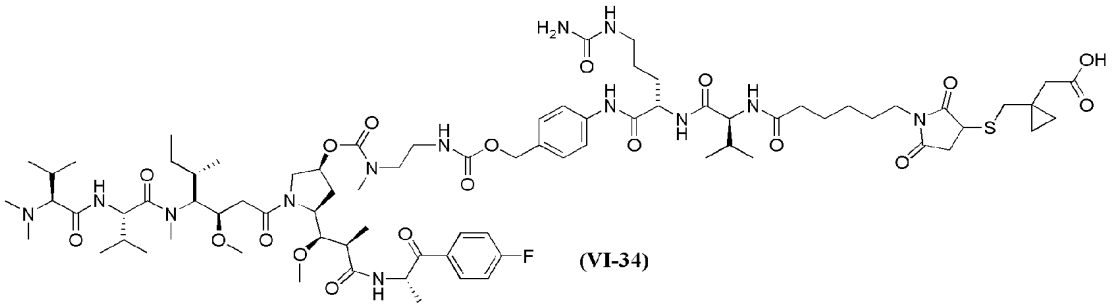
[960] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.64-0.79 (m, 5 H), 0.79-0.98 (m, 17 H), 0.98-1.14 (m, 4 H), 1.14-1.28 (m, 5 H), 1.28-1.41 (m, 6 H), 1.41-1.65 (m, 14 H), 1.65-1.79 (m, 2 H), 1.83-2.05 (m, 7 H), 2.05-2.32 (m, 13 H), 2.32-2.56 (m, 1 H), 2.57-2.88 (m, 10 H), 2.89-3.06 (m, 6 H), 3.06-3.24 (m, 3 H), 3.69-3.86 (m, 3 H), 3.86-4.09 (m, 3 H), 4.09-4.28 (m, 1 H), 4.29-4.48 (m, 2 H), 4.48-4.78 (m, 2 H), 5.04 (s, 2 H), 5.42 (s, 2 H), 6.03 (brs, 1 H), 7.01 (brs, 2 H), 7.14-7.45 (m, 2 H), 7.45-7.70 (m, 1 H), 7.83-8.06 (m, 2 H), 10.08 (brs, 1 H)

[961] LC-MS *m/z* : 1514.4 $[M+H]^+$, 1536.4 $[M+Na]^+$

[962]

[963] **제조예 4-30: 화합물 (VI-34)의 제조**

[964]



(VI-34)

[965] 제조예 3-24에서 수득한 화합물 (IV-28)(137 mg, 0.093 mmol)에 메틸알코올 10

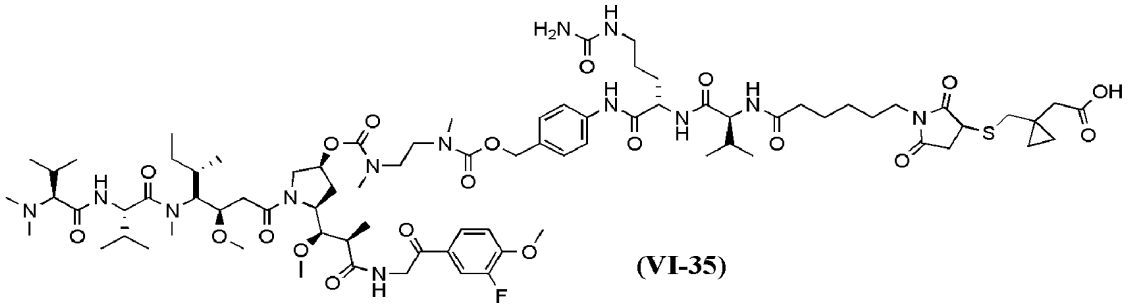
mL를 넣고 무수 디메틸포름아미드 3 mL를 가하여 용해시키고, 2-(1-(머캅토메틸)시클로프로판)아세트산(41 mg, 0.28 mmol)를 가하여 실온에서 15시간 동안 교반하였다. 반응혼합물을 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(25 mg, 17%)를 고체로 얻었다

[966] LC-MS *m/z* : 1609.3 $[M+H]^+$

[967]

[968] **제조예 4-31: 화합물 (VI-35)의 제조**

[969]



(VI-35)

[970] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-25에서 수득한 화합물 (IV-29) (0.19 g, 0.128 mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.085 g, 41%)를 얻었다.

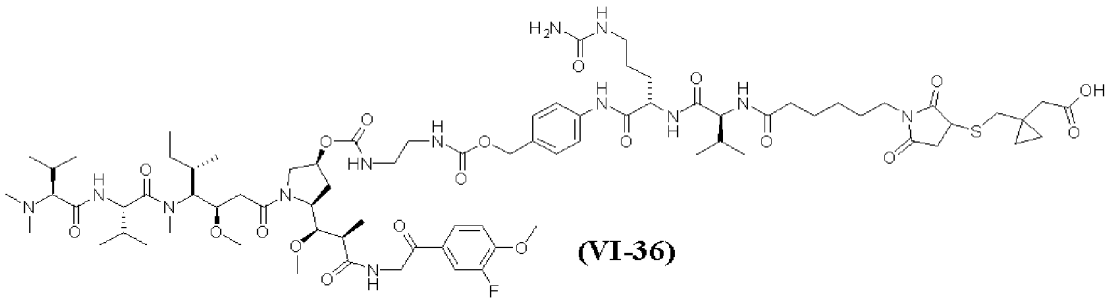
[971] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.42-0.55 (m, 6H), 0.66-0.75 (m, 8H), 0.81 -0.87 (m, 14H), 1.10-1.23 (m, 6H), 1.37-1.49 (m, 6H), 1.54-1.79 (m, 3H), 1.91 -1.98 (m, 7H), 2.27 (s, 1H), 2.30-2.37 (m, 1H), 2.38-2.42 (m, 1H), 2.44-2.45 (m, 1H), 2.56-2.64 (m, 2H), 2.75-2.86 (m, 6H), 2.93-2.99 (m, 4H), 3.12-3.24 (m, 4H), 3.31-3.35 (m, 17H), 3.91-4.06 (m, 6H), 4.14-4.86 (m, 8H), 4.98 (s, 2H), 5.43 (s, 2H), 5.97-5.98 (t, 1H), 7.29-7.30 (m, 2H), 7.58-7.60 (d, 2H), 7.77-7.90 (m, 2H), 7.93-8.10 (m, 2H), 8.11-8.13 (d, 2H), 8.46 (brs, 1H), 10.01 (s, 1H), 12.12 (brs, 1H)

[972] LC-MS *m/z* : 1639.0 $[M+H]^+$, 1661.0 $[M+Na]^+$

[973]

[974] **제조예 4-32: 화합물 (VI-36)의 제조**

[975]



(VI-36)

[976] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-26에서 수득한 화합물 (IV-30) (0.18 g, 0.119mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.12 g, 61%)를 얻었다.

[977] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.44-0.52 (m, 5H), 0.66-0.75 (m, 7H), 0.82 -0.92

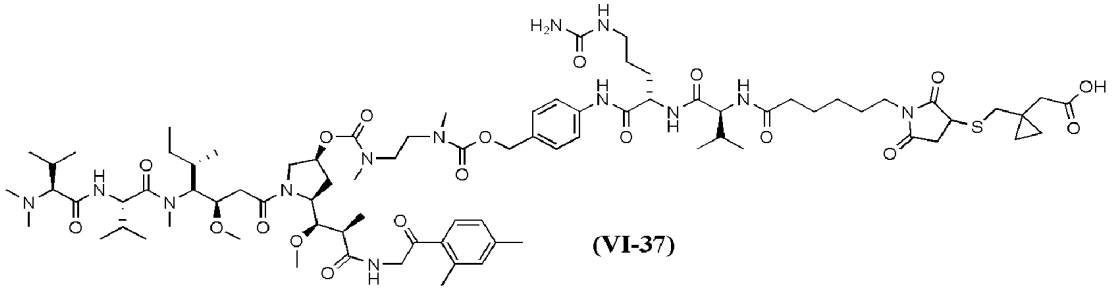
(m, 12H), 1.09-1.23 (m, 6H), 1.43-1.47 (m, 5H), 1.54-1.83 (m, 3H), 1.94 -1.97 (m, 4H), 2.10-2.22 (m, 7H), 2.25-2.26 (d, 1H), 2.38-2.46 (m, 4H), 2.56 -2.67 (m, 2H), 2.78-2.81 (m, 2H), 2.86-2.91 (m, 2H), 2.96-3.04 (m, 5H), 3.10 -3.24 (m, 4H), 3.32-3.35 (m, 17H), 3.91-3.93 (m, 3H), 3.97-4.00 (m, 2H), 4.13 -4.65 (m, 8H), 4.80-4.86 (m, 2H), 4.94 (s, 2H), 5.44 (s, 2H), 6.00 (t, 1H), 7.22-7.32 (m, 4H), 7.54-7.56 (d, 2H), 7.78-7.81 (d, 1H), 7.85-8.00 (m, 2H), 8.09-8.23 (m, 2H), 8.47 (brs, 1H), 10.04 (s, 1H), 12.12 (brs, 1H)

[978] LC-MS *m/z* : 1610.8 $[M+H]^+$, 1632.8 $[M+Na]^+$

[979]

[980] 제조예 **4-33:** 화합물 **(VI-37)**의 제조

[981]



(VI-37)

[982] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-27에서 수득한 화합물 (IV-31) (0.2 g, 0.138 mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.17 g, 77%)를 얻었다.

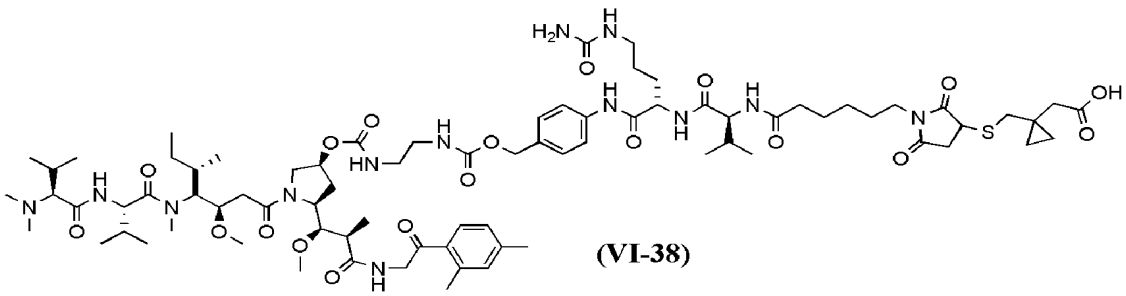
[983] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.00-0.57 (m, 6H), 0.69-0.76 (m, 8H), 0.82 -0.87 (m, 14H), 0.95-1.08 (m, 2H), 1.09-1.18 (m, 2H), 1.20-1.24 (m, 2H), 1.43 -1.49 (m, 6H), 1.54-1.64 (m, 1H), 1.81-2.05 (m, 4H), 2.20-2.23 (m, 6H), 2.27 -2.38 (m, 9H), 2.56-2.69 (m, 1H), 2.70-2.81 (m, 2H), 2.82-2.86 (m, 7H), 2.96 -3.00 (m, 5H), 3.15-3.20 (m, 5H), 3.27 (s, 3H), 3.32-3.34 (m, 15H), 3.89-4.00 (m, 3H), 4.14-4.25 (m, 2H), 4.26-4.82 (m, 8H), 4.98 (s, 2H), 5.43 (s, 2H), 6.00- 6.03 (t, 1H), 7.06-7.16 (m, 2H), 7.28-7.30 (m, 2H), 7.59-7.69 (m, 3H), 7.84-7.86 (d, 1H), 7.97-8.05 (m, 1H), 8.14-8.15 (m, 1H), 8.23-8.28 (m, 1H), 8.49 (brs, 1H), 10.02 (s, 1H)

[984] LC-MS *m/z* : 1619.6 $[M+H]^+$, 1641.6 $[M+Na]^+$

[985]

[986] 제조예 **4-34:** 화합물 **(VI-38)**의 제조

[987]



(VI-38)

[988] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-28에서 수득한 화합물 (IV-32) (0.2 g, 0.137 mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.17 g, 76%)를 얻었다.

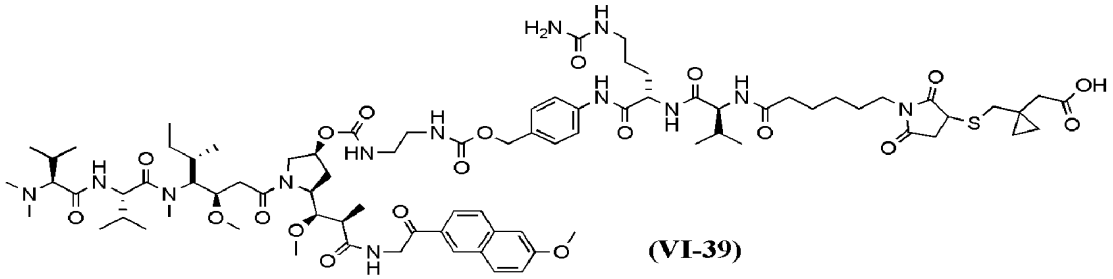
[989] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.50-0.52 (m, 4H), 0.69-0.76 (m, 8H), 0.82 -0.92 (m, 14H), 1.03-1.05 (m, 2H), 1.11-1.12 (m, 1H), 1.18-1.24 (m, 3H), 1.42 -1.49 (m, 6H), 1.52-1.64 (m, 1H), 1.66-1.83 (m, 3H), 1.86-2.02 (m, 4H), 2.10 -2.20 (m, 6H), 2.28-2.38 (m, 8H), 2.45-2.49 (m, 1H), 2.56-2.68 (m, 1H), 2.78 -2.86 (m, 3H), 2.95-3.10 (m, 8H), 3.15-3.17 (m, 4H), 3.28 (s, 3H), 3.34-3.35 (m, 10H), 3.95-4.00 (m, 3H), 4.02-4.88 (m, 8H), 4.94 (s, 2H), 5.43 (s, 2H), 6.03 (t, 1H), 7.06-7.15 (m, 2H), 7.22-7.29 (m, 4H), 7.54-7.69 (m, 3H), 7.89 (brs, 1H), 7.87-7.99 (d, 1H), 8.03-8.05 (d, 1H), 8.19 (brs, 1H), 8.30 (m, 1H), 8.50 (m, 1H), 10.04 (s, 1H)

[990] LC-MS *m/z* : 1591.7 $[M+H]^+$, 1613.7 $[M+Na]^+$

[991]

[992] **제조예 4-35: 화합물 (VI-39)의 제조**

[993]



(VI-39)

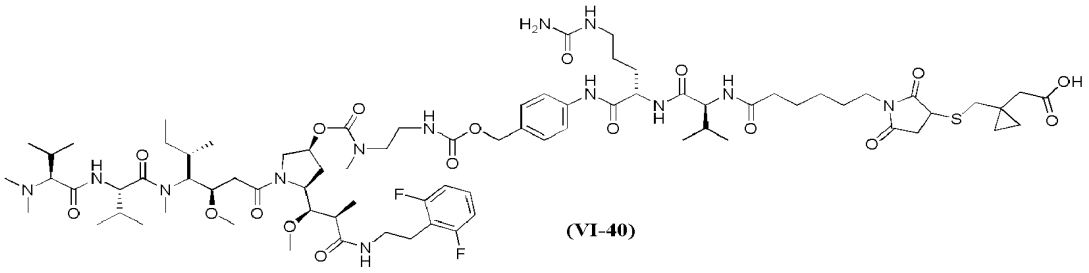
[994] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-29에서 수득한 화합물 (IV-33) (0.15 g, 0.099 mmol)을 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.11 g, 70%)를 얻었다.

[995] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.41-0.54 (m, 6H), 0.58-0.65 (m, 2H), 0.68 -0.76 (m, 3H), 0.82-0.95 (m, 14H), 1.09-1.23 (m, 8H), 1.32-1.49 (m, 5H), 1.59 -1.61 (m, 1H), 1.68-1.71 (m, 3H), 1.87-2.04 (m, 3H), 2.09-2.23 (m, 10H), 2.32 -2.39 (m, 3H), 2.62-2.68 (m, `H), 2.74-3.25 (m, 15H), 3.33-3.50 (m, 14H), 3.90-3.97 (m, 3H), 3.99-4.14 (m, 2H), 4.16-4.89 (m, 8H), 4.90 (s, 2H), 5.45 (s, 2H), 6.10 (t, 1H), 7.22-7.28 (m, 3H), 7.38-7.41 (d, 2H), 7.55-7.85 (d, 2H), 7.86-8.01 (m, 2H), 8.03-8.05 (m, 1H), 8.19-8.20 (brs, 1H), 8.29 (brs, 1H), 8.59 (brs, 1H), 8.84 (s, 1H), 10.08 (s, 1H)

[996] LC-MS *m/z* : 1642.8 $[M+H]^+$, 1664.8 $[M+Na]^+$

[997]

[998] **제조예 4-36: 화합물 (VI-40)의 제조**

[999] 
(VI-40)

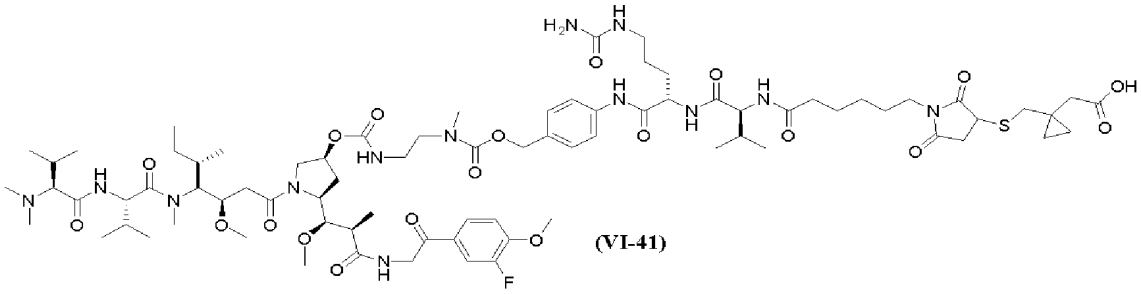
[1000] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-30에서 수득한 화합물 (IV-34) (1.3 g, 0.895mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(1.1 g, 74%)를 얻었다.

[1001] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.40-0.50 (m, 7H), 0.70-0.77 (m, 8H), 0.82 -0.89 (m, 18H), 1.02-0.07 (m, 3H), 1.18-1.29 (m, 3H), 1.44-1.47 (m, 6H), 1.54 -1.76 (m, 4H), 1.89-1.94 (m, 5H), 2.10-2.23 (m, 12H), 2.32-2.44 (m, 3H), 2.61 -2.64 (m, 2H), 2.74-3.00 (m, 10H), 3.13-3.17 (m, 3H), 3.20-3.33 (m, 10H), 3.90-4.00 (m, 4H), 4.16-4.84 (m, 8H), 4.95 (s, 2H), 5.41 (s, 2H), 6.01-6.02 (t, 1H), 7.01-7.05 (m, 2H), 7.28-7.29 (m, 4H), 7.59-7.60 (d, 2H), 7.84-7.99 (m, 2H), 8.13-8.16 (m, 1H), 8.23 (brs, 1H), 10.04 (s, 1H)

[1002] LC-MS *m/z* : 1598.9 $[M+H]^+$, 1621.9 $[M+Na]^+$

[1003]

[1004] **제조예 4-37: 화합물 (VI-41)의 제조**

[1005] 
(VI-41)

[1006] 제조예 3-24에서 수득한 화합물 (IV-28) 대신에 제조예 3-31에서 수득한 화합물 (IV-35) (0.14 g, 0.095mmol)를 사용하여 제조예 4-30과 동일한 방법으로 표제화합물(0.072 g, 47%)를 얻었다.

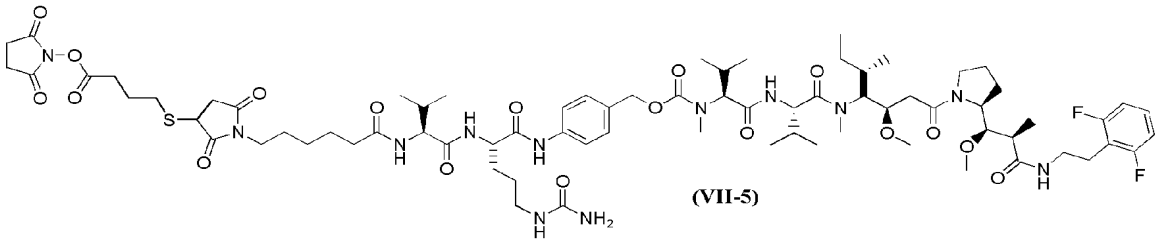
[1007] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43-0.50 (m, 6H), 0.66-0.76 (m, 8H), 0.82 -0.92 (m, 16H), 1.09-1.23 (m, 6H), 1.44-1.48 (m, 6H), 1.65-1.81 (m, 3H), 1.84 -1.97 (m, 4H), 2.01-2.30 (m, 10H), 2.34-2.38 (m, 3H), 2.56-2.64 (m, 2H), 2.80 -2.88 (m, 5H), 2.91-3.04 (m, 5H), 3.14-3.17 (m, 6H), 3.27-3.39 (m, 17H), 3.92 -3.98 (m, 3H), 4.15-4.86 (m, 8H), 4.98 (s, 2H), 5.42 (s, 2H), 6.02-6.03 (t, 1H), 7.21-7.36 (m, 4H), 7.55-7.63 (d, 2H), 7.77-8.01 (m, 4H), 8.03-8.05 (d, 1H), 8.13 (brs, 1H), 8.45 (brs, 1H), 10.01 (s, 1H)

[1008] LC-MS *m/z* : 1624.9 $[M+H]^+$, 1646.9 $[M+Na]^+$

[1009]

[1010] **제조예 5: 화학식 VII-1, VII-2, VII-3 또는 VII-4의 화합물의 제조**

[1011] **제조예 5-1: 화합물 (VII-5)의 제조**

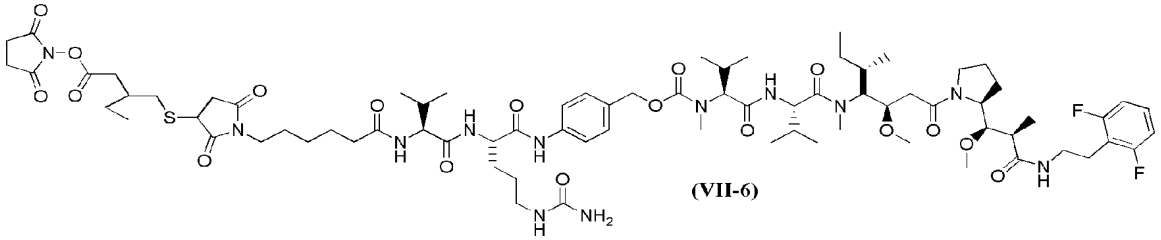
[1012] 
(VII-5)

[1013] 제조예 4-1에서 수득한 화합물 (VI-5) (290 mg, 0.20 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 10 mL에 녹인 후, N-히드록시숙신이미드 (35 mg, 0.30 mmol)를 가하였다. 반응혼합물을 0 ℃로 냉각한 후 EDC·HCl (58 mg, 0.30 mmol)을 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 에틸아세테이트 50 mL에 용해시켜 1M HCl 5 mL를 가하고 증류수 30 mL, 포화식염수 30 mL로 순차적으로 세척한 다음, 무수 황산나트륨으로 건조 후 감압 농축하여 유기용매를 제거하였다. 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물 (226 mg, 73%)를 얻었다.

[1014] MALDI-TOF MS *m/z* : 1562.9 $[M+Na]^+$

[1015]

[1016] **제조예 5-2: 화합물 (VII-6)의 제조**

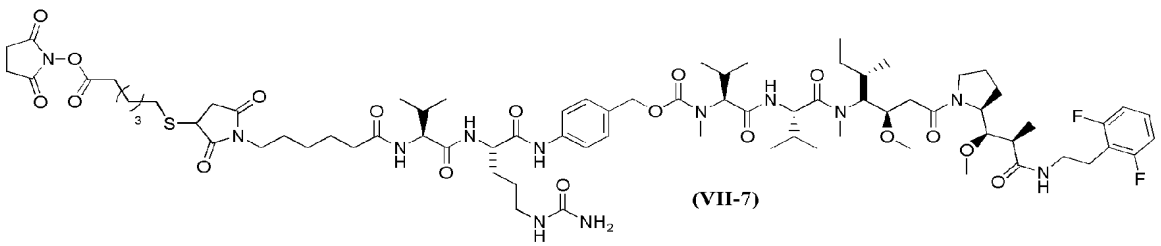
[1017] 
(VII-6)

[1018] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-2에서 수득한 화합물 (VI-6) (60 mg, 0.041 mmol)를 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (53 mg, 83%)을 미백색 고체로 얻었다.

[1019] MALDI-TOF MS *m/z* : 1586.7 $[M+Na]^+$

[1020]

[1021] **제조예 5-3: 화합물 (VII-7)의 제조**

[1022] 
(VII-7)

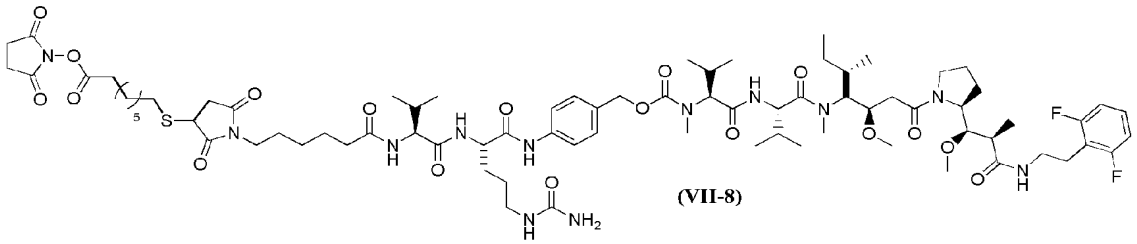
[1023] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-3에서 수득한 화합물 (VI-7) (54 mg, 0.037 mmol)을 사용하여 제조예 5-1과 동일한 방법으로

표제화합물 (57 mg, 98%)을 미백색 고체로 얻었다.

[1024] MALDI-TOF MS *m/z* : 1607.4 $[M+K]^+$

[1025]

[1026] **제조예 5-4: 화합물 (VII-8)의 제조**

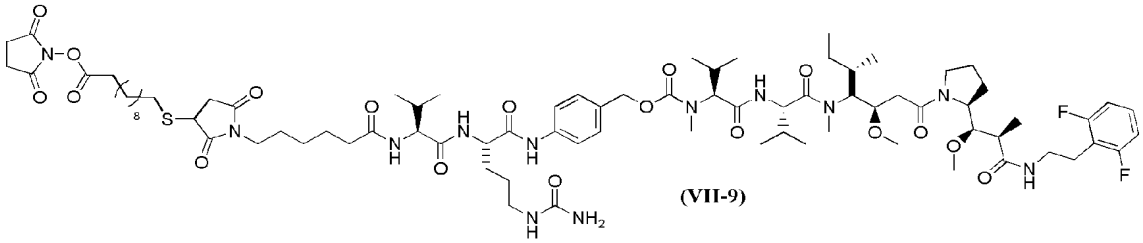
[1027] 

[1028] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-4에서 수득한 화합물 (VI-8) (66 mg, 0.044 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (59 mg, 84%)을 미백색 고체로 얻었다.

[1029] MALDI-TOF MS *m/z* : 1617.0 $[M+Na]^+$

[1030]

[1031] **제조예 5-5: 화합물 (VII-9)의 제조**

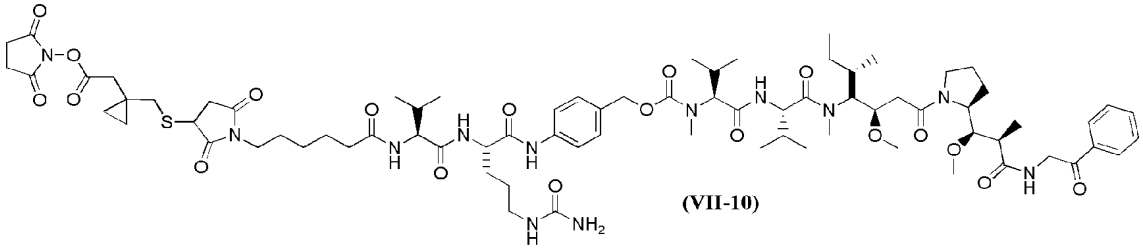
[1032] 

[1033] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-5에서 수득한 화합물 (VI-9) (71 mg, 0.046 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (66 mg, 88%)을 미백색 고체로 얻었다.

[1034] MALDI-TOF MS *m/z* : 1660.9 $[M+Na]^+$, 1677.1 $[M+K]^+$

[1035]

[1036] **제조예 5-6: 화합물 (VII-10)의 제조**

[1037] 

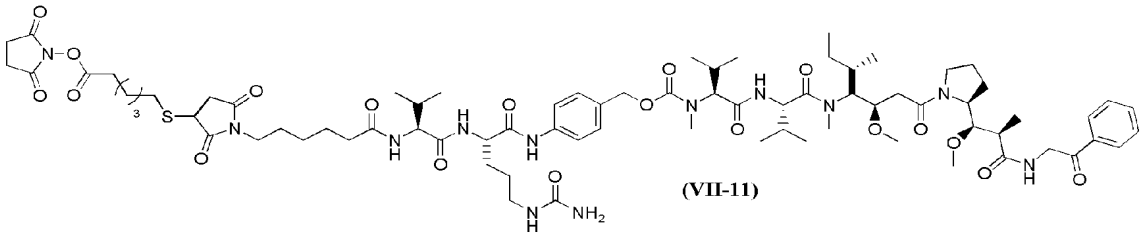
[1038] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-6에서 수득한 화합물 (VI-10) (53 mg, 0.037 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (38 mg, 67%)을 미백색 고체로 얻었다.

[1039] MALDI-TOF MS *m/z* : 1565.5 $[M+Na]^+$

[1040]

[1041] **제조예 5-7: 화합물 (VII-11)의 제조**

[1042]



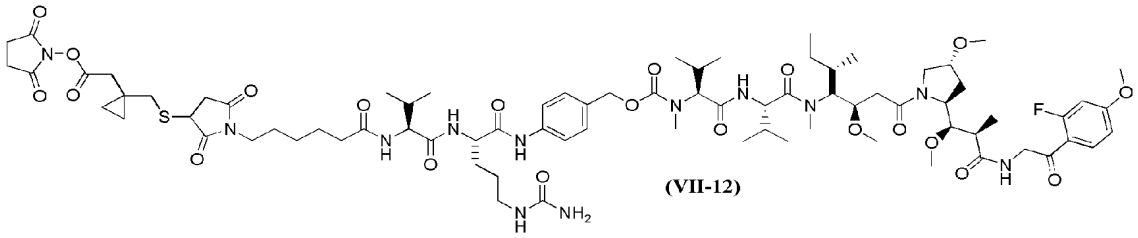
[1043] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-7에서 수득한 화합물 (VI-11) (46 mg, 0.032 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (30 mg, 61%)을 미백색 고체로 얻었다.

[1044] MALDI-TOF MS *m/z* : 1568.9 $[M+Na]^+$, 1585.0 $[M+K]^+$

[1045]

[1046] **제조예 5-8: 화합물 (VII-12)의 제조**

[1047]



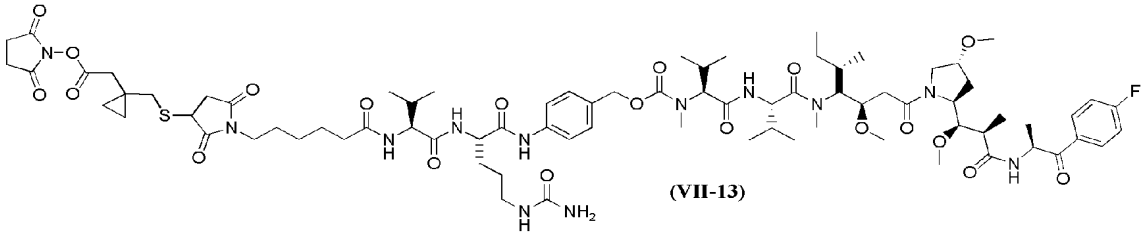
[1048] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-8에서 수득한 화합물 (VI-12) (38 mg, 0.025 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (31 mg, 78%)을 미백색 고체로 얻었다.

[1049] MALDI-TOF MS *m/z* : 1643.7 $[M+Na]^+$, 1660.2 $[M+K]^+$

[1050]

[1051] **제조예 5-9: 화합물 (VII-13)의 제조**

[1052]

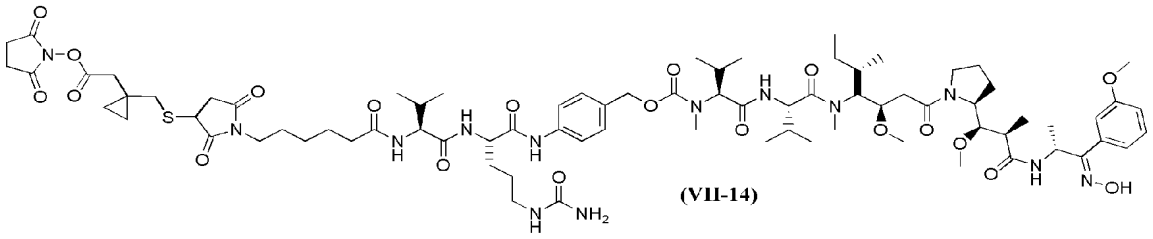


[1053] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-9에서 수득한 화합물 (VI-13) (34 mg, 0.023 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (27 mg, 75%)을 미백색 고체로 얻었다.

[1054] MALDI-TOF MS *m/z* : 1627.7 $[M+Na]^+$, 1644.8 $[M+K]^+$

[1055]

[1056] **제조예 5-10: 화합물 (VII-14)의 제조**

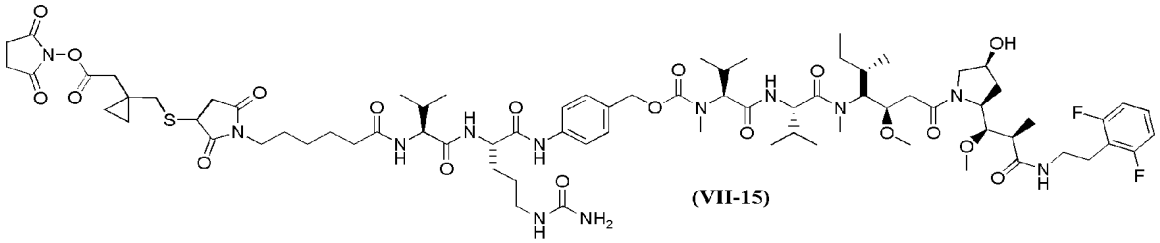
[1057] 
(VII-14)

[1058] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-10에서 수득한 화합물 (VI-14) (29 mg, 0.019 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (8 mg, 27%)을 미백색 고체로 얻었다.

[1059] MALDI-TOF MS *m/z* : 1625.4 $[M+Na]^+$, 1642.0 $[M+K]^+$

[1060]

[1061] **제조예 5-11: 화합물 (VII-15)의 제조**

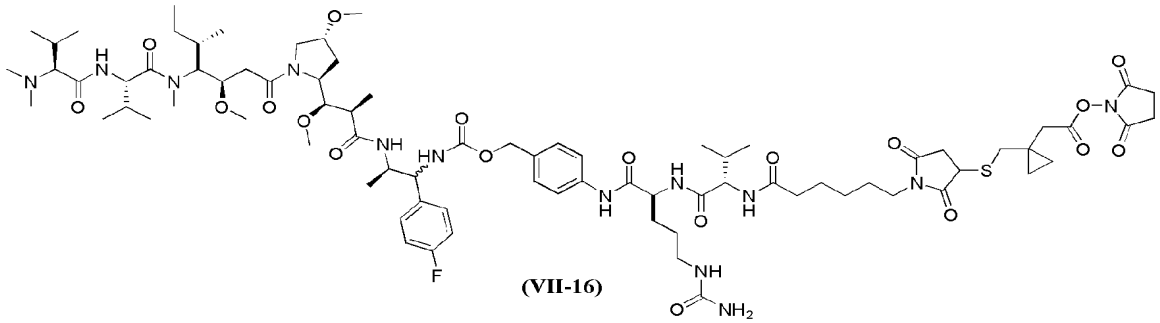
[1062] 
(VII-15)

[1063] 제조예 4-1에서 수득한 화합물 (VI-5) 대신에 제조예 4-11에서 수득한 화합물 (VI-15) (77 mg, 0.052 mmol)을 사용하여 제조예 5-1과 동일한 방법으로 표제화합물 (34 mg, 41%)을 미백색 고체로 얻었다.

[1064] MALDI-TOF MS *m/z* : 1603.8 $[M+Na]^+$, 1621.2 $[M+K]^+$

[1065]

[1066] **제조예 5-12: 화합물 (VII-16)의 제조**

[1067] 
(VII-16)

[1068] 제조예 4-12에서 수득한 화합물 (VI-16)(60 mg, 0.039 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 3 mL에 녹인 후, N-히드록시숙신이미드(18.1 mg, 0.157 mmol)를 가하였다. 반응혼합물을 0 ℃로 냉각한 후 EDC·HCl (30.2 mg, 0.157 mmol)을 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 에틸아세테이트 50 mL에 용해시켜 1M HCl 5 mL를 가하고 증류수 30 mL, 포화식염수 30 mL로 순차적으로 세척한 다음, 무수 황산나트륨으로 건조 후 감압 농축하여 유기용매를 제거하였다. 얻어진 잔사를

실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(37 mg, 57%)을 얻었다.

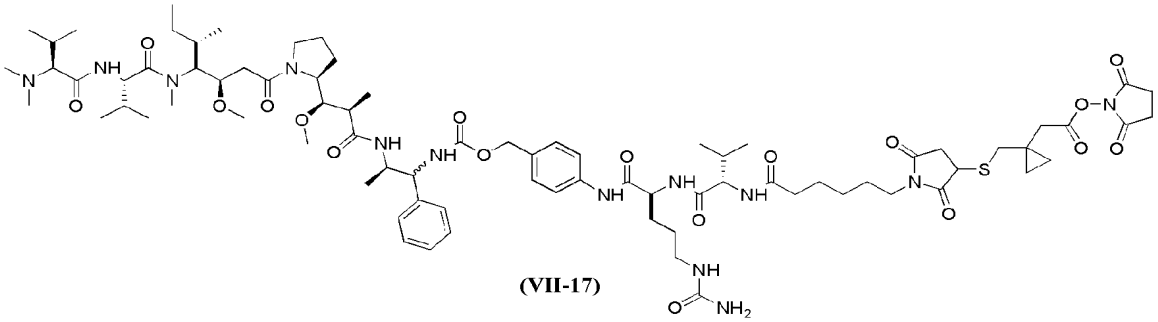
[1069] LC-MS *m/z* : 1620.8 $[M^+]^+$, 1643.7 $[M+Na]^+$

[1070] ^1H NMR (400 MHz, $CDCl_3$) δ 0.48 (m, 2H), 0.64 (m, 2H), 0.71 (m, 6H), 0.78 -0.88 (m, 17H), 0.92-0.97 (m, 4H), 1.02- 1.03 (d, 2H), 1.16 (m, 3H), 1.40-1.45 (m, 5H), 1.50-1.78 (m, 4H), 1.82-2.01 (m, 5H), 2.16 (S, 6H), 2.06- 2.28 (m, 3H), 2.39 (m, 1H), 2.47(S, 3H), 2.44-2.50 (m, 1H), 2.60 (m, 1H), 2.70 (m, 1H), 2.77 (S, 3H), 2.82-2.94 (m, 4H), 2.96-3.00 (m, 3H), 3.10-3.17 (m, 7H), 3.18-3.52 (m, 13H), 3.54-3.70 (m, 2H), 3.76(m, 1H), 3.84-3.99 (m, 3H), 4.00-4.16 (m, 2H), 4.33 (m, 1H), 4.49(t, 1H), 4.54-4.68 (m, 2H), 4.92 (m, 2H), 5.38(s, 2H), 5.95 (m, 1H), 7.00-7.06 (m, 2H), 7.22-7.27 (m, 3H), 7.50-7.55 (m, 2H), 7.70-7.78 (m, 1H), 7.98-8.06 (m, 1H), 9.95 (S, 1H)

[1071]

[1072] **제조예 5-13: 화합물 (VII-17)의 제조**

[1073]



(VII-17)

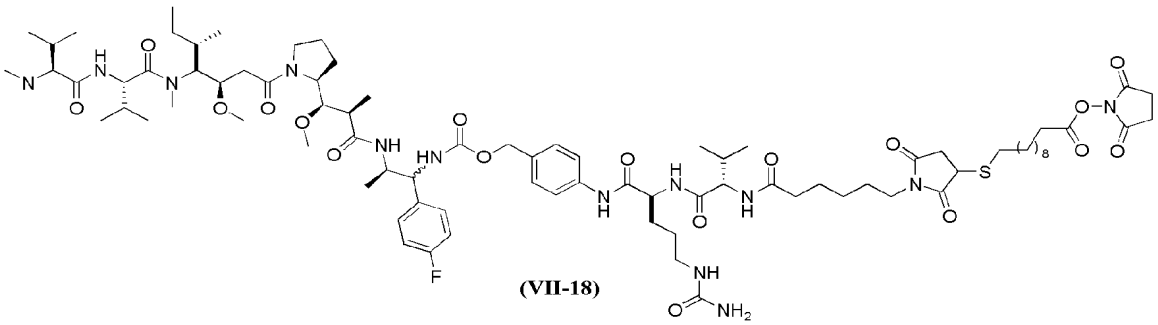
[1074] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-13에서 수득한 화합물 (VI-17)(39 mg, 0.026 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(32 mg, 78%)을 얻었다.

[1075] MALDI-TOF MS *m/z* : 1595.8 $[M+Na]^+$

[1076]

[1077] **제조예 5-14: 화합물 (VII-18)의 제조**

[1078]



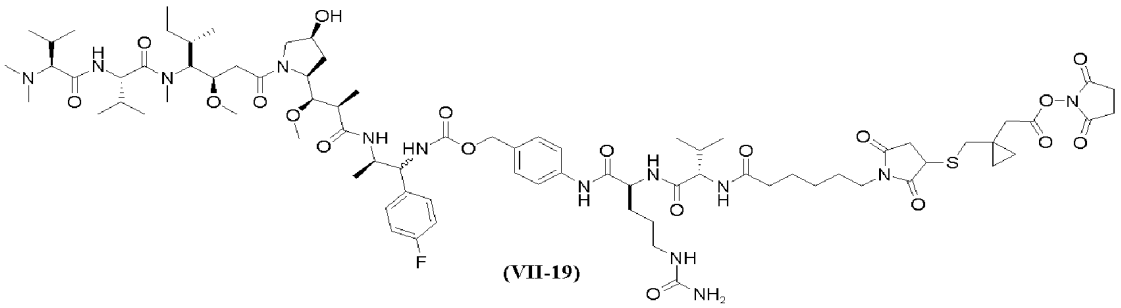
(VII-18)

[1079] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-14에서 수득한 화합물(VI-18)(266 mg, 0.170 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(37 mg, 57%)을 얻었다.

[1080] LC-MS *m/z* : 1590 $[M^+]^+$

[1081]

[1082] **제조예 5-15: 화합물 (VII-19)의 제조**

[1083] 
(VII-19)

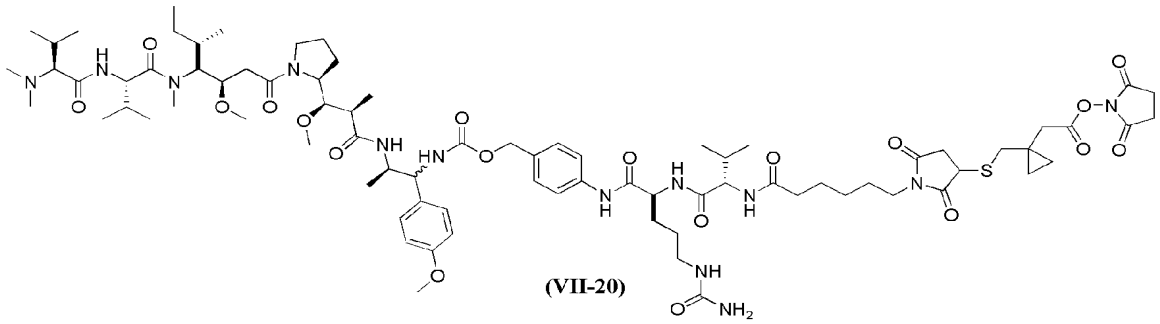
[1084] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-15에서 수득한 화합물 (VI-19)(247 mg, 0.16 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(197 mg, 77%)을 얻었다.

[1085] LC-MS *m/z* : 1607 $[M]^+$

[1086] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.54(m, 1H), 0.65(m, 3H),0.70-0.78(m, 5H), 0.80-1.00(m, 18H), 1.00-1.08(m, 6H), 1.20(m, 3H), 1.47(m, 5H), 1.60(m, 2H), 1.94(m, 5H), 2.08-2.20(m, 3H), 2.25(d, 6H), 2.40(m, 1H), 2.60(s, 6H), 2.73-3.02(m, 13H), 2.84-3.42(m, 17H, 2*OCH_3),3.60(m, 1H), 3.70(m, 1H), 3.80-4.06(m, 3H), 4.20(t, 2H), 4.38(m, 2H), 4.53(t, 1H), 4.66(m, 2H), 4.78(m, 1H), 4.88-5.10(m, 3H), 5.42(s, 2H), 6.00(m, 1H), 7.07(m, 1H), 7.27-7.37(m, 3H), 7.50-7.58(m, 2H), 7.65-7.85(m, 1H), 8.10(m, 1H), 9.95(s, 1H)

[1087]

[1088] **제조예 5-16: 화합물 (VII-20)의 제조**

[1089] 
(VII-20)

[1090] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-16에서 수득한 화합물 (VI-20)(38 mg, 0.025 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(25 mg, 62%)을 얻었다.

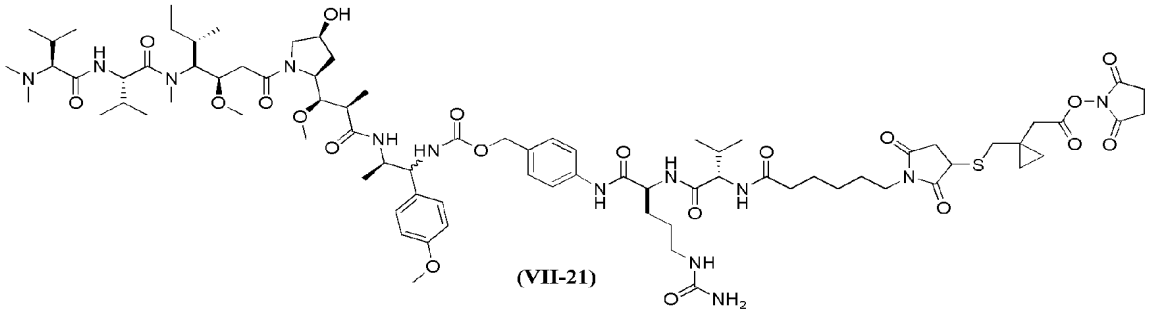
[1091] LC-MS *m/z* : 1603 $[M]^+$

[1092] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48 (m, 1H), 0.62 (m, 2H), 0.65-0.78 (m, 6H), 0.71- 1.02 (m, 25 H), 1.16 (m, 2H), 1.30-1.52 (m, 5H), 1.53-1.82 (m, 5H), 1.83-2.12 (m, 5H), 2.14 (d, 6H), 2.54 (s, 3H), 2.58-3.02 (m, 14H), 3.10-3.16 (m, 6H), 3.18-3.20 (m, 7H), 3.28-3.70 (m, 11H), 3.72-3.86 (m, 1H), 3.90-4.04 (m, 1H), 4.03-4.29 (m, 2H),

4.32 (m, 1H), 4.42-4.78 (m, 3H), 4.82-4.98 (m, 2H), 5.06 (m, 1H), 5.39 (s, 2H), 5.96 (m, 1H), 6.78 (m, 1H), 7.03-7.26 (m, 4H), 7.58 (m, 2H), 7.79(d, 1H), 8.00 (d, 1H), 8.07(d, 1H), 9.95 (S, 1H)

[1093]

[1094] **제조예 5-17: 화합물 (VII-21)의 제조**

[1095] 
(VII-21)

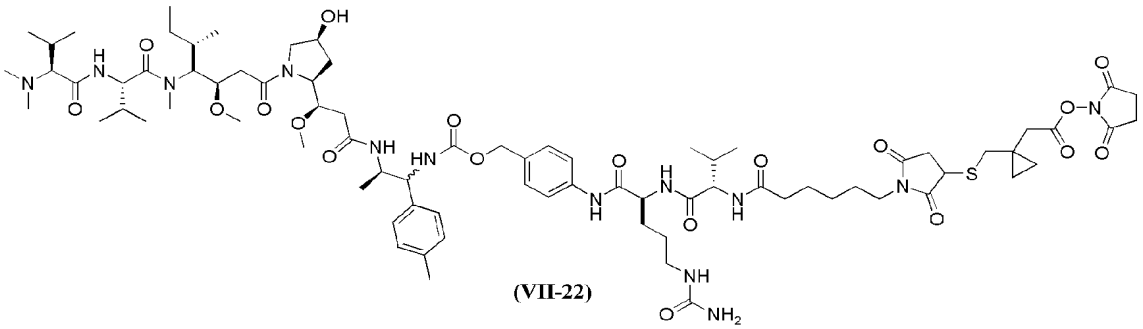
[1096] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-17에서 수득한 화합물 (VI-21)(132 mg, 0.09 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(136 mg, 93%)을 얻었다.

[1097] LC-MS *m/z* : 1619 $[M]^+$

[1098] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.54(m, 1H), 0.63-0.68(m, 3H), 0.70-0.80(m, 5H), 0.81-0.90(m, 17H), 1.00-1.1.08(m, 8H), 1.22(m, 3H), 1.40-1.54(m, 6H), 1.56-1.74(m, 3H), 1.80-2.00(m, 3H), 2.26-2.30(m, 7H), 2.51(m, 6H), 2.56(s, 1H), 2.64-3.04(m, 14H), 3.13-3.30(m, 6H), 3.36(m, 6H), 3.62(s, 1H), 3.69(s, 2H), 3.87-4.04(m, 4H), 4.19(t, 2H0, 4.37(m, 2H), 4.52(t, 1H), 4.62(m, 1H), 4.68(m, 1H), 4.76(m, 1H), 4.89(m, 1H), 4.979m, 2H), 5.08(d, 1H), 5.42(s, 2H), 5.98(m, 1H), 6.81(d, 1H), 7.17(d, 1H), 7.30(m, 1H), 7.60(m, 3H), 7.82(d, 1h), 8.10(m, 1H), 10.00(d, 1H),

[1099]

[1100] **제조예 5-18: 화합물 (VII-22)의 제조**

[1101] 
(VII-22)

[1102] 제조예 4-12에서 수득한 화합물 (VI-16) 대신에 제조예 4-18에서 수득한 화합물 (VI-22)(296 mg, 0.198 mmol)을 사용하여 제조예 5-12와 동일한 방법으로 표제화합물(132 mg, 83%)을 얻었다.

[1103] LC-MS *m/z* : 1589 $[M]^+$

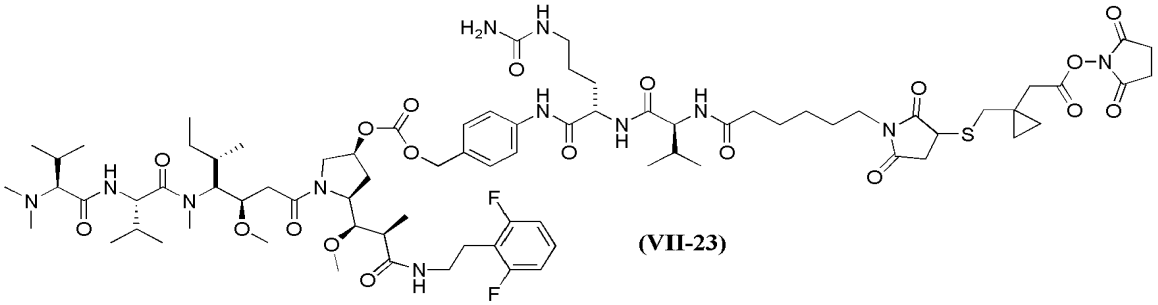
[1104] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48 (m, 1H), 0.60 (m, 2H), 0.71 (m, 5H), 0.71-

0.88 (m, 17 H), 0.98 (d, 3H), 0.93- 1.08 (m, 3H), 1.16 (m, 3H), 1.37-1.45 (m, 5H), 1.50-1.86 (m, 4H), 1.91 (m, 3H), 2.0-2.42 (m, 11H), 2.68 (m, 1H), 2.77 (S, 3H), 2.82-3.02 (m, 5H), 3.10-3.19 (m, 6H), 3.21-3.30 (m, 7H), 3.36-3.50 (m, 16H), 3.54-3.72 (m, 3H), 3.80-4.00 (m, 4H), 4.15 (t, 1H), 4.33 (m, 1H), 4.49-4.71 (m, 3H), 4.86 (m, 2H), 5.04 (m, 1H), 5.39 (s, 2H), 5.96 (m, 1H), 7.03-7.21 (m, 5H), 7.55 (m, 2H), 7.79 (d, 1H), 8.06 (d, 1H) 9.95 (S, 1H)

[1105]

[1106] **제조예 5-19: 화합물 (VII-23)의 제조**

[1107]



(VII-23)

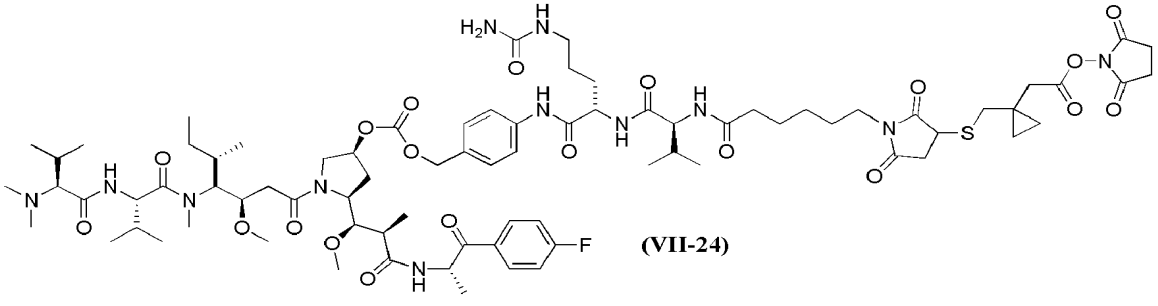
[1108] 제조예 4-19에서 수득한 화합물 (VI-23) (33.4 mg, 0.022 mmol)을 아르곤 기류 하에서 무수 디메틸포름아미드 3 mL에 녹인 후, N-히드록시숙신이미드 (7.7 mg, 0.066 mmol)를 가하였다. 반응혼합물을 0 ℃로 냉각한 후 EDC·HCl (12.8 mg, 0.066 mmol)을 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 에틸아세테이트 50 mL에 용해시켜 1M HCl 5 mL를 가하고 증류수 30 mL, 포화식염수 30 mL로 순차적으로 세척한 다음, 무수 황산나트륨으로 건조 후 감압 농축하여 유기용매를 제거하였다. 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(32 mg, 91%)를 얻었다.

[1109] LC-MS *m/z* : 1596 $[M^+]^+$, 1619 $[M+Na]^+$

[1110]

[1111] **제조예 5-20: 화합물 (VII-24)의 제조**

[1112]



(VII-24)

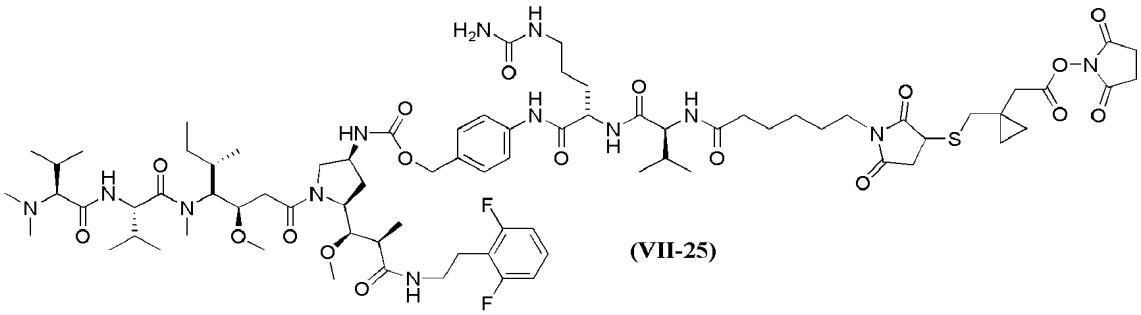
[1113] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-20에서 수득한 화합물 (VI-24) (55 mg, 0.037 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(49 mg, 84%)을 미백색 고체로 얻었다.

[1114] LC-MS *m/z* : 1606 $[M^+]^+$

[1115]

[1116] **제조예 5-21: 화합물 (VII-25)의 제조**

[1117]



(VII-25)

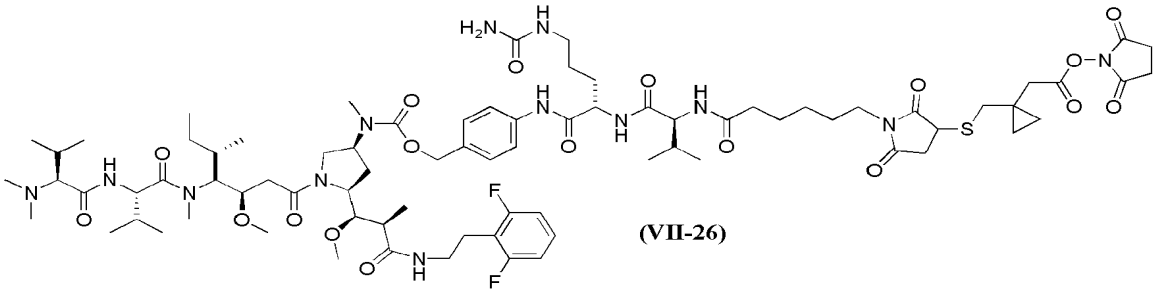
[1118] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-21에서 수득한 화합물 (VI-25) (65 mg, 0.043 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(56 mg, 82%)을 미백색 고체로 얻었다.

[1119] LC-MS *m/z* : 1592 $[M^+]^+$

[1120]

[1121] **제조예 5-22: 화합물 (VII-26)의 제조**

[1122]



(VII-26)

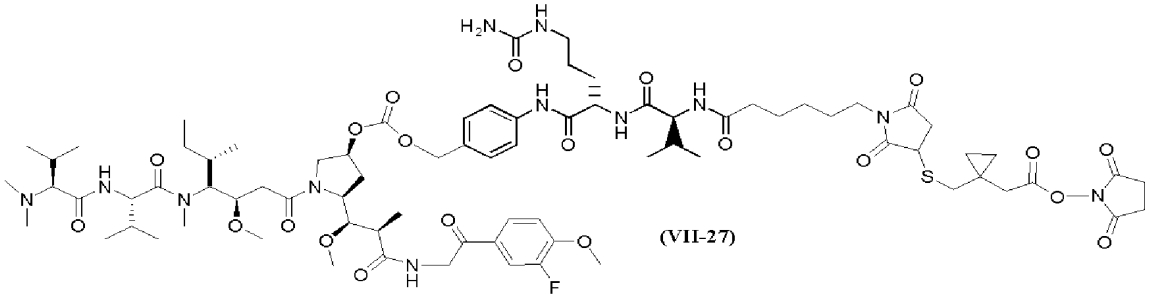
[1123] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-22에서 수득한 화합물 (VI-26) (114 mg, 0.075 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(94 mg, 78%)을 미백색 고체로 얻었다.

[1124] LC-MS *m/z* : 1609 $[M^+]^+$

[1125]

[1126] **제조예 5-23: 화합물 (VII-27)의 제조**

[1127]



(VII-27)

[1128] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-23에서 수득한 화합물 (VI-27) (91 mg, 0.0597 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(25 mg, 25%)을 미백색 고체로 얻었다.

[1129] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.62-0.71 (m, 7H), 0.78 -0.87

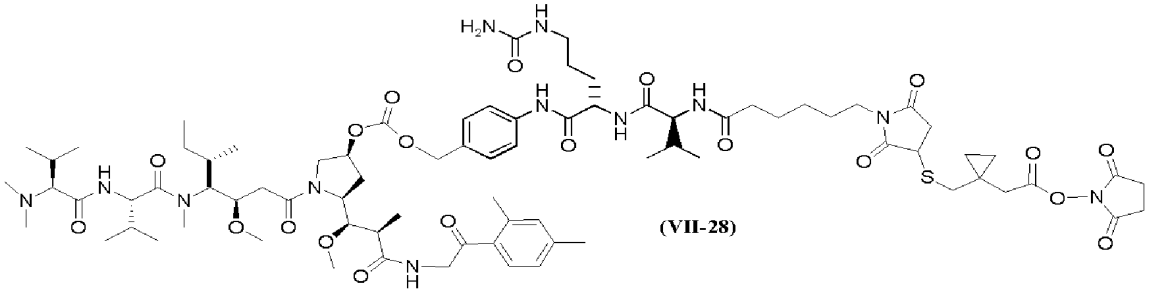
(m, 12H), 1.05-1.17 (m, 4H), 1.38-1.43 (m, 4H), 1.84-1.98 (m, 3H), 2.10 -2.14 (m, 5H), 2.79-3.04 (m, 10H), 3.09-3.13 (m, 3H), 3.25-3.36 (m, 10H), 3.38-3.49 (m, 19H), 3.87-3.89 (m, 4H), 3.98-4.00 (m, 2H), 4.12-4.99 (m, 8H), 5.05 (s, 2H), 5.39 (s, 2H), 6.00-6.03 (t, 1H), 7.23-7.31 (m, 3H), 7.56-7.58 (d, 2H), 7.72-7.80 (m, 3H), 8.06-8.08 (d, 2H), 8.16-8.41 (m, 1H), 10.01 (s, 1H)

[1130] LC-MS *m/z* : 1621.5 $[M^+]^+$

[1131]

[1132] **제조예 5-24: 화합물 (VII-28)의 제조**

[1133]



[1134] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-24에서 수득한 화합물 (VI-28) (60 mg, 0.0399 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(14 mg, 23%)을 미백색 고체로 얻었다.

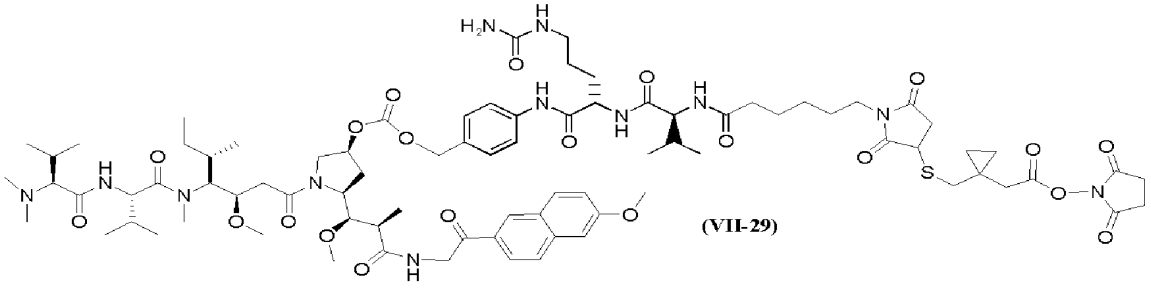
[1135] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.62-0.71 (m, 7H), 0.78 -0.87 (m, 12H), 1.05-1.17 (m, 4H), 1.38-1.43 (m, 4H), 1.84-1.98 (m, 3H), 2.14 -2.15 (m, 7H), 2.24-2.33 (m, 6H), 2.79-3.04 (m, 10H), 3.09-3.13 (m, 3H), 3.25 -3.36 (m, 10H), 3.38-3.49 (m, 19H), 3.87-3.89 (m, 4H), 3.98-4.00 (m, 2H), 4.12-4.99 (m, 8H), 5.05 (s, 2H), 5.39 (s, 2H), 5.97 (t, 1H), 7.02-7.11 (m, 2H), 7.28-7.29 (m, 1H), 7.57-7.65 (d, 2H), 7.88-7.98 (m, 1H), 8.07-8.08 (m, 1H), 8.22-8.44 (m, 1H), 10.00 (s, 1H)

[1136] LC-MS *m/z* : 1601.7 $[M^+]^+$

[1137]

[1138] **제조예 5-25: 화합물 (VII-29)의 제조**

[1139]



[1140] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-25에서 수득한 화합물 (VI-29) (83 mg, 0.053 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(23 mg, 25%)을 미백색 고체로 얻었다.

[1141] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 2H), 0.62-0.71 (m, 5H), 0.78 -0.87 (m, 9H), 1.05-1.17 (m, 4H), 1.38-1.43 (m, 4H), 1.84-1.98 (m, 3H), 2.14 -2.15 (m, 4H),

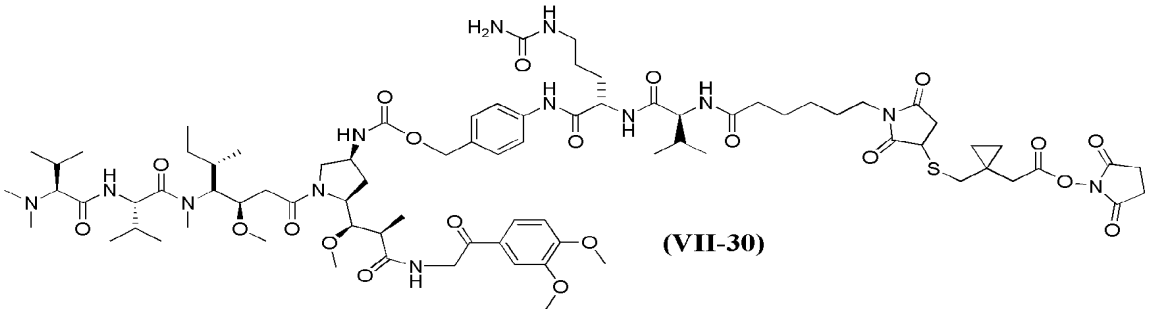
2.79-3.04 (m, 10H), 3.09-3.13 (m, 3H), 3.25-3.36 (m, 10H), 3.38-3.49 (m, 19H), 3.87-3.89 (m, 4H), 3.98-4.00 (m, 2H), 4.12-4.99 (m, 8H), 5.05 (s, 2H), 5.39 (s, 2H), 5.97 (t, 1H), 7.18-7.24 (m, 1H), 7.29-7.37 (m, 2H), 7.57-7.58 (m, 1H), 7.78-8.07 (m, 5H), 8.22-8.46 (m, 1H), 8.58 (s, 1H), 10.02 (s, 1H)

[1142] LC-MS *m/z* : 1653.4 $[M^+]^+$

[1143]

[1144] **제조예 5-26: 화합물 (VII-30)의 제조**

[1145]



(VII-30)

[1146] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-26에서 수득한 화합물 (VI-30) (0.48 g, 0.31 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물 (0.44 g, 86%)을 미백색 고체로 얻었다.

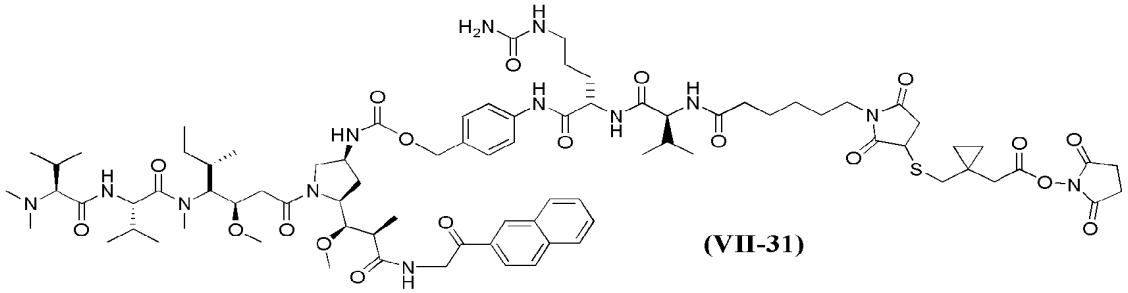
[1147] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.44-0.54 (m, 1 H), 0.55-0.75 (m, 10 H), 0.75-0.96 (m, 17 H), 0.96-1.04 (m, 1 H), 1.04-1.25 (m, 6 H), 1.27 1.51 (m, 6 H), 1.51 1.82 (m, 3 H), 1.82 2.02 (m, 3 H), 2.02-2.21 (m, 6 H), 2.21-2.36 (m, 3 H), 2.52-2.64 (m, 1 H), 2.65-2.75 (m, 4 H), 2.75-2.80 (m, 5 H), 2.80-2.84 (m, 2 H), 2.84-2.93 (m, 4 H), 2.93-3.06 (m, 4 H), 3.06-3.22 (m, 4 H), 3.25-3.28 (m, 3 H), 3.38-3.46 (m, 2 H), 3.48-3.64 (m, 1 H), 3.71-3.86 (m, 7 H), 3.86-4.04 (m, 4 H), 4.04-4.21 (m, 2 H), 4.21-4.31 (m, 1 H), 4.31-4.44 (m, 2 H), 4.44-4.56 (m, 1 H), 4.56-4.71 (m, 2 H), 4.71-4.87 (m, 1 H), 4.93 (s, 2 H), 5.38 (s, 2 H), 5.87-6.01 (t, 1 H), 6.95-7.09 (d, 1 H), 7.18-7.33 (m, 2 H), 7.37-7.54 (m, 1 H), 7.54-7.68 (m, 3 H), 7.71-7.85 (d, 1 H), 8.02-8.12 (d, 1 H), 9.96 (brs, 1 H)

[1148] LC-MS *m/z* : 1633 $[M^+]^+$

[1149]

[1150] **제조예 5-27: 화합물 (VII-31)의 제조**

[1151]



(VII-31)

[1152] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-27에서 수득한 화합물

(VI-31) (0.18 g, 0.12 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물 (0.13 g, 68%)을 미백색 고체로 얻었다.

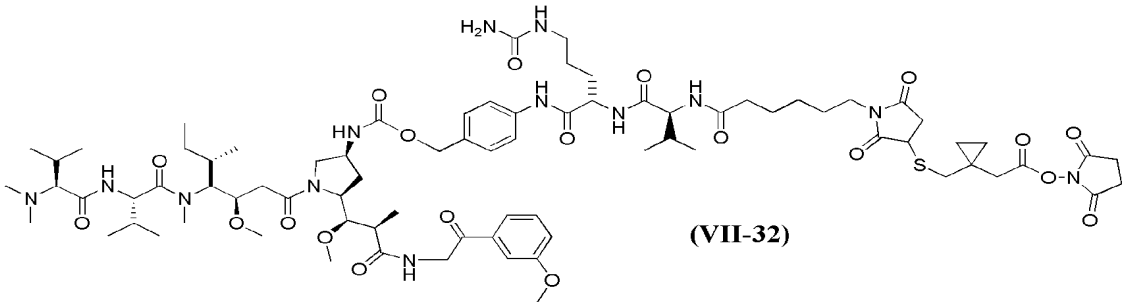
[1153] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.38-0.59 (m, 8 H), 0.60-0.79 (m, 8 H), 0.79-0.98 (m, 16 H), 1.05-1.36 (m, 7 H), 1.36-1.52 (m, 6 H), 1.54-1.86 (m, 5 H), 1.86-2.02 (m, 3 H), 2.04-2.28 (m, 10 H), 2.28-2.44 (m, 3 H), 2.59-2.68 (m, 1 H), 2.69-2.86 (m, 6 H), 2.86-3.09 (m, 8 H), 3.10-3.25 (m, 6 H), 3.25-3.43 (m, 3 H), 3.71-3.86 (m, 1 H), 3.88-4.07 (m, 2 H), 4.07-4.26 (m, 2 H), 4.31-4.43 (m, 1 H), 4.48-4.74 (m, 2 H), 4.74-4.90 (m, 1 H), 4.97 (s, 2 H), 5.41 (m, 2 H), 5.86-6.13 (t, 1 H), 7.16-7.43 (m, 2 H), 7.43-7.55 (m, 1 H), 7.56-7.75 (m, 2 H), 7.75-7.92 (m, 1 H), 7.92-8.21 (m, 4 H), 8.21-8.44 (m, 1 H), 8.44-8.66 (m, 1 H), 8.72 (s, 1 H), 9.38-9.82 (brs, 1 H), 10.01 (brs, 1 H)

[1154] LC-MS *m/z* : 1622 $[M^+]^+$

[1155]

[1156] **제조예 5-28: 화합물 (VII-32)의 제조**

[1157]



(VII-32)

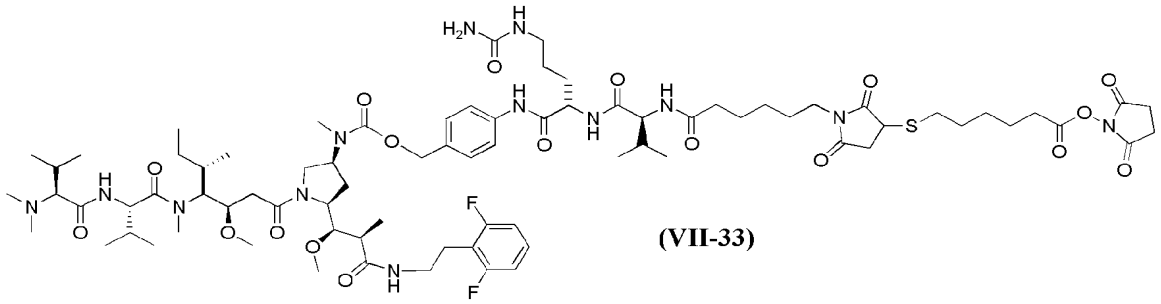
[1158] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-28에서 수득한 화합물 (VI-32) (0.21 g, 0.14 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물 (0.19 g, 88%)을 미백색 고체로 얻었다.

[1159] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43-0.59 (m, 2 H), 0.59-0.78 (m, 8 H), 0.79-1.00 (m, 16 H), 1.00-1.08 (m, 3 H), 1.08-1.29 (m, 5 H), 1.29-1.64 (m, 9 H), 1.64-1.73 (m, 3 H), 1.74-2.04 (m, 4 H), 2.04-2.44 (m, 10 H), 2.56-2.64 (m, 2 H), 2.65-2.92 (m, 12 H), 2.92-3.08 (m, 6 H), 3.08-3.23 (m, 5 H), 3.23-3.32 (m, 3 H), 3.42-3.54 (m, 1 H), 3.55-3.64 (m, 1 H), 3.68-3.88 (m, 3 H), 3.88-4.06 (m, 2 H), 4.06-4.26 (m, 2 H), 4.26-4.46 (m, 2 H), 4.46-4.71 (m, 2 H), 4.74-4.88 (m, 1 H), 4.96 (s, 2 H), 5.41 (s, 2 H), 5.90-6.08 (t, 1 H), 6.74-7.04 (m, 1 H), 7.10-7.38 (m, 3 H), 7.38-7.68 (m, 4 H), 7.72-7.88 (d, 1 H), 8.01-8.18 (d, 1 H), 8.18-8.35 (m, 1 H), 10.00 (brs, 1 H)

[1160] LC-MS *m/z* : 1602 $[M^+]^+$

[1161]

[1162] **제조예 5-29: 화합물 (VII-33)의 제조**

[1163] 
(VII-33)

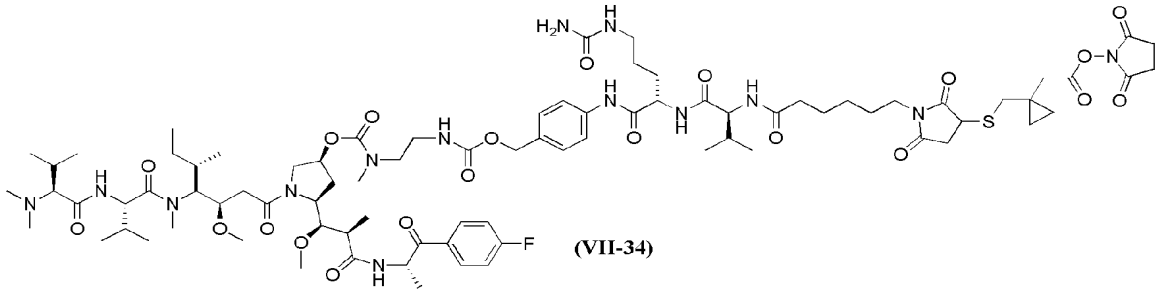
[1164] 제조예 4-19에서 수득한 화합물 (VI-23) 대신에 제조예 4-29에서 수득한 화합물 (VI-33) (0.26 g, 0.17 mmol)을 사용하여 제조예 5-19와 동일한 방법으로 표제화합물(0.14 g, 52%)을 미백색 고체로 얻었다.

[1165] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.53-0.94 (m, 28 H), 0.95-1.28 (m, 9 H), 1.28-1.74 (m, 18 H), 1.76-2.25 (m, 13 H), 2.27-2.43 (m, 1 H), 2.54-2.84 (m, 16 H), 2.84-3.03 (m, 5 H), 3.03-3.21 (m, 7 H), 3.60-3.82 (m, 2 H), 3.82-4.00 (m, 3 H), 4.05-4.24 (m, 1 H), 4.29-4.45 (m, 1 H), 4.45-4.78 (m, 2 H), 5.01 (s, 2 H), 5.39 (s, 2 H), 5.90-6.09 (t, 1 H), 6.97 (brs, 2 H), 7.09-7.43 (m, 2 H), 7.43-7.70 (m, 2 H), 7.70-7.87 (m, 1 H), 8.00-7.33 (m, 2 H), 10.01 (brs, 1 H)

[1166] LC-MS *m/z* : 1610 $[M^+]^+$

[1167]

[1168] **제조예 5-30: 화합물 (VII-34)의 제조**

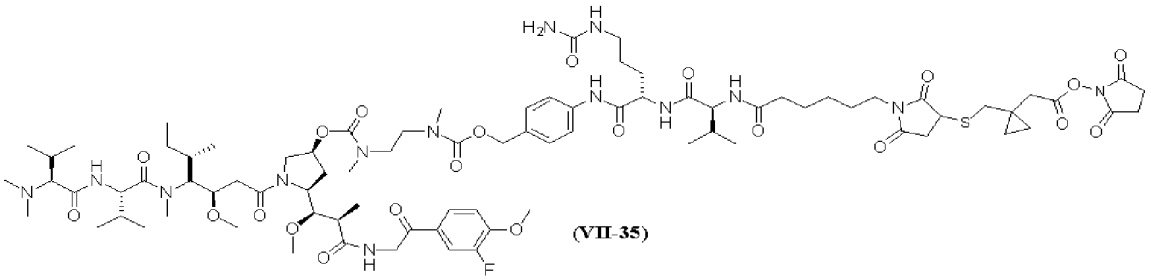
[1169] 
(VII-34)

[1170] 제조예 4-30에서 수득한 화합물 (VI-34) (25 mg, 0.016 mmol)를 아르곤 기류 하에서 무수 디메틸포름아미드 3 mL에 녹인 후, N-히드록시숙신이미드 (7.2 mg, 0.062 mmol)를 가하였다. 반응혼합물을 0 ℃로 냉각한 후 EDC·HCl (11.9 mg, 0.062 mmol)을 가하고 상온에서 15시간 동안 교반하였다. 반응 완결 후 고진공 하에 감압 농축하여 얻어진 잔사를 실리카겔 컬럼크로마토그래피로 정제하여 표제화합물(20 mg, 76%)을 얻었다.

[1171] LC-MS *m/z* : 1706.9 $[M+H]^+$, 1728.9 $[M+Na]^+$

[1172]

[1173] **제조예 5-31: 화합물 (VII-35)의 제조**

[1174] 
(VII-35)

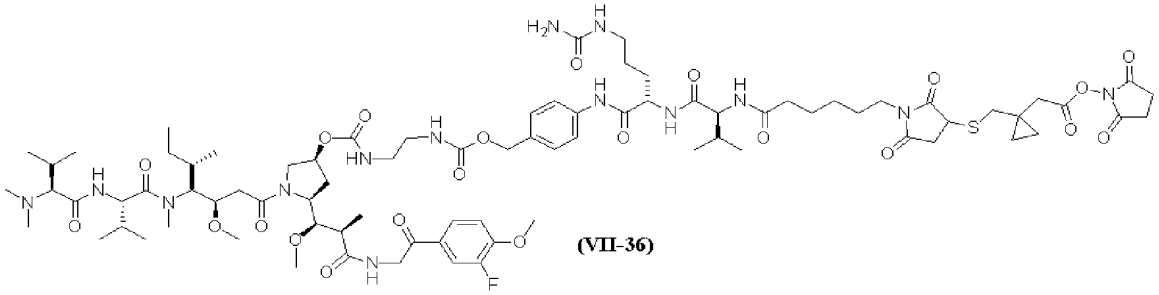
[1175] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-31에서 수득한 화합물 (VI-35) (0.074 g, 0.0452 mmol)를 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.049 g, 62.4%)를 얻었다.

[1176] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.63-0.76 (m, 12H), 0.81-0.86 (m, 18H), 1.02-1.04 (d, 4H), 1.11-1.14 (m, 4H), 1.18-1.22 (m, 2H), 1.42-1.49 (m, 6H), 1.94-1.97 (m, 3H), 2.18-2.19 (m, 6H), 2.73 2.89 (m, 13H), 2.93-2.99 (m, 5H), 3.14-3.21 (m, 5H), 3.31-3.39 (m, 17H), 3.91-4.03 (m, 5H), 4.14-4.85 (m, 8H), 4.98 (s, 2H), 5.42 (s, 2H), 5.96-5.98 (t, 1H), 7.27-7.30 (m, 3H), 7.58-7.59 (d, 2H), 7.77-7.87 (m, 2H), 7.94-8.03 (m, 2H), 8.08-8.11 (d, 1H), 8.46 (brs, 1H), 10.01 (s, 1H)

[1177] LC-MS *m/z* : 1735.4 $[M+H]^+$, 1758.4 $[M+Na]^+$

[1178]

[1179] **제조예 5-32: 화합물 (VII-36)의 제조**

[1180] 
(VII-36)

[1181] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-32에서 수득한 화합물 (VI-36) (0.11 g, 0.0664mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.079 g, 69.6%)를 얻었다.

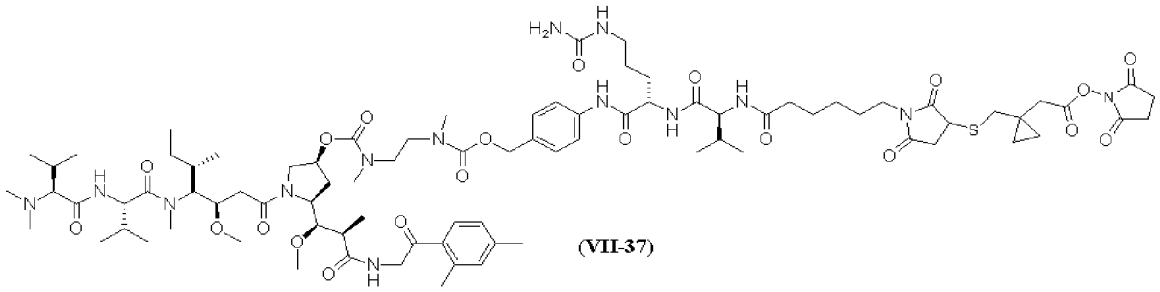
[1182] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.63-0.76 (m, 7H), 0.82 -0.92 (m, 12H), 1.02-1.04 (d, 1H), 1.09-1.24 (m, 7H), 1.42-1.49 (m, 8H), 1.91 -1.99 (m, 4H), 2.11-2.19 (m, 8H), 2.81-2.83 (m, 10H), 2.99-3.14 (m, 6H), 3.17 -.21 (m, 5H), 3.32-3.35 (m, 20H), 3.92-3.93 (m, 4H), 3.97-4.04 (m, 2H), 4.13 -4.85 (m, 8H), 4.94 (s, 2H), 5.43 (s, 2H), 6.00-6.03 (t, 1H), 7.27-7.32 (m, 5H), 7.58-7.61 (d, 2H), 7.77-7.96 (m, 3H), 8.10-8.12 (m, 1H), 8.47 (brs, 1H), 10.02 (s, 1H)

[1183] LC-MS *m/z* : 1708.4 $[M+H]^+$, 1730.4 $[M+Na]^+$

[1184]

[1185] **제조예 5-33: 화합물 (VII-37)의 제조**

[1186]



(VII-37)

[1187] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-33에서 수득한 화합물 (VI-37) (0.16 g, 0.096mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.12 g, 71%)를 얻었다.

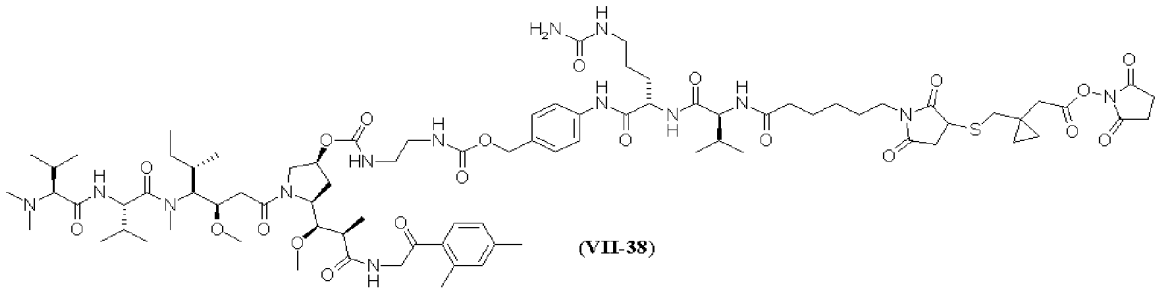
[1188] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.70-0.75 (m, 8H), 0.82 -0.87 (m, 14H), 1.03-1.05 (m, 4H), 1.08-1.14 (m, 1H), 1.18-1.23 (m, 4H), 1.44 -1.49 (m, 7H), 2.08-2.20 (m, 10H), 2.28-2.37 (m, 8H), 2.77-2.89 (m, 13H), 2.93-3.00 (m, 6H), 3.14-3.20 (m, 5H), 3.28 (s, 3H), 3.32-3.34 (m, 18H), 3.88 -4.04 (m, 4H), 4.08-4.87 (m, 8H), 4.97 (s, 2H), 5.42 (s, 2H), 5.99-6.00 (t, 1H), 7.05-7.15 (m, 2H), 7.28-7.30 (m, 2H), 7.59-7.69 (m, 2H), 7.80-7.83 (d, 1H), 7.97-8.05 (m, 1H), 8.10-8.11 (m, 1H), 8.23-8.28 (m, 1H), 8.49 (brs, 1H), 10.02 (s, 1H)

[1189] LC-MS *m/z* : 1716.5 $[M+H]^+$, 1740.5 $[M+Na]^+$

[1190]

[1191] **제조예 5-34: 화합물 (VII-38)의 제조**

[1192]



(VII-38)

[1193] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-34에서 수득한 화합물 (VI-38) (0.15 g, 0.093 mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.1 g, 67%)를 얻었다.

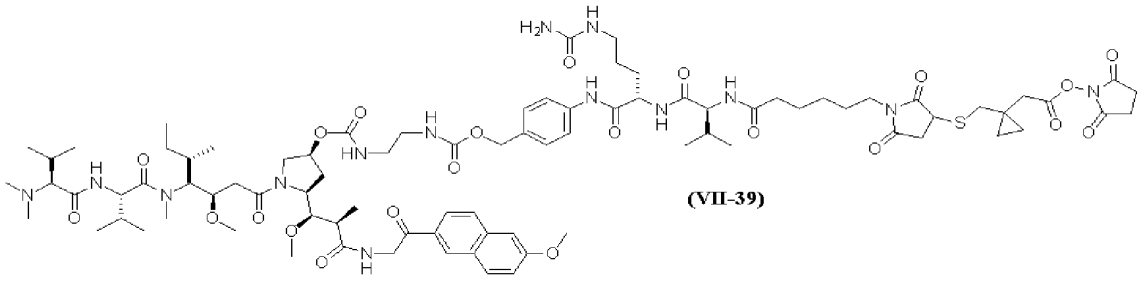
[1194] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.63-0.66 (m, 3H), 0.69 -0.76 (m, 8H), 0.82-0.92 (m, 14H), 1.03-1.05 (m, 2H), 1.11-1.12 (m, 1H), 1.18 -1.24 (m, 3H), 1.44-1.49 (m, 7H), 2.13-2.20 (m, 9H), 2.28-2.37 (m, 7H), 2.73 -2.89 (m, 7H), 2.93-3.03 (m, 9H), 3.15-3.19 (m, 5H), 3.28 (s, 3H), 3.31-3.35 (m, 15H), 3.85-3.87 (m, 1H), 3.95-4.04 (m, 3H), 4.16-4.88 (m, 8H), 4.94 (s, 2H), 5.42 (s, 2H), 5.99-6.02 (t, 1H), 7.06-7.15 (m, 2H), 7.27-7.29 (m, 4H), 7.58-7.63 (m, 3H), 7.81-7.83 (d, 1H), 7.87-8.04 (m, 1H), 8.10-8.11 (d, 1H), 8.30-8.50 (m, 1H), 10.02 (s, 1H)

[1195] LC-MS *m/z* : 1687.6 $[M+H]^+$, 1709.5 $[M+Na]^+$

[1196]

[1197] **제조예 5-35: 화합물 (VII-39)의 제조**

[1198]



(VII-39)

[1199] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-35에서 수득한 화합물 (VI-39) (0.12 g, 0.073mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물 (0.058 g, 46%)를 얻었다.

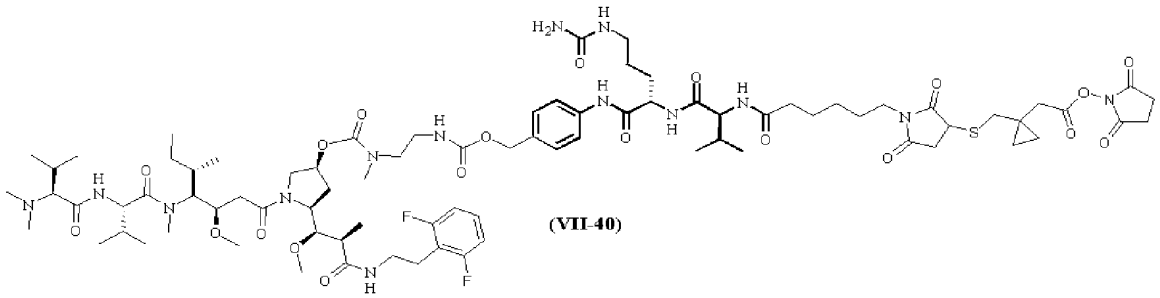
[1200] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.48-0.53 (m, 1H), 0.58-0.59 (m, 1H), 0.64 -0.76 (m, 8H), 0.82-0.92 (m, 15H), 1.03-1.04 (d, 2H), 1.11-1.22 (m, 6H), 1.42 -1.49 (m, 6H), 1.86-1.97 (m, 3H), 2.11-2.20 (m, 9H), 2.73-2.95 (m, 10H), 2.98 -3.13 (m, 6H), 3.14-3.21 (m, 5H), 3.33-3.37 (m, 2H), 3.90-3.92 (m, 4H), 3.98 -4.04 (m, 2H), 4.14-4.80 (m, 8H), 4.94 (s, 2H), 5.42 (s, 2H), 6.00-6.01 (t, 1H), 7.22-7.29 (m, 4H), 7.38-7.42 (d, 1H), 7.59-7.61 (d, 2H), 7.81-7.83 (d, 1H), 7.89-7.96 (m, 2H), 8.00-8.03 (d, 1H), 8.09-8.12 (d, 1H), 8.28-8.51 (m, 1H), 8.64 (s, 1H), 10.02 (s, 1H),

[1201] LC-MS *m/z* : 1740.4 $[M+H]^+$, 1762.3 $[M+Na]^+$

[1202]

[1203] **제조예 5-36: 화합물 (VII-40)의 제조**

[1204]



(VII-40)

[1205] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-36에서 수득한 화합물 (VI-40) (0.1 g, 0.063mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.072 g, 68%)를 얻었다.

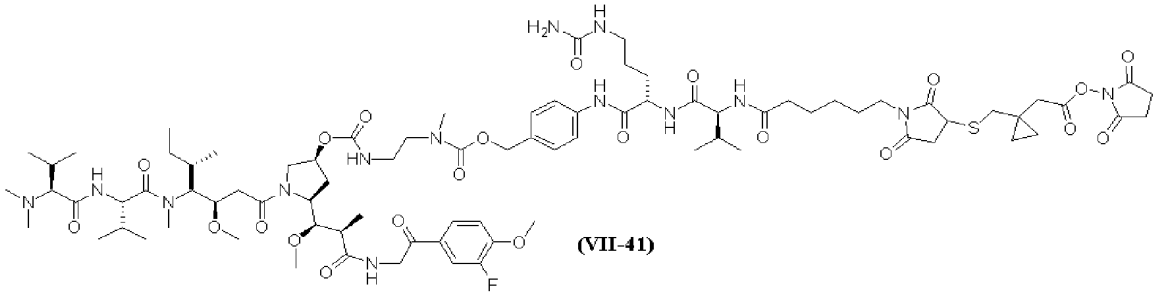
[1206] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.62-0.69 (m, 2H), 0.70 -0.77 (m, 7H), 0.82-0.89 (m, 18H), 1.02-0.07 (m, 3H), 1.18-1.29 (m, 3H), 1.44 -1.47 (m, 6H), 2.19-2.21 (m, 10H), 2.61-2.64 (m, 2H), 2.74-3.00 (m, 10H), 3.17-3.18 (m, 7H), 3.25-3.33 (m, 25H), 3.90-4.01 (m, 5H), 4.16-4.84 (m, 8H), 4.95 (s, 2H), 5.40 (s, 2H), 6.01-6.02 (t, 1H), 7.01-7.05 (m, 2H), 7.26-7.29 (m, 4H), 7.58-7.60 (d, 2H), 7.79-7.82 (m, 1H), 7.94-7.99 (m, 2H), 8.08- 8.10(m, 1H), 10.00 (s, 1H)

[1207] LC-MS *m/z* : 1697.2 $[M+H]^+$, 1719.1 $[M+Na]^+$

[1208]

[1209] **제조예 5-37: 화합물 (VII-41)의 제조**

[1210]



[1211] 제조예 4-30에서 수득한 화합물 (VI-34) 대신에 제조예 4-37에서 수득한 화합물 (VI-41) (0.062 g, 0.038mmol)을 사용하여 제조예 5-30과 동일한 방법으로 표제화합물(0.037 g, 56%)를 얻었다.

[1212] ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.51-0.53 (m, 1H), 0.63-0.77 (m, 13H), 0.81-0.91 (m, 17H), 1.03-1.04 (d, 2H), 1.09-1.22 (m, 6H), 1.42-1.49 (m, 6H), 1.95-1.97 (m, 3H), 2.11-2.19 (m, 7H), 2.77-2.89 (m, 13H), 3.13-3.17 (m, 7H), 3.32-3.36 (m, 18H), 3.92-3.93 (m, 4H), 4.00-4.04 (m, 2H), 4.14-4.85 (m, 8H), 4.99 (s, 2H), 5.42 (s, 2H), 5.88-6.00 (t, 1H), 7.28-7.41 (m, 4H), 7.58-7.65 (d, 2H), 7.77-8.06 (m, 4H), 8.10-8.11 (d, 1H), 8.21-8.45 (m, 1H), 10.00 (s, 1H)

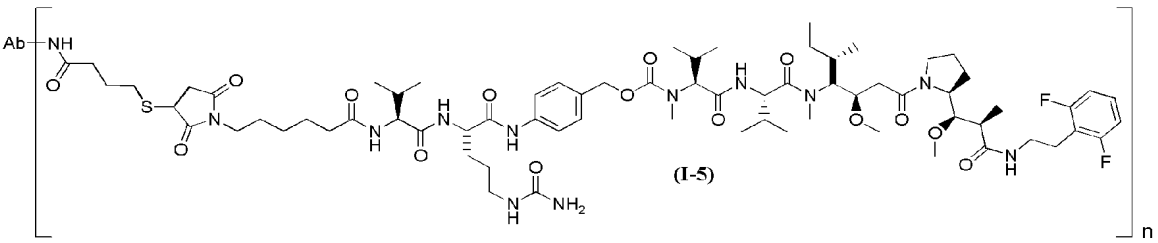
[1213] LC-MS *m/z* : 1722.5 $[M+H]^+$, 1744.3 $[M+Na]^+$

[1214]

[1215] **실시예: 화학식 I-1, I-2, I-3 또는 I-4의 항체-링커-약물 결합체의 제조**

[1216] **실시예 1: 결합체 (I-5)의 제조**

[1217]

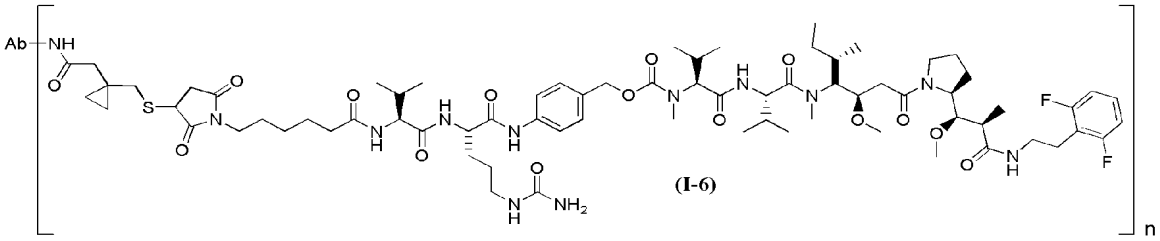


[1218] pH 7.5의 PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl 포함)로 교환 처리된 트라스투주맙(허셉틴) 용액 5 mL (4.0 g/mL, 27 μM)에 추가적으로 pH 7.5의 PBS 2.5 ml와 pH 8.5의 0.05 M 소듐보레이트 버퍼 7.5 ml를 가하고, 20% DMSO 3.75 mL에 용해된 제조예 5-1에서 수득한 화합물 (VII-5) (2 mg, 0.001 mmol, 10 당량)를 가한 후, 25 ℃에서 4시간 동안 교반하였다. 반응이 종결되면 반응혼합물을 0.45 ㎛ 여과기로 여과하고, 여액을 원심여과기(centrifugal filter, Amicon Ultra-15 30K)에 담아 유기용매와 미반응 물질이 제거될 때까지 pH 7.5의 PBS를 다시 가하여 2 - 3회 반복 농축하였다. 농축액을 pH 7.5의 PBS 중에 평형화시킨 Sephadex G-25 수지 충진 컬럼 (30 x 300 mm)을 사용하여 MPLC (280 nm, UV range 0.08, flow rate 10 mL/min)로 분리 정제하여 표제화합물 13.5 mg

(1.28 mg/mL, 총 부피 10.5 mL, 68%)를 얻었다. 이때 얻어진 화합물의 농도는 UV 분광계로 세가지 파장(280 nm, 320 nm, 350 nm) 각각에서의 흡광도를 측정하여 결정하였다.

[1219]

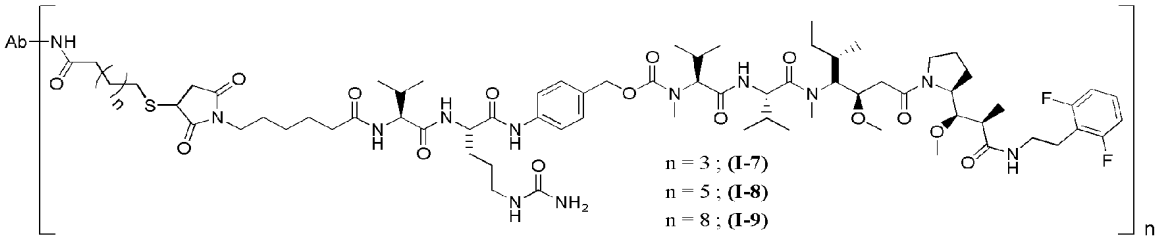
[1220] **실시예 2: 결합체 (I-6)의 제조**

[1221] 

[1222] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-2에서 수득한 화합물 (VII-6) (4.2 mg, 0.003 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 12.1 mg (1.1 mg/mL, 총 부피 11 mL, 45%)를 얻었다.

[1223]

[1224] **실시예 3: 결합체 (I-7)의 제조**

[1225] 

[1226] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-3에서 수득한 화합물 (VII-7) (3.12 mg, 0.002 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 13.7 mg (1.71 mg/mL, 총 부피 8 mL, 68%)를 얻었다.

[1227]

[1228] **실시예 4: 결합체 (I-8)의 제조**

[1229] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-4에서 수득한 화합물 (VII-8) (3.20 mg, 0.002 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 13.9 mg (1.54 mg/mL, 총 부피 9 mL, 68%)를 얻었다.

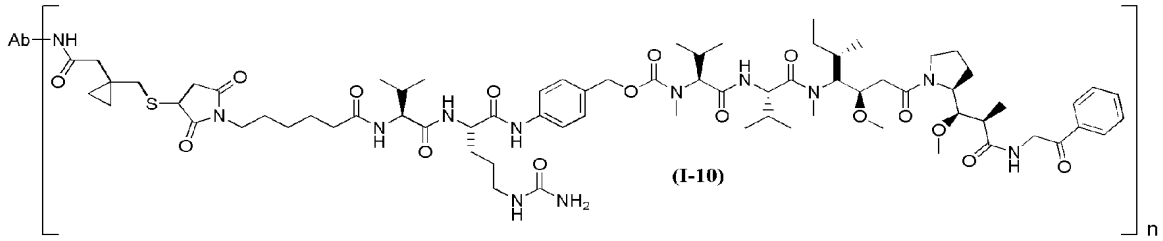
[1230]

[1231] **실시예 5: 결합체 (I-9)의 제조**

[1232] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-5에서 수득한 화합물 (VII-9) (3.27 mg, 0.002 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 15.3 mg (1.53 mg/mL, 총 부피 10 mL, 77%)를 얻었다.

[1233]

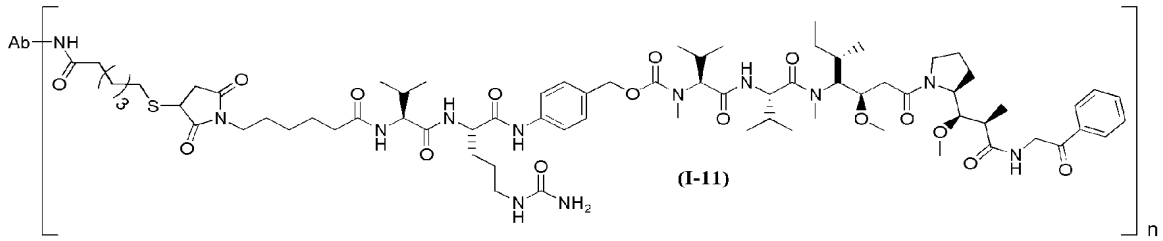
[1234] **실시예 6: 결합체 (I-10)의 제조**

[1235] 

[1236] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-6에서 수득한 화합물 (VII-10) (4.17 mg, 0.003 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 14.2 mg (1.29 mg/mL, 총 부피 11 mL, 53%)를 얻었다.

[1237]

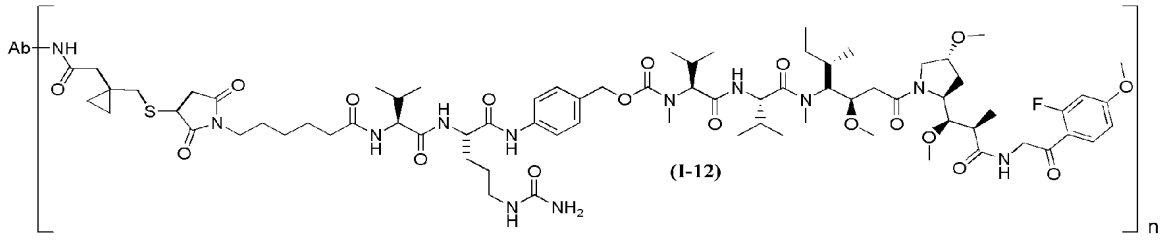
[1238] **실시예 7: 결합체 (I-11)의 제조**

[1239] 

[1240] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-7에서 수득한 화합물 (VII-11) (3.08 mg, 0.002 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 9.5 mg (1.19 mg/mL, 총 부피 8 mL, 48%)를 얻었다.

[1241]

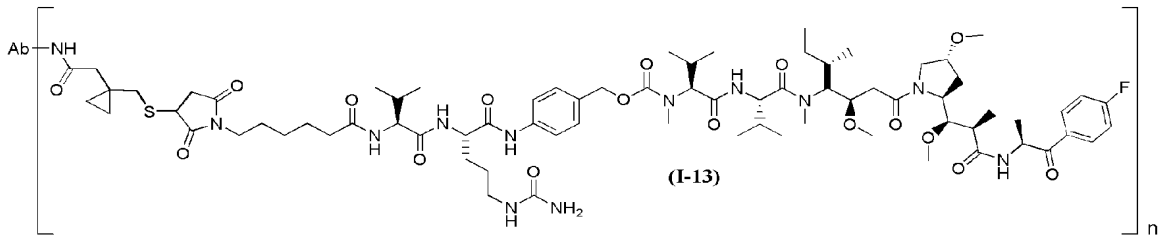
[1242] **실시예 8: 결합체 (I-12)의 제조**

[1243] 

[1244] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-8에서 수득한 화합물 (VII-12) (5.4 mg, 0.003 mmol, 10 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 50 mg (2.5 mg/mL, 총 부피 20 mL, 100%)를 얻었다.

[1245]

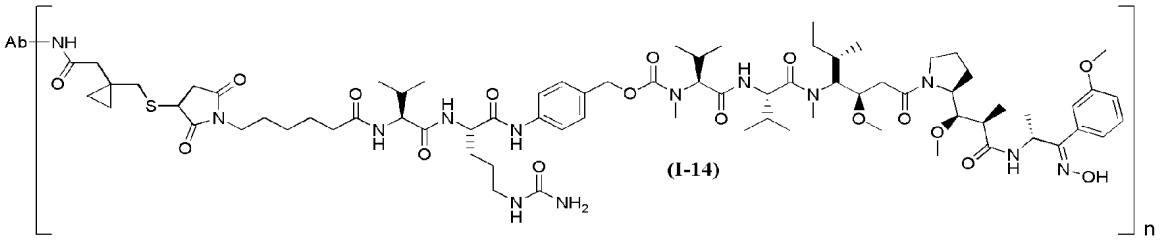
[1246] **실시예 9: 결합체 (I-13)의 제조**

[1247] 

[1248] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-9에서 수득한 화합물 (VII-13) (5.7 mg, 0.004 mmol, 20 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 15.2 mg (1.01 mg/mL, 총 부피 15 mL, 56%)를 얻었다.

[1249]

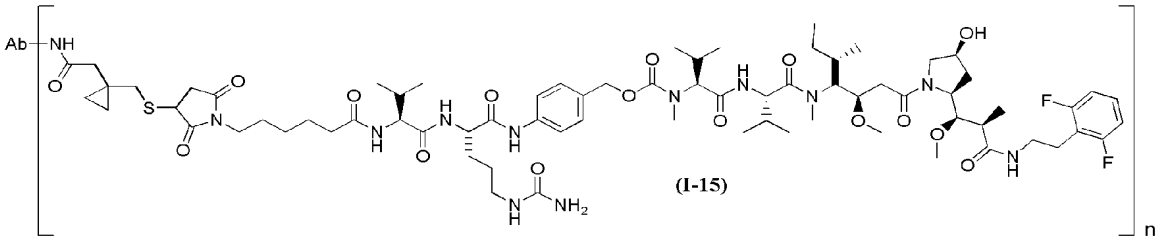
[1250] **실시예 10: 결합체 (I-14)의 제조**

[1251] 

[1252] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-10에서 수득한 화합물 (VII-14) (4.3 mg, 0.003 mmol, 15 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 19.4 mg (1.55 mg/mL, 총 부피 12.5 mL, 72%)를 얻었다.

[1253]

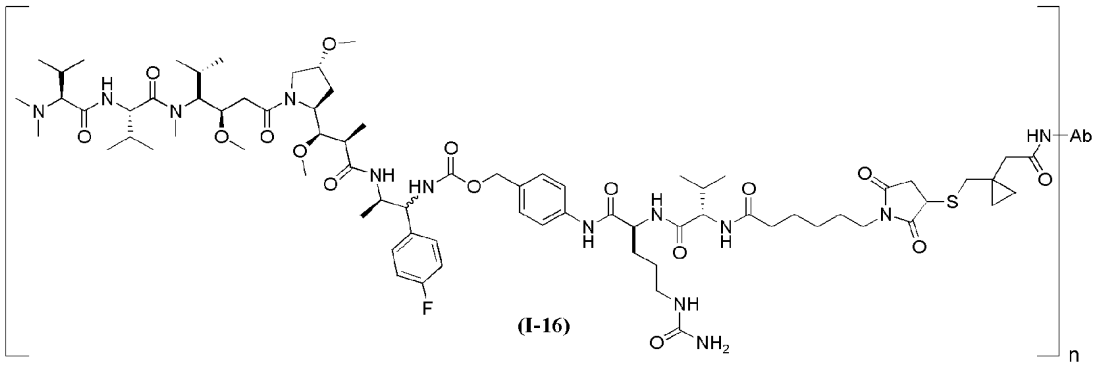
[1254] **실시예 11: 결합체 (I-15)의 제조**

[1255] 

[1256] 제조예 5-1에서 수득한 화합물 (VII-5) 대신에 제조예 5-11에서 수득한 화합물 (VII-15) (2.3 mg, 0.0015 mmol, 10 당량)을 사용하여 실시예 1과 동일한 방법으로 표제화합물 20.1 mg (1.6 mg/mL, 총 부피 12.5 mL, 92%)를 얻었다.

[1257]

[1258] **실시예 12: 결합체 (I-16)의 제조**

[1259] 

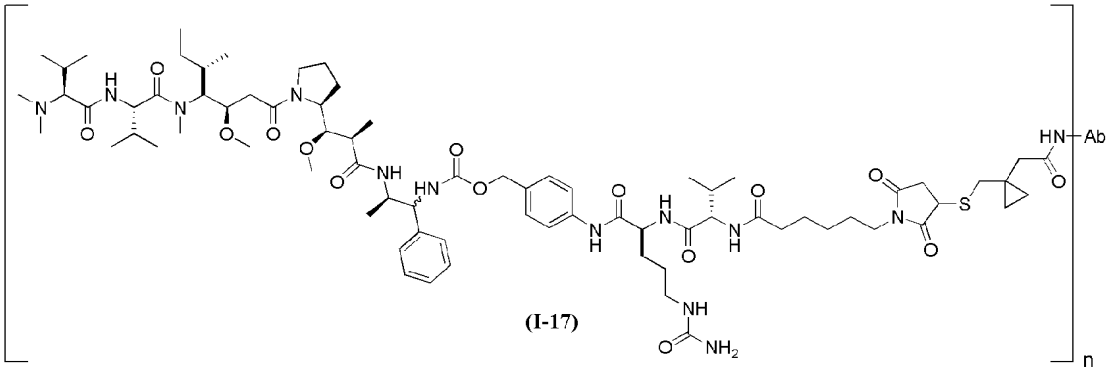
[1260] pH 7.5의 PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl 포함)로 교환 처리된 트라스투주맙 용액 5 mL (5.4 g/mL, 36 μM)에 추가적으로 pH 7.5의 PBS 2.5 ml와 pH 8.5의 0.05 M 소듐보레이트 버퍼 7.5 mL 를 가하고, 20% DMSO 3.75

mL에 용해된 제조예 5-12에서 수득한 화합물 (VII-16) (4.4 mg, 0.003 mmol, 15 당량)을 가한 후, 25 ℃에서 4시간 동안 교반하였다. 반응이 종결되면 반응혼합물을 0.45 ㎛ 여과기로 여과하고, 여액을 원심여과기(centrifugal filter, Amicon Ultra-15 30K)에 담아 유기용매와 미반응 물질이 제거될 때까지 pH 7.5의 PBS를 다시 가하여 2 3회 반복 농축하였다. 농축액을 pH 7.5의 PBS 중에 평형화시킨 Sephadex G-25 수지 충진 컬럼 (30 x 300 mm)을 사용하여 MPLC (280 nm, UV range 0.08, flow rate 10 mL/min)로 분리 정제하여 표제화합물 22.5 mg (1.73 mg/mL, 총 부피 13 mL, 83%)를 얻었다. 이때 얻어진 화합물의 농도는 UV 분광계로 세가지 파장(280 nm, 320 nm, 350 nm) 각각에서의 흡광도를 측정하여 결정하였다.

[1261]

[1262] **실시예 13: 결합체 (I-17)의 제조**

[1263]

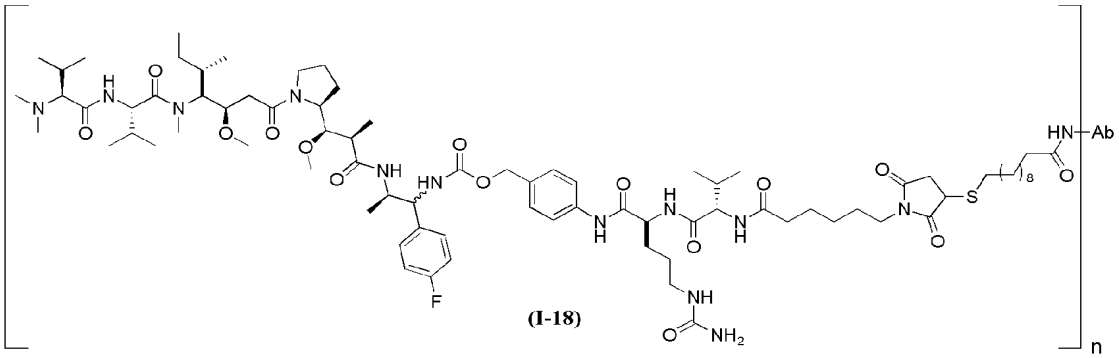


[1264] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-13에서 수득한 화합물 (VII-17) (5.6 mg, 0.004 mmol, 20 당량)을 사용하여 실시예 12와 동일한 방법으로 표제화합물 25.6mg (2.05 mg/mL, 총 부피 12.5 mL, 95%)를 얻었다.

[1265]

[1266] **실시예 14: 결합체 (I-18)의 제조**

[1267]

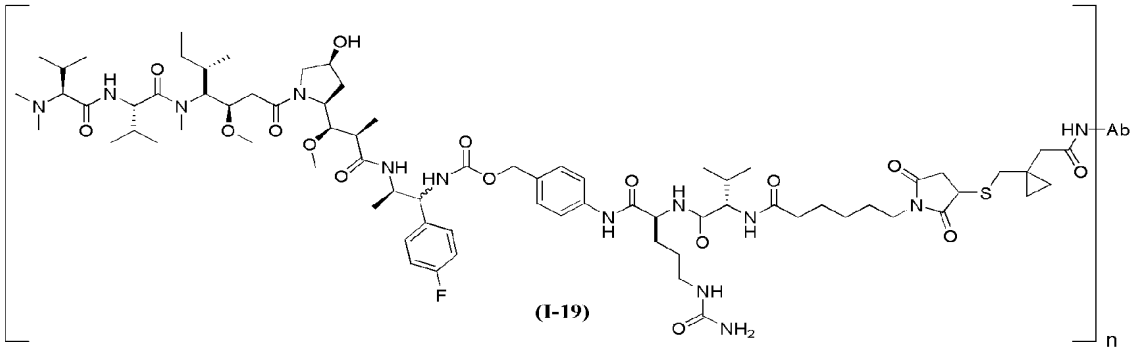


[1268] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-14에서 수득한 화합물 (VII-18) (4.0 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 12와 동일한 방법으로 표제화합물 23.8 mg (1.98 mg/mL, 총 부피 12 mL, 99%)를 얻었다.

[1269]

[1270] **실시예 15: 결합체 (I-19)의 제조**

[1271]

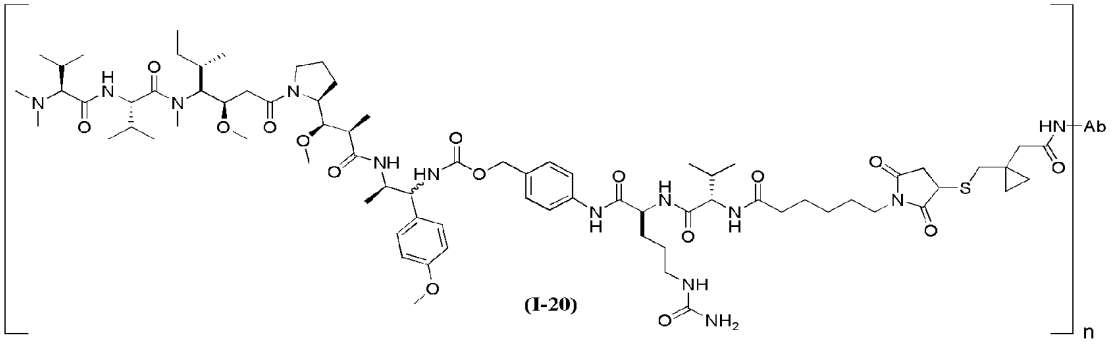


[1272] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-15에서 수득한 화합물 (VII-19) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 12와 동일한 방법으로 표제화합물 20.4 mg (1.86 mg/mL, 총 부피 11 mL, 82%)를 얻었다

[1273]

[1274] **실시예 16: 결합체 (I-20)의 제조**

[1275]

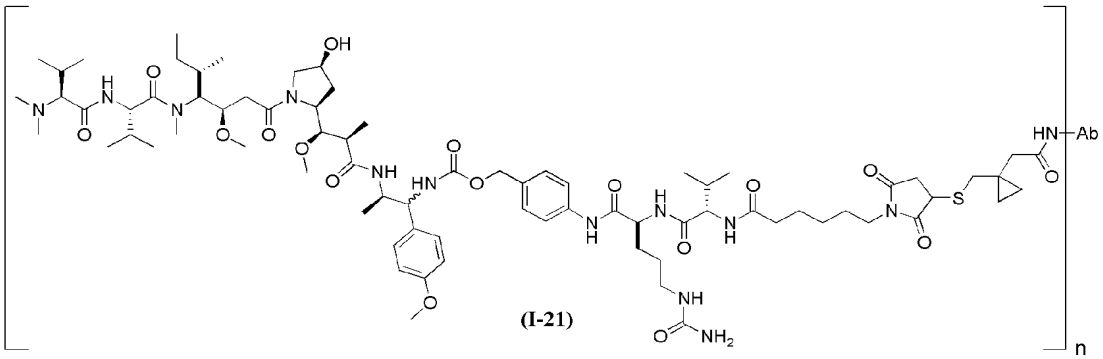


[1276] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-16에서 수득한 화합물 (VII-20) (3.8 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 12와 동일한 방법으로 표제화합물 18.9 mg (1.72 mg/mL, 총 부피 11 mL, 79%)를 얻었다

[1277]

[1278] **실시예 17: 결합체 (I-21)의 제조**

[1279]



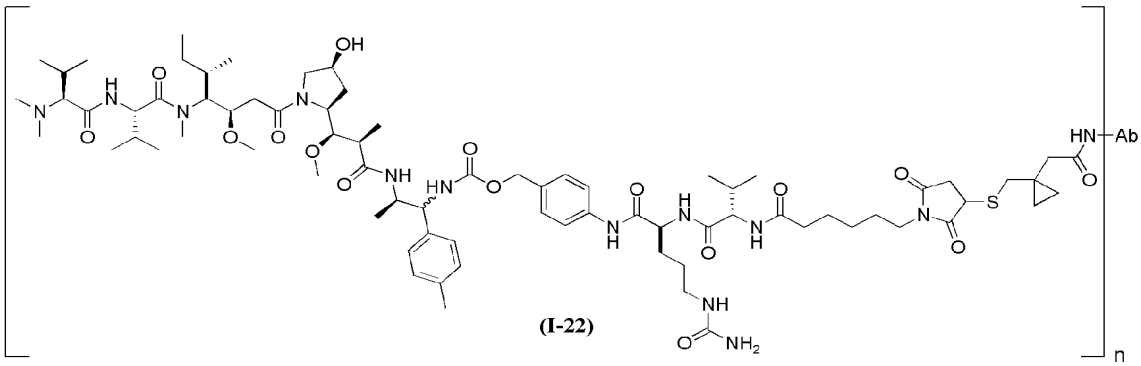
[1280] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-17에서 수득한 화합물 (VII-21) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 12와 동일한

방법으로 표제화합물 19.8 mg (1.8 mg/mL, 총 부피 11 mL, 80%)를 얻었다

[1281]

[1282] **실시예 18: 결합체 (I-22)의 제조**

[1283]



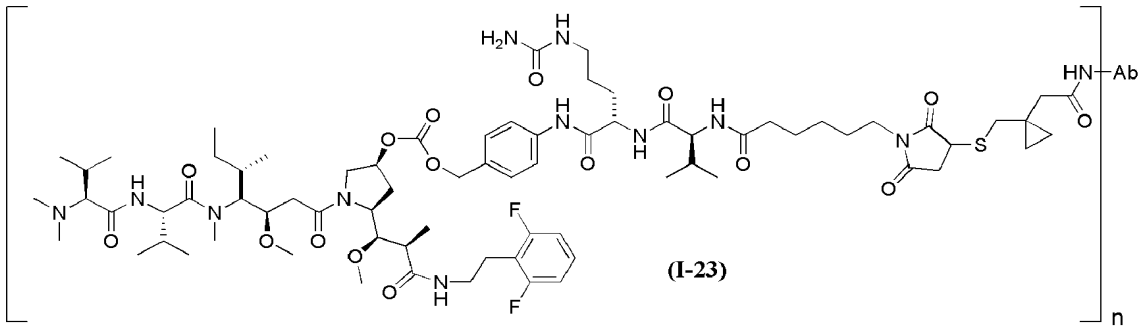
(I-22)

[1284] 제조예 5-12에서 수득한 화합물 (VII-16) 대신에 제조예 5-18에서 수득한 화합물 (VII-22) (3.8 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 12와 동일한 방법으로 표제화합물 13.0 mg (1.18 mg/mL, 총 부피 11 mL, 54%)를 얻었다

[1285]

[1286] **실시예 19: 결합체 (I-23)의 제조**

[1287]

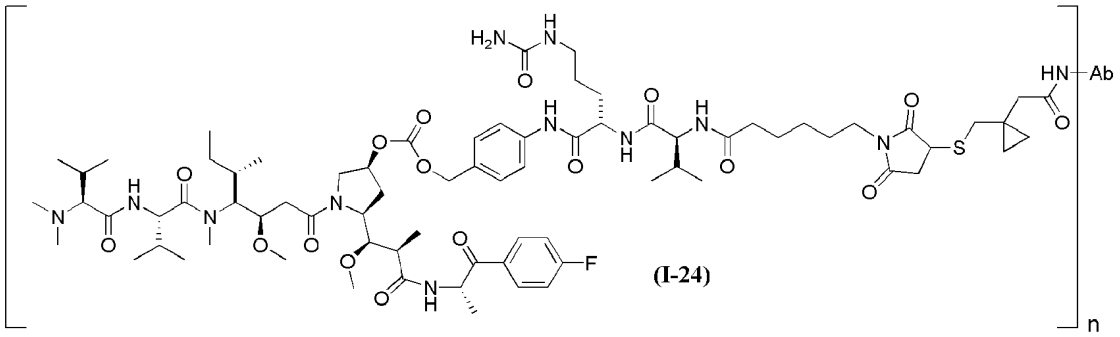


(I-23)

[1288] pH 7.5의 PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl 포함)로 교환 처리된 트라스투주맙(허셉틴) 용액 5 mL (5.4 g/mL, 36 μM)에 추가적으로 pH 7.5의 PBS 2.5 ml와 pH 8.5의 0.05 M 소듐보레이트 버퍼 7.5 mL를 가하고, 20% DMSO 3.75 mL에 용해된 제조예 5-19에서 수득한 화합물 (VII-23) (5.7 mg, 0.0036 mmol, 20 당량)를 가한 후, 25 ℃에서 4시간 동안 교반하였다. 반응이 종결되면 반응혼합물을 0.45 ㎛ 여과기로 여과하고, 여액을 원심여과기(centrifugal filter, Amicon Ultra-15 30K)에 담아 유기용매와 미반응 물질이 제거될 때까지 pH 7.5의 PBS를 다시 가하여 2-3회 반복 농축하였다. 농축액을 pH 7.5의 PBS 중에 평형화시킨 Sephadex G-25 수지 충진 컬럼 (30 x 300 mm)을 사용하여 MPLC (280 nm, UV range 0.08, flow rate 10 mL/min)로 분리 정제하여 표제화합물 16.9 mg (1.21 mg/mL, 총 부피 15 mL, 67%)를 얻었다. 이때 얻어진 화합물의 농도는 UV 분광계로 세가지 파장(280 nm, 320 nm, 350 nm) 각각에서의 흡광도를 측정하여 결정하였다.

[1289]

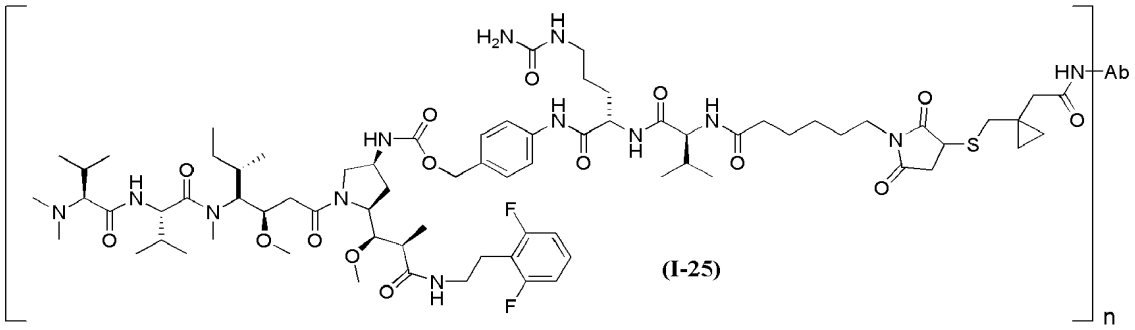
[1290] **실시예 20: 결합체 (I-24)의 제조**

[1291] 

[1292] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-20에서 수득한 화합물 (VII-24) (4.3 mg, 0.0027 mmol, 15 당량)를 사용하여 실시예 19와 동일한 방법으로 표제화합물 19.2 mg (1.54 mg/mL, 총 부피 11.5 mL, 71%)를 얻었다.

[1293]

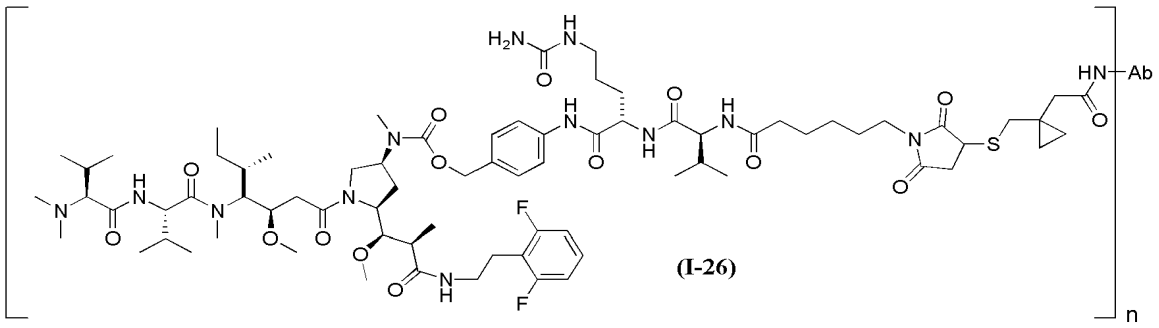
[1294] **실시예 21: 결합체 (I-25)의 제조**

[1295] 

[1296] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-21에서 수득한 화합물 (VII-25) (5.74 mg, 0.0036 mmol, 20 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 14.7 mg (1.23 mg/mL, 총 부피 12 mL, 54%)를 얻었다.

[1297]

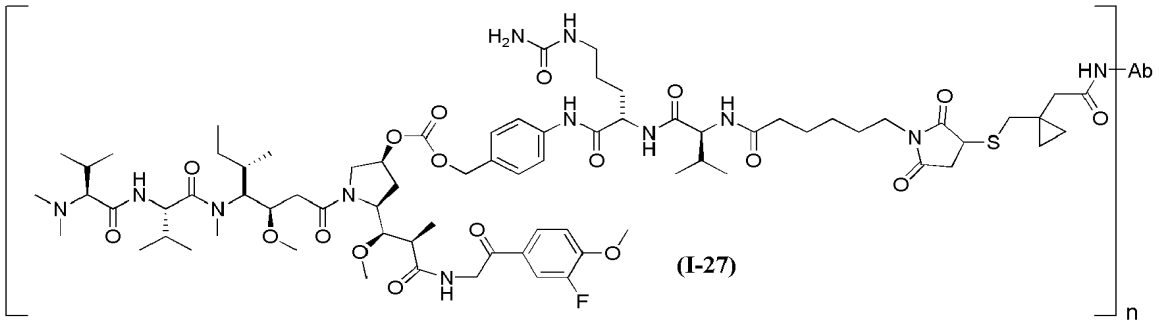
[1298] **실시예 22: 결합체 (I-26)의 제조**

[1299] 

[1300] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-22에서 수득한 화합물 (VII-26) (4.38 mg, 0.0027 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 23.7 mg (1.58 mg/mL, 총 부피 14 mL, 88%)를 얻었다.

[1301]

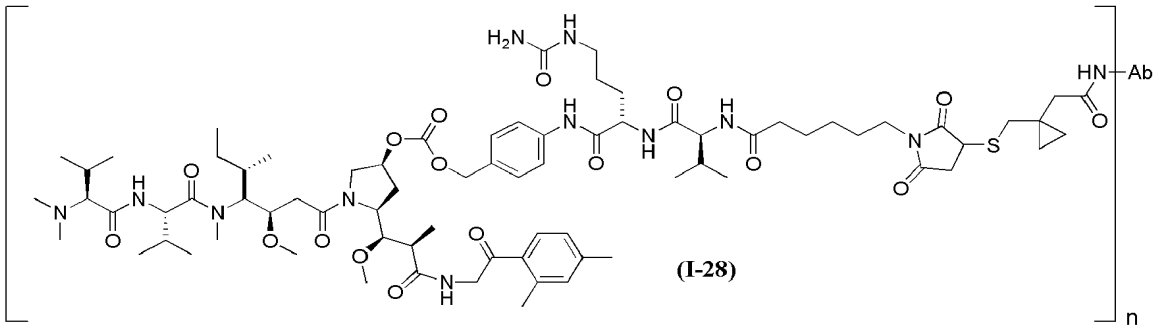
[1302] **실시예 23: 결합체 (I-27)의 제조**

[1303] 

[1304] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-23에서 수득한 화합물 (VII-27) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 21.1 mg (1.15 mg/mL, 총 부피 14 mL, 85%)를 얻었다

[1305]

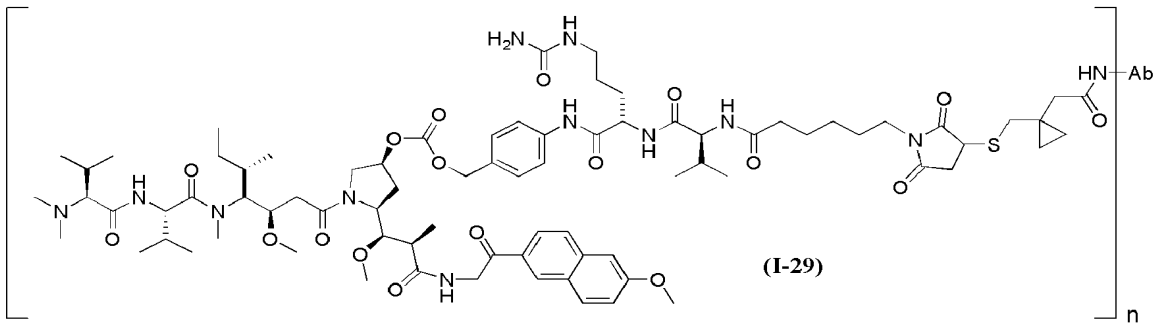
[1306] **실시예 24: 결합체 (I-28)의 제조**

[1307] 

[1308] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-24에서 수득한 화합물 (VII-28) (3.9 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 17.3 mg (1.58 mg/mL, 총 부피 13 mL, 70%)를 얻었다

[1309]

[1310] **실시예 25: 결합체 (I-29)의 제조**

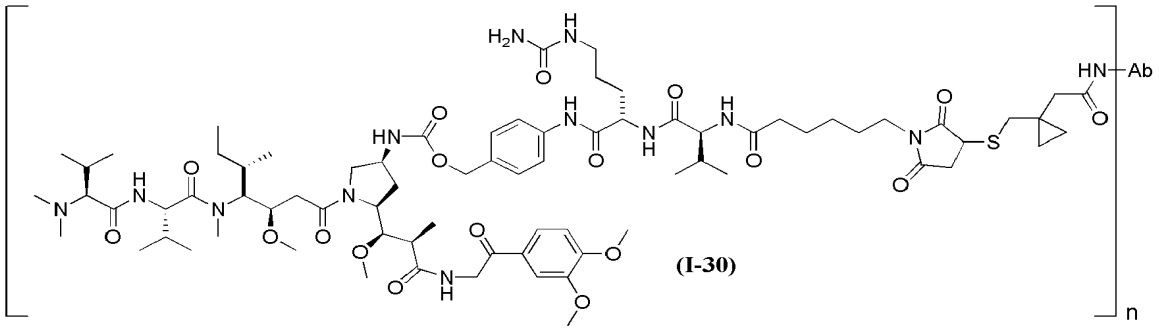
[1311] 

[1312] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-25에서 수득한 화합물 (VII-29) (4.1 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 9.2 mg (0.92 mg/mL, 총 부피 10 mL, 24.8%)를 얻었다

[1313]

[1314] **실시예 26: 결합체 (I-30)의 제조**

[1315]

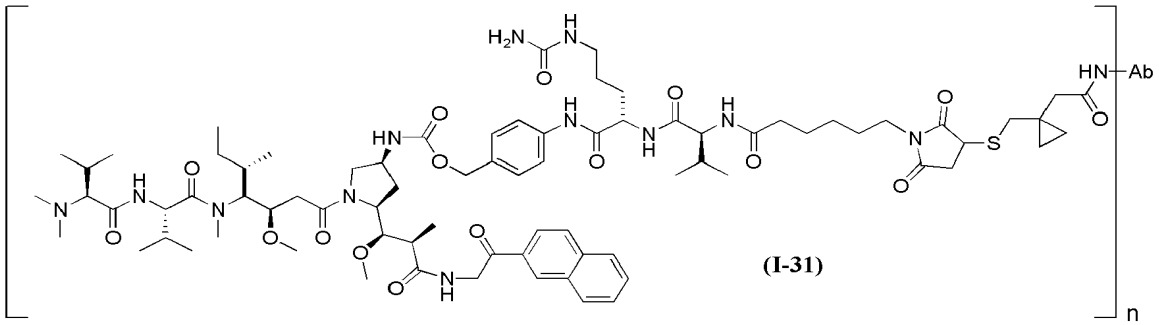


[1316] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-26에서 수득한 화합물 (VII-30) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 16.7 mg (1.19 mg/mL, 총 부피 14 mL, 67%)를 얻었다.

[1317]

[1318] **실시예 27: 결합체 (I-31)의 제조**

[1319]

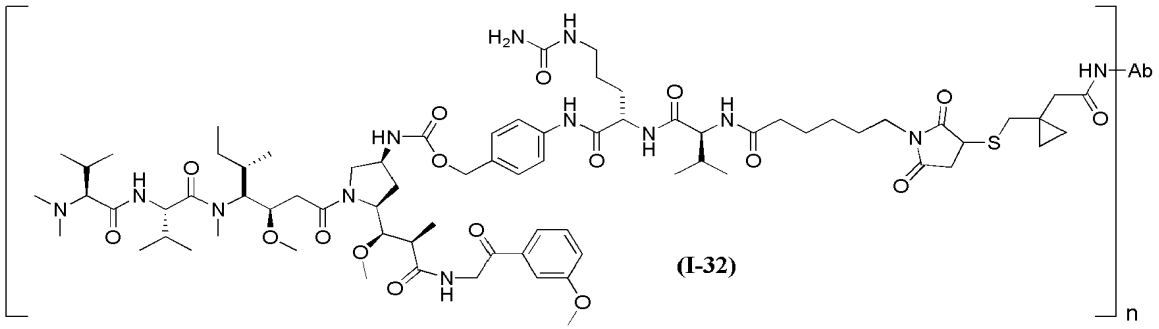


[1320] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-27에서 수득한 화합물 (VII-31) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 21 mg (1.61 mg/mL, 총 부피 13 mL, 85%)를 얻었다.

[1321]

[1322] **실시예 28: 결합체 (I-32)의 제조**

[1323]

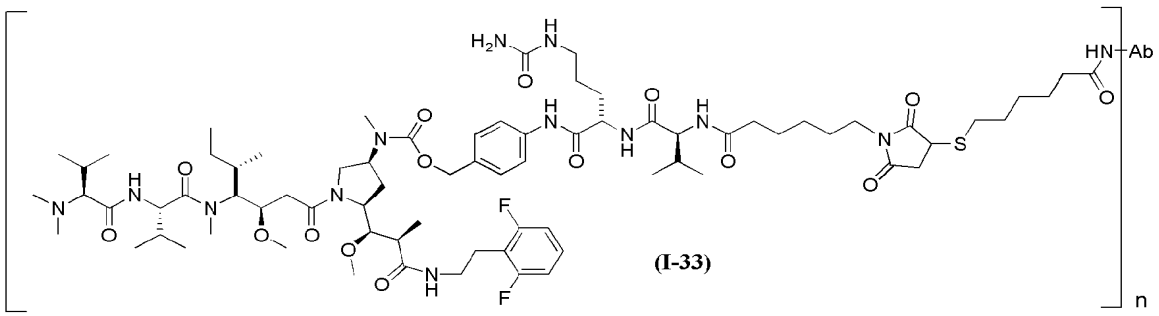


[1324] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-28에서 수득한 화합물 (VII-32) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 12.3 mg (0.88 mg/mL, 총 부피 14 mL, 50%)를 얻었다.

[1325]

[1326] **실시예 29: 결합체 (I-33)의 제조**

[1327]

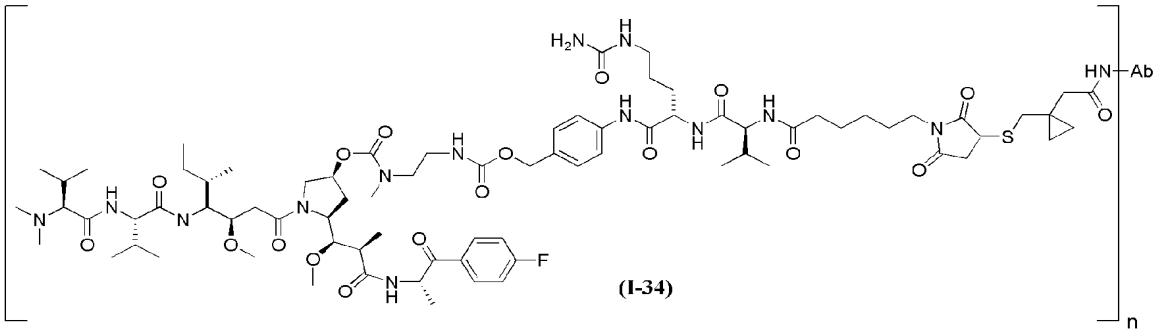


[1328] 제조예 5-19에서 수득한 화합물 (VII-23) 대신에 제조예 5-29에서 수득한 화합물 (VII-33) (4.0 mg, 0.0025 mmol, 15 당량)을 사용하여 실시예 19와 동일한 방법으로 표제화합물 15.3 mg (1.18 mg/mL, 총 부피 13 mL, 62%)를 얻었다.

[1329]

[1330] **실시예 30: 결합체 (I-34)의 제조**

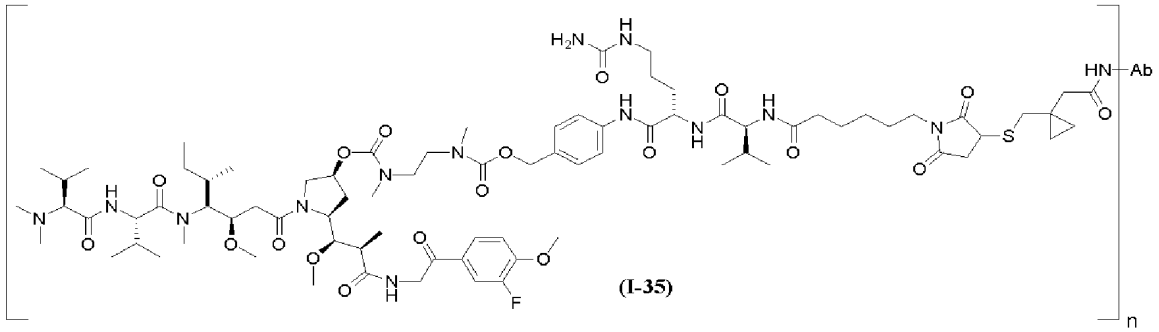
[1331]



[1332] pH 7.5의 PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl 포함)로 교환 처리된 트라스투주맙(허셉틴) 용액 5 mL (5.4 g/mL, 36 μM)에 추가적으로 pH 7.5의 PBS 2.5 ml와 pH 8.5의 0.05 M 소듐보레이트 버퍼 7.5 mL를 가하고, 20% DMSO 3.75 mL에 용해된 제조예 5-30에서 수득한 화합물 (VII-34) (5.7 mg, 0.0036 mmol, 20 당량)를 가한 후, 25℃에서 18시간 동안 교반하였다. 반응이 종결되면 반응혼합물을 0.45 ㎛ 여과기로 여과하고, 여액을 원심여과기(centrifugal filter, Amicon Ultra-15 30K)에 담아 유기용매와 미반응 물질이 제거될 때까지 pH 7.5의 PBS를 다시 가하여 2-3회 반복 농축하였다. 농축액을 pH 7.5의 PBS 중에 평형화시킨 Sephadex G-25 수지 충진 컬럼 (30 x 300 mm)을 사용하여 MPLC (280 nm, UV range 0.08, flow rate 10 mL/min)로 분리 정제하여 표제화합물 26.4 mg (2.20 mg/mL, 총 부피 12 mL, 98%)를 얻었다. 이때 얻어진 화합물의 농도는 UV 분광계로 세가지 파장(280 nm, 320 nm, 350 nm) 각각에서의 흡광도를 측정하여 결정하였다.

[1333]

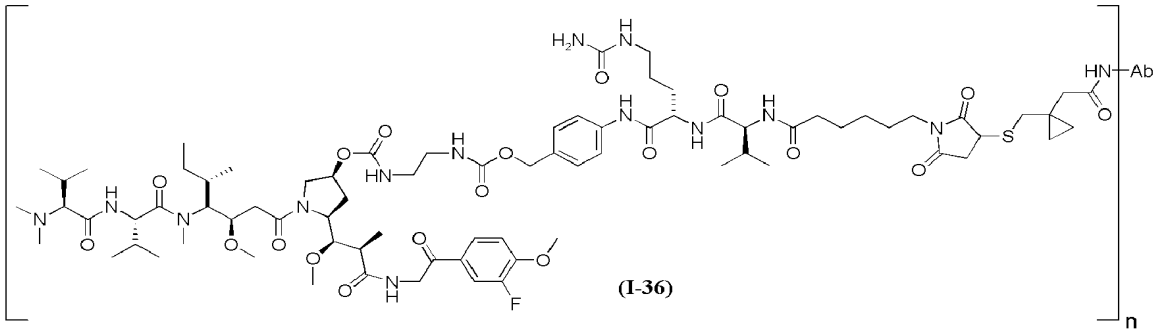
[1334] **실시예 31: 결합체 (I-35)의 제조**

[1335] 

[1336] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-31에서 수득한 화합물 (VII-35) (4.2 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 14.5 mg (1.21 mg/mL, 총 부피 12 mL, 61%)를 얻었다.

[1337]

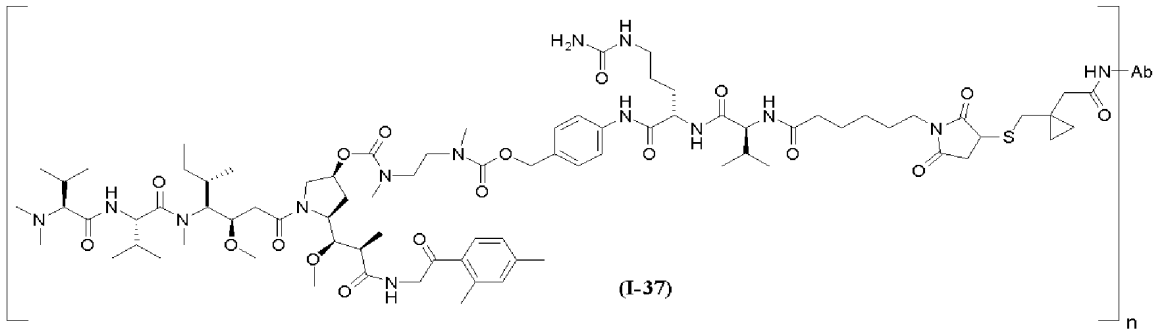
[1338] **실시예 32: 결합체 (I-36)의 제조**

[1339] 

[1340] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-32에서 수득한 화합물 (VII-36) (4.1 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 17.9 mg (1.63 mg/mL, 총 부피 11 mL, 75%)를 얻었다.

[1341]

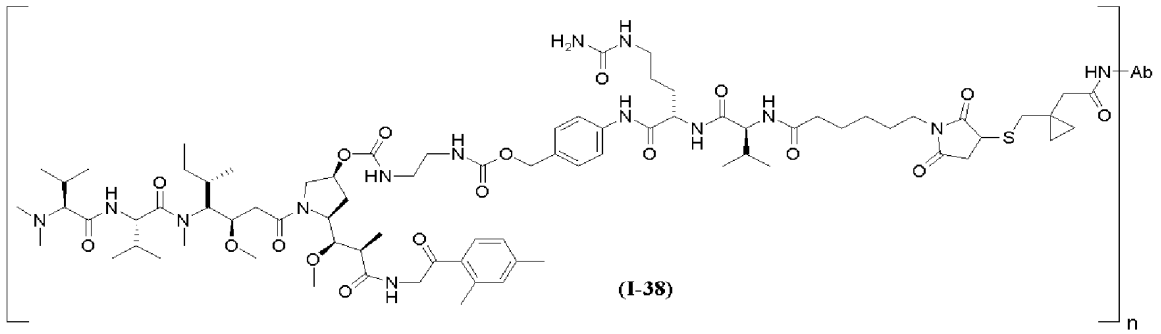
[1342] **실시예 33: 결합체 (I-37)의 제조**

[1343] 

[1344] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-33에서 수득한 화합물 (VII-37) (4.1 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 11.8 mg (1.48 mg/mL, 총 부피 8 mL, 50%)를 얻었다

[1345]

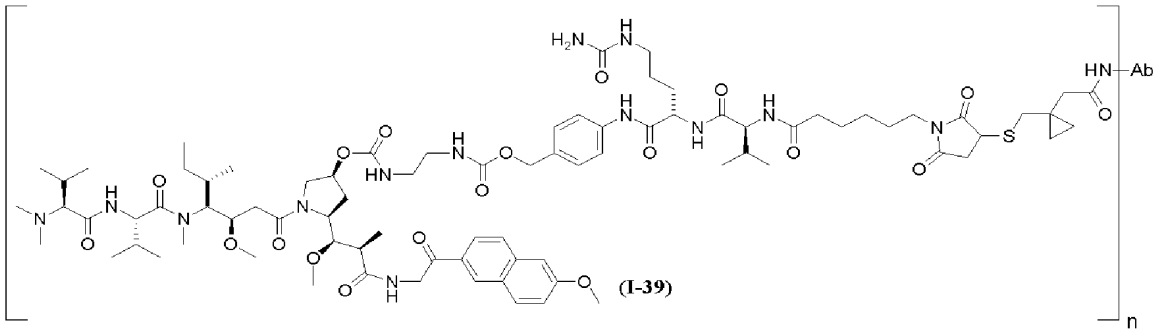
[1346] **실시예 34: 결합체 (I-38)의 제조**

[1347] 

[1348] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-34에서 수득한 화합물 (VII-38) (4.0 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 15.8 mg (1.21 mg/mL, 총 부피 13 mL, 66%)를 얻었다

[1349]

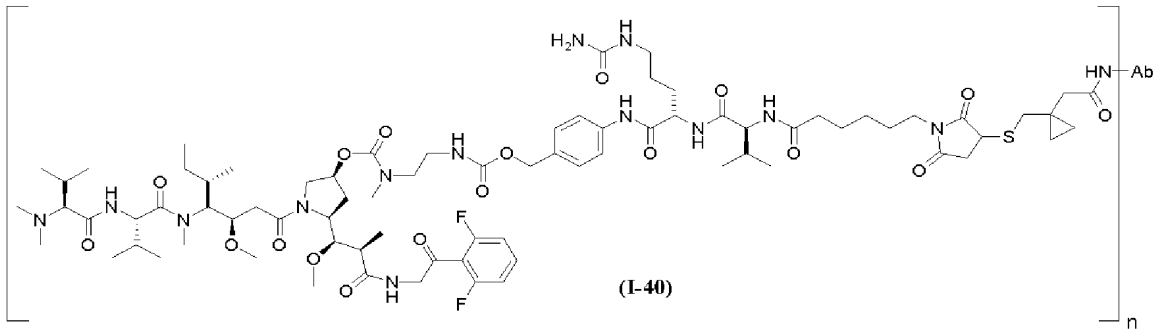
[1350] **실시예 35: 결합체 (I-39)의 제조**

[1351] 

[1352] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-35에서 수득한 화합물 (VII-39) (4.2 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 18.3 mg (1.52 mg/mL, 총 부피 12 mL, 77%)를 얻었다.

[1353]

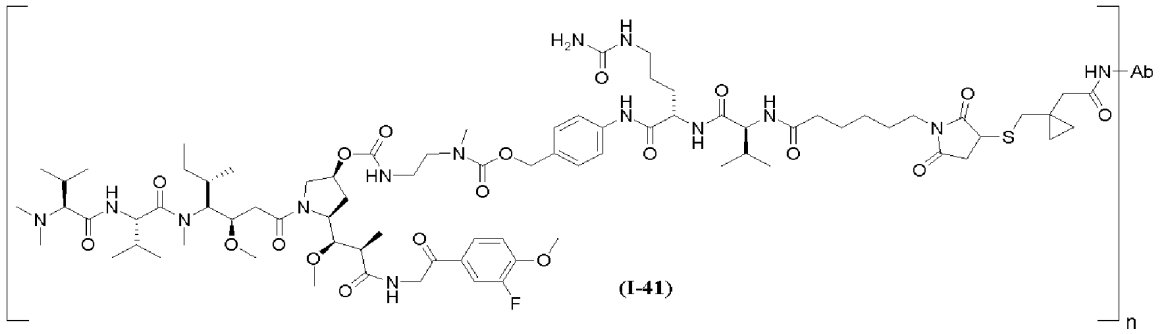
[1354] **실시예 36: 결합체 (I-40)의 제조**

[1355] 

[1356] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-36서 수득한 화합물 (VII-40) (4.6 mg, 0.0027 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 12.7 mg (1.15 mg/mL, 총 부피 11 mL, 47%)를 얻었다.

[1357]

[1358] **실시예 37: 결합체 (I-41)의 제조**

[1359] 

[1360] 제조예 5-30에서 수득한 화합물 (VII-34) 대신에 제조예 5-37에서 수득한 화합물 (VII-41) (4.1 mg, 0.0024 mmol, 15 당량)을 사용하여 실시예 30과 동일한 방법으로 표제화합물 16.1 mg (1.24 mg/mL, 총 부피 13 mL, 67%)를 얻었다

[1361]

[1362] **시험예 1: 단백질 질량분석법을 이용한 DAR 결정**

[1363]

[1364] 실시예로 제조된 항체-링커-약물 결합체 각각에 PNGase F 엔자임을 37 ℃에서 15±3시간 동안 처리하여 N-결합형 당을 제거하였다. 당이 제거된 항체-링커-약물 결합체를 Agilent 1200 HPLC와 Agilent 6530 Q-TOF 질량분석기(mass spectrometer)로 구성된 LC-ESI-MS를 이용하여 분석하였다. Agilent Zorbax 컬럼(300SB-C18, 2.1 mm x 75 mm, 5 ㎛)과 0.1% 포름산 수용액/ 0.1% 포름산 아세토니트릴 용액의 구배 용리(gradient elution)을 이용하여 항체-링커-약물 결합체를 분리하고, m/z(mass to charge) 데이터를 초당 1 스펙트라의 스캔속도로 900 내지 4000 m/z의 범위에서 획득한 후, 디콘볼루션(deconvolution)하여 분자량과 인테그레이션(integration)된 각 피크(D0, D1, D2 .. Dn)의 존재비(abundance) 정보를 이용하여 DAR을 계산하였다.

[1365] 각 피크의 상대 존재비(relative abundance)는 하기 식을 이용하여 계산하였다. 각 피크의 존재비를 모든 피크의 존재비의 합으로 나누어 각 피크의 % 상대 존재비를 계산하였다.

[1366] $$\text{각 피크의 \% 상대 존재비} = \left(\frac{\text{Abundance of each peak}}{\text{Total abundance of peaks (D0+D1+..+Dn)}}\right) \times 100$$

[1367] 항체-링커-약물 결합체의 DAR은 % 상대 존재비와 하기 식을 이용하여 계산하였고, 그 결과를 하기 표 1 내지 표 4에 나타내었다.

[1368] $$\text{DAR} = \left(\frac{\text{Abundance of D0} \times 0 + \text{Abundance of D1} \times 1 + .. + \text{Abundance of Dn} \times n}{100}\right)$$

[1369] 표 1

[Table 1]

결합체 (I-6)										
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**Sum**
Mass (Da)	145179.9	146631.2	148080.8	149535.2	150983.3	152436.5	153888.3			
Area	3080	5170	6730	5764	4659	2430	1059			28892
%Area	**10.7**	**17.9**	**23.3**	**20.0**	**16.1**	**8.4**	**3.7**			100.0
DAR	**2.53**									
결합체 (I-10)										
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**Sum**
Mass (Da)	145170.3	146616.1	148043.7	149473.9	150904.2	152340	153771.2	155201.9	156631.2	
Area	474	2570	5670	6155	6765	5567	3940	2430	655	34226
%Area	**1.4**	**7.5**	**16.6**	**18.0**	**19.8**	**16.3**	**11.5**	**7.1**	**1.9**	100.0
DAR	**3.89**									
결합체 (I-11)										
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**Sum**
Mass (Da)	145173.9	146631.36	148048.1	149481	150912.6	152348	153781.7	155215.2	156641.7	
Area	330	1788	4947	6111	6769	7133	4975	3005	784	35842
%Area	**0.9**	**5.0**	**13.8**	**17.0**	**18.9**	**19.9**	**13.9**	**8.4**	**2.2**	100.0
DAR	**4.18**									

[1370]

[1371] 표 2

[Table 2]

결합체 **(I-16)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145183.4	146693.5	148201.3	149712	151221.6	152733.2	154245	155747.8	157256	158782.5	
Area	102	1424	3455	4709	5143	4244	3996	2766	1683	762	28284
%Area	0.4	5.0	12.2	16.6	18.2	15.0	14.1	9.8	6.0	2.7	100.0
DAR	**4.52**										
결합체 **(I-19)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146700	148195.7	149700.2	151191.8	152675.1	154214.2				
Area	0	659	2225	3104	2632	1969	1031				11620
%Area	0	5.7	19.1	26.7	22.7	16.9	8.9				100.0
DAR	**3.53**										
결합체 **(I-20)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146682.2	148171.4	149661.6	151149.2	152635.6	154120.6	155616.1			
Area	0	1192	4051	5884	5670	4260	2553	1374			24984
%Area	0	4.8	16.2	23.6	22.7	17.1	10.2	5.5			100.0
DAR	**3.84**										
결합체 **(I-21)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146696.3	148185.3	149697.7	151205.5	152699.3	154228.8	155742.3			
Area	0	204	832	1650	1996	906	480	135			6203
%Area	0	3.3	13.4	26.6	32.2	14.6	7.7	2.2			100.0

DAR	**3.73**										
결합체 **(I-22)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146696.3	148150.3	149640.7	151168	152613.7	15087.2	15554.7	157093.4	158600	
Area	0	204	1040	1925	2478	2635	1932	886	929	328	12333
%Area	0	1.7	8.2	15.6	20.1	21.4	15.7	7.2	7.5	2.7	100.0
DAR	**4.81**										

[1372]

[1373] 표 3

[Table 3]

결합체 (I-23)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145188.9	146664	148148.6	149631.3	151108.4	152593.1	154080	155567.8			
Area	2036	5337	7002	5759	4434	2120	1133	207			28028
%Area	7.3	19.0	25.0	20.0	15.8	7.6	4.0	0.7			100.0
DAR	**2.56**										
결합체 (I-24)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145188.9	146664	148148.6	149631.3	151108.4	152593.1	154080				
Area	2036	5337	7002	5759	4434	2120	1133				27821
%Area	7.3	19.2	25.2	20.7	15.9	7.6	4.1				100.0
DAR	**2.58**										
결합체 (I-25)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	148184.2	146662.2	148139.3	149624.8	151109.1	152587.6	154068.8	155555.1	158517.7	158517.7	
Area	977	4474	8992	9929	9912	7176	4842	2313	977	240	49832
%Area	**2.0**	9.0	18.0	19.9	19.9	14.4	9.7	4.6	2.0	0.5	100.0
DAR	**3.67**										
결합체 (I-26)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145188	146689.5	148201.7	149713.7	151226.1	152731.6	154241.5	155753.7	157258.4	158773.3	

Area	136	1285	3717	5699	5756	5280	4541	3287	2348	955	33004
%Area	0.4	3.9	11.3	17.3	17.4	16.0	13.8	10.0	7.1	2.9	100.0
DAR	**4.63**										
결합체 **(I-27)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145182.4	146692.2	148195.4	149705.6	151213.7	152718.3	154222.6	155731.2			
Area	2008	3562	5553	4675	3576	1662	1105	456			22597
%Area	8.9	15.8	24.6	20.7	15.8	7.4	4.9	2			100.0
DAR	**2.71**										
결합체 **(I-28)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145185.4	146672.2	148158.1	149641.9	151128.8	152626	154108				
Area	789	4780	5411	3780	3281	1342	428				19811
%Area	4.0	24.1	27.3	19.1	16.6	6.8	2.2				100.0
DAR	**2.49**										
결합체 **(I-30)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146711.4	148226.3	149742.3	151267.9	152788.8	154311.3	155837.3			
Area	0	144	968	1227	2001	1550	519	153			6562
%Area	0	2.2	14.8	18.7	30.5	23.6	7.9	2.3			**100.0**
DAR	**3.92**										
결합체 **(I-31)**											

	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	Sum
Mass (Da)	145181.5	146691.4	148203.8	149717.6	151228.5						
Area	6412	9396	6548	3337	1388						
%Area	23.7	34.7	24.2	12.3	5.1						
DAR	**1.41**										
결합체 **(I-32)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145185.5	146679.8	148182.7	149670.4	151166.1	152687.2	154165.9	155657.3	157164.6		
Area	240	696	1841	2965	3326	3067	2007	897	260		15299
%Area	1.6	4.5	12.0	19.4	21.7	20.0	13.1	5.9	1.7		100.0
DAR	**4.07**										
결합체 **(I-33)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145177.6	146681.5	148183.2	149668.3	151168.4	152663	154161.2	155656.4	157868.3		
Area	149	432	1535	2946	4174	3528	2278	1803	375		17220
%Area	0.9	2.5	8.9	17.1	24.2	20.5	13.2	10.5	2.2		100.0
DAR	**4.41**										

[1374]

[1375] 표 4

[Table 4]

결합체 (I-34)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145177	146777.9	148378.3	149972.4	151563	153163.7					
Area	5864	10420	8579	5125	1867	849					32704
%Area	17.9	31.9	26.2	15.7	5.7	2.6					100.0
DAR	**1.67**										
결합체 (I-35)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146805.8	148427.5	150051.7	151669	153291.4	154912.5	156539.5	158132.1	159784.1	
Area	0	1791	7950	11577	11722	9767	7214	4651	1852	949	57473
%Area	0.0	3.1	13.8	20.1	20.4	17.0	12.6	8.1	3.2	1.7	100.0
DAR	**4.30**										
결합체 (I-36)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146784.9	148374.9	149964.2	151552.3	153144	154742.7	156334.3	157940.4	159524.4	
Area	0	232	2467	5116	6483	4829	3222	1457	716	356	24878
%Area	0.0	0.9	9.9	20.6	26.1	19.4	13.0	5.9	2.9	1.4	100.0
DAR	**4.38**										
결합체 (I-37)											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146787.7	148387.9	149985.8	151588.4	153191.5	154798.6	156392.1	157995.3		

Area	0	3495	7843	10015	8988	6940	**4126**	**1274**	**389**		**43070**
%Area	0.0	8.1	18.2	23.3	20.9	**16.1**	**9.6**	**3.0**	**0.9**		**100.0**
DAR	**3.64**										
결합체 **(I-38)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146759	148331.1	149903	151476.4	153055.9	154631	156203.6			
Area	0	2221	7557	9075	7933	5512	3044	968			
%Area	0.0	3.1	20.8	25.0	21.8	15.2	8.4	2.7			
DAR	**3.55**										
결합체 **(I-39)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145187.1	146810.7	148434.4	150059.8	151687.1	153320.3	154948.4				
Area	1463	5565	11616	11593	6224	2476	710				39647
%Area	3.7	14.0	29.3	29.2	15.7	6.2	1.8				100.0
DAR	**2.65**										
결합체 **(I-40)**											
	D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**Sum**
Mass (Da)	145180	146770.5	148351.3	149928.2	151506.8	153095	154671.9	156259.1	157840.3	159429.5	
Area	0	1848	6545	10020	11504	9447	6600	4246	2260	661	53131
%Area	0.0	3.5	12.3	18.9	21.6	17.8	12.4	7.99	4.25	1.26	100.0
DAR	**4.36**										
결합체 **(I-41)**											

	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	Sum
Mass (Da)	145180	146792.1	148401	150007	151601.6	153213.6	154823.1				
Area	0	236	1671	3110	3322	2200	1234				11773
%Area	0.0	2.0	14.2	26.4	28.2	18.7	10.5				100.0
DAR	**3.79**										

[1376]

[1377] **시험예 2: SEC-HPLC 분석**

[1378] 크기 배제 크로마토그래피법(Size Exclusion Chromatography, SEC-HPLC)을 이용하여, 항체와 약물을 결합하는 과정에서 항체의 비정상적인 파편(fragment)이나 응집(aggregation)의 발생 유무를 평가하였다. 이런 비정상적인 단백질 구조는 본래 항체가 가지고 있는 항원 특이적인 결합능, 생체 내 약물동력학에 영향을 주기 때문에, 제조된 항체-링커-약물 결합체의 우수성을 간접적으로 확인할 수 있다.

[1379] 실시예에서 제조된 항체-링커-약물 결합체 각각에 대해 SEC-HPLC를 이용하여 정상 항체 구조 비율을 분석하여, 그 결과를 하기 표 5 내지 표 8에 나타내었다.

[1380] 표 5

[Table 5]

시료	Main peak [1] (%)	HMW [2] (%)	LMW [3] (%)
결합전 항체	99.16	0.65	0.19
결합체 (I-6)	98.55	1.07	0.38
결합체 (I-10)	98.21	1.40	0.39
결합체 (I-11)	98.42	1.16	0.42
[1] Main peak: 정상적인 항체 크기에 해당하는 물질의 비율 [2] HMW (High molecular weight): 응집 현상으로 정상 항체보다 큰 크기의 물질의 비율 [3] LMW (Low molecular weight): 파편화 현상으로 정상 항체보다 작은 크기의 물질의 비율			

[1381] 실시예 2, 6 및 7에서 제조된 항체-링커-약물 결합체들은 약물을 접합하기 이전의 항체(99.16%)와 비교하여, 98.21 내지 98.55% 범위의 정상 항체 구조 비율을 보였다.

[1382]

[1383] 표 6

[Table 6]

시료	Main peak [1] (%)	HMW [2] (%)	LMW [3] (%)
결합전 항체	99.48	0.27	0.25
결합체 (I-16)	99.24	0.40	0.35
결합체 (I-17)	99.25	0.38	0.32
결합체 (I-18)	98.44	1.38	0.18
결합체 (I-19)	93.56	6.25	0.18
결합체 (I-20)	94.06	5.73	0.20
결합체 (I-21)	97.27	2.52	0.20
결합체 (I-22)	92.43	7.37	0.19
[1] Main peak: 정상적인 항체 크기에 해당하는 물질의 비율 [2] HMW (High molecular weight): 응집 현상으로 정상 항체보다 큰 크기의 물질의 비율 [3] LMW (Low molecular weight): 파편화 현상으로 정상 항체보다 작은 크기의 물질의 비율			

[1384] 실시예 12 내지 18에서 제조된 항체-링커-약물 결합체들은 약물을 접합하기 이전의 항체(99.48%)와 비교하여, 92.43 내지 99.25% 범위의 정상 항체 구조 비율을 보였다.

[1385]

[1386] 표 7

[Table 7]

시료	Main peak [1] (%)	HMW [2] (%)	LMW [3] (%)
결합전 항체	99.48	0.27	0.25
결합체 (I-23)	99.13	0.53	0.34
결합체 (I-24)	97.77	1.71	0.52
결합체 (I-25)	96.94	2.71	0.35
결합체 (I-26)	98.39	1.30	0.31
결합체 (I-27)	97.12	2.63	0.24
결합체 (I-28)	97.44	2.32	0.24
결합체 (I-29)	97.07	2.68	0.25
결합체 (I-30)	90.68	9.08	0.24
결합체 (I-31)	93.80	6.00	0.20
결합체 (I-32)	94.27	5.51	0.22
결합체 (I-33)	97.09	2.60	0.32

[1] Main peak: 정상적인 항체 크기에 해당하는 물질의 비율 [2] HMW (High molecular weight): 응집 현상으로 정상 항체보다 큰 크기의 물질의 비율 [3] LMW (Low molecular weight): 파편화 현상으로 정상 항체보다 작은 크기의 물질의 비율

[1387] 실시예 19내지 29에서 제조된 항체-링커-약물 결합체들은 약물을 접합하기 이전의 항체(99.48%)와 비교하여, 90.68 내지 99.13% 범위의 정상 항체 구조 비율을 보였다.

[1388]

[1389] 표 8

[Table 8]

시료	Main peak [1] (%)	HMW [2] (%)	LMW [3] (%)
결합전 항체	99.48	0.27	0.25
결합체 (I-34)	98.63	1.02	0.35
결합체 (I-35)	98.93	0.77	0.29
결합체 (I-36)	98.33	1.43	0.24
결합체 (I-37)	98.85	0.87	0.28
결합체 (I-38)	98.31	1.44	0.25
결합체 (I-39)	97.00	2.75	0.24
결합체 (I-40)	98.15	1.49	0.36
결합체 (I-41)	97.61	2.15	0.24
[1] Main peak: 정상적인 항체 크기에 해당하는 물질의 비율 [2] HMW (High molecular weight): 응집 현상으로 정상 항체보다 큰 크기의 물질의 비율 [3] LMW (Low molecular weight): 파편화 현상으로 정상 항체보다 작은 크기의 물질의 비율			

[1390] 실시예 30 내지 37에서 제조된 항체-링커-약물 결합체들은 약물을 접합하기 이전의 항체(99.48%)와 비교하여, 97.00 내지 98.93% 범위의 정상 항체 구조 비율을 보였다.

[1391]

[1392] **시험예 3: IEF 분석**

[1393] 등전위점 초점화(isoelectric focusing: IEF) 분석을 이용하여, 실시예 2, 4, 6 및 7에서 제조된 항체-링커-약물 결합체 (I-6), (I-8), (I-10) 및 (I-11) 각각의 등전점(pI) 값을 분석하여 그 결과를 하기 표 9에 나타내었다.

[1394] 표 9

[Table 9]

Lane	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
시료	결합전 항체	결합체(I-8)	결합체(I-6)	결합체(I-11)	결합체(I-11)	결합체(I-10)	결합체(I-10)	결합전 항체
Band 1	8.96	8.87	8.90	8.78	8.80	8.78	8.78	8.98
Band 2	8.85	8.78	8.82	8.71	8.72	8.70	8.69	8.86
Band 3	8.76	8.69	8.74	8.62	8.62	8.6	8.59	8.77
Band 4	8.68	8.59	8.63	8.51	8.51	8.49	8.48	8.68
Band 5	-	8.48	8.52	8.39	8.4	8.38	8.37	-
Band 6	-	-	8.41	8.29	8.3	8.27	8.26	-
Band 7	-	-	-	8.21	8.2	8.18	8.17	-
* Lane 1 and 10 are pI marker.								

[1395] 상기 표 9에서 보듯이, DAR 결과와 마찬가지로 더 많은 약물이 결합된 결합체(I-10)과 (I-11)에서 더 낮은 pI 밴드(band)가 확인되었으며, 이로부터 결합된 약물의 수가 증가하였음을 알 수 있다.

[1396]

[1397] **시험예 4: 시험관내 세포독성 시험**

[1398] 항체-링커-약물 결합체의 항암 효능(potency)은 HER-2 과발현 유방암세포주(BT-474)를 이용하여, 세포생장 저해능(anti-proliferation activity)을 시험관내 실험법으로 평가하였다. 대조군으로는 항체-링커-약물 결합체의 제조에 사용된 트라스투주맙이 사용되었다. 트라스투주맙은 유방암세포에서 특이적으로 발현되는 HER-2 항원에 대한 모노클로날 항체이다.

[1399] 96웰 마이크로 플레이트에서 웰 당 1 X 10^4 개의 BT-474 세포주를 준비하고, 대조군인 트라스투주맙과 각 실시예에서 제조된 항체-링커-약물 결합체를 각각 농도 별로 처리한 후, 5일 배양 후에 살아있는 세포 수를 발색시약(CCK-8)으로 처리한 후 흡광도를 측정하여 간접 확인하였다. 농도-효능 간의 관계식은 4차 곡선 함수로 구하고, EC_{50}(effective concentration of 50%, 반수영향농도)를 비교하여, 상대적인 효능을 평가하였다. 그 결과를 도 1 내지 17 및 표 10 내지 13에 나타내었다.

[1400]

[1401] 표 10에서 보듯이, 대조군인 트라스투주맙과 비교하여, 실시예 2, 6 및 7에서 제조된 항체-링커-약물 결합체들은 13.2 내지 17.8 배의 낮은 EC_{50}값을 보였고, 이는 더 적은 양을 사용해도 동등한 효능을 보이며 부작용이 줄어드는 효과를 기대할 수 있음을 나타낸다.

[1402] 또한, 도 1 내지 도 3에서 보듯이 실시예 2, 4, 6 및 7에서 제조된 항체-링커-약물

결합체들은 대조군인 트라스투주맙과 비교하여 4차 곡선 함수에서 더 낮은 흡광도까지 도달하는데, 이는 세포생장 저해능이 대조군인 트라스투주맙보다 더 뛰어남을 의미한다. 즉, 항체가 본래 가지는 종양세포 저해효능에 접합된 세포독성 약물의 기작에 의해 추가적으로 종양 세포가 감소했음을 나타낸다.

[1403] 표 10

[Table 10]

시료	EC_{50}(1st) (ng/ml)	EC_{50}(2nd) (ng/ml)	EC_{50}(Mean) (ng/ml)	상대효능 (배)
트라스투주맙	125	-	125	1
결합체 (I-6)	9.47	-	9.47	13.2
트라스투주맙	88.2	-	88.2	1
결합체 (I-10)	6.28	6.28	6.28	14.0
트라스투주맙	85.1	-	85.1	1
결합체 (I-11)	4.66	4.88	4.77	17.8

[1404]

[1405] 표 11에서 보듯이, 대조군인 트라스투주맙과 비교하여, 실시예 1 내지 7에서 제조된 항체-링커-약물 결합체들은 7.7배 내지 34.2 배의 낮은 EC_{50}값을 보였고, 이는 더 적은 양을 사용해도 동등한 효능을 보이며 부작용이 줄어드는 효과를 기대할 수 있음을 나타낸다.

[1406] 또한, 도 4내지 도 7에서 보듯이 실시예 1 내지 7에서 제조된 항체-링커-약물 결합체들은 대조군인 트라스투주맙과 비교하여 4차 곡선 함수에서 더 낮은 흡광도까지 도달하는데, 이는 세포생장 저해능이 대조군인 트라스투주맙보다 더 뛰어남을 의미한다. 즉, 항체가 본래 가지는 종양세포 저해효능에 접합된 세포독성 약물의 기작에 의해 추가적으로 종양 세포가 감소했음을 나타낸다.

[1407] 표 11

[Table 11]

시료	EC_{50} (ng/ml)	상대효능 (배)
트라스투주맙	98.6	1
결합체(I-16)	2.88	34.2
트라스투주맙	110	1
결합체(I-18)	14.3	7.7
트라스투주맙	96.6	1
결합체(I-19)	5.18	18.6
트라스투주맙	92.3	1
결합체(I-20)	5.72	16.1
트라스투주맙	96.6	1
결합체(I-21)	7.09	13.6
트라스투주맙	92.3	1
결합체(I-22)	6.7	13.8

[1408]

[1409] 표 12에서 보듯이, 대조군인 트라스투주맙과 비교하여, 실시예 19 내지 29에서 제조된 항체-링커-약물 결합체들은 0.5 배 내지 33.6 배의 낮은 EC_{50}값을 보였다. 항체-링커-약물 결합체 (I-23), (I-25), (I-26) 및 (I-33)의 경우, 대조군과 비교하여, 20 배 이상의 낮은 EC_{50}값을 보였고, 이는 20배 이상 더 적은 양을 사용해도 동등한 효능을 보이며 부작용이 줄어드는 효과를 기대할 수 있음을 나타낸다.

[1410] 또한, 도 8 내지 도 13에서 보듯이 실시예 19 내지 29에서 제조된 항체-링커-약물 결합체들은 대조군인 트라스투주맙과 비교하여 4차 곡선 함수에서 더 낮은 흡광도까지 도달하는데, 이는 세포생장 저해능이 대조군인 트라스투주맙보다 더 뛰어남을 의미한다. 즉, 항체가 본래 가지는 종양세포 저해효능에 접합된 세포독성 약물의 기작에 의해 추가적으로 종양 세포가 감소했음을 나타낸다.

[1411] 표 12

[Table 12]

시료	EC_{50} (ng/mL)	상대효능 (배)
트라스투주맙	**102**	**1**
결합체 (I-23)	**4.73**	**21.6**
트라스투주맙	**106**	**1**
결합체 (I-24)	**68.4**	**1.5**
트라스투주맙	**107**	**1**
결합체 (I-25)	**4.75**	**22.2**
트라스투주맙	**101**	**1**
결합체 (I-26)	**4.17**	**24.2**
트라스투주맙	**97.9**	**1**
결합체 (I-32)	**12.7**	**7.7**
트라스투주맙	**97**	**1**
결합체 (I-33)	**2.89**	**33.6**

[1412]

[1413] 표 13에서 보듯이, 대조군인 트라스투주맙과 비교하여, 실시예 30 내지 36에서 제조된 항체-링커-약물 결합체들은 8.9 배 내지 22.6 배의 낮은 EC_{50}값을 보였고, 이는 더 적은 양을 사용해도 동등한 효능을 보이며 부작용이 줄어드는 효과를 기대할 수 있음을 나타낸다.

[1414] 또한, 도 14 내지 도 17에서 보듯이 실시예 30내지 36에서 제조된 항체-링커-약물 결합체들은 대조군인 트라스투주맙과 비교하여 4차 곡선 함수에서 더 낮은 흡광도까지 도달하는데, 이는 세포생장 저해능이 대조군인 트라스투주맙보다 더 뛰어남을 의미한다. 즉, 항체가 본래 가지는 종양세포 저해효능에 접합된 세포독성 약물의 기작에 의해 추가적으로 종양 세포가 감소했음을 나타낸다.

[1415] 표 13

[Table 13]

시료	EC_{50} (ng/ml)	상대효능 (배)
트라스투주맙	**104**	**1**
결합체(I-34)	**11.3**	**9.2**
트라스투주맙	**104**	**1**
결합체(I-35)	**10.3**	**10.1**
트라스투주맙	**104**	**1**
결합체(I-36)	**5.74**	**18.1**
트라스투주맙	**116**	**1**
결합체(I-37)	**13**	**8.9**
트라스투주맙	**116**	**1**
결합체(I-38)	**5.13**	**22.6**
트라스투주맙	**94.7**	**1**
결합체(I-39)	**7**	**13.5**
트라스투주맙	**94.7**	**1**
결합체(I-40)	**4.42**	**21.4**

[1416]

[1417] **시험예 5: 약물동태 시험**

[1418] 실시예 2 및 4에서 제조된 항체-링커-약물 결합체 (I-6) 및 (I-8)와 대조군인 트라스투주맙을 각각 Balb/c Slc-nu 마우스에 20 mg/kg의 용량으로 정맥 내 1회 투여한 후, 1개월 동안 혈액 내 약물동태(pharmacokinetics: PK) 시험을 수행하였다.

[1419] 항체-링커-약물 결합체 (I-8) 및 (I-6)의 반감기는 각각 8.3일과 7.9일로 대조군인 트라스투주맙의 반감기 8.6일과 비교하여 통계적으로 유의한 차이를 보이지 않았으며, 이는 접합 과정 동안 항체 자체의 PK에 영향을 미치는 부위(FcRn)에는 세포독성 약물이 접합되지 않았음을 나타낸다.

[1420] 표 14

[Table 14]

파라미터	결합체 (I-8)	결합체 (I-6)	트라스투주맙
AUC_{all} (hr*μg/mL)	507280	575495	607582
C_0 (μg/mL)	375.2	403.7	379.9
$T_{1/2}$ (day) [1)]	8.3	7.9	8.6
[1)] User specified lambda-z range (4m~27day, 9 points)			

[1421]

[1422] **시험예 6: 동물모델 효능 시험**

[1423] 동물모델 효능 시험은 HER-2 과발현 유방암세포주(BT-474)를 이종이식(xenograft)한 누드마우스에 실시예 2, 6 및 7에서 제조된 항체-링커-약물 결합체 (I-6), (I-10) 및 (I-11)를 각각 3종의 용량(1.25, 5, 20 mg/kg)으로 단회 정맥 투여한 후, 56일 동안의 종양의 부피와 최종 무게를 비교 평가하여 수행하였다. 음성대조군으로는 생리식염수(PBS)를, 양성대조군으로는 트라스투주맙(허셉틴®)을 20 mg/kg로 투여하였다. 그 결과를 도 18 및 도 19에 나타내었다.

[1424] 도 18 및 도 19로부터 트라스투주맙(허셉틴®)을 투여한 양성대조군은 음성대조군과 비교하여 유의적으로 종양이 감소하고, 항체-링커-약물 결합체 (I-6), (I-10) 및 (I-11)이 투여된 군들은 양성대조군 보다 더 현저하게 종양이 감소하고, 28일 이후로는 이식된 종양이 완전히 소실되고 다시 재발하지 않음을 확인할 수 있었다.

[1425]

[1426] 또한, HER-2 과발현 유방암세포주(BT-474)를 이종이식(xenograft)한 누드마우스에 실시예 12에서 제조된 항체-링커-약물 결합체 (I-16)을 3종의 용량(0.05, 0.5, 5 mg/kg)으로 단회 정맥 투여한 후, 28일 동안의 종양의 부피와 최종 무게를 비교 평가하여 수행하였다. 음성대조군으로는 생리식염수(PBS)를, 양성대조군으로는 트라스투주맙(허셉틴®)을 5 mg/kg로 투여하였다. 그 결과를 도 20및 도 21에 나타내었다.

[1427] 도 20 및 도 21로부터 트라스투주맙(허셉틴®)을 투여한 양성대조군은 음성대조군과 비교하여 유의적으로 종양이 감소하고, 항체-링커-약물 결합체 (I-16)이 투여된 군들은 양성대조군 보다 더 현저하게 종양이 감소하였음을 확인할 수 있었다.

[1428]

[1429] 또한, HER-2 과발현 유방암세포주(BT-474)를 이종이식(xenograft)한 누드마우스에 실시예 19, 21 및 22에서 제조된 항체-링커-약물 결합체 (I-23), (I-25) 및 (I-26)을 각각 3종의 용량(0.05, 0.5, 5 mg/kg)으로 단회 정맥 투여한 후, 28일 동안의 종양의 부피와 최종 무게를 비교 평가하여 수행하였다. 음성대조군으로는 생리식염수(PBS)를, 양성대조군으로는 트라스투주맙(허셉틴

®)을 5 mg/kg로 투여하였다. 그 결과를 도 22 내지 도 27에 나타내었다.

[1430] 도 22 내지 도 27로부터 트라스투주맙(허셉틴®)을 투여한 양성대조군은 음성대조군과 비교하여 유의적으로 종양이 감소하고, 항체-링커-약물 결합체 (I-23), (I-25) 및 (I-26)이 투여된 군들은 양성대조군 보다 더 현저하게 종양이 감소하였음을 확인할 수 있었다.

[1431]

[1432] 또한, HER-2 과발현 유방암세포주(BT-474)를 이종이식(xenograft)한 누드마우스에 실시예 30에서 제조된 항체-링커-약물 결합체 (I-34)를 3종의 용량(0.05, 0.5, 5 mg/kg)으로 단회 정맥 투여한 후, 28일 동안의 종양의 부피와 최종 무게를 비교 평가하여 수행하였다. 음성대조군으로는 생리식염수(PBS)를, 양성대조군으로는 트라스투주맙(허셉틴®)을 5 mg/kg로 투여하였다. 그 결과를 도 28 및 도 29에 나타내었다.

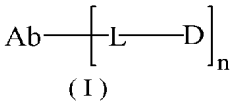
[1433] 도 28 및 도 29로부터 트라스투주맙(허셉틴®)을 투여한 양성대조군은 음성대조군과 비교하여 유의적으로 종양이 감소하고, 항체-링커-약물 결합체 (I-34)가 투여된 군들은 양성대조군 보다 더 현저하게 종양이 감소하였음을 확인할 수 있었다.

[1434]

청구범위

[청구항 1] 하기 화학식 I의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

[화학식 I]



(I)

상기 식에서,

Ab는 항체이고,

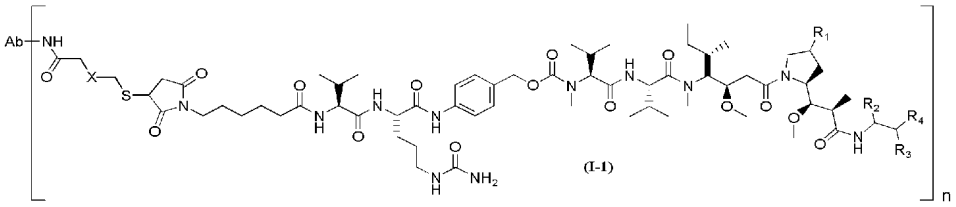
L은 링커이며,

D는 약물이고,

n은 1 내지 5이다.

[청구항 2] 제1항에 있어서, 하기 화학식 I-1의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

[화학식 I-1]



(I-1)

상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1은 수소, 히드록시, C_1-C_4의 알콕시기 또는 옥소(=O)이고,

R_2는 수소 또는 C_1-C_4의 알킬기이며,

R_3는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

R_4는 아릴기이고,

n은 1 내지 5이다.

[청구항 3] 제2항에 있어서,

Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1은 수소, 히드록시 또는 C_1-C_4의 알콕시기이고,

R_2는 수소 또는 C_1-C_4의 알킬기이며,

R_3는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

R_4는 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐인 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의

약제학적으로 허용되는 염.

[청구항 4] 제2항에 있어서,

Ab가 트라스투주맙(trastuzumab)이고,

X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

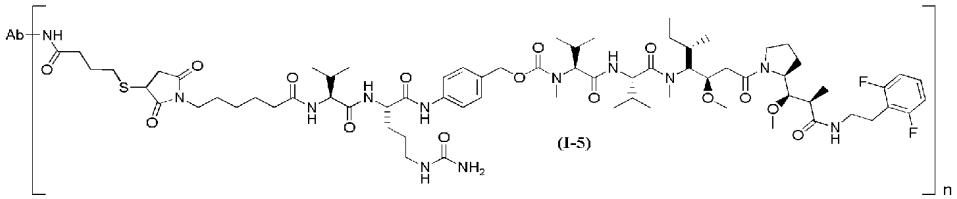
R_1은 수소, 히드록시 또는 메톡시이고,

R_2는 수소 또는 메틸이며,

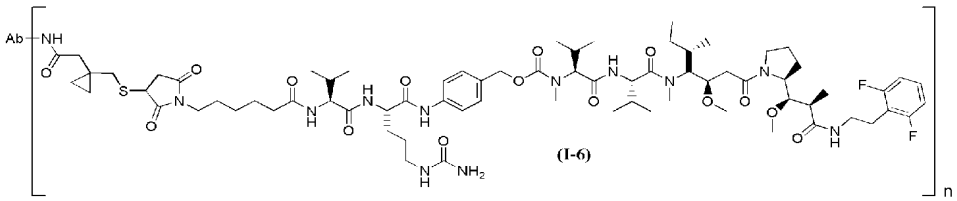
R_3는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

R_4는 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐인 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

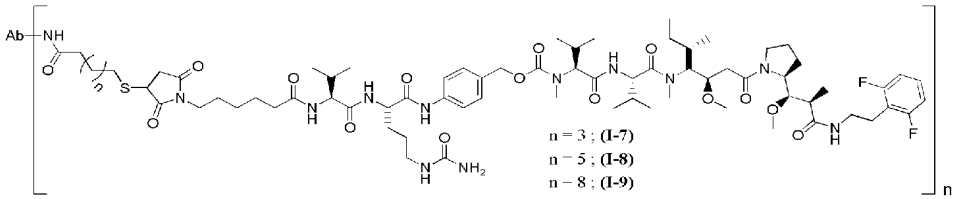
[청구항 5] 제2항에 있어서, 하기 화합물로부터 선택되는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:



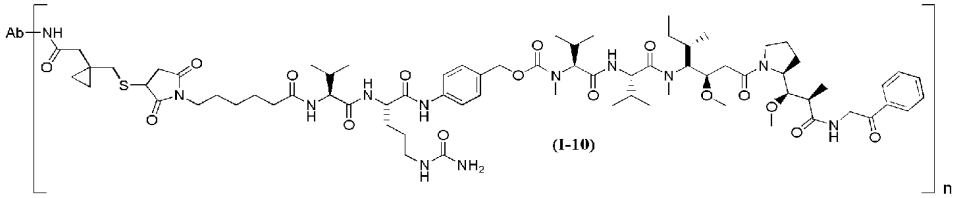
(I-5)



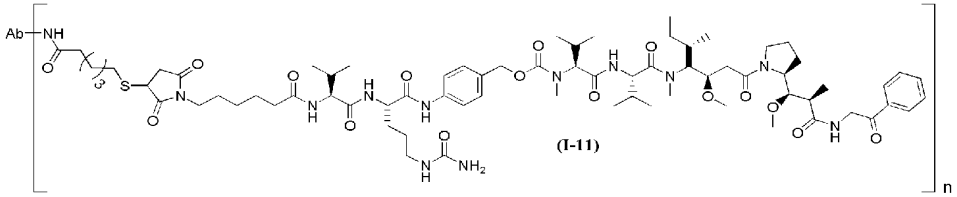
(I-6)



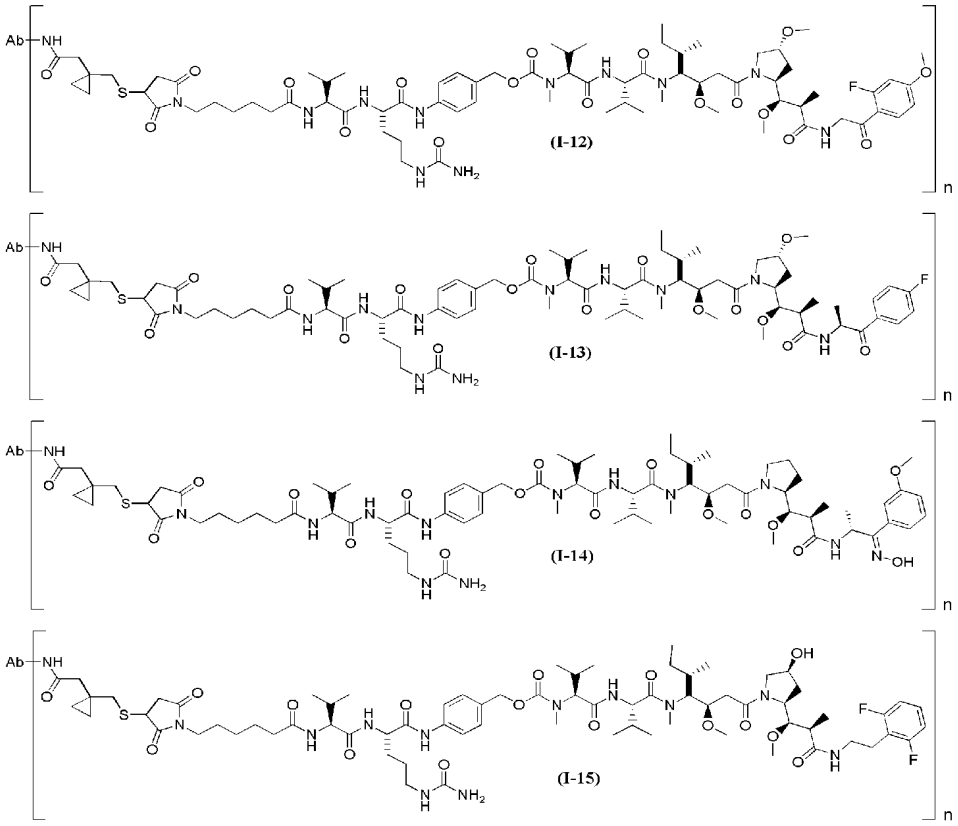
n = 3 ; (I-7)
n = 5 ; (I-8)
n = 8 ; (I-9)



(I-10)



(I-11)



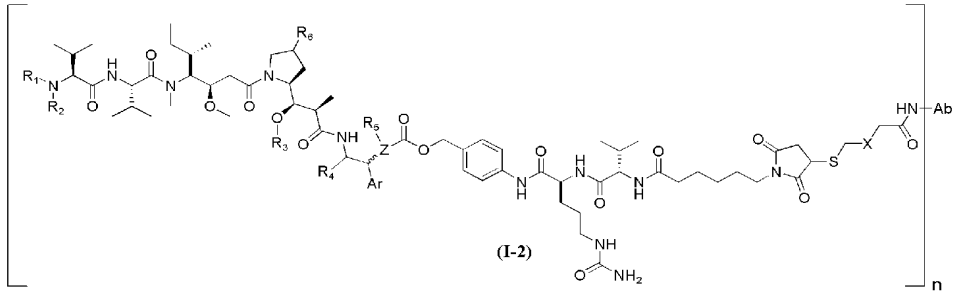
상기 식에서, n은 1 내지 5이다.

[청구항 6] 제2항 내지 제5항 중 어느 한 항에 있어서, 링커-약물이 항체의 라이신 잔기에 직접 결합된 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 7] 제2항 내지 제5항 중 어느 한 항에 있어서, 항체가 98% 이상의 정상 항체 구조 비율을 가지는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 8] 제1항에 있어서, 하기 화학식 I-2의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

[화학식 I-2]



상기 식에서,

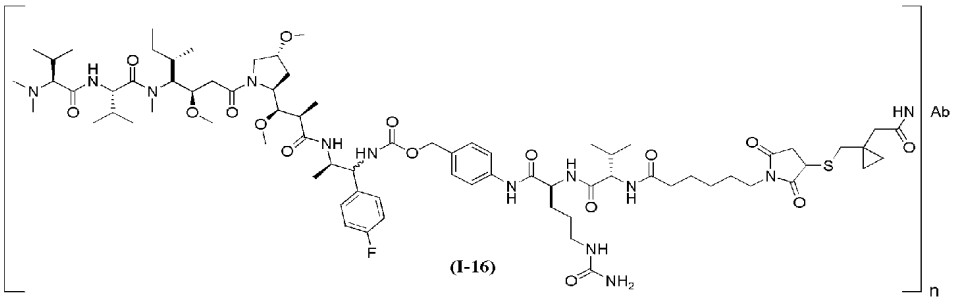
Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,
R_1, R_2, R_3 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,
Ar은 아릴기이며,
Z는 질소 또는 산소이고,
R_5는 Z가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Z가 산소일 때 존재하지 않으며,
R_6는 수소, 히드록시, C_1-C_4의 알콕시기 또는 옥소(=O)이고,
n은 1 내지 5이다.

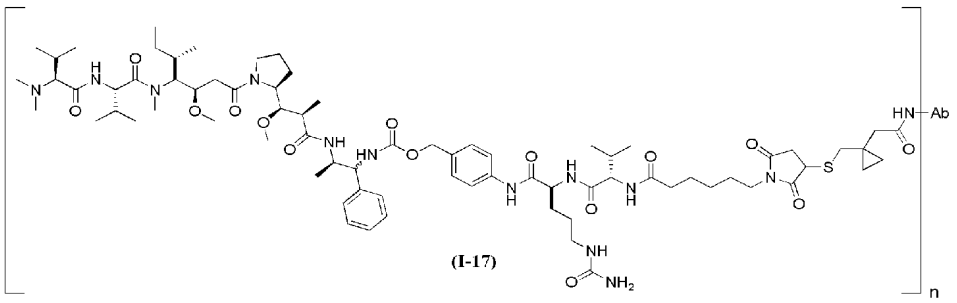
[청구항 9] 제8항에 있어서,
Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,
X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,
R_1, R_2 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,
R_3는 C_1-C_4의 알킬기이며,
Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐이고,
Z는 질소이며,
R_5는 수소 또는 C_1-C_4의 알킬기이고,
R_6는 수소, 히드록시 또는 C_1-C_4의 알콕시기인 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 10] 제8항에 있어서,
Ab가 트라스투주맙(trastuzumab)이고,
X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,
R_1, R_2 및 R_3는 메틸이고,
R_4는 수소 또는 메틸이며,
Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐이고,
Z는 질소이며,
R_5는 수소 또는 메틸이고,
R_6는 수소, 히드록시 또는 메톡시인 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

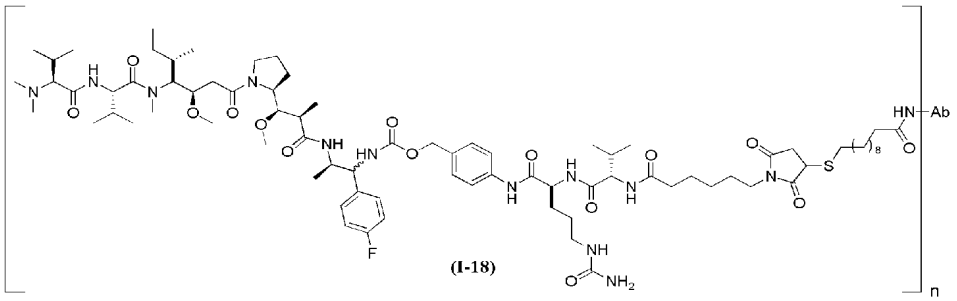
[청구항 11] 제8항에 있어서, 하기 화합물로부터 선택되는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:



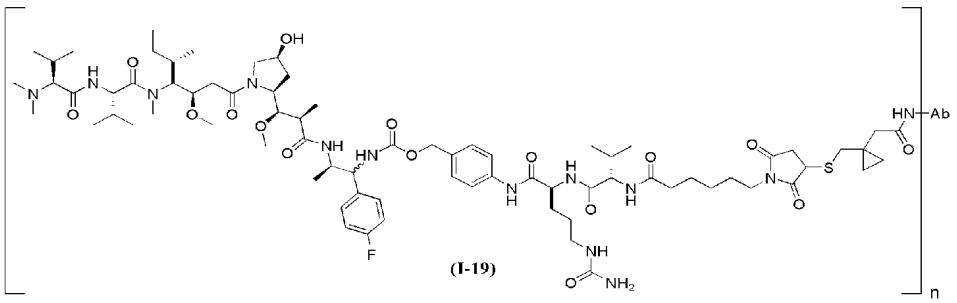
(I-16)



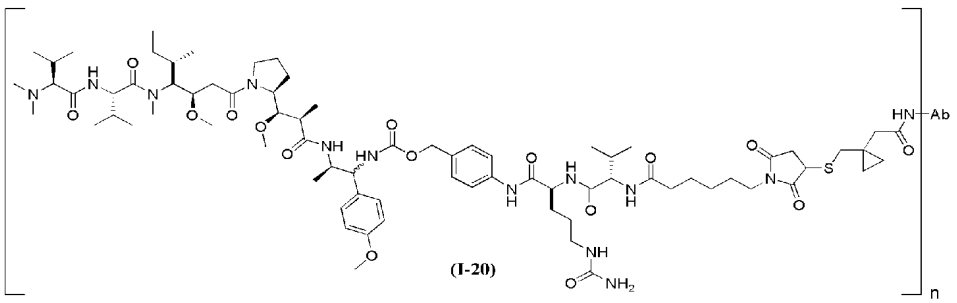
(I-17)



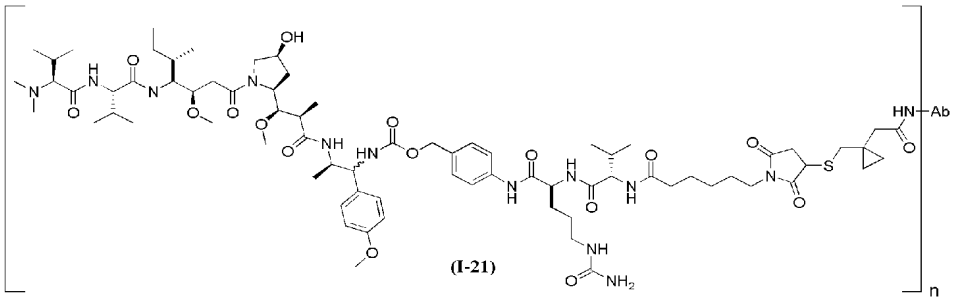
(I-18)

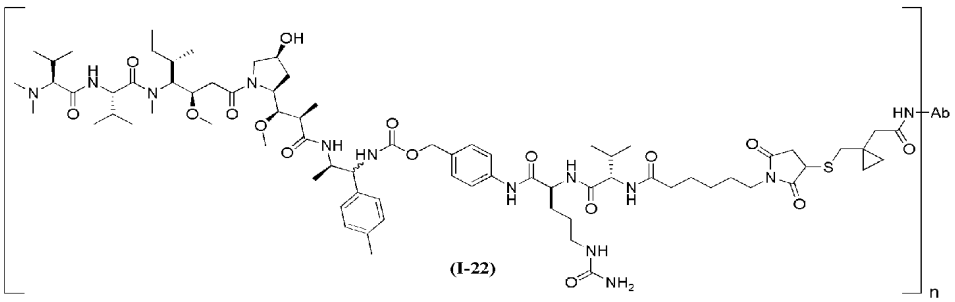


(I-19)



(I-20)





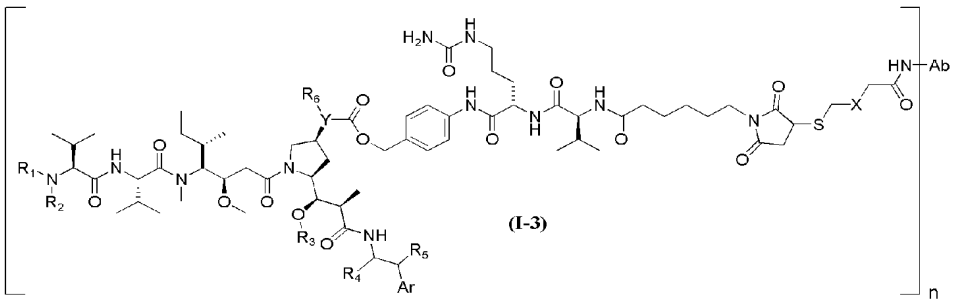
상기 식에서, n은 1 내지 5이다.

[청구항 12] 제8항 내지 제11항 중 어느 한 항에 있어서, 링커-약물이 항체의 라이신 잔기에 직접 결합된 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 13] 제8항 내지 제11항 중 어느 한 항에 있어서, 항체가 92% 이상의 정상 항체 구조 비율을 가지는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 14] 제1항에 있어서, 하기 화학식 I-3의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

[화학식 I-3]



상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1, R_2, R_3 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

R_5는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

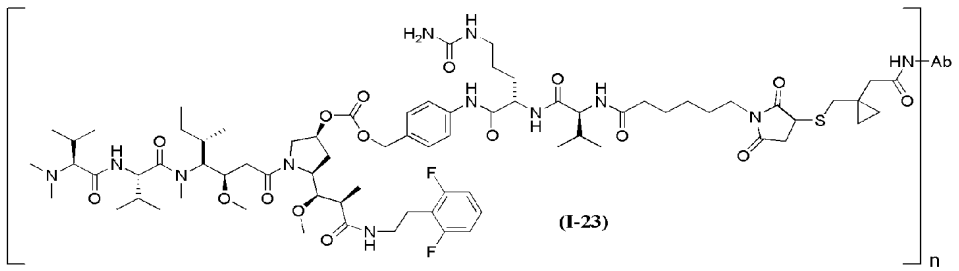
Ar은 아릴기이고,

Y는 질소 또는 산소이며,
R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않으며,
n은 1 내지 5이다.

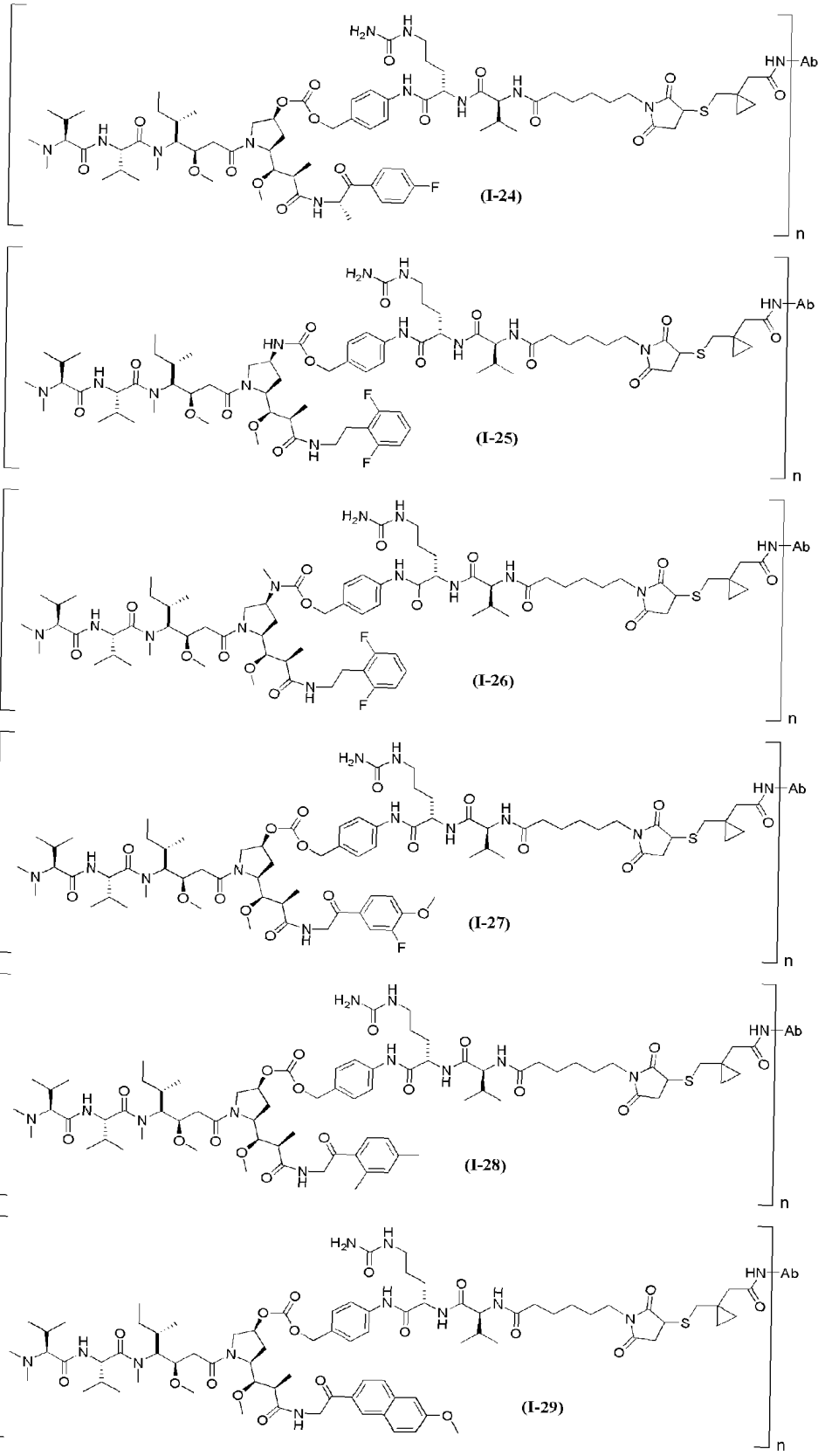
[청구항 15] 제14항에 있어서,
Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,
X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,
R_1, R_2 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,
R_3는 C_1-C_4의 알킬기이며,
R_5는 수소, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,
Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,
Y는 질소 또는 산소이고,
R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

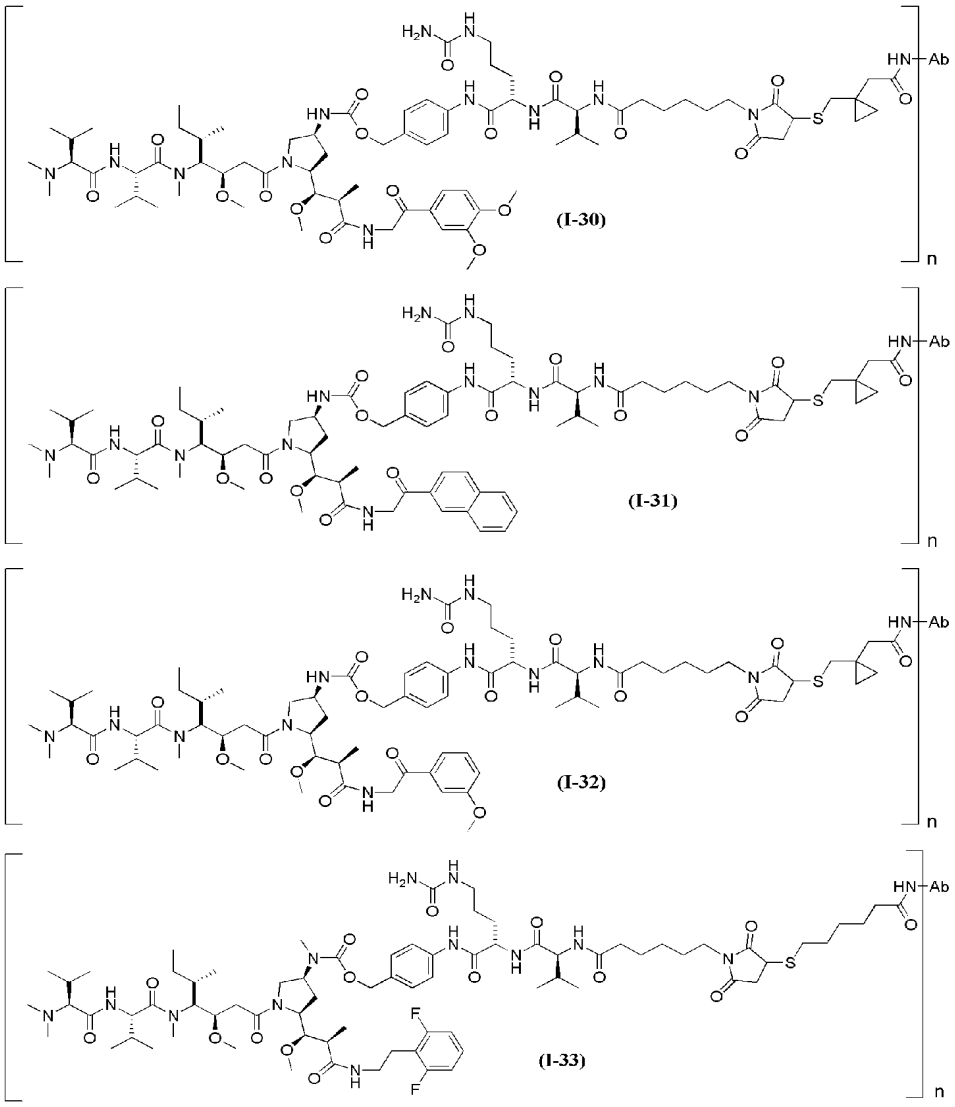
[청구항 16] 제14항에 있어서,
Ab가 트라스투주맙(trastuzumab)이고,
X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,
R_1, R_2 및 R_3는 메틸이고,
R_4는 수소 또는 메틸이며,
R_5는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,
Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,
Y는 질소 또는 산소이고,
R_6는 Y가 질소일 때 수소 또는 메틸이고, Y가 산소일 때 존재하지 않는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 17] 제14항에 있어서, 하기 화합물로부터 선택되는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:



(I-23)


(I-24)
(I-25)
(I-26)
(I-27)
(I-28)
(I-29)
HN—Ab
n



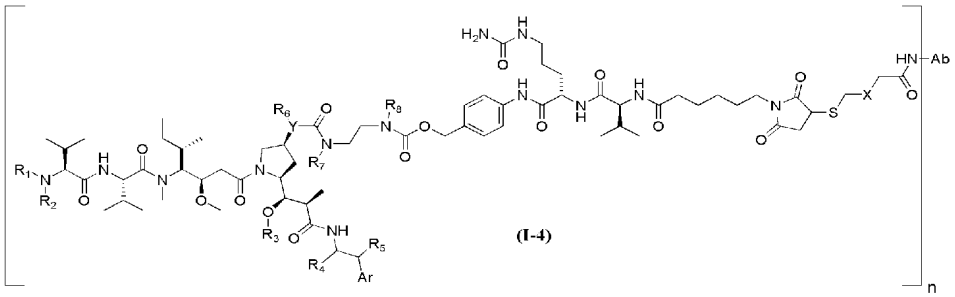
상기 식에서, n은 1 내지 5이다.

[청구항 18] 제14항 내지 제17항 중 어느 한 항에 있어서, 링커-약물이 항체의 라이신 잔기에 직접 결합된 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 19] 제14항 내지 제17항 중 어느 한 항에 있어서, 항체가 90% 이상의 정상 항체 구조 비율을 가지는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 20] 제1항에 있어서, 하기 화학식 I-4의 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

[화학식 I-4]



(I-4)

상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1, R_2, R_3, R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

R_5는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

Ar은 아릴기이고,

Y는 질소 또는 산소이며,

R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않으며,

n은 1 내지 5이다.

[청구항 21] 제20항에 있어서,

Ab가 암세포 항원에 면역특이적으로 결합하는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1, R_2, R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

R_3는 C_1-C_4의 알킬기이며,

R_5는 수소, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,

Ar은 C_1-C_4의 알킬기, C_1-C_4의 알콕시기 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,

Y는 질소 또는 산소이고,

R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 22] 제20항에 있어서,

Ab가 트라스투주맙(trastuzumab)이고,

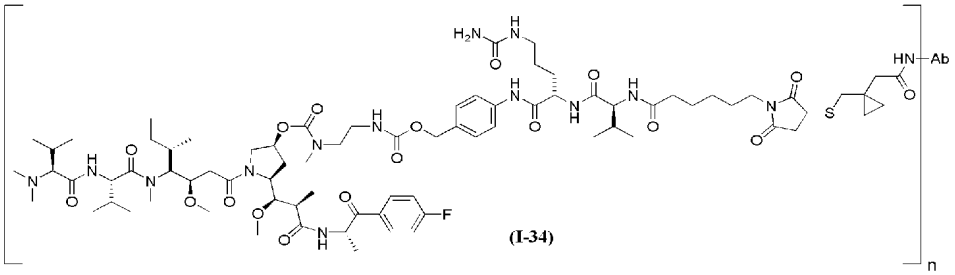
X는 메틸, n-프로필, n-펜틸, n-옥틸 또는 시클로프로필이며,

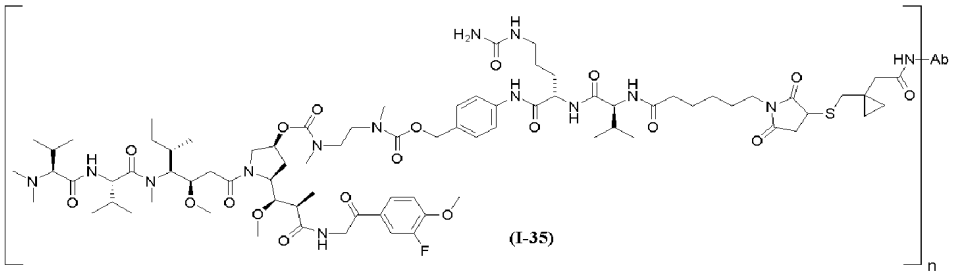
R_1, R_2 및 R_3는 메틸이고,

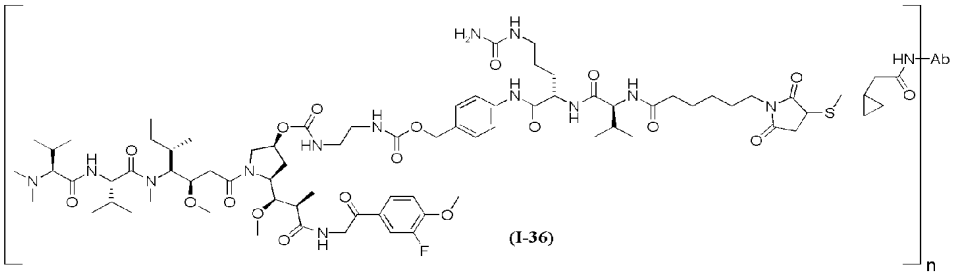
R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 메틸이며,

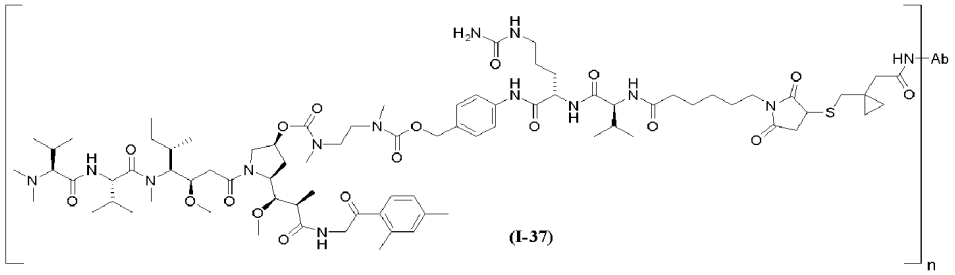
R_5는 수소, 옥소(=O) 또는 히드록시이미노(=N-OH)이고,
Ar은 메틸, 메톡시 및 할로겐으로 구성된 군으로부터 선택된 하나 이상의 치환기로 치환되거나 치환되지 않은 페닐 또는 나프틸이며,
Y는 질소 또는 산소이고,
R_6는 Y가 질소일 때 수소 또는 메틸이고, Y가 산소일 때 존재하지 않는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

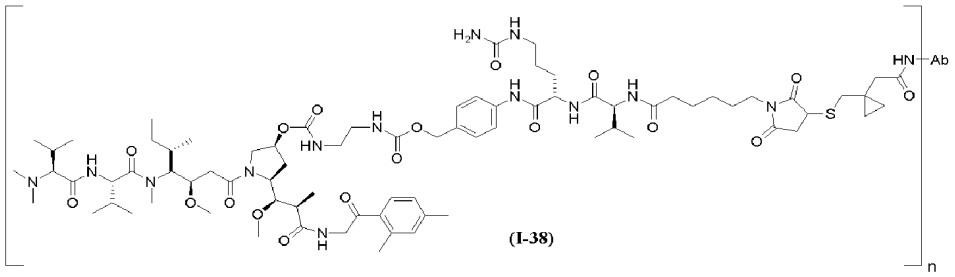
[청구항 23] 제20항에 있어서, 하기 화합물로부터 선택되는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염:

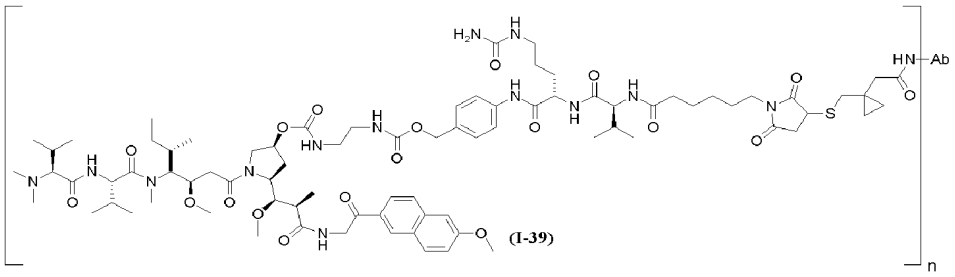


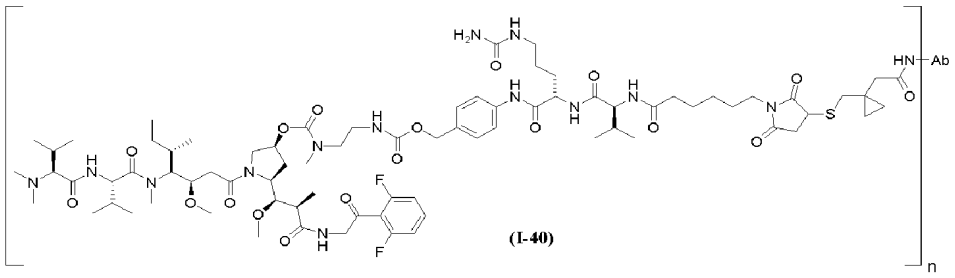


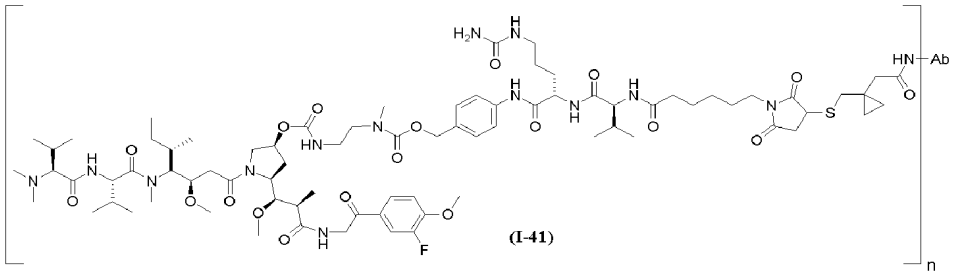












상기 식에서, n은 1 내지 5이다.

[청구항 24] 제20항 내지 제23항 중 어느 한 항에 있어서, 링커-약물이 항체의 라이신 잔기에 직접 결합된 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

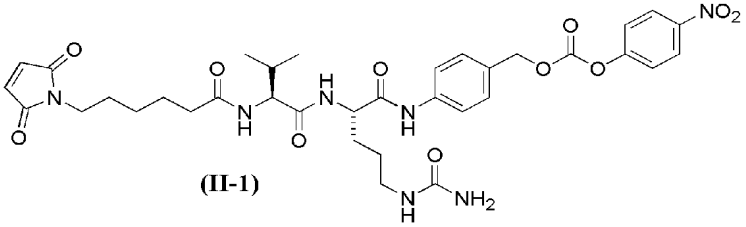
[청구항 25] 20항 내지 제23항 중 어느 한 항에 있어서, 항체가 97% 이상의 정상 항체 구조 비율을 가지는 것을 특징으로 하는 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염.

[청구항 26] (i) 하기 화학식 II-1의 화합물과 하기 화학식 III-1의 돌라스타틴 10 유도체를 축합반응시켜 하기 화학식 IV-1의 화합물을 수득하는 단계;

(ii) 하기 화학식 IV-1의 화합물에 하기 화학식 V의 화합물을 첨가반응시켜 하기 화학식 VI-1의 화합물을 수득하는 단계;

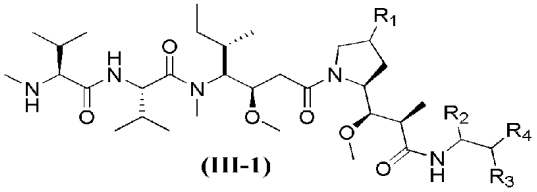
(iii) 하기 화학식 VI-1의 화합물과 N-히드록시숙신이미드를 축합반응시켜 하기 화학식 VII-1의 화합물을 수득하는 단계; 및
(iv) 하기 화학식 VII-1의 화합물과 항체를 반응시키는 단계를 포함하는 하기 화학식 I-1의 항체-링커-약물 결합체의 제조방법:

[화학식 II-1]



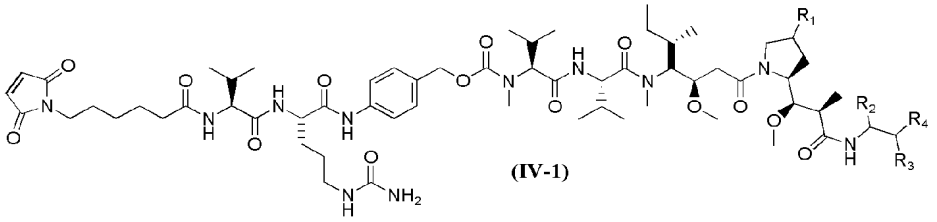
(II-1)

[화학식 III-1]



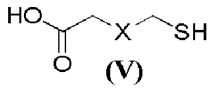
(III-1)

[화학식 IV-1]



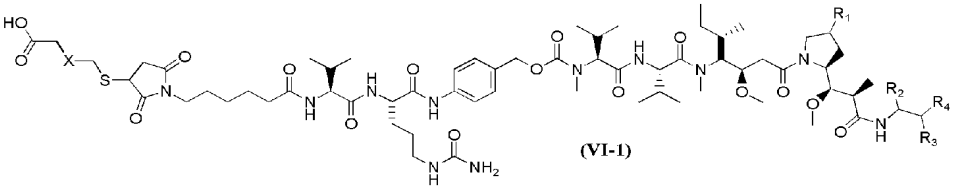
(IV-1)

[화학식 V]



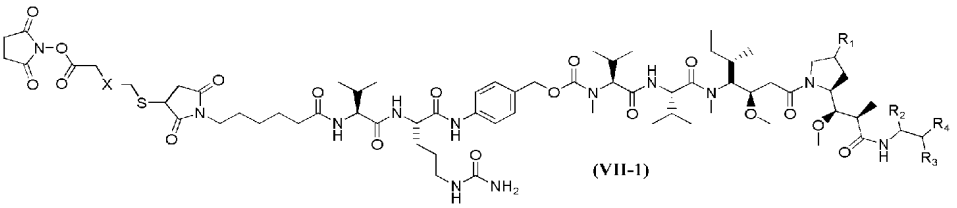
(V)

[화학식 VI-1]



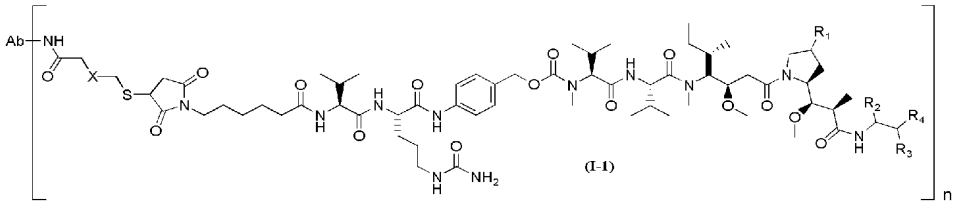
(VI-1)

[화학식 VII-1]



(VII-1)

[화학식 I-1]



(I-1)

상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1은 수소, 히드록시, C_1-C_4의 알콕시기 또는 옥소(=O)이고,

R_2는 수소 또는 C_1-C_4의 알킬기이며,

R_3는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

R_4는 아릴기이고,

n은 1 내지 5이다.

[청구항 27] 제26항에 있어서, 단계 (i)의 축합반응이 히드록시벤조트리아졸(HOBt), 히드록시아자벤조트리아졸(HOAt) 및 히드록시숙신이미드(HOSu)로 구성된 군으로부터 선택된 축합제와 피리딘 및 디이소프로필에틸아민으로 구성된 군으로부터 선택된 유기 염기의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 28] 제26항에 있어서, 단계 (iii)의 축합반응이 디시클로헥실카르보디이미드(DCC) 및 (3-디메틸아미노프로필)카르보디이미드(EDC)로 구성된 군으로부터 선택된 축합제의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 29] 제26항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼인 것을 특징으로 하는 제조방법.

[청구항 30] 제26항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼와 유기용매의 혼합용매인 것을 특징으로 하는 제조방법.

[청구항 31] 제30항에 있어서, 혼합용매 중 유기용매의 비율은 50% 이하인 것을 특징으로 하는 제조방법.

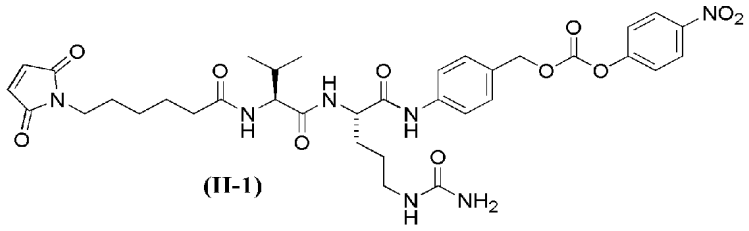
[청구항 32] 제26항에 있어서, 단계 (iv)에서 화학식 VII-1의 화합물이 항체를 기준으로 3 내지 25 당량 사용되는 것을 특징으로 하는 제조방법.

[청구항 33] (i) 하기 화학식 II-1의 화합물과 하기 화학식 III-2의 돌라스타틴 10 유도체를 축합반응시켜 하기 화학식 IV-2의 화합물을 수득하는 단계;

(ii) 하기 화학식 IV-2의 화합물에 하기 화학식 V의 화합물을

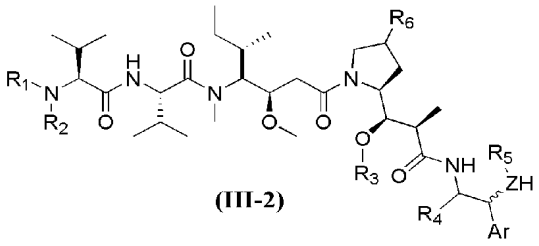
첨가반응시켜 하기 화학식 VI-2의 화합물을 수득하는 단계;
(iii) 하기 화학식 VI-2의 화합물과 N-히드록시숙신이미드를 축합반응시켜 하기 화학식 VII-2의 화합물을 수득하는 단계; 및
(iv) 하기 화학식 VII-2의 화합물과 항체를 반응시키는 단계를 포함하는 하기 화학식 I-2의 항체-링커-약물 결합체의 제조방법:

[화학식 II-1]



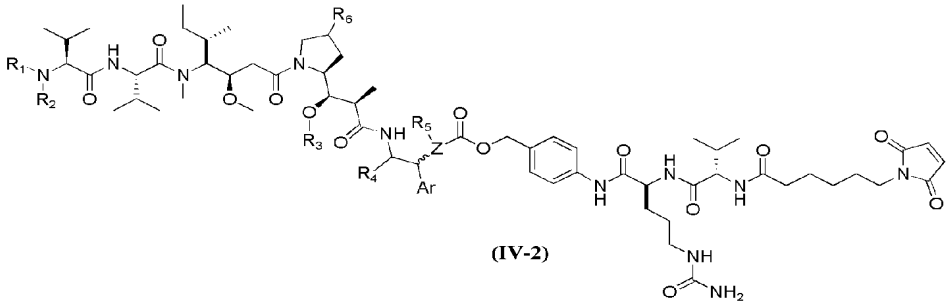
(II-1)

[화학식 III-2]



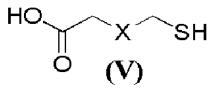
(III-2)

[화학식 IV-2]



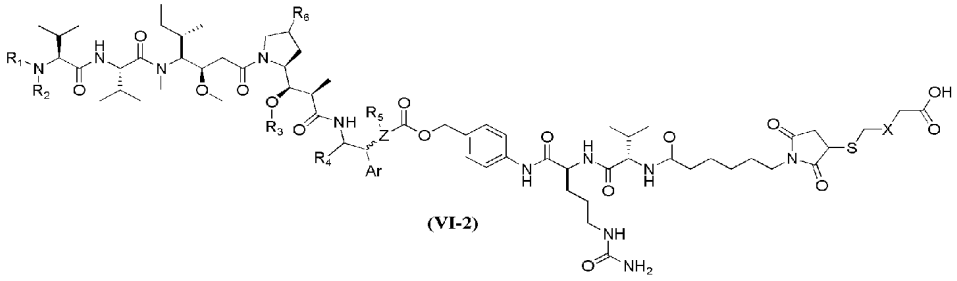
(IV-2)

[화학식 V]



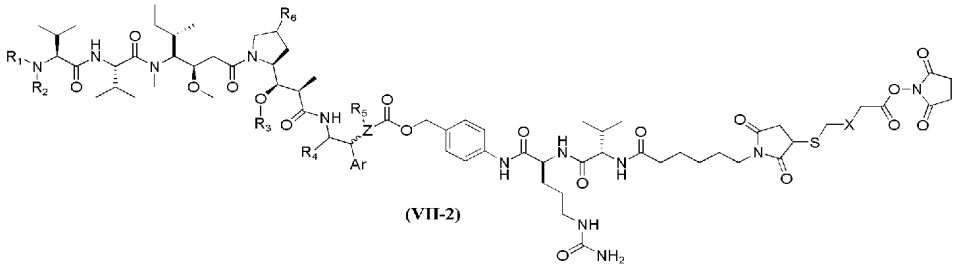
(V)

[화학식 VI-2]



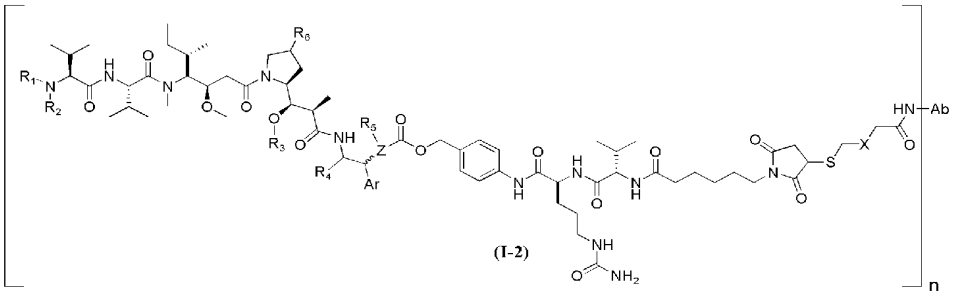
(VI-2)

[화학식 VII-2]



(VII-2)

[화학식 I-2]



(I-2)

상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1, R_2, R_3 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

Ar은 아릴기이며,

Z는 질소 또는 산소이고,

R_5는 Z가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Z가 산소일 때 존재하지 않으며,

R_6는 수소, 히드록시, C_1-C_4의 알콕시기 또는 옥소(=O)이고,

n은 1 내지 5이다.

[청구항 34] 제33항에 있어서, 단계 (i)의 축합반응이 히드록시벤조트리아졸(HOBt), 히드록시아자벤조트리아졸(HOAt) 및 히드록시숙신이미드(HOSu)로 구성된 군으로부터 선택된 축합제와 피리딘 및 디이소프로필에틸아민으로 구성된 군으로부터 선택된 유기 염기의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 35] 제33항에 있어서, 단계 (iii)의 축합반응이 디시클로헥실카르보디이미드(DCC) 및 (3-디메틸아미노프로필)카르보디이미드(EDC)로 구성된 군으로부터 선택된 축합제의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 36] 제33항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼인 것을 특징으로 하는 제조방법.

[청구항 37] 제33항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의

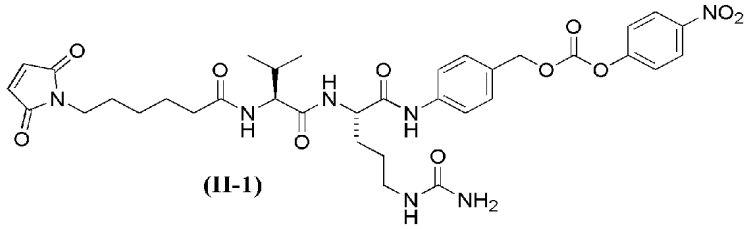
포스페이트 버퍼와 유기용매의 혼합용매인 것을 특징으로 하는 제조방법.

[청구항 38] 제37항에 있어서, 혼합용매 중 유기용매의 비율은 50% 이하인 것을 특징으로 하는 제조방법.

[청구항 39] 제33항에 있어서, 단계 (iv)에서 화학식 VII-2의 화합물이 항체를 기준으로 3 내지 25 당량 사용되는 것을 특징으로 하는 제조방법.

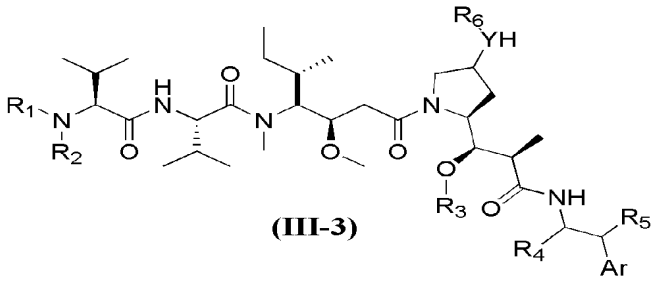
[청구항 40] (i) 하기 화학식 II-1의 화합물과 하기 화학식 III-3의 돌라스타틴 10 유도체를 축합반응시켜 하기 화학식 IV-3의 화합물을 수득하는 단계;
(ii) 하기 화학식 IV-3의 화합물에 하기 화학식 V의 화합물을 첨가반응시켜 하기 화학식 VI-3의 화합물을 수득하는 단계;
(iii) 하기 화학식 VI-3의 화합물과 N-히드록시숙신이미드를 축합반응시켜 하기 화학식 VII-3의 화합물을 수득하는 단계; 및
(iv) 하기 화학식 VII-3의 화합물과 항체를 반응시키는 단계를 포함하는 하기 화학식 I-3의 항체-링커-약물 결합체의 제조방법:

[화학식 II-1]



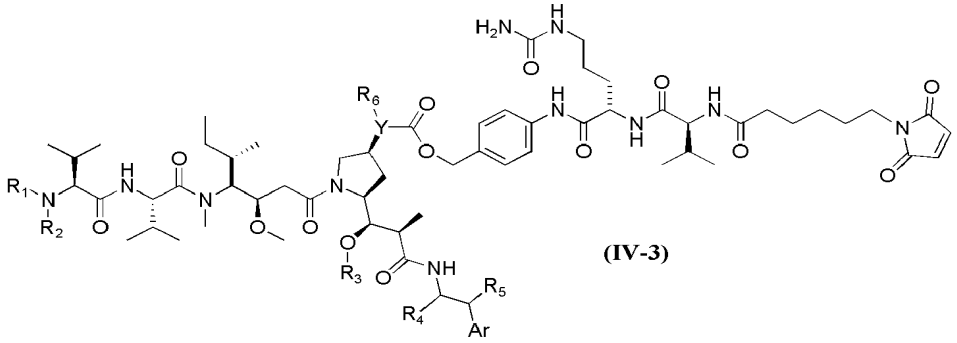
(II-1)

[화학식 III-3]



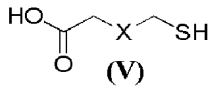
(III-3)

[화학식 IV-3]



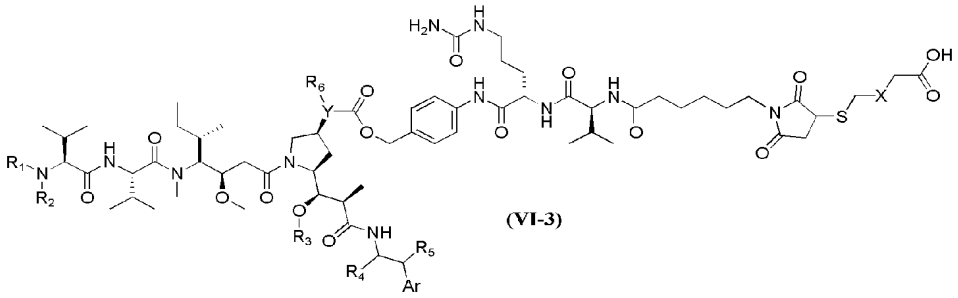
(IV-3)

[화학식 V]



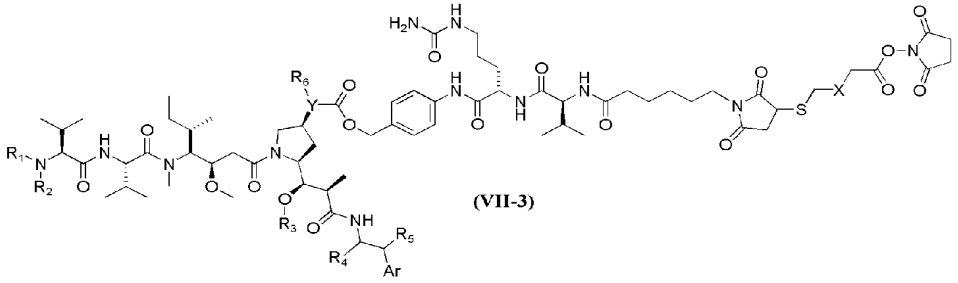
(V)

[화학식 VI-3]



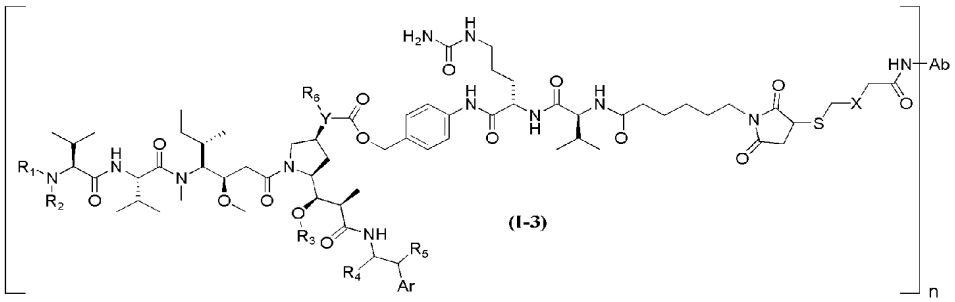
(VI-3)

[화학식 VII-3]



(VII-3)

[화학식 I-3]



(I-3)

상기 식에서,

Ab는 항체이고,

X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,

R_1, R_2, R_3 및 R_4는 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,

R_5는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,

Ar은 아릴기이고,

Y는 질소 또는 산소이며,

R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않으며,

n은 1 내지 5이다.

[청구항 41] 제40항에 있어서, 단계 (i)의 축합반응이 히드록시벤조트리아졸(HOBt), 히드록시아자벤조트리아졸(HOAt) 및 히드록시숙신이미드(HOSu)로 구성된 군으로부터 선택된 축합제와 피리딘 및 디이소프로필에틸아민으로 구성된 군으로부터 선택된 유기 염기의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 42] 제40항에 있어서, 단계 (iii)의 축합반응이 디시클로헥실카르보디이미드(DCC) 및 (3-디메틸아미노프로필)카르보디이미드(EDC)로 구성된 군으로부터 선택된 축합제의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 43] 제40항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼인 것을 특징으로 하는 제조방법.

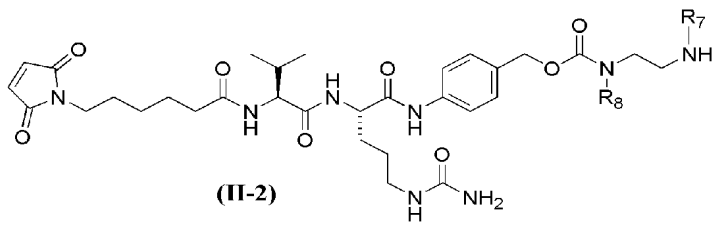
[청구항 44] 제40항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼와 유기용매의 혼합용매인 것을 특징으로 하는 제조방법.

[청구항 45] 제44항에 있어서, 혼합용매 중 유기용매의 비율은 50% 이하인 것을 특징으로 하는 제조방법.

[청구항 46] 제40항에 있어서, 단계 (iv)에서 화학식 VII-3의 화합물이 항체를 기준으로 3 내지 25 당량 사용되는 것을 특징으로 하는 제조방법.

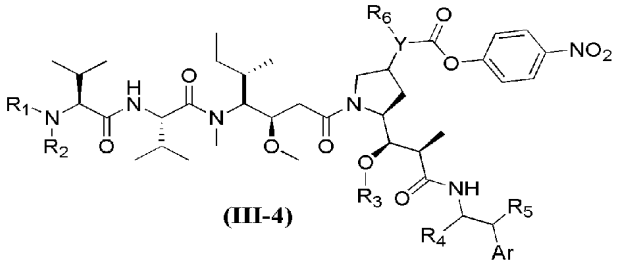
[청구항 47] (i) 하기 화학식 II-2의 화합물과 하기 화학식 III-4의 돌라스타틴 10 유도체를 축합반응시켜 하기 화학식 IV-4의 화합물을 수득하는 단계;
(ii) 하기 화학식 IV-4의 화합물에 하기 화학식 V의 화합물을 첨가반응시켜 하기 화학식 VI-4의 화합물을 수득하는 단계;
(iii) 하기 화학식 VI-4의 화합물과 N-히드록시숙신이미드를 축합반응시켜 하기 화학식 VII-4의 화합물을 수득하는 단계; 및
(iv) 하기 화학식 VII-4의 화합물과 항체를 반응시키는 단계를 포함하는 하기 화학식 I-4의 항체-링커-약물 결합체의 제조방법:

[화학식 II-2]



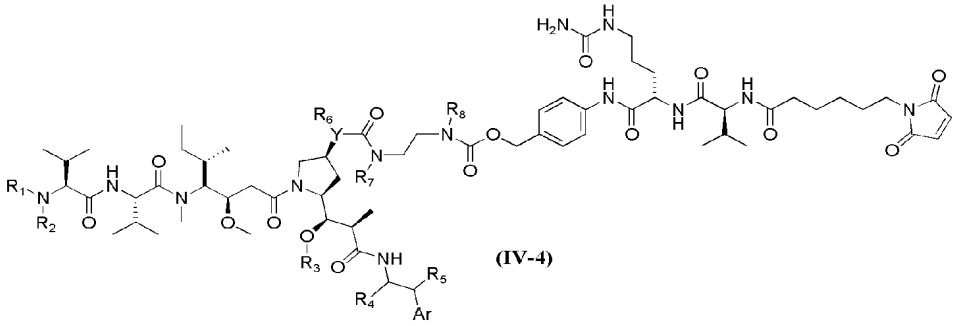
(II-2)

[화학식 III-4]



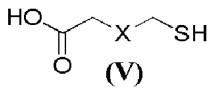
(III-4)

[화학식 IV-4]



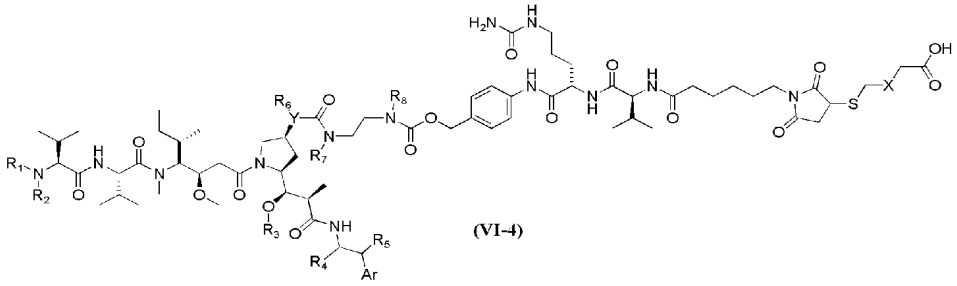
(IV-4)

[화학식 V]



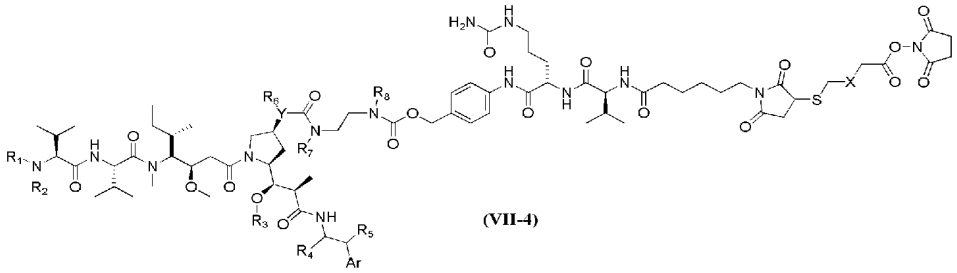
(V)

[화학식 VI-4]



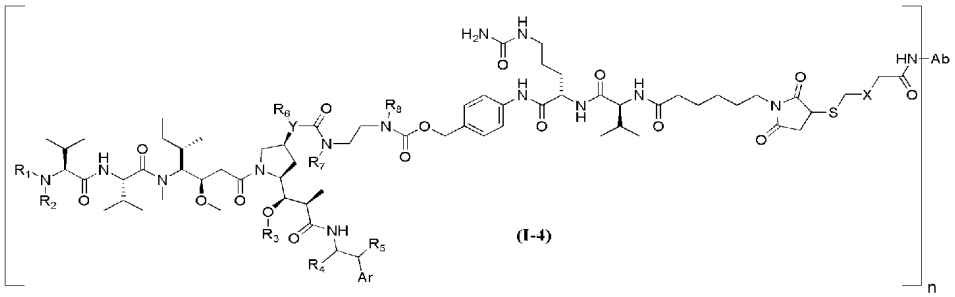
(VI-4)

[화학식 VII-4]



(VII-4)

[화학식 I-4]



(I-4)

상기 식에서,
Ab는 항체이고,
X는 C_1-C_8의 알킬기 또는 C_3-C_6의 시클로알킬기이며,
R_1, R_2, R_3, R_4, R_7 및 R_8은 각각 독립적으로 수소 또는 C_1-C_4의 알킬기이고,
R_5는 수소, 히드록시, C_1-C_4의 알콕시기, 아미노, 옥소(=O) 또는 히드록시이미노(=N-OH)이며,
Ar은 아릴기이고,
Y는 질소 또는 산소이며,
R_6는 Y가 질소일 때 수소 또는 C_1-C_4의 알킬기이고, Y가 산소일 때 존재하지 않으며,
n은 1 내지 5이다.

[청구항 48] 제47항에 있어서, 단계 (i)의 축합반응이 히드록시벤조트리아졸(HOBt), 히드록시아자벤조트리아졸(HOAt) 및 히드록시숙신이미드(HOSu)로 구성된 군으로부터 선택된 축합제와 피리딘 및 디이소프로필에틸아민으로 구성된 군으로부터 선택된 유기 염기의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 49] 제47항에 있어서, 단계 (iii)의 축합반응이 디시클로헥실카르보디이미드(DCC) 및 (3-디메틸아미노프로필)카르보디이미드(EDC)로 구성된 군으로부터 선택된 축합제의 존재 하에 수행되는 것을 특징으로 하는 제조방법.

[청구항 50] 제47항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼인 것을 특징으로 하는 제조방법.

[청구항 51] 제47항에 있어서, 단계 (iv)의 반응용매가 pH 6.0 내지 8.0의 포스페이트 버퍼와 유기용매의 혼합용매인 것을 특징으로 하는 제조방법.

[청구항 52] 제51항에 있어서, 혼합용매 중 유기용매의 비율은 50% 이하인 것을 특징으로 하는 제조방법.

[청구항 53] 제47항에 있어서, 단계 (iv)에서 화학식 VII-4의 화합물이 항체를

기준으로 3 내지 25 당량 사용되는 것을 특징으로 하는 제조방법.

[청구항 54] 제1항 내지 제5항 중 어느 한 항에 따른 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염을 약제학적으로 허용가능한 담체와 함께 포함하는 항암제 조성물.

[청구항 55] 제54항에 있어서, 항암제가 유방암 치료에 적용되는 것을 특징으로 하는 항암제 조성물.

[청구항 56] 제8항 내지 제11항 중 어느 한 항에 따른 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염을 약제학적으로 허용가능한 담체와 함께 포함하는 항암제 조성물.

[청구항 57] 제56항에 있어서, 항암제가 유방암 치료에 적용되는 것을 특징으로 하는 항암제 조성물.

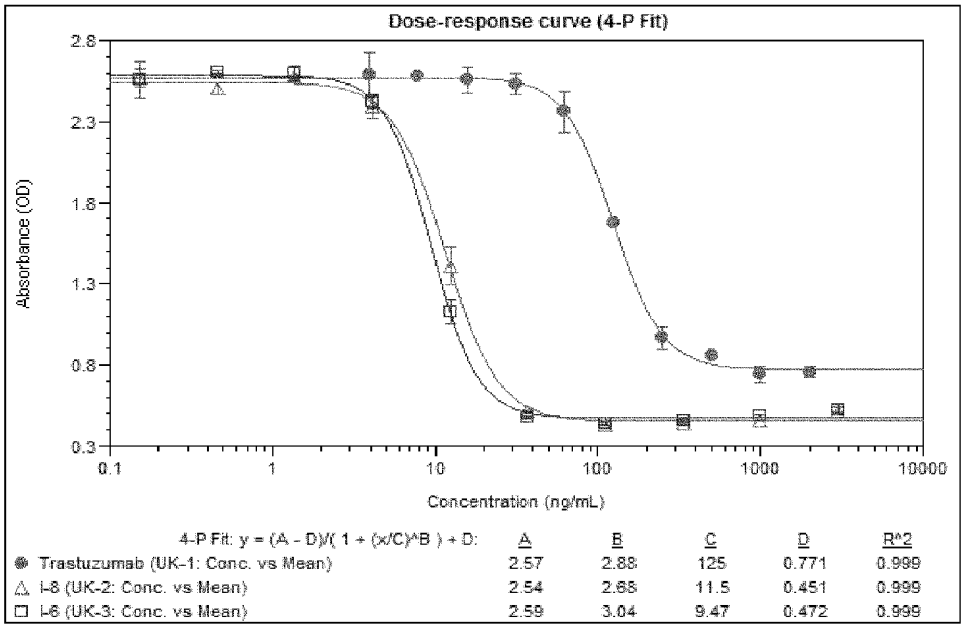
[청구항 58] 제14항 내지 제17항 중 어느 한 항에 따른 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염을 약제학적으로 허용가능한 담체와 함께 포함하는 항암제 조성물.

[청구항 59] 제58항에 있어서, 항암제가 유방암 치료에 적용되는 것을 특징으로 하는 항암제 조성물.

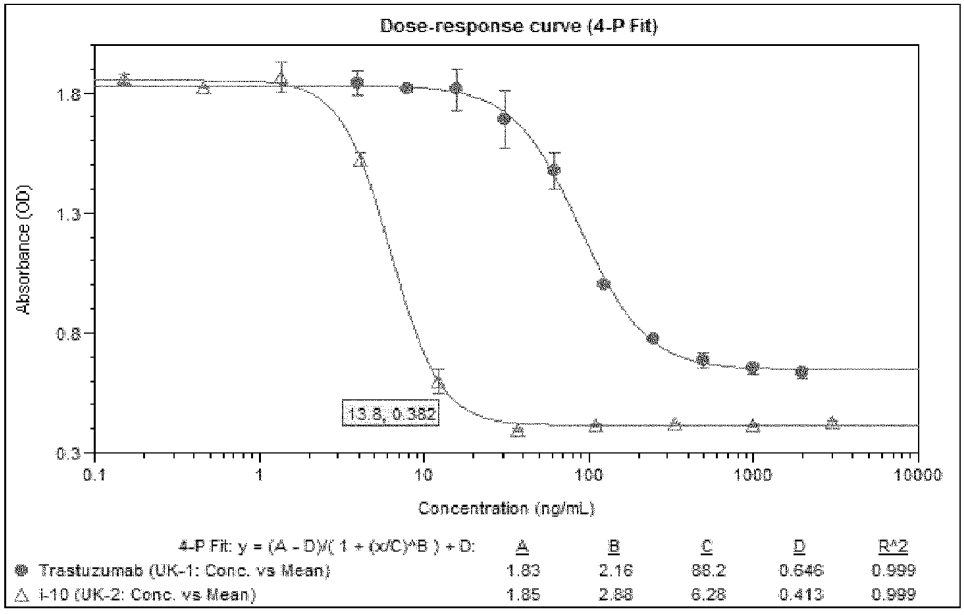
[청구항 60] 제20항 내지 제23항 중 어느 한 항에 따른 항체-링커-약물 결합체 또는 그의 약제학적으로 허용되는 염을 약제학적으로 허용가능한 담체와 함께 포함하는 항암제 조성물.

[청구항 61] 제60항에 있어서, 항암제가 유방암 치료에 적용되는 것을 특징으로 하는 항암제 조성물.

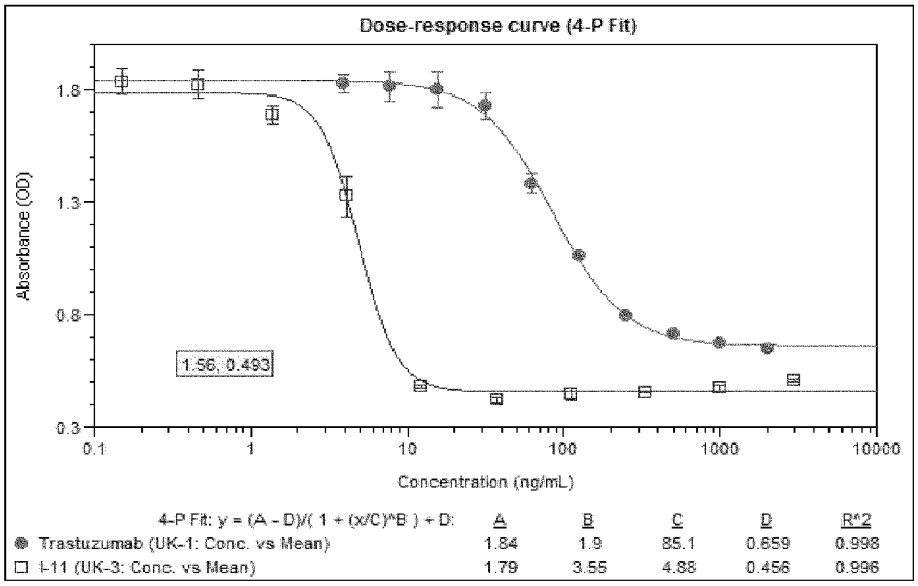
[Fig. 1]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 2.57 2.88 125 0.771 0.999
I-8 (UK-2: Conc. vs Mean) 2.54 2.68 11.5 0.451 0.999
I-6 (UK-3: Conc. vs Mean) 2.59 3.04 9.47 0.472 0.999

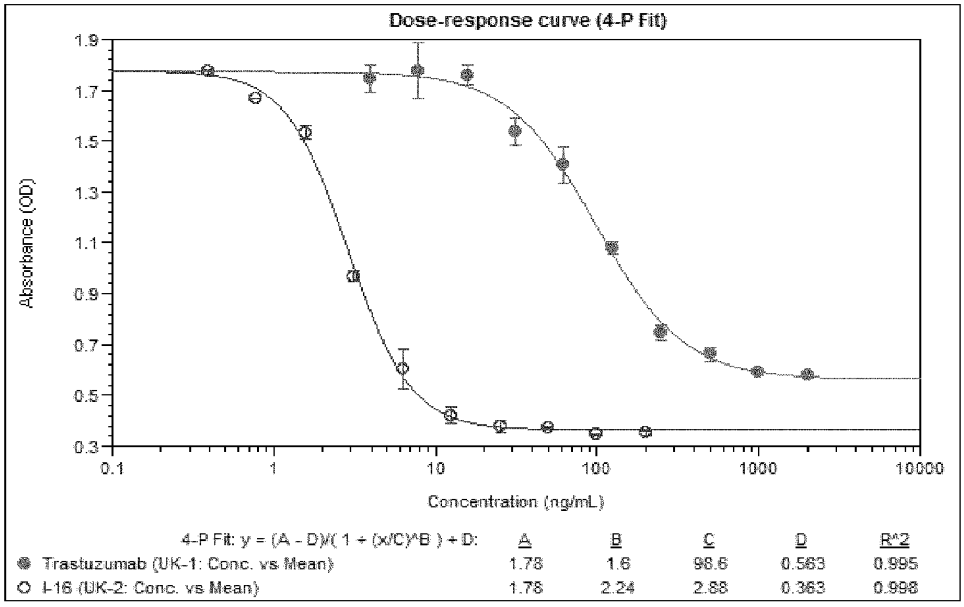
[Fig. 2]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
13.8, 0.382
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.83 2.16 88.2 0.646 0.999
I-10 (UK-2: Conc. vs Mean) 1.85 2.88 6.28 0.413 0.999

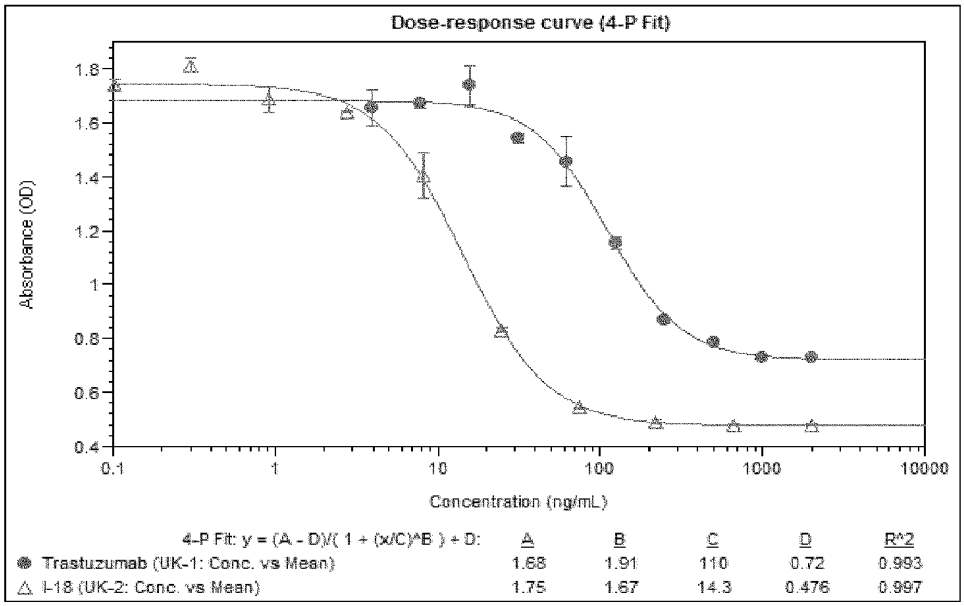
[Fig. 3]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
1.56, 0.493
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean)
1.84
1.9
85.1
0.659
0.998
I-11 (UK-3: Conc. vs Mean)
1.79
3.55
4.88
0.456
0.996

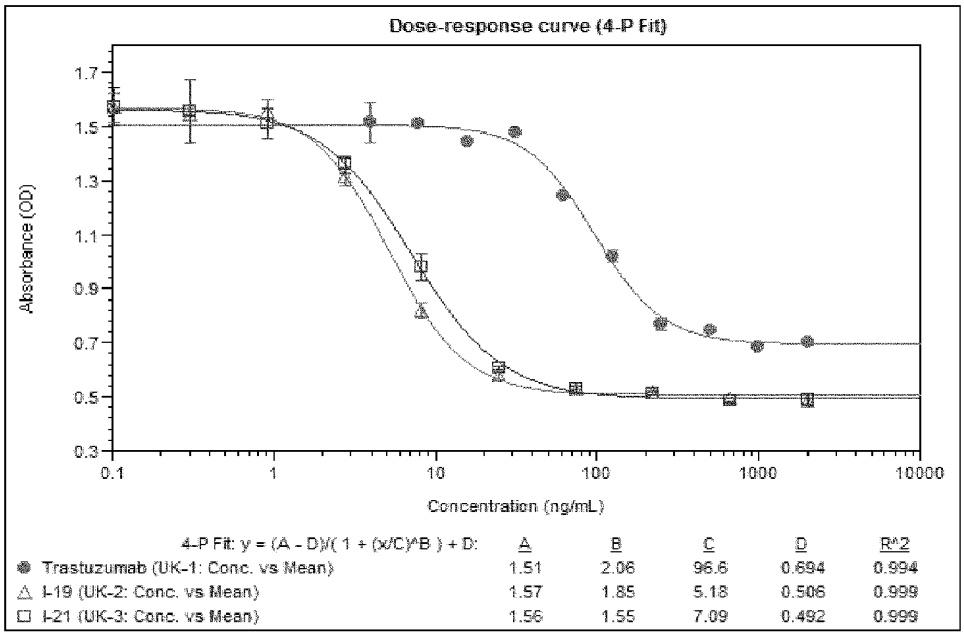
[Fig. 4]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean)
1.78
1.6
98.6
0.563
0.995
I-16 (UK-2: Conc. vs Mean)
1.78
2.24
2.88
0.363
0.998

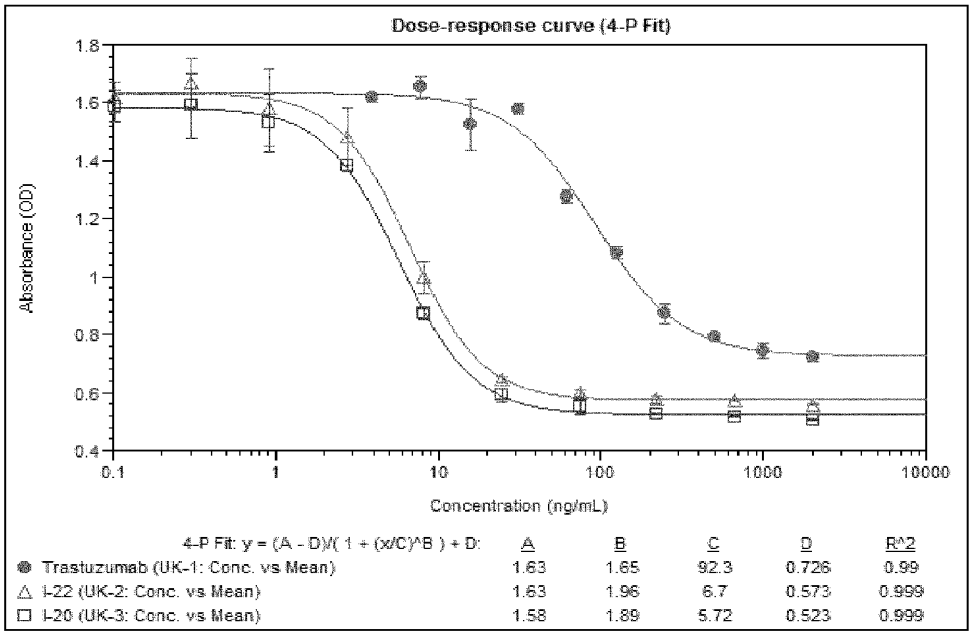
[Fig. 5]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.68 1.91 110 0.72 0.993
I-18 (UK-2: Conc. vs Mean) 1.75 1.67 14.3 0.476 0.997

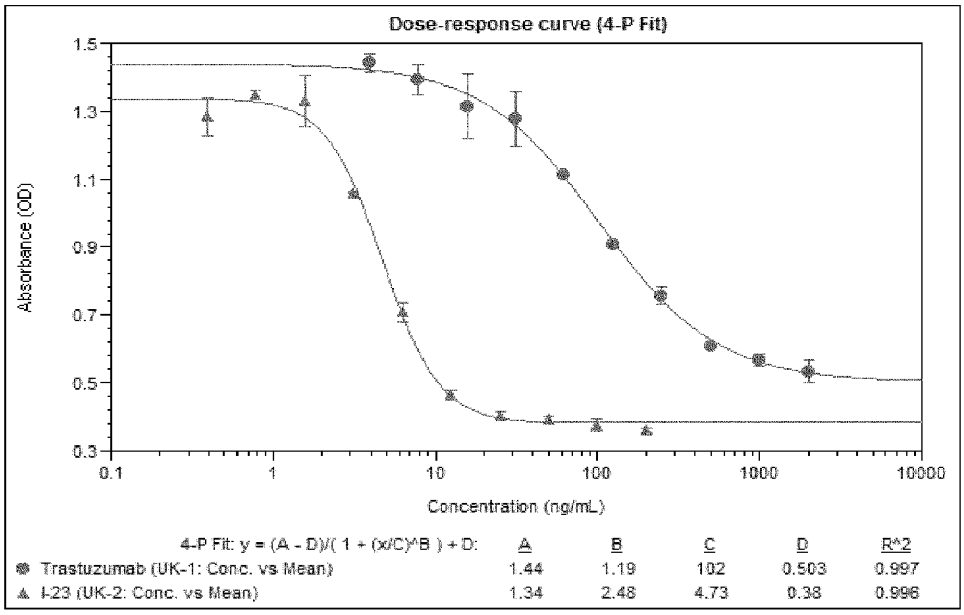
[Fig. 6]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.51 2.06 96.6 0.694 0.994
I-19 (UK-2: Conc. vs Mean) 1.57 1.85 5.18 0.506 0.999
I-21 (UK-3: Conc. vs Mean) 1.56 1.55 7.09 0.492 0.999

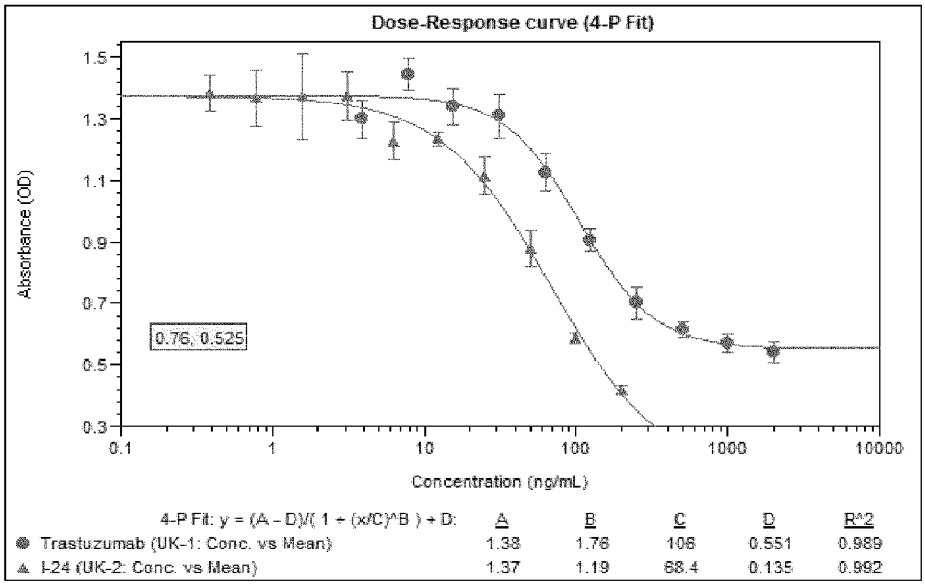
[Fig. 7]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.63 1.65 92.3 0.726 0.99
I-22 (UK-2: Conc. vs Mean) 1.63 1.96 6.7 0.573 0.999
I-20 (UK-3: Conc. vs Mean) 1.58 1.89 5.72 0.523 0.999

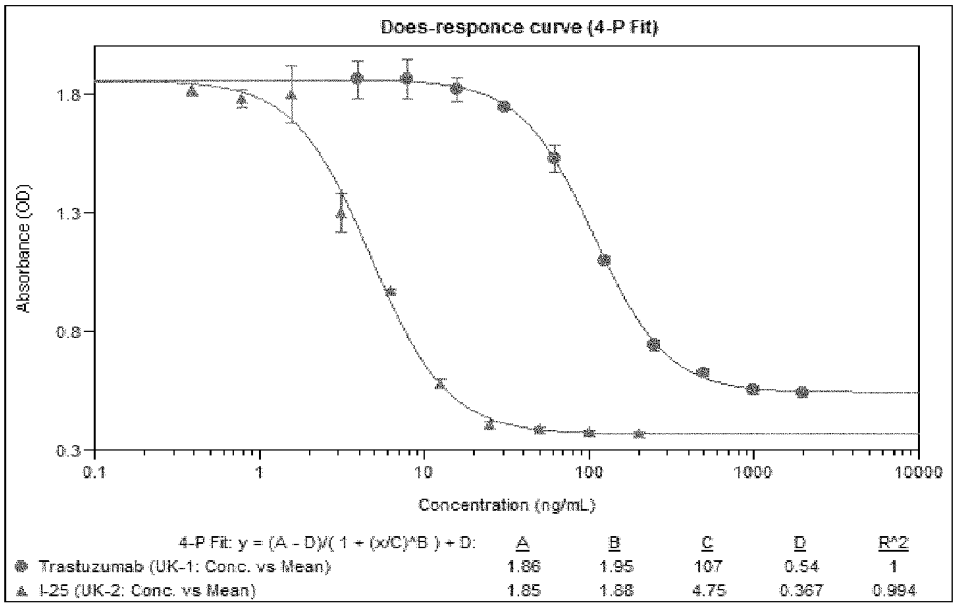
[Fig. 8]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.44 1.19 102 0.503 0.997
I-23 (UK-2: Conc. vs Mean) 1.34 2.48 4.73 0.38 0.996

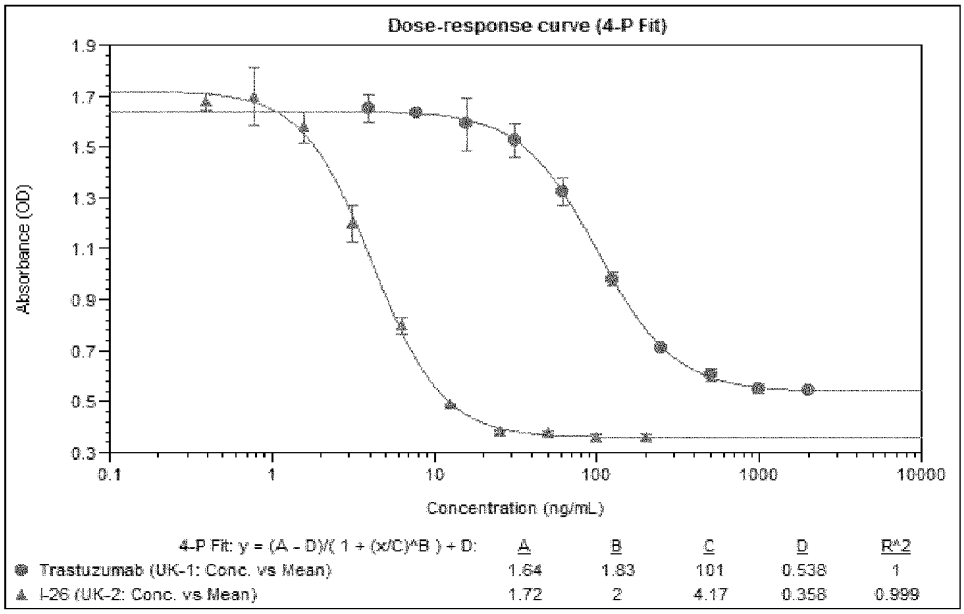
[Fig. 9]


Dose-Response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
0.76, 0.525
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean) 1.38 1.78 108 0.551 0.989
I-24 (UK-2: Conc. vs Mean) 1.37 1.19 68.4 0.135 0.992

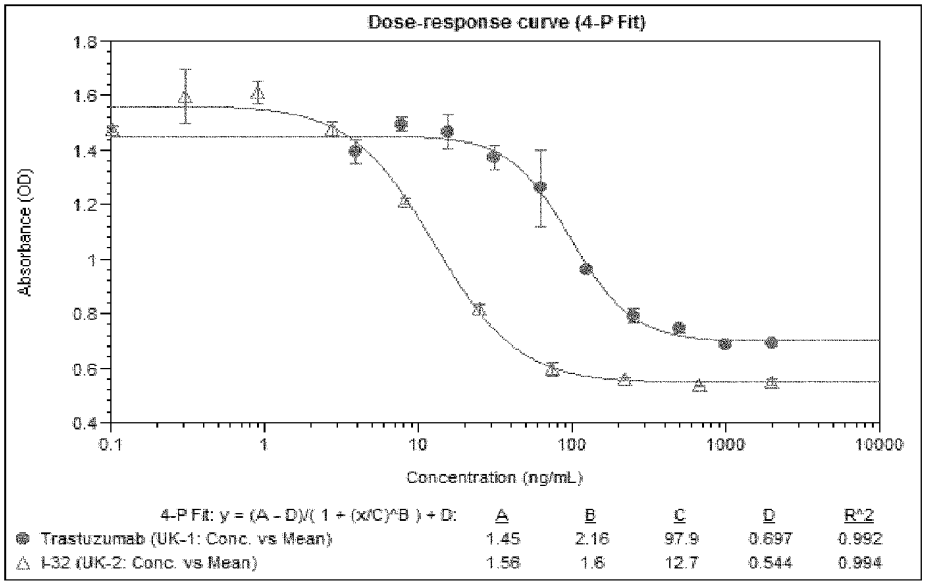
[Fig. 10]


Does-responce curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean) 1.86 1.95 107 0.54 1
I-25 (UK-2: Conc. vs Mean) 1.85 1.88 4.75 0.367 0.994

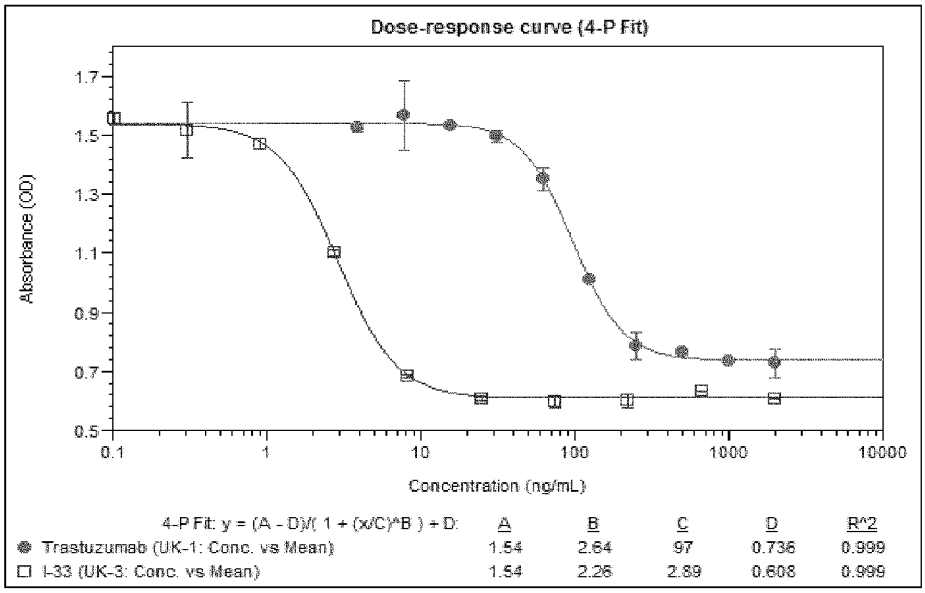
[Fig. 11]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean)
1.64
1.83
101
0.538
1
I-26 (UK-2: Conc. vs Mean)
1.72
2
4.17
0.358
0.999

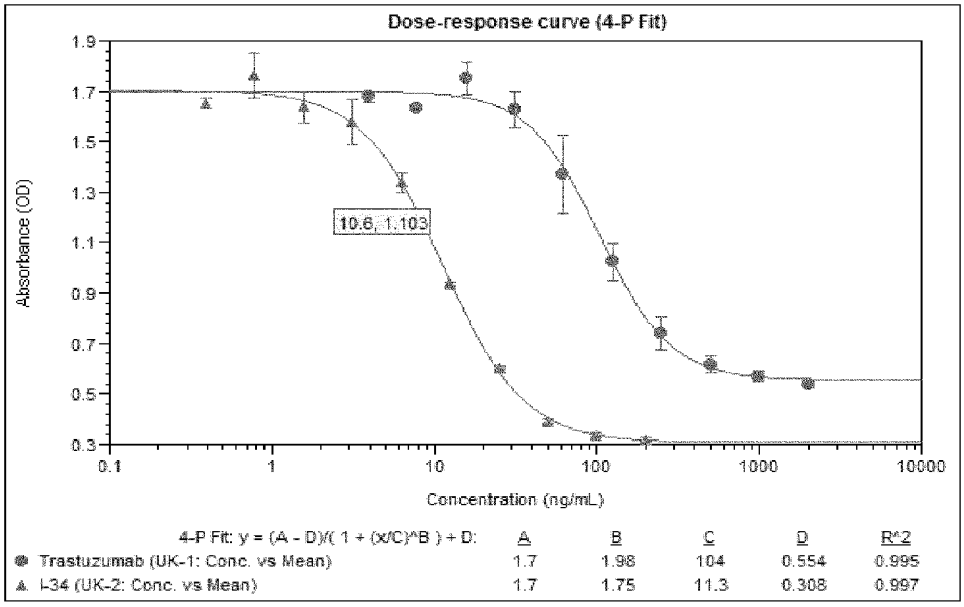
[Fig. 12]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
Trastuzumab (UK-1: Conc. vs Mean)
1.45
2.16
97.9
0.697
0.992
I-32 (UK-2: Conc. vs Mean)
1.56
1.6
12.7
0.544
0.994

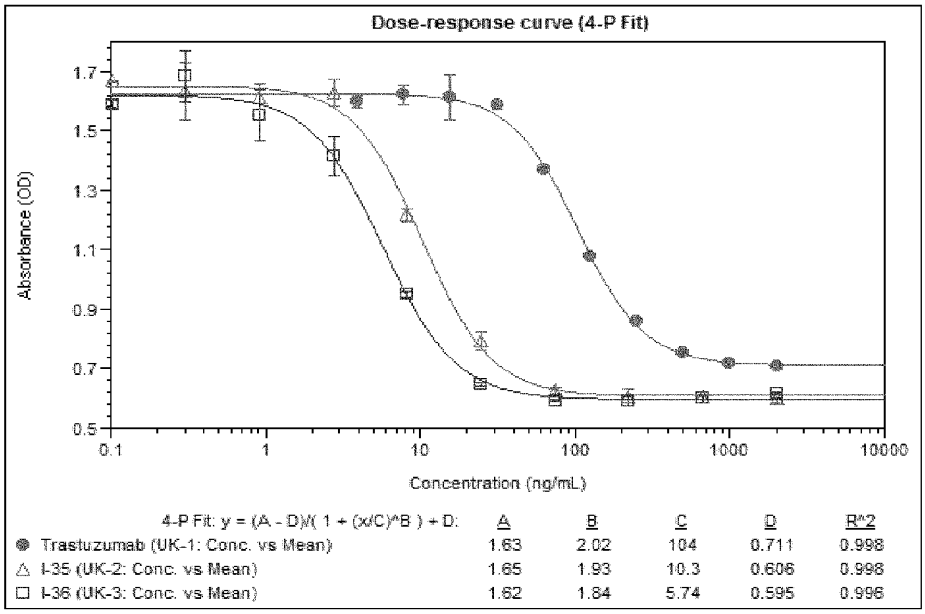
[Fig. 13]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A B C D R^2
Trastuzumab (UK-1: Conc. vs Mean) 1.54 2.64 97 0.736 0.999
I-33 (UK-3: Conc. vs Mean) 1.54 2.26 2.89 0.608 0.999

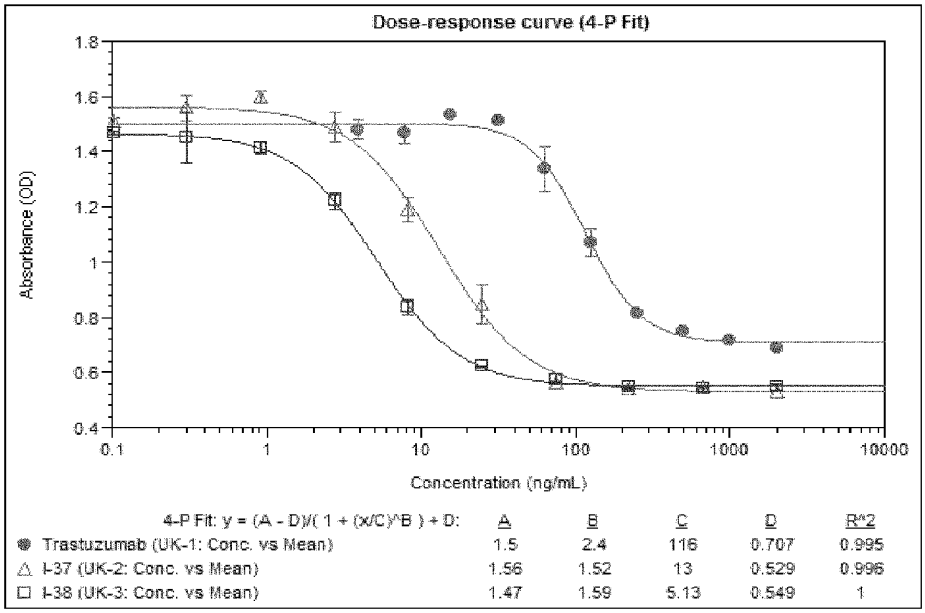
[Fig. 14]


Dose-response curve (4-P Fit)
Absorbance (OD)
10.6, 1.103
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A B C D R^2
Trastuzumab (UK-1: Conc. vs Mean) 1.7 1.98 104 0.554 0.995
I-34 (UK-2: Conc. vs Mean) 1.7 1.75 11.3 0.308 0.997

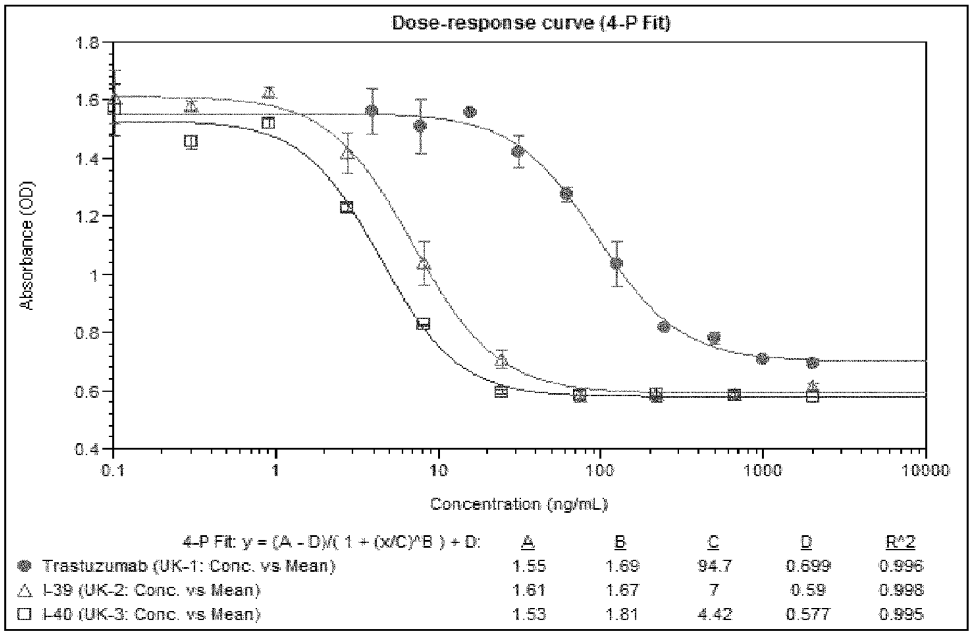
[Fig. 15]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.63 2.02 104 0.711 0.998
I-35 (UK-2: Conc. vs Mean) 1.65 1.93 10.3 0.606 0.998
I-36 (UK-3: Conc. vs Mean) 1.62 1.84 5.74 0.595 0.996

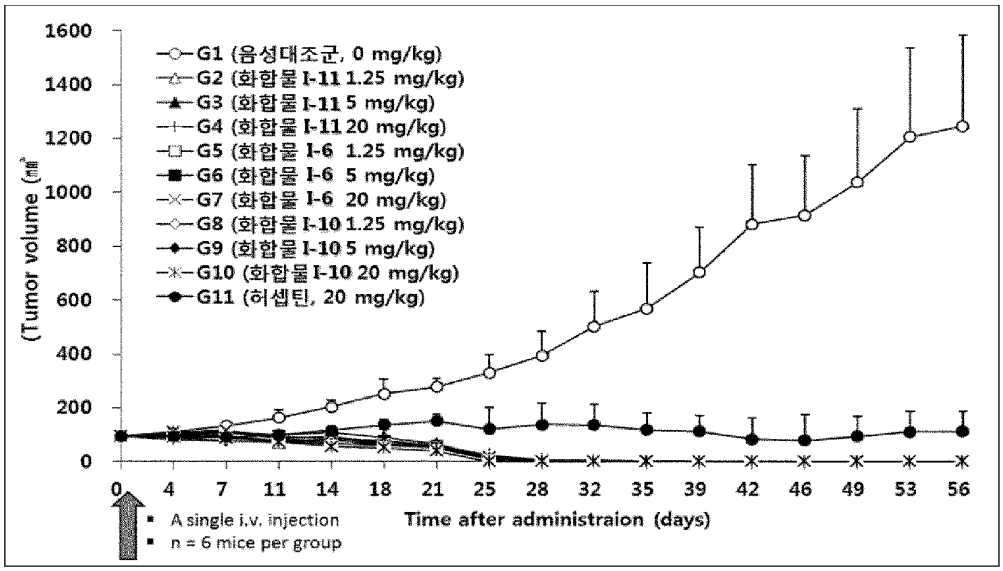
[Fig. 16]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.5 2.4 116 0.707 0.995
I-37 (UK-2: Conc. vs Mean) 1.56 1.52 13 0.529 0.996
I-38 (UK-3: Conc. vs Mean) 1.47 1.59 5.13 0.549 1

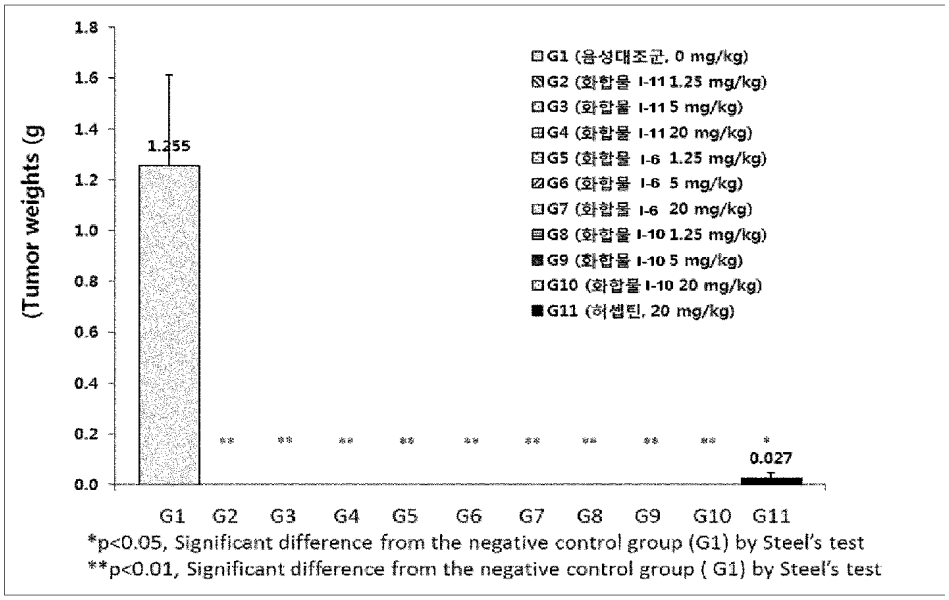
[Fig. 17]


Dose-response curve (4-P Fit)
Absorbance (OD)
Concentration (ng/mL)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
Trastuzumab (UK-1: Conc. vs Mean) 1.55 1.69 94.7 0.699 0.996
I-39 (UK-2: Conc. vs Mean) 1.61 1.67 7 0.59 0.998
I-40 (UK-3: Conc. vs Mean) 1.53 1.81 4.42 0.577 0.995

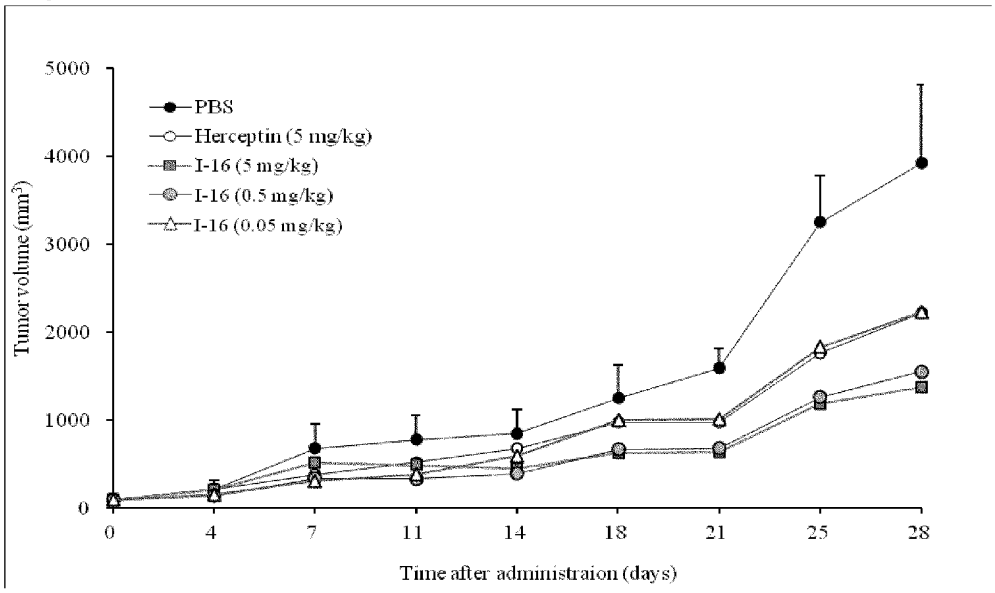
[Fig. 18]


(Tumor volume (㎣)
G1 (음성대조군, 0 mg/kg)
G2 (화합물 I-11 1.25 mg/kg)
G3 (화합물 I-11 5 mg/kg)
G4 (화합물 I-11 20 mg/kg)
G5 (화합물 I-6 1.25 mg/kg)
G6 (화합물 I-6 5 mg/kg)
G7 (화합물 I-6 20 mg/kg)
G8 (화합물 I-10 1.25 mg/kg)
G9 (화합물 I-10 5 mg/kg)
G10 (화합물 I-10 20 mg/kg)
G11 (허셉틴, 20 mg/kg)
A single i.v. injection
n = 6 mice per group
Time after administraion (days)

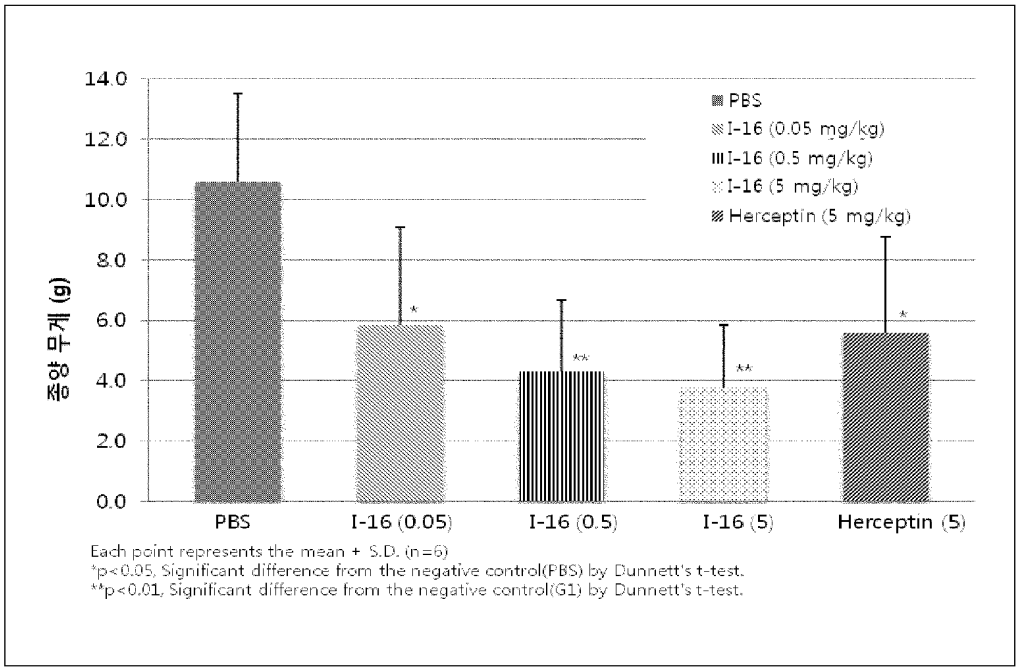
[Fig. 19]


(Tumor weights (g
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.255
0.027
**
*
G1 (음성대조군, 0 mg/kg)
G2 (화합물 I-11 1.25 mg/kg)
G3 (화합물 I-11 5 mg/kg)
G4 (화합물 I-11 20 mg/kg)
G5 (화합물 I-6 1.25 mg/kg)
G6 (화합물 I-6 5 mg/kg)
G7 (화합물 I-6 20 mg/kg)
G8 (화합물 I-10 1.25 mg/kg)
G9 (화합물 I-10 5 mg/kg)
G10 (화합물 I-10 20 mg/kg)
G11 (허셉틴, 20 mg/kg)
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11
*p<0.05, Significant difference from the negative control group (G1) by Steel's test
**p<0.01, Significant difference from the negative control group (G1) by Steel's test

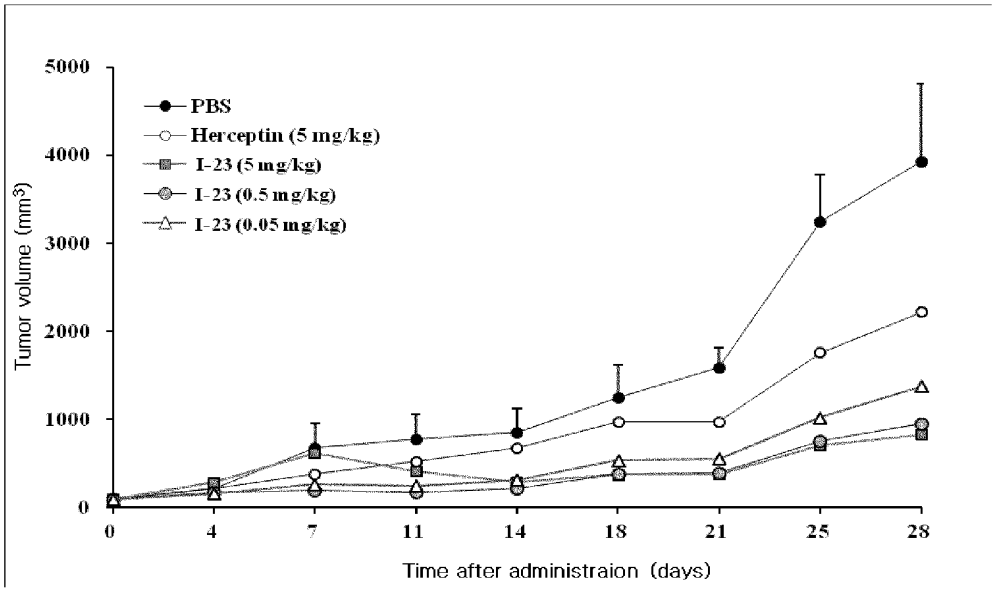
[Fig. 20]


Tumor volume (mm3)
5000
4000
3000
2000
1000
0
PBS
Herceptin (5 mg/kg)
I-16 (5 mg/kg)
I-16 (0.5 mg/kg)
I-16 (0.05 mg/kg)
0 4 7 11 14 18 21 25 28
Time after administraion (days)

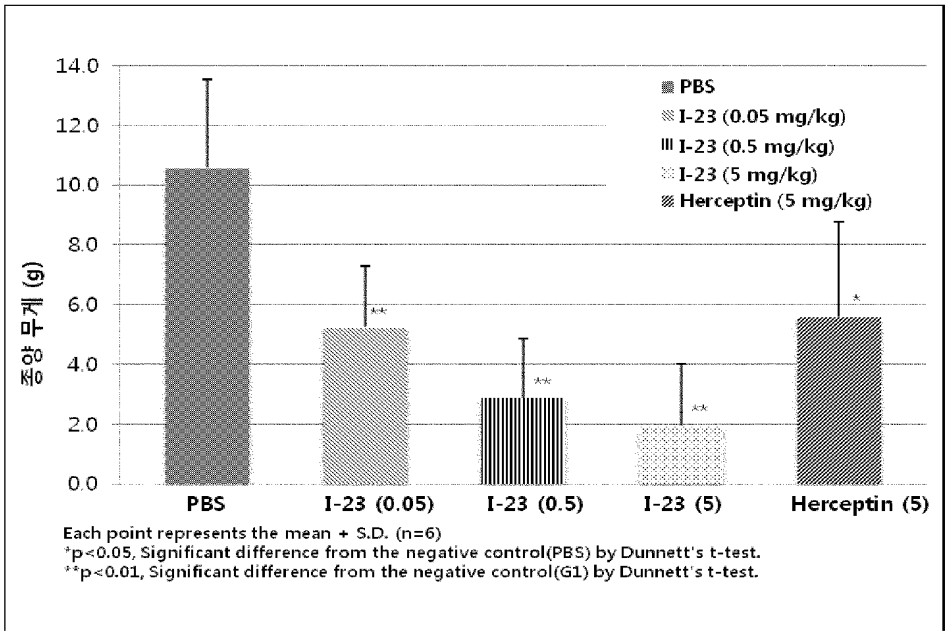
[Fig. 21]


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
종양 무게 (g)
PBS
I-16 (0.05 mg/kg)
I-16 (0.5 mg/kg)
I-16 (5 mg/kg)
Herceptin (5 mg/kg)
*
**
**
*
PBS
I-16 (0.05)
I-16 (0.5)
I-16 (5)
Herceptin (5)
Each point represents the mean + S.D. (n=6)
*p<0.05, Significant difference from the negative control(PBS) by Dunnett's t-test.
**p<0.01, Significant difference from the negative control(G1) by Dunnett's t-test.

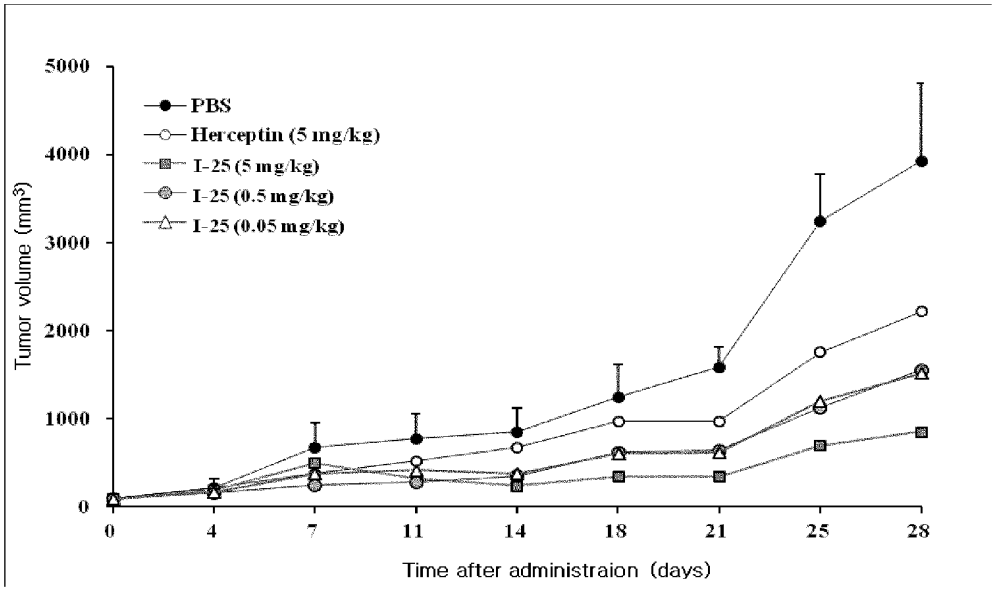
[Fig. 22]


5000
4000
3000
2000
1000
0
Tumor volume (mm3)
PBS
Herceptin (5 mg/kg)
I-23 (5 mg/kg)
I-23 (0.5 mg/kg)
I-23 (0.05 mg/kg)
0
4
7
11
14
18
21
25
28
Time after administraion (days)

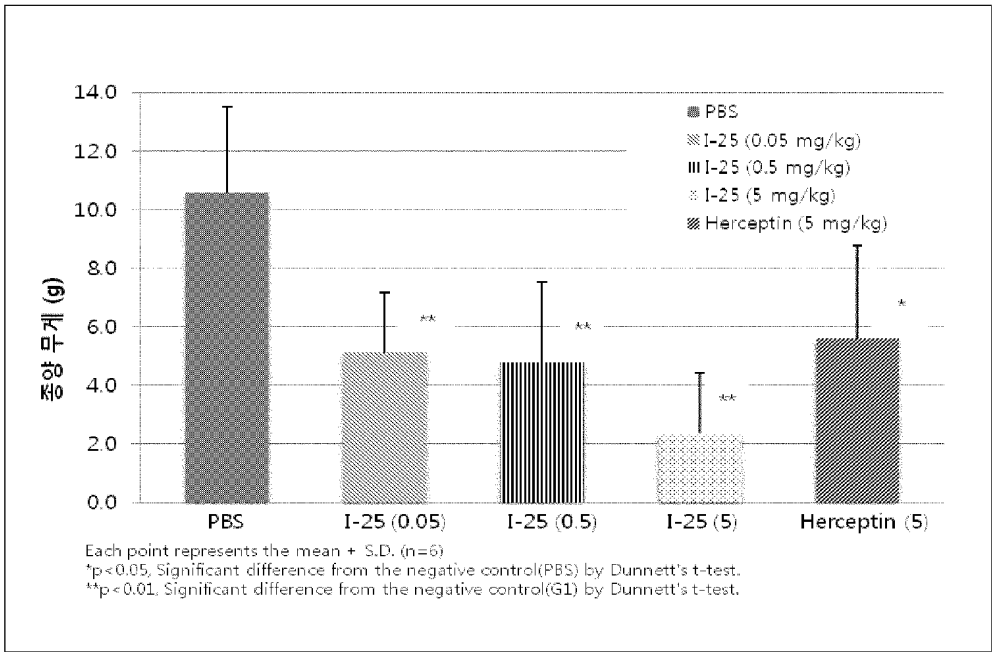
[Fig. 23]


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
종양 무게 (g)
PBS
I-23 (0.05 mg/kg)
I-23 (0.5 mg/kg)
I-23 (5 mg/kg)
Herceptin (5 mg/kg)
PBS
I-23 (0.05)
I-23 (0.5)
I-23 (5)
Herceptin (5)
Each point represents the mean + S.D. (n=6)
*p<0.05, Significant difference from the negative control(PBS) by Dunnett's t-test.
**p<0.01, Significant difference from the negative control(G1) by Dunnett's t-test.

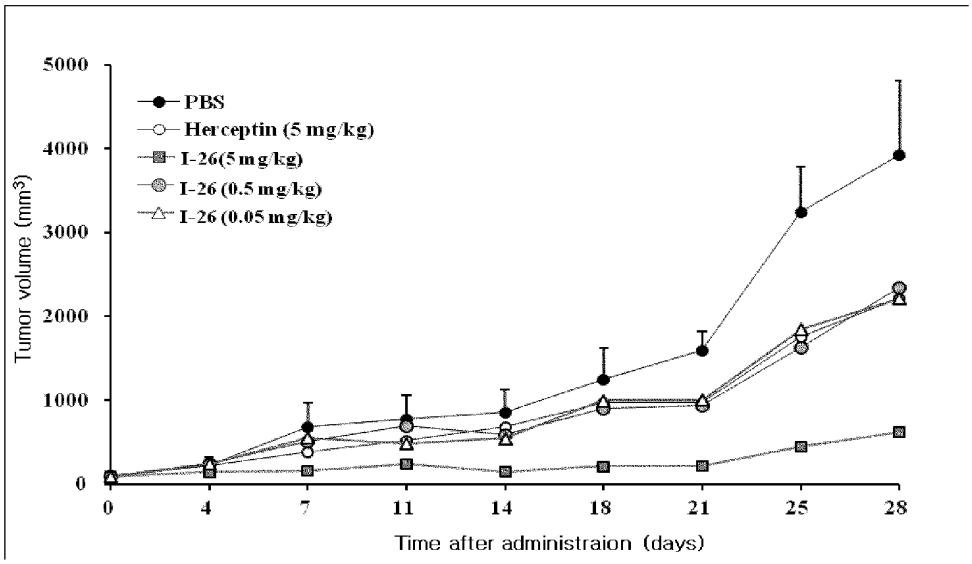
[Fig. 24]


5000
4000
3000
2000
1000
0
Tumor volume (mm3)
PBS
Herceptin (5 mg/kg)
I-25 (5 mg/kg)
I-25 (0.5 mg/kg)
I-25 (0.05 mg/kg)
0
4
7
11
14
18
21
25
28
Time after administraion (days)

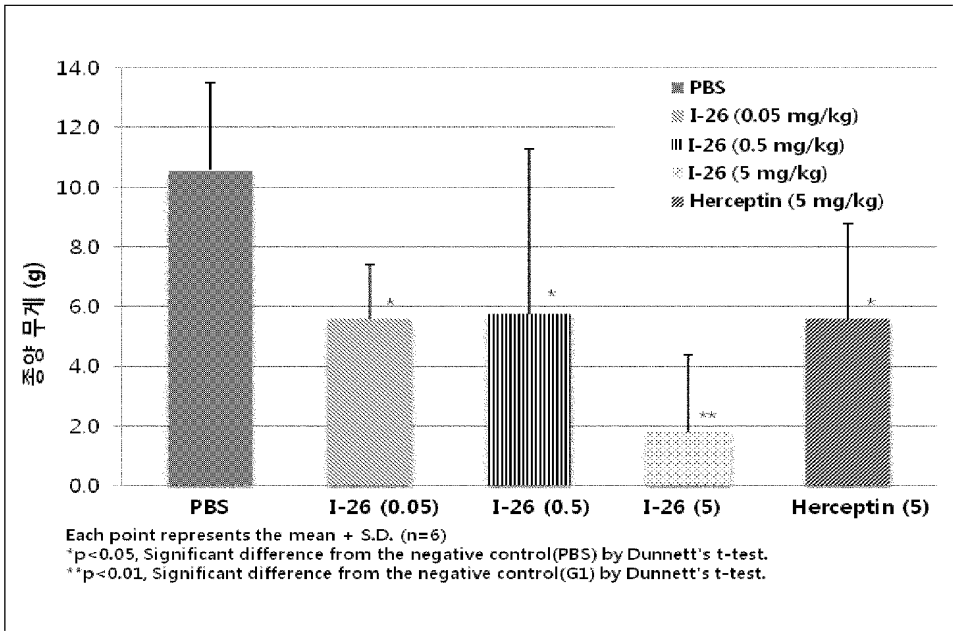
[Fig. 25]


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
종양 무게 (g)
PBS
I-25 (0.05 mg/kg)
I-25 (0.5 mg/kg)
I-25 (5 mg/kg)
Herceptin (5 mg/kg)
**
**
**
*
PBS
I-25 (0.05)
I-25 (0.5)
I-25 (5)
Herceptin (5)
Each point represents the mean + S.D. (n=6)
*p<0.05, Significant difference from the negative control(PBS) by Dunnett's t-test.
**p<0.01, Significant difference from the negative control(G1) by Dunnett's t-test.

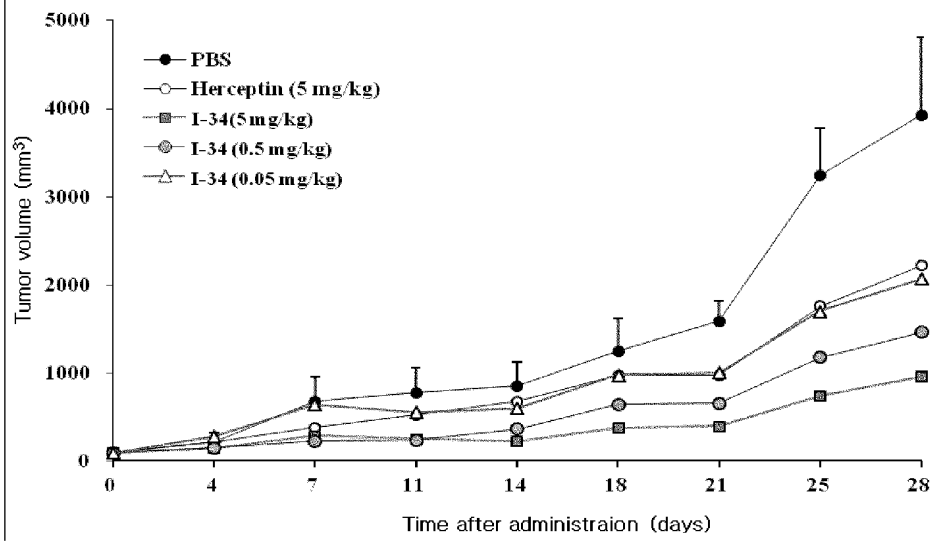
[Fig. 26]


5000
4000
3000
2000
1000
0
Tumor volume (mm3)
PBS
Herceptin (5 mg/kg)
I-26(5 mg/kg)
I-26 (0.5 mg/kg)
I-26 (0.05 mg/kg)
0
4
7
11
14
18
21
25
28
Time after administraion (days)

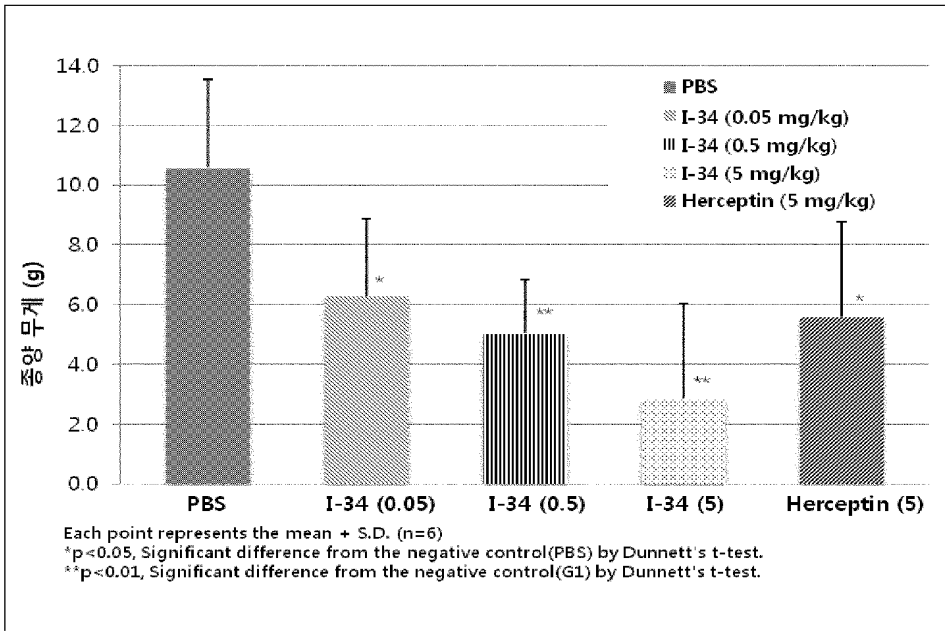
[Fig. 27]


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
종양 무게 (g)
PBS
I-26 (0.05 mg/kg)
I-26 (0.5 mg/kg)
I-26 (5 mg/kg)
Herceptin (5 mg/kg)
*
*
**
*
PBS
I-26 (0.05)
I-26 (0.5)
I-26 (5)
Herceptin (5)
Each point represents the mean + S.D. (n=6)
*p<0.05, Significant difference from the negative control(PBS) by Dunnett's t-test.
**p<0.01, Significant difference from the negative control(G1) by Dunnett's t-test.

[Fig. 28]


5000
4000
3000
2000
1000
0
Tumor volume (mm3)
PBS
Herceptin (5 mg/kg)
I-34(5 mg/kg)
I-34 (0.5 mg/kg)
I-34 (0.05 mg/kg)
0
4
7
11
14
18
21
25
28
Time after administraion (days)

[Fig. 29]


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
종양 무게 (g)
PBS
I-34 (0.05 mg/kg)
I-34 (0.5 mg/kg)
I-34 (5 mg/kg)
Herceptin (5 mg/kg)
*
**
**
*
PBS
I-34 (0.05)
I-34 (0.5)
I-34 (5)
Herceptin (5)
Each point represents the mean + S.D. (n=6)
*p<0.05, Significant difference from the negative control(PBS) by Dunnett's t-test.
**p<0.01, Significant difference from the negative control(G1) by Dunnett's t-test.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2014/000005

A. CLASSIFICATION OF SUBJECT MATTER

C07D 403/12(2006.01)i, A61K 39/395(2006.01)i, A61K 39/44(2006.01)i, A61K 47/48(2006.01)i

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)

C07D 403/12; C07K 5/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

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

eKOMPASS (KIPO internal) & Keywords: antibody-drug conjugate, adc, anticancer drug

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUCRY, LAURENT et al., "Antibody-Drug Conjugates: Linking Cytotoxic Payloads to Monoclonal Antibodies", Bioconjugate Chem. 2010, vol. 21, pages 5-13 See figure 1, the entire chart, the entire document	1,54,55
A		2-53,56-61
X	ALLEY, STEPHEN C. et al., "Contribution of linker stability to the activities of anticancer immunoconjugates", Bioconjugate Chem. 2008, vol. 19, pages 759-765 See figure 1, the entire document	1,54,55
A		2-53,56-61
X	US 7750116 B1 (DORONINA, Svetlana O. et al.) 06 July 2010 See figure 1, scheme 1	1,54,55
A		2-53,56-61

☐ 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
29 APRIL 2014 (29.04.2014)	**30 APRIL 2014 (30.04.2014)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer

Telephone No.

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Ex. 1038

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2014/000005

Patent document cited in search report	Publication date	Patent family member	Publication date
US 7750116 B1	06/07/2010	US 2010-0260786 A1	14/10/2010
		US 8512707 B2	20/08/2013

국 제 조 사 보 고 서

국제출원번호 Ex. 1038
PCT/KR2014/000005

A. 발명이 속하는 기술분류(국제특허분류(IPC))

C07D 403/12(2006.01)i, A61K 39/395(2006.01)i, A61K 39/44(2006.01)i, A61K 47/48(2006.01)i

B. 조사된 분야

조사된 최소문헌(국제특허분류를 기재)
C07D 403/12; C07K 5/00

조사된 기술분야에 속하는 최소문헌 이외의 문헌
한국등록실용신안공보 및 한국공개실용신안공보: 조사된 최소문헌란에 기재된 IPC
일본등록실용신안공보 및 일본공개실용신안공보: 조사된 최소문헌란에 기재된 IPC

국제조사에 이용된 전산 데이터베이스(데이터베이스의 명칭 및 검색어(해당하는 경우))
eKOMPASS(특허청 내부 검색시스템) & 키워드:항체약물결합체,adc,항암제

C. 관련 문헌

카테고리*	인용문헌명 및 관련 구절(해당하는 경우)의 기재	관련 청구항
X	DUCRY, LAURENT et al., `Antibody-Drug Conjugates: Linking Cytotoxic Payloads to Monoclonal Antibodies`, Bioconjugate Chem. 2010, Vol21, pages 5-13 Figure1, 전체 chart, 전체 문헌 참조	1,54,55
A		2-53,56-61
X	ALLEY, STEPHEN C. et al., `Contribution of linker stability to the activities of anticancer immunoconjugates`, Bioconjugate Chem. 2008, Vol.19, pages 759-765 Figure1, 전체 문헌 참조	1,54,55
A		2-53,56-61
X	US 7750116 B1 (DORONINA SVETLANA O. 외 1명) 2010.07.06 Figure1, scheme1 참조	1,54,55
A		2-53,56-61

☐ 추가 문헌이 C(계속)에 기재되어 있습니다.

☒ 대응특허에 관한 별지를 참조하십시오.

* 인용된 문헌의 특별 카테고리:

"A" 특별히 관련이 없는 것으로 보이는 일반적인 기술수준을 정의한 문헌

"E" 국제출원일보다 빠른 출원일 또는 우선일을 가지나 국제출원일 이후에 공개된 선출원 또는 특허 문헌

"L" 우선권 주장에 의문을 제기하는 문헌 또는 다른 인용문헌의 공개일 또는 다른 특별한 이유(이유를 명시)를 밝히기 위하여 인용된 문헌

"O" 구두 개시, 사용, 전시 또는 기타 수단을 언급하고 있는 문헌

"P" 우선일 이후에 공개되었으나 국제출원일 이전에 공개된 문헌

"T" 국제출원일 또는 우선일 후에 공개된 문헌으로, 출원과 상충하지 않으며 발명의 기초가 되는 원리나 이론을 이해하기 위해 인용된 문헌

"X" 특별한 관련이 있는 문헌. 해당 문헌 하나만으로 청구된 발명의 신규성 또는 진보성이 없는 것으로 본다.

"Y" 특별한 관련이 있는 문헌. 해당 문헌이 하나 이상의 다른 문헌과 조합하는 경우로 그 조합이 당업자에게 자명한 경우 청구된 발명은 진보성이 없는 것으로 본다.

"&" 동일한 대응특허문헌에 속하는 문헌

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(302-701) 대전광역시 서구 청사로 189,
4동 (둔산동, 정부대전청사)
팩스 번호 +82-42-472-7140

심사관
신창훈
전화번호 +82-42-481-8406

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대응특허에 관한 정보

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(71) Applicant: **DAIICHI SANKYO COMPANY, LIMITED** [JP/JP]; 3-5-1, Nihonbashi Honcho, Chuo-ku, Tokyo, 1038426 (JP).

(72) Inventors: **HETTMANN, Thore**; c/o U3 Pharma GmbH, Fraunhoferstr. 22, Martinsried, 82152 (DE). **ABRAHAM, Reimar**; c/o U3 Pharma GmbH, Fraunhoferstr. 22, Martinsried, 82152 (DE). **BLUM, Sabine**; c/o U3 Pharma GmbH, Fraunhoferstr. 22, Martinsried, 82152 (DE). **UENO, Suguru**; c/o DAIICHI SANKYO COMPANY, LIMITED, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo, 1408710 (JP).

(74) Agents: **OHNO, Seiji** et al.; OHNO & PARTNERS, Marunouchi Kitaguchi Building 21F, 6-5, Marunouchi 1-chome, Chiyoda-ku, Tokyo, 1000005 (JP).

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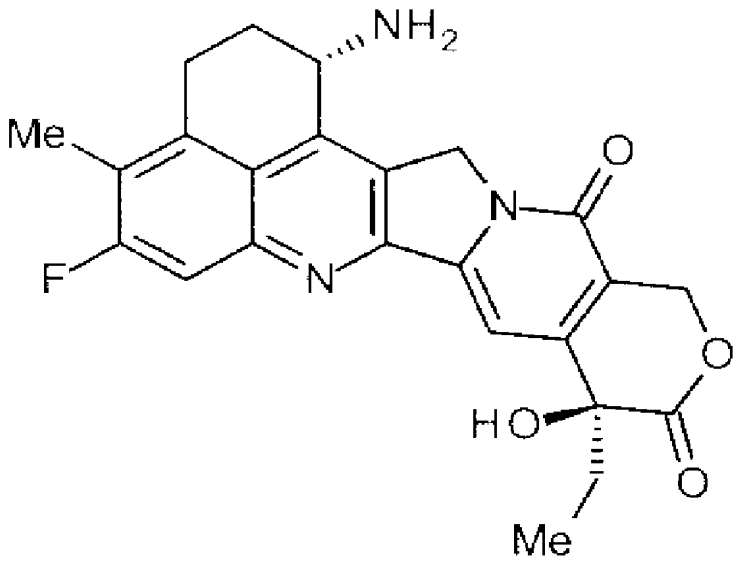
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(54) **Title**: ANTI-HER3 ANTIBODY-DRUG CONJUGATE



(57) **Abstract**: To provide an antitumor drug having excellent therapeutic effect, which is excellent in terms of antitumor effect and safety. Provided is an antibody-drug conjugate in which an antitumor compound represented by the following formula is conjugated to an anti-HER3 antibody via a linker having a structure represented by the formula: -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- or -L^1-L^2-L^P- (the anti-HER3 antibody is connected to the terminal of L^1, the antitumor compound is connected to the carbonyl group of -$(CH_2)n^2$-C(=O)- moiety or the C terminal of L^P, with the nitrogen atom of the amino group at position 1 as a connecting position).

WO 2015/155998 A1

Description

Title of Invention: ANTI-HER3 ANTIBODY-DRUG CONJUGATE

Technical Field

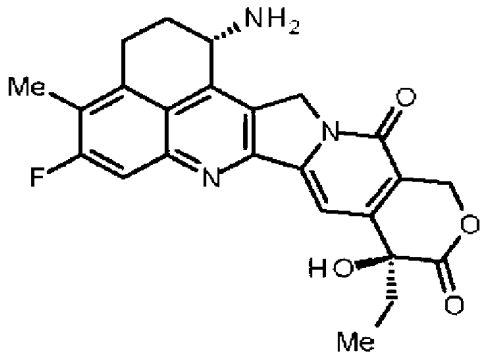
[0001] The present invention relates to an antibody-drug conjugate having an anti-HER3 antibody and an antitumor drug conjugated to each other via a linker structure moiety, the conjugate being useful as an antitumor drug.

Background Art

[0002] An antibody-drug conjugate (ADC) having a drug with cytotoxicity conjugated to an antibody which binds to an antigen expressed on a surface of cancer cells and capable of cellular internalization (the antibody which binds to the angtigen is also capable of cellular internalization), can deliver the drug selectively to the cancer cells and is thus expected to cause accumulation of the drug in the cancer cells and to kill the cancer cells (see, Non Patent Literatures 1 to 3). As an ADC, Mylotarg (registered trademark; Gemtuzumab ozogamicin) in which calicheamicin is conjugated to an anti-CD33 antibody is approved as a therapeutic agent for acute myeloid leukemia. Further, Adcetris (registered trademark; Brentuximab vedotin), in which auristatin E is conjugated to an anti-CD30 antibody, has recently been approved as a therapeutic agent for Hodgkin's lymphoma and anaplastic large cell lymphoma (see, Non Patent Literature 4). The drugs contained in ADCs which have been approved until now target DNA or tubulin.

[0003] As an antitumor, low-molecular-weight compounds, camptothecin derivatives, which inhibit topoisomerase I to exhibit an antitumor effect, are known. Among them, an antitumor compound represented by the formula below (exatecan, chemical name: (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10,13(9H,15H)-dione) is a water soluble derivative of camptothecin (Patent Literature 1 and 2).

[0004] [Chem.1]



[0005] Unlike irinotecan currently used in clinical settings, this compound does not require activation by an enzyme for exerting its antitumor effect. Further, compared to SN-38 as a main pharmaceutically active ingredient of irinotecan and topotecan also used in clinical settings, it has higher inhibitory activity on topoisomerase I and has higher cytocidal activity in vitro against various cancer cells. In particular, it exhibits the effect against cancer cells which have resistance to SN-38 or the like due to expression of P-glycoprotein. Further, in a mouse model with a human tumor subcutaneously transplanted, it exhibited a potent antitumor effect, and thus has undergone the clinical studies, but has not been put on the market yet (see, Non Patent Literatures 5 to 10). It remains unclear whether or not exatecan functions effectively as an ADC.

[0006] DE-310 is a complex in which exatecan is conjugated to a biodegradable carboxymethyldextran polyalcohol polymer via a GGFG peptide spacer (Patent Literature 3). By making exatecan into a form of a polymer prodrug, a high blood retention property can be maintained and also a high penetration property to a tumor area is passively increased by utilizing the increased permeability of newly formed tumor vessels and retention property in tumor tissues. With DE-310, the peptide spacer is cleaved by an enzyme to continuously release exatecan as a main active ingredient and exatecan with glycine bonded to an amino group, and as a result, the pharmacokinetics are improved. According to various tumor evaluation models in non-clinical studies, it was found that higher effectiveness was obtained by DE-310 than exatecan administered alone even though the total amount of exatecan contained therein is lower than the case of administration of exatecan alone. A clinical study was conducted for DE-310, and effective cases were confirmed. There is also a report suggesting that the main active ingredient accumulates in a tumor than in normal tissues. However, there is also a report indicating that the accumulation of DE-310 and the main active ingredient in a tumor is not much different from the accumulation in normal tissues, and thus no passive targeting is observed in humans (see, Non Patent Literatures 11 to 14). As a result, DE-310 was not also commercialized, and it remains unclear whether or not exatecan effectively functions as a drug oriented for such targeting.

[0007] As a compound relating to DE-310, a complex in which a structure moiety represented by -NH-$(CH_2)_4$-C(=O)- is inserted between -GGFG -spacer and exatecan to form -GGFG-NH-$(CH_2)_4$-C(=O)- used as a spacer structure is also known (Patent Literature 4). However, the antitumor effect of the complex is not known at all.

[0008] The human epidermal growth factor receptor 3 (also known as HER3 and ErbB3) is a receptor protein tyrosine kinase and belongs to the epidermal growth factor receptor (EGFR) subfamily of receptor protein tyrosine kinases, which also includes HER1 (also known EGFR), HER2, and HER4 (see Non Patent Literatures 15 to 17). As with the prototypical epidermal growth factor receptor, the transmembrane receptor HER3

consists of an extracellular ligand-binding domain (ECD), a dimerization domain within the ECD, a transmembrane domain, and a carboxyl-terminal phosphorylation domain. HER1, HER2, and HER4 carry an intracellular protein tyrosine kinase domain (TKD) in addition to these domains, while HER3 lacks this domain and is thus unable to be autophosphorylated.

The ligand Heregulin (HRG) binds to the extracellular domain of HER3 and activates the receptor-mediated signaling pathway by promoting dimerization with other human epidermal growth factor receptor (HER) family members and transphosphorylation of its intracellular domain. The dimer formation of HER3 with other HER family members expands the signaling potential of HER3 and serves as means not only for signal diversification but also for signal amplification. For example, the HER2/ HER3 heterodimer induces one of the most important mitogenic signals among HER family members. HER3 is overexpressed in several types of cancers such as breast, gastrointestinal, and pancreatic cancers. Interestingly, a correlation between the expression of HER2/HER3 and the progression from a non-invasive stage to an invasive stage has been shown (see Non Patent Literatures 18 to 20). Accordingly, agents that interfere with HER3-mediated signaling are desirable. Anti-HER3 antibodies and immunoconjugates thereof have been reported in, for example, Patent Literatures 5 to 10, respectively.

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[0009] [PTL 1] Japanese Patent Laid-Open No. 5-59061

[PTL 2] Japanese Patent Laid-Open No. 8-337584

[PTL 3] International Publication No. WO 1997/46260

[PTL 4] International Publication No. WO 2000/25825

[PTL 5] U.S. Patent No. 5968511

[PTL 6] U.S. Patent No. 5480968

[PTL 7] International Publication No. WO 2003/013602

[PTL 8] International Publication No. WO 2007/077028

[PTL 9] International Publication No. WO 2008/100624

[PTL 10] International Publication No. WO 2012/019024

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[NPL 16] Kraus et al., Proc. Natl. Acad. Sci. U.S.A. (1989) 86, 9193-9197.
[NPL 17] Kraus et al., Proc. Natl. Acad. Sci. U.S.A. (1993) 90, 2900-2094.
[NPL 18] Alimandi et al., Oncogene (1995) 10, 1813-1821.
[NPL 19] DeFazio et al., Int. J. Cancer (2000) 87, 487-498.
[NPL 20] Nadiu et al., Br. J. Cancer (1998) 78, 1385-1390.

Summary of Invention

Technical Problem

[0011] With regard to the treatment of tumor using an antibody, an insufficient antitumor effect may be observed even when the antibody recognizes an antigen and binds to tumor cells, and thus a more effective antitumor antibody is sometimes needed. Further, many antitumor low-molecular-weight compounds have a problem in safety like side effect and toxicity even the compounds have an excellent antitumor effect. As such, it remains as a subject to achieve a superior therapeutic effect by further enhancing the safety. Thus, an object of the present invention is to provide an antitumor drug having an excellent therapeutic effect, which is excellent in terms of antitumor effect and safety.

Solution to Problem

[0012] The inventors thought that, since the anti-HER3 antibody is an antibody capable of targeting tumor cells, that is, it is an antibody having a property of recognizing tumor cells, a property of binding to tumor cells, a property of internalizing in tumor cells, a cytocidal activity against tumor cells, or the like, when exatecan as an antitumor compound is converted into an antibody-drug conjugate by conjugation to the antibody via a linker structure moiety, the antitumor compound can be more surely delivered to tumor cells to specifically exhibit the antitumor effect of the compound in tumor cells, and thus the antitumor effect can be surely exhibited and also an enhanced cytocidal

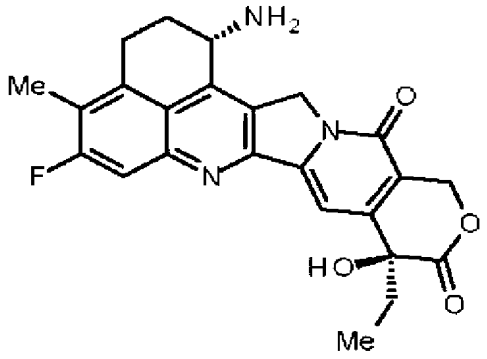
effect of the anti-HER3 antibody is expected, and a dose of the antitumor compound can be reduced compared to a case of administering the compound alone, and thus an influence of the antitumor compound on normal cells can be alleviated so that higher safety can be achieved.

In this connection, the inventors created a linker with a specific structure and succeeded in obtaining an antibody-drug conjugate in which the anti-HER3 antibody and exatecan are conjugated to each other via the linker, and confirmed an excellent antitumor effect exhibited by the conjugate to thereby complete the present invention.

[0013] Specifically, the present invention relates to the followings.

[1] An antibody-drug conjugate wherein an antitumor compound represented by the following formula

[Chem.2]



is conjugated to an anti-HER3 antibody by a thioether bond which is formed at a disulfide bond moiety present in a hinge part of the anti-HER3 antibody via a linker having a structure represented by the following formula:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-(CH_2)n^2-C(=O)-$ or $-L^1-L^2-L^P-$.

[0014] Here, the anti-HER3 antibody is connected to the terminal of L^1, the antitumor compound is connected to the carbonyl group of $-(CH_2)n^2-C(=O)-$ moiety or the C terminal of L^P, with the nitrogen atom of the amino group at position 1 as a connecting position.

In the formula, n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^1 represents -(Succinimid-3-yl-N)-$(CH_2)n^3$-C(=O)-,

wherein n^3 represents an integer of 2 to 8,

L^2 represents $-NH-(CH_2CH_2-O)n^4-CH_2CH_2-C(=O)-$ or a single bond,

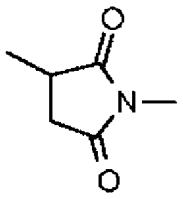
wherein n^4 represents an integer of 1 to 6,

L^P represents a peptide residue consisting of 2 to 7 amino acids,

L^a represents -O- or a single bond,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

[Chem.3]



which is connected to the anti-HER3 antibody at position 3 thereof and is connected on the nitrogen atom at position 1 to a methylene group in the linker structure containing this structure.

[0015] The present invention further relates to each of the followings.

[2] The antibody-drug conjugate according to [1], wherein the peptide residue of L^P is a peptide residue comprising an amino acid selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid.

[3] The antibody-drug conjugate according to [1] or [2], wherein L^P is a peptide residue selected from the following group

-GGF-,
-DGGF-,
-(D-)D-GGF-,
-EGGF-,
-GGFG-,
-SGGF-,
-KGGF-,
-DGGFG-,
-GGFGG-,
-DDGGFG-,
-KDGGFG-, and
-GGFGGGF-;

(wherein, "(D-)D" represents D-aspartic acid).

[4] The antibody-drug conjugate according to [1] or [2], wherein L^P is a peptide residue comprising 4 or 5 amino acids.

[5] The antibody-drug conjugate according to any one of [1] to [4], wherein L^P is -GGFG- or -DGGFG-.

[6] The antibody-drug conjugate according to any one of [1] to [4], wherein L^P is -GGFG-.

[0016] [7] The antibody-drug conjugate according to any one of [1] to [6], wherein n^3 is an integer of 2 to 5 and L^2 is a single bond.

[8] The antibody-drug conjugate according to any one of [1] to [7], wherein the

linker is -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-.

[9] The antibody-drug conjugate according to [8], wherein n^3 is an integer of 2 to 5, L^2 is -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)-, and n^4 is 2 or 4.

[10] The antibody-drug conjugate according to [8] or [9], wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is a partial structure having chain length of 4 to 7 atoms.

[11] The antibody-drug conjugate according to [8] or [9], wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is a partial structure having chain length of 5 or 6 atoms.

[12] The antibody-drug conjugate described in [10] or [11], wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is

-NH-CH_2CH_2-C(=O)-,

-NH-$CH_2CH_2CH_2$-C(=O)-,

-NH-$CH_2CH_2CH_2CH_2$-C(=O)-,

-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-,

-NH-CH_2-O-CH_2-C(=O)-, or

-NH-CH_2CH_2-O-CH_2-C(=O)-.

[13] The antibody-drug conjugate according to [12], wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is any one of the followings:

-NH-$CH_2CH_2CH_2$-C(=O)-,

-NH-CH_2-O-CH_2-C(=O)-, or

-NH-CH_2CH_2-O-CH_2-C(=O)-.

[14] The antibody-drug conjugate according to any one of [1] to [5], wherein the linker is -L^1-L^2-L^P-.

[15] The antibody-drug conjugate according to [14], wherein L^P is -DGGFG-.

[16] The antibody-drug conjugate according to [15], wherein n^3 is an integer of 2 to 5 and L^2 is a single bond.

[0017] [17] The antibody-drug conjugate according to [1], wherein the drug-linker structure moiety in which a drug is bound to -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- or -L^1-L^2-L^P- is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-(NH-DX),

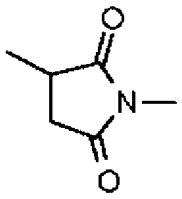
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[0018] In the above, -(Succinimid-3-yl-N)- has a structure represented by the following formula:

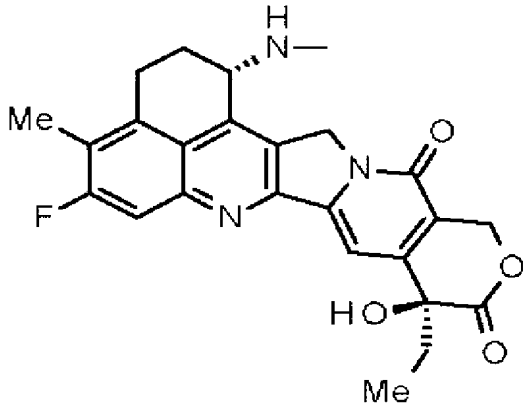
[Chem.4]



which is connected to the anti-HER3 antibody at position 3 and is connected to a methylene group in the linker structure containing it on the nitrogen atom at position 1,

-(NH-DX) represents a group represented by the following formula, wherein the nitrogen atom of the amino group at position 1 is the connectig position,

[Chem.5]



-GGFG- represents a tetrapeptide residue of -Gly-Gly-Phe-Gly- and -DGGFG- represents a pentapeptide residue of -Asp-Gly-Gly-Phe-Gly-.

[0019] [18] The antibody-drug conjugate described in [1], wherein the drug-linker structure moiety having a drug bonded to -L^1-L^2-L^p-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

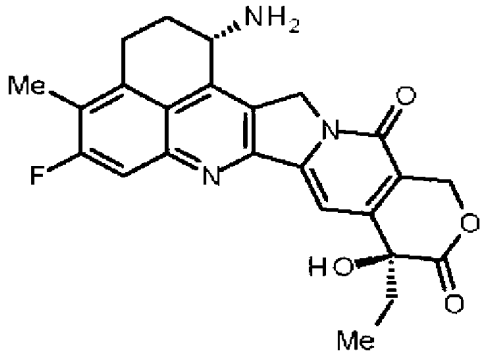
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[0020] Here, -(Succinimid-3-yl-N)-, -(NH-DX), -GGFG-, and -DGGFG- are as described above.

[0021] [19] An antibody-drug conjugate comprising an antitumor compound represented by the following formula:

[Chem.6]



conjugated to an anti-HER3 antibody by a thioether bond which is formed at a disulfide bond moiety present in a hinge part of the anti-HER3 antibody via a linker having a structure represented by the following formula:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-(CH_2)n^2-C(=O)-$.

Here, the anti-HER3 antibody is connected to the terminal of L^1 and the antitumor compound is connected to the carbonyl group of $-(CH_2)n^2-C(=O)-$ moiety.

In the formula, n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^1 represents -(Succinimid-3-yl-N)-$(CH_2)n^3$-C(=O)-,

wherein n^3 represents an integer of 2 to 8,

L^2 represents $-NH-(CH_2CH_2-O)n^4-CH_2CH_2-C(=O)-$ or a single bond,

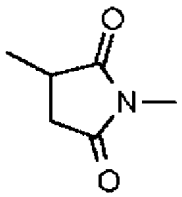
wherein n^4 represents an integer of 1 to 6,

L^P represents a tetrapeptide residue of -GGFG-,

L^a represents -O- or a single bond,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

[Chem.7]



which is connected to the anti-HER3 antibody at position 3 thereof and binds on the nitrogen atom at position 1 to a methylene group in a linker structure containing this structure.

[0022] [20] The antibody-drug conjugate according to [19], wherein n^1 is 3, n^2 is 0, n^3 is 2, L^2 is $-NH-(CH_2CH_2-O)n^4-CH_2CH_2-C(=O)-$, n^4 is 2, and L^a is a single bond, or

n^1 is 1, n^2 is 1, n^3 is 5, L^2 is a single bond, and L^a is -O-, or

n^1 is 2, n^2 is 1, n^3 is 5, L^2 is a single bond, and L^a is -O-.

[21] The antibody-drug conjugate according to [19] or [20], wherein n^3 is 2 or 5 and L^2 is a single bond.

[22] The antibody-drug conjugate according to [19] or [20], wherein n^3 is 2 or 5, L^2 is -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)-, and n^4 is 2 or 4.

[23] The antibody-drug conjugate described in any one of [19] to [22], wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is

-NH-$CH_2CH_2CH_2$-C(=O)-,

-NH-CH_2-O-CH_2-C(=O)-, or

-NH-CH_2CH_2-O-CH_2-C(=O)-.

[0023] [24] The antibody-drug conjugate described in any one of [19] to [23], wherein the drug-linker structure moiety having a drug bonded to -L^1-L^2-L^p-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

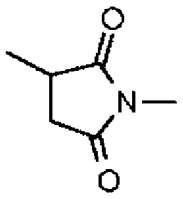
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX);

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

[0024] In the above, -(Succinimid-3-yl-N)- has a structure represented by the following formula:

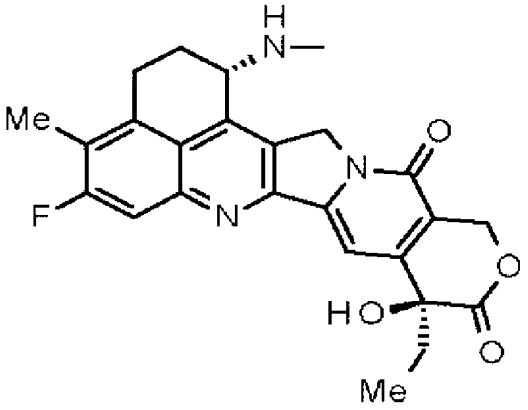
[Chem.8]



which is connected to the anti-HER3 antibody at position 3 thereof and is connected on the nitrogen atom at position 1 to a methylene group in a linker structure containing this structure.

-(NH-DX) represents a group represented by the following formula, wherein the nitrogen atom of the amino group at position 1 is the connectig position:

[Chem.9]



-GGFG- represents a tetrapeptide residue of -Gly-Gly-Phe-Gly-.

[0025] [25] The antibody-drug conjugate described in any one of [19] to [23], wherein the drug-linker structure moiety having a drug connected to -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX).

In the above, -(Succinimid-3-yl-N)-, -(NH-DX), and -GGFG- are as defined above.

[0026] [26] The antibody-drug conjugate according to any one of [1] to [25], wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 1 to 10.

[27] The antibody-drug conjugate according to any one of [1] to [25], wherein the

average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 2 to 8.

[28] The antibody-drug conjugate according to any one of [1] to [25], wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 3 to 8.

[0027] [29] A medicine comprising the antibody-drug conjugate according to any one of [1] to [28], a salt thereof or a hydrate thereof.

[30] An antitumor medicine and/or anticancer medicine comprising the antibody-drug conjugate according to any one of [1] to [28], a salt thereof or a hydrate thereof.

[31] The antitumor medicine and/or anticancer medicine according to [30], which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[32] A pharmaceutical composition comprising the antibody-drug conjugate according to any one of [1] to [28], a salt thereof or a hydrate thereof as an active component, and a pharmaceutically acceptable formulation component.

[33] The pharmaceutical composition according to [32], which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[34] A method for treating a tumor and/or cancer comprising administering the antibody-drug conjugate according to any one of [1] to [28], a salt thereof or a hydrate thereof.

[35] The medicine according to [29], the antitumor medicine and/or anticancer medicine according to [30] or [31], the pharmaceutical composition according to [32] or [33], or the treatment method according to [34], which is used in administration in combination with an additional medicine.

[36] The pharmaceutical composition according to [32] or [33], further comprising

even an additional medicine as an active ingredient.

[0028] [35] A method for producing an antibody-drug conjugate comprising reacting a compound represented by the following formula:

(maleimid-N-yl)-$(CH_2)n^3$-C(=O)-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-(NH-DX) or (maleimid-N-yl)-$(CH_2)n^3$-C(=O)-L^2-L^P-(NH-DX)

with an anti-HER3 antibody or a reactive derivative thereof and conjugating a drug-linker moiety to the antibody by a method for forming a thioether bond on a disulfide bond moiety present at a hinge part of the antibody.

[0029] In the formula, n^3 represents an integer of 2 to 8,

L^2 represents -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)- or a single bond wherein n^4 represents an integer of 1 to 6,

L^P represents a peptide residue consisting of 2 to 7 amino acids selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid,

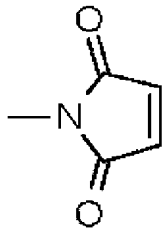
n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^a represents -O- or a single bond,

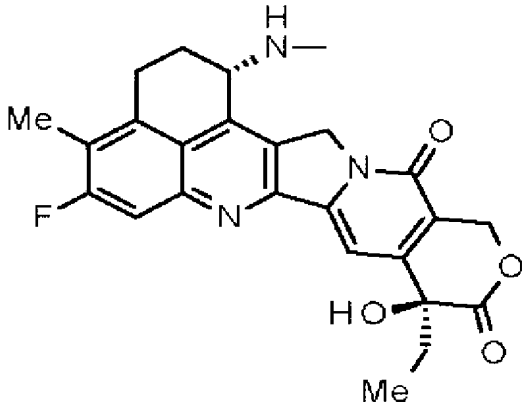
(maleimid-N-yl)- is a group represented by the following formula and has a nitrogen atom as a connecting position.

[Chem.10]



-(NH-DX) represents a group represented by the following formula, wherein the nitrogen atom of the amino group at position 1 is the connectig position:

[Chem.11]



[0030] [36] The production method described in [35], wherein the method for conjugating a drug-linker moiety to an anti-HER3 antibody is a method of reducing the antibody for conversion into a reactive derivative.

[0031] [37] The production method described in [35] or [36], wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in the range of from 1 to 10.

[38] The production method described in [35] or [36], wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in the range of from 2 to 8.

[39] The production method described in [35] or [36], wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in the range of from 3 to 8.

[40] An antibody-drug conjugate obtained by the production method according to any one of [35] to [39].

[0032] [41] An antibody-drug conjugate obtained by forming a thioether bond at a disulfide bond site present in a hinge part of an anti-HER3 antibody, wherein the anti-HER3 antibody is treated in a reducing condition and thereafter reacted with a compound selected from the compound group shown below:

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

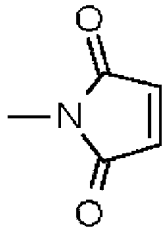
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-CH_2CH_2-C(=O)-(NH-DX), (maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$ -C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(

=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),

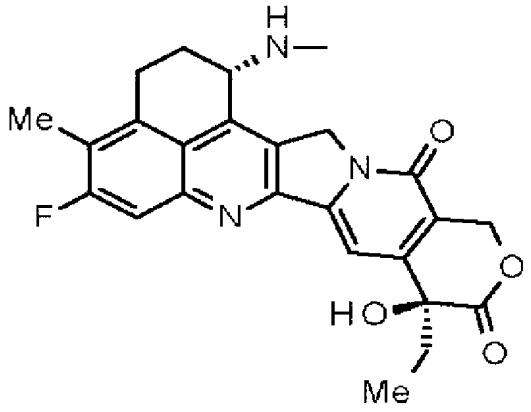
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX), or
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[0033] In the above, (maleimid-N-yl)- is a group represented by the following formula:
[Chem.12]



which has a nitrogen atom as a connecting position.

-(NH-DX) represents a group represented by the following formula, the nitrogen atom of the amino group at position 1 being a connecting position.
[Chem.13]



-GGFG- represents a tetrapeptide residue of -Gly-Gly-Phe-Gly- and -DGGFG- represents pentapeptide residue of -Asp-Gly-Gly-Phe-Gly-.

[0034] [42] An antibody-drug conjugate obtained by forming a thioether bond at a disulfide bond site present in a hinge part of an anti-HER3 antibody, and characterized by treating the anti-HER3 antibody with a reducing condition and thereafter reacting with a compound selected from the compound group shown below:

(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX), or

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX).

In the above, (maleimid-N-yl)-, -(NH-DX), and -GGFG- are as defined above.

[0035] [43] The antibody-drug conjugate according to [41] or [42], wherein an average conjugated number of the selected one drug-linker structure per antibody is in a range of from 1 to 10.

[44] The antibody-drug conjugate according to [41] or [42], wherein an average conjugated number of the selected one drug-linker structure per antibody is in a range of from 2 to 8.

[45] The antibody-drug conjugate according to [41] or [42], wherein an average conjugated number of the selected one drug-linker structure per antibody is in a range of from 3 to 8.

[46] A medicine comprising the antibody-drug conjugate according to any one of [40] to [45], a salt thereof or a hydrate thereof.

[47] An antitumor medicine and/or anticancer medicine comprising the antibody-drug conjugate according to any one of [40] to [45], a salt thereof or a hydrate thereof.

[48] The antitumor medicine and/or anticancer medicine according to [47], which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[49] A pharmaceutical composition comprising the antibody-drug conjugate according to any one of [40] to [45], a salt thereof or a hydrate thereof as an active component, and a pharmaceutically acceptable formulation component.

[50] The pharmaceutical composition according to [49], which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[51] A method for treating a tumor and/or cancer comprising administering the

antibody-drug conjugate according to any one of [40] to [45], a salt thereof or a hydrate thereof.

[52] The medicine according to [46], the antitumor medicine and/or anticancer medicine according to [47] or [48], the pharmaceutical composition according to [49] or [50], or the treatment method according to [51], which is used in administration in combination with an additional medicine.

[53] The pharmaceutical composition according to [49] or [50], further comprising even an additional medicine as an active ingredient.

Advantageous Effects of Invention

[0036] With an anti-HER3 antibody-drug conjugate having an antitumor compound exatecan conjugated via a linker with a specific structure, an excellent antitumor effect and safety can be achieved.

Brief Description of Drawings

[0037] [fig.1]Figure 1 shows the full-length amino acid sequence of a heavy chain of anti-HER3 human antibody U1-59 (SEQ ID NO: 583).

[fig.2]Figure 2 shows the full-length amino acid sequence of a light chain of anti-HER3 human antibody U1-59 (SEQ ID NO: 584).

[fig.3]Figure 3 shows the mean fluorescence intensity of HCC1569 treated with serial dilutions of U1-59 or each antibody-drug conjugate. KD and Bmax values were calculated using GraphPad Prism Software.

[fig.4]A549 cells were cultured for 2 days with U1-59 or varied antibody-drug conjugates. HER3 or phosphorylated HER3 was evaluated by Western blotting. pan-Actin was detected as an electrophoresis control.

[fig.5]Figure 5 shows an average value of reduction in HER3 expression on the surface of HCC1569 cells treated with U1-59 or each antibody-drug conjugate (37C ("C" represents "degrees Celsius"), 1 hr).

[fig.6]Figure 6 shows results of a test on the inhibition of mitogenic or survival signals by each HER3 antibody-drug conjugate in a human breast cancer line (HCC1569). Figure 6A shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The data is indicated by mean +/- standard deviation of triplicates. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. Figure 6B shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[fig.7]Figure 7 shows results of a test on the inhibition of mitogenic or survival signals by each HER3 antibody-drug conjugate in a human breast cancer line (MDA-MB 453).

Figure 7A shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. Figure 7B shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[fig.8]Figure 8 shows results of a test on the inhibition of mitogenic or survival signals by each HER3 antibody-drug conjugate in a human melanoma line (A375). Figure 8A shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. Figure 8B shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[fig.9]Figure 9 shows results of a test on the inhibition of mitogenic or survival signals by each HER3 antibody-drug conjugate in a human colorectal cancer line (HT29). Figure 9A shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. Figure 9B shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[fig.10]Figure 10 shows results of a test on the inhibition of mitogenic or survival signals by each HER3 antibody-drug conjugate in a human lung cancer line (A549). Figure 10A shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. Figure 10B shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[fig.11]Figure 11 shows results of comparing the rate of inhibition of cell growth or survival between the antibody-drug conjugate (3) and the antibody-drug conjugate (4). The left diagram shows the rate of inhibition of cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts luminescence indicating the ATP activity of each sample. The abscissa depicts the con-

centration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. The right diagram shows the comparison of the rate of reduction in luminescence caused by antibody-drug conjugate treatment between high drug loading (HDL) and middle drug loading (MDL) when the luminescence of an untreated group was defined as 100%.

[fig.12]Figure 12 shows results of comparing the rate of inhibition of cell growth or survival between the antibody-drug conjugate (10) and the antibody-drug conjugate (11). The left diagram shows the rate of inhibition of cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts luminescence indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. The right diagram shows the comparison of the rate of reduction in luminescence caused by antibody-drug conjugate treatment between high drug loading (HDL) and middle drug loading (MDL) when the luminescence of an untreated group was defined as 100%.

[fig.13]Figure 13 shows results of comparing the rate of inhibition of cell growth or survival between the antibody-drug conjugate (13) and the antibody-drug conjugate (14). The left diagram shows the rate of inhibition of cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts luminescence indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. The right diagram shows the comparison of the rate of reduction in luminescence caused by antibody-drug conjugate treatment between high drug loading (HDL) and middle drug loading (MDL) when the luminescence of an untreated group was defined as 100%.

[fig.14]Figure 14 shows results of a human breast cancer (HCC1569) antitumor test using the antibody-drug conjugate (3), (10), or (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.15]Figure 15 shows results of a human melanoma (HT-144) antitumor test using the antibody-drug conjugate (3), (10), or (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.16]Figure 16 shows results of a human breast cancer (MDA-MB-453) antitumor

test using the antibody-drug conjugate (3), (10), or (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from administration. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.17]Figure 17 shows results of a human colorectal cancer line (HT-29) antitumor test using the antibody-drug conjugate (3), (10), or (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from administration. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.18]Figure 18 shows results of a human lung cancer line (A549) antitumor test using the antibody-drug conjugate (3), (10), or (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.19]Figure 19 shows results of a human triple-negative breast cancer line (MDA-MB-468) antitumor test using the antibody-drug conjugate (13). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.20]Figure 20 shows results of a human luminal breast cancer line (MCF-7) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.21]Figure 21 shows results of a human melanoma line (WM-266-4) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.22]Figure 22 shows results of a human ovarian cancer line (OVCAR-8) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values

are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.23]Figure 23 shows results of a human bladder cancer line (SW-780) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.24]Figure 24 shows results of a human breast cancer line (MDA-MB-453) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.25]Figure 25 shows results of a human breast cancer line (MDA-MB-453) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.26]Figure 26 shows results of a human breast cancer line (JIMT-1) antitumor test using the antibody-drug conjugate (15). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.27]Figure 27 shows results of a human lung cancer line (PC9) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.28]Figure 28 shows results of a human triple-negative breast cancer line (MDA-MB-468) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive

data (mean and standard deviation) using Microsoft Excel 2009.

[fig.29]Figure 29 shows results of a human head and neck cancer line (Fadu) antitumor test using the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

[fig.30]Figure 30 shows results of an antitumor test using a human stomach cancer patient-derived tumor section (NIBIO-G016) and the antibody-drug conjugate (16a). The ordinate depicts an average tumor volume. The abscissa depicts the number of days from cell transplantation. All values are indicated by mean +/- standard deviation. The initial tumor volume and the initial mouse weight were analyzed on the basis of descriptive data (mean and standard deviation) using Microsoft Excel 2009.

Description of Embodiments

[0038] Hereinbelow, the preferred embodiments for carrying out the present invention are explained in view of the drawings. Meanwhile, the embodiments explained below are the examples of the representative embodiments of the present invention and the scope of the present invention shall not be narrowly interpreted based on them.

[0039] The present invention provides HER3 binding protein-drug conjugate. Preferably, the HER3 binding protein of the invention is a scaffold protein having an antibody like binding activity or an antibody, i.e. an anti-HER3 antibody.

[0040] The anti-HER3 antibody-drug conjugate of the present invention is an antitumor medicine in which an anti-HER3 antibody is conjugated to an antitumor compound via a linker structure moiety and explained in detail hereinbelow.

Within the context of the present invention, the term "scaffold protein", as used herein, 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 invention are 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 Pluckthun, Curr Opin Biotechnol, 16, 459-69). 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 invention. 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 (Skerra, J. Mol. Recog., 2000; Binz and Pluckthun, 2005). 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. Said 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.

[0041] {Antibody}

Moreover, the term "antibody" or "anti-HER3 antibody", as used herein, means a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody (Jones et al., Nature 321 (1986), 522-525; Riechmann et al., Nature 332 (1988), 323-329; and Presta, Curr. Op. Struct. Biol. 2 (1992), 593-596), a chimeric antibody (Morrison et al., Proc. Natl. Acad. Sci. U.S.A. 81 (1984), 6851-6855), a human antibody and fully human antibody, (Tomizuka, K. et al., Nature Genetics (1997) 16, p.133-143,; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, p.3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol.10, p.69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999.; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p.722-727, International Publication No. WO 2007/077028, and so on), a multispecific antibody (e.g. a bispecific antibody) formed from at least two antibodies, or an antibody fragment thereof. The term "antibody fragment" comprises any portion of the afore-mentioned antibodies, preferably their antigen binding region or variable regions. Examples of antibody fragments include Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, diabodies (Hollinger et al., Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 6444-6448), single chain antibody molecules (Pluckthun in: The Pharmacology of Monoclonal Antibodies 113, Rosenburg and Moore, EDS, Springer Verlag, N.Y. (1994), 269-315) and other fragments as long as they exhibit the desired capability of binding to HER3.

[0042] In addition, the term "antibody" or "anti-HER3 antibody", as used herein, may include 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 VH-only or VL-only domains derived either from natural sources such as camelids (Muyldermans et al., Reviews in Molecular Biotechnology

74, 277-302) or through in vitro display of libraries from humans, camelids or other species (Holt et al., Trends Biotechnol., 21, 484-90).

[0043] In accordance with the present invention, the "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- 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.

[0044] In another preferred embodiment of the present invention, the anti-HER3 antibody of the invention is an anti-HER3 antibody directed against the extracellular domain (ECD) of HER3.

[0045] The anti-HER3 antibody used in an anti-HER3 antibody-drug conjugate of the present invention may be derived from any species. Preferred examples of the species can include humans, rats, mice, and rabbits. The anti-HER3 antibody derived from other than human species is preferably chimerized or humanized using a well known technique. The antibody of the present invention may be a polyclonal antibody or a monoclonal antibody and is preferably a monoclonal antibody.

The anti-HER3 antibody is may be those which are capable of targeting tumor cells and thus possesses the property of being capable of recognizing tumor cells, the property of being capable of binding to tumor cells, the property of being internalized into tumor cells, and cytocidal activity against tumor cells, etc. The anti-HER3 antibody can be conjugated with a compound having antitumor activity via a linker to form an antibody-drug conjugate.

The binding activity of the antibody against tumor cells can be confirmed using flow cytometry. The internalization of the antibody into tumor cells can be confirmed using (1) an assay of visualizing an antibody incorporated in cells under a fluorescence microscope using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Cell Death and Differentiation (2008) 15, 751-761), (2) an assay of

measuring the amount of fluorescence incorporated in cells using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Molecular Biology of the Cell, Vol. 15, 5268-5282, December 2004), or (3) a Mab-ZAP assay using an immunotoxin binding to the therapeutic antibody wherein the toxin is released upon incorporation into cells to inhibit cell growth (Bio Techniques 28: 162-165, January 2000). A recombinant complex protein of a diphtheria toxin catalytic domain and protein G may be used as the immunotoxin.

The antitumor activity of the antibody can be confirmed in vitro by determining inhibitory activity against cell growth. For example, a cancer cell line overexpressing a target protein for the antibody is cultured, and the antibody is added at varying concentrations into the culture system to determine inhibitory activity against focus formation, colony formation, and spheroid growth. The antitumor activity can be confirmed in vivo, for example, by administering the antibody to a nude mouse with a transplanted tumor cell line highly expressing the target protein, and determining change in the cancer (tumor) cells.

Since the compound conjugated in the antibody-drug conjugate exerts an antitumor effect, it is preferred but not essential that the antibody itself should have an antitumor effect. For the purpose of exerting the cytotoxicity of the antitumor compound specifically and selectively for tumor cells, it is important and also preferred that the antibody should have the property of being internalized to migrate into tumor cells.

[0046] The anti-HER3 antibody can be obtained using a method usually carried out in the art, which involves immunizing animals with an antigenic polypeptide and collecting and purifying antibodies produced in vivo. The origin of the antigen is not limited to humans, and the animals may be immunized with an antigen derived from a non-human animal such as a mouse or a rat and the like. In this case, the cross-reactivity of antibodies binding to the obtained heterologous antigen with human antigens can be tested to screen for an antibody applicable to a human disease.

Alternatively, antibody-producing cells which produce antibodies against the antigen are fused with myeloma cells according to a method known in the art (e.g., Kohler and Milstein, Nature (1975) 256, p. 495-497; and Kennet, R. ed., Monoclonal Antibodies, p. 365-367, Plenum Press, N.Y. (1980)) to establish hybridomas, from which monoclonal antibodies can in turn be obtained.

The antigen can be obtained by genetically engineering host cells to produce a gene encoding the antigenic protein. Specifically, vectors that permit expression of the antigen gene are prepared and transferred to host cells so that the gene is expressed. The antigen thus expressed can be purified. The antibody can also be obtained by use of a method which involves immunizing animals with the genetically engineered antigen-expressing cells or a cell line with an expressed antigen.

The anti-HER3 antibody can be obtained by means known in the art.

[0047] The anti-HER3 antibody that can be used in the present invention is not particularly limited and is desirably, for example, any of antibodies having properties as described below.

(1) An anti-HER3 antibody having the following properties:

(a) specifically binding to HER3, and/or

(b) having the activity of being internalized into HER3-expressing cells through binding to HER3.

(2) The antibody according to (1), wherein the antibody binds to the extracellular domain of HER3.

(3) The antibody according to (1) or (2), wherein the antibody is a monoclonal antibody.

(4) The antibody according to any of (1) to (3), wherein the antibody has antibody-dependent cell-mediated cytotoxicity (ADCC) activity and/or complement-dependent cytotoxicity (CDC) activity.

(5) The antibody according to any of (1) to (4), wherein the antibody is a mouse monoclonal antibody, a chimeric monoclonal antibody, a humanized monoclonal antibody, or a human or fully human (monoclonal) antibody.

(6) The antibody according to any of (1) to (5), wherein the antibody is a humanized monoclonal antibody comprising a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 1 and a light chain comprising the amino acid sequence represented by SEQ ID NO: 2.

(7) The antibody according to any of (1) to (6), wherein the antibody lacks a lysine residue at the carboxy terminus of the heavy chain.

(8) The antibody according to (7), wherein the antibody comprises a heavy chain variable region represented by the amino acid sequence represented by SEQ ID NO: 70 and a light chain variable region represented by the amino acid sequence represented by SEQ ID NO: 72.

(9) An antibody obtained by a method for producing the antibody according to any of (1) to (8), the method comprising the steps of: culturing a host cell transformed with an expression vector comprising a polynucleotide encoding the antibody; and collecting the antibody of interest from the cultures obtained in the preceding step.

[0048] Hereinafter, the anti-HER3 antibody used in the present invention will be described.

In the present specification, the terms "cancer" and "tumor" are used interchangeably.

In the present specification, the term "gene" includes not only DNA but its mRNA, cDNA, and cRNA thereof.

In the present specification, the term "polynucleotide" is used interchangeably with a nucleic acid and also includes DNA, RNA, probes, oligonucleotides, and primers.

In the present specification, the terms "polypeptide" and "protein" are used interchangeably.
In the present specification, the term "cell" also includes cells in animal individuals and cultured cells.
In the present specification, the term "HER3" is used interchangeably with HER3 protein.
In the present specification, the term "CDR" means a complementarity determining region (CDR). An antibody molecule is known to have three CDRs in each of heavy and light chains. CDRs, also called hypervariable domains, are located in the variable regions of the antibody heavy and light chains. These sites have a particularly highly variable primary structure and are separated at three positions on the respective primary structures of heavy and light chain polypeptide strands. In the present specification, the antibody CDRs are referred to as CDRH1, CDRH2, and CDRH3 from the amino terminus of the heavy chain amino acid sequence as to heavy chain CDRs and as CDRL1, CDRL2, and CDRL3 from the amino terminus of the light chain amino acid sequence as to light chain CDRs. These sites are proximal to each other on the three-dimensional structure and determine specificity for the antigen to be bound.
In the present invention, the phrase "hybridizing under stringent conditions" refers to hybridization at 68C in a commercially available hybridization solution ExpressHyb Hybridization Solution (manufactured by Clontech Laboratories, Inc.), or identifiable hybridization under conditions involving hybridization at 68C in the presence of 0.7 to 1.0 M NaCl using a DNA-immobilized filter, followed by washing at 68C using 0.1 to 2 ´ SSC solution (1 ´ SSC is composed of 150 mM NaCl and 15 mM sodium citrate), or hybridization under conditions equivalent thereto.

[0049] 1. HER3

The human epidermal growth factor receptor 3 (HER3, also known as ErbB3) is a receptor protein tyrosine kinase and belongs to the epidermal growth factor receptor (EGFR) subfamily of receptor protein tyrosine kinases, which also includes HER1 (also known as EGFR), HER2, and HER4. HER3 is a transmembrane receptor and consists of an extracellular ligand-binding domain (ECD), a dimerization domain within the ECD, a transmembrane domain, an intracellular protein tyrosine kinase domain (TKD) and a C-terminal phosphorylation domain. HER3 has been found to be overexpressed in several types of cancer such as breast, gastrointestinal and pancreatic cancers. A correlation between the expression of HER2/HER3 and the progression from a non-invasive to an invasive stage has been shown.

The HER3 protein used in the present invention can be used after direct purification from HER3-expressing human or non-human mammalian (rat, mouse, etc.) cells or can be used by preparing cell membrane fractions of the cells. Alternatively, HER3 may be

synthesized in vitro or may be produced from host cells by genetic engineering. In the genetic engineering, specifically, HER3 cDNA is integrated into vectors that permit expression thereof, and HER3 can then be expressed by synthesis in a solution containing enzymes necessary for transcription and translation, substrates, and energy substances or by transformation of other host prokaryotic cells or host eukaryotic cells to yield the protein. Alternatively, the genetically engineered HER3-expressing cells described above or a cell line with expressed HER3 may be used as the HER3 protein. An RNA sequence, a cDNA sequence, and an amino acid sequence of HER3 are available in public database, and can be referred to by an accession number such as AAA35979 (precursor including a signal sequence consisting of amino terminus 19 amino acid residue), M34309 (NCBI), for example.

The above amino acid sequence of HER3 consists of an amino acid sequence which is subjected to replacements, deletions, additions and/or insertions of at least one amino acid, and proteins having a biological activity equivalent to that of the protein are also included in HER3.

[0050] 2. Production of anti HER3 antibody

The antibody against HER3 of the present invention can be obtained by immunizing an animal with HER3 or an arbitrary polypeptide selected from the amino acid sequence of HER3, and collecting and purifying the antibody produced in vivo according to a method usually carried out in the art. The biological species of HER3 to be used as an antigen is not limited to being human, and an animal can be immunized with HER3 derived from an animal other than humans such as a mouse or a rat. In this case, by examining the cross-reactivity between an antibody binding to the obtained heterologous HER3 and human HER3, an antibody applicable to a human disease can be selected.

Further, a monoclonal antibody can be obtained from a hybridoma established by fusing antibody-producing cells which produce an antibody against HER3 with myeloma cells according to a known method (for example, Kohler and Milstein, Nature, (1975) 256, pp. 495-497; Kennet, R. ed., Monoclonal Antibodies, pp. 365-367, Plenum Press, N.Y. (1980)).

HER3 to be used as an antigen can be obtained by expressing HER3 gene in a host cell using genetic engineering.

Specifically, a vector capable of expressing HER3 gene is produced, and the resulting vector is transfected into a host cell to express the gene, and then, the expressed HER3 is purified.

It is also possible to use HER3 expressing cells obtained by the genetic engineering or a cell line expressing HER3 as HER3 protein. Hereinbelow, a method for obtaining an antibody against HER3 is explained specifically.

[0051] (1) Preparation of antigen

Examples of the antigen to be used for producing the anti HER3 antibody include HER3, a polypeptide consisting of a partial amino acid sequence comprising at least 6 consecutive amino acids of HER3, and a derivative obtained by adding a given amino acid sequence or carrier thereto.

HER3 can be purified directly from human tumor tissues or tumor cells and used. Further, HER3 can be obtained by synthesizing it in vitro or by producing it in a host cell by genetic engineering.

With respect to the genetic engineering, specifically, after HER3 cDNA is integrated into a vector capable of expressing HER3 cDNA, HER3 can be obtained by synthesizing it in a solution containing an enzyme, a substrate and an energy substance required for transcription and translation, or by expressing HER3 in another prokaryotic or eucaryotic transformed host cell.

Further, the antigen can also be obtained as a secretory protein by expressing a fusion protein obtained by ligating the extracellular domain of HER3, which is a membrane protein, to the constant region of an antibody in an appropriate host-vector system.

HER3 cDNA can be obtained by, for example, a so-called PCR method in which a polymerase chain reaction (referred to as "PCR"; see Saiki, R. K., et al., Science, (1988) 239, pp. 487-489) is performed using a cDNA library expressing HER3 cDNA as a template and primers which specifically amplify HER3 cDNA.

As the in vitro synthesis of the polypeptide, for example, Rapid Translation System (RTS) manufactured by Roche Diagnostics, Inc. can be exemplified, but it is not limited thereto.

Examples of the prokaryotic host cells include Escherichia coli and Bacillus subtilis. In order to transform the host cells with a target gene, the host cells are transformed by a plasmid vector comprising a replicon, i.e., a replication origin derived from a species compatible with the host, and a regulatory sequence. Further, the vector preferably has a sequence capable of imposing phenotypic selectivity on the transformed cell.

Examples of the eucaryotic host cells include vertebrate cells, insect cells, and yeast cells. As the vertebrate cells, for example, simian COS cells (Gluzman, Y., Cell, (1981) 23, pp. 175-182, ATCC CRL-1650; ATCC: American Type Culture Collection), murine fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L. A., Proc. Natl. Acad. Sci. USA (1980) 77, pp. 4126-4220) of Chinese hamster ovarian cells (CHO cells; ATCC: CCL-61); and the like are often used, however, the cells are not limited thereto.

The thus obtained transformant can be cultured according to a method usually carried out in the art, and by the culturing of the transformant, a target polypeptide is produced intracellularly or extracellularly.

A suitable medium to be used for the culturing can be selected from various commonly used culture media depending on the employed host cells. If Escherichia coli is employed, for example, an LB medium supplemented with an antibiotic such as ampicillin or IPMG as needed can be used.

A recombinant protein produced intracellularly or extracellularly by the transformant through such culturing can be separated and purified by any of various known separation methods utilizing the physical or chemical property of the protein. Specific examples of the methods include treatment with a common protein precipitant, ultrafiltration, various types of liquid chromatography such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, and affinity chromatography, dialysis, and a combination thereof. Further, by attaching a tag of six histidine residues to a recombinant protein to be expressed, the protein can be efficiently purified with a nickel affinity column. Alternatively, by attaching the IgG Fc region to a recombinant protein to be expressed, the protein can be efficiently purified with a protein A column.

By combining the above-described methods, a large amount of a target polypeptide can be easily produced in high yield and high purity.

It is also possible to use the aforementioned transformant itself as an antigen. It is also possible to use a cell line expressing HER3 as an antigen. Examples of the cell line include. However, as long as HER3 is expressed, it is not limited to those cell lines.

[0052] (2) Production of anti HER3 monoclonal antibody

Examples of the antibody specific binding to HER3 include a monoclonal antibody specific binding to HER3, and a method of obtaining the antibody is as described below.

The production of a monoclonal antibody generally requires the following operational steps of:

(a) Purification of a biopolymer used as an antigen or preparation of cells expressing antigen;

(b) preparing antibody-producing cells by immunizing an animal by injection of the antigen, collecting the blood, assaying its antibody titer to determine when the spleen is excised;

(c) preparing myeloma cells (hereinafter referred to as "myeloma");

(d) fusing the antibody-producing cells with the myeloma;

(e) screening a group of hybridomas producing a desired antibody;

(f) dividing the hybridomas into single cell clones (cloning);

(g) optionally, culturing the hybridoma or rearing an animal implanted with the hybridoma for producing a large amount of a monoclonal antibody;

(h) examining the thus produced monoclonal antibody for biological activity and

binding specificity, or assaying the same for properties as a labeled reagent; and the like.

Hereinafter, the method of producing a monoclonal antibody will be described in detail following the above steps, however, the method is not limited thereto, and, for example, antibody-producing cells other than spleen cells and myeloma can be used.

[0053] (a) Purification of antigen

As the antigen, HER3 prepared by the method as described above or a partial peptide thereof can be used.

Further, a membrane fraction prepared from recombinant cells expressing HER3 or the recombinant cells expressing HER3 themselves, and also a partial peptide of the protein of the invention chemically synthesized by a method known to those skilled in the art can also be used as the antigen.

Further, a cell line expressing HER3 can be also used as an antigen.

[0054] (b) Preparation of antibody-producing cells

The antigen obtained in the step (a) is mixed with an adjuvant such as Freund's complete or incomplete adjuvant or aluminum potassium sulfate and the resulting mixture is used as an immunogen to immunize an experimental animal. In an alternative method, a test animal is immunized with cells expressing antigen as an immunogen. As the experimental animal, any animal used in a known hybridoma production method can be used without any trouble. Specifically, for example, a mouse, a rat, a goat, sheep, cattle, a horse, or the like can be used. However, from the viewpoint of ease of availability of myeloma cells to be fused with the extracted antibody-producing cells, a mouse or a rat is preferably used as the animal to be immunized.

Further, the strain of a mouse or a rat to be used is not particularly limited, and in the case of a mouse, for example, various strains such as A, AKR, BALB/c, BDP, BA, CE, C3H, 57BL, C57BL, C57L, DBA, FL, HTH, HT1, LP, NZB, NZW, RF, R III, SJL, SWR, WB, and 129 and the like can be used, and in the case of a rat, for example, Wistar, Low, Lewis, Sprague, Dawley, ACI, BN, Fischer and the like can be used.

These mice and rats are commercially available from breeders/distributors of experimental animals, for example, CLEA Japan, Inc. and Charles River Laboratories Japan, Inc.

As the animal to be immunized, in consideration of compatibility of fusing with myeloma cells described below, in the case of a mouse, BALB/c strain, and in the case of a rat, Wistar and Low strains are particularly preferred.

Further, in consideration of antigenic homology between humans and mice, it is also preferred to use a mouse having decreased biological function to remove autoantibodies, that is, a mouse with an autoimmune disease.

The age of such mouse or rat at the time of immunization is preferably 5 to 12 weeks of age, more preferably 6 to 8 weeks of age.

In order to immunize an animal with HER3 or a recombinant thereof, for example, a known method described in detail in, for example, Weir, D. M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987); Kabat, E. A. and Mayer, M. M., Experimental Immunochemistry, Charles C Thomas Publisher Springfield, Illinois (1964) or the like can be used.

Among these immunization methods, a preferred specific method in the invention is, for example, as follows.

That is, first, a membrane protein fraction serving as the antigen or cells caused to express the antigen is/are intradermally or intraperitoneally administrated to an animal. However, the combination of both routes of administration is preferred for increasing the immunization efficiency, and when intradermal administration is performed in the first half and intraperitoneal administration is performed in the latter half or only at the last dosing, the immunization efficiency can be particularly increased.

The administration schedule of the antigen varies depending on the type of animal to be immunized, individual difference or the like. However, in general, an administration schedule in which the frequency of administration of the antigen is 3 to 6 times and the dosing interval is 2 to 6 weeks is preferred, and an administration schedule in which the frequency of administration of the antigen is 3 to 4 times and the dosing interval is 2 to 4 weeks is more preferred.

Further, the dose of the antigen varies depending on the type of animal, individual differences or the like, however, the dose is generally set to 0.05 to 5 mg, preferably about 0.1 to 0.5 mg.

A booster immunization is performed 1 to 6 weeks, preferably 1 to 4 weeks, more preferably 1 to 3 weeks after the administration of the antigen as described above. When the immunogen is a cell, $1\acute{}10^6$ to $1\acute{}10^7$ cells are used.

The dose of the antigen at the time of performing the booster immunization varies depending on the type or size of animal or the like, however, in the case of, for example, a mouse, the dose is generally set to 0.05 to 5 mg, preferably 0.1 to 0.5 mg, more preferably about 0.1 to 0.2 mg. When the immunogen is a cell, $1\acute{}10^6$ to $1\acute{}10^7$ cells are used.

Spleen cells or lymphocytes including antibody-producing cells are aseptically removed from the immunized animal 1 to 10 days, preferably 2 to 5 days, more preferably 2 to 3 days after the booster immunization. At this time, the antibody titer is measured, and if an animal having a sufficiently increased antibody titer is used as a supply source of the antibody-producing cells, the subsequent procedure can be carried out more efficiently.

Examples of the method of measuring the antibody titer to be used here include an RIA method and an ELISA method, but the method is not limited thereto. For example, if an ELISA method is employed, the measurement of the antibody titer in the invention can be carried out according to the procedures as described below.

First, a purified or partially purified antigen is adsorbed to the surface of a solid phase such as a 96-well plate for ELISA, and the surface of the solid phase having no antigen adsorbed thereto is covered with a protein unrelated to the antigen such as bovine serum albumin (hereinafter referred to as "BSA"). After washing the surface, the surface is brought into contact with a serially-diluted sample (for example, mouse serum) as a primary antibody to allow the antibody in the sample to bind to the antigen.

Further, as a secondary antibody, an antibody labeled with an enzyme against a mouse antibody is added and is allowed to bind to the mouse antibody. After washing, a substrate for the enzyme is added and a change in absorbance which occurs due to color development induced by degradation of the substrate or the like is measured and the antibody titer is calculated based on the measurement.

The separation of the antibody-producing cells from the spleen cells or lymphocytes of the immunized animal can be carried out according to a known method (for example, Kohler et al., Nature (1975), 256, p. 495; Kohler et al., Eur. J. Immunol. (1977), 6, p. 511; Milstein et al., Nature (1977), 266, p. 550; Walsh, Nature (1977), 266, p. 495). For example, in the case of spleen cells, a general method in which the antibody-producing cells are separated by homogenizing the spleen to yield the cells through filtration with a stainless steel mesh and suspending the cells in Eagle's Minimum Essential Medium (MEM) can be employed.

[0055] (c) Preparation of myeloma cells (hereinafter referred to as "myeloma")

The myeloma cells to be used for cell fusion are not particularly limited and suitable cells can be selected from known cell lines. However, in consideration of convenience when a hybridoma is selected from fused cells, it is preferred to use an HGPRT (hypoxanthine-guanine phosphoribosyl transferase) deficient strain whose selection procedure has been established.

More specifically, examples of the HGPRT-deficient strain include X63-Ag8(X63), NS1-ANS/1(NS1), P3X63-Ag8.U1(P3U1), X63-Ag8.653(X63.653), SP2/0-Ag14(SP2/0), MPC11-45.6TG1.7(45.6TG), FO, S149/5XXO, and BU.1 derived from mice; 210.RSY3.Ag.1.2.3(Y3) derived from rats; and U266AR(SKO-007), GM1500×GTG-A12(GM1500), UC729-6, LICR-LOW-HMy2(HMy2) and 8226AR/NIP4-1(NP41) derived from humans. These HGPRT-deficient strains are available from, for example, ATCC or the like.

These cell strains are subcultured in an appropriate medium such as an 8-azaguanine

medium [a medium obtained by adding 8-azaguanine to an RPMI 1640 medium supplemented with glutamine, 2-mercaptoethanol, gentamicin, and fetal calf serum (hereinafter referred to as "FBS")], Iscove's Modified Dulbecco's Medium; IMDM), or Dulbecco's Modified Eagle Medium (hereinafter referred to as "DMEM"). In this case, 3 to 4 days before performing cell fusion, the cells are subcultured in a normal medium [for example, an ASF104 medium (manufactured by Ajinomoto Co., Ltd.) containing 10% FCS] to ensure not less than 2 x 10^7 cells on the day of cell fusion.

[0056] (d) Cell fusion

Fusion between the antibody-producing cells and the myeloma cells can be appropriately performed according to a known method (Weir, D. M. Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987); Kabat, E. A. and Mayer, M. M., Experimental Immunochemistry, Charles C Thomas Publisher, Springfield, Illinois (1964), etc.), under conditions such that the survival rate of cells is not excessively reduced.

As such a method, for example, a chemical method in which the antibody-producing cells and the myeloma cells are mixed in a solution containing a polymer such as polyethylene glycol at a high concentration, a physical method using electric stimulation, or the like can be used. Among these methods, a specific example of the chemical method is as described below.

That is, in the case where polyethylene glycol is used in the solution containing a polymer at a high concentration, the antibody-producing cells and the myeloma cells are mixed in a solution of polyethylene glycol having a molecular weight of 1500 to 6000, more preferably 2000 to 4000 at a temperature of from 30 to 40C, preferably from 35 to 38C for 1 to 10 minutes, preferably 5 to 8 minutes.

[0057] (e) Selection of a group of hybridomas

The method of selecting hybridomas obtained by the above-described cell fusion is not particularly limited. Usually, an HAT (hypoxanthine, aminopterin, thymidine) selection method (Kohler et al., Nature (1975), 256, p. 495; Milstein et al., Nature (1977), 266, p. 550) is used.

This method is effective when hybridomas are obtained using the myeloma cells of an HGPRT-deficient strain which cannot survive in the presence of aminopterin. That is, by culturing unfused cells and hybridomas in an HAT medium, only hybridomas resistant to aminopterin are selectively allowed to survive and proliferate.

[0058] (f) Division into single cell clone (cloning)

As a cloning method for hybridomas, a known method such as a methylcellulose method, a soft agarose method, or a limiting dilution method can be used (see, for example, Barbara, B. M. and Stanley, M. S.: Selected Methods in Cellular Immunology, W. H. Freeman and Company, San Francisco (1980)). Among these

methods, particularly, a three-dimensional culture method such as a methylcellulose method is preferred. For example, the group of hybridomas produced by cell fusion are suspended in a methylcellulose medium such as ClonaCell-HY Selection Medium D (manufactured by StemCell Technologies, inc., #03804) and cultured. Then, the formed hybridoma colonies are collected, whereby monoclonal hybridomas can be obtained. The collected respective hybridoma colonies are cultured, and a hybridoma which has been confirmed to have a stable antibody titer in an obtained hybridoma culture supernatant is selected as an anti-HER3 monoclonal antibody-producing hybridoma strain.

[0059] (g) Preparation of monoclonal antibody by culturing hybridoma

By culturing the thus selected hybridoma, a monoclonal antibody can be efficiently obtained. However, prior to culturing, it is preferred to perform screening of a hybridoma which produces a target monoclonal antibody.

In such screening, a known method can be employed.

The measurement of the antibody titer in the invention can be carried out by, for example, an ELISA method explained in item (b) described above.

The hybridoma obtained by the method described above can be stored in a frozen state in liquid nitrogen or in a freezer at -80C or below.

After completion of cloning, the medium is changed from an HT medium to a normal medium, and the hybridoma is cultured.

Large-scale culture is performed by rotation culture using a large culture bottle or by spinner culture. From the supernatant obtained by the large-scale culture, a monoclonal antibody which specifically binds to the protein of the invention can be obtained by purification using a method known to those skilled in the art such as gel filtration.

Further, the hybridoma is injected into the abdominal cavity of a mouse of the same strain as the hybridoma (for example, the above-described BALB/c) or a Nu/Nu mouse to proliferate the hybridoma, whereby the ascites containing a large amount of the monoclonal antibody of the invention can be obtained.

In the case where the hybridoma is administrated in the abdominal cavity, if a mineral oil such as 2,6,10,14-tetramethyl pentadecane (pristane) is administrated 3 to 7 days prior thereto, a larger amount of the ascites can be obtained.

For example, an immunosuppressant is previously injected into the abdominal cavity of a mouse of the same strain as the hybridoma to inactivate T cells. 20 days thereafter, 10^6 to 10^7 hybridoma clone cells are suspended in a serum-free medium (0.5 mL), and the suspension is administrated in the abdominal cavity of the mouse. In general, when the abdomen is expanded and filled with the ascites, the ascites is collected from the mouse. By this method, the monoclonal antibody can be obtained at a concentration which is about 100 times or much higher than that in the culture solution.

The monoclonal antibody obtained by the above-described method can be purified by a method described in, for example, Weir, D. M.: Handbook of Experimental Immunology Vol. I, II, III, Blackwell Scientific Publications, Oxford (1978).
The thus obtained monoclonal antibody has high antigen specificity for HER3.

[0060] (h) Assay of monoclonal antibody

The isotype and subclass of the thus obtained monoclonal antibody can be determined as follows.

First, examples of the identification method include an Ouchterlony method, an ELISA method, and an RIA method.

An Ouchterlony method is simple, but when the concentration of the monoclonal antibody is low, a condensation operation is required.

On the other hand, when an ELISA method or an RIA method is used, by directly reacting the culture supernatant with an antigen-adsorbed solid phase and using antibodies corresponding to various types of immunoglobulin isotypes and subclasses as secondary antibodies, the isotype and subclass of the monoclonal antibody can be identified.

In addition, as a simpler method, a commercially available identification kit (for example, Mouse Typer Kit manufactured by Bio-Rad Laboratories, Inc.) or the like can also be used.

Further, the quantitative determination of a protein can be performed by the Folin Lowry method and a method of calculation based on the absorbance at 280 nm [1.4 (OD 280) = Immunoglobulin 1 mg/mL].

Further, even when the monoclonal antibody is separately and independently obtained by performing again the steps of (a) to (h) in (2), it is possible to yield an antibody having a cytotoxic activity equivalent to that of the anti-HER3 antibody. As one example of such an antibody, an antibody which binds to the same epitope as the anti-HER3 antibody can be exemplified. If a newly produced monoclonal antibody binds to a partial peptide or a partial tertiary structure to which the anti-HER3 antibody binds, it can be determined that the monoclonal antibody binds to the same epitope as the anti-HER3 antibody. Further, by confirming the competition by the monoclonal antibody for binding of the anti-HER3 antibody to HER3 (binding between the anti-HER3 antibody and HER3 is interfered by the monoclonal antibody), it can be determined that the monoclonal antibody binds to the same epitope as the anti-HER3 antibody even though a specific sequence or structure of the epitope has not been identified. Once the epitope is confirmed to be the same, it is strongly expected that the monoclonal antibody has the same antigen binding capacity or biological activity as the anti-HER3 antibody.

[0061] (3) Other antibodies

The antibody of the invention includes not only the above-described monoclonal antibody against HER3 but also a recombinant antibody obtained by artificial modification for the purpose of decreasing heterologous antigenicity to humans such as a chimeric antibody, a humanized antibody and a human antibody. These antibodies can be produced using a known method.

As the chimeric antibody, an antibody in which antibody variable and constant regions are derived from different species, for example, a chimeric antibody in which a mouse- or rat-derived antibody variable region is connected to a human-derived constant region can be exemplified (see Proc. Natl. Acad. Sci. USA, 81, 6851-6855, (1984)).

As the humanized antibody, an antibody obtained by integrating only a complementarity determining region (CDR) into a human-derived antibody (see Nature (1986) 321, pp. 522-525), and an antibody obtained by grafting a part of the amino acid residues of the framework as well as the CDR sequence to a human antibody by a CDR-grafting method (WO 90/07861) can be exemplified.

The term "several" as used herein refers to 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 or 2.

[0062] In accordance with the present invention, it is to be understood, that the amino acid sequence of the binding protein of the invention is not limited to the twenty conventional amino acids (See Immunology - A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference). For example, the amino acids may include stereoisomers (e.g. D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as alpha-,alpha-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 protein of the invention, include: 4-hydroxyproline, gamma-carboxyglutamate, epsilon-N,N,N-trimethyllysine, epsilon-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, sigma-N-methylarginine, and other similar amino acids and imino acids, e.g. 4-hydroxyproline.

[0063] As the amino acid substitution in this specification, a conservative amino acid substitution is preferred. The conservative amino acid substitution refers to a substitution occurring within a group of amino acids related to amino acid side chains. Preferred amino acid groups are as follows: an acidic group (aspartic acid and glutamic acid); a basic group (lysine, arginine, and histidine); a non-polar group (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan); and an uncharged polar family (glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine). More preferred amino acid groups are as follows: an aliphatic hydroxy group (serine and threonine); an amide-containing group (asparagine and glutamine);

an aliphatic group (alanine, valine, leucine, and isoleucine); and an aromatic group (phenylalanine, tryptophan, and tyrosine). Such an amino acid substitution is preferably performed within a range which does not impair the properties of a substance having the original amino acid sequence. When the heavy and light chains of the antibody of the present invention have glutamate as the N-terminal amino acid, it may be cyclized (in the form of pyroglutamate). In the present invention, such pyroglutamate is not differentiated from normal glutamine on amino acid sequences. In the heavy and light chains of the antibody of the present invention, cysteine may be in the form of cysteinyl. In the present invention, such a cysteinyl form is not differentiated from normal cysteine on amino acid sequences.

[0064] Further, the antibody of the invention includes a human antibody which binds to the HER3. An anti HER3 human antibody refers to a human antibody having only a gene sequence of an antibody derived from a human chromosome. The anti HER3 human antibody can be obtained by a method using a human antibody-producing mouse having a human chromosome fragment comprising heavy and light chain genes of a human antibody (see Tomizuka, K. et al., Nature Genetics (1997) 16, pp. 133-143; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, pp. 3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol. 10, pp. 69-73 (Kitagawa, Y., Matuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, pp. 722-727, etc.).

[0065] Such a human antibody-producing mouse can be created specifically as follows. A genetically modified animal in which endogenous immunoglobulin heavy and light chain gene loci have been disrupted, and instead, human immunoglobulin heavy and light chain gene loci have been introduced via a yeast artificial chromosome (YAC) vector or the like is created by producing a knockout animal and a transgenic animal and mating these animals.

Further, according to a recombinant DNA technique, by using cDNAs encoding each of such a heavy chain and a light chain of a human antibody, and preferably a vector comprising such cDNAs, eukaryotic cells are transformed, and a transformant cell which produces a recombinant human monoclonal antibody is cultured, whereby the antibody can also be obtained from the culture supernatant.

Here, as the host, for example, eukaryotic cells, preferably mammalian cells such as CHO cells, lymphocytes, or myeloma cells can be used.

With regard to preparation of a human antibody, detailed descriptions are given in International Publication No. WO 2007/077028. The contents of International Publication No. WO 2007/077028 are incorporated herein by reference.

[0066] Further, a method of obtaining a phage display-derived human antibody selected from a human antibody library (see Wormstone, I. M. et al., Investigative Oph-

thalmology & Visual Science. (2002) 43 (7), pp. 2301-2308; Carmen, S. et al., Briefings in Functional Genomics and Proteomics (2002), 1 (2), pp. 189-203; Siriwardena, D. et al., Ophthalmology (2002) 109 (3), pp. 427-431, etc.) is also known. For example, a phage display method in which a variable region of a human antibody is expressed on the surface of a phage as a single-chain antibody (scFv), and a phage which binds to an antigen is selected (Nature Biotechnology (2005), 23, (9), pp. 1105-1116) can be used.

By analyzing the gene of the phage selected based on the binding to an antigen, a DNA sequence encoding the variable region of a human antibody which binds to an antigen can be determined.

If the DNA sequence of scFv which binds to an antigen is determined, a human antibody can be obtained by preparing an expression vector comprising the sequence and introducing the vector into an appropriate host to express it (WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438, WO 95/15388; Annu. Rev. Immunol. (1994) 12, pp. 433-455; Nature Biotechnology (2005) 23 (9), pp. 1105-1116).

[0067] One aspect of the present invention relates to an isolated protein that binds to HER3. In one embodiment of the present invention, an isolated HER3-binding protein of the invention comprises a heavy chain variable region amino acid sequence comprising: (a) CDRH1 comprised in the amino acid sequence represented by SEQ ID NO: 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 or 230, (b) CDRH2 comprised in the amino acid sequence represented by SEQ ID NO: 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 or 230, and (c) CDRH3 comprised in the amino acid sequence represented by SEQ ID NO: 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 or 230, and a light chain variable region amino acid sequence comprising: (d) CDRL1 comprisedin the amino acid sequence represented by SEQ ID NO: 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 or 232, (e) CDRL2 comprised in the amino acid sequence represented by SEQ ID NO: 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 or 232, and (f) CDRL3 comprisedin the amino acid sequence represented by SEQ ID NO: 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 or 232.

[0068] The isolated HER3-binding protein of the present invention preferably comprises a heavy chain amino acid sequence comprising (a) CDRH1 comprising the amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 236, 251, 252, and 256; (b) CDRH2 comprising the amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 258, 278, 280, and 282; and (c) CDRH3 comprising the amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 283, 285, 309, 313, and 315, and a light chain amino acid sequence comprising (d) CDRL1 cmprisingthe amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 320, 334, 337, and 340; (e) CDRL2 comprising the amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 343, 356, 351, and 344; and (f) CDRL3 comprising the amino acid sequence represented by one selected from the group consisting of SEQ ID NOs: 360, 381, 385, and 387.

[0069] In another embodiment of the present invention, an isolated binding protein of the invention comprises a heavy chain variable region 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 variable region 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.

[0070] In yet another embodiment of the present invention, an isolated binding protein of the invention comprises a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by 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 or 230 and 232, or, a heavy chain variable region amino acid sequence represented by SEQ ID NO: 34, 40, 60, 62 or 120 and a light chain variable region amino acid sequence represented by SEQ ID NO: 58 or 64, respectively.

The isolated HER3-binding protein of the present invention more preferably comprises a heavy chain variable region amino acid sequence represented by SEQ ID NO: 42, 54, 70, 92, or 96 and a light chain variable region amino acid sequence represented by SEQ ID NO: 44, 56, 72, 94, or 98.

[0071] An antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 2 and 4 is referred to as "U1-39", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 6 and 8 is referred to as "U1-40", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 10 and 12 is referred to as "U1-38", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 14 and 16 is referred to as "U1-41", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 18 and 20 is referred to as "U1-42", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 22 and 24 is referred to as "U1-43", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 26 and 28 is referred to as "U1-44", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 30 and 32 is referred to as "U1-45", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 36 and 38 is referred to as "U1-47", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 42 and 44 is referred to as "U1-49", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 46 and 48 is referred to as "U1-50", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 50 and 52 is referred to as "U1-51", an antibody comprising a heavy chain variable region amino acid sequence and a light

chain variable region amino acid sequence represented by SEQ ID NOs: 54 and 56 is referred to as "U1-53", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 60 and 58 is referred to as "U1-55", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 62 and 64 is referred to as "U1-57", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 66 and 68 is referred to as "U1-58", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 70 and 72 is referred to as "U1-59", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 74 and 76 is referred to as "U1-52", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 78 and 82 is referred to as "U1-61", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 80 and 82 is referred to as "U1-61.1", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 84 and 86 is referred to as "U1-62", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 88 and 90 is referred to as "U1-2", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 92 and 94 is referred to as "U1-7", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 96 and 98 is referred to as "U1-9", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 100 and 102 is referred to as "U1-10", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 104 and 106 is referred to as "U1-12", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 108 and 110 is referred to as "U1-13", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 112 and 114 is referred to as "U1-14", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by

SEQ ID NOs: 116 and 118 is referred to as "U1-15", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 122 and 124 is referred to as "U1-20", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 126 and 128 is referred to as "U1-21", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 130 and 132 is referred to as "U1-22", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 134 and 136 is referred to as "U1-23", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 138 and 140 is referred to as "U1-24", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 142 and 144 is referred to as "U1-25", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 146 and 148 is referred to as "U1-26", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 150 and 152 is referred to as "U1-27", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 154 and 156 is referred to as "U1-28", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 158 and 160 is referred to as "U1-31", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 162 and 164 is referred to as "U1-32", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 166 and 168 is referred to as "U1-35", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 170 and 172 is referred to as "U1-36", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 174 and 176 is referred to as "U1-37", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 178 and 180 is referred to as "U1-34", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 182 and 184 is referred to as "U1-1", an

antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 186 and 188 is referred to as "U1-3", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 190 and 192 is referred to as "U1-4", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 194 and 196 is referred to as "U1-5", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 198 and 200 is referred to as "U1-6", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 202 and 204 is referred to as "U1-8", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ
ID NOs: 206 and 208 is referred to as "U1-11", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 210 and 212 is referred to as "U1-16", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 214 and 216 is referred to as "U1-17", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 218 and 220 is referred to as "U1-18", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 222 and 224 is referred to as "U1-33", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 226 and 228 is referred to as "U1-29", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 230 and 232 is referred to as "U1-30", an antibody comprising a heavy chain variable region amino acid sequence represented by SEQ ID NO: 34 is referred to as "U1-46", an antibody comprising a heavy chain variable region amino acid sequence represented by SEQ ID NO: 40 is referred to as "U1-48", an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 60 and 58 is referred to as "U1-55.1", an antibody comprising a heavy chain variable region amino acid sequence represented by SEQ ID NO: 120 is referred to as "U1-19", and an antibody comprising a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 62 and 64 is

referred to as "U1-57.1". These antibodies are described in detail in Examples. The isolated HER3-binding protein of the present invention even more preferably comprises a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 42 and 44, respectively, a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 54 and 56, respectively, a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 70 and 72, respectively, a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 92 and 94, respectively, or a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence represented by SEQ ID NOs: 96 and 98, respectively, and still even more preferably, the HER3-binding proteins is U1-49, U1-53, U1-59, U1-7, or U1-9, which are an anti-HER3 antibody.

[0072]

[Chem.14]

Sequence Listing

Antibody U1-39

1 Heavy Chain DNA:
GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGATCCAGCCTGGGGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGGTTCACCGTCAGTAGCAACTACATGAGCTGGGTCCGCCAGGCT
CCAGGGAAGGGGCTGGATTGGGTCTCAGTTATTTATAGCGGTGGTAGCACATACTACGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTT
CAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGGCAGTGG
CTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

2 Heavy Chain Protein:
EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLDWVSVIYSGGSTYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGQWLDVWGQGTTVTVSS

3 Light Chain DNA:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCAAGTCAGAGCCTCCTGCATAGTAATGGATACAACTATTTGGATTGG
TACCTGCAGAGGCCAGGGCAGTCTCCACAACTCCTGTTCTATTTGGGTTTTCATCGGGCC
TCCGGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCAGGCAAGCTCTACAAACTCCG
CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

4 Light Chain Protein:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQRPGQSPQLLFYLGFHRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCRQALQTPLTFGGGTKVEIK

Antibody U1-40

5 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGTACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTCCAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
AGGGAACTGGAACTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTC

6 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYSSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDRELELYYYYYGMDVWGQGTT
VTVS

7 Light Chain DNA:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGTATAGTAATGGATACAACTATTTGGATTGG
TACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCC
TCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGATTTATTACTGCATGCAAGCTCTACAAACTCCG
CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

8 Light Chain Protein:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQLLIYLGSNRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTPLTFGGGTKVEIK

Antibody U1-38

9 Heavy Chain DNA:
CAGATCACCTTGAAGGAGTCTGGTCCTACGCTGGTGAAACCCACACAGACCCTCACGCTG
ACCTGCACCTTCTCTGGGTTCTCACTCAGCACTAGTGGAGTGGGTGTGGGCTGGATCCGT
CAGCCCCCAGGAAAGGCCCTGGACTGGCTTGCACTCATTTATTGGAATGATGATAAGCGC

TACAGCCCATCTCTGAAGAGCAGGCTCACCATCACCAAGGACACCTCCAAAAACCAGGTG
GTCCTTACAATGACCAACATGGATCTTGTGGACACAGCCACATATTACTGTGTACACAGA
GACGAAGTTCGAGGGTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

10 Heavy Chain Protein:
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALDWLALIYWNDDKR
YSPSLKSRLTITKDTSKNQVVLTMTNMDLVDTATYYCVHRDEVRGFDYWGQGTLVTVSS

11 Light Chain DNA:
GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAAAGCCTCGTATACAGTGATGGATACACCTACTTGCATTGG
TTTCAGCAGAGGCCAGGCCAATCTCCAAGGCGCCTTATTTATAAGGTTTCTAACTGGGAC
TCTGGGGTCCCAGACAGATTCAGCGGCAGTGGGTCAGGCACTGATTTCACACTGAAAATC
AGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGGTGCACACTGGCCG
ATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA

12 Light Chain Protein:
DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGYTYLHWFQQRPGQSPRRLIYKVSNWD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGAHWPITFCQGTRLEIK

Antibody U1-41

13 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGGTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTTCTGTGCGAGAGAT
CGGGAACTTGAGGGTTACTCCAACTACTACGGTGTGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTC

14 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYFCARDRELEGYSNYYGVDVWGQGTT
VTVS

15 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGCCATTAGCAACTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCAACAGAATAATAGTCTCCCGATCACCTTCGGCCAA
GGGACACGACTGGAGATTAAA

16 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQAISNYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQNNSLPITFGQGTRLEIK

Antibody U1-42

17 Heavy Chain DNA:
GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATC
TCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATG
CCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATAC
AGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGCCTAC
CTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACATGAA
AACTACGGTGACTACAACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

18 Heavy Chain Protein:
EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRY
SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARHENYGDYNYWGQGTLVTVSS

19 Light Chain DNA:

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTCGCAGCTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCTATGCTGCTTCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCACTTTACTGCTGTCAACAGAGTAACGGTTCCCCGCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCAAA

20 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSIRSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFALYCCQQSNGSPLTFGGGTKVEIK

Antibody U1-43

21 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAGGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
AGAGAGAGAGAGTGGGATGATTACGGTGACCCCCAAGGTATGGACGTCTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTC

22 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLRSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDRREREWDDYGDPQGMDVWGQG
TTVTVS

23 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTACATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCCATGCTGCATCCAGTTTACAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGTAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTAACCCGCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCCAA

24 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLHWYQQKPGKAPKLLIHAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSNPLTFGGGTKVEIQ

Antibody U1-44

25 Heavy Chain DNA:
GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATC
TCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATG
CCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTGGCCTGGTGACTCTGATACCATATAC
AGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGCCTAC
CTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACATGAA
AACTACGGTGACTACAACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

26 Heavy Chain Protein:
EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIWPGDSDTIY
SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARHENYGDYNYWGQGTLVTVSS

27 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTCGAAGTTATTTAAATTGGTATCAGCAGAAACCG
GGGAATGCCCCTAAACTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCACTTTACTACTGTCAACAGAGTATCAGTTCCCCGCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCAAA

28 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSIRSYLNWYQQKPGNAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFALYYCQQSISSPLTFGGGTKVEIK

Antibody (U1-45)
29 Heavy Chain DNA:
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGATACACCTTCACCAGTTATGATATCAACTGGGTGCGACAGGCC
ACTGGACAAGGGCTTGAGTGGATGGGATGGATGAACCCTAACAGTGGTGACACTGGCTAT
GCACAGGTGTTCCAGGGCAGAGTCACCATGACCTGGAACACCTCCATAAGCACAGCCTAC
ATGGAACTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGATTTGGG
GATCTCCCGTATGACTACAGTTACTACGAATGGTTCGACCCCTGGGGCCAGGGAACCCTG
GTCACCGTCTCCTC
30 Heavy Chain Protein:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQGLEWMGWMNPNSGDTGY
AQVFQGRVTMTWNTSISTAYMELSSLRSEDTAVYYCARFGDLPYDYSYYEWFDPWGQGTL
VTVS
31 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGCCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAGACCA
GGGAAAGCCCCTAAGCTCCTGATCTATGCAGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCGCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCAAA
32 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQRPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK

Antibody (U1-46
33 Heavy Chain DNA:
CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTC
ACCTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGG
CAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTAT
AATGATTATGCAGTATCTGTGAAAAGTCGAATAACCATCAACCCAGACACATCCAAGAAC
CAGTTCTCCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCA
AGAGATCTCTACGATTTTTGGAGTGGTTATCCCTACTACTACGGTATGGACGTCTGGGGC
CAAGGGACCACGGTCACCGTCTCCTC
34 Heavy Chain Protein:
QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWY
NDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDLYDFWSGYPYYYGMDVWG
QGTTVTVS

Antibody U1-47
35 Heavy Chain DNA:
CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTC
ACCTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGG
CAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTAT
AATGATTATGCAGTATCTGTGAAAAGTCGAATAACCATCAACCCAGACACATCCAAGAAC
CAGTTCTCCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCA
AGAGATTACTATGGTTCGGGGAGTTTCTACTACTACTACGGTATGGACGTCTGGGGCCAA
GGGACCACGGTCACCGTCTCCTC
36 Heavy Chain Protein:
QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWY

NDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDYYGSGSFYYYYGMDVWGQ
GTTVTVS

37 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGGTCCTGATCTATGCTGCATCCAATTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

38 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKVLIYAASNLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPRTFGQGTKVEIK

Antibody U1-48

39 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAGCTGGATCCGGCAGCCC
GCCGGGAAGGGACTGGAGTGGATTGGGCATATCTATACCAGTGGGAGCACCAACTACAAC
CCCTCCCTCAAGAGTCGAGTCACCATGTCAGTAGACACGTCCAAGAACCAGTTCTCCCTG
AAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAAGCGATT
TTTGGAGTGGGCCCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTC

40 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPAGKGLEWIGHIYTSGSTNYN
PSLKSRVTMSVDTSKNQFSLKLSSVTAADTAVYYCAREAIFGVGPYYYYGMDVWGQGTTV
TVS

Antibody U1-49

41 Heavy Chain DNA:
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCTAATATTGGTGGCACAAACTGT
GCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTAC
ATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGGGGGA
CGGTATAGCAGCAGCTGGTCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACC
ACGGTCACCGTCTCCTC

42 Heavy Chain Protein:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNIGGTNC
AQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGRYSSSWSYYYYGMDVWGQGT
TVTVS

43 Light Chain DNA:
GATATTCTGATGACCCAGACTCCACTCTCTCTGTCCGTCACCCCTGGACAGCCGGCCTCC
ATCTCCTGCAAGTCTAGTCAGAGCCTCCTGCTTAGTGATGGAGGGACCTATTTGTATTGG
TACCTGCAGAAGCCAGGCCAGCCTCCACAGCTCCTGATCTATGAAGTTTCCAACCGGTTC
TCTGGAGTGCCAGATAGGTTCAGTGGCAGCGGGTCAGGGACAGATTTCACACTGAAAATC
AGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAGTATGCAGCTTCCG
ATCACCTTCGGCCAAGGGACACGACTGGAAATTAAA

44 Light Chain Protein:
DILMTQTPLSLSVTPGQPASISCKSSQSLLLSDGGTYLYWYLQKPGQPPQLLIYEVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSMQLPITFGQGTRLEIK

Antibody U1-50

45 Heavy Chain DNA:

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCGTCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGG
CAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACAGTGGGAGCACCAAC
TACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGGG
GGGGACAGTAACTACGAGGATTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTC

46 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGGYYWSWIRQPPGKGLEWIGYIYYSGSTN
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGDSNYEDYYYYYGMDVWGQG
TTVTVS

47 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTAGCATCTATTTACATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCTTGATCTCTGCTGCATCCAGTTTGCAAAGTGGGGTCCCGTCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGAAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACACTTCCCCGATCACCTTCGGCCAA
GGGACACGACTGGAGATTAAA

48 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISIYLHWYQQKPGKAPKLLISAASSLQSGVPS
RFSGSGSGTDFTLTIRSLQPEDFATYYCQQSYTSPITFGQGTRLEIK

49 Antibody U1-51
Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAGCTGGATCCGGCAGCCC
CCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACAGTGGGAGCACCAACTACAAC
CCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGCACCAGTTCTCCCTG
AAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGATTCGAGT
TACTATGATAGTAGTGGTTATTACTTATACTACTACGCTATGGACGTCTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTC

50 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYN
PSLKSRVTISVDTSKHQFSLKLSSVTAADTAVYYCARDSSYYDSSGYYLYYYAMDVWGQG
TTVTVS

51 Light Chain DNA:
GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACC
ATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAACTACTTAGCT
TGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTCCTGGGCATCTACCCGG
GAATCCGGGGTCCCTGACCGATTCAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACC
ATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATACTACT
CCTCTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA

52 Light Chain Protein:
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLISWASTR
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTTPLTFGPGTKVDIK

Antibody U1-53
53 Heavy Chain DNA:
GAGGTGCAACTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTCAGTATCTATAGCATGAACTGGGTCCGCCAGGCT
CCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTAGTAGTACCATATACTAC
GCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTAT
CTGCAAATGAACAGCCTGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGATAGG

GGTGACTTCGATGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCA

54 Heavy Chain Protein:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMNWVRQAPGKGLEWVSYISSSSSTIYY
ADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARDRGDFDAFDIWGQGTMVTVSS

55 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCAGGCGAGTCAGGACATTACCAACTATTTGAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCATCA
AGGTTCAGTGGAAGTGGATCTGGGACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCT
GAAGATATTGCAACATATAACTGTCAACAGTGTGAAAATTTCCCGATCACCTTCGGCCAA
GGGACACGACTGGAGATTAAA

56 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTFTISSLQPEDIATYNCQQCENFPITFGQGTRLEIK

Antibody U1-55

57 Light Chain DNA:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGTATAGTAATGGATACAAGTATTTGGATTGG
TACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCC
TCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTATTGCATGCAGGCTCTACAAACTCCG
ATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA

58 Light Chain Protein:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYKYLDWYLQKPGQSPQLLIYLGSNRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPITFGQGTRLEIK

Antibody (U1-55.1)

59 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCGTCAGCAGTGGTGGTTACTACTGGAACTGGATCCGG
CAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCAATTACAGTGGGAGCACCAAC
TACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACCGCTGCCGCACACGGCCGTGTATTACTGTGCGAGAGAT
CGAGAACTGGAACTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTC

60 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGGYYWNWIRQPPGKGLEWIGYINYSGSTN
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDRELELYYYYYGMDVWGQGTT
VTVS

Antibody (U1-57)

61 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCTGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCGTCAGCAGTGGTGGTTACTACTGGAACTGGATCCGG
CAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCAATTACAGTGGGAGCACCAAC
TACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
CGAGAACTGGAACTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTC

62 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGGYYWNWIRQPPGKGLEWIGYINYSGSTN
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDRELELYYYYYGMDVWGQGTT

Antibody U1-57.1

63 Light Chain DNA:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGTATAGTAATGGATACAAGTATTTGGATTGG
TACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCATGATCTATTTGGGTTCTAATCGGGCC
TCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTATTGCATGCAGGCTCTACAAACTCCG
ATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA

64 Light Chain Protein:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYKYLDWYLQKPGQSPQLMIYLGSNRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPITFGQGTRLEIK

Antibody U1-58

65 Heavy Chain DNA:
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGCAGCT
CGCCTTGACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC
TCA

66 Heavy Chain Protein:
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAARLDYYYGMDVWGQGTTVTVS
S

67 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCTCC
ATCACTTGCCGGGCAAGTCAGAGCATTAACAGCTATTTAAATTGGTTTCAGCAGAAGCCA
GGGAAAGCCCCTCAGCTCCTGATCTTTGGTGCATCCGGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAACAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTTCCCCGCTCACCTTCGGCCAA
GGGACACGACTGGAGATTAAA

68 Light Chain Protein:
DIQMTQSPSSLSASVGDRVSITCRASQSINSYLNWFQQKPGKAPQLLIFGASGLQSGVPS
RFSGSGSGTDFTLTINSLQPEDFATYYCQQSYSSPLTFGQGTRLEIK

Antibody U1-59

69 Heavy Chain DNA:
CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCC
CCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAAC
CCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGAAACGTCCAAGAACCAGTTCTCCCTG
AAGCTGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGATAAGTGG
ACCTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCCTCA

70 Heavy Chain Protein:
QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYN
PSLKSRVTISVETSKNQFSLKLSSVTAADTAVYYCARDKWTWYFDLWGRGTLVTVSS

71 Light Chain DNA:
GACATCGAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACC
ATCAACTGCAGGTCCAGCCAGAGTGTTTTATACAGCTCCAGCAATAGGAACTACTTAGCT
TGGTACCAGCAGAACCCAGGACAGCCTCCTAAGCTGCTCATTTACTGGGCTTCTACCCGG

GAATCCGGGGTCCCTGACCGATTCAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACC
ATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATAGTACT
CCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA

72 Light Chain Protein:
DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSNRNYLAWYQQNPGQPPKLLIYWASTR
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFGQGTKVEIK

Antibody U1-52

73 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATGGGGAACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTGAGAACCAGTTC
TCCCTGAAGCTGAACTCTGTGACTGCCGCGGACACGGCCGTATATTACTGTGCGAGAGGG
GGAACTGGAACCAATTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTC

74 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWMGNIYYSGSTY
YNPSLKSRVTISVDTSENQFSLKLNSVTAADTAVYYCARGGTGTNYYYYYGMDVWGQGTT
VTVS

75 Light Chain DNA:
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCTGGGCCACTGGCATCCCA
AACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGC
GGAGGGACCAAGGTGGAGATCAAA

76 Light Chain Protein:
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSWATGIP
NRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIK

Antibody U1-61

77 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGTCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGATGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGAAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
TCCGAGTCCGAGTATAGCAGCTCGTCGAACTACGGTATGGACGTCTGGGGCCAAGGGACC
ACGGTCACCGTCTCCTC

78 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGVSISSGGYYWSWIRQHPGMGLEWIGYIYYSGSTY
YNPSLKSRVTISEDTSKNQFSLKLSSVTAADTAVYYCARDSESEYSSSSNYGMDVWGQGT
TVTVS

Antibody U1-61.1

79 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGTCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGATGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGAAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
TCCGAGTCCGAGTATAGCAGCTCGTCGAACTACGGTATGGACGTCTGGGGCCAAGGGACC

ACGGTCACCGTCTCCTC

80 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGVSISSGGYYWSWIRQHPGMGLEWIGYIYYSGSTY
YNPSLKSRVTISEDTSKNQFSLKLSSVTAADTAVYYCARDSESEYSSSSNYGMDVWGQGT
TVTVS

81 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAATCACC
ATCACTTGCCGGGCAAGTCAGACCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAGGTGGGGTCCCATCA
AGGTTCAGTGGCAGTGTATCTGGGACAGATTTCACCCTCACCGTCAGCAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTAACCCGCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCAAA

82 Light Chain Protein:
DIQMTQSPSSLSASVGDRITITCRASQTISSYLNWYQQKPGKAPKLLIYAASSLQGGVPS
RFSGSVSGTDFTLTVSSLQPEDFATYYCQQSYSNPLTFGGGTKVEIK

Antibody U1-62 (2.9.1)

83 Heavy Chain DNA:
GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATC
TCCTGTAAGGGTTCTGGATACAGTTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATG
CCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATAC
AGCCCGTCCTTCCAAGGCCAGGTCACCATGTCAGCCGACAAGTCCATCAGTACCGCCTAC
CTGCAGCTGAGCAGCCATGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACAGAT
GGCTGGAAACTACGTACATCACGGGTGATCGAGACGTCCTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTC

84 Heavy Chain Protein:
EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRY
SPSFQGQVTMSADKSISTAYLQLSSHEGLGHRHVLLCETDGWKLRTSRVIETSWGQGTTV
TVS

85 Light Chain DNA:
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTATCAGCATCTACTTAGCCTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGTGCAGTTTTGGC
CAGGGGACCAAACTGGAGATCAAA

86 Light Chain Protein:
EIVLTQSPGTLSLSPGERATLSCRASQSVISIYLAWYQQKPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPCSFGQGTKLEIK

Antibody U1-2

87 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAGGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

88 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLRSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

89 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGATACCT
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAACAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATGGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

90 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQIPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTINSLQPEDFATYYCLQHNGYPWTFGQGTKVEIK

Antibody U1-7

91 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

92 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

93 Light Chain DNA:
GACTTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGACATTCGAAATGATTTAGGCTGGTATCGGCAGAAACCT
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

94 Light Chain Protein:
DFQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYRQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-9

95 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAATAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAATGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

96 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISIDTSKNQFSLKLSSVTAADTAVYYCARADYDFWNGYFDYWGQGTLVTV
SS

97 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTATCGGCAGAAACCT
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT

GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

98 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYRQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-10

99 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTACACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGACTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCA
GATTACGATTTTTGGAGTGGTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

100 Heavy Chain Protein:
QVQLQESGPGLVKPTQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

101 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAATTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

102 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPWTFGQGTKVEIK

Antibody U1-12

103 Heavy Chain DNA
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGTTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

104 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

105 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAATTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

106 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPWTFGQGTKVEIK

Antibody U1- 13

107 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAG
GACGACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

108 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDDGMDVWGQGTTVTVSS

109 Light Chain DNA:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATTTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAATGGATACAACTATTTGGAATGG
TACCTGCAGAAGCCAGGGCAGTCCCCACAGTTCATGATTTATTTGGGGTCTAATCGGGCC
TCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACTCCG
ATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA

110 Light Chain Protein:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLEWYLQKPGQSPQFMIYLGSNRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPITFGQGTRLEIK

Antibody U1-14

111 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGTACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGGTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

112 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQYPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLRSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

113 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATACTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

114 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNTYPWTFGQGTKVEIK

Antibody U1-15

115 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCGTCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGG
CAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACAGTGGGAGCACCAAC
TACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTC

TCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
GGGGACGTGGATACAGCTATGGTCGATGCTTTTGATATCTGGGGCCAAGGGACAATGGTC
ACCGTCTCCTCA

116 Heavy Chain Protein:
QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGGYYWSWIRQPPGKGLEWIGYIYYSGSTN
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGDVDTAMVDAFDIWGQGTMV
TVSS

117 Light Chain DNA:
GAAATTGTATTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTTTAAGCGGCAACTACTTAGCCTGGTACCAGCAGAAG
CCTGGCCAGGCTCCCAGGCTCATCATCTGTGGTGCATCCAGCAGGGCCACTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCACAAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGATAGGTCACCGCTCACTTTCGGC
GGAGGGACCAAGGTGGAGATCAAA

118 Light Chain Protein:
EIVLTQSPGTLSLSPGERATLSCRASQSLSGNYLAWYQQKPGQAPRLIICGASSRATGIP
DRFSGSGSGTDFTLTITRLEPEDFAVYYCQQYDRSPLTFGGGTKVEIK

Antibody U1-19

119 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGGA
GATTACGATTTTTGGAGTGGAGAGTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

120 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGDYDFWSGEFDYWGQGTLVTV
SS

Antibody U1-20

121 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATGACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGGTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAT
CAGGGGCAGGACGGATACAGCTATGGTTACGGCTACTACTACGGTATGGACGTCTGGGGC
CAAGGGACCACGGTCACCGTCTCCTC

122 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYDSGSTY
YNPSLKSRVTISVDTSKNQFSLKLRSVTAADTAVYYCARDQGQDGYSYGYGYYYGMDVWG
QGTTVTVS

123 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCAGGCGAGTCAGGACATTAGCAATTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAACTCCTGATCTACGTTGCATCCAATTTGGAAACAGGGGTCCCATCA
AGGTTCAGTGGAAGTGGATCTGGGACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCT
GAAGATATTGCAACATATTACTGTCAACAGTGTGATAATCTCCCTCTCACTTTCGGCGGA
GGGACCAAGGTGGAGATCAAA

124 Light Chain Protein:

124 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYVASNLETGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQCDNLPLTFGGGTKVEIK

Antibody U1-21

125 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTC

126 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
S

127 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTATCGGCAGAAACCT
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCCGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

128 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYRQKPGKAPKRLIYAASRLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-22

129 Heavy Chain DNA:
CAGGTGCAGCTGCAGCAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

130 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

131 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAATGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

132 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQNGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-23

Antibody U1-23

133 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAATCCTGGTCACCGTC
TCCTC

134 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGILVTV
S

135 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATTTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

136 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-24

137 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGTTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAATGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

138 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWNGYFDYWGQGTLVTV
SS

139 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAATTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

140 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPWTFGQGTKVEIK

Antibody U1-25

141 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC

TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

142 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

143 Light Chain DNA:
GACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAATGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

144 Light Chain Protein:
DIQLTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQNGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-26

145 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGTACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGGGCTCTGTGACTGCCGCGGACACGGCCGTGTATTTCTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTTCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTC

146 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQYPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLGSVTAADTAVYFCARADYDFWSGYFDFWGQGTLVTV
S

147 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATGGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

148 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNGYPWTFGQGTKVEIK

Antibody U1-27

149 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGTACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGGGCTCTGTGACTGCCGCGGACACGGCCGTGTATTTCTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTTCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTC

150 Heavy Chain Protein:

QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQYPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLGSVTAADTAVYFCARADYDFWSGYFDFWGQGTLVTV
S

151 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATGGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

152 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNGYPWTFGQGTKVEIK

Antibody U1-28

153 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCG
GATTACGATTTTTGGAGTGGTTATTTTGACTCCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

154 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDSWGQGTLVTV
SS

155 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGATACCT
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATGGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

156 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQIPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNGYPWTFGQGTKVEIK

Antibody U1-31

157 Heavy Chain DNA:
CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCAACTATGGTATCAGCTGGGTGCGGCAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACGATGGTTACAGAAACTAT
GCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGACCACTGCCTAC
ATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATGTT
CAAGACTACGGTGACTACGACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

158 Heavy Chain Protein:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYDGYRNY
AQKLQGRVTMTTDTSTTTAYMELRSLRSDDTAVYYCARDVQDYGDYDYFDYWGQGTLVTV
SS

159 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC

ATCACTTGCCGGGCAAGTCAGAGCATTAGCAGTTATTTAAATTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAACCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGATTCAGGGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCCATCACCTTCGGCCAA
GGGACACGACTGGAGATTAAA

160 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYAASSLQSGVPS
RFRGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGQGTRLEIK

Antibody U1-32

161 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTTACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGACCACCTAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
GCCCTGAAGCTGAACTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTTATTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

162 Heavy Chain Protein:
QVQLQESGPGLVKPLQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGTTY
YNPSLKSRVTISVDTSKNQFALKLNSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTV
SS

163 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAGGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTCAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCTCTCTCACAATCTCCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAAC

164 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRAGQGIRNDLGWYQQKPGKAPQRLIYAASSLQSGVPS
RFSGSGSGTEFSLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-35

165 Heavy Chain DNA:
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCCGCCAGGCT
CCAGGGAAGGGGCTGGAGTGGGTTTCATATATTAGTAGTAGTGGTAATAACATATACCAC
GCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGAGAGA
TATAGTGGCTACGACGACCCTGATGGTTTTGATATCTGGGGCCAAGGGACAATGGTCACC
GTCTCTTCA

166 Heavy Chain Protein:
QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSGNNIYH
ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARERYSGYDDPDGFDIWGQGTMVT
VSS

167 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAGTTGGTTTCAGCAGAAACCA
GGGAAAGCCCCTAAGCTCCTGATCCACGATGCATCCAATTTGGAAACAGGGGTCCCTTCA
AGGTTCAGTGGAAGTGGATCTGGGACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCT
GAAGATATTGCAACATATTACTGTCAACAGTATGATAATCCCCCGTGCAGTTTTGGCCAG
GGGACCAAGCTGGAGATCAAA

168 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLSWFQQKPGKAPKLLIHDASNLETGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNPPCSFGQGTKLEIK

Antibody U1-36

169 Heavy Chain DNA:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTTATTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGACCACCTAC
TACAATCCGTCCTTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAACTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGCC
GATTACGATTTTTGGAGTGGTCACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCA

170 Heavy Chain Protein:
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGYYYWSWIRQHPGKGLEWIGYIYYSGTTY
YNPSFKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGHFDYWGQGTLVTV
SS

171 Light Chain DNA:
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCA
GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAA

172 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-37

173 Heavy Chain DNA:
CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCC
CCTGGACAAGGACTTGAGTGGATGGGATGGATCAGCGCTTACGATGGTCACACAAACTAT
GCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAACACAGCCTAC
ATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACCCC
CATGACTACAGTAACTACGAGGCTTTTGACTTCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTC

174 Heavy Chain Protein:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYDGHTNY
AQKLQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYCARDPHDYSNYEAFDFWGQGTLVTV
S

175 Light Chain DNA
atgaggtcccctgctcagctcctggggctcctgctactctggctccgaggtgccagatgtg
acatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccat
cacttgccgggcaagtcagagcattagcagttatttaaattggtatcagcagaaaccaggg
aaagcccctaacctcctgatctatgctgcatccagtttgcaaagtggggtcccatcaagat
tcagtggcagtggatctgggacagatttcactctcaccatcagcagtctgcaacctgaaga
ttttgcaacttactactgtcaacagagttacagtacccccatcaccttcggccaagggaca
cgactggagattaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatg
agcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagaga
ggccaaagtacagtggaaggtggataacgcc

176 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGQGTRLEIK

Antibody U1-34

177 Heavy Chain DNA:
accatggactggacctggagggtccttttcttggtggcagcagcaacaggtgcccactccca
ggttcagctggtgcagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcct
gcaaggcttctggttacacctttaccaactatggtatcagctgggtgcggcaggcccctgga
caagggcttgagtggatgggatggatcagcgcttacgatggttacagaaactatgcacagaa
gctccagggcagagtcaccatgaccacagacacatccacgaccactgcctacatggagctga
ggagcctgagatctgacgacacggccgtgtattactgtgcgagagatgttcaagactacggt
gactacgactactttgactactggggccagggaaccctggtcaccgtctcctcagcttccac
caagggcccatccgtcttccccctggtgccctgctccaggagcacctccgagagcacagccg
ccctgggctgcctggtcaaggactacttccccgaaccg

178 Heavy Chain Protein
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYDGYRNYA
QKLQGRVTMTTDTSTTTAYMELRSLRSDDTAVYYCARDVQDYGDYDYFDYWGQGTLVTVSS

179 Light Chain DNA:
cagctcctggggctcctgctactctggctccgaggtgccagatgtgacatccagatgaccc
agtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaag
tcagagcattagcagttatttaaattggtatcagcagaaaccagggaaagcccctaacctc
ctgatctatgctgcatccagtttgcaaagtggggtcccatcaagattcagtggcagtggat
ctgggacagatttcactctcaccatcagcagtctgcaacctgaagattttgcaacttacta
ctgtcaacagagttacagtaccccatcaccttcggccaagggacacgactggagattaaa
cgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctg
gaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtg
gaaggtggataacgcc

180 Light Chain Protein:
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGQGTRLEIK

Antibody U1-1

181 Heavy Chain DNA:
catctgtggttcttcctcctgctggtggcagctcccagatgggtcctgtcccaggtgcagc
tgcaggagtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgt
ctctggtggctccatcaacagtggtgattactactggagctggatccgccagcacccaggg
aagggcctggagtggattgggtacatctattacagtgggagcacctactacaacccgtccc
tcaagagtcgagttaccatatcagtagacacgtctaagaaccagttctccctgaagctgag
ctctgtgactgccgcggacacggccgtgtattactgtgcgagagcagattacgatttttgg
agtggttactttgactactggggccagggaaccctggtcaccgtctcctcagcctccacca
agggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacaacggc
cctgg

182 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSINSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTVSS

183 Light Chain DNA:
atgagggtccctgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca
cttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccagggaaa
gcccctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag
cggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttg
caacttattactgtctacagcataatagttacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagtt
gaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaag
tacagtggaaggtggataacgc

184 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR
FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-3
185 Heavy Chain DNA:
tggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagga
gtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctggtg
gctccatcagcagtggtggttactactggagctggatccgccagcacccagggaagggcctg
gagtggattgggtacatctattacagtgggagcacctactacaacccgtccctcaagagtcg
agttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgtgactg
ccgcggacacggccgtgtattactgtgcgagagatggctatgatagtagtggttattaccac
ggctactttgactactggggccagggaaccctggtcaccgtctcctcagcctccaccaaggg
cc

186 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGYDSSGYYHGYFDYWGQGTLVT
VSS

187 Light Chain DNA:
H3_130_1N1K
caggtcttcatttctctgttgctctggatctctggtgcctacggggacatcgtgatgaccc
agtctccagactccctggctgtgtctctgggcgagagggccaccatcaactgcaagtccag
ccagagtgttttatacagctccaacaataagaactacttagcttggtaccagcagaaacca
ggacagcctcctaagctgctcatttactgggcatctacccgggaatccggggtccctgacc
gattcagtggcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctga
agatgtggcagtttattactgtcagcaatattatagtactccgctcactttcggcggaggg
accaaggtggagatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctg
atgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccag
agaggccaaagtacagtggaaggtggataacgc

188 Light Chain Protein:
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRE
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGGTKVEIK

Antibody U1-4
H3_133_1N1G1
189 Heavy Chain DNA
ctgtggttcttcctcctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgca
ggagtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctg
gtggctccatcagtagtggtgattactactggagctggatccgccagcacccagggaagggc
ctggagtggattgggtacatctattacagtgggagcacctactacaacccgtccctcaagag
tcgagttaccatatcagtagacacgtctaagaaccagttctccctgaagttgagctctgtga
ctgccgcggacacggccgtgtattactgtgcgagagccgattacgatttttggagtggttat
tttgactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggcccatc
ggtcttcccccctggcaccctc

190 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTVSS

191 Light Chain DNA
H3_133_1N1K
gtgcccgctcagcgcctggggctcctgctgctctggttcccaggtgccaggtgtgacatcc
agatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttg
ccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccagggaaagcc
cctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggttcagcg
gcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttgc
aacttattactgtctacagcataataattacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagt
tgaaatctggaactg

192 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRF
SGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPWTFGQGTKVEIK

Antibody U1-5
193 Heavy Chain DNA:
H3_138_1N1G1
tggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagga
gtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctggtg
gctccatcagcagtggtgattactactggagctggatccgccagcacccagggaagggcctg
gagtggattgggtacatctattacagtgggagcacctactacaacccgtccctcaagagtcg
agttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgtgactg
ccgcggacacggccgtgtatttctgtgcgagagccgattacgatttttggagtggttatttt
gactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggcc

194 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYYN
PSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYFCARADYDFWSGYFDYWGQGTLVTVSS

195 Light Chain DNA:
H3_138_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca
cttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccagggaaa

gcccctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag
cggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttg
caacttattactgtctacagcataatacttacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagtt
gaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaag
tacagtggaaggtggataacgc

196 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNTYPWTFGQGTKVEIK

Antibody U1-6
197 Heavy Chain DNA:
H3_162_1N1G1
tggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagga
gtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctggtg
gctccatcagcagtggtgattactactggagctggatccgccagcacccagggaagggcctg
gagtggattgggtacatctattacagtgggagcacctactacaacccgtccctcaagagtcg
agttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgtgactg
ccgcggacacggccgtgtatttctgtgcgagagccgattacgatttttggaatggttatttt
gactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggccc

198 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYFCARADYDFWNGYFDYWGQGTLVTV
SS

199 Light Chain DNA:
H3_162_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca
cttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccagggaaa
gcccctaagcgcctgatctatgctgcttccagtttgcaaagtggggtcccatcaaggttcag
cggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttg
caacttattactgtctacagcataatacttacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagtt
gaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaag
tacagtggaaggtggataacgcc

200 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNTYPWTFGQGTKVEIK

Antibody U1-8
201 Heavy Chain DNA:
H3_174_1N1G1
ttggtggcagcagctacaggcacccacgcccaggtccagctggtacagtctggggctgaggt
gaagaagcctggggcctcagtgaaggtctcctgcaaggtttccggatacaccctcactgaat
tatccatgtactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttgat

cctgaagatggtgaaacaatctacgcacagaagttccagggcagagtcaccatgaccgagga
cacatctacagacacagcctacatggagctgagcagcctgagatctgaggacacggccgtgt
attactgtgcaactgggtggaactacgtctttgactactggggccagggaaccctggtcacc
gtctcctcagcctccaccaagggccc

202 Heavy Chain Protein
QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMYWVRQAPGKGLEWMGGFDPEDGETIYA
QKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGWNYVFDYWGQGTLVTVSS

203 Light Chain DNA:
H3_174_1N1K
ggatccagtggggatattgtgatgactcagtctccactctccctgcccgtcacccctggaga
gccggcctccatctcctgcaggtccagtcagagcctcctgcatagtaatggatacaactatt
tggattggtacctgcagaagccagggcagtctccacagctcctgatctatttggattctcat
cgggcctccggggtccctgacaggttcagtggcagtggatcaggcacagattttacactgaa
aatcagcagagtggaggctgaggatgttggggtttattactgcatgcaagctctacaaactc
cgctcactttcggcggagggaccaaggtggagatcaaacgaactgtggctgcaccatctgtc
ttcatcttcccgccat

204 Light Chain Protein
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLDSHRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPLTFGGGTKVEIK

Antibody U1-11
205 Heavy Chain DNA:
H3_178_1N1G1
tggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagga
gtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctggtg
gctccatcagcagtggtgattactactggagctggatccgccagcacccagggaagggcctg
gagtggattgggtacatctattacagtgggagcacctactacaacccgtccctcaagagtcg
agttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgtgactg
ccgcggacacggccgtgtatttctgtgcgagagccgattacgatttttggagtggttatttt
gactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggcccatcgag
tcttccccctgg

206 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTY
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYFCARADYDFWSGYFDYWGQGTLVTV
SS

207 Light Chain DNA:
H3_178_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtg
acatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccat
cacttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccaggg
aaagcccctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggt
tcagcggcagtggatctgggacaaaattcactctcactatcagcagcctgcagcctgaaga
ttttgcaacttattactgtctacagcataatacttacccgtggacgttcggccaagggacc
aaggtggaaatcagacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatg
agcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagaga
ggccaaagtacagtggaaggtggataacgcc

208 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVP
SRFSGSGSGTKFTLTISSLQPEDFATYYCLQHNTYPWTFGQGTKVEIR

Antibody U1-16
209 Heavy Chain DNA:
H3_221_1N1G1
accatgaaacatctgtggttcttcctcctgctggtggcagctcccagatgggtcctgtccc
aggtgcagctgcaggagtcgggcccaggactggtgaagccttcacagaccctgtccctcac
ctgcactgtctctggtggctccatcagcagtggtgattactactggagctggatccgccag
cacccagggaagggcctggagtggattgggtacatctattacagtgggagcacctactaca
acccgtccctcaagagtcgagttaccatatcagtagacacgtctaagaaccagttctccct
gaagctgagctctgtgactgccgcggacacggccgtgtattactgtgcgagagcggattac
gatttttggagtggttattttgactactggggccagggaatcctggtcaccgtctcctcag
cctccaccaagggcccatcggtcttcccctggcaccctcctccaagaacacctctgggggg
cacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcctgg
aactcaggcgccctg

210 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGILVTVSS

211 Light Chain DNA:
H3_221_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgt
gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcacc
atcacttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaacca
gggaaagcccctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatca
aggttcagcggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcct
gaagattttgcaacttattactgtctacagcataatagttacccgtggacgttcggccaa
gggaccaaggtggaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgcca
tctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctat
cccagagaggccaaagtacagtggaaggtggataacgcc

212 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR
FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-17
213 Heavy Chain DNA:
H3_224_1N1G1
tggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagg
agtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctgg
tggctccatcagcagtggtgattactactggagctggatccgccagcacccagggaagggc
ctggagtggattggatacatctattacagtgggagcacctactacaattcgtccctcaaga
gtcgagttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgt
gactgccgcggacacggccgtgtattactgtgcgagagcggattacgatttttggagtggt
tattttgactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggcc
catcg

214 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NSSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTVSS

215 Light Chain DNA:
H3_224_1N1K
ggtgccaggtgtgacatccagatgacccagtctccatcctccctgtctgcatctgtaggag
acagagtcaccatcacttgccgggcaagtcagggcattagaaatgatttaggctggtatca
gcagaaacctgggaaagcccctaagcgcctgatctatgctgcatccagtttgcaaagtggg
gtcccatcaaggttcagcggcagtggatctgggacagaattcactctcacaatcagcagcc
tgcagcctgaagattttgcaacttattactgtctacagcacaatagttacccgtggacgtt
cggccaagggaccaaggtggaaatcaaacgaactgtggctgcaccatctgtcttcatcttc
ccgcca

216 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-18
217 Heavy Chain DNA:
H3_227_1N1G1
aggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgcagg
agtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctctgg
tggctccatcagcagtggtgattactactggagctggatccgccagcacccagggaagggc
ctggagtggattggatacatctattacagtgggagcacctactacaacccgtccctcaaga
gtcgagttaccatatcagtagacacgtctaagaaccagttctccctgaagctgagctctgt
gactgccgcggacacggccgtgtattactgtgcgagagccgattacgatttttggagtggt
tattttgactactggggccagggaaccctggtcaccgtctcctcagcctccaccaagggcc
catcggtcttcccctggcaccctcctccaagagcacctctgggggcacagcggccctggg
ctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccct

218 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTVSS

219 Light Chain DNA:
H3_227_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca
cttgccgggcaagtcagggcattagaaatgatttaggctggtatcagcagaaaccagggaaa
gcccctaagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag
cggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttg
caacttattactgtctacagcataatagttacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagtt
gaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaag
tacagtggaaggtggataacg

220 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

Antibody U1-33

221 Heavy Chain DNA:
H4_14_1N1G4
ctgtggttcttccttctgctggtggcagctcccagatgggtcctgtcccaggtgcagctgc
aggagtcgggcccaggactggtgaagccttcacagaccctgtccctcacctgcactgtctc
tggtggctccatcagcagtggtgattactactggagctggatccgccagcacccagggaag
ggcctggagtggattgggtacatctattacagtgggagcacctactacaacccgtccctca
agagtcgagttaccatgtcagtagacacgtctaagaaccagttctccctgaagctgagctc
tgtgactgccgcggacacggccgtgtattactgtgcgagagccgattacgatttttggagt
ggtcactttgactgctggggccagggaaccctggtcaccgtctcctcagcttccaccaagg
gccccatccgtcttccccc

222 Heavy Chain Protein
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGSTYY
NPSLKSRVTMSVDTSKNQFSLKLSSVTAADTAVYYCARADYDFWSGHFDCWGQGTLVTVSS

223 Light Chain DNA:
H4_14_1N1K
atgagggtccccgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca
cttgccgggcaagtcagggcattagagatgatttaggctggtatcagcagaaaccagggaaa
gcccctaagcgcctgatctatgctgaatccagtttgcaaagtggggtcccatcaaggttcag
cggcagtggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttg
caacttattactgtctacagcatcatagttacccgtggacgttcggccaagggaccaaggtg
gaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgcc

224 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRASQGIRDDLGWYQQKPGKAPKRLIYAESSLQSGVPSR
FSGSGSGTEFTLTISSLQPEDFATYYCLQHHSYPWTFGQGTKVEIK

Antibody U1-29

225 Heavy Chain DNA:
H4_107_1N1G4
tggctgagctgggttttcctcgttgctcttttaagaggtgtccagtgtcaggtgcagctgg
tggagtctgggggaggcgtggtccagcctgggaggtccctgagactctcctgtgcagcgtc
tggattcaccttcaatagctatgacatgcactgggtccgccaggctccaggcaaggggctg
gagtgggtggcagttatatggtatgatggaagtaataaatactatgcagactccgtgaagg
gccgattcaccatctctagagacaattccaagaacacgctgtatctgcaaatgaacagcct
gagagccgaggacacggctgtgtattactgtgcgagagaccgcttgtgtactaatggtgta
tgctatgaagactacggtatggacgtctggggccaagggaccacggtcaccgtctcctcag
cttccaccaagggcccatccgtcttccccctggcgccctgctccaggagcacctccgagag
cacagccgccctgggc

226 Heavy Chain Protein
QVQLVESGGGVVQPGRSLRLSCAASGFTFNSYDMHWVRQAPGKGLEWVAVIWYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLCTNGVCYEDYGMDVWGQGTTV
TVSS

227 Light Chain DNA:
H4_107_1N1K
atgagggtccctgctcagctcctggggctcctgctgctctggctctcaggtgccagatgtga
catccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatca

cttgccaggcgagtcaggacattagcaactatttaaattggtatcagcagaaaccagggaaa
gcccctaaggtcctgatctacgatgcatccaatttggaaacaggggtcccatcaaggttcag
tggaagtggatctgggacagattttactttcaccatcagcagcctgcagcctgaagatgttg
caacatattactgtcaacactatgatactctcccgctcactttcggcggagggaccaaggtg
gagatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagtt
gaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaag
tacagtgg

228 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYDASNLETGVPSR
FSGSGSGTDFTFTISSLQPEDVATYYCQHYDTLPLTFGGGTKVEIK

Antibody U1-30
229 Heavy Chain DNA:
H4_116_1_1N1G4
ggactgtgcaagaacatgaaacacctgtggttcttcctcctgctggtggcagctcccagatg
ggtcctgtcccaggtgcagctgcaggagtcgggcccaggactggtgaagcctttacagaccc
tgtccctcacctgcactgtctctggtggctccatcagcagtggtgattactactggagctgg
atccgccagcacccagggaagggcctggagtggattgggtacatctattacagtgggaccac
ctactacaacccgtccctcaagagtcgagttaccatatcagtagacacgtctaagaaccagt
tcgccctgaagctgaactctgtgactgccgcggacacggccgtgtattactgtgcgagagcc
gattacgatttttggagtggttattttgactactggggccagggaaccctggtcaccgtctc
ctcagcttccaccaagggcccatccgtcttccccctgg

230 Heavy Chain Protein
QVQLQESGPGLVKPLQTLSLTCTVSGGSISSGDYYWSWIRQHPGKGLEWIGYIYYSGTTYY
NPSLKSRVTISVDTSKNQFALKLNSVTAADTAVYYCARADYDFWSGYFDYWGQGTLVTVSS

231 Light Chain DNA:
H4_116_1_1N1K
atgagggtccctgctcagctcctggggctcctgctgctctggttcccaggtgccaggtgtg
acatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccat
cacttgccgggcaggtcagggcattagaaatgatttaggctggtatcagcagaaaccaggg
aaagcccctcagcgcctgatctatgctgcatccagtttgcaaagtggggtcccatcaaggt
tcagcggcagtggatctgggacagaattctctctcacaatctccagcctgcagcctgaaga
ttttgcaacttattactgtctacagcataatagttacccgtggacgttcggccaagggacc
aaggtggaaatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatg
agcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagaga
ggccaaagtacagtggaaggtggataacgcccttccaatcggg

232 Light Chain Protein
DIQMTQSPSSLSASVGDRVTITCRAGQGIRNDLGWYQQKPGKAPQRLIYAASSLQSGVPSR
FSGSGSGTEFSLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

[0073]

[Chem.15]

Sequence List of ODR

Chain antibodies	Pat. number:	CDR1	CDR2	CDR3
Heavy	U1-1	GGSINSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-2	GGSISSGDYYWS	YIYYSGSTYYNPSLRS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNGYPWT
Heavy	U1-3	GGSISSGGYYWS	YIYYSGSTYYNPSLKS	DGYDSSGYYHGYFDY
Light		KSSQSVLYSSNNKNYLA	WASTRES	QQYYSTPLT
Heavy	U1-4	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNNYPWT
Heavy	U1-5	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNTYPWT
Heavy	U1-6	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWNGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNTYPWT
Heavy	U1-7	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQDIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-8	GYTLTELSMY	GFDPEDGETIYAQKFQG	GWNYVFDY
Light		RSSQSLLHSNGYNYLD	LDSHRAS	MQALQTPLT
Heavy	U1-9	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWNGYFDY
Light		RASQDIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-10	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNNYPWT
Heavy	U1-11	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNTYPWT
Heavy	U1-12	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYPDY
Light		RASQGIRNDLG	AASSLQS	LQHNNYPWT
Heavy	U1-13	GGSISSGGYYWS	YIYYSGSTYYNPSLKS	EDDGMDV
Light		RSSQSLLHSNGYNYLE	LGSNRAS	MQALQTPIT
Heavy	U1-14	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNTYPWT

Heavy	U1-15	GGSVSSGGYYWS	YIYYSGSTNYNPSLKS	DGDVDTAMVDAFDI
Light		RASQSLSGNYLA	GASSRAT	QQYDRSPLT
Heavy	U1-16	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-17	GGSISSGDYYWS	YIYYSGSTYYNSSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-18	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-19	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	GDYDFWSGEFDY
Light		sequence not available		
Heavy	U1-20	GGSISSGGYYWS	YIYDSGSTYYNPSLKS	DQGQDGYSYGYGYYYGM DV
Light		QASQDISNYLN	VASNLET	QQCDNLPLT
Heavy	U1-21	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQDIRNDLG	AASRLQS	LQHNSYPWT
Heavy	U1-22	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQN	LQHNSYPWT
Heavy	U1-23	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-24	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWNGYFDY
Light		RASQGIRNDLG	AASSLQS	LQHNNYPWT
Heavy	U1-25	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDY
Light		RASQGIRNDLG	AASSLQN	LQHNSYPWT
Heavy	U1-26	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDF
Light		RASQGIRNDLG	AASSLQS	LQHNGYPWT
Heavy	U1-27	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDF
Light		RASQGIRNDLG	AASSLQS	LQHNGYPWT
Heavy	U1-28	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGYFDS
Light		RASQGIRNDLG	AASSLQS	LQHNGYPWT
Heavy	U1-29	GFTFNSYDMH	VIWYDGSNKYYADSVKG	DRLCTNGVCYEDYGMDV
Light		QASQDISNYLN	DASNLET	QHYDTLPLT
Heavy	U1-30	GGSISSGDYYWS	YIYYSGTTYYNPSLKS	ADYDFWSGYFDY
Light		RAGQGIRNDLG	AASSLQS	LQHNSYPWT

Heavy	U1-31	GYTFTNYGIS	WISAYDGYRNYAQKLQG	DVQDYGDYDYFDY
Light		RASQSISSYLN	AASSLQS	QQSYSTPIT
Heavy	U1-32	GGSISSGDYYWS	YIYYSGTTYYNPSLKS	ADYDFWSGYFDY
Light		RAGQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-33	GGSISSGDYYWS	YIYYSGSTYYNPSLKS	ADYDFWSGHFDC
Light		RASQGIRDDLG	AESSLQS	LQHHSYPWT
Heavy	U1-34	GYTFTNYGIS	WISAYDGYRNYAQKLQG	DVQDYGDYDYFDY
Light		RASQSISSYLN	AASSLQS	QQSYSTPIT
Heavy	U1-35	GFTFSDYYMS	YISSSGNNIYHADSVKG	ERYSGYDDPDGFDI
Light		QASQDISNYLS	DASNLET	QQYDNPPCS
Heavy	U1-36	GGSISSGYYYWS	YIYYSGTTYYNPSFKS	ADYDFWSGHFDY
Light		RASQGIRNDLG	AASSLQS	LQHNSYPWT
Heavy	U1-37	GYTFTSYGIS	WISAYDGHTNYAQKLQG	DPHDYSNYEAFDF
Light		RASQSISSYLN	AASSLQS	QQSYSTPIT
Heavy	U1-38	GFSLSTSGVGVG	LIYWNDDKRYSPSLKS	RDEVRGFDY
Light		RSSQSLVYSDGYTYLH	KVSNWDS	MQGAHWPIT
Heavy	U1-39	GFTVSSNYMS	VIYSGGSTYYADSVKG	GQWLDV
Light		RSSQSLLHSNGYNYLD	LGFHRAS	RQALQTPLT
Heavy	U1-40	GGSISSGGYYWS	YIYSSGSTYYNPSLKS	DRELELYYYYYGMDV
Light		RSSQSLLYSNGYNYLD	LGSNRAS	MQALQTPLT
Heavy	U1-41	GGSISSGGYYWS	YIYYSGSTYYNPSLKS	DRELEGYSNYYGVDV
Light		RASQAISNYLN	AASSLQS	QQNNSLPIT
Heavy	U1-42	GYSFTSYWIG	IIYPGDSDTRYSPSFQG	HENYGDYNY
Light		RASQSIRSYLN	AASSLQS	QQSNGSPLT
Heavy	U1-43	GGSISSGGYYWS	YIYYSGSTYYNPSLRS	DRREWDDYGDPQGMDV

Light		RASQSISSYLH	AASSLQS	QQSYSNPLT
Heavy	U1-44	GYSFTSYWIG	IIWPGDSDTIYSPSFQG	HENYGDYNY
Light		RASQSIRSYLN	AASSLQS	QQSISSPLT
Heavy	U1-45	GYTFTSYDIN	WMNPNSGDTGYAQVFQG	FGDLPYDYSYYEWFDP
Light		RASQSISSYLN	AASSLQS	QQSYSTPLT
Heavy	U1-46	GDSVSSNSAAWN	RTYYRSKWYNDYAVSVKS	DLYDFWSGYPYYYGMDV
Light		sequence not available		
Heavy	U1-47	GDSVSSNSAAWN	RTYYRSKWYNDYAVSVKS	DYYGSGSFYYYYGMDV
Light		RASQSISSYLN	AASNLQS	QQSYSTPRT
Heavy	U1-48	GGSISSYYWS	HIYTSGSTNYNPSLKS	EAIFGVGPYYYYGMDV
Light		sequence not available		
重 Heavy	U1-49	GYTFTGYYMH	WINPNIGGTNCAQKFQG	GGRYSSSWSYYYYGMDV
Light		KSSQSLLLSDGGTYLY	EVSNRFS	MQSMQLPIT
Heavy	U1-50	GGSVSSGGYYWS	YIYYSGSTNYNPSLKS	GGDSNYEDYYYYYGMDV
Light		RASQSISIYLH	AASSLQS	QQSYTSPIT
Heavy	U1-51	GGSISSYYWS	YIYYSGSTNYNPSLKS	DSSYYDSSGYYLYYYAMDV
Light		KSSQSVLYSSNNKNYLA	WASTRES	QQYYTTPLT
Heavy	U1-52	GGSISSGGYYWS	NIYYSGSTYYNPSLKS	GGTGTNYYYYYGMDV
Light		RASQSVSSSYLA	GASSWAT	QQYGSSPLT
Heavy	U1-53	GFTFSIYSMN	YISSSSSTIYYADSVKG	DRGDFDAFDI
Light		QASQDITNYLN	DASNLET	QQCENFPIT
Heavy	U1-55.1	GGSVSSGGYYWN	YINYSGSTNYNPSLKS	DRELELYYYYYGMDV
Light		Identical with U1-55		
Heavy	U1-55	Identical with U1-55.1		
Light		RSSQSLLYSNGYKYLD	LGSNRAS	MQALQTPIT
Heavy	U1-57.1	Identical with U1-57		

Light		RSSQSLLYSNGYKYLD	LGSNRAS	MQALQTPIT
Heavy	U1-57	GGSVSSGGYYWN	YINYSGSTNYNPSLKS	DRELELYYYYYGMDV
Light		Identical with U1-57.1		
Heavy	U1-58	GFTFSSYGMH	VIWYDGSNKYYADSVKG	AARLDYYYGMDV
Light		RASQSINSYLN	GASGLQS	QQSYSSPLT
Heavy	U1-59	GGSFSGYYWS	EINHSGSTNYNPSLKS	DKWTWYFDL
Light		RSSQSVLYSSSNRNYLA	WASTRES	QQYYSTPRT
Heavy	U1-61.1	GVSISSGGYYWS	YIYYSGSTYYNPSLKS	DSESEYSSSSNYGMDV
Light		RASQTISSYLN	AASSLQG	QQSYSNPLT
Heavy	U1-61	GVSISSGGYYWS	YIYYSGSTYYNPSLKS	DSESEYSSSSNYGMDV
Light		Identical with U1-61.1		
Heavy	U1-62	GYSFTSYWIG	IIYPGDSDTRYSPSFQG	QMAGNYYYGMDV
Light		RASQSVISIYLA	GASSRAT	QQYGSSPCS

[0074] When a newly produced monoclonal antibody binds to a partial peptide or a partial tertiary structure to which the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody binds, it can be determined that the antibody binds to the same epitope as the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody. Further, by confirming that the antibody competes with the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody for binding to HER3 (that is, the antibody inhibits the binding between the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody and HER3), it can be determined that the antibody binds to the same epitope as the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody even when the specific sequence or structure of an epitope is not defined. Once the epitope is confirmed to be the same, it is strongly expected that the antibody has a biological activity equivalent to that of the U1-49, U1-53, U1-59, U1-7, or U1-9 antibody.

[0075] According to the present invention, the binding protein of the invention interacts with at least one epitope in the extracellular part of HER3. The epitopes are preferably located in domain L1 (aa 19-184), which is the amino terminal domain, in domain S1 (aa 185-327) and S2 (aa 500-632), which are the two Cysteine-rich domains, in domain L2 (328-499), which is flanked by the two Cysteine-rich domains or in a combination of HER3 domains. The epitopes may also be located in combinations of domains such as but not limited to an epitope comprised by parts of L1 and S1. Moreover, the

binding protein of the invention is further characterized in that its binding to HER3 reduces HER3-mediated signal transduction. In accordance with the present invention, a reduction of HER3-mediated signal transduction may, e.g. be caused by a down-regulation 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 which exhibits a lower signal transduction compared to the non-stabilized form. Alternatively, a reduction of HER3-mediated signal transduction may also be caused by influencing, e.g. decreasing or inhibiting, the binding of a ligand or another member of the HER family to HER3, of GRB2 to HER-2 or of GRB2 to SHC, by inhibiting receptor tyrosine phosphorylation, AKT phosphorylation, PYK2 tyrosine phosphorylation or ERK2 phosphorylation, or by decreasing tumor invasiveness. Alternatively, a reduction of HER3 mediated signal transduction may also be caused by influencing, e.g., decreasing or inhibiting, the formation of HER3 containing dinners with other HER family members. One example among others may be the decreasing or inhibiting of the HER3-EGFR protein complex formation.

[0076] Furthermore, in accordance with the present invention, minor variations in the amino acid sequences shown in SEQ ID NOs: 1-232 are contemplated as being encompassed by the present invention, providing that even the variations in the amino acid sequence still maintain at least 75 %, more preferably at least 80 %, 90 %, 95 %, and most preferably 99 % of the sequences shown in SEQ ID NOs: 1-232. The variations may occur within the framework regions (i.e. outside the CDRs), within the CDRs, or within the framework regions and the CDRs. Preferred variations in the amino acid sequences shown in SEQ ID NOs: 1-232, i.e. deletions, insertions and/or replacements of at least one amino acid, 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 to identify protein sequences that fold into a known three-dimensional structure are known. See e.g. 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 (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et at., Nature 354, 105 (1991), which are all incorporated herein by reference. 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 invention. Among antibodies obtained by combining heavy and light chains having variations in such an amino acid sequences, an antibody equivalent to the original antibody (parent

antibody) or more excellent than a parent antibody may be selected. As mentioned above, the HER3-binding protein, the anti-HER3 antibody, and the like of the present invention maintain the HER3-binding activity even if having variations in their amino acid sequences.

In the present invention, the term "homology" has the same meaning as the "identity. The homology between two amino acid sequences can be determined using default parameters of Blast algorithm version 2.2.2 (Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaeffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25: 3389-3402). The Blast algorithm can be used also through the Internet by accessing the site www.ncbi.nlm.nih.gov/blast.

[0077] The chimeric antibody, humanized antibody, or human antibody obtained by the aforementioned method can be subjected to a known method for evaluating the binding property to an antigen for selecting preferable antibodies.

In the anti-HER3 antibody of the present invention, MEHD-7945A (or duligotuzumab), RG-7116, MM-111, MM-121 (or seribantumab, MM-141, LJM-716, huHER3-8, tri-specific anti-EGFR/ErbB3 zybody, GSK-2849330, REGN-1400, KTN-3379, AV-203, monospecific surrobody (ErbB3), lumretuzumab, MP-EV-20, ZW-9, Dimercept™ ,anti-Erb3 surrobody(SL-175 or SL-176), SYM-013, variants, active fragments, modified products thereof, and the like are also included.

[0078] As one example of another index for use in the comparison of the properties of antibodies, the stability of antibodies can be exemplified. The differential scanning calorimetry (DSC) is a device capable of quickly and accurately measuring a thermal denaturation midpoint temperature (Tm) to be used as a favorable index of the relative conformational stability of proteins. By measuring the Tm values using DSC and comparing the values, a difference in thermal stability can be compared. It is known that the storage stability of antibodies shows some correlation with the thermal stability of antibodies (Lori Burton, et. al., Pharmaceutical Development and Technology (2007) 12, pp. 265-273), and a preferred antibody can be selected by using thermal stability as an index. Examples of other indices for selecting antibodies include the following features: the yield in an appropriate host cell is high; and the aggregability in an aqueous solution is low. For example, an antibody which shows the highest yield does not always show the highest thermal stability, and therefore, it is necessary to select an antibody most suitable for the administration to humans by making comprehensive evaluation based on the above-described indices.

[0079] The antibody of the present invention encompasses a modified product of the antibody. The modified variant refers to a variant obtained by subjecting the antibody

of the invention to chemical or biological modification. Examples of the chemically modified variant include variants chemically modified by linking a chemical moiety to an amino acid skeleton, variants chemically modified with an N-linked or O-linked carbohydrate chain, etc. Examples of the biologically modified variant include variants obtained by modification after translation (such as N-linked or O-linked glycosylation, N- or C-terminal processing, deamidation, isomerization of aspartic acid, or oxidation of methionine), and variants in which a methionine residue has been added to the N terminus by being expressed in a prokaryotic host cell. Further, an antibody labeled so as to enable the detection or isolation of the antibody or an antigen of the invention, for example, an enzyme-labeled antibody, a fluorescence-labeled antibody, and an affinity-labeled antibody are also included in the meaning of the modified variant. Such a modified variant of the antibody of the invention is useful for improving the stability and blood retention of the antibody, reducing the antigenicity thereof, detecting or isolating the antibody or the antigen, and so on.

[0080] Further, by regulating the modification of a glycan which is linked to the antibody of the invention (glycosylation, defucosylation, etc.), it is possible to enhance an antibody-dependent cellular cytotoxic activity. As the technique for regulating the modification of a glycan of antibodies, International Publication WO 1999/54342, WO 2000/61739, WO 2002/31140, etc. are known. However, the technique is not limited thereto. In the antibody of the invention, an antibody in which the modification of a glycan is regulated is also included.

In the case where an antibody is produced by first isolating an antibody gene and then introducing the gene into an appropriate host, a combination of an appropriate host and an appropriate expression vector can be used. Specific examples of the antibody gene include a combination of a gene encoding a heavy chain sequence of an antibody and a gene encoding a light chain sequence thereof described in this specification. When a host cell is transformed, it is possible to insert the heavy chain sequence gene and the light chain sequence gene into the same expression vector, and also into different expression vectors separately.

In the case where eukaryotic cells are used as the host, animal cells, plant cells, and eukaryotic microorganisms can be used. As the animal cells, mammalian cells, for example, simian COS cells (Gluzman, Y., Cell, (1981) 23, pp. 175-182, ATCC CRL-1650), murine fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L. A., Proc. Natl. Acad. Sci. USA (1980) 77, pp. 4126-4220) of Chinese hamster ovarian cells (CHO cells; ATCC: CCL-61) can be exemplified.

In the case where prokaryotic cells are used, for example, Escherichia coli and Bacillus subtilis can be exemplified.

By introducing a desired antibody gene into these cells through transformation, and culturing the thus transformed cells in vitro, the antibody can be obtained. In the above-described culture method, the yield may sometimes vary depending on the sequence of the antibody, and therefore, it is possible to select an antibody which is easily produced as a pharmaceutical by using the yield as an index among the antibodies having an equivalent binding activity. Therefore, in the antibody of the invention, an antibody obtained by a method of producing an antibody, characterized by including a step of culturing the transformed host cell and a step of collecting a desired antibody or a functional fragment of the antibody from a cultured product obtained in the culturing step is also included.

[0081] It is known that a lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell could be deleted/eliminated (Journal of Chromatography A, 705: 129-134 (1995)), and it is also known that two amino acid residues (glycine and lysine) at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell could be deleted/eliminated and a proline residue newly located at the carboxyl terminus could be amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion/elimination and modification of the heavy chain sequence do not affect the antigen-binding affinity and the effector function (the activation of a complement, the antibody-dependent cellular cytotoxicity, etc.) of the antibody. Therefore, in the antibody of the invention, an antibody and a functional fragment of the antibody subjected to such modification are also included, and a deletion variant in which one or two amino acids have been deleted at the carboxyl terminus of the heavy chain, a variant obtained by amidation of the deletion variant (for example, a heavy chain in which the carboxyl terminal proline residue has been amidated), and the like are also included. The type of deletion variant having a deletion at the carboxyl terminus of the heavy chain of the antibody according to the invention is not limited to the above variants as long as the antigen-binding affinity and the effector function are conserved. The two heavy chains constituting the antibody according to the invention may be of one type selected from the group consisting of a full-length heavy chain and the above-described deletion variant, or may be of two types in combination selected therefrom. The ratio of the amount of each deletion variant can be affected by the type of cultured mammalian cells which produce the antibody according to the invention and the culture conditions, however, a case where one amino acid residue at the carboxyl terminus has been deleted in both of the two heavy chains contained as main components in the antibody according to the invention can be exemplified. The scope of the whole antibody (in the present invention, also simply referred to as an "antibody") of the present invention also includes deletion variants thereof, mixtures containing one or two or more deletion

variants thereof, etc. The "antibody" of the present invention includes an antibody comprising a heavy or light chain in which N-terminal glutamate is in the form of pyroglutamate by cyclization and/or a heavy or light chain in which a portion of cysteine residues are in the form of cysteinyl.

[0082] In a preferred embodiment of the present invention, the anti-HER3 antibody of the invention is of the IgA, IgD-, IgE_1 IgG- or IgM-type, preferably of the IgG-or IgM-type including, but not limited to, the IgGI-, IgG2-, IgG3-, IgG4-, IgMI-and IgM2-type. In most preferred embodiments, the antibody is of the IgGI-, IgG2- or IgG4- type.

[0083] As the biological activity of the antibody, generally, an antigen-binding activity, activity of internalizing an antigen in cells expressing the antigen by binding with the antigen, an activity of neutralizing the activity of an antigen, an activity of enhancing the activity of an antigen, an antibody-dependent cellular cytotoxicity (ADCC) activity, a complement-dependent cytotoxicity (CDC) activity, and an antibody-dependent cell-mediated phagocytosis (ADCP) can be exemplified. The function of the antibody according to the invention is a binding activity to HER3, preferably, activity of internalizing HER3 in HER3 expressing cells by binding with HER3. Further, the antibody of the invention may have an ADCC activity, a CDC activity and/or an ADCP activity in addition to the cell internalization acitivity.

[0084] In certain respects, e.g. in connection with the generation of antibodies as therapeutic candidates against HER3, it may be desirable that the anti-HER3 antibody of the invention 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 without limitations the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgGI, human IgG3, and human IgA. 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 (Idusogie et al., J Immunol., 166, 2571-2575) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (see e.g. U.S. Patent No. 4,816,397), cell-cell fusion techniques (see e.g. U.S. Patent Nos. 5,916,771 and 6,207,418), 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 IgM, human IgGI 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 fixing complement and participating in CDC.

[0085] Moreover, it may also be desirable for the anti-HER3 antibody of the invention to be capable of binding to Fc receptors on effector cells, such as monocytes and natural killer (NK) cells, and participate in antibody-dependent cellular cytotoxicity (ADCC). There are a number of isotypes of antibodies that are capable of the same, including without limitations the following: murine IgG2a, murine IgG2b, murine IgG3, human IgGI and human IgG3. 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 ADCC over naturally occurring variants (Shields et al. J Biol Chem., 276, 6591-6604) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (see e.g. U.S. Patent No. 4,816,397), cell-cell fusion techniques (see e.g. U.S. Patent Nos. 5,916,771 and 6,207,418), 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 IgGI 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 FcyR on effectors cells and participating in ADCC.

[0086] The obtained antibody can be purified to be homogeneous. The separation and purification of the antibody may be performed employing a conventional protein separation and purification method. For example, the antibody can be separated and purified by appropriately selecting and combining column chromatography, filter

filtration, ultrafiltration, salt precipitation, dialysis, preparative polyacrylamide gel electrophoresis, isoelectric focusing electrophoresis, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but the method is not limited thereto.

Examples of such chromatography include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, and adsorption chromatography.

Such chromatography can be performed employing liquid chromatography such as HPLC or FPLC.

As a column to be used in affinity chromatography, a Protein A column and a Protein G column can be exemplified. For example, as a column using a Protein A column, Hyper D, POROS, Sepharose FF (Pharmacia Corp.) and the like can be exemplified.

Further, by using a carrier having an antigen immobilized thereon, the antibody can also be purified utilizing the binding property of the antibody to the antigen.

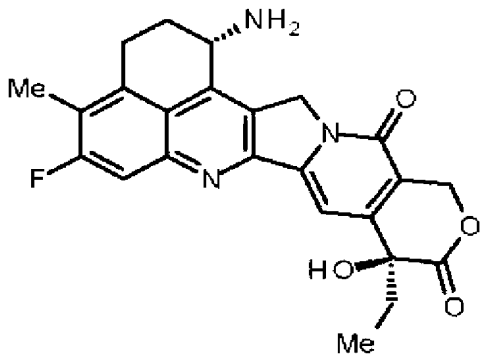
[0087] {Antitumor compound}

The antitumor compound to be conjugated to the anti-HER3 antibody-drug conjugate of the present invention is explained. The antitumor compound used in the present invention is not particularly limited if it is a compound having an antitumor effect and a substituent or a partial structure allowing connecting to a linker structure. When a part or whole linker of the antitumor compound is cleaved in tumor cells, the antitumor compound moiety is released to exhibit the antitumor effect. As the linker is cleaved at a connecting position with a drug, the antitumor compound is released in its un-modified structure to exhibit its intrinsic antitumor effect.

As an antitumor compound used in the present invention, exatecan, a camptothecin derivative ((1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10,13(9H,15H)-dione shown in the following formula) can be preferably used.

[0088]

[Chem.16]



[0089] Although having an excellent antitumor effect, exatecan has not been commercialized as an antitumor drug. The compound can be easily obtained by a known method and the amino group at position 1 can be preferably used as a connecting position to the linker structure. Further, exatecan can be also released in tumor cells while part of the linker is still attached thereto. However, it is an excellent compound exhibiting an excellent antitumor effect even in such structure.

Because exatecan has a camptothecin structure, it is known that the equilibrium shifts to a structure with a closed lactone ring (closed ring) in an acidic aqueous medium (for example, pH 3 or so) but it shifts to a structure with an open lactone ring (open ring) in a basic aqueous medium (for example, pH 10 or so). A drug conjugate being introduced with an exatecan residue corresponding to the closed ring structure and the open ring structure is also expected to have the same antitumor effect and it is needless to say that any of them is within the scope of the present invention.

[0090] Examples of other antitumor compounds include doxorubicin, daunorubicin, mitomycin C, bleomycin, cyclocytidine, vincristine, vinblastine, methotrexate, platinum-based antitumor agent (cisplatin or derivatives thereof), taxol or derivatives thereof, and other camptothecin or derivatives thereof (antitumor agent described in Japanese Patent Laid-Open No. 6-87746).

[0091] With regard to the antibody-drug conjugate, the number of conjugated drug molecules per antibody molecule is a key factor having an influence on the efficacy and safety. Production of the antibody-drug conjugate is performed by defining the reaction condition including the amounts of use of raw materials and reagents for reaction so as to have a constant number of conjugated drug molecules, a mixture containing different numbers of conjugated drug molecules is generally obtained unlike the chemical reaction of a low-molecular-weight compound. The number of drugs conjugated in an antibody molecule is expressed or specified by the average value, that is, the average number of conjugated drug molecules. Unless specifically described otherwise as a principle, the number of conjugated drug molecules means an

average value except in a case in which it represents an antibody-drug conjugate having a specific number of conjugated drug molecules that is included in an antibody-drug conjugate mixture having different number of conjugated drug molecules.
The number of exatecan molecules conjugated to an antibody molecule is controllable, and as an average number of conjugated drug molecules per antibody, about 1 to 10 exatecans can be bound. Preferably, it is 2 to 8, and more preferably 3 to 8.
Meanwhile, a person skilled in the art can design a reaction for conjugating a required number of drug molecules to an antibody molecule based on the description of the Examples of the present application and can obtain an antibody-drug conjugate with a controlled number of conjugated exatecan molecules.
The antibody-drug conjugate of the present invention is unlikely to have an occurrence of aggregation, insolubility, fragmentation, or the like, even when the number of conjugated drug molecules per antibody molecule is increased.

[0092] {Linker structure}

With regard to the anti-HER3 antibody-drug conjugate of the present invention, the linker structure for conjugating an antitumor compound to the anti-HER3 antibody is explained. The linker has the following structure:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-(CH_2)n^2-C(=O)-$ or $-L^1-L^2-L^P-$,

the antibody is connected to the terminal of L^1 (opposite terminal to which L^2 is connected), and the antitumor compound is connected to the carbonyl group of $-L^a-(CH_2)n^2-C(=O)-$ moiety or the C terminal of L^P.

n^1 represents an integer of 0 to 6, preferably, an integer of 1 to 5, and more preferably 1 to 3.

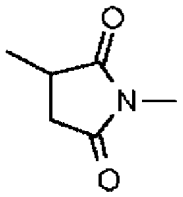
[0093] 1. L^1

L^1 is represented by a structure shown below:

$-$(Succinimid-3-yl-N)$-(CH_2)n^3-C(=O)-$

In the above, n^3 is an integer of 2 to 8, and "-(Succinimid-3-yl-N)-" has a structure represented by the following formula:

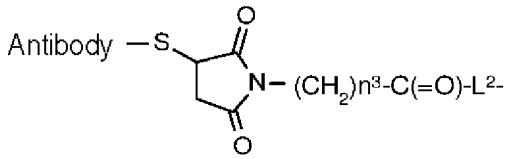
[0094] [Chem.17]



[0095] Position 3 of the above partial structure is the connecting position to the anti-HER3 antibody. The connection to the antibody at position 3 is characterized by forming a thioether bond. The nitrogen atom at position 1 of the structure moiety is connected to

the carbon atom of methylene which is present within the linker including the structure. Specifically, -(Succinimid-3-yl-N)-$(CH_2)n^3$-C(=O)-L^2- is a structure represented by the following formula (herein, "antibody -S-" is derived from an antibody).

[0096] [Chem.18]



[0097] In the formula, n^3 is an integer of 2 to 8, and preferably 2 to 5.

[0098] Specific examples of L^1 include the followings.

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-

[0099] 2. L^2

L^2 has a structure represented by the following formula:

-NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)-

L^2 may not be present, and in such a case, L^2 is a single bond. In the drug-linker structure of the present invention, in particular, L^P may be directly connected to a drug, and in such a case, L^2 is particularly preferably a single bond. n^4 is an integer of 1 to 6, and preferably 2 to 4. L^2 is connected to L^1 at its terminal amino group and is connected to L^P at the carbonyl group of the opposite terminal.

[0100] Specific examples of L^2 include the followings.

-NH-CH_2CH_2-O-CH_2CH_2-C(=O)-,

-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-,

-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-,

-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-,

-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-,

-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-

[0101] 3. L^P

L^P is a peptide residue consisting of 2 to 7 amino acids. Specifically, it consists of an oligopeptide residue in which 2 to 7 amino acids are linked by a peptide bond. L^P is connected to L^2 at N terminal and it is connected to the amino group of -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- moiety of the linker at C terminal.

[0102] The amino acid constituting L^P is not particularly limited, and the examples thereof include an L- or a D-amino acid, preferably an L-amino acid. Further, it can be an amino acid having a structure such as beta-alanine, epsilon-aminocaproic acid, or

gamma-aminobutyric acid in addition to an alpha-amino acid, further, it can be a non-natural type amino acid such as N-methylated amino acid.

Sequence of the amino acid of L^P is not particularly limited, but examples of the constituting amino acid include phenylalanine (Phe; F), tyrosine (Tyr; Y), leucine (Leu; L), glycine (Gly; G), alanine (Ala; A), valine (Val; V), lysine (Lys; K), citrulline (Cit), serine (Ser; S), glutamic acid (Glu; E), and aspartic acid (Asp; D). Among them, preferred examples include phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid. Depending on the type of the amino acid, drug release pattern can be controlled. The number of the amino acid can be between 2 to 7.

[0103] Specific examples of L^P include the followings.

-GGF-,
-DGGF-,
-(D-)D-GGF-,
-EGGF-,
-GGFG-,
-SGGF-,
-KGGF-,
-DGGFG-,
-GGFGG-,
-DDGGFG-,
-KDGGFG-,
-GGFGGGF-

The "(D-)D" described above means D-aspartic acid. Examples of the particularly preferred L^P of the antibody-drug conjugate of the present invention include -GGFG- and -DGGFG- peptide residue. Further, in the drug-linker structure of the present invention, L^P may be directly connected to the drug, and for such a case, preferred examples of L^P include a pentapeptide residue of -DGGFG-.

[0104] 4. L^a-$(CH_2)n^2$-C(=O)-

L^a in L^a-$(CH_2)n^2$-C(=O)- is a structure of -O- or a single bond. n^2 is an integer of 0 to 5, preferably, 0 to 3, and more preferably 0 or 1.

Examples of L^a-$(CH_2)n^2$-C(=O)- include the followings.

-O-CH_2-C(=O)-,
-O-CH_2CH_2-C(=O)-,
-O-$CH_2CH_2CH_2$-C(=O)-,
-O-$CH_2CH_2CH_2CH_2$-C(=O)-,
-O-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-,
-CH_2-C(=O)-,
-CH_2CH_2-C(=O)-,

-$CH_2CH_2CH_2$-C(=O)-,
-$CH_2CH_2CH_2CH_2$-C(=O)-,
-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-.
Among them, those with
-O-CH_2-C(=O)-,
-O-CH_2CH_2-C(=O)-
or those in which L^a is a single bond and n^2 is 0 are preferable.

[0105] Specific examples of the linker structure represented by -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- include the followings.
-NH-CH_2-C(=O)-,
-NH-CH_2CH_2-C(=O)-,
-NH-CH_2-O-CH_2-C(=O)-,
-NH-CH_2CH_2-O-C(=O)-,
-NH-CH_2CH_2-O-CH_2-C(=O)-,
-NH-$CH_2CH_2CH_2$-C(=O)-,
-NH-$CH_2CH_2CH_2CH_2$-C(=O)-,
-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-

[0106] Among them, the examples are more preferably the followings.
-NH-$CH_2CH_2CH_2$-C(=O)-,
-NH-CH_2-O-CH_2-C(=O)-,
-NH-CH_2CH_2-O-C(=O)-

[0107] As for the linker -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-, the chain length of 4 to 7 atoms is preferable, and more preferably, are those having the chain length of 5 or 6 atoms.

[0108] With regard to the anti-HER3 antibody-drug conjugate of the present invention, when it is transferred to the inside of tumor cells, it is thought that the linker moiety is cleaved and the drug derivative having a structure represented by NH_2-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-(NH-DX) is released to express an antitumor action. Examples of the antitumor derivative exhibiting an antitumor effect by releasing from the antibody-drug conjugate of the present invention include an antitumor derivative having a structure moiety in which the terminal of the structure represented by -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- of the linker is an amino group, and the particularly preferred include the followings.
NH_2-CH_2CH_2-C(=O)-(NH-DX),
NH_2-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
NH_2-CH_2-O-CH_2-C(=O)-(NH-DX),
NH_2-CH_2CH_2-O-CH_2-C(=O)-(NH-DX).
Meanwhile, in case of NH_2-CH_2-O-CH_2-C(=O)-(NH-DX), it was confirmed that, as the aminal structure in the molecule is unstable, it again undergoes a self- degradation

to release the following HO-CH_2-C(=O)-(NH-DX). Those compounds can be also preferably used as a production intermediate of the antibody-drug conjugate of the present invention.

Further, in the drug-linker structure of the present invention, there arises a case in which L^P may be directly connected to the drug. In such a case, when the C terminal of L^P is glycine, the antitumor drug to be released is exatecan itself or a compound having glycine bonded to the amino group of exatecan.

[0109] For the antibody-drug conjugate of the present invention in which exatecan is used as a drug, the drug-linker structure moiety having the following structure

-L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-(NH-DX) or

-L^1-L^2-L^P-(NH-DX)

to which the antibody is connceted is preferable. The conjugated number of these drug-linker structure moiety may be from 1 to 10 as the average conjugated number per antibody, preferably, 2 to 8, and more preferably 3 to 8.

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

Among them, the more preferred are the followings.
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

The still more preferred are the followings.
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

The particularly preferred are the followings.
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX).

[0110] With regard to the linker structure for conjugating the anti-HER3 antibody and a

drug in the antibody-drug conjugate of the present application, the preferred linker can be constructed by connecting preferred structures shown for each part of the linker explained above. As for the linker structure, those with the following structure can be preferably used. Meanwhile, the left terminal of the structure is a connecting position to the antibody and the right terminal is a connecting position to the drug.

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-.

Among them, more preferred are the followings.

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-,

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-,

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GG

FG-NH-$CH_2CH_2CH_2$-C(=O)-,
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-,
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-.
Still more preferred are the followings.
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-,
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-,
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-,
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-.
Particularly preferred are the followings.
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-,
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-,
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-.

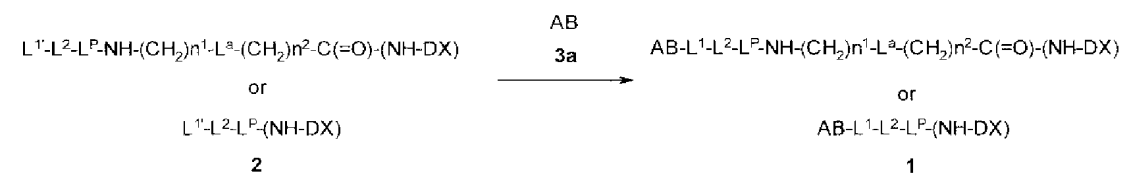
[0111] {Production method}

Next, explanations are given for the representative method for producing the antibody-drug conjugate of the present invention or a production intermediate thereof. Meanwhile, the compounds are hereinbelow described with the number shown in each reaction formula. Specifically, they are referred to as a "compound of the formula (1)", a "compound (1)", or the like. The compounds with numbers other than those are also described similarly.

[0112] 1. Production method 1

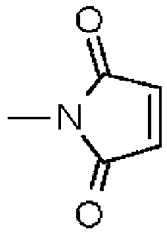
The antibody-drug conjugate represented by the formula (1) in which the antibody is conjugated to the drug-linker structure via thioether can be produced by the following method, for example.

[0113] [Chem.19]



[0114] [in the formula, AB represents an antibody with a sulfhydryl group and $L^{1'}$ corresponds to L^1 having a structure in which the linker terminal is converted to a maleimidyl group (formula shown below).

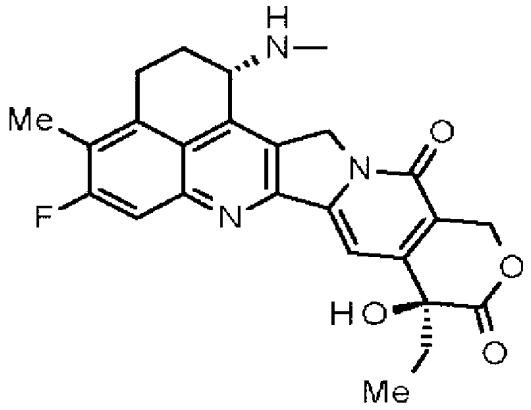
[0115] [Chem.20]



[0116] (in the formula, the nitrogen atom is the connecting position)

Specifically, it represents a linker having a structure which, within the structure of L^1 represented as -(Succinimid-3-yl-N)-$(CH_2)n^2$-C(=O)-, said -(Succinimid-3-yl-N)- moiety is converted into a maleimidyl group. Further, the -(NH-DX) represents a structure represented by the following formula:

[0117] [Chem.21]



[0118] and it represents a group that is derived by removing one hydrogen atom of the amino group at position 1 of exatecan.]

[0119] Further, the compound of the formula (1) in the above reaction formula can be interpreted as a structure in which one structure moiety from drug to the linker terminal is connected to one antibody. However, it is only the description given for the sake of convenience, and there are actually many cases in which a plurality of said structure moieties is connected to one antibody molecule. The same applies to the explanation of the production method described below.

[0120] Specifically, the antibody-drug conjugate (1) can be produced by reacting the compound (2), which is obtainable by the method described below, with the antibody (3a) having a sulfhydryl group.

The antibody (3a) having a sulfhydryl group can be obtained by a method well known in the art (Hermanson, G.T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996)). Examples include: Traut's reagent is reacted with the amino group of the antibody; N-succinimidyl S-acetylthioalkanoates are reacted with the

amino group of the antibody followed by reaction with hydroxylamine; after reacting with N-succinimidyl 3-(pyridyldithio)propionate, it is reacted with a reducing agent; the antibody is reacted with a reducing agent such as dithiothreitol, 2-mercaptoethanol, and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to reduce the disulfide bond at a hinge part in the antibody to form a sulfhydryl group, but it is not limited thereto. Specifically, using 0.3 to 3 molar equivalents of TCEP as a reducing agent per disulfide bonds at hinge part in the antibody and reacting with the antibody in a buffer solution containing a chelating agent, the antibody which the disulfide bonds at hinge part in the antibody is partially or completely reduced can be obtained. Examples of the chelating agent include ethylenediamine tetraacetic acid (EDTA) and diethylene-triamine pentaacetic acid (DTPA). It can be used at concentration of 1 mM to 20 mM. Examples of the buffer solution which may be used include a solution of sodium phosphate, sodium borate, or sodium acetate. Specifically, by reacting the antibody with TCEP at 4C to 37C for 1 to 4 hours, the antibody (3a) having partially or completely reduced sulfhydryl groups can be obtained.

Meanwhile, by performing an addition reaction of a sulfhydryl group to a drug-linker moiety, the drug-linker moiety can be conjugated by a thioether bond.

Using 2 to 20 molar equivalents of the compound (2) per the antibody (3a) having a sulfhydryl group, the antibody-drug conjugate (1) in which 2 to 8 drug molecules are conjugated per antibody can be produced. Specifically, it is sufficient that the solution containing the compound (2) dissolved therein is added to a buffer solution containing the antibody (3a) having a sulfhydryl group for the reaction. Herein, examples of the buffer solution which may be used include sodium acetate solution, sodium phosphate, and sodium borate. pH for the reaction is 5 to 9, and more preferably the reaction is performed near pH 7. Examples of the solvent for dissolving the compound (2) include an organic solvent such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), and N-methyl-2-pyrrolidone (NMP).

The reaction may be carried out by adding the organic solvent solution containing the compound (2) dissolved therein at 1 to 20% v/v to a buffer solution containing the antibody (3a) having a sulfhydryl group. The reaction temperature is 0 to 37C, more preferably 10 to 25C, and the reaction time is 0.5 to 2 hours. The reaction can be terminated by deactivating the reactivity of unreacted compound (2) with a thiol-containing reagent. Examples of the thiol-containing reagent include cysteine and N-acetyl-L-cysteine (NAC). More specifically, by adding 1 to 2 molar equivalents of NAC to the compound (2) used and, by incubating at room temperature for 10 to 30 minutes, the reaction can be terminated.

The produced antibody-drug conjugate (1) can be subjected to, after concentration, buffer exchange, purification, and measurement of antibody concentration and average

number of conjugated drug molecules per antibody molecule according to common procedures described below, to make an identification of the antibody-drug conjugate (1).

[0121] Common procedure A: Concentration of aqueous solution of antibody or antibody-drug conjugate

To a Amicon Ultra (50,000 MWCO, Millipore Corporation) container, a solution of antibody or antibody-drug conjugate was added and the solution of the antibody or antibody-drug conjugate was concentrated by centrifugation (centrifuge for 5 to 20 minutes at 2000 G to 3800 G) using a centrifuge (Allegra X-15R, Beckman Coulter, Inc.)

[0122] Common procedure B: Measurement of antibody concentration

Using a UV detector (Nanodrop 1000, Thermo Fisher Scientific Inc.), measurement of the antibody concentration was performed according to the method defined by the manufacturer. Here, 280 nm absorption coefficient can be estimated from the amino acid sequence of an antibody using a known calculation method (Protein Science, 1995, vol. 4, 2411-2423), and 280 nm absorption coefficient different for each antibody was used (1.3 $mLmg^{-1}cm^{-1}$ to 1.8 $mLmg^{-1}cm^{-1}$). In the case of U1-59, 280 nm absorption coefficient of 1.768 $mLmg^{-1}cm^{-1}$ was used as an estimated value according to its amino acid sequence.

[0123] Common procedure C: Buffer Exchange for antibody

NAP-25 column (Cat. No.17-0852-02, GE Healthcare Japan Corporation) using Sephadex G-25 carrier was equilibrated with phosphate buffer (10 mM, pH 6.0; it is referred to as PBS6.0/EDTA in the specification) containing sodium chloride (137 mM) and ethylene diamine tetraacetic acid (EDTA, 5 mM) according to the method defined by the manufacturer. Aqueous solution of the antibody was applied in an amount of 2.5 mL to single NAP-25 column, and then the fraction (3.5 mL) eluted with 3.5 mL of PBS6.0/EDTA was collected. The resulting fraction was concentrated by the Common procedure A. After measuring the concentration of the antibody using the Common procedure B, the antibody concentration was adjusted to 10 mg/mL using PBS6.0/EDTA.

[0124] Common procedure D: Purification of antibody-drug conjugate

NAP-25 column was equilibrated with acetate buffer containing sorbitol (5%) (10 mM, pH 5.5; it is referred to as ABS in the specification). Aqueous reaction solution of the antibody-drug conjugate (about 2.5 mL) was applied to the NAP-25 column, and then eluted with the buffer in an amount as defined by the manufacturer to collect the antibody fraction. By conducting a gel filtration purification process, in which said collected fraction was again applied to the NAP-25 column and eluted with buffer, was repeated 2 to 3 times in total, the antibody-drug conjugate excluding non-conjugated

drug linker and a low-molecular-weight compound (tris(2-carboxyethyl)phosphine hydrochloride (TCEP), N-acetyl-L-cysteine (NAC), and dimethyl sulfoxide) was obtained.

[0125] Common procedure E: Measurement of antibody concentration in antibody-drug conjugate and average number of conjugated drug molecules per antibody molecule (1)

The conjugated drug concentration in the antibody-drug conjugate can be calculated by measuring UV absorbance of an aqueous solution of the antibody-drug conjugate at two wavelengths of 280 nm and 370 nm, followed by performing the calculation shown below.

Because the total absorbance at any wavelength is equal to the sum of the absorbance of every light-absorbing chemical species that are present in a system [additivity of absorbance], when the molar absorption coefficients of the antibody and the drug remain the same before and after conjugation between the antibody and the drug, the antibody concentration and the drug concentration in the antibody-drug conjugate are expressed with the following equations.

$A_{280} = A_{D,280} + A_{A,280} = E_{D,280}C_D + E_{A,280}C_A$ Equation (1)

$A_{370} = A_{D,370} + A_{A,370} = E_{D,370}C_D + E_{A,370}C_A$ Equation (2)

In the above, A_{280} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 280 nm, A_{370} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 370 nm, $A_{A,280}$ represents the absorbance of an antibody at 280 nm, $A_{A,370}$ represents the absorbance of an antibody at 370 nm, $A_{D,280}$ represents the absorbance of a conjugate precursor at 280 nm, $A_{D,370}$ represents the absorbance of a conjugate precursor at 370 nm, $E_{A,280}$ represents the molar absorption coefficient of an antibody at 280 nm, $E_{A,370}$ represents the molar absorption coefficient of an antibody at 370 nm, $E_{D,280}$ represents the molar absorption coefficient of a conjugate precursor at 280 nm, $E_{D,370}$ represents the molar absorption coefficient of a conjugate precursor at 370 nm, C_A represents the antibody concentration in an antibody-drug conjugate, and C_D represent the drug concentration in an antibody-drug conjugate.

As for $E_{A,280}$, $E_{A,370}$, $E_{D,280}$, and $E_{D,370}$ in the above, previously prepared values (estimated value based on calculation or measurement value obtained by UV measurement of the compound) are used. For example, $E_{A,280}$ can be estimated from the amino acid sequence of an antibody using a known calculation method (Protein Science, 1995, vol. 4, 2411-2423). $E_{A,370}$ is generally zero. In the case of U1-59, $E_{A,280}$ of 259400 was used as an estimated value according to its amino acid sequence. $E_{D,280}$ and $E_{D,370}$ can be obtained based on Lambert-Beer's law (Absorbance = molar concentration ´ molar absorption coefficient ´ cell path length) by measuring the absorbance of a solution in which the conjugate precursor to be used is dissolved at a certain molar concentration. By measuring A_{280} and A_{370} of an aqueous solution of the

antibody-drug conjugate and solving the simultaneous equations (1) and (2) using the values, C_A and C_D can be obtained. Further, by diving C_D by C_A, the average drug binding number per antibody can be obtained.

In the present invention, the method for determining the average number of conjugated drug molecules per antibody as described above is referred to as a "UV method".

[0126] Common procedure F: Measurement of average number of conjugated drug molecules per antibody molecule in antibody-drug conjugate - (2)

The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate can also be determined by high-performance liquid chromatography (HPLC) analysis using the following method, in addition to the aforementioned Common procedure E.

{F-1. Preparation of sample for HPLC analysis (Reduction of antibody-drug conjugate)}

An antibody-drug conjugate solution (about 1 mg/mL, 60 u ("u" represents "micro")L) is mixed with an aqueous solution of dithiothreitol (DTT) (100 mM, 15 uL). By incubating the mixture at 37C for 30 minutes, the disulfide bond between the L and H chains of the antibody-drug conjugate is cleaved. The resulting sample is used in HPLC analysis.

{F-2. HPLC analysis}

The HPLC analysis is carried out under the following measurement conditions.

HPLC system: Agilent 1290 HPLC system (Agilent Technologies)

Detector: Ultraviolet absorption spectrometer (measurement wavelength: 280 nm)

Column: PLRP-S (2.1´50 mm, 8 um, 1000 angstroms; Agilent Technologies, P/N PL1912-1802)

Column temperature: 80C

Mobile phase A: 0.04% aqueous trifluoroacetic acid (TFA) solution

Mobile phase B: Acetonitrile solution containing 0.04% TFA

Gradient program: 29%-36% (0 min.-12.5 min.), 36%-42% (12.5-15 min.), 42%-29% (15 min.-15.1 min.), 29%-29% (15.1 min.-25 min.)

Sample injection: 15 uL

{F-3. Data analysis}

[F-3-1] Compared with non-conjugated antibody L (L_0) and H (H_0) chains, drug-conjugated L (L chain bound to one drug molecule: L_1) and H (H chain bound to one drug molecule: H_1, H chain bound to two drug molecule: H_2, H chain bound to three drug molecules: H_3) chains exhibit higher hydrophobicity in proportion to the number of conjugated drug molecules and thus have a larger retention time. These chains are therefore eluted in the order of L_0 and L_1 or H_0, H_1, H_2, and H_3. Detection peaks can be assigned to any of L_0, L_1, H_0, H_1, H_2, and H_3 by the comparison of retention times with

L_0 and H_0.

[F-3-2] Since the drug linker has UV absorption, peak area values are corrected in response to the number of conjugated drug linker molecules according to the following expression using the molar absorption coefficients of the L or H chain and the drug linker.

[0127] [Math.1]

$$\text{Corrected value of the peak area of the L chain } (Li) = \text{Peak area} \times \frac{\text{Molar absorption coefficient of the L chain}}{\text{Molar absorption coefficient of the L chain} + \text{The number of conjugated drug molecules} \times \text{Molar absorption coefficient of the drug linker}}$$

[0128] [Math.2]

$$\text{Corrected value of the peak area of the H chain } (Hi) = \text{Peak area} \times \frac{\text{Molar absorption coefficient of the H chain}}{\text{Molar absorption coefficient of the H chain} + \text{The number of conjugated drug molecules} \times \text{Molar absorption coefficient of the drug linker}}$$

[0129] Here, a value estimated from the amino acid sequence of the L or H chain of each antibody using a known calculation method (Protein Science, 1995, vol. 4, 2411-2423) can be used as the molar absorption coefficient (280 nm) of the L or H chain of each antibody. In the case of U1-59, a molar absorption coefficient of 34690 and a molar absorption coefficient of 95000 were used as estimated values for the L and H chains, respectively, according to its amino acid sequence. The actually measured molar absorption coefficient (280 nm) of a compound in which the maleimide group has been converted to succinimide thioether by the reaction of each drug linker with mercaptoethanol or N-acetylcysteine was used as the molar absorption coefficient (280 nm) of the drug linker.

[F-3-3] The peak area ratio (%) of each chain is calculated for the total of the corrected values of peak areas.

[0130] [Math.3]

$$\text{Peak area ratio of the L chain} = \frac{A_{Li}}{A_{L0}+A_{L1}} \times 100$$

$$\text{Peak area ratio of the H chain} = \frac{A_{Hi}}{A_{H0}+A_{H1}+A_{H2}+A_{H3}} \times 100$$

Corrected values of respective peak areas of A_{Li}, A_{Hi}:L_i, H_i

[0131] [F-3-4] The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate is calculated according to the following expression.

Average number of conjugated drug molecules = (L_0 peak area ratio x 0 + L_0 peak area ratio x 1 + H_0 peak area ratio x 0 + H_1 peak area ratio x 1 + H_2 peak area ratio x 2 + H_3 peak area ratio x 3) / 100 x 2

[0132] Hereinbelow, production intermediate compounds used in Production method 1 are described. The compound represented by the formula (2) in the production method 1 is a compound represented by the following formula:

[0133] (maleimid-N-yl)-$(CH_2)n^3$-C(=O)-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-(NH-DX) or (maleimid-N-yl)-$(CH_2)n^3$-C(=O)-L^2-L^P-(NH-DX).

In the formula,

n^3 represents an integer of 2 to 8,

L^2 represents -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)- or a single bond,

wherein n^4 represents an integer of 1 to 6,

L^P represents a peptide residue consisting of 2 to 7 amino acids selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid,

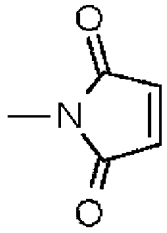
n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^a represents -O- or a single bond,

(maleimid-N-yl)- is a maleimidyl group (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl group) represented by the following formula:

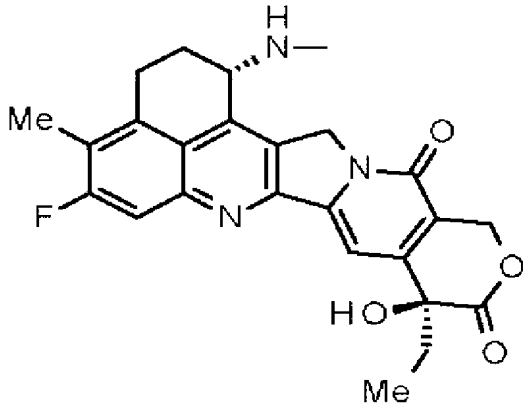
[Chem.22]



[0134] wherein the nitrogen atom is the connecting position, and

-(NH-DX) is a group represented by the following formula:

[Chem.23]



[0135] wherein the nitrogen atom of the amino group at position 1 is the connecting position.

[0136] As for the peptide residue L^P, those consisting of an amino acid selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid is preferred as a production intermediate. Among the peptide residue L^P, those consisting of 4 or 5 amino acids is preferred as a production intermediate. More specifically, those in which L^P is a tetrapeptide residue of -GGFG- or a pentapeptide of -DGGFG- is preferred as a production intermediate, more preferably, -GGFG-.

[0137] Further, as for the -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-, those having -NH-CH_2CH_2-, -NH-$CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2- is preferred as a production intermediate. A compound of -NH-$CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2 is more preferred.

As for n^3, those in which it is an integer of 2 to 8 is preferred as a production intermediate.

As for L^2, those in which it is a single bond or -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)- and n^4 is an integer of 2 to 4 is preferred as a production intermediate.

[0138] Further, those in which n^3 is an integer of 2 to 5, L^2 is a single bond, and -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$- is -NH-CH_2CH_2-, -NH-$CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2- is preferred as a production intermediate. Further, more preferred among them is those in which -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$- is -NH-CH_2CH_2-, -NH-$CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2-. Further, those in which n^3 is an integer of 2 or 5 is preferred.

[0139] Further, those in which n^3 is an integer of 2 to 5, L^2 is -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)-, n^4 is an integer of 2 to 4, and -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$- is -NH-CH_2CH_2-, -NH-$CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2$-, -NH-$CH_2CH_2CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2- is preferred as a production intermediate. More preferred among them is those in which n^4 is an integer of 2 or 4. Further, those in

which -NH-(CH_2)n^1-L^a- is -NH-$CH_2CH_2CH_2$-, -NH-CH_2-O-CH_2-, or -NH-CH_2CH_2-O-CH_2- is preferred.

[0140] Preferred examples of the intermediate that are useful for production of the compound of the present invention include those exemplified below:

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX), or
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[0141] By the reaction of the drug-linker compound selected from the aforementioned group of intermediate compounds with an anti-Her3 antibody or a reactive derivative thereof, a thioether bond can be formed at a disulfide bond moiety present in a hinge part of the anti-Her3 antibody, and as a result, the anti-Her3 antibody-drug conjugate of the present invention can be produced. In this case, it is preferable to use a reactive derivative of an anti-Her3 antibody. A reactive derivative obtained by reducing an anti-Her3 antibody is particularly preferred.

[0142] The followings are a compound which is more preferred as a production intermediate.

(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-

DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
(maleimid-N-yl)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX),
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[0143] Further, among the aforementioned intermediate compound group, the intermediates represented by the following formula are a more preferred compound:

(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX), or

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

Particularly preferred are the compounds that are represented by the following formula:

(maleimid-N-yl)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX), or

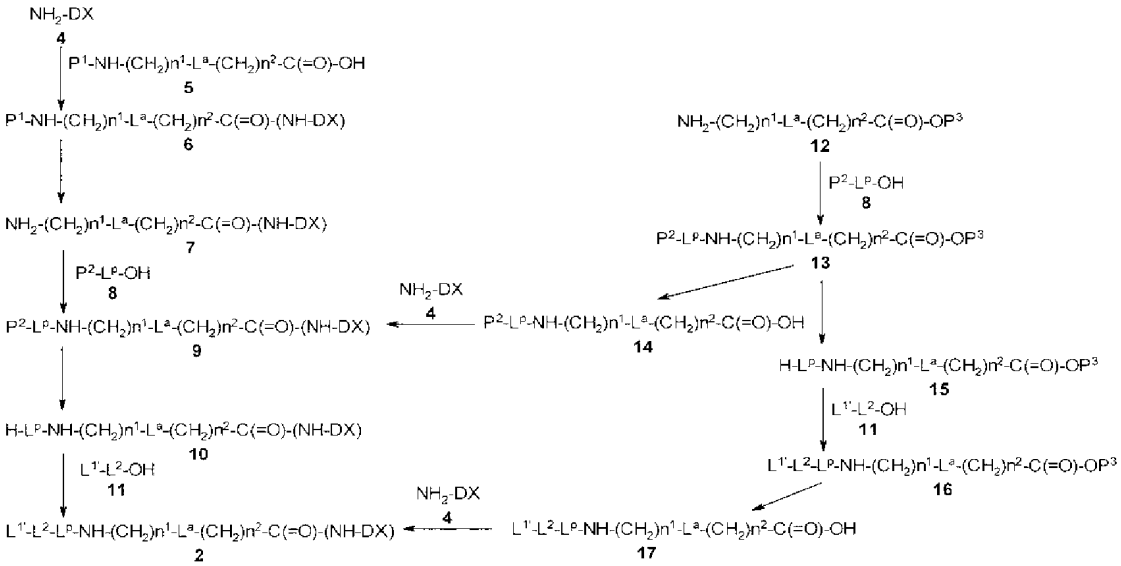
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX).

[0144] 2. Production method 2

The compound represented by the formula (2) or a pharmacologically acceptable salt thereof used as an intermediate in the previous production method can be produced by the following method, for example.

[0145]

[Chem.24]



[0146] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is converted to a maleimidyl group and P^1, P^2, and P^3 represent a protecting group].

[0147] The compound (6) can be produced by derivatizing the carboxylic acid (5) into an active ester, mixed acid anhydride, acid halide, or the like and, in the presense of base, reacting it with NH_2-DX [indicating exatecan; chemical name: (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de] pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10,13(9H,15H)-dione] (4) or a pharmacologically acceptable salt thereof.

Reaction reagents and conditions that are commonly used for peptide synthesis can be employed for the reaction. There are various kinds of active ester,for example, it can be produced by reacting phenols such as p-nitrophenol, N-hydroxy benzotriazole, N-hydroxy succinimide, or the like, with the carboxylic acid (5) using a condensing agent such as N,N'-dicyclohexylcarbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; further, the active ester can be also produced by a reaction of the carboxylic acid (5) with pentafluorophenyl trifluoroacetate or the like; a reaction of the carboxylic acid (5) with 1-benzotriazolyl oxytripyrrolidinophosphonium hexafluorophosphite; a reaction of the carboxylic acid (5) with diethyl cyanophosphonate (Shioiri method); a reaction of the carboxylic acid (5) with triphenylphosphine and 2,2'-dipyridyl disulfide (Mukaiyama method); a reaction of the carboxylic acid (5) with a triazine derivative such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM); or the like. Further, the reaction can be also performed by, e.g., an acid halide method by which the carboxylic acid (5) is treated with acid halide such as thionyl chloride and

oxalyl chloride in the presence of a base.

By reacting the active ester, mixed acid anhydride, or acid halide of the carboxylic acid (5) obtained as above with the compound (4) in the presence of a suitable base in an inert solvent at a reaction temperature of -78C to 150C, the compound (6) can be produced. Meanwhile, "inert solvent" indicates a solvent which does not inhibit a desired reaction for which the solvent is used.

[0148] Specific examples of the base used for each step described above include a carbonate, an alkoxide, a hydroxide or a hydride of an alkali metal or an alkali earth metal such as sodium carbonate, potassium carbonate, sodium ethoxide, potassium butoxide, sodium hydroxide, potassium hydroxide, sodium hydride, or potassium hydride; organometallic base represented by an alkyl lithium such as n-butyl lithium, or dialkylamino lithium such as lithium diisopropylamide; organometallic base such as bissilylamine including lithium bis(trimethylsilyl)amide; and organic base such as pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, N-methyl morpholine, diisopropylethylamine, and diazabicyclo[5.4.0]undec-7-ene (DBU).

[0149] Examples of the inert solvent which is used for the reaction of the present invention include a halogenated hydrocarbon solvent such as dichloromethane, chloroform, and carbon tetrachloride; an ether solvent such as tetrahydrofuran, 1,2-dimethoxyethane, and dioxane; an aromatic hydrocarbon solvent such as benzene and toluene; and an amide solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, and N-methylpyrrolidin-2-one. In addition to them, a sulfoxide solvent such as dimethyl sulfoxide and sulfolane; a ketone solvent such as acetone and methyl ethyl ketone; and an alcohol solvent such as methanol and ethanol may be used in some case. Alternatively, these solvents may be used as a mixed solvent.

[0150] As for the protecting group P^1 for the terminal amino group of the compound (6), a protecting group for an amino group which is generally used for peptide synthesis, for example, tert-butyloxy carbonyl group, 9-fluorenylmethyloxy carbonyl group, and benzyloxy carbonyl group, can be used. Examples of the other protecting group for an amino group include an alkanoyl group such as acetyl group; an alkoxycarbonyl group such as methoxycarbonyl group and ethoxycarbonyl group; an arylmethoxy carbonyl group such as paramethoxybenzyloxy carbonyl group, and para (or ortho)nitroybenzyloxy carbonyl group; an arylmethyl group such as benzyl group and triphenyl methyl group; an aroyl group such as benzoyl group; and an aryl sulfonyl group such as 2,4-dinitrobenzene sulfonyl group and orthonitrobenzene sulfonyl group. The protecting group P^1 can be selected depending on, e.g., properties of a compound having an amino group to be protected.

By deprotecting the protecting group P^1 for the terminal amino group of the compound (6) obtained, the compound (7) can be produced. In this deprotection,

reagents and conditions can be selected depending on the protecting group.
The compound (9) can be produced by derivatizing the peptide carboxylic acid (8) having the N terminal protected with P^2 into an active ester, mixed acid anhydride, or the like and reacting it with the compound (7) obtained. The reaction conditions, reagents, base, and inert solvent used for a peptide bond formation between the peptide carboxylic acid (8) and the compound (7) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^2 can be suitably selected from those described for the protecting group of the compound (6), and the selection can be made based on, e.g., the properties of the compound having an amino group to be protected. As it is generally used for peptide synthesis, by repeating sequentially the reaction and deprotection of the amino acid or peptide constituting the peptide carboxylic acid (8) for elongation, the compound (9) can be also produced.
By deprotecting P^2 as the protecting group for the amino group of the compound (9) obtained, the compound (10) can be produced. In this deprptection, reagents and conditions can be selected depending on the protecting group.
It is possible to produce the compound (2) by derivatizing the carboxylic acid (11) into an active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (10) obtained. The reaction conditions, reagents, base, and inert solvent used for forming a peptide bond between the carboxylic acid (11) and the compound (10) can be suitably selected from those described for the synthesis of the compound (6).

[0151] The compound (9) can be also produced by the following method, for example.

The compound (13) can be produced by derivatizing the peptide carboxylic acid (8) having the N terminal protected with P^2 into active ester, mixed acid anhydride, or the like and reacting it with the amine compound (12) having the carboxy group protected with P^3 in the presence of a base. The reaction conditions, reagents, base, and inert solvent used for forming a peptide bond between the peptide carboxylic acid (8) and the compound (12) can be suitably selected from those described for the synthesis of the compound (6).

The protecting group P^2 for the amino group of the compound (13) is not particularly limited if it is a protecting group which is commonly used. Specifically, examples of the protecting group for a hydroxyl group include an alkoxymethyl group such as methoxymethyl group; an arylmethyl group such as benzyl group, 4-methoxybenzyl group, and triphenylmethyl group; an alkanoyl group such as acetyl group; an aroyl group such as benzoyl group; and a silyl group such as tert-butyl diphenylsilyl group. Carboxy group can be protected by an ester with an alkyl group such as methyl group, ethyl group, and tert-butyl group, an allyl group, or an arylmethyl group such as benzyl group. As for the amino group, an alkyloxy carbonyl group such as tert-butyloxy

carbonyl group, methoxycarbonyl group, and ethoxycarbonyl group; an arylmethoxy carbonyl group such as allyloxycarbonyl group, 9-fluorenylmethyloxy carbonyl group, benzyloxy carbonyl group, paramethoxybenzyloxy carbonyl group, and para (or ortho)nitroybenzyloxy carbonyl group; an alkanoyl group such as acetyl group; an arylmethyl group such as benzyl group and triphenyl methyl group; an aroyl group such as benzoyl group; and an aryl sulfonyl group such as 2,4-dinitrobenzene sulfonyl group or orthonitrobenzene sulfonyl group can be mentioned.

As for the protecting group P^3 for a carboxy group, a protecting group commonly used as a protecting group for a carboxy group in organic synthetic chemistry, in particular, peptide synthesis can be used. A carboxyl group can be protected as an ester with an alkyl group such as a methyl group, an ethyl group, or a tert-butyl, an allyl group, and an arylmethyl group such as a benzyl group.

In such case, it is preferable that the protecting group for an amino group and the protecting group for a carboxy group can be removed by a different method or different conditions. For example, a representative example includes a combination in which P^2 is a tert-butyloxy carbonyl group and P^3 is a benzyl group. The protecting groups can be selected from the aforementioned ones depending on, e.g., the properties of a compound having an amino group and a carboxy group to be protected. For removal of the protecting groups, reagents and conditions can be selected depending on the protecting group.

By deprotecting the protecting group P^3 for the carboxy group of the compound (13) obtained, the compound (14) can be produced. In this deprotection, reagents and conditions are selected depending on the protecting group.

The compound (9) can be produced by derivatizing the compound (14) obtained into active ester, mixed acid anhydride, acid halide, or the like and reacting with the compound (4) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and inert solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0152] The compound (2) can be also produced by the following method, for example.

By deprotecting the protecting group P^2 for the amino group of the compound (13), the compound (15) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (16) can be produced by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (15) obtained in the presence of a base. The reaction conditions, reagents, base, and inert solvent used for forming an amide bond between the peptide carboxylic acid (11) and the compound (15) can be suitably selected from those

described for the synthesis of the compound (6).

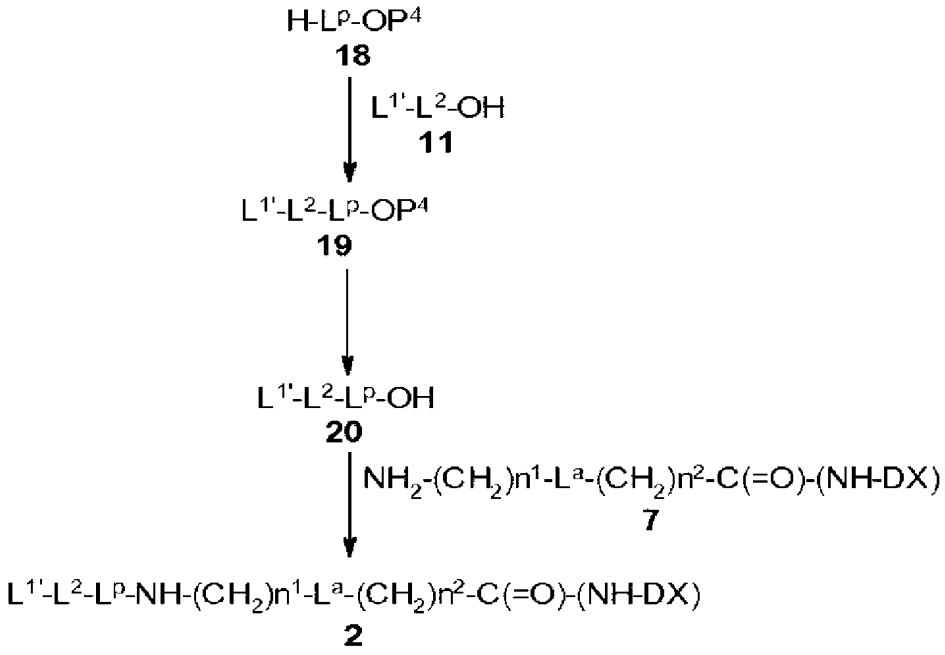
By deprotecting the protecting group for the carboxy group of the compound (16) obtained, the compound (17) can be produced. In this deprotection, it can be carried out similar to deprotecting carboxy group for producing the compound (14).

The compound (2) can be produced by derivatizing the compound (17) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and inert solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0153] 3. Production method 3

The compound represented by the formula (2) used as an intermediate can be also produced by the following method.

[0154] [Chem.25]



[0155] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is converted to a maleimidyl group and P^4 represents a protecting group].

[0156] The compound (19) can be produced by derivatizing the compound (11) into active ester, mixed acid anhydride, or the like and reacting it with the peptide carboxylic acid (18) having the C terminal protected with P^4 in the presence of a base. The reaction conditions, reagents, base, and inert solvent used for forming a peptide bond between the peptide carboxylic acid (18) and the compound (11) can be suitably selected from

those described for the synthesis of the compound (6). The protecting group P^4 for the carboxy group of the compound (18) can be suitably selected from the aforementioned protective groups.

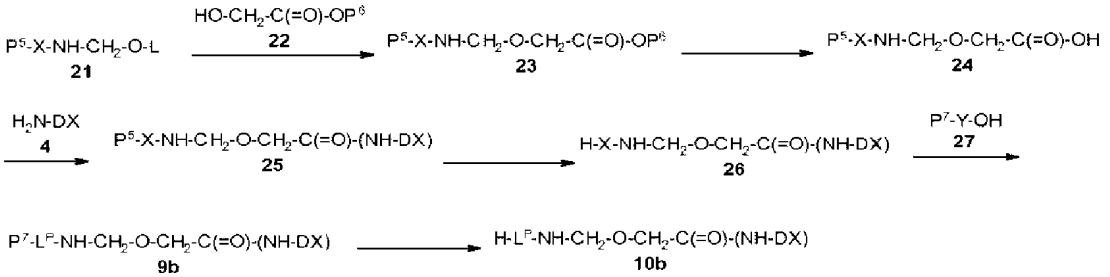
By deprotecting the protecting group for the carboxy group of the compound (19) obtained, the compound (20) can be produced. In this deprotecion, it can be performed similar to the deprotection of the carboxy group for producing the compound (14).

The compound (2) can be produced by derivatizing the compound (20) obtained into active ester, mixed acid anhydride, or the like and reacting it with the compound (7). For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and inert solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0157] 4. Production method 4

Hereinbelow, within the production intermediate (10) described in production method 2, the method for producing the compound (10b) having $n^1 = 1$ and $L^a = 0$ is described in detail. The compound represented by the formula (10b), a salt or a solvate thereof can be produced according to the following method, for example.

[0158] [Chem.26]



[0159] [in the formula, L^P is as defined above, L represents an acyl group including an alkanoyl group such as acetyl group or an aroyl group such as benzoyl group, or represents a hydrogen atom or the like, X and Y represent an oligopeptide consisting of 1 to 3 amino acids, P^5 and P^7 represent a protecting group for an amino group, and P^6 represents a protecting group for a carboxy group].

[0160] A compound represented by the formula (21) can be produced by using or applying the method described in Japanese Patent Laid-Open No. 2002-60351 or the literature (J. Org. Chem., Vol. 51, page 3196, 1986), and if necessary, by removing the protecting groups or modifying the functional groups. Alternatively, it can be also obtained by treating an amino acid with a protected terminal amino group or acid amide of oligopeptide with protected amino group with aldehyde or ketone.

By reacting the compound (21) with the compound (22) having a hydroxyl group

under temperature conditions ranging from under cooling to room temperature in an inert solvent in the presence of an acid or a base, the compound (23) can be produced. Here, examples of the acid which may be used can include inorganic acid such as hydrofluoric acid, hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, and boric acid; an organic acid such as acetic acid, citric acid, paratoluene sulfonic acid, and methane sulfonic acid; and a Lewis acid such as tetrafluoroborate, zinc chloride, tin chloride, aluminum chloride, and iron chloride. Among them, sulfonic acids are preferable, and paratoluene sulfonic acid is particularly preferable. As for the base, any one of the aforementioned base can be suitably selected and used. Preferred examples thereof include an alkali metal alkoxide such as potassium tert-butoxide, an alkali metal or alkaline earth metal hydroxide such as sodium hydroxide and potassium hydroxide; alkali metal hydride such as sodium hydride and potassium hydride; organometallic base represented by dialkylamino lithium such as lithium diisopropylamide; and organometallic base of bissilylamine such as lithium bis(trimethylsilyl)amide.

Examples of the solvent to be used for the reaction include an ether solvent such as tetrahydrofuran and 1,4-dioxane; and an aromatic hydrocarbon solvent such as benzene and toluene. Those solvents can be prepared as a mixture with water.

Further, the protecting group for an amino group as represented as P^5 is not particularly limited if it is a group commonly used for protection of an amino group. Representative examples can include the protecting groups for an amino group that are described in Production method 2. However, the protecting group for an amino group as drepresented as P^5 may be cleaved off within the course of the reaction. In such case, a protecting group can be re-introduced by appropriately performing a reaction with a suitable reagent for protecting an amino group as required.

The compound (24) can be derived by removing the protecting group P^6 of the compound (23). Herein, although the representative examples of the protecting group for a carboxy group as represented as P^6 are described in Production method 2, it can be appropriately selected from these examples. In the compound (23), it is desirable that the protecting group P^5 for an amino group and the protecting group P^6 for a carboxy group are the protecting groups that can be removed by a different method or different conditions. For example, a representative example can include a combination in which P^5 is a 9-fluorenylmethyloxy carbonyl group and P^6 is a benzyl group. The protecting groups can be selected depending on, e.g., the properties of a compound having an amino group and a carboxy group to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group.

The compound (26) can be produced by derivatizing the compound (24) into active

ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) or a pharmacologically acceptable salt thereof in the presence of a base to produce the compound (25) followed by removing the protecting group P^5 of the compound (25) obtained. For the reaction between the compound (4) and the carboxylic acid (24) and the reaction for removing the protecting group P^6, the same reagents and reaction conditions as those described for Production method 2 can be used.

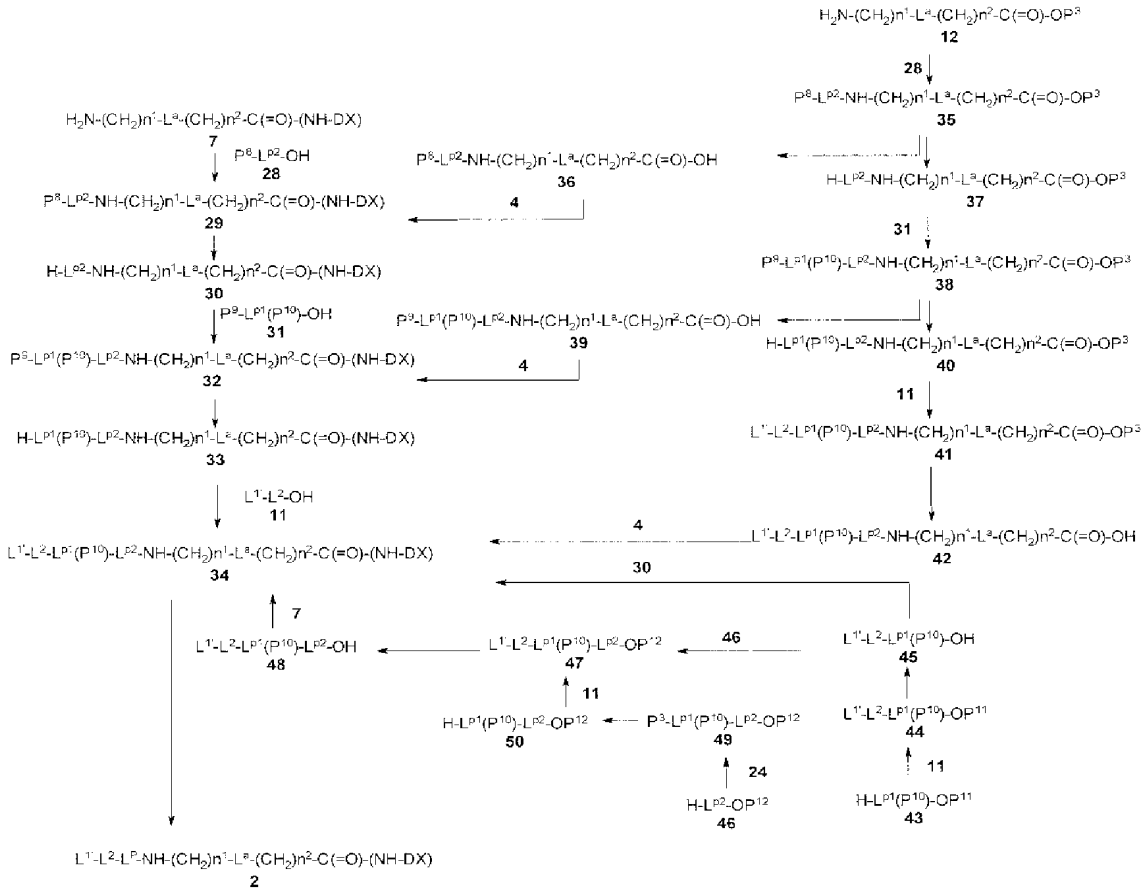
The compound (10b) can be produced by reacting the compound (26) with an amino acid with protected terminal amino group or the oligopeptide (27) with protected amino group to produce the compound (9b) and removing the protective group P^7 of the compound (9b) obtained. The protective group for an amino group as represented as P^7 is not particularly limited if it is generally used for protection of an amino group. Representative examples thereof include the protecting groups for an amino group that are described in Production method 2. For removing the protective group, reagents and conditions are selected depending on the protecting group. For the reaction between the compound (26) and the compound (27), reaction reagents and conditions that are commonly used for peptide synthesis can be employed. The compound (10b) produced by the aforementioned method can be derivatized into the compound (1) of the present invention according to the method described above.

[0161] 5. Production method 5

The compound represented by the formula (2) as an intermediate can be also produced by the method shown below.

[0162]

[Chem.27]



[0163] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is converted to a maleimidyl group, L^P represents a structure consisting of -L^{p1}-L^{p2}-, and P^3, P^8, P^9, P^{10}, P^{11}, and P^{12} represent a protecting group].

[0164] Because L^P is formed by connecting L^{p1} to L^{p2}, the hydrophilic amino acid at N terminal of L^P is derived from L^{p1}, and thus, those that having a hydrophilic amino acid at the N terminal are suitably employed as L^{p1}. Meanwhile, plural hydrophilic amino acids may be present therein. Further, when L^{p2} with hydrophilic amino acid is employed, L^P having plural hydrophilic amino acids at the N terminal of L^P or at the N terminal and at other positions can be produced depending on the location of the hydrophilic amino acid.

[0165] The compound (29) can be produced by derivatizing the peptide or amino acid (28) having the N terminal protected with P^2 into active ester, mixed acid anhydride, or the like and reacting it with the compound (7) obtained. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the peptide or amino acid (28) and the compound (7) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^8 for an amino group can be

suitably selected from those described for the protecting group of the compound (6), and the selection can be made based on the properties of the compound or the like. As it is generally used for peptide synthesis, by repeating sequentially the reaction and deprotection of the amino acid or peptide constituting the peptide or amino acid (28) for elongation, the compound (29) can be also produced.

By deprotection of P^8 as a protecting group of the amino group of the compound (29) obtained, the compound (23) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (32) can be produced by derivatizing the amino acid or peptide (31) having the N terminal protected with P^8 and the protected carboxy group, hydroxy group, or amino group in side chain protected into active ester, mixed acid anhydride, or the like and reacting it with the compound (30) obtained. The reaction conditions, reagents, base, and inert solvent used for forming a peptide bond between the amino acid or peptide (31) and the compound (30) can be suitably selected from those described for the synthesis of the compound (6). As for the protecting groups P^8 and P^9, the protecting groups can be suitably selected from those described as protecting group for an amino group, carboxy group, or hydroxy group of the compound (6). However, in such case, it is necessary that the protecting group P^9 for an amino group and the protecting group P^{10} for a functional group in side chain can be removed by a different method or different conditions. For example, a representative example includes a combination in case P^9 is a 9-fluorenylmethyloxy carbonyl group and P^{10} is a tert-butyl group or the like as a protecting group for a carboxy group, a methoxymethyl group or the like as a protecting group for a hydroxy group, or a tert-butyloxycarbonyl group or the like as a protecting group for an amino group. The protective group P^{10} for a functional group in a side chain is preferably a protecting group which can be deprotected by a treatment under acidic conditions. However, it is not limited thereto, and it can be selected from the aforementioned ones depending on, e.g., the properties of amino group, carboxy group, or a hydroxy group of a compound to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group. As it is generally used for peptide synthesis, by repeating sequentially the reaction and deprotection of the constituting amino acid or peptide for elongation, the compound (32) can be also produced.

By deprotection of P^9 as a protecting group of the terminal amino group of the compound (32) obtained, the compound (33) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

It is possible to produce the compound (34) by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (33) obtained. Herein, the carboxylic acid derivative

(11) is a compound with a structure in which the linker terminal of $L^{1'}$ has a maleimidyl group.

The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the carboxylic acid derivative (11) and the compound (33) can be suitably selected from those described for the synthesis of the compound (6).

By deprotecting the protecting group P^{10} for the carboxy group, hydroxy group, or amino group in the amino acid side chain of the peptide moiety of the compound (34) obtained, the compound (2) can be produced. Reagents and conditions can be selected depending on the protecting group.

[0166] The compound (29) can be also produced by the following method, for example.

The compound (35) can be produced by derivatizing the peptide or amino acid (28) having the N terminal protected with P^8 into active ester, mixed acid anhydride, or the like and reacting it with the amine compound (12) having the terminal carboxy group protected with P^3 in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the peptide or amino acid (28) and the compound (12) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^8 for an amino group of the compound (35) can be suitably selected and used from those described as a protecting group for the compound (6). As for the protecting group P^3 for a carboxy group, a protecting group commonly used as a protecting group for a carboxy group in organic synthetic chemistry, in particular, peptide synthesis can be used. Specific examples include alkyl ester such as methyl group, ethyl group, and tert-butyl, allyl ester, and benzyl ester, and it can be suitably selected and used from the protecting groups that are described for the compound (6). In such case, it is necessary that the protecting group P^8 for an amino group and the protecting group P^3 for a carboxy group can be removed by a different method or different conditions. For example, a representative example includes a combination in which P^8 is a tert-butyloxy carbonyl group and P^3 is a benzyl group. The protecting groups can be selected from the aforementioned ones depending on, e.g., the properties of a compound having an amino group and a carboxy group to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group.

By deprotecting the protecting group P^3 for the carboxy group of the compound (35) obtained, the compound (36) can be produced. In this deprotection, reagents and conditions are selected depending on the protecting group.

The compound (29) can be produced by derivatizing the compound (36) obtained into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the

reaction conditions, reagents, base, and solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0167] The compound (32) can be also produced by the following method, for example.

By deprotecting the protecting group P^8 for the amino group of the compound (35), the compound (37) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (38) can be produced by derivatizing the amino acid or peptide (31) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (37) obtained in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the amino acid or peptide (31) and the compound (37) can be suitably selected from those described for the synthesis of the compound (6). In such case, it is necessary that the protecting group P^9 and P^{10} for the amino acid or peptide (31) and the protecting group P^3 for the compound (37) can be removed by a different method or different conditions. For example, a representative example includes a combination in which P^9 is a 9-fluorenylmethyloxy carbonyl group, P^{10} is a tert-butyloxy carbonyl group, tert-butyl group, or a methoxymethyl group, and P^3 is a benzyl group. Further, the protective group P^{10} for a functional group in a side chain is preferably a protecting group which can be deprotected by a treatment under acidic conditions as described above. However, it is not limited thereto, and it can be selected from the aforementioned ones depending on, e.g., the properties of amino group, carboxy group, or a hydroxy group of a compound to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group.

By deprotecting the protecting group P^3 for the carboxy group of the compound (38) obtained, the compound (39) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (32) can be produced by derivatizing the compound (39) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0168] The compound (34) can be also produced by the following method, for example.

By deprotecting the protecting group P^9 for the amino group of the compound (38), the compound (40) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (41) can be produced by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with

the compound (40) obtained in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the carboxylic acid derivative (11) and the compound (40) can be suitably selected from those described for the synthesis of the compound (6).

By deprotecting the protecting group P^3 for the carboxy group of the compound (41) obtained, the compound (42) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (34) can be produced by derivatizing the compound (42) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

[0169] The compound (34) can be also produced by the following method, for example.

The compound (44) can be produced by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the amino acid or peptide (43) having the carboxy group protected with P^{11} and the carboxy group, hydroxy group, or amino group in side chain protected with P^{10} in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the carboxylic acid derivative (11) and the compound (43) can be suitably selected from those described for the synthesis of the compound (6). As for the protecting groups P^{10} and P^{11} of the compound (44), the protecting groups can be suitably selected from those described as protecting group for a carboxy group, hydroxy group, or amino group of the compound (6). Meanwhile, in such case, it is necessary that the protecting group P^{11} for a carboxy group and the protecting group P^{10} for a functional group in side chain can be removed by a different method or different conditions. For example, a representative example includes a combination in which P^{11} is a benzyl group and P^{10} is a tert-butyl group or the like as a protecting group for a carboxy group, a methoxymethyl group or the like as a protecting group for a hydroxy group, or a tert-butyloxycarbonyl group or the like as a protecting group for an amino group. The protective group P^{10} for a functional group in a side chain is preferably a protecting group which can be deprotected by a treatment under acidic conditions. However, it is not limited thereto, and it can be selected from the aforementioned ones depending on, e.g., the properties of amino group, carboxy group, or a hydroxy group of a compound to be protected. For removing the protecting group, the reagents and conditions can be selected depending on the protecting group.

By deprotecting the protecting group P^{11} for the carboxy group of the compound (44) obtained, the compound (45) can be produced. In this deprotection, reagents and

conditions can be selected depending on the protecting group.

The compound (34) can be produced by derivatizing the compound (45) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (30) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and solvent used for the reaction can be suitably selected from those described for the synthesis of the compound (6).

The compound (47) can be produced by derivatizing the compound (45) into active ester, mixed acid anhydride, acid halide or the like and reacting it with the amino acid or peptide (46) having the carboxy group protected with P^{12} in the presence of a base. For the reaction, the reaction reagents and conditions commonly used for peptide synthesis can be used and the reaction conditions, reagents, base, and solvent can be suitably selected from those described for the synthesis of the compound (6). As for the protecting groups P^{10} and P^{12} of the compound (47), the protecting groups can be suitably selected and used from those described as protecting group for a carboxy group, hydroxy group, or amino group of the compound (6). Meanwhile, in such case, it is necessary that the protecting group P^{12} for a carboxy group and the protecting group P^{10} for a functional group in side chain can be removed by a different method or different conditions. For example, a representative example includes a combination in which P^{12} is a benzyl group and P^{10} is a tert-butyl group or the like as a protecting group for a carboxy group, a methoxymethyl group or the like as a protecting group for a hydroxy group, or a tert-butyloxycarbonyl group or the like as a protecting group for an amino group. The protective group P^{10} for a functional group in a side chain is preferably a protecting group which can be deprotected by a treatment under acidic conditions. However, it is not limited thereto, and it can be selected from the aforementioned ones depending on, e.g., the properties of amino group, carboxy group, or a hydroxy group of a compound to be protected. For removing the protecting group, the reagents and conditions can be selected depending on the protecting group. Further, the compound (47) can be also produced by repeating sequentially the reaction and deprotection of constituting amino acid or peptide for elongation.

By deprotecting the protecting group P^{12} for the carboxy group of the compound (47) obtained, the compound (48) can be produced. Reagents and conditions can be selected depending on the protecting group.

The compound (34) can be produced by derivatizing the compound (48) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (7) in the presence of a base. For the reaction, reaction reagents and conditions that are generally used for peptide synthesis can be also used, and the reaction conditions, reagents, base, and solvent used for the reaction can be suitably selected from those

described for the synthesis of the compound (6).

The compound (47) can be also produced by the following method, for example.

The peptide (49) can be produced by derivatizing the amino acid or peptide (46) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the amino acid or peptide (31) having the N terminal protected with P^9 and the carboxy group, hydroxy group, or amino group in side chain protected with P^{10} in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the amino acid or peptide (46) and the amino acid or peptide (31) can be suitably selected from those described for the synthesis of the compound (6). Meanwhile, in this case, it is necessary that the protecting group P^{12} for a carboxy group of the amino acid or peptide (46) and the protecting group P^9 and P^{10} for the amino acid or peptide (31) can be removed in the same manner as described above but by a different method or different conditions. For example, a representative example includes a combination in which P^9 is a 9-fluorenylmethyloxy carbonyl group, P^{10} is a tert-butyl group or the like as a protecting group for a carboxy group, a methoxymethyl group or the like as a protecting group for a hydroxy group, or a tert-butyloxycarbonyl group as a protecting group or the like for an amino group, and P^{12} is a benzyl group. The protective group P^{10} for a functional group in a side chain is preferably a protecting group which can be deprotected by a treatment under acidic conditions. However, it is not limited thereto, and it can be selected from the aforementioned ones depending on, e.g., the properties of amino group, carboxy group, or a hydroxy group of a compound to be protected. For removing the protecting group, the reagents and conditions can be selected depending on the protecting group.

By deprotecting the protecting group P^9 for the N terminal of the peptide (49) obtained, the compound (50) can be produced. Reagents and conditions can be selected depending on the protecting group.

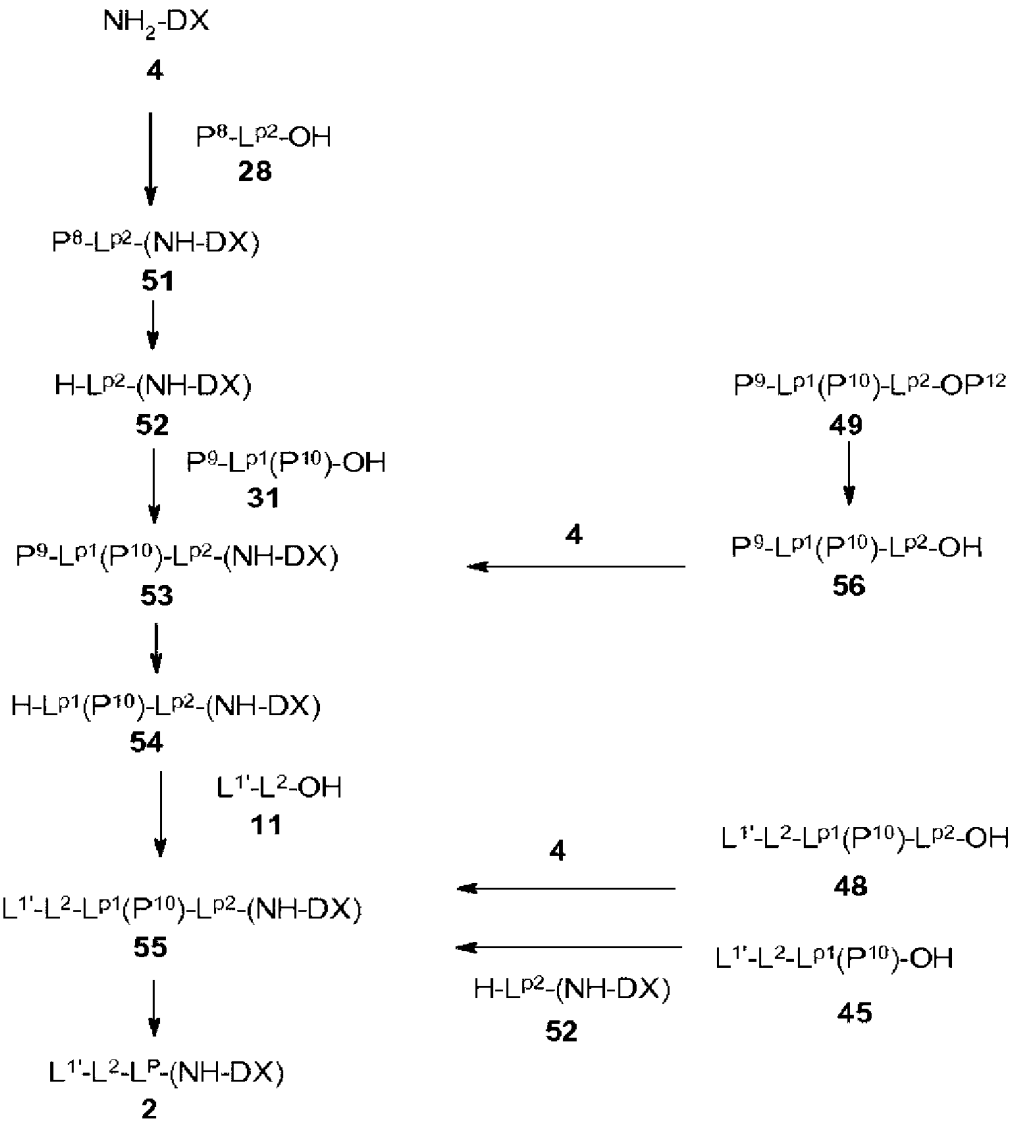
The compound (47) can be produced by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the peptide (50) obtained in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the carboxylic acid derivative (11) and the peptide (50) can be suitably selected from those described for the synthesis of the compound (6).

[0170] 6. Production method 6

Within the production intermediate (2), those inwhich the linker has a structure represented by $-L^1-L^2-L^P-$, and said L^P is the peptide residue containing a hydrophilic amino acid at the N terminal and said hydrophilic amino acid located at the N terminal is other than glycine, can be also produced by the following method.

[0171]

[Chem.28]



[0172] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is modified to maleimidyl group, L^P represents a structure consisting of -L^{p1}-L^{p2}-, and P^8, P^9, P^{10}, and P^{12} represent a protecting group].

[0173] Because L^P is formed by connecting L^{p1} to L^{p2}, the hydrophilic amino acid at N terminal of L^P is derived from L^{p1}, and thus, those that having a hydrophilic amino acid at the N terminal are suitably employed as L^{p1}. Meanwhile, plural hydrophilic amino acids may present therein. Further, when L^{p2} with hydrophilic amino acid is employed, L^P having plural hydrophilic amino acids at the N terminal of L^P or at the N terminal and at other positions can be produced depending on its location of hydrophilic amino acid.

The compound (51) can be produced by derivatizing the peptide or amino acid (28)

described in Production method 5, which has the N terminal protected with P^8, into active ester, mixed acid anhydride, or the like, and reacting with the compound (4) and a salt thereof. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the peptide or amino acid (28) and the compound (4) can be suitably selected from those described for the synthesis of the compound (6). The protective group P^8 can be suitably selected and used from those described as the protecting group for the compound (6), and it can be selected depending on, e.g., a property of the compound having an amino group to be protected. Further, as it is generally used for peptide synthesis, by repeating sequentially the reaction and de-protection of the amino acid or peptide constituting the peptide or amino acid (28) for elongation, the compound (51) can be also produced.

By deprotecting the protecting group P^8 for the amino group of the compound (51) obtained, the compound (52) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (53) can be produced by derivatizing the amino acid or peptide (31) having the N terminal protected with P^9 and the carboxy group, hydroxy group, or amino group in side chain protected with P^{10} as described in Production method 4 into active ester, mixed acid anhydride, or the like and reacting it with the compound (52) obtained. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the amino acid or peptide (31) and the compound (52) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^9 and P^{10} are the same as those described in Production method 5. Further, as it is generally used for peptide synthesis, by repeating sequentially the reaction and deprotection of the constituting amino acid or peptide for elongation, the compound (53) can be also produced.

By deprotection of P^9 as the protecting group of the amino group of the compound (53) obtained, the compound (54) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

It is possible to produce the compound (55) by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (54) obtained. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the carboxylic acid derivative (11) and the compound (54) can be suitably selected from those described for the synthesis of the compound (6).

By deprotecting the protecting group P^{10} for the carboxy group, hydroxy group, or amino group of the compound (55) obtained, the compound (2) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

[0174] The compound (53) can be also produced by the following method, for example.

By deprotecting the protecting group P^{12} for the carboxy group of the compound (49) described in Production method 5, the peptide (56) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (53) can be produced by derivatizing the peptide (56) obtained into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (4) or a salt thereof. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the compound (56) and the compound (4) can be suitably selected from those described for the synthesis of the compound (6).

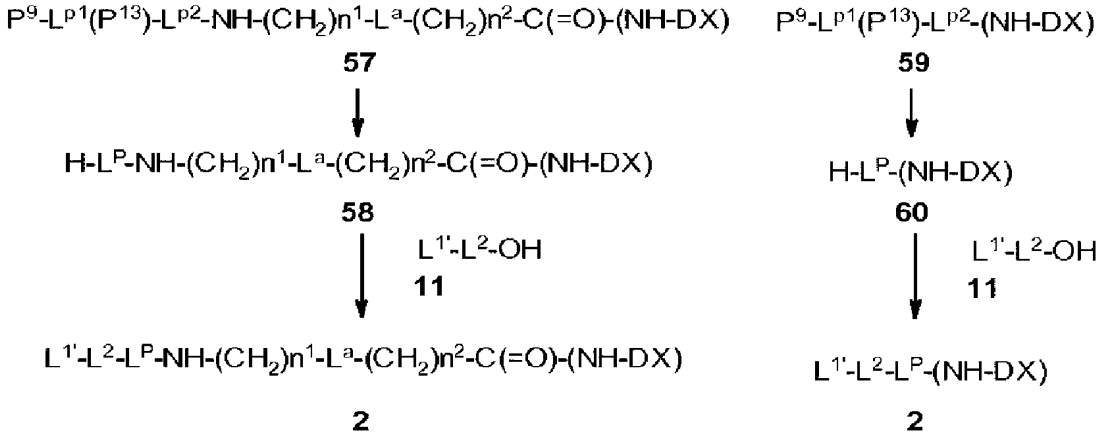
[0175] The compound (55) can be also produced by the following method, for example.

The compound (55) can be produced by derivatizing the compound (48) described in Production method 5 into active ester, mixed acid anhydride, or the like, and reacting it with the compound (4) in the presence of a base, or derivatizing the amino acid or peptide (45) described in Production method 5 into active ester, mixed acid anhydride, or the like, and reacting it with the compound (52) in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming each peptide bond can be suitably selected from those described for the synthesis of the compound (6).

[0176] 7. Production method 7

Within the production intermediate represented by the formula (2), those having the linker structure of $-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-NH-(CH_2)n^2-C(=O)-$, and said L^P is the peptide residue having a hydrophilic amino acid at the N terminal, and said hydrophilic amino acid located at N terminal is other than glycine can be also produced by the following method.

[0177] [Chem.29]



[0178] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is modified to maleimidyl group, L^P represents a structure consisting of $-L^{p1}-L^{p2}-$, and P^9 and P^{13} represent a protecting group].

[0179] The production intermediate represented by the formula (2) includes the following two modes, that is, a structure in which the linker is represented by -L^1-L^2-L^P-NH-(CH_2)n^1-L^a-NH-(CH_2)n^2-C(=O)- and a structure in which the linker is represented by -L^1-L^2-L^P-.

The compound (2) with a structure in which the linker is represented by -L^1-L^2-L^P-NH-(CH_2)n^1-L^a-NH-(CH_2)n^2-C(=O)- can be produced as follows.

The compound (57) can be synthesized in the same manner as the compound (32) described in Production method 5. However, unlike the compound (32), it is not necessary that the protecting group P^9 for the amino group and the protecting group P^{13} for the functional group in side chain can be removed by a different method or different conditions. The functional group in side chain is a carboxy group or a hydroxy group, and the protecting group P^9 for the amino group and the protecting group P^{13} for the carboxy group or hydroxy group in side chain can be simultaneously deprotected. For example, a representative example includes a combination in which P^9 is a tert-butyloxy carbonyl group and P^{13} is a tert-butyl group or a trityl group, or P^3 is a benzyloxy carbonyl group and P^{13} is a benzyl group. The protecting groups can be suitably selected from the aforementioned ones with regard to the protecting groups for the compound (6) depending on, e.g., the properties of an amino group, a carboxy group, or a hydroxy group of the compound to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group. By using the protected amino acid or peptide satisfying above properties, the compound (57) can be synthesized in the same manner as Production method 5.

By sequential or simultaneous deprotection of the protecting group P^9 and P^{13} of the compound (57), the compound (51) can be produced. Reagents and conditions can be selected depending on the protecting group.

A functional group in hydrophilic side chain of L^P in the compound (58) is not particularly protected, however, by reaction with the compound (11) derivatized into active ester, mixed acid anhydride, or the like in the presence of a base, the compound (2) can be produced. The reaction conditions, reagents, base, and solvent used for forming each peptide bond can be suitably selected from those described for the synthesis of the compound (6).

The compound (2) with a structure in which the linker is represented by -L^1-L^2-L^P- can be produced as follows.

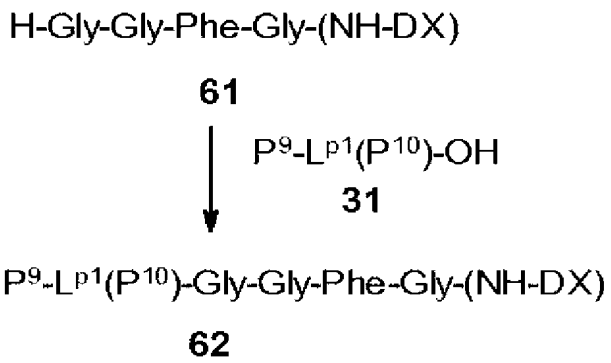
The compound (59) can be also synthesized in the same manner as the compound (53) described in Production method 6. However, unlike the compound (53), it may not be necessary that the protecting group P^3 for the amino group and the protecting group P^8 for the functional group in side chain can be removed by a different method or different conditions. The functional group in side chain is a carboxy group or a

hydroxy group, and the protecting group P^9 for the amino group and the protecting group P^{13} for the carboxy group or hydroxy group in side chain can be simultaneously deprotected. For example, a representative example includes a combination in which P^9 is a tert-butyloxy carbonyl group and P^{13} is a tert-butyl group or a trityl group, or P^3 is a benzyloxy carbonyl group and P^{13} is a benzyl group. The protecting groups can be suitably selected from the aforementioned ones with regard to the protecting groups for the compound (6) depending on, e.g., the properties of an amino group, a carboxy group, or a hydroxy group of the compound to be protected. For removal of the protecting groups, reagents and conditions are selected depending on the protecting group. By using the protected amino acid or peptide satisfying above properties, the compound (59) can be synthesized in the same manner as Production method 6.
By sequential or simultaneous deprotection of the protecting group P^9 and P^{13} of the compound (59), the compound (53) can be produced. Reagents and conditions can be selected depending on the protecting group.
A functional group in hydrophilic side chain of L^p in the compound (60) is not particularly protected. However, by reaction with the compound (11) derivatized into active ester, mixed acid anhydride, or the like in the presence of a base, the compound (2) can be produced. The reaction conditions, reagents, base, and solvent used for forming each peptide bond can be suitably selected from those described for the synthesis of the compound (6).

[0180] 8. Production method 8

The compound (43) shown in Production method 5 in which the linker -L^p- has a structure of -L^{p1}-Gly-Gly-Phe-Gly- can be also produced by the following method.

[0181] [Chem.30]



[0182] [in the formula, P^9 and P^{10} represent a protecting group].

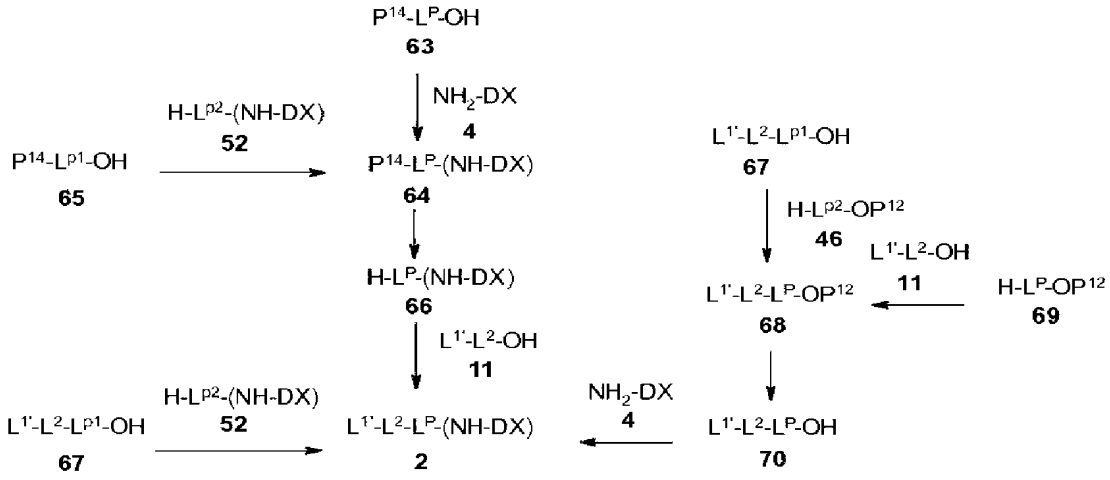
[0183] The compound (62) can be produced by derivatizing the amino acid or peptide (31) described in Production method 5 into active ester, mixed acid anhydride, acid halide, or the like and reacting it with glycylglycyl-L-phenylalanyl-N-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10

,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]glycinamide (that is, free form of the pharmaceutical compound disclosed in International Publication No. WO 1997/46260) (61) or a salt thereof in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the amino acid or peptide (31) and the compound (61) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^3 for N terminal and the protecting group P^{10} for the functional group in side chain are the same as those described in Production method 5. Meanwhile, the protecting group P^{10} for the functional group in side chain may not be present, and by performing the reaction using the amino acid or peptide (31) with N terminal protected only, the compound (62) can be obtained.

[0184] 9. Production method 9

Among the compounds represented by the formula (2), a compound, in which the linker has a structure represented by $-L^1-L^2-L^P-$, and said L^P is the oligopeptide in which the C terminal is composed of 2 or 3 or more glycines and is connected to a drug, and the N terminal of said peptide residue is glycine in case a hydrophilic amino acid is present at N terminal, can be also produced according to the following method.

[0185] [Chem.31]



[0186] [in the formula, $L^{1'}$ corresponds to L^1 having a structure in which the terminal is converted to maleimidyl group, L^P represents a structure consisting of L^{p1}-L^{p2}, and P^{12} and P^{14} represent a protecting group].

[0187] Because L^P is formed by connecting L^{p1} to L^{p2}, the number of glycines for constituting the C terminal of L^P contained therein can be designed in consideration of the number of glycines at C terminal in L^P and the number of repeated use thereof during the reaction.

The peptide (63) is an oligopeptide in which the C terminal is composed of 2 or 3 or

more glycines, and the N terminal is glycine in case the N terminal of said peptide residue is a hydrophilic amino acid, and further, said N terminal is protected with P^{14}.
As commonly employed for peptide synthesis, the peptide (63) can be synthesized by repeating sequentially the condensation reaction of the constituting amino acid or peptide and deprotection.
The compound (64) can be produced by derivatizing the peptide (63) into active ester, mixed acid anhydride, or the like and reacting it with the compound (4) or a salt thereof. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between peptide (63) and the compound (4) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^{14} can be suitably selected and used from those described for synthesis of the compound (6).
The compound (64) can be also produced by derivatizing the amino acid or peptide (65) with the N terminal protected with P^{14} into active ester, mixed acid anhydride, or the like and reacting it with the compound (52) described in Production method 6. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between amino acid or peptide (65) and the compound (52) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^{14} can be suitably selected and used from those described for synthesis of the compound (6).
By deprotecting the protecting group P^{14} for the amino group of the compound (64) obtained, the compound (66) can be produced. Reagents and conditions can be selected depending on the protecting group.
The compound (2) can be produced by derivatizing the carboxylic acid derivative (11) into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (66) obtained. The reaction conditions, reagents, base, and solvent used for forming an amide bond between the carboxylic acid derivative (11) and the compound (66) can be suitably selected from those described for the synthesis of the compound (6).
The compound (2) can be also produced by the following method.
In the compound (67), of which glycine at N terminal of L^{p1} is connected to L^2, and it can be produced in the same manner as the compound (45) described in Production method 5. The compound (68) can be produced by derivatizing the amino acid or peptide (46) described in Production method 5 into active ester, mixed acid anhydride, acid halide, or the like and reacting it with the compound (67). Herein, the amino acid or peptide (46) is an oligopeptide consisting of glycine or having C terminal consisting of 2 or 3 or more glycines, in which the C terminal is protected with P^{12}. The reaction conditions, reagents, base, and solvent used for forming an amide bond between amino acid or peptide (46) and the compound (67) can be suitably selected from those

described for the synthesis of the compound (6).

The compound (68) can be also produced by derivatizing the compound (11) into active ester, mixed acid anhydride, or the like and reacting it with the peptide (69) having the C terminal protected with P^{12}. Herein, the peptide (69) is an oligopeptide in which the C terminal is composed of 2 or 3 or more glycines and the N terminal is glycine in case the N terminal of said peptide residue is a hydrophilic amino acid. As commonly employed for peptide synthesis, the peptide (69) can be produced by repeating sequentially the condensation reaction of the constituting amino acid or peptide and deprotection. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the peptide (69) and the compound (11) can be suitably selected from those described for the synthesis of the compound (6). The protecting group P^{12} is preferably a protecting group which can be deprotected under acidic conditions but it is not limited thereto, and can be suitably selected and used from those described for synthesis of the compound (6).

By deprotecting the protecting group P^{12} for the carboxy group of the compound (68) obtained, the compound (70) can be produced. In this deprotection, reagents and conditions can be selected depending on the protecting group.

The compound (2) can be produced by derivatizing the compound (70) into active ester, mixed acid anhydride, or the like and reacting it with the compound (4) or a salt thereof. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the compound (70) and the compound (4) can be suitably selected from those described for the synthesis of the compound (6).

In addition to above, the compound (2) can be also produced according to the following method.

The compound (2) can be produced by derivatizing the compound (52) described in Production method 6 into active ester, mixed acid anhydride, or the like and reacting it with the compound (67) in the presence of a base. The reaction conditions, reagents, base, and solvent used for forming a peptide bond between the compound (67) and the compound (52) can be suitably selected from those described for the synthesis of the compound (6).

[0188] Meanwhile, it is also possible that all of the intermediate compounds of Production method 1 to Production method 9 may be present as in form of salt and/or hydrate.

[0189] The antibody-drug conjugate of the present invention may absorb moisture to have adsorption water, for example, or turn into a hydrate when it is left in air or subjected to purification procedures such as recrystallization. Such a compound or a salt containing water are also included in the present invention.

A compound labeled with various radioactive or non-radioactive isotopes is also included in the present invention. One or more atoms constituting the antibody-drug

conjugate of the present invention may contain an atomic isotope at non-natural ratio. Examples of the atomic isotope can include deuterium (^{2}H), tritium (^{3}H), iodine-125 (^{125}I), and carbon-14 (^{14}C). Further, the compound of the present invention may be radioactive-labeled with a radioactive isotope such as tritium (^{3}H), iodine-125 (^{125}I), carbon-14 (^{14}C), copper-64 (^{64}Cu), zirconium-89 (^{89}Zr), iodine-124 (^{124}I), fluorine-18 (^{18}F), indium-111 (^{111}I), carbon-11 (^{11}C), or iodine-131 (^{131}I). The compound labeled with a radioactive isotope is useful as a therapeutic or prophylactic agent, a reagent for research such as an assay reagent and an agent for diagnosis such as an in vivo diagnostic imaging agent. Without being related to radioactivity, any isotope variant type of the antibody-drug conjugate of the present invention is within the scope of the present invention.

[0190] {Pharmaceuticals/Medicines}

The anti-HER3 antibody-drug conjugate of the present invention exhibits a cytotoxic activity against cancer cells, and thus, as a medicine, it can be particularly used as a therapeutic agent and/or prophylactic agent for cancer.

Specifically, the anti-HER3 antibody-drug conjugate of the present invention can be selectively used as a medicine for chemotherapy, which is a major method for treating cancer, and as a result, can delay development of cancer cells, inhibit growth thereof, and further destroy the cancer cells. This can allow cancer patients to be free from symptoms caused by cancer or achieve improvement in QOL of cancer patients and attains a therapeutic effect by sustaining the lives of the cancer patients. Even if the anti-HER3 antibody-drug conjugate of the present invention does not reach to destroying cancer cells, it can achieve higher QOL of cancer patients while achieving long-term survival, by inhibiting or controlling the growth of cancer cells.

The anti-HER3 antibody-drug conjugate of the present invention can be used as a medicine alone in such medicinal therapy. In addition, the anti-HER3 antibody-drug conjugate of the present invention can be used as a medicine in combination with an additional therapy in adjuvant therapy and can be combined with surgical operation, radiotherapy, hormone therapy, or the like. Furthermore, the anti-HER3 antibody-drug conjugate of the present invention can also be used as a medicine for drug therapy in neoadjuvant therapy.

In addition to the therapeutic use as described above, the effect of suppressing the growth of tiny metastatic cancer cells and further destroying them can also be expected. Particularly, when the expression of HER3 is confirmed in primary cancer cells, inhibition of cancer metastasis or a prophylactic effect can be expected by administering the anti-HER3 antibody-drug conjugate of the present invention. For example, the effect of inhibiting and destroying cancer cells in a body fluid in the course of metastasis or the effect of, for example, inhibiting and destroying tiny cancer

cells immediately after implantation in any tissue can be expected. Accordingly, inhibition of cancer metastasis or a prophylactic effect can be expected, particularly, after surgical removal of cancer.

The anti-HER3 antibody-drug conjugate of the present invention can be expected to produce a therapeutic effect by administration as systemic therapy to patients as well as by local administration to cancer tissues.

The antibody-drug conjugate (1) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, and anti-melanoma effects in vitro.

The antibody-drug conjugate (2) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-colorectal cancer, and anti-melanoma effects in vitro and stronger anti-breast cancer and anti-melanoma effects in vivo than those of U1-59.

The antibody-drug conjugate (3) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-ovarian, anti-colorectal cancer, and anti-melanoma effects in vitro and stronger anti-breast cancer, anti-lung cancer, anti-stomach cancer, and anti-melanoma effects in vivo than those of U1-59.

The antibody-drug conjugate (4) had excellent antitumor activity, safety, and physical properties and exhibited an anti-breast cancer effect in vitro.

The antibody-drug conjugate (5) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, and anti-melanoma effects in vitro.

The antibody-drug conjugate (6) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, and anti-melanoma effects in vitro and a stronger anti-breast cancer effect in vivo than that of U1-59.

The antibody-drug conjugate (7) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, and anti-melanoma effects in vitro.

The antibody-drug conjugate (8) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, and anti-melanoma effects in vitro and a stronger anti-breast cancer effect in vivo than that of U1-59.

The antibody-drug conjugate (9) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-ovarian cancer, anti-colorectal cancer, and anti-melanoma effects in vitro.

The antibody-drug conjugate (10) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-colorectal cancer, and anti-melanoma effects in vitro and stronger anti-breast cancer, anti-lung cancer,

anti-colorectal cancer, anti-stomach cancer, and anti-melanoma effects in vivo than those of U1-59.

The antibody-drug conjugate (11) had excellent antitumor activity, safety, and physical properties and exhibited an anti-breast cancer effect in vitro.

The antibody-drug conjugate (12) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-ovarian cancer, anti-colorectal cancer, and anti-melanoma effects in vitro.

The antibody-drug conjugate (13) had excellent antitumor activity, safety, and physical properties and exhibited anti-breast cancer, anti-lung cancer, anti-colorectal cancer, and anti-melanoma effects in vitro and stronger anti-breast cancer (including triple-negative breast cancer), anti-lung cancer, anti-stomach cancer, anti-pancreatic cancer, and anti-melanoma effects in vivo than those of U1-59.

The antibody-drug conjugate (14) had excellent antitumor activity, safety, and physical properties and exhibited an anti-breast cancer effect in vitro.

The antibody-drug conjugate (16a) had excellent antitumor activity, safety, and physical properties and exhibited an anti-breast cancer (including luminal and triple negative), anti-melanoma, anti-ovarian cancer, anti-bladder cancer, anti-lung cancer, anti-head and neck cancer, and anti-gastric cancer effects in vivo when it was administered alone or in combination with trastuzumab, gefinitib, cetuximab, panitumumab or pertuzumab.

[0191] Examples of the cancer type to which the anti-HER3 antibody-drug conjugate of the present invention is applied include lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, metastatic breast cancer, luminal breast cancer, melanoma, liver cancer, bladder cancer, gastric (stomach) cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, penis cancer. Chemotherapy is the only current treatment indicated for particularly triple negative breast cancer (that lacks the expression of HER2, estrogen, and progesteron receptors) among breast cancers, which is said to have a poor prognosis. There have been almost no reports of HER3 expression in triple negative breast cancer. However if HER3 expression is observed in patients with triple negative breast cancer, then the anti-HER3 antibody-drug conjugate of the present invention can be used as a therapeutic agent and/or a preventive agent. However, it is not limited to them as long as it is a cancer cell expressing, in a cancer cell as a treatment subject, a protein which the antibody of the antibody-drug conjugate can

recognize.

The treatment using the anti-HER3 antibody-drug conjugate of the present invention can target a cancer cell expressing, in a cancer cell as a treatment subject, HER3 protein which the antibody of the antibody-drug conjugate can recognize. In the present specification, the "cancer expressing HER3 protein" is cancer comprising cells having HER3 protein on their surface or cancer secreting HER3 protein into blood. The HER3 protein is overexpressed in various human tumors and can be evaluated using a method usually carried out, such as immunohistochemical staining method (IHC) for evaluating the overexpression of the HER3 protein in tumor (primary, metastatic) specimens, fluorescence in situ hybridization method (FISH) for evaluating the amplification of the HER3 gene, or enzyme-linked immunosorbent assay (ELISA) for evaluating the overexpression of the HER3 protein in blood specimens.

The anti-HER3 antibody-drug conjugate of the present invention exhibits an antitumor effect by recognizing and further internalizing HER3 protein expressed on cancer cell surface. Thus, the treatment subject of the anti-HER3 antibody-drug conjugate of the present invention is not limited to the "cancer expressing HER3 protein" and can also be, for example, leukemia, malignant lymphoma, plasmacytoma, myeloma, or sarcoma.

[0192] The anti-HER3 antibody-drug conjugate of the present invention can be preferably administered to a mammal, but it is more preferably administered to a human.

[0193] Substances used in a pharmaceutical composition comprising the anti-HER3 antibody-drug conjugate of the present invention can be suitably selected and applied from formulation additives or the like that are generally used in the art, in view of the dosage or administration concentration.

[0194] The anti-HER3 antibody-drug conjugate of the present invention can be administered as a pharmaceutical composition comprising at least one pharmaceutically compatible ingredient. For example, the pharmaceutical composition typically comprises at least one pharmaceutical carrier (for example, sterilized liquid). As described herein, examples of the liquid include water and oil (petroleum oil and oil of animal origin, plant origin, or synthetic origin). The oil may be, for example, peanut oil, soybean oil, mineral oil, sesame oil or the like. Water is a more typical carrier when the pharmaceutical composition is intravenously administered. Saline solution, an aqueous dextrose solution, and an aqueous glycerol solution can be also used as a liquid carrier, in particular, for an injection solution. A suitable pharmaceutical vehicle can be appropriately selected from those known in the art. If desired, the composition may also comprise a trace amount of a moisturizing agent, an emulsifying agent, or a pH buffering agent. Examples of suitable pharmaceutical carrier are disclosed in "Remington's Pharmaceutical Sciences" by E. W. Martin. The formulations correspond

to an administration mode.

[0195] Various delivery systems are known and they can be used for administering the anti-HER3 antibody-drug conjugate of the present invention. Examples of the administration route can include intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes, but not limited thereto. The administration can be made by injection or bolus injection, for example. According to a specific preferred embodiment, the administration of the ligand- drug conjugate is performed by injection. Parenteral administration is a preferred administration route.

[0196] According to a representative embodiment, the pharmaceutical composition is prescribed, as a pharmaceutical composition suitable for intravenous administration to human, according to the conventional procedures. The composition for intravenous administration is typically a solution in a sterile and isotonic aqueous buffer solution. If necessary, the drug may contain a solubilizing agent and local anesthetics to alleviate pain at injection area (for example, lignocaine). Generally, the ingredient is provided individually as any one of lyophilized powder or an anhydrous concentrate contained in a container which is obtained by sealing in an ampoule or a sachet having an amount of the active agent or as a mixture in a unit dosage form. When the pharmaceutical is the form to be administered by injection, it may be administered from an injection bottle containing water or saline of sterile pharmaceutical grade. When the pharmaceutical is administered by injection, an ampoule of sterile water or saline for injection may be provided such that the aforementioned ingredients are admixed with each other before administration.

[0197] The pharmaceutical composition of the present invention may comprise only the anti-HER3 antibody-drug conjugate of the present application as an active ingredient or may comprise the anti-HER3 antibody-drug conjugate and at least one medicine (e.g., cancer-treating agent) other than the conjugate. The anti-HER3 antibody-drug conjugate of the present invention can be administered with another cancer-treating agent, and the anti-cancer effect may be enhanced accordingly. For example, another medicine such as an anti-cancer agent used for such purpose may be administered before administration of the pharmaceutical composition comprising the anti-HER3 antibody-drug conjugate of the present invention as an active ingredient or after administration of the pharmaceutical composition comprising the anti-HER3 antibody-drug conjugate as an active ingredient, or may be administered simultaneously with, separately (individually) from, or subsequently to the antibody-drug conjugate, and it may be administered while varying the administration interval for each. In the present invention, the case where the anti-HER3 antibody-drug conjugate of the present invention is administered simultaneously with another medicine as a single formulation containing the antibody-drug conjugate and the medicine and the case where the anti-

HER3 antibody-drug conjugate of the present invention and another medicine are administered simultaneously or subsequently as separate formulations or administered while varying the administration interval for each are both included in the scope of the "pharmaceutical composition comprising the antibody-drug conjugate and another medicine". Examples of the cancer-treating agent include 5-FU, trastuzumab, trastuzumabb emtansine (T-DM1), cetuximab, gefitinib, panitumumab, pertuzumab, abraxane, erlotinib, carboplatin, cisplatin, gemcitabine, capecitabine, irinotecan (CPT-11), paclitaxel, docetaxel, pemetrexed, sorafenib, vinblastine, vinorelbine, vermurafenib, medicines described in International Publication No. WO 2003/038043, LH-RH analogues (leuprorelin, goserelin, or the like), estramustine phosphate, estrogen antagonist (tamoxifen, raloxifene, or the like), and an aromatase inhibitor (anastrozole, letrozole, exemestane, or the like), but it is not limited as long as it is a medicine having an antitumor activity. These cancer-treating agents can be classified, according to their targets, into: anti-FGER agents such as cetuximab, gefitinib, and panitumumab; anti-HER2 agents such as trastuzumab, T-DM1, and pertuzumab; anti-HER3 agents such as patritumab, MM-121, and MM-111; anti-VEGF agents such as infliximab and adalimumab; etc. Further, they can be classified into: anti-EGFR antibodies such as cetuximab and panitumumab; anti-HER2 antibodies such as trastuzumab and pertuzumab; anti-HER3 antibodies such as patritumab, MM-121, and MM-111; anti-VEGF antibodies such as infliximab and adalimumab; etc. The anti-HER3 antibody-drug conjugate of the present invention exerts an excellent therapeutic effect when administered in combination with an anti-HER2 agent or an anti-HER2 antibody in i) the treatment of stomach cancer, breast cancer, triple-negative breast cancer, or the like or when administered in combination with an anti-EGFR agent or an anti-EGFR antibody in ii) the treatment of lung cancer, head and neck cancer, stomach cancer, breast cancer, triple-negative breast cancer, or the like. One or two or more medicines other than the conjugate can be used, and these medicines may be anticancer agents or may be medicines for alleviating side effect caused by companion medicines.

[0198] In the present invention, the "pharmaceutical composition comprising the anti-HER3 antibody-drug conjugate and another medicine" has the same meaning as a "pharmaceutical composition in which the anti-HER3 antibody-drug conjugate is to be administered in combination with another medicine". In the present invention, the phrase "administered in combination" used for the anti-HER3 antibody-drug conjugate and another medicine means that the anti-HER3 antibody-drug conjugate and another medicine are incorporated in the body of a recipient within a certain period. A single formulation containing the anti-HER3 antibody-drug conjugate and another medicine may be administered, or the anti-HER3 antibody-drug conjugate and another medicine

may be separately formulated and administered as separate formulations. In the case of the separate formulations, the timings of administration thereof are not particularly limited, and the formulations may be administered at the same time or may be administered at different times or different days in a staggered manner. In the case where the anti-HER3 antibody-drug conjugate and another medicine are separately administered at different times or different days, the order of administration thereof is not particularly limited. Since separate formulations are usually administered according to their respective administration methods, the frequency of administration thereof may be the same or may be different. Further, such separate formulations may be administered by the same administration method (administration route) or different administration methods (administration routes). It is not necessary that the anti-HER3 antibody-drug conjugate and another medicine exist in the body at the same time, and it is sufficient that the anti-HER3 antibody-drug conjugate and another medicine are incorporated in the body within a certain period (e.g., 1 month, preferably 1 week, more preferably several days, even more preferably 1 day). Alternatively, when one of the active ingredients is administered, the other active ingredient may have already disappeared from the body.

Examples of the dosage form of the "pharmaceutical composition in which the anti-HER3 antibody-drug conjugate is to be administered in combination with another medicine" can include: 1) the administration of a single formulation comprising the anti-HER3 antibody-drug conjugate and another medicine, 2) the simultaneous administration through the same administration route of two formulations obtained by separately formulating the anti-HER3 antibody-drug conjugate and another medicine, 3) the administration in a staggered manner through the same administration route of two formulations obtained by separately formulating the anti-HER3 antibody-drug conjugate and another medicine, 4) the simultaneous administration through different administration routes of two formulations obtained by separately formulating the anti-HER3 antibody-drug conjugate and another medicine, and 5) the administration in a staggered manner through different administration routes of two formulations obtained by separately formulating the anti-HER3 antibody-drug conjugate and another medicine. The dose, dosing interval, dosage form, formulation, etc., of the "pharmaceutical composition in which the anti-HER3 antibody-drug conjugate is to be administered in combination with another medicine" abide by those of the pharmaceutical composition containing the anti-HER3 antibody-drug conjugate of the present invention, but are not limited thereto.

Such a pharmaceutical composition formulated in two different formulations may be in the form of a kit containing them.

In the present invention, the "combination" of the anti-HER3 antibody-drug conjugate

and another medicine means that the anti-HER3 antibody-drug conjugate and the medicine are "administered in combination".

[0199] The pharmaceutical composition can be formulated into a lyophilization formulation or a liquid formulation as a formulation having desired composition and required purity. When formulated as a lyophilization formulation, it may be a formulation containing suitable formulation additives that are used in the art. Also for a liquid formulation, it can be formulated as a liquid formulation containing various formulation additives that are used in the art.

[0200] Constituents and concentration of the pharmaceutical composition may vary depending on administration method. However, the anti-HER3 antibody-drug conjugate comprised in the pharmaceutical composition of the present invention can exhibit the pharmaceutical effect even at a small dosage when the antibody-drug conjugate has higher affinity for an antigen, that is, higher affinity (= lower Kd value) in terms of the dissociation constant (that is, Kd value) for the antigen. Thus, for determining dosage of the antibody-drug conjugate, the dosage can be determined in view of a situation relating to the affinity between the antibody-drug conjugate and antigen. When the antibody-drug conjugate of the present invention is administered to a human, for example, about 0.001 to 100 mg/kg can be administered once or administered several times with an interval of one time for 1 to 180 days.

Examples

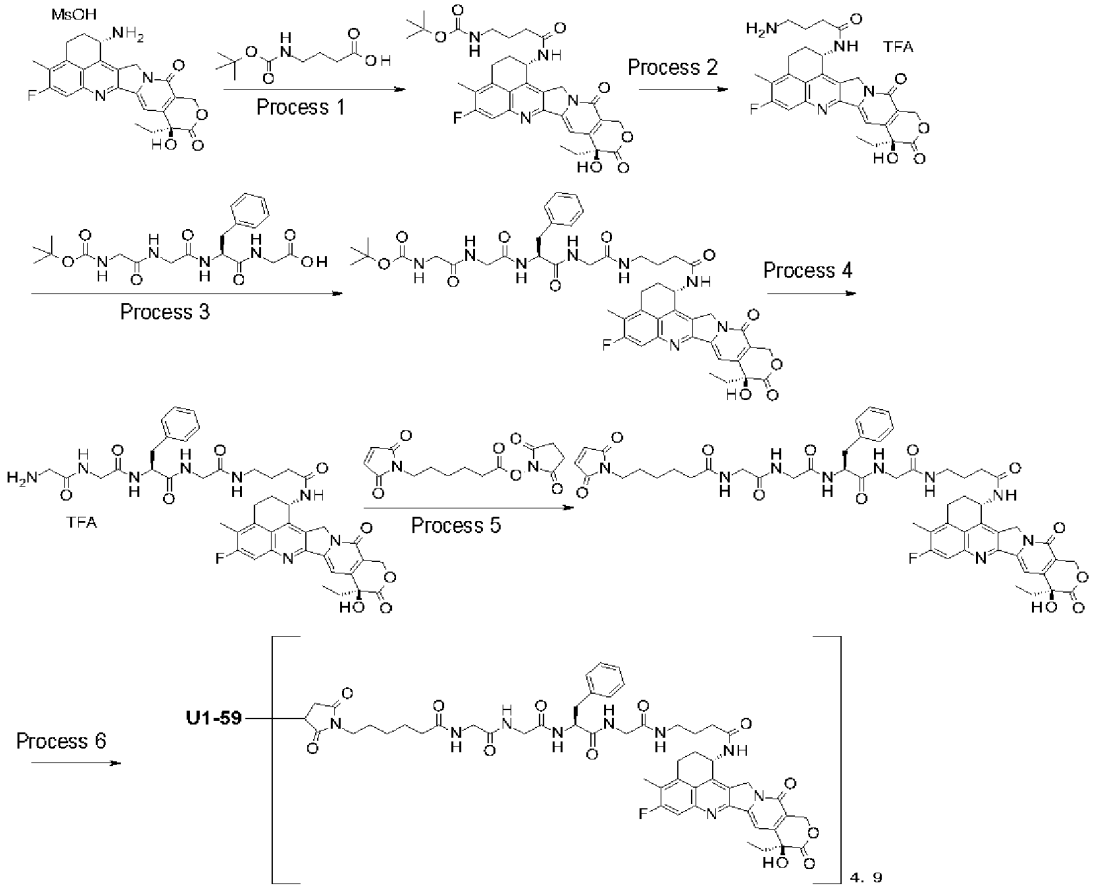
[0201] The present invention is specifically described in view of the examples shown below. However, the present invention is not limited to them. Further, it is by no means interpreted in a limited sense. Further, unless specifically described otherwise, the reagent, solvent, and starting material described in the specification can be easily obtained from a commercial supplier.

[0202] Reference Example 1 Production of U1-59

U1-59 was produced on the basis of the method described in International Publication No. WO 2007/077028.

[0203] Example 1 Antibody-drug conjugate (1)

[Chem.32]



[0204] Process 1: tert-Butyl (4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)carbamate

4-(tert-Butoxycarbonylamino)butanoic acid (0.237 g, 1.13 mmol) was dissolved in dichloromethane (10 mL), charged with N-hydroxysuccinimide (0.130 g, 1.13 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.216 g, 1.13 mmol), and stirred for 1 hour. The reaction solution was added dropwise to an N,N-dimethylformamide solution (10 mL) charged with exatecan mesylate (0.500 g, 0.94 mmol) and triethylamine (0.157 mL, 1.13 mmol), and stirred at room temperature for 1 day. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 8 : 2 (v/v)] to yield the titled compound (0.595 g, quantitative).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J = 7.2 Hz), 1.31 (9H, s), 1.58 (1H, t, J = 7.2 Hz), 1.66 (2H, t, J = 7.2 Hz), 1.89-1.82 (2H, m), 2.12-2.21 (3H, m), 2.39 (3H, s), 2.92 (2H, t, J = 6.5 Hz), 3.17 (2H, s), 5.16 (1H, d, J = 19.2 Hz), 5.24 (1H, d, J

= 18.8 Hz), 5.42 (2H, s), 5.59-5.55 (1H, m), 6.53 (1H, s), 6.78 (1H, t, J = 6.3 Hz), 7.30 (1H, s), 7.79 (1H, d, J = 11.0 Hz), 8.40 (1H, d, J = 8.6 Hz).

MS (APCI) m/z: 621 $(M+H)^+$.

[0205] Process 2:

4-Amino-N-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]butanamide trifluoroacetate

The compound (0.388 g, 0.61 mmol) obtained in above Process 1 was dissolved in dichloromethane (9 mL). After adding trifluoroacetic acid (9 mL), it was stirred for 4 hours. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - partitioned organic layer of chloroform : methanol : water = 7 : 3 : 1 (v/v/v)] to yield the titled compound (0.343 g, quantitative).

^{1}H-NMR (400 MHz, DMSO-d_6) 0.87 (3H, t, J=7.2 Hz), 1.79-1.92 (4H, m), 2.10-2.17 (2H, m), 2.27 (2H, t, J=7.0 Hz), 2.40 (3H, s), 2.80-2.86 (2H, m), 3.15-3.20 (2H, m), 5.15 (1H, d, J=18.8 Hz), 5.26 (1H, d, J=18.8 Hz), 5.42 (2H, s), 5.54-5.61 (1H, m), 6.55 (1H, s), 7.32 (1H, s), 7.72 (3H, brs), 7.82 (1H, d, J=11.0 Hz), 8.54 (1H, d, J=8.6 Hz).

MS (APCI) m/z: 521 $(M+H)^+$.

[0206] Process 3: N-(tert-butoxycarbonyl)glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide

N-(tert-butoxycarbonyl)glycylglycyl-L-phenylalanylglycine (0.081 g, 0.19 mmol) was dissolved in dichloromethane (3 mL), charged with N-hydroxysuccinimide (0.021 g, 0.19 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.036 g, 0.19 mmol), and stirred for 3.5 hours. The reaction solution was added dropwise to an N,N-dimethylformamide solution (1.5 mL) charged with the compound (0.080 g, 0.15 mmol) which has been obtained in above Process 2 and stirred at room temperature for 4 hours. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 8 : 2 (v/v)] to yield the titled compound (0.106 g, 73%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J = 7.4 Hz), 1.36 (9H, s), 1.71 (2H, m), 1.86 (2H, t, J = 7.8 Hz), 2.15-2.19 (4H, m), 2.40 (3H, s), 2.77 (1H, dd, J = 12.7, 8.8 Hz), 3.02 (1H, dd, J = 14.1, 4.7 Hz), 3.08-3.11 (2H, m), 3.16-3.19 (2H, m), 3.54 (2H, d, J = 5.9 Hz), 3.57-3.77 (4H, m), 4.46-4.48 (1H, m), 5.16 (1H, d, J = 19.2 Hz), 5.25 (1H, d, J = 18.8 Hz), 5.42 (2H, s), 5.55-5.60 (1H, m), 6.53 (1H, s), 7.00 (1H, t, J = 6.3 Hz), 7.17-7.26 (5H, m), 7.31 (1H, s), 7.71 (1H, t, J = 5.7 Hz), 7.80 (1H, d, J = 11.0 Hz), 7.92 (1H, t, J = 5.7 Hz), 8.15 (1H, d, J = 8.2 Hz), 8.27 (1H, t, J = 5.5 Hz),

8.46 (1H, d, J = 8.2 Hz).

MS (APCI) m/z: 939 $(M+H)^+$.

[0207] Process 4: Glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide trifluoroacetate

The compound (1.97 g, 2.10 mmol) obtained in above Process 3 was dissolved in dichloromethane (7 mL). After adding trifluoroacetic acid (7 mL), it was stirred for 1 hour. The solvent was removed under reduced pressure, and it was charged with toluene for azeotropic distillation. The obtained residues were purified by silica gel column chromatography [chloroform - partitioned organic layer of chloroform : methanol : water = 7 : 3 : 1 (v/v/v)] to yield the titled compound (1.97 g, 99%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J = 7.4 Hz), 1.71-1.73 (2H, m), 1.82-1.90 (2H, m), 2.12-2.20 (4H, m), 2.40 (3H, s), 2.75 (1H, dd, J = 13.7, 9.4 Hz), 3.03-3.09 (3H, m), 3.18-3.19 (2H, m), 3.58-3.60 (2H, m), 3.64 (1H, d, J = 5.9 Hz), 3.69 (1H, d, J = 5.9 Hz), 3.72 (1H, d, J = 5.5 Hz), 3.87 (1H, dd, J = 16.8, 5.9 Hz), 4.50-4.56 (1H, m), 5.16 (1H, d, J = 19.2 Hz), 5.25 (1H, d, J = 18.8 Hz), 5.42 (2H, s), 5.55-5.60 (1H, m), 7.17-7.27 (5H, m), 7.32 (1H, s), 7.78-7.81 (2H, m), 7.95-7.97 (3H, m), 8.33-8.35 (2H, m), 8.48-8.51 (2H, m).

MS (APCI) m/z: 839 $(M+H)^+$.

[0208] Process 5: N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide

To an N,N-dimethylformamide (1.2 mL) solution of the compound (337 mg, 0.353 mmol) obtained in above Process 4, triethylamine (44.3 mL, 0.318 mmol) and N-succinimidyl 6-maleimidehexanoate (119.7 mg, 0.388 mmol) were added and stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 5 : 1 (v/v)] to yield the titled compound as a pale yellow solid (278.0 mg, 76%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J=7.3 Hz), 1.12-1.22 (2H, m), 1.40-1.51 (4H, m), 1.66-1.76 (2H, m), 1.80-1.91 (2H, m), 2.05-2.21 (6H, m), 2.39 (3H, s), 2.79 (1H, dd, J=14.0, 9.8 Hz), 2.98-3.21 (5H, m), 3.55-3.77 (8H, m), 4.41-4.48 (1H, m), 5.15 (1H, d, J=18.9 Hz), 5.24 (1H, d, J=18.9 Hz), 5.40 (1H, d, J=17.1 Hz), 5.44 (1H, d, J=17.1 Hz), 5.54-5.60 (1H, m), 6.53 (1H, s), 6.99 (2H, s), 7.20-7.27 (5H, m), 7.30 (1H, s), 7.70 (1H, t, J=5.5 Hz), 7.80 (1H, d, J=11.0 Hz), 8.03 (1H, t, J=5.8 Hz),

8.08 (1H, t, J=5.5 Hz), 8.14 (1H, d, J=7.9 Hz), 8.25 (1H, t, J=6.1 Hz), 8.46 (1H, d, J=8.5 Hz).

MS (APCI) m/z: 1032 $(M+H)^+$.

[0209] Process 6: Antibody-drug conjugate (1)

Reducing the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure B and Common procedure C described in Production method 1. The solution (1.00 mL) was added to a 2.0 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.0307 mL; 4.6 equivalents per antibody molecule) and a 1 M aqueous solution of dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.050 mL). After confirming that the solution has pH of 7.4 +/- 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 1 hour.

Conjugation between antibody and drug linker: After incubating the solution in a water bath at 22C for 10 minutes, dimethyl sulfoxide (Sigma-Aldrich Co. LLC; 0.0586 mL) and a dimethyl sulfoxide solution (0.0615 mL; 9.2 equivalents per antibody molecule) containing 10 mM of the compound obtained in above Process 5 were added thereto and incubated in a water bath at 22C for 40 minutes for conjugating the drug linker to the antibody. Next, an aqueous solution (0.0123 mL) of 100 mM NAC (Sigma-Aldrich Co. LLC) was added thereto and stirred by using a tube rotator (MTR-103, manufactured by AS ONE Corporation) at room temperature for 20 minutes to terminate reaction of the drug linker.

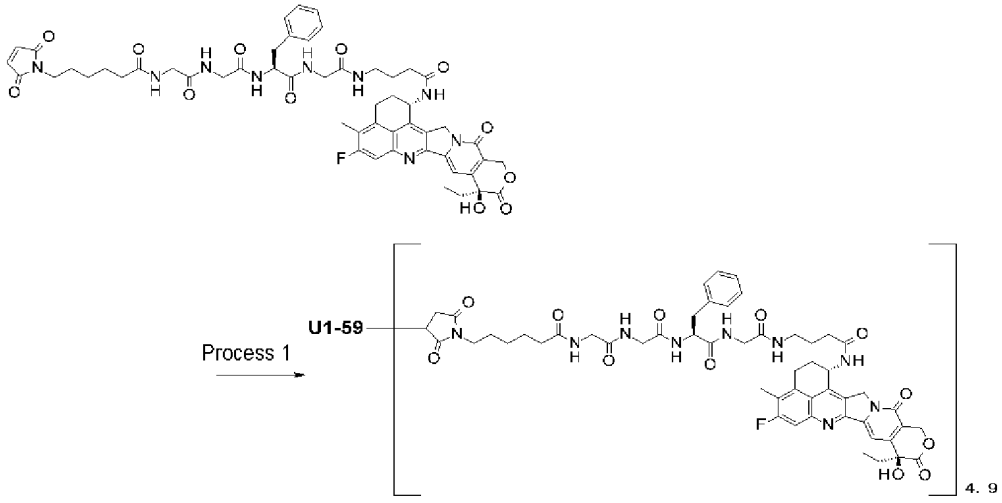
Purification: The above solution was subjected to purification using the Common procedure D (ABS is used as buffer solution) described in Production method 1 to yield 6 mL of a solution containing the titled antibody-drug conjugate.

Physicochemical characterization: By using the Common procedure E described in Production method 1 (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 7280 and $E_{D,370}$ = 23400 were used), the following characteristic values were obtained.

Antibody concentration: 1.29 mg/mL, antibody yield: 7.74 mg (77%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E: 4.9.

[0210] Example 2 Antibody-drug conjugate (2)

[Chem.33]



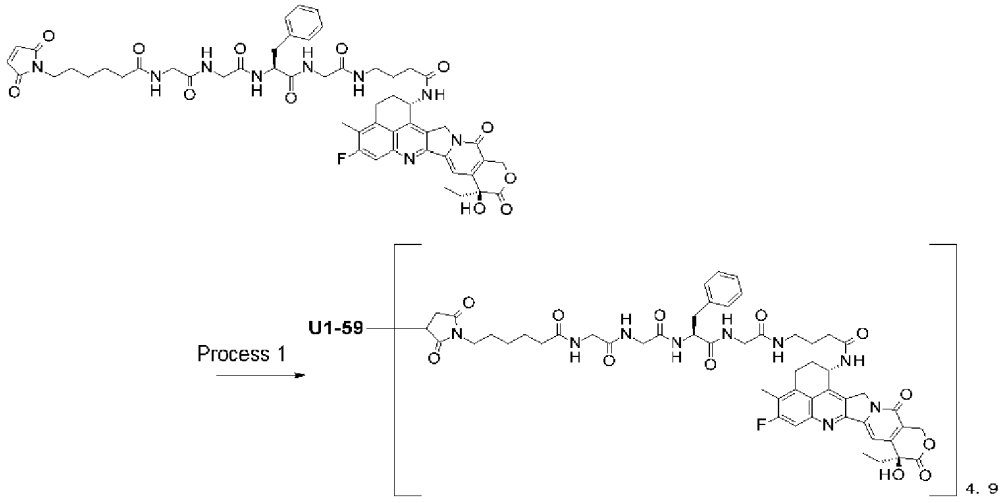
[0211] Process 1: Antibody-drug conjugate (2)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 5 of Example 1, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 12.0 mg/mL, antibody yield: 226.8 mg (91%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E: 4.9.

[0212] Example 3 Antibody-drug conjugate (3)

[Chem.34]



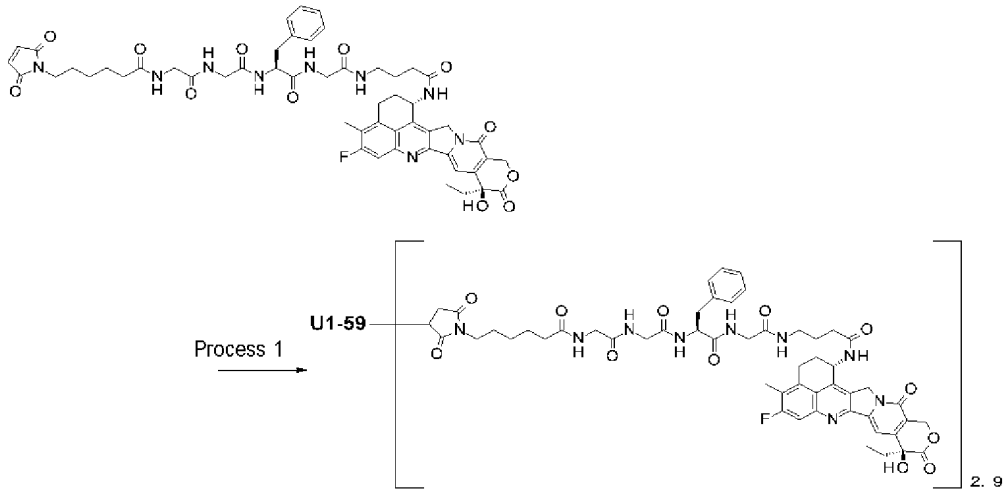
[0213] Process 1: Antibody-drug conjugate (3)

By using U1-59 produced in Reference Example 1 and the compound obtained in Process 5 of Example 1, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 16.9 mg/mL, antibody yield: 219.7 mg (88%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E: 4.9.

[0214] Example 4 Antibody-drug conjugate (4)

[Chem.35]



[0215] Process 1: Antibody-drug conjugate (4)

Reducing the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure B and Common procedure C described in Production method 1. The solution (1.00 mL) was added to a 1.5 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.0187 mL; 2.8 equivalents per antibody molecule) and a 1 M aqueous solution of dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.0170 mL). After confirming that the solution has pH of 7.0 +/- 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 1 hour.

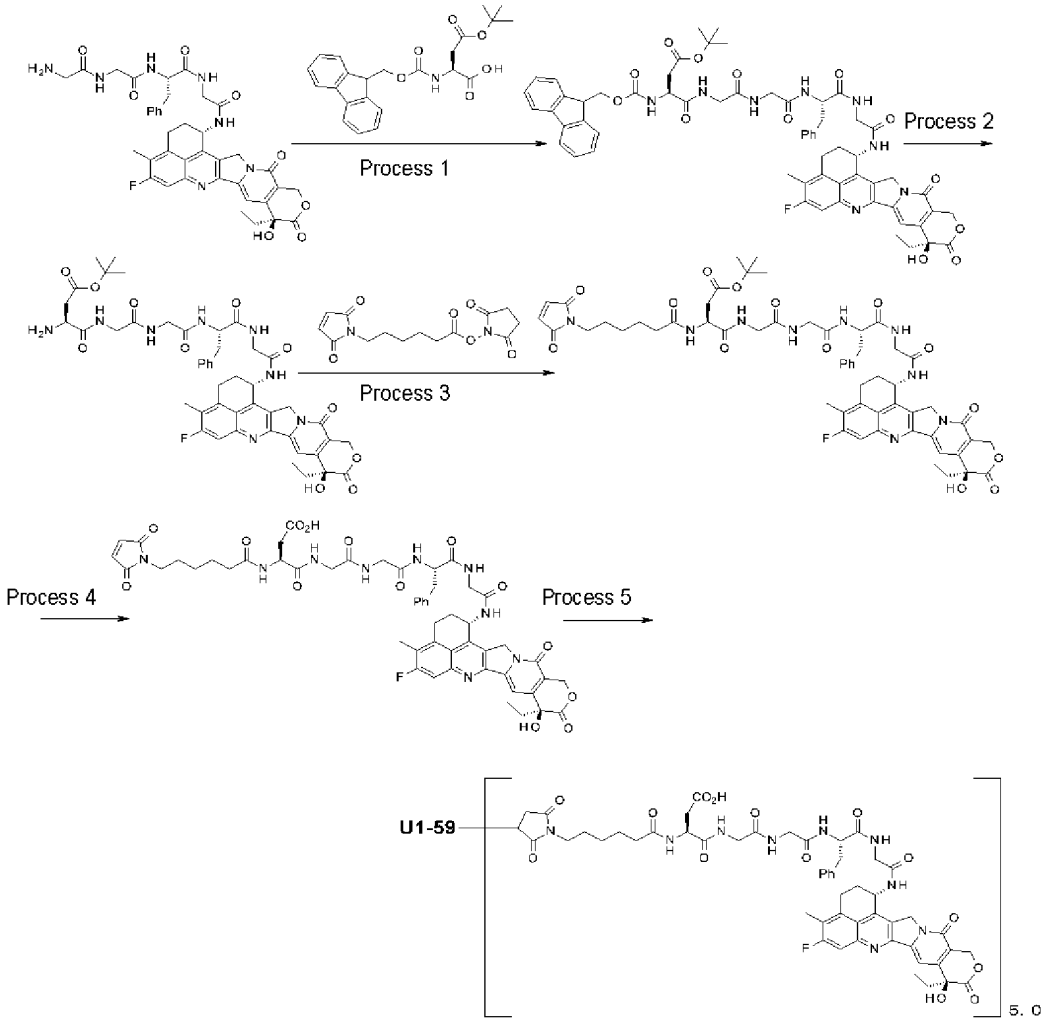
Conjugation between antibody and drug linker: After adding a dimethyl sulfoxide solution (0.0314 mL; 4.7 equivalents per antibody molecule) containing 10 mM of the compound obtained in above Process 5 to the solution at room temperature, it was incubated at 15C for 1 hour for conjugating the drug linker to the antibody. Next, an aqueous solution (0.0123 mL; 18.4 equivalents per antibody molecule) of 100 mM NAC (Sigma-Aldrich Co. LLC) was added thereto and incubatedat room temperature for another 20 minutes to terminate reaction of the drug linker.

Purification: The above solution was subjected to purification using the Common procedure D (ABS is used as buffer solution) described in Production method 1 to yield 6 mL of a solution containing the titled antibody-drug conjugate. After that, the solution was concentrated by the Common procedure A.

Physicochemical characterization: By using the Common procedure E described in Production method 1 (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5000, and $E_{D,370}$ = 19000 were used), the following characteristic values were obtained. Antibody concentration: 1.02 mg/mL, antibody yield: 6.1 mg (61%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E: 2.9; and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5000 were used): 3.2.

[0216] Example 5 Antibody-drug conjugate (5)

[Chem.36]



[0217] Process 1: tert-Butyl (5S,14S)-5-benzyl-1-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-14-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-1,4,7,10,13-pentaoxo-3,6,

9,12-tetraazahexadecan-16-oate

Under ice cooling, to an N,N-dimethylformamide (10.0 mL) solution of glycylglycyl-L-phenylalanyl-N-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]glycinamide (free form of the pharmaceutical compound described in International Publication No. WO 1997/46260; 0.250 g, 0.332 mmol), N-hydroxysuccinimide (57.2 mg, 0.497 mmol), and 4-tert-butyl N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-aspartic acid (0.205 g, 0.497 mmol), N,N'-dicyclohexylcarbodiimide (0.123 g, 0.497 mmol) was added and stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 9 : 1 (v/v)] to yield the titled compound as a pale yellow solid (0.278 g, 73%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.86 (3H, t, J = 7.1 Hz), 1.35 (9H, s), 1.79-1.90 (2H, m), 2.03-2.25 (2H, m), 2.40 (3H, s), 2.40-2.51 (2H, m), 2.64-2.82 (2H, m), 2.98 (1H, dd, J = 13.7, 4.6 Hz), 3.16 (2H, brs), 3.55 (1H, dd, J = 16.7, 5.7 Hz), 3.63-3.80 (4H, m), 4.16-4.34 (3H, m), 4.36-4.50 (2H, m), 5.23 (2H, s), 5.37 (1H, d, J = 16.5 Hz), 5.43 (1H, d, J = 16.5 Hz), 5.51-5.62 (1H, m), 6.52 (1H, s), 7.10-7.25 (5H, m), 7.26-7.33 (3H, m), 7.39 (2H, t, J = 7.3 Hz), 7.65-7.72 (3H, m), 7.80 (1H, d, J = 11.0 Hz), 7.86 (2H, d, J = 7.3 Hz), 7.98 (1H, t, J = 5.5 Hz), 8.07 (1H, d, J = 7.8 Hz), 8.15 (1H, t, J = 5.5 Hz), 8.31 (1H, t, J = 5.5 Hz), 8.41 (1H, d, J = 8.7 Hz).

MS (ESI) m/z: 1147 $(M+H)^+$.

[0218] Process 2: tert-Butyl (5S,14S)-14-amino-5-benzyl-1-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-1,4,7,10,13-pentaoxo-3,6,9,12-tetraazahexadecan-16-oate

To an N,N-dimethylformamide (2.00 mL) solution of the compound (0.279 g, 0.242 mmol) obtained in above Process 1, piperidine (0.240 mL, 2.42 mmol) was added and stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 2 : 1 (v/v)] to yield the titled compound as a pale yellow solid (0.265 g, quantitative).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.88 (3H, t, J = 7.2 Hz), 1.39 (9H, s), 1.81-1.94 (1H, m), 2.07-2.28 (2H, m), 2.37 (1H, dd, J = 15.8, 8.0 Hz), 2.43 (3H, s), 2.60 (1H, dd, J = 15.8, 4.9 Hz), 2.75-2.82 (1H, m), 3.00 (1H, dd, J = 13.9, 4.5 Hz), 3.16-3.25 (2H, m), 3.50-3.61 (2H, m), 3.65-3.81 (5H, m), 4.40-4.51 (1H, m), 5.27 (2H, dd, J = 24.1, 19.0 Hz), 5.43 (2H, dd, J = 21.3, 16.2 Hz), 5.56-5.65 (1H, m), 6.55 (1H, s), 7.15-7.28 (5H, m), 7.33 (1H, s), 7.83 (1H, d, J = 11.0 Hz), 8.04 (1H, t, J = 5.7 Hz), 8.09 (1H, d, J = 8.2 Hz), 8.26-8.39 (2H, m), 8.44 (1H, d, J = 8.2 Hz).

[0219] Process 3: tert-Butyl (5S,14S)-5-benzyl-14-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}-1-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-1,4,7,10,13-pentaoxo-3,6,9,12-tetraazahexadecan-16-oate

To an N,N-dimethylformamide (2.00 mL) solution of the compound (0.100 g, 0.108 mmol) obtained in above Process 2, N-succinimidyl 6-maleimide hexanoate (40.0 mg, 0.130 mmol) was added and stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 9 : 1 (v/v)] to yield the titled compound as a pale yellow solid (80.0 mg, 66%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.88 (3H, t, J = 7.2 Hz), 1.13-1.23 (2H, m), 1.37 (9H, s), 1.42-1.54 (4H, m), 1.80-1.96 (2H, m), 2.08-2.25 (4H, m), 2.35-3.76 (15H, m), 2.43 (3H, s), 4.39-4.49 (1H, m), 4.55-4.67 (1H, m), 5.21-5.34 (2H, m), 5.43 (2H, dd, J = 21.1, 16.4 Hz), 5.56-5.64 (1H, m), 6.55 (1H, s), 7.01 (2H, d, J = 0.8 Hz), 7.16-7.26 (5H, m), 7.33 (1H, s), 7.83 (1H, d, J = 11.3 Hz), 8.04-8.18 (3H, m), 8.30-8.37 (1H, m), 8.43 (1H, d, J = 8.6 Hz).

MS (ESI) m/z: 1118 $(M+H)^+$.

[0220] Process 4: N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alpha-aspartylglycylglycyl-L-phenylalanyl-N-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]glycinamide

Under ice cooling, trifluoroacetic acid (4.00 mL) was added to the compound (70.0 mg, 62.6 u moL) obtained in above Process 3 and stirred at room temperature for 1 hour. The solvent was removed under reduced pressure to yield the titled compound as a pale yellow solid (55.0 mg, 83%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.88 (3H, t, J = 7.4 Hz), 1.14-1.24 (2H, m), 1.41-1.53 (4H, m), 1.79-1.95 (2H, m), 2.08-2.28 (4H, m), 2.37-2.60 (2H, m), 2.42 (3H, s), 2.63-2.82 (2H, m), 2.99 (1H, dd, J = 14.1, 5.1 Hz), 3.12-3.25 (2H, m), 3.29-3.44 (1H, m), 3.52-3.80 (6H, m), 4.38-4.48 (1H, m), 4.56 (1H, dd, J = 13.7, 7.4 Hz), 5.27 (2H, dd, J = 24.3, 18.8 Hz), 5.43 (2H, dd, J = 21.5, 16.4 Hz), 5.57-5.62 (1H, m), 6.55 (1H, s), 7.01 (2H, s), 7.15-7.26 (5H, m), 7.33 (1H, s), 7.82 (1H, d, J = 11.0 Hz), 7.98 (1H, brs), 8.08 (1H, d, J = 6.7 Hz), 8.15 (1H, d, J = 7.8 Hz), 8.34 (1H, brs), 8.44 (1H, d, J = 8.6 Hz), 12.26 (1H, brs).

MS (ESI) m/z: 1062 $(M+H)^+$.

[0221] Process 5: Antibody-drug conjugate (5)

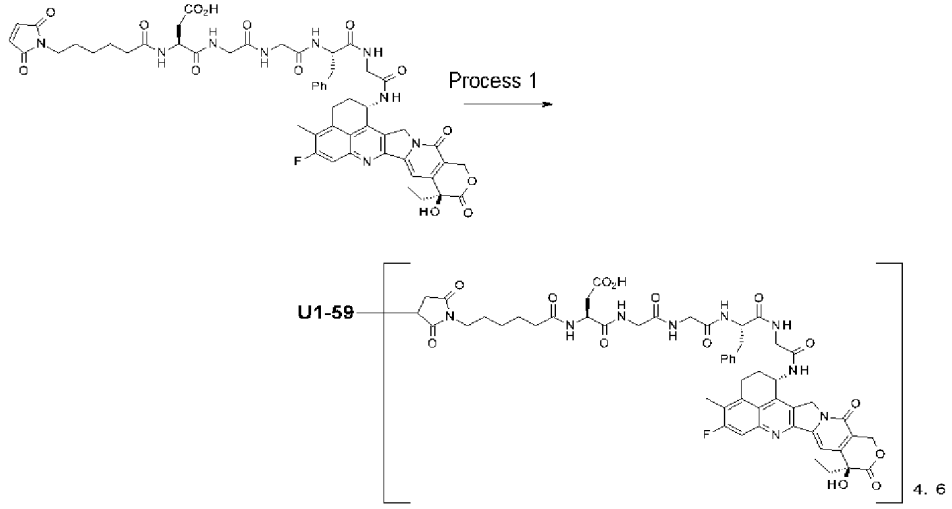
By using U1-59 produced in Reference Example 1 and the compound obtained in

above Process 4, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 1.36 mg/mL, antibody yield: 8.16 mg (82%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 7620, $E_{D,370}$ = 23700 were used): 5.0.

[0222] Example 6 Antibody-drug conjugate (6)

[Chem.37]



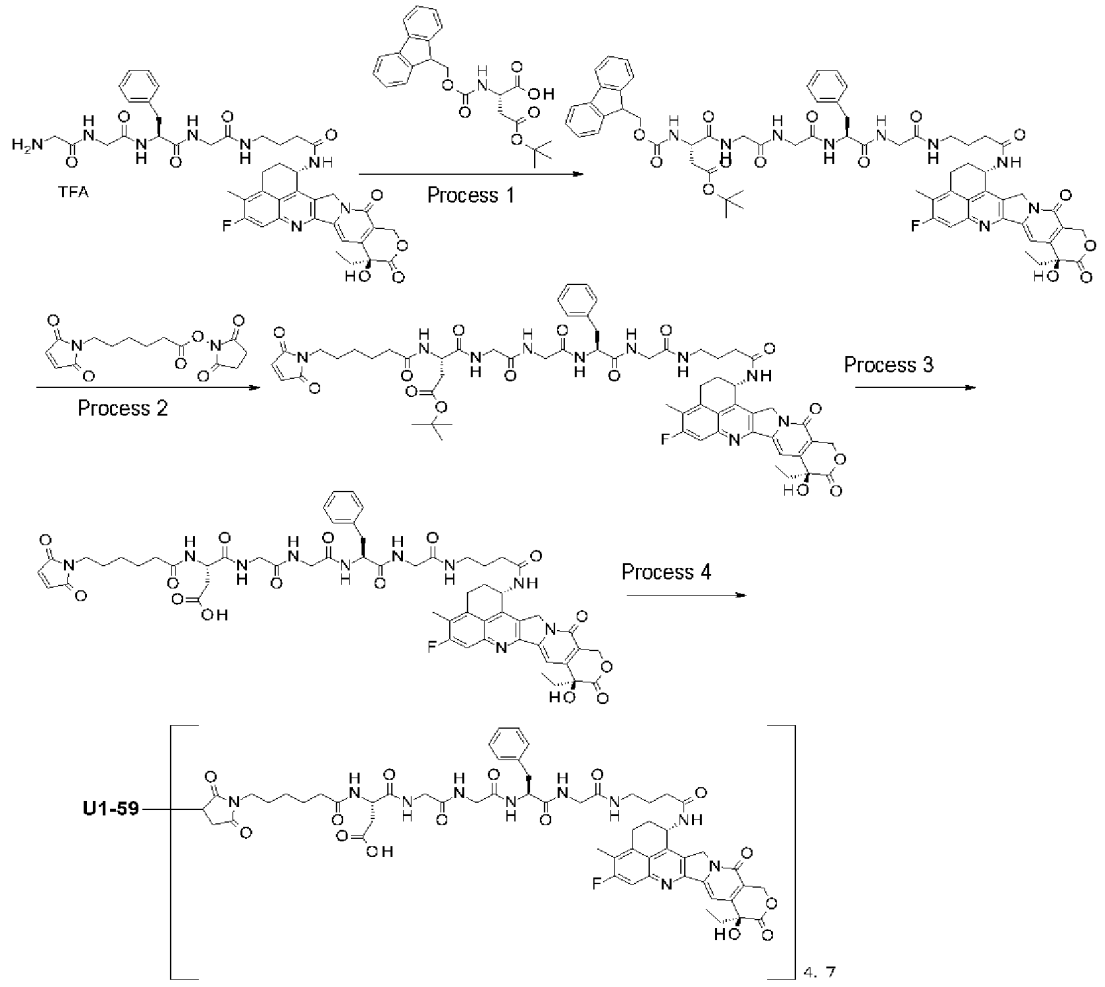
[0223] Process 1: Antibody-drug conjugate (6)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 4 of Example 5, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 11.5 mg/mL, antibody yield: 224.2 mg (90%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 7620, $E_{D,370}$ = 23700 were used): 4.6.

[0224] Example 7 Antibody-drug conjugate (7)

[Chem.38]



[0225] Process 1: tert-Butyl (3S,12S)-12-benzyl-21-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-3-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-4,7,10,13,16,21-hexaoxo-5,8,11,14,17-pentaazahenicosan-1-oate

(2S)-4-tert-Butoxy-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-4-oxobutanoic acid (0.625 g, 1.52 mmol) was dissolved in dichloromethane (10.0 mL), charged with N-hydroxysuccinimide (0.175 g, 1.52 mol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.291 g, 1.52 mmol), and stirred for 1 hour. The reaction solution was added dropwise to an N,N-dimethylformamide solution (10.0 mL) charged with the compound (1.00 g, 1.01 mmol) obtained in above Process 4 of Example 1 and stirred at room temperature for 20 hours. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform :

methanol = 8 : 2 (v/v)] to yield the titled compound as a pale yellow solid (0.873 g, 70%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.88 (3H, t, J=7.4 Hz), 1.37 (9H, s), 1.68-1.78 (2H, m), 1.81-1.93 (2H, m), 2.10-2.23 (4H, m), 2.41 (3H, s), 2.68-2.85 (3H, m), 2.99-3.22 (5H, m), 3.58-3.81 (6H, m), 4.19-4.36 (3H, m), 4.38-4.52 (2H, m), 5.17 (1H, d, J=19.2 Hz), 5.25 (1H, d, J=19.2 Hz), 5.43 (2H, s), 5.54-5.62 (1H, m), 6.55 (1H, s), 7.15-7.34 (8H, m), 7.41 (2H, t, J=7.2 Hz), 7.66-7.75 (4H, m), 7.81 (1H, d, J=11.0 Hz), 7.88 (2H, d, J=7.4 Hz), 8.01-8.06 (1H, m), 8.14 (1H, d, J=8.2 Hz), 8.17-8.22 (1H, m), 8.25-8.30 (1H, m), 8.47 (1H, d, J=8.6 Hz).

MS (APCI) m/z: 1232 $(M+H)^+$.

[0226] Process 2: tert-Butyl (3S,12S)-12-benzyl-3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}-21-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4,7,10,13,16,21-hexaoxo-5,8,11,14,17-pentaazahenicosan-1-oate

The compound (0.800 g, 0.649 mmol) obtained in above Process 1 was dissolved in N,N-dimethylformamide (3.00 mL), charged with piperidine (0.643 mL, 6.49 mmol), and stirred for 1 hour. The solvent was removed to driness under reduced pressure and the obtained residues were dissolved in N,N-dimethylformamide (10 mL). After adding N-succinimidyl 6-maleimide hexanoate (0.300 g, 0.974 mmol), it was stirred for 20 hours. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 8 : 2 (v/v)] to yield the titled compound as a pale yellow solid (0.224 g, 29%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J = 7.6 Hz), 1.15-1.22 (2H, m), 1.35 (9H, s), 1.44-1.47 (4H, m), 1.71-1.73 (2H, m), 1.80-1.91 (2H, m), 2.08 (2H, t, J = 7.6 Hz), 2.13-2.20 (4H, m), 2.40 (3H, s), 2.67 (1H, dt, J = 11.1, 4.8 Hz), 2.78 (1H, dd, J = 13.6, 9.4 Hz), 2.99-3.17 (6H, m), 3.31-3.36 (2H, m), 3.57-3.76 (6H, m), 4.45-4.47 (1H, m), 4.57-4.60 (1H, m), 5.16 (1H, d, J = 18.7 Hz), 5.25 (1H, d, J = 18.7 Hz), 5.42 (2H, s), 5.55-5.60 (1H, m), 6.53 (1H, s), 6.99 (2H, s), 7.15-7.27 (5H, m), 7.31 (1H, s), 7.70 (1H, t, J = 5.4 Hz), 7.80 (1H, d, J = 10.9 Hz), 7.99 (1H, t, J = 5.7 Hz), 8.09-8.12 (3H, m), 8.25 (1H, t, J = 6.0 Hz), 8.45 (1H, d, J = 9.1 Hz).

MS (APCI) m/z: 1203 $(M+H)^+$.

[0227] Process 3: N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alpha-aspartylglycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide

The compound (0.224 g, 0.186 mmol) obtained in above Process 2 was reacted in the same manner as Process 2 of Example 1 to yield the titled compound as a pale yellow solid (21.2 mg, 10%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.87 (3H, t, J = 7.2 Hz), 1.13-1.21 (2H, m), 1.42-1.45 (6H, m), 1.70-1.72 (2H, m), 1.85-1.88 (2H, m), 2.06-2.20 (6H, m), 2.39 (3H, s), 2.63-2.67 (1H, m), 2.78-2.81 (1H, m), 3.04-3.12 (6H, m), 3.63-3.70 (6H, m), 4.46-4.52 (2H, m), 5.16 (1H, d, J = 19.2 Hz), 5.25 (1H, d, J = 18.8 Hz), 5.42 (2H, s), 5.55-5.58 (1H, m), 6.53 (1H, s), 6.99 (2H, s), 7.18-7.23 (6H, m), 7.30 (1H, s), 7.71 (1H, t, J = 5.5 Hz), 7.79 (1H, d, J = 10.9 Hz), 7.99-8.02 (1H, m), 8.10-8.11 (3H, m), 8.27-8.30 (1H, m), 8.47-8.50 (1H, m).

MS (APCI) m/z: 1147 $(M+H)^+$.

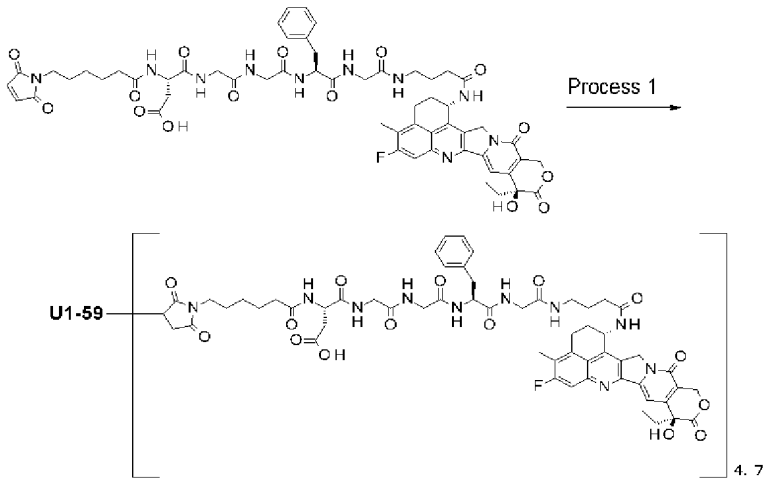
[0228] Process 4: Antibody-drug conjugate (7)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 3, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 1.39 mg/mL, antibody yield: 8.34 mg (83%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 7670, $E_{D,370}$ = 24800 were used): 4.7.

[0229] Example 8 Antibody-drug conjugate (8)

[Chem.39]



[0230] Process 1: Antibody-drug conjugate (8)

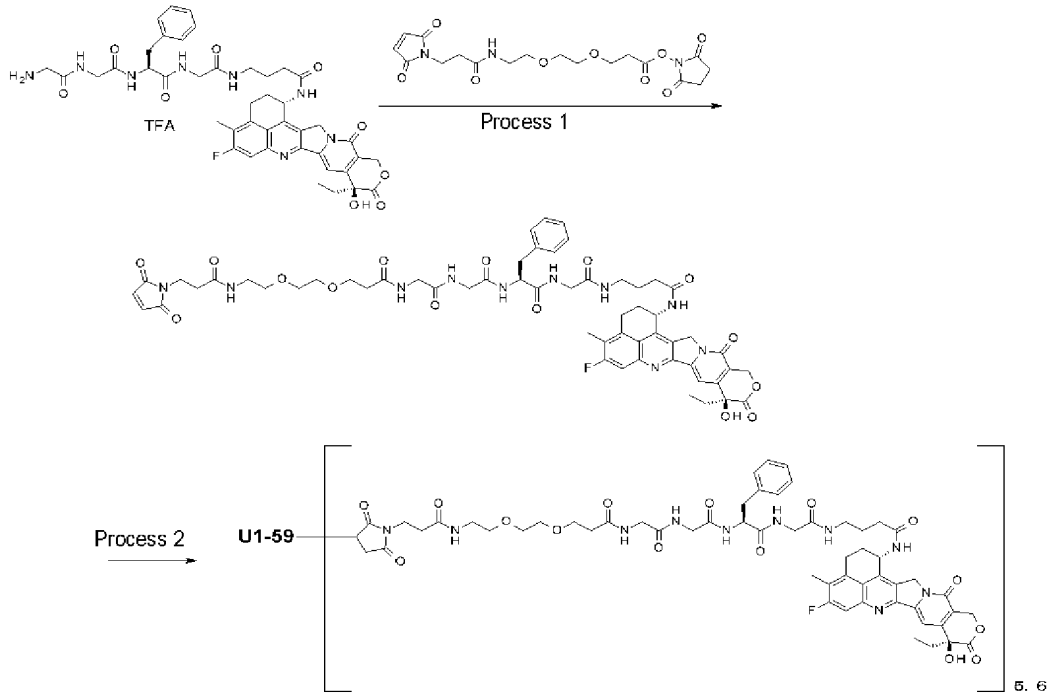
By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 3 of Example 7, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 11.2 mg/mL, antibody yield: 228.5 mg (91%), and average

number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 7670, $E_{D,370}$ = 24800 were used): 4.7.

[0231] Example 9 Antibody-drug conjugate (9)

[0232] [Chem.40]



[0233] Process 1: N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide

The compound (100 mg, 0.119 mmol) obtained in above Process 4 of Example 1 was reacted in the same manner as Process 5 of Example 1 by using N-succinimidyl 3-(2-(2-(3-maleinimidepropanamide)ethoxy)ethoxy)propanoate (50.7 mg, 0.119 mmol) instead of N-succinimidyl 6-maleimide hexanoate to yield the titled compound as a pale yellow solid (66.5 mg, 48%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.85 (3H, t, J = 7.4 Hz), 1.65-1.74 (2H, m), 1.77-1.90 (2H, m), 2.07-2.19 (4H, m), 2.30 (2H, t, J = 7.2 Hz), 2.33-2.36 (2H, m), 2.38 (3H, s), 2.76 (1H, dd, J = 13.7, 9.8 Hz), 2.96-3.18 (9H, m), 3.42-3.44 (4H, m), 3.53-3.76 (10H, m), 4.43 (1H, td, J = 8.6, 4.7 Hz), 5.14 (1H, d, J = 18.8 Hz), 5.23 (1H, d, J = 18.8 Hz), 5.38 (1H, d, J = 17.2 Hz), 5.42 (1H, d, J = 17.2 Hz), 5.52-5.58 (1H, m), 6.52 (1H, s), 6.98 (2H, s), 7.12-7.17 (1H, m), 7.18-7.25 (4H, m), 7.29 (1H, s), 7.69 (1H, t, J = 5.5 Hz), 7.78 (1H, d, J = 11.3 Hz), 7.98-8.03 (2H, m), 8.11 (1H, d, J = 7.8

Hz), 8.16 (1H, t, J = 5.7 Hz), 8.23 (1H, t, J = 5.9 Hz), 8.44 (1H, d, J = 9.0 Hz).
MS (APCI) m/z: 1149 $(M+H)^+$.

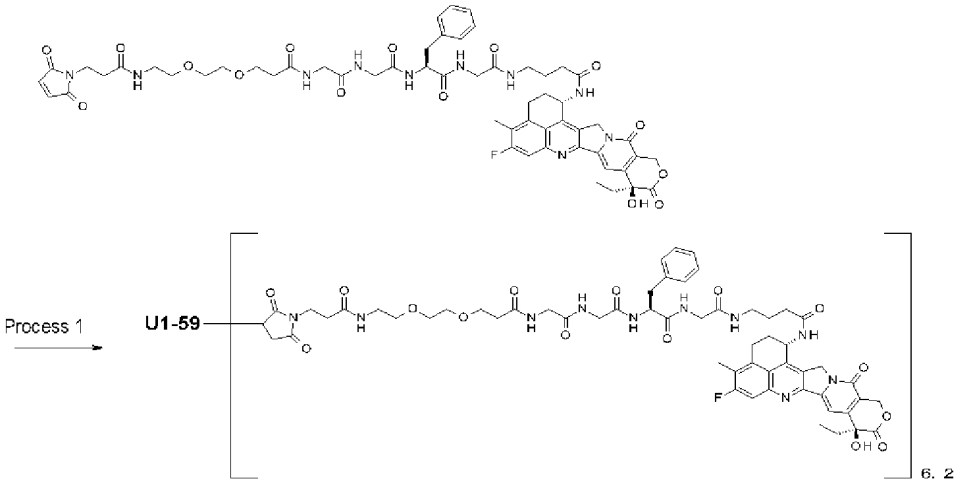
[0234] Process 2: Antibody-drug conjugate (9)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 1, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 2.08 mg/mL, antibody yield: 18.7 mg (94%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 4964, $E_{D,370}$ = 18982 were used): 5.6.

[0235] Example 10 Antibody-drug conjugate (10)

[Chem.41]



[0236] Process 1: Antibody-drug conjugate (10)

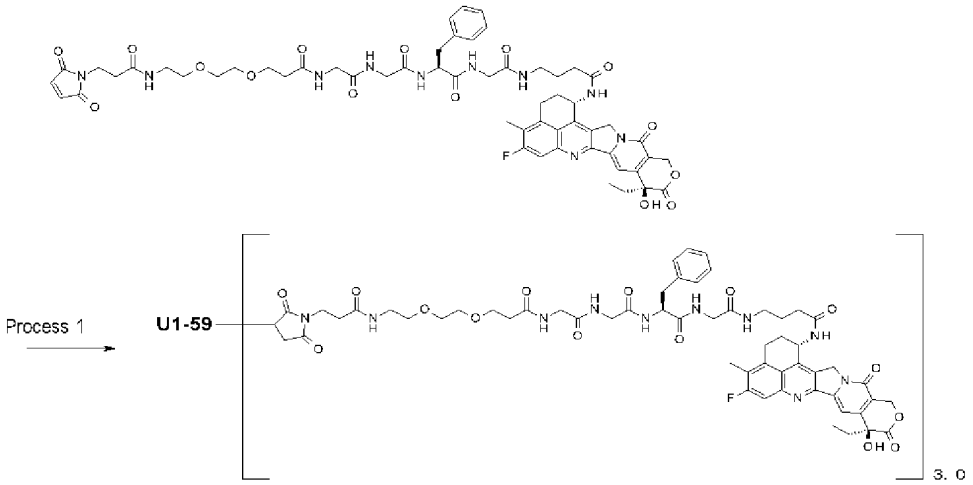
By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 1 of Example 9, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 19.7 mg/mL, antibody yield: 236.4 mg (95%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 4964, $E_{D,370}$ = 18982 were used): 6.2; and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 4964 were used): 6.4.

[0237] Example 11 Antibody-drug conjugate (11)

[0238]

[Chem.42]



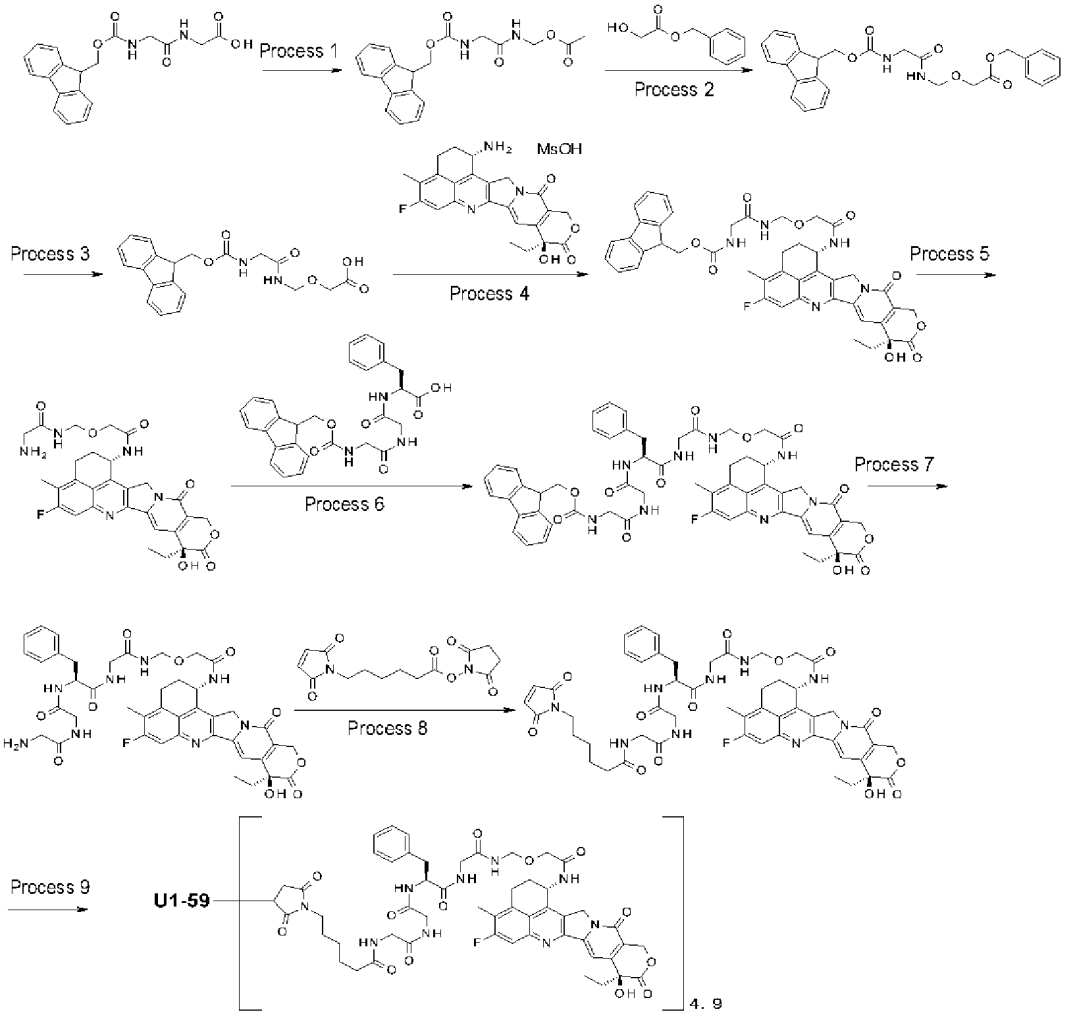
[0239] Process 1: Antibody-drug conjugate (11)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 1 of Example 9, the titled antibody-drug conjugate was obtained in the same manner as Process 1 of Example 4.

Antibody concentration: 0.88 mg/mL, antibody yield: 5.28 mg (53%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 4964, $E_{D,370}$ = 18982 were used): 3.0; and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 4964 were used): 3.3.

[0240] Example 12 Antibody-drug conjugate (12)

[Chem.43]



[0241] Process 1: ({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate

To a mixture containing N-9-fluorenylmethoxycarbonylglycylglycine (4.33 g, 12.2 mmol), tetrahydrofuran (120 ml), and toluene (40.0 ml), pyridine (1.16 mL, 14.7 mmol) and lead tetraacetate (6.84 g, 14.7 mmol) were added and refluxed under heating for 5 hours. After the reaction solution was cooled to room temperature, the insoluble material was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The obtained residues were dissolved in ethyl acetate, washed with water and saturated brine, and then the organic layer was dried over anhydrous magnesium sulfate. After the solvent was removed under reduced pressure, the obtained residues were purified by silica gel column chromatography [hexane : ethyl acetate = 9 : 1 (v/v) - ethyl acetate] to yield the titled compound as colorless solid (3.00 g, 67%).

^{1}H-NMR (400 MHz, $CDCl_3$) delta: 2.07 (3H, s), 3.90 (2H, d, J = 5.1 Hz), 4.23 (1H, t,

J = 7.0 Hz), 4.46 (2H, d, J = 6.6 Hz), 5.26 (2H, d, J = 7.0 Hz), 5.32 (1H, brs), 6.96 (1H, brs), 7.32 (2H, t, J = 7.3 Hz), 7.41 (2H, t, J = 7.3 Hz), 7.59 (2H, d, J = 7.3 Hz), 7.77 (2H, d, J = 7.3 Hz).

[0242] Process 2: Benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate

To a tetrahydrofuran (40.0 mL) solution of the compound (3.68 g, 10.0 mmol) obtained in above Process 1 and benzyl glycolate (4.99 g, 30.0 mmol), potassium tert-butoxide (2.24 g, 20.0 mmol) was added at 0C and stirred at room temperature for 15 minutes. The reaction solution was charged with ethyl acetate and water at 0C and extracted with ethyl acetate and chloroform. The obtained organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure. The obtained residues were dissolved in dioxane (40.0 mL) and water (10.0 mL), charged with sodium hydrogen carbonate (1.01 g, 12.0 mmol) and 9-fluorenylmethyl chloroformate (2.59 g, 10.0 mmol), and stirred at room temperature for 2 hours. The reaction solution was charged with water and extracted with ethyl acetate. The obtained organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [hexane : ethyl acetate = 100 : 0 (v/v) - 0 : 100] to yield the titled compound in colorless oily substance (1.88 g, 40%).

^{1}H-NMR (400 MHz, $CDCl_3$) delta: 3.84 (2H, d, J = 5.5 Hz), 4.24 (3H, t, J = 6.5 Hz), 4.49 (2H, d, J = 6.7 Hz), 4.88 (2H, d, J = 6.7 Hz), 5.15-5.27 (1H, m), 5.19 (2H, s), 6.74 (1H, brs), 7.31-7.39 (7H, m), 7.43 (2H, t, J = 7.4 Hz), 7.61 (2H, d, J = 7.4 Hz), 7.79 (2H, d, J = 7.4 Hz).

[0243] Process 3: [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetic acid

The compound (1.88 g, 3.96 mmol) obtained in above Process 2 was dissolved in ethanol (40.0 mL) and ethyl acetate (20.0 mL). After adding palladium carbon catalyst (376 mg), it was stirred at room temperature under hydrogen atmosphere for 2 hours. The insoluble material was removed by filtration through Celite, and the solvent of the filtrate was removed under reduced pressure to yield the titled compound as colorless solid (1.52 g, quantitative).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 3.62 (2H, d, J = 6.3 Hz), 3.97 (2H, s), 4.18-4.32 (3H, m), 4.60 (2H, d, J = 6.7 Hz), 7.29-7.46 (4H, m), 7.58 (1H, t, J = 5.9 Hz), 7.72 (2H, d, J = 7.4 Hz), 7.90 (2H, d, J = 7.4 Hz), 8.71 (1H, t, J = 6.5 Hz).

[0244] Process 4: 9H-Fluoren-9-ylmethyl(2-{[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]amino}-2-oxoethyl)carbamate

Under ice cooling, to an N,N-dimethylformamide (10.0 mL) solution of exatecan mesylate (0.283 g, 0.533 mmol), N-hydroxysuccinimide (61.4 mg, 0.533 mmol), and the compound (0.205 g, 0.533 mmol) obtained in above Process 3, N,N-diisopropylethylamine (92.9 uL, 0.533 mmol) and N,N'-dicyclohexylcarbodiimide (0.143 g, 0.693 mmol) were added and stirred at room temperature for 3 days. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - partitioned organic layer of chloroform : methanol : water = 7 : 3 : 1 (v/v/v)] to yield the titled compound as a pale brown solid (0.352 g, 82%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.81 (3H, t, J = 7.4 Hz), 1.73-1.87 (2H, m), 2.06-2.20 (2H, m), 2.34 (3H, s), 3.01-3.23 (2H, m), 3.58 (2H, d, J = 6.7 Hz), 3.98 (2H, s), 4.13-4.25 (3H, m), 4.60 (2H, d, J = 6.7 Hz), 5.09-5.22 (2H, m), 5.32-5.42 (2H, m), 5.50-5.59 (1H, m), 6.49 (1H, s), 7.24-7.30 (3H, m), 7.36 (2H, t, J = 7.4 Hz), 7.53 (1H, t, J = 6.3 Hz), 7.66 (2H, d, J = 7.4 Hz), 7.75 (1H, d, J = 11.0 Hz), 7.84 (2H, d, J = 7.4 Hz), 8.47 (1H, d, J = 8.6 Hz), 8.77 (1H, t, J = 6.7 Hz).

MS (ESI) m/z: 802 $(M+H)^+$.

[0245] Process 5: N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide

To an N,N-dimethylformamide (11.0 mL) solution of the compound (0.881 g, 1.10 mmol) obtained in above Process 4, piperidine (1.1 mL) was added and stirred at room temperature for 2 hours. The solvent was removed under reduced pressure to yield a mixture containing the titled compound. The mixture was used for the next reaction without further purification.

[0246] Process 6: N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide

Under ice cooling, to an N,N-dimethylformamide (50.0 mL) solution of the mixture (0.439 mmol) obtained in above Process 5, N-hydroxysuccinimide (0.101 g, 0.878 mmol), and N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanine (the compound described in Japanese Patent Laid-Open No. 2002-60351; 0.440 g, 0.878 mmol), N,N'-dicyclohexylcarbodiimide (0.181 g, 0.878 mmol) was added and stirred at room temperature for 4 days. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 9 : 1 (v/v)] to yield the titled compound as a pale orange solid

(0.269 g, 58%).

MS (ESI) m/z: 1063 $(M+H)^+$.

[0247] Process 7: Glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide

To an N,N-dimethylformamide (4.00 mL) solution of the compound (0.269 g, 0.253 mmol) obtained in above Process 6, piperidine (0.251 mL, 2.53 mmol) was added and stirred at room temperature for 2 hours. The solvent was removed under reduced pressure to yield a mixture containing the titled compound. The mixture was used for the next reaction without further purification.

[0248] Process 8: N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide

To an N,N-dimethylformamide (10.0 mL) solution of the compound (0.253 mmol) obtained in above Process 7, N-succinimidyl 6-maleimide hexanoate (0.156 g, 0.506 mmol) was added and stirred at room temperature for 3 days. The solvent was removed under reduced pressure and the obtained residues were purified by silica gel column chromatography [chloroform - chloroform : methanol = 9 : 1 (v/v)] to yield the titled compound as a pale yellow solid (0.100 g, 38%).

^{1}H-NMR (400 MHz, DMSO-d_6) delta: 0.83 (3H, t, J = 7.2 Hz), 1.09-1.21 (2H, m), 1.33-1.47 (4H, m), 1.75-1.90 (2H, m), 2.00-2.23 (4H, m), 2.36 (3H, s), 2.69-2.81 (1H, m), 2.94-3.03 (1H, m), 3.06-3.22 (2H, m), 3.23-3.74 (6H, m), 3.98 (2H, s), 4.39-4.50 (1H, m), 4.60 (2H, d, J = 6.7 Hz), 5.17 (2H, s), 5.39 (2H, s), 5.53-5.61 (1H, m), 6.50 (1H, s), 6.96 (2H, s), 7.11-7.24 (5H, m), 7.28 (1H, s), 7.75 (1H, d, J = 11.0 Hz), 7.97 (1H, t, J = 5.7 Hz), 8.03 (1H, t, J = 5.9 Hz), 8.09 (1H, d, J = 7.8 Hz), 8.27 (1H, t, J = 6.5 Hz), 8.48 (1H, d, J = 9.0 Hz), 8.60 (1H, t, J = 6.5 Hz).

MS (ESI) m/z: 1034 $(M+H)^+$.

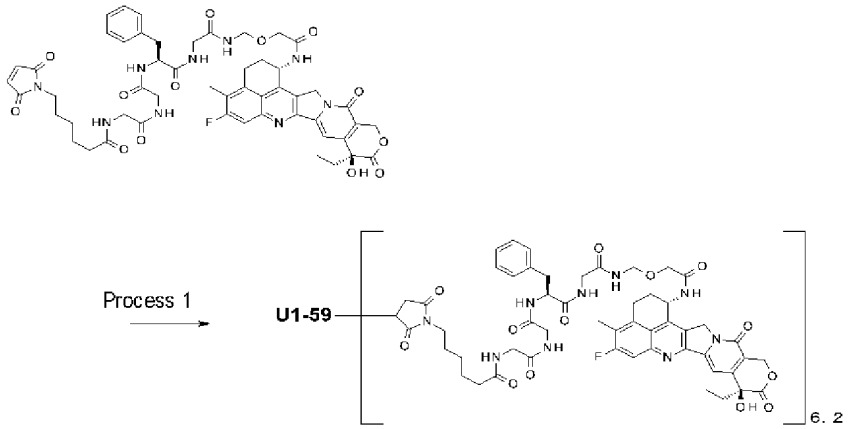
[0249] Process 9: Antibody-drug conjugate (12)

By using U1-59 produced in Reference Example 1 and the compound obtained in aove Process 8, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 2.11 mg/mL, antibody yield: 19.0 mg (95%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178, $E_{D,370}$ = 20217 were used): 4.9.

[0250] Example 13 Antibody-drug conjugate (13)

[Chem.44]



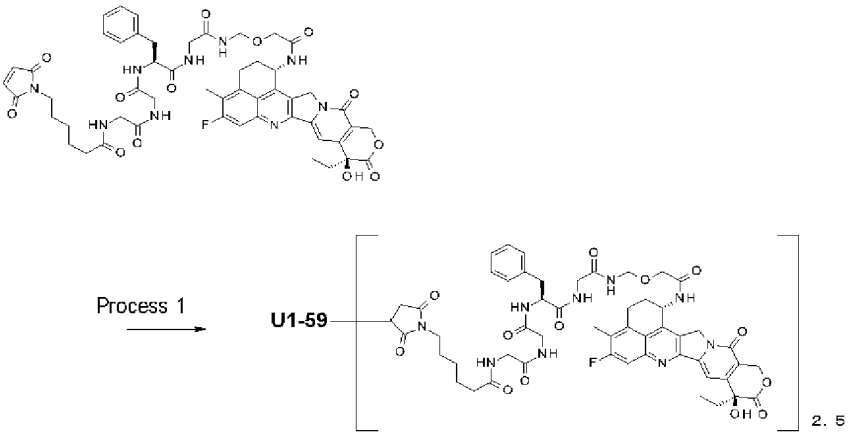
[0251] Process 1: Antibody-drug conjugate (13)

By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 8 of Example 12, the titled antibody-drug conjugate was obtained in the same manner as Process 6 of Example 1.

Antibody concentration: 22.2 mg/mL, antibody yield: 244.2 mg (98%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178, $E_{D,370}$ = 20217 were used): 6.2; and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 were used): 7.0.

[0252] Example 14 Antibody-drug conjugate (14)

[Chem.45]



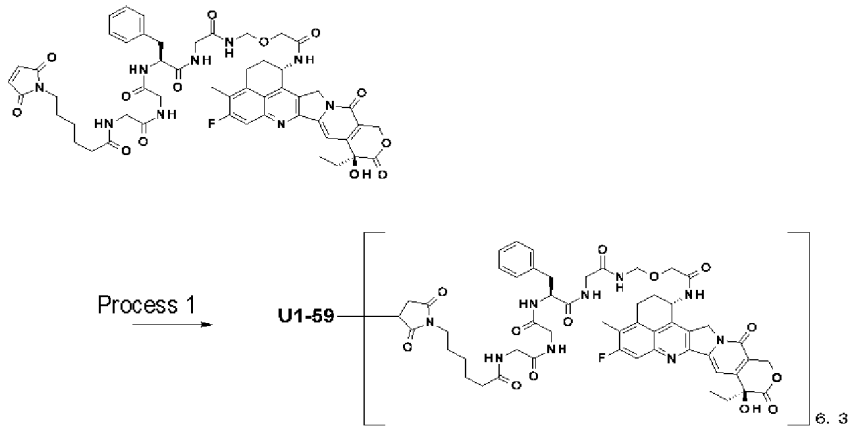
[0253] Process 1: Antibody-drug conjugate (14)

Reduction of the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure B and Common procedure C

described in Production method 1. The solution (1.00 mL) was added to a 2.0 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.0160 mL; 2.4 equivalents per antibody molecule) and a 1 M aqueous solution of dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.0150 mL). After confirming that the solution has pH of 7.0 +/- 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 1 hour.
Conjugation between antibody and drug linker: After incubating the solution in a water bath at 15C for 10 minutes, dimethyl sulfoxide (Sigma-Aldrich Co. LLC; 0.0209 mL) and a dimethyl sulfoxide solution (0.0315 mL; 5.0 equivalents per antibody molecule) containing 10 mM of the compound obtained in above Process 8 of Example 12 was added thereto and incubated in a water bath at 15C for 60 minutes for conjugating the drug linker to the antibody. Next, an aqueous solution (0.0050 mL) of 100 mM NAC (Sigma-Aldrich Co. LLC) was added thereto and stirred by using a tube rotator (MTR-103, manufactured by AS ONE Corporation) at room temperature for another 20 minutes to terminate reaction of the drug linker.
According to the same purification processes and physicochemical characterizations as Process 6 of Example 1, the following characteristics values were obtained.
Antibody concentration: 1.46 mg/mL, antibody yield: 8.76 mg (88%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178, $E_{D,370}$ = 20217 were used): 2.5; and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 were used): 2.9.

[0254] Example 15 Antibody-drug conjugate (15)

[Chem.46]



[0255] Process 1: Antibody-drug conjugate (15)

Reduction of the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with

PBS6.0/EDTA by using the Common procedure B and Common procedure C described in Production method 1. The solution (100 mL) was added to a 250 mL polycarbonate Erlenmeyer flask and charged with a 1 M aqueous solution of dipotassium hydrogen phosphate (1.70 mL) and then an aqueous solution of 10 mM TCEP (4.010 mL; 6.0 equivalents per antibody molecule) at room temperature with stirring using a magnetic stirrer. After confirming that the solution has pH of 7.0 +/- 0.1, the stirring was stopped, and the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 1 hour.

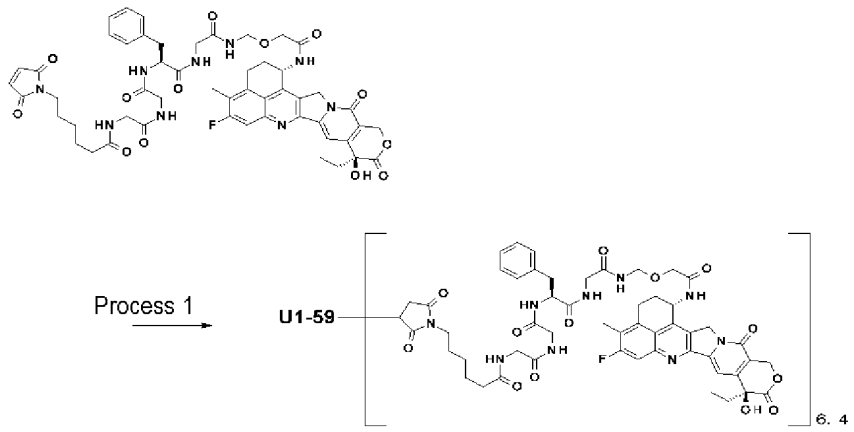
Conjugation between antibody and drug linker: After cooling the above solution to 15C, a DMSO solution (6.684 mL; 10.0 equivalents per antibody molecule) containing 10 mM of the compound obtained in above Process 8 of Example 12 was gradually added thereto with stirring. The mixture was stirred at 15C for the first 30 minutes and, after stopping the stirring, incubated for another 1 hour for conjugating the drug linker to the antibody. Next, an aqueous solution (0.862 mL; 12.9 equivalents per antibody molecule) of 100 mM NAC was added thereto with stirring and incubated at room temperature for 20 minutes to terminate the reaction of unreacted drug linker.

Purification: A 20% aqueous acetic acid solution (about 0.6 mL) and ABS (100 mL) were gradually added to the solution with stirring to adjust pH of the solution to 5.5 +/- 0.1. This solution was subjected to microfiltration (Millipore Corp. Millex-HV filter, 0.45 um, PVDF membrane) to remove whitish matter. This solution was subjected to ultrafiltration purification using a ultrafiltration apparatus constituted by a ultrafiltration membrane (Merck Japan, Ltd., Pellicon XL Cassette, Biomax 50 KDa), a tube pump (Cole-Parmer International, USA, MasterFlex pump model 77521-40, pump head model 7518-00), and a tube (Cole-Parmer International, USA, MasterFlex tube L/S16). Specifically, by adding ABS dropwise (a total of 1600 mL) as a buffer solution for purification to the reaction solution while carrying out ultrafiltration purification, non-conjugated drug linkers and other low-molecular-weight reagents were removed while the buffer solution was replaced with ABS and further the solution was concentrated. The obtained purified solution was subjected to microfiltration (0.22 um (Millipore Corp. Millex-GV filter, PVDF membrane) to yield 37.5 mL of a solution containing the titled antibody-drug conjugate.

Antibody concentration: 26.5 mg/mL, antibody yield: 993.0 mg (90%), average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 and $E_{D,370}$ = 20217 were used): 6.3, and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 was used): 7.3.

[0256] Example 16a Antibody-drug conjugate (16a)

[Chem.47]



[0257] Process 1: Antibody-drug conjugate (16a)

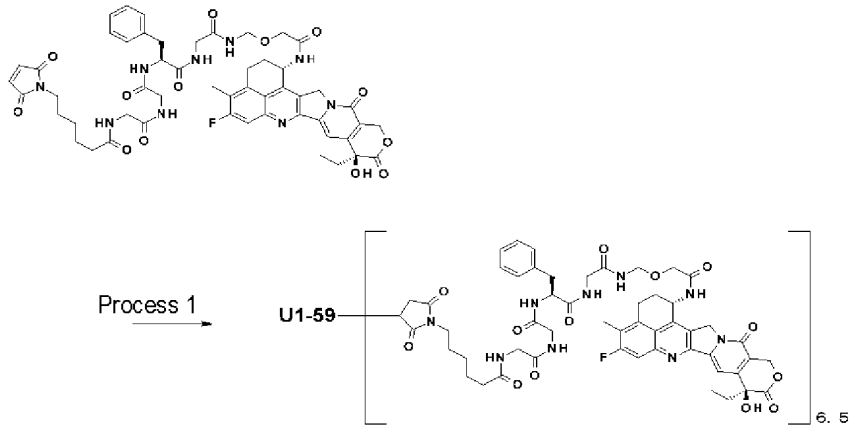
Reduction of the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure B and Common procedure C described in Production method 1. The solution (15 mL) was added to a 50 mL polyethylene terephthalate container and charged with a 1 M aqueous solution of dipotassium hydrogen phosphate (0.255 mL) and then an aqueous solution of 10 mM TCEP (0.601 mL; 6.0 equivalents per antibody molecule) at room temperature with stirring using a magnetic stirrer. After confirming that the solution has pH of 7.0 +/- 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 2 hours.

Conjugation between antibody and drug linker: After cooling the above solution to 15C, a DMSO solution (1.002 mL; 10.0 equivalents per antibody molecule) containing 10 mM of the compound obtained in above Process 8 of Example 12 was gradually added thereto with stirring. The mixture was stirred at 15C for 30 minutes for conjugating the drug linker to the antibody. Next, an aqueous solution (0.129 mL; 12.9 equivalents per antibody molecule) of 100 mM NAC was added thereto with stirring and incubated at room temperature for 20 minutes to terminate the reaction of unreacted drug linker. According to the same purification processes and physicochemical characterizations as Process 6 of Example 1, the following characteristic values were obtained.

Antibody concentration: 2.36 mg/mL, antibody yield: 140 mg (59.5 mL) (94%), average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 and $E_{D,370}$ = 20217 were used): 6.4, and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 was used): 7.7.

[0258] Example 16b Antibody-drug conjugate (16b)

[Chem.48]



[0259] Reduction of the antibody: U1-59 produced in Reference Example 1 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure B and Common procedure C described in Production method 1. The solution (900 mL) was added to a 2000 mL polycarbonate Erlenmeyer flask and charged with a 1 M aqueous solution of dipotassium hydrogen phosphate (15.3 mL) and then an aqueous solution of 10 mM TCEP (36.1 mL; 6.0 equivalents per antibody molecule) at room temperature with stirring using a magnetic stirrer. After confirming that the solution has pH of 7.0 +/- 0.1, the stirring was stopped, and the disulfide bond at hinge part in the antibody was reduced by incubating at 37C for 2 hours.

Conjugation between antibody and drug linker: After cooling the above solution to 15C, a DMSO solution (60.16 mL; 10.0 equivalents per antibody molecule) containing 10 mM of the compound obtained from Process 8 of Example 12 was gradually added thereto with stirring. The mixture was stirred at 15C for 30 minutes for conjugating the drug linker to the antibody. Next, an aqueous solution (7.76 mL; 12.9 equivalents per antibody molecule) of 100 mM NAC was added thereto with stirring and incubatedat room temperature for 20 minutes to terminate the reaction of unreacted drug linker.

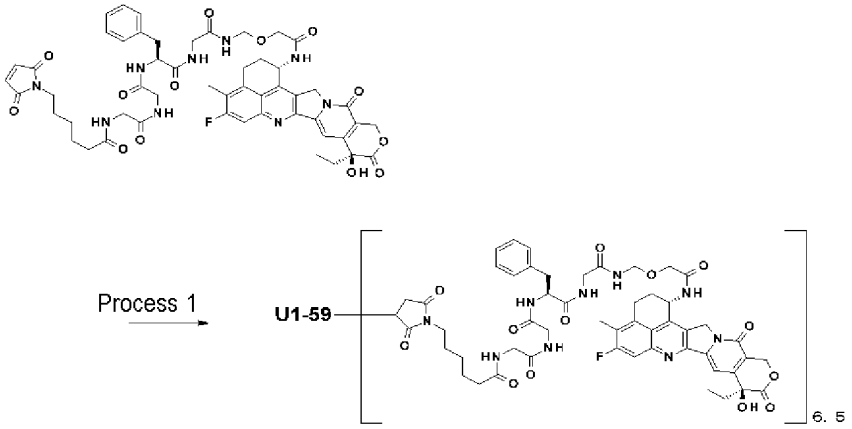
Purification: A 20% aqueous acetic acid solution (about 5 mL) and ABS (1000 mL) were gradually added to the solution with stirring to adjust pH of the solution to 5.5 +/- 0.1. This solution was subjected to microfiltration (Millipore Corp. Stericup, 0.45 um, PVDF membrane) to remove whitish matter. This solution was subjected to ultra-filtration purification using a ultrafiltration apparatus constituted by a ultrafiltration membrane (Merck Japan, Ltd., Pellicon 2 mini cassette, Ultracel 30 KDa, 0.1 m^2), a tube pump (Cole-Parmer International, USA, MasterFlex pump model 7528-20, pump head model 77800-62), and a tube (Cole-Parmer International, USA, MasterFlex tubes L/S24 and 25). Specifically, by adding ABS dropwise (a total of 16 L) as a buffer

solution for purification to the reaction solution while carrying out ultrafiltration purification, non-conjugated drug linkers and other low-molecular-weight reagents were removed while the buffer solution was replaced with ABS and further the solution was concentrated to yield about 500 mL of a solution containing the titled antibody-drug conjugate.

Antibody concentration: 19.66 mg/mL, antibody yield: 9830 mg (109%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 and $E_{D,370}$ = 20217 were used): 6.5.

[0260] Example 16c Antibody-drug conjugate (16c)

[Chem.49]

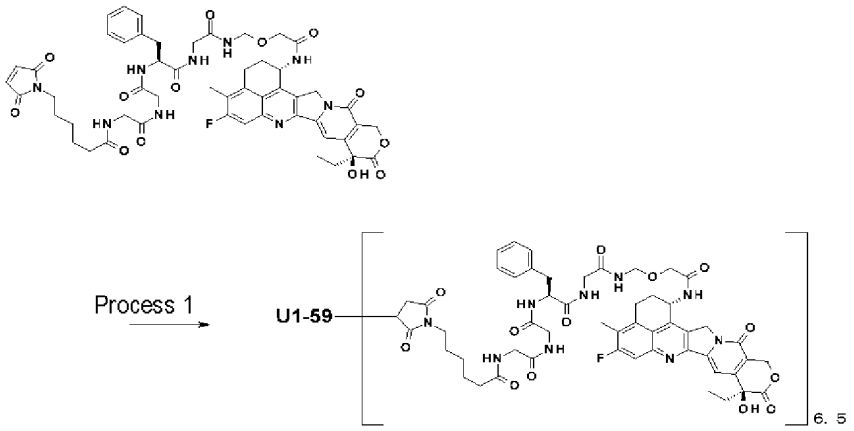


[0261] By using U1-59 produced in Reference Example 1 and the compound obtained in above Process 8 of Example 12, the titled antibody-drug conjugate was obtained in the same manner as Example 16b.

Antibody concentration: 16.21 mg/mL, antibody yield: 9726 mg (600 mL, 108%), and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 and $E_{D,370}$ = 20217 were used): 6.5.

[0262] Example 16d Antibody-drug conjugate (16d)

[Chem.50]



[0263] The antibody-drug conjugates (16a), (16b), and (16c) produced in Examples 16a, 16b, and 16c, respectively, were mixed (a total of about 18 g) and further subjected to ultrafiltration in the same manner as Example 16b (11 L of ABS was used). The obtained purified solution was subjected to microfiltration (Millipore Corp. Stericup, 0.45 um and 0.22 um, PVDF membrane) to yield 745 mL of a solution containing the titled antibody-drug conjugate. By further adding 110 mL of ABS, 855 mL of a solution containing the titled antibody-drug conjugate was obtained.

Antibody concentration: 20.0 mg/mL, antibody yield: 17.1 g (94%), average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure E (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 and $E_{D,370}$ = 20217 were used): 6.5, and average number of conjugated drug molecules (n) per antibody molecule measured by Common procedure F (as molar absorption coefficient of the drug linker, $E_{D,280}$ = 5178 was used): 7.8.

[0264] Test Example 1 HER3 binding affinity of antibody-drug conjugate compared with U1-59

Method:

A human breast cancer cell line HCC1569 (CRL-2330) from ATCC was cultured in an RPMI1640 medium (purchased from Invitrogen Corp., containing 10% bovine serum albumin (manufactured by Invitrogen Corp.) and 2 mM L-glutamine (manufactured by Invitrogen Corp.)). The cells were dissociated from the culture plate using ACCUTASE(R) SOLUTION (Millipore Corp., SCR005) or EDTA (5 mM, phosphate buffered saline (PBS, 137 mM sodium chloride, 2.7 mM potassium chloride, 1.47 mM potassium dihydrogen phosphate, and 10.5 mM disodium hydrogen phosphate)), and the number of living cells was measured by trypan blue treatment. The same numbers of cells suspended in a fluorescence-activated cell sorting (FACS) buffer (PBS containing 3% FBS and 0.004% sodium azide) were inoculated to 96-well U-bottom plates, and the cells were precipitated by centrifugation and suspended in

100 uL of an ice-cooled antibody or antibody-drug conjugate dilution or FACS buffer. The antibody or each antibody-drug conjugate was serially diluted at a ratio of 1/3 with a FACS buffer and adjusted to 30 ug/mL to 5 ng/mL (200 nM to 0.03 nM). Cells treated with a FACS buffer without the addition of a primary antibody were used as a control group.

U1-59, the antibody-drug conjugate (3), the antibody-drug conjugate (10), or the antibody-drug conjugate (13) was evaluated as the antibody or the antibody-drug conjugates.

The cells of each group were reacted with a primary antibody dilution for 45 minutes on ice and then washed with a FACS buffer. Further, 100 uL of a FACS buffer or a reaction solution of a secondary antibody diluted 1/100 (phycoerythrin (PE)-coupled anti human antibody, Dianova GmbH #709-116-149) was added thereto. The cells were treated for 45 minutes on ice in the dark and then washed with a FACS buffer, and dead cells were excluded using a FACS buffer or a FACS buffer supplemented with 7-aminoactinomycin D (7AAD, Sigma-Aldrich Co. LLC, #A9400, 1.1 ug/mL). Fluorescence signals from living cells were evaluated using Accuri C6 Flow cytometer (BD Biosciences/Accuri(R) Cytometers Inc., serial number 1424) and CFlow software (CFlow sampler Version 1.0.264.13).

For the correction of PE- and 7-AAD-derived signals, the fluorescence signals of U1-59 (30 ug/mL) and cells stained with the PE-labeled secondary antibody or 7-AAD were evaluated.

In order to quantify U1-59-specific fluorescence signals in the cells, values obtained by the subtraction of FL-2 signals of cells treated with only the secondary antibody or 7-AAD were used. The equilibrium binding affinity (KD) and the maximum binding strength (Bmax) were calculated using GraphPad Prism software (version 5.04 for Windows(R) (one-site-specific binding)).

[0265] The results are shown in Figure 3 and Table 1. Figure 3 and Table 1 show the mean fluorescence intensity of HCC1569 treated with serial dilutions of U1-59 or each antibody-drug conjugate. The equilibrium binding affinity KD and the maximum binding strength Bmax were calculated using GraphPad Prism software.

[0266] [Table 1]

	U1-59	Antibody-drug conjugate (3)	Antibody-drug conjugate (10)	Antibody-drug conjugate (13)
Bmax	31830	28981	28841	28415
Kd (nM)	1.672	1.653	1.554	2.757

[0267] The antibody-drug conjugate (3) or the antibody-drug conjugate (10) exhibited average binding affinity KD for HCC-1569 equivalent to KD of the non-conjugated

anti-HER3 antibody U1-59. The antibody-drug conjugate (13) also exhibited average binding affinity KD equivalent to KD of the non-conjugated anti-HER3 antibody U1-59 (2.7 nM vs. 1.6 nM). The KD values of the different antibody-drug conjugates suggested that the antibody-drug conjugation processes do not significantly impair the binding affinity of U1-59.

[0268] Test Example 2 Inhibition of HER3 signal by anti-HER3 antibody-drug conjugate

Method:

A human lung cancer cell line A549 (CRS-300114) from Cell Lines Service was dissociated by trypsin treatment, and 50,000 living cells were inoculated to 3 mL of DMEM/F12 (Invitrogen Corp., #21331-020) + 10% FBS (Invitrogen Corp., #10270-106) in each of 6 wells. After culturing the cells for 3 days, the medium was replaced with 2 mL of a fresh medium.

The antibody or each antibody-drug conjugate was added directly to 2 mL of a medium in each of the 6 wells such that the final concentration was 10 ug/mL (20 uL of a stock solution of 1 ug/uL antibody or antibody-drug conjugate was added).

U1-59, the antibody-drug conjugate (3), the antibody-drug conjugate (10), or the antibody-drug conjugate (13) was used as the antibody or the antibody-drug conjugate. An untreated group was used as a control.

The cells were cultured for 2 days, washed once with PBS, and treated with 100 uL of an ice-cooled buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 150 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid (EDTA), 12.5% glycerin, 1% Triton X-100, and 10 mM sodium pyrophosphate tetrabasic supplemented with proteinase inhibitors (Roche Diagnostics, Inc., #11697 498 001), 10 mM sodium fluoride, 1 mM sodium vanadate, 1 mM phenylmethane-sulfonyl-fluoride (PMSF), and 10 ug/mL aprotinin (Sigma-Aldrich Co. LLC, A1153)) for 30 minutes at 4C for lysis. The lysate was washed for 20 minutes at 13000 rpm at 4C, and the supernatant was used in protein concentration measurement by Bradford assay (Sigma-Aldrich Co. LLC, #B6916, BSA standard was from Thermo Fisher Scientific Inc., #23209). To each sample (amount of proteins: 120 ug), a 4-fold concentration of an LDS buffer (Invitrogen Corp., containing DTT (final concentration: 166.67 mM)) was added, and its volume was finally adjusted to 40 uL with water. The sample was boiled for 10 minutes at 70C and added to wells of NuPage Mini Bis-Tris gel (4%-12%, 1.5 mm thick, 10 slots/gel, Invitrogen Corp.). As protein standards, 7.5 uL of Novex(R) sharp ladder (Invitrogen Corp., P/N 57318) was added. The sample was electrophoresed for 70 minutes at 175 V with 1 x MOPS Running buffer (Invitrogen Corp.) containing NuPage antioxidant (Invitrogen Corp., NP0005, Lot 1356629 added to the internal chamber. Proteins separated by the gel electrophoresis were transferred to a nitrocellulose membrane (GE Healthcare Life

Sciences) having a pore size of 0.45 um using NuPage transfer buffer (Invitrogen Corp.) containing 10% methanol and NuPage antioxidant (Invitrogen Corp., NP0005, Lot 1356629, 1:1000 dilution). The proteins were transferred for 80 minutes at a constant volume of 30 V.

The transfer membrane was cut, separated into fractions of 100 kDa or larger and 30 to 100 kDa, washed twice with PBS containing 0.1% Tween-20, and blocked by shaking for 1 hour at room temperature using Odyssey Blocking solution (LI-COR, Inc., #927-40000). The transfer membrane thus blocked was treated overnight at 4C with a solution of a diluted primary antibody (mixture of Odyssey blocking solution and PBS in equal amounts).

An anti-HER3 antibody (Santa Cruz Biotechnology, Inc., SC-81455, dilution 1:500) and an anti-phosphorylated HER antibody (Cell Signaling Technology, Inc., #4791, 1:1000) were used as the primary antibody, and an anti-actin antibody (Neomarkers, #MS1295, dilution 1:3333) was used as an electrophoresis control.

The transfer membrane was washed three times (5 minutes for each) with PBS containing 0.1% Tween-20 and reacted with a dilution containing a secondary antibody (mixture of Odyssey blocking solution and PBS in equal amounts) for 1 hour at room temperature in a dark room.

Goat anti mouse IRDye 680RD (LI-COR, Inc., #926-68070, dilution 1:25000) or goat anti rabbit IR Dye 800CW (LI-COR, Inc., #926-32211, dilution 1:10000) was used as the secondary antibody. The transfer membrane was washed three times (6 minutes for each) with PBS containing 0.1% Tween-20, followed by signal detection using Odyssey infrared imager (LI-COR, Inc.).

[0269] The results are shown in Figure 4. A549 cells were cultured for 2 days with U1-59 or different antibody-drug conjugates. HER3 or phosphorylated HER3 was evaluated by Western blotting. pan-Actin was detected as an electrophoresis control.

[0270] As a result of culturing A549 for 2 days with 10 ug/mL U1-59 or antibody-drug conjugate, HER3 phosphorylation was reduced as compared with untreated cells. This reduction in HER3 phosphorylation was equivalent between U1-59 and the antibody-drug conjugate, suggesting that a plurality of drug conjugation processes did not impair a HER3 signal-inhibiting function derived from U1-59.

When A549 was treated with U1-59 or each antibody-drug conjugate for 2 days, reduction in HER3 expression was also observed as compared with untreated cells. The degree of this reduction in expression was equivalent between U1-59 and the antibody-drug conjugate. This suggests that a plurality of drug conjugation processes did not impair U1-59-mediated internalization (see Test Example regarding internalization) and U1-59-induced HER downregulation (see Test Example regarding signal inhibition).

[0271] Test Example 3 Reduction in expression of HER3 on cell surface by U1-59 and antibody-drug conjugate

Method:

HER3 internalization by U1-59 and each antibody-drug conjugate was evaluated by flow cytometry. 70,000 living cells of HCC1569 (from ATCC) were suspended in 0.5 mL of RPMI1640 (Invitrogen Corp., #31870-025) (containing 10% FBS (Invitrogen Corp., #10270-106 or PAN Biotech GmbH, #1505-P131304) and 2 mM glutamine (Invitrogen Corp., #25030-024)) and inoculated to each well of a 24-well plate. The cells were cultured for 4 days, and the medium was replaced with 0.5 mL of a fresh medium before the start of the internalization test. 5 ug of the antibody or each antibody-drug conjugate was added to 0.5 mL in each well of the 24-well plate such that the final concentration was 10 ug/mL. The cells were cultured for 1 hour at 37C in the presence of the antibody or the antibody-drug conjugate. U1-59, the antibody-drug conjugate (3), the antibody-drug conjugate (10), or the antibody-drug conjugate (13) was used as the antibody or the antibody-drug conjugate. Cells as a positive control or a negative control were untreated in some procedures.

For flow cytometry analysis, the cells were washed once with PBS and dissociated from the plate using 5 mM EDTA (100 uL/well) dissolved in ACCUTASE(R) SOLUTION (Millipore Corp., SCR005) or PBS. The cells were suspended in 200 uL of an ice-cooled FACS buffer (PBS containing 3% FBS and 0.004% sodium azide), then added to each well of a 96-well U-bottom plate, and left on ice. The cells were washed once with a FACS buffer. To each sample, 100 uL of U1-59 (10 ug/mL) diluted with a FACS buffer or only a FACS buffer was added. The cells were treated for 45 minutes with shaking on ice and then washed with a FACS buffer, and 100 uL of a PE-labeled secondary antibody anti-human antibody (Dianova GmbH, 709-116-149) dissolved at a ratio of 1:100 in a FACS buffer, or only a FACS buffer was added to each well. The cells were treated for 45 minutes in a dark room with shaking on ice. The cells were washed with a FACS buffer and treated with a FACS buffer or a FACS buffer containing 7AAD (Sigma-Aldrich Co. LLC, A9400, 1.1 ug to 1.25 ug/mL) for staining dead cells. The fluorescence signals of living cells were measured using Accuri C6 Flow cytometer. For the correction of PE and 7-AAD signals, U1-59 (10 ug/mL) and cells stained with only the PE-labeled secondary antibody or 7-AAD were used.

In order to quantify HER3-specific signals, values of cells stained with only the secondary antibody or 7-AAD were subtracted from FL-2 values of cells stained with the primary antibody and the secondary antibody, and further 7-AAD.

When the FL-2 signals of cells untreated with U1-59 or the antibody-drug conjugate (without internalization) were defined as the maximum value, reduction in HER3

(internalization) on the surface of the cells treated with U1-59 or the antibody-drug conjugate at 37C was calculated.

An average value calculated from 2 to 3 wells was used for the positive control (without treatment at 37C) and the negative control (without the addition of the primary antibody), and internalization in the wells of each treatment group was quantified.

[0272] The results are shown in Figure 5. This diagram shows an average value of reduction in HER3 expression on the surface of HCC1569 cells treated with U1-59 or each antibody-drug conjugate (37C, 1 hr). The HER3 expression of a group without the addition of U1-59 or the antibody-drug conjugate was defined as the maximum value of HER3 expression in cells. The values of groups treated with only the secondary antibody or 7-AAD were used as backgrounds. Groups treated with U1-59 or each antibody-drug conjugate for 1 hour were used as treatment groups. The FL-2 values were almost the same between U1-59 and the antibody-drug conjugate.

[0273] Reduction in generated fluorescence caused by the treatment of HCC-1569 with U1-59 or each antibody-drug conjugate indicates reduction in HER3 expression. As compared with about 50% reduction in HER3 expression by U1-59, reduction in HER3 expression by each antibody-drug conjugate also exhibited a value equivalent to or higher than it, suggesting that the drug conjugation processes of the antibody did not impair the HER3-internalizing function of the antibody.

[0274] Test Example 4 Inhibition of in vitro mitogenic or survival signal by HER3 antibody-drug conjugate in human cancer cell line

Method:

The inhibitory activity of each HER3 antibody-drug conjugate against mitogenic or survival signals was measured in the presence of 10% FBS. The growth and development of cells were evaluated by measuring adenosine triphosphate (ATP) activity in untreated and antibody-drug conjugate-treated groups. Adherent cancer cell lines (human breast cancer cell line HCC1569 (CRL-2330) from ATCC, human breast cancer cell line MDA-MB-453 (CLB-22) from ATCC, and human colorectal cancer cell line HT-29 (CPQ-57) from ProQinase GmbH) were cultured in 2D culture systems, and floating (non-adherent) cancer cell lines (human melanoma cell line A375 (CRL-1619) from ATCC and human lung cancer cell line A549 (CRS-300114) from Cell Lines Service) were cultured in 3D culture systems.

Treatment of adherent cell

Each cancer cell line was suspended in 100 uL of each medium at a low density (500 cells/well for HT-29, 800 cells/well for MDA-MB-453, and 1000 cells/well for HCC-1569) and inoculated to 96-MicroWell Optical Bottom plate (Thermo Fisher Scientific Inc./Nunc, #165306, white wall and clear bottom). As for HCC-1569 and MDA-

MB-453, the cells were cultured in an RPMI1640 medium (Invitrogen Corp., 31870-025) containing 10% FBS (Invitrogen Corp., 10270-106) and 2 mM glutamine (Invitrogen Corp., 25030-024). As for HT-29, the cells were cultured in a McCoy's 5A medium (Invitrogen Corp., 26600-023) containing 10% FBS (Invitrogen Corp., 10270-106) and 2 mM glutamine (Invitrogen Corp., 25030-024). The edge wells of each plate were filled with 100 uL of a medium.

The cells were cultured for 3 days, and the medium was replaced with 95 uL of a fresh medium before antibody-drug conjugate treatment.

The antibody-drug conjugate (3), the antibody-drug conjugate (10), and the antibody-drug conjugate (13) were used. An untreated group was set as a control for measuring normal cell growth.

By adding 5 uL of each antibody-drug conjugate concentrated into a 20-fold concentration to 95 uL of a culture medium (10% FBS) contained in each well of the 96-well plate, the final concentration was established. Only 5 uL of a medium was added to each control well. The test was conducted in triplicate per sample.

In order to calculate the concentration of the antibody-drug conjugate at which the growth or survival of cells was reduced by 50%, concentrations of the antibody-drug conjugate were prepared by 4-fold dilutions (10 ug/mL to 0.15 ng/mL or 40 ug/mL to 0.15 ng/mL), and the cells were treated with these concentrations of the antibody-drug conjugate and compared with the untreated group in terms of ATP activity. Evaluation was carried out by 5-day culture of HT-29 and 7-day culture of HCC-1569 and MDA-MB-453 thus supplemented with the antibody-drug conjugate. CellTiter-Glo(R) Luminescent Cell Viability Assay was used for evaluating the activity of the antibody-drug conjugate. This method involves measuring living cells having metabolic activity on the basis of ATP activity and finally estimating the number of living cells and employed CellTiter-Glo(R) Luminescent Cell (Promega K.K., G7573) as a kit.

100 uL of CellTiter-Glo(R) reagent was added to each well of the 96-well plate and stored for 25 minutes to 65 minutes at room temperature in a dark room before measurement using Wallac Victor2 1420 Multilabel Counter (program luminescence, measurement time: 0.5 s). Wells containing only a culture solution without the inoculation of the cells were assayed as blanks. In order to measure reduction in ATP activity, an average luminescence value of 3 wells was calculated under each condition (Microsoft Excel 2010). In order to remove cell-independent signals, the average luminescence value of the blanks was subtracted from the average luminescence value of the cells treated with the antibody-drug conjugate (Microsoft Excel 2010). The rate of reduction (%) in luminescence was calculated by comparison with the cells of the untreated group (Microsoft Excel 2010). This value was interpreted as the rate of inhibition (%) of cell growth or survival.

Treatment of floating cells

Since A375 and A549 have a faster growth rate than that of other cell lines, growth measurement was carried out in non-adherent 3D culture systems.

Each cancer cell line was suspended in 75 uL of each medium at a low density (500 cells/well for A375 and 1500 cells/well for A549) and inoculated to a 96-well round-bottom non-adherent 3D culture plate (Prime Surface 96U; Sumitomo Bakelite Co, Ltd.; order no. MS-9096U). As for A375, the cells were cultured in a DMEM medium (Invitrogen Corp., 41965-039) containing 10% FBS (Invitrogen Corp., 10270-106) and 2 mM glutamine (Invitrogen Corp., 25030-024). As for A549, the cells were cultured in a DMEM/F12 medium (Invitrogen Corp., 21331-020) containing 10% FBS (Invitrogen Corp., 10270-106) and 2 mM glutamine (Invitrogen Corp., 25030-024). The edge wells of each plate were filled with 150 uL of a medium. The cells were cultured for 3 days, and the final dose was adjusted to 142.5 uL or 150 uL by adding 67.5 or 75 uL of a fresh medium before antibody-drug conjugate addition. The antibody-drug conjugate (3), the antibody-drug conjugate (10), and the antibody-drug conjugate (13) were used. An untreated group was set as a control for measuring normal cell growth.

By adding 7.5 or 8 uL of each antibody-drug conjugate concentrated into a 20-fold concentration to 142.5 or 150 uL of a culture medium (10% FBS) contained in each well of the 96-well plate, the final concentration was established. The final dose was set to 150 uL or 158 uL. Only 7.5 or 8 uL of a medium was added to each control well. The test was conducted in triplicate per sample.

In order to calculate the concentration of the antibody-drug conjugate at which the growth or survival of cells was reduced by 50%, concentrations of the antibody-drug conjugate were prepared by 4-fold dilutions (10 ug/mL to 0.15 ng/mL or 40 ug/mL to 0.15 ng/mL), and the cells were treated with these concentrations of the antibody-drug conjugate and compared with the untreated group in terms of ATP activity. Evaluation was carried out by 7-day culture of the cells thus supplemented with the antibody-drug conjugate. CellTiter-Glo(R) Luminescent Cell Viability Assay was used for evaluating the activity of the antibody-drug conjugate. This method involves measuring living cells having metabolic activity on the basis of ATP activity and finally estimating the number of living cells and employed CellTiter-Glo(R) Luminescent Cell (Promega K.K., G7573) as a kit.

Before measurement, 50 uL of the medium was removed from each well, and 100 uL of CellTiter-Glo(R) reagent was added to each well of the 96-well plate and stored for 30 minutes to 55 minutes at room temperature in a dark room before measurement using Wallac Victor2 1420 Multilabel Counter (program luminescence, measurement time: 0.5 s). Before measurement, 180 uL was collected from each well and transferred

to measurable 96-MicroWell Optical Bottom white plate. Wells containing only a culture solution without the inoculation of the cells were assayed as blanks. The method for calculating the concentration of the antibody-drug conjugate at which the growth or survival of cells was inhibited by 50% was described in the evaluation method as to the adherent cells.

[0275] The results about the human breast cancer lines HCC1569 and MDA-MB453 are shown in Figures 6 and 7, respectively. The results about the human melanoma line A375 is shown in Figure 8. The results about the human colorectal cancer line HT29 are shown in Figure 9. The results about the human lung cancer line A549 are shown in Figure 10. A of each figure shows cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS. The ordinate depicts a luminescence value indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The data is indicated by mean +/- standard deviation of triplicates. B of each figure shows the rate of reduction in luminescence caused by antibody-drug conjugate treatment when the luminescence of an untreated group was defined as 100%.

[0276] Three types of antibody-drug conjugates were added to various human cancer cell lines in the presence of 10% FBS and evaluated for in vitro growth in 2D or 3D systems. The rate of inhibition of cell growth or development in the untreated group or by each antibody-drug conjugate was calculated from the CellTiter-Glo(R) assay of ATP activity. In the evaluation of the ATP activity, these antibody-drug conjugates strongly inhibited the cell growth or survival of two types of breast cancer cell lines (HCC-1569 and MDA-MB-453) and one type of human melanoma line (A375).

The addition of each antibody-drug conjugate and culture (in the presence of 10% FBS) for 7 days reduced the ATP activity by 55 to 75% in HCC1569, by 60 to 83% in MDA-MB-453, and by 60 to 70% in A375. The inhibitory activity of the antibody-drug conjugate against cell growth or survival was not strong in the human colorectal cancer line HT-29 and the human lung cancer line A549 compared with the human breast cancer and human melanoma lines. In HCC-1569 and MDA-MB-453, the antibody-drug conjugate (10) exhibited strong inhibitory activity, which did not largely differ from the inhibitory activity of the antibody-drug conjugate (3) in vitro. By contrast, in both of the human breast cancer lines, the antibody-drug conjugate (13) exhibited low activity and required a concentration of 15 nM for achieving 50% inhibition of cell growth or survival, though the antibody-drug conjugate (3) or the antibody-drug conjugate (10) achieved this inhibition at 1 nM or lower. In the human melanoma line compared with the human breast cancer lines, the activity of the antibody-drug conjugate (13) was equivalent to that of the antibody-drug conjugate (3) or the antibody-drug conjugate (10).

All of the antibody-drug conjugates supported a maximum rate of inhibition on the order of 61 to 68%. The antibody-drug conjugates achieved 50% inhibition of ATP activity at a concentration of 1 to 4 nM.

In addition to the aforementioned test, the inhibitory activity of the antibody-drug conjugate (13) against the cell growth or survival of a human ovarian cancer cell line OVCAR-8 was also confirmed in vitro (data not shown).

[0277] Test Example 5 Comparison of rate of inhibition of in vitro cell growth or survival of human cancer cell line depending on the number of drug molecules (high or medium) loaded on antibody-drug conjugate

Method:

Antibody-drug conjugates differing in the number of loaded drug molecules were evaluated for in vitro inhibitory activity against cell growth or survival. The high drug loading represents the state where 5 to 7 drug molecules are conjugated with an antibody, and the middle drug loading represents the state where about 3 drug molecules are conjugated with an antibody. The average number of drug molecules conjugated with one antibody was measured by the UV method (described in other parts of the present invention).

[0278] The average number of drug molecules conjugated with one antibody:

High drug loading <HDL>

Antibody-drug conjugate (3): 4.9

Antibody-drug conjugate (10): 6.2

Antibody-drug conjugate (13): 6.2

Middle drug loading <MDL>

Antibody-drug conjugate (4): 2.9

Antibody-drug conjugate (11): 3.0

Antibody-drug conjugate (14): 2.5

[0279] The inhibitory activity of each HER3 antibody-drug conjugate against mitogenic or survival signals was measured in the presence of 10% FBS. The growth and development of cells were evaluated by measuring adenosine triphosphate (ATP) activity in untreated and antibody-drug conjugate-treated groups. The cancer cell line was suspended in 100 uL of each medium at a low density (750 cells/well for a human breast cancer cell line MDA-MB-453 (CLB-22)) from ATCC) and inoculated to 96-MicroWell Optical Bottom white plate (Thermo Fisher Scientific Inc./Nunc, #165306). The cells were cultured in an RPMI1640 medium (Invitrogen Corp., 31870-025) containing 10% FBS (Invitrogen Corp., 10270-106) and 2 mM glutamine (Invitrogen Corp., 25030-024). The edge wells of each plate were filled with 100 uL of a medium.

The cells were cultured for 3 days, and the medium was replaced with 95 uL of a

fresh medium before antibody-drug conjugate addition. The antibody-drug conjugate (3), the antibody-drug conjugate (10), the antibody-drug conjugate (13), the antibody-drug conjugate (4), the antibody-drug conjugate (11), and the antibody-drug conjugate (14) were used. An untreated group was set as a control for measuring normal cell growth.

By adding 5 uL of each antibody-drug conjugate concentrated into a 20-fold concentration to 95 uL of a culture medium (10% FBS) contained in each well of the 96-well plate, the final concentration was established. Only 5 ul of a medium was added to each control well. The test was conducted in triplicate per sample. In order to calculate the concentration of the antibody-drug conjugate at which the growth or survival of cells was reduced by 50%, concentrations of the antibody-drug conjugate were prepared by 4-fold dilutions (10 ug/mL to 0.15 ng/mL), and the cells were treated with these concentrations of the antibody-drug conjugate and compared with the untreated group in terms of ATP activity. Evaluation was carried out by 7-day culture of the cells thus supplemented with the antibody-drug conjugate. CellTiter-Glo(R) Luminescent Cell Viability Assay was used for evaluating the activity of the antibody-drug conjugate. This method involves measuring living cells having metabolic activity on the basis of ATP activity and finally estimating the number of living cells and employed CellTiter-Glo(R) Luminescent Cell (Promega K.K., G7573) as a kit. 100 uL of CellTiter-Glo(R) reagent was added to each well of the 96-well plate and stored for 25 minutes to 55 minutes at room temperature in a dark room before measurement using Wallac Victor2 1420 Multilabel Counter (program luminescence, measurement time: 0.5 s). Wells containing only a culture solution without the inoculation of the cells were assayed as blanks. In order to measure reduction in ATP activity, an average luminescence value of 3 wells was calculated under each condition (Microsoft Excel 2010). In order to remove cell-independent signals, the average luminescence value of the blanks was subtracted from the average luminescence value of the cells treated with the antibody-drug conjugate (Microsoft Excel 2010). The rate of reduction (%) in luminescence was calculated by comparison with the cells of the untreated group (Microsoft Excel 2010). This value was interpreted as the rate of inhibition (%) of cell growth or survival.

[0280] The results are shown in Figures 11 to 13. Figure 11 shows the results of comparing the antibody-drug conjugate (3) with the antibody-drug conjugate (4). Figure 12 shows the results of comparing the antibody-drug conjugate (10) with the antibody-drug conjugate (11). Figure 13 shows the results of comparing the antibody-drug conjugate (13) with the antibody-drug conjugate (14). In each figure, the left diagram shows the rate of inhibition of cell growth or survival derived from the antibody-drug conjugate in the presence of 10% FBS in one of the triplicate tests. The ordinate depicts lumi-

nescence indicating the ATP activity of each sample. The abscissa depicts the concentration of each antibody-drug conjugate. The right diagram shows the comparison of the rate of reduction in luminescence caused by antibody-drug conjugate treatment between high drug loading (HDL) and middle drug loading (MDL) when the luminescence of an untreated group was defined as 100%.

[0281] The high drug loading and middle drug loading antibody-drug conjugates inhibited cell growth or survival through the treatment and 7-day culture of MDA-MB-453. As already shown in the results about the high drug loading, the middle drug loading antibody-drug conjugate (11) exhibited high activity at the same level as that of the antibody-drug conjugate (4). In the comparison between the numbers of loaded drug molecules, the antibody-drug conjugates having a high number of loaded drug molecules exhibited higher reduction in ATP than that of the middle drug loading ones. The antibody-drug conjugate (3), the antibody-drug conjugate (10), and the antibody-drug conjugate (13) having a high number of loaded drug molecules exhibited rates of inhibition of 68%, 76%, and 56%, respectively, at a concentration of 10 ug/mL, whereas the antibody-drug conjugate (4), the antibody-drug conjugate (11), and the antibody-drug conjugate (14) having a middle number of loaded drug molecules merely exhibited rates of inhibition of 44%, 47%, and 27%, respectively, at this concentration. The antibody-drug conjugates having a high number of loaded drug molecules were superior in 50% inhibition concentration of cancer cell growth or survival to the antibody-drug conjugates having a middle number of loaded drug molecules. The antibody-drug conjugate (3) or the antibody-drug conjugate (10) having a high number of loaded drug molecules required 15 ng/mL (1 nM) of the antibody-drug conjugate for reducing the ATP activity value by 50%. The antibody-drug conjugate (13) reduced the ATP activity by 50% at least at the highest concentration tested. By contrast, reduction in ATP activity corresponding to this was not observed at 1000 ng/mL (67 nM) or lower within the range of concentrations of the evaluated antibody-drug conjugates having a middle number of loaded drug molecules. The in vitro comparison between the high number of loaded drug molecules and the middle number of loaded drug molecules suggested that an antibody-drug conjugate having a high number of loaded drug molecules is also superior in in vivo inhibitory activity against the growth of cancer cells.

[0282] Test Example 6 Antibody-drug conjugates (3), (10), and (13) exhibited antitumor effect in in vivo antitumor test using human breast cancer

Five-week-old female BALB/C nude mice having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every

other day, and the behaviors of the animals were recorded every day. $5 \acute{} 10^6$ cells of a human breast cancer cell line HCC1569 (CRL-2330) from ATCC were suspended in a solution prepared from 50 uL of PBS and Matrigel (PBS: PAA #H21-002, Matrigel: BD #354230) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each BALB/C nude mouse using a 29 G needle.

The body weights were measured using a weight scale (Mettler Toledo PB602-L). The major axis and minor axis of the tumor were measured twice a week using an electronic digital caliper (manual caliper, OMC Fontana), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression (the same holds true for Test Examples described below).

Tumor volume (mm^3) = 1/2 ´ Major axis (mm) ´ [Minor axis (mm)]^2

At Day 19 when the tumor size reached about 150 mm^3, 70 animals were randomly divided into 7 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (3), (10), or (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0283] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (3) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (10) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 3 or 10 mg/kg.

[0284] All data were indicated by mean +/- SEM. The tumor sizes and the body weights were evaluated by mean +/- SEM. All data were analyzed using Microsoft Excel 2009 (the same holds true for Test Examples described below).

[0285] The results are shown in Figure 14. The PBS administration group was euthanized at Day 53 after the transplantation, because the tumor sizes exceeded the acceptable maximum level. The inhibition of tumor growth of the human breast cancer cell line was observed in all of the antibody-drug conjugate administration groups compared with the control group. No weight loss was observed in the mice of the treated groups.

[0286] Test Example 7 Antibody-drug conjugates (3), (10), and (13) exhibited antitumor effect in antitumor test using human melanoma

Five- to 6-week-old female NMRI nude mice having a body weight of 22 to 26 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

$5 \acute{} 10^6$ cells of a human melanoma cell line HT-144 (HTB-63) from ATCC were suspended in a solution prepared from 50 uL of PBS and Matrigel (PBS: PAA #H21-002, Matrigel: BD #354230) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each NMRI nude mouse using a 29 G needle.

The measurement of the body weights and the tumor sizes and the measurement and calculation of the tumor volumes were carried out in the same manner as Test Example 6.

At Day 22 when the tumor size reached about 150 mm^3, 80 animals were randomly divided into 8 groups on the basis of their tumor sizes. At the same day, U1-59 or the antibody-drug conjugate (3), (10), or (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0287] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: U1-59 was subcutaneously injected twice a week at 25 mg/kg.

Administration group: The antibody-drug conjugate (3) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (10) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 3 or 10 mg/kg.

[0288] The results are shown in Figure 15. The PBS and U1-59 administration groups were euthanized at Days 48 and 52, respectively, after the transplantation, because the tumor sizes exceeded the acceptable maximum level. The inhibition of tumor growth of the human melanoma cell line was observed in all of the antibody-drug conjugate administration groups compared with the control group and the U1-59 administration group.

[0289] Test Example 8 Antibody-drug conjugates (3), (10), and (13) exhibited antitumor effect in antitumor test using human breast cancer line

Sixteen-week-old female SCID nude mice having a body weight of 17 to 25.5 g after acclimation were used. The mice were placed in individually ventilated cages which were kept at room temperature and a constant humidity.

For solid tumors derived from a human breast cancer cell line MDA-MB-453 (CLB-22) from ATCC, MDA-MB-453 in the first passage (Batch 1089) was transplanted to 3 mice (two areas per mouse: right and left side areas of the body). After 13 to 17 weeks, tumor sections were recovered and cryopreserved. For the second passage, the tumor section ($2 \acute{} 2 \acute{} 2$ mm) of the first passage was further subcutaneously transplanted (10 mice, two areas per mouse: right and left side areas of the body) and allowed to grow for tumor formation for 7 weeks. The tumor thus formed

was prepared into a tumor section (2 ´ 2 ´ 2 mm, second passage) and transplanted to the right side area of the body of each SCID nude mouse.

The body weights were measured using a weight scale. The major axis (length) and diameter of the tumor were measured using an electronic digital caliper (Pro-Max 150 mm hand-held calipers, Fred V. Fowler Co., Inc.), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = pi/6 ´ Major axis (mm) ´ [Diameter (mm)]^2

At Day 40 when the tumor size reached about 143 mm^3, 72 animals were randomly divided into 8 groups on the basis of their tumor sizes. At the same day, U1-59 or the antibody-drug conjugate (3), (10), or (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0290] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: U1-59 was subcutaneously injected twice a week at 25 mg/kg.

Administration group: The antibody-drug conjugate (3) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (10) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 3 or 10 mg/kg.

[0291] The results are shown in Figure 16. The inhibition of tumor growth of the human breast cancer cell line was observed in all of the antibody-drug conjugate administration groups compared with the control group and the U1-59 administration group. No weight loss was observed in the mice of the treated groups. Further, in other antitumor test using human breast cancer cell line HCC1954 or JIMT1-PR10 (trastzumab-,pertuzumab- and T-DM1- resistant), the inhibition of tumor growth was also observed in the antibody-drug conjugate (16a) administration group compared with the control group.

[0292] Test Example 9 Antibody-drug conjugates (3), (10), and (13) exhibited antitumor effect in antitumor test using human colorectal cancer line

Five- to 6-week-old female NMRI nude mice having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

4´10^6 cells of a human colorectal cancer cell line HT-29 (CPQ-57) from ProQinase GmbH were suspended in a solution prepared from PBS and Matrigel (PBS: PAA #H21-002, Matrigel: BD #354230) mixed at a ratio of 1:1, and subcutaneously

transplanted to the right side area of the body of each NMRI nude mouse using a 29 G needle.

The measurement of the body weights and the tumor sizes and the measurement and calculation of the tumor volumes were carried out in the same manner as Test Example 6.

At Day 8 when the tumor size reached about 150 mm^3, 70 animals were randomly divided into 7 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (3), (10), or (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0293] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (3) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (10) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 3 or 10 mg/kg.

[0294] The results are shown in Figure 17. The PBS administration group (6 out of the 10 mice), the groups given the antibody-drug conjugate (3) at 3 mg/kg (3 out of the 10 mice) and at 10 mg/kg (4 out of the 10 mice), the groups given the antibody-drug conjugate (10) at 3 mg/kg (2 out of the 10 mice) and at 10 mg/kg (2 out of the 10 mice), and the group given the antibody-drug conjugate (13) at 3 mg/kg (2 out of the 10 mice) were euthanized at Day 50 after the transplantation, because the tumor sizes exceeded the acceptable maximum level or ulcer was formed. The inhibition of tumor growth of the human colorectal cancer cell line was observed in all of the antibody-drug conjugate administration groups compared with the control group. The antibody-drug conjugate (13) exhibited stronger antitumor activity than that of the antibody-drug conjugate (3) or the antibody-drug conjugate (10). No weight loss was observed in the mice of the treated groups.

[0295] Test Example 10 Antibody-drug conjugates (3), (10), and (13) exhibited antitumor effect in antitumor test using human lung cancer line

Five- to 6-week-old female CD1 nude mice having a body weight of 24 to 28 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

$5 \acute{} 10^6$ cells of a human lung cancer cell line A549 (CRS-300114) from Cell Lines Service were suspended in a solution prepared from 200 uL of PBS and Matrigel (PBS:

PAA #H21-002, Matrigel: BD #354230) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each CD1 nude mouse using a 29 G needle.

The measurement of the body weights and the tumor sizes and the measurement and calculation of the tumor volumes were carried out in the same manner as Test Example 6.

At Day 38 when the tumor size reached about 200 mm^3, 70 animals were randomly divided into 7 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (3), (10), or (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0296] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (3) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (10) was intravenously injected once a week at 3 or 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 3 or 10 mg/kg.

[0297] The results are shown in Figure 18. The inhibition of tumor growth of the human lung cancer cell line was observed in all of the antibody-drug conjugate administration groups compared with the control group. No weight loss was observed in the mice of the treated groups.

[0298] Test Example 11 Antibody-drug conjugate (13) exhibited antitumor effect in in vivo antitumor test using human triple-negative breast cancer line

The triple-negative breast cancer refers to a breast cancer that neither expresses hormone receptors (estrogen receptor and progesterone receptor) nor expresses HER2. Since these receptors are not expressed, hormone treatment (tamoxifen, etc.) or anti-HER2 treatment (trastuzumab, trastuzumab emtansine, or pertuzumab) cannot be applied to the cancer. This breast cancer therefore leads to low survival rates, and many therapeutic agents are still under clinical trial. As the expression of HER3 was confirmed in a human triple-negative breast cancer line MDA-MB-468 (data not shown), the antitumor activity of the antibody-drug conjugate was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj 〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

5×10^6 cells of a human triple-negative breast cancer cell line MDA-MB-468 (CRL-2322) from ATCC were suspended in a solution prepared from 200 uL of PBS and Matrigel (PBS: PAA #10010-023, Matrigel: BD #354234) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 20 when the tumor size reached about 170 mm^3, 18 animals were randomly divided into 3 groups on the basis of their tumor sizes. At the same day, U1-59 or the antibody-drug conjugate (13) or PBS for a control group were administered into the tail vein of each animal at the following doses.

[0299] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: U1-59 was intravenously injected once a week at 10 mg/kg.

Administration group: The antibody-drug conjugate (13) was intravenously injected once a week at 10 mg/kg.

[0300] The results are shown in Figure 19. The inhibition of tumor growth of the human triple-negative breast cancer line was observed in the antibody-drug conjugate administration group compared with the control group and the U1-59 administration group. No weight loss was observed in the mice of the treated groups.

[0301] Test Example 12 Antibody-drug conjugate (16a) exhibited antitumor effect in in vivo antitumor test using human luminal breast cancer line

The human luminal breast cancer refers to a breast cancer that expresses hormone receptors (estrogen receptor), but expresses no HER2. Since these receptors are not expressed, anti-HER2 treatment (trastuzumab, trastuzumab emtansine, pertuzumab) cannot be applied to the cancer. This breast cancer therefore leads to low survival rates, and many therapeutic agents are still under clinical trial. As the expression of HER3 was confirmed in a human luminal breast cancer line MCF-7 (data not shown), the antitumor activity of the antibody-drug conjugate was evaluated.

Five- to 6-week-old female athymic nude mice Nude-Foxn1^{nu} (ProQinase GmbH) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

$5 \acute{} 10^6$ cells of a human luminal breast cancer cell line MCF-7 (CRQ-#327) were suspended in a solution prepared from 200 uL of PBS and Matrigel (PBS: PAA #10010-023, Matrigel: BD #354234) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L). The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 ´ Major axis (mm) ´ [Minor axis (mm)]^2

At Day 11 when the tumor size reached about 250 mm^3, 20 animals were randomly divided into 2 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a) or PBS for a control group were administered at the following doses.

[0302] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 10 mg/kg.

[0303] The results are shown in Figure 20. The inhibition of tumor growth of the human luminal breast cancer line was observed in the antibody-drug conjugate administration group compared with the control group. No weight loss was observed in the mice of the treated groups.

[0304] Test Example 13 Antibody-drug conjugate (16a) exhibited antitumor effect in in vivo antitumor test using human melanoma line

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj 〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

$3 \acute{} 10^6$ cells of a human melanoma cell line WM-266-4 (CRL-1676) from ATCC were mixed and suspended in Matrigel (BD #354234) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 ´ Major axis (mm) ´ [Minor axis (mm)]^2

At Day 19 when the tumor size reached about 220 mm^3, 8 animals were randomly

divided into 2 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a) or PBS for a control group were administered at the following doses.

[0305] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 10 mg/kg.

[0306] The results are shown in Figure 21. The inhibition of tumor growth of the human melanoma line was observed in the antibody-drug conjugate administration group compared with the control group. No weight loss was observed in the mice of the treated groups. In other human melanoma model C32, the inhibition of tumor growth was also observed in the antibody-drug conjugate (16a) administration group compared with the control group.

[0307] Test Example 14 Antibody-drug conjugate (16a) exhibited antitumor effect in in vivo antitumor test using human ovarian cancer line

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

5×10^6 cells of a human ovarian cancer cell line OVCAR-8 (HTB-161) from ATCC were mixed and suspended in Matrigel (BD #354234) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 21 when the tumor size reached about 140 mm^3, 8 animals were randomly divided into 2 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a) or PBS for a control group were administered at the following doses.

[0308] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 10 mg/kg.

[0309] The results are shown in Figure 22. The inhibition of tumor growth of the human

ovarian cancer line was observed in the antibody-drug conjugate administration group compared with the control group. No weight loss was observed in the mice of the treated groups.

[0310] Test Example 15 Antibody-drug conjugate (16a) exhibited antitumor effect in in vivo antitumor test using human bladder cancer line

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

8×10^6 cells of a human bladder cancer cell line SW-780 (CRL-2169) from ATCC were mixed and suspended in Matrigel (BD #354234) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 7 when the tumor size reached about 190 mm^3, 10 animals were randomly divided into 2 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a) or PBS for a control group were administered at the following doses.

[0311] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 10 mg/kg.

[0312] The results are shown in Figure 23. The inhibition of tumor growth of the human bladder cancer line was observed in the antibody-drug conjugate administration group compared with the control group. No weight loss was observed in the mice of the treated groups.

[0313] Test Example 16 Antibody-drug conjugate (16a) exhibited HER3-dependent antitumor effect in in vivo antitumor test using human breast cancer line

The human breast cancer line MDA-MB-453 expresses HER3 and responds to the antibody-drug conjugate (13) as described in Test Example 8. However, as it had not been demonstrated that this pharmaceutical effect was mediated by HER3 yet, HER3 was veiled by administering U1-59 beforehand, and whether or not to reduce the pharmaceutical effect was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

1×10^7 cells of a human breast cancer cell line MDA-MB-453 (CLB-22) from ATCC were mixed and suspended in Matrigel (BD #354234) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 11 when the tumor size reached about 130 mm^3, 16 animals were randomly divided into 4 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a) and/or U1-59 or PBS for a control group were administered at the following doses.

[0314] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: U1-59 was intravenously injected at a single dose of 30 mg/kg.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 3 mg/kg.

Administration group: 30 minutes after administration (intravenous injection at a single dose) of U1-59, the antibody-drug conjugate (16a) was intravenously injected at a single dose of 3 mg/kg.

[0315] The results are shown in Figure 24. The inhibition of tumor growth of the human breast cancer line was observed in the antibody-drug conjugate administration group compared with the control group, whereas this tumor inhibitory effect was attenuated by administering U1-59 beforehand. These results demonstrated that the tumor inhibitory effect of the antibody-drug conjugate is a pharmaceutical effect mediated by HER3. No weight loss was observed in the mice of the treated groups.

[0316] Test Example 17 Antibody-drug conjugate (16a) exhibited antitumor effect in combined use with trastuzumab in in vivo antitumor test using human breast cancer line

Trastuzumab has been approved as a therapeutic agent for human HER2-positive breast cancer. However, trastuzumab resistance is known, and a mutation in PIK3CA

<H1047R or H420R> has been reported to participate in one of the mechanisms underlying this resistance. In this test, whether or not the combined use of trastuzumab and the antibody-drug conjugate was effective for a trastuzumab-resistant breast cancer line was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj 〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

1×10^7 cells of a human breast cancer cell line MDA-MB-453 (CLB-22, H1047R mutation in PIK3CA) from ATCC were mixed and suspended in Matrigel (PBS: PAA #10010-023, Matrigel: BD #354234) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 11 when the tumor size reached about 130 mm^3, 16 animals were randomly divided into 4 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a), trastuzumab, combined use of the conjugate and trastuzumab, or PBS for a control group were administered at the following doses.

[0317] Administration group: PBS was intravenously injected at the same single dose as the antibody-drug conjugate.

Administration group: Trastuzumab (Roche Diagnostics, Inc.) was intravenously injected at a single dose of 1 mg/kg.

Administration group: The antibody-drug conjugate (16a) was intravenously injected at a single dose of 3 mg/kg.

Administration group: 30 minutes after administration (intravenous injection at a single dose) of trastuzumab (Roche Diagnostics, Inc.), the antibody-drug conjugate (16a) was intravenously injected at a single dose of 3 mg/kg.

[0318] The results are shown in Figure 25. The antitumor effect brought about by combined use on the human breast cancer line (PIK3CA H1047R) was observed in the administration of trastuzumab and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with trastuzumab. No weight loss was observed in the mice of the treated groups. In other

antitumor test ucing a human breast cancer cell line HCC1954(PIK3CA H1047R), the combined antitumor effect was also observed in the administration of trastuzumab and the antibody-drug conjugate (16a) compared with the administration of each medicine alone.

[0319] Test Example 18 Antibody-drug conjugate (15) exhibited antitumor effect in combined use with trastuzumab in in vivo antitumor test using human breast cancer line

Trastuzumab has been approved as a therapeutic agent for human HER2-positive breast cancer. However, trastuzumab resistance is known, and a mutation in PIK3CA <H1047R or H420R> has been reported to participate in one of the mechanisms underlying this resistance. In this test, whether or not the combined use of trastuzumab and the antibody-drug conjugate was effective for a trastuzumab-resistant breast cancer line was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

5×10^6 cells of a human breast cancer cell line JIMT-1 (ACC-589, H420R mutation in PIK3CA) from ATCC were suspended in PBS (PAA #10010-023) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 10 when the tumor size reached about 200 mm^3, 24 animals were randomly divided into 4 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (15), trastuzumab, combined use of the conjugate and trastuzumab, or PBS for a control group were administered at the following doses.

[0320] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: Trastuzumab (Roche Diagnostics, Inc.) was intravenously injected once a week at 10 mg/kg.

Administration group: The antibody-drug conjugate (15) was intravenously injected once a week at 10 mg/kg.

Administration group: 30 minutes after administration (intravenous injection once a

week) of trastuzumab (Roche Diagnostics, Inc.), the antibody-drug conjugate (15) was intravenously injected once a week at 10 mg/kg.

[0321] The results are shown in Figure 26. The antitumor effect brought about by combined use on the human breast cancer line (PIK3CA H420R) was observed in the administration of trastuzumab and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with trastuzumab. No weight loss was observed in the mice of the treated groups.

[0322] Test Example 19 Antibody-drug conjugate (16a) exhibited antitumor effect in combined use with gefitinib in in vivo antitumor test using human lung cancer line

Gefitinib has been approved as a therapeutic agent for human lung cancer. In this test, whether or not the combined use of gefitinib and the antibody-drug conjugate was effective was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

3×10^6 cells of a human lung cancer cell line PC-9 (RCB0446) from ATCC were suspended in PBS (PAA #10010-023) and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 14 when the tumor size reached about 270 mm^3, 16 animals were randomly divided into 4 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a), gefitinib, combined use of the conjugate and gefitinib, or PBS for a control group were administered at the following doses.

[0323] Administration group: PBS was intravenously injected once a week at the same dose as the antibody-drug conjugate.

Administration group: Gefitinib (AstraZeneca) was orally administered once a day at 6 mg/kg.

Administration group: The antibody-drug conjugate (16a) was intravenously injected once a week at 10 mg/kg.

Administration group: 30 minutes after administration (oral administration once a

day) of gefitinib (AstraZeneca), the antibody-drug conjugate (16a) was intravenously injected once a week at 10 mg/kg.

[0324] The results are shown in Figure 27. The antitumor effect brought about by combined use on the human lung cancer line was observed in the administration of gefitinib and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with gefitinib. No weight loss was observed in the mice of the treated groups.

[0325] Test Example 20 Antibody-drug conjugate (16a) exhibited antitumor effect in combined use with cetuximab or panitumumab in in vivo antitumor test using human triple-negative breast cancer line

An anti-EGFR antibody cetuximab or panitumumab is under clinical trial against human triple-negative breast cancer. In this test, whether or not the combined use of cetuximab or panitumumab and the antibody-drug conjugate was effective was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj 〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

5×10^6 cells of a human triple-negative breast cancer cell line MDA-MB-468 (CRL-2322) from ATCC were suspended in a solution prepared from 200 uL of PBS and Matrigel (PBS: PAA #10010-023, Matrigel: BD #354234) mixed at a ratio of 1:1, and subcutaneously transplanted to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 × Major axis (mm) × [Minor axis (mm)]^2

At Day 21 when the tumor size reached about 160 mm^3, 30 animals were randomly divided into 6 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a), cetuximab or panitumumab, combined use of the conjugate and cetuximab or panitumumab, or PBS for a control group were administered at the following doses.

[0326] Administration group: PBS was administered at the same single dose as the antibody-drug conjugate.

Administration group: Cetuximab (Bristol-Myers Squibb Company) was administered at a single dose of 10 mg/kg.
Administration group: Panitumumab (Amgen Inc.) was administered at a single dose of 10 mg/kg.
Administration group: The antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.
Administration group: 30 minutes after administration (administration at a single dose) of cetuximab (Bristol-Myers Squibb Company), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.
Administration group: 30 minutes after administration (administration at a single dose) of panitumumab (Amgen Inc.), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

[0327] The results are shown in Figure 28. The antitumor effect brought about by combined use on the human triple-negative breast cancer line was observed in the administration of cetuximab (Figure 28A) or panitumumab (Figure 28B) and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with cetuximab or panitumumab. No weight loss was observed in the mice of the treated groups. The antitumor effect brought about by combined use on the other human triple-negative breast cancer line in MDA-MB-231 was also observed in the administration of cetuximab or panitumumab and the antibody-drug conjugate (16a) compared with the administration of each medicine alone.

[0328] Test Example 21 Antibody-drug conjugate (16a) exhibited antitumor effect in combined use with cetuximab or panitumumab in in vivo antitumor test using human head and neck cancer line

An anti-EGFR antibody cetuximab has been approved against human head and neck cancer, while panitumumab is under clinical trial against this cancer. In this test, whether or not the combined use of cetuximab or panitumumab and the antibody-drug conjugate was effective was evaluated.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj 〔Foxn1nu/Foxn1nu〕 nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

4×10^6 cells of a human head and neck cancer cell line Fadu (HTB-43) from ATCC were suspended in 200 uL of PBS (PAA #10010-023) and subcutaneously transplanted

to the right side area of the body of each nude mouse using a 29 G needle. The body weights were measured using a weight scale (Mettler Toledo PB602-L).
The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.
Tumor volume (mm^3) = 1/2 ´ Major axis (mm) ´ [Minor axis (mm)]^2
At Day 6 when the tumor size reached about 330 mm^3, 30 animals were randomly divided into 6 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a), cetuximab or panitumumab, combined use of the conjugate and cetuximab or panitumumab, or PBS for a control group were administered at the following doses.

[0329] Administration group: PBS was administered at the same single dose as the antibody-drug conjugate.

Administration group: Cetuximab (Bristol-Myers Squibb Company) was administered at a single dose of 5 mg/kg.

Administration group: Panitumumab (Amgen Inc.) was administered at a single dose of 5 mg/kg.

Administration group: The antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

Administration group: 30 minutes after administration (administration at a single dose) of cetuximab (Bristol-Myers Squibb Company), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

Administration group: 30 minutes after administration (administration at a single dose) of panitumumab (Amgen Inc.), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

[0330] The results are shown in Figure 29. The antitumor effect brought about by combined use on the human head and neck cancer line was observed in the administration of cetuximab (Figure 29A) or panitumumab (Figure 29B) and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with cetuximab or panitumumab. No weight loss was observed in the mice of the treated groups.

[0331] Test Example 22 Antibody-drug conjugate (16a) exhibited antitumor effect in combined use with cetuximab or pertuzumab in in vivo antitumor test by transplantation of tumor from stomach cancer patient

An anti-EGFR antibody cetuximab and an anti-HER2 antibody pertuzumab are under clinical trial against human stomach cancer. In this test, whether or not the combined use of cetuximab or pertuzumab and the antibody-drug conjugate was effective was

evaluated. The evaluation was carried out by conducting an antitumor test close to clinical condition involving the stroma of a patient using the patient-derived tumors transplanted in mice instead of the general evaluation system using a human cancer line.

Five- to 6-week-old female CAnN.Cg-Foxn1[nu]/CrlCrlj〔Foxn1nu/Foxn1nu〕nude mice (Charles River Laboratories Japan, Inc.) having a body weight of 15 to 20 g after acclimation were used. The mice were placed in individually ventilated cages (IVC, 4 mice at maximum per cage) which were kept at room temperature and a constant humidity. After randomization, the body weights of the mice were measured every other day, and the behaviors of the animals were recorded every day.

A stomach cancer patient-derived tumor NIBIO-G016 from National Institute of Biomedical Innovation was subcutaneously transplanted to the right side area of the body of each nude mouse. The body weights were measured using a weight scale (Mettler Toledo PB602-L).

The major axis and minor axis of the tumor were measured using an electronic digital caliper (CD-15CX, Mitsutoyo Corp), and the tumor volume (mm^3) was calculated. The calculation was carried out according to the following expression.

Tumor volume (mm^3) = 1/2 ´ Major axis (mm) ´ [Minor axis (mm)]^2

At Day 56 when the tumor size reached about 220 mm^3, 30 animals were randomly divided into 6 groups on the basis of their tumor sizes. At the same day, the antibody-drug conjugate (16a), cetuximab or pertuzumab, combined use of the conjugate and cetuximab or pertuzumab, or PBS for a control group were administered at the following doses.

[0332] Administration group: PBS was administered at the same single dose as the antibody-drug conjugate.

Administration group: Cetuximab (Bristol-Myers Squibb Company) was administered at a single dose of 10 mg/kg.

Administration group: Pertuzumab (Roche Diagnostics, Inc.) was administered at a single dose of 10 mg/kg.

Administration group: The antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

Administration group: 30 minutes after administration (administration at a single dose) of cetuximab (Bristol-Myers Squibb Company), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

Administration group: 30 minutes after administration (administration at a single dose) of pertuzumab (Roche Diagnostics, Inc.), the antibody-drug conjugate (16a) was administered at a single dose of 10 mg/kg.

[0333] The results are shown in Figure 30. The antitumor effect brought about by combined

use on the stomach cancer patient-derived tumor model was observed in the administration of cetuximab (Figure 30A) or pertuzumab (Figure 30B) and the antibody-drug conjugate compared with the administration of each medicine alone. These results demonstrated that the pharmaceutical effect of the antibody-drug conjugate is potentiated by the combined use thereof with cetuximab or panitumumab in the cancer patient-derived tumor model. No weight loss was observed in the mice of the treated groups. Further, in an antitumor test using a human pancreatic cancer cell line BxPC3, the antibody-drug conjugate (13) exhibited a stronger antitumor effect as compared with the PBS administration group or the U1-59 administration group. The antibody-drug conjugate also exhibited a strong antitumor effect on in vivo antitumor model using a HER2-positive breast cancer cell line JIMT1 which had acquired resistance to combined use of trastuzumab and pertuzumab or to trastuzumab emtansine.

[0334] Test Example 23 Safety of antibody-drug conjugate for non-human animal

The anti-HER3 antibody-drug conjugate of the present invention and the pharmaceutical composition containing this anti-HER3 antibody-drug conjugate have excellent safety as a therapeutic or prophylactic agent for a disease. For example, when the antibody-drug conjugate (5), (10), or (13) was administered up to 30 mg/kg to a cross-breed rat, two times in total with an interval of once per week, no serious toxicity findings were observed from any of the antibody-drug conjugates as a result of observation until Day 7 after the final administration. Further, when the antibody-drug conjugate (5), (10), or (13) was administered up to 30 mg/kg to a cross-breed monkey, no remarkable toxicity findings were observed from any of the antibody-drug conjugates as a result of observation for 7 days.

Further, when antibody-drug conjugate (5), (10), or (13) was administered at a plurality of doses to a monkey (3-week intervals), no remarkable toxicity findings were observed from any of the antibody-drug conjugates as a result of observation. Accordingly, the antibody-drug conjugate of the present invention has excellent safety as a pharmaceutical composition for treatment or prevention of a disease.

Sequence Listing Free Text

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SEQ ID NO: 163 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 164 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 165 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 166 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 167 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 168 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 169 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-36
SEQ ID NO: 170 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-36
SEQ ID NO: 171 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-36
SEQ ID NO: 172 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-36
SEQ ID NO: 173 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-37
SEQ ID NO: 174 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-37
SEQ ID NO: 175 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 176 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-37
SEQ ID NO: 177 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 178 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 179 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 180 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 181 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 182 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 183 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 184 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 185 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 186 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 187 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 188 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 189 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-4
SEQ ID NO: 190 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-4
SEQ ID NO: 191 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-4
SEQ ID NO: 192 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-4
SEQ ID NO: 193 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-5
SEQ ID NO: 194 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 195 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-5
SEQ ID NO: 196 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-5
SEQ ID NO: 197 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-6
SEQ ID NO: 198 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-6
SEQ ID NO: 199 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-6
SEQ ID NO: 200 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-6
SEQ ID NO: 201 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-8
SEQ ID NO: 202 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-8
SEQ ID NO: 203 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-8
SEQ ID NO: 204 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-8
SEQ ID NO: 205 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 206 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 207 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 208 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 209 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 210 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 211 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 212 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 213 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-17

SEQ ID NO: 214 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 215 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 216 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 217 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 218 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 219 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 220 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 221 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 222 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 223 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 224 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 225 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 226 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 227 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 228 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 229 - Nucleotide sequence encoding a heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 230 - Heavy chain variable region amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 231 - Nucleotide sequence encoding a light chain variable region amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 232 - Light chain variable region amino acid sequence of the anti-HER3 human antibody U1-30

SEQ ID NO: 233 - Primer
SEQ ID NO: 234 - Primer
SEQ ID NO: 235 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 236 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 237 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 238 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 239 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 240 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-1
SEQ ID NO: 241 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 242 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 243 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 244 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 245 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 246 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-2
SEQ ID NO: 247 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 248 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 249 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 250 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 251 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-3
SEQ ID NO: 252 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-3

SEQ ID NO: 253 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 254 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 255 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 256 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 257 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 258 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-4

SEQ ID NO: 259 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 260 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 261 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 262 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 263 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 264 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-5

SEQ ID NO: 265 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 266 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 267 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 268 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 269 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 270 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-6

SEQ ID NO: 271 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 272 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 273 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 274 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 275 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 276 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-7

SEQ ID NO: 277 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 278 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 279 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 280 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 281 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 282 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-8

SEQ ID NO: 283 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 284 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 285 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 286 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 287 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 288 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-9

SEQ ID NO: 289 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-10

SEQ ID NO: 290 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-10

SEQ ID NO: 291 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-10
SEQ ID NO: 292 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-10
SEQ ID NO: 293 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-10
SEQ ID NO: 294 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-10
SEQ ID NO: 295 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 296 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 297 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 298 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 299 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 300 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-11
SEQ ID NO: 301 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 302 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 303 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 304 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 305 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 306 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-12
SEQ ID NO: 307 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-13
SEQ ID NO: 308 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-13
SEQ ID NO: 309 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-13

SEQ ID NO: 310 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-13
SEQ ID NO: 311 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-13
SEQ ID NO: 312 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-13
SEQ ID NO: 313 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 314 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 315 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 316 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 317 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 318 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-14
SEQ ID NO: 319 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 320 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 321 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 322 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 323 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 324 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-15
SEQ ID NO: 325 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 326 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 327 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 328 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-16

SEQ ID NO: 329 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 330 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-16
SEQ ID NO: 331 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 332 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 333 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 334 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 335 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 336 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-17
SEQ ID NO: 337 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 338 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 339 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 340 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 341 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 342 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-18
SEQ ID NO: 343 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-19
SEQ ID NO: 344 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-19
SEQ ID NO: 345 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-19
SEQ ID NO: 346 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-20
SEQ ID NO: 347 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-20

SEQ ID NO: 348 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-20
SEQ ID NO: 349 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-20
SEQ ID NO: 350 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-20
SEQ ID NO: 351 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-20
SEQ ID NO: 352 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 353 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 354 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 355 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 356 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 357 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-21
SEQ ID NO: 358 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 359 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 360 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 361-ight chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 362 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 363 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-22
SEQ ID NO: 364 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-23
SEQ ID NO: 365 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-23
SEQ ID NO: 366 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-23

SEQ ID NO: 367 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-23
SEQ ID NO: 368 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-23
SEQ ID NO: 369 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-23
SEQ ID NO: 370 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 371 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 372 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 373 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 374 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 375 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-24
SEQ ID NO: 376 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 377 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 378 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 379 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 380 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 381 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-25
SEQ ID NO: 382 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-26
SEQ ID NO: 383 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-26
SEQ ID NO: 384 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-26
SEQ ID NO: 385 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-26

SEQ ID NO: 386 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-26
SEQ ID NO: 387 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-26
SEQ ID NO: 388 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 389 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 390 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 391 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 392 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 393 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-27
SEQ ID NO: 394 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 395 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 396 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 397 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 398 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 399 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-28
SEQ ID NO: 400 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 401 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 402 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 403 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 404 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-29

SEQ ID NO: 405 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-29
SEQ ID NO: 406 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 407 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 408 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 409 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 410 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 411 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-30
SEQ ID NO: 412 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 413 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 414 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 415 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 416 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 417 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-31
SEQ ID NO: 418 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 419 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 420 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 421 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 422 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-32
SEQ ID NO: 423 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-32

SEQ ID NO: 424 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 425 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 426 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 427 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 428 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 429 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-33
SEQ ID NO: 430 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 431 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 432 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 433 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 434 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 435 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-34
SEQ ID NO: 436 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 437 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 438 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 439 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 440 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 441 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-35
SEQ ID NO: 442 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 443 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 444 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 445 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 446 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 447 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-36

SEQ ID NO: 448 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 449 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 450 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 451 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 452 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 453 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-37

SEQ ID NO: 454 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 455 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 456 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 457 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 458 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 459 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-38

SEQ ID NO: 460 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-39

SEQ ID NO: 461 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-39

SEQ ID NO: 462 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-39
SEQ ID NO: 463 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-39
SEQ ID NO: 464 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-39
SEQ ID NO: 465 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-39
SEQ ID NO: 466 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-40
SEQ ID NO: 467 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-40
SEQ ID NO: 468 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-40
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SEQ ID NO: 470 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-40
SEQ ID NO: 471 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-40
SEQ ID NO: 472 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 473 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 474 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 475 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 476 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 477 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-41
SEQ ID NO: 478 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-42
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SEQ ID NO: 481 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-42
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SEQ ID NO: 486 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-43
SEQ ID NO: 487 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-43
SEQ ID NO: 488 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-43
SEQ ID NO: 489 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-43
SEQ ID NO: 490 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 491 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 492 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 493 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 494 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 495 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-44
SEQ ID NO: 496 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-45
SEQ ID NO: 497 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-45
SEQ ID NO: 498 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-45
SEQ ID NO: 499 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-45

SEQ ID NO: 500 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-45
SEQ ID NO: 501 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-45
SEQ ID NO: 502 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-46
SEQ ID NO: 503 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-46
SEQ ID NO: 504 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-46
SEQ ID NO: 505 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-47
SEQ ID NO: 506 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-47
SEQ ID NO: 507 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-47
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SEQ ID NO: 513 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-48
SEQ ID NO: 514 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-49
SEQ ID NO: 515 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-49
SEQ ID NO: 516 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-49
SEQ ID NO: 517 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-49
SEQ ID NO: 518 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-49

SEQ ID NO: 519 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-49
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SEQ ID NO: 527 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-51
SEQ ID NO: 528 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-51
SEQ ID NO: 529 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-51
SEQ ID NO: 530 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-51
SEQ ID NO: 531 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-51
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SEQ ID NO: 533 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-52
SEQ ID NO: 534 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-52
SEQ ID NO: 535 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-52
SEQ ID NO: 536 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-52
SEQ ID NO: 537 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-52

SEQ ID NO: 538 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-53
SEQ ID NO: 539 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-53
SEQ ID NO: 540 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-53
SEQ ID NO: 541 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-53
SEQ ID NO: 542 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-53
SEQ ID NO: 543 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-53
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SEQ ID NO: 546 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-55.1
SEQ ID NO: 547 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-55
SEQ ID NO: 548 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-55
SEQ ID NO: 549 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-55
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SEQ ID NO: 551 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-57.1
SEQ ID NO: 552 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-57.1
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SEQ ID NO: 554 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-57
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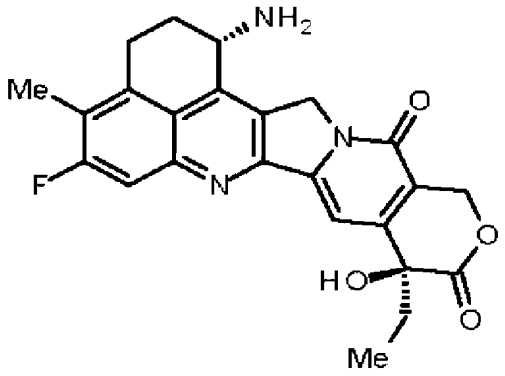
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SEQ ID NO: 560 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-58
SEQ ID NO: 561 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-58
SEQ ID NO: 562 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 563 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 564 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 565 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 566 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 567 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-59
SEQ ID NO: 568 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 569 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 570 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 571 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 572 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 573 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-61.1
SEQ ID NO: 574 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-61
SEQ ID NO: 575 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-61

SEQ ID NO: 576 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-61
SEQ ID NO: 577 - Heavy chain CDRH1 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 578 - Heavy chain CDRH2 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 579 - Heavy chain CDRH3 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 580 - Light chain CDRL1 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 581 - Light chain CDRL2 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 582 - Light chain CDRL3 amino acid sequence of the anti-HER3 human antibody U1-62
SEQ ID NO: 583 - Full-length amino acid sequence of a heavy chain of anti-HER3 human antibody U1-59
SEQ ID NO: 584 - Full-length amino acid sequence of a light chain of anti-HER3 human antibody U1-59

Claims

[Claim 1] An antibody-drug conjugate wherein an antitumor compound represented by the following formula

[Chem.1]



is conjugated to an anti-HER3 antibody by a thioether bond which is formed at a disulfide bond moiety present in a hinge part of the anti-HER3 antibody via a linker having a structure represented by the following formula:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-(CH_2)n^2-C(=O)-$ or $-L^1-L^2-L^P-$

wherein, the anti-HER3 antibody is connected to the terminal of L^1,

the antitumor compound is connected to the carbonyl group of $-(CH_2)n^2-C(=O)-$ moiety or the C terminal of L^P, with the nitrogen atom of the amino group at position 1 as a connecting position,

wherein, n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^1 represents -(Succinimid-3-yl-N)-$(CH_2)n^3$-C(=O)-,

wherein n^3 represents an integer of 2 to 8,

L^2 represents $-NH-(CH_2CH_2-O)n^4-CH_2CH_2-C(=O)-$ or a single bond,

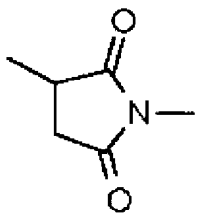
wherein n^4 represents an integer of 1 to 6,

L^P represents a peptide residue consisting of 2 to 7 amino acids,

L^a represents -O- or a single bond,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

[Chem.2]



which is connected to the anti-HER3 antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1.

[Claim 2] The antibody-drug conjugate according to claim 1, wherein the peptide residue of L^P is a peptide residue comprising an amino acid selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid.

[Claim 3] The antibody-drug conjugate according to claim 1 or 2, wherein L^P is a peptide residue selected from the following group:
-GGF-,
-DGGF-,
-(D-)D-GGF-,
-EGGF-,
-GGFG-,
-SGGF-,
-KGGF-,
-DGGFG-,
-GGFGG-,
-DDGGFG-,
-KDGGFG-,
-GGFGGGF-;
wherein, "(D-)D" represents D-aspartic acid.

[Claim 4] The antibody-drug conjugate according to claim 1 or 2, wherein L^P is a peptide residue comprising 4 or 5 amino acids.

[Claim 5] The antibody-drug conjugate according to any one of claims 1 to 4, wherein L^P is -GGFG- or -DGGFG-.

[Claim 6] The antibody-drug conjugate according to any one of claims 1 to 4, wherein L^P is -GGFG-.

[Claim 7] The antibody-drug conjugate according to any one of claims 1 to 6, wherein n^3 is an integer of 2 to 5 and L^2 is a single bond.

[Claim 8] The antibody-drug conjugate according to any one of claims 1 to 7, wherein the linker is -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)-.

[Claim 9] The antibody-drug conjugate according to claim 8, wherein n^3 is an integer of 2 to 5, L^2 is -NH-$(CH_2CH_2$-O$)n^4$-CH_2CH_2-C(=O)-, and n^4 is 2 or 4.

[Claim 10] The antibody-drug conjugate according to claim 8 or 9, wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is a partial structure having a chain length of 4 to 7 atoms.

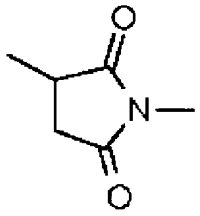
[Claim 11] The antibody-drug conjugate according to claim 8 or 9, wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is a partial structure having a chain length of 5 or 6 atoms.

[Claim 12] The antibody-drug conjugate according to claim 10 or 11, wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is any one of the followings:
-NH-CH_2CH_2-C(=O)-,
-NH-$CH_2CH_2CH_2$-C(=O)-,
-NH-$CH_2CH_2CH_2CH_2$-C(=O)-,
-NH-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-,
-NH-CH_2-O-CH_2-C(=O)-,
-NH-CH_2CH_2-O-CH_2-C(=O)-.

[Claim 13] The antibody-drug conjugate according to claim 12, wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is any one of the followings:
-NH-$CH_2CH_2CH_2$-C(=O)-,
-NH-CH_2-O-CH_2-C(=O)-,
-NH-CH_2CH_2-O-CH_2-C(=O)-.

[Claim 14] The antibody-drug conjugate according to claim 1, wherein the drug-linker structure moiety in which a drug is connected to -L^1-L^2-L^P-NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- or -L^1-L^2-L^P- is one drug-linker structure selected from the following group:
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),

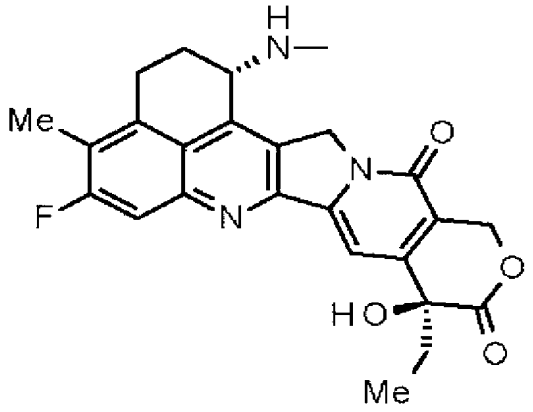
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-(NH-DX),
-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-DGGFG-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-(NH-DX),
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX);
wherein, -(Succinimid-3-yl-N)- has a structure represented by the following formula:
[Chem.3]



which is connected to the anti-HER3 antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,
-(NH-DX) represents a group represented by the following formula,

with the nitrogen atom of the amino group at position 1 being a connecting position,

[Chem.4]



-GGFG- represents a tetrapeptide residue of -Gly-Gly-Phe-Gly- and -DGGFG- represents a pentapeptide residue of -Asp-Gly-Gly-Phe-Gly-.

[Claim 15] The antibody-drug conjugate according to claim 14, wherein a drug-linker structure moiety is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

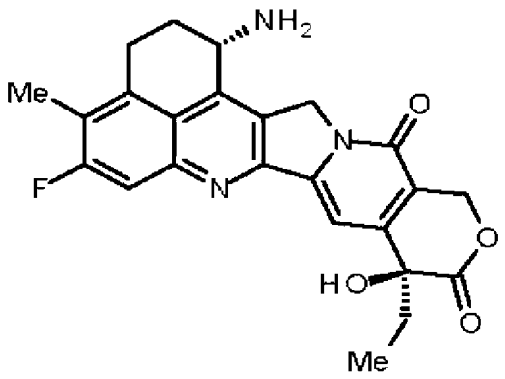
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-DGGFG-(NH-DX).

[Claim 16] An antibody-drug conjugate wherein an antitumor compound represented by the following formula

[Chem.5]



is conjugated to an anti-HER3 antibody by a thioether bond which is formed at a disulfide bond moiety present in a hinge part of the anti-HER3 antibody via a linker having a structure represented by the following formula:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-(CH_2)n^2-C(=O)-$

wherein, the anti-HER3 antibody is connected to the terminal of L^1, the antitumor compound is connected to the carbonyl group of $-(CH_2)n^2-C(=O)-$ moiety,

wherein, n^1 represents an integer of 0 to 6,

n^2 represents an integer of 0 to 5,

L^1 represents -(Succinimid-3-yl-N)-$(CH_2)n^3$-C(=O)-,

wherein n^3 represents an integer of 2 to 8,

L^2 represents $-NH-(CH_2CH_2-O)n^4-CH_2CH_2-C(=O)-$ or a single bond,

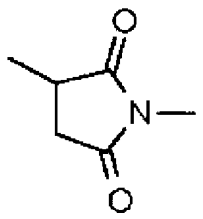
wherein n^4 represents an integer of 1 to 6,

L^P represents a tetrapeptide residue of -GGFG-,

L^a represents -O- or a single bond,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

[Chem.6]



which is connected to the anti-HER3 antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this

structure on the nitrogen atom at position 1.

[Claim 17] The antibody-drug conjugate according to claim 16, wherein n^1 is 3, n^2 is 0, n^3 is 2, L^2 is -NH-$(CH_2CH_2-O)n^4$-CH_2CH_2-C(=O)-, n^4 is 2, and L^a is a single bond, or
n^1 is 1, n^2 is 1, n^3 is 5, L^2 is a single bond, and L^a is -O-, or
n^1 is 2, n^2 is 1, n^3 is 5, L^2 is a single bond, and L^a is -O-.

[Claim 18] The antibody-drug conjugate according to claim 16 or 17, wherein n^3 is 2 or 5 and L^2 is a single bond.

[Claim 19] The antibody-drug conjugate according to claim 16 or 17, wherein n^3 is 2 or 5, L^2 is -NH-$(CH_2CH_2-O)n^4$-CH_2CH_2-C(=O)-, and n^4 is 2 or 4.

[Claim 20] The antibody-drug conjugate according to any one of claims 16 to 19, wherein -NH-$(CH_2)n^1$-L^a-$(CH_2)n^2$-C(=O)- is any one of the followings:
-NH-$CH_2CH_2CH_2$-C(=O)-,
-NH-CH_2-O-CH_2-C(=O)-,
-NH-CH_2CH_2-O-CH_2-C(=O)-.

[Claim 21] The antibody-drug conjugate according to any one of claims 1 to 20, wherein an average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 1 to 10.

[Claim 22] The antibody-drug conjugate according to any one of claims 1 to 20, wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 2 to 8.

[Claim 23] The antibody-drug conjugate according to any one of claims 1 to 20, wherein the average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 3 to 8.

[Claim 24] A medicine comprising the antibody-drug conjugate according to any one of claims 1 to 23, a salt thereof or a hydrate thereof.

[Claim 25] An antitumor medicine and/or anticancer medicine comprising the antibody-drug conjugate according to any one of claims 1 to 23, a salt thereof or a hydrate thereof.

[Claim 26] The antitumor medicine and/or anticancer medicine according to claim 25, which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer,

vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[Claim 27] A pharmaceutical composition comprising the antibody-drug conjugate according to any one of claims 1 to 23, a salt thereof or a hydrate thereof as an active ingredient, and a pharmaceutically acceptable formulation ingredient.

[Claim 28] The pharmaceutical composition according to claim 27, which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[Claim 29] A method for treating tumor and/or cancer comprising administering the antibody-drug conjugate according to any one of claims 1 to 23, a salt thereof or a hydrate thereof.

[Claim 30] The medicine according to claim 24, the antitumor medicine and/or anticancer medicine according to claim 25 or 26, the pharmaceutical composition according to claim 27 or 28, or the treatment method according to claim 29, which is used in administration in combination with an additional medicine.

[Claim 31] The pharmaceutical composition according to claim 27 or 28, further comprising even an additional medicine as an active ingredient.

[Claim 32] The antibody-drug conjugate according to any one of claims 1 to 23, wherein the antibody comprises the CDRH1 to CDRH3 and CDRL1 to CDRL3 of U1-49, U1-53, U1-59, U1-7 or U1-9 in the heavy and light chains, respectively.

[Claim 33] The antibody-drug conjugate according to any one of claims 1 to 23 and 32, wherein the antibody comprises the heavy chain variable region and the light chain variable chain of U1-49, U1-53, U1-59, U1-7 or U1-9 on the heavy and light chains, respectively.

[Claim 34] The antibody-drug conjugate according to any one of claims 1 to 23, 32 and 33, wherein the antibody comprises the amino acid sequences represented by or comprising SEQ ID Nos: 42 and 33, SEQ ID Nos: 54

and 56, SEQ ID Nos: 70 and 72, SEQ ID Nos: 92 and 94, or, SEQ ID Nos: 96 and 98, in the heavy and light chains, respectively.

[Claim 35] The antibody-drug conjugate according to any one of claims 1 to 23 and 32 to 34, wherein the antibody comprises the amino acid sequences represented by or comprising SEQ ID Nos: 583 (Figure 1) and 584 (Figure 2) in the heavy and light chains, respectively.

[Claim 36] A pharmaceutical composition comprising the antibody-drug conjugate according to any one of claims 32 to 35, a salt thereof or a hydrate thereof as an active ingredient, and a pharmaceutically acceptable formulation ingredient.

[Claim 37] A medicine comprising the antibody-drug conjugate according to any one of claims 32 to 35, a salt thereof or a hydrate thereof.

[Claim 38] The composition according to claim 36 or medicine according to claim 37, which is applied to lung cancer, kidney cancer, urothelial cancer, colorectal cancer, prostate cancer, glioblastoma multiforme, ovarian cancer, pancreatic cancer, breast cancer, melanoma, liver cancer, bladder cancer, stomach cancer, gastrointestinal stromal tumor, cervical cancer, head and neck cancer, esophageal cancer, epidermoid cancer, peritoneal cancer, adult glioblastoma multiforme, hepatic cancer, hepatocellular carcinoma, colon cancer, rectal cancer, colon and rectal cancer, endometrial cancer, uterus cancer, salivary cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anus carcinoma, or penis cancer.

[Fig. 1]

Full-length amino acid sequence of heavy chain of anti-HER3 human antibody U1-59 (SEQ ID NO: 583)

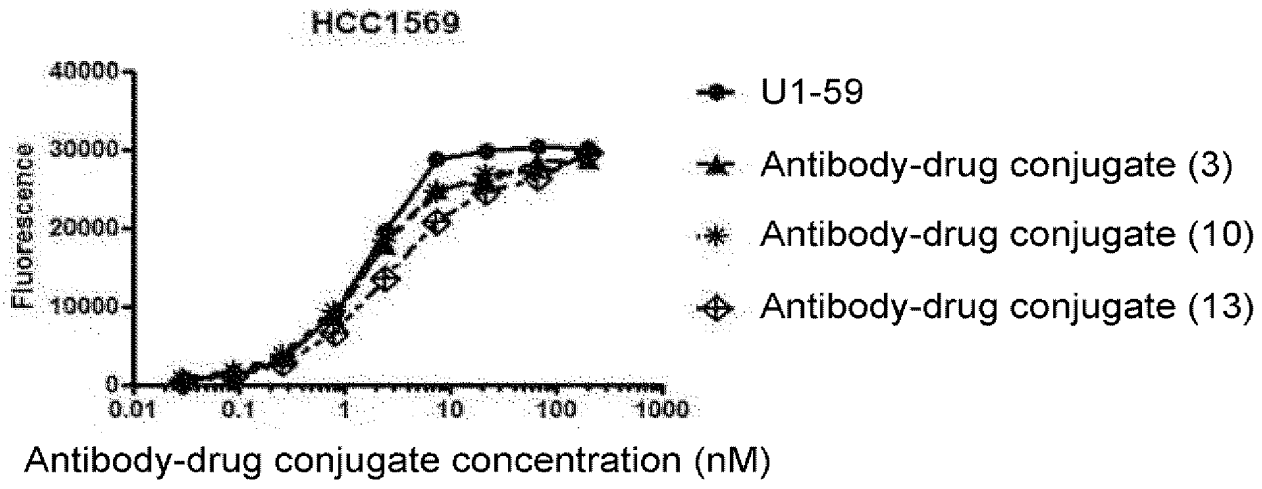
Q V Q L Q Q W G A G L L K P S E T L S L T C A V Y G G S F S G Y Y W S W
I R Q P P G K G L E W I G E I N H S G S T N Y N P S L K S R V T I S V E
T S K N Q F S L K L S S V T A A D T A V Y Y C A R D K W T W Y F D L W G
R G T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C
L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y
S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E
P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M
I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K
T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S
N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K
N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P
V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L
H N H Y T Q K S L S L S P G K

[Fig. 2]

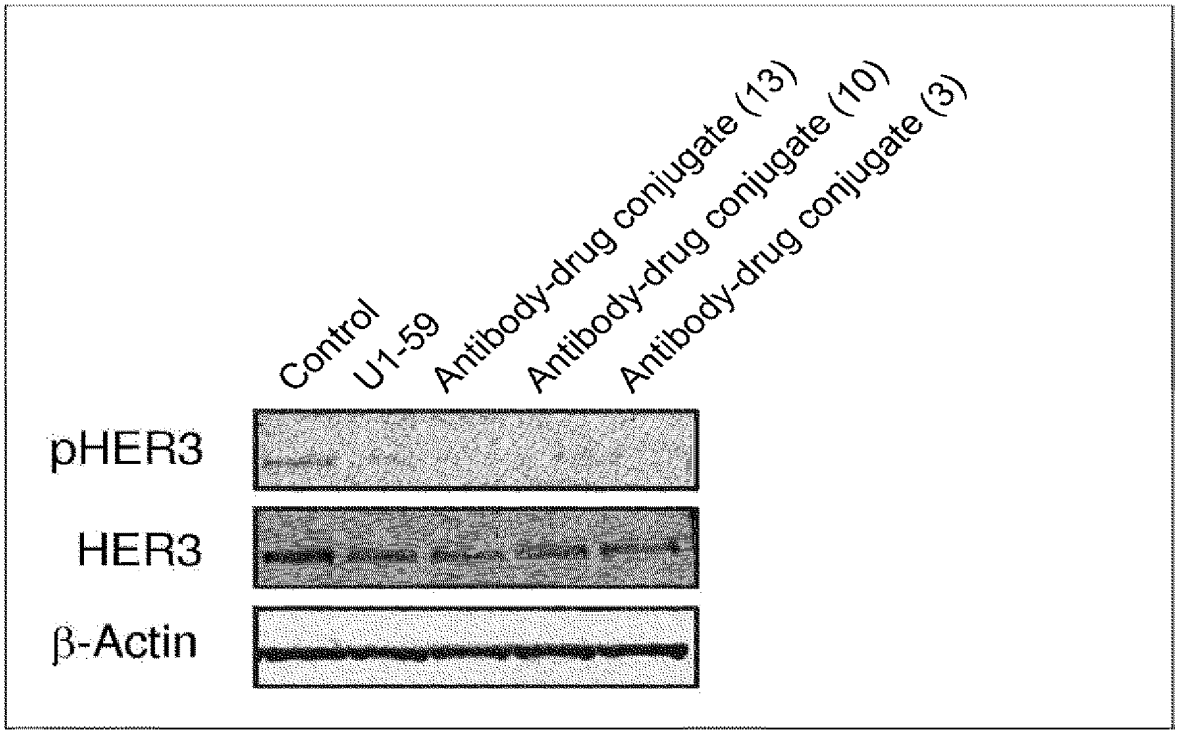
Full-length amino acid sequence of light chain of anti-HER3 human antibody U1-59 (SEQ ID NO: 584)

D I E M T Q S P D S L A V S L G E R A T I N C R S S Q S V L Y S S S N R
N Y L A W Y Q Q N P G Q P P K L L I Y W A S T R E S G V P D R F S G S G
S G T D F T L T I S S L Q A E D V A V Y Y C Q Q Y Y S T P R T F G Q G T
K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N N
F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S
L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N
R G E C

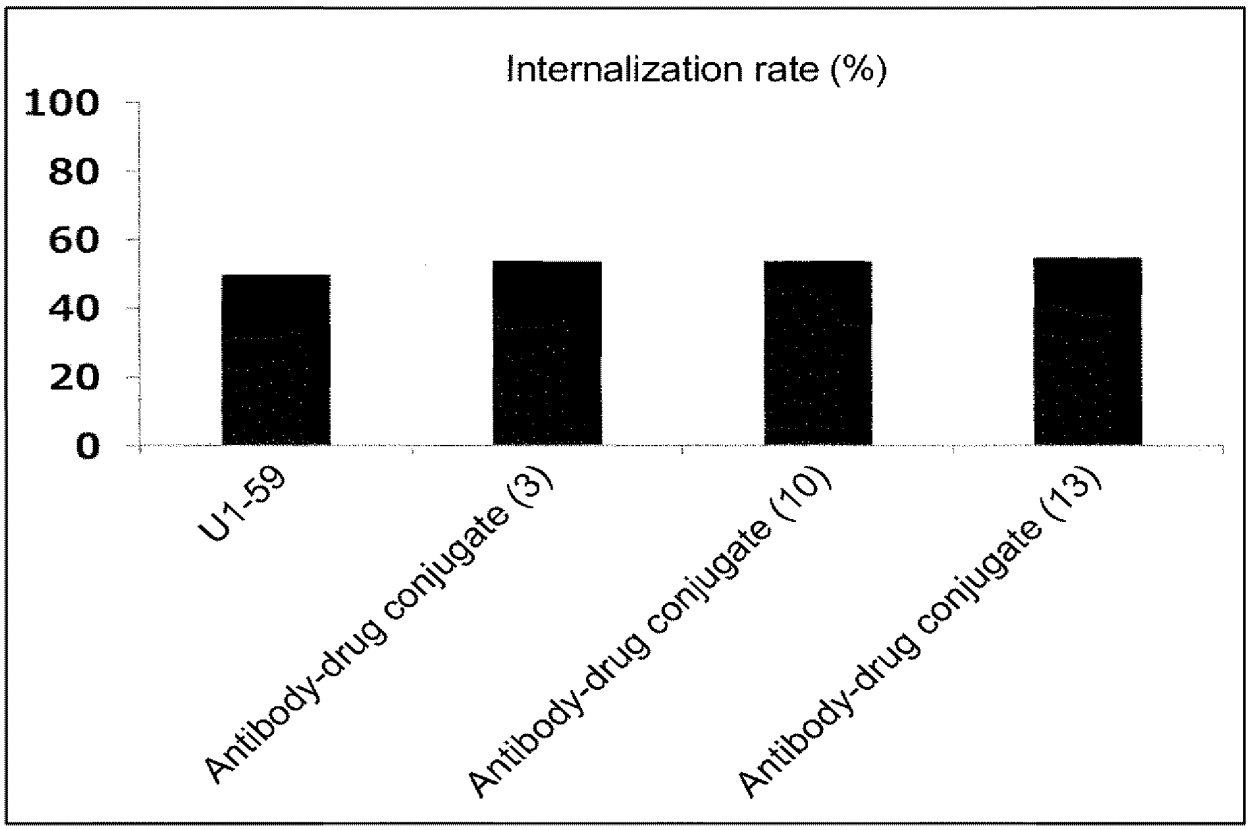
[Fig. 3]



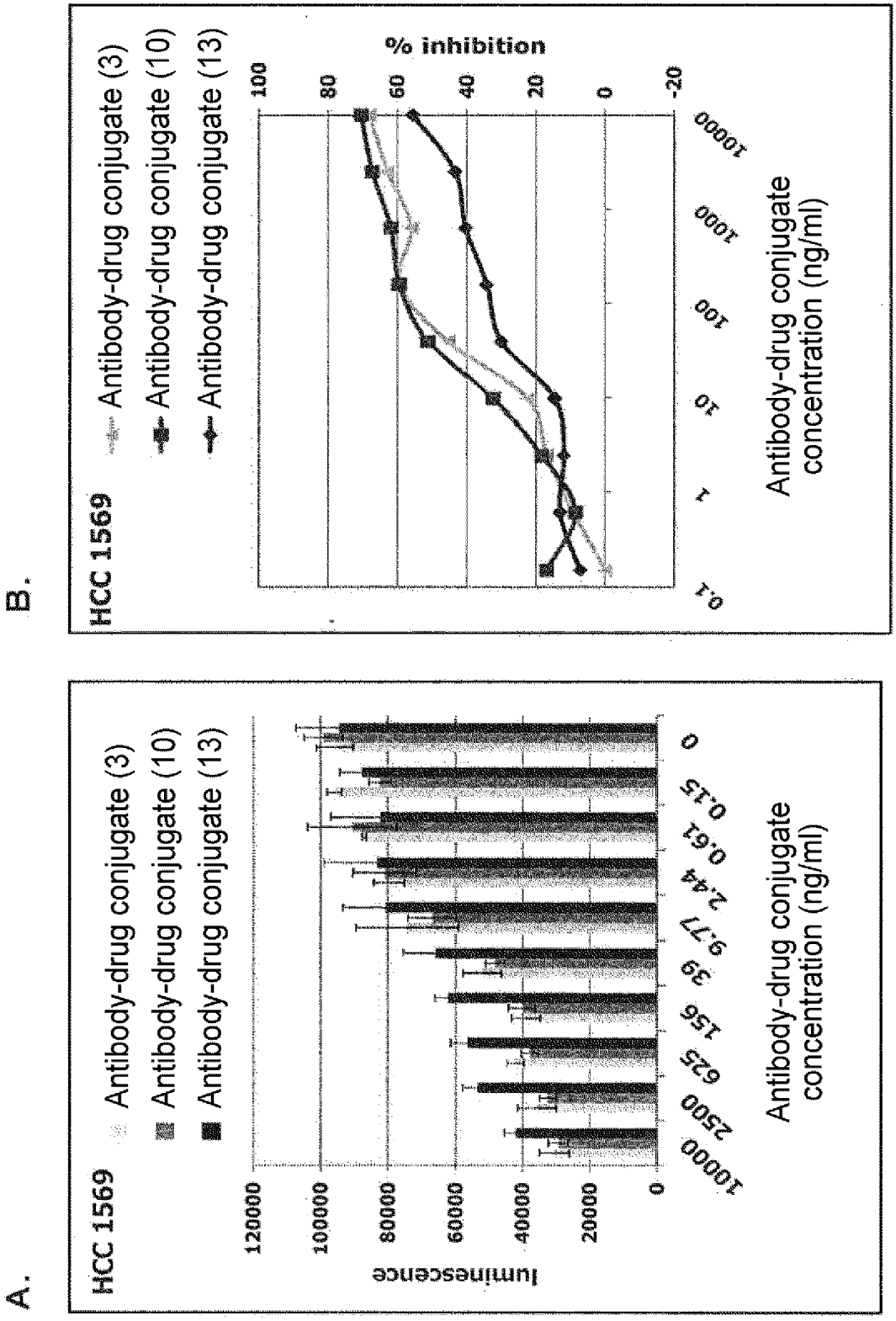
[Fig. 4]


Control
U1-59
Antibody-drug conjugate (13)
Antibody-drug conjugate (10)
Antibody-drug conjugate (3)
pHER3
HER3
β-Actin

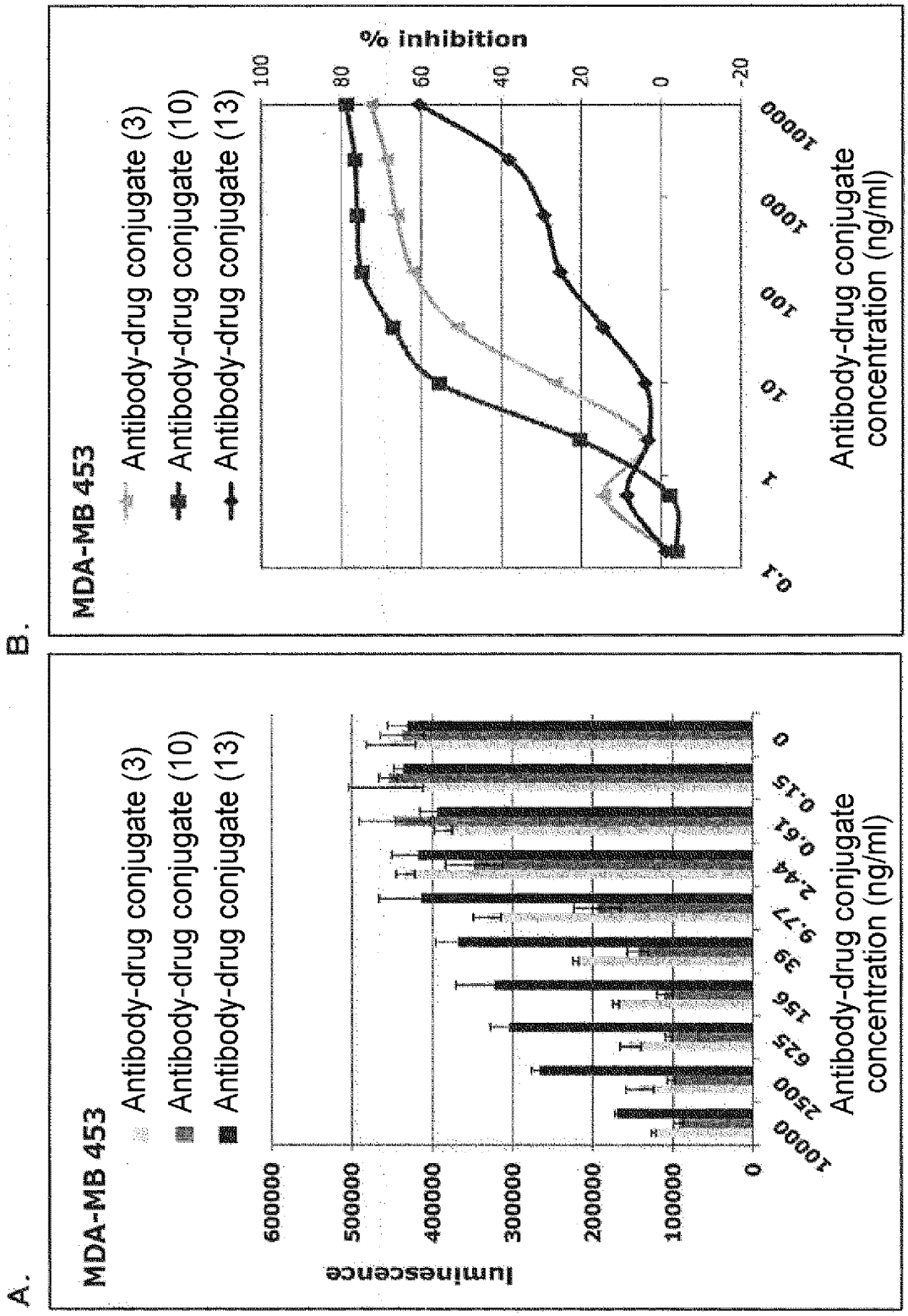
[Fig. 5]


Internalization rate (%)
100
80
60
40
20
0
U1-59
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)

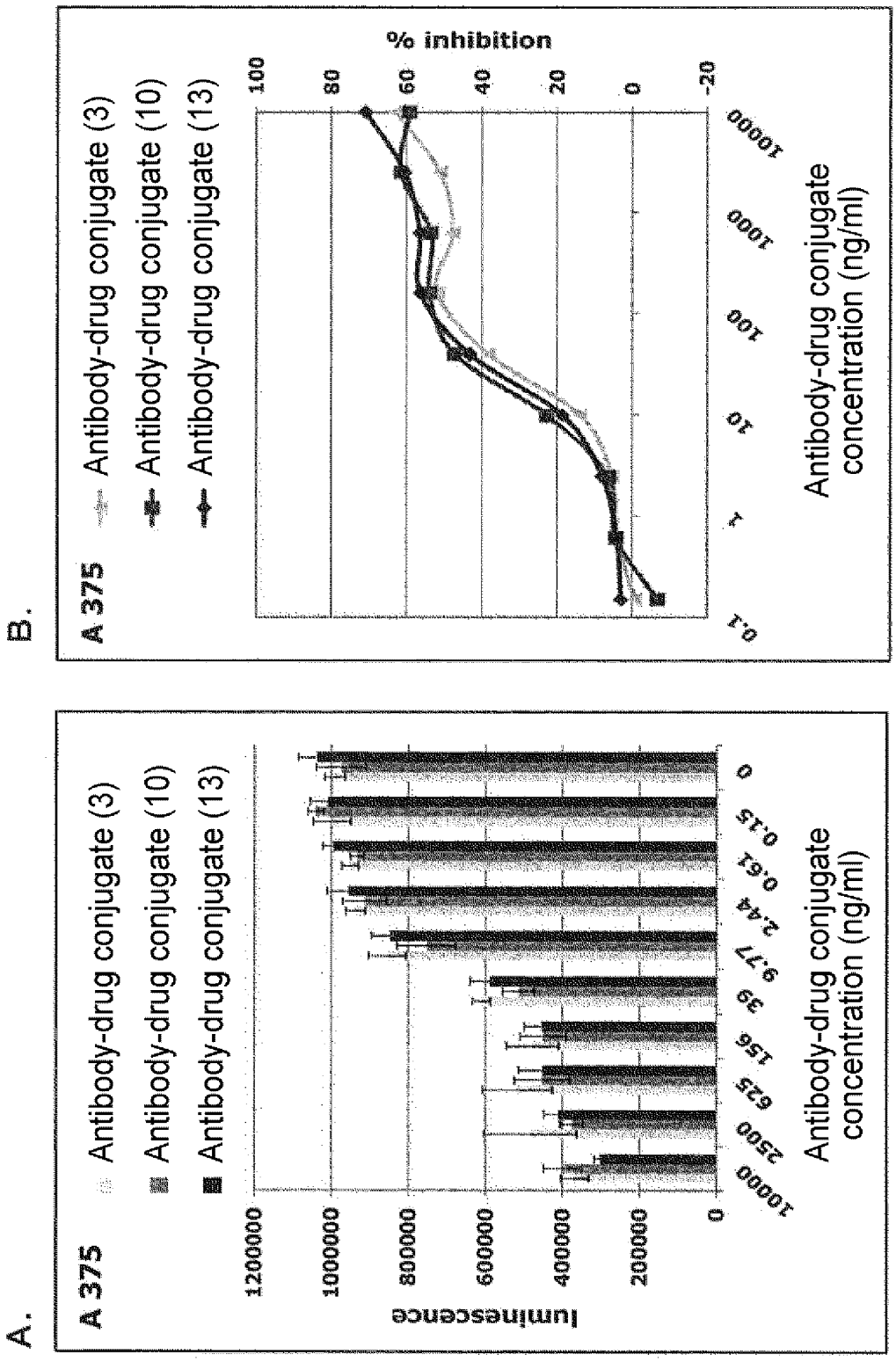
[Fig. 6]


A.
HCC 1569
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
luminescence
120000
100000
80000
60000
40000
20000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)
B.
HCC 1569
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
% inhibition
100
80
60
40
20
0
-20
10000
1000
100
10
1
0.1
Antibody-drug conjugate concentration (ng/ml)

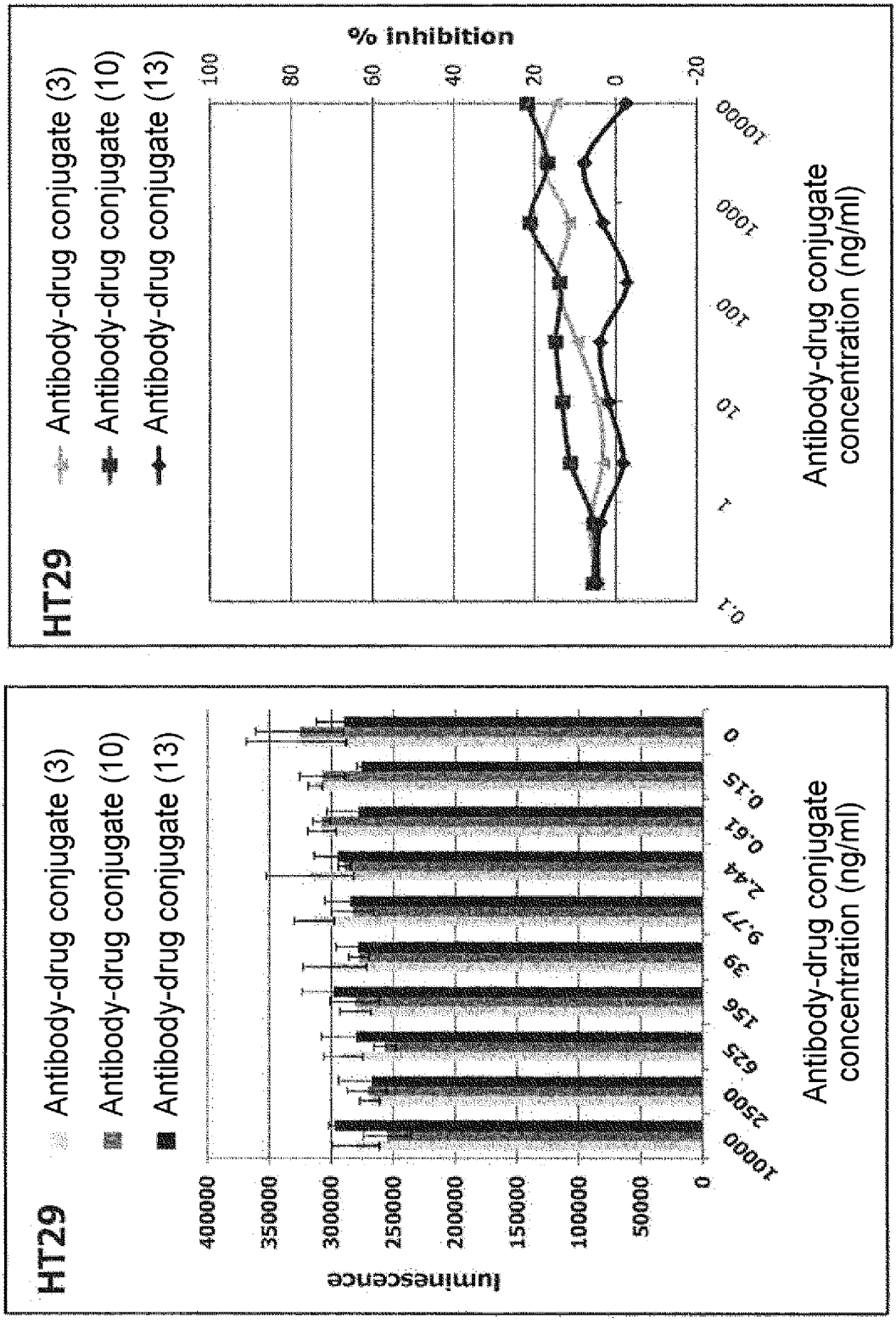
[Fig. 7]


A.
MDA-MB 453
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
luminescence
600000
500000
400000
300000
200000
100000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)
B.
MDA-MB 453
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
% inhibition
100
80
60
40
20
0
-20
10000
1000
100
10
1
0.1
Antibody-drug conjugate concentration (ng/ml)

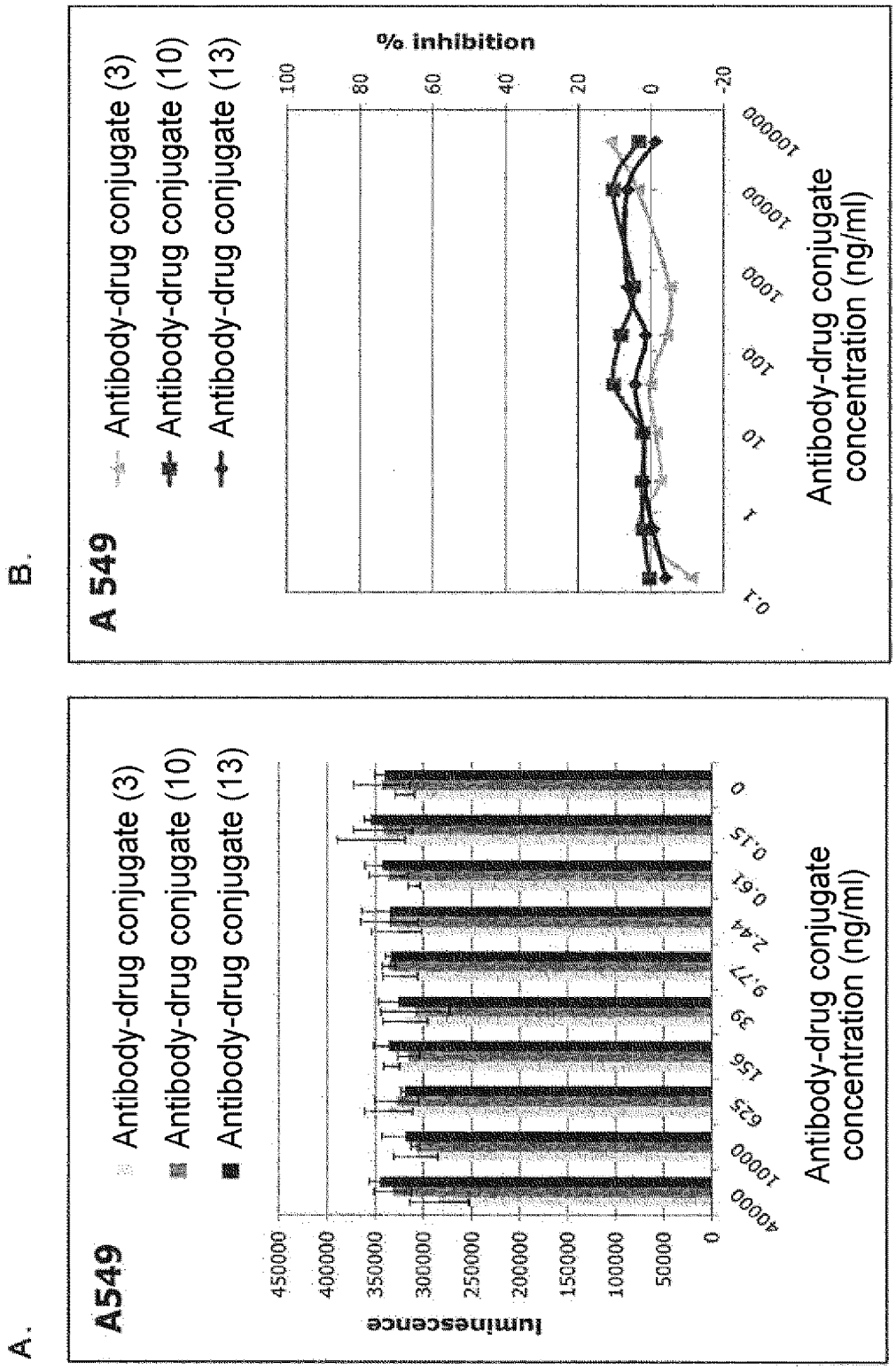
[Fig. 8]


A.
A 375
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
luminescence
1200000
1000000
800000
600000
400000
200000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)
B.
A 375
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
% inhibition
100
80
60
40
20
0
-20
10000
1000
100
10
1
0.1
Antibody-drug conjugate concentration (ng/ml)

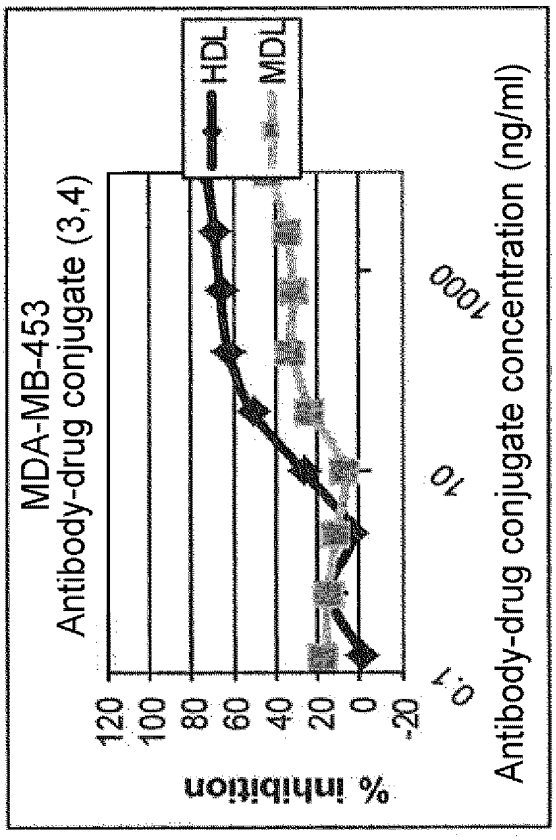
[Fig. 9]

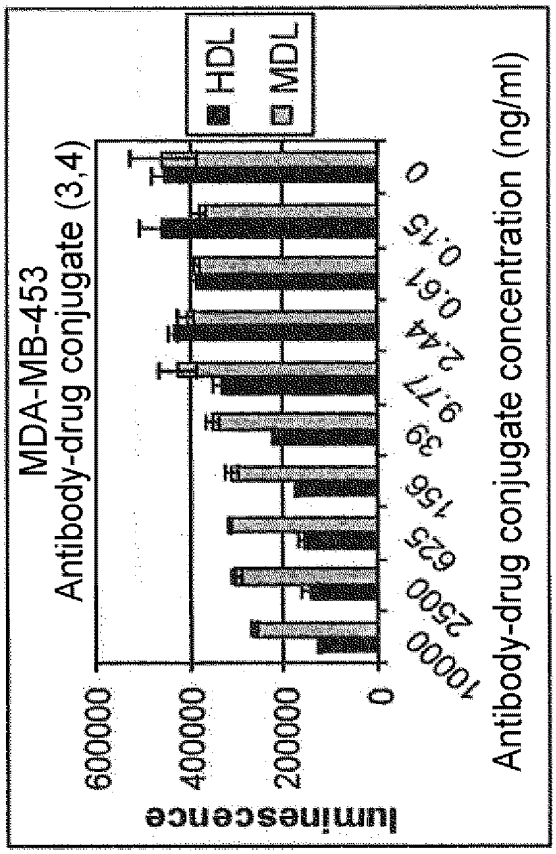

A.
HT29
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
luminescence
400000
350000
300000
250000
200000
150000
100000
50000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)
B.
HT29
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
% inhibition
100
80
60
40
20
0
-20
10000
1000
100
10
1
0.1
Antibody-drug conjugate concentration (ng/ml)

[Fig. 10]

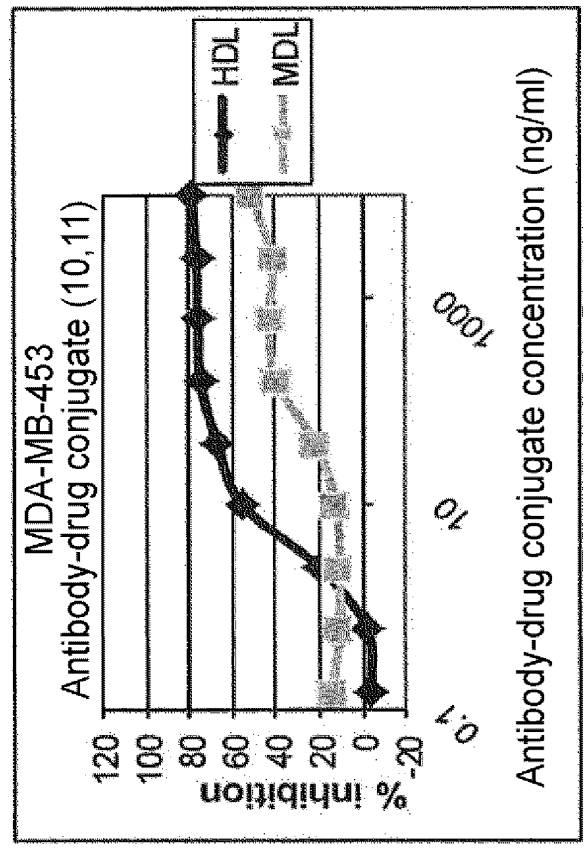

A.
A549
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
luminescence
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
40000
10000
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)
B.
A 549
Antibody-drug conjugate (3)
Antibody-drug conjugate (10)
Antibody-drug conjugate (13)
% inhibition
100
80
60
40
20
0
-20
100000
10000
1000
100
10
1
0.1
Antibody-drug conjugate concentration (ng/ml)

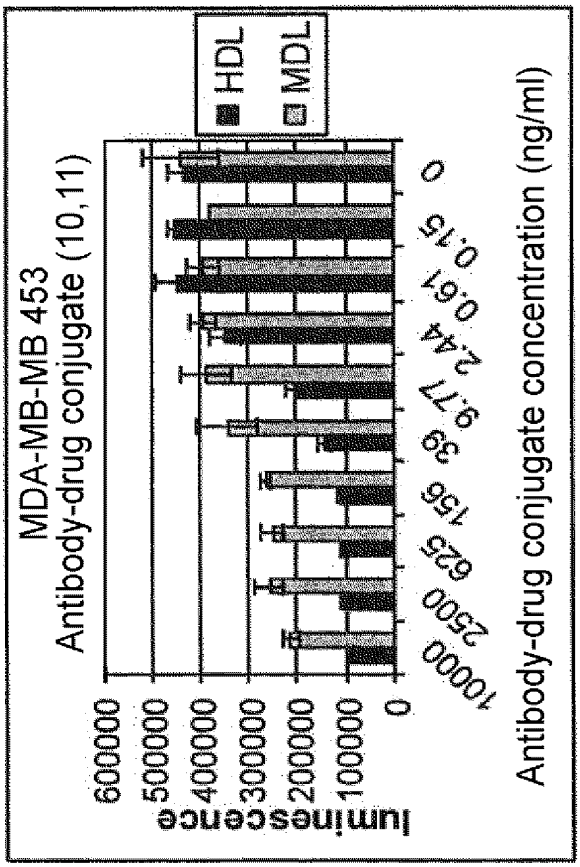
[Fig. 11]


MDA-MB-453
Antibody-drug conjugate (3,4)
HDL
MDL
% inhibition
120
100
80
60
40
20
0
-20
0.1
10
1000
Antibody-drug conjugate concentration (ng/ml)

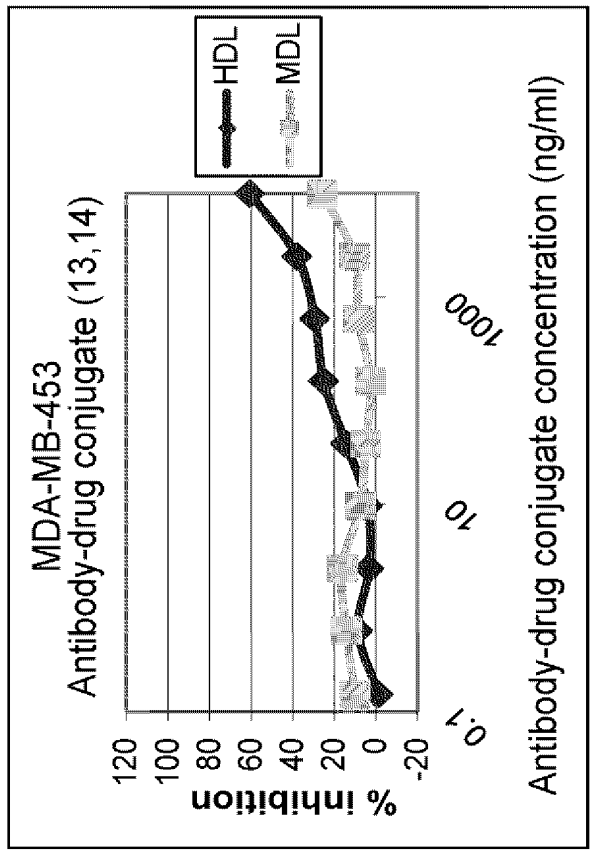

MDA-MB-453
Antibody-drug conjugate (3,4)
HDL
MDL
luminescence
600000
400000
200000
0
10000
2500
625
156
39
9.77
2.44
0.67
0.15
0
Antibody-drug conjugate concentration (ng/ml)

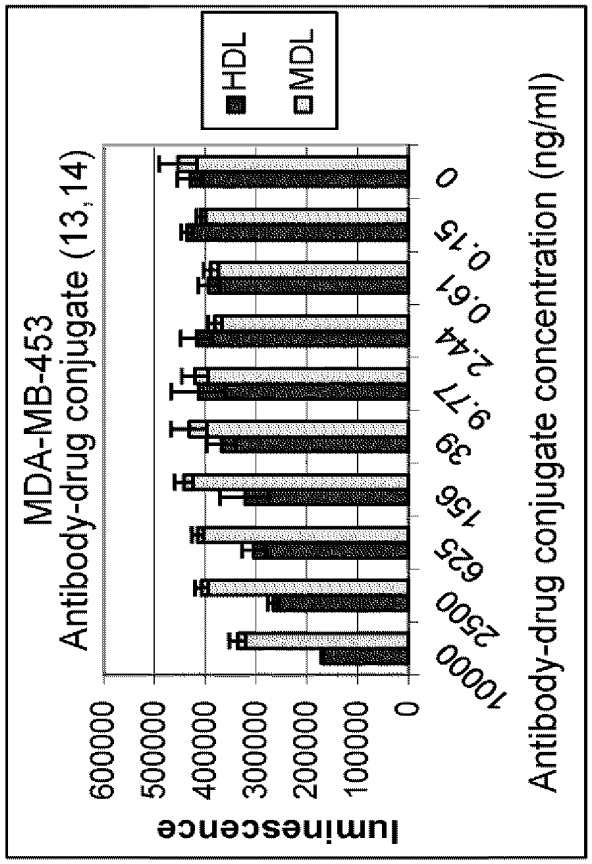
[Fig. 12]


MDA-MB-453
Antibody-drug conjugate (10,11)
HDL
MDL
% inhibition
120
100
80
60
40
20
0
-20
0.1
10
1000
Antibody-drug conjugate concentration (ng/ml)

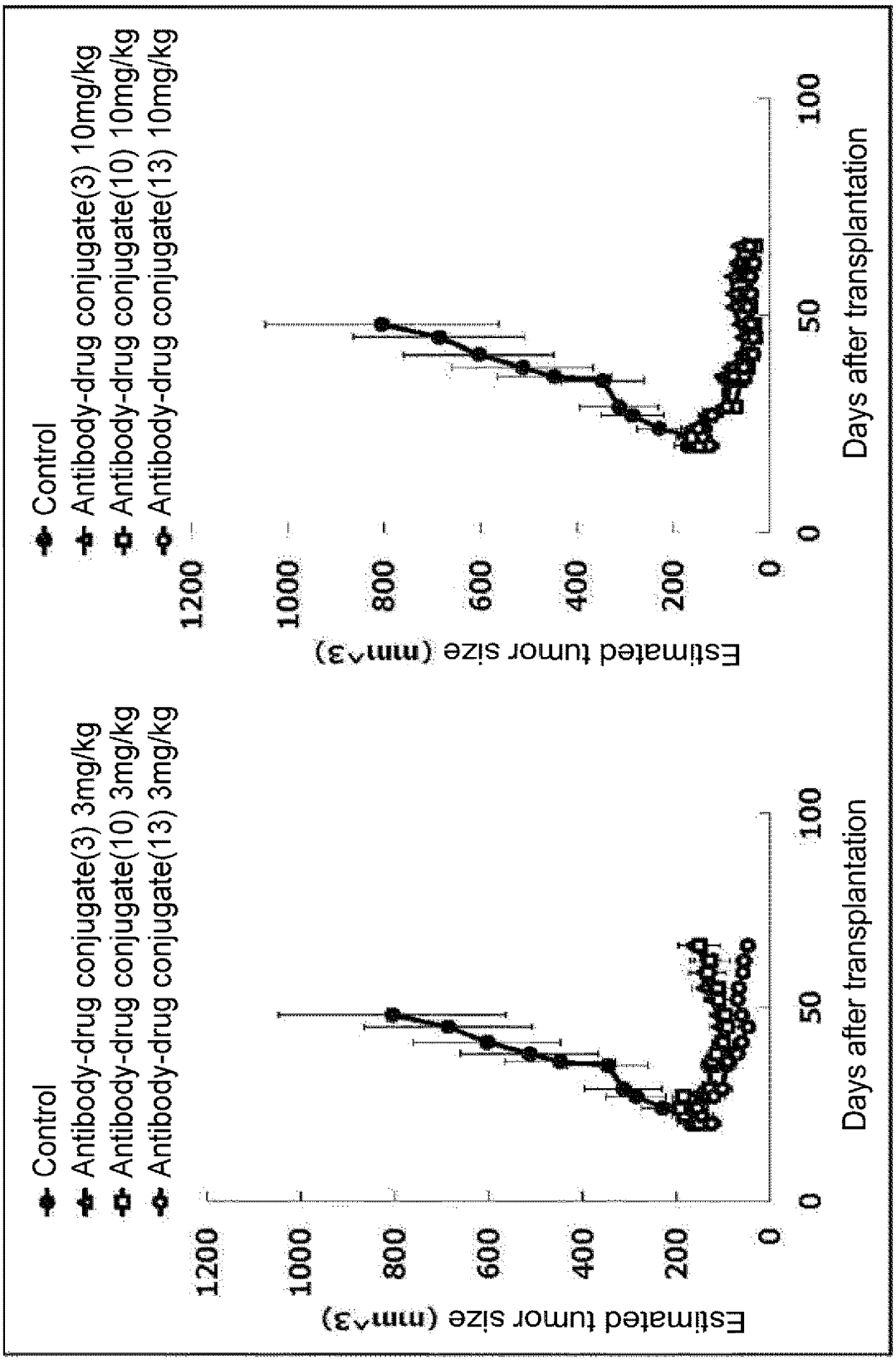

MDA-MB-MB 453
Antibody-drug conjugate (10,11)
HDL
MDL
luminescence
600000
500000
400000
300000
200000
100000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)

[Fig. 13]

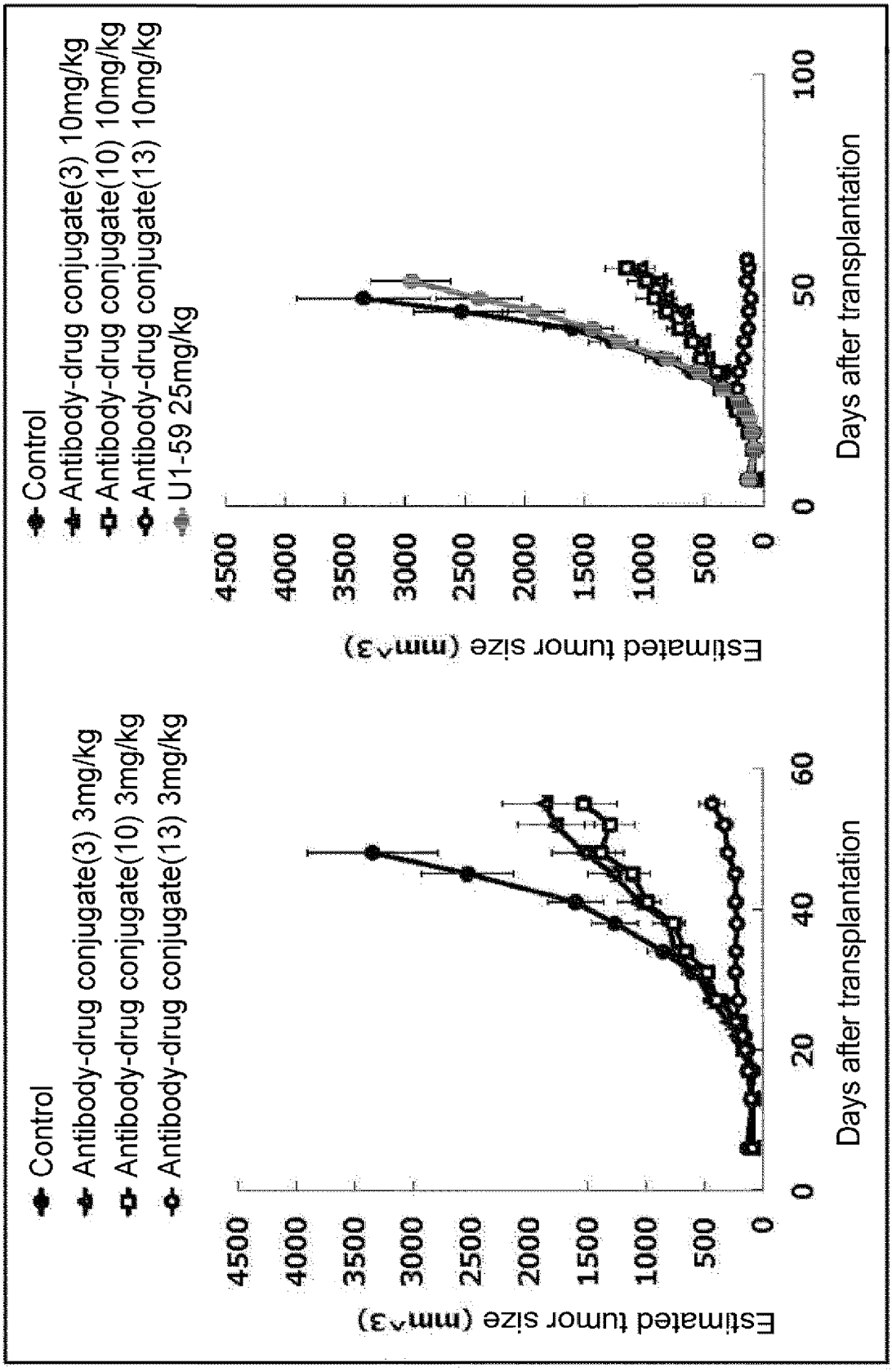

MDA-MB-453
Antibody-drug conjugate (13,14)
HDL
MDL
% inhibition
120
100
80
60
40
20
0
-20
0.1
10
1000
Antibody-drug conjugate concentration (ng/ml)


MDA-MB-453
Antibody-drug conjugate (13,14)
HDL
MDL
luminescence
600000
500000
400000
300000
200000
100000
0
10000
2500
625
156
39
9.77
2.44
0.61
0.15
0
Antibody-drug conjugate concentration (ng/ml)

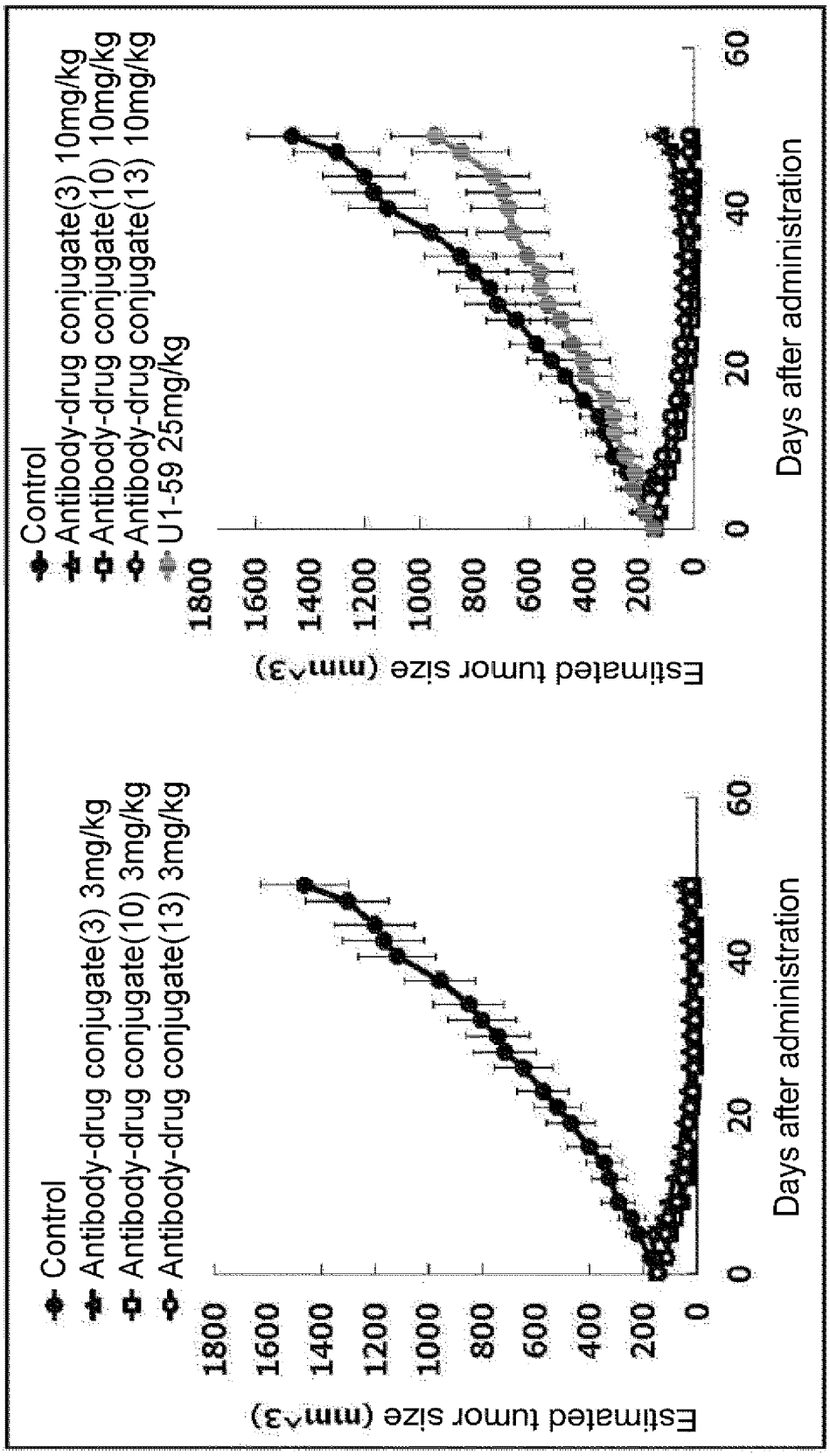
[Fig. 14]


Control
Antibody-drug conjugate(3) 10mg/kg
Antibody-drug conjugate(10) 10mg/kg
Antibody-drug conjugate(13) 10mg/kg
Estimated tumor size (mm^3)
Days after transplantation
Control
Antibody-drug conjugate(3) 3mg/kg
Antibody-drug conjugate(10) 3mg/kg
Antibody-drug conjugate(13) 3mg/kg
Estimated tumor size (mm^3)
Days after transplantation

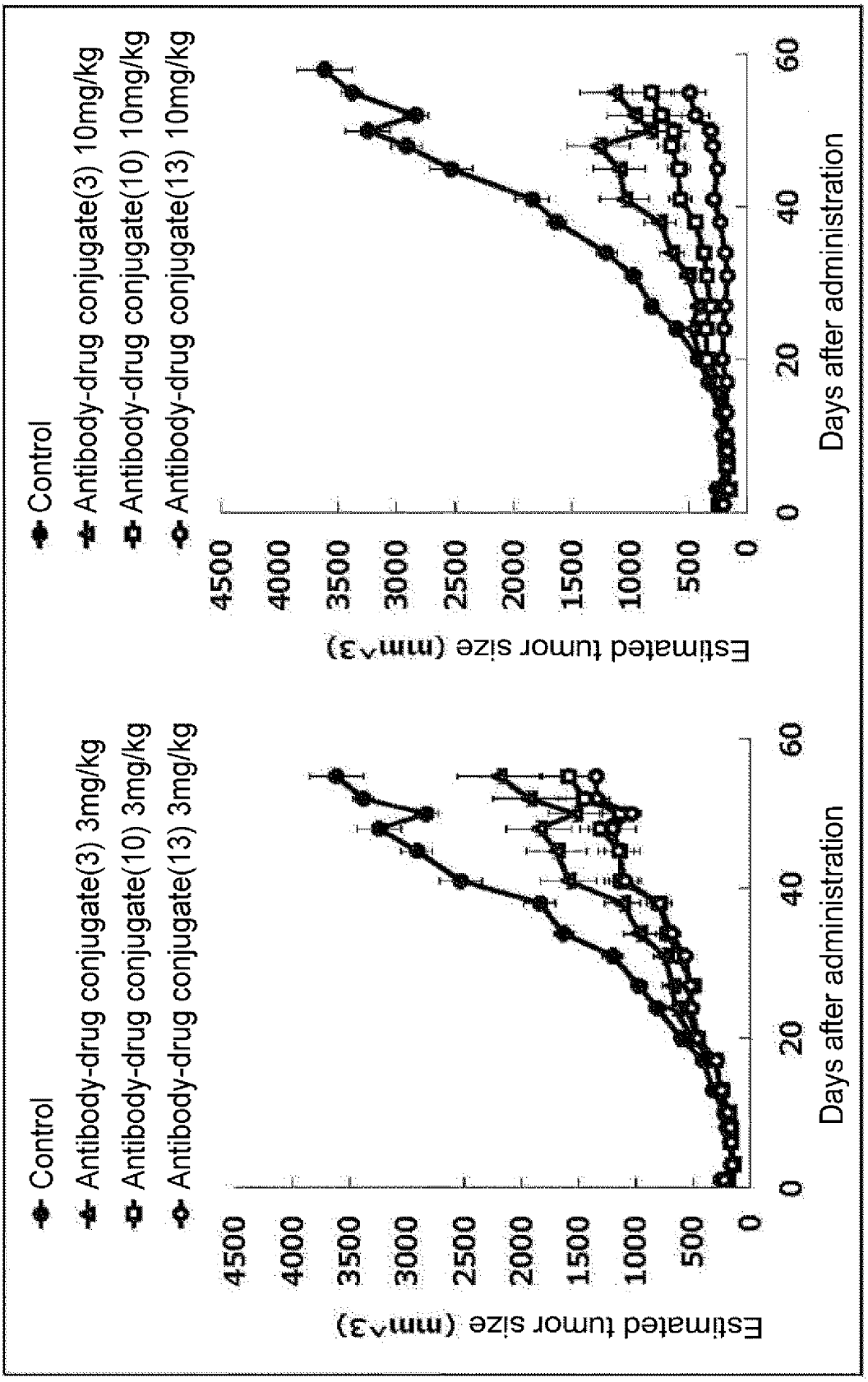
[Fig. 15]


Control
Antibody-drug conjugate(3) 3mg/kg
Antibody-drug conjugate(10) 3mg/kg
Antibody-drug conjugate(13) 3mg/kg
Estimated tumor size (mm^3)
Days after transplantation
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
20
40
60
Control
Antibody-drug conjugate(3) 10mg/kg
Antibody-drug conjugate(10) 10mg/kg
Antibody-drug conjugate(13) 10mg/kg
U1-59 25mg/kg
Estimated tumor size (mm^3)
Days after transplantation
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
50
100

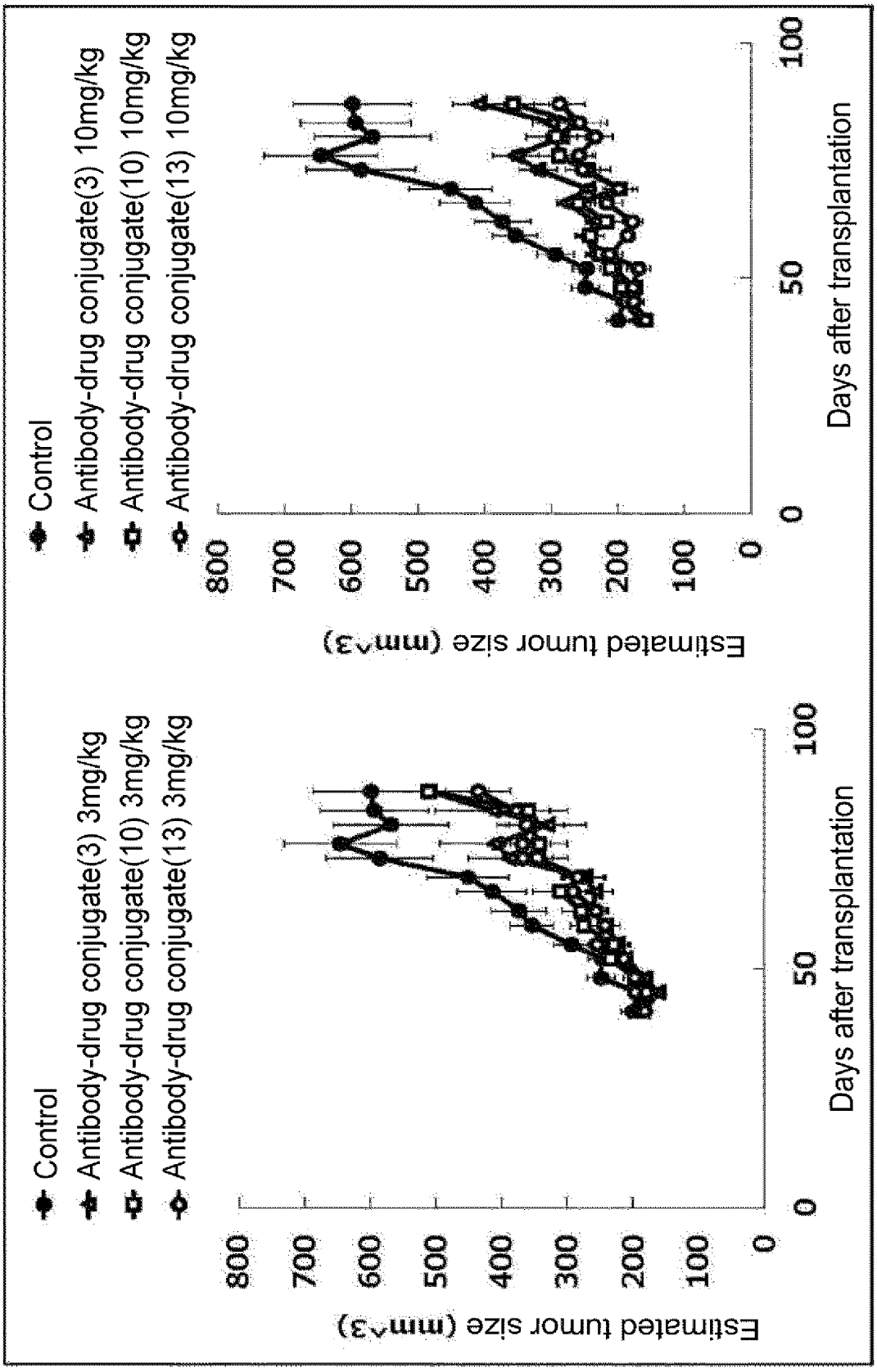
[Fig. 16]


Control
Antibody-drug conjugate(3) 3mg/kg
Antibody-drug conjugate(10) 3mg/kg
Antibody-drug conjugate(13) 3mg/kg
Estimated tumor size (mm^3)
Days after administration
0
200
400
600
800
1000
1200
1400
1600
1800
0
20
40
60
Control
Antibody-drug conjugate(3) 10mg/kg
Antibody-drug conjugate(10) 10mg/kg
Antibody-drug conjugate(13) 10mg/kg
U1-59 25mg/kg
Estimated tumor size (mm^3)
Days after administration

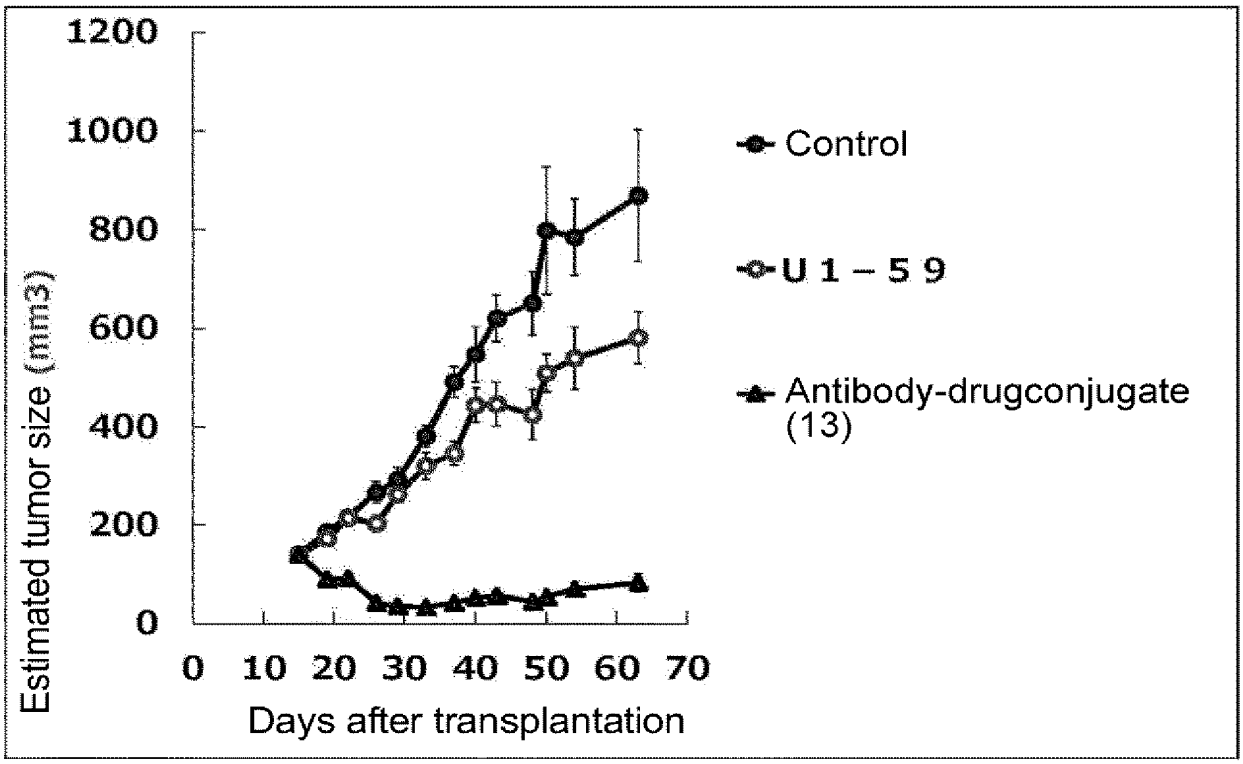
[Fig. 17]


Control
Antibody-drug conjugate(3) 3mg/kg
Antibody-drug conjugate(10) 3mg/kg
Antibody-drug conjugate(13) 3mg/kg
Estimated tumor size (mm^3)
Days after administration
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
20
40
60
Control
Antibody-drug conjugate(3) 10mg/kg
Antibody-drug conjugate(10) 10mg/kg
Antibody-drug conjugate(13) 10mg/kg
Estimated tumor size (mm^3)
Days after administration

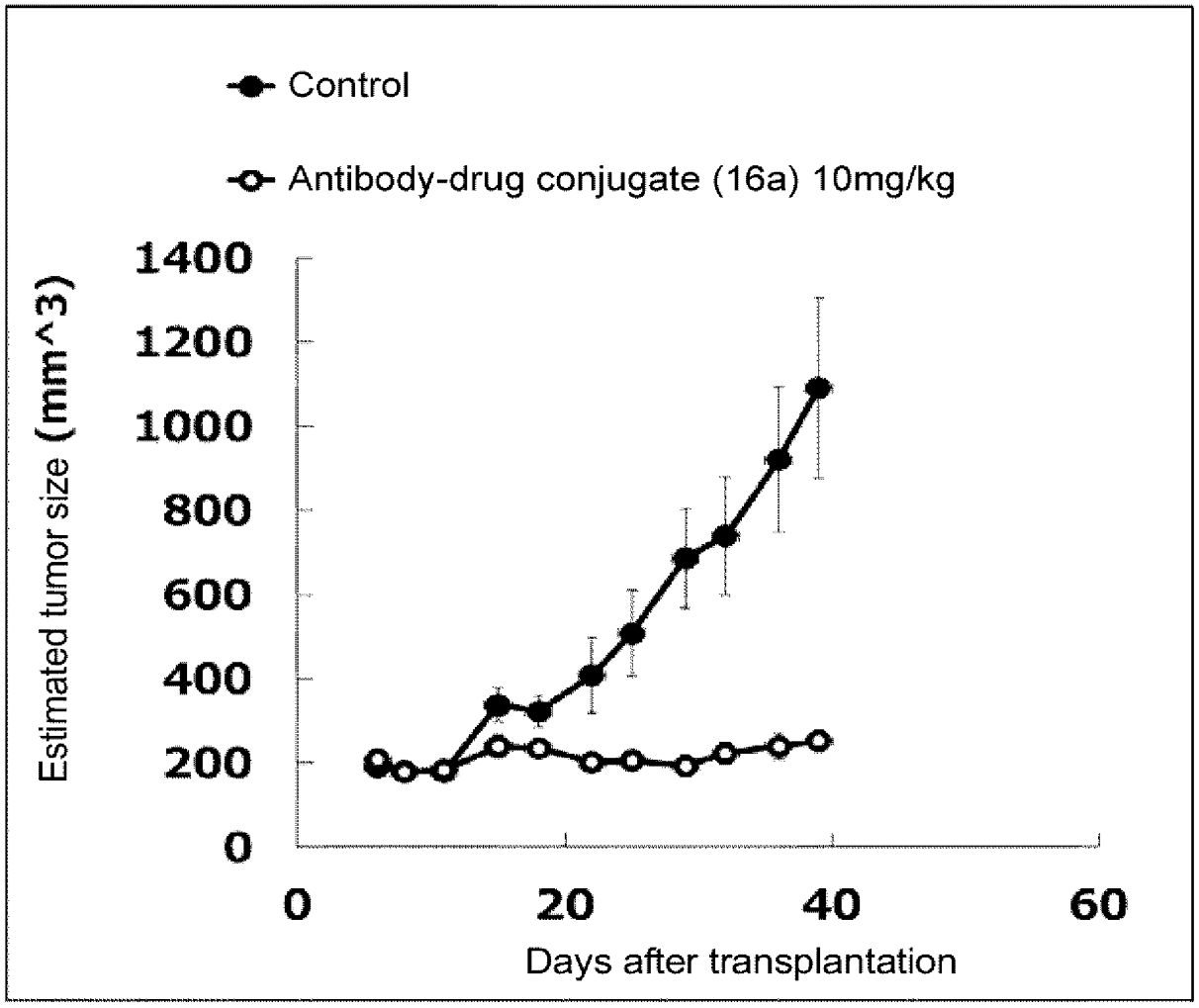
[Fig. 18]


Control
Antibody-drug conjugate(3) 3mg/kg
Antibody-drug conjugate(10) 3mg/kg
Antibody-drug conjugate(13) 3mg/kg
Estimated tumor size (mm^3)
Days after transplantation
0
50
100
100
200
300
400
500
600
700
800
Control
Antibody-drug conjugate(3) 10mg/kg
Antibody-drug conjugate(10) 10mg/kg
Antibody-drug conjugate(13) 10mg/kg
Estimated tumor size (mm^3)
Days after transplantation

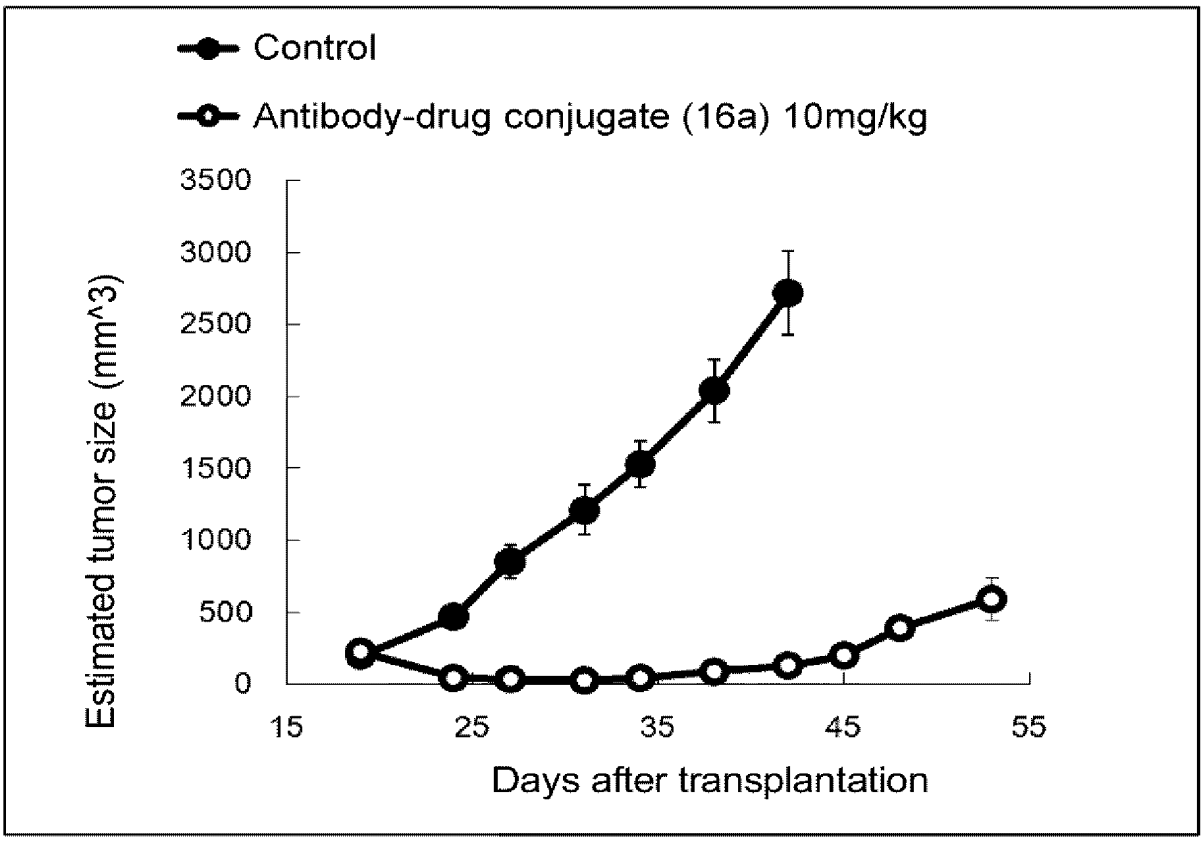
[Fig. 19]


1200
1000
800
600
400
200
0
Estimated tumor size (mm3)
0 10 20 30 40 50 60 70
Days after transplantation
Control
U 1 – 5 9
Antibody-drugconjugate (13)

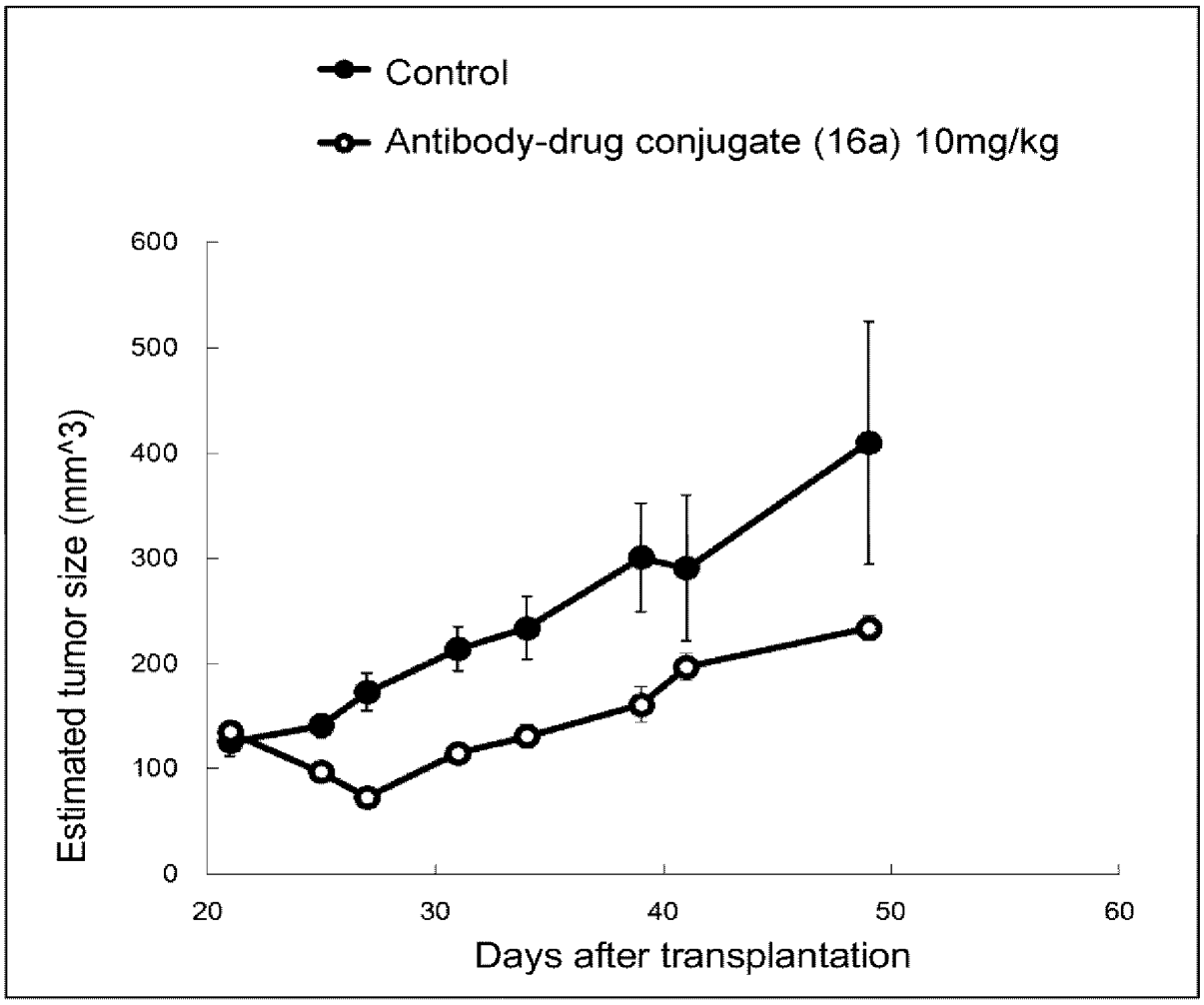
[Fig. 20]


Control
Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1400
1200
1000
800
600
400
200
0
0
20
40
60
Days after transplantation

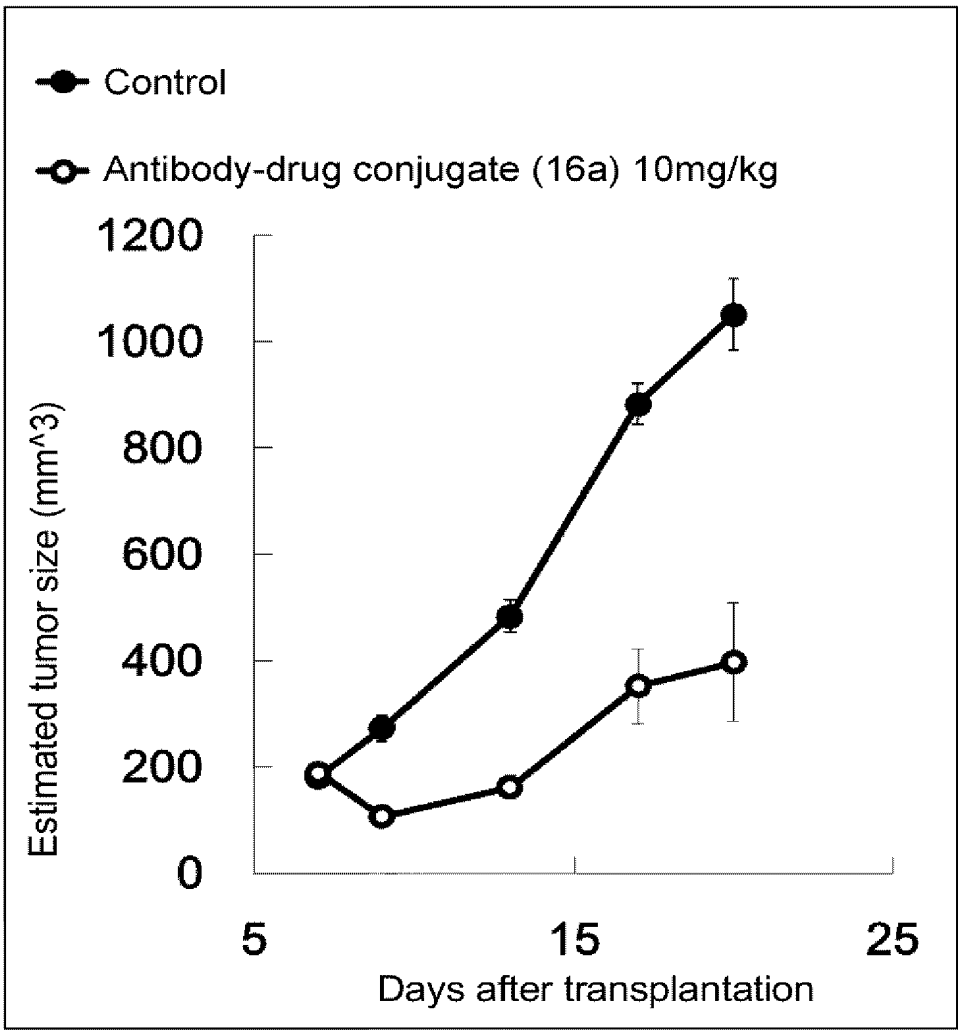
[Fig. 21]


Control
Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
3500
3000
2500
2000
1500
1000
500
0
15
25
35
45
55
Days after transplantation

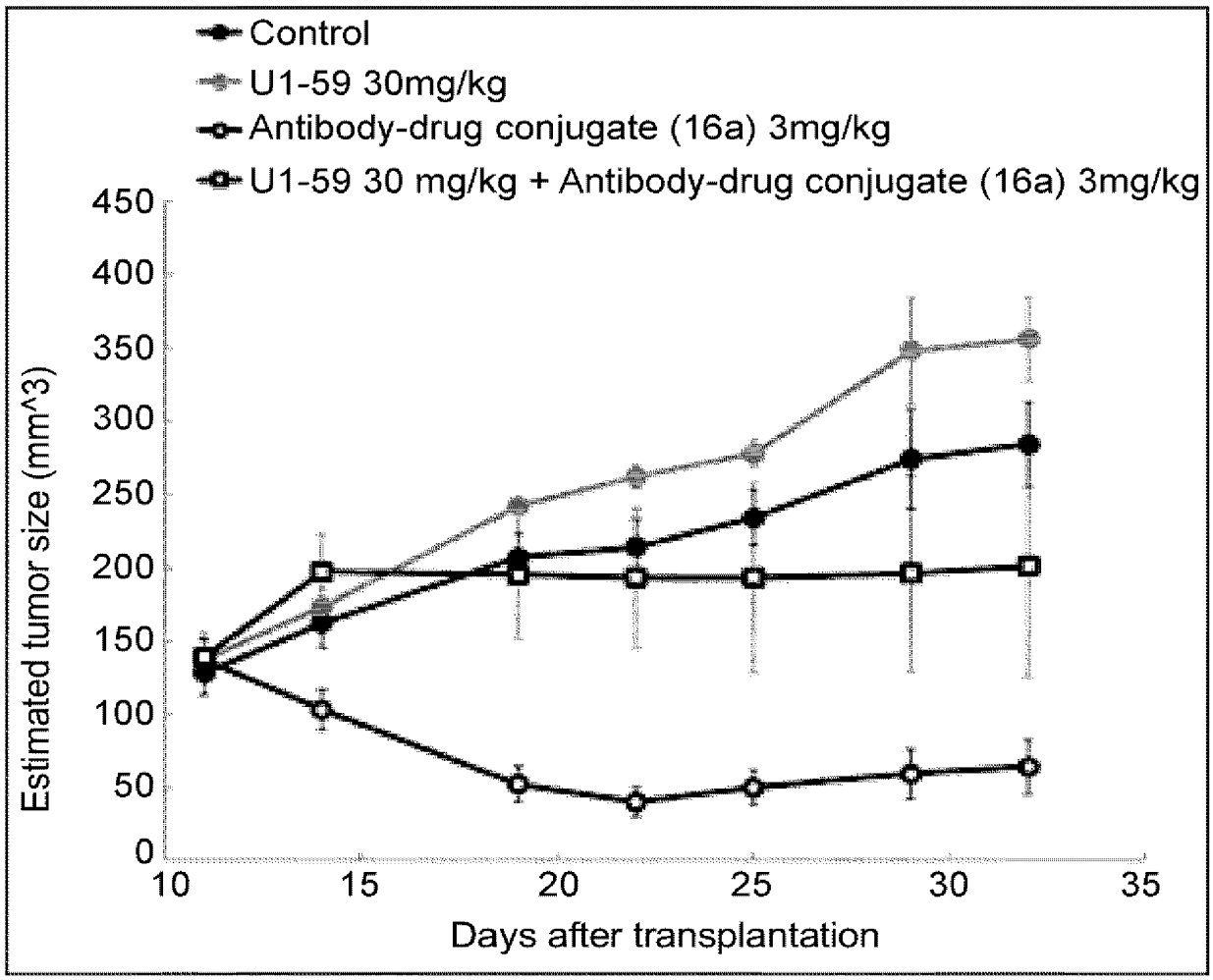
[Fig. 22]


Control
Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
600
500
400
300
200
100
0
20
30
40
50
60
Days after transplantation

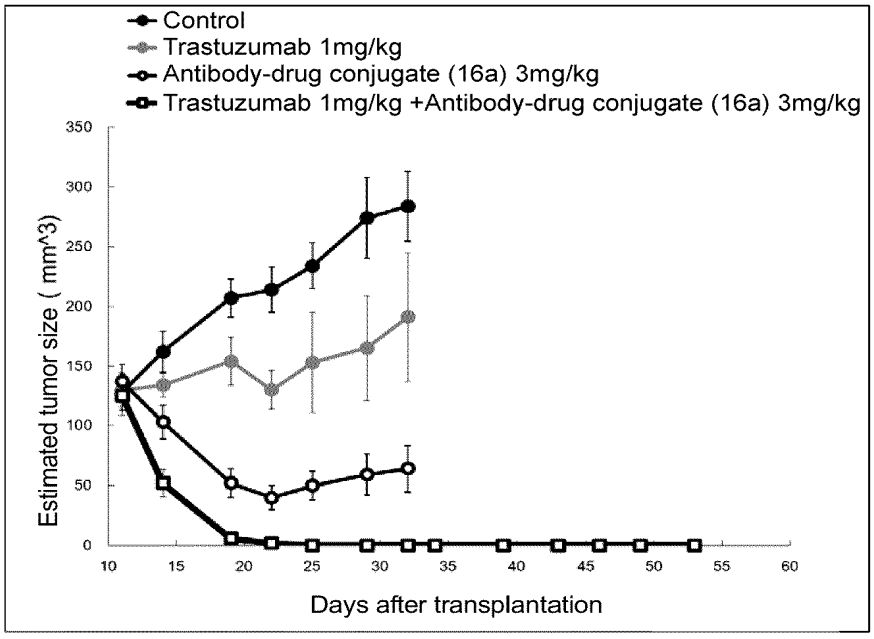
[Fig. 23]


Control
Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1200
1000
800
600
400
200
0
5
15
25
Days after transplantation

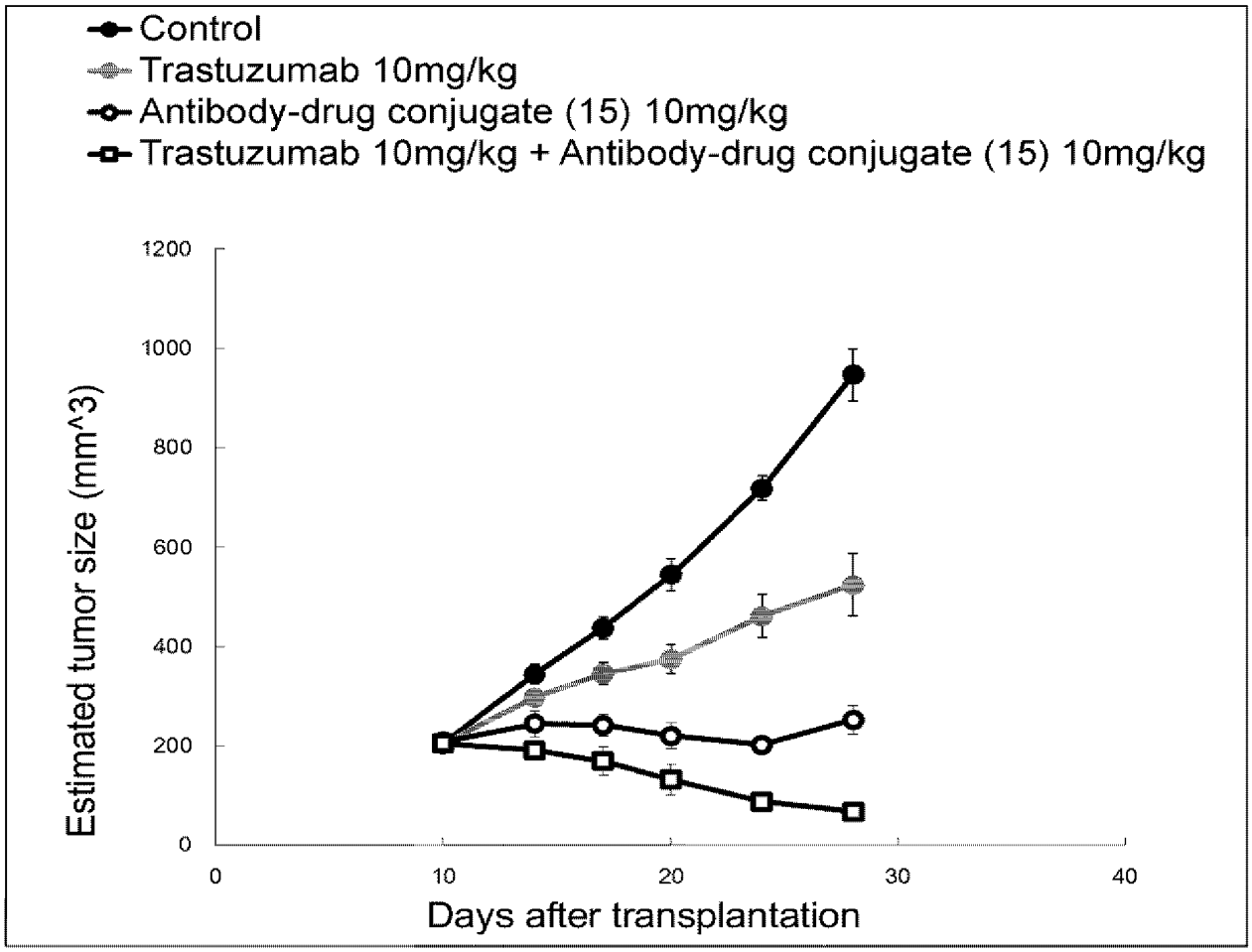
[Fig. 24]


Control
U1-59 30mg/kg
Antibody-drug conjugate (16a) 3mg/kg
U1-59 30 mg/kg + Antibody-drug conjugate (16a) 3mg/kg
Estimated tumor size (mm^3)
450
400
350
300
250
200
150
100
50
0
10
15
20
25
30
35
Days after transplantation

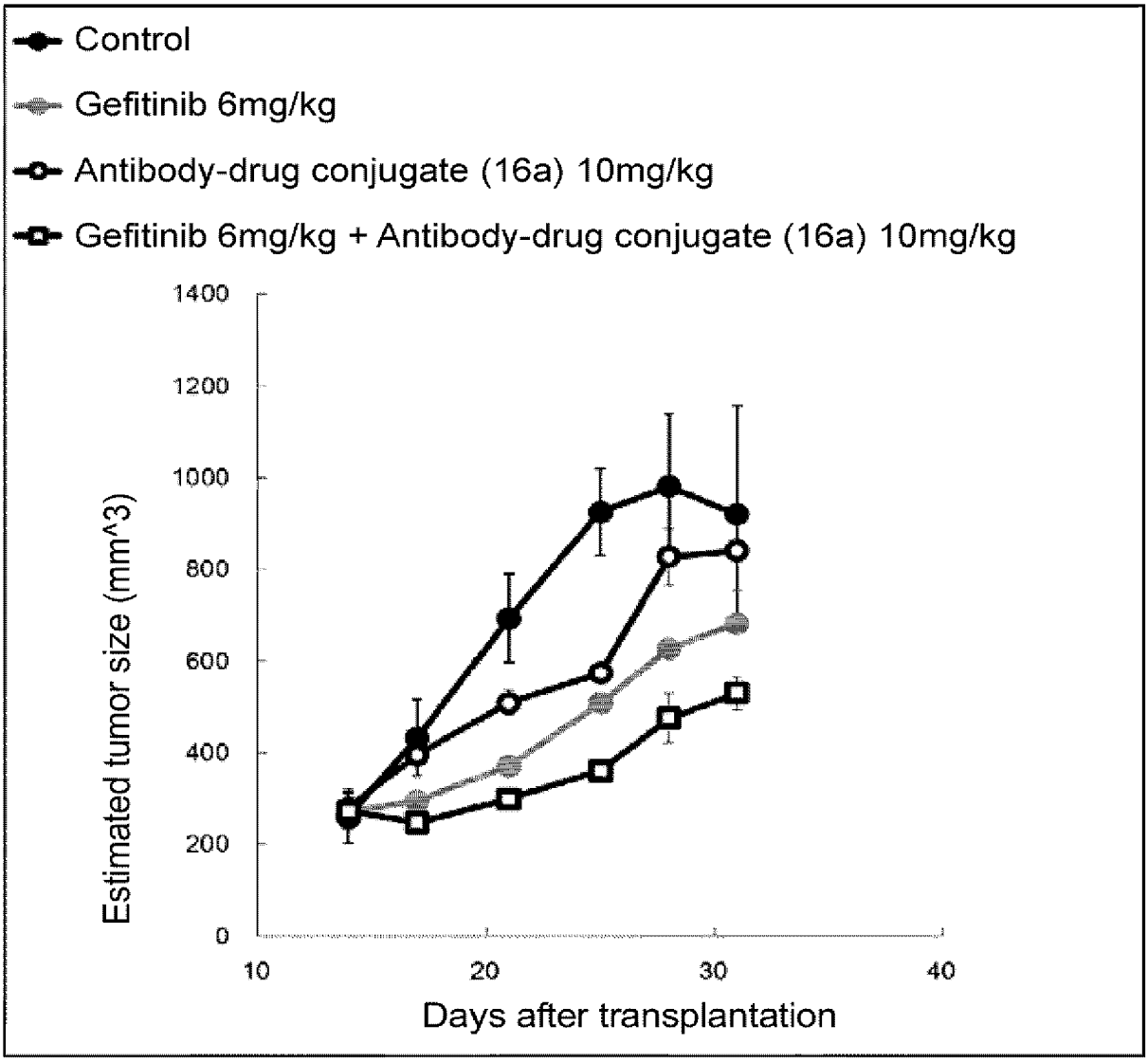
[Fig. 25]


Control
Trastuzumab 1mg/kg
Antibody-drug conjugate (16a) 3mg/kg
Trastuzumab 1mg/kg +Antibody-drug conjugate (16a) 3mg/kg
Estimated tumor size (mm^3)
350
300
250
200
150
100
50
0
10
15
20
25
30
35
40
45
50
55
60
Days after transplantation

[Fig. 26]

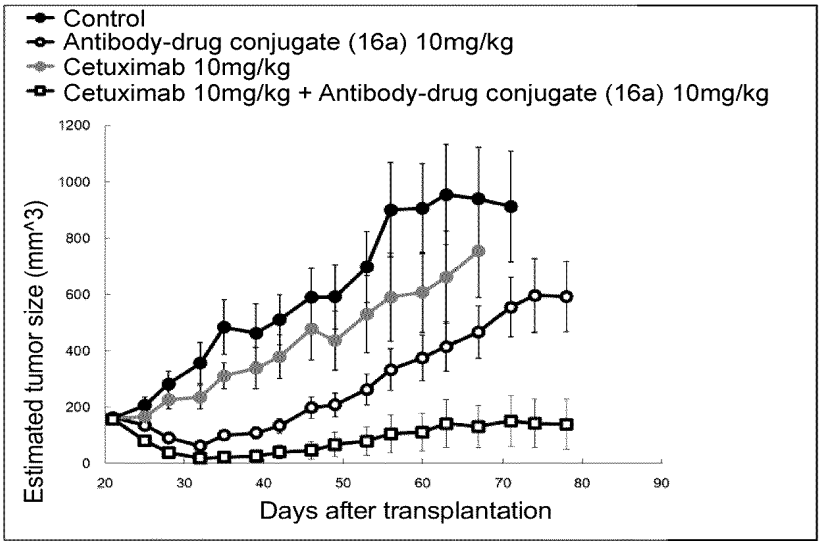

Control
Trastuzumab 10mg/kg
Antibody-drug conjugate (15) 10mg/kg
Trastuzumab 10mg/kg + Antibody-drug conjugate (15) 10mg/kg
Estimated tumor size (mm^3)
1200
1000
800
600
400
200
0
0
10
20
30
40
Days after transplantation

[Fig. 27]

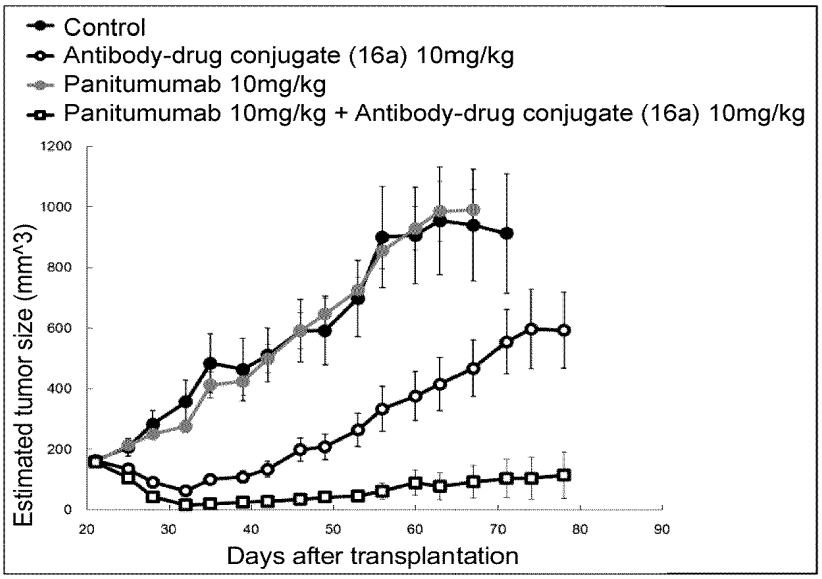

Control
Gefitinib 6mg/kg
Antibody-drug conjugate (16a) 10mg/kg
Gefitinib 6mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1400
1200
1000
800
600
400
200
0
10
20
30
40
Days after transplantation

[Fig. 28]

(A)

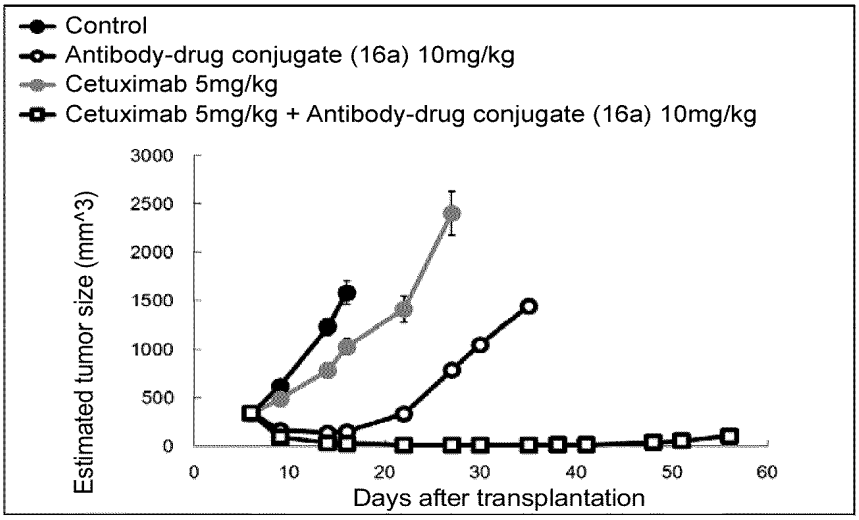

Control
Antibody-drug conjugate (16a) 10mg/kg
Cetuximab 10mg/kg
Cetuximab 10mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
Days after transplantation
0
200
400
600
800
1000
1200
20
30
40
50
60
70
80
90

(B)

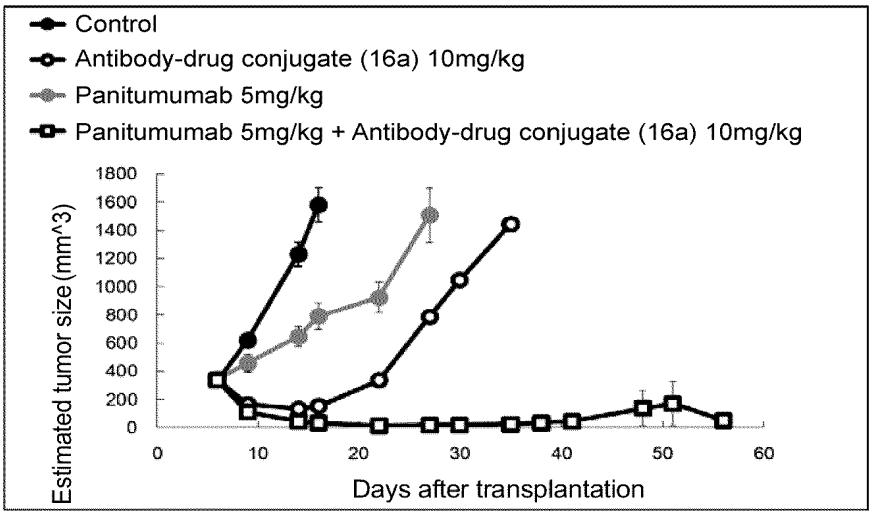

Control
Antibody-drug conjugate (16a) 10mg/kg
Panitumumab 10mg/kg
Panitumumab 10mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
Days after transplantation
0
200
400
600
800
1000
1200
20
30
40
50
60
70
80
90

[Fig. 29]

(A)

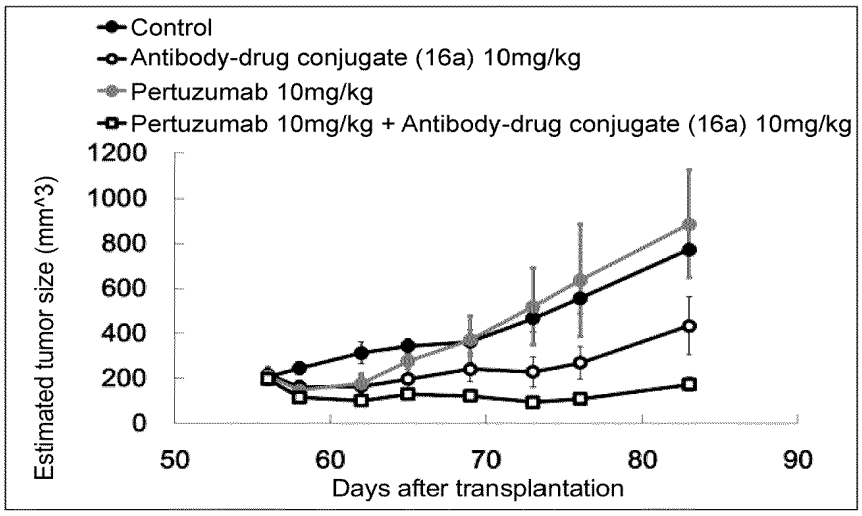

Control
Antibody-drug conjugate (16a) 10mg/kg
Cetuximab 5mg/kg
Cetuximab 5mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Days after transplantation

(B)

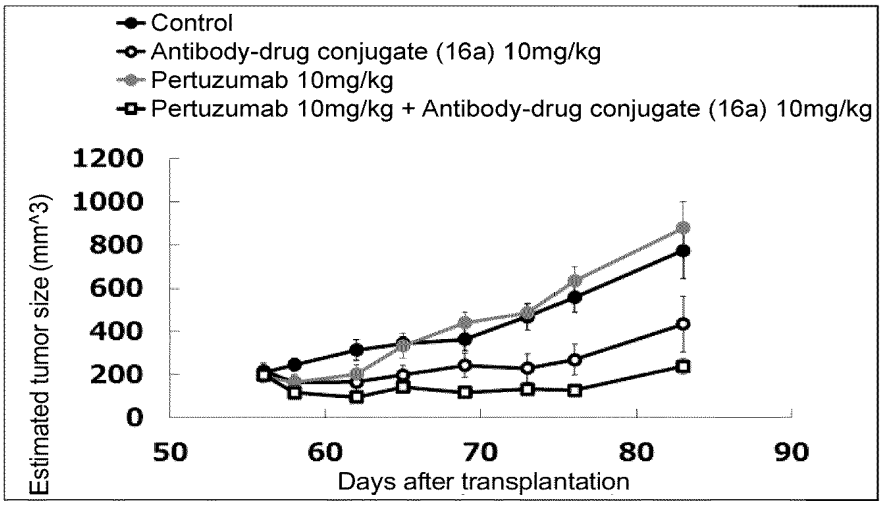

Control
Antibody-drug conjugate (16a) 10mg/kg
Panitumumab 5mg/kg
Panitumumab 5mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Days after transplantation

[Fig. 30]

(A)


Control
Antibody-drug conjugate (16a) 10mg/kg
Pertuzumab 10mg/kg
Pertuzumab 10mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1200
1000
800
600
400
200
0
50
60
70
80
90
Days after transplantation

(B)


Control
Antibody-drug conjugate (16a) 10mg/kg
Pertuzumab 10mg/kg
Pertuzumab 10mg/kg + Antibody-drug conjugate (16a) 10mg/kg
Estimated tumor size (mm^3)
1200
1000
800
600
400
200
0
50
60
70
80
90
Days after transplantation

INTERNATIONAL SEARCH REPORT

International application No
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.

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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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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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特許協力条約に基づいて公開された国際出願

(51) 国際特許分類6 **A61K 47/48**	**A1**	(11) 国際公開番号	**WO97/46260**
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(22) 国際出願日 1997年6月5日(05.06.97)

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特願平8/144421 1996年6月6日(06.06.96) JP

(71) 出願人（米国を除くすべての指定国について）
第一製薬株式会社
(DAIICHI PHARMACEUTICAL CO., LTD.)[JP/JP]
〒103 東京都中央区日本橋三丁目14番10号 Tokyo, (JP)
株式会社 ディ・ディ・エス研究所
(DRUG DELIVERY SYSTEM INSTITUTE, LTD.)[JP/JP]
〒134 東京都江戸川区北葛西1丁目16番13号 Tokyo, (JP)

(72) 発明者；および
(75) 発明者／出願人（米国についてのみ）
井上和泓(INOUE, Kazuhiro)[JP/JP]
洲崎 浩(SUSAKI, Hiroshi)[JP/JP]
池田政浩(IKEDA, Masahiro)[JP/JP]
久我 洋(KUGA, Hiroshi)[JP/JP]
熊澤栄治(KUMAZAWA, Eiji)[JP/JP]
東郷明子(TOGO, Akiko)[JP/JP]
〒134 東京都江戸川区北葛西1丁目16番13号
第一製薬株式会社 東京研究開発センター内 Tokyo, (JP)
(74) 代理人
弁理士 今村正純, 外(IMAMURA, Masazumi et al.)
〒103 東京都中央区八重洲一丁目8番12号
藤和八重洲一丁目ビル7階 Tokyo, (JP)

(81) 指定国 AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, TT, UA, US, UZ, VN, YU, ARIPO特許 (GH, KE, LS, MW, SD, SZ, UG), ユーラシア特許 (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), 欧州特許 (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI特許 (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

添付公開書類
国際調査報告書

(54)Title: DRUG COMPLEXES

(54)発明の名称 薬物複合体

(57) Abstract

Drug complexes wherein a carboxy (C_{1-4})alkyldextran polyalcohol, which has been treated under such conditions as to allow the substantially complete formation of the polyalcohol, is bonded to the residue of a medicinal compound such as an antitumor agent via a spacer consisting of one amino acid or a spacer consisting of two to eight amino acids bonded to each other via peptide bonds. The complexes are excellent in the tumor site selectivity and thus can exhibit a high antitumor effect with relieved expression of toxicity.

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（５７）要約

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実質的に完全にポリアルコール化可能な条件下で処理されてなるカルボキシC_{1-4}アルキルデキストランポリアルコールと抗腫瘍剤などの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。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する。

参考情報
ＰＣＴに基づいて公開される国際出願のパンフレット第一頁に記載されたＰＣＴ加盟国を同定するために使用されるコード

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明　細　書

薬物複合体

技術分野

本発明は医薬として有用な薬物複合体に関するものである。さらに具体的にいうと、本発明は、多糖誘導体であるカルボキシC_{1-4}アルキルデキストランポリアルコールと抗腫瘍剤や抗炎症剤等の医薬化合物とをスペーサーを介して結合させた薬物複合体に関するものである。

背景技術

肺癌や消化器癌などの固形癌や白血病などの血液癌の治療に際して用いられる抗腫瘍剤は、静脈内投与や経口投与などの投与経路により全身的に投与された後、特定の腫瘍部位に移行して癌細胞の増殖を阻害ないし抑制することにより治療効果を発揮する。しかしながら、全身投与された抗腫瘍剤は、血中から肝臓・網内系臓器に速やかに取り込まれたり、あるいは速やかに尿中排泄されるために、血中濃度が低下して腫瘍部位への移行が十分でない場合がある。また、通常の抗腫瘍剤自体では腫瘍部位への移行選択性（腫瘍選択性）が低いために、抗腫瘍剤が全身の様々な細胞や組織に満遍なく分布してしまい、正常な細胞や組織に対しても細胞毒として作用するので、嘔吐、発熱、あるいは脱毛などの副作用を極めて高率に発生させるという問題がある。従って、抗腫瘍剤を効率的かつ選択的に腫瘍部位に移行させる手段の開発が求められている。

このような手段の一つとして、多糖高分子に抗腫瘍剤を結合させて、抗腫瘍剤の血中からの消失を遅延させ、かつ、癌組織への指向性を高める方法が提案されている。例えば、特公平7-84481 号公報には、カルボキシメチル化されたマンノグルカン誘導体にシッフ塩基や酸アミド結合を介してダウノルビシン、ドキソルビシン、マイトマイシンC、又はブレオマイシンなどを導入した薬物複合体が開示されている。この発明におけるマンノグルカン誘導体としては、カルボキシメチル化されたマンノグルカン・ポリアルコールも用いられている。しかしながら、

マンノグルカン誘導体は枝分かれが多いので構造が複雑であり、医薬品の製造に適する均一な品質のものを入手することが困難であった。

また、国際公開 WO94/19376 号には、カルボキシル基を有する多糖のカルボキシル基にペプチド鎖（アミノ酸数 1～8)が結合されており、さらにこのペプチド鎖を介してドキソルビシン、ダウノルビシン、マイトマイシンC、又はブレオマイシンなどを結合した薬物複合体が開示されている。カルボキシル基を有する多糖としては、本来的にその構造中にカルボキシル基を有する多糖（例えば、ヒアルロン酸等）のほか、本来的にその構造中にカルボキシル基を有しない多糖（例えば、プルラン、デキストラン、キチン等）の水酸基にカルボキシC_{1-4}アルキル基を導入するか、あるいはマロン酸やコハク酸などの多塩基性酸をエステル結合させてカルボキシル基を導入した多糖類などが例示されている。この薬物複合体は、ドキソルビシンなどの薬剤と上記多糖部分とがスペーサーを介して結合していることを構造上の特徴としており、ドキソルビシンより優れた抗腫瘍効果が得られるとともに、毒性・副作用が軽減されている。

その他、ポリアルコール化多糖誘導体を薬物の送達キャリアーとして用いた薬物複合体に関する技術について、「多糖－ペプチド－ドキソルビシン複合体に関する研究・多糖キャリアーの血中安定性と抗腫瘍効果の関係」（第10回日本ＤＤＳ学会講演要旨集，279，1994)；「多糖－ペプチド－ドキソルビシン複合体に関する研究・体内動態と抗腫瘍効果」（第９回日本薬物動態学会年会講演要旨集，292，1994)；第19回研究開発動向セミナー（医薬品機構主催）要旨集，D-9，1995 ；及び「多糖キャリアーによる腫瘍への薬物送達に関する研究」（第12回コロイド・界面技術シンポジウム，日本化学会，講演要旨集，51，1995）などの報告がある。

発明の開示

本発明の課題は、抗腫瘍剤や抗炎症剤などの有効成分を腫瘍部位などに対して部位選択的に移行させることができる薬物複合体を提供することにある。より具体的には、抗腫瘍剤や抗炎症剤などの医薬化合物を部分構造として含む薬物複合体であって、血中に長時間滞留することができ、かつ、腫瘍部位や炎症部位に対して部位選択的に該医薬化合物を送り込むことができる薬物複合体を提供するこ

とが本発明の課題である。また、本発明の別の課題は、上記の特徴を有する薬物複合体の製造方法を提供することにある。

本発明者らは上記の課題を解決すべく国際公開 WO94/19376 号に開示された薬物複合体の改良を試みた結果、カルボキシル基を有する多糖類に替えて、デキストランをポリアルコール化したデキストラン誘導体をカルボキシC_{1-4}アルキル化したものを多糖部分として用いると、投与後の医薬が長時間にわたって高濃度で維持されるとともに、腫瘍部位や炎症部位に対する部位選択性を大幅に改善できることを見い出した。また、このような化合物では抗腫瘍効果などの主薬効が顕著に増強されている一方、毒性は低減されていることを見いだした。本発明はこれらの知見を基にして完成されたものである。

すなわち本発明は、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を提供するものである。また本発明の別の態様により、上記の薬物複合体からなる医薬；及び上記の薬物複合体を有効成分として含む医薬組成物、例えばバイアルに充填された凍結乾燥品の形態の注射用若しくは点滴用の製剤などが提供される。さらに本発明の別の態様によれば、上記の薬物複合体の製造方法が提供される。

上記の発明の好ましい態様として、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上記薬物複合体；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上記薬物複合体；医薬化合物が抗腫瘍剤又は抗炎症剤である上記薬物複合体；医薬化合物が濃度に依存した抗腫瘍作用を発現する抗腫瘍剤（より高い濃度でより強い抗腫瘍作用を発現する抗腫瘍剤：本明細書において濃度依存型の抗腫瘍剤という場合がある）である上記薬物複合体；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（より長い作用時間でより強い抗腫瘍作用を発現する抗腫瘍剤：本明細書において時間依存型の抗腫瘍剤という場合がある）である上記薬物複合体；

並びに、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)- ジオンである上記薬物複合体が提供される。

また、同様に好ましい態様として、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意味する）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上記薬物複合体；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上記薬物複合体；スペーサーが（N 末端)-Gly-Gly-Phe-Gly-である上記薬物複合体；並びに、抗腫瘍剤の残基の導入量が 1～15 重量% 、好ましくは 3～10重量% 、さらに好ましくは 5～6 重量% の範囲である上記薬物複合体が提供される。

本発明の特に好ましい態様として、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上記薬物複合体；（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% の範囲である上記薬物複合体；並びに、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 5,000～500,000 の範囲、好ましくは50,000～450,000 の範囲、より好ましくは 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構成糖残基あたり0.01～2.0 の範囲、好ましくは 0.1～1.0 の範囲、より好ましくは 0.3～0.5 の範囲である上記薬物複合体が提供される。

本発明の別の態様によれば、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が提供される。この発明の好ましい態様では、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、好ましくは50,000～450,000 の範囲、より好ましくは 200,000～400,000 の範囲であり、カルボキシC_{1-4}アルキル化度が構成糖残基あたり0.01～2.0 の範囲、好ましくは 0.1～1.0 の範囲、より好ましくは 0.3～0.5 の範囲である。カルボキシメチルデキストランポリアルコールが最も好ましいキャリアーとして提供される。この発明の別の観点からは、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が提供される。

この発明の好ましい態様として、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；並びに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が提供される。

図面の簡単な説明

第1図は、本発明の薬物複合体（例8で製造したもの）のGPC チャートを示す図である。

第2図は、本発明の薬物複合体（例8で製造したもの）の紫外線吸収スペクトルを示す図である。

第3図は、本発明の薬物複合体（例9で製造したもの）のGPC チャートを示す図である。

第4図は、本発明の薬物複合体（例9で製造したもの）の紫外線吸収スペクトルを示す図である。

第5図は、本発明の薬物複合体（例10で製造したもの）の紫外線吸収スペクトルを示す図である。

第6図は、本発明の薬物複合体（例15で製造したもの）のGPC チャートを示す図

である。

第７図は、本発明の薬物複合体（例15で製造したもの）の紫外線吸収スペクトルを示す図である。

第８図は、本発明の薬物複合体（例28で製造したもの）のGPC チャートを示す図である。

第９図は、本発明の薬物複合体（例28で製造したもの）の紫外線吸収スペクトルを示す図である。

第10図は、本発明の薬物複合体（例29で製造したもの）のGPC チャートを示す図である。

第11図は、本発明の薬物複合体（例29で製造したもの）の紫外線吸収スペクトルを示す図である。

第12図は、本発明の薬物複合体（例34で製造したもの）のGPC チャートを示す図である。

第13図は、本発明の薬物複合体（例34で製造したもの）の紫外線吸収スペクトルを示す図である。

第14図は、本発明の薬物複合体（例39で製造したもの）のGPC チャートを示す図である。

第15図は、本発明の薬物複合体（例39で製造したもの）の紫外線吸収スペクトルを示す図である。

第16図は、本発明の薬物複合体（例41で製造したもの）のGPC チャートを示す図である。

第17図は、本発明の薬物複合体（例41で製造したもの）の紫外線吸収スペクトルを示す図である。

第18図は、本発明の薬物複合体（例44で製造したもの）のGPC チャートを示す図である。

第19図は、本発明の薬物複合体（例44で製造したもの）の紫外線吸収スペクトルを示す図である。

第20図は、本発明の薬物複合体（例47で製造したもの）のGPC チャートを示す図である。

第21図は、本発明の薬物複合体（例47で製造したもの）の紫外線吸収スペクトルを示す図である。

第22図は、本発明の薬物複合体（例55で製造したもの）のGPC チャートを示す図である。

第23図は、本発明の薬物複合体（例55で製造したもの）の紫外線吸収スペクトルを示す図である。

第24図は、本発明の薬物複合体（例15で製造したもの）の体内動態を示す図である。図中の各点は３例の平均値を示す。

発明を実施するための最良の形態

本発明の薬物複合体は、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している。

本発明の薬物複合体に含まれる医薬化合物の残基は、例えば、抗腫瘍剤、抗炎症剤、抗菌剤などの医薬としてヒトを含む哺乳類の病気の治療及び／又は予防に用いられる医薬化合物に由来し、その部分構造により構成される。もっとも、該残基が由来する医薬化合物は上記のものに限定されることはない。また、医薬化合物としてはスペーサーとの結合に関与できる１又は２以上の反応性官能基（例えば、アミノ基、カルボキシル基、水酸基、チオール基、エステル基など）を有するものであればいかなるものを用いてもよい。本明細書において医薬化合物という場合には、それ自体が医薬作用を有する化合物の主要構造をその部分構造として含み生体内で該化合物を再生することができるプロドラッグ化合物も含まれる。

より具体的には、本明細書において医薬化合物の残基とは、スペーサーと医薬化合物残基との結合が医薬化合物中の反応性官能基とスペーサー中の反応性官能基との反応（例えば脱水縮合など）により形成されたと仮定した場合において、結合後の化合物中に存在する医薬化合物に由来する部分構造を意味している。例えば、医薬化合物が D-NH_2, D-COOH, D-COOR, D-OH, D-SH, D-$CONH_2$, D-NH-COOR

（R は低級アルキル基等）で表される場合、医薬化合物の残基はそれぞれ D-NH-（D-NH-CO-Q など）, D-CO-（D-CO-NH-Q, D-CO-O-Q, D-CO-S-Q など）, D-CO-（D-CO-NH-Q, D-CO-O-Q, D-CO-S-Q など）, D-O-（D-O-CO-Q, D-O-Qなど）, D-S-（D-S-CO-Q, D-S-Qなど）, D-CONH-（D-CO-NH-CO-Qなど）, D-NH-CO-（D-NH-CO-O-Q, D-NH-CO-NH-Qなど）で表される（カッコ内はスペーサーと医薬化合物残基との結合を示し、Q はスペーサーから反応性官能基を除いた残りの部分構造を示す）。もっとも、スペーサーと医薬化合物残基との結合の種類は上記のものに限定されることはない。医薬化合物の残基は、スペーサーのＮ末端アミノ基又はＣ末端カルボキシル基のほか、スペーサーを構成するアミノ酸に存在する反応性官能基に結合していてもよい。

医薬化合物の残基としては、例えば、ドキソルビシン、ダウノルビシン、マイトマイシンＣ、ブレオマイシン、シクロシチジン、ビンクリスチン、ビンブラスチン、メトトレキセート、白金系抗腫瘍剤（シスプラチン若しくはその誘導体）、タキソール若しくはその誘導体、カンプトテシン若しくはその誘導体（特開平6-87746 号公報に記載された抗腫瘍剤、好ましくは請求項２に記載された（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)- ジオン等）などの抗腫瘍剤の残基を好適に用いることができる。また、例えば、コハク酸ヒドロコルチゾン、コハク酸プレドニゾロンなどのステロイド系抗炎症剤、又はメフェナム酸、フルフェナム酸、ジクロフェナク、イブプロフェン、チノリジンなどの非ステロイド系抗炎症薬の残基も好適である。

医薬化合物の残基と結合するスペーサーとしては、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用いることができる。より具体的には、スペーサーは、１個のアミノ酸の残基（アミノ酸のアミノ基及び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意味する）、又はペプチド結合した２ないし８個のアミノ酸からなるオリゴペプチドの残基（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意味する）の形態を有している。

好ましいスペーサーは２～６個のアミノ酸からなるオリゴペプチドの残基である。スペーサーを構成するアミノ酸の種類は特に限定されないが、例えば、L-又はD-アミノ酸、好ましくはL-アミノ酸を用いることができ、α－アミノ酸のほか、β－アラニン、ε－アミノカプロン酸、γ－アミノ酪酸などを用いてもよい。このようなα－アミノ酸以外のアミノ酸は、多糖誘導体に近接した位置に配置されることが好ましい。

スペーサーの結合方向は特に限定されないが、一般的には、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カルボキシル基にスペーサーのＮ末端を酸アミド結合によって結合し、医薬化合物のアミノ基にスペーサーのＣ末端を結合することができる。また、例えば、ペプチドスペーサの構成単位としてリジン残基を含めておき、リジン残基のα－アミノ基及びε－アミノ基をそれぞれ他のアミノ酸のカルボキシル基と酸アミド結合させると、ペプチドスペーサーの両末端がＮ末端になるので、医薬化合物のカルボキシル基を結合することが可能になる。さらに、スペーサー中に１個又は２個以上のジアミン化合物またはジカルボン酸化合物の残基（例えばエチレンジアミンなどのジアミンの残基やコハク酸などのジカルボン酸の残基）を構成単位として含めておき、それぞれ両末端がＮ末端のスペーサー及び両末端がＣ末端のスペーサーを利用してもよい。

スペーサーのアミノ酸配列は特に限定されないが、例えば、スペーサーが -X-Z-で表されるジペプチドの残基（X は疎水性アミノ酸の残基を示し、Z は親水性アミノ酸の残基を示し、-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たジペプチドの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意味する）であるか、又は該ジペプチドの残基を部分ペプチド配列として含むスペーサーを好適に用いることができる。疎水性アミノ酸としては、例えば、フェニルアラニン、チロシン、ロイシンなどを用いることができ、親水性アミノ酸としては、例えば、グリシン、アラニンなどを用いることができる。スペーサーがこのようなジペプチド残基の繰り返し配列（例えば-X-Z-X-Z-, -X-Z-X-Z-X-Z-など）を有していてもよい。

このようなジペプチド構造を含むスペーサーを用いると、スペーサーがペプチ

ダーゼが豊富であると考えられる腫瘍部位や炎症部位で加水分解され、当該部位において医薬化合物が高濃度に遊離する。上記ジペプチドを含むスペーサーと医薬化合物とが結合して形成される部分構造は本発明の薬物複合体の好ましい部分構造である。医薬化合物の残基として、例えば、濃度依存型の抗腫瘍剤（例えば、ドキソルビシン）などを用いる場合には、-X-Z- で示される上記のジペプチド残基からなるスペーサー又は該ジペプチド残基を部分ペプチド配列として含むスペーサーを用いることが特に好ましい。

また、医薬化合物の残基として、一定の濃度以上で作用時間の持続を必要とする時間依存型の抗腫瘍剤を用いる場合にも、上記のスペーサーを用いることによって高い抗腫瘍効果を達成できる場合があり、例えば、特開平6-87746号公報に記載された抗腫瘍剤、好ましくは請求項２に記載された抗腫瘍剤が挙げられる。一般的には、上記のスペーサーに限定されることなく、抗腫瘍剤の作用機作、体内動態や毒性発現の特徴、体内での抗腫瘍剤の遊離性などの観点から好ましいスペーサーを選択する必要がある。なお、一般的に、増殖の速い癌種に対しては、短時間に高濃度の医薬化合物を遊離することができる上記のスペーサーを選択することが好ましい。

スペーサーの具体例を以下の表に示すが、本発明の薬物複合体に用いられるスペーサーは以下のものに限定されることはなく、医薬化合物の至適な遊離速度を与えるように当業者が適宜選択可能であることはいうまでもない。表中、ペプチド配列は左側がＮ末端であり、Ｃ末端側に医薬化合物の残基が結合する。D-PheはD-フェニルアラニン残基を示し、その他のアミノ酸はL-アミノ酸を示す。なお、遊離速度の大小はドキソルビシンを結合した薬物複合体の Walker 256 担癌ラットに対する薬効の発現の程度、または Walker 256 担癌ラットの腫瘍部位における遊離ドキソルビシン濃度によって判定したものである。これらのスペーサーのうち、ドキソルビシンに対しては（N 末端)-Gly-Gly-Phe-Gly-等の短時間に高濃度の医薬化合物を遊離することができるスペーサーを用いることが好ましい。

表 1

(a) 遊離速度が大きいスペーサー

-Leu-Gly-

-Tyr-Gly-

-Phe-Gly-

-Gly-Phe-Gly-

-Gly-Gly-Phe-Gly-

-Gly-Phe-Gly-Gly-

-Phe-Gly-Gly-Gly-

-Phe-Phe-Gly-Gly-

-Gly-Gly-Gly-Phe-Gly-

(b) 遊離速度が比較的大きいスペーサー

-Gly-Gly-Phe-Phe-

-Gly-Gly-Gly-Gly-Gly-Gly-

(c) 遊離速度が比較的小さいスペーサー

-Phe-Phe-

-Ala-Gly-

-Pro-Gly-

-Gly-Gly-Gly-Phe-

(d) 遊離速度が小さいスペーサー

-Gly-

-D-Phe-Gly-

-Gly-Phe-

-Ser-Gly-

-Gly-Gly-

-Gly-Gly-Gly-

-Gly-Gly-Gly-Gly-

本発明の薬物複合体の多糖誘導体部分を構成するカルボキシC_{1-4}アルキルデキストランポリアルコールのポリアルコール化度は特に限定されないが、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において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が好ましい。例えば、デキストランに大過剰の過ヨウ素酸ナトリウムと水素化ホウ素ナトリウムとを順次作用させてデキストランを実質的に完全にポリアルコール化したデキストランポリアルコールを本発明の薬物複合体の原料化合物として好適に用いることができる。もっとも、デキストランのポリアルコール化の方法は上記のものに限定されることはなく、当業者に利用可能なものであればいかなる方法を採用してもよい。

デキストランの種類は特に限定されず、α-D-1,6- 結合を任意の割合で含んでいてもよい。例えば、α-D-1,6- 結合の割合が 85%以上、90% 以上、又は95% 以上のデキストランなどを用いることができる。デキストランの分子量は特に限定されないが、例えば 10,000 程度から 2,000,000程度のもの、好ましくは 50,000 程度から 800,000程度のものを用いることができる。カルボキシC_{1-4}アルキル基を構成するC_{1-4}アルキルとしては、直鎖又は分枝鎖のC_{1-4}アルキル、具体的にはメチル基、エチル基、n-プロピル基、イソプロピル基、n-ブチル基、sec-ブチル基などを用いることができるが、好ましくはメチル基を用いることができる。カルボキシC_{1-4}アルキル化は、例えば、デキストランポリアルコールの水酸基に対してクロル酢酸、ブロム酢酸、α－クロルプロピオン酸、α－メチル－α－クロルプロピオン酸、β－クロルプロピオン酸、α－メチル－β－クロルプロピオン酸、α－クロル酪酸、β－クロル酪酸、γ－クロル酪酸などのハロゲン化C_{1-4}アルキルカルボン酸、好ましくはクロル酢酸を反応させて水酸基を部分的又は完全にカルボキシC_{1-4}アルキル化することにより行うことができる。

例えば、デキストランポリアルコールを反応に関与しない不活性溶媒（例えば、水、N,N-ジメチルホルムアミド、ジメチルスルホキシド等）に溶解し、塩基（例えば、水酸化ナトリウムや水酸化カリウム等）の存在下にハロゲン化C_{1-4}アルキルカルボン酸またはその塩を添加し、氷冷下ないし100 ℃程度の温度範囲で数分ないし数日間反応させればよい。カルボキシC_{1-4}アルキル基の導入の程度は、例えば、カ

ルボキシC_{1-4}アルキル化の反応温度や試薬として用いるハロゲン化C_{1-4}アルキルカルボン酸及び塩基の量を適宜選択することにより容易に調節可能であり、そのような手段は当業者に周知である。デキストランポリアルコールの水酸基に対するカルボキシC_{1-4}アルキル化の程度は特に限定されないが、例えば、構成糖残基あたり0.01～2.0 の範囲、好ましくは 0.1～1.0 、より好ましくは 0.3～0.5 の範囲である。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は、ゲルろ過法で測定した場合に 5,000から 500,000程度、好ましくは50,000～450,000 程度、より好ましくは200,000 ～400,000 程度である。

上記のカルボキシC_{1-4}アルキルデキストランポリアルコールは、薬物送達のキャリアーとして有用である。医薬化合物とカルボキシC_{1-4}アルキルデキストランポリアルコールとを結合させた薬物複合体は、例えば、腫瘍選択性などの優れた選択性を有しており、また高い血中濃度を長時間維持できるという特徴がある。医薬化合物とカルボキシC_{1-4}アルキルデキストランポリアルコールとの結合としては、例えば、エステル結合などで両者を直接結合させる方法、上記に説明したスペーサーなどの適宜のスペーサーを介して両者を結合させる方法などを採用することができる。

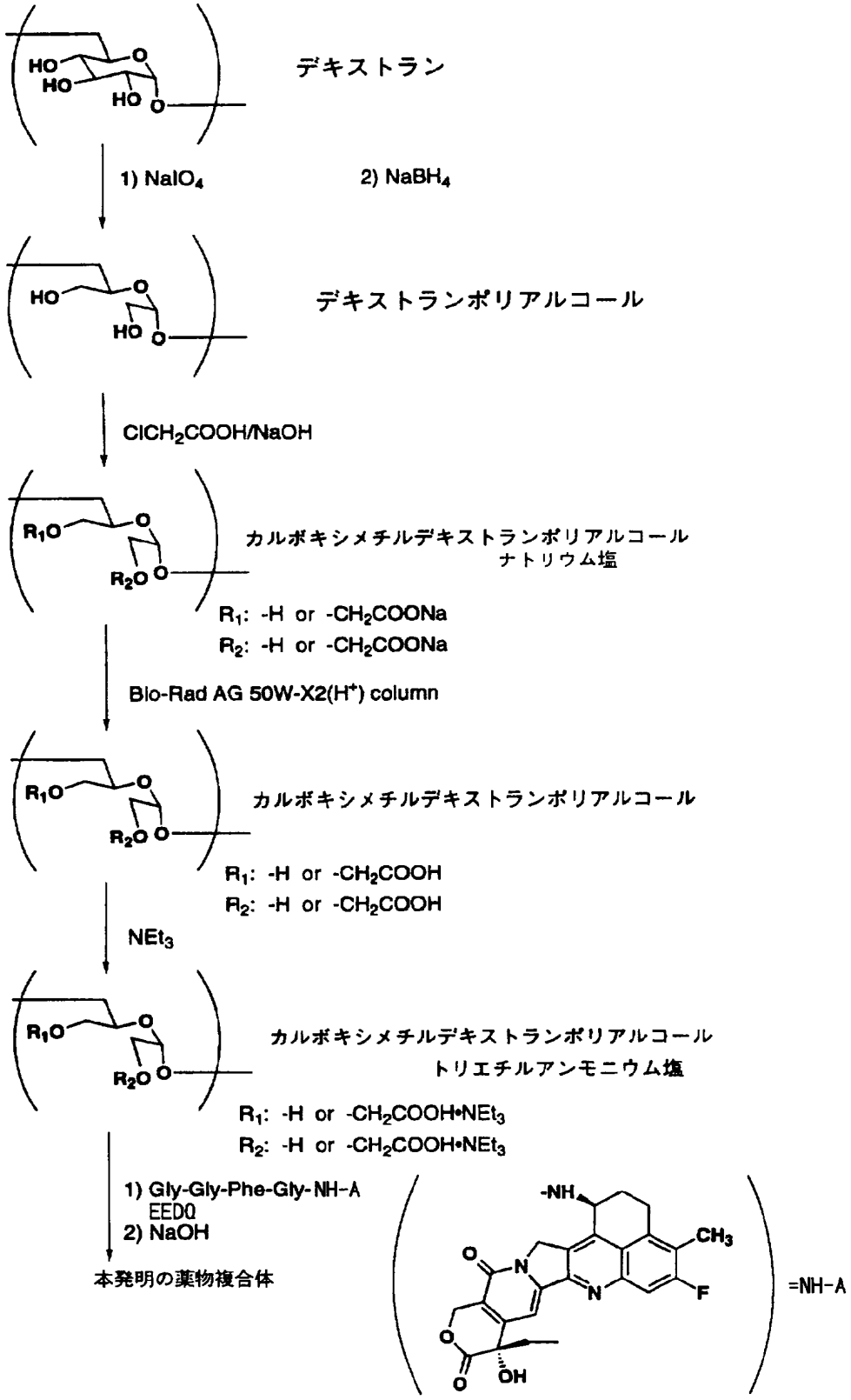
スペーサーを介して結合する薬物複合体に関しては、上記のようにして得られるカルボキシメチルデキストランポリアルコールのカルボキシル基に対して、医薬化合物の残基と結合させたスペーサーを結合することにより本発明の薬物複合体を製造することができる。スペーサーとカルボキシメチルデキストランポリアルコールのカルボキシル基との結合は、一般的には、スペーサーのＮ末端アミノ基とカルボキシメチルデキストランポリアルコールのカルボキシル基とを酸アミド結合により結合させることにより形成できる。もっとも、スペーサーとカルボキシメチルデキストランポリアルコールのカルボキシル基との結合は上記のものに限定されることはなく、他の化学結合や１又は２以上のスペーサーを利用した結合であってもよい。例えば、スペーサーのＣ末端カルボキシル基と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とにより酸無水物を形成させてもよく、また、エチレンジアミン等のジアミン化合物をスペーサーとして用いてそれぞれのカルボキシル基をジアミンの各アミノ基に酸アミド結合させても

よい。

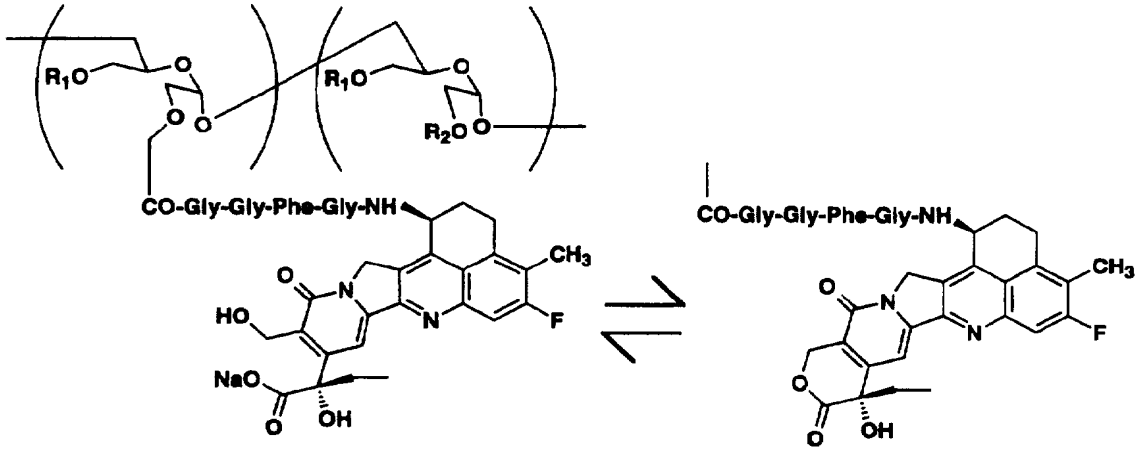
スペーサーのN末端アミノ基とカルボキシメチルデキストランポリアルコールのカルボキシル基とを酸アミド結合により結合させる場合には、ペプチド鎖の合成に用いる通常の脱水縮合剤、例えば、 N,N'-ジシクロヘキシルカルボジイミド(DCC) のような N,N'-ジシクロアルキルカルボジイミド類、1-エチル-3-(3-ジメチルアミノプロピル) カルボジイミド(EDAPC) 等のカルボジイミド誘導体、1-ヒドロキシベンゾトリアゾール(HOBT)のようなベンゾトリアゾール誘導体のほか、1-エトキシカルボニル-2- エトキシ-1,2- ジヒドロキシキノリン(EEDQ)などを用いることができる。また、活性エステル法や酸ハライド法などにより反応を行ってもよい。

カルボキシメチルデキストランポリアルコールに導入する医薬化合物残基の量は特に限定されないが、医薬化合物残基の物理化学的性質、並びに本発明の薬物複合体の体内動態、薬効、及び毒性などの観点から適宜選択すべきである。一般的には、0.1 ～30重量%、好ましくは 1～15重量%、さらに好ましくは 3～10重量%、特に好ましくは 5～6 重量%程度の範囲を選択することができる。カルボキシメチルデキストランポリアルコールに導入された医薬化合物残基の割合は、例えば、吸光度分析などにより容易に決定することが可能である。

本発明の薬物複合体の製造方法の一例として、特開平6-87746 号公報の請求項2に記載された抗腫瘍剤である医薬化合物の残基を導入する場合について、製造方法を以下のスキームに示すが、本発明の薬物複合体及びその製造方法はこのスキームに示されたものに限定されることはない。下記スキームにおいて、医薬化合物残基の導入量は、例えば 1～15重量%、好ましくは 2～10重量%程度である。また、下記スキーム中には、ポリアルコール類の構成単位のうち1個又は2個のカルボキシメチル基が導入された構成単位のみを例示的に記載したが、本発明の薬物複合体の多糖誘導体部分は上記構成単位の繰り返しによって構成されるものではないことを理解すべきである。


デキストラン
1) NaIO4
2) NaBH4
デキストランポリアルコール
ClCH2COOH/NaOH
カルボキシメチルデキストランポリアルコール
ナトリウム塩
R1: -H or -CH2COONa
R2: -H or -CH2COONa
Bio-Rad AG 50W-X2(H+) column
カルボキシメチルデキストランポリアルコール
R1: -H or -CH2COOH
R2: -H or -CH2COOH
NEt3
カルボキシメチルデキストランポリアルコール
トリエチルアンモニウム塩
R1: -H or -CH2COOH•NEt3
R2: -H or -CH2COOH•NEt3
1) Gly-Gly-Phe-Gly-NH-A
EEDQ
2) NaOH
本発明の薬物複合体
=NH-A

本発明の薬物複合体



上記の医薬化合物は、酸性水性媒体中（例えばpH 3程度）ではラクトン環を形成した化合物（閉環体）に平衡が偏り、一方、塩基性水性媒体中（例えばpH 10 程度）ではラクトン環が開環した化合物（開環体）に平衡が偏ることが知られているが、このような閉環体及び開環体に対応する残基を導入した薬物複合体は同等の抗腫瘍効果を有しており、いずれも本発明の範囲に包含されることはいうまでもない。なお、反応系中にラクトン環の開環した反応種が存在すると、ラクトン環に由来するカルボキシル基とスペーサー由来のアミノ基との間で縮合反応が進行して著しく反応収率が低下するだけでなく、目的とする均一な薬物複合体が得られない場合がある。このような副反応は反応種として選択的に閉環体を用いることによって回避することができる。

すなわち、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をトリエチルアンモニウム塩に変換した後、非水系（水を含まない有機溶媒中）で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対して、上記の医薬化合物の残基を結合させたスペーサーのN末端アミノ基を縮合させることにより、副反応を抑制することができ、効率的に目的物を製造することが可能になる。有機溶媒に溶解可能なカルボキシメチルデキストランポリアルコールの塩としては、例えば、トリエチルアンモニウム塩やトリメチルアンモニウム塩などのトリアルキルアンモニウム塩のほか、N-メチルピロリジン、N-メチルモルホリン、ジメチルアミノピリジン（DMAP）などの有機塩基の塩を用いることが可能である。

有機溶媒としては、N,N-ジメチルホルムアミド、ジメチルスルホキシドなどを用いることができる。

本発明の薬物複合体は、医薬化合物の残基の種類（例えば、抗腫瘍剤または抗炎症剤などの医薬化合物の残基）に応じて、所望の医薬活性を腫瘍部位や炎症部位などの局所において特異的に発現させることができ、かつ、医薬化合物自体の有する毒性を低減できるという特徴を有する。いかなる特定の理論に拘泥するわけではないが、本発明の薬物複合体の多糖誘導体部分（例えばカルボキシメチルデキストランポリアルコール）は極めて優れた血中滞留性及び腫瘍・炎症部位への集積性を有するので薬物送達のキャリアーとして有用であり、本発明の薬物複合体は腫瘍選択性及び炎症部位選択性を有している。また、腫瘍部位や炎症部位ではプロテアーゼ（ペプチダーゼ）が発現されていると考えられるので、本発明の薬物複合体のスペーサーは容易に加水分解され、遊離した医薬化合物が薬効を発揮する。

本発明の薬物複合体を含む医薬は、通常、凍結乾燥品などの形態でバイアル等に充填することができ、用時溶解型の注射用または点滴用製剤等の非経口投与用製剤として臨床に提供されるが、本発明の医薬の製剤形態は上記態様に限定されることはない。上記製剤の製造には、例えば、溶解補助剤、pH調節剤、安定化剤など当業界で利用可能な製剤用添加物を用いることができる。本発明の医薬の投与量は特に限定されないが、通常は、医薬化合物残基を構成する医薬化合物の投与量、本発明の薬物複合体中に導入された医薬化合物の残基の量、患者の状態や疾患の種類などを勘案して決定すべきである。例えば、特開平6-87746 号公報の請求項２に記載された抗腫瘍剤の残基が約 6重量% 程度の割合で導入された本発明の薬物複合体を非経口投与する場合には、一般に一日あたり体表面積 1 m^2 につき約 1～500 mg程度、好ましくは約10～100 mg の範囲で一回投与し、 3～4 週毎に繰り返すことが好ましい。

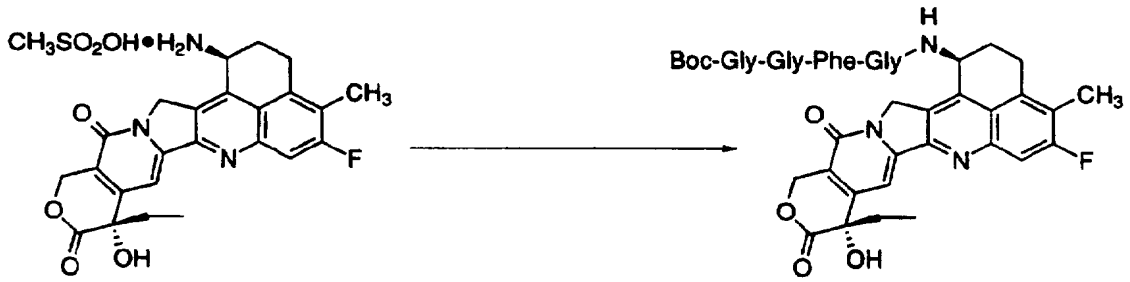
実施例

以下、本発明を実施例によりさらに具体的に説明するが、本発明の範囲は下記の実施例に限定されることはない。実施例中、「A-NH- 」は、特開平6-87746 号公

報の請求項２に記載された医薬化合物（実施例中で「DX-8951 」という場合がある）などのようにラクトン環を有する医薬化合物において、ラクトン環が閉環した医薬化合物を A-NH_2で表した場合の医薬化合物残基を表し、その一例は、上記のスキーム中にA-NH- で示した基（ラクトン環を形成したもの）である。また、A'-NH-は、A-NH- で表される医薬化合物残基中のラクトン環が閉環型若しくは開環型のいずれか又はそれらの混合形態であることを示し、-DXRはドキソルビシン由来の残基を示し、-D51-7059は例55に示されるタキソール誘導体由来の残基を示す。

また、実施例中、特に言及しない場合には、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メチル化度（構成糖残基あたりのカルボキシメチル基の置換度）は、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を遊離酸型に変換した後、 0.1N 水酸化ナトリウム水溶液に溶解して 0.1N 塩酸で滴定することにより求めた。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の水溶液を Bio-Rad AG50W-x 2(H^+ 型）カラムに付して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 0.1N 水酸化ナトリウム水溶液に溶解し、フェノールフタレンを指示薬として 0.1N 塩酸で滴定した。試料の採取量を s(mg)、 0.1N 水酸化ナトリウム水溶液の所定過剰量を a(ml)、 0.1N 塩酸の滴定量を b(ml)とし、カルボキシメチル化度を 13.4(a-b)/[s-5.8(a-b)] の式により求めた。また、薬物の導入量（重量%）は、薬物の特性吸収を利用した吸光度分析（362 nm付近）から求めた。さらに、ゲル濾過法は次の条件に従って行った（カラム：TSK gel G4000 PW_{XL}、溶離液： 0.1M NaCl、流速：0.8 ml/min、カラム温度：40℃）。

例１：3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951)



Boc-Gly-Gly-Phe-Gly (600 mg)および N- ヒドロキシスクシンイミド (160 mg) を N,N- ジメチルホルムアミド (20 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 (280 mg) を添加した。この溶液に特開平6-87746号公報の請求項2に記載された医薬化合物のメタンスルホン酸塩 (600 mg: 上記公報の実施例50に記載された化合物) とトリエチルアミン (0.16 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 (1.0 g)を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.40 (d, 1H, J=8.3 Hz), 8.10-8.17 (m, 2H), 7.91-8.01 (m, 1H), 7.78 (d, 1H, J=10.75 Hz), 7.32 (s, 1H), 6.94-6.96 (m, 1H), 6.50 (s, 1H), 5.57 (t, 1H, J= 4.5Hz), 5.43 (s, 2H), 5.23 (s, 2H), 3.77 (dd, 2H, J= 5.85Hz, J = 8.80Hz), 3.70 (d, 2H, J= 4.40Hz), 3.65 (d, 2H, J= 5.35Hz), 3.56 (d, 2H, J= 5.85), 3.15-3.25 (m, 2H), 2.40 (s, 3H), 2.05-2.25 (m, 1H), 1.86 (m, 2H), 1.35 (s, 9H), 0.88 (t, 3H, J= 7.35).

Mass (FAB): m/e 854 (M+1)

例2：3'-N-(Boc-Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951)の合成

Boc-Gly-Gly-Gly-Phe (600 mg)およびN-ヒドロキシスクシンイミド (160 mg) をN,N-ジメチルホルムアミド (20 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 (280 mg) を添加した。この溶液に DX-8951 のメタンスルホン酸塩 (600 mg) とトリエチルアミン (0.16 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 (700 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ: 8.57 (d, 1H, J= 7.8Hz), 8.19 (d, 1H), 8.05-8.07 (m, 2H), 7.79 (d, 1H, J= 11.2Hz), 7.32 (s, 1H), 7.10 (d, 2H, J= 7.8Hz), 6.93-7.03 (m, 4H), 6.51 (s, 1H), 5.52-5.55 (m, 1H), 5.44 (s, 2H), 5.18 (d, 1H, J= 18.5Hz), 4.84

(d, 1H, J= 18.5Hz), 4.57-4.59 (m, 1H), 3.57-3.71 (m, 6H), 3.15-3.25 (m, 2H), 3.00-3.02 (m, 1H), 2.80-2.90 (m, 1H), 2.40 (s, 3H), 2.05-2.25 (m, 1H), 1.86 (m, 2H), 1.35 (s, 9H), 0.88 (t, 3H, J= 7.35Hz).

Mass (FAB) ; m/e 854 (M+1)

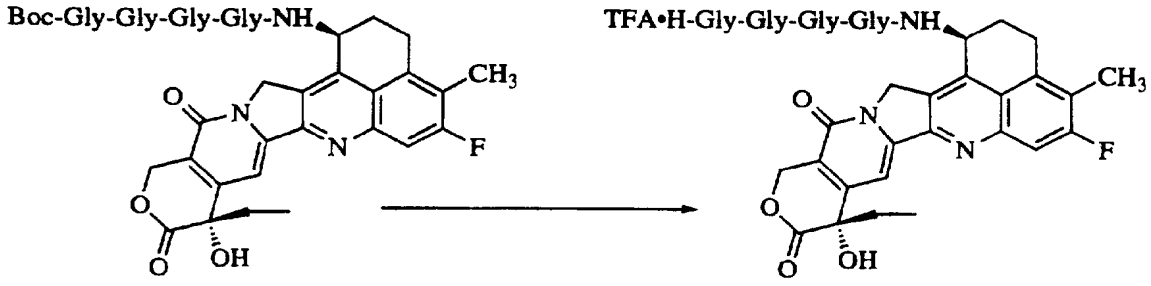
例３ : 3'-N-(Boc-Gly-Gly-Gly-Gly)-NH-A (A-NH_2 =DX-8951)の合成

Boc-Gly-Gly-Gly-Gly (120 mg)およびN-ヒドロキシスクシンイミド (39 mg)をN, N-ジメチルホルムアミド (20 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 (70 mg)を添加した。この溶液に DX-8951 のメタンスルホン酸塩 (150 mg) とトリエチルアミン (0.039 ml) を溶解したN, N-ジメチルホルムアミド (10 ml)溶液を加えて、遮光下に室温で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：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 (100 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ : 8.40 (d, 1H, J= 8.3 Hz), 8.10-8.17 (m, 2H), 7.91-8.01 (m, 1H), 7.78 (d, 1H, J= 10.75 Hz), 7.32 (s, 1H), 6.94-6.96 (m, 1H), 6.50 (s, 1H), 5.57 (t, 1H, J= 4.5Hz), 5.43 (s, 2H), 5.23 (s, 2H), 3.77 (dd, 2H, J= 5.85Hz, J = 8.80Hz), 3.70 (d, 2H, J= 4.40Hz), 3.65 (d, 2H, J= 5.35Hz), 3.56 (d, 2H, J= 5.85Hz), 3.15-3.25 (m, 2H), 2.40 (s, 3H), 2.05-2.25 (m, 1H), 1.86 (m, 2H), 1.35 (s, 9H), 0.88 (t, 3H, J= 7.35Hz).

Mass (FAB); m/e 764 (M+1)

例４ : 3'-N-(Gly-Gly-Gly-Gly)-NH-A (A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成



3'-N-(Boc-Gly-Gly-Gly-Gly)-NH-A (A-NH_2 =DX-8951) (79 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: 8.59-8.61 (m, 1H), 8.50 (d, 1H, J= 8.3Hz), 8.21-8.27 (m, 2H), 7.91-8.01 (br, 3H), 7.81 (d, 1H, J= 11.2Hz), 7.32 (s, 1H), 6.50-6.52 (br, 1H), 5.57-5.59 (m, 1H), 5.43 (s, 2H), 5.23 (s, 2H), 3.80-3.82 (m, 3H), 3.70-3.75 (m, 3H), 3.15-3.25 (m, 2H), 2.41 (s, 3H), 2.05-2.25 (m, 1H), 1.86-1.88 (m, 2H), 0.88 (t, 3H, J= 7.35Hz).

例5：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T2000(10 g, ファルマシア社製, 平均分子量 2,000,000）を 0.1 M酢酸緩衝液（pH 5.5, 1,000 ml）に溶解し、過ヨウ素酸ナトリウム（33.0 g）の水溶液（1000 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（7.0 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（14 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 pH5.5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（ミリポア社製）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を得た。このデキストランポリアルコールをpH 3.0で1時間処理した後、バイオマックス-50 膜で低分子画分を除去し、次いで高分子画分をバイオマックス-100膜で除去し、凍結乾燥して精製デキストランポリアルコール（2.0 g)を得た。この物質の分子量（ゲル濾過法、デキストラン標準）は、220 K であった。

この精製デキストランポリアルコール（1.8 g)を、水酸化ナトリウム（10.5 g）を水（45 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（15 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8 に調整した後、バイオマックス-10 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、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（1.8 g)を得た。この物質の分子量（ゲル濾過、デキストラン標準）は330 K であり、カルボキシメチル化度は0.8 であった。

上記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（300 mg）を水に溶解し、Bio-Rad AG50W-X2（200-400 メッシュ、H^+ 型）カラム（1.5 × 8.6 cm)にのせ、水で溶出した。この溶出液にトリエチルアミン（0.5 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（380 mg）を得た。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（各 300 mg)を上記と同様にカラム処理して、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（380 mg, 400 mg）を得た。

例６：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合成

上記例５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（0.15 g）を、水酸化ナトリウム（1.05 g）を水（4.5 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（1.5 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、90 ml のメタノール中に滴下した後、3 M 塩化ナトリウム水溶液（0.15 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。この沈澱をメタノールで洗浄した後、水（5 ml）に溶解し、3 M 塩化ナトリウム水溶液（0.15 ml)を加えた。この水溶液をミリポアフィルター（0.45μm)で濾過し、濾液を35 mlのエタノール中に滴下して、析出した沈澱を遠心分離（3500 rpm, ８分）により集めた。この沈澱をエタノールで洗浄した後、水に溶解し、透析膜（スペクトラポア１、カットオフ分子量 6,000-8,000）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)で濾過した後に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（0.18 g）を得た。この物質の糖残基あたりのカルボキシメチル化度（アルカリ滴定法）は1.2 であった。

例７：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合成

例５で得た精製デキストランポリアルコール（0.2 g)を、水酸化ナトリウム（0.84 g）を水（6 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（1.2 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を8 に調整し、120 mlのメタノール中に滴下した後、3 M 塩化ナトリウム水溶液（0.2 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。この沈澱をメタノールで洗浄した後、水（5 ml）に溶解し、3 M 塩化ナトリウム水溶液（0.2 ml）を加えた。この水溶液をミリポアフィルター（0.45μm)で濾過し、濾液を 35 mlのエタノール中に滴下して、析出した沈澱を遠心分離（3500 rpm, 8分）により集めた。この沈澱をエタノールで洗浄した後、水に溶解し、透析膜（スペクトラポア１、カットオフ分子量 6,000-8,000）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、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（0.20 g）を得た。この物質の糖残基あたりのカルボキシメチル化度（アルカリ滴定法）は0.4 であった。

例８：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５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（380 mg, カルボキシメチル化度 0.8）をN,N-ジメチルホルムアミド（30 ml)に溶解した。この溶液に、3'-N-(Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951)のトリフルオロ酢酸塩（49 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（0.017 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（380 mg）を順次加え、室温で一晩撹拌しながら反応させた。この反応液を1 M 水酸化ナトリウム水溶液で pH 10に調整した後、 25 mlのエタノール中に 5 ml ずつ滴下した。この混合物に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 6,000-8,000）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（0.22μm)で濾過した後、凍結乾燥した。得られた粗生成物を水（30 ml)に溶解し、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。この処理液を上記と同様に透析した後、透析内液をミリポアフィルター（0.22μm)で濾過し、凍結

乾燥して標記化合物を 289 mg 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：東ソー株式会社製 TSK Gel PW-4000XL、溶媒：0.1 M 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, 0.25 mg/ml）をそれぞれ図１及び図２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 5.3%（W/W）であった。

例９：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)合成

3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A (A-NH_2 =DX-8951)（50 mg)から例４と同様の方法により脱 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を、例８と同様の方法に従って、例５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（380 mg）に導入して標記化合物（300 mg）を合成した。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：東ソー株式会社製 TSK Gel PW-4000XL、溶媒：0.1 M 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, 0.19 mg/ml）をそれぞれ図３及び図４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 5.3%（W/W）であった。

例10：カルボキシメチルデキストランポリアルコール-Gly-Gly-Gly-Gly-NH-A'(A-NH_2 =DX-8951)の合成

3'-N-(Boc-Gly-Gly-Gly-Gly)-NH-A (A-NH_2 =DX-8951)（41 mg)から例４と同様の方法により脱 Boc化して得られる 3'-N-(Gly-Gly-Gly-Gly)-NH-Aのトリフルオロ酢酸塩を、例８と同様の方法に従って、例５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（380 mg）に導入して標記化合物（190 mg）を合成した。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, 0.34 mg/ml）を図５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 5.3%（W/W）であった。

例11：本発明の薬物複合体の抗腫瘍作用

マウス線維肉腫 Meth A 細胞 1×10^6 個を BALB/c 系の雄マウス（7 週齢）の右鼠蹊部皮下に移植して Meth A 担癌マウスを作成した（1群7匹）。7 日目に注射用蒸留水に溶解した例9の薬物複合体を Meth A 担癌マウスの尾静脈内に4日毎に4回投与した。移植後21日目に腫瘍を摘出して重量を測定し、腫瘍増殖抑制率を次式：腫瘍増殖抑制率(%) =[1-(検体投与群の平均腫瘍重量／コントロール群の平均腫瘍重量)]×100 により算出した。その結果、例9で得られた本発明の薬物複合体は、毒性（体重減少）を発現することなく、上記医薬化合物自体（スペーサー及び多糖誘導体なし）に比較して抗腫瘍効果が大幅に増強されていた。多糖誘導体自体（例5）及びスペーサーのみを導入した医薬化合物残基（例1の化合物から例4の方法に従って脱BOC 化して得られたる H_2N-Gly-Gly-Phe-Gly-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩）は無効であった。

表2

被検化合物	投与量(mg/kg)	抑制率(%)
医薬化合物自体	7.5 × 4	76
	1.875 × 4	46
	0.9375× 4	36
例9の化合物	1.4[1] × 4	94
	0.7[1] × 4	59
	0.35[1]× 4	41

[1]医薬化合物換算量

例12：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）、7 日目に例8及び例9の薬物複合体を単回投与した場合の抗腫瘍作用を比較した。その結果、抗腫瘍作用の程度は（多糖誘導体)-Gly-Gly-Phe-Gly-NH-A' ＞（多糖誘導体)-Gly-Gly-Gly-Phe-NH-A' ＞医薬化合物自体であった。スペーサーを介することなく医

薬化合物残基を例５の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直接結合した化合物（医薬化合物残基の導入量： 6.2重量% ）は無効であった。

表３

被検化合物	投与量(mg/kg)	抑制率(%)
医薬化合物自体	60	77
	20	59
例８の化合物	10[1)]	85
	5[1)]	76
例９の化合物	5[1)]	98
	2.5[1)]	87

[1)]医薬化合物換算量

例13：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（10 g, ファルマシア社製, 分子量 500K ）を0.1 M 酢酸緩衝液（pH5.5, 1000 ml）に溶解し、過ヨウ素酸ナトリウム（33 g）の水溶液（1000 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（7.0 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を 7.5に調整した。水素化ホウ素ナトリウム（14 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でpH 7.5に調整して溶液１を得た。別に、デキストランT500（10 g, ファルマシア社製, 分子量500K）について、上記の一連の操作を行い、溶液２を得た。さらに、デキストランT250（各10 g, ファルマシア社製, 分子量250K ）について、上記の一連の操作を行い、溶液３と溶液４を得た。これらの溶液１～４を合して、バイオマックス-50 膜を用いて限外濾過法により、低分子画分

の除去を行った。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（25 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は、163Kであった。

このデキストランポリアルコール（11 g）を、水酸化ナトリウム（46.2 g）を水（330 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（66 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９に調整した後、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（13 g）を得た。この物質の分子量（ゲル濾過、プルラン標準）は 228K 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（600 mg）を水に溶解し、Bio-Rad AG 50W-X2 （200-400 メッシュ、H^+ 型）カラム（直径44mm、長さ210mm)にのせ、水で溶出した。この溶出液にトリエチルアミン（0.93 ml)を加えた後、凍結乾燥して標記化合物（690 mg）を得た。

例14：3'-N-(Gly-Gly-Phe-Gly)-NH-A（A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成

例１で得た3'-N-(Boc-Gly-Gly-Phe-Gly)-NH-A（A-NH_2 =DX-8951）（79 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: 8.53 (d, 1H, J= 8.3Hz), 8.40-8.48 (m, 2H), 8.28 (d, 1H, J= 8.3Hz), 7.95-8.07 (br, 3H), 7.81 (d, 1H, J= 10.2Hz), 7.30-7.37 (m, 2H), 7.15-7.30 (m, 5H), 6.50-6.55 (br, 1H), 5.50-5.57 (m, 1H), 5.41 (d, 2H, J= 7.82Hz), 5.25 (s, 2H), 4.55-4.62 (m, 1H), 3.55-3.92 (m, 6H), 3.15-3.25 (br, 2H), 2.98-3.03 (m, 1H), 2.73-2.82 (m, 1H), 2.40 (s, 3H), 2.05-2.25 (m, 1H), 1.84-1.92 (m, 2H), 0.88 (t, 3H, J= 7.35Hz).

例15：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ナトリウム塩（400 mg）をトリエチルアンモニウム塩（470 mg）に変換し、N,N-ジメチルホルムアミド(30 ml）に溶解させた。この溶液に、例14で得た3'-N-(Gly-Gly-Phe-Gly)-NH-

A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (62 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(0.02 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 (470 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 塩化ナトリウム水溶液に溶解し、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、凍結乾燥して標記化合物 (600 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： 0.1 M 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、0.1 mg/ml) をそれぞれ図6及び図7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16：3'-N-(Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成

例2で得た3'-N-(Boc-Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951) (79 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： 8.62-8.66 (m,2H), 8.23 (d,1H,J= 8.3Hz), 8.18-8.20 (m,1H), 7.98-8.10 (br,2H), 7.79 (d,1H,J= 10.7Hz), 7.32 (s,1H), 7.09 (d,2H,J= 7.3Hz), 6.93-7.03 (m,4H), 6.50-6.60 (br,1H), 5.52-5.55 (m,1H), 5.44(s,2H), 5.18 (d,1H,J= 18.5Hz), 4.80 (d,1H,J= 18.5Hz), 4.57-4.59 (m,1H), 3.57-3.71 (m,6H), 3.15-3.25 (m,2H), 3.00-3.02 (m,1H), 2.80-2.90 (m,1H), 2.50 (s,3H), 2.05-2.25 (m,1H), 1.86-2.00 (m,2H), 0.88 (t,3H,J= 7.35Hz).

例17：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ナトリウム塩 (1.

0 g)をトリエチルアンモニウム塩(1.2 g) に変換し、N,N-ジメチルホルムアミド(90 ml) に溶解させた。この溶液に、例16で得た3'-N-(Gly-Gly-Gly-Phe)-NH-A (A-NH_2 =DX-8951) のトリフルオロ酢酸塩 (158 mg) のN,N-ジメチルホルムアミド(15 ml) 溶液、トリエチルアミン(0.05 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(1.2 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 塩化ナトリウム水溶液に溶解し、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、凍結乾燥して標記化合物 (1.4 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 5.2% (W/W) であった。

例18：Boc-Gly-Phe-Leu-OHの合成

H-Gly-Phe-Leu-OH (3.0 g)を 50%ジオキサン水溶液(48 ml) に加えて氷冷した。この溶液に1 N 水酸化ナトリウム水溶液(9.45 ml) と $(Boc)_2O$ (2.27 g) を含むジオキサン(24 ml) 溶液を加え、1晩撹拌した。反応液に1 N 塩酸(9.45 ml) を加え、溶媒を溜去した。得られ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＝5 ：1溶液) で精製して標記化合物(2.5 g) を得た。

例19：Boc-Gly-Phe-Leu-Gly-OBzlの合成

例18で得たBoc-Gly-Phe-Leu-OH (2.4 g)およびN-ヒドロキシスクシンイミド (656 mg) をN,N-ジメチルホルムアミド(50 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 (1.17 g) を添加し、2時間撹拌した。この溶液に H-Gly-OBzl のトシル酸塩(1.9 g) とトリエチルアミン(0.79 ml) を溶解したN,N-ジメチルホルムアミド(40 ml) 溶液を加えて、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：ジクロロメタン：メタノール＝50：1溶液) で精製して標記化合物(2.0 g) を得た。

^{1}H-NMR(DNSO-d_6) δ : 8.20-8.30 (m, 1H), 8.12 (d, 1H, J= 8.3Hz), 7.83 (d, 1H, J= 8.3Hz), 7.32-7.37 (m, 5H), 6.89-6.95 (m, 1H), 5.12 (s, 1H), 4.52-4.59 (br, 1H), 4.34 (dd, 1H, J= 7.3Hz, J= 15.1Hz), 3.93 (dd, 1H, J= 5.5Hz, J= 17.2Hz), 3 .84 (dd, 1H, J= 5.5Hz, J= 17.2Hz), 3.54 (dd, 1H, J= 5.9Hz, J= 16.7Hz), 3. 42 (dd, J= 5.9Hz, J= 16.7Hz), 3.00 (dd, 1H, J= 4.4Hz, 13.7Hz), 2.78 (dd, 1H, J= 8.8Hz, J= 13.2Hz), 1.50-1.65 (m, 1H), 1.45 (t, 2H, J= 7.3Hz), 1.36 (s, 9H), 0.86 (d, 3H, J= 6.4Hz), 0.82 (d, 3H, J= 6.4Hz).

例20：Boc-Gly-Phe-Leu-Gly-OHの合成

例19で得たBoc-Gly-Phe-Leu-OBzl(1.7 g) を酢酸エチル(30 ml) とメタノール(30 ml) の混合溶液に溶解させた後、5 % Pd-C (1.5 g)を加え、接触還元を行った。反応液を濾過し、濾液を減圧乾固して、標記化合物 (1.15 g) を得た。

例21：3'-N-(Boc-Gly-Phe-Leu-Gly)-NH-A (A-NH_2 =DX-8951)の合成

例20で得たBoc-Gly-Phe-Leu-Gly-OH (200 mg) およびN-ヒドロキシスクシンイミド(58 mg) をN,N-ジメチルホルムアミド (5 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 (104 mg) を加えて溶解させた。この溶液に DX-8951 のメタンスルホン酸塩 (224 mg) とトリエチルアミン (0.059 ml) を溶解したN,N-ジメチルホルムアミド (5 ml) 溶液を加えて、遮光下に室温で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 (200 mg) を得た。

^{1}H-NMR (DMSO-d_6) δ : 8.35 (d, 1H, J=7.8Hz), 8.08-8.18 (m, 2H), 7.75-7.85 (m, 2H), 7.32 (s, 1H), 7.10 (d, 2H, J=6.8Hz), 7.08-7.13 (m, 3H), 6.85-6.95 (br, 1H), 6.40-6.65 (br, 1H), 5.52-5.55 (m, 1H), 5.46 (d, 1H, J=18.5Hz), 5.37(d, 1H, J=18.5Hz), 5.24 (s, 2H), 4.44-4.52 (m, 1H), 4.15-4.25 (m, 1H), 3.68-3.72 (m, 2H), 3.40-3.52 (m, 2H), 3.15-3.25 (br, 2H), 2.85-2.95 (m, 1H), 2.65-2.75 (m, 1H), 2.40 (s, 3H), 2.05-2.25 (m, 1H), 1.80-1.91 (m, 2H), 1.50-1.65 (m, 1H), 1.45 (t, 2H, J=7.3Hz), 1.35 (s, 9H), 0.88 (t, 3H, J=7.4), 0.86 (d, 3H, J=6.4Hz), 0.82 (d, 3H, J=6.4Hz).

例22：3'-N-(Gly-Phe-Leu-Gly)-NH-A (A-NH_2 =DX-8951)トリフルオロ酢酸塩の合成