There remains a need, however, for improved processes for production of Gcg and GLP-1 dual agonist peptides, such processes having a combination of advantages including commercially desired purity. Similarly, there is a need for efficient and environmentally "green" processes, including stable compounds to provide Gcg and GLP-1 dual agonist peptides with fewer or simpler purification steps. The preparation of large-scale, pharmaceutically-elegant Gcg and GLP-1 dual agonist peptides presents a number of technical challenges that may affect the overall yield and purity. There is also a need for processes to avoid the use of harsh reaction conditions that are incompatible with peptide synthesis.

The present disclosure seeks to meet these needs by providing novel processes useful in the manufacture of a Gcg and GLP-1 dual agonist peptide (SEQ ID NO:1), or a pharmaceutically acceptable salt thereof. The improved manufacturing processes of the present disclosure provide compounds and process reactions embodying a combination of advances, including an efficient route having fewer steps, while at the same time maintaining high quality and purity. Importantly, the improved processes and compounds decrease resource intensity.

The improved processes described herein provide various compounds useful for production of a Gcg and GLP-1 dual agonist peptide.

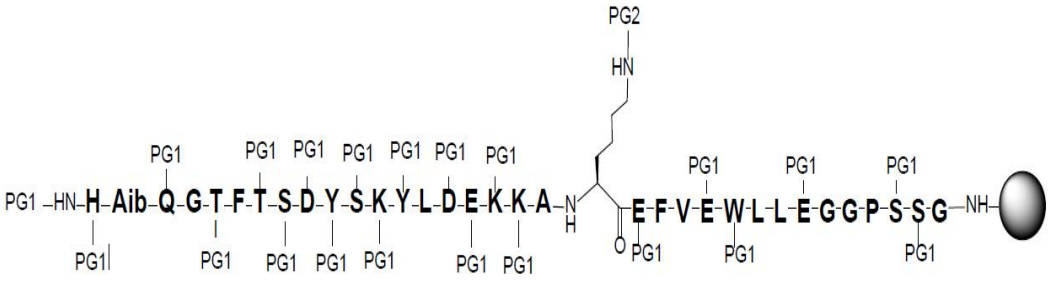
In an aspect, there is provided a process for the preparation of a compound of the following formula:

H_2N-**H-Aib-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-<u>K</u>-E-F-V-E-W-L-L-E-G-G-P-S-S-G**-NH_2

wherein Lys at position 20 is chemically modified by conjugation of the epsilon-amino group of the Lys side chain with ([2-(2-aminoethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}CO_2H$ (SEQ ID NO: 1),

said process comprising the steps of:

(i) solid-phase synthesis of a compound of the following formula


PG2
HN
PG1 –HN–H–Aib–Q–G–T–F–T–S–D–Y–S–K–Y–L–D–E–K–K–A–
N
H
E–F–V–E–W–L–L–E–G–G–P–S–S–G–NH–
PG1
O
PG1
PG1
PG1

impurity profile for the acylation reaction. Moreover, the improved acylation process facilitates an easier purification and isolation of the desired acylated peptide product that results in higher yields and purity.

Selective de-protection of the Lys at position 20 is facilitated by use of an ivDde, Dde or Alloc side-chain protecting group at position 20 and base stable side-chain protecting groups at other positions. De-protection conditions are selected wherein the ivDde, Dde or Alloc side-chain protecting group at position 20 is removed but the base-stable side-chain protecting groups (PG1) remain in place.

A variety of base-stable protecting groups are known in the art and may be used in the process of the present disclosure. In an example of the present disclosure, the base-stable side-chain protecting groups PG1 used in the synthesis of the compound are (a) tert-butyloxycarbonyl (Boc) for Trp and Lys, (b) tert-butyl ester (O^tBu) for Asp and Glu, (c) tert-butyl (^tBu) for Ser, Thr and Tyr, (d) triphenylmethyl (trityl)(Trt) for Gln, and (e) Boc(Boc) or Boc(Dnp) for His.

In a preferred example of the process of the present disclosure, the side-chain protecting group at Lys at position 20 is ivDde.

In an alternative example of the process of the present disclosure, the side-chain protecting group at the Lys at position 20 is Dde.

Dde is a protecting group stable to most conventional bases and is, therefore, stable to Fmoc removal conditions. ivDde is a derivative of Dde and is also stable to Fmoc removal conditions. An additional advantage of ivDde is that its steric hindrance makes it less prone to migrate to other free Lys residues. Both Dde and ivDde are commonly removed by hydrazinolysis.

Preferably, when PG2 is ivDde or Dde, the Lys at position 20 is selectively de-protected by contacting the compound with a solution comprising hydrazine hydrate.

Further preferably, the solution comprises 1% - 15% w/w hydrazine hydrate in DMF, NMP, NBP or DMSO.

Still further preferably, the solution comprises 8% w/w hydrazine hydrate in DMF.

In an alternative example of the process of the present disclosure, the side-chain protecting group at the Lys at position 20 is Alloc.

Alloc is a base-labile protecting group. It is commonly removed by a palladium catalyst in the presence of a scavenger to capture the generated carbocation. The use of Alloc side-chain protecting group is compatible with the Boc/Bn and Fmoc/^{t}Bu strategies and allows tandem removal-acylation reactions when the palladium-catalyzed amino deblocking is performed in the presence of acylating agents. This approach prevents diketopiperazine (DKP) formation.

Preferably, when the side-chain protecting group at Lys at position 20 is Alloc, Lys at position 20 is selectively de-protected by contacting the compound with a palladium catalyst in the presence of scavengers,

Further preferably, the Alloc side-chain protecting group at Lys at position removed by contacting the compound with $Pd(PPh_3)_4$ in the presence of H_3N•BH3, Me_2NH•BH3, or $PhSiH_3$.

The de-protected (at position 20) compound may be washed, de-swelled, isolated, dried and packaged. The de-protected (at position 20) compound is re-swelled prior to coupling with sidechain

In a preferred example of the process of the present disclosure, PG1 is Boc for Trp and Lys, O^{t}Bu for Asp and Glu, ^{t}Bu for Ser, Thr and Tyr, Trt for Gln and Boc(Boc) for His, PG2 is ivDde, and the solid-phase synthesis of the compound (SEQ ID NO: 3) of step (i) is performed on a Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following:

Fmoc-L-Gly-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Pro-OH, Fmoc-L-Gly-OH, Fmoc-L-Gly-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Leu-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Val-OH, Fmoc-L-Phe-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-Lys(ivDde)-OH, Fmoc-L-Ala-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Thr(^{t}Bu)-OH, Fmoc-L-Phe-OH, Fmoc-Gly-Thr($\psi^{Me,Me}$Pro)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-Aib-OH; and Boc-L-His(Boc)-OH.

In an alternative example of the process of the present disclosure, PG1 is Boc(Dnp) for His and the solid-phase synthesis of the compound of step (i) is performed as described above.

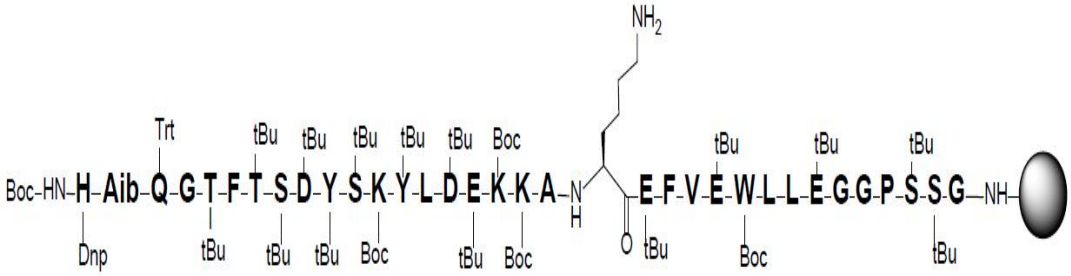
Solid phase synthesis of the compound is performed on a Fmoc amide resin solid support wherein the first step is Fmoc deprotection of the amide resin followed by sequential coupling of the Fmoc amino acids of the peptide. A glycine-threonine pseudoproline dipeptide is used in place of individual Fmoc-L-Gly and Fmoc-L-Thr amino acids for coupling at positions 4 and 5. In these examples, the Thr residue at position 5 is reversibly protected as a proline-like acid-labile oxazolidine. As such, there is no requirement to protect that particular Thr residue with a PG1. A substantial benefit is realized in that the reaction proceeds to completion for the glycine-threonine pseudoproline dipeptide. In contrast, coupling individual Fmoc-L-Gly and Fmoc-L-Thr amino acids result in high levels of peptide impurities having a Thr5 deletion.

In an alternative preferred example of the process of the present disclosure, PG1 is Boc for Trp and Lys, O^{t}Bu for Asp and Glu, ^{t}Bu for Ser, Thr and Tyr, Trt for Gln, and Boc(Dnp) for His, PG2 is ivDde, and the solid-phase synthesis of the compound (SEQ ID NO: 4) of step (i) is performed on a Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following:

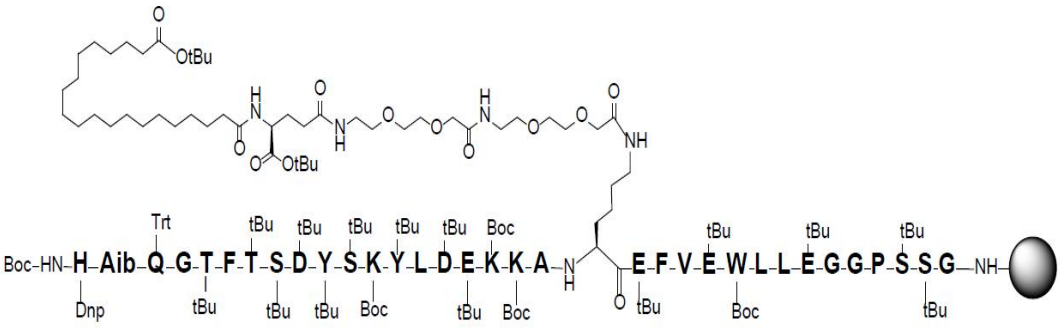
Fmoc-L-Gly-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Pro-OH, Fmoc-L-Gly-OH, Fmoc-L-Gly-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Leu-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Val-OH, Fmoc-L-Phe-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-Lys(ivDde)-OH, Fmoc-L-Ala-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Thr(^{t}Bu)-OH, Fmoc-L-Phe-OH, and Boc-His(Dnp)-Aib-Gln(Trt)-Gly-Thr(^{t}Bu)-OH.

Solid phase synthesis of the compound is performed on a Fmoc amide resin solid support wherein the first step is Fmoc deprotection of the amide resin followed by sequential coupling of the Fmoc amino acids of the peptide. A Boc-His(Dnp)-Aib-Gln(Trt)-Gly-Thr(^{t}Bu)-OH pentamer (SEQ ID NO: 14) is coupled as a single fragment to Phe6 of the H_2N-6-34 intermediate (SEQ ID NO: 10). A substantial benefit realized by this preferred example is improved purity due to minimization of histidine racemization.

The compound of SEQ ID NO: 4 may be selectively de-protected at the lysine at position 20 as described herein. The resulting compound has the following formula (SEQ ID NO: 18):



The compound of SEQ ID NO: 18 may be coupled with the ^{t}BuO-C_{20}-γGlu(^{t}Bu)-AEEA-AEEA-OH sidechain as an intact fragment as described herein. The resulting compound has the following formula (SEQ ID NO: 19):



In a further alternative preferred example of the process of the present disclosure, PG1 is: (a) Boc for Trp and Lys, (b) O^{t}Bu for Asp and Glu, (c) ^{t}Bu for Ser, Thr

and Tyr, (d) Trt for Gln, and (e) Boc(Dnp) for His, PG2 is ivDde, and the solid-phase synthesis of the compound (SEQ ID NO: 4) of step (i) is performed on a Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following:

Fmoc-L-Gly-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Pro-OH, Fmoc-L-Gly-OH, Fmoc-L-Gly-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Leu-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Val-OH, Fmoc-L-Phe-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-Lys(ivDde)-OH, Fmoc-L-Ala-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Leu-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Ser(^{t}Bu)-OH, Fmoc-L-Thr(^{t}Bu)-OH, Fmoc-L-Phe-OH, Fmoc-L-Thr(^{t}Bu)-OH; and Boc-His(Dnp)-Aib-Gln(Trt)-Gly-OH.

Solid phase synthesis of the compound is performed on a Fmoc amide resin solid support wherein the first step is Fmoc deprotection of the amide resin followed by sequential coupling of the Fmoc amino acids of the peptide. A Boc-His(Dnp)-Aib-Gln(Trt)-Gly-OH tetramer (SEQ ID NO: 16) is coupled as a single fragment to Thr5 of the $_{2}$HN-5-34 intermediate (SEQ ID NO: 12). A substantial benefit realized by this preferred example is improved purity due to minimization of histidine racemization.

The compound of SEQ ID NO: 4 may be selectively de-protected at the lysine at position 20 as described herein. The resulting compound has the formula of SEQ ID NO: 18.

The compound of SEQ ID NO: 18 may be coupled with the ^{t}BuO-C$_{20}$-γGlu(^{t}Bu)-AEEA-AEEA-OH sidechain as an intact fragment as described herein. The resulting compound has the formula of SEQ ID NO: 19.

In a preferred example of the process of the present disclosure, the resin solid support is a Fmoc amide resin solid support and the solid phase synthesis comprises Fmoc deprotection of the resin.

Further preferably, the Fmoc amide resin solid support is a Sieber resin.

In an example of the present disclosure, step (iii) further comprises adjusting the pH of a solution comprising the cleaved and deprotected compound to 7.0 – 8.0, stirring for 1-24 hours, subsequently adjusting the pH of the solution to 1.0 - 3.0, and stirring for 1-24 hours.

Adjusting the pH to 7.0 - 8.0 neutralizes the solution and converts any depsi-peptide ester serine and threonine impurities to the desired compound.

Subsequent adjustment of the pH to 1.0 – 3.0 decarboxylates the Trp residue and converts the Trp CO_2 salt to the desired product.

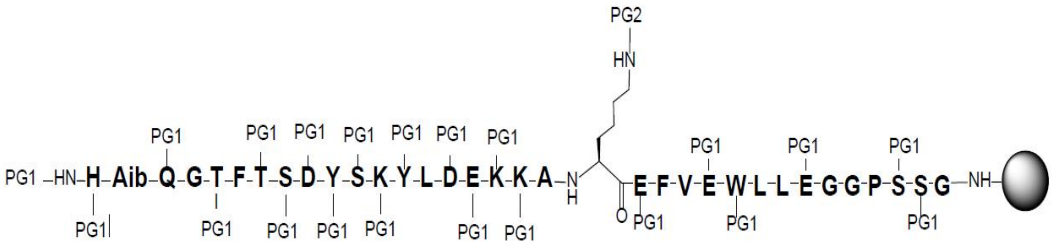
In an exmaple of the process of the disclosure, the purification of the compound comprises subjecting the crude solution of the compound of step (iii) to chromatographic purification.

Preferably, the chromatographic purification is HPLC or reverse phase HPLC.

Still further preferably, the purification further comprises the steps of (i) adding the chromatographic eluent to a solution comprising aqueous sodium hydroxide or aqueous sodium bicarbonate to form a sodium salt of the compound in solution, (ii) precipitating the sodium salt of the compound from solution and (iii) filtering, washing and drying the precipitated sodium salt of the compound.

The sodium salt imparts improved solubility of the compound relative to the zwitterion or actetate forms. Furthermore, precipitation of the sodium salt of the compound replaces expensive lyophilization procedures.

In a further aspect of the present disclosure, there is provided a process for the preparation of a compound of the following formula:

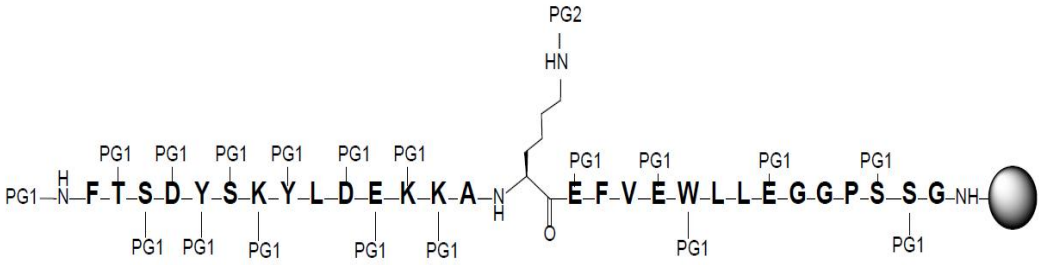


wherein PG1 is a base stable side-chain protecting group,

wherein PG2 is an ivDde, Dde or Alloc side-chain protecting group (SEQ ID NO: 17),

and wherein said process comprises the steps of:

(i) solid-phase synthesis of a compound of the following formula:



wherein PG1 is a base stable side-chain protecting group,
and wherein PG2 is an ivDde, Dde or Alloc side-chain protecting group (SEQ ID NO: 9); and

(ii) coupling the compound of step (i) with a pentamer of the following formula:

PG1-His(PG1)-Aib-Gln(PG1)-Gly-Thr(PG1)-OH

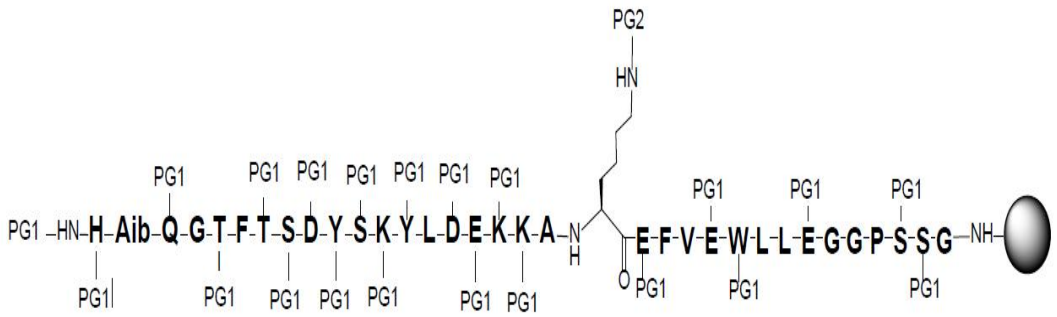
wherein PG1 is a base stable side-chain protecting group (SEQ ID NO: 13).

In a preferred example of the process of the present disclosure, PG1 is Boc for Trp and Lys, O^{t}Bu for Asp and Glu, ^{t}Bu for Ser, Thr and Tyr, Trt for Gln, and Boc(Dnp) for His.

In a further preferred example of the process of the present disclosure, PG2 is ivDde.

In an alternative preferred example of the process of the process disclosure, PG2 is Dde.

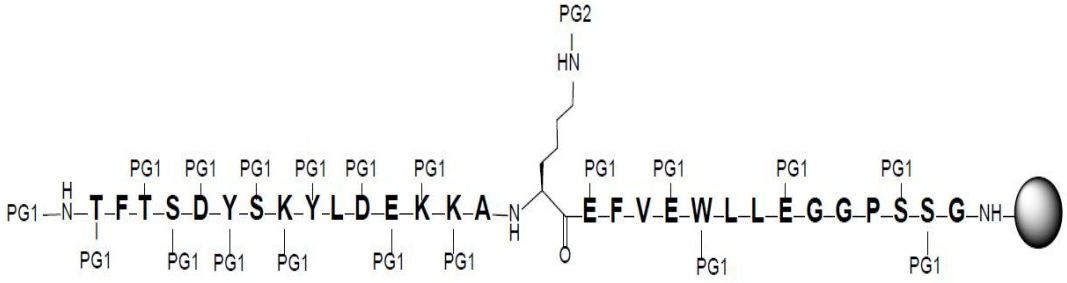
In a further aspect of the present disclosure, there is provided a process for the preparation of a compound of the following formula:



wherein PG1 is a base stable side-chain protecting group,
and wherein PG2 is an ivDde, Dde or Alloc side-chain protecting group (SEQ ID NO: 17)

said process comprising the steps of:

(i) solid-phase synthesis of a compound of the following formula:



wherein PG1 is a base stable side-chain protecting group,
and wherein PG2 is an ivDde, Dde or Alloc side-chain protecting group (SEQ ID NO: 11); and

(ii) coupling the compound of step (i) with a tetramer of the following formula:

PG1-His(PG1)-Aib-Gln(PG1)-Gly-OH

wherein PG1 is a base stable side-chain protecting group (SEQ ID NO: 15).

In a preferred example of the process of the present disclosure, PG1 is Boc for Trp and Lys, O^{t}Bu for Asp and Glu, ^{t}Bu for Ser, Thr and Tyr, Trt for Gln, and Boc(Dnp) for His.

In a further preferred example of the process of the present disclosure, PG2 is ivDde.

In an alternative preferred example of the process of the present disclosure, PG2 is Dde.

In a further example of the present disclosure, there is provided a process for the preparation of a sodium salt of the compound of the following formula:

H_2N-**H-Aib-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-K-E-F-V-E-W-L-L-E-G-G-P-S-S-G**-NH_2

wherein lysine (Lys/K) at position 20 is chemically modified by conjugation of the epsilon-amino group of the lysine side chain with ([2-(2-aminoethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}CO_2H$ (SEQ ID NO: 1)

said process comprising the steps of:

(i) adding aqueous sodium hydroxide or aqueous sodium bicarbonate to a solution comprising the compound to form a sodium salt of the compound in solution;

(ii) precipitating the sodium salt of the compound from solution; and

(iii) filtering, washing and drying the precipitated sodium salt of the compound.

In another aspet, there is provided a process for the preparation of a sodium salt of the compound of the following formula:

H_2N-**H-Aib-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-K-E-F-V-E-W-L-L-E-G-G-P-S-S-G**-NH_2

wherein lysine (Lys/K) at position 20 is chemically modified by conjugation of the epsilon-amino group of the lysine side chain with ([2-(2-aminoethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}CO_2H$ (SEQ ID NO: 1)

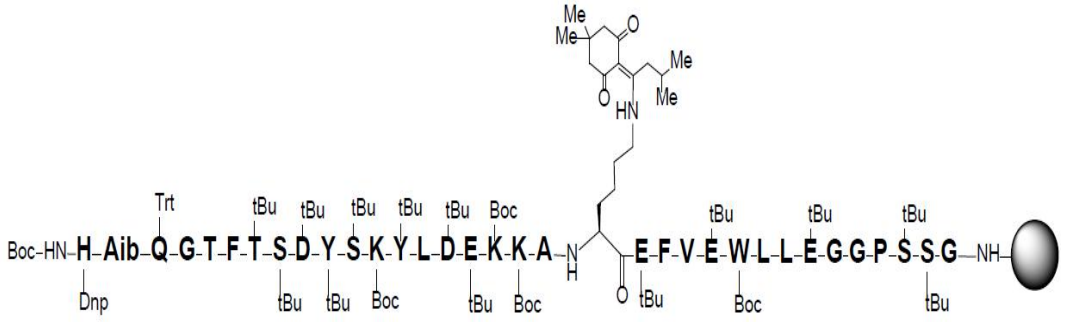
said process comprising the steps of:

(i) adding aqueous sodium hydroxide or aqueous sodium bicarbonate to a solution comprising the compound of SEQ ID NO: 1 to form a sodium salt of the compound in solution;

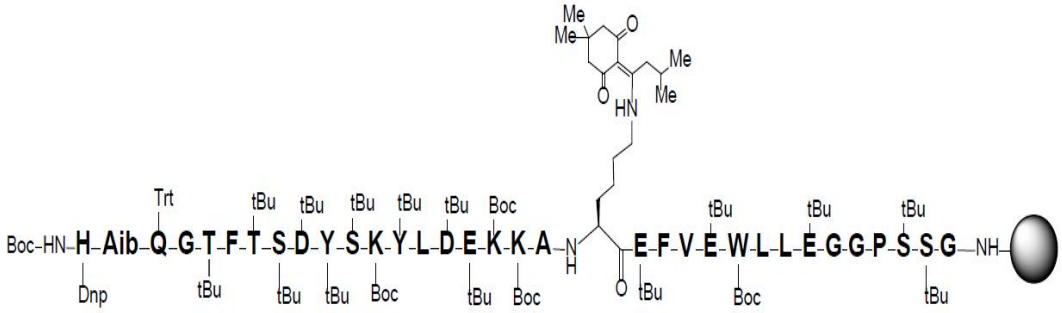
(ii) precipitating the sodium salt of the compound from solution; and

(iii) filtering, washing and drying the precipitated sodium salt of the compound of SEQ ID NO: 1.

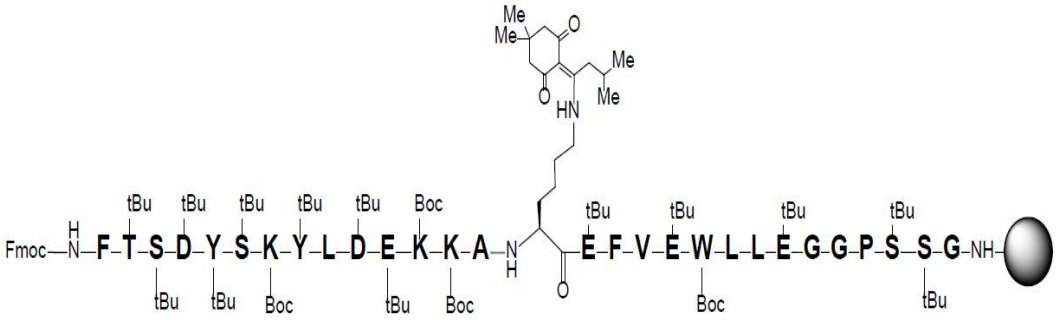
In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 3):



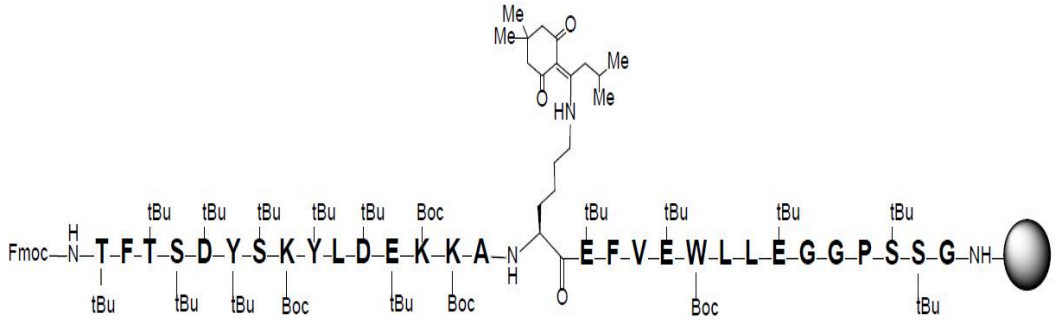
In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 4):



In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 10):



In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 12):



In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 13):

PG1-His(PG1)-Aib-Gln(PG1)-Gly-Thr(PG1)-OH

wherein PG1 is a base stable side-chain protecting group.

Preferably, PG1 is ^{t}Bu for Thr, Trt for Gln, and Boc(Dnp) for His.

In a further aspect of the present disclosure, there is provided a compound having the following formula (SEQ ID NO: 15):

PG1-His(PG1)-Aib-Gln(PG1)-Gly-OH

wherein PG1 is a base stable side-chain protecting group.

Preferably, PG1 is Trt for Gln and Boc(Dnp) for His.