

PROCESS FOR PREPARING A GLP-1/GLUCAGON DUAL AGONIST

The present invention provides processes for synthesizing, via hybrid solid liquid phase synthesis (hybrid SPPS-LPPS, also referred herein as HSLPS), a glucagon (GCG) and GLP-1 dual agonist peptide, or a pharmaceutically acceptable salt thereof.

Over the past several decades, the prevalence of diabetes has continued to rise. Type 2 diabetes mellitus ("T2D") is the most common form of diabetes, accounting for about 90% of all diabetes. T2D is characterized by high blood glucose levels caused by insulin resistance. Uncontrolled diabetes leads to several conditions that impact morbidity and mortality of patients. The leading cause of death for diabetic patients is cardiovascular complications. One of the main risk factors for type 2 diabetes is obesity. It is documented that a decrease in body adiposity will lead to improvement in obesity-associated co-morbidities including hyperglycemia and cardiovascular events. Therefore, therapies effective in glucose control and weight reduction are needed for better disease management.

GCG helps maintain the level of glucose in the blood by binding to GCG receptors on hepatocytes, causing the liver to release glucose - stored in the form of glycogen - through glycogenolysis. As these stores become depleted, GCG stimulates the liver to synthesize additional glucose by gluconeogenesis. This glucose is released into the bloodstream, preventing the development of hypoglycemia.

GLP-1 has different biological activities compared to GCG. The actions of GLP-1 include stimulation of insulin synthesis and secretion, inhibition of GCG secretion and inhibition of food intake. GLP-1 has been shown to reduce hyperglycemia in diabetics. Several GLP-1 agonists have been approved for use in the treatment of T2D in humans, including exenatide, liraglutide, lixisenatide, albiglutide and dulaglutide. Such GLP-1 agonists are effective in glycemic control with favorable effects on weight without the risk of hypoglycemia. However, the weight loss is modest due to dose-dependent gastrointestinal side-effects.

GCG and GLP-1 dual agonist peptides that may be useful in the treatment of T2D and obesity are described and claimed in US Patent No. 9,938,335 B2. A process for the production of such GCG and GLP-1 dual agonist peptides is described therein.

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.

- 5 Intl. Patent Application Publication No. WO2021/252829 describes a method of synthesizing GCG and GLP-1 dual agonist peptides using linear solid phase peptide synthesis (SPPS). There is a need, however, for alternative methods for production of GCG and GLP-1 dual agonist peptides and intermediates thereof to enable pharmaceutically elegant production with commercially desired purity and yield.
- 10 Likewise, there is a need for methods and stable intermediates to provide GCG and GLP-1 dual agonist peptides efficiently with reduced purification steps.

- The present invention seeks to meet these needs by providing novel intermediates and 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 processes of the present
- 15 invention provide intermediates and process reactions embodying a combination of advances, including an efficient route, while at the same time maintaining high quality and purity, and decreasing resource intensity and minimizing waste streams.

 In one embodiment, there is provided a process for the preparation of a compound of the formula:

- 20 $\text{H}_2\text{N-H-Aib-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-}\underline{\text{K}}\text{-E-F-V-E-W-L-L-E-G-G-P-S-S-G-NH}_2$

 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-(CH₂)₁₈.CO₂H (SEQ ID NO:1).

- 25 The processes of the invention include HSLPS methods, where such methods use two to four intermediate preparations to make the compound of SEQ ID NO:1. The term “preparation” as used herein refers to a compound such as a peptide fragment or a fatty acid moiety that is used in the synthesis of the compound of SEQ ID NO:1.

- In one embodiment, the methods can include at least a step of coupling three
- 30 intermediate preparations, where such preparations have a structure as recited in SEQ ID NO’S:2, 3, and 5, or pharmaceutically acceptable salts thereof.

Alternatively, the methods can include at least a step of coupling three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:2, 10, and 12, or pharmaceutically acceptable salts thereof.

Alternatively, the methods can include at least a step of coupling three
5 intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:17, 18 and 12, or pharmaceutically acceptable salts thereof.

Alternatively, the methods can include at least a step of coupling three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:17, 21 and 5, or pharmaceutically acceptable salts thereof.

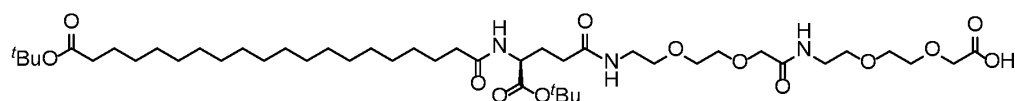
10 Alternatively, the methods can include at least a step of coupling three intermediate preparations, where such intermediate preparations have a structure as recited in SEQ ID NO'S:36, 37 and 12, or pharmaceutically acceptable salts thereof.

Alternatively, the methods can include at least a step of coupling two intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:24 and
15 25, or pharmaceutically acceptable salts thereof.

In the methods above, the fatty acid moiety and linker can be attached to one intermediate preparation before the various intermediate preparations are coupled (*i.e.*, acylation can occur before complete synthesis).

Alternatively, the fatty acid moiety can be attached to the peptide after the various
20 intermediate preparations have been coupled (*i.e.*, acylation can occur after complete synthesis and selective Lys 20 deprotection).

For example, the methods can include at least a step of coupling three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:2, 7, and 5, or pharmaceutically acceptable salts thereof, followed by coupling of a
25 fatty acid moiety having a structure of:



(Preparation 30).

Alternatively, the methods can include at least a step of coupling three intermediate preparations, where such preparations have a structure as recited in SEQ ID

NO'S:2, 14, and 12, or pharmaceutically acceptable salts thereof, followed by coupling of a fatty acid moiety having a structure of Preparation 30.

Alternatively, the methods can include at least a step of coupling two intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:27 and
5 25, or pharmaceutically acceptable salts thereof, followed by coupling of a fatty acid moiety having a structure of Preparation 30.

Alternatively, the methods can include at least a step of coupling a preparation having a structure as recited in SEQ ID NO:30, or pharmaceutically acceptable salt thereof, followed by coupling of a fatty acid moiety having a structure of Preparation 30.

10 The methods above also can include a step of synthesizing the intermediate preparations prior to the coupling step.

In the methods above, the intermediate preparations therefore can be chemically coupled or enzymatically coupled to one another to obtain the compound of SEQ ID NO:1.

15 In addition to the above methods, embodiments herein also include the intermediate preparations themselves (*e.g.*, SEQ ID NO'S:2-39), as well as compositions including the same.

An advantage of the methods herein includes several process improvements such as, for example, shorter peptide fragments initially produced via solid phase peptide
20 synthesis or SPPS allow for generally increased purity and higher yields via HSLPS.

An advantage of the methods herein includes that efficiency of the coupling in SPPS not only is dependent on the actual residues involved in the chemical transformation but also is impacted by structure attached to the resin (*i.e.*, solubility/aggregation issues are well known for certain sequences, in particular GCG);
25 with shorter fragments, more route flexibility is available for couplings of complicated amino acid residues, and an ability to redesign fragment structures to address more difficult transformations.

An advantage of the methods herein includes an improved control strategy for impurities during the synthesis, which can enable an improved final impurity profile for
30 the crude peptide and simplify/reduce chromatography burden resulting in the cost savings.

An advantage of the methods herein includes that synthesis of shorter fragments via SPPS can allow for reduced washing cycles, for reduced volumes of reagents, and for use of greener solvent(s) leading to a reduced process mass intensity (PMI).

5 An advantage of the methods herein includes that with shorter fragments, risks of failure typical in linear builds of a long molecule, in particular molecules containing GCG like sequences, are significantly reduced.

10 An advantage of the methods herein includes that a combination of liquid and solid phase synthesis is more amenable to new manufacturing platforms such as continuous chemistry and introducing other innovative technologies. An advantage of the methods herein includes flexibility in supply chain and logistics of the manufacturing process by using several independent fragments. An advantage of the methods herein includes that use parallel manufacturing of fragments can provide reduced manufacturing cycles by parallel processing of the fragments. An advantage of the methods herein includes that current good manufacturing practice (cGMP) Liquid Phase steps can be
15 executed at a standard manufacturing facility without a need for specialized equipment.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the disclosure pertains. Although any methods and materials similar to or equivalent to those described herein can be used in the synthesis, the preferred methods and materials are described
20 herein.

Moreover, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one element is present, unless the context clearly requires that there be one and only one element. The indefinite article “a” or “an” thus usually means “at least one.”
25

Abbreviations and Definitions

Certain abbreviations are defined as follows: “AEEA” refers to 2-[2-(2-amino-ethoxy)-ethoxy]-acetyl, “Aib” refers to α -amino isobutyric acid, “Alloc” means allyloxycarbonyl, “Boc” refers to tert-butoxycarbonyl, “Bu” refers to butyl, “t-Bu” refers
30 to tert-Butyl, “CTC” refers to chlorotrityl chloride, “DCM” refers to dichloromethane, “DIC” refers to diisopropylcarbodiimide, “DMF” refers to dimethylformamide, “DMSO” refers to dimethyl sulfoxide, “Dnp” means 2,4-dinitrophenyl, “DTT” refers to

dithiothreitol, “Fmoc” refers to fluorenylmethyloxycarbonyl chloride, “hr” refers to hour(s), “HFIP” means hexafluoroisopropanol, “IPA” refers to isopropanol, “IPAc” refers to isopropyl acetate, “min” refers to minute(s), “Me” refers to methyl, “MTBE” refers to methyl-tert-butyl ether, “MTT” refers to 4-methyl Trityl, “oxyma” refers to ethyl
5 cyanohydroxyiminoacetate, “OtBu” means tert-butyl ester “PG” refers to protecting group, “Pip” refers to piperidine, “PyBOP” means (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), “SPPS” refers to solid phase peptide synthesis, “TFA” refers to trifluoroacetic acid, “TIPS” refers to triisopropylsilane, and “Trt” refers to trityl.

10 As used herein, “about” means within a statistically meaningful range of a value or values such as, for example, a stated concentration, length, molecular weight, pH, sequence identity, time frame, temperature or volume. Such a value or range can be within an order of magnitude typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation
15 encompassed by “about” will depend upon the particular system under study, and can be readily appreciated by one of skill in the art.

As used herein, the term “protected” means that a protecting group is attached to at the indicated position. The artisan will recognize that a variety of protecting groups are well known, and alternative protecting groups may be suitable for a particular process.

20 The disclosure provides and therefore encompasses novel intermediate compounds such as peptide fragments and fatty acid moieties (referred herein as “preparations” or “intermediate preparations”) useful in the synthesis of the compound of SEQ ID NO:1 and methods of synthesizing the compound of SEQ ID NO:1 or pharmaceutically acceptable salts thereof. The intermediate preparations herein can be
25 prepared by a variety of techniques known in the art. For example, a method using standard solid phase peptide synthesis for two or more intermediate preparations followed by coupling thereof in the liquid phase is illustrated in the Examples below. The specific synthetic steps for each of the routes or schemes described may be combined in different ways to prepare the compounds described herein. The reagents and starting materials are
30 readily available to one of skill in the art.

Methods

Standard Solid Phase Peptide Synthesis of Intermediate Preparations:

The intermediate peptide fragments herein can be made via any number of standard peptide synthesis methods known in the art, especially SPPS. SPPS builds are accomplished using standard Fmoc peptide chemistry techniques employing sequential
5 couplings with an automated peptide synthesizer such as Gyros Protein Technologies Symphony X Synthesizer. Methods of SPPS are well known in the art and need not be exhaustively described herein. *See generally*, “Fmoc Solid Phase Peptide Synthesis: A Practical Approach” (Chan & White ed., Oxford University Press 2000), and Merrifield (1963) *J. Am. Chem. Soc.* 85:2149-2154.

10 For deprotection, a resin is swelled with DMF, and then deprotected using 20% Pip/DMF (3 x 30 min). Subsequent Fmoc deprotections use sequential 20% Pip/DMF treatments, additional treatment sequences being used for more difficult deprotections.

After deprotection, the resin is washed with DMF. Amino acid pre-activation uses DIC/Oxyma DMF solutions at room temperature for 30 min. Coupling of the activated
15 amino acid to the resin-bound peptide occurs for a specified time for each individual amino acid. After couplings are completed DMF washes are performed after each coupling to remove excess reagents.

For isolation of the final product, the resin-bound product is washed with DCM to remove DMF. The resin is washed with IPA to displace DCM, then washed with MTBE,
20 and then the product is dried at 40°C under vacuum. The resin-bound product is stored cold (-20°C).

Soft Cleavage Procedure for peptides Keeping Protecting groups on:

Peptide on resin intermediate is treated with 30% HFIP in DCM solution. The
25 spent resin is filtered off, then washed with DCM. The combined filtrate is poured into 7-10 volumes of cold (0°C) MTBE. The suspension is aged for 30 min at 0°C, the resulting precipitate is centrifuged, and the clear solution is decanted. The residue is suspended in the same volume of MTBE, and the resulting suspension is again centrifuged and decanted. After decanting the clear MTBE solution, the precipitated peptide is dried *in*
30 *vacuo* at 40°C overnight.

An alternative soft cleavage process is as follows: Peptide is soft cleaved from the resin with a 1-5% TFA/DCM cocktail. The resin is swelled with DCM (10 vol, 1 x 20

min) and drained. 1-5% TFA/DCM (10 vol) is added to the pre-swelled resin, and the suspension stirred for 10 minutes at room temperature. The solution is filtered, and the filtrate treated with pyridine (equimolar to TFA added). The resin is treated twice more with 1-5% TFA/DCM (10 vol) and the filtrates combined and treated with pyridine (equimolar to TFA) each time. The filtrate is concentrated under reduced pressure and the resultant residue dissolved in DMF and precipitated from cold (0 °C) water (10 vol wrt to DMF). The precipitate is filtered, washed with additional water (4-5 vol wrt DMF), and dried *in vacuo* at 40 °C overnight.

10 Hard Cleavage Procedure Removing Protecting Groups:

Peptide is cleaved from the resin with an acidic cocktail of TFA/H₂O/TIPS/DTT in the following ratio: (0.93v/0.04v/0.03v/0.03w). The resin is swelled with DCM (4-5 vol, 3 x 30 min) and drained. Cleavage cocktail (4-5 vol) is added to the pre-swelled resin, and the suspension is stirred for 2 hr at room temperature. The solution is filtered, and then the resin is washed with a small amount of DCM and combined with the cleavage solution. The resulting solution is poured into 7-10 volumes of cold (0°C) MTBE. The suspension is aged for 30 min at 0°C, the resulting precipitate is centrifuged, and the clear solution is decanted. The residue is suspended in the same volume of MTBE, and the resulting suspension is again centrifuged and decanted. After decanting, the clear MTBE solution of the precipitated peptide is dried *in vacuo* at 40°C overnight.

Hybrid Solid Liquid Phase Synthesis

Intermediate peptide fragments prepared via SPPS as described above can be combined in the liquid phase to obtain the dual agonist of SEQ ID NO:1. Methods of HSLPS are well known in the art and need not be exhaustively described herein. *See generally*, US Patent Application Publication No. 2011/0046349; and Albericio *et al.* (1997) *Methods Enzymol.* 289:313-336, Bray *et al.* (2003) *Nature Rev. Drug Discovery* 2:587-593, Dalcol *et al.* (1995) *J. Org. Chem.* 7575-60:7581, Gauthier *et al.* (1991) *Tetrahedron Lett.* 32: 577-580, Schneider *et al.* (2005) *J. Peptide Sci.* 11:744-753, Smith, *Organic Synthesis* (Academic Press 4th ed. 2016), and Zhang *et al.* (2008) *Org. Process Res. Dev.* 12:101-110.

Briefly, HSLPS involves independent intermediate peptide fragment synthesis in solid phase and coupling them in liquid phase.

Applied here, one method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following three intermediate fragments or preparations, where
5 such preparations have a structure as recited in SEQ ID NOS:2, 3 and 5. In some instances, the fragments can be coupled in the following order: SEQ ID NO:2 to SEQ ID NO:3 to SEQ ID NO:5 (*i.e.*, from C-terminus to N-terminus). In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

10 A typical coupling protocol is combining near equimolar amounts of two fragments in a polar solvent such as DMSO. The acid bearing fragment may be activated with stoichiometric amounts of PYBOP and Hunigs base or with related activation systems. After suitable reaction time DEA is used for Fmoc deprotection. Water is then added inducing precipitation of the resulting peptide coupled product which is filtered,
15 isolated and dried.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:2, 10, and 12. In some instances, the fragments are coupled in the following order: SEQ ID NO:2 to SEQ ID NO:10 to SEQ
20 ID NO:12 (*i.e.*, from C-terminus to N-terminus). In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:17, 18, and 12. In some instances, the
25 fragments are coupled in the following order: SEQ ID NO:17 to SEQ ID NO:18 to SEQ ID NO:12 (*i.e.*, from C-terminus to N-terminus). In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following three intermediate preparations, where such preparations have a
30 structure as recited in SEQ ID NO'S:17, 21, and 5. In some instances, the fragments are coupled in the following order: SEQ ID NO:17 to SEQ ID NO:21 to SEQ ID NO:5 (*i.e.*,

from C-terminus to N-terminus). In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following three intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S: 36, 37, and 12. In some instances, the fragments are coupled in the following order: SEQ ID NO:36 to SEQ ID NO:37 to SEQ ID NO:12 (*i.e.*, from C-terminus to N-terminus). In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following two intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S:24 and 25.

Alternatively, other methods of making the compound of SEQ ID NO:1 use the same disconnections as described above but instead couple all amino acid fragments of the backbone first, and then introduce the fatty acid side moiety as the last chemical transformation followed by global deprotection. Here, for example, the corresponding PG can be implemented at Lys20, which can be selectively removed in presence of other PGs (*e.g.*, Boc, Dnp, tBu and/or Trt).

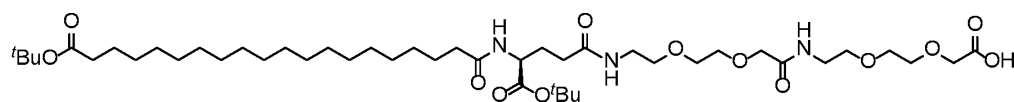
In some instances, a method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S: 2, 7, and 5, as well as preparation 30. In some instances, the fragments are coupled in the following order: SEQ ID NO:2 to SEQ ID NO:7 to SEQ ID NO:5 (*i.e.*, from C-terminus to N-terminus), followed by coupling with preparation 30. In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S: 2, 14, and 12, as well as preparation 30. In some instances, the fragments are coupled in the following order: SEQ ID NO:2 to SEQ ID NO:14 to SEQ ID NO:12 (*i.e.*, from C-terminus to N-terminus), followed by coupling with preparation 30. In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

Another method of making the compound of SEQ ID NO:1 includes at least a step of coupling the following intermediate preparations, where such preparations have a structure as recited in SEQ ID NO'S: 27 and 25, as well as preparation 30. In some instances, the fragments are coupled in the following order: SEQ ID NO:27 to SEQ ID NO:25, followed by coupling with preparation 30. In other instances, and with an appropriate protecting group strategy, the fragments can be coupled in a different order.

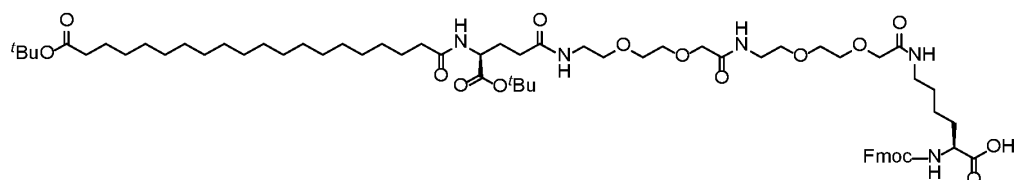
In some instances, a method of making the compound of SEQ ID NO:1 includes at least a step of coupling an intermediate preparation having the structure as recited in SEQ ID NO:30 and intermediate preparation 30.

For effective synthesis of intermediates of SEQ ID NO'S:3, 10, 18, 21, 24, and 37 (acylated peptide fragments) the following is synthesized using fatty acid side chain



Preparation 30

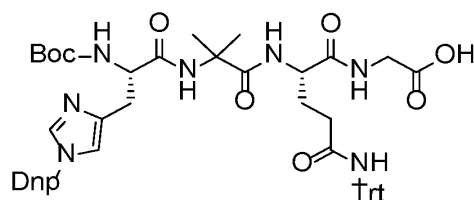
and Fmoc-*L*-Lys-OH amino acid attached with fatty side chain:



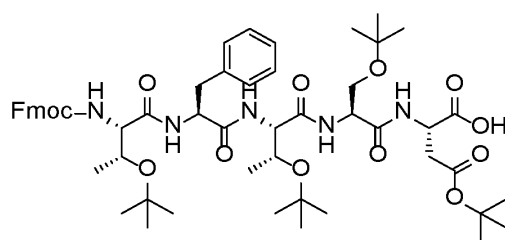
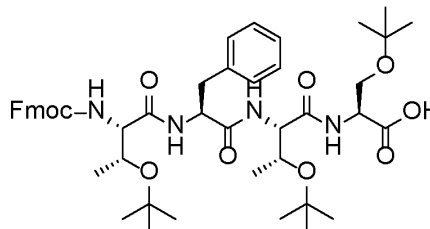
Preparation 31

For improved purity and efficiency of the SPPS, the following tetramers and pentamer (SEQ ID NO'S:31, 32, 33, 34 and 35) can be used for preparing intermediate preparations 4, 11, 24 and 28 (SEQ ID NO'S:5, 12, 25 and 29), where the structures that follow can be synthesized using amino acid building block via SPPS or liquid phase synthesis:

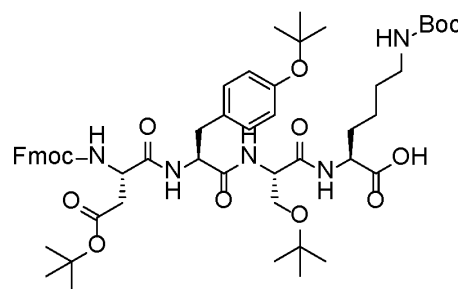
SEQ ID NO:31



SEQ ID NO:32

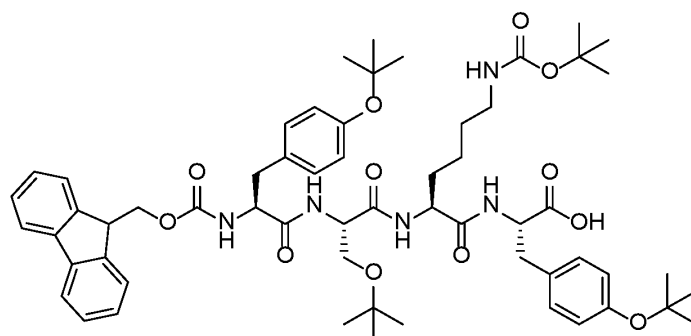


SEQ ID NO:34



SEQ ID NO:33

5



SEQ ID NO:35

Accordingly, in one embodiment, SEQ ID NO:5 is prepared by coupling SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33. In another embodiment, SEQ ID NO:5 is prepared by coupling SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35. In another embodiment, SEQ ID NO:12 is prepared by coupling SEQ ID NO:31 and SEQ ID NO:34. In another embodiment, SEQ ID NO:25 is prepared by coupling SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33. In another embodiment, SEQ ID NO:25 is prepared by coupling SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35. In another embodiment, SEQ ID NO:29 is prepared by coupling SEQ ID NO:31, SEQ ID NO:32 and SEQ ID

NO:33. In another embodiment, SEQ ID NO:29 is prepared by coupling SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35.

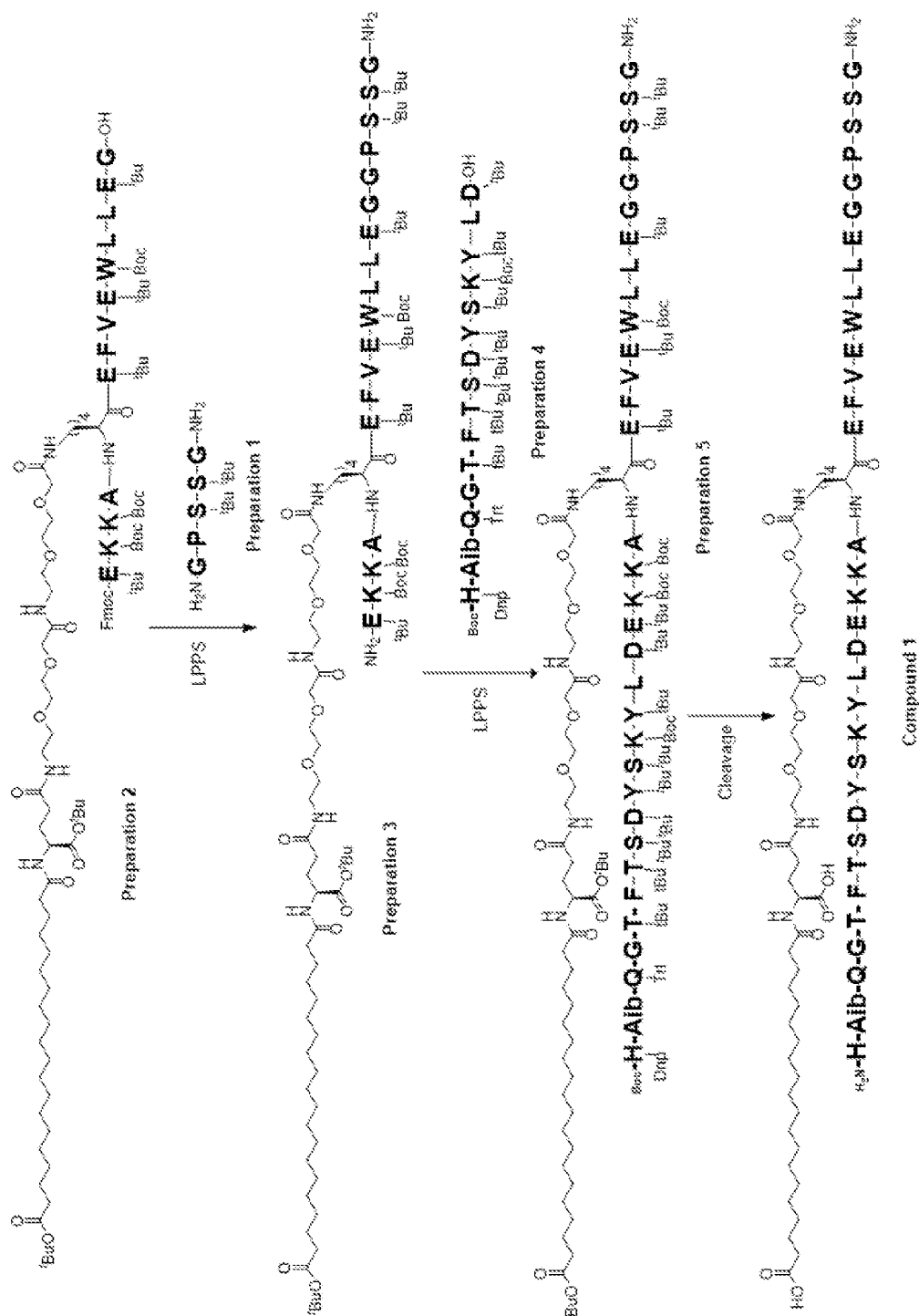
The artisan will recognize that a variety of protecting groups are well known, and alternative protecting groups may be used for the amino acids. For instance, in Boc-
5 H(Dnp) -Aib-Q(Trt)-G (SEQ ID NO:31), instead of Dnp, trityl or Boc may be present on Histidine.

The compound of SEQ ID NO:1 herein can be used in a number of therapeutic applications, for example, in methods of treating obesity, type 2 diabetes, nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH) in an individual.

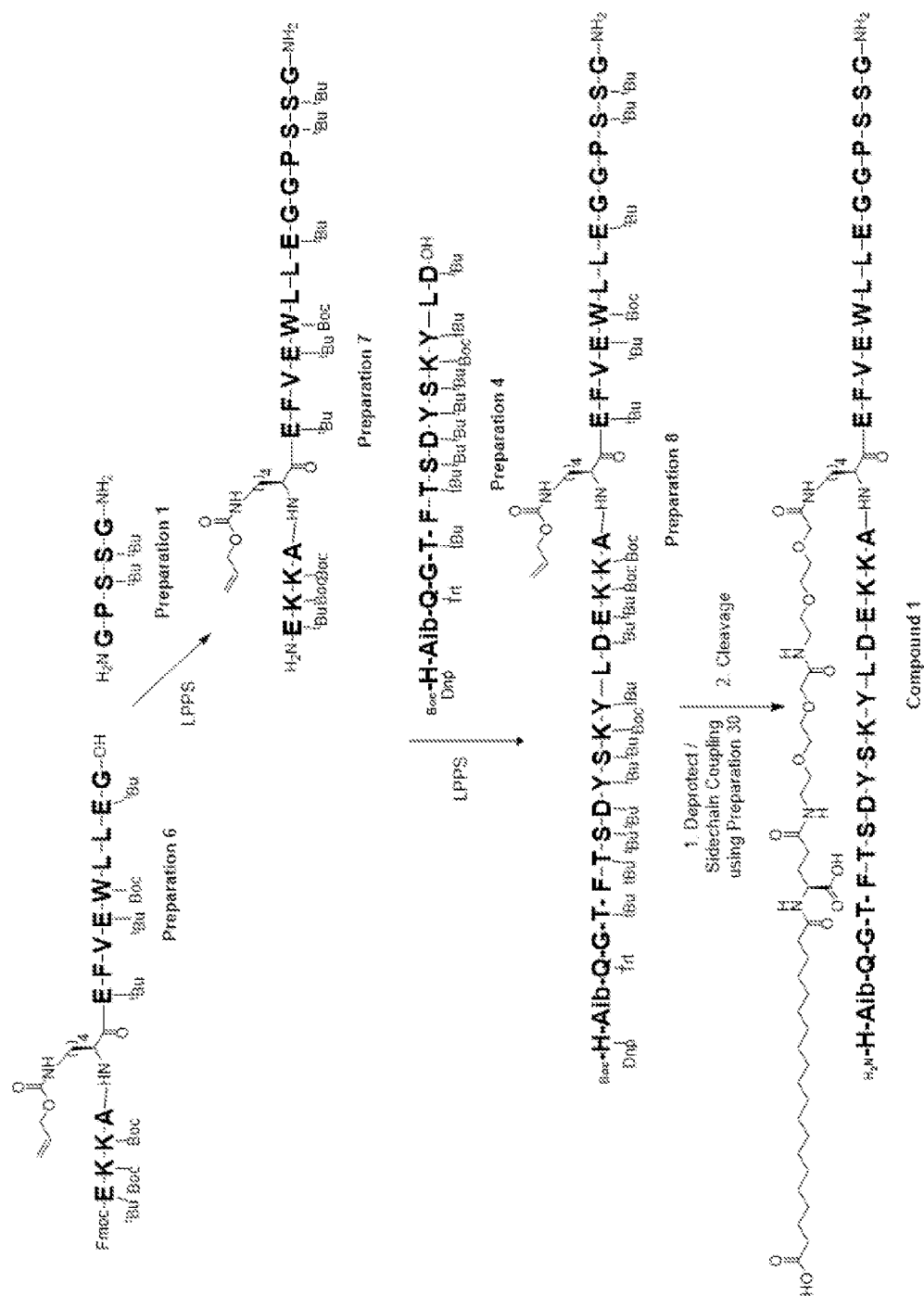
10 The compound of SEQ ID NO:1 may be prepared as described in the hybrid synthesis Schemes 1-10 shown below. The examples are offered for purposes of illustration, not limitation.

EXAMPLES

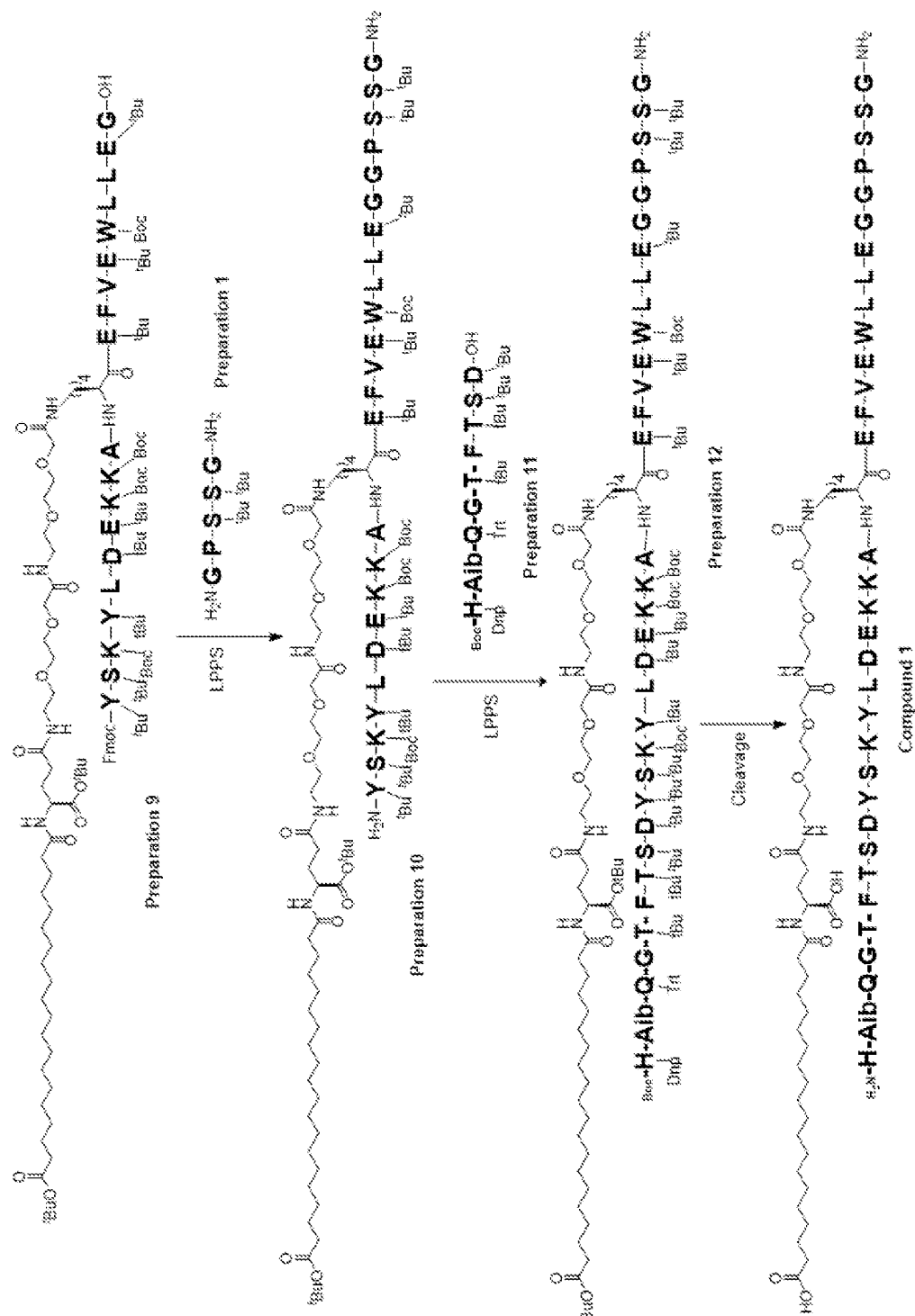
Example 1 (Scheme 1):



Example 2 (Scheme 2):

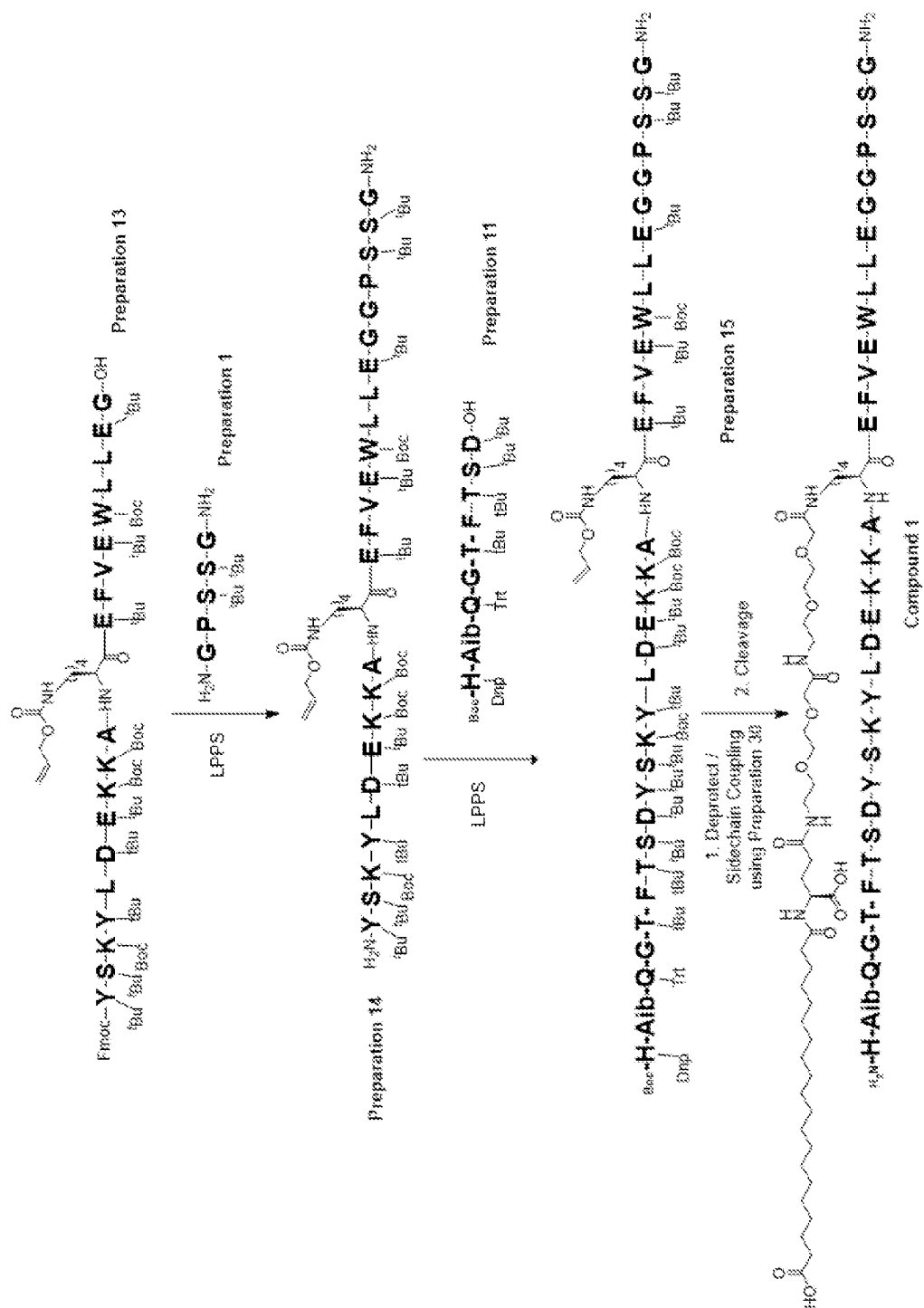


Example 3 (Scheme 3):

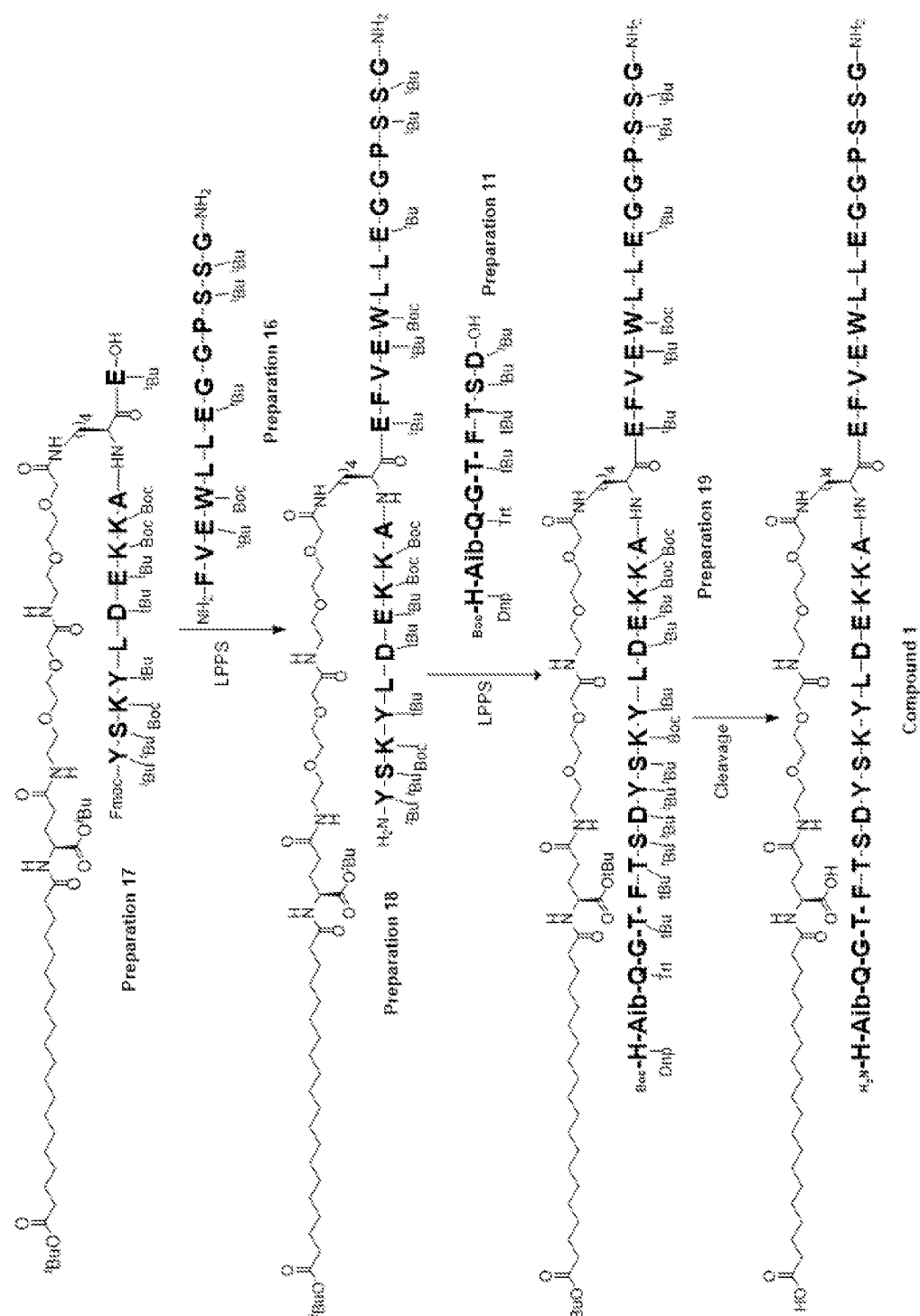


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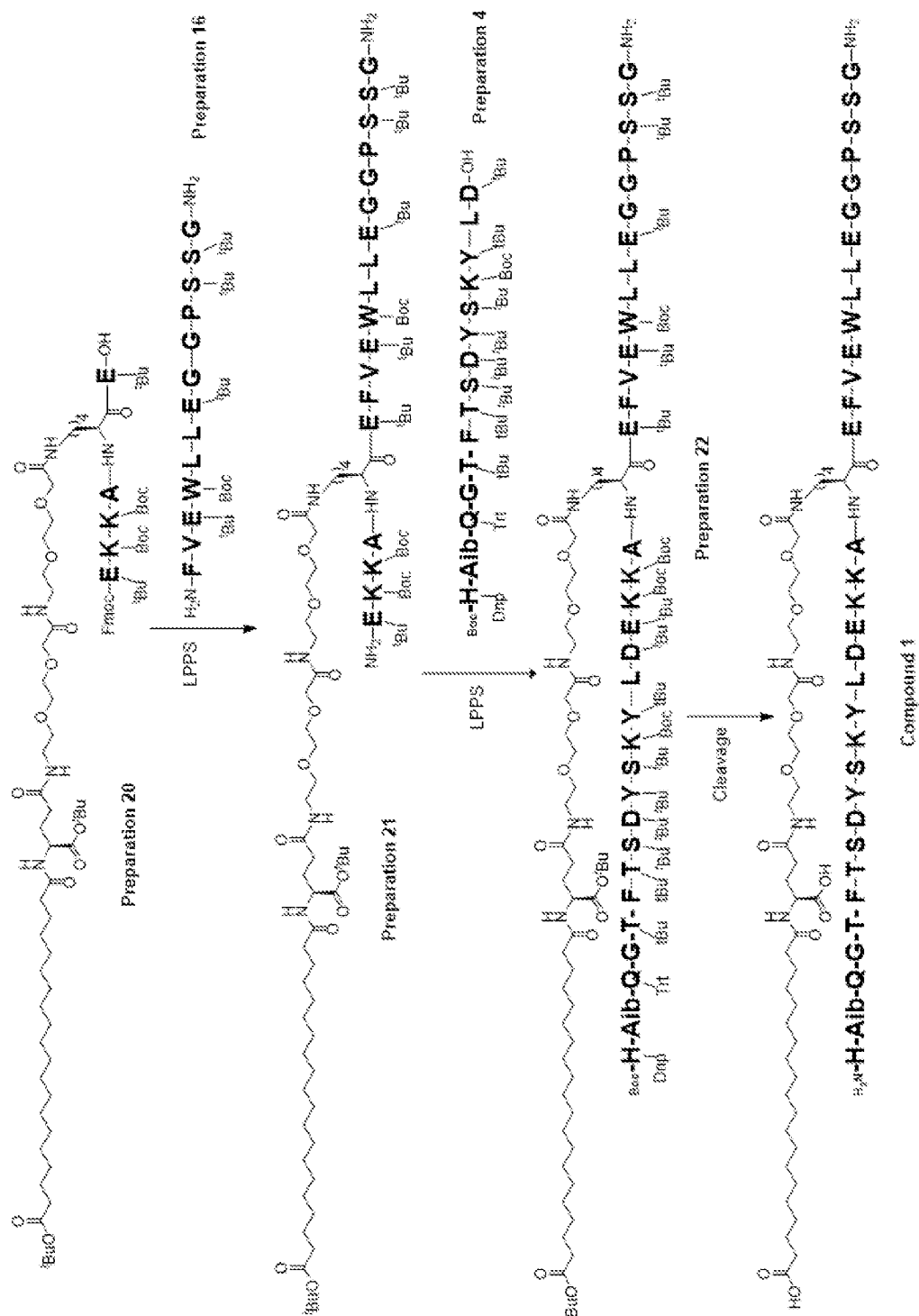
Example 4 (Scheme 4):



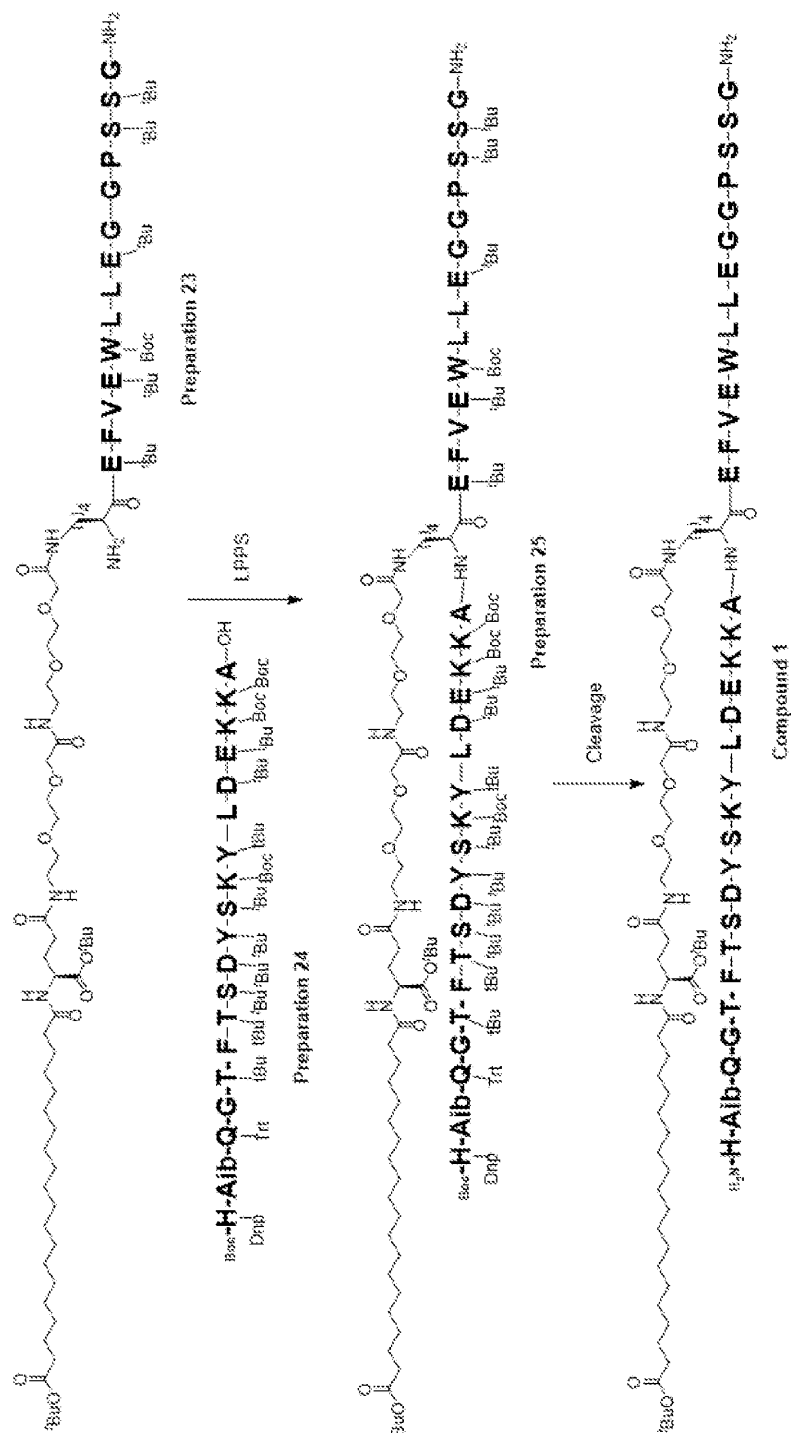
Example 5 (Scheme 5):



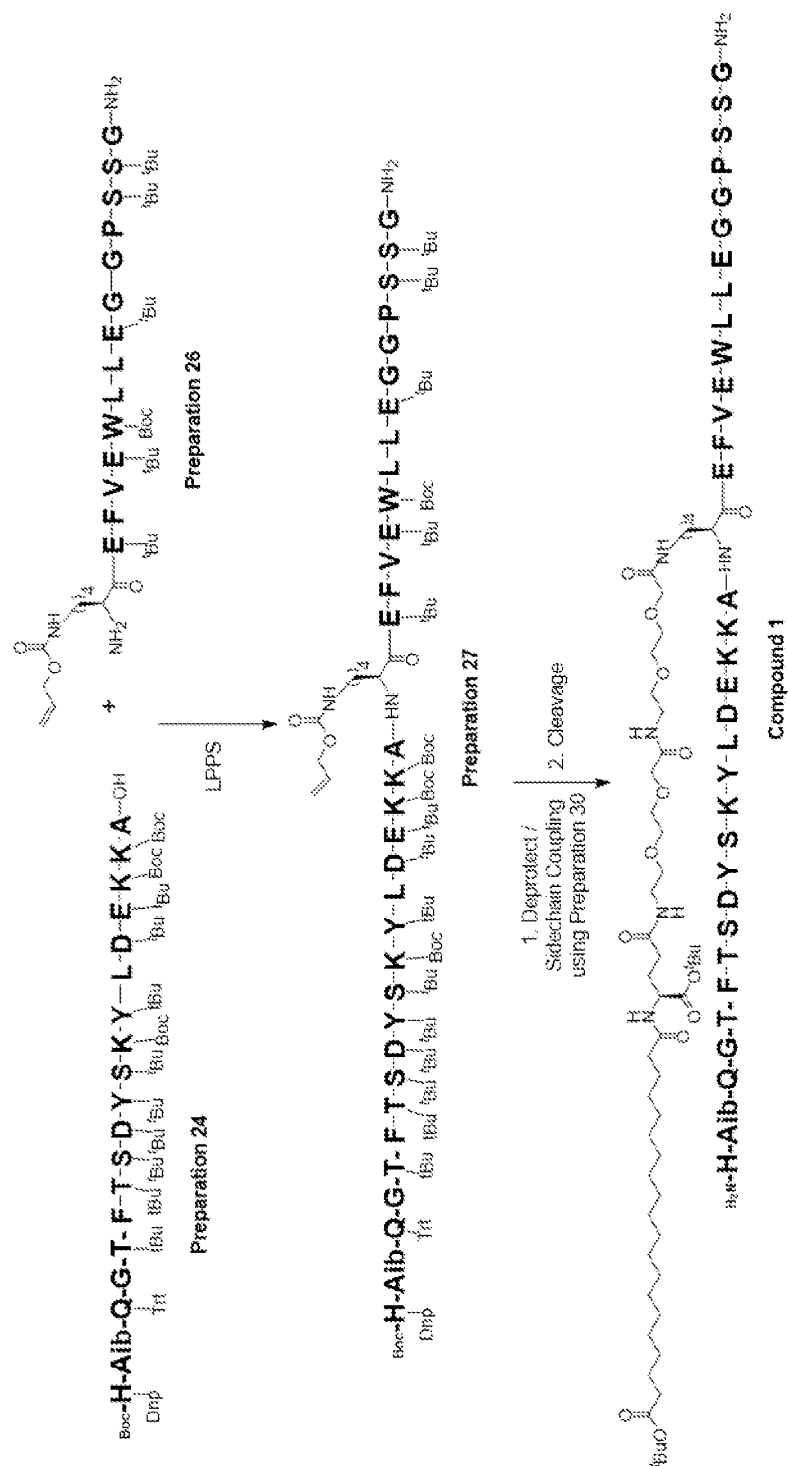
Example 6 (Scheme 6):



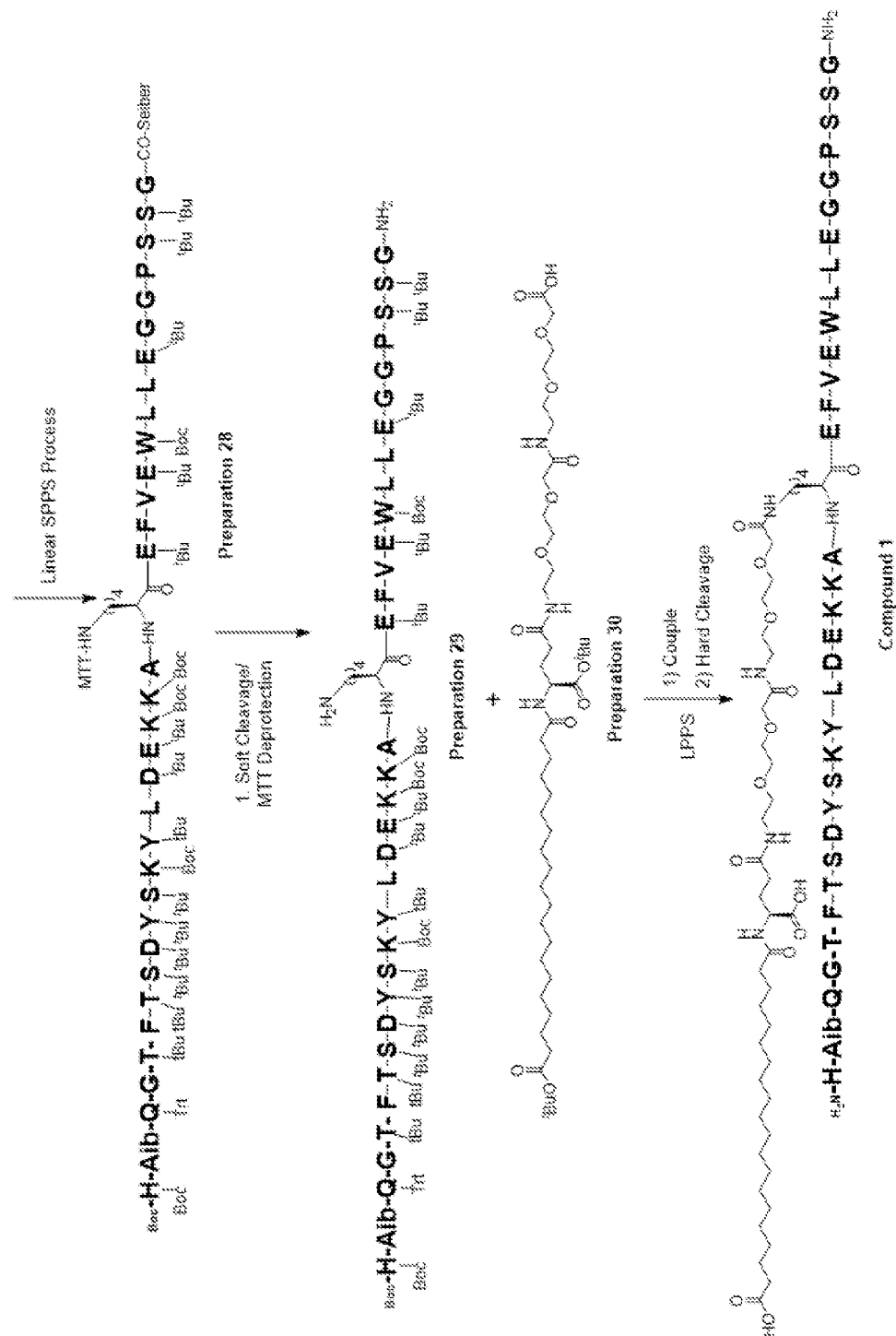
Example 7 (Scheme 7):



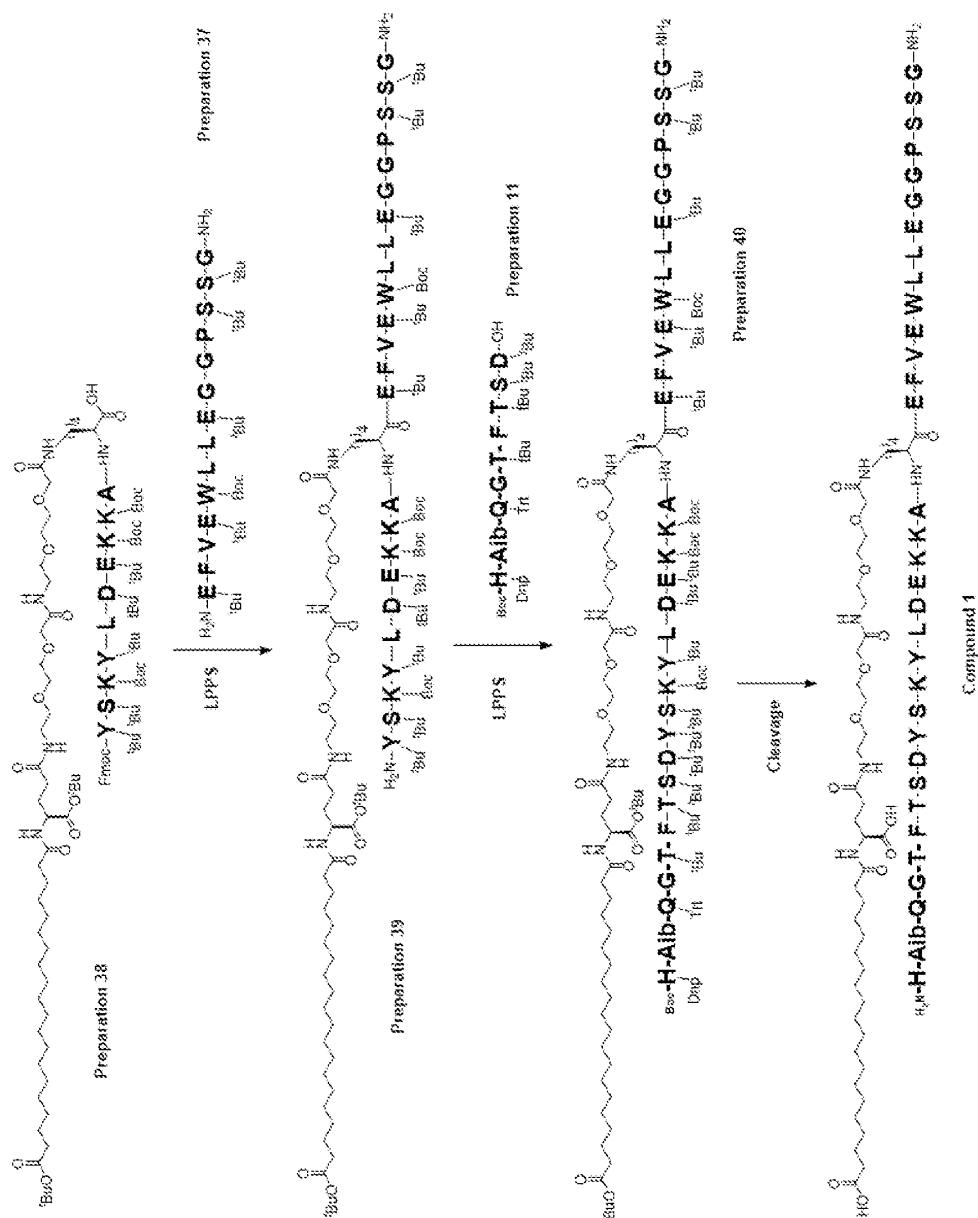
Example 8 (Scheme 8):



Example 9 (Scheme 9):



Example 30 (Scheme 10)



In Example 1 (Scheme 1), Preparations 1 (SEQ ID NO:2), 2 (SEQ ID NO:3) and 4 (SEQ ID NO:5) are prepared by SPPS; Preparations 3 (SEQ ID NO:4), 5 (SEQ ID NO:6) are prepared by LPPS; and Compound 1 (SEQ ID NO:1) is prepared via deprotection of Preparation 5 (SEQ ID NO:6).

5 In Example 2 (Scheme 2), Preparations 1 (SEQ ID NO:2), 6 (SEQ ID NO:7), and 4 (SEQ ID NO:5) are prepared by SPPS; Preparations 7 (SEQ ID NO:8), 8 (SEQ ID NO:9) are prepared by LPPS; Preparation 30 is coupled to the Lys side chain of Preparation 8 followed by deprotection step to yield Compound 1 (SEQ ID NO:1).

10 In Example 3 (Scheme 3), Preparations 1 (SEQ ID NO:2), 9 (SEQ ID NO:10) and 11 (SEQ ID NO:12) are prepared by SPPS; Preparations 10 (SEQ ID NO:11), 12 (SEQ ID NO:13) are prepared by LPPS; and Compound 1 (SEQ ID NO:1) is prepared via deprotection of Preparation 12 (SEQ ID NO:13).

15 In Example 4 (Scheme 4), Preparations 1 (SEQ ID NO:2), 13 (SEQ ID NO:14) and 11 (SEQ ID NO:12) are prepared by SPPS; Preparations 14 (SEQ ID NO:15), 15 (SEQ ID NO:16) are prepared by LPPS; Preparation 30 may be coupled to the Lys side chain of Preparation 15, followed by deprotection step to yield Compound 1 (SEQ ID NO:1).

20 In Example 5 (Scheme 5), Preparations 16 (SEQ ID NO:17), 17 (SEQ ID NO:18) and 11 (SEQ ID NO:12) are prepared by SPPS; Preparations 18 (SEQ ID NO:19), 19 (SEQ ID NO:20) are prepared by LPPS; and Compound 1 (SEQ ID NO:1) is prepared via deprotection of Preparation 19 (SEQ ID NO:20).

25 In Example 6 (Scheme 6), Preparations 16 (SEQ ID NO:17), 20 (SEQ ID NO:21), and 4 (SEQ ID NO:5) are prepared by SPPS; Preparations 21 (SEQ ID NO:22), 22 (SEQ ID NO:23) are prepared by LPPS; and Compound 1 (SEQ ID NO:1) is prepared via deprotection of Preparation 22 (SEQ ID NO:23).

In Example 7 (Scheme 7), Preparations 23 (SEQ ID NO:24), 24 (SEQ ID NO:25) are prepared by SPPS; Preparation 25 (SEQ ID NO:26) is prepared by LPPS and then deprotected to form Compound 1 (SEQ ID NO:1).

30 In Example 8 (Scheme 8), Preparations 24 (SEQ ID NO:25), 26 (SEQ ID NO:27) are prepared by SPPS; Preparation 27 (SEQ ID NO:28) is prepared by LPPS; Preparation 30 is coupled to the Lys side chain of Preparation 27 followed by deprotection to yield Compound 1 (SEQ ID NO:1).

In Example 9 (Scheme 9), Preparation 28 (SEQ ID NO:29) are prepared by SPPS; soft deprotection performed to remove MTT to form Preparation 29 (SEQ ID NO:30); Preparation 30 is coupled to the Lys side chain of Preparation 29, followed by deprotection step to yield Compound 1 (SEQ ID NO:1).

5 In Example 30 (Scheme 10), Preparations 37 (SEQ ID NO:36), 38 (SEQ ID NO:37) and 11 (SEQ ID NO:12) are prepared by SPPS; Preparations 39 (SEQ ID NO:38) and 40 (SEQ ID NO:39) are prepared by LPPS and Compound 1 (SEQ ID NO:1) is prepared via deprotection of Preparation 40 (SEQ ID NO:39).

10 **General Procedures:**

Swelling of the resin: The resin (0.500 mmol) is charged to the reactor and swelled with DMF (3 x 10 mL x 20 min).

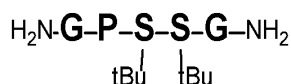
Washing after DeFmoc: Following deprotection, the resin is washed with DMF (5 x 10 mL x 2 min).

15 Washing after coupling: Following coupling, the resin is washed with DMF (5 x 10 mL x 2 min).

Washing and drying of the resin: Following final coupling or deprotection, the resin is washed with DMF (5 x 10 mL x 2 min), followed by DCM (5 x 10 mL x 2 min), and drain dried under N₂ atmosphere to constant weight.

20

Example 10: Synthesis of Preparation 1 (SEQ ID NO:2) by Solid Phase Peptide Synthesis



Preparation 1

25 Preparation 1 (SEQ ID NO:2), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Sieber Amide Resin (0.75 mmol/g loading ratio) with the conditions set forth below.

30

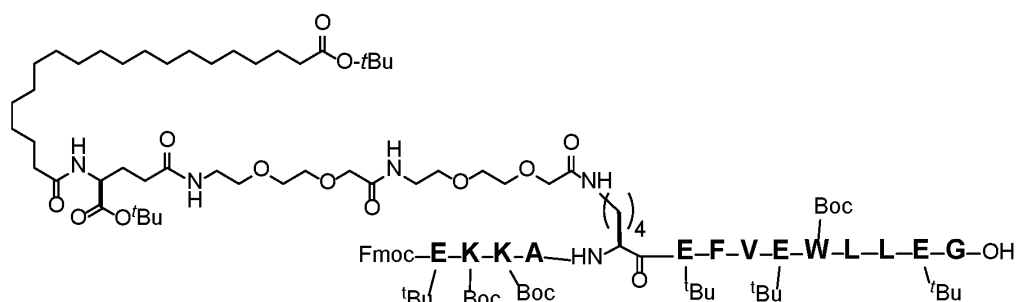
Table 1

Cycle	Fmoc-AA-OH	Gly(30)-Gly(34)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13v DMF	4h	Start with Sieber Amide Resin; 0.75 mmol/g loading
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13v DMF	4h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13v DMF	4h	
4	Fmoc-L-Pro-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13v DMF	4h	
5	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13v DMF	8h	Following coupling, Fmoc is removed using 20%PIP/DMF, 5+20+30 min, 25°C

Cleavage from the resin:

5% TFA/DCM (10 vol) is added to the resin and the reactor is stirred for 30 minutes. The reactor is drained and the resin washed with DCM (2 x 5v). The filtrate is added to pre-cooled MTBE:Heptane (1:1, 10 vol with respect to (wrt) cleavage solution) and then centrifuged (3000 rpm x 10 min). The supernatant is discarded and fresh cold MTBE:Heptane (5 vol) is added and the mixture centrifuged (3000 rpm x 5 min). The supernatant is discarded and the process repeated once more with fresh MTBE:Heptane. The supernatant is discarded and the resultant material placed in a vacuum oven for 14 hours at 34°C to afford Preparation 1. Mass found: 515.8 [M+H].

Example 11: Synthesis of Preparation 2 (SEQ ID NO:3) by Solid Phase Peptide Synthesis



Preparation 2

Preparation 2 (SEQ ID NO:3), or a pharmaceutically acceptable salt thereof, is
5 synthesized by standard SPPS using Fmoc-Gly-CTC resin (0.84 mmol/g loading ratio)
with the conditions set forth below.

Table 2

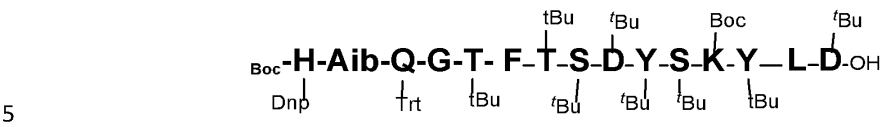
Cycle	Fmoc-AA-OH	Glu(16)-Gly(29) w/Preparation 31			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-CTC	N/A	0.84 mmol/g loading	N/A	Charge 595 mg resin/reactor (0.500 mmol)
2	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
6	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	

7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
8	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
9	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC=3.0/3.0/3.3 14v DMF	4h	
10	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	6h	
11	Preparation 31	20%PIP/DMF 5+20+30 min, 25°C	AA/PyBOP/DIEA 2.0/2.0/4.0 14v DMF	12h	Prepare Preparation 31, PyBOP, DIEA solution in DMF and pre-activate for 30 minutes at RT and then add directly to RV
12	Fmoc-L-Ala-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
13	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
14	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
15	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	

Cleavage from the resin:

Peptide intermediate is soft cleaved from the resin using a 20% HFIP/DCM solution. The peptide intermediate on resin is swelled with DCM (2 x 15 min x 10 vol). The resin is then treated with 20% HFIP/DCM (5 x 20 min x 5 vol) and the filtrates combined. The filtrate is concentrated to 1/3 vol at 30 °C and the volume replaced by chloroform (x 3). The solution is then concentrated under reduced pressure to form a viscous residue that is then added dropwise to n-heptane cooled to -15 °C with vigorous stirring. The precipitate is filtered off and the residue washed with n-heptane (x 3). The resultant solid is then dried *in vacuo* for at least 18 h. Mass Found: 3306.98

Example 12: Synthesis of Preparation 4 (SEQ ID NO:5) by Solid Phase Peptide Synthesis



Preparation 4

Preparation 4 (SEQ ID NO:5), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Asp(OtBu)-CTC resin (0.67 mmol/g loading ratio) with the conditions set forth below.

Table 3

Cycle	Fmoc-AA-OH	His(1)-Asp(15)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Asp(OtBu)-CTC	N/A	0.67 mmol/g loading	N/A	Charge 746 mg resin/reactor (0.500 mmol)
2	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
3	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
5	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
6	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	

7	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
8	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
9	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
10	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
11	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
12	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
13	Fmoc-L-Gln(Trt)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	10h	
14	Fmoc-Aib-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	10h	
15	Boc-L-His(Dnp)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	8h	Prepare 4 mL of 0.375 M His solution with 1.50 mmol His and DMF and charge directly to RV. Charge 2 mL 0.750 M Oxyma followed by 2.5 mL 0.660 M DIC and then resume mixing.

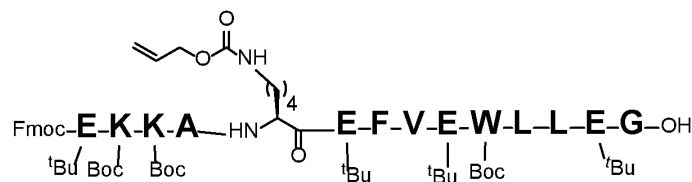
Cleavage from the resin:

Peptide intermediate is soft cleaved from the resin using a 20% HFIP/DCM solution. The peptide intermediate on resin is swelled with DCM (2 x 15 min x 10 vol). The resin is then treated with 20% HFIP/DCM (5 x 20 min x 5 vol) and the filtrates combined. The filtrate is concentrated to 1/3 vol at 30 °C and the volume replaced by chloroform (x 3). The solution is then concentrated under reduced pressure to form a viscous residue that is then added dropwise to n-heptane cooled to -15 °C with vigorous stirring. The precipitate

is filtered off and the residue washed with n-heptane (x 3). The resultant solid is then dried *in vacuo* for at least 18 h. Mass found: 2802.51

Example 13: Synthesis of Preparation 6 (SEQ ID NO:7) by Solid Phase Peptide

5 **Synthesis**



Preparation 6

Preparation 6 (SEQ ID NO:7), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-Gly-CTC resin (0.84 mmol/g loading ratio) with the conditions set forth below.

Table 4

Cycle	Fmoc-AA-OH	Glu(16)-Gly(29) w/Lys(Alloc)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-CTC	N/A	0.84 mmol/g loading	N/A	Charge 595 mg resin/reactor (0.500 mmol)
2	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	

6	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	2h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	4h	
8	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	4h	
9	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC=3. 0/3.0/3.3 14v DMF	4h	
10	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	6h	
11	Fmoc-L-Lys(Alloc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC 3.0/3.0/3.3 14v DMF	12h	
12	Fmoc-L-Ala-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	2h	
13	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	4h	
14	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	4h	
15	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 14v DMF	4h	

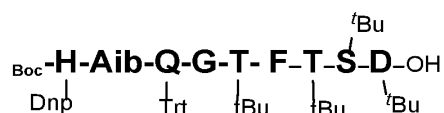
Cleavage from the resin:

Peptide intermediate is soft cleaved from the resin using a 20% HFIP/DCM solution. The peptide intermediate on resin is swelled with DCM (2 x 15 min x 10 vol). The resin is then treated with 20% HFIP/DCM (5 x 20 min x 5 vol) and the filtrates combined. The filtrate is concentrated to 1/3 vol at 30 °C and the volume replaced by chloroform (x 3). The solution is then concentrated under reduced pressure to form a viscous residue that is then added dropwise to n-heptane cooled to -15 °C with vigorous stirring. The precipitate

is filtered off and the residue washed with n-heptane (x 3). The resultant solid is then dried *in vacuo* for at least 18 h. Mass found: 2535.41

Example 14: Synthesis of Preparation 11 (SEQ ID NO:12) by Solid Phase Peptide

5 Synthesis



Preparation 11

Preparation 11 (SEQ ID NO:12), or a pharmaceutically acceptable salt thereof, is
10 synthesized by standard SPPS using Fmoc-L-Asp(OtBu)-CTC resin (0.67 mmol/g loading
ratio) with the conditions set forth below.

Table 5

Cycle	Fmoc-AA-OH	His(1)-Asp(9)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Asp(OtBu)-CTC	N/A	0.67 mmol/g loading	N/A	Charge 746 mg resin/reactor (0.500 mmol)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	6h	
3	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	12h	
4	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
5	Fmoc-Gly-Thr(ψMe, MePro)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	7h	
6	Fmoc-L-Gln(Trt)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	14h	

7	Fmoc-Aib-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	5h	
8	Boc-L-His(Dnp)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	8h	Prepare 4 mL of 0.375 M His solution with 1.50 mmol His and DMF and charge directly to RV. Charge 2 mL 0.750 M Oxyma followed by 2.5 mL 0.660 M DIC and then resume mixing.

*Boc-L-His(Boc)-OH may be used in cycle 8 to prepare Boc-His(Boc) containing fragment.

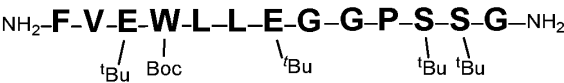
Cleavage from the resin:

To the resin, in a fritted reactor equipped with an overhead stirrer, is added DCM (10v), and the reactor stirred for 10 minutes to swell the resin. The reactor is drained and 1% TFA/DCM (10vol) is added to the resin. The reactor is stirred for 10 minutes and the reactor drained and filtrate collected. To the reactor is added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor is drained and combined with the previous filtrate. To the reactor is again added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the filtrate is added additional pyridine (1:1 equiv. to TFA added). The reactor is drained and the filtrates combined. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor with the spent resin is washed with DCM (2 x 10 vol) and combined with the previous filtrates. The filtrate is concentrated under reduced pressure. The resultant residue is dissolved in minimal DMF and added dropwise to cold water (15 vol with respect to DMF added to dissolve residue) to precipitate the peptide. The suspension is filtered through a fritted funnel and the solids rinsed with cold water (2 x 10 vol). The resultant solids are placed in a vacuum oven for 14 hours at 34 °C to afford Preparation 11 fragment.

Boc-His(Boc) containing: M/Z found = 1642.8911 and 1542.8396 [M-Boc].

Boc-His(Dnp) containing: M/Z found = 1708.8410

Example 15: Synthesis of Preparation 16 (SEQ ID NO:17) by Solid Phase Peptide Synthesis



5 Preparation 16

Preparation 16 (SEQ ID NO:17), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Sieber Amide Resin (0.75 mmol/g loading ratio) with the conditions set forth below.

Table 6

Cycle	Fmoc-AA-OH	Phe(22)-Gly(34)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	Prepare from Sieber Amide Resin (0.75 mmol/g loading); charge 667 mg resin to reactor (0.500 mmol)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
4	Fmoc-L-Pro-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
6	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	

8	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
9	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC=3. 0/3.0/3.3 14v DMF	10h	
10	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
11	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC 3.0/3.0/3.3 14v DMF	2h	
12	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
13	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	20h	Following coupling, Fmoc is removed using 20%PIP/DMF, 5+20+30 min, 25 °C

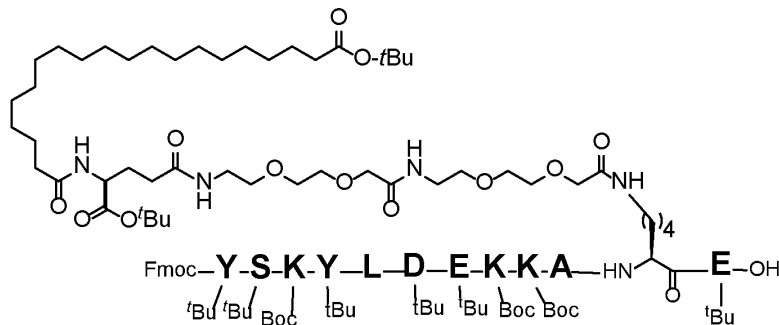
Cleavage from the resin:

- The resin (3 g) is charged to a fritted reactor equipped with an overhead stirrer. The resin is suspended in DCM (28.5 mL) and TFA (1.5 mL) is added to the suspension.
- 5 The resultant suspension is mixed for 30 minutes and the reactor is drained. The resin bed is washed with DCM (20 mL) and the filtrate poured into pre-cooled MTBE:Heptane (1:1, 300 mL). The suspension is centrifuged (3000 rpm x 10 min) and the supernatant discarded. Fresh, pre-cooled MTBE:Heptane (1:1, 300 mL) is added and the suspension centrifuged again (3000 rpm x 5 min). The supernatant is discarded and the washing
- 10 repeated once more with fresh MTBE:Heptane (1:1, 300 mL). Following centrifugation, the supernatant is discarded and the resultant solids placed in the vacuum oven for 14 hours at 34 °C to afford Preparation 16. Mass found: 1699.9829.

Example 16: Synthesis of Preparation 17 (SEQ ID NO:18) by Solid Phase Peptide

15 Synthesis

Preparation 17 (SEQ ID NO:18), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using the conditions set forth below.



Preparation 17

5 Preparation 17 (SEQ ID NO:18), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Glu(OtBu)-CTC resin (0.655 mmol/g loading ratio) with the conditions set forth below.

Table 7

Cycle	Fmoc-AA-OH	Tyr(10)-Glu(21) w/Preparation 31			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Glu(OtBu)-CTC	N/A	0.655 mmol/g loading	N/A	Charge 763 mg to each reactor (0.500 mmol)
2	Preparation 31	20%PIP/DMF 5+20+30+30 min, 25°C	AA/PyBOP/DIEA = 3.0/3.0/6.0 12v DMF	14h	Charge 7.928 g of 24.23% w/w Preparation 31 solution in DMF to vial, add 781 mg PyBOP followed by 0.523 mL DIEA and make up solution to 9 mL. Pre-activate at rt for 30 minutes then add to RV.
3	Fmoc-L-Ala-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	2h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	14h	

5	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	24h	
6	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	16h	
7	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	16h	
8	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	4h	
9	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	14h	
10	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	7h	
11	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	12h	
12	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	5h	

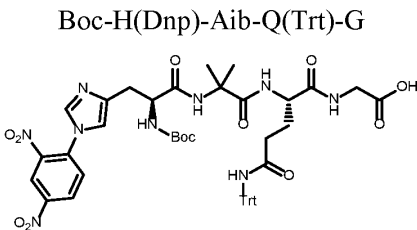
Cleavage from the resin:

To the resin, in a fritted reactor equipped with an overhead stirrer, is added DCM (10v), and the reactor stirred for 10 minutes to swell the resin. The reactor is drained and 1% TFA/DCM (10vol) is added to the resin. The reactor is stirred for 10 minutes and the reactor drained and filtrate collected. To the reactor is added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor is drained and combined with the previous filtrate. To the reactor is again added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the filtrate is added additional pyridine (1:1 equiv. to TFA added). The reactor is drained and the filtrates combined. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor with the spent resin is washed with DCM (2 x 10 vol) and combined with the previous filtrates. The filtrate is concentrated under reduced pressure. The resultant residue is dissolved in minimal DMF and added dropwise to cold water (15 vol with

respect to DMF added to dissolve residue) to precipitate the peptide. The suspension is filtered through a fritted funnel and the solids rinsed with cold water (2 x 10 vol). The resultant solids are placed in a vacuum oven for 14 hours at 34 °C to afford Preparation 17. Mass found: 1609.1 [M+H]

5

Example 17: Synthesis of Preparation 32 (SEQ ID NO:31) by Solid Phase Peptide Synthesis



10

Preparation 32 (SEQ ID NO:31)

Preparation 32 (SEQ ID NO:31), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-Gly-CTC resin (1.04 mmol/g loading ratio) with the conditions set forth below.

15

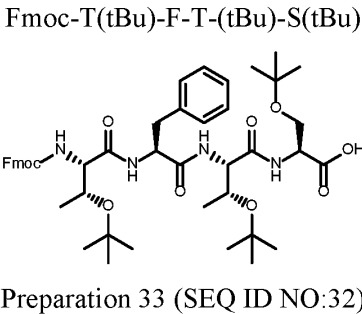
Table 8

Cycle	Fmoc-AA-OH	His(1)-Gly(4)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	N/A	CTC resin AA eq/DIEA eq= 1.2/4.0 7 vol DCM	6h	Loading of Fmoc-Gly-OH on CTC resin, measure resin loading by qNMR (1.04 mmol/g)
2	Fmoc-L-Gln(trt)-OH	20%PIP/DMF 4 x 30 min, 25°C	AA eq/Oxyma eq/DIC eq= 3.0/3.0/3.3 13 vol DMF	12h	
3	Fmoc-Aib-OH	20%PIP/DMF 4 x 30 min, 25°C	AA eq/Oxyma eq/DIC eq= 3.0/3.0/3.3 13 vol DMF	12h	
4	Boc-L-His(DNP)-OH	20%PIP/DMF 4 x 30 min, 25°C	AA eq/Oxyma eq/DIC eq= 3.0/3.0/3.3 13v DMF	18h	

Cleavage from the resin:

Peptide is soft cleaved from the resin using a 30% HFIP/DCM solution. The pre-swelled resin containing the Preparation 33 peptide is treated with 30% HFIP/DCM (10 vol) and agitated for 2 hours. The suspension is filtered, and the resin cake washed with DCM (4-5 vol). The filtrate is concentrated under reduced pressure and then stripped with ACN three times to afford Preparation 32 peptide as a dry foam.

Example 18: Synthesis of Preparation 33 (SEQ ID NO:32) by Solid Phase Peptide Synthesis



Preparation 33 (SEQ ID NO:32), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Ser(tBu)-CTC resin (0.71 mmol/g loading ratio) with the conditions set forth below.

Table 9

Cycle	Fmoc-AA-OH	Thr(5)-Ser(8)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Ser(tBu)-OH	N/A	CTC resin AA eq/DIEA eq= 0.8/3.0 7 vol DCM	18h	Loading of Fmoc-L-Ser(tBu)-OH on CTC resin, measure resin loading by qNMR (0.71 mmol/g)
2	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
3	Fmoc-L-Phe-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	

4	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
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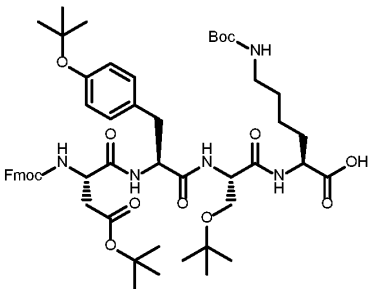
Cleavage from the resin:

Peptide is soft cleaved from the resin using a 30% HFIP/DCM solution. The pre-swelled resin containing the Preparation 33 peptide is treated with 30% HFIP/DCM (10 vol) and agitated for 2 hours. The suspension is filtered, and the resin cake washed with DCM (4-5 vol). The filtrate is concentrated under reduced pressure and then stripped with ACN three times to afford Preparation 33 peptide as a dry foam.

Example 19: Synthesis of Preparation 34 (SEQ ID NO:33) by Solid Phase Peptide

Synthesis

Fmoc-D(tBu)-Y(tBu)-S(tBu)-K(Boc)



Preparation 34 (SEQ ID NO:33)

Preparation 34 (SEQ ID NO:33), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Lys(Boc)-CTC resin (0.92 mmol/g loading ratio) with the conditions set forth below.

Table 10

Cycle	Fmoc-AA-OH	Asp(9)-Lys(12)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Lys(Boc)-OH	N/A	CTC resin AA eq/DIEA eq= 1.0/3.5 7 vol DCM	6h	Loading of Fmoc-L-Lys(Boc)-OH on CTC resin, measure resin loading by qNMR (0.92 mmol/g)

2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
3	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
4	Fmoc-L-Asp(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	

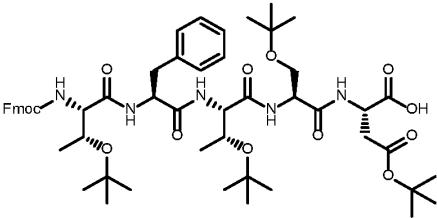
Cleavage from the resin:

The pre-swelled resin containing the Preparation 34 peptide is treated with 30% HFIP/DCM (10 vol) and agitated for 2 hours. The suspension is filtered, and the resin cake washed with DCM (4-5 vol). The filtrate is concentrated under reduced pressure and then stripped with ACN three times to afford Preparation 34 peptide as a dry foam.

Example 20: Synthesis of Preparation 35 (SEQ ID NO:34) by Solid Phase Peptide Synthesis

10

Fmoc-T(tBu)-F-T(tBu)-S(tBu)-D(tBu)



Preparation 35 (SEQ ID NO:34)

Preparation 35 (SEQ ID NO:34), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Asp(tBu)-CTC resin (0.67 mmol/g loading ratio) with the conditions set forth below.

Table 11

Cycle	Fmoc-AA-OH	Thr(5)-Asp(9)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments

1	Fmoc-L-Asp(tBu)-OH	N/A	CTC resin AA eq/DIEA eq= 0.8/3.0 7 vol DCM	16h	Loading of Fmoc-L-Asp(tBu)-OH on CTC resin, measure resin loading by qNMR (0.67 mmol/g)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
3	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
4	Fmoc-L-Phe-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
5	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	

Cleavage from the resin:

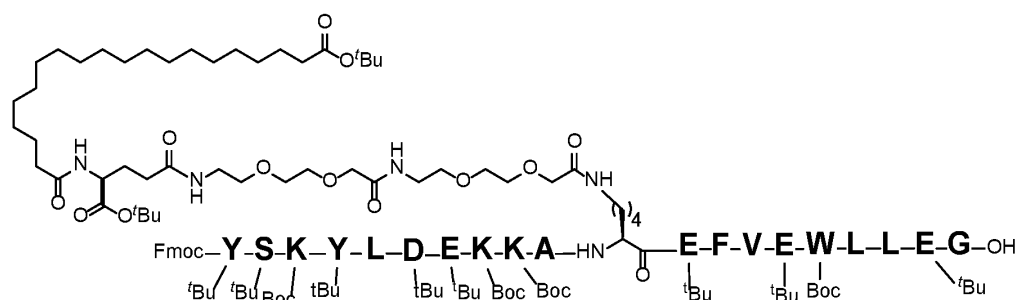
The pre-swelled resin containing the Preparation 35 peptide is treated with 30% HFIP/DCM (10 vol) and agitated for 2 hours. The suspension is filtered, and the resin cake washed with DCM (4-5 vol). The filtrate is concentrated under reduced pressure and then stripped with ACN three times to afford Preparation 35 peptide as a dry foam.

Example 21: Synthesis of Preparation 31

Preparation 31 or ((52S)-52-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-25-(tert-butoxycarbonyl)-2,2-dimethyl-4,23,28,37,46-pentaoxo-3,32,35,41,44-pentaoxa-24,29,38,47-tetraazatripentacontan-53-oic acid) is prepared as described in WO2020/159949.

Example 22: Synthesis of Preparation 9 (SEQ ID NO:10) by Solid Phase Peptide

Synthesis



Preparation 9

Preparation 9 (SEQ ID NO:10), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using H-Gly-CTC resin with the conditions set forth

5 below.

Table 12

Cycle	AA	Deprotection Conditions	Coupling Conditions	Coupling Time (h)	Comments
1	H-Gly-CTC	-	-	-	Preloaded resin with first AA
2	Fmoc-L-Glu(OtBu)-OH	-	AA/TBTU/DIPEA = 2 equiv. AA/ 1.8 equiv. TBTU/ 3.0 equiv. DIPEA 10 mL DMF per g starting resin	2 h	
3	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyma/DIC= 2 equiv. AA/ 3.1 equiv. Oxyma/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
4	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyma/DIC= 2 equiv. AA/ 3.1 equiv. Oxyma/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
5	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyma/DIC= 2 equiv. AA/ 3.1 equiv. Oxyma/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
6	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyma/DIC= 2 equiv. AA/ 3.1 equiv. Oxyma/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	

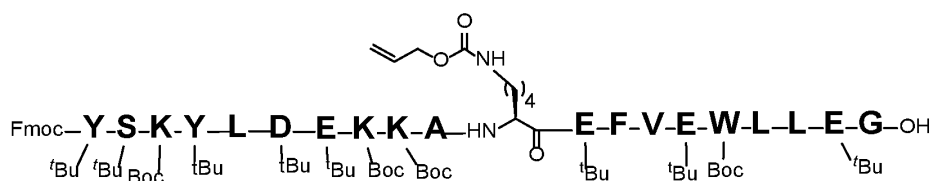
7	Fmoc-L-Val-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
8	Fmoc-L-Phe-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
9	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
10	Preparation 31	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
11	Fmoc-L-Ala-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
12	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
13	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
15	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	4 h	
16	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3.3 h	
17	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3.25 h	

18	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3.5 h	
19	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3.25 h	
20	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3.25 h	

Cleavage from the resin:

Peptide intermediate is soft cleaved from the resin using a 20% HFIP/DCM solution. The peptide intermediate on resin is swelled with DCM (2 x 15 min x 10 vol). The resin is then treated with 20% HFIP/DCM (5 x 20 min x 5 vol) and the filtrates combined. The filtrate is concentrated to 1/3 vol at 30 °C and the volume replaced by chloroform (x 3). The solution is then concentrated under reduced pressure to form a viscous residue that is then added dropwise to n-heptane cooled to -15 °C with vigorous stirring. The precipitate is filtered off and the residue washed with n-heptane (x 3). The resultant solid is then dried *in vacuo* for at least 18 h. Mass found: 4400.64

Example 23: Synthesis of Preparation 13 (SEQ ID NO:14) by Solid Phase Peptide Synthesis



Preparation 13

Preparation 13 (SEQ ID NO:14), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using H-Gly-CTC resin with the conditions set forth below.

Table 13

Cycle	AA	Deprotection Conditions	Coupling Conditions	Coupling Time (h)	Comments
1	H-Gly-CTC	-	-	-	Preloaded resin with first AA
2	Fmoc-L-Glu(OtBu)-OH	-	AA/TBTU/DIPEA = 2 equiv. AA/ 1.8 equiv. TBTU/ 3.0 equiv. DIPEA 10 mL DMF per g starting resin	2 h	
3	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
4	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
5	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
6	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
7	Fmoc-L-Val-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
8	Fmoc-L-Phe-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
9	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	

10	Fmoc-L-Lys(Aloc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
11	Fmoc-L-Ala-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
12	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
13	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	3 h	
15	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	4.5 h	
16	Fmoc-L-Leu-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	4.5 h	
17	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	4.5 h	
18	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL DMF per g starting resin	4.5 h	

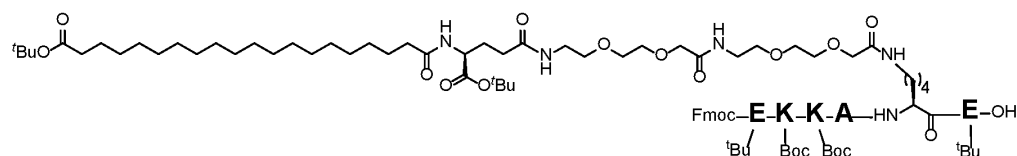
19	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL solvent per g starting resin: -solvent = DMF (first coupling) -solvent = NMP (second coupling)	2 x 4.5 h (repetitive coupling)	
20	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF Time (10 + 20 min), ambient temp.	AA/Oxyrna/DIC= 2 equiv. AA/ 3.1 equiv. Oxyrna/ 3.9 equiv. DIC 10 mL solvent per g starting resin: -solvent = DMF (first coupling) -solvent = NMP (second coupling)	2 x 6 h (repetitive coupling)	Additional Fmoc-protection of the whole peptide is performed using 1.5 equiv. Fmoc-Cl/2.0 equiv. pyridine, in DCM (7 mL DCM per g starting resin), reaction time 5 h

Cleavage from the resin:

- Peptide intermediate is soft cleaved from the resin using a 20% HFIP/DCM solution. The peptide intermediate on resin is swelled with DCM (2 x 15 min x 10 vol).
- 5 The resin is then treated with 20% HFIP/DCM (5 x 20 min x 5 vol) and the filtrates combined. The filtrate is concentrated to 1/3 vol at 30 °C and the volume replaced by chloroform (x 3). The solution is then concentrated under reduced pressure to form a viscous residue that is then added dropwise to n-heptane cooled to -15 °C with vigorous stirring. The precipitate is filtered off and the residue washed with n-heptane (x 3). The
- 10 resultant solid is then dried *in vacuo* for at least 18 h. Mass Found: 3629.07

Example 24: Synthesis of Preparation 20 (SEQ ID NO:21) by Solid Phase Peptide Synthesis

15



Preparation 20

Preparation 20 (SEQ ID NO:21), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-L-Glu(OtBu)-CTC resin (0.920 mmol/g loading ratio) with the conditions set forth below.

Table 14

Cycle	Fmoc-AA-OH	Glu(16)-Glu(21) w/ Preparation 31			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-L-Glu(OtBu)-CTC	N/A	0.920 mmol/g loading	N/A	Charge 54.4 g of resin to the reactor
2	Preparation 31	20%PIP/DMF (8v) 120 min, 25°C	AA/PyBOP/DIE A= 2.0/2.0/4.0 6v DMF	4h	
3	Fmoc-L-Ala-OH	20%PIP/DMF (8v) 120 min, 25°C	AA/Oxyma/DIC= 2.0/2.0/2.2 6v DMF	4h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF (8v) 120 min, 25°C	AA/Oxyma/DIC= 2.0/2.0/2.2 6v DMF	4h	
5	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF (8v) 120 min, 25°C	AA/Oxyma/DIC= 2.0/2.0/2.2 6v DMF	4h	
6	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF (8v) 120 min, 25°C	AA/Oxyma/DIC= 2.0/2.0/2.2 6v DMF	4h	

5

Cleavage from the resin:

To the resin, in a fritted reactor equipped with an overhead stirrer, is added DCM (10v), and the reactor stirred for 10 minutes to swell the resin. The reactor is drained and 1% TFA/DCM (10vol) is added to the resin. The reactor is stirred for 10 minutes and the reactor drained and filtrate collected. To the reactor is added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor is drained and combined with the previous filtrate. To the reactor is again added fresh 1% TFA/DCM (10vol) and the reactor stirred for 10 minutes. To the

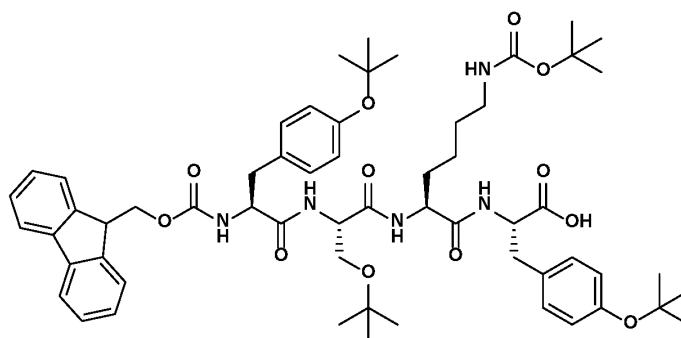
10

filtrate is added additional pyridine (1:1 equiv. to TFA added). The reactor is drained and the filtrates combined. To the filtrate is added pyridine (1:1 equiv. to TFA added). The reactor with the spent resin is washed with DCM (2 x 10 vol) and combined with the previous filtrates. The filtrate is concentrated under reduced pressure. The resultant
 5 residue is dissolved in minimal DMF and added dropwise to cold water (15 vol with respect to DMF added to dissolve residue) to precipitate the peptide. The suspension is filtered through a fritted funnel and the solids rinsed with cold water (2 x 10 vol). The resultant solids are placed in a vacuum oven for 14 hours at 34 °C to afford the Fragment of Preparation 20.

10 Mass found: 2121.2953

Example 25: Synthesis of Preparation 36 (SEQ ID NO:35) by Solid Phase Peptide Synthesis

Fmoc-Y(t-Bu)-S(t-Bu)-K(Boc)-Y(t-Bu)



15

Preparation 36 (SEQ ID NO:35)

Preparation 36 (SEQ ID NO:35), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using CTC resin (0.671 mmol/g loading ratio) with the
 20 conditions set forth below.

Table 15

Cycle	Fmoc-AA-OH	Tyr(10)-Tyr(13)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments

1	Fmoc-L-Tyr(OtBu)-OH	N/A	CTC resin AA eq/DIEA eq= 1.2/7.8 7 vol DCM	14h	Loading of Fmoc-L-Tyr(OtBu)-OH on CTC resin, measure resin loading by qNMR
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	Start with Fmoc-L-Tyr(OtBu) on CTC Resin; 0.671 mmol/g loading
3	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	
4	Fmoc-L-Tyr(OtBu)-OH	20%PIP/DMF 3 x 30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 13 vol DMF	4h	

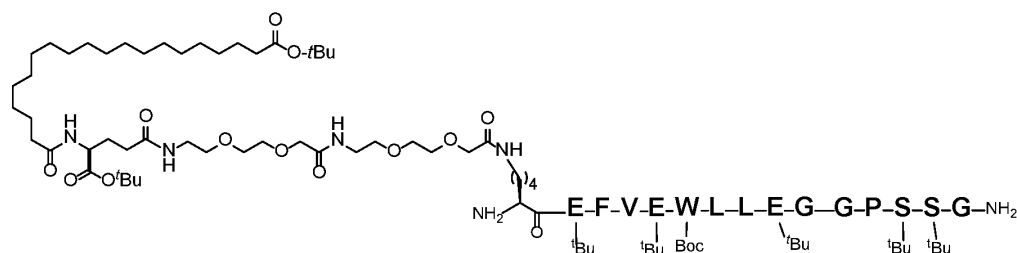
Cleavage from the resin:

The pre-swelled resin containing the Preparation 36 peptide is treated with 30% HFIP/DCM (10 vol) and agitated for 2 hours. The suspension is filtered, and the resin cake washed with DCM (4-5 vol). The filtrate is concentrated under reduced pressure and then stripped with ACN three times to afford Preparation 36 peptide as a dry foam.

Example 26: Synthesis of Preparation 23 (SEQ ID NO:24) by Solid Phase Peptide

Synthesis

10



Preparation 23

Preparation 23 (SEQ ID NO:24), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Sieber Amide Resin (0.75 mmol/g loading ratio) with the conditions set forth below.

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Table 16

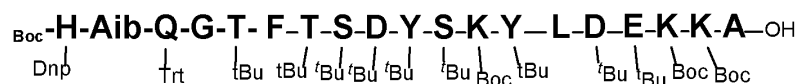
Cycle	Fmoc-AA-OH	Lys(20)-Gly(34) w/ Preparation 31			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	Prepare from Sieber Amide Resin (0.75 mmol/g loading); charge 667 mg resin to reactor (0.500 mmol)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
4	Fmoc-L-Pro-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
6	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
8	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
9	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
10	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	

11	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC 3.0/3.0/3.3 14v DMF	2h	
12	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
13	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	20h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	6h	
15	Preparation 31	20%PIP/DMF 5+20+30 min, 25°C	AA/PyBOP/DIE A=3.0/3.0/6.0 14v DMF	14h	Following coupling, Fmoc is removed using 20%PIP/DMF, 5+20+30 min, 25 °C

Cleavage from the resin:

A sample of the resin was taken for hard cleavage to confirm the success of the build. To the resin (200 mg) is added 2.5 mL of cleavage cocktail (2.32 mL TFA, 63 mg DTT, 0.063 mL TIPS, 0.063 mL H₂O) and the suspension mixed for 2.5 hours. The resin is filtered off and rinsed with 1 mL of TFA and the filtrate poured into pre-cooled MTBE (17.5 mL). The suspension is aged for 30 minutes at 0 °C and then centrifuged (3000 rpm x 10 min). The supernatant is discarded and the solids washed with fresh MTBE (18 mL), centrifuged (3000 rpm x 5 min), and decanted. This is repeated once more with fresh MTBE and the resultant solids dried *in vacuo* for 14 hours. Mass found: 1300.7 [M+2/2]

Example 27: Synthesis of Preparation 24(SEQ ID NO:25) by Solid Phase Peptide Synthesis

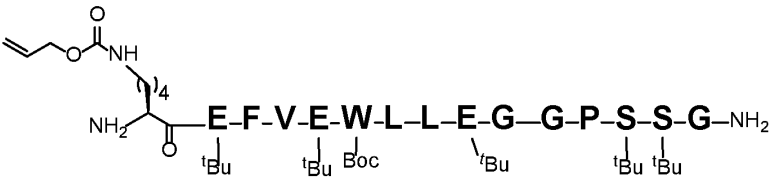


15

Preparation 24

Preparation 24 (SEQ ID NO:25), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Fmoc-Ala-CTC resin followed by soft cleavage from the resin.

Example 28: Synthesis of Preparation 26 (SEQ ID NO:27) by Solid Phase Peptide Synthesis



Preparation 26

Preparation 26 (SEQ ID NO:27), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Sieber Amide Resin (0.75 mmol/g loading ratio) with the conditions set forth below.

Table 17

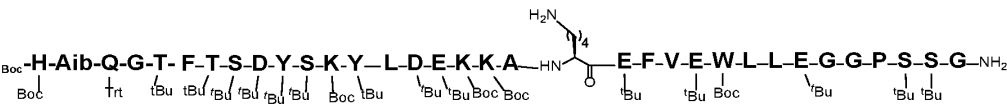
Cycle	Fmoc-AA-OH	Lys(20)-Gly(34) w/alloc			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	Prepare from Sieber Amide Resin (0.75 mmol/g loading); charge 667 mg resin to reactor (0.500 mmol)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	

4	Fmoc-L-Pro-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	
6	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	1h	
8	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	2h	
9	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	10h	
10	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	2h	
11	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC 3.0/3.0/3.3 14v DMF	2h	
12	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	4h	
13	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	20h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	6h	
15	Fmoc-L-Lys(alloc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC = 3.0/3.0/3.3 14v DMF	14h	Following coupling, Fmoc is removed using 20%PIP/DMF, 5+20+30 min, 25 °C

Cleavage from the resin: A sample of the resin was taken for hard cleavage to confirm the success of the build. To the resin (200 mg) was added 2.5 mL of cleavage cocktail

(2.32 mL TFA, 63 mg DTT, 0.063 mL TIPS, 0.063 mL H₂O) and the suspension mixed for 2.5 hours. The resin was filtered off and rinsed with 1 mL of TFA and the filtrate poured into pre-cooled MTBE (17.5 mL). The suspension was aged for 30 minutes at 0 °C and then centrifuged (3000 rpm x 10 min). The supernatant was discarded and the solids washed with fresh MTBE (18 mL), centrifuged (3000 rpm x 5 min), and decanted. This was repeated once more with fresh MTBE and the resultant solids dried *in vacuo* for 14 hours. Mass found: 1940.7

Example 29: Synthesis of Preparation 29 (SEQ ID NO:30) by Solid Phase Peptide Synthesis



Preparation 29

Preparation 29 (SEQ ID NO:30), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Sieber resin (0.75 mmol/g loading ratio) the conditions set forth below.

Table 18

Cycle	Fmoc-AA-OH	His(1)-Gly(34)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	Charge Sieber resin to reactor (loading = 0.75 mmol/g)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	

4	Fmoc-L-Pro-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	10h	
5	Fmoc-Gly-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	
6	Fmoc-Gly-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	4h	
8	Fmoc-L-Leu-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	4h	
9	Fmoc-L-Leu-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	10h	
10	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	4h	
11	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	4h	
12	Fmoc-L-Val-OH	20%PIP/DMF 360 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	8h	
13	Fmoc-L-Phe-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	6h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	6h	
15	Fmoc-Lys(Mtt)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	14h	
16	Fmoc-Ala-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	2h	
17	Fmoc-Lys(Boc)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	8h	

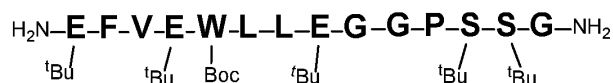
18	Fmoc-Lys(Boc)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	16h	
19	Fmoc-Glu(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	16h	
20	Fmoc-Asp(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	16h	
21	Fmoc-L-Leu-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	4h	
22	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	14h	
23	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	8h	
24	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	12h	
25	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	6h	
26	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	14h	
27	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	6h	
28	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	12h	
29	Fmoc-L-Phe-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	4h	
30	Fmoc-L-Thr(tBu)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	10h	
31	Fmoc-Gly-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyrna/DIC = 2.0/2.0/2.2 5v DMF	4h	

32	Fmoc-L-Gln(Trt)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	14h	
33	Fmoc-Aib-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	6h	
34	Boc-His(Boc)-OH	20%PIP/DMF 120 min, 25°C	AA/Oxyma/DIC = 2.0/2.0/2.2 5v DMF	8h	Make Histidine solution fresh before addition; no pre-activation; add His solution followed by Oxyma and DIC solutions directly to reactor and begin 8 hour stir

Soft Cleavage of the peptide from resin:

To a fritted reactor is charged the resin (20 g) followed by DCM (10v). The resultant suspension is stirred for 20 minutes with an overhead stirrer to swell the resin. The reactor is drained and 20v of 5% TFA/DCM solution is charged to the reactor and the resultant suspension stirred for 30 minutes. The reactor is drained into a round bottomed flask and the resin cake washed with DCM (3 x 5v). To the filtrate is added pyridine (equimolar to TFA added), and then the filtrate is concentrated under reduced pressure. The resultant red-orange residue is taken up in 25 mL of DMF and added dropwise to cold water (200 mL) to precipitate the peptide. The round bottomed flask is rinsed with 2 x 5 mL portions of DMF and added dropwise to the water/peptide suspension. The resultant suspension is placed in the refrigerator for 30 minutes and then filtered through a fritted funnel. The solids are washed with additional cold water and then placed in a vacuum oven at 35 overnight to afford 15.9 g of desired peptide. Masses found: 5444.1861 [M]; 5344.1325 [M-Boc]

Example 31: Synthesis of Preparation 37 (SEQ ID NO:36) by Solid Phase Peptide Synthesis



Preparation 37

Preparation 37 (SEQ ID NO:36), or a pharmaceutically acceptable salt thereof, is
 5 synthesized by standard SPPS using Sieber Amide Resin (0.75 mmol/g loading ratio)
 with the conditions set forth below.

Table 19

Cycle	Fmoc-AA-OH	Glu(21)-Gly(34)			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	Prepare from Sieber Amide Resin (0.75 mmol/g loading); charge 667 mg resin to reactor (0.500 mmol)
2	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
3	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
4	Fmoc-L-Pro-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
5	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
6	Fmoc-Gly-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
7	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	1h	
8	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	

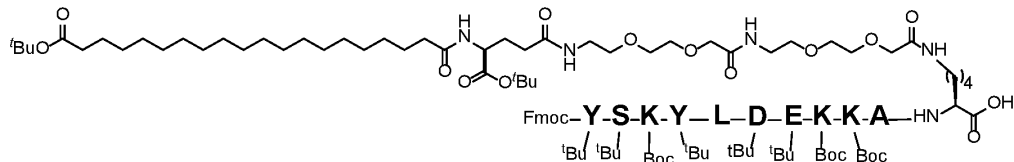
9	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	10h	
10	Fmoc-L-Trp(Boc)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	2h	
11	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC 3.0/3.0/3.3 14v DMF	2h	
12	Fmoc-L-Val-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	4h	
13	Fmoc-L-Phe-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	20h	
14	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 14v DMF	6h	Following coupling, Fmoc is removed using 20%PIP/DMF, 5+20+30 min, 25 °C

Cleavage from the resin:

The resin (2 g) was charged to a fritted reactor equipped with an overhead stirrer. The resin was suspended in DCM (19 mL) and TFA (1 mL) was added to the suspension. The resultant suspension was mixed for 30 minutes and the reactor was drained. The resin bed was washed with DCM (10 mL) and the filtrate poured into pre-cooled MTBE:Heptane (1:1, 200 mL). The suspension was centrifuged (3000 rpm x 10 min) and the supernatant discarded. Fresh, pre-cooled MTBE:Heptane (1:1, 200 mL) was added and the suspension centrifuged again (3000 rpm x 5 min). The supernatant was discarded and the washing repeated once more with fresh MTBE:Heptane (1:1, 200 mL). Following centrifugation, the supernatant was discarded and the resultant solids placed in the vacuum oven for 14 hours at 34 °C to afford Preparation 37. Mass found: 1885.097

Example 32: Synthesis of Preparation 38 (SEQ ID NO:37) by Solid Phase Peptide

15 Synthesis



5

Preparation 38

Preparation 38 (SEQ ID NO:37)), or a pharmaceutically acceptable salt thereof, is synthesized by standard SPPS using Preparation 31-CTC resin (0.3574 mmol/g loading ratio) with the conditions set forth below.

10

Table 20

Cycle	Fmoc-AA-OH	Tyr(10)-Lys(20) w/ Preparation 31			
		DeFmoc reagents & DeFmoc condition	Coupling reagents	Coupling Time	Comments
1	Preparation 31-CTC	N/A	0.3574 mmol/g loading	N/A	Charge 1.40 g Preparation 31-CTC to reactor (0.500 mmol)
2	Fmoc-L-Ala-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	2h	
3	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	14h	
4	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	24h	
5	Fmoc-L-Glu(OtBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	16h	
6	Fmoc-L-Asp(OtBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyma/DIC= 3.0/3.0/3.3 12v DMF	16h	

7	Fmoc-L-Leu-OH	20%PIP/DMF 5+20+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 12v DMF	4h	
8	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 12v DMF	14h	
9	Fmoc-L-Lys(Boc)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 12v DMF	7h	
10	Fmoc-L-Ser(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 12v DMF	12h	
11	Fmoc-L-Tyr(tBu)-OH	20%PIP/DMF 5+20+30+30 min, 25°C	AA/Oxyrna/DIC= 3.0/3.0/3.3 12v DMF	5h	

Cleavage from the resin:

To a 20 mL scintillation vial is charged the resin (1 g). The resin is suspended in DCM (7 mL) and to the suspension is added HFIP (3 mL). The vial is capped and placed on a rotary wheel for 2 hours. The resin is filtered, and the resin cake washed with DCM (10 mL). The filtrate is concentrated under reduced pressure and the resultant residue is taken up in MeCN and concentrated to dryness (x 3) to afford the Preparation 38 as a sticky, yellow solid. Mass found: 3029.8636

10 Example 33: Synthesis of Compound 1 using Scheme 5

Liquid Phase Coupling of Preparation 16 and Preparation 17 to form Preparation 18:

To a 2.5 wt% solution of Preparation 16 (97 mg) in DMF is added a 2.5 wt% solution of Preparation 17 (1.3 equiv.) in DMF. The resultant solution is mixed and to the mixture is added a 2.5 wt% solution of PyAOP (2.1 equiv.) in MeCN followed by DIPEA (8.7 equiv.). The resultant reaction mixture is stirred at room temperature for 2 hours before the addition of DEA (11.5 equiv.). The mixture is stirred at room temperature for 1 hour. Mass found: 1559.8 [M+3/3]

Liquid Phase Coupling of Preparation 18 and Preparation 11 to form Preparation 19:

To a 5 wt% solution of Preparation 18 (40 mg) in DMF is added a 5 wt% solution of Preparation 11 (1.6 equiv.) in DMF. The solutions are mixed, and to the solution is added

a 5 wt% solution of PyAOP (3.0 equiv.) in MeCN followed by a 5 wt% solution of DIPEA (8 equiv.) in DMF. The resultant reaction mixture is stirred at room temperature for 2h 40 minutes and then a 1.0 mL solution of 17% NaCl/0.5% NaHCO₃ is added and stirred for 5 minutes. Then, 1.0 mL of cold DI water is added and the resultant mixture
5 stirred at 0 C for 1 hour. The white precipitate is then filtered through a fritted funnel and washed with DI water (2 mL). The resultant solids are dried in a vacuum oven overnight at 30 C to afford the desired material as an off-white solid.

Global deprotection of Preparation 19 to form Compound 1 (SEQ ID NO:1):
10 To the solid obtained above is added TFA (0.424 mL), TIPS (11.5 µL), water (11.5 µL), and DTT (11.5 mg). The resultant mixture is mixed on a rotary wheel for 2 hours and then poured into 11 mL of cold MTBE. The resultant precipitate is centrifuged at 3000 rpm for 3 minutes and washed twice with MTBE and centrifuged each time. The solids are dried at room temperature. Mass found: 1521.9 [M+3/3]

15

Example 34: Synthesis of Compound 1 using Scheme 6

Liquid Phase Coupling of Preparation 16 and Preparation 20 to form Preparation 21:
To a 250 mL round bottomed flask equipped with a magnetic stir bar is charged Preparation 16 (1.26 g, 1.02 equiv.). To the flask is added DMF/THF (85/15, 20 mL) in 5
20 mL portions. The material is left to stir for ~5 minutes to fully dissolve the material. Meanwhile, to a 20 mL scintillation vial is charged Preparation 20 (2.10 g, 1.00 equiv.) and PyAOP (450 mg, 1.56 equiv.), and the material is dissolved in DMF/THF (85/15, 10 mL) and then added to the round bottomed flask. The vial is rinsed with 2 x 5 mL portions of DMF/THF (85/15). To the reaction mixture is added DIPEA (0.67 mL, 7.00
25 equiv.) and the resultant reaction mixture is stirred at room temperature. After 18 hours, DEA (0.57 mL, 10 equiv.) is added and the resultant solution is stirred for 1 hour. After 1 hour, the reaction mixture is added dropwise to pre-cooled aq. 17% NaCl/0.5% NaHCO₃ (400 mL) to precipitate the peptide. The suspension is filtered and the solids washed with 2 x 50 mL cold DI water. The solids are dried in a vacuum oven at 34 C overnight. Mass
30 found: 3581.2002

Liquid Phase Coupling of Preparation 21 and Preparation 4 To form Preparation 22:

To a solution of Preparation 21 (522 mg, 1.05 equiv.) in DMF/THF (85/15, 8 mL) is added a solution of Preparation 4 (w/His(Dnp)) (385 mg, 1.02 equiv.) and PyAOP (66 mg, 1.53 equiv.) in DMF/THF (85/15, 3 mL). The solution is allowed to mix for 5 minutes to dissolve the materials and then DIPEA (0.044 mL, 3.00 equiv.) is added to the reaction mixture. The resultant reaction mixture is allowed to stir at room temperature for 4 hours and then is added dropwise into 110 mL of pre-cooled aqueous 17% NaCl/0.5% NaHCO₃ to precipitate the peptide. The suspension is then filtered through a fritted funnel and the solids washed with 2 x 25 mL DI water. The solids are then dried for 14 hours at 34 C in a vacuum oven. Mass found: 6365.6991

10

Global Deprotection of Preparation 22 to form Compound 1 (SEQ ID NO:1):

To the solid obtained above is added TFA (0.424 mL), TIPS (11.5 µL), water (11.5 µL), and DTT (11.5 mg). The resultant mixture is mixed on a rotary wheel for 2 hours and then poured into 11 mL of cold MTBE. The resultant precipitate is centrifuged at 3000 rpm for 3 minutes and washed twice with MTBE and centrifuged each time. The solids are dried at room temperature. Mass found: 1521.9 [M+3/3]

15

Example 35: Synthesis of Compound 1 using Scheme 9

Liquid Phase Coupling of Preparation 29 with Preparation 30 to form Compound 1 (SEQ ID NO:1):

20

Coupling of Preparation 30 to Preparation 29: To the reaction vessel equipped with a magnetic stir bar is charged Preparation 29 (2 g) and DMF (15 mL). The resultant mixture is allowed to mix until complete dissolution of the peptide (approx. 10 min). A solution of Preparation 30 (2.0 equiv.) and PyBOP (2.0 equiv.) in DMF (5 mL) is prepared and then added to the peptide solution. To the reaction mixture is charged DIPEA (4.0 equiv.) and the resultant reaction mixture is allowed to stir for 18 hours. The reaction mixture is then added dropwise into 200 mL of pre-cooled aq. 17% NaCl/0.5% NaHCO₃ to precipitate the peptide. The resultant suspension is aged for 30 minutes in the refrigerator and then filtered through a fritted funnel. The solids are washed with additional cold water and then dried in a vacuum oven overnight to afford the desired peptide (2.16 g). Masses found: 6299.7459 [M]; 6199.6908 [M-Boc]

25

30

Global Deprotection: To the protected peptide (1 g) is added 10v of cleavage cocktail (92.5% TFA:2.5% TIPS:2.5% DTT:2.5% H₂O). The resultant mixture is allowed to mix for 2 hours and then added dropwise into pre-cooled MTBE (7v) to precipitate the peptide. The suspension is centrifuged (3000 rpm x 10 min) and then the supernatant
5 discarded. The solids are then suspended in fresh MTBE (7v) and centrifuged again (3000 rpm x 5 min). The supernatant is discarded and the process repeated once more with fresh MTBE (7v). Following centrifugation, the supernatant is discarded and the resultant solids are dried in a vacuum oven overnight to afford the crude peptide. Mass found: 4560.2610.

10

Example 36: Synthesis of Compound 1 using Scheme 10

Liquid Phase Coupling of Preparation 37 and 38 to form Preparation 39:

To a solution of Preparation 37 (54 mg, 1.18 equiv.) in DMF/THF (85/15, 0.5 mL) is added a solution of Preparation 38 (73 mg, 1.00 equiv.) and PyAOP (26 mg, 2.10 equiv.)
15 in DMF/THF (85/15, 3 mL). The mixture is allowed to stir for ~5 minutes to allow the solids to dissolve and then DIPEA (0.03 mL, 7.89 equiv.) is added and the resultant reaction mixture allowed to stir. After 4 hours, DEA (0.03 mL, 11.3 equiv.) is added and the resultant mixture stirred for one hour at room temperature. The reaction mixture is then added dropwise to pre-cooled aqueous 17% NaCl/0.5% NaHCO₃ (35 mL) to
20 precipitate the peptide. The suspension is filtered through a fritted funnel and the solids washed with 2 x 10 mL of DI water. The solids are dried in a vacuum oven at 34 C for 14 hours to afford the Preparation 39 in 49% crude yield. Mass found: 4674.8656

Liquid Phase Coupling of Preparation 39 and Preparation 11 to form Preparation 40:

25 Preparation 39 and 11 are coupled in liquid Phase using coupling conditions as described in Scheme 5, second coupling step (Example 33).

Global Deprotection of Preparation 40 to form Compound 1 (SEQ ID NO:1):

Global deprotection of the Preparation 40 is conducted using conditions as described in
30 Example 33.

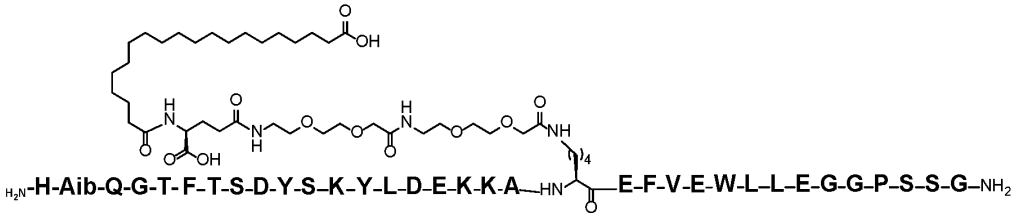
Example 37: Synthesis of Preparation 11 using tetramers Preparation 32 and Preparation 33

Fmoc-Asp(OtBu)-CTC (0.500 mmol, 0.67 mmol/g) resin is charged to a solid phase reactor and then swelled with DMF (3 x 10 mL x 20 min), then deprotected with 20% Piperidine/DMF (3 x 10 mL x 30 min). To a pre-activation vessel is added 4 mL of a 0.375 M solution of Preparation 33 (Fmoc-T(tBu)-F-T(tBu)-S(tBu)-OH) in DMF, followed by 2 mL of a 0.750 M solution of Oxyma in DMF, and 2.5 mL of a 0.660 M solution of DIC in DMF. The resultant solution is mixed for 30 minutes with N₂ bubbling and then transferred to the reactor containing the resin and coupled for 12 hours. The reactor is drained and then the resin washed with DMF (5 x 10 mL x 2 min). The Fmoc is removed with 20% Piperidine/DMF (3 x 10 mL x 30 min) and then the resin washed with DMF (5 x 10 mL x 2 min). To the pre-activation vessel is added 4 mL of a 0.375 M solution of Preparation 32 (Boc-His(dnp)-Aib-Q(Trt)-G-OH) in DMF, followed by 2 mL of a 0.750 M solution of Oxyma in DMF, and 2.5 mL of a 0.660 M solution of DIC in DMF. The resultant solution is mixed for 30 minutes with N₂ bubbling and then transferred to the reactor containing the resin and coupled for 12 hours. The reactor is then drained, and the resin washed with DMF (5 x 10 mL x 2 min) followed by DCM (5 x 10 mL x 2 min), and then drain dried under a N₂ sweep for 8 hours. A sample of the resin is then hard cleaved (see hard cleavage procedure) to confirm the success of the build. Mass found: 1143.3.

SEQUENCES

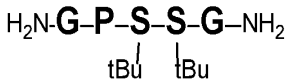
The following sequences are referred to in the disclosure and are provided below for reference.

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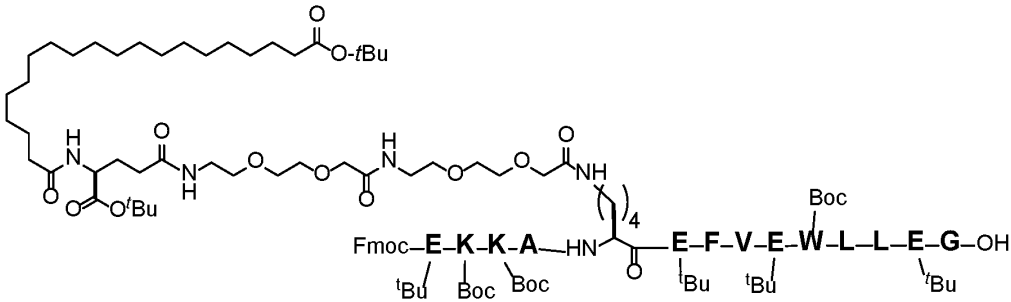


H-Aib-QGTFTSDYSKYLDEKKAK((2-[2-(2-aminoethoxy)-ethoxy]-acetyl)₂-(γ-Glu)-CO-(CH₂)₁₈-CO₂H)EFVEWLLEGGPSSG-NH₂

10 SEQ ID NO:2



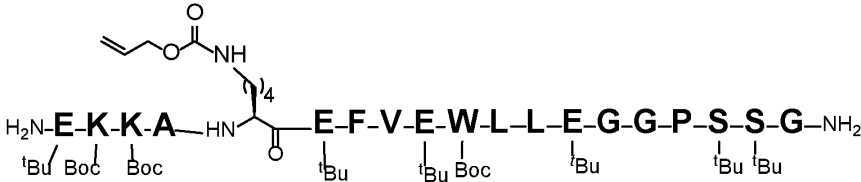
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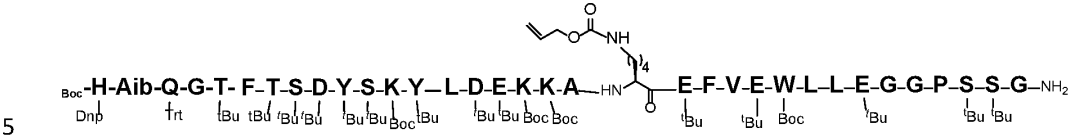
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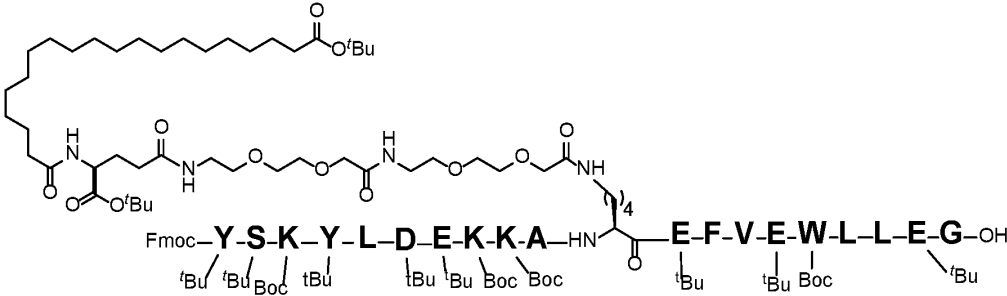
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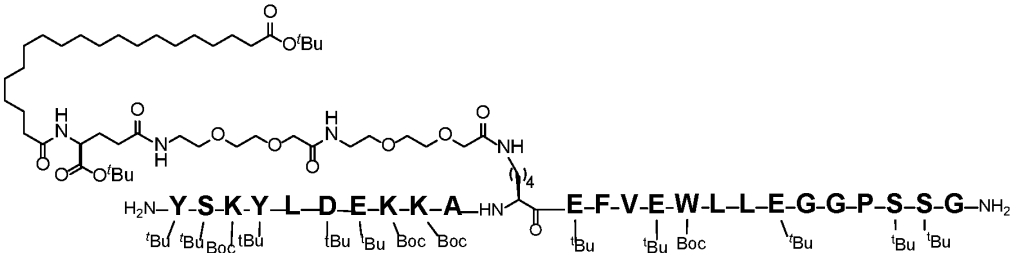
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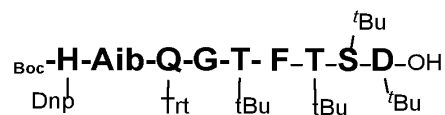


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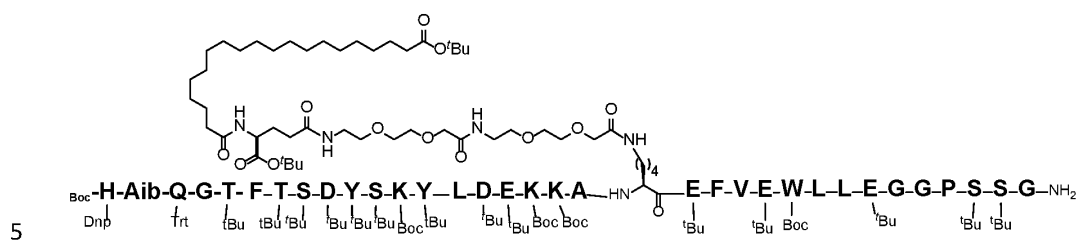


10 SEQ ID NO:11

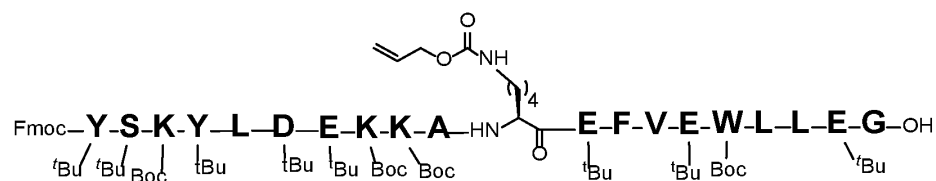




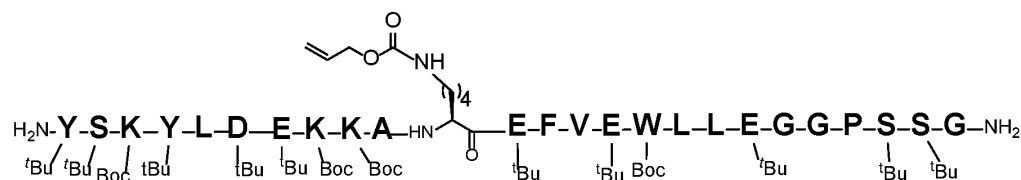
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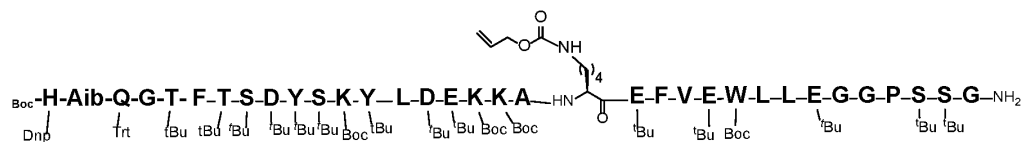
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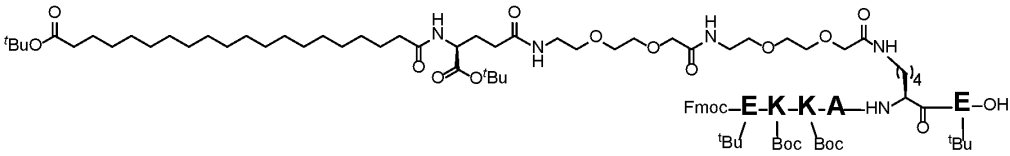
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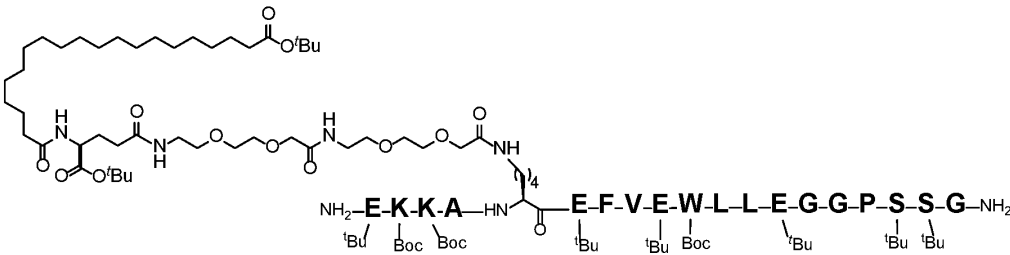
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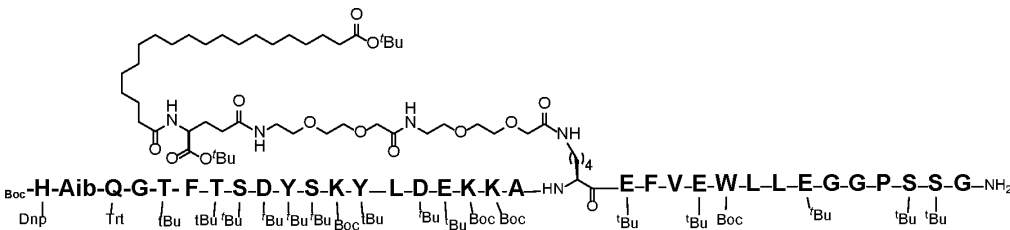
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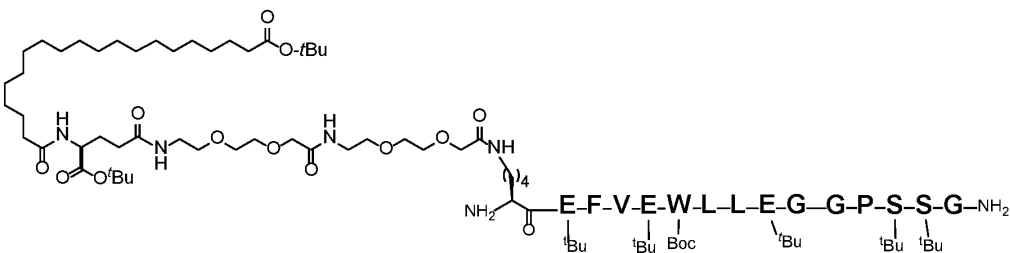
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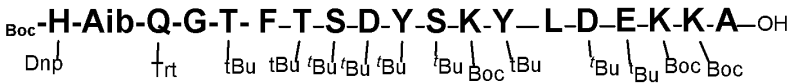
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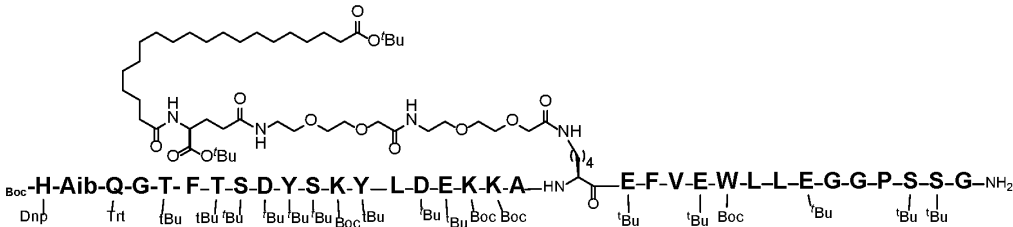
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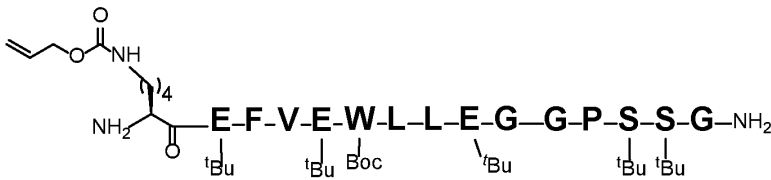
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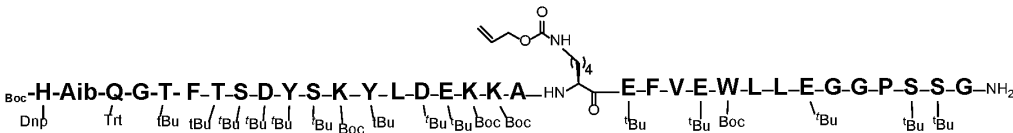
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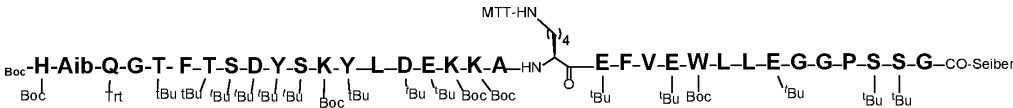


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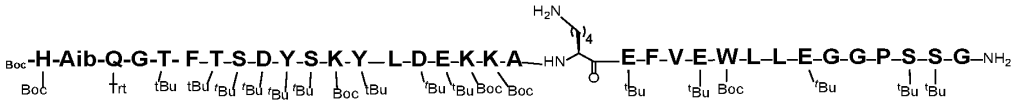


10

SEQ ID NO:29



SEQ ID NO:30



15

SEQ ID NO:31

Boc-H(Dnp)-Aib-Q(Trt)-G

SEQ ID NO:32

Fmoc-T(tBu)-F-T-(tBu)-S(tBu)

5 SEQ ID NO:33

Fmoc-D(tBu)-Y(tBu)-S(tBu)-K(Boc)

SEQ ID NO:34

Fmoc-T(tBu)-F-T(tBu)-S(tBu)-D(tBu)

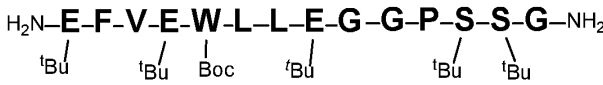
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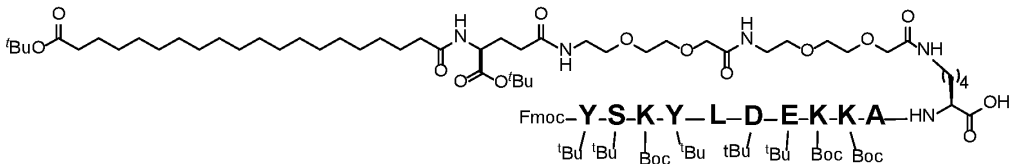
Fmoc-Y(t-Bu)-S(t-Bu)-K(Boc)-Y(t-Bu)

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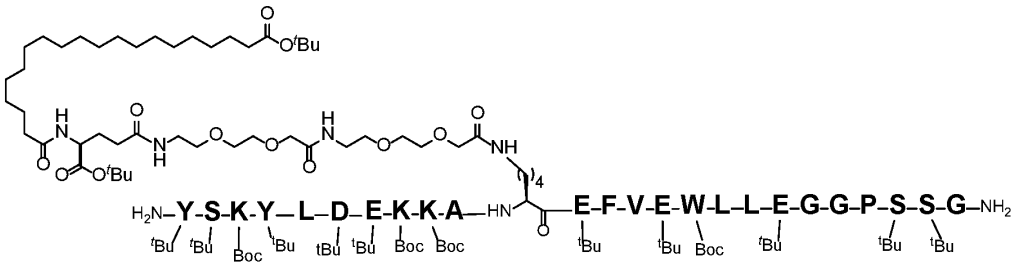
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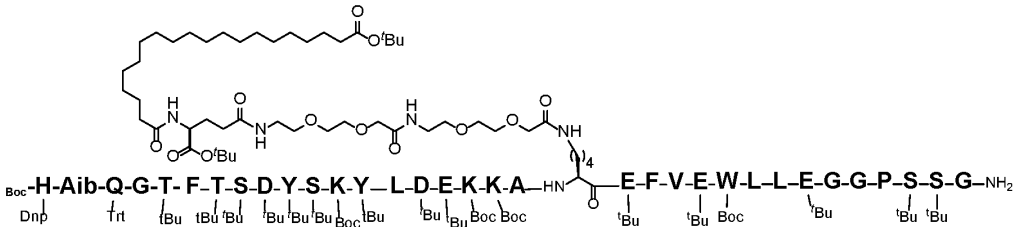
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20 SEQ ID NO:38



SEQ ID NO:39



CLAIMS

The invention claimed is:

1. A method of making a compound of SEQ ID NO:1 or a pharmaceutically
5 acceptable salt thereof, the method comprising coupling intermediate preparations
selected from the groups consisting of:
 - a. SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:12;
 - b. SEQ ID NO:17, SEQ ID NO:21 and SEQ ID NO:5;
 - c. SEQ ID NO:36, SEQ ID NO:37 and SEQ ID NO:12;
 - 10 d. SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:5;
 - e. SEQ ID NO:2, SEQ ID NO:10 and SEQ ID NO:12;
 - f. SEQ ID NO:24 and SEQ ID NO:25;
 - g. SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:5 and Preparation 30;
 - h. SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:12 and Preparation 30;
 - 15 i. SEQ ID NO:27, SEQ ID NO:25 and Preparation 30; and
 - j. SEQ ID NO:30 and Preparation 30.
2. The method of claim 1, wherein the method comprises coupling SEQ ID NO:17,
SEQ ID NO:18 and SEQ ID NO:12.
20
3. The method of claim 1, wherein the method comprises coupling SEQ ID NO:17,
SEQ ID NO:21 and SEQ ID NO:5.
4. The method of claim 1, wherein the method comprises coupling SEQ ID NO:36,
25 SEQ ID NO:37 and SEQ ID NO:12.
5. The method of claim 1, wherein the method comprises coupling SEQ ID NO:2,
SEQ ID NO:3 and SEQ ID NO:5.
- 30 6. The method of claim 1, wherein the method comprises coupling SEQ ID NO:2,
SEQ ID NO:10 and SEQ ID NO:12.

7. The method of claim 1, wherein the method comprises coupling SEQ ID NO:24 and SEQ ID NO:25.
8. The method of claim 1, wherein the method comprises coupling SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:5 and Preparation 30.
9. The method of claim 1, wherein the method comprises coupling SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:12 and Preparation 30.
10. The method of claim 1, wherein the method comprises coupling SEQ ID NO:27, SEQ ID NO:25 and Preparation 30.
11. The method of claim 1, wherein the method comprises coupling SEQ ID NO:30 and Preparation 30.
12. The method of claim 2, wherein the method comprises:
synthesizing each of SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:12 by solid phase peptide synthesis and coupling them in liquid phase.
13. The method of claim 3, wherein the method comprises:
synthesizing each of SEQ ID NO:17, SEQ ID NO:21 and SEQ ID NO:5 by solid phase peptide synthesis and coupling them in liquid phase.
14. The method of claim 4, wherein the method comprises:
synthesizing each of SEQ ID NO:36, SEQ ID NO:37 and SEQ ID NO:12 by solid phase peptide synthesis and coupling them in liquid phase.
15. The method of claim 5, wherein the method comprises:
synthesizing each of SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:5 by solid phase peptide synthesis and coupling them in liquid phase.
16. The method of claim 6, wherein the method comprises:

synthesizing each of SEQ ID NO:2, SEQ ID NO:10 and SEQ ID NO:12 by solid phase peptide synthesis and coupling them in liquid phase.

17. The method of claim 7, wherein the method comprises:

5 synthesizing each of SEQ ID NO:24 and SEQ ID NO:25 by solid phase peptide synthesis and coupling them in liquid phase.

18. The method of claim 8, wherein the method comprises:

10 synthesizing each of SEQ ID NO:2, SEQ ID NO:7 and SEQ ID NO:5 by solid phase peptide synthesis and coupling them in liquid phase, followed by coupling with Preparation 30 in liquid phase.

19. The method of claim 9, wherein the method comprises:

15 synthesizing each of SEQ ID NO:2, SEQ ID NO:14 and SEQ ID NO:12 by solid phase peptide synthesis and coupling them in liquid phase, followed by coupling with Preparation 30 in liquid phase.

20. The method of claim 10, wherein the method comprises:

20 synthesizing each of SEQ ID NO:27 and SEQ ID NO:25 by solid phase peptide synthesis and coupling them in liquid phase, followed by coupling with Preparation 30 in liquid phase.

21. The method of claim 11, wherein the method comprises:

25 synthesizing SEQ ID NO:30 by solid phase peptide synthesis and coupling it with Preparation 30 in liquid phase.

22. An intermediate preparation selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or a pharmaceutically acceptable salt thereof.

30

23. An intermediate preparation selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:21 and SEQ ID NO:22, or a pharmaceutically acceptable salt thereof.
- 5
24. The method of claim 1, comprising preparing SEQ ID NO:5 by coupling:
- a. SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33; or
 - b. SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35.
- 10
25. The method of claim 1, comprising preparing SEQ ID NO:12 by coupling SEQ ID NO:31 and SEQ ID NO:34.
26. The method of claim 1, comprising preparing SEQ ID NO:25 by coupling:
- a. SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33; or
 - 15 b. SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35.
27. The method of claim 1, comprising preparing SEQ ID NO:29 by coupling:
- a. SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33; or
 - 20 b. SEQ ID NO:31, SEQ ID NO:34 and SEQ ID NO:35