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NOVEL LINKERS FOR SUSTAINED DELIVERY OF THERAPEUTICS

SEQUENCE LISTING

[0001] The present application is being filed along with a Sequence Listing in ST.26 XML format. The Sequence Listing is provided as a file titled "30359 Sequence Listing.xml" created 9-Dec-2022 and is 14.6 kilobytes in size. The Sequence Listing information in the ST.26 XML format is incorporated herein by reference in its entirety.

FIELD

[0002] The present invention relates to novel compounds comprising novel linkers for the improved duration of action of therapeutics, such as peptides. The present invention also relates to novel conjugate moieties comprising a linker, an Fc region, and optionally therapeutic. The present invention also relates to novel compounds comprising two or more maleimide functional groups that can be useful to connect to regions with two or more nucleophiles, such as Fc regions of an antibody.

BACKGROUND

[0003] Peptide therapeutics, such as insulin, have been previously used to treat diseases like diabetes. However, the duration of action of many peptides can be quite short. Thus, patients are required to dose every few hrs., typically by injection, which can be burdensome and time-consuming for the patient.

[0004] One strategy to increase duration of action is to use antibody drug conjugates (ADCs) in which the drug component is the peptide therapeutic. However, such ADCs are often difficult to produce and the production methods may require the peptide therapeutic to be exposed to harsh, degrading conditions. One way to shield a peptide therapeutic during ADC formation is to modify and/or protect the peptide therapeutic, but such modifications can impact the efficacy of the peptide therapeutic. Known ADC production methods have also not been able to use peptide therapeutics incorporating synthetic amino acids.

[0005] Accordingly, there is a need for ADCs that can incorporate unprotected/unmodified peptide therapeutics with or without non-native amino acids.

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[0006] The identity of the linker can influence the reaction conditions that the therapeutic must experience during production of the ADC. Accordingly, there is also a need for improved linkers that can incorporate unprotected/unmodified peptide therapeutics with or without non-native amino acids.

DETAILED DESCRIPTION

[0007] The present invention relates to novel linkers, including novel conjugates comprising the novel linkers, for the delivery of a therapeutic to an animal. The novel conjugates can also include an Fc region and a therapeutic, such as a peptide therapeutic.

[0008] It has been surprisingly found that by attaching a therapeutic to one of the disclosed novel linkers and another moiety, such as an Fc region of an antibody, the duration of action of the therapeutic can be improved and/or lengthened. While not wishing to be bound by theory, it is believed that the attachment of the therapeutic to the linker and Fc region can increase the duration of action of the therapeutic by enlarging the overall moiety and preventing the breakdown of the therapeutic. The reaction conditions for the conjugation reaction between the novel linkers and the Fc region are relatively mild, so the payload or therapeutic does not need to be modified to be protected during the reaction.

[0009] It has also been surprisingly found that the disclosed novel linkers can produce an ADC that can incorporate unprotected/unmodified peptide therapeutics with or without non-native amino acids.

Fc-L-Z

Formula I-A.

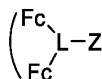
[00010] Disclosed herein are novel conjugate compounds of Formula I-A, wherein Fc comprises an Fc region, such as an Fc region of an antibody, L comprises a linker, and Z comprises a therapeutic, a functional group suitable for the later attachment of a therapeutic, or a functional group suitable for the conjugate compound to exist without attaching a therapeutic.

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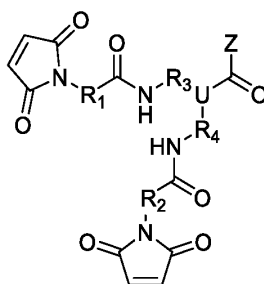
Formula I-B.

[00011] Also disclosed herein are novel conjugate compounds of Formula I-B, wherein Fc comprises one or more Fc regions, L comprises a linker, and Z comprises a therapeutic, a functional group suitable for the later attachment of a therapeutic, or a functional group suitable for the conjugate compound to exist without attaching a therapeutic.



Formula I-C.

[00012] Also disclosed herein are novel conjugate compounds of Formula I-C, wherein Fc comprises a single Fc region, such as an Fc region of an antibody, L comprises a linker, and Z comprises a therapeutic, a functional group suitable for the later attachment of a therapeutic, or a functional group suitable for the conjugate compound to exist without attaching a therapeutic.

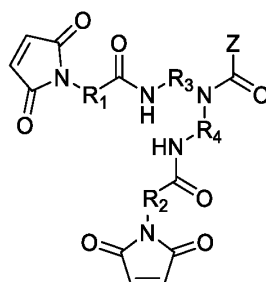


Formula II-A.

[00013] Also disclosed herein are novel compounds of Formula II-A, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-\text{NH}_2$, $-\text{CH}_2\text{NH}_2$, and $-\text{CH}_2\text{CH}_2\text{NH}_2$; R_3 and R_4 are independently C_1 to C_5 alkyl or absent; U is tertiary amine or 1,3,5-substituted phenyl; Z comprises H, OH, NH_2 , NH, a therapeutic,

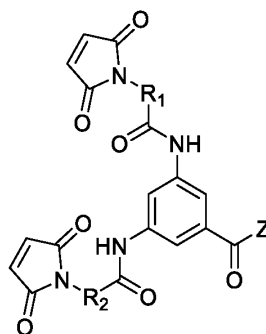
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C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.



Formula II-B.

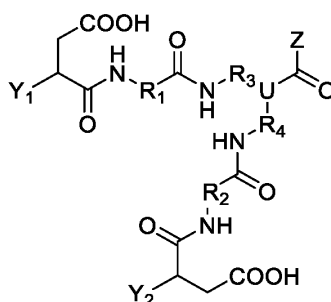
[00014] Also disclosed herein are novel compounds of Formula II-B, wherein R₁ and R₂ are independently C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, or -CH₂CH₂NH₂; R₃ and R₄ are independently C₁ to C₅ alkyl; Z comprises H, OH, NH₂, NH, a therapeutic, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.



Formula II-C.

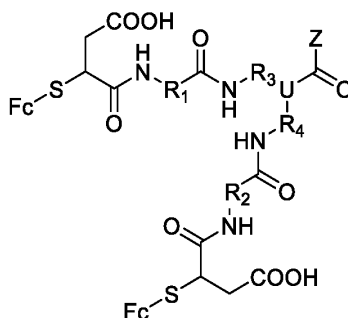
[00015] Also disclosed herein are novel compounds of Formula II-C, wherein R₁ and R₂ are independently C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, or -CH₂CH₂NH₂; Z comprises H, OH, NH₂, NH, a therapeutic, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

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Formula III-A

[00016] Also disclosed herein are novel compounds of Formula III-A, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of NH_2 , CH_2NH_2 , and $CH_2CH_2NH_2$; R_3 and R_4 are independently C_1 to C_5 alkyl or absent; U is tertiary amine or 1,3,5-substituted phenyl; Y_1 and Y_2 independently comprise an amide, amine, imine, sulfonamide, thiourea, urea, thioether, or combinations thereof; Z comprises H , OH , NH_2 , NH , a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

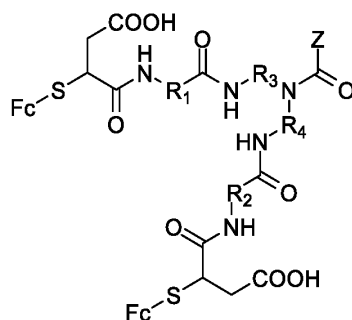


Formula III-B.

[00017] Also disclosed herein are novel compounds of Formula III-B, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of NH_2 , CH_2NH_2 , and $CH_2CH_2NH_2$; R_3 and R_4 are independently C_1 to C_5 alkyl or absent; U is tertiary amine or 1,3,5-substituted phenyl; Z comprises H , OH , NH_2 , NH , a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number

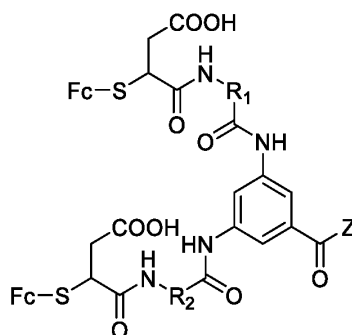
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integer from 1 to 30; Fc comprises an Fc region; S is a sulfur atom from the Fc region; and pharmaceutically acceptable salts thereof.



Formula III-C.

[00018] Also disclosed herein are the novel compounds of Formula III-C, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-NH_2$, $-CH_2NH_2$, and $-CH_2CH_2NH_2$; R_3 and R_4 are independently C_1 to C_5 alkyl or absent; Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; Fc comprises an Fc region; S is a sulfur atom from the Fc region; and pharmaceutically acceptable salts thereof.

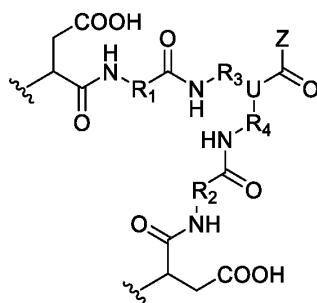


Formula III-D

[00019] Also disclosed herein are the novel compounds of Formula III-D, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-NH_2$, $-CH_2NH_2$, and $-CH_2CH_2NH_2$; Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl,

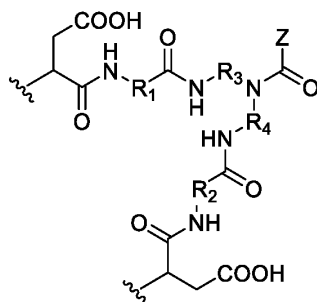
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$(\text{OCH}_2\text{CH}_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; Fc comprises an Fc region; S is a sulfur atom from the Fc region; and pharmaceutically acceptable salts thereof.



Formula IV-A.

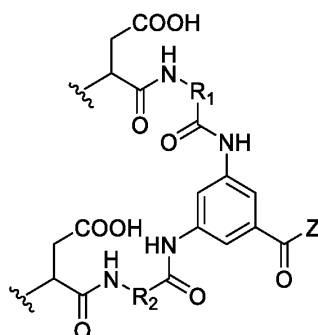
[00020] Also disclosed herein are the novel compounds of Formula IV-A, wherein: R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-\text{NH}_2$, $-\text{CH}_2\text{NH}_2$, and $-\text{CH}_2\text{CH}_2\text{NH}_2$; R_3 and R_4 are independently C_1 to C_5 alkyl or absent; U is tertiary amine or 1,3,5-substituted phenyl; Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(\text{OCH}_2\text{CH}_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.



Formula IV-B.

[00021] Also disclosed herein are the novel compounds of Formula IV-B, wherein: R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-\text{NH}_2$, $-\text{CH}_2\text{NH}_2$, or $-\text{CH}_2\text{CH}_2\text{NH}_2$; R_3 and R_4 is C_1 to C_5 alkyl; and Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(\text{OCH}_2\text{CH}_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

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Formula IV-C.

[00022] Also disclosed herein are the novel compounds of Formula IV-C, wherein R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of $-NH_2$, $-CH_2NH_2$, or $-CH_2CH_2NH_2$; Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

Linker

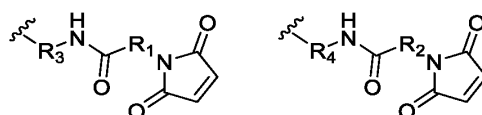
[00023] The present invention includes a linker, L, to connect Z , *via* a nucleophilic conjugate addition reaction, to another moiety, such as one or more cysteine amino acids in an Fc region of an antibody, as shown in Formula I-A, I-B, and/or I-C, or to another suitable nucleophile, such as an amide, amine, imine, sulfonamide, thiourea, urea, thioether, thiol, cysteine, or combinations thereof, as shown in Formula III-A, III-B, and/or III-C.

[00024] The linker can include a central trivalent linking unit, U, as shown in Formula II-A, III-A, and IV-A. One of the portions of the linker can be attached to Z . The other two portions of the linkers comprise a maleimide functional group, an electrophile, which can be used to selectively connect Z to two nucleophiles in a nucleophilic conjugate addition reaction.

[00025] As such, as disclosed herein, the linker of the present invention comprises two or more maleimide functional groups, preferably two maleimide functional groups, prior to the nucleophilic conjugate addition reaction.

[00026] Suitable trivalent linking units can include any atom or molecules that can be tri-substituted, such as tri-substituted C5 to C8 aryl, tri-substituted C5 to C8 heteroaryl with 1 to 3 heteroatoms in the ring system, tri-substituted C5 to C8 cycloalkyl, tri-substituted C5 to C8 heterocycle with 1 to 3 heteroatoms in the ring system, tertiary amine, tertiary phosphine, or combinations thereof. Preferably, U is 1,3,5-trisubstituted phenyl or tertiary amine.

[00027] Each arm that is attached to U and the maleimide functional group can be represented by Formula V.



Formula V.

[00028] In the first of the two arms represented by Formula V, R₁ can be C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, and -CH₂CH₂NH₂ and R₃ can be C₁ to C₅ alkyl or absent. In the second of the two arms represented by Formula V, R₂ can be C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, and -CH₂CH₂NH₂; and R₄ can be C₁ to C₅ alkyl or absent.

[00029] R₁ and/or R₂ can preferably be C₂ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, or -CH₂CH₂NH₂. R₃ and/or R₄ can preferably be C₂ alkyl when U is a tertiary amine and absent when U is 1,3,5-trisubstituted phenyl.

[00030] When both arms are attached to U, R₁ and R₂ can be identical, or they can be independently C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, and -CH₂CH₂NH₂. When both arms are attached to U, R₃ and R₄ can be identical, or they can be independently C₁ to C₅ alkyl or absent. When R₃ and/or R₄ are absent, the amide nitrogen in Formula V is directly connected to U, such as when U is 1,3,5-trisubstituted phenyl as in Formula IV-C.

[00031] The third of the three arms attached to U includes Z. Z can comprise H, OH, NH₂, NH, a therapeutic, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30. Z can be connected directly to U

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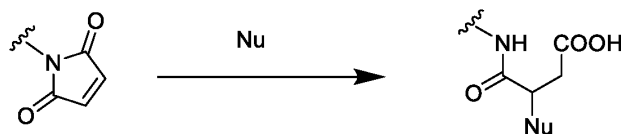
or Z can be connected to a carbonyl functional group. The carbon atom of the carbonyl functional group can be directly attached to U and Z, as shown in Formula II-IV.

[00032] Accordingly, disclosed herein are novel linkers, as shown in Formula II-A, Formula II-B, and Formula II-C, with two maleimide functional groups prior to any nucleophilic conjugate addition reaction.

[00033] Nucleophilic Conjugate Addition Reactions with Maleimide Functional Groups

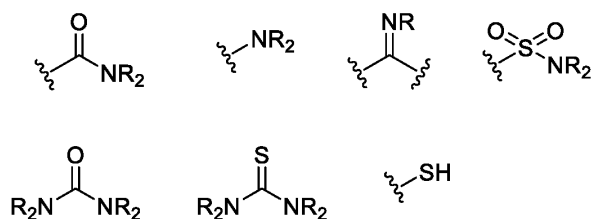
[00034] As disclosed herein, a linker L, can be attached to Z, to yield a linked payload, or if Z comprises a therapeutic, a linked therapeutic. The linker, as disclosed herein, comprises two or more, or preferably two, maleimide functional groups, as shown in Formula V. The maleimide functional groups can be used to attach Z to a different moiety, such as an Fc region, through a nucleophilic conjugate addition reaction.

[00035] In the nucleophilic conjugate addition reaction, the maleimide acts as an electrophile and reacts with a nucleophile to form a new covalent bond between the nucleophilic atom and an electrophilic carbon atom, which can lead to an opening of the maleimide functional group, as shown in Formula VI.



Formula VI.

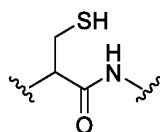
[00036] A variety of nucleophiles can be used to react with the electrophilic maleimide functional group. Suitable nucleophiles include moieties comprising one or more nitrogen and/or sulfur atoms in a functional group where the nitrogen and/or sulfur atom can serve as an electron-rich nucleophile. Suitable examples of nucleophiles include moieties comprising one or more of the following functional groups: amide, amine, imine, sulfonamide, thiol, thiourea, urea, or combinations thereof, which are also shown in Formula VII.



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Formula VII.

[00037] In particular, the amino acid cysteine, which is found in many amino acid sequences in peptides, antibodies, proteins, or the like, as shown in Formula VIII within a peptide chain, includes a thiol functional group, which can be used as a nucleophile to form a thioether between the amino acid sequence and the linker, the linked payload, and/or the linked therapeutic.



Formula VIII.

[00038] Specific conditions for conjugate nucleophilic conjugate addition reactions, are well known to a person of ordinary skill in the art. The specific conditions can vary according to the nucleophile and electrophile used. Accordingly, disclosed herein are novel compounds and/or conjugate compounds comprising a linked payload that has been attached to another moiety through a nucleophilic conjugate addition reaction, as shown in Formula III-A, Formula III-B, Formula III-C, Formula III-D, Formula IV-A, Formula IV-B, and/or Formula IV-C.

[00039] Additionally, use of the novel linkers disclosed herein can be advantageous because the reaction conditions are mild relative to other nucleophilic conjugate addition reactions that have been disclosed with other linkers. For example, the disclosed linkers can be combined with the Fc region of an antibody with very little heating. Specifically, the disclosed linkers can be conjugated to the Fc region of an antibody by mixing the linked therapeutic and a reduced Fc region for no more than 2 hrs., at no more than 30 °C or no more than 25 °C to yield a conjugate.

[00040] As the reaction conditions for the conjugation is relatively mild, the payload or therapeutic does not need to be modified to be protected during the reaction. Other linkers have required that the peptide therapeutic, such as insulin, be modified with a recombinant extension. The disclosed conjugate compounds are still efficacious with a longer duration of action without having to modify the therapeutic.

Fc Region

[00041] In some embodiments, the nucleophile that can react with the two or more maleimide functional groups on the linker, is an Fc region. An Fc region can also be known as a fragment crystallizable region. The Fc region is the tail region of an antibody that interacts with some cell surface receptors called Fc receptors. The Fc region can include a peptide, protein, or portion of an antibody, or an entire monoclonal antibody with the capability of reacting with Fc receptors. In particular, the Fc region of certain antibodies, such as IgA, IgD, IgE, IgG, IgM, or combinations thereof, can have two or more cysteine amino acids forming a disulfide bridge.

[00042] In some embodiments, the Fc region of an antibody can be modified prior to attachment to the linker *via* a nucleophilic conjugate addition reaction. Suitable modifications may include modifications to the antibody to minimize reactivity between the antibody and linker other than the desired nucleophilic conjugation addition reaction, as further described herein.

[00043] In some embodiments, the Fc region can be a modified Fc region of an antibody. Suitable modifications include blunting and/or removal of certain portions of the Fc region, such as hinge regions.

[00044] The disulfide bridge present in suitable Fc regions can be reduced and the two reduced thiols can be suitable nucleophiles that can undergo nucleophilic conjugate addition reactions with the two maleimide functional groups of the linkers of Formula II-A, Formula II-B, and/or Formula II-C to result in the compounds of Formula IV-A, Formula IV-B, and/or Formula IV-C.

Therapeutic

[00045] In some embodiments, Z can comprise a therapeutic. A therapeutic is a drug or active agent that can treat a targeted disease if administered in patient with the targeted disease.

[00046] Suitable therapeutics can comprise one or more amino acids or a peptide with from 2 to 500 about amino acids, from 2 to 250 about amino acids, or from 2 to about 100 amino acids.

[00047] Suitable therapeutics can comprise peptide hormones, such as adrenocorticotrophic hormone (ACTH), adropin, amylin, angiotensin, atrial natriuretic peptide (ANP), calcitonin, cholecystokinin (CCK), gastrin, ghrelin, glucagon, growth hormone, follicle-stimulating hormone (FSH), insulin, leptin, luteinizing hormone (LH), melanocyte-stimulating hormone (MSH), oxyntomodulin, oxytocin, parathyroid hormone (PTH), prolactin, renin, somatostatin, thyroid-stimulating hormone (TSH), thyrotropin-releasing hormone (TRH), vasopressin, also called arginine vasopressin (AVP) or anti-diuretic hormone (ADH), vasoactive intestinal peptide (VIP), combinations thereof, and/or analogs thereof. In some embodiments, the therapeutic can be insulin, oxyntomodulin, and/or analogs thereof.

[00048] Other suitable therapeutics include comprises a monoclonal antibody, an interleukin, an interferon, a protein kinase inhibitor, a hematopoietic growth factor, a protein, a fused protein, oligonucleotide, or combinations thereof.

Conjugate

[00049] In some embodiments, the novel compounds can be a conjugate and/or an antibody-drug conjugate (ADC) when Z comprises a therapeutic and the linker is conjugated to an Fc region of at least a portion of an antibody. As described herein, it was surprisingly found that when a therapeutic, such as oxyntomodulin, was attached to an Fc region of an antibody via the novel linkers disclosed here, that the therapeutic had an improved duration of action relative to the therapeutic alone.

Method of Treatment

[00050] Also disclosed herein are methods of increasing the duration of action of a therapeutic. The method can comprise the step of attaching the therapeutic to a trivalent linker to yield a linker therapeutic, such as the compounds of Formula II-A, Formula II-B, and/or Formula II-C, wherein Z comprises the therapeutic.

[00051] The method can also comprise the step of attaching the linked therapeutic to an Fc region of at least a portion of an antibody, such as IgG, through a nucleophilic conjugate addition reaction between the linked therapeutic and two nucleophiles in the Fc region, such as two sulfur atoms and/or anions, to yield a conjugate compound.

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[00052] The conjugate compound can comprise a therapeutic attached to the novel linkers disclosed herein, which is in turn conjugated to the Fc region of at least a portion of the antibody. The conjugate compound can have a duration of action that is longer than the therapeutic that is unbound. The duration of action of the conjugate compound can be at least 12 hrs, 1 day, 2 days, or 3 days longer than the duration of action of the therapeutic.

[00053] Additionally, use of the novel linkers disclosed herein can be advantageous because the reaction conditions are mild relative to other nucleophilic conjugate addition reactions that have been disclosed with other linkers. For example, the disclosed linkers can be combined with the Fc region of an antibody with very little heating. Specifically, the disclosed linkers can be conjugated to the Fc region of an antibody by mixing the linked therapeutic and a reduced Fc region for no more than 2 hrs. at no more than 30 °C or no more than 25 °C to yield a conjugate

Formulation

[00054] The compounds disclosed herein can be included in a pharmaceutical formulation. The pharmaceutical formulation can comprise one or more carriers, diluents, and excipients that are compatible with the compounds and other components of the composition or formulation and not deleterious to the patient. Examples of pharmaceutical compositions and processes for their preparation can be found in “Remington: The Science and Practice of Pharmacy”, Loyd, V., *et al.* Eds., 22nd Ed., Mack Publishing Co., 2012.

Definitions

[00055] As used herein, the terms “a,” “an,” “the,” and similar terms used in the context of the present disclosure (especially in the context of the claims) are to be construed to cover both the singular and plural unless otherwise indicated herein or clearly contradicted by the context.

[00056] As used herein, the term “alkyl” means saturated linear or branched-chain monovalent or bivalent hydrocarbon radical, containing the indicated number of carbon atoms. For example, “C₁-C₂₀ alkyl” means a radical having 1-20 carbon atoms in a linear or branched arrangement.

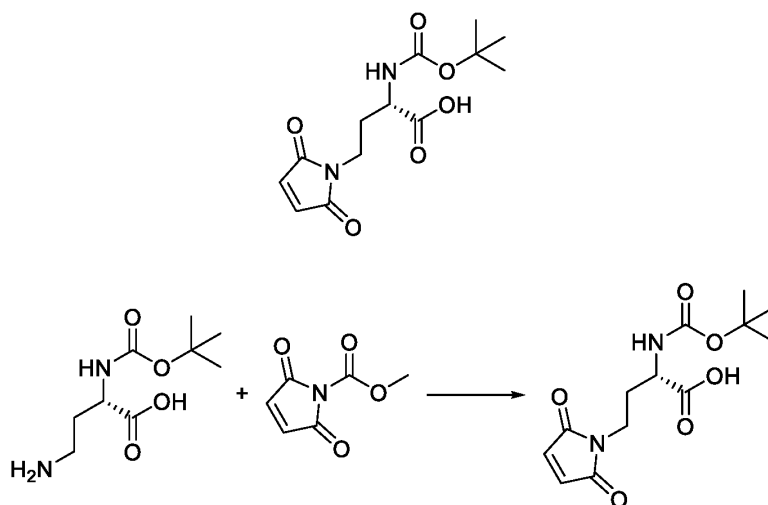
[00057] Certain abbreviations used herein are defined as follows: “BEA” refers to 5-methyl-1,2,4-triazolo[3,4-b]benzothiazole; “Boc” refers to *tert*-butoxycarbonyl; “DABA” refers to 3,5-diaminobenzoic acid; “DCM” refers to dichloromethane; “DIC” refers to diisopropylcarbodiimide; “DIEA” refers to diisopropylethylamine; “DMAP” refers to 4-dimethylaminopyridine; “EtOAc” refers to ethyl acetate; “Fmoc” refers to fluorenylmethoxycarbonyl; “hr/hrs” refers to hour/s; “FPLC” refers to fast protein liquid chromatography; “MalDab” refers to (S)-2-((*tert*-butoxycarbonyl)amino)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid; “Maldap” refers to (S)-3-((*tert*-butoxycarbonyl)amino)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid; Beta-MalDap refers to (S)-2-((*tert*-butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid; “MeOH” refers to methanol; “Min” refers to minute/minutes; “MTT” refers to methylthiazole tetrazolium; “OXM” refers to Oxyntomodulin; “Oxyma” refers to ethyl cyanohydroxyiminoacetate; “PEG” refers to polyethylene glycol; “PyBOP” refers to benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; “t-Bu” refers to tertiary butyl; “TCEP” refers to tris(2-carboxyethyl)phosphine hydrochloride; and “THF” refers to tetrahydrofuran.

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PREPARATIONS

Preparation 1

(S)-2-((*tert*-Butoxycarbonyl)amino)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid

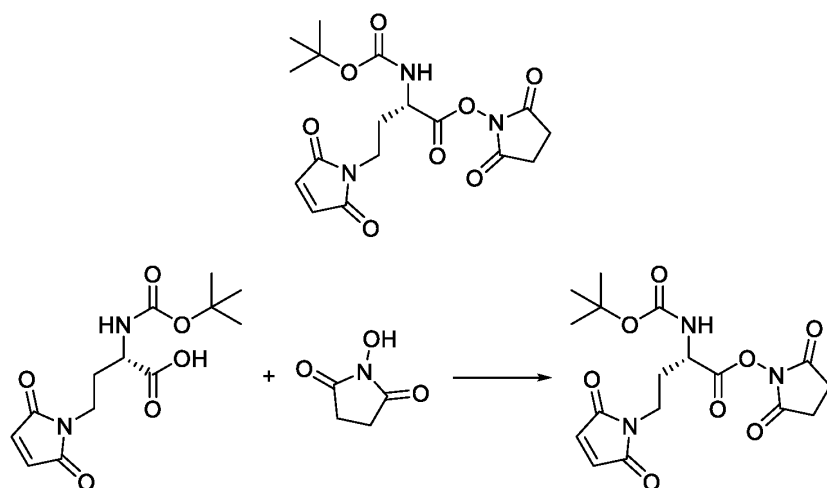


[00058] Added triethylamine (1 mL, 7.17 mmol) to a mixture of (2S)-4-amino-2-(*tert*-butoxycarbonylamino)butanoic acid (500 mg, 2.29 mmol) in 1,4-dioxane (10 mL), THF (5 mL) and water (5 mL). Stirred until a homogenous mixture was obtained, then added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (370 mg, 2.31 mmol). Mixed at ambient temperature for 1 hour. Diluted with 50 mL of water, adjusted pH to ~6 by adding 5N HCl. Extracted the aqueous with EtOAc (30 mL) and chloroform/iso-propanol (3 x 30 mL). Combined the organic phases, dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purified the resulting residue via silica gel column chromatography eluting with 0-30% MeOH in DCM to give the titled compound (580 mg, 81%). ES/MS *m/z*: 199 (M- tBu).

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Preparation 2

2,5-Dioxopyrrolidin-1-yl (S)-2-((*tert*-butoxycarbonyl)amino)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoate

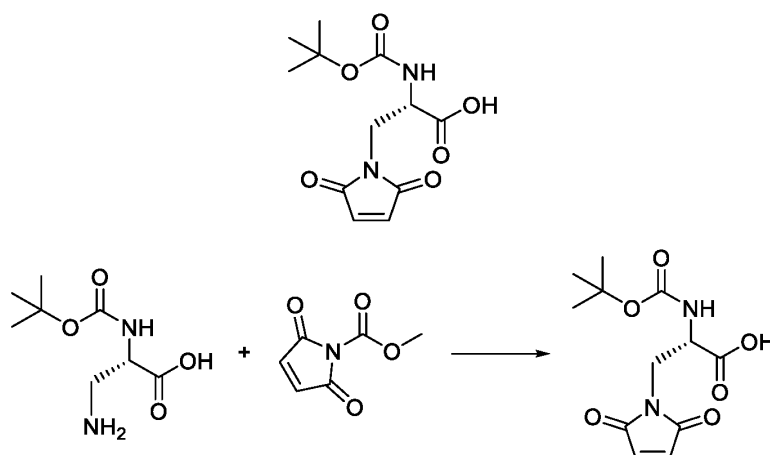


[00059] Added dicyclohexylcarbodiimide (320 mg, 1.53 mmol) to a solution of (S)-2-((*tert*-butoxycarbonyl)amino)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid (400 mg, 1.27 mmol) and N-hydroxysuccinimide (180 mg, 1.53 mmol) in THF (6 mL). Stirred at ambient temperature for 3 hrs. Filtered to remove solids, then concentrated the filtrate under reduced pressure to provide the title compound (605 mg, 84%) which was used without purification. ES/MS *m/z*: 296 (M-tBu).

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Preparation 3

(S)-2-((*tert*-Butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid

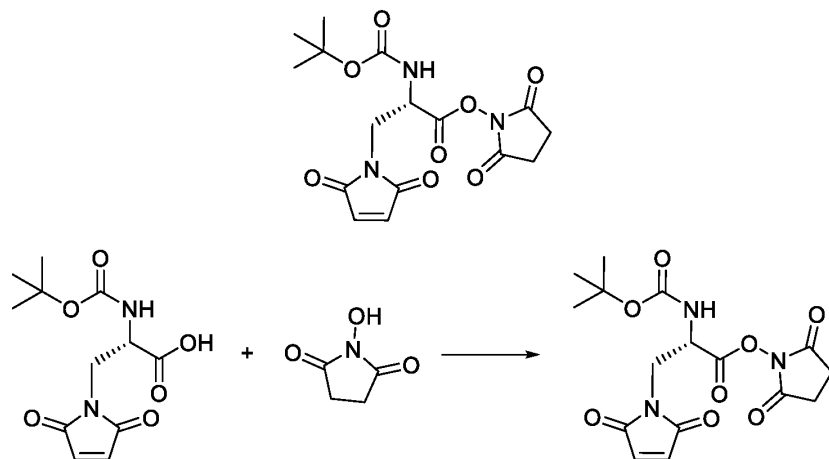


[00060] Mixed (2S)-3-amino-2-(*tert*-butoxycarbonylamino)propanoic acid (5.00 g, 24.5 mmol) with 1M aqueous solution of sodium bicarbonate (130 mL, 130 mmol). Stirred at ambient temperature for 10 min until a clear solution was formed. Cooled the solution in an ice-water bath, and added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (4.00 g, 25.0 mmol) as in three portions over 15 min. Continued stirring at 0 °C for 3 hrs. Added 100 mL of EtOAc and stirred under cooling, then added concentrated HCl to adjust pH to 1. Separated the layers and extracted the aqueous with DCM (4 x 50mL). Combined the organic layers, washed with saturated aqueous NaCl, and dried over sodium sulfate. Removed the solvent under reduced pressure. Purified via silica gel column chromatography eluting with 0-40% MeOH in DCM to give the title compound (5.70 g, 66%). ES/MS *m/z*: 185 (M-*t*Bu).

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Preparation 4

2,5-Dioxopyrrolidin-1-yl (S)-2-((*tert*-butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate

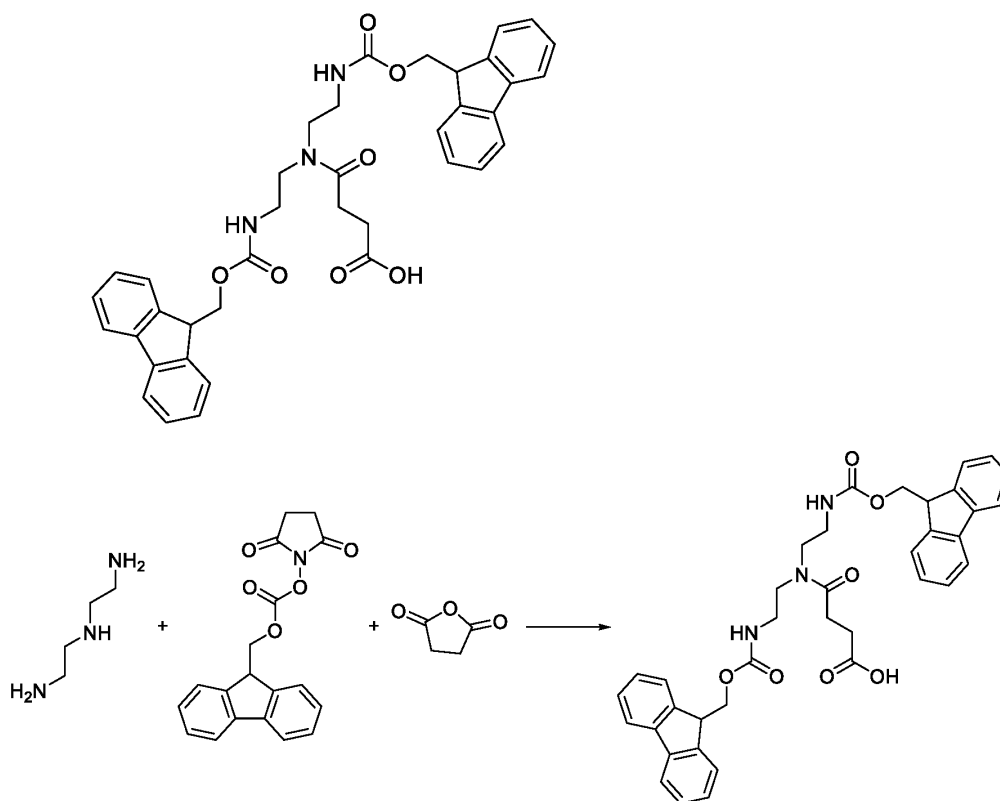


[00061] Added dicyclohexylcarbodiimide (484 mg, 2.32 mmol) to a mixture of (S)-2-((*tert*-butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (550 mg, 1.54 mmol) and N-hydroxysuccinimide (272 mg, 2.31 mmol) in THF (5 mL). Stirred at ambient temperature until a precipitate appeared. Removed the solids by filtration and washed with DCM. Concentrated the filtrate under reduced pressure to give the title compound (1.18 g, 85%). ES/MS *m/z*: 282 (*M*+*H*-Boc).

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Preparation 5

4-(Bis(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)amino)-4-oxobutanoic acid

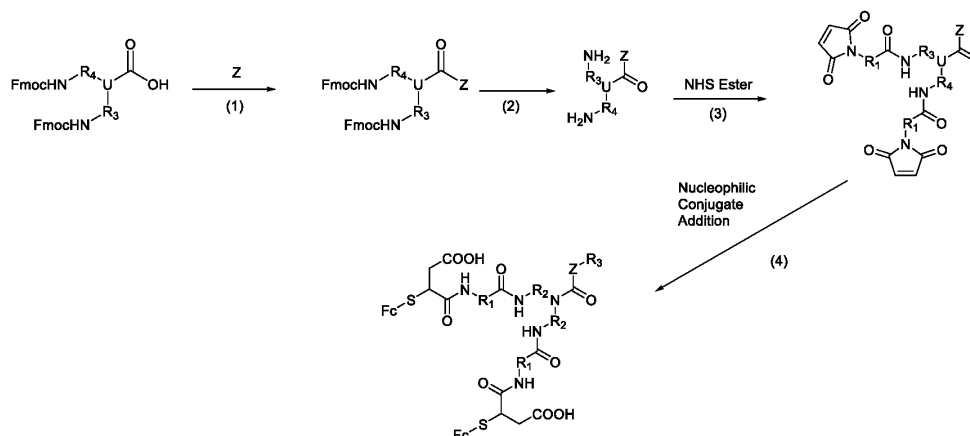


[00062] Added a solution of N-(9-fluorenylmethoxycarbonyloxy) succinimide (19.4 g, 56.4 mmol) in DCM (80 mL) over 45 min to a solution of diethylenetriamine (3.1 mL, 28 mmol) in DCM (30 mL) cooled at -78 °C. After 2 hrs., warmed the mixture to ambient temperature, and added succinic anhydride (9.91 g, 98 mmol) and DMAP (691 mg, 5.60 mmol). Stirred the mixture at ambient temperature for 15 hrs. Adjusted the pH to 5 by slow addition of 1N HCl (20 mL). Separated the phases and extracted the aqueous with DCM (2 x 100 mL). Combined the organic layers and washed with saturated aqueous NaCl, dried over magnesium sulfate concentrated under reduced pressure, and purified by silica gel column chromatography eluting with 0-10% MeOH in DCM to give the title compound (911.84 g, 65%) as a white powder. ES/MS *m/z*: 648 (M+1).

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Examples

Scheme 1



Generic Synthesis of Conjugate Including Fc Region, Linker, and Peptide

[00063] A summary of the general synthesis of the linker-peptide is provided in Scheme 1. In each case, the Fmoc protected (on two of the three available arms) trivalent core of either 1,3,5-trisubstituted phenyl (commercially available) or tertiary amine (Preparation 5) was initially reacted with a peptide on a resin in reaction (1). The Fmoc groups were removed in reaction (2). In reaction (3), the primary amine functional groups that result from the removal of the Fmoc protecting groups were reacted with NHS esters including the MalDap (commercially available), MapDab (Preparation 2), or Beta-MalDap (Preparation 4) and the peptide was released from the resin. In reaction (4), the bismaleimide functional groups reacted with two reduced thiol functional groups in an Fc region in a nucleophilic conjugate addition reaction to yield the conjugate including the Fc region, linker, and therapeutic.

[00064] The linker-peptide is generated by solid-phase peptide synthesis using Fmoc/t-Bu strategy on a SymphonyX Automated Peptide Synthesizer (PTI Protein Technologies Inc.) starting from RAPP AM-Rink Amide resin (H40023 Polystyrene AM RAM, Rapp polymere GmbH). Amino acid couplings are performed using 5 equivalents of amino

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acid, 1.2 M DIC and 0.9 M Oxyma in DMF for 2 hrs., at 25 °C. Deprotections are carried out using 25% piperidine solutions in DMF.

[00065] The N terminal amino acid is a boc protected proline to allow Fmoc chemistry to be carried out at the C terminal K ϵ -amine. The MTT protecting group present in K at the C terminus is removed using 30% Hexafluoroisopropanol (HFIP) in DCM. Additional coupling/deprotection cycles using a Fmoc/t-Bu strategy to extend the side chain involve Fmoc-Peg24-OH (Broadpharm, catalog#BP-22036), Fmoc protected BEA (bisethylamine) aliphatic linker, and Boc-Maldap(CAS No. 1491152-23-8). For Peg coupling, 2 equivalents of Fmoc-Peg24-OH, 2equivalent of PyBOP and 4 equivalents of DIEA was coupled for 2 hrs. at 37 °C. BEA was coupled using 3 equivalents of BEA with 3 equivalents of PyBop and 6 equivalents of DIEA for 3 hrs. at 37 °C. Boc-Maldap was coupled to each of the free amines of BEA using 5 equivalents of Boc-Maldap, 0.9M Oxyma and 1.2M DIC in DMF for 6 hrs. at 25 °C. Deprotections of PEG and BEA were carried out using 25% piperidine solutions in DMF. Concomitant cleavage from the resin and side chain protecting group removal are carried out in a solution containing trifluoroacetic acid (TFA): triisopropylsilane: MilliQ H₂O: thioanisole 85:5:5:5 (v/v) for 2 hrs. at 25 °C followed by precipitation with cold ether. Crude peptide is purified by reverse-phase HPLC on a Waters Symmetry Prep C18 column (19x250mm 100A, 5 μ m), where suitable fractions are pooled and lyophilized.

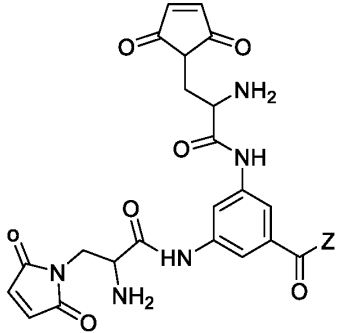
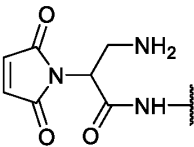
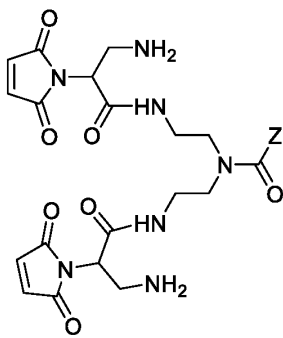
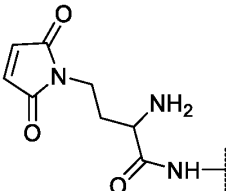
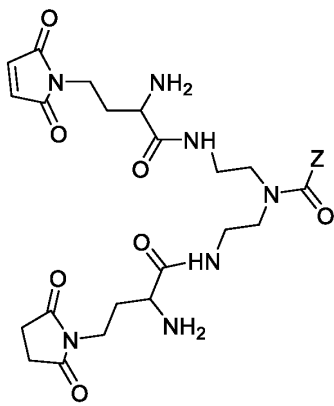
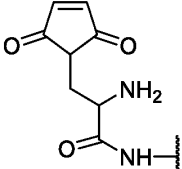
[00066] Once the linker-therapeutic is cleaved from the resin, it can be reacted with a reduced Fc region to form the conjugate, which includes the Fc region, the linker, and the therapeutic. A summary of the possible conjugates is provided in TABLE 1.

[00067] The following linkers were synthesized through the process described above:

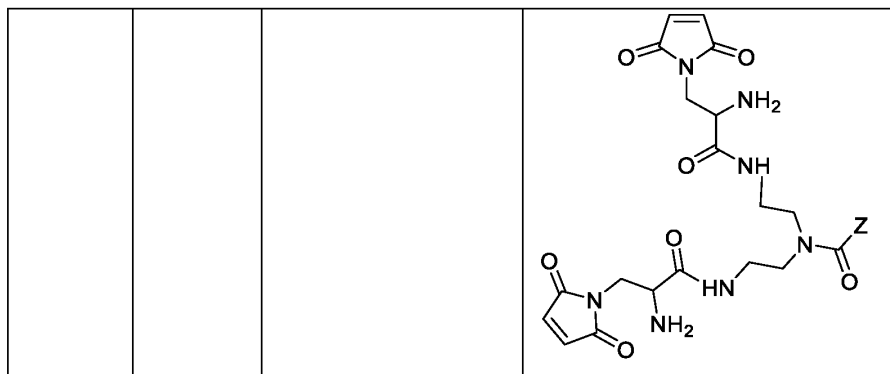
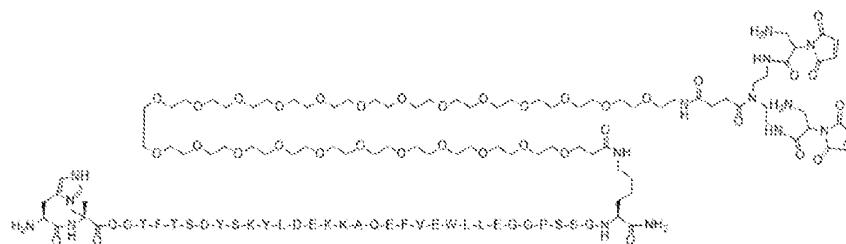
TABLE 1. Conjugates

Example	Trivalent Core	Amine	Linker
A	1,3,5-phenyl		
B	1,3,5-phenyl		
C	1,3,5-phenyl		

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D	Tertiary Amine		
E	Tertiary Amine		
F	Tertiary Amine		

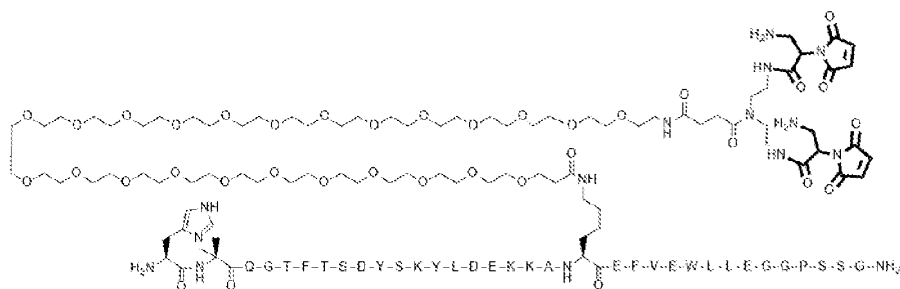
-25-

[00068] SEQ ID NO. 7: H-Aib-QGTFTSDYSKYLDEKKAQEFVEWLLEGGPSSGK(Peg24-BEA-Maldap2)-NH₂

[00069] In this sequence the N terminus is a free, and the C terminus is amidated as a primary amide. The K at the C terminus is chemically modified through conjugation to the ε-amino group of the K sidechain with Peg24-BEA-Maldap2. This was synthesized using the general synthesis above, with the amine being MalDap, the tertiary core being tertiary amine, and the bridge between the peptide and the linker was PEG24.

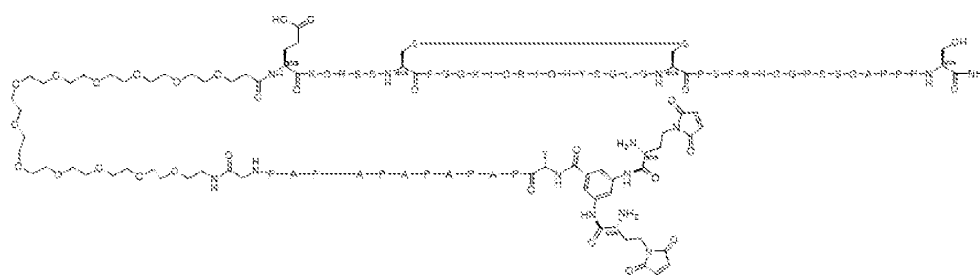
-26-

SEQ ID NO. 8: H-Aib-QGTFTSDYSKYLDEKKAK(Peg24-BEA-Maldap2)EFVEWLLEGGPSSG-NH₂



[00070] In this sequence the N terminus is a free, and the C terminus is amidated as a primary amide. The K at position 20 is chemically modified through conjugation to the ϵ -amino group of the K sidechain with Peg24-BEA-Maldap2. This was synthesized using the general synthesis above, with the amine being MalDap, the tertiary core being tertiary amine, and the bridge between the peptide and the linker was PEG24.

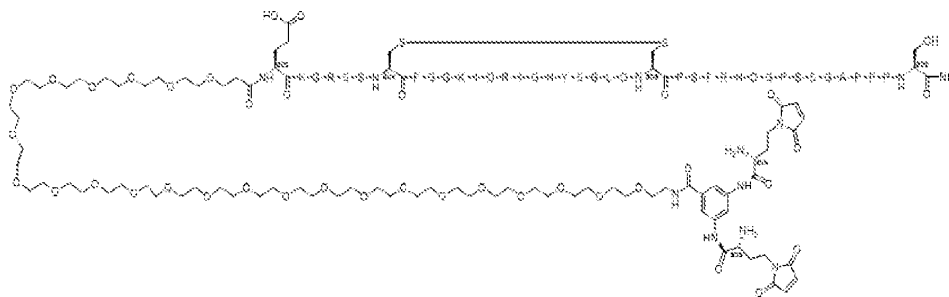
SEQ ID NO 9: NH₂-SPPAGSSPGG-H-RFSPCGLGSYHGIRDIKGGFCSSRGKE-(PEG12-G-PAPAPAPAPAPA)-DABA-(Maldab)2



[00071] SEQ ID NO 9 was prepared using the general synthesis similarly to sequences 7, 8, and 9 with the amine being MalDab, the tertiary core being 1,3,5-phenyl, and the bridge between the peptide and the linker was PEG12.

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SEQ ID NO 10: NH₂-SPPPAGSSPPGG-H-RFSPCGLGSYHGIRDIKGGFCSSRGKE-
(PEG24-DABA-(Maldab)2



[00072] SEQ ID NO. 10 was prepared using the general synthesis similarly to sequences 7, 8, and 9 with the amine being MalDab, the tertiary core being 1,3,5-phenyl, and the bridge between the peptide and the linker was PEG24.

[00073] The peptides from SEQ ID NO 7-12 were generated by solid-phase peptide synthesis using Fmoc/t-Bu strategy on a SymphonyX Automated Peptide Synthesizer (PTI Protein Technologies Inc.) starting from RAPP AM-Rink Amide resin (H40023 Polystyrene AM RAM, Rapp polymere GmbH). Amino acid couplings were performed using 5 equivalents of amino acid, 1.2 M DIC and 0.9 M Oxyma in DMF for 2 hrs. at 25 °C. Deprotections are carried out using 25% piperidine solutions in DMF.

[00074] The N terminal amino acid is a Boc protected proline to allow fmoc chemistry to be carried out at K ε-amine. The MTT protecting group present in K at the position 20 is removed using 30% Hexafluoroisopropanol (HFIP) in DCM. Additional coupling/deprotection cycles using a Fmoc/t-Bu strategy to extend the side chain involve Fmoc-Peg24-OH (Broadpharm, catalog#BP-22036), Fmoc protected BEA (bisethylamine) aliphatic linker, and Boc-Maldap (CAS No. 1491152-23-8). For Peg coupling, 2 eq of Fmoc-Peg24-OH, 2 equivalents of PyBOP and 4 equivalents of DIEA was coupled for 2 hrs. at 37°C. BEA was coupled using 3 equivalents of BEA with 3 equivalents of PyBop and 6 equivalents of DIEA for 3 hrs. at 37 °C. Boc-Maldap was coupled to each of the free amines of BEA using 5 equivalents of Boc-Maldap, 0.9M Oxyma and 1.2M DIC in DMF for 6 hrs. at 25 °C. Deprotections of Peg and BEA were carried out using 25% piperidine solutions in DMF.

Fc Region of IgG4

[00075] The human IgG4 Fc region was prepared and purified by well-known methods. Chinese hamster ovarian cells (CHO) were transiently or stably transfected with an expression system for secreting the Fc using a single vector encoding the blunted Fc heavy chain nucleotide sequence. Clarified media, into which the blunted Fc has been secreted, was purified using the commonly used techniques known to a person of ordinary skill in the art.

[00076] The Fc region of IgG4 included a human IgG4 Fc region with a blunted hinge. To improve the expression and minimize the potential for an unstructured peptide that may interfere with the conjugation process, a very short dipeptide comprised of alanine and glycine was incorporated upstream of the 229 cysteine (EU index numbering). The traditional human IgG4 hinge (SEQ ID NO 1), was blunted, removing the cysteine at position 226 (EU index numbering), involved in the upper hinge disulfide bond, and was replaced with a shortened hinge comprised of an alanine-glycine n-terminal dipeptide followed by the single disulfide from the native cysteine at position 229 (SEQ ID NO 2). Removing the upper hinge disulfide simplifies the conjugation process by eliminating potential mixed conjugation species by limiting the available cysteine thiols which may react with the maleimides. The complete human IgG4 Fc containing a blunted hinge with a single disulfide is shown in SEQ ID NO 3.

[00077] Two alternative human IgG4 blunted Fc variants and combination thereof have been explored: 1) mutations focused on increasing the isoelectric point(pI) of the Fc to potentially improve the biophysical properties of final conjugate, included Q274K, Q355R and E419Q (EU index numbering) (SEQ ID NO 4) mutations to potentially improve the half-life of the conjugate which included M252Y, S254T and T256E, which are known to enhance Neonatal Fc Receptor (FcRn) binding at low pH, which in turns results in higher levels of antibody recycling (SEQ ID NO 5). SEQ ID NO 6 is a combination of both the Q274K, Q355R, E419Q and the M252Y, S254T, T256E mutations.

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Attachment of Linker-Peptide to Fc Region

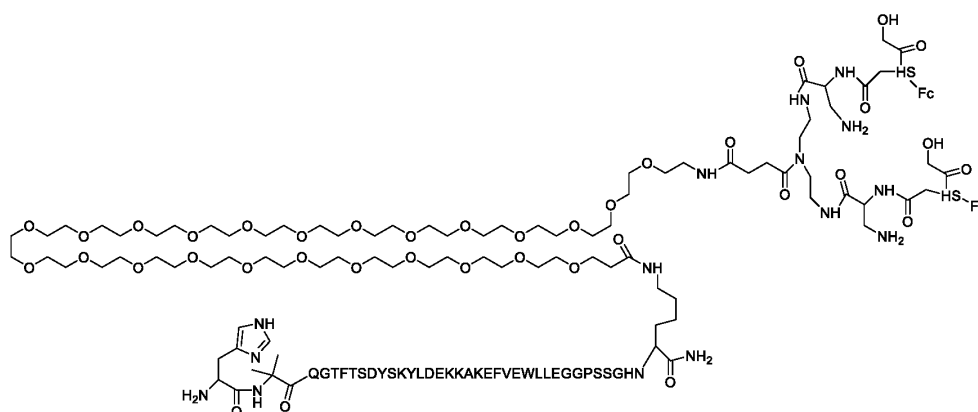
[00078] The disulfide bond in the hinge of the Fc was reduced by adding 2 equivalents of TCEP prepared in PBS to the Fc and incubating at 37°C for 1h. Reduction was tracked using an LCMS-TOF using a Zorbax 300 SB-C3 analytical column, RRHD, 2.1 x 100mm and analyzed using MassHunter software. Once the ~52kD peak was fully reduced to a ~26kD peak, the Fc was ready to be re-bridged.

[00079] Rebridge reaction was done by diluting the reduced Fc five-fold into a 50mM Na Acetate pH 5.5 buffer containing 20% acetonitrile. 2eq of peptide was dissolved in cold MilliQ H₂O containing 20% acetonitrile and 0.1%TFA then immediately added to the buffered Fc solution. Reaction was monitored via LCMS-TOF. The ~26kD peak was converted to a ~58kD peak as each of the peptide maleimides rebridge the reduced cysteine.

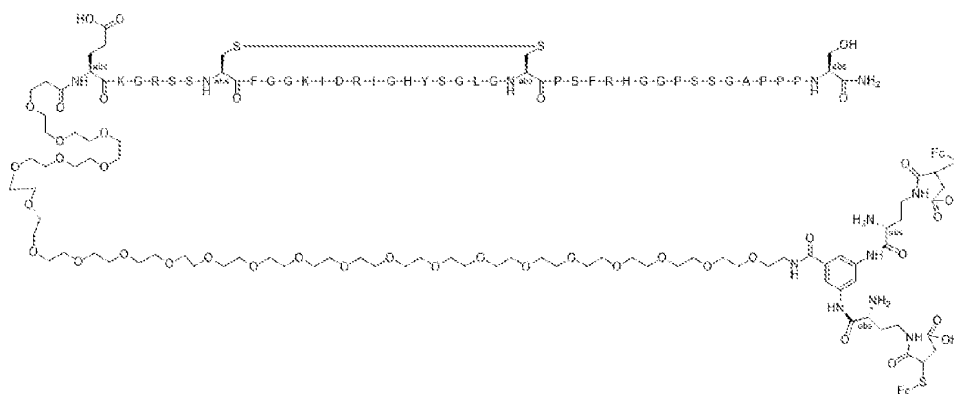
[00080] Fc-peptide rebridged conjugate was purified in two steps using an AKTA FPLC. First the reaction was loaded over a HiTrapTM MabSelect XtraTM protein A column (Cytiva) in a two-step purification. Reaction mixture was loaded to the system equilibrated in PBS pH7.2 to bind the Fc containing product and remove the peptide. Once the peptide was removed, the Fc containing material was collected by changing the mobile phase to 20mM Citrate pH3, disrupting the Fc interaction with the column. 1M T8 was added to raise the pH of the fractions upon elution, and a buffer exchange into 20mM Tris pH8 was done by centrifugation using 10,000mw cutoff amicon-ultra vials.

[00081] If needed, a second purification step was performed remove the unconjugated Fc components via strong anion exchange with a Mono-Q 5/50 GL (Cytiva). Sample was loaded using 20mM T8 and eluted over an increasing gradient with 20mM T8 + 1M NaCl. LCMS-TOF was used to determine fractions with the desired material, which were then again buffer exchanged to remove the NaCl and have a final solution of 20mM T8.

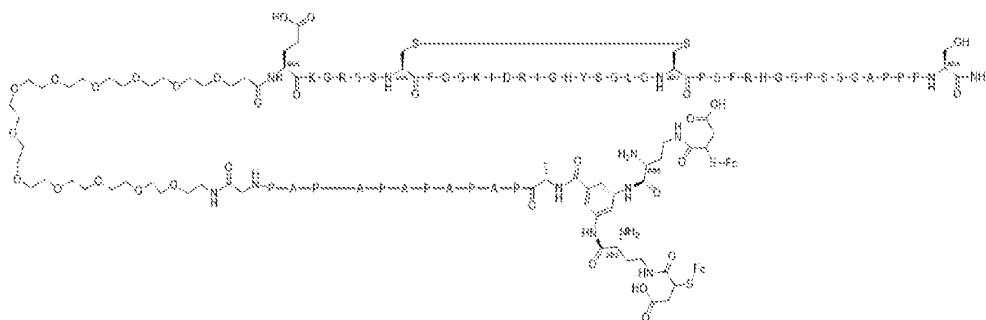
-30-

Example 1: OXMP-2Fc

[00082] Example 1 was synthesized using the procedure described above for attaching a peptide to a linker and then conjugating the peptide-linker to an Fc region. Example 1 is a conjugate including the peptide of SEQ ID NO 11 and the Fc region of SEQ ID NO 6. The linker used to connect SEQ ID NO 11 to SEQ ID NO 6 was Example D from TABLE 1.

Example 2: Natriuretic Peptide

[00083] Example 2 was synthesized using the procedure described above for attaching a peptide to a linker and then conjugating the peptide-linker to an Fc region. Example 2 is a conjugate including the peptide-linker of SEQ ID NO 10 to the Fc region of SEQ ID NO 6.

Example 3: Natriuretic Peptide

[00084] Example 3 was synthesized using the procedure described above for attaching a peptide to a linker and then conjugating the peptide-linker to an Fc region. Example 3 is a conjugate including the peptide-linker of SEQ ID NO 9 attached to the Fc region of SEQ ID NO 6.

Example 4

[00085] Example 4 was synthesized using the procedure described above for attaching a peptide to a linker and then conjugating the peptide-linker to an Fc region. Example 4 is a conjugate including the peptide of SEQ ID NO 12 and the Fc region of SEQ ID NO 6. The linker used to connect SEQ ID NO 12 to SEQ ID NO 6 was Example E from TABLE 1.

Example 5

[00086] Example 5 was synthesized using the procedure described above for attaching a peptide to a linker and then conjugating the peptide-linker to an Fc region. Example 3 is a conjugate including the peptide of SEQ ID NO 12 and the Fc region of SEQ ID NO 6. The linker used to connect SEQ ID NO 12 to SEQ ID NO 6 was Example F from TABLE 1.

Hydrolysis of Maleimide Functional Groups

[00087] Maleimide hydrolysis occurs on these constructs when the $\text{pH} \geq 8$ and causes the maleimide ring to open, accounting for an increase in mass of 18 daltons. The rebridged

constructs each have 2 maleimides, which account for a total mass shift of 36 daltons. The ring opening(hydrolysis) is tracked by mass using an LCMS-TOF. Self-Hydrolysis of Linkers

[00088] Self-hydrolysis of the linkers were assessed by incubating the linkers in triple phosphate buffer with corresponding pH 5.0, 6.0, 7.0, and 8.0. Lyophilized linker powders were solubilized in 100% DMSO to make up 80 mM stock solution. Each of the linkers were incubated in the buffer at final linker concentration of 1.5mM to determine succinimide hydrolysis at room temperature for 1 hour. Effect of pH on linker self-hydrolysis was assessed at particular intervals by Reverse Phase-HPLC mass-spec analysis. TABLEs 2A and 2B show the effect of pH on particular conjugates.

[00089] Surprisingly, TABLEs 2A and 2B show that linkers including β Maldap were more stable to higher pH conditions. Example 4 included a conjugate comprising natriuretic peptide (SEQ ID NO 12) connected to an Fc region (SEQ ID NO 6) through the Maldap linker of Example E of TABLE 1. Example 5 included a conjugate comprising natriuretic peptide (SEQ ID NO 12) connected to an Fc region (SEQ ID NO 6) through the Maldap linker of Example F of TABLE 1.

TABLE 2A. pH Stability of Example4

Tim (min)	pH 5 (% hydrolyzed)	pH 6 (% hydrolyzed)	pH 7 (% hydrolyzed)
5	16	27	100
20	28	51	100
60	40	81	100
120	65	100	100

TABLE 2B. pH stability of Example 5

Tim (min)	pH 5 (% hydrolyzed)	pH 6 (% hydrolyzed)	pH 7 (% hydrolyzed)	pH 8 (% hydrolyzed)
1	13	13	13	18
10	13	17	33	70
30	17	55	83	88
60	33	65	95	100
90	60	90	100	100
180	70	100	100	100

Duration of Action of Therapeutics

[00090] DIO mice were weighed and given a fresh TD95217 diet and were acclimated to handling and daily body weight and food intake prior to the start of the study. Animals were randomized by block order by BW. For acute study, a single injection of each peptide/conjugate shown in TABLE 3 at 10 nmol/kg was given. Daily food intake and body weight tracked for 15 days following injection. For chronic study each dose group (0.3nmol/kg, 1nmol/kg, 3nmol/kg, 10nmol/kg and 30nmol/kg) was given an injection every week for 4 weeks, food intake and body weight were tracked until 3 weeks after the final injection. QNMR was measured every 2 weeks.

[00091] TABLE 3 shows the impact of the conjugation of a therapeutic to an Fc region through the disclosed linkers. Two different injections were given to two populations of randomized DIO mice. The first group was given a control injection with no therapeutic. The second group was injected with an oxyntomodulin analog that is attached a blunted Fc region of IgG4 (Example 1).

[00092] The daily food intake of DIO mice that were given the control dose on was not changed over time (2.7-3.1 g of food per day).

[00093] Surprisingly, the daily food intake of DIO mice that were injected with Example 1 (oxyntomodulin analog attached to Fc region of IgG4 through a linker) was decreased

to 0.94 g by Day 2 and stayed at 1 g or lower until Day 5. By Day 7, the food intake of the DIO mice injected with Example 1 had returned to levels equivalent to the mice given an injection of the control.

[00094] Notably, it is well known to one of ordinary skill in the art, that an equivalent oxyntomodulin analog of Example 1 that is not bound to an Fc region and/or the disclosed linkers, would have almost no extended duration of action beyond the initial action immediately following administration. See, for example, Camacho *et al.* Conjugation of a peptide to an antibody engineered with free cysteines dramatically improves half-life and activity. *Mabs.* 2020 Jan-Dec;12(1):1794687. doi: 10.1080/19420862.2020.1794687, which stated, “endogenous peptide hormones have very short half-lives, making them impractical as therapeutics.” . Accordingly, while not wishing to being bound by theory, it is believed that the attachment of the oxyntomodulin analog to the Fc region through the disclosed linkers extended the duration of action of the oxyntomodulin analog by nearly 7 days.

[00095] As such, conjugation to an Fc region through one of the disclosed linkers led to an increase in the duration of action of a therapeutic by a week.

TABLE 3. Impact of Conjugation to Fc Region through Linker

	Daily Food Intake Post Injection (g)													
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	2.8	2.5	2.7	2.9	2.7	2.9	2.7	2.9	2.8					2.7
	4	8	4	6	6	6	6	4	8	3.1	2.7	2.7	2.7	8
Ex. 1		0.9	0.4	0.7	1.0	2.2	3.0	3.9	3.9	3.9	3.6	3.4	3.4	
	2.9	4	2	8	4	6	6	8	8	8	6	8	8	3.2

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SEQUENCES

SEQ ID NO 1:

ESKYGPPCPSCP

SEQ ID NO 2:

AGCP

SEQ ID NO 3: Blunted Human IgG4 Fc with single hinge disulfide

AGCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSFGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL
SLG

SEQ ID NO 4: Blunted Human IgG4 Fc with single hinge disulfide (Q274K, Q355R and E419Q mutant)

AGCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVKFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSFGSFFLYSRLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SLG

SEQ ID NO 5: Blunted Human IgG4 Fc with single hinge disulfide (M252Y, S254T and T256E mutant)

AGCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSFGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL
SLG

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SEQ ID NO 6: Blunted Human IgG4 Fc with single hinge disulfide (Q274K, Q355R, E419Q and M252Y, S254T, T256E combination mutant)

AGCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVKFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSL
SLG

SEQ ID NO 7: *H-Aib-QGTFTSDYSKYLDEKKAQEFVEWLLEGGPSSGK(Peg24-BEA-Maldap2)-NH₂*

SEQ ID NO 8: H-Aib-QGTFTSDYSKYLDEKKAK(Peg24-BEA Maldap2)
EFVEWLLEGGPSSG-NH₂

SEQ ID NO 9: NH₂-SPPPAGSSPGG-H-RFSPCGLGSYHGIRDIKGGFCSSRGKE-
(PEG12-G-PAPAPAPAPAPA)-DABA-(Maldab)₂

SEQ ID NO 10 NH₂-SPPPAGSSPGG-H-RFSPCGLGSYHGIRDIKGGFCSSRGKE-
(PEG24-DABA-(Maldab)₂

SEQ ID NO 11 OXMP-2

H-Aib-QGTFTSDYSKYLDEKKAQEFVEWLLEGGPSSGK

SEQ ID NO 12 Natriuretic peptide

EKGRSSCFGGKIDRIGHYSGLGCPFSFR-H-GGPSSGAPPPS

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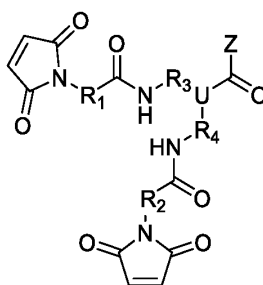
|-----|

forms an intramolecular bond with cysteine at position 33

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CLAIMS

What is claimed is:



1. A compound of the formula:

wherein:

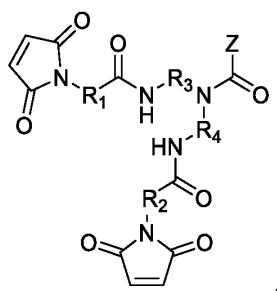
R₁ and R₂ are independently C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, and -CH₂CH₂NH₂;

R₃ and R₄ are independently C₁ to C₅ alkyl or absent;

U is tertiary amine or 1,3,5-substituted phenyl;

Z comprises H, OH, NH₂, NH, a therapeutic, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

2. The compound of claim 1, wherein the compound is of the formula:



wherein:

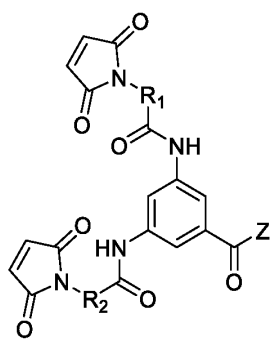
R₁ and R₂ are independently C₁ to C₅ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, or -CH₂CH₂NH₂;

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R_3 and R_4 are C_1 to C_5 alkyl;

Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

3. The compound of claim 1, wherein the compound is of the formula:

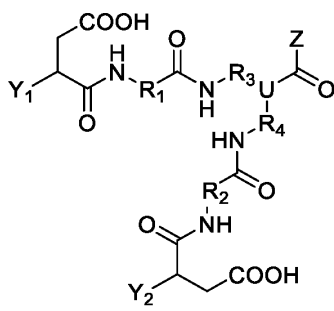


wherein:

R_1 and R_2 are independently C_1 to C_5 linear alkyl, optionally substituted with one or more of $-NH_2$, $-CH_2NH_2$, or $-CH_2CH_2NH_2$;

Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

4. A compound of the formula:



wherein:

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R_1 and R_2 are independently C_1 to C_5 alkyl, optionally substituted with one or more of NH_2 , CH_2NH_2 , and $CH_2CH_2NH_2$;

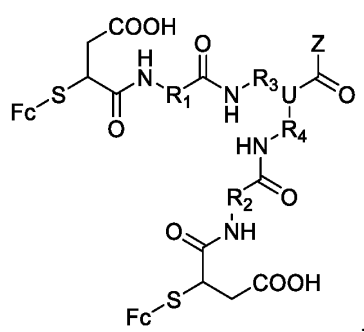
R_3 and R_4 are independently C_1 to C_5 alkyl or absent;

U is tertiary amine or 1,3,5-substituted phenyl;

Y_1 and Y_2 independently comprise an amide, amine, imine, sulfonamide, thiourea, urea, or thioether, ;

Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30; and pharmaceutically acceptable salts thereof.

5. The compound of claim 4, wherein the compound is of the formula:



wherein:

R_1 and R_2 are independently C_1 to C_5 linear alkyl, optionally substituted with one or more of NH_2 , CH_2NH_2 , and $CH_2CH_2NH_2$;

R_3 and R_4 are independently C_1 to C_5 linear alkyl or absent;

U is tertiary amine or 1,3,5-substituted phenyl;

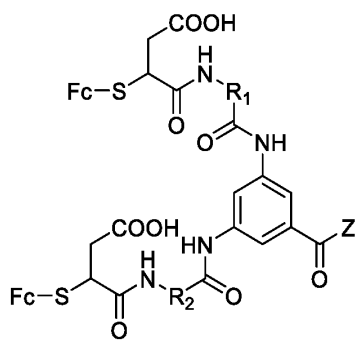
Z comprises H, OH, NH_2 , NH, a therapeutic, C_1 to C_{30} alkyl, $(OCH_2CH_2)_m$, or combinations thereof, and wherein m is a whole number integer from 1 to 30;

Fc comprises an Fc region;

S is a sulfur atom from the Fc region; and

6. The compound of claim 4, wherein the compound is of the formula:

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wherein:

R₁ and R₂ are independently C₁ to C₅ linear alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, and -CH₂CH₂NH₂;

Z comprises H, OH, NH₂, NH, a therapeutic, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and wherein m is a whole number integer from 1 to 30;

Fc comprises an Fc region;

S is a sulfur atom from the Fc region; and

pharmaceutically acceptable salts thereof.

7. The compound of claim 1 to 6, wherein R₁ and R₂ are independently C₂ alkyl, optionally substituted with one or more of -NH₂, -CH₂NH₂, or -CH₂CH₂NH₂, or pharmaceutically acceptable salts thereof.

8. The compound of any one of claims 1 to 7, wherein R₁ and R₂ are identical, or pharmaceutically acceptable salts thereof.

9. The compound of any one of claims 1, 2, 4, 5, 7 or 8, wherein R₃ and R₄ are identical, or pharmaceutically acceptable salts thereof.

10. The compound of any one of claims 1, 2, 4, 5, or 7 to 9, wherein R₃ and R₄ are C₂ alkyl, or pharmaceutically acceptable salts thereof.

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11. The compound of any one of claims 1 to 10, wherein Z comprises NH, C₁ to C₃₀ alkyl, (OCH₂CH₂)_m, or combinations thereof, and a therapeutic, or pharmaceutically acceptable salts thereof.
12. The compound of any one of claims 1 to 11, wherein Z is H, OH, NH₂, a therapeutic, or C₁ to C₃₀ alkyl, or pharmaceutically acceptable salts thereof.
13. The compound of any one of claims 1 to 12, wherein Z comprises a therapeutic.
14. The compound of 13, wherein the therapeutic comprises an amino acid or a peptide, or pharmaceutically acceptable salts thereof.
15. The compound of claim 14, wherein the peptide comprises from 2 to 100 amino acids, or pharmaceutically acceptable salts thereof.
16. The compound of any one of claims 13 to 15, wherein the therapeutic comprises adrenocorticotrophic hormone (ACTH), adropin, amylin, angiotensin, atrial natriuretic peptide (ANP), calcitonin, cholecystokinin (CCK), gastrin, ghrelin, glucagon, growth hormone, follicle-stimulating hormone (FSH), insulin, leptin, luteinizing hormone (LH), melanocyte-stimulating hormone (MSH), natriuretic peptide, oxyntomodulin, oxytocin, parathyroid hormone (PTH), prolactin, renin, somatostatin, thyroid-stimulating hormone (TSH), thyrotropin-releasing hormone (TRH), vasopressin, also called arginine vasopressin (AVP) or anti-diuretic hormone (ADH), vasoactive intestinal peptide (VIP), analogs thereof, or combinations thereof, or pharmaceutically acceptable salts thereof.
17. The compound of any one of claims 13 to 16, wherein the therapeutic comprises oxyntomodulin, natriuretic peptide, analogs thereof, or combinations thereof, or pharmaceutically acceptable salts thereof.
18. The compound of any one of claims 13 to 16 wherein the therapeutic comprises SEQ ID NO 11 or SEQ ID NO 12, or pharmaceutically acceptable salts thereof.

19. The compound of claim 13, wherein the therapeutic comprises a monoclonal antibody, an interleukin, an interferon, a protein kinase inhibitor, a hematopoietic growth factor, a protein, a fused protein, oligonucleotide, or combinations thereof, or pharmaceutically acceptable salts thereof.
20. The compound of claim 4, wherein Y_1 and Y_2 comprise an Fc region.
21. The compound of any one of claims 5 to 20, wherein the compound is connected to a single Fc region, or pharmaceutically acceptable salts thereof.
22. The compound of any one of claims 5 to 21, wherein the Fc region comprises an Fc region of a monoclonal antibody, or pharmaceutically acceptable salts thereof.
23. The compound of any one of claims 5 to 22, wherein the Fc region comprises at least a portion of IgA, IgD, IgE, IgG, IgM, or combinations thereof, or pharmaceutically acceptable salts thereof.
24. The compound of any one of claims 5 to 23, wherein the Fc region comprises SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, or SEQ ID NO 6, or pharmaceutically acceptable salts thereof.
25. The compound of claim 24, wherein the Fc region comprises SEQ ID NO 6.
26. The compound of claim 25, wherein Z comprises SEQ ID NO 11 or SEQ ID NO 12.