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GQ, GW, KM, ML, MR, NE, SN, TD, TG).**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: PROCESS FOR PREPARING BULEVIRTIDE

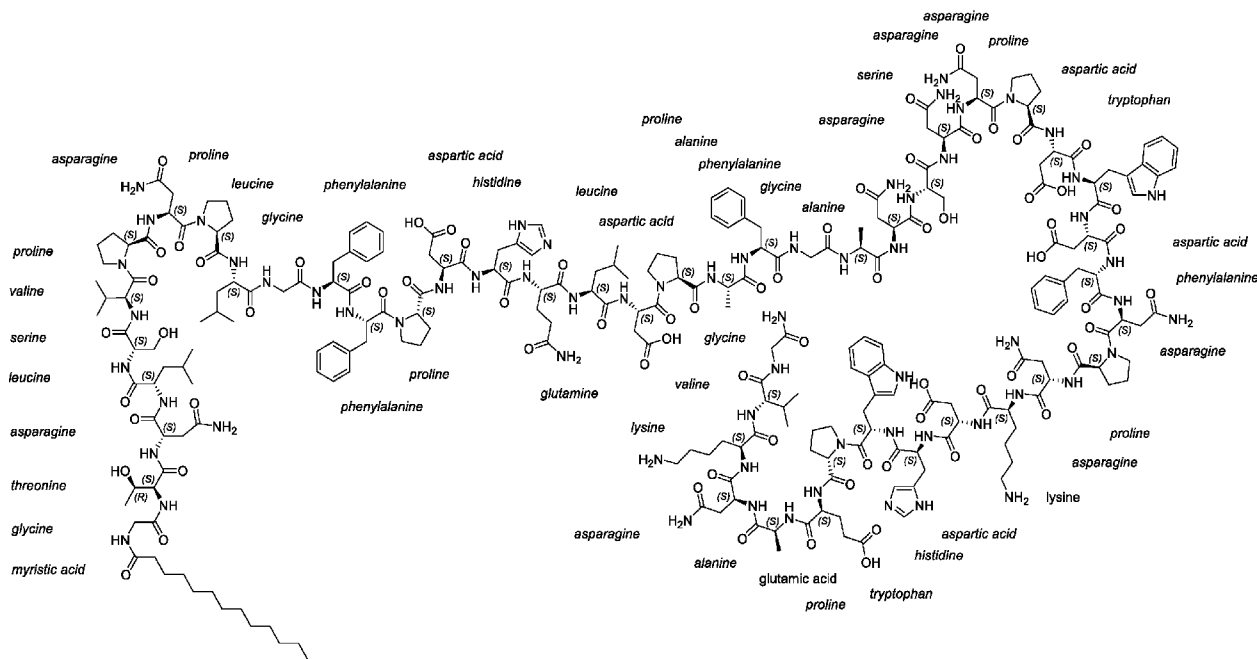


FIG. 1

(57) Abstract: The present application provides process for preparing an acetic acid salt of bulevirtide, which is useful for the inhibition of hepatitis B virus (HBV) and/or hepatitis D virus (HDV) infection, prevention of primary HBV and/or HDV infection, as well as treatment of (chronic) hepatitis B and/or D.

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PROCESS FOR PREPARING BULEVIRTIDE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/492,105, filed on March 24, 2023, the entire contents of which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

The present application provides a process for preparing an acetic acid salt of bulevirtide, which is useful for the inhibition of hepatitis B virus (HBV) and/or hepatitis D virus (HDV) infection, prevention of primary HBV and/or HDV infection, as well as treatment of (chronic) hepatitis B and/or D.

BACKGROUND

Hepatitis B virus (HBV) is the prototype of a family of small, enveloped DNA viruses of mammals and birds (Seeger et al, *Microbiol. Mol. Biol. Rev.* 64, 51-68 (2000)). Hepatitis D virus (HDV) is a hybrid virus and uses the HBV surface antigen as its envelope protein. The HBV envelope encloses three proteins termed L-(large), M-(middle) and S-(small). They share the C-terminal S-domain with four transmembrane regions. The M- and L-protein carry additional N-terminal extensions of 55 (preS2) and, genotype-dependent, 107 or 118 aa (preS1). In virions the stoichiometric ratio of L, M and S is about 1:1:4, while the more abundantly secreted non-infectious subviral particles (SVPs) contain almost exclusively S- and only traces of L-protein (Nassal, M. *Curr. Top. Microbiol. Immunol.* 214, 297-337 (1996)). During synthesis, the preS1 domain of L is myristoylated and translocated through the ER. This modification is essential for HBV infectivity (Gripon et al, *Virology*, 213, 292-299; and Le Seyec et al, *J. Virol.* 73, 2052-2057 (1999)).

The present application is directed to processes for preparing an acetic acid salt of bulevirtide, which is useful for the inhibition, prevention, and/or treatment of HBV and/or HDV infection, and other HBV- and/or HDV-related diseases.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

The instant application contains a Sequence Listing that has been submitted electronically as an XML file named "35648-0289WO1_SL.xml." The XML file, created on

March 6, 2024, is 22523 bytes in size. The material in the XML file is hereby incorporated by reference in its entirety.

SUMMARY

The present application provides, *inter alia*, a process of preparing an acetic acid salt of the compound of Formula I:

Myr-Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-Phe-Pro-Asp-His-Gln-Leu-Asp-Pro-Ala-Phe-Gly-Ala-Asn-Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-Asp-His-Trp-Pro-Glu-Ala-Asn-Lys-Val-Gly-NH₂

I

comprising reacting, for example, a tetrafluoroboric acid salt of the compound of Formula I with acetic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structure of bulevirtide.

DETAILED DESCRIPTION

The description below is made with the understanding that the present disclosure is to be considered as an exemplification of the claimed subject matter, and is not intended to limit the appended claims to the specific embodiments illustrated. The headings used throughout this disclosure are provided for convenience and are not to be construed to limit the claims in any way. Embodiments illustrated under any heading may be combined with embodiments illustrated under any other heading.

The present application provides a process of preparing an acetic acid salt of bulevirtide. Bulevirtide is a hydrophobic modified preS-derived peptide of HBV having the structure of Formula I:

Myr-Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-Phe-Pro-Asp-His-Gln-Leu-Asp-Pro-Ala-Phe-Gly-Ala-Asn-Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-Asp-His-Trp-Pro-Glu-Ala-Asn-Lys-Val-Gly-NH₂

I.

As used herein, “Myr” refers to a coupled myristic acid group (*i.e.*, $C_{13}H_{27}C(O)-$). As used herein, bulevirtide may also be referred to as “the compound of Formula I”, “Myr-(SEQ ID NO.: 1)-NH₂”, or “ $C_{13}H_{27}C(O)-(SEQ ID NO.: 1)-NH_2$ ”. The structure of bulevirtide is shown in FIG. 1.

As used herein, SEQ ID NO.: 1 refers to the following sequence:

Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-Phe-Pro-Asp-His-Gln-Leu-Asp-Pro-Ala-Phe-Gly-Ala-Asn-Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-Asp-His-Trp-Pro-Glu-Ala-Asn-Lys-Val-Gly (SEQ ID NO.: 1).

Additional methods of preparing bulevirtide can be found, for example, in U.S. Patent No.: 9,562,076, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, the process of preparing the acetic acid salt of bulevirtide comprises reacting a tetrafluoroboric acid salt of the compound of Formula I with acetic acid. In some embodiments, the reaction is a salt exchange reaction.

In some embodiments, the process of preparing the acetic acid salt of bulevirtide comprises reacting a trifluoroacetic acid salt of the compound of Formula I with acetic acid. In some embodiments, the reaction is a salt exchange reaction.

In some embodiments, the reacting is performed in the presence of a solvent comprising water. In some embodiments, the reacting is performed in the presence of a solvent comprising water and acetonitrile. In some embodiments, the reacting is performed in the presence of water. In some embodiments, the reacting is performed in the presence of water and an alcohol. In some embodiments, the reacting is performed in the presence of water and methanol. In some embodiments, the reacting is performed in the presence of water and ethanol.

In some embodiments, the reacting is performed in the presence of a buffer agent. In some embodiments, the reacting is performed in the presence of a buffer agent comprising ammonium acetate, an amine base, and an inorganic acid.

In some embodiments, the reacting comprises mixing the compound of Formula I with an amine base and an inorganic acid to form a first mixture and subsequently mixing the first mixture with ammonium acetate.

In some embodiments, the buffer agent comprises an ammonium salt. In some embodiments, the buffer agent comprises ammonium acetate.

In some embodiments, the buffer agent comprises an amine base. In some embodiments, the buffer agent comprises a tertiary amine base. In some embodiments, the buffer agent

comprises a tri(C₁₋₆ alkyl)amine base. Example tri(C₁₋₆ alkyl)amine bases include, but are not limited to, trimethylamine, triethylamine, triisopropylamine, tributylamine, and the like. In some embodiments, the buffer agent comprises triethylamine.

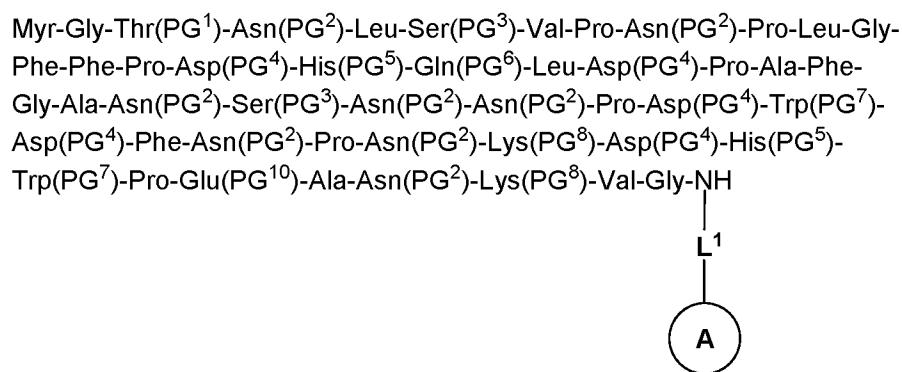
In some embodiments, the buffer agent comprises a weak inorganic acid. In some embodiments, the inorganic acid is phosphoric acid.

In some embodiments, amine base is triethylamine and the inorganic acid is phosphoric acid

In some embodiments, the reacting is performed in a liquid chromatography system.

In some embodiments, the process further comprises substantially isolating the acetic acid salt of bulevirtide. As used herein, the term “substantially isolated” is meant that the compound or salt (*e.g.*, the acetic acid salt of bulevirtide), is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound or salt of the invention. Substantial separation can include compositions containing from at least about 50% to at least about 99% by weight of the compound or salt, for example, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compound or salt. In some embodiments, the isolating comprises lyophilization. In some embodiments, the isolating comprises chromatography (*e.g.*, preparative chromatography).

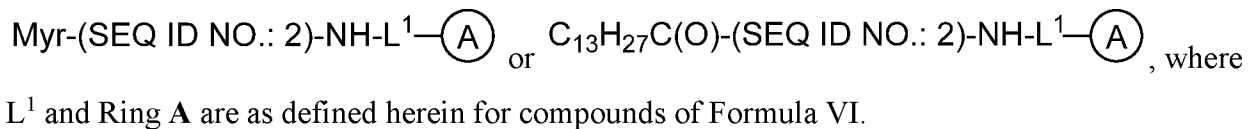
In some embodiments, the tetrafluoroboric acid salt of bulevirtide (*i.e.*, the tetrafluoroboric acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula VI:



VI

in the presence of tetrafluoroboric acid, wherein L¹ is a linking group, Ring A refers to a solid support (*e.g.*, a resin), and PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, and PG¹⁰ are each independently absent or a protecting group.

As used herein, the compound of Formula VI may also be referred to as

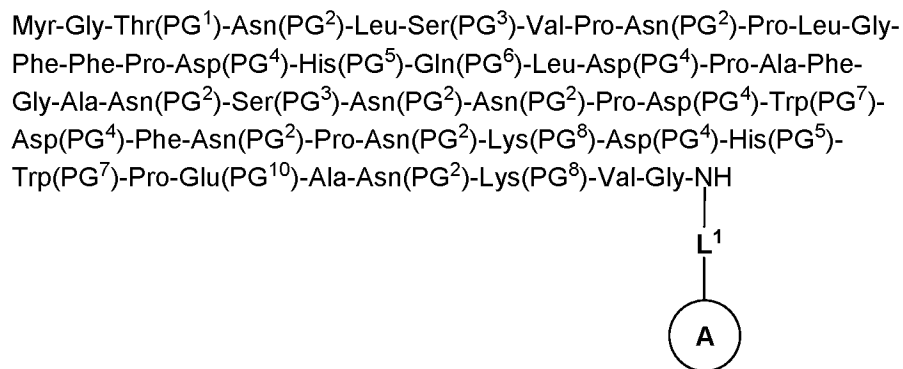


As used herein, SEQ ID NO.: 2 refers to the following sequence:

Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly (SEQ ID NO.: 2)

wherein PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, and PG¹⁰ are each independently absent or a protecting group.

In some embodiments, the trifluoroacetic acid salt of bulevirtide (*i.e.*, the trifluoroacetic acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula VI:



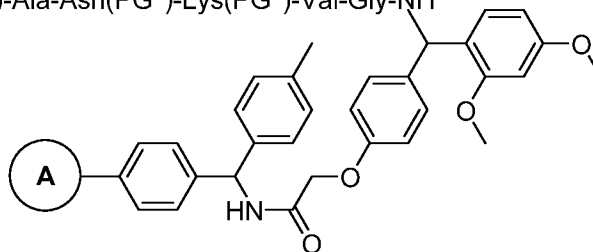
VI

in the presence of trifluoroacetic acid, wherein L¹ is a linking group and Ring A refers to a solid support (*e.g.*, a resin).

In some embodiments, Ring A refers to a resin. In some embodiments, the L¹-Resin group is a group that results in C terminal amide groups when cleaved. In some embodiments, the linking group is a 4-((2,4-dimethoxyphenyl)(Fmoc amino)methyl)phenoxyacetic acid linking group (*i.e.*, a Rink amide linker). In some embodiments, the resin is selected from a 4-methylbenzhydrylamine HCl salt resin, a PAL Resin, a Sieber amide resin, and a Ramage resin. In some embodiments, the resin is a 4-methylbenzhydrylamine HCl salt resin.

In some embodiments, the compound of Formula VI is a compound of Formula II:

Myr-Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH

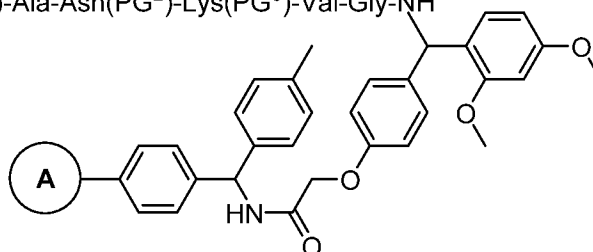


II

wherein PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, and PG¹⁰ are each independently absent or a protecting group.

In some embodiments, the tetrafluoroboric acid salt of bulevirtide (*i.e.*, the tetrafluoroboric acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula II:

Myr-Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



II

in the presence of tetrafluoroboric acid, wherein:

wherein PG², PG⁶, PG⁷ are each independently absent or a protecting group; and

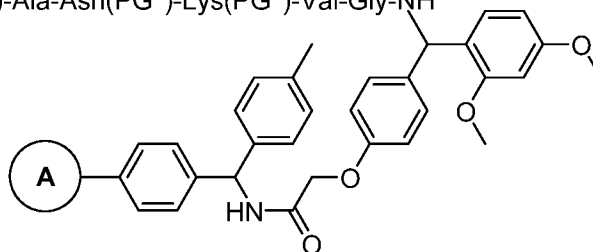
PG¹, PG³, PG⁴, PG⁵, PG⁸, and PG¹⁰ are each an independently selected protecting group;

and

Ring **A** refers to a resin.

In some embodiments, the trifluoroacetic acid salt of bulevirtide (*i.e.*, the trifluoroacetic acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula II:

Myr-Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



II

in the presence of trifluoroacetic acid, wherein:

wherein PG², PG⁶, PG⁷ are each independently absent or a protecting group; and

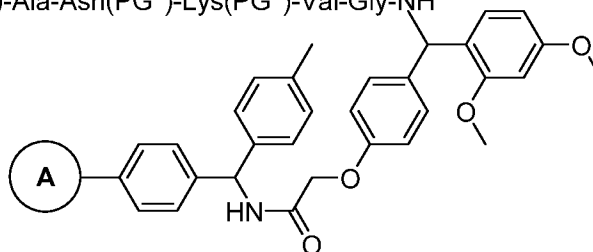
PG¹, PG³, PG⁴, PG⁵, PG⁸, and PG¹⁰ are each an independently selected protecting group;

and

Ring A refers to a resin.

In some embodiments, the tetrafluoroboric acid salt of bulevirtide (*i.e.*, the tetrafluoroboric acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula II:

Myr-Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



II

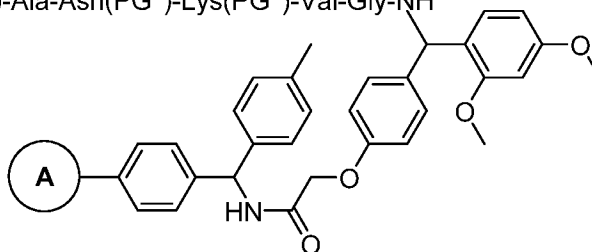
in the presence of tetrafluoroboric acid, wherein:

PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, and PG¹⁰ are each an independently selected protecting group; and

Ring A refers to a resin.

In some embodiments, the trifluoroacetic acid salt of bulevirtide (*i.e.*, the trifluoroacetic acid salt of the compound of Formula I) is prepared by deprotecting a compound of Formula II:

Myr-Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



II

in the presence of trifluoroacetic acid, wherein:

PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, and PG¹⁰ are each an independently selected protecting group; and

Ring **A** refers to a resin.

As used herein, the term “protecting groups” (*e.g.*, amino protecting groups) refers to a moiety that may be used to prevent unwanted reactions of a group (*e.g.*, an amino group) while performing a desired transformation. Protecting groups (*e.g.*, amino protecting groups) allow easy covalent attachment to an atom (*e.g.*, a nitrogen atom) as well as selective cleavage from the atom (*e.g.*, a nitrogen atom). Suitable protecting groups, such as alkoxycarbonyl (such as ethoxycarbonyl, *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), 9-fluorenylmethyloxycarbonyl (Fmoc), and the like), acyl (such as acetyl (Ac), benzoyl (Bz), and the like), sulfonyl (such as methanesulfonyl, trifluoromethanesulfonyl, and the like), arylalkyl (such as benzyl, 4-methoxybenzyl, diphenylmethyl, triphenylmethyl (trityl), and the like), alkenylalkyl (such as allyl, prenyl, and the like), diarylmethyleneyl (such as (C₆H₅)₂C=, and the like), and silyl (such as *tert*-butyldimethylsilyl, triisopropylsilyl, and the like), would be readily known to a skilled artisan. The chemistry of protecting groups can be found, *e.g.*, in Wuts and Greene, *Greene's Protective Groups in Organic Synthesis*, 4th Ed., pp 696-926, John Wiley & Sons: New York, 2006, which is incorporated herein by reference in its entirety.

In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of tetrafluoroboric acid. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of trifluoroacetic acid. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of trifluoroacetic acid

and water. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of $(\text{CH}_3\text{O})_3\text{SiCl}$.

In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of one or more additives. In some embodiments, the one or more additives comprise water, one or more silyl compounds, one or more thiol compounds, and one or more aromatic compounds, or any combination thereof. In some embodiments, the one or more additives comprise one or more silyl compounds, one or more thiol compounds, and one or more aromatic compounds, or any combination thereof.

In some embodiments, the one or more silyl compounds are each an independently selected tri(C_{1-6} alkyl) silane compound. In some embodiments, the one or more silyl compounds are each independently selected from triisopropyl silane (TIPS) and triethylsilane (TES).

In some embodiments, the one or more thiol compounds are each an independently selected dithiol compound. In some embodiments, the one or more thiol compounds are each independently selected from dioxo-1,8-octane-dithiol, dithioethane (DTE), 1,2-ethanedithiol (EDT), and dithiothreitol (DTT). In some embodiments, the thiol compound is dioxo-1,8-octane-dithiol.

In some embodiments, the one or more aromatic compounds are each independently selected from phenol and anisole.

In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of 2,2'-(ethylenedioxy)diethanethiol. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of thioanisole. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of phenol. In some embodiments, the deprotecting of the compound of Formula II is performed in the presence of 2,2'-(ethylenedioxy)diethanethiol, thioanisole, and phenol.

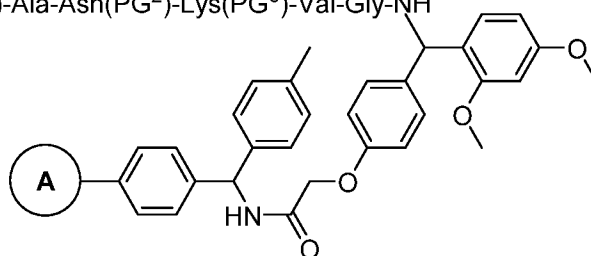
In some embodiments, the deprotecting of the compound of Formula II is performed at a temperature of from about 10°C to about 35°C . In some embodiments, the deprotecting of the compound of Formula II is performed at a temperature of from about 15°C to about 25°C . In some embodiments, the deprotecting of the compound of Formula II is performed at a temperature of from about 19°C to about 22°C .

In some embodiments, the deprotecting further comprises substantially isolating the tetrafluoroboric acid salt of bulevirtide (*e.g.*, in the presence of an organic ether). In some

embodiments, the deprotecting further comprises substantially isolating the trifluoroacetic acid salt of bulevirtide (*e.g.*, in the presence of an organic ether). In some embodiments, the isolating comprises precipitating the trifluoroacetic acid salt of bulevirtide. In some embodiments, the isolating comprises precipitating the trifluoroacetic acid salt of bulevirtide in the presence of diethyl ether.

In some embodiments, the compound of Formula II is prepared by reacting a compound of Formula IIIa:

Gly-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



IIIa

with myristic acid under coupling conditions.

In some embodiments, the coupling conditions comprise reacting the compound of Formula IIIa with myristic acid in the presence of an amino coupling agent and an amino coupling additive.

In some embodiments, the amino coupling agent comprises one or more benzotriazole compounds (see *e.g.*, *J. Org. Chem.* 2019, 84, 4615–4628), one or more uronium or aminium salts (*e.g.*, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), tetramethylfluoroformamidinium hexafluorophosphate (TFFH), N'-tetramethylchloroformamidinium hexafluorophosphate (TCFH), and the like), propylphosphonic anhydride, one or more phosphonium salts (*e.g.*, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) (PyAOP), 6-chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyClock), and the like), one or more carbodiimide compounds (*e.g.*, N,N'-diisopropylcarbodiimide (DIC), N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide-methyl-p-

toluenesulfonate (CMC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and the like), or any combination thereof.

In some embodiments, the amino coupling agent is 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

In some embodiments, the amino coupling additive is selected from 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), and ethyl cyano(hydroxyimino)acetate (oxyma pure). In some embodiments, the amino coupling additive is 1-hydroxybenzotriazole.

In some embodiments, the coupling conditions comprise reacting the compound of Formula IIIa with myristic acid in the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and 1-hydroxybenzotriazole.

In some embodiments, the process further comprises mixing the myristic acid, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, and 1-hydroxybenzotriazole, prior to reacting with the compound of Formula IIIa.

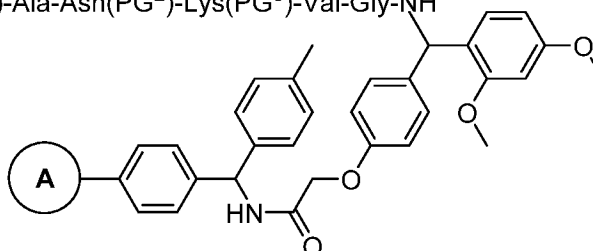
In some embodiments, the reacting of the compound of Formula IIIa with myristic acid is performed in the presence of an amine base. In some embodiments, the amine base is selected from triethylamine, ethyldiisopropylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base is N-methylmorpholine.

In some embodiments, the reacting of the compound of Formula IIIa and myristic acid is performed a temperature of from about 5°C to about 40°C. In some embodiments, the reacting of the compound of Formula IIIa and myristic acid is performed a temperature of from about 15°C to about 30°C. In some embodiments, the reacting of the compound of Formula IIIa and myristic acid is performed a temperature of from about 17°C to about 27°C.

In some embodiments, the reacting of the compound of Formula IIIa and myristic acid is performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), or any combination thereof. In some embodiments, the solvent comprises N,N-dimethylformamide.

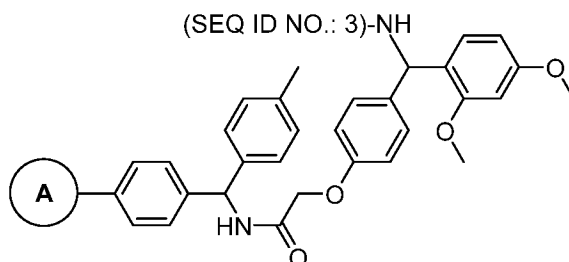
In some embodiments, the compound of Formula IIIa is prepared by deprotecting a compound of Formula III:

Gly(PG⁹)-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



III.

As used herein, the compound of Formula III may also be referred to as



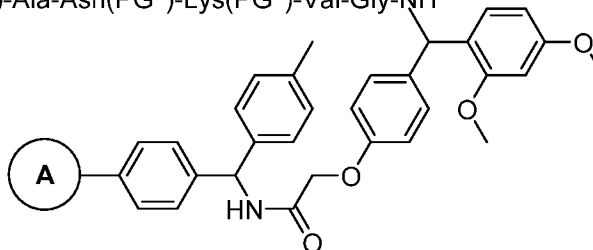
As used herein, SEQ ID NO.: 3 refers to the following sequence:

Gly(PG⁹)-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly (SEQ ID NO.: 3)

wherein PG¹, PG², PG³, PG⁴, PG⁵, PG⁶, PG⁷, PG⁸, PG⁹, and PG¹⁰ are each independently absent or a protecting group.

In some embodiments, the compound of Formula IIIa is prepared by deprotecting a compound of Formula III:

Gly(PG⁹)-Thr(PG¹)-Asn(PG²)-Leu-Ser(PG³)-Val-Pro-Asn(PG²)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(PG⁴)-His(PG⁵)-Gln(PG⁶)-Leu-Asp(PG⁴)-Pro-Ala-Phe-Gly-Ala-Asn(PG²)-Ser(PG³)-Asn(PG²)-Asn(PG²)-Pro-Asp(PG⁴)-Trp(PG⁷)-Asp(PG⁴)-Phe-Asn(PG²)-Pro-Asn(PG²)-Lys(PG⁸)-Asp(PG⁴)-His(PG⁵)-Trp(PG⁷)-Pro-Glu(PG¹⁰)-Ala-Asn(PG²)-Lys(PG⁸)-Val-Gly-NH



III

wherein PG⁹ is a protecting group.

In some embodiments, the deprotecting comprises reacting the compound of Formula III with an amine base. In some embodiments, the amine base is a tertiary amine base or a secondary amine base. In some embodiments, the amine base is selected from piperidine, morpholine, 4-methylpiperidine, piperazine, pyrrolidine, triethylamine, ethyldiisopropylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base comprises piperidine.

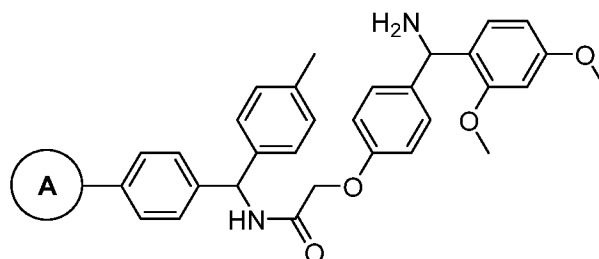
In some embodiments, the deprotecting is performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), or any combination thereof. In some embodiments, the deprotecting is performed in the presence of a solvent comprising N,N-dimethylformamide.

In some embodiments, the deprotecting is performed in the presence of an additive. In some embodiments, the additive is selected from a reagent that suppresses racemization and aspartimide formation (see *e.g.*, www.peptide.com/resources/solid-phase-peptide-synthesis/amino-acid-derivatives-for-peptide-synthesis/, and all references cited therein; and *Chem. Rev.* 2009, 109, 6, 2455–2504). In some embodiments, the additive is selected from an organic acid (*e.g.*, formic acid) and a heterocyclic compound (*e.g.*, 1-hydroxybenzotriazole (HOBt), 6-chloro-1-hydroxybenzotriazole (Cl-HOBt), and the like).

In some embodiments, the deprotecting is performed at a temperature of from about 5°C to about 40°C. In some embodiments, the deprotecting is performed at a temperature of from

about 15°C to about 30°C. In some embodiments, the deprotecting is performed at a temperature of from about 17°C to about 27°C. In some embodiments, the deprotecting is performed at a temperature of from about 19°C to about 25°C.

In some embodiments, the compound of Formula III is prepared by sequentially coupling the compound of Formula IV:

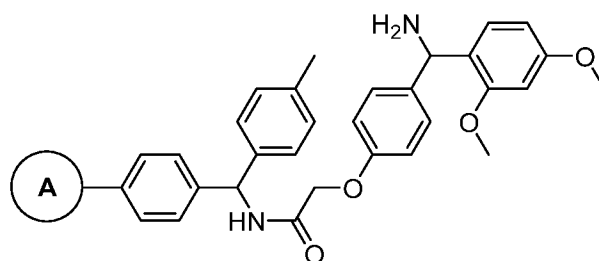


IV

with one or more protected amino acids selected from PG⁹-Gly-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-Leu-OH, Fmoc-Thr(PG¹)-OH, Fmoc-Asn(PG²)-OH, Fmoc-Ser(PG³)-OH, Fmoc-Asp(PG⁴)-OH, Fmoc-His(PG⁵)-OH, Fmoc-Gln(PG⁶)-OH, Fmoc-Trp(PG⁷)-OH, Fmoc-Lys(PG⁸)-OH, and Fmoc-Glu(PG¹⁰)-OH, under coupling conditions, to afford the compound of Formula III, wherein PG², PG⁶, PG⁷ are each independently absent or a protecting group; and

PG¹, PG³, PG⁴, PG⁵, PG⁸, PG⁹, and PG¹⁰ are each an independently selected protecting group.

In some embodiments, the compound of Formula III is prepared by sequentially coupling the compound of Formula IV:



IV

with one or more protected amino acids selected from PG⁹-Gly-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-Leu-OH, Fmoc-Thr(PG¹)-OH, Fmoc-Asn(PG²)-OH, Fmoc-Ser(PG³)-OH, Fmoc-Asp(PG⁴)-OH, Fmoc-His(PG⁵)-OH, Fmoc-Gln(PG⁶)-OH, Fmoc-Trp(PG⁷)-OH, Fmoc-Lys(PG⁸)-OH, and Fmoc-Glu(PG¹⁰)-OH, under coupling conditions, to afford the compound of Formula III, wherein PG¹⁰ is a protecting group.

In some embodiments, the coupling conditions comprise reacting the compound of Formula IV with the protected amino acid in the presence of an amino coupling agent and an amino coupling additive, and subsequently deprotecting the Fmoc group in the presence of an amine base.

In some embodiments, the coupling and deprotecting steps are repeated one or more times.

In some embodiments, the amino coupling agent comprises one or more benzotriazole compounds (see *e.g.*, *J. Org. Chem.* 2019, 84, 4615–4628), one or more uronium or aminium salts (*e.g.*, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), tetramethylfluoroformamidinium hexafluorophosphate (TFFH), N'-tetramethylchloroformamidinium hexafluorophosphate (TCFH), and the like), propylphosphonic anhydride, one or more phosphonium salts (*e.g.*, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) (PyAOP), 6-chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyClock), and the like), one or more carbodiimide compounds (*e.g.*, N,N'-diisopropylcarbodiimide (DIC), N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide-methyl-p-toluenesulfonate (CMC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and the like), or any combination thereof. In some embodiments, the amino coupling agent is N,N-diisopropylcarbodiimide.

In some embodiments, the amino coupling additive is 1-hydroxybenzotriazole.

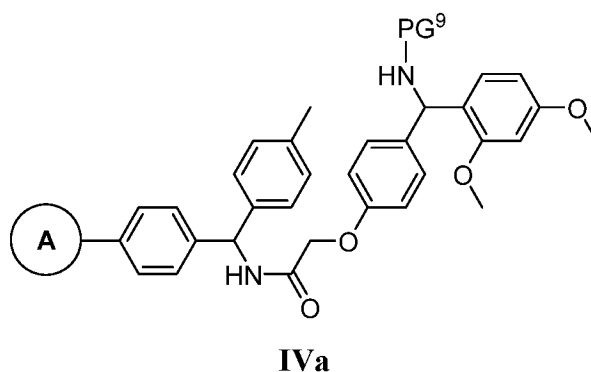
In some embodiments, the deprotecting of the Fmoc group is performed in the presence of an amine base, which is a tertiary amine base or a secondary amine base. In some embodiments, the amine base is selected from piperidine, morpholine, 4-methylpiperidine, piperazine, pyrrolidine, triethylamine, ethyldiisopropylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base comprises piperidine.

In some embodiments, the coupling and deprotecting steps are performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-

methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), or any combination thereof. In some embodiments, the coupling and deprotecting steps are performed in the presence of a solvent comprising N,N-dimethylformamide.

In some embodiments, the coupling and deprotecting steps are performed at a temperature of from about 5°C to about 40°C. In some embodiments, the coupling further comprises as pre-activation step performed at about -10°C to about 10°C. performed at a temperature of from about 5°C to about 40°C. In some embodiments, the coupling and deprotecting steps are performed at a temperature of from about 15°C to about 30°C. In some embodiments, the coupling and deprotecting steps are performed at a temperature of from about 17°C to about 27°C.

In some embodiments, the compound of Formula IV is prepared by deprotecting a compound of Formula IVa:



in the presence of an amine base.

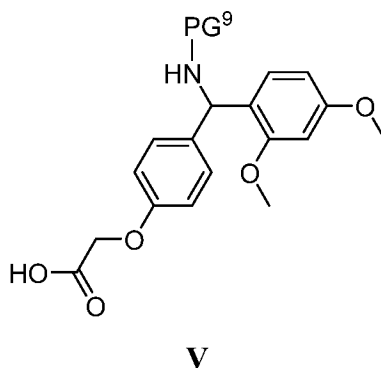
In some embodiments, the amine base is a tertiary amine base or a secondary amine base. In some embodiments, the amine base is selected from piperidine, morpholine, 4-methylpiperidine, piperazine, pyrrolidine, triethylamine, ethyldiisopropylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base comprises piperidine.

In some embodiments, the deprotecting is performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane,

chloroform, and the like), or any combination thereof. In some embodiments, the deprotecting is performed in the presence of a solvent comprises N,N-dimethylformamide.

In some embodiments, the deprotecting is performed at a temperature of from about 5°C to about 40°C. In some embodiments, the deprotecting is performed at a temperature of from about 15°C to about 30°C. In some embodiments, the deprotecting is are performed at a temperature of from about 17°C to about 27°C. In some embodiments, the deprotecting is are performed at a temperature of from about 19°C to about 25°C.

In some embodiments, the compound of Formula IVa is prepared by reacting 4-methylbenzhydrylamine resin with a compound of Formula V:



in the presence of an amine base and an amino coupling agent.

In some embodiments, the amine base is selected from N-methylmorpholine, N,N-diisopropylethylamine (DIPEA), triethylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base comprises N-methylmorpholine.

In some embodiments, the amino coupling agent comprises one or more benzotriazole compounds (see *e.g.*, *J. Org. Chem.* 2019, 84, 4615–4628), one or more uronium or aminium salts (*e.g.*, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), tetramethylfluoroformamidinium hexafluorophosphate (TFFH), N'-tetramethylchloroformamidinium hexafluorophosphate (TCFH), and the like), propylphosphonic anhydride, one or more phosphonium salts (*e.g.*, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) (PyAOP), 6-chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyClock), and the like), one or more carbodiimide compounds (*e.g.*, N,N'-

diisopropylcarbodiimide (DIC), N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide-methyl-p-toluenesulfonate (CMC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and the like), or any combination thereof. In some embodiments, the amino coupling agent is 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

In some embodiments, the reacting of 4-methylbenzhydrylamine resin with the compound of Formula V is performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), or any combination thereof. In some embodiments, the reacting is performed in the presence of a solvent comprising N,N-dimethylformamide.

In some embodiments, the reacting is performed at a temperature of from about 5°C to about 40°C. In some embodiments, the reacting is performed at a temperature of from about 15°C to about 30°C. In some embodiments, the reacting is performed at a temperature of from about 17°C to about 27°C.

In some embodiments, the process further comprises reacting the compound of Formula IVa with an anhydride (*e.g.*, acetic anhydride, propionic anhydride, phenoxyacetic anhydride, benzoic anhydride, and the like) or an acid chloride (*e.g.*, acetyl chloride, propionyl chloride, benzoyl chloride, and the like). In some embodiments, the process further comprises reacting the compound of Formula IVa with an anhydride.

In some embodiments, the process further comprises acetylating the compound of Formula IVa. In some embodiments, the acetylating comprises reacting the compound of Formula IVa with acetic anhydride. In some embodiments, the acetylating comprises reacting the compound of Formula IVa with acetic anhydride in the presence of an amine base.

In some embodiments, the amine base is selected from N-methylmorpholine, triethylamine, ethyldiisopropylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, 1,5-diazabicyclo-4.3.0]non-5-ene, pyridine, and 2,6-lutidine. In some embodiments, the amine base is N-methylmorpholine.

In some embodiments, the acetylating is performed in the presence of a solvent. In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide

(DMSO), pyridine, and the like), an ether (*e.g.*, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), or any combination thereof. In some embodiments, the acetylating is performed in the presence of a solvent comprising N,N-dimethylformamide.

In some embodiments, the acetylating is performed at a temperature of from about 5°C to about 40°C. In some embodiments, the acetylating is performed at a temperature of from about 15°C to about 30°C. In some embodiments, the acetylating is performed at a temperature of from about 17°C to about 27°C.

In some embodiments, the process further comprises mixing the 4-methylbenzhydrylamine resin with an amine base and solvent prior to the reacting with the compound of Formula V. In some embodiments, the amine base is selected from N-methylmorpholine, N,N-diisopropylethylamine (DIPEA), triethylamine, pyrrolidine, 1,4-diazabicyclo[2.2.2]-octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and 1,5-diazabicyclo-4.3.0]non-5-ene. In some embodiments, the amine base is N-methylmorpholine.

In some embodiments, the solvent comprises a polar aprotic solvent (*e.g.*, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), pyridine, and the like), an ether (*e.g.*, methyl *tert*-butyl ether, tetrahydrofuran, 2-methyltetrahydrofuran, dimethoxyethane, and the like), a chlorinated solvent (*e.g.*, dichloromethane, dichloroethane, chloroform, and the like), an aromatic solvent (*e.g.*, benzene, toluene, xylenes, and the like), an alcohol (*e.g.*, methanol, ethanol, 2-propanol, and the like), an acid (*e.g.*, trifluoroacetic acid), and water, or any combination thereof. In some embodiments, the 4-methylbenzhydrylamine resin is mixed with an amine base, which is N-methylmorpholine and a solvent comprising N,N-dimethylformamide prior to the reacting with the compound of Formula V.

In some embodiments, the reacting is performed at a temperature of from about 0°C to about 50°C. In some embodiments, the reacting is performed at a temperature of from about 10°C to about 30°C.

In some embodiments of the Formulas described herein, each PG¹ is independently selected from *tert*-butyl and trityl. In some embodiments of the Formulas described herein, each PG¹ is *tert*-butyl.

In some embodiments of the Formulas described herein, each PG² is trityl.

In some embodiments of the Formulas described herein, each PG³ is independently selected from *tert*-butyl and trityl. In some embodiments of the Formulas described herein, each PG³ is *tert*-butyl.

In some embodiments of the Formulas described herein, each PG⁴ is independently selected from *tert*-butoxy, 2-phenylisopropyl ester (O-2-PhiPr), allyl ester (OAll), and β -3-methylpent-3-yl (Mpe). In some embodiments of the Formulas described herein, each PG⁴ is *tert*-butoxy.

In some embodiments of the Formulas described herein, each PG⁵ is independently selected from trityl, *p*-monomethoxytrityl (Mmt), and 4-methyltrityl (Mtt). In some embodiments of the Formulas described herein, each PG⁵ is trityl.

In some embodiments of the Formulas described herein, each PG⁶ is trityl.

In some embodiments of the Formulas described herein, each PG⁷ is *tert*-butoxycarbonyl (Boc).

In some embodiments of the Formulas described herein, PG⁸ is independently selected from *tert*-butoxycarbonyl, 2-chlorobenzoyloxycarbonyl (Cl-Z), 4-methyltrityl (Mtt), *p*-monomethoxytrityl (Mmt), dimethoxytrityl (Dmt), allyloxycarbonyl (Alloc). In some embodiments of the Formulas described herein, each PG⁸ is *tert*-butoxycarbonyl (Boc).

In some embodiments of the Formulas described herein, PG⁹ is 9-fluorenylmethoxycarbonyl (Fmoc).

In some embodiments of the Formulas described herein, each PG¹⁰ is independently selected from *tert*-butoxycarbonyl (Boc), 2-chlorobenzoyloxycarbonyl (Cl-Z), 4-methyltrityl (Mtt), *p*-Monomethoxytrityl (Mmt), dimethoxytrityl (Dmt), allyloxycarbonyl (Alloc). In some embodiments of the Formulas described herein, each PG¹⁰ is *tert*-butoxycarbonyl (Boc).

In some embodiments of the Formulas described herein:

each PG¹ is independently selected from *tert*-butyl and trityl;

each PG² is trityl;

each PG³ is independently selected from *tert*-butyl and trityl;

each PG⁴ is independently selected from *tert*-butoxy, 2-phenylisopropyl ester (O-2-PhiPr), allyl ester (OAll), and β -3-methylpent-3-yl (Mpe);

each PG⁵ is independently selected from trityl, *p*-monomethoxytrityl (Mmt), and 4-methyltrityl (Mtt);

each PG⁶ is trityl;

each PG⁷ is *tert*-butoxycarbonyl (Boc);

each PG⁸ is independently selected from *tert*-butoxycarbonyl (Boc), 2-chlorobenzyloxycarbonyl (Cl-Z), 4-methyltrityl (Mtt), *p*-monomethoxytrityl (Mmt), dimethoxytrityl (Dmt), allyloxycarbonyl (Alloc);

each PG⁹ is 9-fluorenylmethoxycarbonyl (Fmoc); and

each PG¹⁰ is independently selected from *tert*-butoxycarbonyl (Boc), 2-chlorobenzyloxycarbonyl (Cl-Z), 4-methyltrityl (Mtt), *p*-Monomethoxytrityl (Mmt), dimethoxytrityl (Dmt), allyloxycarbonyl (Alloc).

In some embodiments of the Formulas described herein:

each PG¹ and PG³ is *tert*-butyl;

each PG², PG⁵, and PG⁶ is trityl;

each PG⁴ is *tert*-butoxy;

each PG⁷, PG⁸, and PG¹⁰ is *tert*-butoxycarbonyl (Boc); and

each PG⁹ is 9-fluorenylmethoxycarbonyl (Fmoc).

As used herein, the term “reacting” is used as known in the art and generally refers to the bringing together of chemical reagents in such a manner so as to allow their interaction at the molecular level to achieve a chemical or physical transformation. In some embodiments, the reacting involves two reagents, wherein one or more equivalents of second reagent are used with respect to the first reagent. The reacting steps of the processes described herein can be conducted for a time and under conditions suitable for preparing the identified product.

The reactions of the processes described herein can be carried out in suitable solvents which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, *e.g.*, temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

The reactions described herein can be carried out at appropriate temperatures which can be readily determined by the skilled artisan. Reaction temperatures will depend on, for example, the melting and boiling points of the reagents and solvent, if present; the thermodynamics of the reaction (*e.g.*, vigorously exothermic reactions may need to be carried out at reduced

temperatures); and the kinetics of the reaction (*e.g.*, a high activation energy barrier may need elevated temperatures).

The reactions of the processes described herein can be carried out in air or under an inert atmosphere. Typically, reactions containing reagents or products that are substantially reactive with air can be carried out using air-sensitive synthetic techniques that are well known to the skilled artisan.

The processes described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means (*e.g.*, nuclear magnetic resonance spectroscopy (NMR), such as ^1H -NMR and/or ^{13}C -NMR) or by chromatography (*e.g.*, high performance liquid chromatography (HPLC)).

As used herein, the singular forms "a" and "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, *e.g.*, reference to "the compound" includes a plurality of such compounds and reference to "the assay" includes reference to one or more assays, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

Except as expressly defined otherwise, the present disclosure includes all tautomers of compounds detailed herein, even if only one tautomer is expressly represented (*e.g.*, both tautomeric forms are intended and described by the presentation of one tautomeric form where a pair of two tautomers may exist). For example, if reference is made to a compound containing an amide (*e.g.*, by structure or chemical name), it is understood that the corresponding imidic acid tautomer is included by this disclosure and described the same as if the amide were expressly recited either alone or together with the imidic acid. Where more than two tautomers may exist, the present disclosure includes all such tautomers even if only a single tautomeric form is depicted by chemical name and/or structure.

It is understood by one skilled in the art that this disclosure also includes any compound disclosed herein (*e.g.*, bulevirtide, a salt thereof, such as an acetic acid salt of bulevirtide as described herein) that may be enriched at any or all atoms above naturally occurring isotopic ratios with one or more isotopes such as, but not limited to, deuterium (^2H or D).

Disclosed are also compounds in which from 1 to n hydrogen atoms attached to a carbon atom may be replaced by a deuterium atom or D, in which n is the number of hydrogen atoms in the molecule. As known in the art, the deuterium atom is a non-radioactive isotope of the

hydrogen atom. Such compounds may increase resistance to metabolism, and thus may be useful for increasing the half-life of the compounds when administered to a mammal. *See, e.g.*, Foster, “Deuterium Isotope Effects in Studies of Drug Metabolism”, *Trends Pharmacol. Sci.*, 5(12):524-527 (1984). Such compounds are synthesized by means well known in the art, for example by employing starting materials in which one or more hydrogen atoms have been replaced by deuterium.

Examples of isotopes that can be incorporated into the disclosed compounds also include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine, and iodine, such as ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{13}N , ^{15}N , ^{15}O , ^{17}O , ^{18}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , ^{36}Cl , ^{123}I , and ^{125}I , respectively. Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. An isotopically-labeled compound of Formula I can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Examples as set out below using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

Additionally, in some embodiments, isotopically-labeled compounds of Formula I can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Examples as set out below using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

Compounds described herein may have chiral centers and/or geometric isomeric centers (E- and Z- isomers), and it is to be understood that all such optical, enantiomeric, diastereoisomeric and geometric isomers are encompassed. Where compounds are represented in their chiral form, it is understood that the embodiment encompasses, but is not limited to, the specific diastereomerically or enantiomerically enriched form. Where chirality is not specified but is present, it is understood that the embodiment is directed to either the specific diastereomerically or enantiomerically enriched form; or a racemic or scalemic mixture of such compound(s). As used herein, “scalemic mixture” is a mixture of stereoisomers at a ratio other than 1:1.

The following abbreviations may be used throughout the present application: alanine (Ala), asparagine (Asn), aspartic acid (Asp), glycine (Gly), glutamic acid (Glu), glutamine (Gln), histidine (His), leucine (Leu), myristic acid (Myr), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), valine (Val), 2-chlorobenzyloxycarbonyl (Cl-Z),

N,N-dimethylformamide (DMF), *tert*-butoxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), *tert*-butyl (tBu), trityl (Trt), *tert*-butoxy (OtBu), 2-phenylisopropyl ester (O-2-PhiPr), allyl ester (OAll), p-Monomethoxytrityl (Mmt), β -3-methylpent-3-yl (Mpe), 4-methyltrityl (Mtt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), tetramethylfluoroformamidinium hexafluorophosphate (TFFH), N'-tetramethylchloroformamidinium Hexafluorophosphate (TCFH), propylphosphonic anhydride (T3P), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) (PyAOP), 6-chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyClock), N,N'-diisopropylcarbodiimide (DIC), N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide-methyl-p-toluenesulfonate (CMC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), 6-chloro-1-hydroxybenzotriazole (Cl-HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), ethyl cyano(hydroxyimino)acetate (oxyma pure), N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMAc), and dimethyl sulfoxide (DMSO).

Methods of Use

The present application further provides a method of inhibiting an HBV and/or HDV infection in a subject in need thereof. In some embodiments, the method comprises administering to the subject an acetic acid salt of bulevirtide (*e.g.*, a therapeutically effective amount of an acetic acid salt of bulevirtide), as described herein.

The present application further provides a method of preventing a primary HBV and/or HDV infection in a subject in need thereof, comprising administering to the subject an acetic acid salt of bulevirtide as described herein.

The present application further provides methods of treating and/or preventing hepatitis B and/or D (*e.g.*, chronic hepatitis B and/or D) in a subject in need thereof, comprising administering to the subject an acetic acid salt of bulevirtide as described herein. In some embodiments, the acetic acid salt of bulevirtide is prepared according to one or more of the processes described herein.

The present application further provides methods of treating and/or preventing chronic hepatitis B in a subject in need thereof, comprising administering to the subject an acetic acid salt of bulevirtide as described herein. In some embodiments, the method provided herein is a method of treating chronic hepatitis B. In some embodiments, the method provided herein is a method of preventing chronic hepatitis B.

The present application further provides methods of treating and/or preventing chronic hepatitis D in a subject in need thereof, comprising administering to the subject an acetic acid salt of bulevirtide as described herein. In some embodiments, the method provided herein is a method of preventing chronic hepatitis B. In some embodiments, the method provided herein is a method of preventing chronic hepatitis B.

The present application further provides bulevirtide, or an acetic acid salt of bulevirtide, for use in any of the methods described herein. In some embodiments, the use further comprises preparing the acetic acid salt of bulevirtide according to one or more of the processes described herein.

The present application further provides bulevirtide, or an acetic acid salt of bulevirtide, for the preparation of a medicament for use in any of the methods described herein. In some embodiments, the use further comprises preparing the acetic acid salt of bulevirtide according to one or more of the processes described herein.

The terms “subject” and “subjects” refers to humans, domestic animals (*e.g.*, dogs and cats), farm animals (*e.g.*, cattle, horses, sheep, goats and pigs), laboratory animals (*e.g.*, mice, rats, hamsters, guinea pigs, pigs, rabbits, dogs, and monkeys), and the like.

As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results. For purposes of the present disclosure, beneficial or desired results include, but are not limited to, alleviation of a symptom and/or diminishment of the extent of a symptom and/or preventing a worsening of a symptom associated with a disease or condition. In one embodiment, “treatment” or “treating” includes one or more of the following: a) inhibiting the disease or condition (*e.g.*, decreasing one or more symptoms resulting from the disease or condition, and/or diminishing the extent of the disease or condition); b) slowing or arresting the development of one or more symptoms associated with the disease or condition (*e.g.*, stabilizing the disease or condition, delaying the worsening or progression of the disease or condition); and/or c) relieving the disease or condition, *e.g.*, causing the regression of clinical symptoms,

ameliorating the disease state, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival.

As used herein, “prevention” or “preventing” refers to a regimen that protects against the onset of the disease or disorder such that the clinical symptoms of the disease do not develop. Thus, “prevention” relates to administration of a therapy (e.g., administration of a therapeutic substance) to a subject before signs of the disease are detectable in the subject (e.g., administration of a therapeutic substance to a subject in the absence of detectable infectious agent (e.g., virus) in the subject). The subject may be an individual at risk of developing the disease or disorder, such as an individual who has one or more risk factors known to be associated with development or onset of the disease or disorder. Thus, the terms “preventing HBV infection” and “preventing HDV infection” refer to administering to a subject who does not have a detectable HBV or HDV infection an anti- HBV or HDV therapeutic substance (e.g., an acetic acid salt of bulevirtide as described herein). It is understood that the subject for preventative bulevirtide therapy may be an individual at risk of contracting the HBV and/or HDV virus. Further, it is understood that prevention may not result in complete protection against onset of the disease or disorder. In some instances, prevention includes reducing the risk of developing the disease or disorder. The reduction of the risk may not result in complete elimination of the risk of developing the disease or disorder.

As used herein, an “at risk” individual is an individual who is at risk of developing a condition to be treated. An individual “at risk” may or may not have detectable disease or condition, and may or may not have displayed detectable disease prior to the treatment of methods described herein. “At risk” denotes that an individual has one or more so-called risk factors, which are measurable parameters that correlate with development of a disease or condition and are known in the art. An individual having one or more of these risk factors has a higher probability of developing the disease or condition than an individual without these risk factor(s).

As used herein, the term “therapeutically effective amount” or “effective amount” refers to an amount that is effective to elicit the desired biological or medical response, including the amount of a compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease or to an amount that is effective to protect against the contracting or onset of a disease. The effective amount will vary depending on the compound, the disease, and its severity and the age, weight, etc., of the subject to be treated. The effective

amount can include a range of amounts. As is understood in the art, an effective amount may be in one or more doses, *i.e.*, a single dose or multiple doses may be required to achieve the desired treatment outcome. An effective amount may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable or beneficial result may be or is achieved. Suitable doses of any co-administered compounds may optionally be lowered due to the combined action (*e.g.*, additive or synergistic effects) of the compounds.

Pharmaceutical Formulations

Pharmaceutical compositions disclosed herein comprise a compound or salt (*e.g.*, an acetic acid salt of bulevirtide) described herein, together with one or more pharmaceutically acceptable excipients and optionally other therapeutic agents. Pharmaceutical compositions containing the active ingredient may be in any form suitable for the intended method of administration. In some embodiments, the compound or salt is prepared according to one or more of the processes described herein.

In some embodiments, the pharmaceutical compositions disclosed herein comprise bulevirtide or an acetic acid salt of bulevirtide prepared according to one or more of the processes described herein, together with one or more pharmaceutically acceptable excipients and optionally other therapeutic agents. Pharmaceutical compositions containing the active ingredient may be in any form suitable for the intended method of administration.

“Pharmaceutically acceptable” refers to compounds, salts, compositions, dosage forms and other materials which are useful in preparing a pharmaceutical composition that is suitable for veterinary or human pharmaceutical use.

“Pharmaceutically acceptable excipient” includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

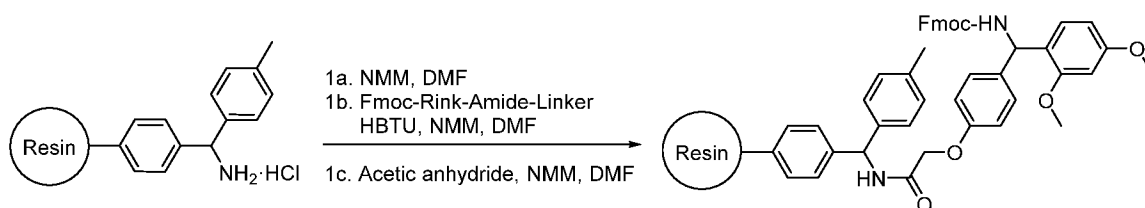
Pharmaceutical compositions provided herein may be prepared with conventional carriers (*e.g.*, inactive ingredient or excipient material) which may be selected in accord with ordinary practice. Tablets may contain excipients including glidants, fillers, binders and the like. Aqueous compositions may be prepared in sterile form, and when intended for delivery by

other than oral administration generally may be isotonic. All compositions may optionally contain excipients such as those set forth in the Rowe et al, Handbook of Pharmaceutical Excipients, 5th edition, American Pharmacists Association, 1986. Excipients can include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like.

EXAMPLES

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters which can be changed or modified to yield essentially the same results.

Example 1. Synthesis of Side-Chain Protected Peptide Resin



Step 1a. Swelling the MBHA resin

A reaction vessel was charged with MBHA resin (1 equiv amine functionality, scaling factor based on a known resin substitution between 0.28 to 0.60 mmol/g).

N,N-Dimethylformamide (DMF, approximately 5-8 mL of per gram of the resin) and N-methylmorpholine (NMM, 10 equiv.) were added. The resin was swelled for about 2 hours and the liquid was then drained. The resin was washed twice with DMF (about 2-3 times the volume of the resin bed). The resin was then used in the subsequent step.

Step 1b. 4-((2,4-Dimethoxyphenyl)(Fmoc amino)methyl)phenoxyacetic acid Coupling and Optional Recoupling

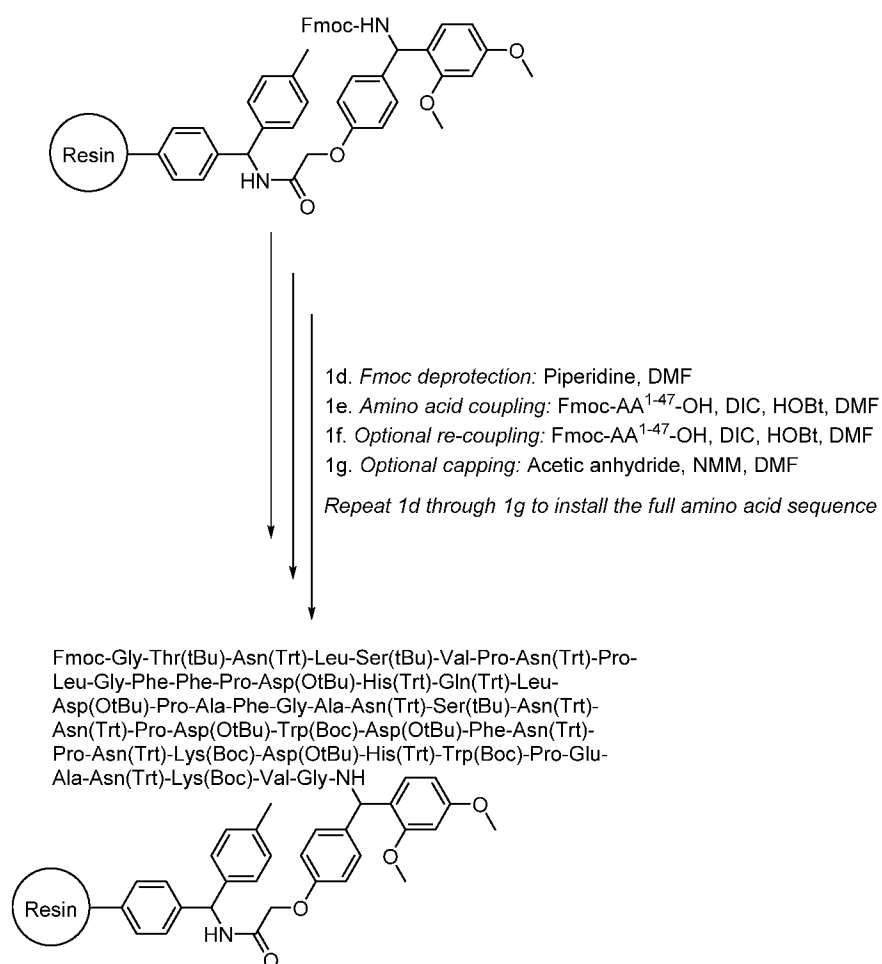
A reaction vessel was charged with 4-((2,4-dimethoxyphenyl)(Fmoc amino)methyl)phenoxyacetic acid (2 equiv.), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1.9 equiv), and excess DMF. The resulting solution was added to the swollen MBHA resin. N-Methyl Morpholine (NMM, 4 equiv.) and optional DMF was added to achieve a thin slurry

and the mixture was agitated for about 2 hours at about 22 °C. The liquid was drained from the resin. If the reaction was complete, then the resulting material is used for Step 1c.

If the reaction was incomplete, re-coupling may be performed one time according to the following procedures. In a reaction vessel, the resin bed was rinsed three times with DMF using 2 times the resin bed volume per rinse. In another vessel was combined 4-((2,4-dimethoxyphenyl)(Fmoc amino)methyl)phenoxyacetic acid (2 equiv.), HBTU (1.9 equiv), and excess DMF. The resulting solution was added to the resin bed. NMM (4 equiv.) and optional DMF was added to achieve a thin slurry and the mixture was agitated at about 22 °C for about 2 hours. The liquid was drained and the resin was used in the acetylation step (as described in step 1c).

Step 1c. Acetylation

In a reaction vessel, the resin bed was rinsed five times with DMF using approximately 2 times the resin bed volume per rinse. In another vessel was combined acetic anhydride, NMM, and DMF (10:6:84 v:v:v). A portion of the resulting solution was then added to the resin (approximately 2-3 times the volume of the resin bed). The mixture was agitated for about 30 min and the liquid was drained. The resin bed was rinsed five times with DMF using approximately 2-3 times the resin bed volume per rinse.

Example 2. Synthesis of Protected Bulevirtide**Step 1d. Removal of Fmoc Protecting Group**

The following procedure was used for removal of Fmoc protecting group of all the amino acids except for the removal of Fmoc-Asp³⁹(OtBu)-peptide, Fmoc-Asp³³(OtBu)-peptide, Fmoc-Asp³¹(OtBu)-peptide, Fmoc-Asp²⁰(OtBu)-peptide, and Fmoc-Asp¹⁶(OtBu)-peptide.

In a reaction vessel, the resin bed was treated with 20% piperidine in DMF (approximately 2-3 times the volume of the resin bed) and stirred for about 30 min. After draining the liquid, the resin was washed five times with DMF (approximately 2-3 times the resin bed volume per rinse) and drained.

The following procedure was used for removal of Fmoc group of Fmoc-Asp³⁹(OtBu)-peptide, Fmoc-Asp³³(OtBu)-peptide, Fmoc-Asp³¹(OtBu)-peptide, Fmoc-Asp²⁰(OtBu)-peptide, and Fmoc-Asp¹⁶(OtBu)-peptide:

In a reaction vessel, the resin bed was treated with 20% piperidine in DMF (approximately 2-3 times the volume of the resin bed) for about 10 min. After draining, the resin was then re-treated with 20% piperidine in DMF again (approximately 2-3 times the volume of

the resin bed) and stirred for about 10 min. After draining the liquid, the resin was washed five times with DMF (approximately 2-3 times the volume of the resin volume per rinse) and drained.

Steps 1e-1f. Amino Acid Coupling and Optional Recoupling

The following procedure was used for amino coupling of all amino acids except Fmoc-Gly-OH:

In a reaction vessel was combined the amino acid derivative (3 equiv.), 1-hydroxybenzotriazole (HOBt, 3 equiv.), and excess DMF and cooled to about 0 °C. To the mixture was added N,N-diisopropylcarbodiimide (DIC, 3 equiv.). The mixture was kept in an ice-bath for about 10 min and then added to the resin bed. The resulting mixture was stirred for about 2 hours at about 22 °C before the liquid was drained. If the reaction was incomplete, amino acid re-coupling was performed one time. If the reaction was complete, the resin was washed three times with DMF (approximately 2-3 times the volume of the resin bed) before proceeding to Fmoc deprotection and subsequent amino acid coupling or myristic acid coupling.

The following procedure was used for the amino acid coupling of Fmoc-Gly-OH:

In a reaction vessel was combined the amino acid derivative (3 equiv.), 1-hydroxybenzotriazole (HOBt, 3 equiv.), and excess DMF. To the mixture was added N,N-diisopropylcarbodiimide (DIC, 3 equiv.). The mixture was reacted at about 22 °C for about 5 min and then added to the resin bed. The resulting mixture was stirred for about 2 hours at about 22°C before the liquid was drained. If the reaction was incomplete, amino acid re-coupling was performed one time. If the reaction was complete, the resin was washed three times with DMF (approximately 2-3 times the volume of the resin bed) before proceeding to Fmoc deprotection and subsequent amino acid coupling or myristic acid coupling.

Optional Re-Coupling Steps

The following procedure was used, if necessary, for re-coupling of all amino acids except Fmoc-Gly-OH:

In a reaction vessel, the resin bed was rinsed three times with DMF using approximately 2 times the resin bed volume per rinse. In a separate reaction vessel was combined the amino acid derivative (3 equiv.), 1-hydroxybenzotriazole (HOBt, 3 equiv.), and excess DMF and cooled to about 0 °C. To the mixture was added N,N-diisopropylcarbodiimide (DIC, 3 equiv.). The

mixture was kept in an ice-bath for about 10 min and then added to the resin bed. The resulting mixture was stirred for about 2 hours at about 22 °C before the liquid was drained. If the reaction was incomplete, acetylation (step 1c) was performed. If the reaction was complete, the resin was washed three times with DMF (approximately 2-3 times the volume of the resin bed) before proceeding to Fmoc deprotection and subsequent amino acid coupling or myristic acid coupling.

The following procedure was used for the amino acid re-coupling of Fmoc-Gly-OH:

In a reaction vessel, the resin bed was rinsed three times with DMF using approximately 2 times the resin bed volume per rinse. In a separate reaction vessel was combine the amino acid derivative (3 equiv.), 1-hydroxybenzotriazole (HOBt, 3 equiv.), and excess DMF. To the mixture was added N,N-diisopropylcarbodiimide (DIC, 3 equiv.). The mixture was reacted at about 22 °C for about 5 min and then added to the resin bed. The resulting mixture was stirred for about 2 hours at about 22 °C before the liquid was drained. If the reaction was incomplete, acetylation (step 1c) is performed. If the reaction was complete, the resin is washed three times with DMF (approximately 2-3 times the volume of the resin bed) before proceeding to Fmoc deprotection and subsequent amino acid coupling or myristic acid coupling.

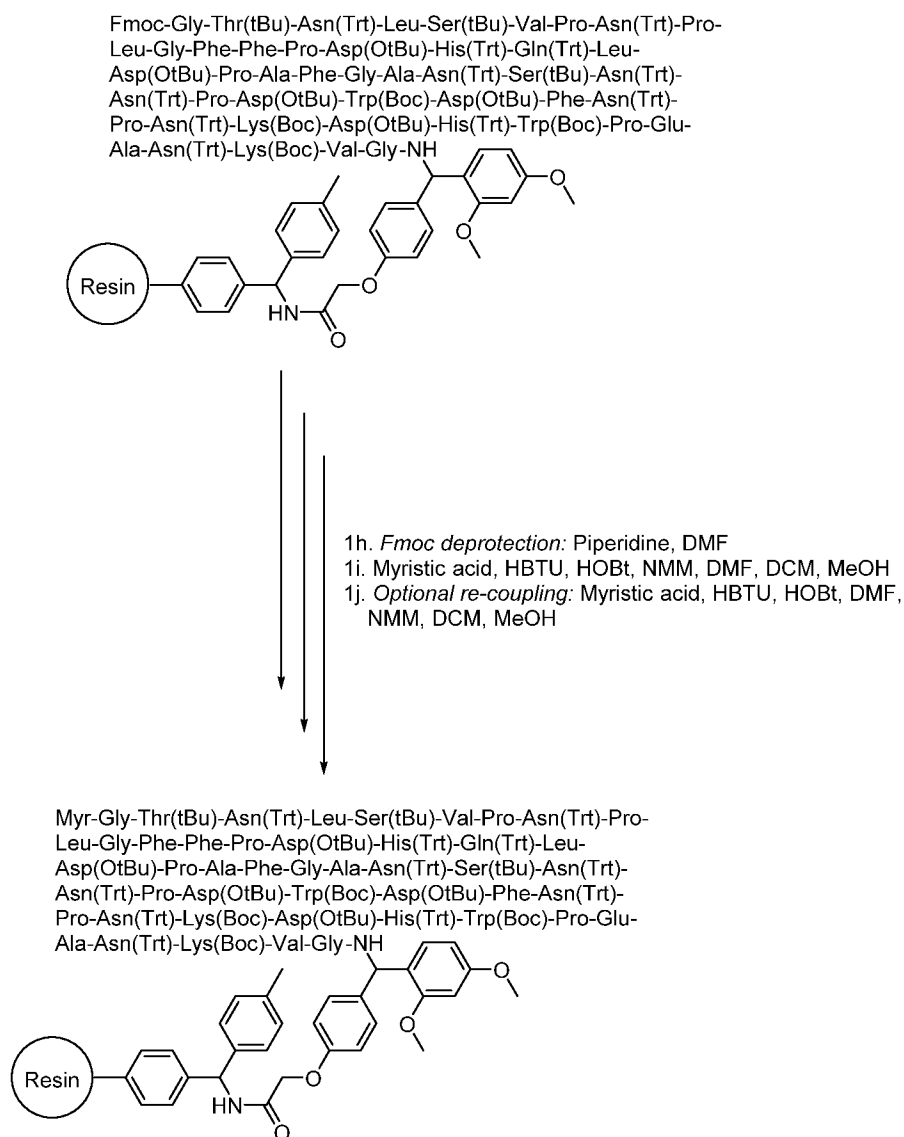
The above amino acid coupling (and optional re-coupling) steps were performed in the following sequence to afford the protected bulevirtide.

Table 1. Protected Amino Acid Coupling Order

Order	Protected Amino acid	Order	Protected Amino Acid	Order	Protected Amino Acid
1	Fmoc-Gly ⁴⁸ -OH	17	Fmoc-Trp ³² (Boc)-OH	33	Fmoc-Asp ¹⁶ (OtBu)-OH
2	Fmoc-Val ⁴⁷ -OH	18	Fmoc-Asp ³¹ (OtBu)-OH	34	Fmoc-Pro ¹⁵ -OH
3	Fmoc-Lys ⁴⁶ (Boc)-OH	19	Fmoc-Pro ³⁰ -OH	35	Fmoc-Phe ¹⁴ -OH
4	Fmoc-Asn ⁴⁵ (Trt)-OH	20	Fmoc-Asn ²⁹ (Trt)-OH	36	Fmoc-Phe ¹³ -OH
5	Fmoc-Ala ⁴⁴ -OH	21	Fmoc-Asn ²⁸ (Trt)-OH	37	Fmoc-Gly ¹² -OH
6	Fmoc-Glu ⁴³ (OtBu)-OH	22	Fmoc-Ser ²⁷ (tBu)-OH	38	Fmoc-Leu ¹¹ -OH
7	Fmoc-Pro ⁴² -OH	23	Fmoc-Asn ²⁶ (Trt)-OH	39	Fmoc-Pro ¹⁰ -OH
8	Fmoc-Trp ⁴¹ (Boc)-OH	24	Fmoc-Ala ²⁵ -OH	40	Fmoc-Asn ⁹ (Trt)-OH
9	Fmoc-His ⁴⁰ (Trt)-OH	25	Fmoc-Gly ²⁴ -OH	41	Fmoc-Pro ⁸ -OH
10	Fmoc-Asp ³⁹ (OtBu)-OH	26	Fmoc-Phe ²³ -OH	42	Fmoc-Val ⁷ -OH
11	Fmoc-Lys ³⁸ (Boc)-OH	27	Fmoc-Ala ²² -OH	43	Fmoc-Ser ⁶ (tBu)-OH
12	Fmoc-Asn ³⁷ (Trt)-OH	28	Fmoc-Pro ²¹ -OH	44	Fmoc-Leu ⁵ -OH
13	Fmoc-Pro ³⁶ -OH	29	Fmoc-Asp ²⁰ (OtBu)-OH	45	Fmoc-Asn ⁴ (Trt)-OH

14	Fmoc-Asn ³⁵ (Trt)-OH	30	Fmoc-Leu ¹⁹ -OH	46	Fmoc-Thr ³ (tBu)-OH
15	Fmoc-Phe ³⁴ -OH	31	Fmoc-Gln ¹⁸ (Trt)-OH	47	Fmoc-Gly ² -OH
16	Fmoc-Asp ³³ (OtBu)-OH	32	Fmoc-His ¹⁷ (Trt)-OH		

Example 3. Myristic Acid Coupling and Optional Re-Coupling



Step 1h. Fmoc Deprotection

In a reaction vessel, the resin bed was treated with 20% piperidine in DMF (approximately 2-3 times the volume of the resin bed) and stirred for about 30 min. After draining the liquid, the resin was washed five times with DMF (approximately 2-3 times the resin bed volume per rinse) and drained.

Step. 1i. Myristic Acid Coupling

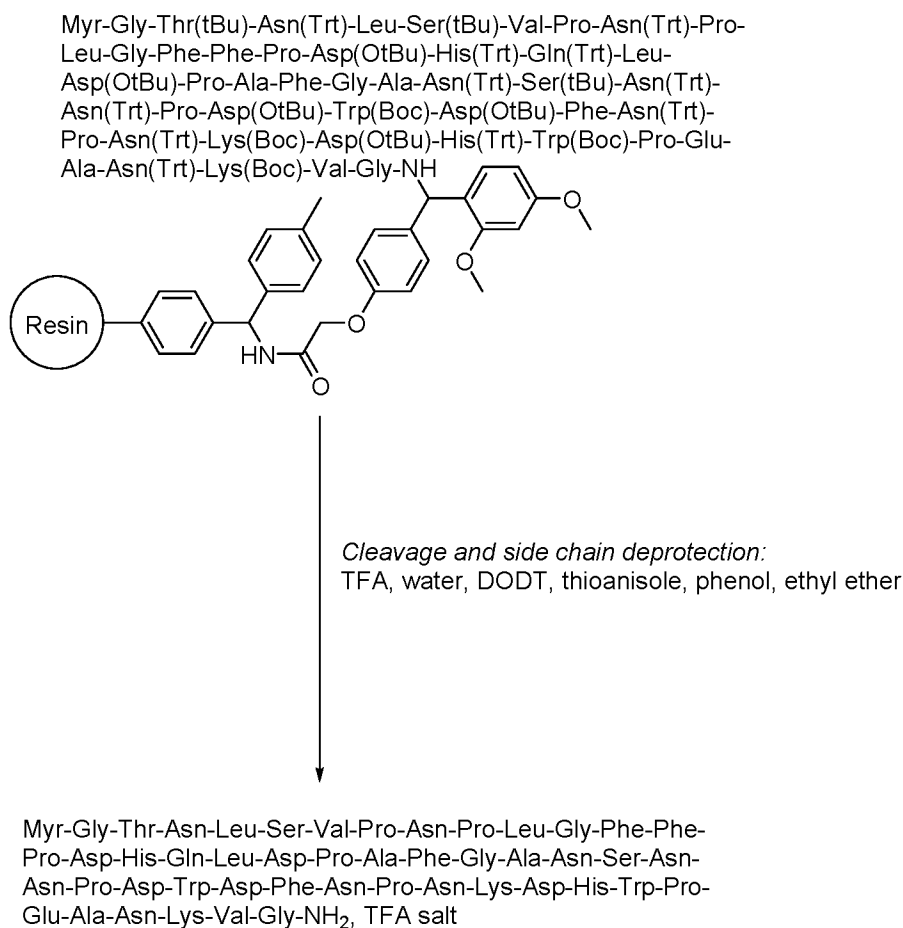
In a separate reaction vessel was combined myristic acid (3 equiv.), HBTU (2.85 equiv) and HOBt (3 equiv) in excess DMF. The mixture was added to the resin bed, followed by NMM (6 equiv) and optional DMF to achieve a thin slurry. The resulting mixture was stirred for about 2 hours at about 22 °C before the liquid was drained. If the reaction was incomplete, the myristic acid re-coupling was performed one time. If the reaction was complete, the resin was rinsed three times with DMF, twice with DCM, and three times with MeOH using approximately 2 times the resin bed volume per rinse.

Step 1j. Optional Myristic Acid Re-Coupling

In a reaction vessel, the resin bed was rinsed three times with DMF using approximately 2 times the resin bed volume per rinse. In a separate reaction vessel was combined myristic acid (3 equiv.), HBTU (2.85 equiv) and HOBt (3 equiv) in excess DMF. The mixture was added to the resin bed, followed by NMM (6 equiv) and optional DMF to achieve a thin slurry. The resulting mixture was stirred for about 2 hours at about 22 °C before the liquid was drained. If the reaction was incomplete, acetylation is performed. If the reaction was complete, rinse the resin three times with DMF, twice with DCM, and three times with MeOH using approximately 2 times the resin bed volume per rinse.

Step 1k. Drying Under Vacuum

The resulting peptide resin was dried under vacuum until the protected peptide resin reaches a constant weight to obtain the protected resin: Myristoyl-Gly-Thr(tBu)-Asn(Trt)-Leu-Ser(tBu)-Val-Pro-Asn(Trt)-Pro-Leu-Gly-Phe-Phe-Pro-Asp(OtBu)-His(Trt)-Gln(Trt)-Leu-Asp(OtBu)-Pro-Ala-Phe-Gly-Ala-Asn(Trt)-Ser(tBu)-Asn(Trt)-Asn(Trt)-Pro-Asp(OtBu)-Trp(Boc)-Asp(OtBu)-Phe-Asn(Trt)-Pro-Asn(Trt)-Lys(Boc)-Asp(OtBu)-His(Trt)-Trp(Boc)-Pro-Glu(OtBu)-Ala-Asn(Trt)-Lys(Boc)-Val-Gly-Rink Amide MBHA Resin.

Example 4. Synthesis of Bulevirtide Trifluoroacetic Acid Salt**Step. 2**

In a reaction vessel was charged the peptide resin (1 equiv., scaling factor). In a separate reaction vessel, combine trifluoroacetic acid (TFA), water, 2,2'-(ethylenedioxy)diethanethiol (DODT), thioanisole, and phenol in a ratio of 87.5:5.0:2.5:2.5:2.5 (v:v:v:v:v) (10 to 12 mL per gram of peptide resin). The solution was cooled and then added to the peptide resin and stirred about 3 hours at about 20 °C. The reaction mixture was filtered, and the resin was washed twice with TFA (1.0 mL per gram of resin). The filtrates were combined and treated with pre-chilled ether while stirring, in a ratio of filtrate to ether of 1/(10-12) (v/v). The mixture was held at about 22 °C for about 30 minutes and filtered. The precipitate was rinsed three times with excess pre-chilled ethyl ether and then dried under vacuum until the crude peptide reaches a constant weight.

Example 5. Synthesis of Bulevirtide Acetic Acid Salt

Myr-Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-
Phe-Pro-Asp-His-Gln-Leu-Asp-Pro-Ala-Phe-Gly-Ala-Asn-
Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-
Asp-His-Trp-Pro-Glu-Ala-Asn-Lys-Val-Gly-NH₂, TFA salt

↓
3a. *Purification*: water, acetonitrile, TFA
3b. *Salt exchange*: water, acetonitrile, ammonium acetate,
acetic acid, triethylamine, phosphoric acid
4. *Lyophilization*

Myr-Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-Phe-
Pro-Asp-His-Gln-Leu-Asp-Pro-Ala-Phe-Gly-Ala-Asn-Ser-Asn-
Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-Asp-His-Trp-Pro-
Glu-Ala-Asn-Lys-Val-Gly-NH₂, acetate salt

Step 3a. Filtration and Trifluoroacetic Acid Purification

The crude peptide obtained from the process described in Example 4 was dissolved at a concentration of 20 mg/mL in a mixture of Buffer J and acetonitrile (70:30 v/v) and was filtered with a 0.45 µm filter membrane.

A Luna C18(3) resin (10 µm in particle diameter) or equivalent column was equilibrated with a mixture of Buffer J and Buffer K, loaded with the dissolved crude peptide, equilibrated with a mixture of Buffer J and Buffer K, and eluted with a solvent gradient with the composition and time as described in Table 3. The composition of Buffers J and K are provided in Table 2.

Table 2. Solution Composition for TFA Purification

Item	Composition
Buffer J	1 mL TFA per 1 L H ₂ O
Buffer K	1 mL TFA per 800 mL acetonitrile and 200 mL H ₂ O

Table 3. Elution Gradient for TFA Purification

Time (min)	Buffer J (%)	Buffer K (%)
0	57	43
60	37	63

Step 3b. Salt Exchange Chromatography

A Luna C18(3) resin (10 µm in particle diameter) or equivalent column was equilibrated with water and was loaded with the fractions obtained by the TFA purification step. The

solutions used for the salt exchange steps are described in Table 4. After loading, the column was equilibrated with Buffer L and Buffer M, equilibrated with 77 g/L AA, equilibrated with mobile phase A and mobile phase B, and eluted with a solvent gradient with the composition and time as described in Table 5.

Table 4. Solution Composition for Salt Exchange

Item	Composition
Mobile phase A	3 mL AcOH (acetic acid) per 1 L H ₂ O
Mobile phase B	3 mL AcOH per solution of 800 mL acetonitrile and 200 mL H ₂ O
Buffer L	10 mL TEA and 10 mL H ₃ PO ₄ per 1 L H ₂ O
Buffer M	200 mL Buffer L per 800 mL acetonitrile
77 g/L ammonium acetate solution	77 g/L ammonium acetate per 900 mL H ₂ O and 100 mL acetonitrile

Table 5. Elution Gradient for Salt Exchange

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	63	37
40	13	87

Step 4. Lyophilization of the Bulevirtide Acetic Acid Salt

The fractions obtained from the process described in Step 3b were filtered through a 0.2 µm filter membrane and transfer to lyophilization plates with a plate volume of not more than 2.5 L at a height of approximately 1.5 cm. The filtrates were frozen and freeze-dried according to the parameters described in Table 6. The resulting solids obtained from the lyophilization were then blended to afford the desired bulevirtide acetic acid salt.

(C₂₄₈H₃₅₅N₆₅O₇₂) 5398.9, Observed: 5399.2

Table 6. Freeze-Drying Parameters

Experimental step	Temperature	Time
Freezing (step 2)	Adjust temperature to -45±2 °C over 30 minutes	Hold for NLT 180 minutes
First-drying (step 3)	Adjust temperature from -45±2 °C to 20±2 °C over 60 minutes	Hold for NLT 36 hours

Parsing-desiccation (step 4)	Adjust temperature from 20 ± 2 °C to 25 ± 2 °C over 30 minutes	Hold for NLT 12 hours
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Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference, including all patent, patent applications, and publications, cited in the present application is incorporated herein by reference in its entirety.

WHAT IS CLAIMED IS:

1. A process of preparing an acetic acid salt of the compound of Formula I:
Myr-Gly-Thr-Asn-Leu-Ser-Val-Pro-Asn-Pro-Leu-Gly-Phe-Phe-Pro-Asp-His-Gln-Leu-
Asp-Pro-Ala-Phe-Gly-Ala-Asn-Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe-Asn-Pro-Asn-Lys-
Asp-His-Trp-Pro-Glu-Ala-Asn-Lys-Val-Gly-NH₂

I

comprising reacting a tetrafluoroboric acid salt of the compound of Formula I with acetic acid.

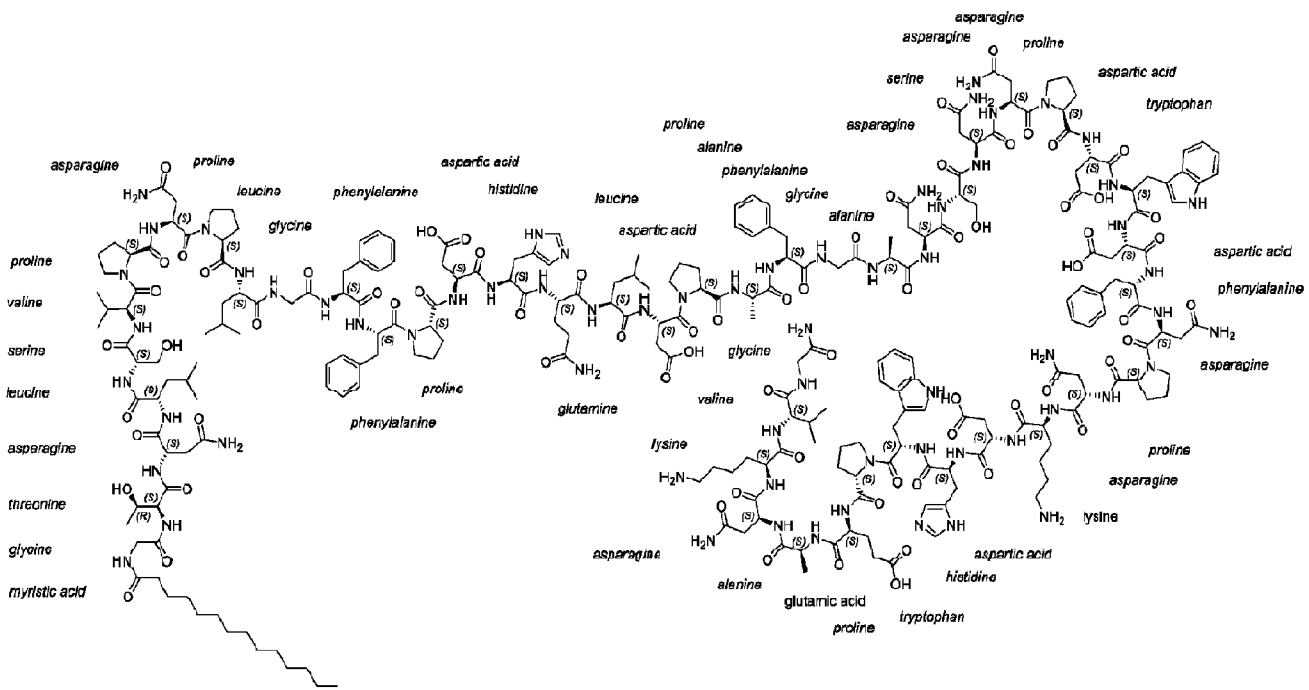


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/021120

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 115 746 102 A (SHENZHEN JX BIO PHARMACEUTICAL CO LTD) 7 March 2023 (2023-03-07) embodiment 26 -----	1
X	CN 115 746 104 A (SHENZHEN JX BIO PHARMACEUTICAL CO LTD) 7 March 2023 (2023-03-07) example 37 -----	1
X	WO 2009/092396 A1 (UNIVERSITAETSKLINIKUM HEIDELBE [DE]; MIER WALTER [DE] ET AL.) 30 July 2009 (2009-07-30) claims; examples -----	1
X	US 8 603 975 B2 (YAMANAKA TOSHIO [JP]; OHKI HIDENORI [JP] ET AL.) 10 December 2013 (2013-12-10) claims; examples -----	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

6 June 2024

Date of mailing of the international search report

19/06/2024

Name and mailing address of the ISA/

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West, Nuki

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/021120

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2024/021120

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 115746102	A	07-03-2023	NONE
CN 115746104	A	07-03-2023	NONE
WO 2009092396	A1	30-07-2009	AU 2009207806 A1 30-07-2009
		BR PI0906710 A2	04-08-2015
		CA 2713189 A1	30-07-2009
		CA 3018860 A1	30-07-2009
		CN 101970464 A	09-02-2011
		EP 2247605 A1	10-11-2010
		ES 2441118 T3	31-01-2014
		HK 1150168 A1	04-11-2011
		JP 5791900 B2	07-10-2015
		JP 2011510035 A	31-03-2011
		JP 2015086227 A	07-05-2015
		KR 20100136960 A	29-12-2010
		RU 2010135519 A	27-02-2012
		US 2011020397 A1	27-01-2011
		WO 2009092396 A1	30-07-2009
		WO 2009092611 A1	30-07-2009
		ZA 201005331 B	28-04-2011
US 8603975	B2	10-12-2013	AU 2008250254 A1 20-11-2008
		BR PI0810203 A2	14-10-2014
		CA 2684557 A1	20-11-2008
		CN 101687012 A	31-03-2010
		EP 2150269 A1	10-02-2010
		EP 2455098 A1	23-05-2012
		ES 2396170 T3	19-02-2013
		JP 5459545 B2	02-04-2014
		JP 2010526024 A	29-07-2010
		KR 20100017547 A	16-02-2010
		RU 2009144538 A	10-06-2011
		TW 200911839 A	16-03-2009
		US 2010120672 A1	13-05-2010
		WO 2008139986 A1	20-11-2008