

AROMATIC BORON-CONTAINING COMPOUNDS AND RELATED INSULIN ANALOGS

CROSS REFERENCES TO RELATED APPLICATIONS

- 5 This application claims priority from U.S. Provisional Patent Application No. 63/364,893, filed May 18, 2022, which is incorporated herein by reference.

FIELD OF THE DISCLOSURE

- 10 The present disclosure relates to novel compounds that include one or more aromatic boron-containing groups. The disclosure relates to the use of the novel compounds to bind glucose. The present disclosure further relates to kits and the use of the compounds and/or pharmaceutical compositions comprising the disclosed compounds for the treatment of disorders characterized by elevated glucose levels, such as hyperglycemia, type 2 diabetes, impaired glucose tolerance, and type 1 diabetes, as well as obesity, metabolic syndromes, neurological
15 diseases, mood disorders, and psychiatric disorders.

SEQUENCE LISTING

- The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file titled 02021_00153-00304_SL.xml, created April 7,
20 2023, and is 6,324,718 bytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

- Boronic acids are generally considered Lewis acids that have a tendency to bind to
25 hydroxyls, because, as Lewis acids, boronic acids can form complexes with Lewis bases such as, for example, hydroxide anions. Thus, molecules containing boronates including boronic acids have a general tendency to bind hydroxyl groups. This binding tendency can be used for detection of hydroxyl-containing groups by boronated labeling reagents wherein the boronate groups bind to the hydroxyls and, depending on the solvent and buffer conditions, the boronates
30 can form hydrolysable boronate-ester bonds to the hydroxyl groups of hydroxyl containing molecules, such as the hydroxyl groups present in diols (e.g., glucose). Although boron-containing compounds can bind to diol containing molecules, achieving selectivity using boron-containing compounds has been challenging because of their ability to bind various diols, including cis diols, to varying degrees. While improved binding affinity of boron-containing
35 compounds towards a specific vicinal diol of interest may be achieved, this may result in a loss of selectivity.

Glucose is the main fuel for the human body, and blood glucose values are tightly regulated in healthy individuals. For example, between meals, blood glucose is near 5 mmol/L (mM), and when blood glucose concentrations rise after a meal, the value is quickly adjusted back toward 5 mM by the action of insulin. The hormone insulin is secreted from pancreatic beta cells, and when insulin binds to insulin receptors on cells in the body (for example muscle and fat), the cells are stimulated to absorb glucose by translocation of glucose transporters from storage vesicles to the cell surface (GLUT4). (Huang, S.H. et al. (2007) Cell Metabolism 5:237-252.)

People with diabetes may lose their ability to produce insulin due to autoimmunity against beta cells (type 1) or have low sensitivity to insulin in combination with impaired insulin secretion (type 2). For example, those with type 1 diabetes may rely on multiple daily insulin injections, both for basal coverage, typically once a day, and with meals (bolus) to control their glucose levels. (Polonsky, K.S. et al. (1988) The Journal of Clinical Investigation 81: 442-448.) Because glucose values can fluctuate unpredictably, perfect insulin dosing day after day is extremely difficult. Indeed, despite many technological advances in diabetes treatment, researchers are currently observing, partly due to lifestyle problems, a worsening of long-term glucose control and/or overall metabolic health.

Glucose sensors and glucose sensing insulin analogs are known in the art. *See*, e.g., WO 2016/179568 A1 and WO 2021/202802 A1. However, there is a need in the art to develop glucose sensors and glucose sensing insulins that have improved properties, such as binding to the insulin receptor and be proportionately responsive to different glucose concentrations and provide a graded and reversible response to changes in glucose levels under physiological conditions.

Thus, there is an unmet medical need for novel compounds, such as compounds which bind glucose and may be utilized in glucose-responsive insulin analogues/conjugates, that can control blood glucose levels.

Here, we disclose glucose sensors and glucose sensing insulin analogs that have improved properties, such as improved affinity for glucose at relatively low concentrations, improved affinity for the insulin receptor, and improved activation of the insulin receptor.

SUMMARY OF THE DISCLOSURE

The present disclosure provides, according to some embodiments, novel Z1c compounds (e.g., FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226) comprising aromatic boron-containing functionalities (e.g., F2, F5, and F10). In some embodiments, the present disclosure provides indirect linkers represented by

Formula (X'')_{n1} where X'' is as defined herein and each n1 is 1, 2, 3, 4, or 5 (e.g., as exemplified in Formulae FL3, FL5, FL5A, FL5B, and FL20-FL75). In some embodiments, the present disclosure provides novel Z1c-Linker(s) (e.g., Formulae FFL-1 to FFL-101) comprising aromatic boron-containing functionalities (e.g., F2, F5, and F10).

5 In some embodiments, the present disclosure provides compounds that comprise a drug substance (e.g., X1) and at least one Z1c with aromatic boron-containing functionalities. In some embodiments, the compounds disclosed comprise one or more scaffolds (e.g., FF scaffolds and Z1c-Linker scaffolds as exemplified in Formulae FFL-1 to FFL-101). In some embodiments, the compounds comprise a drug substance. In at least some embodiments, the drug substance is a
10 polypeptide or a small-molecule.

In at least some embodiments, the disclosed compounds have at least two aromatic boron-containing functionalities. In some embodiments, the compounds are selective towards specific sugars, such as glucose, while showing reduced affinity for other sugars and wherein there is at least two aromatic boron-containing functionalities in the aromatic boron-containing
15 portion of the compounds. In certain embodiments, the aromatic boron-containing portion may be covalently conjugated, directly or indirectly, to a drug substance comprising an amine, a drug substance that is covalently conjugated to an amine containing linker, or NH₂, or OH (e.g., where X1 is NH₂ or OH). In some embodiments, the drug substance is covalently conjugated to an amine containing linker and the amine group is conjugated to the aromatic boron-containing
20 portion (Z1c). In certain embodiments, the aromatic boron-containing portion (Z1c) has an architecture comprising of tethering groups (FF formulae) and aromatic boron containing groups that collectively make the aromatic boron-containing portion of the disclosed compounds. In some embodiments, the aromatic boron-containing portion (Z1c) is covalently conjugated to an indirect linker (e.g., Formulae FL3, FL5, FL5A, FL5B, and FL20-FL75) and has an architecture
25 comprising tethering groups (FF formulae), and aromatic boron containing groups that collectively make the aromatic boron-containing portion of the disclosed compounds.

In some embodiments, one or more Z1c may be linked (e.g., conjugated, connected) to a drug substance (e.g., X1) via one or more indirect linkers (e.g., Formulae FL3, FL5, FL5A, FL5B, and FL20-FL75 and/or one or more L- or D-amino acids connected together using amide
30 or peptide bonds). In at least some embodiments, rotational constraining of the boron functionalities via the tethering group (e.g., FF formulae) enhances the binding affinity of the compounds (e.g., conjugates) towards specific diols (such as glucose) and away from other diols in the body and thereby provides selectivity to specific diols. In some embodiments, the compounds may exhibit therapeutic pharmacokinetics and/or pharmacodynamics in response to
35 endogenous and/or exogenous small molecules in the body, such as glucose. Changes in the

physiological concentration of glucose, may result in the activation and/or release of the drug molecule or conversely, may result in the deactivation and/or sequestering of the drug molecule (e.g., a peptide hormone), and/or modulation of the activity of the drug molecule, through interaction of glucose with the boron-containing architectures conjugated to the drug molecule.

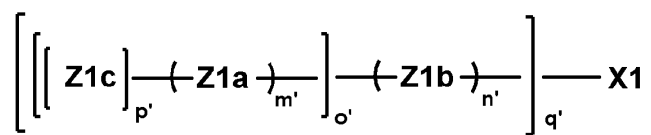
5 In some embodiments, the present disclosure provides a compound comprising X1 and one or more Z1c, or a tautomer, stereoisomer or a mixture of stereoisomers, or a pharmaceutically acceptable salt, or hydrate, or isotopic derivative thereof, wherein:

X1 comprises:

- i. a drug substance comprising an amine;
- 10 ii. an amine configured to be covalently conjugated to a drug substance; or
- iii. NH₂ or OH (e.g. X1 is NH₂ or OH);

wherein each Z1c is independently selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, 15 FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226; and wherein each Z1c is covalently conjugated, directly or indirectly, to an amine in X1 or to NH₂ which X1 is NH₂ or to OH when X1 is OH.

In some embodiments, the present disclosure provides a compound represented by Formula I, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt 20 thereof:



(Formula I)

wherein:

X1 comprises:

- 25 i. a drug substance comprising an amine;
- ii. an amine configured to be covalently conjugated to a drug substance; or
- iii. NH₂ or OH (e.g. X1 is NH₂ or OH);

each Z1a independently comprises 1 to 50 amino acids connected together using 30 amide or peptide bonds;

each Z1b is independently a small-molecule linker;

each Z1c is independently selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B,

FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226, wherein each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH;

each m' is 0 or 1;

5 each n' is 0;

each o' is 1;

each p' is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and

each q' is 1, 2, 3, 4, or 5, wherein when p' or q' is 2 or more, each corresponding

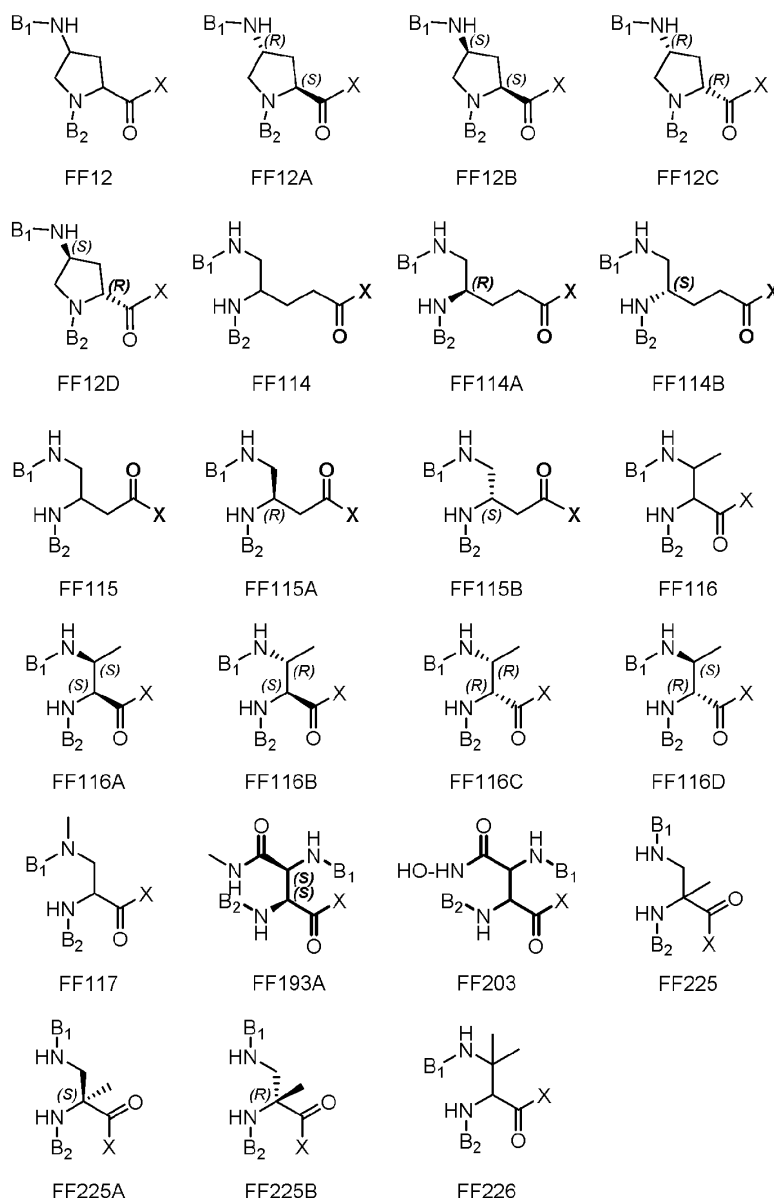
Z1c is independently selected and may be the same or different and wherein when q' is 2 or

10 more, each corresponding Z1a is independently selected and may be the same or different;

when m'=0 and n'=0, each Z1c is covalently conjugated either directly or via an indirect linker to an amine of X1 or to NH₂ when X1 is NH₂, or to OH when X1 is OH, and for m'=1 each Z1a is covalently conjugated via an amide or peptide bond to X1 and each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to X1;

15 and

wherein in Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226 are:



wherein X represents a point of covalent attachment either directly to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1; and wherein B₁ and B₂, which may be identical or different, each independently represent an aromatic boron-containing group; and wherein one or more positions of the compound of Formula I may comprise an isotope (e.g., ²H or ¹⁴C).

In some embodiments, X1 is OH or NH₂. In some embodiments, the compound disclosed herein (e.g., Formula I, Formula II) is conjugated directly or via an optional linker to a drug substance.

In some embodiments, the compound of Formula I comprises:

at least one Z1c covalently conjugated via the indirect linker to X1 or to Z1a, wherein the indirect linker is represented by Formula (X'')_{n1},

each n1 is independently selected from 1, 2, 3, 4, and 5, and

5 each X'' is independently selected from:

(i) an L- or D-amino acid, wherein an amine functional group of the L- or D-amino acid is covalently conjugated, directly or indirectly, to Z1c and an acid functional group of the L- or D- amino acid is conjugated, directly or indirectly, to X1 or to Z1a; and

10 (ii) Formulae FL3, FL5, FL5A, FL5B, FL20-FL68, and FL71-FL75 which comprise substituents R'' and Z'', and Formulae FL69 and FL70, which comprise substituents R'', Z'', A', and A'', and stereoisomers thereof;

wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

15 Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, an alkyl group (e.g., C₁-C₆alkyl group), a saturated fatty acid, an unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a haloalkyl group (e.g., C₁-C₆haloalkyl group), an aryl group, and a heteroaryl group; and

20 A'' is selected from one of an alkyl group (e.g., C₁-C₆alkyl group), a substituted acyl group, acyl group terminating in an acid group, a saturated fatty acid, an unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a haloalkyl group (e.g., C₁-C₆ haloalkyl group), an aryl group, and a heteroaryl group;

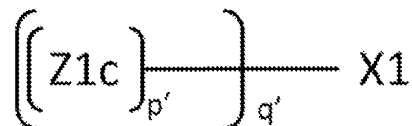
p is 1, 2, 3, 4, or 5,

q is 1, 2, 3, 4, or 5, and

25 any primary amine is optionally acetylated or alkylated.

In some embodiments, n1 is 1.

In some embodimentsof the compound of Formula I, m'=0, n'=0, o'=0, and the compound is represented by Formula IC:



; wherein:

30

(Formula IC)

each p' is 1, 2, 3, or 4; and

each q' is 1, 2, 3, 4, or 5.

In some embodiments, each B_1 and B_2 is Formula F2.

In some embodiments, each Z1c is covalently conjugated to an indirect linker and each of the Z1c and the indirect linker in combination is selected from Formulae FFL-1 to FFL-101,
5 which comprise substituent X; wherein X represents a point of covalent attachment either directly to an amine of X1 or to NH_2 when X1 is NH_2 or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1 or to an amine of Z1a.

In some embodiments, X1 is a polypeptide comprising an insulin receptor agonist having an A-chain and a B-chain.

10 In some embodiments, at least one Z1c comprises an alpha methyl group or a beta methyl group.

Also disclosed herein are methods of treatment. The disclosed methods may comprise treating a subject in need thereof, wherein the subject has a disease characterized by elevated glucose levels. The methods may comprise administering to a subject in need thereof a
15 compound of Formula I, a pharmaceutically acceptable salt of a compound of Formula I, a pharmaceutical composition comprising a compound of Formula I, or a pharmaceutical composition comprising a pharmaceutically acceptable salt of a compound of Formula I.

Also disclosed herein is a method of treating or preventing an endocrine and/or metabolic disease, in a subject in need thereof, comprising administering to said subject a compound of
20 Formula I, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of Formula (I) comprises a drug substance, wherein the drug substance is a polypeptide human hormone, an endocrine hormone, insulin, human insulin, glucagon, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide,
25 glucose-dependent insulinotropic polypeptide, a hybrid peptide comprising sequences from two or more human polypeptide hormones, or an analogue thereof.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, is administered simultaneously with a second agent or
30 therapy. In some embodiments, the second agent is a drug substance, wherein the drug substance is a polypeptide human hormone, an endocrine hormone, insulin, human insulin, glucagon, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide, glucose-dependent insulinotropic polypeptide, a hybrid peptide comprising sequences from two or more human polypeptide hormones, or an analogue thereof. In some embodiments, the compound of
35 Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition

comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, is administered concurrently with the second agent or therapy. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, is administered sequentially with the second agent or therapy (e.g., where a compound of Formula (I) is administered initially and the second agent or therapy is administered subsequently or where the second agent or therapy is administered initially and the compound of Formula (I) is administered subsequently). In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, is administered in the same unit dosage form as the second agent or therapy. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, is administered in a different unit dosage form from the second agent or therapy.

In at least one embodiment, the pharmaceutical composition of the present disclosure may be for use in (or in the manufacture of medicaments for) the treatment of diseases characterized by elevated glucose in a subject such as diabetes. In at least one embodiment, a therapeutically-effective amount of a pharmaceutical composition of the present disclosure may be administered to a subject diagnosed with diabetes or metabolic disease. In at least one embodiment, the pharmaceutical composition of the present disclosure comprises at least one compound disclosed herein (e.g., Formula I, Z1c, Z1c-Linker) and at least one additional component selected from pharmaceutically acceptable carriers, pharmaceutically acceptable vehicles, and pharmaceutically acceptable excipients.

In some embodiments, the disclosed compounds comprise an insulin receptor agonist selected from insulin and an insulin analogue (e.g., where X1 is insulin or an insulin analogue). In some embodiments, X1 comprises an insulin having an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, 24051, and 24052, and the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24061, 24062, 24063, 24064, and 25000–25397.

In some embodiments, X1 comprises an insulin having an A-chain and a B-chain, wherein:

the A-chain comprises a sequence selected from SEQ ID NOs 24051 and 24052,
the B-chain comprises a sequence selected from SEQ ID NOs 25095, 25229, 25232, 25236, 25305, 25308, 25312, and 25380–25397;

each Z1c is independently selected from FF12A, FF12B, FF12C, FF12D, FF116A, FF116B, FF116C, FF116D, and covalently conjugated either directly or via the indirect linker to one or more lysine residues in X1;

B₁ and B₂ is each F2; and

5 the indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL20-FL75, and stereoisomers thereof.

In some embodiments, the compound is selected from an insulin comprising an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 24501 and 24502; and wherein the B-chain comprises a sequence selected from SEQ ID NOs 25229, 25232, 10 25305, 25308, 25312, 25236, 25095, and 25380-25397.

In some embodiments, the present disclosure provides a compound represented by Formula II, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt thereof:

Z1c-Linker

15 (Formula II)

wherein the Z1c-Linker is selected from Formulae FFL-1 to FFL-101;

wherein X is selected from a leaving group (e.g., halogen, a N-hydroxysuccinimide (NHS) group, a 2,3,5,6-tetrafluorophenol (TFP) group, a pentafluorophenol (Pfp) group, or a sulfonate ester), NH₂, OH, and H; and

20 wherein B₁ and B₂, which may be identical or different, each independently represents an aromatic boron-containing group.

In some embodiments of the compound of Formula I, X1 comprises an insulin receptor agonist that is insulin or an insulin analogue having an A-chain and a B-chain. In some embodiments, one or more lysine residues and/or the N-terminus of the A- or B-chain are covalently conjugated as described by Formula I. In some embodiments the insulins or insulin analogues described herein are used as compounds (e.g., intermediate compounds) for the manufacture of conjugates described by Formula I. In some embodiments, the insulins or insulin analogues described herein are used in methods for preventing and/or treating an endocrine and/or metabolic disease, for example comprising administering any compound (e.g., modified insulin) of the embodiments described herein to a subject in need thereof, thereby treating the endocrine and/or metabolic disease.

DETAILED DESCRIPTION OF THE DISCLOSURE

While aromatic boron-containing compounds (e.g., groups) can bind to diol containing molecules, achieving selectivity using aromatic boron-containing compounds (which can act as

molecular sensors) is challenging because of their ability to bind various diols, including cis diols, to varying degrees. Improved binding affinity of aromatic boron-containing compounds (which can act as sensors) towards a specific vicinal diol of interest may result in a loss of selectivity.

5 Scaffolds that position the boron functionality (e.g., sensor) of the aromatic boron-containing compounds in a specific or particular ensemble of geometries can increase selectivity towards a specific vicinal diol while simultaneously maintaining affinity for the diol of interest. According to some embodiments, aromatic boron-containing compounds disclosed herein have different pendant groups on the aromatic boron-based scaffolds along with specific scaffold
10 geometries that impact binding to hydroxyl containing molecules.

 According to some embodiments, the compounds of the present disclosure comprise aromatic boron-containing compounds that orient the boron functionalities in three dimensional space, so that the boron-containing compounds are spatially oriented to engage hexoses containing vicinal diols, such that the boron groups can appropriately engage the hydroxyls in
15 the vicinal diol molecule and provide enhancement of selectivity. In some embodiments, the aromatic boron-containing compounds are modified with specific functional groups on the aromatic ring that, together with an appropriate or suitable scaffold, may provide higher selectivity and/or affinity for binding towards a vicinal diol of interest and away from other diols in the body.

20 In some embodiments, the aromatic boron-containing compounds are conjugated to a drug substance (e.g., small-molecule, polypeptide) wherein the aromatic boron-containing compounds provide intramolecular and/or intermolecular interactions with the drug substance and/or with proteins in the body, such as circulating proteins in the blood and/or plasma including albumin and/or globulins. In some embodiments, aromatic boron-containing
25 compounds exhibit reversible binding to glycosylated proteins in the body, such as glycosylated albumin, and this binding is reversibly influenced by the levels of blood sugar or plasma sugar molecules. In some embodiments, the selective binding of the sensors to specific vicinal diols changes the extent of those intramolecular and/or intermolecular bindings and thereby modulates the pharmacokinetics and overall activity of the drug substance in the body; this effect can be
30 controlled by the level of the vicinal diols present.

 In some embodiments, the drug substance is a peptide hormone. In some embodiments, the peptide hormone is a human peptide hormone such as insulin or an insulin analogue, glucagon, or another incretin hormone. In some embodiments the sensors are selective towards the vicinal diols in glucose, and this selectivity is enhanced while maintaining affinity to glucose
35 and simultaneously reducing affinity to other sugars in the blood. In some embodiments, the

scaffolds as well as (e.g., in combination with) the pendant groups on the aromatic core of the boron-containing compounds enable controlling the overall activity and/or pharmacokinetics of the conjugated drug substances based on levels of glucose and/or other vicinal diols in the blood.

In some embodiments, the aromatic boron-containing compounds comprise specific scaffold molecules (e.g., FF structures, FFL-1 to FFL-101, DSL-1 (Diboronate Scaffold Linker) to DSL-112) with conjugated boron functionalities (i.e., F2, F5, and F10), wherein the scaffolds have been used to orient the boron functionalities in three dimensional geometries so that the boron functionalities are oriented near each other and within a distance that helps engage specific hydroxyl orientations of select hexoses such as glucose.

Without wishing to be bound by theory, it is believed that the aromatic boron-containing compounds (e.g., molecules) disclosed herein enhance selectivity through at least one or more of the following three mechanisms: (1) the FF scaffold facilitates matching the orientation of the hydroxyl and/or alkoxy groups on boron groups in the aromatic boron-containing compounds and the hydroxyls in the vicinal diol molecule which enhances selectivity; (2) further selectivity gain is obtained by identifying specific functional groups attached to, or near, for example, the aromatic core of the boron-containing compound which impact the electronic structure of the aromatic boron-containing compound and thereby favor reversible binding to the vicinal diols at physiological pH; and (3) functional groups attached to the aromatic boron-containing compound (e.g., the sensor scaffold) help to provide steric hindrance to reduce binding to unwanted hexoses while maintaining binding to the sugar of interest such as glucose. In some embodiments, the FF scaffolds provide glucose binding. In some embodiments, the combination of the FF scaffold and the indirect linker provides affinity to plasma proteins, such as but not limited to glycosylated proteins, and the combination of the FF scaffold and the indirect linker provides a reversible interaction with plasma proteins that is controlled through the binding of sugar molecules to the FF scaffolds under physiological conditions. These effects as combined together (e.g., compounds of Formula I and II) in the present disclosure provide desired or suitable selectivity of binding towards a vicinal diol-containing molecule of interest and away from other diols in the body.

In some embodiments, the aromatic boron-containing compounds are conjugated to a drug substance wherein the aromatic boron-containing compounds provide intramolecular and/or intermolecular interactions with proteins in the body. Such proteins may include circulating proteins in the blood and/or human plasma such as albumin, glycosylated proteins and/or immunoglobulins, glycosylated proteins including glycosylated plasma proteins such as glycosylated albumin. In some embodiments the selective binding of the sensors to specific vicinal diols in a molecule of interest changes the extent of intramolecular and intermolecular bindings and

thereby modulates the pharmacokinetics and overall activity of the drug substance in the body. In some embodiments, the drug substance is a peptide hormone and in certain embodiments thereof the peptide hormone is an incretin hormone such as insulin and the vicinal diol containing molecule is glucose, but the present disclosure is not limited thereto.

5

Definitions

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. It will be further understood that terms, such as those defined in
10 commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and/or the present specification, and should not be interpreted in an idealized or overly formal sense, unless expressly so defined herein.

Unless specifically described herein, functional groups, functional moieties, and reactions referred to herein are understood to have meanings consistent with standard descriptions in
15 and/or general principles of organic chemistry, for example, as described in Organic Chemistry, Thomas Sorrell, University Science Books, Sausalito, 1999; Larock, Comprehensive Organic Transformations, VCH Publishers, Inc., New York, 1989; Carruthers, Some Modern Methods of Organic Synthesis, 3rd Edition, Cambridge University Press, Cambridge, 1987; Smith and March, March's Advanced Organic Chemistry, 5th Edition, John Wiley & Sons, Inc., New York, 2001.
20 Generic functional groups (such as alkyl, aryl, acetyl, etc.) encompass specific examples or species falling within those functional group categories as generally defined in the field of organic chemistry, and those having ordinary skill in the art are capable of identifying specific example embodiments of functional groups.

Unless specifically described herein, chemical terms, functional groups, and general
25 terms used throughout the specification are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover. In certain embodiments, the terms "a," "an," and "the" and similar referents used herein are to be construed to cover both the singular and the plural unless their usage in context indicates otherwise. The term "CAS #" as used herein is also referred to as CASRN or CAS Number, is a
30 unique numerical identifier assigned by Chemical Abstracts Service (CAS) to every chemical substance described in the open scientific literature.

As used herein, nomenclature for compounds including organic compounds, can be given using common names, IUPAC, IUBMB, or CAS recommendations for nomenclature. One of skill in the art can readily ascertain the structure of a compound if given a name, either by

systemic reduction of compound structure using naming conventions, or by commercially available software, such as CHEMDRAW™ (Cambridgesoft Corporation, U.S.A.).

The terminology used herein is for the purpose of describing embodiments and is not intended to be limiting of the present disclosure. It will be further understood that the terms

5 “comprises,” “comprising,” “includes,” and “including,” when used in this specification, specify the presence of the stated features, integers, acts, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, acts, operations, elements, components, and/or groups thereof. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items. Expressions such as “at least one

10 of,” when preceding a list of elements, modify the entire list of elements and do not modify the individual elements of the list.

As used herein, the terms “substantially,” “about,” and similar terms are used as terms of approximation and not as terms of degree, and are intended to account for the inherent deviations in measured or calculated values that would be recognized by those of ordinary skill in the art.

15 The term “about” used throughout is used to describe and account for small variations. For instance, “about” may mean the numeric value may be modified by $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, $\pm 1\%$, $\pm 0.5\%$, $\pm 0.4\%$, $\pm 0.3\%$, $\pm 0.2\%$, $\pm 0.1\%$ or $\pm 0.05\%$. Numeric values modified by the term “about” include the specific identified value. For example, “about 5.0” includes 5.0.

Further, the use of “may” when describing embodiments of the present disclosure refers

20 to “one or more embodiments of the present disclosure.” As used herein, the terms “use,” “using,” and “used” may be considered synonymous with the terms “utilize,” “utilizing,” and “utilized,” respectively. Also, the term “exemplary” is intended to refer to an example or illustration.

Also, any numerical range recited herein is intended to include all sub-ranges of the same

25 numerical precision subsumed within the recited range. For example, a range of “1 to 10” is intended to include all subranges between (and including) the recited minimum value of 1 and the recited maximum value of 10, that is, having a minimum value equal to or greater than 1 and a maximum value equal to or less than 10, such as, for example, 2 to 7. Any maximum numerical limitation recited herein is intended to include all lower numerical limitations subsumed therein,

30 and any minimum numerical limitation recited in this specification is intended to include all higher numerical limitations subsumed therein. Accordingly, Applicant reserves the right to amend this specification, including the claims, to expressly recite any sub-range subsumed within the ranges expressly recited herein.

As used herein, “aromatic boron-containing group” refers to a compound having at least

35 one boron atom covalently bonded to an aromatic group and/or a compound having at least one

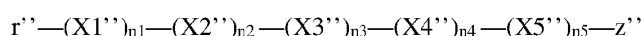
boron atom covalently incorporated within an aromatic group. The term “aromatic” as used herein may include “heterocycle,” “heterocyclyl,” or “heterocyclic.” As used herein the terms “heterocycle,” “heterocyclyl,” or “heterocyclic” each refer to an unsaturated 3- to 18-membered ring containing one, two, three, or four heteroatoms independently selected from nitrogen, oxygen, phosphorus, and sulfur. In some embodiments, the term “aromatic” may include an “aryl.” The term “aryl” as used herein refers to a mono-, bi-, or other multi carbocyclic, aromatic ring system with 5 to 14 ring atoms. The aryl group can optionally be fused to one or more rings selected from aryls, cycloalkyls, heteroaryls, and heterocyclyls. Exemplary aryl groups also include but are not limited to a monocyclic aromatic ring system, wherein the ring comprises 6 carbon atoms.

The term “heteroaryl” as used herein refers to a mono-, bi-, or multi-cyclic, aromatic ring system containing one or more heteroatoms, for example 1-3 heteroatoms, such as nitrogen, oxygen, and sulfur. Heteroaryls can be substituted with one or more substituents. Heteroaryls can also be fused to non-aromatic rings. Exemplary heteroaryl groups include, but are not limited to, a monocyclic aromatic ring, wherein the ring comprises 2-5 carbon atoms and 1-3 heteroatoms. In some embodiments, the aromatic boron-containing group may include but is not limited to aryl- and heteroaryl boronic acids, aryl and heteroaryl boronate esters, and/or boroxoles. Exemplary aromatic boron-containing groups useful according to certain embodiments, include, e.g., those described herein as FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, FF226, F2, F5, F10, and FFL-1 to FFL-101, and further include, e.g., those as disclosed in patent application PCT/US2021/025261 (filed Mar. 31, 2021) as compounds F1-F9, F12-F43, F500-F520 and PCT/US2021/059802 (filed Nov. 18 2021) as compounds FF1-FF224 and F1-F10; the disclosures of which are herein expressly incorporated by reference in their entirety.

The term “small-molecule linker” as used herein refers to a chemical group (e.g., scaffold, moiety) comprising a first attachment point toward X1 and a second attachment point toward Z1b, Z1a, or Z1c. In some embodiments, the first attachment point is toward X1 and the second attachment point is toward Z1c. In some embodiments, the first attachment point is toward X1 and the second attachment point is toward Z1a. In some embodiments, the small molecule linker is a moiety/chemical group selected from Formulae IIa-IIai, Formulae IIIa-IIIai, and FL1-FL19 disclosed in patent application PCT/US2021/025261, the relevant disclosure of which is expressly incorporated herein by reference.

The term “indirect linker” as used herein refers to a chemical group (e.g., scaffold, moiety) comprising a first attachment point toward X1 and a second attachment point toward

Z1b, Z1a, or Z1c. In some embodiments, the first attachment point is toward X1 and the second attachment point is toward Z1c. In some embodiments, the indirect linker is a moiety/chemical group selected from Formulae (i) FL3, FL5, FL5A, FL5B, and FL20-FL75, stereoisomers thereof; and (ii) an L- or D- natural or unnatural amino acid. In some embodiments, at least one
 5 Z1c is covalently conjugated via one or more indirect linkers to X1 or to Z1a, wherein the indirect linker is represented by Formula (X'')_{n1}, and each n1 is independently selected from 1, 2, 3, 4, and 5. For example, at least one Z1c is covalently conjugated via the indirect linker to X1, wherein the indirect linker is represented by Formula III:



10 (Formula III)

wherein

z'' represents an attachment point to an amine in X1;

r'' represents an attachment point to Z1c;

each of n1, n2, n3, n4 and n5 is independently 0 or 1 ;

15 each X1'', X2'', X3'', X4'', X5'' is independently selected from an L- or D-amino acid, wherein the amine functional group of the amino acid is covalently conjugated towards r'' in Formula III, and the acid functional group of the amino acid is covalently conjugated towards z'' in Formula III, FL3, FL5, FL5A, FL5B, and FL20-FL75.

As used herein, "amino acid" includes proteinogenic (or natural) amino acids (amongst
 20 those the 20 standard amino acids), as well as non-proteinogenic (or non-natural) amino acids. Proteinogenic amino acids are those which are naturally incorporated into proteins. The standard amino acids are those encoded by the genetic code. Non-proteinogenic amino acids are either not found in proteins, or not produced by standard cellular machinery (e.g., they may have been subject to post-translational modification). In general, amino acid residues (peptide/protein
 25 sequences) may be identified by their full name, their one-letter code, and/or their three-letter code. These three ways are fully equivalent. In what follows, each amino acid of the compounds of the disclosure for which the optical isomer is not stated is to be understood to mean the L-isomer (unless otherwise specified). Amino acids are molecules containing an amino group and a carboxylic acid group, and, optionally, one or more additional groups, often referred to as a side
 30 chain.

As used herein, the term "amino acid residue" is an amino acid from which, formally, a hydroxy group has been removed from a carboxy group and/or from which, formally, a hydrogen atom has been removed from an amino group. As is apparent from the below examples, amino acid residues may be identified by their full name, their one-letter code, and/or their three-letter
 35 code. These three ways are fully equivalent and interchangeable.

The term “alkyl” as used herein refers to a saturated straight or branched hydrocarbon, such as a straight or branched group of 1-30 carbon atoms, referred to herein as C₁₋₃₀ alkyl. In some embodiments, the alkyl group is a C_{1-C22} alkyl group. In some embodiments, the alkyl group is a C_{1-C20} alkyl group. In some embodiments, the alkyl group is a C_{1-C18} alkyl group. In some embodiments, the alkyl group is a C_{1-C16} alkyl group. In some embodiments, the alkyl group is a C_{1-C14} alkyl group. In some embodiments, the alkyl group is a C_{1-C12} alkyl group. In some embodiments, the alkyl group is a C_{1-C10} alkyl group. In some embodiments, the alkyl group is a C_{1-C8} alkyl group. In some embodiments, the alkyl group is a C_{1-C6} alkyl group. In some embodiments, the alkyl group is a C_{1-C4} alkyl group. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, and octyl. In some embodiments, “alkyl” is a straight-chain hydrocarbon. In some embodiments, “alkyl” is a branched hydrocarbon.

The term “cycloalkyl” as used herein refers to a saturated or unsaturated cyclic, bicyclic, or bridged bicyclic hydrocarbon group of 3-16 carbons, or 3-8 carbons, referred to herein as “(C_{3-C8})cycloalkyl,” derived from a cycloalkane. In some embodiments, the cycloalkyl is a (C_{3-C6})cycloalkyl. Exemplary cycloalkyl groups include, but are not limited to, cyclohexanes, cyclohexenes, cyclopentanes, and cyclopentenes. Cycloalkyl groups may be substituted with alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, ketone, nitro, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide and thioketone. Cycloalkyl groups can be fused to other cycloalkyl (saturated or partially unsaturated), aryl, or heterocyclyl groups, to form a bicycle, tetracycle, etc. The term “cycloalkyl” also includes bridged and spiro-fused cyclic structures which may or may not contain heteroatoms.

The term “acyl” as used herein refers to R-C(O)- groups such as, but not limited to, (alkyl)-C(O)-, (alkenyl)-C(O)-, (alkynyl)-C(O)-, (aryl)-C(O)-, (cycloalkyl)-C(O)-, (heteroaryl)-C(O)-, and (heterocyclyl)-C(O)-, wherein the group is attached to the parent molecular structure through the carbonyl functionality. In some embodiments, it is a C₁₋₁₀ acyl radical which refers to the total number of chain or ring atoms of the, for example, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, or heteroaryl, portion plus the carbonyl carbon of acyl. For example, a C₄-acyl has three other ring or chain atoms plus carbonyl. In some embodiments, it is a C_{1-C22}acyl group. In some embodiments, it is a C_{1-C20}acyl group. In some embodiments, it is a C_{1-C18}acyl group. In some embodiments, it is a C_{1-C16}acyl group. In some embodiments, it is a C_{1-C14}acyl group. In

some embodiments, it is a C₁-C₁₂acyl group. In some embodiments, it is a C₁-C₁₀acyl group. In some embodiments, it is a C₁-C₈acyl group.

The term “haloalkyl” as used herein refers to an alkyl group substituted with one or more halogens. Examples of haloalkyl groups include, but are not limited to, trifluoromethyl, difluoromethyl, pentafluoroethyl, trichloromethyl, etc. In some embodiments, it is a C₁-C₂₂ haloalkyl group. In some embodiments, it is a C₁-C₂₀ haloalkyl group. In some embodiments, it is a C₁-C₁₈ haloalkyl group. In some embodiments, it is a C₁-C₁₆ haloalkyl group. In some embodiments, it is a C₁-C₁₄ haloalkyl group. In some embodiments, it is a C₁-C₁₂ haloalkyl group. In some embodiments, it is a C₁-C₁₀ haloalkyl group. In some embodiments, it is a C₁-C₈ haloalkyl group.

The term “aryl” as used herein refers to a mono-, bi-, or other multi-carbocyclic, aromatic ring system with 5 to 14 ring atoms. The aryl group can optionally be fused to one or more rings selected from aryls, cycloalkyls, heteroaryls, and heterocyclyls. The aryl groups of this present disclosure can be substituted with groups selected from alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, ketone, nitro, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone. Exemplary aryl groups include, but are not limited to, phenyl, tolyl, anthracenyl, fluorenyl, indenyl, azulenyl, and naphthyl, as well as benzo-fused carbocyclic moieties such as 5,6,7,8-tetrahydronaphthyl. Exemplary aryl groups also include but are not limited to a monocyclic aromatic ring system, wherein the ring comprises 6 carbon atoms.

As used herein, a “leaving group” is an atom (or a group of atoms) that can be displaced as stable species taking with it the bonding electrons. For example, leaving groups can be anions (e.g. Cl⁻) or neutral molecules (e.g. H₂O). In some embodiments, a leaving group is a halogen, a N-hydroxysuccinimide (NHS) group, a 2,3,5,6-tetrafluorophenol (TFP) group, a pentafluorophenol (Pfp) group, or a sulfonate ester.

“Isomers” means compounds having the same number and kind of atoms, and hence the same molecular weight, but differing with respect to the arrangement or configuration of the atoms in space.

“Stereoisomer” or “optical isomer” means a stable isomer that has at least one chiral atom or restricted rotation giving rise to perpendicular dissymmetric planes (e.g., certain biphenyls, allenes, and spiro compounds) and can rotate plane-polarized light. Because asymmetric centers and other chemical structure exist in the compounds of the disclosure which may give rise to stereoisomerism, the disclosure contemplates stereoisomers and mixtures thereof. The compounds of the disclosure and their salts include asymmetric carbon atoms and

may therefore exist as single stereoisomers, racemates, and as mixtures of enantiomers and diastereomers. In some embodiments, such compounds will be prepared as a racemic mixture. In some embodiments, such compounds can be prepared or isolated as pure stereoisomers, e.g., as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. As discussed in more detail below, individual stereoisomers of compounds may be prepared by synthesis from optically active starting materials containing the desired chiral centers or by preparation of mixtures of enantiomeric products followed by separation or resolution, such as conversion to a mixture of diastereomers followed by separation or recrystallization, chromatographic techniques, use of chiral resolving agents, or direct separation of the enantiomers on chiral chromatographic columns. Starting compounds of particular stereochemistry are either commercially available or are made by the methods described below and resolved by techniques well-known in the art.

As used herein, a “fatty acid” is a carboxylic acid with an aliphatic chain. A saturated fatty acid has a saturated aliphatic chain whereas an unsaturated fatty acid has an unsaturated aliphatic chain. In some embodiments, the fatty acid is a C₃-C₂₆ fatty acid. In some embodiments, the fatty acid is a C₄-C₂₀ fatty acid. In some embodiments, the fatty acid is a saturated fatty acid selected from CH₃CH₂COOH, CH₃(CH₂)₂COOH, CH₃(CH₂)₃COOH, CH₃(CH₂)₄COOH, CH₃(CH₂)₅COOH, CH₃(CH₂)₆COOH, CH₃(CH₂)₇COOH, CH₃(CH₂)₈COOH, CH₃(CH₂)₉COOH, CH₃(CH₂)₁₀COOH, CH₃(CH₂)₁₁COOH, and CH₃(CH₂)₁₂COOH, CH₃(CH₂)₁₃COOH, CH₃(CH₂)₁₄COOH, CH₃(CH₂)₁₅COOH, CH₃(CH₂)₁₆COOH, CH₃(CH₂)₁₇COOH, and CH₃(CH₂)₁₈COOH. In some embodiments, the fatty acid is an unsaturated fatty acid selected from α-linoleic acid, stearidonic acid, eicosapentaenoic acid, cervonic acid, linoleic acid, linolelaidic acid, γ-Linolenic acid, oleic acid, elaidic acid, and gondoic acid.

The term “pharmaceutically acceptable salt(s)” refers to salts of acidic or basic groups that may be present in compounds used in the present compositions.

As used herein, “drug substance” refers to small-molecule compounds and/or polypeptide containing compounds. According to some embodiments, a drug substance suitable for use in the compounds and methods described herein is a therapeutically, prophylactically and/or diagnostically active drug substance.

It will be understood that, although terms such as “first,” “second,” “third,” etc., may be used herein to describe various elements (such as molecules, components, groups, and/or moieties, etc.), those elements should not be limited by these terms. These terms are merely used to distinguish one element from another element. Thus, a first element described below could be termed a second element without departing from the spirit and scope of the present disclosure. It

will be understood that when an element or group is referred to as being “connected to,” “conjugated with,” “linked,” or “coupled to” another element or group, the two elements may be directly connected, or one or more intervening elements may be present. It will be understood that conjugations and linkages described herein have the option of being direct conjugations or direct linkages, unless expressly excluded or precluded by the context.

As used herein, the terms “directly” or “directly covalently conjugated” or “covalently conjugated directly” may be interchangeably used to indicate that a first group is “directly” or “directly covalently conjugated” or “covalently conjugated directly” to a second group, which means the first and second groups are covalently bonded together without additional intervening groups.

As used herein, the terms “indirectly” or “indirectly covalently conjugated” or “covalently conjugated indirectly” may be interchangeably used to indicate that a first group is “indirectly” or “indirectly covalently conjugated” or “covalently conjugated indirectly” to a second group, which means the first and second groups are covalently bonded together with at least one additional intervening group (e.g., a small-molecule, a linker, a spacer, a linear sequence of amino acids and/or nonlinear sequence of amino acids).

The terms “insulin receptor agonist,” “compound having agonist potency” in the context of having potency against an insulin receptor, and “agonist potency” in the context of a compound having agonist potency against an insulin receptor refer to a compound (e.g., a compound of Formula (I), a protein, a fusion protein as described in, e.g., US 2016/0324932) that binds to and/or activates an insulin receptor.

The term “short-acting insulin receptor agonist” and “short-acting insulin” refer to an insulin receptor agonist having an onset of activity equal to or faster than insulin isophane human and insulin human, sold under the tradename Humulin®. For example, a short-acting of insulin may have an onset of action that occurs within 10 minutes of injection. As another example, a short-acting of insulin may have an onset of action that occurs within 20 minutes of injection. In some embodiments, a short-acting insulin may have a maximum effectiveness (peak) within 1-4 hours post injection. In some embodiments, short-acting insulin can be administered before or during and/or shortly after a meal.

The term “long-acting insulin receptor agonist” and “long-acting insulin” refer to an insulin receptor agonist having an onset of action slower than Humulin®. For example, a long-acting insulin may have an onset of action between 1-2 hours, or longer. In certain embodiments, a long-acting insulin may have a maximum effectiveness (peak) within between 6-20 hours and or have prolonged action. In some embodiments, a long-acting insulin can be administered at a frequency of once daily or, for example, once weekly.

When used herein in connection with an insulin receptor agonist, the term “suitable for meal dosing” refers to a short acting insulin receptor agonist suitable for controlling blood glucose levels during and/or shortly after a meal.

When used herein in connection with an insulin receptor agonist, the term “suitable for once- weekly dosing” refers to an insulin receptor agonist with a pharmacokinetic and pharmacodynamic profile that is sufficiently prolonged to control blood glucose levels throughout the day when administered no more frequently than once weekly. Examples of such molecules include fusion proteins as described in US2016/0324932, including BIF. BIF, also known as insulin efsitora alfa, comprises a dimer of an insulin receptor agonist fused to a human IgG Fc region, wherein the insulin receptor agonist comprises an insulin B-chain analogue fused to an insulin A-chain analogue through the use of a first peptide linker and wherein the C-terminal residue of the insulin A-chain analogue is directly fused to the N-terminal residue of a second peptide linker, and the C-terminal residue of the second peptide linker is directly fused to the N-terminal residue of the human IgG Fc region. BIF is identified by CAS registry number 2131038-11-2, which provides the following chemical names: (1) Insulin [16-glutamic acid, 25-histidine, 27-glycine, 28- glycine, 29-glycine, 30-glycine] (human B-chain) fusion protein with peptide (synthetic 7-amino acid linker) fusion protein with insulin [47-threonine, 51-aspartic acid, 58- glycine] (human A-chain) fusion protein with peptide (synthetic 20-amino acid linker) fusion protein with immunoglobulin G2 (human Fc fragment), dimer; and (2) Homo sapiens Insulin B-chain [Y16>Y(16), F25>H(25), TPKT27-30>GGGG(27-30)] (1-30) fusion protein with di glycyl seryl tetraglycyl (31-37) Insulin A-chain [I10>T(47), Y14>D(51), N21>G(58)] (38-58) fusion protein with tris(tetraglycylglutaminy)pentaglycyl (59-78) Homo sapiens Immunoglobulin heavy constant gamma 2 {del-CH1, hinge-(7-12), CH2, CH3[K¹⁰⁷>del(300)]} (79-299), dimer (80-80':83-83')-bisdisulfide, expressed in CHO cells, alfa glycosylated.

As used herein, “glucose sensing insulin” refers to an insulin receptor agonist having an onset of activity and/or level of activity that depends on blood sugar level. Examples of such molecules include compounds of Formula (I) disclosed herein, such as Examples 2, 5, 13, 22, 28, 32, 35, 50, 56, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 126, 128, 132, 134, 135, 150, and 154.

The term “incretin-based therapy” includes any treatment which comprises administration of, or promotes, enables, enhances and/or simulates the effects of, a group of metabolic hormones known as incretins, which group includes, but is not limited to, GLP-1, gastric inhibitory peptide (GIP), and glucagon. Incretin-based therapies which are currently available include GLP-1R agonists.

A “DPP-4 inhibitor” is a compound that blocks the DPP-4 enzyme, which is responsible for the degradation of incretins. Currently available DPP-4 inhibitors include sitagliptin (Januvia®) and linagliptin (Tradjenta®).

A “GLP-1R agonist” and “Glucagon receptor agonist” is defined as a compound comprising the amino acid sequence of native human GLP-1 (SEQ ID NO:25) or human glucagon, as well as a compound that maintains full or partial activity at the GLP-1 receptor that is a GLP-1 analogue, GLP-1 derivative or GLP-1 fusion protein. GLP-1R activity may be measured by methods known in the art, including using in vivo experiments and in vitro assays that measure GLP-1 receptor binding activity or receptor activation, e.g., assays employing pancreatic islet cells or insulinoma cells, as described in EP 619,322 and U.S. Pat. No. 5,120,712, respectively. A GLP-1 analogue is a molecule having a modification including one or more amino acid substitutions, deletions, inversions, or additions when compared with the amino acid sequence of native human GLP-1 (SEQ ID NO:25). A GLP-1 derivative is a molecule having the amino acid sequence of native human GLP-1 (SEQ ID NO:25) or of a GLP-1 analogue, but additionally having at least one chemical modification of one or more of its amino acid side groups, α -carbon atoms, terminal amino group, or terminal carboxylic acid group. A GLP-1 fusion protein is a heterologous protein comprising GLP-1, a GLP-1 analogue or a GLP-1 derivative portion and a second polypeptide. Currently available GLP-1R agonists include exenatide (Byetta® and Bydureon®), liraglutide (Victoza®), albiglutide (Tanzeum®) and dulaglutide (Trulicity®), the structures of which are known in the art. See, e.g., U.S. Pat. No. 5,424,286 (exenatide); U.S. Pat. No. 6,268,343 (liraglutide); US 20140447 I 7 (albiglutide); and U.S. Pat. No. 7,452,966 (dulaglutide).

The term “excipient” means any substance added to the composition other than the fusion protein or any other additional active ingredient(s). Examples of such excipients that may be used in the compositions of the present invention include buffering agents, surfactants, isotonicity agents and preservatives. A “pharmaceutically acceptable excipient” refers to an excipient that is compatible with the other ingredients in the composition and that is suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits. In some embodiments the excipients may be used to stabilize the agonist while in solution or to increase the onset and maximum effectiveness. Such excipients may include mannitol, sorbitol, m-cresol, EDTA, and citrate.

A “buffering agent” is a substance which resists changes in pH by the action of its acid-base conjugate components. In certain embodiments, the composition of the present invention

has a pH from about 5.5 to about 9.0, preferably, between about 7.0 and about 8.0, more preferably between about 7.2 and 7.8. Buffering agents suitable for controlling the pH of the compositions of the present invention in the desired range include, but are not limited to agents such as phosphate, acetate, citrate, or acids thereof, arginine, TRIS, HEPES, and histidine
5 buffers, as well as combinations thereof. “TRIS” refers to 2-amino-2-hydroxymethyl-1,3,-propanediol, and to any pharmacologically acceptable salt thereof. The free base and the hydrochloride form (i.e., TRIS-HCl) are two common forms of TRIS. TRIS is also known in the art as trimethylol aminomethane, tromethamine, and tris(hydroxymethyl) aminomethane. Preferred buffering agents in the composition of the present invention are citrate, or citric acid,
10 phosphate, and TRIS.

The compounds disclosed herein may comprise one or more protein components (e.g., where X1 comprises one or more protein components). In some embodiments, the compounds disclosed herein comprise two or more fused protein components or two or more conjugated protein components. The term “fusion protein” as used herein refers to the combination of two
15 or more distinct proteins that are linked together directly via a peptide bond between the C-terminus of one protein and the N-terminus of another protein or indirectly via a linker (i.e., through chemical linkers such as biofunctionalized PEG linkers) or through a continuous amino acid chain) joining the C-terminus or the N-terminus or a side chain of one protein and the C-terminus or the N-terminus or a side chain of another protein. The term “conjugate protein” as
20 used herein refers to the combination of two or more distinct proteins that are linked together chemically either directly via a chemical bond or indirectly via a chemical linker. In some instances, the two or more proteins may act on the same or similar receptors (i.e., a fusion of two insulin agonist peptides) or may act upon separate and distinct receptors (i.e., one protein is an insulin receptor agonist, and another protein is a glucagon receptor agonist). In some
25 instances, the fusion protein may contain only one agonist protein while the other protein is a human IgG Fc region or a single domain antibody (nanobody) or a variable-heavy chain sequence (V_HH).

The phrase “composition comprising a fusion protein” encompasses compositions comprising a monomer, homodimer, heterodimer, or multimer of a fusion protein. In certain
30 embodiments, a pharmaceutical composition of the present disclosure is a composition comprising a fusion protein in a concentration of at least 1 mg/mL, at least 2 mg/mL, at least 5 mg/mL, at least 10 mg/mL, at least 20 mg/mL, at least 25 mg/mL, at least 30 mg/mL, at least 35 mg/mL, at least 50 mg/mL, at least 55 mg/mL, at least 50 mg/mL, at least 65 mg/mL, at least 75 mg/mL, at least 100 mg/mL or greater. In some embodiments, the fusion protein is present in a
35 concentration of 10-100 mg/mL. In some embodiments, the fusion protein is present in a

concentration of 15-75 mg/mL, and in some embodiments, the fusion protein is present in a concentration of 20-65 mg/mL.

The pharmaceutical compositions of the present disclosure may also contain a “surfactant,” meaning a substance that lowers the surface tension of a liquid. Examples of surfactants used in pharmaceutical compositions and which may be used in certain compositions of the present disclosure include polysorbate 20, polysorbate 80, polyethylene glycols (e.g., PEG 400, PEG 3000, TRITON X-100), polyethylene glycol alkyl ethers (e.g., BRIJ), polypropylene glycols, block copolymers (e.g., poloxamer, PLURONIC F68; poloxamer 407, PLURONIC F127; TETRONICS), sorbitan alkyl esters (e.g., SPAN), polyethoxylated castor oil (e.g., KOLLIPHOR, CREMOPHOR), and trehalose.

The pharmaceutical compositions of the present invention may also contain a preservative. The term “preservative” refers to a compound added to a pharmaceutical formulation to act as an anti-microbial agent. Among preservatives known in the art as being effective and acceptable in parenteral formulations are benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methyl- or propyl-paraben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. Phenolic preservative includes the compounds phenol, m-cresol, o-cresol, p-cresol, chlorocresol, methylparaben, benzyl alcohol, and mixtures thereof. If a preservative is necessary, the preservative used in compositions of the present invention is preferably a phenolic preservative, preferably either m-cresol, phenol, and/or benzyl alcohol. Certain phenolic preservatives, such as phenol and m-cresol, are known to bind to insulin and insulin hexamers and thereby stabilize a conformational change that increases either physical or chemical stability, or both. In compositions comprising other proteins, however, such preservatives may contribute to the formation of protein aggregates, or high molecular weight polymers (HMWP). See, e.g., Maa Y F and Hsu C C, *Int J Pharm* 140: 155-168 (1996); Fransson J, et al., *Pharm. Res.*, 14: 606-612 (1997); Lam X M, et al., *Pharm. Res.*, 14: 725-729 (1997); Remmele R L Jr, et al., *Pharm Res* 15: 200-208. (1998); Thirumangalathu R, et al., *J Pharm Sci* 95: 1480-1497 (2006). In some instances, protein aggregates in therapeutic formulations can be undesirable due to their tendency to induce an immune response.

As used herein, “combination therapy” or administration “in combination with” one or more further therapeutic agents involves administration of two or more active agents (e.g., two or more pharmacological agents) intended to treat a predetermined indication and/or conditions associated therewith. The administration can be simultaneous (concurrent) or consecutive (sequential) in any order. The two or more agents of the “combination therapy” may be formulated as separate compositions (e.g., formulations) or may be formulated as a single

composition (formulation). “Combination therapy” encompasses administration of agents having different mechanisms of action or targeting different indications and/or conditions as well as agents having similar mechanisms of actions or targeting similar indications and/or conditions. For example, because Type 1 diabetes patients produce little or no insulin, effective insulin therapy for Type 1 diabetics can involve the use of two types of exogenously administered insulin: a rapid-acting, mealtime insulin provided by bolus injections, and a long-acting, basal insulin, administered once or twice daily to control blood glucose levels between meals. Treatment of patients with Type 2 diabetes typically begins with prescribed weight loss, exercise, and a diabetic diet; but when these measures fail to control elevated blood sugars, then oral medications and incretin-based therapy, such as administration of glucagon-like peptide-1 (GLP-1) receptor agonists and/or dipeptidyl peptidase 4 (DPP-4) inhibitors that enable increased incretin levels, may be necessary. When these medications are still insufficient, treatment with insulin may be considered. Type 2 diabetes patients whose disease has progressed to the point that insulin therapy is required are may also be started on a single daily injection of a long-acting, basal insulin, although mealtime injections of rapid-acting insulins may be included, as necessary, in some cases. In some embodiments, the present disclosure provides a combination therapy comprising administering a rapid acting insulin and a basal insulin, and/or a fusion protein comprising, for example, one or more diboronate sensors disclosed herein.

The terms “administer,” “administering,” or “administration” include any method or act of delivery of a pharmacological agent (e.g., a medicament) to an intended subject (e.g., a patient). The pharmacological agent may be any suitable therapeutic agent, such as a biologic agent, such as an antibody or an antigen-binding fragment thereof (e.g., a pharmaceutical composition comprising such an antibody or antigen-binding fragment), a peptide agent (e.g., a hormone or a modified analogue thereof), or a low molecular weight agent (e.g., a structurally-defined small molecule or chemical entity). The administration of a pharmacological agent can be systemic or by local administration. In some embodiments, administration may involve one or more pharmacological agents that can be administered concurrently, simultaneously, or sequentially.

The terms “simultaneous” and “concurrently” are used interchangeably and are used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time or where the administration of second therapeutic agent falls within a short period of time, no longer than necessary to start the subsequent administration, after administration of a first therapeutic agent. For example, concurrent administration would include administering a second agent after a first agent without added delay beyond the time necessary to complete the first administration and start the second administration.

The terms “sequentially” and “consecutively” are used interchangeably and are used herein to refer to administration of two or more therapeutic agents where there is a period of delay between administering one therapeutic agent and administering another agent(s). For example, sequential administration would include administration of the two or more therapeutic agents are administered with a time separation of more than about 15 minutes, such as about any of 20, 30, 40, 50, or 60 minutes, 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, or 1 month, or longer.

As used herein, the terms “basal insulin” and “basal insulins” may refer to several types of basal insulins (e.g., long-acting insulin). For example, insulin glargine, sold under the tradename LANTUS®, comprises a modified insulin structure in which the asparagine at position 21 in the insulin A-chain is replaced with glycine, and two arginines are added to the C-terminus of the B-chain. Another example, insulin glargine-algr, sold under the tradename Rezvoglar™, similarly comprises a modified insulin structure in which the asparagine at position 21 in the insulin A-chain is replaced with glycine, and two arginines are added to the C-terminus of the B-chain. As yet another example, insulin detemir, sold under the tradename LEVEMIR®, comprises a modified insulin structure in which the threonine at position 30 of the B-chain has been deleted and the lysine at position 29 of the B-chain has been derivatized through the covalent linkage of a 14-carbon, myristoyl fatty acid to the E-amine group of lysine at B29. Insulin degludec, available in Europe and Japan under the tradename TRESIBA®, comprises a modified insulin structure in which the threonine at position 30 of the B-chain has been deleted, and the ε-amino group of the lysine at position 29 of the B-chain is covalently derivatized with hexadecandioic acid via a γ-L-glutamic acid linker. All of these insulins are indicated for once-daily administration. In some embodiments, the present disclosure provides the use of one or more compounds (e.g., Formula I, fusion proteins) in the manufacture of a medicament for the treatment of a disease (e.g., diabetes mellitus, obesity, dyslipidemia or metabolic syndrome), wherein the medicament is to be administered simultaneously, separately or sequentially in combination with another active ingredient. The compounds and combinations disclosed herein (e.g., compounds of Formula I, fusion proteins) are effective in treating a disease and/or condition in a subject in need thereof by administering to a patient in need thereof a therapeutically effective amount of a compound and/or composition of the present disclosure.

As used herein, the phrase “therapeutically effective amount” and “prophylactically effective amount” refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of a disease or an overt symptom of the disease. The therapeutically effective amount may treat a disease or condition, a symptom of disease, or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate,

improve, or affect the disease, the symptoms of disease, or the predisposition toward disease. The set or specific amount that is therapeutically effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, *e.g.* the type of disease, the patient's history and age, the stage of disease, and the administration of other therapeutic agents. In some embodiments, the phrase "therapeutically effective amount" refers to that amount of a compound and/or pharmaceutical composition and/or combination of actives disclosed herein that is sufficient to regulate blood glucose in a patient without causing unacceptable side effects. A therapeutically effective amount of the compound and/or pharmaceutical composition and/or combination of actives disclosed herein administered to a subject will depend on the type and severity of the disease and on the characteristics of the subject, such as general health, age, sex, body weight, and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. For example, a therapeutically effective amount of a fusion protein of the present disclosure when administered once weekly ranges from about 0.01 nmol/kg to about 100 nmol/kg. In some embodiments, a therapeutically effective amount of a fusion protein of the present disclosure when administered once weekly ranges from about 1 nmol/kg to about 50 nmol/kg. In some embodiments, a therapeutically effective amount of a fusion protein of the present disclosure when administered once weekly ranges from about 16 nmol/kg to about 25 nmol/kg. In some embodiments, a therapeutically effective amount of a fusion protein of the present disclosure when administered once weekly ranges from about 1 mg to about 200 mg. In some embodiments, a therapeutically effective amount of a fusion protein of the present disclosure when administered once weekly ranges from about 25 mg to about 175 mg. In some embodiments, a therapeutically effective amount of a fusion protein of the disclosure when administered once weekly ranges from about 100 mg to about 160 mg.

The terms "subject" and "patient" are herein used interchangeably and refer to a party receiving a therapy or treatment. In some embodiments, the "subject" and "patient" is a human.

The terms "blood sugar level" and "glycemia" are herein used interchangeably and refer to the concentration of sugar present in the blood. A blood sugar meter or glucometer can be used to measure the amount of sugar in a sample of blood. Blood sugar levels are generally measured as mg of sugar per dL blood (mg/dL). The blood sugar level can refer to the level of any of the sugars disclosed herein. For example, the blood sugar level would include the concentration of glucose in the blood, otherwise known as the blood glucose level/concentration.

As used herein, the term "steady state" refers to a constant/fixed condition with no increases or decreases in a variable of interest or with minimal (*i.e.*, less than 10% or lower) variation. By way of context, in some embodiments, an insulin receptor agonist is infused

intravenously (IV) in a fixed dose bolus, and blood glucose levels are maintained at some predetermined “steady state” level (e.g. 100 mg/dL for euglycemic state; 200 mg/dL or 400 mg/dL for hyperglycemic) by continuous infusion of glucose at a variable rate (mg/kg/min). The amount of glucose infused to maintain a “steady state” blood sugar level is equal to whole-body glucose uptake and utilization.

The term “hyperglycemia” is used herein to refer to physiologically high blood glucose levels. Hyperglycemic refers to a state of physiologically high blood glucose levels. Blood glucose levels that are considered hyperglycemic will vary based on the species of the subject. For example, in rats, hyperglycemia refers to a blood glucose level ≥ 200 mg/dL. In humans, hyperglycemia refers to a blood glucose level > 180 mg/dL.

The term “euglycemia” is used herein to refer to physiologically normal blood glucose level. Euglycemic refers to a state of normal blood glucose levels. Blood glucose levels that are considered euglycemic will vary based on the species of the subject. For example, in rats, euglycemia refers to a blood glucose level < 200 mg/dL. In humans, euglycemia refers to blood glucose levels of between 70-180 mg/dL.

The phrase “glucose infusion rate” is used herein to refer to the rate at which glucose is infused into a subject. The glucose infusion rate (GIR), in units of mg/kg/min, is calculated as follows: $(\text{Infusion rate (mL/hr)} \times \text{Glucose concentration (g/dL)} \times 1000 \text{ (mg/g)}) / \text{Weight (kg)} \times 60 \text{ (min/hr)} \times 100 \text{ (mL/dL)}$, wherein “infusion rate” refers to the rate at which glucose is infused into the subject, “glucose concentration” refers to the concentration of glucose that is being infused, and “weight” refers to the subject’s weight. In some embodiments, the GIR corresponds to the rate of glucose infusion that is required to maintain a specified blood glucose level.

The phrase “relative glucose infusion rate difference” as used herein, refers to the difference in the amount of glucose infused between two different conditions, experiments, or measurements. In some embodiments, it may be measured by taking the difference between (a) the area under the curve (AUC) for one recorded GIR for maintaining a specified blood glucose concentration and (b) the AUC for another recorded GIR for maintaining a different blood glucose concentration. Generally, the AUC for a GIR for maintaining a lower blood glucose concentration is subtracted from the AUC for a GIR for maintaining a higher blood glucose concentration. For example, where the AUC for a first GIR (providing a blood glucose concentration of 100 mg/dL) is 600 mg/kg/min.min and the AUC for a second GIR (providing a blood glucose concentration of 200 mg/dL) is 800 mg/kg/min.min, subtracting the AUC for the first GIR from the AUC for the second GIR provides a relative glucose infusion rate difference of 200 mg/kg/min.min.

As used herein, the phrase “relative glucose infusion rate ratio” refers to a ratio of the amount of glucose infused between two different conditions, experiments, or measurements. In some embodiments, it may be measured by taking the ratio between (a) the area under the curve (AUC) for one recorded GIR for maintaining a blood glucose concentration and (b) the AUC for another recorded GIR for maintaining a different glucose sugar concentration. Generally, the AUC for a GIR for maintaining a higher blood glucose concentration is divided by an AUC for a GIR for maintaining a lower blood glucose concentration. For example, where the AUC for a first GIR (providing a blood glucose concentration of 100 mg/dL) is 600 mg/kg/min.min and the AUC for a second GIR (providing a blood glucose concentration of 200 mg/dL) is 800 mg/kg/min.min, dividing the AUC for the second GIR by the AUC for the first GIR provides a relative glucose infusion rate ratio of 1.33

As used herein, the phrase “area under the curve” refers to the area bounded by a curve, the axis, and two boundary points, whether plotted or mathematically represented. In some embodiments, the curve used to calculate the area under the curve (AUC) is a measure of GIR as a function of time where the X-axis would correspond to time and the Y-axis would correspond the GIR, with the origin is at 0 on the Y-axis (i.e., the X-axis and Y-axis intersect at 0 on the Y-axis). For example, a curve may be a recorded GIR over a period of time, and the boundary points are the GIR at the start and end of the infusion in the experiment. The AUC would then be calculated for the area between the plotted curve and the X-axis, between the starting and ending boundary points. Mathematically, AUC can be calculated according to methods known to those skilled in the art, for example, as in Tai M.M. (1994) Diabetes Care, 17(2):152-154, the contents of which, are hereby incorporated by reference in its entirety. In some embodiments, area under the curve (AUC) is calculated using the trapezoid rule and with baseline correction applied. In some embodiments, the AUC is calculated using GraphPad Prism v9. For the baseline correction, an averaged GIR value from 30 minutes prior to injections ($x = -30$ min) to the time of injections ($x=0$) can be subtracted from each GIR value from time 0 ($x = 0$) to time the last timepoint measured ($x=300$). The trapezoidal calculation is as follows:

$$\int_a^b f(x) dx \approx (b - a) \cdot \frac{1}{2} (f(a) + f(b)).$$

Where a is the first time point (e.g., 0 min) and b is the last time point (e.g., 300 min).

The area was calculated as a subdivision of small trapezoids as follows:

$$\int_a^b f(x) dx \approx \frac{\Delta x}{2} (f(x_0) + 2f(x_1) + 2f(x_2) + 2f(x_3) + 2f(x_4) + \dots + 2f(x_{N-1}) + f(x_N)).$$

Such that Δx is the difference between each time point (5-minute intervals).

As used herein, the term “EC50” refers to the half maximal effective concentration of a compound in a dose-response assay. The EC50 is a measure of the concentration of a compound that is required to produce half of the maximum possible effect as a result of exposure to the compound. The EC50 can be calculated using known methods in the art. In some embodiments, the EC50 can be calculated using a four-parameter logistic regression curve using the following equation: $Y = \text{Bottom} + (X^{\text{Hillslope}} * (\text{Top} - \text{Bottom}) / (X^{\text{Hillslope}} + \text{EC50}^{\text{Hillslope}}))$, wherein hillslope refers to the slope of the sigmoidal curve between the top and bottom plateaus of the dose-response curve. In some embodiments, the EC50 may be calculated using GraphPad Prism v7, 8, or 9. When a compound has its EC50 measured at two or more concentrations of a sugar (e.g., glucose), (a) the term “a first sugar concentration” refers to the first, lower, concentration of the sugar, for example where the concentration is about 3 mM or about 5 mM, and (b) “a second sugar concentration” refers to the second, higher, concentration of the sugar, for example where the concentration is about 10 mM, about 20 mM, or about 30 mM. In some embodiments, for example, the EC50 of dose-response curves were compared to assess fold change in insulin receptor phosphorylation (IR Phosphorylation) activity of the exemplary compounds of Formula I from low (e.g., about 3 mM, or about 4 mM) to high glucose concentration (e.g., about 10 mM, about 20 mM, or about 30 mM). This fold activity change was determined by dividing the EC50 of a compound (e.g., a compound of Formula I) at “low” glucose concentration (e.g., about 3 mM) by the EC50 of that compound at “high” glucose concentration (e.g., about 20 mM), with all other conditions held constant. See Example titled “*In Vitro* Demonstration of Activity for Compounds of Formula I.”

As used herein, the term “Kd” refers to the dissociation constant, and is reflective of the binding affinity between a ligand (e.g., a diboronate sensor as described herein) and its target (e.g., a sugar, for example, glucose). For example, “glucose Kd” and “average glucose Kd” in the context of diboronate sensors described herein refer to the affinity of the diboronate sensor for glucose where the Kd is measured before the diboronate sensor is conjugated to the insulin molecule to generate the compounds described herein. The binding of a diboronate sensor described herein to glucose may be measured through an Alizarin red S (ARS) displacement assay and is recorded prior to the diboronate sensor being conjugated (e.g., covalently bound) to the insulin molecule (e.g., compounds of Formula I). ARS displacement assays are known in the art, for example, in Springsteen and Wang (2001) Chem. Comm., 1608-1609, the content of which is incorporated by reference herein in its entirety. In the ARS displacement assay, a diboronate sensor as disclosed herein is incubated with ARS and a fluorescence emission is recorded. A composition of the diboronate sensor as described herein and ARS is then titrated against serial dilutions of a sugar (e.g., glucose), and fluorescence emission is then measured

after incubation to determine displacement of ARS when the diboronate sensor binds to the sugar. The changes of intensity (fluorescence emission with and without the sugar) can be plotted against concentration of sugar to generate an associate constant for the binding of sugar. When a diboronate sensor has a higher association constant for the sugar, there is an increased

5 Kd. Additionally, diboronate sensors as described herein include those with selective affinity/binding to sugars. For example, a diboronate sensor as described herein can include sensors having increased affinity for (binding to) glucose but not for other sugars, such as lactate and/or fructose. *See* Example titled "Procedure for determination of the glucose, fructose, and lactate binding (Kd) using ARS displacement assay."

10 As used herein the terms "clamp," "clamp assay," and "clamped" refer to hyperglycemic/euglycemic clamp (glucose clamp), which has been recognized as the "gold standard" method for detecting insulin activity via glucose utilization in experimental animals and in humans. In a hyperglycemic/euglycemic clamp, the plasma (blood) insulin concentration is acutely raised and, in certain embodiments, maintained by a continuous infusion of insulin

15 while the plasma glucose concentration is held constant at predetermined hyperglycemic or euglycemic levels by a variable glucose infusion rate (GIR). When the steady-state blood glucose level is achieved, the glucose infusion rate equals insulin receptor-stimulated glucose uptake by all the tissues in the body and is therefore a measure of insulin activity at a specific blood glucose level. For example, in some embodiments, glucose is continuously infused to a

20 subject over the course of a study and the glucose infusion rate is adjusted to maintain a constant blood glucose level in response to administration of a compound (e.g., a compound of Formula I). Changes in the sugar infusion rate in response to administration of a compound at a specific dose level or concentration are recorded and used to determine the sugar infusion rate. In some embodiments, the sugar is glucose. *See, e.g.*, Lauth W.W., et al. (1998) Canadian Journal of

25 Physiology and Pharmacology. 76(12): 1080-1086, the content of which is hereby incorporated by reference in its entirety. The clamp technique/assay referred to and used herein is a modified version of the technique known and disclosed in the art. *See* Example titled "*In Vivo* Demonstration of Activity for Compounds of Formula I."

In at least some embodiments, one or more groups (e.g., X1a, Z1a, Z1c) are covalently

30 conjugated directly or indirectly (e.g., via indirect linker(s)) to each other. For example, according to certain embodiments Z1c is covalently conjugated, directly or indirectly (e.g., via one or more indirect linkers), to an amine in X1. As another example, according to certain embodiments one or more drug substances (X1) are covalently conjugated to one or more amine containing linkers. In some embodiments, X represents a point of covalent attachment either

35 directly to an amine in X1 or to an amine that is covalently conjugated directly or indirectly (e.g.,

via the indirect linker(s) to X1. In certain embodiments, each Z1c is independently covalently conjugated, directly or indirectly (e.g., via the indirect linker(s) of Formula (X'')_{n1}), to an amine of Z1a or to an amine of X1. In some embodiments, at least one Z1c is covalently conjugated via the indirect linker (e.g., Formula (X'')_{n1}) to X1 or to Z1a.

5 As used herein, terms such as “attachment point toward [group],” “attachment to,” and “covalent linkage toward [group]” express that the indicated atom, attachment, or linkage is closer to the indicated group than the other attachment point or covalent linkage variables within the structure formula. In some embodiments, an attachment point or covalent linkage may be directly adjacent to the indicated group, and in some embodiments other atoms or groups may be
10 present therebetween.

 As used herein, the term “percentage homology” refers to the percentage of sequence identity between two sequences after optimal alignment. Identical sequences have a percentage homology of 100%. Optimal alignment may be performed by homology alignment algorithms described by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*
15 85:2444 (1988), by general methods described for search for similarities by Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), including implementation of these algorithms or visual comparison. As used herein, “insulin A-chain” is the chain of insulin that has the highest percentage homology to the A-chain of wild-type human insulin. As used herein, “insulin B-chain” is the chain of insulin that has the highest percentage homology to the B-chain of wild-
20 type human insulin.

 In some embodiments, the terms “covalently connected,” “covalently conjugated,” or “through a covalent conjugation” may be interchangeably used to indicate that two or more atoms, groups, or chemical moieties are bonded or connected via a chemical linkage. In some embodiments, the chemical linkage (which in some embodiments may be referred to as a
25 covalent linkage) may be (e.g., consist of) one or more shared electron pairs (e.g., in a single bond, a double bond, or a triple bond) between two atoms, groups, or chemical moieties.” In some embodiments, the chemical (covalent) linkage may further include one or more atoms or functional groups, and may be referred to using the corresponding name of that functional group in the art. For example, a covalent linkage including a –S–S– group may be referred to as a
30 disulfide linkage; a covalent linkage including a –(C=O)– group may be referred to as a carbonyl linkage; a covalent linkage including a –(CF₂)– group may be referred to as a difluoromethylene linkage, etc. The type of linkage or functional group within the covalent bond is not limited unless expressly stated, for example when it is described as including or being selected from certain groups. The types or kinds of suitable covalent linkages will be understood from the
35 description and/or context.

In some embodiments, the insulin receptor agonist may be bound or associated with human serum albumin (HSA) before binding to the insulin receptor. For example, insulin receptor agonists that contain any one of DSL-1 to DSL-112 may associate or bind either through hydrogen bonds, ionic associations, or hydrolysable boron ester bonds to HSA. These interactions may occur along the surface of HSA such as salt bridges to lysine residues, cleavable boron ester bonds formed with serine, threonine, and/or tyrosine hydroxyl groups, and/or to specific locations on HSA such as the Site I or Site II small molecule drug binding sites or one or more of the seven fatty acid binding sites (Yamasaki, K. et. al. (2013) *Biochimica et Biophysica Acta* 12:5435-5443).

In some embodiments, side chains of amino acids may be covalently connected (e.g., linked or cross-linked) through any number of chemical bonds (e.g., bonding moieties) as generally described in *Bioconjugate Techniques* (Third edition), edited by Greg T. Hermanson, Academic Press, Boston, 2013. For example, the side chains may be covalently connected through an amide, ester, ether, thioether, isourea, imine, triazole, or any suitable covalent conjugation chemistry available in the art for covalently connecting one peptide, protein, or synthetic polymer to a second peptide, protein, or synthetic polymer. The term polymer includes polypeptide. The term "covalent conjugation chemistry" may refer to one or more functional groups included in the bonding moiety, and/or the chemical reactions used to form the bonding moiety.

The term "vicinal diol" refers to a group of molecules in which two hydroxyl groups occupy vicinal positions, that is, they are attached to adjacent atoms. Such molecules may include, but are not limited to, sugars such as hexoses, glucose, mannose and fructose.

In some embodiments, the term "albumin" means human serum albumin or a protein with at least 60% percentage homology to human serum albumin protein. It is to be understood that in some embodiments the albumin may be further chemically modified for the purposes of conjugation. In some embodiments, modifications may include one or more covalently connected linkers. In some embodiments, the term "albumin" means human serum albumin or a protein with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% percentage homology to human serum albumin protein. In some embodiments, the term "albumin" means human serum albumin or a protein with at least 90% percentage homology to human serum albumin protein. In some embodiments, the term "albumin" means human serum albumin or a protein with at least 95% percentage homology to human serum albumin protein. In some embodiments, the term "albumin" means human serum albumin or a protein with at least 99% percentage homology to human serum albumin protein. In some embodiments, albumin is unmodified human serum albumin.

The term "treatment" is meant to include both the prevention and minimization of the referenced disease, disorder, or condition (i.e., "treatment" refers to both prophylactic and therapeutic administration of a compound of the present invention or a composition comprising a compound of the present invention unless otherwise indicated or clearly contradicted by context). The route of administration may be any route which effectively transports a compound of this disclosure to the desired or appropriate place in the body, such as parenterally, for example, subcutaneously, intramuscularly, orally, or intravenously. For parenterally administration, a compound of the disclosure is formulated analogously with the formulation of known insulins. Furthermore, for parenteral administration, a compound of this disclosure is administered analogously with the administration of known insulins and the physicians are familiar with this procedure. The amount of a compound of this disclosure to be administered, the determination of how frequently to administer a compound of this disclosure, and the election of which compound or compounds of this disclosure to administer, optionally together with another antidiabetic compound, is decided in consultation with a practitioner who is familiar with the treatment of the condition (e.g. diabetes) to be treated.

In some embodiments, "therapeutic composition" and "pharmaceutical composition" as used herein means a composition that is intended to have a therapeutic effect such as pharmaceutical compositions, genetic materials, biologics, and other substances. Pharmaceutical compositions may be configured to function in the body with therapeutic qualities; concentration may be altered to reduce the frequency of replenishment, and the like. In some embodiments, "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of a disease or an overt symptom of the disease. The therapeutically effective amount may treat a disease or condition, a symptom of disease, or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of disease, or the predisposition toward disease. The set or specific amount that is therapeutically effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, *e.g.* the type of disease, the patient's history and age, the stage of disease, and the administration of other therapeutic agents. In some embodiments, modified insulins described herein are delivered to the body by injection or inhalation, or by other routes, and can reversibly bind to soluble glucose in a non-depot form. In some embodiments, modified insulins described herein are released over an extended period of time from a local depot in the body or from bound forms to proteins in the serum such as albumin. In some embodiments, the release of modified insulin is accelerated at elevated glucose levels, and in some embodiments such release rate may be dependent on blood sugar levels or levels of

other small molecules in the blood including diol containing molecules. In some embodiments the release, bioavailability, and/or solubility of modified insulins described herein is controlled as a function of blood or serum glucose concentrations or concentrations of other small molecules in the body.

5 Additionally, unless otherwise stated, structures described herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium (^2H) or tritium (^3H), or the replacement of a carbon by a ^{13}C - or ^{14}C -carbon atom are within the scope of this disclosure. Such compounds may be useful as, for example,
10 analytical tools, probes in biological assays, or therapeutic agents.

 In some embodiments, functional groups can be covalently conjugated or linked via any suitable covalent conjugation chemistry (linker) that can be used to covalently conjugate one functional group or amino acid side chain to another functional group, non-limiting examples include an amide, an ester, an ether, a thioether, an isourea, an imine, and a triazole linker. In
15 some embodiments, functional groups are covalently conjugated through click chemistry reactions as defined in the art. These include, for example, cycloaddition reactions including but not limited to 3+2 cycloadditions, strain-promoted alkyne-nitrone cycloaddition, reactions of strained alkenes, alkene and tetrazine inverse-demand Diels-Alder, Copper(I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC), thiol-maleimide addition, Strain-promoted azide-alkyne
20 cycloaddition, Staudinger ligation, nucleophilic ring-opening reactions, and additions to carbon-carbon multiple bonds. Some of these reactions are described for example by H. C. Kolb, M. G. Finn and K. B. Sharpless (2001); Click Chemistry: Diverse Chemical Function from a Few Good Reactions, *Angewandte Chemie International Edition* 40 (11): 2004–2021; Kolb and Sharpless, *Drug Discovery Today* 8:1128-1137, 2003; Huisgen, R. *Angew. Chem. Int. Ed. Engl.* 1963, 2,
25 565; Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* 2006, 1, 644. One skilled in the art will be capable of selecting suitable buffers, pH and reaction conditions for such click reactions. In some embodiments, covalent conjugation is the result of a “bioorthogonal reaction” as defined in the art. Such reactions are, for example, described by Sletten, Ellen M.; Bertozzi, Carolyn R. (2009). Bioorthogonal Chemistry: Fishing for Selectivity
30 in a Sea of Functionality, *Angewandte Chemie International Edition* 48 (38): 6974–98.; Prescher, Jennifer A; Bertozzi, Carolyn R (2005). Chemistry in living systems, *Nature Chemical Biology* 1 (1): 13–21.

 In some embodiments, functional groups may be linked using native chemical ligation as described for example by Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. (1994)
35 Synthesis of proteins by native chemical ligation, *Science* 266 (5186): 776–778. As used herein,

terms such as “linkage,” “covalent conjugation,” etc. may refer to any of the chemistries described above in some embodiments. The terms “amine,” “amino group,” and/or “amine group,” when used to describe part of a covalent bond or connectivity, may be interchangeably used to indicate an amino group or an amine group to which the described element is covalently
5 linked. In some embodiments, the amino group or amine group may be a primary amine, a secondary amine, or a fragment such as $\text{NH---}\oplus$ to which a conjugation is made and described.

In some embodiments, the amino group or amine group may be the NH_2 group at the N-terminus of a peptide or peptide chain, or the NH_2 group of a lysine side chain, but embodiments of the present disclosure are not limited thereto. In some embodiments, the connectivity of a first
10 group to a second group is described by reference to an amine or amino group, originating from the second group, that is part of a covalent linkage between the first group and the second group. For example, an amine of a lysine side chain on X1 may be referred to as an amine, and furthermore may be described as being conjugated through an amide bond in order to specify the structure and connectivity of the functional groups that constitute the covalent bond. If a covalent
15 linkage is via an amine bond or amine linkage, then it is referred to as an amine linkage. It is to be understood that a carbonyl connected to an amine (e.g., a $(\text{C}=\text{O})\text{-NH}$ moiety) constitutes an amide bond, and thus by definition, an amine linkage is not directly connected to a carbonyl group. Stated another way, the terms “amide bond” and/or “amide linkage,” when used to describe a covalent bond or connectivity, may be interchangeably used to indicate a carbonyl
20 connected to an amine (e.g., a $(\text{C}=\text{O})\text{-NH}$ moiety).

In some embodiments, further modifications include attachment of a chemical entity (e.g., moiety or functional group) such as a carbohydrate group, one or more cis-diol containing groups, one or more phosphate groups, one or more catechol groups, a farnesyl group, an isofarnesyl group, a fatty acid group, or a linker for conjugation, functionalization, or other
25 modifications meant to impact the pharmacokinetics, pharmacodynamics, and/or biophysical solution characteristics of insulin.

In some embodiments, a compound includes a human peptide hormone (e.g., as X1). In some embodiments, the peptide hormone is a polypeptide hormone of the human pancreas. In some embodiments, a compound, such as a compound of Formula I, includes a human insulin or
30 a human insulin analogue. In some embodiments, two different amine groups in insulin are covalently conjugated to as described by Formula I.

It will be understood that human peptide hormone, polypeptide hormone of the human pancreas, insulin, human insulin, modified insulin, and human insulin analogue may be used interchangeably in some of the described embodiments; that is, for example, in certain
35 embodiments “human insulin analogue” may instead be used in embodiments described as using

human insulin. In some embodiments, a compound, such as a compound of Formula I, includes a human insulin or a human insulin analogue. In some embodiments, a compound of Formula I includes a human insulin or a human insulin analogue as described by Formula I for $p'=1$ wherein a single amino group in insulin is conjugated to as described by Formula I. In some
5 embodiments, the amino group is the N-terminus of the B-chain of insulin or an amino group of the side chain of a lysine. In some embodiments, two or more different amine groups in insulin are each independently covalently conjugated to as described by Formula I. In some embodiments, at least one amine group is the N-terminus of the B-chain of insulin. In some embodiments, amino groups comprise amino groups of side chains of lysine residues in insulin.

10 Various suitable modifications of the peptide hormone (e.g., human polypeptide hormone, for example, Insulin), known to those having ordinary skill in the art, are included in the scope of the disclosure. In some embodiments, the polypeptide of Z1a or the optionally extended polypeptide at the N-terminus of B-chain or C-terminus of A-chain of insulin contain sequences with up to 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence
15 homology to a human polypeptide sequence. In some embodiments, the polypeptide of Z1a or the optionally extended polypeptide at the N-terminus of B-chain or C-terminus of A-chain of insulin contain one or more lysine residues that are optionally next to a proline residue, such that the proline is C-terminal to lysine. In some embodiments, the amino group of lysine residues is each independently conjugated as described by Formula I.

20 In some embodiments, insulin is further modified through conjugation to a sugar- or diol-containing molecule. In some embodiments, the human polypeptide hormone is a dual or triple hybrid peptide comprising sequences of two or more human peptide hormones and which can act through multiple receptors; for example, a glucose-dependent insulintropic polypeptide (GIP) and GLP-1 receptor agonist or GLP-1/GIP/glucagon triple agonist. In some embodiments, the
25 human polypeptide hormone is a gut hormone. In some embodiments, the human polypeptide hormone is chosen from c-peptide, adrenocorticotrophic hormone (ACTH), amylin, angiotensin, atrial natriuretic peptide (ANP), calcitonin, cholecystokinin (CCK), gastrin, ghrelin, glucagon, growth hormone, follicle-stimulating hormone (FSH), insulin, leptin, melanocyte-stimulating hormone (MSH), oxytocin, parathyroid hormone (PTH), prolactin, renin, somatostatin, thyroid-
30 stimulating hormone (TSH), thyrotropin-releasing hormone (TRH), vasopressin, vasoactive intestinal peptide, a neuropeptide, a peptide hormone that impacts cardiovascular health or appetite, a hybrid of one or more of these peptides, and an analogue of one of these peptides. In some embodiments, compounds comprise a human polypeptide hormone further modified, for example, through the covalent conjugation to polymers, XTEN protein sequences or aliphatic
35 chains. In some embodiments, polymer modified compounds have a longer circulation time in

the blood. In some embodiments, polymer modified compounds have a circulation time in the blood such that they are suitable for injection once a day injection, once a week injection, or once a month injection. In some embodiments, a human polypeptide hormone or the analogue thereof includes one or more L- or D- natural or unnatural amino acids that are each

5 independently one of the twenty canonical amino acids or a non-canonical amino acid.

In some embodiments, the insulin receptor agonist is an insulin analogue comprising an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, 24051, and 24052, and the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24061, 24062, 24063, 24064, and 25000 – 25397. In some embodiments, an insulin analogue
10 comprises an A-chain comprising sequence 24051 and a B-chain comprising a sequence selected from 25000, 25001, 25006-25009, 25076, 25077, 25082-25085, 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313. In some embodiments, an insulin analogue comprises an A-chain comprising sequence 24051 and a B-chain comprises a sequence selected from 25011, 25012, 25017-25020, 25087, 25088, 25093-25096, 25229, 25239, 25232, 25240,
15 25245-25248, 25305, 25308, 25315, 25316, and 25321-25324. In some embodiments, an insulin analogue comprises an A-chain comprising sequence 24051 and a B-chain comprising a sequence selected from 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313. In some embodiments, an insulin analogue comprises an A-chain comprising sequence 24051 and a B-chain comprising a sequence selected from 25011, 25012, 25017-25020, 25087,
20 25088, 25093-25096, 25229, 25232, 25305 and 25308. In some embodiments, an insulin analogue comprises a human insulin with an A-chain and a B-chain wherein up to six residues have been mutated, deleted or additionally inserted into each of the A-chain and/ or the B-chain. In some embodiments thereof, the insulin analogue includes insulins containing A- and B-chains with a connecting peptide connecting the C-terminus for the B-chain to the N-terminus for the A-
25 chain, and the connecting peptide includes the natural proinsulin C-peptide as well as shorter version of the C-peptide as is known in the art, wherein shorter versions of the C-peptide allow the single chain insulin to retain biological activity and/or potency.

The words analog and analogue are alternative spellings of the same word, are interchangeably used herein and have the same meaning. In the context of a human hormone, an
30 endocrine hormone, insulin, human insulin, glucagon, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide, glucose-dependent insulinotropic polypeptide, a hybrid peptide comprising sequences from two or more human polypeptide hormones, an analogue means any related sequences resulting from the parent sequence that includes up to 8 mutations, deletions or insertions of amino acids and/or additional chemical modifications. In
35 some embodiments, as is known in the art such analogs may enhance biological activity,

pharmacokinetics, pharmacodynamics, potency, stability and or chemical, and physical characteristics.

In some embodiments, a human hormone analog includes one or more residues that are 2-aminoisobutyric acid and/or other artificial (i.e., unnatural) amino acids.

5 In some embodiments, in an insulin analog the C-terminus of the B-chain of insulin is covalently conjugated to the N-terminus of the A-chain. In some embodiments, the C-terminus of the B-chain of insulin is covalently conjugated to the N-terminus of the A-chain and the connecting peptide is a C-peptide. In some embodiments, the C-terminus of the B-chain of insulin is covalently conjugated to the N-terminus of the A-chain and the connecting peptide is a
10 C-peptide and further includes any intermediate compounds that comprise a conjugate of Formula I.

In some embodiments, the insulin analog includes insulin lispro, or a glargine-type of modification, or any suitable modification to human insulin that impacts the pharmacokinetics or half-life of insulin in the body. In some embodiments, the insulin lispro used to prepare the
15 PEGylated insulin lispro compounds of the present invention may be prepared by any of a variety of recognized peptide synthesis techniques including solution-phase methods, solid-phase methods, semi-synthetic methods, and recombinant DNA methods. For example, U.S. Pat. No. 5,700,662 (Chance, et al.) and European Patent No. 214 826 (Brange, et al.), disclose the preparation of various insulin analogs. The A- and B-chains of insulin lispro may also be
20 prepared via a proinsulin-like precursor molecule using recombinant DNA techniques. In some embodiments, a proinsulin-like precursor is used to make the insulin lispro used to make the PEGylated insulin lispro of the present invention.

In some embodiments, the insulin portion of the present compounds may be prepared via production of a precursor protein molecule using recombinant DNA techniques. The DNA,
25 including cDNA and synthetic DNA, may be double-stranded or single-stranded. The coding sequences that encode the precursor protein molecule described herein may vary as a result of the redundancy or degeneracy of the genetic code. The DNA may be introduced into a host cell in order to produce the precursor protein of the present invention. An appropriate host cell is either transiently or stably transfected or transformed with an expression system for producing
30 the precursor protein. The host cells may be bacterial cells such as K12 or B strains of Escherichia coli, fungal cells such as yeast cells, or mammalian cells such as Chinese hamster ovary ("CHO") cells. The expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline, neomycin, and dihydrofolate reductase, to
35 permit selection of those cells transformed with the desired DNA sequences.

In some embodiments, the polypeptide hormone is glucagon. In some embodiments, glucagon has additional mutations and modifications that are known to impact solubility and solution stability of glucagon. In some embodiments, a compound, such as a compound of Formula I, comprises a conjugation of a Z1a to the N-terminus of the B-chain of insulin through a peptide bond, and at least one additional conjugation described by Formula I to insulin. In some embodiments, the additional conjugation is to a lysine residue in insulin. In some embodiments, at least one such lysine is a residue between position 15 and the C-terminus of the B-chain of insulin. In some embodiments, the lysine residue is optionally next to a proline, glycine, arginine, threonine or serine. In some embodiments, one or more amino acids in Formula I is a D-amino acid. In some embodiments, any secondary or primary amine in a compound, such as a compound represented by Formula I, is each independently optionally acetylated. In some embodiments, a compound of Formula I has a polypeptide hormone X1 further conjugated to a drug molecule, an imaging agent, a chelator, a contrast agent, a radioactive isotope or a molecule that engages immune cells.

In some embodiments, X1 is a polypeptide hormone comprising a peptide ligand that binds to an extracellular protein receptor. In some embodiments, X1 comprises a polypeptide analogue of a human polypeptide hormone that has at least 50% homology to a natural human polypeptide hormone. In some embodiments, X1 comprises a polypeptide analogue of a human polypeptide hormone that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% homology to a natural human polypeptide hormone. In some embodiments, X1 comprises a polypeptide analogue of a human polypeptide hormone that has at least 90% homology to a natural human polypeptide hormone. In some embodiments, X1 comprises a polypeptide analogue of a human polypeptide hormone that has at least 95% homology to a natural human polypeptide hormone. In some embodiments, X1 comprises a polypeptide analogue of a human polypeptide hormone that has at least 99% homology to a natural human polypeptide hormone. In some embodiments, X1 is an analogue of human insulin with up to 10 additional residues added to the A-chain or the B-chain of insulin.

In some embodiments, the term “glucose responsiveness” refers to the change in activity in the presence and absence of glucose or in a difference of lower levels and higher levels of glucose (e.g., 3 mM glucose vs 20 mM glucose). In some embodiments the activity of a conjugated insulin is assessed by the concentration of insulin (in nanomolar units (nM) of insulin) required to induce the half maximum response (EC50) in a cell-based assay. Lower EC50 concentrations of conjugated insulins have a higher activity than insulins with higher EC50 concentrations (e.g., an insulin with an EC50 of 3 nM is more active than an insulin with an EC50 of 50 nM). A “glucose response” is observed when the insulin changes from a less

active EC50 (higher nM) to a more active EC50 (lower nM) in the absence and presence of glucose or in lower and higher levels of glucose, respectively.

In some embodiments, the compound of Formula I comprises one or more L- or D-artificial amino acids which are not one of the twenty naturally occurring amino acids. In some
5 embodiments, the side chains of such artificial amino acids can be covalently conjugated through a number of reactions, including bio-orthogonal reactions such as, for example, described by: Rostovtsev, V.V., Green, L.G., Fokin, V.V. & Sharpless, K.B. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem. Int. Ed.* 41, 2596-2599 (2002), or by: Liang, Y., Mackey, J.L., Lopez, S.A., Liu, F. &
10 Houk, K.N. Control and design of mutual orthogonality in bioorthogonal cycloadditions. *J. Am. Chem. Soc.* 134, 17904-17907 (2012). In some embodiments, Z1a contains one or more L- or D-artificial amino acids that are not one of the twenty naturally occurring amino acids. In some embodiments, the side chains of two amino acids in Z1a are covalently conjugated together through a triazole bond.

15 Insulin hormone is an important regulator of blood glucose (sugar) levels. In a normal individual, insulin is present and, when released by the pancreas, it acts to reduce blood sugar levels, for example, by binding to and activating the insulin receptor, triggering glucose absorption by liver, fat, and skeletal muscle cells. Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases characterized by the persistence of high blood sugar
20 levels over a prolonged period.

As used herein, "insulin" encompasses both wild-type and altered forms of insulin capable of binding to and activating the insulin receptor, or capable of causing a measurable reduction in blood glucose when administered in vivo and encompasses both wild-type and altered forms of human insulin capable of binding to and activating the human insulin receptor,
25 or capable of causing a measurable reduction in blood glucose when administered in vivo to a human.

In some embodiments, insulin includes insulin from any species whether in purified, synthetic, or recombinant form and includes human insulin, porcine insulin, bovine insulin, sheep insulin and rabbit insulin. In some embodiments, insulin has two chains: a B- and an A-chain.
30 In some embodiments, the chains are connected together through peptides such as, for example, c-peptide as is known in the art, or a shortened version of the c-peptide, and in other embodiments the insulin may be provided as a proinsulin (insulin precursor) that can be further processed into mature insulin. A variety of altered forms of insulin are known in the art and may be chemically altered such as by addition of a chemical moiety such as a PEG group or a fatty
35 acyl chain. Altered insulins may be mutated including additions, deletions or substitutions of

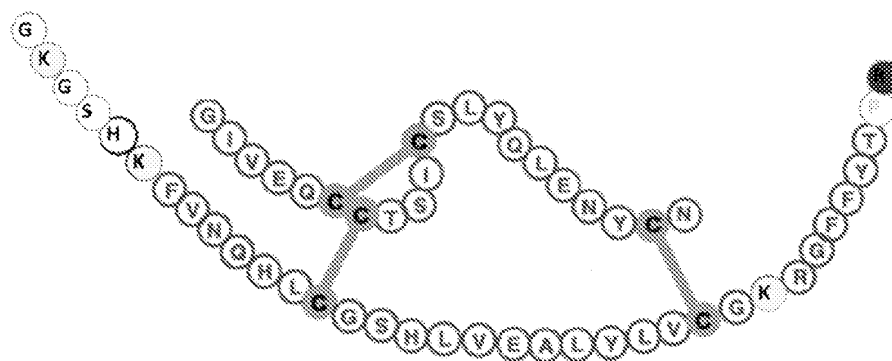
amino acids. In some embodiments the term “desB30” refers to an insulin lacking the B30 amino acid residue.

As used herein, the term “insulin analogue” means a modified human insulin having insulin receptor agonist activity wherein from one to 10 amino acid residues of the insulin have been modified (e.g., substituted, deleted, added (i.e. extended), inserted, and any combination thereof) relative to human insulin. In this context, an insertion or addition of one or more amino acids is considered a single modification. For example, an insulin analogue may have from one to 9 modified amino acid residues. As yet another example, an insulin analogue may have from one to 8 modified amino acid residues. As yet another example, an insulin analogue may have from one to 7 modified amino acid residues. As yet another example, an insulin analogue may have from one to 6 modified amino acid residues. As yet another example, an insulin analogue may have from one to 5 modified amino acid residues. As yet another example, an insulin analogue may have from one to 4 modified amino acid residues. As yet another example, an insulin analogue may have from one to 3 modified amino acid residues. As yet another example, an insulin analogue may have from one to 2 modified amino acid residues. In some embodiments, an insulin analogue may have 2, 3, 4, 5, 6, 7, 8, or 9 modified amino acid residues.

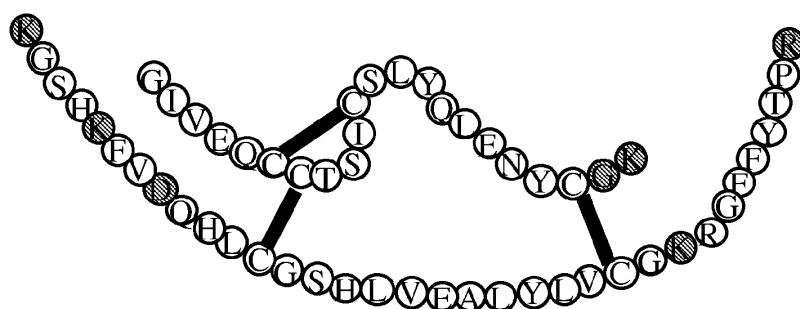
Modifications in the insulin molecule are denoted stating the chain (A or B), the position, and the one or three letter code for the amino acid residue substituting the native amino acid residue. Herein terms like “A1”, “A2” and “A3” etc. indicates the amino acid in position 1, 2 and 3 etc., respectively, in the A chain of insulin (counted from the N-terminal end). Similarly, terms like B1, B2 and B3 etc. indicates the amino acid in position 1, 2 and 3 etc., respectively, in the B chain of insulin (counted from the N-terminal end). Using the one letter codes for amino acids, terms like A21A, A21G and A21Q designates that the amino acid in the A21 position is A, G and Q, respectively. Using the three letter codes for amino acids, the corresponding expressions are A21Ala, A21 Gly, and A21 Gln, respectively.

Thus, for example, an insulin analogue having 4 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25737); and wherein the B-chain comprises sequence GKGSHK FVNQHLCGSHLVEALYLVC GKRGFFYTPR (SEQ ID NO: 25738), where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with GKGSHK (SEQ ID NO: 25726) (i.e. 6 amino acids have been added/appended to the N-terminus), and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K) in the B chain is substituted with R, and the amino acid in position 30 (i.e. Thr) in the B

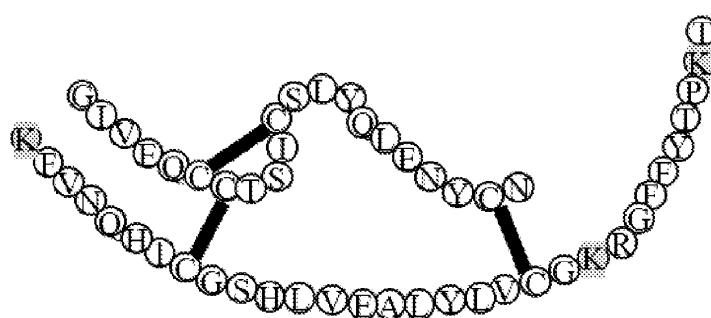
chain is deleted (as illustrated below (SEQ ID NOS 25737 and 25738, respectively, in order of appearance)).



- 5 As yet another example, an insulin analogue having 7 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCGK (SEQ ID NO: 25739); and wherein the B-chain comprises sequence KGSHKFVDQHLGSHLVEALYLVCGRGFFYTPR (SEQ ID NO: 25740), where in the A chain the amino acid in position 21 (i.e. N) is substituted with G, and the A chain is
- 10 extended at the C-terminal with K (i.e. 1 amino acid has been added/appended to the C-terminus), and the B chain has been extended at the N-terminal with KGSHK (SEQ ID NO: 25727) (i.e. 5 amino acids have been added/appended to the N-terminus), and where the amino acid in position 3 (i.e. N) in the B chain is substituted with D, and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K)
- 15 in the B chain is substituted with R, and the amino acid in position 30 (i.e. T) in the B chain is deleted (as illustrated below (SEQ ID NOS 25739 and 25740, respectively, in order of appearance)).

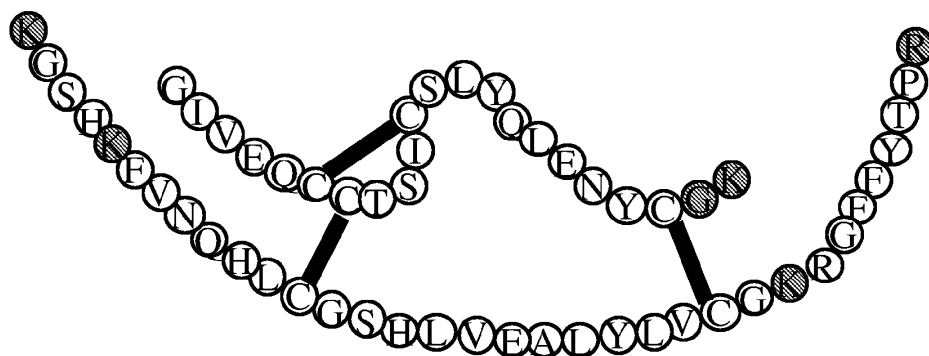


As yet another example, an insulin analogue having 2 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25731); and wherein the B-chain comprises sequence K FVNQHLCGSHLVEALYLVCCKRGFFYTPKT (SEQ ID NO: 25741), where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with K (i.e. 1 amino acid has been added/appended to the N-terminus), and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K (as illustrated below (SEQ ID NOS 25731 and 25741, respectively, in order of appearance)).



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As yet another example, an insulin analogue having 6 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCGK (SEQ ID NO: 25732); and wherein the B-chain comprises sequence KGSHK FVNQHLCGSHLVEALYLVCCKRGFFYTPR (SEQ ID NO: 25742), where in the A chain the amino acid in position 21 (i.e. N) is substituted with G, and the A chain is extended at the C-terminal with K (i.e. 1 amino acid has been added/appended to the C-terminus), and the B chain has been extended at the N-terminal with KGSHK (SEQ ID NO: 25727) (i.e. 5 amino acids have been added/appended to the N-terminus), and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K) in the B chain is substituted with R, and the amino acid in position 30 (i.e. T) in the B chain is deleted (as illustrated below (SEQ ID NOS 25732 and 25742, respectively, in order of appearance)).

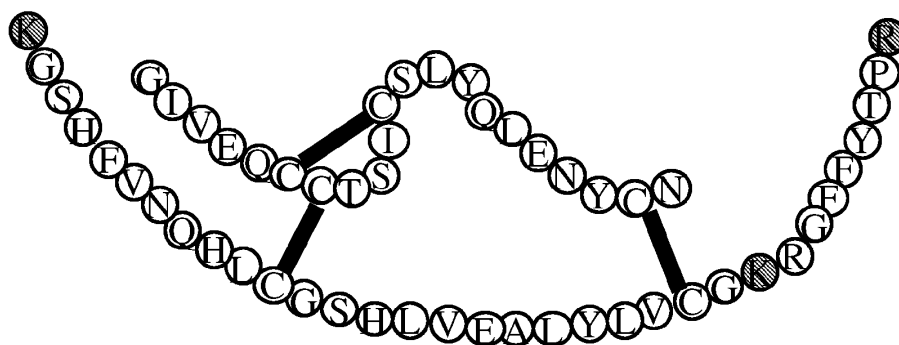


As yet another example, an insulin analogue having 4 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25733); and wherein the B-chain comprises sequence

5 KGSHFVNQHLVCGSHLVEALYLVCGRGFFYTTPR (SEQ ID NO: 25743); where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with KGSH (SEQ ID NO: 25728) (i.e. 4 amino acids have been added/appended to the N-terminus), and where the amino acid in position

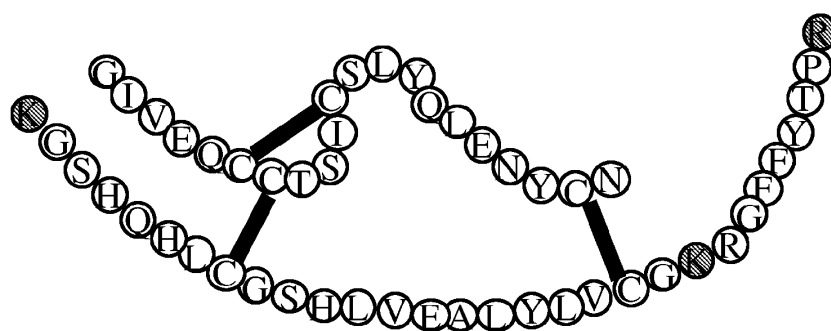
10 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K) in the B chain is substituted with R, and the amino acid in position 30 (i.e. Thr) in the B chain is deleted (as illustrated below (SEQ ID NOS 25733 and 25743, respectively, in order of appearance)).

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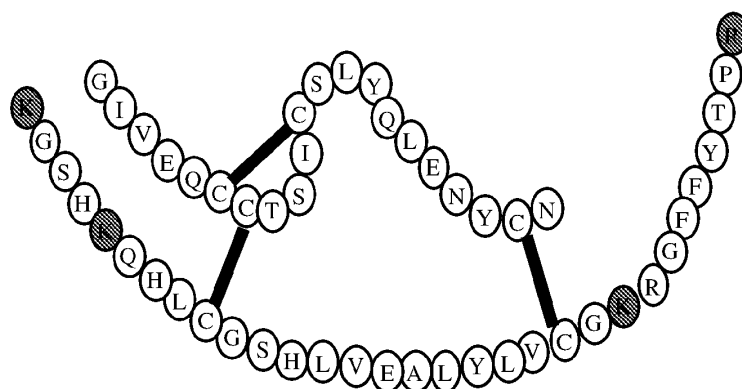


As yet another example, an insulin analogue having 5 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25734); and wherein the B-chain comprises sequence KGSHQHLGSHLVEALYLVCGRGFFYTPR (SEQ ID NO: 25744);

- 5 where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with KGSH (SEQ ID NO: 25728) (i.e. 4 amino acids have been added/appended to the N-terminus), and the first 3 residue (FVN) has been deleted, and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K) in the B chain is substituted with R, and the amino acid in position 30 (i.e. Thr) in the B chain is deleted (as illustrated below (SEQ ID NOS 25734 and 25744, respectively, in order of appearance)).

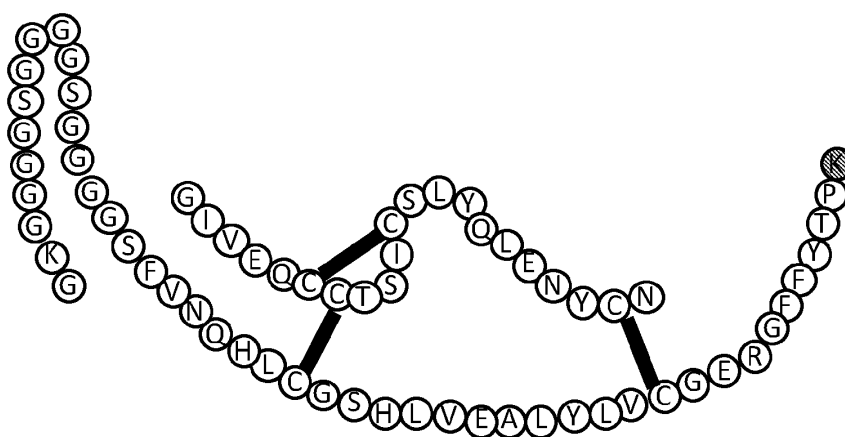


- 15 As yet another example, an insulin analogue having 5 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25735); and wherein the B-chain comprises sequence KGSHKQHLGSHLVEALYLVCGRGFFYTPR (SEQ ID NO: 25745); where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with KGSHK (SEQ ID NO: 25727) (i.e. 5 amino acids have been added/appended to the N-terminus), and the first 3 residue (FVN) has been deleted, and where the amino acid in position 21 (i.e. E) in the B chain is substituted with K, and the amino acid in position 29 (i.e. K) in the B chain is substituted with R, and the amino acid in position 30 (i.e. Thr) in the B chain is deleted (as illustrated below (SEQ ID NOS 25735 and 25745, respectively, in order of appearance)).
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As yet another example, an insulin analogue having 2 modifications comprises an A-chain and a B-chain, wherein the A-chain comprises sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 25736); and wherein the B-chain comprises sequence

- 5 GKGGGSGGGGSGGGGSGGGGSFVNQHLCSHLEALYLVCGERFFYTPK (SEQ ID NO: 25746), where the A chain has the wild-type sequence of chain A of human insulin (e.g. no mutations, deletions, additions) and the B chain has been extended at the N-terminal with GKGGGSGGGGSGGGGS (SEQ ID NO: 25729) (i.e. 17 amino acids have been added/appended to the N-terminus), and the amino acid in position 30 (i.e. Thr) in the B chain is
- 10 deleted (as illustrated below (SEQ ID NOS 25736 and 25746, respectively, in order of appearance)).



- In some embodiments, insulin analogues include insulin that is chemically altered as
- 15 compared to wild type human insulin, such as, but not limited to, by addition of a chemical moiety such as a PEG group or a fatty acyl chain. In some embodiments, altered insulins/ insulin

analogues/analogs, which are used herein interchangeably, may be mutated including additions, deletions or substitutions of amino acids. Different protomers of insulin may result from these changes and be incorporated into some embodiments. In some embodiments, active forms of insulins have fewer than 11 such modifications (e.g., 1-4, 1-3, 1-9, 1-8, 1-7, 1-6, 2-6, 2-5, 2-4, 1-5, 1-2, 2-9, 2-8, 2-7, 2-3, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-9, 4-8, 4-7, 4-6, 4-5, 5-9, 5-8, 5-7, 5-6, 6-9, 6-8, 6-7, 7-9, 7-8, 8-9, 9, 8, 7, 6, 5, 4, 3, 2 or 1). As used herein, the wild-type sequence of human insulin (A-chain and B-chain), has an A-chain with the amino acid sequence GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 1), and a B-chain having the amino acid sequence FVNQHLCGSHLVEALYLVCGERGFFYTPKT (SEQ ID NO: 2). In some

10 embodiments, an insulin analogue has at least 70% sequence homology to a wild-type human insulin. In some embodiments, an insulin analogue has at least 80% sequence homology to a wild-type human insulin. In some embodiments, an insulin analogue has at least 90% sequence homology to a wild-type human insulin. In some embodiments, an insulin analogue has at least 95%, 96%, 97%, or 95% sequence homology to a wild-type human insulin. In some

15 embodiments, an insulin analogue has at least 99% sequence homology to a wild-type human insulin.

Human insulin differs from rabbit, porcine, bovine, and sheep insulin in amino acids A8, A9, A10, and B30, which are in order the following: Thr, Ser, Ile, Thr for human; Thr, Ser, Ile, Ser for rabbit; Thr, Ser, Ile, Ala for porcine; Ala, Gly, Val, Ala for sheep; and Ala, Ser, Val, Ala

20 for bovine. In some embodiments, a modified insulin may be mutated at position B1, B2, B28 or B29, or at positions B28 and B29 of the B-chain. In some embodiments, a modified insulin may be mutated at A1, A2, A21 or other positions of the A-chain. For example, insulin lispro is a fast-acting modified insulin in which the lysine and proline residues on the C-terminal end of the B-chain have been reversed. Insulin aspart is a fast-acting modified insulin in which proline has

25 been substituted with aspartic acid at position B28. It is contemplated in some embodiments of the present disclosure that insulins mutated at B28 and B29 may further include additional mutations. For example, insulin glulisine is a fast-acting modified insulin in which aspartic acid has been replaced by a lysine residue at position B3, and lysine has been replaced by a glutamic acid residue at position B29. In some embodiments, longer acting and higher stability insulin

30 analogs are covalently modified as described by Formula I, and may contain mutations such as tyrosine at A14 replaced with glutamic acid, the tyrosine at B16 replaced with histidine, and the phenylalanine at B25 replaced with a histidine.

In some embodiments, the isoelectric point of insulins herein may be shifted relative to wild-type human insulin using any suitable method, for example by addition or substitution of

35 suitable amino acids. In some embodiments, the isoelectric point of the modified insulins may be

modulated by glucose (e.g., by interaction with glucose). For example, insulin glargine is a basal insulin in which two arginine residues have been added to the C-terminus of the B-peptide, and A21 has been replaced by glycine. In some embodiments, the insulin may not have one or more of the residues B1, B2, B3, B26, B27, B28, B29, and B30 (e.g., the insulin may be a deletion mutant at one or more of the listed residues). In some embodiments, the insulin molecule contains up to five additional amino acid residues on the N- or C-terminus of the A-chain or B-chain. In some embodiments, one or more amino acid residues are located at positions A1, A21, B1, B29, B30 and/or B31 or are missing. In some embodiments, an insulin molecule of the present disclosure is mutated such that one or more amino acids are replaced (substituted) with their acidic forms. In some embodiments, an asparagine is replaced with aspartic acid or glutamic acid. In some embodiments, glutamine is replaced with aspartic acid or glutamic acid. In some embodiments, A21 may be an aspartic acid, B3 may be an aspartic acid, or both positions may contain an aspartic acid. One skilled in the art will recognize that it is possible to make any previously reported, or widely accepted mutations or modifications to insulin that retains biological activity, and that such an insulin analogue can be used in embodiments of the present disclosure. In some embodiments, an insulin may be linked at any position to a fatty acid, or acylated with a fatty acid at any amino group, including those on lysine side chains and the alpha-amino group on the N-terminus of insulin, and the fatty acid may include a C8, C9, C10, C11, C12, C14, C15, C16, C17, or C18 chain. In some embodiments, the fatty acid chain is 8-20 carbons long. In some embodiments, is an insulin detemir, in which a myristic acid is covalently conjugated to lysine at B29, and B30 is deleted or absent. In some embodiments, position B28 of the insulin molecule is lysine and the epsilon(ϵ)-amino group of this lysine is conjugated to a fatty acid.

In some embodiments, the N- or C-terminal end of the A- or B-chain of the modified insulin is ligated using a peptide ligase. In some embodiments, a polypeptide is added to the C-terminus of the insulin A- and/or B-chain or to the N-terminus of insulin A- and/or B-chain using a protein ligase, and in some embodiments thereof the ligase is chosen from sortases, butelases, Trypsilgases, Subtilisins, Peptilgases or enzymes having at least 75% homology to these ligases. In some embodiments, ligation is achieved through expressed protein ligation as described in: Muir TW, Sondhi D, Cole PA. "Expressed protein ligation: a general method for protein engineering." *Proc Natl Acad Sci U S A*. 1998; 95(12):6705–6710. In some embodiments, the polypeptide is linked to the modified insulin using Staudinger ligation, utilizing the Staudinger reaction and as described for example in Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. (2000). "Staudinger ligation: A peptide from a thioester and azide". *Org. Lett.* 2 (13): 1939–1941. In some embodiments, a polypeptide is conjugated to the modified insulin

using Ser/Thr ligation as, for example, described in: Zhang Y, Xu C, Kam HY, Lee CL, Li X. 2013, "Protein chemical synthesis by serine/threonine ligation." *Proc. Natl. Acad. Sci. USA*. 17:6657-6662. In some embodiments, the B-chain itself has less than 32 amino acids or 34 amino acids, and in some embodiments the insulin has 4 disulfide bonds instead of 3. There are

5 disulfide bonds present in the A and B chains of insulin. For example, a disulfide bond exists between the cysteine at position 6 of SEQ ID NO:1 and the cysteine at position 11 of SEQ ID NO:1, a disulfide bond exists between the cysteine at position 7 of SEQ ID NO:1 and the cysteine at position 7 of SEQ ID NO:2, and a disulfide bond exists between the cysteine at position 20 of SEQ ID NO:1 and the cysteine at position 19 of SEQ ID NO:2.

10 In some embodiments, a modified insulin of the present disclosure comprises one or more mutations and/or chemical modifications including, but not limited to one of the following insulin molecules: N^{εB29}-octanoyl-Arg^{B0}Gly^{A21}Asp^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Arg^{A0}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Gly^{A21}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Gly^{A21}Gln^{B3}Lys^{B28}Pro^{B30}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Arg^{A0}Gly^{A21}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Arg^{A0}Gly^{A21}Gln^{B3}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Arg^{A0}Gly^{A21}Asp^{B3}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-myristoyl-Arg^{A0}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Gly^{A21}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Gly^{A21}Gln^{B3}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Arg^{A0}Gly^{A21}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB29}-palmitoyl-HI, N^{εB29}-myristoyl-HI, N^{εB28}-palmitoyl-Lys^{B28}Pro^{B29}-HI, N^{εB28}-myristoyl-Lys^{B28}Pro^{B29}-HI, N^{εB29}-palmitoyl-des(B30)-HI, N^{εB30}-myristoyl-Thr^{B29}Lys^{B30}-HI, N^{εB30}-palmitoyl-Thr^{B29}Lys^{B30}-HI, N^{εB29}-(N-palmitoyl-γ-glutamyl)-des(B30)-HI, N^{εB29}-(N-lithocolyl-γ-glutamyl)-des(B30)-HI, N^{εB29}-(ω-carboxyheptadecanoyl)-des(B30)-HI, N^{εB29}-(ω-carboxyheptadecanoyl)-HI, N^{εB29}-octanoyl-HI, N^{εB29}-myristoyl-Gly^{A21}Arg^{B31}Arg^{B31}-HI, N^{εB29}-myristoyl-Gly^{A21}Gln^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB29}-myristoyl-Arg^{A0}Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-Arg^{A0}Gly^{A21}Gln^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB29}-myristoyl-Arg^{A0}Gly^{A21}Asp^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB29}-myristoyl-Arg^{B31}Arg^{B32}-HI, N^{εB29}-myristoyl-Arg^{A0}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Gly^{A21}Gln^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Arg^{A0}Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-Arg^{A0}Gly^{A21}Gln^{B3}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Arg^{A0}Gly^{A21}Asp^{B3}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI, N^{εB28}-octanoyl-Arg^{A0}Lys^{B28}Pro^{B29}Arg^{B31}Arg^{B32}-HI. N^{εB29}-pentanoyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{αB1}-hexanoyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{αA1}-heptanoyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-octanoyl-N^{αB1}-octanoyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-propionyl-N^{αA1}-propionyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{αA1}-acetyl-N^{αB1}-acetyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-

35

formyl-N^{αA1}-formyl-N^{αB1}-formyl-Gly^{A21}Arg^{B31}Arg^{B32}-HI, N^{εB29}-formyl-des(B26)-HI, N^{αB1}-
 acetyl-Asp^{B28}-HI, N^{εB29}-propionyl-N^{αA1}-propionyl-N^{αB1}-propionyl-Asp^{B1}Asp^{B3}Asp^{B21}-HI, N^{εB29}-
 pentanoyl-Gly^{A21}-HI, N^{αB1}-hexanoyl-Gly^{A21}-HI, N^{αA1}-heptanoyl-Gly^{A21}-HI, N^{εB29}-octanoyl-N^{αB1}-
 octanoyl-Gly^{A21}-HI, N^{εB29}-propionyl-N^{αA1}-propionyl-Gly^{A21}-HI, N^{αA1}-acetyl-N^{αB1}-acetyl-Gly^{A21}-
 5 HI, N^{εB29}-formyl-N^{αA1}-formyl-N^{αB1}-formyl-Gly^{A21}-HI, N^{εB29}-butyryl-des(B30)-HI, N^{αB31}-
 butyryl-des(B30)-HI, N^{αA1}-butyryl-des(B30)-HI, N^{εB29}-butyryl-N^{αB31}-butyryl-des(B30)-HI,
 N^{εB29}-butyryl-N^{αA1}-butyryl-des(B30)-HI, N^{αA1}-butyryl-N^{αB31}-butyryl-des(B30)-HI, N^{εB29}-
 butyryl-N^{αA1}-butyryl-N^{αB31}-butyryl-des(B30)-HI, Lys^{B28}Pro^{B29}-HI (insulin lispro), Asp^{B28}-HI
 (insulin aspart), Lys^{B3}Glu^{B29}-HI (insulin glulisine), Arg^{B31}Arg^{B32}-HI (insulin glargine), N^{εB29}-
 10 myristoyl-des(B30)-HI (insulin detemir), Ala^{B26}-HI, Asp^{B1}-HI, Arg^{A0}-HI, Asp^{B1}Glu^{B13}-HI,
 Gly^{A21}-HI, Gly^{A21}Arg^{B31}Arg^{B32}-HI, Arg^{A0}Arg^{B31}Arg^{B32}-HI, Arg^{A0}Gly^{A21}Arg^{B31}Arg^{B32}-HI,
 des(B30)-HI, des(B27)-HI, des(B28-B30)-HI, des(B1)-HI, des(B1-B3)-HIN^{εB29}-tridecanoyl-
 des(B30)-HI, N^{εB29}-tetradecanoyl-des(B30)-HI, N^{εB29}-decanoyl-des(B30)-HI, N^{εB29}-dodecanoyl-
 des(B30)-HI, N^{εB29}-tridecanoyl-Gly^{A21}-des(B30)-HI, N^{εB29}-tetradecanoyl-Gly^{A21}-des(B30)-HI,
 15 N^{εB29}-decanoyl-Gly^{A21}-des(B30)-HI, N^{εB29}-dodecanoyl-Gly^{A21}-des(B30)-HI, N^{εB29}-tridecanoyl-
 Gly^{A21}Gln^{B3}-des(B30)-HI, N^{εB29}-tetradecanoyl-Gly^{A21}Gln^{B3}-des(B30)-HI, N^{εB29}-decanoyl-
 Gly^{A21}-Gln^{B3}-des(B30)-HI, N^{εB29}-dodecanoyl-Gly^{A21}-Gln^{B3}-des(B30)-HI, N^{εB29}-tridecanoyl-
 Ala^{A21}-des(B30)-HI, N^{εB29}-tetradecanoyl-Ala^{A21}-des(B30)-HI, N^{εB29}-decanoyl-Ala^{A21}-des(B30)-
 HI, N^{εB29}-dodecanoyl-Ala^{A21}-des(B30)-HI, N^{εB29}-tridecanoyl-Ala^{A21}-Gln^{B3}-des(B30)-HI, N^{εB29}-
 20 tetradecanoyl-Ala^{A21}Gln^{B3}-des(B30)-HI, N^{εB29}-decanoyl-Ala^{A21}Gln^{B3}-des(B30)-HI, N^{εB29}-
 dodecanoyl-Ala^{A21}Gln^{B3}-des(B30)-HI, N^{εB29}-tridecanoyl-Gln^{B3}-des(B30)-HI, N^{εB29}-
 tetradecanoyl-Gln^{B3}-des(B30)-HI, N^{εB29}-decanoyl-Gln^{B3}-des(B30)-HI, N^{εB29}-dodecanoyl-Gln^{B3}-
 des(B30)-HI, N^{εB29}-Z1-Gly^{A21}-HI, N^{εB29}-Z2-Gly^{A21}-HI, N^{εB29}-Z4-Gly^{A21}-HI, N^{εB29}-Z3-Gly^{A21}-
 HI, N^{εB29}-Z1-Ala^{A21}-HI, N^{εB29}-Z2-Ala^{A21}-HI, N^{εB29}-Z4-Ala^{A21}-HI, N^{εB29}-Z3-Ala^{A21}-HI, N^{εB29}-Z1-
 25 Gly^{A21}Gln^{B3}-HI, N^{εB29}-Z2-Gly^{A21}Gln^{B3}-HI, N^{εB29}-Z4-Gly^{A21}Gln^{B3}-HI, N^{εB29}-Z3-Gly^{A21}Gln^{B3}-HI,
 N^{εB29}-Z1-Ala^{A21}Gln^{B3}-HI, N^{εB29}-Z2-Ala^{A21}Gln^{B3}-HI, N^{εB29}-Z4-Ala^{A21}Gln^{B3}-HI, N^{εB29}-Z3-
 Ala^{A21}Gln^{B3}-HI, N^{εB29}-Z1-Gln^{B3}-HI, N^{εB29}-Z2-Gln^{B3}-HI, N^{εB29}-Z4-Gln^{B3}-HI, N^{εB29}-Z3-Gln^{B3}-HI,
 N^{εB29}-Z1-Glu^{B30}-HI, N^{εB29}-Z2-Glu^{B30}-HI, N^{εB29}-Z4-Glu^{B30}-HI, N^{εB29}-Z3-Glu^{B30}-HI, N^{εB29}-Z1-
 Gly^{A21}Glu^{B30}-HI, N^{εB29}-Z2-Gly^{A21}Glu^{B30}-HI, N^{εB29}-Z4-Gly^{A21}Glu^{B30}-HI, N^{εB29}-Z3-Gly^{A21}Glu^{B30}-
 30 HI, N^{εB29}-Z1-Gly^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z2-Gly^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z4-
 Gly^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z3-Gly^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z1-Ala^{A21}Glu^{B30}-HI, N^{εB29}-Z2-
 Ala^{A21}Glu^{B30}-HI, N^{εB29}-Z4-Ala^{A21}Gln^{B30}-HI, N^{εB29}-Z3-Ala^{A21}Glu^{B30}-HI, N^{εB29}-Z1-
 Ala^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z2-Ala^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z4-Ala^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-
 Z3-Ala^{A21}Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z1-Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z2-Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z4-

Gln^{B3}Glu^{B30}-HI, N^{εB29}-Z3-Gln^{B3}Glu^{B30}-HI and where Z1 is tridecanoyl, Z2 is tetradecanoyl, Z3 is dodecanoyl, Z4 is decanoyl, and HI is human insulin.

In some embodiments, insulin includes one or more of the following mutations and/or chemical modifications: N^{εB28}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{αB1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{αA1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{εB28}-XXXXX-N^{αB1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{εB28}-XXXXX-N^{αA1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{αA1}-XXXXX-N^{αB1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{εB28}-XXXXX-N^{αA1}-XXXXX-N^{αB1}-XXXXX-Lys^{B28}Pro^{B29}-HI, N^{εB29}-XXXXX-HI, N^{αB1}-XXXXX-HI, N^{αA1}-XXXXX-HI, N^{εB29}-XXXXX-N^{αB1}-XXXXX-HI, N^{εB29}-XXXXX-N^{αA1}-XXXXX-HI, N^{αA1}-XXXXX-N^{αB1}-XXXXX-HI, N^{εB29}-XXXXX-N^{αA1}-XXXXX-N^{αB1}-XXXXX-HI, N^{εB29}-YYYYY-HI, N^{αB1}-YYYYY-HI, N^{αA1}-YYYYY-HI, N^{εB29}-YYYYY-N^{αB1}-YYYYY-HI, N^{εB29}-YYYYY-N^{αA1}-YYYYY-HI, N^{αA1}-YYYYY-N^{αB1}-YYYYY-HI, N^{εB29}-YYYYY-N^{αA1}-YYYYY-N^{αB1}-YYYYY-HI, N^{εB28}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{εB21}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{αA1}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{εB28}-YYYYY-N^{αB1}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{εB28}-YYYYY-N^{αA1}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{αA1}-YYYYY-N^{αB1}-YYYYY-Lys^{B28}Pro^{B29}-HI, N^{εB28}-YYYYY-N^{αA1}-YYYYY-N^{αB1}-YYYYY-Lys^{B28}Pro^{B29}-HI, and where YYYYY is one of acetyl or formyl and where XXXXX is one of: propionyl, butyryl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl or decanoyl and HI is human insulin.

In some embodiments, insulin may be conjugated through a reactive moiety that is naturally present within the insulin structure or is added prior to conjugation, including, for example, carboxyl or reactive ester, amine, hydroxyl, aldehyde, sulfhydryl, maleimidyl, alkynyl, azido, etc. moieties. Insulin naturally includes reactive alpha-terminal amine and epsilon-amine lysine groups to which NHS-ester, isocyanates or isothiocyanates can be covalently conjugated. In some embodiments, a modified insulin may be employed in which a suitable amino acid (e.g., a lysine or a non-natural amino acid) has been added or substituted into the amino acid sequence in order to provide an alternative point of conjugation in addition to the modified amino acids of the embodiments described herein. In some embodiments, the conjugation process may be controlled by selectively blocking certain reactive moieties prior to conjugation. In some embodiments, insulin may include any combination of modifications and the present disclosure also encompasses modified forms of non-human insulins (e.g., porcine insulin, bovine insulin, rabbit insulin, sheep insulin, etc.) that comprise any one of the aforementioned modifications. It is understood that some embodiments include these and other previously described modified insulins such as those described in United States patent numbers 5,474,978; 5,461,031; 4,421,685; 7,387,996; 6,869,930; 6,174,856; 6,011,007; 5,866,538; 5,750,4976; 906,028; 6,551,992; 6,465,426; 6,444,641; 6,335,316; 6,268,335; 6,051,551; 6,034,054; 5,952,297; 5,922,675; 5,747,642; 5,693,609; 5,650,486; 5,547,929; 5,504,188; US 2015/0353619, including

non-natural amino acids described or referenced herein and including such modifications to the non-human insulins described herein. It is also to be understood that in some embodiments the insulin may be covalently conjugated to polyethylene glycol polymers, such as polyethylene glycol polymers of no more than Mn 60,000, or covalently conjugated either through permanent
5 or reversible bonds to albumin.

In some embodiments, a compound of the present disclosure (e.g., Formula I) is conjugated to a chelator, and in some embodiments the chelator can be used to capture a radioactive payload, such as gallium 68, copper 64, lutetium 177, or actinium 225. In some embodiments, the chelator is based on DOTA, NOTA, TETA, or 4-arm DOTA, and in some
10 embodiments, the chelator can be linked to the peptide using a PEG linker through amide bonds to the chelator and to the peptide.

In some embodiments, the activity, bioavailability, solubility, isoelectric point, charge and/or hydrophobicity of the modified insulins can be controlled through chemical modifications and/or as result of interaction of a small molecule such as a sugar with the compounds, such as
15 the compounds described herein which are either covalently linked or mixed with insulin.

In some embodiments one or more elements, functional groups, or atoms may be specifically omitted or excluded from a depicted structure (e.g., a terminal functional group may be replaced by a hydrogen atom, or a linking group may be replaced by a bond), for example in Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A,
20 FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226, and it will be understood that such omitted or excluded elements make these groups (structures) distinct and non-equivalent. For example, if an alternative version (variation) of a formula structure does not have a nitro group in R1 for B1 or B2, that variation is not equivalent (e.g., is structurally and chemically inequivalent) to a structure that includes the
25 nitro group, at least because the nitro group changes the pKa of B1 and B2 in physiological conditions and hence the overall affinity of Z1c for glucose.

Rotationally Constrained Tethered Boron Conjugates.

In some embodiments, aromatic boron-containing compounds and/or aromatic boron-
30 containing groups are rotationally constrained tether boron conjugates. In some embodiments, rotationally constrained tether boron conjugates presented in this disclosure contain scaffolds that are rotationally hindered by disfavored steric interactions (e.g. gauche vs anti interactions of substituents), hindered rotation due to bond hybridization (e.g., cis- vs trans- amide rotations), or through rigid covalent bonds (e.g., (E) vs (Z) configurations for alkene moieties). For example,
35 formula FF116 contains alkyl functionalities geminal (e.g., attached to the same atom) to the

amine groups that are covalently conjugated to the boronic acid functionalized moieties. Alkyl functionalities may limit the accessible dihedral angles and the rotation freedom around the C-C or C-X bond (commonly referred to as χ (chi) dihedral angles in amino acids). For example, the hydroxyl sidechain on a serine residue can access dihedral angles of 60°, 180°, or 240° (-60°) with near equal distribution while the hydroxyl sidechain of threonine may only adopt dihedral angles of 180° or 240° (-60°). The presence of a methyl group geminal to the hydroxy on threonine may provide steric bulk, creating unfavorable interactions when other bulky substituents are in a gauche conformation relative to the methyl. Formula FF116 contains geminal alkyl substituents which may limit the accessible dihedral angles that the boron conjugated amines adopt, influencing adopted dihedral angles and placing the boronic functionalized groups closer together and allowing for increased binding of the conjugates to target molecules such as proteins or sugars.

In some embodiments, the activity (glucose responsiveness) of the insulin agonist is controlled by the three-dimensional structure of the sensor. For example, FF116A, FF115A, FF225A are constitutional isomers (contain the same molecular formula but different linkages between atoms). In some embodiments, when used with the same linker (FL3) and same insulin backbone (i.e., same A chain (i.e., seq: 1) and same B chain (i.e., seq: 24060)) in Example 23, Example 82, and Example 27, these compounds exhibit different responsiveness to differences in glucose, with Example 23 showing the largest fold change (responsiveness) to glucose over Example 82 and Example 27. In some embodiments, the presence of a methyl group on the FF scaffold (e.g., FF116A, FF225A) has greater potency (e.g., lower EC₅₀) than the non-methyl FF scaffold (e.g., FF115A). Example 23 has a fold change of about 7 to 8-fold in response to glucose change of 3 mM to 20 mM glucose, whereas example 27 has a fold change of about 6 and example 82 has a fold change of about 4 in the same range of glucose change. Therefore, the presence of the methyl group in the scaffolds FF116A (having a beta methyl) and FF225A (having an alpha methyl) provide improvement in glucose responsiveness over FF115A (no methyl on the FF scaffold), and moreover glucose responsiveness provided by scaffold FF116A is better than FF225A. As another comparison, a compound having an insulin backbone (i.e., A chain seq: 24051 and B chain seq: 24061) with scaffold FF116A and linker FL3, as shown in example 156, has a diboronate scaffold 3-((2*S*,3*S*)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid which provides a fold change of 4.0 in response to glucose changes of 3 mM to 20 mM. Whereas if in the same example 156 the diboronate sensor is changed to (*S*)-3-(2,3-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)propanamido)propanoic acid, the fold change observed is 3.6 in response to glucose changes of 3 mM to 20 mM. This shows that the

diboronate sensor 3-((2*S*,3*S*)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid (having a methyl on the FF scaffold) provides superior glucose responsiveness on insulin to an insulin containing the sensor (*S*)-3-(2,3-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)propanamido)propanoic acid (no methyl on the FF scaffold).

In some embodiments, the stereochemistry of isomeric structures (e.g., the stereochemistry of a compound (for example within the Z1c moiety)) may selectively increase the affinity of the conjugate (e.g., the Z1c moiety) for a specific target diol, such as glucose. For example, in some embodiments one or more stereoisomers (e.g., *cis*- or *trans*-, (*R*) or (*S*), and (*E*) or (*Z*)) of Z1c may be selected to increase or decreases the affinity of Z1c (and the molecular architecture or conjugate as a whole) for glucose. In some embodiments, the *cis* form of Formulae FF12, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable (e.g., Z1c includes a structure having *cis* stereochemistry). In some embodiments, the *trans* form of Formulae FF12, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when these Formulae include two stereocenters linked by a bond, (e.g., Z1c includes a structure having *trans* stereochemistry). In some embodiments, the *R* form of Formulae FF12, FF114, FF114A, FF115, FF115A, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include at least one stereocenter, (e.g., Z1c includes a structure having *R* stereochemistry). In some embodiments, the *S* form of Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include at least one stereocenter, (e.g., Z1c includes a structure having *S* stereochemistry). In some embodiments, the *S,S* form of Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include two stereocenters linked by a bond, (e.g., Z1c includes a structure having *S,S* stereochemistry). In some embodiments, the *S,R* form of Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include two stereocenters linked by a bond, (e.g., Z1c includes a structure having *S,R* stereochemistry). In some embodiments, the *R,R* form of Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include two stereocenters linked by a bond, (e.g., Z1c includes a structure having *R,R* stereochemistry). In some embodiments, the *R,S* form

of Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 is used when applicable, e.g., when Formulae FF12, FF114, FF115, FF116, FF117, FF193A, FF203, FF225, and FF226 include two stereocenters linked by a bond, (e.g., Z1c includes a structure having *R,S* stereochemistry). In some embodiments, a compound includes one or more tautomers
5 of a compound disclosed herein. In some embodiments, a compound includes one or more stereoisomers or a mixture of stereoisomers of a compound disclosed herein.

In some embodiments, a compound is covalently conjugated to glucagon, GLP-1, GLP-2 or a variation of any of these (e.g., any variation with deletions, insertions and/or replacements of one or more amino acids). In some embodiments any suitable chemical modifications made to
10 insulin discussed herein can be made to glucagon. In some embodiments the conjugate is mixed with a second or drug substance or one or more compounds chosen from: aminoethylglucose, aminoethylbimannose, aminoethyltrimannose, D-glucose, D-galactose, D-Allose, D-Mannose, D-Gulose, D-Idose, D-Talose, N-Azidomannosamine (ManNAz) or N-Azidogalactoseamine (GalNAz) or N-azidoglucoseamine (GlcNAz), 2'-fluororibose, 2'-deoxyribose, glucose, sucrose,
15 maltose, mannose, derivatives of these (e.g., glucosamine, mannosamine, methylglucose, methylmannose, ethylglucose, ethylmannose, etc.), sorbitol, inositol, galactitol, dulcitol, xylitol, arabitol and/or higher order combinations of these (such as linear and/or branched bimannose, linear and/or branched trimannose), molecules containing cis-diols, catechols, tris, and DOPA molecules such as L-DOPA or L-3,4-dihydroxyphenylalanine.

Moreover, one skilled in the art will recognize that in some embodiments, one or more of suitable proteinogenic artificial amino acids can be used (included) in Z1a. For example, in some
20 embodiments one or more of the following artificial amino acids can be used based on the methods described in and referenced through, and the list of amino acids provided in: Liu, C. C.; Schultz, P. G. (2010). "Adding new chemistries to the genetic code." *Annual Review of Biochemistry* 79: 413–44. One skilled in the art will recognize that, in some embodiments,
25 artificial amino acids can be incorporated by peptide synthesis in Z1a which is then covalently conjugated to the drug or insulin, and these include the amino acids referenced herein as well as previously reported non-proteinogenic amino acids. In some embodiments, artificial amino acids exist (e.g., may be included) in the insulin, and in some embodiments thereof, proteinogenic
30 artificial amino acids can be incorporated through recombinant protein expression using suitable methods and approaches, including those described in United States patent and patent applications including: US 2008/0044854, US 8518666, US 8980581, US 2008/0044854, US 20140045261, US 2004/0053390, US 7229634, US 8236344, US 2005/0196427, US 2010/0247433, US 7198915, US 7723070, US 2002/0042097, US 2004/0058415, US
35 2008/0026422, US 2008/0160609, US 2010/0184193, US 2012/0077228, US 2014/025599, US

7198915, US 7632492, US 7723070, and other proteinogenic artificial amino acids may be introduced recombinantly using methods and approaches described in: US 7736872, US 7816320, US 7829310, US 7829659, US 7883866, US 8097702, US 8946148.

In some embodiments, cyclic amino acids such as 3-hydroxyproline, 4-hydroxyproline, aziridine-2-carboxylic acid, azetidine-2-carboxylic acid, piperidine-2-carboxylic acid, 3-carboxymorpholine, 3-carboxy-thiomorpholine, 4-oxaproline, pyroglutamic acid, 1,3-oxazolidine-4-carboxylic acid, 1,3-thiazolidine-4-carboxylic acid, 3-thiaproline, 4-thiaproline, 3-selenoproline, 4-selenoproline, 4-ketoproline, 3,4-dehydropyridine, 4-aminoproline, 4-fluoroproline, 4,4-difluoroproline, 4-chloroproline, 4,4-dichloroproline, 4-bromoproline, 4,4-dibromoproline, 4-methylproline, 4-ethylproline, 4-cyclohexylproline, 3-phenylproline, 4-phenylproline, 3,4-phenylproline, 4-azidoproline, 4-carboxyproline, α -methylproline, α -ethylproline, α -propylproline, α -allylproline, α -benzylproline, α -(4-fluorobenzyl)-proline, α -(2-chlorobenzyl)-proline, α -(3-chlorobenzyl)-proline, α -(2-bromobenzyl)-proline, α -(4-bromobenzyl)-proline, α -(4-methylbenzyl)-proline, α -(diphenylmethyl)-proline, α -(naphthylmethyl)-proline, D-proline, or S-homoproline, (2S, 4S)-4-fluoro-L-proline, (2S, 4R)-4-fluoro-L-proline, (2S)-3,4-dehydro-L-proline, (2S, 4S)-4-hydroxy-L-proline, (2S, 4R)-4-hydroxy-L-proline, (2S,4S)-4-azido-L-proline, (2S)-4,4-difluoro-L-proline, (2S)-azetidine-2-carboxylic acid, (2S)-piperidine-2-carboxylic acid, or (4R)-1,3-thiazolidine-4-carboxylic acid can be used in the molecular architecture that is conjugated to insulin.

It is to be understood that in some embodiments, a specific orientation of amino acids is achieved using, for example, methods of Albericio, F. (2000). *Solid-Phase Synthesis: A Practical Guide* (1 ed.). Boca Raton: CRC Press. p. 848. In some embodiments a compound of the present disclosure, such as a compound of Formula I or Formula II, can bind to a diol, a catechol, a hexose sugar, glucose, xylose, fucose, galactosamine, glucosamine, mannosamine, galactose, mannose, fructose, galacturonic acid, glucuronic acid, iduronic acid, mannuronic acid, acetyl galactosamine, acetyl glucosamine, acetyl mannosamine, acetyl muramic acid, 2-keto-3-deoxyglycero-galacto-nononic acid, acetyl neuraminic acid, glycolyl neuraminic acid, a neurotransmitter, dopamine, or a disaccharide or polymer of saccharides or diols.

In some embodiments, modifications or intermediates may include the use of an N-methyliminodiacetic acid (MIDA) group to make a MIDA conjugated boronate or a MIDA boronate; such modifications can be used during preparation of boronates towards the final structures of use (e.g., in embodiments of methods for preparing the conjugates described herein). In some embodiments boronic acid pinacol esters are used towards the final structures wherein the pinacol group can be readily removed using standard techniques by one skilled in the art. The MIDA-protected boronate esters are easily handled, stable under air, compatible with

chromatography, and unreactive under standard anhydrous cross-coupling conditions and easily deprotected at room temperature under mild aqueous basic conditions such as 1M NaOH, or even NaHCO₃, or as described by Lee, S. J. et al. (2008). *J. Am. Chem. Soc.* 130:466.

The biological mechanism by which wild type insulin binds to the insulin receptor is previously reported in Menting, J.G. et al. (2013). *Nature* 493, 241-245; and Menting, J.G. et al. (2014). "Protective hinge in insulin opens to enable its receptor engagement." *Proc. Natl. Acad. Sci. U.S.A.* 111, E3395-3404. The activity of such insulin can be measured using any suitable technique, for example, by using *in vitro* insulin receptor binding with TyrA14-¹²⁵I human insulin as tracer and utilizing antibody binding beads with an insulin receptor monoclonal antibody. In some embodiments, animal models can be used for *in vivo* assessment of insulin activity during glucose challenge using methods that are known to one skilled in the art. In some embodiments, a compound disclosed herein is partially or fully expressed along with a recombinant protein of interest such as insulin. The processes for expression of insulin in *E. coli* are known and can be easily performed by one skilled in the art e.g., by using the procedures outlined in Jonasson (1996). *Eur. J. Biochem.* 236:656-661; Cowley (1997). *FEBS Lett.* 402:124-130; Cho (2001). *Biotechnol. Bioprocess Eng.* 6: 144- 149; Tikhonov (2001). *Protein Exp. Pur.* 21: 176-182; Malik (2007). *Protein Exp. Pur.* 55: 100-111; and Min (2011). *J. Biotech.* 151:350-356. In the most common process, the protein is expressed as a single-chain proinsulin construct with a fission protein or affinity tag. The compound (e.g., a compound of Formula I) that includes Z1a when Z1a is present can be expressed as part of proinsulin, then modified chemically to conjugate, through amide linkages, to structures of interest. This approach provides good yield and reduces experimental complexity by decreasing the number of processing steps and allows refolding in a native-like insulin, see for example, Jonasson, *Eur. J. Biochem.* 236:656-661 (1996); Cho, *Biotechnol. Bioprocess Eng.* 6: 144- 149 (2001); Tikhonov, *Protein Exp. Pur.* 21 : 176-182 (2001); Min, *J. Biotech.* 151 :350-356 (2011)). When expressed in *E. coli*, proinsulin is usually found in inclusion bodies and can be easily purified by one skilled in the art.

In some embodiments, proinsulin can be expressed using standard IPTG (isopropylthio- β -galactoside) induction of IPTG inducible expression constructs and vectors in *e coli* strains such as B21 strain. As an example, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation.

Expression of the desired proinsulin can be performed *via* known procedures in the art. *See, e.g.*, U.S. Pat. Nos. 5457066, 5700662, 5514646, 9050371, and 10400021.

In some embodiments, a compound of the present disclosure (e.g. Formula I, Formula II) may be formulated for injection. For example, it may be formulated for injection into a subject, such as a human. In some embodiments, the composition may be a pharmaceutical composition, such as a sterile, injectable pharmaceutical composition. In some embodiments, the composition may be formulated for subcutaneous injection. In some embodiments, the composition is formulated for transdermal, intradermal, transmucosal, nasal, inhalable or intramuscular administration. In some embodiments, the composition may be formulated in an oral dosage form or a pulmonary dosage form. Pharmaceutical compositions suitable for injection may include sterile aqueous solutions containing, for example, sugars, polyalcohols such as mannitol and sorbitol, phenol, *meta*-cresol, and sodium chloride and dispersions may be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils and the carrier can, for example, be a solvent or dispersion medium containing, for example, water, saccharides, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. One skilled in the art recognizes that specific formulations can be developed to best suit the application and method of use of the molecular architectures of the invention. General considerations in the formulation and manufacture of pharmaceutical compositions, routes of administering and including suitable pharmaceutically acceptable carriers may be found, for example, in *Remington's Pharmaceutical Sciences*, 19th ed., Mack Publishing Co., Easton, Pa., 1995. In some embodiments, the pharmaceutical composition may include zinc, *e.g.*, Zn^{2+} along with insulin if the compound (e.g. a compound of Formula I) comprises insulin. Such zinc formulations are, for example, described in United States Patent No. 9,034,818. For example, the pharmaceutical composition may comprise zinc at a molar ratio to the modified insulin of about M:N where M is 1-11 and N is 6-1. In some embodiments, such modified insulins may be stored in a pump, and that pump being either external or internal to the body releases the modified insulins. In some embodiments, a pump may be used to release a constant amount of modified insulin wherein the insulin is glucose responsive and can automatically adjust activity based on the levels of glucose in the blood and/or the release rate from the injection site. In some embodiments, the compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. In some embodiments, the pharmaceutical composition may further include a second insulin type to provide fast-acting or basal-insulin in addition to the effect afforded by the molecular architecture. In some embodiments, a compound of the present disclosure (e.g., a compound of Formula I) is injected

separately from insulin but modulates the activity of insulin by binding to insulin, and in some embodiments this activity change is dependent on glucose.

In some embodiments, the pharmaceutical composition comprises one or more of the compounds disclosed herein and at least one additional component selected from
5 pharmaceutically acceptable carriers, pharmaceutically acceptable vehicles, and pharmaceutically acceptable excipients. In some embodiments, the pharmaceutical composition comprises a compound of Formula I and at least one additional component selected from pharmaceutically acceptable carriers, pharmaceutically acceptable vehicles, and pharmaceutically acceptable excipients.

10 In some embodiments, the present disclosure includes compounds that can be part of a kit, wherein the kit includes a compound of Formula I comprising modified insulin, as well as a pharmaceutically acceptable carrier, and for injections may include a syringe or pen. In some embodiments, a kit may include a syringe or pen that is pre-filled with a pharmaceutical composition that includes the compound of Formula I with a liquid carrier. Alternatively, a kit
15 may include a separate container such as a vial with a pharmaceutical composition that includes the compound of Formula I with a dry carrier and an empty syringe or pen. In some embodiments, such a kit may include a separate container that has a liquid carrier, which can be used to reconstitute a given composition that can then be taken up into the syringe or pen. In some embodiments, a kit may include instructions. In some embodiments, the kit may include
20 blood glucose measuring devices that either locally or remotely calculate an appropriate dose of the modified insulin that is to be injected at a given point in time, or at regular intervals. Such a dosing regimen is unique to the patient and may, for example, be provided as instruction to program a pump either by a person or by a computer. The kit may include an electronic device which transfers blood glucose measurements to a second computer, either locally or elsewhere
25 (for example, in the cloud) which then calculate the correct amount of compound of Formula I comprising, e.g., a modified insulin that needs to be used by the patient at a certain time.

In some embodiments, the invention relates to a method for treating a disease or condition in a subject, comprising administering to the subject a composition including a compound described herein. In some embodiments, the disease or condition may be
30 hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, metabolic syndrome X, or dyslipidemia, diabetes during pregnancy, pre-diabetes, Alzheimer's disease, MODY 1, MODY 2 or MODY 3 diabetes, mood disorders and psychiatric disorders. It will be appreciated that this combination approach may also be used with insulin resistant patients who are receiving an insulin sensitizer or a secondary drug for diabetes (such as, for example, a

biguanide such as metformin, a glitazone) or/and an insulin secretagogue (such as, for example, a sulfonylurea, GLP-1, exendin-4 etc.) or amylin.

In some embodiments, a compound of the present disclosure (e.g. a compound of Formula I) may be administered to a patient who is receiving at least one additional therapy or taking at least one additional drug or therapeutic protein. In some embodiments, the at least one additional therapy is intended to treat the same disease or disorder as the administered compound (e.g. a compound of Formula I). In some embodiments, the at least one additional therapy is intended to treat a side-effect of the compound (e.g. a compound of Formula I) or as an adjuvant. The timeframe of the two therapies may differ or be the same; they may be administered on the same or different schedules as long as there is a period when the patient is receiving a benefit from both therapies. The two or more therapies may be administered within the same or different formulations as long as there is a period when the patient is receiving a benefit from both therapies. Any of these approaches may be used to administer more than one anti-diabetic drug to a subject.

In some embodiments a therapeutically effective amount of the compound (e.g. a compound of Formula I) which is sufficient amount to treat (meaning for example to ameliorate the symptoms of, delay progression of, prevent recurrence of, or delay onset of) the disease or condition at a reasonable benefit to risk ratio will be used. In some embodiments, this may involve balancing of the efficacy and additional safety to toxicity. By additional safety, it is meant that, for example, the compound (e.g. a compound of Formula I) can be responsive to changes in blood glucose levels or level of other molecules, even when the patient is not actively monitoring the levels of that molecule, such as blood glucose levels at a given timeframe, for example during sleep. In some embodiments, therapeutic efficacy and toxicity may be determined by standard pharmacological procedures in cell cultures or in vivo with experimental animals, and for example measuring ED₅₀ and LD₅₀ for therapeutic index of the drug. In some embodiments, the average daily dose of insulin with the molecular architecture is in the range of 5 to 400 U, (for example 30-150 U where 1 Unit of insulin is about 0.04 mg). In some embodiments, an amount of compound (e.g. a compound of Formula I) with these insulin doses is administered on a daily basis or bi-daily basis or by every three days or by every 4 days. In some embodiments, the basis is determined by an algorithm, which can be computed by a computer. In some embodiments, an amount of compound, such as a compound of Formula I, with 5 to 10 times these doses is administered on a weekly basis or at regular intervals. In some embodiments, an amount of conjugate with 10 to 20 times these doses is administered on a bi-weekly basis or at regular intervals. In some embodiments, an amount of compound (e.g. a compound of Formula I) with 20 to 40 times these doses is administered on a monthly basis or at

regular intervals. In some embodiments, the C-terminus of the A-chain of insulin may be further extended with a peptide (amino acid sequence) including 1-20 amino acid residues. In some embodiments, the insulin analogue is a desB30 insulin.

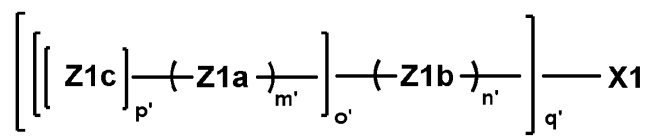
In some embodiments, Z1a is an amino acid or a peptide. In at least some embodiments, the Z1a includes (is composed of) 1-50 amino acid residues, for example, 1 residue, 50 residues, or any intermediate number of residues (such as e.g., 10, 15, 25, 30, 42 residues, etc.). In some embodiments, the Z1a includes 1-15 amino acids. In at least some embodiments, the peptide Z1a includes 1-8 amino acids. In some embodiments, Z1a includes 5 to 6 amino acids. In some embodiments, Z1a comprises at least one amino acid independently selected from: Alanine (A), Asparagine (N), Glutamine (Q), Threonine (T), Methionine (M), Histidine (H), Cysteine (C), Valine (V), Isoleucine (I), Lysine (K), and Leucine (L), and the rest of the amino acids are each independent selected from any of the twenty naturally occurring amino acids. In some embodiments, Z1a may include diaminopropionic acid, diaminobutyric acid, or ornithine.

In some embodiments, Z1a includes 1 to 5 lysine residues (K). In some embodiments, Z1a includes 1 to 3 K amino acids. In some embodiments Z1a includes 5 to 6 amino acids and at least one or more amino acids are K. In some embodiments, Z1a includes 5 to 6 amino acids and 1 to 3 amino acids are K.

In some embodiments Z1a is appended to the N-terminus and/or C-terminus, and/or inserted into the sequence of the A-chain or B-chain of insulin.

Compounds

In some embodiments, provided herein are compounds of Formula I, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt thereof:



(Formula I)

wherein:

X1 comprises:

- i. a drug substance comprising an amine;
 - ii. an amine configured to be covalently conjugated to a drug substance;
- or

- iii. NH₂ or OH (e.g. X1 is NH₂ or OH);

each Z1a independently comprises 1 to 50 amino acids connected together using amide or peptide bonds;

each Z1b is independently a small-molecule linker;

each Z1c is independently selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226, wherein each
 5 Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH;

each m' is 0 or 1;

each n' is 0;

each o' is 1;

10 each p' is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and

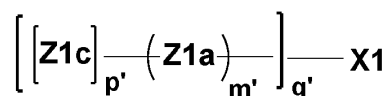
each q' is 1, 2, 3, 4, or 5, wherein when p' or q' is 2 or more, each corresponding Z1c is independently selected and may be the same or different and wherein when q' is 2 or more, each corresponding Z1a is independently selected and may be the same or different; and

15 when m'=0, each Z1c is covalently conjugated either directly or via an indirect linker to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH, and for m'=1 each Z1a is covalently conjugated via an amide or peptide bond to X1 and each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to X1.

20 In some embodiments, the compound is selected from a compound represented by Formula I, a stereoisomer thereof, a mixture of stereoisomers thereof, and pharmaceutically acceptable salt thereof, with the proviso that the compound is not any of Examples 1-880 disclosed in PCT/US2021/059802.

In some embodiments, X1 is a polypeptide comprising an insulin receptor agonist having an A-chain and a B-chain.

25 In some embodiments, the present disclosure is directed to a compound of the following formula, or a stereoisomer or a mixture of stereoisomers, or a pharmaceutically acceptable salt thereof:



wherein:

30 X1 comprises a polypeptide comprising an insulin receptor agonist having an A-chain and a B-chain;

each Z1a independently comprises 1 to 50 amino acids connected together using amide or peptide bonds;

each Z1c is independently selected from Formulae FF12A, FF12B, FF114A, FF115A, FF116A, and FF225A, wherein each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to an amine of X1 or to OH when X1 is OH;

each m' is 0 or 1;

5 each p' is 1, 2, 3, 4, or 5; and

each q' is 1, 2, 3, 4, or 5, wherein when p' or q' is 2 or more, each corresponding Z1c is independently selected and may be the same or different and wherein when q' is 2 or more, each corresponding Z1a is independently selected and may be the same or different;

when m'=0, each Z1c is covalently conjugated either directly or via an indirect linker to an amine of X1 or to OH when X1 is OH, when m'=1 each Z1a is covalently conjugated via an amide or peptide bond to X1 and each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a; and

wherein one or more positions of the compound may comprise an isotope.

In some embodiments, at least one Z1c comprises an alpha methyl group or a beta methyl group. In some embodiments, Z1c comprises an alpha methyl group. In some embodiments, Z1c comprises a beta methyl group.

In some embodiments, the compound comprises:

at least one Z1c is covalently conjugated via the indirect linker to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine of Z1a,

20 the indirect linker is represented by Formula (X'')_{n1}, wherein

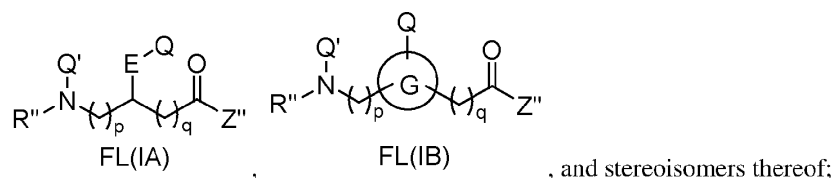
each n1 is independently selected from 1, 2, 3, 4, and 5, and

each X'' is independently selected from:

i. an L- or D-amino acid, wherein an amine functional group of the L- or D-amino acid is covalently conjugated, directly or indirectly, to Z1c and an acid functional group of the L- or D- amino acid is conjugated, directly or indirectly, to X1 or to Z1a; and

ii. Formulae FL(IA), FL(IB), FL69, and FL70;

wherein Formula FL(IA) and FL(IB) are:



30 wherein:

G is selected from a 3- to 6-membered cycloalkyl group, a 3- to 10-membered heterocyclyl group, a heteroaryl group, and an aryl group, wherein each group is

optionally substituted with 1-3 groups independently selected from hydroxy, amino, halogen, cycloalkyl (e.g., (C₃-C₆)cycloalkyl), alkoxy, and alkyl (e.g., C₁-C₆alkyl);

E is absent or is an alkylene group optionally substituted with 1-3 groups independently selected from halogen, hydroxy, and amino;

5 Q is absent or is selected from hydrogen, alkyl (e.g., C₁-C₆alkyl), halo, cyano, alkoxy, carboxylic acid, amino, hydroxy, amide, halo alkyl (e.g., C₁-C₆haloalkyl), cycloalkyl (e.g., (C₃-C₆)cycloalkyl), heterocycle, heteroaryl, and aryl, wherein the alkyl, alkoxy, cycloalkyl, heterocycle, heteroaryl, and aryl is each optionally substituted with 1-5 groups independently selected from alkyl (e.g., C₁-C₆alkyl), amino, amide, halo, hydroxy, cyano, halo alkyl (e.g., C₁-C₆haloalkyl), and alkoxy;

Q' is selected from hydrogen, alkyl (e.g., C₁-C₆alkyl), and an acyl group;

Q and Q', together with the carbon and nitrogen atom to which they are attached, optionally form a 4-membered heterocyclyl, a 5-membered heterocyclyl, a 6-membered heterocyclyl, a 9-membered bicyclic heterocyclyl, or a 10-membered bicyclic heterocyclyl, wherein the 4-membered heterocyclyl, 5-membered heterocyclyl, 6-membered heterocyclyl, 9-membered bicyclic heterocyclyl, and 10-membered bicyclic heterocyclyl are each optionally substituted with 1-5 groups independently selected from alkyl (e.g., C₁-C₆alkyl), amino, halo, hydroxy, cyano, amide, halo alkyl (e.g., C₁-C₆haloalkyl), and alkoxy;

20 p is 0, 1, 2, 3, 4, or 5;

q is 0, 1, 2, 3, 4, or 5;

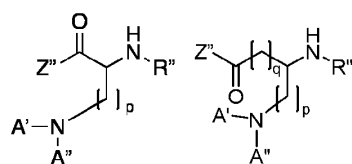
R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a; and

any primary amine is optionally acetylated or alkylated;

25

wherein Formulae FL69, and FL70 are:



FL69

FL70

, and stereoisomers thereof; wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, an alkyl (e.g., C₁-C₆alkyl), a saturated fatty acid, an unsaturated fatty acid, a cycloalkyl (e.g., (C₃-C₆)cycloalkyl), a haloalkyl (e.g., C₁-C₆ haloalkyl), an aryl, and a heteroaryl; and

A'' is selected from an alkyl (e.g., C₁-C₆alkyl), a substituted acyl, acyl terminating in an acid, a saturated or unsaturated fatty acid, a cycloalkyl (e.g., (C₃-C₆)cycloalkyl), a haloalkyl (e.g., C₁-C₆haloalkyl), an aryl, and a heteroaryl;

p is 1, 2, 3, 4, or 5,

q is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

In some embodiments, the compound comprises:

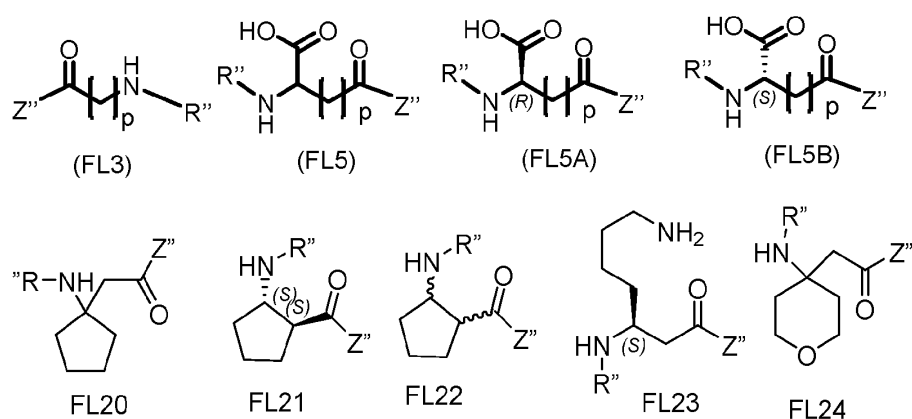
at least one Z1c covalently conjugated via the indirect linker to X1 or to Z1a, wherein the indirect linker is represented by Formula (X'')_{n1},

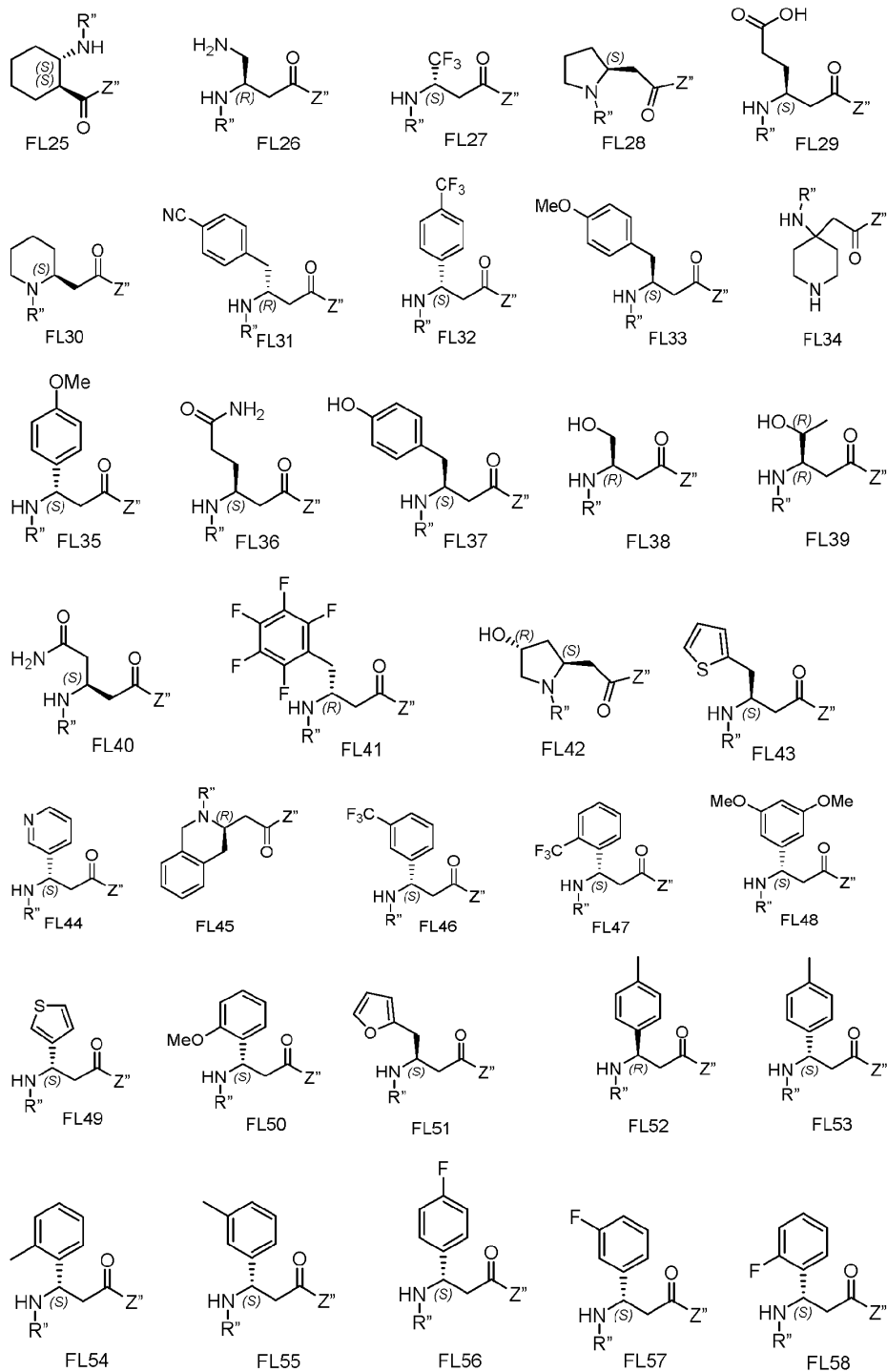
each n1 is independently selected from 1, 2, 3, 4, or 5, and

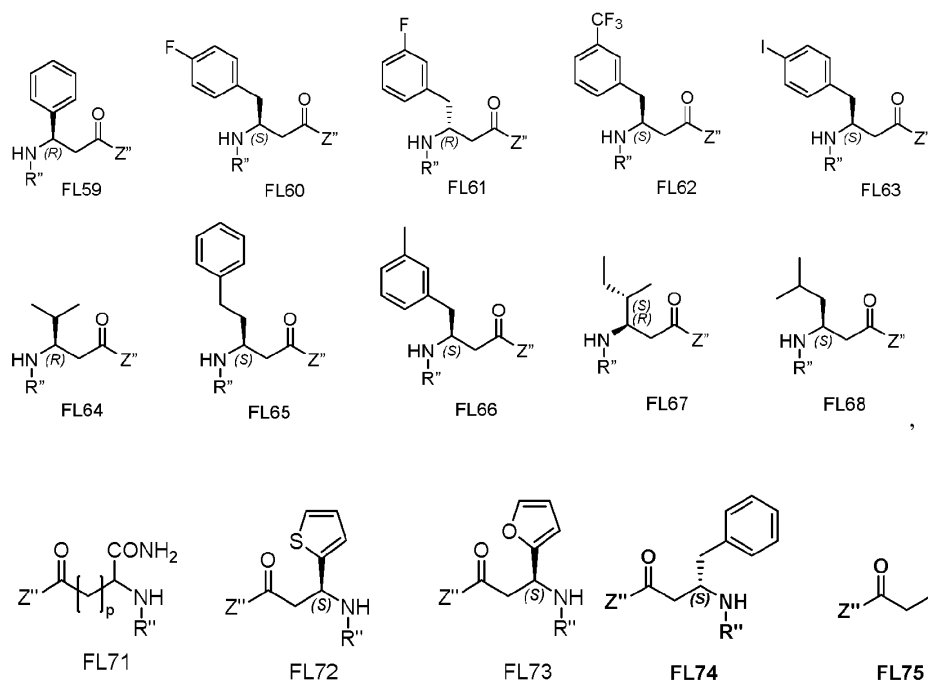
each X'' is independently selected from:

an L- or D-amino acid, wherein an amine functional group of the L- or D-amino acid is covalently conjugated, directly or indirectly, to Z1c and an acid functional group of the L- or D- amino acid is conjugated, directly or indirectly, to X1 or to Z1a; and

Formulae FL(IA), FL(IB), FL69, and FL70, wherein Formulae FL(IA) and FL(IB) are selected from Formulae FL3, FL5, FL5A, FL5B, FL20-FL68, and FL71-FL75:







5 and stereoisomers thereof;

wherein:

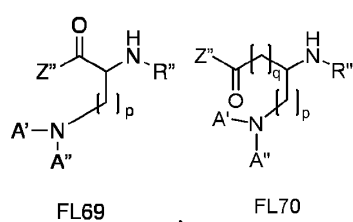
p is 1, 2, 3, 4, or 5,

q is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

10

Wherein Formulae FL69, and FL70 are:



, and stereoisomers thereof; wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, an alkyl group (e.g., C₁-C₆alkyl group), a saturated or unsaturated fatty acid, a cycloalkyl group (e.g., C₃-C₆cycloalkyl group), a haloalkyl group (e.g., C₁-C₆haloalkyl group), an aryl group, and a heteroaryl group; and

15

A'' is selected from alkyl group (e.g., C₁-C₆alkyl group), a substituted acyl group, acyl group terminating in an acid group, a saturated or unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl group), a haloalkyl group (e.g., C₁-C₆haloalkyl group), an aryl group, and a heteroaryl group;

5 p is 1, 2, 3, 4, or 5,

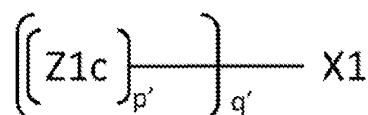
q is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

In some embodiments, the stereochemistry of FL70 can be *S* or *R* at the stereogenic carbon. In some embodiments, the stereochemistry of FL70 is *S* at the stereogenic carbon. In some embodiments, the stereochemistry of FL70 is *R* at the stereogenic carbon.

In some embodiments, n₁ is 1.

In some embodiments, the compound is represented by Formula IC:



15

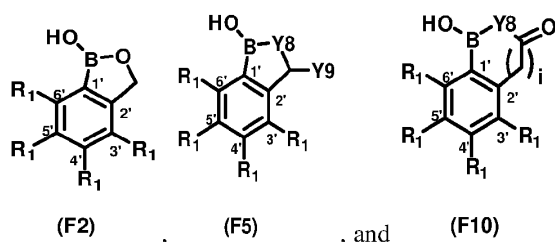
IC.

each p' is 1, 2, 3, or 4; and

each q' is 1, 2, 3, 4, or 5.

In some embodiments, each B₁ and B₂ is independently selected from Formulae F2, F5, and F10,

wherein Formulae F2, F5, and F10 are:



wherein:

25 one R₁ represents (C=O)---* or (CH₂)_m(C=O)---*, wherein ---* represents the attachment point to the rest of Z1c, and m is 1, 2, 3, 4, 5, 6, or 7; and

each remaining R_1 is independently selected from H, F, Cl, Br, I, OH, $\text{CH}_2\text{-NH}_2$, NH_2 , $(\text{C=O})\text{-NH}_2$, CH=O , SO_2CH_3 , SO_2CF_3 , CF_3 , CHF_2 , NO_2 , CH_3 , OCH_3 , $\text{O}(\text{CH}_2)_{m''}\text{CH}_3$, $(\text{SO}_2)\text{NH-CH}_3$, $(\text{SO}_2)\text{NH}(\text{CH}_2)_{m''}\text{CH}_3$, and OCF_3 , wherein m'' is 1, 2, 3, 4, 5, 6, or 7;

Y8 is O, N, or NR, wherein R is an alkyl group (e.g., $\text{C}_1\text{-C}_6$ alkyl group) or H;

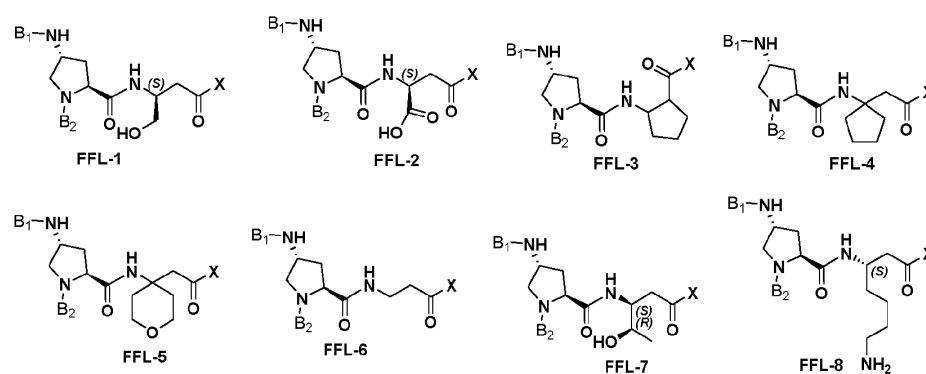
5 Y9 is H, CH_3 , or an alkyl group (e.g., $\text{C}_1\text{-C}_6$ alkyl group), provided that when Y8 is O, the Y9 is a CH_3 or an alkyl group (e.g., $\text{C}_1\text{-C}_6$ alkyl group); and

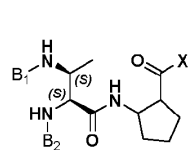
i is 1, 2, or 3.

In some embodiments, the B_1 and the B_2 are selected from Formula F2, and each remaining R_1 is independently selected from H, CF_3 and F; and either each remaining R_1 is H, or
10 one or two of the each remaining R_1 is F or CF_3 .

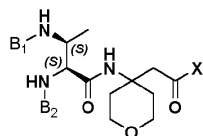
In some embodiments, the at least one Z1c is selected from FF12, FF12A-D, FF116, and FF116A-D. In some embodiments, both B_1 and B_2 are selected from F2, wherein all remaining R_1 are H; and the indirect linker is selected from a relatively hydrophobic indirect linker. In some embodiments, the relatively hydrophobic indirect linker may be selected from FL3, FL20-
15 22, FL25, FL27-28, FL30-33, FL35, FL37, FL41, FL43-70, and amino acids having a hydrophobic side chain. In some embodiments, both B_1 and B_2 are selected from F2, wherein one of the remaining R_1 is CF_3 and the remaining R_1 is H; and the indirect linker is selected from a relatively hydrophilic indirect linker. In some embodiments, the relatively hydrophilic indirect linker may be selected from FL5, FL5A, FL5B, FL23-24, FL26, FL29, FL34, FL36,
20 FL38-40, FL42, and amino acids having a hydrophilic side chain.

In some embodiments, each Z1c is covalently conjugated to an indirect linker and each of the Z1c and the indirect linker in combination (i.e., a Z1c-Linker) is selected from Formulae FFL-1 to FFL-101:

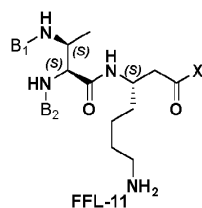




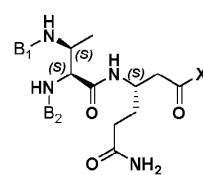
FFL-9



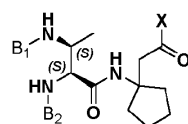
FFL-10



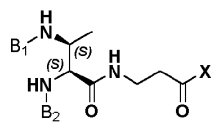
FFL-11



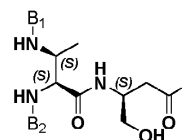
FFL-12



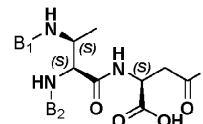
FFL-13



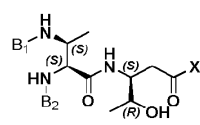
FFL-14



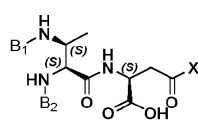
FFL-15



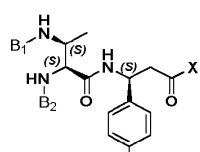
FFL-16



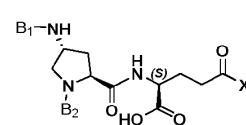
FFL-17



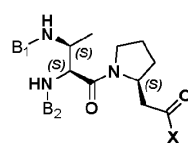
FFL-18



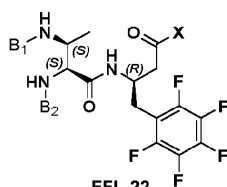
FFL-19 OMe



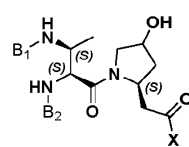
FFL-20



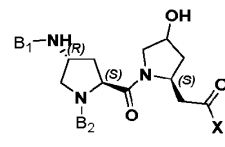
FFL-21



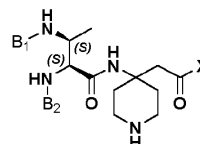
FFL-22



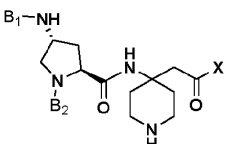
FFL-23



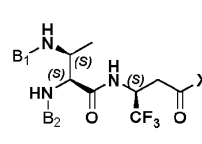
FFL-24



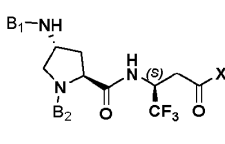
FFL-25



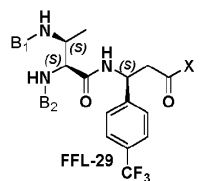
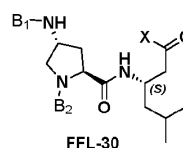
FFL-26



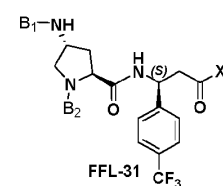
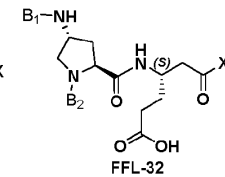
FFL-27



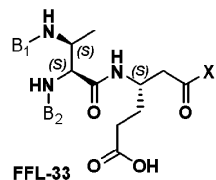
FFL-28

FFL-29 CF₃

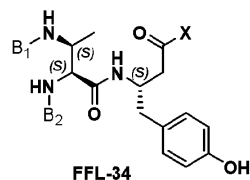
FFL-30

FFL-31 CF₃

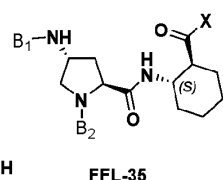
FFL-32



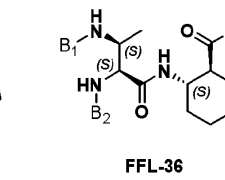
FFL-33



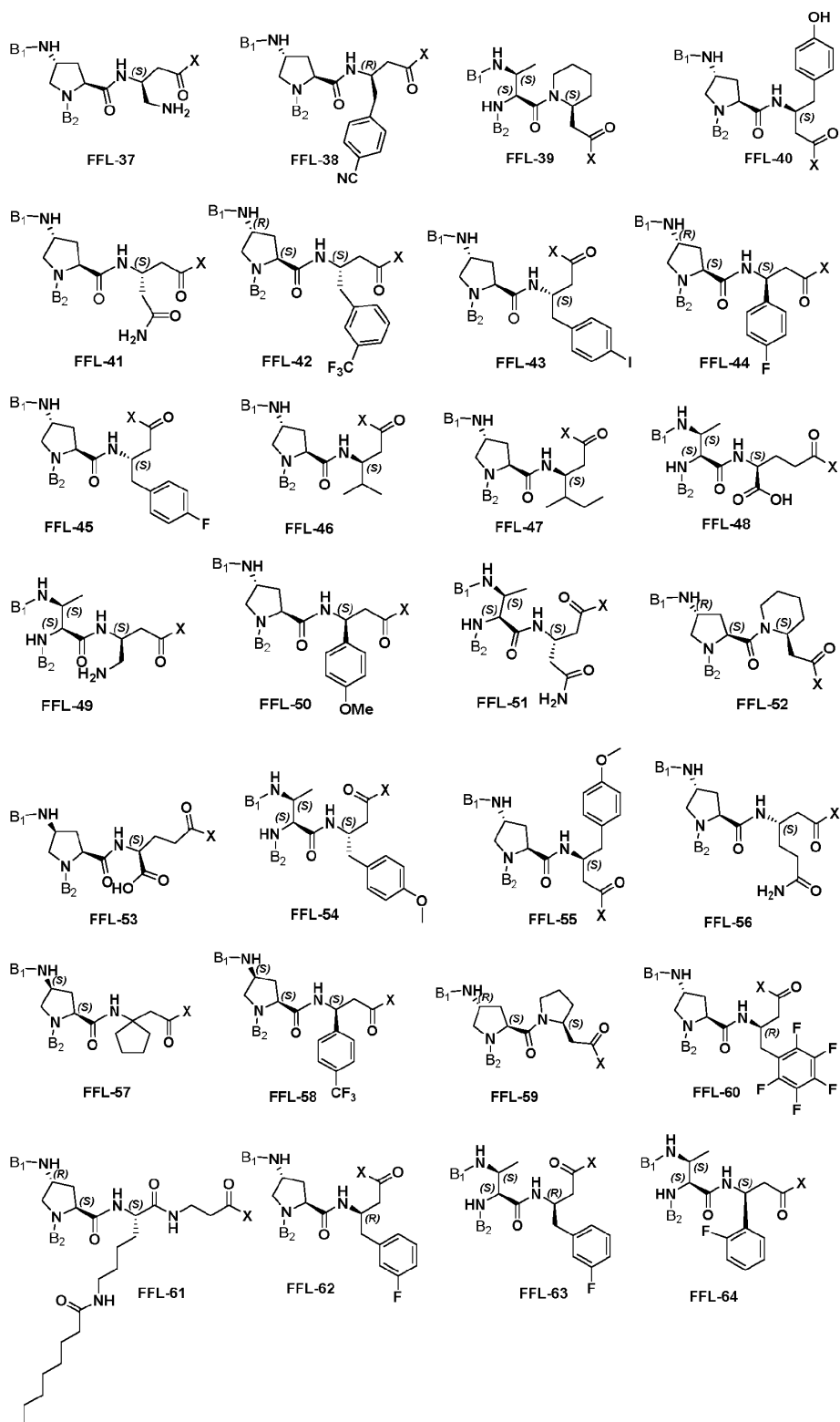
FFL-34

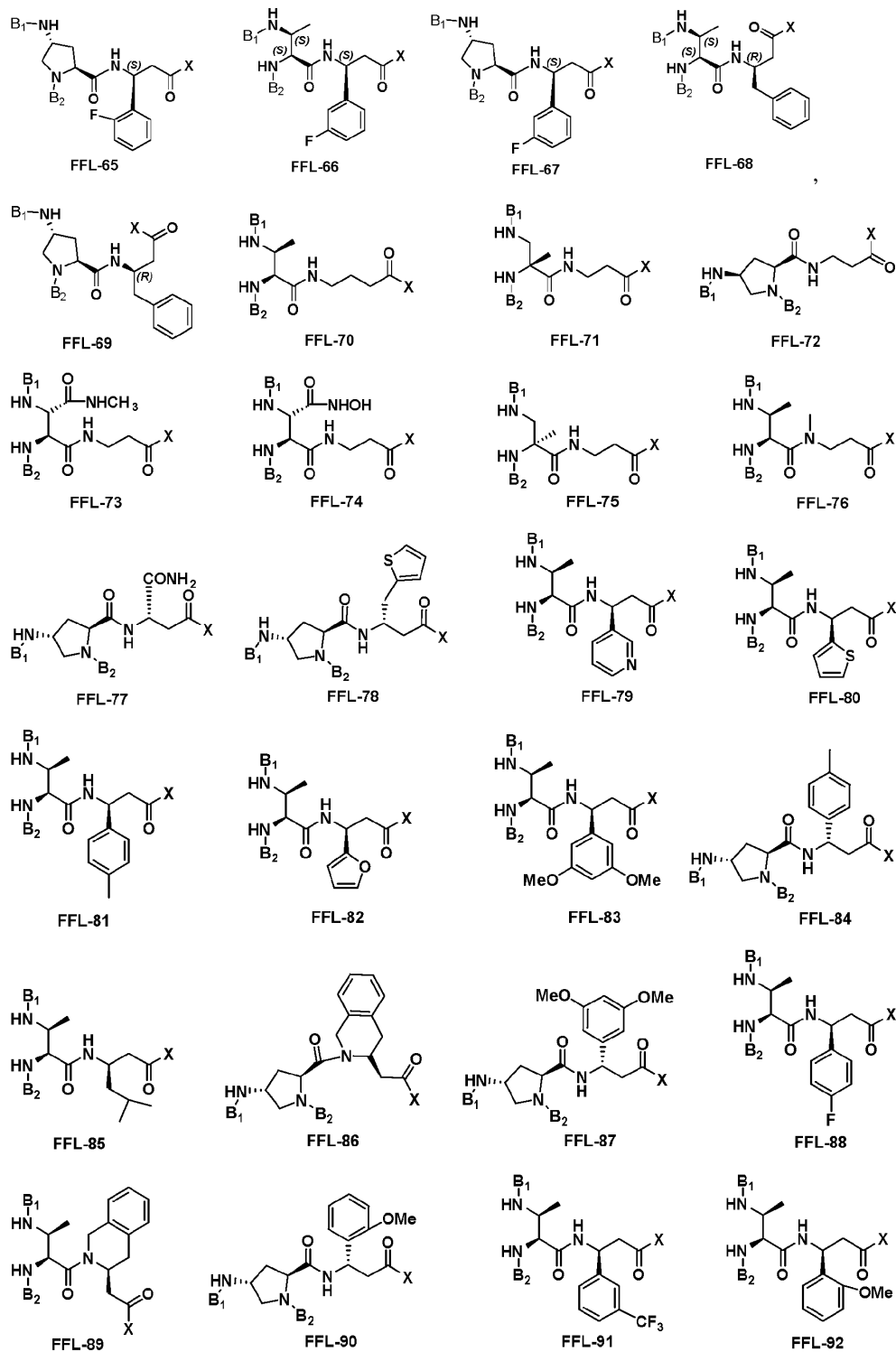


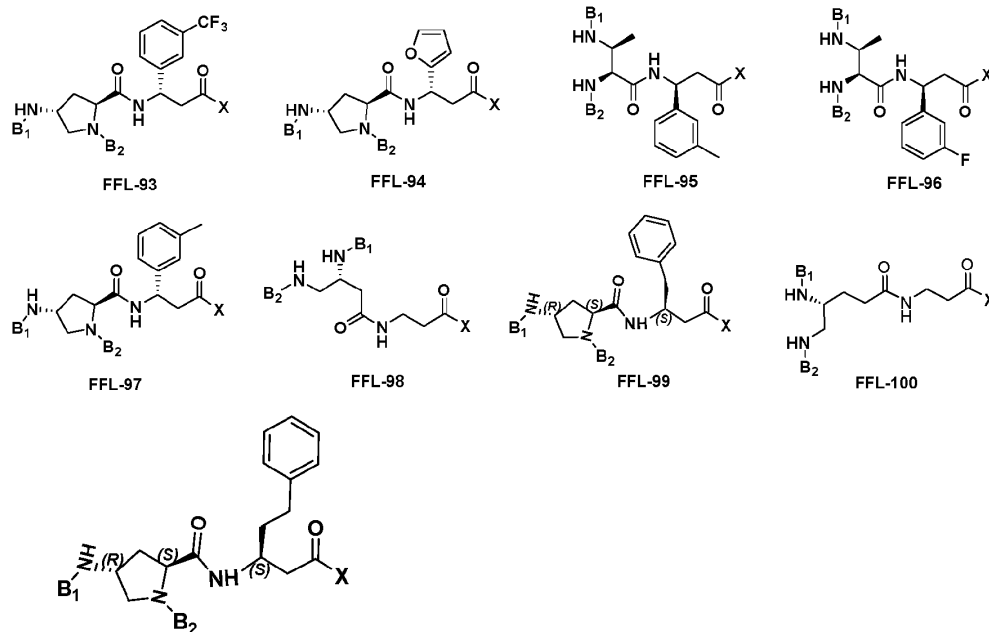
FFL-35



FFL-36



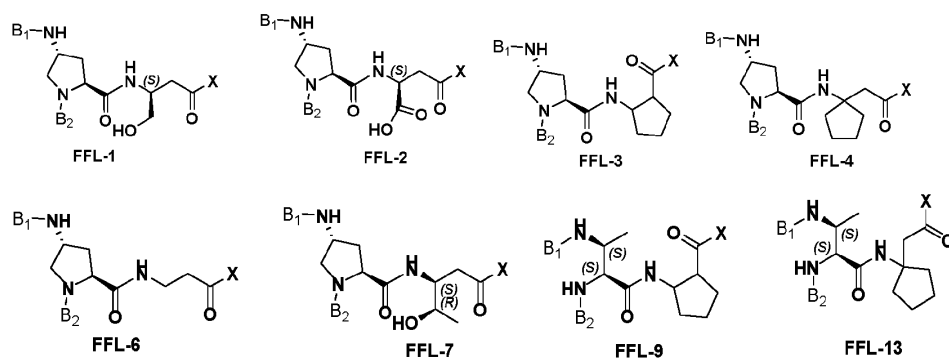


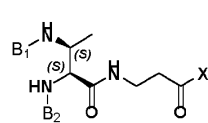


and FFL-101, and stereoisomers thereof;

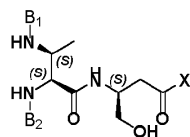
wherein X represents a point of covalent attachment either directly to an amine of X1 or to
 5 NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated
 directly or indirectly to X1 or to an amine of Z1a.

In some embodiments, B₁ and the B₂ are selected from Formula F2 wherein each
 remaining R₁ is independently selected from H, CF₃ and F; wherein each Z1c is covalently
 conjugated to an indirect linker and each of the Z1c and the indirect linker in combination is
 10 selected from Formulae FFL-1-4, 6-7, 9, 13-16, 20-23, 28-29, 31-33, 39, 42-45, 48, 53, 57-58,
 60-62, 65, and 67:

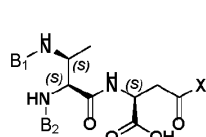




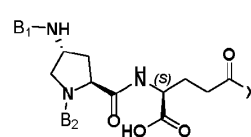
FFL-14



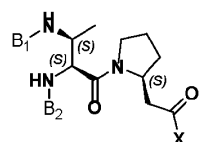
FFL-15



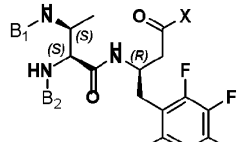
FFL-16



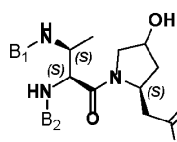
FFL-20



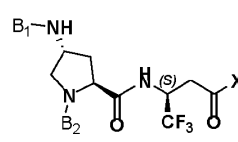
FFL-21



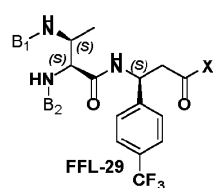
FFL-22



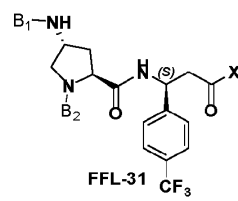
FFL-23



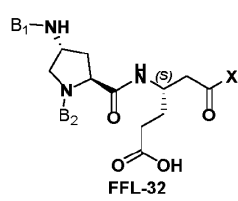
FFL-28



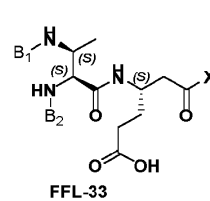
FFL-29



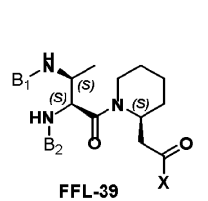
FFL-31



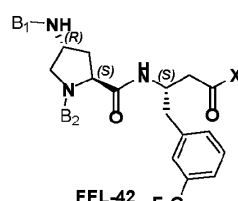
FFL-32



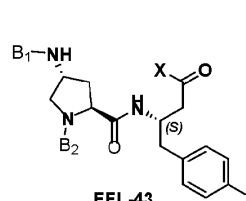
FFL-33



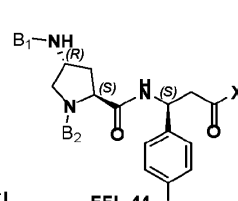
FFL-39



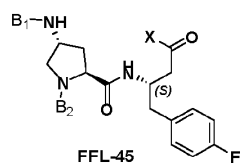
FFL-42



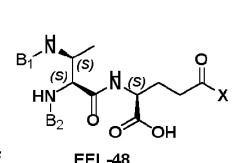
FFL-43



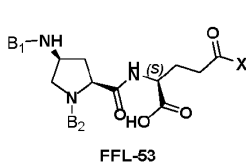
FFL-44



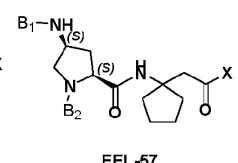
FFL-45



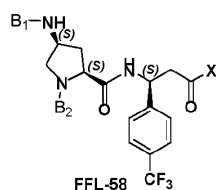
FFL-48



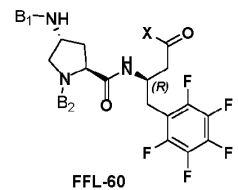
FFL-53



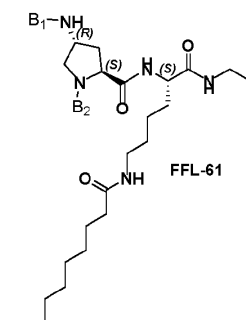
FFL-57



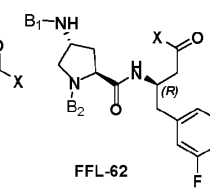
FFL-58



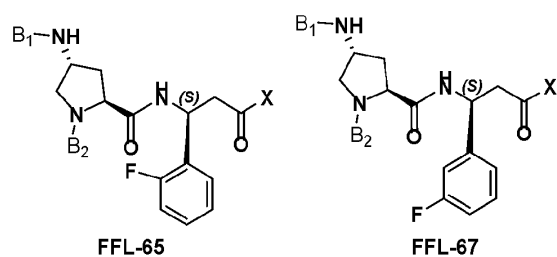
FFL-60



FFL-61



FFL-62



wherein X represents a point of covalent attachment either directly to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1 or to an amine of Z1a.

5 In some embodiments, X1 is a polypeptide drug substance comprising one or more lysine amino acids each independently conjugated, directly or via an indirect linker, to a Z1c.

In some embodiments, X1 comprises a polypeptide human hormone, an endocrine hormone, insulin, human insulin, glucagon, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide, glucose-dependent insulinotropic polypeptide, a
10 hybrid peptide comprising sequences from two or more human polypeptide hormones, or an analogue of any thereof.

In some embodiments, X1 comprises an insulin having an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, 24051, and 24052, and the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24061, 24062, 24063,
15 24064, and 25000–25397.

In some embodiments, X1 comprises an insulin having an A-chain and a B-chain, wherein:

the A-chain comprises a sequence selected from SEQ ID NOs 24051 and 24052,
the B-chain comprises a sequence selected from SEQ ID NOs 25095, 25229, 25232,
20 25236, 25305, 25308, 25312, and 25380-25397;

each Z1c is independently selected from FF12A, FF12B, FF12D, FF116A, FF116B, FF116C, and FF116D, and covalently conjugated either directly or via the indirect linker to one or more lysine residues in X1;

B₁ and B₂ is each selected from F2; and

25 the indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL20-FL75, and stereoisomers thereof.

In some embodiments, X1 is a polypeptide comprising an insulin receptor agonist having an A-chain and a B-chain, wherein:

the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, and 24051,

the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24063, 25228, 25313, 25393, 25396, and 25397;

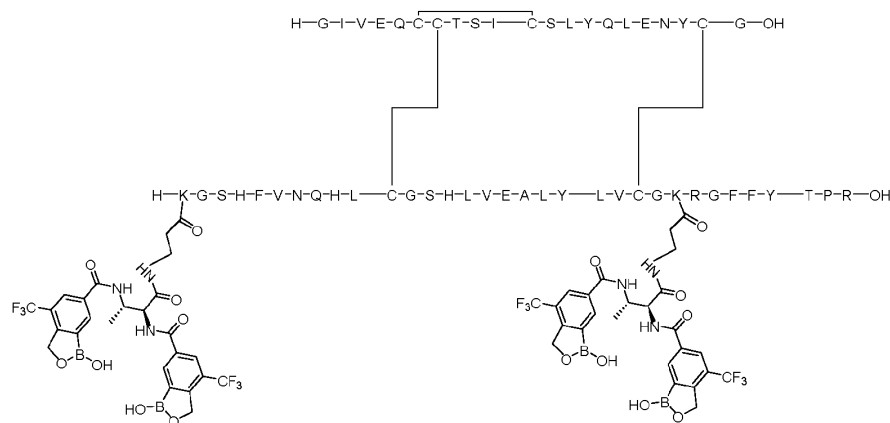
each Z1c is independently selected from FF12A, FF12B, FF114A, FF115A, FF116A, and FF225A, and covalently conjugated either directly or via the indirect linker to one or more lysine
5 residues in X1;

B₁ and B₂ is each selected from F2; and

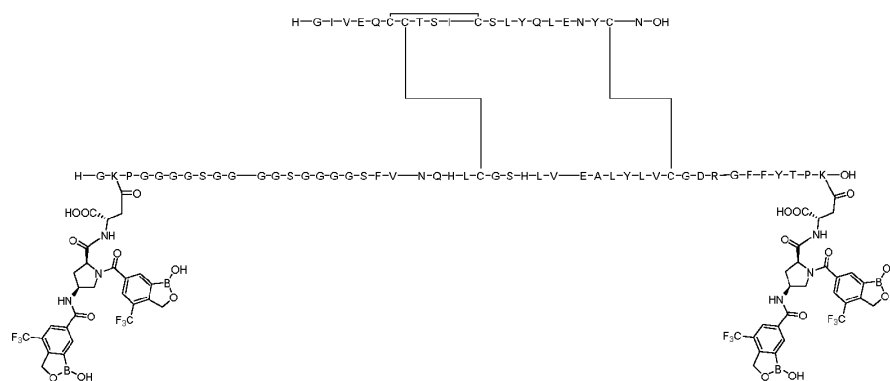
the indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL21, and stereoisomers thereof.

10 In some embodiments, the compound is selected from:

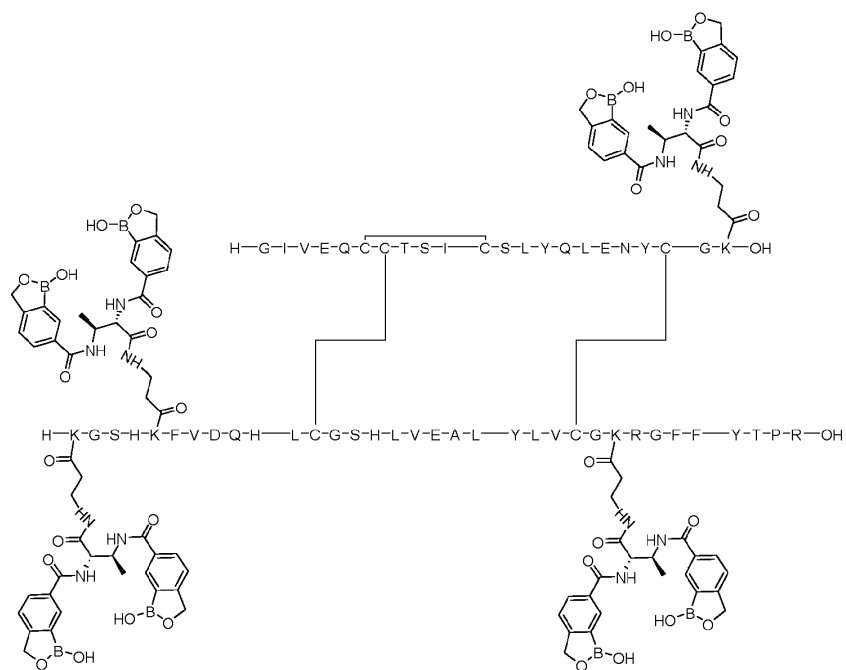
Example 1 (SEQ ID NOS 25398 and 25399, respectively, in order of appearance):



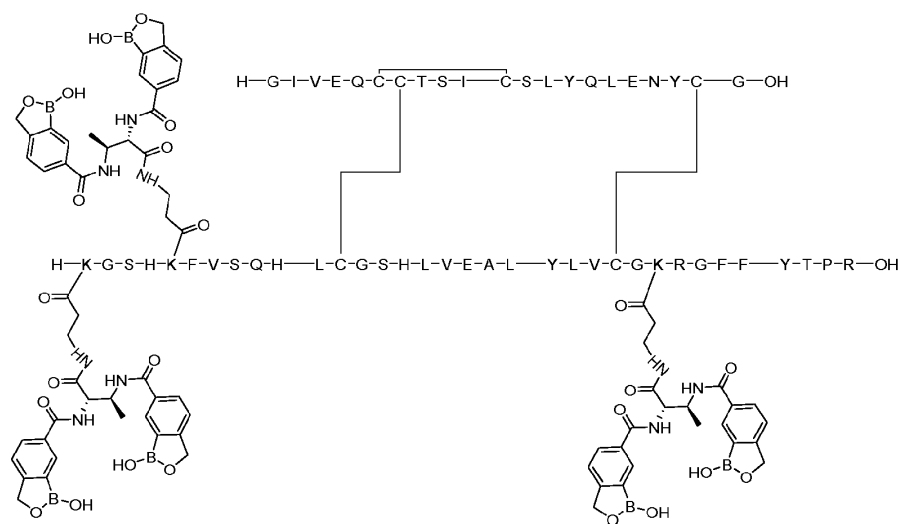
Example 2 (SEQ ID NOS 25400 and 25401, respectively, in order of appearance):



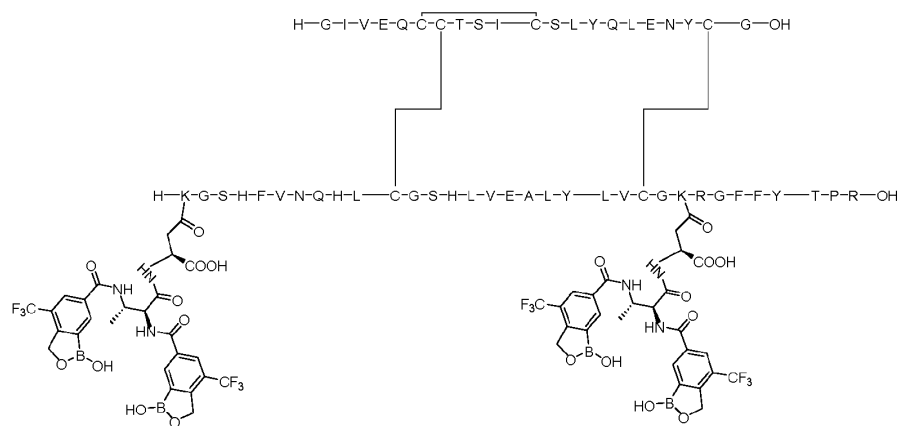
Example 3 (SEQ ID NOS 25402 and 25403, respectively, in order of appearance):



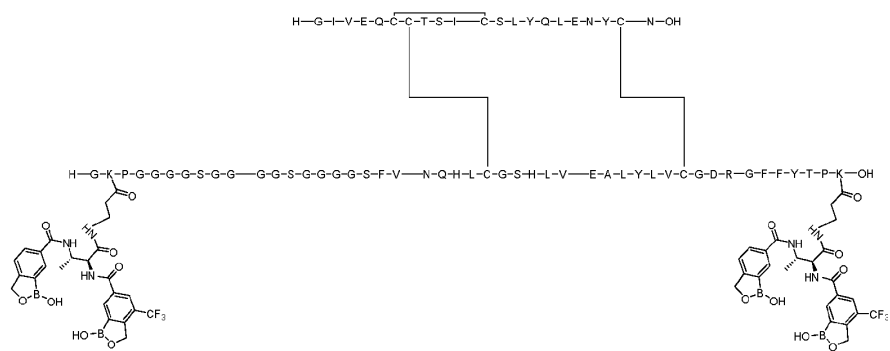
Example 4 (SEQ ID NOS 25404 and 25405, respectively, in order of appearance):



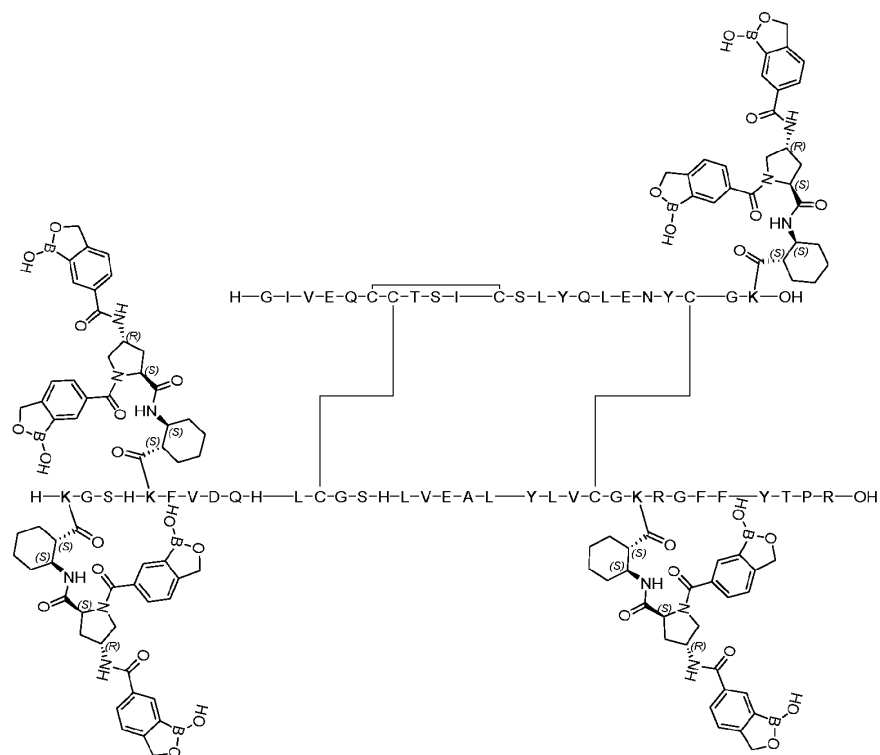
Example 5 (SEQ ID NOS 25406 and 25407, respectively, in order of appearance):



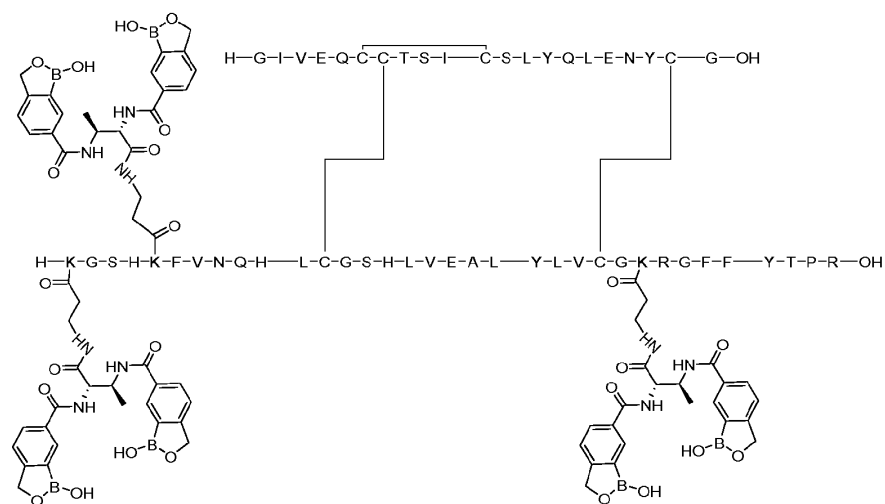
Example 6 (SEQ ID NOS 25408 and 25409, respectively, in order of appearance):



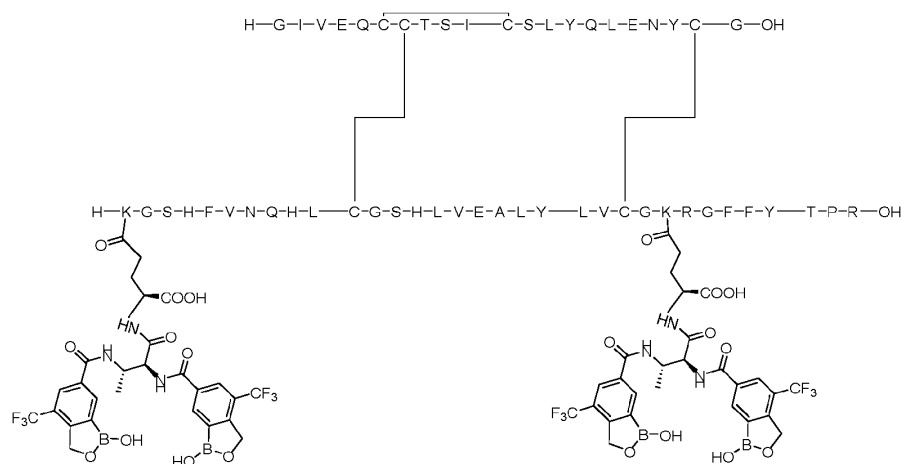
Example 7 (SEQ ID NOS 25410 and 25411, respectively, in order of appearance):



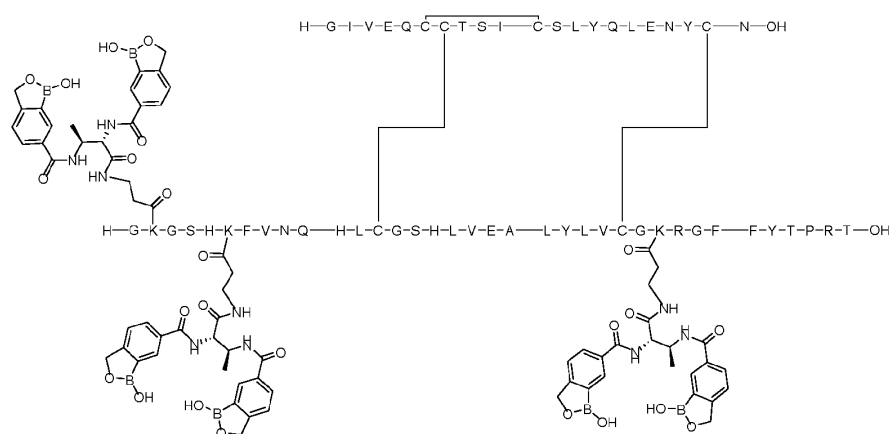
Example 8 (SEQ ID NOS 25412 and 25413, respectively, in order of appearance):



Example 9 (SEQ ID NOS 25414 and 25415, respectively, in order of appearance):

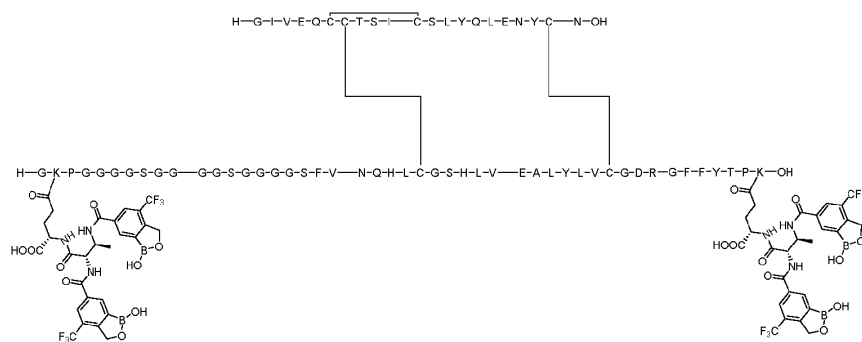


Example 10 (SEQ ID NOS 25416 and 25417, respectively, in order of appearance):

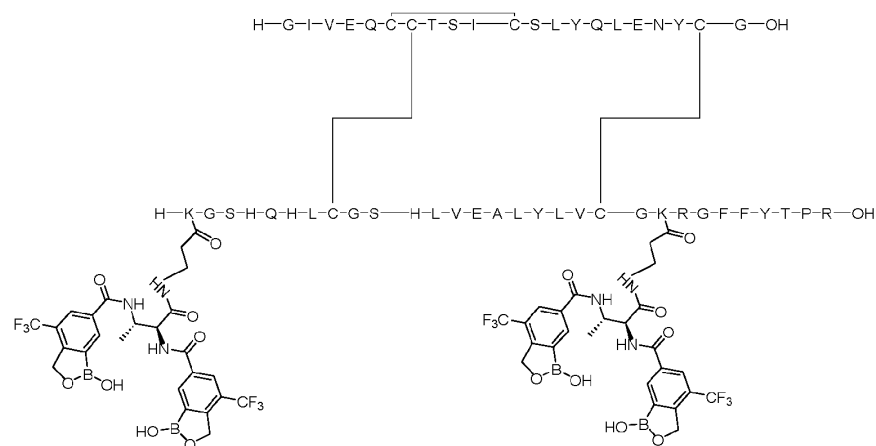


5

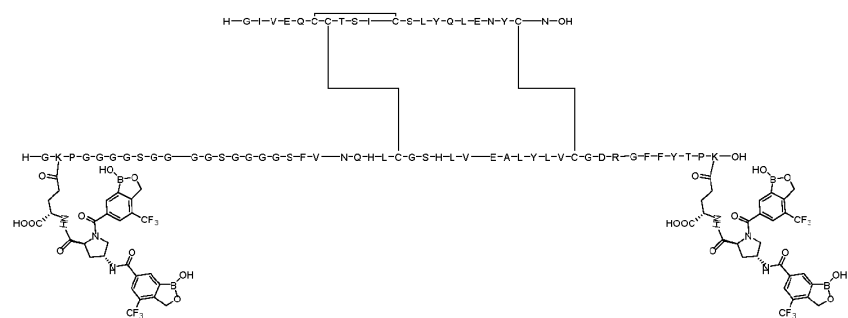
Example 11 (SEQ ID NOS 25418 and 25419, respectively, in order of appearance):



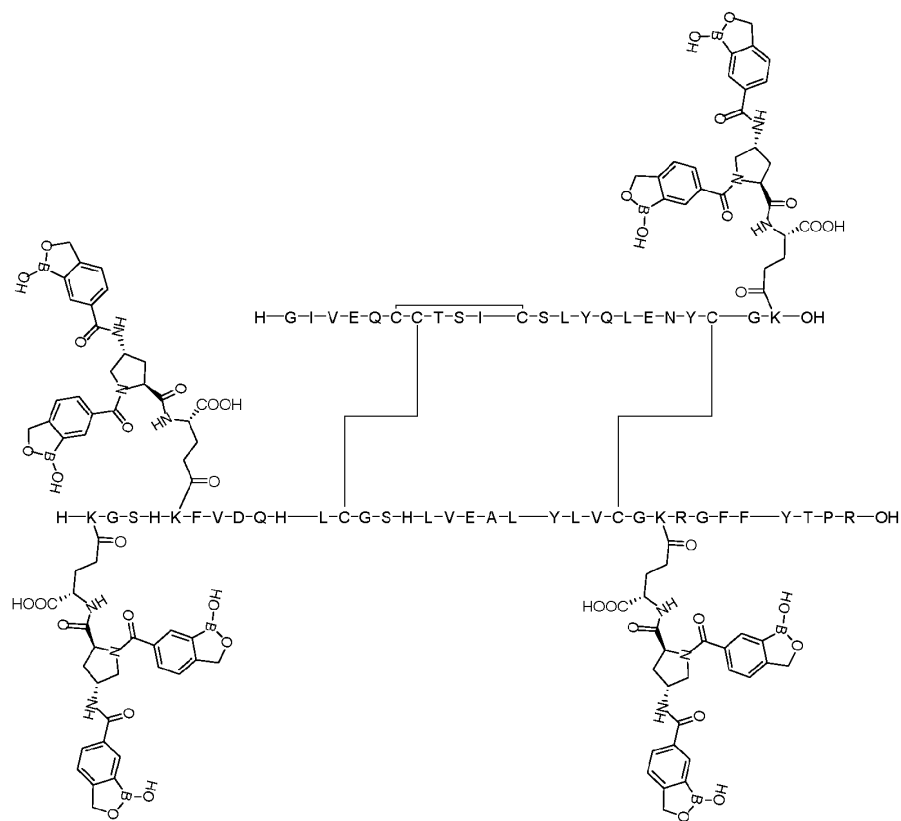
Example 12 (SEQ ID NOS 25420 and 25421, respectively, in order of appearance):



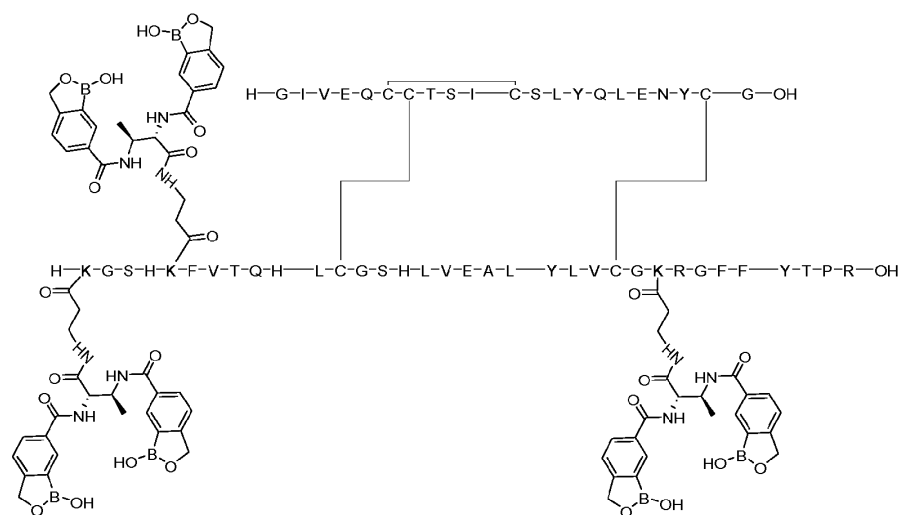
Example 13 (SEQ ID NOS 25422 and 25423, respectively, in order of appearance):



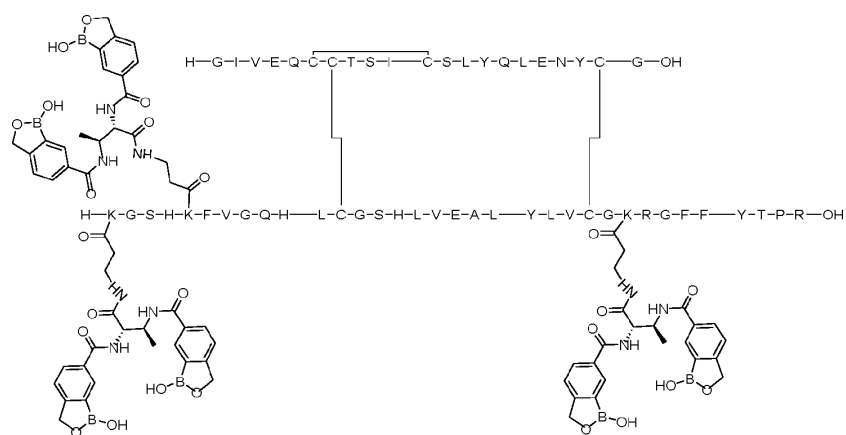
Example 14 (SEQ ID NOS 25424 and 25425, respectively, in order of appearance):



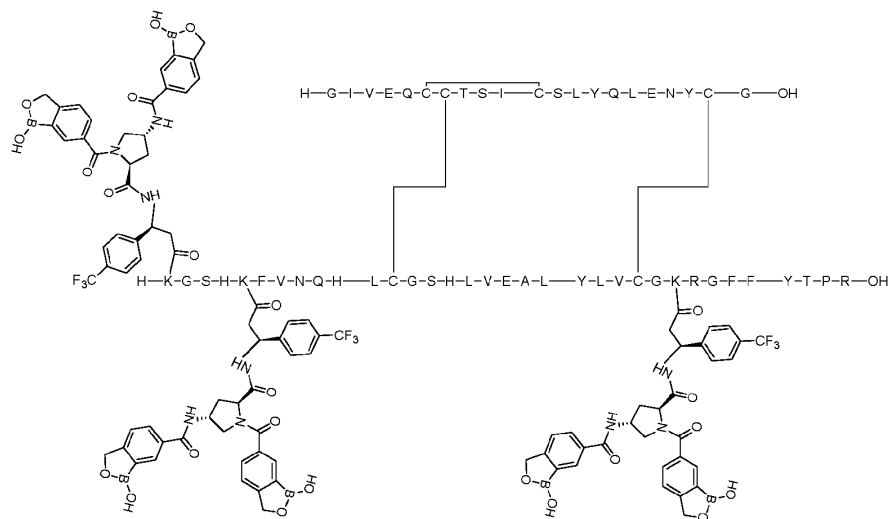
Example 15 (SEQ ID NOS 25426 and 25427, respectively, in order of appearance):



Example 16 (SEQ ID NOS 25428 and 25429, respectively, in order of appearance):

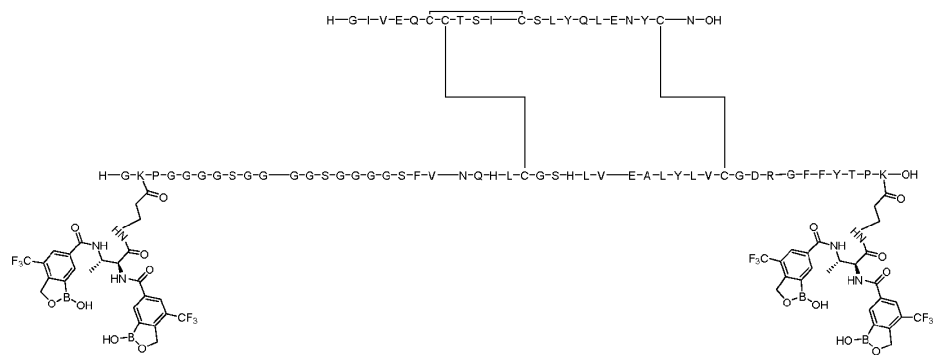


Example 17 (SEQ ID NOS 25430 and 25431, respectively, in order of appearance):

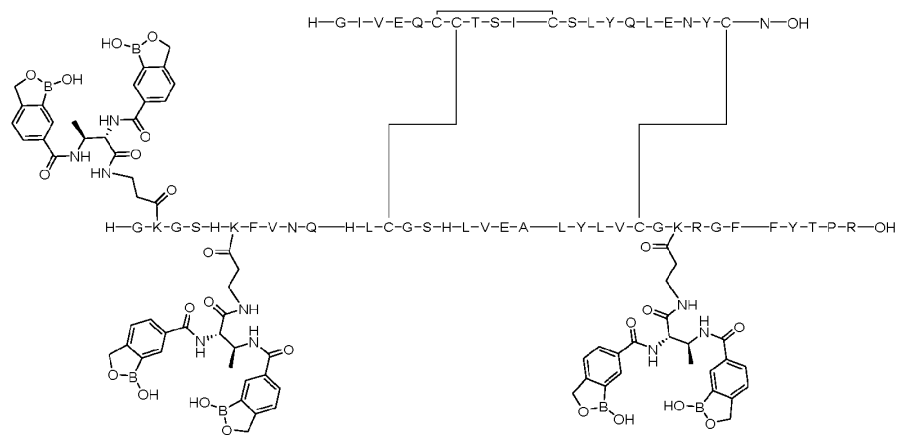


5

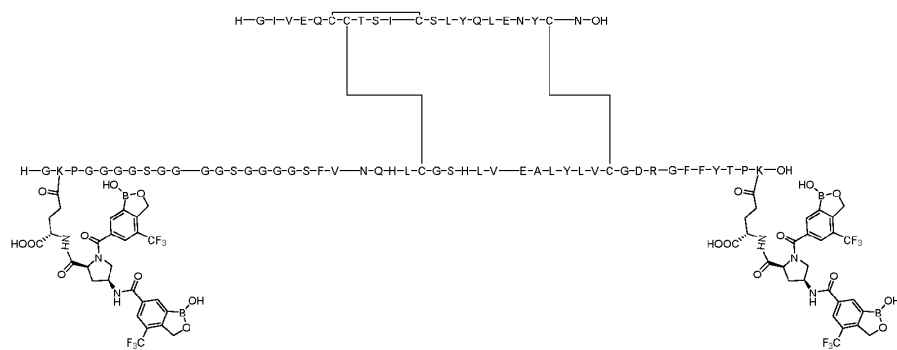
Example 18 (SEQ ID NOS 25432 and 25433, respectively, in order of appearance):



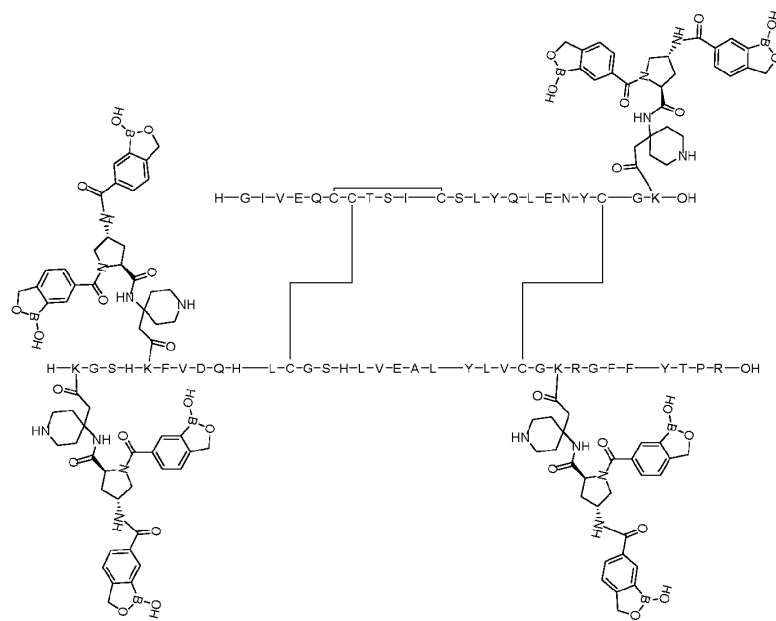
Example 19 (SEQ ID NOS 25434 and 25435, respectively, in order of appearance):



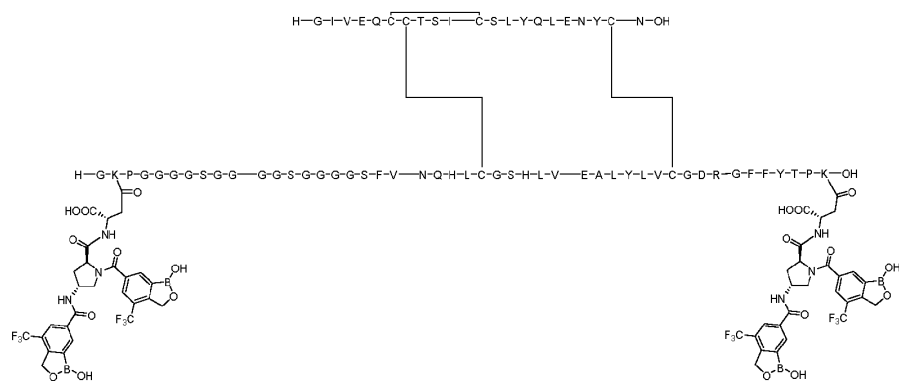
5 Example 20 (SEQ ID NOS 25436 and 25437, respectively, in order of appearance):



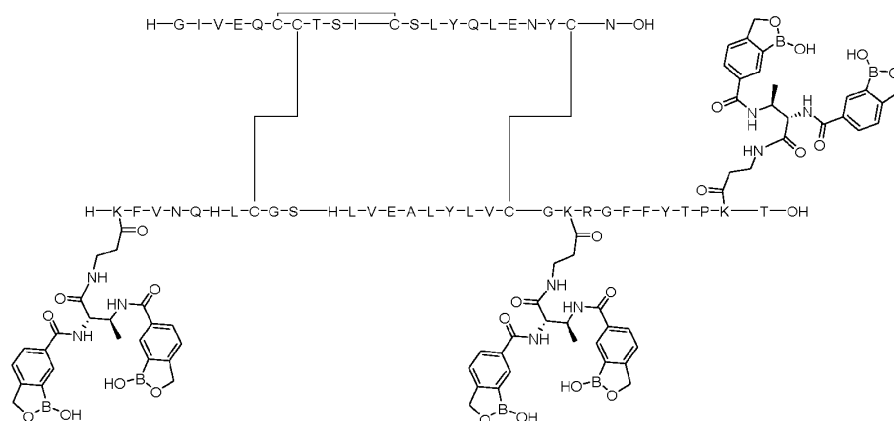
Example 21 (SEQ ID NOS 25438 and 25439, respectively, in order of appearance):



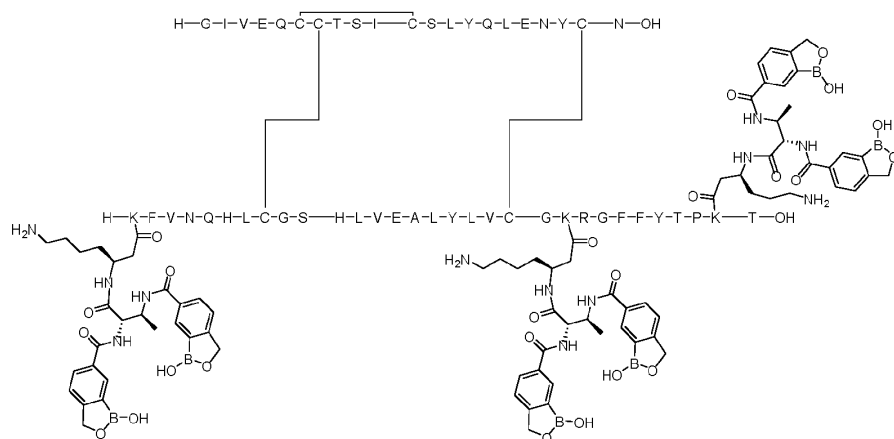
Example 22 (SEQ ID NOS 25422 and 25423, respectively, in order of appearance):



Example 23 (SEQ ID NOS 25440 and 25441, respectively, in order of appearance):

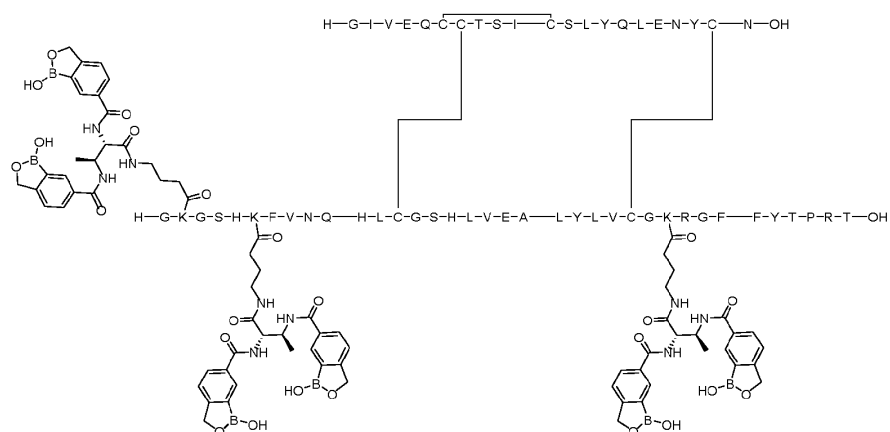


Example 24 (SEQ ID NOS 25442 and 25443, respectively, in order of appearance):

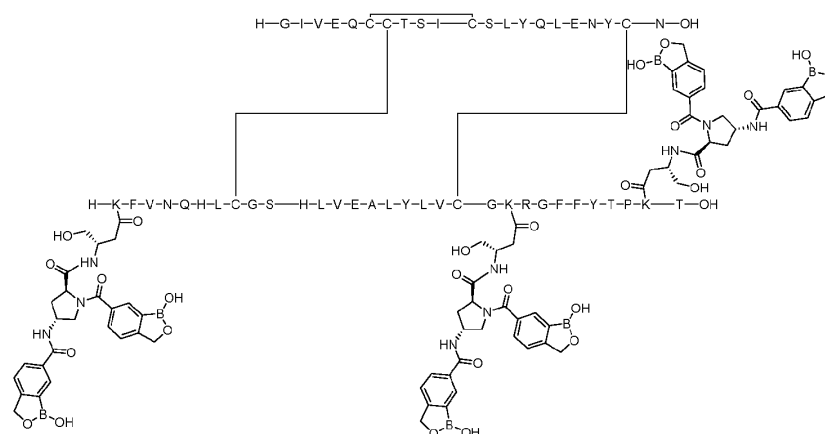


5

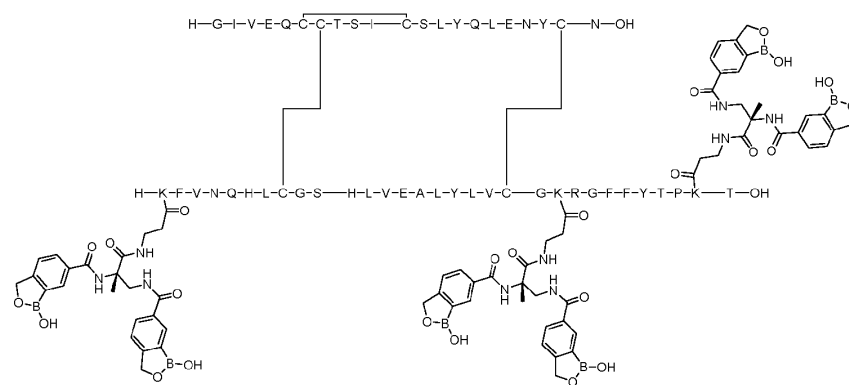
Example 25 (SEQ ID NOS 25444 and 25445, respectively, in order of appearance):



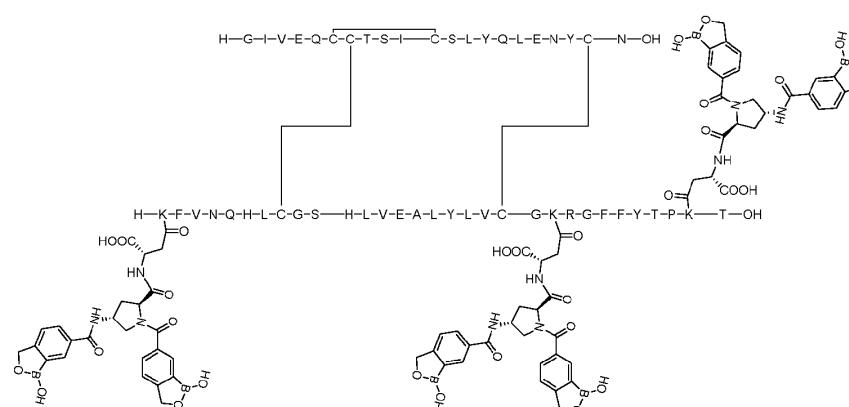
Example 26 (SEQ ID NOS 25446 and 25447, respectively, in order of appearance):



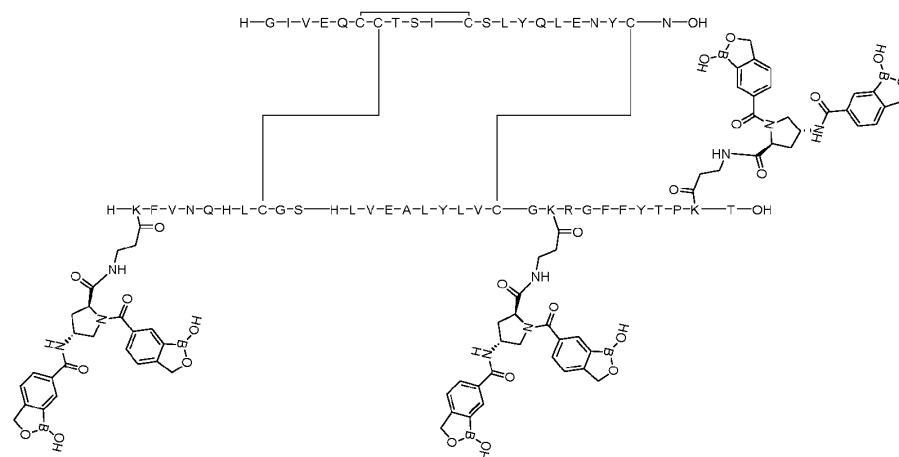
5 Example 27 (SEQ ID NOS 25448 and 25449, respectively, in order of appearance):



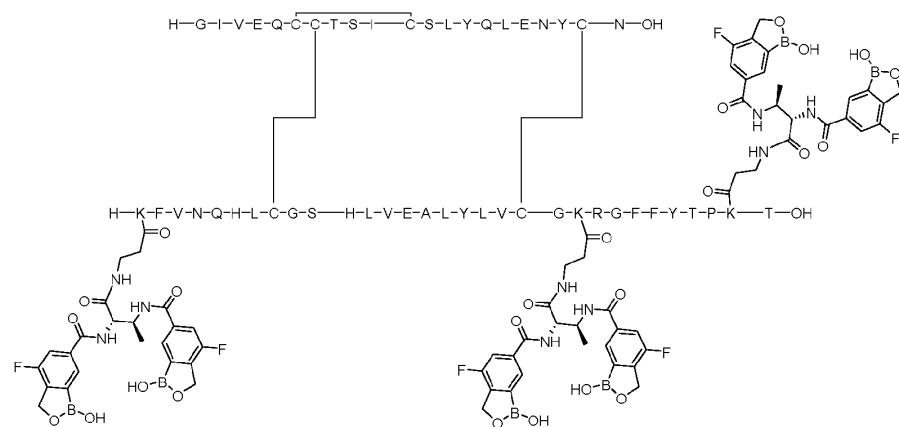
Example 28 (SEQ ID NOS 25450 and 25451, respectively, in order of appearance):



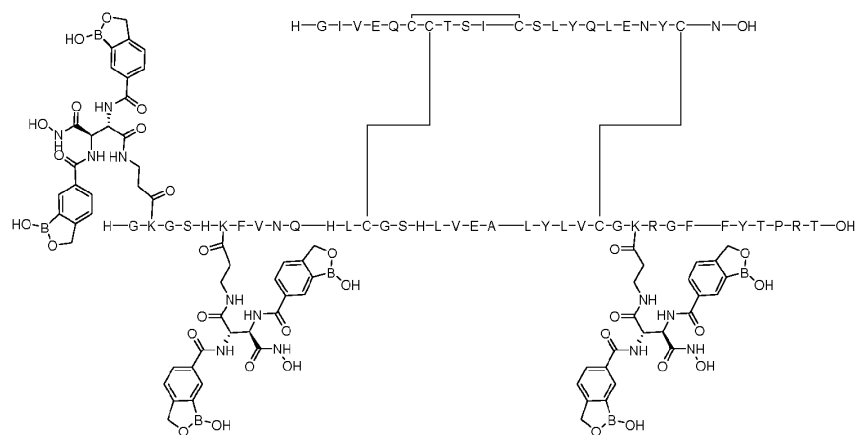
Example 29 (SEQ ID NOS 25452 and 25453, respectively, in order of appearance):



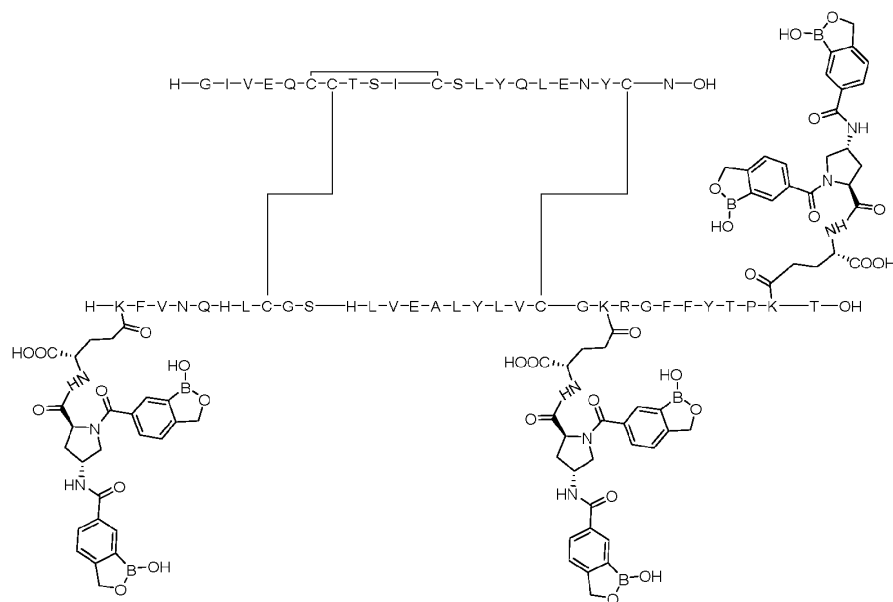
5 Example 30 (SEQ ID NOS 25454 and 25455, respectively, in order of appearance):



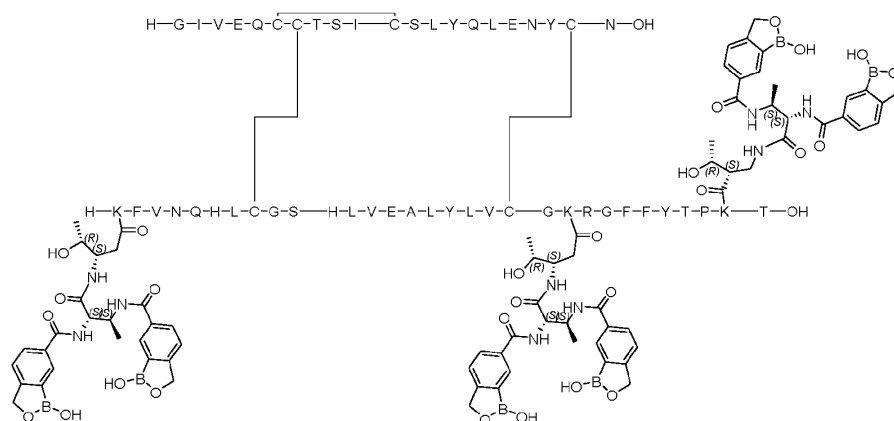
Example 31 (SEQ ID NOS 25456 and 25457, respectively, in order of appearance):



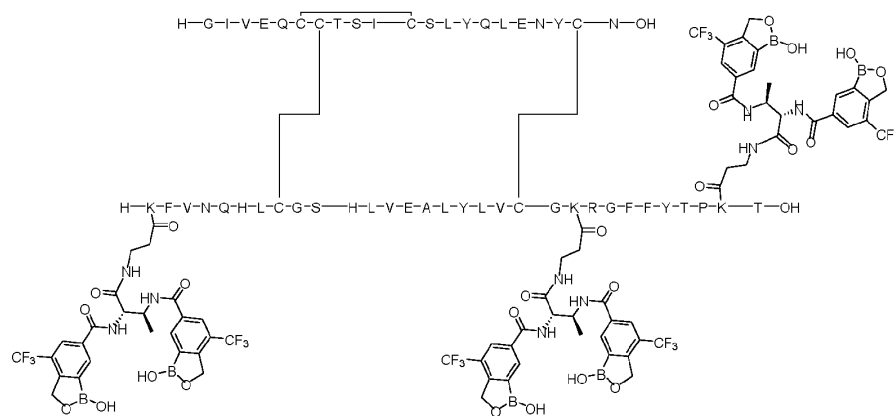
Example 32 (SEQ ID NOS 25458 and 25459, respectively, in order of appearance):



Example 33 (SEQ ID NOS 25460 and 25461, respectively, in order of appearance):

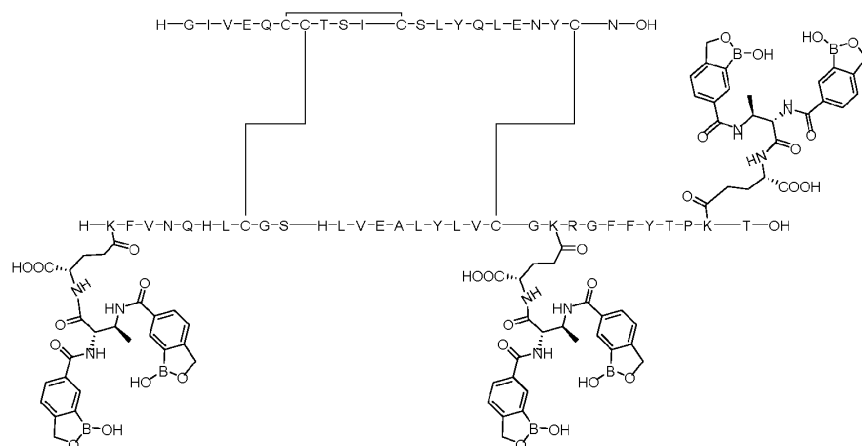


Example 34 (SEQ ID NOS 25462 and 25463, respectively, in order of appearance):

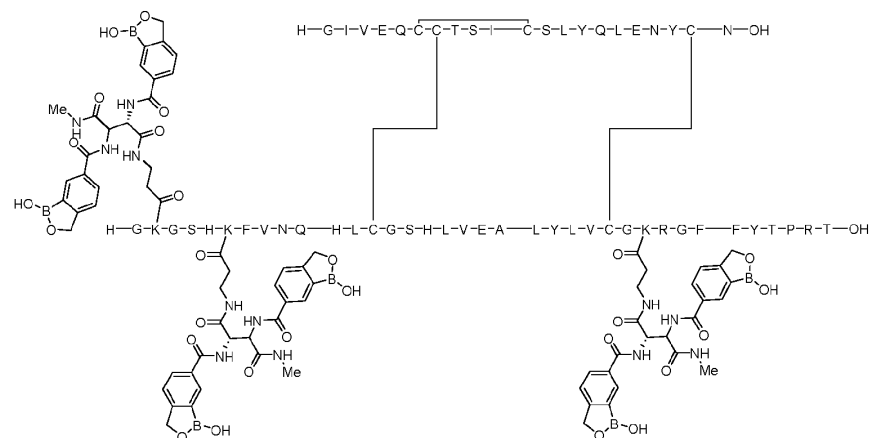


5

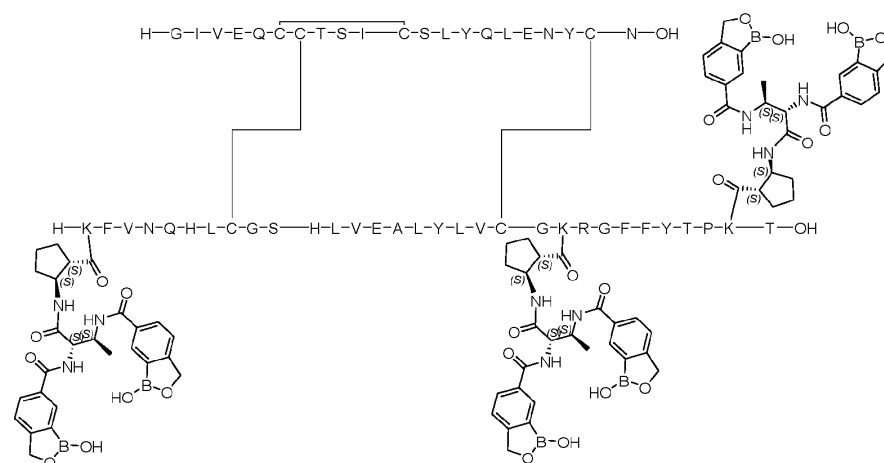
Example 35 (SEQ ID NOS 25464 and 25465, respectively, in order of appearance):



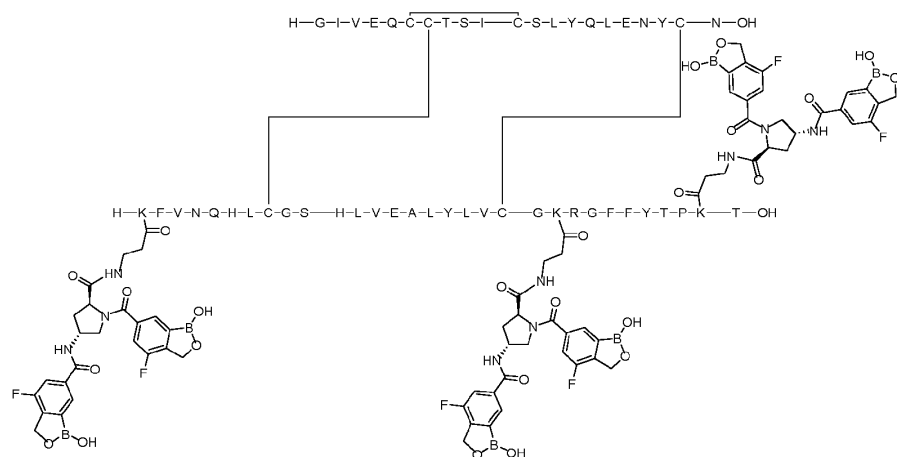
Example 36 (SEQ ID NOS 25466 and 25467, respectively, in order of appearance):



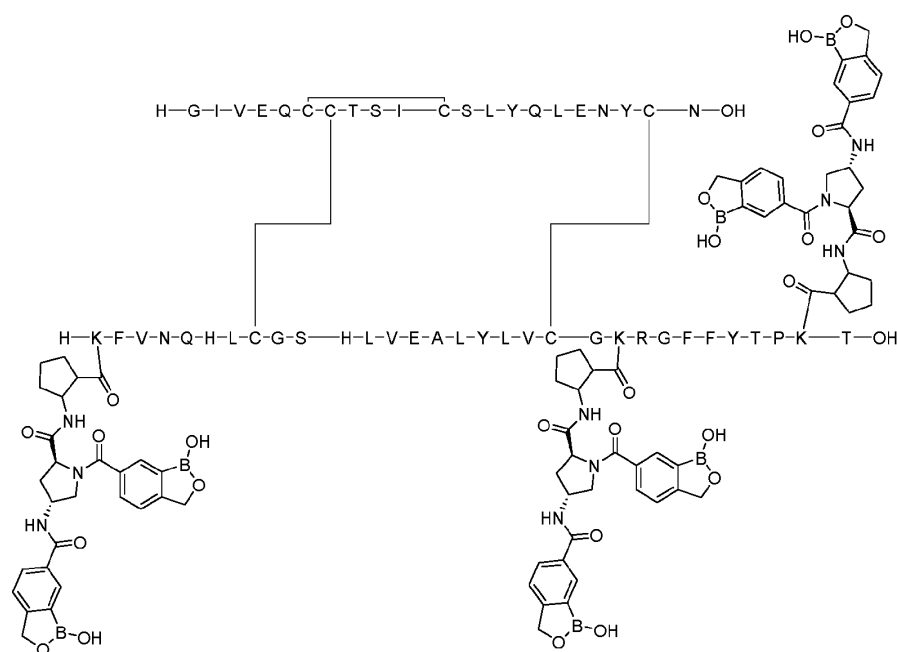
5 Example 37 (SEQ ID NOS 25468 and 25469, respectively, in order of appearance):



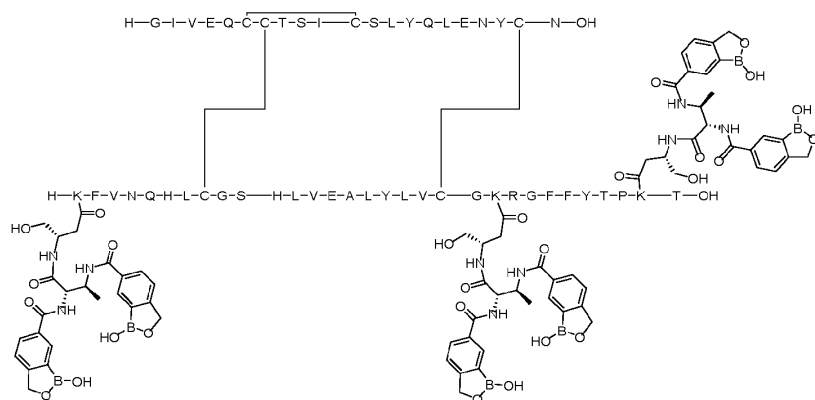
Example 38 (SEQ ID NOS 25470 and 25471, respectively, in order of appearance):



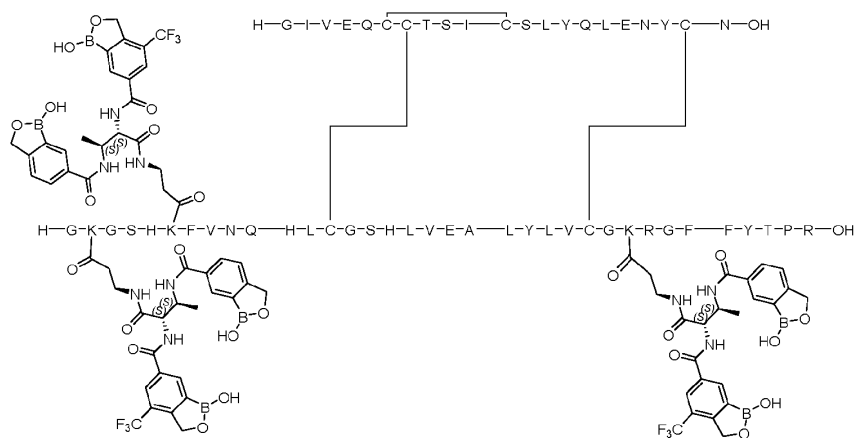
Example 39 (SEQ ID NOS 25472 and 25473, respectively, in order of appearance):



Example 40 (SEQ ID NOS 25474 and 25475, respectively, in order of appearance):

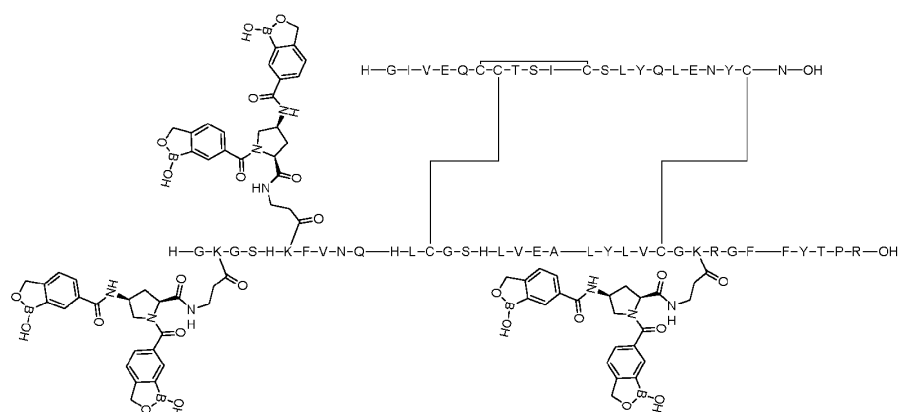


Example 41 (SEQ ID NOS 25476 and 25477, respectively, in order of appearance):

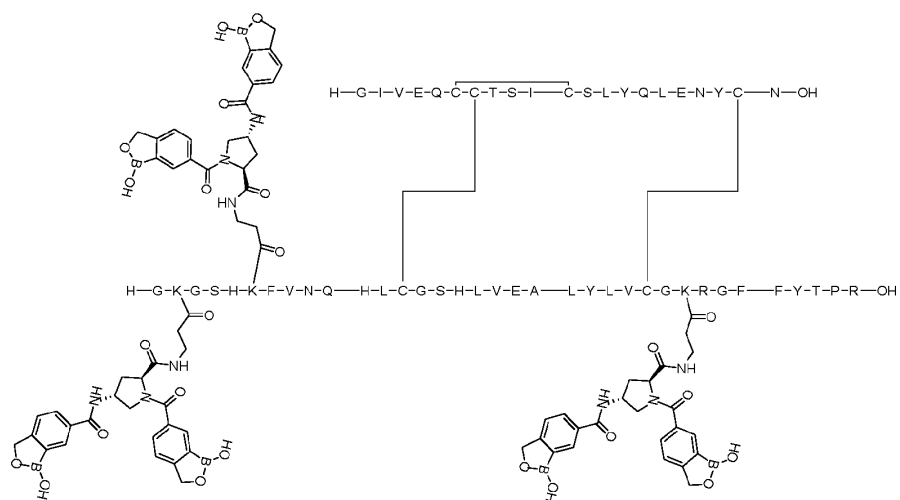


5

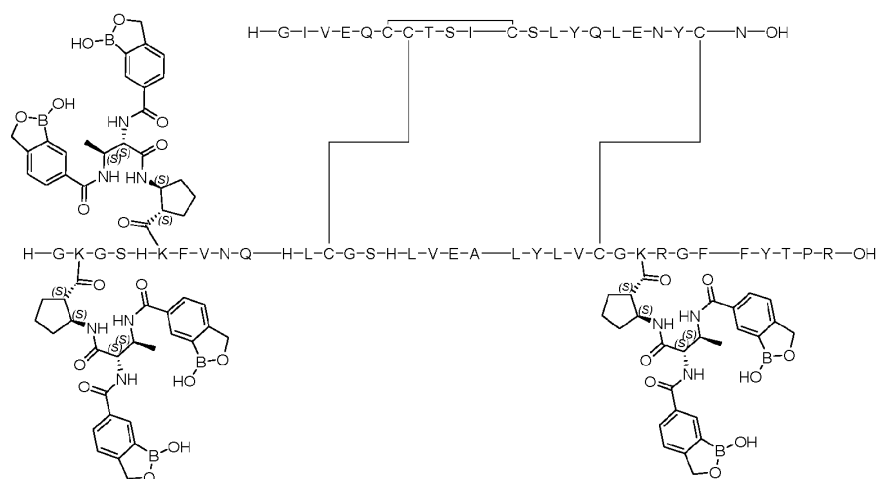
Example 42 (SEQ ID NOS 25478 and 25479, respectively, in order of appearance):



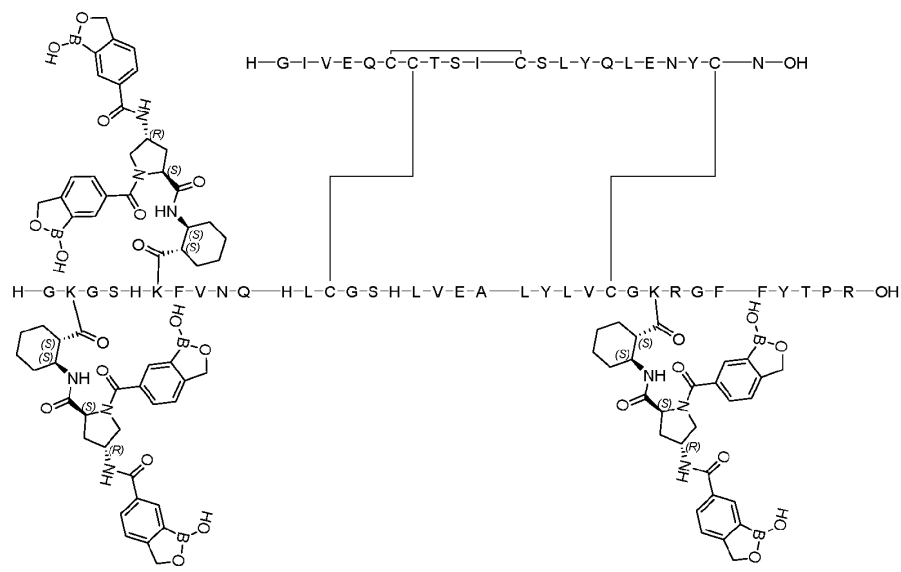
Example 43 (SEQ ID NOS 25480 and 25481, respectively, in order of appearance):



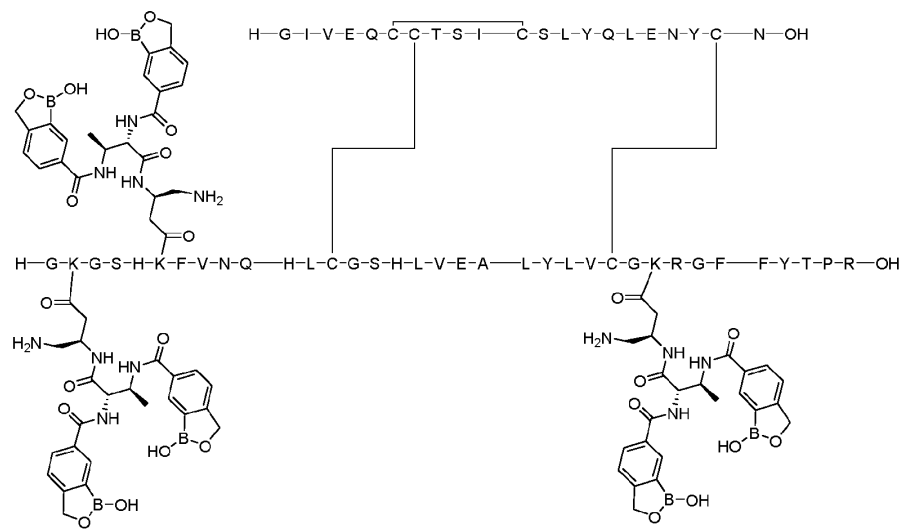
Example 44 (SEQ ID NOS 25482 and 25483, respectively, in order of appearance):



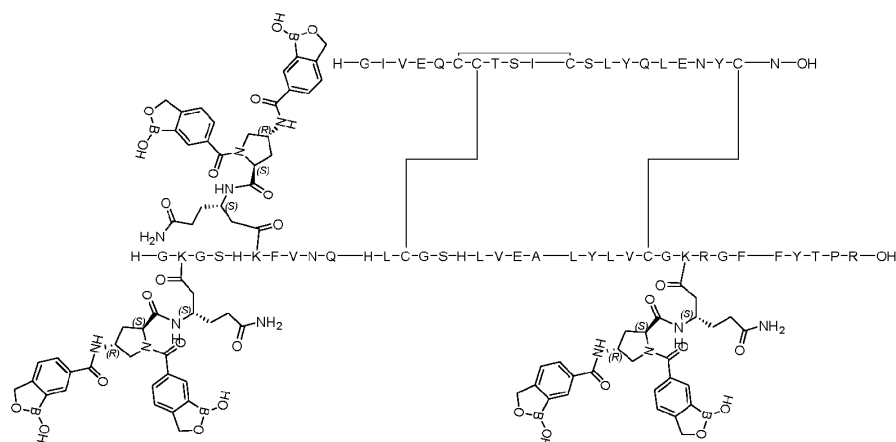
Example 45 (SEQ ID NOS 25484 and 25485, respectively, in order of appearance):



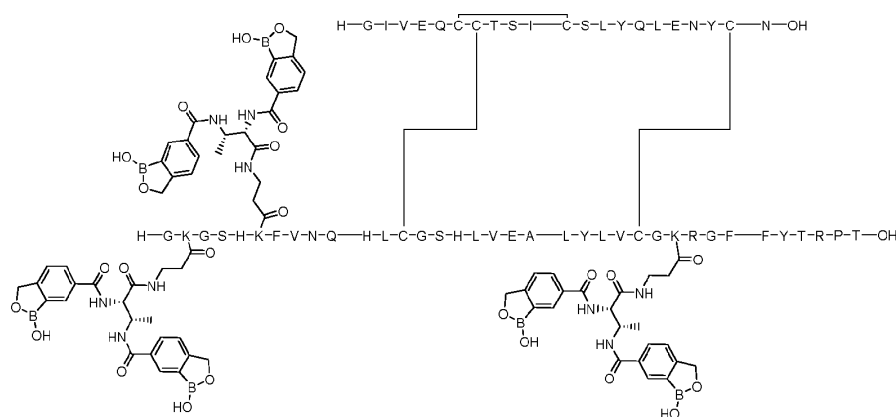
Example 46 (SEQ ID NOS 25486 and 25487, respectively, in order of appearance):



Example 47 (SEQ ID NOS 25488 and 25489, respectively, in order of appearance):

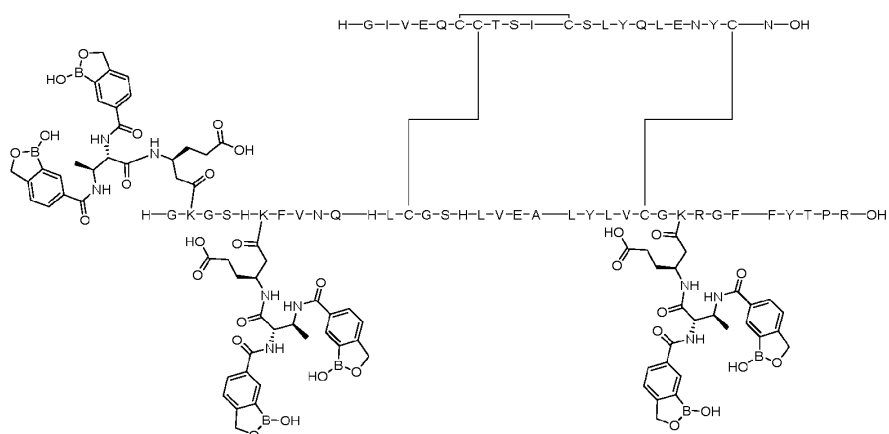


Example 48 (SEQ ID NOS 25490 and 25491, respectively, in order of appearance):

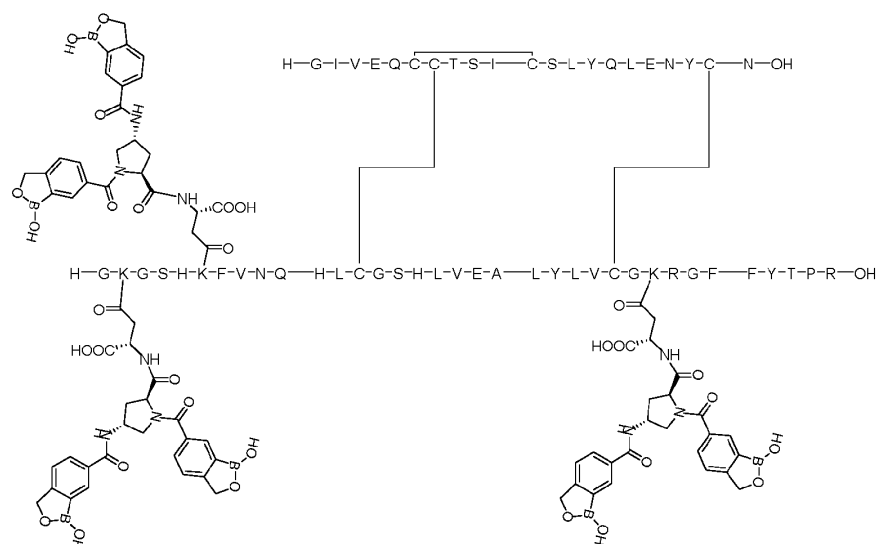


5

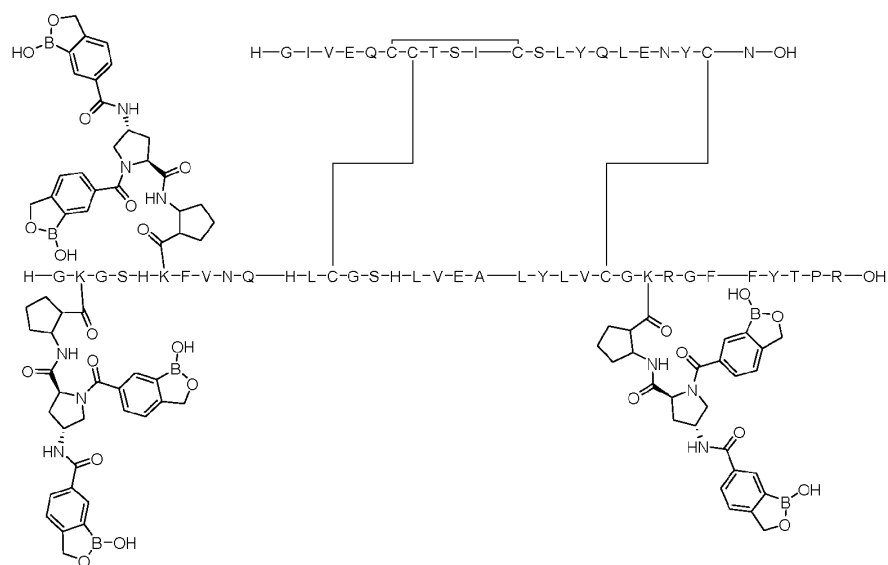
Example 49 (SEQ ID NOS 25492 and 25493, respectively, in order of appearance):



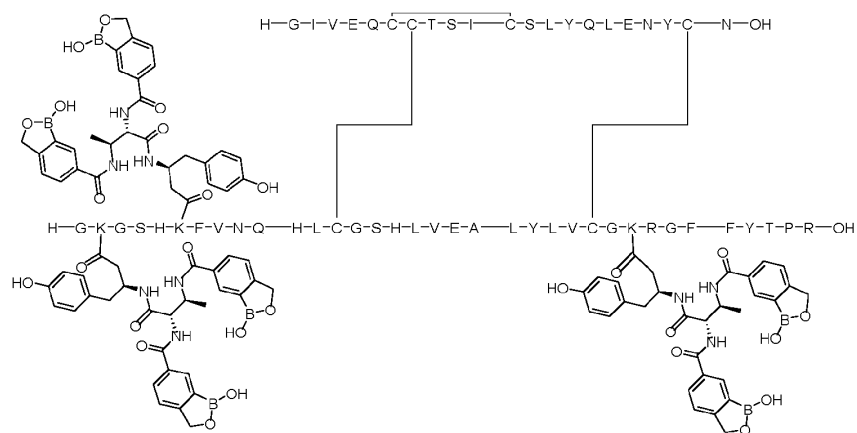
Example 50 (SEQ ID NOS 25494 and 25495, respectively, in order of appearance):



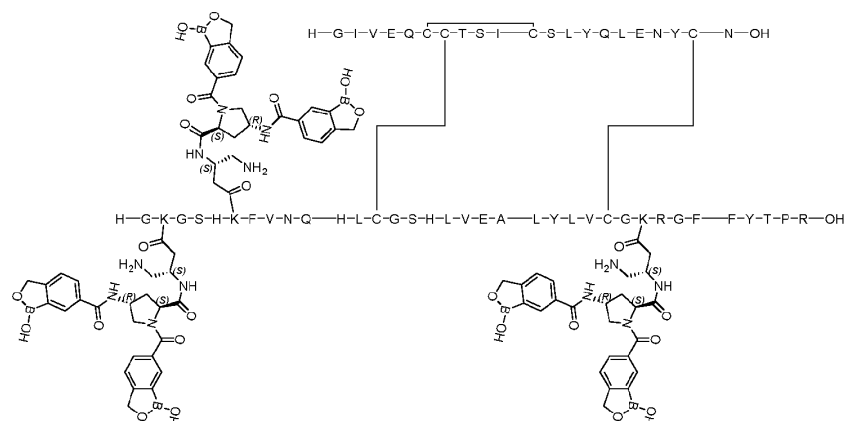
5 Example 51 (SEQ ID NOS 25496 and 25497, respectively, in order of appearance)



Example 52 (SEQ ID NOS 25498 and 25499, respectively, in order of appearance):

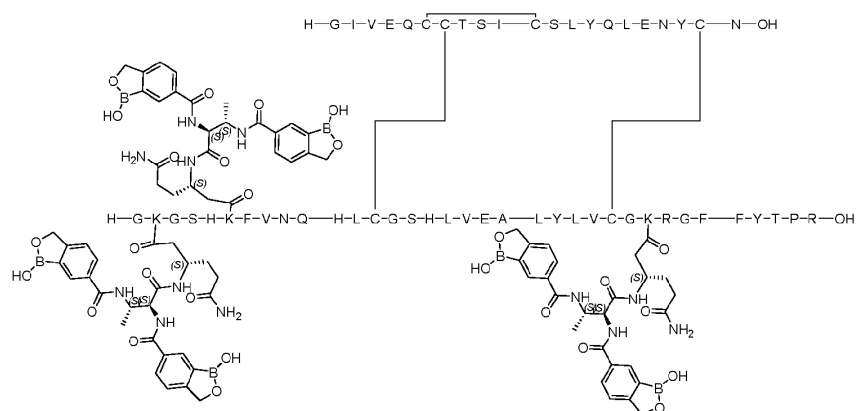


Example 53 (SEQ ID NOS 25500 and 25501, respectively, in order of appearance):

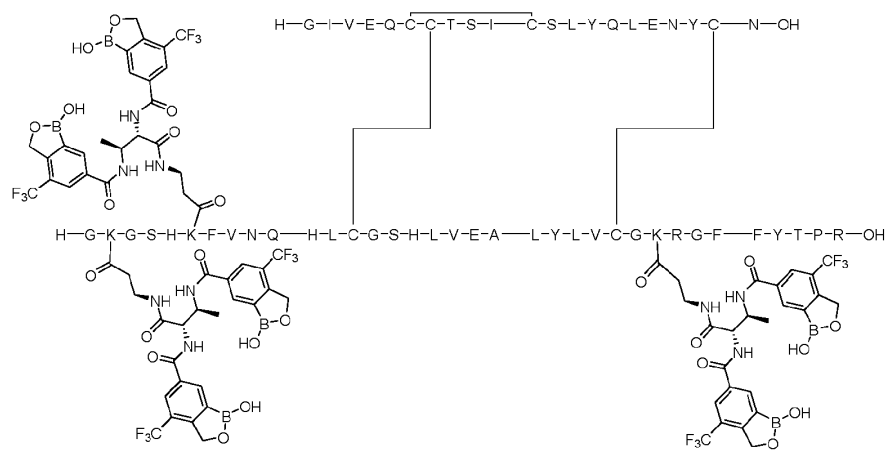


5

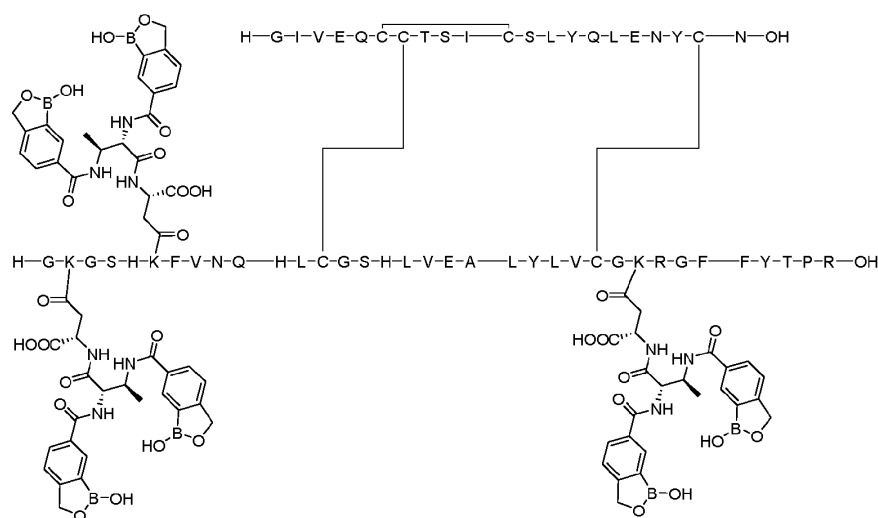
Example 54 (SEQ ID NOS 25502 and 25503, respectively, in order of appearance):



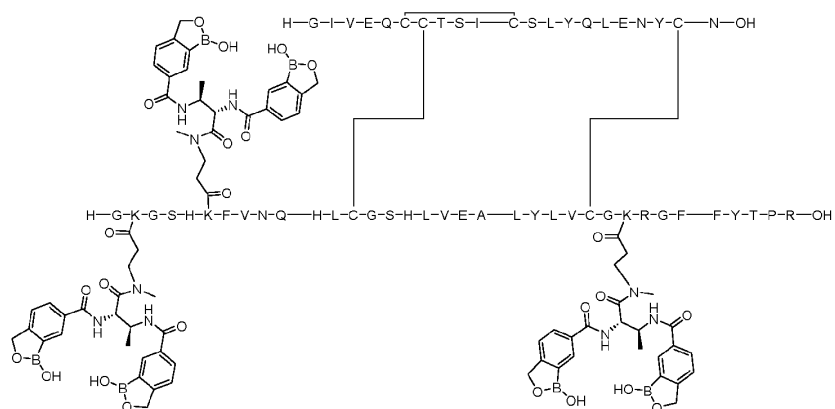
Example 55 (SEQ ID NOS 25504 and 25505, respectively, in order of appearance):



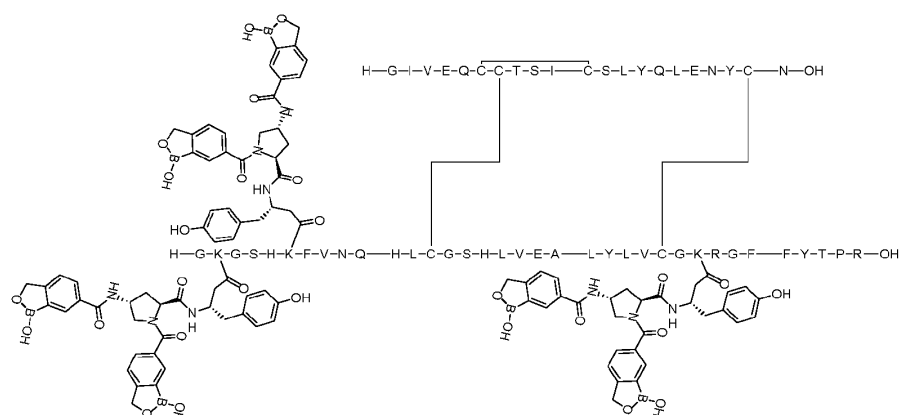
5 Example 56 (SEQ ID NOS 25506 and 25507, respectively, in order of appearance):



Example 57 (SEQ ID NOS 25508 and 25509, respectively, in order of appearance):

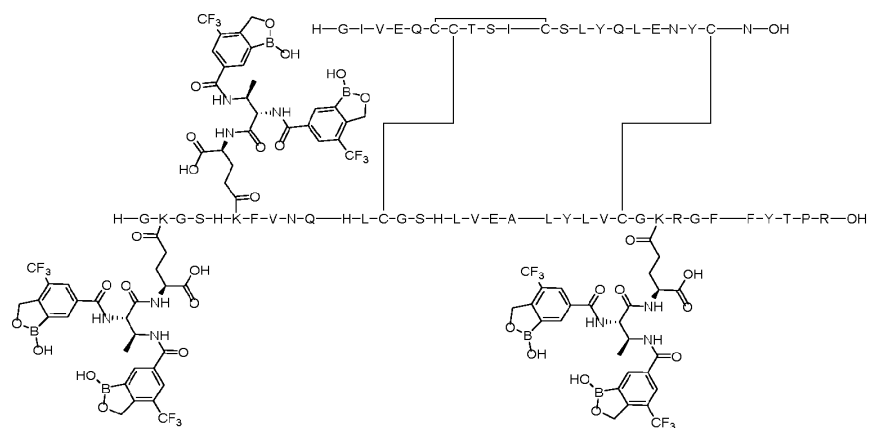


Example 58 (SEQ ID NOS 25510 and 25511, respectively, in order of appearance):

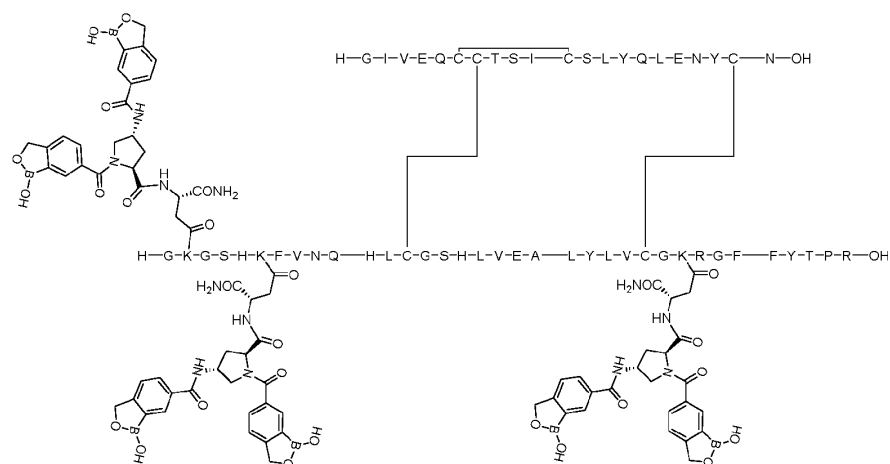


5

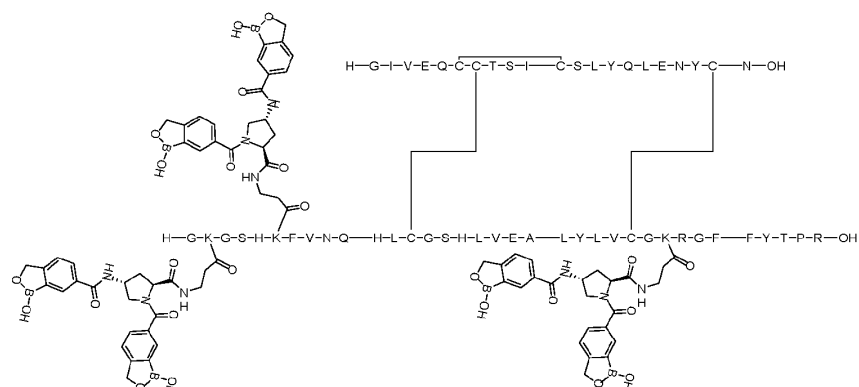
Example 59 (SEQ ID NOS 25512 and 25513, respectively, in order of appearance):



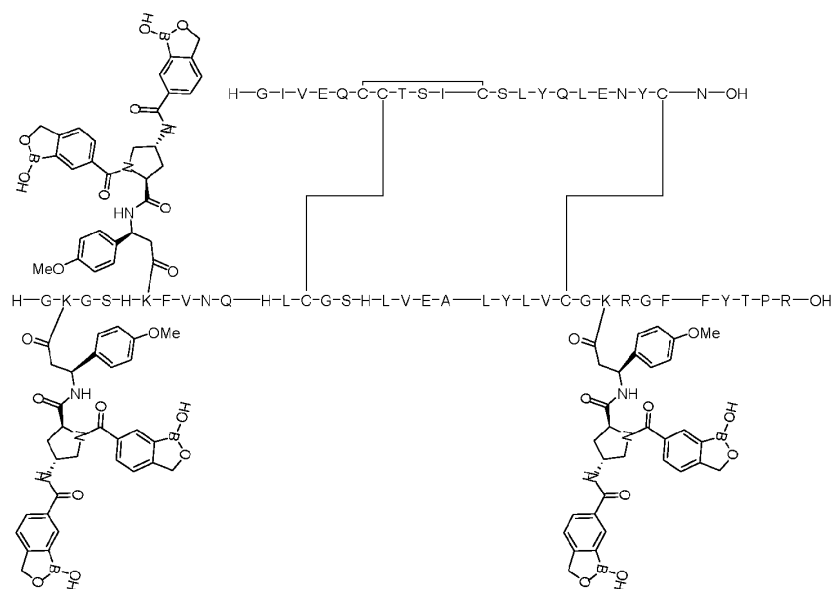
Example 60 (SEQ ID NOS 25514 and 25515, respectively, in order of appearance):



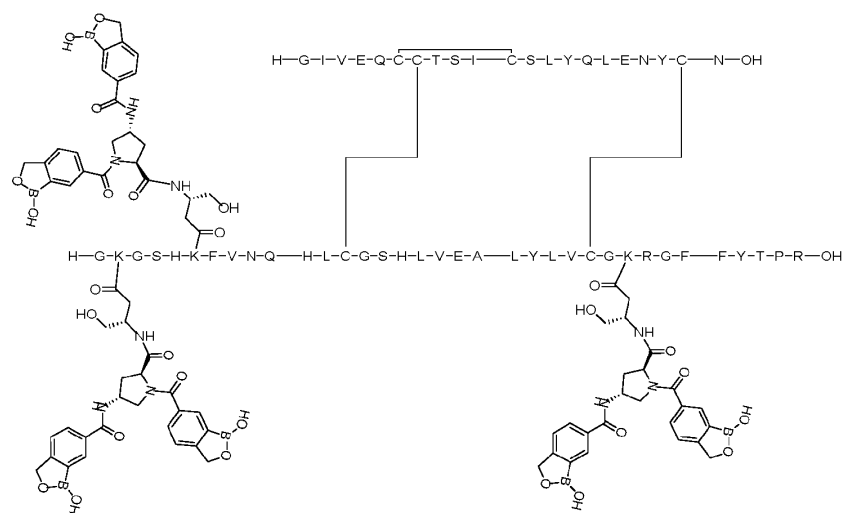
Example 61 (SEQ ID NOS 25480 and 25481, respectively, in order of appearance):



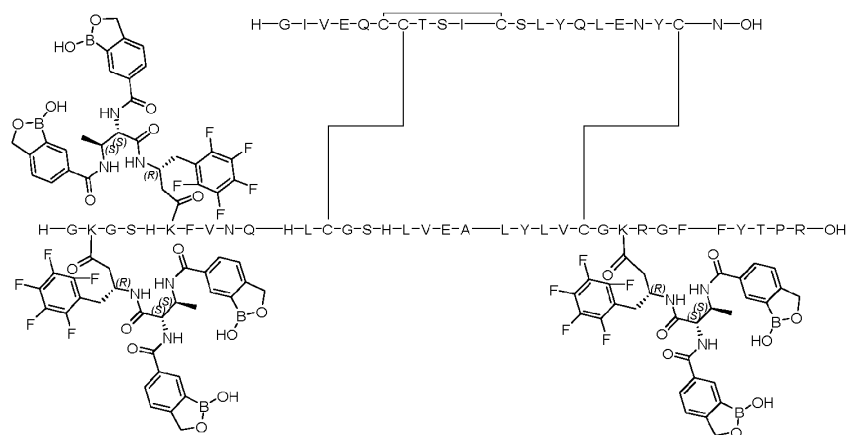
Example 62 (SEQ ID NOS 25516 and 25517, respectively, in order of appearance):



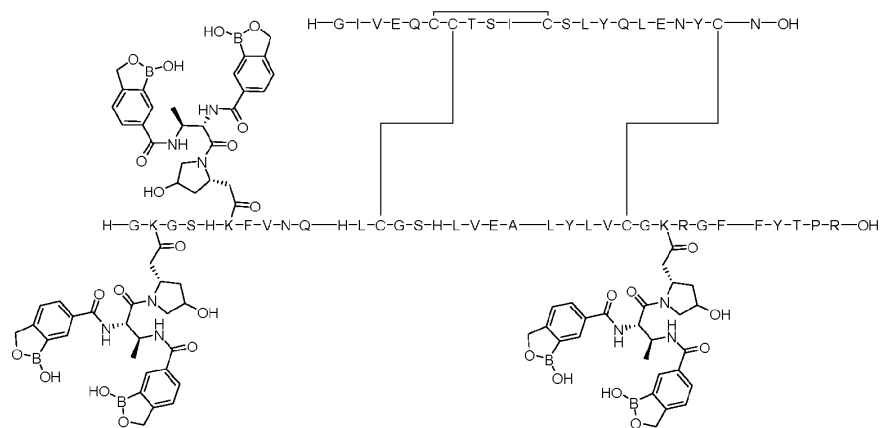
Example 63 (SEQ ID NOS 25518 and 25519, respectively, in order of appearance):



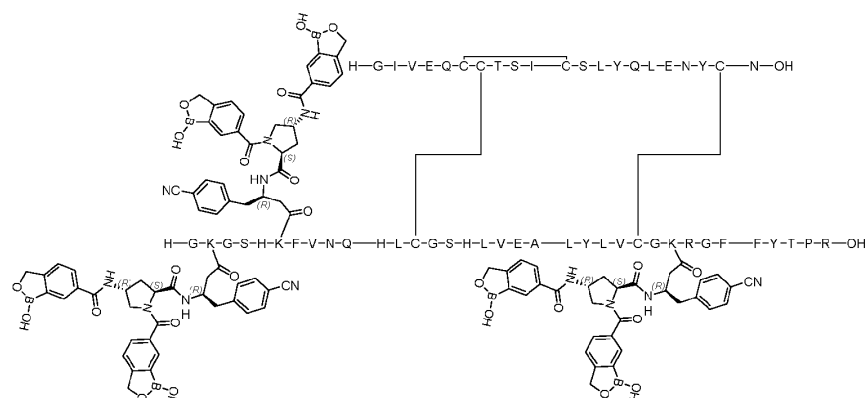
Example 64 (SEQ ID NOS 25520 and 25521, respectively, in order of appearance):



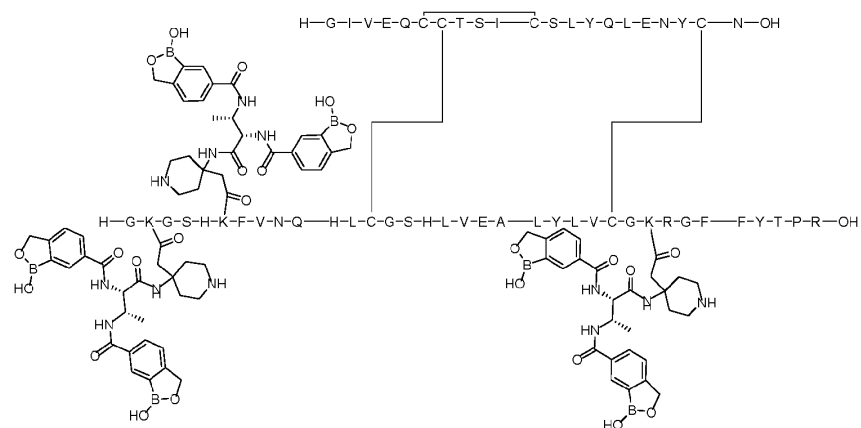
Example 65 (SEQ ID NOS 25522 and 25523, respectively, in order of appearance):



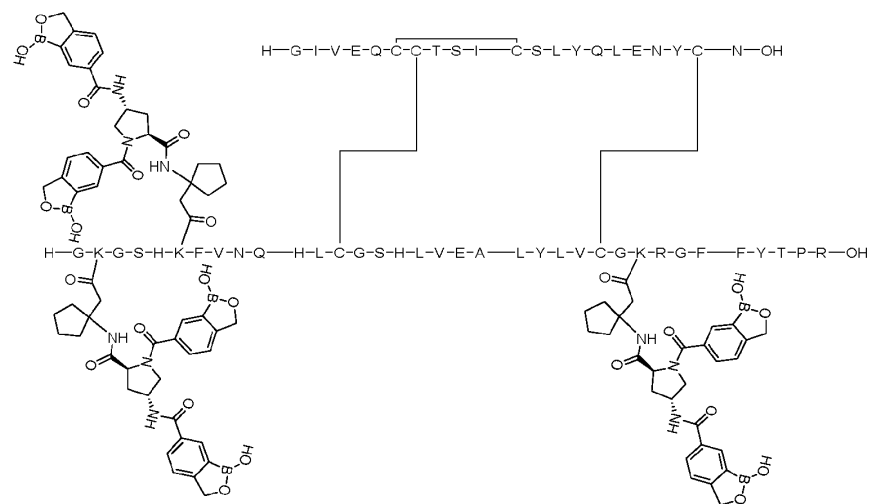
Example 66 (SEQ ID NOS 25524 and 25525, respectively, in order of appearance):



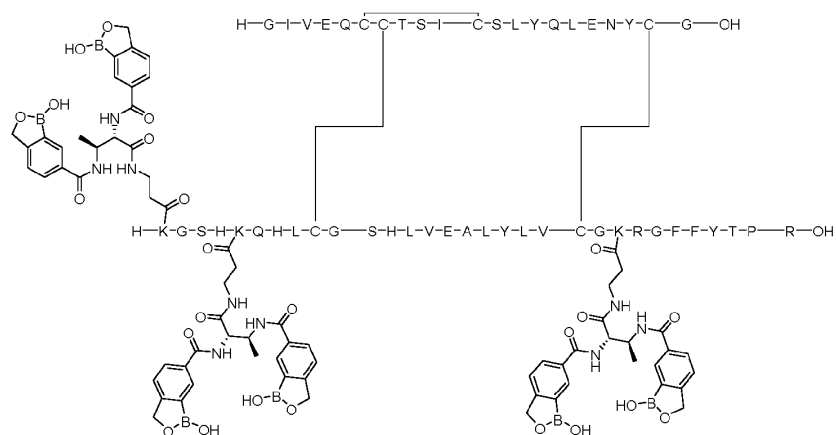
Example 67 (SEQ ID NOS 25526 and 25527, respectively, in order of appearance):



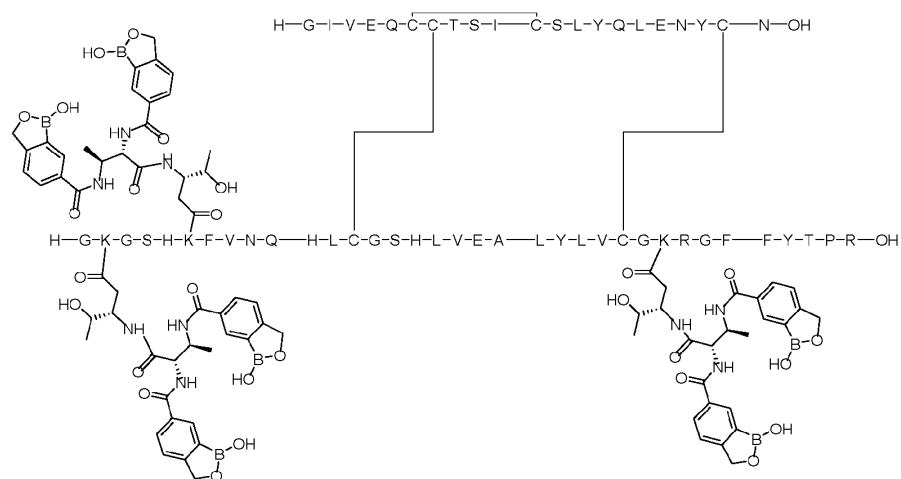
5 Example 68 (SEQ ID NOS 25528 and 25529, respectively, in order of appearance):



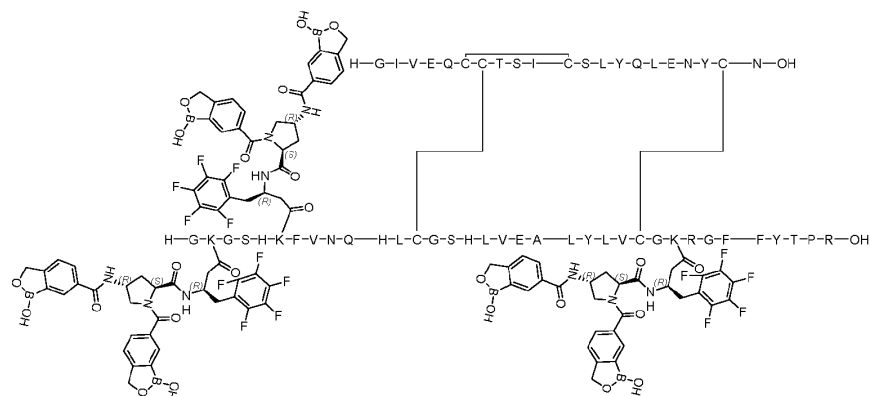
Example 69 (SEQ ID NOS 25530 and 25531, respectively, in order of appearance):



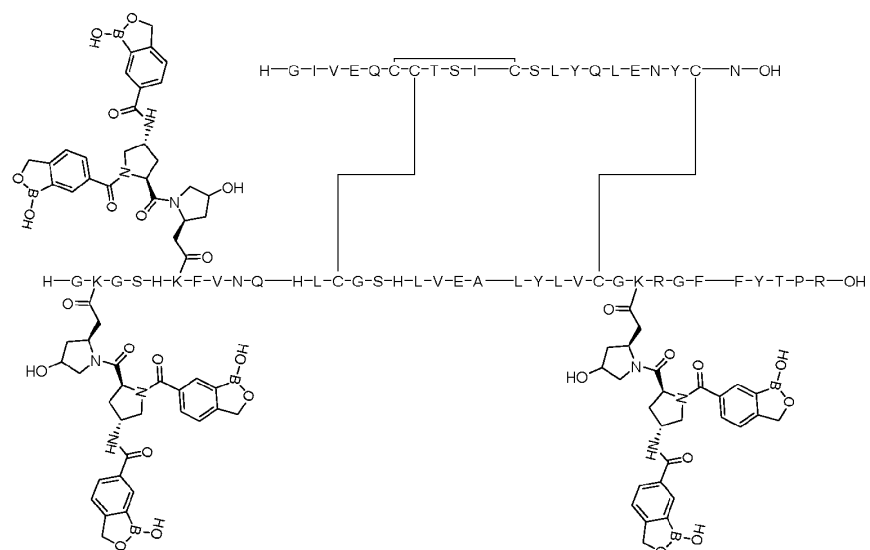
Example 70 (SEQ ID NOS 25532 and 25533, respectively, in order of appearance):



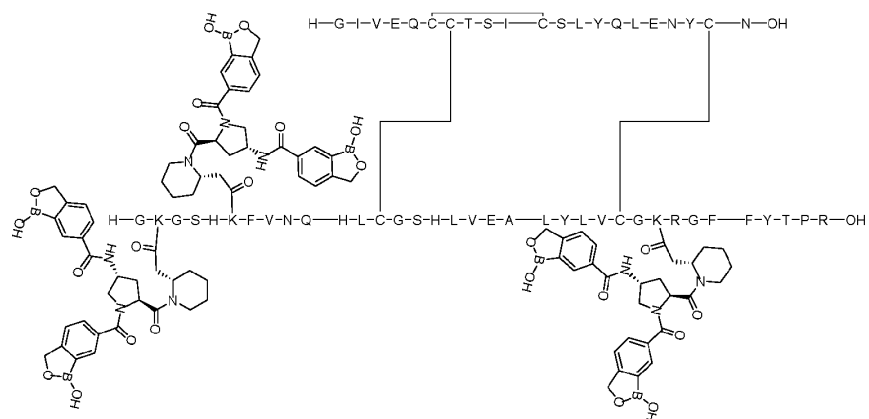
Example 71 (SEQ ID NOS 25534 and 25535, respectively, in order of appearance):



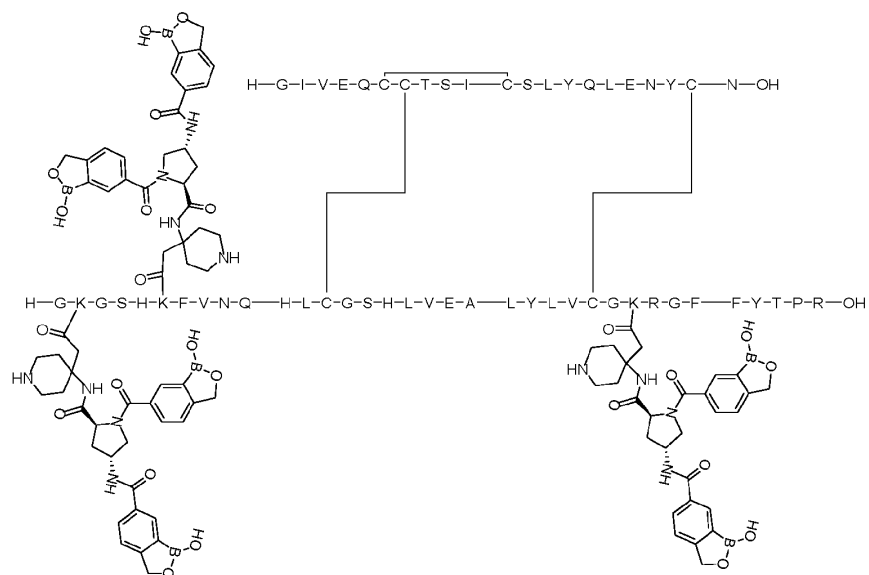
Example 72 (SEQ ID NOS 25536 and 25537, respectively, in order of appearance):



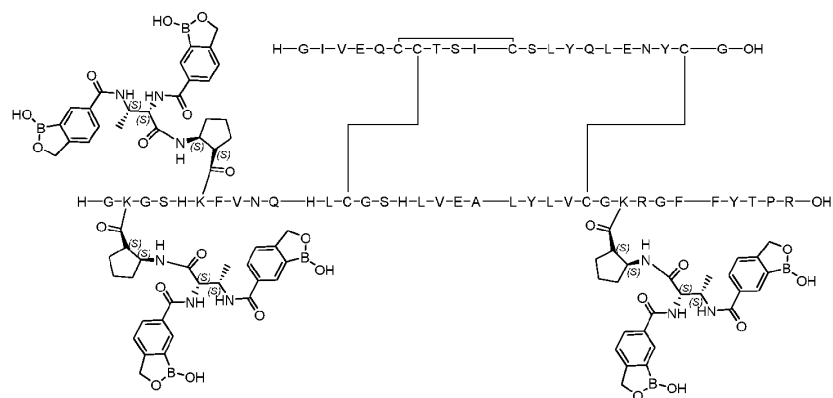
Example 73 (SEQ ID NOS 25538 and 25539, respectively, in order of appearance):



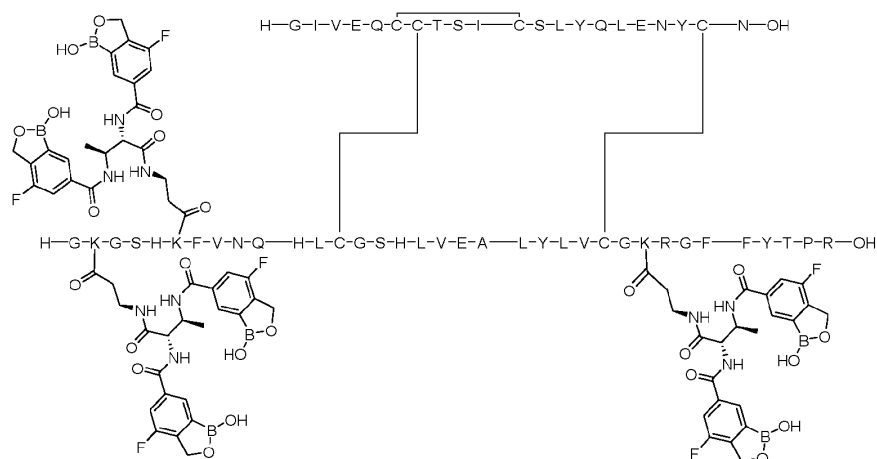
Example 74 (SEQ ID NOS 25540 and 25541, respectively, in order of appearance):



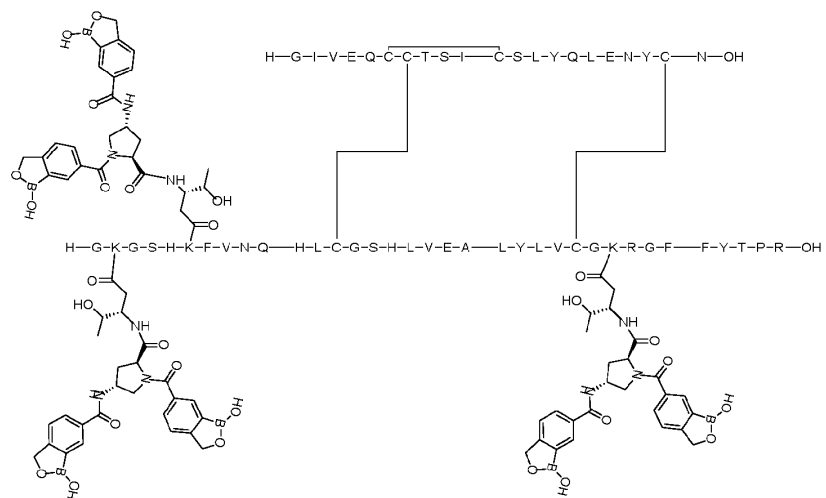
Example 75 (SEQ ID NOS 25542 and 25543, respectively, in order of appearance):



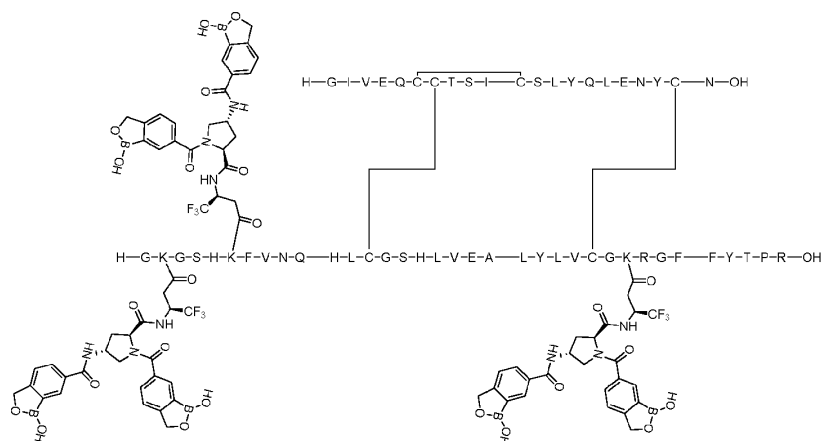
Example 76 (SEQ ID NOS 25544 and 25545, respectively, in order of appearance):



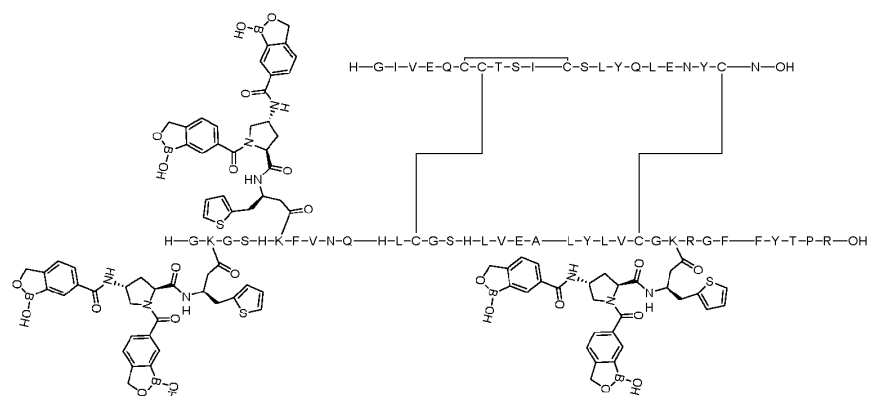
Example 77 (SEQ ID NOS 25546 and 25547, respectively, in order of appearance):



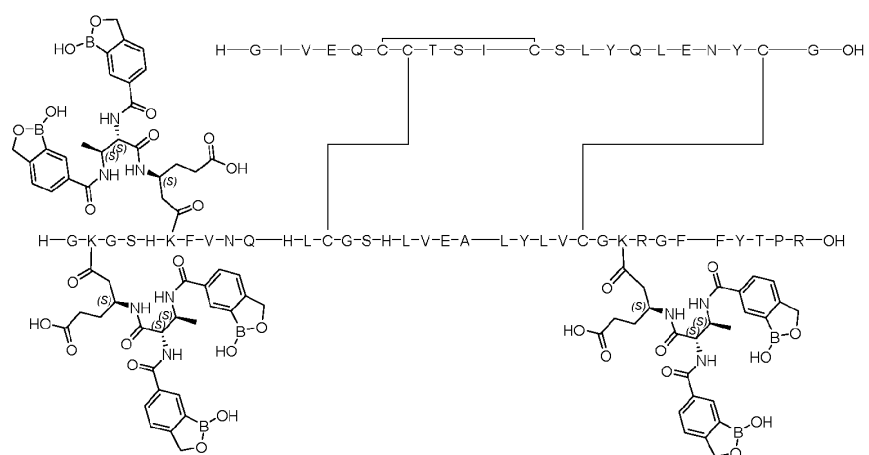
Example 78 (SEQ ID NOS 25548 and 25549, respectively, in order of appearance):



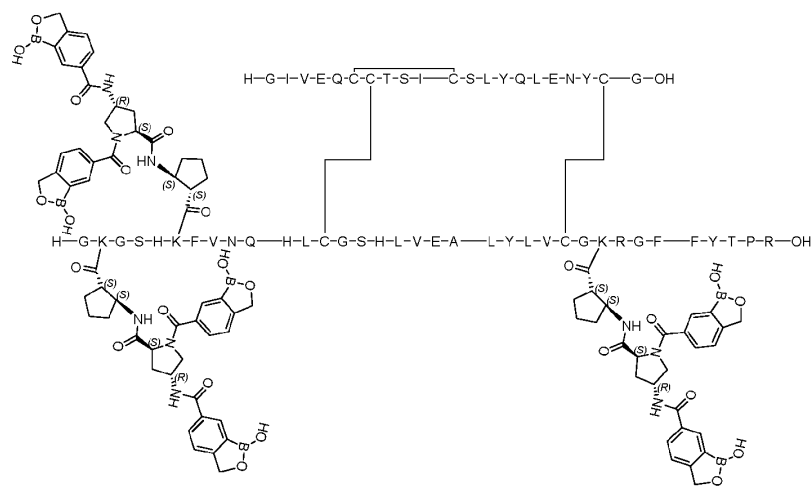
Example 79 (SEQ ID NOS 25550 and 25551, respectively, in order of appearance):



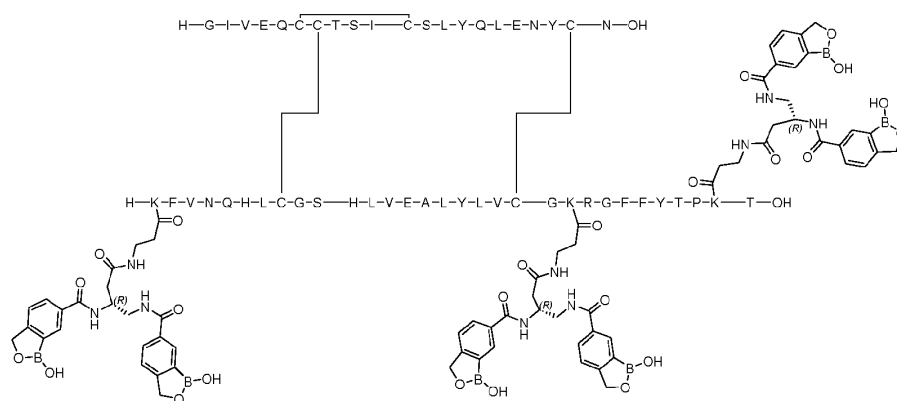
Example 80 (SEQ ID NOS 25552 and 25553, respectively, in order of appearance):



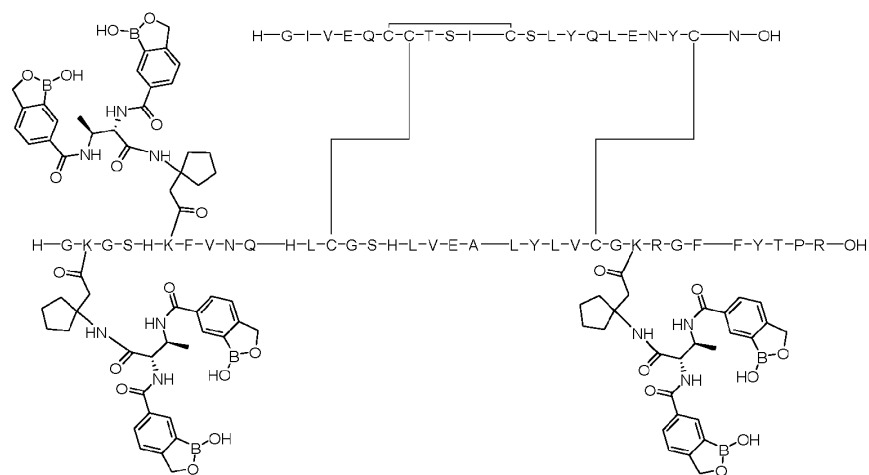
Example 81 (SEQ ID NOS 25554 and 25555, respectively, in order of appearance):



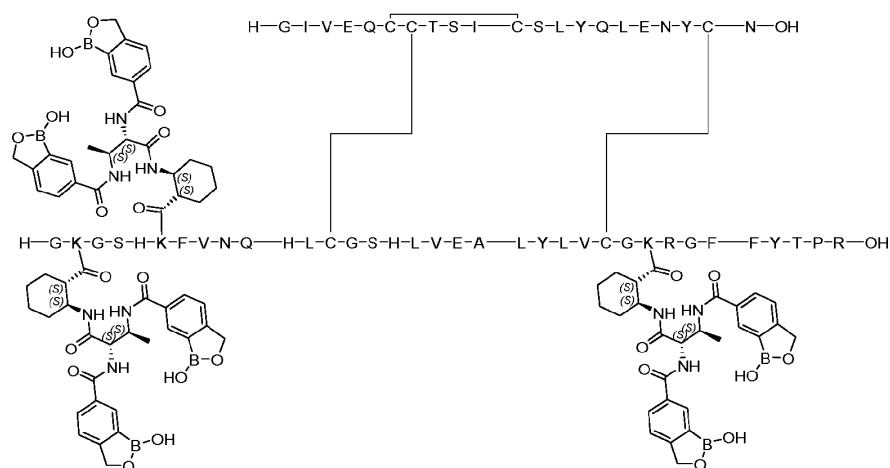
Example 82 (SEQ ID NOS 25556 and 25557, respectively, in order of appearance):



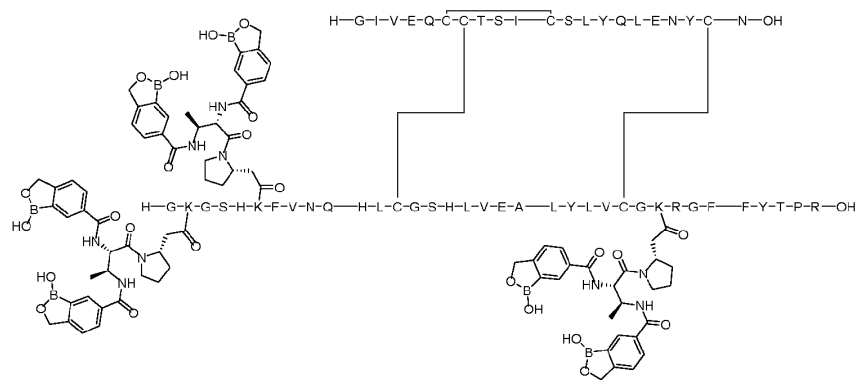
Example 83 (SEQ ID NOS 25558 and 25559, respectively, in order of appearance):



Example 84 (SEQ ID NOS 25560 and 25561, respectively, in order of appearance):

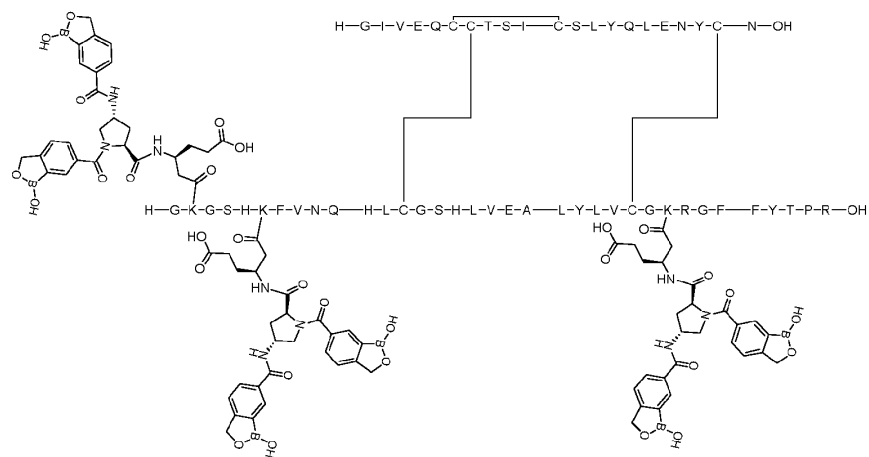


Example 85 (SEQ ID NOS 25562 and 25563, respectively, in order of appearance):

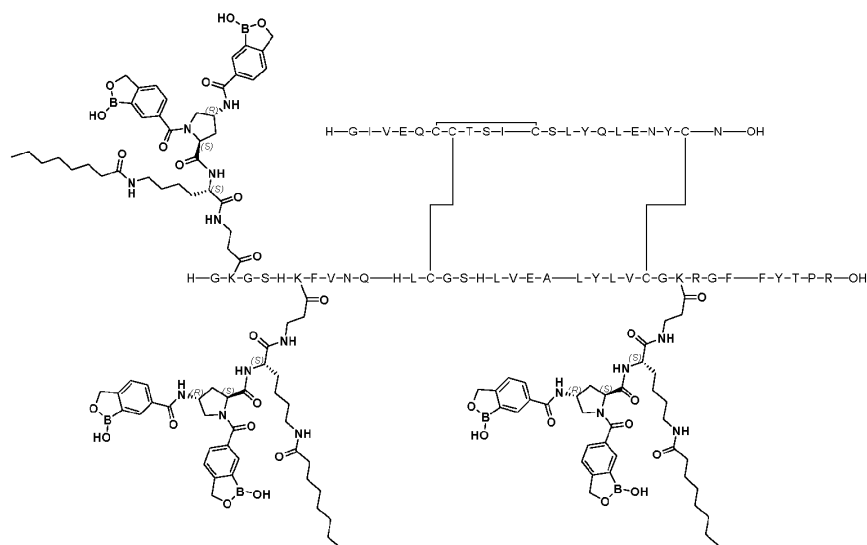


Example 86 (SEQ ID NOS 25564 and 25565, respectively, in order of appearance):

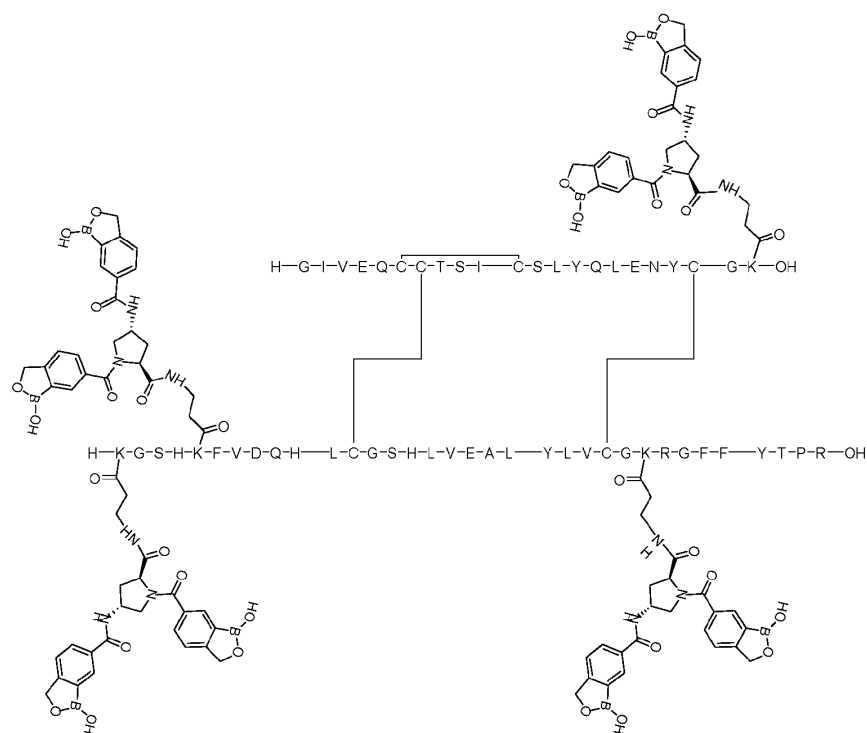
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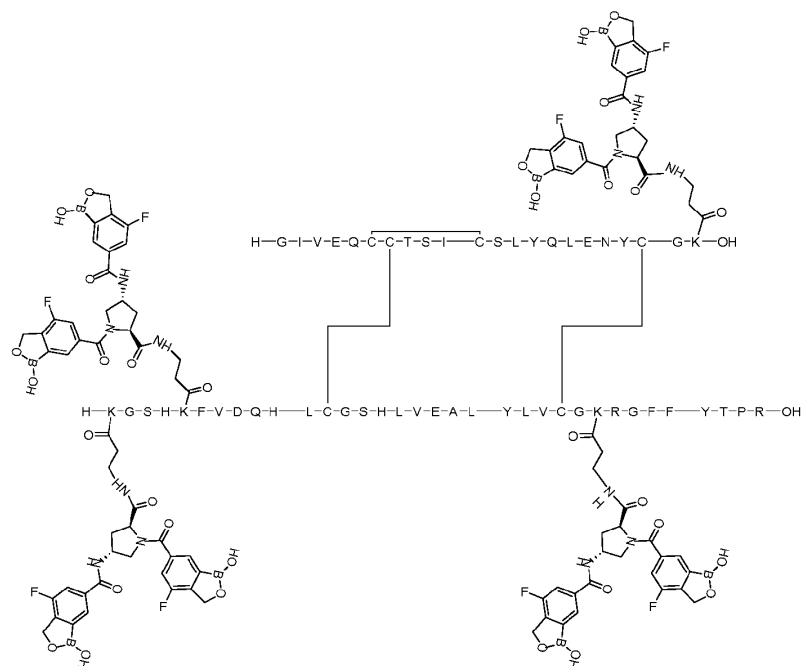
Example 87 (SEQ ID NOS 25566 and 25567, respectively, in order of appearance):



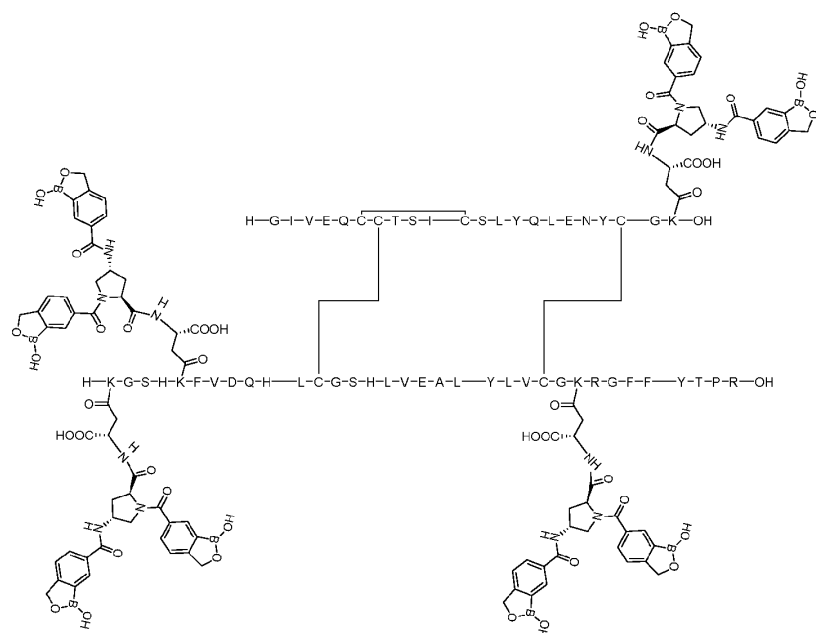
Example 88 (SEQ ID NOS 25568 and 25569, respectively, in order of appearance):



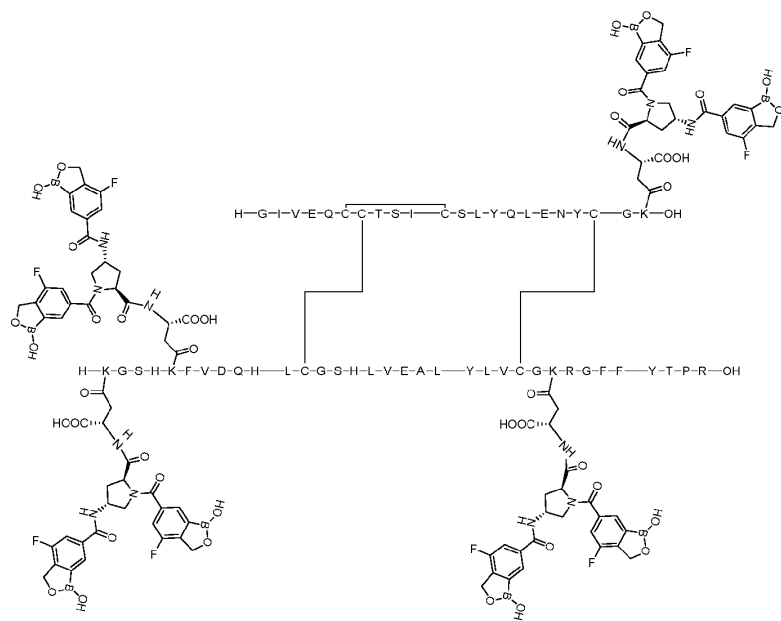
Example 89 (SEQ ID NOS 25570 and 25571, respectively, in order of appearance):



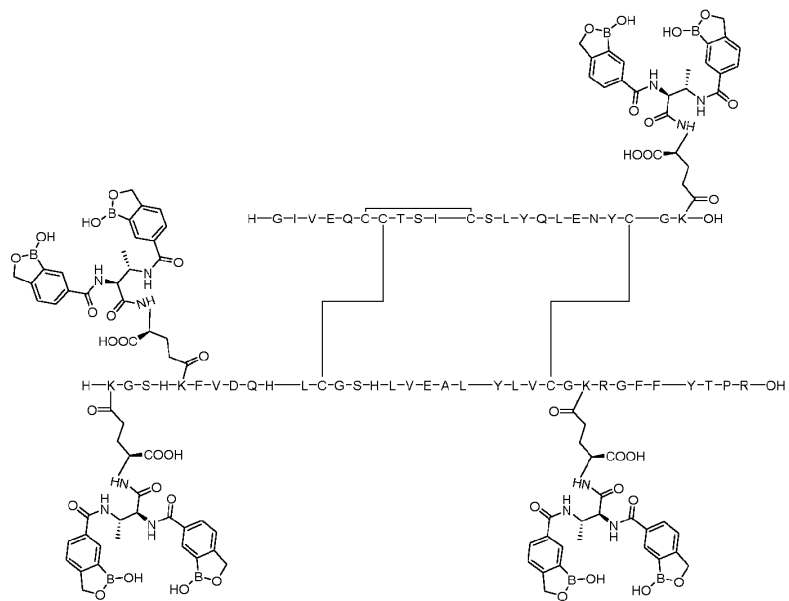
Example 90 (SEQ ID NOS 25572 and 25573, respectively, in order of appearance):



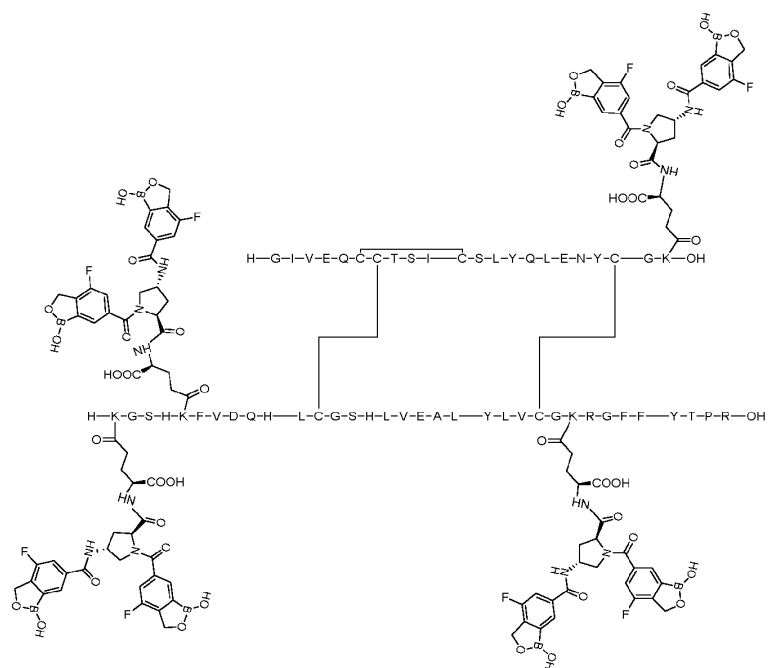
Example 91 (SEQ ID NOS 25574 and 25575, respectively, in order of appearance):



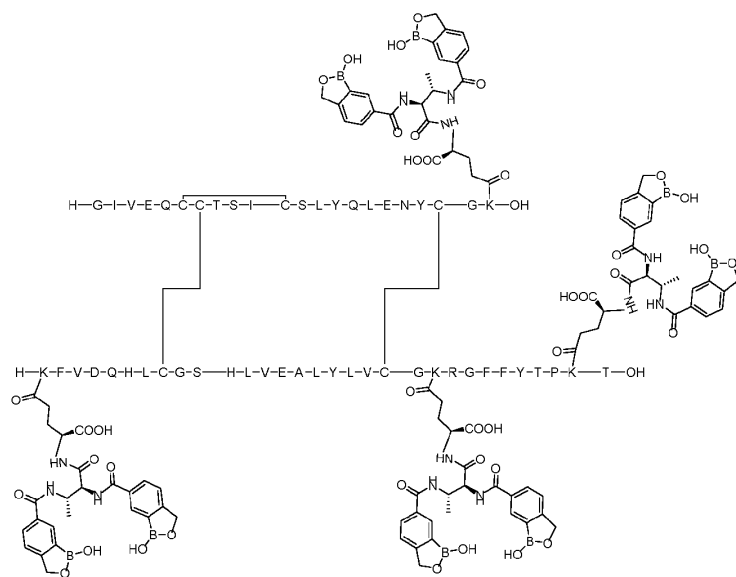
Example 92 (SEQ ID NOS 25576 and 25577, respectively, in order of appearance):



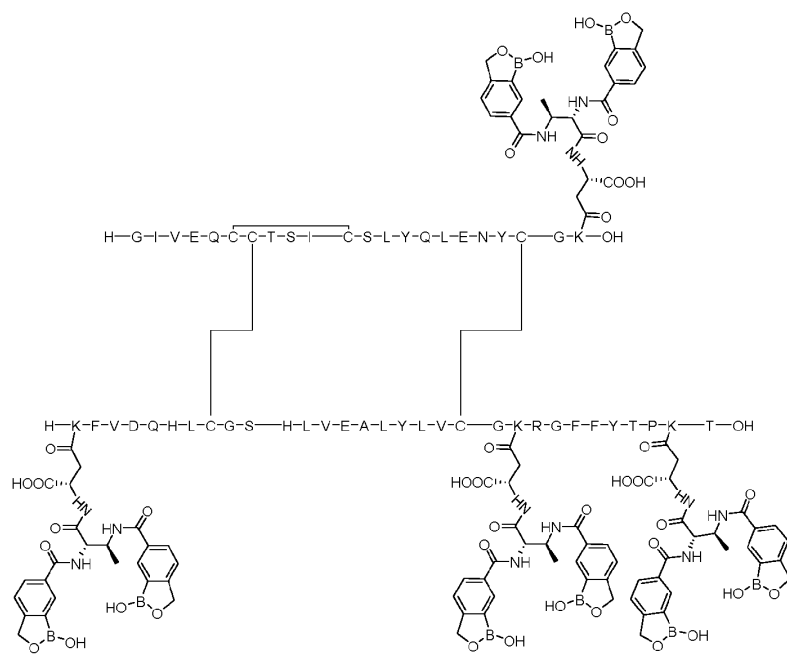
Example 93 (SEQ ID NOS 25578 and 25579, respectively, in order of appearance):



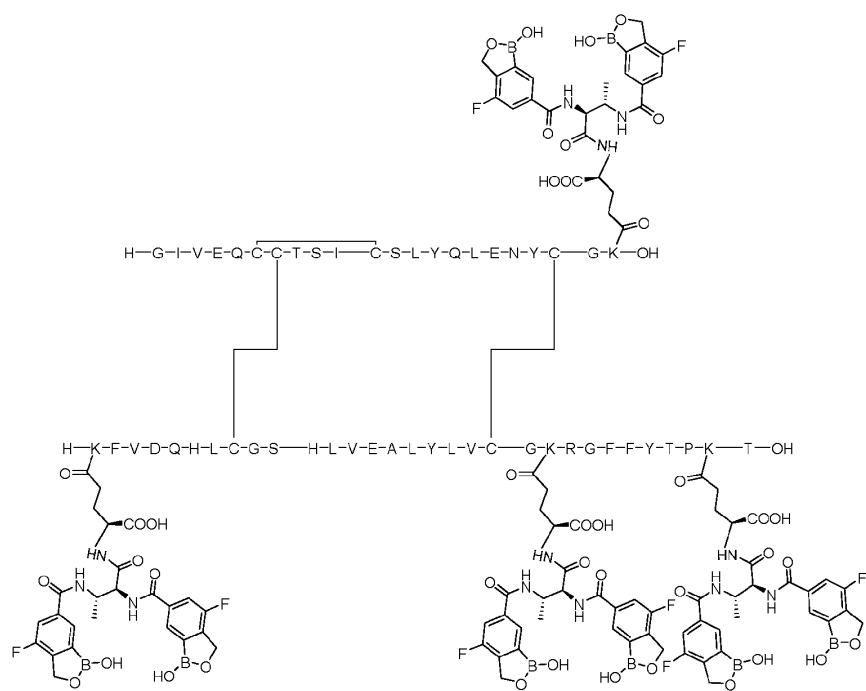
Example 94 (SEQ ID NOS 25580 and 25581, respectively, in order of appearance):



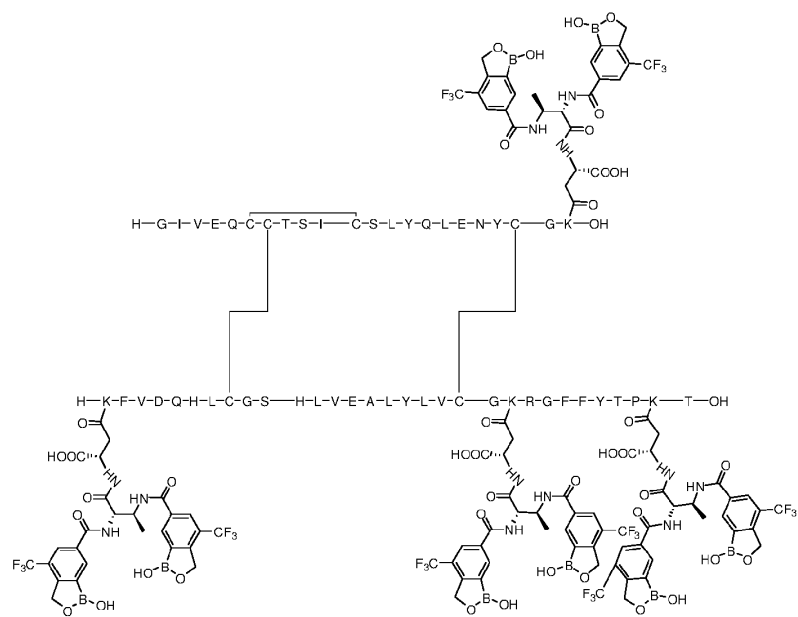
Example 95 (SEQ ID NOS 25582 and 25583, respectively, in order of appearance):



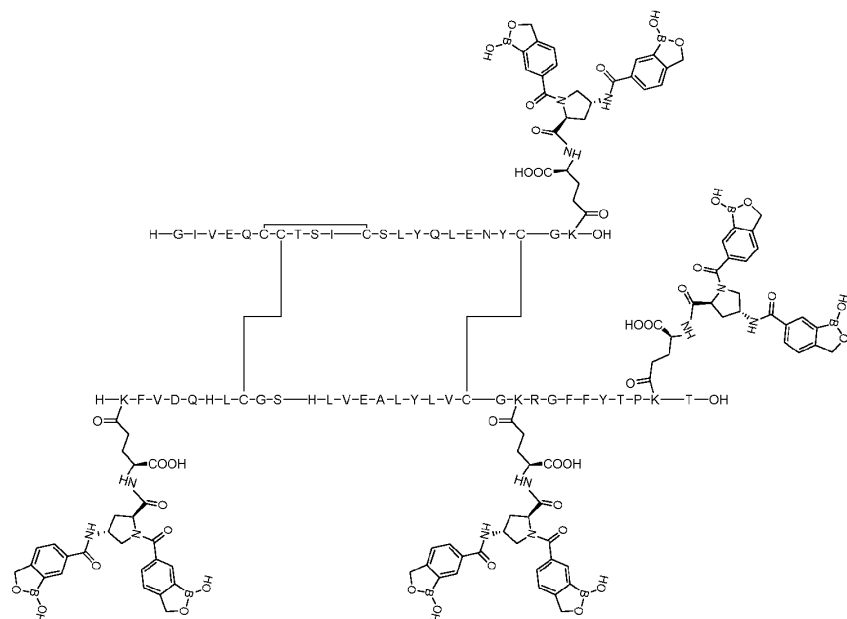
Example 96 (SEQ ID NOS 25584 and 25585, respectively, in order of appearance):



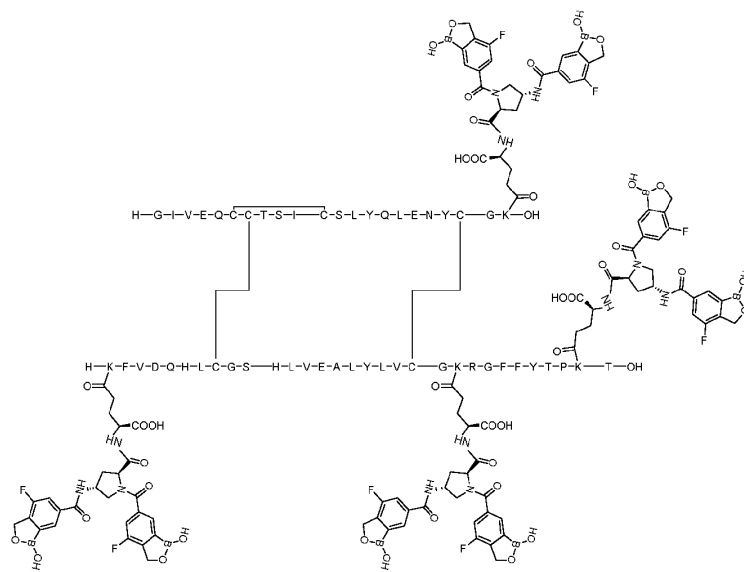
Example 97 (SEQ ID NOS 25586 and 25587, respectively, in order of appearance):



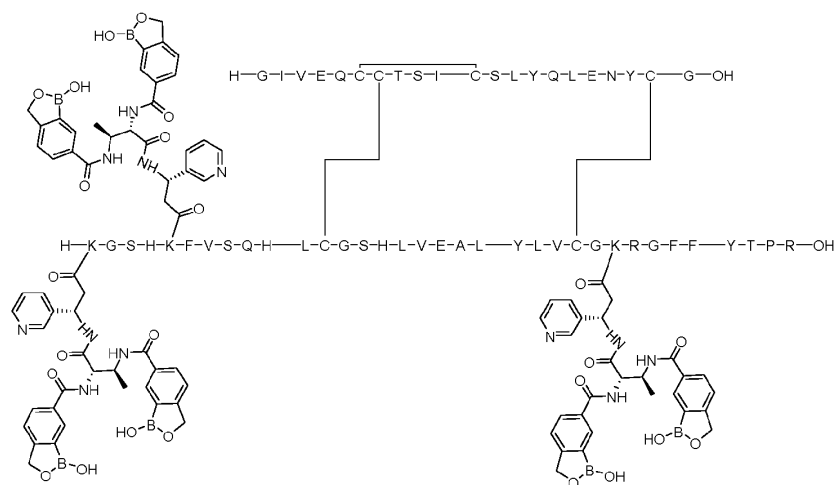
Example 98 (SEQ ID NOS 25588 and 25589, respectively, in order of appearance):



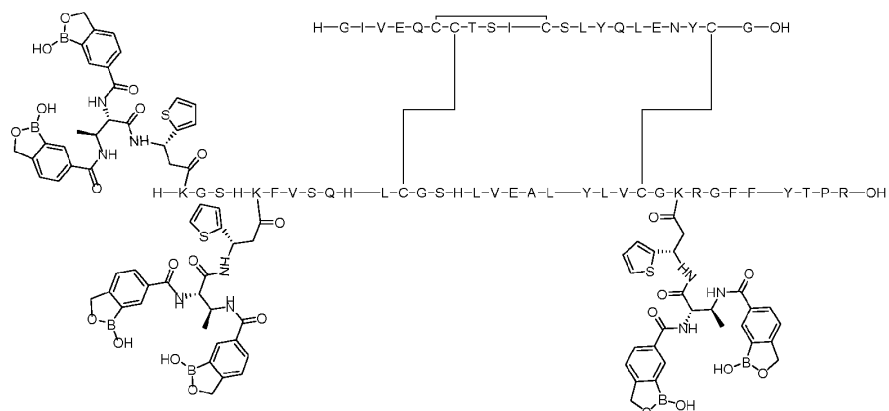
Example 99 (SEQ ID NOS 25590 and 25591, respectively, in order of appearance):



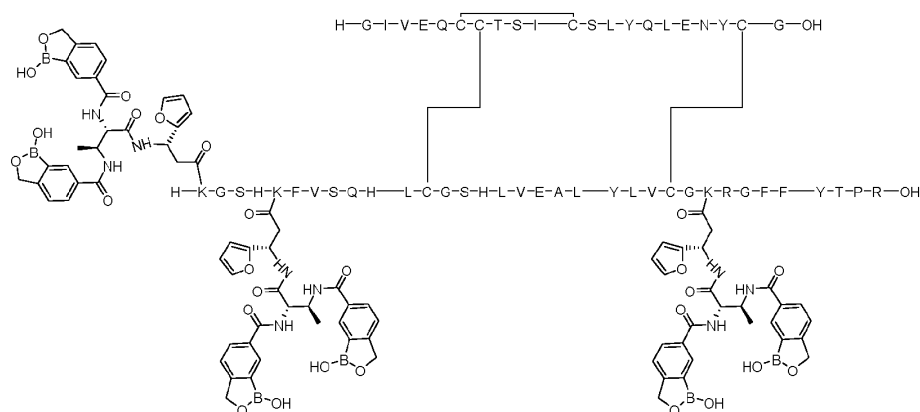
Example 100 (SEQ ID NOS 25592 and 25593, respectively, in order of appearance):



Example 101 (SEQ ID NOS 25594 and 25595, respectively, in order of appearance):

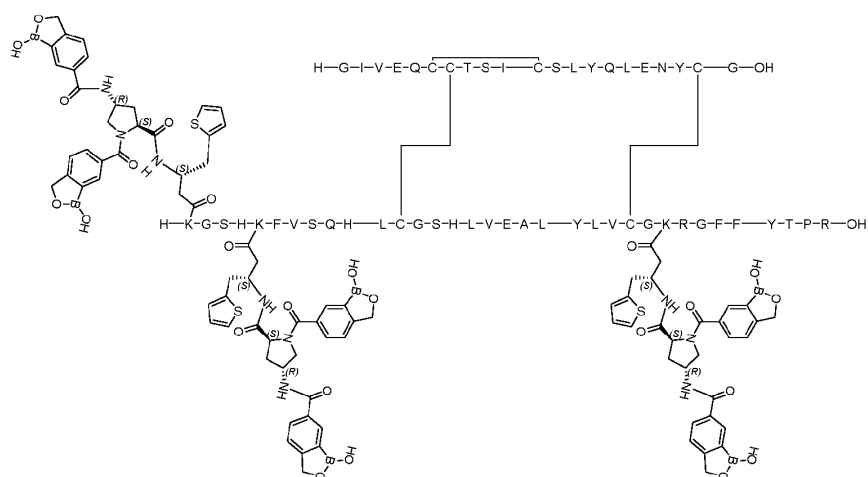


Example 102 (SEQ ID NOS 25596 and 25597, respectively, in order of appearance):

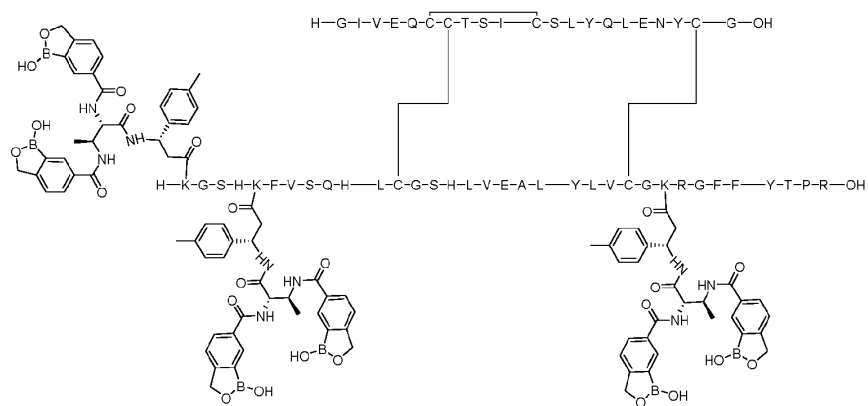


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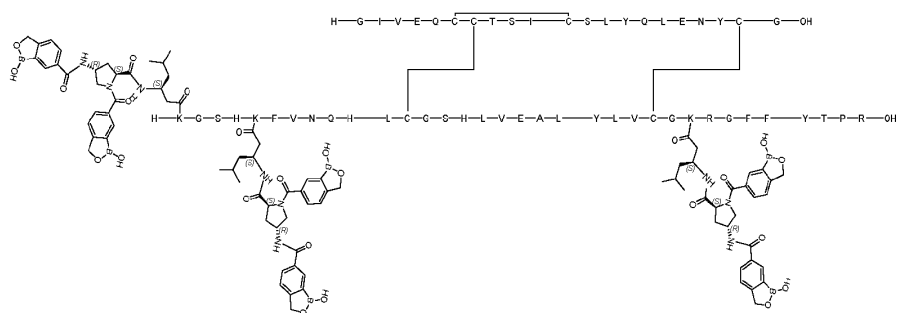
Example 103 (SEQ ID NOS 25598 and 25599, respectively, in order of appearance):



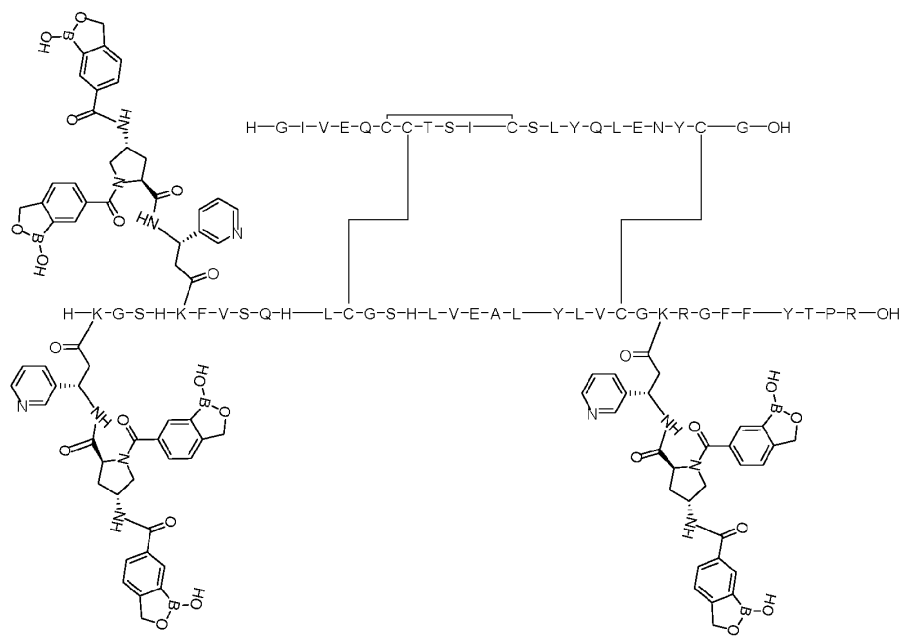
Example 104 (SEQ ID NOS 25600 and 25601, respectively, in order of appearance):



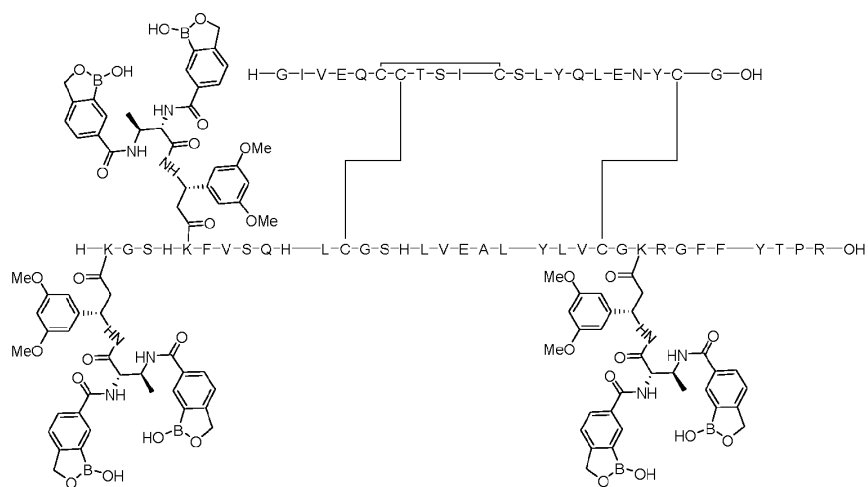
5 Example 105 (SEQ ID NOS 25602 and 25603, respectively, in order of appearance):



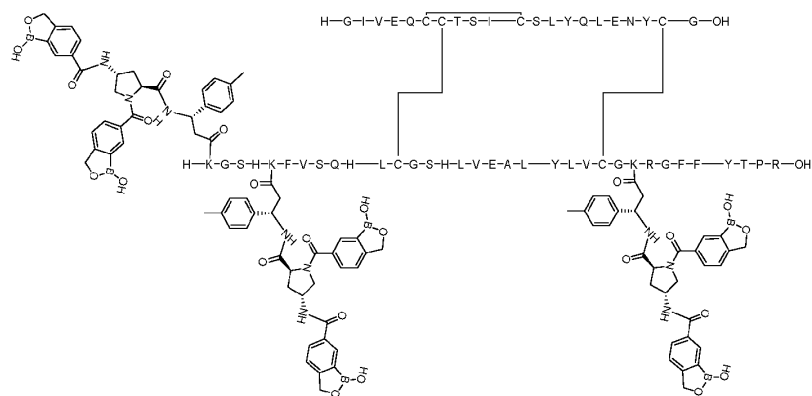
Example 106 (SEQ ID NOS 25604 and 25605, respectively, in order of appearance):



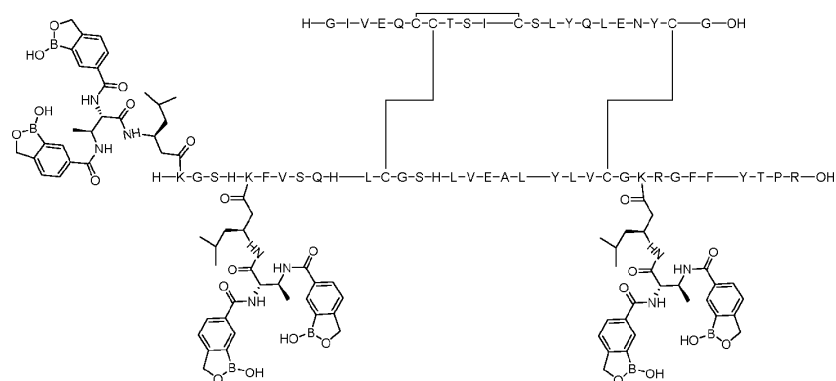
Example 107 (SEQ ID NOS 25606 and 25607, respectively, in order of appearance):



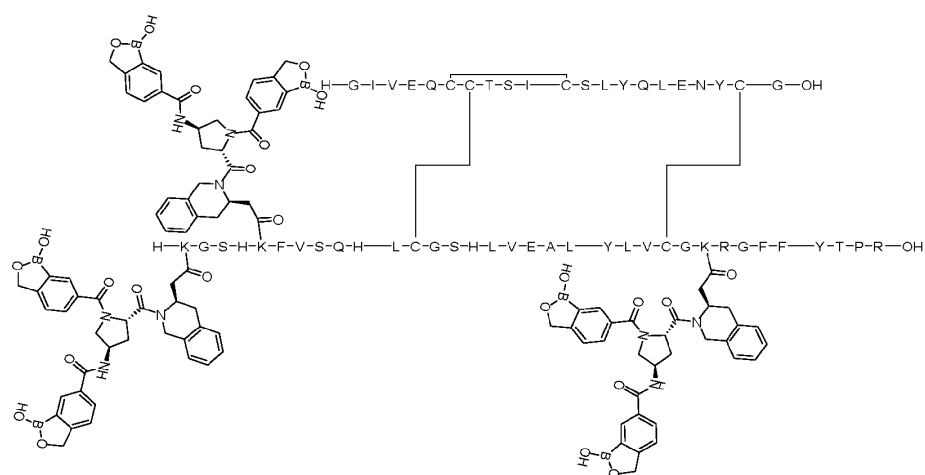
Example 108 (SEQ ID NOS 25608 and 25609, respectively, in order of appearance):



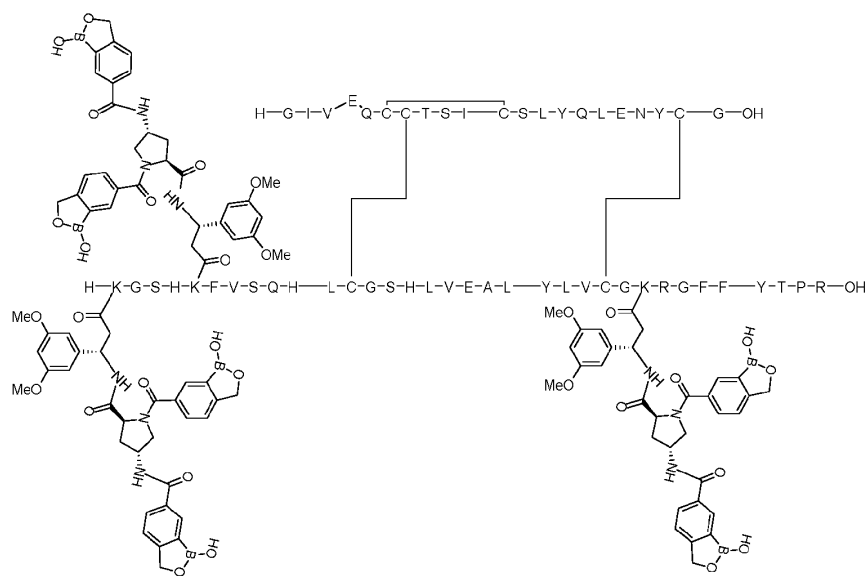
Example 109 (SEQ ID NOS 25610 and 25611, respectively, in order of appearance):



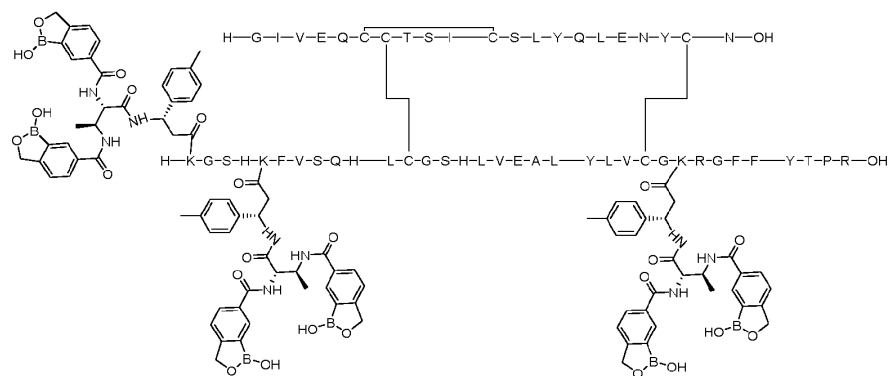
Example 110 (SEQ ID NOS 25612 and 25613, respectively, in order of appearance):



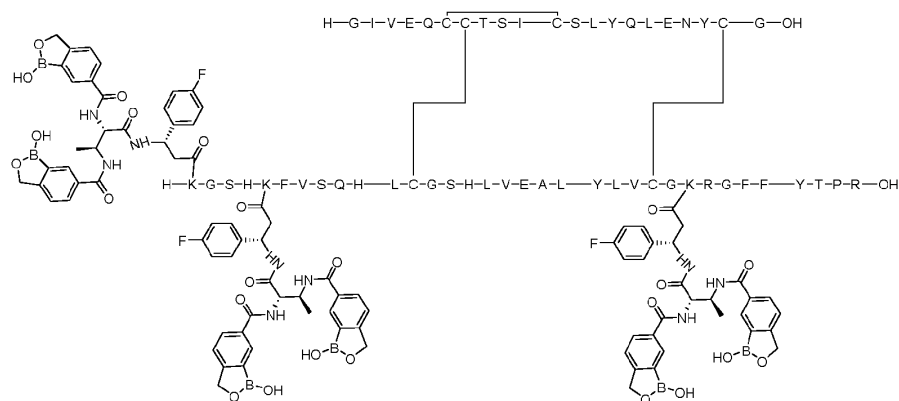
Example 111 (SEQ ID NOS 25614 and 25615, respectively, in order of appearance):



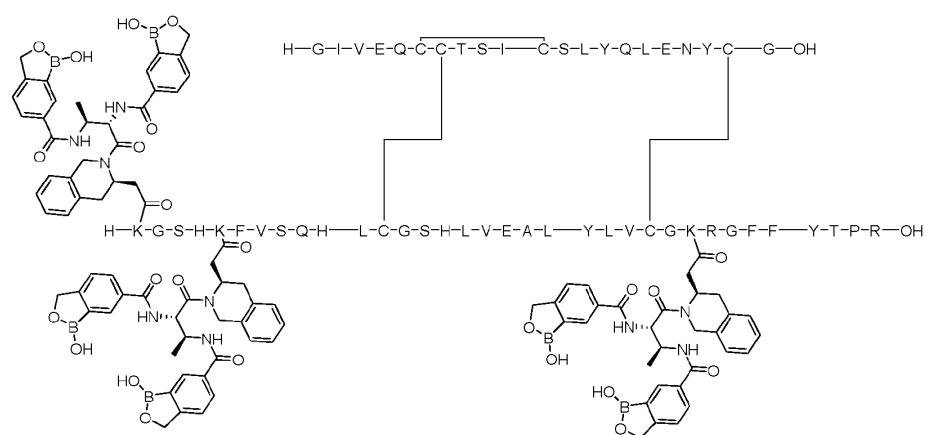
Example 112 (SEQ ID NOS 25616 and 25617, respectively, in order of appearance):



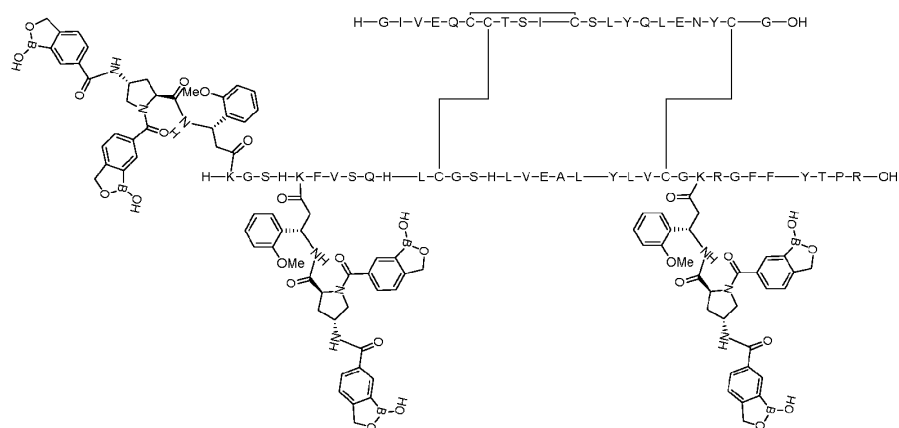
Example 113 (SEQ ID NOS 25618 and 25619, respectively, in order of appearance):



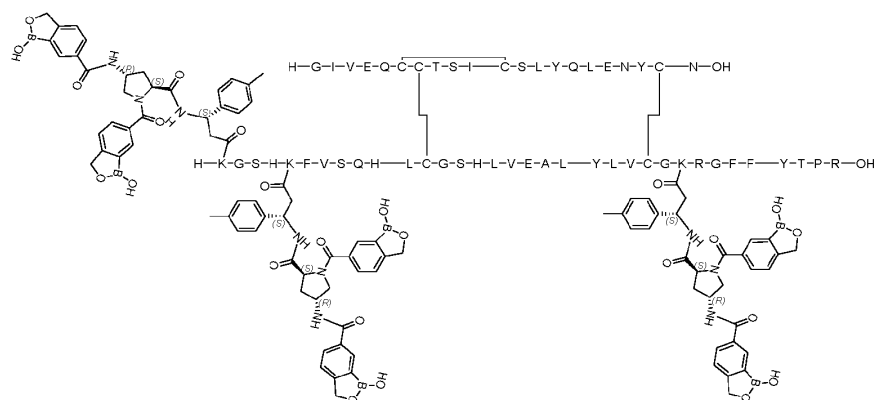
5 Example 114 (SEQ ID NOS 25620 and 25621, respectively, in order of appearance):



Example 115 (SEQ ID NOS 25622 and 25623, respectively, in order of appearance):

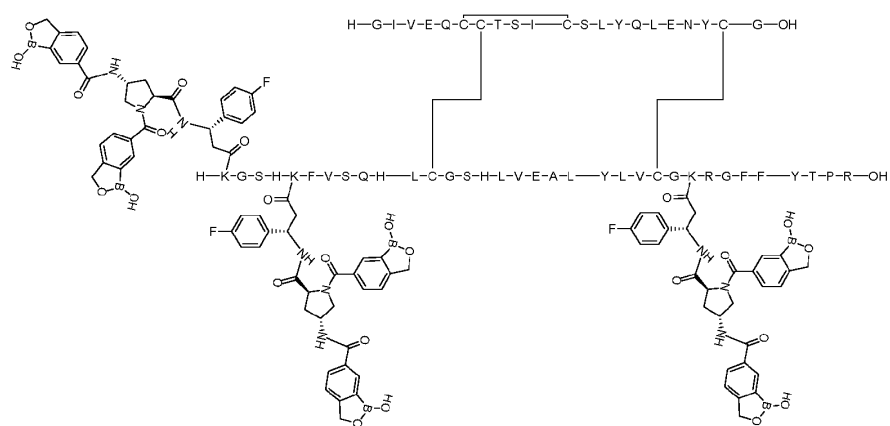


Example 116 (SEQ ID NOS 25624 and 25625, respectively, in order of appearance):

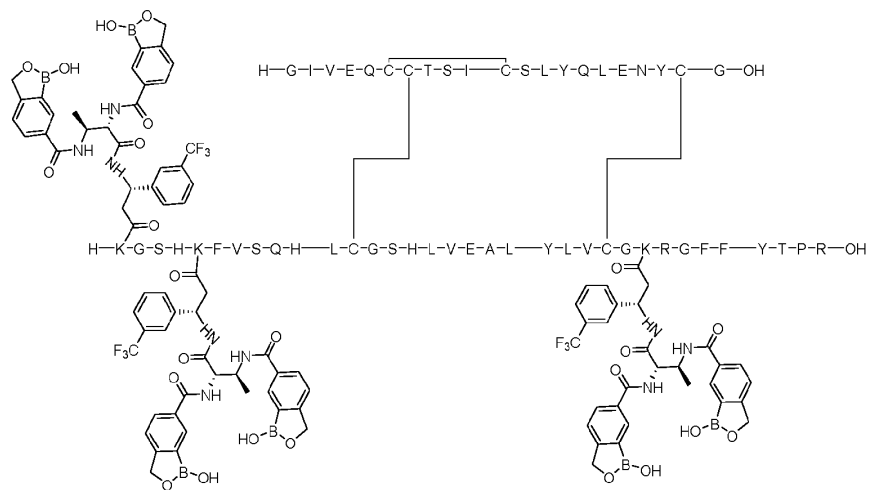


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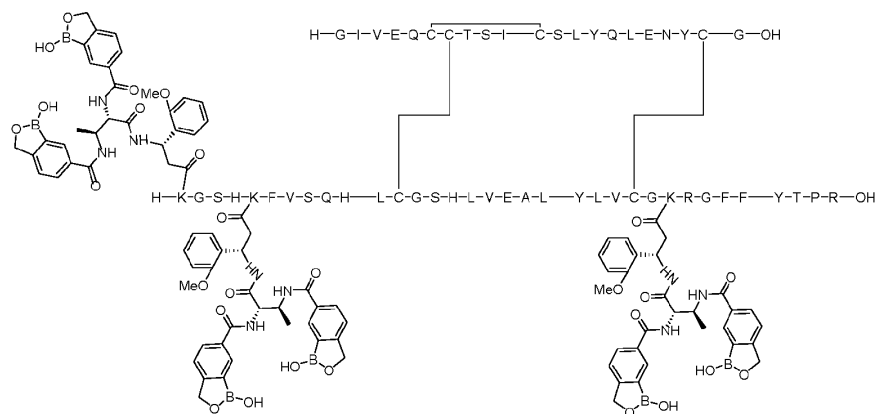
Example 117 (SEQ ID NOS 25626 and 25627, respectively, in order of appearance):



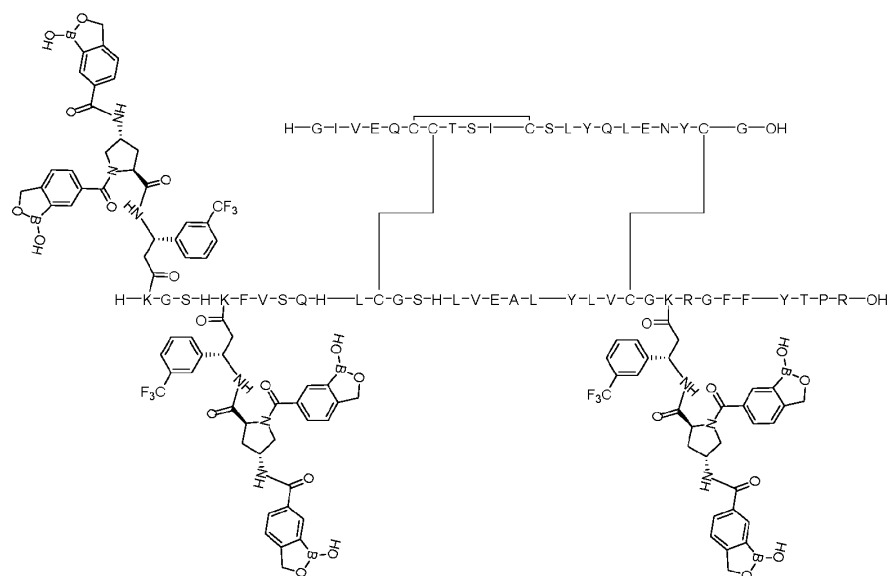
Example 118 (SEQ ID NOS 25628 and 25629, respectively, in order of appearance):



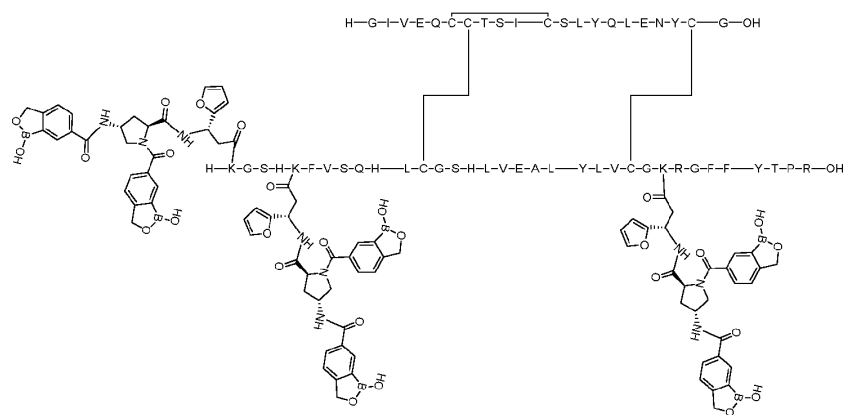
5 Example 119 (SEQ ID NOS 25630 and 25631, respectively, in order of appearance):



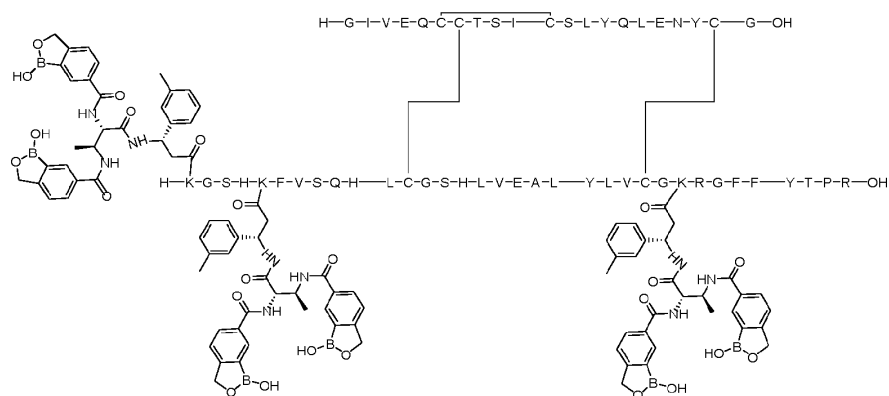
Example 120 (SEQ ID NOS 25632 and 25633, respectively, in order of appearance):



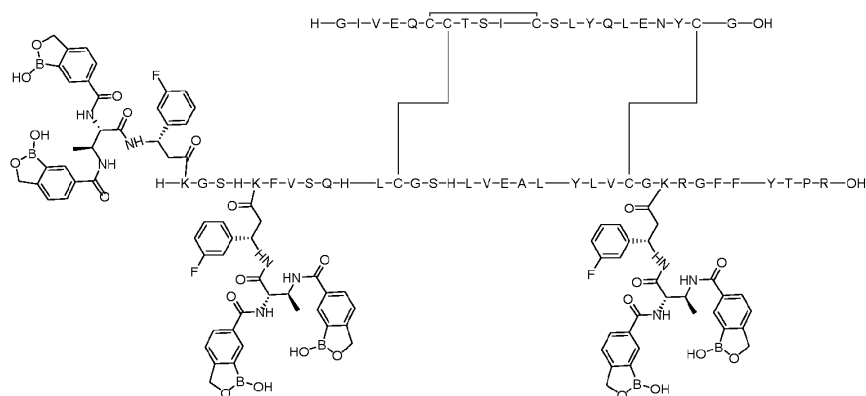
Example 121 (SEQ ID NOS 25634 and 25635, respectively, in order of appearance):



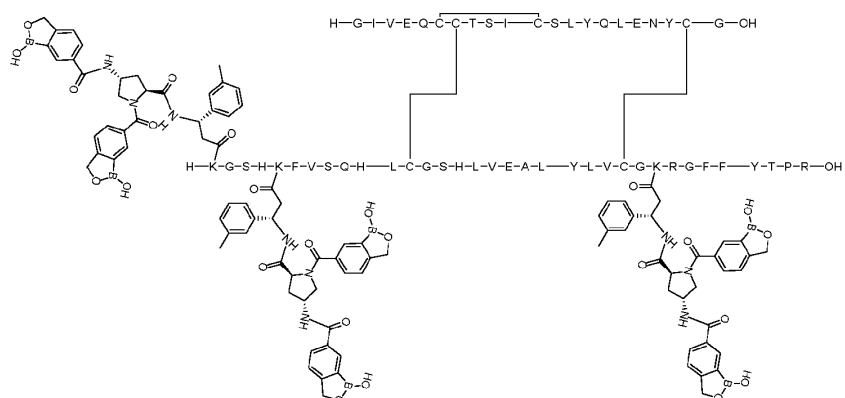
Example 122 (SEQ ID NOS 25636 and 25637, respectively, in order of appearance):



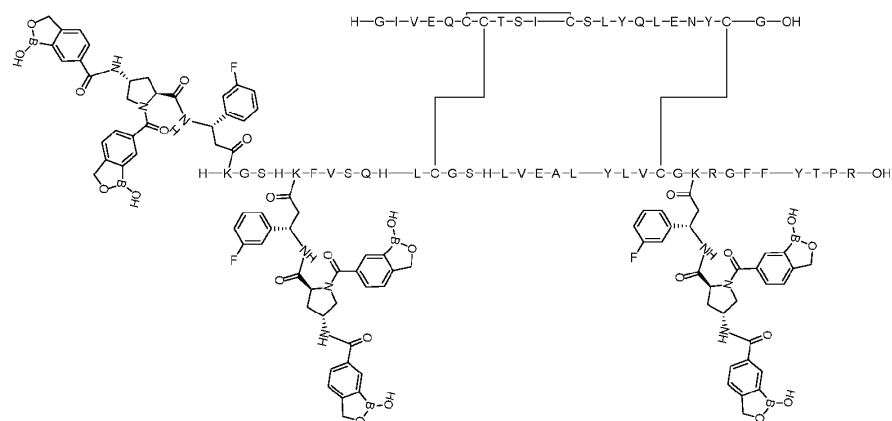
Example 123 (SEQ ID NOS 25638 and 25639, respectively, in order of appearance):



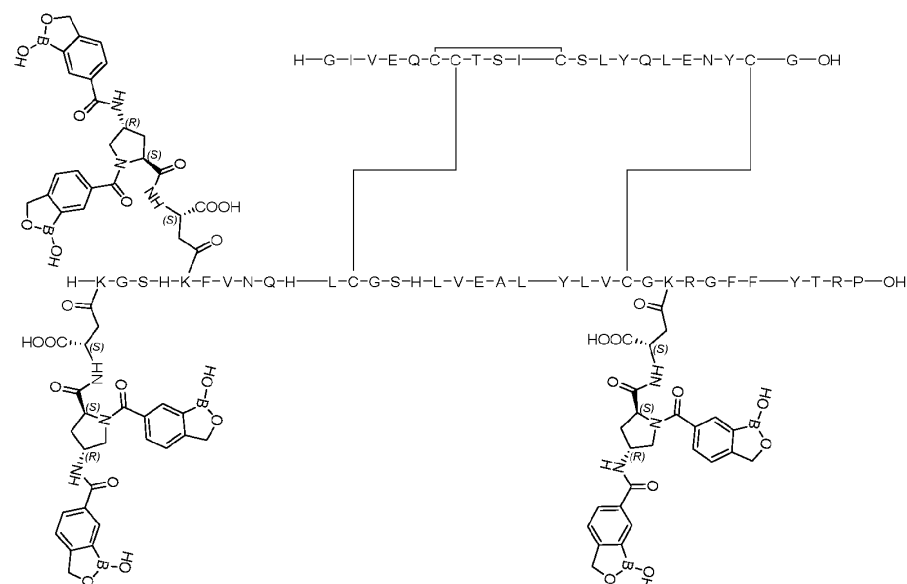
Example 124 (SEQ ID NOS 25640 and 25641, respectively, in order of appearance):



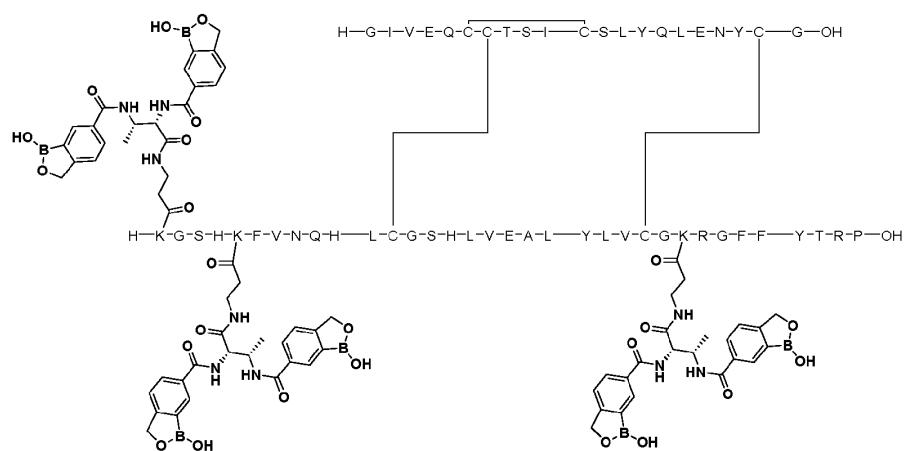
Example 125 (SEQ ID NOS 25642 and 25643, respectively, in order of appearance):



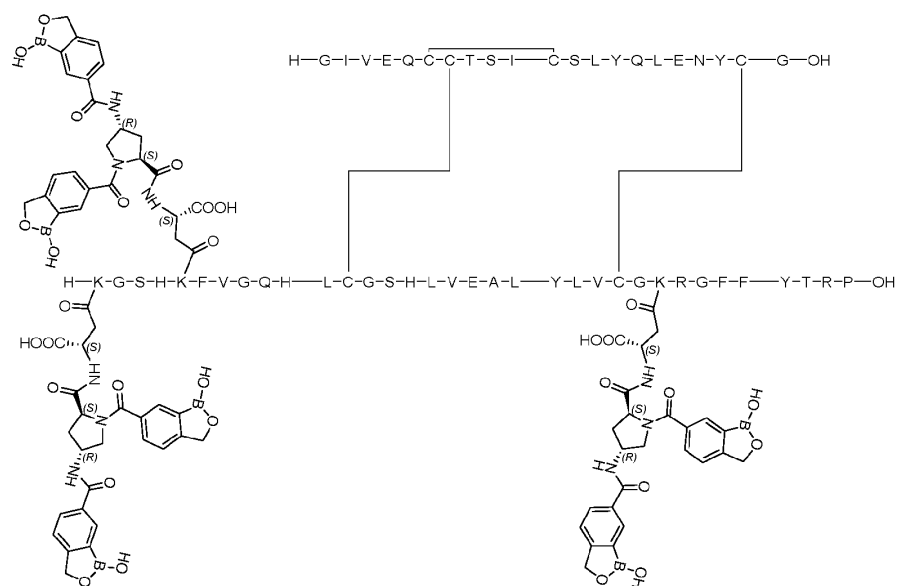
Example 126 (SEQ ID NOS 25644 and 25645, respectively, in order of appearance):



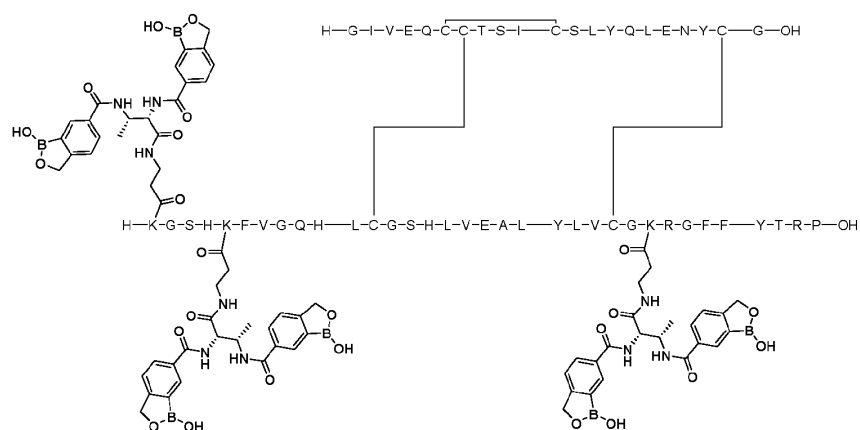
Example 127 (SEQ ID NOS 25646 and 25647, respectively, in order of appearance):



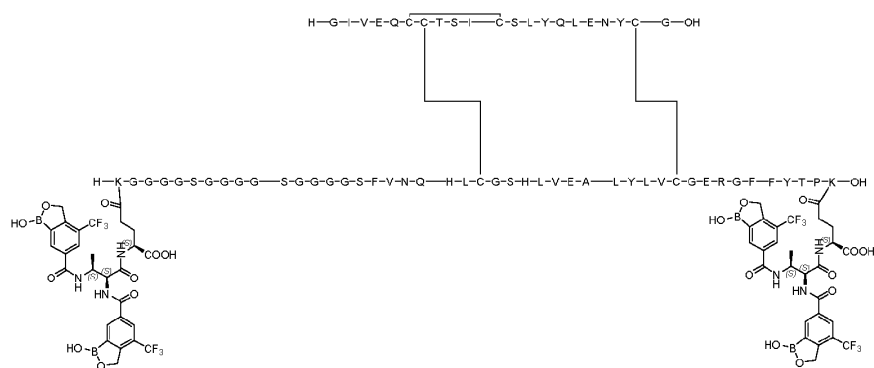
Example 128 (SEQ ID NOS 25648 and 25649, respectively, in order of appearance):



Example 129 (SEQ ID NOS 25650 and 25651, respectively, in order of appearance):

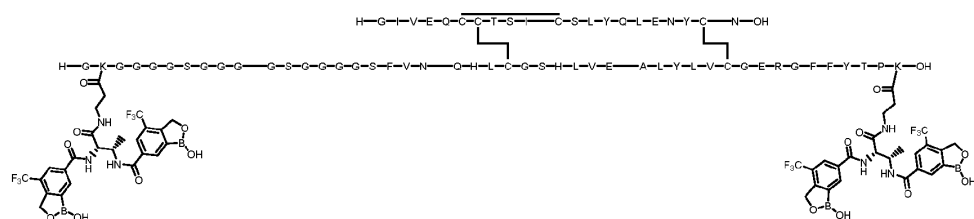


Example 130 (SEQ ID NOS 25652 and 25653, respectively, in order of appearance):

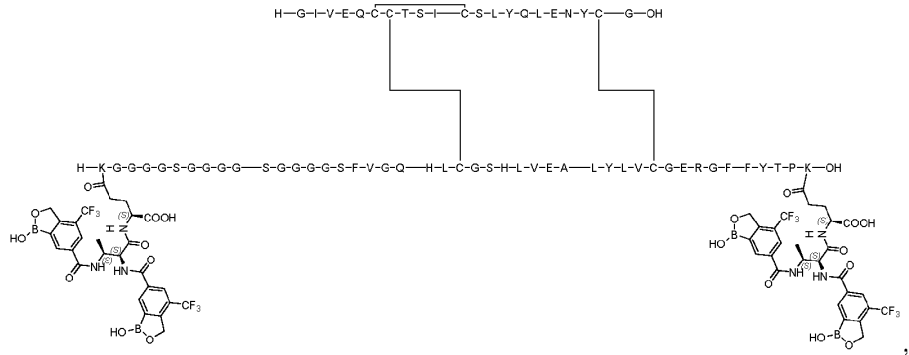


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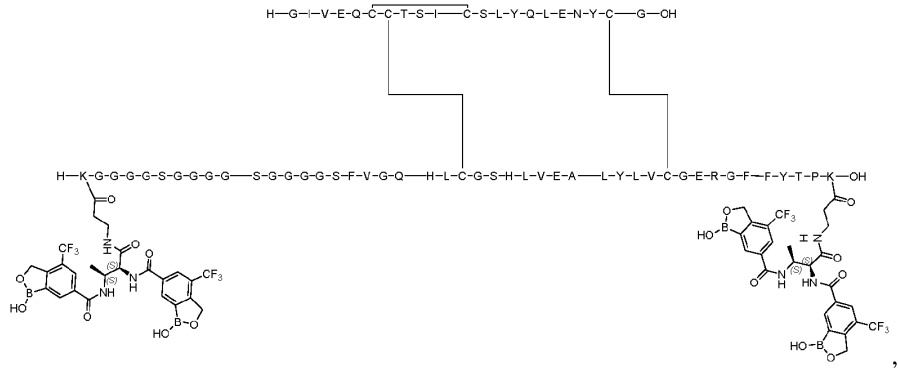
Example 131 (SEQ ID NOS 25654 and 25655, respectively, in order of appearance):



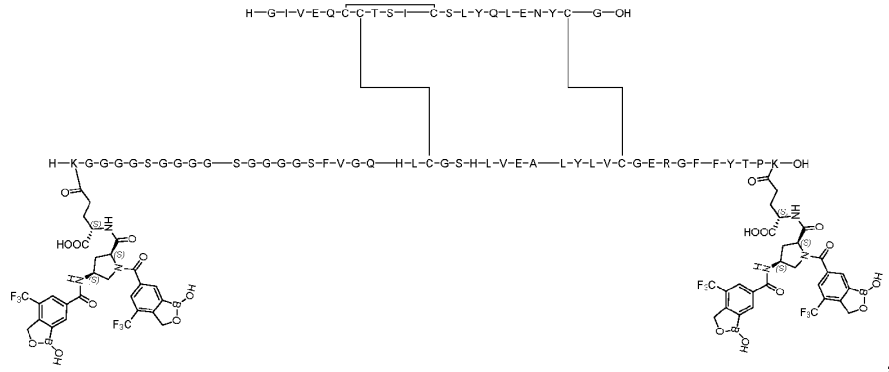
Example 132 (SEQ ID NOS 25656 and 25657, respectively, in order of appearance):



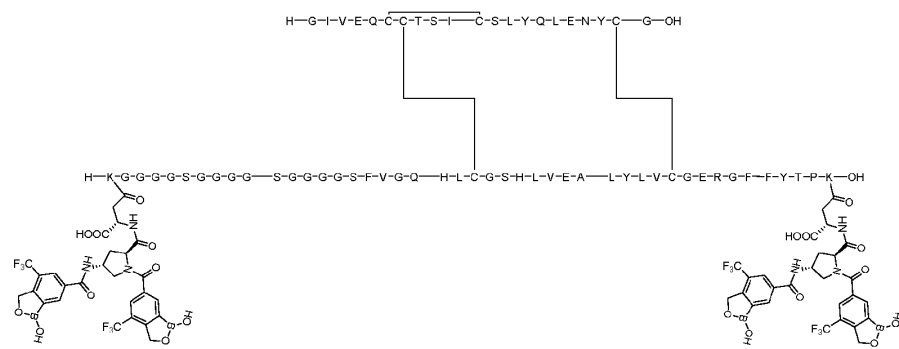
5 Example 133 (SEQ ID NOS 25658 and 25659, respectively, in order of appearance):



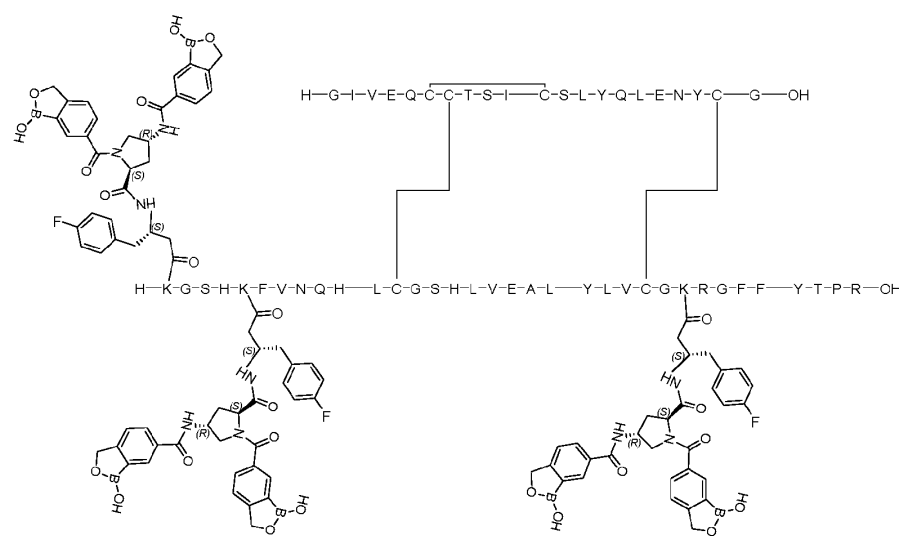
Example 134 (SEQ ID NOS 25660 and 25661, respectively, in order of appearance):



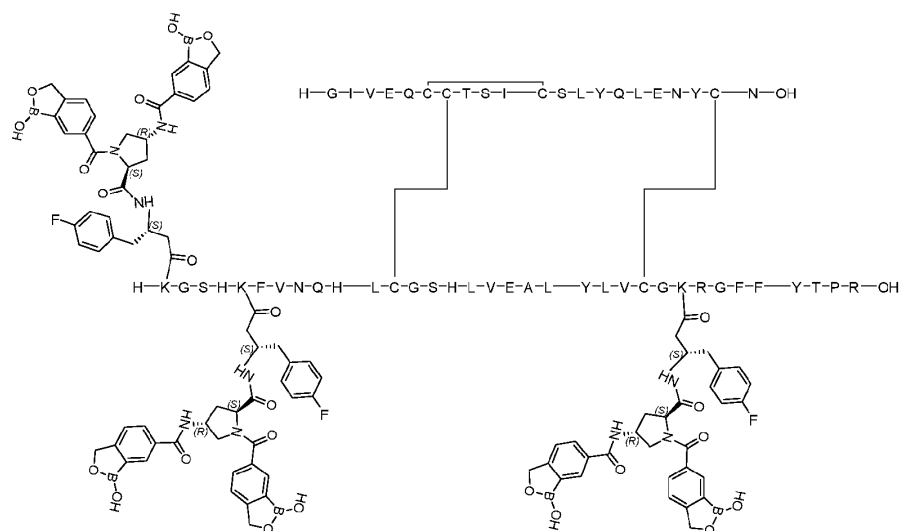
Example 135 (SEQ ID NOS 25662 and 25663, respectively, in order of appearance):



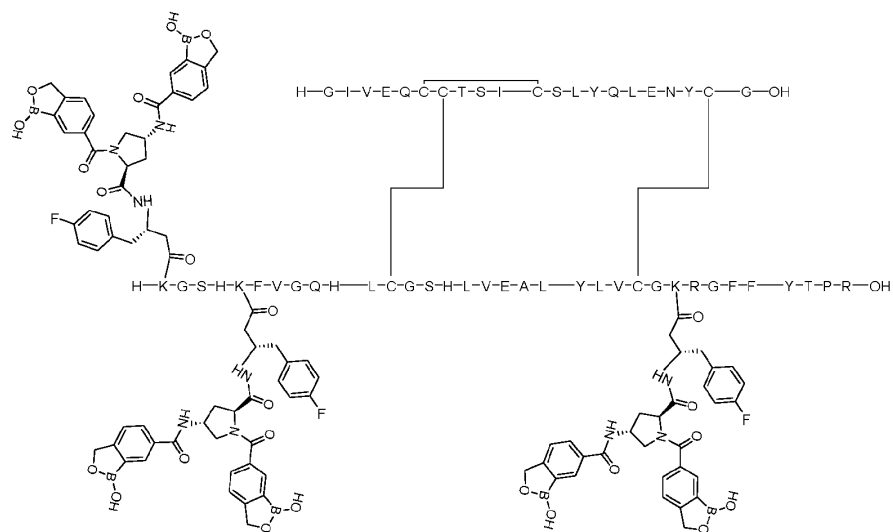
Example 136 (SEQ ID NOS 25664 and 25665, respectively, in order of appearance):



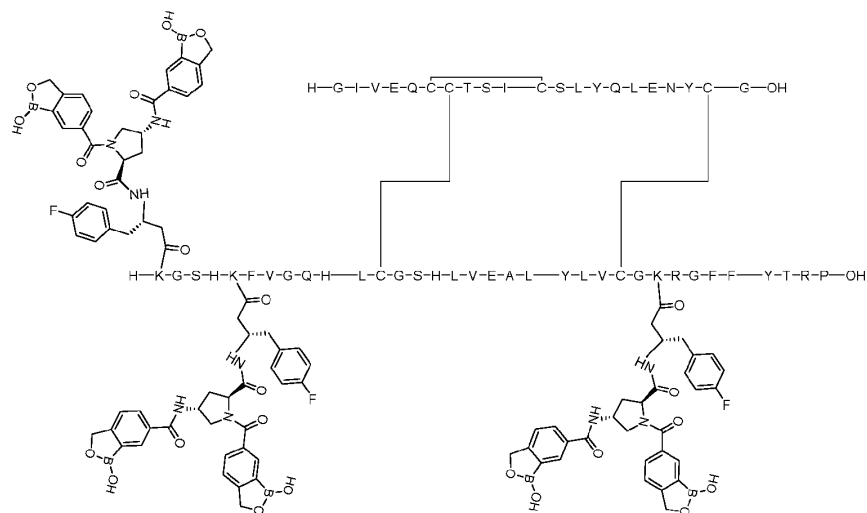
Example 137 (SEQ ID NOS 25666 and 25667, respectively, in order of appearance):



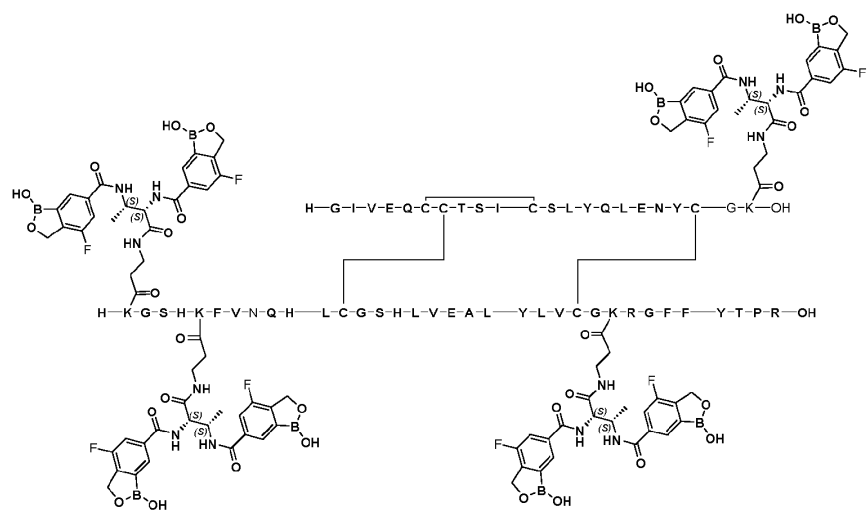
Example 138 (SEQ ID NOS 25668 and 25669, respectively, in order of appearance):



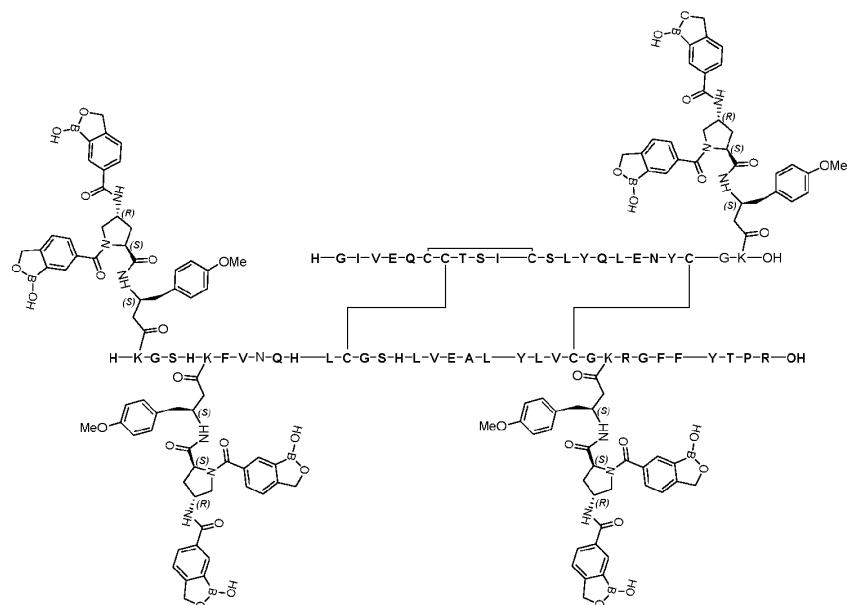
Example 139 (SEQ ID NOS 25670 and 25671, respectively, in order of appearance):



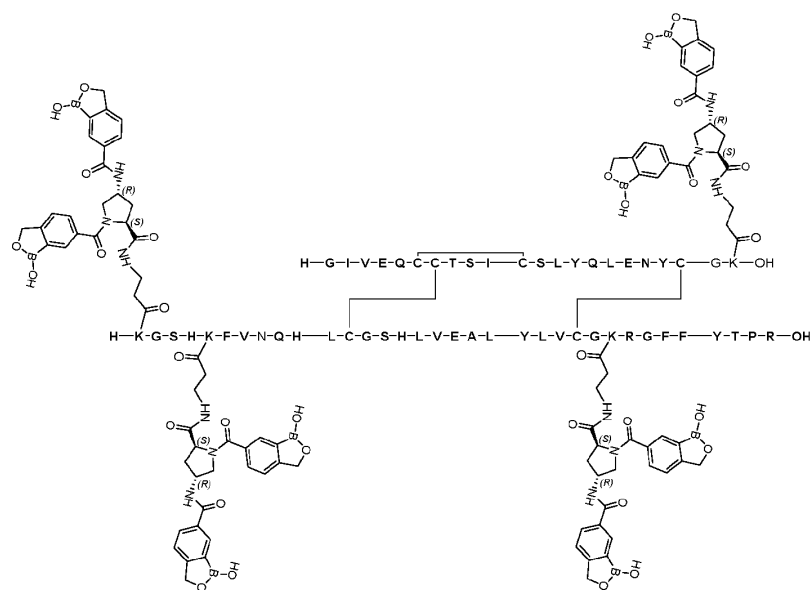
Example 140 (SEQ ID NOS 25672 and 25673, respectively, in order of appearance):



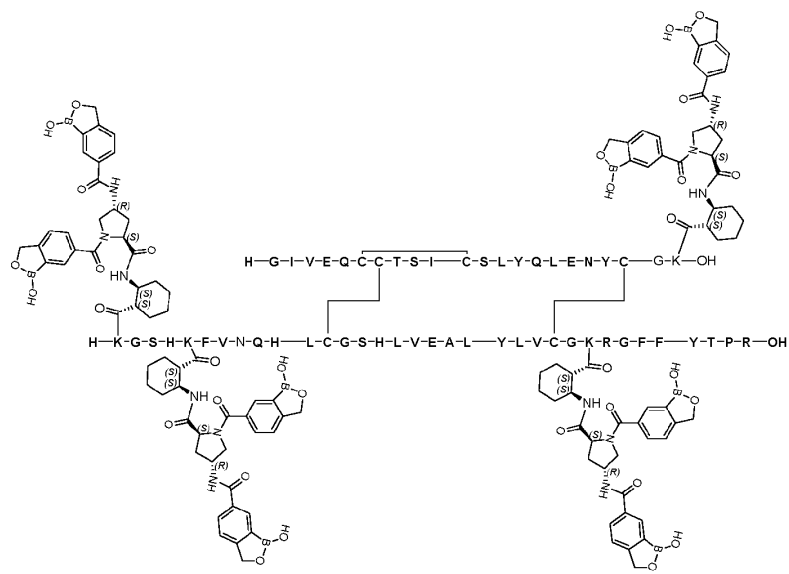
Example 141 (SEQ ID NOS 25674 and 25675, respectively, in order of appearance):



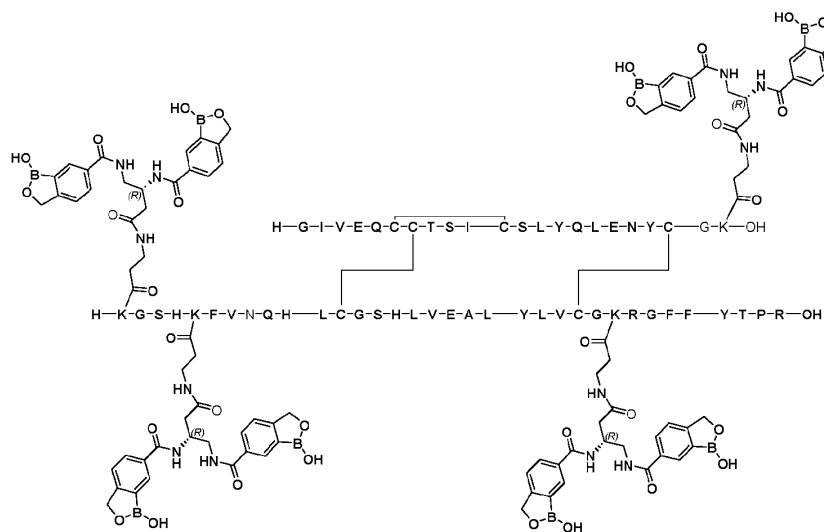
Example 142 (SEQ ID NOS 25676 and 25677, respectively, in order of appearance):



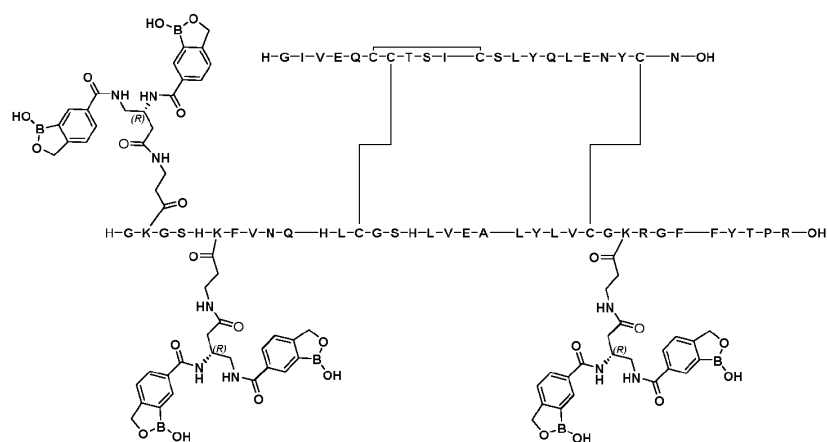
Example 143 (SEQ ID NOS 25678 and 25679, respectively, in order of appearance):



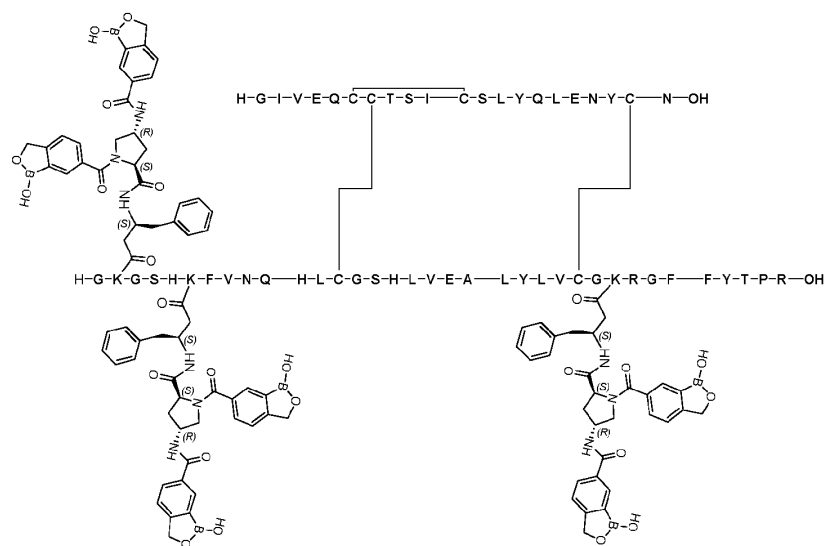
Example 144 (SEQ ID NOS 25680 and 25681, respectively, in order of appearance):



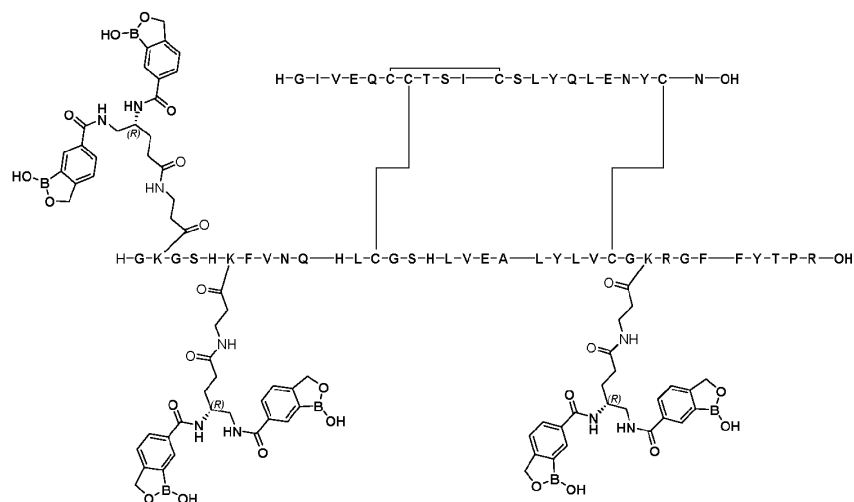
Example 145 (SEQ ID NOS 25682 and 25683, respectively, in order of appearance):



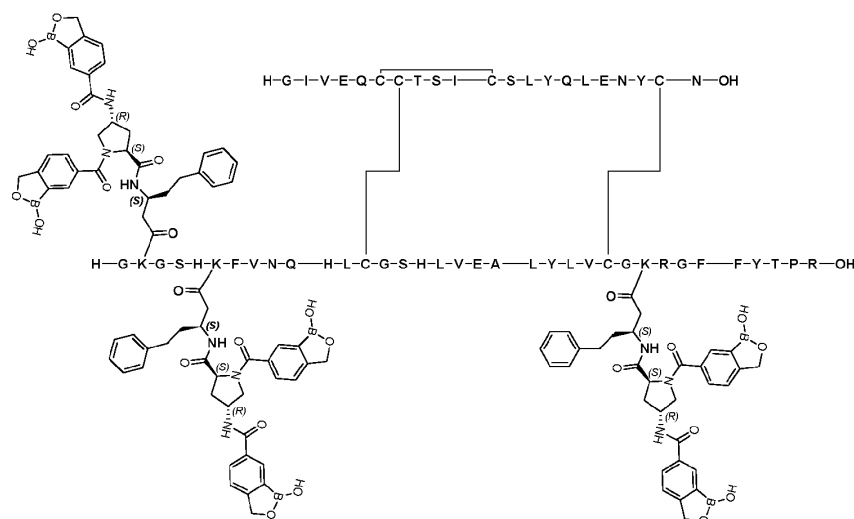
Example 146 (SEQ ID NOS 25684 and 25685, respectively, in order of appearance):



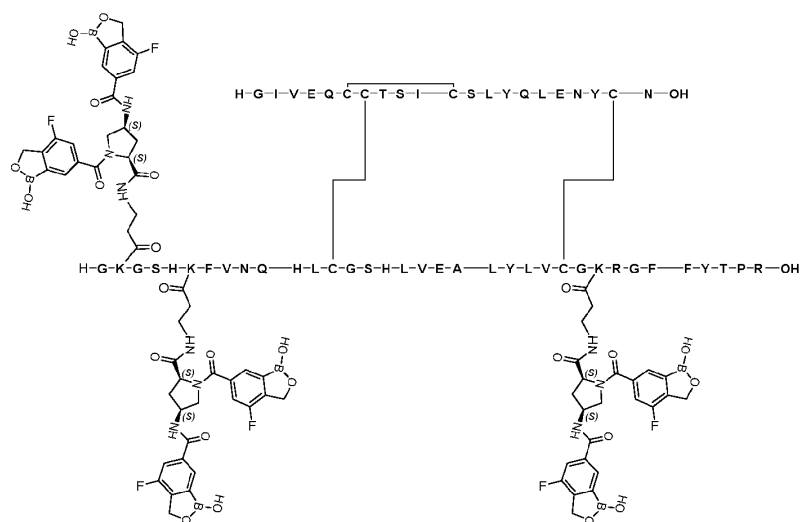
Example 147 (SEQ ID NOS 25686 and 25687, respectively, in order of appearance):



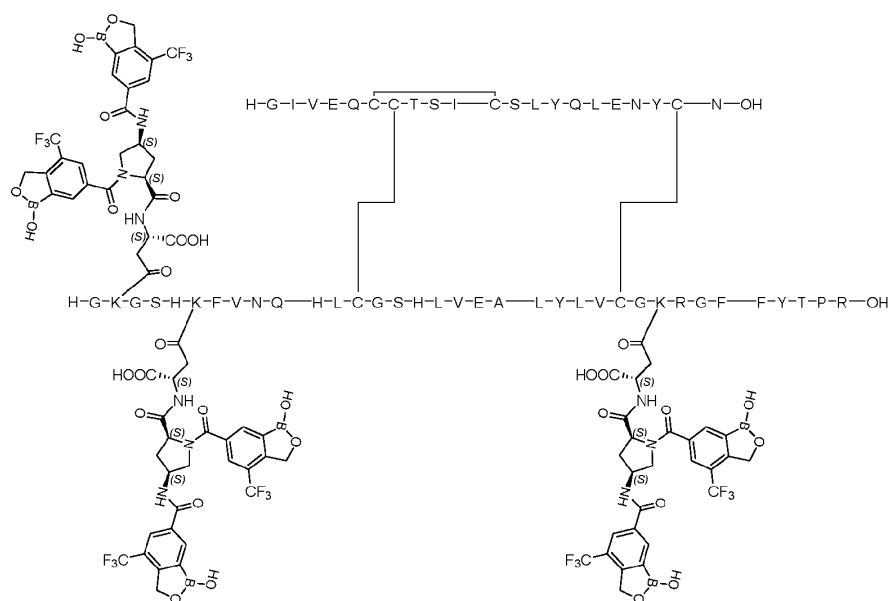
Example 148 (SEQ ID NOS 25688 and 25689, respectively, in order of appearance):



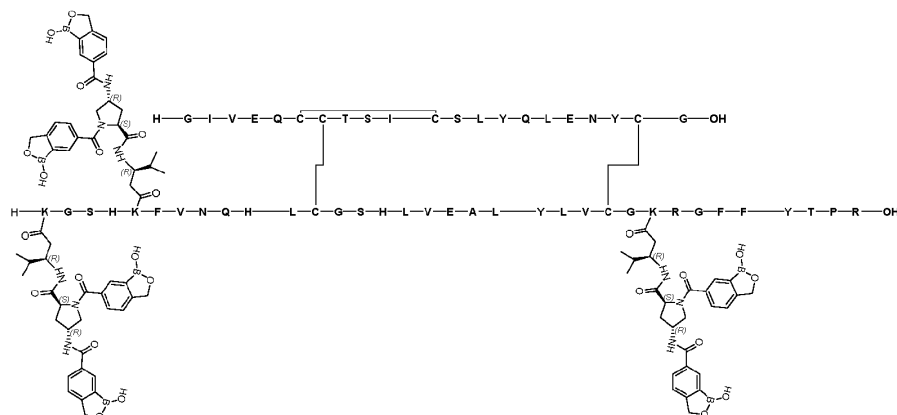
Example 149 (SEQ ID NOS 25690 and 25691, respectively, in order of appearance):



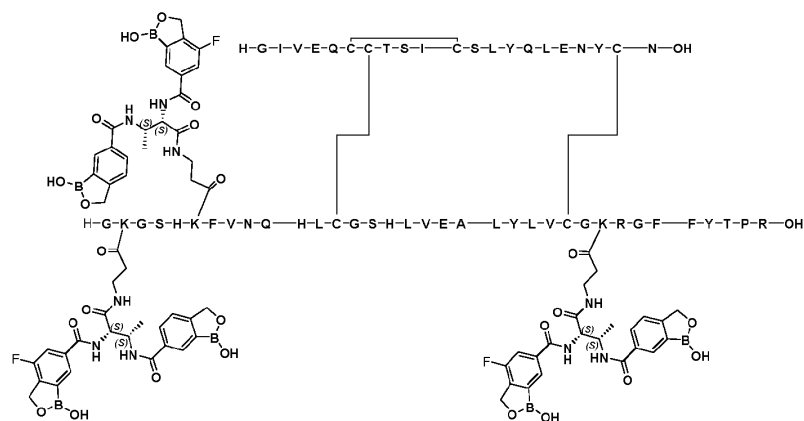
Example 150 (SEQ ID NOS 25692 and 25693, respectively, in order of appearance):



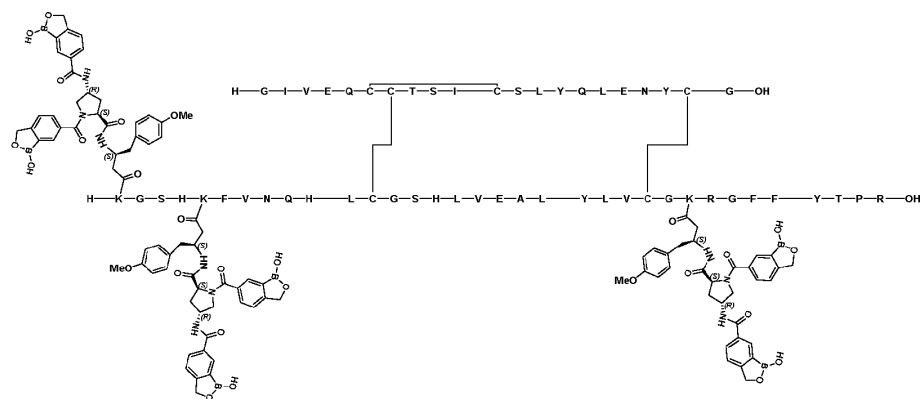
Example 151 (SEQ ID NOS 25694 and 25695, respectively, in order of appearance):



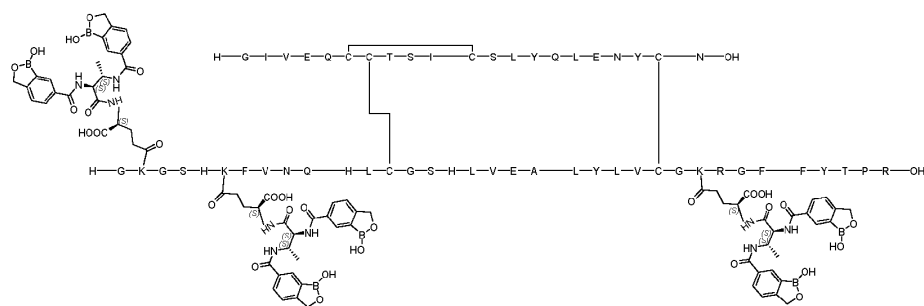
Example 152 (SEQ ID NOS 25696 and 25697, respectively, in order of appearance):



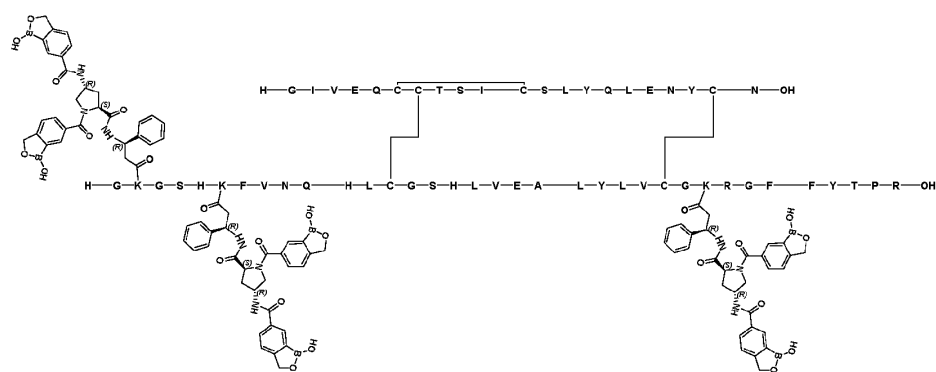
5 Example 153 (SEQ ID NOS 25698 and 25699, respectively, in order of appearance):



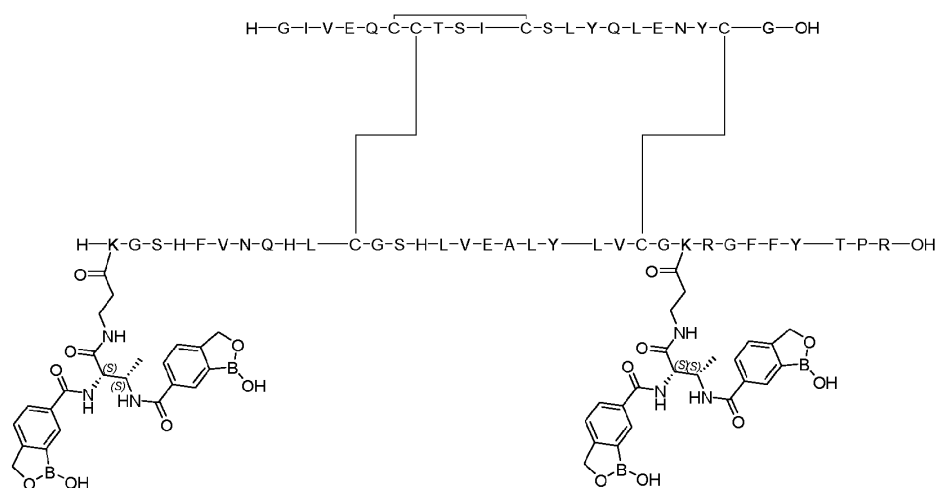
Example 154 (SEQ ID NOS 25700 and 25701, respectively, in order of appearance):



Example 155 (SEQ ID NOS 25702 and 25703, respectively, in order of appearance):



Example 156 (SEQ ID NOS 25704 and 25705, respectively, in order of appearance):



a stereoisomer thereof, a pharmaceutically acceptable salt thereof, an isotope thereof, and combinations thereof.

10 In some embodiments, provided herein are compounds selected from the group consisting of an insulin comprising an A-chain and a B-chain, wherein the A-chain comprises a

sequence selected from SEQ ID NOs 24501 and 24502; and wherein the B-chain comprises a sequence selected from SEQ ID NOs 25229, 25232, 25305, 25308, 25312, 25236, 25095, and 25380-25397.

In some embodiments, each indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL32, FL41, FL62, FL63, and stereoisomers thereof.

In some embodiments, the B₁ and the B₂ are selected from Formula F2, and each remaining R₁ is independently selected from H, F, Cl, I, Br, and CF₃.

In some embodiments, the B₁ and the B₂ are selected from Formula F2, and each remaining R₁ is H.

In some embodiments, X1 comprises at least one lysine and an N-terminus; each Z1c is independently selected from FF12A, FF12B, FF12C, FF12D, FF116, FF116A, FF116B, FF116C, FF116D, wherein each B₁ and B₂ is F2; each Z1c is conjugated, either directly or via the indirect linker, to an amine side chain in the lysine in X1 or to the N-terminus in X1; and each indirect linker is independently selected from FL3, FL5, FL5A, FL5B, and FL20-FL75, and stereoisomers thereof, wherein in FL3 and FL5 each p is independently 1, 2, or 3.

In some embodiments, at least one Z1c is conjugated via the indirect linker to an amine side chain in the lysine in the B-chain or to the B chain N-terminus.

In some embodiments, X1 comprises two or more lysine amino acids.

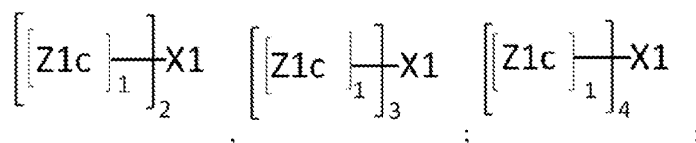
In some embodiments, the B-chain comprises at least two lysine amino acids each independently conjugated either directly or via an indirect linker to a Z1c.

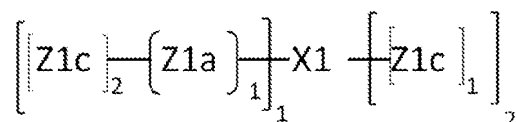
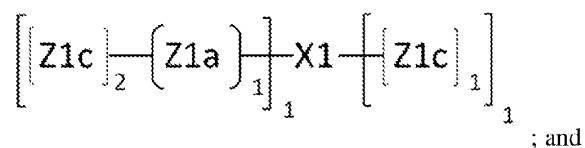
In some embodiments, the B-chain comprises at least three lysine amino acids each independently covalently conjugated via the indirect linker to a Z1c.

In some embodiments, the A-chain comprises one or more lysine amino acids each independently covalently conjugated either directly or via an indirect linker to a Z1c.

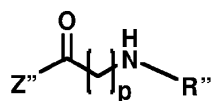
In some embodiments, X1 comprises an A-chain having at least one lysine covalently conjugated via the indirect linker to a Z1c.

In some embodiments, the compound of Formula I is selected from:





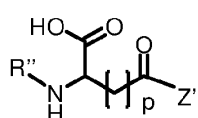
In some embodiments, at least one Z1c is covalently conjugated to X1 via the indirect linker, and wherein the indirect linker is independently selected from:



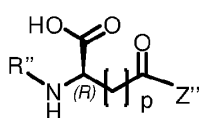
(FL3)

Formula FL3,

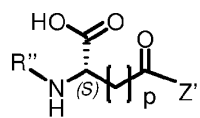
wherein p is 2 or 3;



(FL5)



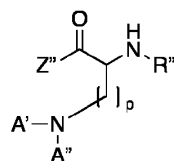
(FL5A)



(FL5B)

Formulae FL5, FL5A, and FL5B,

wherein p is 1 or 2; and



FL69

, and stereoisomers thereof;

wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

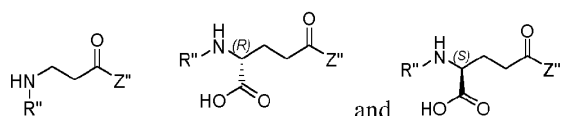
Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, an alkyl group (e.g., C₁-C₆alkyl group), a saturated fatty acid, an unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a haloalkyl group (e.g., C₁-C₆ haloalkyl group), an aryl group, and a heteroaryl group; and

A'' is selected from an alkyl group (e.g., C₁-C₆alkyl group), a substituted acyl group, acyl group terminating in an acid group, a saturated or unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a haloalkyl group (e.g., C₁-C₆haloalkyl group), an aryl group, and a heteroaryl group;

- 5 p is 1, 2, 3, 4, or 5, and
any primary amine is optionally acetylated or alkylated.

In some embodiments, the indirect linker is selected from:



10

In some embodiments, X1 is an insulin further comprising from 1 to 5 residues replaced, inserted, appended, or mutated to an amino acid that has a free amine conjugated directly or indirectly to a Z1c.

- 15 In some embodiments, at least one Z1c is conjugated to a free amine side chain of an amino acid that has been replaced, inserted, or mutated on an insulin.

In some embodiments, the compound is covalently conjugated either directly or via a linker to a molecule that can bind to at least one protein present in human plasma.

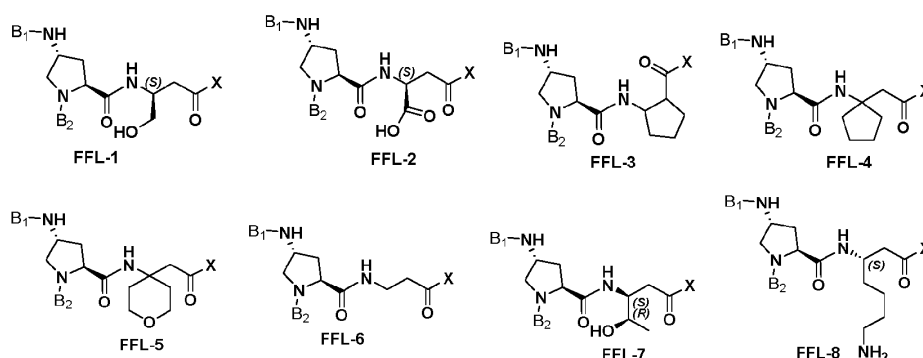
In some embodiments, in each of Formulae F2, F5, and F10 one R₁ is (C=O)---*.

- 20 In some embodiments, a compound is represented by Formula II, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt thereof:

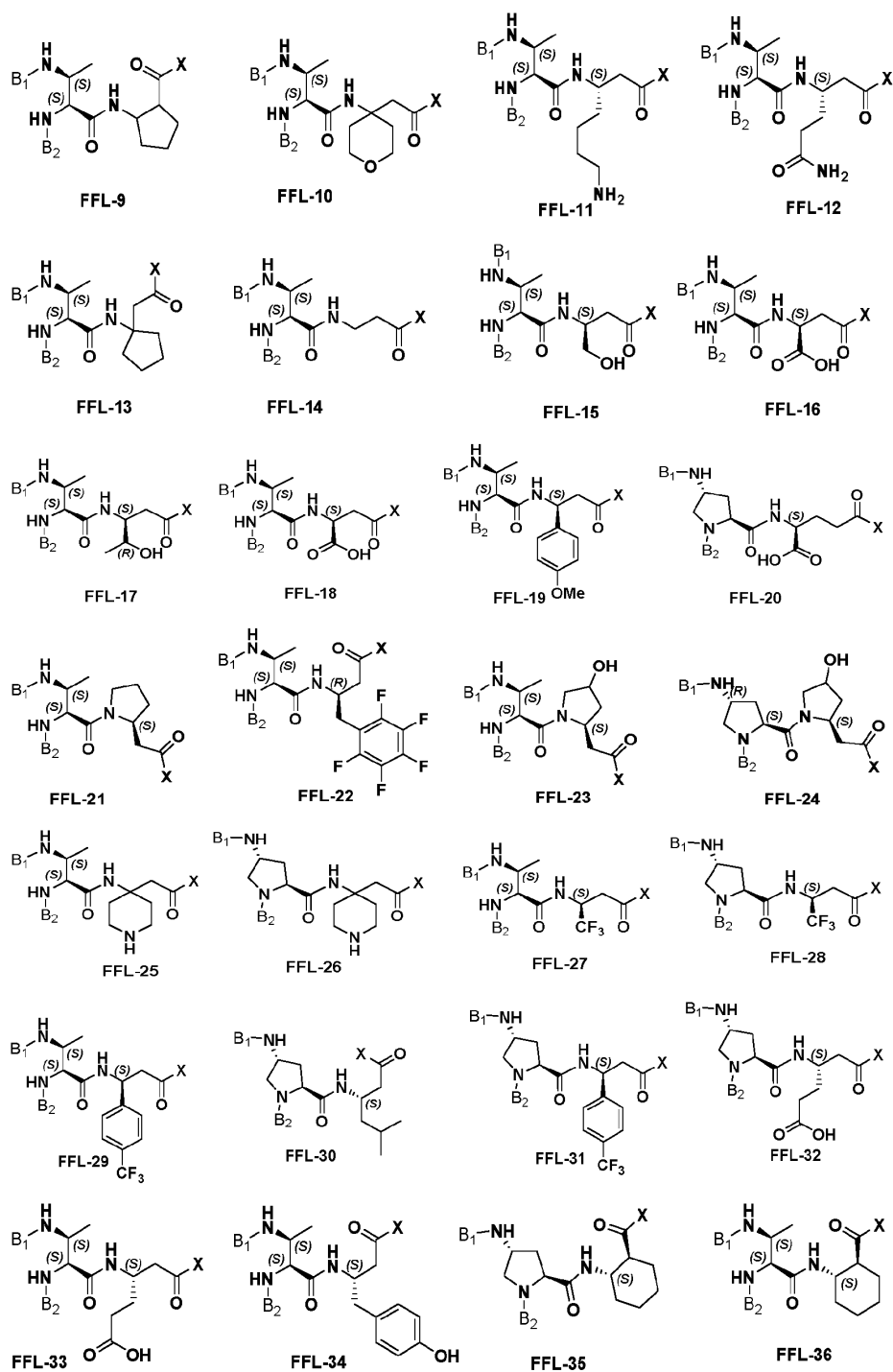
Z1c-Linker

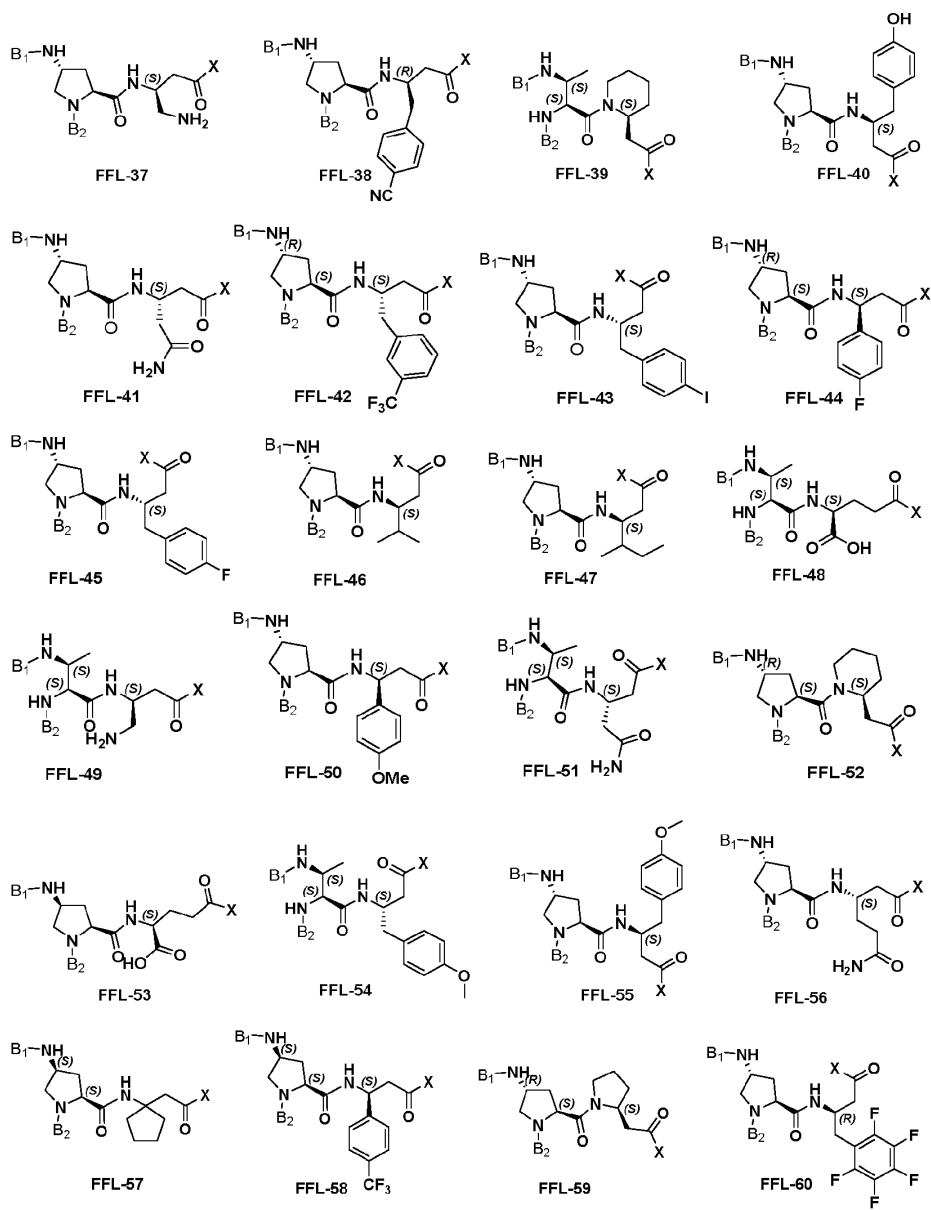
(Formula II)

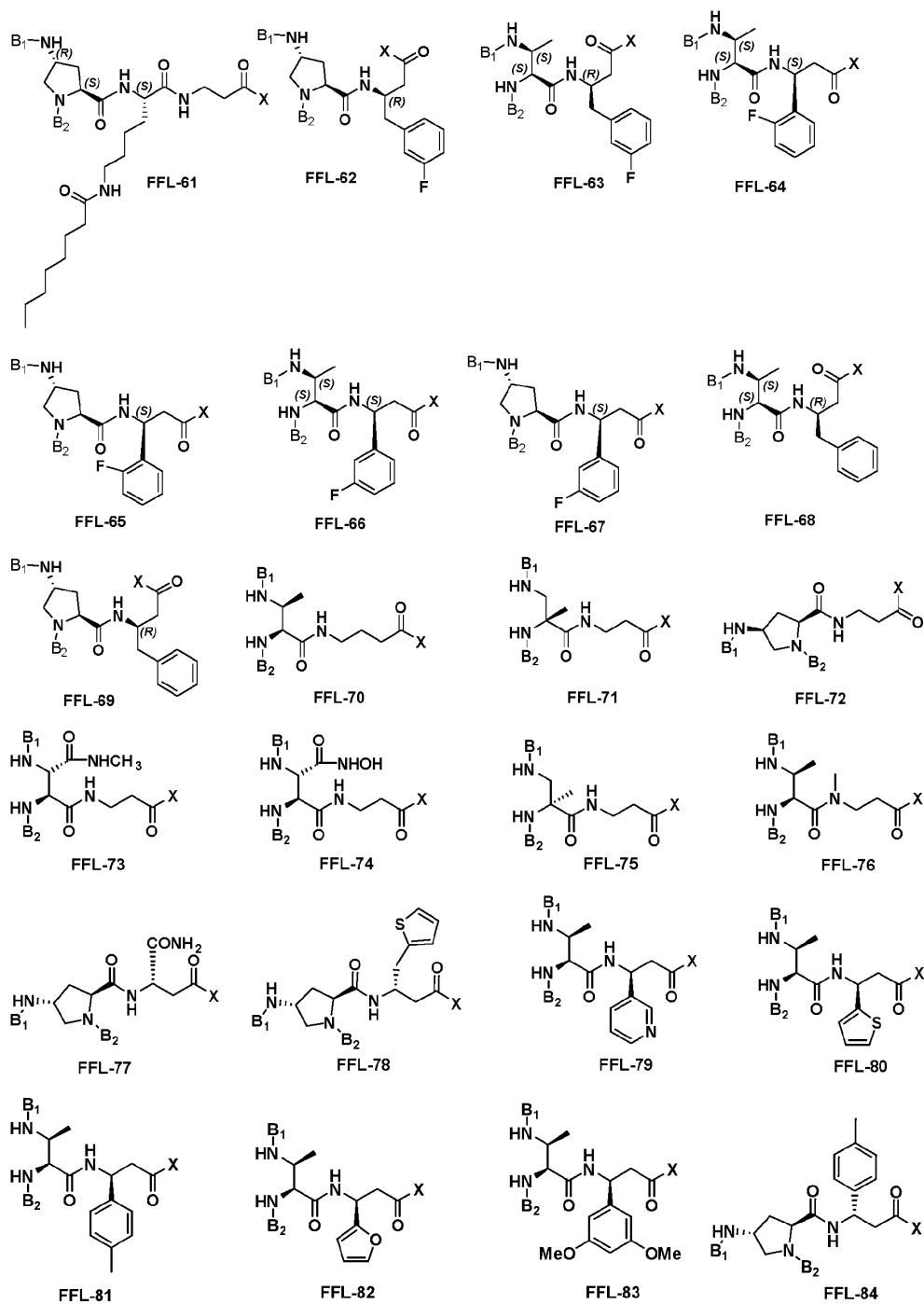
wherein the Z1c-Linker is selected from:

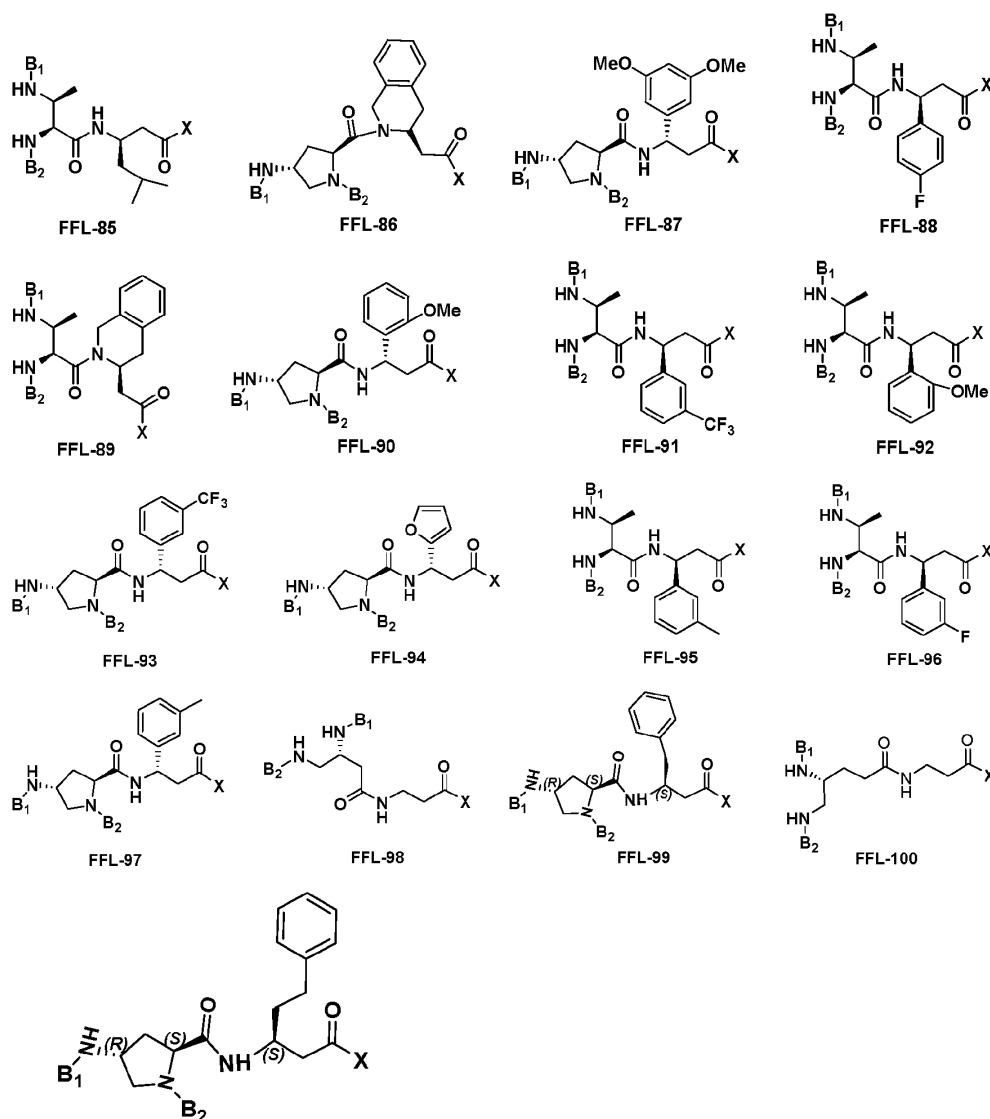


25





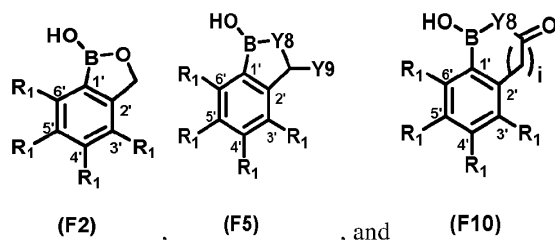




5 and **FFL-101** ; wherein X is selected from a leaving group, NH₂, OH, and H; and

B₁ and B₂, which may be identical or different, each independently represents an aromatic boron-containing group.

In some embodiments, the compound represented by Formula (II) comprises at least one
 10 B₁ or B₂ independently selected from Formulae F2, F5, and F10,
 wherein Formulae F2, F5, and F10 are:



wherein:

one R₁ represents (C=O)---* or (CH₂)_m(C=O)---*, wherein ---* represents the attachment point to the rest of Z1c, and m is 1, 2, 3, 4, 5, 6, or 7; and

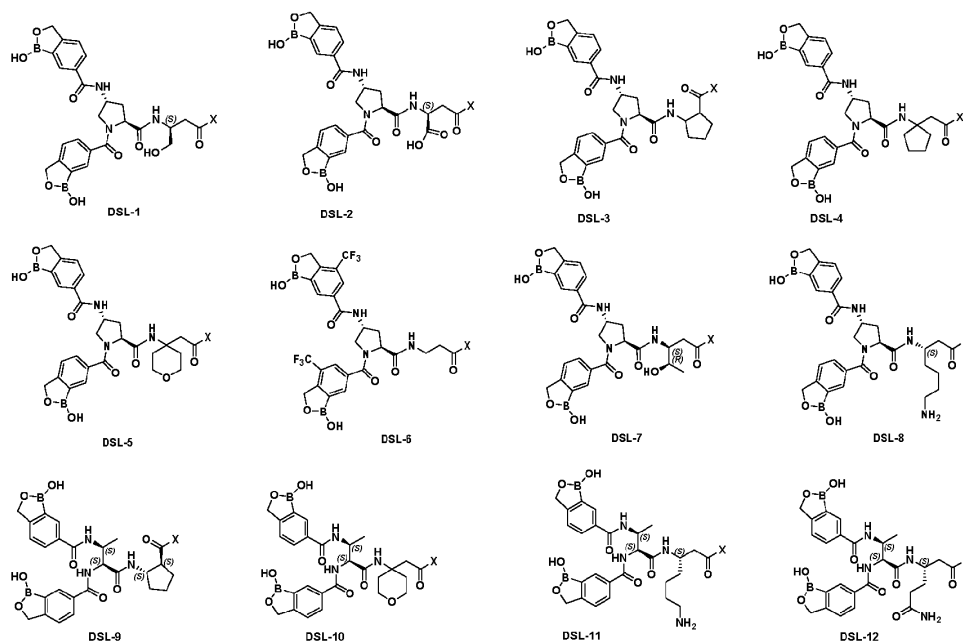
- 5 each remaining R₁ is independently selected from H, F, Cl, Br, I, OH, CH₂-NH₂, NH₂, (C=O)-NH₂, CH=O, SO₂CH₃, SO₂CF₃, CF₃, CHF₂, NO₂, CH₃, OCH₃, O(CH₂)_{m'}CH₃, (SO₂)NH-CH₃, (SO₂)NH(CH₂)_{m'}CH₃, and OCF₃, wherein m' is 1, 2, 3, 4, 5, 6, or 7;

Y₈ is O, N, and NR, wherein R is an alkyl group (e.g., C₁-C₆alkyl group) or H;

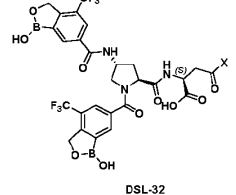
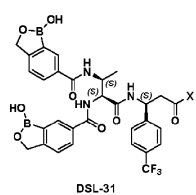
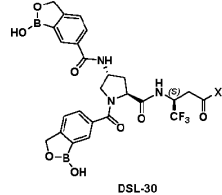
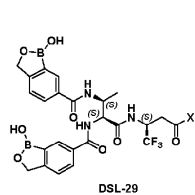
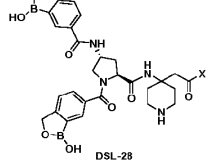
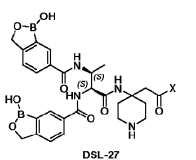
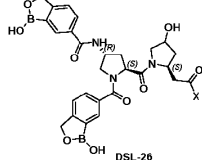
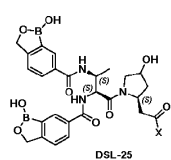
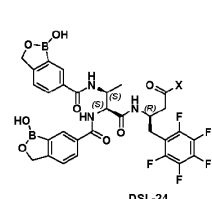
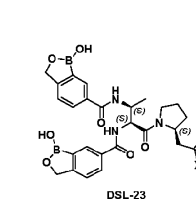
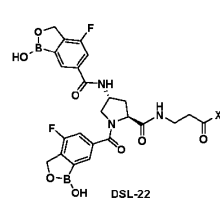
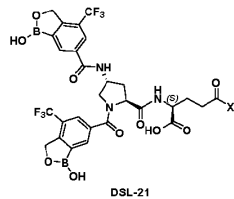
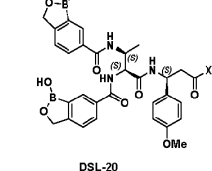
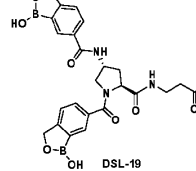
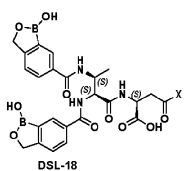
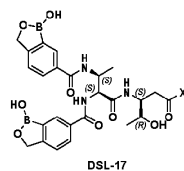
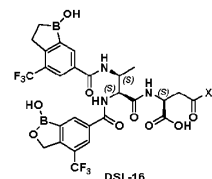
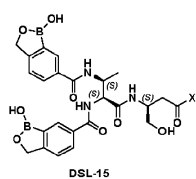
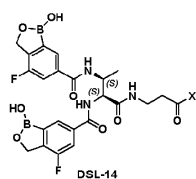
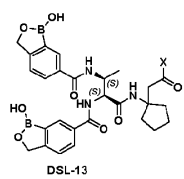
- 10 Y₉ is H, CH₃, or an alkyl group (e.g., C₁-C₆alkyl group); and

i is 1, 2, or 3.

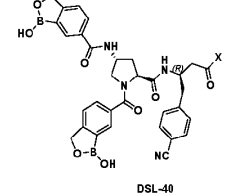
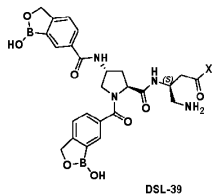
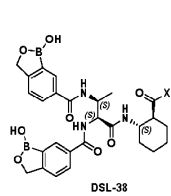
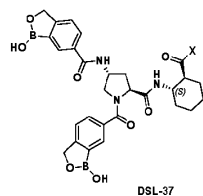
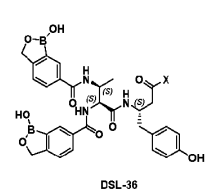
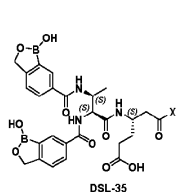
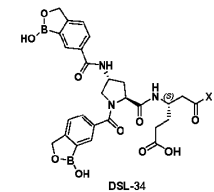
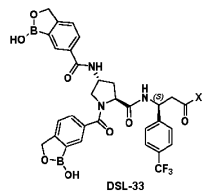
In some embodiments, the Z1c-Linker (i.e., Formula II) is selected from:

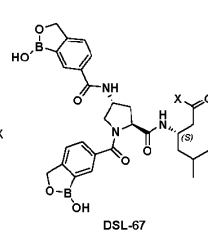
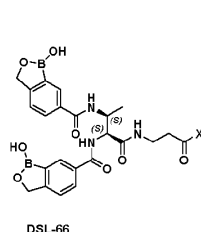
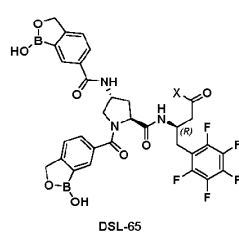
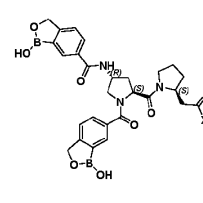
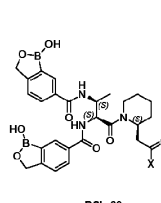
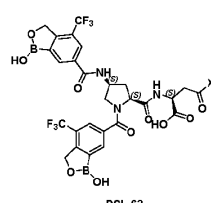
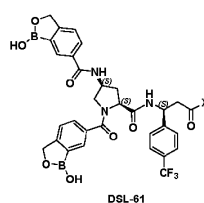
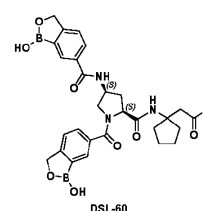
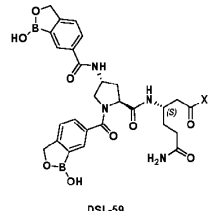
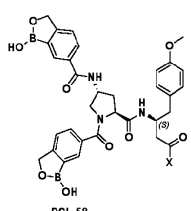
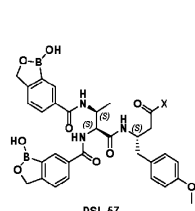
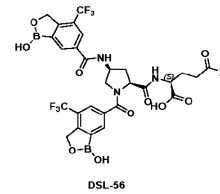
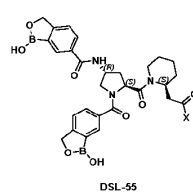
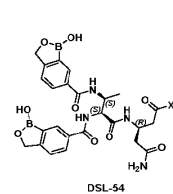
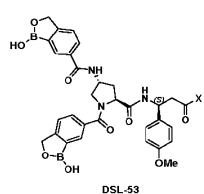
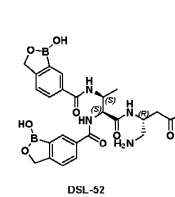
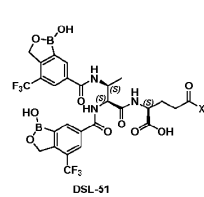
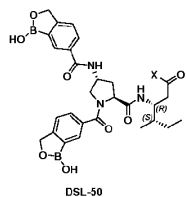
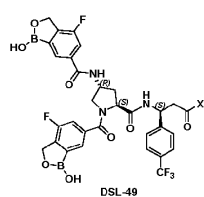
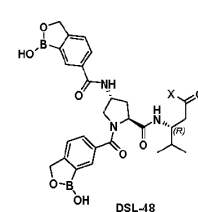
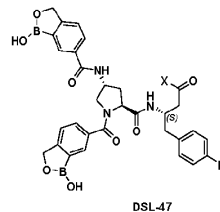
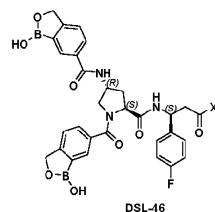
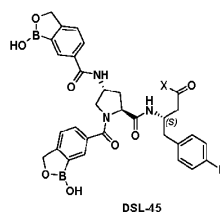
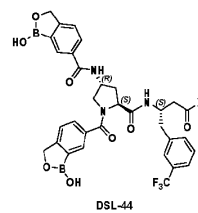
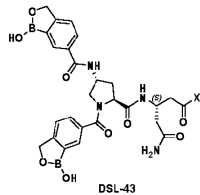
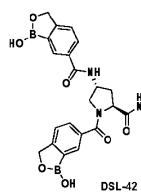
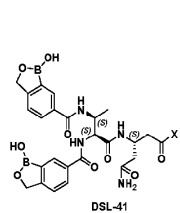


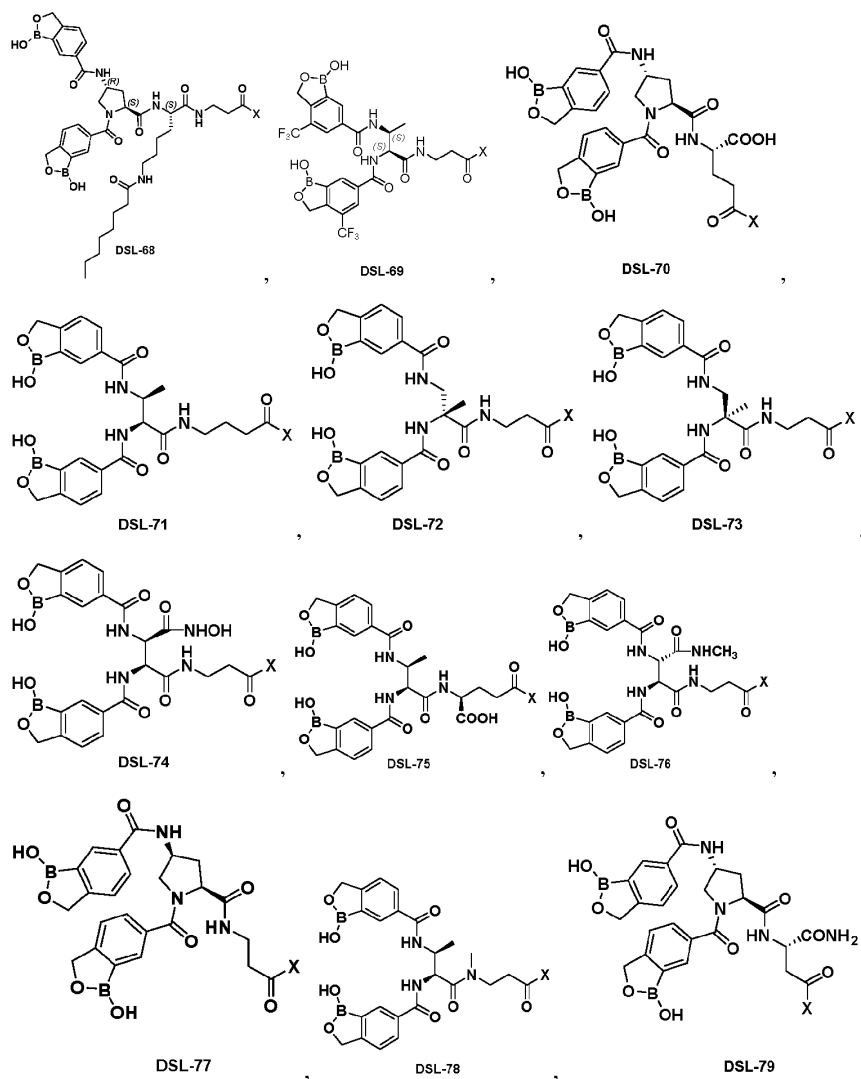
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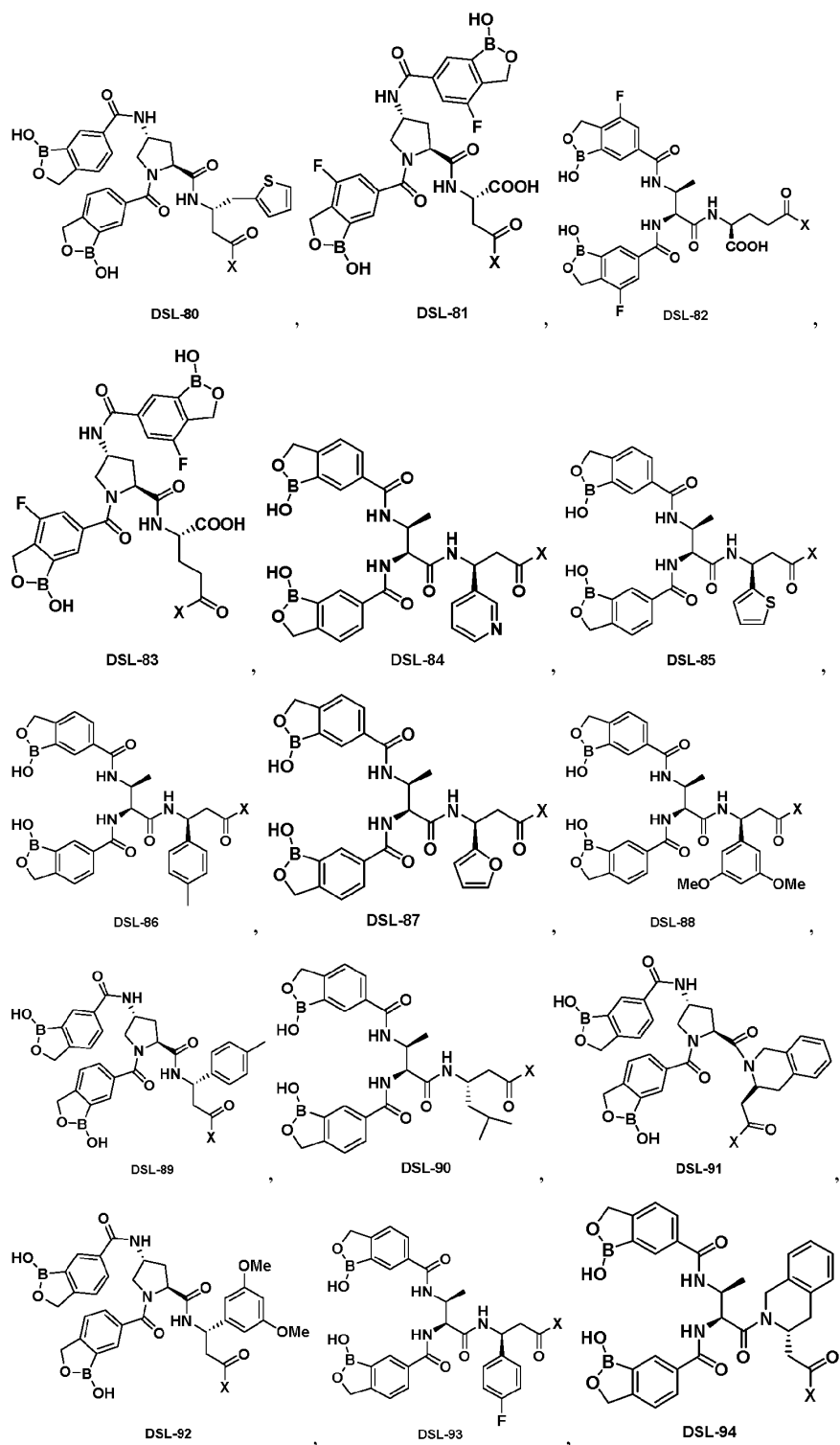


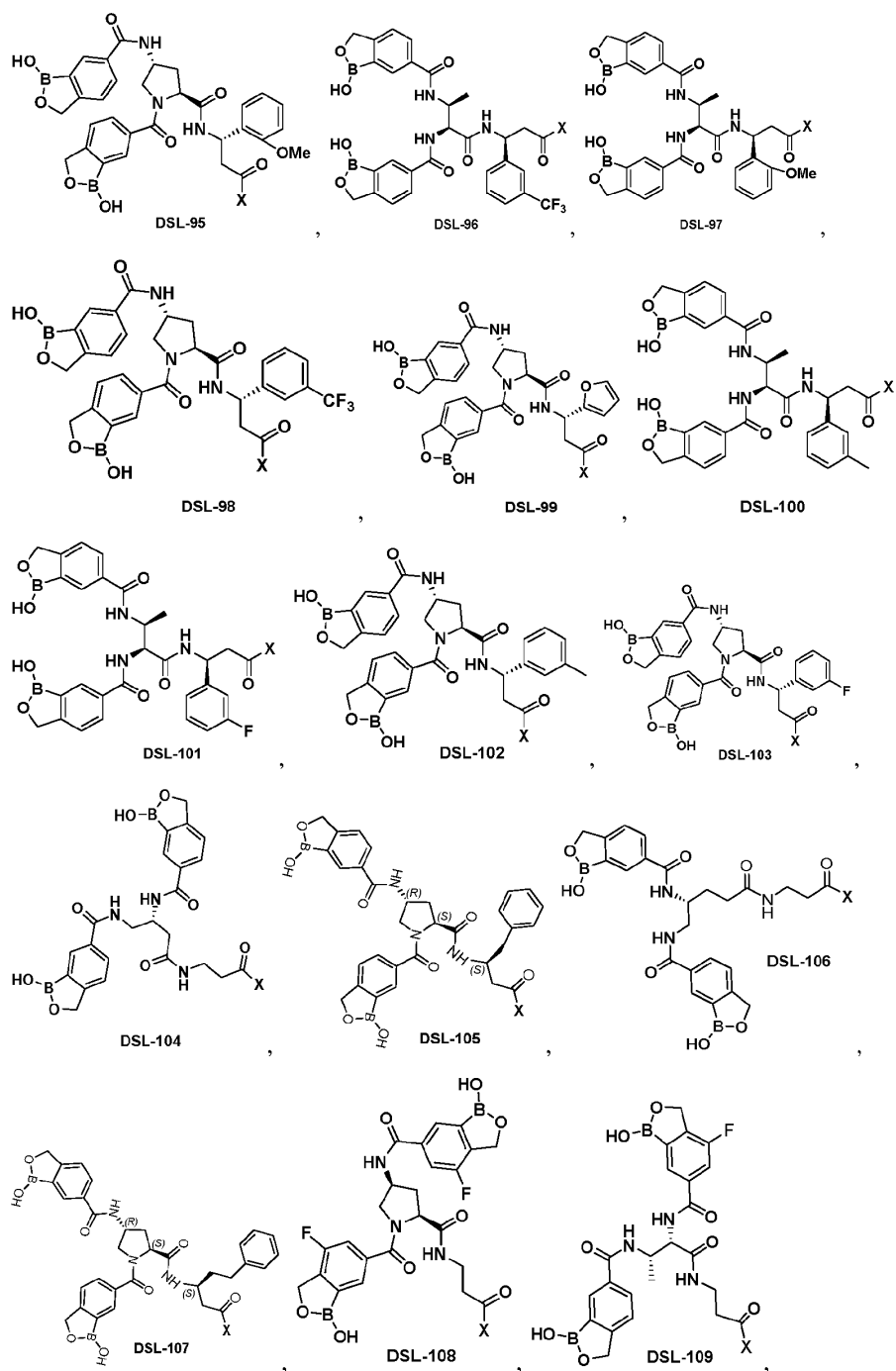
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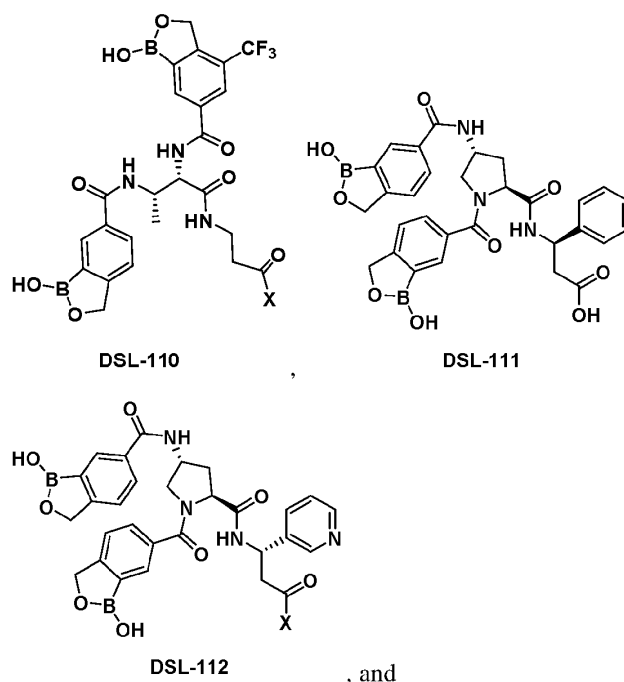






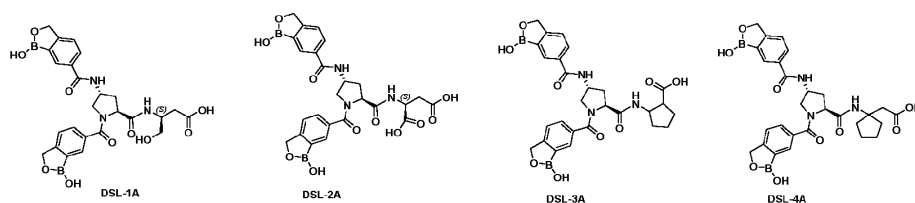


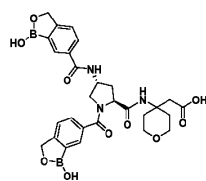




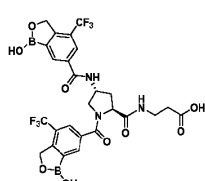
wherein X is a leaving group or represents a point of covalent attachment directly to X1 of Formula I.

- 5 In some embodiments, when X is a leaving group, X is selected from N-oxysuccinimide, 2,3,5,6-tetrafluorophenoxy (TFP), pentafluorophenoxy (Pfp), OH, halogen, maleimide alkyl amino, maleimide amido polyethylene glycol amino, and maleimide polyethylene glycol amino. In some embodiments, X is selected from N-oxysuccinimide and OH. In some embodiments, X is OH. In some embodiments, X is N-oxysuccinimide. In some embodiments, the maleimide
- 10 polyethylene glycol amino is selected from Mal-PEG2-amine, Mal-PEG4-amine, and Mal-PEG5-amine, or a pharmaceutically acceptable salt thereof. In some embodiments, the maleimide alkyl amino is selected from Mal-C6-amine and N-(2-Aminoethyl)maleimide, or a pharmaceutically acceptable salt thereof. In some embodiments, the maleimide amido polyethylene glycol amino is selected from Mal-amido-PEG9-amine, Mal-amido-PEG11-amine,
- 15 Mal-amido-PEG23-amine, 4-Mal-methyl-cyclohexanecarboxamide-methyl-[1,2,3]triazole-PEG8-amine, or a pharmaceutically acceptable salt thereof. In some embodiments, the Z1c-Linker is selected from:

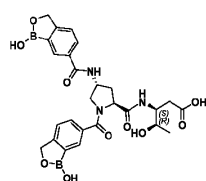




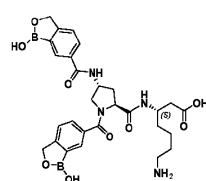
DSL-5A



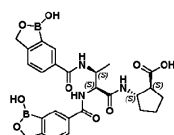
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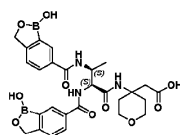
DSL-7A



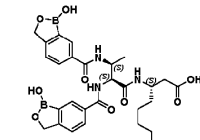
DSL-8A



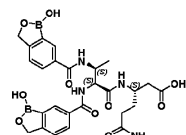
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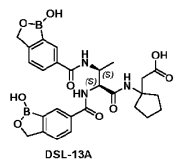
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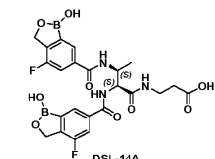
DSL-11A



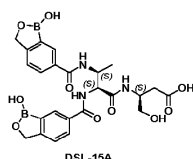
DSL-12A



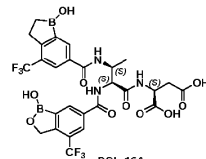
DSL-13A



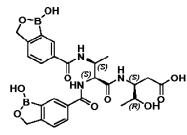
DSL-14A



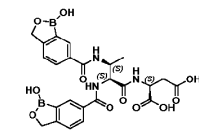
DSL-15A



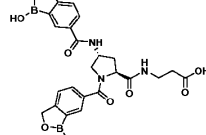
DSL-16A



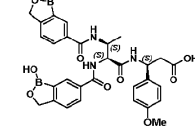
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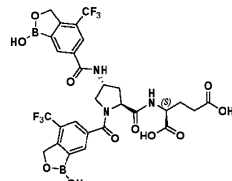
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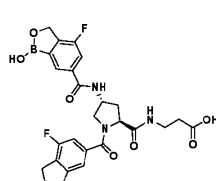
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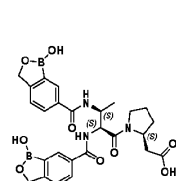
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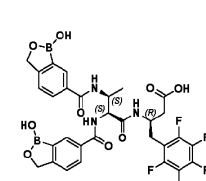
DSL-21A



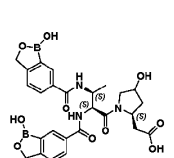
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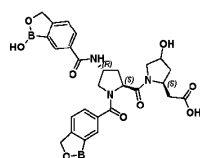
DSL-23A



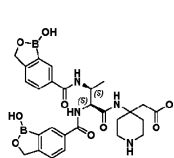
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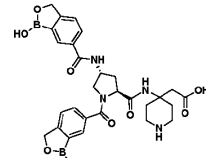
DSL-25A



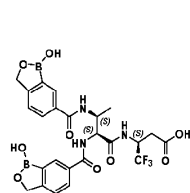
DSL-26A



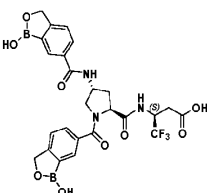
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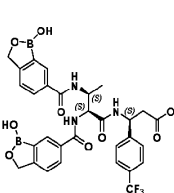
DSL-28A



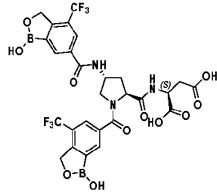
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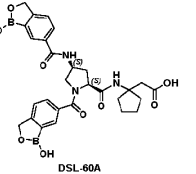
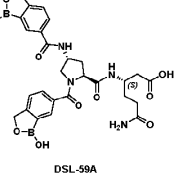
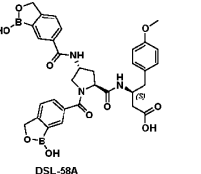
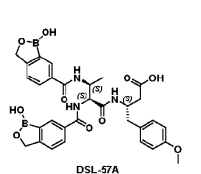
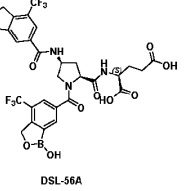
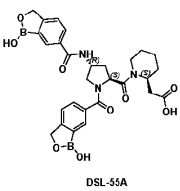
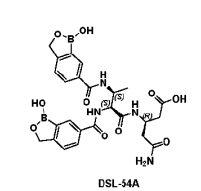
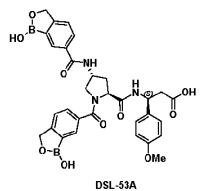
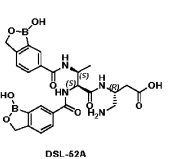
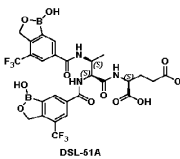
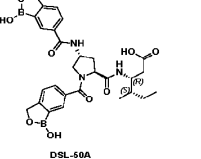
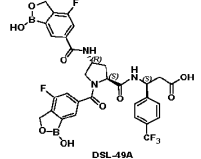
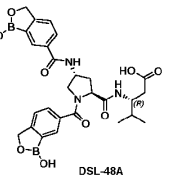
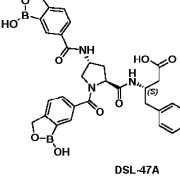
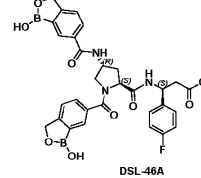
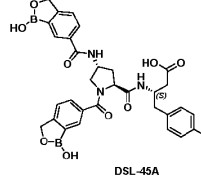
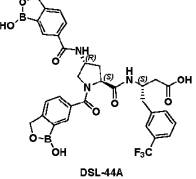
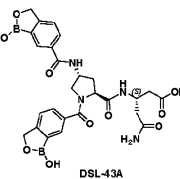
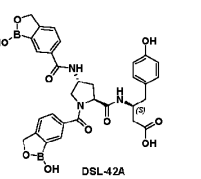
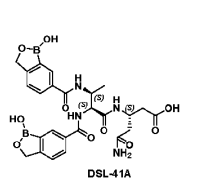
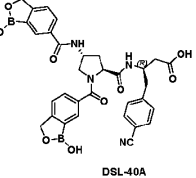
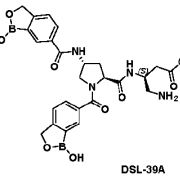
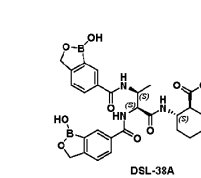
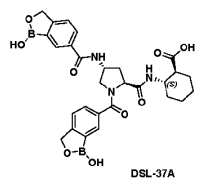
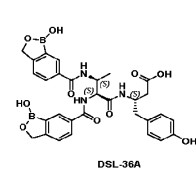
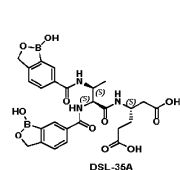
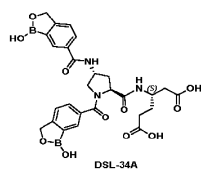
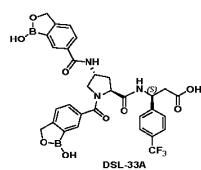
DSL-30A

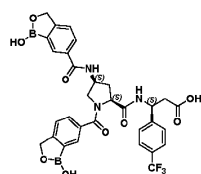


DSL-31A

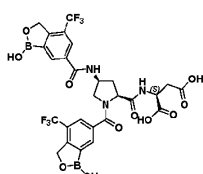


DSL-32A

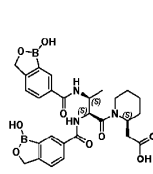




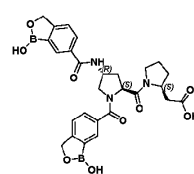
DSL-61A



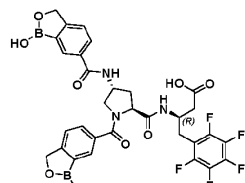
DSL-62A



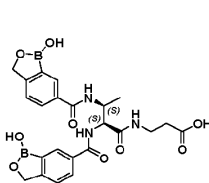
DSL-63A



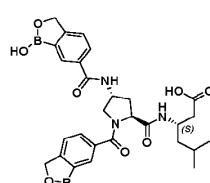
DSL-64A



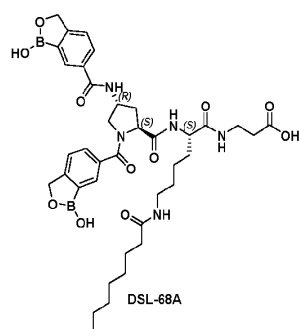
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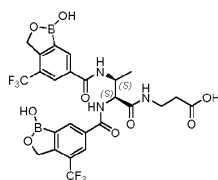
DSL-66A



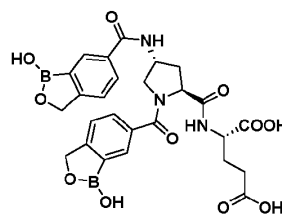
DSL-67A



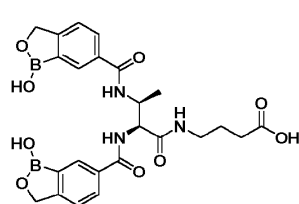
DSL-68A



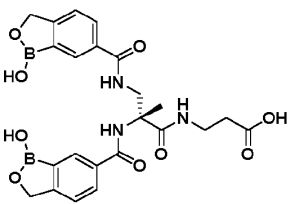
DSL-69A



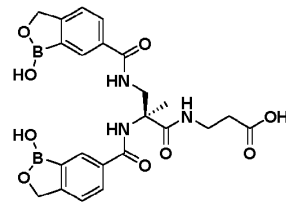
DSL-70A



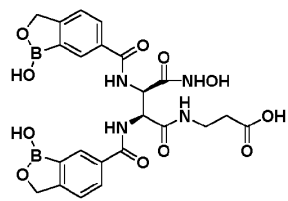
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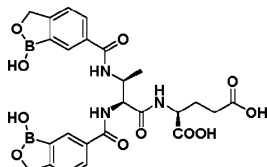
DSL-72A



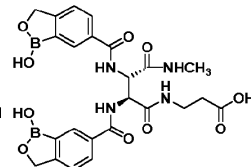
DSL-73A



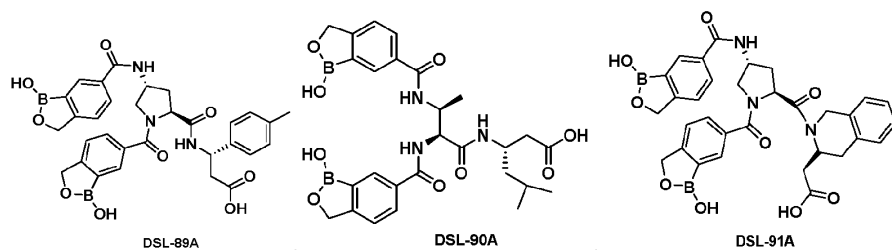
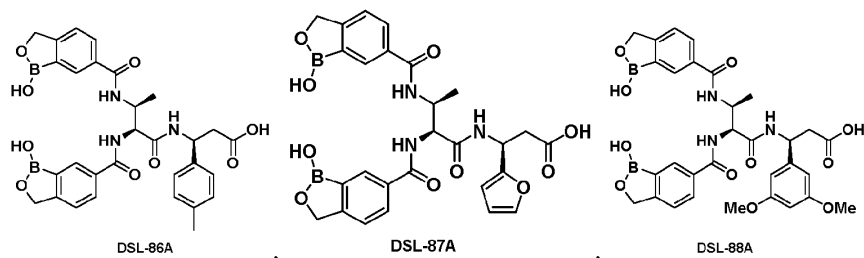
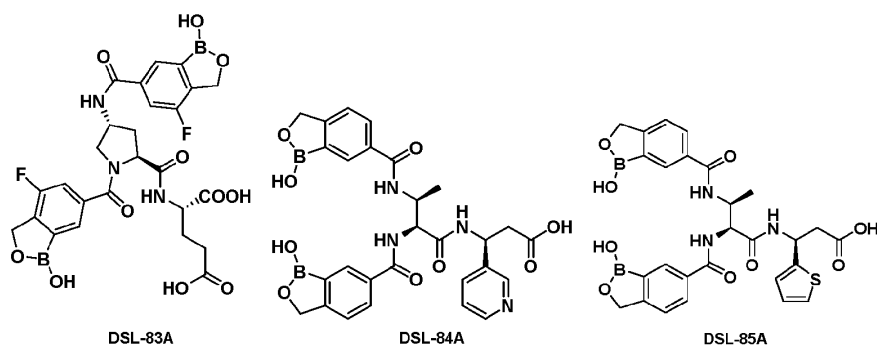
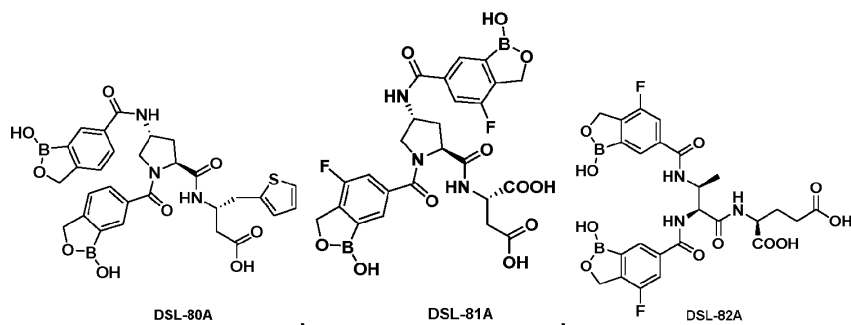
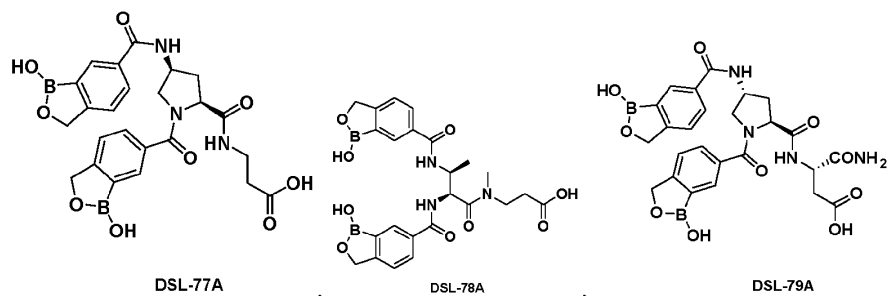
DSL-74A

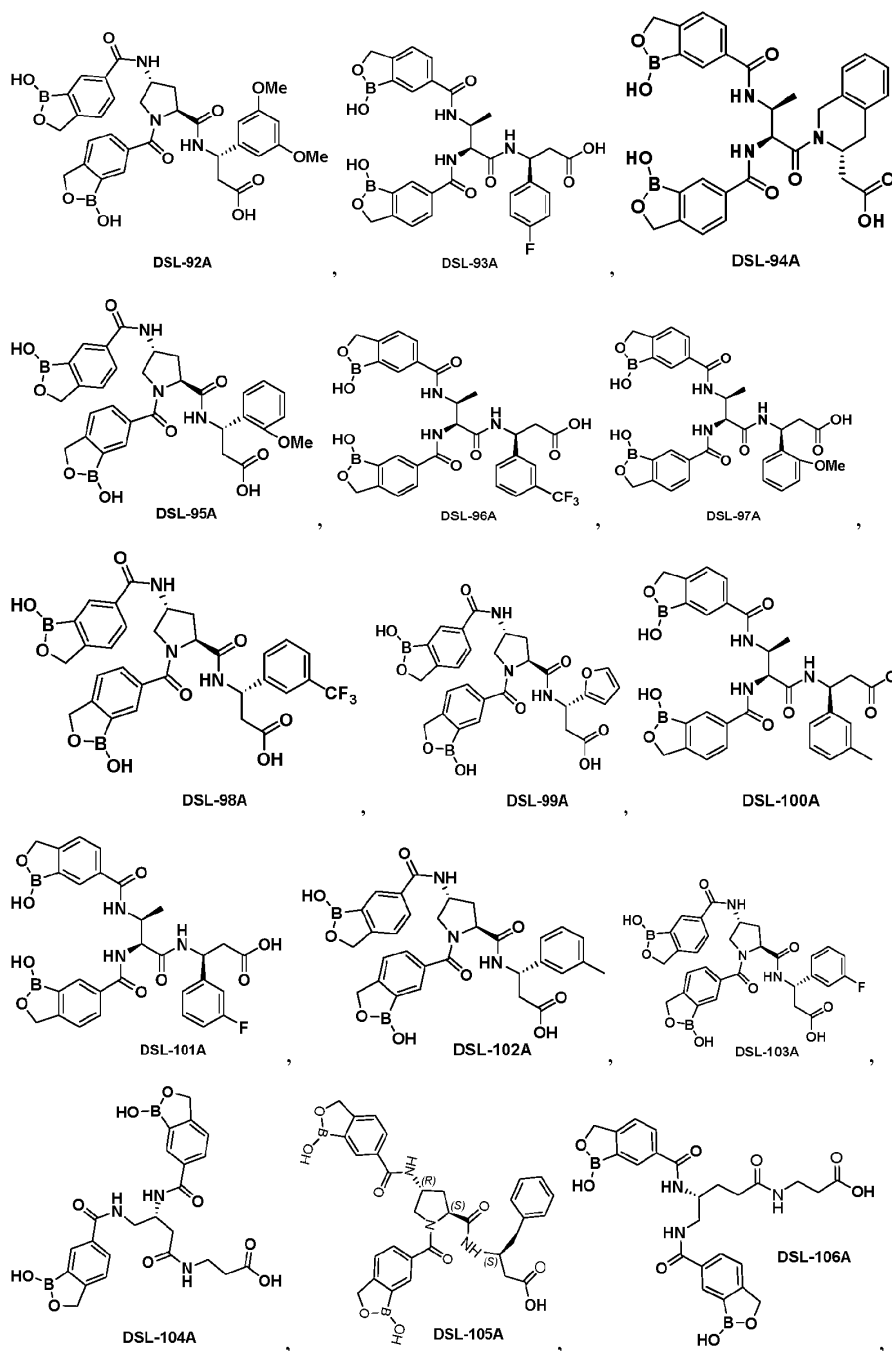


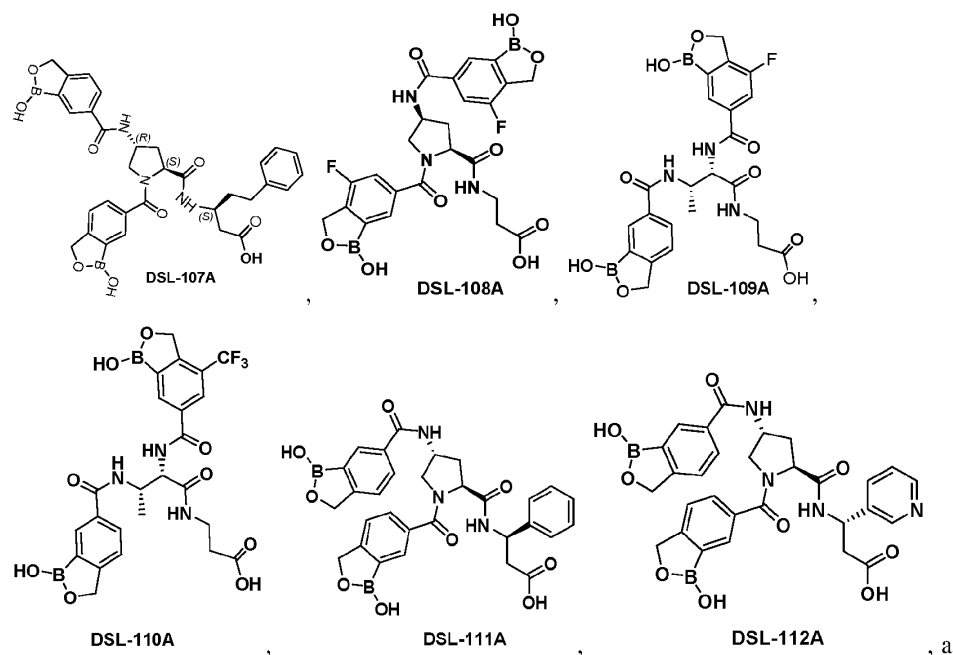
DSL-75A



DSL-76A

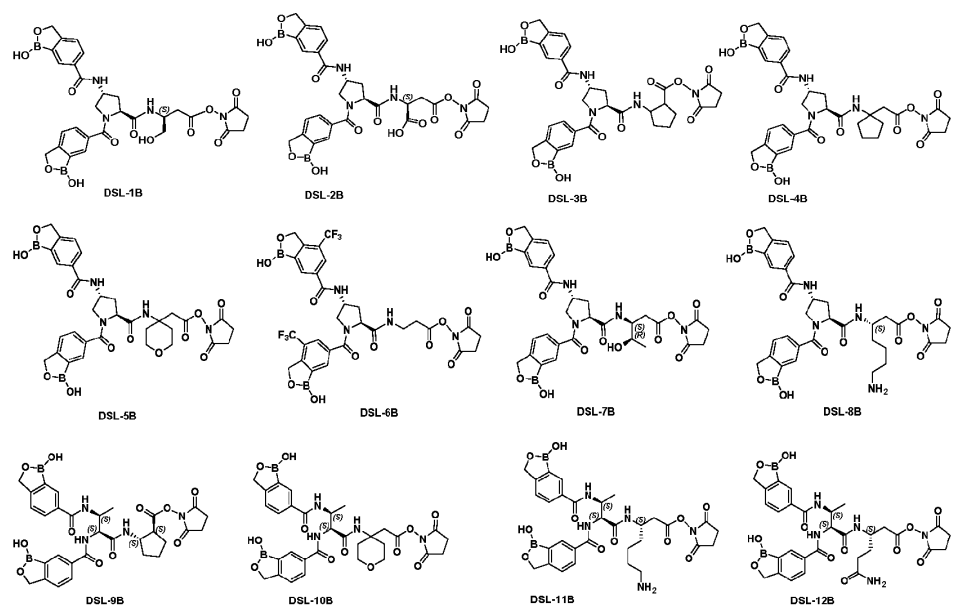


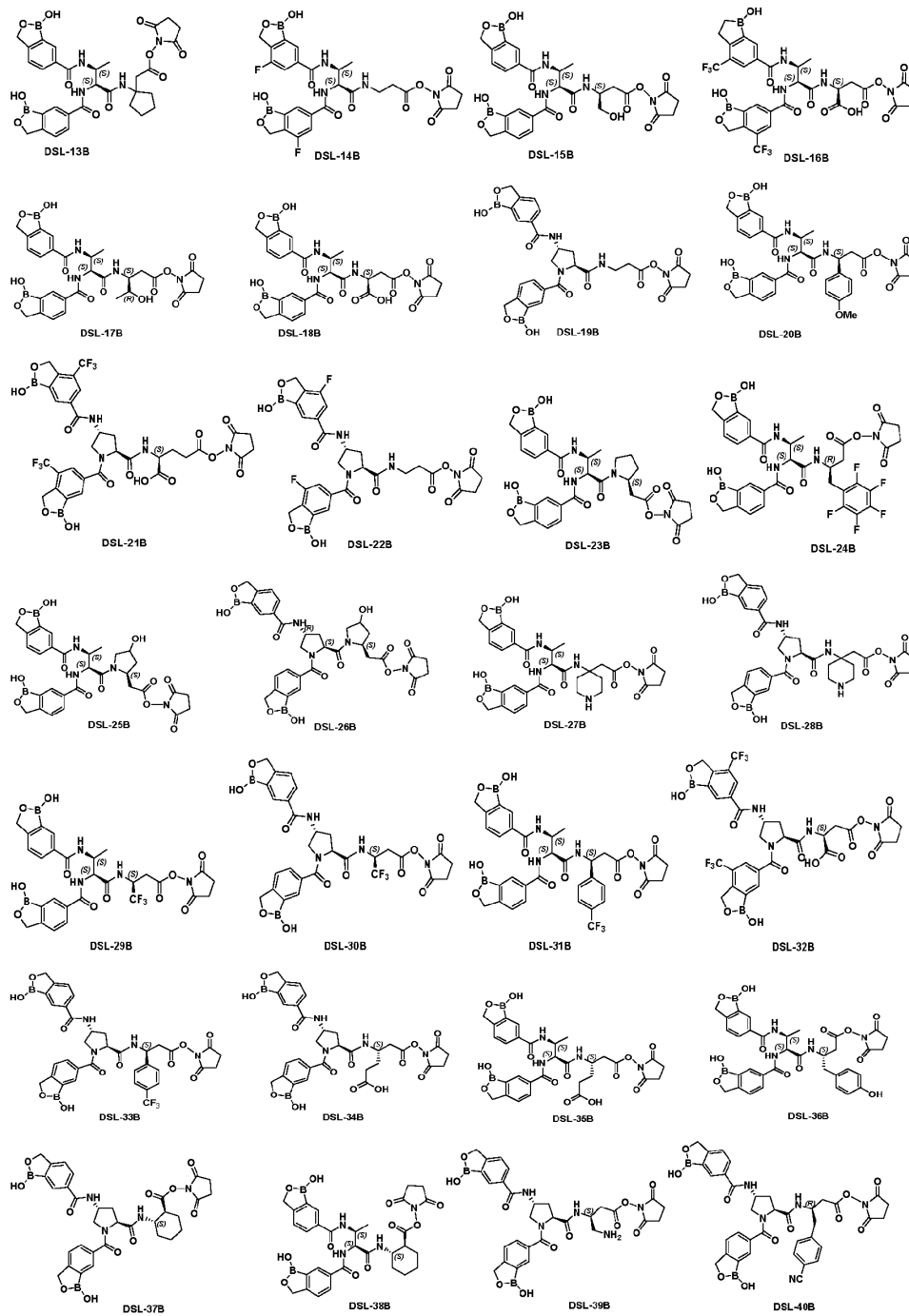


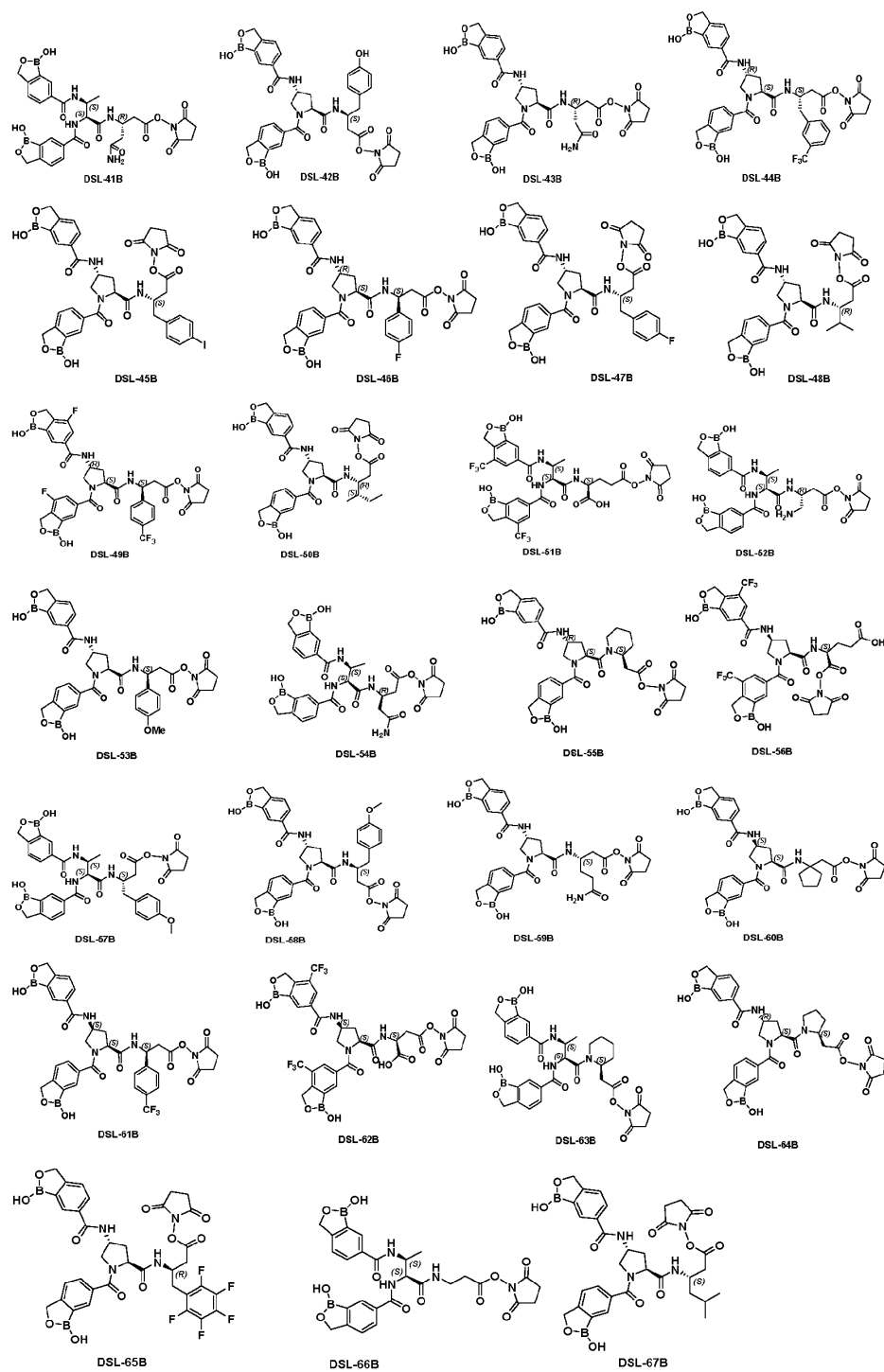


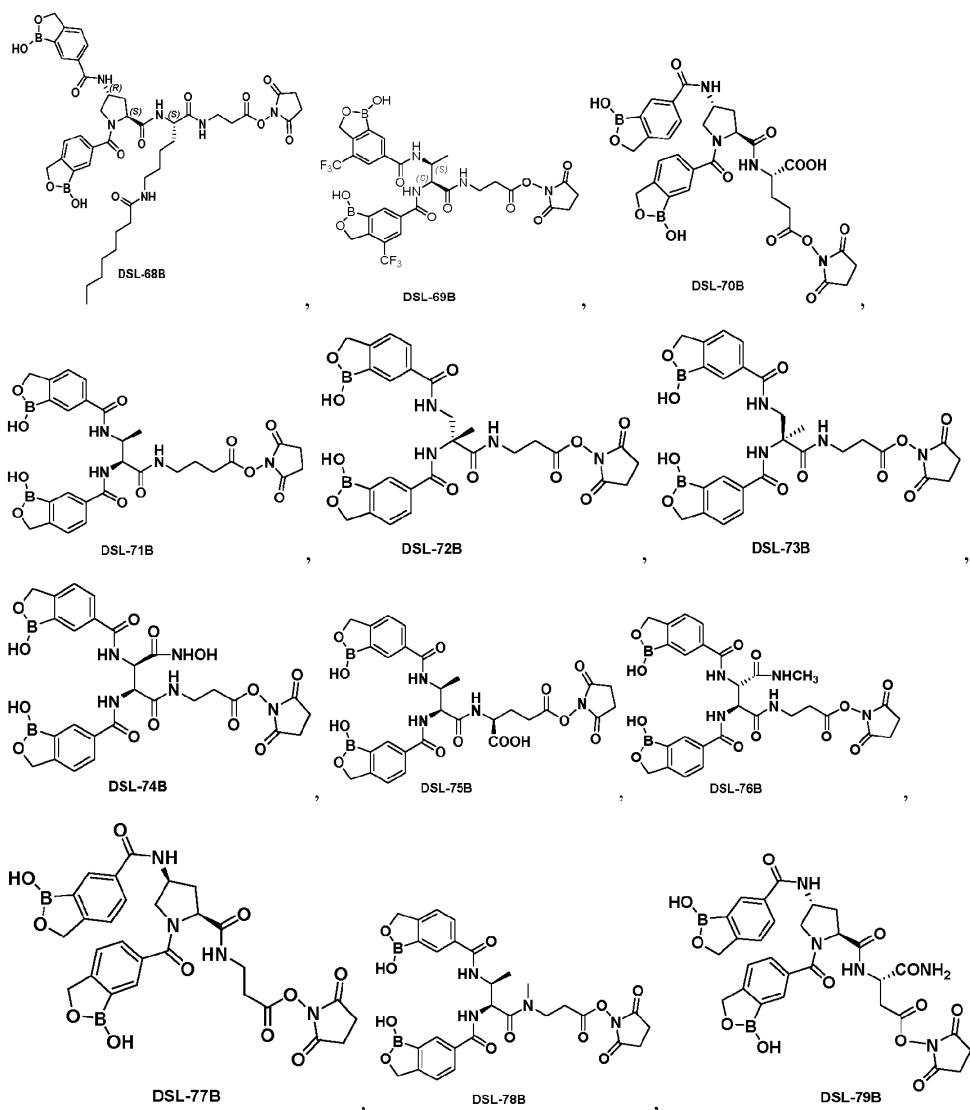
stereoisomer or a mixture of stereoisomers, a pharmaceutically acceptable salt thereof, and combinations thereof. In some embodiments, the Z1c-Linker is selected from:

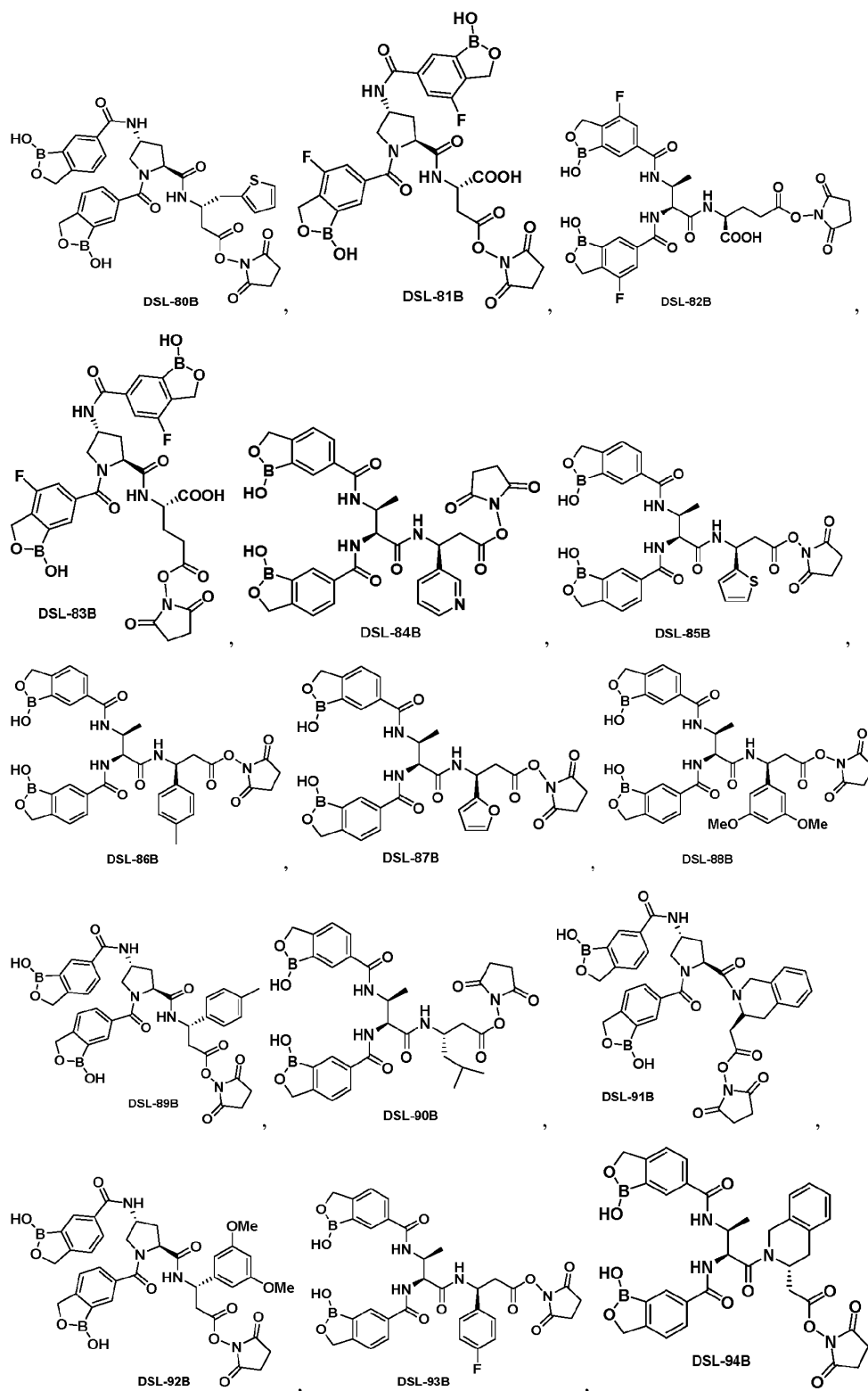
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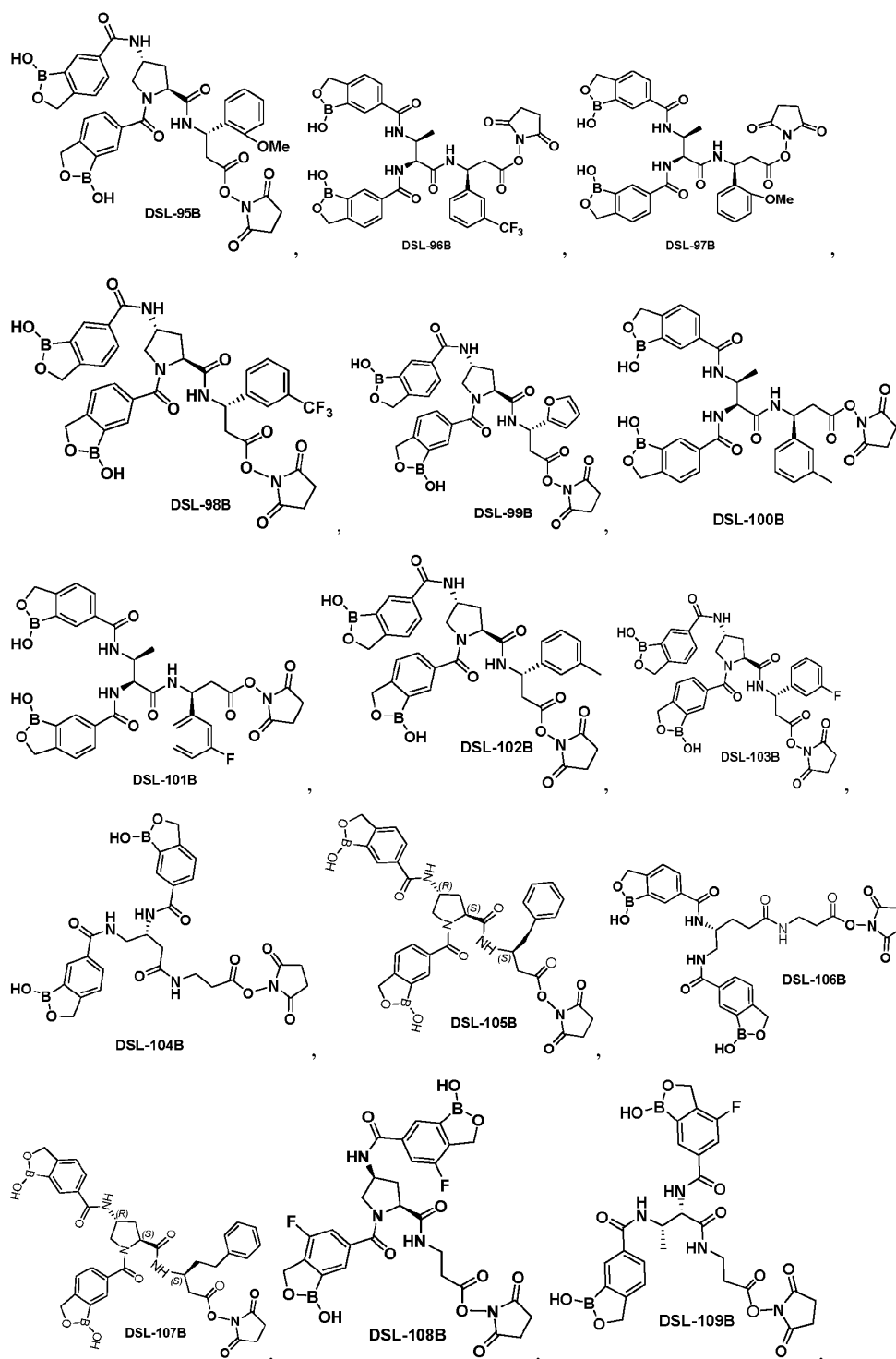


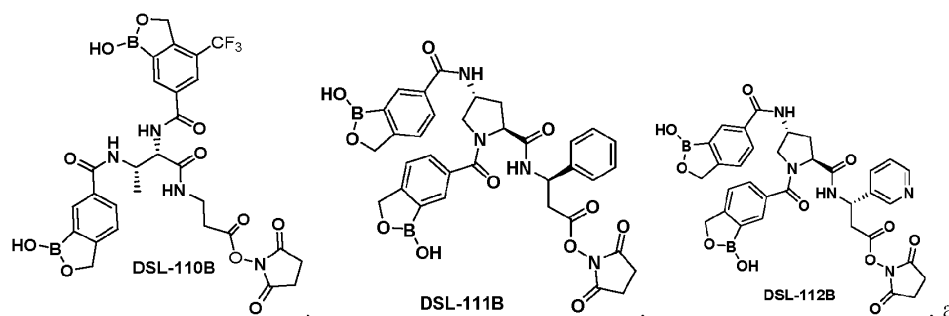












stereoisomer or a mixture of stereoisomers, a pharmaceutically acceptable salt thereof, and combinations thereof.

5 In some embodiments, a therapeutically-effective amount of a pharmaceutical composition of the present disclosure may be administered to a subject. In some embodiments, the pharmaceutical composition comprises at least one compound disclosed herein (e.g., Formula I) and a pharmaceutically acceptable carrier.

In some embodiments, a compound disclosed herein is used as a medicament.

10 In some embodiments, the disclosure provides a method of treatment or prevention of diabetes, impaired glucose tolerance, hyperglycemia or metabolic syndrome, comprises administering to a subject in need thereof a therapeutically effective amount of a compound disclosed herein or a pharmaceutical composition disclosed herein.

In some embodiments, the pharmaceutical composition comprises one or more
15 polyalcohols.

In some embodiments, the polyalcohols are selected from mannitol, sorbitol, erythritol, isomalt, lactitol, glucose, and maltitol.

In some embodiments, the pharmaceutical composition comprises at least one compound disclosed herein for use as a medicament for the treatment of diabetes or obesity, for control of
20 blood sugar levels, or for control of release of a drug.

In some embodiments, the present disclosure provides a method of administering the compounds disclosed herein or a pharmaceutical composition disclosed herein to a subject as a therapeutic or prophylactic agent.

In some embodiments, the disclosure provides a method of making a compound as
25 disclosed herein comprising at least one alkylation and/or amidation step.

In some embodiments, the disclosure provides a method of treating a subject by administering a device or formulation comprising a compound as disclosed herein, such as Examples 1-156. For example, the device can be a fixed dose injector, microdosing injector, an internal or external patch.

In some embodiments, the compound of the present disclosure may be used, as an intermediate in the manufacture of a drug substance or a therapeutic of a prophylactic compound.

In another aspect, the disclosure provides a human insulin analogue, comprising an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID 24051 and 24052; and wherein the B-chain comprises a sequence selected from SEQ ID NOs 25000 – 25397.

In some embodiments, the A-chain comprises sequence 24051 and the B-chain comprises a sequence selected from 25000, 25001, 25006-25009, 25076, 25077, 25082-25085, 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313.

In some embodiments, the A-chain comprises sequence 24051, and the B-chain comprises a sequence selected from 25011, 25012, 25017-25020, 25087, 25088, 25093-25096, 25229, 25239, 25232, 25240, 25245-25248, 25305, 25308, 25315, 25316, and 25321-25324.

In some embodiments, the A-chain comprises sequence 24051 and the B-chain comprises a sequence selected from 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313.

In some embodiments, the A-chain comprises sequence 24051 and the B-chain comprises a sequence selected from 25011, 25012, 25017-25020, 25087, 25088, 25093-25096, 25229, 25232, 25305, and 25308.

In some embodiments, the agonist potency of a compound disclosed herein is determined by measuring the compound's potency for activation of a receptor (e.g., insulin receptor). In some embodiments, the present disclosure provides a compound having agonist potency for an insulin receptor comprising at least one aromatic boron-containing group, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 10 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1.2 to about 14, about 1.2 to about 10, about 2 to about 9, about 2 to about 8, about 3 to about 7, or about 3 to about 6.

In some embodiments, the present disclosure is directed to a compound comprising at least one aromatic boron-containing group attached to a FF scaffold (e.g. FF116A, FF225A) comprising a methyl group, the compound having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 10 mM the compound has an insulin receptor agonist potency ratio of the first

EC50 to the second EC50 of about 2.5 to about 3.7, about 2.5 to about 3.5, about 2.5 to about 3, or about 3 to about 3.5. In some embodiments, the methyl group is a beta methyl group, e.g. FF116A. In some embodiments, the methyl group is an alpha methyl group, e.g. FF225A.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 10 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1 to about 15, about 1 to about 10, about 2 to about 9, about 2 to about 8, about 3 to about 7, or about 3 to about 6. In some embodiments, when the first glucose concentration is 3 mM and the second glucose concentration is 10 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 20 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 2 to about 25, about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 10, about 2 to about 9, or about 2 to about 8. In some embodiments, when the first glucose concentration is 3 mM and the second glucose concentration is 20 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25.

In some embodiments, the present disclosure is directed to a compound comprising at least one aromatic boron-containing group comprising a methyl group (e.g., an alpha methyl group diboronate sensor or a beta methyl group diboronate sensor, such as DSL-9, DSL-14, DSL-18, DSL-66, DSL-69, DSL-72, DSL-75, DSL-109, DSL-110, wherein X represents a point of covalent attachment directly to the amine in X1 of Formula I) and having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 20 mM the compound has an insulin receptor agonist potency

ratio of the first EC50 to the second EC50 of about 1.5 to about 21, about 1.6 to about 21, about 1.7 to about 20, and about 3 to about 20, about 4 to about 15, or about 10 to about 15. In some embodiments, when the first glucose concentration is 3 mM and the second glucose concentration is 20 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose concentration is 20 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1 to about 25, about 2 to about 24, about 2 to about 24, about 4 to about 20, about 5 to about 18, or about 10 to about 15.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1.2 to about 5, about 1.3 to about 5, about 1.4 to about 5, about 1.5 to about 5, about 2 to about 4, or about 3 to about 4. In some embodiments, when the first glucose concentration is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 1.2, 1.3, 1.4, 1.5, 2, 3, 4, or 5.

In some embodiments, the present disclosure is directed to a compound comprising at least one aromatic boron-containing group comprising a methyl group (e.g., an alpha methyl group diboronate sensor or a beta methyl group diboronate sensor, such as DSL-9, DSL-14, DSL-18, DSL-66, DSL-69, DSL-72, DSL-75, DSL-109, DSL-110) having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1.5 to about 3.5, about 1.5 to about 3, about 2 to about 3, or about 2.5 to about 3. In some embodiments, when the first glucose concentration

is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 1.2, 1.3, 1.4, 1.5, 2, or 3.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 1 to about 6, about 2 to about 6, about 3 to about 5, or about 3 to about 4. In some embodiments, when the first glucose concentration is 5.6 mM and the second glucose concentration is 11.1 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of at least or up to about 1, 2, 3, 4, 5, or 6.

In some embodiments, the present disclosure provides a compound comprising at least one diboronate sensor having binding affinity to glucose, wherein the compound has a glucose Kd ranging from 0.01 mM to 3 mM, 0.01 mM to 2.5 mM, 0.01 mM to 2 mM, 0.01 mM to 1.5 mM, 0.01 mM to 1 mM, or 0.01 mM to 0.5 mM. In some embodiments, the compound has a glucose Kd of less than about 3, 2.5, 2, 1.5, 1, 0.5, or 0.01 mM. In some embodiments, the compound has a glucose Kd of less than about 3, 2.5, 2, 1.5, 1, 0.5, or 0.01 mM and the compound has agonist activity for the insulin receptor.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having binding affinity for glucose, wherein when administered at a dose of 30 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100 mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200 mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of 50 to 2500 and/or a relative glucose infusion rate ratio of 0.2 to 5. In some embodiments, the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of at least about 50, 100, 200, 300, 400, 500, 1000, 1500, 2000, or 2500 and/or a relative glucose infusion rate ratio of at least about 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5.

In some embodiments, the present disclosure is directed to a compound comprising at least one aromatic boron-containing group comprising a methyl group (e.g., an alpha methyl group diboronate sensor or a beta methyl group diboronate sensor, such as DSL-9, DSL-14, DSL-18, DSL-66, DSL-69, DSL-72, DSL-75, DSL-109, DSL-110) and having agonist potency for an insulin receptor, wherein administered at a dose of 30 nmol/kg to a first group of rats with

a first glucose infusion rate to provide a blood glucose concentration of 100 mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200 mg/dL, the compound has a relative glucose infusion rate difference (mg/kg/min.min) of about 700 to about 2500, about 750 to about 2500, about 800 to about 2500, about 850 to about 2500, about 900 to about 2500, and a relative glucose infusion rate ratio of about 2 to about 4, about 2 to about 3, or about 3 to about 4.

In some embodiments, the present disclosure provides a compound having agonist potency for glucose comprising at least one aromatic boron-containing group having binding affinity for glucose, wherein when administered at a dose of 30 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100 mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200 mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of about 1 to about 2500, about 1 to about 2000, about 1 to about 1500, about 100 to about 1500, and about 1000 to about 1500, and a relative glucose infusion rate ratio of about 0.1 to about 5, about 0.2 to about 4.5, about 0.5 to about 4, about 0.5 to about 3.5, or about 1 to about 3. In some embodiments, the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of at least about 50, 100, 200, 300, 400, 500, 1000, 1500, 2000, or 2500 and/or a relative glucose infusion rate ratio of at least or up to about 2, 2.5, 3, 3.5, 4, 4.5, or 5.

In some embodiments, the present disclosure provides a compound comprising at least one aromatic boron-containing group having binding affinity for glucose, wherein when administered at a dose of 20 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of 150 to 1000 and a relative glucose infusion rate ratio of about 1 to about 3. In some embodiments, the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of at least about 150, 200, 250, 300, 450, 500, 600, 700, 800, 900, or 1000, and/or a relative glucose infusion rate ratio of at least or up to about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5.

In some embodiments, the present disclosure is directed to a compound comprising at least one aromatic boron-containing group comprising a methyl group (e.g., an alpha methyl group diboronate sensor or a beta methyl group diboronate sensor, such as DSL-9, DSL-14, DSL-18, DSL-66, DSL-69, DSL-72, DSL-75, DSL-109, DSL-110) having binding affinity for glucose. In some embodiments, the compound is administered at a dose of 20 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100

mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200 mg/dL, the compound has a relative glucose infusion rate difference (mg/kg/min.min) of about 10 to about 1000, about 10 to about 900, about 20 to about 800, about 50 to about 700, about 100 to about 600, and about 200 to about 500, and a relative
5 glucose infusion rate ratio of about 1 to about 2, about 1 to about 1.5, or about 2 to about 2.5.

In some embodiments, the present disclosure provides a compound having agonist potency for glucose comprising at least one aromatic boron-containing group, wherein when administered at a dose of 20 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100 mg/dL and to a second group of rats with a second
10 glucose infusion rate to provide a blood glucose concentration of 200 mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of about 100 to about 2500, about 100 to about 1000, about 100 to about 900, about 200 to about 800, about 300 to about 700, about 400 to about 600, and about 400 to about 500, and a relative glucose infusion rate ratio of about 1 to about 2.5, about 1 to about 2, about 1 to about 1.5, about 0.5 to about 1.5, or
15 about 1 to about 1.5.

In some embodiments, the present disclosure provides a compound having binding affinity for glucose comprising at least one aromatic boron-containing group having binding affinity to glucose, wherein when administered at a dose of 10 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose concentration of 100mg/dL and to a
20 second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of 60 to 2400 and a relative glucose infusion rate ratio of 0.5 to about 4.

In some embodiments, the present disclosure provides a compound having binding affinity for glucose comprising at least one aromatic boron-containing group comprising at least
25 one diboronate sensor having affinity to glucose.

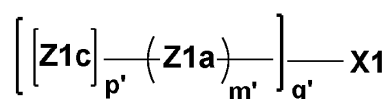
In some embodiments, the present disclosure is directed to a compound having agonist potency for an insulin receptor comprising at least one aromatic boron-containing group (e.g., diboronate sensor, such as DSL-1 to DSL-112), wherein one or more aromatic boron-containing groups comprise a methyl group (e.g., beta methyl, alpha methyl). In some embodiments, the
30 compound is selected from Example 1, Example 3, Example 4, Example 5, Example 6, Example 8, Example 9, Example 10, Example 11, Example 12, Example 15, Example 16, Example 18, Example 19, Example 23, Example 24, Example 25, Example 27, Example 30, Example 33, Example 34, Example 35, Example 37, Example 40, Example 41, Example 44, Example 46, Example 48, Example 49, Example 52, Example 54, Example 55, Example 56, Example 57,
35 Example 59, Example 64, Example 65, Example 67, Example 69, Example 70, Example 75,

Example 76, Example 80, Example 82, Example 83, Example 84, Example 85, Example 92, Example 94, Example 95, Example 96, Example 97, Example 100, Example 101, Example 102, Example 104, Example 107, Example 109, Example 112, Example 113, Example 114, Example 118, Example 119, Example 122, Example 123, Example 127, Example 129, Example 130, Example 131, Example 132, Example 133, Example 140, Example 152, and Example 154. In some embodiments, at least one aromatic boron-containing group (e.g. diboronate sensor) has binding affinity to glucose. In some embodiments, a compound comprising two or more diboronate sensors has higher affinity to glucose. In some embodiments, a compound comprising three or more diboronate sensors has higher affinity to glucose.

In some embodiments, the binding constants of DSL compounds, such as DSL-1A to DSL-112A, to glucose, fructose, and/or lactate can be tested and calculated. In some embodiments, DSL compounds disclosed herein have binding affinity to glucose with a K_d value ranging from about 0.01 mM to about 3 mM. Some exemplary DSL compounds disclosed herein have binding affinity to glucose with a K_d value ranging from about 0.01 mM to about 2.5 mM. Some exemplary DSL compounds disclosed herein have binding affinity to glucose with a K_d value ranging from about 0.5 mM to about 1.5 mM. In some embodiments, exemplary DSL compounds have a binding affinity to glucose with a K_d value of less than about 3, 2.5, 2, 1.5, 1, 0.5, or 0.01 mM.

The present disclosure may also be defined according to any one of the following numbered embodiments:

1. A compound of the following formula, or a stereoisomer or a mixture of stereoisomers, or a pharmaceutically acceptable salt thereof:



wherein:

X1 comprises a polypeptide comprising an insulin receptor agonist having an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, and 24051, and the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24061, 24063, 25228, 25313, 25393, 25396, and 25397;

each Z1a independently comprises 1 to 50 amino acids connected together using amide or peptide bonds;

each Z1c is independently selected from Formulae FF12A, FF12B, FF114A, FF115A, FF116A, and FF225A, wherein each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to an amine of X1 or to OH when X1 is OH;

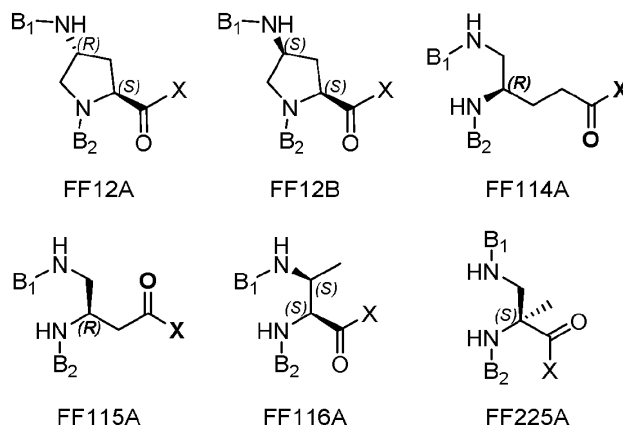
each m' is 0 or 1;

5 each p' is 1, 2, 3, 4, or 5; and

each q' is 1, 2, 3, 4, or 5, wherein when p' or q' is 2 or more, each corresponding Z1c is independently selected and may be the same or different and wherein when q' is 2 or more, each corresponding Z1a is independently selected and may be the same or different;

when m'=0, each Z1c is covalently conjugated either directly or via an indirect linker to an amine of X1 or to OH when X1 is OH, when m'=1 each Z1a is covalently conjugated via an amide or peptide bond to X1 and each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a;

and wherein Formulae FF12A, FF12B, FF114A, FF115A, FF116A, and FF225A are:



15 wherein X represents a point of covalent attachment either directly to an amine of X1 or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1; and wherein B₁ and B₂, which may be identical or different, each independently represent an aromatic boron-containing group; and

wherein one or more positions of the compound may comprise an isotope.

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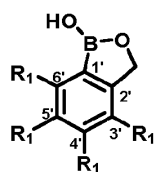
2. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof, wherein at least one Z1c comprises a beta methyl group.

3. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof, wherein at least one Z1c comprises an alpha methyl group.

25

4. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof, wherein the B₁ and the B₂ are each independently Formula F2,

wherein Formula F2 is:



(F2) ;

5 wherein:

a first R₁ represents (C=O)---* or (CH₂)_m(C=O)---*, wherein ---* represents the attachment point to the rest of Z1c, and m is 1, 2, 3, 4, 5, 6, or 7; and

each remaining R₁ is independently selected from H, F, Cl, Br, I, OH, CH₂-NH₂, NH₂, (C=O)-NH₂, CH=O, SO₂CH₃, SO₂CF₃, CF₃, CHF₂, NO₂, CH₃, OCH₃, O(CH₂)_{m'}CH₃, (SO₂)NH-
10 CH₃, (SO₂)NH(CH₂)_{m'}CH₃, and OCF₃, wherein m' is 1, 2, 3, 4, 5, 6, or 7.

5. The compound of embodiment 4, or a pharmaceutically acceptable salt thereof, wherein the first R₁ is (C=O)---*.

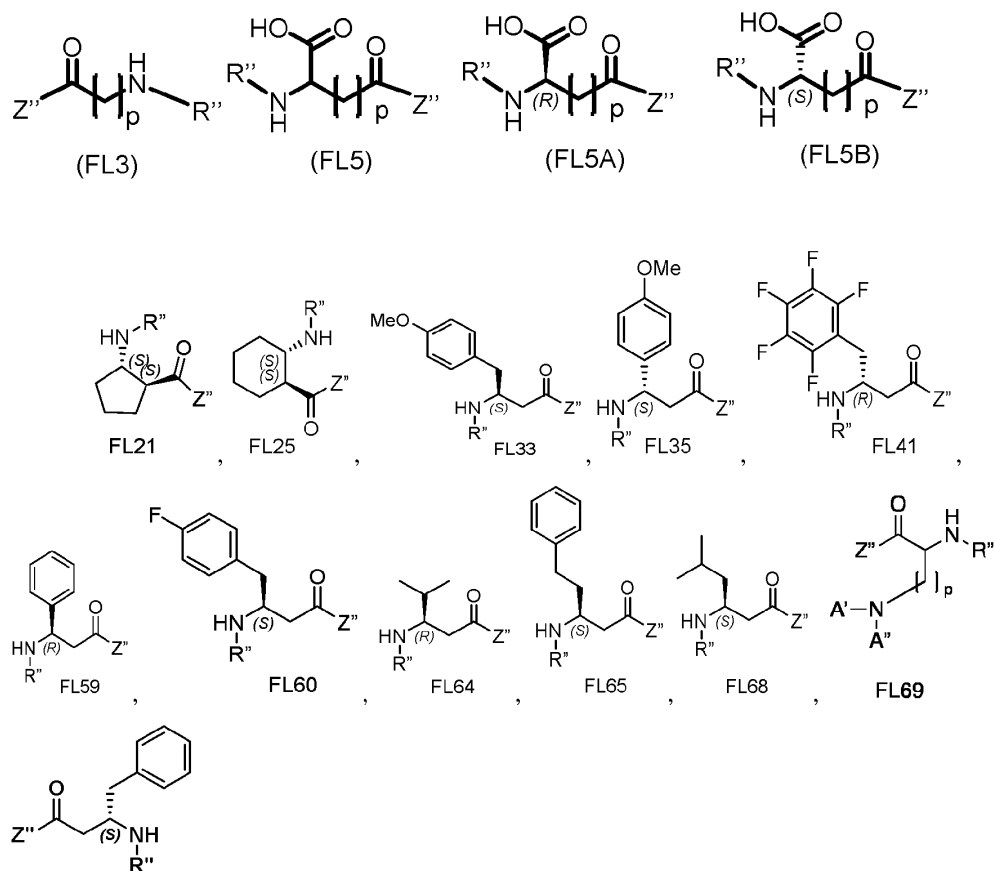
15 6. The compound of embodiment 4, or a pharmaceutically acceptable salt thereof, wherein (a) each remaining R₁ is independently selected from (i) H, CF₃, and F; or (b) two remaining R₁ are H and one remaining R₁ is CF₃ and F.

20 7. The compound of embodiment 6, or a pharmaceutically acceptable salt thereof, wherein each remaining R₁ is H.

8. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof, wherein one or more Z1c is covalently conjugated via the indirect linker to X1 or to Z1a, the indirect linker is represented by Formula (X'')_{n1},

25 each n1 is independently selected from 1, 2, 3, 4, and 5, and

each X'' is independently selected from Formulae FL3, FL5, FL5A, FL5B, FL21, FL25, FL33, FL41, FL59, FL60, FL64, FL65, FL68, FL69, and FL74:



5 (FL74), and stereoisomers thereof;

wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

10 A' is selected from H, an alkyl group (e.g., C₁-C₆alkyl group), a saturated fatty acid, an unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a C₁-C₆ haloalkyl group, an aryl group, and a heteroaryl group; and

15 A'' is selected from an alkyl group (e.g., C₁-C₆alkyl group), a substituted acyl group, acyl group terminating in an acid group, a saturated or unsaturated fatty acid, a cycloalkyl group (e.g., (C₃-C₆)cycloalkyl), a haloalkyl group (e.g., C₁-C₆haloalkyl group), an aryl group, and a heteroaryl group;

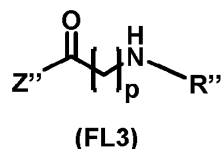
p is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

9. The compound of embodiment 8, or a pharmaceutically acceptable salt thereof,

wherein each said indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL33, FL64, FL65, FL69, and stereoisomers thereof.

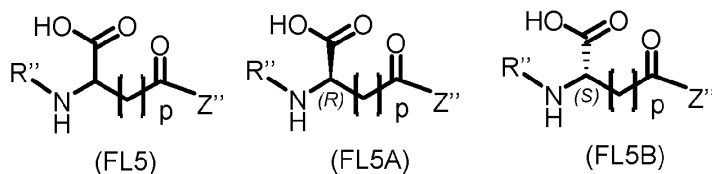
10. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof,
 5 wherein one or more Z1c is covalently conjugated to X1 via the indirect linker, and wherein the indirect linker is independently selected from



Formula FL3,

wherein p is 2 or 3; and

10

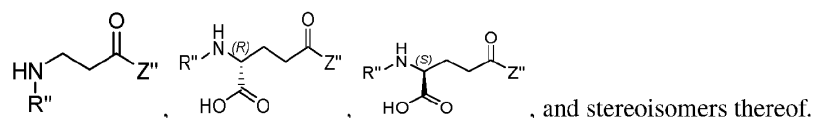


Formulae FL5, FL5A, and FL5B,

wherein p is 1 or 2.

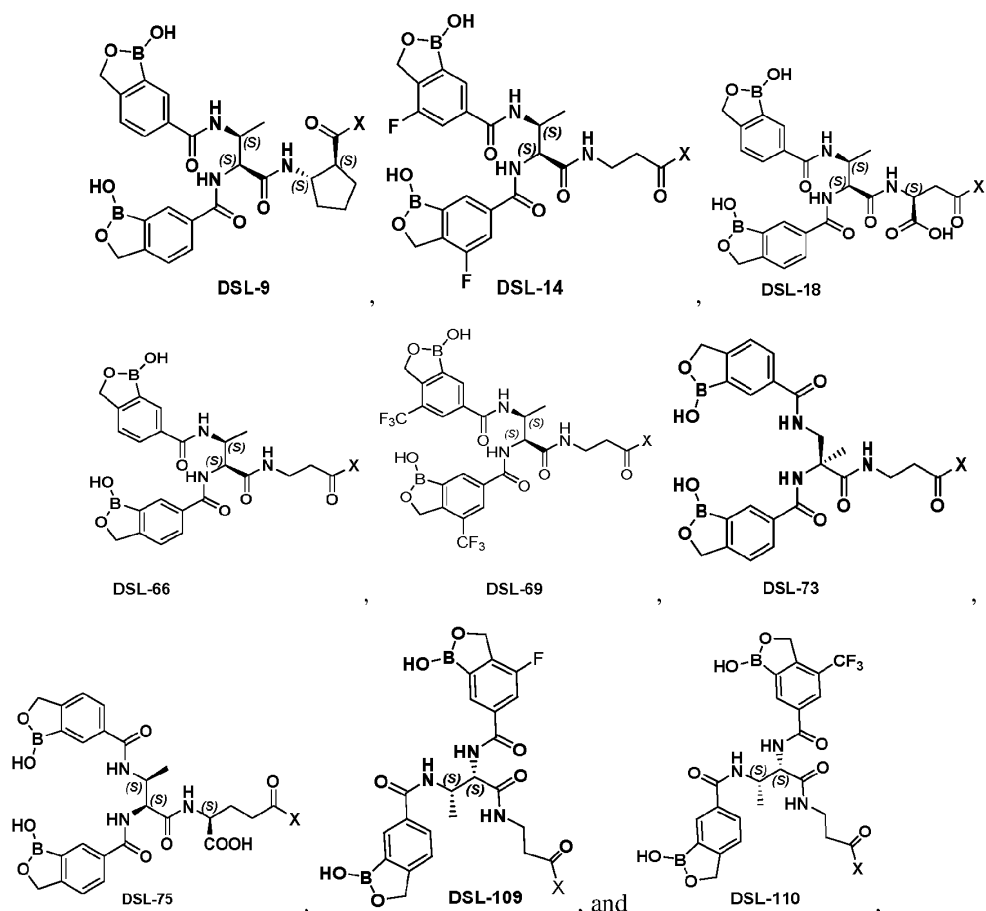
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11. The compound of embodiment 10, or a pharmaceutically acceptable salt thereof,
 wherein the indirect linker is selected from:



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12. The compound of embodiment 1, or a pharmaceutically acceptable salt thereof,
 wherein each of the Z1c is covalently conjugated to the indirect linker to form a Z1c-Linker
 independently selected from:



wherein X represents a point of covalent attachment directly to the amine in X1 or to OH
 5 when X1 is OH.

13. The compound of embodiment 1, wherein the compound is selected from
 Example 3, Example 19, Example 22, Example 23, Example 27, Example 41, Example 42,
 Example 44, Example 45, Example 48, Example 50, Example 55, Example 56, Example 69,
 10 Example 71, Example 76, Example 87, Example 93, Example 105, Example 131, Example 136,
 Example 140, Example 141, Example 142, Example 143, Example 144, Example 145, Example
 146, Example 147, Example 148, Example 149, Example 151, Example 152, Example 153,
 Example 154, and Examples 155; a pharmaceutically acceptable salt thereof, an isotope thereof,
 and combinations thereof.

15

14. The compound of embodiment 1, wherein the compound is selected from Example 3,

Example 19, Example 23, Example 27, Example 41, Example 44, Example 48, Example 55, Example 56, Example 69, Example 76, Example 131, Example 140, Example 152, Example 154, and Example 156; a pharmaceutically acceptable salt thereof, an isotope thereof, and combinations thereof.

5

15. A pharmaceutical composition comprising at least one compound according to embodiment 1, or a stereoisomer or a mixture of stereoisomers, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

10

16. A pharmaceutical composition comprising at least one compound according to embodiment 13, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

15

17. A pharmaceutical composition comprising at least one compound according to embodiment 14, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

20

18. A method of treating diabetes, impaired glucose tolerance, hyperglycemia or metabolic syndrome, comprising administering to a subject in need thereof a therapeutically effective amount of the compound of embodiment 1, or a stereoisomer or mixture of stereoisomers, or a pharmaceutically acceptable salt thereof.

25

19. A method of treating diabetes, impaired glucose tolerance, hyperglycemia or metabolic syndrome, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition according to embodiment 15.

30

20. A method of treating type 1 diabetes or type 2 diabetes comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition according to any one of embodiments 15-17.

35

21. A method of treating type 2 diabetes comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition according to embodiment 16 or 17.

22. A compound represented by Formula II, or a stereoisomer or a mixture of

stereoisomers, or a pharmaceutically acceptable salt thereof:

Z1c-Linker

(Formula II)

wherein the Z1c-Linker is selected from DSL-2, DSL-8, DSL-14, DSL-18, DSL-19,
5 DSL-21, DSL-32, DSL-37, DSL-47, DSL-48, DSL-58, DSL-65, DSL-66, DSL-67, DSL-68,
DSL-69, DSL-73, DSL-75, DSL-77, DSL-83, DSL-104, DSL-105, DSL-106, DSL-107, DSL-
108, DSL-109, DSL-110, DSL-111; and wherein X is a leaving group, and
wherein one or more positions of the compound of Formula II may comprise an isotope.

10 23. The compound of embodiment 22, wherein X is selected from
N-hydroxysuccinimide (NHS), 2,3,5,6-tetrafluorophenol (TFP), pentafluorophenol (Pfp), OH,
and halogen.

24. The compound of embodiment 22, wherein the compound is selected from DSL-
15 2A, DSL-9A, DSL-14A, DSL-18A, DSL-19A, DSL-21A, DSL-32A, DSL-37A, DSL-47A,
DSL-48A, DSL-58A, DSL-65A, DSL-66A, DSL-67A, DSL-68A, DSL-69A, DSL-73A, DSL-
75A, DSL-77A, DSL-83A, DSL-104A, DSL-105A, DSL-106A, DSL-107A, DSL-108A, DSL-
109A, DSL-110A, DSL-111A, a stereoisomer or a mixture of stereoisomers, a pharmaceutically
acceptable salt thereof, and combinations thereof.

20 25. The compound of embodiment 22, wherein the compound is selected from DSL-
2B, DSL-9B, DSL-14B, DSL-18B, DSL-19B, DSL-21B, DSL-32B, DSL-37B, DSL-47B, DSL-
48B, DSL-49B, DSL-58B, DSL-65B, DSL-66B, DSL-67B, DSL-68B, DSL-69B, DSL-73B,
DSL-75B, DSL-77B, DSL-83B, DSL-104B, DSL-105B, DSL-106B, DSL-107B, DSL-108B,
25 DSL-109B, DSL-110B, DSL-111B, a stereoisomer or a mixture of stereoisomers, a
pharmaceutically acceptable salt thereof, and combinations thereof.

Methods of Preparation

In some embodiments, the present disclosure provides a method to prepare a compound
30 comprising an aromatic boron-containing compound and/or an aromatic boron-containing group
(e.g., Z1c, Z1c-Linker (Formula II), Formula I) or a pharmaceutical preparation comprising one
or more compounds of the present disclosure.

In some embodiments, the disclosure provides a method for preparing rotationally
constrained tether boron conjugates that contain scaffolds (Z1c, Z1c-Linker) that are rotationally
35 hindered by disfavored steric interactions (e.g. gauche vs anti interactions of substituents),

hindered rotation due to bond hybridization (e.g., cis- vs trans- amide rotations), or through rigid covalent bonds (e.g., (*E*) vs (*Z*) configurations for alkene moieties). For example, Formulae FF116, FF116A, FF116B, FF116C, and FF116D contain alkyl functionalities geminal (e.g., attached to the same atom) to the amine groups that are covalently conjugated to the boronic acid functionalized moieties. As another example, one or more of Formulae FF116, FF116A, FF116B, FF116C, and FF116D contain geminal alkyl substituents which may limit the accessible dihedral angles that the boron conjugated amines adopt, influencing adopted dihedral angles and placing the boronic functionalized groups closer together and allowing for increased binding of the conjugates to target molecules such as proteins or sugars.

10 In some embodiments, a compound as disclosed herein is further modified through connection (e.g., conjugation, fusion, etc.) to a second agent or therapy to form a fusion protein. In some embodiments, the second agent or therapy is a protein or peptide as herein described. In some embodiments, the second agent is a drug substance, wherein the drug substance is a polypeptide human hormone, an endocrine hormone, insulin, human insulin, glucagon or a glucagon analog, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide, glucose-dependent insulinotropic polypeptide, a hybrid peptide comprising sequences from two or more human polypeptide hormones, or an analogue thereof. In some embodiments, the fusion protein comprises one or more diboronate sensor(s) as described herein. In some embodiments, two or more agents are connected (e.g., conjugation, fusion, etc.) to produce a fusion protein comprising one or more diboronate sensor(s). In some embodiments, the two or more agents are proteins or peptides as herein described. The biological activity (e.g., agonist potency, bioavailability, etc.) of the compounds and compositions or methods of treatment described herein may be evaluated according to methods known by those skilled in the art. In some embodiments, the biological activity of a compound is determined by evaluating the EC50 of a compound. In some embodiments, the biological activity of a compound is determined using an insulin receptor phosphorylation (IR Phosphorylation) assay disclosed herein. In some embodiments, the biological activity of a compound is determined by evaluating the binding affinity (*K_d*) of a compound for its target. In some embodiments, the biological activity of a compound is determined by assessing the relative glucose rate difference. In some embodiments, the biological activity of a compound is determined by assessing the relative glucose rate ratio.

Methods of Treatment

35 In some embodiments, the disclosure provides a method of treating a subject suffering from, or susceptible to, a disease that is beneficially treated by a compound disclosed herein or

a pharmaceutical preparation comprising one or more of the compounds disclosed herein. In some embodiments, the method comprises the step of administering to a subject in need thereof an effective amount of a pharmaceutical preparation/composition of the present disclosure. In at least one embodiment, the compound(s) and/or pharmaceutical preparations of the present disclosure may be for use in (or in the manufacture of medicaments for) the treatment or prevention of disorders, including hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, metabolic syndrome X, or dyslipidemia, diabetes during pregnancy, pre-diabetes, Alzheimer's disease, MODY 1, MODY 2 or MODY 3 diabetes, neurological diseases, mood disorders, and psychiatric disorders. In at least one embodiment, a therapeutically-effective amount of a compound and/or pharmaceutical preparation of the present disclosure is administered to a subject suffering from diabetes. In some embodiments, the diabetes is type 1 diabetes or type 2 diabetes. In some embodiments, the diabetes is type 1 diabetes.

In some embodiments, when a first active agent is administered with a second (another) active agent the dose may be adjusted so that the activity of the two treatments combined is sufficient to regulate blood glucose levels in a patient. Thus, the amount of a first active agent or second active agent that can be administered to regulate blood glucose levels in such combinations may be less than would be required if the first active agent or second active agent were administered as a monotherapy.

20

The following examples and experimental data are provided for illustrative purposes only, and do not limit the scope of the embodiments of the present disclosure.

The following abbreviations have the definitions set forth below:

Abbreviation	Full Name
Acm	s-Acetamidomethyl group
ACN	Acetonitrile
ARS	Alizarin Red S
Boc	tert-Butyloxycarbonyl
DCM	Dichloromethane
Dde	1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
DIPEA, DIEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DTDP	2,2,-Dithiopyridine
EDC	3-(Ethyliminomethyleneamino)-N,N-dimethylpropan-1-amine
Fmoc	Fluorenylmethyloxycarbonyl Chloride
GLP-1	Glucagon-Like Peptide 1

Abbreviation	Full Name
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HCl	Hydrochloric Acid
IGF1	Insulin-like Growth Factor 1
IPTG	Isopropylthio- β -galactoside
IBs	Inclusion Bodies
LC-MS	Liquid Chromatography-Mass Spectrometry
MeOH	Methanol
MIDA	N-methyliminodiacetic acid
MODY	Maturity Onset Diabetes of the Young
NHS	N-Hydroxysuccinimide
Oxyma	Ethyl cyanohydroxyiminoacetate
RAM	Rink Amide Matrix
PEG	Polyethylene Glycol
SDS	Sodium Dodecyl Sulfate
tBu	tert-Butyl
TFA	Trifluoroacetic Acid
TFP	2,3,5,6-tetrafluorophenol
TMB	3,3',5,5'-tetramethybenzidine
Pfp	pentafluorophenol
CaCl ₂	Calcium Chloride
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
SnCl ₂	Tin Chloride

Examples

A. Preparation of aromatic boron-containing compounds.

The disclosed compounds can be prepared according to the following schemes. The following schemes represent the general methods used in preparing these compounds. However, the synthesis of these compounds is not limited to these representative methods, as they can also be prepared through various other methods by those skilled in the art of synthetic chemistry.

DSL Synthesis Method 1:

On Resin Synthesis of Diboronated Sensors:

2-Chlorotriptyl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (0.93mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated 20% piperidine with DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of 2,3-bis((((9H-fluoren-9-yl)methoxy)carbonyl) amino)butanoic acid,

4 (0.224g, 0.4mmol), DIPEA (0.14ml, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **5**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol),
 5 HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **7**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

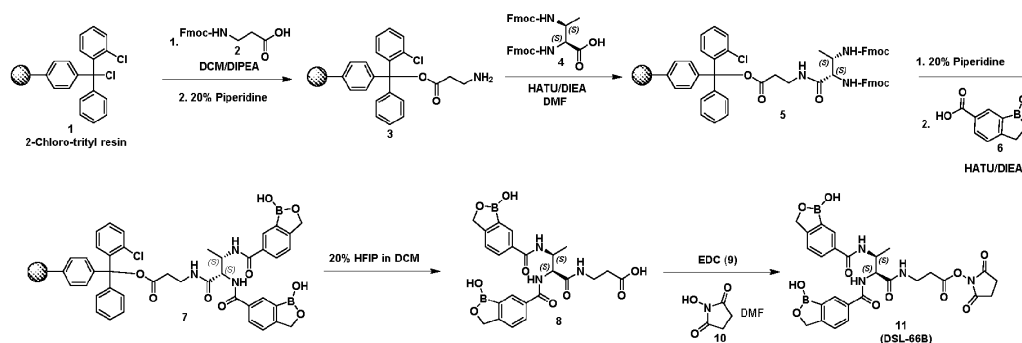
Cleavage of diboronated sensor from resin

Compound **7** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under
 10 reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **8** (DSL-66A) which was used in the next step without purification.

NHS-activation of diboronated sensor:

Crude diboronated sensor acid **8** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and
 20 fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate **11** (DSL-66B) in ~33% yield (20mg). Calculated mass
 25 (M+H)⁺ = 607.2, Observed mass (M+H)⁺ = 606.9.

Method 1



The MS data for DSL-66B is listed in Table I. The other examples listed in Table I below were synthesized under similar conditions.

5

Table I

DSL-B Examples	Mass Calculated [M+H] ⁺	Mass Observed [M+H] ⁺
DSL-9B	647.24	647
DSL-14B	643.19	643.07
DSL-66B	607.20	607.08
DSL-73B	607.20	
DSL-112B	743.18	742.90

DSL Synthesis Method 2:

On Resin synthesis of diboronated sensor:

- 2-Chlorotriptyl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5 mL) for 30 mins.
- 10 Solvent was removed with nitrogen and a solution of 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (0.93mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol)) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5 mL) then DMF (3 x 5 mL). The
- 15 resin was treated 20% piperidine with DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of (2S,3S)-(Fmoc-amino)-3-azidobutyric acid **4** (0.146g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) was added to the resin and heated at 50 °C for 30 min to give **5**. Resin was washed with DMF (4 x 5mL) and THF (3 x 5mL) and treated with 5mL solution of 0.2M SnCl₂ (189mg), 0.8M thiophenol (440ul) and 1M DIEA (870ul) in
- 20 THF and gently mix it for 1hr on shaker. After 1h, washed the resin with THF (3 x 5mL) and DMF (3 x 5mL). Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL) to obtain **6a**. Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35ml, 2mmol) was added to the resin (**6a**) and heated at 50 °C for 30 min to get **7**. The resin was washed with DMF
- 25 (4 x 5mL) and DCM (3 x 5mL).

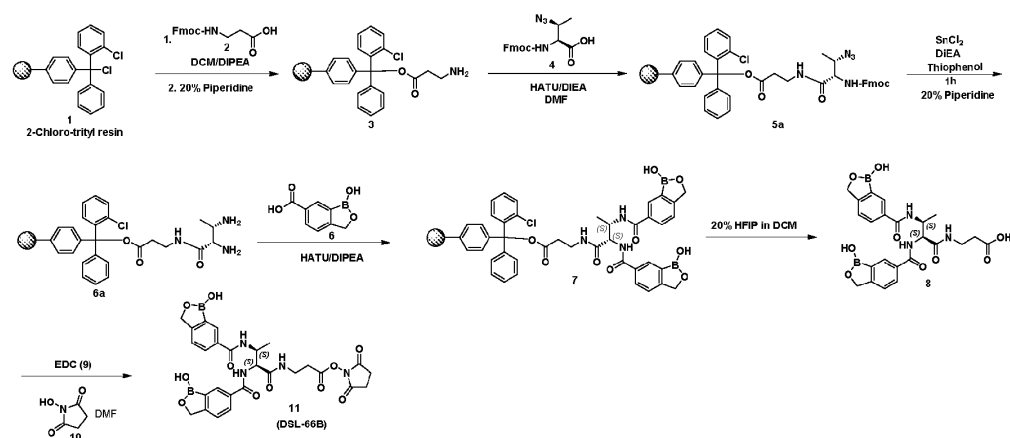
Cleavage of diboronated sensor from resin

- Compound **7** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield
- 30 diboronated sensor acid **8** (**DSL-66A**) which was used in the next step without purification. Similar procedures were followed for the synthesis of **DSL-14A**, **DSL-15A**, **DSL-68A**.

NHS-activation of diboronated sensor:

Diboronated sensor acid **8** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and then was gently mixed overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and dried under vacuum, dissolved in DMSO (100uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes gave the desired product as analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5-dioxypyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate **11** (DSL-66B) in 29% yield (17.4mg). Calculated mass (M+H)⁺ = 607.2, Observed mass (M+H)⁺ = 606.9

Similar procedures were followed for the synthesis of **DSL-14B**, **DSL-15B**, and **DSL-68B**.

Method 2**DSL Synthesis Method 3:****On Resin synthesis of diboronated sensor:**

2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of (S)-3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(tert-butoxy)butanoic acid **40** (119mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a

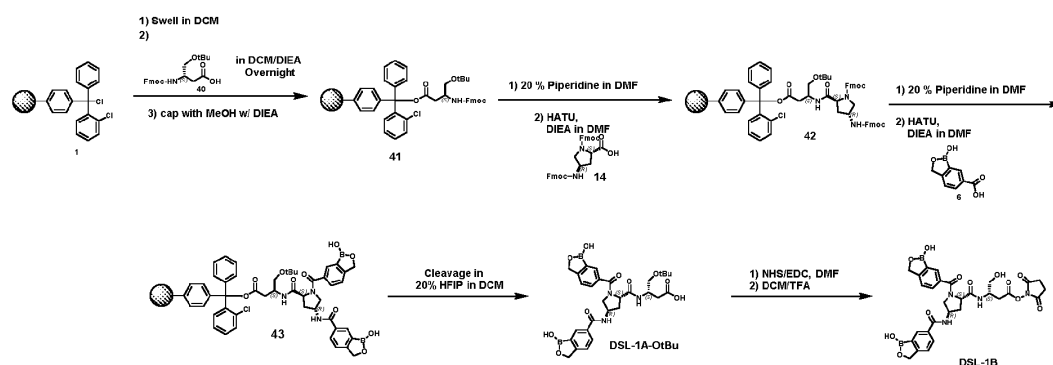
solution of DCM and DIPEA (1M) and mixed for 1hr to yield **41**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (2*S*,4*R*)-1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-4-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-2-carboxylic acid, **14** (0.229g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **42**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3*H*-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **43**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

Cleavage of diboronated sensor from resin

Compound **43** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-1A-OtBu** which was used in the next step without purification.

NHS-activation of diboronated sensor:

Crude diboronated sensor acid **DSL-1A-OtBu** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum. The tert-butyl protecting group was removed with TFA in DCM (50%), allowed to mix for 1 hour, reduced under vacuum then dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give **DSL-1B** as a white powder (25 mg).



The MS data for DSL-1B is listed in Table II. The other examples listed in Table II below were synthesized under similar conditions.

5

Table II

DSL-B Examples	Mass Calculated $[M+H]^+$	Mass Observed $[M+H]^+$
DSL-1B	648.20	
DSL-2B	663.19	718.93 (with tBu group)
DSL-70B	677.21	733.19 (with tBu group)

DSL Synthesis Method 4:

On Resin synthesis of diboronated sensor:

2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of (S)-3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino-4-(tert-butoxy)butanoic acid **40** (119mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **44**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (2S,4R)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-2-carboxylic acid, **14** (0.229g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **45**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **43**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

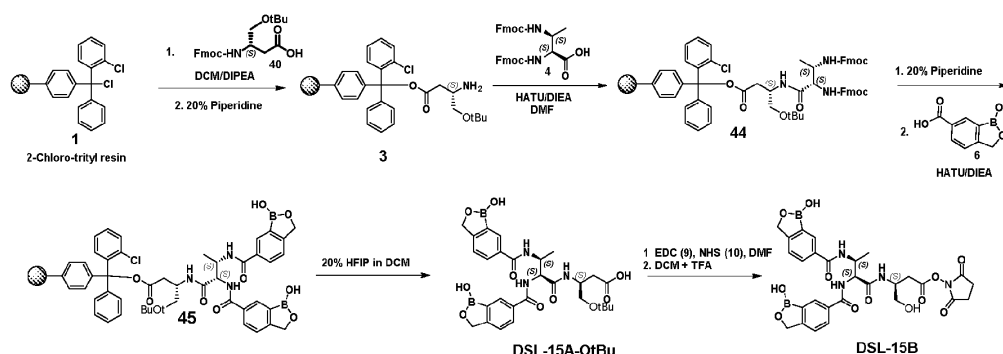
Cleavage of diboronated sensor from resin

Compound **45** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-15A-OtBu** which was used in the next step without purification.

NHS-activation of diboronated sensor:

Crude diboronated sensor acid **DSL-15A-OtBu** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum. The tert-butyl protecting group was removed with TFA in DCM (50%), allowed to mix for 1 hour, reduced under vacuum then dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give **DSL-15B** as a white powder (21 mg).

Method 1



The MS data for DSL-15B is listed in Table III. The other examples listed in Table III below were synthesized under similar conditions.

Table III

DSL-B Examples	Mass Calculated [M+H] ⁺	Mass Observed [M+H] ⁺
DSL-15B	636.20	
DSL-51B	801.18	856.45 (with tBu group)
DSL-75B	665.21	734.75 (with tBu group)

DSL Synthesis Method 5:**On Resin synthesis of diboronated sensor:**

- 2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins.
- 5 Solvent was removed with nitrogen and a solution of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-methylhexanoic acid **12** (110mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **13**. The resin was washed with DCM (3 x 10 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (2*S*,4*R*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-2-carboxylic acid, **14** (0.229g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **15**. Resin 15 was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3*H*-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **16**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

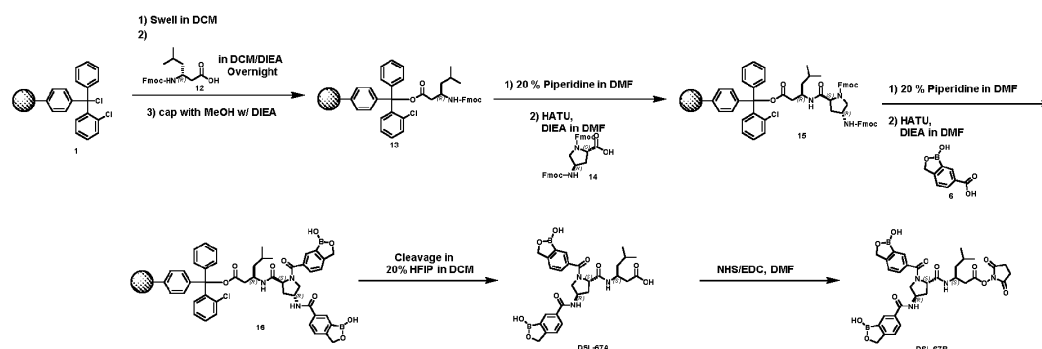
Cleavage of diboronated sensor from resin

- 20 Compound **16** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-67A** which was used in the next step without purification.

5b: NHS-activation of diboronated sensor:

- Crude diboronated sensor acid **DSL-67A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted 30 with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5- 35 dioxopyrrolidin-1-yl (*S*)-3-((2*S*,4*R*)-1-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-

carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-5-methylhexanoate **DSL-67B** in ~28% yield (18.7mg). Calculated mass (M+H)⁺ = 675.2, Observed mass (M+H)⁺ = 675.0.



- 5 The MS data for DSL-67B is listed in Table IV. The other examples listed in Table IV below were synthesized under similar conditions.

Table IV

DSL-B Examples	Mass Calculated [M+H] ⁺	Mass Observed [M+H] ⁺
DSL-19B	619.20	
DSL-37B	673.25	672.99
DSL-47B	727.24	727.88
DSL-48B	661.25	661.12
DSL-58B	739.26	738.95
DSL-65B	799.20	798.83
DSL-67B	675.27	675.07
DSL-105B	709.25	709.03
DSL-107B	723.27	722.93
DSL-111B	695.24	695.09

DSL Synthesis Method 6:

- 10 2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of 2-(1-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)cyclopentyl)acetic acid **17** (109mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **18**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (2*S*,4*S*)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-2-carboxylic acid, **19** (0.229g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **20**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x

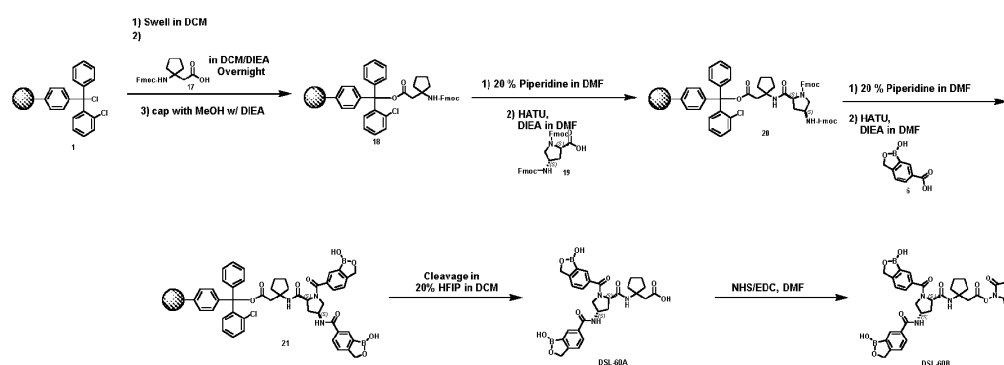
5mL). Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **21**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

Cleavage of diboronated sensor from resin

Compound **21** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid (**DSL-60A**) which was used in the next step without purification.

Crude diboronated sensor acid **DSL-60A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5-dioxypyrrolidin-1-yl 2-(1-((2*S*,4*S*)-1-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclopentyl)acetate **DSL-60B** in ~22% yield (14.7mg). Calculated mass (M+H)⁺ = 673.2, Observed mass (M+H)⁺ = 673.0

Similar procedures were followed for the synthesis of **DSL-61B** to **DSL-77B**, and **DSL-108B**.



25 The MS data for DSL-60B is listed in Table V. The other examples listed in Table V below were synthesized under similar conditions.

Table V

DSL-B Examples	Mass Calculated [M+H] ⁺	Mass Observed [M+H] ⁺
DSL-60B	672.24	
DSL-77B	619.20	619.11
DSL-108B	655.19	

DSL Synthesis Method 7:

2-Chlorotriethyl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of 3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (94mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of *N*²-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*⁶-((allyloxy)carbonyl)-*L*-lysine **22** (0.181g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **23** followed by treatment with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of (2*S*,4*R*)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-2-carboxylic acid, **14** (0.229g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **24**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **25**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL) and treated with Tetrakis(triphenylphosphine)palladium (20 mol%, 46mg) and phenylsilane (493ul, 4mmol) in 6mL DCM was agitated at room temperature for 1h and washed with DCM (3 x 5mL) and DMF (3 x 5mL). Solution of octanoic acid **26** (63ul, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **27**.

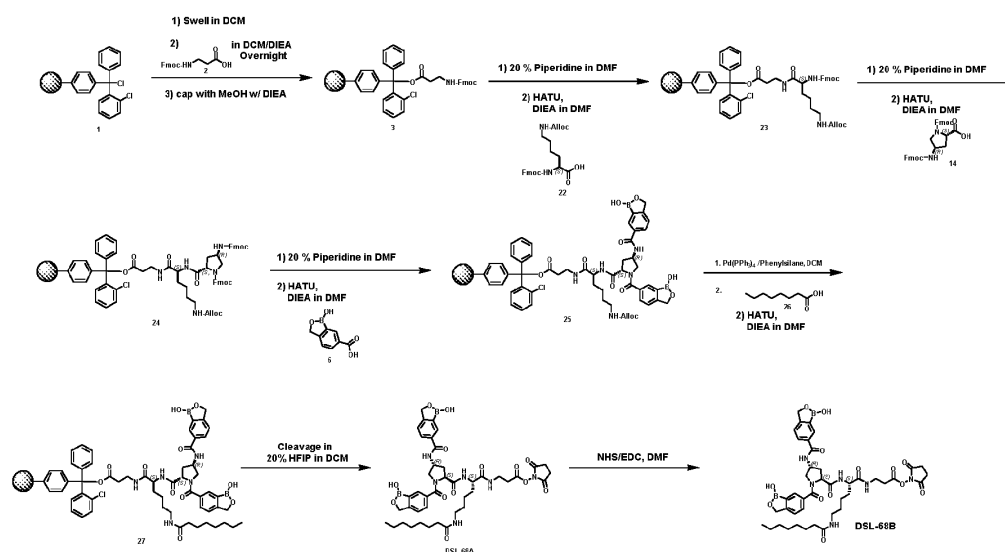
Cleavage of diboronated sensor from resin

Compound **27** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-68A** which was used in the next step without purification.

7b: NHS-activation of diboronated sensor:

Crude diboronated sensor acid **DSL-68A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5-dioxopyrrolidin-1-yl 3-((*S*)-2-((2*R*,4*S*)-1-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-6-octanamido)hexanamido)propanoate **DSL-68B** in ~30% yield (26.1mg).

Calculated mass (M+H)⁺ = 873.4, Observed mass (M+H)⁺ = 873.2

**DSL Synthesis Method 8:****20 Synthesis of (*R*)-3,4-bis(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)butanoic acid**

In 100mL round bottom flask (3*R*)-3-[(tert-butoxy)carbonyl]amino}-4-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butanoic acid (1g, 2.27mmol) was treated with 1:1 TFA:DCM (15mL) and stirred at room temperature for 1h. After completion of the reaction, solvent was

removed under reduced pressure and further co-evaporated with DCM (15mL x 3). The oily product was dissolved in 5:1 10% sodium carbonate and THF (50mL) followed by the addition of Fmoc-OSu (0.76g, 2.27mmol) in THF (10mL) and stirred for 15h. The reaction mixture was diluted with ethyl acetate (35mL) and acidified with 1N HCl to pH ~3. The organic layer was separated and aqueous layer was extracted with ethyl acetate (2 x 15mL) washed with brine, dried over anhydrous sodium sulphate and evaporated under reduced pressure to get (R)-3,4-bis(((9H-fluoren-9-yl)methoxy)carbonyl)amino)butanoic acid (1.2g, 95%).

Synthesis of 2,5-dioxopyrrolidin-1-yl (R)-3-(3,4-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate

- 2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of 3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (94mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIPEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (R)-3,4-bis(((9H-fluoren-9-yl)methoxy)carbonyl)amino)butanoic acid **30** (0.224g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to obtain **31**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and heated at 50 °C for 30 min to get **32**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

25 Cleavage of diboronated sensor from resin

Compound **32** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-104A** which was used in the next step without purification.

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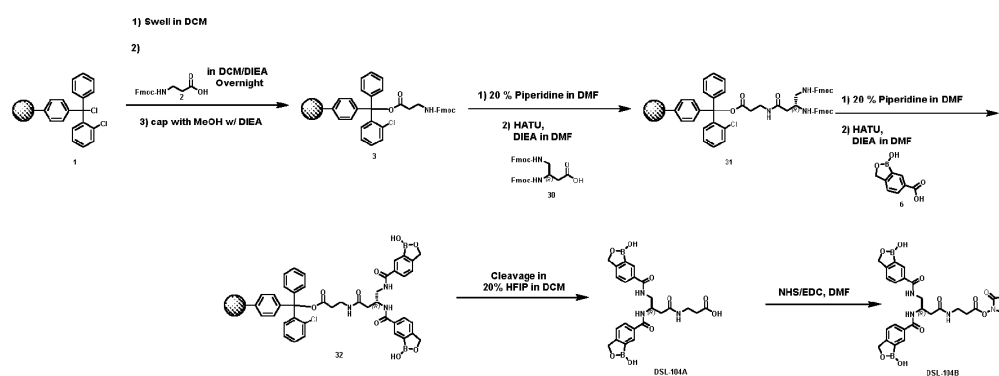
8b: NHS-activation of diboronated sensor:

- Crude diboronated sensor acid **DSL-104A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted

35

with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give

5 **DSL-104B** 2,5-dioxopyrrolidin-1-yl (*R*)-3-(3,4-bis(1-hydroxy-1,3-dihydrobenzo[*c*] [1,2] oxaborole-6-carboxamido)butanamido)propanoate ~22% yield (13mg). Calculated mass (M+H)⁺ = 607.2, Observed mass (M+H)⁺ = 607.1



10

DSL Synthesis Method 9:

Synthesis of 2,5-dioxopyrrolidin-1-yl (*R*)-3-(4,5-bis(1-hydroxy-1,3-dihydrobenzo[*c*] [1,2] oxaborole-6-carboxamido)pentanamido)propanoate

15 2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5mL) for 30 mins. Solvent was removed with nitrogen and a solution of 3-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (94mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol) was added immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and

20 DIPEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5mL) then DMF (3 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL), washed with DMF (4 x 5mL). Solution of (*R*)-4,5-bis(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pentanoic acid **33** (0.224g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) in DMF (5 mL) was added to the resin and heated at 50 °C for 30 min, washed with DMF (4 x 5mL) to

25 obtain **34**. Resin was treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL). Solution of 1-hydroxy-3*H*-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) were added to the resin and

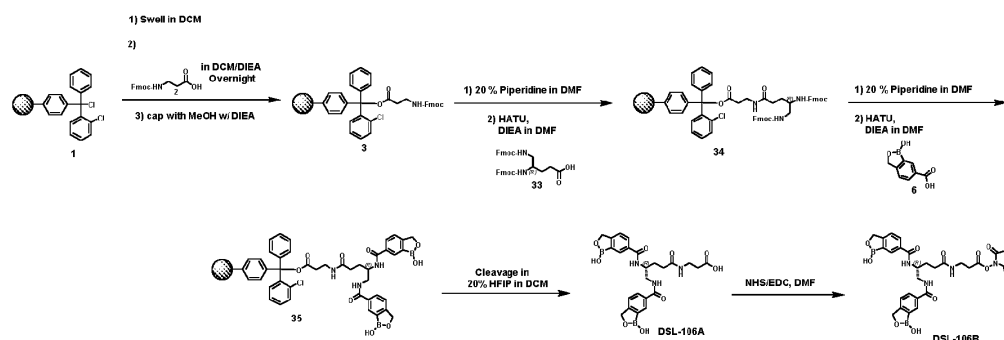
heated at 50 °C for 30 min to get **35**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

Cleavage of diboronated sensor from resin

Compound **35** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-106A** which was used in the next step without purification.

9b: NHS-activation of diboronated sensor:

Crude diboronated sensor acid **DSL-106A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and gently mixed for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum, dissolved in DMSO (100 uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes and analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give **DSL-106B** 2,5-dioxopyrrolidin-1-yl (*R*)-3-(4,5-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)pentanamido)propanoate ~30% yield (18.6mg). Calculated mass (M+H)⁺ = 621.2, Observed mass (M+H)⁺ = 621.1



25 DSL Synthesis Method 10:

2-Chlorotrityl resin **1** (300 mg, 0.3 mmol) was swelled in dry DCM (5 mL) for 30 mins. Solvent was removed with nitrogen and a solution of 3-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid **2** (0.93mg, 0.3mmol) in DCM with DIPEA (0.3mL, 1.7mmol)) was added

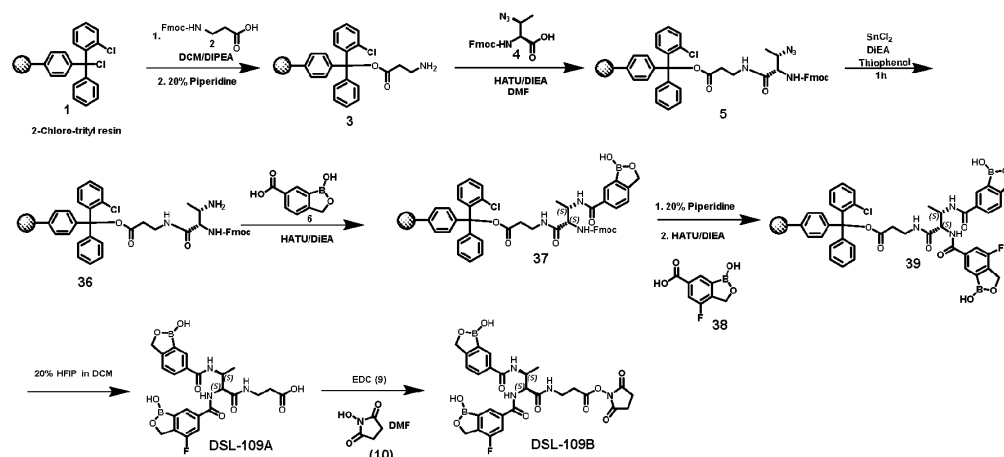
immediately and gently mixed for overnight. The mixture was washed with DCM and unreacted sites were capped with a solution of 20% MeOH in a solution of DCM and DIEA (1M) and mixed for 1hr to yield **3**. The resin was washed with DCM (3 x 5 mL) then DMF (3 x 5 mL). The resin was treated 20% piperidine with DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5 mL). Solution of (2S,3S)-(Fmoc-amino)-3-azidobutyric acid **4** (0.146g, 0.4mmol), DIPEA (0.14mL, 0.8mmol) and HATU (0.152g, 0.4mmol) was added to the resin and heated at 50 °C for 30 min to give **5**. Resin was washed with DMF (4 x 5mL) and THF (3 x 5mL) and treated with 5mL solution of 0.2M SnCl₂ (189mg), 0.8M thiophenol (440ul) and 1M DIEA (870ul) in THF and gently mix it for 1hr on shaker. After 1h, washed the resin with THF (3 x 5mL) and DMF (3 x 5mL) to give **36**. Solution of 1-hydroxy-3H-2,1-benzoxaborole-6-carboxylic acid **6** (0.178g, 1mmol), HATU (0.380g, 1mmol) and DIPEA (0.35mL, 2mmol) was added to the resin (6a) and heated at 50 °C for 30 min to get **37**. Resin was washed with DMF (5 x 5mL) and treated with 20% piperidine in DMF for 5 minutes (3 x 5 mL) and washed with DMF (4 x 5mL) followed by coupling with **38** to give **39**. The resin was washed with DMF (4 x 5mL) and DCM (3 x 5mL).

Cleavage of diboronated sensor from resin

Compound **39** on resin was treated with 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in DCM (7mL) and gently mixed for 1hr. The resin was filtered, and filtrate was evaporated under reduced pressure. Residue was further suspended with DCM (2 x 5mL) and evaporated to yield diboronated sensor acid **DSL-109A** which was used in the next step without purification.

10b: NHS-activation of diboronated sensor:

Diboronated sensor acid **DSL-109A** was dissolved in DMF (2mL) and treated with 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride **9** (EDC) (0.096g, 0.5mmol), N-hydroxysuccinimide **10** (NHS) (0.115g, 1mmol), and then was gently mixed overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, reaction mixture was diluted with ethyl acetate (20mL) and washed with 100mmol HCl (10mL). The organic layer was dried over anhydrous Na₂SO₄ and dried under vacuum, dissolved in DMSO (100uL) and fractionated by reverse-phase (RP) flash chromatography on a C18 column with a gradient of 20% ACN in water with 0.1% TFA to 60% ACN in water with 0.1% TFA over 10 minutes gave the desired product as analyzed by LCMS. Pure fractions were isolated, combined, frozen, and lyophilized to give 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate **DSL-109B** in 20% yield (12.5mg). Calculated mass (M+H)⁺ = 625.1, Observed mass (M+H)⁺ = 625.1



The MS data for DSL-109B is listed in Table VI. The example listed in Table VI below was synthesized under similar conditions.

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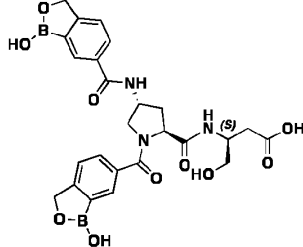
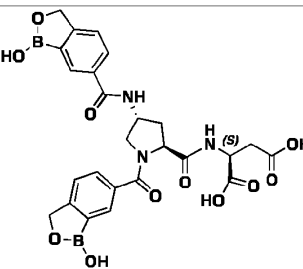
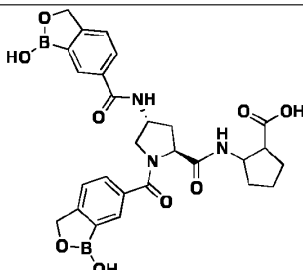
Table VI

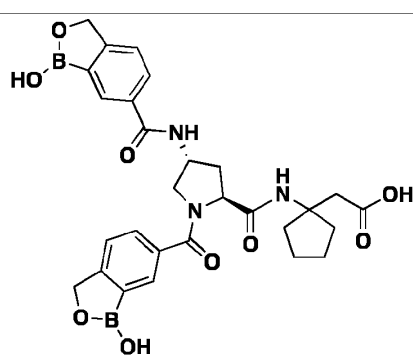
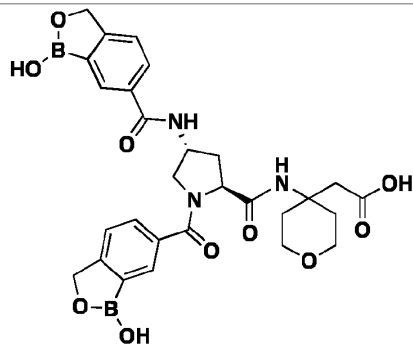
DSL-B Examples	Mass Calculated [M+H] ⁺	Mass Observed [M+H] ⁺
DSL-109B	624.18	
DSL-110B	675.19	675.07

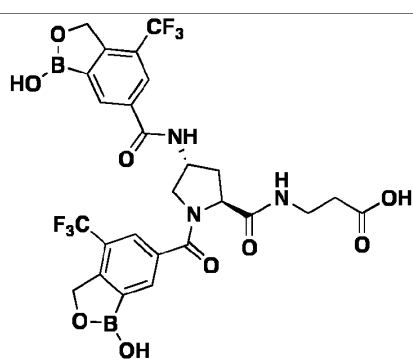
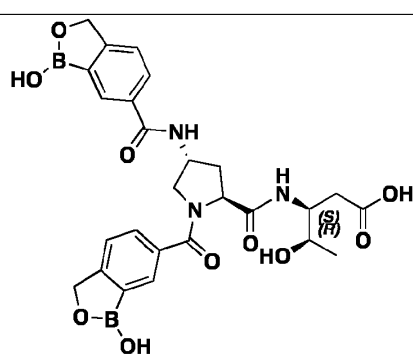
The chemical structure and IUPAC name of **DSL-1A** to **DSL-112A** are summarized in Table 1 below.

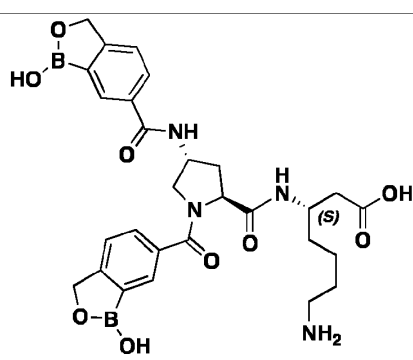
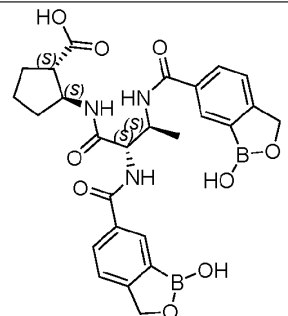
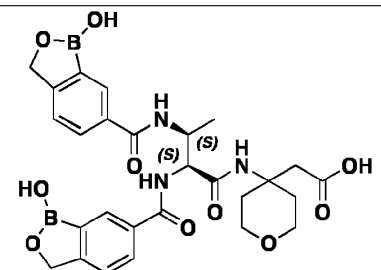
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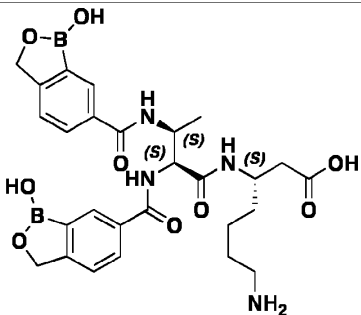
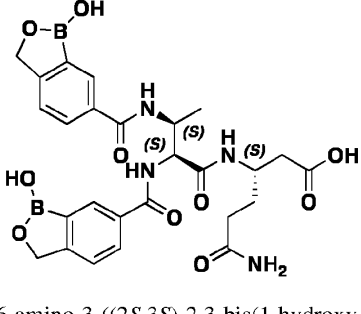
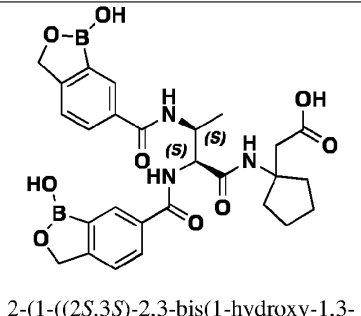
Table 1

Compound #	FF	FL	F	Structure/IUPAC
DSL-1A	FF12A	FL38	F2	 (S)-4-hydroxy-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)butanoic acid
DSL-2A	FF12A	FL5B	F2	 ((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-aspartic acid
DSL-3A	FF12A	FL22	F2	 2-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclopentane-1-carboxylic acid

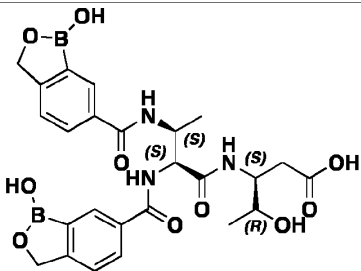
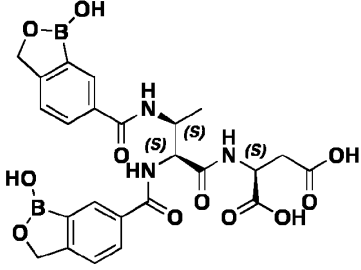
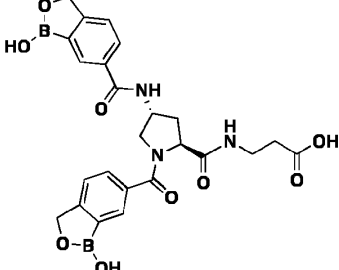
Compound #	FF	FL	F	Structure/IUPAC
DSL-4A	FF12A	FL20	F2	 2-(1-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclopentyl)acetic acid
DSL-5A	FF12A	FL24	F2	 2-(4-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)tetrahydro-2<i>H</i>-pyran-4-yl)acetic acid

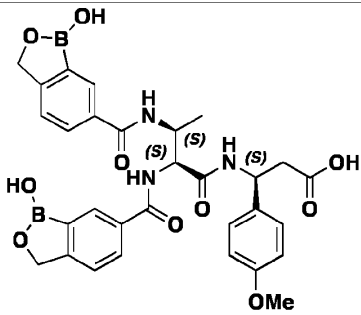
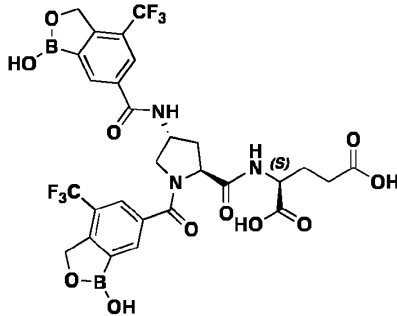
Compound #	FF	FL	F	Structure/IUPAC
DSL-6A	FF12A	FL3	F2	 3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-7A	FF12A	FL39	F2	 (3<i>S</i>,4<i>R</i>)-4-hydroxy-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)pentanoic acid

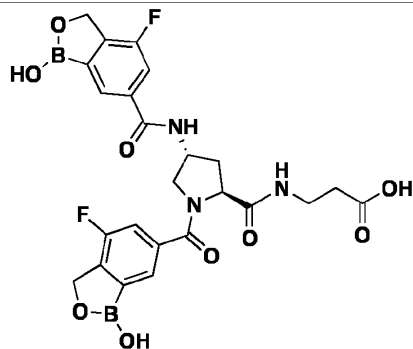
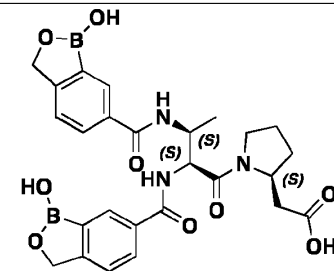
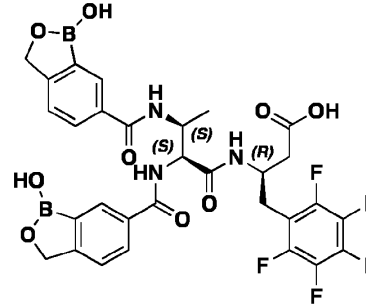
Compound #	FF	FL	F	Structure/IUPAC
DSL-8A	FF12A	FL23	F2	 (S)-7-amino-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)heptanoic acid
DSL-9A	FF116A	FL21	F2	 (1S,2S)-2-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)cyclopentane-1-carboxylic acid
DSL-10A	FF116A	FL24	F2	 2-(4-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)tetrahydro-2H-pyran-4-yl)acetic acid

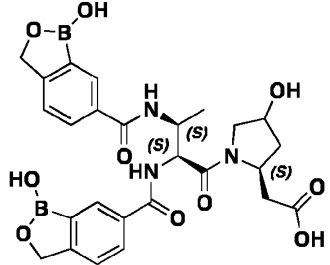
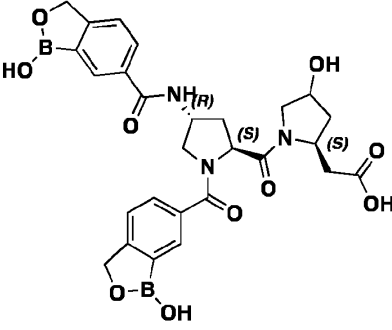
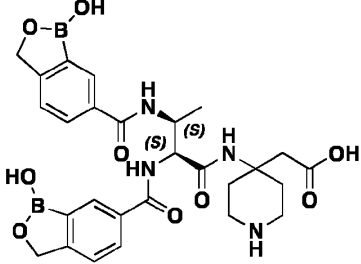
Compound #	FF	FL	F	Structure/IUPAC
DSL-11A	FF116A	FL23	F2	 (S)-7-amino-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)heptanoic acid
DSL-12A	FF116A	FL36	F2	 (S)-6-amino-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-6-oxohexanoic acid
DSL-13A	FF116A	FL20	F2	 2-(1-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)cyclopentyl)acetic acid

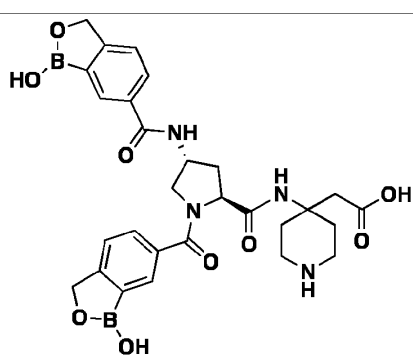
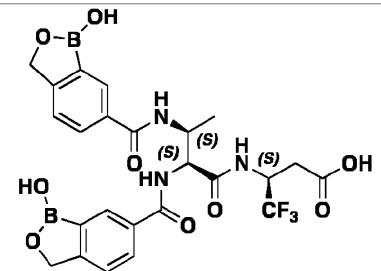
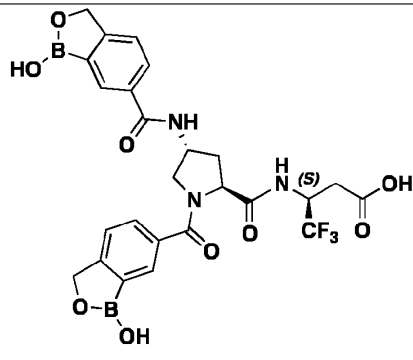
Compound #	FF	FL	F	Structure/IUPAC
DSL-14A	FF116A	FL3	F2	3-((2<i>S</i>,3<i>S</i>)-2,3-bis(4-fluoro-1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid
DSL-15A	FF116A	FL38	F2	(<i>S</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4-hydroxybutanoic acid
DSL-16A	FF116A	FL5B	F2	((2<i>S</i>,3<i>S</i>)-2-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)-3-(1-hydroxy-4-(trifluoromethyl)-2,3-dihydro-1<i>H</i>-benzo[<i>b</i>]borole-6-carboxamido)butanoyl)-<i>L</i>-aspartic acid

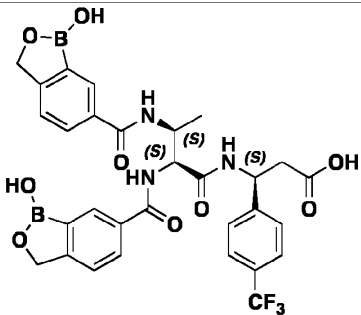
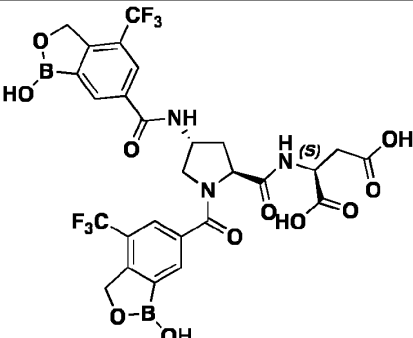
Compound #	FF	FL	F	Structure/IUPAC
DSL-17A	FF116A	FL39	F2	 (3<i>S</i>,4<i>R</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4-hydroxypentanoic acid
DSL-18A	FF116A	FL5B	F2	 ((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanoyl)-<i>L</i>-aspartic acid
DSL-19A	FF12A	FL3	F2	 3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid

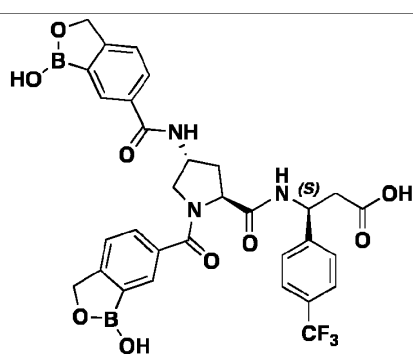
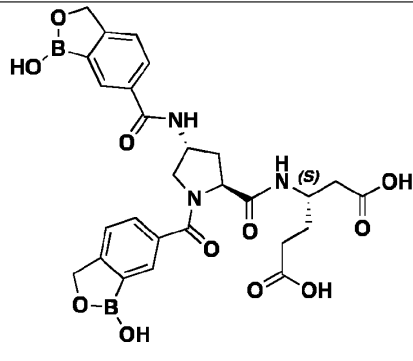
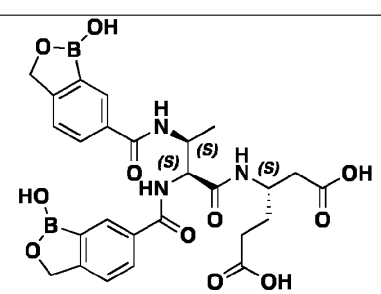
Compound #	FF	FL	F	Structure/IUPAC
DSL-20A	FF116A	FL35	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(4-methoxyphenyl)propanoic acid
DSL-21A	FF12A	FL5B	F2	 ((2S,4R)-1-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-glutamic acid

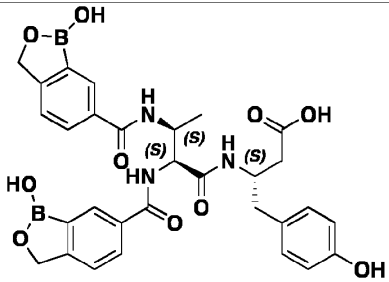
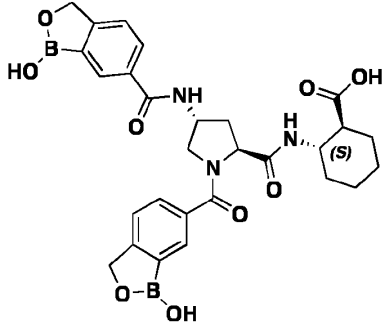
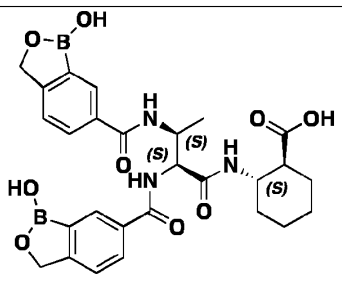
Compound #	FF	FL	F	Structure/IUPAC
DSL-22A	FF12A	FL3	F2	 3-((2<i>S</i>,4<i>R</i>)-1-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-23A	FF116A	FL28	F2	 2-((<i>S</i>)-1-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanoyl)pyrrolidin-2-yl)acetic acid
DSL-24A	FF116A	FL41	F2	 (<i>R</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4-(perfluorophenyl)butanoic acid

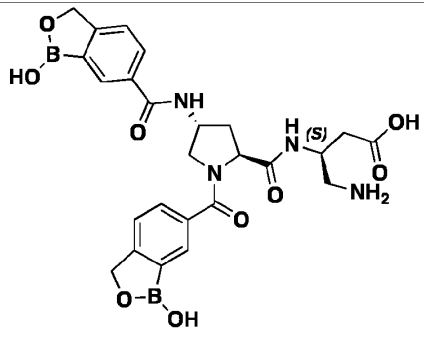
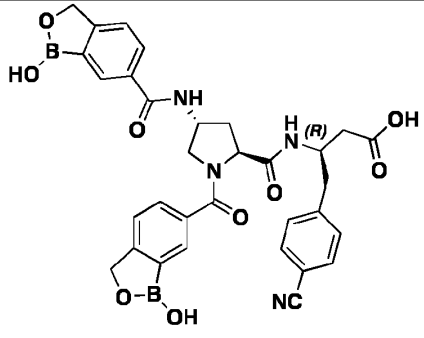
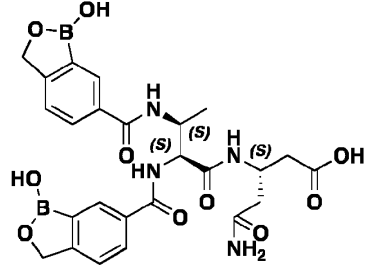
Compound #	FF	FL	F	Structure/IUPAC
DSL-25A	FF116A	FL42	F2	 2-((2<i>S</i>)-1-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanoyl)-4-hydroxypyrrolidin-2-yl)acetic acid
DSL-26A	FF12A	FL42	F2	 2-((2<i>S</i>)-4-hydroxy-1-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)pyrrolidin-4-yl)acetic acid
DSL-27A	FF116A	FL34	F2	 2-(4-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)piperidin-4-yl)acetic acid

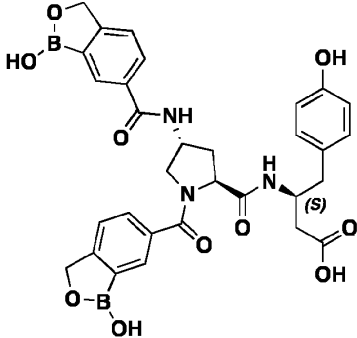
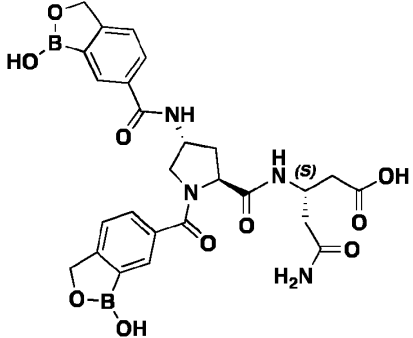
Compound #	FF	FL	F	Structure/IUPAC
DSL-28A	FF12A	FL34	F2	 2-(4-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)piperidin-4-yl)acetic acid
DSL-29A	FF116A	FL27	F2	 (<i>S</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4,4,4-trifluorobutanoic acid
DSL-30A	FF12A	FL27	F2	 (<i>S</i>)-4,4,4-trifluoro-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)butanoic acid

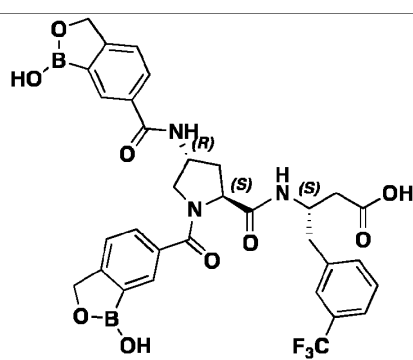
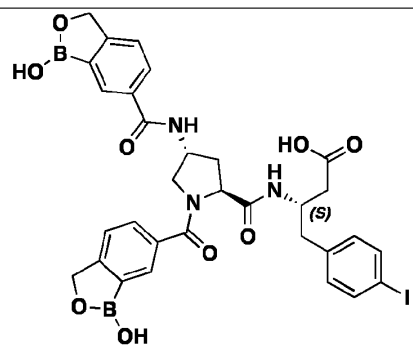
Compound #	FF	FL	F	Structure/IUPAC
DSL-31A	FF116A	FL32	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(4-(trifluoromethyl)phenyl)propanoic acid
DSL-32A	FF12A	FL5B	F2	 ((2S,4R)-1-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-aspartic acid

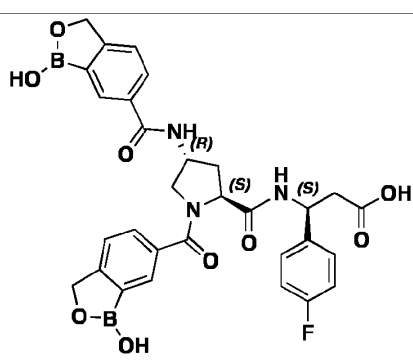
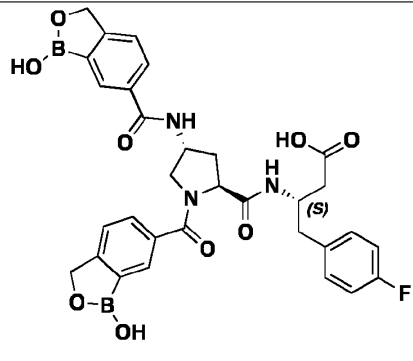
Compound #	FF	FL	F	Structure/IUPAC
DSL-33A	FF12A	FL32	F2	 (<i>S</i>)-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanoic acid
DSL-34A	FF12A	FL29	F2	 (<i>S</i>)-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)hexanedioic acid
DSL-35A	FF116A	FL29	F2	 (<i>S</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)hexanedioic acid

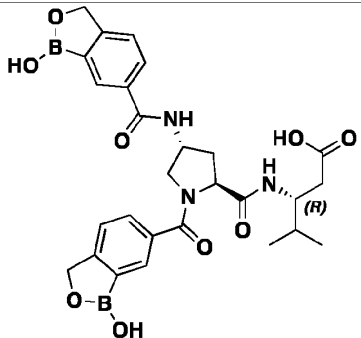
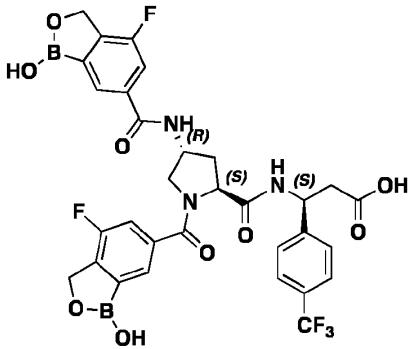
Compound #	FF	FL	F	Structure/IUPAC
DSL-36A	FF116A	FL37	F2	 (<i>S</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4-(4-hydroxyphenyl)butanoic acid
DSL-37A	FF12A	FL25	F2	 (1<i>S</i>,2<i>S</i>)-2-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclohexane-1-carboxylic acid
DSL-38A	FF116A	FL25	F2	 (1<i>S</i>,2<i>S</i>)-2-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)cyclohexane-1-carboxylic acid

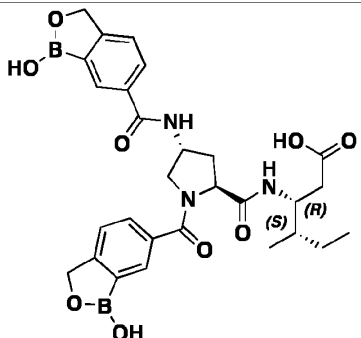
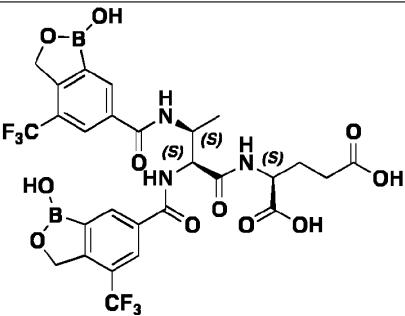
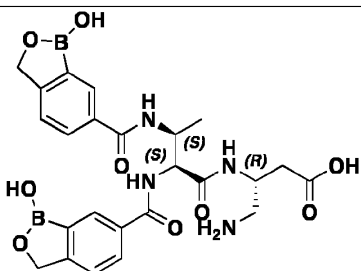
Compound #	FF	FL	F	Structure/IUPAC
DSL-39A	FF12A	FL26	F2	 (S)-4-amino-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)butanoic acid
DSL-40A	FF12A	FL31	F2	 (R)-4-(4-cyanophenyl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)butanoic acid
DSL-41A	FF116A	FL40	F2	 (S)-5-amino-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-5-oxopentanoic acid

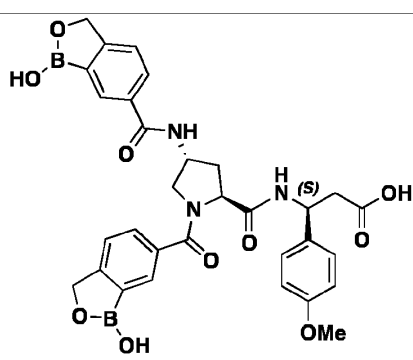
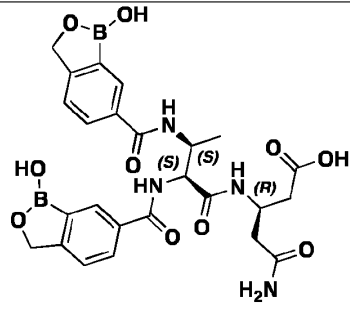
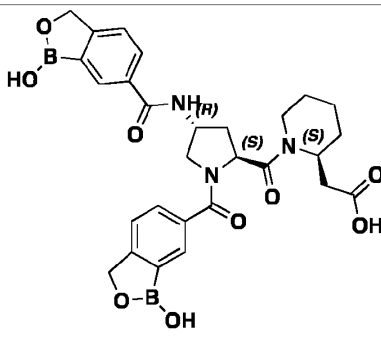
Compound #	FF	FL	F	Structure/IUPAC
DSL-42A	FF12A	FL37	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(4-hydroxyphenyl)butanoic acid
DSL-43A	FF12A	FL40	F2	 (S)-5-amino-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-5-oxopentanoic acid

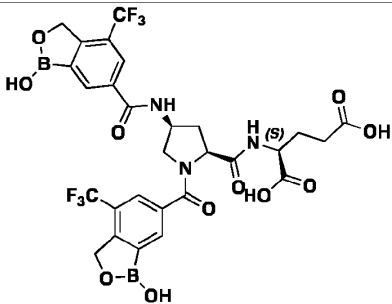
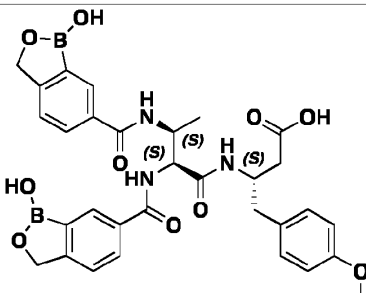
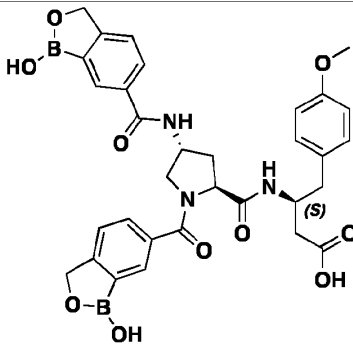
Compound #	FF	FL	F	Structure/IUPAC
DSL-44A	FF12A	FL62	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(3-(trifluoromethyl)phenyl)butanoic acid
DSL-45A	FF12A	FL63	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(4-iodophenyl)butanoic acid

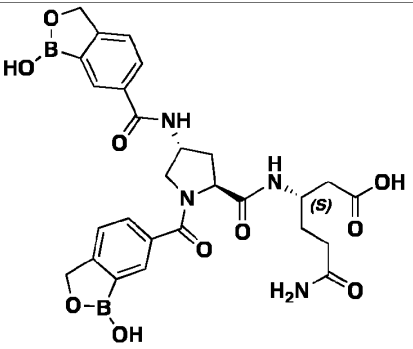
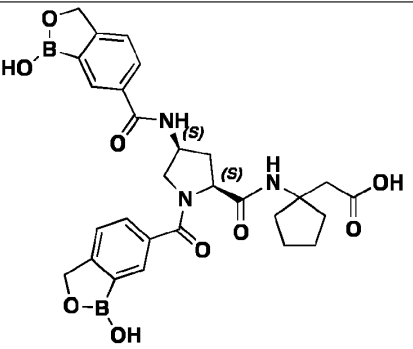
Compound #	FF	FL	F	Structure/IUPAC
DSL-46A	FF12A	FL56	F2	 (S)-3-(4-fluorophenyl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-47A	FF12A	FL60	F2	 (S)-4-(4-fluorophenyl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)butanoic acid

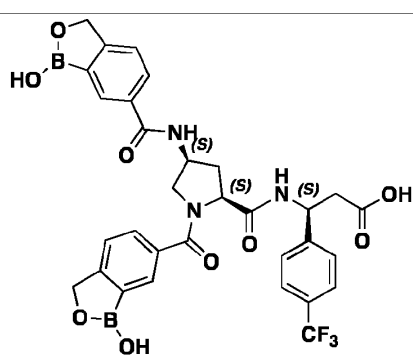
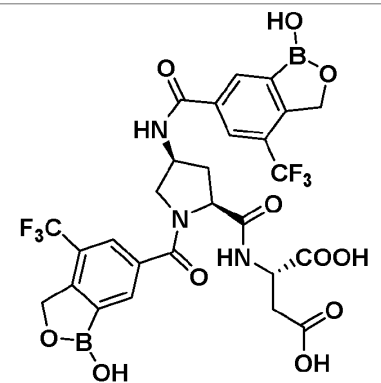
Compound #	FF	FL	F	Structure/IUPAC
DSL-48A	FF12A	FL64	F2	 (R)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-methylpentanoic acid
DSL-49A	FF12A	FL32	F2	 (S)-3-((2S,4R)-1-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanoic acid

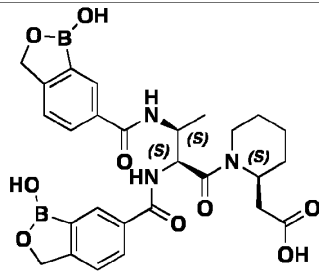
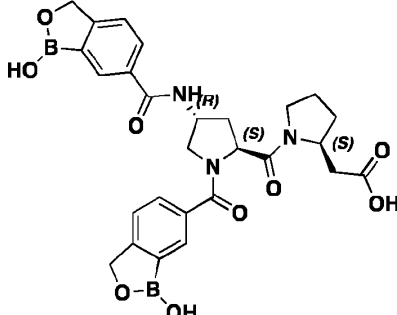
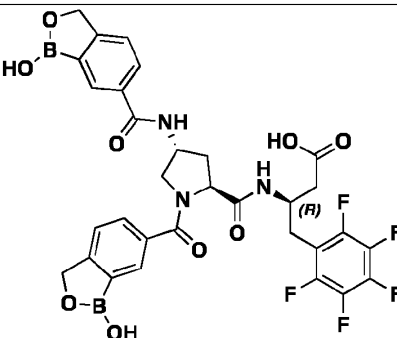
Compound #	FF	FL	F	Structure/IUPAC
DSL-50A	FF12A	FL67	F2	 (3R)-3-(3R,4S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-methylhexanoic acid
DSL-51A	FF116A	FL5B	F2	 ((2S,3S)-2,3-bis(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanoyl)-L-glutamic acid
DSL-52A	FF116A	FL26	F2	 (R)-4-amino-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanoyl)-L-glutamic acid

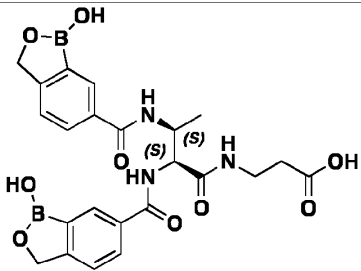
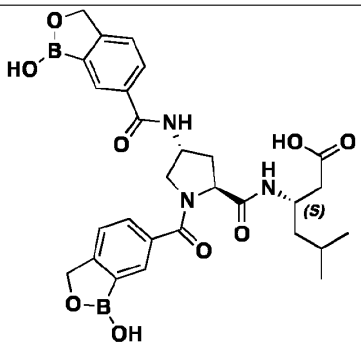
Compound #	FF	FL	F	Structure/IUPAC
DSL-53A	FF12A	FL35	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(4-methoxyphenyl)propanoic acid
DSL-54A	FF116A	FL40	F2	 (R)-5-amino-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamide)-5-oxopentanoic acid
DSL-55A	FF12A	FL30	F2	 2-((S)-1-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)piperidin-2-yl)acetic acid

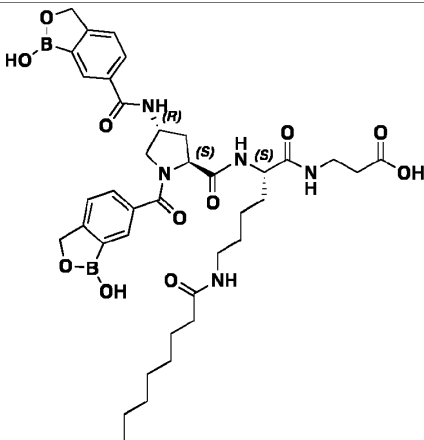
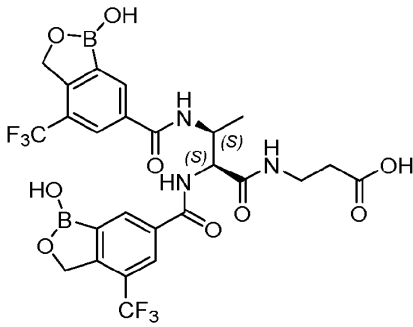
Compound #	FF	FL	F	Structure/IUPAC
DSL-56A	FF12A	FL5B	F2	 ((2<i>S</i>,4<i>S</i>)-1-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-<i>L</i>-glutamic acid
DSL-57A	FF116A	FL33	F2	 (<i>S</i>)-3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)-4-(4-methoxyphenyl)butanoic acid
DSL-58A	FF12A	FL33	F2	 (<i>S</i>)-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(4-methoxyphenyl)butanoic acid

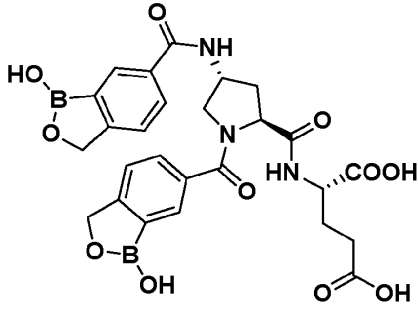
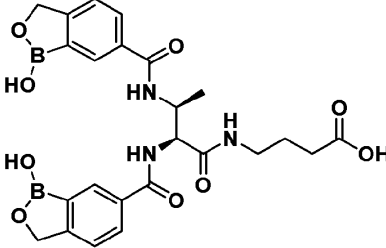
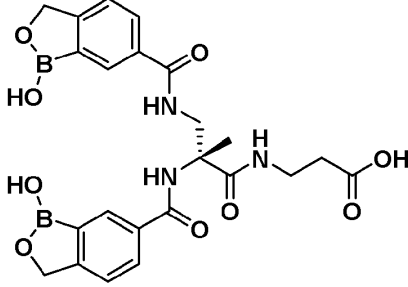
Compound #	FF	FL	F	Structure/IUPAC
DSL-59A	FF12A	FL36	F2	 (S)-6-amino-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-6-oxohexanoic acid
DSL-60A	FF12B	FL20	F2	 2-(1-((2S,4S)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclopentyl)acetic acid

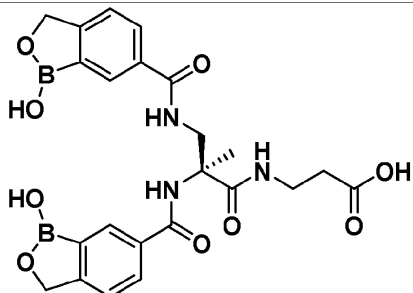
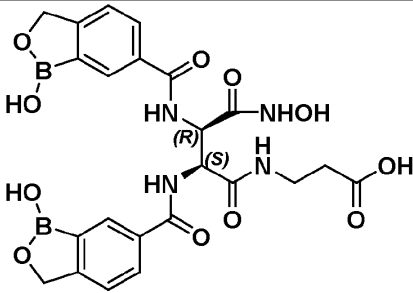
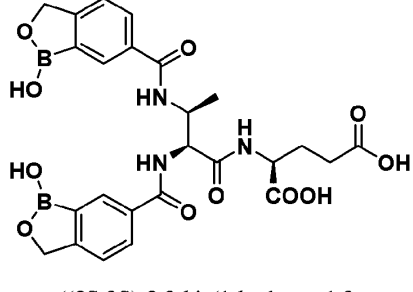
Compound #	FF	FL	F	Structure/IUPAC
DSL-61A	FF12B	FL32	F2	 (S)-3-((2S,4S)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanoic acid
DSL-62A	FF12B	FL5B	F2	 ((2S,4S)-1-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-aspartic acid

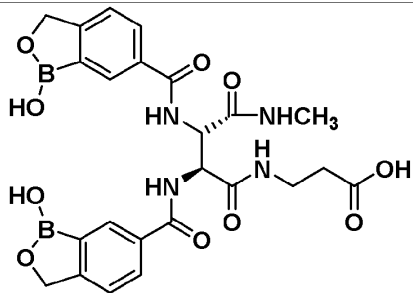
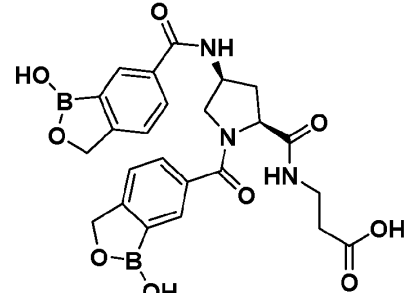
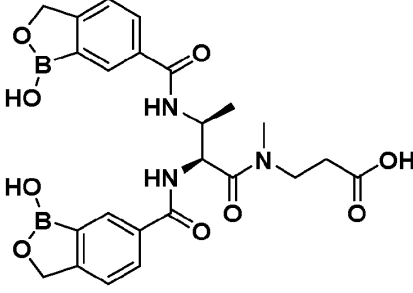
Compound #	FF	FL	F	Structure/IUPAC
DSL-63A	FF116A	FL30	F2	 2-((<i>S</i>)-1-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanoyl)piperidin-2-yl)acetic acid
DSL-64A	FF12A	FL28	F2	 2-((<i>S</i>)-1-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)pyrrolidin-2-yl)acetic acid
DSL-65A	FF12A	FL41	F2	 (<i>R</i>)-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(perfluorophenyl)butanoic acid

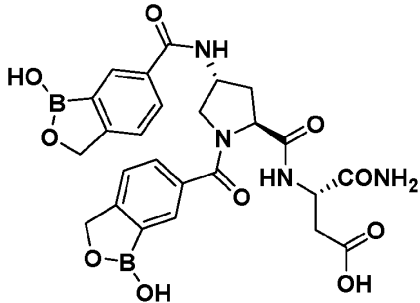
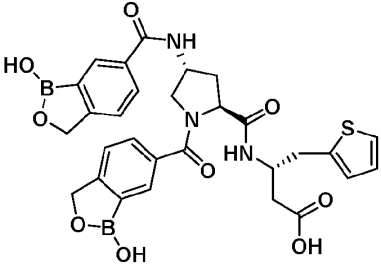
Compound #	FF	FL	F	Structure/IUPAC
DSL-66A	FF116A	FL3	F2	 3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid
DSL-67A	FF12A	FL68	F2	 (<i>S</i>)-3-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-5-methylhexanoic acid

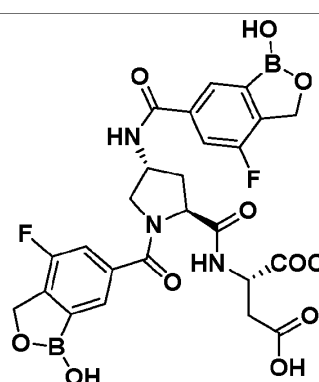
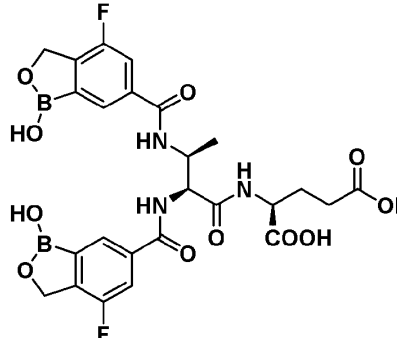
Compound #	FF	FL	F	Structure/IUPAC
DSL-68A	FF12A	FL3, FL69	F2	 3-((<i>S</i>)-2-((2<i>S</i>,4<i>R</i>)-1-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-6-octanamido)hexanamido)propanoic acid
DSL-69A	FF116A	FL3	F2	 3-((2<i>S</i>,3<i>S</i>)-2,3-bis(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[<i>c</i>][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid

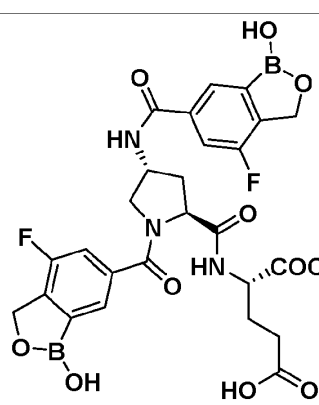
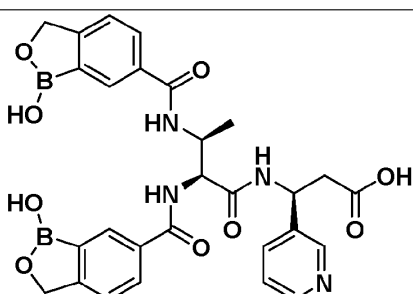
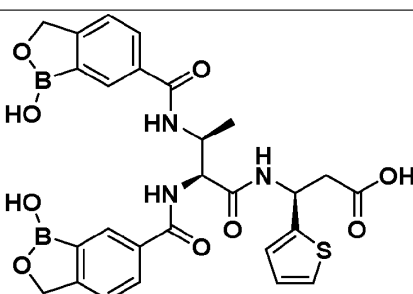
Compound #	FF	FL	F	Structure/IUPAC
DSL-70A	FF12A	FL5B	F2	 ((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-glutamic acid
DSL-71A	FF116A	FL3	F2	 4-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)butanoic acid
DSL-72A	FF225B	FL3	F2	 (R)-3-(2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-2-methylpropanamido)propanoic acid

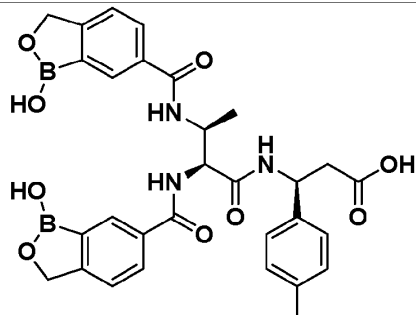
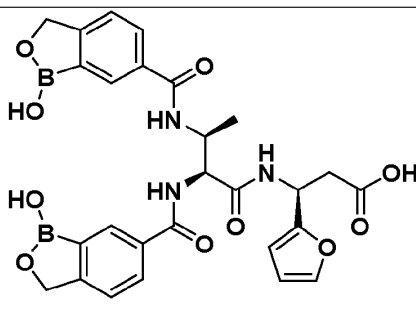
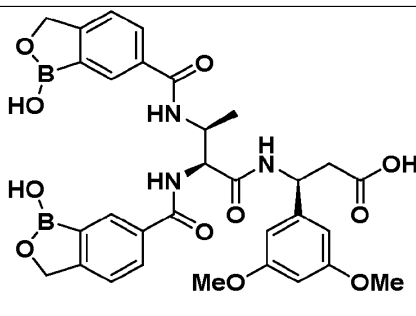
Compound #	FF	FL	F	Structure/IUPAC
DSL-73A	FF225A	FL3	F2	 (S)-3-(2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-2-methylpropanamido)propanoic acid
DSL-74A	FF203	FL3	F2	 3-((2S,3R)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-4-(hydroxyamino)-4-oxobutanamido)propanoic acid
DSL-75A	FF116A	FL5B	F2	 ((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanoyl)-L-glutamic acid

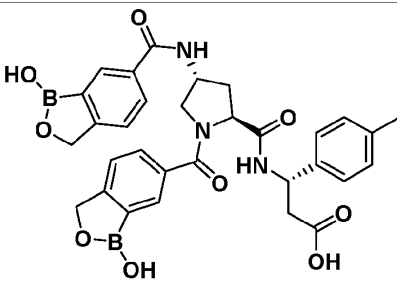
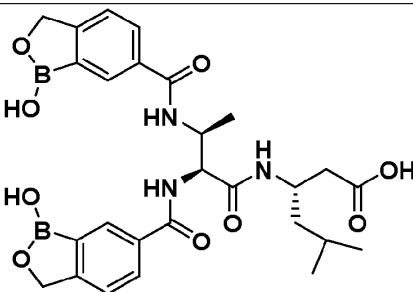
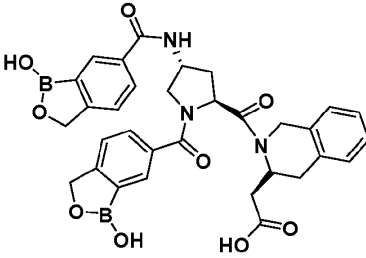
Compound #	FF	FL	F	Structure/IUPAC
DSL-76A	FF193A	FL3	F2	 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-carboxamido)-4-(methylamino)-4-oxobutanamido)propanoic acid
DSL-77A	FF12B	FL3	F2	 3-((2S,4S)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-78A	FF116A	FL75	F2	 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-N-methylbutanamido)propanoic acid

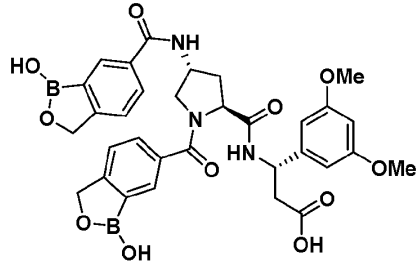
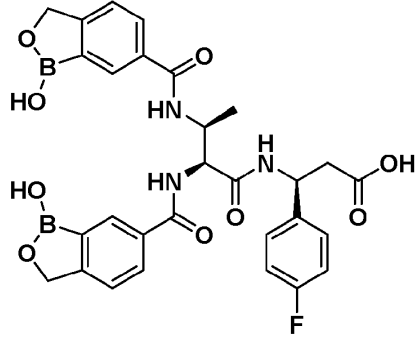
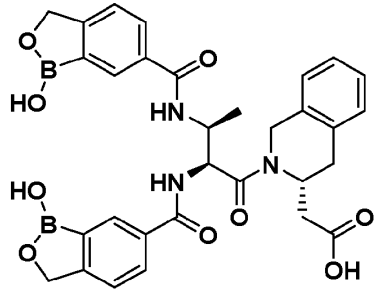
Compound #	FF	FL	F	Structure/IUPAC
DSL-79A	FF12A	FL71	F2	 (S)-4-amino-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-oxobutanoic acid
DSL-80A	FF12A	FL43	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(thiophen-2-yl)butanoic acid

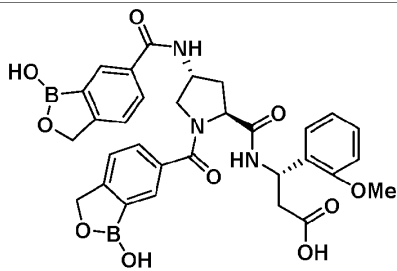
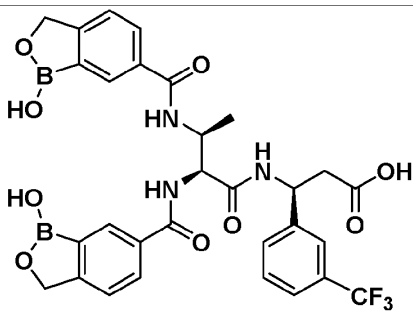
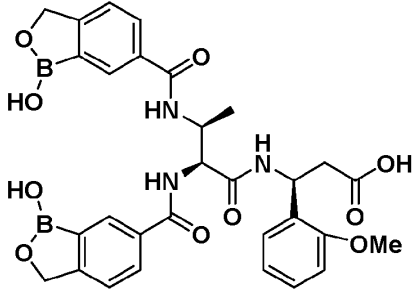
Compound #	FF	FL	F	Structure/IUPAC
DSL-81A	FF12A	FL5B	F2	 ((2S,4R)-1-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-aspartic acid
DSL-82A	FF116A	FL5B	F2	 ((2S,3S)-2,3-bis(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanoyl)-L-glutamic acid

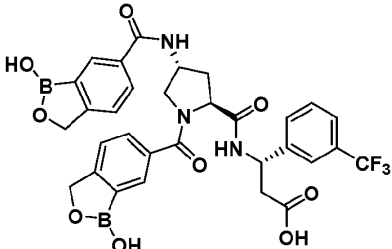
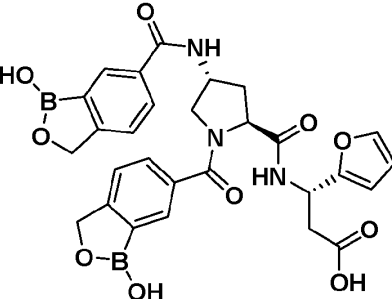
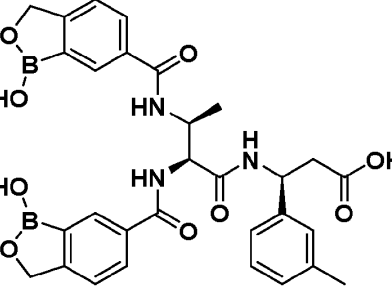
Compound #	FF	FL	F	Structure/IUPAC
DSL-83A	FF12A	FL5B	F2	 ((2S,4R)-1-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-L-glutamic acid
DSL-84A	FF116A	FL44	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(pyridin-3-yl)propanoic acid
DSL-85A	FF116A	FL72	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(thiophen-2-yl)propanoic acid

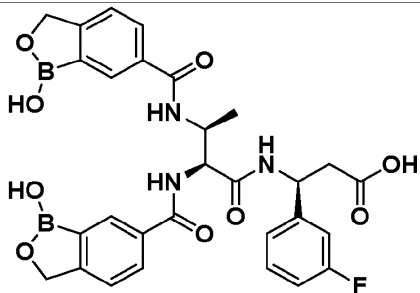
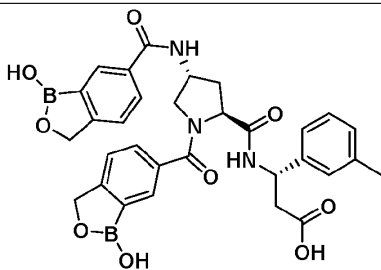
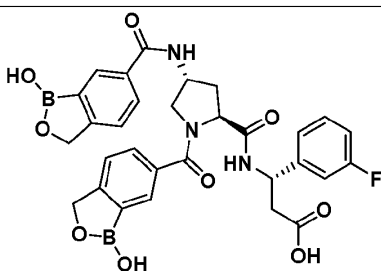
Compound #	FF	FL	F	Structure/IUPAC
DSL-86A	FF116A	FL53	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(p-tolyl)propanoic acid
DSL-87A	FF116A	FL73	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(furan-2-yl)propanoic acid
DSL-88A	FF116A	FL48	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(3,5-dimethoxyphenyl)propanoic acid

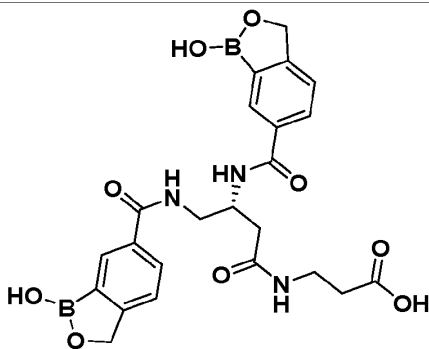
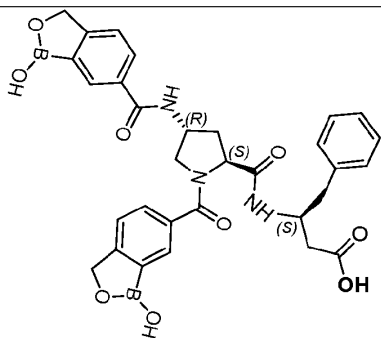
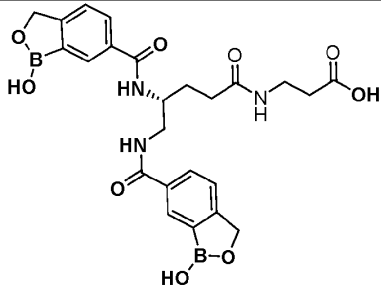
Compound #	FF	FL	F	Structure/IUPAC
DSL-89A	FL12A	FL53	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(p-tolyl)propanoic acid
DSL-90A	FF116A	FL68	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-5-methylhexanoic acid
DSL-91A	FF12A	FL45	F2	 2-((R)-2-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carbonyl)-1,2,3,4-tetrahydroisoquinolin-3-yl)acetic acid

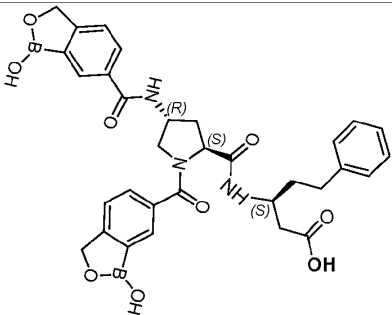
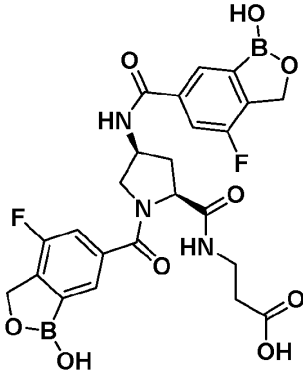
Compound #	FF	FL	F	Structure/IUPAC
DSL-92A	FF12A	FL48	F2	 (S)-3-(3,5-dimethoxyphenyl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-93A	FF116A	FL56	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamide)-3-(4-fluorophenyl)propanoic acid
DSL-94A	FF116A	FL45	F2	 2-((R)-2-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanoyl)-1,2,3,4-tetrahydroisoquinolin-3-yl)acetic acid

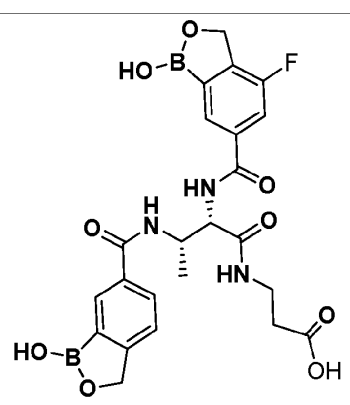
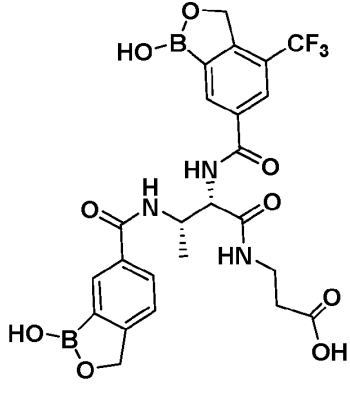
Compound #	FF	FL	F	Structure/IUPAC
DSL-95A	FF12A	FL50	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(2-methoxyphenyl)propanoic acid
DSL-96A	FF116A	FL46	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(3-(trifluoromethyl)phenyl)propanoic acid
DSL-97A	FF116A	FL50	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(2-methoxyphenyl)propanoic acid

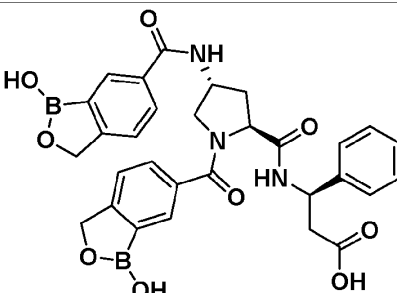
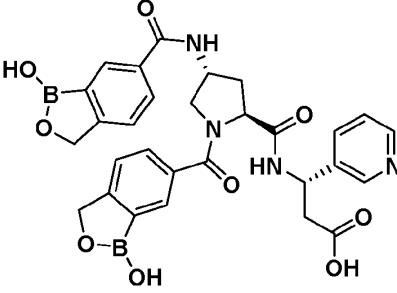
Compound #	FF	FL	F	Structure/IUPAC
DSL-98A	FF12A	FL46	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(3-(trifluoromethyl)phenyl)propanoic acid
DSL-99A	FF12A	FL73	F2	 (S)-3-(furan-2-yl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid
DSL-100A	FF116A	FL55	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(m-tolyl)propanoic acid

Compound #	FF	FL	F	Structure/IUPAC
DSL-101A	FF116A	FL57	F2	 (S)-3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-3-(3-fluorophenyl)propanoic acid
DSL-102A	FF12A	FL55	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(m-tolyl)propanoic acid
DSL-103A	FF12A	FL57	F2	 (S)-3-(3-fluorophenyl)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid

Compound #	FF	FL	F	Structure/IUPAC
DSL-104A	FF115A	FL3	F2	 (R)-3-(3,4-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid
DSL-105A	FF12A	FL74	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-phenylbutanoic acid
DSL-106A	FF114A	FL3	F2	 (R)-3-(4,5-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pentanamido)propanoic acid

Compound #	FF	FL	F	Structure/IUPAC
DSL-107A	FF12A	FL65	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-5-phenylpentanoic acid
DSL-108A	FF12B	FL3	F2	 3-((2S,4S)-1-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)propanoic acid

Compound #	FF	FL	F	Structure/IUPAC
DSL-109A	FF116A	FL3	F2	 3-((2S,3S)-2-(4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid
DSL-110A	FF116A	FL3	F2	 3-((2S,3S)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-2-(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoic acid

Compound #	FF	FL	F	Structure/IUPAC
DSL-111A	FF12A	FL59	F2	 (R)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-phenylpropanoic acid
DSL-112A	FF12A	FL44	F2	 (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-3-(pyridin-3-yl)propanoic acid

Synthesis of Compounds of Formula I

Synthesis of Compounds of Formula I

Illustrative synthesis protocols are provided that can be used to synthesize the examples described.

The lines connecting cysteine residues are disulfide bonds. For the sake of clarity, the H- at the N-terminus of the A- and B-chain of insulin is not histidine, it is the hydrogen of the N-terminus. The -OH shown at the C-terminal end of the A- and B-chain is the C-terminus of the respective chain.

Insulin expression and conjugation Method 1

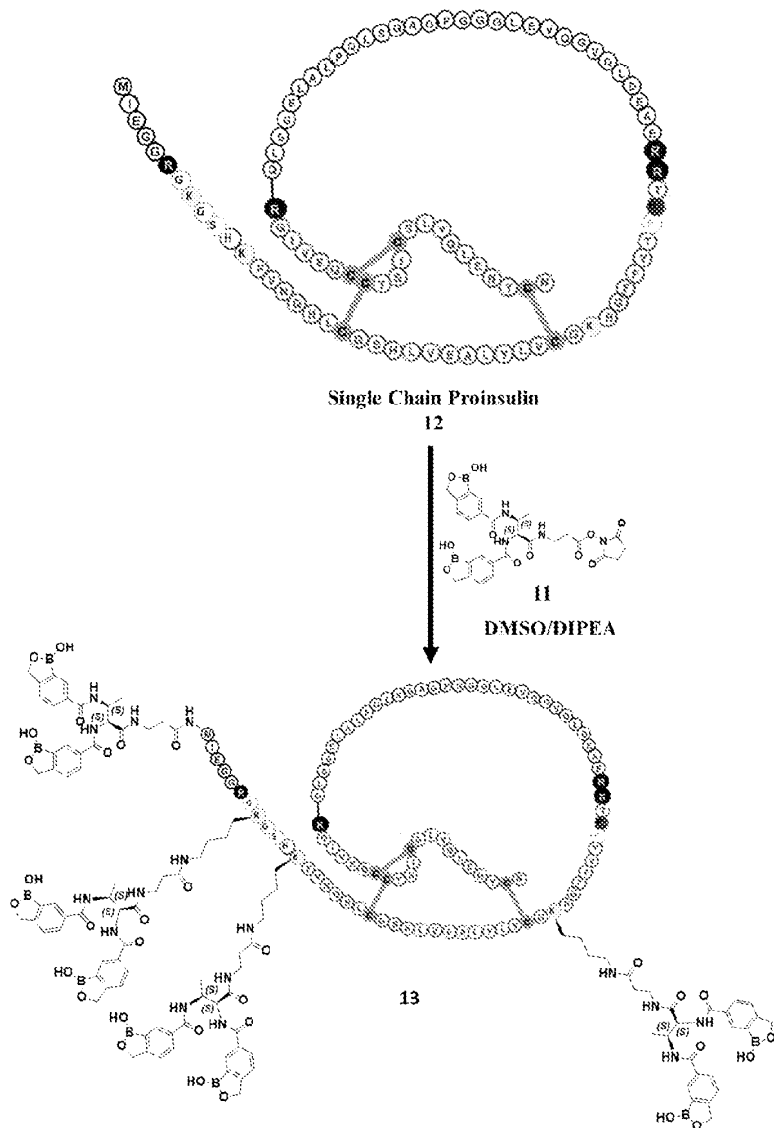
1a. Expression of proinsulin

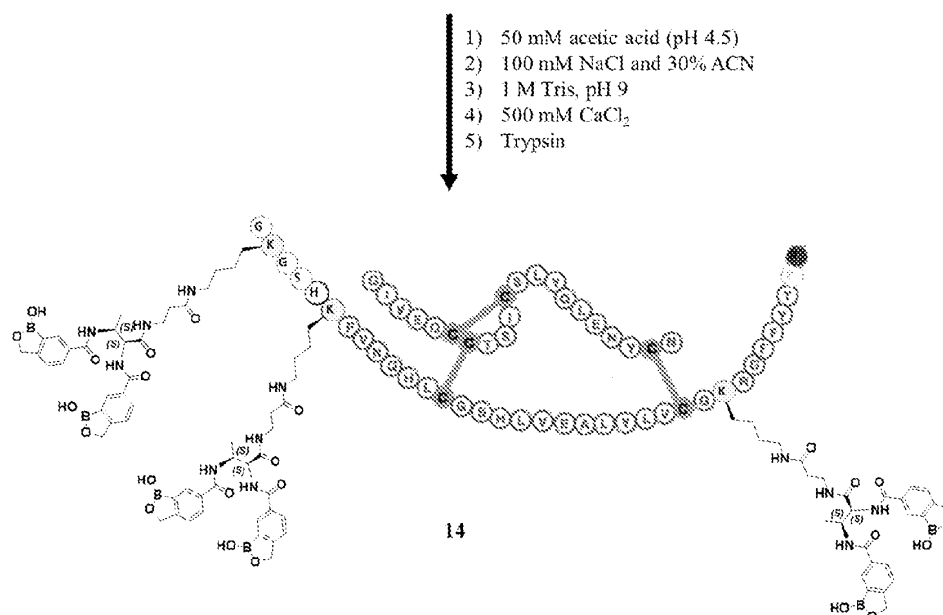
Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for
5 expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation. Expression of the desired single-chain proinsulin can be performed *via* known procedures in the art. See, e.g., U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

1b. Conjugation of diboronate sensor 11 with proinsulin.

10 To a solution of single-chain proinsulin **12** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl 3-((2*S*,3*S*)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carboxamido)butanamido)propanoate (**11**, **DSL-66B**, 4.4mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate the proinsulin
15 conjugate intermediate **13**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass
20 to insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Crude solution was allowed to stir at r.t for 16 hours. The crude conjugated insulin conjugate was precipitated with TFA (~200uL), centrifuged, decanted. Then crude precipitate was washed with water (2x 500uL) then dissolved in DMSO (100-200uL), diluted with 20% ACN/Water (5-10mL), and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield a white powder
25 (5-10 mg) of **14** (Example 19).

Scheme 1. Conjugation of diboronate sensor (11) with proinsulin (12) (SEQ ID NOS 25706-25707 and 25434-25435, respectively, in order of appearance).





The MS data for Example 19 (compound 14) is listed in Table A. The other examples listed in Table A below were synthesized under similar conditions.

Table A

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H-3H ₂ O] ⁵⁺	Mass Calculated [M+6H-3H ₂ O] ⁶⁺	Mass Observed [M+6H-3H ₂ O] ⁶⁺
Example 19	7797.47	1549.695	1549.4580	1291.581	1291.4125
Example 41	8001.44	1590.489	1590.4195	1325.576	1325.4336
Example 42	7833.47	1556.895	1556.5677	1297.581	1297.3340
Example 43	7833.47	1556.895		1297.581	
Example 44	7917.57	1573.715	1573.7895	1311.598	1311.6387
Example 45	7995.61	1589.323	1589.1750	1324.604	1324.5053
Example 46	7884.55	1567.111		1306.094	
Example 47	8046.58	1599.517		1333.099	
Example 49	8013.54	1592.909		1327.593	
Example 50	7965.44	1583.289	1583.2360	1319.576	1319.5296
Example 51	7953.57	1580.915		1317.598	
Example 52	8115.60	1613.321		1344.603	
Example 53	7920.55	1574.311		1312.094	
Example 54	8010.58	1592.317		1327.099	
Example 55	8205.40	1631.281	1631.4997	1359.569	1359.5781
Example 56	7929.44	1576.089		1313.576	
Example 57	7839.52	1558.105		1298.589	
Example 58	8151.60	1620.521		1350.603	
Example 59	8379.41	1666.083		1388.571	
Example 60	7962.49	1582.699		1319.084	
Example 61	7833.47	1556.895		1297.581	
Example 62	8151.60	1620.521		1350.603	
Example 63	7923.51	1574.903		1312.588	
Example 64	8337.47	1657.695		1381.581	
Example 65	7965.55	1583.311		1319.594	

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H-3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 66	8178.60	1625.921		1355.103	
Example 67	8004.65	1591.131		1326.111	
Example 68	7995.61	1589.323		1324.604	
Example 70	7929.55	1576.111		1313.594	
Example 71	8373.47	1664.895	1664.8339	1387.581	1387.5842
Example 72	8001.55	1590.511		1325.594	
Example 73	7995.61	1589.323		1324.604	
Example 74	8040.65	1598.331		1332.111	
Example 76	7905.42	1571.285	1571.3102	1309.573	1309.5893
Example 77	7965.55	1583.311		1319.594	
Example 78	8037.44	1597.689		1331.576	
Example 79	8121.48	1614.497		1345.583	
Example 83	7959.61	1582.123		1318.604	
Example 84	7959.61	1582.123		1318.604	
Example 85	7917.57	1573.715		1311.598	
Example 86	8049.54	1600.109		1333.593	
Example 87	8596.07	1709.415	1709.4965	1424.681	1424.7392
Example 145	7797.47	1549.695	1549.6064	1291.581	1291.4941
Example 146	8103.61	1610.923	1611.0307	1342.604	1342.5192
Example 147	7839.52	1558.105	1558.0749	1298.589	1298.5543
Example 148	8145.66	1619.333	1619.5586	1349.613	1349.6242
Example 149	7941.42	1578.485	1578.3760	1315.573	1315.5098
Example 152	7851.45	1560.491	1560.3640	1300.578	1300.4802
Example 154	7971.49	1584.499	1584.6594	1320.584	1320.56091
Example 155	8061.57	1602.515	1602.4331	1335.598	1335.4232

Insulin expression and conjugation Method 2

2a. Expression of proinsulin

- Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation.
- Expression of the desired single-chain proinsulin can be performed *via* known procedures in the art. See, e.g., 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

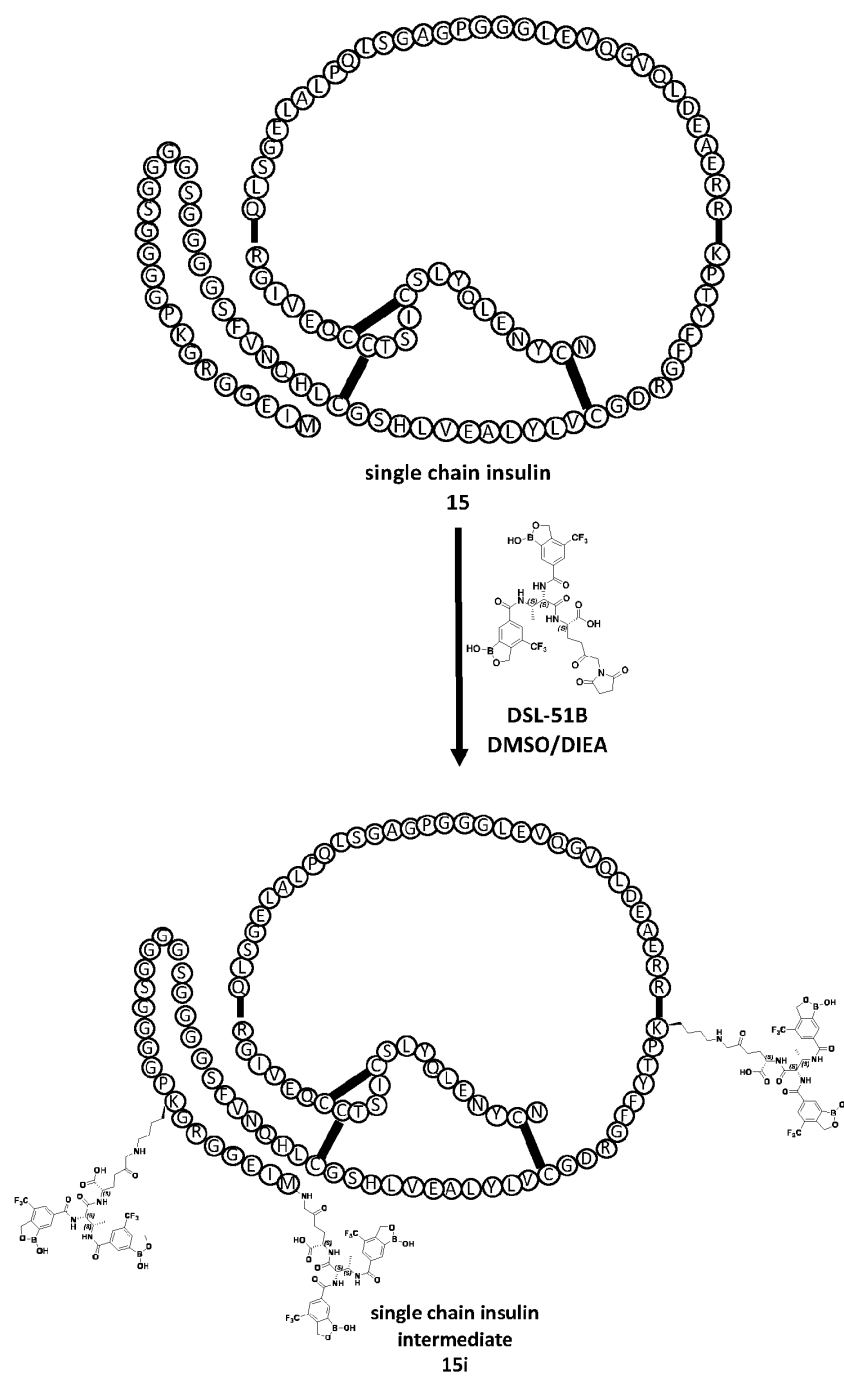
2b. Conjugation of diboronate sensor DSL-51 with proinsulin.

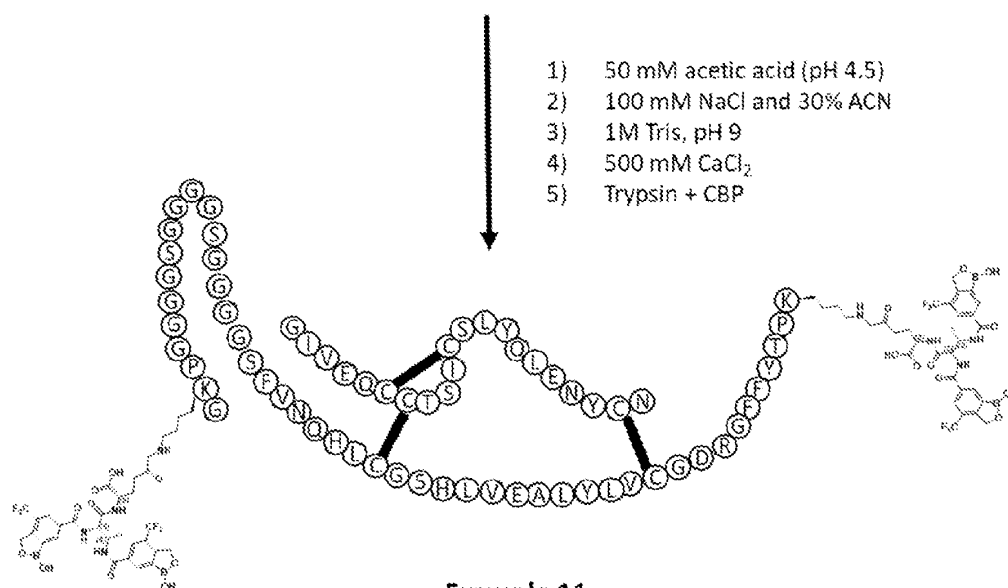
- To a solution of single-chain proinsulin **15** (20 mg) in DMSO (200uL) was added (S)-2-((2S,3S)-2,3-bis(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-6-(2,5-dioxopyrrolidin-1-yl)-5-oxohexanoic acid (**DSL-51B**, 6.2 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to

precipitate proinsulin conjugate intermediate **15i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) and carboxy peptidase (CBP, 1:1000 carboxy peptidase to insulin mass) was added. Insulin mixture was allowed to stir at r.t for 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL), diluted into 20% ACN/Water (5-10mL), and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield a white powder (6 mg) of Example 11.

Alternatively, proinsulin **15** was treated with tert-butyl (S)-2-((2S,3S)-2,3-bis(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)-6-(2,5-dioxopyrrolidin-1-yl)-5-oxohexanoate (**DSL-51-OtBu**) under similar conditions as described above. After digestions and precipitation, the crude conjugate was dissolved in TFA:H₂O (95:5), allowed to stir for 1 hour, concentrated under reduced pressure, dissolved in DMSO (100-200uL), diluted into 20% ACN/Water (5-10mL), and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 11.

Scheme 2. Conjugation of **DSL-51B** with proinsulin **15** (SEQ ID NOS 25708-25709 and 25418-25419, respectively, in order of appearance).





Example 11

The MS data for Example 2 is listed in Table B. The other examples listed in Table B below were synthesized under similar conditions.

5

Table B

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 2	8170.38	1627.88	1627.8299	1356.73	1356.6901
Example 6	8034.41	1597.083		1331.071	
Example 11	8286.39	1647.479	1647.4054	1373.068	1373.0243
Example 13	8310.39	1652.279		1377.068	
Example 18	8170.38	1624.277		1353.733	
Example 20	8310.39	1652.279		1377.068	
Example 22	8282.36	1646.673		1372.396	

Insulin expression and conjugation Method 3

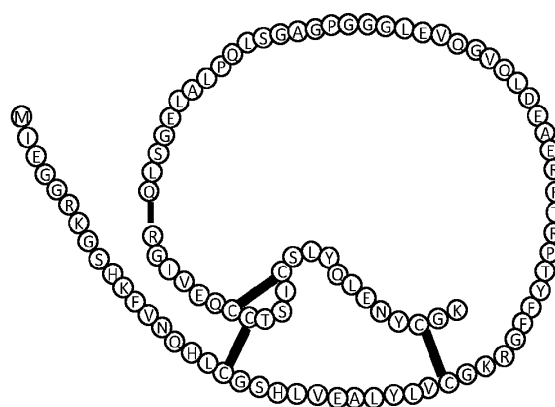
3a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation.

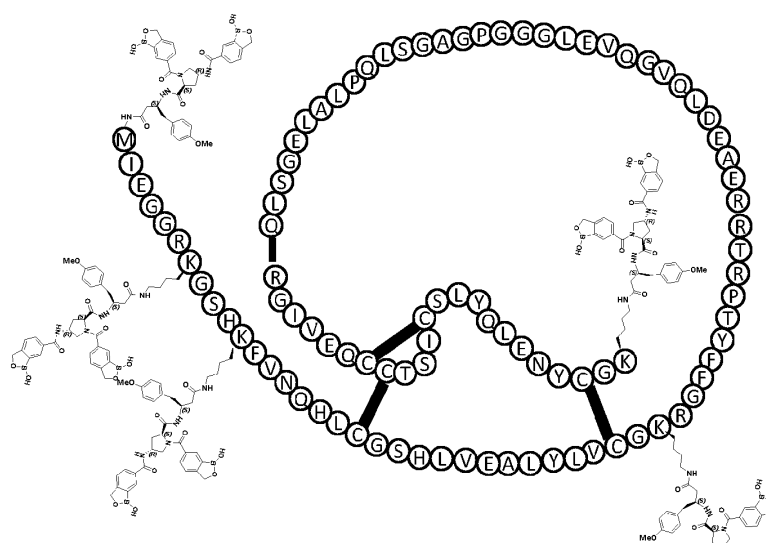
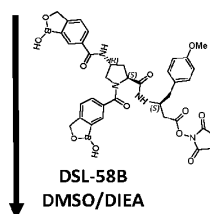
Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. *See, e.g.*, U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

3b. Conjugation of diboronate sensor DSL-58B with proinsulin.

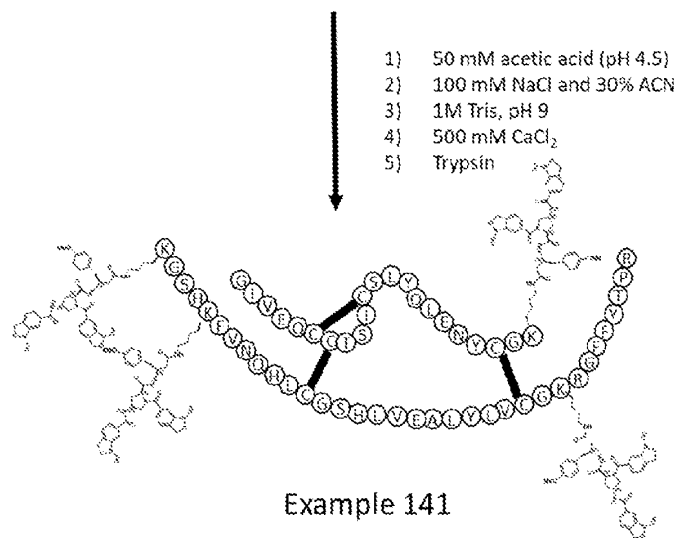
- To a solution of one-chain proinsulin **16** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(4-methoxyphenyl)butanoate (**DSL-58B**, 5.3 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate
- 10 **16i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to
- 15 insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 141 as a white powder (8.2 mg).
- 20 Scheme 3. Conjugation of **DSL-58B** with proinsulin **16** (SEQ ID NOS 25710-25711 and 25674-25675, respectively, in order of appearance).



single chain insulin
16



single chain insulin
intermediate
16i



The MS data for Example 141 is listed in Table C. The other examples listed in Table C below were synthesized under similar conditions.

5

Table C

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 140	8446.62	1679.525	1679.0139	1399.773	1399.1890
Example 141	8830.92	1756.385	1756.3025	1463.823	1463.6154
Example 142	8350.69	1660.339	1660.1569	1383.784	1383.6989
Example 143	8566.88	1703.577	1703.8767	1419.816	1419.0679
Example 144	8302.69	1650.739	1650.3696	1375.784	1375.6896

Insulin expression and conjugation Method 4

4a. Expression of proinsulin

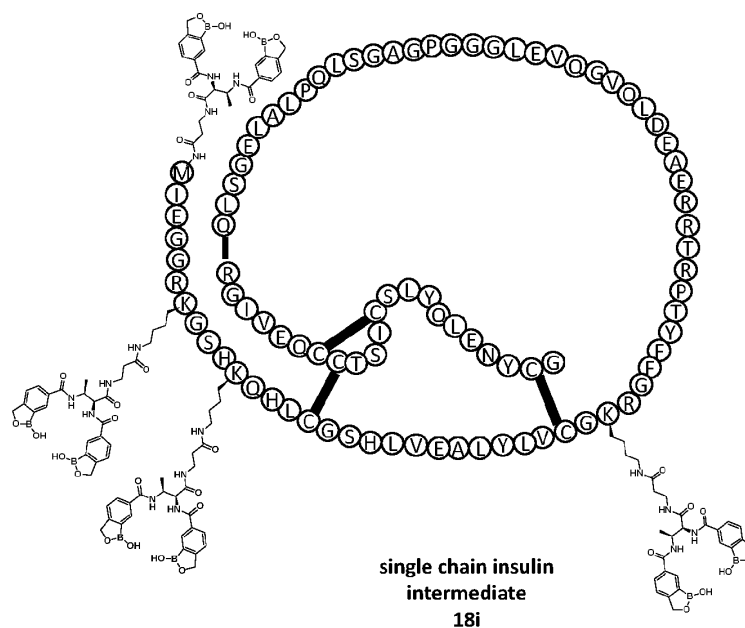
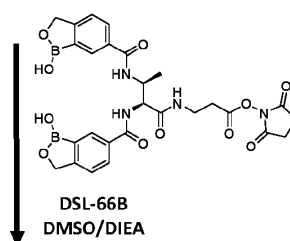
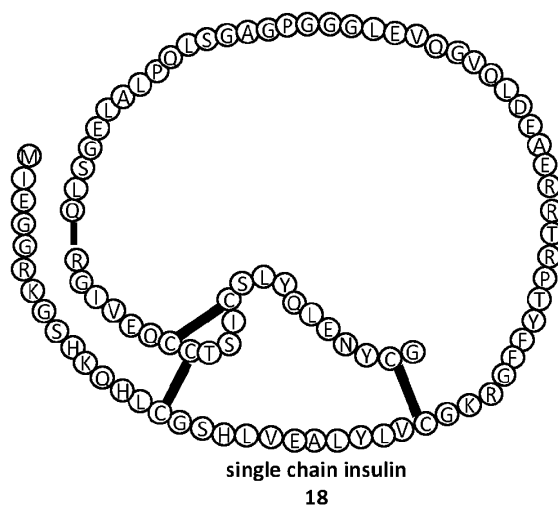
Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation.

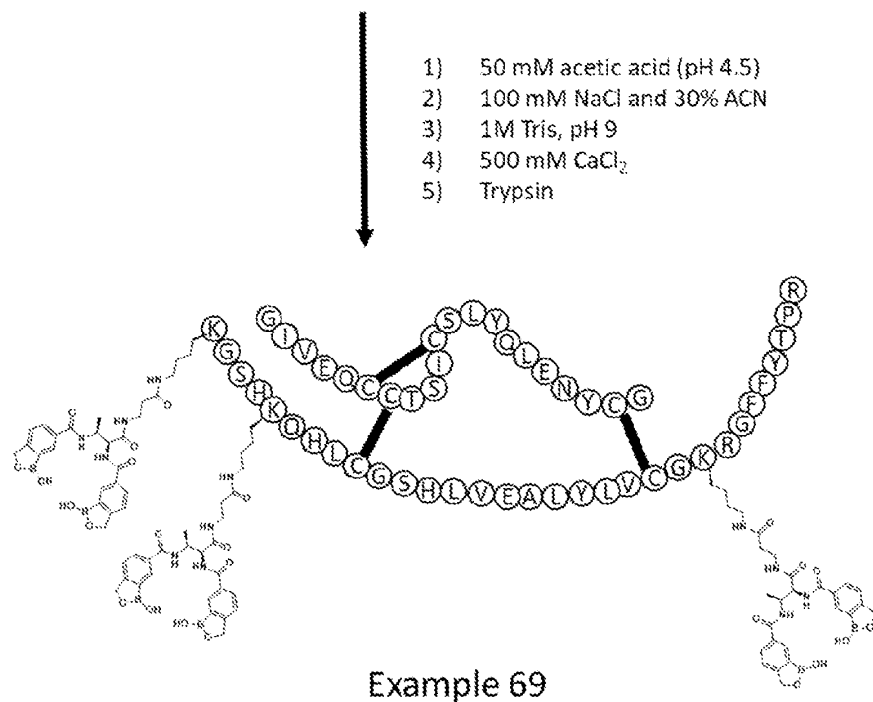
Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. See, e.g., U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

4b. Conjugation of diboronate sensor DSL-66 with proinsulin 18.

- To a solution of one-chain proinsulin **18** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate (**DSL-66B**, 6.2 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr.
- 5 Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **18i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The
- 10 pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions
- 15 were collected, frozen, and lyophilized to yield Example 69 as a white powder (7 mg).

Scheme 4. Conjugation of **DSL-66B** with proinsulin **18** (SEQ ID NOS 25712-25713 and 25530-25531, respectively, in order of appearance).





The MS data for Example 69 is listed in Table D. The other examples listed in Table D below were synthesized under similar conditions.

Table D

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 69	7323.25	1454.85	1454.8797	1212.54	1212.5711

5

Insulin expression and conjugation Method 5

5a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct

consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of

EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for

expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured,

washed and further purified, for example via an existing his-tag before sensor conjugation.

Expression of the desired one chain proinsulin can be performed *via* known procedures in the art.

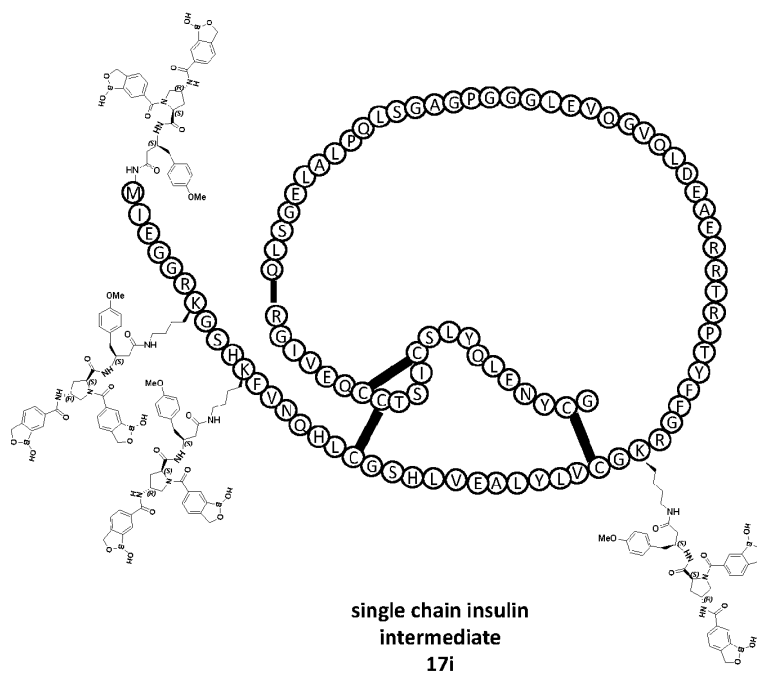
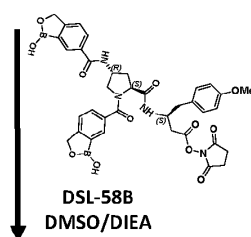
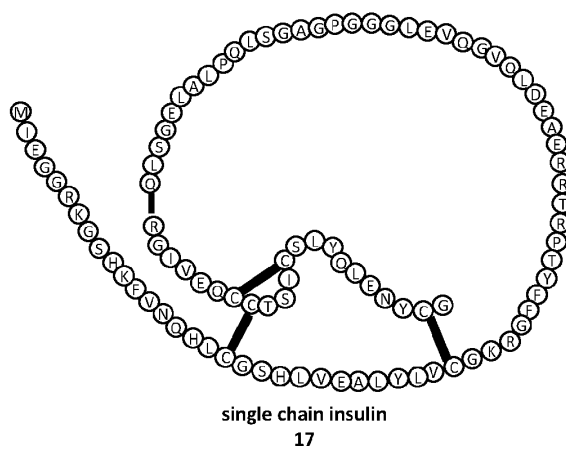
See, e.g., U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

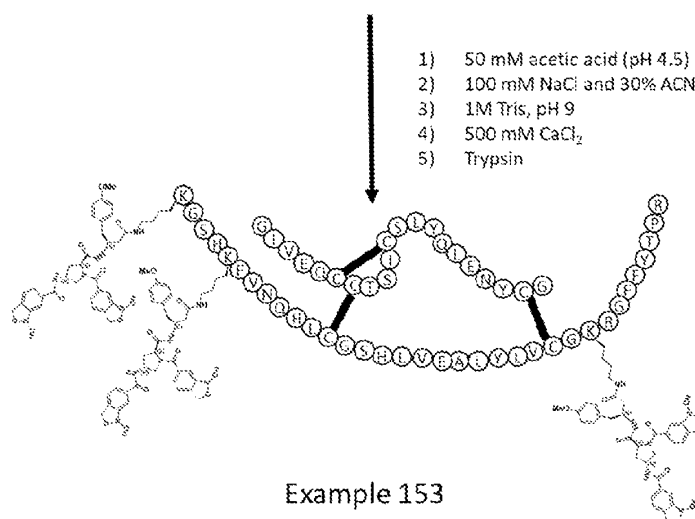
5b. Conjugation of diboronate sensor DSL-58B with proinsulin 17.

260

To a solution of one-chain proinsulin **17** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl (S)-3-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)-4-(4-methoxyphenyl)butanoate (**DSL-58B**, 5.8 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate **17i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 153 as a white powder (11 mg).

Scheme 5. Conjugation of **DSL-58B** with proinsulin **17** (SEQ ID NOS 25714-25715 and 25698-25699, respectively, in order of appearance).





The MS data for Example 153 is listed in Table E. The other examples listed in Table E below were synthesized under similar conditions.

Table E

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 8	7683.43	1526.887		1272.574	
Example 17	8151.49	1620.499		1350.584	
Example 105	7887.62	1567.725	1567.7978	1306.60	1306.4987
Example 136	8043.54	1598.909	1598.9497	1332.593	1332.4593
Example 138	7986.52	1587.505		1323.089	
Example 151	7845.57	1559.315	1559.3305	1299.598	1299.4420
Example 153	8079.6	1606.121	1606.1023	1338.603	1338.6067

5

Insulin expression and conjugation Method 6

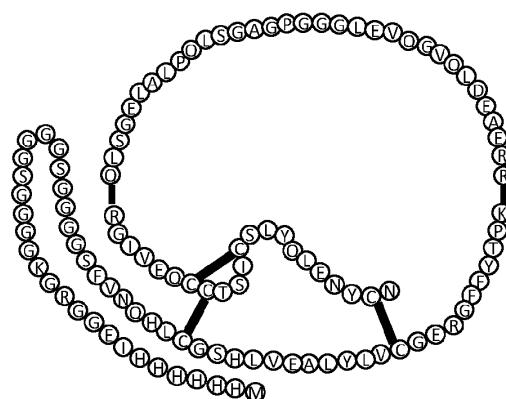
6a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation. Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. See, e.g., U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

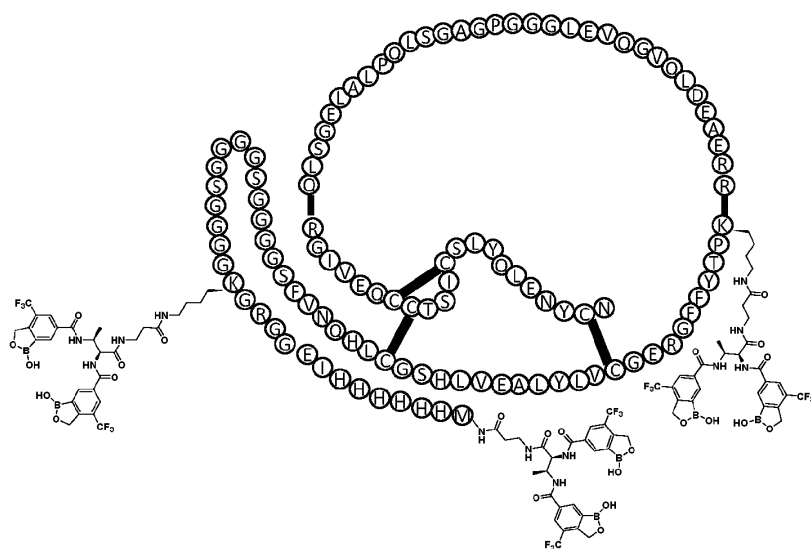
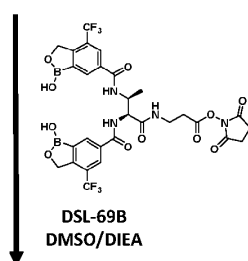
6b. Conjugation of diboronate sensor DSL-69B with proinsulin 19.

To a solution of one-chain proinsulin **19** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-4-(trifluoromethyl)-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate (**DSL-69B**, 5 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **19i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 then trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) and carboxy peptidase (CBP, 1:1000 carboxy peptidase to insulin mass) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 131 as a white powder (8 mg).

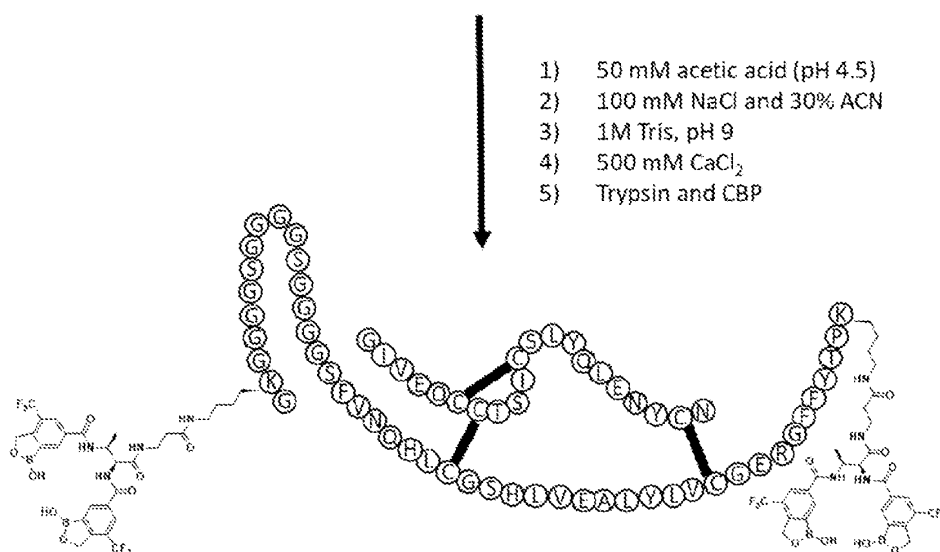
Scheme 6. Conjugation of **DSL-51B** with proinsulin **19** (SEQ ID NOS 25716-25717 and 25654-25655, respectively, in order of appearance).



single chain insulin
19



single chain insulin
19i



Example 131

The MS data for Example 131 is listed in Table F. The other examples listed in Table F below were synthesized under similar conditions.

Table F

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H-2H ₂ O] ⁶⁺	Mass Observed [M+6H- 2H ₂ O] ⁶⁺
Example 130	8089.31	1608.06		1343.22	
Example 131	8088.35	1608.063	1607.8112	1323.888	1342.8357
Example 132	8032.29	1596.659		1333.719	
Example 133	7916.28	1573.457		1314.384	
Example 134	8056.29	1601.459		1337.719	
Example 135	8028.26	1595.853		1333.048	

5

Insulin expression and conjugation Method 7

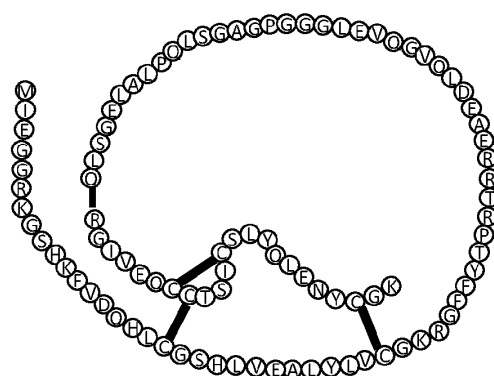
7a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct
10 consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation. Expression of the desired one chain proinsulin can be performed *via* known procedures in the art.
15 *See, e.g.*, U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

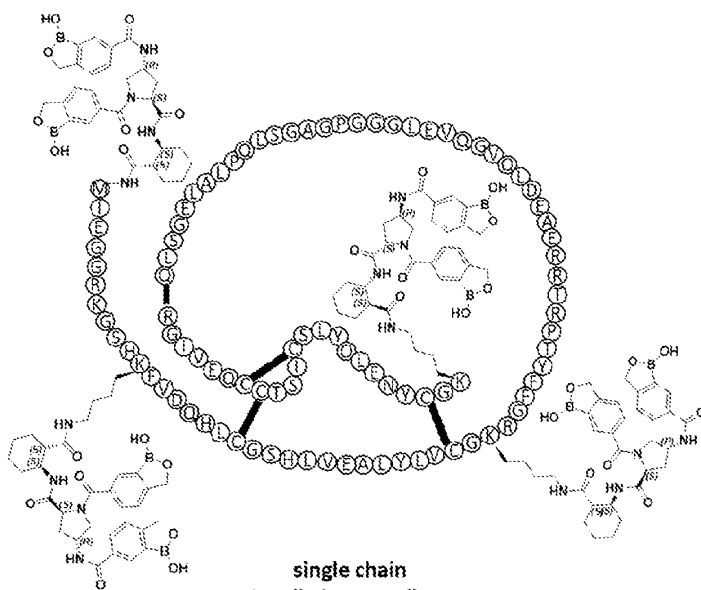
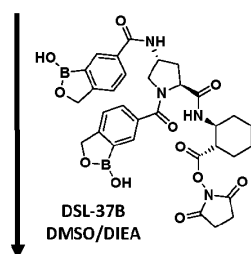
7b. Conjugation of diboronate sensor DSL-37B with proinsulin 20.

To a solution of one-chain proinsulin **20** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl (1S,2S)-2-((2S,4R)-1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbonyl)-4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)pyrrolidine-2-carboxamido)cyclohexane-1-carboxylate (**DSL-37B**, 7.5mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **20i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 7 as a white powder (10 mg).

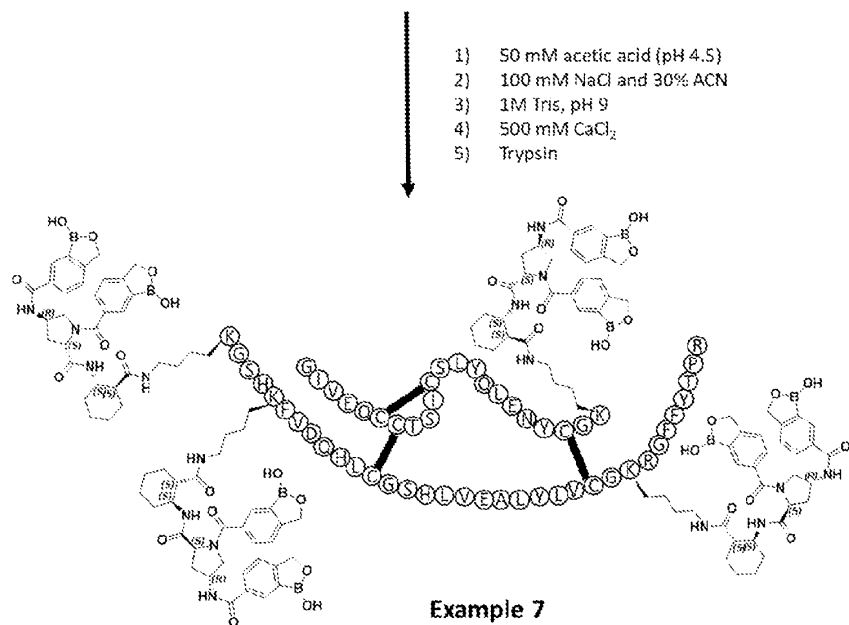
Scheme 7. Conjugation of **DSL-37B** with proinsulin **20** (SEQ ID NOS 25718-25719 and 25410-25411, respectively, in order of appearance).



single chain insulin
20



single chain
insulin intermediate
201



The MS data for Example 7 is listed in Table G. The other examples listed in Table G below were synthesized under similar conditions.

Table G

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H-3H ₂ O] ⁵⁺	Mass Calculated [M+6H-3H ₂ O] ⁶⁺	Mass Observed [M+6H-3H ₂ O] ⁶⁺
Example 3	8303.68	1650.937	1650.9410	1375.949	1375.7885
Example 7	8567.86	1703.773	1703.6046	1422.98	1422.5018
Example 14	8583.70	1706.941		1422.619	
Example 21	8627.91	1715.783		1429.988	
Example 88	8351.68	1660.537		1383.949	
Example 89	8495.60	1689.321		1407.936	
Example 90	8527.64	1695.729		1413.276	
Example 91	8673.56	1724.913		1437.596	
Example 92	8535.70	1697.341		1414.619	
Example 93	8727.62	1735.725		1446.606	

5

Insulin expression and conjugation Method 8

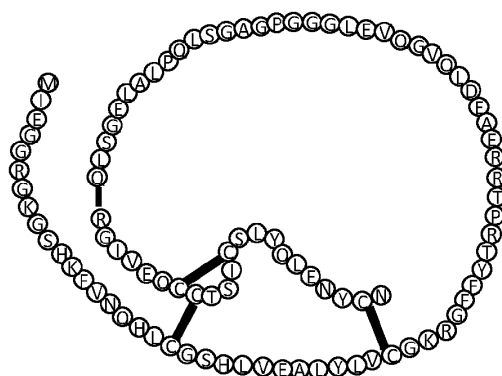
8a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct
10 consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of
EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for
expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured,
washed and further purified, for example via an existing his-tag before sensor conjugation.

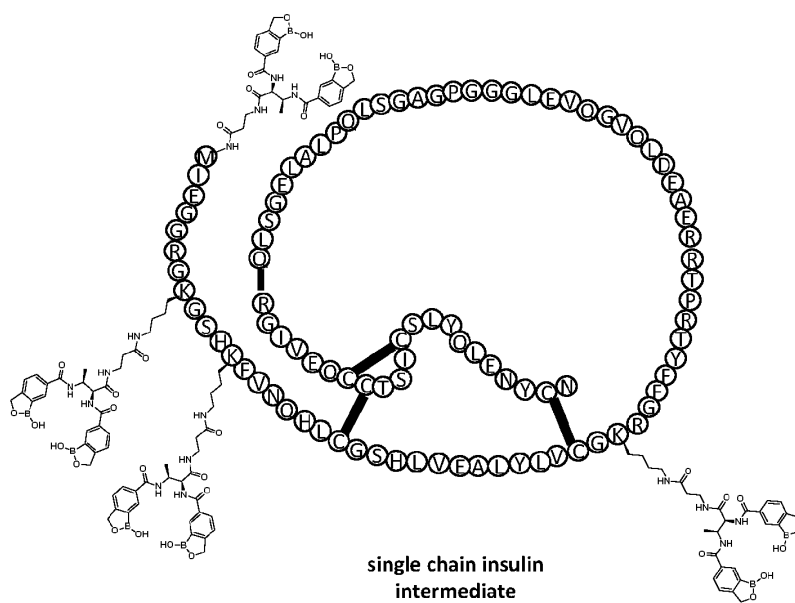
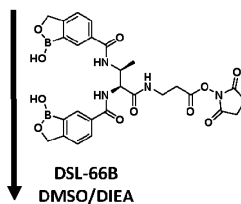
Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. *See, e.g.*, U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

8b. Conjugation of diboronate sensor DSL-66B with proinsulin 21.

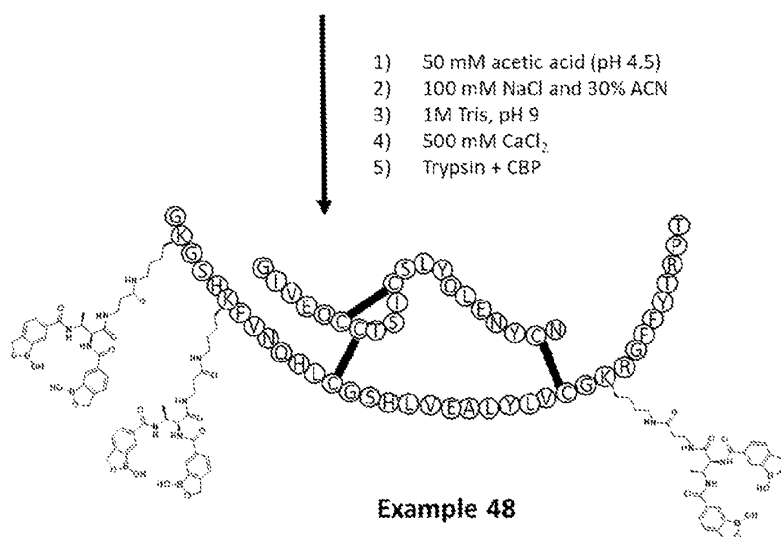
- To a solution of one-chain proinsulin **21** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate (**DSL-66B**, 6.4 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **21i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) and carboxy peptidase (CBP, 1:1000 carboxy peptidase to insulin mass) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 48 as a white powder (9 mg).
- Scheme 8. Conjugation of **DSL-66B** with proinsulin **21** (SEQ ID NOS 25720-25721 and 25490-25491, respectively, in order of appearance).



single chain insulin
21



single chain insulin
intermediate
21i



The MS data for Example 48 is listed in Table H.

Table H

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H-3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H- 3H ₂ O] ⁶⁺
Example 48	7898.52	1569.905	1569.8236	1308.423	1308.1837

5 Insulin expression and conjugation Method 9

9a. Expression of proinsulin

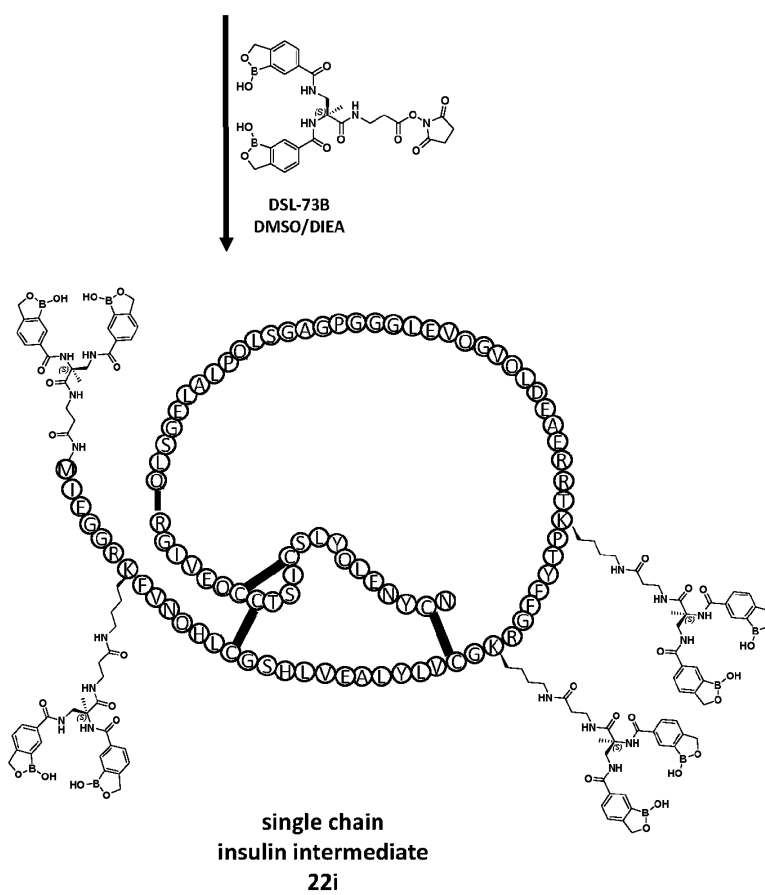
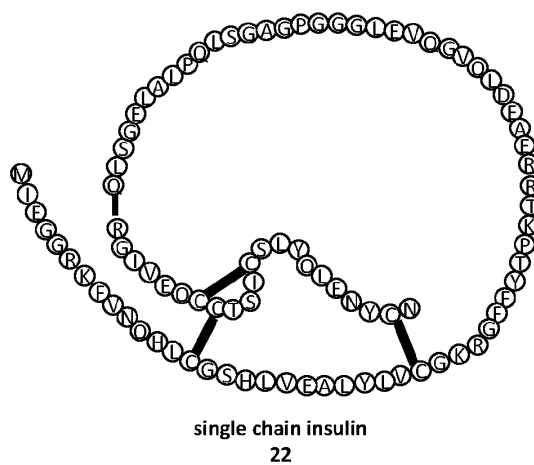
Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of
10 EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation. Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. *See, e.g.*, U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

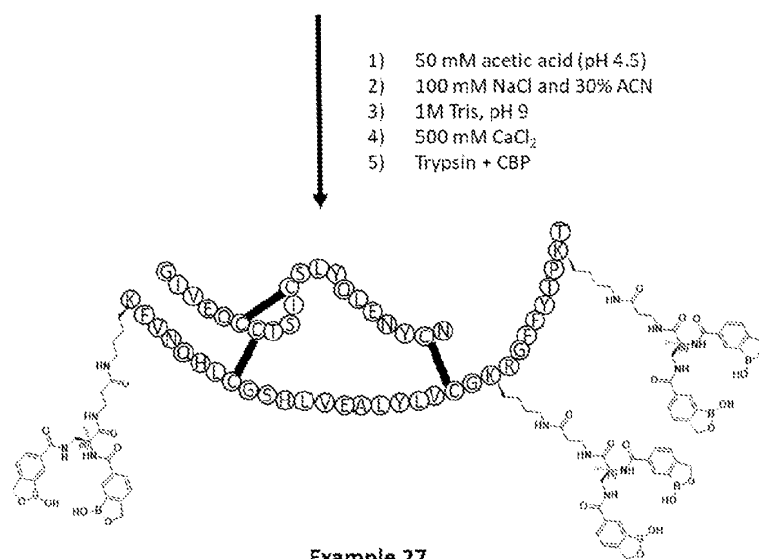
15 **9b. Conjugation of diboronate sensor DSL-73B with proinsulin 22.**

To a solution of one-chain proinsulin **22** (20 mg) in DMSO (200uL) was added 2,5-dioxypyrrolidin-1-yl (S)-3-(2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)-2-methylpropanamido)propanoate (**DSL-73B**, 5.5 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr. Trifluoroacetic acid

(TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **22i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) and carboxy peptidase (CBP, 1:1000 carboxy peptidase to insulin mass) was added was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions were collected, frozen, and lyophilized to yield Example 27 as a white powder (10 mg).

Scheme 9. Conjugation of **DSL-73B** with proinsulin **22** (SEQ ID NOS 25722-25723 and 25448-25449, respectively, in order of appearance).





Example 27

The MS data for Example 27 is listed in Table I.

Table I

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H-3H ₂ O] ⁶⁺
Example 23	7404.29	1471.059	1470.9639	1226.05	1225.9504
Example 24	7603.49	1510.899		1259.251	
Example 26	7530.32	1496.265		1247.056	
Example 27	7404.29	1471.059	1471.2545	1226.05	1226.2500
Example 28	7572.26	1504.653		1254.046	
Example 29	7440.29	1478.259		1232.051	
Example 30	7512.23	1492.647		1244.041	
Example 32	7614.30	1513.061		1261.053	
Example 33	7536.37	1497.475		1248.064	
Example 34	7812.21	1552.643		1294.038	

5 Insulin expression and conjugation Method 10

10a. Expression of proinsulin

Proinsulin can be expressed using standard IPTG induction of IPTG inducible expression constructs and vectors in e coli strains such as B21 strain. Briefly, the expression construct consists of the B-chain, C-peptide, A-chain. For example, the c-peptide sequence of

10 EAEDLQVGQVELGGGPGAGSLQPLALEGSLQR (SEQ ID NO: 25730) can be used for

expression of the proinsulin. Proinsulin is expressed in inclusion bodies (IBs), IBs are captured, washed and further purified, for example via an existing his-tag before sensor conjugation. Expression of the desired one chain proinsulin can be performed *via* known procedures in the art. *See, e.g.*, U.S. Pat. Nos. 5,457,066, 5,700,662, 5,514,646, 9,050,371, and 10,400,021.

5 **10b. Conjugation of diboronate sensor DSL-66B with proinsulin 22.**

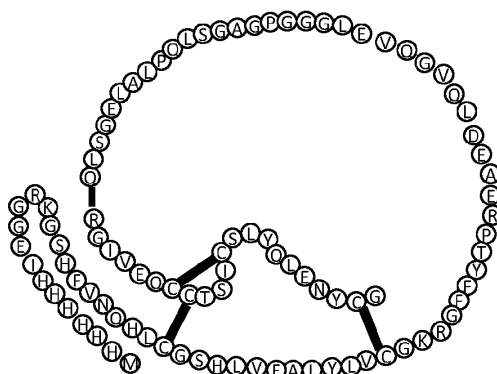
To a solution of one-chain proinsulin **22** (20 mg) in DMSO (200uL) was added 2,5-dioxopyrrolidin-1-yl 3-((2S,3S)-2,3-bis(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxamido)butanamido)propanoate (**DSL-66B**, 4.5 mg) in DMSO (50uL) and diisopropylethylamine (DIPEA, 20uL). The reaction was stirred at room temperature for 1 hr.

10 Trifluoroacetic acid (TFA, 40uL) was added to the reaction mixture to precipitate proinsulin conjugate intermediate **22i**. Reaction mixture was centrifuged (11000 RPM, 5 min), decanted, and supernatant was resuspended in 100 uL solution of 50mM acetic acid (pH 4.5), 100 mM NaCl and 30% ACN. Solution was diluted with 500 uL of water then 30 uL of 1M Tris pH 9 (50 mM final concentration), 6 uL of 500 mM CaCl₂ (5 mM final concentration) were added. The

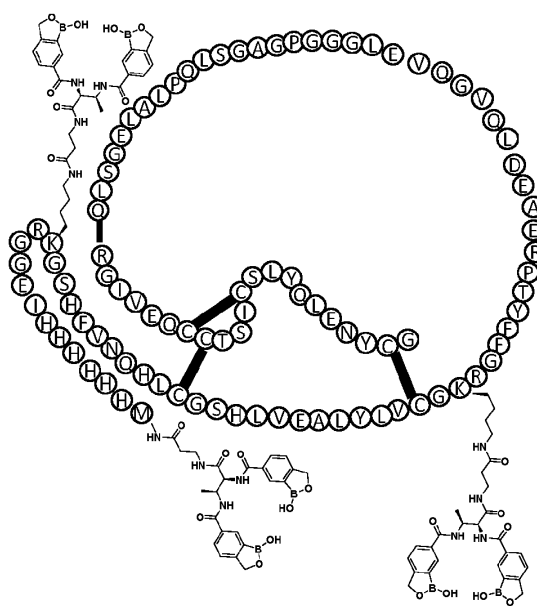
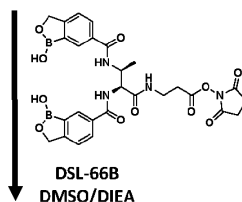
15 pH was adjusted with 0.5M NaOH (5-10 uL) to ~9-9.5 and trypsin (1:500 to 1:2000 trypsin mass to insulin mass ~10uL of 0.2 ug/mL stock solution) was added. Insulin mixture was allowed to stir at r.t 16 hr. The crude conjugated insulin was precipitated with TFA (~200uL) and the solution was centrifuged, decanted, and the precipitate was dissolved in DMSO (100-200uL) then diluted into 20% ACN/Water (5-10mL) and purified by reverse-phase HPLC. Fractions

20 were collected, frozen, and lyophilized to yield Example 156 as a white powder (6 mg).

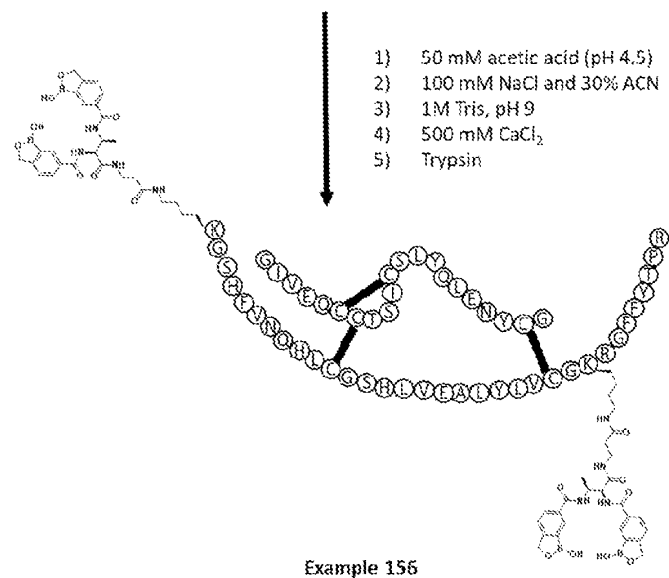
Scheme 10. Conjugation of **DSL-66B** with proinsulin **22** (SEQ ID NOS 25724-25725 and 25704-25705, respectively, in order of appearance).



single chain insulin
23



single chain insulin
23i



The MS data for **Example 156** is listed in Table J.

Table J

Example	Mass Expected (whole mass)	Mass Calculated [M+5H-3H ₂ O] ⁵⁺	Mass Observed [M+5H- 3H ₂ O] ⁵⁺	Mass Calculated [M+6H- 3H ₂ O] ⁶⁺	Mass Observed [M+6H-3H ₂ O] ⁶⁺
Example 156	7064.17	1403.03	1403.0438	1169.36	1169.3714

5

B. Testing of Compounds for Activity in Biological Assays

Exemplary compounds (e.g. diboronated sensors) of the present disclosure were tested using an alizarin red S (ARS) displacement assay.

B.1 Procedure for determination of the glucose, fructose, and lactate binding (Kd) using ARS displacement assay

The association constant for the binding event between Alizarin Red S (ARS) and the exemplary compounds tested was determined using standard methods in the art. Triplicate titrations of 10⁻⁵ M ARS in 0.1 M phosphate buffer, pH 7.4, were performed in a 96-well plate against serial dilutions of example compounds, ranging in concentration from 0 – 0.1M at 25°C.

The example compound-ARS solution was incubated for 5-45 minutes at 25 °C, and fluorescence intensity was measured using excitation wavelength 468 nm and emission wavelength 585 nm.

Changes in intensity were plotted against the concentration of the example compound, and the intensity data was fitted to yield an association constant for ARS binding.

The association constant for the binding between a target sugar compound (e.g., glucose) and the tested aromatic boron-containing groups (DSL compounds) was determined via the displacement of ARS bound to the example compounds. Triplicate wells of 10^{-5} M ARS and 0.1 M example compounds in 0.1 M phosphate buffer, pH 7.4, was titrated in a 96-well plate against serial dilutions of the target sugar compound, ranging in concentration from 0 – 2.0 M at 25 °C. The boron-ARS-carbohydrate solution was incubated for 30-60 minutes at 25 °C and the intensity of each well was measured in a plate reader at excitation wavelength 468 nm and emission wavelength 585 nm.

Changes in intensity were plotted against concentration of the target sugar compound, and the data was fitted to a one -site competition equation:

$$y = \min(y) + (\max(y) - \min(y)) / (1 + 10^{x - \log EC_{50}})$$

to yield an association constant for the boron compound-target sugar compound binding event.

The binding constants of DSL compounds, such as DSL-1A to DSL-112A, to glucose, fructose, and/or lactate can be tested and calculated. Exemplary DSL compounds disclosed herein have binding affinity to glucose with a Kd value ranging from about 0.01 mM to about 3 mM. Some exemplary DSL compounds disclosed herein have binding affinity to glucose with a Kd value ranging from about 0.01 mM to about 2.5 mM. Some exemplary DSL compounds disclosed herein have binding affinity to glucose with a Kd value ranging from about 0.5 mM to about 1.5 mM.

B.2 In Vitro Demonstration of Activity for Compounds of Formula I

Chinese hamster ovary (CHO) cells constitutively expressing Human Insulin Receptor Isoform β were seeded in a 96-well tissue culture microplate at 35,000 cells/well and grown overnight in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with Glutamine and 10% Fetal Bovine Serum (growth media). The next morning, growth media was replaced with fresh growth media.

A separate microplate of spiking media was prepared with a stepwise serial dilution of compounds of Formula I in Dulbecco's Modified Eagle Medium (DMEM) media (without glucose, without phenol red, with 4% w/v serum albumin). Wells of serially diluted compounds of Formula I were prepared in triplicate with an appropriate "high" (e.g., 20 mM) and "low" (e.g., 3 mM) concentration of glucose to determine change in potency of compounds of Formula I at various potential blood glucose levels. Wild type insulin served as a positive control and media without any compounds served as a negative control.

Growth media on cells in the 96-well tissue culture microplate was then replaced with DMEM media (without glucose, without phenol red) and the plate was allowed to sit for 5 minutes at 37°C. The media was aspirated and replaced with the contents of the separate prepared plate (spiking media) for 10 minutes at 37°C. After 10 minutes, the spiking media was aspirated and the cells were fixed with 10% neutral buffered formalin for 10 minutes at room temperature. The neutral buffered formalin was then aspirated, and the microplate was stringently washed with PBS, pH 7.4. The microplate was then permeabilized and blocked with PBS, pH 7.4 supplemented with 10% v/v Fetal Bovine Serum and 0.1% Triton X-100 for 30 minutes. The plate was then stained at 4°C overnight with 5% FBS in PBS + 1:680 ratio (v:v) of Rabbit (Rb) α -phospho-Y1150/Y1151 IR antibody (*Cell Signaling Technologies #3024*). After overnight incubation followed by stringent washes with PBS, pH 7.4, the microplate was incubated at 37°C in 5% FBS in PBS + 1:1000 ratio (v/v) of HRP α -Rabbit antibody (*Cell Signaling Technologies, #7074*) for 100 minutes. The plate was stringently washed with PBS, pH 7.4, and colorimetric readout was developed for 15 minutes at 37°C using TMB substrate. Color development was stopped with the addition of 0.1 M hydrochloric acid and absorbance measured at 450 nm. Triplicate absorbance values were plotted in GraphPad Prism and analyzed using a four-parameter logistic regression to generate dose-response curves. The EC₅₀ of the dose-response curves were compared to assess fold activation of the exemplary compounds of Formula I from low to high glucose concentration. The ratio of EC₅₀ values for GSI at high and low glucose concentrations were taken to calculate fold-change in insulin activity. The EC₅₀ was calculated using the following equation:

$$Y = \text{Bottom} + (X^{\text{Hillslope}} * (\text{Top} - \text{Bottom}) / (X^{\text{Hillslope}} + \text{EC}_{50}^{\text{Hillslope}}).$$

Examples 1-156 have an insulin receptor phosphorylation (IR Phosphorylation) (fold change) ranging from ≥ 1.2 to 45. Some examples disclosed herein had an insulin receptor phosphorylation (IR Phosphorylation) (fold change) ranging from ≥ 1.2 to 10.

B.3 *In Vivo* Demonstration of Activity for Compounds of Formula I

Male Sprague-Dawley rats underwent surgical cannulation of a carotid artery and jugular vein for blood sampling and infusions and were allowed to recover for one week. Cannulated rats were treated with streptozotocin (STZ, 55 mg/kg, 7 days) to induce a diabetic state (blood glucose concentration $>300\text{mg/dL}$). Diabetic rats were fasted overnight, then placed in a solo experimental chamber and connected to sampling/infusion lines. Prior to injection of insulin examples, glucose was infused to raise the blood glucose level (BG) or phloridzin is used to lower the BG of each rat to achieve a cohort of rats in a hyperglycemic state (200 or 400 mg/dL, N=6-8) or euglycemic state (100 mg/dL, N=6-8). Blood glucose and the glucose infusion rate

(GIR) required to maintain clamped BG were measured every 5 minutes and the GIR was adjusted to maintain the desired BG level. Somatostatin (5 ug/kg/min) was continuously infused throughout the study beginning with the administration of phloridzin or glucose. At time 0 min, a bolus dose (1 nmol/kg to 60 nmol/kg) of a compound of Formula I was infused over a 10 second period, and glucose was continuously infused at variable rates to maintain the hyperglycemia (200 or 400 mg/dL) or euglycemia steady state (100 mg/dL) for 300 minutes. The total area-under-the-curve (AUC) value of total glucose infusion post-compound injection until the GIR returned to its pre-compound injection value or 300 minutes was achieved was calculated for both hyperglycemic (200 or 400 mg/dL) and euglycemic (100 mg/dL) levels. Area under the curve (AUC) was calculated using the trapezoid rule and with baseline correction applied. Averaged GIR values from 30 minutes prior to injections to the injections were subtracted from each GIR value from time 0 to time 600 min for baseline correction. The trapezoidal calculation is as follows:



Where a is the first time point (0 min) and b is the last time point (300 min). The area was calculated as a subdivision of small trapezoids as follows:

$$\int_a^b f(x) dx \approx \frac{\Delta x}{2} (f(x_0) + 2f(x_1) + 2f(x_2) + 2f(x_3) + 2f(x_4) + \dots + 2f(x_{N-1}) + f(x_N)).$$

Such that Δx is the difference between each time point.

Compounds of Formula I require greater GIR and total glucose infusion AUC to maintain clamped hyperglycemic BG than euglycemic BG, demonstrating a glucose-responsive increase in compound glucose-lowering action at increased BG. Compounds exhibited AUC values ranging from 250 to-5000 under hyperglycemic conditions and 200-to-4500 under euglycemic levels. The ratio of hyperglycemic AUC to euglycemic AUC ranged from 0.01-to-5 in 400 mg/dL to 100 mg/dL and ranged from 0.2-to-4.5 in 200 mg/dL to 100 mg/dL.

It is also observed in cell-based experiments on compounds containing formulae FF116, FF116A-D, FF225, FF225A, FF225B, and FF226 that sensors with geminal alkyl substituent on the same carbon as the nitrogen conjugated to the boroxole or boronates provided between 5-56% higher glucose responsiveness in the range of 3-20 mM glucose in comparison to variants that do not have the geminal alkyl substituents. For example, when a Z1C represented by one of formulae FF12, FF12A-D, FF116, FF116A-D, FF225, FF225A, FF225B, and FF226 is conjugated to lysine residues in insulin wherein the boronates (B1, B2) in formulae FF12, FF12A-D, FF116, FF116A-D, FF225, FF225A, FF225B, and FF226 are represented by F2, the resulting insulin is observed to be between 11-56% more responsive to changes in glucose levels

between 3-20 mM glucose than if instead of one of the formulae FF12, FF12A-D, FF116, FF116A-D, FF225, FF225A, FF225B, and FF226, one uses 2,3-diaminopropionic acid. This data shows that the presence of the geminal alkyl substituent on the same carbon as the nitrogen conjugated to the boroxole or boronates improves glucose responsiveness of the resulting insulin conjugate, and in tested variations in the 3-20 mM glucose range.

Without wishing to be bound by theory, it is believed that this general principle extends to other formulae FF12, FF12A-D, FF114, FF115, FF116, FF116A-D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226 providing a framework for enhancement of glucose responsiveness by at least 5%, at least 10%, at least 20%, or at least 40% in the 3-20mM or 2-50mM glucose ranges.

Without wishing to be bound by theory, it is believed based on observations of glucose responsiveness trends, that the presence of the carbonyl group adjacent to- or within less than two carbon centers away from the amine groups in FF formulae (to which aromatic boron-containing groups are conjugated) enhances glucose responsiveness through impacting ability to turn off activity of drug substance through plasma protein interactions such as with albumin and that this is independent of glucose affinity such that glucose affinity is not impacted by the position of this carbonyl group.

Without wishing to be bound by theory, it is believed that the pharmacokinetics of the molecules and potential albumin or blood proteins binding is impacted by the position of this carbonyl group, and thereby enhances overall glucose responsiveness whilst the absolute glucose affinity is maintained or nearly identical. Therefore, in certain embodiments of the present invention, the carbonyl group (as part of an acid, amid or linkage to X in FF formulae) is placed within less than three, or within less than two-carbon centers away from one of the two amines to which the boron-containing compounds are conjugated. In certain embodiments, the placement of amines within two carbon centers from each other enables the spatial and geometric constraining of the aromatic boron containing groups to enhance glucose binding and selectively, and furthermore the presence of a carbonyl group (for example, as part of an amide linkage) which is within less than two carbon centers, from one of the two amines (to which aromatic boron containing groups are attached) ensures differential albumin binding in a manner that results in the compound exhibiting glucose responsiveness in the blood and in the body. In some embodiments, the combination of geometrical constraining of the two amines to which the aromatic boron containing groups are conjugated, as well as the presence of the carbonyl within one to two carbon centers from one of the amines provides the necessary requirements for glucose responsiveness in physiological blood and plasma glucose levels.

Without wishing to be bound by theory, it is believed based on observations of glucose responsiveness trends, that the interaction of the indirect linker with the Z1c helps further enhance glucose responsiveness. For example, enhanced glucose responsiveness may result in some embodiments where Z1c is selected from FF12, FF12A-D, FF116, FF116A-D, B1 and B2
5 are represented by F2, wherein each of the remaining R1 is H, and wherein the indirect linker is selected from a relatively hydrophobic amino acid or contains a relatively hydrophobic molecule (e.g., linker) selected from FL3, FL20-22, FL25, FL27-28, FL30-33, FL35, FL37, FL41, FL43, FL45-70. For example, enhanced glucose responsiveness may result in some embodiments where Z1c is selected from FF12, FF12A-D, FF116, FF116A-D, B1 and B2 are represented by
10 F2 wherein one of the remaining R1 is CF₃ and the remaining R1 is H, and wherein the indirect linker is selected from a hydrophilic amino acid or contains a hydrophilic molecule (e.g., linker) selected from FL5, FL5A, FL5B, FL23-24, FL26, FL29, FL34, FL36, FL38-40, and FL42.

Without wishing to be bound by theory, in some embodiments glucose responsiveness at physiological ranges of 3-10mM, 4-10mM, 3-20mM or 4-20mM glucose is further enhanced
15 either by (i) a combination of a relatively hydrophobic indirect linker (e.g., FL3, FL20-22, FL25, FL27-28, FL30-33, FL35, FL37, FL41, FL43-70) and a Z1c in which both B1 and B2 are selected from F2, wherein all remaining R1 are H, or (ii) a combination of a relatively hydrophilic indirect linker (e.g., FL5, FL5A, FL5B, FL23-24, FL26, FL29, FL34, FL36, FL38-40, FL42) and a Z1c in which both B1 and B2 are selected from F2, wherein one of the
20 remaining R1 is CF₃.

Without wishing to be bound by theory, in some embodiments glucose responsiveness of an insulin at physiological ranges of 3-10mM, 4-10mM, 3-20mM or 4-20mM glucose is further enhanced either by (i) a combination of a relatively hydrophobic indirect linker and a Z1c in which both B1 and B2 are selected from F2, wherein all remaining R1 are H, wherein at least
25 three or at least four lysine residues in the insulin are conjugated to a Z1c or (ii) a combination of a relatively hydrophilic indirect linker and a Z1c in which both B1 and B2 are selected from F2, wherein one of the remaining R1 is CF₃, and wherein at least two or at least three lysine residues in the insulin are conjugated to a Z1c.

Without wishing to be bound by theory, in some embodiments glucose responsiveness is
30 enhanced through selection of combination of the indirect linker and Z1c is selected from (i) a combination of a relatively hydrophobic indirect linker and a Z1c in which both B1 and B2 are selected from F2, wherein all remaining R1 are H, or (ii) a combination of a relatively hydrophilic indirect linker and a Z1c in which both B1 and B2 are selected from F2, wherein one of the remaining R1 is CF₃ and the remaining R1 is H.

Without wishing to be bound by theory, in some embodiments glucose responsiveness is enhanced through selection of combination of the indirect linker and Z1c is selected from (i) a combination of a relatively hydrophobic indirect linker and a Z1C selected from FF12, FF12A-D, FF116, FF116A-D, in which both B1 and B2 are selected from F2, wherein all remaining R₁ are H, or (ii) a combination of a relatively hydrophilic indirect linker and a Z1c selected from FF12, FF12A-D, FF116, FF116A-D, in which both B1 and B2 are selected from F2, wherein one of the remaining R₁ is CF₃ and the remaining R₁ is H.

Experiments on cell-based assays of insulins with lysine residues conjugated with one of formulae FF12, FF12A-D, FF114, FF115, FF116, FF116A-D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226 demonstrated that the enhanced glucose responsiveness of the insulins is increased when one or more lysine residues are modified as described by Formula I using one of formulae FF12, FF12A-D, FF114, FF115, FF116, FF116A-D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226, and wherein the lysine residues are present in insulin (as insertions or mutations) or in a polypeptide that is appended to the N- or C- terminus of the B-chain of insulin or the C-terminus of the A-chain of insulin, and wherein there are additional lysine residues within the insulin sequence that are similarly modified. The results are further corroborated by testing of the compounds of Formula I in STZ diabetic mouse models wherein the activity of the insulin is measured through bolus injections of the compounds of Formula I followed by glucose challenges and measurements of blood glucose, or through glucose clamp assays in which activity of the insulins is measured as a function of blood glucose levels. The results further showed that exemplary compounds of Formula I disclosed herein function in the body and are responsive to physiological changes in blood glucose and provide dynamic insulin action in the body in response to changes in blood glucose levels. In certain embodiments one or more lysine residues near the N-terminus of a polypeptide appended to the N-terminus of the B-chain of insulin, as well as at least one additional lysine in the B-chain of insulin are conjugated as described by Formula IC.

In certain embodiments, a sequence is appended to the N-terminus and/or C-terminus, and/or inserted into the sequence of the A-chain of insulin, wherein the A-chain of insulin comprises one of the following sequences, optionally with up to four additional deletions and/or mutations:

GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 1);
 GIVEQCCTSICSLYQLENYCGK (SEQ ID NO: 25);
 GIVEQCCTSICSLYQLENYCG (SEQ ID NO: 24051);
 GIVEQCCTSICSLEQLENYCG (SEQ ID NO: 24052);

and/or a sequence is appended to the N-terminus and/or C-terminus, and/or inserted into the sequence of the B-chain of insulin, wherein the B-chain of insulin comprises one of following sequences, and optionally with up to four additional deletions and/or mutations:

- KFVNQHLCGSHLVEALYLVCCKRGFFYTPKT (SEQ ID NO: 24060);
- 5 KGSFVNQHLCGSHLVEALYLVCCKRGFFYTPR (SEQ ID NO: 24061);
KGSQHLCGSHLVEALYLVCCKRGFFYTPR (SEQ ID NO: 24062);
KGSQHLGSHLVEALYLVCCKRGFFYTPR (SEQ ID NO: 24063);
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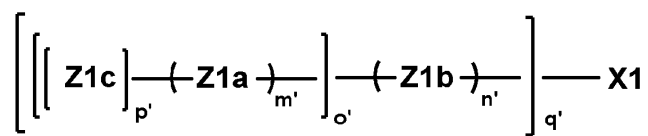
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- 30

WHAT IS CLAIMED IS:

1. A compound represented by Formula I, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt thereof:



(Formula I)

wherein:

X1 comprises

- i. a drug substance comprising an amine;
- ii. an amine configured to be covalently conjugated to a drug substance; or
- iii. NH₂ or OH;

each Z1a independently comprises 1 to 50 amino acids connected together using amide or peptide bonds;

each Z1b is independently a small-molecule linker;

- each Z1c is independently selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226, wherein each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH;

each m' is 0 or 1;

each n' is 0;

each o' is 1;

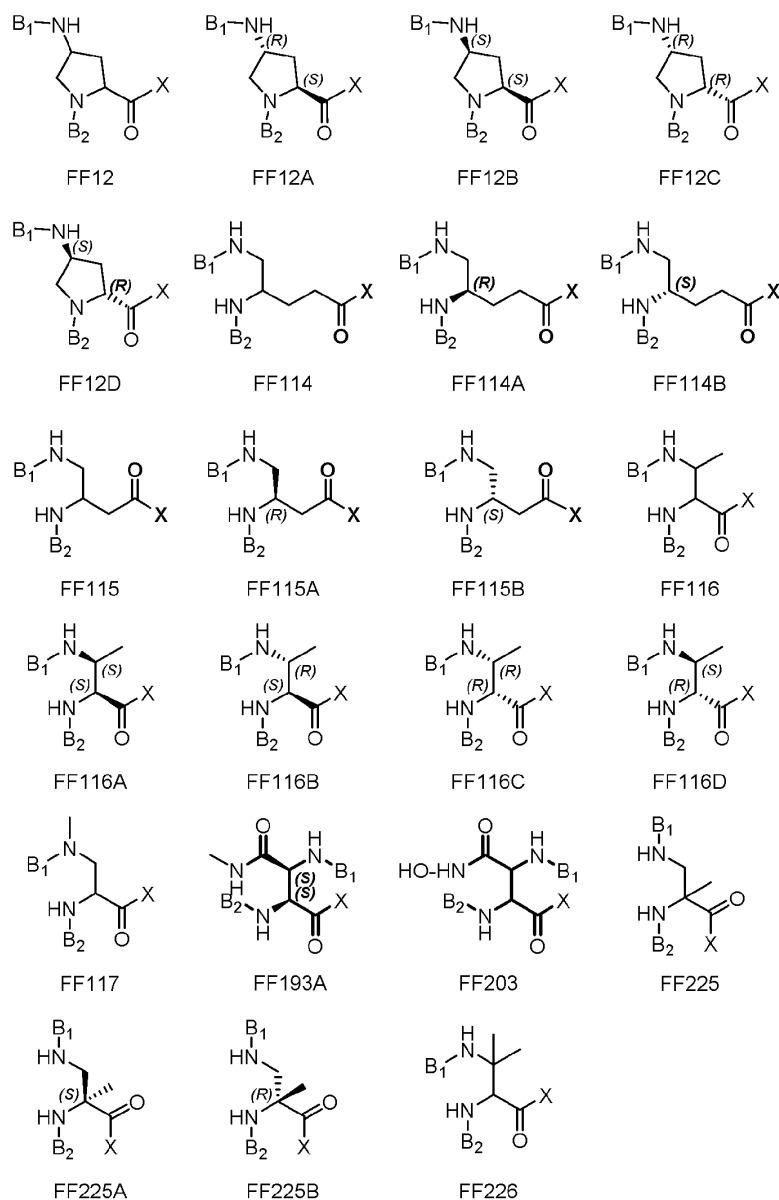
each p' is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and

each q' is 1, 2, 3, 4, or 5, wherein when p' or q' is 2 or more, each corresponding Z1c is independently selected and may be the same or different and wherein when q' is 2 or more, each corresponding Z1a is independently selected and may be the same or different;

when m'=0 and n'=0, each Z1c is covalently conjugated either directly or via an indirect linker to an amine of X1 or to NH₂ when X1 is NH₂, or to OH when X1 is OH, and for m'=1 each Z1a is covalently conjugated via an amide or peptide bond to X1 and each Z1c is covalently conjugated either directly or via an indirect linker to an amine of Z1a or to X1;

and

wherein Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226 are:



wherein X represents a point of covalent attachment either directly to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1; and wherein B₁ and B₂, which may be identical or different, each

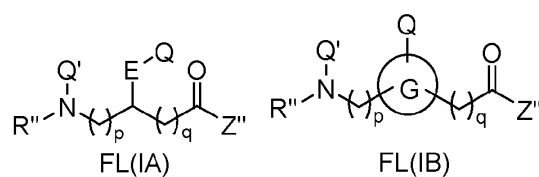
5 independently represent an aromatic boron-containing group; and

wherein one or more positions of the compound of Formula I may comprise an isotope.

2. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein at least one Z1c is covalently conjugated via the indirect linker to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine of Z1a,

the indirect linker is represented by Formula (X'')_{n1}, wherein each n1 is independently selected from 1, 2, 3, 4, and 5, and each X'' is independently selected from:

- i. an L- or D-amino acid, wherein an amine functional group of the L- or D-amino acid is covalently conjugated, directly or indirectly, to Z1c and an acid functional group of the L- or D- amino acid is conjugated, directly or indirectly, to X1 or to Z1a; and
- ii. Formulae FL(IA), FL(IB), FL69, and FL70; wherein Formula FL(IA) and FL(IB) are:



wherein:

G is selected from a 3- to 6-membered cycloalkyl group, a 3- to 10-membered heterocyclyl group, a heteroaryl group, and an aryl group, wherein each group is optionally substituted with 1-3 groups independently selected from hydroxy, amino, halogen, (C₃-C₆)cycloalkyl, alkoxy, and C₁-C₆alkyl;

E is absent or is an alkylene group optionally substituted with 1-3 groups independently selected from halogen, hydroxy, and amino;

Q is absent or is selected from hydrogen, C₁-C₆alkyl, halo, cyano, alkoxy, carboxylic acid, amino, hydroxy, amide, C₁-C₆haloalkyl, (C₃-C₆)cycloalkyl, heterocycle, heteroaryl, and aryl, wherein the alkyl, alkoxy, cycloalkyl, heterocycle, heteroaryl, and aryl is each optionally substituted with 1-5 groups independently selected from C₁-C₆alkyl, amino, amide, halo, hydroxy, cyano, C₁-C₆haloalkyl, and alkoxy;

Q' is selected from hydrogen, C₁-C₆alkyl, and an acyl group;

Q and Q', together with the carbon and nitrogen atom to which they are attached, optionally form a 4-membered heterocyclyl, a 5-membered heterocyclyl, a 6-membered heterocyclyl, a 9-membered bicyclic heterocyclyl, or a 10-membered bicyclic heterocyclyl, wherein the 4-membered heterocyclyl, 5-membered heterocyclyl, 6-membered heterocyclyl, 9-membered bicyclic heterocyclyl, and 10-membered bicyclic heterocyclyl are each optionally substituted with 1-5 groups independently selected from C₁-C₆alkyl, amino, halo, hydroxy, cyano, amide, C₁-C₆haloalkyl, and alkoxy;

p is 0, 1, 2, 3, 4, or 5;

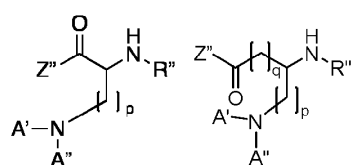
q is 0, 1, 2, 3, 4, or 5;

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a; and

any primary amine is optionally acetylated or alkylated;

5 wherein Formulae FL69 and FL70 are:



FL69 , FL70 , and stereoisomers thereof; wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, a C₁-C₆alkyl, a saturated fatty acid, an unsaturated fatty
10 acid, a (C₃-C₆)cycloalkyl, a haloalkyl, an aryl, and a heteroaryl; and

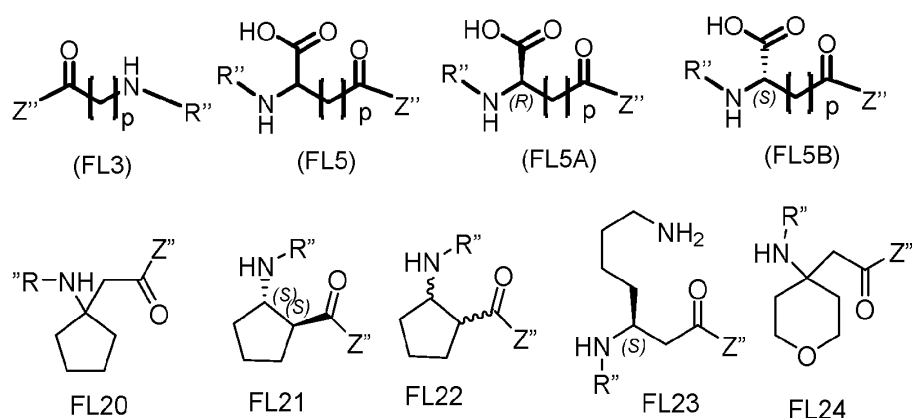
A'' is selected from a C₁-C₆alkyl, a substituted acyl, acyl terminating in an acid, a
saturated or unsaturated fatty acid, a (C₃-C₆)cycloalkyl, a haloalkyl, an aryl, and a
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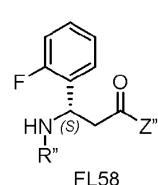
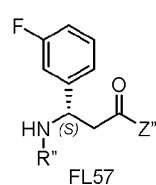
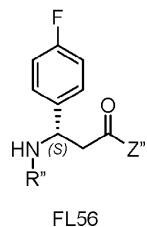
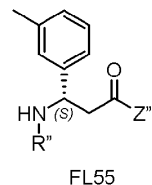
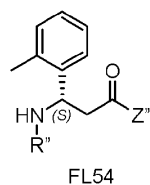
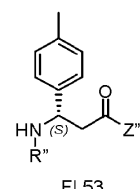
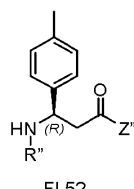
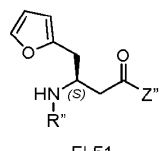
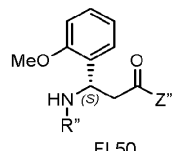
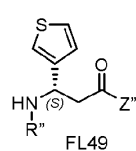
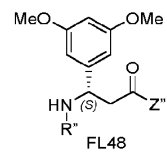
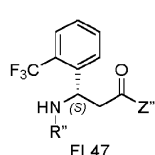
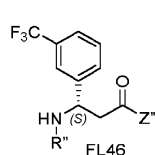
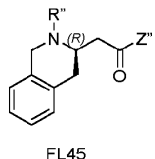
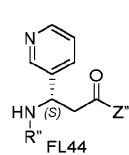
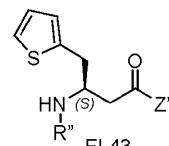
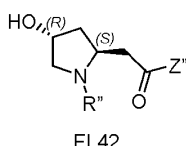
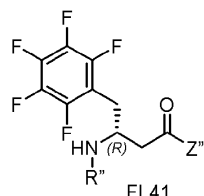
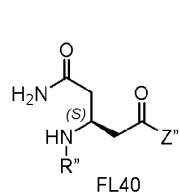
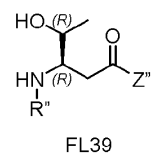
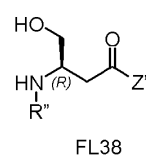
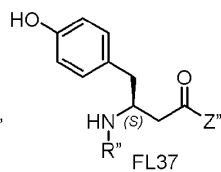
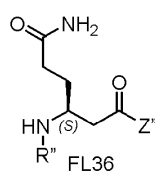
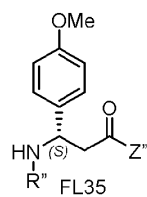
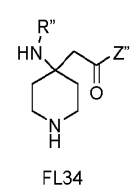
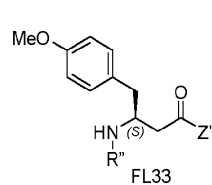
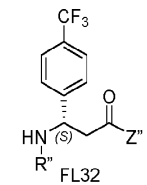
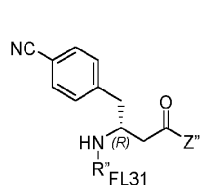
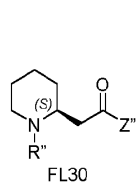
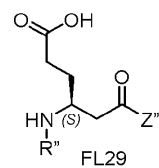
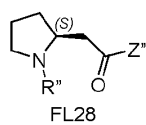
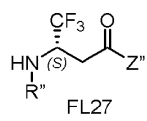
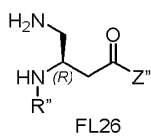
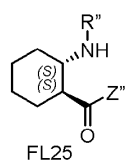
p is 1, 2, 3, 4, or 5,

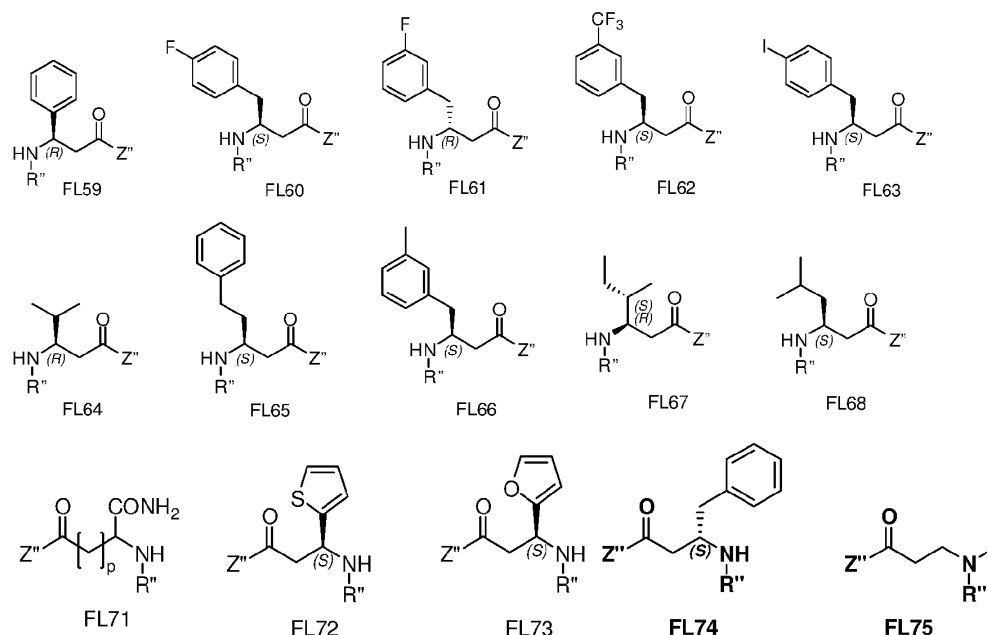
15 q is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

3. The compound of claim 2, or a pharmaceutically acceptable salt thereof, wherein
Formulae FL(IA) and FL(IB) are selected from Formulae FL3, FL5, FL5A, FL5B, FL20-FL68,
20 and FL71-FL75:







and stereoisomers thereof;

5 wherein:

p is 1, 2, 3, 4, or 5,

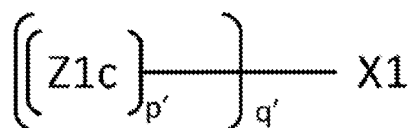
q is 1, 2, 3, 4, or 5, and

any primary amine is optionally acetylated or alkylated.

4. The compound of claim 2, or a pharmaceutically acceptable salt thereof, wherein

10 n1 is 1.

5. The compound of any one of claims 1-4, or a pharmaceutically acceptable salt thereof, wherein the compound is represented by Formula IC:



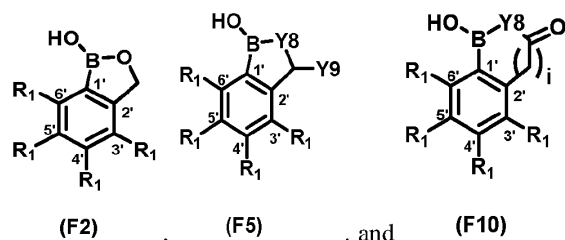
(Formula IC)

15 each p' is 1, 2, 3, or 4; and

each q' is 1, 2, 3, 4, or 5.

6. The compound of any one of claims 1-5, or a pharmaceutically acceptable salt thereof, wherein each B₁ and B₂ is independently selected from Formulae F2, F5, and F10, wherein Formulae F2, F5, and F10 are:

20



wherein:

one R_1 represents $(C=O)---*$ or $(CH_2)_m(C=O)---*$, wherein $---*$ represents the attachment point to the rest of Z1c, and m is 1, 2, 3, 4, 5, 6, or 7; and

- 5 each remaining R_1 is independently selected from H, F, Cl, Br, I, OH, CH_2-NH_2 , NH_2 , $(C=O)-NH_2$, $CH=O$, SO_2CH_3 , SO_2CF_3 , CF_3 , CHF_2 , NO_2 , CH_3 , OCH_3 , $O(CH_2)_{m''}CH_3$, $(SO_2)NH-CH_3$, $(SO_2)NH(CH_2)_{m''}CH_3$, and OCF_3 , wherein m'' is 1, 2, 3, 4, 5, 6, or 7;

Y_8 is O, N, or NR, wherein R is a C_1 - C_6 alkyl group or H;

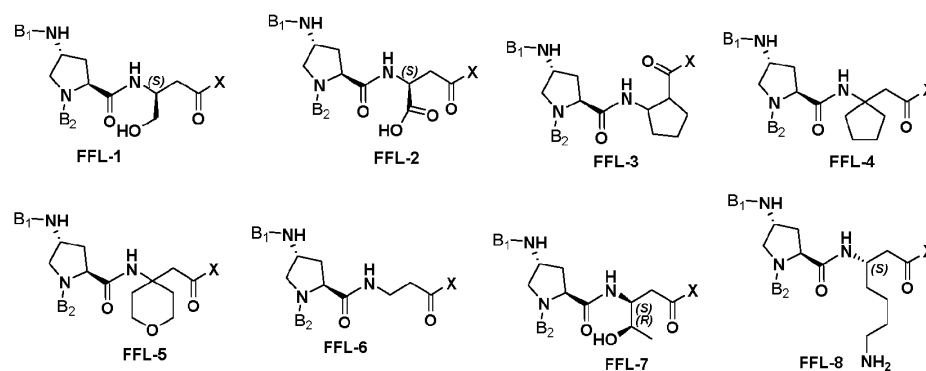
- 10 Y_9 is H, CH_3 , or a C_1 - C_6 alkyl group, provided that when Y_8 is O, the Y_9 is a CH_3 or a C_1 - C_6 alkyl group; and

i is 1, 2, or 3.

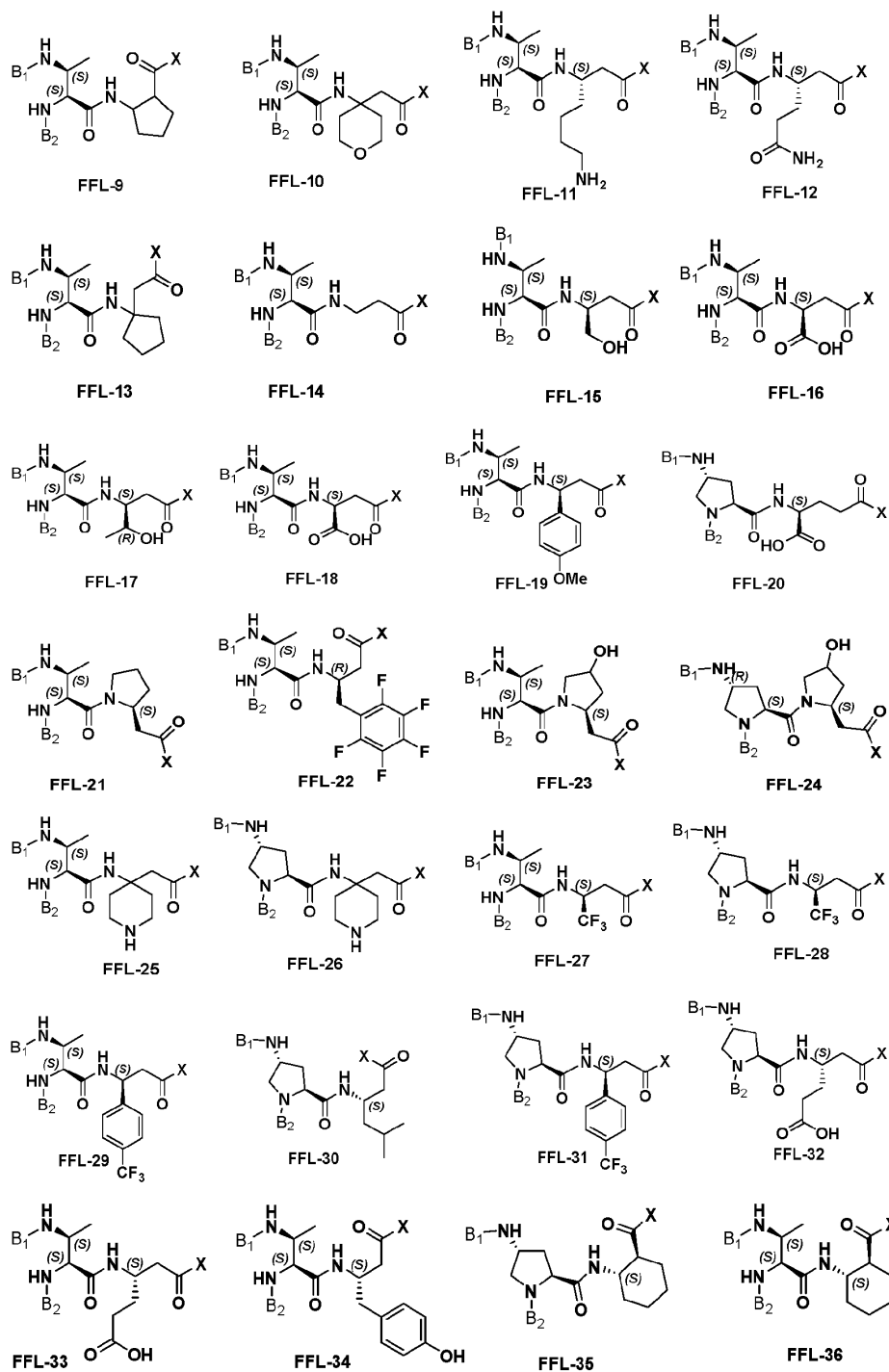
7. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein (a) each remaining R_1 is independently selected from (i) H, CF_3 , and F; or (b) two remaining R_1 are H and one remaining R_1 is CF_3 or F.

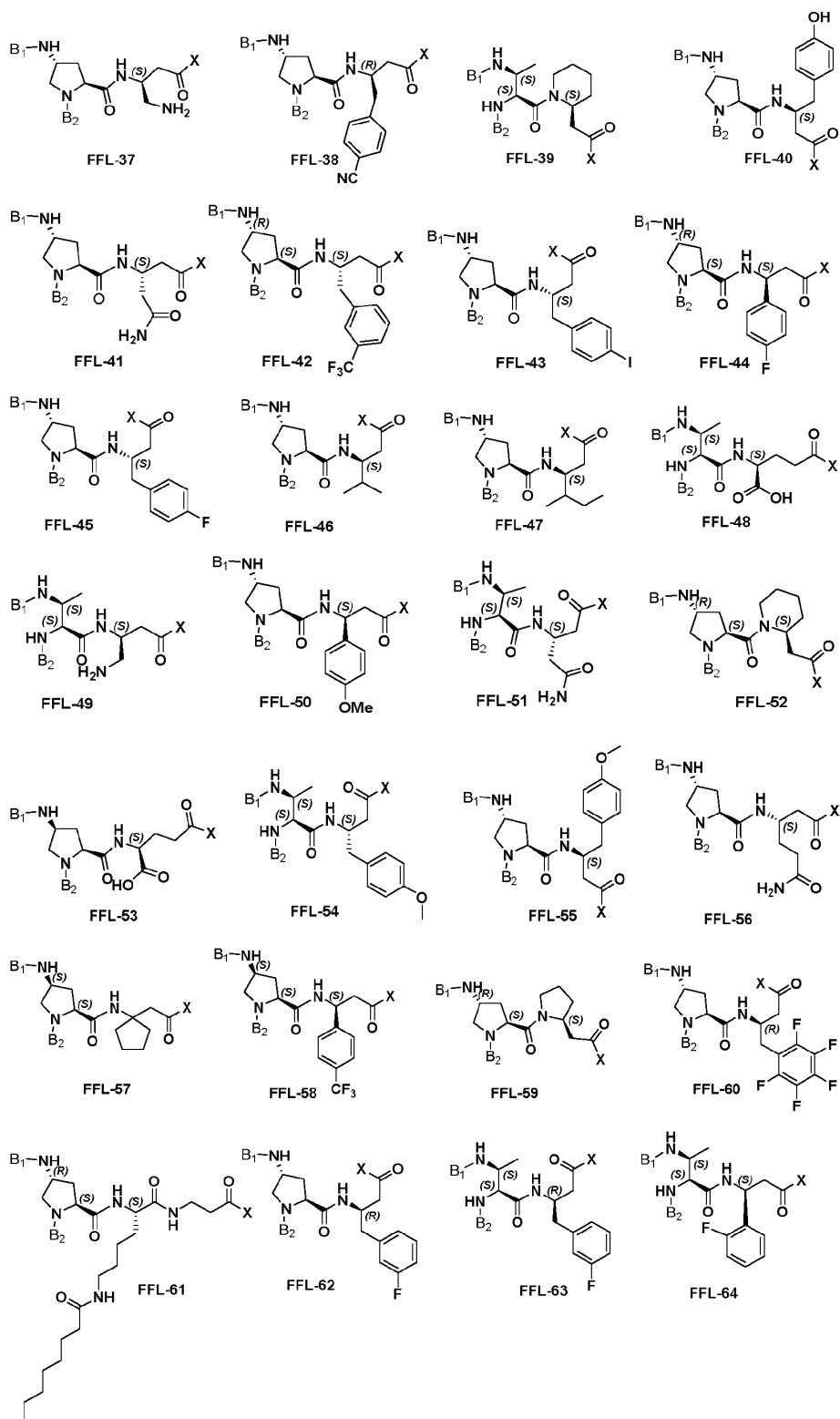
- 15 8. The compound of any one of claims 1-7, or a pharmaceutically acceptable salt thereof, wherein each Z1c is covalently conjugated to an indirect linker and each of the Z1c and the indirect linker in combination is

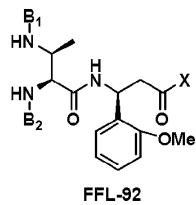
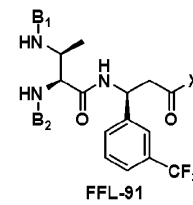
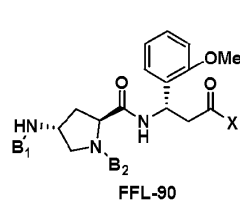
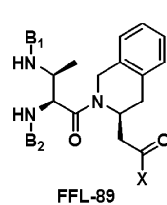
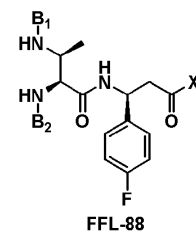
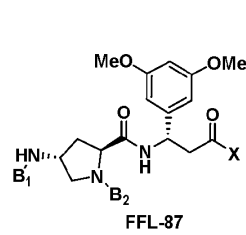
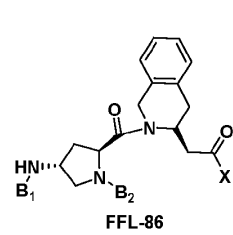
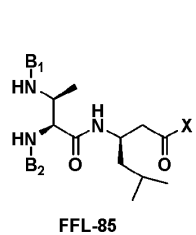
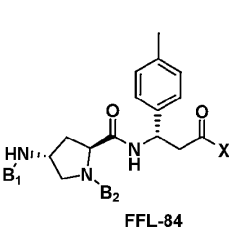
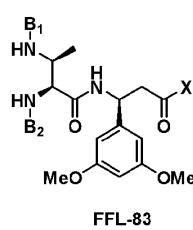
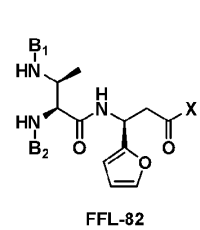
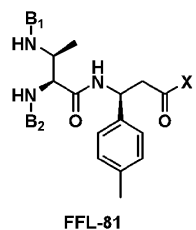
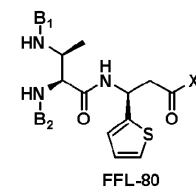
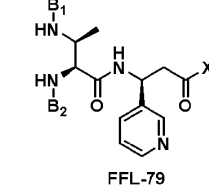
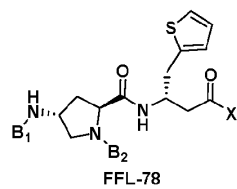
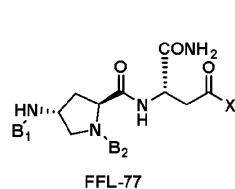
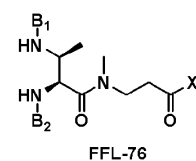
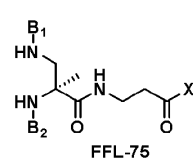
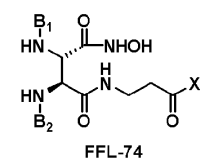
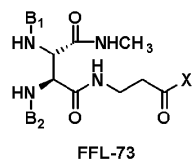
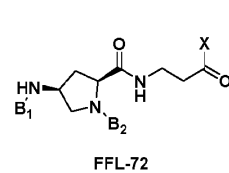
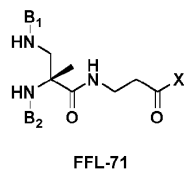
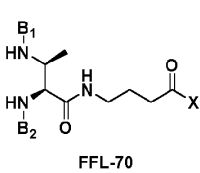
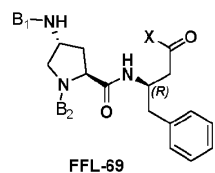
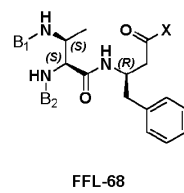
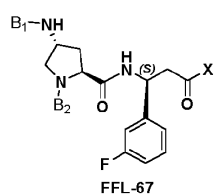
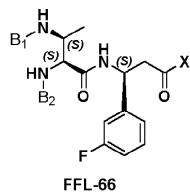
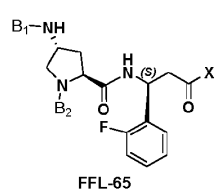
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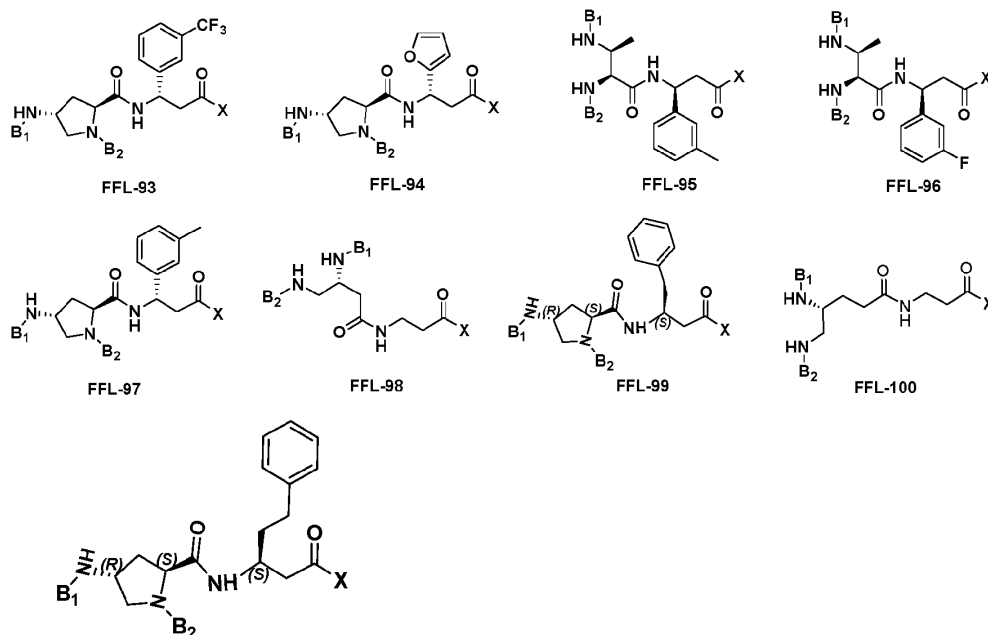


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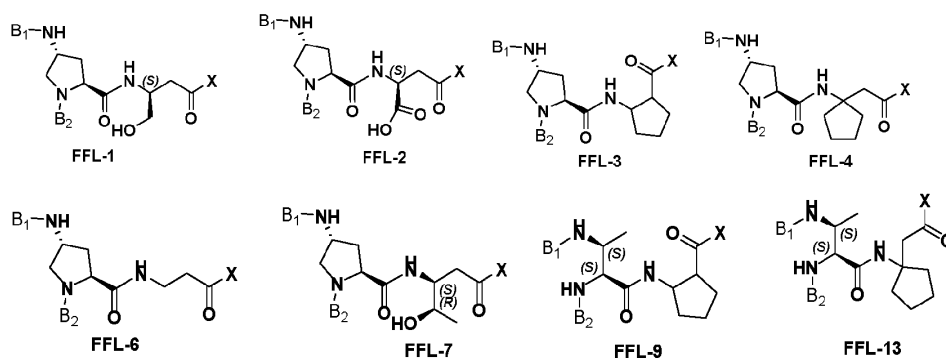




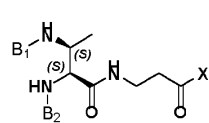
and FFL-101, and stereoisomers thereof;

wherein X represents a point of covalent attachment either directly to an amine of X1 or to
 5 NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated
 directly or indirectly to X1 or to an amine of Z1a.

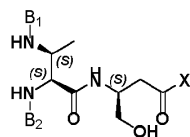
9. The compound of claim 6 or 7, or a pharmaceutically acceptable salt thereof,
 wherein B₁ and the B₂ are each independently Formula F2, wherein each remaining R₁ is
 10 independently selected from H, CF₃, and F; wherein each Z1c is covalently conjugated to an
 indirect linker and each of the Z1c and the indirect linker in combination is selected from
 Formulae FFL-1-4, 6-7, 9, 13-16, 20-23, 28-29, 31-33, 39, 42-45, 48, 53, 57-58, 60-62, 65, and
 67:



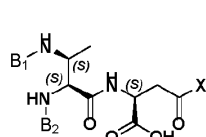
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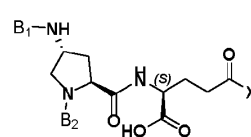
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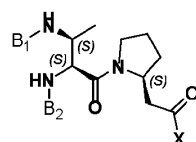
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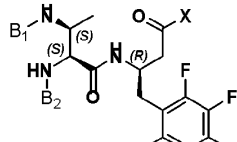
FFL-16



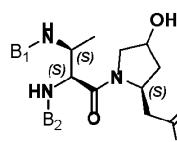
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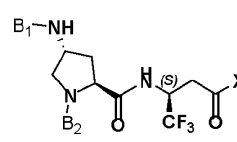
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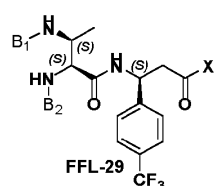
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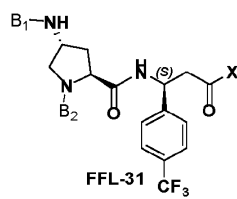
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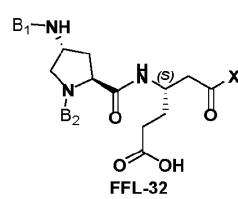
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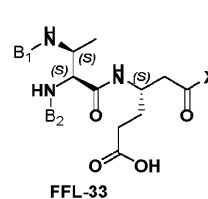
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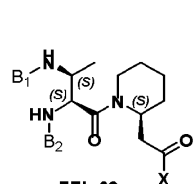
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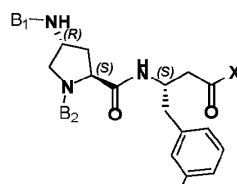
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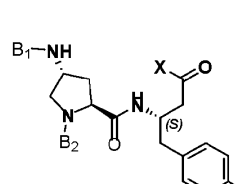
FFL-33



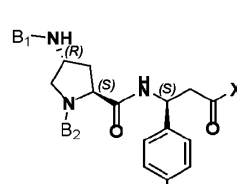
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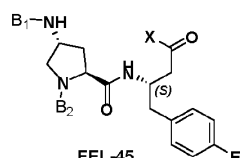
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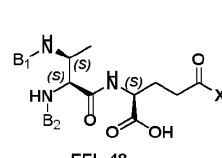
FFL-43



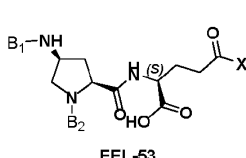
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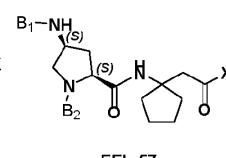
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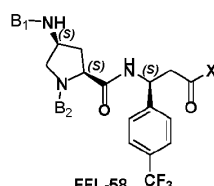
FFL-48



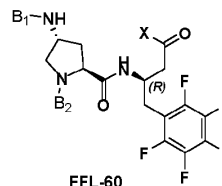
FFL-53



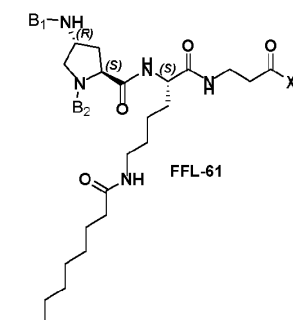
FFL-57



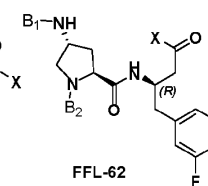
FFL-58



FFL-60

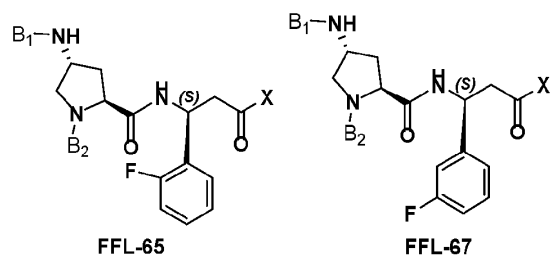


FFL-61



FFL-62

5



, and stereoisomers thereof;

wherein X represents a point of covalent attachment either directly to an amine of X1 or to NH₂ when X1 is NH₂ or to OH when X1 is OH or to an amine that is covalently conjugated directly or indirectly to X1 or to an amine of Z1a.

10. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein X1 is a polypeptide drug substance comprising one or more lysine amino acids each independently conjugated, directly or via an indirect linker, to a Z1c.

11. The compound of any one of claims 1-10, or a pharmaceutically acceptable salt thereof, wherein X1 comprises a polypeptide human hormone, an insulin receptor agonist, an endocrine hormone, insulin, human insulin, glucagon, amylin, relaxin, GLP-1, oxyntomodulin, somatostatin, gastric inhibitory polypeptide, glucose-dependent insulinotropic polypeptide, a hybrid peptide comprising sequences from two or more human polypeptide hormones, or an analogue of any thereof.

12. The compound of any one of claims 1-11, or a pharmaceutically acceptable salt thereof, wherein:

X1 comprises an insulin having an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 1, 25, 24051, and 24052, and the B-chain comprises a sequence selected from SEQ ID NOs 24060, 24061, 24062, 24063, 24064, and 25000-25397.

13. The compound of claim 12, or a pharmaceutically acceptable salt thereof, wherein:

X1 comprises an insulin having an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from SEQ ID NOs 24051 and 24052, and

wherein the B-chain comprises a sequence selected from SEQ ID NOs 25095, 25229, 25232, 25236, 25305, 25308, 25312, and 25380-25397;

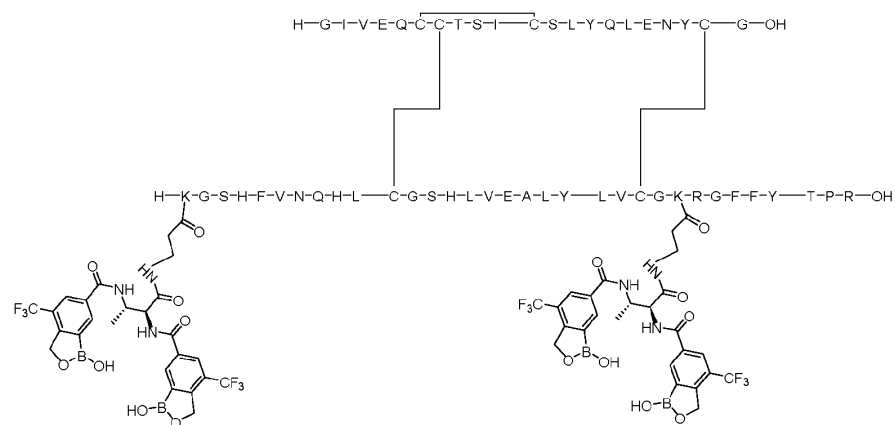
each Z1c is independently selected from FF12A, FF12B, FF12C, FF12D, FF116A, FF116B, FF116C, and FF116D, and covalently conjugated either directly or via the indirect linker to one or more lysine residues in X1;

B₁ and B₂ is each independently Formula F2; and

the indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL20-FL75, and stereoisomers thereof.

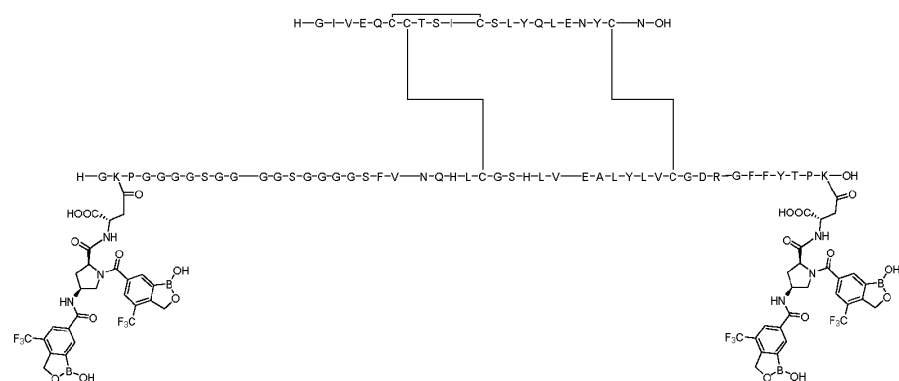
14. The compound of claim 11, wherein the compound is selected from:

Example 1 (SEQ ID NOS 25398 and 25399, respectively, in order of appearance):

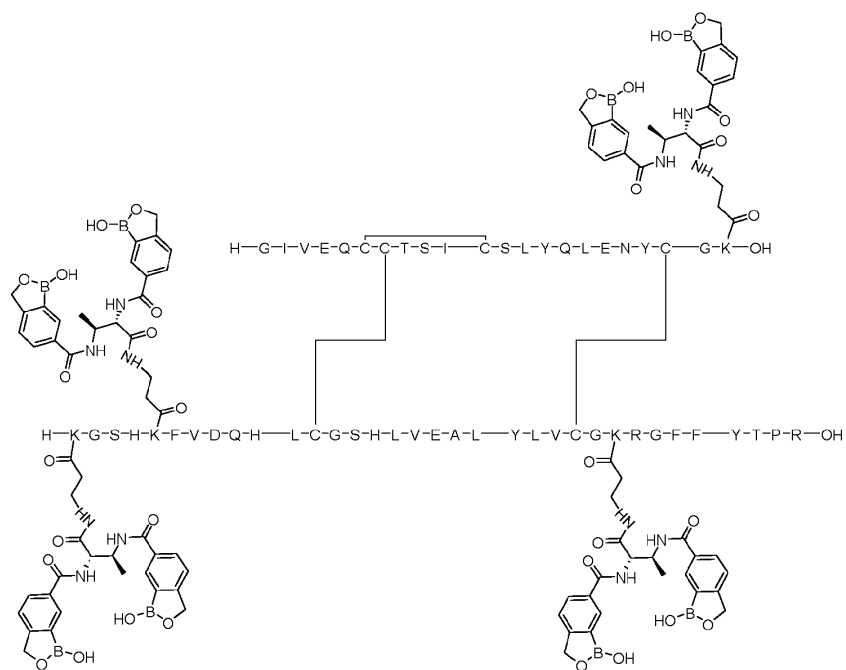


5

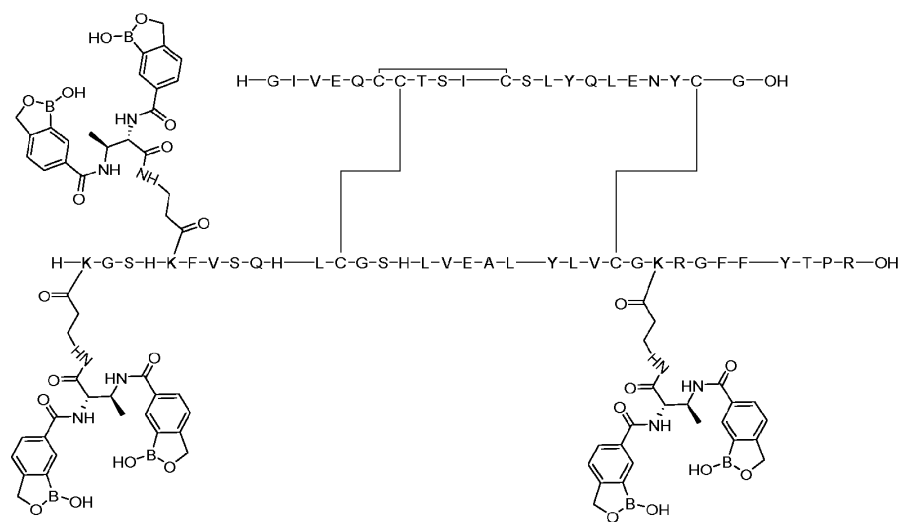
Example 2 (SEQ ID NOS 25400 and 25401, respectively, in order of appearance):



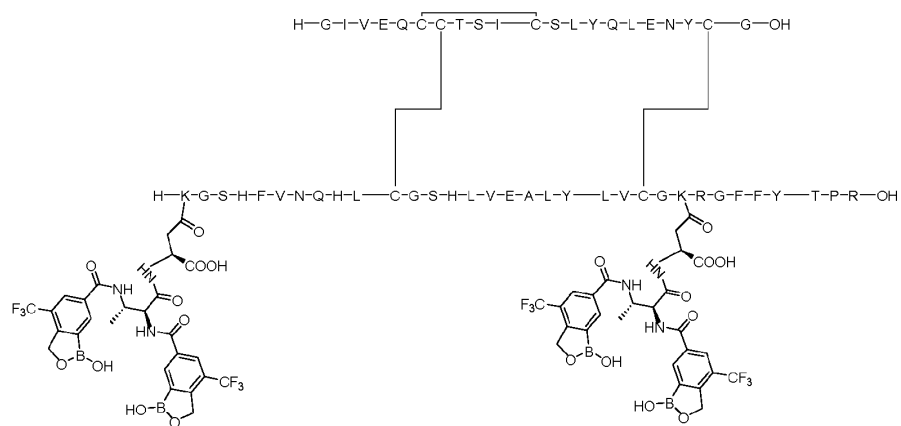
Example 3 (SEQ ID NOS 25402 and 25403, respectively, in order of appearance):



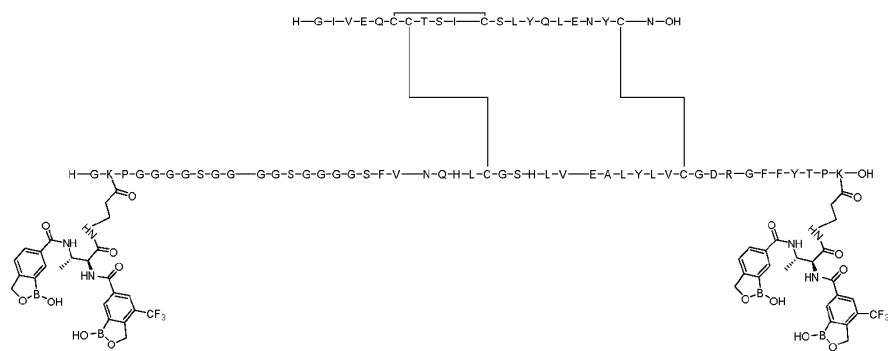
Example 4 (SEQ ID NOS 25404 and 25405, respectively, in order of appearance):



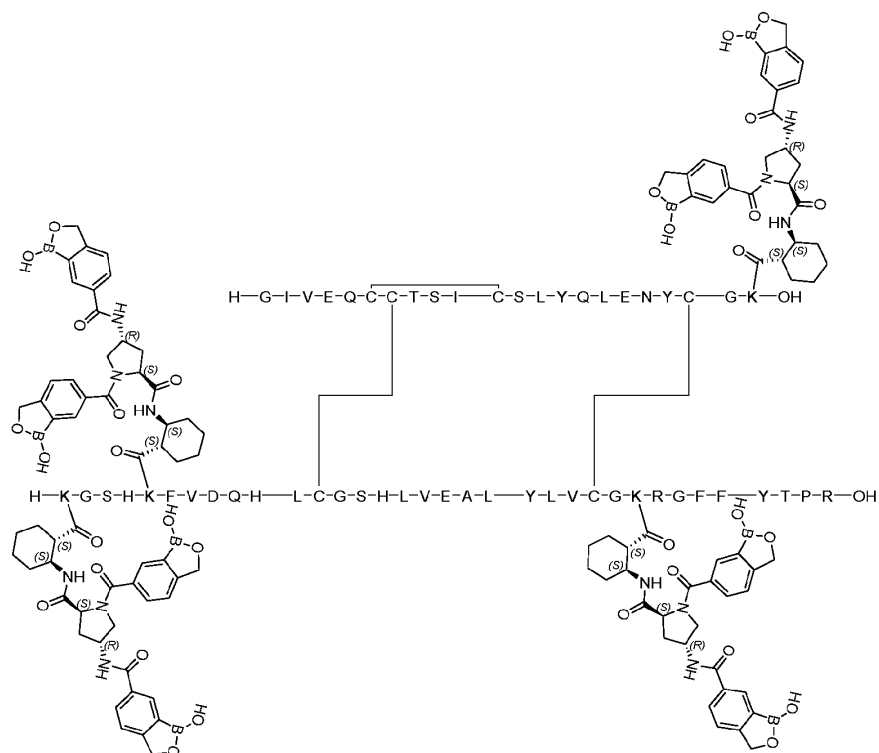
Example 5 (SEQ ID NOS 25406 and 25407, respectively, in order of appearance):



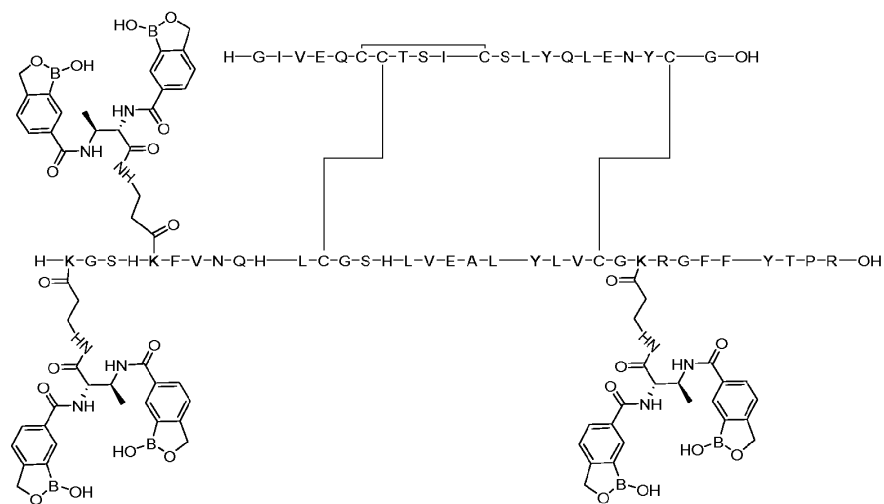
Example 6 (SEQ ID NOS 25408 and 25409, respectively, in order of appearance):



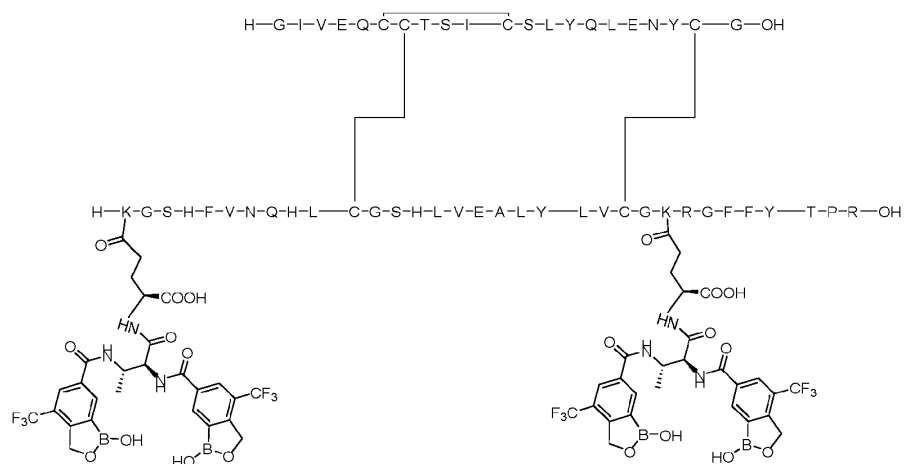
Example 7 (SEQ ID NOS 25410 and 25411, respectively, in order of appearance):



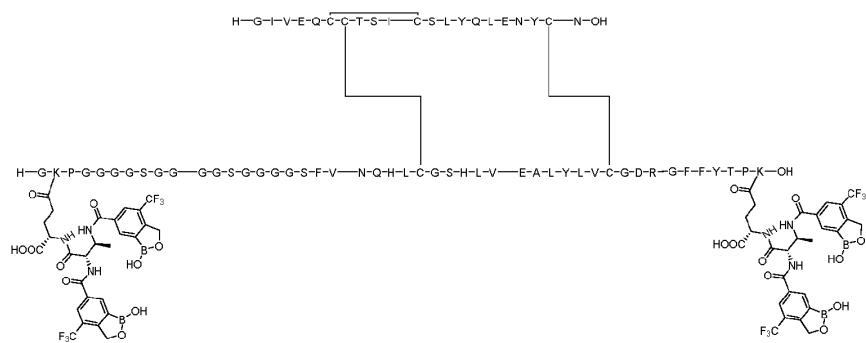
Example 8 (SEQ ID NOS 25412 and 25413, respectively, in order of appearance):



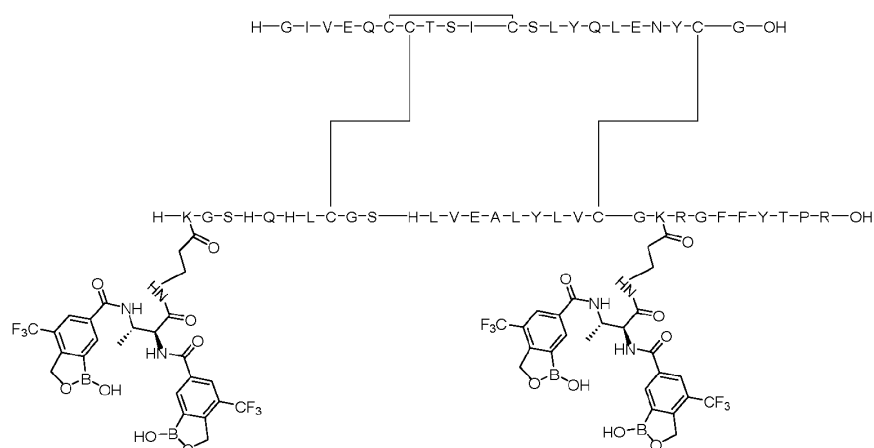
Example 9 (SEQ ID NOS 25414 and 25415, respectively, in order of appearance):



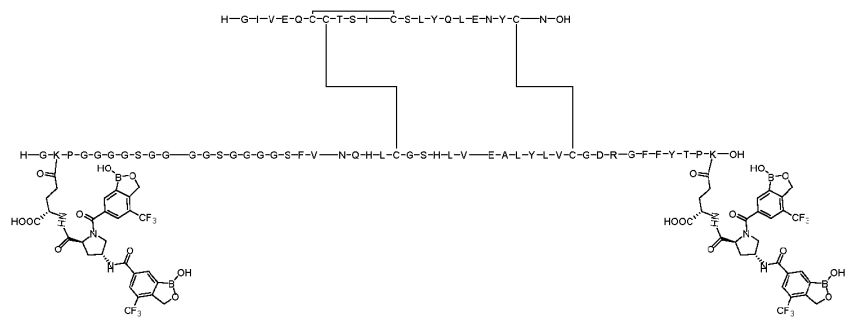
5 Example 11 (SEQ ID NOS 25418 and 25419, respectively, in order of appearance):



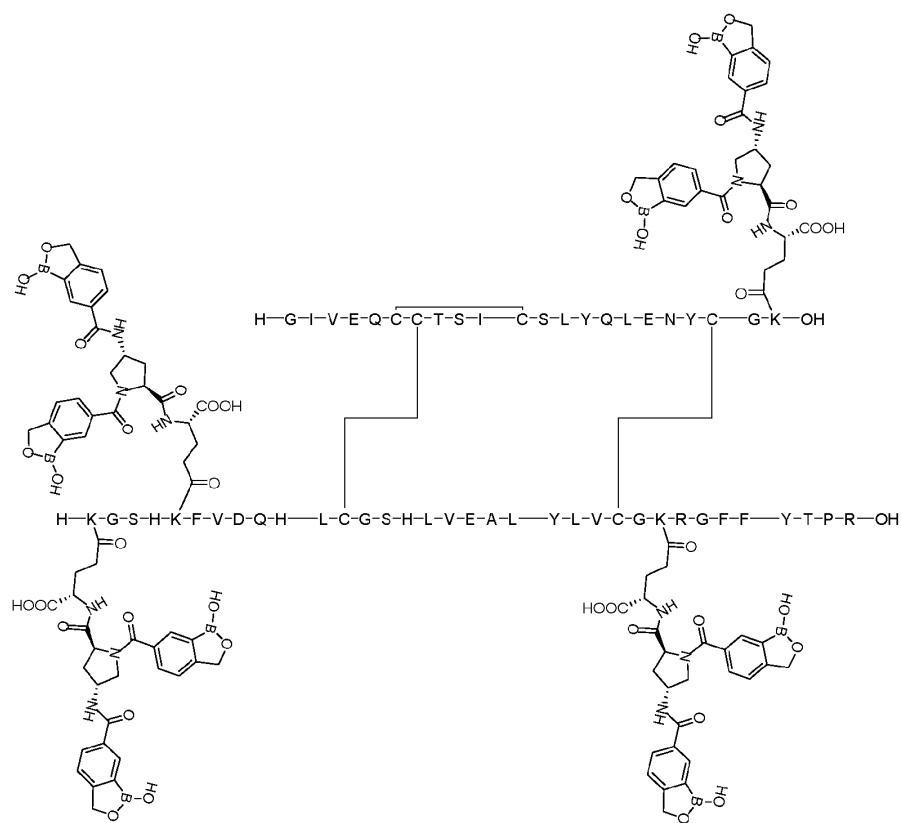
Example 12 (SEQ ID NOS 25420 and 25421, respectively, in order of appearance):



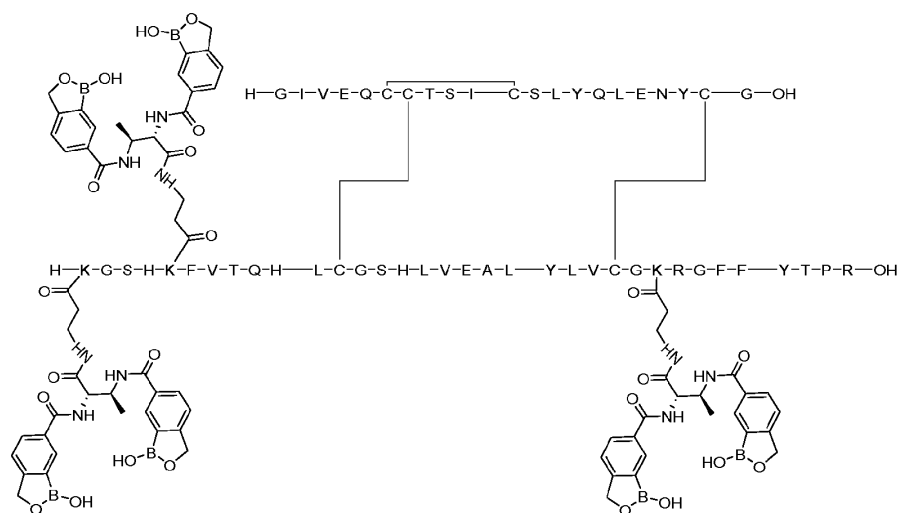
Example 13 (SEQ ID NOS 25422 and 25423, respectively, in order of appearance):



5 Example 14 (SEQ ID NOS 25424 and 25425, respectively, in order of appearance):



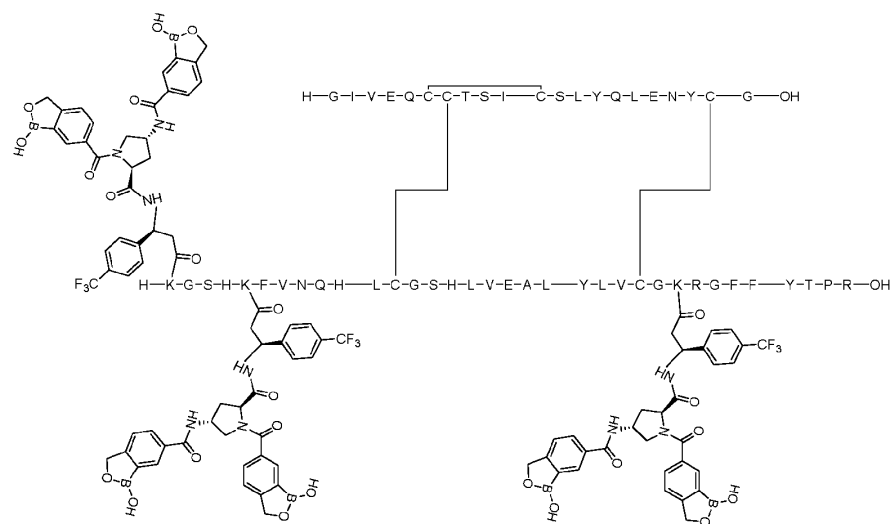
Example 15 (SEQ ID NOS 25426 and 25427, respectively, in order of appearance):



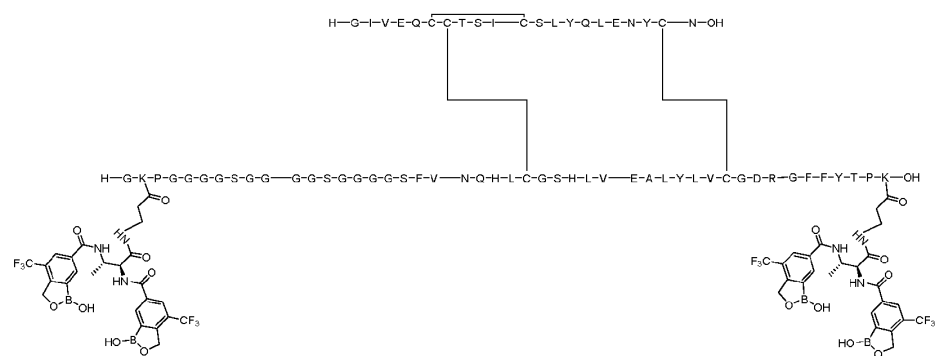
Example 16 (SEQ ID NOS 25428 and 25429, respectively, in order of appearance):



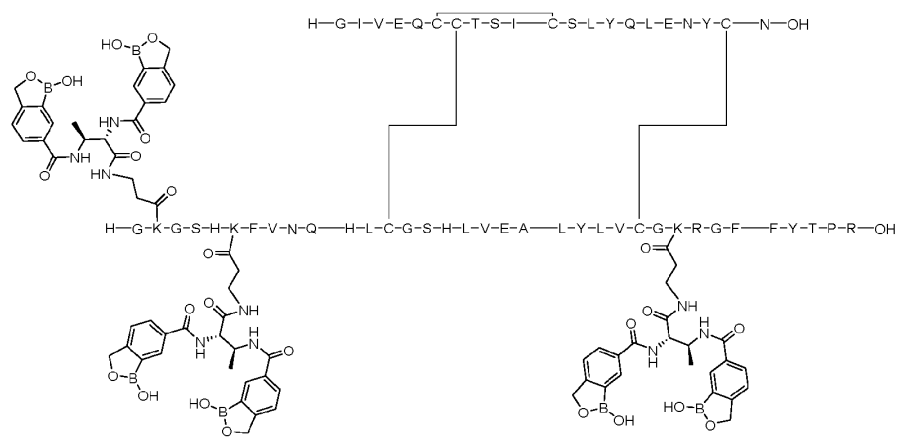
Example 17 (SEQ ID NOS 25430 and 25431, respectively, in order of appearance):



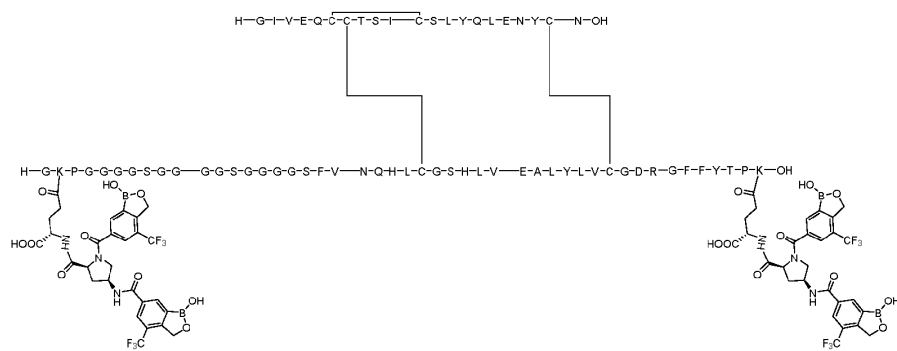
Example 18 (SEQ ID NOS 25432 and 25433, respectively, in order of appearance):



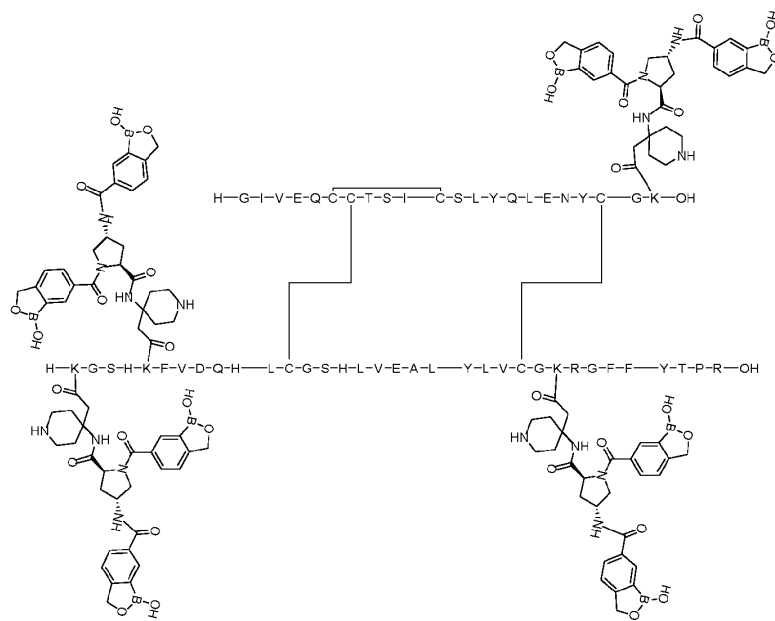
Example 19 (SEQ ID NOS 25434 and 25435, respectively, in order of appearance):



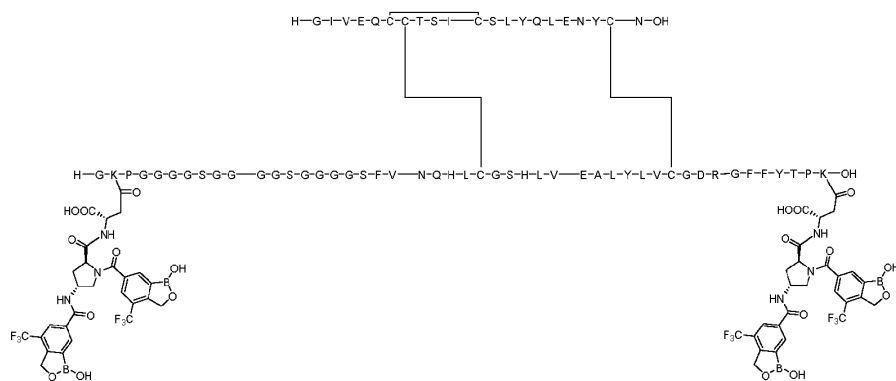
Example 20 (SEQ ID NOS 25436 and 25437, respectively, in order of appearance):



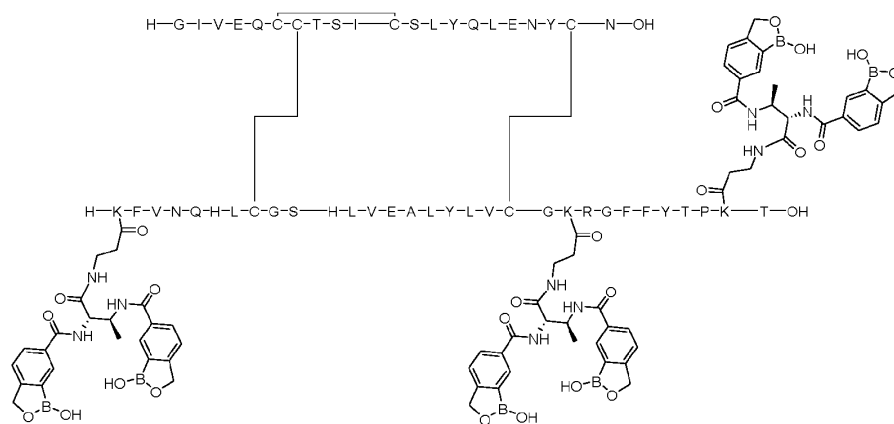
Example 21 (SEQ ID NOS 25438 and 25439, respectively, in order of appearance):



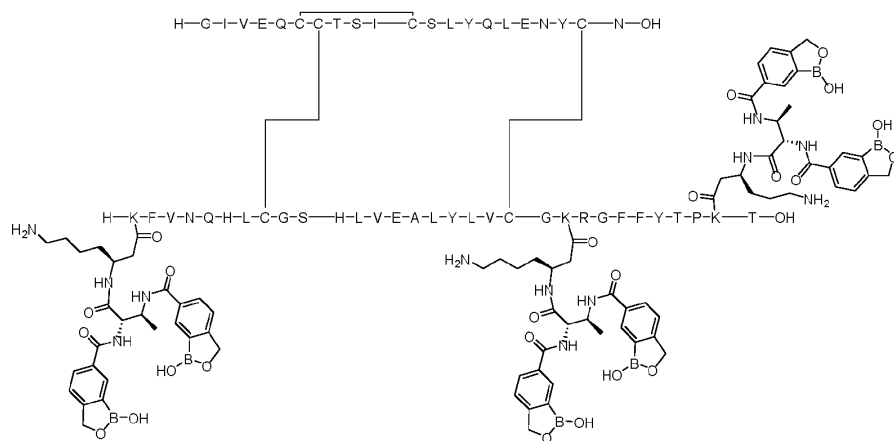
Example 22 (SEQ ID NOS 25422 and 25423, respectively, in order of appearance):



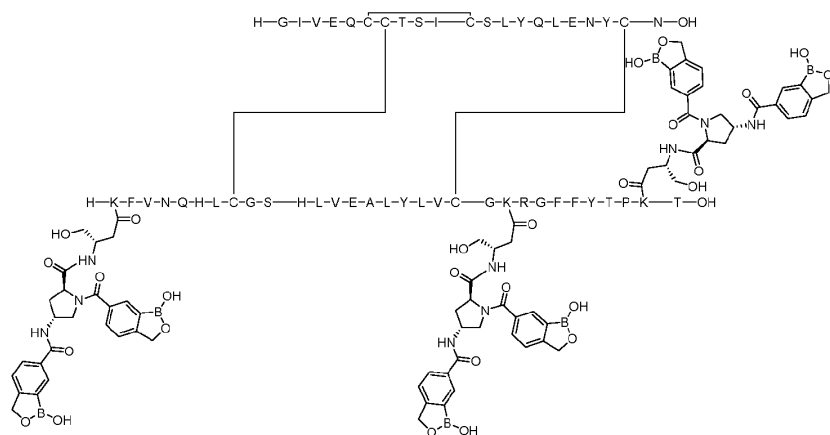
Example 23 (SEQ ID NOS 25440 and 25441, respectively, in order of appearance):



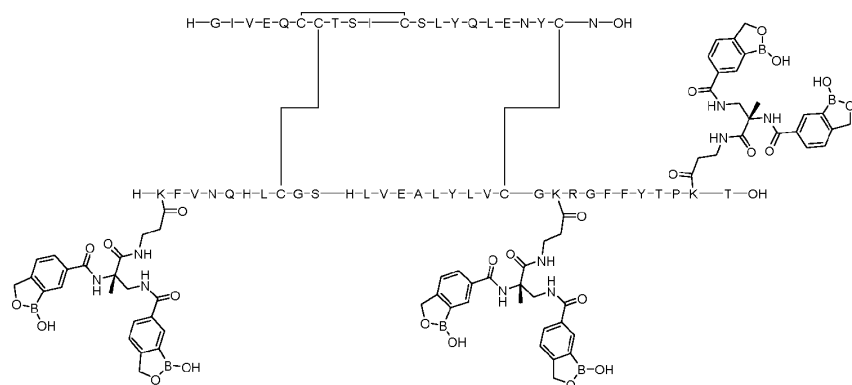
Example 24 (SEQ ID NOS 25442 and 25443, respectively, in order of appearance):



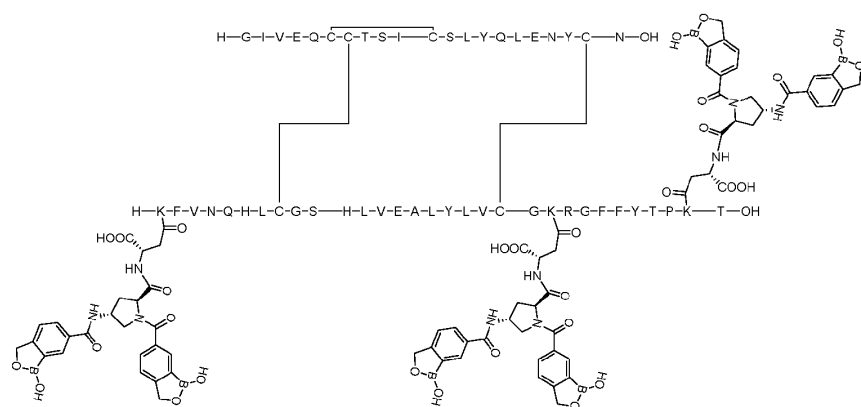
Example 26 (SEQ ID NOS 25446 and 25447, respectively, in order of appearance):



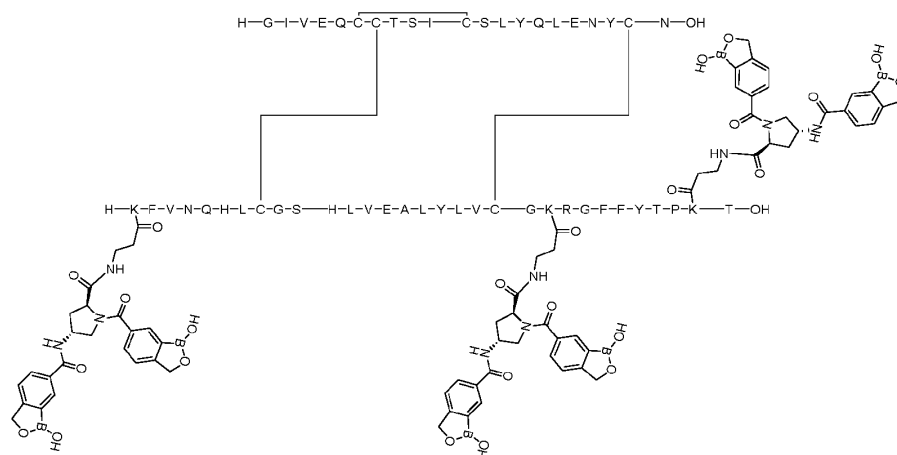
Example 27 (SEQ ID NOS 25448 and 25449, respectively, in order of appearance):



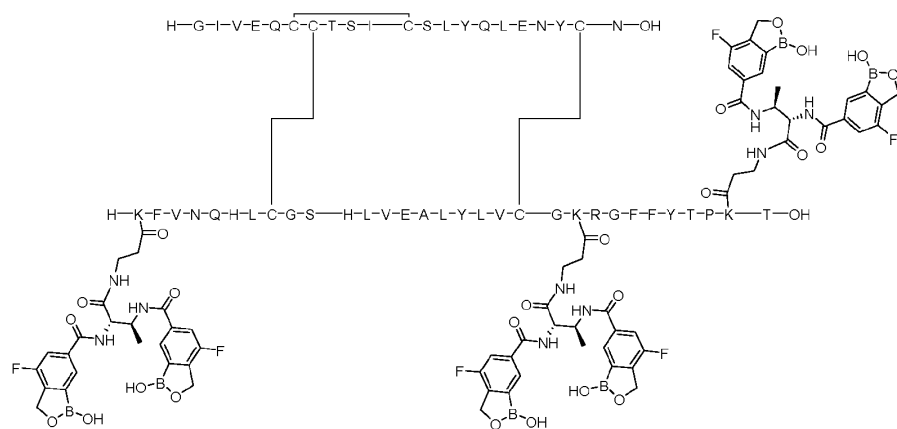
Example 28 (SEQ ID NOS 25450 and 25451, respectively, in order of appearance):



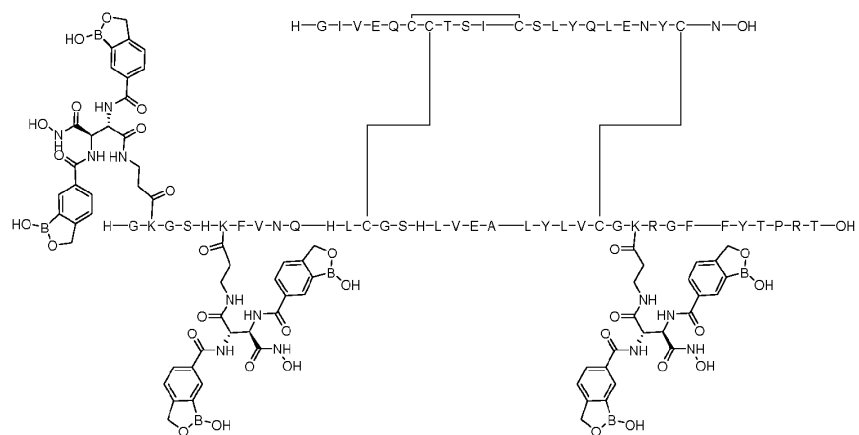
Example 29 (SEQ ID NOS 25452 and 25453, respectively, in order of appearance):



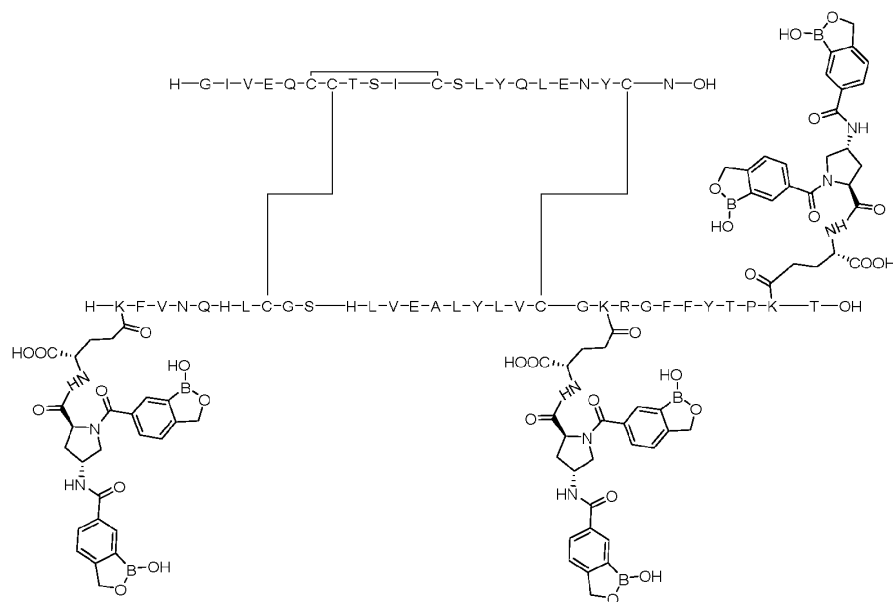
Example 30 (SEQ ID NOS 25454 and 25455, respectively, in order of appearance):



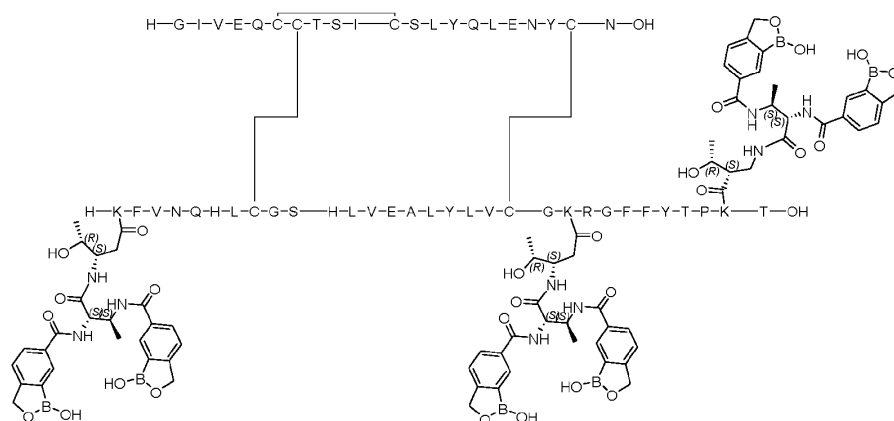
Example 31 (SEQ ID NOS 25456 and 25457, respectively, in order of appearance):



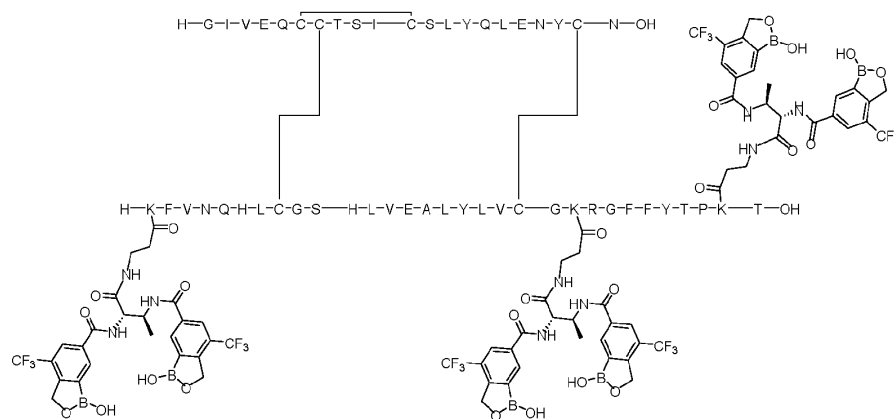
Example 32 (SEQ ID NOS 25458 and 25459, respectively, in order of appearance):



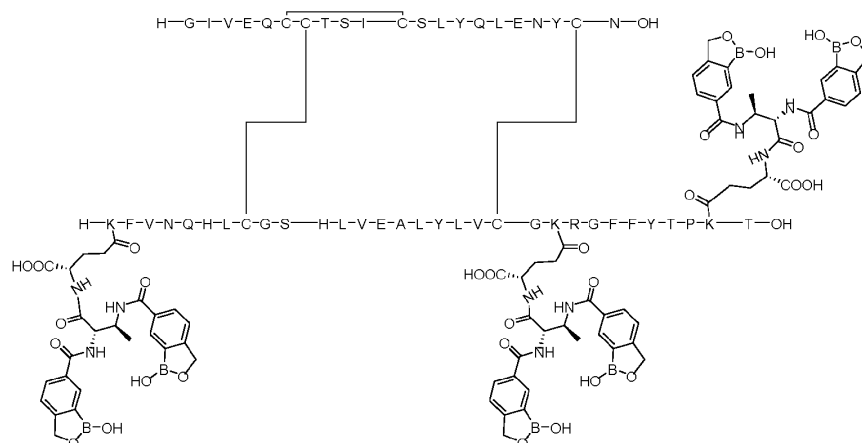
Example 33 (SEQ ID NOS 25460 and 25461, respectively, in order of appearance):



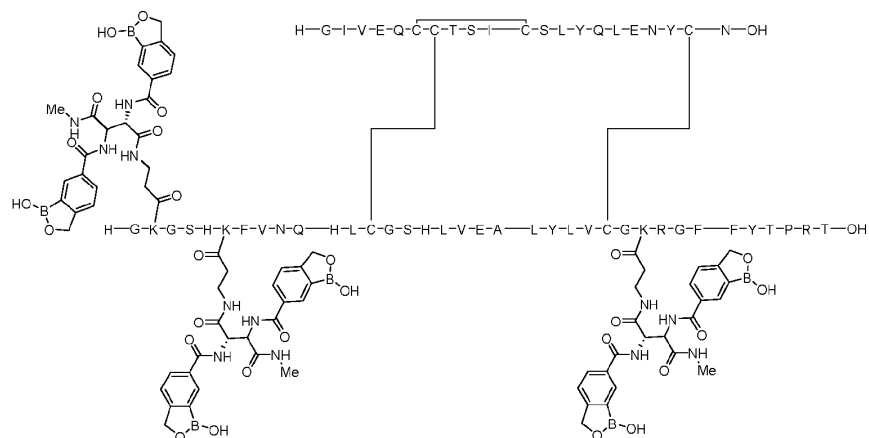
Example 34 (SEQ ID NOS 25462 and 25463, respectively, in order of appearance):



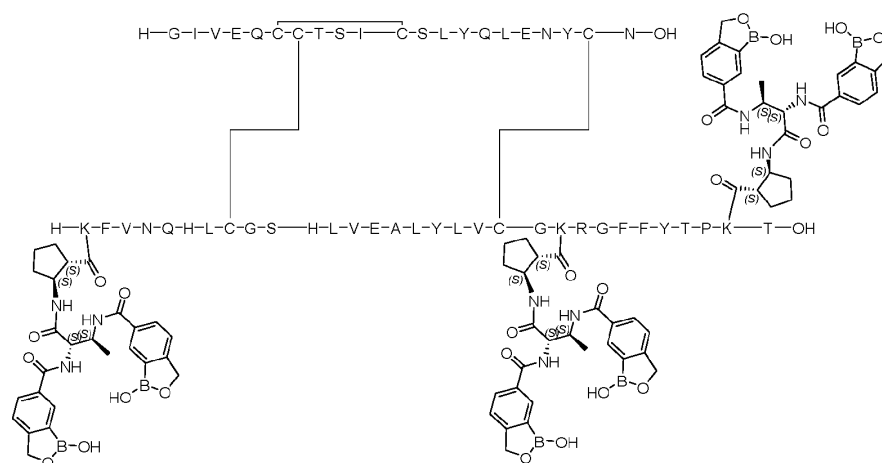
Example 35 (SEQ ID NOS 25464 and 25465, respectively, in order of appearance):



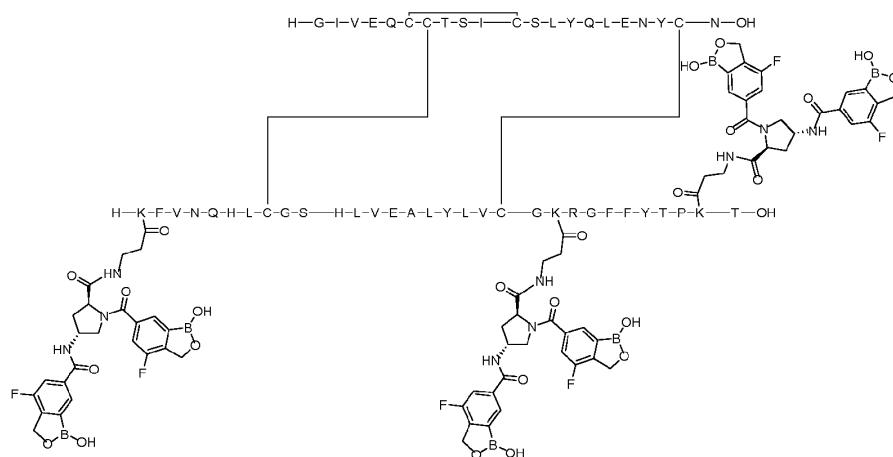
Example 36 (SEQ ID NOS 25466 and 25467, respectively, in order of appearance):



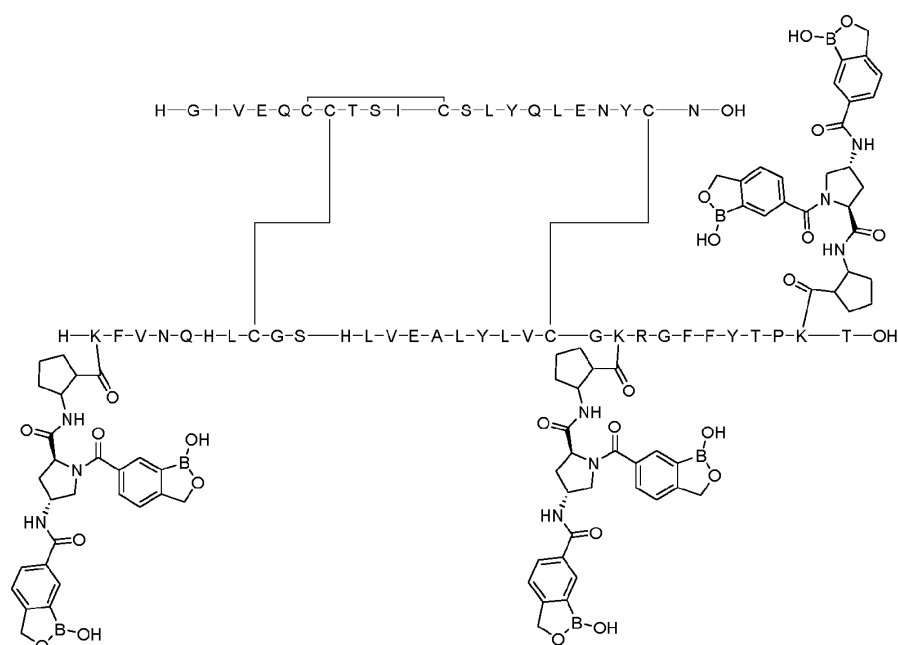
5 Example 37 (SEQ ID NOS 25468 and 25469, respectively, in order of appearance):



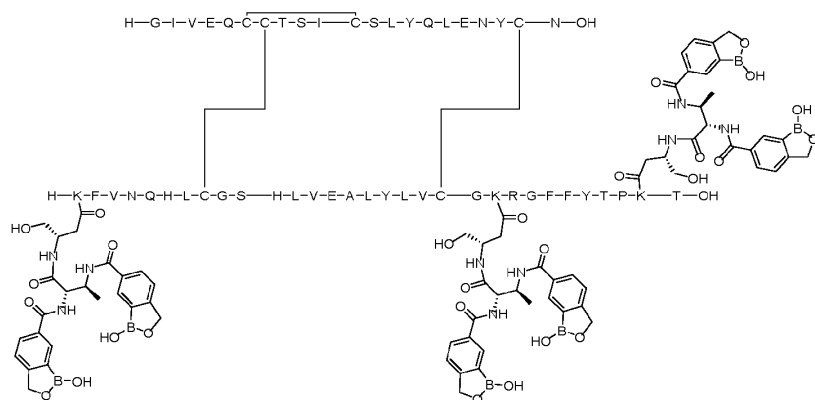
Example 38 (SEQ ID NOS 25470 and 25471, respectively, in order of appearance):



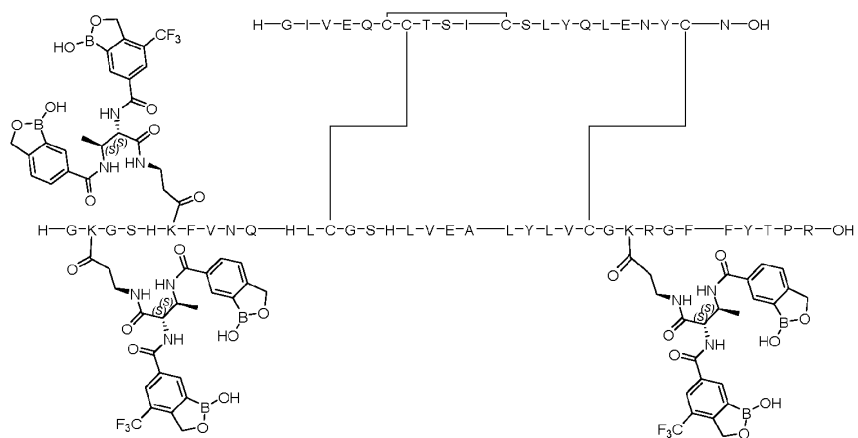
Example 39 (SEQ ID NOS 25472 and 25473, respectively, in order of appearance):



Example 40 (SEQ ID NOS 25474 and 25475, respectively, in order of appearance):

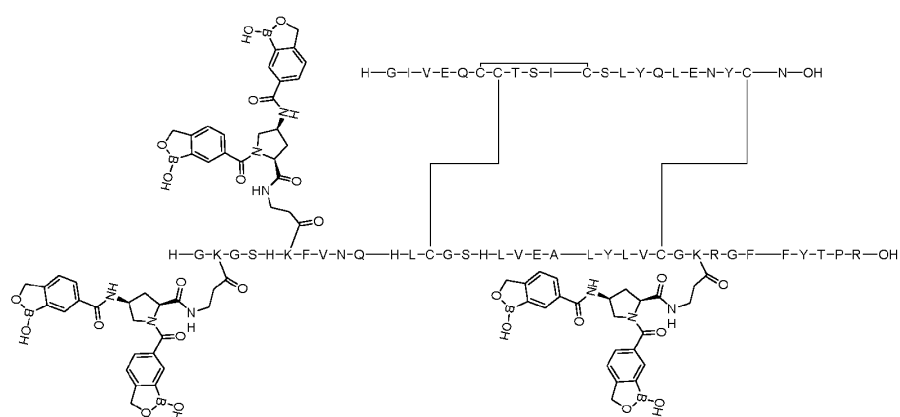


Example 41 (SEQ ID NOS 25476 and 25477, respectively, in order of appearance):

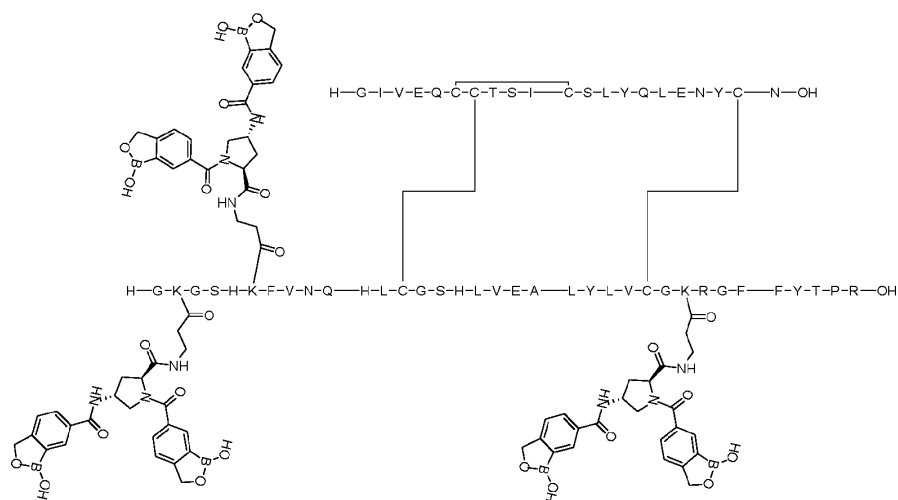


5

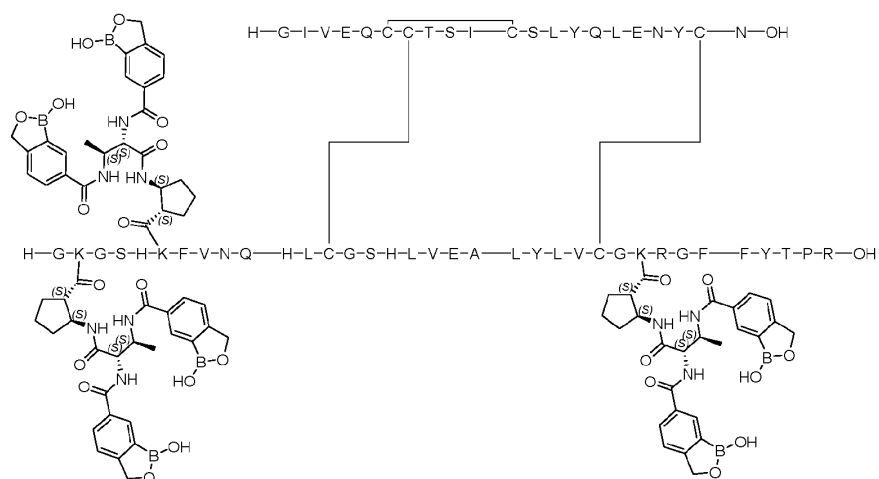
Example 42 (SEQ ID NOS 25478 and 25479, respectively, in order of appearance):



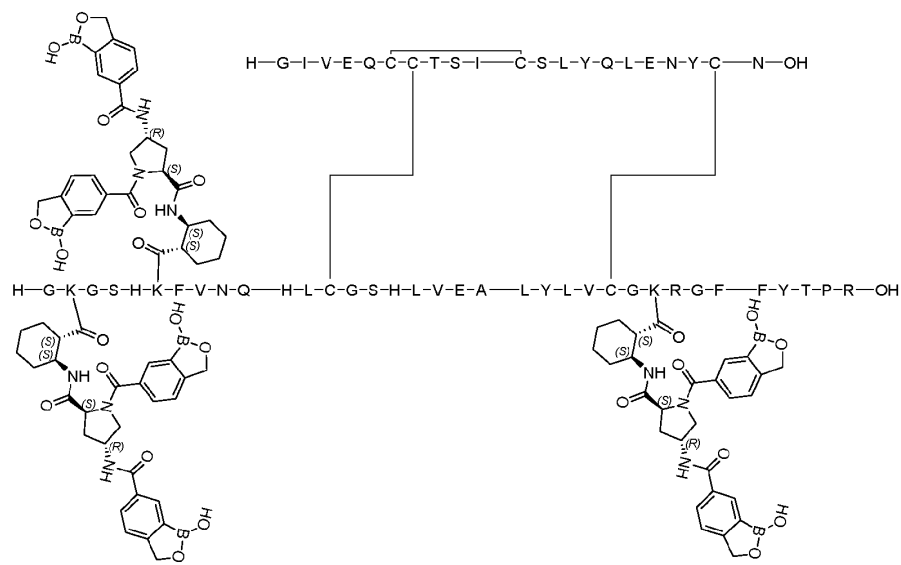
Example 43 (SEQ ID NOS 25480 and 25481, respectively, in order of appearance):



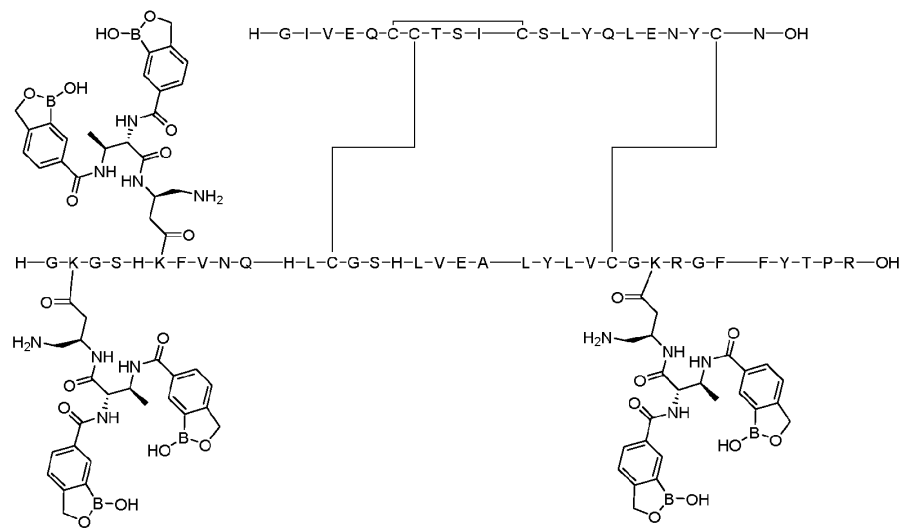
Example 44 (SEQ ID NOS 25482 and 25483, respectively, in order of appearance):



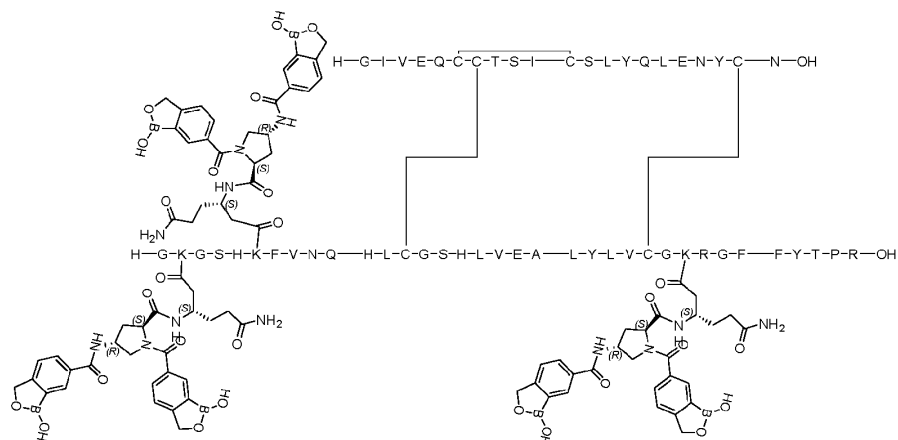
Example 45 (SEQ ID NOS 25484 and 25485, respectively, in order of appearance):



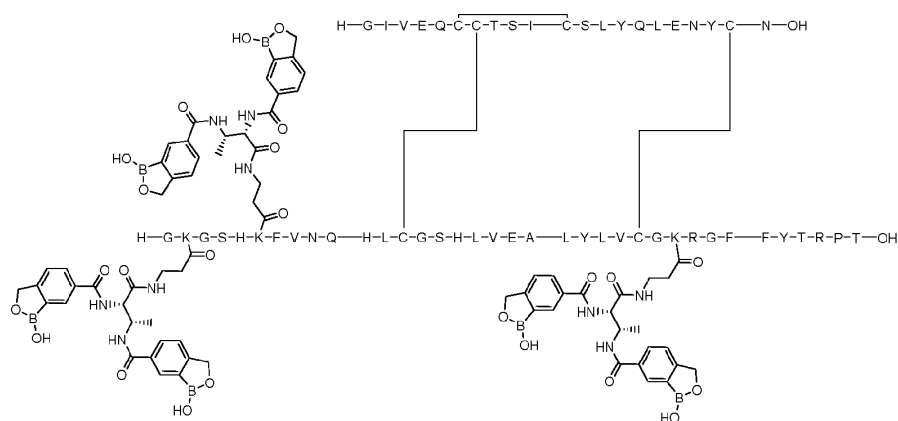
Example 46 (SEQ ID NOS 25486 and 25487, respectively, in order of appearance):



Example 47 (SEQ ID NOS 25488 and 25489, respectively, in order of appearance):

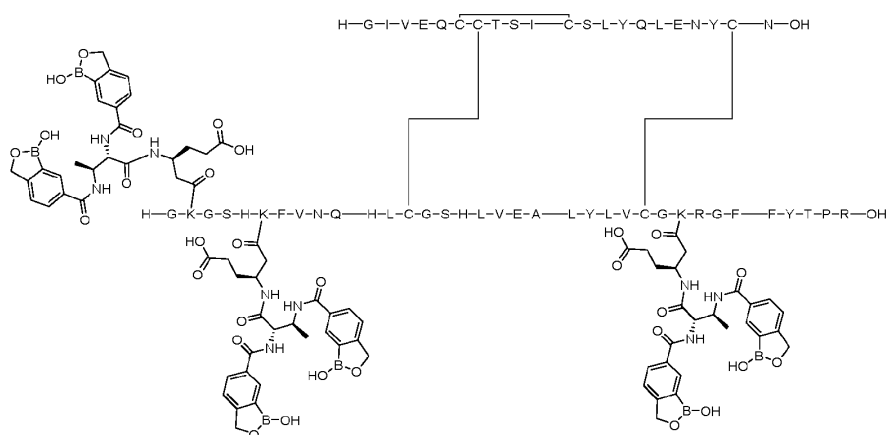


Example 48 (SEQ ID NOS 25490 and 25491, respectively, in order of appearance):

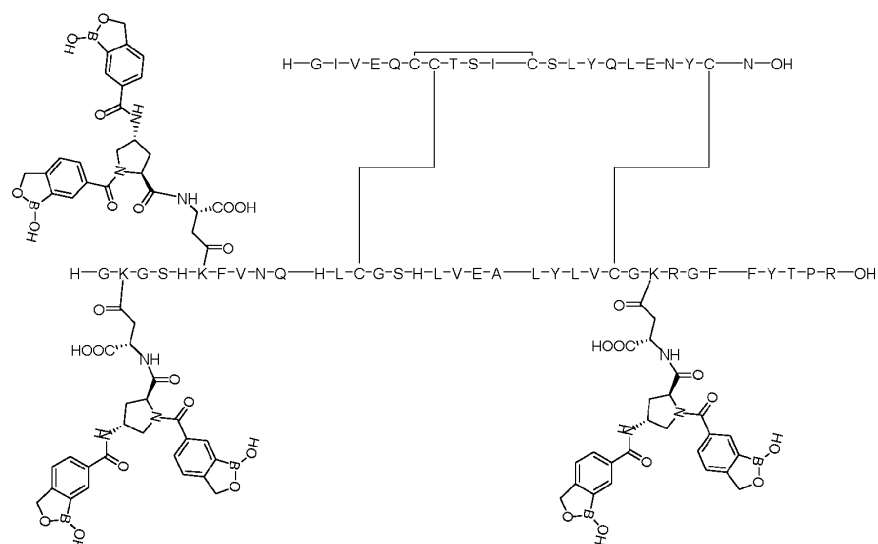


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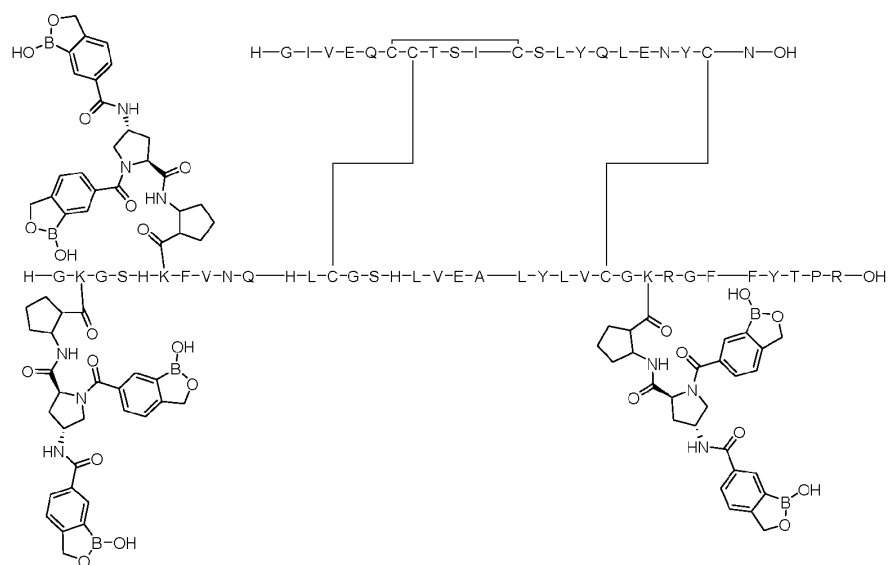
Example 49 (SEQ ID NOS 25492 and 25493, respectively, in order of appearance):



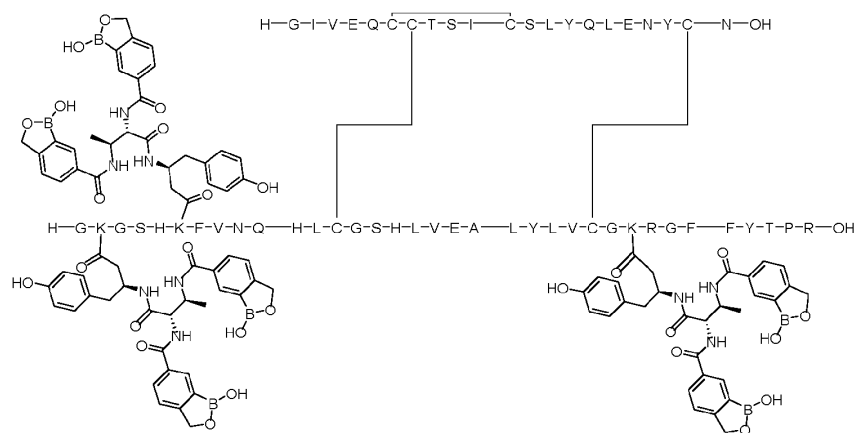
Example 50 (SEQ ID NOS 25494 and 25495, respectively, in order of appearance):



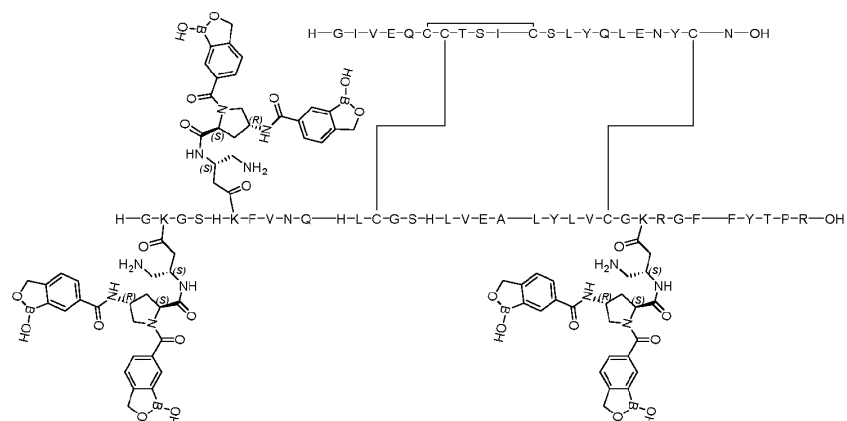
5 Example 51 (SEQ ID NOS 25496 and 25497, respectively, in order of appearance)



Example 52 (SEQ ID NOS 25498 and 25499, respectively, in order of appearance):

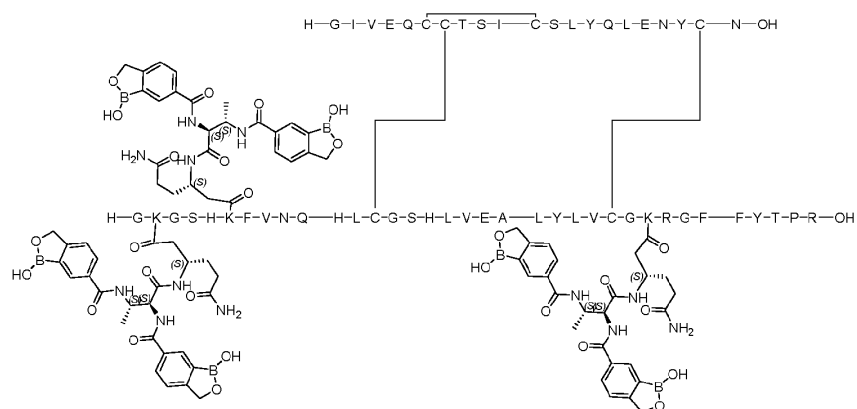


Example 53 (SEQ ID NOS 25500 and 25501, respectively, in order of appearance):

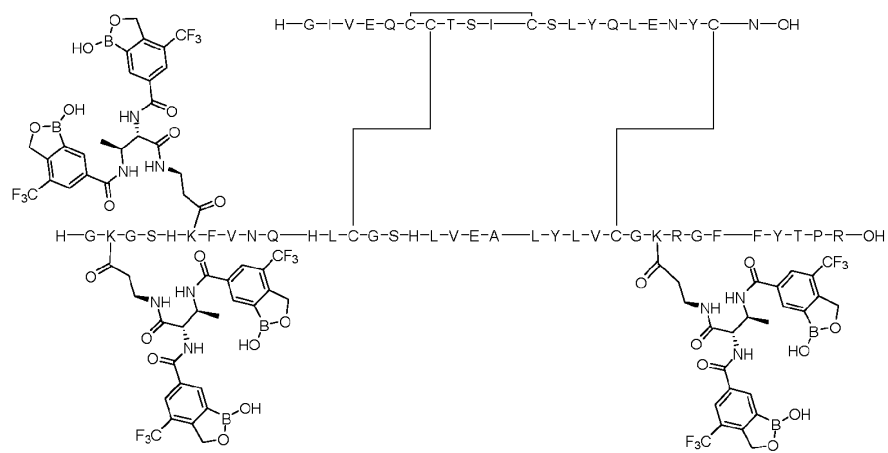


5

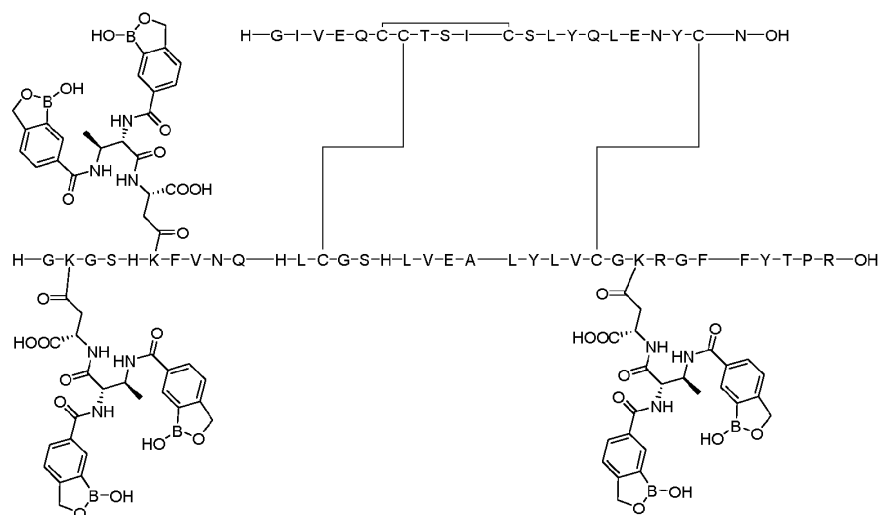
Example 54 (SEQ ID NOS 25502 and 25503, respectively, in order of appearance):



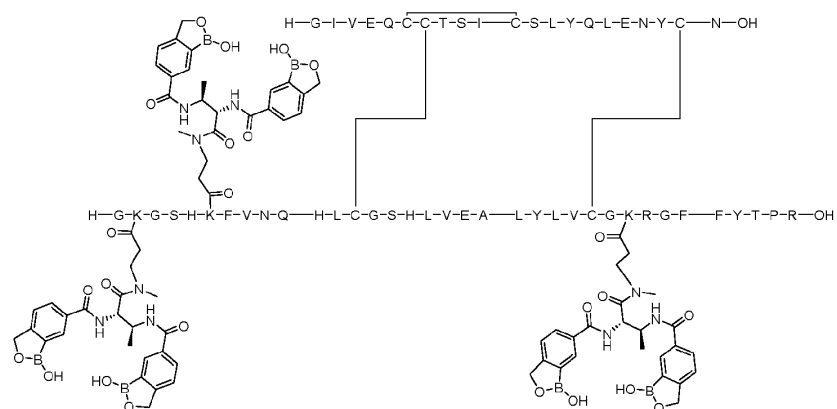
Example 55 (SEQ ID NOS 25504 and 25505, respectively, in order of appearance):



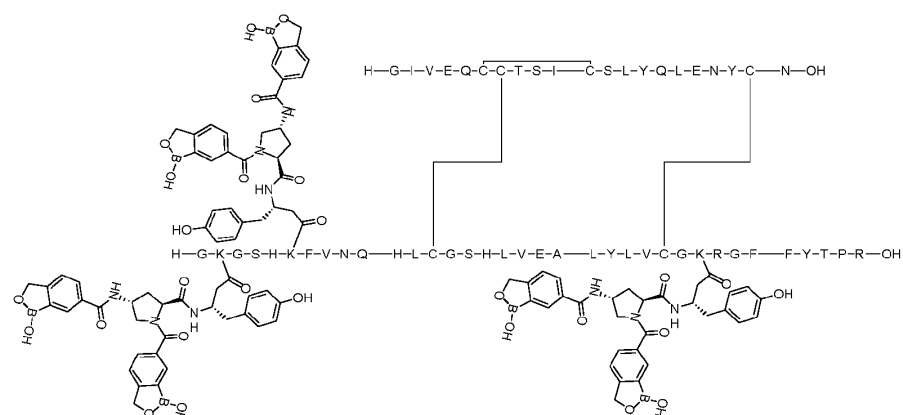
5 Example 56 (SEQ ID NOS 25506 and 25507, respectively, in order of appearance):



Example 57 (SEQ ID NOS 25508 and 25509, respectively, in order of appearance):

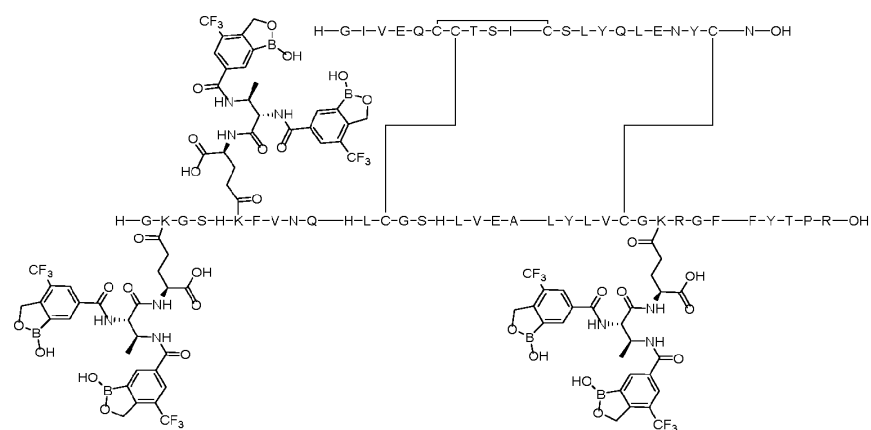


Example 58 (SEQ ID NOS 25510 and 25511, respectively, in order of appearance):

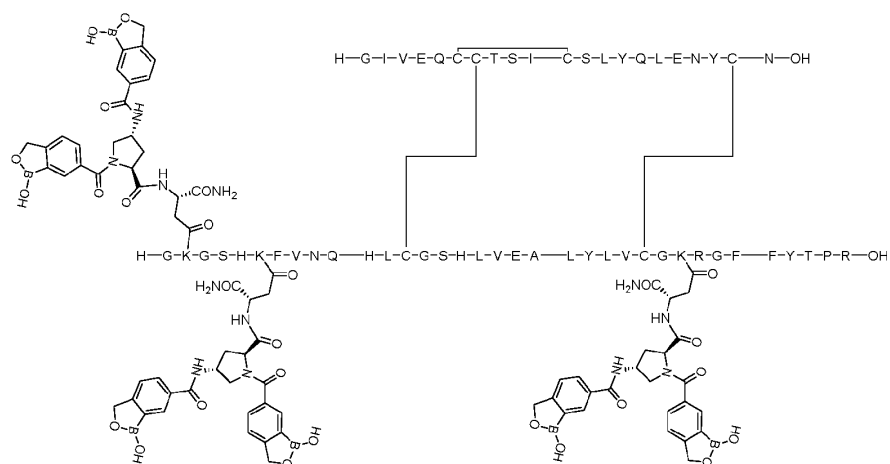


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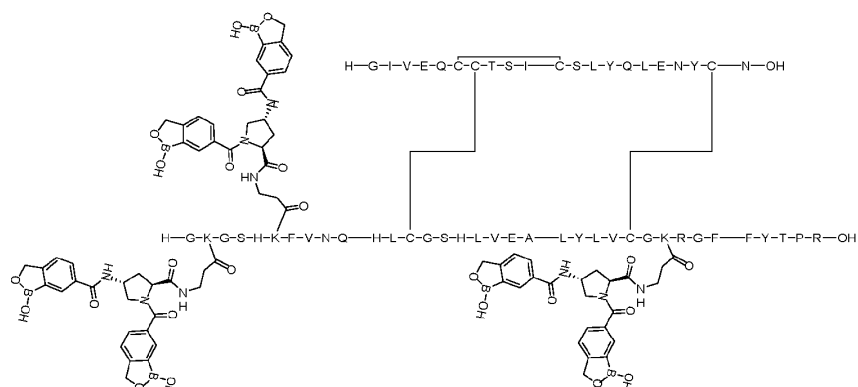
Example 59 (SEQ ID NOS 25512 and 25513, respectively, in order of appearance):



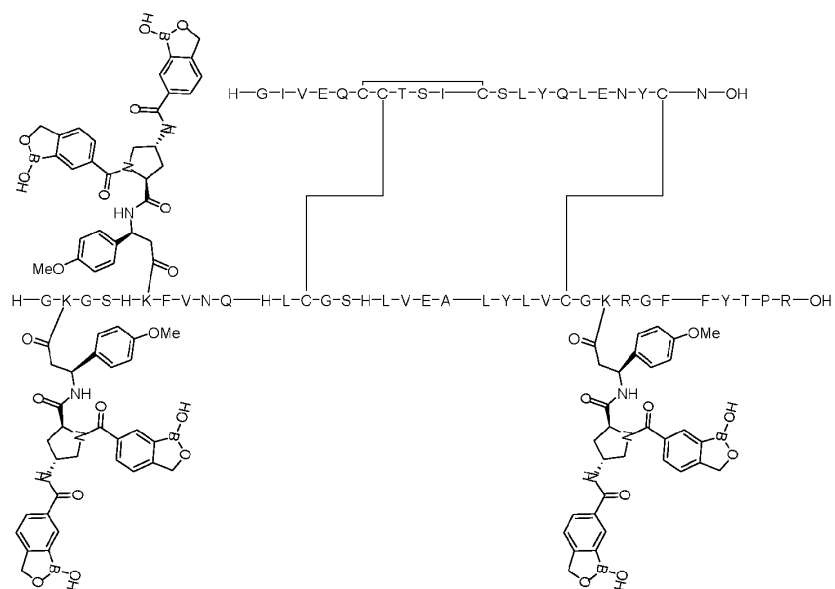
Example 60 (SEQ ID NOS 25514 and 25515, respectively, in order of appearance):



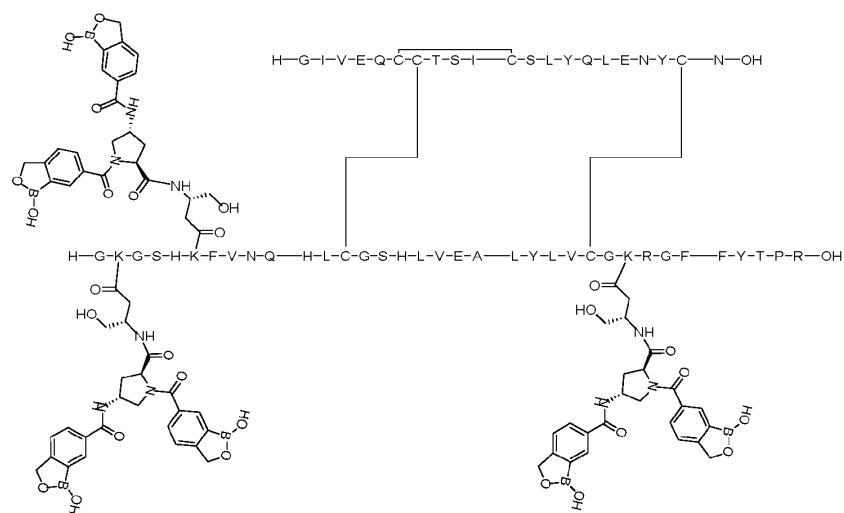
Example 61 (SEQ ID NOS 25480 and 25481, respectively, in order of appearance):



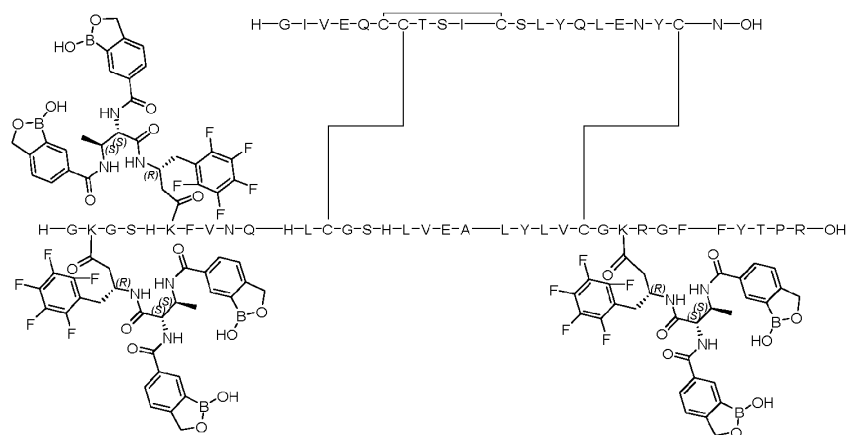
Example 62 (SEQ ID NOS 25516 and 25517, respectively, in order of appearance):



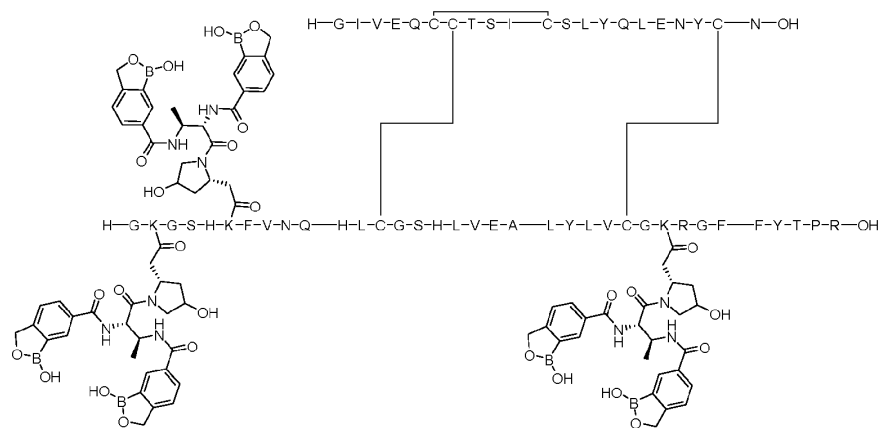
Example 63 (SEQ ID NOS 25518 and 25519, respectively, in order of appearance):



Example 64 (SEQ ID NOS 25520 and 25521, respectively, in order of appearance):

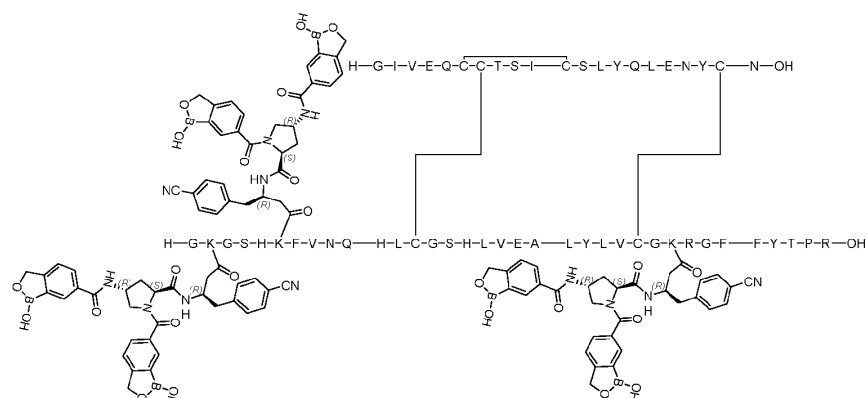


Example 65 (SEQ ID NOS 25522 and 25523, respectively, in order of appearance):

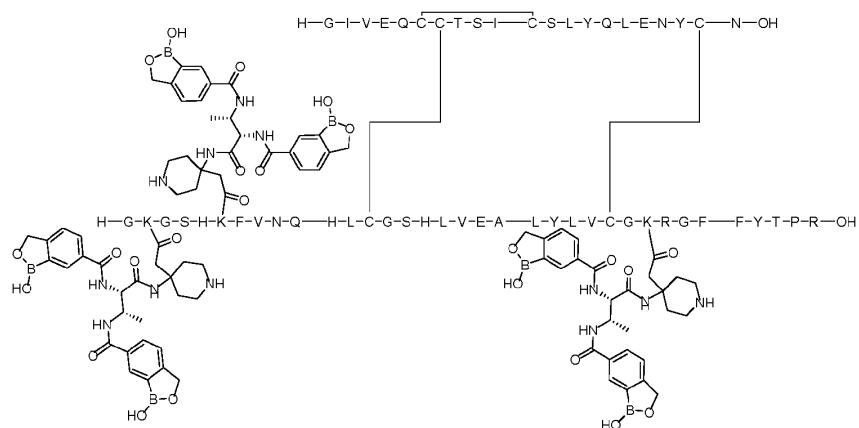


5

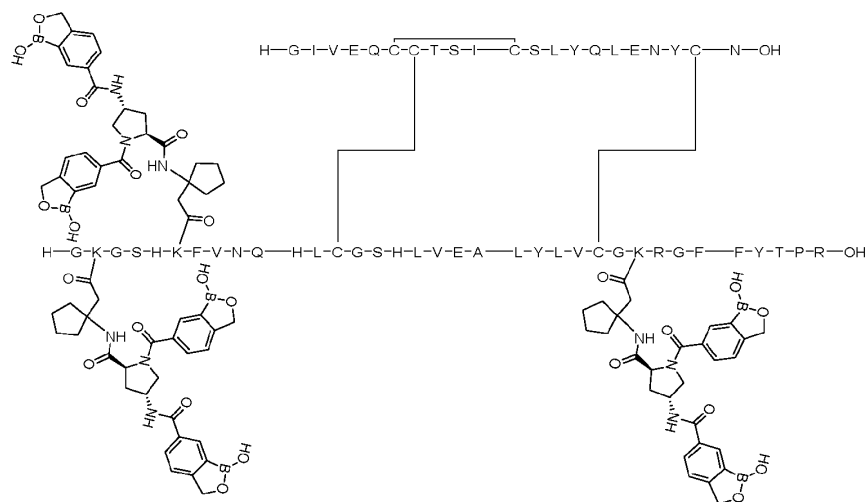
Example 66 (SEQ ID NOS 25524 and 25525, respectively, in order of appearance):



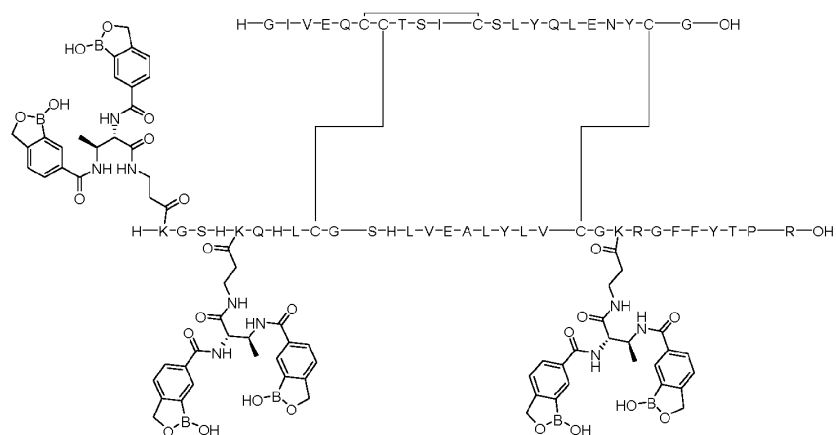
Example 67 (SEQ ID NOS 25526 and 25527, respectively, in order of appearance):



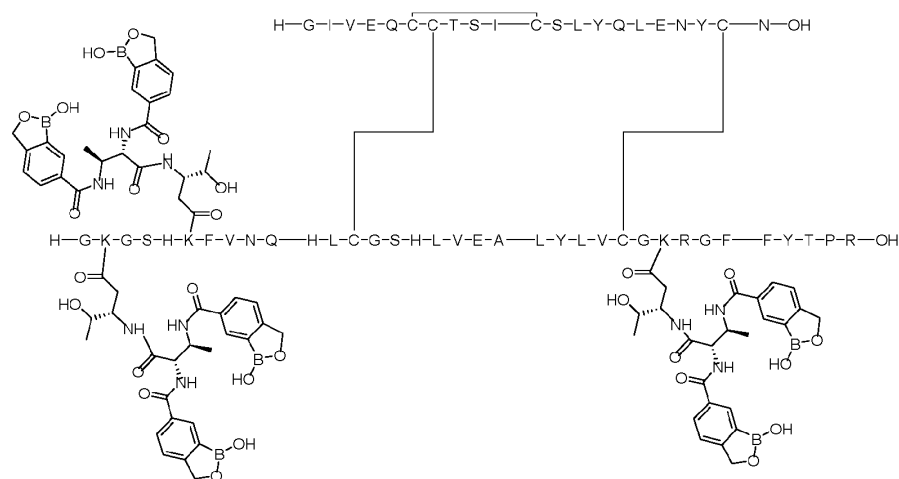
5 Example 68 (SEQ ID NOS 25528 and 25529, respectively, in order of appearance):



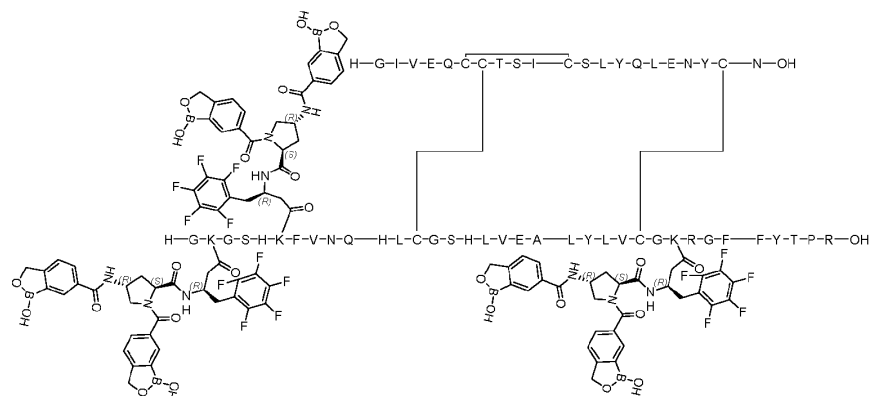
Example 69 (SEQ ID NOS 25530 and 25531, respectively, in order of appearance):



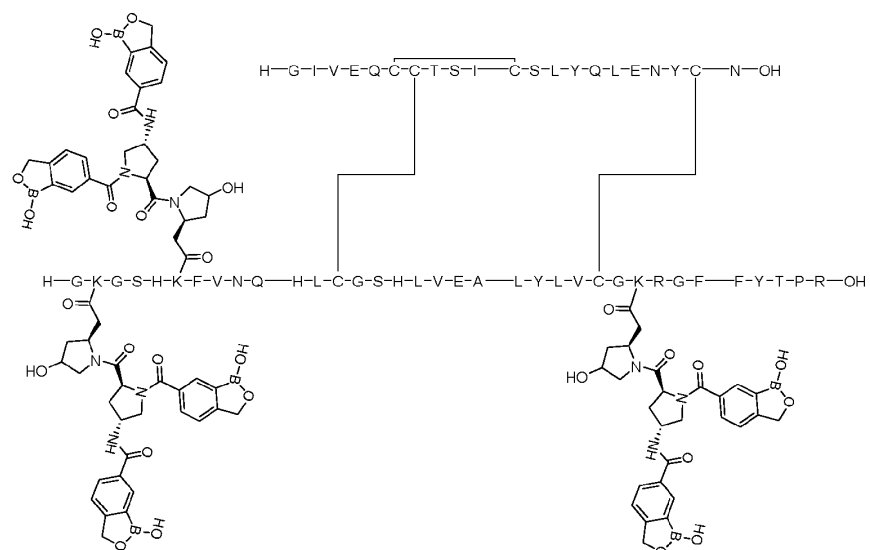
Example 70 (SEQ ID NOS 25532 and 25533, respectively, in order of appearance):



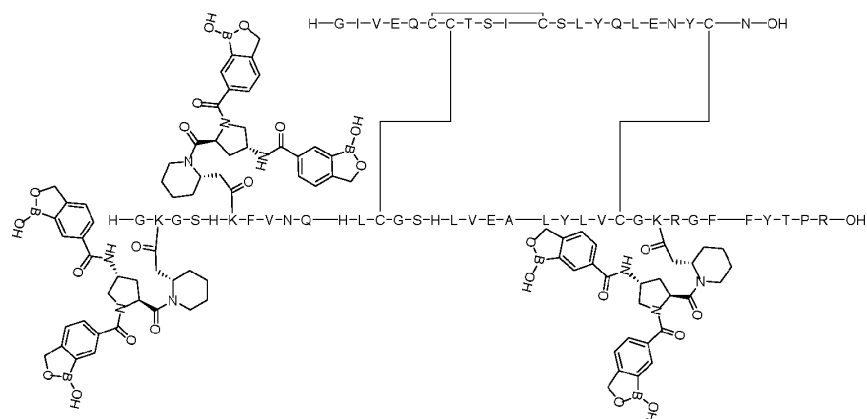
Example 71 (SEQ ID NOS 25534 and 25535, respectively, in order of appearance):



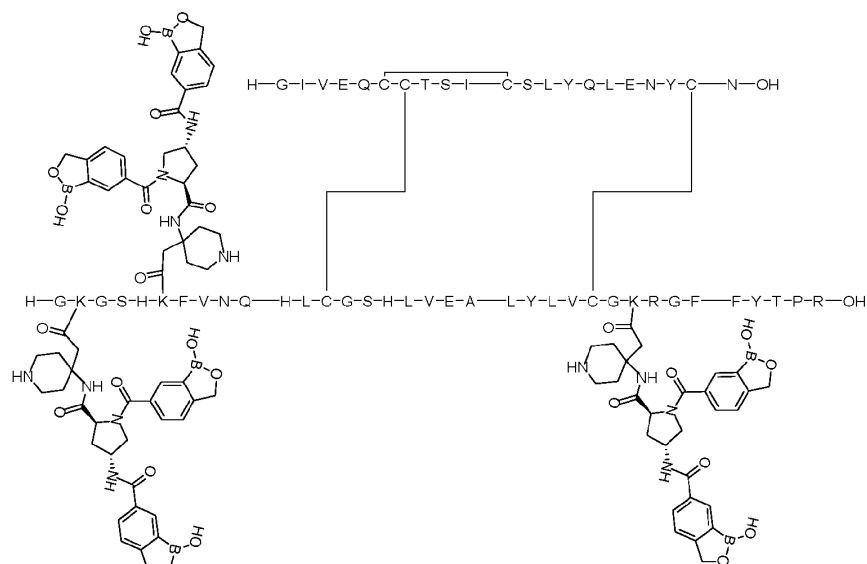
Example 72 (SEQ ID NOS 25536 and 25537, respectively, in order of appearance):



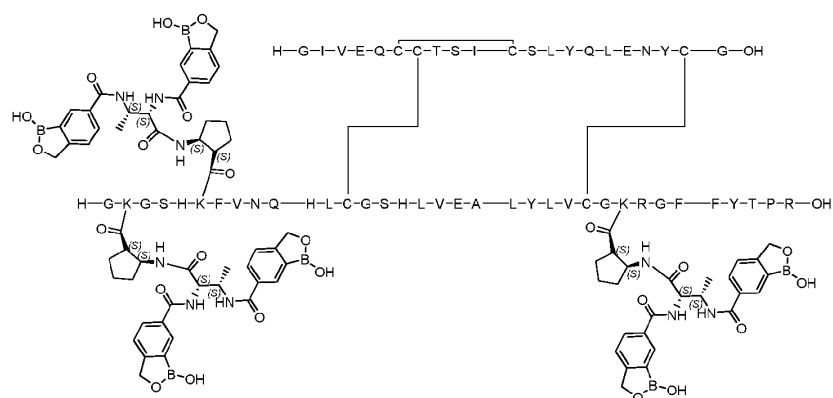
Example 73 (SEQ ID NOS 25538 and 25539, respectively, in order of appearance):



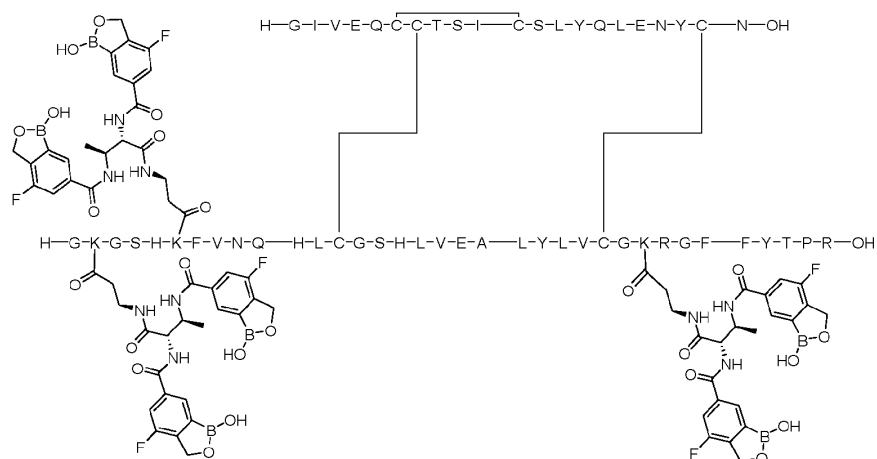
Example 74 (SEQ ID NOS 25540 and 25541, respectively, in order of appearance):



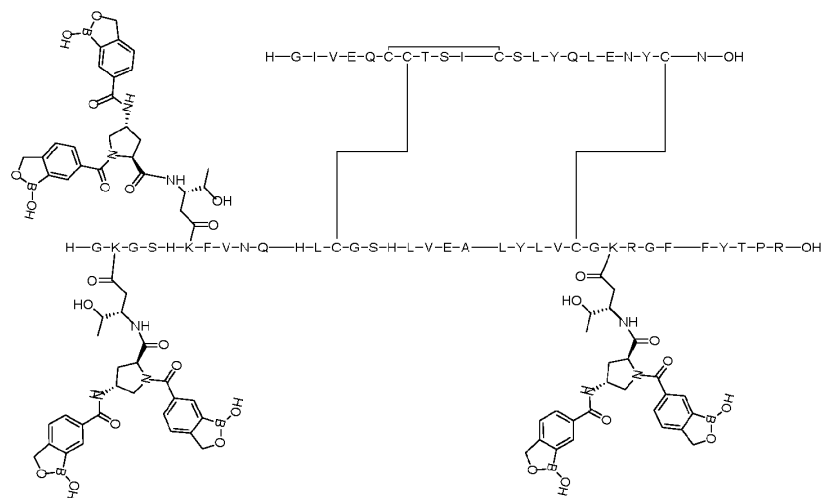
Example 75 (SEQ ID NOS 25542 and 25543, respectively, in order of appearance):



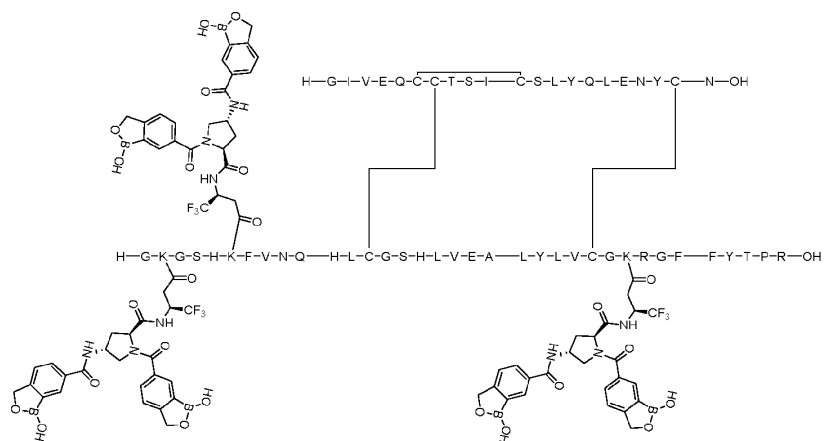
Example 76 (SEQ ID NOS 25544 and 25545, respectively, in order of appearance):



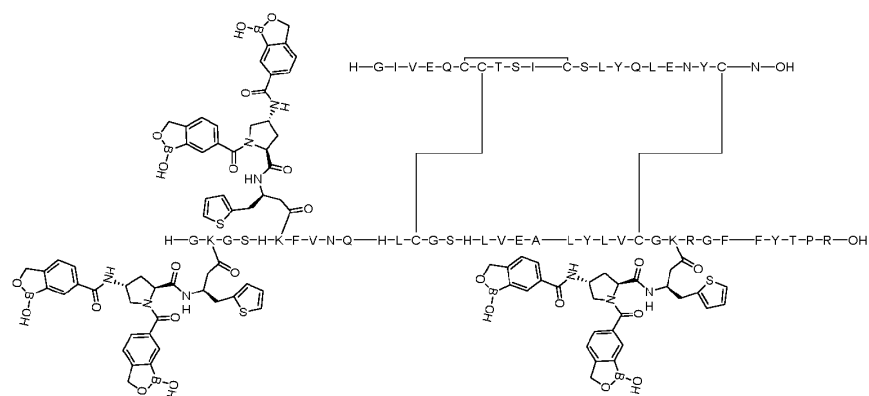
Example 77 (SEQ ID NOS 25546 and 25547, respectively, in order of appearance):



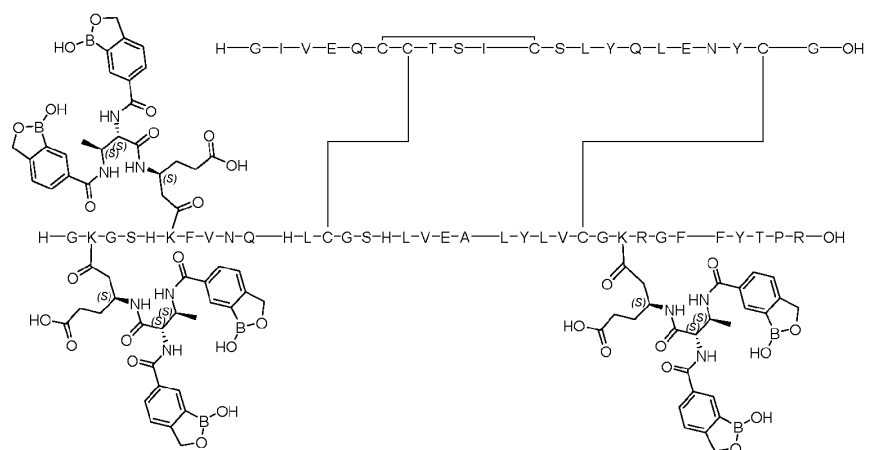
Example 78 (SEQ ID NOS 25548 and 25549, respectively, in order of appearance):



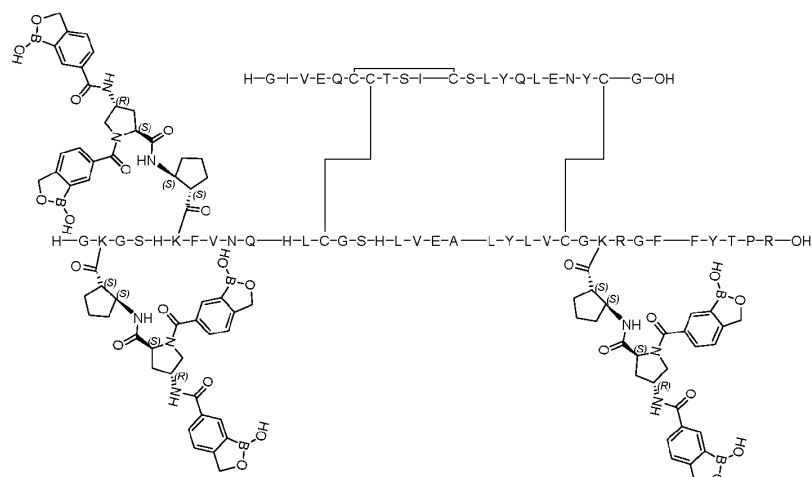
Example 79 (SEQ ID NOS 25550 and 25551, respectively, in order of appearance):



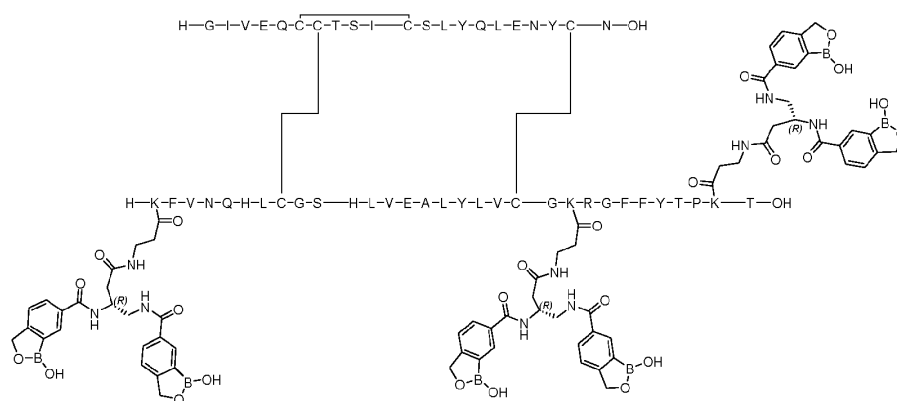
Example 80 (SEQ ID NOS 25552 and 25553, respectively, in order of appearance):



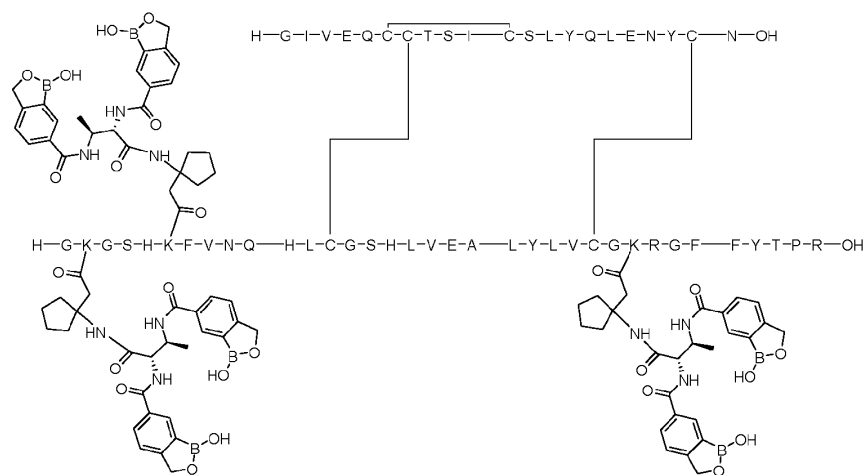
Example 81 (SEQ ID NOS 25554 and 25555, respectively, in order of appearance):



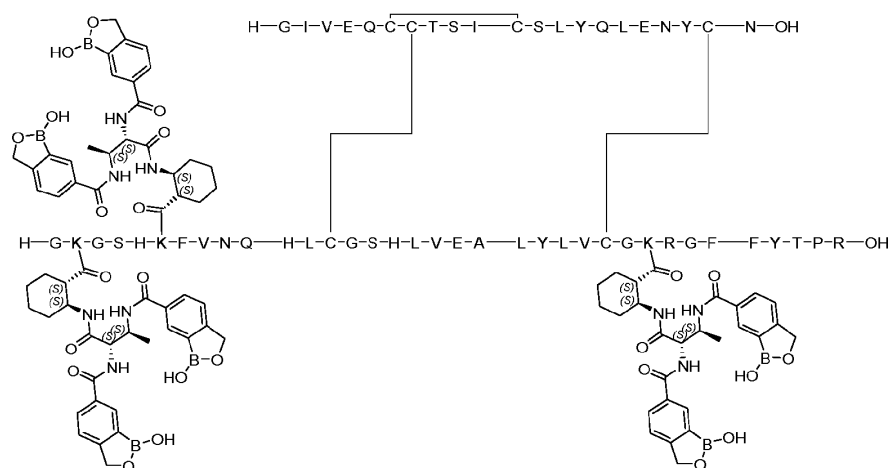
Example 82 (SEQ ID NOS 25556 and 25557, respectively, in order of appearance):



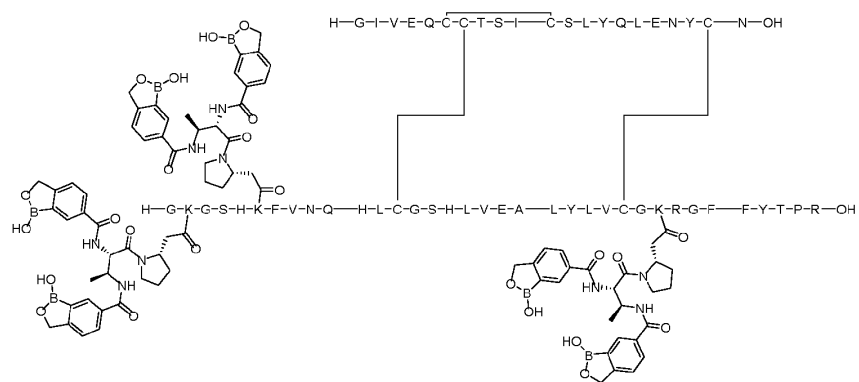
Example 83 (SEQ ID NOS 25558 and 25559, respectively, in order of appearance):



Example 84 (SEQ ID NOS 25560 and 25561, respectively, in order of appearance):



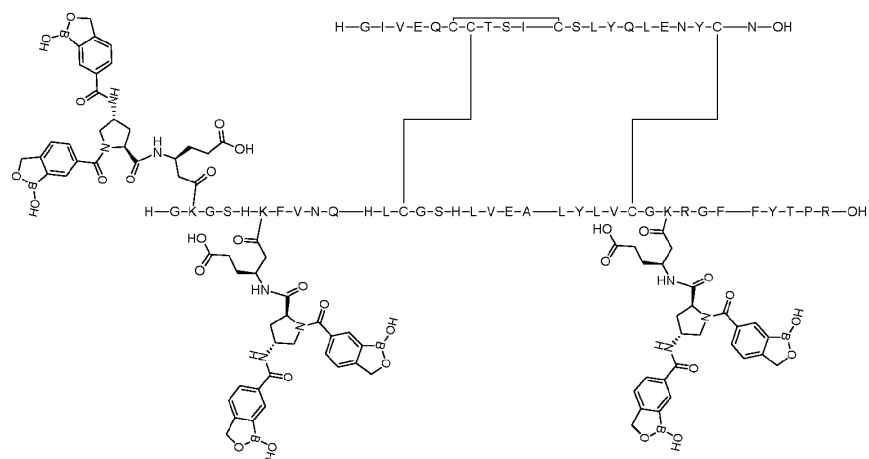
Example 85 (SEQ ID NOS 25562 and 25563, respectively, in order of appearance):



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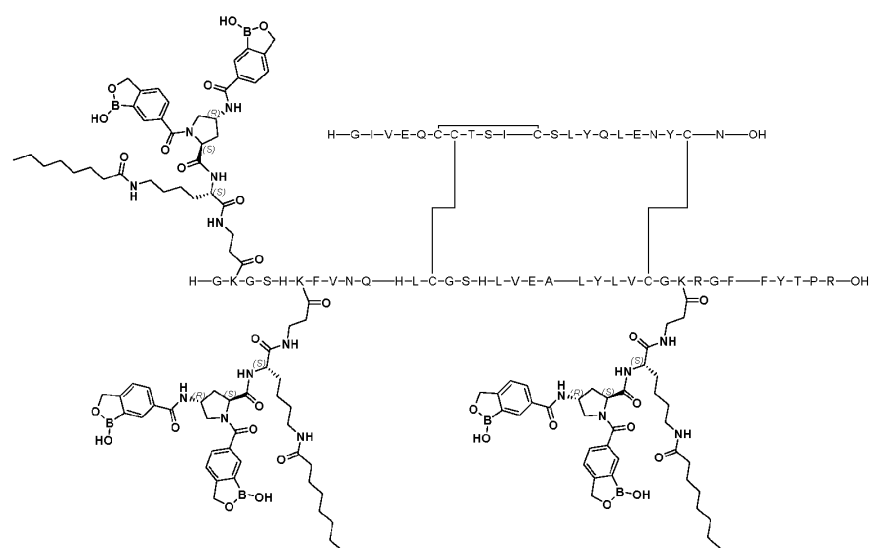
10

Example 86 (SEQ ID NOS 25564 and 25565, respectively, in order of appearance):

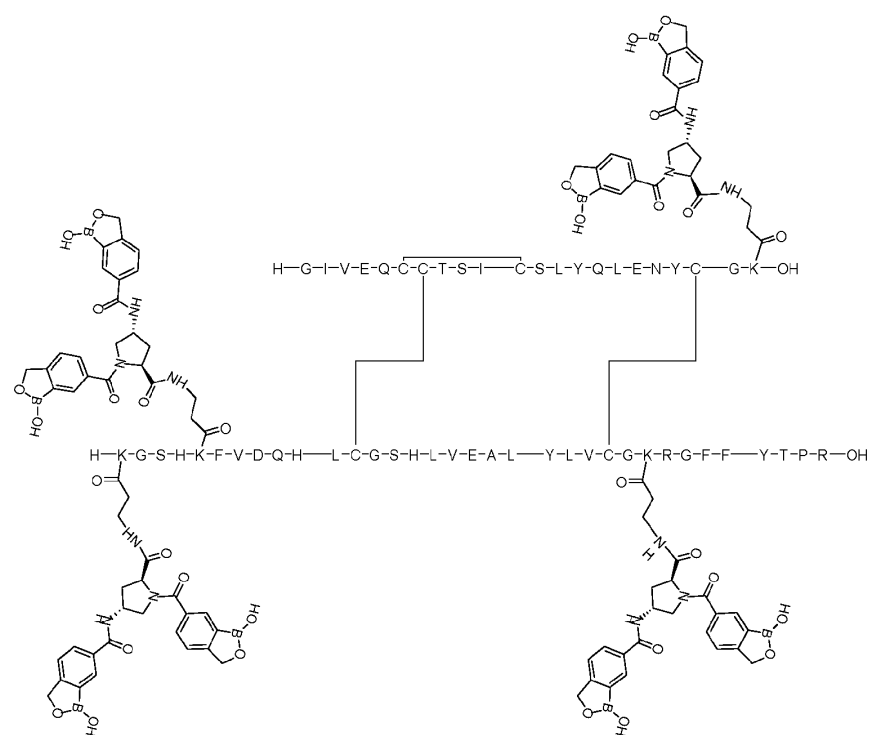


15

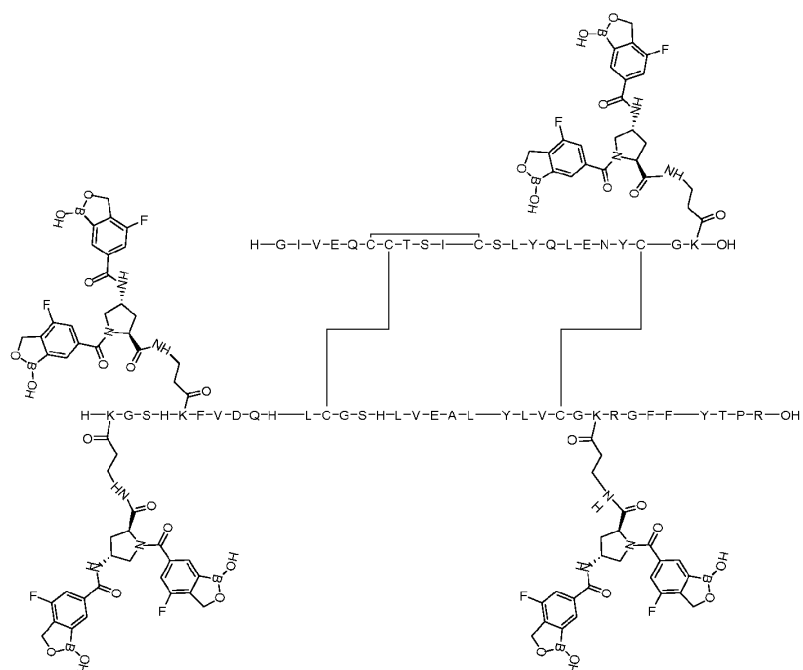
Example 87 (SEQ ID NOS 25566 and 25567, respectively, in order of appearance):



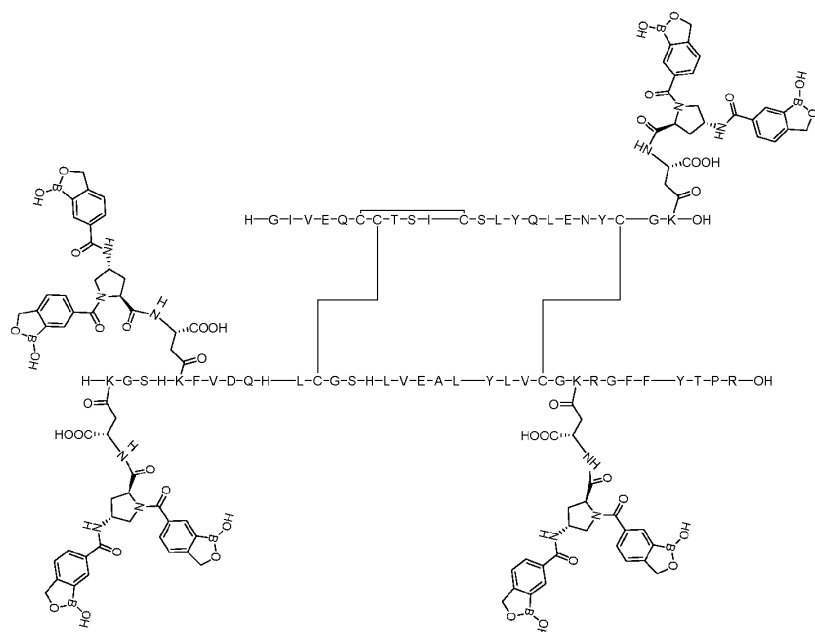
Example 88 (SEQ ID NOS 25568 and 25569, respectively, in order of appearance):



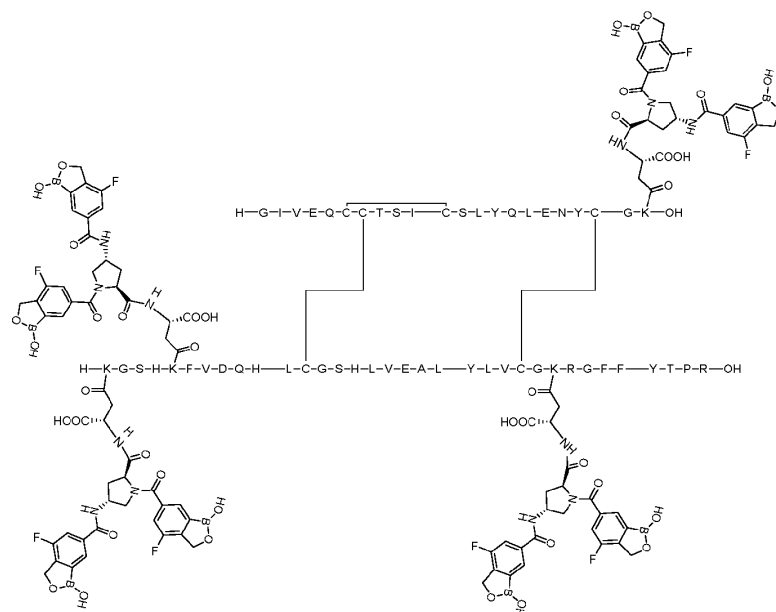
Example 89 (SEQ ID NOS 25570 and 25571, respectively, in order of appearance):



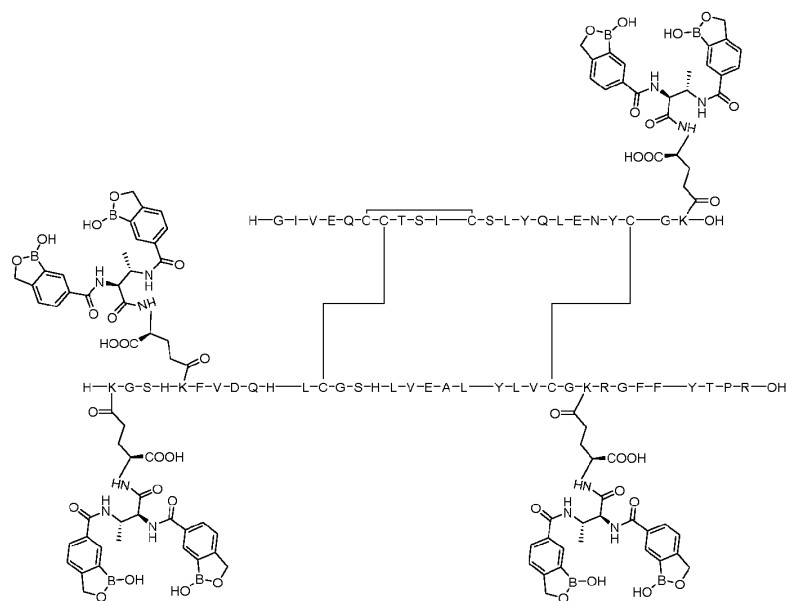
Example 90 (SEQ ID NOS 25572 and 25573, respectively, in order of appearance):



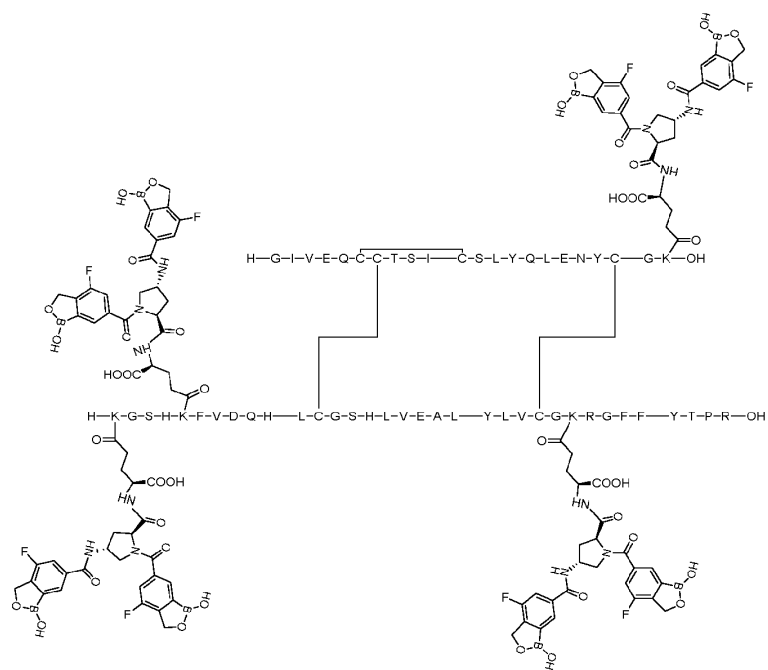
Example 91 (SEQ ID NOS 25574 and 25575, respectively, in order of appearance):



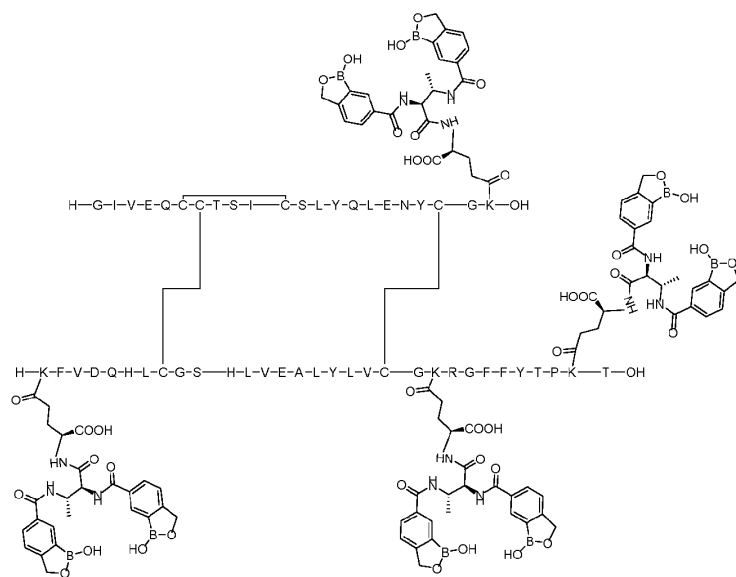
Example 92 (SEQ ID NOS 25576 and 25577, respectively, in order of appearance):



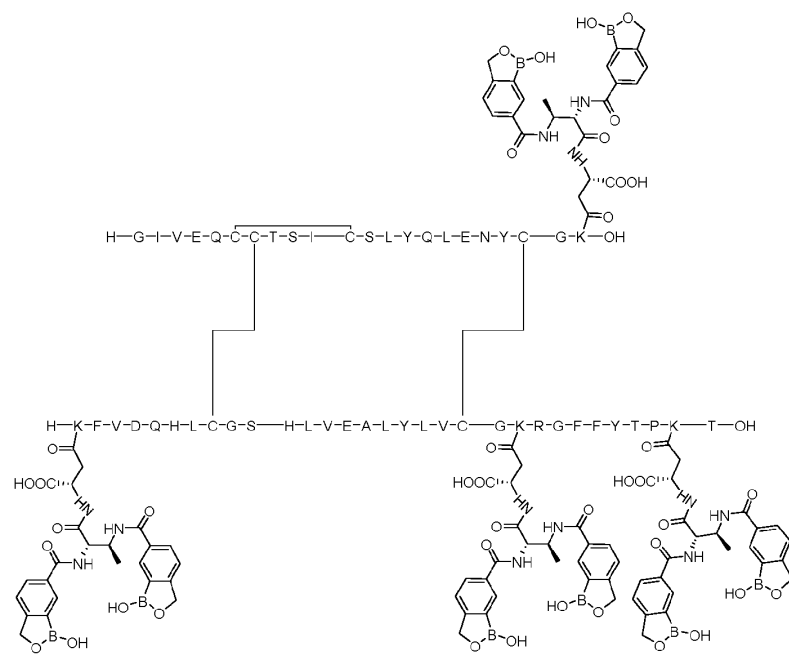
Example 93 (SEQ ID NOS 25578 and 25579, respectively, in order of appearance):



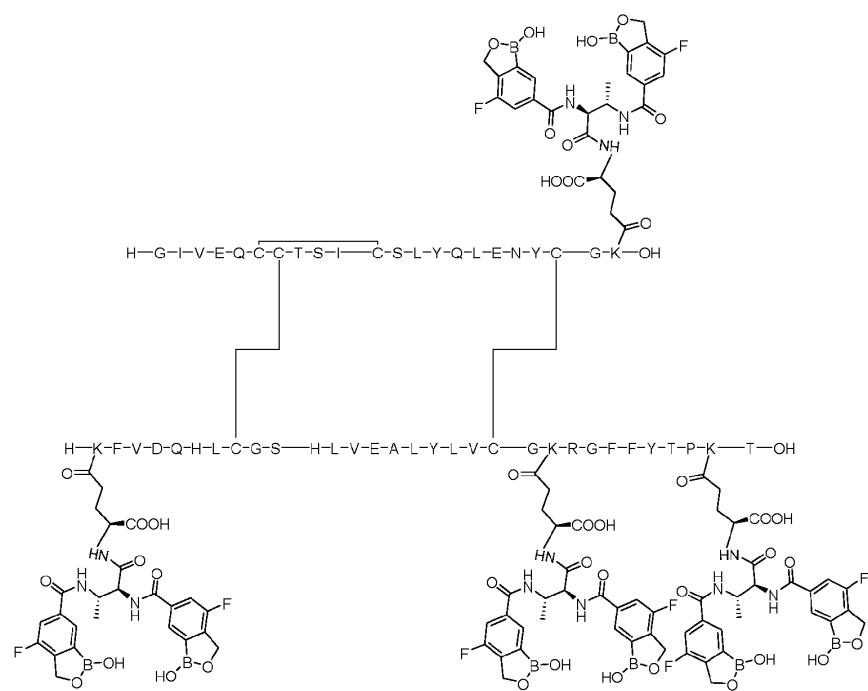
Example 94 (SEQ ID NOS 25580 and 25581, respectively, in order of appearance):



Example 95 (SEQ ID NOS 25582 and 25583, respectively, in order of appearance):

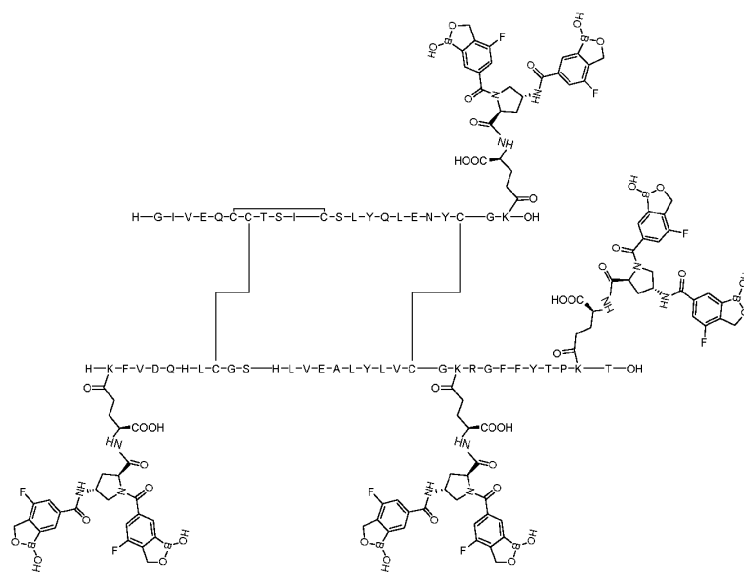


Example 96 (SEQ ID NOS 25584 and 25585, respectively, in order of appearance):

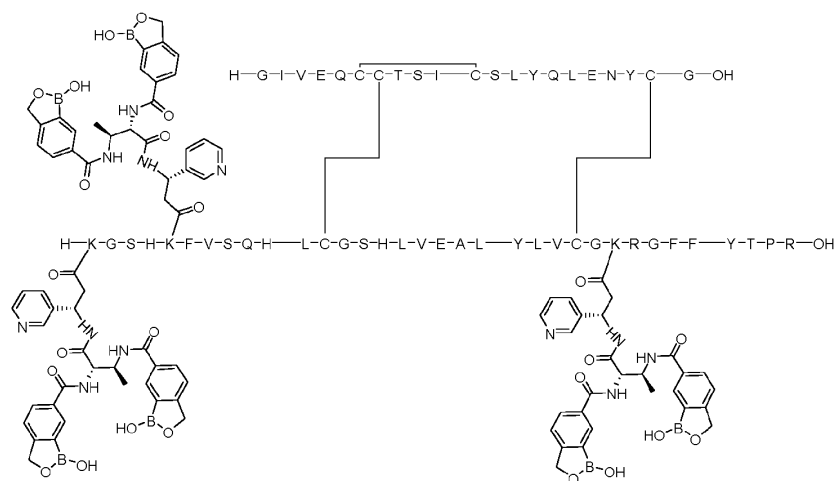


The 12th peptide sequence is: H-G-I-V-E-Q-C-C-T-S-I-C-S-L-Y-Q-L-E-N-Y-C-G-K-OH. The side chains are detailed at positions 1, 10, and 11. Position 1 is a tryptophan derivative with a 2,4,6-trifluorophenyl group and a boronate ester. Position 10 is a tryptophan derivative with a 2,4,6-trifluorophenyl group and a boronate ester. Position 11 is a tryptophan derivative with a 2,4,6-trifluorophenyl group and a boronate ester.

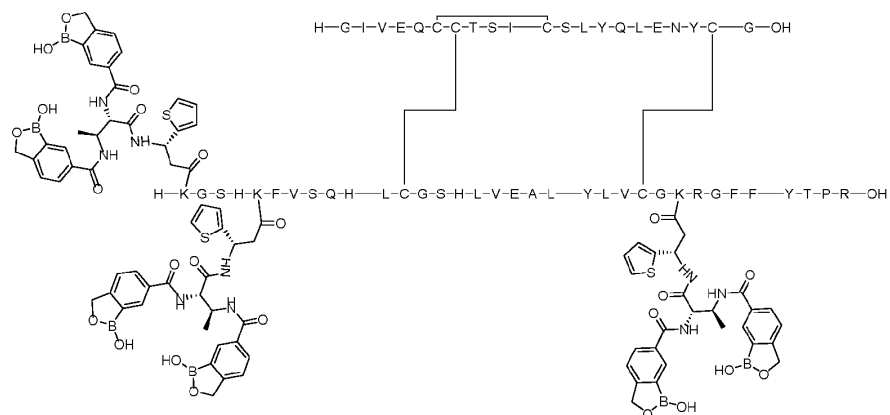
Example 99 (SEQ ID NOS 25590 and 25591, respectively, in order of appearance):



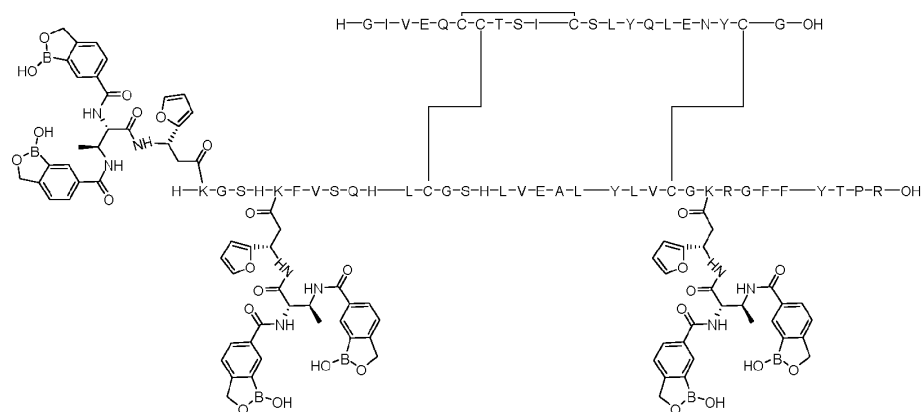
Example 100 (SEQ ID NOS 25592 and 25593, respectively, in order of appearance):



Example 101 (SEQ ID NOS 25594 and 25595, respectively, in order of appearance):

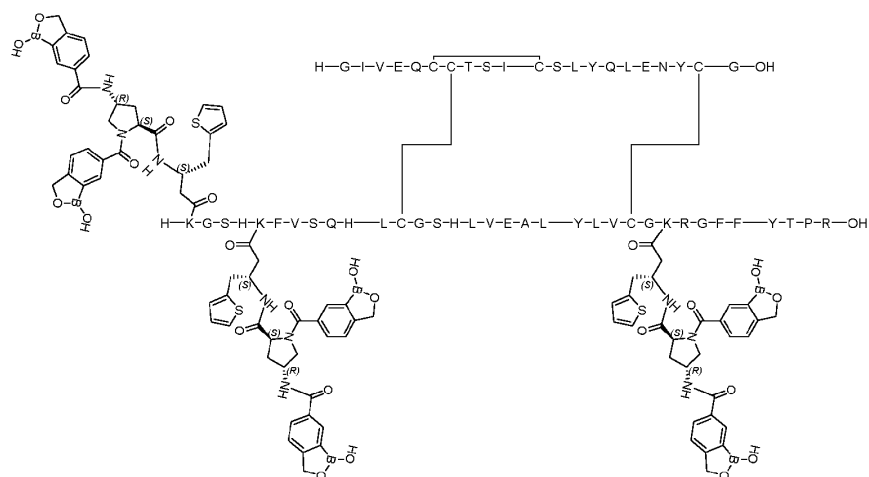


Example 102 (SEQ ID NOS 25596 and 25597, respectively, in order of appearance):

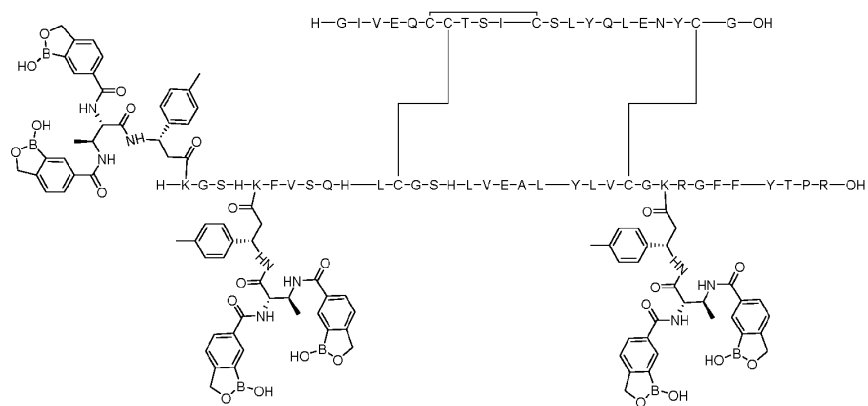


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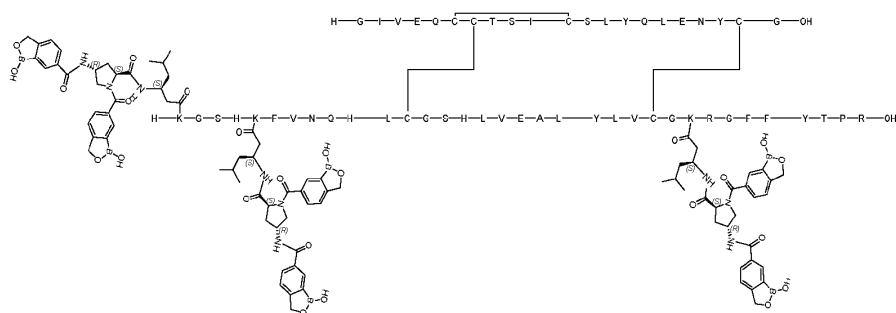
Example 103 (SEQ ID NOS 25598 and 25599, respectively, in order of appearance):



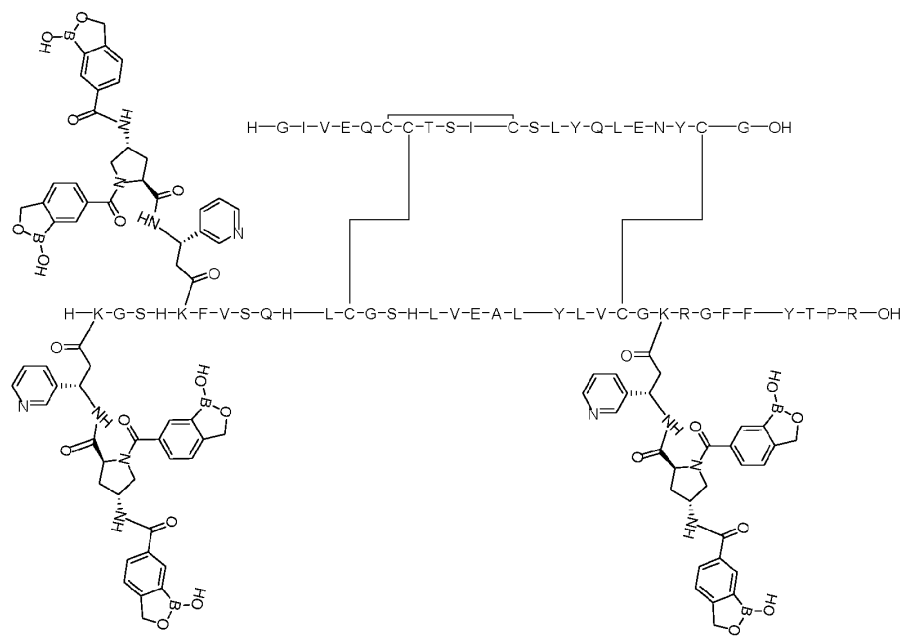
Example 104 (SEQ ID NOS 25600 and 25601, respectively, in order of appearance):



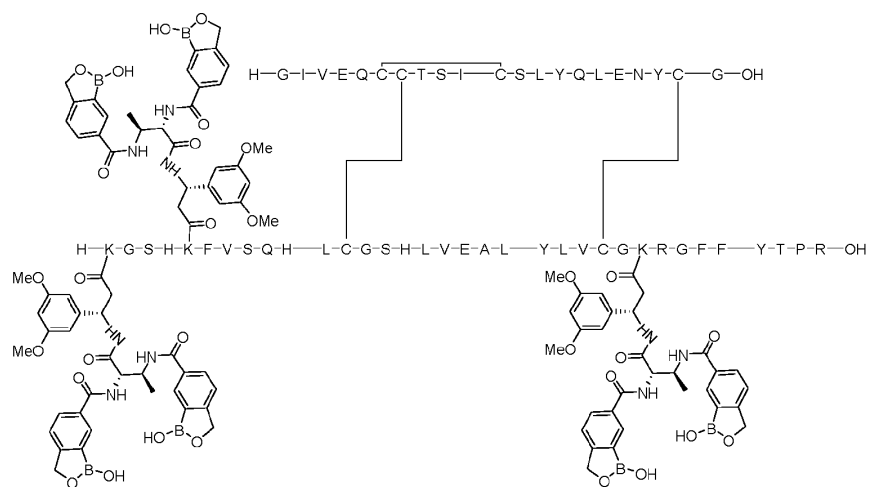
5 Example 105 (SEQ ID NOS 25602 and 25603, respectively, in order of appearance):



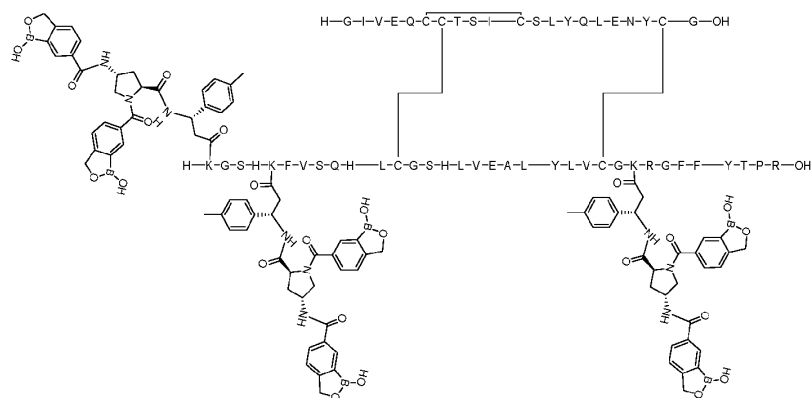
Example 106 (SEQ ID NOS 25604 and 25605, respectively, in order of appearance):



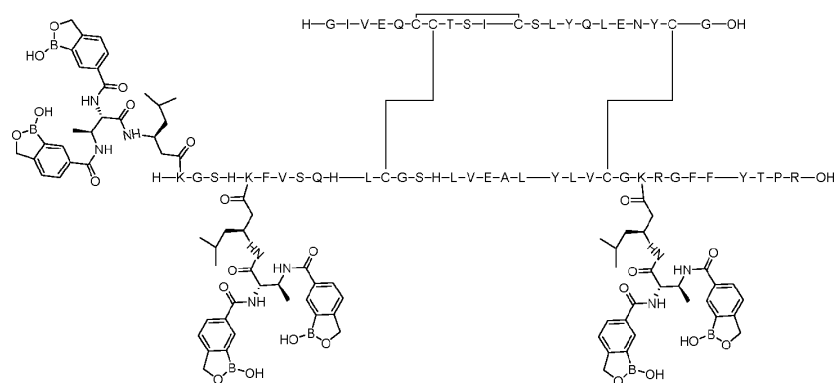
Example 107 (SEQ ID NOS 25606 and 25607, respectively, in order of appearance):



Example 108 (SEQ ID NOS 25608 and 25609, respectively, in order of appearance):

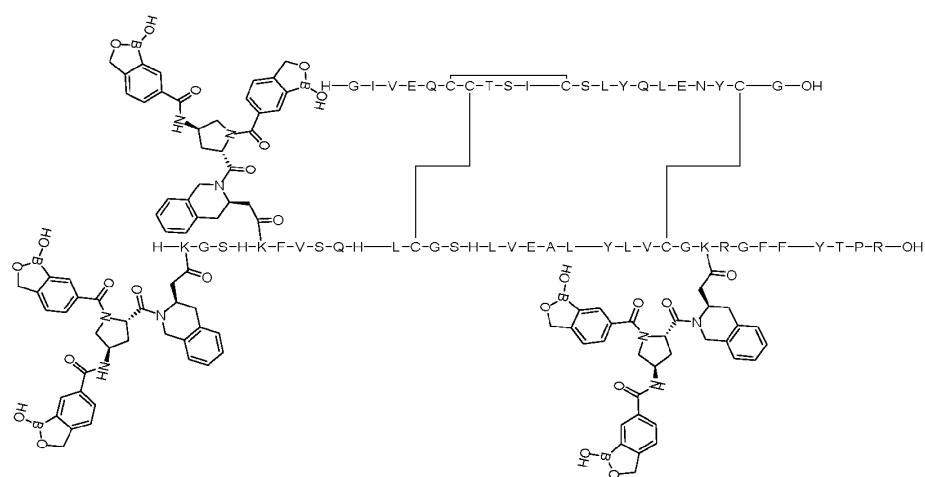


Example 109 (SEQ ID NOS 25610 and 25611, respectively, in order of appearance):

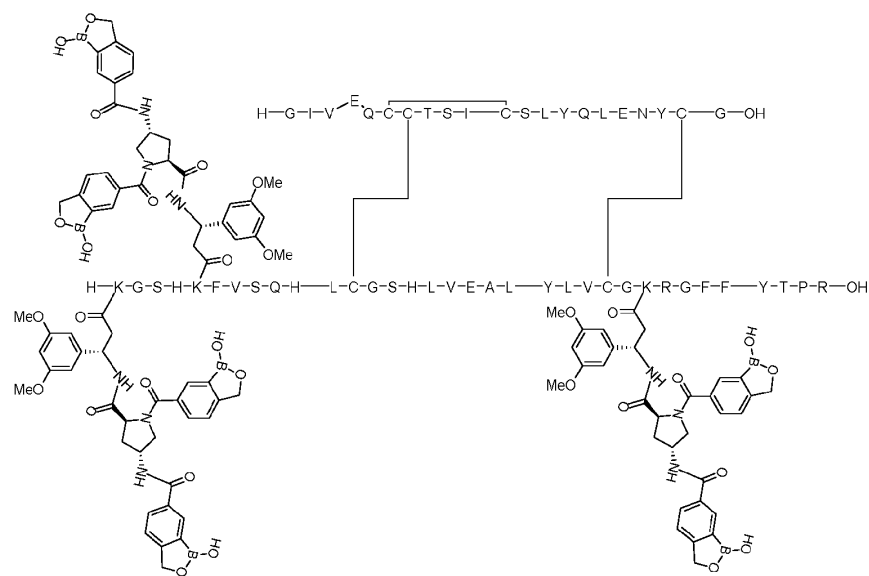


5

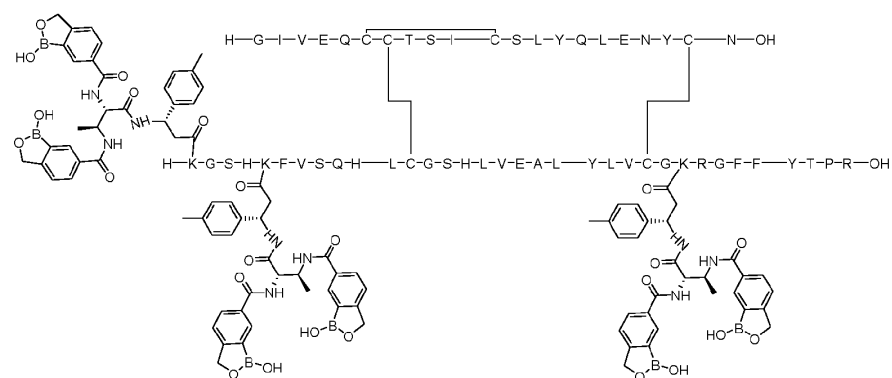
Example 110 (SEQ ID NOS 25612 and 25613, respectively, in order of appearance):



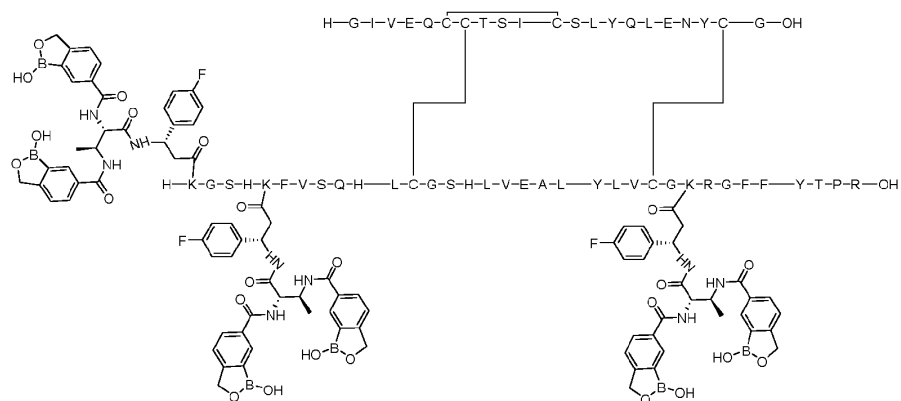
Example 111 (SEQ ID NOS 25614 and 25615, respectively, in order of appearance):



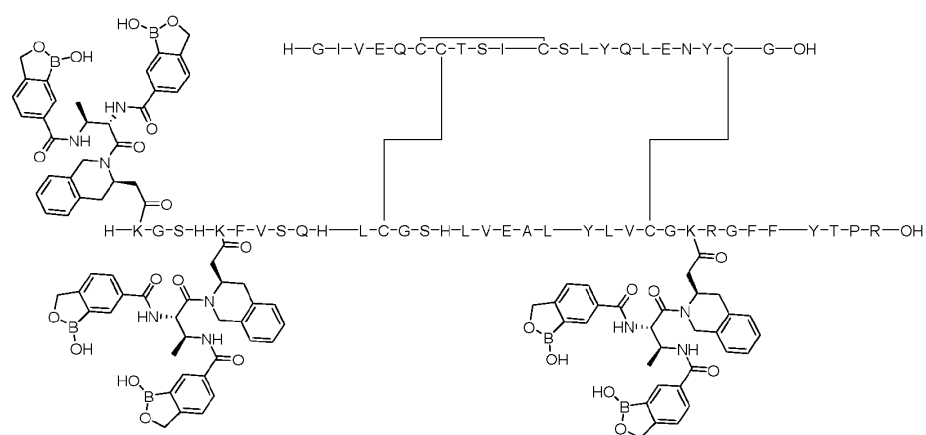
Example 112 (SEQ ID NOS 25616 and 25617, respectively, in order of appearance):



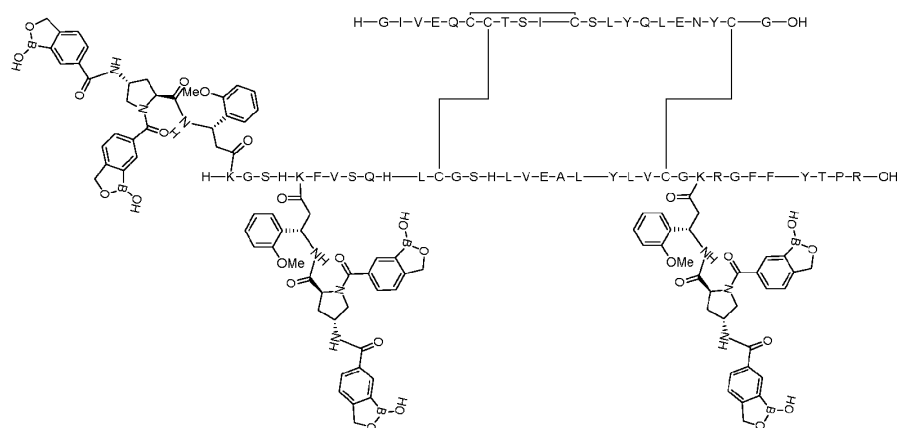
Example 113 (SEQ ID NOS 25618 and 25619, respectively, in order of appearance):



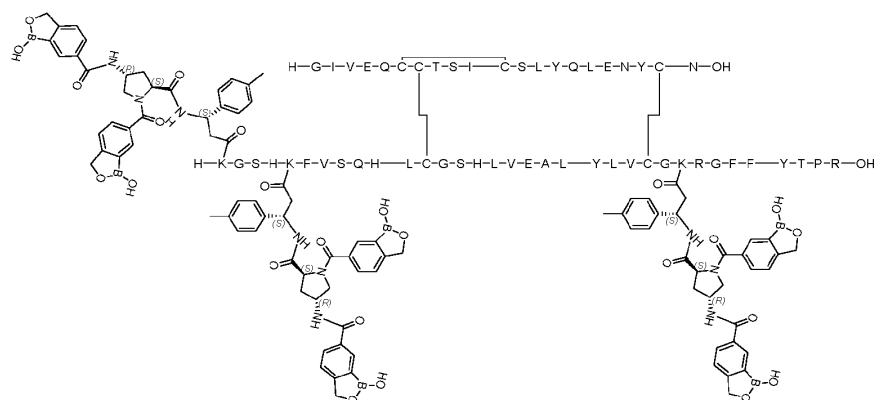
5 Example 114 (SEQ ID NOS 25620 and 25621, respectively, in order of appearance):



Example 115 (SEQ ID NOS 25622 and 25623, respectively, in order of appearance):

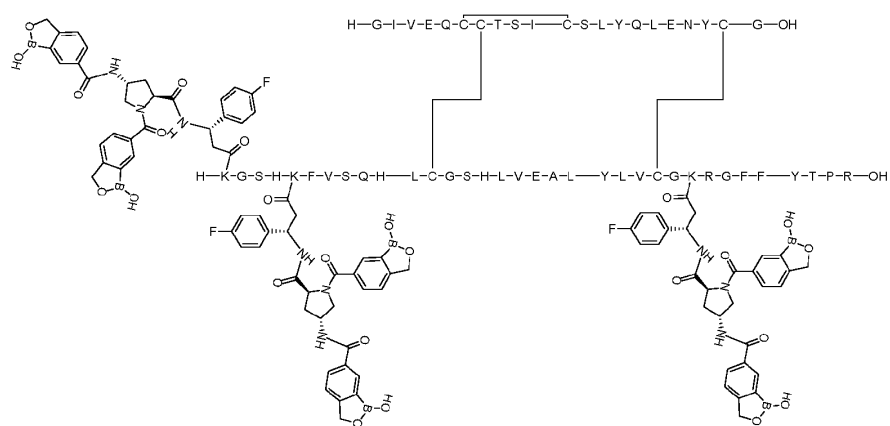


Example 116 (SEQ ID NOS 25624 and 25625, respectively, in order of appearance):

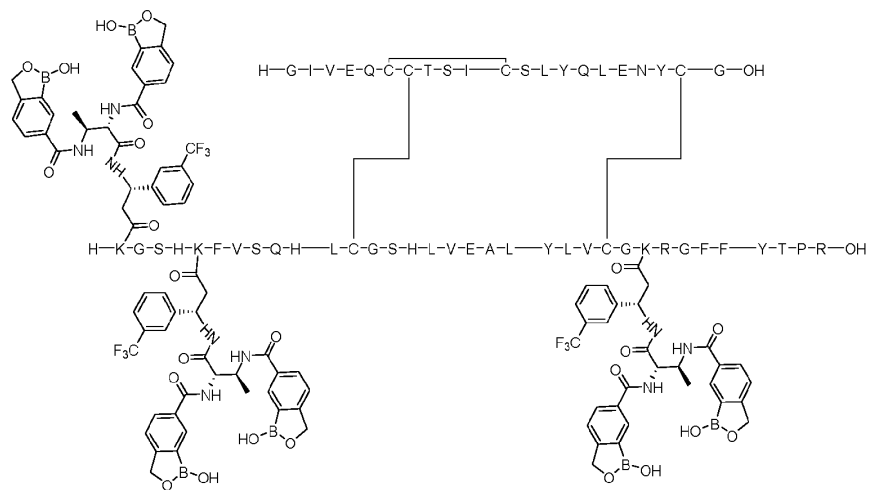


5

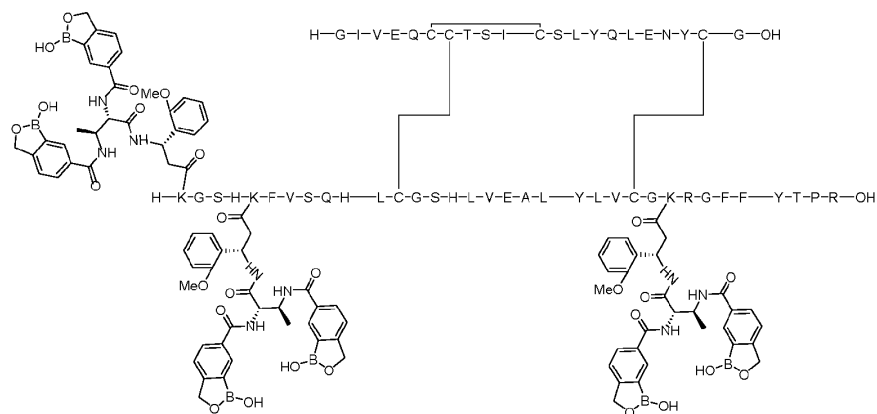
Example 117 (SEQ ID NOS 25626 and 25627, respectively, in order of appearance):



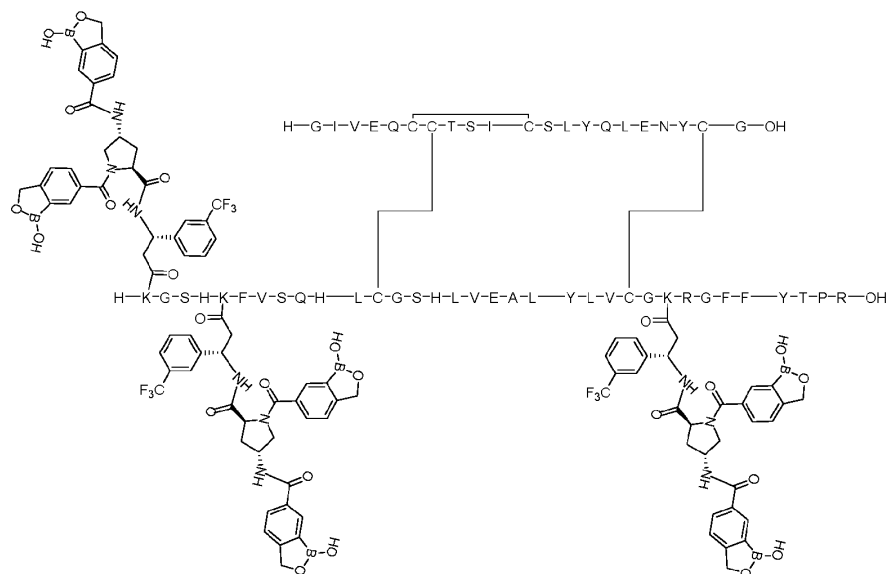
Example 118 (SEQ ID NOS 25628 and 25629, respectively, in order of appearance):



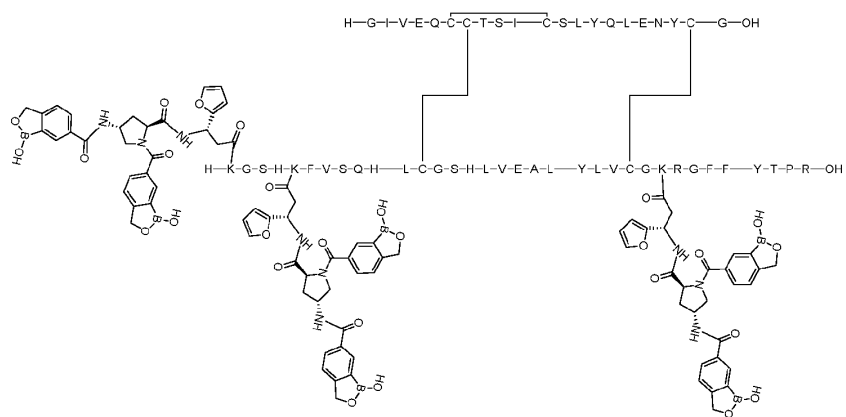
5 Example 119 (SEQ ID NOS 25630 and 25631, respectively, in order of appearance):



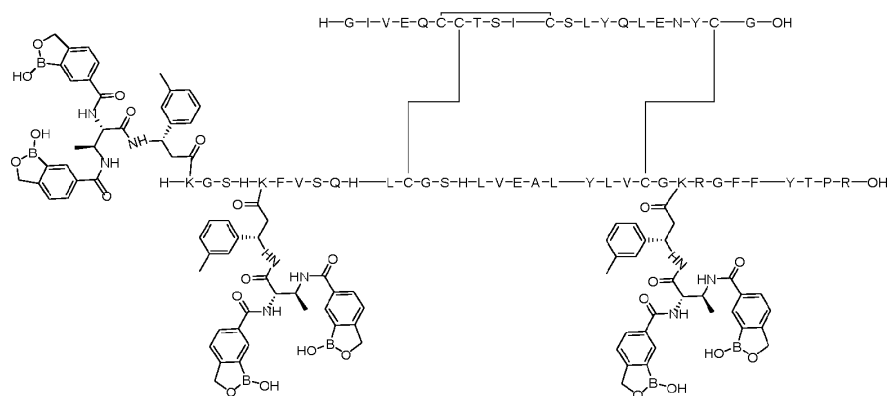
Example 120 (SEQ ID NOS 25632 and 25633, respectively, in order of appearance):



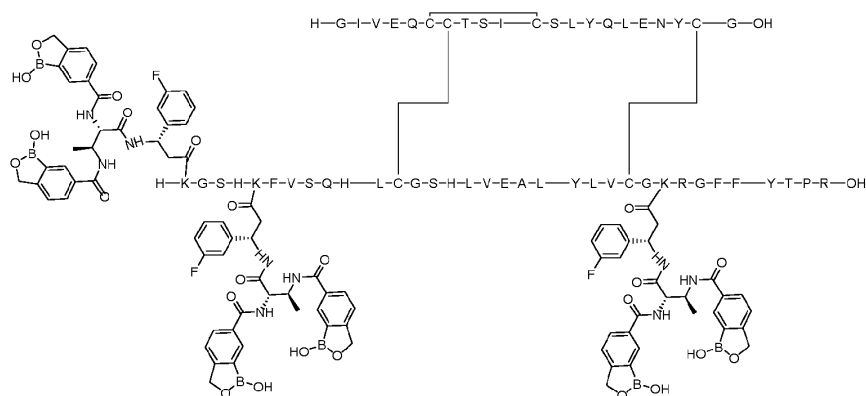
Example 121 (SEQ ID NOS 25634 and 25635, respectively, in order of appearance):



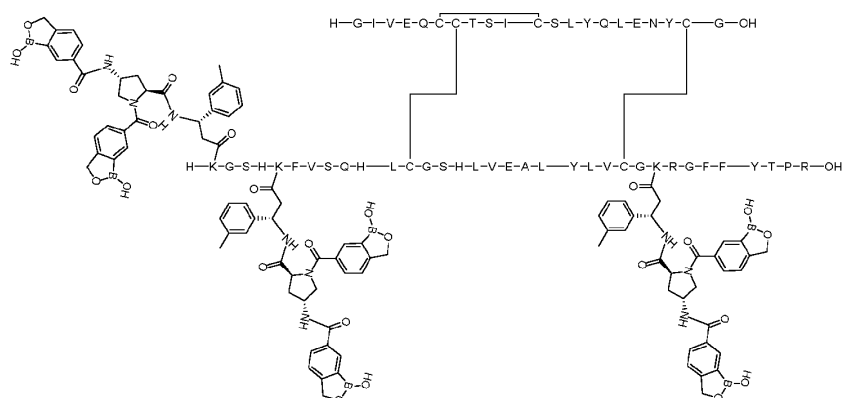
Example 122 (SEQ ID NOS 25636 and 25637, respectively, in order of appearance):



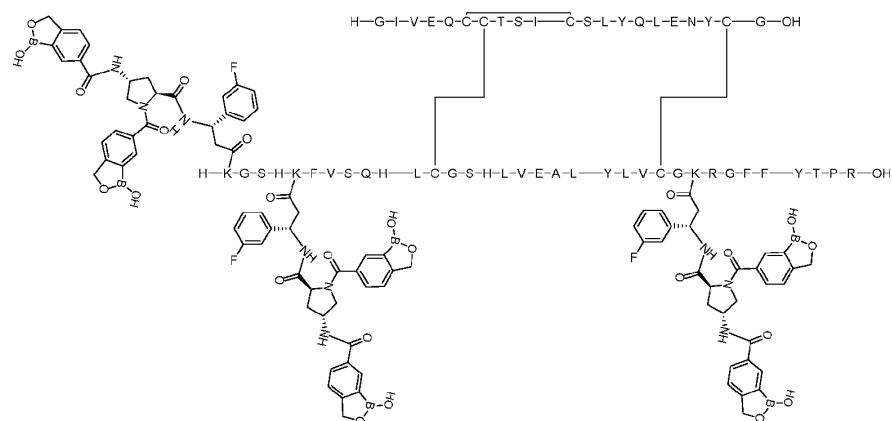
Example 123 (SEQ ID NOS 25638 and 25639, respectively, in order of appearance):



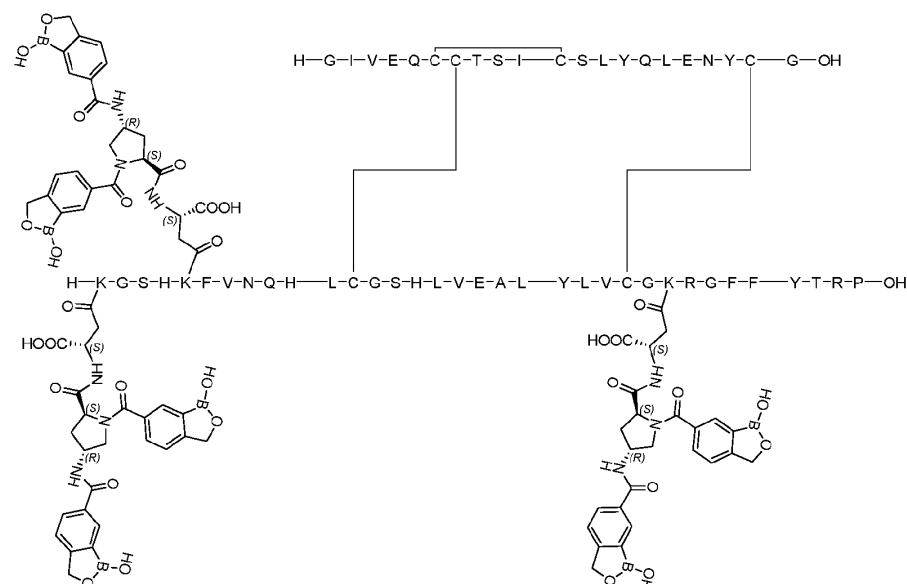
Example 124 (SEQ ID NOS 25640 and 25641, respectively, in order of appearance):



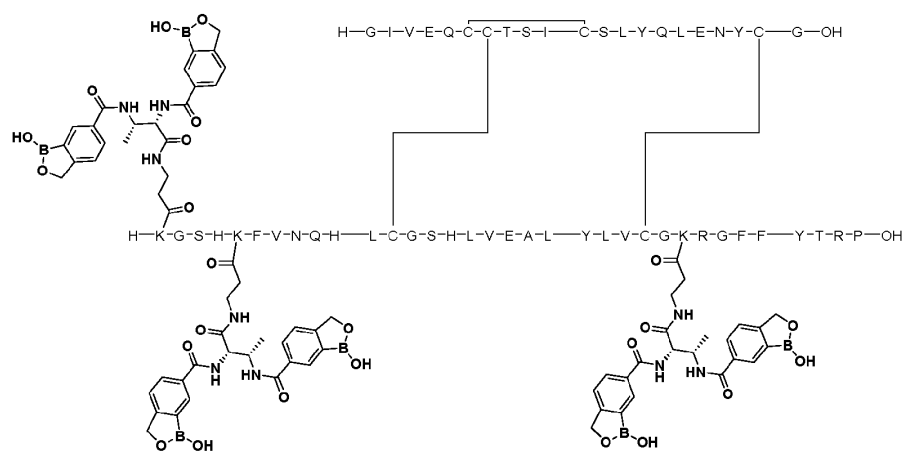
Example 125 (SEQ ID NOS 25642 and 25643, respectively, in order of appearance):



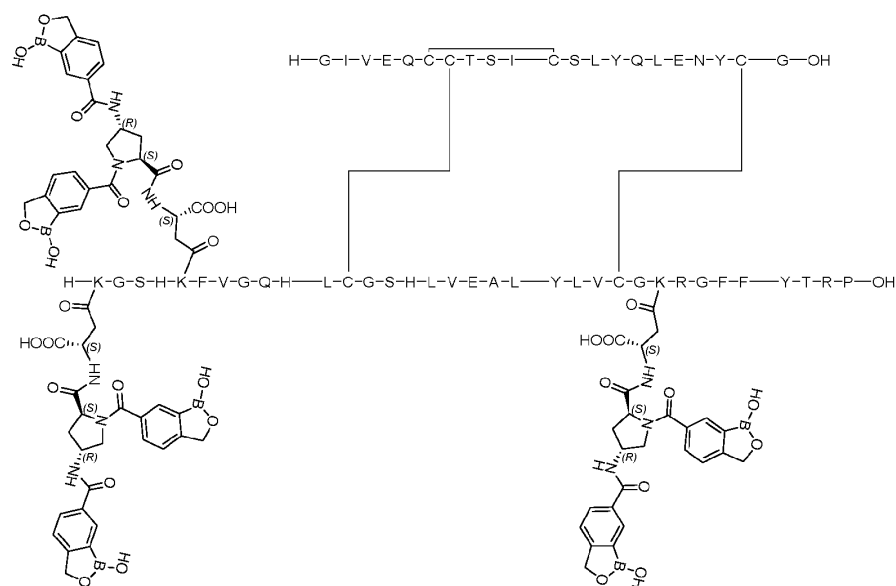
Example 126 (SEQ ID NOS 25644 and 25645, respectively, in order of appearance):



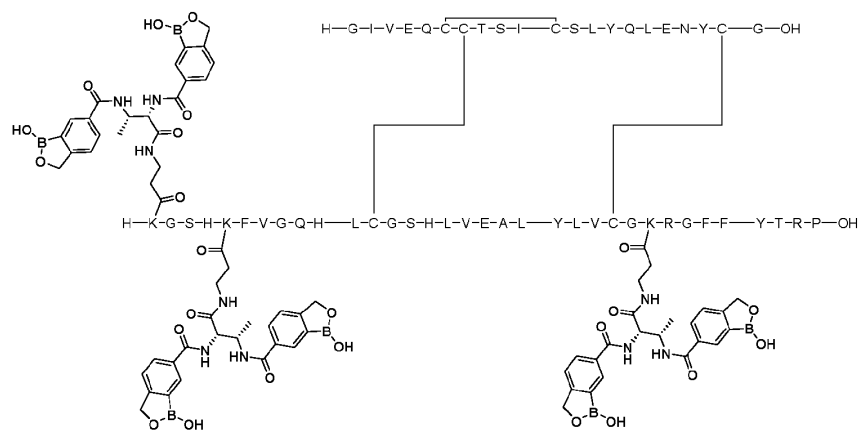
Example 127 (SEQ ID NOS 25646 and 25647, respectively, in order of appearance):



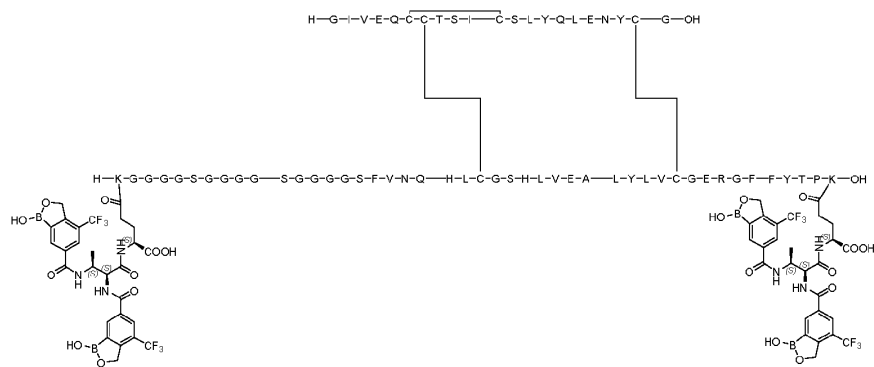
Example 128 (SEQ ID NOS 25648 and 25649, respectively, in order of appearance):



Example 129 (SEQ ID NOS 25650 and 25651, respectively, in order of appearance):

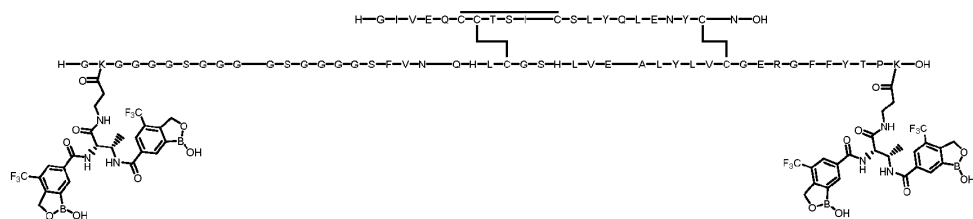


Example 130 (SEQ ID NOS 25652 and 25653, respectively, in order of appearance):

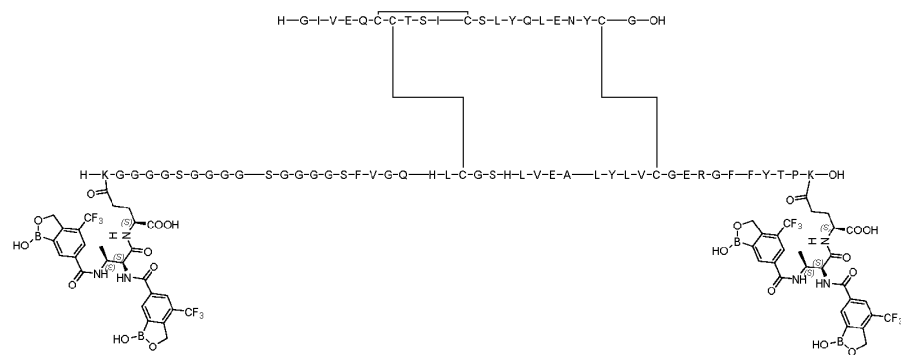


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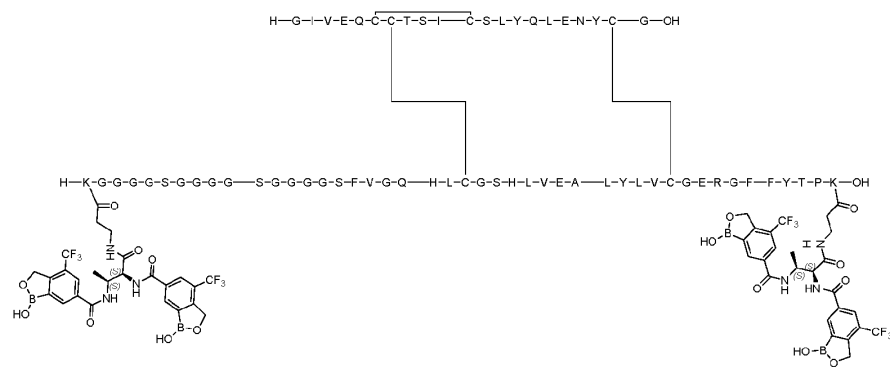
Example 131 (SEQ ID NOS 25654 and 25655, respectively, in order of appearance):



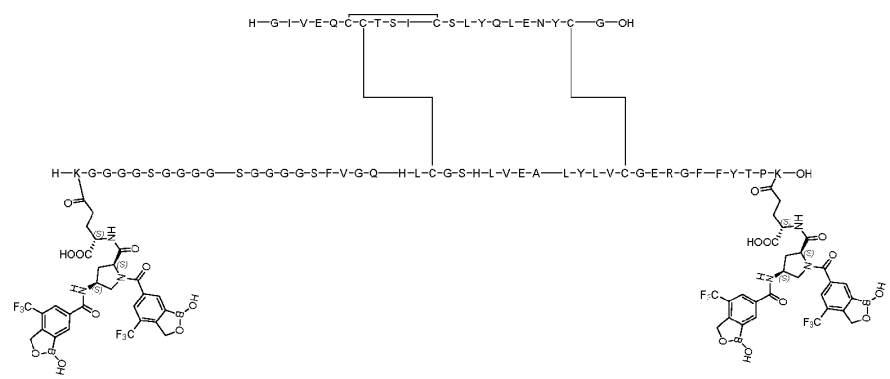
Example 132 (SEQ ID NOS 25656 and 25657, respectively, in order of appearance):



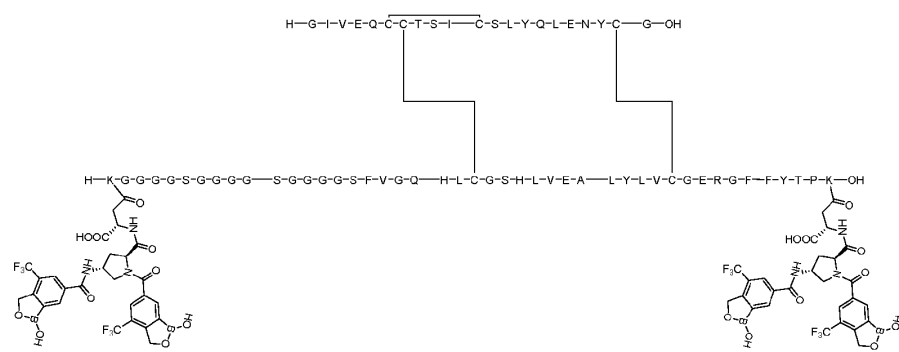
5 Example 133 (SEQ ID NOS 25658 and 25659, respectively, in order of appearance):



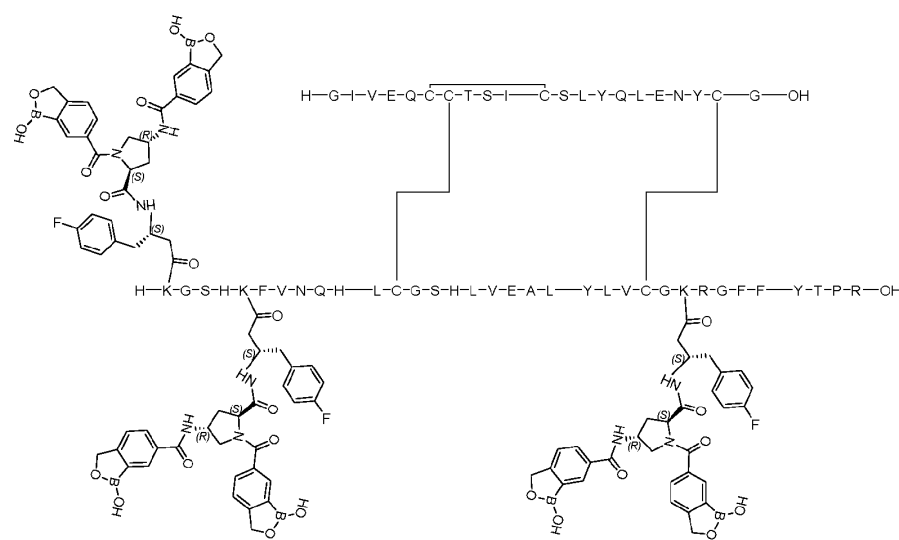
Example 134 (SEQ ID NOS 25660 and 25661, respectively, in order of appearance):



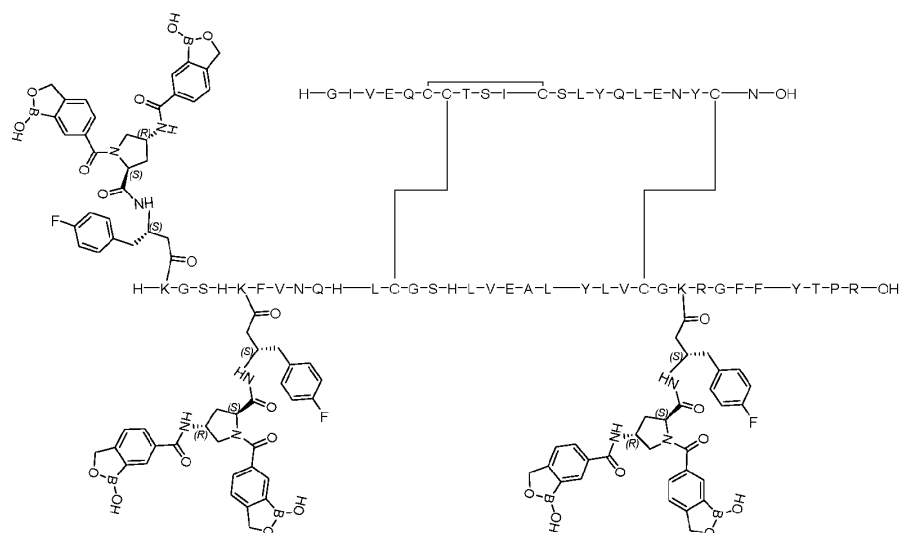
Example 135 (SEQ ID NOS 25662 and 25663, respectively, in order of appearance):



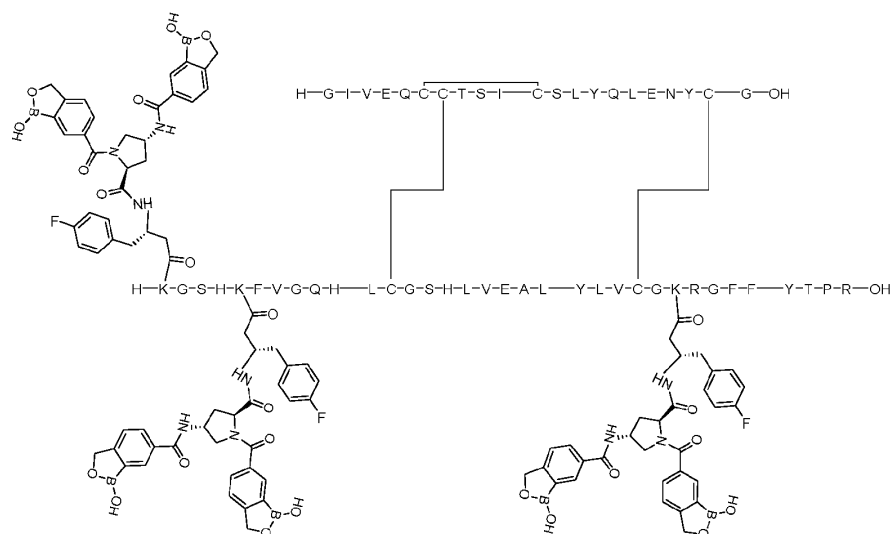
Example 136 (SEQ ID NOS 25664 and 25665, respectively, in order of appearance):



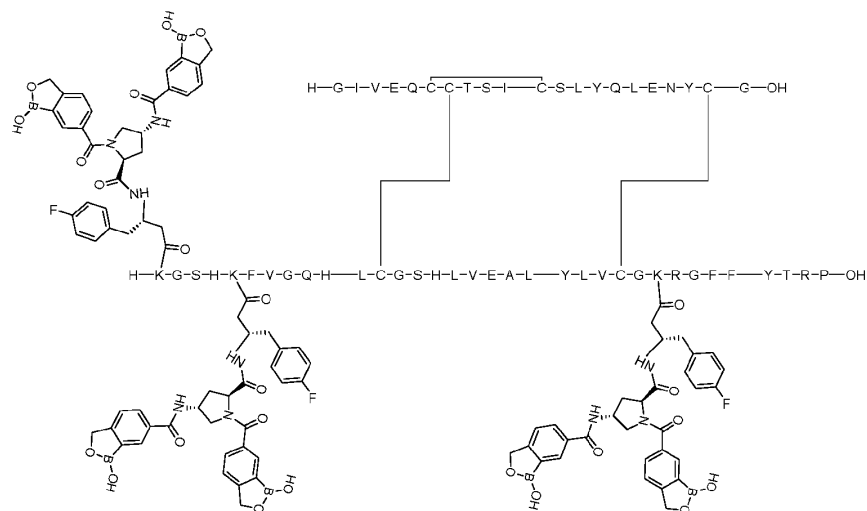
Example 137 (SEQ ID NOS 25666 and 25667, respectively, in order of appearance):



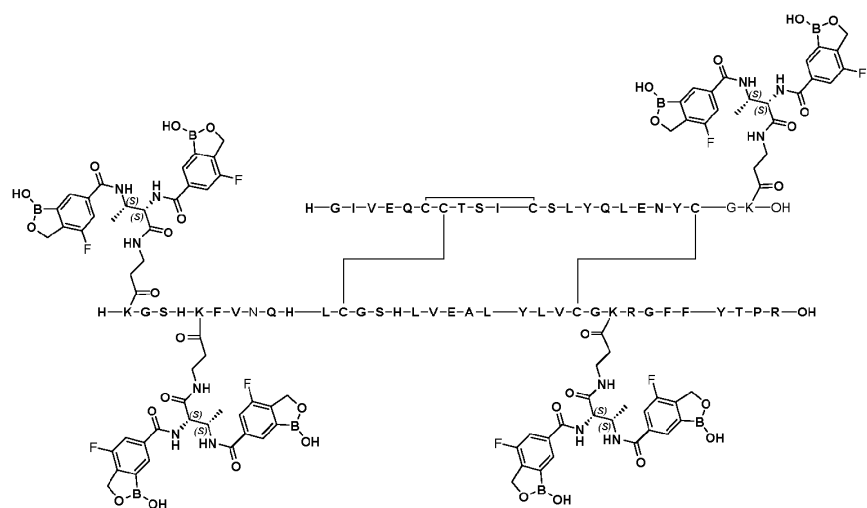
Example 138 (SEQ ID NOS 25668 and 25669, respectively, in order of appearance):



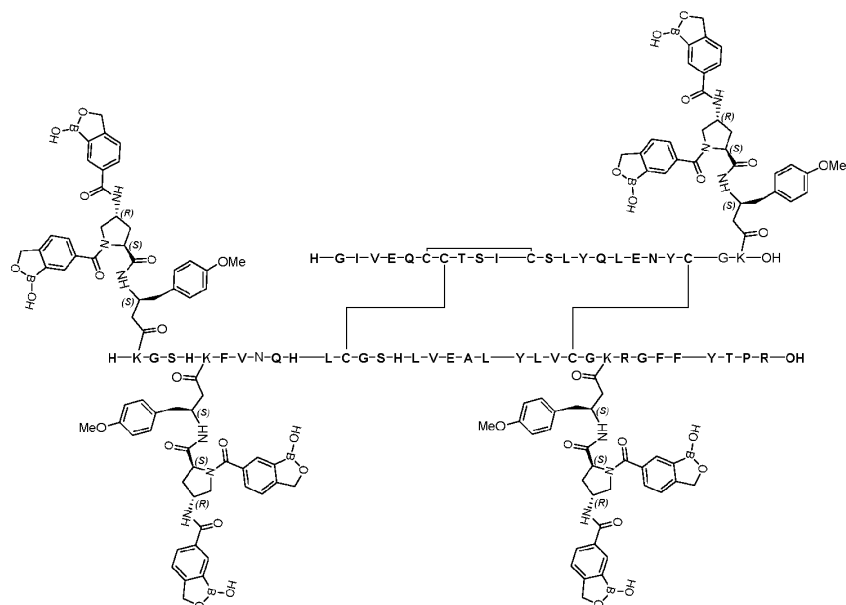
Example 139 (SEQ ID NOS 25670 and 25671, respectively, in order of appearance):



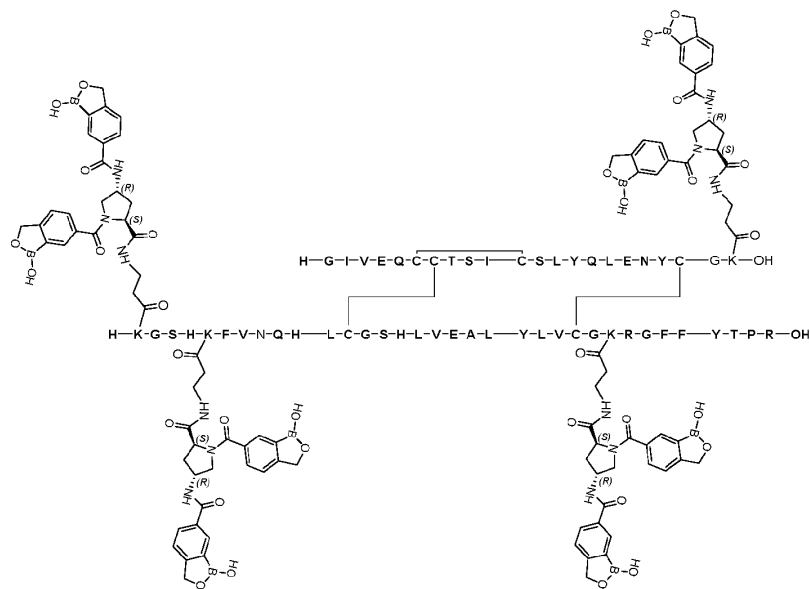
Example 140 (SEQ ID NOS 25672 and 25673, respectively, in order of appearance):



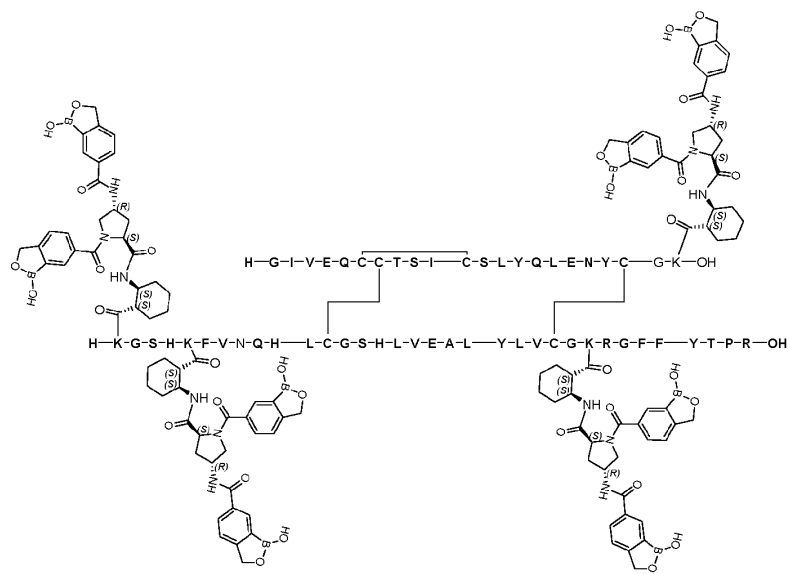
Example 141 (SEQ ID NOS 25674 and 25675, respectively, in order of appearance):



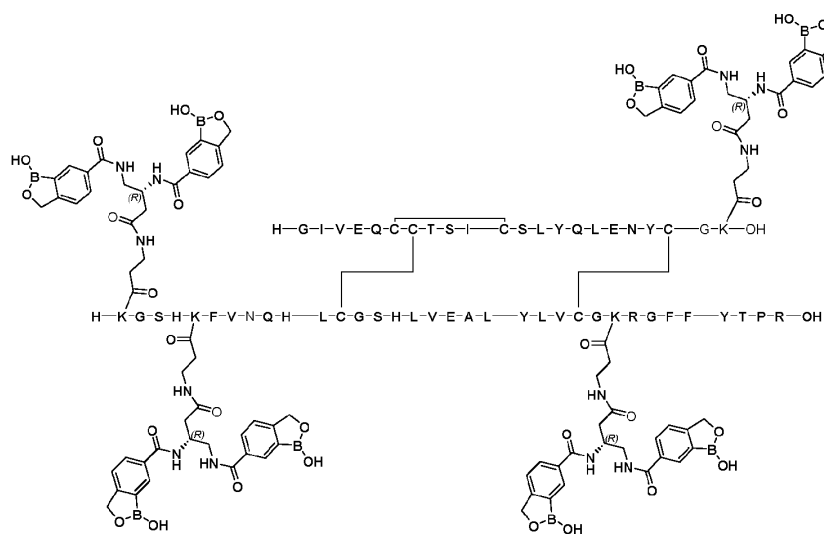
Example 142 (SEQ ID NOS 25676 and 25677, respectively, in order of appearance):



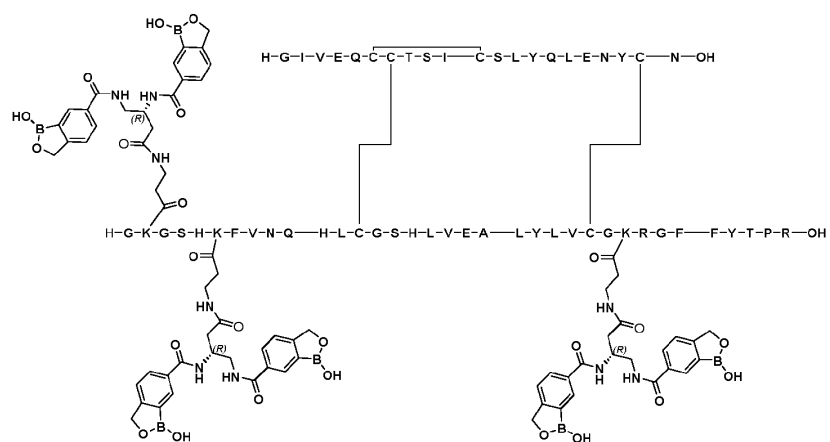
Example 143 (SEQ ID NOS 25678 and 25679, respectively, in order of appearance):



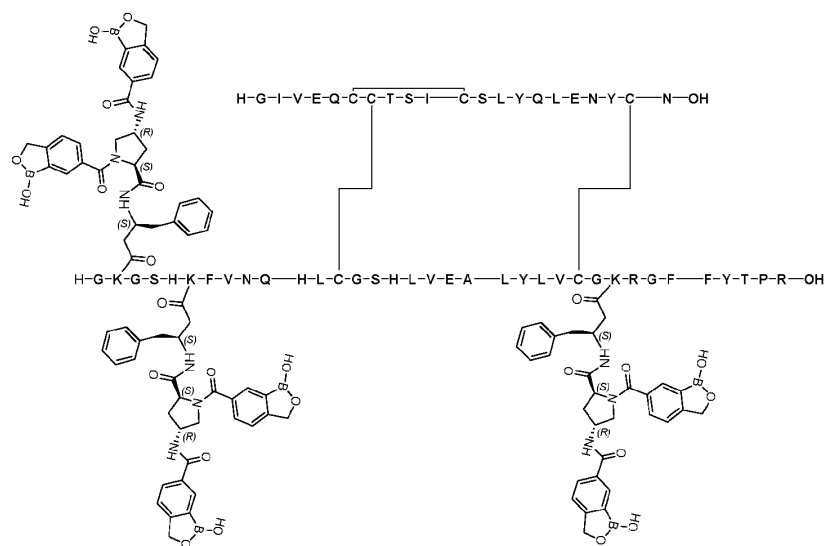
Example 144 (SEQ ID NOS 25680 and 25681, respectively, in order of appearance):



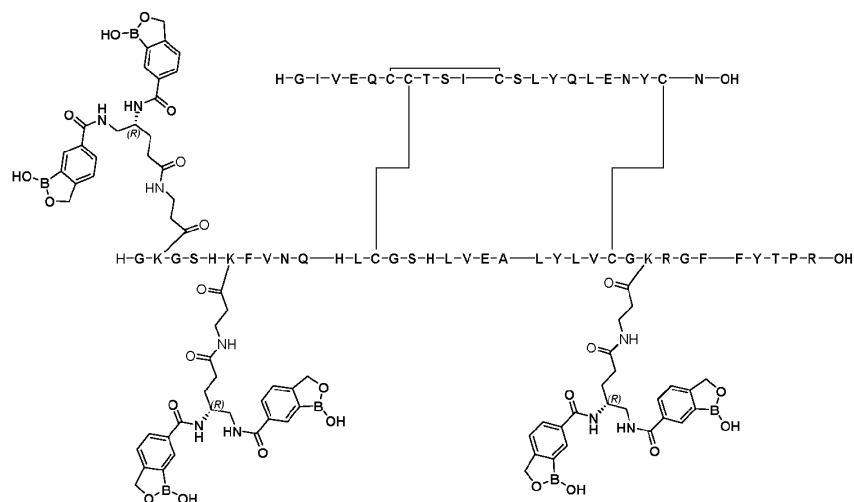
Example 145 (SEQ ID NOS 25682 and 25683, respectively, in order of appearance):



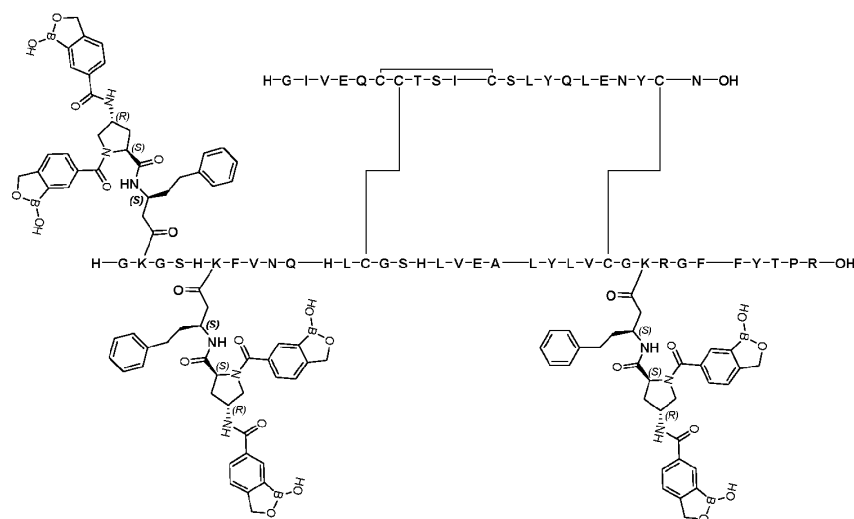
Example 146 (SEQ ID NOS 25684 and 25685, respectively, in order of appearance):



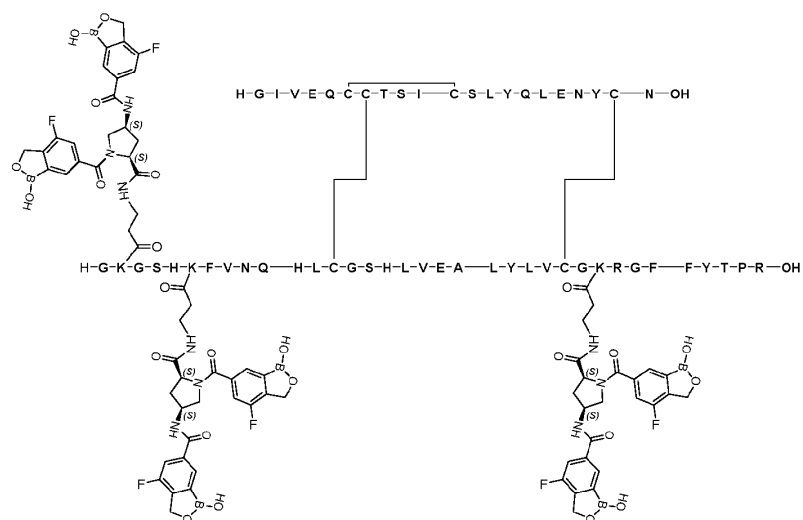
Example 147 (SEQ ID NOS 25686 and 25687, respectively, in order of appearance):



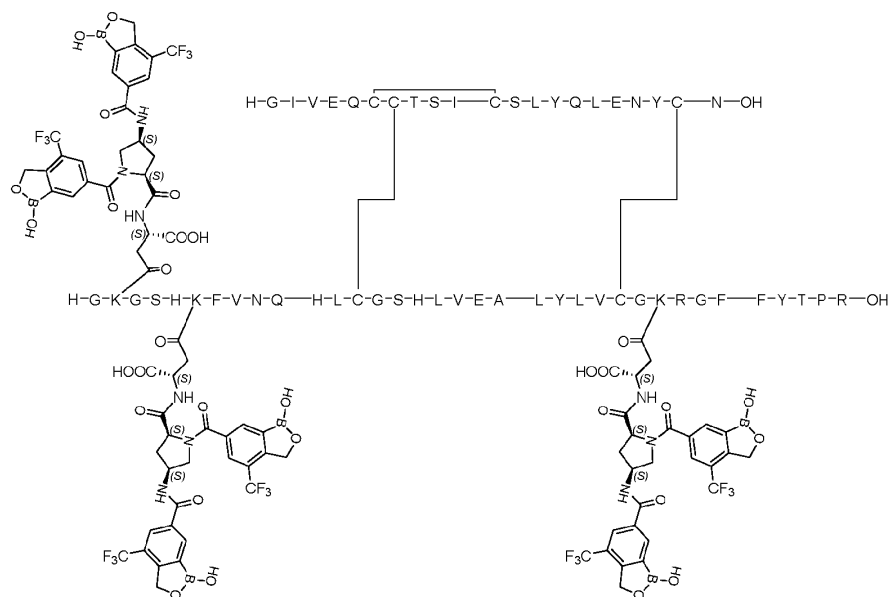
Example 148 (SEQ ID NOS 25688 and 25689, respectively, in order of appearance):



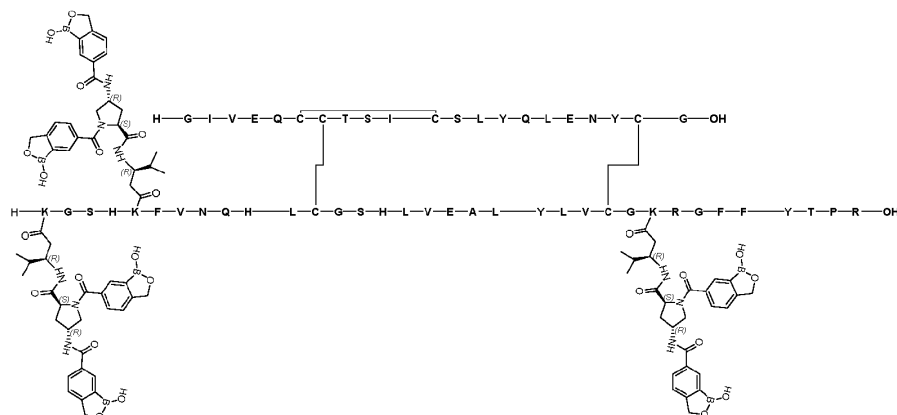
Example 149 (SEQ ID NOS 25690 and 25691, respectively, in order of appearance):



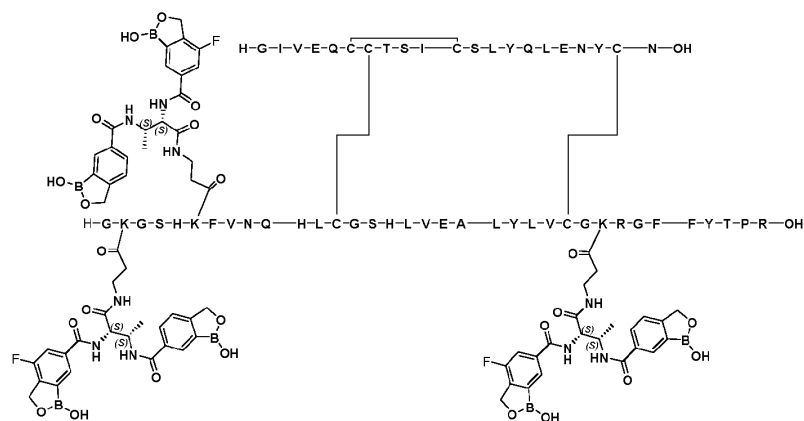
Example 150 (SEQ ID NOS 25692 and 25693, respectively, in order of appearance):



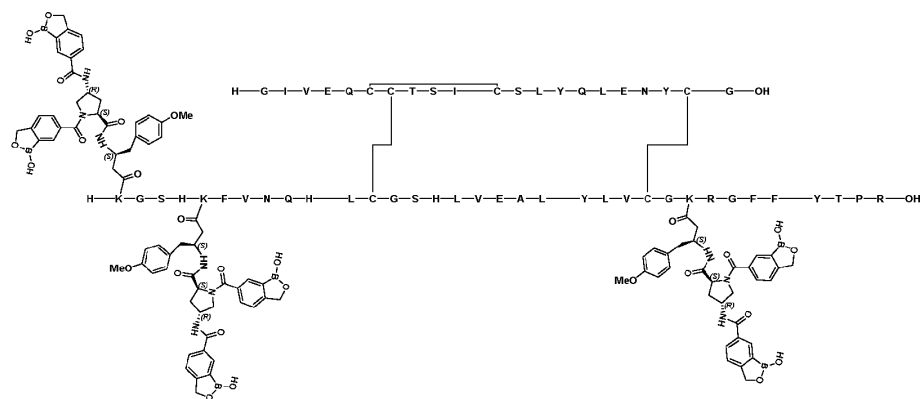
Example 151 (SEQ ID NOS 25694 and 25695, respectively, in order of appearance):



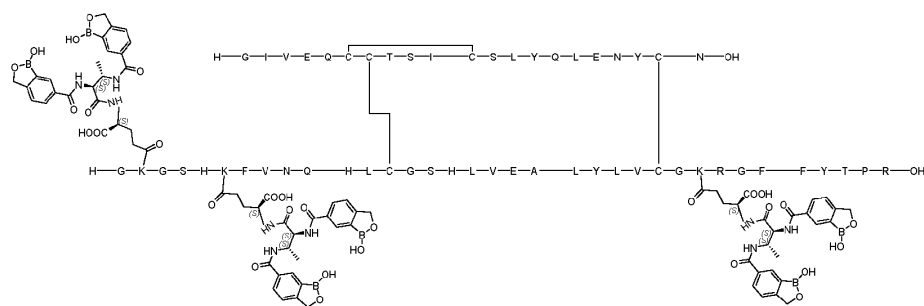
Example 152 (SEQ ID NOS 25696 and 25697, respectively, in order of appearance):



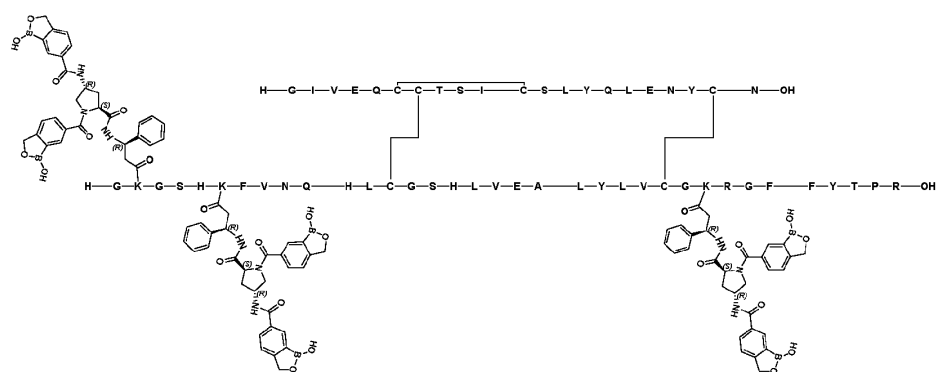
5 Example 153 (SEQ ID NOS 25698 and 25699, respectively, in order of appearance):



Example 154 (SEQ ID NOS 25700 and 25701, respectively, in order of appearance):



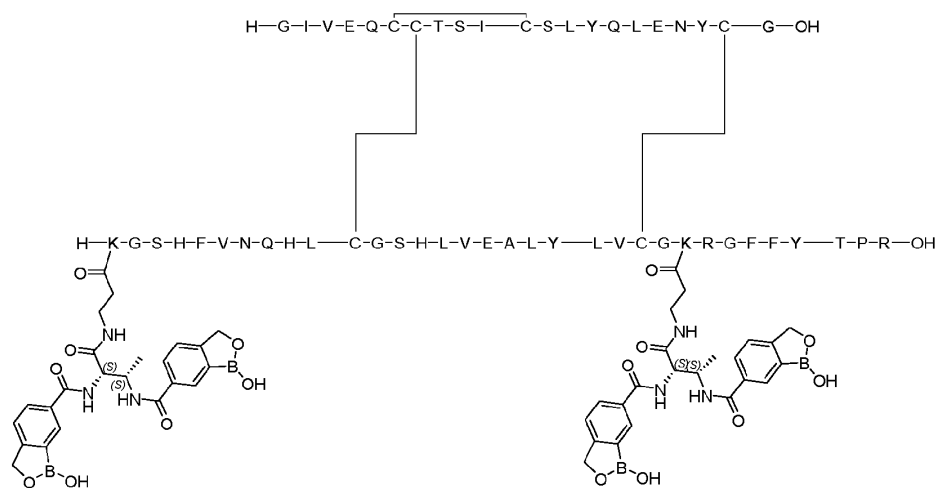
Example 155 (SEQ ID NOS 25702 and 25703, respectively, in order of appearance):



, and

5

Example 156 (SEQ ID NOS 25704 and 25705, respectively, in order of appearance):



; or

a pharmaceutically acceptable salt thereof, an isotope thereof, and combinations thereof.

15. A compound selected from the group consisting of an insulin comprising an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from 24501 and 24502;

10

and wherein the B-chain comprises a sequence selected from SEQ ID NOs 25229, 25232, 25305, 25308, 25312, 25236, 25095, and 25380-25397.

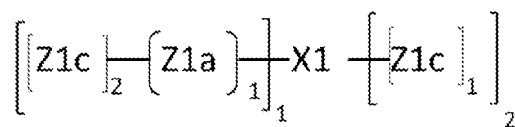
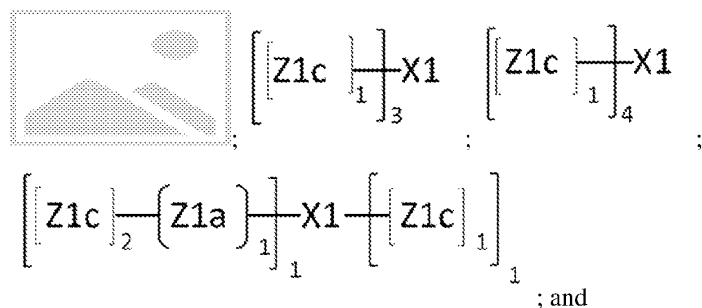
16. A pharmaceutical composition comprising at least one compound or a pharmaceutically acceptable salt thereof according to any one of claims 1-15 and a pharmaceutically acceptable carrier.
17. A compound according to any one of claims 1-15, for use as a medicament.
18. A method of treatment or prevention of diabetes, impaired glucose tolerance, hyperglycemia or metabolic syndrome, wherein the method comprises administering to a subject in need thereof the compound of any one of claims 1-15, or a pharmaceutical composition according to claim 16.
19. The compound of claim 4, or a pharmaceutically acceptable salt thereof, wherein each said indirect linker is independently selected from FL3, FL5, FL5A, FL5B, FL32, FL41, FL62, FL63, and stereoisomers thereof.
20. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein the B₁ and the B₂ are each independently Formula F2, and each remaining R₁ is independently selected from H, F, Cl, I, Br, and CF₃.
21. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein the B₁ and the B₂ are each independently Formula F2, and each remaining R₁ is H.
22. The compound of any one of claims 2-7, or a pharmaceutically acceptable salt thereof, wherein:
- X1 comprises at least one lysine and an N-terminus;
- each Z1c is independently selected from FF12A, FF12B, FF12C, FF12D, FF116, FF116A, FF116B, FF116C, and FF116D, wherein each B₁ and B₂ is F2;
- each Z1c is conjugated, either directly or via the indirect linker, to an amine side chain in the lysine in X1 or to the N-terminus in X1; and
- each indirect linker is independently selected from FL3, FL5, FL5A, FL5B, and FL20-FL75, and stereoisomers thereof, wherein in FL3 and FL5 each p is independently 1, 2, or 3.
23. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein at least one Z1c is conjugated via the indirect linker to an amine side chain in the lysine in the B-chain or to the B chain N-terminus.
24. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein X1 comprises two or more lysine amino acids.
25. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein the B-chain comprises at least two lysine amino acids each independently conjugated either directly or via an indirect linker to a Z1c.

26. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein the B-chain comprises at least three lysine amino acids each independently covalently conjugated via the indirect linker to a Z1c.

27. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein the A-chain comprises one or more lysine amino acids each independently covalently conjugated either directly or via an indirect linker to a Z1c.

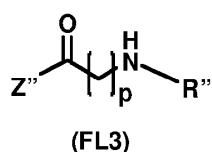
28. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein X1 comprises an A-chain having at least one lysine covalently conjugated via the indirect linker to a Z1c.

29. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein the compound is selected from:



15

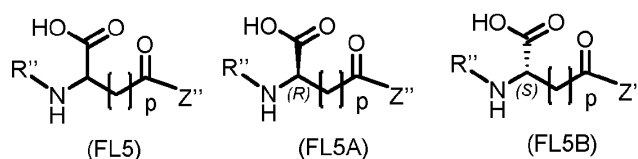
30. The compound of claim 4 or 5, wherein at least one Z1c is covalently conjugated to X1 via the indirect linker, and wherein each indirect linker is independently selected from



Formula FL3,

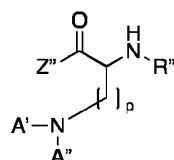
20

wherein p is 2 or 3;



Formulae FL5, FL5A, and FL5B,

wherein p is 1 or 2, and



FL69, and stereoisomers thereof;

5 wherein:

R'' represents a covalent bond, directly or indirectly, to Z1c;

Z'' represents a covalent bond, directly or indirectly, to X1 or to Z1a;

A' is selected from H, a C₁-C₆alkyl group, a saturated fatty acid, an unsaturated fatty acid, a (C₃-C₆)cycloalkyl group, a C₁-C₆haloalkyl group, an aryl group, and a heteroaryl group;

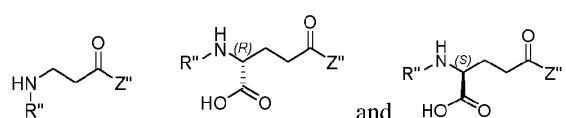
10 and

A'' is selected from a C₁-C₆alkyl group, a substituted acyl group, acyl group terminating in an acid group, a saturated or unsaturated fatty acid, a (C₃-C₆)cycloalkyl group, a C₁-C₆haloalkyl group, an aryl group, and a heteroaryl group;

p is 1, 2, 3, 4, or 5, and

15 any primary amine is optionally acetylated or alkylated.

31. The compound of claim 30, or a pharmaceutically acceptable salt thereof, wherein each indirect linker is selected from:



32. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt thereof, wherein X1 is an insulin further comprising from 1 to 5 residues replaced, inserted, appended, or mutated to an amino acid that has a free amine conjugated directly or indirectly to a Z1c.

33. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt thereof, wherein at least one Z1c is conjugated to a free amine side chain of an amino acid that has been replaced, inserted, or mutated on an insulin.

34. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt thereof, wherein the compound is covalently conjugated either directly or via a linker to a molecule that can bind to at least one protein present in human plasma.

35. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition comprises one or more polyalcohols.

36. The pharmaceutical composition of claim 35, wherein the polyalcohols are selected from mannitol, sorbitol, erythritol, isomalt, lactitol, glucose, and maltitol.

37. A composition comprising at least one compound of any one of claims 1-15 and 19-34 for use as a medicament for the treatment of diabetes or obesity, for control of blood sugar levels, or for control of release of a drug.

38. A method of administering the compound of any one of claims 1-15 or a pharmaceutical composition according to claims 16 or 35-36 to a subject, wherein the method comprises administering to the subject the compound or the composition as a therapeutic or prophylactic agent.

39. A method of making a compound of any one of claims 1-15, wherein the method comprises at least one alkylation and/or amidation step.

40. A method of treating a subject by administering a device or formulation comprising a compound of any one of claims 1-15.

41. The compound of any one of claims 1-15, or a pharmaceutically acceptable salt thereof, wherein the compound is used as an intermediate in the manufacture of a drug substance or a therapeutic of a prophylactic compound.

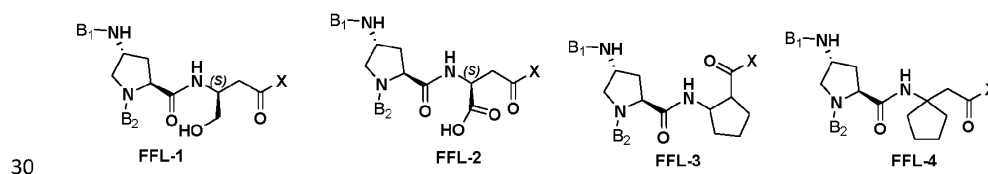
42. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein in each Formulae F2, F5, and F10 one R₁ is (C=O)---*.

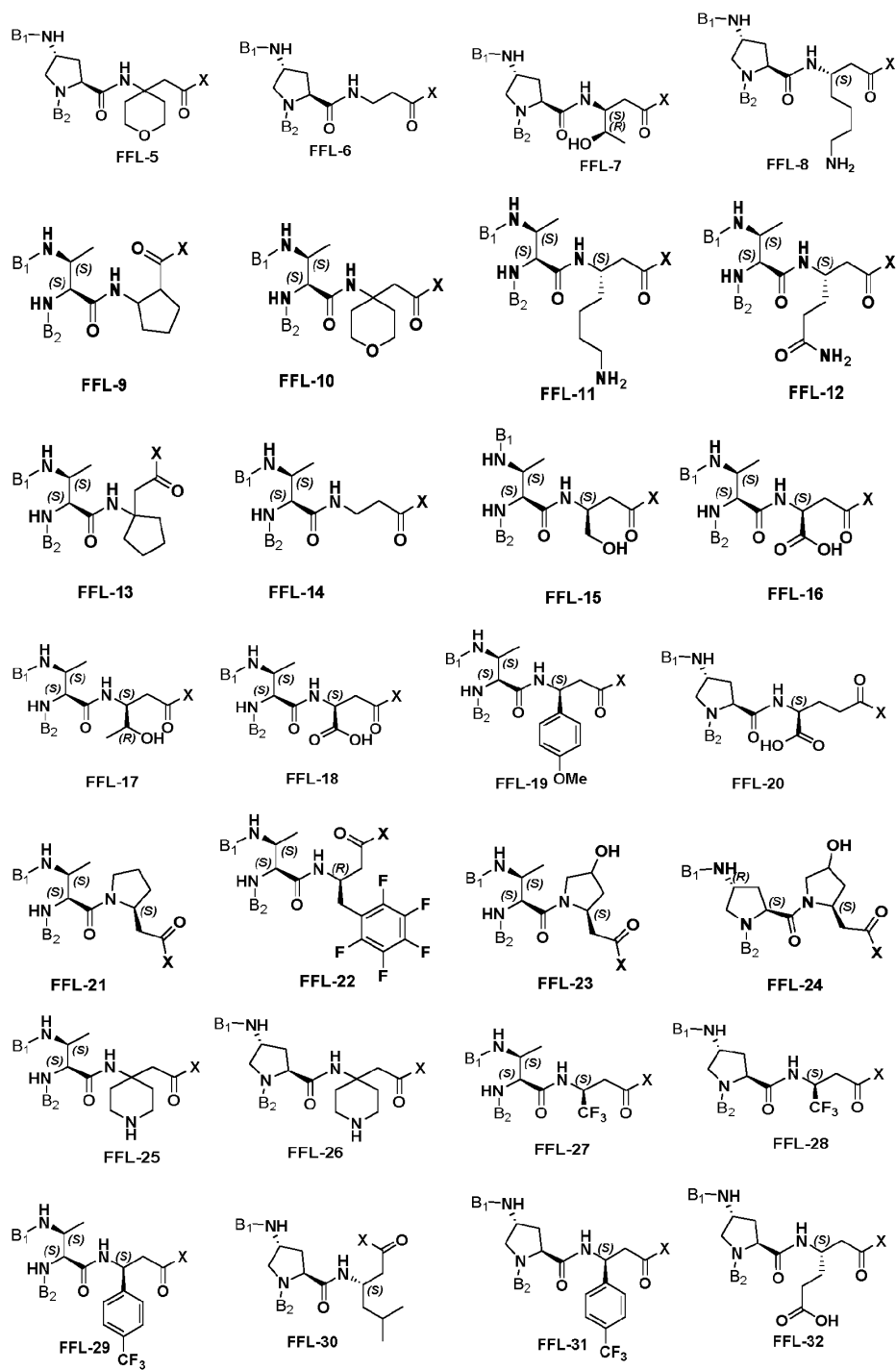
43. A compound represented by Formula II, or a stereoisomer or a mixture of stereoisomers, or pharmaceutically acceptable salt thereof:

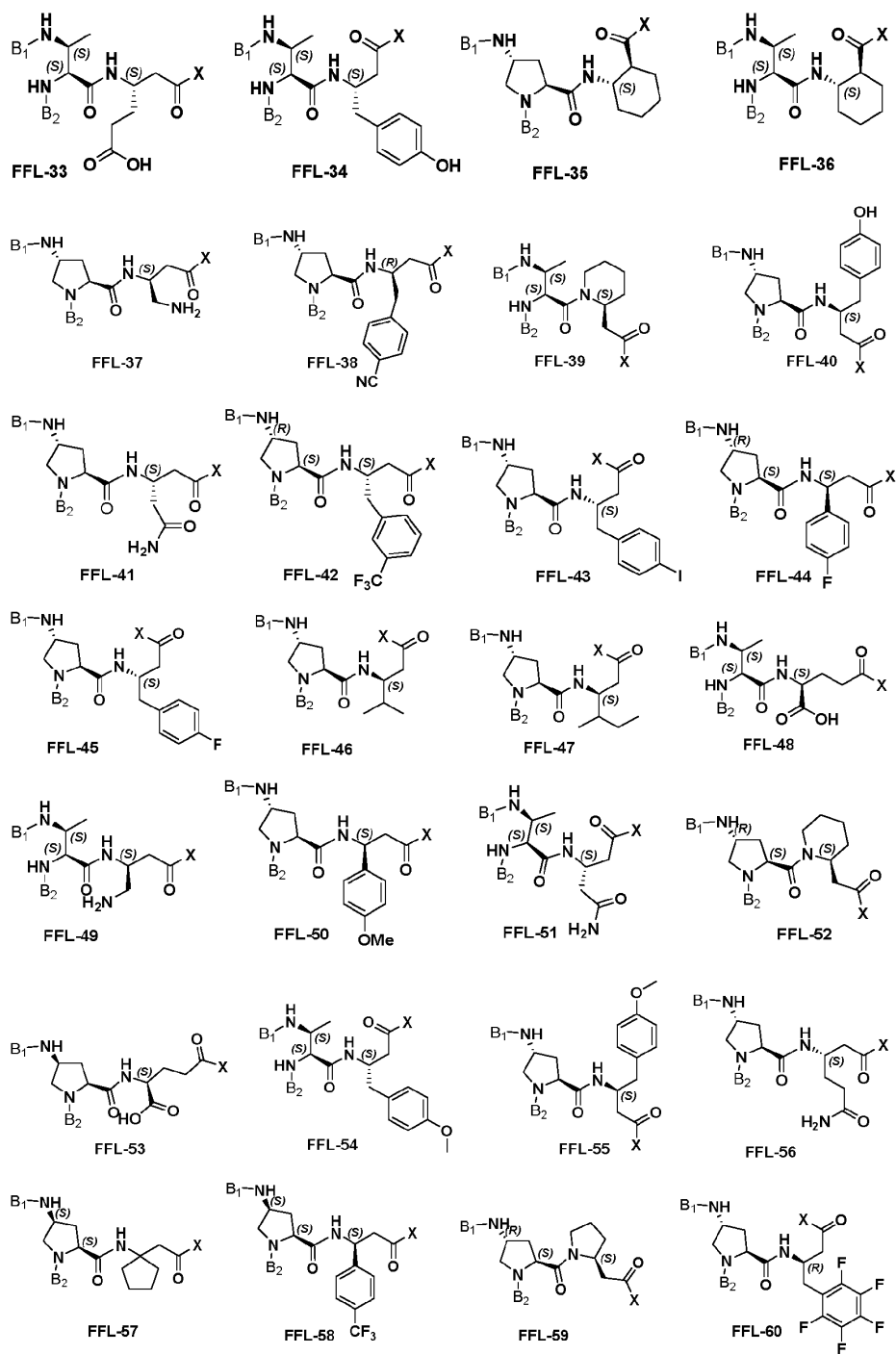
Z1c-Linker

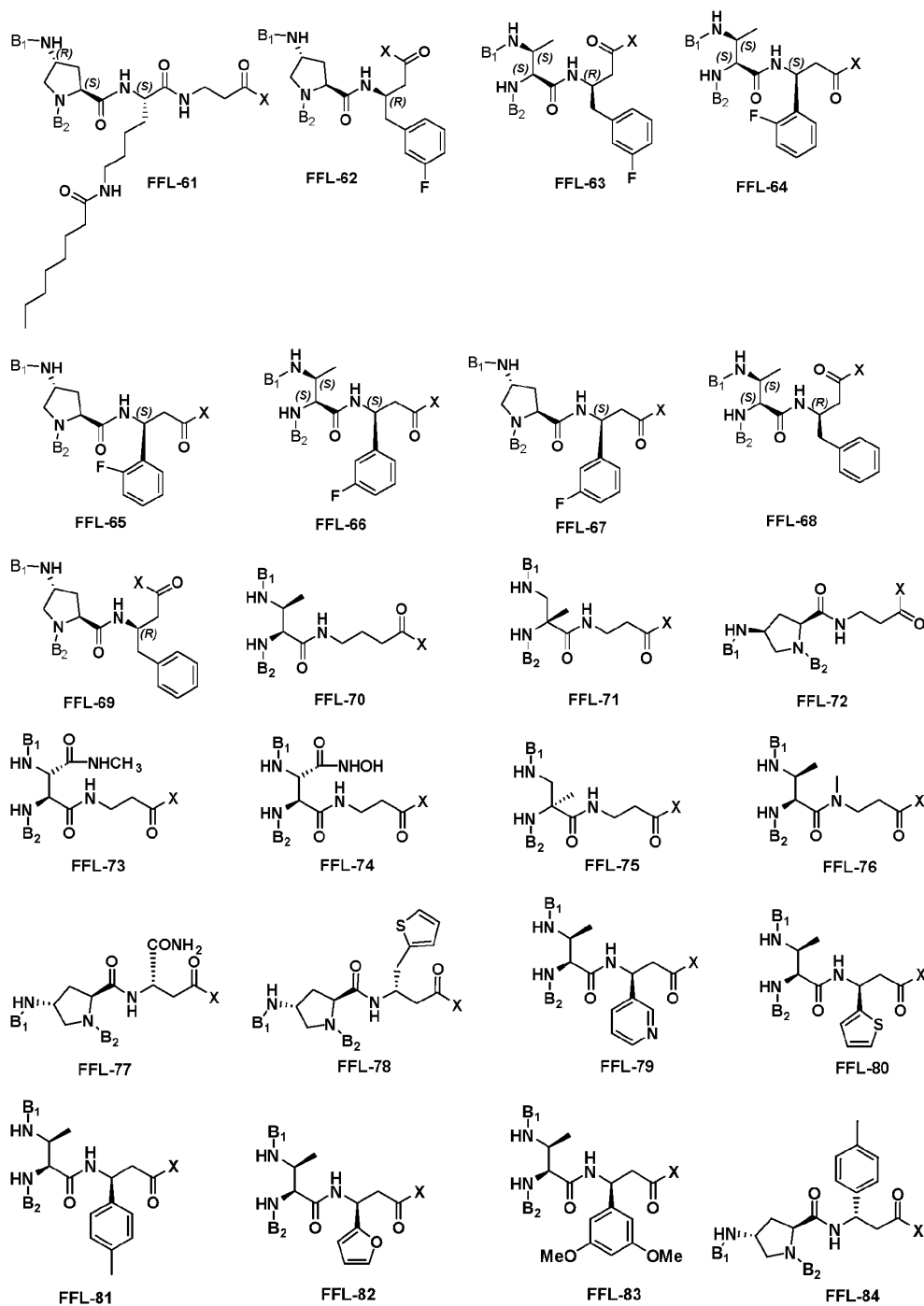
(Formula II)

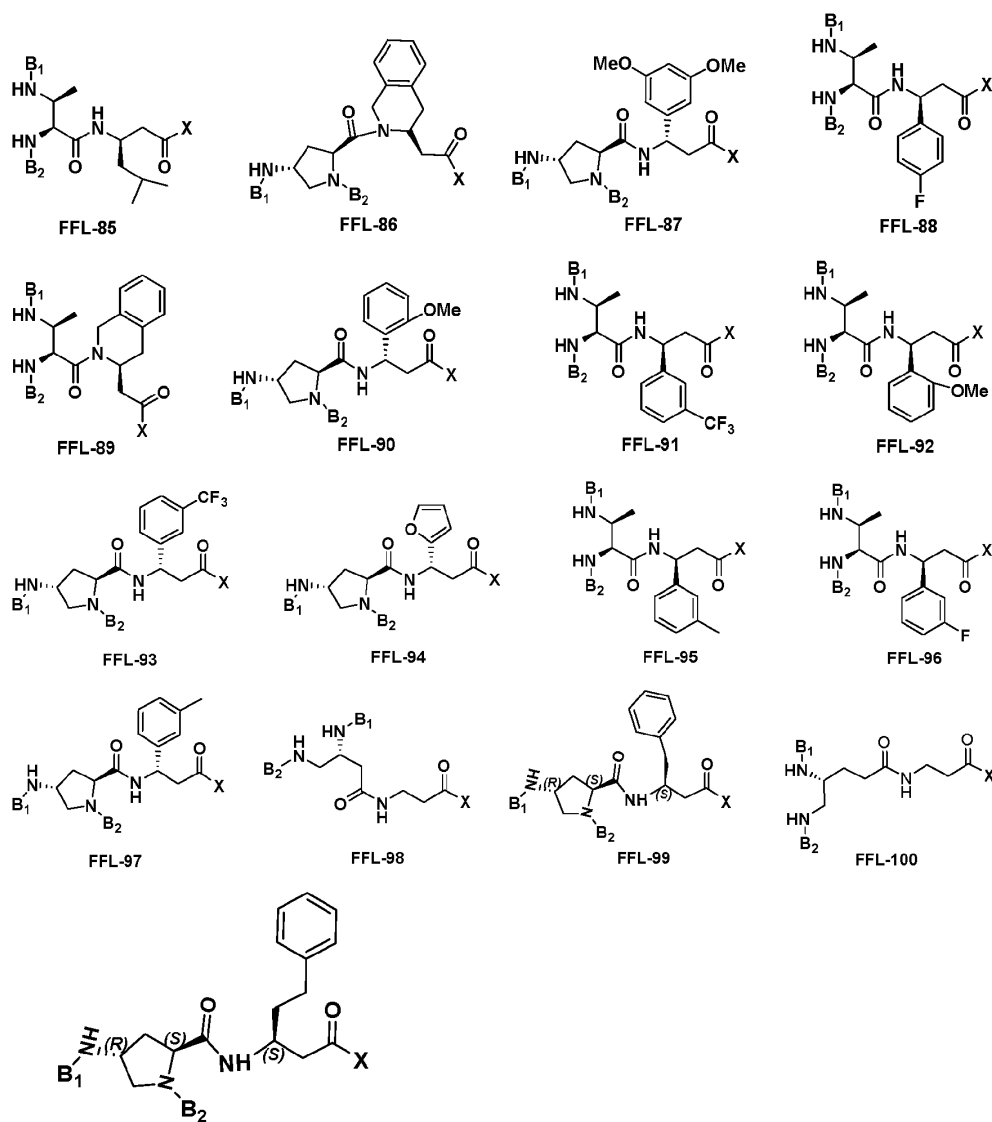
wherein the Z1c-Linker is selected from:









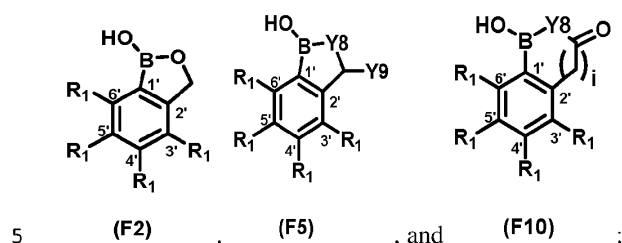


5 and FFL-101 ; wherein X is selected from a leaving group, NH₂, and H;
and

B₁ and B₂, which may be identical or different, each independently represents an aromatic boron-containing group.

44. The compound of claim 43, comprising at least one B₁ or B₂ independently selected from Formulae F2, F5, and F10,

wherein Formulae F2, F5, and F10 are:



wherein:

one R₁ represents (C=O)---* or (CH₂)_m(C=O)---*, wherein ---* represents the attachment point to the rest of Z1c, and m is 1, 2, 3, 4, 5, 6, or 7; and

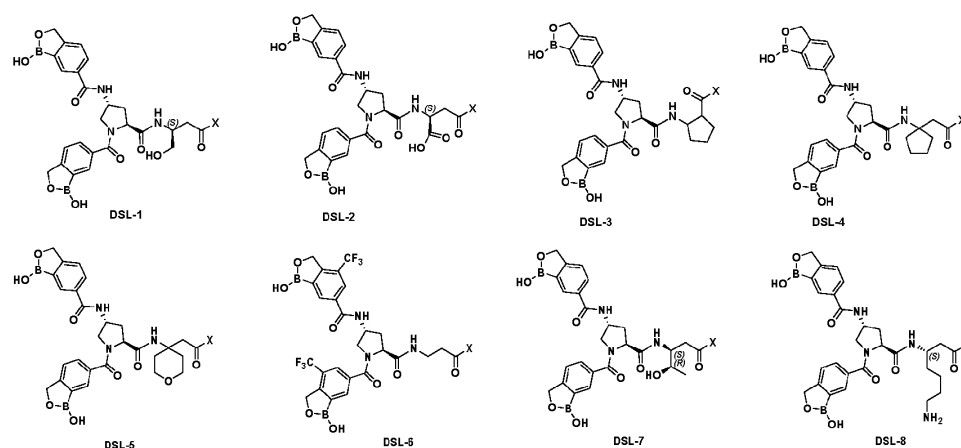
each remaining R₁ is independently selected from H, F, Cl, Br, I, OH, CH₂-NH₂, NH₂,
10 (C=O)-NH₂, CH=O, SO₂CH₃, SO₂CF₃, CF₃, CHF₂, NO₂, CH₃, OCH₃, O(CH₂)_{m'}CH₃, (SO₂)NH-CH₃, (SO₂)NH(CH₂)_{m'}CH₃, and OCF₃, wherein m' is 1, 2, 3, 4, 5, 6, or 7;

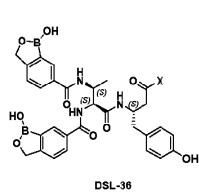
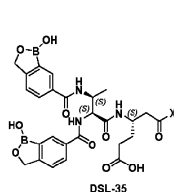
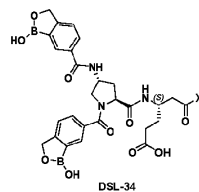
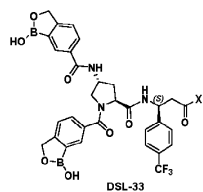
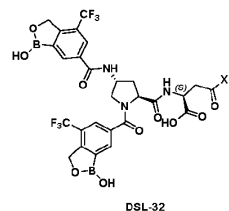
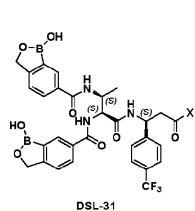
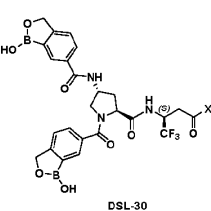
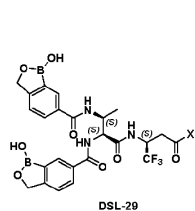
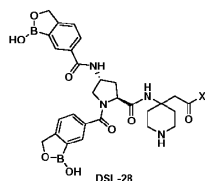
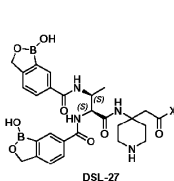
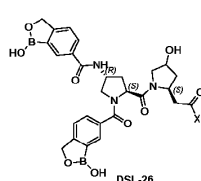
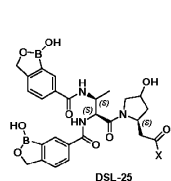
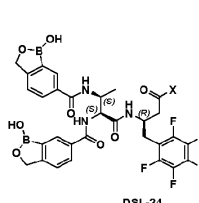
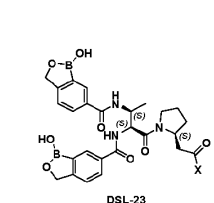
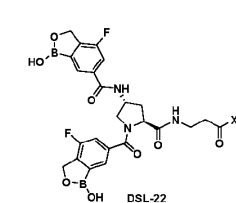
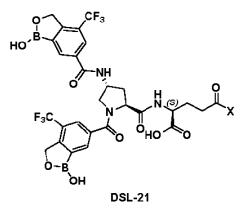
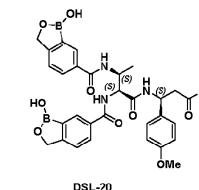
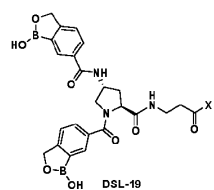
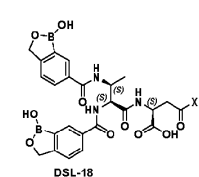
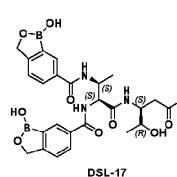
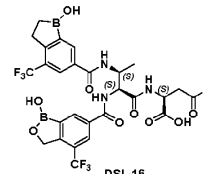
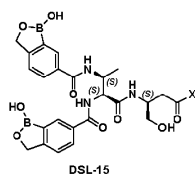
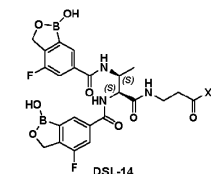
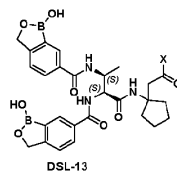
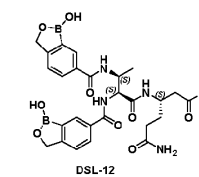
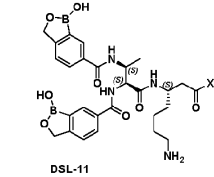
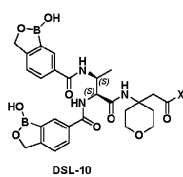
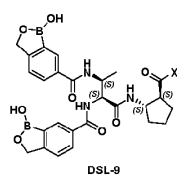
Y8 is O, N, and NR, wherein R is a C₁-C₆alkyl group or H;

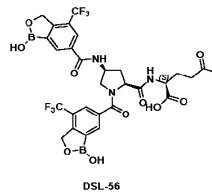
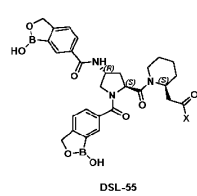
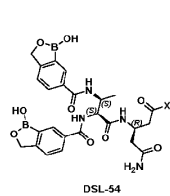
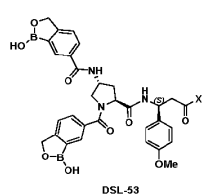
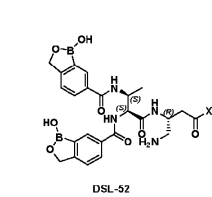
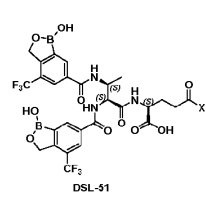
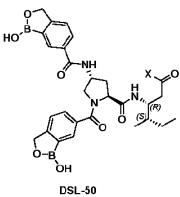
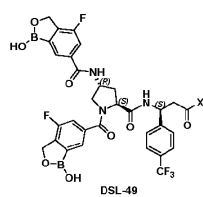
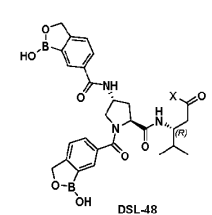
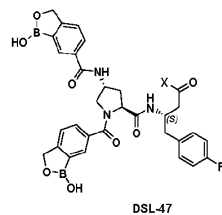
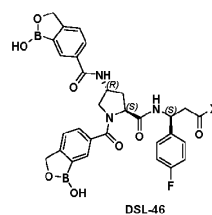
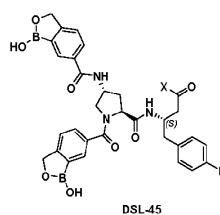
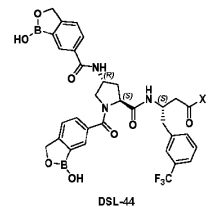
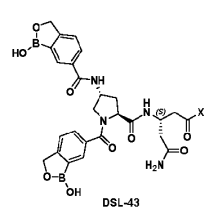
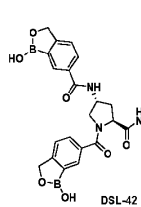
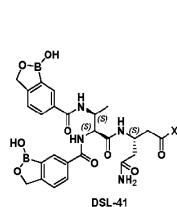
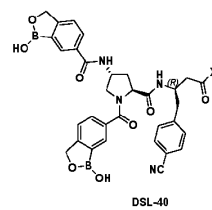
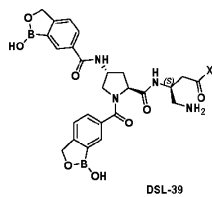
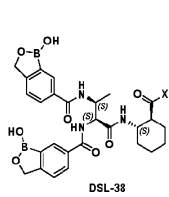
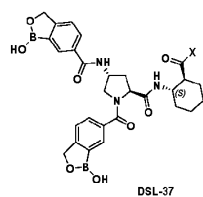
Y9 is H, CH₃, or a C₁-C₆alkyl group, provided that when Y8 is O, Y9 is a CH₃ or a C₁-C₆alkyl group; and

15 i is 1, 2, or 3.

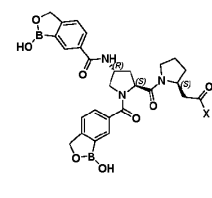
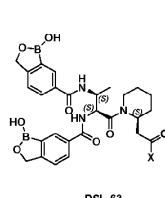
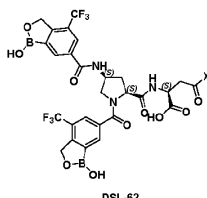
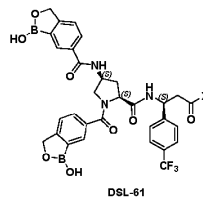
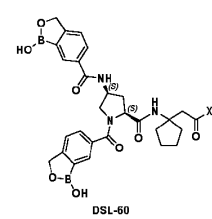
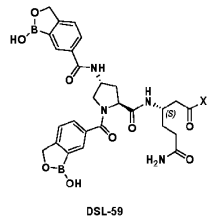
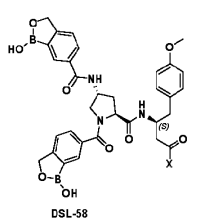
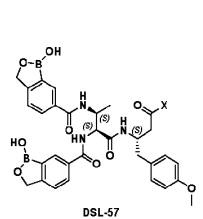
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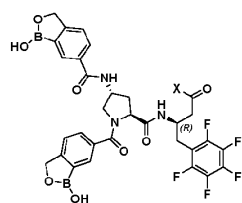




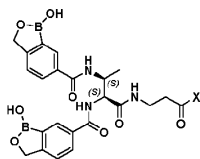


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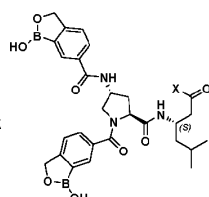




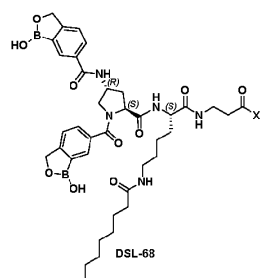
DSL-65



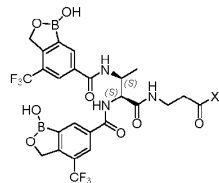
DSL-66



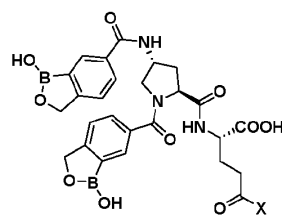
DSL-67



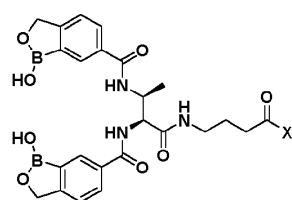
DSL-68



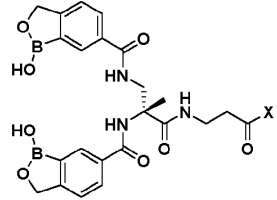
DSL-69



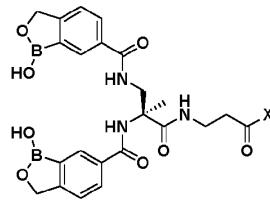
DSL-70



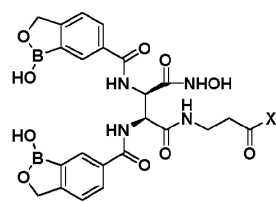
DSL-71



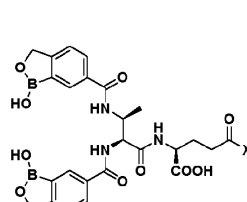
DSL-72



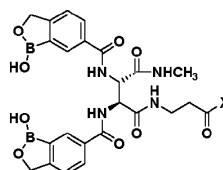
DSL-73



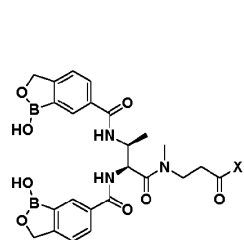
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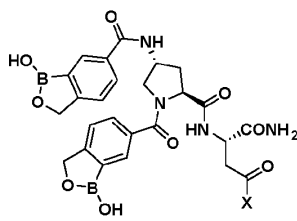
DSL-75



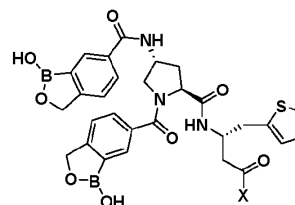
DSL-76



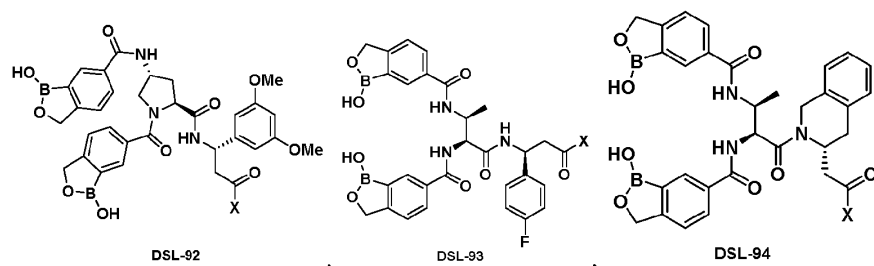
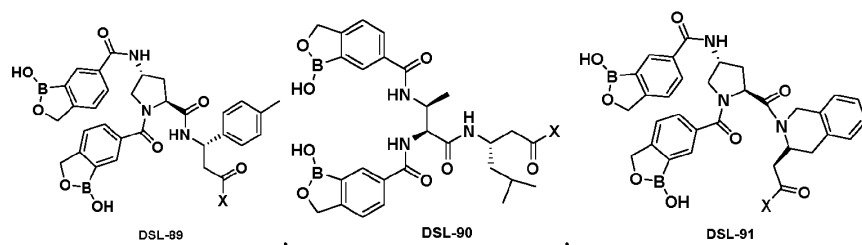
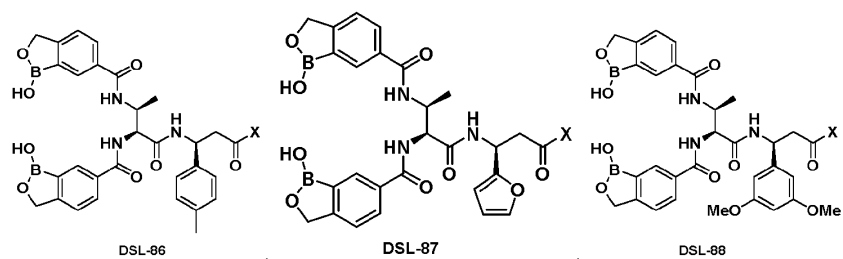
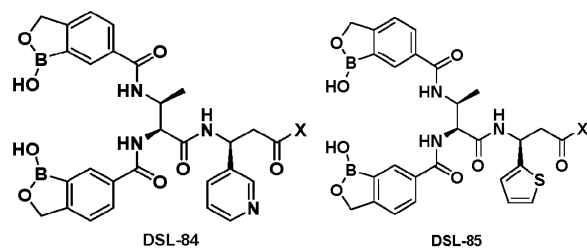
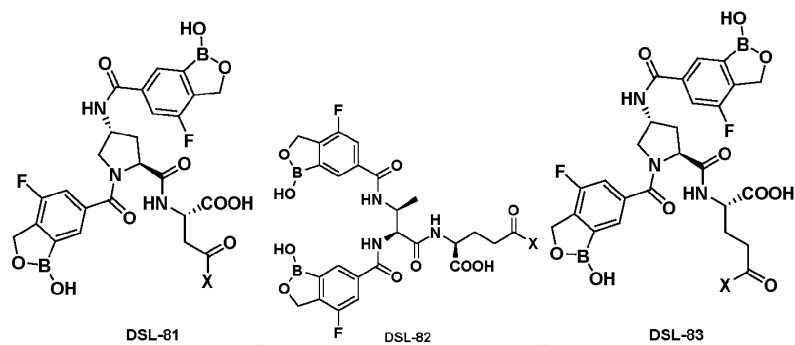
DSL-78

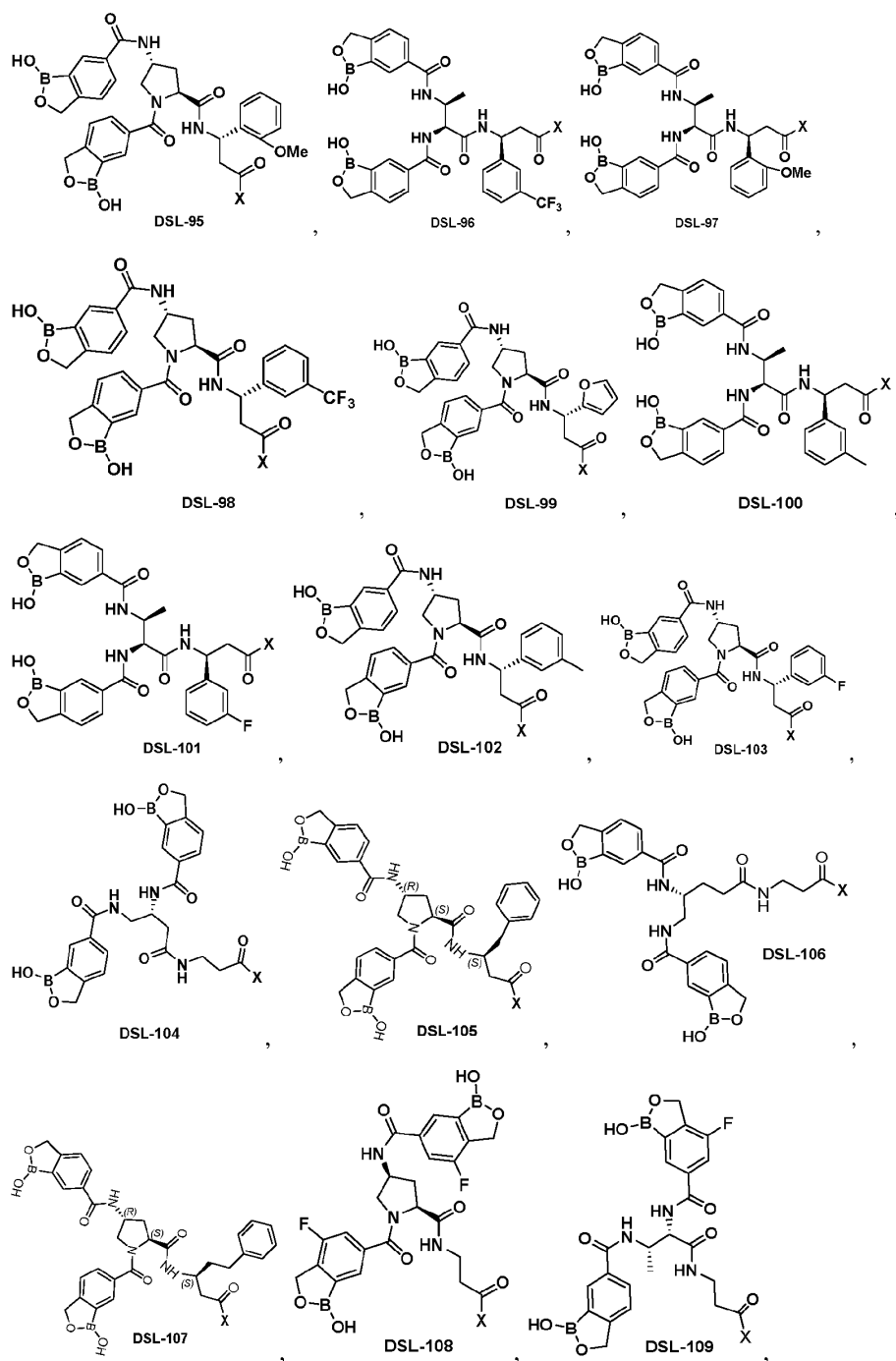


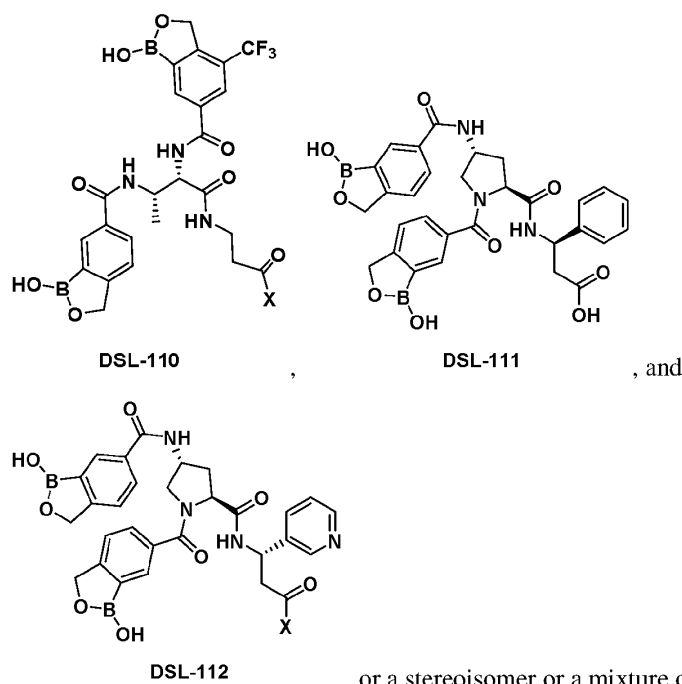
DSL-79



DSL-80



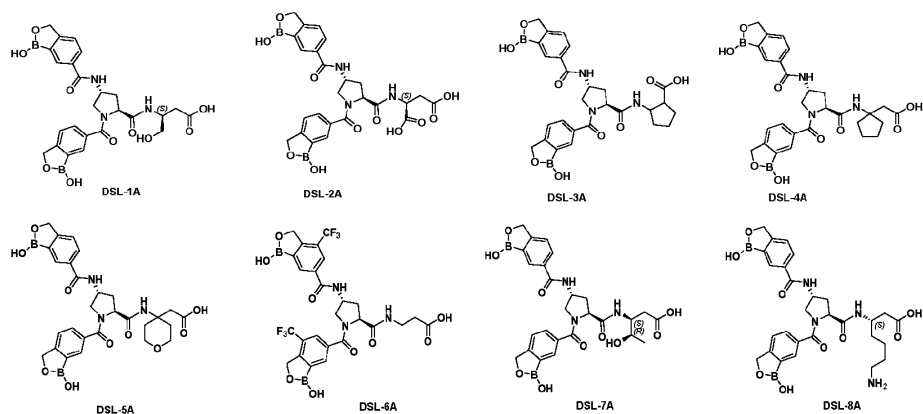


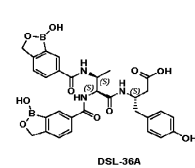
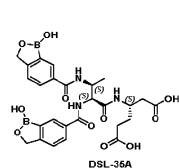
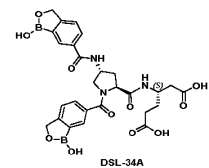
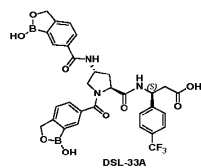
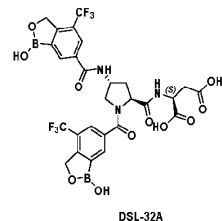
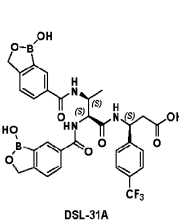
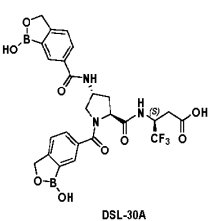
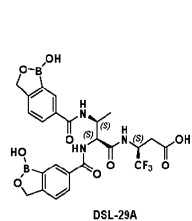
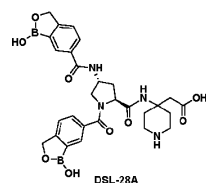
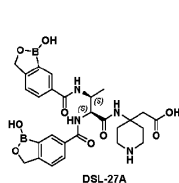
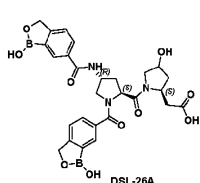
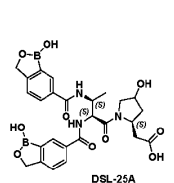
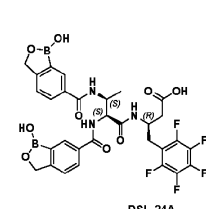
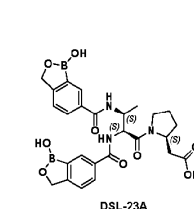
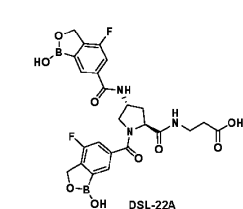
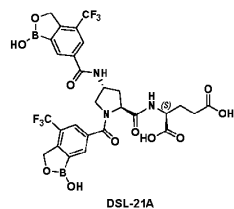
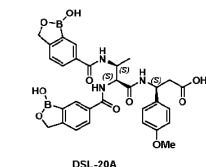
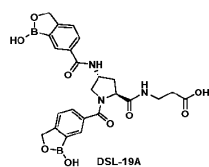
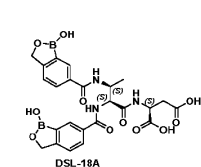
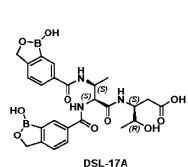
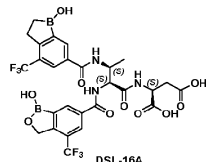
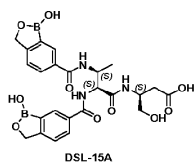
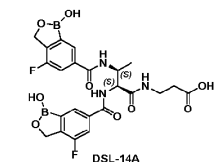
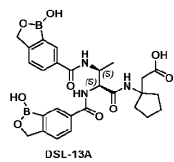
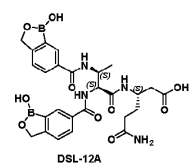
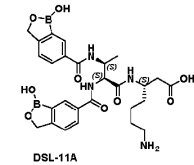
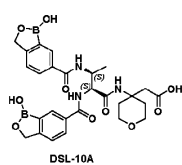
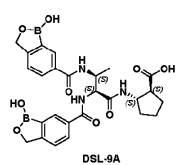


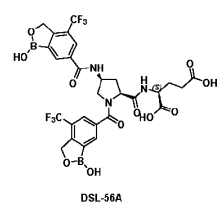
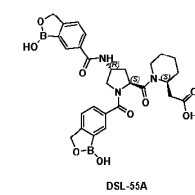
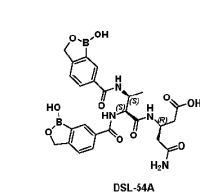
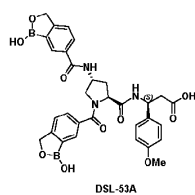
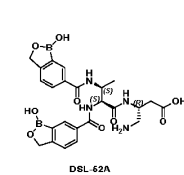
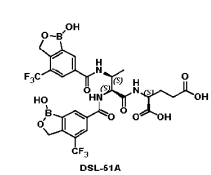
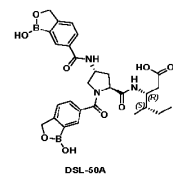
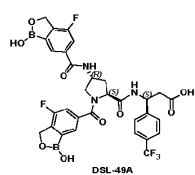
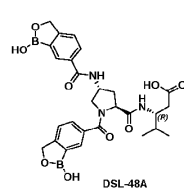
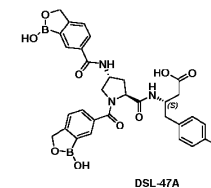
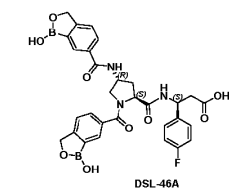
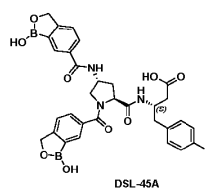
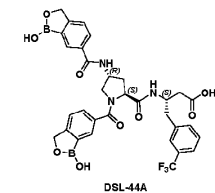
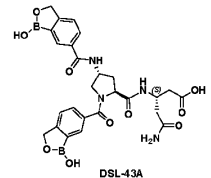
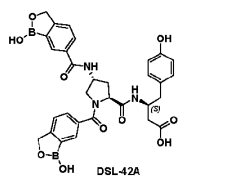
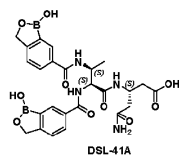
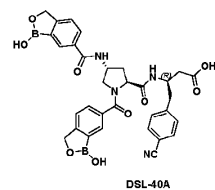
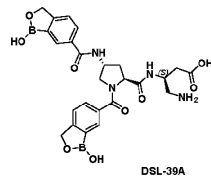
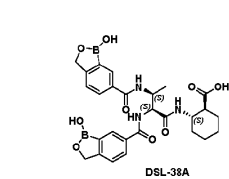
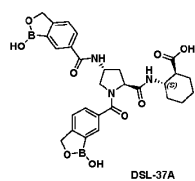
5

46. The compound of claim 45, or a pharmaceutically acceptable salt thereof, wherein X is selected from N-oxysuccinimide (NHS), 2,3,5,6-tetrafluorophenoxy (TFP), pentafluorophenoxy (Pfp), OH, halogen, maleimide alkyl amino, maleimide amido polyethylene glycol amino, and maleimide polyethylene glycol amino.

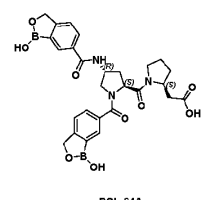
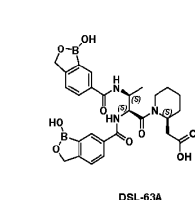
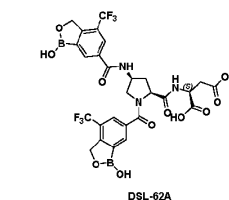
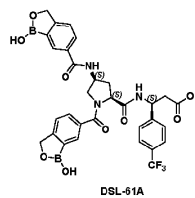
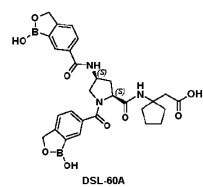
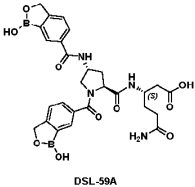
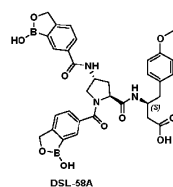
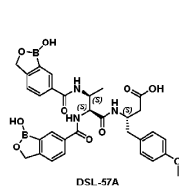
10 47. The compound of claim 46, wherein the compound is selected from:

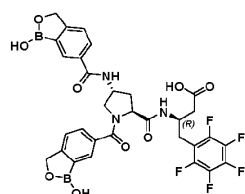




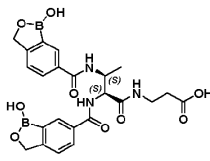


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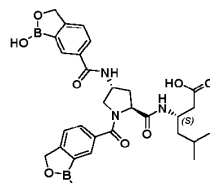




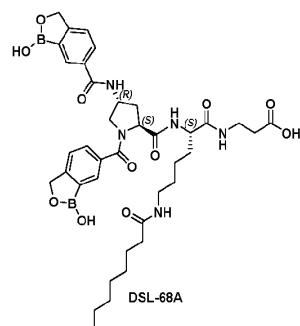
DSL-65A



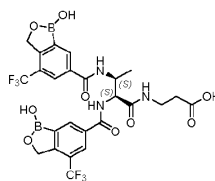
DSL-66A



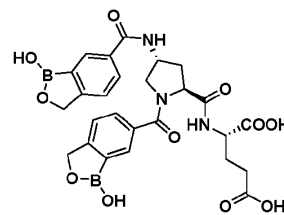
DSL-67A



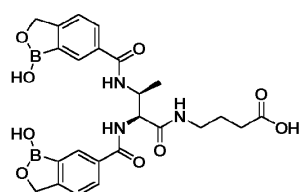
DSL-68A



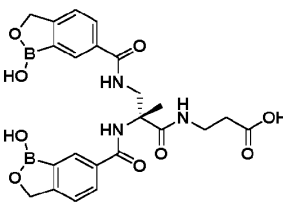
DSL-69A



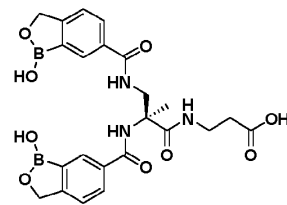
DSL-70A



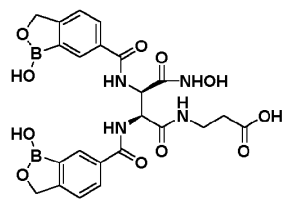
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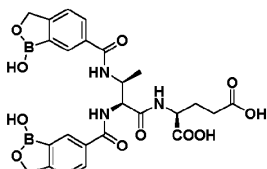
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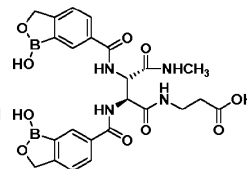
DSL-73A



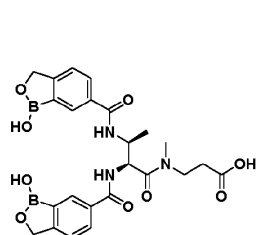
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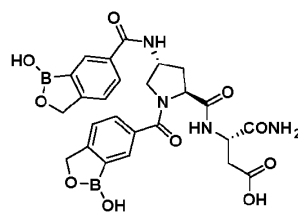
DSL-75A



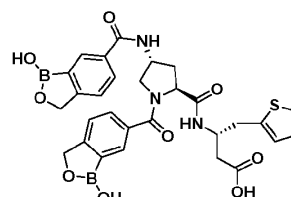
DSL-76A



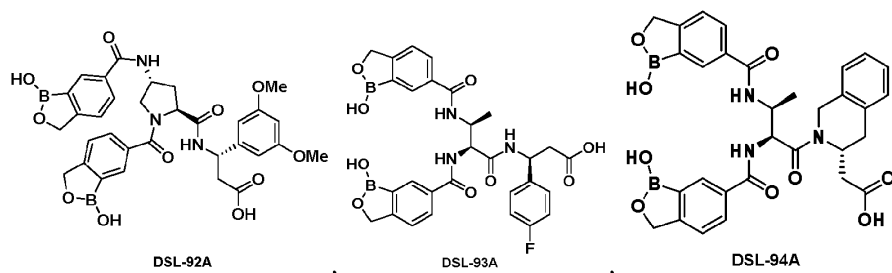
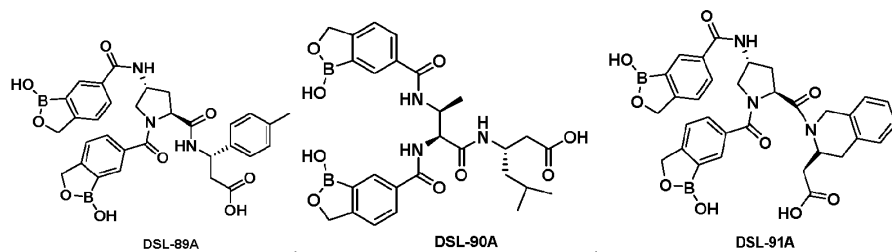
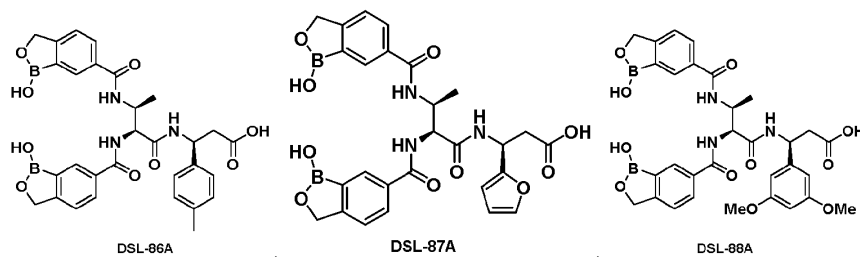
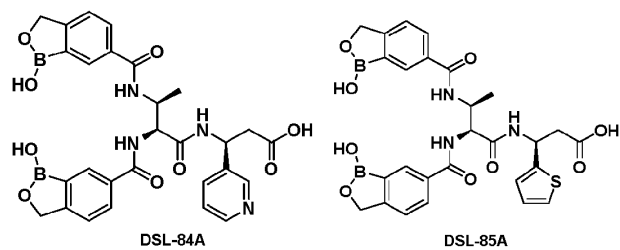
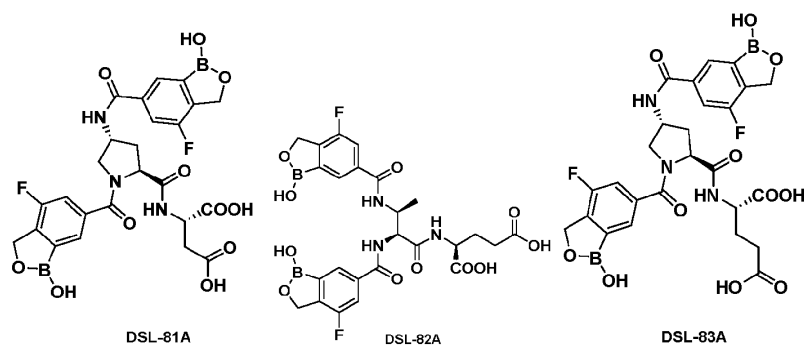
DSL-78A

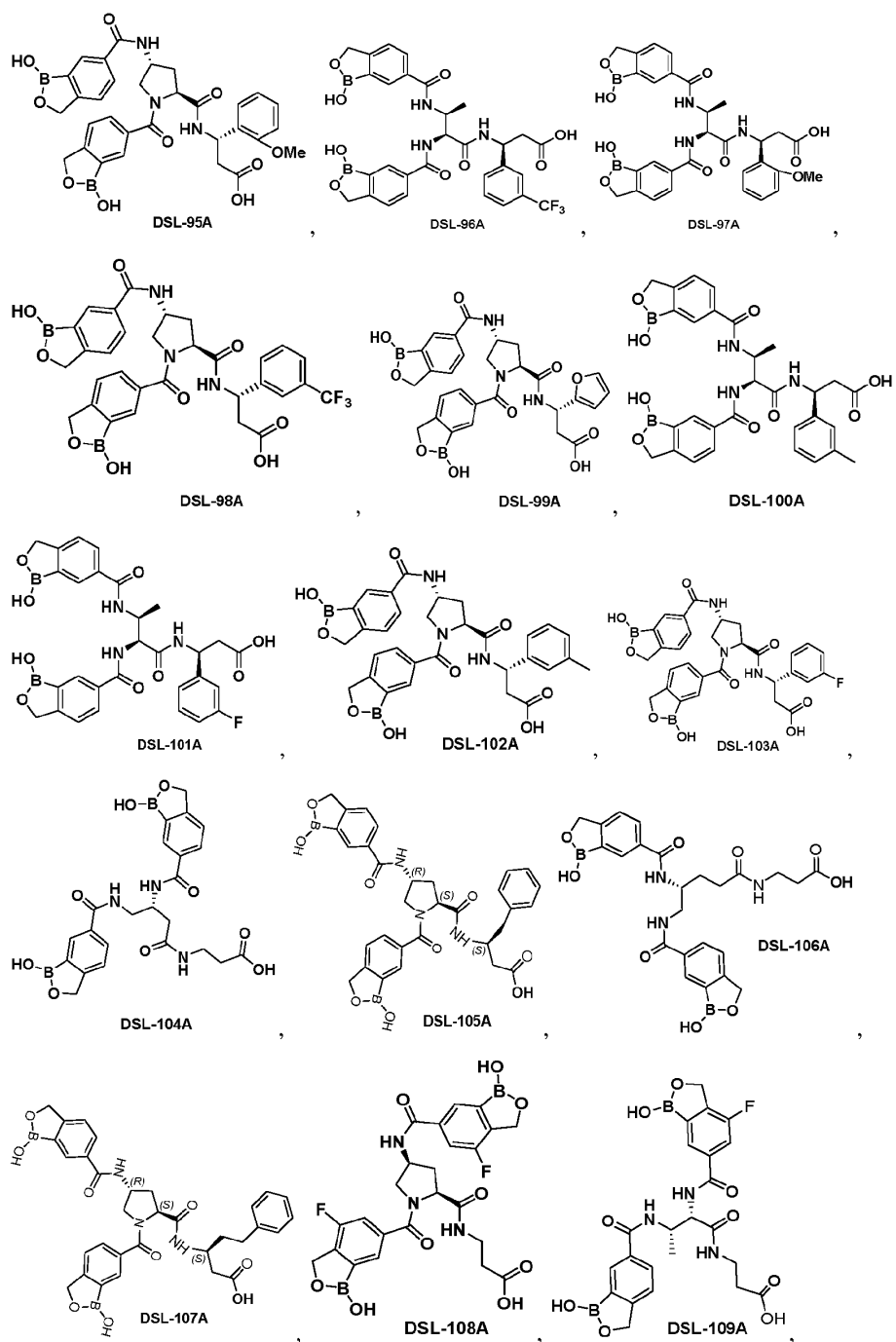


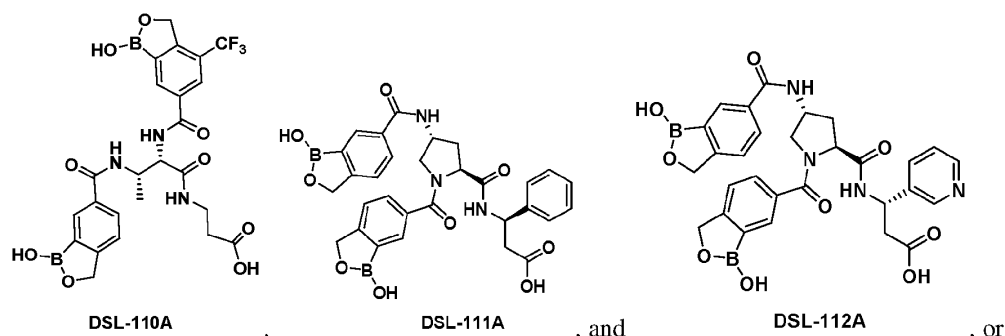
DSL-79A



DSL-80A







a stereoisomer or a mixture of stereoisomers, a pharmaceutically acceptable salt thereof, and combinations thereof.

48. An insulin analogue comprising an A-chain and a B-chain, wherein the A-chain comprises a sequence selected from 24051 and 24052; and wherein the B-chain comprises a sequence selected from 25000 – 25397.

49. The insulin analogue of claim 48, wherein the A-chain comprises sequence 24051; and

wherein the B-chain comprises a sequence selected from 25000, 25001, 25006-25009, 25076, 25077, 25082-25085, 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313.

50. The insulin analogue of claim 48, wherein the A-chain comprises sequence 24051; and

wherein the B-chain comprises a sequence selected from 25011, 25012, 25017-25020, 25087, 25088, 25093-25096, 25229, 25239, 25232, 25240, 25245-25248, 25305, 25308, 25315, 25316, and 25321-25324.

51. The insulin analogue of claim 48, wherein the A-chain comprises sequence 24051; and

wherein the B-chain comprises a sequence selected from 25228, 25229, 25232, 25234-25237, 25304, 25305, 25308, and 25310-25313.

52. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein in the at least one Z1c both B1 and B2 are each independently Formula F2, wherein all remaining R1 are H; and the indirect linker is selected from FL3, FL20-22, FL25, FL27-28, FL30-33, FL35, FL37, FL41, FL43-70, and amino acids having a hydrophobic side chain.

53. The compound of claim 6, or a pharmaceutically acceptable salt thereof, wherein in the at least one Z1c both B1 and B2 are each independently Formula F2, wherein one of the remaining R1 is CF₃ and the remaining R1 is H; and the indirect linker is selected from FL5,

FL23-24, FL26, FL29, FL34, FL36, FL38-40, FL42, and amino acids having a hydrophilic side chain.

54. The compound of claim 52 or 53, or a pharmaceutically acceptable salt thereof, wherein the at least one Z1c is selected from FF12, FF12A-D, FF116, and FF116A-D.

5 55. A compound having agonist potency for an insulin receptor comprising at least one aromatic boron-containing group having agonist potency for an insulin receptor, the compound having a first EC50 potency for activating the insulin receptor at a first glucose concentration and a second EC50 potency for activating the insulin receptor at a second glucose concentration, wherein when the first glucose concentration is 3 mM and the second glucose
10 concentration is 20 mM the compound has an insulin receptor agonist potency ratio of the first EC50 to the second EC50 of about 2 to about 25, about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 10, about 2 to about 9, or about 2 to about 8, and

wherein the least one aromatic boron-containing group is attached to an FF scaffold, and wherein the FF scaffold is selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D,
15 FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226.

56. A compound having agonist potency for glucose comprising at least one aromatic boron-containing group having binding affinity for glucose, wherein when administered at a dose of 30 nmol/kg to a first group of rats with a first glucose infusion rate to provide a blood glucose
20 concentration of 100 mg/dL and to a second group of rats with a second glucose infusion rate to provide a blood glucose concentration of 200 mg/dL the compound provides a relative glucose infusion rate difference (mg/kg/min.min) of about 1 to about 2500, about 1 to about 2000, about 1 to about 1500, about 100 to about 1500, and about 1000 to about 1500, and a relative glucose infusion rate ratio of about 0.1 to about 5, about 0.2 to about 4.5, about 0.5 to about 4, about 0.5
25 to about 3.5, or about 1 to about 3, and

wherein the least one aromatic boron-containing group is attached to an FF scaffold, and wherein the FF scaffold is selected from Formulae FF12, FF12A, FF12B, FF12C, FF12D, FF114, FF114A, FF114B, FF115, FF115A, FF115B, FF116, FF116A, FF116B, FF116C, FF116D, FF117, FF193A, FF203, FF225, FF225A, FF225B, and FF226.

30 57. The compound of claim 55 or 56, wherein the FF scaffold is selected from Formulae FF116, FF116A, FF116B, FF116C, FF116D, FF225, FF225A, and FF225B.

58. A method of administering the compound of any one of claims 55-57 or a pharmaceutical composition according to claims 16 or 35-36 to a subject, wherein the method comprises administering to the subject the compound or the composition as a therapeutic or
35 prophylactic agent.

59. A method of making a compound of any one of claims 55-57, wherein the method comprises at least one alkylation and/or amidation step.

60. A method of treating a subject by administering a device or formulation comprising a compound of any one of claims 55-57.

5