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(54) Title: GLP-1 RECEPTOR AGONIST COMPOUNDS WITH A MODIFIED N-TERMINUS

(57) Abstract: The invention relates to GLP-1 receptor agonist compounds with a modified N- terminus. The compounds are of the formula Chem. 1: Y-Z-P, wherein P represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues; and Y-Z represents novel His-Ala mimetics. Examples of GLP-1 receptor agonist compounds are derived from human GLP-1 (7-37), exendin-4(1-39), or GLP-1 A (1-37). The invention also relates to derivatives of these compounds, in particular compounds with one or more albumin binding side chains capable of protracting the duration of action in vivo of these compounds. The peptides and derivatives of the invention have a good potency, a protracted pharmacokinetic profile, are stable against degradation by gastro intestinal enzymes, and/or have a high oral bioavailability. These properties are of importance in the development of GLP-1 receptor agonist compounds for subcutaneous, intravenous, and/or in particular oral administration. The invention also relates to intermediate products for use in the preparation of the GLP-1 receptor agonist compounds of the invention.

MPI EXHIBIT 1057 PAGE 1

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Ex. 1057, p. 1 of 96

GLP-1 RECEPTOR AGONIST COMPOUNDS WITH A MODIFIED N-TERMINUS

FIELD OF THE INVENTION

The present invention relates to analogues and derivatives of GLP-1 receptor agonist peptides, and their pharmaceutical use. In the GLP-1 receptor agonist peptides of the invention, such as Glucagon-Like Peptide-1 (GLP-1), exendins and analogues thereof, the two N-terminal amino acids have been replaced by N-terminal mimetics.

INCORPORATION-BY-REFERENCE OF THE SEQUENCE LISTING

The Sequence Listing, entitled "SEQUENCE LISTING", is 1770 bytes, was created on 01-DEC-2010, and is incorporated herein by reference.

BACKGROUND OF THE INVENTION

WO 2004/067548 A2 relates to chemically modified metabolites of regulatory peptides and methods of producing and using same.

Liraglutide, a GLP-1 derivative for once daily administration which is marketed by Novo Nordisk A/S, is disclosed in Example 37 of WO 98/08871.

Semaglutide, a GLP-1 derivative for once weekly administration which is under development by Novo Nordisk A/S, is disclosed in Example 4 of WO 06/097537.

SUMMARY OF THE INVENTION

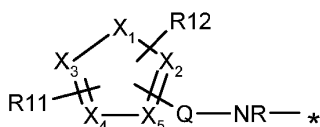
The invention relates to GLP-1 receptor agonist compounds comprising a modified N-terminus.

Preferred compounds have the formula Chem. 1: Y-Z-P, wherein P represents a fragment of a GLP-1 receptor agonist peptide lacking the N-terminus; and Y-Z represents a group mimicing the N-terminus of the peptide. The new N-terminal is preferably a His-Ala, a His-Gly, and/or a His-Ser mimetic.

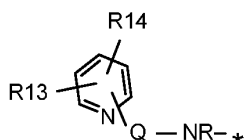
More in particular the invention relates to a GLP-1 receptor agonist peptide having the formula Chem. 1: Y-Z-P, wherein P represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues; Z represents a group of the formula

Chem. 2: $\begin{array}{c} * \\ \diagup \\ \text{C} \\ \parallel \\ \text{O} \end{array} \text{W} \begin{array}{c} \diagdown \\ \text{C} \\ \parallel \\ \text{O} \\ * \end{array}$, wherein W represents a group of formula Chem. 3: $\begin{array}{c} * \\ | \\ \text{R1}-\text{C}-\text{R2} \\ | \\ * \end{array}$, wherein R1 and R2 independently represent (i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl,

halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or R1 and R2 together form (ii) cyclo alkyl, heterocyclyl, or heteroaryl; and Y



represents a group of formula Chem. 4: , or Chem. 5:



5 , wherein X_1 is N, O, or S; X_2 , X_3 , X_4 , and X_5 independently represent C, or N, with the proviso that at least one of X_2 , X_3 , X_4 and X_5 is C; R11, R12, R13, and R14 independently represent hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl;

10 Q represents a bond, or a group of formula Chem. 6: $^*-(C(R15)(R16))_q-^*$, wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl; and R represents hydrogen, or alkyl; or a pharmaceutically acceptable salt, amide, or ester thereof.

15 The invention also relates to a derivative of this peptide, and a pharmaceutically acceptable salt, amide, or ester thereof.

The invention also relates to the pharmaceutical use of these compounds, preferably for the treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid

20 parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

Finally, the invention relates to intermediate products corresponding to the new N-terminus, as well as to the peptide fragments, i.e. before attachment of the new N-terminus, both relevant for the preparation of the peptides of the invention.

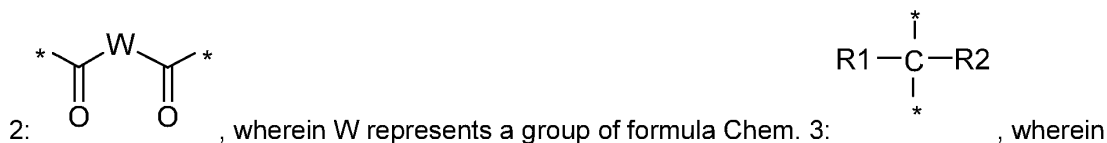
25 The peptides and derivatives of the invention are biologically active, preferably of a high potency. Also, or alternatively, they have a protracted pharmacokinetic profile. Also, or alternatively, they are stable against degradation by gastro intestinal enzymes. Also, or alternatively, they have a high oral bioavailability. These properties are of importance in the

development of next generation GLP-1 compounds for subcutaneous, intravenous, and/or in particular oral administration.

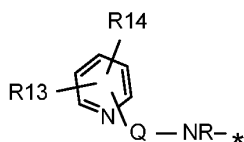
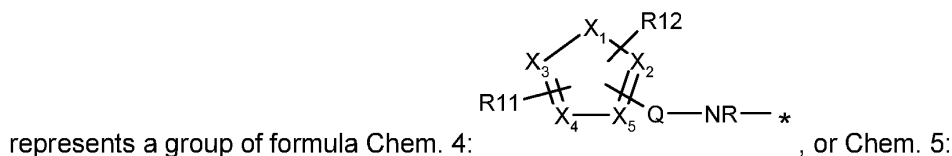
DESCRIPTION OF THE INVENTION

The invention relates to a GLP-1 receptor agonist peptide having the formula Chem.

- 5 1: Y-Z-P, wherein P represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues; Z represents a group of the formula Chem.

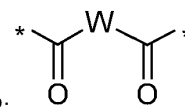


- 10 R1 and R2 independently represent (i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or R1 and R2 together form (ii) cyclo alkyl, heterocyclyl, or heteroaryl; and Y

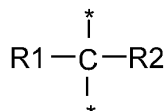


- 15 , wherein X_1 is N, O, or S; X_2 , X_3 , X_4 , and X_5 independently represent C, or N, with the proviso that at least one of X_2 , X_3 , X_4 and X_5 is C; R11, R12, R13, and R14 independently represent hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl; Q represents a bond, or a group of formula Chem. 6: $^{*}-(C(R15)(R16))_q-^{*}$, wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl; and R represents hydrogen, or alkyl; or a pharmaceutically acceptable salt, amide, or ester thereof.

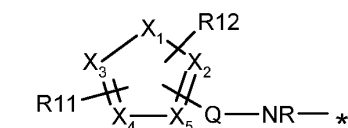
In a first aspect, R1 and R2 do not both represent hydrogen, and the invention accordingly relates to a GLP-1 receptor agonist peptide having the formula Chem. 1: Y-Z-P, wherein P represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-



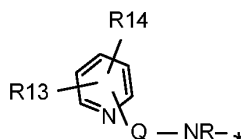
terminal amino acid residues; Z represents a group of the formula Chem. 2:



wherein W represents a group of formula Chem. 3: $\begin{array}{c} \text{*} \\ | \\ \text{R1} - \text{C} - \text{R2} \\ | \\ \text{*} \end{array}$, wherein R1 and R2 independently represent (i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or R1 and R2 together form (ii) cyclo alkyl, heterocyclyl, or heteroaryl, with the proviso that (iii) R1 and R2 do not both represent hydrogen; and Y represents a group of formula Chem.



4: $\begin{array}{c} \text{X}_1 \quad \text{R12} \\ \diagup \quad \diagdown \\ \text{X}_3 \quad \text{X}_2 \\ \diagdown \quad \diagup \\ \text{R11} \quad \text{X}_4 \quad \text{X}_5 \quad \text{Q} - \text{NR} - \text{*} \end{array}$, or Chem. 5:



wherein X₁ is N, O, or S; X₂, X₃, X₄, and X₅ independently represent C, or N, with the proviso that at least one of X₂, X₃, X₄ and X₅ is C; R11, R12, R13, and R14 independently represent hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl; Q represents a bond, or a group of formula Chem. 6: $\text{*} - (\text{C}(\text{R15})(\text{R16}))_q - \text{*}$, wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl; and R represents hydrogen, or alkyl; or a pharmaceutically acceptable salt, amide, or ester thereof.

In a second aspect, R1 and R2 may both represent hydrogen, and Q-NR-* is not attached to a nitrogen atom of Chem. 4.

In a third aspect, R1 and R2 may both represent hydrogen, and Q-NR-* is attached to a carbon atom of Chem. 4.

The invention also relates to a derivative of each of these peptides, and to pharmaceutically acceptable salts, amides, or esters thereof.

The invention also relates to the pharmaceutical use of these compounds, preferably for the treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid

parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

Finally, the invention relates to intermediate products corresponding to the new N-terminus, as well as to the peptide fragments, i.e. before attachment of the new N-terminus, both relevant for the preparation of the peptides of the invention.

In what follows, Greek letters may be represented by their symbol or the corresponding written name, for example: α = alpha; β = beta; ε = epsilon; γ = gamma; ω = omega; etc. Also, the Greek letter of μ may be represented by "u", e.g. in $\mu\text{L}=\text{uL}$, or in $\mu\text{M}=\text{uM}$.

An asterisk (*) in a chemical formula designates i) a point of attachment, ii) a radical, and/or iii) an unshared electron.

GLP-1 receptor agonist

The GLP-1 receptor agonist compounds of the invention may be derived, or are derivable, from human GLP-1(7-37), exendin-4(1-39), and/or GLP-1A(1-37). The amino acid sequences of these peptides may be found in the UniProt Knowledgebase (UniProtKB) - SwissProt section (www.uniprot.org) with the following accession numbers, sequence identifiers, and sequence names: UNIPROT:P01275_8, GLUC_HUMAN, Glucagon-like peptide 1(7-37); UNIPROT:P26349_3, EXE4_HELSU, Exendin-4, or exenatide; and UNIPROT:O42143_5, GLUC1_XENLA, Glucagon-like peptide 1A; respectively.

The sequences of the corresponding fragments lacking the two N-terminal amino acids, viz. GLP-1(9-37), exendin-4(3-39), and GLP-1A(3-37), are included in the appended sequence listing as SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively.

Another example of a GLP-1 receptor agonist fragment from which the compounds of the invention may be derived, or are derivable, is the peptide designated exendin-3(3-39) which is the D3 analogue of SEQ ID NO: 2, i.e. identical to SEQ ID NO: 2 except for having aspartic acid (D, Asp) at position 3, the first amino acid residue.

The sequence of the GLP-1 receptor may be found in the UniProtKB database referred to above with the following accession number, identifier, and name: UNIPROT:P43220, GLP1R_HUMAN, Glucagon-like peptide 1 receptor, GLP-1 receptor, GLP-1-R, or GLP-1R.

The term "GLP-1 receptor agonist" as used herein refers to a compound which is an agonist of the human GLP-1 receptor, i.e. a compound that stimulates the formation of cAMP in a medium containing the human GLP-1 receptor. GLP-1 receptor agonism, or potency, is determined as described below, in the section headed "Potency", see also Example 13 herein.

Amino acids and peptides

The term "peptide", as e.g. used in the context of the GLP-1 receptor agonist peptides of the invention, refers to a compound which comprises a series of amino acids
5 interconnected by amide (or peptide) bonds.

In a particular embodiment the peptide is to a large extent, or predominantly, composed of amino acids interconnected by amide bonds (e.g., at least 50%, 60%, 70%, 80%, or at least 90%, by molar mass). In another particular embodiment the peptide consists of amino acids interconnected by peptide bonds.

10 The peptides of the invention comprise at least five constituent amino acids connected by peptide bonds. In particular embodiments the peptide comprises at least 10, preferably at least 15, more preferably at least 20, even more preferably at least 25, or most preferably at least 28 amino acids.

In particular embodiments, the peptide is composed of at least five constituent
15 amino acids, preferably composed of at least 10, at least 15, at least 20, at least 25, or most preferably composed of at least 28 amino acids.

In additional particular embodiments, the peptide is a) composed of, or b) consists of, i) 29, ii) 30, iii) 31, or iv) 32 amino acids.

In still further particular embodiments, the peptide is a) composed of, or b) consists
20 of, i) 33, ii) 34, iii) 35, or iv) 36 amino acids.

In a still further particular embodiment the peptide consists of amino acids interconnected by peptide bonds.

Amino acids are molecules containing an amine group and a carboxylic acid group, and, optionally, one or more additional groups, often referred to as a side chain.

25 The term "amino acid" includes proteogenic amino acids (encoded by the genetic code, including natural amino acids, and standard amino acids), as well as non-proteogenic (not found in proteins, and/or not coded for in the standard genetic code), and synthetic amino acids. Thus, the amino acids may be selected from the group of proteinogenic amino acids, non-proteinogenic amino acids, and/or synthetic amino acids.

30 Non-limiting examples of amino acids which are not encoded by the genetic code are gamma-carboxyglutamate, ornithine, and phosphoserine. Non-limiting examples of synthetic amino acids are the D-isomers of the amino acids such as D-alanine and D-leucine, Aib (α -aminoisobutyric acid), β -alanine, and des-amino-histidine (desH, alternative name imidazopropionic acid, abbreviated Imp).

In what follows, all amino acids for which the optical isomer is not stated is to be understood to mean the L-isomer (unless otherwise specified).

GLP-1 receptor agonist peptides, fragments, analogues, residue numbering, identity

5 A "GLP-1 receptor agonist peptide" is a peptide as defined above, and also a GLP-1 receptor agonist as defined above.

The peptides of the invention are GLP-1 receptor agonist peptides.

Additional examples of GLP-1 receptor agonist peptides are the following known compounds: Human GLP-1(7-37), exendin-4(1-39), exendin-3(1-39), and GLP-1A(1-37).

10 In a particular embodiment, the GLP-1 receptor agonist compound of the invention may be derived, or is derivable, from any one or more of these known GLP-1 receptor agonist peptides.

The term "fragment" as it refers to a GLP-1 receptor agonist peptide means a peptide which is shorter than the peptide referred to.

15 In a particular embodiment corresponding to the definition of group P in formula I, the fragment lacks the two N-terminal amino acids as compared to the corresponding full-length peptide being a GLP-1 receptor agonist.

In another particular embodiment this particular fragment is not in itself a GLP-1 receptor agonist, due to a i) substantial, ii) preferably almost complete, or iii) more preferably
20 for all practical purposes complete, loss of biological activity (i.e., GLP-1 receptor agonism).

Particular examples of P (fragments of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues) are the following: GLP-1(9-37), exendin-4(3-39), and GLP-1A(3-37), which are included in the appended sequence listing as SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively. Another example of P is exendin-3(3-39) which is
25 variant D3 of SEQ ID NO: 2.

In the sequence listing, the first amino acid residue of the fragment of GLP-1(9-37) (SEQ ID NO: 1), which is glutamic acid, is assigned no. 1. However, in what follows - according to established practice in the art - this glutamic acid residue is referred to as no. 9, and subsequent amino acid residues are numbered accordingly, ending with glycine no. 37.

30 Likewise, in the sequence listing, the first amino acid residue of exendin-4(3-39) (SEQ ID NO: 2), which is also glutamic acid, is assigned no. 1. However, in what follows - according to established practice in the art - this glutamic acid residue is referred to as no. 3, and subsequent amino acid residues are numbered accordingly, ending with serine as no. 39.

Likewise, in the sequence listing, the first amino acid residue of GLP-1A(3-37) (SEQ ID NO: 3), which is aspartic acid, is assigned no. 1. However, in what follows - according to established practice in the art - this aspartic acid residue is referred to as no. 3, and subsequent amino acid residues are numbered accordingly, ending with serine as no. 37.

5 Therefore, generally, any reference herein to an amino acid residue number or a position number in the context of the peptides of SEQ ID NO: 1, 2, or 3 or analogues thereof is to the sequence starting with Glu at position 9, Glu at position 3, or Asp at position 3, respectively; and ending with Gly at position 37, Ser at position 39, or Ser at pos. 37, respectively.

10 Additional particular examples of P are analogues of SEQ ID NO: 1, SEQ ID NO: 2, and/or SEQ ID NO: 3.

An "analogue" as used herein in the context of SEQ ID NO: 1, 2, or 3 refers to a peptide, or a compound, which is a variant of any one or more of SEQ ID NO: 1, 2, or 3.

15 In a particular embodiment, the analogue of SEQ ID NO: 1 refers to a modification of SEQ ID NO: 1 in which a number of amino acid residues have been exchanged as compared to SEQ ID NO: 1. These exchanges, or modifications, may represent, independently, one or more amino acid substitutions, additions, and/or deletions. Additions at the N-terminus are, however, preferably excluded. Analogues of SEQ ID NO: 2, and SEQ ID NO: 3 are defined similarly, by analogy to the definition of analogues of SEQ ID NO: 1.

20 Analogues may be described by reference to a reference sequence, the number of the amino acid residue in the reference sequence corresponding to the one which is modified, i.e., its position, and to the actual modification.

In particular embodiments, the reference sequence is i) GLP-1(9-37) (SEQ ID NO: 1); ii) exendin-4(3-39) (SEQ ID NO: 2); or iii) GLP-1A(3-37) (SEQ ID NO: 3).

25 The following are non-limiting, illustrative examples of suitable analogue nomenclature, as used herein:

N^9 -[2-[2-(1H-imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl]-[Lys¹⁸,Glu²²,Gln³⁴]GLP-1(9-37) peptide is a GLP-1 receptor agonist peptide of the invention derivable from GLP-1(9-37) (SEQ ID NO: 1), i.e. P in Chem. 1 is an analogue of SEQ ID NO: 30 1, viz. the analogue in which the serine at position 18 has been substituted with lysine, the glycine at position 22 has been substituted with glutamic acid, and the lysine at position 34 has been substituted with glutamine;

N^9 -[2-[2-(1H-imidazol-4-yl)propylcarbamoyl]-2-methyl-propionyl]-[Glu³⁰,Lys³⁶]GLP-1(9-37)Glu³⁸-peptide is a GLP-1 receptor agonist peptide of the invention derivable from 35 GLP-1(9-37) (SEQ ID NO: 1), i.e. P in Chem. 1 is an analogue of SEQ ID NO: 1, viz. the

analogue in which the alanine at position 30 has been substituted with glutamic acid, the arginine at position 36 has been substituted with lysine, and a glutamic acid has been added at the C-terminus, viz. at position 38; and

N^3 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}-

- 5 [Arg¹⁷,Arg²⁰,Arg³³,Lys³⁸]GLP-1A(3-37)-peptide is a GLP-1 receptor agonist peptide of the invention derivable from GLP-1A(3-37) (SEQ ID NO: 3), i.e. P in Chem. 1 is an analogue of SEQ ID NO: 3, viz. the analogue in which the lysines at position 17, 20, and 33 have been substituted with arginine, and a lysine has been added at the C-terminus, viz. at position 38.

- 10 This peptide, by the way, is also derivable from SEQ ID NO: 1, and it can therefore also be designated as analogue (17T, 18Q, 19Q, 21D, 22E, 23R, 26R, 30D, 33I, 34N, 36G, 37P, 38S, 39R, 40E, 41I, 42I, 43S, 44K) of GLP-1(9-37) (SEQ ID NO: 1), having {2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}- attached to the N-terminus, N^9 .

- 15 As another example, a GLP-1 receptor agonist peptide of the invention which "comprises at least one of the following substitutions as compared to GLP-1(9-37) (SEQ ID NO: 1): 18K; 22E; 30E; 31H; 34Q,R; 36K; 37K; and/or 38E" refers to a GLP-1 receptor agonist peptide in which P of Chem. 1 is considered an analogue of SEQ ID NO: 1, which analogue has a lysine at position 18, a glutamic acid at position 22, a glutamic acid at position 30, a histidine at position 31, a glutamine at position 34, a lysine at position 36, a lysine at position 37, and/or a glutamic acid at position 38, and which analogue may
20 comprise further modifications as compared to SEQ ID NO: 1.

As is apparent from the above examples, amino acid residues may be identified by their full name, their one-letter code, and/or their three-letter code. These three ways are fully equivalent.

- 25 The expressions "a position equivalent to" or "corresponding position" may be used to characterise the site of modification in a modified GLP-1 receptor agonist peptide sequence by reference to any one or more of SEQ ID NO: 1, 2, or 3. Equivalent or corresponding positions, as well as the number of modifications, are easily deduced, e.g. by simple handwriting and eyeballing; and/or a standard protein or peptide alignment program may be used, such as "align" which is a Needleman-Wunsch alignment. The algorithm is
30 described in Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48: 443-453, and the align program by Myers and W. Miller in "Optimal Alignments in Linear Space" CABIOS (computer applications in the biosciences) (1988) 4:11-17. For the alignment, the default scoring matrix BLOSUM50 and the default identity matrix may be used, and the penalty for the first residue in a gap may be set at -12, or preferably at -10, and
35 the penalties for additional residues in a gap at -2, or preferably at -0.5.

This algorithm may also suitably be used for determining the degree of identity of the P-group of a GLP-1 receptor agonist peptide of the invention to each of SEQ ID NO: 1, 2, and 3, e.g. with a view to determining which of these three sequences has the highest percentage of identity to the P-group in question, and thus for determining the number of amino acid residues that have been exchanged as compared to the closest related sequence of SEQ ID NOs: 1-3 (the one with the highest percentage of identity). If the percentages of identity of a given P group of a GLP-1 receptor agonist of the invention to SEQ ID NO: 1, 2, and 3, respectively, should happen to be the same, any of those having the same highest percentage of identity may be used for the determination.

An example of such alignment is inserted hereinbelow, in which sequence no. 1 is SEQ ID NO: 1, and sequence no. 2 is SEQ ID NO: 3:

```
# 1: GLP_1(9-37)
# 2: GLP-1A(3-37)
# Matrix: EBLSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 35
# Identity:      18/35 (51.4%)
# Similarity:    22/35 (62.9%)
# Gaps:          6/35 (17.1%)
# Score: 92.0

GLP_1(9-37)      1 EGTFTSDVSSYLEGQAAKEFIAWLVKGRG----- 29
                  ||||| | | | : . . | : . : || | | | | . | | : . | . .
GLP-1A(3-37)    1 EGTFTSDVTQQLDEKAAKEFIDWLINGGPSKEIIS 35
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The above alignment is just for illustration, as typically an analogue of one of SEQ ID NOs: 1, 2, or 3 will be compared with either of these reference sequences.

In case of analogues comprising non-natural amino acids such as Imp, and/or Aib being included in the sequence, these may, for alignment purposes, be replaced with X. If desired, X can later be manually corrected.

Derivatives

The terms "derivative" as used herein in the context of the GLP-1 receptor agonist peptides of the invention means a chemically modified peptide or analogue, in which one or more substituents have been covalently attached to the peptide. The substituent(s) may also be referred to as side chain(s). In a particular embodiment, the derivative of the invention has one side chain. In another particular embodiment it has two side chains. For the purpose of this definition, the group Y-Z- of formula I is preferably not considered a substituent/side chain.

In particular embodiments, the side chain has at least 10 carbon atoms, or at least 15, 20, 25, 30, 35, 40, or at least 43 carbon atoms. In further particular embodiments, the side chain may further include at least 5 hetero atoms, in particular O and N, for example at least 7, 9, 10, 12, 15, 17, or at least 20 hetero atoms, such as at least 1, 2, 3, or 4 N-atoms, and/or at least 3, 4, 6, 9, 12, 13, or 15 O-atoms.

Non-limiting examples of GLP-1 receptor agonist derivatives include heterologous fusion proteins or conjugates of the GLP-1 receptor agonist peptides of the invention, with e.g. the Fc portion of an immunoglobulin such as IgG, with human albumin, with antibodies such as a glucagon binding antibody heavy chain variable region, or with fragments or analogues of any of these (see, e.g., US 2007/0161087, WO 2005/058958, and WO 2007/124463 A2). Additional examples include PEGylated peptides (see, e.g., WO 2005/058954, WO 2004/093823, and WO 2006/124529), as well as acylated peptides (see, e.g., WO 98/08871, WO2005/027978, WO 2006/097537, and WO 2009/030771).

In a preferred embodiment, the side chain is capable of forming non-covalent aggregates with albumin, thereby promoting the circulation of the derivative with the blood stream, and also having the effect of protracting the time of action of the derivative, due to the fact that the aggregate of the derivative and albumin is only slowly disintegrated to release the active pharmaceutical ingredient. Thus, a preferred substituent, or side chain, as a whole may be referred to as an albumin binding moiety.

In another particular embodiment, the albumin binding moiety comprises a portion which is particularly relevant for the albumin binding and thereby the protraction, which portion may accordingly be referred to as a protracting moiety. The protracting moiety may be at, or near, the opposite end of the albumin binding moiety, relative to its point of attachment to the peptide.

In a still further particular embodiment, the albumin binding moiety comprises a portion in-between the protracting moiety and the point of attachment to the peptide, which portion may be referred to as a linker, linker moiety, spacer, or the like. The presence of a linker is optional; hence if no linker is present the albumin binding moiety may be identical to the protracting moiety.

In particular embodiments, the albumin binding moiety and/or the protracting moiety is lipophilic, and/or negatively charged at physiological pH (7.4).

The albumin binding moiety, the protracting moiety, or the linker may be covalently attached to a lysine residue of the GLP-1 receptor agonist peptide by conjugation chemistry such as by alkylation, acylation, ester formation, or amide formation; or to a cysteine residue, such as by maleimide or haloacetamide (such as bromo-/fluoro-/iodo-) coupling.

In a preferred embodiment, an active ester of the albumin binding moiety and/or the protracting moiety, optionally with a linker, is covalently linked to an amino group of a lysine residue, preferably the epsilon amino group thereof, under formation of an amide bond (this process being referred to as acylation).

5 Unless otherwise stated, when reference is made to an acylation of a lysine residue, it is understood to be to the epsilon-amino group thereof.

In one embodiment, the invention relates to a derivative of a GLP-1 receptor agonist peptide which comprises, preferably has, an albumin binding moiety attached to one or more of 18K, 26K, 36K, and/or 37K, wherein reference may be had to the sequence of GLP-1(9-
10 37) (SEQ ID NO: 1). As explained above, each residue number refers to the corresponding position in GLP-1(9-37) (SEQ ID NO: 1). Furthermore, as also explained above, ordinary script may be used instead of superscript to designate the position number. E.g., "K¹⁸" is fully equivalent to "18K".

Corresponding position numbers are preferably identified by handwriting and
15 eyeballing, or by using a suitable alignment program, as explained above.

For the present purposes, the terms "albumin binding moiety", "protracting moiety", and "linker" include the un-reacted as well as the reacted forms of these molecules. Whether or not one or the other form is meant is clear from the context in which the term is used.

In one aspect the albumin binding moiety comprises, or consists of, a protracting
20 moiety selected from

Chem. 8: $\text{HOOC}-(\text{CH}_2)_x-\text{CO}-^*$

Chem. 9: $\text{HOOC}-\text{C}_6\text{H}_4-\text{O}-(\text{CH}_2)_y-\text{CO}-^*$

Chem. 10: $\text{R}^{18}-\text{C}_6\text{H}_4-(\text{CH}_2)_z-\text{CO}-^*$

in which x is an integer in the range of 6-18, y is an integer in the range of 3-17, z is
25 an integer in the range of 1-5, and R¹⁸ is a group having a molar mass not higher than 150 Da.

In one embodiment, $^*(\text{CH}_2)_x-$ refers to straight or branched, preferably straight, alkylene in which x is an integer in the range of 6-18.

In another embodiment, $^*(\text{CH}_2)_y-$ refers to straight or branched, preferably straight,
30 alkylene in which y is an integer in the range of 3-17.

In a third embodiment, $^*(\text{CH}_2)_z-$ refers to straight or branched, preferably straight, alkylene in which z is an integer in the range of 1-5.

The molar mass (M) of a chemical substance (such as the group R¹) is the mass of one mole of the substance. The molar mass is quoted in dalton, symbol Da, with the
35 definition 1 Da = 1 g/mol.

Molar mass may be calculated from standard atomic weights, and is often listed in chemical catalogues. The molar mass of a compound is given by the sum of the standard atomic weights of the atoms which form the compound multiplied by the molar mass constant, M_u which equals 1 g/mol. As an example, the molecular mass of tert. butyl (C_4H_9) is

5 $M(C_4H_9) = ([4 \times 12.01] + [9 \times 1.008]) \times 1 \text{ g/mol} = 57 \text{ Da}$.

Standard atomic weights are published by the International Union of Pure and Applied Chemistry (IUPAC), and also reprinted in a wide variety of textbooks, commercial catalogues, wallcharts etc.

For the attachment to the GLP-1 receptor agonist peptide, the acid group of the fatty acid, or one of the acid groups of the fatty diacid, forms an amide bond with the epsilon amino group of a lysine residue in the GLP-1 receptor agonist peptide.

10

The term "fatty acid" refers to aliphatic monocarboxylic acids having from 4 to 28 carbon atoms, it is preferably unbranched, and/or even numbered, and it may be saturated or unsaturated.

15 The term "fatty diacid" refers to fatty acids as defined above but with an additional carboxylic acid group in the omega position. Thus, fatty diacids are dicarboxylic acids.

The nomenclature is as is usual in the art, for example $^*-\text{COOH}$, as well as $\text{HOOC}-^*$, refers to carboxy; $^*-\text{C}_6\text{H}_4-^*$ to phenylene; $^*-\text{CO}-^*$, as well as $^*-\text{OC}-^*$, to carbonyl ($\text{O}=\text{C}<^*^*$); and $\text{C}_6\text{H}_5-\text{O}-^*$ to phenoxy.

20 In particular embodiments, the aromatics, such as the phenoxy and the phenylene radicals, may be, independently, ortho, meta, or para.

In a preferred embodiment the linker moiety, if present, has from 5 to 30 C-atoms. In additional preferred embodiments, the linker moiety, if present, has from 4 to 20 hetero atoms. H-atoms are not hetero atoms.

25 In another embodiment, the linker comprises at least one OEG molecule, at least one glutamic acid residue, and/or at least one piperidine molecule, optionally substituted, or rather the corresponding radicals (OEG designates 8-amino-3,6-dioxaoctanic acid, i.e. this di-radical: $^*-\text{NH}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{O}-\text{CH}_2-\text{CO}-^*$).

The amino acid glutamic acid comprises two carboxylic acid groups. Its gamma-carboxy group is preferably used for forming an amide bond with the epsilon-amino group of lysine, or with an amino group of an OEG molecule, if present, or with the amino group of another Glu residue, if present. The amino group of Glu in turn forms an amide bond with the carboxy group of the protracting moiety, or with the carboxy group of an OEG molecule, if present, or with the gamma-carboxy group of another Glu, if present. This way of inclusion of

30

35 Glu is occasionally briefly referred to as "gamma-Glu".

The derivatives of the invention may exist in different stereoisomeric forms having the same molecular formula and sequence of bonded atoms, but differing only in the three-dimensional orientation of their atoms in space. The stereoisomerism of the exemplified derivatives of the invention is indicated in the experimental section, in the names as well as the structures, using standard nomenclature. Unless otherwise stated the invention relates to all stereoisomeric forms of the claimed derivative.

The concentration in plasma of the GLP-1 receptor agonist peptides and derivatives of the invention may be determined using any suitable method. For example, LC-MS (Liquid Chromatography Mass Spectroscopy) may be used, or immunoassays such as RIA (Radio Immuno Assay), ELISA (Enzyme-Linked Immuno Sorbent Assay), and LOCI (Luminescence Oxygen Channeling Immunoassay). General protocols for suitable RIA and ELISA assays are found in, e.g., WO09/030738 on p. 116-118. A preferred assay is the LOCI assay in which the plasma concentrations of the compounds are determined using a Luminescence Oxygen Channeling Immunoassay (LOCI), generally as described for the determination of insulin by Poulsen and Jensen in Journal of Biomolecular Screening 2007, vol. 12, p. 240-247. The donor beads are coated with streptavidin, while acceptor beads are conjugated with a monoclonal antibody recognising a mid-/C-terminal epitope of the peptide. Another monoclonal antibody, specific for the N-terminus, is biotinylated. The three reactants are combined with the analyte and form a two-sited immuno-complex. Illumination of the complex releases singlet oxygen atoms from the donor beads, which are channeled into the acceptor beads and trigger chemiluminescence which is measured in an Envision plate reader. The amount of light is proportional to the concentration of the compound.

Pharmaceutically acceptable salt, amide, or ester

The GLP-1 receptor agonist peptides, derivatives, and intermediate products of the invention may be in the form of a pharmaceutically acceptable salt, amide, or ester.

Salts are e.g. formed by a chemical reaction between a base and an acid, e.g.: $\text{NH}_3 + \text{H}_2\text{SO}_4 \rightarrow (\text{NH}_4)_2\text{SO}_4$.

The salt may be a basic salt, an acid salt, or it may be neither nor (i.e. a neutral salt). Basic salts produce hydroxide ions and acid salts hydronium ions in water.

The salts of the peptides and derivatives of the invention may be formed with added cations or anions that react with anionic or cationic groups, respectively. These groups may be situated in the peptide moiety, and/or in the side chain of the compounds of the invention.

Non-limiting examples of anionic groups of the compounds of the invention include free carboxylic groups in the side chain, if any, as well as in the peptide moiety. The peptide

moiety often includes a free carboxylic acid group at the C-terminus, and it may also include free carboxylic groups at internal acid amino acid residues such as Asp and Glu.

Non-limiting examples of cationic groups in the peptide moiety include the free amino group at the N-terminus, if present, as well as any free amino group of internal basic amino acid residues such as His, Arg, and Lys.

The ester of the peptides and derivatives of the invention may, e.g., be formed by the reaction of a free carboxylic acid group with an alcohol or a phenol, which leads to replacement of at least one hydroxyl group by an alkoxy or aryloxy group

The ester formation may involve the free carboxylic group at the C-terminus of the peptide, and/or any free carboxylic group in the side chain.

The amide of the peptides and derivatives of the invention may, e.g., be formed by the reaction of a free carboxylic acid group with an amine or a substituted amine, or by reaction of a free or substituted amino group with a carboxylic acid.

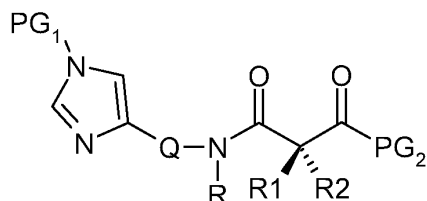
The amide formation may involve the free carboxylic group at the C-terminus of the peptide, any free carboxylic group in the side chain, the free amino group at the N-terminus of the peptide, and/or any free or substituted amino group of the peptide in the peptide and/or the side chain.

In a particular embodiment, the peptide or derivative is in the form of a pharmaceutically acceptable salt. In another particular embodiment, the peptide or derivative is in the form of a pharmaceutically acceptable amide, preferably with an amide group at the C-terminus of the peptide. In a still further particular embodiment, the peptide or derivative is in the form a pharmaceutically acceptable ester.

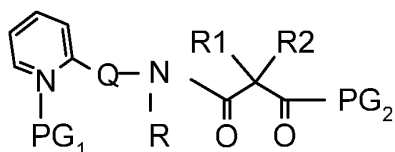
Intermediate compounds

The invention also relates to an intermediate product of the formula Chem. 50 or Chem. 51:

Chem. 50:



Chem. 51:



wherein Q, R, R1, and R2 are as defined for the GLP-1 receptor agonist peptide of the invention, having the formula Chem. 1, and each of PG₁ and PG₂ represents a protection group.

5 Non-limiting examples of PG₁ groups are Boc, Trt, Mtt, Mmt, and Fmoc.

Non-limiting examples of PG₂ groups are -OH, or groups functionalised as an activated ester, for example, without limitation, OPfp, OPnp, and OSuc.

Other suitable activated esters may be selected, e.g., according to the teaching of M. Bodanszky, "Principles of Peptide Synthesis", 2nd ed., Springer Verlag, 1993.

10

Functional properties

In a first functional aspect, the GLP-1 receptor agonist peptides and/or derivatives of the invention have a good potency. Also, or alternatively, in a second functional aspect, they have a protracted pharmacokinetic profile. Also, or alternatively, in a third functional aspect,
15 they are stable against degradation by gastro intestinal enzymes. Also, or alternatively, in a fourth functional aspect, they have a high oral bioavailability.

Biological activity (potency)

According to the first functional aspect, the GLP-1 receptor agonist derivatives are
20 biologically active, or have a good potency.

Surprisingly, they have an improved potency as compared to the comparative compound of Example 11 (Chem. 40) herein, which is based on one of the most potent compounds of the prior art, viz. compound 215 of WO2004/067548.

Also, or additionally, the derivatives of the invention have a high binding affinity to
25 the GLP-1 receptor at low albumin concentration (0.005%), i.e. a low IC₅₀ value, which is discussed further below under the heading of receptor binding.

As regards potency, the term half maximal effective concentration (EC₅₀) generally refers to the concentration which induces a response halfway between the baseline and maximum, by reference to the dose response curve. EC₅₀ is used as a measure of the
30 potency of a compound and represents the concentration where 50% of its maximal effect is observed.

The in vitro potency of the derivatives of the invention may be determined as described hereinbelow, and the EC₅₀ of the derivative in question determined. The lower the EC₅₀, the better the potency.

In a particular embodiment, the derivatives of the invention are at least 3 times more potent than Chem. 40; preferably at least 4 times more potent; even more preferably at least 5 times more potent; or most preferably at least 6 times more potent than Chem. 40.

In another particular embodiment, the derivatives of the invention are at least 7 times more potent than Chem. 40; preferably at least 8 times more potent; even more preferably at least 9 times more potent; or most preferably at least 10 times more potent than Chem. 40.

In another particular embodiment, the derivatives of the invention are at least 20 times more potent than Chem. 40; preferably at least 50 times more potent; even more preferably at least 100 times more potent; still more preferably at least 200 times more potent; or most preferably at least 400 times more potent than Chem. 40.

Potency is preferably determined as described below, and it is noted that a, e.g., three times more potent compound has an EC₅₀ which is three times lower.

In a particular embodiment, potency and/or activity refers to in vitro potency, i.e. performance in a functional GLP-1 receptor assay, more in particular to the capability of stimulating cAMP formation in a cell line expressing the cloned human GLP-1 receptor.

The stimulation of the formation of cAMP in a medium containing the human GLP-1 receptor may preferably be determined using a stable transfected cell-line such as BHK467-12A (tk-ts13), and/or using for the determination of cAMP a functional receptor assay, e.g. based on competition between endogenously formed cAMP and exogenously added biotin-labelled cAMP, in which assay cAMP is more preferably captured using a specific antibody, and/or wherein an even more preferred assay is the AlphaScreen cAMP Assay, most preferably the one described in Example 13.

In an additional particular embodiment, the medium has the following composition (final in-assay concentrations):): 50 mM TRIS-HCl; 5 mM HEPES; 10 mM MgCl₂, 6H₂O; 150 mM NaCl; 0.01% Tween; 0.1% BSA ; 0.5 mM IBMX; 1 mM ATP; 1 uM GTP; pH 7.4.

In a further particular embodiment, the GLP-1 receptor agonist has an EC₅₀ below 2000pM, preferably below 1800pM, more preferably below 1700pM, even more preferably below 1600pM, or most preferably below 1500pM.

In another particular embodiment the derivatives of the invention are potent in vivo, which may be determined as is known in the art in any suitable animal model, as well as in

clinical trials. The diabetic db/db mouse is one example of a suitable animal model, and the blood glucose lowering effect may be determined in such mice in vivo, e.g. as described in Example 43 of WO09/030738.

5 Protraction - receptor binding - high and low albumin

According to the second functional aspect, the derivatives of the invention are protracted.

A suitable assay for determining receptor binding of the peptides and derivatives of the invention at high and low albumin concentration is disclosed in Example 14 herein.

10 Generally, the binding to the GLP-1 receptor at low albumin concentration should be as good as possible, corresponding to a low IC_{50} value.

The IC_{50} value at high albumin concentration is a measure of the influence of albumin on the binding of the compound to the GLP-1 receptor. As is known, the peptides GLP-1 receptor agonist peptide derivatives of the invention also bind to albumin. This is a
15 generally desirable effect, which extends their lifetime in plasma. Therefore, the IC_{50} value at high albumin will generally be higher than the IC_{50} value at low albumin, corresponding to a reduced binding to the GLP-1 receptor, caused by albumin binding competing with the binding to the GLP-1 receptor.

A high ratio (IC_{50} value (high albumin) / IC_{50} value (low albumin)) may therefore be
20 taken as an indication that the derivative in question binds well to albumin (may have a long half-life), and also *per se* binds well to the GLP-1 receptor (the IC_{50} value (high albumin) is high, and the IC_{50} value (low albumin) is low). On the other hand, albumin binding may not always be desirable, or the binding to albumin may become too strong. Therefore, the desirable ranges for IC_{50} (low albumin), IC_{50} (high albumin) /, and the ratio high/low may vary
25 from compound to compound, depending on the intended use and the circumstances surrounding such use, and on other compound properties of potential interest.

In a particular embodiment, the peptides and derivatives of the invention have a high binding affinity to the GLP-1 receptor at low albumin concentration (0.005%), i.e. a low IC_{50} value.

30 In a particular embodiment, the GLP-1 receptor binding affinity (IC_{50}) in the presence of 0.005% HSA (low albumin) is below 600.00 nM, preferably below 500.00 nM, more preferably below 200.00 nM, even more preferably below 100.00 nM, or most preferably below 45.00 nM.

35 Degradation by gastro intestinal enzymes

According to the third functional aspect, the GLP-1 receptor agonist peptides and/or derivatives of the invention are stable, or stabilised, against degradation by one or more gastro intestinal enzymes.

5 Gastro intestinal enzymes include, without limitation, exo and endo peptidases, such as pepsin, trypsin, chymotrypsin, elastases, and carboxypeptidases. The stability may be tested against these gastro intestinal enzymes in the form of purified enzymes, or in the form of extracts from the gastrointestinal system.

10 In a particular embodiment, the derivative of the invention has an in vitro half-life ($T_{1/2}$), in an extract of rat small intestines, divided by the corresponding half-life ($T_{1/2}$) of GLP-1(7-37), of above 1.0, preferably above 2.0, more preferably above 3.0, even more preferably above 4.0, or most preferably above 5.0. In other words, a ratio(SI) may be defined for each derivative, viz. as the in vitro half-life ($T_{1/2}$) of the derivative in question, in an extract of rat small intestines, divided by the corresponding half-life ($T_{1/2}$) of GLP-1(7-37).

15 A suitable assay for determining in vitro half-life in an extract of rat small intestines is disclosed in Example 57 of a PCT application entitled "Double-acylated GLP-1 derivatives" filed with the EPO as receiving office, by Novo Nordisk A/S, on the same date as the present application.

Protraction - half life in vivo in rats

20 According to the second functional aspect, the derivatives of the invention are protracted. In a particular embodiment, protraction may be determined as half-life ($T_{1/2}$) in vivo in rats after i.v. administration. In additional embodiments, the half-life is at least 4 hours, preferably at least 5 hours, even more preferably at least 6 hours, or most preferably at least 8 hours.

25 A suitable assay for determining half-life in vivo in rats after i.v. administration is disclosed in Example 58 of a PCT application entitled "Double-acylated GLP-1 derivatives" filed with the EPO as receiving office, by Novo Nordisk A/S, on the same date as the present application.

Protraction - half life in vivo in minipigs

30 According to the second functional aspect, the derivatives of the invention are protracted. In a particular embodiment protraction may be determined as half-life ($T_{1/2}$) in vivo in minipigs after i.v. administration. In additional embodiments, the half-life is at least 12 hours, preferably at least 24 hours, more preferably at least 36 hours, even more preferably at least 48 hours, or most preferably at least 60 hours.

35

A suitable assay for determining half-life in vivo in minipigs after i.v. administration is disclosed in Example 16 herein.

Oral bioavailability

5 According to the fourth functional aspect, the derivatives of the invention have a high oral bioavailability.

The oral bioavailability of commercial GLP-1 receptor agonist peptide derivatives is very low. The oral bioavailability of such derivatives under development for i.v. or s.c. administration is also low.

10 Accordingly, there is a need in the art for derivatives of an improved oral bioavailability. Such derivatives could be suitable candidates for oral administration, as long as their potency is generally satisfactory, and/or as long as their half-life is also generally satisfactory.

The present inventors identified a novel class of GLP-1 receptor agonist peptide derivatives, which have a high oral bioavailability, and at the same time a satisfactory potency, and/or half-life.

Also, or alternatively, these derivatives have a high oral bioavailability, and at the same time a high binding affinity (i.e. a low IC_{50} value) to the GLP-1 receptor at a low concentration of albumin.

20 These features are of importance with a view to obtaining a low daily oral dose of the active pharmaceutical ingredient, which is desirable for various reasons, including, e.g., economy of production, likelihood of potential safety issues, as well as administration comfort issues, and environmental concerns.

Generally, the term bioavailability of a GLP-1 receptor agonist compound of the invention refers to the fraction of an administered dose of the compound that reaches the systemic circulation unchanged. By definition, when a medication is administered intravenously, its bioavailability is 100%. However, when a medication is administered via other routes (such as orally), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Knowledge about bioavailability is essential when calculating dosages for non-intravenous routes of administration.

30 Absolute oral bioavailability compares the bioavailability (estimated as the area under the curve, or AUC) of the active drug in systemic circulation following oral administration, with the bioavailability of the same drug following intravenous administration. It is the fraction of the drug absorbed through non-intravenous administration compared with the corresponding intravenous administration of the same drug. The comparison must be

dose normalised if different doses are used; consequently, each AUC is corrected by dividing the corresponding dose administered.

A plasma drug concentration vs time plot is made after both oral and intravenous administration. The absolute bioavailability (F) is the dose-corrected AUC-oral divided by
5 AUC-intravenous.

The GLP-1 receptor agonist compounds of the invention have an absolute oral bioavailability which is higher than that of a) liraglutide, and/or b) semaglutide; preferably at least 10% higher, more preferably at least 20% higher, even more preferably at least 30% higher, or most preferably at least 40% higher. Before testing oral bioavailability the GLP-1
10 receptor agonist compounds of the invention may suitably be formulated as is known in the art of oral formulations of insulinotropic compounds, e.g. using any one or more of the formulations described in WO 2008/145728.

A test has been developed, described in Example 15, which was found to be a very good prediction of oral bioavailability. According to this test, after direct injection of a GLP-1
15 derivative into the intestinal lumen of rats, the concentration (exposure) thereof in plasma is determined, and the ratio of plasma concentration (pmol/l) divided by the concentration of the dosing solution (umol/l) is calculated for t=30 min. This ratio is a measure of intestinal bioavailability, and it has shown to correlate nicely with actual oral bioavailability data.

20 Additional particular embodiments of the derivatives of the invention are described in the section headed "particular embodiments" before the experimental section.

PRODUCTION PROCESSES

The production of peptides like for example GLP-1(7-37) and analogues thereof is well known in the art.

25 The fragment P of the peptides of formula I of the invention may for instance be produced by classical peptide synthesis, e.g., solid phase peptide synthesis using t-Boc or Fmoc chemistry or other well established techniques, see, e.g., Greene and Wuts, "Protective Groups in Organic Synthesis", John Wiley & Sons, 1999, Florencio Zaragoza Dörwald, "Organic Synthesis on solid Phase", Wiley-VCH Verlag GmbH, 2000, and "Fmoc
30 Solid Phase Peptide Synthesis", Edited by W.C. Chan and P.D. White, Oxford University Press, 2000.

Also, or alternatively, it may be produced by recombinant methods, viz. by culturing a host cell containing a DNA sequence encoding the fragment and capable of expressing the peptide in a suitable nutrient medium under conditions permitting the expression of the

peptide. Non-limiting examples of host cells suitable for expression of these peptides are: Escherichia coli, Saccharomyces cerevisiae, as well as mammalian BHK or CHO cell lines.

The complete GLP-1 receptor agonist peptides of the invention incorporating, viz. adding Y-Z to P of formula I, may e.g. be produced as described in the experimental part. Or
5 see Hodgson et al: "The synthesis of peptides and proteins containing non-natural amino acids", Chemical Society Reviews, vol. 33, no. 7 (2004), p. 422-430; and in WO 2009/083549 A1 entitled "Semi-recombinant preparation of GLP-1 analogues".

Derivatives of the invention may be prepared as is known in the art, and specific examples of methods of preparing a number of derivatives of the invention are included in
10 the experimental part herein.

PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions comprising a peptide or a derivative of the invention; or a pharmaceutically acceptable salt, amide, or ester thereof, and a pharmaceutically acceptable excipient may be prepared as is known in the art.

15 The term "excipient" broadly refers to any component other than the active therapeutic ingredient(s). The excipient may be an inert substance, an inactive substance, and/or a not medicinally active substance.

The excipient may serve various purposes, e.g. as a carrier, vehicle, diluent, tablet aid, and/or to improve administration, and/or absorption of the active substance.

20 The formulation of pharmaceutically active ingredients with various excipients is known in the art, see e.g. Remington: The Science and Practice of Pharmacy (e.g. 19th edition (1995), and any later editions).

Non-limiting examples of excipients are: Solvents, diluents, buffers, preservatives, tonicity regulating agents, chelating agents, and stabilisers.

25 Examples of formulations include liquid formulations, i.e. aqueous formulations comprising water. A liquid formulation may be a solution, or a suspension. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 80%, or even at least 90% w/w of water.

Alternatively, a pharmaceutical composition may be a solid formulation, e.g. a
30 freeze-dried or spray-dried composition, which may be used as is, or whereto the physician or the patient adds solvents, and/or diluents prior to use.

The pH in an aqueous formulation may be anything between pH 3 and pH 10, for example from about 7.0 to about 9.5; or from about 3.0 to about 7.0.

A pharmaceutical composition may comprise a buffer. The buffer may e.g. be selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, 5 tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid, and mixtures thereof. A pharmaceutical composition may comprise a preservative. The preservative may e.g. be selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, bronopol, 10 benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol), and mixtures thereof. The preservative may be present in a concentration from 0.1 mg/ml to 20 mg/ml. A pharmaceutical composition may comprise an isotonic agent. The isotonic agent may e.g. be selected from the group consisting of a salt (e.g. sodium chloride), 15 a sugar or sugar alcohol, an amino acid (e.g. glycine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (e.g. glycerol (glycerine), 1,2-propanediol (propyleneglycol), 1,3-propanediol, 1,3-butanediol) polyethyleneglycol (e.g. PEG400), and mixtures thereof. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, 20 lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, alfa and beta HPCD, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na may be used. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one -OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment, the sugar alcohol additive is mannitol. A pharmaceutical composition may 25 comprise a chelating agent. The chelating agent may e.g. be selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. A pharmaceutical composition may comprise a stabiliser. The stabiliser may e.g. be one or more oxidation inhibitors, aggregation inhibitors, surfactants, and/or one or more protease inhibitors. Non-limiting examples of these various kinds of stabilisers are disclosed in the 30 following.

The term "aggregate formation" refers to a physical interaction between the peptide molecules resulting in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. Aggregate formation by a peptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that 35 polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition.

Furthermore, aggregate formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

A pharmaceutical composition may comprise an amount of an amino acid base
5 sufficient to decrease aggregate formation of the polypeptide during storage of the composition. The term "amino acid base" refers to one or more amino acids (such as methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), or analogues thereof. Any amino acid may be present either in its free base form or in its salt form. Any stereoisomer (i.e., L, D, or a mixture thereof) of the amino acid base
10 may be present.

Methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. Any stereoisomer of methionine (L or D) or combinations
15 thereof can be used.

A the pharmaceutical composition may comprise a stabiliser selected from the group of high molecular weight polymers or low molecular compounds. The stabiliser may e.g. be selected from polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivatives thereof (e.g. HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (e.g. sodium chloride). A pharmaceutical composition may comprise additional stabilising agents such as, but not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a nonionic surfactant, which protects the polypeptide against aggregation associated
25 with freeze-thawing or mechanical shearing.

A pharmaceutical composition may comprise one or more surfactants, preferably a surfactant, at least one surfactant, or two different surfactants. The term "surfactant" refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant may e.g. be selected from the group consisting of
30 anionic surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants.

A pharmaceutical composition may comprise one or more protease inhibitors, such as, e.g., EDTA (ethylenediamine tetraacetic acid), and/or benzamidineHCl.

Additional, optional, ingredients of a pharmaceutical composition include, e.g., wetting agents, emulsifiers, antioxidants, bulking agents, metal ions, oily vehicles, proteins

(e.g., human serum albumin, gelatine), and/or a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine).

Still further, a pharmaceutical composition may be formulated as is known in the art of oral formulations of insulinotropic compounds, e.g. using any one or more of the
5 formulations described in WO 2008/145728.

An administered dose may contain from 0.01 mg - 100 mg of the GLP-1 receptor agonist derivative, or from 0.01-50 mg, or from 0.01-20 mg, or from 0.01-10 mg of the GLP-1 receptor agonist derivative.

The derivative may be administered in the form of a pharmaceutical composition. It
10 may be administered to a patient in need thereof at several sites, for example, at topical sites such as skin or mucosal sites; at sites which bypass absorption such as in an artery, in a vein, or in the heart; and at sites which involve absorption, such as in the skin, under the skin, in a muscle, or in the abdomen.

The route of administration may be, for example, lingual; sublingual; buccal; in the
15 mouth; oral; in the stomach; in the intestine; nasal; pulmonary, such as through the bronchioles, the alveoli, or a combination thereof; parenteral, epidermal; dermal; transdermal; conjunctival; uretal; vaginal; rectal; and/or ocular. A composition may be an oral composition, and the route of administration is per oral.

A composition may be administered in several dosage forms, for example as a
20 solution; a suspension; an emulsion; a microemulsion; multiple emulsions; a foam; a salve; a paste; a plaster; an ointment; a tablet; a coated tablet; a chewing gum; a rinse; a capsule such as hard or soft gelatine capsules; a suppository; a rectal capsule; drops; a gel; a spray; a powder; an aerosol; an inhalant; eye drops; an ophthalmic ointment; an ophthalmic rinse; a vaginal pessary; a vaginal ring; a vaginal ointment; an injection solution; an in situ
25 transforming solution such as in situ gelling, setting, precipitating, and in situ crystallisation; an infusion solution; or as an implant. A composition may be a tablet, optionally coated, a capsule, or a chewing gum.

A composition may further be compounded in a drug carrier or drug delivery system, e.g. in order to improve stability, bioavailability, and/or solubility. In a particular embodiment a
30 composition may be attached to such system through covalent, hydrophobic, and/or electrostatic interactions. The purpose of such compounding may be, e.g., to decrease adverse effects, achieve chronotherapy, and/or increase patient compliance.

A composition may also be used in the formulation of controlled, sustained, protracting, retarded, and/or slow release drug delivery systems.

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal, or intravenous injection by means of a syringe, optionally a pen-like syringe, or by means of an infusion pump.

5 A composition may be administered nasally in the form of a solution, a suspension, or a powder; or it may be administered pulmonally in the form of a liquid or powder spray.

Transdermal administration is a still further option, e.g. by needle-free injection, from a patch such as an iontophoretic patch, or via a transmucosal route, e.g. buccally.

10 A composition may be a stabilised formulation. The term "stabilised formulation" refers to a formulation with increased physical and/or chemical stability, preferably both. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

The term "physical stability" refers to the tendency of the polypeptide to form biologically inactive and/or insoluble aggregates as a result of exposure to thermo-mechanical stress, and/or interaction with destabilising interfaces and surfaces (such as hydrophobic surfaces). The physical stability of an aqueous polypeptide formulation may be evaluated by means of visual inspection, and/or by turbidity measurements after exposure to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Alternatively, the physical stability may be evaluated using a spectroscopic agent or probe of the conformational status of the polypeptide such as e.g. Thioflavin T or "hydrophobic patch" probes.

20 The term "chemical stability" refers to chemical (in particular covalent) changes in the polypeptide structure leading to formation of chemical degradation products potentially having a reduced biological potency, and/or increased immunogenic effect as compared to the intact polypeptide. The chemical stability can be evaluated by measuring the amount of chemical degradation products at various time-points after exposure to different environmental conditions, e.g. by SEC-HPLC, and/or RP-HPLC.

The treatment with a derivative according to the present invention may also be combined with one or more additional pharmacologically active substances, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity. Examples of these pharmacologically active substances are : Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose

uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the β -cells; Cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; β -blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α -blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonists, Y2 receptor agonists, Y4 receptor agonists, mixed Y2/Y4 receptor agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β 3 agonists, oxyntomodulin and analogues, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR β agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

The treatment with a derivative according to this invention may also be combined with a surgery that influences the glucose levels, and/or lipid homeostasis such as gastric banding or gastric bypass.

PHARMACEUTICAL INDICATIONS

The present invention also relates to a GLP-1 receptor agonist peptide of the invention, and a derivative thereof, for use as a medicament.

In particular embodiments, these compounds may be used for the following medical treatments, all preferably relating one way or the other to diabetes:

(i) prevention and/or treatment of all forms of diabetes, such as hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent diabetes,

MODY (maturity onset diabetes of the young), gestational diabetes, and/or for reduction of HbA1C;

(ii) delaying or preventing diabetic disease progression, such as progression in type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin requiring type 2 diabetes, and/or delaying the progression of non-insulin requiring type 2 diabetes to insulin requiring type 2 diabetes;

(iii) improving β -cell function, such as decreasing β -cell apoptosis, increasing β -cell function and/or β -cell mass, and/or for restoring glucose sensitivity to β -cells;

(iv) prevention and/or treatment of cognitive disorders;

(v) prevention and/or treatment of eating disorders, such as obesity, e.g. by decreasing food intake, reducing body weight, suppressing appetite, inducing satiety; treating or preventing binge eating disorder, bulimia nervosa, and/or obesity induced by administration of an antipsychotic or a steroid; reduction of gastric motility; and/or delaying gastric emptying;

(vi) prevention and/or treatment of diabetic complications, such as neuropathy, including peripheral neuropathy; nephropathy; or retinopathy;

(vii) improving lipid parameters, such as prevention and/or treatment of dyslipidemia, lowering total serum lipids; lowering HDL; lowering small, dense LDL; lowering VLDL; lowering triglycerides; lowering cholesterol; increasing HDL; lowering plasma levels of lipoprotein a (Lp(a)) in a human; inhibiting generation of apolipoprotein a (apo(a)) in vitro and/or in vivo;

(viii) prevention and/or treatment of cardiovascular diseases, such as syndrome X; atherosclerosis; myocardial infarction; coronary heart disease; stroke, cerebral ischemia; an early cardiac or early cardiovascular disease, such as left ventricular hypertrophy; coronary artery disease; essential hypertension; acute hypertensive emergency; cardiomyopathy; heart insufficiency; exercise tolerance; chronic heart failure; arrhythmia; cardiac dysrhythmia; syncope; atherosclerosis; mild chronic heart failure; angina pectoris; cardiac bypass reocclusion; intermittent claudication (atherosclerosis obliterans); diastolic dysfunction; and/or systolic dysfunction;

(ix) prevention and/or treatment of gastrointestinal diseases, such as inflammatory bowel syndrome; small bowel syndrome, or Crohn's disease; dyspepsia; and/or gastric ulcers;

(x) prevention and/or treatment of critical illness, such as treatment of a critically ill patient, a critical illness poly-nephropathy (CIPNP) patient, and/or a potential CIPNP patient; prevention of critical illness or development of CIPNP; prevention, treatment and/or cure of

systemic inflammatory response syndrome (SIRS) in a patient; and/or for the prevention or reduction of the likelihood of a patient suffering from bacteraemia, septicaemia, and/or septic shock during hospitalisation; and/or

(xi) prevention and/or treatment of polycystic ovary syndrome (PCOS).

5 In a particular embodiment, the indication is selected from the group consisting of (i)-(iii) and (v)-(iix), such as indications (i), (ii), and/or (iii); or indication (v), indication (vi), indication (vii), and/or indication (iix).

In another particular embodiment, the indication is (i). In a further particular embodiment the indication is (v). In a still further particular embodiment the indication is (iix).

10 The following indications are particularly preferred: Type 2 diabetes, and/or obesity.

PARTICULAR EMBODIMENTS

The following are particular embodiments of the invention:

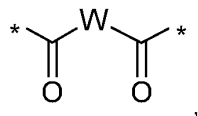
1. A GLP-1 receptor agonist peptide having the formula Chem. 1:

Chem. 1: Y-Z-P, wherein

15 P represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues;

Z represents a group of the formula Chem. 2:

Chem. 2:

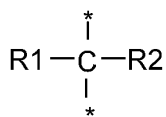


20

wherein

W represents a group of formula Chem. 3:

Chem. 3:



wherein

25

R1 and R2 independently represent

(i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide,

alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or

R1 and R2 together form

(ii) cyclo alkyl, heterocyclyl, or heteroaryl,

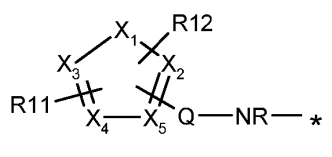
with the proviso that

(iii) R1 and R2 do not both represent hydrogen;

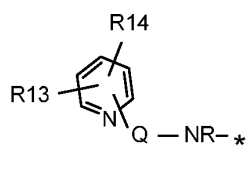
and

Y represents a group of formula Chem. 4 or Chem. 5:

Chem. 4:



Chem. 5:



wherein

X₁ is N, O, or S; X₂, X₃, X₄, and X₅ independently represent C, or N,

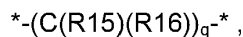
with the proviso that at least one of X₂, X₃, X₄ and X₅ is C;

R₁₁, R₁₂, R₁₃, and R₁₄ independently represent

hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl;

Q represents a bond, or a group of formula Chem. 6:

Chem. 6:



wherein

q is 1-6, and

R₁₅ and R₁₆ independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl; and

R represents hydrogen, or alkyl;

or a pharmaceutically acceptable salt, amide, or ester thereof.

2. The peptide of embodiment 1, wherein R1 and R2 independently represent

hydrogen, alkyl, aryl, halogen, hydroxyl, hydroxylalkyl, amino, aminoalkyl, carboxyl,

5 carboxylalkyl, alkoxy, aryloxy, carboxamide, alkyl ester, or aryl ester; wherein preferably alkyl, hydroxylalkyl, aminoalkyl, carboxylalkyl, alkoxy, and/or alkyl ester contains lower alkyl, straight or branched, more preferably having 1-6 C-atoms.

3. The peptide of any one of embodiments 1-2, wherein wherein R1 and R2

independently represent hydrogen, lower alkyl, or lower alkoxy, wherein the lower alkyl and

10 lower alkoxy, independently, have 1-5 C-atoms, preferably 1-4 C-atoms, or most preferably 1-3 C-atoms.

4. The peptide of any one of embodiments 1-3, wherein R1 and R2 independently

represent hydrogen, alkyl having 1-2 C-atoms (ethyl, or methyl), or alkoxy having 1-2 C-atoms (ethoxy, methoxy).

15 5. The peptide of any one of embodiments 1-4, wherein R1 and R2 are methyl or methoxy, preferably methyl.

6. The peptide of embodiment 1, wherein R1 and R2 independently represent alkyl,

aryl, halogen, hydroxyl, hydroxylalkyl, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, alkyl ester, or aryl ester; wherein preferably alkyl, hydroxylalkyl,

20 aminoalkyl, carboxylalkyl, alkoxy, and/or alkyl ester contains lower alkyl, straight or branched, more preferably having 1-6 C-atoms.

7. The peptide of any one of embodiments 1 and 6, wherein wherein R1 and R2

independently represent lower alkyl, or lower alkoxy, wherein the lower alkyl and lower

25 alkoxy, independently, have 1-5 C-atoms, preferably 1-4 C-atoms, or most preferably 1-3 C-atoms.

8. The peptide of any one of embodiments 1, and 6-7, wherein R1 and R2

independently represent alkyl having 1-2 C-atoms (ethyl, or methyl), or alkoxy having 1-2 C-atoms (ethoxy, methoxy).

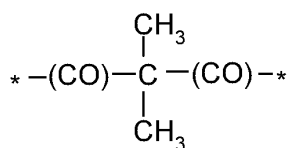
9. The peptide of any one of embodiments 1, and 6-8, wherein R1 and R2 are methyl

30 or methoxy, preferably methyl.

10. The peptide of any one of embodiments 1-9, wherein Z is a group of formula Chem.

7):

Chem. 7:



11. The peptide of any one of embodiments 1-10, wherein Y is Chem. 4.
12. The peptide of any one of embodiments 1-11, wherein X₁ is N.
13. The peptide of any one of embodiments 1-12, wherein one of X₂, X₃, X₄, and X₅ is N.
- 5 14. The peptide of any one of embodiments 1-13, preferably the peptide of embodiment 12, wherein i) X₃ is N; ii) X₄ is N; iii) X₅ is N; or X₂ is N.
15. The peptide of any one of embodiments 1-14, wherein R₁₁ and R₁₂ independently represent hydrogen, alkyl, aryl, halogen, hydroxyl, hydroxylalkyl, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, alkyl ester, or aryl ester;
- 10 wherein preferably alkyl, hydroxylalkyl, aminoalkyl, carboxylalkyl, alkoxy, and/or alkyl ester contains lower alkyl, straight or branched, more preferably having 1-6 C-atoms.
16. The peptide of any one of embodiment 1-15, wherein R₁₁ and R₁₂ independently represent hydrogen, lower alkyl, or lower alkoxy, wherein the lower alkyl and lower alkoxy, independently, have 1-5 C-atoms, preferably 1-4 C-atoms, or most preferably
- 15 1-3 C-atoms.
17. The peptide of any one of embodiments 1-16, wherein R₁₁ and R₁₂ independently represent hydrogen, alkyl having 1-2 C-atoms (ethyl, or methyl), or alkoxy having 1-2 C-atoms (ethoxy, methoxy).
18. The peptide of any one of embodiments 1-17, wherein R₁₁ and R₁₂ are methyl or
- 20 hydrogen, preferably hydrogen.
19. The peptide of any one of embodiments 1-18, wherein Y is a derivative of 1H-imidazole, preferably 1H-imidazol-4-yl, optionally substituted at one or two of positions 2, 3, and/or 5, wherein the position numbering of imidazole is according to IUPAC, and/or as shown for imidazole on wikipedia on 03-DEC-2010 at 18:00 DK time.
- 25 20. The peptide of any one of embodiments 1-19, wherein Q is attached to i) X₂, ii) X₃, X₄, or X₅, preferably to X₂ or X₄, most preferably to X₄.
21. The peptide of any one of embodiments 1-20, wherein q is 1-5, preferably 1-4, more preferably 1-3, even more preferably 1, 2, or 3.
22. The peptide of any one of embodiments 1-21, wherein R₁₅ and R₁₆ independently
- 30 of each other and independently for each value of q represent hydrogen.
23. The peptide of any one of embodiments 1-22, wherein R₁₅ and R₁₆ both represent hydrogen.

24. The peptide of any one of embodiments 1-23, wherein R is hydrogen.

25. The peptide of any one of embodiments 1-24, wherein Y is a derivative of an imidazole, such as 1H-imidazol, being substituted with a group of formula Chem. 7:
Chem. 7:

5 *-(R17)-N-*,

wherein R17 represents alkylene, straight or branched, having 1-6 C-atoms, preferably 1-5 C-atoms, more preferably 1-4 C-atoms, or most preferably 1-3 C-atoms.

26. The peptide of any one of embodiments 1-10, wherein Y is Chem. 5.

27. The peptide of embodiment any one of embodiments 1-10 and 26, wherein R13 and
10 R14 independently represent hydrogen, alkyl, aryl, halogen, hydroxyl, hydroxylalkyl, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, alkyl ester, or aryl ester; wherein preferably alkyl, hydroxylalkyl, aminoalkyl, carboxylalkyl, alkoxy, and/or alkyl ester contains lower alkyl, straight or branched, more preferably having 1-6 C-atoms.

28. The peptide of any one of embodiment 1-10 and 26-27, wherein wherein R13 and
15 R14 independently represent hydrogen, lower alkyl, or lower alkoxy, wherein the lower alkyl and lower alkoxy, independently, have 1-5 C-atoms, preferably 1-4 C-atoms, or most preferably 1-3 C-atoms.

29. The peptide of any one of embodiments 1-10 and 26-28, wherein R13 and R14
20 independently represent hydrogen, alkyl having 1-2 C-atoms (ethyl, or methyl), or alkoxy having 1-2 C-atoms (ethoxy, methoxy).

30. The peptide of any one of embodiments 1-10 and 26-29, wherein R13 and R14 are methyl or hydrogen, preferably hydrogen.

31. The peptide of any one of embodiments 1-10 and 26-30, wherein Y is a derivative of pyridine, preferably pyridin-2-yl, optionally substituted at one or two of positions 3, 4, 5,
25 and/or 6, where the position numbering of pyridine is according to IUPAC, and/or as shown for pyridine on wikipedia on 03-DEC-2010 at 18:00 DK time.

32. The peptide of any one of embodiments 1-10 and 26-31, wherein Q is attached to position 2, 3, 4, 5, or 6 of the pyridine ring, preferably to position 2, where the position numbering of pyridine is as defined in embodiment 31.

30 33. The peptide of any one of embodiments 1-32, wherein q is as defined in embodiment 21.

34. The peptide of any one of embodiments 1-33, wherein R15 and R16 are as defined in any one of embodiments 22-23.

35. The peptide of any one of embodiments 1-34, wherein R is hydrogen.

36. The peptide of any one of embodiments 1-35, wherein the GLP-1 receptor agonist peptide of which P is a fragment lacking the two N-terminal amino acid residues is an agonist of the human GLP-1 receptor, wherein agonist activity is preferably determined as stimulation of the formation of cAMP in a medium containing the human GLP-1 receptor.

5 37. The peptide of embodiment 36, wherein the medium is a suitable medium, such as a medium containing the human GLP-1 receptor and having the following composition (final in-assay concentrations): 50 mM Tris-HCl, 1 mM EGTA, 1.5 mM MgSO₄, 1.7 mM ATP, 20 mM GTP, 2 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01 % Tween-20, pH 7.4; more preferably the following composition (final in-assay concentrations): 50 mM TRIS-HCl; 5 mM HEPES;
10 10 mM MgCl₂, 6H₂O; 150 mM NaCl; 0.01% Tween; 0.1% BSA ; 0.5 mM IBMX; 1 mM ATP; 1 uM GTP; pH 7.4.

38. The peptide of any one of embodiments 1-37, wherein the GLP-1 receptor agonist peptide of which P is a fragment lacking the two N-terminal amino acid residues has a potency corresponding to an EC₅₀ of below 1000 pM, preferably below 500 pM, more
15 preferably below 250 pM, even more preferably below 125 pM, or most preferably below 50 pM.

39. The peptide of any one of embodiments 1-38, wherein the GLP-1 receptor agonist peptide of which P is a fragment lacking the two N-terminal amino acid residues is selected from His-Ala-"P", His-Gly-"P", and "His-Ser-P".

20 40. The peptide of any one of embodiments 1-39, wherein the GLP-1 receptor agonist peptide of which P is a fragment lacking the two N-terminal amino acid residues is selected from His-Ala-"P" and His-Gly-"P".

41. The peptide of any one of embodiments 1-40, wherein P is selected from i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), iii) GLP-1A(3-37) (SEQ ID NO:
25 3), and iv) analogues of i), ii), or iii) having a maximum of eight amino acid residues exchanged as compared to the respective sequence i), ii), or iii) with which the analogue has the highest similarity, or, preferably, percentage of identity.

42. The peptide of any one of embodiments 1-41, wherein P is GLP-1(9-37) (SEQ ID NO: 1), or an analogue thereof having a maximum of eight amino acid residues exchanged
30 as compared to SEQ ID NO: 1.

43. The peptide of any one of embodiments 1-42, wherein P has a) a maximum of seven, six, or five; preferably b) a maximum of four, or c) most preferably a maximum of three amino acid residues exchanged, as compared to one of the sequences of i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO:

3), with which P has the highest similarity, wherein preferably the comparison is made with GLP-1(9-37) (SEQ ID NO: 1), or with GLP-1A(3-37), most preferably with GLP-1(9-37)..

44. The peptide of any one of embodiments 1-43, wherein P has a maximum of two, preferably a maximum of one, or most preferably no amino acid residues exchanged as compared to one of the sequences of i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3), with which P has the highest similarity, wherein preferably the comparison is made with GLP-1(9-37) (SEQ ID NO: 1), or with GLP-1A(3-37), most preferably with GLP-1(9-37).

45. The peptide of any one of embodiments 1-44, which has a C-terminal amide.

10 46. The peptide of any one of embodiments 1-45, which has a C-terminal -COOH group.

47. The peptide of any one of embodiments 1-46, which comprises at least one of the following substitutions as compared to GLP-1(9-37) (SEQ ID NO: 1): 18K; 22E; 30E; 31H; 34Q,R; 36K; 37K; and/or 38E.

48. The peptide of any one of embodiments 1-47, which comprises 18K.

15 49. The peptide of any one of embodiments 1-48, which comprises 22E.

50. The peptide of any one of embodiments 1-49, which comprises 30E.

51. The peptide of any one of embodiments 1-50, which comprises 31H.

52. The peptide of any one of embodiments 1-51, which comprises 34Q or 34R, preferably 34R.

20 53. The peptide of any one of embodiments 1-52, which comprises 36K.

54. The peptide of any one of embodiments 1-53, which comprises 37K.

55. The peptide of any one of embodiments 1-54, which comprises 38E.

56. The peptide of any one of embodiments 1-55, which comprises 34R and 37K.

57. The peptide of any one of embodiments 1-56, which comprises 30E and 36K.

25 58. The peptide of any one of embodiments 1-57, which comprises 31H and 34Q.

59. The peptide of any one of embodiments 56-57, which further comprises 38E.

60. The peptide of any one of embodiments 1-59 which comprises the following substitutions: (i) 18K, 22E, 34Q; (ii) 31H, 34Q; (iii) 30E, 36K; (iv) 30E, 36K, 38E; (v) 34R; (vi) 34R, 37K; or (vii) 34R, 37K, 38E.

30 61. The peptide of any one of embodiments 1-60, which has the following substitutions as compared to GLP-1(9-37) (SEQ ID NO: 1), all other amino acid residues being as in SEQ ID NO: 1: (i) 18K, 22E, 34Q; (ii) 31H, 34Q; (iii) 30E, 36K; (iv) 30E, 36K, 38E; (v) 34R; (vi) 34R, 37K; of (vii) 34R, 37K, 38E.

62. The peptide of any one of embodiments 1-46, which comprises at least one of the following substitutions as compared to GLP-1A(3-37) (SEQ ID NO: 3): 17Q,R; 20R; 33R; and/or 38K.

63. The peptide of any one of embodiments 1-46, and 62, which has the following substitutions, as compared to GLP-1A(3-37) (SEQ ID NO: 3), all other amino acid residues being as in SEQ ID NO: 3: 17R, 20R, 33R, and 38K.

64. A GLP-1 receptor agonist peptide selected from the following:

- (i) N^9 -[2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl]-[Lys¹⁸,Glu²²,Gln³⁴]GLP-1(9-37)-peptide;
- 10 (ii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[His³¹,Gln³⁴]GLP-1(9-37)-peptide;
- (iii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶]GLP-1(9-37)-Glu³⁸-peptide amide;
- (iv) N^9 -{2-[2-(1H-Imidazol-4-yl)propylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶]GLP-1(9-37)Glu³⁸-peptide amide;
- 15 (v) N^9 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶]GLP-1(9-37)-Glu³⁸-peptide amide;
- (vi) N^3 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}-[Arg¹⁷,Arg²⁰,Arg³³,Lys³⁸]GLP-1A(3-37)-peptide;
- 20 (vii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴,Lys³⁷]GLP-1(9-37)-peptide;
- (viii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴,Lys³⁷]GLP-1(9-37)Glu³⁸-peptide;
- (ix) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴,Lys³⁷]GLP-1(9-37)-peptide;
- 25 (x) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴]GLP-1(9-37)-peptide; and
- (xii) N^9 -[2,2-dimethyl-3-oxo-3-(pyridin-2-ylmethylamino)propanoyl]-[Arg³⁴,Lys³⁷]-GLP-1-(9-37)-peptide;
- 30 or a pharmaceutically acceptable salt, amide, or ester thereof.

64. The peptide of any one of embodiments 1-63 which has a maximum of two K residues.

65. The peptide of any one of embodiments 1-64, which has a maximum of one K residue.

35 66. The peptide of any one of embodiments 1-65, wherein

a) the position corresponding to any of the indicated positions of i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3); and/or

b) the number of amino acid modifications as compared to i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3), is/are identified by handwriting and eyeballing.

67. The peptide of any one of embodiments 1-66, wherein

a) the position corresponding to any of the indicated positions of i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3); and/or

b) the number of amino acid modifications as compared to i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3), is/are identified by use of a standard protein or peptide alignment program.

68. The peptide of embodiment 67, wherein the alignment program is a Needleman-Wunsch alignment.

69. The peptide of any one of embodiments 67-68, wherein the default scoring matrix and the default identity matrix is used.

70. The peptide of any one of embodiments 67-69, wherein the scoring matrix is BLOSUM62.

71. The peptide of any one of embodiments 67-70, wherein the penalty for the first residue in a gap is -10 (minus ten).

72. The peptide of any one of embodiments 67-71, wherein the penalties for additional residues in a gap is -0.5 (minus point five).

73. The peptide of any one of embodiments 1-72, wherein, a residue number, preferably any residue number, be it in superscript after an amino acid residue, or in ordinary script before or after the amino acid residue in question, refers to the corresponding position in one of the sequences of i) GLP-1(9-37) (SEQ ID NO: 1), ii) exendin-4(3-39) (SEQ ID NO: 2), and iii) GLP-1A(3-37) (SEQ ID NO: 3)..

74. A derivative of a peptide of any one of embodiments 1-73, or a pharmaceutically acceptable salt, amide, or ester thereof.

75. The derivative of embodiment 74 which comprises, preferably has, an albumin binding moiety attached to a lysine residue, more preferably to the epsilon-amino group thereof, via an amide bond.

76. The derivative of embodiment 75, which comprises, preferably has, an albumin binding moiety attached to one or more of 18K, 26K, 36K, and/or 37K; preferably one

albumin binding moiety attached to 18K, 26K, or 36K; or two albumin binding moieties attached to 26K and 37K; wherein reference may be had to the sequence of GLP-1(9-37) (SEQ ID NO: 1).

77. The derivative of embodiment 75, which has an albumin binding moiety attached to one or more of 17K, 20K, 33K, and/or 38K; preferably one albumin binding moiety attached to 38K; or two albumin binding moieties attached to 20K and 33K; wherein reference may be had to the sequence of GLP-1A(3-37) (SEQ ID NO: 3).

78. The derivative of any one of embodiments 75-77, in which the albumin binding moiety comprises a protracting moiety.

79. The derivative of embodiment 78, wherein the protracting moiety is selected from Chem. 8, Chem. 9, and Chem. 10:

Chem. 8: $\text{HOOC}-(\text{CH}_2)_x-\text{CO}-^*$

Chem. 9: $\text{HOOC}-\text{C}_6\text{H}_4-\text{O}-(\text{CH}_2)_y-\text{CO}-^*$

Chem. 10: $\text{R}^{18}-\text{C}_6\text{H}_4-(\text{CH}_2)_z-\text{CO}-^*$

in which x is an integer in the range of 6-18, y is an integer in the range of 3-17, z is an integer in the range of 1-5, and R^{18} is a group having a molar mass not higher than 150 Da.

80. The derivative of embodiment 79, in which the protracting moiety is Chem. 8, in which x is an even number, preferably in the range of 14-18, more preferably 16-18, or most preferably x is 16.

81. The derivative of embodiment 79, in which the protracting moiety is Chem. 9, in which y is an odd number, preferably in the range of 7-15.

82. The derivative of embodiment 79, in which the protracting moiety is Chem. 10, in which z is an odd number, preferably 3; and R^{18} is tert. butyl.

83. The derivative of any one of embodiments 79 and 81, in which y is 9.

84. The derivative of any one of embodiments 79, 81, and 83, in which the -COOH group is in the para-position.

85. The derivative of any one of embodiments 79, and 82, in which R^{18} is in the para-position.

86. The derivative of any one of embodiments 75-84 which comprises a linker.

87. The derivative of any one of embodiments 75-86, wherein the albumin binding moiety comprises a linker.

88. The derivative of any one of embodiments 75-87, wherein the albumin binding moiety further comprises a linker.

89. The derivative of any one of embodiments 86-88, wherein the linker is a di-radical which comprises an N radical and a CO radical, wherein

i) the N-radical is represented by a first $^*\text{-NR}^{19}\text{R}^{20}$ group, where R^{19} and R^{20} may, independently, designate hydrogen, carbon, or sulphur, optionally substituted; and

5 ii) the CO radical is represented by a first $^*\text{-CO}$ group,

and wherein, preferably, the first $^*\text{-NR}^{19}\text{R}^{20}$ group is capable of forming an amide bond with a second $^*\text{-CO}$ group, and the first $^*\text{-CO}$ group is capable of forming an amide bond with a second $^*\text{-NR}^{19}\text{R}^{20}$ group, wherein the second $^*\text{-NR}^{19}\text{R}^{20}$ group and the second $^*\text{-CO}$ group are defined as the first $^*\text{-NR}^{19}\text{R}^{20}$ group and the first $^*\text{-CO}$ group, respectively, and form part,
10 independently, of the structure of i) the analogue, ii) the protracting moiety, and/or iii) another linker.

90. The derivative of any one of embodiments 74-89, which comprises at least one linker selected from the group consisting of Chem. 11, Chem. 12, Chem. 13, and Chem. 14:

Chem. 11: $^*\text{-NH-CH}_2\text{-CH}_2\text{-(O-CH}_2\text{-CH}_2\text{)}_k\text{-O-(CH}_2\text{)}_n\text{-CO-}^*$

15 Chem. 12: $^*\text{-NH-C(COOH)-(CH}_2\text{)}_2\text{-CO-}^*$

Chem. 13: $^*\text{-N-C((CH}_2\text{)}_2\text{COOH)-CO-}^*$

Chem. 14: $^*\text{-NC}_5\text{H}_8\text{-CO-}^*$

wherein k is an integer in the range of 1-5, and n is an integer in the range of 1-5; and wherein Chem. 12 and Chem. 13 are di-radicals of Glu.

20 91. The derivative of embodiment 90, wherein the linker comprises Chem. 11, and wherein preferably Chem. 11 is a first linker element.

92. The derivative of any one of embodiments 90-91, wherein k is 1.

93. The derivative of any one of embodiments 90-92, wherein n is 1.

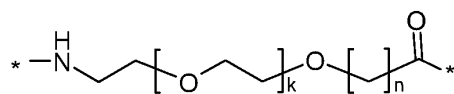
94. The derivative of any one of embodiments 90-93, wherein Chem. 11 is included m
25 times, wherein m is an integer in the range of 1-10.

95. The derivative of embodiment 94, wherein m is an integer in the range of 1-6; preferably in the range of 1-4; more preferably m is 1 or 2; even more preferably m is 1; or most preferably m is 2.

96. The derivative of any one of embodiments 94-95, wherein, when m is different from
30 1, the Chem. 11 elements are interconnected via amide bond(s).

97. The derivative of any one of embodiments 90-96, wherein the linker consists of one or more Chem. 11 elements.

98. The derivative of any one of embodiments 90-97, wherein Chem. 11 is represented by Chem. 11a:



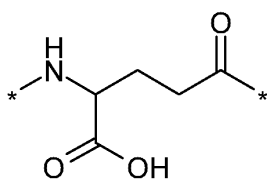
Chem. 11a:

wherein k and n are as defined in any one of embodiments 90-97.

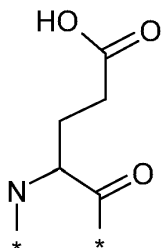
99. The derivative of any one of embodiments 90-99, wherein the linker comprises a Glu di-radical, such as Chem. 12, and Chem. 13.

- 5 100. The derivative of any one of embodiments 90-99, wherein Chem. 12 and Chem. 13, independently, may be represented by Chem. 12a and Chem. 13a, respectively:

Chem. 12a:



Chem. 13a:



10

most preferably by Chem. 12a.

101. The derivative of any one of embodiments 90-101, wherein the Glu di-radical, such as Chem. 12, and/or Chem. 13, independently, is included p times, wherein p is an integer in the range of 1-3.

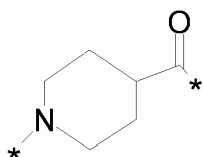
- 15 102. The derivative of embodiment 101, wherein p is 1, 2, or 3; preferably 1 or 2, or most preferably 1.

103. The derivative of any one of embodiments 90-102, wherein the Glu di-radical is a radical of L-Glu or D-Glu, preferably of L-Glu.

- 20 104. The derivative of any one of embodiments 90-103, wherein the linker consists of a Glu di-radical, preferably Chem. 12, more preferably Chem. 12a.

105. The derivative of any one of embodiments 90-104, wherein the linker comprises Chem. 14.

106. The derivative of any one of embodiments 90-105, where Chem. 14 is represented by Chem. 14a:



107. The derivative of any one of embodiments 90-106, wherein the linker consists of Chem. 11, being connected at its *-NH end to * the *-CO end of the protracting moiety, and at its *-CO end to the epsilon amino group of a lysine residue of the peptide.

5 108. The derivative of any one of embodiments 90-106, wherein the linker consists of one time Chem. 12 and two times Chem. 11, interconnected via amide bonds and in the sequence indicated, the linker being connected at its *-NH end to the *-CO end of the protracting moiety, and at its *-CO end to the epsilon amino group of a lysine residue of the peptide.

10 109. The derivative of any one of embodiments 90-106, wherein the linker consists of one time Chem. 14, one time Chem. 12, and two times Chem. 11, interconnected via amide bonds and in the sequence indicated, the linker being connected at its *-NH end to the *-CO end of the protracting moiety, and at its *-CO end to the epsilon amino group of a lysine residue of the peptide.

15 110. The derivative of any one of embodiments 90-109, wherein the one or more linker(s) are interconnected via amide bond(s).

111. A compound selected from the following: Chem. 30, Chem. 31, Chem. 32, Chem. 33, Chem. 34, Chem. 35, Chem. 36, Chem. 37, Chem. 38, Chem. 39, and Chem. 41; or a pharmaceutically acceptable salt, amide, or ester thereof.

20 112. A compound characterised by its name, and selected from a listing of each of the names of the compounds of Examples 1-10, and 12 herein; or a pharmaceutically acceptable salt, amide, or ester thereof.

113. The compound of embodiment 112, which is a compound of embodiment 111.

114. The compound of any one of embodiments 111-113, which is a derivative of any
25 one of embodiments 74-110.

115. The peptide or derivative of any one of embodiments 1-114, which has GLP-1 activity.

116. The peptide or derivative of embodiment 115, wherein GLP-1 activity refers to the capability of activating the human GLP-1 receptor.

30 117. The peptide or derivative of embodiment 116, wherein activation of the human GLP-1 receptor is measured in an in vitro assay, as the potency of cAMP production.

118. The peptide or derivative of any one of embodiments 1-117, which has a potency corresponding to an EC_{50} at or below 4500pM, preferably below 4500pM, more preferably below 4000pM, even more preferably below 3500pM, or most preferably below 3000pM.

119. The peptide or derivative of any one of embodiments 1-118, which has a potency
5 corresponding to an EC_{50} below 2500pM, preferably below 2000pM, more preferably below 1500pM, even more preferably below 1000pM, or most preferably below 800pM.

120. The peptide or derivative of any one of embodiments 1-119 which has a potency corresponding to an EC_{50} below 600pM, preferably below 500pM, more preferably below 400pM, even more preferably below 300pM, or most preferably below 200pM.

10 121. The peptide or derivative of any one of embodiments 1-120 which has a potency corresponding to an EC_{50} below 180pM, preferably below 160pM, more preferably below 140pM, even more preferably below 120pM, or most preferably below 100pM.

122. The peptide or derivative of any one of embodiments 1-121 which has a potency corresponding to an EC_{50} below 80pM, preferably below 60pM, more preferably below 50pM,
15 even more preferably below 40pM, or most preferably below 30pM.

123. The peptide or derivative of any one of embodiments 1-122, wherein the potency is determined as EC_{50} for the dose-response curve showing dose-dependent formation of cAMP in a medium containing the human GLP-1 receptor, preferably using a stable transfected cell-line such as BHK467-12A (tk-ts13), and/or using for the determination of
20 cAMP a functional receptor assay, e.g. based on competition between endogenously formed cAMP and exogenously added biotin-labelled cAMP, in which assay cAMP is more preferably captured using a specific antibody, and/or wherein an even more preferred assay is the AlphaScreen cAMP Assay, most preferably the one described in Example 13.

124. The peptide or derivative of any one of embodiments 1-123, the EC_{50} of which is
25 less than 10 times the EC_{50} of semaglutide, preferably less than 8 times the EC_{50} of semaglutide, more preferably less than 6 times the EC_{50} of semaglutide, even more preferably less than 4 times the EC_{50} of semaglutide, or most preferably less than 2 times the EC_{50} of semaglutide.

125. The peptide or derivative of any one of embodiments 1-124, the EC_{50} of which is
30 less than the EC_{50} of semaglutide, preferably less than 0.8 times the EC_{50} of semaglutide, more preferably less than 0.6 times the potency of semaglutide, even more preferably less than 0.4 times the EC_{50} of semaglutide, or most preferably less than 0.2 times the EC_{50} of semaglutide.

126. The peptide or derivative of any one of embodiments 1-125, the EC_{50} of which is
35 less than 10 times the EC_{50} of liraglutide, preferably less than 8 times the EC_{50} of liraglutide,

more preferably less than 6 times the EC_{50} of liraglutide, even more preferably less than 4 times the EC_{50} of liraglutide, or most preferably less than 2 times the EC_{50} of liraglutide.

127. The peptide or derivative of any one of embodiments 1-126, the EC_{50} of which is less than the EC_{50} of liraglutide, preferably less than 0.8 times the EC_{50} of liraglutide, more preferably less than 0.6 times the EC_{50} of liraglutide, even more preferably less than 0.5 times the EC_{50} of liraglutide, or most preferably less than or at 0.4 times the EC_{50} of liraglutide.

128. The derivative of any one of embodiments 74-127, for which the ratio [GLP-1 receptor binding affinity (IC_{50}) in the presence of 2.0% HSA (high albumin), divided by GLP-1 receptor binding affinity (IC_{50}) in the presence of 0.005% HSA (low albumin)] is:

- a) at least 0.5, preferably at least 1.0, more preferably at least 10, even more preferably at least 20, or most preferably at least 30;
- b) at least 40, preferably at least 50, more preferably at least 60, even more preferably at least 70, or most preferably at least 80;
- 15 c) at least 90, preferably at least 100, more preferably at least 110, or most preferably at least 120;
- d) at least 20% of the ratio of semaglutide, preferably at least 50% of the ratio of semaglutide, more preferably at least 75% of the ratio of semaglutide, even more preferably at least equal to the ratio of semaglutide, or most preferably at least twice the ratio of semaglutide; or
- 20 e) at least equal to the ratio of liraglutide, preferably at least twice the ratio of liraglutide, more preferably at least three times the ratio of liraglutide, even more preferably at least 5 times the ratio of liraglutide, or most preferably at least 10 times the ratio of liraglutide.

25 129. The derivative of any one of embodiments 74-128, for which the GLP-1 receptor binding affinity (IC_{50}) in the presence of 0.005% HSA (low albumin) is

- a) below 600.00 nM, preferably below 500.00 nM, more preferably below 200.00 nM, even more preferably below 100.00 nM, or most preferably below 45.00 nM; or
- b) below 20.00 nM, preferably below 10.00 nM, more preferably below 5.00 nM, even
- 30 more preferably below 2.00 nM, or most preferably below 1.00 nM.

130. The derivative of any one of embodiments 1-129, for which the GLP-1 receptor binding affinity (IC_{50}) in the presence of 2.0% HSA (high albumin) is

- a) below 900 nM, more preferably below 800 nM, even more preferably below 700 nM, or most preferably below 600 nM; or

b) below 400.00 nM, preferably below 300.00 nM, more preferably below 200.00 nM, even more preferably below 100.00 nM, or most preferably below 50.00 nM.

131. The derivative of any one of embodiments 1-130, wherein the binding affinity to the GLP-1 receptor is measured by way of displacement of ^{125}I -GLP-1 from the receptor,

5 preferably using a SPA binding assay.

132. The derivative of any one of embodiments 1-131, wherein the GLP-1 receptor is prepared using a stable, transfected cell line, preferably a hamster cell line, more preferably a baby hamster kidney cell line, such as BHK tk-ts13.

133. The analogue or derivative of any one of embodiments 1-132, wherein the IC_{50} value is determined as the concentration which displaces 50% of ^{125}I -GLP-1 from the receptor.

134. The derivative of any one of embodiments 74-133, which has an oral bioavailability, preferably an absolute oral bioavailability, which is higher than that of liraglutide; and/or higher than that of semaglutide.

15 135. The derivative of embodiment 134, wherein oral bioavailability is measured in vivo in rats, as exposure in plasma after direct injection into the intestinal lumen.

136. The derivative of any one of embodiments 74-135, for which the plasma concentration (pM) of the derivative, determined 30 minutes after injection of a solution of the derivative in the jejunum of rat, divided by the concentration (μM) of the injected solution (dose-corrected exposure at 30 min) is at least 15, preferably at least 30, more preferably at least 48, still more preferably at least 62, even more preferably at least 80, or most preferably at least 100.

137. The derivative of any one of embodiments 74-136, for which the plasma concentration (pM) of the derivative, determined 30 minutes after injection of a solution of the derivative in the jejunum of rat, divided by the concentration (μM) of the injected solution (dose-corrected exposure at 30 min) is at least 30, preferably at least 40, more preferably at least 50, still more preferably at least 60, even more preferably at least 70, or most preferably at least 80.

138. The derivative of any one of embodiments 74-137, wherein the Derivative is tested in a concentration of 1000 μM in admixture with 55 mg/ml sodium caprate.

139. The derivative of any one of embodiments 74-138, wherein male Sprague Dawley rats are used, preferably with a body weight upon arrival of approximately 240 g.

140. The derivative of any one of embodiments 74-139, wherein the rats are fasted for approximately 18 hours before the experiment.

141. The derivative of any one of embodiments 74-140, wherein the rats are taken into general anaesthesia after having fasted and before the injection of the derivative in the jejunum.

142. The derivative of any one of embodiments 74-141, wherein the derivative is administered in the proximal part of the jejunum (10 cm distal for the duodenum) or in the mid-intestine (50 cm proximal for the cecum).

143. The derivative of any one of embodiments 74-142, wherein 100 µl of the derivative is injected into the jejunal lumen through a catheter with a 1 ml syringe, and subsequently 200 µl of air is pushed into the jejunal lumen with another syringe, which is then left connected to the catheter to prevent flow back into the catheter.

144. The derivative of any one of embodiments 74-143, wherein blood samples (200 µl) are collected into EDTA tubes from the tail vein at desired intervals, such as at times 0, 10, 30, 60, 120 and 240 min, and centrifuged 5 minutes, 10000G, at 4°C within 20 minutes.

145. The derivative of any one of embodiments 74-144, wherein plasma (75µl) is separated, immediately frozen, and kept at -20°C until analyzed for plasma concentration of the derivative.

146. The derivative of any one of embodiments 74-145, wherein LOCI (Luminescent Oxygen Channeling Immunoassay) is used for analyzing the plasma concentration of the derivative.

147. The derivative of any one of embodiments 74-145, which has a more protracted profile of action than liraglutide.

148. The derivative of embodiment 147, wherein protraction means half-life in vivo in a relevant animal species, such as db/db mice, rat, pig, and/or, preferably, minipig; wherein the derivative is administered i) s.c., and/or, preferably, ii) s.c.

149. The derivative of any one of embodiments 74-148, wherein the terminal half-life ($T_{1/2}$) after i.v. administration in minipigs is

a) at least 12 hours, preferably at least 24 hours, more preferably at least 36 hours, even more preferably at least 48 hours, or most preferably at least 60 hours; or

b) at least 0.2 times the half-life of semaglutide, preferably at least 0.4 times the half-life of semaglutide, more preferably at least 0.6 times the half-life of semaglutide, even more preferably at least 0.8 times the half-life of semaglutide, or most preferably at least the same as the half-life of semaglutide.

150. The derivative of embodiment 149, wherein the minipigs are male Göttingen minipigs.

151. The derivative of any one of embodiments 149-150, wherein the minipigs are 7-14 months of age, and preferably weighing from 16-35 kg.

152. The derivative of any one of embodiments 149-151, wherein the minipigs are housed individually, and fed once or twice daily, preferably with SDS minipig diet.

5 153. The derivative of any one of embodiments 149-152, wherein the derivative is dosed, i.v., after at least 2 weeks of acclimatisation.

154. The derivative of any one of embodiments 149-153, wherein the animals are fasted for approximately 18 h before dosing and for at least 4 h after dosing, and have ad libitum access to water during the whole period.

10 155. The derivative of any one of embodiments 149-154, wherein the Derivative is dissolved in 50 mM sodium phosphate, 145 mM sodium chloride, 0.05% tween 80, pH 7.4 to a suitable concentration, preferably from 20-60 nmol/ml.

156. The derivative of any one of embodiments 149-155, wherein intravenous injections of the derivative are given in a volume corresponding to 1-2 nmol/kg.

15 157. The derivative of any one of embodiments 74-156, which is not the compound of Example 2, preferably not Chem. 31.

158. The derivative of any one of embodiments 74-157, which is not the compound of Examples 7, 8, 9, and 12; preferably not Chem. 36, Chem. 37, Chem. 38, and Chem. 41.

20 158. A peptide according to any one of embodiments 1-73 and 115-127, for use as a medicament.

159. A derivative according to any one of embodiments 74-157, for use as a medicament.

160. A peptide according to any one of embodiments 1-73 and 115-127, for use in the treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical
25 illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

161. A derivative according to any one of embodiments 74-157, for use in the treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell
30 function, and/or for delaying or preventing diabetic disease progression.

162. Use of a peptide according to any one of embodiments 1-73 and 115-127 in the manufacture of a medicament for treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal
35 diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for

improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

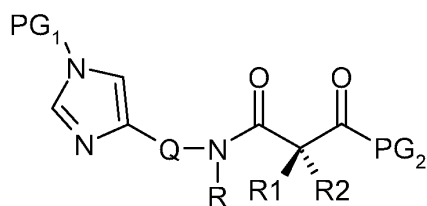
163. Use of a derivative according to any one of embodiments 74-157, in the manufacture of a medicament for treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

164. A method of treating or preventing all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression, by administering a pharmaceutically active amount of a peptide according to any one of embodiments 1-73 and 115-127.

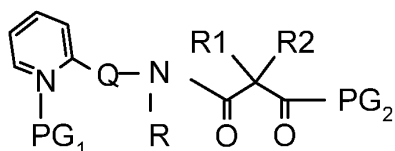
165. A method of treating or preventing all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression, by administering a pharmaceutically active amount of a derivative according to any one of embodiments 74-157.

166. An intermediate product of the formula Chem. 50 or Chem. 51:

Chem. 50:

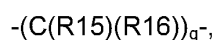


Chem. 51:



wherein Q represents a bond, or a group of formula Chem. 6:

Chem. 6:



wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl;

R represents hydrogen, or alkyl;

R1 and R2 independently represent (i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl,
5 halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or (ii) R1 and R2 together form cyclo alkyl, heterocyclyl, or heteroaryl; and each of PG₁ and PG₂ represents a protection group;

with the optional proviso (iii) that R1 and R2 do not both represent hydrogen;

10 or a pharmaceutically acceptable salt, ester, or amide thereof.

167. The intermediate product of embodiment 166, wherein PG₁ is a group that reversibly renders the N-atom to which it is attached unreactive, and that can be removed selectively.

168. The intermediate product of any one of embodiments 165-167, wherein PG₁ is selected from the group consisting of Boc, Trt, Mtt, Bzl, Tos, Clt, Mmt, Bom, and Fmoc.

15 169. The intermediate product of any one of embodiments 166-168, wherein PG₂ is a group that reversibly renders the -CO group to which it is attached unreactive, and that can be removed selectively.

170. The intermediate product of any one of embodiments 165-169, wherein PG₂ is i) -OH, or ii) functionalised as an activated ester.

20 171. The intermediate product of embodiment 170, wherein the activated ester is an ester of p-nitrophenol; 2,4,5-trichlorophenol; N-hydroxysuccinimide; N-hydroxysulfosuccinimide; 3,4-dihydro-3-hydroxy-1,2,3-benzotriazine-4-one; 5-chloro-8-hydroxyquinoline; N-hydroxy-5-norbornene-2,3-dicarboxylic acid imide; pentafluorophenol; p-sulfotetrafluorophenol; N-hydroxyphthalimide; 1-hydroxybenzotriazole; 1-hydroxy-7-azabenzotriazole; N-hydroxymaleimide; 4-hydroxy-3-nitrobenzene sulfonic acid; or any other activated ester
25 known in the art.

172. The intermediate product of any one of embodiments 165-171, wherein PG₂ is selected from OPfp, OPnp, and OSuc.

173. The intermediate product of any one of embodiments 166-172, wherein, PG₁ is Trt.

30 174. The intermediate product of any one of embodiments 166-173, wherein q is 1-5, preferably 1-4, more preferably 1-3.

175. The intermediate product of any one of embodiments 166-174, wherein R15 and R16 independently of each other and independently for each value of q represent hydrogen.

35 176. The intermediate product of any one of embodiments 166-175, wherein R15 and R16 both represent hydrogen.

177. The intermediate product of any one of embodiments 166-176, wherein Q is $-(CH_2)_n$, wherein n is 1, 2, or 3.

178. The intermediate product of any one of embodiments 166-177, wherein R is hydrogen.

5 179. The intermediate product of any one of embodiments 166-178, wherein R1 and R2 independently represent hydrogen, alkyl, aryl, halogen, hydroxyl, hydroxylalkyl, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, alkyl ester, or aryl ester; wherein preferably alkyl, hydroxylalkyl, aminoalkyl, carboxylalkyl, alkoxy, and/or alkyl ester contains lower alkyl, straight or branched, more preferably having 1-6 C-atoms.

10 180. The intermediate product of any one of embodiments 166-179, wherein R1 and R2 independently represent hydrogen, lower alkyl, or lower alkoxy, wherein the lower alkyl and lower alkoxy, independently, have 1-5 C-atoms, preferably 1-4 C-atoms, or most preferably 1-3 C-atoms.

181. The intermediate product of any one of embodiments 166-180, wherein R1 and R2
15 independently represent alkyl having 1-2 C-atoms (ethyl, or methyl), or alkoxy having 1-2 C-atoms (ethoxy, or methoxy).

182. The intermediate product of any one of embodiments 166-181, wherein R1 and R2 are methyl or methoxy, preferably methyl.

183. The intermediate product of any one of embodiments 166-182, wherein R1 and R2
20 are both methyl.

184. The intermediate product of any one of embodiments 166-183, wherein PG₂ is OH.

185. An intermediate product of formula Chem. 50 or Chem. 51:

wherein Q represents a bond, or a group of formula Chem. 6:

Chem. 6:

25 $-(C(R15)(R16))_q-$, wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl; R represents hydrogen, or alkyl; R1 and R2 independently represent (i) alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester,
30 alkyl sulfonyl, or aryl sulfonyl, or (ii) R1 and R2 together form cyclo alkyl, heterocyclyl, or heteroaryl; and each of PG₁ and PG₂ represents a protection group, preferably as defined in any one of embodiments 167-173;
or a pharmaceutically acceptable salt, amide, or ester thereof.

186. An intermediate compound selected from Chem. 23, Chem. 24, Chem. 25, Chem. 26, Chem. 27, Chem. 28, and Chem. 29; or a pharmaceutically acceptable salt, amide, or ester thereof.

187. The compound of Chem. 40; or a pharmaceutically acceptable salt, amide, or ester thereof.

188. A compound characterised by the name of the compound of Example 11 herein; or a pharmaceutically acceptable salt, amide, or ester thereof.

189. The compound of embodiment 188, which is a compound of embodiment 187.

190. A peptide intermediate product, which is selected from the following analogues of GLP-1(9-37) (SEQ ID NO: 1): (i) (18K, 22E, 34Q); (ii) (30E, 36K, 38E); (iii) (31H, 34Q); (iv) 34R; (v) (34R, 37K); and (vi) (34R, 37K, 38E); or a pharmaceutically acceptable salt, amide, or ester thereof.

191. A peptide intermediate product, which is the following analogue of GLP-1A(3-37) (SEQ ID NO: 3): (17R, 20R, 33R, 38K); or a pharmaceutically acceptable salt, amide, or ester thereof.

EXAMPLES

This experimental part starts with a list of abbreviations, and is followed by a section including general methods for synthesising and characterising peptides and derivatives of the invention. Then follows a number of examples which relate to the preparation of specific GLP-1 peptide derivatives, and at the end a number of examples have been included relating to the activity and properties of these peptides and derivatives (section headed pharmacological methods).

The examples serve to illustrate the invention.

Abbreviations

The following abbreviations are used in the following, in alphabetical order:

Aib:	aminoisobutyric acid (α -aminoisobutyric acid)
API:	Active Pharmaceutical Ingredient
AUC:	Area Under the Curve
BG:	Blood Glucose
BHK	Baby Hamster Kidney
Boc:	<i>t</i> -butyloxycarbonyl
Bom:	benzyloxymethyl

	BW:	Body Weight
	Bzl:	benzyl
	Clt:	2-chlorotrityl
	collidine:	2,4,6-trimethylpyridine
5	cpm:	counts per minute
	DCM:	dichloromethane
	Dde:	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl
	DIC:	diisopropylcarbodiimide
	DIPEA:	diisopropylethylamine
10	DMAP:	4-dimethylaminopyridine
	DMEM:	Dulbecco's Modified Eagle's Medium (DMEM)
	EDTA:	ethylenediaminetetraacetic acid
	EGTA:	ethylene glycol tetraacetic acid
	FCS:	Fetal Calf Serum
15	Fmoc:	9-fluorenylmethyloxycarbonyl
	HATU:	(O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate)
	HBTU:	(2-(1H-benzotriazol-1-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate)
	HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
20	HFIP	1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroisopropanol
	HOAt:	1-hydroxy-7-azabenzotriazole
	HOBt:	1-hydroxybenzotriazole
	HPLC:	High Performance Liquid Chromatography
	HSA:	Human Serum Albumin
25	IBMX:	3-isobutyl-1-methylxanthine
	Imp:	Imidazopropionic acid (also referred to as des-amino histidine, DesH)
	i.v.	intravenously
	ivDde:	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
	IVGTT:	Intravenous Glucose Tolerance Test
30	LCMS:	Liquid Chromatography Mass Spectroscopy
	LYD:	Landrace Yorkshire Duroc
	MALDI-MS:	See MALDI-TOF MS
	MALDI-TOF MS:	Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectroscopy
35	MeOH:	methanol

	Mmt:	4-methoxytrityl
	Mtt:	4-methyltrityl
	NMP:	N-methyl pyrrolidone
	OBz	benzoyl ester
5	OEG:	8-amino-3,6-dioxaoctanic acid
	OPfp:	pentafluorophenoxy
	OPnp:	para-nitrophenoxy
	OSu:	O-succinimidyl esters (hydroxysuccinimide esters)
	OSuc:	2,5-dioxo-pyrrolidin-1-yl
10	OtBu:	tert butyl ester
	PBS:	Phosphate Buffered Saline
	PD:	Pharmacodynamic
	Pen/Strep:	Pencillin/Streptomycin
	PK:	Pharmacokinetic
15	RP:	Reverse Phase
	RP-HPLC:	Reverse Phase High Performance Liquid Chromatography
	RT:	Room Temperature
	Rt:	Retention time
	s.c.:	Subcutaneously
20	SD:	Standard Deviation
	SEC-HPLC:	Size Exclusion High Performance Liquid Chromatography
	SPPS:	Solid Phase Peptide Synthesis
	tBu:	tert. butyl
	TFA:	trifluoroacetic acid
25	TIS:	triisopropylsilane
	TLC:	Thin Layer Chromatography
	Tos:	tosylate (or para-toluenesulfonyl)
	Tris:	tris(hydroxymethyl)aminomethane or 2-amino-2-hydroxymethyl-propane-1,3-diol
30	Trt:	triphenylmethyl, or trityl
	Trx:	tranexamic acid
	UPLC:	Ultra Performance Liquid Chromatography

METHODS OF PREPARATION

A. General methods

This section relates to methods for solid phase peptide synthesis (SPPS methods, including methods for de-protection of amino acids, methods for cleaving the peptide from the resin, and for its purification), as well as methods for detecting and characterising the resulting peptide (LCMS, MALDI, and UPLC methods). The solid phase synthesis of peptides may in some cases be improved by the use of di-peptides protected on the di-peptide amide bond with a group that can be cleaved under acidic conditions such as, but not limited to, 2-Fmoc-oxy-4-methoxybenzyl, or 2,4,6-trimethoxybenzyl. In cases where a serine or a threonine is present in the peptide, pseudoproline di-peptides may be used (available from, e.g., Novabiochem, see also W.R. Sampson (1999), J. Pep. Sci. 5, 403). The protected amino acid derivatives used were standard Fmoc-amino acids (supplied from e.g. Anaspec, IRIS, or Novabiochem). The N-terminal amino acid was Boc protected at the alpha amino group (e.g. Boc-His(Boc)-OH, or Boc-His(Trt)-OH for peptides with His at the N-terminus). The epsilon amino group of lysines in the sequence were either protected with Mtt, Mmt, Dde, ivDde, or Boc, depending on the route for attachment of the albumin binding moiety and spacer. The albumin binding moiety and/or linker can be attached to the peptide either by acylation of the resin bound peptide or by acylation in solution of the unprotected peptide. In case of attachment of the albumin binding moiety and/or linker to the protected peptidyl resin, the attachment can be modular using SPPS and suitably protected building blocks such as but not limited to Fmoc-OEG-OH (Fmoc-8-amino-3,6-dioxaoctanoic acid), Fmoc-Trx-OH (Fmoc-tranexamic acid), Fmoc-Glu-OtBu, octadecanedioic acid mono-tert-butyl ester, nonadecanedioic acid mono-tert-butyl ester, or 4-(9-carboxynonyloxy) benzoic acid tert-butyl ester.

1. Synthesis of resin bound peptide

SPPS method A

SPPS method A refers to the synthesis of a protected peptidyl resin using Fmoc chemistry on an Applied Biosystems 433 peptide synthesiser (also designated ABI433A synthesiser) in 0.25 mmol or 1.0 mmol scale using the manufacturer's FastMoc UV protocols which employ HBTU or HATU mediated couplings in NMP, and UV monitoring of the de-protection of the Fmoc protection group.

The starting resin used for the synthesis of peptide amides was a suitable Rink-Amide resin (for peptide amides), or (for peptides with a carboxy C-terminus) either a suitable Wang resin or a suitable chlorotrityl resin. Suitable resins are commercially available from, e.g., Novabiochem.

SPPS method B

SPPS method B refers to the synthesis of a protected peptidyl resin using Fmoc chemistry on a microwave-based Liberty peptide synthesiser (CEM Corp., North Carolina). A suitable resin is a pre-loaded, low-load Wang resin available from Novabiochem (e.g. low load Fmoc-Lys(Mtt)-Wang resin, 0.35 mmol/g). Fmoc-deprotection was with 5% piperidine in NMP at up to 70 or 75°C. The coupling chemistry was DIC/HOAt in NMP. Amino acid/HOAt solutions (0.3 M in NMP at a molar excess of 3-10 fold) were added to the resin followed by the same molar equivalent of DIC (0.75M in NMP). For example, the following amounts of 0.3M amino acid/HOAt solution were used per coupling for the following scale reactions: Scale/ml, 0.10 mmol/2.5 ml, 0.25 mmol/5 ml, 1 mmol/15 ml. Coupling times and temperatures were generally 5 minutes at up to 70 or 75°C. Longer coupling times were used for larger scale reactions, for example 10 min. Histidine amino acids were double coupled at 50°C, or quadruple coupled if the previous amino acid was sterically hindered (e.g. Aib). Arginine amino acids were coupled at RT for 25 min then heated to 70 or 75°C for 5 min. Some amino acids such as but not limited to Aib, were "double coupled", meaning that after the first coupling (e.g. 5 min at 75°C), the resin is drained and more reagents are added (amino acid, HOAt and DIC), and the mixture is heated again (e.g. 5 min at 75°C). When a chemical modification of a lysine side chain was desired, the lysine was incorporated as Lys(Mtt). The Mtt group was removed by washing the resin with DCM and suspending the resin in neat (undiluted) hexafluoroisopropanol for 20 minutes followed by washing with DCM and NMP. The chemical modification of the lysine was performed either by manual synthesis (see SPPS method D) or by one or more automated steps on the Liberty peptide synthesiser as described above, using suitably protected building blocks (see General methods), optionally including a manual coupling.

SPPS method D

SPPS method D refers to synthesis of the protected peptidyl resin using manual Fmoc chemistry. This was typically used for the attachment of the linkers and side chains to the peptide backbone. The following conditions were employed at 0.25 mmol synthesis scale. The coupling chemistry was DIC/HOAt/collidine in NMP at a 4-10 fold molar excess. Coupling conditions were 1-6 h at room temperature. Fmoc-deprotection was performed with 20-25% piperidine in NMP (3 x 20 ml, each 10 min) followed by NMP washings (4 x 20 mL). Dde- or ivDde-deprotection was performed with 2% hydrazine in NMP (2 x 20 ml, each 10 min) followed by NMP washings (4 x 20 ml). Mtt- or Mmt-deprotection was performed with 2% TFA and 2-3% TIS in DCM (5 x 20 ml, each 10 min) followed by DCM (2 x 20 ml), 10% MeOH and 5% DIPEA in DCM (2 x 20 ml) and NMP (4 x 20 ml) washings, or by treatment with neat hexafluoroisopropanol (5 x 20 ml, each 10 min) followed by washings as above. The

albumin binding moiety and/or linker can be attached to the peptide either by acylation of the resin bound peptide or acylation in solution of the unprotected peptide (see the routes described below). In case of attachment of the albumin binding moiety and/or linker to the protected peptidyl resin the attachment can be modular using SPPS and suitably protected building blocks (see General methods).

Attachment to resin bound peptide - Route I: Activated (active ester or symmetric anhydride) albumin binding moiety or linker such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.* EP511600, 4 molar equivalents relative to resin bound peptide) was dissolved in NMP (25 mL), added to the resin and shaken overnight at room temperature. The reaction mixture was filtered and the resin was washed extensively with NMP, DCM, 2-propanol, methanol and diethyl ether.

Attachment to resin bound peptide - Route II: The albumin binding moiety was dissolved in NMP/DCM (1:1, 10 ml). The activating reagent such as HOBt (4 molar equivalents relative to resin) and DIC (4 molar equivalents relative to resin) was added and the solution was stirred for 15 min. The solution was added to the resin and DIPEA (4 molar equivalents relative to resin) was added. The resin was shaken 2 to 24 hours at room temperature. The resin was washed with NMP (2 x 20 ml), NMP/DCM (1:1, 2 x 20ml) and DCM (2 x 20 ml).

Attachment to peptide in solution - Route III: Activated (active ester or symmetric anhydride) albumin binding moiety or linker such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.* EP511600) 1-1.5 molar equivalents relative to the peptide was dissolved in an organic solvent such as acetonitrile, THF, DMF, DMSO or in a mixture of water/organic solvent (1-2 ml) and added to a solution of the peptide in water (10-20ml) together with 10 molar equivalents of DIPEA. In case of protecting groups on the albumin binding residue such as *tert*-butyl, the reaction mixture was lyophilised overnight and the isolated crude peptide deprotected afterwards. In case of *tert*-butyl protection groups the deprotection was performed by dissolving the peptide in a mixture of trifluoroacetic acid, water and triisopropylsilane (90:5:5). After for 30min the mixture was evaporated *in vacuo* and the crude peptide purified by preparative HPLC as described later.

SPPS method E

SPPS method E refers to peptide synthesis by Fmoc chemistry on a Prelude Solid Phase Peptide Synthesiser from Protein Technologies (Tucson, AZ 85714 U.S.A.). A suitable resin is a pre-loaded, low-load Wang resin available from Novabiochem (e.g. low load fmoc-Lys(Mtt)-Wang resin, 0.35 mmol/g). Fmoc-deprotection was with 25% piperidine in NMP for 2 x 10 min. The coupling chemistry was DIC/HOAt/collidine in NMP. Amino acid/HOAt

solutions (0.3 M in NMP at a molar excess of 3-10 fold) were added to the resin followed by the same molar equivalent of DIC (3 M in NMP) and collidine (3 M in NMP). For example, the following amounts of 0.3M amino acid/HOAt solution were used per coupling for the following scale reactions: Scale/ml, 0.10 mmol/2.5 ml, 0.25 mmol/5 ml. Coupling times were generally 5 60 minutes. Some amino acids including, but not limited to arginine, Aib or histidine were "double coupled", meaning that after the first coupling (e.g. 60 min), the resin is drained and more reagents are added (amino acid, HOAt, DIC, and collidine), and the mixture allowed to react again (e.g. 60 min). Some amino acids and fatty acid derivatives including but not limited to Fmoc-Oeg-OH, Fmoc-Trx-OH, Fmoc-Glu-OtBu, octadecanedioic acid mono-tert-butyl 10 ester, nonadecanedioic acid mono-tert-butyl ester, or 4-(9-carboxynonyloxy) benzoic acid tert-butyl ester were coupled for prolonged time, for example 6 hours. When a chemical modification of a lysine side chain was desired, the lysine was incorporated as Lys(Mtt). The Mtt group was removed by washing the resin with DCM and suspending the resin in hexafluoroisopropanol/DCM (75:25) for 3 x 10 minutes followed by washings with DCM, 20% 15 piperidine and NMP. The chemical modification of the lysine was performed either by manual synthesis (see SPPS method D) or by one or more automated steps on the Prelude peptide synthesiser as described above using suitably protected building blocks (see General methods).

20 2. Cleavage of peptide from the resin and purification

After synthesis the resin was washed with DCM, and the peptide was cleaved from the resin by a 2-3 hour treatment with TFA/TIS/water (95/2.5/2.5 or 92.5/5/2.5) followed by precipitation with diethylether. The peptide was dissolved in a suitable solvent (such as, e.g., 30% acetic acid) and purified by standard RP-HPLC on a C18, 5µM column, using 25 acetonitrile/water/TFA. The fractions were analysed by a combination of UPLC, MALDI and LCMS methods, and the appropriate fractions were pooled and lyophilised.

3. Methods for detection and characterisation

LCMS methods

30 LCMS Method 1 (LCMS1)

An Agilent Technologies LC/MSD TOF (G1969A) mass spectrometer was used to identify the mass of the sample after elution from an Agilent 1200 series HPLC system. The de-convolution of the protein spectra was calculated with Agilent's protein confirmation software.

35 Eluents:

A: 0.1% Trifluoro acetic acid in water

B: 0.1% Trifluoro acetic acid in acetonitrile

Column: Zorbax 5u, 300SB-C3, 4.8x50mm

5 Gradient: 25% - 95 % acetonitrile over 15 min

LCMS Method 2 (LCMS2)

A Perkin Elmer Sciex API 3000 mass spectrometer was used to identify the mass of the sample after elution from a Perkin Elmer Series 200 HPLC system.

10 Eluents:

A: 0.05% Trifluoro acetic acid in water

B: 0.05% Trifluoro acetic acid in acetonitrile

Column: Waters Xterra MS C-18 X 3 mm id 5 µm

15 Gradient: 5% - 90 % acetonitrile over 7.5 min at 1.5ml/min

LCMS Method 3 (LCMS3)

A Waters Micromass ZQ mass spectrometer was used to identify the mass of the sample after elution from a Waters Alliance HT HPLC system.

20 Eluents:

A: 0.1% Trifluoro acetic acid in water

B: 0.1% Trifluoro acetic acid in acetonitrile

Column: Phenomenex, Jupiter C4 50 X 4.60 mm id 5 µm

25 Gradient: 10% - 90% B over 7.5 min at 1.0 ml/min

LCMS Method 4 (LCMS4)

LCMS4 was performed on a setup consisting of Waters Acquity UPLC system and LCT Premier XE mass spectrometer from Micromass. The UPLC pump was connected to two eluent reservoirs containing:

30

A: 0.1% Formic acid in water

B: 0.1% Formic acid in acetonitrile

The analysis was performed at RT by injecting an appropriate volume of the sample (preferably 2-10µl) onto the column which was eluted with a gradient of A and B.

35 The UPLC conditions, detector settings and mass spectrometer settings were:

Column: Waters Acquity UPLC BEH, C-18, 1.7µm, 2.1mm x 50mm

Gradient: Linear 5% - 95% acetonitrile during 4.0 min (alternatively 8.0 min) at 0.4ml/min

Detection: 214 nm (analogue output from TUV (Tunable UV detector))

MS ionisation mode: API-ES

- 5 Scan: 100-2000 amu (alternatively 500-2000 amu), step 0.1 amu

UPLC and HPLC methods

Method 05_B5_1

- UPLC (method 05_B5_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C. The UPLC system was connected to two eluent reservoirs containing:
- A: 0.2 M Na₂SO₄, 0.04 M H₃PO₄, 10 % CH₃CN (pH 3.5)
B: 70 % CH₃CN, 30 % H₂O
- 15 The following linear gradient was used: 60 % A, 40 % B to 30 % A, 70 % B over 8 minutes at a flow-rate of 0.40 ml/min.

Method 05_B7_1

- UPLC (method 05_B7_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C. The UPLC system was connected to two eluent reservoirs containing:
- A: 0.2 M Na₂SO₄, 0.04 M H₃PO₄, 10 % CH₃CN (pH 3.5)
B: 70 % CH₃CN, 30 % H₂O
- 25 The following linear gradient was used: 80 % A, 20 % B to 40 % A, 60 % B over 8 minutes at a flow-rate of 0.40 ml/min.

Method 04_A2_1

- UPLC (method 04_A2_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C. The UPLC system was connected to two eluent reservoirs containing:
- A: 90 % H₂O, 10 % CH₃CN, 0.25 M ammonium bicarbonate
B: 70 % CH₃CN, 30 % H₂O
- 30

The following linear gradient was used: 90 % A, 10 % B to 60 % A, 40 % B over 16 minutes at a flow-rate of 0.40 ml/min.

Method 04_A3_1

5 UPLC (method 04_A3_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C.

The UPLC system was connected to two eluent reservoirs containing:

A: 90 % H₂O, 10 % CH₃CN, 0.25 M ammonium bicarbonate

10 B: 70 % CH₃CN, 30 % H₂O

The following linear gradient was used: 75 % A, 25 % B to 45 % A, 55 % B over 16 minutes at a flow-rate of 0.40 ml/min.

Method 04_A4_1

15 UPLC (method 04_A4_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40 °C.

The UPLC system was connected to two eluent reservoirs containing:

A: 90 % H₂O, 10 % CH₃CN, 0.25 M ammonium bicarbonate

20 B: 70 % CH₃CN, 30 % H₂O

The following linear gradient was used: 65 % A, 35 % B to 25 % A, 65 % B over 16 minutes at a flow-rate of 0.40 ml/min.

Method 08_B2_1

25 UPLC (method 08_B2_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C.

The UPLC system was connected to two eluent reservoirs containing:

A: 99.95 %H₂O, 0.05 % TFA

30 B: 99.95 % CH₃CN, 0.05 % TFA

The following linear gradient was used: 95 % A, 5 % B to 40 % A, 60 % B over 16 minutes at a flow-rate of 0.40 ml/min.

Method 08_B4_1

UPLC (method 08_B4_1): The RP-analysis was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C.

The UPLC system was connected to two eluent reservoirs containing:

5 A: 99.95 %H₂O, 0.05 % TFA

B: 99.95 % CH₃CN, 0.05 % TFA

The following linear gradient was used: 95 % A, 5 % B to 95 % A, 5 % B over 16 minutes at a flow-rate of 0.40 ml/min.

10 Method 05_B10_1

UPLC (Method 05_B10_1): The RP-analyses was performed using a Waters UPLC system fitted with a dual band detector. UV detections at 214nm and 254nm were collected using an ACQUITY UPLC BEH130, C18, 130Å, 1.7µm, 2.1 mm x 150 mm column, 40°C.

The UPLC system was connected to two eluent reservoirs containing:

15 A: 0.2 M Na₂SO₄, 0.04 M H₃PO₄, 10 % CH₃CN (pH 3.5)

B: 70 % CH₃CN, 30 % H₂O

The following linear gradient was used: 40 % A, 60 % B to 20 % A, 80 % B over 8 minutes at a flow-rate of 0.40 ml/min.

20 Method 02_B4_4

HPLC (Method 02_B4_4): The RP-analysis was performed using a Alliance Waters 2695 system fitted with a Waters 2487 dualband detector. UV detections at 214nm and 254nm were collected using a Symmetry300 C18 , 5 µm, 3.9 mm x 150 mm column, 42°C. Eluted with a linear gradient of 5-95% acetonitrile, 90-0% water, and 5% trifluoroacetic acid (1.0%) in water over 15 minutes at a flow-rate of 1.0 ml/min.

Method 01_B4_1

30 HPLC (Method 01_B4_1): The RP-analysis was performed using a Waters 600S system fitted with a Waters 996 diode array detector. UV detections were collected using a Waters 3 mm x 150 mm 3.5µm C-18 Symmetry column. The column was heated to 42°C and eluted with a linear gradient of 5-95% acetonitrile, 90-0% water, and 5% trifluoroacetic acid (1.0%) in water over 15 minutes at a flow-rate of 1 ml/min.

35 **MALDI-MS method**

Molecular weights were determined using matrix-assisted laser desorption and ionisation time-of-flight mass spectroscopy, recorded on a Microflex or Autoflex (Bruker). A matrix of alpha-cyano-4-hydroxy cinnamic acid was used.

5 NMR method

Proton NMR spectra were recorded using a Bruker Avance DPX 300 (300 MHz) with tetramethylsilane as an internal standard. Chemical shifts (δ) are given in ppm and splitting patterns are designated as follows: s, singlet; d, doublet; dd, double doublet; dt, double triplet; t, triplet; tt, triplet of triplets; q, quartet; quint, quintet; sext, sextet; m, multiplet, and br = broad.

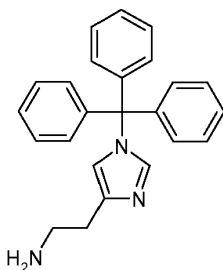
B. Synthesis of intermediates

1. Synthesis of mono esters of fatty diacids

Overnight reflux of the C12, C14, C16 and C18 diacids with Boc-anhydride, DMAP, and *t*-butanol in toluene gives predominately the *t*-butyl mono ester. Obtained is after work-up a mixture of mono acid, diacid and diester. Purification is carried out by washing, short plug silica filtration and crystallisation.

2. Synthesis of 2-(1-Trityl-1H-imidazol-4-yl)-ethyl amine

Chem. 23:



Histamine dihydrochloride (20.47 g; 0.111 mol) and triethylamine (48 mL; 0.345 mol) in absolute methanol (400 mL) were stirred at room temperature for 10 min. Trifluoroacetic acid ethyl ester (14.6 mL; 0.122 mol) in methanol (30 mL) was added dropwise over 30 min at 0 °C. Reaction mixture was stirred for 3.5 hrs at room temperature and then it was evaporated to dryness *in vacuo*. The residue was dissolved in dichloromethane (450 mL) and triethylamine (31 mL; 0.222 mol) was added. Then trityl chloride (34.1 g; 0.122 mol) was added piecewise and mixture was stirred over night at room temperature. Chloroform (400 mL) and water (600 mL) were poured into reaction mixture. Aqueous layer was separated and extracted with chloroform (3 x 400 mL). The combined organic layers were

dried over anhydrous magnesium sulfate. Solvent was removed and the beige solid was triturated with hexanes (1000 mL). Suspension was filtered to yield 2,2,2-trifluoro-N-[2-(1-trityl-1H-imidazol-4-yl)-ethyl]-acetamide as white solid.

Yield: 45.54 g (91 %).

- 5 ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 8.44 (bs, 1 H); 7.43 (s, 1 H); 7.41 - 7.33 (m, 9 H); 7.19 - 7.10 (m, 6 H); 6.65 (s, 1 H); 3.66 (q, J=5.9 Hz, 2 H); 2.79 (t, J=5.9 Hz, 2 H).

The above amide (45.54 g; 0.101 mmol) was dissolved in tetrahydrofuran (1000 mL) and methanol (1200 mL). A solution of sodium hydroxide (20.26 g; 0.507 mol) in water (500 mL) was added. Mixture was stirred for 2 hrs at room temperature and then it was
10 concentrated *in vacuo*. The residue was separated between chloroform (1200 mL) and water (800 mL). Aqueous layer was extracted with chloroform (3 x 400 mL). Organic layers were combined and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded brown oil, which was dried for 3 days *in vacuo* to give the title product as beige solid.

Yield: 32.23 g (90 %).

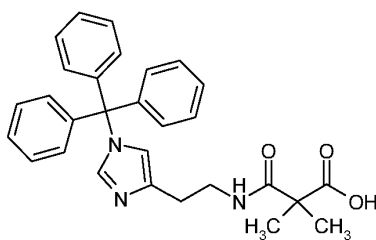
- 15 Overall yield: 82 %.

M.p.: 111-113 °C.

¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.39 (d, J=1.3, 1 H); 7.38 - 7.32 (m, 9 H); 7.20 - 7.12 (m, 6 H); 6.61 (s, 1 H); 3.00 (t, J=6.6 Hz, 2 H); 2.70 (t, J=6.5 Hz, 2 H); 1.93 (bs, 2 H).

20 **3. Synthesis of 2,2-Dimethyl-N-[2-(1-trityl-1H-imidazol-4-yl)-ethyl]-malonamic acid**

Chem. 24:



- A mixture of Meldrum's acid (5.52 g, 38.3 mmol), potassium carbonate (26.5 g, 191 mmol) and methyl iodide (7.15 mL, 115 mmol) in acetonitrile (75 mL) was heated at 75 °C in
25 a sealed tube for 7 hrs. The mixture was cooled to room temperature, diluted with dichloromethane (300 mL), filtered and the filtrate evaporated to dryness *in vacuo*. Ethyl acetate (75 mL), hexanes (75 mL) and water (50 mL) were added and phases were separated. The organic layer was washed with 10% aqueous solution of sodium thiosulfate (50 mL) and water (50 mL); dried over anhydrous magnesium sulfate and solvent removed in
30 *vacuo* to give 2,2,5,5-tetramethyl-[1,3]dioxane-4,6-dione as white solid.

Yield: 6.59 g (79%).

R_F (SiO₂, chloroform/ethyl acetate, 98:2): 0.60.

¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 1.76 (s, 6 H); 1.65 (s, 6 H).

A solution of 2-(1-Trityl-1H-imidazol-4-yl)-ethyl amine (5.00 g, 14.2 mmol) prepared
5 as described above and triethylamine (9.86 mL, 70.7 mmol) in toluene (80 mL) was added
dropwise over 50 min to a solution of the above dione compound (3.65 g, 21.2 mmol) in
toluene (40 mL) at 75 °C. The mixture was stirred at this temperature for additional 3 hrs
(until the starting amine was detected on TLC), then it was evaporated to dryness. The
residue was redissolved in chloroform (300 mL) and washed with 10% aqueous solution of
10 citric acid (200 mL). The aqueous phase was extracted with chloroform (2 x 60 mL); the
chloroform phases were combined, dried over anhydrous magnesium sulfate and solvent
removed *in vacuo*. The residue was triturated with hot chloroform (140 mL); hexanes (70 mL)
were added and the suspension was stirred at room temperature overnight. Solids were
filtered off, washed with chloroform/hexanes mixture (1:1, 2 x 50 mL) and dried *in vacuo* to
15 give the title product.

Yield: 6.73 g (88%).

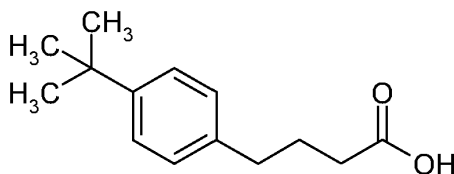
M.p.: 161-162 °C.

R_F (SiO₂, chloroform/methanol, 85:15): 0.40.

¹H NMR spectrum (300 MHz, DMSO-d₆, δ_H): 12.45 (bs, 1 H); 7.66 (t, J=5.1 Hz, 1 H); 7.57-
20 7.31 (m, 9 H); 7.26 (s, 1 H); 7.20-7.02 (m, 6 H); 6.66 (s, 1 H); 3.25 (m, 2 H); 2.57 (t, J=7.3 Hz,
2 H); 1.21 (s, 6 H).

4. Synthesis of 4-(4-*tert*-Butyl-phenyl)-butyric acid

Chem. 25:



25

Aluminum chloride powder (80.0 g, 600 mmol) was added in portions to a stirred
mixture of *tert*-butylbenzene (40.0 g, 300 mmol) and succinic anhydride (26.7 g, 267 mmol)
and 1,1,2,2-tetrachloroethane (100 mL). After all the aluminum chloride had been added, the
mixture was poured into a mixture of ice (500 mL) and concentrated hydrochloric acid (100
30 mL). The organic layer was separated, washed with water (500 mL) and the solvent distilled
off. Solid residue was dissolved in hot 15% aqueous solution of sodium carbonate (1000
mL), filtered, cooled and the acid was precipitated with hydrochloric acid (acidified to pH=1).

The crude acid was filtered, dried on air and recrystallised from benzene (500 mL) to give 4-(4-*tert*-butyl-phenyl)-4-oxo-butiric acid as colorless crystals.

Yield: 36.00 g (58%).

M.p.: 117-120 °C.

- 5 ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.93 (dm, J=8.3 Hz, 2 H); 7.48 (dm, J=8.3 Hz, 2 H); 3.30 (t, J=6.6 Hz, 2 H); 2.81 (t, J=6.6 Hz, 2 H); 1.34 (s, 9 H).

A mixture of the above acid (36.0 g, 154 mmol), potassium hydroxide (25.8 g, 462 mmol), hydrazine hydrate (20 mL, 400 mmol) and ethylene glycol (135 mL) was refluxed for 3 hrs, and then distilled until the temperature of the vapor had risen to 196-198 °C. After a further 14 hrs reflux, the mixture was allowed to cool slightly, and was then poured into cold water (200 mL). The mixture was acidified with concentrated hydrochloric acid (to pH=1) and extracted with dichloromethane (2 x 400 mL). The organic extracts were combined, dried over anhydrous magnesium sulfate, solvent removed *in vacuo* and the residue was purified by column chromatography (Silicagel 60A, 0.060–0.200 mm; eluent: hexanes/ethyl acetate 10:1-6:1) to give the title product as off white solid.

Yield: 16.25 g (48%).

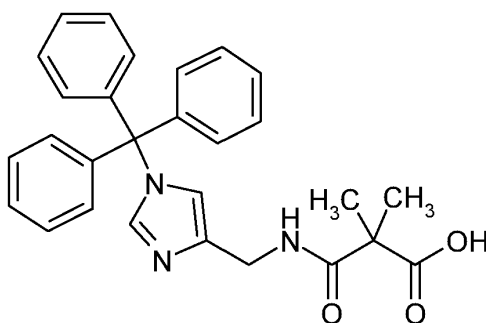
M.p.: 59-60 °C.

R_F (SiO₂, ethyl acetate): 0.60.

- ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.31 (dm, J=8.1 Hz, 2 H); 7.12 (dm, J=8.1 Hz, 2 H); 2.64 (t, J=7.6 Hz, 2 H); 2.38 (t, J=7.4 Hz, 2 H); 1.96 (m, 2 H); 1.31 (s, 9 H).

5. Synthesis of 2,2-Dimethyl-N-(1-trityl-1H-imidazol-4-ylmethyl)-malonamic acid

Chem. 26:



- 25 Hydroxylamine hydrochloride (15.9 g, 229 mmol) was added to a solution of 4(5)-imidazolecarboxaldehyde (20.0 g, 209 mmol) and sodium carbonate (12.1 g, 114 mmol) in water (400 mL) and the resulting solution was stirred at room temperature overnight. The mixture was evaporated to 100 mL and cooled in an ice bath. The solids were separated by

filtration and the filtrate was concentrated to 40 mL. After cooling to 0 °C, another portion of crystals was obtained. The solids (23 g) were combined and recrystallised from ethanol (approx. 160 mL) to afford imidazole-4(5)-carbaldehyde oxime as colorless crystals.

Yield: 15.98 g (69%).

- 5 ¹H NMR spectrum (300 MHz, acetone-d₃+D₂O, δ_H): 7.78 (bs, 1 H); 7.74 (d, J=0.9 Hz, 1 H); 7.43 (s, 1 H).

Acetyl chloride (51.0 mL, 718 mmol) was added dropwise to methanol (670 mL) at 0 °C under argon. After 30 min, the cooling bath was removed and the above oxime (16.0 g, 144 mmol) was added, followed by palladium on carbon (5 wt%, 6.1 g). The mixture was
10 hydrogenated at atmospheric pressure for 17 hrs, then it was filtered through Celite and the solvent evaporated to give pure 4-(aminomethyl)-imidazole dihydrochloride as colorless crystals.

Yield: 23.92 g (98%).

¹H NMR spectrum (300 MHz, D₂O, δ_H): 8.72 (s, 1 H); 7.60 (s, 1 H); 4.33 (s, 2 H).

- 15 The above amine dihydrochloride (18.9 g; 111 mmol) and triethylamine (93 mL; 667 mmol) in methanol (1000 mL) were stirred at room temperature for 10 min. Trifluoroacetic acid ethyl ester (13.3 mL; 111 mmol) in methanol (30 mL) was added dropwise over 40 min at 0 °C. Reaction mixture was stirred for 18 hrs at room temperature and then it was evaporated to dryness *in vacuo*. The residue was dissolved in dry dichloromethane (2000 mL) and
20 triethylamine (31 mL; 222 mmol) was added. Then trityl chloride (31.6 g; 113 mmol) was added and the mixture was stirred overnight at room temperature. Chloroform (1000 mL) and water (1000 mL) were poured into the reaction mixture. Aqueous layer was separated and extracted with chloroform (2 x 300 mL). The combined organic layers were dried over anhydrous magnesium sulfate. Solvent was removed and the beige solid was triturated with
25 hexanes (1000 mL). Suspension was filtered to yield 2,2,2-trifluoro-N-(1-trityl-1H-imidazol-4-ylmethyl)-acetamide as white solid.

Yield: 46.59 g (96%).

R_F (SiO₂, dichloromethane/methanol 95:5): 0.35.

- ¹H NMR spectrum (300 MHz, DMSO-d₆, δ_H): 9.77 (t, J=5.7 Hz, 1 H); 7.47-7.34 (m, 9 H); 7.33
30 (d, J=1.5 Hz, 1 H); 7.13-7.03 (m, 6 H); 6.80 (d, J=0.8 Hz, 1 H); 4.25 (d, J=5.7 Hz, 2 H).

The above amide (46.6 g; 107 mmol) was dissolved in tetrahydrofuran (600 mL) and ethanol (310 mL). A solution of sodium hydroxide (21.4 g; 535 mmol) in water (85 mL) was added. Mixture was stirred for 5 hrs at room temperature and then it was concentrated *in vacuo*. The residue was separated between chloroform (1600 mL) and water (800 mL). Aqueous layer
35 was extracted with chloroform (4 x 200 mL). Organic layers were combined and dried over

anhydrous magnesium sulfate. Evaporation of the solvent yielded (1-trityl-1*H*-imidazol-4-yl)-methylamine as off white solid.

Yield: 36.30 g (100%).

¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.38 (d, J=1.3, 1 H); 7.36-7.30 (m, 9 H); 7.18-7.10 (m, 6 H); 6.69 (m, 1 H); 3.77 (s, 2 H); 1.80 (bs, 2 H).

A solution of the above amine (10.0 g, 29.5 mmol) and triethylamine (20.5 mL, 147 mmol) in toluene (220 mL) was added dropwise over 45 min to a solution of 2,2,5,5-tetramethyl-[1,3]dioxane-4,6-dione (3.65 g, 21.2 mmol) in toluene (80 mL) at 75 °C. The mixture was stirred at this temperature for additional 3 hrs (until the starting amine was detected on TLC), then it was evaporated to dryness. The residue was redissolved in chloroform (500 mL) and washed with 10% aqueous solution of citric acid (300 mL). The aqueous phase was extracted with chloroform (100 mL); the chloroform phases were combined, washed with water (150 mL) dried over anhydrous magnesium sulfate and solvent removed *in vacuo*. The residue was purified by flash column chromatography (silica gel Fluka 60, dichloromethane/methanol 98:2 to 9:1) and crystallised from chloroform/hexanes mixture to give the title product as beige crystals.

Yield: 9.80 g (73%).

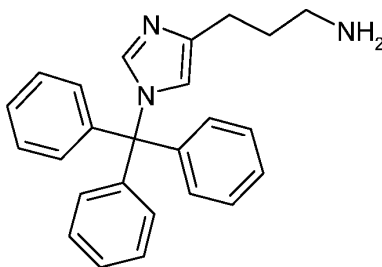
M.p.: 174-175 °C.

R_F (SiO₂, chloroform/methanol, 85:15): 0.35.

¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 8.45 (t, J=5.8 Hz, 1 H); 7.53 (s, 1 H); 7.40-7.28 (m, 9 H); 7.14-7.01 (m, 6 H); 6.84 (s, 1 H); 4.39 (d, J=5.8 Hz, 2 H); 1.44 (s, 6 H).

6. Synthesis of 3-(1-Trityl-1*H*-imidazol-4-yl)-propyl amine

Chem. 27:



Ethyl 3-(1-trityl-4-imidazolyl)propionate (93.0 g, 223 mmol) in tetrahydrofuran/diethyl ether (1:1, 100 mL) was added dropwise to a suspension of lithium aluminium hydride (17.0 g, 446 mmol) during 1 hr. The mixture was refluxed for 3 hrs, then treated with water (100 mL), 20% sodium hydroxide (100 mL) and water (100 mL) under cooling with ice/water, filtered and the

solid washed with tetrahydrofuran. The organic phase was dried over anhydrous potassium carbonate, filtered and evaporated to give 3-(1-trityl-4-imidazolyl)propanol as white solid.

Yield: 68.0 g (82%).

M.p.: 127-129 °C.

- 5 ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.40-7.24 (m, 10 H); 7.17-7.06 (m, 6 H); 6.55 (s, 1 H); 3.72 (t, J=5.3 Hz, 2 H); 2.68 (t, J=6.6 Hz, 2 H); 1.86 (m, 2 H).

Methanesulfonyl chloride (8 mL, 104 mmol) was added dropwise to a solution of the above alcohol (32.0 g, 86.8 mmol) in dichloromethane (400 mL) and triethyl amine (15.5 mL) at 0°C during 1 hr. The mixture was stirred without cooling for an additional 1 hr; then it was washed
10 with 5% sodium bicarbonate and dried over anhydrous magnesium sulfate. Dichloromethane was evaporated at 30°C *in vacuo* and the residual oily mesylate was used directly in the next step.

Yield: 31.2 g (80%).

- 15 ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.37-7.30 (m, 10 H); 7.16-7.09 (m, 6 H); 6.58 (s, 1 H); 4.24 (t, J=6.3 Hz, 2 H); 2.96 (s, 3 H); 2.67 (m, 2 H); 2.10 (m, 2 H).

A mixture of the above mesylate (30.0 g, 67 mmol), potassium phthalimide (18.0 g, 100 mmol), sodium iodide (4.0 g, 26.7 mmol) and dimethylformamide (200 mL) was stirred overnight at ambient temperature and then treated with water (2 L) and benzene (2 L). The
20 organic phase was dried over anhydrous magnesium sulfate, filtered and solvent evaporated giving a residue, which was recrystallised from benzene yielding 1-trityl- 4-(3-phthalimidopropyl)imidazole as white solid.

Yield: 17.2 g (52%).

M.p.: 211-214 °C.

- 25 ¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.83 (m, 2 H); 7.72 (m, 2 H); 7.39-7.27 (m, 10 H); 7.18-7.07 (m, 6 H); 6.60 (d, J=0.9 Hz, 1 H); 3.72 (t, J=7.4 Hz, 2 H); 2.60 (t, J=7.5 Hz, 2 H); 1.99 (m, 2 H).

The above imidazole derivative (26.6 g, 53.5 mmol) was dissolved in ethanol (300 mL) and tetrahydrofuran (150 mL) at 60°C, hydrazine hydrate (50 g, 1 mol) was added and the
30 solution was refluxed for 6 hrs and then heated at 70 °C overnight. The solid was removed by filtration and the filtrate was treated with 25% aqueous solution of ammonia (2.5 l) and dichloromethane (2.5 L). The organic layer was dried over anhydrous potassium carbonate and evaporated to give a residue, which was purified by column chromatography on silica gel (Fluka 60, chloroform saturated with ammonia/methanol) giving the title compound as white
35 solid.

Yield: 14.2 g (72%).

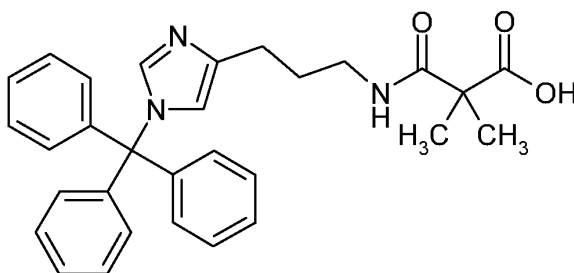
M.p.: 112-113 °C.

R_F (SiO₂, chloroform saturated with ammonia/methanol 9:1): 0.30.

¹H NMR spectrum (300 MHz, CDCl₃, δ_H): 7.37-7.28 (m, 10 H); 7.18-7.09 (m, 6 H); 6.53 (d, J=1.3 Hz, 1 H); 2.74 (t, J=6.9 Hz, 2 H); 2.59 (t, J=7.4 Hz, 2 H); 1.95 (bs, 2 H); 1.78 (m, 2 H).

7. Synthesis of 2,2-Dimethyl-N-[3-(1-trityl-1H-imidazol-4-yl)-propyl]-malonamic acid

Chem. 28:



10 2-Chlorotrityl chloride resin (2.3 g, 3.0 mmol) was swelled in DCM for 20 mins and filtered. Dimethylmalonic acid (2 eq; 6.0 mmol; 793 mg) was dissolved i DCM:DMF 1:1 (10 mL) and added to the resin followed by DIPEA (6 eq; 18.0 mmol; 3.14 mL) and DCM (10 mL). The resin was shaken overnight at RT. The resin was filtered and washed with DCM:MeOH:DIPEA (17:2:1), DCM, NMP og DCM (2 x 25 mL of each). The resin was
15 swelled in DMF for 20 mins and filtered. HOAt (3 eq; 9.0 mmol; 1.23 g), DIC (3 eq; 9.0 mmol; 1.40 mL) and DMF (25 mL) was added and the resin was shaken for 90 min at RT. The resin was filtrered and 3-(1-Trityl-1H-imidazol-4-yl)-propyl amine (1.8 eq; 5.40 mmol; 1.84 g), DIPEA (4 eq; 6.0 mmol; 2.09 mL), and DMF (10 mL) was added. The resin was shaken for 2 days. The resin was filtered and washed with NMP (5 x 20 mL) and DCM (10 x 20 mL).
20 2,2,2-Trifluoroethanol/dichlormethan 1:1 (20 mL) was added to the resin and it was shaken for 2 hrs. The resin was washed with 2,2,2-Trifluoroethanol/dichlormethan 1:1 (10 mL) and the combined filtrates were collected and concentrated *in vacuo* to yield the title compound. Yield: 600 mg (41%).

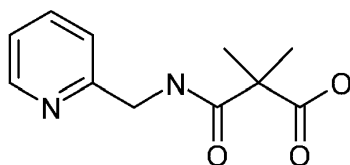
LCMS4: m/z = 482 (M+1)

25 UPLC (method 02_B4_4): Rt = 8.07 min

¹H NMR spectrum (300 MHz, DMSO-*d*₆, δ_H): 7.36-7.44 (9H, m), 7.07-7.12 (6H, m), 6.62 (1H, s), 3.02-3.09 (2H, q), 2.38-2.43 (2H, t), 1.61-1.69 (2H, m), 1.26 (6H, s)

8. Synthesis of 2,2-Dimethyl-N-pyridin-2-ylmethylmalonamic acid

Chem. 29:



Chlorotriyl chloride resin (2.3 g, 3.0 mmol) was swelled in DCM for 20 mins and filtered. Dimethylmalonic acid (2 eq; 6.0 mmol; 793 mg) was dissolved in DCM:NMP 1:1 (10 mL) and added to the resin followed by DIPEA (6 eq; 18.0 mmol; 3.14 mL) and DCM (10 mL). The resin was shaken overnight at RT. The resin was filtered and washed with DCM:MeOH:DIPEA (17:2:1), DCM, NMP and DCM (2 x 25 mL of each). The resin was swelled in NMP for 20 mins and filtered. HOAt (3 eq; 9.0 mmol; 1.23 g), DIC (3 eq; 9.0 mmol; 1.40 mL) and NMP (25 mL) was added and the resin was shaken for 90 min at RT. The resin was filtered and 2-(Aminomethyl)pyridine (2 eq; 6 mmol; 659 mg), DIPEA (4 eq; 6.0 mmol; 2.09 mL), and NMP (10 mL) was added. The resin was shaken for overnight. The resin was filtered and washed with NMP (5 x 20 mL) and DCM (10 x 20 mL). TFA/TIS/water (95:2.5:2.5; 30 mL) was added to the resin and it was shaken for 1 hr, filtered and concentrated *in vacuo* to yield the title compound.

Yield: 600 mg (41%).

LCMS4: m/z = 223 (M+1)

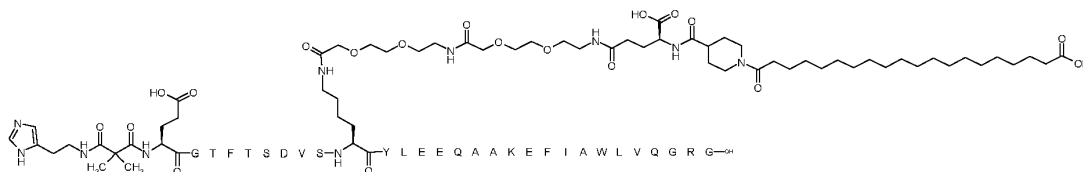
UPLC (method 08_B4_1): Rt = 1.79 min

C. Synthesis of compounds of the invention

Example 1

N^9 -[2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl]- N^{c18} -[2-(2-[2-(2-[2-(2-((S)-3-[1-(19-Carboxy-nonadecanoyl)-piperidine-4-carboxyamino]-3-carboxypropionylamino)ethoxy)ethoxy]acetyl)amino)ethoxy]ethoxy]acetyl][Lys¹⁸, Glu²², Gln³⁴]GLP-1(9-37)-peptide

Chem. 30:

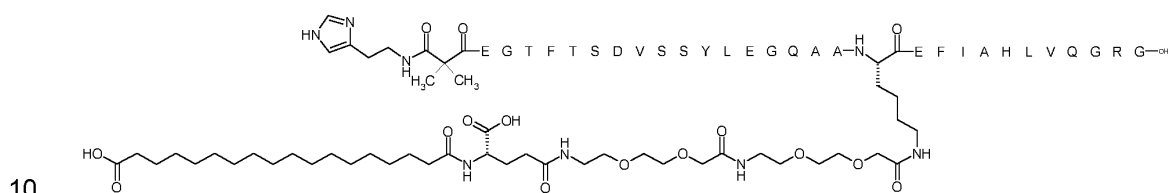


Preparation method: SPPS method E. 2,2-Dimethyl-*N*-[2-(1-trityl-1*H*-imidazol-4-yl)-ethyl]-malonic acid was coupled using the same coupling condition as an Aib amino acid
LCMS4: m/z = 1441.63 ($m/3$), 1081.62 ($m/4$)

5 Example 2

N^9 -{2-[2-(1*H*-imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}- $N^{\epsilon 26}$ -[2-(2-{2-[2-(2-{[(*S*)-4-Carboxy-4-(17-carboxyheptadecanoylamino)butyrylamino]ethoxy}ethoxy)acetyl]amino]ethoxy}ethoxy)acetyl][His³¹,Gln³⁴]GLP-1(9-37)-peptide

Chem. 31:



Preparation method: SPPS method B. 2,2-Dimethyl-*N*-[2-(1-trityl-1*H*-imidazol-4-yl)-ethyl]-malonic acid, Fmoc-Oeg-OH, Fmoc-Glu-OtBu, and octadecanedioic acid mono-*tert*-butyl ester were coupled using the same coupling condition as an Aib amino acid.

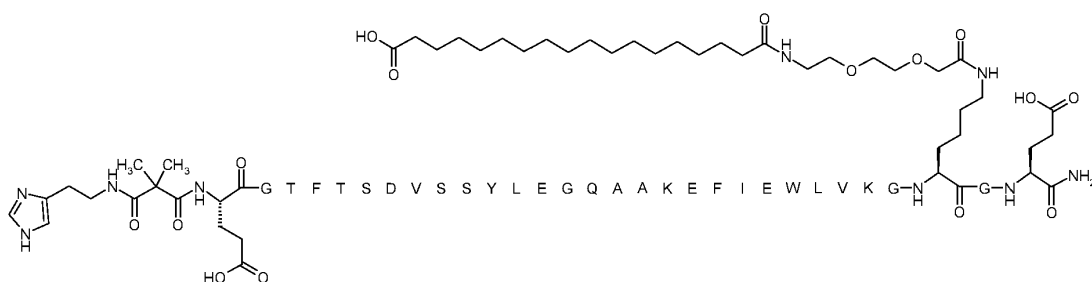
LCMS4: R_t = 2.23 min. m/z = 1341 ($m/3$), 1006 ($m/4$)

15

Example 3

N^9 -{2-[2-(1*H*-imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}- $N^{\epsilon 36}$ -(2-{2-[2-(17-carboxyheptadecanoylamino)ethoxy]ethoxy}acetyl) (Glu³⁰,Lys³⁶)GLP-1(9-37) Glu³⁸-peptide amide

20 Chem. 32:



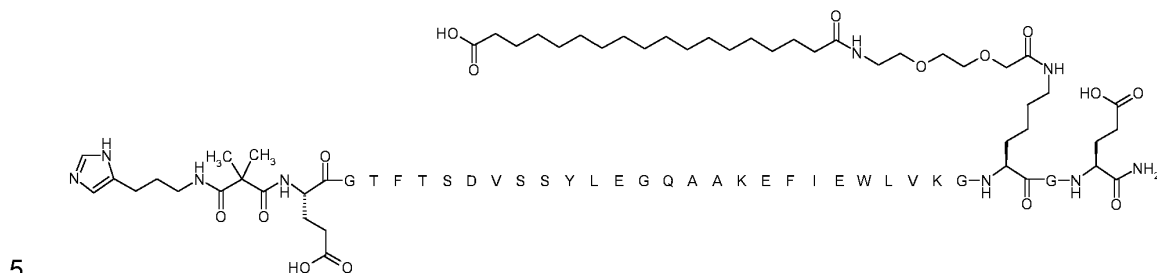
Preparation method: As in Example 2

LCMS4: R_t = 6.90 min. m/z = 1319 ($m/3$), 990 ($m/4$)

25 Example 4

N^9 -{2-[2-(1H-Imidazol-4-yl)propylcarbamoyl]-2-methyl-propionyl}- $N^{\epsilon 36}$ -(2-{2-[2-(17-carboxyheptadecanoylamino)ethoxy]ethoxy}acetyl) [Glu³⁰,Lys³⁶]GLP-1(9-37)Glu³⁸-peptide amide

Chem. 33:



Preparation method: SPPS method B. 2,2-Dimethyl- N -[3-(1-trityl-1H-imidazol-4-yl)-propyl]-malonic acid, Fmoc-Oeg-OH, and octadecanedioic acid mono-*tert*-butyl ester were coupled using the same coupling condition as an Aib amino acid

LCMS4: m/z = 1324 ($m/3$), 993 ($m/4$)

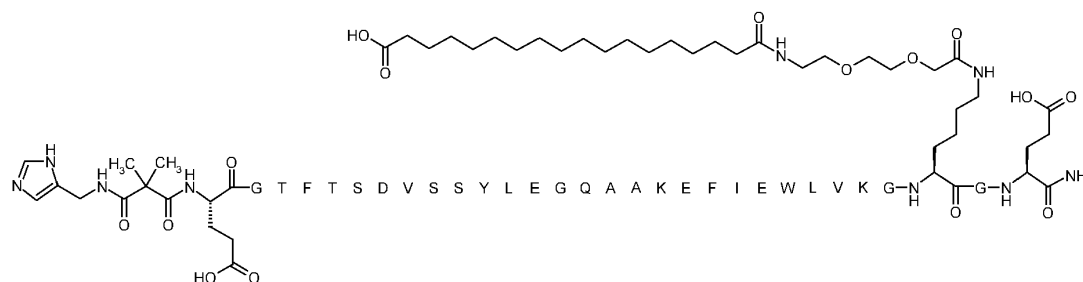
10 UPLC (method 04_A3_1): R_t = 12.55 min

Example 5

N^9 -{2-[2-(1H-Imidazol-4-yl)methylcarbamoyl]-2-methyl-propionyl}- $N^{\epsilon 36}$ -(2-{2-[2-(17-carboxyheptadecanoylamino)ethoxy]ethoxy}acetyl) [Glu³⁰,Lys³⁶] GLP-1(9-37) Glu³⁸-peptide amide

15

Chem. 34:



Preparation method: SPPS method B. 2,2-Dimethyl- N -(1-trityl-1H-imidazol-4-ylmethyl)-malonic acid, Fmoc-Oeg-OH, and octadecanedioic acid mono-*tert*-butyl ester were

20 coupled using the same coupling condition as an Aib amino acid

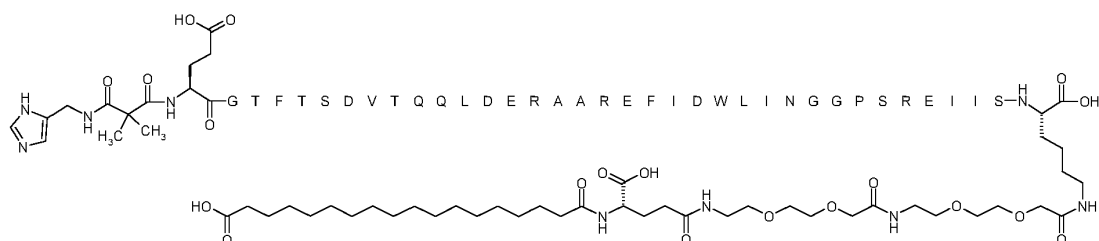
LCMS4: m/z = 1313 ($m/3$), 975 ($m/4$)

UPLC (method 04_A3_1): R_t = 12.44 min

Example 6

N^2 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}- [2-(2-{2-[2-(2-{(S)-4-Carboxy-4-(17-carboxyheptadecanoylamino)-butyrylamino]ethoxy}ethoxy)-acetylaminol]ethoxy}-ethoxy)acetyl][Arg¹⁷,Arg²⁰,Arg³³,Lys³⁸]GLP-1A(3-37)-peptide

Chem. 35:



5

Preparation method: SPPS method B. 2,2-Dimethyl-*N*-(1-trityl-1*H*-imidazol-4-ylmethyl)-malonic acid, Fmoc-Oeg-OH, Fmoc-Glu-OtBu, and octadecanedioic acid mono-*tert*-butyl ester were coupled using the same coupling condition as an Aib amino acid

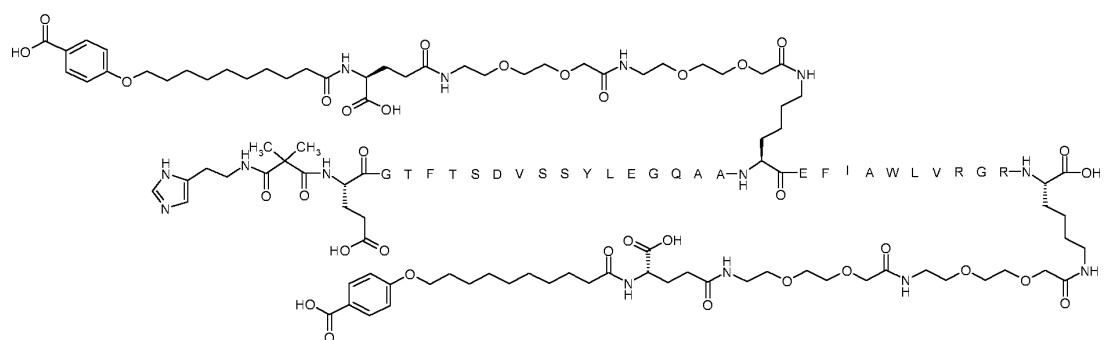
10 LCMS4: m/z = 1663 ($m/3$), 1247 ($m/4$), 998 ($m/5$)

UPLC (method 04_A3_1): R_t = 11.26 min

Example 7

15 N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}- N^{e26} -{2-[2-(2-{2-[2-(2-{(S)-4-Carboxy-4-[10-(4-carboxy-phenoxy)-decanoylamino]-butyrylamino}-ethoxy)-ethoxy]-acetylaminol]ethoxy}-ethoxy)-acetyl], N^{e37} -{2-[2-(2-{2-[2-(2-{(S)-4-Carboxy-4-[10-(4-carboxy-phenoxy)-decanoylamino]-butyrylamino}-ethoxy)-ethoxy]-acetylaminol]ethoxy}-ethoxy)-acetyl] [Arg³⁴,Lys³⁷]GLP-1(9-37)-peptide

Chem. 36:



20

Preparation method: SPPS method B. 2,2-Dimethyl-*N*-[2-(1-trityl-1*H*-imidazol-4-yl)-ethyl]-malonic acid was coupled using the same coupling condition as an Aib amino acid. 8-(9-fluorenylmethyloxycarbonyl-amino)-3,6-dioxaoctanoic acid (commercially available from Iris Biotech), Fmoc-Glu-OtBu and 4-(9-carboxy-nonyloxy)-benzoic acid *tert*-butyl ester (prepared

as described in Example 25, step 2 of WO 2006/082204) were coupled using SPPS method D.

UPLC (method 08_B4_1): Rt = 8.81 min.

LCMS4: Rt = 2.29 min. m/z = 1625 (m/3), 1219 (m/4), 975 (m/5)

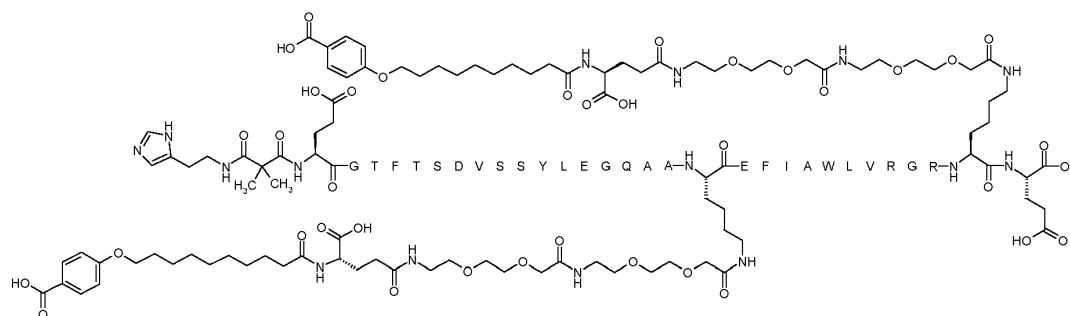
5

Example 8

N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl} $N^{\epsilon 26}$ -{2-[2-(2-[2-(2-[2-(2-[2-(S)-4-Carboxy-4-[10-(4-carboxy-phenoxy)-decanoylamino]-butyrylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl}, $N^{\epsilon 37}$ -{2-[2-(2-[2-(2-[2-(2-[2-(S)-4-Carboxy-4-[10-(4-carboxy-phenoxy)-decanoylamino]-butyrylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl} [Arg³⁴, Lys³⁷]GLP-1(9-37)Glu³⁸-peptide

10

Chem. 37:



Preparation method: SPPS method B. 2,2-Dimethyl- N -[2-(1-trityl-1H-imidazol-4-yl)-ethyl]-

15

malonamic acid was coupled using the same coupling condition as an Aib amino acid. 8-(9-fluorenylmethyloxycarbonyl-amino)-3,6-dioxaoctanoic acid (commercially available from Iris Biotech), Fmoc-Glu-OtBu, and 4-(9-carboxy-nonyloxy)-benzoic acid *tert*-butyl ester (prepared as described in Example 25, step 2 of WO 2006/082204) were coupled using SPPS method D.

20

UPLC (method 04_A3_1): Rt = 9.32 min.

LCMS4: Rt = 2.29 min., m/z = 1669 (m/3), 1252 (m/4), 1001 (m/5)

Example 9

N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}- $N^{\epsilon 26}$ -{2-[2-(2-[2-(2-[2-(2-[2-(S)-4-[4-(4-*tert*-Butyl-phenyl)-butyrylamino]-4-carboxy-butrylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl}, $N^{\epsilon 37}$ -{2-[2-(2-[2-(2-[2-(2-[2-(S)-4-[4-(4-*tert*-Butyl-phenyl)-butyrylamino]-4-carboxy-butrylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl}[Arg³⁴, Lys³⁷]GLP-1(9-37)-peptide

25

Chem. 38:

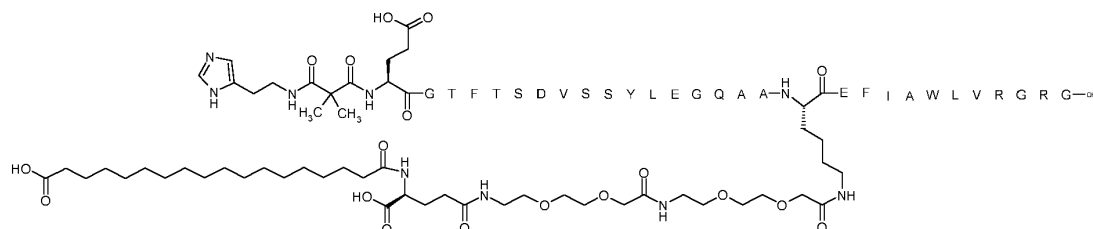
5 Iris Biotech), Fmoc-Glu-OtBu and 4-(4-*t*-butylphenyl)butyric acid were coupled using SPPS method D.

LCMS4: Rt = 2.40 min. m/z = 940(m/5), 1174(m/4), 1565(m/3)

10 Example 10

N^9 -[2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl] N^{26} -[2-(2-{2-[2-(2-{2-[(S)-4-Carboxy-4-(17-carboxyheptadecanoylamino)butyrylamino]ethoxy}ethoxy)acetyl]amino}ethoxy)ethoxy)acetyl][Arg³⁴]GLP-1(9-37)-peptide

Chem. 39:

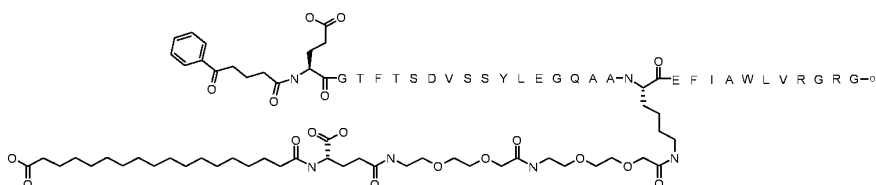


Preparation method: SPPS method E. 2,2-Dimethyl-*N*-[2-(1-trityl-1*H*-imidazol-4-yl)-ethyl]-malonic acid was coupled using the same coupling condition as an Aib amino acid
LCMS4: $m/z = 1367.30$ ($m/3$), 1025.60 ($m/4$)

20 **Example 11 (Comparative compound)**

N^9 -(5-oxo-5-phenylpentanoyl), $N^{\epsilon 26}$ -[2-[2-[2-[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-[Arg³⁴]-GLP-1-(9-37)-peptide

Chem. 40:



Preparation method: SPPS method B

UPLC (method 01_B4_1: Rt = 11.48

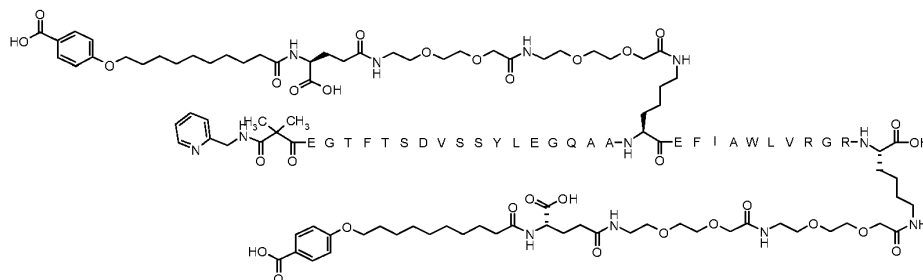
LCMS2: Rt = 5.60 m/z 1356 (m/3)

5

Example 12

N^9 -[2,2-dimethyl-3-oxo-3-(pyridin-2-ylmethylamino)propanoyl], $N^{\epsilon 26}$ -[2-[2-[2-[[2-[2-[2-[(4S)-4-carboxy-4-[10-(4-carboxyphenoxy)decanoylamino]butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl], $N^{\epsilon 37}$ -[2-[2-[2-[[2-[2-[2-[(4S)-4-carboxy-4-[10-(4-carboxyphenoxy)decanoylamino]butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-[Arg³⁴, Lys³⁷]-GLP-1-(9-37)-peptide

Chem. 41:



- 15 Preparation method: SSPS method B. 2,2-Dimethyl-N-pyridin-2-ylmethyl-malonamic acid was coupled using the same coupling condition as used for 2,2-Dimethyl-N-[2-(1-trityl-1H-imidazol-4-yl)-ethyl]-malonamic acid in the previous examples. Fmoc-Glu-OtBu and 4-(9-carboxy-nonyloxy)-benzoic acid *tert*-butyl ester (prepared as described in Example 25, step 2 of WO 2006/082204) were coupled using SPPS method D
- 20 UPLC (method 08_B4_1): Rt = 8.98 min
LCMS4: Rt = 2.23 min. m/z = 1624(m/3), 1218 (m/4)

PHARMACOLOGICAL METHODS

Example 13: In vitro potency

The purpose of this example is to test the activity, or potency, of the GLP-1 receptor agonist derivatives in vitro.

5 The potencies of the GLP-1 receptor agonist derivatives of Examples 1-12 were determined as described below, i.e. as the stimulation of the formation of cyclic AMP (cAMP) in a medium containing membranes expressing the human GLP-1 receptor.

It is noted that the compound of Example 11 (Chem. 40) is a comparative compound based on compound 215 (p. 24) of WO 2004/067548, which according to Fig. 1 of this WO
10 publication is one of the most potent compounds of this publication

Principle

Purified plasma membranes from a stable transfected cell line, BHK467-12A (tk-ts13), expressing the human GLP-1 receptor were stimulated with the GLP-1 receptor agonist derivative in question, and the potency of cAMP production was measured using the
15 AlphaScreen™ cAMP Assay Kit from Perkin Elmer Life Sciences. The basic principle of The AlphaScreen Assay is a competition between endogenous cAMP and exogenously added biotin-cAMP. The capture of cAMP is achieved by using a specific antibody conjugated to acceptor beads.

Cell culture and preparation of membranes

20 A stable transfected cell line and a high expressing clone were selected for screening. The cells were grown at 5% CO₂ in DMEM, 5% FCS, 1% Pen/Strep (Penicillin/Streptomycin) and 0.5 mg/ml of the selection marker G418.

Cells at approximate 80% confluence were washed 2X with PBS and harvested with Versene (aqueous solution of the tetrasodium salt of ethylenediaminetetraacetic acid),
25 centrifuged 5 min at 1000 rpm and the supernatant removed. The additional steps were all made on ice. The cell pellet was homogenised by the Ultrathurax for 20-30 sec. in 10 ml of Buffer 1 (20 mM Na-HEPES, 10 mM EDTA, pH=7.4), centrifuged 15 min at 20,000 rpm and the pellet resuspended in 10 ml of Buffer 2 (20 mM Na-HEPES, 0.1 mM EDTA, pH=7.4). The suspension was homogenised for 20-30 sec and centrifuged 15 min at 20,000 rpm.
30 Suspension in Buffer 2, homogenisation and centrifugation was repeated once and the membranes were resuspended in Buffer 2. The protein concentration was determined and the membranes stored at -80°C until use.

The assay was performed in ½-area 96-well plates, flat bottom (Costar cat. no:3693). The final volume per well was 50 µl.

Solutions and reagents

AlphaScreen cAMP Assay Kit from Perkin Elmer Life Sciences (cat. No: 6760625M); containing Anti-cAMP Acceptor beads (10 U/ μ l), Streptavidin Donor beads (10 U/ μ l) and Biotinylated-cAMP (133 U/ μ l).

- 5 AlphaScreen Buffer, pH=7.4: 50 mM TRIS-HCl (Sigma, cat.no: T3253); 5 mM HEPES (Sigma, cat.no: H3375); 10 mM $MgCl_2$, 6H₂O (Merck, cat.no: 5833); 150 mM NaCl (Sigma, cat.no: S9625); 0.01% Tween (Merck, cat.no: 822184). The following was added to the AlphaScreen Buffer prior to use (final concentrations indicated): BSA (Sigma, cat. no. A7906): 0.1%; IBMX (Sigma, cat. no. I5879): 0.5 mM; ATP (Sigma, cat. no. A7699): 1 mM;
- 10 GTP (Sigma, cat. no. G8877): 1 μ M.

cAMP standard (dilution factor in assay = 5): cAMP Solution: 5 μ L of a 5 mM cAMP-stock + 495 μ L AlphaScreen Buffer.

- Suitable dilution series in AlphaScreen Buffer were prepared of the cAMP standard as well as the GLP-1 analogue or derivative to be tested, e.g. the following eight
- 15 concentrations of the GLP-1 compound: 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} and 10^{-14} M, and a series from, e.g., 10^{-6} to 3×10^{-11} of cAMP.

Membrane/Acceptor beads

Use hGLP-1/ BHK 467-12A membranes; 6 μ g/well corresponding to 0.6 mg/ml (the amount of membranes used pr. well may vary)

- 20 “No membranes”: Acceptor Beads (15 μ g/ml final) in AlphaScreen buffer
- “6 μ g/well membranes”: membranes + Acceptor Beads (15 μ g/ml final) in AlphaScreen buffer

Add 10 μ l “No membranes” to the cAMP standard (per well in duplicates) and the positive and negative controls

- 25 Add 10 μ l “6 μ g/well membranes” to GLP-1 and analogues (per well in duplicates/triplicates)

Pos. Control: 10 μ l “no membranes” + 10 μ l AlphaScreen Buffer

Neg. Control: 10 μ l “no membranes” + 10 μ l cAMP Stock Solution (50 μ M)

- As the beads are sensitive to direct light, any handling was in the dark (as dark as
- 30 possible), or in green light. All dilutions were made on ice.

Procedure

1. Make the AlphaScreen Buffer.
2. Dissolve and dilute the GLP-1/Analogues/cAMP standard in AlphaScreen Buffer.
3. Make the Donor Beads solution and incubate 30 min. at R.T.

4. Add the cAMP/GLP-1/Analogues to the plate: 10 µl per well.
5. Prepare membrane/Acceptor Beads solution and add this to the plates: 10 µl per well.
6. Add the Donor Beads: 30 µl per well.
- 5 7. Wrap the plate in aluminum foil and incubate on the shaker for 3 hours (very slowly) at RT.
8. Count on AlphaScreen – each plate pre incubates in the AlphaScreen for 3 minutes before counting.

If desired, the fold variation in relation to GLP-1 may be calculated as $EC_{50} \text{ (GLP-1)} / EC_{50} \text{ (analogue)} - 3693.2$.

Results

The EC_{50} [pM] values were calculated using the Graph-Pad Prism software (version 5).

15 All tested derivatives of the invention had a good in vitro potency corresponding to an EC_{50} of 2100 pM or below; eight derivatives were even more potent having an EC_{50} at 1000 pM or below; five derivatives had a still further improved potency corresponding to an EC_{50} at 500 pM or below; four derivatives were very potent corresponding to an EC_{50} at 300 pM or below; and one derivative had a very good potency corresponding to an EC_{50} at 100 pM or below.

20 The comparative compound of Example 11 was much less potent, namely with an EC_{50} of above 8000 pM.

Example 14: GLP-1 receptor binding

The purpose of this experiment is to investigate the binding to the GLP-1 receptor of the GLP-1 agonist derivatives, and how the binding is potentially influenced by the presence
25 of albumin. This is done in an in vitro experiment as described below.

The binding affinity of the GLP-1 receptor agonist derivatives of Examples 1-12 to the human GLP-1 receptor was measured by way of their ability to displace of ^{125}I -GLP-1 from the receptor.

It is noted that the compound of Example 11 (Chem. 40) is a comparative compound
30 based on compound 215 (p. 24) of WO 2004/067548, which according to Fig. 1 of this WO publication is one of the most potent compounds of this publication.

In order to test the binding of the derivatives to albumin, the assay was performed with a low concentration of albumin (0.005% - corresponding to the residual amount thereof in the tracer), as well as with a high concentration of albumin (2.0% added).

A shift in the binding affinity, IC_{50} , is an indication that the peptide in question binds to albumin, and thereby a prediction of a potential protracted pharmacokinetic profile of the peptide in question in animal models.

Conditions

- 5 Species (in vitro): Hamster
 Biological End Point: Receptor Binding
 Assay Method: SPA
 Receptor: GLP-1 receptor
 Cell Line: BHK tk-ts13

10 Cell culture and membrane purification

A stable transfected cell line and a high expressing clone were selected for screening. The cells were grown at 5% CO_2 in DMEM, 10% FCS, 1% Pen/Strep (Penicillin/Streptomycin) and 1.0 mg/ml of the selection marker G418.

- The cells (approx. 80% confluence) were washed twice in PBS and harvested with
15 Versene (aqueous solution of the tetrasodium salt of ethylenediaminetetraacetic acid), following which they were separated by centrifugation at 1000 rpm for 5 min. The cells/cell pellet must be kept on ice to the extent possible in the subsequent steps. The cell pellet was homogenised with Ultrathurrax for 20-30 seconds in a suitable amount of Buffer 1 (depending on the amount of cells, but e.g. 10 ml). The homogenate was centrifuged at
20 20000 rpm for 15 minutes. The pellet was resuspended (homogenised) in 10 ml Buffer 2 and re-centrifuged. This step was repeated once more. The resulting pellet was resuspended in Buffer 2, and the protein concentration was determined. The membranes were stored at minus 80°C.

 Buffer 1: 20 mM Na-HEPES + 10mM EDTA, pH 7.4

- 25 Buffer 2: 20 mM Na-HEPES + 0.1mM EDTA, pH 7.4

Binding assay:

 SPA:

- Test compounds, membranes, SPA-particles and [^{125}I]-GLP-1(7-36)NH₂ were diluted in assay buffer. 25 ul (micro liter) of test compounds were added to Optiplate. HSA ("high
30 albumin" experiment containing 2% HSA), or buffer ("low albumin" experiment containing 0.005% HSA), was added (50 ul). 5-10 ug protein/sample was added (50ul) corresponding to 0.1 - 0.2 mg protein/ml (to be preferably optimised for each membrane preparation). SPA-particles (Wheatgerm agglutinin SPA beads, Perkin Elmer, #RPNQ0001) were added in an amount of 0.5 mg/well (50ul). The incubation was started with [^{125}I]-GLP-1-(7-36)NH₂ (final
35 concentration 0.06 nM corresponding to 49.880 DPM, 25ul). The plates were sealed with

PlateSealer and incubated for 120 minutes at 30°C while shaking. The plates were centrifuged (1500 rpm, 10min) and counted in Topcounter.

Assay buffer:

50 mM HEPES
5 mM EGTA
5 mM MgCl₂
0.005% Tween 20
pH 7.4
HSA was SIGMA A1653

10 Calculations

The IC₅₀ value was read from the curve as the concentration which displaces 50% of ¹²⁵I-GLP-1 from the receptor, and the ratio of [(IC₅₀/nM) high HSA] / [(IC₅₀/nM) low HSA] was determined.

15 Generally, the binding to the GLP-1 receptor at low albumin concentration should be as good as possible, corresponding to a low IC₅₀ value.

The IC₅₀ value at high albumin concentration is a measure of the influence of albumin on the binding of the derivative to the GLP-1 receptor. As is known, the GLP-1 receptor agonist derivatives also bind to albumin. This is a generally desirable effect, which extends their lifetime in plasma. Therefore, the IC₅₀ value at high albumin will generally be
20 higher than the IC₅₀ value at low albumin, corresponding to a reduced binding to the GLP-1 receptor, caused by albumin binding competing with the binding to the GLP-1 receptor.

A high ratio (IC₅₀ value (high albumin) / IC₅₀ value (low albumin)) may therefore be taken as an indication that the derivative in question binds well to albumin (may have a long half-life), and also *per se* binds well to the GLP-1 receptor (the IC₅₀ value (high albumin) is
25 high, and the IC₅₀ value (low albumin) is low).

Results

The following results were obtained, where "ratio" refers to [(IC₅₀/nM) high HSA] / [(IC₅₀/nM) low HSA]:

30 All derivatives had a ratio above 10; ten were above 20; six derivatives were above 30; four derivatives were above 50; and one derivative was above 100. The comparative compound of Example 11, had a ratio above 300.

Furthermore as regards IC₅₀ (low albumin), all derivatives, except the comparative compound of Example 11, had an IC₅₀ (low albumin) below 40 nM; all but one below 20 nM;

all but four were below 10.0 nM; five were below 5.00 nM; and three derivatives were below 1.00 nM.

Finally as regards IC₅₀ (high albumin), all derivatives of the invention had an IC₅₀ (high albumin) below 900.00 nM; nine were below 500.00 nM; four were below 100.00 nM; and two derivatives were below 50.00 nM. The IC₅₀ (high albumin) for the comparative compound of Example 11 was above 800.00 nM.

Example 15: Estimate of oral bioavailability

The purpose of this experiment is to estimate the oral bioavailability of the GLP-1 receptor agonist derivatives.

To this end, the exposure in plasma after direct injection into the intestinal lumen of the compounds is studied in vivo in rats, as described in the following.

The compounds are tested in a concentration of 1000 uM in a solution of 55 mg/ml sodium caprate.

32 male Sprague Dawley rats with a body weight upon arrival of approximately 240 g are obtained from Taconic (Denmark) and assigned to the different treatments by simple randomisation, 4 rats per group. The rats are fasted for approximately 18 hours before the experiment and taken into general anaesthesia (Hypnorm/Dormicum).

The compounds are administered in the jejunum either in the proximal part (10 cm distal for the duodenum) or in the mid-intestine (50 cm proximal for the cecum). A PE50-catheter, 10 cm long is inserted into the jejunum, forwarded at least 1.5 cm into the jejunum, and secured before dosing by ligature around the gut and the catheter with 3/0 suture distal to tip to prevent leak or catheter displacement. Catheter is placed without syringe and needle and 2 ml saline is administered into abdomen before closing the incision with wound clips.

100 µl of the respective compound is injected into the jejunal lumen through the catheter with a 1 ml syringe. Subsequently, 200 µl of air is pushed into the jejunal lumen with another syringe to "flush" the catheter. This syringe is leaved connected to the catheter to prevent flow back into the catheter.

Blood samples (200 ul) are collected at desired intervals (usually at times 0, 10, 30, 60, 120 and 240 min) into EDTA tubes from the tail vein and centrifuged 5 minutes, 10000G, at 4°C within 20 minutes. Plasma (75ul) is separated to Micronic tubes, immediately frozen, and kept at -20°C until analyzed for plasma concentration of the respective GLP-1 receptor agonist derivative with LOCI (Luminescent Oxygen Channeling Immunoassay), generally as described for the determination of insulin by Poulsen and Jensen in Journal of Biomolecular Screening 2007, vol. 12, p. 240-247. The donor beads are coated with streptavidin, while

acceptor beads are conjugated with a monoclonal antibody recognising a mid-/C-terminal epitope of the peptide. Another monoclonal antibody, specific for the N-terminus, is biotinylated. The three reactants are combined with the analyte and formed a two-sited immuno-complex. Illumination of the complex released singlet oxygen atoms from the donor beads, which are channeled into the acceptor beads and triggered chemiluminescence which is measured in an Envision plate reader. The amount of light is proportional to the concentration of the compound.

After the blood sampling the rats are sacrificed under anaesthesia and the abdomen is opened to verify correct catheter placement.

The mean (n=4) plasma concentrations (pmol/l) are determined as a function of time. The ratio of plasma concentration (pmol/l) divided by the concentration of the dosing solution ($\mu\text{mol/l}$) is calculated for each treatment, and the results for $t = 30$ min (30 minutes after the injection of the compound in the jejunum) are assessed (dose-corrected exposure at 30 min) as a surrogate measure of intestinal bioavailability. The dose-corrected exposure has been shown to correlate significantly with the actual bioavailability.

The results may be given as dose-corrected exposure at 30 min which refers to (the plasma concentration 30 minutes after injection of the compound in the jejunum (pM)), divided by (the concentration of the compound in the dosing solution (μM)).

Example 16: Pharmacokinetics in minipigs

The purpose of this study is to determine the protraction in vivo of the GLP-1 receptor agonist derivatives after i.v. administration to minipigs, i.e. the prolongation of their time of action. This is done in a pharmacokinetic (PK) study, where the terminal half-life of the derivative in question is determined. By terminal half-life is generally meant the period of time it takes to halve a certain plasma concentration, measured after the initial distribution phase.

Male Göttingen minipigs obtained from Ellegaard Göttingen Minipigs (Dalmose, Denmark) approximately 7-14 months of age and weighing from approximately 16-35 kg are used in the studies. The minipigs are housed individually and fed restrictedly once or twice daily with SDS minipig diet (Special Diets Services, Essex, UK). After at least 2 weeks of acclimatisation two permanent central venous catheters are implanted in vena cava caudalis or cranialis in each animal. The animals are allowed 1 week recovery after the surgery, and are then used for repeated pharmacokinetic studies with a suitable wash-out period between dosings.

The animals are fasted for approximately 18 h before dosing and for at least 4 h after dosing, but had ad libitum access to water during the whole period.

The compounds are dissolved in 50 mM sodium phosphate, 145 mM sodium chloride, 0.05% tween 80, pH 7.4 to a concentration of usually from 20-60 nmol/ml.

5 Intravenous injections (the volume corresponding to usually 1-2 nmol/kg, for example 0.033ml/kg) of the compounds are given through a catheter, and blood is sampled at predefined time points for up till 13 days post dosing (preferably through the other catheter). Blood samples (for example 0.8 ml) are collected in EDTA buffer (8mM) and then centrifuged at 4°C and 1942G for 10 minutes. Plasma is pipetted into Micronic tubes on dry ice, and
10 kept at -20°C until analyzed for plasma concentration of the respective GLP-1 compound using ELISA or a similar antibody based assay or LC-MS. Individual plasma concentration-time profiles are analyzed by a non-compartmental model in WinNonlin v. 5.0 (Pharsight Inc., Mountain View, CA, USA), and the resulting terminal half-lives (harmonic mean) determined.

15 While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

20

CLAIMS

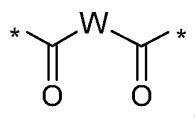
1. A GLP-1 receptor agonist peptide having the formula Chem. 1:

Chem. 1: Y-Z-P, wherein

5 **P** represents a fragment of a GLP-1 receptor agonist peptide lacking the two N-terminal amino acid residues;

Z represents a group of the formula Chem. 2:

Chem. 2:

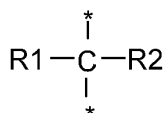


10

wherein

W represents a group of formula Chem. 3:

Chem. 3:



wherein

15

R1 and R2 independently represent

(i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or

20

R1 and R2 together form

(ii) cyclo alkyl, heterocyclyl, or heteroaryl,

with the proviso that

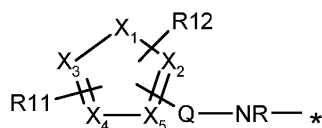
25

(iii) R1 and R2 do not both represent hydrogen;

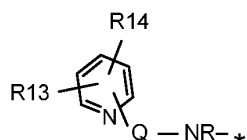
and

Y represents a group of formula Chem. 4 or Chem. 5:

Chem. 4:



Chem. 5:



wherein

- 5 X_1 is N, O, or S; X_2 , X_3 , X_4 , and X_5 independently represent C, or N,
with the proviso that at least one of X_2 , X_3 , X_4 and X_5 is C;

R_{11} , R_{12} , R_{13} , and R_{14} independently represent

- hydrogen, alkyl, aryl, heterocyclyl, heteroaryl, halogen,
hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl,
10 carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide,
substituted carboxamide, alkyl ester, aryl ester, alkyl
sulfonyl, or aryl sulfonyl;

Q represents a bond, or a group of formula Chem. 6:

Chem. 6:

- 15 $^{*-(C(R_{15})(R_{16}))_q-^{*}}$,

wherein

q is 1-6, and

R_{15} and R_{16} independently of each other and
independently for each value of q represent
20 hydrogen, alkyl, carboxyl, or hydroxyl; and

R represents hydrogen, or alkyl;

or a pharmaceutically acceptable salt, amide, or ester thereof.

2. A GLP-1 receptor agonist peptide selected from the following:

- 25 (i) N^9 -[2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl]-[Lys¹⁸, Glu²², Gln³⁴]GLP-1(9-37)-peptide;
(ii) N^9 -[2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl]-[His³¹, Gln³⁴]GLP-1(9-37)-peptide;

(iii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶]GLP-1(9-37)-Glu³⁸-peptide amide;

(iv) N^9 -{2-[2-(1H-Imidazol-4-yl)propylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶]GLP-1(9-37)-Glu³⁸-peptide amide;

5 (v) N^9 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}-[Glu³⁰,Lys³⁶] GLP-1(9-37)-Glu³⁸-peptide amide;

(vi) N^3 -{2-[2-(1H-Imidazol-4-yl)-methylcarbamoyl]-2-methyl-propionyl}-[Arg¹⁷,Arg²⁰,Arg³³,Lys³⁸] GLP-1A(3-37)-peptide;

10 (vii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴,Lys³⁷]GLP-1(9-37)-peptide;

(viii) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}[Arg³⁴,Lys³⁷]GLP-1(9-37)-Glu³⁸-peptide;

(ix) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}-[Arg³⁴,Lys³⁷]GLP-1(9-37)-peptide;

15 (x) N^9 -{2-[2-(1H-Imidazol-4-yl)-ethylcarbamoyl]-2-methyl-propionyl}[Arg³⁴]GLP-1(9-37)-peptide; and

(xii) N^9 -[2,2-dimethyl-3-oxo-3-(pyridin-2-ylmethylamino)propanoyl]-[Arg³⁴,Lys³⁷]-GLP-1-(9-37)-peptide;

or a pharmaceutically acceptable salt, amide, or ester thereof.

20

3. A derivative of a peptide of any one of claims 1-2, or a pharmaceutically acceptable salt, amide, or ester thereof.

4. The derivative of claim 3 which has an albumin binding moiety attached to a lysine residue of the peptide.

25

5. The derivative of claim 4, in which the albumin binding moiety comprises a protracting moiety selected from Chem. 8, Chem. 9, and Chem. 10:

Chem. 8: $\text{HOOC}-(\text{CH}_2)_x-\text{CO}-^*$

30 Chem. 9: $\text{HOOC}-\text{C}_6\text{H}_4-\text{O}-(\text{CH}_2)_y-\text{CO}-^*$

Chem. 10: $\text{R}^{18}-\text{C}_6\text{H}_4-(\text{CH}_2)_z-\text{CO}-^*$

in which x is an integer in the range of 6-18, y is an integer in the range of 3-17, z is an integer in the range of 1-5, and R^{18} is a group having a molar mass not higher than 150 Da.

35

6. The derivative of claim 5, wherein the albumin binding moiety further comprises a linker selected from Chem. 11, Chem. 12, Chem. 13, and Chem. 14:

Chem. 11: $^*-\text{NH}-\text{CH}_2-\text{CH}_2-(\text{O}-\text{CH}_2-\text{CH}_2)_k-\text{O}-(\text{CH}_2)_n-\text{CO}-^*$

Chem. 12: $^*-\text{NH}-\text{C}(\text{COOH})-(\text{CH}_2)_2-\text{CO}-^*$

5 Chem. 13: $^*-\text{N}-\text{C}((\text{CH}_2)_2\text{COOH})-\text{CO}-^*$

Chem. 14: $^*-\text{NC}_5\text{H}_8-\text{CO}-^*$

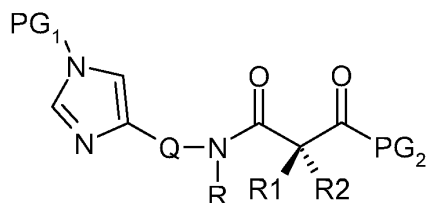
wherein k is an integer in the range of 1-5, and n is an integer in the range of 1-5.

7. A derivative of a GLP-1 receptor agonist peptide selected from the following: Chem.

10 30, Chem. 31, Chem. 32, Chem. 33, Chem. 34, Chem. 35, Chem. 36, Chem. 37, Chem. 38, Chem. 39, and Chem. 41; or a pharmaceutically acceptable salt, amide, or ester thereof.

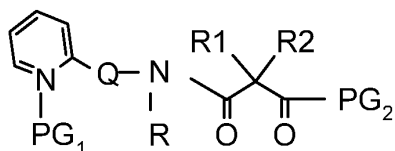
8. An intermediate compound of the formula Chem. 50 or Chem. 51:

Chem. 50:



15

Chem. 51:



wherein Q represents a bond, or a group of formula Chem. 6:

Chem. 6:

20 $-(\text{C}(\text{R}15)(\text{R}16))_q-$,

wherein q is 1-6, and R15 and R16 independently of each other and independently for each value of q represent hydrogen, alkyl, carboxyl, or hydroxyl;

R represents hydrogen, or alkyl;

R1 and R2 independently represent (i) hydrogen, alkyl, aryl, heterocyclyl, heteroaryl,

25 halogen, hydroxyl, hydroxylalkyl, cyano, amino, aminoalkyl, carboxyl, carboxylalkyl, alkoxy, aryloxy, carboxamide, substituted carboxamide, alkyl ester, aryl ester, alkyl sulfonyl, or aryl sulfonyl, or (ii) R1 and R2 together form cyclo alkyl, heterocyclyl, or heteroaryl; and

each of PG₁ and PG₂ represents a protection group;

or a pharmaceutically acceptable salt, ester, or amide thereof.

9. An intermediate compound selected from Chem. 23, Chem. 24, Chem. 25, Chem. 26, Chem. 27, Chem. 28, and Chem. 29;

5 or a pharmaceutically acceptable salt, amide, or ester thereof.

10. The compound of Chem. 40; or a pharmaceutically acceptable salt, amide, or ester thereof.

10 11. A peptide intermediate product, which is selected from
a) the following analogues of GLP-1(9-37) (SEQ ID NO: 1): (i) (18K, 22E, 34Q); (ii) (30E, 36K, 38E); (iii) (31H, 34Q); (iv) 34R; (v) (34R, 37K); and (vi) (34R, 37K, 38E); and
b) the following analogue of GLP-1A(3-37) (SEQ ID NO: 3): (17R, 20R, 33R, 38K);
or a pharmaceutically acceptable salt, amide, or ester thereof.

15

12. A peptide or derivative according to any one of claims 1-7, for use as a medicament.

13. A peptide or derivative according to any one of claims 1-7, for use in the treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders,
20 cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

14. Use of a peptide or derivative according to any one of claims 1-7, in the manufacture
25 of a medicament for treatment and/or prevention of all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease progression.

30

15. A method of treating or preventing all forms of diabetes and related diseases, such as eating disorders, cardiovascular diseases, gastrointestinal diseases, diabetic complications, critical illness, and/or polycystic ovary syndrome; and/or for improving lipid parameters, improving β -cell function, and/or for delaying or preventing diabetic disease

progression, by administering a pharmaceutically active amount of a peptide or a derivative according to any one of claims 1-7.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2010/069929

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. (means)
- ☐ on paper
- ☒ in electronic form
- b. (time)
- ☒ in the international application as filed
- ☐ together with the international application in electronic form
- ☐ subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet (1)) (July 2009)

MPI EXHIBIT 1057 PAGE 91

DR. REDDY'S LABORATORIES, INC.

IPR2024-00009

Ex. 1057, p. 91 of 96

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/069929

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)

MPI EXHIBIT 1057 PAGE 92

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/069929

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/48 C07D233/64 C07K14/605
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 C07D C07K

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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, 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.
Y	WO 2004/067548 A2 (THERATECHNOLOGIES INC [CA]; PERI KRISHNA [CA]; HABI ABDELKRIM [CA]; GR) 12 August 2004 (2004-08-12) Table 5, Compounds 290 and 215; claims -----	1-15
Y	WO 99/43707 A1 (NOVO NORDISK AS [DK]) 2 September 1999 (1999-09-02) claims -----	1-15
X	WO 2009/083549 A1 (NOVO NORDISK AS [DK]; LAU JESPER FAERGEMAN [DK]; ANDERSEN ASSER SLOTH) 9 July 2009 (2009-07-09) claims 5, 7; example 2 -----	11
Y	----- -/--	1-15



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 document but published on or after the international filing date

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

"O" document referring to an 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

8 February 2011

Date of mailing of the international search report

24/02/2011

Name and mailing address of the ISA/

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Authorized officer

Huber, Angelika

Form PCT/ISA/210 (second sheet) (April 2005)

page 1 of 2

MPI EXHIBIT 1057 PAGE 93

DR. REDDY'S LABORATORIES, INC.

IPR2024-00009

Ex. 1057, p. 93 of 96

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/069929

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOLENSKY B ET AL: "New Building Blocks for Fluorinated Imidazole Derivatives: Preparation of α -Fluoro- and α,α -Difluorohistamine", JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY, EASTON.; US, vol. 66, no. 13, 29 June 2001 (2001-06-29), pages 4687-4691, XP002564373, ISSN: 0022-3263, DOI: DOI:10.1021/J00102415 [retrieved on 2001-06-06] page 4691, right-hand column, paragraph 2 -----	9
A	WO 2006/127948 A2 (SQUIBB BRISTOL MYERS CO [US]; EWING WILLIAM R [US]; MAPELLI CLAUDIO [U]) 30 November 2006 (2006-11-30) claims -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/069929

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004067548	A2	12-08-2004	NONE
WO 9943707	A1	02-09-1999	AT 466028 T 15-05-2010 AU 2610899 A 15-09-1999 EP 1062240 A1 27-12-2000 JP 2002506792 T 05-03-2002
WO 2009083549	A1	09-07-2009	CN 101910193 A 08-12-2010 EP 2238153 A1 13-10-2010 US 2010317057 A1 16-12-2010
WO 2006127948	A2	30-11-2006	AR 053495 A1 09-05-2007 AU 2006249869 A1 30-11-2006 CN 101282991 A 08-10-2008 EP 1883652 A2 06-02-2008 JP 2008542292 T 27-11-2008 KR 20080026117 A 24-03-2008 PE 14482006 A1 17-12-2006

Form PCT/ISA/210 (patent family annex) (April 2005)

MPI EXHIBIT 1057 PAGE 95

DR. REDDY'S LABORATORIES, INC.

IPR2024-00009

Ex. 1057, p. 95 of 96

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9, 11-15

GLP-1 receptor agonist peptide of the formula Y-Z-P and derivative thereof; intermediate compound for preparing the GLP-1 receptor agonist peptide of Claims 1-7; peptide intermediate products selected from analogues of GLP-1(9-37) and GLP-1A(3-37); use of the peptide or derivative as medicament for treating e.g. diabetes.

2. claim: 10

Compound of Chem. 40
