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(54) Title: A PROCESS FOR PREPARING A GLUCAGON-LIKE PEPTIDE

(57) Abstract: A process for preparing a GLP-1 or GLP-2 peptide, said process comprising coupling in solution at least a first fragment and at least a second fragment, wherein the coupling comprises reacting the carboxy terminal amino acid of the first fragment with the amino terminal amino acid of the second fragment, and wherein the carboxy terminal amino acid of the first fragment is other than a Gly residue

A PROCESS FOR PREPARING A GLUCAGON-LIKE PEPTIDE

FIELD OF THE INVENTION

The present invention describes a process for the synthesis of glucagon-like peptides and analogues and variants thereof, more specifically, the GLP-1 peptide analogues Liraglutide and Semaglutide, and the GLP-2 peptide analogue Teduglutide. The method is based on the condensation of two or more fragments in solution.

BACKGROUND OF THE INVENTION

The glucagon-like peptides (GLP-1 and GLP-2) are processed from the proglucagon polypeptide in the gut. GLP-2 is co-encoded with GLP-1 inside the pro-glucagon gene, and secreted in a 1:1 ratio with GLP-1 from intestinal cells [Endocrinology; 1986 Oct;119(4):1467-75]. GLP-1 and GLP-2 are co-secreted in equimolar amounts upon nutrient ingestion, but have opposite effects on chylomicron (CM) production, with GLP-1 significantly reducing and GLP-2 increasing postprandial chylomicronemia.

Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide with the sequence (in humans):

H - His - Ala - Asp - Gly - Ser - Phe - Ser - Asp - Glu - Met - Asn - Thr - Ile - Leu - Asp - Asn - Leu - Ala - Ala - Arg - Asp - Phe - Ile - Asn - Trp - Leu - Ile - Gln - Thr - Lys - Ile - Thr - Asp - OH

"GLP-2"

GLP-2 is created by specific post-translational proteolytic cleavage of proglucagon in a process that also liberates the related glucagon-like peptide-1 (GLP-1). GLP-2 is produced by the intestinal endocrine L cell and by various neurons in the central nervous system.

When externally administered, GLP-2 produces a number of effects in humans and rodents, including intestinal growth, enhancement of intestinal function, reduction in bone breakdown and neuroprotection. GLP-2 may act in an endocrine fashion to link intestinal growth and metabolism with nutrient intake. GLP-2 and related analogues

may be treatments for short bowel syndrome, Crohn's disease, osteoporosis and as adjuvant therapy during cancer chemotherapy.

Teduglutide is a 33-membered polypeptide and GLP-2 analogue having the sequence:

H - His - Gly - Asp - Gly - Ser - Phe - Ser - Asp - Glu - Met - Asn - Thr - Ile - Leu - Asp - Asn - Leu - Ala - Ala - Arg - Asp - Phe - Ile - Asn - Trp - Leu - Ile - Gln - Thr - Lys - Ile - Thr - Asp – OH

Teduglutide

Teduglutide is used for the treatment of short bowel syndrome and works by promoting mucosal growth and possibly restoring gastric emptying and secretion [*"Teduglutide, a novel glucagon-like peptide 2 analog, in the treatment of patients with short bowel syndrome*"; Therap Adv Gastroenterol. **5** (3): 159–71].

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide hormone derived from the tissue-specific post-translational processing of the proglucagon gene, having the sequence:

H-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-NH_2.

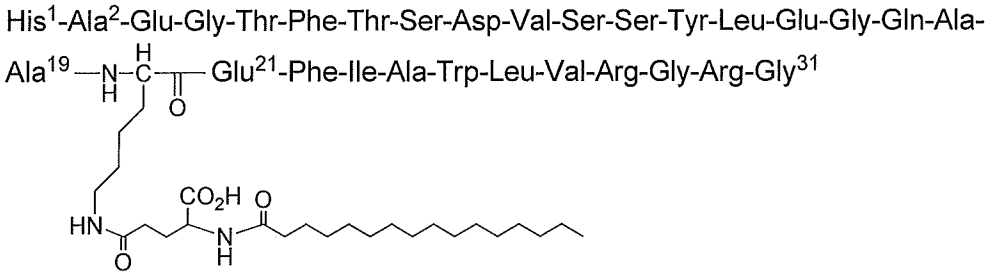
"GLP-1"

GLP-1 is produced and secreted by intestinal enteroendocrine L-cells and certain neurons within the nucleus of the solitary tract in the brainstem upon food consumption. The initial product GLP-1 (1–37) is susceptible to amidation and proteolytic cleavage which gives rise to the two truncated and equipotent biologically active forms, GLP-1 (7–36) amide and GLP-1 (7–37). Active GLP-1 composes two α-helices from amino acid position 13–20 and 24–35 separated by a linker region

GLP-1 possesses several physiological properties making it (and its functional analogues) a subject of intensive investigation as a potential treatment of diabetes mellitus. These properties include the potentiation of the glucose-induced secretion of insulin, increased insulin expression, inhibition of the apoptosis of beta-cells, the reduction of glucagon secretion, and the promotion of satiety. These advantageous

properties have prompted intensive research and development into several therapeutic GLP-1 analogues, including Liraglutide and Semaglutide.

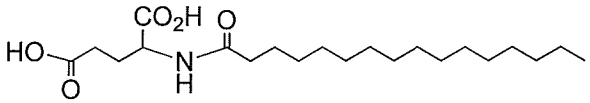
Liraglutide is a GLP-1 peptide analogue having a 31 amino acid backbone with the structure shown below:



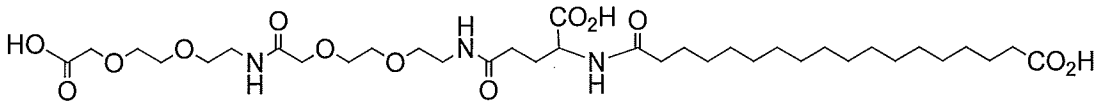
Liraglutide

Semaglutide shares a similar backbone to Liraglutide, but differs in the amino acid in the 2-position (Aib in Semglutide; Ala in Liraglutide) and in the modification of the Lys^{20} side chain.

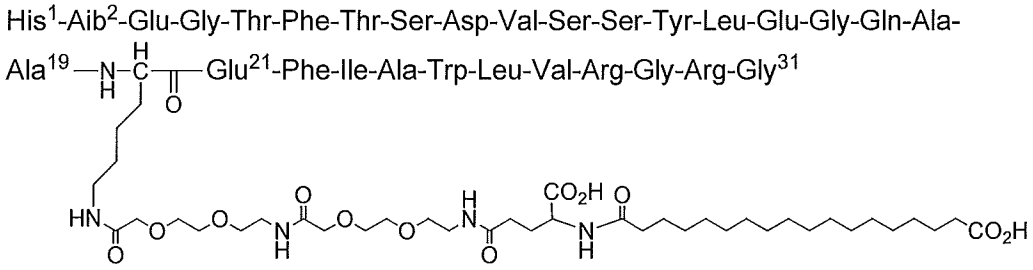
The Lys^{20} in Liraglutide is modified with 2-palmitamidopentanedioic acid



whereas in Semaglutide, it is modified with 9,18,23-trioxo-2,5,11,14-tetraoxa-8,17,22-triazanonatriacontane-1,21,39-tricarboxylic acid.



Semaglutide has the structure shown below:



Semaglutide

Liraglutide and Semaglutide are used for the treatment of dibetes type 2, obesity and also have promising applications in the treatment of Alzheimer's disease.

In the step-by-step solid-phase synthesis of GLP-1 analogues, Liraglutide and Semaglutide are known to contain positions in their sequences where the coupling and deprotection steps become difficult. This results in the formation of several undesirable byproducts which lower the yield and make purification more difficult. Similar difficulties apply in relation to the synthesis of Teduglutide. Several methods have been developed in an attempt to overcome these disadvantages. However, to date, none have proved completely satisfactory.

One particular difficulty with the synthesis lies in the incorporation of Lys(20) into the growing peptide chain of Liraglutide and Semaglutide. Indeed, difficulties arise both before and after modification of the side chain. Especially after Lys incorporation, the coupling reactions become very slow, resulting in racemization and the formation of failure sequences (i.e. those having an amino acid less or more) in a higher degree than expected.

The synthesis of Liraglutide is described in US 6,268,343, US 6,458,924 and US 6,451,974. Recombinant DNA synthesis is used to prepare the peptide intermediate (1-31), which contains two free amino groups, one at the N-terminus, and the other in the side chain of Lys(20). The Palmitoyl-glutamyl (Pal-Glu) unit is then introduced into the Lys side chain. However, this coupling reaction cannot be performed with high selectivity at the Lys side chain amino group, and gives rise to the formation of N-alpha-Pal-Glu-GLP-1(1-31), as well as the doubly acylated form. These side products, together with the complexity of using recombinant DNA technique for large scale

synthesis, considerably lowers the yield and complicates purification of the desired GLP-1 analogues.

US 8,445,433 describes a method of synthesizing GLP-1 analogues by step-by-step solid phase synthesis using Fmoc-pseudoproline dipeptides for the assembly of the peptide chain instead of Fmoc-amino acids. Similarly, WO 2007/090496 discloses a method of synthesizing GLP-1 peptide agonists by linear sequential synthesis, using an Fmoc-pseudoproline dipeptide unit at the relevant position in order to prepare the Val-Ser or Ser-Ser segment of the peptide chain. The remaining sequence is then prepared by stepwise sequential synthesis. Pseudoprolines are known to improve the reaction rates in difficult regions, for example, where β-turns and β-sheets prevent the reagents from reaching the required reaction sites, thereby leading to the formation of failure sequences (Sampson W. R. *et al*: "*The synthesis of 'difficult' peptides using 2-hydroxy-4-methoxybenzyl or pseudoproline amino acid building blocks: A comparative study*" Journal of Peptide Science, vol. 5, no. 9, 1999, pages 403-409).

CN 102286092 A and EP20120831927 describe the solid phase sequential synthesis of Liraglutide using Fmoc-Lys(Alloc)-OH. After the Liraglutide chain is assembled, the Alloc-group is removed catalytically using $Pd(PPh_3)_4$ and Pal-Glu-O^{t}Bu is reated with the liberated Lys side chain, before deprotection and cleavage from the resin. However, $Pd(PPh_3)_4$ is unsuitable for large scale synthesis in view of its toxicity, handling difficulties and high cost. Moreover, it is difficult to assemble full length peptides in an acceptable yield and purity using sequential solid phase synthesis.

CN 103145828 describes the solid phase synthesis of Liraglutide using Fmoc-Lys(ivDde). After the Liraglutide chain is assembled, the Dde-group is removed by hydrazine treatment of the resin-bound peptide. Pal-Glu-O^{t}Bu is then reacted with the liberated Lys side chain, before deprotection cleavage from the resin. However, hydrazines are unsuitable for large scale synthesis in view of their toxicity, and the formation of side products caused by the cleavage of sensitive peptide bonds and/or the hydrolysis of amide bonds.

CN 103864918 discloses the solid phase synthesis of Liraglutide by coupling a fragment containing amino acid residues (1-10) with a fragment containing amino acid residues (11-31), removing the resin and protecting groups, before purifying and freeze

drying the resulting product. The Lys residue is incorporated using Fmoc-Lys(Mtt)-OH, with the Mtt side chain being removed selectively in the presense of the (21-31) resin bound peptide before introduction of the Pal-Glu group. The resulting (20-31)-Pal-Glu fragment - still bound on the Wang resin - is condensed with the (11-19) fragment, and then with the (1-10) fragment. Alternatively, the (1-10) fragment is condensed first with the (11-19) fragment, and the resulting (1-19) fragment is then condensed with the resin-bound (20-31) fragment. The peptide is then deprotected and cleaved from the resin. However, this synthesis has several important drawbacks. Firstly, peptide fragment condensations on the resin are slow, which necessitates the use of an excess of costly peptide fragments in order to allow the condensation to proceed with an acceptable speed. Secondly, because fragment condensation is slow, racemization occurs if the C-terminal amino acid is not Gly or Pro, and increases considerably with the prolongation of the condensation time. Thus, extensive racemization is expected if the fragments (1-10) and (11-19) are used for fragment condensation on the resin. Thirdly, Val is the most sterically hindered amino acid. Thus, if Val is used for fragment condensation on the resin, very slow condensation rates and extensive racemization are expected, more so than for any other amino acid contained in the Liraglutide sequence. Fourthly, the Pal-Glu on-resin derivatization also requires an excess of activated Pal-Glu derivative to be used in order to drive the reaction to completion. As this derivative is costly, this again is a significant drawback to the synthesis. Fifthly, the removal of the Mtt function requires the use of 5% TFA, which causes the partial removal of side chain protecting groups such as ^{t}Bu, Boc and Pbf functions. In turn, this causes the formation of further side products. Finally, resin-bound alkoxybenzyl cations formed during the cleavage of the peptide from the Wang resin and the deprotection react and bind irreversibly to resin peptides containing Trp in their peptide chain, which significantly reduces the yield of the desired peptide.

CN 104004083 discloses the solid phase synthesis of Liraglutide from peptide fragments containing amino acid residues (1-4), (15-16) and (17-31). More specifically, the method involves coupling the (15-16) fragment with the (17-31) fragment, and sequentially adding amino acids thereto before coupling with the (1-4) fragment, removing the resin and protecting groups, and purifying the resulting product. However, this synthetic approach requires an excess of costly fragments to drive the condensation reactions on the resin to completion. Moreover, it also requires an excess of the costly activated Pal-Glu derivative to modify the side chain of the Lys

residue. Furthermore, the conditions required for removal of the Mtt function and detachment from the resin lead to the same difficulties outlined above in relation to CN 103145828.

WO 2016/046753 discloses methods for synthesizing GLP-1 peptides, including Liraglutide and Semaglutide, which comprise a final coupling step in which at least two fragments are coupled at a terminal Gly residue, wherein at least one of the fragments is prepared by the coupling of at least two sub-fragments. By way of example, WO 2016/046753 discloses coupling fragment (1-4) and fragment (5-31) in solid state or in solution. Fragment (5-31) can be prepared by coupling fragment (5-16) with fragment (17-31). Fragment (5-16) itself can be prepared by coupling fragment (5-12) with fragment (13-16). Coupling with a terminal Gly, for example, at Gly4 or Gly16, avoids racemization.

A method for the preparation of Teduglutide is described in CN104817638 and involves synthesizing fragments (1-2), (3-4) and (5-33) and coupling said fragments together. CN104418949 describes the synthesis of Teduglutide from fragments (1-3) and (4-33). CN104072603 descrbes the synthesis of Teduglutide by coupling a His residue with a fragment (2-33). CN104072605 describes the synthesis of Teduglutide from an Asp-Gly dipeptide starting material by preparing a C-end fragment and a middle fragment. Further methods for preparing Teduglutide are described in CN106749614.

To date, none of the existing methods for the bulk production of GLP-1 peptides (such as Liraglutide and Semaglutide) and GLP-2 peptides (such as Tedulglutide) are completely satisfactory. The present invention therefore seeks to provide alternative methods for the synthesis of GLP-1 and GLP-2 peptides, ideally methods that are more efficient, and lead to improved yields and/or purity. In particular, there is a need to provide methods that are suitable for industrial scale-up, and which avoid the use of toxic or otherwise undesirable reagents.

STATEMENT OF INVENTION

A first aspect of the invention relates to a process for preparing a GLP peptide or an analogue or variant thereof, said process comprising coupling in solution at least a first fragment and at least a second fragment, wherein the coupling comprises reacting the

carboxy terminal amino acid of the first fragment with the amino terminal amino acid of the second fragment, and wherein the carboxy terminal amino acid of the first fragment is other than a Gly residue.

The present invention enables the coupling of the peptide fragments in solution, i.e. without the need for a hydrophobic solid support. The Applicant has synthesized various fragments of Liraglutide and Semaglutide with C-terminal amino acids other than Gly. Specifically, the fragments (1-19), (1-18), (1-17) were synthesized and condensed with the corresponding fragments (20-31), (19-31), (18-31) in a solution phase reaction. It was expected that the fragments would racemize to give a mixture of D- and L-diastereomeric peptides at the condensation positions (for example, DL-Ala(19) Liraglutide, DL-Ala(19) Semaglutide, DL-Ala(18) Liraglutide, DL-Ala(18) Semaglutide, DL-Gln(17) etc). This is because it is well established in the art that protected fragments containing amino acids other than Gly or Pro at the C-terminal racemize extensively during their condensation. However, contrary to expectations, studies by the Applicant showed that certain fragments led to unexpectedly low levels of racemization. Similar results were observed for the synthesis of Teduglutide using fragments (1-14) and (15-33), (1-17) and (18-33), (1-18) and (19-33), and (1-19) and (20-33).

In another approach for preparing Liraglutide/Semaglutide, smaller fragments were synthesized (such as the (2-19), (3-19)……(17-19) fragments) and after condensation with the (20-31) fragment in solution, the synthesis was continued by the step by step method, or by fragment condensation, to obtain Liraglutide or Semaglutide. Similar observations were made in relation to the lower than expected levels of racemization. For example, solution phase coupling of fragment (20-31) with fragment (1-19) or fragment (2-19) racemized to a very low extent (< 5 %).

DETAILED DESCRIPTION

The present invention relates to a process for preparing a GLP peptide or an analogue or variant thereof, said process comprising coupling in solution at least a first fragment and at least a second fragment, wherein the coupling comprises reacting the carboxy terminal amino acid of the first fragment with the amino terminal amino acid of the second fragment, and wherein the carboxy terminal amino acid of the first fragment is other than a Gly residue.

The present synthetic approach allows introduction of the Pal-Glu unit (for Liraglutide) and the N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy)ethoxy] acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl (for Semaglutide) on the side chain of Lys at an early stage of the synthesis.

Resin-bound fragments (20-31), (19-31), (18-31) etc were synthesized by the step by step method on 2-chlorotrityl resin or the Wang resin using Fmoc-Lys^{20}(Pal-Glu-OtBu)-OH or Fmoc-Lys(C18-Glu-PEG2)-OH. Alternatively, the fragments were prepared by introducing Lys(20) with Fmoc-Lys(Mmt)-OH on 2-chlorotrityl resin. The fragments were then cleaved from the resin with simultaneous removal of the Mmt group from the side chain of Lys(20). Pal-Glu or C18-Glu-PEG2 was then incorporated using Fmoc or C18-Glu(OSu)-PEG2 or the corresponding Pfp derivatives. The formed Lys^{20}(Pal-Glu-OtBu)- or Lys^{20}(C18Glu-PEG2)-(20-31) fragments were then esterified in solution using a suitable method with a trityl type group, a diphenyl methyl type group or with ^{t}Bu.

The protected esters (20-31, 19-31, 18-31 etc) were then condensed in solution with the L-Ala(1-19), L-Ala(1-18), L-Gln(1-17) protected fragments using methods known in the art. Preferably, dehydrating agents such as EDAC/DIPEA, DIC, HBTU and an acidic catalyst such as HOBt, HOAtu, PfpOH are used to facilitate the condensation reaction. Solid phase couplings were also carried out for comparison.

As expected, the condensations performed on solid-phase, using either the Wang or the 2-chlorotrityl resin proceeded with extensive racemization. In this regard, it was determined by HPLC that >33% D-isomer was formed in all cases, even though the condensation conditions were chosen to be as mild as possible. Lowering the condensation temperature led to a similar degree of racemization to when the condensation was performed at room temperature. Without wishing to be bound by theory, it is believed that at lower temperatures, the condensation becomes very slow so that the racemization increases concurrently.

However, surprisingly, when carried out in solution, the degree of racemization proved to be much lower (<7 %) than expected compared to the condensation results on solid phase. In particular, at the positions Ala(19), Ala(20), Gln(18), and Leu(14), the racemization was observed to be less than 3%.

In one preferred embodiment, the racemization at the coupling position between the first and second fragments is less than 10 %, more preferably less than 9 %, more preferably less than 8 %, more preferably less than 7 %, more preferably less than 6 %, more preferably less than 5 %, more preferably less than 4 %, more preferably less than 3 %.

In one preferred embodiment, the carboxyl terminal amino acid of the first fragment is an Ala residue. For example, for Liraglutide/Semaglutide, the process comprises coupling a fragment (1-19) with a fragment (20-31), or a fragment (1-18) with a fragment (19-31).

In another preferred embodiment, the carboxyl terminal amino acid of the first fragment is a Gln residue. For example, for Liraglutide/Semaglutide, the process comprises coupling a fragment (1-17) with a fragment (18-31).

In another preferred embodiment, the carboxyl terminal amino acid of the first fragment is a Leu residue. For example, for Liraglutide/Semaglutide, the process comprises coupling a fragment (1-14) with a fragment (15-31).

In one preferred embodiment, the carboxy terminal residue of the first fragment is an amino acid ester or an amino acid amide. In an even more preferred embodiment, the amino acid ester group is selected from a trityl type group, a diphenylmethyl group and a tert-butyl group.

In addition to the specific peptides mentioned herein, the invention also encompasses variants, derivatives, analogues, homologues and fragments thereof.

As used herein, a "variant" of any given sequence is a sequence in which the specific sequence of amino acid residues has been modified in such a manner that the peptide in question retains at least one of its endogenous functions. A variant sequence can be obtained by addition, deletion, substitution, modification, replacement and/or variation of at least one residue present in the naturally occurring peptide.

The term "derivative" as used herein in relation to peptides described herein includes any substitution of, variation of, modification of, replacement of, deletion of and/or

addition of one (or more) amino acid residues from or to the sequence, providing that the resultant peptide retains at least one of its endogenous functions.

The term "analogue" as used herein in relation to peptides includes any mimetic, that is, a chemical compound that possesses at least one of the endogenous functions of the peptides which it mimics.

Typically, amino acid substitutions may be made, for example from 1, 2 or 3, to 10 or 20 substitutions, provided that the modified sequence retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

Peptides described herein may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent peptide. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine and tyrosine.

Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R H
AROMATIC		F W Y

The term "homologue" as used herein means an entity having a certain homology with the wild type amino acid sequence. The term "homology" can be equated with "identity".

In the present context, a homologous sequence is taken to include an amino acid sequence which may be at least 50%, 55%, 65%, 75%, 85% or 90% identical, preferably at least 95%, 96% or 97% or 98% or 99% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Preferably, reference to a sequence which has a percent identity to any one of the SEQ ID NOs detailed herein refers to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percent homology or identity between two or more sequences.

Percent homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the amino acid sequence may cause the following residues or codons to be put out of alignment, thus potentially resulting in a large reduction in percent homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids or nucleotides, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the

existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum percent homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, USA; Devereux et al. (1984) Nucleic Acids Research 12: 387). Examples of other software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al. (1999) ibid – Ch. 18), FASTA (Atschul et al. (1990) J. Mol. Biol. 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al. (1999) ibid, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. Another tool, BLAST 2 Sequences, is also available for comparing protein and nucleotide sequences (FEMS Microbiol. Lett. (1999) 174(2):247-50; FEMS Microbiol. Lett. (1999) 177(1):187-8).

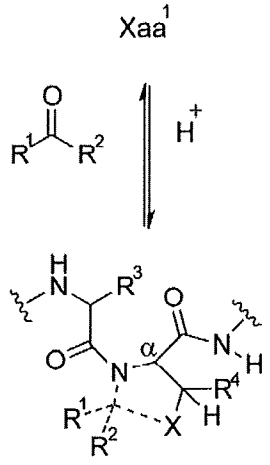
Although the final percent homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix (the default matrix for the BLAST suite of programs). GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see the user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate percent homology, preferably percent sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

"Fragments" are also variants and the term typically refers to a selected region of the peptide that is of interest either functionally or, for example, in an assay. "Fragment" thus refers to an amino acid sequence that is a portion of a full-length peptide.

More preferably, the term "variant" includes any variation wherein wherein (a) one or more amino acid residues are replaced by a naturally or non-naturally occurring amino acid residue (b) the order of two or more amino acid residues is reversed, (c) one, two or three amino acids are deleted, (d) a spacer group is present between any two amino acid residues, (e) one or more amino acid residues are in peptoid form, (f) the (N-C-C) backbone of one or more amino acid residues of the peptide has been modified, (g) one or more additional amino acids are present at the N-terminus and/or the C-terminus, or any of (a)-(g) in combination. Preferably, the variants arise from one of (a), (b) or (c).

The present invention also encompasses amino acid sequences modified by the incorporation of one or more pseudoprolines (denoted Ψ). Pseudoprolines are artificially created dipeptides that minimize aggregation during FMOC solid phase synthesis of peptides. Pseudoprolines consist of serine- (Oxa) or threonine-derived oxazolidines [Oxa(5-Me)] and Cysteine-derived thiazolidines (THz) with Proline-like ring structures (see below).



"pseudoproline"

Xaa^1 = Ser, Thr, Cys

Due to the preference for a cis-amide bond with the preceding residue of C2-substituted pseudoprolines, their incorporation results in a kink conformation of the

peptide backbone, thereby preventing peptide aggregation, self-association, or β-structure formation. Hence, pseudoprolines fulfil two functions simultaneously: firstly, they serve as temporary side-chain protection for Ser, Thr, and Cys, and secondly they act as solubilizing building blocks to increase solvation and coupling rates during peptide synthesis and in subsequent chain assembly.

Pseudoprolines are obtained by reacting the free amino acids with aldehydes or ketones. Pseudoproline dipeptides can be introduced in the same manner as other amino acid derivatives. Preferably the pseudoproline is derived from a Ser-X, Thr-X or Cys-X group, where X is a natural or unnatural amino acid. The routine use of pseudoproline (oxazolidine) dipeptides in the FMOC solid phase peptide synthesis (SPPS) of serine- and threonine-containing peptides leads to significant improvements in quality and yield of crude products. Once the peptide is deprotected, the pseuoproline becomes a conventional dipeptide of the form X-Ser, X-Thr or X-Cys, wherein X is a natural or unnatural amino acid.

More preferably, the variant has one to five, or one to four, or one to three amino acids residues substituted by one or more other amino acid residues. Even more preferably, two amino acid residues are substituted by another amino acid residue. More preferably still, one amino acid residue is substituted by another amino acid residue. Preferably, the substitution is homologous.

Homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) may occur, i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine, diaminobutyric acid ornithine, norleucine ornithine, pyridylalanine, thienylalanine, naphthylalanine and phenylglycine, a more detailed list of which appears below. More than one amino acid residue may be modified at a time.

Suitable spacer groups that may be inserted between any two amino acid residues of the carrier moiety include alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β-alanine residues. A further form of

variation, type (e), involving the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino acid residues wherein the α-carbon substituent group is on the residue's nitrogen atom rather than the α-carbon. Processes for preparing peptides in the peptoid form are known in the art, for example, Simon RJ *et al.*, PNAS (1992) 89(20), 9367-9371 and Horwell DC, Trends Biotechnol. (1995) 13(4), 132-134. Type (f) modification may occur by methods such as those described in International Application PCT/GB99/01855 (WO 99/64574).

It is preferable for amino acid variation, preferably of type (a) or (b), to occur independently at any position. As mentioned above more than one homologous or non-homologous substitution may occur simultaneously. Further variation may occur by virtue of reversing the sequence of a number of amino acid residues within a sequence.

In one embodiment the replacement amino acid residue is a natural amino acid selected from the residues of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

The replacement amino acid residue may additionally be selected from unnatural amino acids. As used herein, the term "non-natural amino acid" or "unnatural amino acid" includes alpha and alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, halide derivatives of natural amino acids such as trifluorotyrosine, p-Cl-phenylalanine, p-F-phenylalanine, p-Br-phenylalanine, p-NO_2-phenylalanine, phenylglycine, sarcosine, penicillamine, D-2-methyltryptophan, phosphoserine, phosphothreonine, phosphotyrosine, p-I-phenylalanine, L-allyl-glycine, ß-alanine, ß-aspartic acid, ß-cyclohexylalanine, citrulline, homoserine, homocysteine, pyroglutamic acid, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, α-cyclohexylglycine, diaminobutyric acid, diaminopimelic acid, N-ε-dinitrophenyl-lysine, L-1-naphthylalanine, L-2-naphthylalanine, 3-(2-pyridyl)-L-alanine, 3-(3-pyridyl)-L-alanine, 3-(4-pyridyl)-L-alanine, N-ε-methyl-lysine, N,N-ε-dimethyl-lysine, N,N,N-ε-trimethyl-lysine, 3-mercaptopropionic acid, L-ε-amino caproic acid, 7-amino heptanoic acid, 6-amino hexanoic acid L-methionine sulfone, ornithine, L-norleucine, L-norvaline, p-nitro-

L-phenylalanine, L-hydroxyproline, γ-glutamic acid, γ-amino butyric acid L-thioproline, methyl derivatives of phenylalanine (Phe) such as 4-methyl-Phe, pentamethyl-Phe, L-Phe (4-amino), L-Tyr (methyl), L-Phe (4-isopropyl), L-Tic (1,2,3,4tetrahydroiso-quinoline-3-carboxyl acid), L-diaminopropionic acid and L-Phe (4-benzyl).

In one preferred embodiment, the GLP peptide is a GLP-1 peptide, or an analogue or variant thereof.

In one preferred embodiment, the GLP-1 peptide is Liraglutaride, or an analogue or variant thereof.

Preferably, for this embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala [**SEQ ID NO: 2**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 3**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-Ala-O-P2 [**SEQ ID NO: 4**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 5**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 59**], (where ΨSer is a pseudoproline); and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 62**], (where ΨSer and ΨThr are pseudoprolines).

In one preferred embodiment, the second peptide has the formula:
Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 6**]

wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt **[SEQ ID NO: 7]** or H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu **[SEQ ID NO: 63]**.

In one highly preferred embodiment for preparing Liraglutide, the first peptide is **[SEQ ID NO: 62]** and the second peptide is **[SEQ ID NO: 63]**.

In another preferred embodiment, the GLP-1 peptide or analogue or variant thereof is semaglutide or a variant thereof.

Preferably, for this embodiment, the GLP-1 peptide is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala **[SEQ ID NO: 9]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 10**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-Ala-O-P2 [**SEQ ID NO: 11**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 12**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 60**], (where ΨSer is a pseudoproline); and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 61**] (where ΨSer and ΨThr are pseudoprolines).

In one preferred embodiment, the second peptide has the formula:
Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 13**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.
In a more preferred embodiment, the second peptide is H-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 14**], or H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 64]**.

In one highly preferred embodiment for preparing Semaglutide, the first peptide is **[SEQ ID NO: 61]** and the second peptide is **[SEQ ID NO: 64]**.

In another embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala **[SEQ ID NO: 15]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH **[SEQ ID NO: 16]**

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-O-P2 **[SEQ ID NO: 17]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH **[SEQ ID NO: 18]**;
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH **[SEQ ID NO: 65]**; and

Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH [**SEQ ID NO: 66**].

In one preferred embodiment, the second peptide has the formula:

Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 19**]

wherein each P represents a side chain protecting group which may be the same or different; and

P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 20**] or H-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 67**].

In another embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala [**SEQ ID NO: 21**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 22**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-O-P2 [**SEQ ID NO: 23**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 24**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 68**]; and

Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 69**].

In one preferred embodiment, the second peptide has the formula:

Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 25**]

wherein:

W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

each P represents a side chain protecting group which may be the same or different; and

P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 26]** or H-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 70]**.

In another embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln
[**SEQ ID NO: 27**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Gln carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH
[**SEQ ID NO: 28**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln-O-P2 [**SEQ ID NO: 29**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Gln residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:

Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 30**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ψ Ser-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 71**]; and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)- Ψ Ser-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 72**].

In one preferred embodiment, the second peptide has the formula:
Ala-Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 31**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 32**] or H-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 73**].

In one preferred embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln **[SEQ ID NO: 33]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and
the Gln carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH **[SEQ ID NO: 34]**

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:
P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln-O-P2 **[SEQ ID NO: 35]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Gln residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:

Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 36**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 74**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe- ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 75**].

In one preferred embodiment, the second peptide has the formula:
Ala-Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 37**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 38**], or H-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 76**].

In another embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu [**SEQ ID NO: 39**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Glu-Gly-Gln-Ala-Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 40**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:

P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-O-P2 [**SEQ ID NO:41**]

wherein:

P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);

each P represents a side chain protecting group which may be the same or different; and

P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:

Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH [**SEQ ID NO: 42**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 77**]; and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 78**].

In one preferred embodiment, the second peptide has the formula:
Glu(P)-Gly-Gln(P)-Ala-Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 43**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 44**], or H-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 79**].

In one preferred embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu [**SEQ ID NO: 45**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Glu-Gly-Gln-Ala-Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 46**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, the first peptide has the formula:

P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-O-P2 [**SEQ ID NO: 47**]

wherein:

P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);

each P represents a side chain protecting group which may be the same or different; and

P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

In a more preferred embodiment, the first peptide is selected from:

Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH [**SEQ ID NO: 48**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 80**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe- ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 81**].

In one preferred embodiment, the second peptide has the formula:
Glu(P)-Gly-Gln(P)-Ala-Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 49**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In a more preferred embodiment, the second peptide is H-Glu(tBu)-Gly-Gln(Trt)-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 50]** or or H-Glu(tBu)-Gly-Gln(Trt)-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 82].**

In one preferred embodiment the GLP peptide is a GLP-2 peptide or an analogue or variant thereof.

In one highly preferred embodiment the GLP-2 peptide or analogue or variant thereof is Teduglutide, or an analogue or variant thereof.

In one preferred embodiment, the GLP-2 peptide is a variant of Teduglutide in which the Gly residue in the 2-position is substituted with Aib ("Aib2-Ted"), i.e. **SEQ ID NO: 108**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Aib	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

In one preferred embodiment the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu [**SEQ ID NO: 84**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 85**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

In one preferred embodiment the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu-OP2 [**SEQ ID NO: 86**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

In one preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 83**, and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-OH [**SEQ ID NO: 87**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In another preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 108**, and the first peptide is Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH [(**Boc-Aib^{2}Ted(1-14)-OH**] , where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In one preferred embodiment the second peptide has the formula:
Asp(P)-Asn(P)-Leu-Ala-Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 [**SEQ ID NO: 88**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In one preferred embodiment the second peptide is selected from:

Asp(tBu)-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 89**]; and

H-Asp(tBu)^{15}-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(15-33)-O-R**],
wherein R = H, Clt, Dpm, tBu,
and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

In one preferred embodiment the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu [**SEQ ID NO: 90**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 91**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

In one preferred embodiment the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-O-P2 [**SEQ ID NO: 92**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

In one preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 83**, and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-OH [**SEQ ID NO: 93**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In another preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 108**, and the first peptide is Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH [**(Boc- Aib^{2}Ted(1-17)-OH**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In one preferred embodiment the second peptide has the formula:
Ala-Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 [**SEQ ID NO: 94**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In one preferred embodiment the second peptide is selected from:

Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 95**]; and

H-Ala^{18}-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(18-33)-O-R**], wherein R = H, Clt, Dpm, tBu,

where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

In one preferred embodiment the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala [**SEQ ID NO: 96**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 97**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

In one preferred embodiment the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-Ala-O-P2 **[SEQ ID NO: 98]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In one preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 83**, and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-OH [**SEQ ID NO: 99**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In another preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 108**, and the first peptide is Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH [**(Boc-Aib^{2}Ted(1-18)-OH**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In one preferred embodiment the second peptide has the formula:
Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 **[SEQ ID NO: 100]**
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In one preferred embodiment the second peptide is selected from:

Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 101**]; and

H-Ala^{19}-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(19-33)-O-R**], wherein R = H, Clt, Dpm, tBu,

and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

In one preferred embodiment the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala [**SEQ ID NO: 102**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 103**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

In one preferred embodiment the first peptide has the formula:

P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-Ala-Ala-O-P2 [**SEQ ID NO: 104**]

wherein:

P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);

each P represents a side chain protecting group which may be the same or different; and

P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

In one preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 83** and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-Ala-OH [**SEQ ID NO: 105**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In one preferred embodiment, the GLP-2 peptide is of **SEQ ID NO: 108** and the first peptide is Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)- Asn(Trt)-Leu-Ala-Ala^{19}-OH [(**Boc- Aib^{2}Ted(1-19)-OH**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

In one preferred embodiment the second peptide has the formula:

Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 [**SEQ ID NO: 106**]

wherein each P represents a side chain protecting group which may be the same or different; and

P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

In one preferred embodiment the second peptide is selected from:

Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 107**]; and

H-Arg(Pbf)^{20}-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(20-33)-O-R**], wherein R = H, Clt, Dpm, tBu, and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

Preferably, the first fragment is prepared from:

Boc-His(Trt)^{1}- Gly-Asp(OtBu)-Gly^{4}-OH [(**Boc-Ted(1-4)-OH**]
Boc-His(Trt)^{1}- Aib-Asp(OtBu)-Gly^{4}-OH [(**Boc-Aib^{2}Ted(1-4)-OH**]
Boc-His(Trt)^{1}- Gly-Asp(OtBu)-Gly^{4}-O-Pfp [(**Boc-Ted(1-4)-O-Pfp**]
Boc-His(Trt)^{1}- Aib-Asp(OtBu)-Gly^{4}-O-Pfp [(**Boc-Aib^{2}Ted(1-4)-O-Pfp**]
by coupling on solid-phase (R = 2-chlorotrityl resin) or in solution (R = H) with one of the following fragments:

H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-O-**R [H-Ted(5-14)-O-R]**
H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu^{17}-O-**R [H-Ted(5-17)-O-R]**
H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu-Ala^{18}-O-**R [H-Ted(5-18)-O-R]**
H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu-Ala-Ala^{19}-O-**R [H-Ted(5-19)-O-R]**

to give a first fragment of Teduglutide (or its Aib^{2}-analogue) as set out below:

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH **[Boc-Ted(1-14)-OH]**
Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH **[Boc-Ted(1-17)-OH]**
Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH **[Boc-Ted(1-18)-OH]**

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-Ala^{19}-OH **[Boc-Ted(1-19)-OH]**

Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH [(**Boc-Aib^{2}Ted(1-14)-OH**]

Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH [(**Boc- Aib^{2}Ted(1-17)-OH**]

Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH [(**Boc- Aib^{2}Ted(1-18)-OH**]

Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)- Asn(Trt)-Leu-Ala-Ala^{19}-OH [(**Boc- Aib^{2}Ted(1-19)-OH**]

(where Phe-Ser(tBu)- can also be -Phe-ΨSer-).

Preferably, the first fragment of Teduglutide obtained above is then coupled with the corresponding second fragment selected from:

H-Asp(tBu)^{15}-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(15-33)-O-R**], wherein R = H, Clt, Dpm, tBu as described for Liraglutide and Semaglutide;

H-Ala^{18}-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(18-33)-O-R**];

H-Ala^{19}-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(19-33)-O-R**];

H-Arg(Pbf)^{20}-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(20-33)-O-R**];

where R = 2-chlorotrityl resin or H, and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-

to form Teduglutide (1-33).

For all the embodiments described herein, preferably, the first fragment is prepared on solid phase or in solution. Where the first fragment is prepared on solid phase, it is cleaved from the resin before coupling with the second fragment in solution.

For all the embodiments described herein, preferably the second fragment is prepared on solid phase or in solution. Where the second fragment is prepared on solid phase, it is cleaved from the resin before coupling with the first fragment in solution.

For all the embodiments described herein, preferably the second fragment is prepared by coupling two or more sub-fragments.

In one preferred embodiment, the crude GLP peptide is purified by preparative HPLC using various buffers in water/acetonitrile or water/methanol.

In another preferred embodiment, the crude GLP peptide is purified by reverse phase chromatography, for example, reverse phase HPLC.

In one preferred embodiment, the reverse phase chromatography is carried out using C18, C8 or C4 modified silica, for example, "RP-18" (octadecyl carbon chain C18-bonded silica), or "RP-82 (C8-bonded silica). Suitable mobile phases are as described in WO 2016/046753. For example, in one embodiment, the crude peptide is subjected to reverse phase chromatography on a C8 or C8 column using a mobile phase A, comprising water, and a mobile phase B, comprising acetonitrile and at least one C_{1-4}-alcohol, and optionally repeating. The resulting fractions are then subjected to reverse phase chromatography on a C8 or C8 column using a mobile phase C, comprising water, and a mobile phase D, comprising acetonitrile, and optionally repeating. The resulting fractions are then dried.

In one preferred embodiment, the crude peptides were first treated with RP-18 or RP-8 material in a buffer (RP-18 = octadecyl carbon chain, C18-bonded silica; RP-8 = C8-bonded silica) where early eluting impurities remained in solution while the required peptides remained bound on the silica. After washing the RP-material with this buffer, the RP-material was treated with a buffer that elutes >95% of the main product but retains the more lypophilic impurities on the silica. By this method >90-95% crude Liraglutide and Semaglutide and >80% crude Teduglutide were obtained and subjected to usual HPLC purification with RP-4, RP-8 or RP-18 reverse phase silica. Among other buffers ammonium acetate or ammonium carbonate or ammonium formate were used preferentially giving products of >99% purity with no single impurity

exceeding the 0.15%. Such products are well suited to be used in pharmaceutical preparations.

For Liraglutide, in one preferred embodiment, the second peptide is prepared by solid phase synthesis using Lys(Pal-Glu-O^{t}Bu)-OH. In a more preferred embodiment, the second peptide is prepared by the steps of (i) solid phase synthesis solid phase synthesis using Fmoc-Lys(Mmt)-OH, (ii) cleaving from the resin and simultaneously removing the Mmt group from the Lys side chain, and (iii) treating with Pal-Glu(OSu)O^{t}Bu or Pal-Glu(OPfp)O^{t}Bu, (iv) esterifying in solution with a trityl type group, a diphenylmethyl group or a tert-butyl group.

For Semglutide, in one preferred embodiment, the second peptide is prepared by solid phase synthesis using Lys(C18-Glu-PEG2)-OH. In a more preferred embodiment, the second peptide is prepared by the steps of (i) solid phase synthesis solid phase synthesis using Fmoc-Lys(Mmt)-OH, (ii) cleaving from the resin and simultaneously removing the Mmt group from the Lys side chain, and (iii) treating with C18-Glu(OSu)-PEG2 or C18-Glu(OPfp)-PEG2, (iv) esterifying in solution with a trityl type group, a diphenylmethyl group or a tert-butyl group.

In one preferred embodiment, the peptide of **[SEQ ID NO: 2]** is prepared from His-Ala-Glu-Gly **[SEQ ID NO: 51]** by stepwise solid phase synthesis.

In a more preferred embodiment, the peptide of **[SEQ ID NO: 2]** is prepared by fragment condensation of P1-His-Ala-Glu-Gly-OH **[SEQ ID NO: 52]** and H-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-O-P3 **[SEQ ID NO: 53]**, wherein P1 is a protecting group for the N-terminal, and P3 is a protecting group for the C-terminal, and where one or more amino acid residues in the peptides may be unprotected or protected.

In one preferred embodiment, the peptide of **[SEQ ID NO: 9]** is prepared from His-Aib-Glu-Gly **[SEQ ID NO: 54]** by stepwise solid phase synthesis.
In a more preferred embodiment, the peptide of **[SEQ ID NO: 9]** is prepared by fragment condensation of P1-His-Aib-Glu-Gly-OH **[SEQ ID NO: 55]** and H-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-O-P3 **[SEQ ID NO: 56]**, wherein

P1 is a protecting group for the N-terminal, and P3 is a protecting group for the C-terminal, and where one or more amino acid residues in the peptides may be unprotected or protected.

In one preferred embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

$X_n...X_{18}$-Ala [**SEQ ID NO: 57**]

wherein:

$X_n...X_{18}$ represents amino acid residues n to 18 of liraglutide, where n is 1 to 17;

the N-terminal is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 3**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in the first and second peptides can be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, n is 2 to 17, and wherein after solution phase coupling with **SEQ ID NO:3**, said process further comprises the stepwise addition of one or more amino acids, or condensation with a sub-fragment, to give **SEQ ID NO: 1**.

In one particularly preferred embodiment, n is 15.

In one preferred embodiment, the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

$Y_n...Y_{18}$-Ala [**SEQ ID NO: 58**]

wherein:

$Y_n...Y_{18}$ represents amino acid residues n to 18 of semaglutide, where n is 1 to 17;

the N-terminal is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 10**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

In one preferred embodiment, n is 2 to 17, and wherein after solution phase coupling with **SEQ ID NO: 10**, said process further comprises the stepwise addition of one or more amino acids, or condensation with a sub-fragment, to give **SEQ ID NO: 8**.

In one particularly preferred embodiment, n is 15.

Another aspect of the invention relates to one or more fragments as described, for example, a peptide selected from **SEQ ID NOS: 2-7** and **9-58**.

Another aspect of the invention relates to the use of one or more peptide fragments as described herein in the synthesis of a GLP-1 peptide or analogue or variant thereof, more preferably, Liraglutide or Semaglutide. In one preferred embodiment, the invention relates to the use of a peptide selected from **SEQ ID NOS: 2-7** and **9-58** in the synthesis of a GLP-1 peptide.

Another aspect of the invention relates to the use of one or more peptide fragments as described herein in the synthesis of a GLP-2 peptide or analogue or variant thereof, more preferably, Teuglutide. In one preferred embodiment, the invention relates to the use of a peptide selected from **SEQ ID NOS: 84-107** in the synthesis of a GLP-2 peptide.

The present invention is further described by way of the following non-limiting examples.

EXAMPLES

Abbreviations

AIB (or Aib)	2-Aminoisobutyric acid or α-aminoisobutyric acid
Boc or t-Boc	t-butyloxycarbonyl
Bt	benzotriazole
	carboxybenzyl
Bz	benzyl
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
EDC.HCl	1-ethyl-3-(3 '-dimethyl-aminopropyl)carbodiimide hydrochloride#
DPM	diphenylmethyl
MeDPM	methyl-diphenylmethyl
MeODPM	methoxy-diphenylmethyl
ivDde	1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
Fmoc	9-fluorenylmethoxycarbonyl
HPLC	High Performance Liquid Chromatography
Mmt	monomethoxytrityl [(4-methoxyphenyl)diphenylmethyl]
Mtt	4-methyltrityl
Pfp	pentafluorophenyl
Su	succinimide
tBu	tert-butyl
Pal	palmitoyl
Pbf	2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl
TFA	trifluoroacetic acid
Trt	trityl
Clt	chlorotrityl

Materials & Methods

The (1-19), (1-18) and (1-17) fragments of Liraglutide/Semaglutide were obtained either by the step-by-step method on 2-chlorotrityl resin using Fmoc-amino acids or by the fragment condensation method.

Resin-bound fragments (20-31), (19-31), (18-31) of Liraglutide/Semaglutide etc were synthesized by the step by step method on 2-chlorotrityl resin or the Wang resin using Fmoc-Lys^{20}(Pal-Glu-OtBu)-OH or Fmoc-Lys(C18-Glu-PEG2)-OH. Alternatively, the fragments were prepared by introducing Lys(20) with Fmoc-Lys(Mmt)-OH on 2-chlorotrityl resin. The fragments were then cleaved from the resin with simultaneous removal of the Mmt group from the side chain of Lys(20). Pal-Glu or C18-Glu-PEG2 was then incorporated using Fmoc or C18-Glu(OSu)-PEG2 or the corresponding Pfp derivatives. The formed Lys^{20}(Pal-Glu-OtBu) or Lys^{20}(C18Glu-PEG2)20-31 fragments were then esterified in solution using a suitable method with a trityl type group, a diphenyl methyl type group or with ^{t}Bu.

The protected esters ((20-31), (19-31), (18-31) etc) were then condensed in solution with the L-Ala(1-19), L-Ala(1-18), L-Gln(1-17) protected fragments using methods known in the art. Dehydrating agents such as EDAC/DIPEA, DIC, HBTU and an acidic catalyst such as HOBt, HOAtu, PfpOH are used to facilitate the condensation reaction. Similar methods were used to prepare the fragments of Teduglutide.

General Procedures for the preparation of Protected Fragments

Resin Loading

CLTR-Cl is charged to a peptide reactor and swelled with DCM for 10 min at RT. The resin is drained and a solution of Fmoc-amino acid and DIEA in DCM is added. The mixture is stirred under nitrogen for 2 hours at RT. Then, remaining active sites on CLTR are end-capped with addition of MeOH and stirring for 1 hour. The resin is drained, washed three times, 5 min each, with a mixture of DCM/DIEA/MeOH (80:5:15) and twice with NMP. After draining, the resin is treated twice with 15% piperidine in NMP for 30 min and then four times with NMP, four times with IPA, drained and dried to a constant weight in vacuum. A loading of 0.3 mmol/g resin was obtained.

Coupling of the second Amino Acid

The resin-bound amino acid is washed with NMP (3 x 6 mL/g resin) and drained. Then a mixture of preactivated Fmoc-amino acid with HBTU/HOBt/DIEA in the 3:3:3:6 molar ratio over the resin-bound amino acid in NMP (0.6M) is added. The mixture is shaken until negative Kaiser test, drained and washed with NMP (5 x 6 mL/g resin).

Chain Elongation

The resin-bound peptide is reacted with 0.6M solution in NMP of 2.5 fold molar excess of the Fmoc-amino acid with respect to the resin-bound peptide. The amino acid was preactivated for 10 min at 0°C and 10 min at 15°C with DIC/HOBt in a 1.05:1.2 molar ratio in relation to the applied Fmoc-amino acid. After each coupling, the resin is washed with NMP and reaction completion is verified with Kaiser test.

Cleavage of the protected peptides from the Resin

After the completion of the chain assembly, the resin-bound peptides are washed with DCM (x 10). The resin is treated twice with 2% TFA in DCM and the resin is washed with DCM (x 5). The combined filtrates are extracted with water (x5), concentrated and protected peptides are precipitated.

Chlorotrityl chloride protection of *C*-terminal Glycine of Fmoc-(20-31)-OH of Liraglutide

The Fmoc-(20-31)-OH protected peptide (1g, 0.365 µmol) is dissolved in 62 mL DCM under stirring and 2-CLT chloride (0.571g, 1.825 µmol) is added. The reaction is left for 10 min and DIPEA (0.629 mL, 3.65 µmol) is added. The reaction is left to stand for 4h. The DCM solution is concentrated and precipitated with diethyl ether (30 mL). The protected peptided is washed with ether (6 x 10 mL).

Chlorotrityl chloride protection of *C*-terminal Glycine of Fmoc-(20-31)-OH of Semaglutide

The Fmoc-(20-31)-OH protected peptide (5g, 1.27 mmol) is dissolved in 300 mL DCM under stirring and 2-CLT chloride (2g, 6.35 mmol) is added. The reaction is left for 10 min and DIPEA (2.2 mL, 12.7 mmol) is added. The reaction is left to stand for 4h. The DCM solution is concentrated and precipitated with diethyl ether (120 mL). The protected peptided is washed with ether (6 x 50 mL).

N^a deprotection of Fmoc-(20-31)-O-Clt of Liraglutide

The protected peptide (0.9 g, 0.298 µmol) is dissolved in NMP (30 mL). After 10 min, piperidine (0.147 mL, 1.49 µmol) is added under stirring for 2.5 h. To the resulting solution DCM (90 mL) is added and the mixture is exctracted with water (8 x 100 mL). The final DCM-peptide solution is concentrated and precipitated.

N^{α} deprotection of Fmoc-(20-31)-O-Clt of Semaglutide

The protected peptide (5.4 g, 1.27 mmol) is dissolved in NMP (120 mL). After 10 min, piperidine (0.76 mL, 7.62 mmol) is added under stirring for 2.5 h. To the resulting solution DCM (360 mL) is added and the mixture is exctracted with water (8 x 350 mL). The final DCM-peptide solution is concentrated and precipitated.

Liquid Phase Fragment Condensation of Boc-(1-19)-OH and H-(20-31)-O-Clt of Liraglutide

The protected peptide H-(20-31)-O-CLt (160 mg, 56 µmol) is dissolved in 7.5 mL NMP and cooled down to 5°C. The protected peptide Boc-(1-19)-OH (195 mg, 64 µmol) is dissolved in 2.5 mL NMP and HOBt.H2O (11.8 mg, 77 µmol) is added. The resulting solution is cooled down to 5°C and EDAC.HCl (13.4 mg, 70 µmol) is added. The solutions containing the protected fragments are mixed together under stirring for 1 h and then 13.4 mg EDAC.HCl (70 µmol) and DIPEA (10 λ, 58 µmol) is added. The reaction is left under stirring for 20 h at ambient conditions. DCM (30 mL) is added and the organic phase is exctracted 5 times with water. The DCM solution is concentrated, precipitated and washed with Hexane (3 x 10 mL).

Liquid Phase Fragment Condensation of Boc-(1-19)-OH and H-(20-31)-O-Clt of Semaglutide

The protected peptide H-(20-31)-O-CLt (3 g, 0.75 mmol) is dissolved in 130 mL NMP and cooled down to 5°C. The protected peptide Boc-(1-19)-OH (2.65 g, 0.86 µmol) is dissolved in 32 mL NMP and HOBt.H2O (158.0 mg, 1.32 mmol) is added. The resulting solution is cooled down to 5°C and EDAC.HCl (181.3 mg, 0.94 mmol) is added. The solutions containing the protected fragments are mixed together under stirring for 1 h and then 181.3 mg EDAC.HCl (0.94 mmol) and DIPEA (129 λ, 0.75 mmol) is added. The reaction is left under stirring for 20 h at ambient conditions. DCM (480 mL) is added and the organic phase is exctracted 5 times with water. The DCM solution is concentrated, precipitated and washed with Hexane (3 x 180 mL).

One highly preferred embodiment of the invention involves the condensation of the 1-19 fragment (Fragment 1) with the 20-31-OtBu Fragment 2. For example:

For Liraglutide:

Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 62**], (where ΨSer and ΨThr is a pseudoproline) + H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu **[SEQ ID NO: 63]**.

For Semaglutide:

Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 61**], (where ΨSer and ΨThr is a pseudoproline) + H-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 64]**.

Preparation of 20-31-OtBu Fragment

The 20-31-OtBu fragment was prepared by the condensation of the 20-29 Fragment + 30-31 Fragment.

Synthesis of the 20-31 Fragment 2 of Liraglutide

Fmoc-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-OH + H-Arg(Pbf)-Gly-O-tBu followed by Fmoc-removal of the product.

Synthesis of the 20-31 Fragment 2 of Semaglutide

Fmoc-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-OH + H-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl, followed by Fmoc-removal of the product.

Side-chain Deprotection

To 75 mg of the final protected peptides a mixture of TFA/H_2O/DTT (3 mL) in a molar ratio of 94:3:3 is added at ambient conditions for 3 h . The TFA solution is concentrated and precipitated with prechilled diethylether 2 mL. The globally deprotected peptide is washed with DEE (5 x 0.5 mL) and left to dry to a constant weight.

Purification

Crude Liraglutide, Semaglutide and Teduglutide can be purified by preparative HPLC using various buffers in water/acetonitrile or water/methanol. In one preferred embodiment, Liraglutide, Semaglutide and Teduglutide were purified in >99.0% purity applying buffers of ammonium acetate at pH 6.5-9.5, of tetrabutyl ammonium hydraxide at pH 7.0-9.5, of ammonium formate at pH 6.5-9.5 and of 0.5-3% acetic acid. Preferably 1% acetic acid and ammonium formate were used as the buffers. The overall purification yield was 70-90% and the total yield was 40-75%.

In one preferred embodiment, the crude peptides were first treated with RP-18 or RP-8 material in a buffer (RP-18 = octadecyl carbon chain, C18-bonded silica; RP-8 = C8-bonded silica) where early eluting impurities remained in solution while the required peptides remained bound on the silica. After washing the RP-material with this buffer, the RP-material was treated with a buffer that elutes >95% of the main product but retains the more lypophilic impurities on the silica. By this method >90-95% crude Liraglutide and Semaglutide and >80% crude Teduglutide were obtained and subjected to usual HPLC purification with RP-4, RP-8 or RP-18 reverse phase silica. Among other buffers ammonium acetate or ammonium carbonate or ammonium formate were used preferentially giving products of >99% purity with no single impurity exceeding the 0.15%. Such products are well suited to be used in pharmaceutical preparations.

For the purification of the crude peptides preparative HPLC Knauer K1800 was used with a column of 50 mm packed with Kromasil C18, 13 µm, 100 A.

A two step approach either with basic and acidic conditions and acetonitrile as the organic modifier, at ambient temperature was used for the preparation of highly pure peptides.

Results

A summary of the results is given in Table 1. As expected, the condensations performed on solid-phase, using either the Wang or the 2-chlorotrityl resin proceeded with extensive racemization. In this regard, it was determined by HPLC that >33% D-isomer was formed in all cases, even though the condensation conditions were chosen to be as mild as possible. Lowering the condensation temperature led to a similar degree of racemization to when the condensation was performed at room temperature.

However, when carried out in solution, surprisingly at several positions the racemization proved to be much lower (<7 %) than expected compared to the condensation results on solid-phase. In particular at the positions Ala(19), Ala(18), Gln(17), and Leu(14) the observed racemization was less than 3%.

In addition to that observation, many of the diastereomeric D-peptides were surprisingly well separated from the corresponding L-diastereomers using analytical and preparative HPLC. Accordingly, the formed diastereomers could be easily separated from the main product.

The results for Teduglutide are shown in Table 2.

Semaglutide and Liraglutide

The following fragments were prepared by methods described herein:

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-Gly-Y

Y = OH, NH_2

Acids

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-OH (1-4)

Boc-His(Trt)^{1}-Aib^{2}-Glu(tBu)-Gly-OH (1-4)

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH (1-19)

Boc-His(Trt)^{1}-Aib^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH (1-19)

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH (1-18)

Boc-His(Trt)^{1}-Aib^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH (1-18)

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH (1-17)

Boc-His(Trt)^{1}-Aib^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-OH (1-17)

Boc-His(Trt)^{1}-Aib^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH (1-17)

Fmoc-Gln(Trt)-Ala-Ala-OH (17-19)

Fmoc-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH (15-19)

Boc-His(Trt)^{1}-Ala^{2}-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu^{14}-OH (1-14)

Boc-His(Trt)^{1}-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu^{14}-OH (1-14)

Fmoc-Glu(tBu)^{21}-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-OH (21-31) Precursor fragment used to make 15-31 + (15-20)

Fmoc-Glu(tBu)^{15}-Gly-Gln(Trt)-Ala-Ala-Lys(W)^{20}-OH (15-20) Precursor fragment used to make 15-31 (+ 21-31)

Fmoc-Glu(tBu)^{15}-Gly-Gln(Trt)-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31} –OH (15-31)

Esters

H-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-Gly-O-Pr_1 (20-31)

H-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-Gly-O-Pr_1 (19-31)

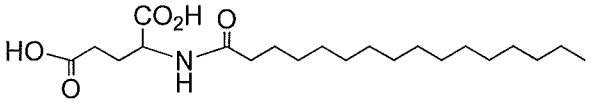
H-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-Gly-O-Pr_1 (18-31)

H-Gln(Trt)-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31}-Gly-O-Pr_1 (17-31)

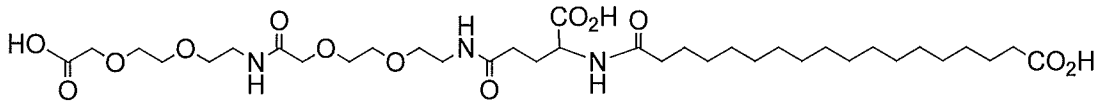
H-Glu(tBu)^{15}-Gly-Gln(Trt)-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)^{31} –O Pr_1 (15-31)

Pr_1 = Clt, Trt, tBu, DPM, MeDPM, MeODPM etc.

W =



and Semaglutide by the 9,18,23-trioxo-2,5,11,14-tetraoxa-8,17,22-triazanonatriacontane-1,21,39-tricarboxylic acid.



See Table 1 for results.

Teduglutide Acids (synthesis of the first fragment)

A protected (1-4) fragment of Teduglutide (or its Aib^2 analogue) selected from the following:

Boc-His(Trt)^1- Gly-Asp(OtBu)-Gly^4-OH [(**Boc-Ted(1-4)-OH**] for condensation on solid-phase or

Boc-His(Trt)^1- Aib-Asp(OtBu)-Gly^4-OH [(**Boc-Aib^{2}Ted(1-4)-OH**] for condensation on solid-phase or

Boc-His(Trt)^1- Gly-Asp(OtBu)-Gly^4-O-Pfp [(**Boc-Ted(1-4)-O-Pfp**] for condensation on liquid-phase or

Boc-His(Trt)^1- Aib-Asp(OtBu)-Gly^4-O-Pfp [(**Boc-Aib^{2}Ted(1-4)-O-Pfp**] for condensation in liquid-phase

was coupled on solid-phase (R = 2-chlorotrityl resin) or in solution (R = H) with one of the following fragments:

H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-O-**R [H-Ted(5-14)-O-R]**

H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu^{17}-O-**R [H-Ted(5-17)-O-R]**

H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu-Ala^{18}-O-**R [H-Ted(5-18)-O-R]**

H-Ser(tBu)^{5}-Phe-Ser(tBu)-Asp(OtBu)-Glu(OtBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(OtBu)- Asn(Trt)-Leu-Ala-Ala^{19}-O-**R [H-Ted(5-19)-O-R]**

to give a first fragment of Teduglutide (or its Aib^{2}-analogue) as set out below, after cleavage from the protected peptide from the resin, or directly:

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH **[Boc-Ted(1-14)-OH]**

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH **[Boc-Ted(1-17)-OH]**

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH **[Boc-Ted(1-18)-OH]**

Boc-His(Trt)^{1}-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-Ala^{19}-OH **[Boc-Ted(1-19)-OH]**

Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH [(**Boc-Aib^{2}Ted(1-14)-OH**]

Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH [(**Boc- Aib^{2}Ted(1-17)-OH**]

Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH [(**Boc- Aib^{2}Ted(1-18)-OH**]

Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)- Asn(Trt)-Leu-Ala-Ala^{19}-OH [(**Boc- Aib^{2}Ted(1-19)-OH**]
(where Phe-Ser(tBu)- can also be -Phe-ΨSer-).

The first fragment of Teduglutide obtained above was then coupled with the corresponding second fragment selected from:

H-Asp(tBu)^{15}-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(15-33)-O-R**],
wherein R = H, Clt, Dpm, tBu as described for Liraglutide and Semaglutide
H-Ala^{18}-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(18-33)-O-R**]

H-Ala^{19}-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(19-33)-O-R**]

H-Arg(Pbf)^{20}-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(20-33)-O-R**]

where R = 2-chlorotrityl resin or H, and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-

to form Teduglutide (1-33), after the cleavage from the resin and deprotection, or direct deprotection. See Table 2 for results.

Various modifications and variations of the described aspects of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of

the described modes of carrying out the invention which are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

Table 1: Racemization and yield determined during the fragment condensation synthesis of Liraglutide and Semaglutide under various conditions. Scale: 0.01 mmol; Ratio: Fragment 1/Fragment 2 (1/1.05).

Run No.	Fragment 1	Fragment 2	Condensing agent	Solvent	Temp [°C]	%-D[1)]	Yield [%][2)]
1	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = Wang resin	DIC/HOBt	NMP	22	37.4*	13.4
2	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = CTC resin	DIC/HOBt	NMP	22	35.6*	16.8
3	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = H	DIC/HOBt	NMP	22	5.4	27.4
4	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = H	DIC/HOBt	NMP	22	6.1	22.7
5	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = H	DIC/HOBt	NMP	10	3.4	22.1
6	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = H	DIC/HOBt	NMP	10	3.7	25.8
7	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = Clt	DIC/HOBt	NMP	22	4.6	34.5
8	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = Clt	DIC/HOBt	NMP	22	4.2	36.7
9	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = Clt	DIC/HOBt	NMP	10	3.3	35.6
10	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = Clt	DIC/HOBt	NMP	10	3.5	37.2
11	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = Dpm	DIC/HOBt	NMP	22	4.5	32.5
12	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = Dpm	DIC/HOBt	NMP	22	4.4	33.5
13	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = Dpm	DIC/HOBt	NMP	10	3.1	36.4
14	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = Dpm	DIC/HOBt	NMP	10	2.9	37.7
15	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	22	3.9	54.5
16	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	22	4.6	52.2
17	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	10	3.3	77.7
18	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	10	3.0	75.4
19	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	10	3.3	76.3
20	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	NMP	2-5	2.1	81.7
21	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DMF	2-5	2.4	82.2
22	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DMF	2-5	2.3	83.1
23	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DMF/DCM (1:1)	2-5	2.7	76.4
24	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DMF/DCM	2-5	2.6	77.7

				(1:1)			
25	Boc-Lir(1-19)-OH; [SEQ ID NO: 5]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DCM	2-5	2.8	62.4
26	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	DIC/HOBt	DCM	2-5	2.2	66.4
27	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	EDAC/HOBt/ DIPEA	DMF	2-5	1.9	84.7
28	Boc-Lir(1-19)-OH; [SEQ ID NO: 59]	[SEQ ID NO: 6]; P3 = tBu	HBTU/HOBt/ DIPEA	DMF	2-5	4.9	83.2
29	Boc-Lir(1-19)-OSu [SEQ ID NO: 4]; P2 = Su	[SEQ ID NO: 6]; P3 = tBu	EDAC/HOBt/ DIPEA	DMF	2-5	13.7	34.4
30	Boc-Lir(1-19)-OPfp [SEQ ID NO: 4]; P2 = Pfp	[SEQ ID NO: 6]; P3 = tBu	EDAC/HOBt/ DIPEA	DMF	2-5	14.4	29.2
32	Boc-Sem(1-19)-OH [SEQ ID NO: 12]	[SEQ ID NO: 13]; P3 = tBu	EDAC/HOBt/ DIPEA	DMF	2-5	2.4	81.3
33	Boc-Sem(1-19)-OH [SEQ ID NO: 60] ΨSer	[SEQ ID NO: 13]; P3 = tBu	EDAC/HOBt/ DIPEA	DMF	2-5	2.1	82.7
34	Boc-Lir(1-18)-OH [SEQ ID NO: 18]	[SEQ ID NO: 20] H-Lir(19-31)-O-Clt	EDAC/HOBt/ DIPEA	DMF	2-5	2.9	67.6
35	Boc-Lir(1-17)-OH [SEQ ID NO: 30]	[SEQ ID NO: 32] H-Lir(18-31)-O-Clt	EDAC/HOBt/ DIPEA	DMF	2-5	2.6	44.4
36	Boc-Lir(1-14)-OH [SEQ ID NO: 42]	H-Lir(15-31)-O-Clt [SEQ ID NO: 44]	EDAC/HOBt/ DIPEA	DMF	2-5	2.7	24.3

[1)]The percentage of D-peptide was determined by HPLC setting the area of the peaks of L-peptide + D-peptide = 100%.

[2)]The yield percentage was determined by seting the total peaks area = 100% and comparing with area of the peak of the D-peptide.

*The condensation could not be completed even by applying a five molar excess of Fragment 1.

Table 2: Racemization and yield determined during the fragment condensation synthesis of Teduglutide under various conditions. Scale: 0.01 mmol; Ratio: Fragment 1/Fragment 2 (1/1.05).

Run Nr.	Fragment 1	Fragment 2	Condensing agent	Solvent	Temp [°C]	%-D[1)]	Yield [%][2)]
1	[Boc-Ted(1-14)-OH]	H-Ted(15-33)-O-R R = 2-chlorotrityl	DIC/HOBt	DMF	2-5	28.3	26.5
2	[Boc-Ted(1-14)-OH]	H-Ted(15-33)-O-R R = H	DIC/HOBt	DMF	2-5	3.2	58.2
3	Boc-Ted(1-17)-OH	H-Ted(18-33)]-O-R R = 2-chlorotrityl	DIC/HOBt	DMF	2-5	24.7	23,5
4	Boc-Ted(1-17)-OH	H-Ted(18-33)]-O-R R =H	DIC/HOBt	DMF	2-5	3.4	55.5
5	Boc-Ted(1-18)-OH	H-Ted(19-33)]-O-R R = 2-chlorotrityl	DIC/HOBt	DMF	2-5	26.2	24.8
6	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R =H	DIC/HOBt	DMF	2-5	2.9	62.2
7	Boc-Ted(1-19)-OH	H-Ted(20-33)-O-R R = 2-chlorotrityl	DIC/HOBt	DMF	2-5	25.6	18.7
8	Boc-Ted(1-19)-OH	H-Ted(20-33)-O-R R =H	DIC/HOBt	DMF	2-5	3.1	59.4
9	[Boc-Ted(1-14)-OH]	H-Ted(15-33)-O-R R = 2-chlorotrityl	HBTU/DIPEA/HOBt	DMF	2-5	19.4	27.2

10	[Boc-Ted(1-14)-OH]	H-Ted(15-33)-O-R R = H	HBTU/DIPEA/HOBt	DMF	2-5	1.8	65.7
11	Boc-Ted(1-17)-OH	H-Ted(18-33)-O-R R = H	HBTU/DIPEA/HOBt	DMF	2-5	2.2	64.2
12	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R = H	HBTU/DIPEA/HOBt	DMF	2-5	1.9	59.9
13	Boc-Ted(1-19)-OH	H-Ted(20-33)-O-R R =H	HBTU/DIPEA/HOBt	DMF	2-5	2.4	66.3
14	Boc-Aib2Ted(1-18)-OH	H-Ted(19-33)-O-R R = H	HBTU/DIPEA/HOBt	DMF	2-5	2.4	62.7
15	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R = Clt	DIC/HOBt	DMF	2-5	2.8	62.2
16	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R = Dpm	DIC/HOBt	DMF	2-5	2.9	57.5
17	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R = tBu	DIC/HOBt	DMF	2-5	2.2	68.2
18	Boc-Ted(1-18)-OH	H-Ted(19-33)-O-R R = tBu; Ser5 = ΨSer	DIC/HOBt	DMF	2-5	2.4	69.9
19	Boc-Aib2Ted(1-18)-OH	H-Ted(19-33)-O-R R = H; Ser5 = ΨSer	DIC/HOBt	DMF	2-5	2.4	59.8

[1)]The percentage of D-peptide was determined by HPLC setting the area of the peaks of L-peptide + D-peptide = 100%.

[2)]The yield percentage was determined by seting the total peaks area = 100% and comparing with area of the peak of the D-peptide.

CLAIMS

1. A process for preparing a glucagon-like peptide (GLP), or an analogue or variant thereof, said process comprising coupling in solution at least a first fragment and at least a second fragment, wherein the coupling comprises reacting the carboxy terminal amino acid of the first fragment with the amino terminal amino acid of the second fragment, and wherein the carboxy terminal amino acid of the first fragment is other than a Gly residue.

2. A process according to claim 1 wherein the carboxyl terminal amino acid of the first fragment is an Ala residue.

3. A process according to claim 1 wherein the carboxyl terminal amino acid of the first fragment is a Gln residue.

4. A process according to claim 1 wherein the carboxyl terminal amino acid of the first fragment is a Leu residue.

5. A process according to any preceding claim wherein the carboxy terminal residue of the first fragment is an amino acid ester or an amino acid amide.

6. A process according to claim 5 wherein the amino acid ester group is selected from a trityl type group, a diphenylmethyl type group and a tert-butyl group.

7. A process according to any preceding claim wherein the glucagon-like peptide is a GLP-1 peptide or an analogue or variant thereof.

8. A process according to claim 7 wherein the GLP-1 peptide or analogue or variant thereof is Liraglutaride, or an analogue or variant thereof.

9. A process according to claim 8 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala [**SEQ ID NO: 2**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 3**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

10. A process according to claim 9 wherein the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-Ala-O-P2 [**SEQ ID NO: 4**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);

each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

11. A process according to claim 10 wherein the first peptide is selected from: Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 5**]; Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 59**]; and Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 62**].

12. A process according to any one of claims 9 to 11 wherein the second peptide has the formula:
Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 6**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

13. A process according to claim 12 wherein the second peptide is H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 7**] or H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu **[SEQ ID NO: 63]**.

14. A process according to claim 7 wherein the GLP-1 peptide is Semaglutide, or an analogue or variant thereof.

15. A process according to claim 14 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala [**SEQ ID NO: 9**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 10**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

16. A process according to claim 15 wherein the first peptide has the formula:
P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-Ala-O-P2 [**SEQ ID NO: 11**]

wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

17. A process according to claim 16 wherein the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 12**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 60**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 61**].

18. A process according to any one of claims 15 to 17 wherein the second peptide has the formula:
Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 13**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

19. A process according to claim 18 wherein the second peptide is H-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 14**], or H-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 64]**.

20. A process according to claim 8 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala
[**SEQ ID NO: 15**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH
[**SEQ ID NO: 16**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in the first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

21. A process according to claim 20 wherein the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-O-P2 [**SEQ ID NO: 17**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

22. A process according to claim 21 wherein the first peptide is selected from:
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH [**SEQ ID NO: 18**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH [**SEQ ID NO: 65**]; and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-OH [**SEQ ID NO: 66**].

23. A process according to any one of claims 20 to 22 wherein the second peptide has the formula:
Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 19**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

24. A process according to claim 23 wherein the second peptide is H-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 20**] or H-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 67**].

25. A process according to claim 14 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala **[SEQ ID NO: 21]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH **[SEQ ID NO: 22]**

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

26. A process according to claim 25 wherein the first peptide has the formula:

P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln(P)-Ala-O-P2 [**SEQ ID NO: 23**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

27. A process according to claim 26 wherein the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 24**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 68**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-OH [**SEQ ID NO: 69**].

28. A process according to any one of claims 25 to 27 wherein the second peptide has the formula:
Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 25**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

29. A process according to claim 28 wherein the second peptide is H-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 26**], or H-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 70**].

30. A process according to claim 8 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln **[SEQ ID NO: 27]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Gln carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH **[SEQ ID NO: 28]**

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in the first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

31. A process according to claim 30 wherein the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln-O-P2 [**SEQ ID NO: 29**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Gln residue (preferably Su, Bt or Pfp).

32. A process according to claim 31 wherein the first peptide is selected from:
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 30**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 71**]; and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)- Ψ Ser-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 72**].

33. A process according to any one of claims 30 to 32 wherein the second peptide has the formula:
Ala-Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 31**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

34. A process according to claim 33 wherein the second peptide is H-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 32**] or H-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 73**].

35. A process according to claim 14 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln **[SEQ ID NO: 33]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Gln carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH **[SEQ ID NO: 34]**

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

36. A process according to any claim 35 wherein the first peptide has the formula:

P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-Glu(P)-Gly-Gln-O-P2 [**SEQ ID NO: 35**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Gln residue (preferably Su, Bt or Pfp).

37. A process according to claim 36 wherein the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 36**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 74**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe- ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-Glu(tBu)-Gly-Gln-OH [**SEQ ID NO: 75**].

38. A process according to any one of claims 35 to 37 wherein the second peptide has the formula:
Ala-Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 37**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

39. A process according to claim 38 wherein the second peptide is H-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 38**] or H-Ala-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 76**].

40. A process according to claim 8 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu [**SEQ ID NO: 39**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Glu-Gly-Gln-Ala-Ala-Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 40**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

41. A process according to claim 40 wherein the first peptide has the formula:
P1-His(P)-Ala-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-O-P2 [**SEQ ID NO:41**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

42. A process according to claim 41 wherein the first peptide is selected from:
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH [**SEQ ID NO: 42**];
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 77**]; and
Boc-His(Trt)-Ala-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 78**].

43. A process according to any one of claims 40 to 42 wherein the second peptide has the formula:
Glu(P)-Gly-Gln(P)-Ala-Ala-Lys(Pal-Glu)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 43**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

44. A process according to claim 43 wherein the second peptide is H-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt [**SEQ ID NO: 44**] or H-Glu(tBu)-Gly-Gln(Trt)-Ala-Ala-Ala-Lys(Pal-Glu)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-tBu [**SEQ ID NO: 79**].

45. A process according to claim 14 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu [**SEQ ID NO: 45**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Glu-Gly-Gln-Ala-Ala-Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 46**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in said first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

46. A process according to claim 45 wherein the first peptide has the formula:
P1-His(P)-Aib-Glu(P)-Gly-Thr(P)-Phe-Thr(P)-Ser(P)-Asp(P)-Val-Ser(P)-Ser(P)-Tyr(P)-Leu-O-P2 [**SEQ ID NO: 47**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

47. A process according to claim 46 wherein the first peptide is selected from:
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH [**SEQ ID NO: 48**];
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 80**]; and
Boc-His(Trt)-Aib-Glu(tBu)-Gly-Thr(tBu)-Phe-ΨThr-Ser(tBu)-Asp(tBu)-Val-Ser(tBu)-ΨSer-Tyr(tBu)-Leu-OH [**SEQ ID NO: 81**].

48. A process according to any one of claims 45 to 47 wherein the second peptide has the formula:
Glu(P)-Gly-Gln(P)-Ala-Ala-Lys(W)-Glu(P)-Phe-Ile-Ala-Trp(P)-Leu-Val-Arg(P)-Gly-Arg(P)-Gly-O-P3 [**SEQ ID NO: 49**]
wherein:
W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
each P represents a side chain protecting group which may be the same or different; and
P3 is a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

49. A process according to claim 48 wherein the second peptide is H-Glu(tBu)-Gly-Gln(Trt)-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-O-Clt, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl [**SEQ ID NO: 50**] or H-Glu(tBu)-Gly-Gln(Trt)-Ala-Lys(W)-Glu(tBu)-Phe-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-

O-tBu, where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl **[SEQ ID NO: 82].**

50. A process for preparing a GLP-1 peptide of **SEQ ID NO: 1** according to any one of claims 9, 20, 30 or 40, wherein the second peptide is prepared by solid phase synthesis using Lys(Pal-Glu-O^{t}Bu)-OH.

51. A process for preparing a GLP-1 peptide of **SEQ ID NO: 1** according to any one of claims 9, 20, 30 or 40, wherein the second peptide is prepared by the steps of (i) solid phase synthesis using Fmoc-Lys(Mmt)-OH, (ii) cleaving from the resin and simultaneously removing the Mmt group from the Lys side chain, and (iii) treating with Pal-Glu(OSu)-O^{t}Bu or Pal-Glu(OPfp)-O^{t}Bu, (iv) esterifying in solution with a trityl type group, a diphenylmethyl group or a tert-butyl group.

52. A process for preparing a GLP-1 peptide of **SEQ ID NO: 8** according to any one of claims 15, 25, 35 or 45, wherein the second peptide is prepared by solid phase synthesis using Lys(C18-Glu-PEG2)-OH.

53. A process for preparing a GLP-1 peptide of **SEQ ID NO: 8** according to any one of claims 15, 25, 35 or 45, wherein the second peptide is prepared by the steps of (i) solid phase synthesis solid phase synthesis using Fmoc-Lys(Mmt)-OH, (ii) cleaving from the resin and simultaneously removing the Mmt group from the Lys side chain, and (iii) treating with C18-Glu(OSu)-PEG2 or C18-Glu(OPfp)-PEG2, (iv) esterifying in solution with a trityl type group, a diphenylmethyl group or a tert-butyl group.

54. A method according to claim 9 wherein the peptide of **[SEQ ID NO: 2]** is prepared from His-Ala-Glu-Gly **[SEQ ID NO: 51]** by stepwise solid phase synthesis.

55. A method according to claim 9 wherein the peptide of **[SEQ ID NO: 2]** is prepared by fragment condensation of P1-His-Ala-Glu-Gly-OH **[SEQ ID NO: 52]** and H-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-O-P3 **[SEQ ID NO: 53]**, wherein P1 is a protecting group for the N-terminal, and P3 is a protecting group for the C-terminal, and where one or more amino acid residues in the peptide fragments may be unprotected or protected.

56. A method according to claim 15 wherein the peptide of **[SEQ ID NO: 9]** is prepared from His-Aib-Glu-Gly **[SEQ ID NO: 54]** by stepwise solid phase synthesis.

57. A method according to claim 15 wherein the peptide of **[SEQ ID NO: 9]** is prepared by fragment condensation of P1-His-Aib-Glu-Gly-OH **[SEQ ID NO: 55]** and H-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-O-P3 **[SEQ ID NO: 56]**, wherein P1 is a protecting group for the N-terminal, and P3 is a protecting group for the C-terminal, and where one or more amino acid residues in the peptide fragments may be unprotected or protected.

58. A process according to claim 8 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 1**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(Pal-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

and said process comprises:

(i) coupling a first peptide having the sequence:

$X_n...X_{18}$-Ala [**SEQ ID NO: 57**]

wherein:

$X_n...X_{18}$ represents amino acid residues n to 18 of liraglutide, where n is 1 to 17;

the N-terminal is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(Pal-Glu-OX)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 3**]

wherein:

X is H or a protecting group for the Glu carboxylic acid group;

and wherein one or more of the amino acid residues in the first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

59. A process according to claim 58, where n is 2 to 17, and wherein after solution phase coupling with **SEQ ID NO: 3**, said process further comprises the stepwise addition of one or more amino acids, or condensation with a sub-fragment, to give **SEQ ID NO: 1**.

60. A process according to claim 58 or claim 59 where n is 15.

61. A process according to claim 14 wherein the GLP-1 peptide or analogue or variant thereof is of **SEQ ID NO: 8**:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

20 21 22 23 24 25 26 27 28 29 30 31

Lys(W)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

where W is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;
and said process comprises:

(i) coupling a first peptide having the sequence:

$Y_n...Y_{18}$-Ala [**SEQ ID NO: 58**]

wherein:

$Y_n...Y_{18}$ represents amino acid residues n to 18 of semaglutide, where n is 1 to 17;

the N-terminal is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Lys(W1)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH [**SEQ ID NO: 10**]

wherein:

W1 is N-(17-carboxy-1-oxoheptadecyl)-L-γ-glutamyl-2-[2-(2-aminoethoxy) ethoxy]acetyl-2-[2-(2-aminoethoxy)ethoxy]acetyl;

and wherein one or more of the amino acid residues in the first and second peptides and W1 may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-1 peptide.

62. A process according to claim 61, where n is 2 to 17, and wherein after solution phase coupling with **SEQ ID NO:10**, said process further comprises the stepwise addition of one or more amino acids, or condensation with a sub-fragment, to give **SEQ ID NO: 8**.

63. A process according to claim 61 or claim 62 where n is 15.

64. A process according to any one of claims 1 to 6 wherein the glucagon-like peptide is a GLP-2 peptide or an analogue or variant thereof.

65. A process according to claim 64 wherein the GLP-2 peptide or analogue or variant thereof is Teduglutide, or an analogue or variant thereof.

66. A process according to claim 65 wherein the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu [**SEQ ID NO: 84**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 85**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

67. A process according to claim 66 wherein the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu-OP2 [**SEQ ID NO: 86**]
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

68. A process according to claim 66 wherein the GLP-2 peptide is of **SEQ ID NO: 83**, and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-OH [**SEQ ID NO: 87**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-; or
the GLP-2 peptide is of **SEQ ID NO: 108**, and the first peptide is Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu^{14}-OH [(**Boc-Aib^{2}Ted(1-14)-OH**] , where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

69. A process according to any one of claims 66 to 68 wherein the second peptide has the formula:
Asp(P)-Asn(P)-Leu-Ala-Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 [**SEQ ID NO: 88**]
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

70. A process according to claim 69 wherein the second peptide is selected from:

Asp(tBu)-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 89**]; and

H-Asp(tBu)[15]-Asn(Trt)-Leu-Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)[33]-O-**R** [(**H-Ted(15-33)-O-R**], wherein R = H, Clt, Dpm, tBu,
and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

71. A process according to claim 65 wherein the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu **[SEQ ID NO: 90]**

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Leu carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH **[SEQ ID NO: 91]**

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

72. A process according to claim 71 wherein the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-O-P2 **[SEQ ID NO: 92]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Leu residue (preferably Su, Bt or Pfp).

73. A process according to claim 71 wherein the GLP-2 peptide is of **SEQ ID NO: 83**, and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-OH **[SEQ ID NO: 93]**, where Phe-Ser(tBu)- can also be -Phe-ΨSer-; or
the GLP-2 peptide is of **SEQ ID NO: 108**, and the first peptide is Boc-His(Trt)^{1}-Aib-Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu^{17}-OH **[(Boc- Aib^{2}Ted(1-17)-OH]**, where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

74. A process according to any one of claims 71 to 73 wherein the second peptide has the formula:
Ala-Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 **[SEQ ID NO: 94]**
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

75. A process according to claim 74 wherein the second peptide is selected from:

Ala-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 95**]; and

H-Ala^{18}-Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(18-33)-O-R**], wherein R = H, Clt, Dpm, tBu,
where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

76. A process according to claim 65 wherein the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala [**SEQ ID NO: 96**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 97**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

77. A process according to claim 76 wherein the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-Ala-O-P2 **[SEQ ID NO: 98]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

78. A process according to claim 76 wherein the GLP-2 peptide is of **SEQ ID NO: 83** and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-OH [**SEQ ID NO: 99**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-; or
the GLP-2 peptide is of **SEQ ID NO: 108** and the first peptide is Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala^{18}-OH [**(Boc- Aib^{2}Ted(1-18)-OH**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

79. A process according to any one of claims 76 to 78 wherein the second peptide has the formula:
Ala-Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 **[SEQ ID NO: 100]**
wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

80. A process according to claim 79 wherein the second peptide is selected from:

Ala-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH [**SEQ ID NO: 101**];

H-Ala^{19}-Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [(**H-Ted(19-33)-O-R**], wherein R = H, Clt, Dpm, tBu,
and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

81. A process according to claim 65 wherein the GLP-2 peptide or analogue or variant thereof is of **SEQ ID NO: 83**:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
His	- Gly	- Asp	- Gly	- Ser	- Phe	- Ser	- Asp	- Glu	- Met	- Asn	- Thr	- Ile	- Leu	- Asp

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
- Asn	- Leu	- Ala	- Ala	- Arg	- Asp	- Phe	- Ile	- Asn	- Trp	- Leu	- Ile	- Gln	- Thr	- Lys

31	32	33
- Ile	- Thr	- Asp

or a variant thereof,

and said process comprises:

(i) coupling a first peptide having the sequence:

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala [**SEQ ID NO: 102**]

wherein:

the N-terminal of His is optionally protected with a protecting group, preferably Boc, or Fmoc; and

the Ala carboxylic acid group is optionally in the form of an activated carboxylic acid derivative;

in solution with a second peptide having the sequence:

Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH [**SEQ ID NO: 103**]

wherein one or more of the amino acid residues in said first and second peptides may be unprotected or protected, preferably with an acid-cleavable protecting group;

(ii) optionally removing any protecting groups;

(iii) optionally purifying the GLP-2 peptide.

82. A process according to claim 81 wherein the first peptide has the formula:
P1-His(P)-Gly-Asp(P)-Gly-Ser(P)-Phe-Ser(P)-Asp(P)-Glu(P)-Met-Asn(P)-Thr(P)-Ile-Leu- Asp(P)-Asn(P)-Leu-Ala-Ala-O-P2 **[SEQ ID NO: 104]**
wherein:
P1 is a protecting group for the N-terminal of His (preferably Boc, or Fmoc);
each P represents a side chain protecting group which may be the same or different; and
P2 is H or an activated carboxylic ester of the Ala residue (preferably Su, Bt or Pfp).

83. A process according to claim 81 wherein the GLP-2 peptide is of **SEQ ID NO: 83** and the first peptide is Boc-His(Trt)-Gly-Asp(tBu)-Gly-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)-Asn(Trt)-Leu-Ala-Ala-OH **[SEQ ID NO: 105]**, where Phe-Ser(tBu)- can also be -Phe-ΨSer; or
the GLP-2 peptide is of **SEQ ID NO: 108** and the first peptide is Boc-His(Trt)^{1}-Aib -Asp(tBu)-Gly^{4}-Ser(tBu)-Phe-Ser(tBu)-Asp(tBu)-Glu(tBu)-Met-Asn(Trt)-Thr(tBu)-Ile-Leu-Asp(tBu)- Asn(Trt)-Leu-Ala-Ala^{19}-OH [**(Boc- Aib^{2}Ted(1-19)-OH**], where Phe-Ser(tBu)- can also be -Phe-ΨSer-.

84. A process according to any one of claims 81 to 83 wherein the second peptide has the formula:
Arg(P)-Asp(P)-Phe-Ile-Asn(P)-Trp(P)-Leu-Ile-Gln(P)-Thr(P)-Lys(P)-Ile-Thr(P)-Asp(P)-O-P3 **[SEQ ID NO: 106]**

wherein each P represents a side chain protecting group which may be the same or different; and
P3 is H or a carboxy protecting group, preferably selected from Clt, Trt, tBu, DPM, MeDPM and MeODPM.

85. A process according to claim 84 wherein the second peptide is selected from:

Arg(Pbf)-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)-OH **[SEQ ID NO: 107]**; and

H-Arg(Pbf)^{20}-Asp(tBu)-Phe-Ile-Asn(Trt)-Trp(Boc)-Leu-Ile-Gln(Trt)-Thr(tBu)-Lys(Boc)-Ile-Thr(tBu)-Asp(tBu)^{33}-O-**R** [**(H-Ted(20-33)-O-R**], wherein R = H, Clt, Dpm, tBu, and where -Gln(Trt)-Thr(tBu)- can also be -Gln(Trt)-ΨThr-.

86. A process according to any preceding claim wherein the first fragment is prepared on solid phase or in solution.

87. A process according to any preceding claim wherein the second fragment is prepared by coupling two or more sub-fragments.

88. A process according to any preceding claim wherein the crude GLP peptide or analogue or variant thereof is purified by reverse phase chromatography.

89. A process according to any preceding claim wherein the reverse phase chromatography is carried out using C18, C8 or C4 modified silica.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2018/057736

A. CLASSIFICATION OF SUBJECT MATTER
INV. 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)
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 practicable, search terms used)

EPO-Internal, INSPEC, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WU, JIAN ET AL.: "Synthesis of human GLP-1 (7-36) by chemoselective .alpha.-ketoacid-hydroxylamine peptide ligation of unprotected fragments", CHEMICAL SCIENCE, vol. 2, no. 10, 2011, pages 1976-1979, XP002787085,	1,7
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2018	18/12/2018

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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