PYRAZOLOAZEPINE IMMUNOCONJUGATES, AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This non-provisional application claims the benefit of priority to U.S. Provisional Application No. 63/065,219, filed 13 August 2020, which is incorporated by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 5, 2021, is named 17019_009WO1_SL.txt and is 63,469 bytes in size.

FIELD OF THE INVENTION

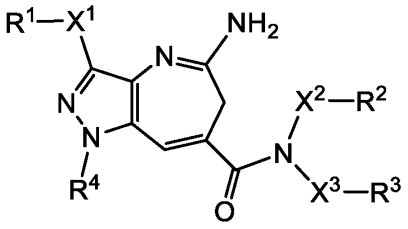
The invention relates generally to an immunoconjugate comprising an antibody conjugated to one or more pyrazoloazepine molecules.

BACKGROUND OF THE INVENTION

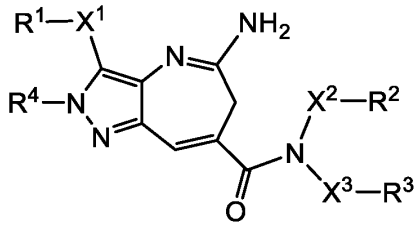
New compositions and methods for the delivery of antibodies and immune adjuvants are needed in order to reach inaccessible tumors and/or to expand treatment options for cancer patients and other subjects. The invention provides such compositions and methods.

SUMMARY OF THE INVENTION

The invention is generally directed to immunoconjugates comprising an antibody linked by conjugation to one or more pyrazoloazepine derivatives. The invention is further directed to pyrazoloazepine derivative intermediate compositions comprising a reactive functional group. Such intermediate compositions are suitable substrates for formation of immunoconjugates wherein an antibody may be covalently bound by a linker L to a pyrazoloazepine (PAZ) moiety having the formulas:



IIa



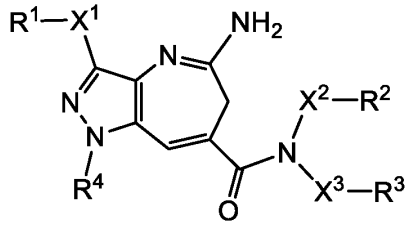
IIb;

where one of R^1, R^2, R^3 and R^4 is attached to L. The X^1, X^2, and X^3 and R^1, R^2, R^3 and R^4 substituents are defined herein.

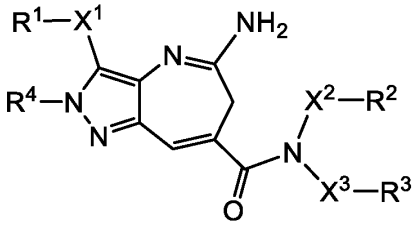
The invention is further directed to use of such an immunoconjugates in the treatment of an illness, in particular cancer.

An aspect of the invention is an immunoconjugate comprising an antibody covalently attached to a linker which is covalently attached to one or more pyrazoloazepine moieties.

Another aspect of the invention is a 5-aminopyrazoloazepine-linker compound selected from formulas IIa and IIb:



IIa



IIb;

where one of R^1, R^2, R^3, and R^4 is attached to L.

Another aspect of the invention is a method for treating cancer comprising administering a therapeutically effective amount of an immunoconjugate comprising an antibody linked by conjugation to one or more pyrazoloazepine moieties.

Another aspect of the invention is a use of an immunoconjugate comprising an antibody linked by conjugation to one or more pyrazoloazepine moieties for treating cancer.

Another aspect of the invention is a method of preparing an immunoconjugate by conjugation of one or more pyrazoloazepine moieties with an antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a graph of HEK human TLR7 activity at 24 hours of pyrazoloazepine compounds PAZ-2, PAZ-4 and PAZ-11, versus comparator adjuvant compounds C-1 and C-2.

Figure 2 shows a graph of HEK human TLR8 activity at 24 hours of pyrazoloazepine compounds PAZ-2, PAZ-4 and PAZ-11, versus comparator adjuvant compounds C-1 and C-2.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the invention as defined by the claims.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The invention is in no way limited to the methods and materials described.

DEFINITIONS

The term "immunoconjugate" refers to an antibody construct that is covalently bonded to an adjuvant moiety via a linker. the term "adjuvant" refers to a substance capable of eliciting an immune response in a subject exposed to the adjuvant. The phrase "adjuvant moiety" refers to an adjuvant that is covalently bonded to an antibody construct, e.g., through a linker, as described herein. The adjuvant moiety can elicit the immune response while bonded to the antibody construct or after cleavage (e.g., enzymatic cleavage) from the antibody construct following administration of an immunoconjugate to the subject. Immunoconjugates allow targeted delivery of an active adjuvant moiety while the target antigen is bound.

"Adjuvant" refers to a substance capable of eliciting an immune response in a subject exposed to the adjuvant. The phrase "adjuvant moiety" refers to an adjuvant that is covalently bonded to an antibody construct, e.g., through a linker, as described herein. The adjuvant moiety can elicit the immune response while bonded to the antibody construct or after cleavage (e.g., enzymatic cleavage) from the antibody construct following administration of an immunoconjugate to the subject.

The terms "Toll-like receptor" and "TLR" refer to any member of a family of highly-conserved mammalian proteins which recognizes pathogen-associated molecular patterns and acts as key signaling elements in innate immunity. TLR polypeptides share a characteristic structure that includes an extracellular domain that has leucine-rich repeats, a transmembrane domain, and an intracellular domain that is involved in TLR signaling.

The terms "Toll-like receptor 7" and "TLR7" refer to nucleic acids or polypeptides sharing at least about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or more sequence identity to a publicly-available TLR7 sequence, e.g., GenBank accession number AAZ99026 for human TLR7 polypeptide, or GenBank accession number AAK62676 for murine TLR7 polypeptide.

The terms "Toll-like receptor 8" and "TLR8" refer to nucleic acids or polypeptides sharing at least about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or more sequence identity to a publicly-available TLR7 sequence, e.g., GenBank accession number AAZ95441 for human TLR8 polypeptide, or GenBank accession number AAK62677 for murine TLR8 polypeptide.

A "TLR agonist" is a substance that binds, directly or indirectly, to a TLR (e.g., TLR7 and/or TLR8) to induce TLR signaling. Any detectable difference in TLR signaling can indicate that an agonist stimulates or activates a TLR. Signaling differences can be manifested, for example, as changes in the expression of target genes, in the phosphorylation of signal transduction components, in the intracellular localization of downstream elements such as nuclear factor-κB (NF-κB), in the association of certain components (such as IL-1 receptor associated kinase (IRAK)) with other proteins or intracellular structures, or in the biochemical activity of components such as kinases (such as mitogen-activated protein kinase (MAPK)).

"Antibody" refers to a polypeptide comprising an antigen binding region (including the complementarity determining region (CDRs)) from an immunoglobulin gene or fragments thereof. The term "antibody" specifically encompasses monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa) connected by disulfide bonds. Each chain is composed of structural domains, which are referred to as immunoglobulin domains. These domains are classified into different categories by size and function, e.g., variable domains or regions on the light and heavy chains (V_L and V_H, respectively) and constant domains or regions on the light and heavy chains (C_L and C_H, respectively). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids, referred to as the paratope, primarily responsible for antigen recognition, i.e., the antigen binding domain. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. IgG antibodies are large molecules of about 150 kDa composed of four peptide chains. IgG antibodies contain two identical class γ heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure. The two heavy chains are linked to each other and to a light chain each by disulfide bonds. The resulting tetramer has two identical halves, which together form the Y-like shape. Each end of the fork contains an identical antigen binding domain. There are four IgG subclasses (IgG1, IgG2, IgG3, and IgG4) in humans, named in order of their abundance in serum (i.e., IgG1 is the most abundant). Typically, the antigen binding domain of an antibody will be most critical in specificity and affinity of binding to cancer cells.

An antibody that targets a particular antigen includes a bispecific or multispecific antibody with at least one antigen binding region that targets the particular antigen. In some

embodiments, the targeted monoclonal antibody is a bispecific antibody with at least one antigen binding region that targets tumor cells. Such antigens include but are not limited to: mesothelin, prostate specific membrane antigen (PSMA), HER2, TROP2, CEA, EGFR, 5T4, Nectin4, CD19, CD20, CD22, CD30, CD70, B7H3, B7H4 (also known as 08E), protein tyrosine kinase 7 (PTK7), glypican-3, RG1, fucosyl-GMl, CTLA-4, and CD44 (WO 2017/196598).

"Antibody construct" refers to an antibody or a fusion protein comprising (i) an antigen binding domain and (ii) an Fc domain.

In some embodiments, the binding agent is an antigen-binding antibody "fragment," which is a construct that comprises at least an antigen-binding region of an antibody, alone or with other components that together constitute the antigen-binding construct. Many different types of antibody "fragments" are known in the art, including, for instance, (i) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L, and CH_1 domains, (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a Fab' fragment, which results from breaking the disulfide bridge of an $F(ab')_2$ fragment using mild reducing conditions, (v) a disulfide-stabilized Fv fragment (dsFv), and (vi) a single chain Fv (scFv), which is a monovalent molecule consisting of the two domains of the Fv fragment (i.e., V_L and V_H) joined by a synthetic linker which enables the two domains to be synthesized as a single polypeptide chain.

The antibody or antibody fragments can be part of a larger construct, for example, a conjugate or fusion construct of the antibody fragment to additional regions. For instance, in some embodiments, the antibody fragment can be fused to an Fc region as described herein. In other embodiments, the antibody fragment (e.g., a Fab or scFv) can be part of a chimeric antigen receptor or chimeric T-cell receptor, for instance, by fusing to a transmembrane domain (optionally with an intervening linker or "stalk" (e.g., hinge region)) and optional intercellular signaling domain. For instance, the antibody fragment can be fused to the gamma and/or delta chains of a t-cell receptor, so as to provide a T-cell receptor like construct that binds PD-L1. In yet another embodiment, the antibody fragment is part of a bispecific T-cell engager (BiTEs) comprising a CD1 or CD3 binding domain and linker.

"Epitope" means any antigenic determinant or epitopic determinant of an antigen to which an antigen binding domain binds (i.e., at the paratope of the antigen binding domain). Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

The terms "Fc receptor" or "FcR" refer to a receptor that binds to the Fc region of an antibody. There are three main classes of Fc receptors: (1) FcγR which bind to IgG, (2) FcαR which binds to IgA, and (3) FcεR which binds to IgE. The FcγR family includes several members, such as FcγI (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16A), and FcγRIIIB (CD16B). The Fcγ receptors differ in their affinity for IgG and also have different affinities for the IgG subclasses (e.g., IgG1, IgG2, IgG3, and IgG4).

Nucleic acid or amino acid sequence "identity," as referenced herein, can be determined by comparing a nucleic acid or amino acid sequence of interest to a reference nucleic acid or amino acid sequence. The percent identity is the number of nucleotides or amino acid residues that are the same (i.e., that are identical) as between the optimally aligned sequence of interest and the reference sequence divided by the length of the longest sequence (i.e., the length of either the sequence of interest or the reference sequence, whichever is longer). Alignment of sequences and calculation of percent identity can be performed using available software programs. Examples of such programs include CLUSTAL-W, T-Coffee, and ALIGN (for alignment of nucleic acid and amino acid sequences), BLAST programs (e.g., BLAST 2.1, BL2SEQ, BLASTp, BLASTn, and the like) and FASTA programs (e.g., FASTA3x, FASTM, and SSEARCH) (for sequence alignment and sequence similarity searches). Sequence alignment algorithms also are disclosed in, for example, Altschul et al., *J. Molecular Biol.*, 215(3): 403-410 (1990), Beigert et al., *Proc. Natl. Acad. Sci. USA*, *106*(10): 3770-3775 (2009), Durbin et al., eds., *Biological Sequence Analysis: Probalistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, 21(7): 951-960 (2005), Altschul et al., *Nucleic Acids Res.*, 25(17): 3389-3402 (1997), and Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997)). Percent (%) identity of sequences can be also calculated, for example, as 100 x [(identical positions)/min(TG_A, TG_B)], where TG_A and TG_B are the sum of the number of residues and internal gap positions in peptide sequences A and B in the alignment that minimizes TG_A and TG_B. See, e.g., Russell et al., *J. Mol Biol.*, 244: 332-350 (1994).

The binding agent comprises Ig heavy and light chain variable region polypeptides that together form the antigen binding site. Each of the heavy and light chain variable regions are polypeptides comprising three complementarity determining regions (CDR1, CDR2, and CDR3) connected by framework regions. The binding agent can be any of a variety of types of binding agents known in the art that comprise Ig heavy and light chains. For instance, the binding agent can be an antibody, an antigen-binding antibody "fragment," or a T-cell receptor.

"Biosimilar" refers to an approved antibody construct that has active properties similar to, for example, a PD-L1-targeting antibody construct previously approved such as atezolizumab

(TECENTRIQ™, Genentech, Inc.), durvalumab (IMFINZI™, AstraZeneca), and avelumab (BAVENCIO™, EMD Serono, Pfizer); a HER2-targeting antibody construct previously approved such as trastuzumab (HERCEPTIN™, Genentech, Inc.), and pertuzumab (PERJETA™, Genentech, Inc.); or a CEA-targeting antibody such as labetuzumab (CEA-CIDE™, MN-14, hMN14, Immunomedics) CAS Reg. No. 219649-07-7).

"Biobetter" refers to an approved antibody construct that is an improvement of a previously approved antibody construct, such as atezolizumab, durvalumab, avelumab, trastuzumab, pertuzumab, and labetuzumab. The biobetter can have one or more modifications (e.g., an altered glycan profile, or a unique epitope) over the previously approved antibody construct.

"Amino acid" refers to any monomeric unit that can be incorporated into a peptide, polypeptide, or protein. Amino acids include naturally-occurring α-amino acids and their stereoisomers, as well as unnatural (non-naturally occurring) amino acids and their stereoisomers. "Stereoisomers" of a given amino acid refer to isomers having the same molecular formula and intramolecular bonds but different three-dimensional arrangements of bonds and atoms (e.g., an L-amino acid and the corresponding D-amino acid). The amino acids can be glycosylated (e.g., *N*-linked glycans, *O*-linked glycans, phosphoglycans, *C*-linked glycans, or glypication) or deglycosylated. Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

Naturally-occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and *O*-phosphoserine. Naturally-occurring α-amino acids include, without limitation, alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), arginine (Arg), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), and combinations thereof. Stereoisomers of naturally-occurring α-amino acids include, without limitation, D-alanine (D-Ala), D-cysteine (D-Cys), D-aspartic acid (D-Asp), D-glutamic acid (D-Glu), D-phenylalanine (D-Phe), D-histidine (D-His), D-isoleucine (D-Ile), D-arginine (D-Arg), D-lysine (D-Lys), D-leucine (D-Leu), D-methionine (D-Met), D-asparagine (D-Asn), D-proline (D-Pro), D-glutamine (D-Gln), D-serine (D-Ser), D-threonine (D-Thr), D-valine (D-Val), D-tryptophan (D-Trp), D-tyrosine (D-Tyr), and combinations thereof.

Naturally-occurring amino acids include those formed in proteins by post-translational modification, such as citrulline (Cit).

Unnatural (non-naturally occurring) amino acids include, without limitation, amino acid analogs, amino acid mimetics, synthetic amino acids, *N*-substituted glycines, and *N*-methyl amino acids in either the L- or D-configuration that function in a manner similar to the naturally-occurring amino acids. For example, "amino acid analogs" can be unnatural amino acids that have the same basic chemical structure as naturally-occurring amino acids (i.e., a carbon that is bonded to a hydrogen, a carboxyl group, an amino group) but have modified side-chain groups or modified peptide backbones, e.g., homoserine, norleucine, methionine sulfoxide, and methionine methyl sulfonium. "Amino acid mimetics" refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally-occurring amino acid.

"Linker" refers to a functional group that covalently bonds two or more moieties in a compound or material. For example, the linking moiety can serve to covalently bond an adjuvant moiety to an antibody construct in an immunoconjugate.

"Linking moiety" refers to a functional group that covalently bonds two or more moieties in a compound or material. For example, the linking moiety can serve to covalently bond an adjuvant moiety to an antibody in an immunoconjugate. Useful bonds for connecting linking moieties to proteins and other materials include, but are not limited to, amides, amines, esters, carbamates, ureas, thioethers, thiocarbamates, thiocarbonates, and thioureas.

"Divalent" refers to a chemical moiety that contains two points of attachment for linking two functional groups; polyvalent linking moieties can have additional points of attachment for linking further functional groups. Divalent radicals may be denoted with the suffix "diyl". For example, divalent linking moieties include divalent polymer moieties such as divalent poly(ethylene glycol), divalent cycloalkyl, divalent heterocycloalkyl, divalent aryl, and divalent heteroaryl group. A "divalent cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group" refers to a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group having two points of attachment for covalently linking two moieties in a molecule or material. Cycloalkyl, heterocycloalkyl, aryl, or heteroaryl groups can be substituted or unsubstituted. Cycloalkyl, heterocycloalkyl, aryl, or heteroaryl groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy.

A wavy line (" ~~~ ") represents a point of attachment of the specified chemical moiety. If the specified chemical moiety has two wavy lines (" ~~~ ") present, it will be understood that the chemical moiety can be used bilaterally, i.e., as read from left to right or from right to left. In some embodiments, a specified moiety having two wavy lines (" ~~~ ") present is considered to be used as read from left to right.

"Alkyl" refers to a straight (linear) or branched, saturated, aliphatic radical having the number of carbon atoms indicated. Alkyl can include any number of carbons, for example from one to twelve. Examples of alkyl groups include, but are not limited to, methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$, 1-heptyl, 1-octyl, and the like. Alkyl groups can be substituted or unsubstituted. "Substituted alkyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The term "alkyldiyl" refers to a divalent alkyl radical. Examples of alkyldiyl groups include, but are not limited to, methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), and the like. An alkyldiyl group may also be referred to as an "alkylene" group.

"Alkenyl" refers to a straight (linear) or branched, unsaturated, aliphatic radical having the number of carbon atoms indicated and at least one carbon-carbon double bond, *sp*2. Alkenyl can include from two to about 12 or more carbons atoms. Alkenyl groups are radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations. Examples include, but are not limited to, ethylenyl or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$). butenyl, pentenyl, and isomers thereof. Alkenyl groups can be substituted or unsubstituted. "Substituted alkenyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The terms "alkenylene" or "alkenyldiyl" refer to a linear or branched-chain divalent hydrocarbon radical. Examples include, but are not limited to, ethylenylene or vinylene ($-CH=CH-$), allyl ($-CH_2CH=CH-$), and the like.

"Alkynyl" refers to a straight (linear) or branched, unsaturated, aliphatic radical having the number of carbon atoms indicated and at least one carbon-carbon triple bond, *sp*. Alkynyl can include from two to about 12 or more carbons atoms. For example, C_2-C_6 alkynyl includes, but is not limited to ethynyl ($-C\equiv CH$), propynyl (propargyl, $-CH_2C\equiv CH$), butynyl, pentynyl,

hexynyl, and isomers thereof Alkynyl groups can be substituted or unsubstituted. "Substituted alkynyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The term "alkynylene" or "alkynyldiyl" refer to a divalent alkynyl radical.

The terms "carbocycle", "carbocyclyl", "carbocyclic ring" and "cycloalkyl" refer to a saturated or partially unsaturated, monocyclic, fused bicyclic, or bridged polycyclic ring assembly containing from 3 to 12 ring atoms, or the number of atoms indicated. Saturated monocyclic carbocyclic rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Saturated bicyclic and polycyclic carbocyclic rings include, for example, norbornane, [2.2.2] bicyclooctane, decahydronaphthalene and adamantane. Carbocyclic groups can also be partially unsaturated, having one or more double or triple bonds in the ring. Representative carbocyclic groups that are partially unsaturated include, but are not limited to, cyclobutene, cyclopentene, cyclohexene, cyclohexadiene (1,3- and 1,4-isomers), cycloheptene, cycloheptadiene, cyclooctene, cyclooctadiene (1,3-, 1,4- and 1,5-isomers), norbornene, and norbornadiene.

The term "cycloalkyldiyl" refers to a divalent cycloalkyl radical.

"Aryl" refers to a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms (C_6–C_{20}) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system.. Aryl groups can be monocyclic, fused to form bicyclic or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. Other aryl groups include benzyl, having a methylene linking group. Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl.

The terms "arylene" or "aryldiyl" mean a divalent aromatic hydrocarbon radical of 6-20 carbon atoms (C_6–C_{20}) derived by the removal of two hydrogen atom from a two carbon atoms of a parent aromatic ring system. Some aryldiyl groups are represented in the exemplary structures as "Ar". Aryldiyl includes bicyclic radicals comprising an aromatic ring fused to a saturated, partially unsaturated ring, or aromatic carbocyclic ring. Typical aryldiyl groups include, but are not limited to, radicals derived from benzene (phenyldiyl), substituted benzenes, naphthalene, anthracene, biphenylene, indenylene, indanylene, 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthyl, and the like. Aryldiyl groups are also referred to as "arylene", and are optionally substituted with one or more substituents described herein.

The terms "heterocycle," "heterocyclyl" and "heterocyclic ring" are used interchangeably herein and refer to a saturated or a partially unsaturated (i.e., having one or more double and/or triple bonds within the ring) carbocyclic radical of 3 to about 20 ring atoms

in which at least one ring atom is a heteroatom selected from nitrogen, oxygen, phosphorus and sulfur, the remaining ring atoms being C, where one or more ring atoms is optionally substituted independently with one or more substituents described below. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 4 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 6 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system. Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566. "Heterocyclyl" also includes radicals where heterocycle radicals are fused with a saturated, partially unsaturated ring, or aromatic carbocyclic or heterocyclic ring. Examples of heterocyclic rings include, but are not limited to, morpholin-4-yl, piperidin-1-yl, piperazinyl, piperazin-4-yl-2-one, piperazin-4-yl-3-one, pyrrolidin-1-yl, thiomorpholin-4-yl, S-dioxothiomorpholin-4-yl, azocan-1-yl, azetidin-1-yl, octahydropyrido[1,2-a]pyrazin-2-yl, [1,4]diazepan-1-yl, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, homopiperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinylimidazolinyl, imidazolidinyl, 3-azabicyco[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 3H-indolyl quinolizinyl and N-pyridyl ureas. Spiro heterocyclyl moieties are also included within the scope of this definition. Examples of spiro heterocyclyl moieties include azaspiro[2.5]octanyl and azaspiro[2.4]heptanyl. Examples of a heterocyclic group wherein 2 ring atoms are substituted with oxo (=O) moieties are pyrimidinonyl and 1,1-dioxo-thiomorpholinyl. The heterocycle groups herein are optionally substituted independently with one or more substituents described herein.

The term "heterocyclyldiyl" refers to a divalent, saturated or a partially unsaturated (i.e., having one or more double and/or triple bonds within the ring) carbocyclic radical of 3 to about 20 ring atoms in which at least one ring atom is a heteroatom selected from nitrogen, oxygen, phosphorus and sulfur, the remaining ring atoms being C, where one or more ring atoms is optionally substituted independently with one or more substituents as described. Examples of 5-membered and 6-membered heterocyclyldiyls include morpholinyldiyl, piperidinyldiyl,

piperazinyldiyl, pyrrolidinyldiyl, dioxanyldiyl, thiomorpholinyldiyl, and S-dioxothiomorpholinyldiyl.

The term "heteroaryl" refers to a monovalent aromatic radical of 5-, 6-, or 7-membered rings, and includes fused ring systems (at least one of which is aromatic) of 5-20 atoms, containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur. Examples of heteroaryl groups are pyridinyl (including, for example, 2-hydroxypyridinyl), imidazolyl, imidazopyridinyl, pyrimidinyl (including, for example, 4-hydroxypyrimidinyl), pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxadiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. Heteroaryl groups are optionally substituted independently with one or more substituents described herein.

The term "heteroaryldiyl" refers to a divalent aromatic radical of 5-, 6-, or 7-membered rings, and includes fused ring systems (at least one of which is aromatic) of 5-20 atoms, containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur. Examples of 5-membered and 6-membered heteroaryldiyls include pyridyldiyl, imidazolyldiyl, pyrimidinyldiyl, pyrazolyldiyl, triazolyldiyl, pyrazinyldiyl, tetrazolyldiyl, furyldiyl, thienyldiyl, isoxazolyldiyldiyl, thiazolyldiyl, oxadiazolyldiyl, oxazolyldiyl, isothiazolyldiyl, and pyrrolyldiyl.

The heterocycle or heteroaryl groups may be carbon (carbon-linked), or nitrogen (nitrogen-linked) bonded where such is possible. By way of example and not limitation, carbon bonded heterocycles or heteroaryls are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline.

By way of example and not limitation, nitrogen bonded heterocycles or heteroaryls are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline.

The terms "halo" and "halogen," by themselves or as part of another substituent, refer to a fluorine, chlorine, bromine, or iodine atom.

The term "carbonyl," by itself or as part of another substituent, refers to C(=O) or –C(=O)–, i.e., a carbon atom double-bonded to oxygen and bound to two other groups in the moiety having the carbonyl.

As used herein, the phrase "quaternary ammonium salt" refers to a tertiary amine that has been quaternized with an alkyl substituent (e.g., a C_1-C_4 alkyl such as methyl, ethyl, propyl, or butyl).

The terms "treat," "treatment," and "treating" refer to any indicia of success in the treatment or amelioration of an injury, pathology, condition (e.g., cancer), or symptom (e.g., cognitive impairment), including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the symptom, injury, pathology, or condition more tolerable to the patient; reduction in the rate of symptom progression; decreasing the frequency or duration of the symptom or condition; or, in some situations, preventing the onset of the symptom. The treatment or amelioration of symptoms can be based on any objective or subjective parameter, including, for example, the result of a physical examination.

The terms "cancer," "neoplasm," and "tumor" are used herein to refer to cells which exhibit autonomous, unregulated growth, such that the cells exhibit an aberrant growth phenotype characterized by a significant loss of control over cell proliferation. Cells of interest for detection, analysis, and/or treatment in the context of the invention include cancer cells (e.g., cancer cells from an individual with cancer), malignant cancer cells, pre-metastatic cancer cells, metastatic cancer cells, and non-metastatic cancer cells. Cancers of virtually every tissue are known. The phrase "cancer burden" refers to the quantum of cancer cells or cancer volume in a subject. Reducing cancer burden accordingly refers to reducing the number of cancer cells or the cancer cell volume in a subject. The term "cancer cell" as used herein refers to any cell that is a cancer cell (e.g., from any of the cancers for which an individual can be treated, e.g., isolated from an individual having cancer) or is derived from a cancer cell, e.g., clone of a cancer cell. For example, a cancer cell can be from an established cancer cell line, can be a primary cell isolated from an individual with cancer, can be a progeny cell from a primary cell isolated from an individual with cancer, and the like. In some embodiments, the term can also refer to a portion of a cancer cell, such as a sub-cellular portion, a cell membrane portion, or a cell lysate of a cancer cell. Many types of cancers are known to those of skill in the art, including solid tumors such as carcinomas, sarcomas, glioblastomas, melanomas, lymphomas, and myelomas, and circulating cancers such as leukemias.

As used herein, the term "cancer" includes any form of cancer, including but not limited to, solid tumor cancers (e.g., skin, lung, prostate, breast, gastric, bladder, colon, ovarian, pancreas, kidney, liver, glioblastoma, medulloblastoma, leiomyosarcoma, head & neck squamous cell carcinomas, melanomas, and neuroendocrine) and liquid cancers (e.g., hematological cancers); carcinomas; soft tissue tumors; sarcomas; teratomas; melanomas; leukemias; lymphomas; and brain cancers, including minimal residual disease, and including both primary and metastatic tumors.

"PD-L1 expression" refers to a cell that has a PD-L1 receptor on the cell's surface. As used herein "PD-L1 overexpression" refers to a cell that has more PD-L1 receptors as compared to corresponding non-cancer cell.

"HER2" refers to the protein human epidermal growth factor receptor 2.

"HER2 expression" refers to a cell that has a HER2 receptor on the cell's surface. For example, a cell may have from about 20,000 to about 50,000 HER2 receptors on the cell's surface. As used herein "HER2 overexpression" refers to a cell that has more than about 50,000 HER2 receptors. For example, a cell 2, 5, 10, 100, 1,000, 10,000, 100,000, or 1,000,000 times the number of HER2 receptors as compared to corresponding non-cancer cell (e.g., about 1 or 2 million HER2 receptors). It is estimated that HER2 is overexpressed in about 25% to about 30% of breast cancers.

The "pathology" of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression, or aggravation of inflammatory or immunological response, neoplasia, premalignancy, malignancy, and invasion of surrounding or distant tissues or organs, such as lymph nodes.

As used herein, the phrases "cancer recurrence" and "tumor recurrence," and grammatical variants thereof, refer to further growth of neoplastic or cancerous cells after diagnosis of cancer. Particularly, recurrence may occur when further cancerous cell growth occurs in the cancerous tissue. "Tumor spread," similarly, occurs when the cells of a tumor disseminate into local or distant tissues and organs, therefore, tumor spread encompasses tumor metastasis. "Tumor invasion" occurs when the tumor growth spread out locally to compromise the function of involved tissues by compression, destruction, or prevention of normal organ function.

As used herein, the term "metastasis" refers to the growth of a cancerous tumor in an organ or body part, which is not directly connected to the organ of the original cancerous tumor. Metastasis will be understood to include micrometastasis, which is the presence of an

undetectable amount of cancerous cells in an organ or body part that is not directly connected to the organ of the original cancerous tumor. Metastasis can also be defined as several steps of a process, such as the departure of cancer cells from an original tumor site, and migration and/or invasion of cancer cells to other parts of the body.

The phrases "effective amount" and "therapeutically effective amount" refer to a dose or amount of a substance such as an immunoconjugate that produces therapeutic effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th Edition (McGraw-Hill, 2006); and *Remington: The Science and Practice of Pharmacy*, 22nd Edition, (Pharmaceutical Press, London, 2012)). In the case of cancer, the therapeutically effective amount of the immunoconjugate may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the immunoconjugate may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR)

"Recipient," "individual," "subject," "host," and "patient" are used interchangeably and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired (e.g., humans). "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, camels, etc. In certain embodiments, the mammal is human.

The phrase "synergistic adjuvant" or "synergistic combination" in the context of this invention includes the combination of two immune modulators such as a receptor agonist, cytokine, and adjuvant polypeptide, that in combination elicit a synergistic effect on immunity relative to either administered alone. Particularly, the immunoconjugates disclosed herein comprise synergistic combinations of the claimed adjuvant and antibody construct. These synergistic combinations upon administration elicit a greater effect on immunity, e.g., relative to when the antibody construct or adjuvant is administered in the absence of the other moiety. Further, a decreased amount of the immunoconjugate may be administered (as measured by the total number of antibody constructs or the total number of adjuvants administered as part of the

immunoconjugate) compared to when either the antibody construct or adjuvant is administered alone.

As used herein, the term "administering" refers to parenteral, intravenous, intraperitoneal, intramuscular, intratumoral, intralesional, intranasal, or subcutaneous administration, oral administration, administration as a suppository, topical contact, intrathecal administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to the subject.

The terms "about" and "around," as used herein to modify a numerical value, indicate a close range surrounding the numerical value. Thus, if "X" is the value, "about X" or "around X" indicates a value of from 0.9X to 1.1X, e.g., from 0.95X to 1.05X or from 0.99X to 1.01X. A reference to "about X" or "around X" specifically indicates at least the values X, 0.95X, 0.96X, 0.97X, 0.98X, 0.99X, 1.01X, 1.02X, 1.03X, 1.04X, and 1.05X. Accordingly, "about X" and "around X" are intended to teach and provide written description support for a claim limitation of, e.g., "0.98X."

ANTIBODY TARGETS

In some embodiments, the antibody of an immunoconjugate is capable of binding one or more targets selected from (e.g., specifically binds to a target selected from) 5T4, ABL, ABCF1, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, ADORA2A, Aggrecan, AGR2, AICDA, AIF1, AIGI, AKAP1, AKAP2, AMH, AMHR2, ANGPT1, ANGPT2, ANGPTL3, ANGPTL4, ANPEP, APC, APOC1, AR, aromatase, ATX, AX1, AZGP1 (zinc-a-glycoprotein), B7.1, B7.2, B7-H1, BAD, BAFF, BAG1, BAI1, BCR, BCL2, BCL6, BDNF, BLNK, BLR1 (MDR15), BIyS, BMP1, BMP2, BMP3B (GDFIO), BMP4, BMP6, BMP8, BMPRTA, BMPR1B, BMPR2, BPAG1 (plectin), BRCA1, C19orflO (IL27w), C3, C4A, C5, C5R1, CANT1, CAPRIN-1, CASP1, CASP4, CAV1, CCBP2 (D6/JAB61), CCLI (1-309), CCLI1 (eotaxin), CCL13 (MCP-4), CCL15 (MIP-Id), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19 (MIP-3b), CCL2 (MCP-1), MCAF, CCL20 (MIP-3a), CCL21 (MEP-2), SLC, exodus-2, CCL22(MDC/STC-1), CCL23 (MPIF-I), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26 (eotaxin-3), CCL27 (CTACK/ILC), CCL28, CCL3 (MIP-Ia), CCL4 (MIPIb), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (mcp-2), CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CCR1 (CKR1/HM145), CCR2 (mcp-IRB/RA), CCR3 (CKR3/CMKBR3), CCR4, CCR5 (CMKBR5/ChemR13), CCR6 (CMKBR6/CKR-L3/STRL22/DRY6), CCR7 (CKR7/EBI1), CCR8 (CMKBR8/TERI/CKR-L1), CCR9 (GPR-9-6), CCRL1 (VSHK1), CCRL2 (L-CCR), CD164, CD19, CDIC, CD2, CD20, CD21, CD200, CD-22, CD24, CD27, CD28, CD3, CD33, CD35, CD37, CD38, CD3E, CD3G, CD3Z, CD4, CD38, CD40, CD40L, CD44, CD45RB,

CD47, CD52, CD69, CD72, CD74, CD79A, CD79B, CD8, CD80, CD81, CD83, CD86, CD137, CD152, CD274, CDH1 (Ecadherin), CDH1O, CDH12, CDH13, CDH18, CDH19, CDH2O, CDH5, CDH7, CDH8, CDH9, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, CDKN1A (p21Wap1/Cip1), CDKN1B (p27Kip1), CDKN1C, CDKN2A (p16INK4a), CDKN2B, CDKN2C, CDKN3, CEBPB, CERI, CHGA, CHGB, Chitinase, CHST1O, CKLFSF2, CKLFSF3, CKLFSF4, CKLFSF5, CKLFSF6, CKLFSF7, CKLFSF8, CLDN3, CLDN7 (claudin-7), CLDN18.2 (claudin 18.2), CLN3, CLU (clusterin), CMKLR1, CMKOR1 (RDC1), CNR1, COL18A1, COLIA1, COL4A3, COL6A1, CR2, Cripto, CRP, CSF1 (M-CSF), CSF2 (GM-CSF), CSF3 (GCSF), CTL8, CTNNB1 (b-catenin), CTSB (cathepsin B), CX3CL1 (SCYD1), CX3CR1 (V28), CXCL1 (GRO1), CXCL1O (IP-IO), CXCLI1 (1-TAC/IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL16, CXCL2 (GRO2), CXCL3 (GRO3), CXCL5 (ENA-78/LIX), CXCL6 (GCP-2), CXCL9 (MIG), CXCR3 (GPR9/CKR-L2), CXCR4, CXCR6 (TYMSTR/STRL33/Bonzo), CYB5, CYC1, CYSLTR1, DAB2IP, DES, DKFZp451J0118, DNCL1, DPP4, E2F1, Engel, Edge, Fennel, EFNA3, EFNB2, EGF, EGFR, ELAC2, ENG, Enola, ENO2, ENO3, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA9, EPRA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB5, EPHB6, EPHRIN-A1, EPHRIN-A2, EPHRINA3, EPHRIN-A4, EPHRIN-A5, EPHRIN-A6, EPHRIN-B1, EPHRIN-B2, EPHRIN-B3, EPHB4, EPG, ERBB2 (Her-2), EREG, ERK8, Estrogen receptor, Earl, ESR2, F3 (TF), FADD, famesyltransferase, FasL, FASNf, FCER1A, FCER2, FCGR3A, FGF, FGF1 (aFGF), FGF10, FGF11, FGF12, FGF12B, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2 (bFGF). FGF20, FGF21, FGF22, FGF23, FGF3 (int-2), FGF4 (HST), FGF5, FGF6 (HST-2), FGF7 (KGF), FGF8, FGF9, FGFR3, FIGF (VEGFD), FILI (EPSILON), FBL1 (ZETA), FLJ12584, FLJ25530, FLRT1 (fibronectin), FLT1, FLT-3, FOS, FOSL1 (FRA-1), FY (DARC), GABRP (GABAa), GAGEB1, GAGEC1, GALNAC4S-6ST, GATA3, GD2, GDF5, GFI1, GGT1, GM-CSF, GNAS1, GNRH1, GPR2 (CCR10), GPR31, GPR44, GPR81 (FKSG80), GRCC1O (C1O), GRP, GSN (Gelsolin), GSTP1, HAVCR2, HDAC, HDAC4, HDAC5, HDAC7A, HDAC9, Hedgehog, HGF, HIF1A, HIP1, histamine and histamine receptors, HLA-A, HLA-DRA, HLA-E, HM74, HMOXI, HSP90, HUMCYT2A, ICEBERG, ICOSL, ID2, IFN-a, IFNA1, IFNA2, IFNA4, IFNA5, EFNA6, BFNA7, IFNB1, IFNgamma, IFNW1, IGBP1, IGF1, IGFIR, IGF2, IGFBP2, IGFBP3, IGFBP6, DL-1, ILIO, ILIORA, ILIORB, IL-1, IL1R1 (CD121a), IL1R2 (CD121b), IL-IRA, IL-2, IL2RA (CD25), IL2RB (CD122), IL2RG (CD132), IL-4, IL-4R (CD123), IL-5, IL5RA (CD125), IL3RB (CD131), IL-6, IL6RA, (CD126), IR6RB (CD130), IL-7, IL7RA (CD127), IL-8, CXCR1 (IL8RA), CXCR2, (IL8RB/CD128), IL-9, IL9R (CD129), IL-10, IL10RA (CD210), IL10RB (CDW210B), IL-11, IL11RA, IL-12, IL-12A, IL-12B, IL-12RB1, IL-12RB2, IL-13, IL13RA1, IL13RA2, IL14, IL15, IL15RA, IL16, IL17,

IL17A, IL17B, IL17C, IL17R, IL18, IL18BP, IL18R1, IL18RAP, IL19, ILIA, ILIB, ILIF10, ILIF5, IL1F6, ILIF7, IL1F8, DL1F9, ILIHYI, ILIR1, ILIR2, ILIRAP, ILIRAPLI, ILIRAPL2, ILIRL1, IL1RL2, ILIRN, IL2, IL20, IL20RA, IL21R, IL22, IL22R, IL22RA2, IL23, DL24, IL25, IL26, IL27, IL28A, IL28B, IL29, IL2RA, IL2RB, IL2RG, IL3, IL30, IL3RA, IL4, IL4, IL6ST (glycoprotein 130), ILK, INHA, INHBA, INSL3, INSL4, IRAK1, IRAK2, ITGA1, ITGA2, ITGA3, ITGA6 (.alpha.6 integrin), ITGAV, ITGB3, ITGB4 (.beta.4 integrin), JAG1, JAK1, JAK3, JTB, JUN, K6HF, KAI1, KDR, KITLG, KLF5 (GC Box BP), KLF6, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, KRT1, KRT19 (Keratin 19), KRT2A, KRTHB6 (hair-specific type II keratin), LAMA5, LEP (leptin), Lingo-p75, Lingo-Troy, LPS, LTA (TNF-b)), LTB, LTB4R (GPR16), LTB4R2, LTBR, MACMARCKS, MAG or OMgp, MAP2K7 (c-Jun), MCP-1, MDK, MIB1, midkine, MIF, MISRII, MJP-2, MK, MKI67 (Ki-67), MMP2, MMP9, MS4A1, MSMB, MT3 (metallothionectin-UI), mTOR, MTSS1, MUC1 (mucin), MYC, MYD88, NCK2, neurocan, Nectin-4, NFKBI, NFKB2, NGFB (NGF), NGFR, NgR-Lingo, NgRNogo66, (Nogo), NgR-p75, NgR-Troy, NMEI (NM23A), NOTCH, NOTCH1, NOX5, NPPB, NROB1, NROB2, NRID1, NR1D2, NR1H2, NR1H3, NR1H4, NR112, NR113, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR2F6, NR3C1, NR3C2, NR4A1, NR4A2, NR4A3, NR5A1, NR5A2, NR6A1, NRP1, NRP2, NT5E, NTN4, ODZI, OPRDI, P2RX7, PAP, PART1, PATE, PAWR, PCA3, PCDGF, PCNA, PDGFA, PDGFB, PDGFRA, PDGFRB, PECAMI, peg-asparaginase, PF4 (CXCL4), PGF, PGR, phosphacan, PIAS2, PI3 Kinase, PIK3CG, PLAU (uPA), PLG, PLXDCI, PKC, PKC-beta, PPBP (CXCL7), PPID, PR1, PRKCQ, PRKD1, PRL, PROC, PROK2, PSAP, PSCA, PTAFR, PTEN, PTGS2 (COX-2), PIN, RAC2 (P21Rac2), RANK, RANK ligand, RARB, RGS1, RGS13, RGS3, RNFI1O (ZNF144), Ron, ROBO2, RXR, S100A2, SCGB 1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SCYE1 (endothelial Monocyte-activating cytokine), SDF2, SERPENA1, SERPINA3, SERPINB5 (maspin), SERPINEI (PAI-I), SERPINFI, SHIP-1, SHIP-2, SHB1, SHB2, SHBG, SfcAZ, SLC2A2, SLC33A1, SLC43A1, SLIT2, SPP1, SPRR1B (Spr1), ST6GAL1, STAB1, STATE, STEAP, STEAP2, TB4R2, TBX21, TCP1O, TDGF1, TEK, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFBI, TGEBR1, TGFBR2, TGFBR3, THIL, THBS1 (thrombospondin-1), THBS2, THBS4, THPO, TIE (Tie-1), TIMP3, tissue factor, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TNF, TNF-a, TNFAIP2 (B94), TNFAIP3, TNFRSF11A, TNFRSF1A, TNFRSF1B, TNFRSF21, TNFRSF5, TNFRSF6 (Fas), TNFRSF7, TNFRSF8, TNFRSF9, TNFSF1O (TRAIL), TNFSF11 (TRANCE), TNFSF12 (APO3L), TNFSF13 (April), TNFSF13B, TNSF14 (HVEM-L), TNFRSF14 (HVEM), TNFSF15 (VEGI), TNFSF18, TNFSF4 (OX40 ligand), TNFSF5 (CD40 ligand). TNFSF6 (FasL), TNFSF7 (CD27 ligand), TNFSF8

(CD30 ligand), TNFSF9 (4-1BB ligand), TOLLIP, Toll-like receptors, TOP2A (topoisomerase 1ia), TP53, TPM1, TPM2, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, TRKA, TREM1, TREM2, TROP2, TRPC6, TSLP, TWEAK, Tyrosinase, uPAR, VEGF, VEGFB, VEGFC, versican, VHL C5, VLA-4, Wnt-1, XCL1 (tymphotactin), XCL2 (SCM-Ib), XCRI (GPR5/CCXCR1), YYI, ZFPM2, CLEC4C (BDCA-2, DLEC, CD303, CLECSF7), CLEC4D (MCL, CLECSF8), CLEC4E (Mincle), CLEC6A (Dectin-2). CLEC5A (MDL-1, CLECSF5), CLEC1B (CLEC-2), CLEC9A (DNGR-1), CLEC7A (Dectin-1), PDGFRa, SLAMF7, GP6 (GPVI), LILRA1 (CD85I), LILRA2 (CD85H, ILT1), LILRA4 (CD85G, ILT7), LILRA5 (CD85F, ILT11), LILRA6 (CD85b, ILT8), NCR1 (CD335, LY94, NKp46), NCR3 (CD335, LY94, NKp46), NCR3 (CD337, NKp30), OSCAR, TARM1, CD300C, CD300E, CD300LB (CD300B), CD300LD (CD300D), KIR2DL4 (CD158D), KIR2DS, KLRC2 (CD159C, NKG2C), KLRK1 (CD314, NKG2D), NCR2 (CD336, NKp44), PILRB, SIGLEC1 (CD169, SN), SIGLEC14, SIGLEC15 (CD33L3), SIGLEC16, SIRPB1 (CD172B), TREM1 (CD354), TREM2, and KLRF1 (NKp80).

In some embodiments, the antibody binds to an FcR.gamma-coupled receptor. In some embodiments, the FcR.gamma-coupled receptor is selected from the group consisting of GP6 (GPVI), LILRA1 (CD85I), LILRA2 (CD85H, ILT1), LILRA4 (CD85G, ILT7), LILRA5 (CD85F, ILT11), LILRA6 (CD85b, ILT8), NCR1 (CD335, LY94, NKp46), NCR3 (CD335, LY94, NKp46), NCR3 (CD337, NKp30), OSCAR, and TARM1.

In some embodiments, the antibody binds to a DAP12-coupled receptor. In some embodiments, the DAP12-coupled receptor is selected from the group consisting of CD300C, CD300E, CD300LB (CD300B), CD300LD (CD300D), KIR2DL4 (CD158D), KIR2DS, KLRC2 (CD159C, NKG2C), KLRK1 (CD314, NKG2D), NCR2 (CD336, NKp44). PILRB, SIGLEC1 (CD169, SN), SIGLEC14, SIGLEC15 (CD33L3), SIGLEC16, SIRPB1 (CD172B), TREM1 (CD354), and TREM2.

In some embodiments, the antibody binds to a hemITAM-bearing receptor. In some embodiments, the hemITAM-bearing receptor is KLRF1 (NKp80).

In some embodiments, the antibody is capable of binding one or more targets selected from CLEC4C (BDCA-2, DLEC, CD303, CLECSF7), CLEC4D (MCL, CLECSF8), CLEC4E (Mincle), CLEC6A (Dectin-2), CLEC5A (MDL-1, CLECSF5), CLEC1B (CLEC-2), CLEC9A (DNGR-1), and CLEC7A (Dectin-1). In some embodiments, the antibody is capable of binding CLEC6A (Dectin-2) or CLEC5A. In some embodiments, the antibody is capable of binding CLEC6A (Dectin-2).

In some embodiments, the antibody is capable of binding one or more targets selected from (e.g., specifically binds to a target selected from): ATP5I (Q06185), OAT (P29758),

AIFM1 (Q9Z0X1), AOFA (Q64133), MTDC (P18155), CMC1 (Q8BH59), PREP (Q8K411), YMEL1 (O88967), LPPRC (Q6PB66), LONM (Q8CGK3), ACON (Q99KI0), ODO1 (Q60597), IDHP (P54071), ALDH2 (P47738), ATPB (P56480), AATM (P05202), TMM93 (Q9CQW0), ERGI3 (Q9CQE7), RTN4 (Q99P72), CL041 (Q8BQR4), ERLN2 (Q8BFZ9), TERA (Q01853), DAD1 (P61804), CALX (P35564), CALU (O35887), VAPA (Q9WV55), MOGS (Q80UM7), GANAB (Q8BHN3), ERO1A (Q8R180), UGGG1 (Q6P5E4), P4HA1 (Q60715), HYEP (Q9D379), CALR (P14211), AT2A2 (O55143), PDIA4 (P08003), PDIA1 (P09103), PDIA3 (P27773), PDIA6 (Q922R8), CLH (Q68FD5), PPIB (P24369), TCPG (P80318), MOT4 (P57787), NICA (P57716), BASI (P18572), VAPA (Q9WV55), ENV2 (P11370), VAT1 (Q62465), 4F2 (P10852), ENOA (P17182), ILK (O55222), GPNMB (Q99P91), ENV1 (P10404), ERO1A (Q8R180), CLH, (Q68FD5), DSG1A (Q61495), AT1A1 (Q8VDN2), HYOU1 (Q9JKR6), TRAP1 (Q9CQN1), GRP75 (P38647), ENPL (P08113), CH60 (P63038), and CH10 (Q64433). In the preceding list, accession numbers are shown in parentheses.

In some embodiments, the antibody binds to an antigen selected from CDH1, CD19, CD20, CD29, CD30, CD38, CD40, CD47, EpCAM, MUC1, MUC16, EGFR, Her2, SLAMF7, and gp75. In some embodiments, the antigen is selected from CD19, CD20, CD47, EpCAM, MUC1, MUC16, EGFR, and Her2. In some embodiments, the antibody binds to an antigen selected from the Tn antigen and the Thomsen-Friedenreich antigen.

In some embodiments, the antibody or Fc fusion protein is selected from: abagovomab, abatacept (also known as ORENCIA®), abciximab (also known as REOPRO®), c7E3 Fab), adalimumab (also known as HUMIRA®), adecatumumab, alemtuzumab (also known as CAMPATH®), MabCampath or Campath-1H), altumomab, afelimomab, anatumomab mafenatox, anetumumab, anrukizumab, apolizumab, arcitumomab, aselizumab, atlizumab, atorolimumab, bapineuzumab, basiliximab (also known as SIMULECT®), bavituximab, bectumomab (also known as LYMPHOSCAN®), belimumab (also known as LYMPHO-STAT-B®), bertilimumab, besilesomab, bevacizumab (also known as AVASTIN®), biciromab brallobarbital, bivatuzumab mertansine, campath, canakinumab (also known as ACZ885), cantuzumab mertansine, capromab (also known as PROSTASCINT®), catumaxomab (also known as REMOVAB®), cedelizumab (also known as CIMZIA®), certolizumab pegol, cetuximab (also known as ERBITUX®), clenoliximab, dacetuzumab, dacliximab, daclizumab (also known as ZENAPAX®), denosumab (also known as AMG 162), detumomab, dorlimomab aritox, dorlixizumab, duntumumab, durimulumab, durmulumab, ecromeximab, eculizumab (also known as SOLIRIS®), edobacomab, edrecolomab (also known as Mab17-1A, PANOREX®), efalizumab (also known as RAPTIVA®), efungumab (also known as MYCOGRAB®), elsilimomab, enlimomab pegol, epitumomab cituxetan, efalizumab, epitumomab, epratuzumab,

erlizumab, ertumaxomab (also known as REXOMUN®), etanercept (also known as ENBREL®), etaracizumab (also known as etaratuzumab, VITAXIN®, ABEGRIN®), exbivirumab, fanolesomab (also known as NEUTROSPEC®), faralimomab, felvizumab, fontolizumab (also known as HUZAF®), galiximab, gantenerumab, gavilimomab (also known as ABXCBL®), gemtuzumab ozogamicin (also known as MYLOTARG®), golimumab (also known as CNTO 148), gomiliximab, ibalizumab (also known as TNX-355), ibritumomab tiuxetan (also known as ZEVALIN®), igovomab, imciromab, infliximab (also known as REMICADE®), inolimomab, inotuzumab ozogamicin, ipilimumab (also known as MDX-010, MDX-101), iratumumab, keliximab, labetuzumab, lemalesomab, lebrilizumab, lerdelimumab, lexatumumab (also known as, HGS-ETR2, ETR2-ST01), lexitumumab, libivirumab, lintuzumab, lucatumumab, lumiliximab, mapatumumab (also known as HGSETR1, TRM-1), maslimomab, matuzumab (also known as EMD72000), mepolizumab (also known as BOSATRIA®), metelimumab, milatuzumab, minretumomab, mitumomab, morolimumab, motavizwnab (also known as NUMAX®), muromonab (also known as OKT3), nacolomab tafenatox, naptumomab estafenatox, natalizumab (also known as TYSABRI®, ANTEGREN®), nebacumab, nerelimomab, nimotuzumab (also known as THERACIM hR3®, THERA-CIM-hR3®, THERALOC®), nofetumomab merpentan (also known as VERLUMA®), ocrelizumab, odulimomab, ofatumumab, omalizumab (also known as XOLAIR®), oregovomab (also known as OVAREX®), otelixizumab, pagibaximab, palivizumab (also known as SYNAGIS®), panitumumab (also known as ABX-EGF, VECTIBIX®), pascolizumab, pemtumomab (also known as THERAGYN®), pertuzumab (also known as 2C4, OMNITARG®), pexelizumab, pintumomab, priliximab, pritumumab, ranibizumab (also known as LUCENTIS®), raxibacumab, regavirumab, reslizumab, rituximab (also known as RITUXAN®, MabTHERA®), rovelizumab, ruplizumab, satumomab, sevirumab, sibrotuzumab, siplizumab (also known as MEDI-507), sontuzumab, stamulumab (also known as MYO-029), sulesomab (also known as LEUKOSCAN®), tacatuzumab tetraxetan, tadocizumab, talizumab, taplitumomab paptox, tefibazumab (also known as AUREXIS®), telimomab aritox, teneliximab, teplizumab, ticilimumab, tocilizumab (also known as ACTEMRA®), toralizumab, tositumomab, trastuzumab (also known as HERCEPTIN®), tremelimumab (also known as CP-675,206), tucotuzumab celmoleukin, tuvirumab, urtoxazumab, ustekinumab (also known as CNTO 1275), vapaliximab, veltuzumab, vepalimomab, visilizumab (also known as NUVION®), volociximab (also known as M200), votumumab (also known as HUMASPECT®), zalutumumab, zanolimumab (also known as HuMAX-CD4), ziralimumab, zolimomab aritox, daratumumab, elotuxumab, obintunzumab, olaratumab, brentuximab vedotin, afibercept, abatacept, belatacept,

afibercept, etanercept, romiplostim, SBT-040 (sequences listed in US 2017/0158772. In some embodiments, the antibody is rituximab.

ANTIBODIES

The immunoconjugate of the invention comprises an antibody. Included in the scope of the embodiments of the invention are functional variants of the antibody constructs or antigen binding domain described herein. The term "functional variant" as used herein refers to an antibody construct having an antigen binding domain with substantial or significant sequence identity or similarity to a parent antibody construct or antigen binding domain, which functional variant retains the biological activity of the antibody construct or antigen binding domain of which it is a variant. Functional variants encompass, for example, those variants of the antibody constructs or antigen binding domain described herein (the parent antibody construct or antigen binding domain) that retain the ability to recognize target cells expressing, for example but not limited to, PD-L1, HER2, CEA or TROP2, to a similar extent, the same extent, or to a higher extent, as the parent antibody construct or antigen binding domain.

In reference to the antibody construct or antigen binding domain, the functional variant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the antibody construct or antigen binding domain.

A functional variant can, for example, comprise the amino acid sequence of the parent antibody construct or antigen binding domain with at least one conservative amino acid substitution. Alternatively, or additionally, the functional variants can comprise the amino acid sequence of the parent antibody construct or antigen binding domain with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent antibody construct or antigen binding domain.

The antibodies comprising the immunoconjugates of the invention include Fc engineered variants. In some embodiments, the mutations in the Fc region that result in modulated binding to one or more Fc receptors can include one or more of the following mutations: SD (S239D), SDIE (S239D/I332E), SE (S267E), SELF (S267E/L328F), SDIE (S239D/I332E), SDIEAL (S239D/I332E/A330L), GA (G236A), ALIE (A330L/I332E), GASDALIE (G236A/S239D/A330L/I332E), V9 (G237D/P238D/P271G/A330R), and V11

(G237D/P238D/H268D/P271G/A330R), and/or one or more mutations at the following amino acids: E345R, E233, G237, P238, H268, P271, L328 and A330. Additional Fc region modifications for modulating Fc receptor binding are described in, for example, US 2016/0145350; US 7416726; and US 5624821, which are hereby incorporated by reference in their entireties herein.

The antibodies comprising the immunoconjugates of the invention include glycan variants, such as afucosylation. In some embodiments, the Fc region of the binding agents are modified to have an altered glycosylation pattern of the Fc region compared to the native non-modified Fc region.

Amino acid substitutions of the inventive antibody constructs or antigen binding domains are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (e.g., Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (e.g., Asn, Gln, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (e.g., Ile, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (e.g., His, Phe, Trp, and Tyr), etc.

The antibody construct or antigen binding domain can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, e.g., other amino acids, do not materially change the biological activity of the antibody construct or antigen binding domain functional variant.

In some embodiments, the antibodies in the immunoconjugates contain a modified Fc region, wherein the modification modulates the binding of the Fc region to one or more Fc receptors.

In some embodiments, the antibodies in the immunoconjugates (e.g., antibodies conjugated to at least two adjuvant moieties) contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region that results in modulated binding (e.g., increased binding or decreased binding) to one or more Fc receptors (e.g., FcγRI (CD64),

FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a), and/or FcγRIIIB (CD16b)) as compared to the native antibody lacking the mutation in the Fc region. In some embodiments, the antibodies in the immunoconjugates contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region that reduce the binding of the Fc region of the antibody to FcγRIIB. In some embodiments, the antibodies in the immunoconjugates contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region of the antibody that reduce the binding of the antibody to FcγRIIB while maintaining the same binding or having increased binding to FcγRI (CD64), FcγRIIA (CD32A), and/or FcRγIIIA (CD16a) as compared to the native antibody lacking the mutation in the Fc region. In some embodiments, the antibodies in the immunoconjugates contain one of more modifications in the Fc region that increase the binding of the Fc region of the antibody to FcγRIIB.

In some embodiments, the modulated binding is provided by mutations in the Fc region of the antibody relative to the native Fc region of the antibody. The mutations can be in a CH2 domain, a CH3 domain, or a combination thereof. A "native Fc region" is synonymous with a "wild-type Fc region" and comprises an amino acid sequence that is identical to the amino acid sequence of an Fc region found in nature or identical to the amino acid sequence of the Fc region found in the native antibody (e.g., cetuximab). Native sequence human Fc regions include a native sequence human IgG1 Fc region, native sequence human IgG2 Fc region, native sequence human IgG3 Fc region, and native sequence human IgG4 Fc region, as well as naturally occurring variants thereof. Native sequence Fc includes the various allotypes of Fcs (Jefferis et al., (2009) mAbs, 1(4):332-338).

In some embodiments, the Fc region of the antibodies of the immunoconjugates are modified to have an altered glycosylation pattern of the Fc region compared to the native non-modified Fc region. Human immunoglobulin is glycosylated at the Asn297 residue in the Cγ2 domain of each heavy chain. This N-linked oligosaccharide is composed of a core heptasaccharide, N-acetylglucosamine4Mannose3 (GlcNAc4Man3). Removal of the heptasaccharide with endoglycosidase or PNGase F is known to lead to conformational changes in the antibody Fc region, which can significantly reduce antibody-binding affinity to activating FcγR and lead to decreased effector function. The core heptasaccharide is often decorated with galactose, bisecting GlcNAc, fucose, or sialic acid, which differentially impacts Fc binding to activating and inhibitory FcγR. Additionally, it has been demonstrated that α2,6-sialyation enhances anti-inflammatory activity in vivo, while afucosylation leads to improved FcγRIIIa binding and a 10-fold increase in antibody-dependent cellular cytotoxicity and antibody-

dependent phagocytosis. Specific glycosylation patterns, therefore, can be used to control inflammatory effector functions.

In some embodiments, the modification to alter the glycosylation pattern is a mutation. For example, a substitution at Asn297. In some embodiments, Asn297 is mutated to glutamine (N297Q). Methods for controlling immune response with antibodies that modulate FcγR-regulated signaling are described, for example, in U.S. Patent 7,416,726 and U.S. Patent Application Publications 2007/0014795 and 2008/0286819, which are hereby incorporated by reference in their entireties.

In some embodiments, the antibodies of the immunoconjugates are modified to contain an engineered Fab region with a non-naturally occurring glycosylation pattern. For example, hybridomas can be genetically engineered to secrete afucosylated mAb, desialylated mAb or deglycosylated Fc with specific mutations that enable increased FcRγIIIa binding and effector function. In some embodiments, the antibodies of the immunoconjugates are engineered to be afucosylated.

In some embodiments, the entire Fc region of an antibody in the immunoconjugates is exchanged with a different Fc region, so that the Fab region of the antibody is conjugated to a non-native Fc region. For example, the Fab region of cetuximab, which normally comprises an IgG1 Fc region, can be conjugated to IgG2, IgG3, IgG4, or IgA, or the Fab region of nivolumab, which normally comprises an IgG4 Fc region, can be conjugated to IgG1, IgG2, IgG3, IgA1, or IgG2. In some embodiments, the Fc modified antibody with a non-native Fc domain also comprises one or more amino acid modification, such as the S228P mutation within the IgG4 Fc, that modulate the stability of the Fc domain described. In some embodiments, the Fc modified antibody with a non-native Fc domain also comprises one or more amino acid modifications described herein that modulate Fc binding to FcR.

In some embodiments, the modifications that modulate the binding of the Fc region to FcR do not alter the binding of the Fab region of the antibody to its antigen when compared to the native non-modified antibody. In other embodiments, the modifications that modulate the binding of the Fc region to FcR also increase the binding of the Fab region of the antibody to its antigen when compared to the native non-modified antibody.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds PD-L1.

Programmed Death-Ligand 1 (PD-L1, cluster of differentiation 274, CD274, B7-homolog 1, or B7-H1) belongs to the B7 protein superfamily, and is a ligand of programmed cell death protein 1 (PD-1, PDCD1, cluster of differentiation 279, or CD279). PD-L1 can also

interact with B7.1 (CD80) and such interaction is believed to inhibit T cell priming. The PD-L1/PD-1 axis plays a large role in suppressing the adaptive immune response. More specifically, it is believed that engagement of PD-L1 with its receptor, PD-1, delivers a signal that inhibits activation and proliferation of T-cells. Agents that bind to PD-L1 and prevent the ligand from binding to the PD-1 receptor prevent this immunosuppression, and can, therefore, enhance an immune response when desired, such as for the treatment of cancers, or infections. PD-L1/PD-1 pathway also contributes to preventing autoimmunity and therefore agonistic agents against PD-L1 or agents that deliver immune inhibitory payloads may help treatment of autoimmune disorders.

Several antibodies targeting PD-L1 have been developed for the treatment of cancer, including atezolizumab (TECENTRIQ™), durvalumab (IMFINZI™), and avelumab (BAVENCIO™). Nevertheless, there continues to be a need for new PD-L1-binding agents, including agents that bind PD-L1 with high affinity and effectively prevent PD-L1/PD-1 signaling and agents that can deliver therapeutic payloads to PD-L1 expressing cells. In addition, there is a need for new PD-L1-binding agents to treat autoimmune disorders and infections.

A method is provided of delivering a pyrazoloazepine derivative payload to a cell expressing PD-L1 comprising administering to the cell, or mammal comprising the cell, an immunoconjugate comprising an anti-PD-L1 antibody covalently attached to a linker which is covalently attached to one or more pyrazoloazepine moieties.

Also provided is a method for enhancing or reducing or inhibiting an immune response in a mammal, and a method for treating a disease, disorder, or condition in a mammal that is responsive to PD-L1 inhibition, which methods comprise administering a PD-L1 immunoconjugate thereof, to the mammal.

The invention provides a PD-L1 antibody comprising an immunoglobulin heavy chain variable region polypeptide and an immunoglobulin light chain variable region polypeptide. The PD-L1 antibody specifically binds PD-L1. The binding specificity of the antibody allows for targeting PD-L1 expressing cells, for instance, to deliver therapeutic payloads to such cells. In some embodiments, the PD-L1 antibody binds to human PD-L1. However, antibodies that bind to any PD-L1 fragment, homolog or paralog also are encompassed.

In some embodiments, the PD-L1 antibody binds PD-L1 without substantially inhibiting or preventing PD-L1 from binding to its receptor, PD-1. However, in other embodiments, the PD-L1 antibody can completely or partially block (inhibit or prevent) binding of PD-L1 to its receptor, PD-1, such that the antibody can be used to inhibit PD-L1/PD-1 signaling (e.g., for therapeutic purposes). The antibody or antigen-binding antibody fragment can be monospecific

for PD-L1, or can be bispecific or multi-specific. For instance, in bivalent or multivalent antibodies or antibody fragments, the binding domains can be different targeting different epitopes of the same antigen or targeting different antigens. Methods of constructing multivalent binding constructs are known in the art. Bispecific and multispecific antibodies are known in the art. Furthermore, a diabody, triabody, or tetrabody can be provided, which is a dimer, trimer, or tetramer of polypeptide chains each comprising a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H -V_L polypeptide chains to generate a multimeric molecule having two, three, or four functional antigen binding sites. Also, bis-scFv fragments, which are small scFv fragments with two different variable domains can be generated to produce bispecific bis-scFv fragments capable of binding two different epitopes. Fab dimers (Fab2) and Fab trimers (Fab3) can be produced using genetic engineering methods to create multispecific constructs based on Fab fragments.

The PD-L1 antibody can be, or can be obtained from, a human antibody, a non-human antibody, a humanized antibody, or a chimeric antibody, or corresponding antibody fragments. A "chimeric" antibody is an antibody or fragment thereof typically comprising human constant regions and non-human variable regions. A "humanized" antibody is a monoclonal antibody typically comprising a human antibody scaffold but with non-human origin amino acids or sequences in at least one CDR (e.g., 1, 2, 3, 4, 5, or all six CDRs).

The PD-L1 antibody can be internalizing, as described in WO 2021/150701 and incorporated by reference herein, or the PD-L1 antibody can be non-internalizing, as described in WO 2021/150702 and incorporated by reference herein.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds HER2.

In certain embodiments, immunoconjugates of the invention comprise anti-HER2 antibodies. In one embodiment of the invention, an anti-HER2 antibody of an immunoconjugate of the invention comprises a humanized anti-HER2 antibody, e.g., huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8, as described in Table 3 of US 5821337, which is specifically incorporated by reference herein. Those antibodies contain human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. The humanized antibody huMAb4D5-8 is also referred to as trastuzumab, commercially available under the tradename HERCEPTIN™ (Genentech, Inc.).

Trastuzumab (CAS 180288-69-1, HERCEPTIN®, huMAb4D5-8, rhuMAb HER2, Genentech) is a recombinant DNA-derived, IgG1 kappa, monoclonal antibody that is a humanized version of a murine anti-HER2 antibody (4D5) that selectively binds with high affinity in a cell-based assay (Kd = 5 nM) to the extracellular domain of HER2 (US 5677171; US 5821337; US 6054297; US 6165464; US 6339142; US 6407213; US 6639055; US 6719971; US 6800738; US 7074404; Coussens et al (1985) *Science* 230:1132-9; Slamon et al (1989) *Science* 244:707-12; Slamon et al (2001) *New Engl. J. Med.* 344:783-792).

In an embodiment of the invention, the antibody construct or antigen binding domain comprises the CDR regions of trastuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises the framework regions of the trastuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises one or both variable regions of trastuzumab.

In another embodiment of the invention, an anti-HER2 antibody of an immunoconjugate of the invention comprises a humanized anti-HER2 antibody, e.g., humanized 2C4, as described in US 7862817. An exemplary humanized 2C4 antibody is pertuzumab (CAS Reg. No. 380610-27-5), PERJETA™ (Genentech, Inc.). Pertuzumab is a HER dimerization inhibitor (HDI) and functions to inhibit the ability of HER2 to form active heterodimers or homodimers with other HER receptors (such as EGFR/HER1, HER2, HER3 and HER4). See, for example, Harari and Yarden, *Oncogene* 19:6102-14 (2000); Yarden and Sliwkowski. *Nat Rev Mol Cell Biol* 2:127-37 (2001); Sliwkowski *Nat Struct Biol* 10:158-9 (2003); Cho et al. *Nature* 421:756-60 (2003); and Malik et al. *Pro Am Soc Cancer Res* 44:176-7 (2003). PERJETA™ is approved for the treatment of breast cancer.

In an embodiment of the invention, the antibody construct or antigen binding domain comprises the CDR regions of pertuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises the framework regions of the pertuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises one or both variable regions of pertuzumab.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds Caprin-1 (Ellis JA, Luzio JP (1995) *J Biol Chem.* 270(35):20717–23; Wang B, et al (2005) *J Immunol.* 175 (7):4274–82; Solomon S, et al (2007) *Mol Cell Biol.* 27(6):2324–42). Caprin-1 is also known as GPIAP1, GPIP137, GRIP137, M11S1, RNG105, p137GPI, and cell cycle associated protein 1.

Cytoplasmic activation/proliferation-associated protein-1 (caprin-1) is an RNA-binding protein that participates in the regulation of cell cycle control-associated genes. Caprin-1

selectively binds to c-Myc and cyclin D2 mRNAs, which accelerates cell progression through the G_1 phase into the S phase, enhances cell viability and promotes cell growth, indicating that it may serve an important role in tumorigenesis (Wang B, et al (2005) *J Immunol*. 175:4274–4282). Caprin-1 acts alone or in combination with other RNA-binding proteins, such as RasGAP SH3-domain-binding protein 1 and fragile X mental retardation protein. In the tumorigenesis process, caprin-1 primarily functions by activating cell proliferation and upregulating the expression of immune checkpoint proteins. Through the formation of stress granules, caprin-1 is also involved in the process by which tumor cells adapt to adverse conditions, which contributes to radiation and chemotherapy resistance. Given its role in various clinical malignancies, caprin-1 holds the potential to be used as a biomarker and a target for the development of novel therapeutics (Yang, Z-S, et al (2019) *Oncology Letters* 18:15-21).

Antibodies that target caprin-1 for treatment and detection have been described (WO 2011/096519; WO 2013/125654; WO 2013/125636; WO 2013/125640; WO 2013/125630; WO 2013/018889; WO 2013/018891; WO 2013/018883; WO 2013/018892; WO 2014/014082; WO 2014/014086; WO 2015/020212; WO 2018/079740).

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds CEA. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) also known as CD66e (Cluster of Differentiation 66e), is a member of the carcinoembryonic antigen (CEA) gene family.

Elevated expression of carcinoembryonic antigen (CEA, CD66e, CEACAM5) has been implicated in various biological aspects of neoplasia, especially tumor cell adhesion, metastasis, the blocking of cellular immune mechanisms, and having antiapoptosis functions. CEA is also used as a blood marker for many carcinomas. Labetuzumab (CEA-CIDE^{TM}, Immunomedics, CAS Reg. No. 219649-07-7), also known as MN-14 and hMN14, is a humanized IgG1 monoclonal antibody and has been studied for the treatment of colorectal cancer (Blumenthal, R. et al (2005) Cancer Immunology Immunotherapy 54(4):315-327). Labetuzumab conjugated to a camptothecin analog (labetuzumab govitecan, IMMU-130) targets carcinoembryonic antigen-related cell adhesion mol. 5 (CEACAM5) and is being studied in patients with relapsed or refractory metastatic colorectal cancer (Sharkey, R. et al, (2018), Molecular Cancer Therapeutics 17(1):196-203; Cardillo, T. et al (2018) Molecular Cancer Therapeutics 17(1):150-160).

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hMN-14/labetuzumab SEQ ID NO. 1 as disclosed in US 6676924, which is incorporated by reference herein for this purpose.

DIQLTQSPSSLSASVGDRVTITCKASQDVGTSVAWYQQKPGKAPKLLIYWTSTRHTGVPSRFSGSGSGTD
FTFTISSLQPEDIATYYCQQYSLYRSFGQGTKVEIK SEQ ID NO. 1

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hMN-14/labetuzumab SEQ ID NO. 2-8 (US 6676924).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQLTQSPSSLSASVGDRVTITC	1 - 23	23	2
CDR-L1	KASQDVGTSVA	24 – 34	11	3
LFR2	WYQQKPGKAPKLLIY	35 – 49	15	4
CDR-L2	WTSTRHT	50 – 56	7	5
LFR3	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	57 – 88	32	6
CDR-L3	QQYSLYRS	89 – 96	8	7
LFR4	FGQGTKVEIK	97 – 106	10	8

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of hMN-14/labetuzumab SEQ ID NO. 9 as disclosed in US 6676924, which is incorporated by reference herein for this purpose.

EVQLVESGGGVVQPGRSLRLSCSSSGFDFTTYWMSWVRQAPGKGLEWVAEIHPDSSTINYAPSLKDRFTI
SRDNSKNTLFLQMDSLRPEDTGVYFCASLYFGFPWFAYWGQGTPVTVSS SEQ ID NO. 9

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hMN-14/labetuzumab SEQ ID NO. 10-16 (US 6676924).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGVVQPGRSLRLSCSSSGFDFT	1 – 30	30	10
CDR-H1	TYWMS	31 – 35	5	11
HFR2	WVRQAPGKGLEWVA	36 – 49	14	12
CDR-H2	EIHPDSSTINYAPSLKD	50 – 66	17	13
HFR3	RFTISRDNSKNTLFLQMDSLRPEDTGVYFCAS	67 – 98	32	14
CDR-H3	LYFGFPWFAY	99 – 108	10	15
HFR4	WGQGTPVTVSS	109 – 119	11	16

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hPR1A3 SEQ ID NO. 17 as disclosed in US 8642742, which is incorporated by reference herein for this purpose.

DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK SEQ ID NO. 17

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hPR1A3 SEQ ID NO. 18-24 (US 8642742).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQMTQSPSSLSASVGDRVTITC	1 - 23	23	18
CDR-L1	KASAAVGTYVA	24 - 34	11	19
LFR2	WYQQKPGKAPKLLIY	35 - 49	15	20
CDR-L2	SASYRKR	50 - 56	7	21
LFR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	57 - 88	32	22
CDR-L3	HQYYTYPLFT	89 - 98	10	23
LFR4	FGQGTKLEIK	99 - 108	10	24

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hPR1A3 SEQ ID NO. 25-31 (US 8642742).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	1 - 30	30	25
CDR-H1	EFGMN	31 - 35	5	26
HFR2	WVRQAPGQGLEWMG	36 - 49	14	27
CDR-H2	WINTKTGEATYVEEFKG	50 - 66	17	28
HFR3	RVTFTTDTSTSTAYMELRSLRSDDTAVYYCAR	67 - 98	32	29
CDR-H3	WDFAYYVEAMDY	99 - 110	12	30
HFR4	WGQGTTVTVSS	111 - 121	11	31

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hMFE-23 SEQ ID NO. 32 as disclosed in US 7232888, which is incorporated by reference herein for this purpose.

ENVLTQSPSSMSASVGDRVNIACSASSSVSYMHWFQQKPGKSPKLWIYSTSNLASGVPSRFSGSGSGTDY
SLTISSMQPEDAATYYCQQRSSYPLTFGGGTKLEIK SEQ ID NO. 32

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hMFE-23 SEQ ID NO. 33-40 (US 7232888). The embodiment includes two variants of LFR1, SEQ ID NO.:33 and SEQ ID NO.:34.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	ENVLTQSPSSMSASVGDRVNIAC	1 – 23	23	33
LFR1	EIVLTQSPSSMSASVGDRVNIAC	1 – 23	23	34
CDR-L1	SASSSVSYMH	24 – 33	10	35
LFR2	WFQQKPGKSPKLWIY	34 – 48	15	36
CDR-L2	STSNLAS	49 – 55	7	37
LFR3	GVPSRFSGSGSGTDYSLTISSMQPEDAATYYC	56 – 87	32	38
CDR-L3	QQRSSYPLT	88 – 96	9	39
LFR4	FGGGTKLEIK	97 – 106	10	40

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of hMFE-23 SEQ ID NO. 41 (US 7232888).

QVKLEQSGAEVVKPGASVKLSCKASGFNIKDSYMHWLRQGPGQRLEWIGWIDPENGDTEYAPKFQGKATF
TTDTSANTAYLGLSSLRPEDTAVYYCNEGTPTGPYYFDYWGQGTLVTVSS SEQ ID NO. 41

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hMFE-23 SEQ ID NO. 42-49 (US 7232888). The embodiment includes two variants of HFR1, SEQ ID NO.:42 and SEQ ID NO.:43.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	QVKLEQSGAEVVKPGASVKLSCKASGFNIK	1 – 30	30	42
HFR1	QVQLVQSGAEVVKPGASVKLSCKASGFNIK	1 – 30	30	43

CDR-H1	DSYMH	31 – 35	5	44
HFR2	WLRQGPGQRLEWIG	36 - 49	14	45
CDR-H2	WIDPENGDTEYAPKFQG	50 - 66	17	46
HFR3	KATFTTDTSANTAYLGLSSLRPEDTAVYYCNE	67 - 98	32	47
CDR-H3	GTPTGPYYFDY	99 - 109	11	48
HFR4	WGQGTLVTVSS	110 - 120	11	49

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of SM3E SEQ ID NO. 50 (US 7232888).

ENVLTQSPSSMSVSVGDRVTIACSASSSVPYMHWLQQKPGKSPKLLIYLTSNLASGVPSRFSGSGSGTDY SLTISSVQPEDAATYYCQQRSSYPLTFGGGTKLEIK SEQ ID NO. 50

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of SM3E SEQ ID NO. 51-56 and 38-39 (US 7232888). The embodiment includes two variants of LFR1, SEQ ID NO.:51 and SEQ ID NO.:52.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	ENVLTQSPSSMSVSVGDRVTIAC	1 - 23	23	51
LFR1	EIVLTQSPSSMSVSVGDRVTIAC	1 - 23	23	52
CDR-L1	SASSSVPYMH	24 - 33	10	53
LFR2	WLQQKPGKSPKLLIY	34 - 48	15	54
CDR-L2	LTSNLAS	49 - 55	7	55
LFR3	GVPSRFSGSGSGTDYSLTISSVQPEDAATYYC	56 - 87	32	56
CDR-L3	QQRSSYPLT	88 - 96	9	39
LFR4	FGGGTKLEIK	97 - 106	10	40

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain of NP-4/arcitumomab SEQ ID NO. 57

QTVLSQSPAILSASPGEKVTMTCRASSSVTYIHWYQQKPGSSPKSWIYATSNLASGVPARFSGSGSGTSY SLTISRVEAEDAATYYCQHWSSKPPTFGGGTKLEIK SEQ ID NO. 57

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of NP-4/arcitumomab SEQ ID NO. 58-64.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	QTVLSQSPAILSASPGEKVTMTC	1 – 23	23	58
CDR-L1	RASSSVTYIH	24 – 33	10	59
LFR2	WYQQKPGSSPKSWIY	34 – 48	15	60
CDR-L2	ATSNLAS	49 – 55	7	61
LFR3	GVPARFSGSGSGTSYSLTISRVEAEDAATYYC	56 – 87	32	62
CDR-L3	QHWSSKPPT	88 – 96	9	63
LFR4	FGGGTKLEIK	97 – 106	10	64

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of NP-4/arcitumomab SEQ ID NO. 65.

EVKLVESGGGLVQPGGSLRLSCATSGFTFTDYYMNWVRQPPGKALEWLGFIGNKANGYTTEYSASVKGRF
TISRDKSQSILYLQMNTLRAEDSATYYCTRDRGLRFYFDYWGQGTTLTVSS SEQ ID NO. 65.

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of NP-4 SEQ ID NO. 66-72.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVKLVESGGGLVQPGGSLRLSCATSGFTFT	1 – 30	30	66
CDR-H1	DYYMN	31 – 35	5	67
HFR2	WVRQPPGKALEWLG	36 – 49	14	68
CDR-H2	FIGNKANGYTTEYSASVKG	50 – 68	19	69
HFR3	RFTISRDKSQSILYLQMNTLRAEDSATYYCTR	69 – 100	32	70
CDR-H3	DRGLRFYFDY	101 – 110	10	71
HFR4	WGQGTTLTVSS	111 – 121	11	72

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of M5A/hT84.66 SEQ ID NO. 73 as disclosed in US 7776330, which is incorporated by reference herein for this purpose.

DIQLTQSPSSLSASVGDRVTITCRAGESVDIFGVGFLHWYQQKPGKAPKLLIYRASNLESGVPSRFSGSG
SRTDFTLTISSLQPEDFATYYCQQTNEDPYTFGQGTKVEIK SEQ ID NO. 73

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of M5A/hT84.66 SEQ ID NO. 74-80 (US 7776330).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQLTQSPSSLSASVGDRVTITC	1 - 23	23	74
CDR-L1	RAGESVDIFGVGFLH	24 - 38	15	75
LFR2	WYQQKPGKAPKLLIY	39 - 53	15	76
CDR-L2	RASNLES	54 - 60	7	77
LFR3	GVPSRFSGSGSRTDFTLTISSLQPEDFATYYC	61 - 92	32	78
CDR-L3	QQTNEDPYT	93 - 101	9	79
LFR4	FGQGTKVEIK	102 - 111	10	80

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of M5A/hT84.66 SEQ ID NO. 81 (US 7776330).

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYMHWVRQAPGKGLEWVARIDPANGNSKYADSVKGRFTI
SADTSKNTAYLQMNSLRAEDTAVYYCAPFGYYVSDYAMAYWGQGTLVTVSS SEQ ID NO. 81

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of M5A/hT84.66 SEQ ID NO. 82-88 (US 7776330).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGGSLRLSCAASGFNIK	1 - 30	30	82
CDR-H1	DTYMH	31 - 35	5	83
HFR2	WVRQAPGKGLEWVA	36 - 49	14	84
CDR-H2	RIDPANGNSKYADSVKG	50 – 66	17	85
HFR3	RFTISADTSKNTAYLQMNSLRAEDTAVYYCAP	67 – 98	32	86

CDR-H3	FGYYVSDYAMAY	99 – 110	12	87
HFR4	WGQGTLVTVSS	111 – 121	11	88

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hAb2-3 SEQ ID NO. 89 as disclosed in US 9617345, which is incorporated by reference herein for this purpose.

DIQMTQSPASLSASVGDRVTITCRASENIFSYLAWYQQKPGKSPKLLVYNTRTLAEGVPSRFSGSGSGTD
FSLTISSLQPEDFATYYCQHHYGTPFTFGSGTKLEIK SEQ ID NO. 89

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hAb2-3 SEQ ID NO. 90-96 (US 9617345).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQMTQSPASLSASVGDRVTITC	1 – 23	23	90
CDR-L1	RASENIFSYLA	24 – 34	11	91
LFR2	WYQQKPGKSPKLLVY	35 – 49	15	92
CDR-L2	NTRTLAE	50 – 56	7	93
LFR3	GVPSRFSGSGSGTDFSLTISSLQPEDFATYYC	57 – 88	32	94
CDR-L3	QHHYGTPFT	89 – 97	9	95
LFR4	FGSGTKLEIK	98 – 107	10	96

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of SEQ ID NO. 97 (US 9617345).

EVQLQESGPGLVKPGGSLSLSCAASGFVFSSYDMSWVRQTPERGLEWVAYISSGGGITYAPSTVKGRFTV
SRDNAKNTLYLQMNSLTSEDTAVYYCAAHYFGSSGPFAYWGQGTLVTVSS SEQ ID NO. 97

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hAb2-3 SEQ ID NO. 98-104.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLQESGPGLVKPGGSLSLSCAASGFVFS	1 - 30	30	98
CDR-H1	SYDMS	31 - 35	5	99
HFR2	WVRQTPERGLEWVA	36 - 49	14	100

CDR-H2	YISSGGGITYAPSTVKG	50 - 66	17	101
HFR3	RFTVSRDNAKNTLYLQMNSLTSEDTAVYYCAA	67 - 98	32	102
CDR-H3	HYFGSSGPFAY	99 - 109	11	103
HFR4	WGQGTLVTVSS	110 - 120	11	104

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of A240VL-B9VH/AMG-211 SEQ ID NO. 105 as disclosed in US 9982063, which is incorporated by reference herein for this purpose.

QAVLTQPASLSASPGASASLTCTLRRGINVGAYSIYWYQQKPGSPPQYLLRYKSDSDKQQGSGVSSRFSA
SKDASANAGILLISGLQSEDEADYYCMIWHSGASAVFGGGTKLTVL SEQ ID NO. 105

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of A240VL-B9VH/AMG-211 SEQ ID NO. 106-112 (US 9982063).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	QAVLTQPASLSASPGASASLTC	1 - 22	22	106
CDR-L1	TLRRGINVGAYSIY	23 - 36	14	107
LFR2	WYQQKPGSPPQYLLR	37 - 51	15	108
CDR-L2	YKSDSDKQQGS	52 - 62	11	109
LFR3	GVSSRFSASKDASANAGILLISGLQSEDEADYYC	63 - 96	34	110
CDR-L3	MIWHSGASAV	97 - 106	10	111
LFR4	FGGGTKLTVL	107 - 116	10	112

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of B9VH SEQ ID NO. 113 (US 9982063).

EVQLVESGGGLVQPGRSLRLSCAASGFTVSSYWMHWVRQAPGKGLEWVGFIRNKANGGTTEYAASVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGLRFYFDYWGQGTTVTVSS SEQ ID NO. 113

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy

chain framework (HFR) sequences of SEQ ID NO. 114-121 (US 9982063). The embodiment includes two variants of CDR-H2, SEQ ID NO.:117 and SEQ ID NO.:118.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGRSLRLSCAASGFTVS	1 – 30	30	114
CDR-H1	SYWMH	31 – 35	5	115
HFR2	WVRQAPGKGLEWVG	36 – 49	14	116
CDR-H2	FIRNKANGGTTEYAASVKG	50 – 68	19	117
CDR-H2	FIRNKANSGTTEYAASVKG	50 – 68	19	118
HFR3	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR	69 – 100	32	119
CDR-H3	DRGLRFYFDY	101 - 110	10	120
HFR4	WGQGTTVTVSS	111 - 121	11	121

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of E12VH SEQ ID NO. 122 (US 9982063).

EVQLVESGGGLVQPGRSLRLSCAASGFTVSSYWMHWVRQAPGKGLEWVGFILNKANGGTTEYAASVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGLRFYFDYWGQGTTVTVSS SEQ ID NO. 122

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of SEQ ID NO. 123-129 (US 9982063).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGRSLRLSCAASGFTVS	1 – 30	30	123
CDR-H1	SYWMH	31 - 35	5	124
HFR2	WVRQAPGKGLEWVG	36 - 49	14	125
CDR-H2	FILNKANGGTTEYAASVKG	50 - 68	19	126
HFR3	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR	69 - 100	32	127
CDR-H3	DRGLRFYFDY	101 - 110	10	128
HFR4	WGQGTTVTVSS	111 - 121	11	129

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of PR1A3 VH SEQ ID NO. 130 (US 8642742).

QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTKTGEATYVEEFKGRVTF
TTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTVTVSS SEQ ID NO. 130

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds TROP2. Tumor-associated calcium signal transducer 2 (TROP-2) is a transmembrane glycoprotein encoded by the TACSTD2 gene (Linnenbach AJ, et al (1993) Mol Cell Biol. 13(3): 1507–15; Calabrese G, et al (2001) Cytogenet Cell Genet. 92(1–2): 164–5). TROP2 is an intracellular calcium signal transducer that is differentially expressed in many cancers and signals cells for self-renewal, proliferation, invasion, and survival. TROP2 is considered a stem cell marker and is expressed in many normal tissues, though in contrast, it is overexpressed in many cancers (Ohmachi T, et al., (2006) Clin. Cancer Res., 12(10), 3057-3063; Muhlmann G, et al., (2009) J. Clin. Pathol., 62(2), 152-158; Fong D, et al., (2008) Br. J. Cancer, 99(8), 1290-1295; Fong D, et al., (2008) Mod. Pathol., 21(2), 186-191; Ning S, et al., (2013) Neurol. Sci., 34(10), 1745-1750). Overexpression of TROP2 is of prognostic significance. Several ligands have been proposed that interact with TROP2. TROP2 signals the cells via different pathways and it is transcriptionally regulated by a complex network of several transcription factors.

Human TROP2 (TACSTD2: tumor-associated calcium signal transducer 2, GA733-1, EGP-1, M1S1; hereinafter, referred to as hTROP2) is a single-pass transmembrane type 1 cell membrane protein consisting of 323 amino acid residues. While the presence of a cell membrane protein involved in immune resistance, which is common to human trophoblasts and cancer cells (Faulk W P, et al., Proc. Natl. Acad. Sci. 75(4):1947-1951 (1978)), has previously been suggested, an antigen molecule recognized by a monoclonal antibody against a cell membrane protein in a human choriocarcinoma cell line was identified and designated as TROP2 as one of the molecules expressed in human trophoblasts (Lipinski M, et al., Proc. Natl. Acad. Sci. 78(8), 5147-5150 (1981)). This molecule was also designated as tumor antigen GA733-1 recognized by a mouse monoclonal antibody GA733 (Linnenbach A J, et al., Proc. Natl. Acad. Sci. 86(1), 27-31 (1989)) obtained by immunization with a gastric cancer cell line or an epithelial glycoprotein (EGP-1; Basu A, et al., Int. J. Cancer, 62 (4), 472-479 (1995)) recognized by a mouse monoclonal antibody RS7-3G11 obtained by immunization with non-small cell lung cancer cells. In 1995, however, the TROP2 gene was cloned, and all of these molecules were confirmed to be identical molecules (Fornaro M, et al., Int. J. Cancer, 62(5), 610-618 (1995)). The DNA

sequence and amino acid sequence of hTROP2 are available on a public database and can be referred to, for example, under Accession Nos. NM_002353 and NP_002344 (NCBI).

In response to such information suggesting the association with cancer, a plurality of anti-hTROP2 antibodies have been established so far and studied for their antitumor effects. Among these antibodies, there is disclosed, for example, an unconjugated antibody that exhibits in itself antitumor activity in nude mouse xenograft models (WO 2008/144891; WO 2011/145744; WO 2011/155579; WO 2013/077458) as well as an antibody that exhibits antitumor activity as ADC with a cytotoxic drug (WO 2003/074566; WO 2011/068845; WO 2013/068946; US 7999083). However, the strength or coverage of their activity is still insufficient, and there are unsatisfied medical needs for hTROP2 as a therapeutic target.

TROP2 expression in cancer cells has been correlated with drug resistance. Several strategies target TROP2 on cancer cells that include antibodies, antibody fusion proteins, chemical inhibitors, nanoparticles, etc. The in vitro studies and pre-clinical studies, using these various therapeutic treatments, have resulted in significant inhibition of tumor cell growth both in vitro and in vivo in mice. Clinical studies have explored the potential application of TROP2 as both a prognostic biomarker and as a therapeutic target to reverse resistance.

Sacituzumab govitecan (TRODELVY®, Immunomedics, IMMU-132), an antibody-drug conjugate comprising a TROP2-directed antibody linked to a topoisomerase inhibitor drug, is indicated for the treatment of metastatic triple-negative breast cancer (mTNBC) in adult patients that have received at least two prior therapies. The TROP2 antibody in sacituzumab govitecan is conjugated to SN-38, the active metabolite of irinotecan (US 2016/0297890; WO 2015/098099).

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences of hRS7 (humanized RS7), SEQ ID NO. 131-133 (US 7238785, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	KASQDVSIAVA	131
CDR-L2	SASYRYT	132
CDR-L3	QQHYITPLT	133

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences of hRS7 (humanized RS7), SEQ ID NO. 134-136 (US 7238785; US 9797907; US 9382329; WO 2020/142659, each incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	NYGMN	134
CDR-H2	WINTYTGEPTYTDDFKG	135
CDR-H3	GGFGSSYWYFDV	136

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences of AR47A6.4.2, SEQ ID NO. 131-133 (US 7420040, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	KASQDVSIAVA	131
CDR-L2	SASYRYT	132
CDR-L3	QQHYITPLT	133

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences of AR47A6.4.2, SEQ ID NO. 134, 137, 138 (US 7420040, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	NYGMN	134
CDR-H2	WINTKTGEPTYAEEFKG	137
CDR-H3	GGYGSSYWYFDV	138

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences of humanized KM4097, SEQ ID NO. 139-141 (US 2012/0237518, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	KSSQSLLNSGNQQNYLA	139
CDR-L2	GASTRES	140

CDR-L3	QSDHIYPYT	141

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences of humanized KM4097, SEQ ID NO. 142-144 (US 2012/0237518, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	IYWLG	142
CDR-H2	NIFPGSAYINYNEKFKG	143
CDR-H3	EGSNSGY	144

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences of hTINA1-H1L1, SEQ ID NO. 132, 133, 145 (US 10,227,417, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	KASQDVSTAVA	145
CDR-L2	SASYRYT	132
CDR-L3	QQHYITPLT	133

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences of hTINA1-H1L1, SEQ ID NO. 146-148 (US 10,227,417, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	TAGMQ	146
CDR-H2	WINTHSGVPKYAEDFKG	147
CDR-H3	SGFGSSYWYFDV	148

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences of hTINA1-H1L1, SEQ ID NO. 149-151 (US 8871908, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	RASKSVSTS(X1)YSYMH where X1 is G, L, or N	149
CDR-L2	LASNLES	150
CDR-L3	QHSRELPYT	151

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences of hTINA1-H1L1, SEQ ID NO. 152-157 (US 8871908, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	SYGVH	152
CDR-H1	GGSISSY	153
CDR-H1	GGSISSYGVH	154
CDR-H2	VIWT(X1)G(X2)TDYNSALM(X3) where X1 is G or S; X2 is S or V; X3 is S or G	155
CDR-H2	WT(X1)G(X2) where X1 is G or S; X2 is S or V	156
CDR-H3	DGDYDRYTMDY	157

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) sequences SEQ ID NO. 150, 151, 158 of hTINA1-H1L1, (US 8871908, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-L1	RASKSVSTSGYSYMH	158
CDR-L2	LASNLES	150
CDR-L3	QHSRELPYT	151

In an embodiment of the invention, the TROP2-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) sequences SEQ ID NO. 152-154, 157, 159, 160 of hTINA1-H1L1, (US 8871908, incorporated by reference herein).

Region	CDR Sequence Fragment	SEQ ID NO.
CDR-H1	SYGVH	152
CDR-H1	GGSISSY	153
CDR-H1	GGSISSYGVH	154
CDR-H2	VIWTSGVTDYNSALMG	159
CDR-H2	WTSGV	160
CDR-H3	DGDYDRYTMDY	157

In some embodiments, the antibody construct further comprises an Fc domain. In certain embodiments, the antibody construct is an antibody. In certain embodiments, the antibody construct is a fusion protein. The antigen binding domain can be a single-chain variable region fragment (scFv). A single-chain variable region fragment (scFv), which is a truncated Fab fragment including the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated using routine recombinant DNA technology techniques. Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology. The antibody construct or antigen binding domain may comprise one or more variable regions (e.g., two variable regions) of an antigen binding domain of an antibody, such as an anti-PD-L1 antibody, an anti-Her2 antibody, an anti-CEA antibody, or an anti-TROP2 antibody, each variable region comprising a CDR1, a CDR2, and a CDR3.

In some embodiments, the antibodies in the immunoconjugates contain a modified Fc region, wherein the modification modulates the binding of the Fc region to one or more Fc receptors.

In some embodiments, the Fc region is modified by inclusion of a transforming growth factor beta 1 (TGFβ1) receptor, or a fragment thereof, that is capable of binding TGFβ1. For example, the receptor can be TGFβ receptor II (TGFβRII). In some embodiments, theTGFβ receptor is a human TGFβ receptor. In some embodiments, the IgG has a C-terminal fusion to a TGFβRII extracellular domain (ECD). An “Fc linker” may be used to attach the IgG to the TGFβRII extracellular domain. The Fc linker may be a short, flexible peptide that allows for the

proper three-dimensional folding of the molecule while maintaining the binding-specificity to the targets. In some embodiments, the N-terminus of the TGFβ receptor is fused to the Fc of the antibody construct (with or without an Fc linker). In some embodiments, the C-terminus of the antibody construct heavy chain is fused to the TGFβ receptor (with or without an Fc linker). In some embodiments, the C-terminal lysine residue of the antibody construct heavy chain is mutated to alanine.

In some embodiments, the antibodies in the immunoconjugates are glycosylated.

In some embodiments, the antibodies in the immunoconjugates is a cysteine-engineered antibody which provides for site-specific conjugation of an adjuvant, label, or drug moiety to the antibody through cysteine substitutions at sites where the engineered cysteines are available for conjugation but do not perturb immunoglobulin folding and assembly or alter antigen binding and effector functions (Junutula, et al., 2008b *Nature Biotech.*, 26(8):925-932; Dornan et al. (2009) *Blood* 114(13):2721-2729; US 7521541; US 7723485; US 2012/0121615; WO 2009/052249). A "cysteine engineered antibody" or "cysteine engineered antibody variant" is an antibody in which one or more residues of an antibody are substituted with cysteine residues. Cysteine-engineered antibodies can be conjugated to the pyrazoloazepine adjuvant moiety as a pyrazoloazepine-linker compound with uniform stoichiometry (e.g., up to two pyrazoloazepine moieties per antibody in an antibody that has a single engineered cysteine site).

In some embodiments, cysteine-engineered antibodies used to prepare the immunoconjugates of Table 3 have a cysteine residue introduced at the 149-lysine site of the light chain (LC K149C). In other embodiments, the cysteine-engineered antibodies have a cysteine residue introduced at the 118-alanine site (EU numbering) of the heavy chain (HC A118C). This site is alternatively numbered 121 by Sequential numbering or 114 by Kabat numbering. In other embodiments, the cysteine-engineered antibodies have a cysteine residue introduced in the light chain at G64C or R142C according to Kabat numbering, or in the heavy chain at D101C, V184C or T205C according to Kabat numbering.

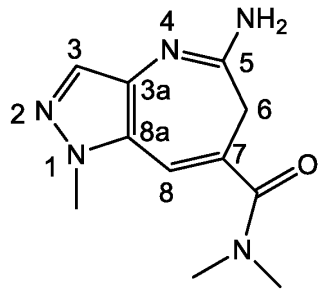
PYRAZOLOAZEPINE ADJUVANT COMPOUNDS

The immunoconjugate of the invention comprises a pyrazoloazepine adjuvant moiety. The adjuvant moiety described herein is a compound that elicits an immune response (i.e., an immunostimulatory agent). Generally, the adjuvant moiety described herein is a TLR agonist. TLRs are type-I transmembrane proteins that are responsible for the initiation of innate immune responses in vertebrates. TLRs recognize a variety of pathogen-associated molecular patterns from bacteria, viruses, and fungi and act as a first line of defense against invading pathogens. TLRs elicit overlapping yet distinct biological responses due to differences in cellular

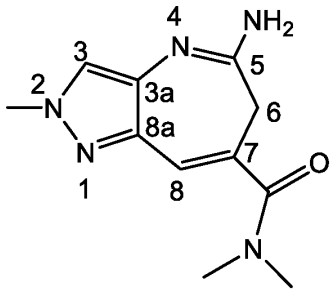
expression and in the signaling pathways that they initiate. Once engaged (e.g., by a natural stimulus or a synthetic TLR agonist), TLRs initiate a signal transduction cascade leading to activation of nuclear factor-κB (NF-κB) via the adapter protein myeloid differentiation primary response gene 88 (MyD88) and recruitment of the IL-1 receptor associated kinase (IRAK). Phosphorylation of IRAK then leads to recruitment of TNF-receptor associated factor 6 (TRAF6), which results in the phosphorylation of the NF-κB inhibitor I-κB. As a result, NF-κB enters the cell nucleus and initiates transcription of genes whose promoters contain NF-κB binding sites, such as cytokines. Additional modes of regulation for TLR signaling include TIR-domain containing adapter-inducing interferon-β (TRIF)-dependent induction of TNF-receptor associated factor 6 (TRAF6) and activation of MyD88 independent pathways via TRIF and TRAF3, leading to the phosphorylation of interferon response factor three (IRF3). Similarly, the MyD88 dependent pathway also activates several IRF family members, including IRF5 and IRF7 whereas the TRIF dependent pathway also activates the NF-κB pathway.

Typically, the adjuvant moiety described herein is a TLR7 and/or TLR8 agonist. TLR7 and TLR8 are both expressed in monocytes and dendritic cells. In humans, TLR7 is also expressed in plasmacytoid dendritic cells (pDCs) and B cells. TLR8 is expressed mostly in cells of myeloid origin, i.e., monocytes, granulocytes, and myeloid dendritic cells. TLR7 and TLR8 are capable of detecting the presence of "foreign" single-stranded RNA within a cell, as a means to respond to viral invasion. Treatment of TLR8-expressing cells, with TLR8 agonists can result in production of high levels of IL-12, IFN-γ, IL-1, TNF-α, IL-6, and other inflammatory cytokines. Similarly, stimulation of TLR7-expressing cells, such as pDCs, with TLR7 agonists can result in production of high levels of IFN-α and other inflammatory cytokines. TLR7/TLR8 engagement and resulting cytokine production can activate dendritic cells and other antigen-presenting cells, driving diverse innate and acquired immune response mechanisms leading to tumor destruction.

Exemplary pyrazoloazepine compounds (PAZ) of the invention are shown in Table 1. Each compound was characterized by mass spectrometry and shown to have the mass indicated. Pyrazoloazepine compounds of the invention include regioisomers A and B, with IUPAC position numbering as shown:



A



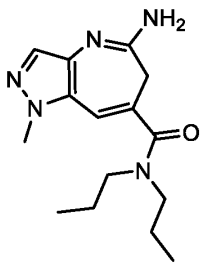
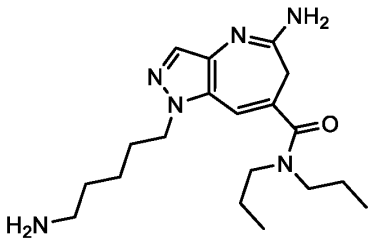
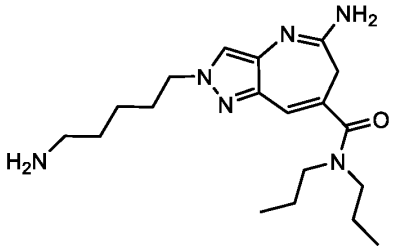
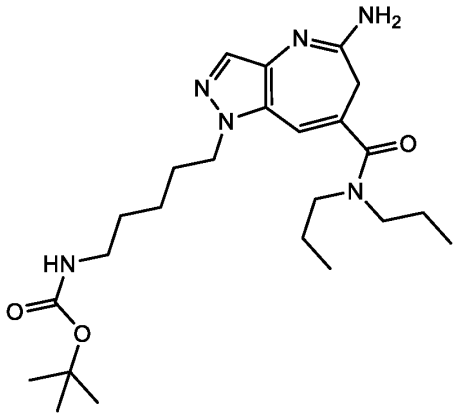
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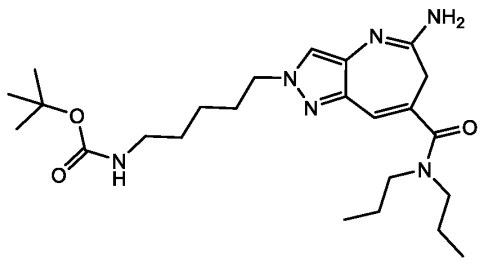
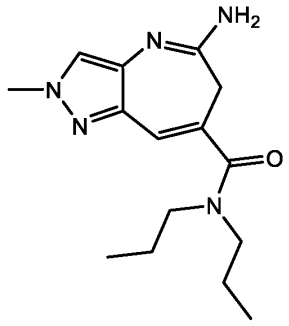
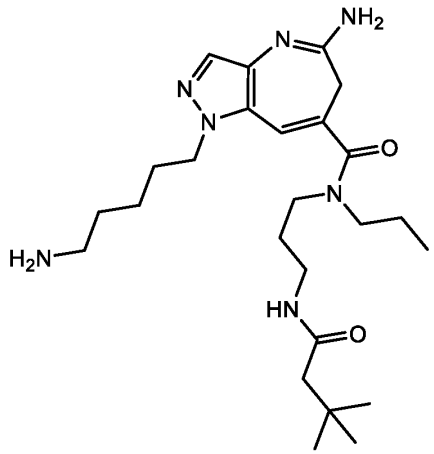
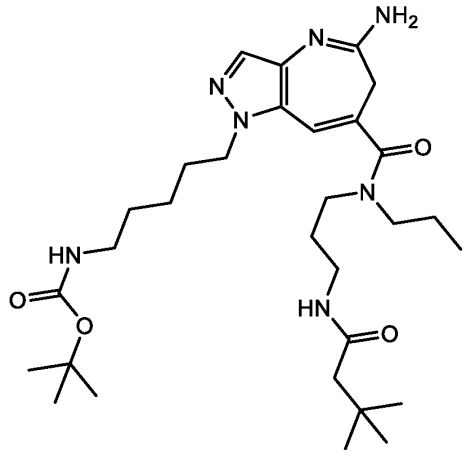
Activity against HEK293 NFKB reporter cells expressing human TLR7 or human TLR8 was measured according to Example 202. The pyrazoloazepine compounds of Table 1 demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

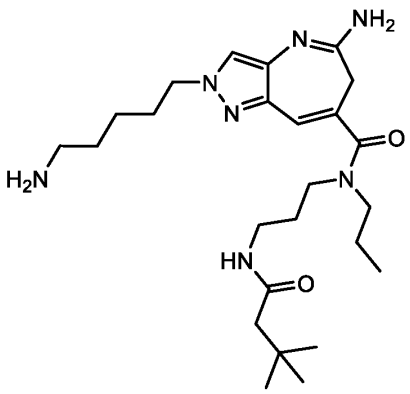
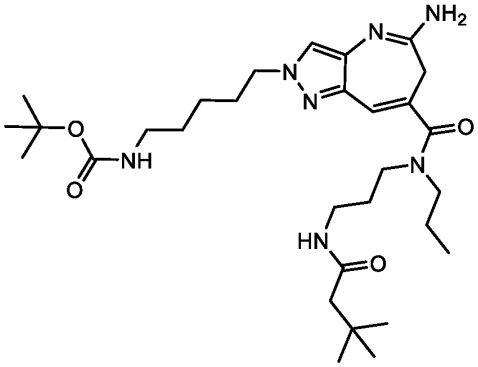
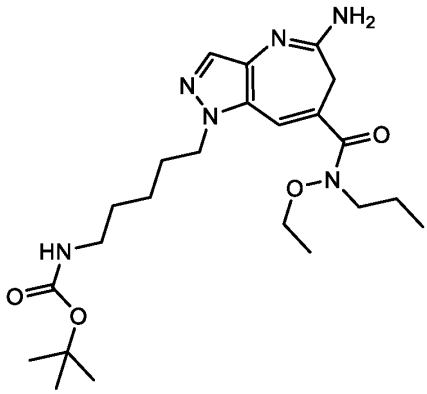
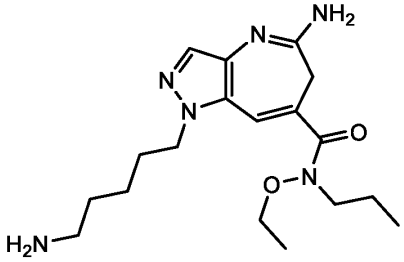
Figure 1 shows a graph of HEK human TLR7 activity at 24 hours of pyrazoloazepine compounds PAZ-2, PAZ-4, and PAZ-11, versus comparator adjuvant compounds C-1 and C-2. PAZ-2 and PAZ-11 have comparable TLR7 activity relative to a known TLR7 adjuvant C-1, all while having very different structural and biophysical features.

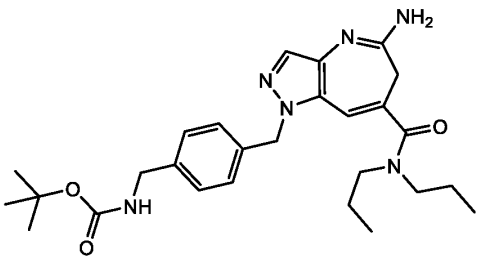
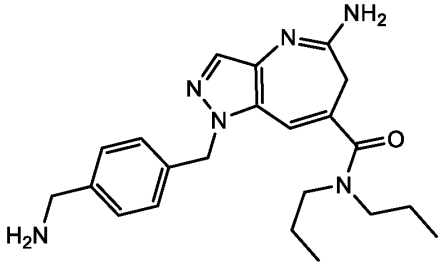
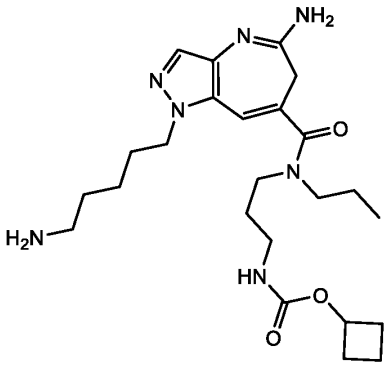
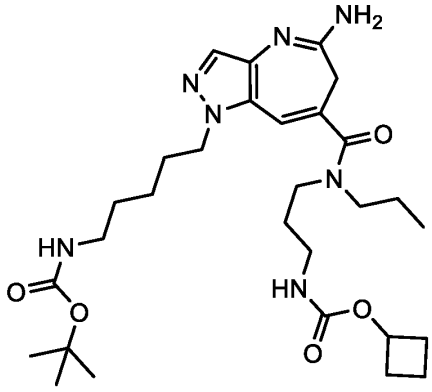
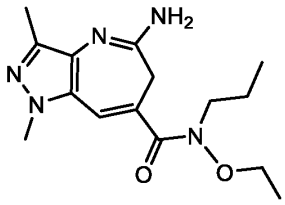
Figure 2 shows a graph of HEK human TLR8 activity at 24 hours of pyrazoloazepine compounds PAZ-1 and PAZ-2, versus comparator adjuvant compounds C-1 and C-2. PAZ-11 has better TLR8 potency relative to known TLR8 adjuvant C-2. Additionally, it possesses improved hydrophilicity relative to C-2. The improved physicochemical properties coupled with increased TLR8 potency, yield a much more efficient adjuvant.

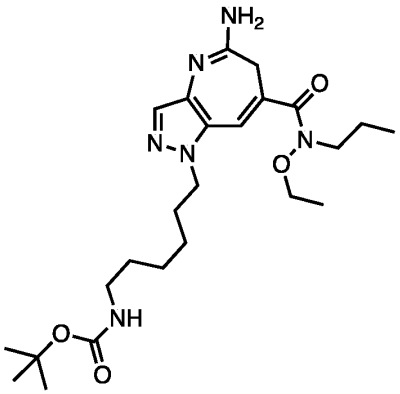
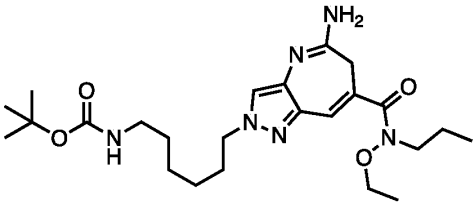
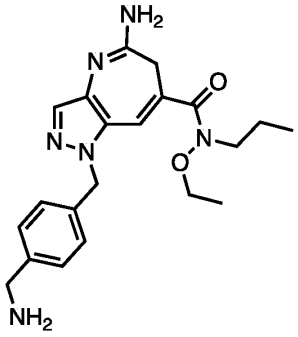
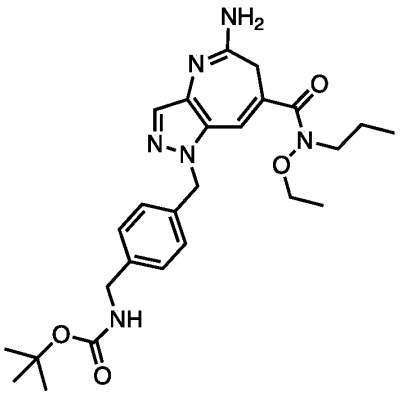
Table 1: Pyrazoloazepine compounds (PAZ)

PAZ No.	Structure	MW	HEK293 hTLR7 EC50 (nM)	HEK293 hTLR8 EC50 (nM)
PAZ-1		289.4	>9000	4152
PAZ-2		360.5	>9000	2955
PAZ-3		360.5	>9000	>9000
PAZ-4		460.6	2920	3096

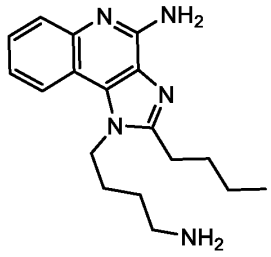
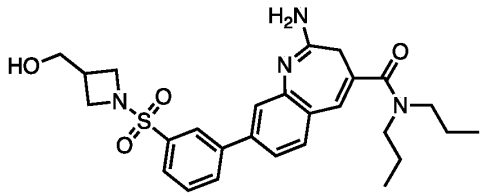
PAZ-5		460.6	>9000	>9000
PAZ-6		289.4	>9000	4046
PAZ-7		473.7	>9000	>9000
PAZ-8		573.8	2749	>9000

PAZ-9		473.7	>9000	>9000
PAZ-10		573.8	>9000	3230
PAZ-11		462.6	795	1490
PAZ-12		362.5	3210	290

PAZ-13		494.6	881	3837
PAZ-14		394.5	5587	>9000
PAZ-15		473.6	>9000	>9000
PAZ-16		573.7		
PAZ-17		305.4		

PAZ-18		476.6		
PAZ-19		476.6		
PAZ-20		369.5		
PAZ-21		496.6		

Comparator compounds:

Comparator No.	Structure	MW	HEK293 hTLR7 EC50 (nM)	HEK293 hTLR8 EC50 (nM)
C-1		311.4	284	4053
C-2		510.7	>9000	1590

PYRAZOLOAZEPINE-LINKER COMPOUNDS

The immunoconjugates of the invention are prepared by conjugation of an antibody with a pyrazoloazepine-linker compound. The pyrazoloazepine-linker compounds comprise a pyrazoloazepine (PAZ) moiety covalently attached to a linker unit. The linker units comprise functional groups and subunits which affect stability, permeability, solubility, and other pharmacokinetic, safety, and efficacy properties of the immunoconjugates. The linker unit includes a reactive functional group which reacts, i.e. conjugates, with a reactive functional group of the antibody. For example, a nucleophilic group such as a lysine side chain amino of the antibody reacts with an electrophilic reactive functional group of the PAZ-linker compound to form the immunoconjugate. Also, for example, a cysteine thiol of the antibody reacts with a maleimide or bromoacetamide group of the PAZ-linker compound to form the immunoconjugate.

Considerations for the design of the immunoconjugates of the invention include: (1) preventing the premature release of the PAZ moiety during in vivo circulation and (2) ensuring that a biologically active form of the PAZ moiety is released at the desired site of action at an adequate rate. The complex structure of the immunoconjugate together with its functional properties requires careful design and selection of every component of the molecule including antibody, conjugation site, linker structure, and the pyrazoloazepine compound. The linker determines the mechanism and rate of adjuvant release.

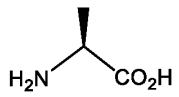
Generally, the linker unit (L) may be cleavable or non-cleavable. Cleavable linker units may include a peptide sequence which is a substrate for certain proteases such as Cathepsins which recognize and cleave the peptide linker unit, separating the PAZ agonist from the antibody (Caculitan NG, et al (2017) *Cancer Res.* 77(24):7027-7037).

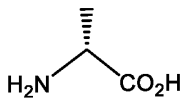
Cleavable linker units may include labile functionality such as an acid-sensitive disulfide group (Kellogg, BA et al (2011) *Bioconjugate Chem.* 22, 717−727; Ricart, A. D. et al (2011) *Clin. Cancer Res.* 17, 6417−6427; Pillow, T., et al (2017) *Chem. Sci.* 8, 366-370; Zhang D, et al (2016) *ACS Med Chem Lett.* 7(11):988-993).

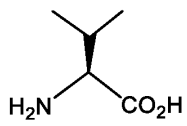
In some embodiments , the linker is non-cleavable under physiological conditions . As used herein , the term "physiological conditions" refers to a temperature range of 20-40 degrees Celsius , atmospheric pressure (i.e. , 1 atm) , a pH of about 6 to about 8 , and the one or more physiological enzymes, proteases, acids , and bases. One advantage of a non-cleavable linker between the antibody and PAZ moiety in an immunoconjugate is minimizing premature payload release and corresponding toxicity.

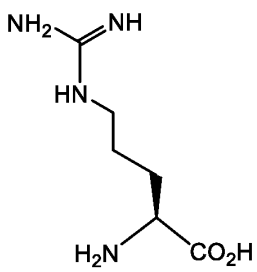
In one embodiment, the invention includes a peptide linking unit, PEP, between the cell-binding agent and the immunostimulatory PAZ moiety, comprising a peptide radical based on a linear sequence of specific amino acid residues which can be selectively cleaved by a protease such as a cathepsin, a tumor-associated elastase enzyme or an enzyme with protease-like or elastase-like activity. The peptide radical may be about two to about twelve amino acids. Enzymatic cleavage of a bond within the peptide linker releases an active form of the immunostimulatory PAZ moiety. This leads to an increase in the tissue specificity of the conjugates according to the invention and thus to an additional decrease of toxicity of the conjugates according to the invention in other tissue types.

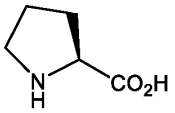
In an exemplary embodiment, PEP is comprised of amino acid residues (AA) of amino acids selected from the group consisting of:

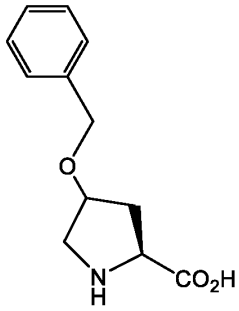
Ala 

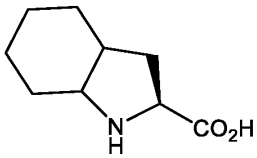
D-Ala 

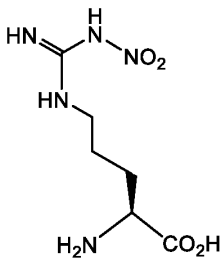
Val 

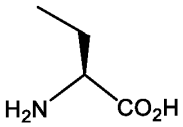
Arg 

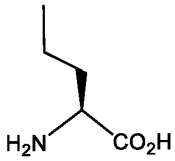
Pro 

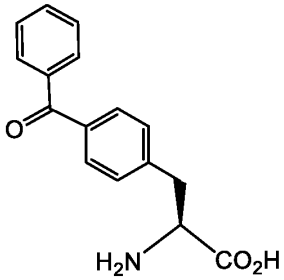
Hyp(O-Bzl) 

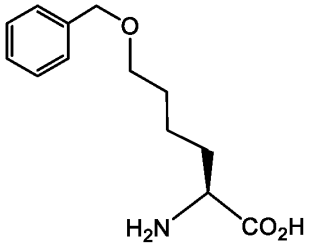
Oic 

Arg(NO_2) 

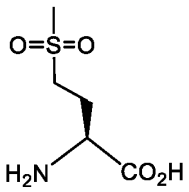
Abu 

Nva 

Bpa 

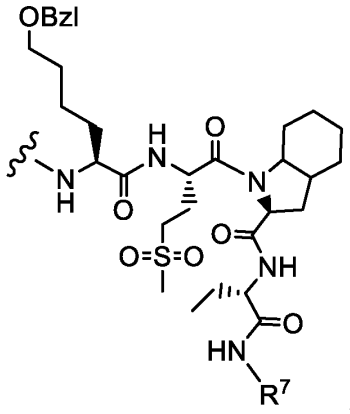
Nle(O-Bzl) 

and

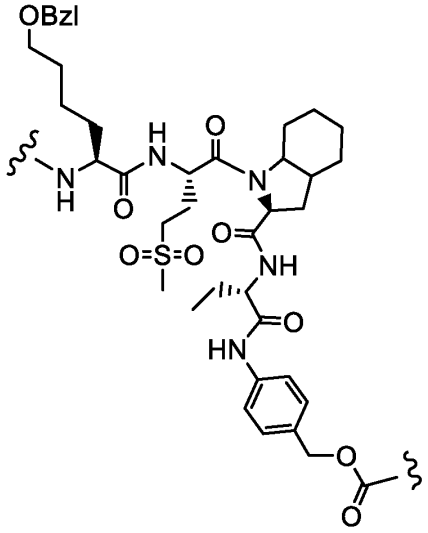
Met(O_2) 

In an exemplary embodiment, PEP is selected from the group consisting of Ala-Pro-Val, Asn-Pro-Val, Ala-Ala-Val, Ala-Ala-Pro-Ala, Ala-Ala-Pro-Val, and Ala-Ala-Pro-Nva.

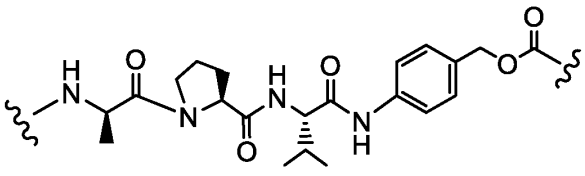
In an exemplary embodiment, PEP has the formula:



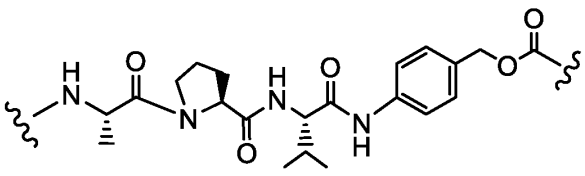
In an exemplary embodiment, PEP has the formula:



In an exemplary embodiment, PEP is selected from the formulas:



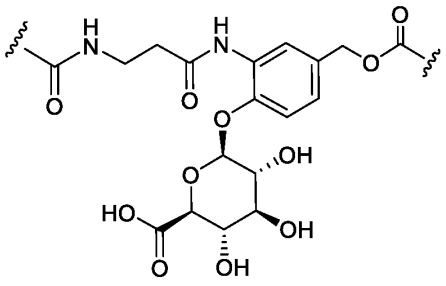
; and



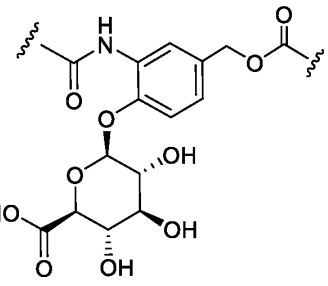
The linker provides sufficient stability of the immunoconjugate in biological media, e.g. culture medium or serum and, at the same time, the desired intracellular action within tumor tissue as a result of its specific enzymatic or hydrolytic cleavability with release of the immunostimulatory PAZ moiety, i.e. “payload”.

The enzymatic activity of a protease, cathepsin, or elastase can catalyze cleavage of a covalent bond of the immunoconjugate under physiological conditions. The enzymatic activity being the expression product of cells associated with tumor tissue. The enzymatic activity on the cleavage site of the targeting peptide converts the immunoconjugate to an active immunostimulatory drug free of targeting peptide and linking group. The cleavage site may be specifically recognized by the enzyme.. Cathepsin or elastase may catalyze the cleavage of a specific peptidyl bond between the C-terminal amino acid residue of the specific peptide and the immunostimulatory moiety of the immunoconjugate.

In one embodiment, the invention includes a linking unit, i.e. L or linker, between the cell-binding agent and the immunostimulatory moiety, comprising a substrate for glucuronidase (Jeffrey SC, et al (2006) *Bioconjug. Chem.* 17(3):831-40), or sulfatase (Bargh JD, et al (2020) *Chem Sci.* 11(9):2375-2380) cleavage. In particular, L include a Gluc unit and comprise a formula selected from:



and



.

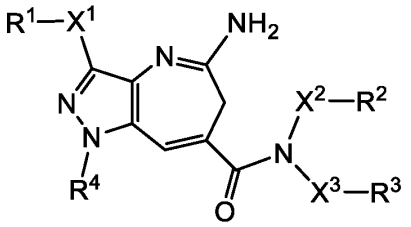
Specific cleavage of the immunoconjugates of the invention takes advantage of the presence of tumor infiltrating cells of the immune system and leukocyte-secreted enzymes, to promote the activation of an anticancer drug at the tumor site.

Electrophilic reactive functional groups suitable for the PAZ-linker compounds include, but are not limited to, N-hydroxysuccinimidyl (NHS) esters and N-hydroxysulfosuccinimidyl (sulfo-NHS) esters (amine reactive); carbodiimides (amine and carboxyl reactive); hydroxymethyl phosphines (amine reactive); maleimides (thiol reactive); halogenated acetamides such as *N*-iodoacetamides (thiol reactive); aryl azides (primary amine reactive); fluorinated aryl azides (reactive via carbon-hydrogen (C-H) insertion); pentafluorophenyl (PFP) esters (amine reactive); tetrafluorophenyl (TFP) esters (amine reactive); imidoesters (amine reactive); isocyanates (hydroxyl reactive); vinyl sulfones (thiol, amine, and hydroxyl reactive); pyridyl disulfides (thiol reactive); and benzophenone derivatives (reactive via C-H bond insertion). Further reagents include, but are not limited, to those described in Hermanson, *Bioconjugate Techniques* 2nd Edition, Academic Press, 2008.

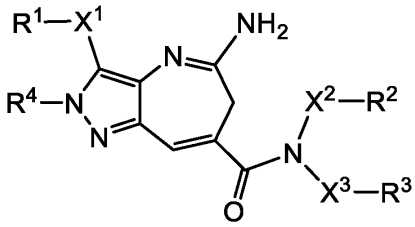
The invention provides solutions to the limitations and challenges to the design, preparation and use of immunoconjugates. Some linkers may be labile in the blood stream,

thereby releasing unacceptable amounts of the adjuvant/drug prior to internalization in a target cell (Khot, A. et al (2015) *Bioanalysis* 7(13):1633–1648). Other linkers may provide stability in the bloodstream, but intracellular release effectiveness may be negatively impacted. Linkers that provide for desired intracellular release typically have poor stability in the bloodstream. Alternatively stated, bloodstream stability and intracellular release are typically inversely related. In addition, in standard conjugation processes, the amount of adjuvant/drug moiety loaded on the antibody, i.e. drug loading, the amount of aggregate that is formed in the conjugation reaction, and the yield of final purified conjugate that can be obtained are interrelated. For example, aggregate formation is generally positively correlated to the number of equivalents of adjuvant/drug moiety and derivatives thereof conjugated to the antibody. Under high drug loading, formed aggregates must be removed for therapeutic applications. As a result, drug loading-mediated aggregate formation decreases immunoconjugate yield and can render process scale-up difficult.

Exemplary embodiments include a 5-aminopyrazoloazepine-linker compound of Formulas IIa and IIb:



IIa



IIb;

wherein X^1, X^2, and X^3 are independently selected from the group consisting of a bond, C(=O), C(=O)N(R^5), O, N(R^5), S, S(O)$_2$, and S(O)$_2$N(R^5);

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} carbocyclyl, C_6-C_{20} aryl, C_2-C_9 heterocyclyl, and C_1-C_{20} heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–OR^5;

–(C_3-C_{12} carbocyclyl);

–(C_3-C_{12} carbocyclyl)–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_3-C_{12} carbocyclyl)–NR^5–C(=NR^5)NR^5–*;

–(C_6-C_{20} aryl);

–(C_6-C_{20} aryldiyl)–*;

–(C_6-C_{20} aryldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–C(=NR^{5a})N(R^5)–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyl)–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_2-C_9 heterocyclyl)–C(=O)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_2-C_9 heterocyclyl)–NR^5–C(=NR^{5a})NR^5–*;

–(C_2-C_9 heterocyclyl)–NR^5–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_2-C_9 heterocyclyl)–(C_6-C_{20} aryldiyl)–*;

–(C_1-C_{20} heteroaryl);

–(C_1-C_{20} heteroaryldiyl)–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{20} heteroaryldiyl)–NR^5–C(=NR^{5a})N(R^5)–*;

–(C_1-C_{20} heteroaryldiyl)–N(R^5)C(=O)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–C(=O)–*;

–C(=O)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–C(=O)N(R^5)$_2$;

–C(=O)N(R^5)–*;

–C(=O)N(R^5)–(C_1-C_{12} alkyldiyl)–*;

–C(=O)N(R^5)–(C_1-C_{12} alkyldiyl)–C(=O)N(R^5)–*;

–C(=O)N(R^5)–(C_1-C_{12} alkyldiyl)–N(R^5)C(=O)R^5;

–C(=O)N(R^5)–(C_1-C_{12} alkyldiyl)–N(R^5)C(=O)N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–N(R^5)CO_2R^5;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–N(R^5)C(=NR^{5a})N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^{5a})R^5;

–C(=O)NR^5–(C$_1$-C$_8$ alkyldiyl)–NR^5(C$_2$-C$_5$ heteroaryl);

–C(=O)NR^5–(C$_1$-C$_{20}$ heteroaryldiyl)–N(R^5)–*;

–C(=O)NR^5–(C$_1$-C$_{20}$ heteroaryldiyl)–*;

–C(=O)NR^5–(C$_1$-C$_{20}$ heteroaryldiyl)–(C$_1$-C$_{12}$ alkyldiyl)–N(R^5)$_2$;

–C(=O)NR^5–(C$_1$-C$_{20}$ heteroaryldiyl)–(C$_2$-C$_{20}$ heterocyclyldiyl)–C(=O)NR^5–(C$_1$-C$_{12}$ alkyldiyl)–NR^5–*;

–N(R^5)$_2$;

–N(R^5)–*;

–N(R^5)C(=O)R^5;

–N(R^5)C(=O)–*;

–N(R^5)C(=O)N(R^5)$_2$;

–N(R^5)C(=O)N(R^5)–*;

–N(R^5)CO$_2$R^5;

–N(R^5)CO$_2$(R^5)–*;

–NR^{5}C(=NR^{5a})N(R^5)$_2$;

–NR^{5}C(=NR^{5a})N(R^5)–*;

–NR^{5}C(=NR^{5a})R^5;

–N(R^5)C(=O)–(C$_1$-C$_{12}$ alkyldiyl)–N(R^5)–*;

–N(R^5)–(C$_2$-C$_5$ heteroaryl);

–N(R^5)–S(=O)$_2$–(C$_1$-C$_{12}$ alkyl);

–O–(C$_1$-C$_{12}$ alkyl);

–O–(C$_1$-C$_{12}$ alkyldiyl)–N(R^5)$_2$;

–O–(C$_1$-C$_{12}$ alkyldiyl)–N(R^5)–*;

–OC(=O)N(R^5)$_2$;

–OC(=O)N(R^5)–*;

–S(=O)$_2$–(C$_2$-C$_{20}$ heterocyclyldiyl)–*;

–S(=O)$_2$–(C$_2$-C$_{20}$ heterocyclyldiyl)–(C$_1$-C$_{12}$ alkyldiyl)–N(R^5)$_2$;

–S(=O)$_2$–(C$_2$-C$_{20}$ heterocyclyldiyl)–(C$_1$-C$_{12}$ alkyldiyl)–NR^5–*; and

–S(=O)$_2$–(C$_2$-C$_{20}$ heterocyclyldiyl)–(C$_1$-C$_{12}$ alkyldiyl)–OH;

or R^2 and R^3 together form a 5- or 6-membered heterocyclyl ring;

R^5 is selected from the group consisting of H, C$_6$-C$_{20}$ aryl, C$_3$-C$_{12}$ carbocyclyl, C$_2$-C$_{20}$ heterocyclyl, C$_6$-C$_{20}$ aryldiyl, C$_1$-C$_{12}$ alkyl, and C$_1$-C$_{12}$ alkyldiyl, or two R^5 groups together form a 5- or 6-membered heterocyclyl ring;

R^{5a} is selected from the group consisting of C_6-C_{20} aryl and C_1-C_{20} heteroaryl;

where the asterisk * indicates the attachment site of L, and where one of R^1, R^2, R^3 and R^4 is attached to L;

L is the linker selected from the group consisting of:

Q–C(=O)–PEG–;

Q–C(=O)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

Q–C(=O)–PEG–O–;

Q–C(=O)–PEG–O–C(=O)–;

Q–C(=O)–PEG–C(=O)–;

Q–C(=O)–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–N(R^6)–;

Q–C(=O)–PEG–N(R^6)–C(=O)–;

Q–C(=O)–PEG–N(R^6)–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–N^+(R^6)$_2$–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

Q–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

Q–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

Q–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–C(=O)–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^5)–C(=O);

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–PEP–;

Q–$(CH_2)_m$–C(=O)N(R^6)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

Q–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

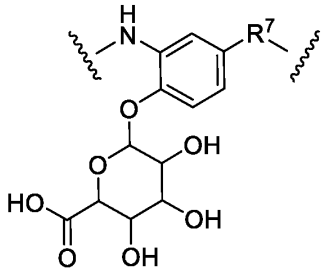
Q–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–; and

Q–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

R^6 is independently H or C_1-C_6 alkyl;

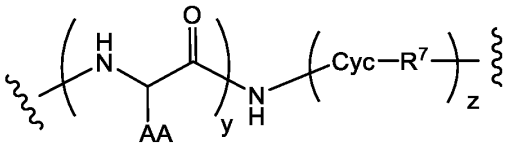
PEG has the formula: –$(CH_2CH_2O)_n$–$(CH_2)_m$–; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:



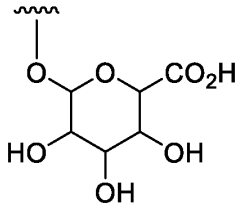
;

PEP has the formula:



where AA is independently selected from a natural or unnatural amino acid side chain, or one or more of AA, and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

Cyc is selected from C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl, optionally substituted with one or more groups selected from F, Cl, NO_2, –OH, $–OCH_3$, and a glucuronic acid having the structure:



;

R^7 is selected from the group consisting of –$CH(R^8)O$–, $–CH_2$–, $–CH_2N(R^8)$–, and –$CH(R^8)O$–C(=O)–, where R^8 is selected from H, C_1-C_6 alkyl, C(=O)–C_1-C_6 alkyl, and –$C(=O)N(R^9)_2$, where R^9 is independently selected from the group consisting of H, C_1-C_{12} alkyl,

and $-(CH_2CH_2O)_n-(CH_2)_m-OH$, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R^9 groups together form a 5- or 6-membered heterocyclyl ring;

y is an integer from 2 to 12;

z is 0 or 1; and

Q is selected from the group consisting of N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, maleimide, and phenoxy substituted with one or more groups independently selected from F, Cl, NO_2, and SO_3^-;

where alkyl, alkyldiyl, alkenyl, alkenyldiyl, alkynyl, alkynyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $-CH_3$, $-CH_2CH_3$, $-CH=CH_2$, $-C\equiv CH$, $-C\equiv CCH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2CH_2OH$, $-C(CH_3)_2OH$, $-CH(OH)CH(CH_3)_2$, $-C(CH_3)_2CH_2OH$, $-CH_2CH_2SO_2CH_3$, $-CH_2OP(O)(OH)_2$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CH_2CHF_2$, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NHC(=NH)H$, $-NHC(=NH)CH_3$, $-NHC(=NH)NH_2$, $-NHC(=O)NH_2$, $-NO_2$, =O, –OH, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein X^1 is a bond, and R^1 is H.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein X^2 is a bond, and R^2 is C_1-C_8 alkyl.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein X^2 and X^3 are each a bond, and R^2 and R^3 are independently selected from C_1-C_8 alkyl, $-O-(C_1$-C_{12} alkyl), $-(C_1$-C_{12} alkyldiyl$)-OR^5$, $-(C_1$-C_8 alkyldiyl$)-N(R^5)CO_2R^5$, $-(C_1$-C_{12} alkyl$)-OC(O)N(R^5)_2$, $-O-(C_1$-C_{12} alkyl$)-N(R^5)CO_2R^5$, and $-O-(C_1$-C_{12} alkyl$)-OC(O)N(R^5)_2$.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^2 is C_1-C_8 alkyl and R^3 is $-(C_1$-C_8 alkyldiyl$)-N(R^5)CO_2R^4$.

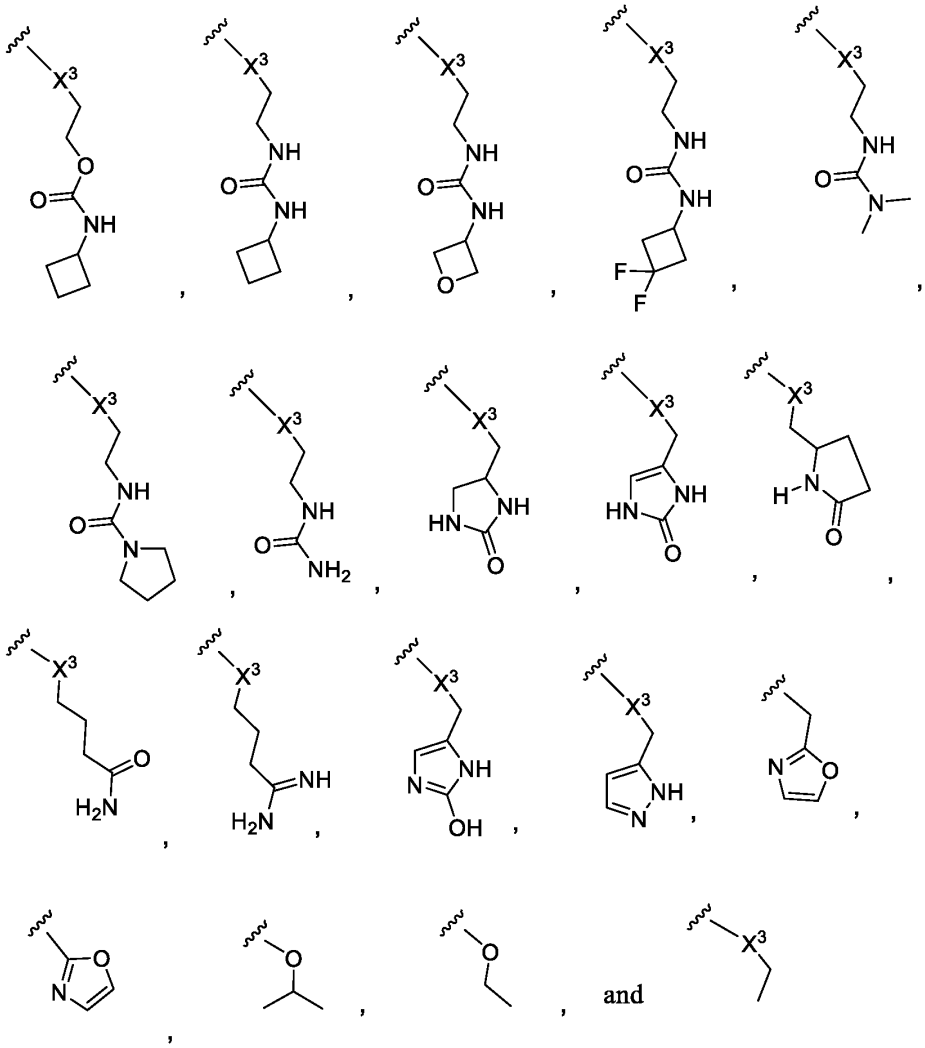
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^2 is $-CH_2CH_2CH_3$ and R^3 is selected from $-CH_2CH_2CH_2NHCO_2$(t-Bu), $-OCH_2CH_2NHCO_2$(cyclobutyl), and $-CH_2CH_2CH_2NHCO_2$(cyclobutyl).

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^2 and R^3 are each independently selected from $-CH_2CH_2CH_3$, $-OCH_2CH_3$, $-OCH_2CF_3$, $-CH_2CH_2CF_3$, $-OCH_2CH_2OH$, and $-CH_2CH_2CH_2OH$.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^2 and R^3 are each $-CH_2CH_2CH_3$.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^2 is $-CH_2CH_2CH_3$ and R^3 is $-OCH_2CH_3$.

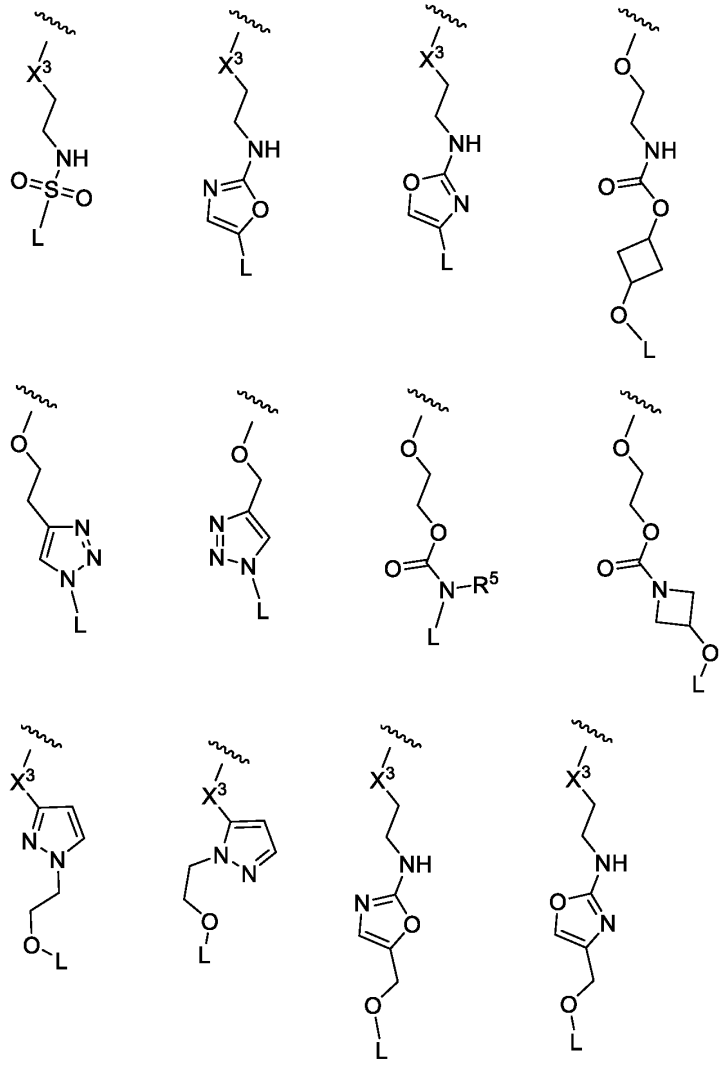
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein X^3-R^3 is selected from the group consisting of:



and

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes where R^2 or R^3 is attached to L.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein X^3–R^3–L is selected from the group consisting of:



where the wavy line indicates the point of attachment to N.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^4 is C_1-C_{12} alkyl.

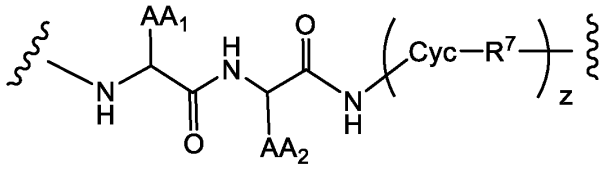
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein R^4 is –(C_1-C_{12} alkyldiyl)–N(R^5)–*; where the asterisk * indicates the attachment site of L.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein L is –C(=O)–PEG– or –C(=O)–PEG–C(=O)–.

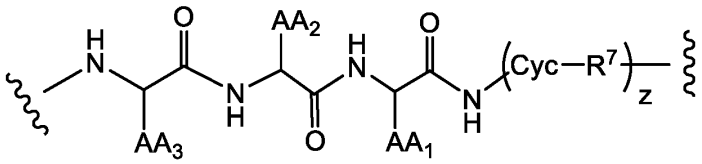
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein for the PEG, m is 1 or 2, and n is an integer from 2 to 10.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein wherein for the PEG, n is 10.

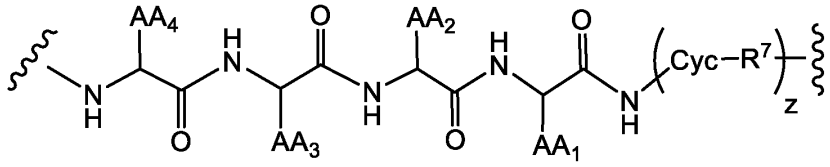
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein L comprises PEP and PEP is a dipeptide and has the formula:



An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein L comprises PEP and PEP is a tripeptide and has the formula:



An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein L comprises PEP and PEP is a tetrapeptide and has the formula:



An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II L wherein L comprises PEP and PEP is a tetrapeptide wherein:

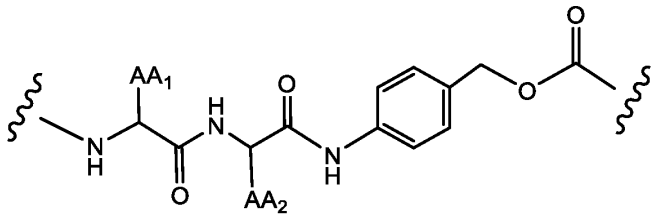
AA_1 is selected from the group consisting of Abu, Ala, and Val;

AA_2 is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

AA_3 is selected from the group consisting of Ala and $Met(O)_2$; and

AA_4 is selected from the group consisting of Oic, $Arg(NO_2)$, Bpa, and Nle(O-Bzl).

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein PEP has the formula:



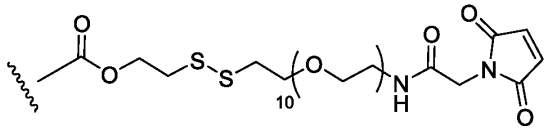
wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

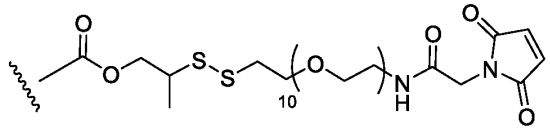
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$; or AA_1 and AA_2 form a 5-membered ring proline amino acid.

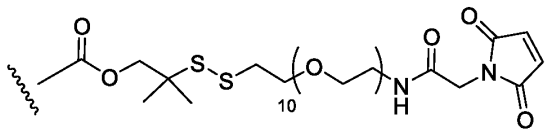
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

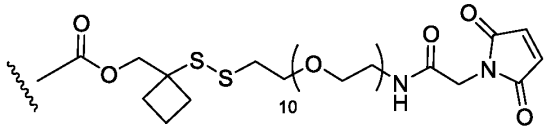
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein L is selected from the structures:



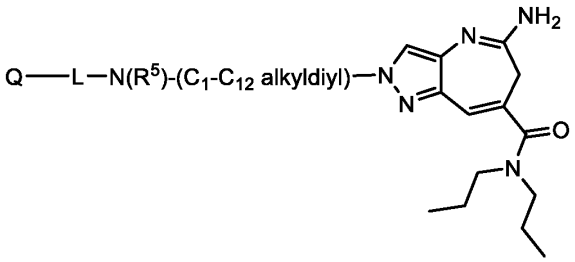




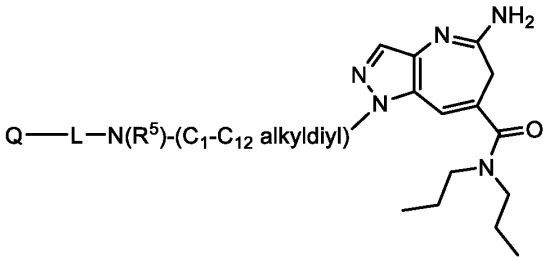


where the wavy line indicates the attachment to one of R^1, R^2, R^3 and R^4.

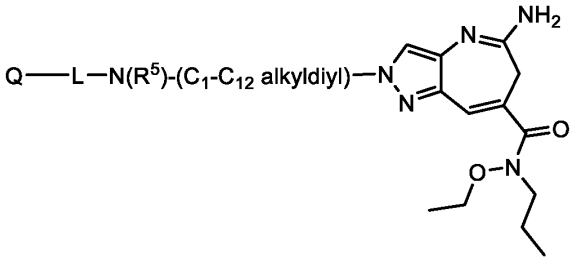
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II is selected from Formulae IIa-IId:



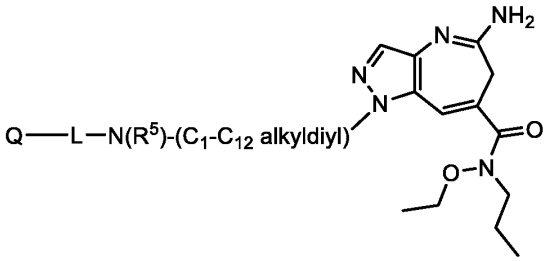
IIa;



IIb;

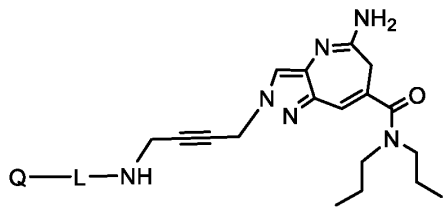


IIc; and

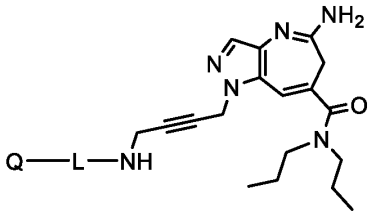


IId.

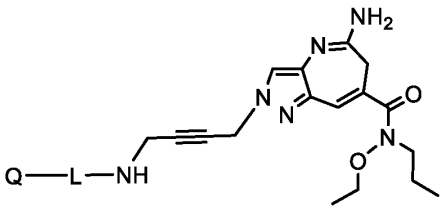
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II is selected from Formulae IIe-IIl:



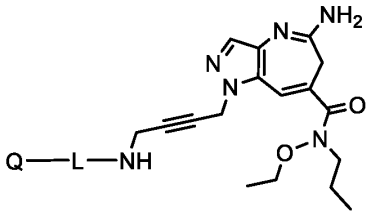
IIe;



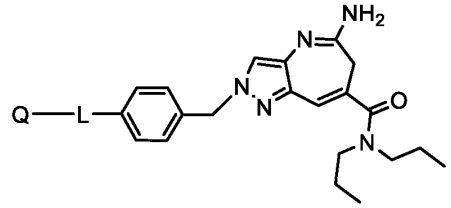
IIf;



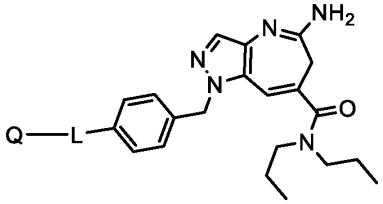
IIg;



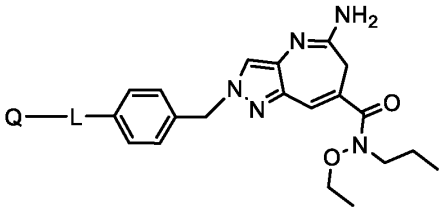
IIh;



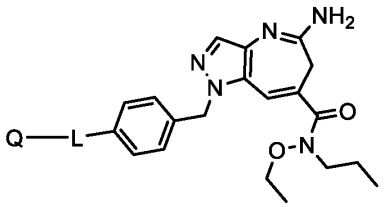
IIi;



IIj;

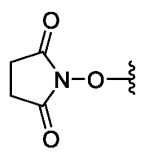


Ik; and

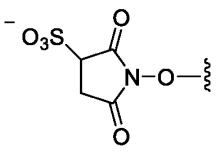


Il.

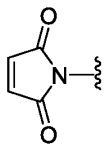
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein Q is selected from:



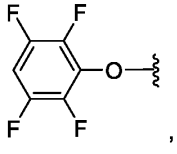
,



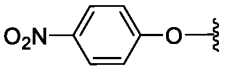
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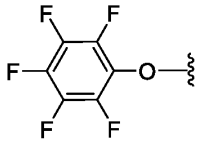
,



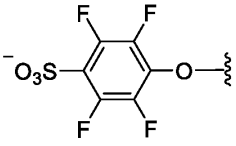
,



,



, and



.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein Q is phenoxy substituted with one or more F.

An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein Q is 2,3,5,6-tetrafluorophenoxy.

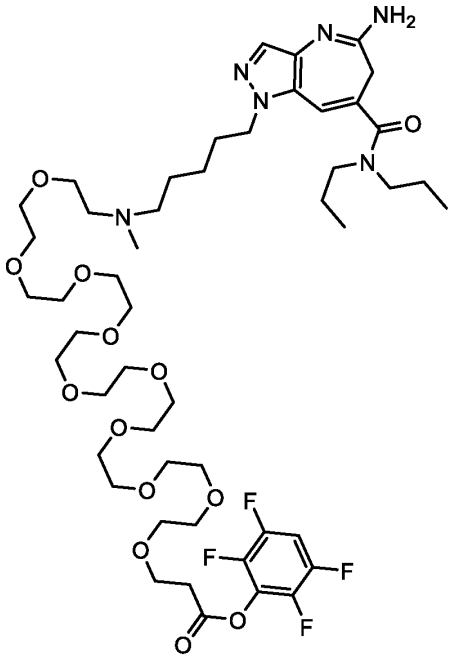
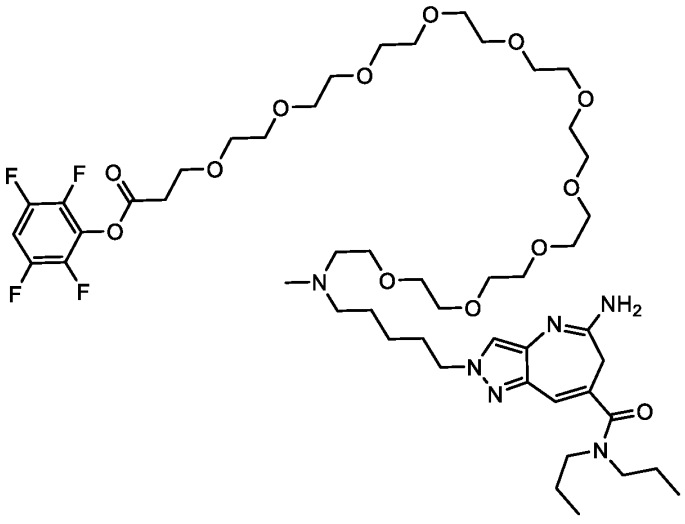
An exemplary embodiment of the pyrazoloazepine-linker compound of Formula II includes wherein Q is maleimide.

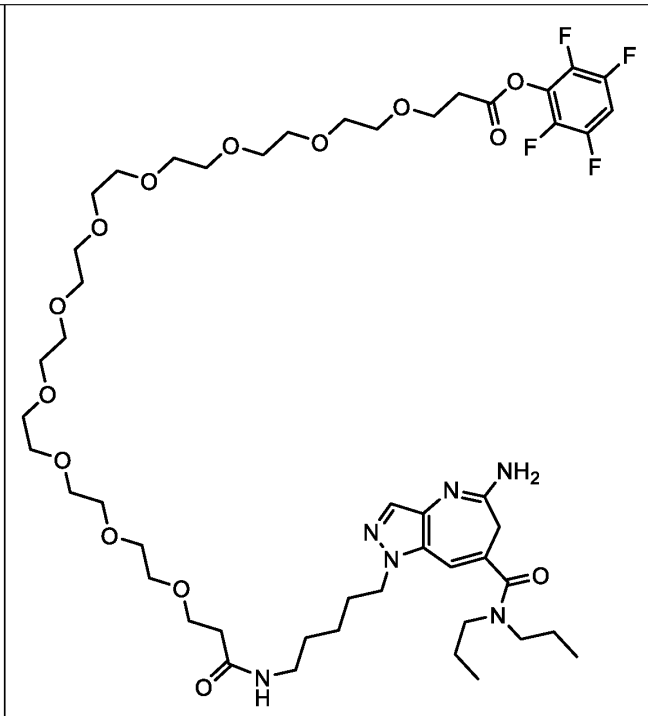
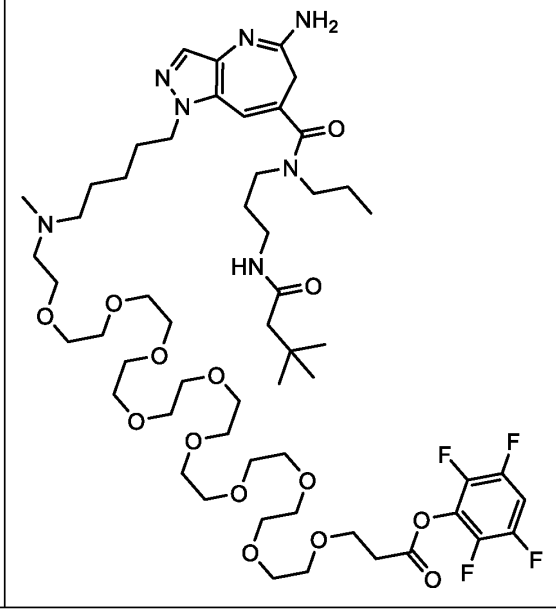
The invention includes all reasonable combinations and permutations of the features of the Formula II embodiments.

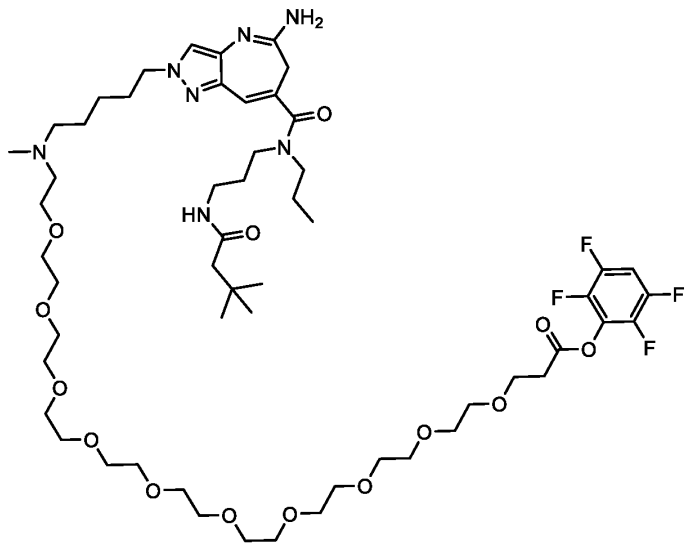
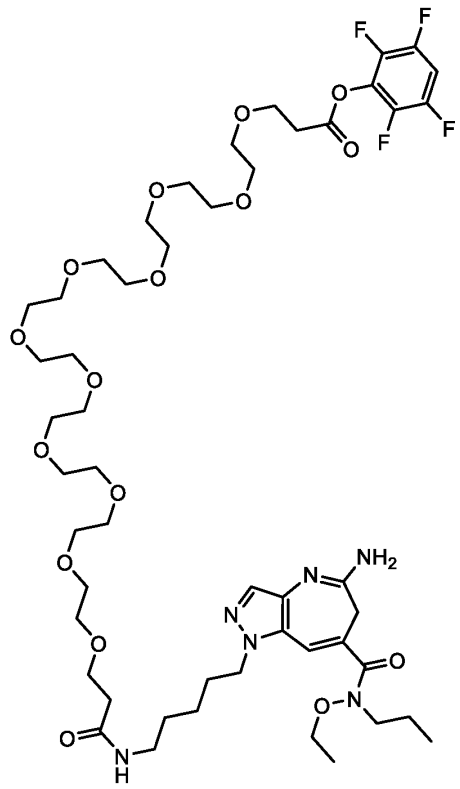
An exemplary embodiment of the pyrazoloazepine-linker compound is selected from Tables 2a and 2b. Each compound was characterized by mass spectrometry and shown to have

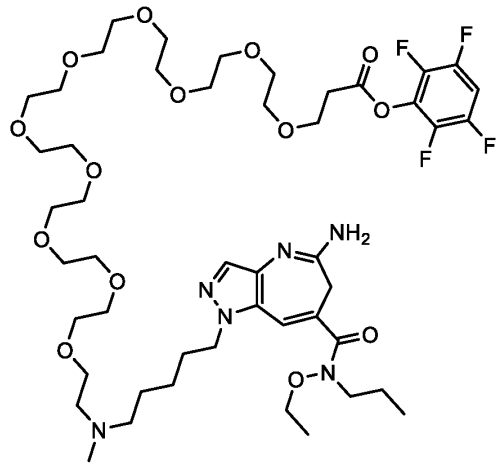
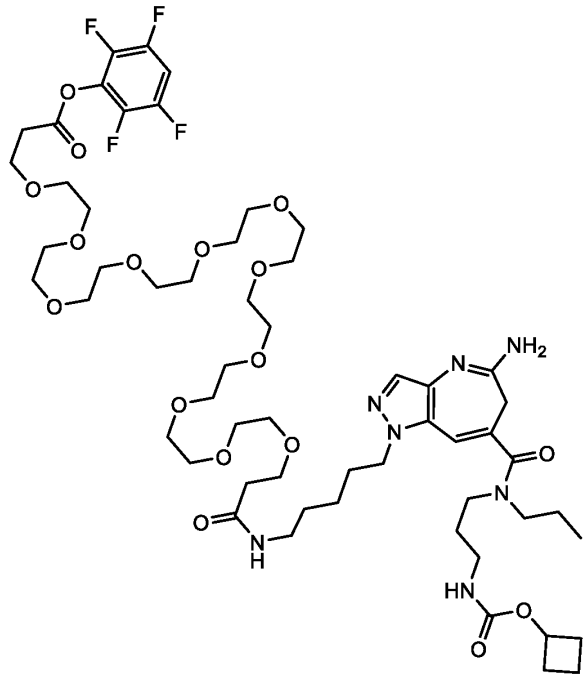
the mass indicated. The pyrazoloazepine-linker compounds of Tables 2a and 2b demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

Table 2a Pyrazoloazepine-linker Formula II compounds (PAZ-L)

PAZ-L No.	Structure	MW
PAZ-L-1		1035.2
PAZ-L-2		1035.2

PAZ-L-3		1049.2
PAZ-L-4		1148.3

PAZ-L-5		1148.3
PAZ-L-6		1095.2

PAZ-L-7		1037.1
PAZ-L-8		1206.3

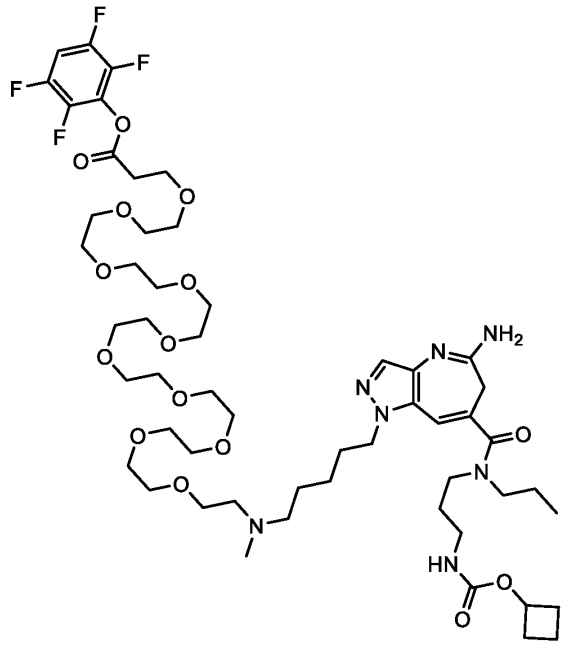
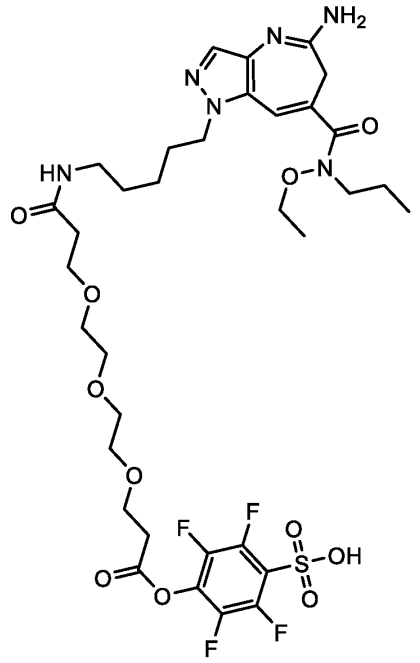
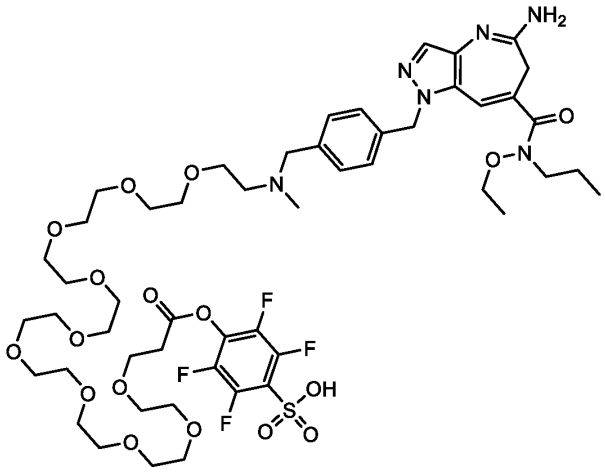
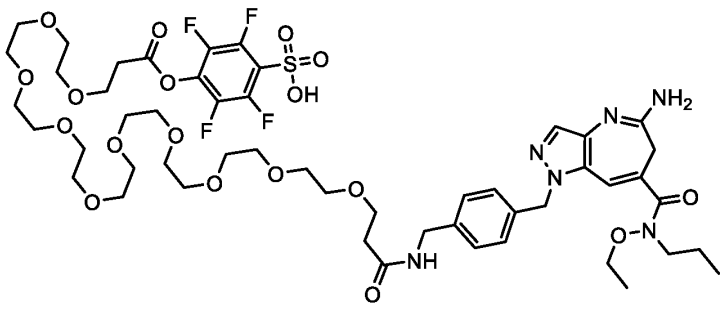
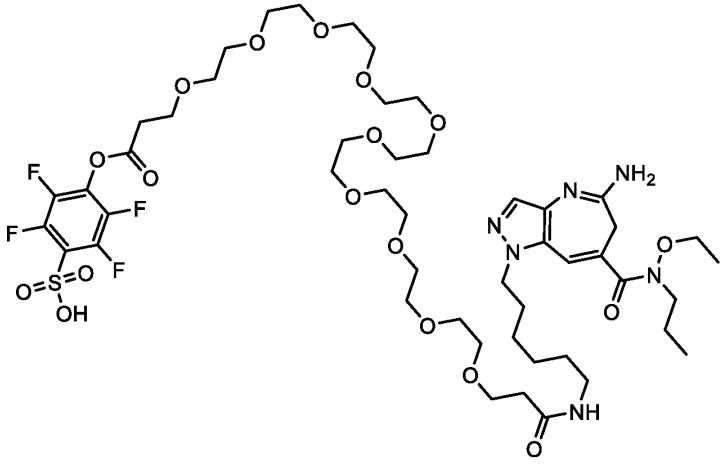
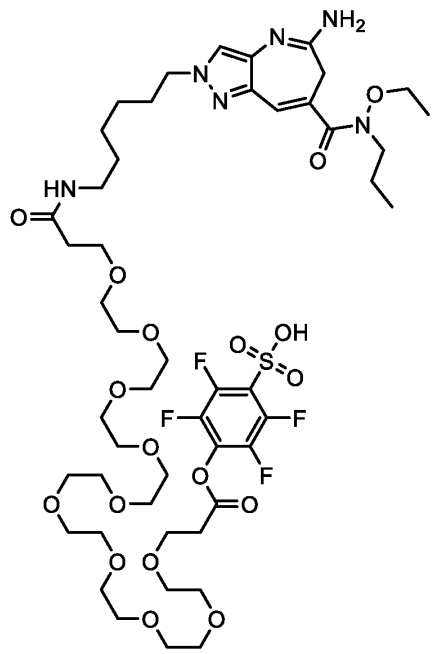
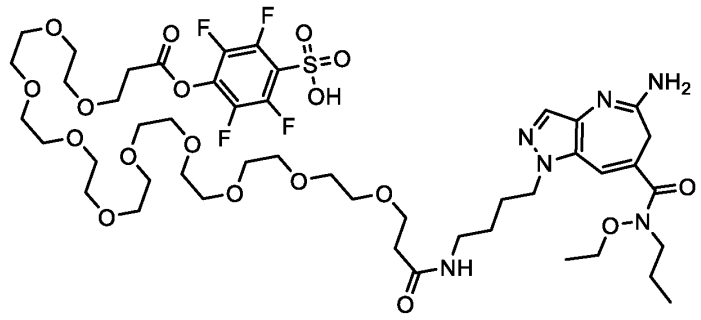
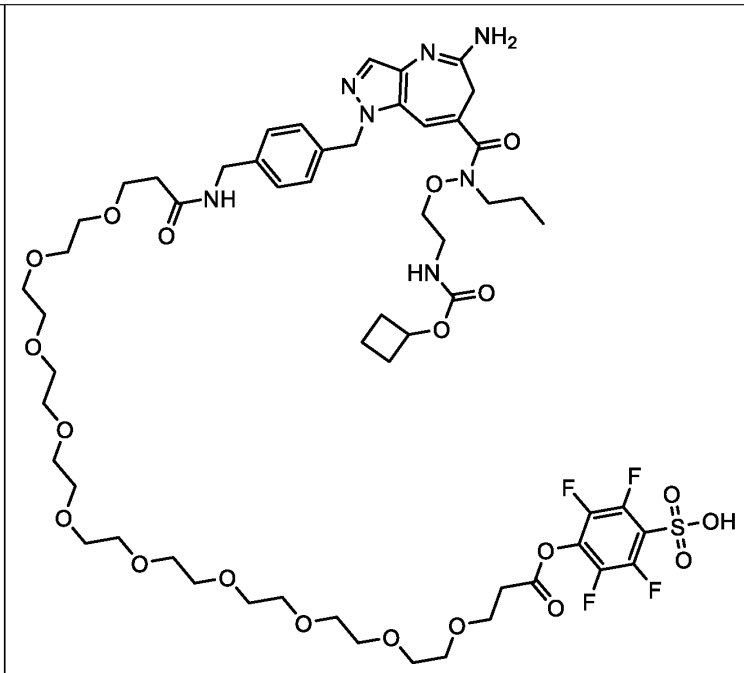
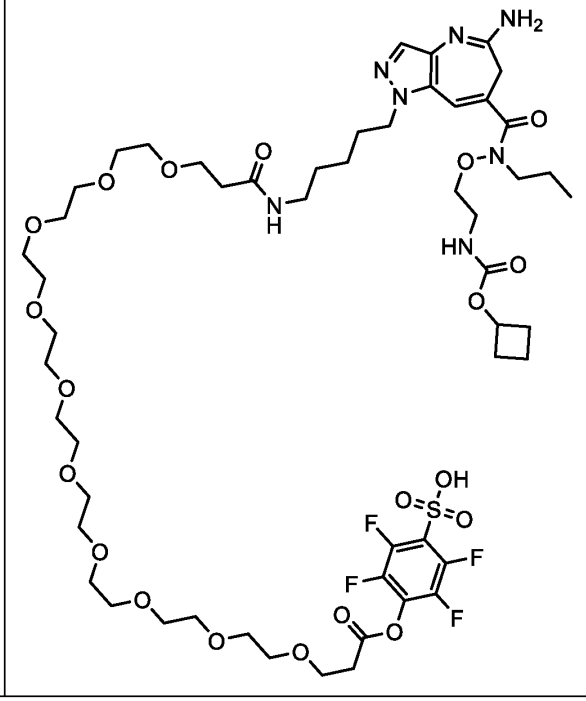
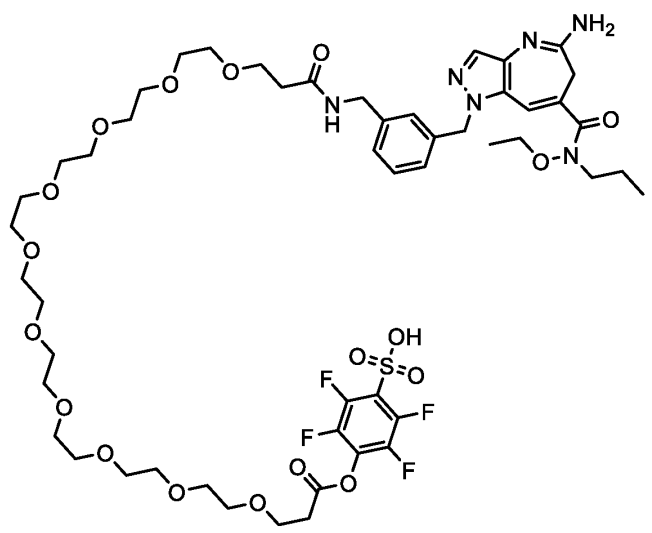
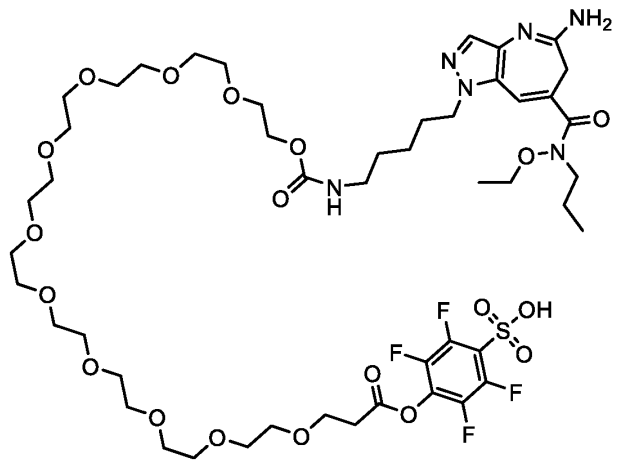
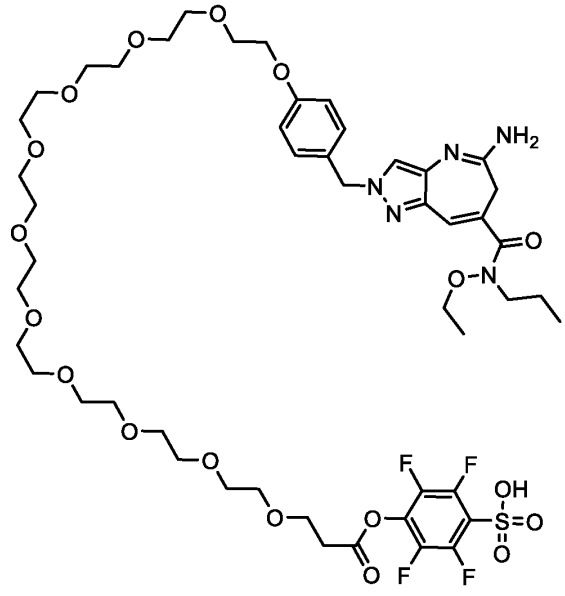
PAZ-L-9		1148.3
PAZ-L-10		822.8

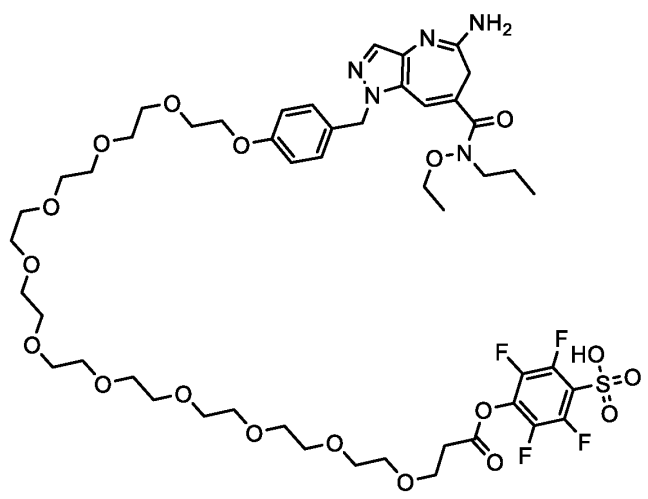
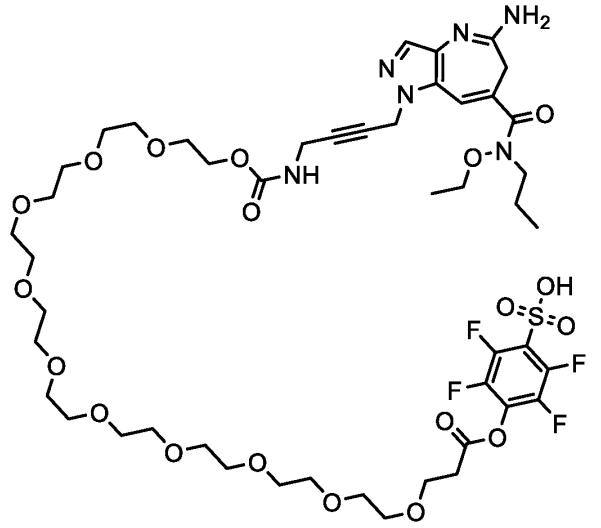
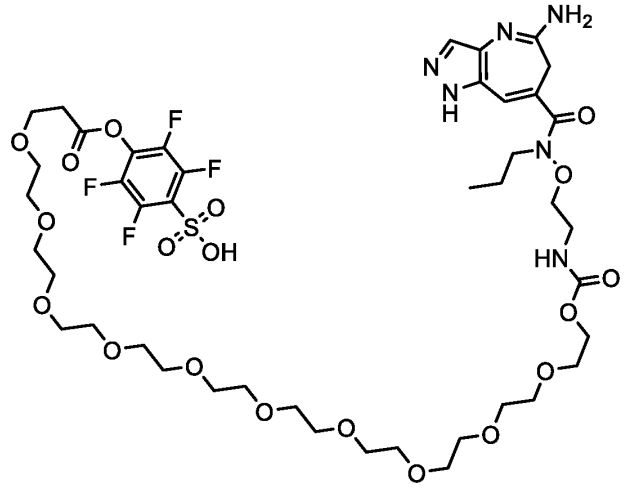
Table 2b Pyrazoloazepine-linker Formula II compounds (PAZ-L)

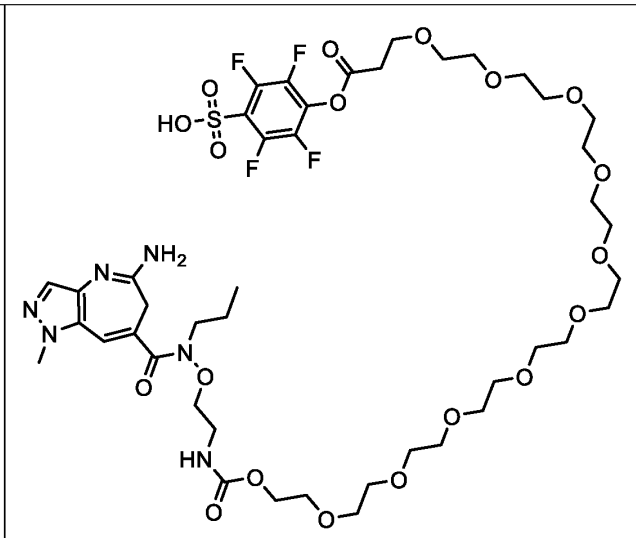
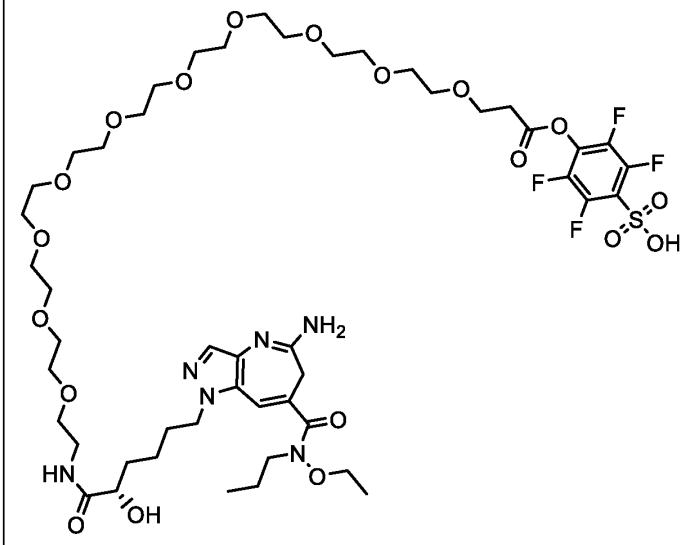
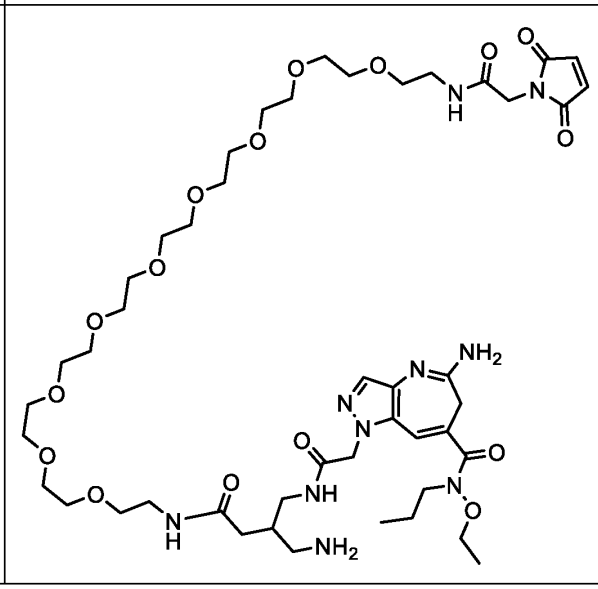
PAZ-L No.	Structure	MW
PAZ-L-11		1151.2
PAZ-L-12		1165.2
PAZ-L-13		1145.2

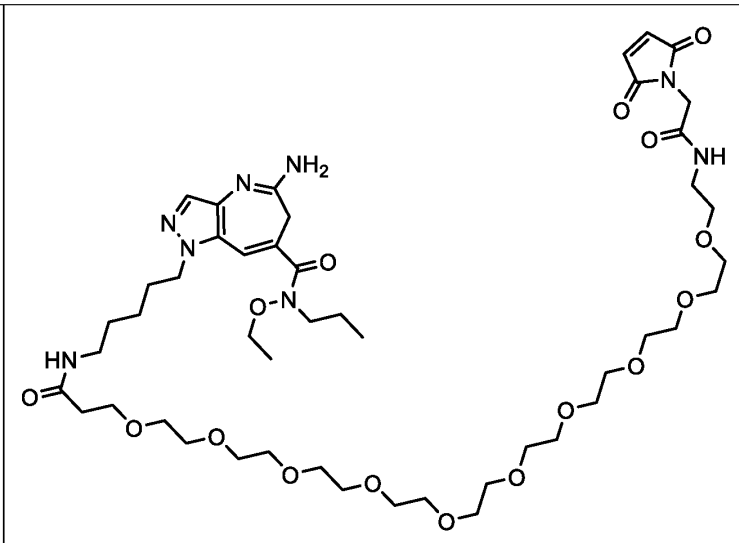
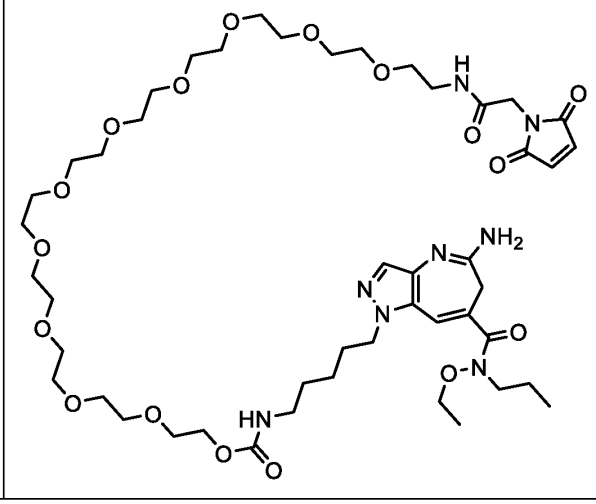
PAZ-L-14		1145.2
PAZ-L-15		1117.2

PAZ-L-16		1278.3
PAZ-L-17		1244.3

PAZ-L-18		1165.2
PAZ-L-19		1147.2
PAZ-L-20		1124.2

PAZ-L-21		1124.2
PAZ-L-22		1129.1
PAZ-L-23		1077.1

PAZ-L-24		1091.1
PAZ-L-25		1147.2
PAZ-L-26		1025.2

PAZ-L-27		1011.2
PAZ-L-28		1027.2

IMMUNOCONJUGATES

Exemplary embodiments of immunoconjugates comprise an antibody covalently attached to one or more 5-aminopyrazoloazepine (PAZ) moieties by a linker, and having Formula I:

$$Ab–[L–PAZ]_p \qquad I$$

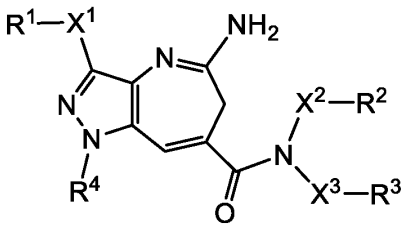
or a pharmaceutically acceptable salt thereof,

wherein:

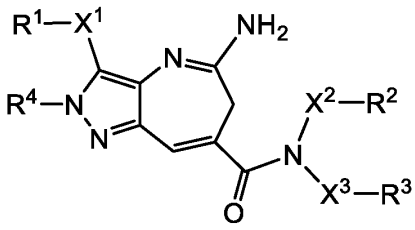
Ab is the antibody;

p is an integer from 1 to 8;

PAZ is the 5-aminopyrazoloazepine moiety selected from formulas IIa and IIb:



IIa



IIb;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, C(=O), C(=O)N(R^5), O, N(R^5), S, S(O)$_2$, and S(O)$_2$N(R^5);

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} carbocyclyl, C_6-C_{20} aryl, C_2-C_9 heterocyclyl, and C_1-C_{20} heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–OR^5;

–(C_3-C_{12} carbocyclyl);

–(C_3-C_{12} carbocyclyl)–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_3-C_{12} carbocyclyl)–NR^5–C(=NR^5)NR^5–*;

–(C_6-C_{20} aryl);

–(C_6-C_{20} aryldiyl)–*;

–(C_6-C_{20} aryldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–C(=NR^{5a})N(R^5)–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyl)–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_2-C_9 heterocyclyl)–C(=O)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_2-C_9 heterocyclyl)–NR^5–C(=NR^{5a})NR^5–*;

–(C_2-C_9 heterocyclyl)–NR^5–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_2-C_9 heterocyclyl)–(C_6-C_{20} aryldiyl)–*;

–(C_1-C_{20} heteroaryl);

–(C_1-C_{20} heteroaryldiyl)–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{20} heteroaryldiyl)–NR^5–$C(=NR^{5a})N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–$N(R^5)C(=O)$–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–*;

–C(=O)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–$C(=O)N(R^5)_2$;

–C(=O)$N(R^5)$–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$C(=O)N(R^5)$–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)R^5$;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)N(R^5)_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–$N(R^5)CO_2R^5$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=NR^{5a})N(R^5)_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^{5a})R^5$;

–C(=O)NR^5–(C_1-C_8 alkyldiyl)–NR^5(C_2-C_5 heteroaryl);

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–$N(R^5)$–*;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–*;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–NR^5–*;

–$N(R^5)_2$;

–$N(R^5)$–*;

–$N(R^5)C(=O)R^5$;

–$N(R^5)C(=O)$–*;

–$N(R^5)C(=O)N(R^5)_2$;

–$N(R^5)C(=O)N(R^5)$–*;

–$N(R^5)CO_2R^5$;

–$N(R^5)CO_2(R^5)$–*;

$-NR^5C(=NR^{5a})N(R^5)_2$;

$-NR^5C(=NR^{5a})N(R^5)-*$;

$-NR^5C(=NR^{5a})R^5$;

$-N(R^5)C(=O)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)-*$;

$-N(R^5)-(C_2\text{-}C_5$ heteroaryl);

$-N(R^5)-S(=O)_2-(C_1\text{-}C_{12}$ alkyl);

$-O-(C_1\text{-}C_{12}$ alkyl);

$-O-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-O-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)-*$;

$-OC(=O)N(R^5)_2$;

$-OC(=O)N(R^5)-*$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-*$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$;

or R^2 and R^3 together form a 5- or 6-membered heterocyclyl ring;

R^5 is selected from the group consisting of H, $C_6\text{-}C_{20}$ aryl, $C_3\text{-}C_{12}$ carbocyclyl, $C_2\text{-}C_{20}$ heterocyclyl, $C_6\text{-}C_{20}$ aryldiyl, $C_1\text{-}C_{12}$ alkyl, and $C_1\text{-}C_{12}$ alkyldiyl, or two R^5 groups together form a 5- or 6-membered heterocyclyl ring;

R^{5a} is selected from the group consisting of $C_6\text{-}C_{20}$ aryl and $C_1\text{-}C_{20}$ heteroaryl;

where the asterisk * indicates the attachment site of L, and where one of R^1, R^2, R^3 and R^4 is attached to L;

L is the linker selected from the group consisting of:

$-C(=O)-PEG-$;

$-C(=O)-PEG-C(=O)N(R^6)-(C_1\text{-}C_{12}$ alkyldiyl$)-C(=O)-Gluc-$;

$-C(=O)-PEG-O-$;

$-C(=O)-PEG-O-C(=O)-$;

$-C(=O)-PEG-C(=O)-$;

$-C(=O)-PEG-C(=O)-PEP-$;

$-C(=O)-PEG-N(R^6)-$;

$-C(=O)-PEG-N(R^6)-C(=O)-$;

$-C(=O)-PEG-N(R^6)-PEG-C(=O)-PEP-$;

$-C(=O)-PEG-N^+(R^6)_2-PEG-C(=O)-PEP-$;

–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–C(=O)–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^5)–C(=O);

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–O–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–O–C(=O)–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–C(=O)–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–N(R^5)–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–N(R^5)–C(=O)–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–C(=O)–PEP–;

–succinimidyl–$(CH_2)_m$–C(=O)N(R^6)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

–succinimidyl–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

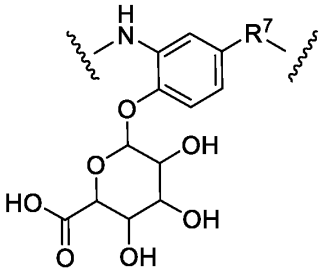
–succinimidyl–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–; and

–succinimidyl–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

R^6 is independently H or C_1-C_6 alkyl;

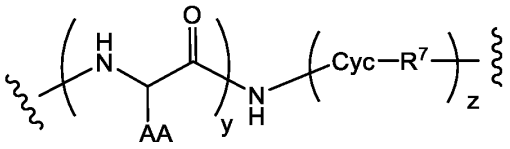
PEG has the formula: $–(CH_2CH_2O)_n–(CH_2)_m–$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:



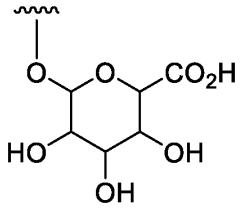
;

PEP has the formula:



where AA is independently selected from a natural or unnatural amino acid side chain, or one or more of AA, and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

Cyc is selected from C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl, optionally substituted with one or more groups selected from F, Cl, NO_2, –OH, $–OCH_3$, and a glucuronic acid having the structure:



;

R^7 is selected from the group consisting of $–CH(R^8)O–$, $–CH_2–$, $–CH_2N(R^8)–$, and $–CH(R^8)O–C(=O)–$, where R^8 is selected from H, C_1-C_6 alkyl, $C(=O)–C_1$-C_6 alkyl, and $–C(=O)N(R^9)_2$, where R^9 is independently selected from the group consisting of H, C_1-C_{12} alkyl, and $–(CH_2CH_2O)_n–(CH_2)_m–OH$, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R^9 groups together form a 5- or 6-membered heterocyclyl ring;

y is an integer from 2 to 12;

z is 0 or 1; and

alkyl, alkyldiyl, alkenyl, alkenyldiyl, alkynyl, alkynyldiyl, aryl, aryldiyl, carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are independently and optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $–CH_3$, $–CH_2CH_3$, $–CH=CH_2$, $–C\equiv CH$, $–C\equiv CCH_3$, $–CH_2CH_2CH_3$, $–CH(CH_3)_2$, $–CH_2CH(CH_3)_2$, $–CH_2OH$, $–CH_2OCH_3$, $–CH_2CH_2OH$, $–C(CH_3)_2OH$, $–CH(OH)CH(CH_3)_2$, $–C(CH_3)_2CH_2OH$, $–CH_2CH_2SO_2CH_3$, $–CH_2OP(O)(OH)_2$, $–CH_2F$, $–CHF_2$, $–CF_3$, $–CH_2CF_3$, –

CH_2CHF_2, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NHC(=NH)H$, $-NHC(=NH)CH_3$, $-NHC(=NH)NH_2$, $-NHC(=O)NH_2$, $-NO_2$, =O, $-OH$, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds PD-L1.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, and avelumab, or a biosimilar or a biobetter thereof.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds HER2.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is selected from the group consisting of trastuzumab and pertuzumab, or a biosimilar or a biobetter thereof.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds CEA.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is labetuzumab, or a biosimilar or a biobetter thereof.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds Caprin-1.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds TROP2.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is sacituzumab, or a biosimilar or a biobetter thereof.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein X^1 is a bond, and R^1 is H.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein X^2 is a bond, and R^2 is C_1-C_8 alkyl.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein X^2 and X^3 are each a bond, and R^2 and R^3 are independently selected from C_1-C_8 alkyl, $-O-(C_1$-

C_{12} alkyl), –(C_1-C_{12} alkyldiyl)–OR^5, –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^5$, –(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$, –O–(C_1-C_{12} alkyl)–$N(R^5)CO_2R^5$, and –O–(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 is C_1-C_8 alkyl and R^3 is –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^4$.

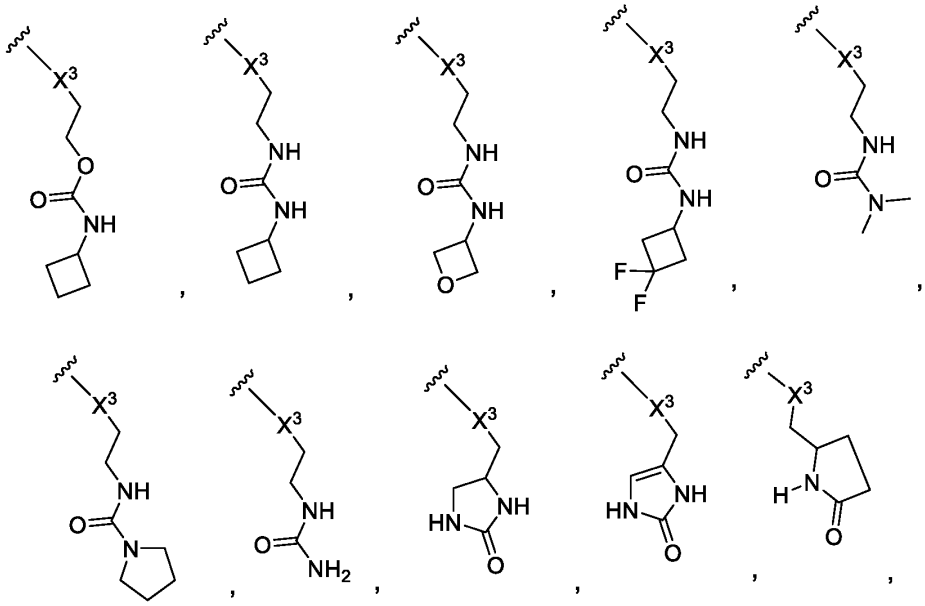
An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 is $–CH_2CH_2CH_3$ and R^3 is selected from $–CH_2CH_2CH_2NHCO_2$(t-Bu), $–OCH_2CH_2NHCO_2$(cyclobutyl), and $–CH_2CH_2CH_2NHCO_2$(cyclobutyl).

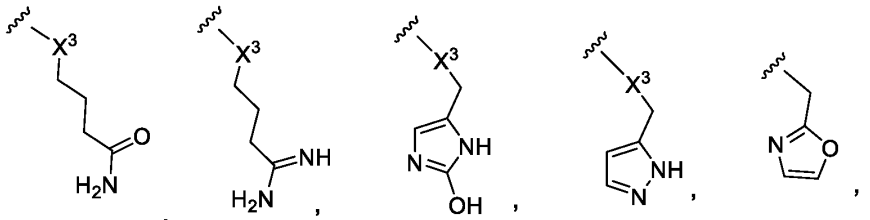
An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 and R^3 are each independently selected from $–CH_2CH_2CH_3$, $–OCH_2CH_3$, $–OCH_2CF_3$, $–CH_2CH_2CF_3$, $–OCH_2CH_2OH$, and $–CH_2CH_2CH_2OH$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 and R^3 are each $–CH_2CH_2CH_3$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 is $–CH_2CH_2CH_3$ and R^3 is $–OCH_2CH_3$.

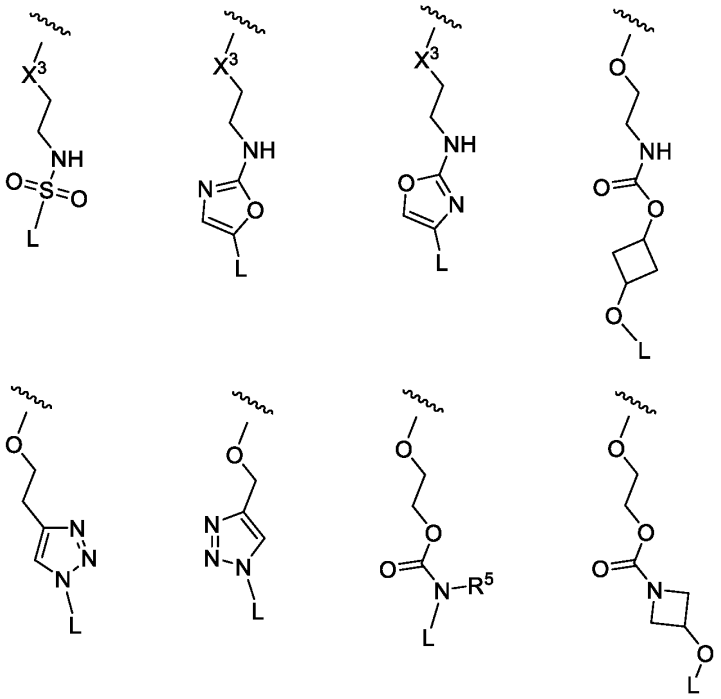
An exemplary embodiment of the immunoconjugate of Formula I includes wherein X^3-R^3 is selected from the group consisting of:

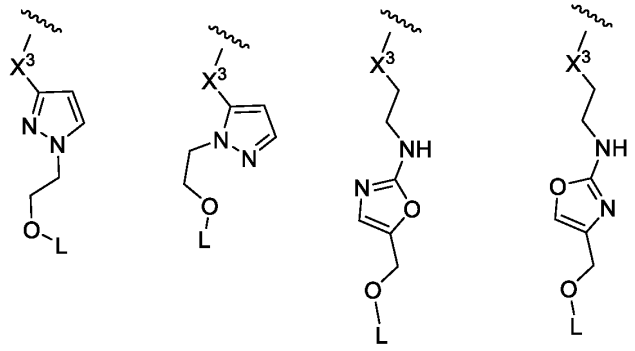




An exemplary embodiment of the immunoconjugate of Formula I includes where R^2 or R^3 is attached to L.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein X^3–R^3–L is selected from the group consisting of:





where the wavy line indicates the point of attachment to N.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^4 is C_1-C_{12} alkyl.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^4 is –(C_1-C_{12} alkyldiyl)–N(R^5)–*; where the asterisk * indicates the attachment site of L.

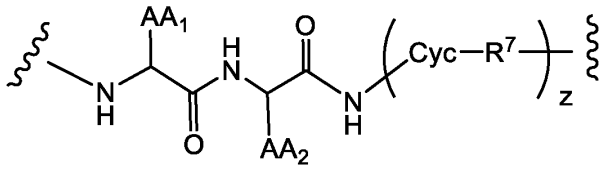
An exemplary embodiment of the immunoconjugate of Formula I includes wherein L is –C(=O)–PEG– or –C(=O)–PEG–C(=O)–.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein L is attached to a cysteine thiol of the antibody.

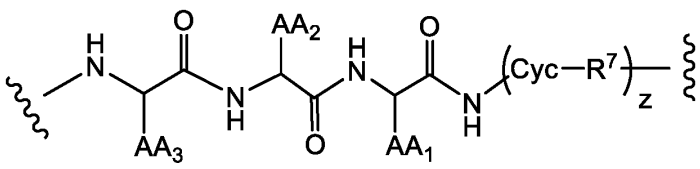
An exemplary embodiment of the immunoconjugate of Formula I includes wherein for the PEG, m is 1 or 2, and n is an integer from 2 to 10.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein for the PEG, n is 10.

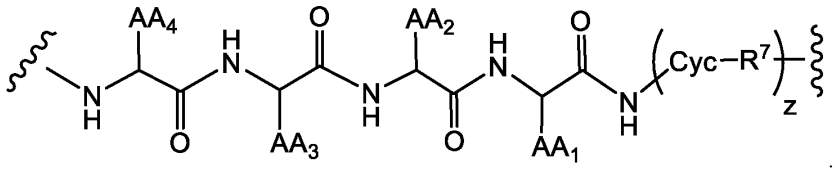
An exemplary embodiment of the immunoconjugate of Formula I includes wherein L comprises PEP and PEP is a dipeptide and has the formula:



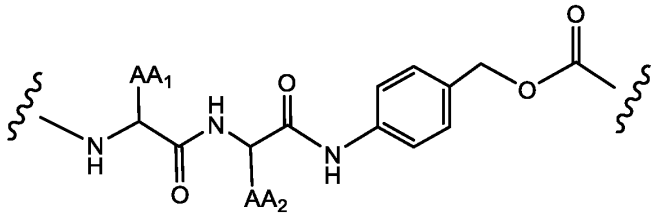
An exemplary embodiment of the immunoconjugate of Formula I includes wherein L comprises PEP and PEP is a tripeptide and has the formula:



An exemplary embodiment of the immunoconjugate of Formula I includes wherein L comprises PEP and PEP is a tetrapeptide and has the formula:



An exemplary embodiment of the immunoconjugate of Formula I includes wherein PEP has the formula:



wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$; or AA_1 and AA_2 form a 5-membered ring proline amino acid.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein A

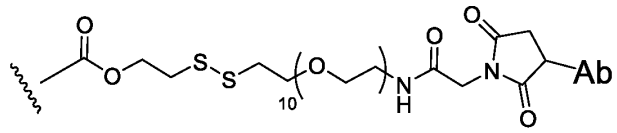
A_1 is selected from the group consisting of Abu, Ala, and Val;

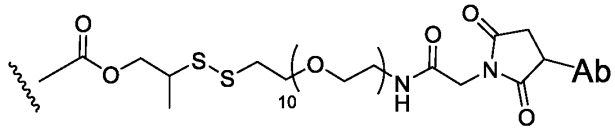
AA_2 is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

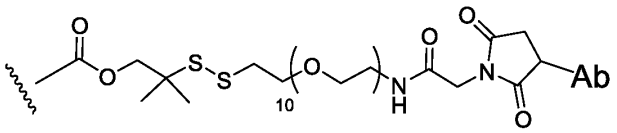
AA_3 is selected from the group consisting of Ala and $Met(O)_2$; and

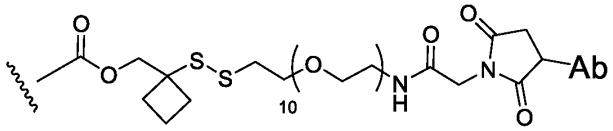
AA_4 is selected from the group consisting of Oic, $Arg(NO_2)$, Bpa, and Nle(O-Bzl).

An exemplary embodiment of the immunoconjugate of Formula I includes wherein L is selected from the structures:



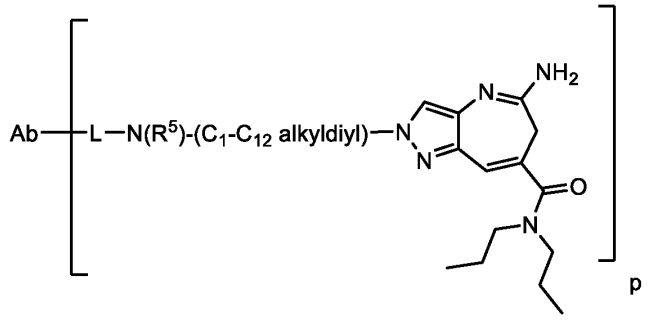




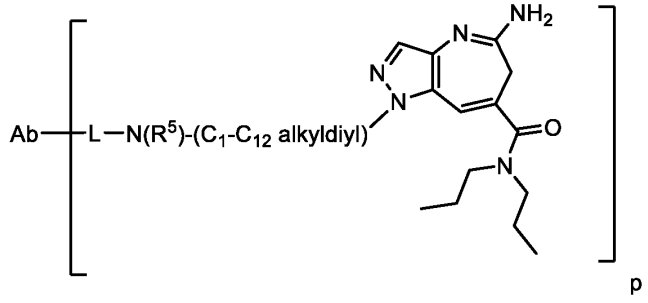


where the wavy line indicates the attachment to R^5.

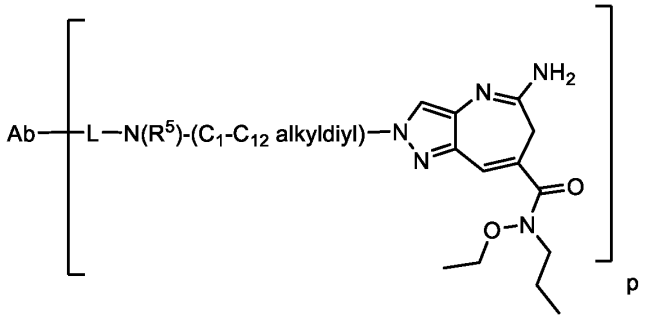
An exemplary embodiment of the immunoconjugate of Formula I is selected from Formulae Ia-Id:



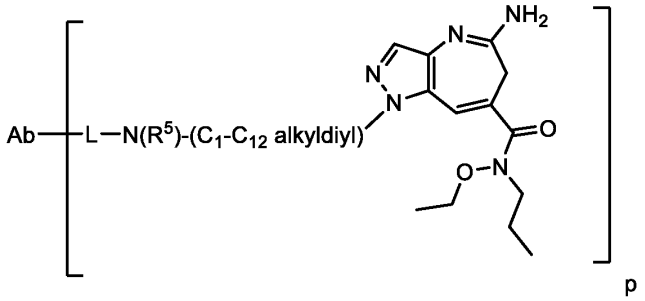
Ia;



Ib;

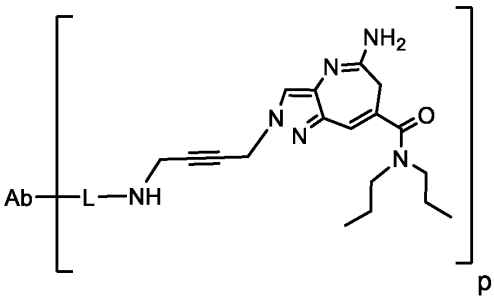


Ic; and

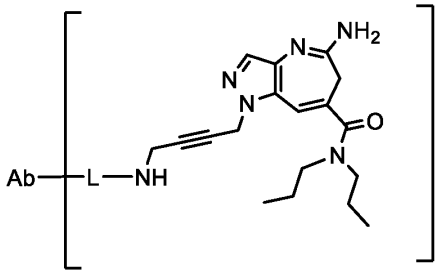


Id.

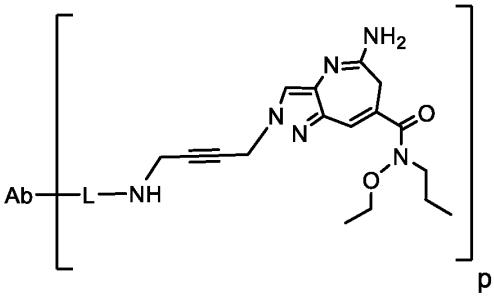
An exemplary embodiment of the immunoconjugate of Formula I is selected from Formulae Ie-Il:



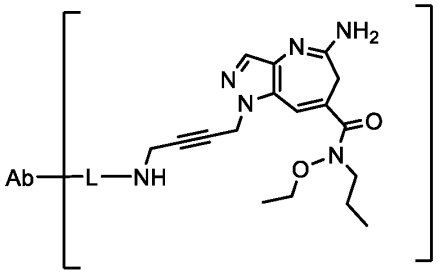
Ie;



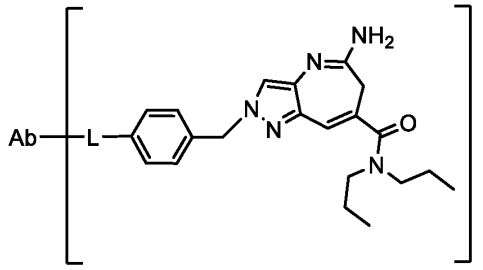
If;



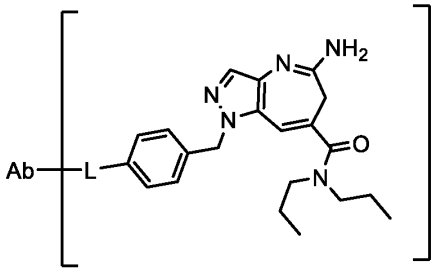
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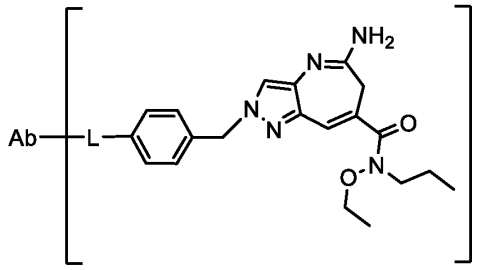
Ih;



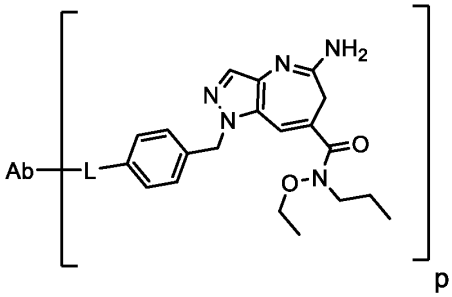
Ii;



Ij;



Ik; and



Il.

The invention includes all reasonable combinations, and permutations of the features, of the Formula I embodiments.

In certain embodiments, the immunoconjugate compounds of the invention include those with immunostimulatory activity. The antibody-drug conjugates of the invention selectively deliver an effective dose of a pyrazoloazepine drug to tumor tissue, whereby greater selectivity (*i.e.*, a lower efficacious dose) may be achieved while increasing the therapeutic index ("therapeutic window") relative to unconjugated pyrazoloazepine.

Drug loading is represented by p, the number of PAZ moieties per antibody in an immunoconjugate of Formula I. Drug (PAZ) loading may range from 1 to about 8 drug moieties (D) per antibody. Immunoconjugates of Formula I include mixtures or collections of antibodies conjugated with a range of drug moieties, from 1 to about 8. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of reactive or available amino acid side chain residues such as lysine and cysteine. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein. In such aspects, p may be 1, 2, 3, 4, 5, 6, 7, or 8, and ranges thereof, such as from 1 to 8 or from 2 to 5. In any such aspect, p and n are equal (i.e., p = n = 1, 2, 3, 4, 5, 6, 7, or 8, or some range there between). Exemplary immunoconjugates of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al. (2012) *Methods in Enzym.* 502:123-138). In some embodiments, one or more free cysteine residues are already present in an antibody forming intrachain disulfide bonds, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the

antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

For some immunoconjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments described herein, an antibody may have only one or a limited number of cysteine thiol groups, or may have only one or a limited number of sufficiently reactive thiol groups, to which the drug may be attached. In other embodiments, one or more lysine amino groups in the antibody may be available and reactive for conjugation with an PAZ-linker compound of Formula II. In certain embodiments, higher drug loading, *e.g.* p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an immunoconjugate ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

The loading (drug/antibody ratio) of an immunoconjugate may be controlled in different ways, and for example, by: (i) limiting the molar excess of the PAZ-linker intermediate compound relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive denaturing conditions for optimized antibody reactivity.

It is to be understood that where more than one nucleophilic group of the antibody reacts with a drug, then the resulting product is a mixture of immunoconjugate compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual immunoconjugate molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, *e.g.* hydrophobic interaction chromatography (*see, e.g.*, McDonagh et al. (2006) *Prot. Engr. Design & Selection* 19(7):299-307; Hamblett et al. (2004) *Clin. Cancer Res.* 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous immunoconjugate with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

An exemplary embodiment of the immunoconjugate of Formula **I** is selected from the Tables 3a and 3b Immunoconjugates. Immunoconjugates of Tables 3a and 3b were tested utilizing methods described in Example 203 with the majority demonstrating activity.

Table 3a Immunoconjugates (IC)

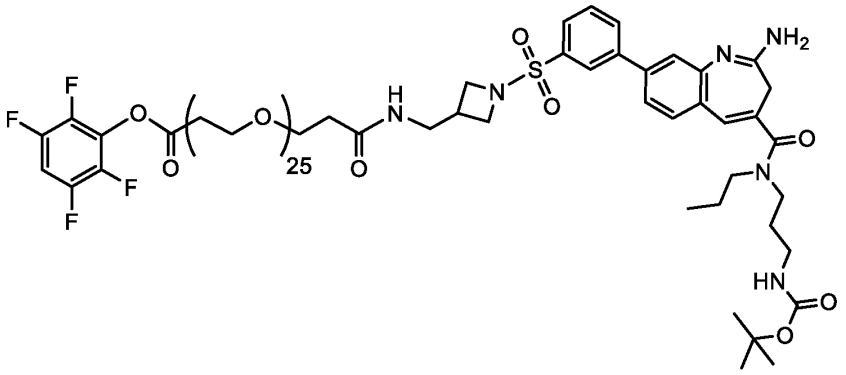
Immunoconjugate No.	PAZ-linker Table 2a	Ab Antigen	DAR	cDC Activation (TNFα Secretion) – EC_{50} (nM)
IC-1	PAZ-L-1	trastuzumab HER2	2.00	
IC-2	PAZ-L-2	trastuzumab HER2	2.76	
IC-3	PAZ-L-6	trastuzumab HER2	2.43	1.6 nM
IC-4	PAZ-L-7	trastuzumab HER2	2.25	
IC-5	PAZ-L-3	trastuzumab HER2	3.52	
IC-6	PAZ-L-10	trastuzumab HER2	2.20	

Table 3b Immunoconjugates (IC)

Immunoconjugate No.	PAZ-linker Table 2b	Ab Antigen	DAR	cDC Activation (TNFα Secretion) – EC_{50} (nM)
IC-7	PAZ-L-12	trastuzumab HER2	2.37	1.0 nM
IC-8	PAZ-L-13	trastuzumab HER2	2.03	
IC-9	PAZ-L-15	trastuzumab HER2	2.4	
IC-10	PAZ-L-17	trastuzumab HER2	2.35	
IC-11	PAZ-L-16	trastuzumab HER2	2.22	
IC-12	PAZ-L-14	trastuzumab	2.29	

		HER2		
IC-13	PAZ-L-17	9-G1fhL2 CEA	2.29	
IC-14	PAZ-L-18	trastuzumab HER2	2.42	
IC-15	PAZ-L-23	trastuzumab HER2	2.39	
IC-16	PAZ-L-20	trastuzumab HER2	2.16	
IC-17	PAZ-L-21	trastuzumab HER2	2.30	
IC-18	PAZ-L-22	trastuzumab HER2	2.37	
IC-19	PAZ-L-19	trastuzumab HER2	2.30	
IC-20	PAZ-L-24	trastuzumab HER2	2.45	
IC-21	PAZ-L-25	trastuzumab HER2	2.38	
IC-22	PAZ-L-28	1-G1f TROP2	3.60	1.2 nM
IC-23	PAZ-L-27	1-G1f TROP2	3.48	
IC-24	PAZ-L-26	1-G1f TROP2	2.67	

Comparator immunoconjugate A was prepared by conjugation of anti-HER2 antibody trastuzumab with linker-adjuvant compound:



COMPOSITIONS OF IMMUNOCONJUGATES

The invention provides a composition, e.g., a pharmaceutically or pharmacologically acceptable composition or formulation, comprising a plurality of immunoconjugates as described herein and optionally a carrier therefor, e.g., a pharmaceutically or pharmacologically acceptable carrier. The immunoconjugates can be the same or different in the composition, i.e., the composition can comprise immunoconjugates that have the same number of adjuvants linked to the same positions on the antibody construct and/or immunoconjugates that have the same number of PAZ adjuvants linked to different positions on the antibody construct, that have different numbers of adjuvants linked to the same positions on the antibody construct, or that have different numbers of adjuvants linked to different positions on the antibody construct.

In an exemplary embodiment, a composition comprising the immunoconjugate compounds comprises a mixture of the immunoconjugate compounds, wherein the average drug (PAZ) loading per antibody in the mixture of immunoconjugate compounds is about 2 to about 5.

A composition of immunoconjugates of the invention can have an average adjuvant to antibody construct ratio (DAR) of about 0.4 to about 10. A skilled artisan will recognize that the number of pyrazoloazepine adjuvants conjugated to the antibody construct may vary from immunoconjugate to immunoconjugate in a composition comprising multiple immunoconjugates of the invention and thus the adjuvant to antibody construct (e.g., antibody) ratio can be measured as an average which may be referred to as the drug to antibody ratio (DAR). The adjuvant to antibody construct (e.g., antibody) ratio can be assessed by any suitable means, many of which are known in the art.

The average number of adjuvant moieties per antibody (DAR) in preparations of immunoconjugates from conjugation reactions may be characterized by conventional means such as mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of immunoconjugates in a composition in terms of p may also be determined. In some instances,

separation, purification, and characterization of homogeneous immunoconjugates where p is a certain value from immunoconjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

In some embodiments, the composition further comprises one or more pharmaceutically or pharmacologically acceptable excipients. For example, the immunoconjugates of the invention can be formulated for parenteral administration, such as IV administration or administration into a body cavity or lumen of an organ. Alternatively, the immunoconjugates can be injected intra-tumorally. Compositions for injection will commonly comprise a solution of the immunoconjugate dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and an isotonic solution of one or more salts such as sodium chloride, e.g., Ringer's solution. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed, including synthetic monoglycerides or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These compositions desirably are sterile and generally free of undesirable matter. These compositions can be sterilized by conventional, well known sterilization techniques. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

The composition can contain any suitable concentration of the immunoconjugate. The concentration of the immunoconjugate in the composition can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. In certain embodiments, the concentration of an immunoconjugate in a solution formulation for injection will range from about 0.1% (w/w) to about 10% (w/w).

METHOD OF TREATING CANCER WITH IMMUNOCONJUGATES

The invention provides a method for treating cancer. The method includes administering a therapeutically effective amount of an immunoconjugate as described herein (e.g., as a composition as described herein) to a subject in need thereof, e.g., a subject that has cancer and is in need of treatment for the cancer. The method includes administering a therapeutically effective amount of an immunoconjugate (IC) selected from Tables 3a and 3b.

It is contemplated that the immunoconjugate of the present invention may be used to treat various hyperproliferative diseases or disorders, e.g. characterized by the overexpression of

a tumor antigen. Exemplary hyperproliferative disorders include benign or malignant solid tumors and hematological disorders such as leukemia and lymphoid malignancies.

In another aspect, an immunoconjugate for use as a medicament is provided. In certain embodiments, the invention provides an immunoconjugate for use in a method of treating an individual comprising administering to the individual an effective amount of the immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described herein.

In a further aspect, the invention provides for the use of an immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer, the method comprising administering to an individual having cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described herein.

Carcinomas are malignancies that originate in the epithelial tissues. Epithelial cells cover the external surface of the body, line the internal cavities, and form the lining of glandular tissues. Examples of carcinomas include, but are not limited to, adenocarcinoma (cancer that begins in glandular (secretory) cells such as cancers of the breast, pancreas, lung, prostate, stomach, gastroesophageal junction, and colon) adrenocortical carcinoma; hepatocellular carcinoma; renal cell carcinoma; ovarian carcinoma; carcinoma in situ; ductal carcinoma; carcinoma of the breast; basal cell carcinoma; squamous cell carcinoma; transitional cell carcinoma; colon carcinoma; nasopharyngeal carcinoma; multilocular cystic renal cell carcinoma; oat cell carcinoma; large cell lung carcinoma; small cell lung carcinoma; non-small cell lung carcinoma; and the like. Carcinomas may be found in prostrate, pancreas, colon, brain (usually as secondary metastases), lung, breast, and skin. In some embodiments, methods for treating non-small cell lung carcinoma include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, methods for treating breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, methods for treating triple-negative breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof).

Soft tissue tumors are a highly diverse group of rare tumors that are derived from connective tissue. Examples of soft tissue tumors include, but are not limited to, alveolar soft part sarcoma; angiomatoid fibrous histiocytoma; chondromyoxid fibroma; skeletal chondrosarcoma; extraskeletal myxoid chondrosarcoma; clear cell sarcoma; desmoplastic small round-cell tumor; dermatofibrosarcoma protuberans; endometrial stromal tumor; Ewing's sarcoma; fibromatosis (Desmoid); fibrosarcoma, infantile; gastrointestinal stromal tumor; bone giant cell tumor; tenosynovial giant cell tumor; inflammatory myofibroblastic tumor; uterine leiomyoma; leiomyosarcoma; lipoblastoma; typical lipoma; spindle cell or pleomorphic lipoma; atypical lipoma; chondroid lipoma; well-differentiated liposarcoma; myxoid/round cell liposarcoma; pleomorphic liposarcoma; myxoid malignant fibrous histiocytoma; high-grade malignant fibrous histiocytoma; myxofibrosarcoma; malignant peripheral nerve sheath tumor; mesothelioma; neuroblastoma; osteochondroma; osteosarcoma; primitive neuroectodermal tumor; alveolar rhabdomyosarcoma; embryonal rhabdomyosarcoma; benign or malignant schwannoma; synovial sarcoma; Evan's tumor; nodular fasciitis; desmoid-type fibromatosis; solitary fibrous tumor; dermatofibrosarcoma protuberans (DFSP); angiosarcoma; epithelioid hemangioendothelioma; tenosynovial giant cell tumor (TGCT); pigmented villonodular synovitis (PVNS); fibrous dysplasia; myxofibrosarcoma; fibrosarcoma; synovial sarcoma; malignant peripheral nerve sheath tumor; neurofibroma; pleomorphic adenoma of soft tissue; and neoplasias derived from fibroblasts, myofibroblasts, histiocytes, vascular cells/endothelial cells, and nerve sheath cells.

A sarcoma is a rare type of cancer that arises in cells of mesenchymal origin, e.g., in bone or in the soft tissues of the body, including cartilage, fat, muscle, blood vessels, fibrous tissue, or other connective or supportive tissue. Different types of sarcoma are based on where the cancer forms. For example, osteosarcoma forms in bone, liposarcoma forms in fat, and rhabdomyosarcoma forms in muscle. Examples of sarcomas include, but are not limited to, askin's tumor; sarcoma botryoides; chondrosarcoma; ewing's sarcoma; malignant hemangioendothelioma; malignant schwannoma; osteosarcoma; and soft tissue sarcomas (e.g., alveolar soft part sarcoma; angiosarcoma; cystosarcoma phyllodesdermatofibrosarcoma protuberans (DFSP); desmoid tumor; desmoplastic small round cell tumor; epithelioid sarcoma; extraskeletal chondrosarcoma; extraskeletal osteosarcoma; fibrosarcoma; gastrointestinal stromal tumor (GIST); hemangiopericytoma; hemangiosarcoma (more commonly referred to as "angiosarcoma"); kaposi's sarcoma; leiomyosarcoma; liposarcoma; lymphangiosarcoma; malignant peripheral nerve sheath tumor (MPNST); neurofibrosarcoma; synovial sarcoma; and undifferentiated pleomorphic sarcoma).

A teratoma is a type of germ cell tumor that may contain several different types of tissue (e.g., can include tissues derived from any and/or all of the three germ layers: endoderm, mesoderm, and ectoderm), including, for example, hair, muscle, and bone. Teratomas occur most often in the ovaries in women, the testicles in men, and the tailbone in children.

Melanoma is a form of cancer that begins in melanocytes (cells that make the pigment melanin). Melanoma may begin in a mole (skin melanoma), but can also begin in other pigmented tissues, such as in the eye or in the intestines.

Merkel cell carcinoma is a rare type of skin cancer that usually appears as a flesh-colored or bluish-red nodule on the face, head or neck. Merkel cell carcinoma is also called neuroendocrine carcinoma of the skin. In some embodiments, methods for treating Merkel cell carcinoma include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, the Merkel cell carcinoma has metastasized when administration occurs.

Leukemias are cancers that start in blood-forming tissue, such as the bone marrow, and cause large numbers of abnormal blood cells to be produced and enter the bloodstream. For example, leukemias can originate in bone marrow-derived cells that normally mature in the bloodstream. Leukemias are named for how quickly the disease develops and progresses (e.g., acute versus chronic) and for the type of white blood cell that is affected (e.g., myeloid versus lymphoid). Myeloid leukemias are also called myelogenous or myeloblastic leukemias. Lymphoid leukemias are also called lymphoblastic or lymphocytic leukemia. Lymphoid leukemia cells may collect in the lymph nodes, which can become swollen. Examples of leukemias include, but are not limited to, Acute myeloid leukemia (AML), Acute lymphoblastic leukemia (ALL), Chronic myeloid leukemia (CML), and Chronic lymphocytic leukemia (CLL).

Lymphomas are cancers that begin in cells of the immune system. For example, lymphomas can originate in bone marrow-derived cells that normally mature in the lymphatic system. There are two basic categories of lymphomas. One category of lymphoma is Hodgkin lymphoma (HL), which is marked by the presence of a type of cell called the Reed-Sternberg cell. There are currently 6 recognized types of HL. Examples of Hodgkin lymphomas include nodular sclerosis classical Hodgkin lymphoma (CHL), mixed cellularity CHL, lymphocyte-depletion CHL, lymphocyte-rich CHL, and nodular lymphocyte predominant HL.

The other category of lymphoma is non-Hodgkin lymphomas (NHL), which includes a large, diverse group of cancers of immune system cells. Non-Hodgkin lymphomas can be further divided into cancers that have an indolent (slow-growing) course and those that have an aggressive (fast-growing) course. There are currently 61 recognized types of NHL. Examples of

non-Hodgkin lymphomas include, but are not limited to, AIDS-related Lymphomas, anaplastic large-cell lymphoma, angioimmunoblastic lymphoma, blastic NK-cell lymphoma, Burkitt's lymphoma, Burkitt-like lymphoma (small non-cleaved cell lymphoma), chronic lymphocytic leukemia/small lymphocytic lymphoma, cutaneous T-Cell lymphoma, diffuse large B-Cell lymphoma, enteropathy-type T-Cell lymphoma, follicular lymphoma, hepatosplenic gamma-delta T-Cell lymphomas, T-Cell leukemias, lymphoblastic lymphoma, mantle cell lymphoma, marginal zone lymphoma, nasal T-Cell lymphoma, pediatric lymphoma, peripheral T-Cell lymphomas, primary central nervous system lymphoma, transformed lymphomas, treatment-related T-Cell lymphomas, and Waldenstrom's macroglobulinemia.

Brain cancers include any cancer of the brain tissues. Examples of brain cancers include, but are not limited to, gliomas (e.g., glioblastomas, astrocytomas, oligodendrogliomas, ependymomas, and the like), meningiomas, pituitary adenomas, and vestibular schwannomas, primitive neuroectodermal tumors (medulloblastomas).

Immunoconjugates of the invention can be used either alone or in combination with other agents in a therapy. For instance, an immunoconjugate may be co-administered with at least one additional therapeutic agent, such as a chemotherapeutic agent. Such combination therapies encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the immunoconjugate can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Immunoconjugates can also be used in combination with radiation therapy.

The immunoconjugates of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof are known to be useful in the treatment of cancer, particularly breast cancer, especially triple negative (test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer, bladder cancer, and Merkel cell carcinoma. The immunoconjugate described herein can be used to treat the same types of cancers as atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof, particularly breast cancer, especially triple negative

(test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer, bladder cancer, and Merkel cell carcinoma.

The immunoconjugate is administered to a subject in need thereof in any therapeutically effective amount using any suitable dosing regimen, such as the dosing regimens utilized for atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof. For example, the methods can include administering the immunoconjugate to provide a dose of from about 100 ng/kg to about 50 mg/kg to the subject. The immunoconjugate dose can range from about 5 mg/kg to about 50 mg/kg, from about 10 μg/kg to about 5 mg/kg, or from about 100 μg/kg to about 1 mg/kg. The immunoconjugate dose can be about 100, 200, 300, 400, or 500 μg/kg. The immunoconjugate dose can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. The immunoconjugate dose can also be outside of these ranges, depending on the particular conjugate as well as the type and severity of the cancer being treated. Frequency of administration can range from a single dose to multiple doses per week, or more frequently. In some embodiments, the immunoconjugate is administered from about once per month to about five times per week. In some embodiments, the immunoconjugate is administered once per week.

In another aspect, the invention provides a method for preventing cancer. The method comprises administering a therapeutically effective amount of an immunoconjugate (e.g., as a composition as described above) to a subject. In certain embodiments, the subject is susceptible to a certain cancer to be prevented. For example, the methods can include administering the immunoconjugate to provide a dose of from about 100 ng/kg to about 50 mg/kg to the subject. The immunoconjugate dose can range from about 5 mg/kg to about 50 mg/kg, from about 10 μg/kg to about 5 mg/kg, or from about 100 μg/kg to about 1 mg/kg. The immunoconjugate dose can be about 100, 200, 300, 400, or 500 μg/kg. The immunoconjugate dose can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. The immunoconjugate dose can also be outside of these ranges, depending on the particular conjugate as well as the type and severity of the cancer being treated. Frequency of administration can range from a single dose to multiple doses per week, or more frequently. In some embodiments, the immunoconjugate is administered from about once per month to about five times per week. In some embodiments, the immunoconjugate is administered once per week.

Some embodiments of the invention provide methods for treating cancer as described above, wherein the cancer is breast cancer. Breast cancer can originate from different areas in the breast, and a number of different types of breast cancer have been characterized. For example, the immunoconjugates of the invention can be used for treating ductal carcinoma *in situ;* invasive ductal carcinoma (*e.g.,* tubular carcinoma; medullary carcinoma; mucinous carcinoma; papillary carcinoma; or cribriform carcinoma of the breast); lobular carcinoma *in*

situ; invasive lobular carcinoma; inflammatory breast cancer; and other forms of breast cancer such as triple negative (test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer. In some embodiments, methods for treating breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding HER2 (e.g. trastuzumab, pertuzumab, biosimilars, or biobetters thereof) and PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars, or biobetters thereof). In some embodiments, methods for treating colon cancer lung cancer, renal cancer, pancreatic cancer, gastric cancer, and esophageal cancer include administering an immunoconjugate containing an antibody construct that is capable of binding CEA, or tumors over-expressing CEA (e.g. labetuzumab, biosimilars, or biobetters thereof).

In some embodiments, the cancer is susceptible to a pro-inflammatory response induced by TLR7 and/or TLR8.

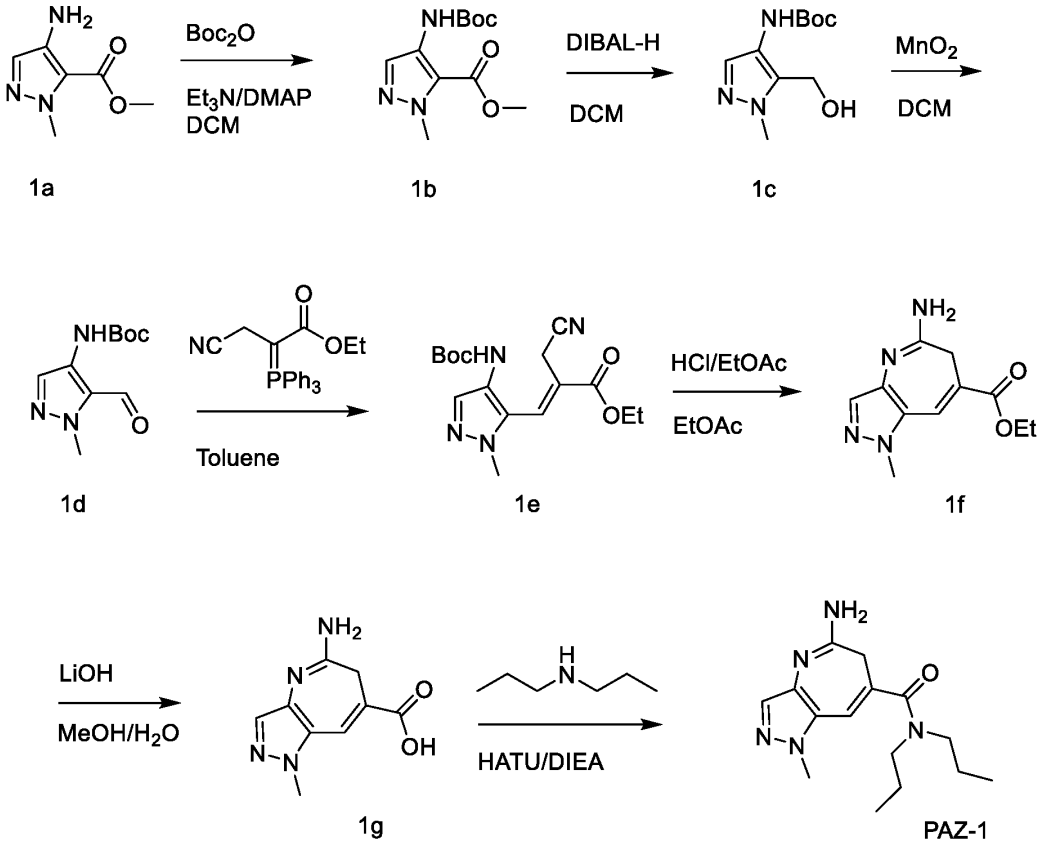
In some embodiments, a therapeutically effective amount of an immunoconjugate is administered to a patient in need to treat cancer wherein the cancer expresses PD-L1, HER2, CEA, or TROP2.

In some embodiments, a therapeutically effective amount of an immunoconjugate is administered to a patient in need to treat cervical cancer, endometrial cancer, ovarian cancer, prostate cancer, pancreatic cancer, esophageal cancer, bladder cancer, urinary tract cancer, urothelial carcinoma, lung cancer, non-small cell lung cancer, Merkel cell carcinoma, colon cancer, colorectal cancer, gastric cancer, or breast cancer. The Merkel cell carcinoma cancer may be metastatic Merkel cell carcinoma. The breast cancer may be triple-negative breast cancer. The esophageal cancer may be gastroesophageal junction adenocarcinoma.

EXAMPLES

Preparation of pyrazoloazepine compounds (PAZ) and intermediates

Example 1 Synthesis of 5-amino-1-methyl-N,N-dipropyl-1,6-dihydropyrazolo[4,3-b]azepine-7-carboxamide, PAZ-1



Preparation of methyl 4-(tert-butoxycarbonylamino)-2-methyl–pyrazole -3-carboxylate, 1b

To a mixture of methyl 4-amino-2-methyl-pyrazole-3-carboxylate, 1a (1 g, 6.45 mmol, 1 eq) in DCM (25 mL) was added TEA (1.96 g, 19.3 mmol, 2.69 mL, 3 eq), DMAP (78.7 mg, 644 umol (micromoles), 0.1 eq) and $(Boc)_2O$ (2.81 g, 12.9 mmol, 2.96 mL, 2 eq) and then stirred at 15°C for 10 h. The mixture was concentrated and purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=1:0 to 1:1) to give 1b (1 g, 3.92 mmol, 60.78% yield) as yellow oil.

Preparation of tert-butyl N-[5-(hydroxymethyl)-1-methyl-pyrazol-4-yl]carbamate, 1c

To a mixture of 1b (800 mg, 3.13 mmol, 1 eq) in DCM (5 mL) was added DIBAL-H (1 M, 12.5 mL, 4 eq) at 0°C and it was stirred at 15°C for 10 h. The reaction mixture was quenched by water (0.5 mL), then dried over Na_2SO_4, and filtered through celite, and the filtrate was concentrated to give 1c (400 mg, 1.76 mmol, 56.2% yield) as yellow oil. LC/MS [M+H] 228.1 (calculated); LC/MS [M+H] 228.0 (observed).

Preparation of tert-butyl N-(5-formyl-1-methyl-pyrazol-4-yl)carbamate, 1d

A mixture of 1c (300 mg, 1.32 mmol, 1 eq) and MnO_2 (1.15 g, 13.2 mmol, 10 eq) in DCM (10 mL) was stirred at 45°C for 23 h. The mixture was filtered through celite, and the

filtrate was concentrated to give 1d (297 mg, 1.32 mmol, 99.9% yield) as yellow oil. ^{1}H NMR (400MHz, $CDCl_3$) δ10.01 (s, 1H), 8.28 (s, 1H), 8.04 (s, 1H), 4.11 (s, 3H), 1.53 (s, 9H)

Preparation of (E)-ethyl 3-(4-((tert-butoxycarbonyl)amino)-1-methyl-1H-pyrazol-5-yl) -2-(cyanomethyl)acrylate, 1e

A mixture of 1d (270 mg, 1.20 mmol, 1 eq) and ethyl 3-cyano-2-(triphenyl-phosphanylidene)propanoate (650 mg, 1.68 mmol, 1.4 eq) in toluene (10 mL) was stirred at 80°C for 10 h. The mixture was concentrated and the crude was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate= 1:0- 1:2) to give 1e (300 mg, 897.21 umol, 74.9 % yield) as yellow oil. LC/MS [M+H] 335.2 (calculated); LC/MS [M+H] 335.2 (observed).

Preparation of ethyl 5-amino -1-methyl -6H-pyrazolo[4,3-b]azepine-7-carboxylate, 1f

A mixture of 1e (280 mg, 837 umol, 1 eq) in HCl/EtOAc (4 M, 5 mL) was stirred at 15°C for 10 min. The mixture was concentrated to give 1f (120 mg, 512 umol, 61.17% yield) as yellow solid.

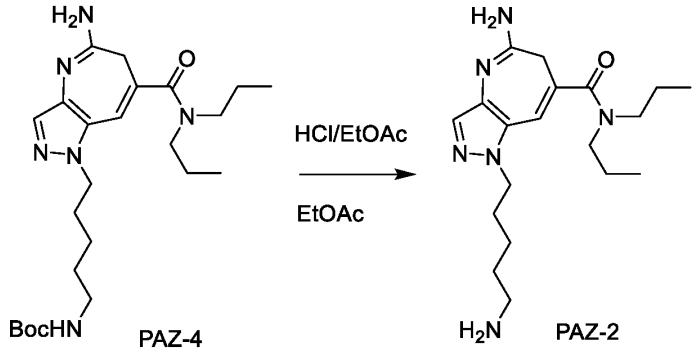
Preparation of 5-amino-1- methyl-6H -pyrazolo[4,3-b] azepine-7-carboxylic acid, 1g

To a mixture of 1f (120 mg, 512 umol, 1 eq) in EtOH (5 mL) and H_2O (1 mL) was added $LiOH.H_2O$ (43 mg, 1.02 mmol, 2 eq) and it was stirred at 25°C for 10 h. The mixture was purified by prep-HPLC(HCl condition: column: Waters Xbridge BEH C18 100*30mm*10um;mobile phase: [water(0.04%HCl)-ACN];B%:1%-20%,9min) to give 1g (90 mg, 436 umol, 85.2 % yield) as yellow solid. LC/MS [M+H] 207.1 (calculated); LC/MS [M+H] 207.1 (observed).

Preparation of 5-amino-1- methyl-6H -pyrazolo[4,3-b] azepine-7-carboxylic acid, PAZ-1

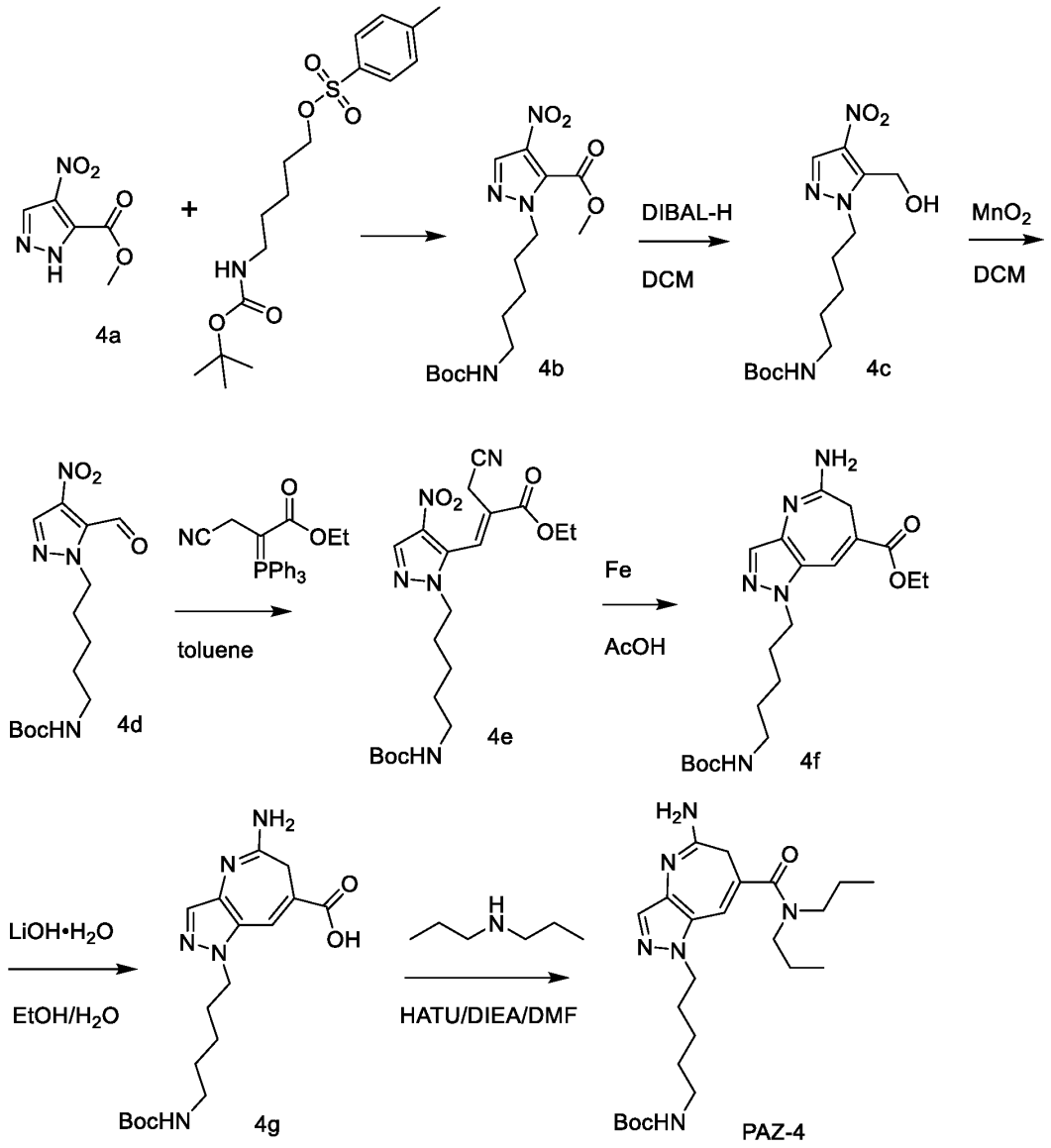
To a mixture of 5-amino-1-methyl-6H-pyrazolo[4,3-b]azepine-7-carboxylic acid (60 mg, 291 umol, 1 eq) in DMF (1 mL) was added 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium, HATU (133 mg, 349 umol, 1.2 eq) and DIEA (188 mg, 1.45 mmol, 253 uL, 5 eq). Then N-propylpropan-1-amine (147 mg, 1.45 mmol, 201 uL, 5 eq) was added into the mixture and it was stirred at 15°C for 10 h. The mixture was concentrated and then purified by prep-HPLC(TFA condition: column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-40%,10min) to give 5-amino-1-methyl-N,N-dipropyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide (14 mg, 48.4 umol, 16.6% yield) as white solid. ^{1}H NMR (400MHz, MeOD-d_4) δ7.61 (s, 1H), 7.11 (s, 1H), 3.51-3.35 (m, 4H), 3.34 (s, 2H), 1.73-1.64 (m, 4H), 1.02-0.85 (m, 6H). LC/MS [M+H] 290.2 (calculated); LC/MS [M+H] 290.2 (observed).

Example 2 Synthesis of 5-amino-1-(5-aminopentyl)-N,N-dipropyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-2



tert-Butyl N-[5-[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl]carbamate, PAZ-4 was prepared by the procedures of Example 4. To a solution of PAZ-4 (30 mg, 65.1 umol, 1.0 eq) in EtOAc (1 mL) was added HCl/EtOAc (4 M, 5 mL), and then stirred for 0.5 hr at 20°C. The mixture was concentrated in vacuum to give 5-amino-1-(5-aminopentyl)-N,N-dipropyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide (14.2 mg, 35.2 umol, 54.09% yield, 98.48% purity, HCl) as white solid. ^{1}H NMR (MeOD, 400 MHz) δ7.66 (s, 1H), 7.14 (s, 1H), 4.27 (t, J = 7.2 Hz, 2H), 3.46-3.42 (m, 4H), 3.38 (s, 2H), 2.90 (t, J = 8.0 Hz, 2H), 1.89-1.86 (m, 2H), 1.72-1.66 (m, 6H), 1.40-1.38 (m, 2H), 0.96-0.89 (m, 6H). LC/MS [M+H] 361.3 (calculated); LC/MS [M+H] 361.2 (observed).

Example 4 Synthesis of tert-butyl N-[5-[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl]carbamate, PAZ-4



Preparation of methyl 2-[5-(tert-butoxycarbonylamino)pentyl]-4-nitro-pyrazole-3-carboxylate, 4b

To a solution of methyl 4-nitro-1H-pyrazole-5-carboxylate, 4a (5 g, 29.2 mmol, 1.0 eq) in DMF (50 mL) was added K_2CO_3 (20.2 g, 146 mmol, 5.0 eq) and 5-(tert-butoxycarbonylamino)pentyl 4-methylbenzenesulfonate (10.5 g, 29.2 mmol, 1.0 eq) at 25°C under N_2. The mixture was stirred for 3 hrs (hours) at 60 °C. Then it was quenched by adding H_2O (200 mL) and extracted with ethyl acetate (200 mL x 3). The combined organic phase was washed with brine (100 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 50/1, 0/1) to give 4b (4.1 g, crude) as yellow oil and regioisomer, methyl 1-[5-(tert-butoxycarbonylamino)pentyl]-

4- nitro-pyrazole-3-carboxylate (6.1 g, crude) as yellow oil. ^{1}H NMR (CDCl3, 400 MHz) δ8.03 (s, 1H), 4.26 (t, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.94-1.86 (m, 2H), 1.53-1.44 (m, 2H), 1.44 (s, 9H), 1.34-1.33 (m, 2H).

Preparation of tert-butyl N-[5-[5-(hydroxymethyl)-4-nitro-pyrazol-1-yl]pentyl]carbamate, 4c

To a solution of 4b (3.6 g, 10.1 mmol, 1.0 eq) in DCM (36 mL) was added into DIBAL-H (1 M, 40.4 mL, 4.0 eq) at 0°C and then stirred for 0.5 hr at 0°C. The mixture was quenched by H_2O 2 mL and stirred for 10 min, then dried by Na_2SO_4, washed with ethyl acetate (50 mL x 4), filtered and concentrated under pressure. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 100/1 to 0/1) to give 4c (2.4 g, 7.31 mmol, 72.35% yield) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ8.09 (s, 1H), 4.98 (d, J = 7.2 Hz, 2H), 4.59 (s, 1H), 4.24 (t, J = 7.2 Hz, 2H), 3.32 (t, J = 6.8 Hz, 1H), 3.12 (q, J = 6.8 Hz, 2H), 1.96-1.92 (m, 2H), 1.53-1.49 (m, 2H), 1.44 (s, 9H), 1.38-1.34 (m, 2H).

Preparation of tert-butyl N-[5-(5-formyl-4-nitro-pyrazol-1-yl)pentyl]carbamate, 4d

To a solution of 4c (2.4 g, 7.31 mmol, 1.0 eq) in DCM (24 mL) was added MnO_2 (6.35 g, 73.1 mmol, 10.0 eq) and then stirred for 12 hrs at 50°C. The mixture was filtered and concentrated under pressure. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 100/1 to 0/1) to afford 2d (0.93 g, 2.85 mmol, 38.99% yield) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ10.51 (s, 1H), 8.13 (s, 1H), 4.56 (t, J = 7.6 Hz, 2H), 3.13-3.10 (m, 2H), 1.91-1.84 (m, 2H), 1.53-1.51 (m, 2H), 1.45 (s, 9H), 1.43-1.36 (m, 2H).

Preparation of (E)-ethyl 3-(1-(5-((tert-butoxycarbonyl)amino)pentyl)-4-nitro-1H-pyrazol-5-yl)-2-(cyanomethyl)acrylate, 4e

To a solution of ethyl 3-cyano-2-(triphenyl-λ5-phosphanylidene)propanoate (1.21 g, 3.13 mmol, 1.10 eq) in toluene (10 mL) was added 2d (0.93 g, 2.85 mmol, 1.0 eq) and then stirred for 3 hrs at 70°C under N_2. After that, it was concentrated to remove toluene (10 mL). The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 100/1 to 0/1 to Ethyl acetate/MeOH = 100/1 to 10/1) to obtain 4e (1.2 g, 2.76 mmol, 96.70% yield) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ8.22 (s, 1H), 7.63 (s, 1H), 4.58 (s, 1H), 4.45-4.40 (m, 2H), 4.10-4.07 (m, 2H), 3.41 (s, 2H), 3.11 (q, J = 6.4 Hz, 2H), 1.93-1.89 (m, 2H), 1.54-1.50 (m, 2H), 1.45-1.42 (m, 12H), 1.34-1.32 (m, 2H)

Preparation of ethyl 5-amino-1-[5-(tert-butoxycarbonylamino)pentyl]-6H-pyrazolo[4,3-b]azepine-7-carboxylate, 4f

To a solution of 4e (600 mg, 1.38 mmol, 1.0 eq) in AcOH (6 mL) was added Fe (385 mg, 6.89 mmol, 5.0 eq), and then stirred for 3 hrs at 70 °C. The mixture was filtered and concentrated to remove AcOH, then added H_2O 5 mL, extracted with ethyl acetate (10 mL x 5). The combined organic phase was washed with brine (5 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 50/1 to 0/1 to Ethyl acetate/MeOH = 50/1 to 1/1) to give 4f (150 mg, 369.92 umol, 26.85% yield) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.69 (s, 1H), 7.58 (s, 1H), 4.36-4.31 (m, 2H), 4.20-4.16 (m, 2H), 3.13 (s, 2H), 3.10-3.09 (m, 2H), 1.89-1.84 (m, 2H), 1.52-1.49 (m, 2H), 1.44 (s, 9H), 1.39 (t, J = 7.2 Hz, 3H), 1.32-1.31 (m, 2H).

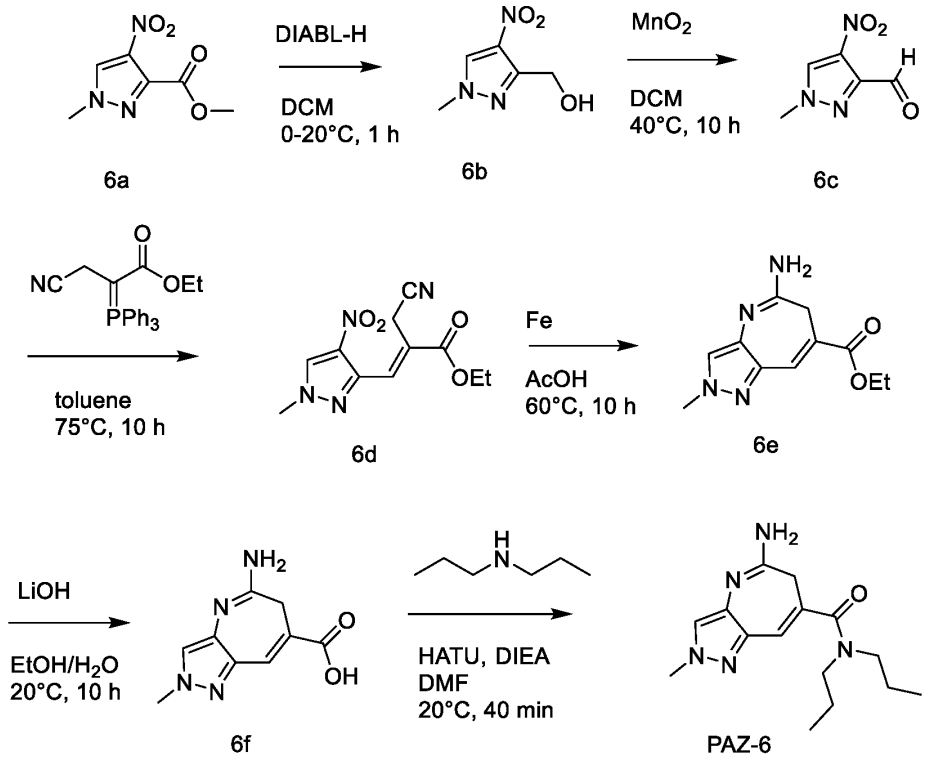
Preparation of 5-amino-1-[5-(tert-butoxycarbonylamino)pentyl]-6H-pyrazolo[4,3-b]azepine-7-carboxylic acid, 4g

To a solution of 4f (130 mg, 321 umol, 1.0 eq) in EtOH (0.5 mL) was added a solution of LiOH.H2O (53.8 mg, 1.28 mmol, 4.0 eq) in H_2O (0.5 mL), then stirred for 3 hrs at 20°C. The pH of the mixture was adjusted to ~7 with HCl(4M), and then extracted with DCM/i-PrOH (3/1, 10 mL x 3). The combined organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum to give 4g (121 mg, 320.58 umol, 99.99% yield) as yellow oil. ^{1}H NMR (MeOD, 400 MHz) δ7.66 (s, 1H), 7.56 (s, 1H), 4.23 (t, J = 7.2 Hz, 2H), 3.41 (s, 2H), 2.99 (t, J = 6.8 Hz, 2H), 1.86-1.82 (m, 2H), 1.48-1.45 (m, 2H), 1.41 (s, 9H), 1.29-1.26 (m, 2H)

Preparation of tert-butyl N-[5-[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1- yl]pentyl]carbamate, PAZ-4

To a solution of 4g (100 mg, 265 umol, 1.0 eq) in DMF (0.5 mL) was added HATU (106 mg, 278 umol, 1.05 eq), DIEA (103 mg, 795 umol, 3.0 eq) and N-propylpropan-1-amine (40.2 mg, 397 umol, 1.50 eq). The mixture was stirred for 0.5 hr at 20°C. Then it was filtered and purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10 um; mobile phase: [water (0.1% TFA)-ACN]; B%: 20%-45%, 9 min) to obtain, PAZ-4 (105 mg, 218.98 umol, 82.65% yield, 96.06% purity) as white solid. ^{1}H NMR (MeOD, 400 MHz) δ7.63 (s, 1H), 7.14 (s, 1H), 4.25 (t, J = 7.2 Hz, 2H), 3.46-3.45 (m, 4H), 3.37 (s, 2H), 2.98 (t, J = 6.4 Hz, 2H), 1.84-1.83 (m, 2H), 1.73-1.67 (m, 4H), 1.45-1.44 (m, 2H), 1.42 (s, 9H), 1.31-1.27 (m, 2H), 0.96-0.89 (m, 6H). LC/MS [M+H] 461.3 (calculated); LC/MS [M+H] 461.3 (observed).

Example 6 Synthesis of 5-amino-2-methyl-N,N-dipropyl-6H- pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-6



Preparation of (1-methyl-4-nitro-pyrazol-3-yl)methanol, 6b

To a solution of methyl 1-methyl-4-nitro-pyrazole-3-carboxylate, 6a (4.00 g, 21.6 mmol, 1.0 eq) in DCM (40 mL) was added DIBAL-H (1 M, 64.8 mL, 3.0 eq) dropwise at 0°C under N_2, and then stirred at 0°C for 1 hour. The reaction mixture was quenched with water (1.2 mL) and filtered and then the filtrate was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=10/1, 1/2) to afford 6b (2.20 g, 14.0 mmol, 64.8% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ8.07 (s, 1H), 4.84 (d, J = 5.6 Hz, 2H), 3.87 (s, 3H), 2.77 (t, J = 5.6Hz, 1H).

Preparation of 1-methyl-4-nitro-pyrazole-3-carbaldehyde, 6c

To a solution of 6b (2.20 g, 14.0 mmol, 1.0 eq) in DCM (20 mL) was added MnO_2 (6.09 g, 70.0 mmol, 5.0 eq) in one portion at 20°C under N_2 and then stirred at 40 °C for 10 hours. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=10/1, 1/4) to afford 6c (1.00 g, 6.45 mmol, 46.0% yield) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ10.38 (s, 1H), 8.15 (s, 1H), 4.01 (s, 3H).

Preparation of (E)-ethyl 2-(cyanomethyl)-3-(1-methyl-4-nitro-1H-pyrazol-3-yl)acrylate, 6d

To a mixture of 6c (1.00 g, 6.45 mmol, 1.0 eq) and ethyl 3-cyano-2-(triphenyl-λ^5-phosphanylidene)propanoate (3.25 g, 8.38 mmol, 1.3 eq) in toluene (10 mL) in one portion at 20°C under N_2 and it was stirred at 75°C for 10 hours. The reaction mixture was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=10/1, 1/1) to afford 6d (1.10 g, 4.16 mmol, 64.5% yield) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ8.27 (s, 1H), 8.17 (s, 1H), 4.30 (q, J = 7.2 Hz, 2H), 3.98 (s, 3H), 3.95 (s, 2H), 1.33 (t, J = 7.2 Hz, 3H).

Preparation of ethyl 5-amino-2-methyl-6H-pyrazolo[4,3-b]azepine-7-carboxylate, 6e

To a solution of 6d (900 mg, 3.41 mmol, 1.0 eq) in AcOH (18 mL) was added Fe (951 mg, 17.0 mmol, 5.0 eq) in one portion at 20°C under N_2 and then stirred at 60°C for 10 hours. The reaction mixture was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=10/1, 0/1 and then Ethyl acetate/Methanol=1/0, 6/1) to afford 6e (270 mg, 1.15 mmol, 33.8% yield) as yellow solid.

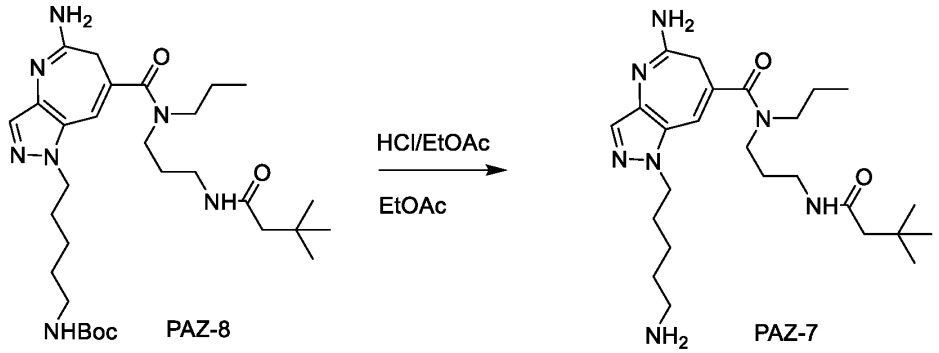
Preparation of 5-amino-2-methyl-6H-pyrazolo[4,3-b]azepine-7-carboxylic acid, 6f

To a solution of PAZ-6e (120 mg, 512 umol, 1.0 eq) in EtOH (10 mL) and H_2O (2 mL) was added $LiOH•H_2O$ (107 mg, 2.56 mmol, 5.0 eq) in one portion at 20°C under N_2 and then stirred at 20°C for 10 hours. The reaction mixture was added with water (5 mL) and adjusted pH to ~7 with HCl (4 M), then the mixture was concentrated in vacuum, filtered and the filter cake was dried to afford 6f (70.0 mg, 339 umol, 66.2 % yield) as light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.56 (s, 1H), 7.19 (br s, 1H), 3.86 (s, 3H), 3.07 (s, 2H).

Preparation of 5-amino-2-methyl-N, N-dipropyl-6H-pyrazolo[4,3-b]azepine-7-carbox - amide, PAZ-6

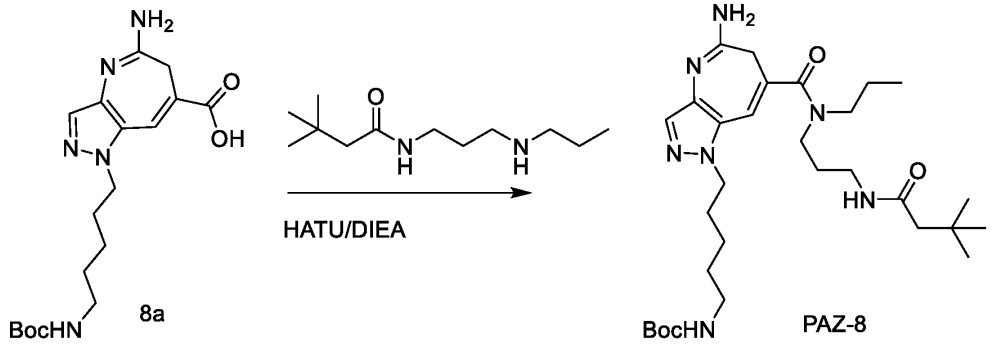
To a solution of 6f (60.0 mg, 291 umol, 1.0 eq) in DMF (1 mL) was added HATU (99.5 mg, 261 umol, 0.9 eq) and DIEA (112 mg, 873 umol, 152 uL, 3.0 eq) at 20°C under N_2. After 10 min, N-propylpropan-1-amine (58.9 mg, 582 umol, 80.2 uL, 2.0 eq) was added and it was stirred for another 0.5 hours at 20°C. The reaction mixture was filtered and the filtrate was purified by prep-HPLC (column: Phenomenex Luna C18 150*30mm*5um;mobile phase: [water(0.1%TFA)-ACN];B%: 5%-40%,12min) to afford PAZ-6 (25.6 mg, 86.7 umol, 29.8% yield, 98.0% purity) as yellow solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ7.86 (s, 1H), 6.96 (s, 1H), 3.99 (s, 3H), 3.50-3.42 (m, 4H), 3.41 (s, 2H), 1.75-1.64 (m, 4H), 0.98-0.92 (m, 6H). LC/MS [M+H] 290.2 (calculated); LC/MS [M+H] 290.2 (observed).

Example 7 Synthesis of 5-amino-1-(5-aminopentyl)-N-[3-(3,3-dimethylbutanoylamino)propyl]-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-7



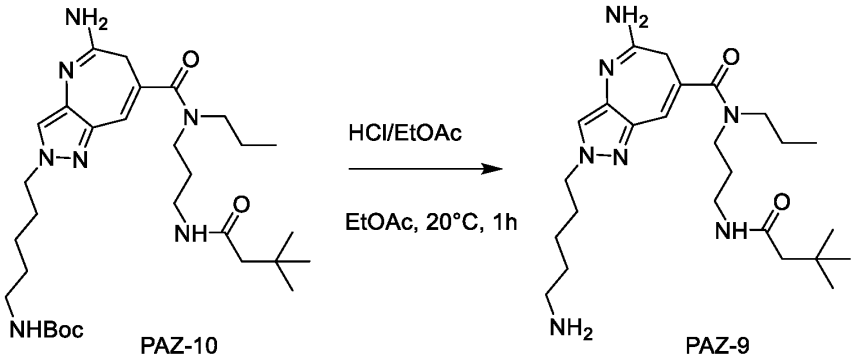
To a solution of PAZ-8 (80 mg, 139 umol, 1.0 eq) in EtOAc (2 mL) was added HCl/EtOAc (4 M, 1.05 mL, 30.0 eq) and then the mixture was stirred for 0.5 hr at 20°C. The mixture was concentrated under pressure to obtain (75 mg, 146 umol, 98 % yield PAZ-7, 2HCl) as yellow solid. ^{1}H NMR (MeOD, 400 MHz) δ7.67 (s, 1H), 7.18 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.52 (t, J = 6.8 Hz, 2H), 3.45-3.41 (m, 4H), 3.32 (d, J = 2.4 Hz, 2H), 2.91 (t, J = 7.6 Hz, 2H), 2.07-2.04 (m, 2H), 1.90-1.85 (m, 4H), 1.69-1.66 (m, 4H), 1.40-1.36 (m, 2H), 1.02 (s, 9H), 0.97-0.87 (m, 3H). LC/MS [M+H] 474.3 (calculated); LC/MS [M+H] 474.3 (observed).

Example 8 Synthesis of tert-butyl N-[5-[5-amino-7-[3-(3,3-dimethylbutanoylamino)propyl-propyl-carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl]carbamate, PAZ-8



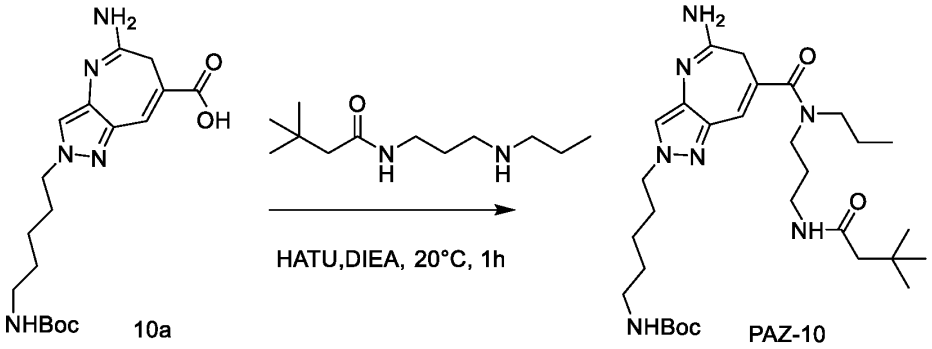
To a solution of 8a (250 mg, 662 umol, 1.0 *eq*) in DMF (3 mL) was added HATU (252 mg, 662 umol, 1.0 *eq*), DIEA (257 mg, 1.99 mmol, 346 uL, 3.0 *eq*) and 3,3-dimethyl-N-[3-(propylamino)propyl]butanamide (149 mg, 695 umol, 1.05 *eq*) at 20 °C and it was stirred for 0.5 hr. The mixture was filtered and purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10 um; mobile phase: [water(0.1%TFA)-ACN]; B%: 15%-45%, 10 min) to give PAZ-8 (120 mg, 195 umol, 29.44% yield, 93.23% purity) as white solid. ^{1}H NMR (MeOD, 400 MHz) δ 7.64 (s, 1H), 7.17 (s, 1H), 4.25 (t, J = 7.2 Hz, 2H), 3.52 (t, J = 7.2 Hz, 2H), 3.46-3.40 (m, 4H), 3.22 (d, J = 1.6 Hz, 2H), 3.00-2.97 (m, 2H), 2.06 (s, 2H), 1.88-1.83 (m, 4H), 1.70-1.68 (m, 2H), 1.47-1.45 (m, 2H), 1.42-1.41 (m, 11H), 1.28-1.27 (m, 2H), 1.02 (s, 9H), 0.93 (s, 3H). LC/MS [M+H] 574.4 (calculated); LC/MS [M+H] 574.4 (observed).

Example 9 Synthesis of 5-amino-2-(5-aminopentyl)-N-[3-(3,3-dimethylbutanoyl amino)propyl]-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-9



To a solution of tert-butyl N-[5-[5-amino-7-[3-(3,3-dimethylbutanoylamino)propyl -propyl-carbamoyl]-6H-pyrazolo[4,3-b]azepin-2-yl]pentyl]carbamate, PAZ-10 from Example 10 (80.0 mg, 139 umol, 1 eq) in EtOAc (2 mL) was added HCl/EtOAc (4 M, 2 mL, 57.0 eq) and it was stirred at 20°C for 1 h. The mixture was concentrated to give PAZ-9 (70 mg, 128 umol, 91.85% yield, 2HCl) as light yellow solid. ^{1}H NMR (MeOD-d_4, 400 MHz) δ7.93 (s, 1H), 6.97 (s, 1H), 4.25 (t, J = 6.8 Hz, 2H), 3.52 (br t, J = 7.2 Hz, 2H), 3.47-3.39 (m, 4H), 3.27-3.16 (m, 2H), 2.92 (br t, J = 7.2 Hz, 2H), 2.08-2.01 (m, 2H), 1.99-1.91 (m, 2H), 1.90-1.80 (m, 2H), 1.75-1.63 (m, 4H), 1.44-1.40 (m, 2H), 1.01 (br s, 9H), 0.94-0.90 (m, 3H). LC/MS [M+H] 474.4 (calculated); LC/MS [M+H] 474.3 (observed).

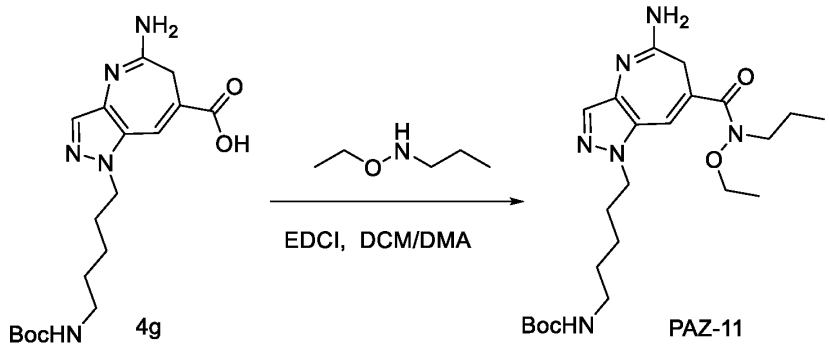
Example 10 Synthesis of tert-butyl N-[5-[5-amino-7-[3-(3,3-dimethylbutanoylamino)propyl-propyl -carbamoyl]-6H-pyrazolo[4,3-b]azepin-2-yl]pentyl]carbamate, PAZ-10



To a solution of 5-amino-2-[5-(tert-butoxycarbonylamino)pentyl]-6H-pyrazolo [4,3-b]azepine-7-carboxylic acid, 10a (250 mg, 662 umol, 1 eq) in DMF (5 mL) was added HATU (252 mg, 662 umol, 1 eq), DIEA (257 mg, 2.00 mmol, 346 uL, 3 eq) and 3,3-dimethyl-N-[3-(propylamino)propyl]butanamide (664 mg, 2.70 mmol, 4 eq, HCl) and then stirred at 20°C for 1 h. The mixture was diluted with water (30 mL) and extracted with EtOAc (30 mL x 3). The

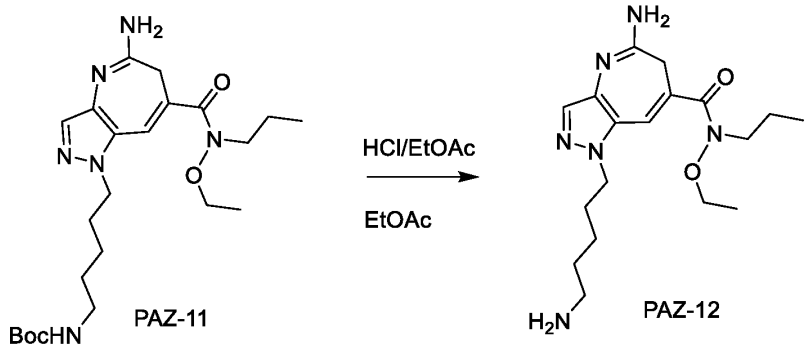
organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%,8min) to give PAZ-10 (90 mg, 131 umol, 19.76% yield, TFA) as light yellow solid. ^{1}H NMR (MeOD-d_4, 400 MHz) δ7.88 (s, 1H), 6.98 (s, 1H), 4.22 (t, J = 7.2 Hz, 2H), 3.52 (br t, J = 7.2 Hz, 2H), 3.48-3.38 (m, 4H), 3.26-3.15 (m, 2H), 3.02 (t, J = 6.8 Hz, 2H), 2.10-1.99 (m, 2H), 1.96-1.79 (m, 4H), 1.72-1.62 (m, 2H), 1.54-1.47 (m, 2H), 1.42 (s, 9H), 1.37-1.28 (m, 2H), 1.01 (s, 9H), 0.95-0.86 (m, 3H). LC/MS [M+H] 574.4 (calculated); LC/MS [M+H] 574.4 (observed).

Example 11 Synthesis of tert-butyl N-[5-[5-amino-7-[ethoxy(propyl)carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl]carbamate, PAZ-11



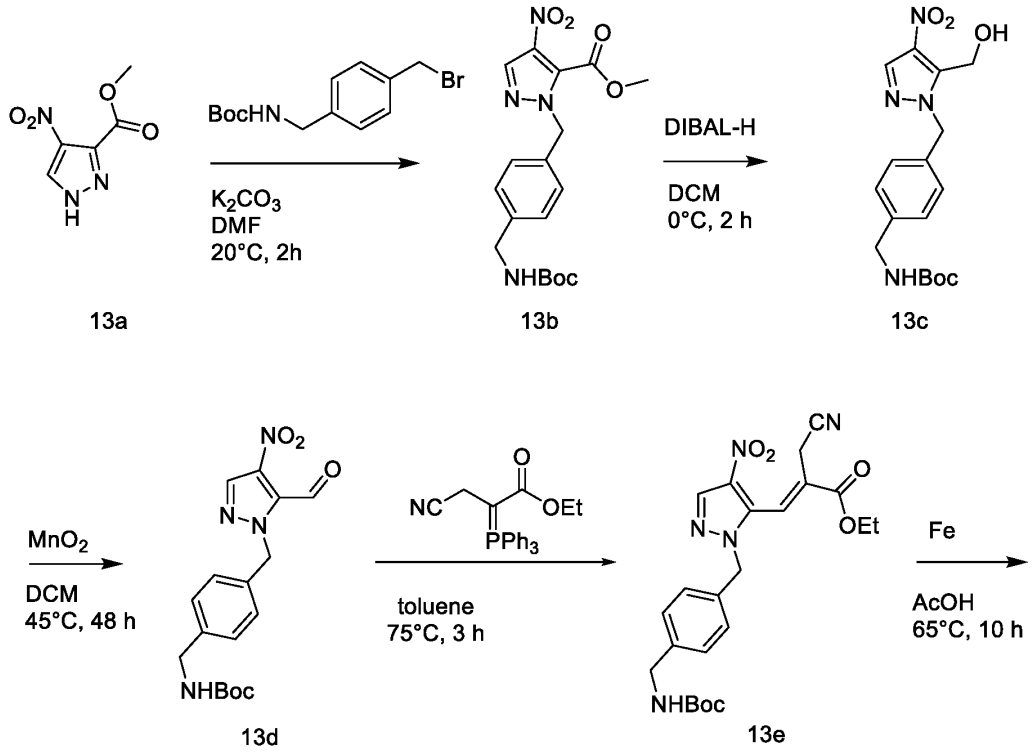
To a solution of 5-amino-1-[5-(tert-butoxycarbonylamino)pentyl]-6H-pyrazolo [4,3-b]azepine-7-carboxylic acid, 4g (220 mg, 582 umol, 1 *eq*) and N-ethoxypropan-1-amine (122 mg, 874 umol, 1.5 *eq*, HCl) in DCM (5 mL) and dimethylacetamide, DMA (5 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, EDCI (447 mg, 2.33 mmol, 4 *eq*) and then stirred at 20°C for 1 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Gemini-NX 150*30mm*5um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-55%,9min) to give PAZ-11 (135 mg, 234.13 umol, 40.17% yield, TFA) as a white solid. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.64 (s, 1H), 7.48 (s, 1H), 4.25 (t, J = 6.8 Hz, 2H), 3.96 (q, J = 7.2 Hz, 2H), 3.74 (t, J = 7.2 Hz, 2H), 3.43 (s, 2H), 2.99 (t, J = 6.8 Hz, 2H), 1.90-1.71 (m, 4H), 1.51-1.37 (m, 11H), 1.33-1.23 (m, 2H), 1.19 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 463.3 (calculated); LC/MS [M+H] 463.3 (observed).

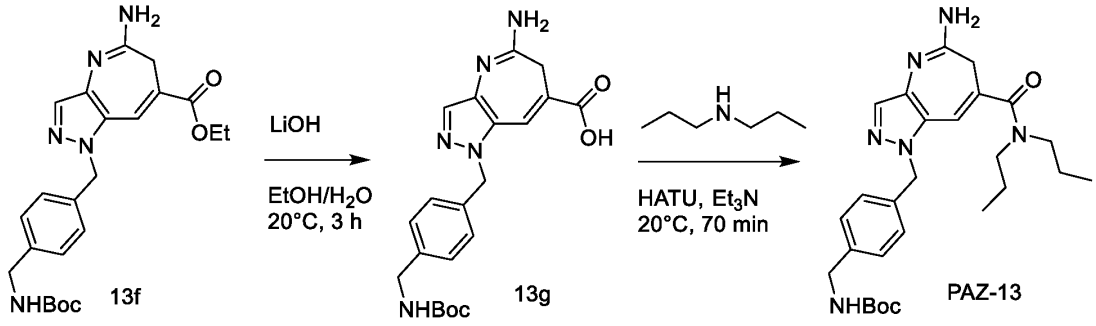
Example 12 Synthesis of 5-amino-1-(5-aminopentyl)-N-ethoxy-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-12



To a solution of PAZ-11 (123 mg, 265.90 umol, 1 *eq*) in EtOAc (1 mL) was added HCl/EtOAc (4 M, 10 mL, 150 *eq*) and it was stirred at 20°C for 0.5 h. The reaction mixture was concentrated under reduced pressure to give PAZ-12 (100.5 mg, 230.83 umol, 86.81% yield, 2HCl) as a light yellow solid. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.66 (s, 1H), 7.46 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.95 (q, J = 7.2 Hz, 2H), 3.74 (t, J = 7.2 Hz, 2H), 3.43 (s, 2H), 2.91 (t, J = 7.6 Hz, 2H), 1.95-1.84 (m, 2H), 1.83-1.73 (m, 2H), 1.70-1.64 (m, 2H), 1.45-1.34 (m, 2H), 1.18 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 363.2 (calculated); LC/MS [M+H] 363.1 (observed).

Example 13 Synthesis of tert-butyl N-[[4-[[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1-yl] methyl]phenyl]methyl]carbamate, PAZ-13





Preparation of methyl 2-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-4-nitro -pyrazole-3-carboxylate, 13b

To a mixture of methyl 4-nitro-1H-pyrazole-5-carboxylate, 13a (200 mg, 1.17 mmol, 1.0 *eq*) and tert-butyl N-[[4-(bromomethyl)phenyl]methyl]carbamate (350 mg, 1.17 mmol, 1.0 *eq*) in DMF (5 mL) was added K_2CO_3 (323 mg, 2.34 mmol, 2.0 *eq*) in one portion at 20°C under N_2 and then stirred at 20°C for 2 hours. Water (20 mL) was added and the aqueous phase was extracted with ethyl acetate (10 mL x 3), the combined organic phase was washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 1/0, 2/1) to afford 13b (100 mg, 256 umol, 21.9 % yield) as white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ8.18 (s, 1H), 7.32-7.21 (m, 4H), 5.50 (s, 2H), 4.23 (s, 2H), 3.92 (s, 3H), 1.46 (s, 9H).

Preparation of tert-butyl N-[[4-[[5-(hydroxymethyl)-4-nitro-pyrazol-1-yl]methyl]phenyl] methyl]carbamate, 13c

To a solution of 13b (1.50 g, 3.84 mmol, 1.0 *eq*) in DCM (20 mL) was added DIBAL-H (1 M, 15.3 mL, 4.0 *eq*) drop-wise at 0°C under N_2, the mixture was stirred at 0°C for 2 hour. The reaction mixture was quenched with ice-water (3 mL), then the mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=1/0, 1/1) to afford 13c (600 mg, 1.66 mmol, 43.1% yield) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$-d) δ8.05 (s, 1H), 7.23-7.20 (m, 2H), 7.14-7.11 (m, 2H), 5.36 (s, 2H), 4.85 (d, J = 6.8 Hz, 2H), 4.22 (d, J = 6.0 Hz, 2H), 1.38 (s, 9H).

Preparation of tert-butyl N-[[4-[(5-formyl-4-nitro-pyrazol-1-yl) methyl] phenyl] methyl] carbamate, 13d

To a solution of 13c (600 mg, 1.66 mmol, 1.0 *eq*) in DCM (10 mL) was added MnO_2 (1.44 g, 16.5 mmol, 10 *eq*) in one portion at 20 °C under N_2 and then the mixture was stirred at 45°C for 48 hours. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm,

diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=1/0, 2/1) to afford 13d (500 mg, 1.39 mmol, 83.8% yield) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ10.33 (s, 1H), 8.53 (s, 1H), 7.25 (s, 4H), 5.72 (s, 2H), 4.14 (d, J = 6.0 Hz, 2H), 1.43 (s, 9H).

Preparation of ethyl (E)-3-[2-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl] -4-nitro-pyrazol-3-yl]-2-(cyanomethyl)prop-2-enoate, 13e

A mixture of PAZ-13d (380 mg, 1.05 mmol, 1.0 *eq*) and ethyl 3-cyano-2-(triphenyl-λ^5-phosphanylidene) propanoate (449 mg, 1.16 mmol, 1.1 *eq*) in toluene (10 mL) was stirred at 75 °C for 3 hours. The reaction mixture was concentrated in vacuum and then the residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=10/1, 2/1) to afford 13e (370 mg, 788 umol, 74.7% yield) as brown solid.

Preparation of ethyl 5-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl] methyl]-6H-pyrazolo[4,3-b]azepine-7-carboxylate, 13f

To a solution of 13e (370 mg, 788 umol, 1.0 *eq*) in AcOH (7 mL) was added Fe (220 mg, 3.94 mmol, 5.0 *eq*) in one portion at 20°C under N_2 and then it was stirred at 65°C for 10 hours. The reaction mixture was diluted with ethyl acetate and then filtered. The filtrate was concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex luna C18 100*40mm*5 um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-40%,8min) to afford 13f (180 mg, 409 umol, 51.9% yield) as yellow solid. ^{1}H NMR (400 MHz, MeOD) δ7.73 (s, 1H), 7.48 (s, 1H), 7.24 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 5.44 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 4.21 (s, 2H), 3.05 (s, 2H), 1.45 (s, 9H), 1.34 (t, J = 7.2 Hz, 3H).

Preparation of 5-amino-1-[[4-[(tert-butoxycarbonylamino) methyl] phenyl] methyl]-6H -pyrazolo[4,3-b]azepine-7-carboxylic acid, 13g

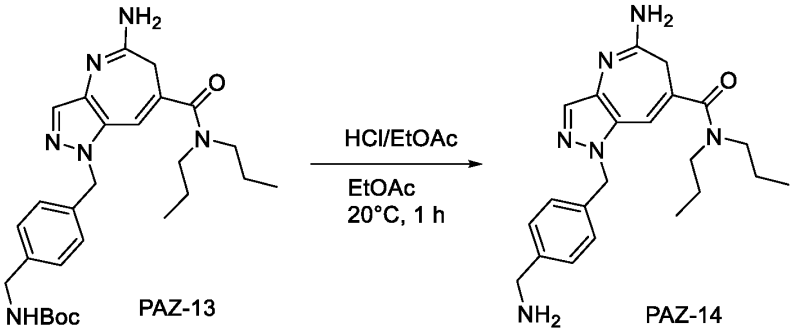
To a solution of PAZ-13f (160 mg, 364 umol, 1.0 *eq*) in EtOH (4 mL) and H_2O (4 mL) was added LiOH•H_2O (61.1 mg, 1.46 mmol, 4.0 *eq*) in one portion at 20°C under N_2 and it was stirred at 20 °C for 3 hours. The reaction mixture was quenched with HCl (4 M) until pH=7, and then concentrated to remove EtOH in vacuum. The precipitation was filtered to afford 13g (120 mg, 291 umol, 80.1% yield) as gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.66 (s, 1H), 7.39 (s, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 5.39 (s, 2H), 4.09 (d, J = 6.0 Hz, 2H), 2.90 (s, 2H), 1.39 (s, 9H).

Preparation of PAZ-13

To a solution of 13g (150 mg, 364 umol, 1.0 *eq*) in DMF (2 mL) was added HATU (138 mg, 364 umol, 1.0 *eq*) and Et_3N (110 mg, 1.09 mmol, 152 uL, 3.0 *eq*) in one portion at 20 °C under N_2. After 10 min, N-propylpropan-1-amine (110 mg, 1.09 mmol, 150 uL, 3.0 *eq*) was added and it was stirred at 20°C for 1 hour. The reaction mixture was filtered and the filtrate

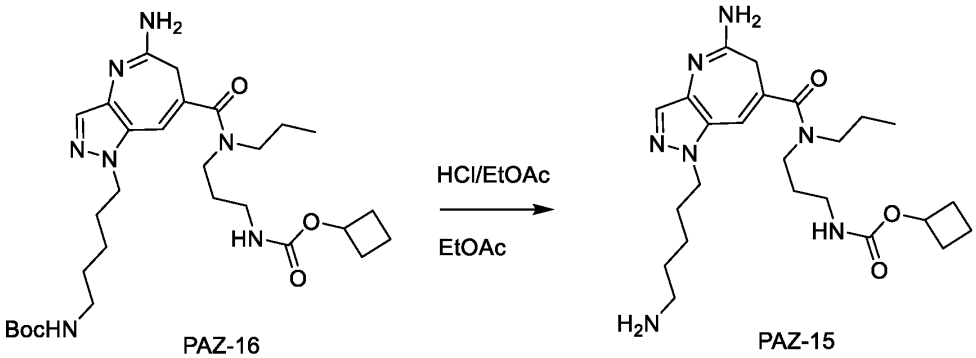
was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 10%-40%, 10min). to afford PAZ-13 (110 mg, 221 umol, 60.8% yield, 99.7% purity) as white solid. ^{1}H NMR (400 MHz, MeOD) δ7.71 (s, 1H), 7.26 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 7.02 (s, 1H), 5.51 (s, 2H), 4.20 (s, 2H), 3.38-3.34 (m, 4H), 3.30 (s, 2H), 1.55-1.50 (m, 4H), 1.45 (s, 9H), 1.04-0.66 (m, 6H). LC/MS [M+H] 495.3 (calculated); LC/MS [M+H] 495.2 (observed).

Example 14 Synthesis of 5-amino-1-[[4-(aminomethyl)phenyl]methyl]-N,N-dipropyl-6H- pyrazolo[4,3-b]azepine -7-carboxamide, PAZ-14



To a solution of tert-butyl N-[[4-[[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin -1-yl]methyl]phenyl]methyl]carbamate, PAZ-13 (100 mg, 202 umol, 1.0 *eq*) in EtOAc (2 mL) was added HCl/EtOAc (4 M, 2.53 mL, 50 *eq*) in one portion at 20°C under N_2 and then the mixture was stirred at 20°C for 1 hour. The reaction mixture was concentrated in vacuum to afford PAZ-14 (87.0 mg, 196 umol, 97.1% yield, 97.2% purity, HCl) as brown oil. ^{1}H NMR (400 MHz, MeOD) δ7.74 (s, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.06 (s, 1H), 5.56 (s, 2H), 4.11 (s, 2H), 3.35 (s, 2H), 3.33-3.31 (m, 4H), 1.72-1.54 (m, 4H), 1.01-0.71 (m, 6H). LC/MS [M+H] 395.2 (calculated); LC/MS [M+H] 395.1 (observed).

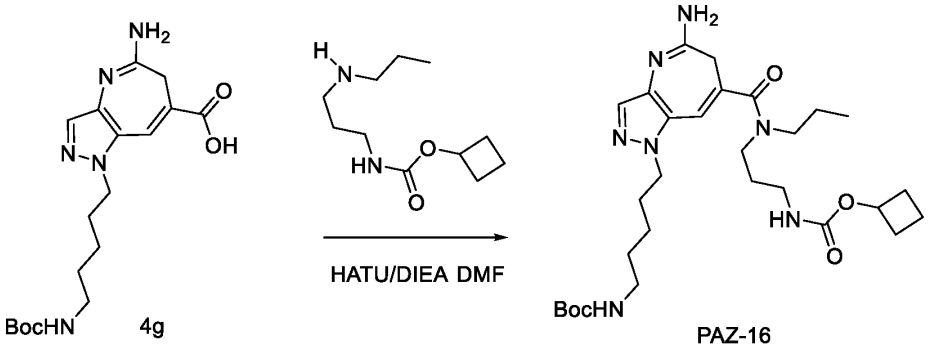
Example 15 Synthesis of cyclobutyl (3-(5-amino-1-(5-aminopentyl)-N-propyl-1,6-dihydropyrazolo[4,3-b]azepine-7-carboxamido)propyl)carbamate, PAZ-15



To a solution of PAZ-16 (200 mg, 349 umol, 1 eq) in EtOAc (3 mL) was added HCl/EtOAc (4 M, 10 mL) and then stirred at 25°C for 1 h. The mixture was concentrated under

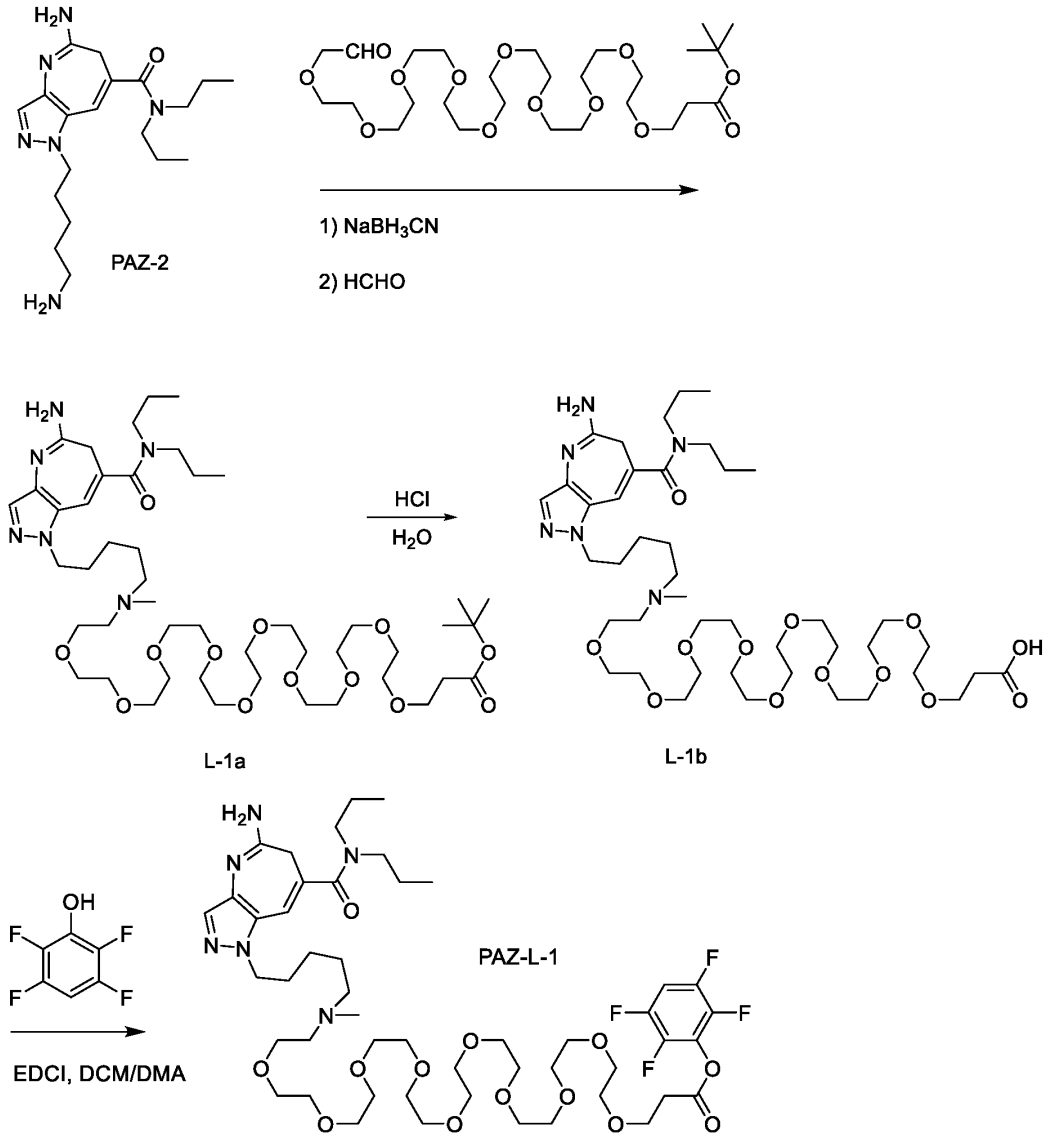
reduced pressure to afford PAZ-15 (170 mg, 333 umol, 95.61% yield, HCl) as a yellow solid. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.67 (s, 1H), 7.17 (br s, 1H), 4.85-4.80 (m, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.51 (br t, J = 7.2 Hz, 2H), 3.47-3.36 (m, 4H), 3.19-3.02 (m, 2H), 2.91 (br t, J = 7.6 Hz, 2H), 2.32-2.20 (m, 2H), 2.04-1.92 (m, 2H), 1.90-1.82 (m, 4H), 1.77-1.57 (m, 6H), 1.45-1.31 (m, 2H), 0.98-0.84 (m, 3H). LC/MS [M+H] 474.3 (calculated); LC/MS [M+H] 474.1 (observed).

Example 16 Synthesis of tert-butyl (5-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)pentyl)carbamate, PAZ-16



To a solution of 5-amino-1-[5-(tert-butoxycarbonylamino)pentyl]-6H-pyrazolo [4,3-b]azepine-7-carboxylic acid, 4g (250 mg, 662 umol, 1 eq) in DMF (0.5 mL) was added HATU (277 mg, 729 umol, 1.1 eq) and DIEA (428 mg, 3.3 mmol, 577 uL, 5 eq), then cyclobutyl N-[3-(propylamino)propyl]carbamate (166 mg, 662 umol, 1 eq, HCl) was added and it was stirred at 25°C for 0.5 h. The mixture was filtered and purified by prep-HPLC (TFA condition; column: Phenomenex Gemini-NX C18 75*30mm*3um;mobile phase: [water(0.1%TFA)-ACN];B%: 30%-50%,8min) to afford PAZ-16 (200 mg, 348.6 umol, 52.63% yield) as a yellow solid. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.42 (s, 1H), 6.95 (s, 1H), 4.84-4.77 (m, 1H), 4.17 (t, J = 7.2 Hz, 2H), 3.48 (br t, J = 7.2 Hz, 2H), 3.42-3.37 (m, 2H), 3.30 (br s, 2H), 3.12-3.02 (m, 2H), 2.98 (t, J = 6.8 Hz, 2H), 2.27 (br s, 2H), 2.07-1.93 (m, 2H), 1.83-1.75 (m, 4H), 1.71-1.55 (m, 4H), 1.47-1.39 (m, 11H), 1.29-1.22 (m, 2H), 0.97-0.86 (m, 3H). LC/MS [M+H] 574.4 (calculated); LC/MS [M+H] 574.4 (observed).

Example L-1 Synthesis of (2,3,5,6-tetrafluorophenyl) 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, PAZ-L-1



Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-(dipropylcarbamoyl)-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-1a

To a solution of 5-amino-1-(5-aminopentyl)-N,N-dipropyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-2 (57 mg, 143.59 umol, 1.0 eq, HCl) in MeOH (2 mL) was added tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-oxoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate (218 mg, 373 umol, 2.60 eq) and $NaBH_3CN$ (27.0 mg, 431 umol, 3.0 eq) and the mixture was stirred for 12 hrs at 20°C, then HCHO (23.3 mg, 287 umol, 21.3 uL, 37% purity, 2.0 eq) was added and it was stirred for another 1 hr at 20°C. The reaction was filtered and purified by prep-HPLC

(column: Phenomenex Synergi C18 150*25*10 um; mobile phase: [water(0.1%TFA)-ACN]; B%: 25%-35%, 10 min) to obtain L-1a (100 mg, 106.02 umol, 73.83% yield) as yellow oil. 1H NMR (MeOD, 400 MHz) δ7.67 (s, 1H), 7.13 (s, 1H), 4.30-4.28 (m, 2H), 3.84-3.83 (m, 2H), 3.71-3.59 (m, 40H), 3.47-3.44 (m, 6H), 3.38 (s, 2H), 2.91 (s, 3H), 2.47 (t, J = 6.0 Hz, 2H), 2.03 (s, 3H), 1.94-1.91 (m, 2H), 1.82-1.63 (m, 6H), 1.45 (s, 9H), 1.39-1.37 (m, 2H), 0.96-0.91 (m, 6H).

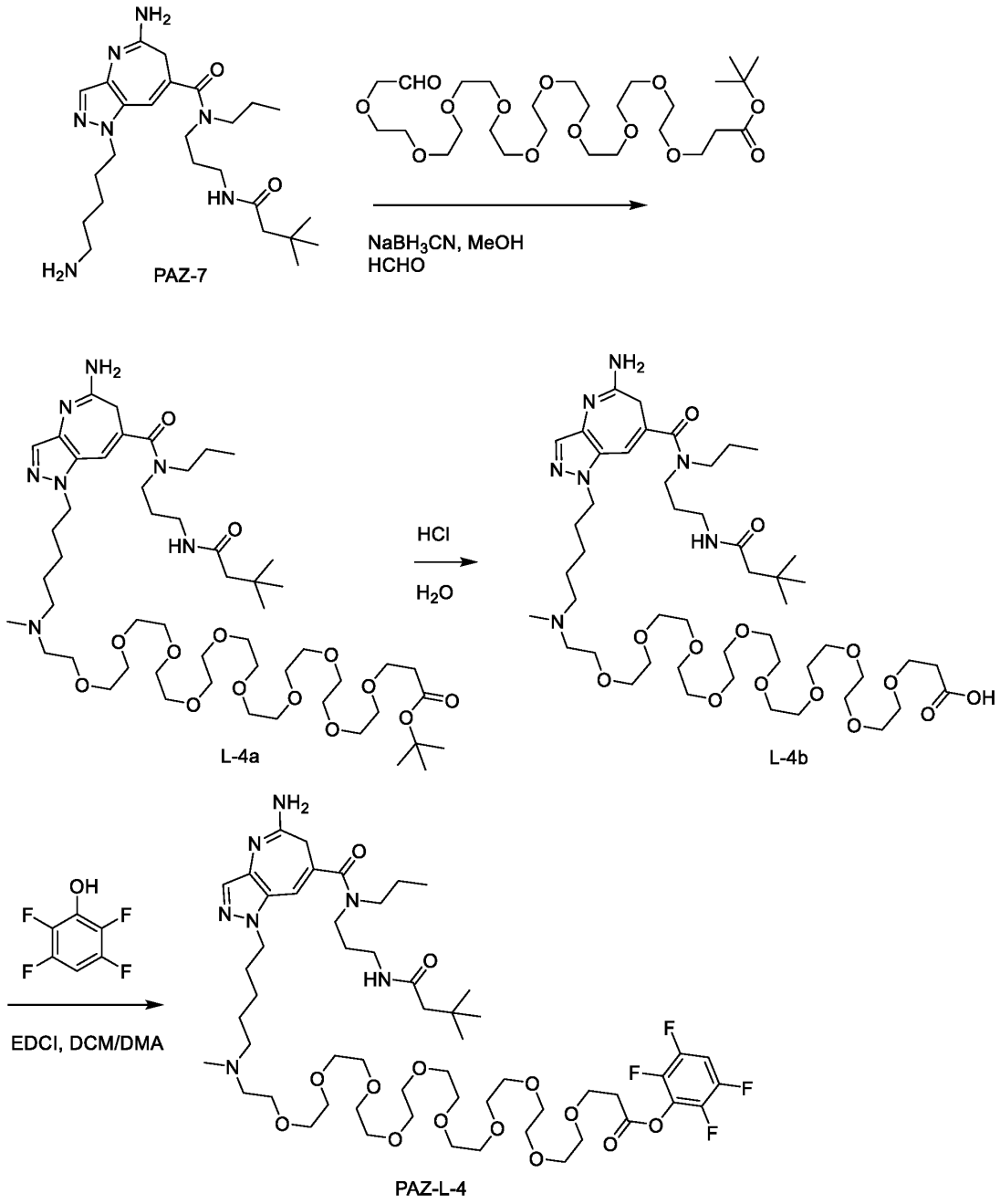
Preparation of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-(dipropylcarbamoyl)- 6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid, L-1b

To a solution of L-1a (90 mg, 95.42 umol, 1.0 eq) in H_2O (0.2 mL) was added HCl (12 M, 159 uL, 20.0 eq) and it was stirred for 1 hr at 80°C. The mixture was concentrated under pressure to give L-1b (60 mg, 67.64 umol, 70.88% yield) as yellow oil.

Preparation of PAZ-L-1

To a solution of L-1b (55 mg, 62.0 umol, 1.0 eq) in DMA (0.1 mL) and DCM (1 mL) was added 2,3,5,6-tetrafluorophenol (82.5 mg, 496 umol, 8 eq) and EDCI (119 mg, 620 umol, 10.0 eq) and then stirred for 0.5 hr at 20°C. The mixture was concentrated at 25°C and purified by (column: Phenomenex Synergi C18 150*25*10 um; mobile phase: [water(0.1%TFA)-ACN]; B%: 20%-50%, 8 min) to obtain PAZ-L-1 as (31.5 mg, 24.94 umol, 40.22% yield, 2TFA) as light yellow oil. ^{1}H NMR (MeOD, 400 MHz) δ7.67 (s, 1H), 7.47-7.42 (m, 1H), 7.13 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.87-3.85 (m, 2H), 3.84-3.82 (m, 2H), 3.71-3.57 (m, 38H), 3.53-3.40 (m, 6H), 3.41 (s, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.91 (s, 3H), 1.90-1.89 (m, 2H), 1.77-1.76 (m, 2H), 1.71-1.66 (m, 4H), 1.38-1.34 (m, 2H), 0.96-0.92 (m, 6H). LC/MS [M+H] 1035.6 (calculated); LC/MS [M+H] 1035.6 (observed).

Example L-4 Synthesis of 2,3,5,6-tetrafluorophenyl 39-(5-amino-7-((3-(3,3-dimethylbutanamido)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, PAZ-L-4



Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-[3-(3,3-dimethylbutanoylamino)propyl-propyl-carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-4a

To a mixture of PAZ-7 (90 mg, 165 umol, 1.0 eq, 2 HCl) in MeOH (4 mL) was added tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-oxoethoxy)ethoxy]ethoxy]

ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate (96.3 mg, 165 umol, 1.0 eq) and $NaBH_3CN$ (20.7 mg, 329.3 umol, 2.0 eq) in one portion at 25°C. The mixture was stirred at 25°C for 12 h. Then formaldehyde, HCHO (66.81 mg, 823 umol, 37% purity, 5 eq) and sodium cyanoborohydride, $NaBH_3CN$ (20.7 mg, 329 umol, 2 eq) was added and it was stirred for another 2 h at 25 °C. The reaction mixture was concentrated and purified by prep-HPLC(column: Phenomenex Gemini-NX 150*30mm*5um;mobile phase: [water (0.1% TFA)-ACN]; B%: 20%-50%, 9 min) to give L-4a (80 mg, 75.73 umol, 45.99% yield) as yellow oil.

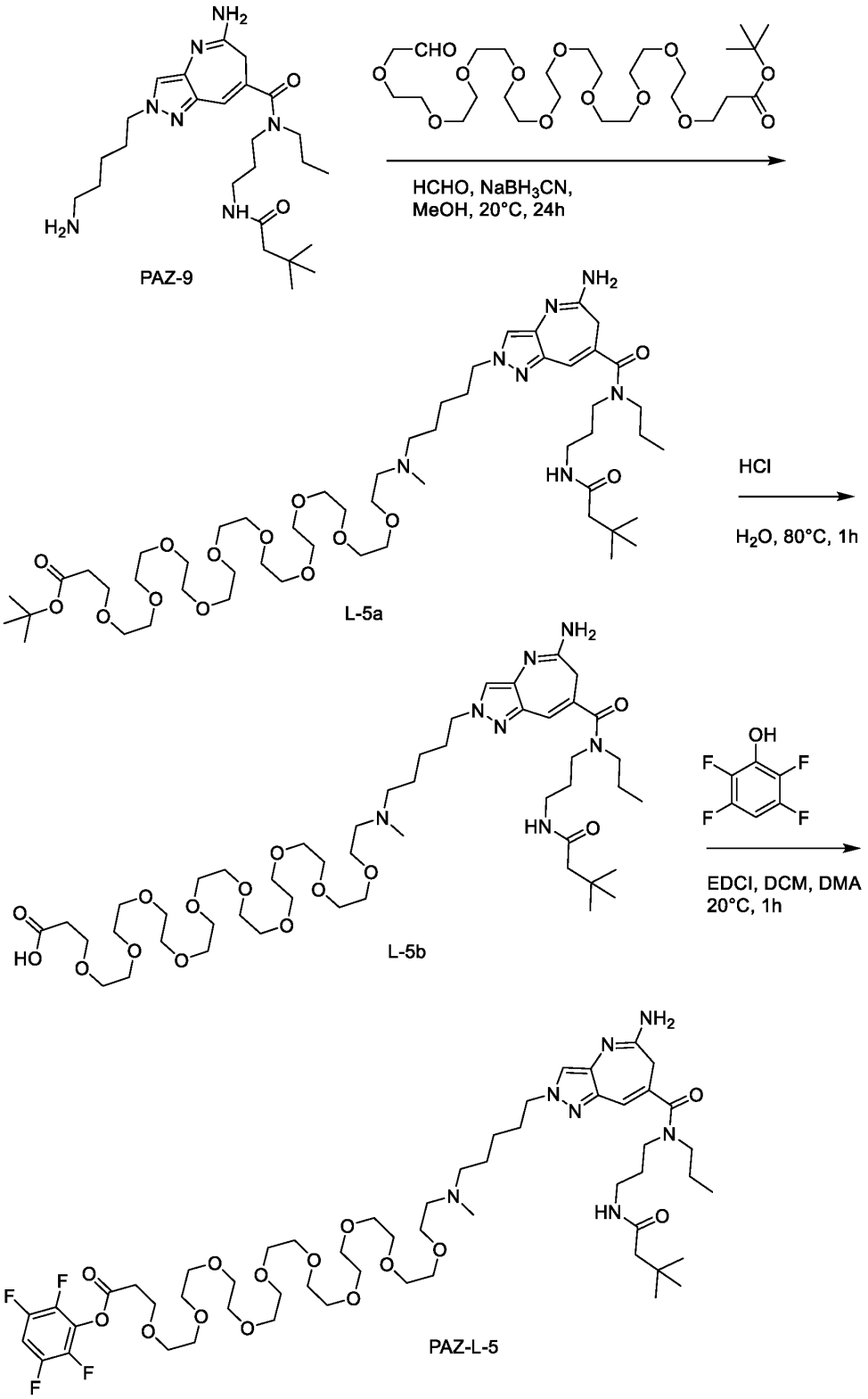
Preparation of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-[3-(3,3-dimethylbutanoyl amino)propyl-propyl-carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid, L-4b

To a mixture of L-4a (75 mg, 71.0 umol, 1.0 eq) in H_2O (2 mL) and CH_3CN (0.5 mL) was added HCl (12 M, 148 uL, 25 eq) in one portion at 25°C. The mixture was stirred at 80°C for 1 h and then concentrated to give L-4b (60 mg, crude, HCl) was obtained as yellow oil.

Preparation of PAZ-L-4

To a mixture of L-4b (55 mg, 54.9 umol, 1.0 eq, HCl) in DCM (2 mL) and DMA (0.4 mL) was added 2,3,5,6-tetrafluorophenol (91.3 mg, 550 umol, 10 eq) and EDCI (105 mg, 550 umol, 10 eq) in one portion at 25°C. The mixture was stirred at 25°C for 1 h and then it was concentrated and purified by prep-HPLC(column: Phenomenex Synergi C18 150*25*10um; mobile phase: [water (0.1% TFA)-ACN]; B%: 20%-50%, 8 min) to give PAZ-L-4 (39.4 mg, 34.31 umol, 62.40% yield) as yellow oil. ^{1}H NMR (MeOD, 400 MHz) δ7.67 (s, 1H), 7.47-7.42 (m, 1H), 7.17 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.87 (t, J = 6.0 Hz, 2H), 3.84-3.51 (m, 2H), 3.71-3.57 (m, 38H), 3.53-3.41 (m, 8H), 3.17-3.05 (m, 2H), 2.98 (t, J = 5.6 Hz, 2H), 2.91 (s, 3H), 2.10-2.06 (m, 2H), 1.96-1.82 (m, 4H), 1.82-1.73 (m, 2H), 1.73-1.62 (m, 2H), 1.39-1.37 (m, 2H), 1.02 (s, 9H), 0.95-0.88 (m, 3H). LC/MS [M+H] 1148.6 (calculated); LC/MS [M+H] 1148.7 (observed).

Example L-5 Synthesis of 2,3,5,6-tetrafluorophenyl 39-(5-amino-7-((3-(3,3-dimethylbutanamido)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-2(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, PAZ-L-5



Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-[3-(3,3-dimethyl butanoylamino)propyl-propyl-carbamoyl]-6H-pyrazolo[4,3-b]azepin-2-yl]pentyl-methyl-

amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-5a

To a solution of 5-amino-2-(5-aminopentyl)-N-[3-(3,3-dimethylbutanoylamino) propyl]-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-9 (55.0 mg, 101 umol, 1 eq, 2HCl) in MeOH (1 mL) was added tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-oxoethoxy)ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate (88.0 mg, 151 umol, 1.5 eq) and $NaBH_3CN$ (10.00 mg, 151.00 umol, 1.5 eq) and then stirred for 23 h. After that, HCHO (50.00 mg, 503.00 umol, 46.00 uL, 30% purity, 5 eq) and $NaBH_3CN$ (10.00 mg, 151.00 umol, 1.5 eq) was added to the mixture and stirred at 25°C for another 1 h. The mixture was filtered and concentrated. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%,8min) to give L-5a (70 mg, 59.81 umol, 59.44% yield, TFA) as colorless oil. LC/MS [M+H] 1056.7 (calculated); LC/MS [M+H] 1056.6 (observed).

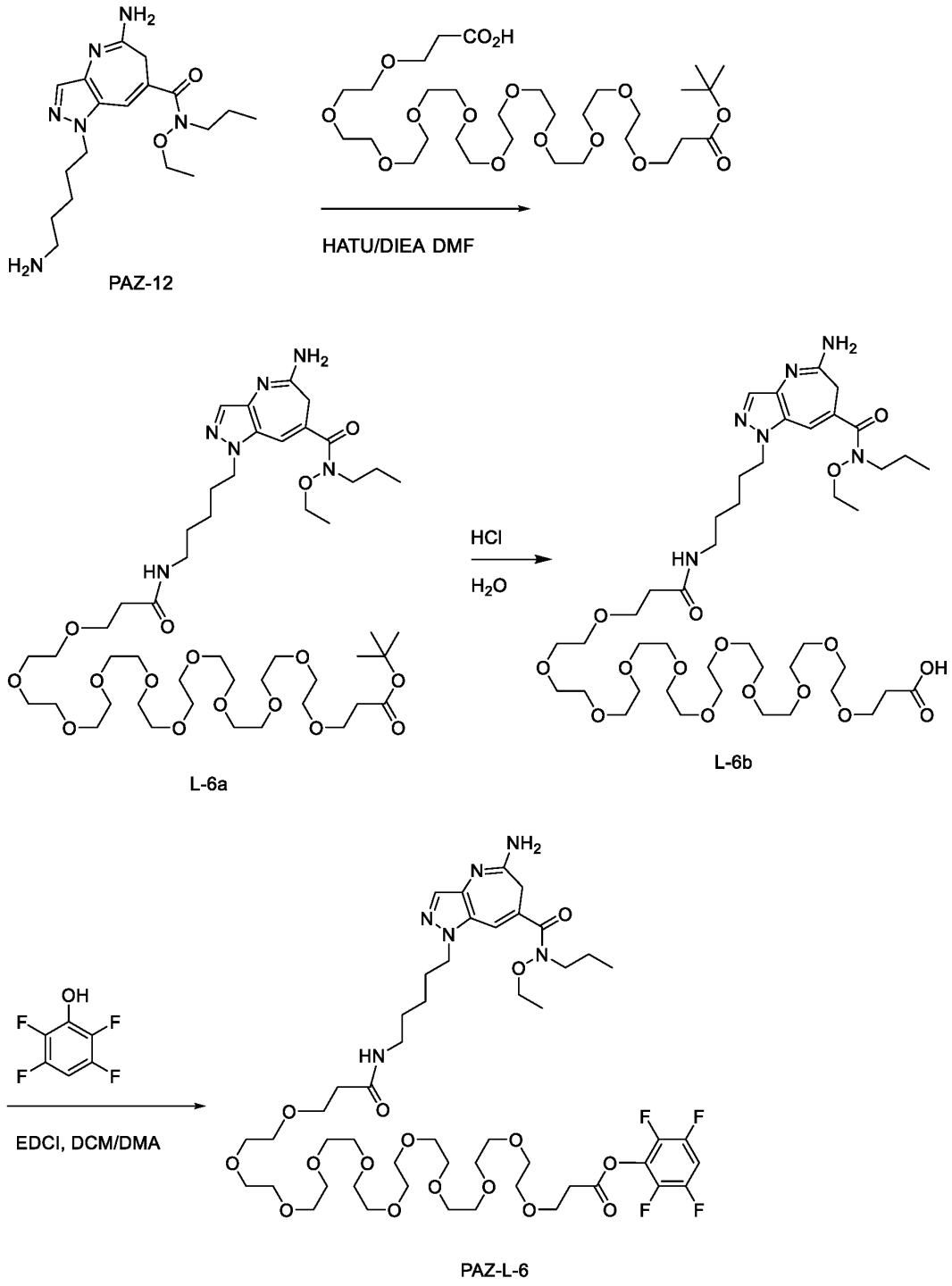
Preparation of 39-(5-amino-7-((3-(3,3-dimethylbutanamido)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-2(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoic acid, L-5b

To a solution of L-5a (70.0 mg, 60.0 umol, 1 eq, TFA) in H_2O (1 mL) was added HCl (12 M, 75.0 uL, 15 eq) at 20°C and then stirred at 80°C for 1 h. The mixture was concentrated to give L-5b (50 mg, 48.2 umol, 80.64% yield, HCl) as light yellow solid. LC/MS [M+H] 1000.7 (calculated); LC/MS [M+H] 1000.6 (observed).

Preparation of PAZ-L-5

To a solution of L-5b (45.0 mg, 43.0 umol, 1 eq, HCl) in DCM (2 mL) and DMA (0.1 mL) was added 2,3,5,6-tetrafluorophenol (58.0 mg, 347 umol, 8 eq) and EDCI (83.0 mg, 434 umol, 10 eq). The mixture was stirred at 20°C for 1 h and then it was concentrated and filtered. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 20%-50%,8min) to give PAZ-L-5 (22 mg, 17.43 umol, 40.15% yield, TFA) as light yellow oil. ^1H NMR (MeOD-d_4, 400 MHz) δ7.92 (s, 1H), 7.50-7.42 (m, 1H), 6.98 (s, 1H), 4.26 (t, J = 6.8 Hz, 2H), 3.87 (t, J = 6.0 Hz, 2H), 3.85-3.80 (m, 2H), 3.71-3.60 (m, 38H), 3.52 (br t, J = 7.2 Hz, 2H), 3.49-3.35 (m, 6H), 3.26-3.07 (m, 4H), 2.98 (t, J = 6.0 Hz, 2H), 2.91 (s, 3H), 2.10-1.92 (m, 4H), 1.89-1.75 (m, 4H), 1.69-1.65 (m, 2H), 1.47-1.36 (m, 2H), 1.02 (br s, 9H), 0.96-0.86 (m, 3H). LC/MS [M+H] 1148.6 (calculated); LC/MS [M+H] 1148.5 (observed).

Example L-6 Synthesis of 2,3,5,6-tetrafluorophenyl 43-(5-amino-7-(ethoxy(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoate, PAZ-L-6



Preparation of tert-butyl 43-(5-amino-7-(ethoxy(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoate, L-6a

To a solution of 2,2-dimethyl-4-oxo-3,7,10,13,16,19,22,25,28,31,34,37-dodecaoxatetracontan-40-oic acid (54.5 mg, 82.7 umol, 1.2 *eq*) in DMF (0.5 mL) was added

HATU (28.8 mg, 75.8 umol, 1.1 *eq*) and DIPEA (44.5 mg, 344 umol, 5 *eq*). After 5 min, 5-amino-1-(5-aminopentyl)-N-ethoxy-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-12 (30 mg, 68.90 umol, 1 *eq*, 2HCl) was added to the reaction mixture and it was stirred at 15°C for 25 min. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%,10min) to give L-6a (40 mg, 35.80 umol, 51.96% yield, TFA) as a light yellow oil. LC/MS [M+H] 1003.6 (calculated); LC/MS [M+H] 1003.8 (observed).

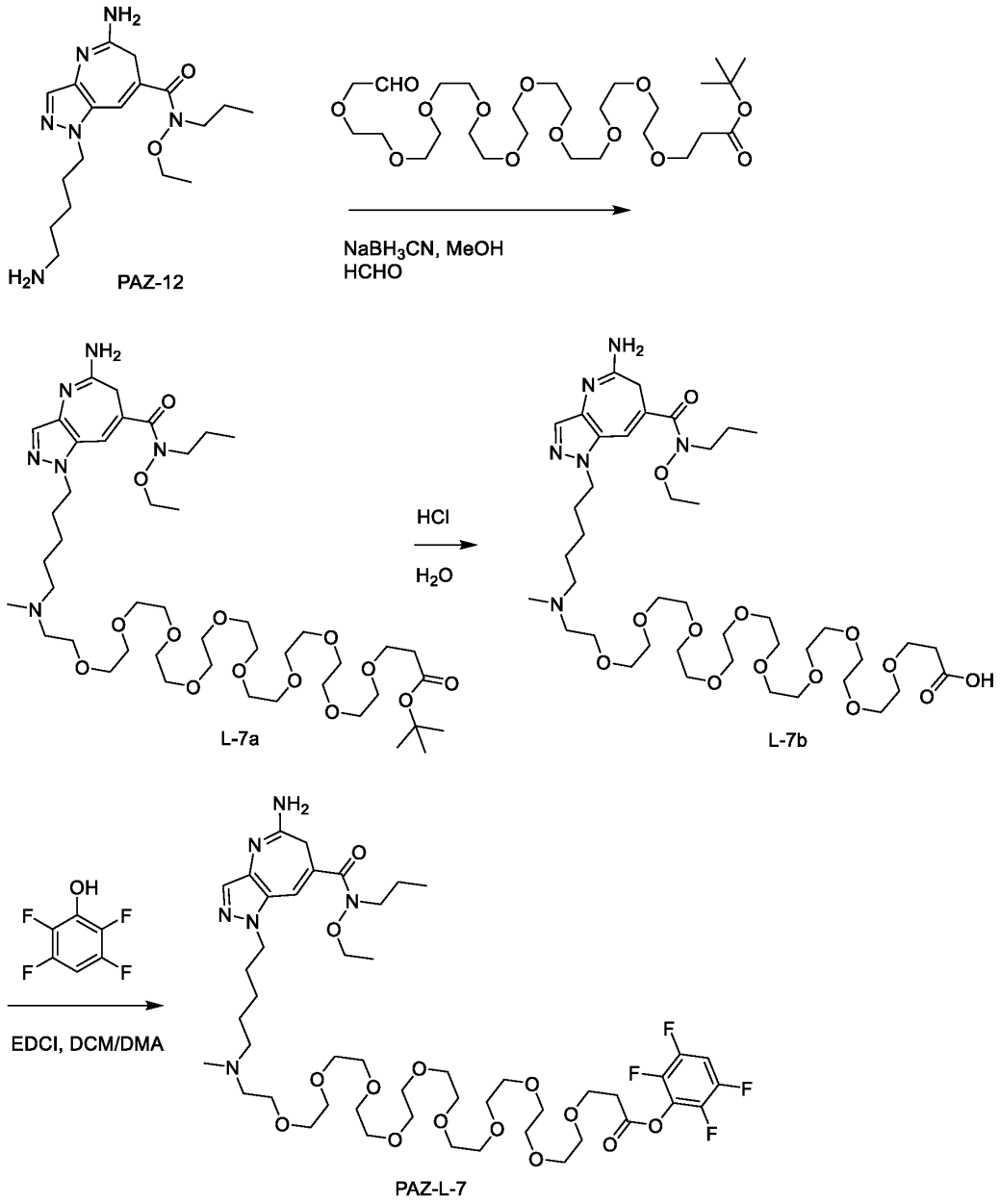
Preparation of 43-(5-amino-7-(ethoxy(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoic acid, L-6b

To a solution of L-6a (40 mg, 35.8 umol, 1 *eq*, TFA) in H_2O (3 mL) was added HCl (12 M, 20 *eq*) and the mixture was stirred at 80 °C for 0.5 h. The reaction mixture was concentrated under reduced pressure to give L-6b (40 mg, crude, HCl) as a light yellow oil. LC/MS [M+H] 947.6 (calculated); LC/MS [M+H] 947.7 (observed).

Preparation of PAZ-L-6

To a solution of L-6b (30 mg, 30.5 umol, 1 *eq*, HCl) and 2,3,5,6-tetrafluorophenol (50.6 mg, 305 umol, 10 *eq*) in DMA (0.2 mL) and DCM (1 mL) was added EDCI (58.5 mg, 305 umol, 10 *eq*) and it was stirred at 15°C for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-50%,8min) to give PAZ-L-6 (13 mg, 10.75 umol, 35.25% yield, TFA) as a light yellow oil. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.66 (s, 1H), 7.48 (s, 1H), 7.47-7.38 (m, 1H), 4.26 (t, J = 6.8 Hz, 2H), 3.97 (q, J = 6.8 Hz, 2H), 3.88 (t, J = 6.0 Hz, 2H), 3.77-3.67 (m, 4H), 3.66-3.64 (m, 4H), 3.64-3.58 (m, 36H), 3.43 (s, 2H), 3.35 (s, 2H), 3.14 (t, J = 6.8 Hz, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.40 (t, J = 6.0 Hz, 2H), 1.91-1.70 (m, 4H), 1.52-1.46 (m, 2H), 1.34-1.24 (m, 2H), 1.20 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 1095.5 (calculated); LC/MS [M+H] 1095.4 (observed).

Example L-7 Synthesis of 2,3,5,6-tetrafluorophenyl 39-(5-amino-7-(ethoxy(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, PAZ-L-7



Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-[ethoxy(propyl) carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-7a

To a solution of 5-amino-1-(5-aminopentyl)-N-ethoxy-N-propyl-6H-pyrazolo[4,3-b] azepine-7-carboxamide, PAZ-12 (30 mg, 68.9 umol, 1 *eq*, 2HCl) in MeOH (10 mL) was added TEA (13.9 mg, 137 umol, 2 *eq*) and tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-oxoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate

(80.6 mg, 138 umol, 2 *eq*) at 15°C. After 30 min, $NaBH_3CN$ (8.66 mg, 137.81 umol, 2 *eq*) was added at 15 °C and the resulting mixture was stirred at this temperature for 12 h. HCHO (41.38 mg, 413.42 umol, 37.97 uL, 30% purity, 6 *eq*) and $NaBH_3CN$ (8.66 mg, 137.81 umol, 2 *eq*) were added to the mixture at 15°C and stirred at 15°C for 2 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-43%,8min) to give L-7a (45 mg, 38.36 umol, 55.67% yield, 2TFA) as a light yellow oil. LC/MS [M+H] 945.6 (calculated); LC/MS [M+H] 945.5 (observed).

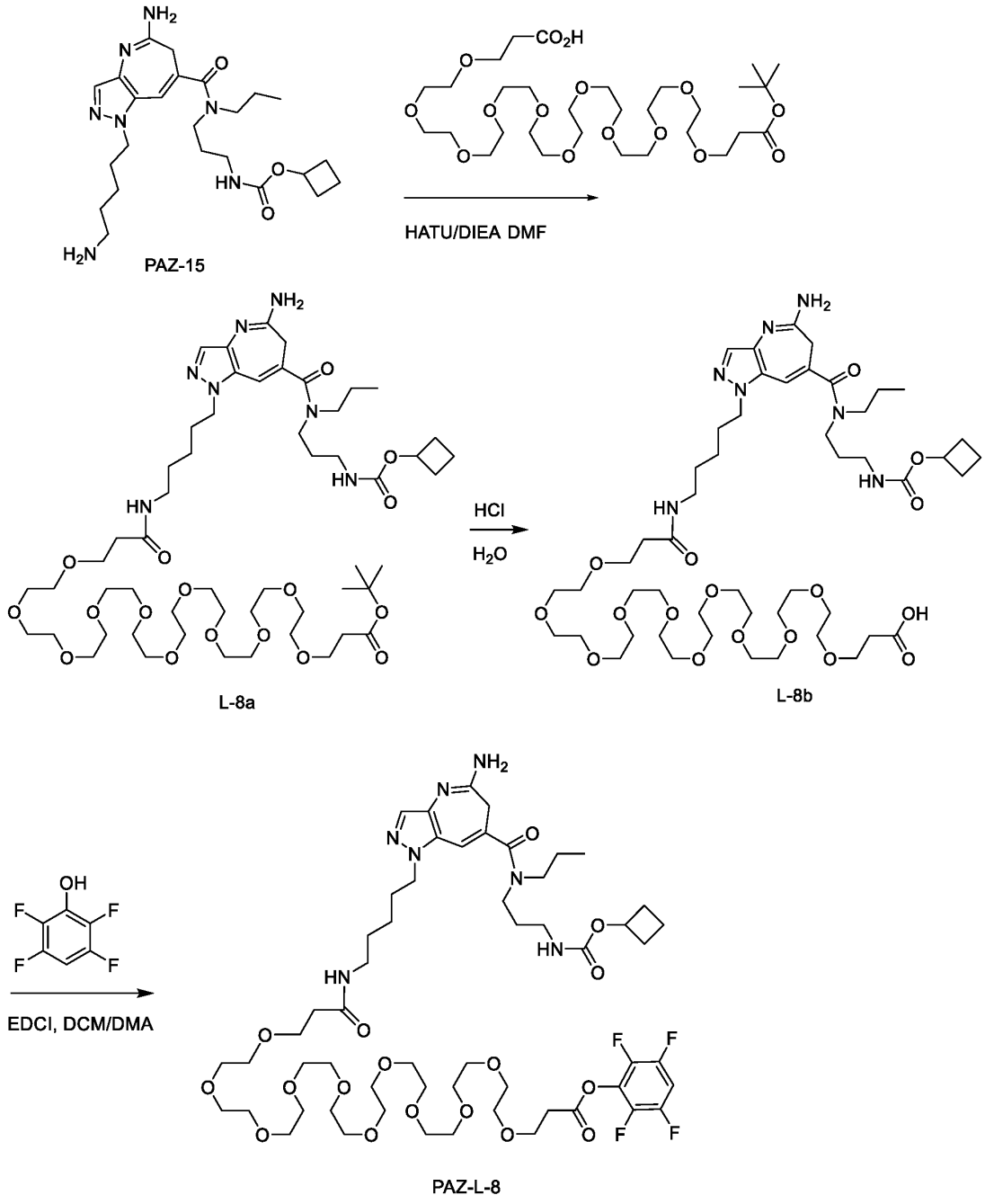
Preparation of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[5-amino-7-[ethoxy(propyl)carbamoyl]-6H-pyrazolo[4,3-b]azepin-1-yl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid, L-7b

To a solution of L-7a (45 mg, 38.36 umol, 1 *eq*, 2TFA) in H_2O (3 mL) was added HCl (12 M, 63.9 uL, 20 *eq*) and then the mixture was stirred at 80°C for 1 h. The reaction mixture was concentrated under reduced pressure to give L-7b (40 mg, crude, 2HCl) as a light yellow oil. LC/MS [M+H] 889.5 (calculated); LC/MS [M+H] 889.6 (observed).

Preparation of PAZ-L-7

To a solution of L-7b (40 mg, 41.58 umol, 1 *eq*, 2HCl) and 2,3,5,6-tetrafluorophenol (69.0 mg, 416 umol, 10 *eq*) in DCM (3 mL) and DMA (0.3 mL) was added EDCI (79.7 mg, 415 umol, 10 *eq*) and then it was stirred at 15°C for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Synergi C18 150*25*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-50%,8min) to give PAZ-L-7 (19.5 mg, 15.41 umol, 37.07% yield, 2TFA) as a light yellow oil. ^1H NMR (MeOD-d_4, 400MHz) δ7.67 (s, 1H), 7.46 (s, 1H), 7.45-7.38 (m, 1H), 4.29 (t, J = 6.8 Hz, 2H), 3.95 (q, J = 7.2 Hz, 2H), 3.88 (t, J = 6.0 Hz, 2H), 3.82 (br d, J = 3.6 Hz, 2H), 3.74 (t, J = 7.2 Hz, 2H), 3.71-3.55 (m, 38H), 3.43 (s, 2H), 3.26-3.03 (m, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.91 (s, 3H), 1.97-1.87 (m, 2H), 1.78-1.74 (m, 4H), 1.44-1.32 (m, 2H), 1.18 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.6 Hz, 3H). LC/MS [M+H] 1037.5 (calculated); LC/MS [M+H] 1037.4 (observed).

Example L-8 Synthesis of 2,3,5,6-tetrafluorophenyl 43-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoate, PAZ-L-8



Preparation of tert-butyl 43-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoate, L-8a

To a solution of 2,2-dimethyl-4-oxo-3,7,10,13,16,19,22,25,28,31,34,37-dodecaoxatetracontan-40-oic acid (77.5 mg, 117 umol, 1 eq) in DMF (0.5 mL) was added HATU (49.2 mg, 129 umol, 1.1 eq) and DIEA (76.0 mg, 588 umol, 102 uL, 5 eq), then cyclobutyl (3-(5-amino-1-(5-aminopentyl)-N-propyl-1,6-dihydropyrazolo[4,3-b]azepine-7-

carboxamido)propyl)carbamate, PAZ-15 (60 mg, 117.6 umol, 1 eq, HCl) was added. The mixture was stirred at 25°C for 0.5 h. The residue was filtered and concentrated under reduced pressure and then purified by prep-HPLC (TFA condition; column: Phenomenex luna C18 100*40mm*5 um;mobile phase: [water(0.1%TFA)-ACN];B%: 10%-45%,8min) to afford L-8a (90 mg, 73.3 umol, 62.29% yield, TFA) as yellow oil. ^{1}H NMR (MeOD-d_4, 400MHz) δ7.66 (s, 1H), 7.16 (br s, 1H), 4.90-4.89 (m, 1H), 4.26 (t, J = 7.2 Hz, 2H), 3.72-3.68 (m, 4H), 3.65-3.57 (m, 44H), 3.55-3.43 (m, 4H), 3.39 (br s, 2H), 3.17-3.11 (m, 2H), 2.47 (t, J = 6.4 Hz, 2H), 2.40 (t, J = 6.0 Hz, 2H), 2.29-2.23 (m, 2H), 2.05-1.99 (m, 2H), 1.90-1.80 (m, 4H), 1.77-1.56 (m, 4H), 1.53-1.41 (m, 12H), 1.32-1.25 (m, 2H), 0.98-0.89 (m, 3H)

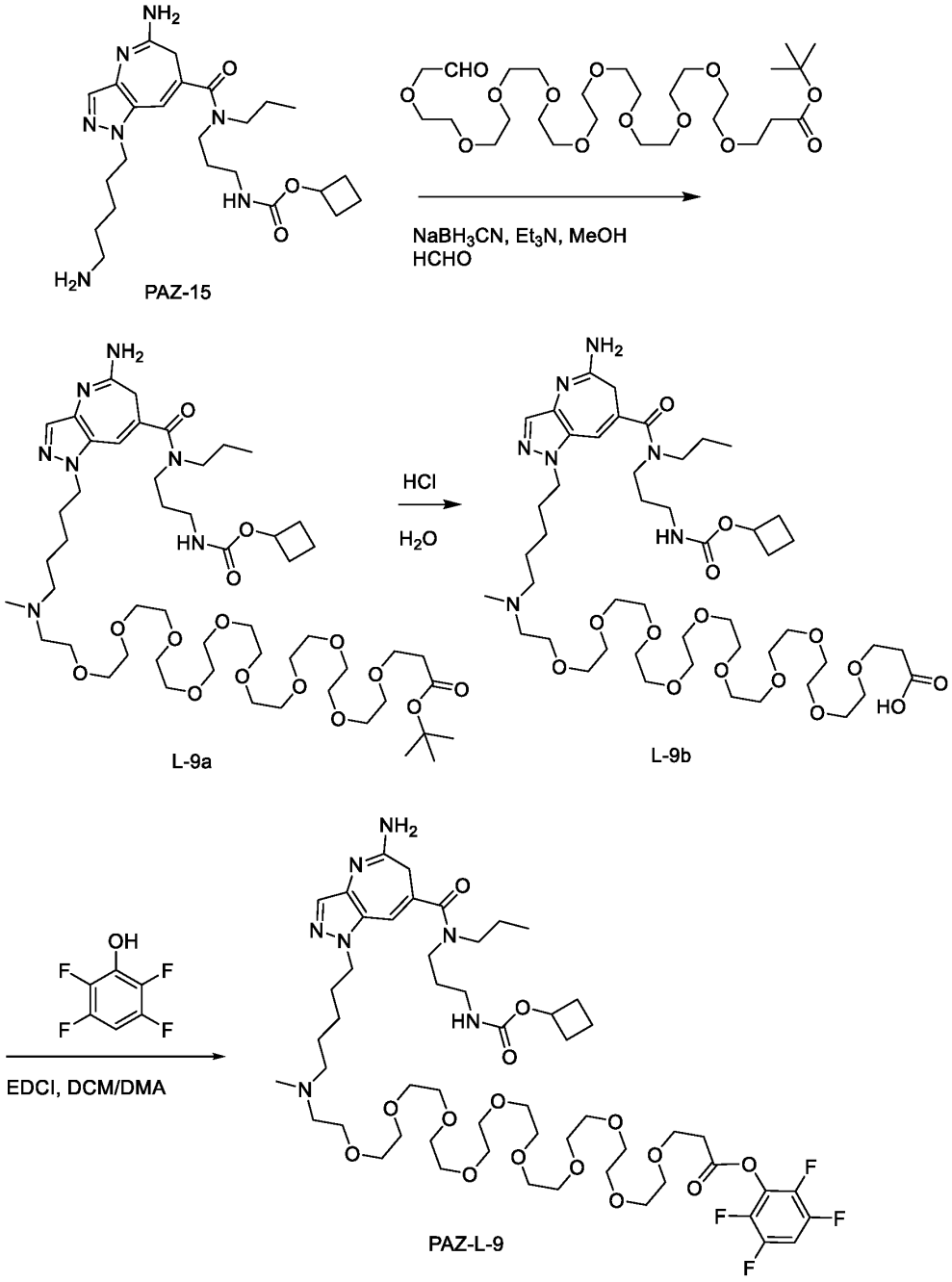
Preparation of 43-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-37-oxo-4,7,10,13,16,19,22,25,28,31,34-undecaoxa-38-azatritetracontanoic acid, L-8b

To a solution of L-8a (50 mg, 44.9 umol, 1 eq, TFA) in water (2 mL) was added HCl (12 M, 74.8 uL, 20 eq) and then the mixture was stirred at 80°C for 0.5 h. The mixture was concentrated under reduced pressure to afford L-8b (40 mg, 37.8 umol, 84.24% yield) as colorless oil.

Preparation of PAZ-L-8.

To a solution of L-8b (40 mg, 34.0 umol, 1 eq, TFA) in DCM (1 mL) and DMA (0.1 mL) was added 2,3,5,6-tetrafluorophenol (45.3 mg, 273 umol, 8 eq) and EDCI (65.4 mg, 341 umol, 10 eq) and it was stirred at 25°C for 0.5 h. The residue was filtered and concentrated under reduced pressure and then purified by prep-HPLC (TFA condition; column: Phenomenex Synergi C18 150*30mm*4um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-50%,8min) to afford PAZ-L-8 (30 mg, 22.7 umol, 66.65% yield, TFA) as a yellow solid. ^{1}H NMR (METHANOL-d_4, 400MHz) δ7.65 (s, 1H), 7.49-7.38 (m, 1H), 7.16 (s, 1H), 4.90-4.89 (m, 1H), 4.25 (t, J = 6.8 Hz, 2H), 3.88 (t, J = 6.0 Hz, 2H), 3.72-3.55 (m, 44H), 3.54-3.44 (m, 4H), 3.38 (br s, 2H), 3.18-3.12 (m, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.40 (t, J = 6.0 Hz, 2H), 2.32-2.24 (m, 2H), 2.04-1.98 (m, 2H), 1.89-1.80 (m, 4H), 1.80-1.56 (m, 4H), 1.55-1.42 (m, 2H), 1.32-1.26 (m, 2H), 0.96-0.89 (m, 3H). LC/MS [M+H] 1206.6 (calculated); LC/MS [M+H] 1206.6 (observed).

Example L-9 Synthesis of 2,3,5,6-tetrafluorophenyl 39-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, PAZ-L-9



Preparation of tert-butyl 39-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, L-9a

To a solution of cyclobutyl (3-(5-amino-1-(5-aminopentyl)-N-propyl-1,6-dihydropyrazolo[4,3-b]azepine-7-carboxamido)propyl)carbamate, PAZ-15 (70 mg, 137 umol, 1 eq, HCl) and tert-butyl 1-oxo-3,6,9,12,15,18,21,24,27,30-decaoxatritriacontan-33-oate (185 mg, 316 umol, 2.3 eq) in MeOH (2 mL) was added $NaBH_3CN$ (17.3 mg, 274.5 umol, 2 eq) and Et_3N

(13.9 mg, 137 umol, 1 eq) and it was stirred at 25°C for 16 h. Then formaldehyde (22.3 mg, 274.5 umol, 20.4 uL, 37% purity, 2 eq) and $NaBH_3CN$ (17.3 mg, 274.5 umol, 2 eq) were added to the mixture and it was stirred at 25°C for another 0.5 h. The residue was filtered and concentrated under reduced pressure then purified by prep-HPLC (TFA condition; column: Phenomenex Gemini-NX C18 75*30mm*3um;mobile phase: [water(0.1%TFA)-ACN];B%: 20%-40%,8min) to afford L-9a (90 mg, 76.90 umol, 56.03% yield, TFA) as yellow oil.

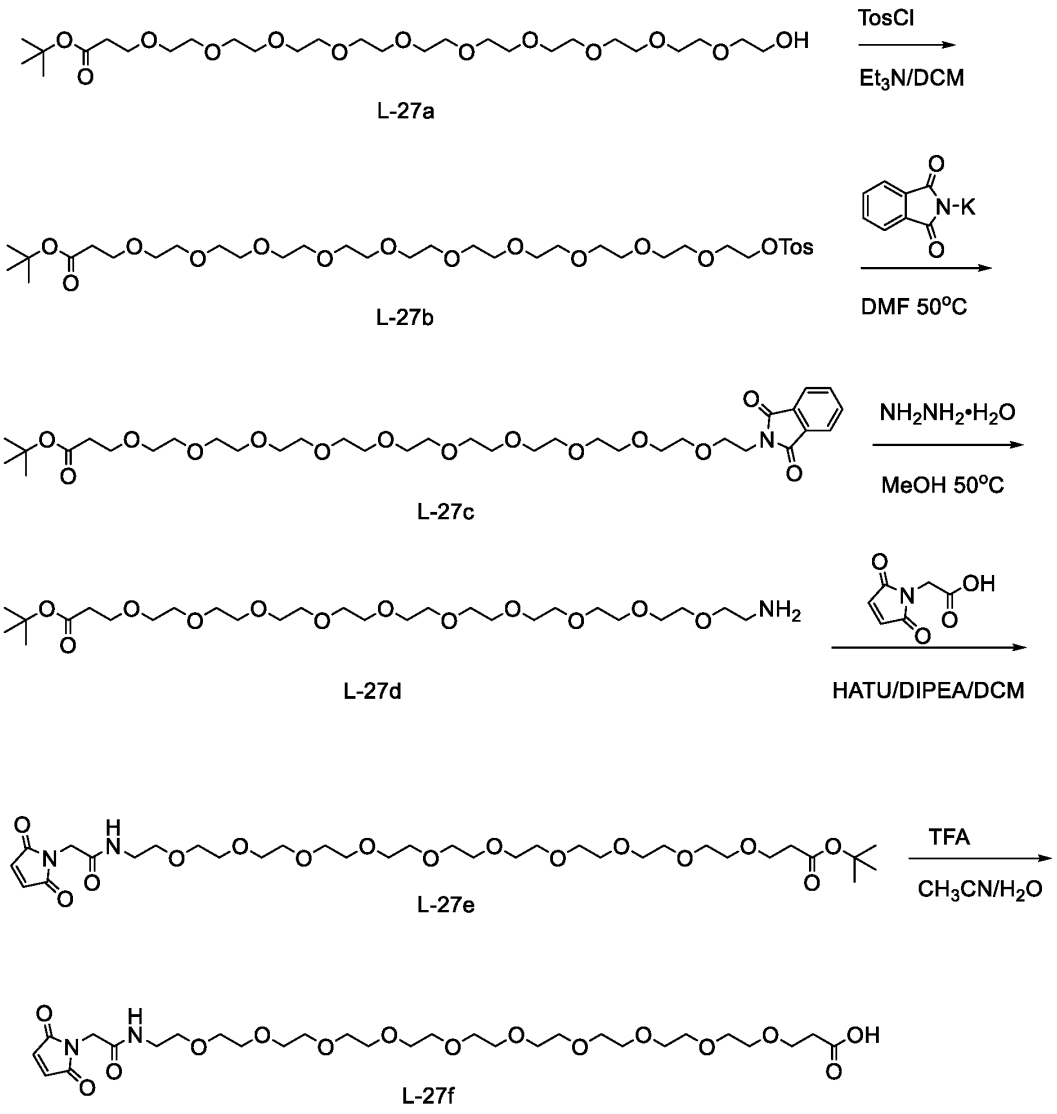
Preparation of 39-(5-amino-7-((3-((cyclobutoxycarbonyl)amino)propyl)(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoic acid, L-9b

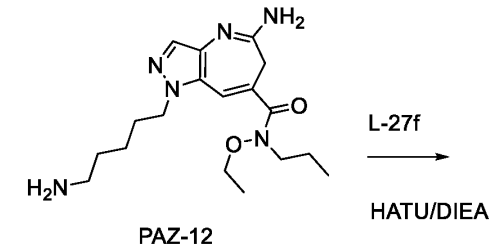
To a solution of L-9a (90 mg, 76.9 umol, 1 eq, TFA) in water (2 mL) was added HCl (12 M, 128 uL, 20 eq) and the mixture was stirred at 80 °C for 0.5 h. The mixture was concentrated under reduced pressure to give L-9b (70 mg, 67.5 umol, 87.81% yield, HCl) as colorless oil.

Preparation of PAZ-L-9

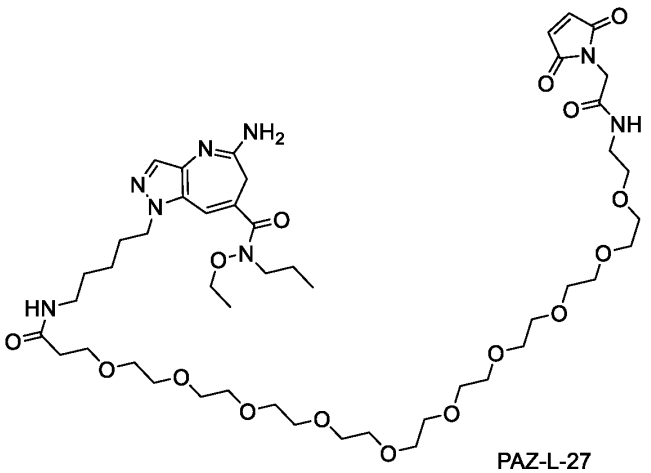
To a solution of L-9b (70 mg, 62.8 umol, 1 eq, TFA) in DCM (2 mL) and DMA (0.1 mL) was added 2,3,5,6-tetrafluorophenol (83.5 mg, 503 umol, 8 eq) and EDCI (120 mg, 628 umol, 10 eq) and then the mixture was stirred at 25 °C for 0.5 h. The residue was filtered and concentrated under reduced pressure and then purified by prep-HPLC (TFA condition; column: Phenomenex Synergi C18 150*30mm*4um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-50%,8min) to afford PAZ-L-9 (40 mg, 31.69 umol, 50.44% yield, TFA) as a yellow solid. ^1H NMR ($MeOD-d_4$, 400MHz) δ7.68 (s, 1H), 7.50-7.39 (m, 1H), 7.16 (br s, 1H), 4.80-4.76 (m, 1H), 4.29 (t, J = 6.8 Hz, 2H), 3.88 (t, J = 6.0 Hz, 2H), 3.83 (br s, 2H), 3.69-3.61 (m, 38H), 3.53-3.48 (m, 2H), 3.44 (br d, J = 7.2 Hz, 2H), 3.38 (br s, 2H), 3.29-3.19 (m, 2H), 3.16-3.05 (m, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.91 (s, 3H), 2.28-2.24 (m, 2H), 2.04-1.98 (m, 2H), 1.96-1.90 (m, 2H), 1.89-1.72 (m, 6H), 1.71-1.62 (m, 2H), 1.42-1.36 (m, 2H), 0.96-0.93 (m, 3H). LC/MS [M+H] 1148.6 (calculated); LC/MS [M+H] 1148.6 (observed).

Example L-27 Synthesis of 5-amino-1-(1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,36-dioxo-6,9,12,15,18,21,24,27,30,33-decaoxa-3,37-diazadotetracontan-42-yl)-N-ethoxy-N-propyl-1,6-dihydropyrazolo[4,3-b]azepine-7-carboxamide, PAZ-L-27


TosCl
Et3N/DCM
L-27a
N-K
DMF 50°C
L-27b
NH2NH2•H2O
MeOH 50°C
L-27c
OH
HATU/DIPEA/DCM
L-27d
TFA
CH3CN/H2O
L-27e
L-27f



PAZ-12



PAZ-L-27

Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(p-tolylsulfonyloxy) ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-27b

To a solution of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-27a (100 g, 170 mmol, 1 eq), TEA (43.1 g, 426 mmol, 59.3 mL, 2.5 eq) and DMAP (2.08 g, 17.0 mmol, 0.1 eq) in DCM (1000 mL) was added TosCl (48.7 g, 255 mmol, 1.5 eq) at 0°C under N_2, and then stirred at 15°C for 12 h. The reaction mixture was quenched by addition of H_2O (2000 mL) at 0°C, and then extracted with DCM (1000 mL x 3). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Petroleum ether:Ethyl acetate = 1:0 to 0:1) and then (SiO_2, EtOAc:MeOH = 1:0 to 10:1) to give L-27b (187.4 g, crude) as a light yellow oil. ^1H NMR ($CDCl_3$, 400 MHz) δ7.81 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.17 (t, J = 4.8 Hz, 2H), 3.74-3.57 (m, 40H), 2.51 (t, J = 6.4 Hz, 2H), 2.46 (s, 3H), 1.45 (s, 9H).

Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(1,3-dioxoisoindolin-2-yl) ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-27c

To a solution of L-27b (127 g, 171 mmol, 1 eq) in DMF (1000 mL) was added(1,3-dioxoisoindolin-2-yl)potassium (41.3 g, 223 mmol, 1.3 eq) at 25°C and then stirred at 50°C for 12 h. The reaction mixture was poured into ice water (3000 mL), and then extracted with EtOAc (800 mL x 6). The combined organic layers were washed with brine (300 mL x 3), dried

over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Petroleum ether:Ethyl acetate = 1:0 to 0:1) and then (SiO_2, EtOAc:MeOH = 1:0 to 10:1) to give L-27c (142 g, crude) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.85 (dd, J = 3.2, 5.6 Hz, 2H), 7.72 (dd, J = 3.2, 5.6 Hz, 2H), 3.96-3.86 (m, 2H), 3.76-3.69 (m, 4H), 3.68-3.55 (m, 36H), 2.51 (t, J = 6.8 Hz, 2H), 1.45 (s, 9H).

Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-27d

To a solution of L-27c (100 g, 140 mmol, 1 eq) in MeOH (1000 mL) was added $NH_2NH_2.H_2O$ (28.54 g, 559 mmol, 27.71 mL, 98% purity, 4 eq) at 25°C and then stirred at 50°C for 8 h. The reaction mixture was cooled to 25°C, and then filtered and the filtrate was concentrated under reduced pressure. The crude product was further triturated with MTBE (500 mL x 3) at 25°C for 30 min, and then filtered and concentrated under reduced pressure to give L-27d (113.7 g, crude) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ3.74-3.58 (m, 38H), 3.51 (t, J = 5.2 Hz, 2H), 2.86 (t, J = 5.2 Hz, 2H), 2.50 (t, J = 6.8 Hz, 2H), 1.45 (s, 9H). LC/MS [M+H] 586.4 (calculated); LC/MS [M+H] 586.4 (observed)

Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl] amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, L-27e

To a solution of L-27d (11.3 g, 19.3 mmol, 1 eq), 2-(2,5-dioxopyrrol-1-yl)acetic acid (3 g, 19.3 mmol, 1 eq) and diisopropylethylamine, DIPEA (10.0 g, 77.4 mmol, 13.5 mL, 4 eq) in DCM (100 mL) was added HATU (8.09 g, 21.3 mmol, 1.1 eq) at 0°C and then stirred at 0°C for 30 min. The reaction mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition; column: Phenomenex luna c18 250mm*100mm*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 25%-55%, 25min) to give L-27e (4.5 g, 6.23 mmol, 32.2% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.88-6.80 (m, 1H), 6.78 (s, 2H), 4.22 (s, 2H), 3.77-3.54 (m, 40H), 3.47 (q, J = 5.2 Hz, 2H), 2.51 (t, J = 6.4 Hz, 2H), 1.46 (s, 9H)

Preparation of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl]amino]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid, L-27f

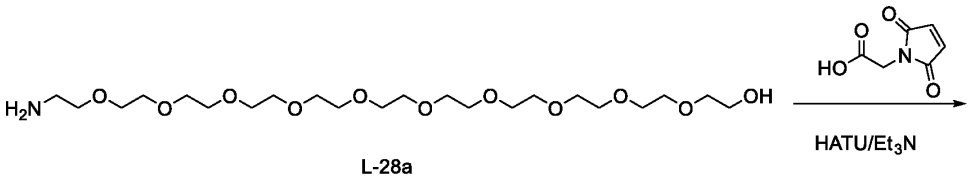
To a solution of L-27e (4.5 g, 6.23 mmol, 1 eq) in CH_3CN (25 mL) and H_2O (25 mL) was added TFA (5.68 g, 49.8 mmol, 3.69 mL, 8 eq), and then stirred at 80 °C for 1 h. The reaction mixture was concentrated under reduced pressure to remove CH_3CN. The residue was extracted with MTBE (10 mL x 3) and discarded. The water phase was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (TFA condition;

column: Phenomenex luna c18 250mm*100mm*10um;mobile phase: [water(0.1%TFA)-ACN];B%: 0%-25%,24min) to give L-27f (1.6 g, 2.40 mmol, 38.6% yield) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.95 (br s, 1H), 6.78 (s, 2H), 4.22 (s, 2H), 3.78 (t, J = 6.4 Hz, 2H), 3.70-3.63 (m, 36H), 3.60-3.54 (m, 2H), 3.46 (q, J = 5.2 Hz, 2H), 2.61 (t, J = 6.0 Hz, 2H). LC/MS [M+H] 667.3 (calculated); LC/MS [M+H] 667.2 (observed).

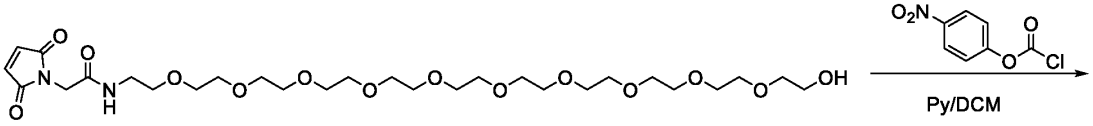
Preparation of PAZ-L-27

To a mixture of L-27f (79.0 mg, 119 umol (micromoles), 1.0 eq) in DMF (0.5 mL) was added HATU (45.1 mg, 119 umol, 1.0 eq), DIEA (61.3 mg, 474 umol, 82.6 uL (microliters), 4.0 eq) and 5-amino-1-(5-aminopentyl)-N-ethoxy-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-12 (70.0 mg, 119 umol, 1.0 eq, 2TFA) at 25 °C and then stirred at this temperature for 0.5 h. The mixture was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3um;mobile phase: [water(TFA)-ACN];B%: 5%-30%,8min) to give PAZ-L-27 (40.4 mg, 39.95 umol, 33.70% yield) as light yellow oil. ^{1}H NMR (MeOD, 400 MHz) δ 7.66 (s, 1H), 7.48 (s, 1H), 6.90 (s, 2H), 4.26 (t, J = 6.8 Hz, 2H), 4.17 (s, 2H), 3.97 (q, J = 7.2 Hz, 2H), 3.74 (t, J = 7.2 Hz, 2H), 3.69 (t, J = 6.0 Hz, 2H), 3.66-3.55 (m, 38H), 3.44 (s, 2H), 3.40-3.35 (m, 2H), 3.14 (t, J = 6.8 Hz, 2H), 2.40 (t, J = 6.0 Hz, 2H), 1.90-1.71 (m, 4H), 1.56-1.45 (m, 2H), 1.34-1.24 (m, 2H), 1.20 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.6 Hz, 3H). LC/MS [M+H] 1011.6 (calculated); LC/MS [M+H] 1011.5 (observed).

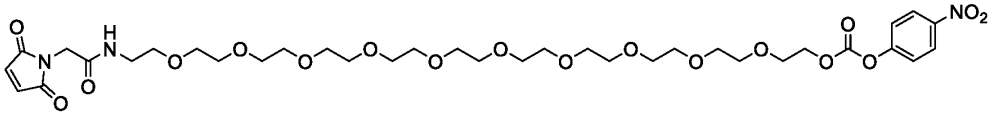
Example L-28 Synthesis of 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15,18,21,24,27,30,33-decaoxa-3-azapentatriacontan-35-yl (5-(5-amino-7-(ethoxy(propyl)carbamoyl)pyrazolo[4,3-b]azepin-1(6H)-yl)pentyl)carbamate, PAZ-L-28



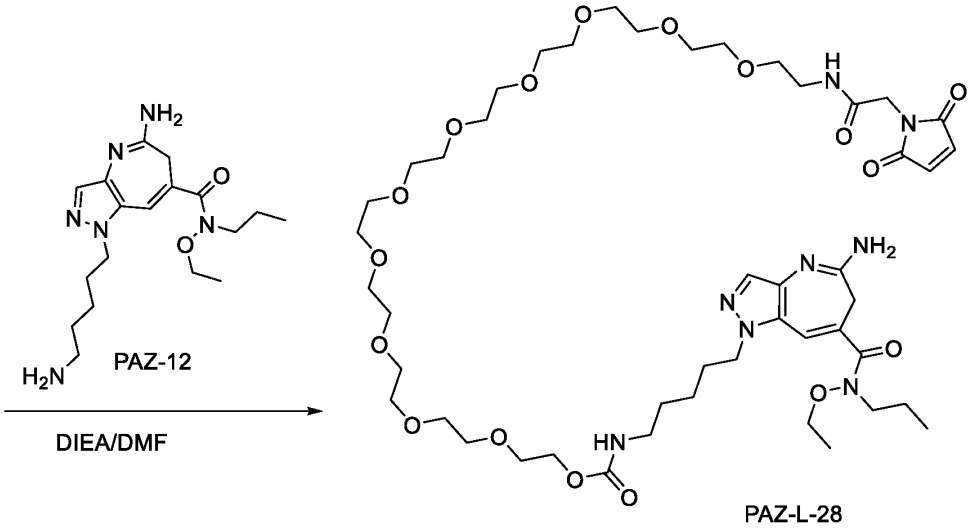
L-28a



L-28b



L-28c



Preparation of 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(32-hydroxy-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)acetamide, L-28b

To a mixture of 2-(2,5-dioxopyrrol-1-yl)acetic acid (309 mg, 1.99 mmol, 1 eq) and 2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethanol, L-28a (1 g, 1.99 mmol, 1 eq) in DCM (5 mL) was added HATU (796 mg, 2.09 mmol, 1.05 eq) and Et_3N (302 mg, 2.99 mmol, 416 uL, 1.5 eq) at 0 °C under N_2 and then stirred at 0 °C for 1 hours. The reaction mixture was washed with H_2O (20 mL*2), the organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum to afford L-28b as colorless oil. ^1H NMR (CDCl3, 400 MHz) δ 6.78 (s, 2H), 6.71-6.76 (m, 1H), 4.21 (s, 2H), 3.55-3.79 (m, 42H), 3.60-3.45 (m, 2H).

Preparation of 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15,18,21,24,27,30,33-decaoxa-3-azapentatriacontan-35-yl (4-nitrophenyl) carbonate, L-28c

To a mixture of L-28b (1 g, 1.57 mmol, 1 eq) and (4-nitrophenyl) carbonochloridate (473 mg, 2.35 mmol, 1.5 eq) in DCM (20 mL) was added pyridine, Py (247 mg, 3.13 mmol, 252 uL, 2 eq) at 25 °C under N_2, and then stirred at 25 °C for 2 hours. The mixture was washed with H_2O (20 mL), and then brine (20 mL), the organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=1/1, 0/1 to EtOAc/MeOH=10/1) to afford L-28c (750 mg, 933.07 umol, 59.59% yield) as light yellow oil. ^1H NMR (CDCl3, 400 MHz) δ 8.23-8.33 (m, 2H), 7.37-7.45 (m, 2H), 6.78 (s, 2H), 6.62-6.69 (m, 1H), 4.41-4.48 (m, 2H), 4.21 (s, 2H), 3.79-3.87 (m, 2H), 3.62-3.73 (m, 36H), 3.56-3.61 (m, 2H), 3.43-3.49 (m, 2H).

Preparation of PAZ-L-28

To a mixture of 5-amino-1-(5-aminopentyl)-N-ethoxy-N-propyl-6H-pyrazolo[4,3-b]azepine-7-carboxamide, PAZ-12 (70 mg, 119 umol, 1.0 *eq*, 2TFA) and L-28c (95.2 mg, 118 umol, 1 *eq*) in DMF (0.5 mL) was added DIEA (61.3 mg, 474 umol, 82.6 uL, 4 .0 *eq*) in one portion at 25 °C and then stirred at 25 °C for 0.5 h. The mixture was filtered, the filtrate was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3um;mobile phase: [water(TFA)-ACN];B%: 5%-30%,8min) to give PAZ-L-28 (23.1 mg, 22.4 umol, 18.9% yield) as light yellow oil. ^{1}H NMR (MeOD, 400 MHz) δ 7.66 (s, 1H), 7.49 (s, 1H), 6.90 (s, 2H), 4.26 (t, J=6.8 Hz, 2H), 4.17 (s, 2H), 4.14-4.09 (m, 2H), 3.97 (q, J=7.2 Hz, 2H), 3.74 (t, J=7.2 Hz, 2H), 3.67-3.60 (m, 38H), 3.55 (t, J=5.6 Hz, 2H), 3.44 (s, 2H), 3.40-3.35 (m, 2H), 3.05 (t, J=6.8 Hz, 2H), 1.91-1.72 (m, 4H), 1.55-1.41 (m, 2H), 1.34-1.23 (m, 2H), 1.20 (t, J=7.2 Hz, 3H), 1.00 (t, J=7.6 Hz, 3H). LC/MS [M+H] 1027.6 (calculated); LC/MS [M+H] 1027.5 (observed).

Example 201 Preparation of Immunoconjugates (IC)

In an exemplary procedure, for preparation for Lysine-based conjugation, an antibody is buffer exchanged into a conjugation buffer containing 100 mM Borate, 50 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid at pH 8.3 using Zeba™ Spin Desalting Columns (Thermo Fisher Scientific). The concentration of the buffer-exchanged antibody was adjusted to approximately 5 – 25 mg/ml using the conjugation buffer and sterile-filtered. The Pyrazoloazepine-linker Formula II compound (PAZ-L) is either dissolved in dimethylsulfoxide (DMSO) or dimethylacetamide (DMA) to a concentration of 5 – 20 mM. For conjugation, the antibody is mixed with 4 – 20 molar equivalents of PAZ-L. In some instances, additional DMA or DMSO up to 20% (v/v), was added to improve the solubility of PAZ-L in the conjugation buffer. The reaction is allowed to proceed for approximately 30 min to 4 hours at 20 °C or 30 °C or 37 °C. The resulting conjugate is purified away from the unreacted PAZ-L using two successive Zeba™ Spin Desalting Columns. The columns are pre-equilibrated with phosphate-buffered saline (PBS), pH 7.2. Adjuvant to antibody ratio (DAR) is estimated by liquid chromatography mass spectrometry analysis using a C4 reverse phase column on an ACQUITY™ UPLC H-class (Waters Corporation, Milford, MA) connected to a XEVO™ G2-XS TOF mass spectrometer (Waters Corporation).

In an exemplary procedure, for preparation for Cysteine-based conjugation, an antibody is buffer exchanged into a conjugation buffer containing PBS, pH 7.2 with 2 mM EDTA using Zeba™ Spin Desalting Columns (Thermo Fisher Scientific). The interchain disulfides are reduced using 2 – 4 molar excess of Tris (2-carboxyethyl) phosphine (TCEP) or dithiothreitol (DTT) at 37 °C for 30 min – 2 hours. Excess TCEP or DTT was removed using a Zeba™ Spin Desalting column pre-equilibrated with the conjugation buffer. The concentration of the buffer-

exchanged antibody was adjusted to approximately 5 – 20 mg/ml using the conjugation buffer and sterile-filtered. The PAZ-L is either dissolved in dimethylsulfoxide (DMSO) or dimethylacetamide (DMA) to a concentration of 5 – 20 mM. For conjugation, the antibody is mixed with 10 – 20 molar equivalents of PAZ-L. In some instances, additional DMA or DMSO up to 20% (v/v), was added to improve the solubility of the PAZ-L in the conjugation buffer. The reaction is allowed to proceed for approximately 30 min to 4 hours at 20 °C. The resulting conjugate is purified away from the unreacted PAZ-L using two successive Zeba™ Spin Desalting Columns. The columns are pre-equilibrated with phosphate-buffered saline (PBS), pH 7.2. Adjuvant to antibody ratio (DAR) is estimated by liquid chromatography mass spectrometry analysis using a C4 reverse phase column on an ACQUITY™ UPLC H-class (Waters Corporation, Milford, MA) connected to a XEVO™ G2-XS TOF mass spectrometer (Waters Corporation).

Following conjugation, to potentially remove unreacted PAZ-L and/or higher-molecular weight aggregate, the conjugates may be purified further using size exclusion chromatography, hydrophobic interaction chromatography, ion exchange chromatography, chromatofocusing, ultrafiltration, centrifugal ultrafiltration, tangential flow filtration, and combinations thereof.

In another exemplary procedure, an antibody is buffer exchanged into a conjugation buffer containing 100 mM boric acid, 50 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid at pH 8.3, using G-25 SEPHADEX™ desalting columns (Sigma-Aldrich, St. Louis, MO). The eluates are then each adjusted to a concentration of about 1-10 mg/ml using the buffer and then sterile filtered. The antibody is pre-warmed to 20-30 °C and rapidly mixed with 2-20 (e.g., 7-10) molar equivalents of PAZ-L. The reaction is allowed to proceed for about 16 hours at 30 °C and the immunoconjugate (IC) is separated from reactants by running over two successive G-25 desalting columns equilibrated in phosphate buffered saline (PBS) at pH 7.2 to provide the Immunoconjugate (IC) of Table 2. Adjuvant-antibody ratio (DAR) is determined by liquid chromatography mass spectrometry analysis using a C4 reverse phase column on an ACQUITY™ UPLC H-class (Waters Corporation, Milford, MA) connected to a XEVO™ G2-XS TOF mass spectrometer (Waters Corporation).

For conjugation, the antibody may be dissolved in a aqueous buffer system known in the art that will not adversely impact the stability or antigen-binding specificity of the antibody. Phosphate buffered saline may be used. The PAZ-L is dissolved in a solvent system comprising at least one polar aprotic solvent as described elsewhere herein. In some such aspects, the PAZ-L is dissolved to a concentration of about 5 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM or about 50 mM, and ranges thereof such as from about 5 mM to about 50mM or from about 10 mM to about 30 mM in pH 8 Tris buffer (e.g., 50 mM Tris). In some aspects, the

PAZ-L is dissolved in DMSO (dimethylsulfoxide), DMA (dimethylacetamide) or acetonitrile, or another suitable dipolar aprotic solvent.

Alternatively in the conjugation reaction, an equivalent excess of PAZ-L solution may be diluted and combined with antibody solution. The PAZ-L solution may suitably be diluted with at least one polar aprotic solvent and at least one polar protic solvent, examples of which include water, methanol, ethanol, n-propanol, and acetic acid. The molar equivalents of PAZ-L to antibody may be about 1.5:1, about 3:1, about 5:1, about 10:1, about 15:1, or about 20:1, and ranges thereof, such as from about 1.5:1 to about 20:1 from about 1.5:1 to about 15:1, from about 1.5:1 to about 10:1,from about 3:1 to about 15:1, from about 3:1 to about 10:1, from about 5:1 to about 15:1 or from about 5:1 to about 10:1. The reaction may suitably be monitored for completion by methods known in the art, such as LC-MS. The conjugation reaction is typically complete in a range from about 1 hour to about 16 hours. After the reaction is complete, a reagent may be added to the reaction mixture to quench the reaction. If antibody thiol groups are reacting with a thiol-reactive group such as maleimide of the PAZ-L, unreacted antibody thiol groups may be reacted with a capping reagent. An example of a suitable capping reagent is ethylmaleimide.

Following conjugation, the immunoconjugates may be purified and separated from unconjugated reactants and/or conjugate aggregates by purification methods known in the art such as, for example and not limited to, size exclusion chromatography, hydrophobic interaction chromatography, ion exchange chromatography, chromatofocusing, ultrafiltration, centrifugal ultrafiltration, tangential flow filtration, and combinations thereof. For instance, purification may be preceded by diluting the immunoconjugate, such in 20 mM sodium succinate, pH 5. The diluted solution is applied to a cation exchange column followed by washing with, e.g., at least 10 column volumes of 20 mM sodium succinate, pH 5. The conjugate may be suitably eluted with a buffer such as PBS.

Example 202 HEK Reporter Assay

HEK293 reporter cells expressing human TLR7 or human TLR8 were purchased from Invivogen and vendor protocols were followed for cellular propagation and experimentation. Briefly, cells were grown to 80-85% confluence at 5% CO_2 in DMEM supplemented with 10% FBS, Zeocin, and Blasticidin. Cells were then seeded in 96-well flat plates at $4x10^4$ cells/well with substrate containing HEK detection medium and immunostimulatory molecules. Activity was measured using a plate reader at 620-655 nm wavelength.

Example 203 Assessment of Immunoconjugate Activity *In Vitro*

This example shows that Immunoconjugates of the invention are effective at eliciting myeloid activation, such as in dendritic cells, and therefore are useful for the treatment of cancer.

Isolation of Human Conventional Dendritic Cells: Human conventional dendritic cells (cDCs) were negatively selected from human peripheral blood obtained from healthy blood donors (Stanford Blood Center, Palo Alto, California) by density gradient centrifugation. Briefly, cells are first enriched by using a ROSETTESEP™ Human CD3 Depletion Cocktail (Stem Cell Technologies, Vancouver, Canada) to remove T cells from the cell preparation. cDCs are then further enriched via negative selection using an EASYSEP™ Human Myeloid DC Enrichment Kit (Stem Cell Technologies).

cDC Activation Assay: 8×10^4 APCs were co-cultured with tumor cells expressing the ISAC target antigen at a 10:1 effector (cDC) to target (tumor cell) ratio. Cells were incubated in 96-well plates (Corning, Corning, NY) containing RPMI-1640 medium supplemented with 10% FBS, and where indicated, various concentrations of the indicated immunoconjugate of the invention (as prepared according to the example above). Following overnight incubation of about 18 hours, cell-free supernatants were collected and analyzed for cytokine secretion (including TNFα) using a BioLegend LEGENDPLEX cytokine bead array.

Activation of myeloid cell types can be measured using various screen assays in addition to the assay described in which different myeloid populations are utilized. These may include the following: monocytes isolated from healthy donor blood, M-CSF differentiated Macrophages, GM-CSF differentiated Macrophages, GM-CSF+IL-4 monocyte-derived Dendritic Cells, conventional Dendritic Cells (cDCs) isolated from healthy donor blood, and myeloid cells polarized to an immunosuppressive state (also referred to as myeloid derived suppressor cells or MDSCs). Examples of MDSC polarized cells include monocytes differentiated toward immunosuppressive state such as M2a MΦ (IL4/IL13), M2c MΦ (IL10/TGFb), GM-CSF/IL6 MDSCs and tumor-educated monocytes (TEM). TEM differentiation can be performed using tumor-conditioned media (e.g. 786.O, MDA-MB-231, HCC1954). Primary tumor-associated myeloid cells may also include primary cells present in dissociated tumor cell suspensions (Discovery Life Sciences).

Assessment of activation of the described populations of myeloid cells may be performed as a mono-culture or as a co-culture with cells expressing the antigen of interest which the ISAC may bind to via the CDR region of the antibody. Following incubation for 18-48 hours, activation may be assessed by upregulation of cell surface co-stimulatory molecules using flow cytometry or by measurement of secreted proinflammatory cytokines. For cytokine

measurement, cell-free supernatant is harvested and analyzed by cytokine bead array (e.g. LegendPlex from Biolegend) using flow cytometry.

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

CLAIMS:

1. An immunoconjugate comprising an antibody covalently attached to one or more 5-aminopyrazoloazepine moieties by a linker, and having Formula I:

$$Ab–[L–PAZ]_p \qquad I$$

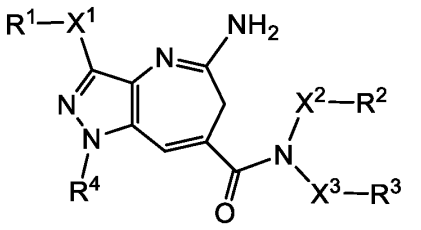
or a pharmaceutically acceptable salt thereof,

wherein:

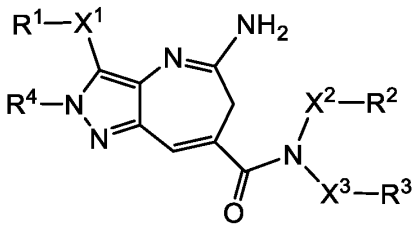
Ab is the antibody;

p is an integer from 1 to 8;

PAZ is the 5-aminopyrazoloazepine moiety selected from formulas IIa and IIb:



IIa



IIb;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, C(=O), C(=O)N(R^5), O, N(R^5), S, S(O)$_2$, and S(O)$_2$N(R^5);

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} carbocyclyl, C_6-C_{20} aryl, C_2-C_9 heterocyclyl, and C_1-C_{20} heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–OR^5;

–(C_3-C_{12} carbocyclyl);

–(C_3-C_{12} carbocyclyl)–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_3-C_{12} carbocyclyl)–NR^5–C(=NR^5)NR^5–*;

–(C_6-C_{20} aryl);

–(C_6-C_{20} aryldiyl)–*;

–(C_6-C_{20} aryldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–$C(=NR^{5a})N(R^5)$–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyl)–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_2-C_9 heterocyclyl)–C(=O)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_2-C_9 heterocyclyl)–NR^5–$C(=NR^{5a})NR^5$–*;

–(C_2-C_9 heterocyclyl)–NR^5–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_2-C_9 heterocyclyl)–(C_6-C_{20} aryldiyl)–*;

–(C_1-C_{20} heteroaryl);

–(C_1-C_{20} heteroaryldiyl)–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{20} heteroaryldiyl)–NR^5–$C(=NR^{5a})N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–$N(R^5)C(=O)$–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–*;

–C(=O)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–$C(=O)N(R^5)_2$;

–C(=O)$N(R^5)$–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$C(=O)N(R^5)$–*;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)R^5$;

–C(=O)$N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)N(R^5)_2$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$N(R^5)CO_2R^5$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=NR^{5a})N(R^5)_2$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^{5a})R^5$;

–$C(=O)NR^5$–(C_1-C_8 alkyldiyl)–NR^5(C_2-C_5 heteroaryl);

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–$N(R^5)$–*;

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–*;

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

–N(R^5)$_2$;

–N(R^5)–*;

–N(R^5)C(=O)R^5;

–N(R^5)C(=O)–*;

–N(R^5)C(=O)N(R^5)$_2$;

–N(R^5)C(=O)N(R^5)–*;

–N(R^5)CO$_2$R^5;

–N(R^5)CO$_2$(R^5)–*;

–NR^{5}C(=NR^{5a})N(R^5)$_2$;

–NR^{5}C(=NR^{5a})N(R^5)–*;

–NR^{5}C(=NR^{5a})R^5;

–N(R^5)C(=O)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–N(R^5)–(C_2-C_5 heteroaryl);

–N(R^5)–S(=O)$_2$–(C_1-C_{12} alkyl);

–O–(C_1-C_{12} alkyl);

–O–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–O–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–OC(=O)N(R^5)$_2$;

–OC(=O)N(R^5)–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

or R^2 and R^3 together form a 5- or 6-membered heterocyclyl ring;

R^5 is selected from the group consisting of H, C_6-C_{20} aryl, C_3-C_{12} carbocyclyl, C_2-C_{20} heterocyclyl, C_6-C_{20} aryldiyl, C_1-C_{12} alkyl, and C_1-C_{12} alkyldiyl, or two R^5 groups together form a 5- or 6-membered heterocyclyl ring;

R^{5a} is selected from the group consisting of C_6-C_{20} aryl and C_1-C_{20} heteroaryl;

where the asterisk * indicates the attachment site of L, and where one of R^1, R^2, R^3 and R^4 is attached to L;

L is the linker selected from the group consisting of:

–C(=O)–PEG–;

–C(=O)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

–C(=O)–PEG–O–;

–C(=O)–PEG–O–C(=O)–;

–C(=O)–PEG–C(=O)–;

–C(=O)–PEG–C(=O)–PEP–;

–C(=O)–PEG–N(R^6)–;

–C(=O)–PEG–N(R^6)–C(=O)–;

–C(=O)–PEG–N(R^6)–PEG–C(=O)–PEP–;

–C(=O)–PEG–N^+(R^6)$_2$–PEG–C(=O)–PEP–;

–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–C(=O)–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^5)–C(=O);

–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–C(=O)–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–C(=O)–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–PEP–;

–succinimidyl–(CH_2)$_m$–C(=O)N(R^6)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

–succinimidyl–(CH_2)$_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

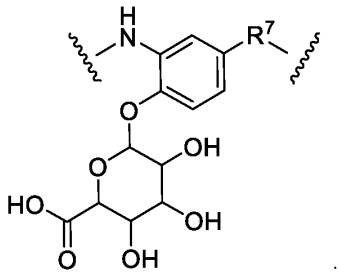
–succinimidyl–(CH_2)$_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–; and

–succinimidyl–$(CH_2)_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

R^6 is independently H or C_1-C_6 alkyl;

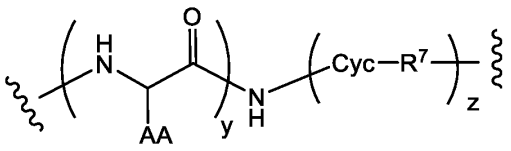
PEG has the formula: –$(CH_2CH_2O)_n$–$(CH_2)_m$–; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:



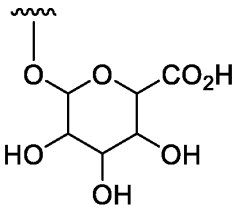
;

PEP has the formula:



where AA is independently selected from a natural or unnatural amino acid side chain, or one or more of AA, and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

Cyc is selected from C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl, optionally substituted with one or more groups selected from F, Cl, NO_2, –OH, $–OCH_3$, and a glucuronic acid having the structure:



;

R^7 is selected from the group consisting of $–CH(R^8)O–$, $–CH_2–$, $–CH_2N(R^8)–$, and $–CH(R^8)O–C(=O)–$, where R^8 is selected from H, C_1-C_6 alkyl, C(=O)–C_1-C_6 alkyl, and $–C(=O)N(R^9)_2$, where R^9 is independently selected from the group consisting of H, C_1-C_{12} alkyl, and –$(CH_2CH_2O)_n$–$(CH_2)_m$–OH, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R^9 groups together form a 5- or 6-membered heterocyclyl ring;

y is an integer from 2 to 12;

z is 0 or 1; and

alkyl, alkyldiyl, alkenyl, alkenyldiyl, alkynyl, alkynyldiyl, aryl, aryldiyl, carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are independently and optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $–CH_3$, $–CH_2CH_3$, $–CH=CH_2$, $–C\equiv CH$, $–C\equiv CCH_3$, $–CH_2CH_2CH_3$, $–CH(CH_3)_2$, $–CH_2CH(CH_3)_2$, $–CH_2OH$, $–CH_2OCH_3$, $–CH_2CH_2OH$, $–C(CH_3)_2OH$, $–CH(OH)CH(CH_3)_2$, $–C(CH_3)_2CH_2OH$, $–CH_2CH_2SO_2CH_3$, $–CH_2OP(O)(OH)_2$, $–CH_2F$, $–CHF_2$, $–CF_3$, $–CH_2CF_3$, $–CH_2CHF_2$, $–CH(CH_3)CN$, $–C(CH_3)_2CN$, $–CH_2CN$, $–CH_2NH_2$, $–CH_2NHSO_2CH_3$, $–CH_2NHCH_3$, $–CH_2N(CH_3)_2$, $–CO_2H$, $–COCH_3$, $–CO_2CH_3$, $–CO_2C(CH_3)_3$, $–COCH(OH)CH_3$, $–CONH_2$, $–CONHCH_3$, $–CON(CH_3)_2$, $–C(CH_3)_2CONH_2$, $–NH_2$, $–NHCH_3$, $–N(CH_3)_2$, $–NHCOCH_3$, $–N(CH_3)COCH_3$, $–NHS(O)_2CH_3$, $–N(CH_3)C(CH_3)_2CONH_2$, $–N(CH_3)CH_2CH_2S(O)_2CH_3$, –NHC(=NH)H, $–NHC(=NH)CH_3$, $–NHC(=NH)NH_2$, $–NHC(=O)NH_2$, $–NO_2$, =O, –OH, $–OCH_3$, $–OCH_2CH_3$, $–OCH_2CH_2OCH_3$, $–OCH_2CH_2OH$, $–OCH_2CH_2N(CH_3)_2$, $–O(CH_2CH_2O)_n–(CH_2)_mCO_2H$, $–O(CH_2CH_2O)_nH$, $–OP(O)(OH)_2$, $–S(O)_2N(CH_3)_2$, $–SCH_3$, $–S(O)_2CH_3$, and $–S(O)_3H$.

2. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds PD-L1.

3. The immunoconjugate of claim 2 wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, and avelumab, or a biosimilar or a biobetter thereof.

4. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds HER2.

5. The immunoconjugate of claim 4 wherein the antibody is selected from the group consisting of trastuzumab and pertuzumab, or a biosimilar or a biobetter thereof.

6. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds CEA.

7. The immunoconjugate of claim 6 wherein the antibody is labetuzumab, or a biosimilar or a biobetter thereof.

8. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds TROP2.

9. The immunoconjugate of claim 8 wherein the antibody is sacituzumab, or a biosimilar or a biobetter thereof.

10. The immunoconjugate of any one of claims 1 to 9 wherein X^1 is a bond, and R^1 is H.

11. The immunoconjugate of any one of claims 1 to 9 wherein X^2 is a bond, and R^2 is C_1-C_8 alkyl.

12. The immunoconjugate of any one of claims 1 to 9 wherein X^2 and X^3 are each a bond, and R^2 and R^3 are independently selected from C_1-C_8 alkyl, –O–(C_1-C_{12} alkyl), –(C_1-C_{12} alkyldiyl)–OR^5, –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^5$, –(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$, –O–(C_1-C_{12} alkyl)–$N(R^5)CO_2R^5$, and –O–(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$.

13. The immunoconjugate of claim 12 wherein R^2 is C_1-C_8 alkyl and R^3 is –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^4$.

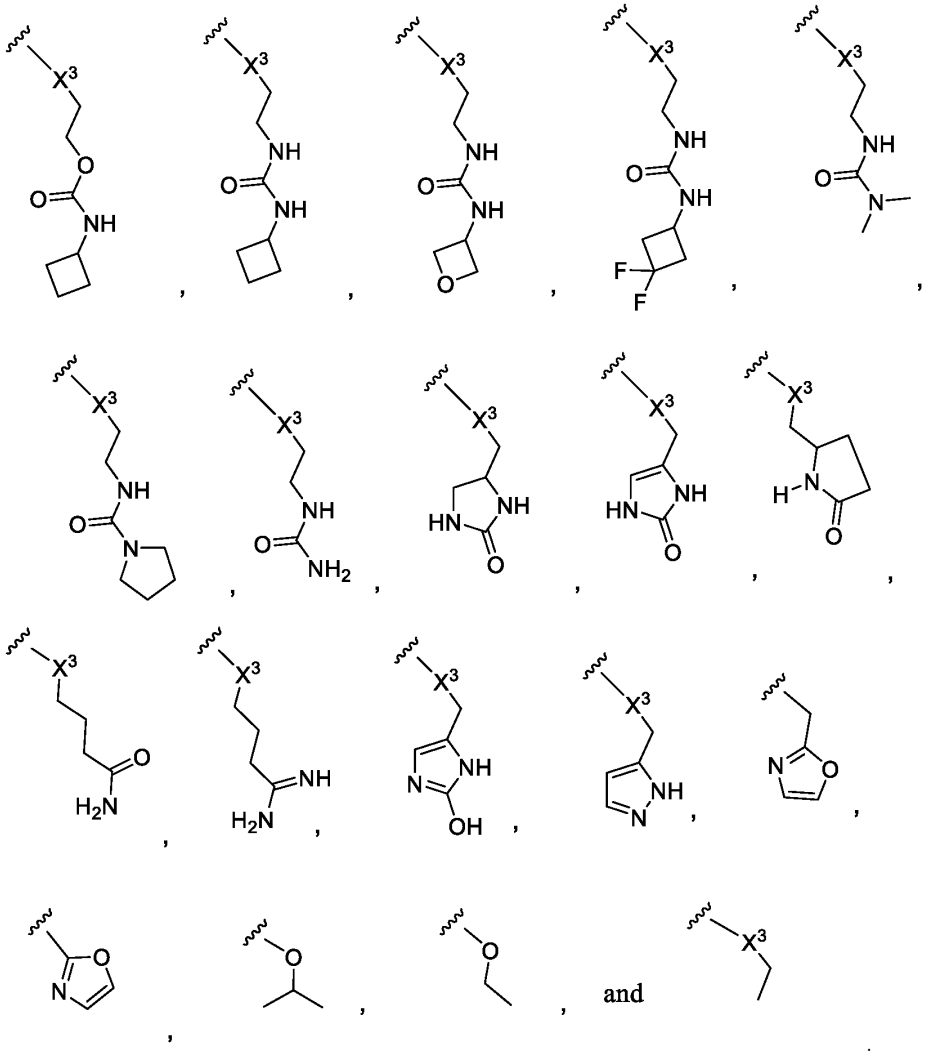
14. The immunoconjugate of claim 12 wherein R^2 is $–CH_2CH_2CH_3$ and R^3 is selected from $–CH_2CH_2CH_2NHCO_2$(t-Bu), $–OCH_2CH_2NHCO_2$(cyclobutyl), and $–CH_2CH_2CH_2NHCO_2$(cyclobutyl).

15. The immunoconjugate of claim 12 wherein R^2 and R^3 are each independently selected from $–CH_2CH_2CH_3$, $–OCH_2CH_3$, $–OCH_2CF_3$, $–CH_2CH_2CF_3$, $–OCH_2CH_2OH$, and $–CH_2CH_2CH_2OH$.

16. The immunoconjugate of claim 12 wherein R^2 and R^3 are each $–CH_2CH_2CH_3$.

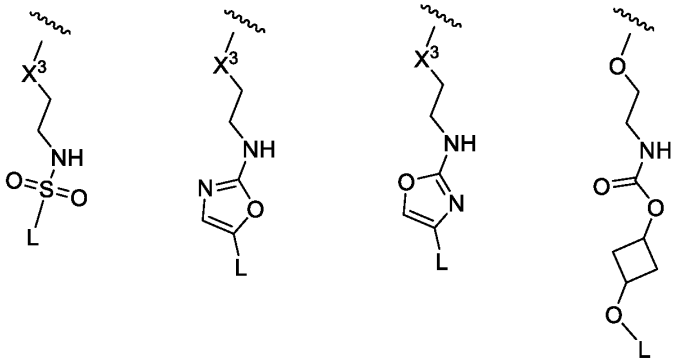
17. The immunoconjugate of claim 12 wherein R^2 is $–CH_2CH_2CH_3$ and R^3 is $–OCH_2CH_3$.

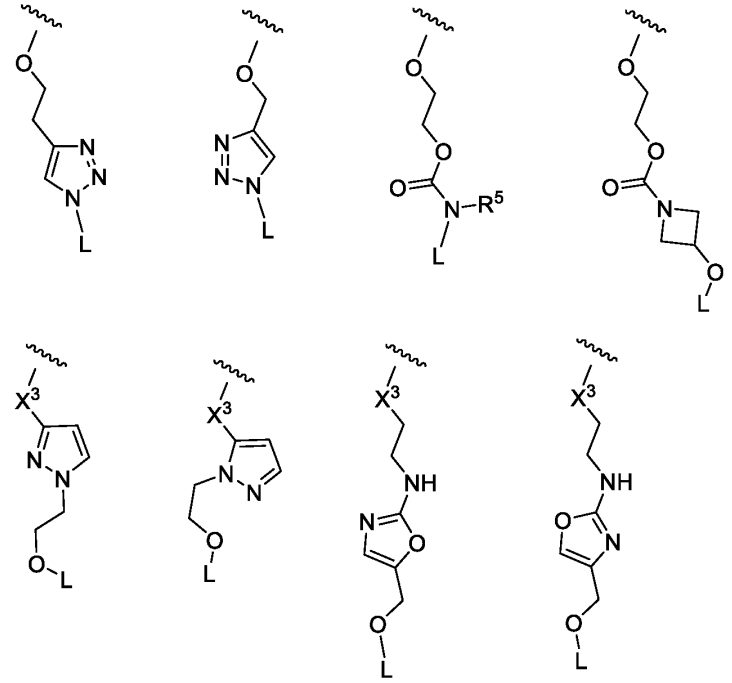
18. The immunoconjugate of any one of claims 1 to 9 wherein X^3-R^3 is selected from the group consisting of:



19. The immunoconjugate of any one of claims 1 to 9 where R^2 or R^3 is attached to L.

20. The immunoconjugate of claim 19 wherein X^3–R^3–L is selected from the group consisting of:





where the wavy line indicates the point of attachment to N.

21. The immunoconjugate of any one of claims 1 to 9 wherein R^4 is C_1-C_{12} alkyl.

22. The immunoconjugate of any one of claims 1 to 9 wherein R^4 is –(C_1-C_{12} alkyldiyl)–N(R^5)–*; where the asterisk * indicates the attachment site of L.

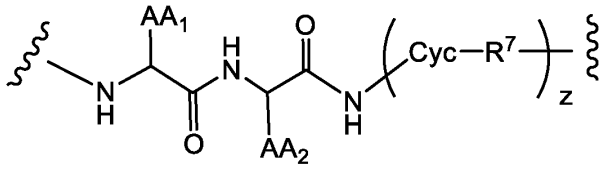
23. The immunoconjugate of any one of claims 1 to 9 wherein L is –C(=O)–PEG– or –C(=O)–PEG–C(=O)–.

24. The immunoconjugate of any one of claims 1 to 9 wherein L is attached to a cysteine thiol of the antibody.

25. The immunoconjugate of any one of claims 1 to 9 wherein for the PEG, m is 1 or 2, and n is an integer from 2 to 10.

26. The immunoconjugate of claim 25 wherein n is 10.

27. The immunoconjugate of any one of claims 1 to 9 wherein L comprises PEP and PEP is a dipeptide and has the formula:

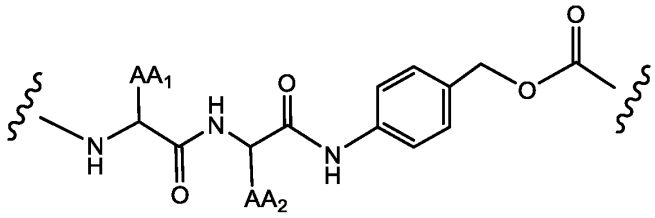


28. The immunoconjugate of claim 27 wherein AA_1 and AA_2 are independently selected from H, $–CH_3$, $–CH(CH_3)_2$, $–CH_2(C_6H_5)$, $–CH_2CH_2CH_2CH_2NH_2$, $–CH_2CH_2CH_2NHC(NH)NH_2$, $–CHCH(CH_3)CH_3$, $–CH_2SO_3H$, and $–CH_2CH_2CH_2NHC(O)NH_2$; or AA_1 and AA_2 form a 5-membered ring proline amino acid.

29. The immunoconjugate of claim 27 wherein AA_1 is $–CH(CH_3)_2$, and AA_2 is $–CH_2CH_2CH_2NHC(O)NH_2$.

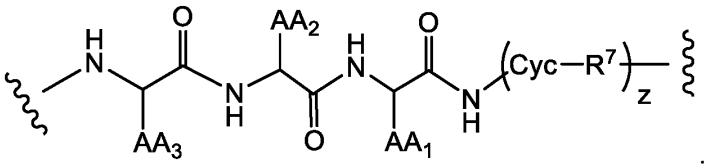
30. The immunoconjugate of claim 27 wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $–CH_2SO_3H$, and $–CH_2OPO_3H$.

31. The immunoconjugate of claim 27 wherein PEP has the formula:

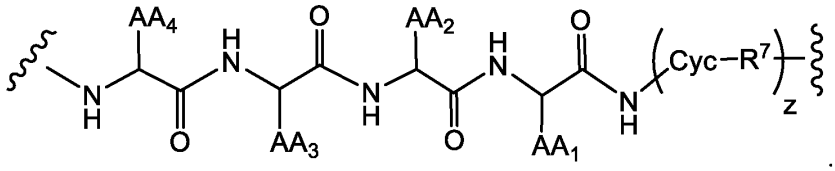


wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

32. The immunoconjugate of any one of claims 1 to 9 wherein L comprises PEP and PEP is a tripeptide and has the formula:



33. The immunoconjugate of any one of claims 1 to 9 wherein L comprises PEP and PEP is a tetrapeptide and has the formula:



34. The immunoconjugate of claim 33 wherein

AA_1 is selected from the group consisting of Abu, Ala, and Val;

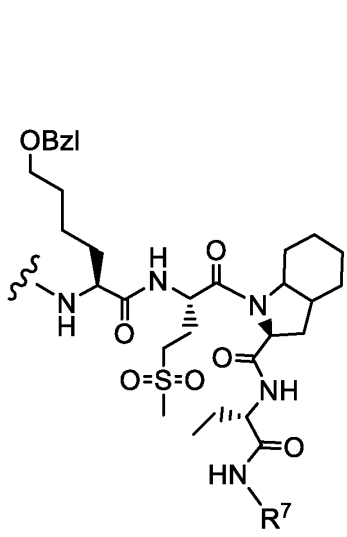
AA_2 is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

AA_3 is selected from the group consisting of Ala and $Met(O)_2$; and

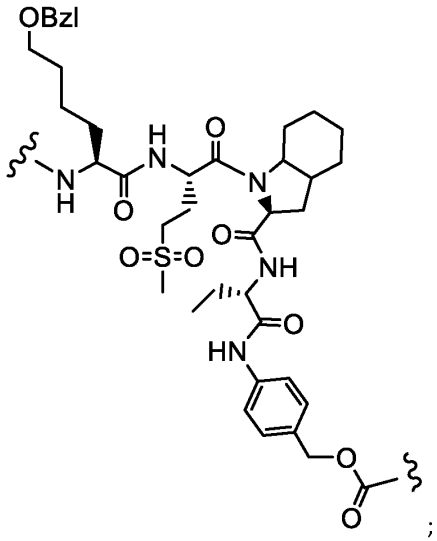
AA_4 is selected from the group consisting of Oic, $Arg(NO_2)$, Bpa, and Nle(O-Bzl).

35. The immunoconjugate of any one of claims 1 to 9 wherein L comprises PEP and PEP is selected from the group consisting of Ala-Pro-Val, Asn-Pro-Val, Ala-Ala-Val, Ala-Ala-Pro-Ala, Ala-Ala-Pro-Val, and Ala-Ala-Pro-Nva.

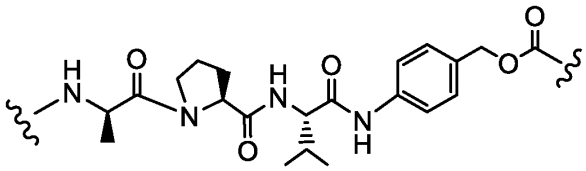
36. The immunoconjugate of any one of claims 1 to 9 wherein L comprises PEP and PEP is selected from the structures:



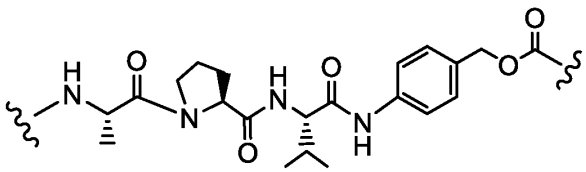
;



;

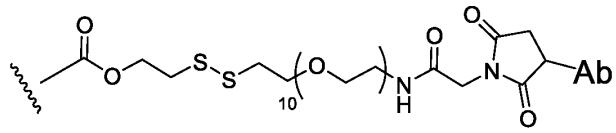


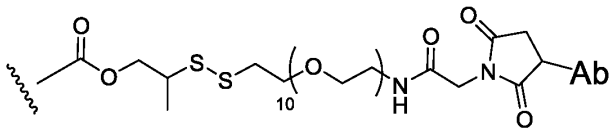
; and

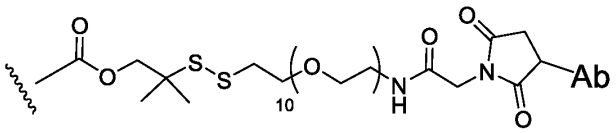


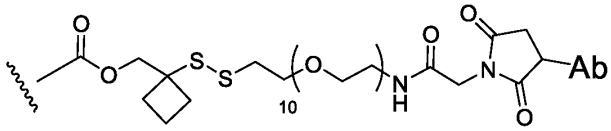
.

37. The immunoconjugate of any one of claims 1 to 9 wherein L is selected from the structures:



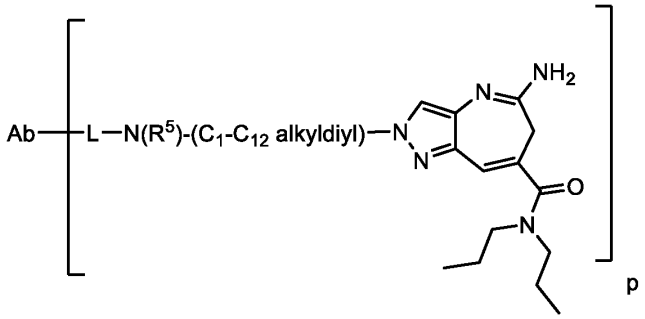




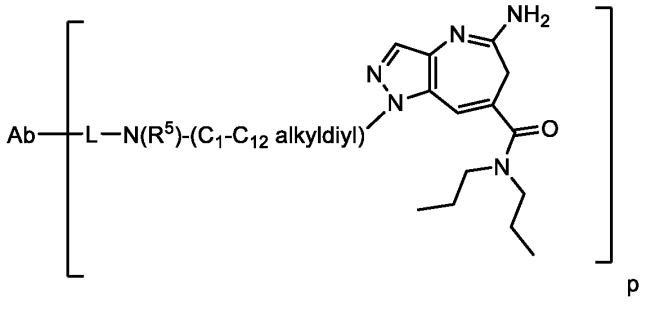


where the wavy line indicates the attachment to R^5.

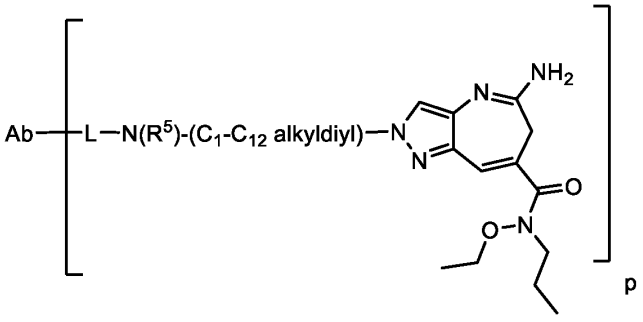
38. The immunoconjugate of any one of claims 1 to 9 selected from Formulae Ia-Id:



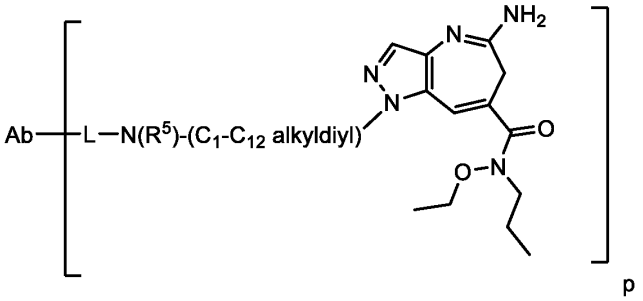
Ia;



Ib;

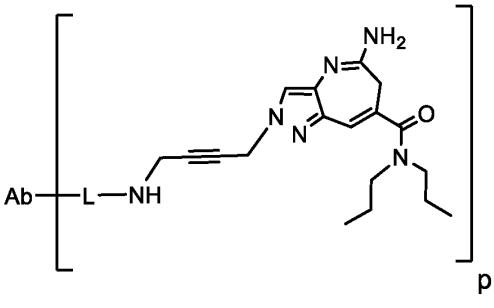


Ic; and

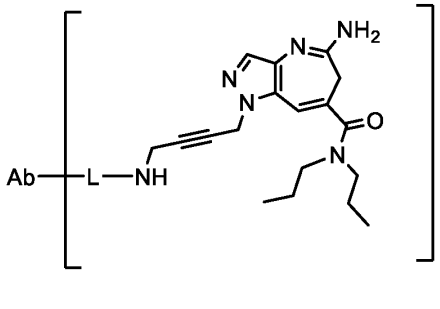


Id.

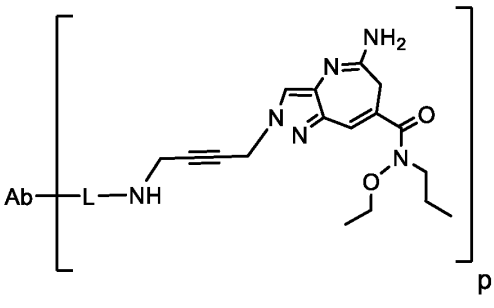
39. The immunoconjugate of any one of claims 1 to 9 selected from Formulae Ie-Il:



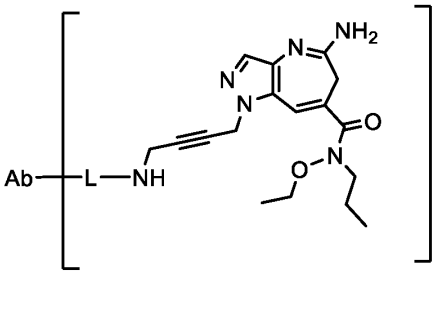
Ie;



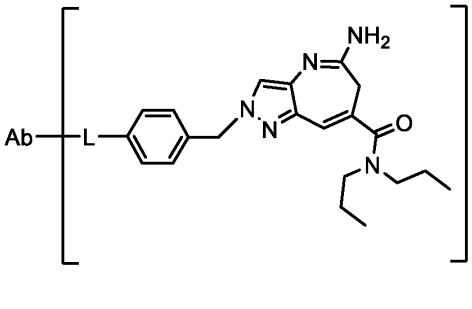
If;



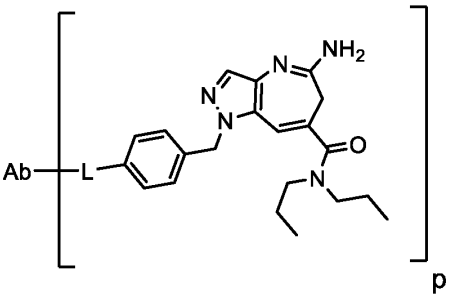
Ig;



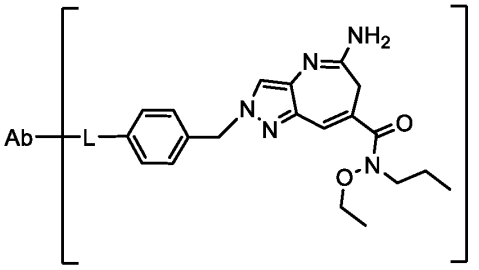
Ih;



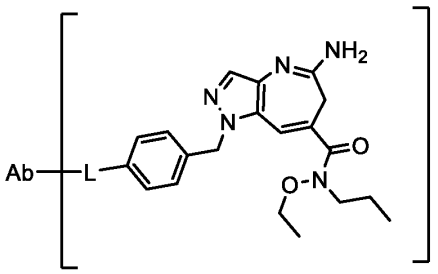
Ii;



Ij;

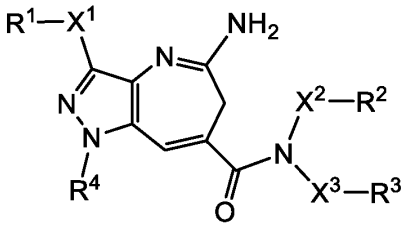


Ik; and

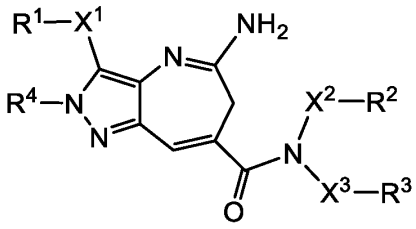


Il.

40. A 5-aminopyrazoloazepine-linker compound selected from formulas IIa and IIb:



IIa



IIb;

wherein X^1, X^2, and X^3 are independently selected from the group consisting of a bond, C(=O), C(=O)N(R^5), O, N(R^5), S, S(O)$_2$, and S(O)$_2$N(R^5);

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} carbocyclyl, C_6-C_{20} aryl, C_2-C_9 heterocyclyl, and C_1-C_{20} heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–OR^5;

–(C_3-C_{12} carbocyclyl);

–(C_3-C_{12} carbocyclyl)–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_3-C_{12} carbocyclyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_3-C_{12} carbocyclyl)–NR^5–C(=NR^5)NR^5–*;

–(C_6-C_{20} aryl);

–(C_6-C_{20} aryldiyl)–*;

–(C_6-C_{20} aryldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–C(=NR^{5a})N(R^5)–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyl)–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_9 heterocyclyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_2-C_9 heterocyclyl)–C(=O)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_2-C_9 heterocyclyl)–NR^5–$C(=NR^{5a})NR^5$–*;

–(C_2-C_9 heterocyclyl)–NR^5–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_2-C_9 heterocyclyl)–(C_6-C_{20} aryldiyl)–*;

–(C_1-C_{20} heteroaryl);

–(C_1-C_{20} heteroaryldiyl)–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{20} heteroaryldiyl)–NR^5–$C(=NR^{5a})N(R^5)$–*;

–(C_1-C_{20} heteroaryldiyl)–$N(R^5)C(=O)$–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–*;

–C(=O)–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–$C(=O)N(R^5)_2$;

–$C(=O)N(R^5)$–*;

–$C(=O)N(R^5)$–(C_1-C_{12} alkyldiyl)–*;

–$C(=O)N(R^5)$–(C_1-C_{12} alkyldiyl)–$C(=O)N(R^5)$–*;

–$C(=O)N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)R^5$;

–$C(=O)N(R^5)$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)N(R^5)_2$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$N(R^5)CO_2R^5$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=NR^{5a})N(R^5)_2$;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^{5a})R^5$;

–$C(=O)NR^5$–(C_1-C_8 alkyldiyl)–NR^5(C_2-C_5 heteroaryl);

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–$N(R^5)$–*;

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–*;

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–$C(=O)NR^5$–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–NR^5–*;

–$N(R^5)_2$;

–$N(R^5)$–*;

–$N(R^5)C(=O)R^5$;

–$N(R^5)C(=O)$–*;

–$N(R^5)C(=O)N(R^5)_2$;

–$N(R^5)C(=O)N(R^5)$–*;

–$N(R^5)CO_2R^5$;

–$N(R^5)CO_2(R^5)$–*;

–$NR^5C(=NR^{5a})N(R^5)_2$;

–$NR^5C(=NR^{5a})N(R^5)$–*;

–$NR^5C(=NR^{5a})R^5$;

–$N(R^5)C(=O)$–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–$N(R^5)$–(C_2-C_5 heteroaryl);

–$N(R^5)$–$S(=O)_2$–(C_1-C_{12} alkyl);

–O–(C_1-C_{12} alkyl);

–O–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–O–(C_1-C_{12} alkyldiyl)–$N(R^5)$–*;

–$OC(=O)N(R^5)_2$;

–$OC(=O)N(R^5)$–*;

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–*;

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

or R^2 and R^3 together form a 5- or 6-membered heterocyclyl ring;

R^5 is selected from the group consisting of H, C_6-C_{20} aryl, C_3-C_{12} carbocyclyl, C_2-C_{20} heterocyclyl, C_6-C_{20} aryldiyl, C_1-C_{12} alkyl, and C_1-C_{12} alkyldiyl, or two R^5 groups together form a 5- or 6-membered heterocyclyl ring;

R^{5a} is selected from the group consisting of C_6-C_{20} aryl and C_1-C_{20} heteroaryl;

where the asterisk * indicates the attachment site of L, and where one of R^1, R^2, R^3 and R^4 is attached to L;

L is the linker selected from the group consisting of:

Q–C(=O)–PEG–;

Q–C(=O)–PEG–$C(=O)N(R^6)$–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

Q–C(=O)–PEG–O–;

Q–C(=O)–PEG–O–C(=O)–;

Q–C(=O)–PEG–C(=O)–;

Q–C(=O)–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–N(R^6)–;

Q–C(=O)–PEG–N(R^6)–C(=O)–;

Q–C(=O)–PEG–N(R^6)–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–N^+(R^6)$_2$–PEG–C(=O)–PEP–;

Q–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

Q–C(=O)–PEG–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

Q–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

Q–C(=O)–PEG–SS–(C_1-C_{12} alkyldiyl)–C(=O)–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^5)–C(=O);

Q–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)N(R^6)–(C_1-C_{12} alkyldiyl)–C(=O)–Gluc–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–O–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–N(R^5)–C(=O)–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–C(=O)–PEP–;

Q–(CH_2)$_m$–C(=O)N(R^6)–PEG–SS–(C_1-C_{12} alkyldiyl)–OC(=O)–;

Q–(CH_2)$_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)–;

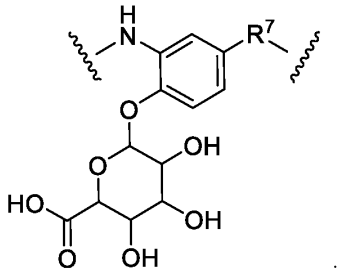
Q–(CH_2)$_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–; and

Q–(CH_2)$_m$–C(=O)–PEP–N(R^6)–(C_1-C_{12} alkyldiyl)N(R^6)C(=O)–(C_2-C_5 monoheterocyclyldiyl)–;

R^6 is independently H or C_1-C_6 alkyl;

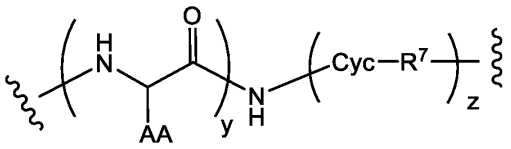
PEG has the formula: $–(CH_2CH_2O)_n–(CH_2)_m–$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:



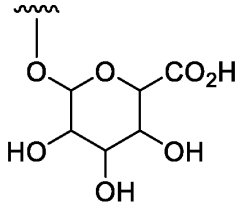
;

PEP has the formula:



where AA is independently selected from a natural or unnatural amino acid side chain, or one or more of AA, and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

Cyc is selected from C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl, optionally substituted with one or more groups selected from F, Cl, NO_2, –OH, $–OCH_3$, and a glucuronic acid having the structure:



;

R^7 is selected from the group consisting of $–CH(R^8)O–$, $–CH_2–$, $–CH_2N(R^8)–$, and $–CH(R^8)O–C(=O)–$, where R^8 is selected from H, C_1-C_6 alkyl, $C(=O)–C_1$-C_6 alkyl, and $–C(=O)N(R^9)_2$, where R^9 is independently selected from the group consisting of H, C_1-C_{12} alkyl, and $–(CH_2CH_2O)_n–(CH_2)_m–OH$, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R^9 groups together form a 5- or 6-membered heterocyclyl ring;

y is an integer from 2 to 12;

z is 0 or 1; and

Q is selected from the group consisting of N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, maleimide, and phenoxy substituted with one or more groups independently selected from F, Cl, NO_2, and SO_3^-;

where alkyl, alkyldiyl, alkenyl, alkenyldiyl, alkynyl, alkynyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, –CH_3, –CH_2CH_3, –CH=CH_2, –C≡CH, –C≡CCH_3, –$CH_2CH_2CH_3$, –$CH(CH_3)_2$, –$CH_2CH(CH_3)_2$, –CH_2OH, –CH_2OCH_3, –CH_2CH_2OH, –$C(CH_3)_2OH$, –$CH(OH)CH(CH_3)_2$, –$C(CH_3)_2CH_2OH$, –$CH_2CH_2SO_2CH_3$, –$CH_2OP(O)(OH)_2$, –CH_2F, –CHF_2, –CF_3, –CH_2CF_3, –CH_2CHF_2, –$CH(CH_3)CN$, –$C(CH_3)_2CN$, –CH_2CN, –CH_2NH_2, –$CH_2NHSO_2CH_3$, –CH_2NHCH_3, –$CH_2N(CH_3)_2$, –CO_2H, –$COCH_3$, –CO_2CH_3, –$CO_2C(CH_3)_3$, –$COCH(OH)CH_3$, –$CONH_2$, –$CONHCH_3$, –$CON(CH_3)_2$, –$C(CH_3)_2CONH_2$, –NH_2, –$NHCH_3$, –$N(CH_3)_2$, –$NHCOCH_3$, –$N(CH_3)COCH_3$, –$NHS(O)_2CH_3$, –$N(CH_3)C(CH_3)_2CONH_2$, –$N(CH_3)CH_2CH_2S(O)_2CH_3$, –NHC(=NH)H, –$NHC(=NH)CH_3$, –$NHC(=NH)NH_2$, –$NHC(=O)NH_2$, –$NO_2$, =O, –OH, –$OCH_3$, –$OCH_2CH_3$, –$OCH_2CH_2OCH_3$, –$OCH_2CH_2OH$, –$OCH_2CH_2N(CH_3)_2$, –$O(CH_2CH_2O)_n$–$(CH_2)_mCO_2H$, –$O(CH_2CH_2O)_nH$, –$OP(O)(OH)_2$, –$S(O)_2N(CH_3)_2$, –$SCH_3$, –$S(O)_2CH_3$, and –$S(O)_3H$.

41. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein X^1 is a bond, and R^1 is H.

42. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein X^2 is a bond, and R^2 is C_1-C_8 alkyl.

43. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein X^2 and X^3 are each a bond, and R^2 and R^3 are independently selected from C_1-C_8 alkyl, –O–(C_1-C_{12} alkyl), –(C_1-C_{12} alkyldiyl)–OR^5, –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^5$, –(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$, –O–(C_1-C_{12} alkyl)–$N(R^5)CO_2R^5$, and –O–(C_1-C_{12} alkyl)–$OC(O)N(R^5)_2$.

44. The 5-amino-pyrazoloazepine-linker compound of claim 43 wherein R^2 is C_1-C_8 alkyl and R^3 is –(C_1-C_8 alkyldiyl)–$N(R^5)CO_2R^4$.

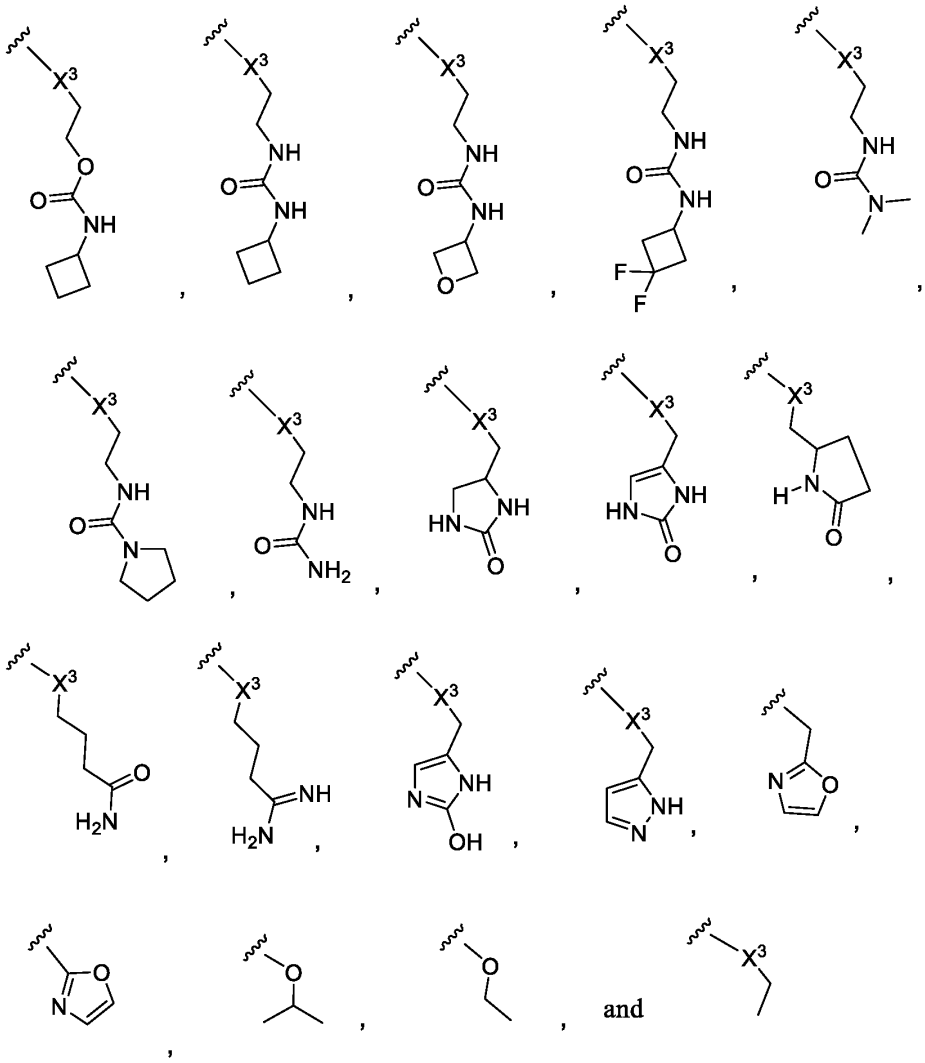
45. The 5-amino-pyrazoloazepine-linker compound of claim 43 wherein R^2 is –$CH_2CH_2CH_3$ and R^3 is selected from –$CH_2CH_2CH_2NHCO_2$(t-Bu), –$OCH_2CH_2NHCO_2$(cyclobutyl), and –$CH_2CH_2CH_2NHCO_2$(cyclobutyl).

46. The 5-amino-pyrazoloazepine-linker compound of claim 43 wherein R^2 and R^3 are each independently selected from $-CH_2CH_2CH_3$, $-OCH_2CH_3$, $-OCH_2CF_3$, $-CH_2CH_2CF_3$, $-OCH_2CH_2OH$, and $-CH_2CH_2CH_2OH$.

47. The 5-amino-pyrazoloazepine-linker compound of claim 43 wherein R^2 and R^3 are each $-CH_2CH_2CH_3$.

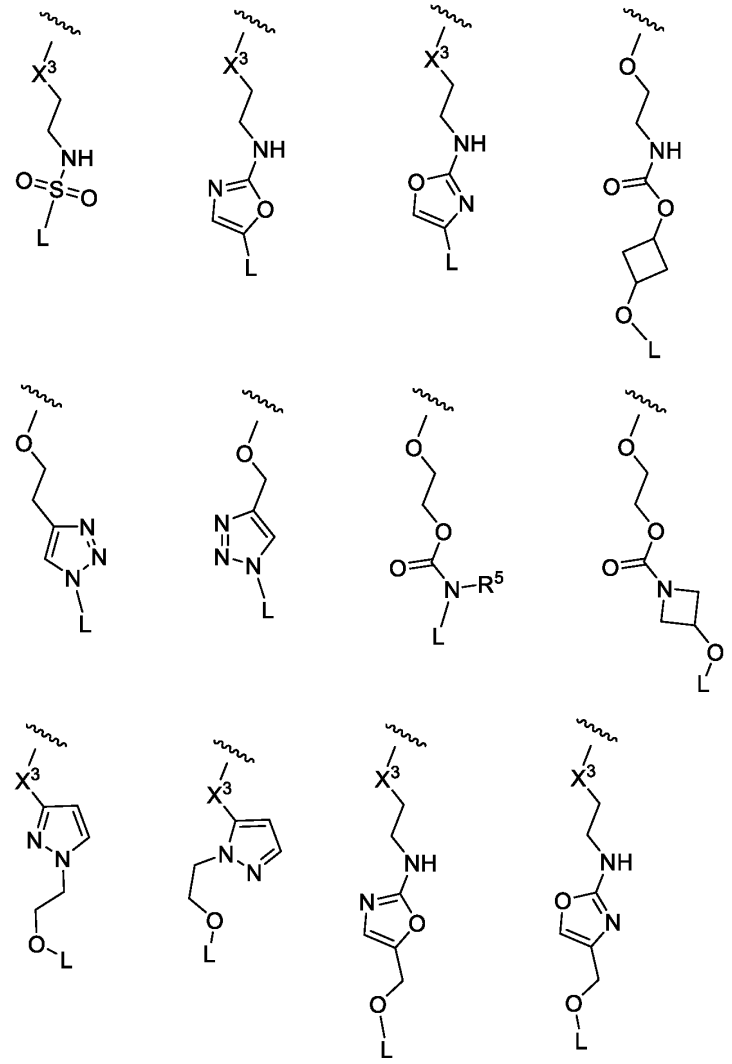
48. The 5-amino-pyrazoloazepine-linker compound of claim 43 wherein R^2 is $-CH_2CH_2CH_3$ and R^3 is $-OCH_2CH_3$.

49. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein X^3-R^3 is selected from the group consisting of:



50. The 5-amino-pyrazoloazepine-linker compound of claim 40 where R^2 or R^3 is attached to L.

51. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein X^3–R^3–L is selected from the group consisting of:



where the wavy line indicates the point of attachment to N.

52. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein R^4 is C_1-C_{12} alkyl.

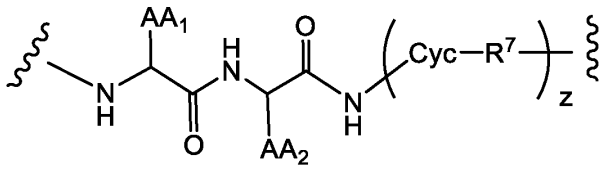
53. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein R^4 is –(C_1-C_{12} alkyldiyl)–N(R^5)–*; where the asterisk * indicates the attachment site of L.

54. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L is –C(=O)–PEG– or –C(=O)–PEG–C(=O)–.

55. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein for the PEG, m is 1 or 2, and n is an integer from 2 to 10.

56. The 5-amino-pyrazoloazepine-linker compound of claim 55 wherein n is 10.

57. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L comprises PEP and PEP is a dipeptide and has the formula:

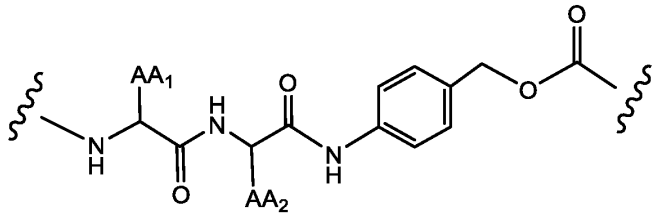


58. The 5-amino-pyrazoloazepine-linker compound of claim 57 wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$; or AA_1 and AA_2 form a 5-membered ring proline amino acid.

59. The 5-amino-pyrazoloazepine-linker compound of claim 58 wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

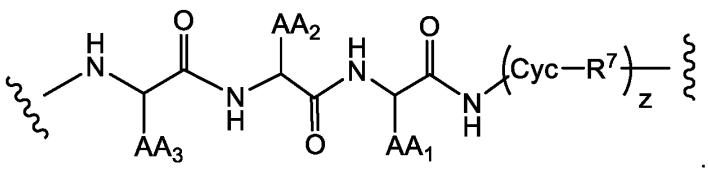
60. The 5-amino-pyrazoloazepine-linker compound of claim 57 wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

61. The 5-amino-pyrazoloazepine-linker compound of claim 57 wherein PEP has the formula:

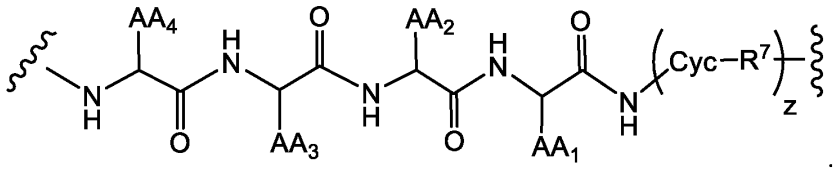


wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

62. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L comprises PEP and PEP is a tripeptide and has the formula:



63. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L comprises PEP and PEP is a tetrapeptide and has the formula:



64. The 5-amino-pyrazoloazepine-linker compound of claim 63 wherein

AA_1 is selected from the group consisting of Abu, Ala, and Val;

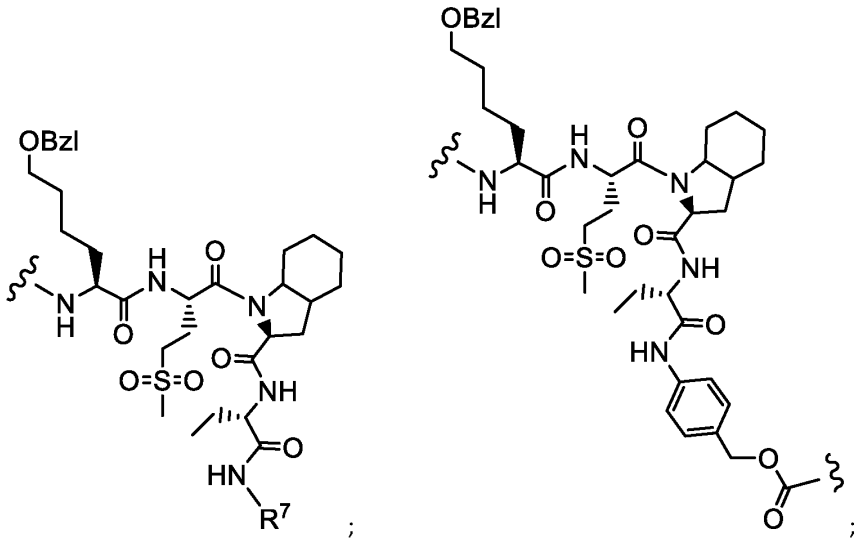
AA_2 is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

AA_3 is selected from the group consisting of Ala and $Met(O)_2$; and

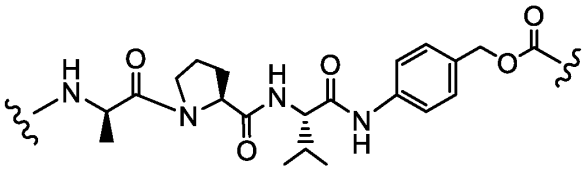
AA_4 is selected from the group consisting of Oic, $Arg(NO_2)$, Bpa, and Nle(O-Bzl).

65. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L comprises PEP and PEP is selected from the group consisting of Ala-Pro-Val, Asn-Pro-Val, Ala-Ala-Val, Ala-Ala-Pro-Ala, Ala-Ala-Pro-Val, and Ala-Ala-Pro-Nva.

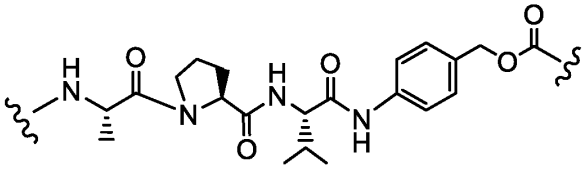
66. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L comprises PEP and PEP is selected from the structures:



;

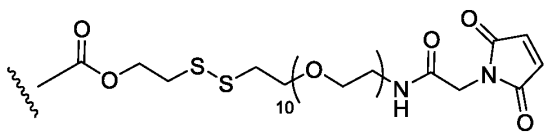


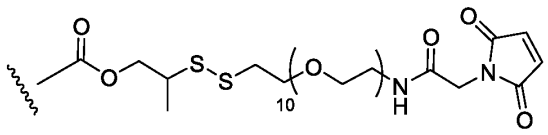
; and

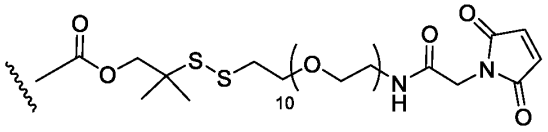


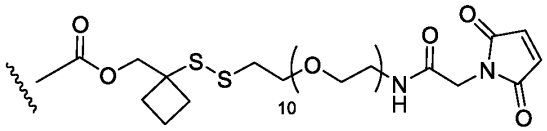
.

67. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein L is selected from the structures:



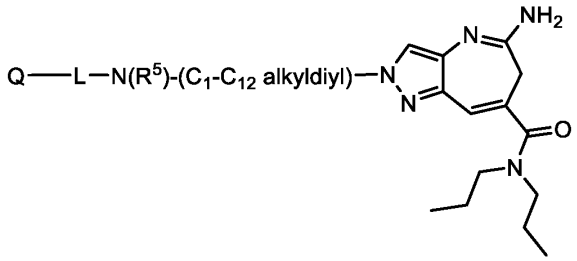




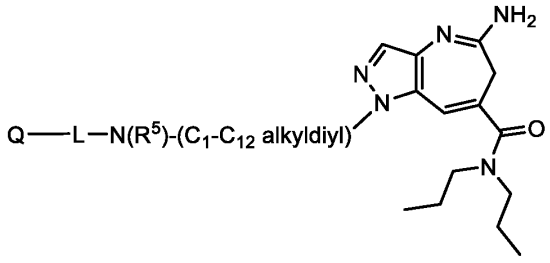


where the wavy line indicates the attachment to one of R^1, R^2, R^3 and R^4.

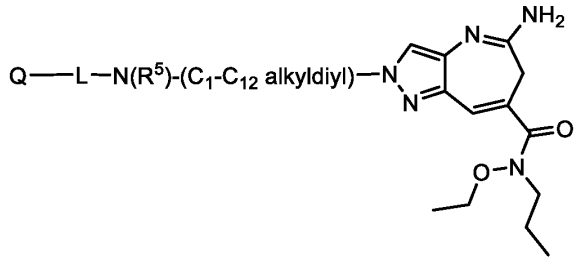
68. The 5-amino-pyrazoloazepine-linker compound of claim 40 selected from Formulae IIa-IId:



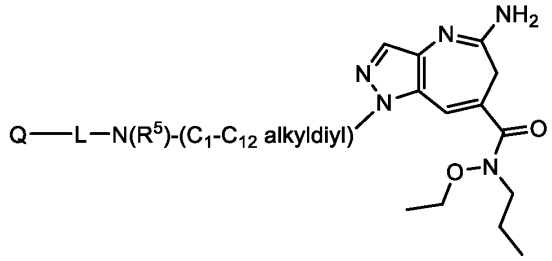
IIa;



IIb;

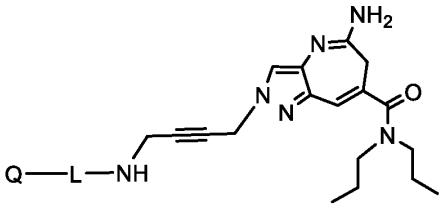


IIc; and

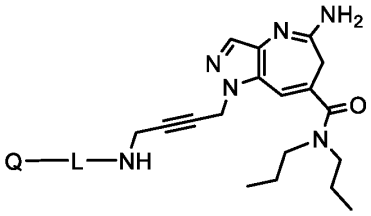


IId.

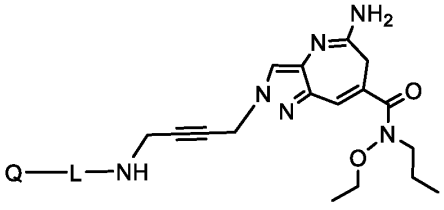
69. The 5-amino-pyrazoloazepine-linker compound of claim 40 selected from Formulae IIe-III:



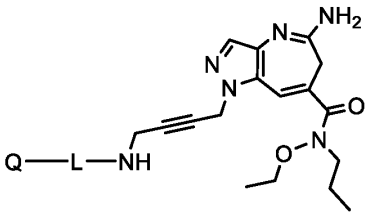
IIe;



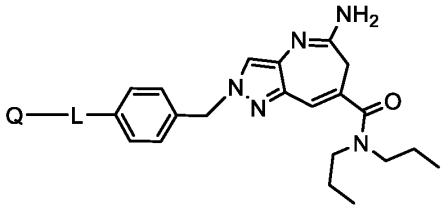
IIf;



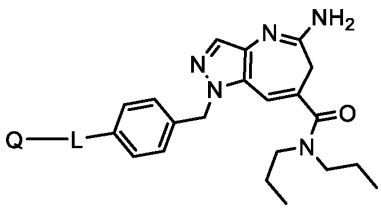
IIg;



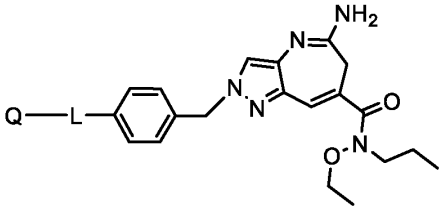
IIh;



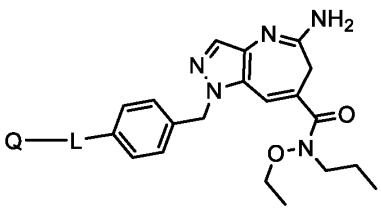
IIi;



IIj;

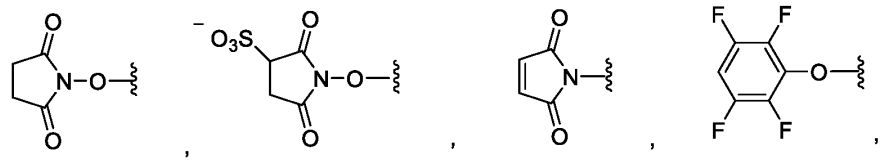


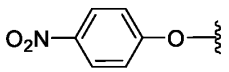
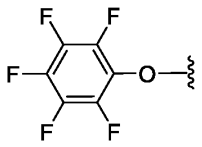
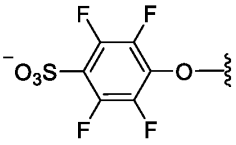
Ik; and



Il.

70. The 5-amino-pyrazoloazepine-linker compound of claim 40 wherein Q is selected from:


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, and 
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71. The 5-amino-pyrazoloazepine-linker compound of claim 70 wherein Q is phenoxy substituted with one or more F.

72. The 5-amino-pyrazoloazepine-linker compound of claim 71 wherein Q is 2,3,5,6-tetrafluorophenoxy.

73. The 5-amino-pyrazoloazepine-linker compound of claim 70 wherein Q is maleimide.

74. The 5-amino-pyrazoloazepine-linker compound of claim 40 selected from Tables 2a and 2b.

75. An immunoconjugate prepared by conjugation of an antibody with a 5-amino-pyrazoloazepine-linker compound of claim 40.

76. A pharmaceutical composition comprising a therapeutically effective amount of an immunoconjugate according to any one of claims 1-39 and one or more pharmaceutically acceptable diluent, vehicle, carrier, or excipient.

77. A method for treating cancer comprising administering a therapeutically effective amount of an immunoconjugate according to any one of claims 1 to 39, to a patient in need thereof.

78. The method of claim 77, wherein the cancer is susceptible to a pro-inflammatory response induced by TLR7 and/or TLR8 agonism.

79. The method of claim 77, wherein the cancer is a PD-L1-expressing cancer.

80. The method of claim 77, wherein the cancer is a HER2-expressing cancer.

81. The method of claim 77 wherein the cancer is a CEA-expressing cancer.

82. The method of claim 77 wherein the cancer is a TROP2-expressing cancer.

83. The method of any one of claims 77-82, wherein the cancer is selected from cervical cancer, endometrial cancer, ovarian cancer, prostate cancer, pancreatic cancer, esophageal cancer, bladder cancer, urinary tract cancer, urothelial carcinoma, lung cancer, non-small cell lung cancer, Merkel cell carcinoma, colon cancer, colorectal cancer, gastric cancer, and breast cancer.

84. The method of claim 83, wherein the breast cancer is triple-negative breast cancer.

85. The method of claim 83, wherein the Merkel cell carcinoma cancer is metastatic Merkel cell carcinoma.

86. The method of claim 83, wherein the gastric cancer is HER2 overexpressing gastric cancer.

87. The method of claim 83, wherein the cancer is gastroesophageal junction adenocarcinoma.

88. Use of an immunoconjugate according to any one of claims 1 to 36 for treating cancer.

89. A method of preparing an immunoconjugate of Formula I of claim 1 wherein a 5-amino-pyrazoloazepine-linker compound of Formula II of claim 40 is conjugated with the antibody.