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(54) **GLP-1 ANALOGUES**

ANALOGUE DES GLUCAGON ÄHNLICHEN PEPTIDES-1 (GLP-1)

ANALOGUES GLP-1

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**EP 1 137 666 B9**

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**Description**Background of the Invention

5 **[0001]** The present invention is directed to peptide analogues of glucagon-like peptide-1, the pharmaceutically-acceptable salts thereof, to methods of using such analogues to treat mammals and to pharmaceutical compositions useful therefor comprising said analogues.

**[0002]** Glucagon-like peptide-1 (7-36) amide (GLP-1) is synthesized in the intestinal L-cells by tissue-specific post-translational processing of the glucagon precursor pre-proglucagon (Varndell, J.M., et al., J. Histochem Cytochem, 1985: 33:1080-6) and is released into the circulation in response to a meal. The plasma concentration of GLP-1 rises from a fasting level of approximately 15 pmol/L to a peak postprandial level of 40 pmol/L. It has been demonstrated that, for a given rise in plasma glucose concentration, the increase in plasma insulin is approximately threefold greater when glucose is administered orally compared with intravenously (Kreymann, B., et al., Lancet 1987:2, 1300-4). This alimentary enhancement of insulin release, known as the incretin effect, is primarily humoral and GLP-1 is now thought to be the most potent physiological incretin in humans. In addition to the insulinotropic effect, GLP-1 suppresses glucagon secretion, delays gastric emptying (Wettergren A., et al., Dig Dis Sci 1993:38:665-73) and may enhance peripheral glucose disposal (D'Alessio, D.A. et al., J. Clin Invest 1994:93:2293-6).

**[0003]** In 1994, the therapeutic potential of GLP-1 was suggested following the observation that a single subcutaneous (s/c) dose of GLP-1 could completely normalize postprandial glucose levels in patients with non-insulin-dependent diabetes mellitus (NIDDM) (Gutniak, M.K., et al., Diabetes Care 1994:17:1039-44). This effect was thought to be mediated both by increased insulin release and by a reduction in glucagon secretion. Furthermore, an intravenous infusion of GLP-1 has been shown to delay postprandial gastric emptying in patients with NIDDM (Williams, B., et al., J. Clin Endo Metab 1996:81:327-32). Unlike sulfonylureas, the insulinotropic action of GLP-1 is dependent on plasma glucose concentration (Holz, G.G. 4th, et al., Nature 1993:361:362-5). Thus, the loss of GLP-1-mediated insulin release at low plasma glucose concentration protects against severe hypoglycemia.

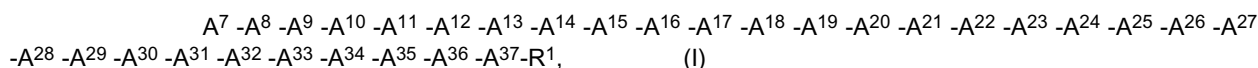
This combination of actions gives GLP-1 unique potential therapeutic advantages over other agents currently used to treat NIDDM.

Numerous studies have shown that when given to healthy subjects, GLP-1 potently influences glycemic levels as well as insulin and glucagon concentrations (Orskov, C, Diabetologia 35:701-711, 1992; Holst, J.J., et al., Potential of GLP-1 in diabetes management in Glucagon III, Handbook of Experimental Pharmacology, Lefevbre PJ, Ed. Berlin, Springer Verlag, 1996, p. 311-326), effects which are glucose dependent (Kreymann, B., et al., Lancet ii: 1300-1304, 1987; Weir, G.C., et al., Diabetes 38:338-342, 1989). Moreover, it is also effective in patients with diabetes (Gutniak, M., N. Engl J Med 226:1316-1322, 1992; Nathan, D.M., et al., Diabetes Care 15:270-276, 1992) normalizing blood glucose levels in type 2 diabetic subjects (Nauck, M.A., et al., Diabetologia 36:741-744, 1993), and improving glycemic control in type 1 patients (Creutzfeldt, W.O., et al., Diabetes Care 19:580-586, 1996), raising the possibility of its use as a therapeutic agent.

**[0004]** GLP-1 is, however, metabolically unstable, having a plasma half-life ( $t_{1/2}$ ) of only 1-2 min *in vivo*. Exogenously administered GLP-1 is also rapidly degraded (Deacon, C.F., et al., Diabetes 44:1126-1131, 1995). This metabolic instability limits the therapeutic potential of native GLP-1. Hence, there is a need for GLP-1 analogues that are more active or are more metabolically stable than native GLP-1. GLP-1 analogs have been described in prior art (see for example WO 91/11457). However, there is a need of new GLP-1 analogs.

Summary of the Invention

45 **[0005]** In one aspect, the present invention is directed to a compound of formula (I),



50 wherein

A<sup>7</sup> is, Ura, Paa, Pta or Hppa,

A<sup>8</sup> is Ala, D-Ala, Aib, Acc, N-Me-Ala, N-Me-D-Ala, Arg or N-Me-Gly;

A<sup>9</sup> is Glu, N-Me-Glu, N-Me-Asp or Asp;

A<sup>10</sup> is Gly, Acc, Ala, D-Ala, Phe or Aib;

55 A<sup>11</sup> is Thr or Ser;

A<sup>12</sup> is Phe, Acc, Aic, Aib, 3-Pal, 4-Pal, β-Nal, Cha, Trp or X<sup>1</sup>-Phe;

A<sup>13</sup> is Thr or Ser;

A<sup>14</sup> is Ser, Thr, Ala or Aib;

EP 1 137 666 B9

A<sup>15</sup> is Asp, Ala, D-Asp or Glu;

A<sup>16</sup> is Val, D-Val, Acc, Aib, Leu, Ile, Tle, Nle, Abu, Ala, D-Ala, Tba or Cha;

A<sup>17</sup> is Ser, Ala, D-Ala, Aib, Acc or Thr;

A<sup>18</sup> is Ser, Ala, D-Ala, Aib, Acc or Thr;

5 A<sup>19</sup> is Tyr, D-Tyr, Cha, Phe, 3-Pal, 4-Pal, Acc, β-Nal, Amp or X<sup>1</sup>-Phe;

A<sup>20</sup> is Leu, Ala, Acc, Aib, Nle, lie, Cha, Tle, Val, Phe or X<sup>1</sup>-Phe;

A<sup>21</sup> is Glu, Ala or Asp;

A<sup>22</sup> is Gly, Acc, Ala, D-Ala, β-Ala or Aib;

A<sup>23</sup> is Gln, Asp, Ala, D-Ala, Aib, Acc, Asn or Glu;

10 A<sup>24</sup> is Ala, Aib, Val, Abu, Tle or Acc;

A<sup>25</sup> is Ala, Aib, Val, Abu, Tle, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>m</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O) or HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O);

A<sup>26</sup> is Lys, Ala, 3-Pal, 4-Pal, Arg, hArg, Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O) or HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O);

A<sup>27</sup> is Glu, Ala, D-Ala or Asp;

A<sup>28</sup> is Phe, Ala, Pal, β-Nal, X<sup>1</sup>-Phe, Aic, Acc, Aib, Cha or Trp;

15 A<sup>28</sup> is lie, Acc, Aib, Leu, Nle, Cha, Tle, Val, Abu, Ala, Tba or Phe;

A<sup>30</sup> is Ala, Aib, Acc or deleted;

A<sup>31</sup> is Trp, Ala, β-Nal, 3-Pal, 4-Pal, Phe, Acc, Aib, Cha, Amp or deleted;

A<sup>32</sup> is Leu, Ala, Acc, Aib, Nle, lie, Cha, Tle, Phe, X<sup>1</sup>-Phe, Ala or deleted;

A<sup>33</sup> is Val, Acc, Aib, Leu, lie, Tle, Nle, Cha, Ala, Phe, Abu, X<sup>1</sup>-Phe, Tba, Gaba or deleted;

20 A<sup>34</sup> is Lys, Arg, hArg, Orn, Amp, Gaba, HN-CH((CH<sub>2</sub>)<sub>m</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) or deleted;

A<sup>35</sup> is Gly or deleted;

A<sup>36</sup> is L- or D-Arg, D- or L-Lys, D- or L-hArg, D- or L-Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) or deleted;

A<sup>37</sup> is Gly or deleted;

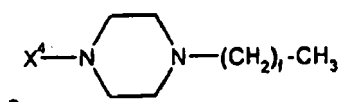
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X<sup>1</sup> for each occurrence is independently selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, OH and halo;

R<sup>1</sup> is OH, NH<sub>2</sub>, (C<sub>1</sub>-C<sub>12</sub>)alkoxy, or NH-X<sup>2</sup>-CH<sub>2</sub>-Z<sup>0</sup>, wherein X<sup>2</sup> is a (C<sub>1</sub>-C<sub>12</sub>)hydrocarbon moiety, and Z<sup>0</sup> is H, OH, CO<sub>2</sub>H or CONH<sub>2</sub>;

X<sup>3</sup> is

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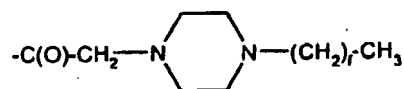
or -C(O)-NHR<sup>12</sup>, wherein X<sup>4</sup> for each occurrence is independently -C(O)-, -NH-C(O)- or -CH<sub>2</sub>-, and f for each occurrence is independently an integer from 1 to 29;

e for each occurrence is independently an integer from 1 to 4;

n for each occurrence is independently an integer from 1-5; and

40 R<sup>10</sup> and R<sup>11</sup> for each occurrence is each independently H, (C<sub>1</sub>-C<sub>30</sub>)alkyl, (C<sub>1</sub>-C<sub>30</sub>)acyl, (C<sub>1</sub>-C<sub>30</sub>)alkylsulfonyl, -C((NH)(NH<sub>2</sub>)) or

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provided that when R<sup>10</sup> is (C<sub>1</sub>-C<sub>30</sub>)acyl, (C<sub>1</sub>-C<sub>30</sub>)alkylsulfonyl, -C((NH)(NH<sub>2</sub>)) or

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R<sup>11</sup> is H or (C<sub>1</sub>-C<sub>30</sub>)alkyl; and

R<sup>12</sup> is (C<sub>1</sub>-C<sub>30</sub>)alkyl;

or a pharmaceutically acceptable salt thereof.

**[0006]** A preferred compound of the immediately foregoing compound of formula (I) is where A<sup>11</sup> is Thr; A<sup>13</sup> is Thr; A<sup>14</sup> is Ser, Aib or Ala; A<sup>17</sup> is Ser, Ala, Aib or D-Ala; A<sup>18</sup> is Ser, Ala, Aib or D-Ala; A<sup>21</sup> is Glu or Ala; A<sup>23</sup> is Gln, Glu, or Ala; and A<sup>27</sup> is Glu or Ala; or a pharmaceutically acceptable salt thereof.

**[0007]** A preferred compound of the immediately foregoing compound of formula (I) is where A<sup>9</sup> is Glu, N-Me-Glu or N-Me-Asp; A<sup>12</sup> is Phe, Acc or Aic; A<sup>16</sup> is Val, D-Val, Acc, Aib, Ala, Tle or D-Ala; A<sup>19</sup> is Tyr, 3-Pal, 4-Pal or D-Tyr; A<sup>20</sup> is Leu, Acc, Cha, Ala or Tle; A<sup>24</sup> is Ala, Aib or Acc; A<sup>25</sup> is Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH(CH<sub>2</sub>)<sub>n</sub>-NH-R<sup>10</sup>-C(O); A<sup>28</sup> is Phe or Ala; A<sup>29</sup> is Ile, Acc or Tle; A<sup>30</sup> is Ala, Aib or deleted; A<sup>31</sup> is Trp, Ala, 3-Pal, 4-Pal or deleted; A<sup>32</sup> is Leu, Acc, Cha, Ala or deleted; A<sup>33</sup> is Val, Acc, Ala, Gaba, Tle or deleted; or a pharmaceutically acceptable salt thereof.

**[0008]** A preferred compound of the immediately foregoing compound of formula (I) is where A<sup>8</sup> is Ala, D-Ala, Aib, A6c, A5c, N-Me-Ala, N-Me-D-Ala or N-Me-Gly; A<sup>10</sup> is Gly, Ala, D-Ala or Phe; A<sup>12</sup> is Phe, A6c or A5c; A<sup>16</sup> is Val, Ala, Tle, A6c, A5c or D-Val; A<sup>20</sup> is Leu, A6c, A5c, Cha, Ala or Tle; A<sup>22</sup> is Gly, Aib, β-Ala, L-Ala or D-Ala; A<sup>24</sup> is Ala or Aib; A<sup>29</sup> is Ile, A6c, A5c or Tle; A<sup>32</sup> is Leu, A6c, A5c, Cha, Ala or deleted; A<sup>33</sup> is Val, A6c, A5c, Ala, Gaba, Tle or deleted; or a pharmaceutically acceptable salt thereof.

**[0009]** A preferred compound of the immediately foregoing compound of formula (I) is where R<sup>1</sup> is OH or NH<sub>2</sub> or a pharmaceutically acceptable salt thereof.

**[0010]** A most preferred compound of formula (I) is where said compound is [Hppa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> or a pharmaceutically acceptable salt thereof.

**[0011]** Another most preferred compound of formula (I) is wherein said compound is [Ura<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; [Paa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; [Pta<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; or a pharmaceutically acceptable salt thereof.

**[0012]** In another aspect, the present invention provides a pharmaceutical composition comprising an effective amount of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.

**[0013]** In still another aspect, the present invention provides a use of compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof in the preparation of a medicament for eliciting an agonist effect from a GLP-1 receptor in a subject in need thereof.

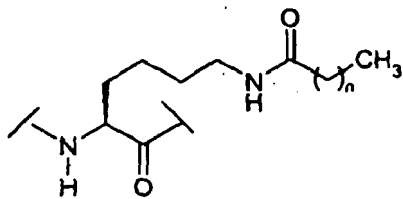
**[0014]** In yet a further aspect, this invention provides a use of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof in the preparation of a medicament for treating a disease selected from the group consisting of Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system disease, restenosis and neurodegenerative disease, renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, and hypertension, in a subject in need thereof which comprises administering to said subject an effective amount. Preferred of the foregoing uses is where the disease is Type I diabetes or Type II diabetes.

**[0015]** With the exception of the N-terminal amino acid, all abbreviations (e.g. Ala) of amino acids in this disclosure stand for the structure of -NH-CH(R)-CO-, wherein R is the side chain of an amino acid (e.g., CH<sub>3</sub> for Ala). For the N-terminal amino acid, the abbreviation stands for the structure of (R<sup>2</sup>R<sup>3</sup>)-N-CH(R)-CO-, wherein R is a side chain of an amino acid and R<sup>2</sup> and R<sup>3</sup> are as defined above except in the case where A<sup>7</sup> is Ura, Paa, Pta or Hppa in which case R<sup>2</sup> and R<sup>3</sup> are not present since Ura, Paa, Pta and Hppa are considered here as des-amino amino acids. The abbreviations: β-Nal, Nle, Cha, Amp, 3-Pal, 4-Pal and Aib stand for the following α-amino acids: β-(2-naphthyl)alanine, norleucine, cyclohexylalanine, 4-amino-phenylalanine, β-(3-pyridinyl)alanine, β-(4-pyridinyl)alanine and α-aminoisobutyric acid, respectively. Other amino acid definitions are: Ura is urocanic acid; Pta is (4-pyridylthio) acetic acid; Paa is trans-3-(3-pyridyl) acrylic acid; Tma-His is N,N-tetramethylamidino-histidine; N-Me-Ala is N-methyl-alanine; N-Me-Gly is N-methyl-glycine; N-Me-Glu is N-methyl-glutamic acid; Tle is *tert*-butylglycine; Abu is α-aminobutyric acid; Tba is *tert*-butylalanine; Orn is ornithine; Aib is α-aminoisobutyric acid; β-Ala is β-alanine; Gaba is γ-aminobutyric acid; Ava is 5-aminovaleric acid; and Aic is 2-aminoindane-2-carboxylic acid.

**[0016]** What is meant by Acc is an amino acid selected from the group of 1-amino-1-cyclopropanecarboxylic acid (A3c); 1-amino-1-cyclobutanecarboxylic acid (A4c); 1-amino-1-cyclopentanecarboxylic acid (A5c); 1-amino-1-cyclohexanecarboxylic acid (A6c); 1-amino-1-cycloheptanecarboxylic acid (A7c); 1-amino-1-cyclooctanecarboxylic acid (A8c); and 1-amino-1-cyclononanecarboxylic acid (A9c). In the above formula, hydroxyalkyl, hydroxyphenylalkyl, and hydroxynaphthylalkyl may contain 1-4 hydroxy substituents. COX<sup>5</sup> stands for -C=O·X<sup>5</sup>. Examples of -C=O·X<sup>5</sup> include, but are not limited to, acetyl and phenylpropionyl.

**[0017]** What is meant by Lys(N<sup>ε</sup>-alkanoyl) is represented by the following structure:

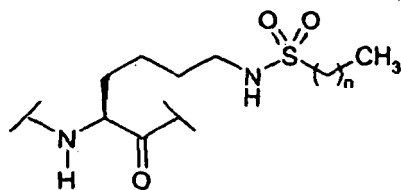
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What is meant by Lys(N<sup>ε</sup>-alkylsulfonyl) is represented by the following structure:

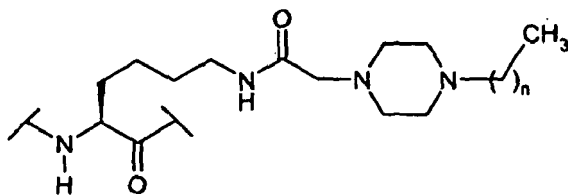
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What is meant by Lys(N<sup>ε</sup>-(2-(4-alkyl-1-piperazine)-acetyl)) is represented by the following structure:

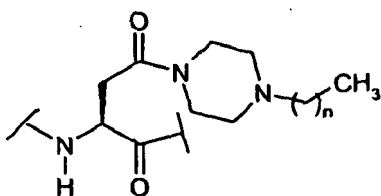
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What is meant by Asp(1-(4-alkyl-piperazine)) is represented by the following structure:

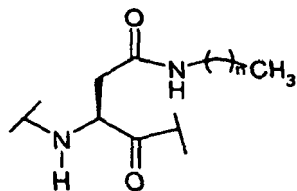
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What is meant by Asp(1-alkylamino) is represented by the following structure:

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55 The variable n in the foregoing structures is 1 to 30.

**[0018]** The full names for other abbreviations used herein are as follows: Boc for t-butyloxycarbonyl, HF for hydrogen fluoride, Fm for formyl, Xan for xanthyl, Bzl for benzyl, Tos for tosyl, DNP for 2,4-dinitrophenyl, DMF for dimethylformamide, DCM for dichloromethane, HBTU for 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, DIEA

for diisopropylethylamine, HOAc for acetic acid, TFA for trifluoroacetic acid, 2CIZ for 2-chlorobenzoyloxycarbonyl and OcHex for O-cyclohexyl.

**[0019]** A peptide of this invention is also denoted herein by another format, e.g., [A5c<sup>8</sup>]hGLP-1 (7-36)NH<sub>2</sub>, with the substituted amino acids from the natural sequence placed between the set of brackets (e.g., A5c<sup>8</sup> for Ala<sup>8</sup> in hGLP-1). The abbreviation GLP-1 means glucagon-like peptide-1, and hGLP-1 means human glucagon-like peptide-1. The numbers between the parentheses refer to the number of amino acids present in the peptide (e.g., hGLP-1 (7-36) is amino acids 7 through 36 of the peptide sequence for human GLP-1). The sequence for hGLP-1 (7-37) is listed in Mojsov, S., *Int. J. Peptide Protein Res.*, 40, 1992, pp. 333-342. The designation "NH<sub>2</sub>" in hGLP-1(7-36)NH<sub>2</sub> indicates that the C-terminus of the peptide is amidated. hGLP-1 (7-36) means that the C-terminus is the free acid.

#### Detailed Description

**[0020]** The peptides of this invention can be prepared by standard solid phase peptide synthesis. See, e.g., Stewart, J.M., et al., *Solid Phase Synthesis* (Pierce Chemical Co., 2d ed. 1984).

**[0021]** When R<sup>1</sup> is NH-X<sup>2</sup>-CH<sub>2</sub>-CONH<sub>2</sub> (i.e., Z<sup>0</sup>=CONH<sub>2</sub>), the synthesis of the peptide starts with BocHN-X<sup>2</sup>-CH<sub>2</sub>COOH which is coupled to the MBHA resin. If R<sup>1</sup> is NH-X<sup>2</sup>-CH<sub>2</sub>-COOH (i.e., Z<sup>0</sup>=COOH) the synthesis of the peptide starts with Boc-HN-X<sup>2</sup>-CH<sub>2</sub>-COOH which is coupled to PAM resin.

**[0022]** The following describes a synthetic method for making a peptide of this invention, which method is well-known to those skilled in the art. Other methods are also known to those skilled in the art.

**[0023]** Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc., Louisville, KY) (0.9 g, 0.3 mmole) in the chloride ion form is placed in a reaction vessel of an Advanced ChemTech Peptide Synthesizer Model 200 programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 15 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% diisopropylethylamine in methylene chloride.

**[0024]** The neutralized resin is stirred with Boc-protected amino acid which is to be the C-terminal amino acid of the desired peptide to be synthesized and diisopropylcarbodiimide (3 mmole each) in methylene chloride for 1 hour and the resulting amino acid resin is then cycled through steps (a) through (f) in the above wash program. The other amino acids (3 mmol) of the desired peptide are then coupled successively by the same procedure. The finished peptide is cleaved from the resin by mixing it with anisole (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at about 0 °C and stirring it for about 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is then dissolved in a minimum volume of dilute acetic acid and eluted on a column (2.5 x 25 cm) of VYDAC® octadecylsilane silica (10 mM) and eluted with a linear gradient of 20-60% acetonitrile over about 1 h in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography (40-70% B at 1%/min, solution B is 80% acetonitrile/water containing 0.1% TFA) and pooled to give maximum purity rather than yield. Repeated lyophilization of the solution from water gives the product as a white, fluffy powder.

**[0025]** The product peptide is analyzed by HPLC. Amino acid analysis of an acid hydrolysate of the product peptide can confirm the composition of the peptide. Laser desorption MS is used to determine the molecular weight of the peptide.

**[0026]** The protected amino acid 1-[N-tert-butoxycarbonyl-amino]-1-cyclohexanecarboxylic acid (Boc-A6c-OH) was synthesized as follows. 19.1 g (0.133 mol) of 1-amino-1-cyclohexanecarboxylic acid (Acros Organics, Fisher Scientific, Pittsburgh, PA) was dissolved in 200 ml of dioxane and 100 ml of water. To it was added 67 ml of 2N NaOH. The solution was cooled in an ice-water bath. 32.0 g (0.147 mol) of di-tert-butylidicarbonate was added to this solution. The reaction mixture was stirred overnight at room temperature. Dioxane was then removed under reduced pressure. 200 ml of ethyl acetate was added to the remaining aqueous solution. The mixture was cooled in an ice-water bath. The pH of the aqueous layer was adjusted to about 3 by adding 4N HCl. The organic layer was separated. The aqueous layer was extracted with ethyl acetate (1 x 100 ml). The two organic layers were combined and washed with water (2 x 150 ml), dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to dryness under reduced pressure. The residue was recrystallized in ethyl acetate/hexanes. 9.2 g of the pure product was obtained. 29% yield.

**[0027]** Boc-A5c-OH was synthesized in an analogous manner to that of Boc-A6c-OH. Other protected Acc amino acids can be prepared in an analogous manner by a person of ordinary skill in the art as enabled by the teachings herein.

**[0028]** In the synthesis of a peptide of this invention containing A5c, A6c and/or Aib, the coupling time is about 2 hrs. for these residues and the residue immediately following them. For the synthesis of [Tma-His<sup>7</sup>]hGLP-1(7-36)NH<sub>2</sub>, HBTU (2 mmol) and DIEA (1.0 ml) in 4 ml DMF were used to react with the N-terminal free amine of the peptide-resin in the last coupling reaction; the coupling time is about 2 hours.

**[0029]** The full names for the abbreviations used above are as follows: Boc for t-butyloxycarbonyl, HF for hydrogen fluoride, Fm for formyl, Xan for xanthyl, Bzl for benzyl, Tos for tosyl, DNP for 2,4-dinitrophenyl, DMF for dimethylformamide, DCM for dichloromethane, HBTU for 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, DIEA for diisopropylethylamine, HOAc for acetic acid, TFA for trifluoroacetic acid, 2CIZ for 2-chlorobenzoyloxycarbonyl, 2BrZ

## EP 1 137 666 B9

for 2-bromobenzyloxycarbonyl and OcHex for O-cyclohexyl.

**[0030]** A compound of the present invention can be tested for activity as a GLP-1 binding compound according to the following procedure.

### 5 Cell Culture:

**[0031]** RIN 5F rat insulinoma cells (ATCC-# CRL-2058, American Type Culture Collection, Manassas, VA), expressing the GLP-1 receptor, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, and maintained at about 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>/95% air.

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### Radioligand Binding:

**[0032]** Membranes were prepared for radioligand binding studies by homogenization of the RIN cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 g / 10 min), and the final pellets were re-suspended in 50 mM Tris-HCl, containing 2.5 mM MgCl<sub>2</sub>, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% BSA. For assay, aliquots (0.4 ml) were incubated with 0.05 nM [<sup>125</sup>I]GLP-1(7-36) (~2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing test peptides. After a 100 min incubation (25 °C), the bound [<sup>125</sup>I]GLP-1(7-36) was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac LKB, Gaithersburg, MD). Specific binding was defined as the total [<sup>125</sup>I]GLP-1(7-36) bound minus that bound in the presence of 1000 nM GLP1 (7-36) (Bachem, Torrance, CA).

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**[0033]** The peptides of this invention can be provided in the form of pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, those formed with organic acids (e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, methanesulfonic, toluenesulfonic, or pamoic acid), inorganic acids (e.g., hydrochloric acid, sulfuric acid, or phosphoric acid), and polymeric acids (e.g., tannic acid, carboxymethyl cellulose, polylactic, polyglycolic, or copolymers of polylactic-glycolic acids). A typical method of making a salt of a peptide of the present invention is well known in the art and can be accomplished by standard methods of salt exchange. Accordingly, the TFA salt of a peptide of the present invention (the TFA salt results from the purification of the peptide by using preparative HPLC, eluting with TFA containing buffer solutions) can be converted into another salt, such as an acetate salt by dissolving the peptide in a small amount of 0.25 N acetic acid aqueous solution. The resulting solution is applied to a semi-prep HPLC column (Zorbax, 300 SB, C-8). The column is eluted with (1) 0.1N ammonium acetate aqueous solution for 0.5 hrs., (2) 0.25N acetic acid aqueous solution 0.5 hrs. and (3) a linear gradient (20% to 100% of solution B over 30 min.) at a flow rate of 4 ml/min (solution A is 0.25N acetic acid aqueous solution; solution B is 0.25N acetic acid in acetonitrile/water, 80:20). The fractions containing the peptide are collected and lyophilized to dryness.

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**[0034]** As is well known to those skilled in the art, the known and potential uses of GLP-1 is varied and multitudinous [See, Todd, J.F., et al., Clinical Science, 1998, 95, pp. 325-329; and Todd, J.F. et al., European Journal of Clinical investigation. 1997, 27, pp.533-536]. Thus, the administration of the compounds of this invention for purposes of eliciting an agonist effect can have the same effects and uses as GLP-1 itself. These varied uses of GLP-1 may be summarized as follows, treatment of: Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system diseases, restenosis and neurodegenerative diseases. GLP-1 analogues of the present invention that elicit an antagonist effect from a subject can be used for treating the following: hypoglycemia and malabsorption syndrome associated with gastroectomy or small bowel resection.

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**[0035]** Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of formula (I) in association with a pharmaceutically acceptable carrier or diluent.

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**[0036]** The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. In general, an effective dosage for the activities of this invention is in the range of 1x10<sup>-7</sup> to 200 mg/kg/day, preferably 1x10<sup>-4</sup> to 100 mg/kg/day, which can be administered as a single dose or divided into multiple doses.

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**[0037]** The compounds of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

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**[0038]** Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as

sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

**[0039]** Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

**[0040]** Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and com oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

**[0041]** Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax.

**[0042]** Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

**[0043]** Further, a compound of this invention can be administered in a sustained release composition such as those described in the following patents and patent applications. U.S. Patent No. 5,672,659 teaches sustained release compositions comprising a bioactive agent and a polyester. U.S. Patent No. 5,595,760 teaches sustained release compositions comprising a bioactive agent in a gelable form. U.S. Application No. 08/929,363 filed September 9, 1997, teaches polymeric sustained release compositions comprising a bioactive agent and chitosan. U.S. Application No. 08/740,778 filed November 1, 1996, teaches sustained release compositions comprising a bioactive agent and cyclodextrin. U.S. Application No. 09/015,394 filed January 29, 1998, teaches absorbable sustained release compositions of a bioactive agent. U.S. Application No. 09/121,653 filed July 23, 1998, teaches a process for making microparticles comprising a therapeutic agent such as a peptide in an oil-in-water process. U.S. Application No. 09/131,472 filed August 10, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a phosphorylated polymer. U.S. Application No. 09/184,413 filed November 2, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a polymer bearing a non-polymerizable lactone.

**[0044]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

**[0045]** The following examples describe synthetic methods for making a peptide of this invention, which methods are well-known to those skilled in the art. Other methods are also known to those skilled in the art. The examples are provided for the purpose of illustration.

#### Example 1

[D-Ala<sup>8</sup>, Ala<sup>17,22,23,27</sup>, 3-Pal<sup>19,31</sup>, Gaba<sup>34</sup>]-GLP-1(7-34)NH<sub>2</sub>

**[0046]** Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc. Louisville, KY) (0.9 g, 0.3 mmole) in the chloride ion form was placed in a reaction vessel of an Advanced ChemTech peptide synthesizer Model 200 programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 15 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% diisopropylethylamine in methylene chloride.

**[0047]** The neutralized resin was stirred with Boc-Gaba and diisopropylcarbodiimide (3 mmole each) in methylene chloride for 1 hour and the resulting amino acid resin was then cycled through steps (a) to (f) in the above wash program. The following amino acids (3 mmole) were then coupled successively by the same procedure: Boc-Val, Boc-Leu, Boc-3-Pal, Boc-Ala, Boc-Ile, Boc-Phe, Boc-Ala, Boc-Lys(2-Cl-Z), Boc-Ala, Boc-Ala, Boc-Ala, Boc-Ala, Boc-Glu(Bzl), Boc-Leu, Boc-3-Pal, Boc-Ser(Bzl), Boc-Ala, Boc-Val, Boc-Asp(Bzl), Boc-Ser(Bzl), Boc-Thr(Bzl), Boc-Phe, Boc-Thr(Bzl), Boc-Gly, Boc-Glu(Bzl), Boc-D-Ala, Boc-His(Bom).

**[0048]** The resin with the completed peptide sequence was mixed with anisole (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at about 0 °C and stirred for about 45 min. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide was then dissolved in a minimum volume of dilute acetic acid and eluted on a column (2.5 x 25 cm) of VYDAC® octadecylsilane silica (10 mM) and eluted with a linear gradient of 20-60% acetonitrile over about 1 h in 0.1% trifluoroacetic acid in water. Fractions were examined by thin layer chromatography and analytical high performance liquid chromatography (40-70%

B at 1%/main: r.t.: 14.1 min) and pooled to give maximum purity rather than yield. Repeated lyophilization of the solution from water gives the product (49.9 mg) as a white, fluffy powder.

**[0049]** The product was found to be homogeneous by HPLC and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide. Laser desorption MS gave a MW of 2880 (Calc. M+H 2873).

### Example 2

#### Synthesis of Peptide Lower-Alkylamides

**[0050]** Peptides are assembled on O-benzyl-polystyrene resin (often referred to as Merrifield resin) using the Boc amino acid protocol described in Example 1, except that Asp and Glu amino acid carboxyl side-chains are protected with an Fm (fluorenylmethyl ester) group. Completed peptide-resins are suspended in dilute DMF solutions of an appropriate lower alkylamine (such as ethylamine, propylamine, phenethylamine, 1,2-diaminoethane, etc.) and stirred at about 60 °C (for about 18 hrs) whereupon filtration, removal of solvents under reduced pressure and trituration of cleaved peptide oil with ether gives a solid, protected alkylamide peptide. This is then subjected to HF cleavage to remove additional side chain protecting groups and HPLC purification as described in Example 1.

### Example 11

**[0051]** Examples 11 as made substantially according to the procedure described for Example 1 but using the appropriate protected amino acid to yield the noted peptide. MS were obtained by laser desorption MS (NA means not available). Example 11: [Hppa<sup>7</sup>]hGLP-1 (7-36)-NH<sub>2</sub>; MS = NA

### Example 12

[Aib<sup>8</sup>, A6c<sup>32</sup>]hGLP-1 (7-36)NH<sub>2</sub>

**[0052]** The title peptide was synthesized on an Applied Biosystems (Foster City, CA) model 430A peptide synthesizer which was modified to do accelerated Boc-chemistry solid phase peptide synthesis. See Schnolzer, et al., Int. J. Peptide Protein Res., 40:180 (1992). 4-Methylbenzhydrylamine (MBHA) resin (Peninsula, Belmont, CA) with the substitution of 0.91 mmol/g was used. The Boc amino acids (Bachem, CA, Torrance, CA; Nova Biochem., LaJolla, CA) were used with the following side chain protection: Boc-Ala-OH, Boc-Arg(Tos)-OH, Boc-Asp(OcHex)-OH, Boc-Tyr(2BrZ)-OH, Boc-His(DNP)-OH, Boc-Val-OH, Boc-Leu-OH, Boc-Gly-OH, Boc-Gln-OH, Boc-ile-OH, Boc-Lys(2ClZ)-OH, Boc-Thr(Bzl)-OH, Boc-A6c-OH, Ser(Bzl)-OH, Boc-Phe-OH, Boc-Aib-OH, Boc-Glu(OcHex)-OH and Boc-Trp(Fm)-OH. The synthesis was carried out on a 0.20 mmol scale. The Boc groups were removed by treatment with 100% TFA for 2 x 1 min. Boc amino acids (2.5 mmol) were pre-activated with HBTU (2.0 mmol) and DIEA (1.0 ml) in 4 ml of DMF and were coupled without prior neutralization of the peptide-resin TFA salt. Coupling times were about 5 min except for the Boc-Aib-OH and Boc-A6c-OH residues and the following residues, Boc-Trp(Fm)-OH and Boc-His(DNP)-OH wherein the coupling times were about 2 hours.

**[0053]** At the end of the assembly of the peptide chain, the resin was treated with a solution of 20% mercaptoethanol/ 10% DIEA in DMF for 2 x 30 min to remove the DNP group on the His side chain. The N-terminal Boc group was then removed by treatment with 100% TFA for 2 x 2 min. After neutralization of the peptide-resin with 10% DIEA in DMF (1 x 1 min), the formal group on the side of the chain of Trp was removed by treatment with a solution of 15% ethanolamine/ 15% water/ 70% DMF for 2 x 30 min. The partially-deprotected peptide-resin was washed with DMF and DCM and dried under reduced pressure. The final cleavage was done by stirring the peptide-resin in 10 ml of HF containing 1 ml of anisole and dithiothreitol (24 mg) at 0 °C for about 75 min. HF was removed with a flow of nitrogen. The residue was washed with ether (6 x 10 ml) and extracted with 4N HOAc (6 x 10 ml).

**[0054]** The peptide mixture in the aqueous extract was purified on a reverse-phase preparative high pressure liquid chromatography (HPLC) using a reverse phase VYDAC® C<sub>18</sub> column (Nest Group, Southborough, MA). The column was eluted with a linear gradient (20% to 50% of solution B over 105 min) at a flow rate of 10 ml/min (Solution A = water containing 0.1% TFA; Solution B = acetonitrile containing 0.1% of TFA). Fractions were collected and checked on analytical HPLC. Those containing pure product were combined and lyophilized to dryness. 92 mg of a white solid was obtained. Purity was >99% based on analytical HPLC analysis. Electro-spray mass spectrometer analysis gave the molecular weight at 3324.2 (the calculated molecular weight is 3323.7).

**[0055]** The synthesis of other compounds of the present invention can be carried out in the same manner as described for the synthesis of [Aib<sup>8</sup>, A6c<sup>32</sup>]hGLP-1(7-36)NH<sub>2</sub> in Example 12 above but using the appropriate protected amino acids depending on the desired peptide.

**[0056]** [(N<sup>α</sup>-HEPES-His)<sup>7</sup>]hGLP-1(7-36)NH<sub>2</sub> {HEPES is (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)} can be

synthesized as follows: After assembly of the peptide long chain on MBHA resin (0.20 mmol), the peptide-resin is treated with 100% TFA (2 x 2 min.) and washed with DMF and DCM. The resin is then neutralized with 10% DIEA in DMF for about 2 min. After washing with DMF and DCM, the resin is treated with 0.23 mmol of 2-chloro-1-ethanesulfonyl chloride and 0.7 mmol of DIEA in DMF for about 1 hour. The resin is washed with DMF and DCM and treated with 1.2 mmol of 2-hydroxyethylpiperazine for about 2 hours. The resin is washed with DMF and DCM and treated with different reagents ((1) 20% mercaptoethanol / 10% DIEA in DMF and (2) 15% ethanolamine / 15% water / 70% DMF) to remove the DNP group from the His side chain and formyl group on the Trp side chain as described above before the final HF cleavage of the peptide from the resin.

**[0057]** [(N<sup>α</sup>-HEPA-His)<sup>7</sup>]hGLP-1(7-36)NH<sub>2</sub>, ([[4-(2-hydroxyethyl)-1-piperazineacetyl]-His<sup>7</sup>]hGLP-1(7-36)NH<sub>2</sub>), can be made substantially according to the procedure described immediately above for making [(N<sup>α</sup>-HEPES-His)<sup>7</sup>]hGLP-1(7-36)NH<sub>2</sub> except that 2-bromo-acetic anhydride is used in place of 2-chloro-1-ethanesulfonyl chloride.

#### Examples 13 - 15

**[0058]** Examples 13-15 were made substantially according to Example 12 but using the appropriate protected amino acid.

Example 13 : [Ura<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; MS = 3279.5; Calc. MW = 3280.7.

Example 14 : [Paa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; MS = 3290.9; Calc. MW = 3291.8.

Example 15 : [Pta<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; MS = 3311.2; Calc. MW = 3311.8.

#### Example 16

[Aib<sup>8</sup>, A6c<sup>32</sup>, Lys<sup>36</sup>(N<sup>ε</sup>-tetradecanoyl)]hGLP-1(7-36)NH<sub>2</sub>

**[0059]** The Boc amino acids to be used are the same as those in the synthesis of [Aib<sup>8</sup>, A6c<sup>32</sup>]hGLP-1(7-36)NH<sub>2</sub> (Example 12) except that Fmoc-Lys(Boc)-OH is used here for the Lys<sup>36</sup>(N<sup>ε</sup>-tetradecanoyl) residue. The first amino acid residue is coupled to the resin manually on a shaker. 2.5 mmol of Fmoc-Lys(Boc)-OH is dissolved in 4 ml of 0.5N HBTU in DMF. To the solution is added 1 ml of DIEA. The mixture is shaken for about 2 min. To the solution is then added 0.2 mmol of MBHA resin (substitution = 0.91 mmol/g). The mixture is shaken for about 1 hr. The resin is washed with DMF and treated with 100% TFA for 2x2 min to remove the Boc protecting group. The resin is washed with DMF. Myristic acid (2.5 mmol) is pre-activated with HBTU (2.0 mmol) and DIEA (1.0 ml) in 4 ml of DMF for 2 min and is coupled to the Fmoc-Lys-resin. The coupling time is about 1 hr. The resin is washed with DMF and treated with 25% piperidine in DMF for 2x20 min to remove the Fmoc protecting group. The resin is washed with DMF and transferred to the reaction vessel of the peptide synthesizer. The remainder of the synthesis and purification procedures of the peptide are the same as those in the synthesis of [Aib<sup>8</sup>, A6c<sup>32</sup>]hGLP-1(7-36)NH<sub>2</sub>.

**[0060]** The syntheses of other compounds containing Lys(N<sup>ε</sup>-alkanoyl) residue are carried out in an analogous manner as described for the synthesis of [Aib<sup>8</sup>, A6c<sup>32</sup>, Lys<sup>36</sup>(N<sup>ε</sup>-octanoyl)]hGLP-1(7-36)NH<sub>2</sub>. Fmoc-Lys(Boc)-OH amino acid is used for the residue of Lys(N<sup>ε</sup>-alkanoyl) in the peptide, while Boc-Lys(2ClZ)-OH amino acid is used for the residue of Lys. If the Lys(N<sup>ε</sup>-alkanoyl) residue is not at the C-terminus, the peptide fragment immediately prior to the Lys(N<sup>ε</sup>-alkanoyl) residue is assembled on the resin on the peptide synthesizer first.

#### Example 17

[Aib<sup>8</sup>, Arg<sup>26,34</sup>, A6c<sup>32</sup>, Lys<sup>36</sup>(N<sup>ε</sup>-tetradecanoyl)]hGLP-1(7-36)-OH

**[0061]** The Boc amino acids to be used are the same as those used in the synthesis of [Aib<sup>8</sup>, A6c<sup>32</sup>, Lys<sup>36</sup>(N<sup>ε</sup>-tetradecanoyl)]hGLP-1(7-36)NH<sub>2</sub> (Example 16). Fmoc-Lys(Boc)-OH (2.5 mmol) is pre-activated with HBTU (2.0 mmol), HOBT (2.0 mmol) and DIEA (2.5 ml) in DMF (4 ml) for about 2 min. This amino acid is coupled to 235 mg of PAM resin (Chem-Impex, Wood Dale, IL; substitution = 0.85 mmol/g) manually on a shaker. The coupling time is about 8 hrs. The remainder of the synthesis and purification procedures for making the peptide are the same as those described in Example 52.

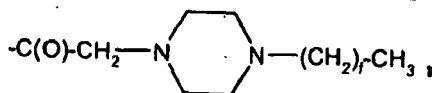
**[0062]** The syntheses of other analogs of hGLP-1(7-36)-OH and hGLP-1(7-37)-OH, which contain Lys(N<sup>ε</sup>-alkanoyl) residue, are carried out in an analogous manner as described for the synthesis of [Aib<sup>8</sup>, Arg<sup>26,34</sup>, A6c<sup>32</sup>, Lys<sup>36</sup>(N<sup>ε</sup>-tetradecanoyl)]hGLP-1(7-36)-OH. Fmoc-Lys(Boc)-OH amino acid is used for the residue of Lys(N<sup>ε</sup>-alkanoyl) in the peptide, while Boc-Lys(2ClZ)-OH amino acid is used for the residue of Lys.



-C((NH)(NH<sub>2</sub>)) or



provided that when R<sup>10</sup> is (C<sub>1</sub>-C<sub>30</sub>)acyl, (C<sub>1</sub>-C<sub>30</sub>)alkylsulfonyl, -C((NH)(NH<sub>2</sub>)) or



R<sup>11</sup> is H or (C<sub>1</sub>-C<sub>30</sub>)alkyl; and

R<sup>12</sup> is (C<sub>1</sub>-C<sub>30</sub>)alkyl,

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 or a pharmaceutically acceptable salt thereof wherein A<sup>11</sup> is Thr; A<sup>13</sup> is Thr; A<sup>14</sup> is Ser, Aib or Ala; A<sup>17</sup> is Ser, Ala, Aib or D-Ala; A<sup>18</sup> is Ser, Ala, Aib or D-Ala; A<sup>21</sup> is Glu or Ala; A<sup>23</sup> is Gln, Glu, or Ala; and A<sup>27</sup> is Glu or Ala.
3. A compound according to claim 2 or a pharmaceutically acceptable salt thereof wherein A<sup>9</sup> is Glu, N-Me-Glu or N-Me-Asp; A<sup>12</sup> is Phe, Acc or Aic; A<sup>16</sup> is Val, D-Val, Acc, Aib, Ala, Tle or D-Ala; A<sup>19</sup> is Tyr, 3-Pal, 4-Pal or D-Tyr, A<sup>20</sup> is Leu, Acc, Cha, Ala or Tle; A<sup>24</sup> is Ala, Aib or Acc; A<sup>25</sup> is Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NH-R<sup>10</sup>)-C(O); A<sup>28</sup> is Phe or Ala; A<sup>29</sup> is Ile, Acc or Tle; A<sup>30</sup> is Ala, Aib or deleted; A<sup>31</sup> is Trp, Ala, 3-Pal, 4-Pal or deleted; A<sup>32</sup> is Leu, Acc, Cha, Ala or deleted; A<sup>33</sup> is Val, Acc, Ala, Gaba, Tle or deleted.
4. A compound according to claim 3 or a pharmaceutically acceptable salt thereof wherein A<sup>8</sup> is Ala, D-Ala, Aib, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c), N-Me-Ala, N-Me-D-Ala or N-Me-Gly; A<sup>10</sup> is Gly, Ala, D-Ala or Phe; A<sup>12</sup> is Phe, 1-amino-1-cyclohexanecarboxylic acid (A6c) or 1-amino-1-cyclopentanecarboxylic acid (A5c); A<sup>16</sup> is Val, Ala, Tle, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c) or D-Val; A<sup>20</sup> is Leu, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c), Cha, Ala or Tle; A<sup>22</sup> is Gly, Aib, β-Ala, L-Ala or D-Ala; A<sup>24</sup> is Ala or Aib; A<sup>29</sup> is Ile, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c) or Tle; A<sup>32</sup> is Leu, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c), Cha, Ala or deleted; A<sup>33</sup> is Val, 1-amino-1-cyclohexanecarboxylic acid (A6c), 1-amino-1-cyclopentanecarboxylic acid (A5c), Ala, Gaba, Tle or deleted.
5. A compound according to claim 4 or a pharmaceutically acceptable salt thereof wherein R<sup>1</sup> is OH or NH<sub>2</sub>.
6. A compound according to claim 1 wherein said compound is [Hppa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>(SEQ ID NO: 87) or a pharmaceutically acceptable salt thereof.
7. A compound according to claim 1 wherein said compound is [Ura<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID NO: 125; [Paa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID NO: 126; or [Pta<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID NO: 127 or a pharmaceutically acceptable salt thereof.
8. A pharmaceutical composition comprising an effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.
9. Use of a compound according to claim 1 or a pharmaceutically acceptable salt thereof in the preparation of a medicament for eliciting an agonist effect from a GLP-1 receptor in a subject in need thereof
10. Use of a compound according to claim 1 or a pharmaceutically acceptable salt thereof in the preparation of a

medicament for treating a disease selected from the group consisting of Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system disease, restenosis and neurodegenerative disease, renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, and hypertension, in a subject in need thereof.

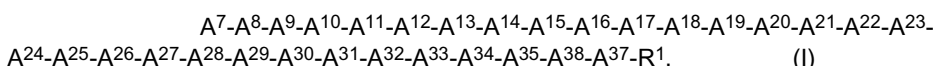
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11. A use according to claim 10 wherein said disease is Type I diabetes or Type II diabetes.

## Patentansprüche

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1. Verbindung mit der Formel (I):



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in der

A<sup>7</sup> Urocansäure (ura), *trans*-3-(3-Pyridyl)acrylsäure (Paa), (4-Pyridylthio)essigsäure (Pta) oder 3-(4-Hydroxyphenyl)propionsäure (Hppa) ist;

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A<sup>8</sup> Ala, D-Ala, Aib, Acc, N-Me-Ala, N-Me-D-Ala, Arg oder N-Me-Gly ist;

A<sup>9</sup> Glu, N-Me-Glu, N-Me-Asp oder Asp ist;

A<sup>10</sup> Gly, Acc, Ala, D-Ala, Phe oder Aib ist;

A<sup>11</sup> Thr oder Ser ist;

A<sup>12</sup> Phe, Acc, Aic, Aib, 3-Pal, 4-Pal, β-Nal, Cha, Trp oder X<sup>1</sup>-Phe ist;

A<sup>13</sup> Thr oder Ser ist;

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A<sup>14</sup> Ser, Thr, Ala oder Aib ist;

A<sup>15</sup> Asp, Ala, D-Asp oder Glu ist;

A<sup>16</sup> Val, D-Val, Acc, Aib, Leu, Ile, Tle, Nle, Abu, Ala, D-Ala, Tba oder Cha ist;

A<sup>17</sup> Ser, Ala, D-Ala, Aib, Acc oder Thr ist;

A<sup>18</sup> Ser, Ala, D-Ala, Aib, Acc oder Thr ist;

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A<sup>19</sup> Tyr, D-Tyr, Cha, Phe, 3-Pal, 4-Pal, Acc, β-Nal, Amp oder X<sup>1</sup>-Phe ist;

A<sup>20</sup> Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tle, Val, Phe oder X<sup>1</sup>-Phe ist;

A<sup>21</sup> Glu, Ala oder Asp ist;

A<sup>22</sup> Gly, Acc, Ala, D-Ala, β-Ala oder Aib ist;

A<sup>23</sup> Gln, Asp, Ala, D-Ala, Aib, Acc, Asn oder Glu ist;

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A<sup>24</sup> Ala, Aib, Val, Abu, Tle oder Acc ist;

A<sup>25</sup> Ala, Aib, Val, Abu, Tle, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O) oder HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) ist;

A<sup>26</sup> Lys, Ala, 3-Pal, 4-Pal, Arg, hArg, Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O) oder HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) ist;

A<sup>27</sup> Glu, Ala, D-Ala oder Asp ist;

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A<sup>28</sup> Phe, Ala, Pal, β-Nal, X<sup>1</sup>-Phe, Aic, Acc, Aib, Cha oder Trp ist;

A<sup>29</sup> Ile, Acc, Aib, Leu, Nle, Cha, Tle, Val, Abu, Ala, Tba oder Phe ist;

A<sup>30</sup> Ala, Aib, Acc ist oder fehlt;

A<sup>31</sup> Trp, Ala, β-Nal, 3-Pal, 4-Pal, Phe, Acc, Aib, Cha, Amp ist oder fehlt;

A<sup>32</sup> Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tle, Phe, X<sup>1</sup>-Phe, Ala ist oder fehlt;

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A<sup>33</sup> Val, Acc, Aib, Leu, Ile, Tle, Nle, Cha, Ala, Phe, Abu, X<sup>1</sup>-Phe, Tba, Gaba ist oder fehlt;

A<sup>34</sup> Lys, Arg, hArg, Orn, Amp, Gaba, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) ist oder fehlt;

A<sup>35</sup> Gly ist oder fehlt;

A<sup>36</sup> L- oder D-Arg, D- oder L-Lys, D- oder L-hArg, D- oder L-Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH((CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O) ist oder fehlt;

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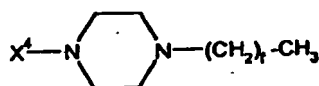
A<sup>37</sup> Gly ist oder fehlt;

X<sup>1</sup> bei jedem Vorkommen unabhängig ausgewählt ist aus der Gruppe bestehend aus (C<sub>1</sub>- bis C<sub>6</sub>)-Alkyl, OH und Halogen;

R<sup>1</sup> OH, NH<sub>2</sub>, (C<sub>1</sub>- bis C<sub>12</sub>-)-Alkoxy oder NH-X<sup>2</sup>-CH<sub>2</sub>-Z<sup>0</sup> ist, wobei X<sup>2</sup> ein (C<sub>1</sub>- bis C<sub>12</sub>-)-Kohlenwasserstoffanteil ist und Z<sup>0</sup> H, OH, CO<sub>2</sub>H oder CONH<sub>2</sub> ist;

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X<sup>3</sup>

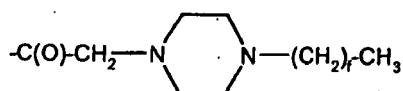


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- X<sup>3</sup> oder -C(O)-NHR<sup>12</sup> ist, wobei X<sup>4</sup> bei jedem Vorkommen unabhängig -C(O)-, -NH-C(O)- oder -CH<sub>2</sub>- ist, und f bei jedem Vorkommen unabhängig eine Zahl von 1 bis 29 ist;  
 e bei jedem Vorkommen unabhängig eine Zahl von 1 bis 4 ist;  
 n bei jedem Vorkommen unabhängig eine Zahl von 1 bis 5 ist;  
 R<sup>10</sup> und R<sup>11</sup> bei jedem Vorkommen jeweils unabhängig H, (C<sub>1</sub>- bis C<sub>30</sub>-)Alkyl, (C<sub>1</sub>- bis C<sub>30</sub>-)Acyl, (C<sub>1</sub>- bis C<sub>30</sub>-)Alkylsulfonyl, -C((NH)(NH<sub>2</sub>)) oder



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sind, mit der Maßgabe, dass, wenn R<sup>10</sup> (C<sub>1</sub>- bis C<sub>30</sub>-)Acyl, (C<sub>1</sub>- bis C<sub>30</sub>-)Alkylsulfonyl, -C((NH)(NH<sub>2</sub>)) oder



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ist, R<sup>11</sup> H oder (C<sub>1</sub>-C<sub>30</sub>-)Alkyl ist und R<sup>12</sup> (C<sub>1</sub>- bis C<sub>30</sub>-)Alkyl ist, oder ein pharmazeutisch annehmbares Salz davon.

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2. Verbindung nach Anspruch 1 oder ein pharmazeutisch annehmbares Salz davon, bei der/dem A<sup>11</sup> Thr ist, A<sup>13</sup> Thr ist, A<sup>14</sup> Ser, Aib oder Ala ist; A<sup>17</sup> Ser, Ala, Aib oder D-Ala ist; A<sup>18</sup> Ser, Ala, Aib oder D-Ala ist; A<sup>21</sup> Glu oder Ala ist; A<sup>23</sup> Gln, Glu oder Ala ist und A<sup>27</sup> Glu oder Ala ist.
  3. Verbindung nach Anspruch 2 oder ein pharmazeutisch annehmbares Salz davon, bei der/dem A<sup>9</sup> Glu, N-Me-Glu oder N-Me-Asp ist; A<sup>12</sup> Phe, Acc oder Aic ist; A<sup>16</sup> Val, D-Val, Acc, Aib, Ala, Tle oder D-Ala ist; A<sup>19</sup> Tyr, 3-Pal, 4-Pal oder D-Tyr ist; A<sup>20</sup> Leu, Acc, Cha, Ala oder Tle ist; A<sup>24</sup> Ala, Aib oder Acc ist; A<sup>25</sup> Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NH-R<sup>10</sup>)-C(O) ist; A<sup>28</sup> Phe oder Ala ist; A<sup>29</sup> Ile, Acc oder Tle ist; A<sup>30</sup> Ala, Aib ist oder fehlt; A<sup>31</sup> Trp, Ala, 3-Pal, 4-Pal ist oder fehlt; A<sup>32</sup> Leu, Acc, Cha, Ala ist oder fehlt; A<sup>33</sup> Val, Acc, Ala, Gaba, Tle ist oder fehlt.
  4. Verbindung nach Anspruch 3 oder ein pharmazeutisch annehmbares Salz davon, bei der/dem A<sup>8</sup> Ala, D-Ala, Aib, 1-Amino-1-cyclohexancarbonsäure (A6c), 1-Amino-1-cyclopentancarbonsäure (A5c), N-Me-Ala, N-Me-D-Ala oder N-Me-Gly ist; A<sup>10</sup> Gly, Ala, D-Ala oder Phe ist; A<sup>12</sup> Phe, 1-Amino-1-cyclohexancarbonsäure (A6c) oder 1-Amino-1-cyclopentancarbonsäure (A5c) ist; A<sup>16</sup> Val, Ala, Tle, 1-Amino-1-cyclohexancarbonsäure (A6c), 1-Amino-1-cyclopentancarbonsäure (A5c) oder D-Val ist; A<sup>20</sup> Leu, 1-Amino-1-cyclohexancarbonsäure (A6c), 1-Amino-1-cyclopentancarbonsäure (A5c), Cha, Ala oder Tle ist; A<sup>22</sup> Gly, Aib, β-Ala, L-Ala oder D-Ala ist; A<sup>24</sup> Ala oder Aib ist; A<sup>29</sup> Ile, 1-Amino-1-cyclohexancarbonsäure (A6c); 1-Amino-1-cyclopentancarbonsäure (A5c) oder Tle ist; A<sup>32</sup> Leu, 1-Amino-1-cyclohexancarbonsäure (A6c), 1-Amino-1-cyclopentancarbonsäure (A5c), Cha, Ala ist oder fehlt; A<sup>33</sup> Val, 1-Amino-1-cyclohexancarbonsäure (A6c), 1-Amino-1-cyclopentancarbonsäure (A5c), Ala, Gaba, Tle ist oder fehlt.
  5. Verbindung nach Anspruch 4 oder ein pharmazeutisch annehmbares Salz davon, bei der/dem R<sup>1</sup> OH oder NH<sub>2</sub> ist.
  6. Verbindung nach Anspruch 1, die [Hppa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> (SEQ ID Nr. 87) oder ein pharmazeutisch annehmbares Salz davon ist.
  7. Verbindung nach Anspruch 1, die [Ura<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID Nr. 125; [Paa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID Nr. 126 oder [Pta<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub>; SEQ ID Nr. 127 oder ein pharmazeutisch annehmbares Salz davon ist.

8. Pharmazeutische Zusammensetzung, die eine wirksame Menge einer Verbindung gemäß Anspruch 1 oder eines pharmazeutisch annehmbaren Salzes davon und einen pharmazeutisch annehmbaren Träger oder ein pharmazeutisch annehmbares Verdünnungsmittel dafür umfasst.

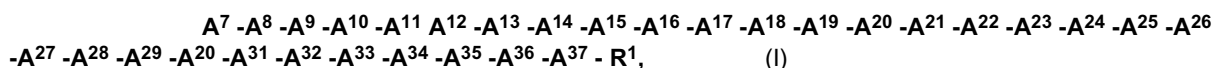
9. Verwendung einer Verbindung nach Anspruch 1 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Auslösung einer Agonistwirkung von einem GLP-1-Rezeptor bei einer Person/einem Subjekt, die/das dessen bedarf.

10. Verwendung einer Verbindung nach Anspruch 1 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Behandlung einer Erkrankung ausgewählt aus der Gruppe bestehend aus Diabetes Typ I, Diabetes Typ II, Fettleibigkeit, Glukagonomen, Sekretionsstörungen der Luftwege, metabolischen Erkrankungen, Arthritis, Osteoporose, Erkrankungen des zentralen Nervensystems, Restenose und neurodegenerativer Erkrankung, Nierenversagen, kongestivem Herzversagen, nephrotischem Syndrom, Zirrhose, Lungenödem und Bluthochdruck bei einer Person/einem Subjekt, die/das dessen bedarf.

11. Verwendung nach Anspruch 10, bei der die Erkrankung Diabetes Typ I oder Diabetes Typ II ist.

### Revendications

1. Composé représenté par la formule (I),



dans laquelle :

- A<sup>7</sup> est acide urocanique (Ura), acide *trans*-3-(3-pyridyl)acrylique (Paa), acide (4-pyridylthio)acétique (Pta) ou acide 3-(4-hydroxyphényl)propionique (Hppa) ;

- A<sup>8</sup> est **Ala, D-Ala, Aib, Acc, N-Me-Ala, N-Me-D-Ala, Arg** ou **N-Me-Gly**;

- A<sup>9</sup> est **Glu, N-Me-Glu, N-Me-Asp** ou **Asp**;

- A<sup>10</sup> est **Gly, Acc, Ala, D-Ala, Phe** ou **Aib**;

- A<sup>11</sup> est **Thr** ou **Ser**;

- A<sup>12</sup> est **Phe, Acc, Alc, Aib, 3-Pal, 4-Pal, β-Nal, Cha, Trp** ou **X<sup>1</sup>-Phe**;

- A<sup>13</sup> est **Thr** ou **Ser**;

- A<sup>14</sup> est **Ser, Thr, Ala** ou **Aib**;

- A<sup>15</sup> est **Asp, Ala, D-Asp** ou **Glu**;

- A<sup>16</sup> est **Val, D-Val, Acc, Aib, Leu, Ile, Tie, Nie, Abu, Ala, D-Ala, Tba** ou **Cha**;

- A<sup>17</sup> est **Ser, Ala, D-Ala, Aib, Acc** ou **Thr**;

- A<sup>18</sup> est **Ser, Ala, D-Ala, Aib, Acc** ou **Thr**;

- A<sup>19</sup> est **Tyr, D-Tyr, Cha, Phe, 3-Pal, 4-Pal, Acc, β-Nal, Amp** ou **X<sup>1</sup>-Phe**;

- A<sup>20</sup> est **Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tie, Val, Phe** ou **X<sup>1</sup>-Phe**;

- A<sup>21</sup> est **Glu, Ala** ou **Asp**;

- A<sup>22</sup> est **Gly, Acc, Ala, D-Ala, β-Ala** ou **Aib**;

- A<sup>23</sup> est **Gln, Asp, Ala, D-Ala, Aib, Acc, Asn** ou **Glu**;

- A<sup>24</sup> est **Ala, Aib, Val, Abu, Tie** ou **Acc**;

- A<sup>25</sup> est **Ala, Aib, Val, Abu, Tie, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O)** ou **HN-CH((CH<sub>2</sub>)<sub>n</sub>-X<sup>3</sup>)-C(O)**;

- A<sup>26</sup> est **Lys, Ala, 3-Pal, 4-Pal, Arg, hArg, Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O)** ou **HN-CH((CH<sub>2</sub>)<sub>n</sub>-X<sup>3</sup>)-C(O)**;

- A<sup>27</sup> est **Glu, Ala, D-Ala** ou **Asp**;

- A<sup>28</sup> est **Phe, Ala, Pal, β-Nal, X<sup>1</sup>-Phe, Aic, Acc, Aib, Cha** ou **Trp**;

- A<sup>29</sup> est **Ile, Acc, Aib, Leu, Nle, Cha, Tie, Val, Abu, Ala, Tba** ou **Phe**;

- A<sup>30</sup> est **Ala, Aib, Acc** ou déléte ;

- A<sup>31</sup> est **Trp, Ala, β-Nal, 3-Pal, 4-Pal, Phe, Acc, Aib, Cha, Amp** ou déléte ;

- A<sup>32</sup> est **Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tie, Phe, X<sup>1</sup>-Phe, Ala** ou déléte ;

- A<sup>33</sup> est **Val, Acc, Aib, Leu, Ile, Tie, Nle, Cha, Ala, Phe, Abu, X<sup>1</sup>-Phe, Tba, Gaba** ou déléte ;

- A<sup>34</sup> est **Lys, Arg, nArg, Orn, Amp, Gaba, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH(CH<sub>2</sub>)<sub>e</sub>-X<sup>3</sup>)-C(O)** ou déléte ;

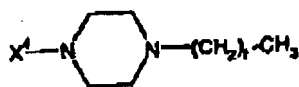
- A<sup>35</sup> est Gly ou délété ;
- A<sup>36</sup> est L- ou D-Arg, D-ou L-Lys, D- ou L-hArg, D- ou L-Orn, Amp, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NR<sup>10</sup>R<sup>11</sup>)-C(O), HN-CH((CH<sub>2</sub>)<sub>n</sub>-X<sup>3</sup>)-C(O) ou délété ;
- A<sup>37</sup> est Gly ou délété ;

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X<sup>1</sup> pour chaque occurrence est indépendamment choisi dans le groupe constitué par alkyle en C<sub>1</sub>-C<sub>6</sub>, OH et halo; R<sup>1</sup> est OH, NH<sub>2</sub>, alcoxy en C<sub>1</sub>-C<sub>12</sub> ou NH-X<sup>2</sup>-CH<sub>2</sub>-Z<sup>0</sup>, où X<sup>2</sup> est une fraction hydrocarbonée en C<sub>1</sub>-C<sub>12</sub> et Z<sup>0</sup> est H, OH, CO<sub>2</sub>H ou CONH<sub>2</sub> ;

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X<sup>3</sup> est



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ou -C(O)-NHR<sup>12</sup>, où X<sup>4</sup> pour chaque occurrence est indépendamment -C(O)-, -NH-C(O)- ou -CH<sub>2</sub>- et f pour chaque occurrence est indépendamment un entier de 1 à 29 ;

e pour chaque occurrence est indépendamment un entier de 1 à 4 ;

n pour chaque occurrence est indépendamment un entier de 1 à 5 ;

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R<sup>10</sup> et R<sup>11</sup> pour chaque occurrence est indépendamment H, alkyle en C<sub>1</sub>-C<sub>30</sub>, acyle en C<sub>1</sub>-C<sub>30</sub>, alkylsulfonyle en C<sub>1</sub>-C<sub>30</sub>, -C((NH)(NH<sub>2</sub>)) ou

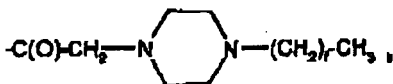
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à la condition que, lorsque R<sup>10</sup> est acyle en C<sub>1</sub>-C<sub>30</sub>, alkylsulfonyle en C<sub>1</sub>-C<sub>30</sub>, -C((NH)(NH<sub>2</sub>)) ou

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R<sup>11</sup> est H ou alkyle en C<sub>1</sub>-C<sub>30</sub> ; et

R<sup>12</sup> est alkyle en C<sub>1</sub>-C<sub>30</sub>

ou un sel pharmaceutiquement acceptable de ce composé.

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2. Composé selon la revendication 1, ou un sel pharmaceutiquement acceptable de celui-ci, dans lequel A<sup>11</sup> est Thr ; A<sup>13</sup> est Thr ; A<sup>14</sup> est Ser, Aib ou Ala ; A<sup>17</sup> est Ser, Ala, Aib ou D-Ala ; A<sup>18</sup> est Ser, Ala, Aib ou D-Ala ; A<sup>21</sup> est Glu ou Ala ; A<sup>23</sup> est Gln, Glu ou Ala ; et A<sup>27</sup> est Glu ou Ala.

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3. Composé selon la revendication 2, ou un sel pharmaceutiquement acceptable de celui-ci, dans lequel A<sup>9</sup> est Glu, N-Me-Glu ou N-Me-Asp ; A<sup>12</sup> est Phe, Acc ou Aic ; A<sup>16</sup> est Val, D-Val, Acc, Aib, Ala, Tle ou D-Ala ; A<sup>19</sup> est Tyr, 3-Pal, 4-Pal ou D-Tyr ; A<sup>20</sup> est Leu, Acc, Cha, Ala ou Tle ; A<sup>24</sup> est Ala, Aib ou Acc ; A<sup>25</sup> est Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH((CH<sub>2</sub>)<sub>n</sub>-NH-R<sup>10</sup>)-C(O) ; A<sup>28</sup> est Phe ou Ala ; A<sup>29</sup> est Ile, Acc ou Tle ; A<sup>30</sup> est Ala, Aib ou délété ; A<sup>31</sup> est Trp, Ala, 3-Pal, 4-Pal ou délété ; A<sup>32</sup> est Leu, Acc, Cha, Ala ou délété ; A<sup>33</sup> est Val, Acc, Ala, Gaba, Tle ou délété.

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4. Composé selon la revendication 3, ou un sel pharmaceutiquement acceptable de celui-ci, dans lequel A<sup>8</sup> est Ala, D-Ala, Aib, acide 1-amino-1-cyclohexanecarboxylique (A6c), acide 1-amino-1-cyclopentanecarboxylique (A5c), N-Me-Ala, N-Me-D-Ala ou N-Me-Gly ; A<sup>10</sup> est Gly, Ala, D-Ala ou Phe ; A<sup>12</sup> est Phe, acide 1-amino-1-cyclohexanecarboxylique (A6c) ou acide 1-amino-1-cyclopentanecarboxylique (A5c) ; A<sup>16</sup> est Val, Ala, Tle, acide 1-amino-1-cyclohexanecarboxylique (A6c), acide 1-amino-1-cyclopentanecarboxylique (A5c) ou D-Val ; A<sup>20</sup> est Leu, acide 1-amino-1-cyclohexanecarboxylique (A6c), acide 1-amino-1-cyclopentanecarboxylique (A5c), Cha, Ala ou Tle ; A<sup>22</sup> est Gly, Aib, β-Ala, L-Ala ou D-Ala ; A<sup>24</sup> est Ala ou Aib ; A<sup>29</sup> est Ile, acide 1-amino-1-cyclohexanecarboxylique (A6c), acide 1-amino-1-cyclopentanecarboxylique (A5c) ou Tle ; A<sup>32</sup> est Leu, acide 1-amino-1-cyclohexanecarboxylique (A6c),

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## EP 1 137 666 B9

acide 1-amino-1-cyclopentanecarboxylique (A5c), Cha, Ala ou délété ; A<sup>33</sup> est Val, acide 1-amino-1-cyclohexanecarboxylique (A6c), acide 1-amino-1-cyclopentanecarboxylique (A5c), Ala, Gaba, Tle ou délété.

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5. Composé selon la revendication 4, ou un sel pharmaceutiquement acceptable de celui-ci, dans lequel R<sup>1</sup> est OH ou NH<sub>2</sub>.
6. Composé selon la revendication 1, dans lequel ledit composé est [Hppa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> (SEQ ID NO:87), ou un sel pharmaceutiquement acceptable de celui-ci.
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7. Composé selon la revendication 1, dans lequel ledit composé est [Ura<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> ; SEQ ID NO:125 ; [Paa<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> ; SEQ ID NO:126 ; ou [Pta<sup>7</sup>]hGLP-1(7-36)-NH<sub>2</sub> ; SEQ ID NO:127 ; ou un sel pharmaceutiquement acceptable de ceux-ci.
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8. Composition pharmaceutique comprenant une quantité efficace d'un composé tel que défini à la revendication 1, ou un sel pharmaceutiquement acceptable de celui-ci, et un support ou diluant pharmaceutiquement acceptable.
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9. Utilisation d'un composé tel que défini à la revendication 1, ou d'un sel pharmaceutiquement acceptable de celui-ci, dans la préparation d'un médicament pour déclencher un effet agoniste à partir d'un récepteur GLP-1 dans un sujet en ayant besoin.
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10. Utilisation d'un composé tel que défini à la revendication 1, ou d'un sel pharmaceutiquement acceptable de celui-ci, dans la préparation d'un médicament pour traiter un maladie choisie dans le groupe constitué par le diabète de Type I, le diabète de Type II, l'obésité, les glucagonomes, les troubles sécrétoires des voies aériennes, un trouble métabolique, l'arthrite, l'ostéoporose, une maladie du système nerveux central, la resténose et une maladie neuro-dégénérative, une insuffisance rénale, une insuffisance cardiaque congestive, le syndrome néphrotique, la cirrhose, un oedème pulmonaire et l'hypertension, dans un sujet en ayant besoin.
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11. Utilisation selon la revendication 10, dans laquelle ladite maladie est le diabète de Type I ou le diabète de Type II.

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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 9111457 A [0004]
- US 5672659 A [0043]
- US 5595760 A [0043]
- US 92936397 A [0043]
- US 74077896 A [0043]
- US 01539498 A [0043]
- US 12165398 A [0043]
- US 13147298 A [0043]
- US 18441398 A [0043]

Non-patent literature cited in the description

- **VARNDELL, J.M. et al.** *J. Histochem Cytochem*, 1985, vol. 33, 1080-6 [0002]
- **KREYMANN, B. et al.** *Lancet*, 1987, vol. 2, 1300-4 [0002]
- **WETTERGREN A. et al.** *Dig Dis Sci*, 1993, vol. 38, 665-73 [0002]
- **D'ALESSIO, D.A. et al.** *J. Clin Invest*, 1994, vol. 93, 2293-6 [0002]
- **GUTNIAK, M.K. et al.** *Diabetes Care*, 1994, vol. 17, 1039-44 [0003]
- **WILLIAMS, B. et al.** *J. Clin Endo Metab*, 1996, vol. 81, 327-32 [0003]
- **HOLZ, G.G. 4 et al.** *Nature*, 1993, vol. 361, 362-5 [0003]
- **ORSKOV, C.** *Diabetologia*, 1992, vol. 35, 701-711 [0003]
- Potential of GLP-1 in diabetes management. **HOLST, J.J. et al.** *Glucagon III, Handbook of Experimental Pharmacology*. Springer Verlag, 1996, 311-326 [0003]
- **KREYMANN, B. et al.** *Lancet*, 1987, vol. ii, 1300-1304 [0003]
- **WEIR, G.C. et al.** *Diabetes*, 1989, vol. 38, 338-342 [0003]
- **GUTNIAK, M.** *N. Engl J Med*, 1992, vol. 226, 1316-1322 [0003]
- **NATHAN, D.M. et al.** *Diabetes Care*, 1992, vol. 15, 270-276 [0003]
- **NAUCK, M.A. et al.** *Diabetologia*, 1993, vol. 36, 741-744 [0003]
- **CREUTZFELDT, W.O. et al.** *Diabetes Care*, 1996, vol. 19, 580-586 [0003]
- **DEACON, C.F. et al.** *Diabetes*, 1995, vol. 44, 1126-1131 [0004]
- **MOJSOV, S.** *Int. J. Peptide Protein Res*, 1992, vol. 40, 333-342 [0019]
- **STEWART, J.M. et al.** *Solid Phase Synthesis*. Pierce Chemical Co, 1984 [0020]
- **TODD, J.F. et al.** *Clinical Science*, 1998, vol. 95, 325-329 [0034]
- **TODD, J.F. et al.** *European Journal of Clinical investigation*, 1997, vol. 27, 533-536 [0034]
- **SCHNOLZER et al.** *Int. J. Peptide Protein Res.*, 1992, vol. 40, 180 [0052]