[0006] The present disclosure seeks to meet these needs by providing novel intermediates and processes useful in the manufacture of tirzepatide (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof. The tirzepatide manufacturing processes of the present disclosure provide intermediates and process reactions embodying a combination of advances, including an efficient route having fewer steps, while at the same time maintaining high quality and purity. In addition, the new processes and intermediates decrease resource intensity and minimize waste streams.

[0007] The new processes described herein provide various embodiments of intermediates useful for production of tirzepetide.

SUMMARY

[0008] The present disclosure describes methods of preparing tirzepatide or a pharmaceutically acceptable salt thereof.

[0009] In an aspect, the present disclosure describes a method of preparing tirzepatide or a pharmaceutically acceptable salt thereof, the method comprising: (a) coupling a peptide of SEQ ID NO: 10 to SEQ ID NO: 11 to form a peptide of SEQ ID NO: 12; and (b) deprotecting the peptide of SEQ ID NO: 12 to obtain tirzepatide or a pharmaceutically acceptable salt thereof. In certain examples, the peptide of SEQ ID NO: 12 from step (a) is isolated prior to the deprotection step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed before the deprotection step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed after the deprotection step (b).

[0010] In another aspect, the present disclosure describes a method of preparing tirzepatide or a pharmaceutically acceptable salt thereof, the method comprising: (a) coupling a peptide of SEQ ID NO: 13 to SEQ ID NO: 14 to form a peptide of SEQ ID NO: 12; and (b) deprotecting the peptide of SEQ ID NO: 12 to obtain tirzepatide or a pharmaceutically acceptable salt thereof. In certain examples, the peptide of SEQ ID NO: 12 from step (a) is isolated prior to step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed before the deprotection step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed after the deprotection step (b).

[0011] In another aspect, the present disclosure describes a method of preparing tirzepatide or a pharmaceutically acceptable salt thereof, the method comprising: (a) coupling a peptide of SEQ ID NO: 15 to SEQ ID NO: 16 to form a peptide of SEQ ID NO: 12; and (b) deprotecting the peptide of SEQ ID NO: 12 to obtain tirzepatide or a pharmaceutically acceptable salt thereof. In certain examples, the peptide of SEQ ID NO: 12 from step (a)

is isolated prior to step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed before the deprotection step (b). In certain examples, the peptide of SEQ ID NO: 12 from step (a) is washed after the deprotection step (b).

[0012] The present disclosure also describes a compound of SEQ ID NO: 10, or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure describes a compound of SEQ ID NO: 10, or a pharmaceutically acceptable salt thereof, wherein the compound comprises one or more protecting groups. In certain examples, the one or more protecting groups are chosen from Fmoc, Boc, *tert*-butyl, and trityl groups. In certain examples, the compound does not comprise one or more of the protecting groups of SEQ ID NO: 10 (i.e., one or more protecting groups are removed from SEQ ID NO: 10). In certain examples, the compound does not comprise any of the protecting groups of SEQ ID NO: 10 (i.e., all the protecting groups are removed from SEQ ID NO: 10).

[0013] The present disclosure also describes a compound of SEQ ID NO: 13, or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure describes a compound of SEQ ID NO: 13, or a pharmaceutically acceptable salt thereof, wherein the compound comprises one or more protecting groups. In certain examples, the one or more protecting groups are chosen from Fmoc, Boc, *tert*-butyl, and trityl groups.

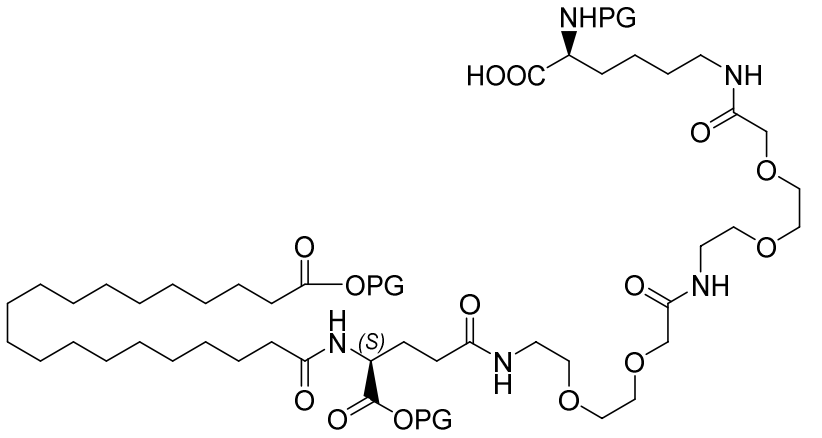
[0014] The present disclosure also describes a compound of SEQ ID NO: 14, or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure describes a compound of SEQ ID NO: 14, or a pharmaceutically acceptable salt thereof, wherein the compound comprises one or more protecting groups. In certain examples, the one or more protecting groups are chosen from Fmoc, Boc, *tert*-butyl, and trityl groups.

[0015] The present disclosure also describes a compound of SEQ ID NO: 15, or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure describes a compound of SEQ ID NO: 15, or a pharmaceutically acceptable salt thereof, wherein the compound comprises one or more protecting groups. In certain examples, the one or more protecting groups are chosen from Fmoc, Boc, *tert*-butyl, and trityl groups. In certain examples, the compound does not comprise one or more of the protecting groups of SEQ ID NO: 15 (i.e., one or more protecting groups are removed from SEQ ID NO: 15). In certain examples, the compound does not comprise any of the

protecting groups of SEQ ID NO: 15 (i.e., all the protecting groups are removed from SEQ ID NO: 15).

[0016] The present disclosure also describes a compound of SEQ ID NO: 16, or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure describes a compound of SEQ ID NO: 16, or a pharmaceutically acceptable salt thereof, wherein the compound comprises one or more protecting groups. In certain examples, the one or more protecting groups are chosen from Fmoc, Boc, *tert*-butyl, and trityl groups. In certain examples, the compound does not comprise one or more of the protecting groups of SEQ ID NO: 16 (i.e., one or more protecting groups are removed from SEQ ID NO: 16). In certain examples, the compound does not comprise any of the protecting groups of SEQ ID NO: 16 (i.e., all the protecting groups are removed from SEQ ID NO: 16).

[0017] In another aspect, the present disclosure describes a process of preparing a compound of formula (I):



(I)

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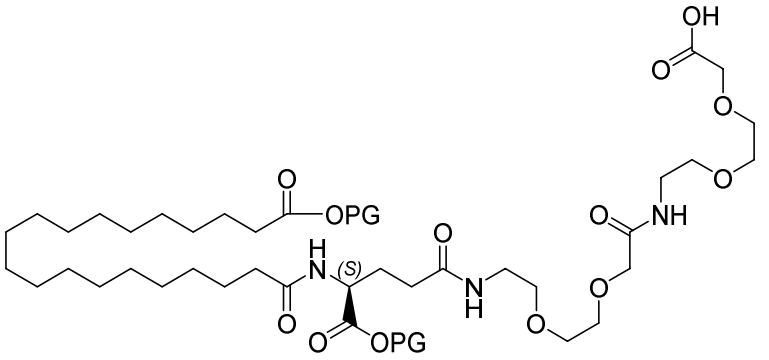
wherein PG is a protecting group, the process comprising:

(a) contacting a compound of formula (Ia) and diisopropylethylamine

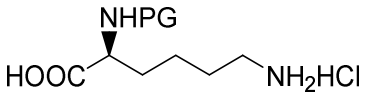


(Ia)

,

(b) contacting the product of step (a) with 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborates in DMF; and

(c) adding a compound of formula (Ib) to the mixture of (b),



(Ib)

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[0018] In certain examples, the protecting group is chosen from Boc and Fmoc. In certain examples, the protecting group is Fmoc. In certain examples, the process is conducted using continuous flow. In certain examples, the process is carried out in flow reactor.

[0019] In certain examples, the present disclosure describes a method that includes filtering tirzepatide or pharmaceutically acceptable salt thereof under one or more of the following conditions: (i) a temperature from 10 to 34 ^{O}C, optionally about 20 ^{O}C; (ii) a turbulent flow / crossflow velocity from 1.94 to 2.46 m/s, optionally about 2.2 m/s; (iii) a laminar flow / shear rate from 2.1 x 10^3 to 2.7 x 10^3 1/s, optionally about 2.4 x 10^3 1/s; (iv) a primary product concentration from 32.8 to 47.2 mg/mL, optionally about 40 mg/mL; and (v) a secondary product concentration from 37 to 53 mg/mL, optionally about 45 mg/mL.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1A, FIG. 1B, and FIG. 1C show representative XRPD patterns for Fmoc-G-P-S(tBu)-S(tBu)-G-OH (SEQ ID NO: 19) pentamer Form A, Form C, and Form D, respectively.

[0021] FIG. 2A, FIG. 2B, FIG. 2C, and FIG. 2D show viscosity measurements for peptide fragment gelation studies of the present disclosure. FIG. 2A shows viscosity measurements