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(54) **COMPOSITIONS AND METHODS
CONCERNING IMMUNE TOLERANCE**

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(57) **ABSTRACT**

The present disclosure provides compositions comprising mannose-fused antigens to target mannose receptors. The compositions may be used to prevent immunity or reduce an immune response protein-based drugs that would otherwise elicit an immune response.

17 Claims, 7 Drawing Sheets

Specification includes a Sequence Listing.

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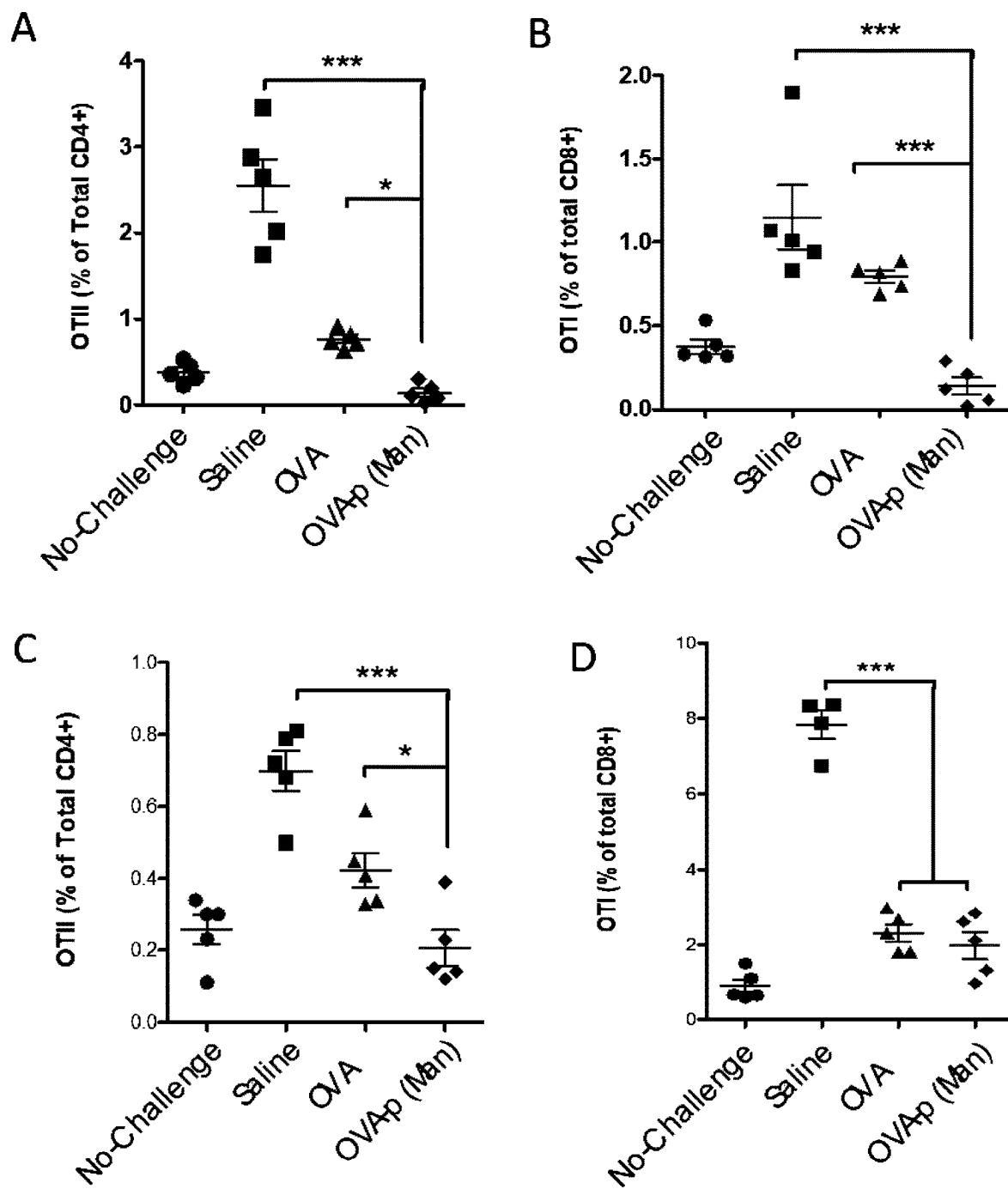


FIG. 1A-D

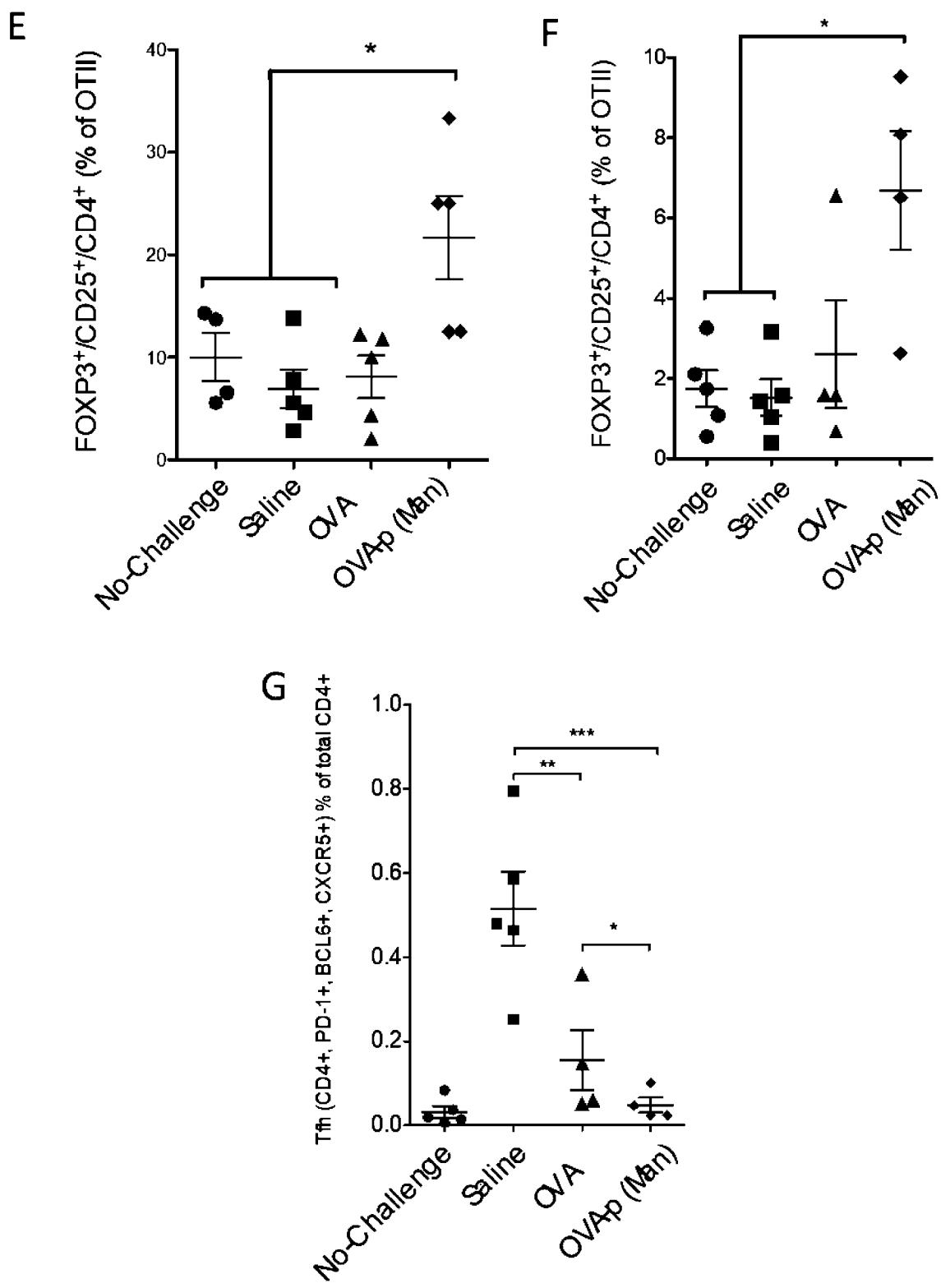


FIG. 1E-G

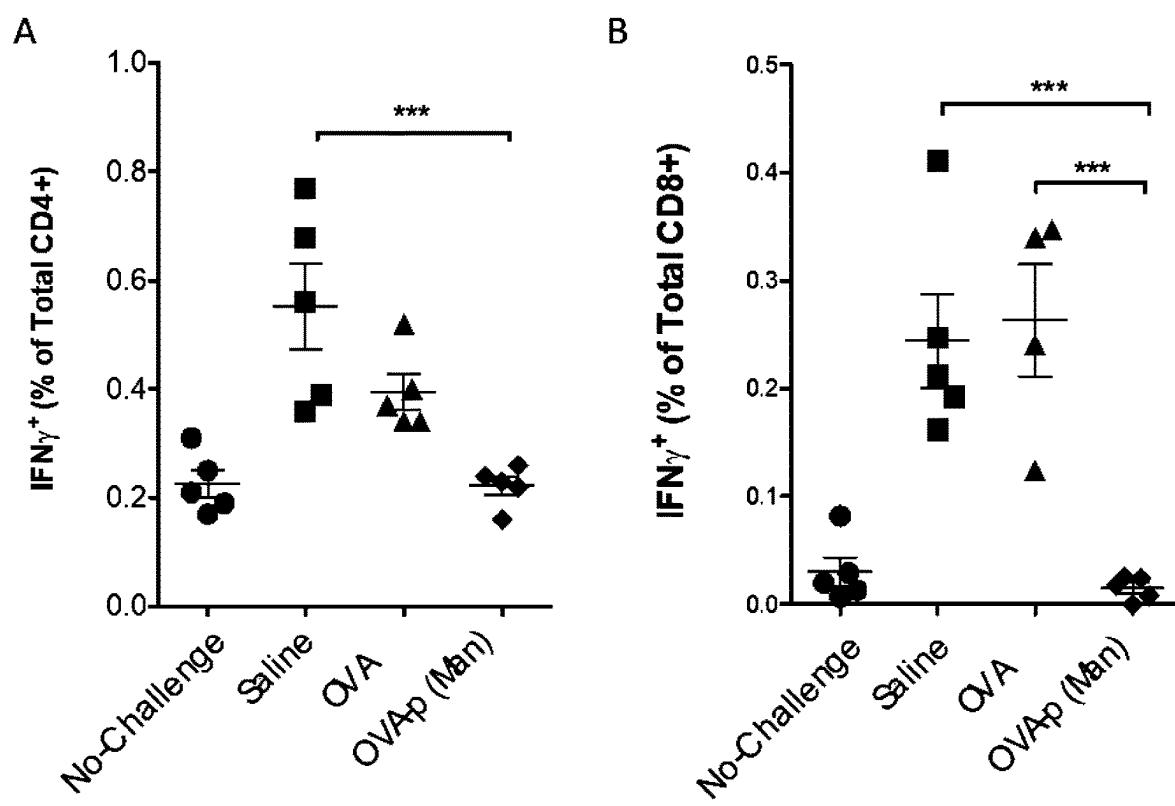


FIG. 2A-B

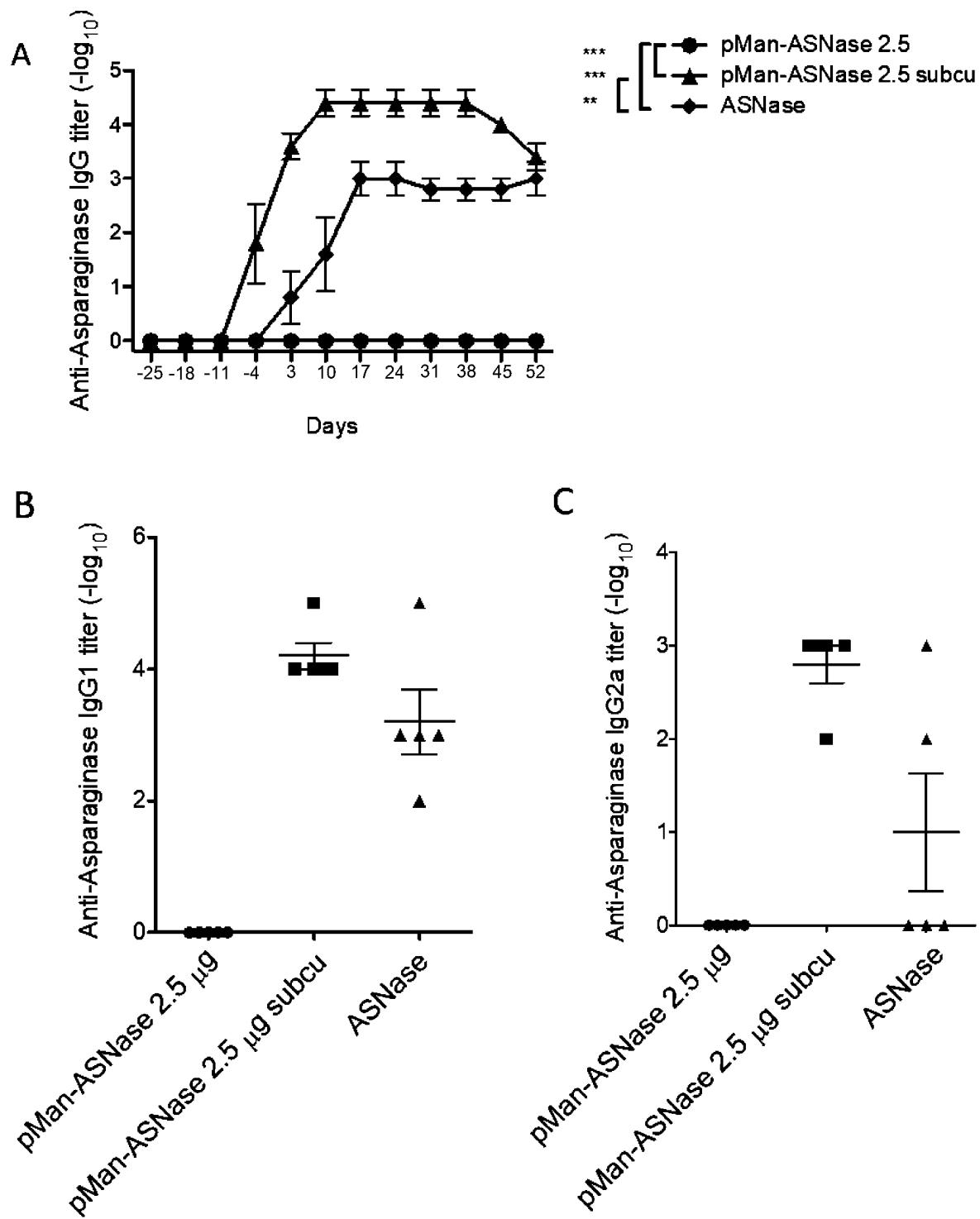


FIG. 3A-C

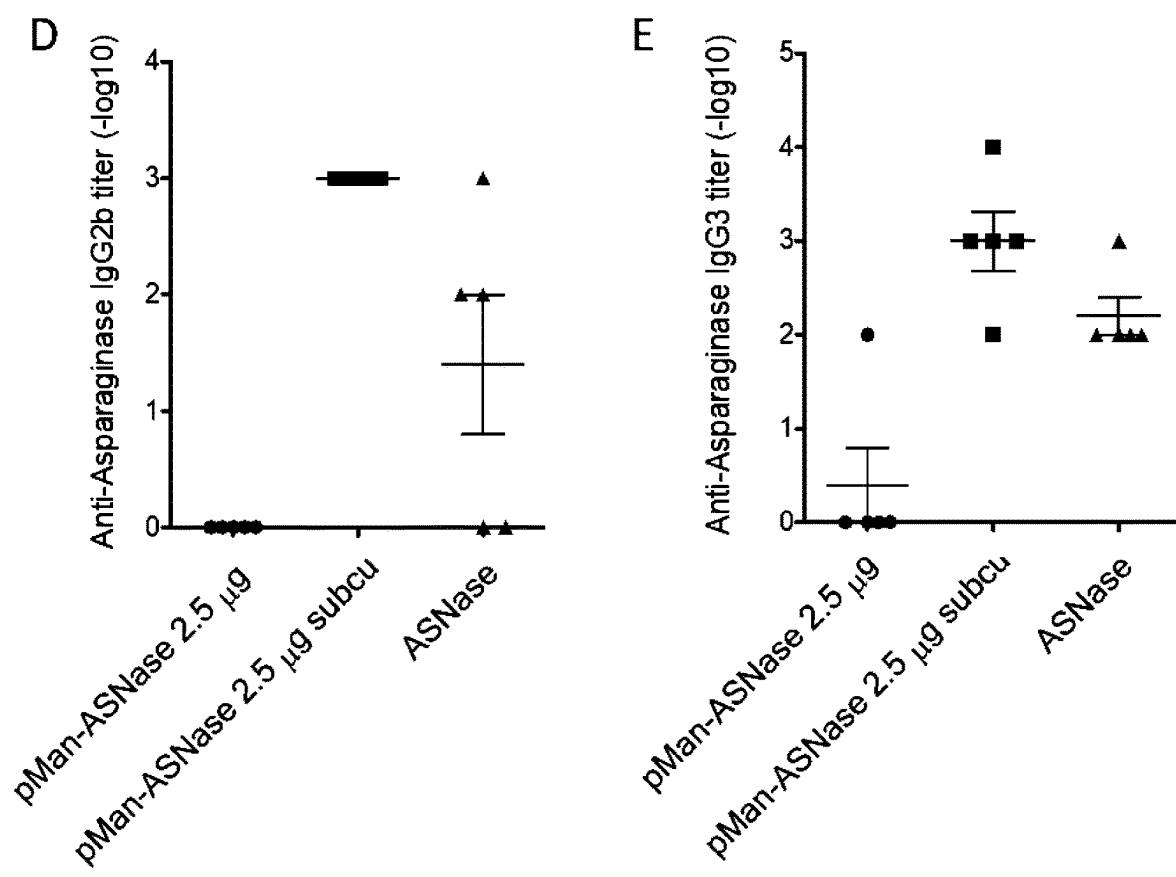


FIG. 3D-E

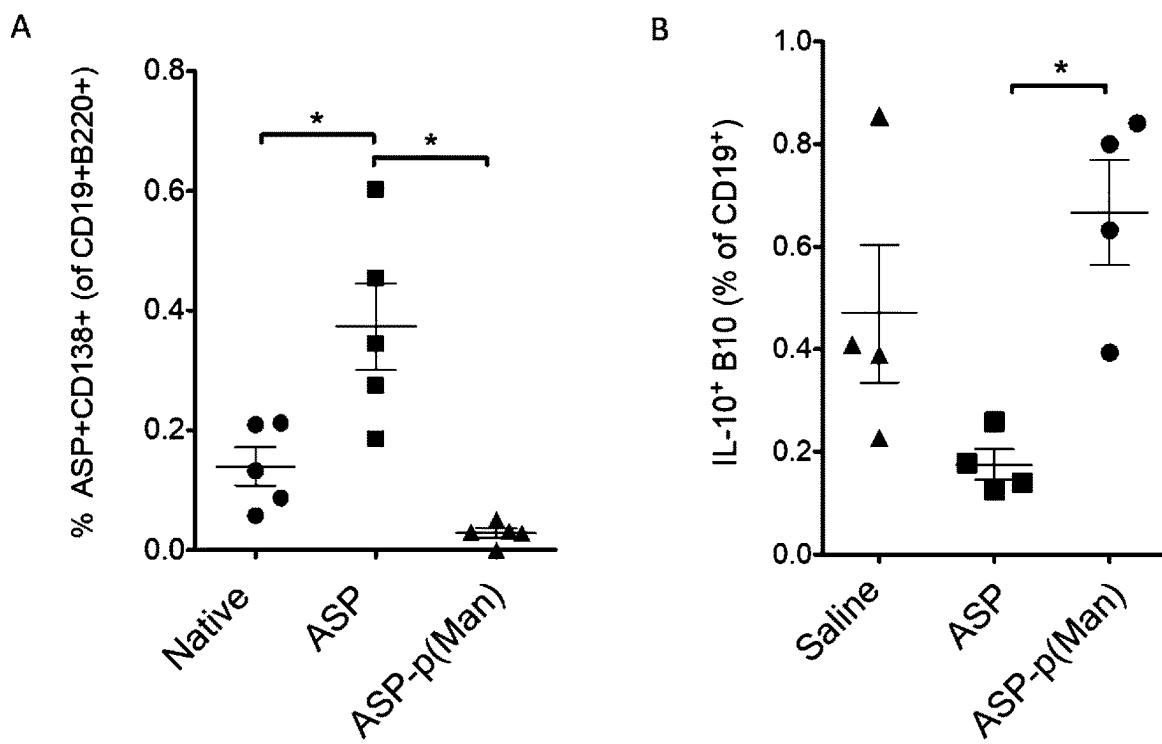


FIG. 4A-B

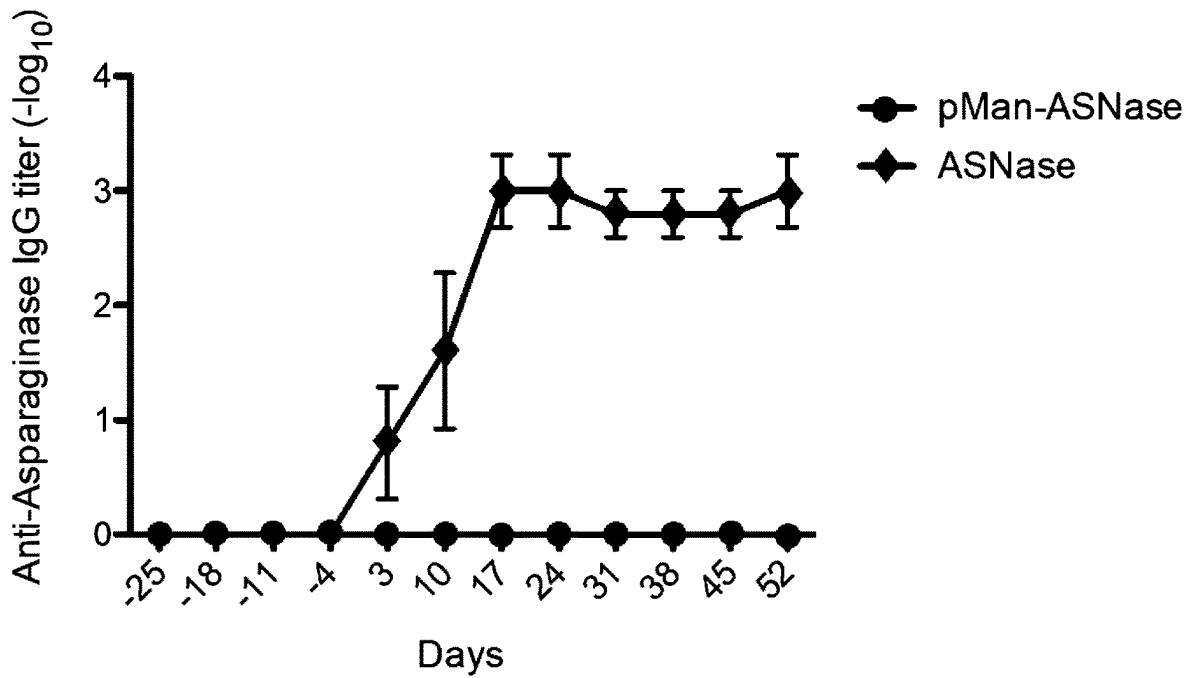


FIG. 5

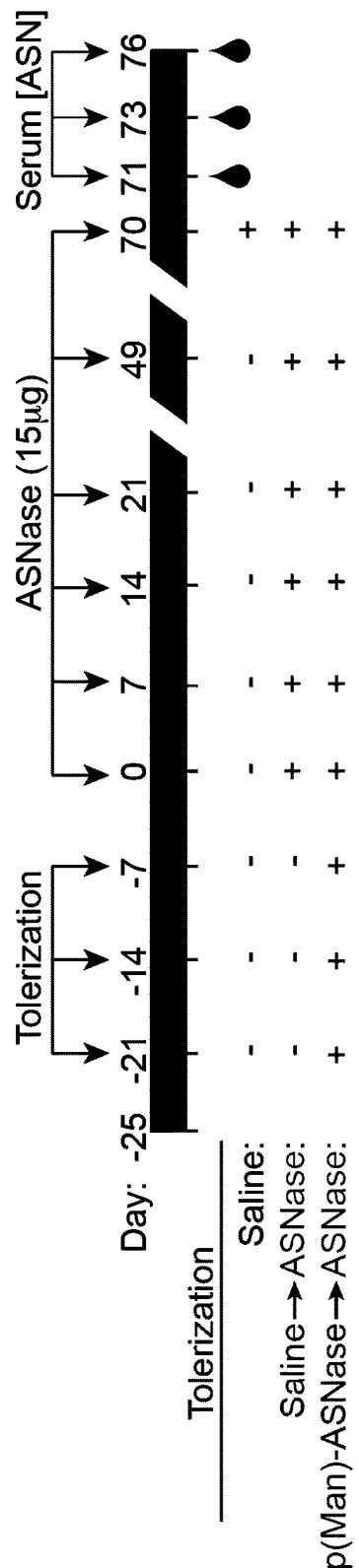


FIG. 6

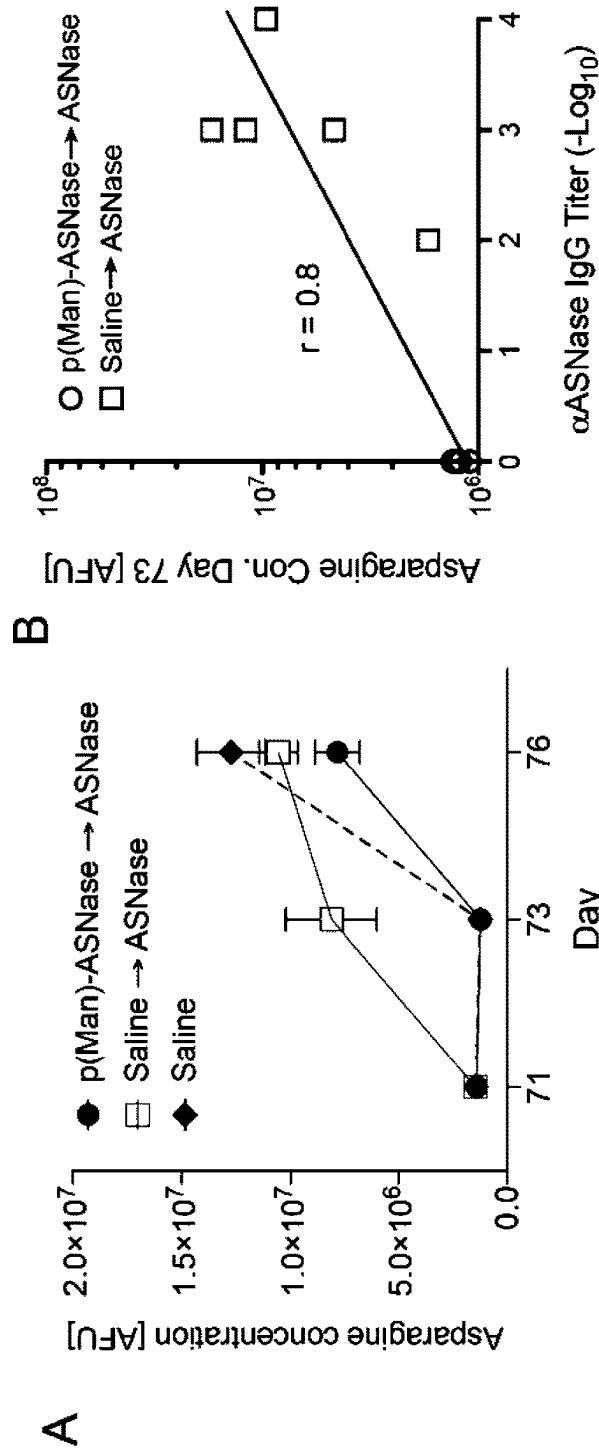


FIG. 7A-B

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COMPOSITIONS AND METHODS
CONCERNING IMMUNE TOLERANCECROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a national phase application under 35 U.S.C. § 371 of International Application No. PCT/US2019/031440 filed May 9, 2019, which claims priority to U.S. Provisional Patent Application No. 62/669,044, filed May 9, 2018, all of which are incorporated in their entirety by reference.

BACKGROUND OF THE INVENTION

1. Field

Embodiments of the present invention relate generally to the fields of organic chemistry, biochemistry, and immunology.

2. Background

Small molecules, e.g., non-peptide or nucleic acid-based molecules having a molecular weight of less than 500 Daltons, have historically dominated the FDA's new molecular entity (NME) approvals. Since the FDA's approval of insulin as the first drug produced by recombinant DNA technology in 1982, protein-based drug approvals have experienced an upward trend, as demonstrated by the almost yearly increase in FDA biologics license application (BLA) approvals in the last 23 years.

A primary difference between a small molecule drug and a protein-based drug lies in the ability of antigen presenting cells to take up proteins, including protein-based drugs, process them, and present them as peptides to the major histocompatibility complex (MHC) class I and II to the immune system. Protein-based drugs are expressed and purified to be free of any pathogen-associated molecular patterns (PAMPs) and danger associated molecules (DAMPs). Notwithstanding the anti-immunogenic design of protein-based drugs, a significant portion of patients who are treated with these drugs develop antibodies against the drugs (anti-drug antibodies).

These and other examples demonstrate that protein-based drug immunogenicity can revert the effect of therapy for certain patients or even render a new approach to treat a disease inefficient. The negative effects provided by anti-drug antibodies highlight the need to develop a platform that allows clinicians induce tolerance to foreign and partly foreign proteins.

Technologies to induce antigen-specific immunological tolerance are still in their infancy, but are needed to prevent immunity to many protein-based drugs, to reverse immunity to allergens, and to prevent and reverse immunity to autoimmune antigens.

SUMMARY

A useful tolerogenic drug would consist of an antigen and a tolerance inducing component. The tolerance inducing component can be a chemical conjugated entity, part of a fusion protein, nanoparticles, or cells that are pulsed with the antigen. Ideally, a tolerance-inducing drug is based on the drug it tries to tolerize against, and can easily be modified.

Disclosed herein are compositions and methods for inducing tolerance towards therapeutic proteins, e.g., protein-

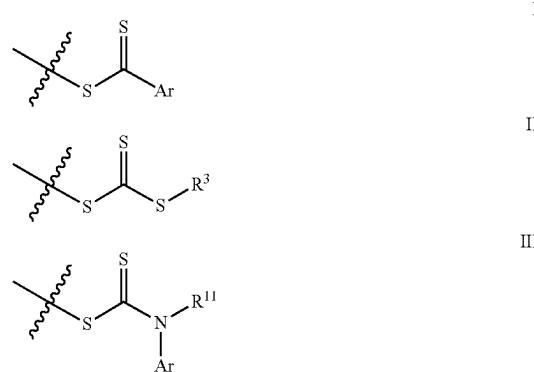
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based drugs. In some aspects, the present disclosure provides therapeutic, polymer-tethered antigens that include mannose monomers or derivatives thereof. In some embodiments, derivatives of mannose include optionally substituted mannose substituents. In several embodiments, the mannose derivative comprises a mannose with a phosphate at the C1, C2, C3, C4, C5, and/or C6 position. In several embodiments, the targeting moiety comprises mannose-6-phosphate. In some embodiments, compositions including a mannose or mannose-derived moiety induce tolerance to immunogenic protein-based therapeutics, delete antigen-specific CD4 and CD8 T cells, elevate levels of regulatory T cell responses and IL-10 producing Breg cells, and/or reduce antigen-specific plasma cells and memory B cells. The compositions disclosed herein may be targeted for delivery to antigen-presenting cells including, but not limited to, hepatocytes, LSECs, Kupffer cells, and stellate cells. In some aspects, the compositions disclosed herein exhibit affinity for and specifically bind to mannose-binding receptors.

In several embodiments, there are provided compounds, as well as compositions comprising such compounds, of Formula 1:



where X comprises an antigen, a tolerogenic portion thereof, or a mimetic thereof, Y comprises a linker moiety, Z comprises a moiety that specifically targets a mannose receptor, p is an integer from about 2 to about 250, m is an integer from about 1 to about 100, and R² is any of functional groups I-III:

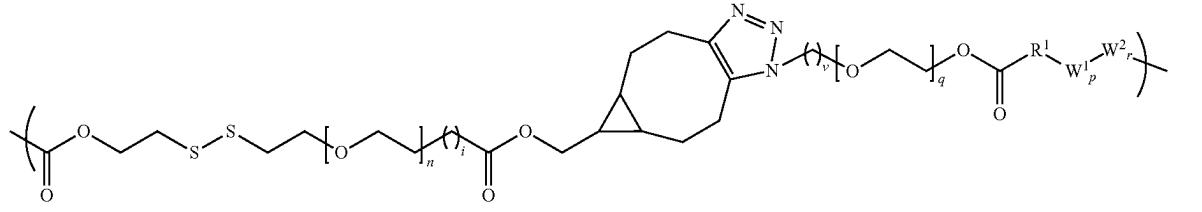
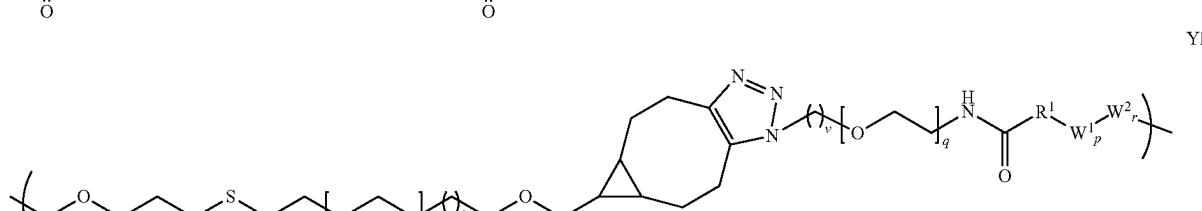
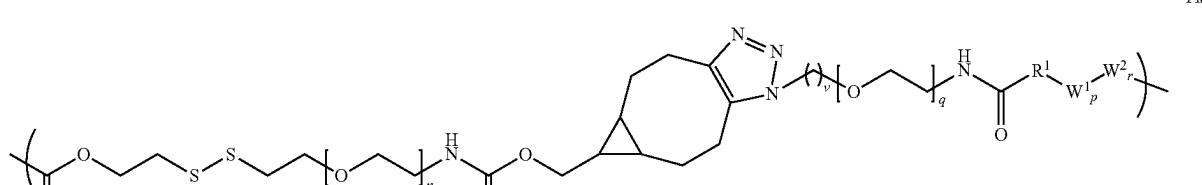
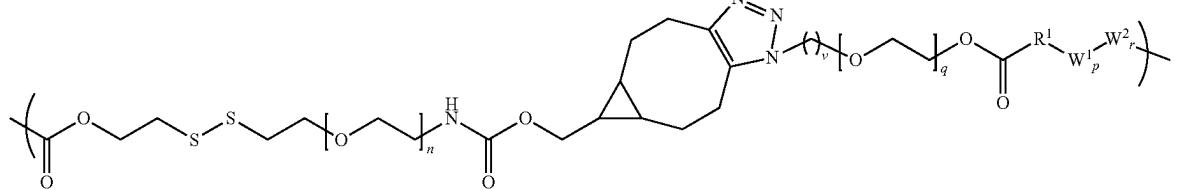
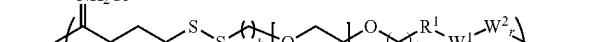
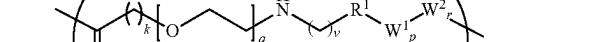
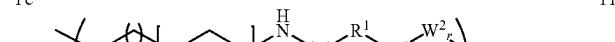
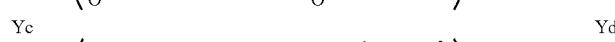
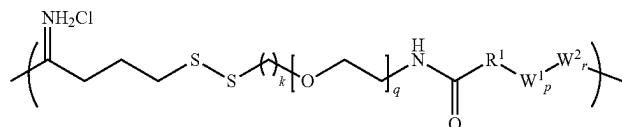
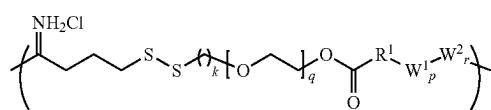
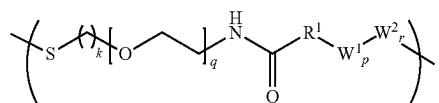
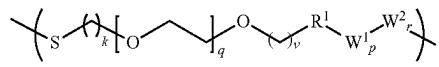
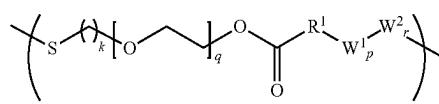


where Ar is a substituted or unsubstituted aromatic group, where R³ is any carbon-containing linear or heterocyclic moiety, and R¹¹ is hydrogen or an alkyl group.

In several embodiments, the moiety that specifically targets a mannose receptor is selected from the group consisting of α -linked mannose, β -linked mannose, substituted mannose, mannose-6-phosphate, N-acetyl mannosamine, and mannan having β (1-4), α (1-6), α (1-2), and/or α (1-3) linkages. In several embodiments, a plurality of these targeting moieties are used in combination in order to enhance targeting of and/or binding to the mannose receptor.

In several embodiments, Y is a linker resulting from reaction of at least one of a N-hydroxysuccinimidyl linker, maleimide linker, PEG linker, vinylsulfone linker, pyridyl di-thiol-poly(ethylene glycol) linker, pyridyl di-thiol linker, n-nitrophenyl carbonate linker, NHS-ester linker, and nitro-phenoxyl poly(ethylene glycol)ester linker. In some embodiments, Y is covalently bound to X.

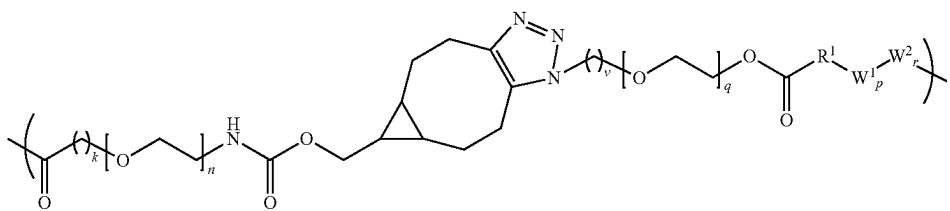
Depending on the embodiment, the following portion of Formula 1 ($-\text{[Y}(Z)\text{p}]-$) is represented by one of Formula Ya to Yr:



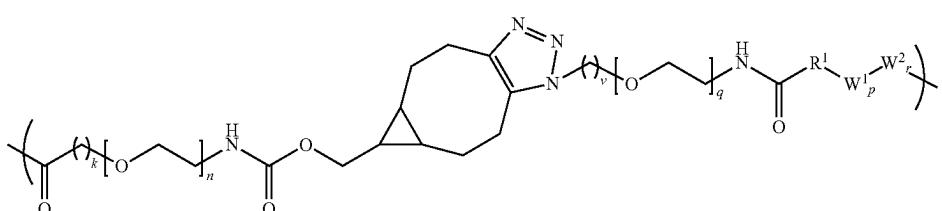
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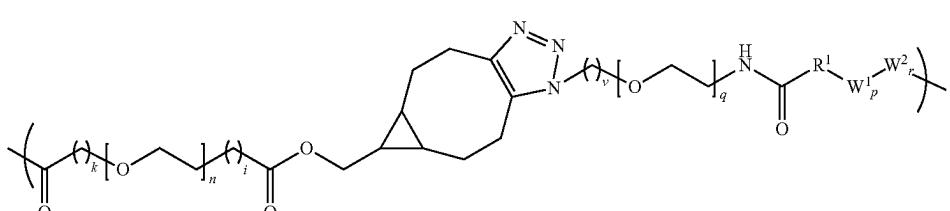
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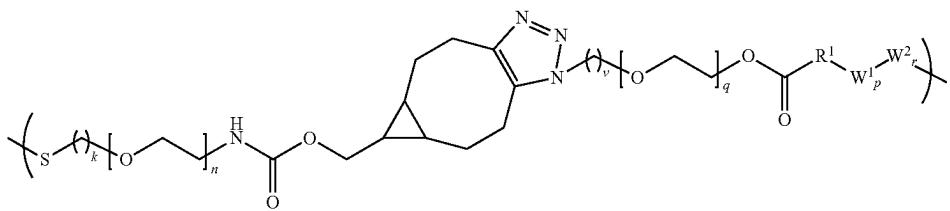
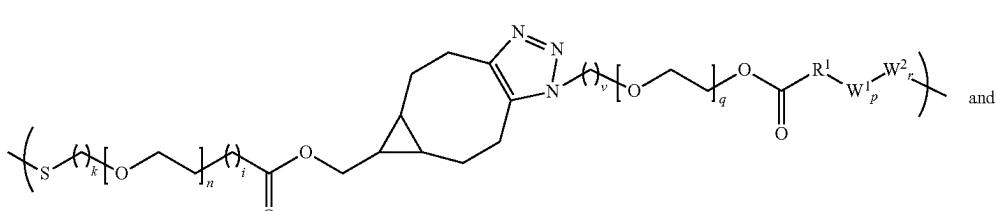
Yn



Yo



Yp



where

n is an integer from about 1 to about 100;

q is an integer from about 1 to about 44;

k is an integer from about 1 to about 12;

i is an integer from 0 to about 20;

v is an integer from about 1 to about 4;

p is an integer from about 2 to about 250;
 r is an integer from 0 to about 250;
 R_1 is $-\text{CH}_2-$, $-(\text{CH}_2)_2-\text{C}(\text{CH}_3)(\text{CN})-$, $-(\text{CH}_2)_2-$
 $-\text{C}(\text{CH}_3)(\text{CH}_3)-$, $-(\text{CH}_2)_2-\text{CH}(\text{CH}_3)-$ or
 $\text{CH}_2=\text{CH}-$

W^1 and W^2 are as defined below:

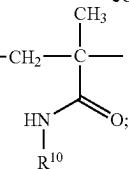
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$$W^1 = \begin{array}{c} & CH_3 \\ & | \\ ---CH_2-C & | \\ & R^9 \\ & | \\ & Z \end{array}$$

-continued



R^9 is a direct bond, $-(CH_2)_2-NH-C(O)-$ (an ethylacetamido group or “EtAcN”) or $-(CH_2)_2-(O-CH_2-CH_2)_t-NH-C(O)-$ (a pegylated ethylacetamido group or “Et-PEG t -AcN”)

60 t is an integer from 1 to 5, Z is mannose or a mannose receptor-targeting moiety; and R¹⁰ is an aliphatic group, an alcohol, an aliphatic amine-containing group, or an aliphatic alcohol.

In several embodiments, Y is an antibody, antibody fragment, peptide or other ligand that binds to X.

In several embodiments, X is an antigen against which a patient may develop or has developed an unwanted immune

response. For example, depending on the embodiment, the antigen may be a foreign transplant antigen, an alloantigen, an autoimmune antigen, a food antigen, an animal antigen, a plant antigen, an environmental antigen, a therapeutic antigen, a synthetic self-antigen, or a tolerogenic (e.g., immunogenic, or capable of inducing an immune response) portion thereof. In several embodiments, X is an asparaginase antigen or an ovalbumin antigen. In several embodiments, the antigen is comprised in a vesicle, cell fragment, or cell.

In several embodiments, the antigen comprises at least one autoimmune antigen or tolerogenic portion thereof. In several embodiments, the at least one autoimmune antigen comprises at least one of an immunogenic fragment or fragments of myelin basic protein (MPB), an immunogenic fragment or fragments of myelin oligodendrocyte glycoprotein (MOG), an immunogenic fragment or fragments of myelin proteolipid protein (PLP), MBP, MOG, or PLP. In several embodiments, the at least one autoimmune antigen comprises at least one of SEQ ID Nos. 23-47. In several embodiments, the at least one autoimmune antigen comprises at least one of SEQ ID Nos. 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 43, 44, 45, 46, and 47. In some embodiments, the compound optionally further comprises at least one of SEQ ID NOS: 29, 38, 39, 40, 41, and 42. In several embodiments, such compounds are for use in treatment of or prevention of multiple sclerosis. In some embodiments, such compounds are administered for the use of preventing multiple sclerosis in a subject predicted to have multiple sclerosis. In some aspects, such compounds are administered to a subject presenting one or more symptoms of multiple sclerosis.

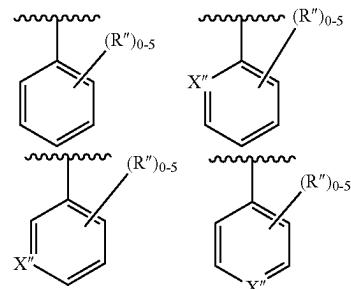
In several embodiments, the at least one autoimmune antigen comprises at least one of insulin, proinsulin, preproinsulin, glutamic acid decarboxylase-65 (GAD-65) or glutamate decarboxylase 2), GAD-67, glucose-6 phosphatase 2, islet-specific glucose 6 phosphatase catalytic subunit related protein (IGRP), insulinoma-associated protein 2 (IA-2), insulinoma-associated protein 2 β (IA-2 β), ICA69, ICA12 (SOX-13), carboxypeptidase H, Imogen 38, GLIMA 38, chromogranin-A, HSP-60, carboxypeptidase E, peripherin, glucose transporter 2, hepatocarcinoma-intestine-pancreas/pancreatic associated protein, S100 β , glial fibrillary acidic protein, regenerating gene II, pancreatic duodenal homeobox 1, dystrophia myotonica kinase, and SST G-protein coupled receptors 1-5, or an immunogenic fragment of any of these antigens. In several embodiments, the autoimmune antigen comprises an immunogenic fragment of proinsulin. Optionally an immunogenic fragment of IA-2, GAD-65, GAD-67, insulin, and/or IGRP is included. In several embodiments, the at least one autoimmune antigen comprises at least one of SEQ ID NOS: 1-19, or an immunogenic fragment of any of SEQ ID NOS: 1-19. In several embodiments, the at least one autoimmune antigen comprises at least one of SEQ ID NOS: 4-19. In several embodiments, such compounds are for use in the treatment or prevention of Type 1 Diabetes. In some embodiments, such compounds are administered for the use of preventing Type 1 Diabetes in a subject predicted to have Type 1 Diabetes. In some aspects, such compounds are administered to a subject presenting one or more symptoms of Type I Diabetes.

In several embodiments, the antigen comprises a food antigen, or a tolerogenic portion thereof. In several embodiments, the antigen comprises at least one of tissue transglutaminase, high molecular weight glutenin, low molecular weight glutenin, gluten, alpha-gliadin, gamma-gliadin, omega-gliadin, hordein, secalin, avenin, and deamidated

forms thereof. In several embodiments, the antigen comprises a tolerogenic portion of at least one of tissue transglutaminase, high molecular weight glutenin, low molecular weight glutenin, gluten, alpha-gliadin, gamma-gliadin, omega-gliadin, hordein, secalin, avenin, and deamidated forms thereof. In several embodiments, the antigen comprises at least one of SEQ ID NOS. 54-61. In several embodiments, such compounds are for use in the treatment or prevention of Celiac Disease. In some embodiments, such compounds are administered for the use of preventing Celiac Disease in a subject predicted to have Celiac Disease. In some aspects, such compounds are administered to a subject presenting one or more symptoms of Celiac Disease.

In several embodiments, the mannose receptor is mannose-6-phosphate receptor. In several embodiments, Y and X are connected through a bond configured to cleave when the compound reaches a target area. Advantageously, this cleavage, in several embodiments, is triggered when the compound is at a target site (e.g., site where the mannose receptor is bound). This allows, in several embodiments, delivery of the free antigen to the target site.

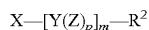
In several embodiments, Ar is selected from:



where each instance of R'', when present, is independently selected from an optionally substituted C1-6-alkyl, optionally substituted C1-6 alkoxy, optionally substituted amino, OH, or halogen and wherein, X'' is a heteroatom. In several embodiments, X'' is N. In several embodiments, R11 is C1-6-alkyl. In several embodiments, R11 is —CH3. In several embodiments, R3 is C1-6-alkyl.

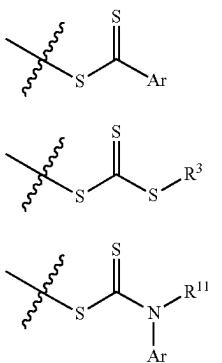
There are also provided herein, compositions comprising the compounds as described above, or elsewhere herein. Likewise, there are provided for herein uses of these compounds for inducing tolerance (or treating an unwanted immune response) to an antigen, a tolerogenic portion (or portions) of one or more antigens, and/or to mimetics of the antigens or portions of antigens. Also provided are uses of such compounds in the preparation of a medicament for inducing tolerance (or treating an unwanted immune response) to an antigen, a tolerogenic portion (or portions) of one or more antigens, and/or to mimetics of the antigens or portions of antigens.

Certain aspects of the disclosure are directed towards compositions comprising a compound of Formula 1:

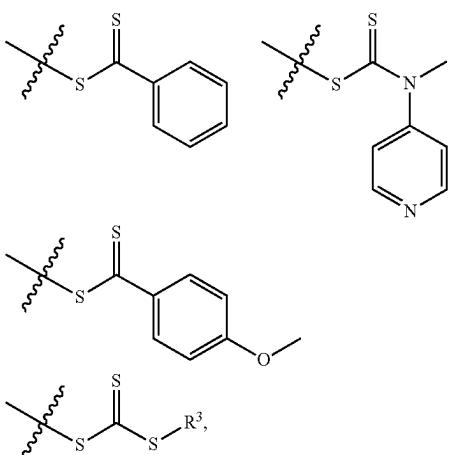


Formula 1

where X comprises an antigen or a tolerogenic portion thereof, Y comprises a linker moiety, Z comprises a moiety that specifically targets a mannose receptor, p is an integer from about 2 to about 250, m is an integer from about 1 to about 100, R² is any of functional groups I-III:



where Ar is a substituted or unsubstituted aromatic group, R³ is any carbon-containing linear or heterocyclic moiety, and R¹¹ is hydrogen or an alkyl group. In some embodiments, R² comprises an end-capping group. In some embodiments, R² when disconnected from the construct, forms a stable or substantially stable free radical. In some embodiments, R² is a reversible addition-fragmentation chain transfer (RAFT) agent for a living polymerization. In some embodiments, R² can be reversibly added and removed to the construct to lengthen the linker region. In some embodiments, R² is a RAFT agent. In some embodiments, R² is not a RAFT agent. In some embodiments, R² is H or is absent. In some embodiments, R² is an optionally substituted dithiobenzoate, a trithiocarbonate, or a xanthate. In some embodiments, R³ or R¹¹ may be hydrogen, optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl. In some embodiments, R³ is hydrogen, optionally substituted C₆-aryl, or C₁₋₆-alkyl (optionally substituted with halogen, or hydroxyl). In some embodiments, Ar as provided above is phenyl (optionally substituted with one or more OH groups, NH₂ groups, and/or halogens). In some embodiments, R² is one of the functional groups:

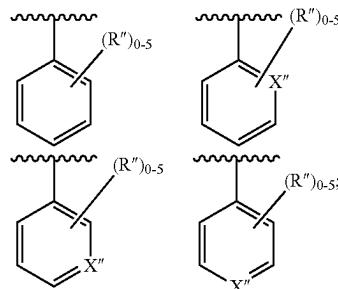


where R³ is as defined above. In several embodiments, Ar in any of functional groups I or II is an optionally substituted C₆-C₁₄ aryl or an optionally substituted heteroaryl having 6 to 14 ring members of which 1-4

I
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II

III
10
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are heteroatoms. In several embodiments, the optionally substituted C₆-C₁₄ aryl is optionally substituted with one or more functional groups selected from C₁-C₆-alkyl, amino, halogen, —OH, or combinations thereof. In several embodiments, Ar is selected from the group consisting of:



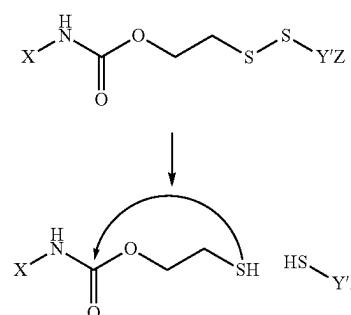
where each instance of R'', when present, is independently selected from an optionally substituted C₁₋₆-alkyl, optionally substituted C₁₋₆-alkoxy, optionally substituted amino, OH, or halogen. In several embodiments, X'' is a heteroatom. In several embodiments, X'' is N. In several embodiments, R¹¹ is C₁₋₆-alkyl. In several embodiments, R¹¹ is —CH₃. In several embodiments, R³ is C₁₋₆-alkyl.

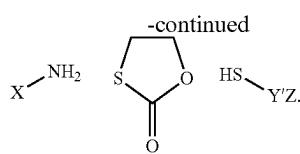
In some embodiments, Y and X are connected via a bond that cleaves or is configured to cleave at a target site for the compound. In several embodiments, Y and X are bonded through a disulfanyl ethyl ester or a disulfide bond. In several embodiments, the bond between Y and X is configured to cleave when the compound of Formula 1 reaches its biological target (e.g., the liver, liver cells, and/or the cytosol of cells in the liver) in a patient. In several embodiments, the Y—X bond cleaves in the presence of a cellular reducing agent (e.g., glutathione). In some embodiments, advantageously, once the bond between X and Y is cleaved, X is left in its native form and/or an active form. In some embodiments, once X is cleaved from Y, it is in a form that is more active than when bound to Y. In some embodiments, di-thiol-containing compounds, particularly disulfanylethyl carbamate-containing links between X and Y (named including a free amine of X, otherwise named a “disulfanyl ethyl ester” without including the free amine of X) are advantageous as having the ability to cleave and release an antigen in its original form once inside a cell, for example as illustrated below (where Y' indicates the remaining portion of the linker and X and Z are as defined)

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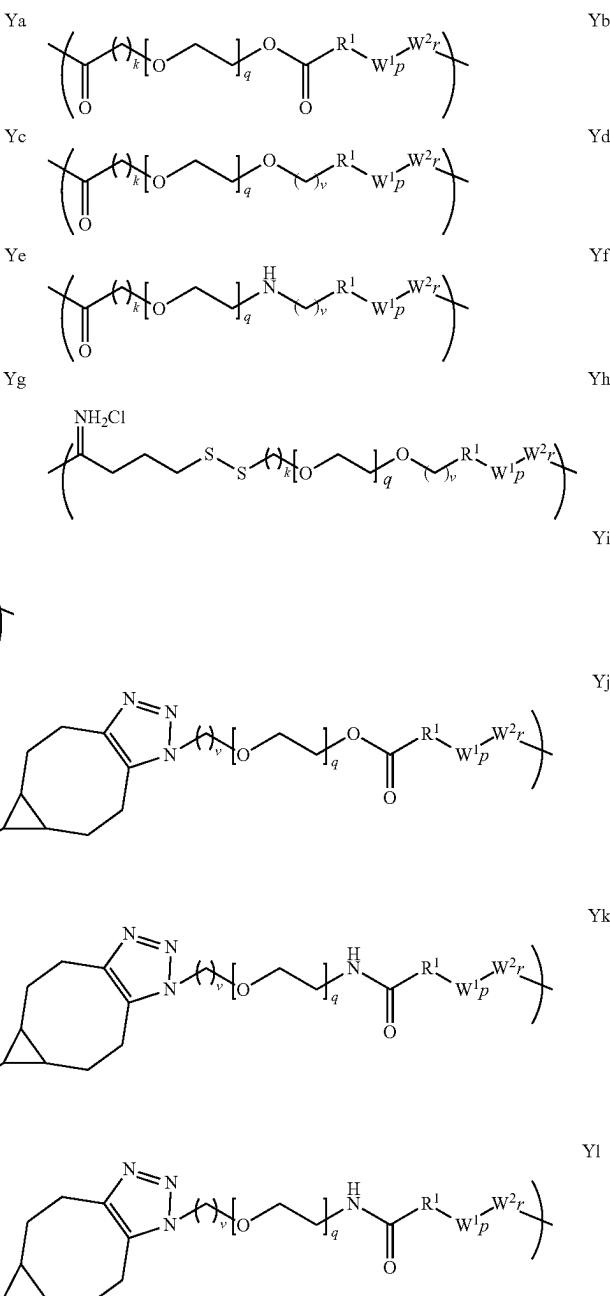
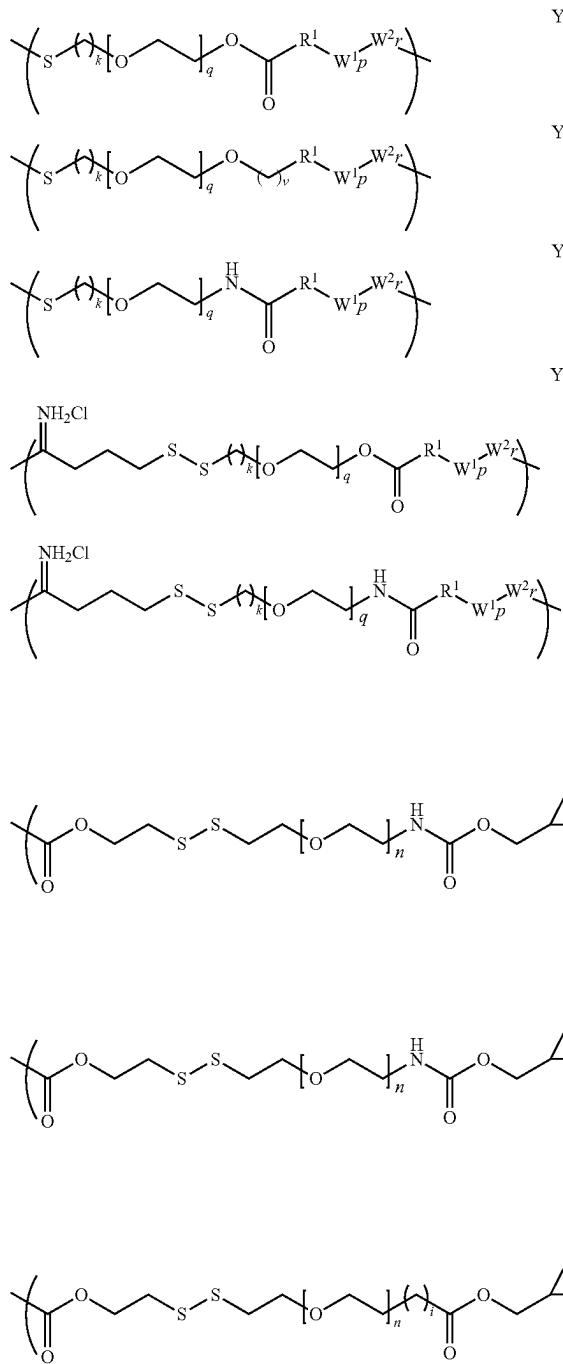
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In several embodiments, the liver targeting moiety of the compound of Formula 1 is not galactose, galactosamine, N-acetylgalactosamine, glucose, glucoseamine and/or N-acetylglucosamine.

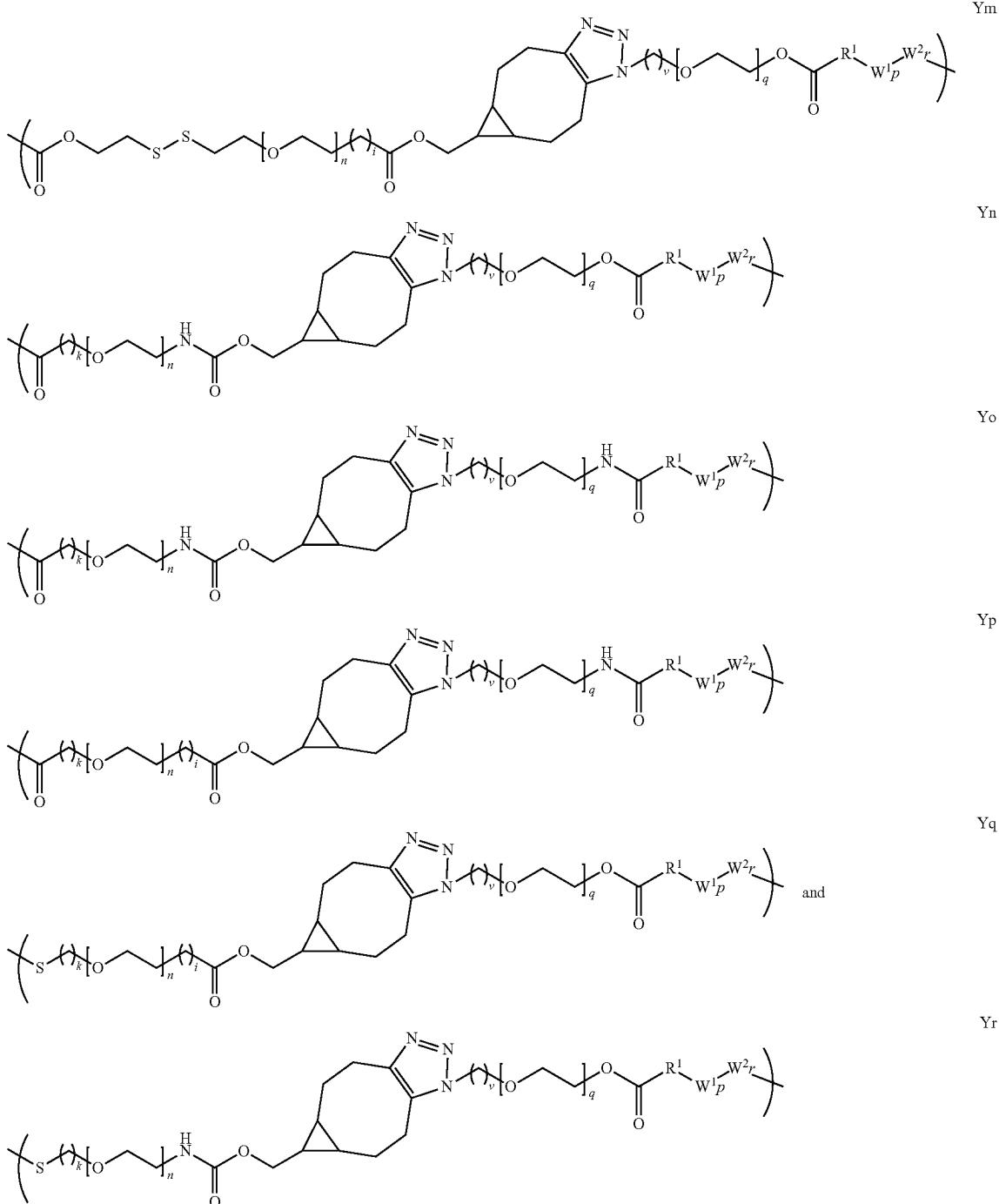
In some embodiments, Y is a linker. In several embodiments, Y is a reaction product resulting from one or more

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reactions involving at least one of the following: N-hydroxysuccinimidyl (NHS) linker, NHS ester linker, PEG linker, maleimide linker, vinylsulfone linker, pyridyl di-thiol-poly(ethylene glycol) linker, pyridyl di-thiol linker, n-nitrophenyl carbonate linker, or a nitrophenoxy poly(ethylene glycol)ester linker. The linker may have one or more mannose moieties or mannose receptor-targeting moieties bound to it. In several embodiments, Y comprises an antibody, an antibody fragment, a peptide, or a disulfanyl ethyl ester to which one or more mannose moieties or mannose receptor-targeting moieties are bound. In some aspects, —[Y(Z)_p]— is a group represented by one of Formula Ya to Yr.



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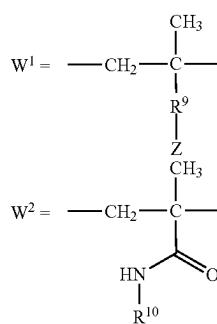


where the left, opening parentheses “(” signifies the location of the bond between X and Y, the right, closing parentheses “)” signifies the location of the bond between Y and R². In several embodiments, n is an integer greater than or equal to about: 1, 10, 20, 40, 50, 75, 100, 150 or ranges including and/or spanning the aforementioned values. In several embodiments, n is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or ranges including and/or spanning the aforementioned values. In several embodiments, q is an integer greater than or equal to about: 1, 10, 20,

40, 50, 75, 100, 150 or ranges including and/or spanning the aforementioned values. In several embodiments, q is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 150, or ranges including and/or spanning the aforementioned values. In several embodiments, k is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, v is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values.

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In several embodiments, k is 2. In several embodiments, v is 2. In several embodiments, n is 4. In several embodiments, n is 44. In several embodiments, q is 3. As used herein, variables disclosed as having structure, a value, or a range of values for one embodiment, may also have those values when the variable is used in another embodiment (even where the variable is not defined with respect to that other embodiment). In several embodiments, n is an integer from 1 to 100. In several embodiments, q is an integer from 1 to 44. In several embodiments, k is an integer from 1 to 12. In several embodiments, i is an integer from 0 to 20. In several embodiments, v is an integer from 1 to 4. In several embodiments, R₁ is —CH₂—, —(CH₂)₂—C(CH₃)(CN)—, —(CH₂)₂—C(CH₃)(CH₃)—, —(CH₂)₂—CH(CH₃)— or —CH(CH₃)—. In several embodiments, W¹ and W² are as depicted below:



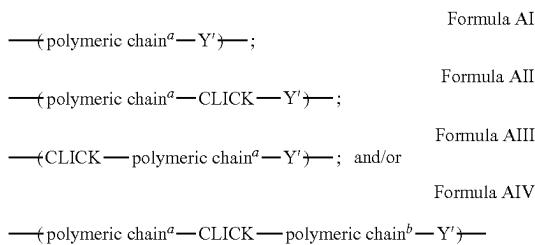
where Z is mannose or a mannose receptor-targeting moiety, R⁹ is a direct bond, —(CH₂)₂—NH—C(O)— (an ethylacetamido group or “EtAcN”) or —(CH₂)₂—(O—CH₂—CH₂)_t—NH—C(O)— (a pegylated ethyl-acetamido group or “Et-PEG_t-AcN”), t is an integer from 1 to 5, p is an integer from 2 to 250, R¹⁰ is an aliphatic group, an alcohol, an aliphatic amine-containing group, or an aliphatic alcohol, and r is an integer from 0 to 250. In several embodiments, —W¹_p—W²_r— (e.g., as provided in —[Y(Z)_p]— or in linker structures) is a random copolymer or block copolymer of W₁ and W₂. In several embodiments, the number of repeat units of W¹ is denoted as p and wherein p is an integer of at least about 1. In several embodiments, the number of repeat units of W² is denoted as r and wherein r is an integer of at least about 1. In some embodiments, R¹⁰ is a C_falkyl or C_falkyloH_g, where f represents the number of carbons in the alkyl group and is an integer between 0 and 10, and g represents the number of hydroxyl groups present on the alkyl group and is an integer between 0 and 10. In some embodiments, R¹⁰ is 2-hydroxyethyl. In some aspects —W¹_p—W²_q— represents a block copolymer or a random copolymer of W¹ and W² monomers.

In several embodiments, the linker comprises a polymeric chain with pendant liver targeting moieties decorating the polymeric chain. In some embodiments, the polymeric chain (or Y) comprises Y' as disclosed elsewhere herein. In several embodiments, the polymeric chain comprises an acrylate portion (e.g., acrylate-based polymers and/or acrylate-based copolymers). In several embodiments, the acrylate portion comprises one or more acrylate units (e.g., acrylate derivatives, including methacrylates and derivatives thereof) comprising a pendant liver targeting agent. In several embodi-

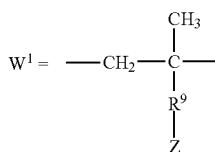
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ments, the polymeric chain comprises a hydrophilic portion and/or region. In several embodiments, the hydrophilic portion comprises a length of one or more regions having —(CH₂CH₂O)_s— where s is an integer from about 1 to about 44. In several embodiments, s is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 150, or ranges including and/or spanning the aforementioned values. In some embodiments, the hydrophilic portion comprises one or more polyethylene glycol (PEG) regions. In some embodiments, the PEG may have polydispersity as measured by the weight average molecular weight in g/mol (M_w) of the PEG divided by the number average molecular weight in g/mol (M_n) of the PEG (e.g., M_w/M_n). In some embodiments, the PEG chains have a number average or weight average molecular weight (g/mol) of equal to or at least about: 500, 1000, 2000, 5000, 10000, or ranges including and/or spanning the aforementioned values. In several embodiments, the polymeric chain is optionally substituted. In some embodiments, the polymeric chain comprises pendant hydrophilic groups such as a —OH, —SO(OH)₂, optionally substituted polyether, optionally substituted polyamino, and the like.

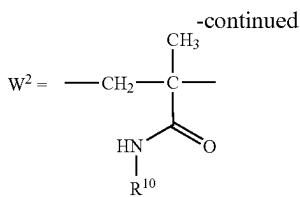
In several embodiments, the antigen and liver targeting portion of the compound are joined using click chemistry, for example, by functionalizing the antigen with a first linker arm comprising an alkynyl group (or an azide), functionalizing the liver targeting moiety with a second linker arm comprising an azide (or an alkynyl group), and clicking them together via “click” chemistry. In some embodiments, an alkynyl group that can be clicked in copper-free conditions is used. In some embodiments, —[Y(—Z)_p]— is a group represented by one or more of Formulae AI-AIV:



where the left, opening parentheses “(” signifies the location of the bond between X and Y, the right, closing parentheses “)” signifies the location of the bond between Y and R², Y' is a random copolymer or block copolymer of two or more different types of repeat units, wherein at least one type of repeat unit comprises a pendant Z group, (or plurality of pendant Z groups) where Z is mannose and/or a mannose receptor-targeting moiety. In some embodiments, Y' is a random copolymer or block copolymer of W¹ and W², where W¹ and W² are as depicted below:



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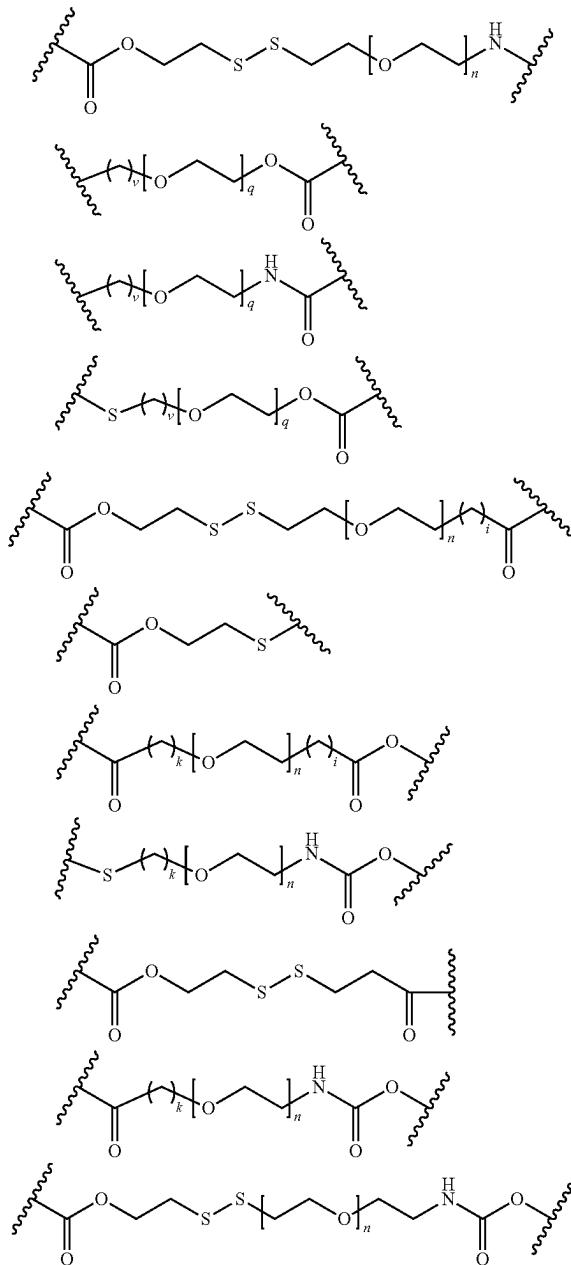
where Z is mannose and/or a mannose receptor-targeting moiety (including, but not limited to, one or more of mannosamine, N-acetylmannosamine, or N-acetylglucosamine), R⁹ is a direct bond, optionally substituted —C(O)—NH—(CH₂)₂— (an ethylacetamido group or “EtAcN”) or optionally substituted —C(O)—NH—(CH₂)₂—(O—CH₂—CH₂)_t— (a pegylated ethylacetamido group or “Et-PEG_t-AcN”), t is an integer from 1 to 5. In some embodiments, t is an integer of equal to or at least about: 1, 2, 3, 4, 5, 10, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, R¹⁰ is an aliphatic group, an alcohol, an aliphatic amine-containing group, or an aliphatic alcohol. In some embodiments, R⁹ or R¹⁰ are independently optionally substituted alkyl, an optionally substituted polyether, or optionally substituted polyamino. In some embodiments, R¹⁰ is an optionally substituted C_falkyl, optionally substituted C_falkylOH_g, or an optionally substituted —(C_falkylOH_g)—O_e—H where f represents the number of carbons in the alkyl group and is an integer between 0 and 10, g represents the number of hydroxyl groups present on the alkyl group and is an integer between 0 and 10, and e represents the number of alkyl/ether repeat units and is an integer between 0 and 10. In some embodiments, e, f, and g are independently selected integers of equal to or at least about: 0, 1, 2, 3, 4, 5, 10, or ranges including and/or spanning the aforementioned values. In some embodiments, R¹⁰ is a 2-hydroxyethyl (e.g., —CH₂CH₂OH). In some embodiments, R¹⁰ is an optionally substituted 2-hydroxyethyl. In some embodiments, R¹⁰ is an optionally substituted polyether.

In some embodiments, Y' is represented as —W¹_p-W²_r—. As noted elsewhere herein, —W¹_p-W²_r— may represent a block copolymer or a random copolymer of W¹ and W² monomers having p repeat units of W¹ and r repeat units of W². In some embodiments, p is an integer equal to or greater than about: 1, 50, 85, 100, 150, 165, 200, 225, 250, 300, 400, or ranges including and/or spanning the aforementioned values. In some embodiments, r is an integer equal to or greater than about: 1, 50, 85, 100, 150, 165, 200, 225, 250, 300, 400, or ranges including and/or spanning the aforementioned values. In some embodiments, Y' is a homopolymer of W¹ or W². In some embodiments, r is 0. In some embodiments, the sum of p and r is an integer equal to or greater than about: 1, 50, 85, 100, 150, 165, 170, 200, 225, 250, 300, 400, 600, 800, or ranges including and/or spanning the aforementioned values.

In some embodiments, polymeric chain^a and polymeric chain^b are present or optionally not present. In some embodiments, where present, polymeric chain^a and polymeric chain^b can independently comprise hydrophilic polymers. In some embodiments, where present, polymeric chain^a and polymeric chain^b can independently comprise one or more optionally substituted —(CH₂CH₂O)_s—, optionally substituted —(CH₂)_u—, or optionally substituted alkylene. In several embodiments, u is an integer less than or equal to about: 1, 5, 10, 20, or ranges including and/or spanning the

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aforementioned values. In some embodiments, polymeric chain^a and polymeric chain^b comprise or consist of one or more of the following structures, or a portion thereof:



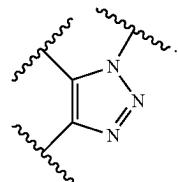
wherein the variables (e.g., i, k, n, q, v, etc.) are as disclosed elsewhere herein. In several embodiments, for example, n is an integer from about 1 to about 100, q is an integer from about 1 to about 100, k is an integer from about 1 to about 20, i is an integer from about 0 to about 20, and v is an integer from about 1 to about 20. In several embodiments, n or q represents the number of repeat units in a PEG chain. In some embodiments, the PEG chain may have some polydispersity. In some embodiments, n and q do not indicate a number of repeat units but instead independently indicate the presence of a PEG polymer chain having a Mn (in g/mol) or Mw (in g/mol) of equal to or at least

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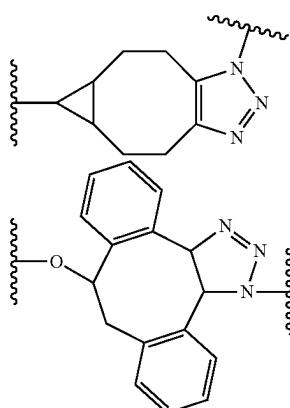
about 500, 1000, 2000, 5000, 10000, or ranges including and/or spanning the aforementioned values. In some embodiments, k, i, and v can each independently comprise an optionally substituted alkylene.

In several embodiments, n is an integer greater than or equal to about: 1, 10, 20, 40, 50, 75, 100, 150 or ranges including and/or spanning the aforementioned values. In several embodiments, n is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or ranges including and/or spanning the aforementioned values. In several embodiments, q is an integer greater than or equal to about: 1, 10, 20, 40, 50, 75, 100, 150 or ranges including and/or spanning the aforementioned values. In several embodiments, q is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, k is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, v is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, k is 2. In several embodiments, v is 2. In several embodiments, n is 4. In several embodiments, n is 44. In several embodiments, q is 3. As used herein, variables disclosed as having structure, a value, or a range of values for one embodiment, may also have those values when the variable is used in another embodiment (even where the variable is not defined with respect to that other embodiment).

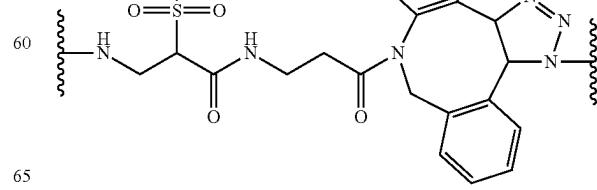
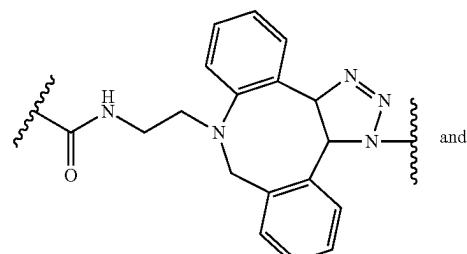
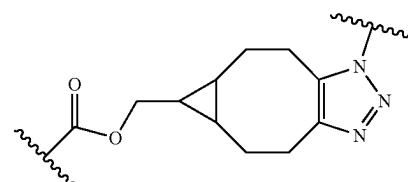
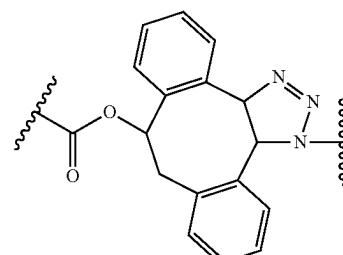
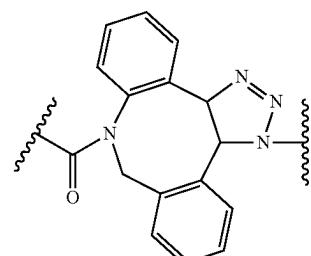
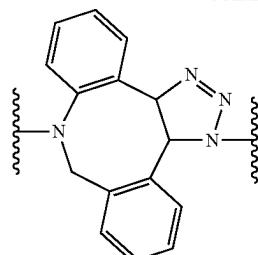
In several embodiments, the "CLICK" group and/or $[-Y(-Z)_p]$, more generally, comprises the following functional unit:



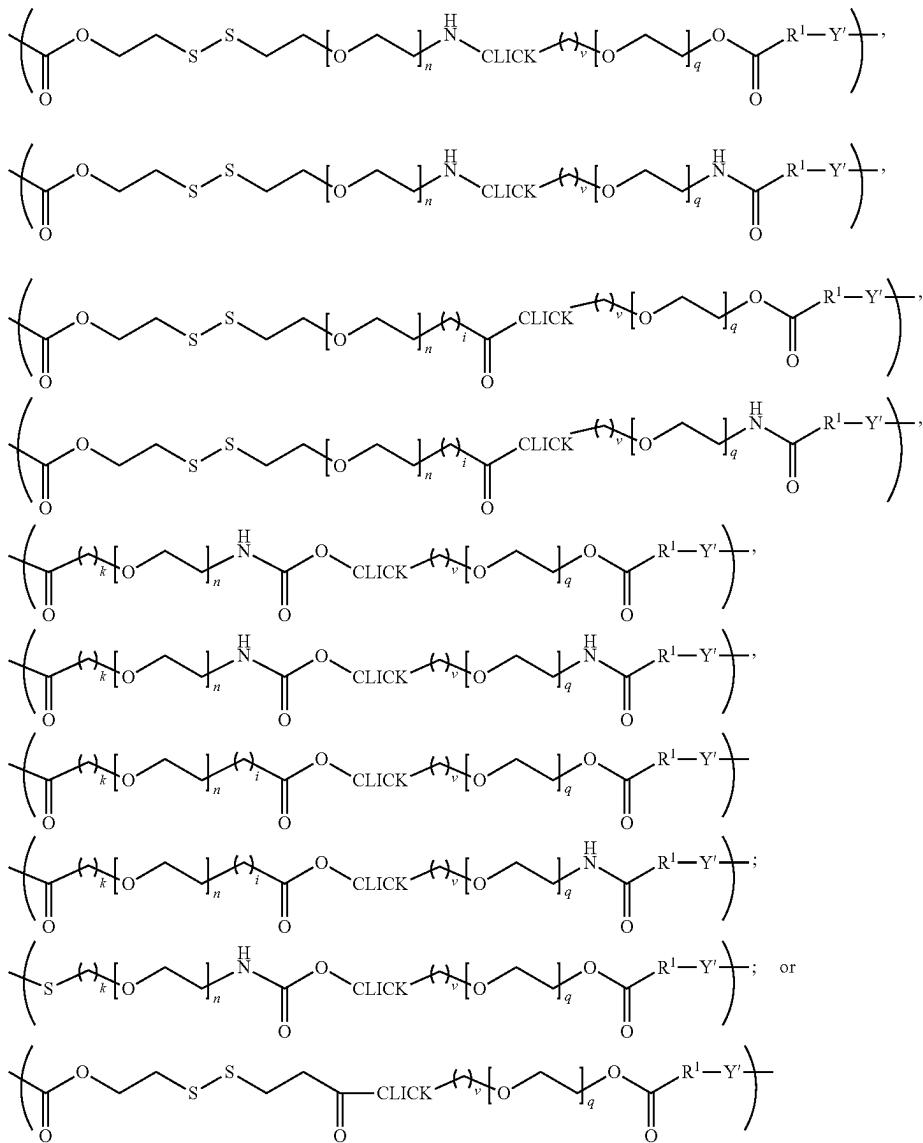
In several embodiments, the "CLICK" group and/or $[-Y(-Z)_p]$, more generally, comprises one or more of the following units (each of which may be optionally substituted):

**20**

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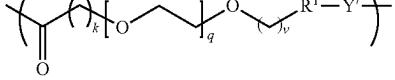
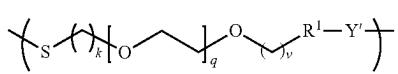
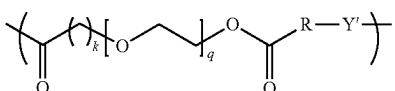
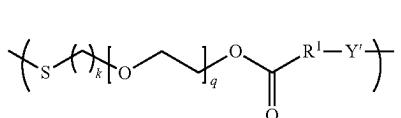
In several embodiments, —[Y(—Z)p]- comprises the one or more of the following functional units:



wherein each variable (e.g., i, k, n, q, v, CLICK, R¹, Y¹, 50 etc.) is as disclosed elsewhere herein. In some embodiments, for example, n is an integer from about 1 to about 44, q is an integer from about 1 to about 44, k is an integer from about 1 to about 12, i is an integer from about 0 to about 20, v is an integer from about 1 to

about 4, and R_1 is $-\text{CH}_2-$, $-(\text{CH}_2)_2-\text{C}(\text{CH}_3)$, $(\text{CN})-$, $-(\text{CH}_2)_2-\text{C}(\text{CH}_3)(\text{CH}_3)-$, $-(\text{CH}_2)_2-\text{CH}(\text{CH}_3)-$, $-\text{CH}(\text{CH}_3)-$, or is absent.

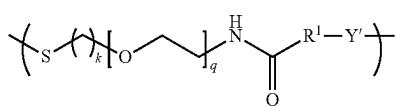
In several embodiments, —[Y(Z)_p]— is a group represented by any one or more of Formula Ya' to Yr':



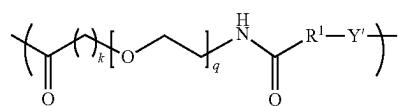
23**24**

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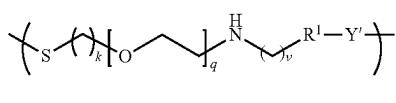
Ye'

**24**

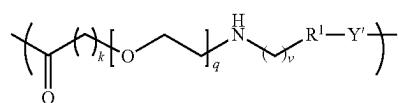
Ye"



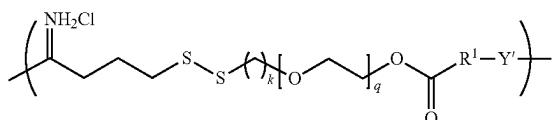
Yf'



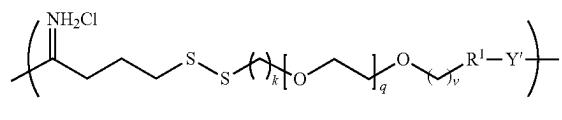
Yf"



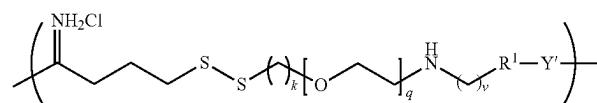
Yg'



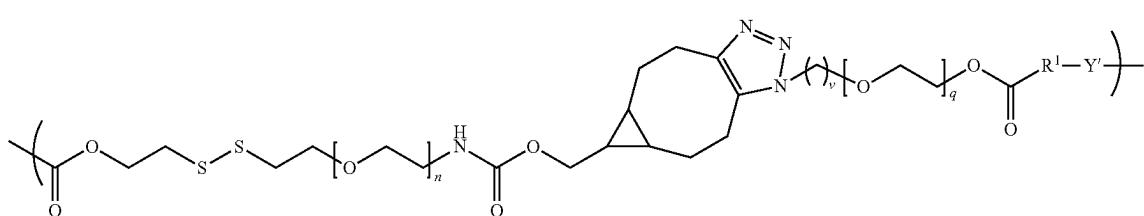
Yh'



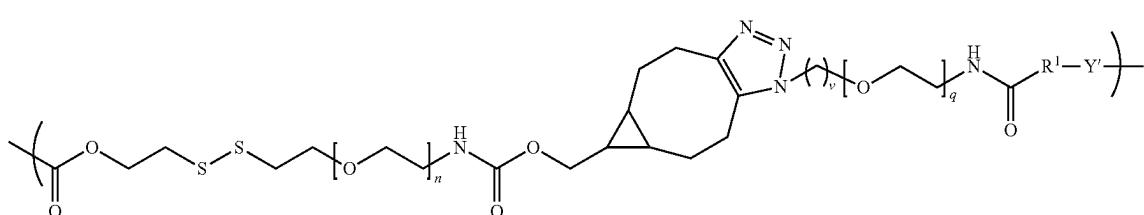
Yi'



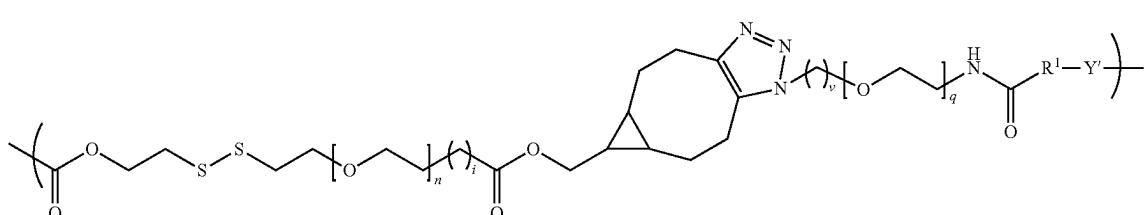
Yj'



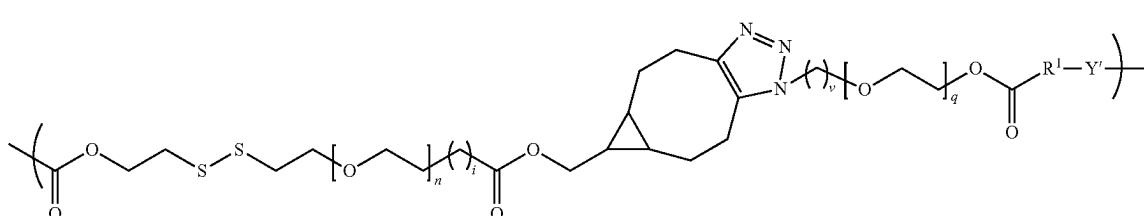
Yk'



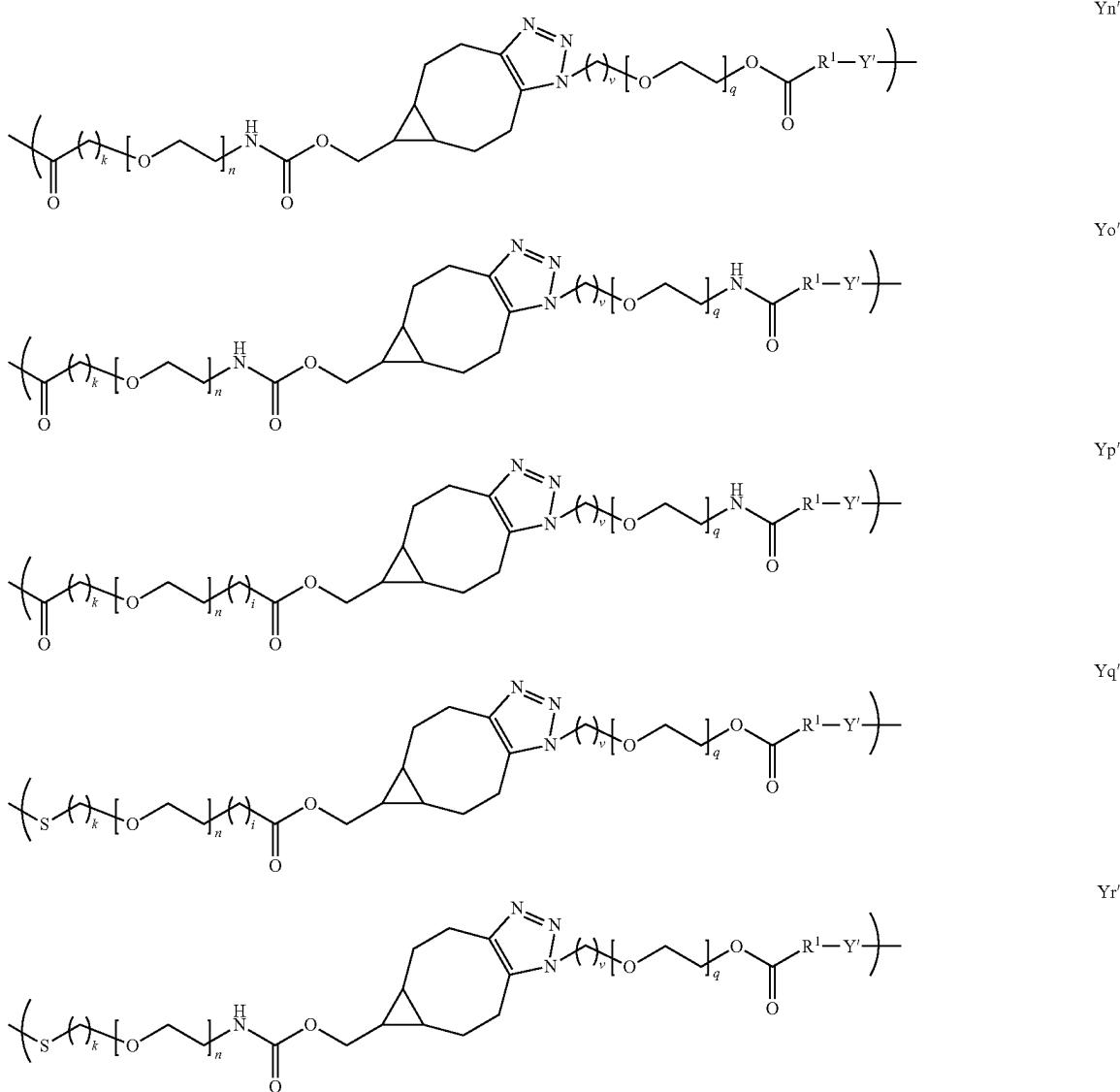
Yl'



Ym'



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wherein the variables (e.g., i, k, n, q, v, R¹, Y', etc.) are as disclosed elsewhere herein. For example, in several embodiments, n is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or ranges including and/or spanning the aforementioned values. In several embodiments, q is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 150, or ranges including and/or spanning the aforementioned values. In several embodiments, k is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, v is an integer greater than or equal to about: 1, 2, 3, 4, 5, 10, 15, 20, or ranges including and/or spanning the aforementioned values. In several embodiments, k is 2. In several embodiments, n is 4. In several embodiments, n is 43 or 44. In several embodiments, q is 3. In several embodiments, R₁ is —CH₂—, —(CH₂)₂—C(CH₃)(CN)—, —(CH₂)₂—C(CH₃)(CH₃)—, —(CH₂)₂—CH(CH₃)— or —CH

(CH₃)—. In some embodiments, Y' is a random copolymer or block copolymer of W¹ and W² having p repeat units of W¹ and r repeat units of W².

In several embodiments, as shown elsewhere herein, the targeting portion comprises one or more pendant liver targeting moieties decorating a portion of the linker. In several embodiments, the portion of the linker is a polymeric chain with pendant targeting agents attached randomly or in blocks along the chain. In some embodiments, the polymeric chain comprises an acrylate portion (e.g., acrylate polymers and/or acrylate copolymers). In several embodiments, the acrylate portion comprises an acrylate unit comprising a pendant liver targeting agent. In several embodiments, the acrylate portion further comprises an acrylate unit not comprising a pendant liver targeting agent.

In some embodiments, Y is a linker resulting from one or more reactions involving at least one of the following: N-hydroxysuccinimydyl (NHS) linker, NHS ester linker, PEG linker, maleimide linker, vinylsulfone linker, pyridyl di-thiol-poly(ethylene glycol) linker, pyridyl di-thiol linker,

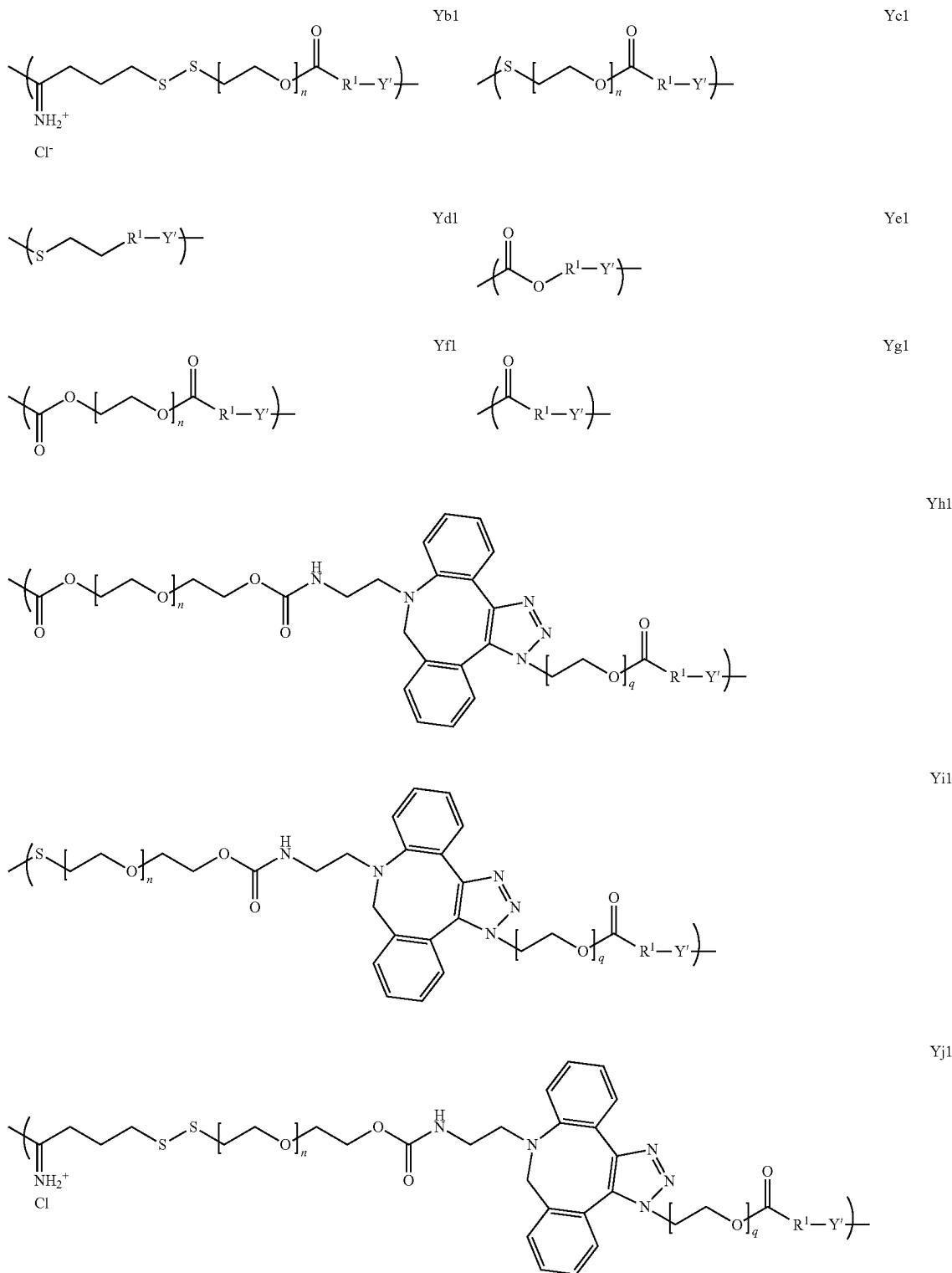
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n-nitrophenyl carbonate linker, or a nitrophenoxy poly(ethylene glycol)ester linker. The linker may have one or more mannose and/or a mannose receptor-targeting moieties (including, but not limited to, one or more of mannosamine, N-acetylmannosamine, or N-acetylglucosamine) bound to it. In embodiments, Y comprises an antibody, an antibody fragment, a peptide, or a disulfanyl ethyl ester to which one

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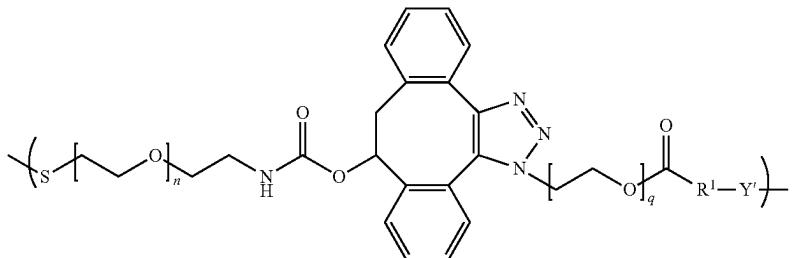
or more mannose and/or mannose receptor-targeting moieties (including, but not limited to, one or more of mannosamine, N-acetylmannosamine, or N-acetylglucosamine) are bound.

In some embodiments, $-[Y(-Z)_p]-$ comprises one of the following structures:

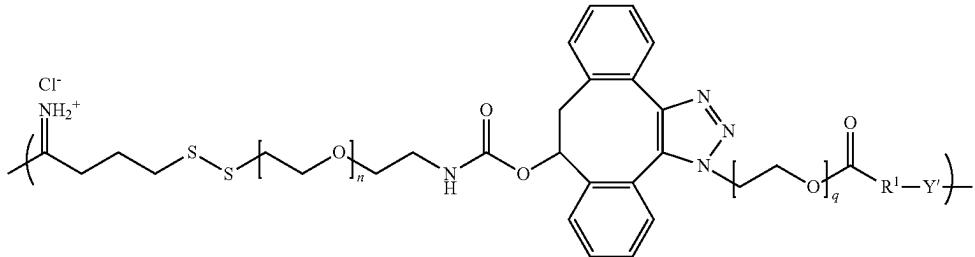


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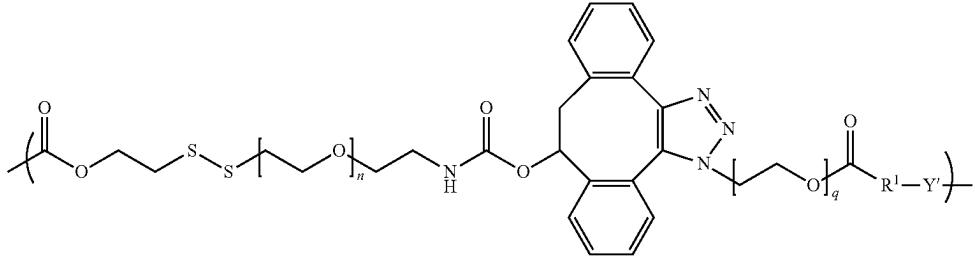
Yk1



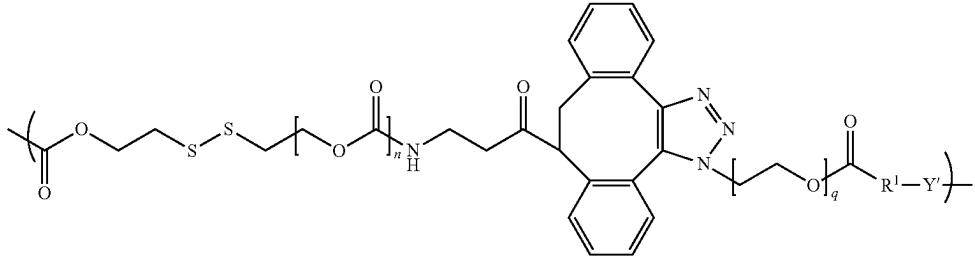
YL1



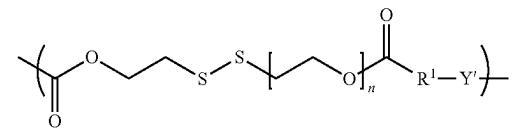
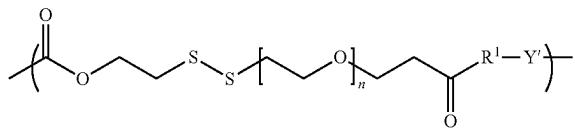
Ym1



Yn1



Yp1



where the variables are as disclosed elsewhere herein.

In some embodiments, other linker structures can be found in U.S. Application Publication Nos. U.S. 2017/0007708A1 and 2016/0243248A1 and International Publication No. WO 2017/046652, each of which is incorporated by reference in its entirety.

In several embodiments, various ratios of W^1 to W^2 are used (e.g., W^1 and W^2 as provided in any of the Formulae disclosed elsewhere herein). In some embodiments, a majority of Y' repeat units comprise W^1 . In some embodiments, the ratio of W^1 to W^2 is equal to or greater than about 50:1, about 25:1, about 10:1, about 5:1, about 4:1, about 2:1, about 1:1, about 1:2, about 1:4, about 1:5, about 1:10, about 1:25, about 1:50, and any ratio in between those listed, including endpoints.

55 In some embodiments, the ratio of p to r is equal to or greater than about 50:1, about 25:1, about 10:1, about 5:1, about 4:1, about 2:1, about 1:1, about 1:2, about 1:4, about 1:5, about 1:10, about 1:25, about 1:50, and any ratio in between those listed, including endpoints. In some embodiments, a homopolymer of W^1 is provided without a W^2 portion.

X may be a foreign transplant antigen, or alloantigen, or autoimmune antigen. In some aspects, X represents an antigen against which a patient may develop or has developed an unwanted immune response. In some embodiments, X is an antigen against which a subject, such as a transplant recipient or autoimmune patient, develops an unwanted immune response. In several embodiments, X is a foreign

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extracellular vesicle, cell fragment, or cell containing alloantigens against which transplant recipients or autoimmune patients develop an unwanted immune response. In still further embodiments, X is a foreign food, animal, plant or environmental antigen against which patients develop an unwanted immune response. In certain aspects, X is a foreign therapeutic agent against which patients develop an unwanted immune response. In a further aspect, X is a synthetic self-antigen to which patients develop an unwanted immune response. In several embodiments, X is a tolerogenic portion of a larger antigen. In certain embodiments, X, or a portion of X, is, is at least, or is at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, or 500 amino acids in length (or any range derivable therein). In some embodiments, X is an asparaginase antigen or an ovalbumin antigen. In several embodiments, X is an immunogenic fragment of one or more of myelin basic protein, myelin oligodendrocyte glycoprotein, proteolipid protein, insulin, proinsulin, preproinsulin, high molecular weight glutenin, low molecular weight glutenin, alpha- or gamma-gliadin, hordein, secalin, or avenin.

In some embodiments, Z, the moiety that specifically targets a mannose receptor, is selected from the group consisting of α -linked mannose, β -linked mannose, substituted mannose, mannose-6-phosphate, N-acetyl mannosamine, and mannose having $\beta(1-4)$, $\alpha(1-6)$, $\alpha(1-2)$, and/or $\alpha(1-3)$ linkages. In some aspects, the mannose receptor is the mannose-6-phosphate receptor.

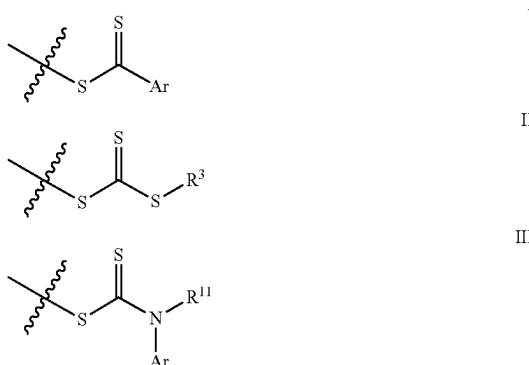
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Some aspects of the disclosure are directed towards the use of a composition as disclosed herein in any method disclosed herein. Some embodiment provide for the use of any composition disclosed herein for the induction of tolerance to the antigen or a tolerogenic portion thereof. It is specifically contemplated that any step or element of an embodiment may be implemented in the context of any other step(s) or element(s) of a different embodiment disclosed herein.

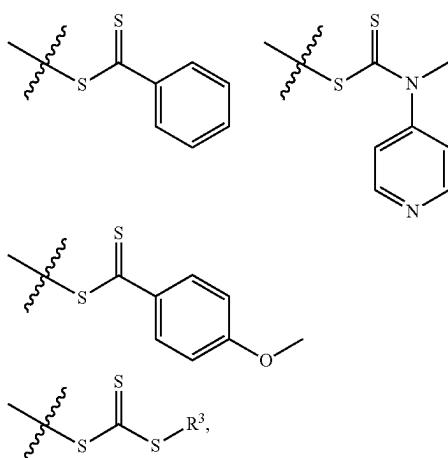
In some embodiments, a method of inducing immunological tolerance to an antigen target is provided. The method comprises administering a composition of Formula 1:



where the variables are as disclosed elsewhere herein. In some embodiments, for example, X comprises an antigen or a tolerogenic portion thereof, Y comprises a linker moiety, Z comprises a moiety that specifically targets a mannose receptor, p is an integer from 2 to 250, m is an integer from 1 to 100, R² is any of functional groups I-III:



where Ar is a substituted or unsubstituted aromatic group, R³ is any carbon-containing linear or heterocyclic moiety, and R¹¹ is hydrogen or an alkyl group. In some embodiments, R² is one of the functional groups:

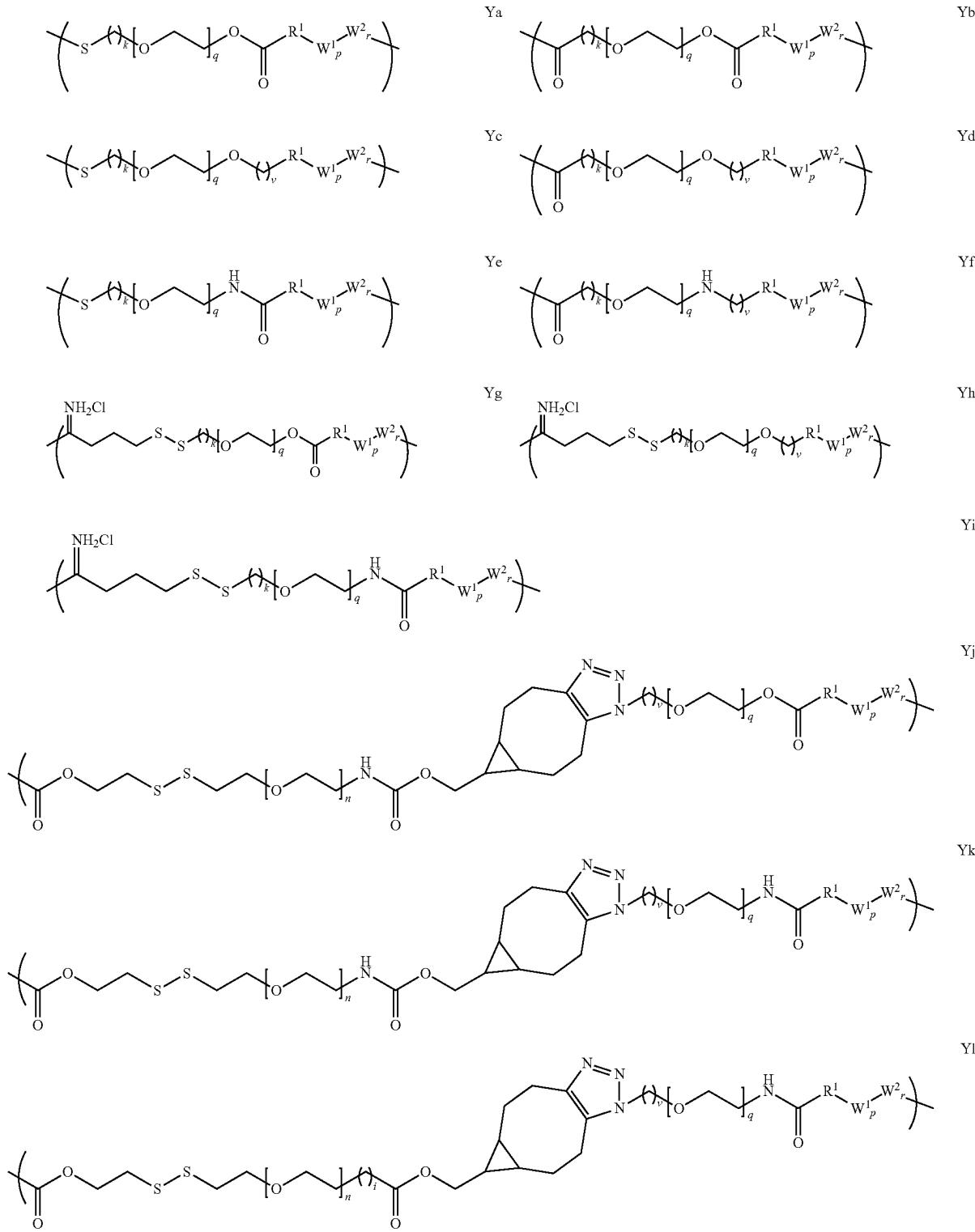


where R³ is as defined above.

In some embodiments, Y is a linker as described elsewhere herein. In several embodiments, Y is a linker resulting

from one or more reactions involving at least one of the following N-hydroxysuccinimidyl (NHS) linker, NHS ester linker, PEG linker, maleimide linker, vinylsulfone linker, pyridyl di-thiol-poly(ethylene glycol) linker, pyridyl di-thiol linker, n-nitrophenyl carbonate linker, or a nitrophenoxy poly(ethylene glycol)ester linker. The linker may have one or more mannose moieties or mannose receptor-targeting

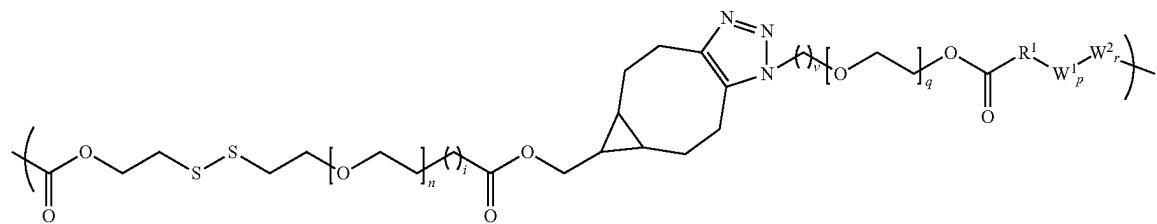
moieties bound to it. In embodiments, Y comprises an antibody, an antibody fragment, a peptide, or a disulfanyl ethyl ester to which one or more mannose moieties or mannose receptor-targeting moieties are bound. In several embodiments, $—[Y(—Z)_p]—$ is as disclosed elsewhere herein. For example, in some aspects, $—[Y(Z)_p]—$ is a group represented by one of sequence Formula Ya to Yr:



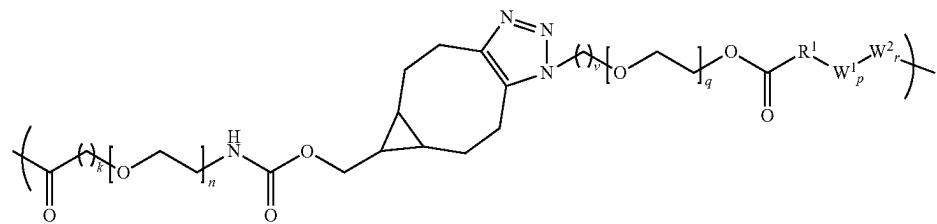
35**36**

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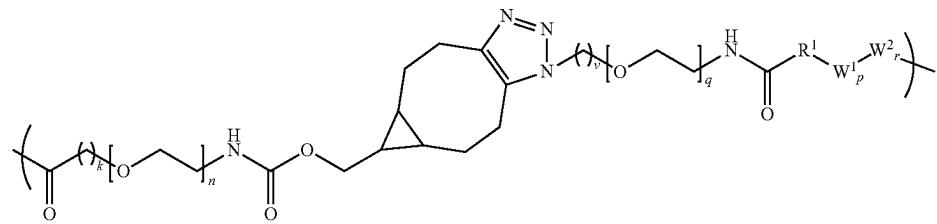
Ym



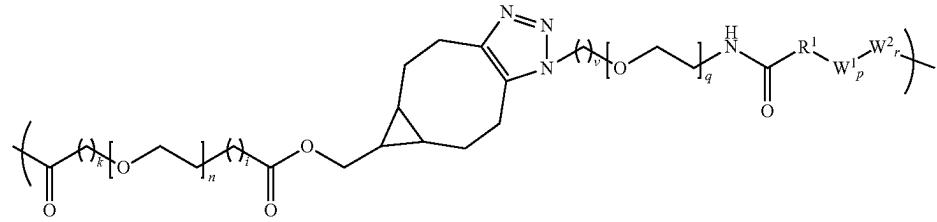
Yn



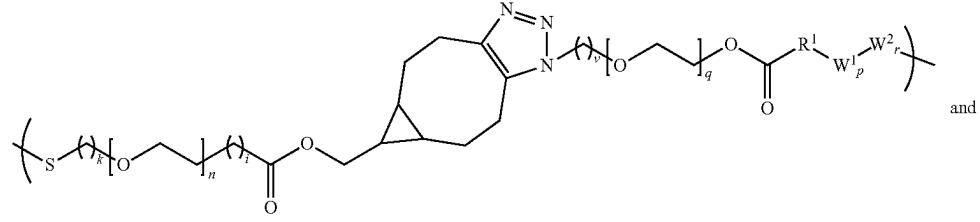
Yo



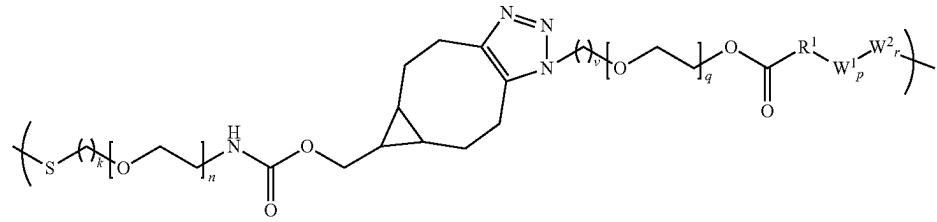
Yp



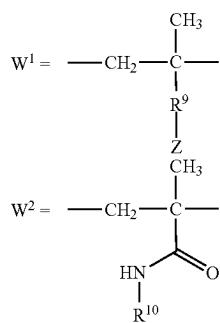
Yq



Yr



where the left, opening parentheses “(” signifies the location of the bond between X and Y, the right, closing parentheses “)” signifies the location of the bond between Y and R², n is an integer from 1 to 100, q is an integer from 1 to 44, k is an integer from 1 to 12, i is an integer from 0 to 20, v is an integer from 1 to 4, R₁ is —CH₂—, —(CH₂)₂—C(CH₃)(CN)—, —(CH₂)₂—C(CH₃)(CH₃)—, —(CH₂)₂—CH(CH₃)— or —CH(CH₃)—, W¹ and W² are as depicted below:



where Z is mannose or a mannose receptor-targeting moiety, R⁹ is a direct bond, —(CH₂)₂—NH—C(O)— (an ethylacetamido group or “EtAcN”) or —(CH₂)₂—(O—CH₂—CH₂)_t—NH—C(O)— (a pegylated ethylacetamido group or “Et-PEG_t-AcN”), t is an integer from 1 to 5, 1 to 3, or 1 or 2, p is an integer from 2 to 250, R¹⁰ is an aliphatic group, an alcohol, an aliphatic amine-containing group, or an aliphatic alcohol, and r is an integer from 0 to 250. In some embodiments, R¹⁰ is a C_falkyl or C_falkyloH_g, where f represents the number of carbons in the alkyl group and is an integer between 0 and 10, and g represents the number of hydroxyl groups present on the alkyl group and is an integer between 0 and 10. In some embodiments, R¹⁰ is 2-hydroxyethyl. In some aspects —W¹_p—W²_q— represents a block copolymer or a random copolymer of W¹ and W² monomers.

X may be a foreign transplant antigen, or alloantigen, or autoimmune antigen. In some aspects, X represents an antigen against which a patient may develop or has developed an unwanted immune response. In some embodiments, X can be an antigen against which a subject, such as a transplant recipient or autoimmune patient, develops an unwanted immune response. In several embodiments, X can be a foreign extracellular vesicle, cell fragment, or cell containing alloantigens against which transplant recipients or autoimmune patients develop and unwanted immune response. In still further embodiments, X can be a foreign food, animal, plant or environmental antigen against which patients develop an unwanted immune response. In certain aspects X can be a foreign therapeutic agent against which patients develop an unwanted immune response. In a further aspect X can be a synthetic self-antigen to which patients develop an unwanted immune response. In several embodiments, X can be a tolerogenic portion of a larger antigen. In certain embodiments, X, or a portion of X, is at least, or is at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98,

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that any embodiment discussed herein can be implemented with respect to any method or composition, and vice versa. Furthermore, compositions and kits can be used to achieve methods disclosed herein.

The term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. The phrase "consisting of" excludes any element, step, or ingredient not specified. The phrase "consisting essentially of" limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments described in the context of the term "comprising" may also be implemented in the context of the term "consisting of" or "consisting essentially of."

The terms "effective amount" or "therapeutically effective amount" refer to that amount of a composition of the disclosure that is sufficient to effect treatment, as defined herein, when administered to a mammal in need of such treatment. This amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the particular composition of the disclosure chosen, the dosing regimen to be followed, timing of administration, manner of administration and the like, all of which can readily be determined by one of ordinary skill in the art.

The "numerical values" and "ranges" provided for the various substituents are intended to encompass all integers within the recited range. For example, when defining n as an integer representing a mixture including from 1 to 100, where the mixture typically encompasses the integer specified as $n \pm 10\%$ (or for smaller integers from 1 to about 25, ± 3), it should be understood that n can be an integer from 1 to 100 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 25, 30, 34, 35, 37, 40, 41, 45, 50, 54, 55, 59, 60, 65, 70, 75, 80, 82, 83, 85, 88, 90, 95, 99, 100, or any between those listed). The terms " $\pm 10\%$ " or " ± 3 " should be understood to disclose and provide specific support for equivalent ranges wherever used.

The term "optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not.

A peptide, protein, or fragment that specifically binds a particular target is referred to as a "ligand" for that target.

A "polypeptide" is a term that refers to a chain of amino acid residues, regardless of post-translational modification (e.g., phosphorylation or glycosylation) and/or complexation with additional polypeptides, and/or synthesis into multisubunit complexes with nucleic acids and/or carbohydrates, or other molecules. Proteoglycans therefore also are referred to herein as polypeptides. A long polypeptide (having over 50 amino acids) is referred to as a "protein." A short polypeptide (having 50 amino acids or fewer) is referred to as a "peptide." Depending upon size, amino acid composition and three dimensional structure, certain polypeptides can be referred to as an "antigen-binding molecule," "antibody," an "antibody fragment" or a "ligand." Polypeptides can be produced by a number of methods, many of which are well known in the art. For example, polypeptides can be obtained by extraction (e.g., from isolated cells), by expression of a recombinant nucleic acid encoding the polypeptide, or by chemical synthesis. Polypeptides can be produced by, for example, recombinant technology, and expression vec-

tors encoding the polypeptide introduced into host cells (e.g., by transformation or transfection) for expression of the encoded polypeptide.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. In several embodiments, these media and agents can be used in combination with pharmaceutically active substances. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "purified" as used herein with reference to a polypeptide refers to a polypeptide that has been chemically or biologically synthesized and is thus substantially uncontaminated by other polypeptides, or has been separated or isolated from most other cellular components by which it is naturally accompanied (e.g., other cellular proteins, nucleic acids, or cellular components such as lipid membrane). An example of a purified polypeptide is one that is at least 70%, by dry weight, free from the proteins and naturally occurring organic molecules with which it naturally associates. A preparation of a purified polypeptide therefore can be, for example, at least 80%, at least 90%, or at least 99%, by dry weight, the polypeptide. Polypeptides also can be engineered to contain a tag sequence (e.g., a polyhistidine tag, a myc tag, a FLAG® tag, a SNAP® tag, or other affinity tag) that facilitates purification or marking (e.g., capture onto an affinity matrix, visualization under a microscope). Thus a purified composition that comprises a polypeptide refers to a purified polypeptide unless otherwise indicated. The term "isolated" indicates that the polypeptides or nucleic acids of the disclosure are not in their natural environment. Isolated products of the disclosure can thus be contained in a culture supernatant, partially enriched, produced from heterologous sources, cloned in a vector or formulated with a vehicle, etc.

The term "sequence identity" is used with regard to polypeptide or polynucleotide sequence comparisons. This expression in particular refers to a percentage of sequence identity, for example at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide. Particularly, the polypeptide in question and the reference polypeptide exhibit the indicated sequence identity over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids or over the entire length of the reference polypeptide. In several embodiments, despite differing sequences from a reference nucleotide (or corresponding polypeptide) a polynucleotide exhibits at least some degree of functional equivalence to the reference sequence, and in some embodiments, enhanced function.

The term "treatment" or "treating" means any treatment of a disease or disorder in a mammal, including: preventing or protecting against the disease or disorder, that is, causing the clinical symptoms not to develop; inhibiting the disease or disorder, that is, arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder, that is, causing the regression of clinical symptoms.

The term "unwanted immune response" refers to a reaction by the immune system of a subject, which in the given situation is not desirable. The reaction of the immune system is unwanted if such reaction does not lead to the prevention,

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reduction, or healing of a disease or disorder but instead causes, enhances or worsens a disorder or disease. Typically, a reaction of the immune system causes, enhances or worsens a disease if it is directed against an inappropriate target. Exemplified, an unwanted immune response includes but is not limited to transplant rejection, immune response against a therapeutic agent, autoimmune disease, and allergy or hypersensitivity.

The term “operatively linked” refers to a situation where two components are combined to form the active complex prior to binding at the target site. For example, a molecule conjugated to one-half of a biotin-streptavidin complex and an antigen complexed to the other one-half of the biotin-streptavidin complex are operatively linked through complementation of the biotin and streptavidin molecules. The term operatively linked is also intended to refer to covalent or chemical linkages that conjugate two molecules together.

Throughout this application, the term “about” is used according to its plain and ordinary meaning in the area of protein chemistry to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

Methods may involve multiple administrations of one or more compounds, compositions, and/or agents. In certain embodiments, cells or a subject are provided with a tolerance inducing agent prior to administering the composition for which a tolerance is being induced. It is contemplated that compounds, compositions, and/or agents may be formulated in a pharmaceutically acceptable formulation in certain embodiments of the invention.

A “disease” is defined as a pathological condition of a body part, an organ, or a system resulting from any cause, such as infection, genetic defect, or environmental stress. A “health-related condition” is defined herein to refer to a condition of a body part, an organ, or a system that may not be pathological, but for which treatment is sought. Examples include conditions for which cosmetic therapy is sought, such as skin wrinkling, skin blemishes, and the like. The disease can be any disease, and non-limiting examples include hyperproliferative diseases such as cancer and pre-malignant lesions, wounds, and infections.

A subject may be “predicted to have” a disease if the subject exhibits a characteristic, condition, or behavior that increases the likelihood of getting a disease. The characteristic, condition, or behavior that increases the likelihood of getting a disease is known as a risk factor, and may be behavioral, physiological, demographic, environmental, or genetic in nature. Behavioral risk factors usually relate to actions that a subject has chosen to take. Demographic risk factors are those that relate to the overall population, such as age or gender. Environmental risk factors include those that are related to exposure to objects in an environment, such as air pollution and access to clean water. Genetic risk factors are based on an individual's genetic makeup, and may reflect interaction between the genes of the individual and environmental factors. A subject having a high number of manifestations of risk factors associated with a disease may be at increased risk of developing the disease, and may be “predicted to have” the disease.

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“Prevention” and “preventing” are used according to their ordinary and plain meaning to mean “acting before” or such an act. In the context of a particular disease or health-related condition, those terms refer to administration or application of an agent, drug, or remedy to a subject or performance of a procedure or modality on a subject for the purpose of blocking the onset of a disease or health-related condition.

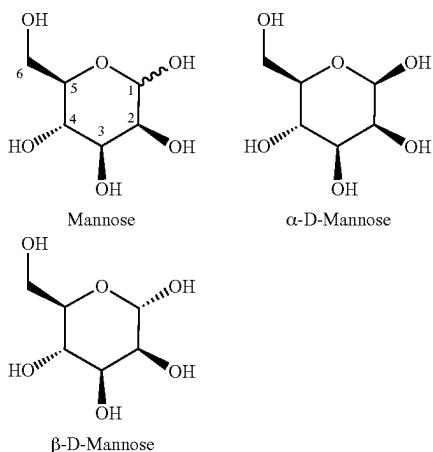
As used herein, an “antigen” is any substance that serves as a target for the receptors of an adaptive immune response, such as the T cell receptor, major histocompatibility complex class I and II, B cell receptor or an antibody. In some embodiments, an antigen may originate from within the body (e.g., “self,” “auto” or “endogenous”). In additional embodiments, an antigen may originate from outside the body (“non-self,” “foreign” or “exogenous”), having entered, for example, by inhalation, ingestion, injection, or transplantation, transdermal, etc. In some embodiments, an exogenous antigen may be biochemically modified in the body. Foreign antigens include, but are not limited to, food antigens, animal antigens, plant antigens, environmental antigens, therapeutic agents, as well as antigens present in an allograft transplant. Non-limiting examples of antigens are provided herein.

An “antigen-binding molecule” as used herein relates to molecules, in particular to proteins such as immunoglobulin molecules, which contain antibody variable regions providing a binding (specific binding in some embodiments) to an epitope. The antibody variable region can be present in, for example, a complete antibody, an antibody fragment, and a recombinant derivative of an antibody or antibody fragment. The term “antigen-binding fragment” of an antibody (or “binding portion”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind a target sequence. Antigen-binding fragments containing antibody variable regions include (without limitation) “Fv”, “Fab”, and “F(ab')₂” regions, “single domain antibodies (sdAb)”, “nanobodies”, “single chain Fv (scFv)” fragments, “tandem scFvs” (V_HA-V_LA-V_HB-V_LB), “diabodies”, “tribodies” or “tribodies”, “single-chain diabodies (scDb)”, and “bi-specific T-cell engagers (BiTEs)”.

An “epitope”, also known as antigenic determinant, is the segment of a macromolecule, e.g. a protein, which is recognized by the adaptive immune system, such as by antibodies, B cells, major histocompatibility complex molecules, or T cells. An epitope is that part or segment of a macromolecule capable of binding to an antibody or antigen-binding fragment thereof. In this context, the term “binding” in particular relates to a specific binding. In the context of several embodiments of the present invention it is preferred that the term “epitope” refers to the segment of protein or polyprotein that is recognized by the immune system.

The term mannose or mannosylating moiety refers to a monosaccharide sugar that exists both in open-chain form and in cyclic form, having D- and L-isomers. In the cyclic form, there are two anomers, namely alpha and beta. In the alpha form, the C1 alcohol group is in the axial position, whereas in the beta form, the C1 alcohol group is in the equatorial position. In particular, “mannose” refers to the cyclic six-membered pyranose, more in particular the D-isomer and even more particularly the alpha-D-form (α -D-mannose). The structure and numbering of mannose on non-limiting examples of stereochemical illustration. In the current formulation, the mannose residue is a single sugar that is connected to the backbone of the polymer via a single site such as the primary alcohols that attach to C1-C4 and C6. Thus, one of the alcohols is used to connect the mannose

to the polymer while the other alcohols remain OH groups at neutral pH. In several embodiments, the advantageous polyfunctionality of the approaches disclosed herein comes, at least in part, from the multiple monomers that are used to decorate the side of the polymer.



Whenever a group is described as being “optionally substituted” that group may be unsubstituted or substituted with one or more of the indicated substituents. Likewise, when a group is described as being “unsubstituted or substituted” if substituted, the substituent(s) may be selected from one or more the indicated substituents. If no substituents are indicated, it is meant that the indicated “optionally substituted” or “substituted” group may be substituted with one or more group(s) individually and independently selected from C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkynyl, C_{1-6} cycloalkyl, C_{1-6} aryl, C_{1-6} heteroaryl, C_{1-6} heterocycl, C_{1-6} alkoxy, C_{1-6} acyl, cyano, hydroxyl, an amino, halogen substituted C_{1-6} alkyl, halogen substituted C_{1-6} alkoxy, and halogen.

As used herein, a “chemical modification” refers to a change in the naturally-occurring chemical structure of one or more amino acids of a polypeptide. Such modifications can be made to a side chain or a terminus, e.g., changing the amino-terminus or carboxyl terminus. In some embodiments, the modifications are useful for creating chemical groups that can conveniently be used to link the polypeptides to other materials, or to attach a therapeutic agent.

“Conservative changes” can generally be made to an amino acid sequence without altering activity. These changes are termed “conservative substitutions” or mutations; that is, an amino acid belonging to a grouping of amino acids having a particular size or characteristic can be substituted for another amino acid. Substitutes for an amino acid sequence can be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, methionine, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such substitutions are not expected to substantially affect apparent molecular weight as determined by polyacrylamide gel electrophoresis or isoelectric point. Conservative substitutions also include substituting optical iso-

mers of the sequences for other optical isomers, specifically d amino acids for l amino acids for one or more residues of a sequence. Moreover, all of the amino acids in a sequence can undergo a d to 1 isomer substitution. Exemplary conservative substitutions include, but are not limited to, Lys for Arg and vice versa to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a free —OH is maintained; and Gln for Asn to maintain a free —NH₂. Yet another type of conservative substitution constitutes the case where amino acids with desired chemical relativities are introduced to impart reactive sites for chemical conjugation reactions, if the need for chemical derivatization arises. Such amino acids include but are not limited to Cys (to insert a sulphydryl group), Lys (to insert a primary amine), Asp and Glu (to insert a carboxylic acid group), or specialized noncanonical amino acids containing ketone, azide, alkyne, alkene, and tetrazine side-chains. Conservative substitutions or additions of free —NH₂ or —SH bearing amino acids can be particularly advantageous for chemical conjugation with the linkers and mannosylating moieties of Formula 1. Moreover, point mutations, deletions, and insertions of the polypeptide sequences or corresponding nucleic acid sequences can in some cases be made without a loss of function of the polypeptide or nucleic acid fragment. Substitutions can include, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more residues (including any number of substitutions between those listed). A variant usable in embodiments herein may exhibit a total number of up to 200 (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200, including any number in between those listed) changes in the amino acid sequence (e.g., exchanges, insertions, deletions, N-terminal truncations, and/or C-terminal truncations). In several embodiments, the number of changes is greater than 200. Additionally, in several embodiments, the variants include polypeptide sequences or corresponding nucleic acid sequences that exhibit a degree of functional equivalence with a reference (e.g., unmodified or native sequence). In several embodiments, the variants exhibit about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99% functional equivalence to an unmodified or native reference sequence (and any degree of functional equivalence between those listed). The amino acid residues described herein employ either the single letter amino acid designator or the three-letter abbreviation in keeping with the standard polypeptide nomenclature, *J. Biol. Chem.*, (1969), 243, 3552-3559. All amino acid residue sequences are represented herein by formulae with left and right orientation in the conventional direction of amino-terminus to carboxy-terminus.

The term “liver-targeting moiety” refers to mannose moieties having the ability to direct, e.g., a polypeptide, to the cells of a liver expressing mannose receptors. The liver comprises different cell types, including but not limited to hepatocytes, sinusoidal epithelial cells, Kupffer cells, stellate cells, and/or dendritic cells. Typically, a liver-targeting moiety directs a polypeptide to one or more of these cells. On the surface of the respective liver cells, receptors are present which recognize and specifically bind the liver-targeting moiety. Liver-targeting can be achieved by chemical conjugation of an antigen or ligand to a mannosylating or mannansylating moiety. Naturally occurring desilylated proteins are not encompassed within the scope of certain embodiments of the present disclosure.

The term “random copolymer” refers to the product of simultaneous polymerization of two or more monomers in

admixture, where the probability of finding a given monomeric unit at any given site in a polymer chain is independent of the nature of the neighboring units at that position (Bernoullian distribution). Thus, when the variable group identified as W_p represents a random copolymer, the chain can comprise any sequence from 2 up to about 150 W₁ and W₂ groups, such as: —W₁-W₂-W₁-W₂-; —W₂-W₁-W₂-W₁-; —W₁-W₁-W₁-W₂-; —W₁-W₁-W₂-W₂-; —W₁-W₂-W₁-W₁-; —W₁-W₂-W₁-W₂-W₂-W₁-W₁-W₂-; and W₂-W₂-W₁-W₂-W₁-W₁-W₂-W₂-W₁; ad infinitum, where Z attached to the various W₁ groups and the W₁ and W₂ groups themselves can be the same or different.

The term "sequence identity" is used with regard to polypeptide (or nucleic acid) sequence comparisons. This expression in particular refers to a percentage of sequence identity, for example at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide. Particularly, the polypeptide in question and the reference polypeptide exhibit the indicated sequence identity over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids (or any range derivable therein) or over the entire length of the reference polypeptide.

"Specific binding," as that term is commonly used in the biological arts, refers to a molecule that binds to a target with a relatively high affinity as compared to non-target tissues, and generally involves a plurality of non-covalent interactions, such as electrostatic interactions, van der Waals interactions, hydrogen bonding, and the like. Specific binding interactions characterize antibody-antigen binding, enzyme-substrate binding, and certain protein-receptor interactions; while such molecules might bind tissues besides their specific targets from time to time, to the extent that such non-target binding is inconsequential, the high-affinity binding pair can still fall within the definition of specific binding.

The term "unwanted immune response" refers to a reaction by the immune system of a subject, which in the given situation is not desirable. The reaction of the immune system is unwanted if such reaction does not lead to the prevention, reduction, or healing of a disease or disorder but instead causes, enhances or worsens, or is otherwise associated with induction or worsening of a disorder or disease. Typically, a reaction of the immune system causes, enhances or worsens a disease if it is directed against an inappropriate target. Exemplified, an unwanted immune response includes but is not limited to transplant rejection, immune response against a therapeutic agent, autoimmune disease, and allergy or hypersensitivity.

The term "variant" is to be understood as a protein (or nucleic acid) which differs in comparison to the protein from which it is derived by one or more changes in its length, sequence, or structure. The polypeptide from which a protein variant is derived is also known as the parent polypeptide or polynucleotide. The term "variant" comprises "fragments" or "derivatives" of the parent molecule. Typically, "fragments" are smaller in length or size than the parent molecule, whilst "derivatives" exhibit one or more differences in their sequence or structure in comparison to the parent molecule. Also encompassed are modified molecules such as but not limited to post-translationally modified proteins (e.g. glycosylated, phosphorylated, ubiquitinated, palmitoylated, or proteolytically cleaved proteins) and

modified nucleic acids such as methylated DNA. Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are encompassed by the term "variant". Naturally occurring and artificially constructed variants are to be understood to be encompassed by the term "variant" as used herein. Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent molecule or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent molecule, e.g., is functionally active. A variant can be characterized by a certain degree of sequence identity to the parent polypeptide from which it is derived. More precisely, a protein variant in the context of the present disclosure may exhibit at least 80% sequence identity to its parent polypeptide. Preferably, the sequence identity of protein variants is over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids. As discussed above, in several embodiments variants exhibit about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99% functional equivalence to an unmodified or native reference sequence (and any degree of functional equivalence between those listed).

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGS. 1A-1G. OVA-p(Man) induces T cell deletion. BLK6 mice were treated with saline, or 10 µg of OVA in the form of free OVA or OVA conjugated to p(Man) (OVA-p(Man)) one day and 7 days after an adoptive transfer of 7.0×10⁵ OTI and OTII T cells. The mice were challenged with an intradermal injection of LSP and OVA on 14 days after the initial OTI and OTII T cell transfer, then the immune response in the draining lymph nodes (dLNs) was assessed on day 19 via flow cytometry. FIG. 1A Fraction of OTII T cells in the dLNs on day 19. FIG. 1B Fraction of OTI T cells in the dLNs on day 19. FIG. 1C Fraction of OTII T cells in the liver on day 19. FIG. 1D Fraction of OTI T cells in the liver on day 19. FIG. 1E Fraction of Tregs in the dLN on day 19. FIG. 1F Fraction of Tregs in the liver on day 19.

FIG. 1G Fraction of T follicular helper cells (Tfh) as a fraction of total CD4 T cells in the dLN_s on day 19.

FIGS. 2A-2B: OVA-p(Man) induces T cells Anergy in DLs. BLK6 mice were treated with saline, or 10 µg of OVA in the form of free OVA or OVA conjugated to p(Man) (OVA-p(Man)) one day and 7 days after an adoptive transfer of 7.0×105 OTI and OTII T cells. The mice were challenged with an intradermal injection of LPS and OVA on 14 days after the initial OTI and OTII T cell transfer, and immune response in the draining lymph nodes (dLN_s) was assessed on day 19. Cells from the dLN were restimulated with OVA (A) or SIINFEKL (peptide) for 6 hours, then the percentage of IFN-γ producing cells was determined by flow cytometry. FIG. 2A Percentage of IFN-γ producing CD4+ T cells in the dLN_s. FIG. 2B Percentage of IFN-γ producing CD8+ T cells in the dLN_s.

FIGS. 3A-3D. Tolerance induction to protein therapeutics. Five BALB/c mice per group were injected with 2.5 µg of asparaginase formulated as free asparaginase (ASNase) or conjugated to p(Man) (ASNase-p(Man)) once a week for 3 weeks and then were switched to 15 µg of ASNase i.v. one a week for 8 weeks. During the initial 3 weeks ASNase-p(Man) was administered via either i.v. or subcutaneous injection. Sera was taken from the mice and monitored weekly for the presence of αASNase. FIG. 3A Pan IgG αASNase titers of mice treated with ASNase and ASNase-p(Man) via i.v. and subcutaneous injection. FIG. 3B αASNase IgG1 titers of treatment groups on day 38. FIG. 3C αASNase IgG2a titers of treatment groups on day 38. FIG. 3D αASNase IgG2b titers of treatment groups on day 38. FIG. 3E. αASNase IgG3 titers of treatment groups on day 38.

FIGS. 4A-4B. Response to p(Man) conjugates. FIG. 4A Bone marrow of animals treated with ASNase-p(Man) had fewer αASNase plasma cells than animals that were treated with ASNase. FIG. 4B Spleens of animals treated with ASNase-p(Man) had a greater percentage of IL-10 producing B regulatory cells.

FIG. 5. Tolerance induction to protein therapeutics. FIG. 5 is a graph depicting tolerance induction to the protein therapeutic asparaginase conjugated to p(Man) linker and mannose. Five BALB/c mice per group were injected with 2.5 µg of asparaginase formulated as free asparaginase (ASNase) or conjugated to p(Man) (ASNase-p(Man)) once a week for 3 weeks and then were switched to 15 µg of ASNase i.v. one a week for 7 weeks. Sera was taken from the mice and monitored weekly for the presence of anti-ASNase. Over the entire time of analysis none of the animals treated with ASNase-p(Man) developed measurable levels of antibodies against ASNase.

FIG. 6 p(Man)-ASNase administration regimen for assessment of p(Man)-protein conjugates on anti-asparaginase (anti-ASNase) humoral immune response.

FIGS. 7A-7B. Assessment of p(Man)-protein conjugates on anti-asparaginase (anti-ASNase) humoral immune response. FIG. 7A is a graph depicting serum asparagine concentration for days 71, 73, and 76. Mice treated with only saline or p(Man)-ASNase have a significantly lower serum asparagine concentration as compared to animals that had been treated with saline and then administered wt ASNase. FIG. 7B is a graph depicting serum asparagine concentration vs the anti-ASNase titer for each animal in the study. A strong correlation ($r=0.8$) between serum asparagine concentration and anti-ASNase titer is evident.

DETAILED DESCRIPTION

Several embodiments disclosed herein overcome the deficiencies of the prior art by providing compositions comprising

ing mannose-fused antigens. The compositions may be used to prevent immunity or reduce an immune response protein-based drugs that would otherwise elicit an immune response.

Multiple mannose binding receptors are expressed by antigen presenting cells (APCs) and serve as gateways for antigen uptake and antigen cross presentation by these cells to T cells. Antigens taken up by APCs and presented to T cells in the absence of co-stimulation leads to T cell deletion, inactivity, and the formation of T regulator cells, that control antigen specific immune responses.

The present disclosure provides, in several embodiments, certain therapeutic compositions that are targeted for delivery to (and for uptake by) antigen presenting cells, particularly hepatocytes, LSECs, Kupffer cells and/or stellate cells, more particularly hepatocytes and/or LSECs, and even more particularly to specifically bind mannose-binding receptors.

Liver targeting facilitates two mechanisms of treatment: tolerization and clearance. Tolerization takes advantage of the liver's role in clearing apoptotic cells and processing their proteins to be recognized by the immune system as "self," as well as the liver's role in sampling peripheral proteins for immune tolerance. Clearance takes advantage of the liver's role in blood purification by rapidly removing and breaking down toxins, polypeptides and the like. Targeting of these compositions to the liver is accomplished by a mannosating moiety. The mannosylating moiety is chemically conjugated. The antigen can be endogenous (a self-antigen) or exogenous (a foreign antigen), including but not limited to: a foreign transplant antigen against which transplant recipients develop an unwanted immune response (e.g., transplant rejection), a foreign food, animal, plant or environmental antigen to which patients develop an unwanted immune (e.g., allergic or hypersensitivity) response, a therapeutic agent to which patients develop an unwanted immune response (e.g., hypersensitivity and/or reduced therapeutic activity), a self-antigen to which patients develop an unwanted immune response (e.g., autoimmune disease), or a tolerogenic portion (e.g., a fragment or an epitope) thereof; these compositions are useful for inducing tolerization to the antigen. Accordingly, the compositions of the present disclosure can be used for treating an unwanted immune response, e.g., transplant rejection, an immune response against a therapeutic agent, an autoimmune disease, and/or an allergy, depending on the embodiment. Also provided are pharmaceutical compositions containing a therapeutically effective amount of a composition of the disclosure admixed with at least one pharmaceutically acceptable excipient. In another aspect, the disclosure provides methods for the treatment of an unwanted immune response, such as transplant rejection, response against a therapeutic agent, autoimmune disease or allergy.

B. CHEMICAL DEFINITIONS

As used herein, a "small molecule" refers to an organic compound that is either synthesized via conventional organic chemistry methods (e.g., in a laboratory) or found in nature. Typically, a small molecule is characterized in that it contains several carbon-carbon bonds, and has a molecular weight of less than about 1500 grams/mole. In certain embodiments, small molecules are less than about 1000 grams/mole. In certain embodiments, small molecules are less than about 550 grams/mole. In certain embodiments, small molecules are between about 200 and about 550 grams/mole. In certain embodiments, small molecules exclude peptides (e.g., compounds comprising 2 or more

amino acids joined by a peptidyl bond). In certain embodiments, small molecules exclude nucleic acids.

As used herein, the term "amino" means —NH₂; the term "nitro" means —NO₂; the term "halo" or "halogen" designates —F, —Cl, —Br or —I; the term "mercapto" means —SH; the term "cyano" means —CN; the term "azido" means —N₃; the term "silyl" means —SiH₃, and the term "hydroxy" means —OH. In certain embodiments, a halogen may be —Br or —I.

As used herein, a "monovalent anion" refers to anions of a —1 charge. Such anions are well-known to those of skill in the art. Non-limiting examples of monovalent anions include halides (e.g., F⁻, Cl⁻, Br⁻ and I⁻), NO₂⁻, NO₃⁻, hydroxide (OH⁻) and azide (N₃⁻).

As used herein, the structure indicates that the bond may be a single bond or a double bond. Those of skill in the chemical arts understand that in certain circumstances, a double bond between two particular atoms is chemically feasible and in certain circumstances, a double bond is not. The present invention therefore contemplates that a double bond may be formed only when chemically feasible.

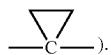
The term "alkyl" includes straight-chain alkyl, branched-chain alkyl, cycloalkyl (cyclic), cyclic alkyl, heteroatom-unsubstituted alkyl, heteroatom-substituted alkyl, heteroatom-unsubstituted C_n-alkyl, and heteroatom-substituted C_n-alkyl. In certain embodiments, lower alkyls are contemplated. Examples of branched alkyl groups include, but are not limited to, iso-propyl, sec-butyl, t-butyl and the like. Examples of straight chain alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl and the like. The alkyl group may have 1 to 30 carbon atoms (whenever it appears herein, a numerical range such as "1 to 30" refers to each integer in the given range; e.g., "1 to 30 carbon atoms" means that the alkyl group may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated). The "alkyl" group may also be a medium size alkyl having 1 to 12 carbon atoms. The term "lower alkyl" refers to alkyls of 1-6 carbon atoms (that is, 1, 2, 3, 4, 5 or 6 carbon atoms). The term "heteroatom-unsubstituted C_n-alkyl" refers to a radical, having a linear or branched, cyclic or acyclic structure, further having no carbon-carbon double or triple bonds, further having a total of n carbon atoms, all of which are nonaromatic, 3 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted C₁-C₁₀-alkyl has 1 to 10 carbon atoms. The groups, —CH₃ (Me), —CH₂CH₃ (Et), —CH₂CH₂CH₃ (n-Pr), —CH(CH₃)₂ (iso-Pr), —CH(CH₂)₂ (cyclopropyl), —CH₂CH₂CH₂CH₃ (n-Bu), —CH(CH₃)CH₂CH₃ (sec-butyl), —CH₂CH(CH₃)₂ (iso-butyl), —C(CH₃)₃ (tert-butyl), —CH₂C(CH₃)₃ (neo-pentyl), cyclobutyl, cyclopentyl, and cyclohexyl, are all non-limiting examples of heteroatom-unsubstituted alkyl groups. The term "heteroatom-substituted C_n-alkyl" refers to a radical, having a single saturated carbon atom as the point of attachment, no carbon-carbon double or triple bonds, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, all of which are nonaromatic, 0, 1, or more than one hydrogen atom, at least one heteroatom, wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₁-C₁₀-alkyl has 1 to 10 carbon atoms. The following groups are all non-limiting examples of heteroatom-substituted alkyl groups: trifluoromethyl, —CH₂F, —CH₂Cl, —CH₂Br,

—CH₂OH, —CH₂OCH₃, —CH₂OCH₂CF₃, —CH₂OC(O)CH₃, —CH₂NH₂, —CH₂NHCH₃, —CH₂N(CH₃)₂, —CH₂CH₂Cl, —CH₂CH₂OH, —CH₂CH₂OC(O)CH₃, —CH₂CH₂NHCO₂C(CH₃)₃, and —CH₂Si(CH₃)₃.

As used herein, the term "alkylene" refers to a bivalent fully saturated straight chain aliphatic hydrocarbon group. Examples of alkylene groups include, but are not limited to, methylene, ethylene, propylene, butylene, pentylene hexylene, heptylene and octylene. An alkylene group may be represented by followed by the number of carbon atoms, followed by a **. For example,



to represent ethylene. The alkylene group may have 1 to 30 carbon atoms (whenever it appears herein, a numerical range such as "1 to 30" refers to each integer in the given range; e.g., "1 to 30 carbon atoms" means that the alkyl group may consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 30 carbon atoms, although the present definition also covers the occurrence of the term "alkylene" where no numerical range is designated). The alkylene group may also be a medium size alkyl having 1 to 12 carbon atoms. The alkylene group could also be a lower alkyl having 1 to 4 carbon atoms. An alkylene group may be substituted or unsubstituted. For example, a lower alkylene group can be substituted by replacing one or more hydrogen of the lower alkylene group and/or by substituting both hydrogens on the same carbon with a C₃-6 monocyclic cycloalkyl group (e.g.,



The term "alkenyl" includes straight-chain alkenyl, branched-chain alkenyl, cycloalkenyl, cyclic alkenyl, heteroatom-unsubstituted alkenyl, heteroatom-substituted alkenyl, heteroatom-unsubstituted C_n-alkenyl, and heteroatom-substituted C_n-alkenyl. In certain embodiments, lower alkenyls are contemplated. The term "lower alkenyl" refers to alkenyls of 1-6 carbon atoms (that is, 1, 2, 3, 4, 5 or 6 carbon atoms). The term "heteroatom-unsubstituted C_n-alkenyl" refers to a radical, having a linear or branched, cyclic or acyclic structure, further having at least one nonaromatic carbon-carbon double bond, but no carbon-carbon triple bonds, a total of n carbon atoms, three or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted C₂-C₁₀-alkenyl has 2 to 10 carbon atoms. Heteroatom-unsubstituted alkenyl groups include: —CH=CH₂ (vinyl), —CH=CHCH₃, —CH=CHCH₂CH₃, —CH₂CH=CHCH₃, and —CH=CH—C₆H₅. The term "heteroatom-substituted C_n-alkenyl" refers to a radical, having a single nonaromatic carbon atom as the point of attachment and at least one nonaromatic carbon-carbon double bond, but no carbon-carbon triple bonds, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, and at least one heteroatom, wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₂-C₁₀-alkenyl has 2 to 10 carbon

atoms. The groups, —CH=CHF, —CH=CHCl and —CH=CHBr, are non-limiting examples of heteroatom-substituted alkenyl groups.

The term “aryl” includes heteroatom-unsubstituted aryl, heteroatom-substituted aryl, heteroatom-unsubstituted C_n-aryl, heteroatom-substituted C_n-aryl, heteroaryl, heterocyclic aryl groups, carbocyclic aryl groups, biaryl groups, and single-valent radicals derived from polycyclic fused hydrocarbons (PAHs). The term “heteroatom-unsubstituted C_n-aryl” refers to a radical, having a single carbon atom as a point of attachment, wherein the carbon atom is part of an aromatic ring structure containing only carbon atoms, further having a total of n carbon atoms, 5 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted C₆-C₁₀-aryl has 6 to 10 carbon atoms. Non-limiting examples of heteroatom-unsubstituted aryl groups include phenyl (Ph), methylphenyl, (dimethyl)phenyl, —C₆H₄CH₂CH₃, —C₆H₄CH₂CH₂CH₃, —C₆H₄CH(CH₃)₂, —C₆H₄CH(CH₂)₂, —C₆H₃(CH₃)CH₂CH₃, —C₆H₄CH=CH₂, —C₆H₄CH=CHCH₃, —C₆H₄C≡CH, —C₆H₄C≡CCH₃, naphthyl, and the radical derived from biphenyl. The term “heteroatom-substituted C_n-aryl” refers to a radical, having either a single aromatic carbon atom or a single aromatic heteroatom as the point of attachment, further having a total of n carbon atoms, at least one hydrogen atom, and at least one heteroatom, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-unsubstituted C₁-C₁₀-heteroaryl has 1 to 10 carbon atoms. Non-limiting examples of heteroatom-substituted aryl groups include the groups: —C₆H₄F, —C₆H₄Cl, —C₆H₄Br, —C₆H₄I, —C₆H₄OH, —C₆H₄OCH₃, —C₆H₄OCH₂CH₃, —C₆H₄OC(O)CH₃, —C₆H₄NH₂, —C₆H₄NHCH₃, —C₆H₄N(CH₃)₂, —C₆H₄CH₂OH, —C₆H₄CH₂OOC(O)CH₃, —C₆H₄CH₂NH₂, —C₆H₄CF₃, —C₆H₄CN, —C₆H₄CHO, —C₆H₄CHO, —C₆H₄C(O)CH₃, —C₆H₄C(O)C₆H₅, —C₆H₄CO₂H, —C₆H₄CO₂CH₃, —C₆H₄CONH₂, —C₆H₄CONHCH₃, —C₆H₄CON(CH₃)₂, furanyl, thieryl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, quinolyl, indolyl, and imidazoyl. In certain embodiments, heteroatom-substituted aryl groups are contemplated. In certain embodiments, heteroatom-unsubstituted aryl groups are contemplated. In certain embodiments, an aryl group may be mono-, di-, tri-, tetra- or penta-substituted with one or more heteroatom-containing substituents.

The term “aralkyl” includes heteroatom-unsubstituted aralkyl, heteroatom-substituted aralkyl, heteroatom-unsubstituted C_n-aralkyl, heteroatom-substituted C_n-aralkyl, heteroaralkyl, and heterocyclic aralkyl groups. In certain embodiments, lower aralkyls are contemplated. The term “lower aralkyl” refers to aralkyls of 7-12 carbon atoms (that is, 7, 8, 9, 10, 11 or 12 carbon atoms). The term “heteroatom-unsubstituted C_n-aralkyl” refers to a radical, having a single saturated carbon atom as the point of attachment, further having a total of n carbon atoms, wherein at least 6 of the carbon atoms form an aromatic ring structure containing only carbon atoms, 7 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted C₇-C₁₀-aralkyl has 7 to 10 carbon atoms. Non-limiting examples of heteroatom-unsubstituted aralkyls are: phenylmethyl (benzyl, Bn) and phenylethyl. The term “heteroatom-substituted C_n-aralkyl” refers to a radical, having a single saturated carbon atom as the point of attachment, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, and at least one heteroatom, wherein at least one of the carbon atoms is incorporated an aromatic ring

structures, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₂-C₁₀-heteroaralkyl has 2 to 10 carbon atoms.

The term “acyl” includes straight-chain acyl, branched-chain acyl, cycloacyl, cyclic acyl, heteroatom-unsubstituted acyl, heteroatom-substituted acyl, heteroatom-unsubstituted C_n-acyl, heteroatom-substituted C_n-acyl, alkylcarbonyl, alkoxy carbonyl and aminocarbonyl groups. In certain embodiments, lower acyls are contemplated. The term “lower acyl” refers to acyls of 1-6 carbon atoms (that is, 1, 2, 3, 4, 5 or 6 carbon atoms). The term “heteroatom-unsubstituted C_n-acyl” refers to a radical, having a single carbon atom of a carbonyl group as the point of attachment, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 1 or more hydrogen atoms, a total of one oxygen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₁-C₁₀-acyl has 1 to 10 carbon atoms. The groups, —CHO, —C(O)CH₃, —C(O)CH₂CH₃, —C(O)CH₂CH₂CH₃, —C(O)CH(CH₃)₂, —C(O)CH(CH₂)₂, —C(O)C₆H₅, —C(O)C₆H₄CH₃, —C(O)C₆H₄CH₂CH₃, and —COC₆H₃(CH₃)₂, are non-limiting examples of heteroatom-unsubstituted acyl groups. The term “heteroatom-substituted C_n-acyl” refers to a radical, having a single carbon atom as the point of attachment, the carbon atom being part of a carbonyl group, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom, in addition to the oxygen of the carbonyl group, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₁-C₁₀-acyl has 1 to 10 carbon atoms. The groups, —C(O)CH₂CF₃, —CO₂H, —CO₂, —CO₂CH₃, —CO₂CH₂CH₃, —CO₂CH₂CH₂CH₃, —CO₂CH(CH₃)₂, —CO₂CH(CH₂)₂, —C(O)NH₂ (carbamoyl), —C(O)NHCH₃, —C(O)NHCH₂CH₃, —CONHCH(CH₃)₂, —CONHCH(CH₂)₂, —CON(CH₃)₂, and —CONHCH₂CF₃, are non-limiting examples of heteroatom-substituted acyl groups.

The term “alkoxy” includes straight-chain alkoxy, branched-chain alkoxy, cycloalkoxy, cyclic alkoxy, heteroatom-unsubstituted alkoxy, heteroatom-substituted alkoxy, heteroatom-unsubstituted C_n-alkoxy, and heteroatom-substituted C_n-alkoxy. In certain embodiments, lower alkoxys are contemplated. The term “lower alkoxy” refers to alkoxys of 1-6 carbon atoms (that is, 1, 2, 3, 4, 5 or 6 carbon atoms). The term “heteroatom-unsubstituted C_n-alkoxy” refers to a group, having the structure —OR, in which R is a heteroatom-unsubstituted C_n-alkyl, as that term is defined above. Heteroatom-unsubstituted alkoxy groups include: —OCH₃, —OCH₂CH₃, —OCH₂CH₂CH₃, —OCH(CH₃)₂, and —OCH(CH₂)₂. The term “heteroatom-substituted C_n-alkoxy” refers to a group, having the structure —OR, in which R is a heteroatom-substituted C_n-alkyl, as that term is defined above. For example, —OCH₂CF₃ is a heteroatom-substituted alkoxy group.

The term “alkenyloxy” includes straight-chain alkenyloxy, branched-chain alkenyloxy, cycloalkenyloxy, cyclic alkenyloxy, heteroatom-unsubstituted alkenyloxy, heteroatom-substituted alkenyloxy, heteroatom-unsubstituted C_n-alkenyloxy, and heteroatom-substituted C_n-alkenyloxy. The term “heteroatom-unsubstituted C_n-alkenyloxy” refers to a group, having the structure —OR, in which R is a heteroatom-unsubstituted C_n-alkenyl, as that term is defined above. The term “heteroatom-substituted C_n-alkenyloxy” refers to

a group, having the structure —OR, in which R is a heteroatom-substituted C_n-alkenyl, as that term is defined above.

The term "alkynyloxy" includes straight-chain alkynyloxy, branched-chain alkynyloxy, cycloalkynyloxy, cyclic alkynyloxy, heteroatom-unsubstituted alkynyloxy, heteroatom-substituted alkynyloxy, heteroatom-unsubstituted C_n-alkynyloxy, and heteroatom-substituted C_n-alkynyloxy. The term "heteroatom-unsubstituted C_n-alkynyloxy" refers to a group, having the structure —OR, in which R is a heteroatom-unsubstituted C_n-alkynyl, as that term is defined above. The term "heteroatom-substituted C_n-alkynyloxy" refers to a group, having the structure —OR, in which R is a heteroatom-substituted C_n-alkynyl, as that term is defined above.

The term "aryloxy" includes heteroatom-unsubstituted aryloxy, heteroatom-substituted aryloxy, heteroatom-unsubstituted Cn-aryloxy, heteroatom-substituted Cn-aryloxy, heteroaryloxy, and heterocyclic aryloxy groups. The term "heteroatom-unsubstituted Cn-aryloxy" refers to a group, having the structure —OAr, in which Ar is a heteroatom-unsubstituted Cn-aryl, as that term is defined above. A non-limiting example of a heteroatom-unsubstituted aryloxy group is —OC₆H₅. The term "heteroatom-substituted Cn-aryloxy" refers to a group, having the structure —OAr, in which Ar is a heteroatom-substituted Cn-aryl, as that term is defined above.

The term "aralkyloxy" includes heteroatom-unsubstituted aralkyloxy, heteroatom-substituted aralkyloxy, heteroatom-unsubstituted Cn-aralkyloxy, heteroatom-substituted Cn-aralkyloxy, heteroaralkyloxy, and heterocyclic aralkyloxy groups. The term "heteroatom-unsubstituted Cn-aralkyloxy" refers to a group, having the structure —OAr, in which Ar is a heteroatom-unsubstituted Cn-aralkyl, as that term is defined above. The term "heteroatom-substituted Cn-aralkyloxy" refers to a group, having the structure —OAr, in which Ar is a heteroatom-substituted Cn-aralkyl, as that term is defined above.

The term “acyloxy” includes straight-chain acyloxy, branched-chain acyloxy, cycloacyloxy, cyclic acyloxy, heteroatom-unsubstituted acyloxy, heteroatom-substituted acyloxy, heteroatom-unsubstituted Cn-acyloxy, heteroatom-substituted Cn-acyloxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, and carboxylate groups. The term “heteroatom-unsubstituted Cn-acyloxy” refers to a group, having the structure —OAc, in which Ac is a heteroatom-unsubstituted Cn-acyl, as that term is defined above. For example, —OC(O)CH₃ is a non-limiting example of a heteroatom-unsubstituted acyloxy group. The term “heteroatom-substituted Cn-acyloxy” refers to a group, having the structure —OAc, in which Ac is a heteroatom-substituted Cn-acyl, as that term is defined above. For example, —OC(O)OCH₃ and —OC(O)NHCH₃ are non-limiting examples of heteroatom-unsubstituted acyloxy groups.

The term "alkylamino" includes straight-chain alkylamino, branched-chain alkylamino, cycloalkylamino, cyclic alkylamino, heteroatom-unsubstituted alkylamino, heteroatom-substituted alkylamino, heteroatom-unsubstituted C_n-alkylamino, and heteroatom-substituted C_n-alkylamino. The term "heteroatom-unsubstituted C_n-alkylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two saturated carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, containing a total of n carbon atoms, all of which are nonaromatic, 4 or more hydrogen atoms, a total of 1 nitrogen atom, and no

additional heteroatoms. For example, a heteroatom-unsubstituted C₁-C₁₀-alkylamino has 1 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-alkylamino" includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-alkyl, as that term is defined above. A heteroatom-unsubstituted alkylamino group would include —NHCH₃, —NHCH₂CH₃, —NHCH₂CH₂CH₃, —NHCH(CH₃)₂, —NHCH(CH₂)₂, —NHCH₂CH₂CH₃, —NHCH(CH₃)CH₂CH₃, —NHCH₂CH(CH₃)₂, —NHC(CH₃)₃, —N(CH₃)₂, —N(CH₃)CH₂CH₃, —N(CH₂CH₃)₂, N-pyrrolidinyl, and N-piperidinyl. The term "heteroatom-substituted C_n-alkylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two saturated carbon atoms attached to the nitrogen atom, no carbon-carbon double or triple bonds, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, all of which are nonaromatic, 0, 1, or more than one hydrogen atom, and at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₁-C₁₀-alkylamino has 1 to 10 carbon atoms. The term "heteroatom-substituted C_n-alkylamino" includes groups, having the structure —NHR, in which R is a heteroatom-substituted C_n-alkyl, as that term is defined above.

The term "alkenylamino" includes straight-chain alkenylamino, branched-chain alkenylamino, cycloalkenylamino, cyclic alkenylamino, heteroatom-unsubstituted alkenylamino, heteroatom-substituted alkenylamino, heteroatom-unsubstituted C_n-alkenylamino, heteroatom-substituted C_n-alkenylamino, dialkenylamino, and alkyl(alkenyl)amino groups. The term "heteroatom-unsubstituted C_n-alkenylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, containing at least one nonaromatic carbon-carbon double bond, a total of n carbon atoms, 4 or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₂-C₁₀-alkenylamino has 2 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-alkenylamino" includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-alkenyl, as that term is defined above. The term "heteroatom-substituted C_n-alkenylamino" refers to a radical, having a single nitrogen atom as the point of attachment and at least one nonaromatic carbon-carbon double bond, but no carbon-carbon triple bonds, further having one or two carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, and at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₂-C₁₀-alkenylamino has 2 to 10 carbon atoms. The term "heteroatom-substituted C_n-alkenylamino" includes groups, having the structure —NHR, in which R is a heteroatom-substituted C_n-alkenyl, as that term is defined above.

The term "alkynylamino" includes straight-chain alkynylamino, branched-chain alkynylamino, cycloalkynylamino, cyclic alkynylamino, heteroatom-unsubstituted alkynylamino, heteroatom-substituted alkynylamino, heteroatom-unsubstituted Cn-alkynylamino, heteroatom-sub-

stituted C_n-alkynylamino, dialkynylamino, alkyl(alkynyl)amino, and alkenyl(alkynyl)amino groups. The term “heteroatom-unsubstituted C_n-alkynylamino” refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, containing at least one carbon-carbon triple bond, a total of n carbon atoms, at least one hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₂-C₁₀-alkynylamino has 2 to 10 carbon atoms. The term “heteroatom-unsubstituted C_n-alkynylamino” includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-alkynyl, as that term is defined above. The term “heteroatom-substituted C_n-alkynylamino” refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two carbon atoms attached to the nitrogen atom, further having at least one nonaromatic carbon-carbon triple bond, further having a linear or branched, cyclic or acyclic structure, and further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, and at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₂-C₁₀-alkynylamino has 2 to 10 carbon atoms. The term “heteroatom-substituted C_n-alkynylamino” includes groups, having the structure —NHR, in which R is a heteroatom-substituted C_n-alkynyl, as that term is defined above.

The term “arylamino” includes heteroatom-unsubstituted arylamino, heteroatom-substituted arylamino, heteroatom-unsubstituted C_n-arylamino, heteroatom-substituted C_n-arylamino, heteroarylamino, heterocyclic arylamino, and alkyl(aryl)amino groups. The term “heteroatom-unsubstituted C_n-arylamino” refers to a radical, having a single nitrogen atom as the point of attachment, further having at least one aromatic ring structure attached to the nitrogen atom, wherein the aromatic ring structure contains only carbon atoms, further having a total of n carbon atoms, 6 or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₆-C₁₀-arylamino has 6 to 10 carbon atoms. The term “heteroatom-unsubstituted C_n-arylamino” includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-aryl, as that term is defined above. The term “heteroatom-substituted C_n-arylamino” refers to a radical, having a single nitrogen atom as the point of attachment, further having a total of n carbon atoms, at least one hydrogen atom, at least one additional heteroatoms, that is, in addition to the nitrogen atom at the point of attachment, wherein at least one of the carbon atoms is incorporated into one or more aromatic ring structures, further wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₆-C₁₀-arylamino has 6 to 10 carbon atoms. The term “heteroatom-substituted C_n-arylamino” includes groups, having the structure —NHR, in which R is a heteroatom-substituted C_n-aryl, as that term is defined above.

The term “aralkylamino” includes heteroatom-unsubstituted aralkylamino, heteroatom-substituted aralkylamino, heteroatom-unsubstituted C_n-aralkylamino, heteroatom-substituted C_n-aralkylamino, heteroaralkylamino, heterocyclic aralkylamino groups, and diaralkylamino groups. The term “heteroatom-unsubstituted C_n-aralkylamino” refers to a radical, having a single nitrogen atom as the point of

attachment, further having one or two saturated carbon atoms attached to the nitrogen atom, further having a total of n carbon atoms, wherein at least 6 of the carbon atoms form an aromatic ring structure containing only carbon atoms, 8 or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₇-C₁₀-aralkylamino has 7 to 10 carbon atoms. The term “heteroatom-unsubstituted C_n-aralkylamino” includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-aralkyl, as that term is defined above. The term “heteroatom-substituted C_n-aralkylamino” refers to a radical, having a single nitrogen atom as the point of attachment, further having at least one or two saturated carbon atoms attached to the nitrogen atom, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein at least one of the carbon atom incorporated into an aromatic ring, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₇-C₁₀-aralkylamino has 7 to 10 carbon atoms. The term “heteroatom-substituted C_n-aralkylamino” includes groups, having the structure —NHR, in which R is a heteroatom-substituted C_n-aralkyl, as that term is defined above.

The term “amido” includes straight-chain amido, branched-chain amido, cycloamido, cyclic amido, heteroatom-unsubstituted amido, heteroatom-substituted amido, heteroatom-unsubstituted C_n-amido, heteroatom-substituted C_n-amido, alkylcarbonylamino, arylcarbonylamino, alkoxy-carbonylamino, aryloxycarbonylamino, acylamino, alkylaminocarbonylamino, arylaminocarbonylamino, and ureido groups. The term “heteroatom-unsubstituted C_n-amido” refers to a radical, having a single nitrogen atom as the point of attachment, further having a carbonyl group attached via its carbon atom to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 1 or more hydrogen atoms, a total of one oxygen atom, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₁-C₁₀-amido has 1 to 10 carbon atoms. The term “heteroatom-unsubstituted C_n-amido” includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-acyl, as that term is defined above. The group, —NHCO(O)CH₃, is a non-limiting example of a heteroatom-unsubstituted amido group. The term “heteroatom-substituted C_n-amido” refers to a radical, having a single nitrogen atom as the point of attachment, further having a carbonyl group attached via its carbon atom to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further having a total of n aromatic or nonaromatic carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom in addition to the oxygen of the carbonyl group, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₁-C₁₀-amido has 1 to 10 carbon atoms. The term “heteroatom-substituted C_n-amido” includes groups, having the structure —NHR, in which R is a heteroatom-unsubstituted C_n-acyl, as that term is defined above. The group, —NHCO₂CH₃, is a non-limiting example of a heteroatom-unsubstituted amido group.

The term “alkylthio” includes straight-chain alkylthio, branched-chain alkylthio, cycloalkylthio, cyclic alkylthio, heteroatom-unsubstituted alkylthio, heteroatom-substituted alkylthio, heteroatom-unsubstituted C_n-alkylthio, and het-

eroatom-substituted Cn-alkylthio. The term “heteroatom-unsubstituted Cn-alkylthio” refers to a group, having the structure —SR, in which R is a heteroatom-unsubstituted Cn-alkyl, as that term is defined above. The group, —SCH₃, is an example of a heteroatom-unsubstituted alkylthio group. The term “heteroatom-substituted Cn-alkylthio” refers to a group, having the structure —SR, in which R is a heteroatom-substituted Cn-alkyl, as that term is defined above.

The term “alkenylthio” includes straight-chain alkenylthio, branched-chain alkenylthio, cycloalkenylthio, cyclic alkenylthio, heteroatom-unsubstituted alkenylthio, heteroatom-substituted alkenylthio, heteroatom-unsubstituted Cn-alkenylthio, and heteroatom-substituted Cn-alkenylthio. The term “heteroatom-unsubstituted Cn-alkenylthio” refers to a group, having the structure —SR, in which R is a heteroatom-unsubstituted Cn-alkenyl, as that term is defined above. The term “heteroatom-substituted Cn-alkenylthio” refers to a group, having the structure —SR, in which R is a heteroatom-substituted Cn-alkenyl, as that term is defined above.

The term “alkynylthio” includes straight-chain alkynylthio, branched-chain alkynylthio, cycloalkynylthio, cyclic alkynylthio, heteroatom-unsubstituted alkynylthio, heteroatom-substituted alkynylthio, heteroatom-unsubstituted Cn-alkynylthio, and heteroatom-substituted Cn-alkynylthio. The term “heteroatom-unsubstituted Cn-alkynylthio” refers to a group, having the structure —SR, in which R is a heteroatom-unsubstituted Cn-alkynyl, as that term is defined above. The term “heteroatom-substituted Cn-alkynylthio” refers to a group, having the structure —SR, in which R is a heteroatom-substituted Cn-alkynyl, as that term is defined above.

The term “arylthio” includes heteroatom-unsubstituted arylthio, heteroatom-substituted arylthio, heteroatom-unsubstituted Cn-arylthio, heteroatom-substituted Cn-arylthio, heteroarylthio, and heterocyclic arylthio groups. The term “heteroatom-unsubstituted Cn-arylthio” refers to a group, having the structure —SAr, in which Ar is a heteroatom-unsubstituted Cn-aryl, as that term is defined above. The group, —SC₆H₅, is an example of a heteroatom-unsubstituted arylthio group. The term “heteroatom-substituted Cn-arylthio” refers to a group, having the structure —SAr, in which Ar is a heteroatom-substituted Cn-aryl, as that term is defined above.

The term “aralkylthio” includes heteroatom-unsubstituted aralkylthio, heteroatom-substituted aralkylthio, heteroatom-unsubstituted Cn-aralkylthio, heteroatom-substituted Cn-aralkylthio, heteroaralkylthio, and heterocyclic aralkylthio groups. The term “heteroatom-unsubstituted Cn-aralkylthio” refers to a group, having the structure —SAr, in which Ar is a heteroatom-unsubstituted Cn-aralkyl, as that term is defined above. The group, —SCH₂C₆H₅, is an example of a heteroatom-unsubstituted aralkyl group. The term “heteroatom-substituted Cn-aralkylthio” refers to a group, having the structure —SAr, in which Ar is a heteroatom-substituted Cn-aralkyl, as that term is defined above.

The term “acylthio” includes straight-chain acylthio, branched-chain acylthio, cycloacetylthio, cyclic acylthio, heteroatom-unsubstituted acylthio, heteroatom-substituted acylthio, heteroatom-unsubstituted Cn-acylthio, heteroatom-substituted Cn-acylthio, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, and carboxylate groups. The term “heteroatom-unsubstituted Cn-acylthio” refers to a group, having the structure —SAC, in which AC is a heteroatom-unsubstituted Cn-acyl, as that term is defined above. The group, —SCOCH₃, is an example of a heteroatom-unsubstituted acylthio group. The term

“heteroatom-substituted Cn-acylthio” refers to a group, having the structure —SAC, in which AC is a heteroatom-substituted Cn-acyl, as that term is defined above.

The term “alkylsilyl” includes straight-chain alkylsilyl, branched-chain alkylsilyl, cycloalkylsilyl, cyclic alkylsilyl, heteroatom-unsubstituted alkylsilyl, heteroatom-substituted alkylsilyl, heteroatom-unsubstituted Cn-alkylsilyl, and heteroatom-substituted Cn-alkylsilyl. The term “heteroatom-unsubstituted Cn-alkylsilyl” refers to a radical, having a single silicon atom as the point of attachment, further having one, two, or three saturated carbon atoms attached to the silicon atom, further having a linear or branched, cyclic or acyclic structure, containing a total of n carbon atoms, all of which are nonaromatic, 5 or more hydrogen atoms, a total of 1 silicon atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C₁-C₁₀-alkylsilyl has 1 to 10 carbon atoms. An alkylsilyl group includes dialkylamino groups. The groups, —Si(CH₃)₃ and —Si(CH₃)₂C(CH₃)₃, are non-limiting examples of heteroatom-unsubstituted alkylsilyl groups. The term “heteroatom-substituted Cn-alkylsilyl” refers to a radical, having a single silicon atom as the point of attachment, further having at least one, two, or three saturated carbon atoms attached to the silicon atom, no carbon-carbon double or triple bonds, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, all of which are nonaromatic, 0, 1, or more than one hydrogen atom, and at least one additional heteroatom, that is, in addition to the silicon atom at the point of attachment, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₁-C₁₀-alkylsilyl has 1 to 10 carbon atoms.

The term “phosphonate” includes straight-chain phosphonate, branched-chain phosphonate, cyclophosphonate, cyclic phosphonate, heteroatom-unsubstituted phosphonate, heteroatom-substituted phosphonate, heteroatom-unsubstituted Cn-phosphonate, and heteroatom-substituted Cn-phosphonate. The term “heteroatom-unsubstituted Cn-phosphonate” refers to a radical, having a single phosphorous atom as the point of attachment, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 2 or more hydrogen atoms, a total of three oxygen atom, and no additional heteroatoms. The three oxygen atoms are directly attached to the phosphorous atom, with one of these oxygen atoms doubly bonded to the phosphorous atom. For example, a heteroatom-unsubstituted C₀-C₁₀-phosphonate has 0 to 10 carbon atoms. The groups, —P(O)(OH)2, —P(O)(OH)OCH₃, —P(O)(OH)OCH₂CH₃, —P(O)(OCH₃)₂, and —P(O)(OH)(OC₆H₅) are non-limiting examples of heteroatom-unsubstituted phosphonate groups. The term “heteroatom-substituted Cn-phosphonate” refers to a radical, having a single phosphorous atom as the point of attachment, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 2 or more hydrogen atoms, three or more oxygen atoms, three of which are directly attached to the phosphorous atom, with one of these three oxygen atoms doubly bonded to the phosphorous atom, and further having at least one additional heteroatom in addition to the three oxygen atoms, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-unsubstituted C₀-C₁₀-phosphonate has 0 to 10 carbon atoms.

The term “phosphinate” includes straight-chain phosphinate, branched-chain phosphinate, cyclophosphinate, cyclic phosphinate, heteroatom-unsubstituted phosphinate, heteroatom-substituted phosphinate, heteroatom-unsubstituted

C_n-phosphinate, and heteroatom-substituted C_n-phosphinate. The term “heteroatom-unsubstituted C_n-phosphinate” refers to a radical, having a single phosphorous atom as the point of attachment, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 2 or more hydrogen atoms, a total of two oxygen atom, and no additional heteroatoms. The two oxygen atoms are directly attached to the phosphorous atom, with one of these oxygen atoms doubly bonded to the phosphorous atom. For example, a heteroatom-unsubstituted C₀-C₁₀-phosphinate has 0 to 10 carbon atoms. The groups, —P(O)(OH)H, —P(O)(OH)CH₃, —P(O)(OH)CH₂CH₃, —P(O)(OCH₃)CH₃, and —P(O)(OC₆H₅)H are non-limiting examples of heteroatom-unsubstituted phosphinate groups. The term “heteroatom-substituted C_n-phosphinate” refers to a radical, having a single phosphorous atom as the point of attachment, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 2 or more hydrogen atoms, two or more oxygen atoms, two of which are directly attached to the phosphorous atom, with one of these two oxygen atoms doubly bonded to the phosphorous atom, and further having at least one additional heteroatom in addition to the two oxygen atoms, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-unsubstituted C₀-C₁₀-phosphinate has 0 to 10 carbon atoms.

Any apparently unfulfilled valency is to be understood to be properly filled by hydrogen atom(s). For example, a compound with a substituent of —O or —N is to be understood to be —OH or —NH₂, respectively.

Any genus, subgenus, or specific compound discussed herein is specifically contemplated as being excluded from any embodiment described herein.

Compounds described herein may be prepared synthetically using conventional organic chemistry methods known to those of skill in the art and/or are commercially available (e.g., ChemBridge Co., San Diego, Calif.).

The claimed invention is also intended to encompass salts of any of the compounds of the present invention. The term “salt(s)” as used herein, is understood as being acidic and/or basic salts formed with inorganic and/or organic acids and bases. Zwitterions (internal or inner salts) are understood as being included within the term “salt(s)” as used herein, as are quaternary ammonium salts such as alkylammonium salts. Nontoxic, pharmaceutically acceptable salts are preferred, although other salts may be useful, as for example in isolation or purification steps during synthesis. Salts include, but are not limited to, sodium, lithium, potassium, amines, tartrates, citrates, hydrohalides, phosphates and the like. A salt may be a pharmaceutically acceptable salt, for example. Thus, pharmaceutically acceptable salts of compounds of the present invention are contemplated.

The term “pharmaceutically acceptable salts,” as used herein, refers to salts of compounds of this invention that are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of a compound of this invention with an inorganic or organic acid, or an organic base, depending on the substituents present on the compounds of the invention.

Non-limiting examples of inorganic acids which may be used to prepare pharmaceutically acceptable salts include: hydrochloric acid, phosphoric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, phosphorous acid and the like. Examples of organic acids which may be used to prepare pharmaceutically acceptable salts include: aliphatic mono- and dicarboxylic acids, such as oxalic acid, carbonic acid,

citric acid, succinic acid, phenyl-heteroatom-substituted alkanoic acids, aliphatic and aromatic sulfuric acids and the like. Pharmaceutically acceptable salts prepared from inorganic or organic acids thus include hydrochloride, hydrobromide, nitrate, sulfate, pyrosulfate, bisulfate, sulfite, bisulfate, phosphate, monohydrogenphosphate, dihydrogen phosphate, metaphosphate, pyrophosphate, hydroiodide, hydrofluoride, acetate, propionate, formate, oxalate, citrate, lactate, p-toluenesulfonate, methanesulfonate, maleate, and the like.

Suitable pharmaceutically acceptable salts may also be formed by reacting the agents of the invention with an organic base such as methylamine, ethylamine, ethanolamine, lysine, ornithine and the like.

Pharmaceutically acceptable salts include the salts formed between carboxylate or sulfonate groups found on some of the compounds of this invention and inorganic cations, such as sodium, potassium, ammonium, or calcium, or such organic cations as isopropylammonium, trimethylammonium, tetramethylammonium, and imidazolium.

Derivatives of compounds of the present invention are also contemplated. In certain aspects, “derivative” refers to a chemically modified compound that still retains the desired effects of the compound prior to the chemical modification. Such derivatives may have the addition, removal, or substitution of one or more chemical moieties on the parent molecule. Non-limiting examples of the types modifications that can be made to the compounds and structures disclosed herein include the addition or removal of lower alkanes such as methyl, ethyl, propyl, or substituted lower alkanes such as hydroxymethyl or aminomethyl groups; carboxyl groups and carbonyl groups; hydroxyls; nitro, amino, amide, and azo groups; sulfate, sulfonate, sulfone, sulphydryl, sulfonyl, sulfoxido, phosphate, phosphono, phosphoryl groups, and halide substituents. Additional modifications can include an addition or a deletion of one or more atoms of the atomic framework, for example, substitution of an ethyl by a propyl; substitution of a phenyl by a larger or smaller aromatic group. Alternatively, in a cyclic or bicyclic structure, heteroatoms such as N, S, or O can be substituted into the structure instead of a carbon atom.

Compounds employed in methods may contain one or more asymmetrically-substituted carbon or nitrogen atoms, and may be isolated in optically active or racemic form. Thus, all chiral, diastereomeric, racemic form, epimeric form, and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated. Compounds may occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. In some embodiments, a single diastereomer is obtained. The chiral centers of the compounds of the present invention can have the S- or the R-configuration, as defined by the IUPAC 1974 Recommendations. Compounds may be of the D- or L-form, for example. It is well known in the art how to prepare and isolate such optically active forms. For example, mixtures of stereoisomers may be separated by standard techniques including, but not limited to, resolution of racemic form, normal, reverse-phase, and chiral chromatography, preferential salt formation, recrystallization, and the like, or by chiral synthesis either from chiral starting materials or by deliberate synthesis of target chiral centers.

In addition, atoms making up the compounds described herein are intended to include all isotopic forms of such atoms. Isotopes, as used herein, include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of

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hydrogen include tritium and deuterium, and isotopes of carbon include ¹³C and ¹⁴C.

As noted above, compounds described herein may exist in prodrug form. As used herein, "prodrug" is intended to include any covalently bonded carriers which release the active parent drug or compounds that are metabolized in vivo to an active drug or other compounds employed in the methods described herein in vivo when such prodrug is administered to a subject. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.), the compounds employed in some methods described herein may, if desired, be delivered in prodrug form. Thus, the invention contemplates prodrugs of compounds described herein as well as methods of delivering prodrugs. Prodrugs of the compounds employed in embodiments may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound.

Accordingly, prodrugs include, for example, compounds described herein in which a hydroxy, amino, or carboxy group is bonded to any group that, when the prodrug is administered to a subject, cleaves to form a free hydroxyl, free amino, or carboxylic acid, respectively. Other examples include, but are not limited to, acetate, formate, and benzoate derivatives of alcohol and amine functional groups; and alkyl, carbocyclic, aryl, and alkylaryl esters such as methyl, ethyl, propyl, iso-propyl, butyl, isobutyl, sec-butyl, tert-butyl, cyclopropyl, phenyl, benzyl, and phenethyl esters, and the like.

It should be recognized that the particular anion or cation forming a part of any salt in any embodiment is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, Selection and Use* (2002), which is incorporated herein by reference.

C. PHARMACEUTICAL FORMULATIONS AND ADMINISTRATION THEREOF

1. Pharmaceutical Formulations and Routes of Administration

Pharmaceutical compositions disclosed herein comprise an effective amount of one or more candidate substance or additional agent dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical" or "pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one candidate substance or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's *Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels,

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binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's *Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The compounds administered according to embodiments disclosed herein may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. Embodiments can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, systemically, subcutaneously, subconjunctival, intravesicularily, mucosally, intrapericardially, intraumbilically, intraocularly, orally, locally, via inhalation (e.g., aerosol inhalation), via injection, via infusion, via continuous infusion, via localized perfusion bathing target cells directly, via a catheter, via a lavage, in creams, in lipid compositions (e.g., liposomes), or by other method or any combination of the foregoing as would be known to one of ordinary skill in the art (see, for example, Remington's *Pharmaceutical Sciences*, 1990).

The actual dosage amount of a composition of embodiments disclosed herein that are administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of a compound of the present disclosure. In other embodiments, the compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited

to parabens (e.g., methylparabens, propylparabens), chlorbutanol, phenol, sorbic acid, thimerosal, or combinations thereof.

The candidate substance may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine, or procaine.

In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, etc.), lipids (e.g., triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or combinations thereof such methods. It may be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof.

In other embodiments, one may use eye drops, nasal solutions or sprays, aerosols or inhalants. Such compositions are generally designed to be compatible with the target tissue type. In a non-limiting example, nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, in certain embodiments the aqueous nasal solutions usually are isotonic or slightly buffered to maintain a pH of about 5.5 to about 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, drugs, or appropriate drug stabilizers, if required, may be included in the formulation. For example, various commercial nasal preparations are known and include drugs such as antibiotics or antihistamines.

In certain embodiments the candidate substance is prepared for administration by such routes as oral ingestion. In these embodiments, the solid composition may comprise, for example, solutions, suspensions, emulsions, tablets, pills, capsules (e.g., hard or soft shelled gelatin capsules), sustained release formulations, buccal compositions, troches, elixirs, suspensions, syrups, wafers, or combinations thereof. Oral compositions may be incorporated directly with the food of the diet. In certain embodiments, carriers for oral administration comprise inert diluents, assimilable edible carriers or combinations thereof. In other aspects, the oral composition may be prepared as a syrup or elixir. A syrup or elixir, and may comprise, for example, at least one active agent, a sweetening agent, a preservative, a flavoring agent, a dye, a preservative, or combinations thereof.

In certain embodiments an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. In certain embodiments, a composition may comprise one or more of the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof, an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium

saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc.; or combinations thereof the foregoing. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both.

Additional formulations which are suitable for other modes of administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum, vagina, or urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides, or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, certain methods of preparation may include vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The preparation of highly concentrated compositions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein.

In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin, or combinations thereof.

2. Combination Therapy

In some embodiments, it is contemplated that the tolerance-inducing compositions disclosed herein may be used in conjunction with the compositions for which a tolerance is being induced as part of a treatment regimen. This process may involve contacting the cell(s) with the agents at the same time or within a period of time wherein separate administration of the agents produces a desired therapeutic benefit. This may be achieved by contacting the cell, tissue or organism with a single composition or pharmacological

formulation that includes two or more agents, or by contacting the cell with two or more distinct compositions or formulations, wherein one composition includes one agent and the other includes another.

Compounds discussed herein may precede, be co-current with and/or follow the other agents by intervals ranging from minutes to weeks. In embodiments where the agents are applied separately to a cell, tissue or organism, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agents would still be able to exert an advantageously combined effect on the cell, tissue or organism. For example, in such instances, it is contemplated that one may contact the cell, tissue or organism with two, three, four or more modalities substantially simultaneously (i.e., within less than about a minute) as the candidate substance. In other aspects, one or more tolerance-inducing compositions may be administered or provided within 1 minute, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, 30 hours, 31 hours, 32 hours, 33 hours, 34 hours, 35 hours, 36 hours, 37 hours, 38 hours, 39 hours, 40 hours, 41 hours, 42 hours, 43 hours, 44 hours, 45 hours, 46 hours, 47 hours, 48 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, or 8 weeks or more, and any range derivable therein, prior to administering the compositions for which a tolerance is being induced. In some embodiments, more than one course of therapy may be employed. It is contemplated that multiple courses may be implemented.

D. ORGANISMS AND CELL SOURCE

Cells that may be used in some methods can be from a variety of sources. Embodiments include the use of mammalian cells, such as cells from monkeys, chimpanzees, rabbits, mice, rats, ferrets, dogs, pigs, humans, and cows. Alternatively, the cells may be from fruit flies, yeast, or *E. coli*, which are all model systems for evaluating homologous recombination.

Methods can involve cells, tissues, or organs involving the heart, lung, kidney, liver, bone marrow, pancreas, skin, bone, vein, artery, cornea, blood, small intestine, large intestine, brain, spinal cord, smooth muscle, skeletal muscle, ovary, testis, uterus, and umbilical cord.

Moreover, methods can be employed in cells of the following type: platelet, myelocyte, erythrocyte, lymphocyte, adipocyte, fibroblast, epithelial cell, endothelial cell, smooth muscle cell, skeletal muscle cell, endocrine cell, glial cell, neuron, secretory cell, barrier function cell, contractile cell, absorptive cell, mucosal cell, limbus cell (from cornea), stem cell (totipotent, pluripotent or multipotent), unfertilized or fertilized oocyte, or sperm.

Moreover, methods can be implemented with or in plants or parts of plants, including fruit, flowers, leaves, stems, seeds, cuttings. Plants can be agricultural, medicinal, or decorative.

E. ANTIGENS

The antigen employed as X in the compositions of Formula 1, or in any of the compositions or methods of the

current disclosure, can be a protein or a peptide, e.g. the antigen may be a complete or partial therapeutic agent, a full-length transplant protein or peptide thereof, a full-length autoantigen or peptide thereof, a full-length allergen or peptide thereof, and/or a nucleic acid, or a mimetic of an aforementioned antigen. Combinations of multiple fragments may also be used, depending on the embodiment. For example, if a longer peptide identified as P has antigenic regions A, B, C, and D, compositions disclosed herein for induction of tolerance to P can comprise any combination of A, B, C, and D, and repeats of any of A, B, C, and D. A listing of any particular antigen in a category or association with any particular disease or reaction does not preclude that antigen from being considered part of another category or associated with another disease or reaction.

In several embodiments, the antigen comprises one or more therapeutic agents that are proteins, peptides, antibodies and antibody-like molecules (including antibody fragments and fusion proteins with antibodies and antibody fragments), and gene therapy vectors. These include human, non-human (such as mouse) and non-natural (e.g., engineered) proteins, antibodies, chimeric antibodies, humanized antibodies, viruses and virus-like particles, and non-antibody binding scaffolds, such as fibronectins, DARPins, knottins, and the like. In several embodiments, human allograft transplantation antigens against which transplant recipients develop an unwanted immune response are used. In several embodiments, the antigen comprises one or more self-antigens that cause an unwanted, autoimmune response. While self-antigens are of an endogenous origin in an autoimmune disease patient, according to several embodiments, the polypeptides employed in the disclosed compositions are, depending on the embodiment, synthesized exogenously (as opposed to being purified and concentrated from a source of origin).

In several embodiments, the antigen to which tolerance is desired comprises one or more foreign antigens, such as food, animal, plant and environmental antigens, against which a patient experiences an unwanted immune response. While a therapeutic protein can also be considered a foreign antigen due to its exogenous origin, for purposes of clarity in the description of the present disclosure such therapeutics are described as a separate group. Similarly, a plant or an animal antigen can be eaten and considered a food antigen, and an environmental antigen may originate from a plant. They are, however, considered foreign antigens. In the interest of simplicity no attempt will be made to describe distinguish and define all of such potentially overlapping groups, as those skilled in the art can appreciate the antigens that can be employed in the compositions of the disclosure, particularly in light of the detailed description and examples.

In several embodiments, X is selected from the group consisting of insulin, proinsulin, preproinsulin, gluten, gliadin, myelin basic protein, myelin oligodendrocyte glycoprotein and proteolipid protein, Factor VIII, Factor IX, asparaginase, uricase and fragments of any of the preceding. In several embodiments, the antigen X is not a full length protein. For example, in some embodiments, the antigen is not full length gliadin, insulin, or proinsulin. In several embodiments, the antigen is not full length myelin basic protein, not full length myelin oligodendrocyte protein, or not full length proteolipid protein. In several embodiments, the antigen X is not a fragment of a protein. As discussed in more detail below, there exist a variety of antigens to which tolerance may be desired. These may include, but are not limited to, exogenous antigens that result in an adverse immune response when a subject is exposed to the antigen.

In several embodiments, the adverse immune response could be a result of ingestion of the antigen, e.g., orally or nasally, or via some other mucosal route. These routes could be the case, for example, with food antigens. In some embodiments, the antigen may be purposefully administered to a subject, for example, with the administration of a therapeutic composition to treat a disease or condition that the subject is affected by. In still additional embodiments, the antigen may be produced by the subject, e.g., an autoimmune antigen. For example, in several embodiments, X comprises a foreign transplant antigen against which transplant recipients develop an unwanted immune response or a tolerogenic portion thereof. In several embodiments, X comprises a foreign food, animal, plant or environmental antigen against which patients develop an unwanted immune response or a tolerogenic portion thereof. In several embodiments, X comprises a foreign therapeutic agent against which patients develop an unwanted immune response or a tolerogenic portion thereof. In several embodiments, X comprises a synthetic self-antigen against the endogenous version of which patients develop an unwanted immune response or a tolerogenic portion thereof.

In further detail to the above, there are provided in several embodiments, compounds where X is a food antigen. In some such embodiments, X is one or more of conarachin (Ara h 1), allergen II (Ara h 2), arachis agglutinin, conglutin (Ara h 6), α -lactalbumin (ALA), lactotransferrin, Pen a 1 allergen (Pen a 1), allergen Pen m 2 (Pen m 2), tropomyosin fast isoform, high molecular weight glutenin, low molecular weight glutenin, alpha-gliadin, gamma-gliadin, omega-gliadin, hordein, seclain, and avenin. Fragment of any of these antigens and/or mimotopes of any of these antigens are also used, in several embodiments. In several embodiments, X is selected from the group consisting of gluten, high molecular weight glutenin, low molecular weight glutenin, alpha-gliadin, gamma-gliadin, omega-gliadin, hordein, seclain, and avenin and fragments thereof. In several embodiments, X is selected from the group consisting of gluten, high molecular weight glutenin, low molecular weight glutenin, alpha-gliadin, gamma-gliadin, and omega-gliadin and fragments thereof. In several embodiments, X is gluten or fragment thereof. In several embodiments, X is gliadin or fragment thereof.

In several embodiments, there are provided compounds where X is a therapeutic agent. In several embodiments, X is selected from the group consisting of Factor VII, Factor IX, asparaginase, and uricase and fragments thereof. In several embodiments, X is a therapeutic agent selected from the group consisting of Factor VII and Factor IX and fragments thereof. In several embodiments, X is a therapeutic agent selected from the group consisting of Factor VIII or fragment thereof. In several embodiments, when X is a therapeutic agent, the compound can be used in the treatment, prevention, reduction, or otherwise amelioration of an immune response developed against a therapeutic agent for hemophilia. As discussed herein, mimotopes of any antigenic portion of the antigens above can be used in several embodiments.

In several embodiments, X comprises asparaginase or a fragment thereof. In several embodiments, X comprises uricase or a fragment thereof. In several such embodiments, the compound can be used in the treatment, prevention, reduction, or otherwise amelioration of an immune response developed against an anti-neoplastic agent. As discussed herein, mimotopes of any antigenic portion of the antigens above can be used in several embodiments.

In several embodiments, X is associated with an autoimmune disease. For example, in several embodiments, the associated autoimmune disease is one or more of Type I diabetes, multiple sclerosis, rheumatoid arthritis, vitiligo, uveitis, pemphigus vulgaris and neuromyelitis optica.

In several embodiments, the autoimmune disease is Type I diabetes and X comprises insulin or a fragment thereof. In several embodiments, the autoimmune disease is Type I diabetes and X comprises proinsulin or a fragment thereof.

10 In several embodiments, the autoimmune disease is Type I diabetes and X comprises preproinsulin or a fragment thereof. As discussed herein, mimotopes of any antigenic portion of the antigens above can be used in several embodiments. In several embodiments, combinations of these antigens can be incorporated into the tolerogenic compound which may aid in reducing immune responses to self-antigens at multiple points along the insulin pathway.

In several embodiments, the autoimmune disease is multiple sclerosis and X comprises myelin basic protein or a fragment thereof. In several embodiments, the autoimmune disease is multiple sclerosis and X comprises myelin oligodendrocyte glycoprotein or a fragment thereof. In several embodiments, the autoimmune disease is multiple sclerosis and X comprises proteolipid protein or a fragment thereof.

20 As discussed herein, mimotopes of any antigenic portion of the antigens above can be used in several embodiments. In several embodiments, combinations of these antigens can be incorporated into the tolerogenic compound (e.g., a mixture of antigens or fragments of MOG, MBP and/or PLP) which may aid in reducing immune responses to self-antigens at multiple points along the enzymatic pathways that control myelination or myelin repair.

As discussed herein, mimotopes of any antigenic portion of the self-antigens above (or otherwise disclosed herein) can be used in several embodiments.

In several embodiments, the pharmaceutically acceptable composition consists of, or consists essentially of a compound wherein X is a food antigen, therapeutic agent, a self antigen, or fragment thereof, a linker Y, and a liver targeting moiety Z selected from mannose and/or a mannose receptor-targeting moiety (including, but not limited to, one or more of mannosamine, N-acetylmannosamine, or N-acetylglucosamine).

The tolerogenic antigen can be a complete protein, a portion of a complete protein, a peptide, or the like, and can be derivatized (as discussed above) for attachment to a linker and/or antigen-binding moiety, can be a variant and/or can contain conservative substitutions, particularly maintaining sequence identity, and/or can be desialylated.

45 In the embodiments where the antigen is a therapeutic protein, peptide, antibody or antibody-like molecule, specific antigens can be selected from: Abatacept, Abciximab, Adalimumab, Adenosine deaminase, Ado-trastuzumab emtansine, Agalsidase alfa, Agalsidase beta, Aldesleukin, Alglicerase, Alglucosidase alfa, α -1-proteinase inhibitor, Anakinra, Anistreplase (anisoylated plasminogen streptokinase activator complex), Antithrombin III, Antithymocyte globulin, Ateplase, Bevacizumab, Bivalirudin, Botulinum toxin type A, Botulinum toxin type B, C1-esterase inhibitor, Canakinumab, Carboxypeptidase G2 (Glucarpidase and Voraxaze), Certolizumab pegol, Cetuximab, Collagenase, Crotalariae immune Fab, Darbepoetin- α , Denosumab, Digoxin immune Fab, Dornase alfa, Eculizumab, Etanercept, Factor VIIa, Factor VIII, Factor IX, Factor XI, Factor XIII, Fibrinogen, Filgrastim, Galsulfase, Golimumab, Histrelin acetate, Hyaluronidase, Idursulphase, Imiglucerase, Infliximab, Insulin [including recombinant human insulin

("rHu insulin") and bovine insulin], Interferon- α 2a, Interferon- α 2b, Interferon- β 1a, Interferon- β 1b, Interferon- γ 1b, I�imumab, L-arginase, L-asparaginase, L-methionase, Lactase, Laronidase, Lepirudin/hirudin, Mecasermin, Mecasermin rinfabate, Methoxy Natalizumab, Octreotide, Ofatumumab, Oprelvekin, Pancreatic amylase, Pancreatic lipase, Papain, Peg-asparaginase, Peg-doxorubicin HCl, PEG-epoetin- β , Pegfilgrastim, Peg-Interferon- α 2a, Peg-Interferon- α 2b, Pegloticase, Pegvisomant, Phenylalanine ammonia-lyase (PAL), Protein C, Rasburicase (uricase), Sacrosidase, Salmon calcitonin, Sargramostim, Streptokinase, Tenecteplase, Teriparatide, Tocilizumab (atilizumab), Trastuzumab, Type 1 alpha-interferon, Ustekinumab, vWF factor. The therapeutic protein can be obtained from natural sources (e.g., concentrated and purified) or synthesized, e.g., recombinantly, and includes antibody therapeutics that are typically IgG monoclonal or fragments or fusions.

Particular therapeutic protein, peptide, antibody or anti-body-like molecules include, but are not limited to, Abciximab, Adalimumab, Agalsidase alfa, Agalsidase beta, Aldeslukin, Alglucosidase alfa, Factor VIII, Factor IX, Infliximab, Insulin (including rHu Insulin), L-asparaginase, Laronidase, Natalizumab, Octreotide, Phenylalanine ammonia-lyase (PAL), or Rasburicase (uricase) and generally IgG monoclonal antibodies in their varying formats.

Some embodiments employ hemostatic agents (e.g., Factor VIII and IX), Insulin (including rHu Insulin), and the non-human therapeutics uricase, PAL and asparaginase.

In several embodiments, therapeutic agents are delivered through the use of, e.g., a gene therapy vector. In some such embodiments, an immune response may be developed against a portion of such vectors and/or their cargo (e.g., the therapeutic agent). Thus, in several embodiments, the antigen to which tolerance is desired comprises a gene therapy vector, including, but are not limited to: adenoviruses and adeno-associated virus (and corresponding variants-1, -2, -5, -6, -8, -9, and/or other parvoviruses), lentiviruses, and retroviruses.

Unwanted immune response in hematology and transplant includes autoimmune aplastic anemia, transplant rejection (generally), and Graft vs. Host Disease (bone marrow transplant rejection). In the embodiments where the tolerogenic antigen is a human allograft transplantation antigen, specific sequences can be selected from: subunits of the various MHC class I and MHC class II haplotype proteins (for example, donor/recipient differences identified in tissue cross-matching), and single-amino-acid polymorphisms on minor blood group antigens including RhCE, Kell, Kidd, Duffy and Ss. Such compositions can be prepared individually for a given donor/recipient pair.

In type 1 diabetes mellitus, antigens include, but are not limited to: insulin, proinsulin, preproinsulin, glutamic acid decarboxylase-65 (GAD-65 or glutamate decarboxylase 2), GAD-67, glucose-6 phosphatase 2 (IGRP or islet-specific glucose 6 phosphatase catalytic subunit related protein), insulinoma-associated protein 2 (IA-2), and insulinoma-associated protein 2 β (IA-2 β); other antigens include ICA69, ICA12 (SOX-13), carboxypeptidase H, Imogen 38, GLIMA 38, chromogranin-A, HSP-60, carboxypeptidase E, peripherin, glucose transporter 2, hepatocarcinoma-intestine-pancreas/pancreatic associated protein, S100 β , glial fibrillary acidic protein, regenerating gene II, pancreatic duodenal homeobox 1, dystrophia myotonica kinase, islet-specific glucose-6-phosphatase catalytic subunit-related protein, and SST G-protein coupled receptors 1-5, or immunogenic fragments or portions of any of such antigens. It should be noted that insulin is an example of an antigen that

can be characterized both as a self-antigen and a therapeutic protein antigen. For example, rHu Insulin and bovine insulin are therapeutic protein antigens (that are the subject of unwanted immune attack), whereas endogenous human insulin is a self-antigen (that is the subject of an unwanted immune attack). Because endogenous human insulin is not available to be employed in a pharmaceutical composition, a recombinant form is employed in certain embodiments of the compositions of the disclosure.

Human insulin, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P01308):

(SEQ ID NO: 1)
 MALWMRLPLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGF
 YTPKTRREAEDLQVGQVELGGPGAGSLQPLALEGSLOQKRGIVEQCCTS
 ICSLYQLENYCN

GAD-65, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT Q05329):

(SEQ ID NO: 2)
 MASPGSGFWSGSEDGSDENPGTARAWCQVAQKFTGGIGNKLCALLY
 GDAEKPAESGGSQPPRAARKAACACDQKPCSCSKVDVNYAFLHATDLL
 30 PACDGERPLTLAQDVNMNILLQYVVKSFDRSTKVIFDHYPNELLQEYNW
 ELADQPQNLEEILMHQCQTLKYAIKTGHPRYFNQLSTGLDMVGLAADWL
 TSTANTNMFTYEIAPVFLLEYVTLLKKMREIIIGWPGGSGDGFSPGGAI
 SNMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLKKGAAALGI
 GTDSVILIKCDERGKMPSDLERRILEAKQKGFPFLVSATAGTTVYGA
 FDPLLAVIDICKYKIWMHVDAAWGGGLMSRKHKWKLGSVERANSVTW
 NPHKMMGVPLQCSALLVREEGLMQNCNQMHASYLFFQQDKHYDLSYDTGD
 40 KALQCGRHVDFVFLWLMWRAKGTTGFEAHVDKCLELAEYLYNIINKREG
 YEMVFDGKPQHTNVCFWYIIPPSLRTLEDNEERMSRLSKVAPIKARMME
 YGTTMVSYQPLGDKVNFPRMVISNPAAATHQDIDFLIEEIERLGQDL

IGRP, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT QN9QR9):

(SEQ ID NO: 3)
 MDFLHRNGVLIIQHLQKDYRAYYTFLNFMNSNVGDPRNIFIYFPLCFQF
 NQTVGTMIVAVIGDWLNLIPIWKWLFHGRPYWWVQETQIYPNHSSPCL
 55 EQFPPTCETGPSPSGHAMGASCWVYVMVTAALSHTVCGMDKSITLHR
 LTWSFLWSVFWLIQISVCISRVFIATHEPHQVILGVIGGMLVAEAFEH
 PGIQTASLGTYLKTNLFLFLFAVGFYLLLRLVNIDLLWSVPIAKKWCAN
 60 PDWIHIDTPFAGLVRNLGVLFGLGFAINSEMFLSCRGNNYTLSFRL
 LCALTSLTILQLYHFLQIPTHEEHLFYVLSFCKSASIPLTVVAFIPYSV
 HMLMKQSGKKSQ.

In several embodiments, human proinsulin, including an exogenously obtained form useful in the tolerogenic compositions of the disclosure, has the following sequence:

(SEQ ID NO: 4)
 FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGPG
 AGSLQPLALEGSLQKRGIVEQCCTSCISLYQLENYC.

Depending on the embodiment, peptides/epitopes useful in the tolerogenic compositions of the disclosure for treating type 1 diabetes include some or all of the following sequences, individually in a tolerogenic composition or together in a cocktail of tolerogenic compositions:

Human Proinsulin 1-70:

(SEQ ID NO: 5)
 FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGPG
 AGSLQPLALEGSLQKRGIVEQ;

Human Proinsulin 9-70:

Human Proinsulin 1-70:

(SEQ ID NO: 6)
 SHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGPGAGSLQPLA
 LEGSLQKRGIVEQ;

Human Proinsulin 9-38:

(SEQ ID NO: 7)
 SHLVEALYLVCGERGFFYTPKTRREAEDLQ;

Human Proinsulin 1-38:

(SEQ ID NO: 8)
 FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQ;

Human Proinsulin 9-23:

(SEQ ID NO: 9)
 SHLVEALYLVCGERG;

Human Proinsulin 45-71 (C13-A6):

(SEQ ID NO: 10)
 GGGPGAGSLQPLALEGSLQKRGIVEQC;

Human Proinsulin C24-A1:

(SEQ ID NO: 11)
 LALEGSLQKRG;

Human Proinsulin C19-A3:

(SEQ ID NO: 12)
 GSLQPLALEGSLQKRGIV;

Human Proinsulin C13-32:

(SEQ ID NO: 13)
 GGGPGAGSLQPLALEGSLQK;

Human Proinsulin B9-C4:

(SEQ ID NO: 14)
 SHLVEALYLVCGERGFFYTPKTRREAED;

Human Proinsulin C22-A5:

(SEQ ID NO: 15)
 QPLALEGSLQKRGIVEQ;

Human IA-2 718-782:

(SEQ ID NO: 16)
 AYQAEPNTCATAQGEGENNIKKNRHPDFLPYDHARIKLKVESSPSRSYIN
 ASPIIEHDPRMPAYIA;

Human IA-2 785-819:

(SEQ ID NO: 17)
 GPLSHTIADFWQM伟WESGCTVIMLTPLVEDGVKQ;

Human IA-2 828-883:

(SEQ ID NO: 18)
 GASLYHVYEVNLVSEHIWCEDFLVRSFYLKVNQTQETRTLQFHFLSWP
 AEGTPAS;

Human IA-2 943-979:

(SEQ ID NO: 19)
 EHVRDQRPGGLVRSKDQFEFALTAVAEEVNAILKALPQCG.

In autoimmune diseases of the thyroid, including Hashimoto's thyroiditis and Graves' disease, main antigens

include, but are not limited to, thyroglobulin (TG), thyroid peroxidase (TPO) and thyrotropin receptor (TSRH); other antigens include sodium iodine symporter (NIS) and megalin. In thyroid-associated ophthalmopathy and dermopathy, in addition to thyroid autoantigens including TSRH, an antigen is insulin-like growth factor 1 receptor. In hypoparathyroidism, a main antigen is calcium sensitive receptor.

In Addison's Disease, main antigens include, but are not limited to, 21-hydroxylase, 17 α -hydroxylase, and P450 side chain cleavage enzyme (P450sc); other antigens include ACTH receptor, P450c21 and P450c17.

In premature ovarian failure, main antigens include, but are not limited to, FSH receptor and α -enolase.

In autoimmune hypophysitis, or pituitary autoimmune disease, main antigens include, but are not limited to, pituitary gland-specific protein factor (PGSF) 1a and 2; another antigen is type 2 iodothyronine deiodinase.

In multiple sclerosis, main antigens include, but are not limited to, myelin basic protein ("MBP"), myelin oligodendrocyte glycoprotein ("MOG") and myelin proteolipid protein ("PLP").

MBP, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P02686):

(SEQ ID NO: 20)
 MGNHAGKRELNAEKASTNSETNRGESEKKRNLGELSRTTSEDNEVPEA
 DANQNNGNTSSQD TAVTD SKRTADPKNAWQDAHPADPGSRPHLIRLFLSRD
 APGR DENTFKDRPSESDELQTIQEDSAATSESLDVMASQKRPSQRHGSK
 YLATASTMDHARHGFLPRHRDTGILD S1 GRFFGGDRGAPKRGSGKDHH
 PARTAHYGS LPQKSHGR TQDENPVVHFFKNIVTPRTPPPSQGKGRLSL
 SRFSWGAEGQRPFGYGRASDYKSAHKGFKGVD AQGTL SKIFKLGGRD
 SRSGSPMARR.

MOG, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT Q16653):

(SEQ ID NO: 21)
 MASLSRPSLPSCLCSFLLLLLQVSSSYAGQFRVIGPRHPIRALVGDEV
 ELP CRIS PGKNATGMEVGWYRPPFSRVVHLYRNGKDQGDQAPEYRGRT
 45 ELLKDAIGEGKVTLRIRNVRFSDEGGFTCF FRDH SYQEEAAMELKVEDP
 FYWVSPGVLVLLAVLPVLLQITVGLIFLCLQYRLRGKLR AEIENLHRT
 FDPHFLRVP CWKITLFVIVPVLGPLVALIICYNWLHRRLAGQFLEELRN
 50 PF.

PLP, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P60201):

(SEQ ID NO: 22)
 MGLLECCARCLVGAPFASL VATGLCFFGVALFCGCHEAL TGTEKLIET
 YFSK NYQDYEY LINVI HAFQYVIYGTASFFF LYGALLAEGFYTTGAVR
 QIFGDYKTTICGKGLSATVTGGQKGRGSRQHQAHSLERVCHCLGKWLG
 HPDKFVGITYALT VVWLLVFA CSAPVYIYFNTWTT CQSIAFPSKTSAS
 60 IGS LCA DARMYGVLPWN AFPGKVC GS NLLSICKTAEFQMTFHLFIAAFV
 GAAATLVSLTFMIAATYNFAVLKLMGRGKTF.

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Peptides/epitopes useful in the compositions of the disclosure for treating multiple sclerosis include some or all of the following sequences, individually in a tolerogenic composition as disclosed herein or together in a combination (e.g., a cocktail) of tolerogenic compositions:

MBP 13-32:
KYLATASTMDHARHGFLPRH;
(SEQ ID NO: 23)

MBP 83-99:
ENPWHTFFKNIVTPRTP;
(SEQ ID NO: 24)

MBP 111-129:
LSRFSWGAEGQRPGFGYGG;
(SEQ ID NO: 25)

MBP 146-170:
AQGTLSKIFKLGGRDSRGSPMARR;
(SEQ ID NO: 26)

MOG 1-20:
GQFRVIGPRHPIRALVGDEV;
(SEQ ID NO: 27)

MOG 35-55:
MEVGWYRPPFSRVHLYRNGK;
(SEQ ID NO: 28)

PLP 139-154:
HCLGKWLGHPDKFVGI;
(SEQ ID NO: 29)

MOG 30-60:
KNATGMVEGVWYRSPFSRVVHLYRNGKDQDAE;
(SEQ ID NO: 30)

MBP 83-99:
ENPVVHFFKNIVTPRTP;
(SEQ ID NO: 31)

MOG 35-55:
MEVGWYRPPFSRVHLYRNGK;
(SEQ ID NO: 32)

MBP 82-98:
DENPVVHFFKNIVTPRTP;
(SEQ ID NO: 33)

MBP 82-99:
DENPVVHFFKNIVTPRTP;
(SEQ ID NO: 34)

MBP 82-106:
DENPVVHFFKNIVTPRTPPPSQGKG;
(SEQ ID NO: 35)

MBP 87-106:
VHFFKNIVTPRTPPPSQGKG;
(SEQ ID NO: 36)

MBP 131-155:
ASDYKSAHKGLGVDAQGTLISKIFK;
(SEQ ID NO: 37)

PLP 41-58:
GTEKLIETYFSKNYQDYE;
(SEQ ID NO: 38)

PLP 89-106:
GFYTTGAVRQIPGQDYKTT;
(SEQ ID NO: 39)

PLP 95-116:
AVRQIFGQDYKTTICGKGLSATV;
(SEQ ID NO: 40)

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-continued

PLP 178-197:
NTWTTTCQSIAFPSKTSASIG;
(SEQ ID NO: 41)

5 PLP 190-209:
SKTSASIGSLCADARMYGVL;
(SEQ ID NO: 42)

MOG 11-30:
PIRALVGDEVELPCRISPGK;
(SEQ ID NO: 43)

MOG 21-40:
ELPCRISPGKNATGMEVGWY;
(SEQ ID NO: 44)

MOG 64-86:
EYRGRTELLKDAIGEGKVTLRIR;
(SEQ ID NO: 45)

MOG 1-62:
GQFRVIGPRHPIRALVGDEVELPCRISPGKNATGMEVGWYRPPFSRVH
LYRNGKDQDQA
(SEQ ID NO: 46)

MBP 76-136:
SHGRQTQDENPVVHFFKNIVTPRTPPPSQGKGRLSLSRFSWGAEGQRPG
FGYGGRADSYKSCG
(SEQ ID NO: 47)

In rheumatoid arthritis, main antigens include, but are not limited to, collagen II, immunoglobulin binding protein, the fragment crystallizable region of immunoglobulin G, double-stranded DNA, and the natural and cirtullinated forms of proteins implicated in rheumatoid arthritis pathology, including fibrin/fibrinogen, vimentin, collagen I and II, and alpha-enolase.

In autoimmune gastritis, a main antigen is H+, K+-AT-Pase.

In pernicious anemia, a main antigen is intrinsic factor.

40 In celiac disease, main antigens include, but are not limited to, tissue transglutaminase and the natural and deamidated forms of gluten or gluten-like proteins, such as alpha-, gamma-, and omega-gliadin, gliutenin, hordein, secalin, and avenin. Those skilled in the art will appreciate, for example, that while the main antigen of celiac disease is alpha gliadin, alpha gliadin turns more immunogenic in the body through deamidation by tissue glutaminase converting alpha gliadin's glutamines to glutamic acid. Thus, while alpha gliadin is originally a foreign food antigen, once it has been modified in the body to become more immunogenic it can be characterized as a self-antigen, depending on the embodiment.

45 In vitiligo, a main antigen is tyrosinase, and tyrosinase related protein 1 and 2.

50 MART1, Melanoma antigen recognized by T cells 1, Melan-A, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT Q16655):

55 (SEQ ID NO: 48)
MPREDAHFIYGYPKKGHGSYTTAEAAAGIGILTVIDGVLLLIGCWYCR
RRNGYRALMDKSLHVGTCALTRRCPOEGFDHRDSKVLQEKNCPEVVP
NAPPAYEKLSAEQSPPPYSP.

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Tyrosinase, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P14679):

(SEQ ID NO: 49)
 MLLAVLYCLLWSFQTSAGHFPRACVSSKNLMEKECCPPWSDRSPCGQL
 SGRGSCQNILLSNAPLGQFPFTGVDDRESWPSVFYNRTCQCSGNFMGF
 NCGNCKPGFWGPNCTERLLVRRNIFDLSAPEKDKFFAYLTLAKHTISS
 DYVIPIGTYQGMKNGSTPMENDINIYDLFVWMHYYVSMDALLGGSEIWR
 DIFDAFAHEAPAFLPWHRLFLLRWEQEIQKLTDENFTIPYWDWRDAEKCD
 ICTDEYMGQHPTNPNLSPASFFSSWQIVCSRLEEEYNSHQSLCNGTPE
 GPLRRNPGNHDKSRTPRLPSSADVEFCLSLTQYESGSMDKAANFSFRNT
 LEGFASPLTGIADASQSSMHNALHIYMNGTMSQVQGSANDPIFLHHAF
 VDSIFEQWLRRHRPLQEVEVYPEANAPIGHNRSEYMVPFIPLYRNGDPFIS
 SKDLYDYSYLQSDPDPSFQDYIKSYLEQASRIWSLLGAAMVGAVLTA
 LLAGLVSSLRCRHKRKQLPEEKQPLLMEEKDYHSLYQSHL

Melanocyte protein PMEL, gp100, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P40967):

(SEQ ID NO: 50)
 MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVRQLRTKAWNRLQY
 PEWTEAQRLCDWRGGQVSLKVSNNDGPTLIGANASFIALNPPGSQKVLP
 DGQVIWVNNTIINGSQVWGGQPVYPQETDDACIFPDGGPCPSGSWSQKR
 SFVYVWKWQYQVILGGPVSGLSIGTGRAMLGTHTMEVTVYHRRGSRS
 YVPLAHSSSAFTITDQVPFSVSVSQLRALDGNNKHFLRNQPLTFALQLH
 DPSGYLAEADLSYTWDPGDSSGTLISRALVVTHTYLEPGPVTAQVVLQA
 AIPLTSCGSSPVPGTTDGHRTAEAPNTTAGQVPTTEVVGTTPGQAPTA
 EPSGTTSVQVPTTEVISTAPVQMPATAESTGMTPEKVPVSEVMGTTLAEM
 STPEATGMPAEVSVIVLSGTTAAQVTTTEWETTARELPIPEPEGPDA
 SSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRYGSFSVTLDIV
 QGIESAEILQAVPSGEGDAFELTVSCQGGLPKEAACMEISSPGCQPPAQR
 LCQVLPSPACQQLVLHQILKGSSGTYCLNVSLADTNSLAVVSTQLIMP
 QEAGLGQVPLIVGILLVLMAVVLASLIYRRRLMKQDFSVPQLPHSSSHW
 LRLPRIFCSCPGENSPLLSGQQV.

In myasthenia gravis, a main antigen is acetylcholine receptor.

In pemphigus vulgaris and variants, main antigens include, but are not limited to, desmoglein 3, 1 and 4; other antigens include pemphaxin, desmocollins, plakoglobin, periplakin, desmplakins, and acetylcholine receptor.

In bullous pemphigoid, main antigens include BP180 and BP230; other antigens include plectin and laminin 5.

In dermatitis herpetiformis Duhring, main antigens include, but are not limited to, endomysium and tissue transglutaminase.

In epidermolysis bullosa acquisita, a main antigen is collagen VII.

In systemic sclerosis, main antigens include, but are not limited to, matrix metalloproteinase 1 and 3, the collagen-

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specific molecular chaperone heat-shock protein 47, fibrillin-1, and PDGF receptor; other antigens include Scl-70, U1 RNP, Th/To, Ku, Jo1, NAG-2, centromere proteins, topoisomerase I, nucleolar proteins, RNA polymerase I, II and III, 5 PM-Slc, fibrillarin, and B23.

In mixed connective tissue disease, a main antigen is U1snRNP.

In Sjogren's syndrome, the main antigens include, but are not limited to, nuclear antigens SS-A and SS-B; other 10 antigens include fodrin, poly(ADP-ribose) polymerase and topoisomerase, muscarinic receptors, and the Fc-gamma receptor IIIb.

In systemic lupus erythematosus, main antigens include nuclear proteins including the "Smith antigen," SS-A, high 15 mobility group box 1 (HMGB1), nucleosomes, histone proteins and double-stranded DNA (against which auto-antibodies are made in the disease process).

In Goodpasture's syndrome, main antigens include, but are not limited to, glomerular basement membrane proteins 20 including collagen IV.

In rheumatic heart disease, a main antigen is cardiac myosin.

In autoimmune polyendocrine syndrome type 1 antigens include aromatic L-amino acid decarboxylase, histidine 25 decarboxylase, cysteine sulfinate acid decarboxylase, tryptophan hydroxylase, tyrosine hydroxylase, phenylalanine hydroxylase, hepatic P450 cytochromes P4501A2 and 2A6, SOX-9, SOX-10, calcium-sensing receptor protein, and the type 1 interferons interferon alpha, beta and omega.

In neuromyelitis optica, a main antigen is AQP4.

Aquaporin-4, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P55087):

35 (SEQ ID NO: 51)
 MSDRPTARRWGKCGPLCTRENIMVAFGVWTQAFWKAVTAEFLAMILFV
 LLSLGSTINWGGTEKPLPVDMVLISLCFGLSIATMVQCFGHISGGHINP
 40 AVTVAMVCTRKSIAKSVFYIAAQCLGAIIGAGILYLVTPPSVVGGLGV
 TMVHGNTLIGHGLLVELIITFQLVFTIFASCDSKRTDVTGSIALAIGFS
 VAIGHLFAINYTGASMNPARSFGPAVIMGNWENHWIYWVGPPIGAVLAG
 45 GLYEYVFCPDVEFKRKFKEAFSKAAQQTGKGSYMEVEDNRSQVETDDLIL
 KPGVVHVIDVDRGEEKKGKDQSGEVLSSV.

In uveitis, main antigens include Retinal S-antigen or "S-arrestin" and interphotoreceptor retinoid binding protein (IRBP) or retinol-binding protein 3.

S-arrestin, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P10523):

55 (SEQ ID NO: 52)
 MAASGKTSKSEPENHVFKKISRDKSVTIYLGNDYIDHVSQVQPVGDGVV
 LVDPDLVKGKKVYVLTCAFRYQGEDIDVIGLTFRRLDLYFSRVQVYPPV
 GAASPTKLQESLLKKLGSNTYPFLTFPDYLPCSVMLQPAQDSGKSC
 60 GVDFEVKAFATDSTDAAEDKIPKKSSVRLLIRKVQHAPLEMGPQPRAEA
 AWQFFMSDKPLHLAVSLNKEIYFHGEPIPVTVTNTNEKTVKKIKAFV
 EQVANVVLVYSSDYYVKPVAMEEAQEKVPPNSTLTKTLPLLLANNRER
 65 RGIALDGKIKHEDTNLASSTIIKEGIDRTVLGILVSYQIKVKLTVSGFL

-continued

GELTSSEVATEVPFRLMHPQPEDPAKESYQDANLVFEFARHNLKDAGE
AEEGKRDKNDVDE.

IRBP, including an exogenously obtained form useful in the compositions of the disclosure, has the following sequence (UNIPROT P10745):

(SEQ ID NO: 53)
 MMREWVLLMSVLLCGLAGPTHLFQPSLVLDAKVLLDNYPFPENLLGMQ
 EAIQQQAISHEILSISDPQTASLAVLTAGVQSSLNDRPLVISYPESTPEP
 PPQVPALTSLSSEEELLAWLQRGLRHEVLEGNVGYLRVDSVPGQEVLSMS
 15 GEFLVAHVWGNLMGTSALVLDLRLHCTGGQVSGIPIISYLHPGNTILHV
 DTIYNRPSNTTTEIWTLPQVILGERYGADKDVVVLTSQTRGVIAEDI
 LKQMRRRAIVVGERTGGGALDLRKLRIGESDFFFTPVPSRSLSGPLGGGSQ
 TWEGSGVLPVCVGTPAEQALEKALAILTLRSALPGVVHCLQEVLKDYYLT
 VDRVPTLQLQHLASMDFSTVVSSEDLVTKLNAGLQAASEDPRLLVRAIGP
 TETPSWPAPAAAEDSPGVAPELPEDAIRQALVDSVFCQSVLPGNVGY
 20 LRFDASFADASVILGVLAPYVLRQWEPLODTEHIMDLRHNPGGPSAVP
 LLLSYFQGPEAGPVHLFTTYDRTTNITQEHFSHMELPGRYSTQRGVYL
 LTSHTATAAEEFAFLMQSLGWATLVGEITAGNLLHTRTVPLLDTPEGS
 25 LALTVPVLTIFDNHGEAWLGGGVPAIDVLAEEALDKAQEVLEFHQSGL
 ALVEGTGHLLAEAHYARPEVVGQTSALLRAKLAQGAYRTAVDLESLASQL
 TADLQEVSQDHRLLVFHSPGELVVEEAPPAPAVPSPEELTYLIEALFK
 TEVLPGQLGYLRFDMAELETVKAVGPQLVRLWQQLVDTAAVIDLRY
 NPGSYSTAIPLLCSYFFEAEPRQHLYSVPDRATSKVTEVWTLQVAGQR
 YGSHKDLYILMSHTSGSAAEAFAHTMQDLQRATVIGEPTAGGALSVGIVY
 QVGSSPLYASMPMTQAMASATTGKAWSLAGVEPDITVPMSEALNSIAQDIV
 ALRAKVPTVLQTAGKLVADNYASAELGAKMATKLSGLQSRYSRVTSEVA
 LAEILGADLQMLSGDPHLKAAHIPENAKDRIPGIVPMQIPSPEVFEELI
 KFSFHTNVLEDNIGYLRDFMDFGDGELLTQVSRLLVEHIWKKIMHTDAMI
 30 IDMRFNIGGPTSSIPILCSYFFFDEGPPVLLDKIYSRPDDSVSELWTHAQ
 VVGERYGSKKSMLTSSVTAGTAEEFTYIMKRLGRALVIGEVTSGGCQ
 PPQTYHVDDTNLYLTIPTARTSGASDGSSWEGVGVTPTHVVPAEEALAR
 AKEMLQHNQLRVKRSPGLQDH.

In the embodiments where the tolerogenic antigen is a foreign antigen against which an unwanted immune response can be developed, such as food antigens, specific antigens include, but are not limited to: from peanut: conarachin (Ara h 1), allergen II (Ara h 2), arachis agglutinin, conglutin (Ara h 6); conarachin, for example has the sequence identified as UNIPROT Q6PSU6; from apple: 31 kDa major allergen/disease resistance protein homolog (Mal d 2), lipid transfer protein precursor (Mal d 3), major allergen Mal d 1.03D (Mal d 1); from milk: α -lactalbumin (ALA), lactotransferrin; from kiwi: actinidin (Act c 1, Act d 1), phytocystatin, thaumatin-like protein (Act d 2), kiwelin (Act d 5); from egg whites: ovomucoid, ovalbumin, ovotransferrin, and lysozyme; from egg yolks: livetin, apovitillin, and vosvetin; from mustard: 2S albumin (Sin a 1),

11S globulin (Sin a 2), lipid transfer protein (Sin a 3), profilin (Sin a 4); from celery: profilin (Api g 4), high molecular weight glycoprotein (Api g 5); from shrimp: Pen a 1 allergen (Pen a 1), allergen Pen m 2 (Pen m 2), tropomyosin fast isoform; from wheat and/or other cereals: high molecular weight glutenin, low molecular weight glutenin, alpha-, gamma- and omega-gliadin, hordein, secalin and/or avenin; peptides/epitopes useful in the compositions of the disclosure for treating Celiac Disease include some or all of the following sequences, individually in a composition of Formula 1 or together in a cocktail of compositions of Formula 1:
 10 DQ-2 relevant, Alpha-gliadin “33-mer” native: LQLQPFPQPQLPYQPQLPYPQPQLPYPQPQPF (SEQ ID NO: 54);
 DQ-2 relevant, Alpha-gliadin “33-mer” deamidated: LQLQPFPQPELPYPQPELPYPQPELPYPQPELPYPQPF (SEQ ID NO: 55);
 15 DQ-8 relevant, Alpha-gliadin: QQYPSGQGSFQPSQQNPQ (SEQ ID NO: 56);
 DQ-8 relevant, Omega-gliadin (wheat, U5UA46): QPFPQPEQPFW (SEQ ID NO: 57);
 Alpha-gliadin “15-mer” fragment: ELQPFQPEL
 20 PYPQP (SEQ ID NO: 58);
 Gliadin linker: GCRGGGPQPKPQFPSQQPY (SEQ ID NO: 59);
 Gliadin extended: GCRGGGPQPKPQFPSQQPYQLQ
 25 QPFPQPKPQQLPYPQPKPQLPYQPKPQPF (SEQ ID NO: 60);
 Gliadin deamidated extended: GCRGGGPQPKPQFPSQQPYQLQ
 ELYPQPQELYPQPQPF (SEQ ID NO: 61); from strawberry: major strawberry allergy Fra a 1-E (Fra a 1); and from banana: profilin (Mus xp 1).
 In the embodiments where the antigen is a foreign antigen against which an unwanted immune response is developed, such as to animal, plant and environmental antigens, specific antigens can, for example, be: cat, mouse, dog, horse, bee,
 30 dust, tree and goldenrod, including the following proteins or peptides derived from: weeds, (including ragweed allergens amb a 1, 2, 3, 5, and 6, and Amb t 5; pigweed Che a 2 and 5; and other weed allergens Par j 1, 2, and 3, and Par o 1); grass (including major allergens Cyn d 1, 7, and 12; Dac g 1, 2, and 5; Hol I 1.01203; Lol p 1, 2, 3, 5, and 11; Mer a 1; Pha a 1; Poa p 1 and 5); pollen from ragweed and other weeds (including curly dock, lambs quarters, pigweed, plantain, sheep sorrel, and sagebrush), grass (including Bermuda, Johnson, Kentucky, Orchard, Sweet vernal, and Timothy grass), and trees (including *catalpa*, elm, hickory, olive, pecan, sycamore, and walnut); dust (including major allergens from species *Dermatophagoides pteronyssinus*, such as Der p 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 18, 20, 21, and 23; from species *Dermatophagoides farina*, such as Der f 1, 2, 3, 6, 7, 10, 11, 13, 14, 15, 16, 18, 22, and 24; from species *Blomia tropicalis* such as Blo t 1, 2, 3, 4, 5, 6, 10, 11, 12, 13, 19, and 21; also allergens Eur m 2 from *Euroglyphus maynei*, Tyr p 13 from *Tyrophagus putrescentiae*, and allergens Bla g 1, 2, and 4; Per a 1, 3, and 7 from cockroach); pets (including cats, dogs, rodents, and farm animals); major cat allergens include Fel d 1 through 8, cat IgA, BLA g 2, and cat albumin; major dog allergens include Can f 1 through 6, and dog albumin); bee stings, including major allergens Api m 1 through 12; and fungus, including allergens derived from, species of *Aspergillus* and *Penicillium*, as well as the species *Alternaria alternata*, *Davidiella tassiana*, and *Trichophyton rubrum*.

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In Parkinson's disease, the main antigen is alpha synuclein. Alpha synuclein, including an exogenously obtained form useful in the tolerogenic compositions of the disclosure, has the following sequence (UNIPROT P37840):

(SEQ ID NO: 62)
MDVFMKGLSKAKEGVVAAAEEKTKQGVAAAGKTKEGVLYVGSKTKEGVV
HGVATVAEKTKEQVTNVGGAVTGTAVAKTVEGAGSIAAATGFVKD
QLGKNEEGAPQEGILEDMMPVDPDNEAYEMPSEEGYQDYEPEA.

The antigen can be a complete protein, a portion of a complete protein, a peptide, or the like, and can be derivatized (as discussed above) for attachment to a linker and/or mannosylating moiety, can be a variant and/or can contain conservative substitutions, particularly maintaining sequence identity, and/or can be desylated.

F. EXAMPLES

The following examples are included to demonstrate non-limiting embodiments of the invention disclosed herein. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the embodiments of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the embodiments of the invention disclosed herein.

Example 1

Polymer and Conjugate Synthesis

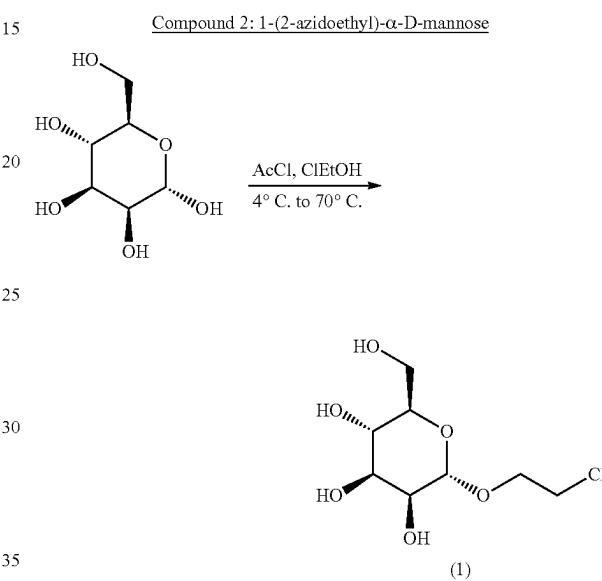
All reactions were carried out at room temperature unless specified. Unless otherwise stated, chemicals were reagent grade and purchased from Sigma-Aldrich (Saint Louis, Mo., USA). Size exclusion chromatography was carried out on an ÄKTA protein purification system (General Electric Healthcare Lifesciences), using a Superdex 200 10/300 column (General Electric Healthcare Lifesciences). All NMR spectra were collected on a Bruker Avance-II 400 MHz NMR, unless otherwise noted, and NMR spectra were analyzed with MnovaNMR (Mestrelab). High pressure size exclusion chromatography was performed on a Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific). Gels were imaged using a Biorad Universal Hood Gel Doc 2000 System (Biorad). Antigens conjugated to polymers as disclosed in the Examples are non-limiting examples of tolerogenic antigens according to embodiments disclosed herein, including embodiments using immunogenic fragments of antigens.

Compound 1: 1-(2-chloroethyl)- α -D-mannose

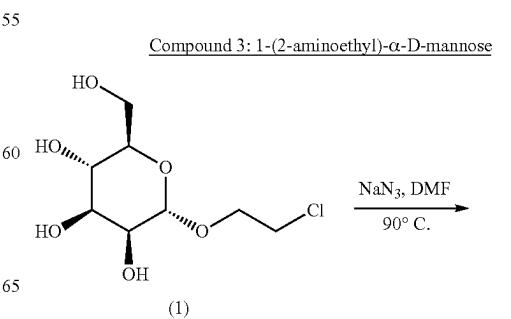
Acetyl chloride (4.35 mL, 61.05 mmol) was added dropwise to the ice-cold solution of D-mannose (10.0 g, 55.51 mmol) in chloroethanol (40 mL, 413.68 mmol). The mixture was stirred for 15 minutes at 4° C. and then was transferred to the oil bath at 70° C. The reaction was then stirred for 4 h. After cooling to room temperature, a dark brown solution was poured into a 400 mL solution of ethyl acetate and DCM (3:1, v/v) in order to remove excess chloroethanol. The mixture was placed at -20° C. for 30 minutes and then a dark brown sticky precipitate was collected from the supernatant.

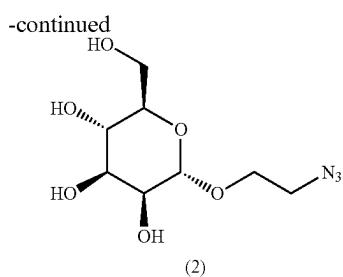
80

The precipitate was dissolved in anhydrous ethanol and 3 spoons of activated charcoal was added. The suspension was mixed for 1.5 h and then filtered through Celite and washed with ethanol. In the last step, ethanol was evaporated via 5 rotary evaporation to provide 12.8 g (95.24%) of product: C₈H₁₅ClO₆, ESI-MS [M+Na]⁺_{theor}=m/z 265.0455, [M+Na]⁺_{found}=m/z 265.0458; ¹H NMR (400 MHz, D₂O) δ 4.86 (s, C1, 1H), 3.92 (dd, C2, 1H), 3.62-3.85 (multiple signals from 7H: C3, C4, C5, C6, C6', ethoxy group); ¹³C NMR (100 MHz, D₂O) δ 99.84, 76.32, 72.94, 70.52, 69.94, 69.65, 67.76, 66.75, 60.96, 43.39.



Compound 1 (12.7 g, 52.48 mmol) was dissolved in 15 mL of N,N-dimethylformamide. To that solution, sodium azide was added (5.0 g, 76.92 mmol) and the suspension was placed in an oil bath and stirred over night at 90° C. After 16 h, the reaction mixture was filtered through Celite and the solvent was then removed via rotary evaporation to provide a oily, brown substance. The residual was adsorbed on silica gel and purified using flash chromatography (DCM:MeOH 92:8, v/v) to yield 5.6 g (42.86%) of pure product: C₈H₁₅N₃O₆, ESI-MS [M+Na]⁺_{theor}=m/z 272.2578, [M+Na]⁺_{found}=m/z 272.0850; ¹H NMR (400 MHz, D₂O) δ 4.84 (s, C1, 1H), 3.91 (dd, C2, 1H), 3.39-3.87 (multiple signals from 7H: C3, C4, C5, C6, C6', ethoxy group); ¹³C NMR (100 MHz, D₂O) δ 99.85, 72.94, 70.44, 69.98, 66.73, 66.34, 50.24.



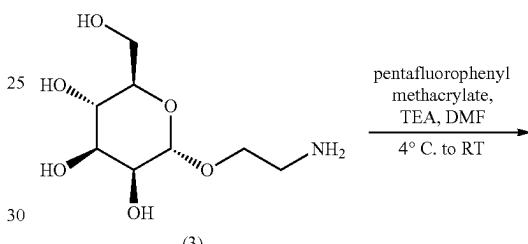
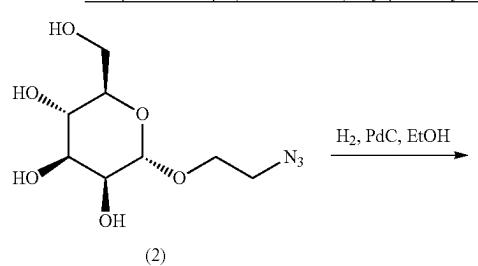
81

A suspension of 2 (5.5 g, 22.09 mmol) and 10% palladium on carbon (one spoon, ca. 500 mg) in 10 mL of ethanol was hydrogenated in a Shlenk flask with an initial pressure of 2 bars of hydrogen gas. The reduction process was monitored by TLC. After 3 h reaction was completed and the suspension was filtered through Celite. The solvent was evaporated in vacuo to give 4.9 g (99.48%) of product: C₈H₁₇NO₆, ESI-MS [M+Na]⁺theor=m/z 246.0954, [M+Na]⁺found=m/z 246.0955; ¹H NMR (400 MHz, D₂O) δ 4.80 (s, C1, 1H), 3.89 (dd, C2, 1H), 3.83-3.44 (multiple signals from 7H: C3, C4, C5, C6, C6', ethoxy group); ¹³C NMR (100 MHz, D₂O) δ 99.87, 72.74, 70.55, 70.03, 68.82, 66.81, 60.97, 39.94.

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and the reaction was allowed to stir at room temperature for the next 4 h. Next, the solvent was evaporated and the residual was adsorbed on silica gel. The purification of crude material using flash chromatography (DCM:MeOH 95:5, v/v) provided 3.8 g (64.73%) of mannose monomer: C₁₂H₂₁NO₇, ESI-MS [M+Na]⁺theor=m/z 314.1216, [M+Na]⁺found=m/z 314.1208; ¹H NMR (400 MHz, D₂O) δ 5.6 (s, 1H), 5.38 (s, 1H), 4.78 (s, 1H), 3.84 (s, C2, 1H), 3.77-3.34 (multiple signals from 7H: C3, C4, C5, C6, C6', ethoxy group), 1.85 (s, 3H); ¹³C NMR (100 MHz, D₂O) δ 172.06, 139.06, 121.00, 99.63, 72.78, 70.47, 69.99, 66.58, 65.73, 60.78, 39.04, 17.68.

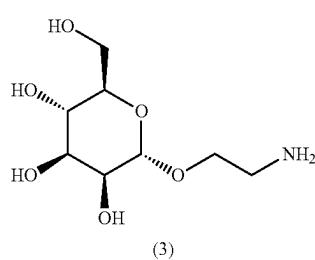
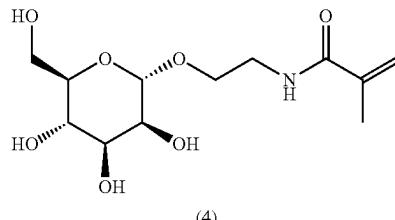
Compound 17: 2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate

Compound 4: N-[2-(α -D-mannose)ethyl] methacrylamide

35

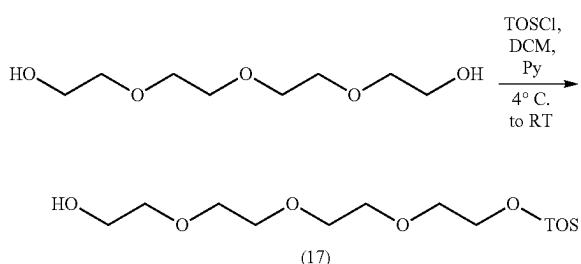
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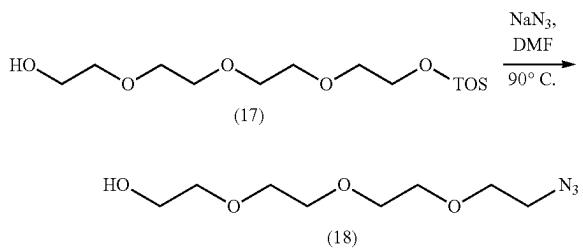


Compound 3 (4.5 g, 20.17 mmol) was dissolved in 10 mL of N,N-dimethylformamide. To that solution, triethylamine (3 mL, 22.28 mmol) was added and the mixture was cooled down to 4° C. Subsequently, pentafluorophenyl methacrylate (4.38 mL, 24.21 mmol) was added drop-wise with constant stirring. After 30 minutes, ice-bath was removed

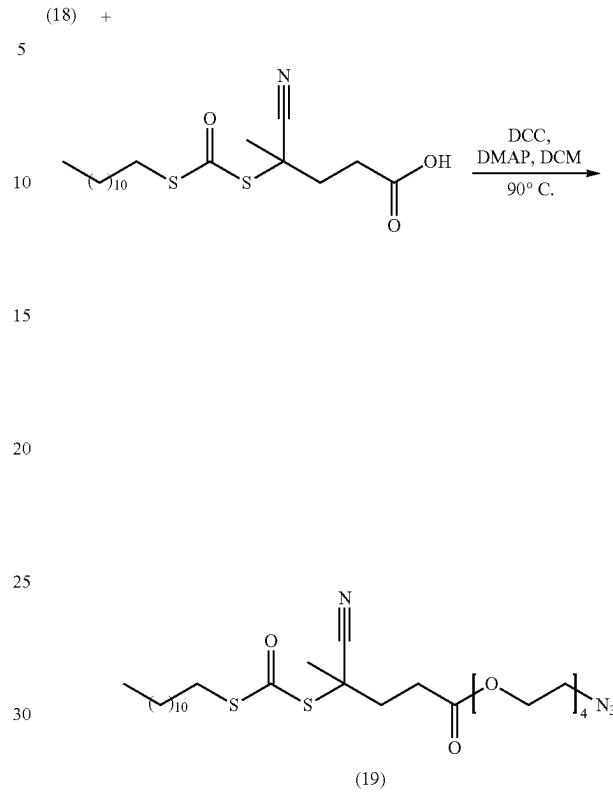
Tetraethylene glycol (2.5 g, 12.9 mmol) and pyridine (1.0 g, 12.6 mmol) were added to 50 mL of DCM and stirred for 20 minutes at 0° C. To that solution, p-toluenesulfonyl chloride (2.37 g, 10 mmol) in 15 mL of DCM was added slowly. The reaction mixture was then stirred for 2 h at 0° C. followed by 4 h at room temperature. After that time, the solvent was evaporated and crude product was purified via flash chromatography (ethyl acetate:hexane 6:4, v/v). The final yield was 1.95 g (72.22%): C₁₅H₂₄O₇S, ESI-MS [M+H]⁺theor=m/z 349.1321, [M+H]⁺found=m/z 349.1325; ¹H NMR (400 MHz, CDCl₃) δ 7.68 (d, 2H), 7.24 (d, 2H), 4.00-4.12 (m, 2H), 3.42-3.70 (m, 14H), 2.89 (t, 1H), 2.33 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 144.68, 132.76, 129.68, 127.73, 72.34, 70.46, 70.41, 70.22, 70.10, 69.17, 68.44, 61.40, 21.41.

Compound 18: 2-(2-(2-azidoethoxy)ethoxyethoxyethan-1-ol

Sodium azide (1.5 g, 23.1 mmol) was added to a solution of 17 (1.5 g, 4.3 mmol) in N,N-dimethylformamide (75 mL) at room temperature. The reaction mixture was stirred overnight at 90° C. The reaction was then filtered and the solvent was removed in vacuo. The resulting viscous liquid was then purified by flash column chromatography (ethyl acetate:hexane 6:4, v/v) to yield a pure product (1.25 g, 83%): C₈H₁₇N₃O₄, ESI-MS [M+Na]⁺ theor=m/z 242.1117, 25 [M+Na]⁺found=m/z 242.1171; ¹H NMR (400 MHz, DMSO-d₆) δ 3.49-3.65 (m, 14H), 3.30 (t, 2H), 2.91 (t, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 72.43, 70.56, 70.52, 70.46, 70.21, 69.91, 61.51, 50.54.

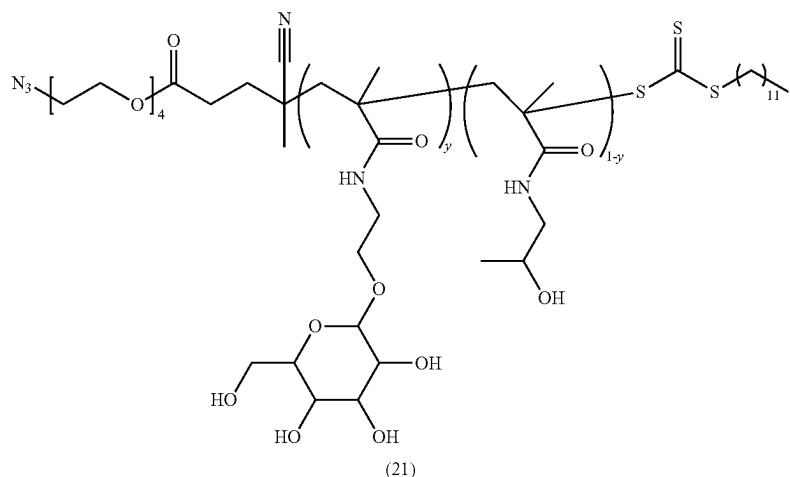
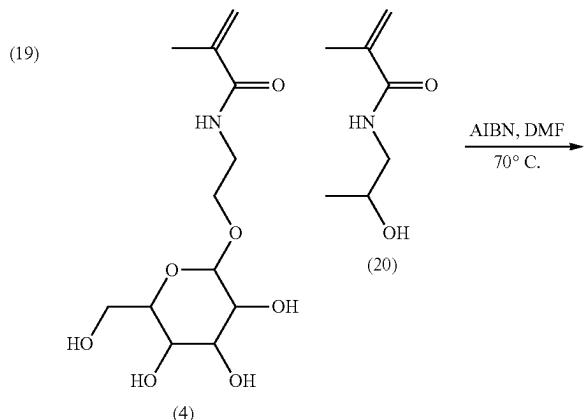
Compound 19: 2-(2-(2-azidoethoxy)ethoxyethyl 4-cyano-4(((dodecylthio)carbonyl)thio)pentanoate

4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (500 mg, 1.24 mmol), compound 18 (226 mg, 1.03 mmol) and DCC (255 mg, 1.24 mmol) were dissolved in 4 mL of DCM. The solution was placed in an ice bath and let stir for 30 minutes. After that time, 4-dimethylaminopyridine (12.6 mg, 0.10 mmol) dissolved in 1 mL of DCM was added drop-wise. The solution was stirred at 0° C. for 2 h and then at room temperature for 1 hour. Once reaction was completed, DCM was evaporated in vacuum. The crude product was purified via column chromatography (DCM: ethyl acetate 97:3, v/v) to yield 300 mg (88.57%) of the pure product: C₂₇H₄₈N₄O₅S₃, ESI-MS [M+H]⁺ theor=m/z 604.2865 [M+H]⁺found=m/z 604.2862; ¹H NMR (400 MHz, CDCl₃) δ 4.26 (t, 2H), 3.76-3.65 (m, 12H), 3.39 (t, 2H), 3.33 (t, 2H), 2.66 (dd, 2H), 2.53 (m, 1H), 2.38 (m, 1H), 1.88 (s, 3H), 1.69 (dt, 2H), 1.38 (m, 2H), 1.26 (s, 16H), 0.88 (t, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 216.92, 171.46, 119.00, 70.72, 70.68, 70.61, 70.07, 68.96, 64.16, 50.69, 46.35, 37.06, 33.81, 31.91, 29.71, 29.62, 29.54, 29.42, 29.34, 29.07, 28.93, 27.68, 24.84, 22.69, 14.13.

Compound 21: p (Man)

The mannose monomer (4) (150.0 mg, 0.51 mmol) and (20) (130.0 mg, 1.03 mmol) were dissolved in 600 μ L of anhydrous DMF and added to a schlenk tube. Then, RAFT agent (19) (18.5 mg, 0.03 mmol) in 100 μ L of DMF and AIBN (1 mg, 0.006 mmol) in 10 μ L of DMF were added to the schlenk tube. The tube was degassed via four freeze-pump-thaw cycles and then immersed in an oil bath pre-heated at 70° C. to initiate polymerization. The reaction was left stirring for 14 hours. After that time, the polymer was precipitated by transferring its viscous solution into 20 mL of cold acetone. The light-yellow suspension was placed in the freezer for 30 minutes. The precipitate was then centrifuged and re-suspended in fresh acetone. The processed was repeated 3 times. In the final step, the resultant glycopolymer (150 mg, 47.6%) was dried in a vacuum oven at reduced pressure and characterized by means of ¹H NMR and GPC. The p(HPMA-TLR7) used in the biological studies had a number average molecular weight of 15,425 Da, as determined by size exclusion chromatography, using a dextran standard, a degree of polymerization of 82.1, and were composed of a 1:2.25 molar ratio of Mannose:HPMA, as determined by ¹H NMR.

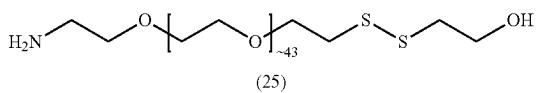
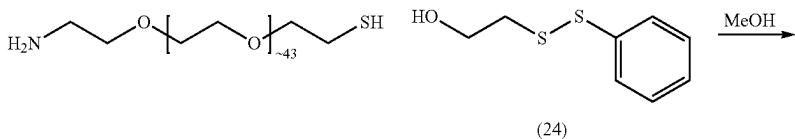
Compound 25: Ethanol disulfanyl polyethylene glycol amine



A solution of thiol polyethylene glycol amine (JenKem Technology, USA) (1.0 g, 0.5 mmol) in DCM (5 ml) was added dropwise to a stirred solution of 2-(2-pyridinylidithio) ethanol (24) (467.5 mg, 2.5 mmol) in MeOH (3 ml). The solution was stirred at room temperature for 10 h then approximately half the solvent was removed via rotary evaporation. The remaining crude product was then decanted into ice cold hexanes (40 ml) and placed at -20° C.

for 4 h. The precipitate and solvent mixture was centrifuged at 2000 g for 3 min. The solvent was then decanted and excess solvent was removed from the pelleted precipitate under reduced pressure. The crude product was then used in the next step without further purification (65% crude yield). The final structure was characterized by ^1H NMR and reverse phase chromatography.

Compound 26: Ethanol disulfanyl polyethylene glycol (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl carbamate



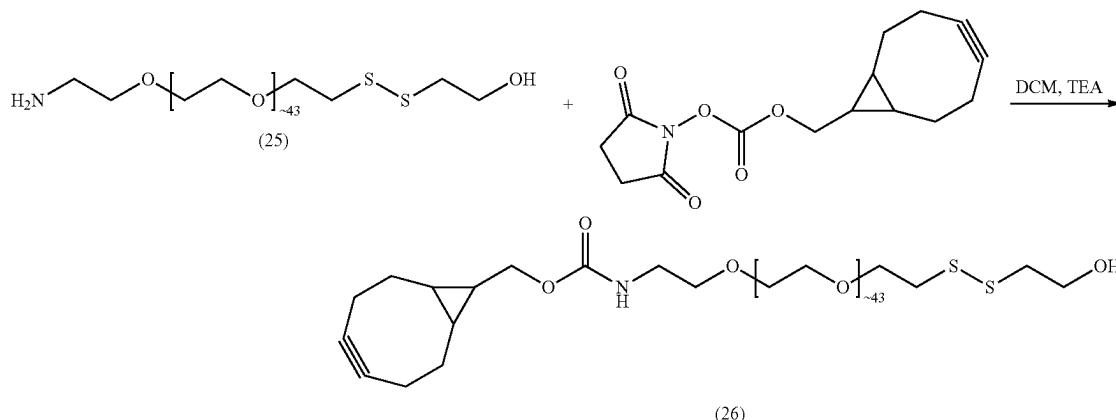
87

A solution of (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate (90 mg, 0.30 mmol) in DCM (0.5 ml) was added dropwise to an ice-cooled stirred solution of ethanol disulfanyl polyethylene glycol amine 25 (0.5 g, 0.24 mmol) and trimethylamine (48 mg, 0.48 mmol) in DCM (5 ml). After the addition of (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate, the reaction was allowed to come to room temperature and stirred for another 6 h. The reaction mixture was then poured

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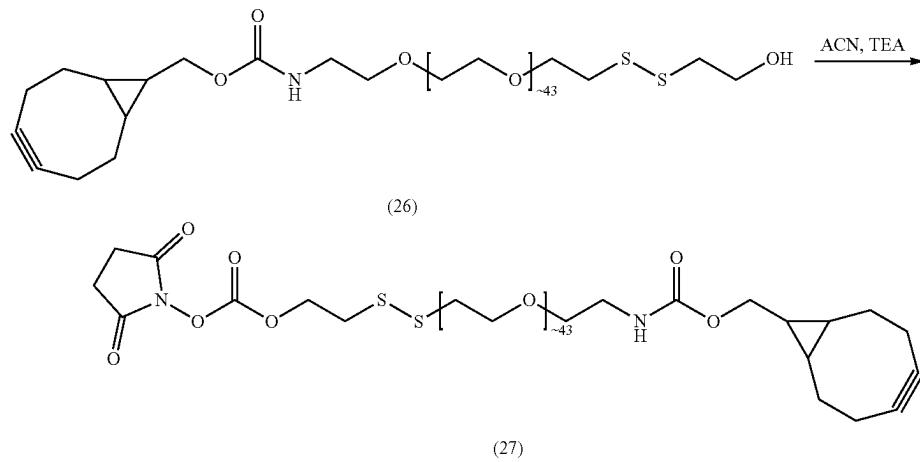
into ice-cold hexanes (40 ml) and placed at -20° C. for 4 h. The precipitate and solvent mixture was centrifuged at 2000 g for 3 min. The solvent was then decanted and excess solvent was removed from the pelleted precipitate under reduced pressure. The crude product was then used in the next step without further purification (75% crude yield). The final structure was characterized by ^1H NMR and reverse phase chromatography.

Compound 27: N-succinimidyl carboamate Ethanol disulfanyl polyethylene glycol (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl carbamate
 (Self-immolative Linker)



A solution of Ethanol disulfanyl polyethylene glycol (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl carbamate (300 mg, 0.13 mmol) in anhydrous acetonitrile (ACN) (1.5 ml) was added dropwise to a stirred solution of N,N'-Disuccinimidyl carbonate 26 (0.5 g, 0.24 mmol) and trimethylamine (48 mg, 0.48 mmol) in anhydrous ACN (5 ml). The reaction mixture was stirred overnight and was then poured into ice-cold hexanes (40 ml) and placed at -20° C. for 4 h. The precipitate and solvent mixture was centrifuged at 2000 g for 3 min. The solvent was then decanted and excess solvent was removed from the pelleted precipitate under reduced pressure. The crude product was purified via silica gel flash chromatography trough a thin pad of silica DCM:MeOH (85:15) (yield: 43%, 129 mg). The final structure was characterized by ¹H NMR and reverse phase chromatography.

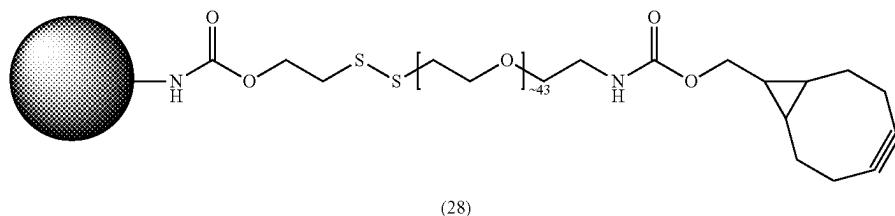
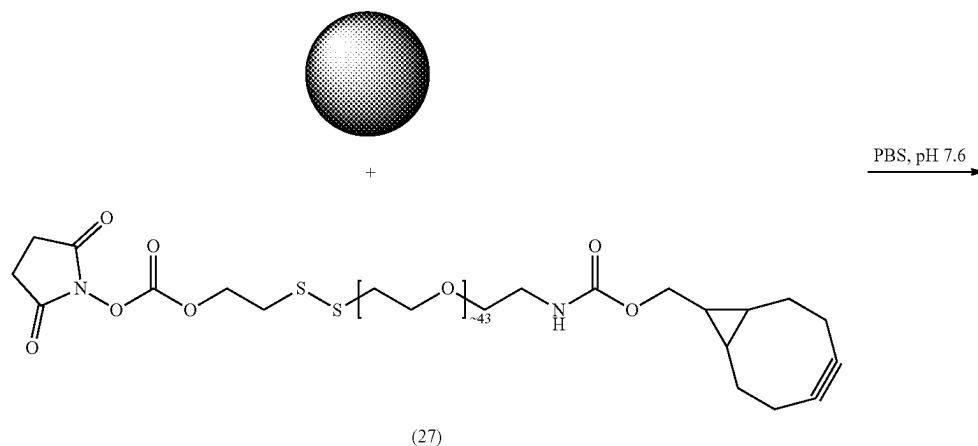
Compound 28: General procedure for OVA-, asparaginase-self-immolative linker conjugates



89**90**

EndoGrade® Ovalbumin (OVA) (Hyglos) (10 mg, 222.2 nmol), recombinant asparaginase (7.2 mg, 222.2 nmol) and self-immolative Linker (27) (5 mg) were added to an endotoxin free tube. Phosphate buffer at pH 7.7 (200 μ L) was 5 added to the tube and the tube was stirred at 1 h at room temperature. The reaction mixture was then filtered (0.22 μ M) and the conjugates were purified via Zeba Spin Desalting Columns with a 30 kDa cutoff limit (Thermo Fisher). 10 Chemical conjugation was verified via gel electrophoresis and high pressure size exclusion chromatography.

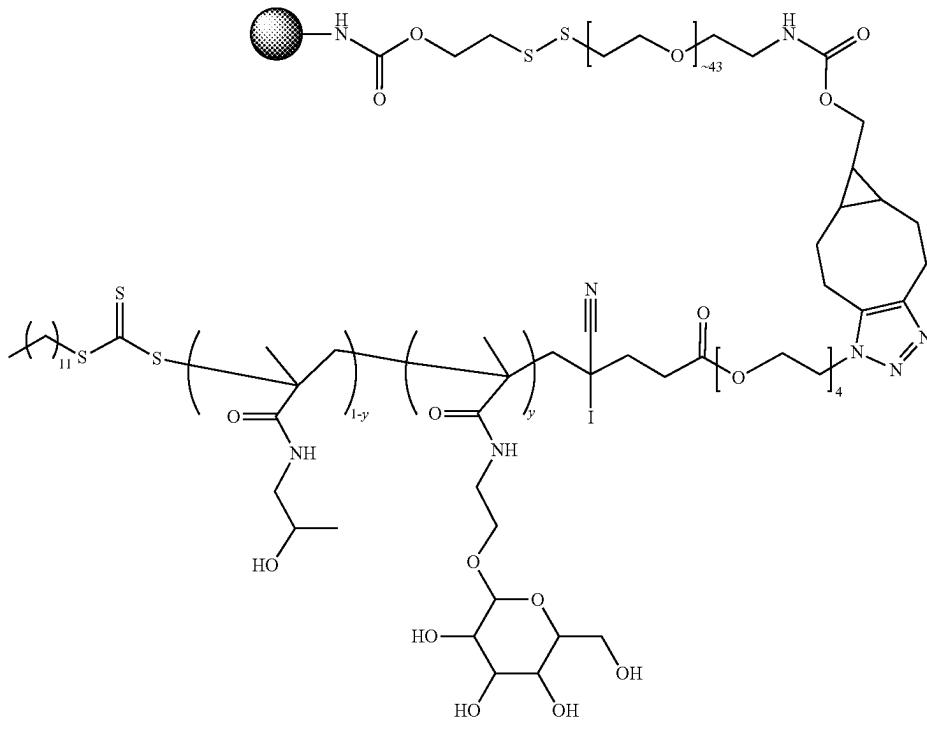
Compound 29



Compound 28 in PBS, prepared as described above, was added to an endotoxin free tube and p(Man) (21) (30 mg) was added and the reaction was stirred for 30 min at room 60 temperature. The reaction mixture was then filtered (0.22 μ M) and the final product was purified via size exclusion chromatography. Chemical conjugation was verified via gel 65 electrophoresis and high pressure size exclusion chromatography.

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Example 2

OTI/OTII Challenge to Tolerance Model

BLK6 mice were treated with saline, or 10 µg of OVA (as a non-limiting example of an immunogenic antigen) in the form of free OVA or OVA conjugated to p(Man) (OVA-p(Man)) one day and 7 days after an adoptive transfer of 7.0×10^5 OTI and OTII T cells. These mice were challenged with an intradermal injection of LPS and OVA 14 days after the initial OTI and OTII T cell transfer and then the immune response in the draining lymph nodes (dLNs) was assessed on day 19 and compared to mice that were treated with saline, but did not receive the challenge of LPS and OVA (No-challenge).

Profound tolerance was induced in the CD4+ T cell compartment, as shown in FIGS. 1-2. In terms of total cell frequencies, both dosing regimens of both OVA-p(Man) resulted in equivalent low levels of OTII cells after challenge, statistically lower than by treatment of OVA (* indicates $p < 0.05$, ** indicates $p < 0.01$), as shown in FIG. 1. When the cells that remained were analyzed by flow cytometry for the presence of the transcription factor FoxP3 and the receptor CD25, the numbers of FoxP3+CD25+ cells (markers of T regulatory cells) was statistically significantly elevated compared to treatment with OVA alone, as shown in FIG. 1. Additionally, the spleens of animals treated with OVA-p(Man) contained a lower percentage of T follicular helper cells (Tfh) as compared to other groups that were challenged with LPS and OVA. When the cells that remained were analyzed by flow cytometry for the expression of IFN-γ after exposure to OVA antigen, the frequency of CD4+ T cells expressing this inflammatory cytokine was decreased in the groups receiving OVA-p(Man), as shown in FIG. 2.

Profound tolerance was also induced in the CD8+ T cell compartment, as shown in FIG. 1. In terms of total cell

35 frequencies, OVA-p(Man) resulted in equivalent low levels of OTI cells after challenge, statistically lower than by treatment of OVA (* indicates $p < 0.05$, ** indicates $p < 0.01$), as shown in FIG. 1. When the cells that remained were analyzed by flow cytometry for the expression of IFN-γ after exposure to SIINFEKLE antigen, the frequency of CD8+ T cells expressing this inflammatory cytokine was decreased in the groups receiving OVA-p(Man), as shown in FIG. 2.

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Example 3

Tolerance Induction to Intravenously Administered Asparaginase

50 Five BALB/c mice per group were injected with 2.5 µg of asparaginase (as a non-limiting example of an immunogenic antigen) formulated as free asparaginase (ASNase) or conjugated to p(Man) (ASNase-p(Man)) once a week for 3 weeks and then, at week 4, were switched to 15 µg of ASNase i.v. once a week for 8 weeks. During the initial 3 weeks ASNase-p(Man) was administered via either i.v. or subcutaneous injection. Sera was taken from the mice and monitored weekly for the presence of αASNase.

55 Upon intravenous injection of ASNase at week 4, animals treated with saline and subcutaneously administered ASNase-p(Man) experienced a rapid increase in serum αASNase IgG (FIG. 3), animals treated with i.v. administered ASNase-p(Man) did not incur an increase in serum αASNase IgG for the duration of the experiment. Furthermore, after 38 days of treatment, animals treated with ASNase-p(Man) via intravenous infusion, experienced significantly lower αASNase IgG subclass titers (FIG. 3).

60 At week 22 (3 weeks of tolerization, 8 weeks of ASNase treatment, and 11 weeks after last dose of ASNase), animals treated with saline and ASNase-p(Man) via intravenous

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infusion were sacrificed and the spleens, livers, and bone marrow of these animals was collected and processed into single cell suspensions. The cells from these organs were stimulated in vitro with recombinant ASNase lacking activity for 3 days. After three days the cells were analyzed via flow cytometry for the existence of α ASNase producing cells (plasma cells) and IL-10 producing B regulatory cells. The results show that the bone marrow of animals treated with ASNase-p(Man) had a fewer α ASNase plasma cells than animals that were treated with ASNase (FIG. 4). On the contrary, the spleens of animals treated with ASNase-p(Man) had a greater percentage of IL-10 producing B regulatory cells.

Example 4

Effect on Anti-Asparaginase (Anti-ASNase)
Humoral Immune Response

To assess the ability of p(Man)-protein conjugates to prevent an anti-asparaginase (anti-ASNase) humoral immune response and thus avoid the loss of efficacy that is the result of rapid antibody-mediated clearance of biological therapeutics from the serum, mice were treated on days -21, -14, and -7 with saline or 2.5 μ g of ASNase conjugated to p(Man) (p(Man)-ASNase) via iv infusion (n=5 animals per group). After 7 days, the mice were then treated with weekly iv infusions of 15 μ g of wt ASNase for 4 weeks. The mice were then treated with 15 μ g of wt ASNase on day 49. On day 70, mice that had been treated with saline on days -21, -14, -7, 0, 14, 21, and 49 were treated with 15 μ g of wt ASNase to assess the efficacy of ASNase in naïve mice (FIG. 6). On day 70, the mice that had received saline or p(Man)-ASNase then treated with wt ASNase were also adminis-

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tered 15 μ g of wt ASNase. The serum asparagine concentration of each animal was assessed on days 71, 73, and 76.

The results show that one day after being treated with ASNase, animals in each group have a similar serum concentration of asparagine. However, three days after being administered asparagine on day 7, animals treated with only saline or p(Man)-ASNase have a significantly lower serum asparagine concentration as compared to animals that had been treated with saline and then administered wt ASNase on days 0, 7, 14, 21, and 49 (FIG. 7A). In addition, animals treated with p(Man)-ASNase had significantly lower asparagine serum concentrations on day 76 than the animals in other groups. When serum asparagine concentration is plotted against the anti-ASNase titer for each animal in the study, a strong correlation ($r=0.8$) between serum asparagine concentration and anti-ASNase titer becomes evident (FIG. 7B). These results demonstrate that p(Man)-ASNase inhibits the loss of efficacy associated with an anti-ASNase immune response.

All of the methods and compositions disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and apparatuses and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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95**96**

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97**98**

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405 410 415

Asn Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His
420 425 430

Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg
435 440 445

His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr
450 455 460

Thr Gly Phe Glu Ala His Val Asp Lys Cys Leu Glu Leu Ala Glu Tyr
465 470 475 480

Leu Tyr Asn Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp
485 490 495

Gly Lys Pro Gln His Thr Asn Val Cys Phe Trp Tyr Ile Pro Pro Ser
500 505 510

Leu Arg Thr Leu Glu Asp Asn Glu Glu Arg Met Ser Arg Leu Ser Lys
515 520 525

Val Ala Pro Val Ile Lys Ala Arg Met Met Glu Tyr Gly Thr Thr Met
530 535 540

Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val
545 550 555 560

Ile Ser Asn Pro Ala Ala Thr His Gln Asp Ile Asp Phe Leu Ile Glu
565 570 575

Glu Ile Glu Arg Leu Gly Gln Asp Leu
580 585

<210> SEQ ID NO 3
<211> LENGTH: 355
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Asp Phe Leu His Arg Asn Gly Val Leu Ile Ile Gln His Leu Gln
1 5 10 15

Lys Asp Tyr Arg Ala Tyr Tyr Thr Phe Leu Asn Phe Met Ser Asn Val
20 25 30

Gly Asp Pro Arg Asn Ile Phe Phe Ile Tyr Phe Pro Leu Cys Phe Gln
35 40 45

Phe Asn Gln Thr Val Gly Thr Lys Met Ile Trp Val Ala Val Ile Gly
50 55 60

Asp Trp Leu Asn Leu Ile Phe Lys Trp Ile Leu Phe Gly His Arg Pro
65 70 75 80

Tyr Trp Trp Val Gln Glu Thr Gln Ile Tyr Pro Asn His Ser Ser Pro
85 90 95

Cys Leu Glu Gln Phe Pro Thr Thr Cys Glu Thr Gly Pro Gly Ser Pro
100 105 110

Ser Gly His Ala Met Gly Ala Ser Cys Val Trp Tyr Val Met Val Thr
115 120 125

Ala Ala Leu Ser His Thr Val Cys Gly Met Asp Lys Phe Ser Ile Thr
130 135 140

Leu His Arg Leu Thr Trp Ser Phe Leu Trp Ser Val Phe Trp Leu Ile
145 150 155 160

Gln Ile Ser Val Cys Ile Ser Arg Val Phe Ile Ala Thr His Phe Pro
165 170 175

His Gln Val Ile Leu Gly Val Ile Gly Gly Met Leu Val Ala Glu Ala
180 185 190

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Phe Glu His Thr Pro Gly Ile Gln Thr Ala Ser Leu Gly Thr Tyr Leu
 195 200 205
 Lys Thr Asn Leu Phe Leu Phe Ala Val Gly Phe Tyr Leu Leu
 210 215 220
 Leu Arg Val Leu Asn Ile Asp Leu Leu Trp Ser Val Pro Ile Ala Lys
 225 230 235 240
 Lys Trp Cys Ala Asn Pro Asp Trp Ile His Ile Asp Thr Thr Pro Phe
 245 250 255
 Ala Gly Leu Val Arg Asn Leu Gly Val Leu Phe Gly Leu Gly Phe Ala
 260 265 270
 Ile Asn Ser Glu Met Phe Leu Leu Ser Cys Arg Gly Gly Asn Asn Tyr
 275 280 285
 Thr Leu Ser Phe Arg Leu Leu Cys Ala Leu Thr Ser Leu Thr Ile Leu
 290 295 300
 Gln Leu Tyr His Phe Leu Gln Ile Pro Thr His Glu Glu His Leu Phe
 305 310 315 320
 Tyr Val Leu Ser Phe Cys Lys Ser Ala Ser Ile Pro Leu Thr Val Val
 325 330 335
 Ala Phe Ile Pro Tyr Ser Val His Met Leu Met Lys Gln Ser Gly Lys
 340 345 350
 Lys Ser Gln
 355

<210> SEQ ID NO 4
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1 5 10 15
 Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
 20 25 30
 Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Pro
 35 40 45
 Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
 50 55 60
 Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
 65 70 75 80
 Leu Glu Asn Tyr Cys Asn
 85

<210> SEQ ID NO 5
<211> LENGTH: 70
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1 5 10 15
 Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
 20 25 30
 Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Pro
 35 40 45
 Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
 50 55 60

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Arg Ile Lys Leu Lys Val Glu Ser Ser Pro Ser Arg Ser Asp Tyr Ile
 35 40 45

Asn Ala Ser Pro Ile Ile Glu His Asp Pro Arg Met Pro Ala Tyr Ile
 50 55 60

Ala
 65

<210> SEQ ID NO 17
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Gly Pro Leu Ser His Thr Ile Ala Asp Phe Trp Gln Met Val Trp Glu
 1 5 10 15

Ser Gly Cys Thr Val Ile Val Met Leu Thr Pro Leu Val Glu Asp Gly
 20 25 30

Val Lys Gln
 35

<210> SEQ ID NO 18
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Gly Ala Ser Leu Tyr His Val Tyr Glu Val Asn Leu Val Ser Glu His
 1 5 10 15

Ile Trp Cys Glu Asp Phe Leu Val Arg Ser Phe Tyr Leu Lys Asn Val
 20 25 30

Gln Thr Gln Glu Thr Arg Thr Leu Thr Gln Phe His Phe Leu Ser Trp
 35 40 45

Pro Ala Glu Gly Thr Pro Ala Ser
 50 55

<210> SEQ ID NO 19
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Glu His Val Arg Asp Gln Arg Pro Gly Leu Val Arg Ser Lys Asp Gln
 1 5 10 15

Phe Glu Phe Ala Leu Thr Ala Val Ala Glu Glu Val Asn Ala Ile Leu
 20 25 30

Lys Ala Leu Pro Gln Cys Gly
 35

<210> SEQ ID NO 20
<211> LENGTH: 304
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Gly Asn His Ala Gly Lys Arg Glu Leu Asn Ala Glu Lys Ala Ser
 1 5 10 15

Thr Asn Ser Glu Thr Asn Arg Gly Glu Ser Glu Lys Lys Arg Asn Leu
 20 25 30

Gly Glu Leu Ser Arg Thr Thr Ser Glu Asp Asn Glu Val Phe Gly Glu

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35	40	45
Ala Asp Ala Asn Gln Asn Asn Gly	Thr Ser Ser Gln Asp Thr Ala Val	
50	55	60
Thr Asp Ser Lys Arg Thr Ala Asp Pro Lys Asn Ala Trp	Gln Asp Ala	
65	70	75
His Pro Ala Asp Pro Gly Ser Arg Pro His Leu Ile Arg	Leu Phe Ser	
85	90	95
Arg Asp Ala Pro Gly Arg Glu Asp Asn Thr Phe Lys Asp	Arg Pro Ser	
100	105	110
Glu Ser Asp Glu Leu Gln Thr Ile Gln Glu Asp Ser Ala	Ala Thr Ser	
115	120	125
Glu Ser Leu Asp Val Met Ala Ser Gln Lys Arg Pro Ser	Gln Arg His	
130	135	140
Gly Ser Lys Tyr Leu Ala Thr Ala Ser Thr Met Asp His	Ala Arg His	
145	150	155
Gly Phe Leu Pro Arg His Arg Asp Thr Gly Ile Leu Asp Ser	Ile Gly	
165	170	175
Arg Phe Phe Gly Asp Arg Gly Ala Pro Lys Arg Gly Ser	Gly Lys	
180	185	190
Asp Ser His His Pro Ala Arg Thr Ala His Tyr Gly Ser	Leu Pro Gln	
195	200	205
Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val	His Phe Phe	
210	215	220
Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Ser Gln Gly	Lys Gly	
225	230	235
Arg Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu	Gly Gln Arg	
245	250	255
Pro Gly Phe Gly Tyr Gly Arg Ala Ser Asp Tyr Lys Ser	Ala His	
260	265	270
Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr Leu Ser	Lys Ile Phe	
275	280	285
Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser Pro Met	Ala Arg Arg	
290	295	300

<210> SEQ ID NO 21

<211> LENGTH: 247

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Met Ala Ser Leu Ser Arg Pro Ser Leu Pro Ser Cys	Leu Cys Ser Phe	
1	5	10
Leu Leu Leu Leu Leu Gln Val Ser Ser Ser Tyr Ala	Gly Gln Phe	
20	25	30
Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val	Gly Asp Glu	
35	40	45
Val Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn	Ala Thr Gly Met	
50	55	60
Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val	Val His Leu Tyr	
65	70	75
Arg Asn Gly Lys Asp Gln Asp Gly Asp Gln Ala Pro	Glu Tyr Arg Gly	
85	90	95
Arg Thr Glu Leu Leu Lys Asp Ala Ile Gly Glu Gly	Lys Val Thr Leu	
100	105	110

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Arg Ile Arg Asn Val Arg Phe Ser Asp Glu Gly Gly Phe Thr Cys Phe
 115 120 125

Phe Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val
 130 135 140

Glu Asp Pro Phe Tyr Trp Val Ser Pro Gly Val Leu Val Leu Leu Ala
 145 150 155 160

Val Leu Pro Val Leu Leu Gln Ile Thr Val Gly Leu Ile Phe Leu
 165 170 175

Cys Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile Glu Asn
 180 185 190

Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp Lys
 195 200 205

Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala Leu
 210 215 220

Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu
 225 230 235 240

Glu Glu Leu Arg Asn Pro Phe
 245

<210> SEQ ID NO 22

<211> LENGTH: 277

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Met Gly Leu Leu Glu Cys Cys Ala Arg Cys Leu Val Gly Ala Pro Phe
 1 5 10 15

Ala Ser Leu Val Ala Thr Gly Leu Cys Phe Phe Gly Val Ala Leu Phe
 20 25 30

Cys Gly Cys Gly His Glu Ala Leu Thr Gly Thr Glu Lys Leu Ile Glu
 35 40 45

Thr Tyr Phe Ser Lys Asn Tyr Gln Asp Tyr Glu Tyr Leu Ile Asn Val
 50 55 60

Ile His Ala Phe Gln Tyr Val Ile Tyr Gly Thr Ala Ser Phe Phe Phe
 65 70 75 80

Leu Tyr Gly Ala Leu Leu Ala Glu Gly Phe Tyr Thr Thr Gly Ala
 85 90 95

Val Arg Gln Ile Phe Gly Asp Tyr Lys Thr Thr Ile Cys Gly Lys Gly
 100 105 110

Leu Ser Ala Thr Val Thr Gly Gly Gln Lys Gly Arg Gly Ser Arg Gly
 115 120 125

Gln His Gln Ala His Ser Leu Glu Arg Val Cys His Cys Leu Gly Lys
 130 135 140

Trp Leu Gly His Pro Asp Lys Phe Val Gly Ile Thr Tyr Ala Leu Thr
 145 150 155 160

Val Val Trp Leu Leu Val Phe Ala Cys Ser Ala Val Pro Val Tyr Ile
 165 170 175

Tyr Phe Asn Thr Trp Thr Thr Cys Gln Ser Ile Ala Phe Pro Ser Lys
 180 185 190

Thr Ser Ala Ser Ile Gly Ser Leu Cys Ala Asp Ala Arg Met Tyr Gly
 195 200 205

Val Leu Pro Trp Asn Ala Phe Pro Gly Lys Val Cys Gly Ser Asn Leu
 210 215 220

Leu Ser Ile Cys Lys Thr Ala Glu Phe Gln Met Thr Phe His Leu Phe
 225 230 235 240

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Ile Ala Ala Phe Val Gly Ala Ala Ala Thr Leu Val Ser Leu Leu Thr
 245 250 255

Phe Met Ile Ala Ala Thr Tyr Asn Phe Ala Val Leu Lys Leu Met Gly
 260 265 270

Arg Gly Thr Lys Phe
 275

<210> SEQ ID NO 23

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Lys Tyr Leu Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe
 1 5 10 15

Leu Pro Arg His
 20

<210> SEQ ID NO 24

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Glu Asn Pro Trp His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
 1 5 10 15

<210> SEQ ID NO 25

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Arg Pro Gly Phe Gly
 1 5 10 15

Tyr Gly Gly

<210> SEQ ID NO 26

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Ala Gln Gly Thr Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp Ser
 1 5 10 15

Arg Ser Gly Ser Pro Met Ala Arg Arg
 20 25

<210> SEQ ID NO 27

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Gly Gln Phe Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val
 1 5 10 15

Gly Asp Glu Val
 20

<210> SEQ ID NO 28

<211> LENGTH: 20

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

```
Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Trp His Leu Tyr
1           5          10          15
Arg Asn Gly Lys
20
```

<210> SEQ ID NO 29
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

```
His Cys Leu Gly Lys Trp Leu Gly His Pro Asp Lys Phe Val Gly Ile
1           5          10          15
```

<210> SEQ ID NO 30
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

```
Lys Asn Ala Thr Gly Met Glu Val Gly Trp Tyr Arg Ser Pro Phe Ser
1           5          10          15
Arg Val Val His Leu Tyr Arg Asn Gly Lys Asp Gln Asp Ala Glu
20          25          30
```

<210> SEQ ID NO 31
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

```
Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr
1           5          10          15
```

Pro

<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

```
Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His Leu
1           5          10          15
Tyr Arg Asn Gly Lys
20
```

<210> SEQ ID NO 33
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

```
Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
1           5          10          15
```

Thr

<210> SEQ ID NO 34
<211> LENGTH: 18

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
1 5 10 15

Thr Pro

<210> SEQ ID NO 35

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
1 5 10 15

Thr Pro Pro Pro Ser Gln Gly Lys Gly
20 25

<210> SEQ ID NO 36

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Ser
1 5 10 15

Gln Gly Lys Gly
20

<210> SEQ ID NO 37

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Ala Ser Asp Tyr Lys Ser Ala His Lys Gly Leu Lys Gly Val Asp Ala
1 5 10 15

Gln Gly Thr Leu Ser Lys Ile Phe Lys
20 25

<210> SEQ ID NO 38

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Gly Thr Glu Lys Leu Ile Glu Thr Tyr Phe Ser Lys Asn Tyr Gln Asp
1 5 10 15

Tyr Glu

<210> SEQ ID NO 39

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

Gly Phe Tyr Thr Thr Gly Ala Val Arg Gln Ile Phe Gly Asp Tyr Lys
1 5 10 15

Thr Thr

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<210> SEQ ID NO 40

<211> LENGTH: 22

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Ala	Val	Arg	Gln	Ile	Phe	Gly	Asp	Tyr	Lys	Thr	Thr	Ile	Cys	Gly	Lys
1															15

Gly	Leu	Ser	Ala	Thr	Val
20					

<210> SEQ ID NO 41

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

Asn	Thr	Trp	Thr	Thr	Cys	Gln	Ser	Ile	Ala	Phe	Pro	Ser	Lys	Thr	Ser
1														15	

Ala	Ser	Ile	Gly
20			

<210> SEQ ID NO 42

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

Ser	Lys	Thr	Ser	Ala	Ser	Ile	Gly	Ser	Leu	Cys	Ala	Asp	Ala	Arg	Met
1														15	

Tyr	Gly	Val	Leu
20			

<210> SEQ ID NO 43

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Pro	Ile	Arg	Ala	Leu	Val	Gly	Asp	Glu	Val	Glu	Leu	Pro	Cys	Arg	Ile
1														15	

Ser	Pro	Gly	Lys
20			

<210> SEQ ID NO 44

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Glu	Leu	Pro	Cys	Arg	Ile	Ser	Pro	Gly	Lys	Asn	Ala	Thr	Gly	Met	Glu
1														15	

Val	Gly	Trp	Tyr
20			

<210> SEQ ID NO 45

<211> LENGTH: 23

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Glu	Tyr	Arg	Gly	Arg	Thr	Glu	Leu	Leu	Lys	Asp	Ala	Ile	Gly	Glu	Gly
1														15	

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Lys Val Thr Leu Arg Ile Arg
20

<210> SEQ ID NO 46
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Gly	Gln	Phe	Arg	Val	Ile	Gly	Pro	Arg	His	Pro	Ile	Arg	Ala	Leu	Val
1				5				10			15				

Gly	Asp	Glu	Val	Glu	Leu	Pro	Cys	Arg	Ile	Ser	Pro	Gly	Lys	Asn	Ala
20				25					30						

Thr	Gly	Met	Glu	Val	Gly	Trp	Tyr	Arg	Pro	Pro	Phe	Ser	Arg	Val	Val
35					40				45						

His	Leu	Tyr	Arg	Asn	Gly	Lys	Asp	Gln	Asp	Gly	Asp	Gln	Ala
50				55				60					

<210> SEQ ID NO 47

<211> LENGTH: 63

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Ser	His	Gly	Arg	Thr	Gln	Asp	Glu	Asn	Pro	Val	Val	His	Phe	Lys
1				5				10			15			

Asn	Ile	Val	Thr	Pro	Arg	Thr	Pro	Pro	Pro	Ser	Gln	Gly	Lys	Gly	Arg
20				25					30						

Gly	Leu	Ser	Leu	Ser	Arg	Phe	Ser	Trp	Gly	Ala	Glu	Gly	Gln	Arg	Pro
35				40					45						

Gly	Phe	Gly	Tyr	Gly	Arg	Ala	Ser	Asp	Tyr	Lys	Ser	Cys	Gly
50				55				60					

<210> SEQ ID NO 48

<211> LENGTH: 118

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Met	Pro	Arg	Glu	Asp	Ala	His	Phe	Ile	Tyr	Gly	Tyr	Pro	Lys	Lys	Gly
1				5				10			15				

His	Gly	His	Ser	Tyr	Thr	Thr	Ala	Glu	Glu	Ala	Ala	Gly	Ile	Gly	Ile
20				25					30						

Leu	Thr	Val	Ile	Leu	Gly	Val	Leu	Leu	Ile	Gly	Cys	Trp	Tyr	Cys
35				40					45					

Arg	Arg	Arg	Asn	Gly	Tyr	Arg	Ala	Leu	Met	Asp	Lys	Ser	Leu	His	Val
50				55				60							

Gly	Thr	Gln	Cys	Ala	Leu	Thr	Arg	Arg	Cys	Pro	Gln	Glu	Gly	Phe	Asp
65				70				75			80				

His	Arg	Asp	Ser	Lys	Val	Ser	Leu	Gln	Glu	Lys	Asn	Cys	Glu	Pro	Val
85				90					95						

Val	Pro	Asn	Ala	Pro	Pro	Ala	Tyr	Glu	Lys	Leu	Ser	Ala	Glu	Gln	Ser
100				105					110						

Pro	Pro	Pro	Tyr	Ser	Pro
115					

<210> SEQ ID NO 49
<211> LENGTH: 529

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<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Met Leu Leu Ala Val Leu Tyr Cys Leu Leu Trp Ser Phe Gln Thr Ser
 1 5 10 15

 Ala Gly His Phe Pro Arg Ala Cys Val Ser Ser Lys Asn Leu Met Glu
 20 25 30

 Lys Glu Cys Cys Pro Pro Trp Ser Gly Asp Arg Ser Pro Cys Gly Gln
 35 40 45

 Leu Ser Gly Arg Gly Ser Cys Gln Asn Ile Leu Leu Ser Asn Ala Pro
 50 55 60

 Leu Gly Pro Gln Phe Pro Phe Thr Gly Val Asp Asp Arg Glu Ser Trp
 65 70 75 80

 Pro Ser Val Phe Tyr Asn Arg Thr Cys Gln Cys Ser Gly Asn Phe Met
 85 90 95

 Gly Phe Asn Cys Gly Asn Cys Lys Phe Gly Phe Trp Gly Pro Asn Cys
 100 105 110

 Thr Glu Arg Arg Leu Leu Val Arg Arg Asn Ile Phe Asp Leu Ser Ala
 115 120 125

 Pro Glu Lys Asp Lys Phe Phe Ala Tyr Leu Thr Leu Ala Lys His Thr
 130 135 140

 Ile Ser Ser Asp Tyr Val Ile Pro Ile Gly Thr Tyr Gly Gln Met Lys
 145 150 155 160

 Asn Gly Ser Thr Pro Met Phe Asn Asp Ile Asn Ile Tyr Asp Leu Phe
 165 170 175

 Val Trp Met His Tyr Tyr Val Ser Met Asp Ala Leu Leu Gly Gly Ser
 180 185 190

 Glu Ile Trp Arg Asp Ile Asp Phe Ala His Glu Ala Pro Ala Phe Leu
 195 200 205

 Pro Trp His Arg Leu Phe Leu Leu Arg Trp Glu Gln Glu Ile Gln Lys
 210 215 220

 Leu Thr Gly Asp Glu Asn Phe Thr Ile Pro Tyr Trp Asp Trp Arg Asp
 225 230 235 240

 Ala Glu Lys Cys Asp Ile Cys Thr Asp Glu Tyr Met Gly Gly Gln His
 245 250 255

 Pro Thr Asn Pro Asn Leu Leu Ser Pro Ala Ser Phe Phe Ser Ser Trp
 260 265 270

 Gln Ile Val Cys Ser Arg Leu Glu Tyr Asn Ser His Gln Ser Leu
 275 280 285

 Cys Asn Gly Thr Pro Glu Gly Pro Leu Arg Arg Asn Pro Gly Asn His
 290 295 300

 Asp Lys Ser Arg Thr Pro Arg Leu Pro Ser Ser Ala Asp Val Glu Phe
 305 310 315 320

 Cys Leu Ser Leu Thr Gln Tyr Glu Ser Met Asp Lys Ala Ala
 325 330 335

 Asn Phe Ser Phe Arg Asn Thr Leu Glu Gly Phe Ala Ser Pro Leu Thr
 340 345 350

 Gly Ile Ala Asp Ala Ser Gln Ser Ser Met His Asn Ala Leu His Ile
 355 360 365

 Tyr Met Asn Gly Thr Met Ser Gln Val Gln Gly Ser Ala Asn Asp Pro
 370 375 380

 Ile Phe Leu Leu His His Ala Phe Val Asp Ser Ile Phe Glu Gln Trp
 385 390 395 400

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Leu Arg Arg His Arg Pro Leu Gln Glu Val Tyr Pro Glu Ala Asn Ala
 405 410 415
 Pro Ile Gly His Asn Arg Glu Ser Tyr Met Val Pro Phe Ile Pro Leu
 420 425 430
 Tyr Arg Asn Gly Asp Phe Phe Ile Ser Ser Lys Asp Leu Gly Tyr Asp
 435 440 445
 Tyr Ser Tyr Leu Gln Asp Ser Asp Pro Asp Ser Phe Gln Asp Tyr Ile
 450 455 460
 Lys Ser Tyr Leu Glu Gln Ala Ser Arg Ile Trp Ser Trp Leu Leu Gly
 465 470 475 480
 Ala Ala Met Val Gly Ala Val Leu Thr Ala Leu Leu Ala Gly Leu Val
 485 490 495
 Ser Leu Leu Cys Arg His Lys Arg Lys Gln Leu Pro Glu Glu Lys Gln
 500 505 510
 Pro Leu Leu Met Glu Lys Glu Asp Tyr His Ser Leu Tyr Gln Ser His
 515 520 525
 Leu

<210> SEQ ID NO 50
 <211> LENGTH: 661
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <400> SEQUENCE: 50

Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
 1 5 10 15
 Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
 20 25 30
 Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
 35 40 45
 Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
 50 55 60
 Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
 65 70 75 80
 Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
 85 90 95
 Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
 100 105 110
 Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
 115 120 125
 Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
 130 135 140
 Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
 145 150 155 160
 Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
 165 170 175
 Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
 180 185 190
 Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
 195 200 205
 Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
 210 215 220
 Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
 225 230 235 240

-continued

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
 245 250 255
 Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
 260 265 270
 Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
 275 280 285
 Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
 290 295 300
 Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
 305 310 315 320
 Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
 325 330 335
 Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
 340 345 350
 Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
 355 360 365
 Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
 370 375 380
 Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
 385 390 395 400
 Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
 405 410 415
 Gln Val Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
 420 425 430
 Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
 435 440 445
 Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
 450 455 460
 Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
 465 470 475 480
 Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
 485 490 495
 Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
 500 505 510
 Thr Val Ser Cys Gln Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
 515 520 525
 Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
 530 535 540
 Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
 545 550 555 560
 Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
 565 570 575
 Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
 580 585 590
 Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
 595 600 605
 Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Leu Met Lys Gln Asp
 610 615 620
 Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
 625 630 635 640
 Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
 645 650 655

-continued

Ser Gly Gln Gln Val
660

<210> SEQ ID NO 51
<211> LENGTH: 323
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Met Ser Asp Arg Pro Thr Ala Arg Arg Trp Gly Lys Cys Gly Pro Leu
1 5 10 15

Cys Thr Arg Glu Asn Ile Met Val Ala Phe Lys Gly Val Trp Thr Gln
20 25 30

Ala Phe Trp Lys Ala Val Thr Ala Glu Phe Leu Ala Met Leu Ile Phe
35 40 45

Val Leu Leu Ser Leu Gly Ser Thr Ile Asn Trp Gly Gly Thr Glu Lys
50 55 60

Pro Leu Pro Val Asp Met Val Leu Ile Ser Leu Cys Phe Gly Leu Ser
65 70 75 80

Ile Ala Thr Met Val Gln Cys Phe Gly His Ile Ser Gly Gly His Ile
85 90 95

Asn Pro Ala Val Thr Val Ala Met Val Cys Thr Arg Lys Ile Ser Ile
100 105 110

Ala Lys Ser Val Phe Tyr Ile Ala Ala Gln Cys Leu Gly Ala Ile Ile
115 120 125

Gly Ala Gly Ile Leu Tyr Leu Val Thr Pro Pro Ser Val Val Gly Gly
130 135 140

Leu Gly Val Thr Met Val His Gly Asn Leu Thr Ala Gly His Gly Leu
145 150 155 160

Leu Val Glu Leu Ile Ile Thr Phe Gln Leu Val Phe Thr Ile Phe Ala
165 170 175

Ser Cys Asp Ser Lys Arg Thr Asp Val Thr Gly Ser Ile Ala Leu Ala
180 185 190

Ile Gly Phe Ser Val Ala Ile Gly His Leu Phe Ala Ile Asn Tyr Thr
195 200 205

Gly Ala Ser Met Asn Pro Ala Arg Ser Phe Gly Pro Ala Val Ile Met
210 215 220

Gly Asn Trp Glu Asn His Trp Ile Tyr Trp Val Gly Pro Ile Ile Gly
225 230 235 240

Ala Val Leu Ala Gly Gly Leu Tyr Glu Tyr Val Phe Cys Pro Asp Val
245 250 255

Glu Phe Lys Arg Arg Phe Lys Glu Ala Phe Ser Lys Ala Ala Gln Gln
260 265 270

Thr Lys Gly Ser Tyr Met Glu Val Glu Asp Asn Arg Ser Gln Val Glu
275 280 285

Thr Asp Asp Leu Ile Leu Lys Pro Gly Val Val His Val Ile Asp Val
290 295 300

Asp Arg Gly Glu Glu Lys Lys Gly Lys Asp Gln Ser Gly Glu Val Leu
305 310 315 320

Ser Ser Val

<210> SEQ ID NO 52
<211> LENGTH: 405
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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-continued

<400> SEQUENCE: 52

Met Ala Ala Ser Gly Lys Thr Ser Lys Ser Glu Pro Asn His Val Ile
 1 5 10 15

Phe Lys Lys Ile Ser Arg Asp Lys Ser Val Thr Ile Tyr Leu Gly Asn
 20 25 30

Arg Asp Tyr Ile Asp His Val Ser Gln Val Gln Pro Val Asp Gly Val
 35 40 45

Val Leu Val Asp Pro Asp Leu Val Lys Gly Lys Lys Val Tyr Val Thr
 50 55 60

Leu Thr Cys Ala Phe Arg Tyr Gly Gln Glu Asp Ile Asp Val Ile Gly
 65 70 75 80

Leu Thr Phe Arg Arg Asp Leu Tyr Phe Ser Arg Val Gln Val Tyr Pro
 85 90 95

Pro Val Gly Ala Ala Ser Thr Pro Thr Lys Leu Gln Glu Ser Leu Leu
 100 105 110

Lys Lys Leu Gly Ser Asn Thr Tyr Pro Phe Leu Leu Thr Phe Pro Asp
 115 120 125

Tyr Leu Pro Cys Ser Val Met Leu Gln Pro Ala Pro Gln Asp Ser Gly
 130 135 140

Lys Ser Cys Gly Val Asp Phe Glu Val Lys Ala Phe Ala Thr Asp Ser
 145 150 155 160

Thr Asp Ala Glu Glu Asp Lys Ile Pro Lys Lys Ser Ser Val Arg Leu
 165 170 175

Leu Ile Arg Lys Val Gln His Ala Pro Leu Glu Met Gly Pro Gln Pro
 180 185 190

Arg Ala Glu Ala Ala Trp Gln Phe Phe Met Ser Asp Lys Pro Leu His
 195 200 205

Leu Ala Val Ser Leu Asn Lys Glu Ile Tyr Phe His Gly Glu Pro Ile
 210 215 220

Pro Val Thr Val Thr Val Thr Asn Asn Thr Glu Lys Thr Val Lys Lys
 225 230 235 240

Ile Lys Ala Phe Val Glu Gln Val Ala Asn Val Val Leu Tyr Ser Ser
 245 250 255

Asp Tyr Tyr Val Lys Pro Val Ala Met Glu Glu Ala Gln Glu Lys Val
 260 265 270

Pro Pro Asn Ser Thr Leu Thr Lys Thr Leu Thr Leu Pro Leu Leu
 275 280 285

Ala Asn Asn Arg Glu Arg Arg Gly Ile Ala Leu Asp Gly Lys Ile Lys
 290 295 300

His Glu Asp Thr Asn Leu Ala Ser Ser Thr Ile Ile Lys Glu Gly Ile
 305 310 315 320

Asp Arg Thr Val Leu Gly Ile Leu Val Ser Tyr Gln Ile Lys Val Lys
 325 330 335

Leu Thr Val Ser Gly Phe Leu Gly Glu Leu Thr Ser Ser Glu Val Ala
 340 345 350

Thr Glu Val Pro Phe Arg Leu Met His Pro Gln Pro Glu Asp Pro Ala
 355 360 365

Lys Glu Ser Tyr Gln Asp Ala Asn Leu Val Phe Glu Glu Phe Ala Arg
 370 375 380

His Asn Leu Lys Asp Ala Gly Glu Ala Glu Glu Gly Lys Arg Asp Lys
 385 390 395 400

Asn Asp Val Asp Glu
 405

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<210> SEQ_ID NO 53
<211> LENGTH: 1247
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

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Met Met Arg Glu Trp Val Leu Leu Met Ser Val Leu Leu Cys Gly Leu
1           5          10          15

Ala Gly Pro Thr His Leu Phe Gln Pro Ser Leu Val Leu Asp Met Ala
20          25          30

Lys Val Leu Leu Asp Asn Tyr Cys Phe Pro Glu Asn Leu Leu Gly Met
35          40          45

Gln Glu Ala Ile Gln Gln Ala Ile Lys Ser His Glu Ile Leu Ser Ile
50          55          60

Ser Asp Pro Gln Thr Leu Ala Ser Val Leu Thr Ala Gly Val Gln Ser
65          70          75          80

Ser Leu Asn Asp Pro Arg Leu Val Ile Ser Tyr Glu Pro Ser Thr Pro
85          90          95

Glu Pro Pro Pro Gln Val Pro Ala Leu Thr Ser Leu Ser Glu Glu Glu
100         105         110

Leu Leu Ala Trp Leu Gln Arg Gly Leu Arg His Glu Val Leu Glu Gly
115         120         125

Asn Val Gly Tyr Leu Arg Val Asp Ser Val Pro Gly Gln Glu Val Leu
130         135         140

Ser Met Met Gly Glu Phe Leu Val Ala His Val Trp Gly Asn Leu Met
145         150         155         160

Gly Thr Ser Ala Leu Val Leu Asp Leu Arg His Cys Thr Gly Gly Gln
165         170         175

Val Ser Gly Ile Pro Tyr Ile Ile Ser Tyr Leu His Pro Gly Asn Thr
180         185         190

Ile Leu His Val Asp Thr Ile Tyr Asn Arg Pro Ser Asn Thr Thr Thr
195         200         205

Glu Ile Trp Thr Leu Pro Gln Val Leu Gly Glu Arg Tyr Gly Ala Asp
210         215         220

Lys Asp Val Val Val Leu Thr Ser Ser Gln Thr Arg Gly Val Ala Glu
225         230         235         240

Asp Ile Ala His Ile Leu Lys Gln Met Arg Arg Ala Ile Val Val Gly
245         250         255

Glu Arg Thr Gly Gly Gly Ala Leu Asp Leu Arg Lys Leu Arg Ile Gly
260         265         270

Glu Ser Asp Phe Phe Phe Val Pro Val Ser Arg Ser Leu Gly Pro
275         280         285

Leu Gly Gly Gly Ser Gln Thr Trp Glu Gly Ser Gly Val Leu Pro Cys
290         295         300

Val Gly Thr Pro Ala Glu Gln Ala Leu Glu Lys Ala Leu Ala Ile Leu
305         310         315         320

Thr Leu Arg Ser Ala Leu Pro Gly Val Val His Cys Leu Gln Glu Val
325         330         335

Leu Lys Asp Tyr Tyr Thr Leu Val Asp Arg Val Pro Thr Leu Leu Gln
340         345         350

His Leu Ala Ser Met Asp Phe Ser Thr Val Val Ser Glu Glu Asp Leu
355         360         365

Val Thr Lys Leu Asn Ala Gly Leu Gln Ala Ala Ser Glu Asp Pro Arg

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370	375	380
Leu Leu Val Arg Ala Ile Gly Pro Thr Glu Thr Pro Ser Trp Pro Ala		
385	390	395
400		
Pro Asp Ala Ala Ala Glu Asp Ser Pro Gly Val Ala Pro Glu Leu Pro		
405	410	415
Glu Asp Glu Ala Ile Arg Gln Ala Leu Val Asp Ser Val Phe Gln Val		
420	425	430
Ser Val Leu Pro Gly Asn Val Gly Tyr Leu Arg Phe Asp Ser Phe Ala		
435	440	445
Asp Ala Ser Val Leu Gly Val Leu Ala Pro Tyr Val Leu Arg Gln Val		
450	455	460
Trp Glu Pro Leu Gln Asp Thr Glu His Leu Ile Met Asp Leu Arg His		
465	470	475
480		
Asn Pro Gly Pro Ser Ser Ala Val Pro Leu Leu Leu Ser Tyr Phe		
485	490	495
Gln Gly Pro Glu Ala Gly Pro Val His Leu Phe Thr Thr Tyr Asp Arg		
500	505	510
Arg Thr Asn Ile Thr Gln Glu His Phe Ser His Met Glu Leu Pro Gly		
515	520	525
Pro Arg Tyr Ser Thr Gln Arg Gly Val Tyr Leu Leu Thr Ser His Arg		
530	535	540
Thr Ala Thr Ala Ala Glu Glu Phe Ala Phe Leu Met Gln Ser Leu Gly		
545	550	555
560		
Trp Ala Thr Leu Val Gly Glu Ile Thr Ala Gly Asn Leu Leu His Thr		
565	570	575
Arg Thr Val Pro Leu Leu Asp Thr Pro Glu Gly Ser Leu Ala Leu Thr		
580	585	590
Val Pro Val Leu Thr Phe Ile Asp Asn His Gly Glu Ala Trp Leu Gly		
595	600	605
Gly Gly Val Val Pro Asp Ala Ile Val Leu Ala Glu Glu Ala Leu Asp		
610	615	620
Lys Ala Gln Glu Val Leu Glu Phe His Gln Ser Leu Gly Ala Leu Val		
625	630	635
640		
Glu Gly Thr Gly His Leu Leu Glu Ala His Tyr Ala Arg Pro Glu Val		
645	650	655
Val Gly Gln Thr Ser Ala Leu Leu Arg Ala Lys Leu Ala Gln Gly Ala		
660	665	670
Tyr Arg Thr Ala Val Asp Leu Glu Ser Leu Ala Ser Gln Leu Thr Ala		
675	680	685
Asp Leu Gln Glu Val Ser Gly Asp His Arg Leu Leu Val Phe His Ser		
690	695	700
Pro Gly Glu Leu Val Val Glu Glu Ala Pro Pro Pro Pro Ala Val		
705	710	715
720		
Pro Ser Pro Glu Glu Leu Thr Tyr Leu Ile Glu Ala Leu Phe Lys Thr		
725	730	735
Glu Val Leu Pro Gly Gln Leu Gly Tyr Leu Arg Phe Asp Ala Met Ala		
740	745	750
Glu Leu Glu Thr Val Lys Ala Val Gly Pro Gln Leu Val Arg Leu Val		
755	760	765
Trp Gln Gln Leu Val Asp Thr Ala Ala Leu Val Ile Asp Leu Arg Tyr		
770	775	780
Asn Pro Gly Ser Tyr Ser Thr Ala Ile Pro Leu Leu Cys Ser Tyr Phe		
785	790	795
800		

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Phe Glu Ala Glu Pro Arg Gln His Leu Tyr Ser Val Phe Asp Arg Ala
805 810 815

Thr Ser Lys Val Thr Glu Val Trp Thr Leu Pro Gln Val Ala Gly Gln
820 825 830

Arg Tyr Gly Ser His Lys Asp Leu Tyr Ile Leu Met Ser His Thr Ser
835 840 845

Gly Ser Ala Ala Glu Ala Phe Ala His Thr Met Gln Asp Leu Gln Arg
850 855 860

Ala Thr Val Ile Gly Glu Pro Thr Ala Gly Ala Leu Ser Val Gly
865 870 875 880

Ile Tyr Gln Val Gly Ser Ser Pro Leu Tyr Ala Ser Met Pro Thr Gln
885 890 895

Met Ala Met Ser Ala Thr Thr Gly Lys Ala Trp Asp Leu Ala Gly Val
900 905 910

Glu Pro Asp Ile Thr Val Pro Met Ser Glu Ala Leu Ser Ile Ala Gln
915 920 925

Asp Ile Val Ala Leu Arg Ala Lys Val Pro Thr Val Leu Gln Thr Ala
930 935 940

Gly Lys Leu Val Ala Asp Asn Tyr Ala Ser Ala Glu Leu Gly Ala Lys
945 950 955 960

Met Ala Thr Lys Leu Ser Gly Leu Gln Ser Arg Tyr Ser Arg Val Thr
965 970 975

Ser Glu Val Ala Leu Ala Glu Ile Leu Gly Ala Asp Leu Gln Met Leu
980 985 990

Ser Gly Asp Pro His Leu Lys Ala Ala His Ile Pro Glu Asn Ala Lys
995 1000 1005

Asp Arg Ile Pro Gly Ile Val Pro Met Gln Ile Pro Ser Pro Glu
1010 1015 1020

Val Phe Glu Glu Leu Ile Lys Phe Ser Phe His Thr Asn Val Leu
1025 1030 1035

Glu Asp Asn Ile Gly Tyr Leu Arg Phe Asp Met Phe Gly Asp Gly
1040 1045 1050

Glu Leu Leu Thr Gln Val Ser Arg Leu Leu Val Glu His Ile Trp
1055 1060 1065

Lys Lys Ile Met His Thr Asp Ala Met Ile Ile Asp Met Arg Phe
1070 1075 1080

Asn Ile Gly Gly Pro Thr Ser Ser Ile Pro Ile Leu Cys Ser Tyr
1085 1090 1095

Phe Phe Asp Glu Gly Pro Pro Val Leu Leu Asp Lys Ile Tyr Ser
1100 1105 1110

Arg Pro Asp Asp Ser Val Ser Glu Leu Trp Thr His Ala Gln Val
1115 1120 1125

Val Gly Glu Arg Tyr Gly Ser Lys Lys Ser Met Val Ile Leu Thr
1130 1135 1140

Ser Ser Val Thr Ala Gly Thr Ala Glu Glu Phe Thr Tyr Ile Met
1145 1150 1155

Lys Arg Leu Gly Arg Ala Leu Val Ile Gly Glu Val Thr Ser Gly
1160 1165 1170

Gly Cys Gln Pro Pro Gln Thr Tyr His Val Asp Asp Thr Asn Leu
1175 1180 1185

Tyr Leu Thr Ile Pro Thr Ala Arg Ser Val Gly Ala Ser Asp Gly
1190 1195 1200

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Ser	Ser	Trp	Glu	Gly	Val	Gly	Val	Thr	Pro	His	Val	Val	Val	Pro
1205					1210						1215			
Ala	Glu	Glu	Ala	Leu	Ala	Arg	Ala	Lys	Glu	Met	Leu	Gln	His	Asn
1220					1225						1230			
Gln	Leu	Arg	Val	Lys	Arg	Ser	Pro	Gly	Leu	Gln	Asp	His	Leu	
1235					1240						1245			

<210> SEQ ID NO 54
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Leu	Gln	Leu	Gln	Pro	Phe	Pro	Gln	Pro	Gln	Leu	Pro	Tyr	Pro	Gln	Pro
1					5					10			15		

Gln	Leu	Pro	Tyr	Pro	Gln	Pro	Gln	Leu	Pro	Tyr	Pro	Gln	Pro	Gln	Pro
			20			25						30			

Phe

<210> SEQ ID NO 55
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Leu	Gln	Leu	Gln	Pro	Phe	Pro	Gln	Pro	Glu	Leu	Pro	Tyr	Pro	Gln	Pro
1					5				10			15			

Glu	Leu	Pro	Tyr	Pro	Gln	Pro	Glu	Leu	Pro	Tyr	Pro	Gln	Pro	Gln	Pro
			20			25						30			

Phe

<210> SEQ ID NO 56
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Gln	Gln	Tyr	Pro	Ser	Gly	Gln	Gly	Ser	Phe	Gln	Pro	Ser	Gln	Gln	Asn
1					5				10			15			

Pro Gln

<210> SEQ ID NO 57
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Gln	Pro	Phe	Pro	Gln	Pro	Glu	Gln	Pro	Phe	Pro	Trp				
1					5				10						

<210> SEQ ID NO 58
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Glu	Leu	Gln	Pro	Phe	Pro	Gln	Pro	Glu	Leu	Pro	Tyr	Pro	Gln	Pro	
1					5				10			15			

<210> SEQ ID NO 59
<211> LENGTH: 18

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-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Gly Cys Arg Gly Gly Pro Gln Pro Gln Pro Phe Pro Ser Gln Gln
1 5 10 15
Pro Tyr

<210> SEQ ID NO 60

<211> LENGTH: 51

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 60

Gly Cys Arg Gly Gly Pro Gln Pro Gln Pro Phe Pro Ser Gln Gln
1 5 10 15
Pro Tyr Leu Gln Leu Gln Pro Phe Pro Gln Pro Gln Leu Pro Tyr Pro
20 25 30
Gln Pro Gln Leu Pro Tyr Pro Gln Pro Gln Leu Pro Tyr Pro Gln Pro
35 40 45
Gln Pro Phe
50

<210> SEQ ID NO 61

<211> LENGTH: 51

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

Gly Cys Arg Gly Gly Pro Gln Pro Gln Pro Phe Pro Ser Gln Gln
1 5 10 15
Pro Tyr Leu Gln Leu Gln Pro Phe Pro Gln Pro Glu Leu Pro Tyr Pro
20 25 30
Gln Pro Glu Leu Pro Tyr Pro Gln Pro Glu Leu Pro Tyr Pro Gln Pro
35 40 45
Gln Pro Phe
50

<210> SEQ ID NO 62

<211> LENGTH: 140

<212> TYPE: PRT

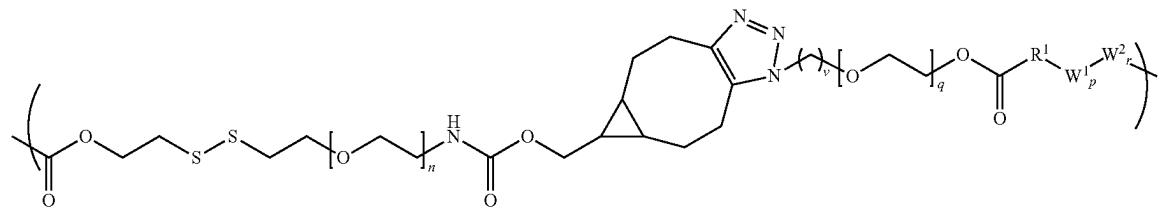
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

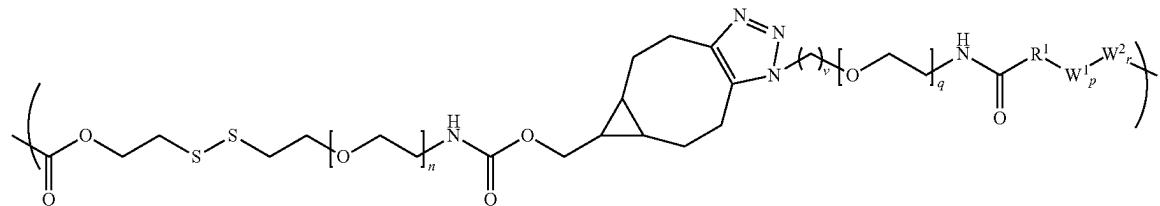
Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15
Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30
Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45
Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60
Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80
Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
85 90 95
Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
100 105 110

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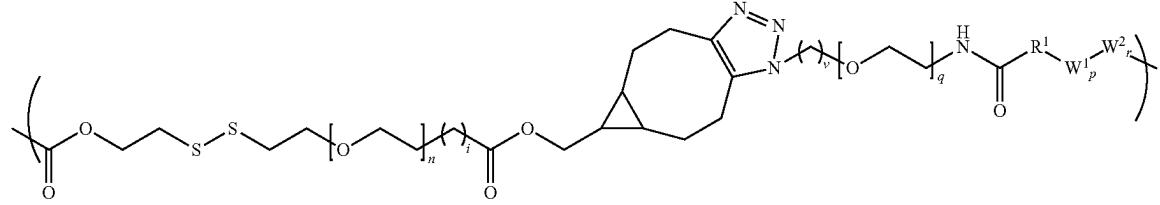
Yj



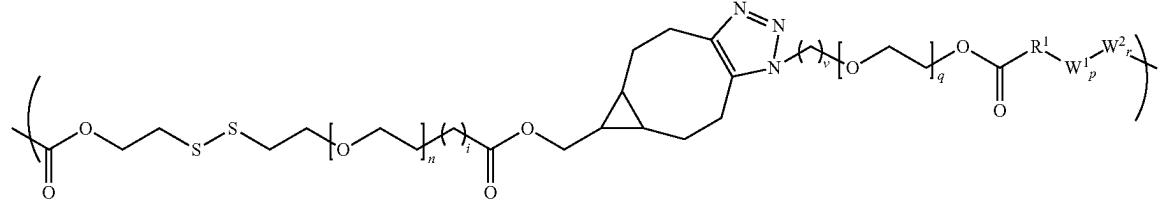
Yk



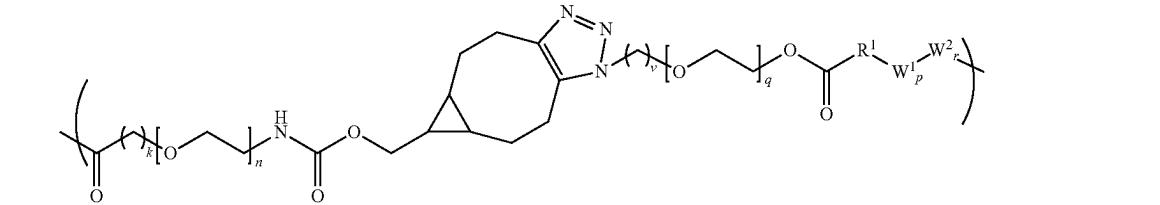
Yl



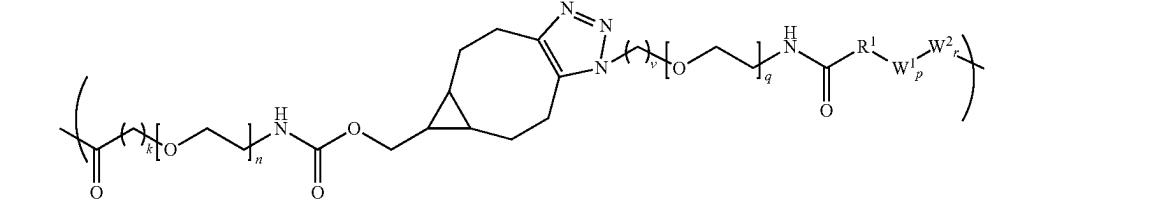
Ym



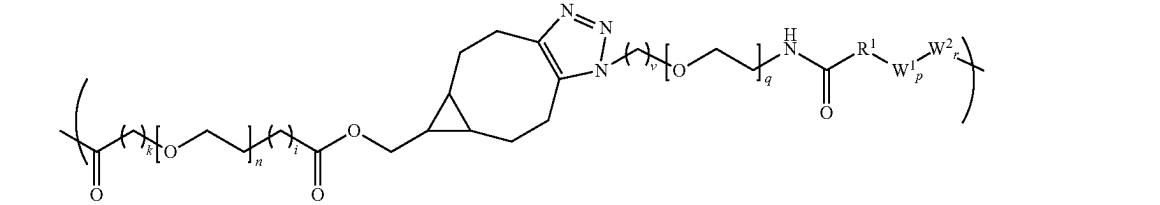
Yn



Yo



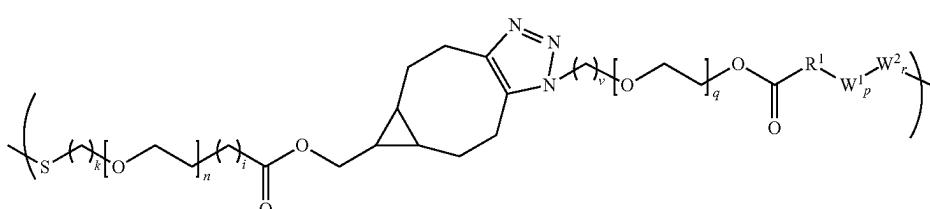
Yp



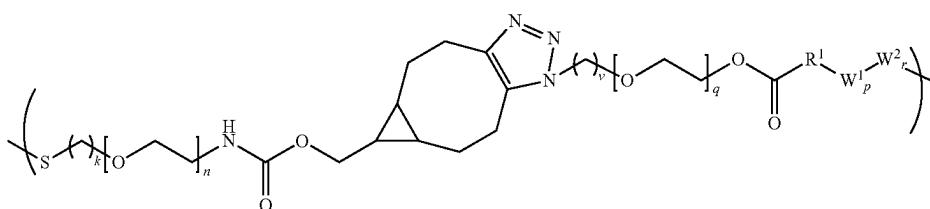
145

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-continued



Yq



Yr

20

where

n is an integer from 1 to 100;

q is an integer from 1 to 44;

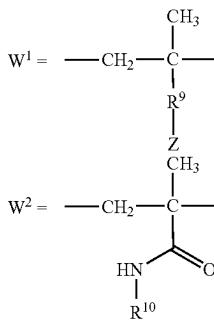
k is an integer from 1 to 12;

i is an integer from 0 to 20;

v is an integer from 1 to 4;

p is an integer from 2 to 250;

r is an integer from 0 to 250;

R₁ is —CH₂—, —(CH₂)₂—C(CH₃)(CN)—, —(CH₂)₂—C(CH₃)(CH₃)—, —(CH₂)₂—CH(CH₃)— or—CH(CH₃)—;W¹ and W² are as defined below:

R⁹ is a direct bond, —(CH₂)₂NH—C(O)— (an ethylacetamido group or “EtAcN”) or —(CH₂)₂(O—CH₂CH₂)_tNH—C(O)— (a pegylated ethylacetamido group or “Et-PEGt-AcN”)

t is an integer from 1 to 5;

Z is mannose or a mannose receptor-targeting moiety; and

R¹⁰ is an aliphatic group, an alcohol, an aliphatic amine-containing group, or an aliphatic alcohol.

6. The compound of claim 1, wherein Y is an antibody, antibody fragment, peptide or other ligand that binds to X.

7. The compound of claim 1, wherein X is an antigen against which a patient may develop or has developed an unwanted immune response.

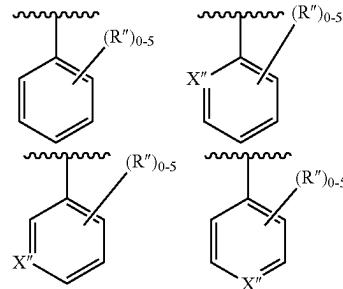
8. The compound of claim 7, wherein the antigen is a foreign transplant antigen, an alloantigen, an autoimmune antigen, a food antigen, an animal antigen, a plant antigen, an environmental antigen, a therapeutic antigen, a synthetic self-antigen, or a tolerogenic portion thereof.

25 9. The compound of claim 8, wherein X is an asparaginase antigen or an ovalbumin antigen.

10. The compound of claim 1, wherein the mannose receptor is mannose-6-phosphate receptor.

11. The compound of claim 1, wherein Y and X are connected through a bond configured to cleave when the compound reaches a target area.

12. The compound of claim 1, wherein Ar is selected from:

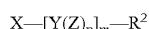
45 where each instance of R'', where present, is independently selected from an optionally substituted C₁₋₆-alkyl, optionally substituted C₁₋₆ alkoxy, optionally substituted amino, OH, or halogen and wherein, X'' is a heteroatom.

13. The compound of claim 12 wherein X'' is N.

14. The compound of claim 1, wherein R¹¹ is —CH₃.

15. A composition comprising the compound of claim 1.

16. A method of inducing immunological tolerance to an antigen target comprising administering to a subject a composition Formula 1:



Formula 1

where:

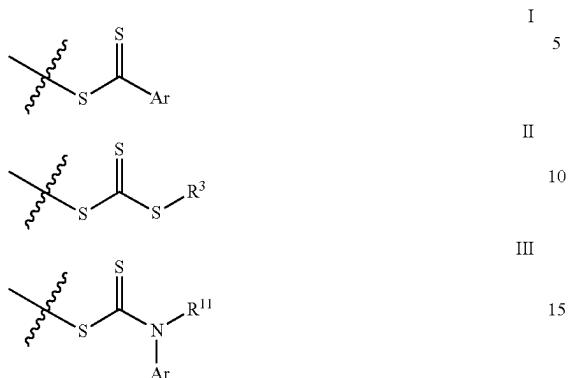
X comprises an antigen, a tolerogenic portion thereof, or a mimetic thereof;

Y comprises a linker moiety;

Z comprises a moiety that specifically targets a mannose receptor;

p is an integer from 2 to 250;

m is an integer from 1 to 100;

147R² is any of functional groups I-III:

where Ar is a substituted or unsubstituted aromatic group 20
and one or more of:

R³ is C₁₋₆-alkyl; orR¹¹ is C₁₋₆- alkyl.

17. The method of claim 16, wherein the moiety that specifically targets a mannose receptor is selected from the 25 group consisting of α -linked mannose, β -linked mannose, substituted mannose, mannose-6-phosphate, N-acetyl mannosamine, and mannan having β (1-4), α (1-6), α (1-2), and/or α (1-3) linkages.

* * * *