

MACROMOLECULE-SUPPORTED 8-SULFONYL-BENZAZEPINE COMPOUNDS
AND THEIR USES

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This non-provisional application claims the benefit of priority to U.S. Provisional Application No. 63/308,268, filed 9 February 2022, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

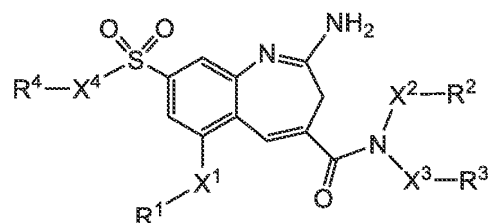
- 10 The invention relates generally to a macromolecule-supported compound comprising a macromolecular support conjugated to one or more 8-sulfonyl-benzazepine molecules.

BACKGROUND OF THE INVENTION

New compositions and methods for the delivery of dendritic cell 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.

15 SUMMARY OF THE INVENTION

The invention is generally directed to macromolecule-supported compounds (MSC) comprising a macromolecular support, covalently attached by a linker to one or more 8-sulfonyl-benzazepine TLR agonist moieties having the formula:



- 20 where one of R¹, R², R³ and R⁴ is attached to L. The various substituents are defined herein.

Another aspect of the invention is a method of preparing a macromolecule-supported compound by conjugation of one or more 8-sulfonyl-benzazepine-linker compounds with a macromolecular support.

- 25 Another aspect of the invention is a pharmaceutical composition comprising a therapeutically effective amount of a macromolecule-supported compound, and one or more pharmaceutically acceptable diluent, vehicle, carrier or excipient.

Another aspect of the invention is an 8-sulfonyl-benzazepine-linker compound.

Another aspect of the invention is a method for treating cancer comprising administering a therapeutically effective amount of a macromolecule-supported compound.

Another aspect of the invention is the use of a macromolecule-supported compound in
5 the treatment of an illness, in particular cancer.

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
10 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
15 invention is in no way limited to the methods and materials described.

DEFINITIONS

As used herein, the phrase “macromolecule-supported compound” refers to a macromolecular support that is covalently bonded to a TLR agonist via a linking moiety. The phrase is used interchangeably here with “MSC”.

20 As used herein, the terms “macromolecule support,” “macromolecular support,” or “macromolecule” can be used interchangeably to refer to an organic or inorganic structure having a chemical moiety on a surface of the structure that can be modified. In some embodiments, the macromolecular support is a resin, bead, probe, tag, well, plate, or any other surface that can be used for therapeutics, diagnostics, or chemical assays. The resin, bead,
25 probe, tag, well, plate, or any other surface can be made of any suitable material so long as the material can be surface modified. In some embodiments, the macromolecular support is a chemical structure (e.g., a biological structure or an inorganic framework) having a molecular weight of at least about 200 Da (e.g., at least about 500 Da, at least about 1,000 Da, at least about 2,000 Da, at least about 5,000 Da, or at least about 10,000 Da). As a singular entity, the
30 macromolecular support can be biologically active or biologically inactive relative to the TLR agonist described herein. However, when used in combination with the TLR agonist, the biological activity of the TLR agonist desirably is enhanced, for example, by providing a targeted effect (i.e., TLR activity), beneficial off-target effects (i.e., biological activity other than TLR activity), improved pharmacokinetic properties (e.g., half-life extension), enhanced

biological delivery (e.g., tumor penetration), or additional biological stimulation, differentiation, up-regulation, and/or down-regulation. In certain embodiments, the biological effect of the macromolecular support and the TLR agonist is synergistic, i.e., greater than the sum of the biological activity of each of the macromolecular support and TLR agonist as singular entities.

5 For example, the macromolecular support can be a biopolymer (e.g., a glycopolymer, a cellulosic polymer, etc.), a nanoparticle (e.g., a carbon nanotube, a quantum dot, a metal nanoparticle (e.g., silver, gold, titanium dioxide, silicon dioxide, zirconium dioxide, aluminum oxide, or ytterbium trifluoride), etc.), a lipid (e.g., lipid vesicles, micelles, liposomes, etc.), a carbohydrate (e.g., sugar, starch, cellulose, glycogen, etc.), a peptide (e.g., a polypeptide, a protein, a peptide mimetic, a glycopeptide, etc.), an antibody construct (e.g., antibody, an antibody-derivative (including Fc fusions, Fab fragments and scFvs), etc.), a nucleotide (e.g., RNA, DNA, antisense, siRNA, an aptamer, etc.), or any combination thereof. In some
10 embodiments, the macromolecular support is a peptide, a nucleotide, a sugar, a lipid, or an antibody. In certain embodiments, the macromolecular support is an immune checkpoint
15 inhibitor.

As used herein, the term “biopolymer” refers to any polymer produced by a living organism. For example, biopolymer can include peptides, polypeptides, proteins, oligonucleotides, nucleic acids (e.g., RNA and DNA) antibodies, polysaccharides, carbohydrates, sugars, peptide hormones, glycoproteins, glycogen, etc. Alternatively, a subunit
20 of a biopolymer, such as a fatty acid, glucose, an amino acid, a succinate, a ribonucleotide, a ribonucleoside, a deoxyribonucleotide, and a deoxyribonucleoside can be used. Illustrative examples include antibodies or fragments thereof; extracellular matrix proteins such as laminin, fibronectin, growth factors, peptide hormones, and other polypeptides. In some embodiments, the biopolymer comprises suberin, melanin, lignin, or cellulose, or the biopolymer is glycosidic.

25 As used herein, the term “nanoparticle” refers to a support structure having a diameter of about 1 nm to about 100 nm. Exemplary structure types include nanopowders, nanoparticles, nanoclusters, nanorods, nanotubes, nanocrystals, nanospheres, nanochains, nanoreefs, nanoboxes, and quantum dots. The nanoparticles can contain an inorganic material (e.g., silver, gold, hydroxyapatite, clay, titanium dioxide, silicon dioxide, zirconium dioxide, carbon
30 (graphite), diamond, aluminum oxide, ytterbium trifluoride, etc.) or an organic material (e.g., micelles, dendrimers, vesicles, liposomes, etc.). Alternatively, the nanoparticle can have a mixture of organic and inorganic material.

As used herein the term “lipid” refers to a hydrophobic or amphiphilic biomolecule. Exemplary lipids include fatty acids, waxes, sterols, fat-soluble vitamins, monoglycerides,
35 diglycerides, triglycerides, phospholipids, sphingolipids, saccharolipids, polyketides, sterol

lipids, glycerophospholipids, prenol lipids, etc. The lipid can exist in any suitable macromolecular structure, for example, a vesicle, a micelle, a liposome, etc.

As used herein, the term “carbohydrate” refers to any chemical entity comprising a monosaccharide, disaccharide, oligosaccharide, or polysaccharide. For example, the chemical entity can comprise a sugar (e.g., fructose, glucose, sucrose, lactose, galactose, etc.), starch, glycogen, or cellulose.

The terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residues is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. The peptide can have any suitable posttranslational modification (e.g., phosphorylation, hydroxylation, sulfonation, palmitoylation, glycosylation, disulfide formation, galactosylation, fucosylation, etc.).

As used herein, the phrase “alternative protein scaffold” refers to a non-immunoglobulin derived protein or peptide. Such proteins and peptides are generally amenable to engineering and can be designed to confer monospecificity against a given antigen, bispecificity, or multispecificity. Engineering of an alternative protein scaffold can be conducted using several approaches. A loop grafting approach can be used where sequences of known specificity are grafted onto a variable loop of a scaffold. Sequence randomization and mutagenesis can be used to develop a library of mutants, which can be screened using various display platforms (e.g., phage display) to identify a novel binder. Site-specific mutagenesis can also be used as part of a similar approach. Alternative protein scaffolds exist in a variety of sizes, ranging from small peptides with minimal secondary structure to large proteins of similar size to a full-sized antibody. Examples of scaffolds include, but are not limited to, cystine knotted miniproteins (also known as knottins), cyclic cystine knotted miniproteins (also known as cyclotides), avimers, affibodies, the tenth type III domain of human fibronectin, DARPins (designed ankyrin repeats), and anticalins (also known as lipocalins). Naturally occurring ligands with known specificity can also be engineered to confer novel specificity against a given target. Examples of naturally occurring ligands that may be engineered include the EGF ligand and VEGF ligand. Engineered proteins can either be produced as monomeric proteins or as multimers, depending on the desired binding strategy and specificities. Protein engineering strategies can be used to fuse alternative protein scaffolds to Fc domains.

As used herein, the term “nucleotide” refers to any chemical entity comprising deoxyribonucleic acid (“DNA”), ribonucleic acid (“RNA”), a deoxyribonucleic acid derivative, or a ribonucleic acid derivative. Exemplary nucleotide-based structures include RNA, DNA,

antisense oligonucleotides, siRNA, aptamers, etc. As used herein, the terms “deoxyribonucleic acid derivative” and “ribonucleic acid derivative” refer to DNA and RNA, respectively, that have been modified, such as, for example, by removing the phosphate backbone, methylating a hydroxyl group, or replacing a hydroxyl group with a thiol group.

5 The term “immunoconjugate” or “immune-stimulating antibody conjugate” 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.

 “Adjuvant moiety” refers to an adjuvant that is covalently bonded to a macromolecule support, e.g., through a linker, as described herein. The adjuvant moiety can elicit the immune response while bonded to the macromolecule support or after cleavage (e.g., enzymatic cleavage) from the macromolecule support following administration of a macromolecule conjugate compound to the subject.

 “Adjuvant” refers to a substance capable of eliciting an immune response in a subject
15 exposed to the adjuvant.

 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
20 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
25 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
30 number AAK62677 for murine TLR8 polypeptide.

 A “TLR agonist” is a compound 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
35 transduction components, in the intracellular localization of downstream elements such as

nuclear factor- κ B (NF- κ B), 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.

“Bispecific” antibodies (bsAbs) are antibodies that bind two distinct epitopes to cancer (Suurs F.V. et al (2019) *Pharmacology & Therapeutics* 201: 103-119). Bispecific antibodies may engage immune cells to destroy tumor cells, deliver payloads to tumors, and/or block tumor signaling pathways. 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, RGI, fucosyl-GMI, CTLA-4, and CD44 (WO 2017/196598).

In some embodiments, the antibody construct is an antigen-binding antibody “fragment,” which comprises at least an antigen-binding region of an antibody, alone or with other components that together constitute the antibody 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. In some embodiments, the antibody construct is an antibody or a fusion protein comprising (i) an antigen binding domain and (ii) an Fc domain.

The antibody or antibody fragment 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.

In some embodiments, the antibody construct 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 anti-CEA antibody, each variable region comprising a CDR1, a CDR2, and a CDR3.

“Cysteine-mutant antibody” is an antibody or antibody construct in which one or more amino acid residues are substituted with cysteine residues. A cysteine-mutant antibody may be prepared from the parent antibody by antibody engineering methods (Junutula, et al., (2008b) *Nature Biotech.*, 26(8):925-932; Doman et al. (2009) *Blood* 114(13):2721-2729; US 7521541; US 7723485; US 2012/0121615; WO 2009/052249). Cysteine residues provide for site-specific conjugation of an adjuvant such as a TLR agonist to the antibody through the reactive cysteine thiol groups at the engineered cysteine sites but do not perturb immunoglobulin folding and assembly or alter antigen binding and effector functions. Cysteine-mutant antibodies can be conjugated to the TLR agonist-linker compound with uniform stoichiometry of the immunoconjugate (e.g., up to two TLR agonist moieties per antibody in an antibody that has a single engineered, mutant cysteine site). The TLR agonist-linker compound has a reactive electrophilic group to react specifically with the free cysteine thiol groups of the cysteine-mutant antibody.

“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),
 5 Durbin et al., eds., *Biological Sequence Analysis: Probabilistic 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 $100 \times$
 10 $[(\text{identical positions})/\min(\text{TG}_A, \text{TG}_B)]$, 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 “antibody construct” or “binding agent” comprises Ig heavy and light chain variable region polypeptides that together form the antigen binding site. Each of the heavy and light
 15 chain variable regions are polypeptides comprising three complementarity determining regions (CDR1, CDR2, and CDR3) connected by framework regions. The antibody construct 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.

20 “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
 25 (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
 30 (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
 35 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
5 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),
10 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),
15 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.

20 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-
25 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
30 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 a macromolecule-supported compound.

“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 a macromolecule in a macromolecule-supported compound. 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, -CH₃), ethyl (Et, -CH₂CH₃), 1-propyl (n-Pr, n-propyl, -CH₂CH₂CH₃), 2-propyl (i-Pr, i-propyl, -CH(CH₃)₂), 1-butyl (n-Bu, n-butyl, -CH₂CH₂CH₂CH₃), 2-methyl-1-propyl (i-Bu, i-butyl, -CH₂CH(CH₃)₂), 2-butyl (s-Bu, s-butyl, -CH(CH₃)CH₂CH₃), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH₃)₃), 1-pentyl (n-pentyl, -CH₂CH₂CH₂CH₂CH₃), 2-pentyl (-CH(CH₃)CH₂CH₂CH₃), 3-pentyl (-CH(CH₂CH₃)₂), 2-methyl-2-butyl (-C(CH₃)₂CH₂CH₃), 3-methyl-2-butyl (-CH(CH₃)CH(CH₃)₂), 3-methyl-1-butyl (-CH₂CH₂CH(CH₃)₂), 2-methyl-1-butyl (-CH₂CH(CH₃)CH₂CH₃), 1-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₃), 2-hexyl (-CH(CH₃)CH₂CH₂CH₂CH₃), 3-hexyl (-CH(CH₂CH₃)(CH₂CH₂CH₃)), 2-methyl-2-pentyl (-C(CH₃)₂CH₂CH₂CH₃), 3-methyl-2-pentyl (-CH(CH₃)CH(CH₃)CH₂CH₃), 4-methyl-2-pentyl (-CH(CH₃)CH₂CH(CH₃)₂), 3-methyl-3-pentyl (-C(CH₃)(CH₂CH₃)₂), 2-methyl-3-pentyl (-CH(CH₂CH₃)CH(CH₃)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.

5 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
10 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
15 ($=O$), 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
20 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, C₂-C₆ 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,
25 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
30 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
35 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 (C₆–C₂₀) 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 (C₆–C₂₀) 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, tetrahydrofuran-yl, dihydrofuran-yl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, homopiperazinyl, azetidiny, 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, dihydrofuran-yl, pyrazolidinylimidazoliny, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 3H-indolyl quinoliziny and N-pyridyl ureas. Spiro heterocycl

moieties are also included within the scope of this definition. Examples of spiro heterocycl 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 morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, dioxanyl, thiomorpholinyl, and S-dioxothiomorpholinyl.

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, benzofuran-yl, cinnolinyl, indazolyl, indoliziny, 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 “heteroaryl” 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 heteroaryls include pyridyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxadiazolyl, oxazolyl, isothiazolyl, and pyrrolyl.

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 C₁-C₄ 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.

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 a macromolecule-supported compound 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 macromolecule-supported compound 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 macromolecule-supported compound 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 macromolecule-supported compounds disclosed herein may comprise synergistic combinations of the claimed adjuvant and macromolecule support. These synergistic combinations upon administration elicit a greater effect on immunity, e.g., relative to when the macromolecule support is administered in the absence of the other moiety. Further, a decreased amount of the macromolecule-supported compound (MSC) may be administered (as measured by the total number of macromolecule supports or the total number of adjuvants administered as part of the MSC) compared to when either the macromolecule support 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.”

8-SULFONYL-2-AMINOBENZAZEPINE ADJUVANT COMPOUNDS

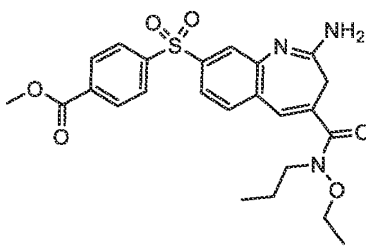
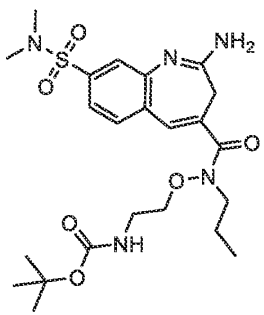
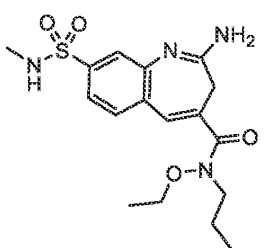
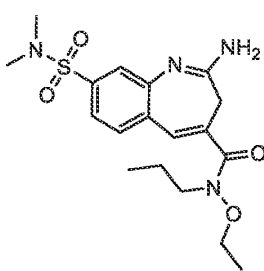
The macromolecule-supported compound (MSC) of the invention comprises an 8-sulfonyl-2-aminobenzazepine (8SO2Bz) adjuvant moiety. The adjuvant moiety described herein is a compound that elicits an immune response (i.e., an immunostimulatory agent). Generally, the adjuvant moiety described herein is a 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

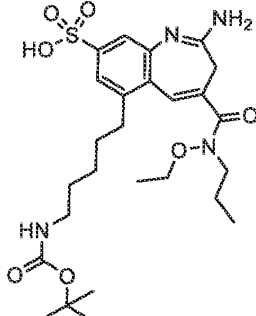
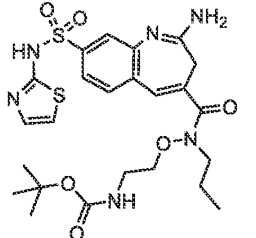
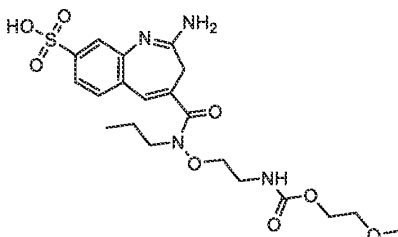
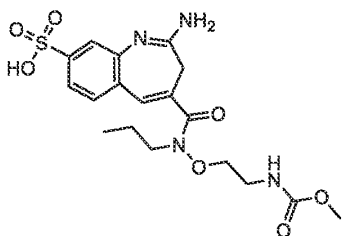
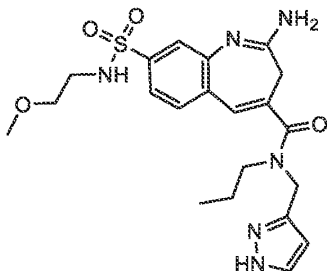
initiate. Once engaged (e.g., by a natural stimulus or a synthetic TLR agonist), TLRs initiate a signal transduction cascade leading to activation of nuclear factor- κ B (NF- κ B) via the adapter protein myeloid differentiation primary response gene 88 (MyD88) and recruitment of the IL-1 receptor associated kinase (IRAK). Phosphorylation of IRAK then leads to recruitment of TNF-receptor associated factor 6 (TRAF6), which results in the phosphorylation of the NF- κ B inhibitor I- κ B. As a result, NF- κ B enters the cell nucleus and initiates transcription of genes whose promoters contain NF- κ B binding sites, such as cytokines. Additional modes of regulation for TLR signaling include TRIF-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- κ B pathway.

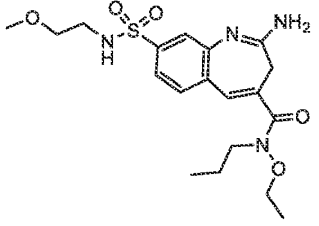
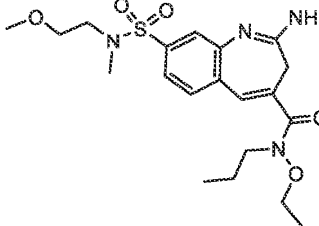
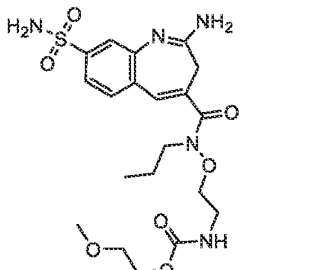
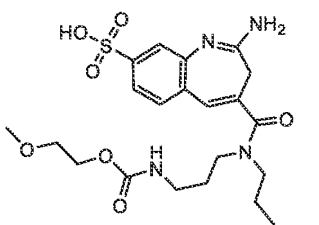
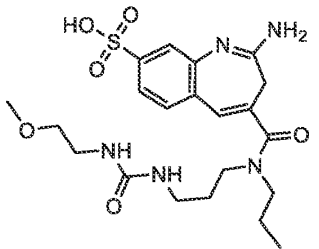
Typically, the adjuvant moiety described herein is a TLR7 and/or TLR8 agonist. TLR7 and TLR8 are both expressed in monocytes and dendritic cells. In humans, TLR7 is also expressed in plasmacytoid dendritic cells (pDCs) and B cells. TLR8 is expressed mostly in cells of myeloid origin, i.e., monocytes, 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.

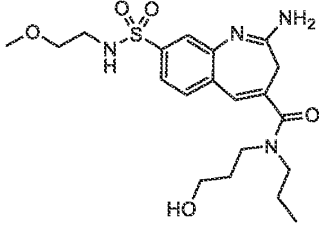
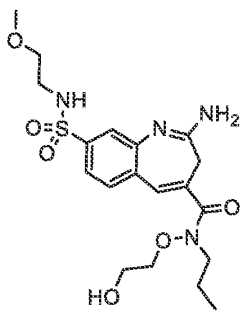
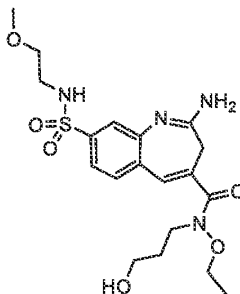
Exemplary 8-sulfonyl-2-aminobenzazepine compounds (8SO2Bz) of the invention were synthesized, purified, and characterized by mass spectrometry and shown to have the expected mass. Additional experimental procedures are found in the Examples. Activity against HEK293 NF κ B reporter cells expressing human TLR7 or human TLR8 was measured according to Example 202. Certain 8-sulfonyl-2-aminobenzazepine compounds demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

Table 1: 8-Sulfonyl-2-aminobenzazepine compounds (8SO2Bz)

8SO2Bz No.	Structure	MW	HEK293 hTLR7 EC50 (nM)	HEK293 hTLR8 EC50 (nM)
8SO2Bz-1		485.6	>9000	1056
8SO2Bz-2		509.6	395	<2.3
8SO2Bz-3		380.5	>5000	202
8SO2Bz-4		394.5		

8SO2Bz-5		552.7		
8SO2Bz-6		564.7		
8SO2Bz-7		484.5		
8SO2Bz-8		440.5		
8SO2Bz-9		460.6		

8SO2Bz-10		424.5		
8SO2Bz-11		438.5		
8SO2Bz-12		483.5		
8SO2Bz-13		482.6		
8SO2Bz-14		481.6		

8SO2Bz-15		438.5		
8SO2Bz-16		440.5		
8SO2Bz-17		440.5		

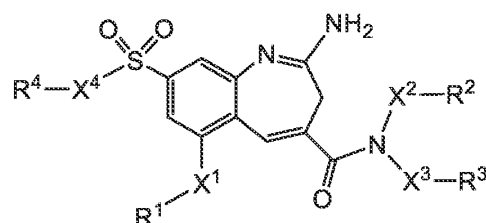
8-SULFONYL-2-AMINOBENZAZEPINE-LINKER COMPOUNDS

- The MSC of the invention are prepared by conjugation of an anti-CEA antibody with a 8-sulfonyl-2-aminobenzazepine-linker compound, 8SO2Bz-L. The 8-sulfonyl-2-aminobenzazepine-linker compounds comprise a 8-sulfonyl-2-aminobenzazepine (8SO2Bz) 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 MSC. The linker unit comprises a polyethyleneoxy (PEG) group. 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 8SO2Bz-L compound to form the MSC. Also, for example, a cysteine thiol of the antibody reacts with a maleimide or bromoacetamide group of the 8SO2Bz-linker compound (8SO2Bz-L) to form the MSC.

Reactive electrophilic functional groups (Q in Formula II) suitable for the 8SO₂Bz-L 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);
 5 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
 10 (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 MSC. 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
 15 (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,
 20 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
 25 aggregate formation decreases MSC yield and can render process scale-up difficult.

Exemplary embodiments include a 8-sulfonyl-2-aminobenzazepine-linker compound of Formula II:



wherein

30 R^1 , R^2 , R^3 , and R^4 are independently selected from the group consisting of H, C₁-C₁₂ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₁₂ carbocyclyl, C₆-C₂₀ aryl, C₂-C₉ heterocyclyl, and

C₁-C₂₀ heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

- (C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- 5 —(C₁-C₁₂ alkylidyl)—OR⁵;
- (C₃-C₁₂ carbocyclyl);
- (C₃-C₁₂ carbocyclyl)—*;
- (C₃-C₁₂ carbocyclyl)—(C₁-C₁₂ alkylidyl)—NR⁵—*;
- (C₃-C₁₂ carbocyclyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- 10 —(C₃-C₁₂ carbocyclyl)—NR⁵—C(=NR⁵)NR⁵—*;
- (C₆-C₂₀ aryl);
- (C₆-C₂₀ arylidyl)—*;
- (C₆-C₂₀ arylidyl)—N(R⁵)—*;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- 15 —(C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—(C₂-C₂₀ heterocyclidyl)—*;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—NR⁵—C(=NR^{5a})N(R⁵)—*;
- (C₂-C₂₀ heterocyclyl);
- (C₂-C₂₀ heterocyclyl)—*;
- 20 —(C₂-C₉ heterocyclyl)—(C₁-C₁₂ alkylidyl)—NR⁵—*;
- (C₂-C₉ heterocyclyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₂-C₉ heterocyclyl)—C(=O)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₂-C₉ heterocyclyl)—NR⁵—C(=NR^{5a})NR⁵—*;
- (C₂-C₉ heterocyclyl)—NR⁵—(C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- 25 —(C₂-C₉ heterocyclyl)—(C₆-C₂₀ arylidyl)—*;
- (C₁-C₂₀ heteroaryl);
- (C₁-C₂₀ heteroarylidyl)—*;
- (C₁-C₂₀ heteroaryl)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₁-C₂₀ heteroaryl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- 30 —(C₁-C₂₀ heteroaryl)—NR⁵—C(=NR^{5a})N(R⁵)—*;
- (C₁-C₂₀ heteroaryl)—N(R⁵)C(=O)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- C(=O)—*;
- C(=O)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;

- $-\text{C}(=\text{O})-(\text{C}_2-\text{C}_{20} \text{ heterocyclidiyl})-*;$
 $-\text{C}(=\text{O})\text{N}(\text{R}^5)_2;$
 $-\text{C}(=\text{O})\text{N}(\text{R}^5)-*;$
 $-\text{C}(=\text{O})\text{N}(\text{R}^5)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{R}^5;$
5 $-\text{C}(=\text{O})\text{N}(\text{R}^5)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{N}(\text{R}^5)_2;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)\text{CO}_2\text{R}^5;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)\text{C}(=\text{NR}^{5a})\text{N}(\text{R}^5)_2;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{R}^5;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_8 \text{ alkylidiyl})-\text{NR}^5(\text{C}_2-\text{C}_5 \text{ heteroaryl});$
10 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{20} \text{ heteroaryldiyl})-\text{N}(\text{R}^5)-*;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{20} \text{ heteroaryldiyl})-*;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{20} \text{ heteroaryldiyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)_2;$
 $-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{20} \text{ heteroaryldiyl})-(\text{C}_2-\text{C}_{20} \text{ heterocyclidiyl})-\text{C}(=\text{O})\text{NR}^5-(\text{C}_1-\text{C}_{12}$
 $\text{alkylidiyl})-\text{NR}^5-*;$
15 $-\text{N}(\text{R}^5)_2;$
 $-\text{N}(\text{R}^5)-*;$
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{R}^5;$
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})-*;$
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{N}(\text{R}^5)_2;$
20 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{N}(\text{R}^5)-*;$
 $-\text{N}(\text{R}^5)\text{CO}_2\text{R}^5;$
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{N}(\text{R}^5)_2;$
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{N}(\text{R}^5)-*;$
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{R}^5;$
25 $-\text{N}(\text{R}^5)\text{C}(=\text{O})-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)-*;$
 $-\text{N}(\text{R}^5)-(\text{C}_2-\text{C}_5 \text{ heteroaryl});$
 $-\text{N}(\text{R}^5)-\text{S}(=\text{O})_2-(\text{C}_1-\text{C}_{12} \text{ alkyl});$
 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl});$
 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)_2;$
30 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)-*;$
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)_2;$
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)-*;$
 $-\text{O}-(\text{R}^5)-*;$

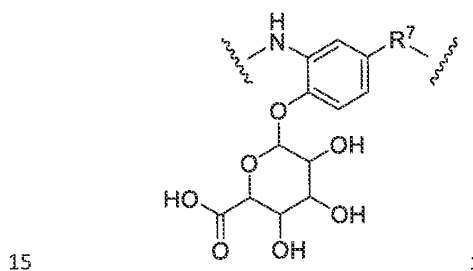
- $-\text{OR}^5$;
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclyldiyl})-*$;
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclyldiyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{N}(\text{R}^5)_2$;
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclyldiyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{NR}^5-*$; and
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclyldiyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{OH}$;
 or R^2 and R^3 together form a 5- or 6-membered heterocyclyl ring;
 X^1 , X^2 , X^3 , and X^4 are independently selected from the group consisting of a bond,
 $\text{C}(=\text{O})$, $\text{C}(=\text{O})\text{N}(\text{R}^5)$, O , $\text{N}(\text{R}^5)$, S , $\text{S}(\text{O})_2$, and $\text{S}(\text{O})_2\text{N}(\text{R}^5)$;
 R^5 is independently selected from the group consisting of H , C_6-C_{20} aryl, C_3-C_{12}
 carbocyclyl, C_6-C_{20} arylidiyl, C_1-C_{12} alkyl, and C_1-C_{12} alkylidiyl, 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:
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{C}(=\text{O})-\text{Gluc}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{O}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{O}-\text{C}(=\text{O})-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-\text{C}(=\text{O})-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{N}^+(\text{R}^6)_2-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})\text{N}(\text{R}^6)\text{C}(=\text{O})-(\text{C}_2-\text{C}_5$
 monoheterocyclyldiyl)-;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{SS}-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{OC}(=\text{O})-$;
 $\text{Q}-\text{C}(=\text{O})-\text{PEG}-\text{SS}-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{C}(=\text{O})-$;
 $\text{Q}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-$;
 $\text{Q}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidiyl})-\text{C}(=\text{O})-\text{Gluc}-$;
 $\text{Q}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{O}-$;

- $Q-(CH_2)_m-C(=O)N(R^6)-PEG-O-C(=O)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-C(=O)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-N(R^5)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-N(PEG-CO_2H)-PEG-N(R^5)-;$
5 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-C(=O)N(PEG-CO_2H)-PEG-N(R^5)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-N(R^5)-C(=O)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-N(PEG-CO_2H)-PEG-C(=O)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-C(=O)N(PEG-CO_2H)-PEG-C(=O)-;$
 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-C(=O)-PEP-;$ and
10 $Q-(CH_2)_m-C(=O)N(R^6)-PEG-SS-(C_1-C_{12} \text{ alkylidyl})-OC(=O)-;$

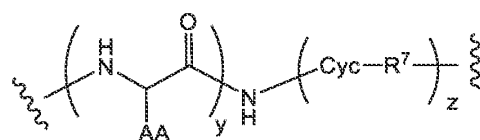
R^6 is independently H or C_1-C_6 alkyl;

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:

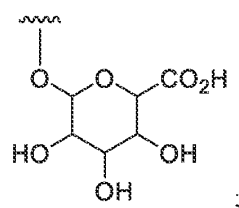


PEP has the formula:



- 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,
 20 and the wavy line indicates a point of attachment;

Cyc is selected from C_6-C_{20} arylidyl and C_1-C_{20} heteroarylidyl, 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 $-\text{CH}(R^8)\text{O}-$, $-\text{CH}_2-$, $-\text{CH}_2\text{N}(R^8)-$, and $-\text{CH}(R^8)\text{O}-\text{C}(=\text{O})-$, where R^8 is selected from H, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}(=\text{O})-\text{C}_1\text{-C}_6$ alkyl, and $\text{C}(=\text{O})\text{N}(R^9)_2$, where R^9 is independently selected from the group consisting of H, $\text{C}_1\text{-C}_{12}$ alkyl, and $-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_m-\text{OH}$, 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 heterocycl ring;

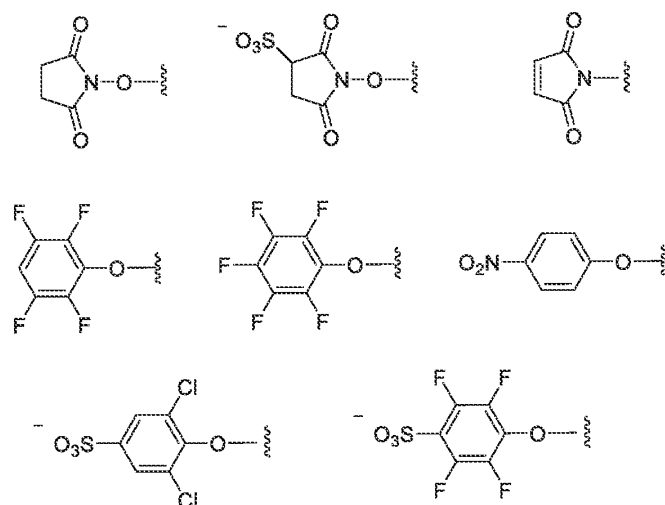
y is an integer from 2 to 12;

z is 0 or 1;

- 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^- ; and

- alkyl, alkylidyl, alkenyl, alkenyldiyl, alkynyl, alkynyldiyl, aryl, aryldiyl, carbocycl, carbocyclidyl, heterocycl, heterocyclidyl, heteroaryl, and heteroaryldiyl are independently and optionally substituted with one or more groups independently selected from F, Cl, Br, I, CN , $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}=\text{CH}_2$, $-\text{C}\equiv\text{CH}$, $-\text{C}\equiv\text{CCH}_3$, $-\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{OCH}_3$, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{C}(\text{CH}_3)_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_3$, $-\text{CH}_2\text{OP}(\text{O})(\text{OH})_2$, $-\text{CH}_2\text{F}$, $-\text{CHF}_2$, $-\text{CF}_3$, $-\text{CH}_2\text{CF}_3$, $-\text{CH}_2\text{CHF}_2$, $-\text{CH}(\text{CH}_3)\text{CN}$, $-\text{C}(\text{CH}_3)_2\text{CN}$, $-\text{CH}_2\text{CN}$, $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{NHSO}_2\text{CH}_3$, $-\text{CH}_2\text{NHCH}_3$, $-\text{CH}_2\text{N}(\text{CH}_3)_2$, $-\text{CO}_2\text{H}$, $-\text{COCH}_3$, $-\text{CO}_2\text{CH}_3$, $-\text{CO}_2\text{C}(\text{CH}_3)_3$, $-\text{COCH}(\text{OH})\text{CH}_3$, $-\text{CONH}_2$, $-\text{CONHCH}_3$, $-\text{CON}(\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_2\text{CONH}_2$, $-\text{NH}_2$, $-\text{NHCH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{NHCOCH}_3$, $-\text{N}(\text{CH}_3)\text{COCH}_3$, $-\text{NHS}(\text{O})_2\text{CH}_3$, $-\text{N}(\text{CH}_3)\text{C}(\text{CH}_3)_2\text{CONH}_2$, $-\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{S}(\text{O})_2\text{CH}_3$, $-\text{NHC}(=\text{NH})\text{H}$, $-\text{NHC}(=\text{NH})\text{CH}_3$, $-\text{NHC}(=\text{NH})\text{NH}_2$, $-\text{NHC}(=\text{O})\text{NH}_2$, $-\text{NO}_2$, $=\text{O}$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{OCH}_2\text{CH}_2\text{OCH}_3$, $-\text{OCH}_2\text{CH}_2\text{OH}$, $-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$, $-\text{O}(\text{CH}_2\text{CH}_2\text{O})_n-$, $(\text{CH}_2)_m\text{CO}_2\text{H}$, $-\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$, $-\text{OCH}_2\text{F}$, $-\text{OCHF}_2$, $-\text{OCF}_3$, $-\text{OP}(\text{O})(\text{OH})_2$, $-\text{S}(\text{O})_2\text{N}(\text{CH}_3)_2$, $-\text{SCH}_3$, $-\text{S}(\text{O})_2\text{CH}_3$, and $-\text{S}(\text{O})_3\text{H}$.

An exemplary embodiment of the 8-sulfonyl-2-aminobenzazepine-linker compound of Formula II includes wherein Q is selected from:

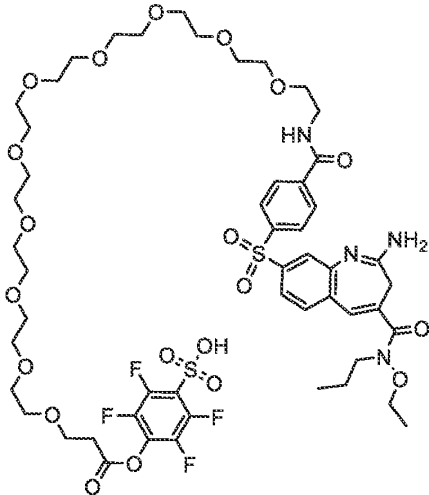
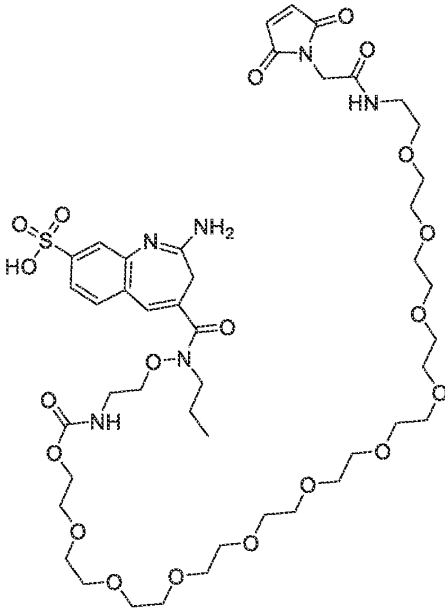


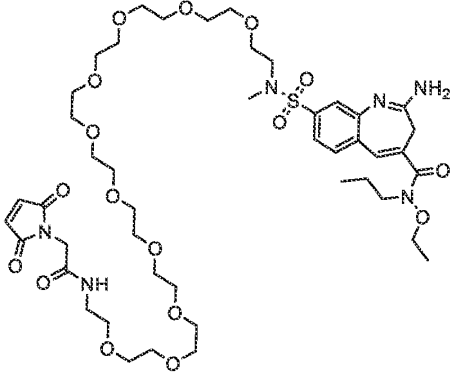
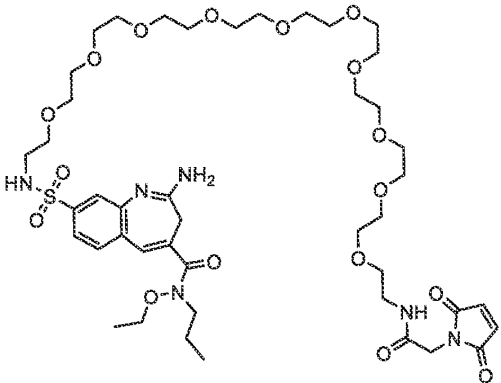
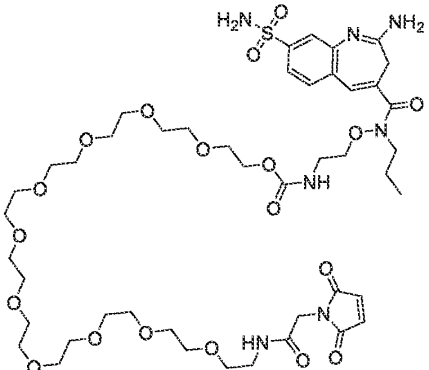
An exemplary embodiment of the 8-sulfonyl-2-aminobenzazepine-linker compound of Formula II includes wherein Q is phenoxy substituted with one or more F.

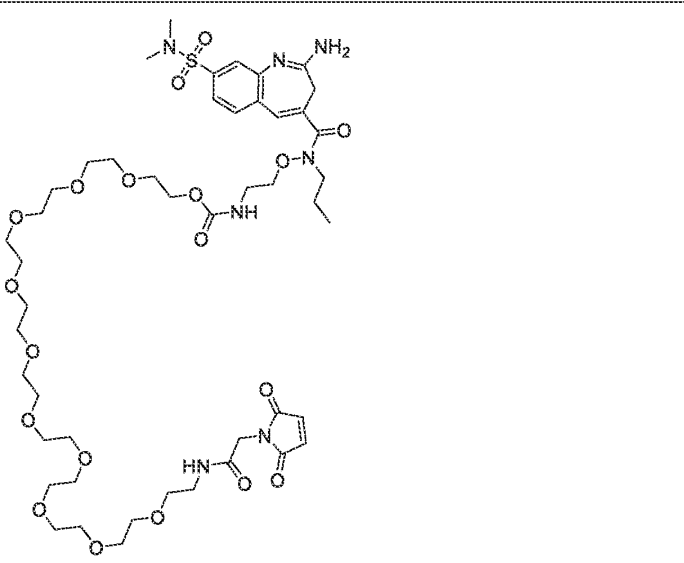
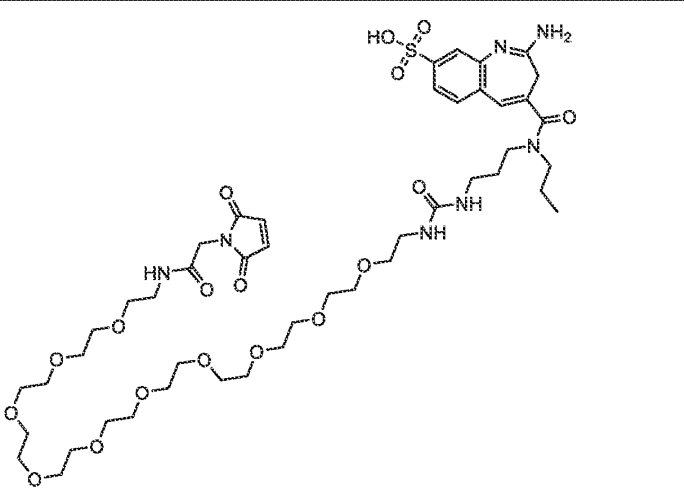
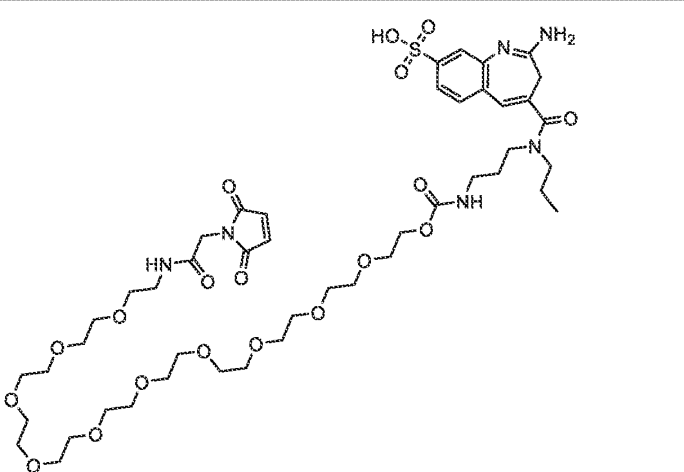
An exemplary embodiment of the 8-sulfonyl-2-aminobenzazepine-linker compound of Formula II includes wherein Q is 2,3,5,6-tetrafluorophenoxy.

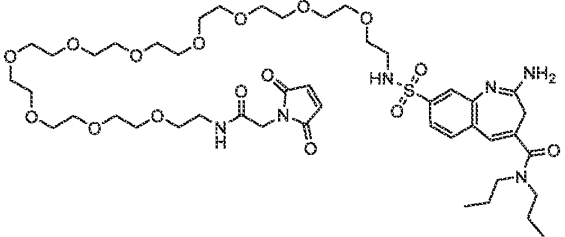
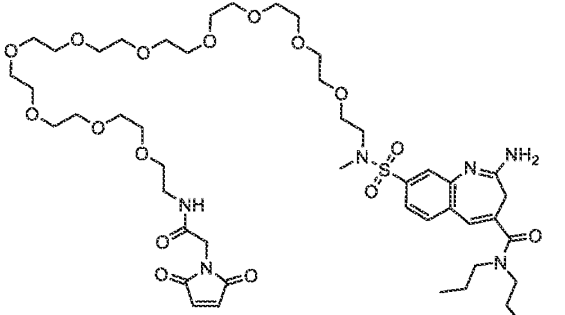
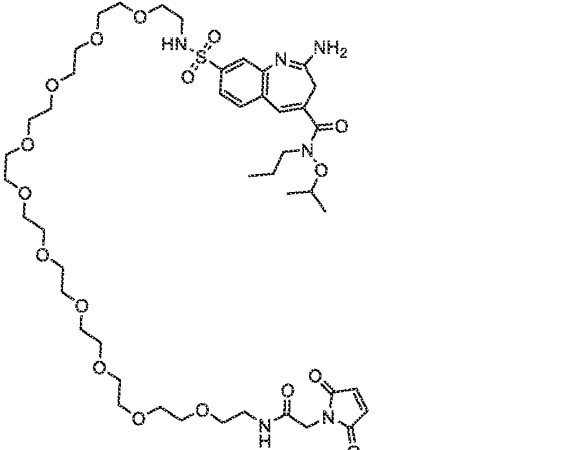
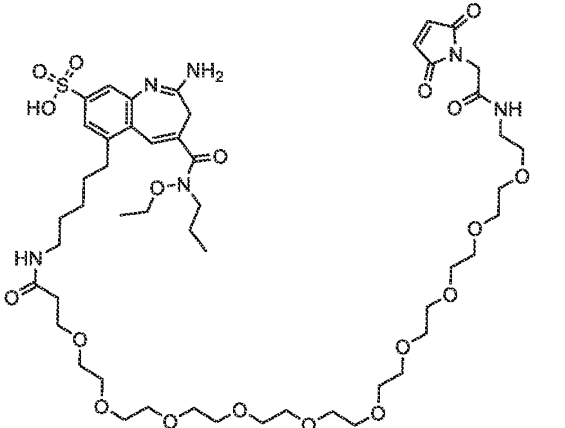
An exemplary embodiment of the 8-sulfonyl-2-aminobenzazepine-linker (8SO2BzL) compound is selected from Table 2. Each compound was synthesized, purified, and characterized by mass spectrometry and shown to have the mass indicated. Additional experimental procedures are found in the Examples. The 8-sulfonyl-2-aminobenzazepine-linker compounds of Table 2 demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders. The 8-sulfonyl-2-aminobenzazepine-linker intermediate, Formula II compounds of Table 2 may be used in conjugation with a macromolecule support by the methods of Example 201 to form the MSC of Formula I.

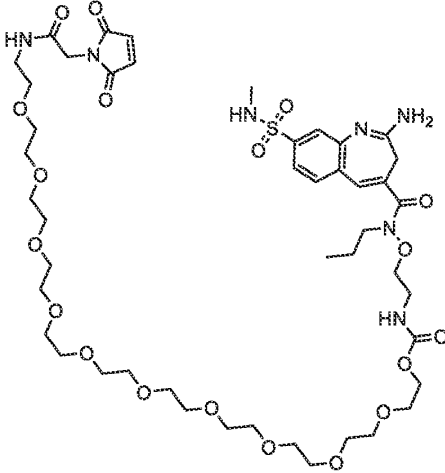
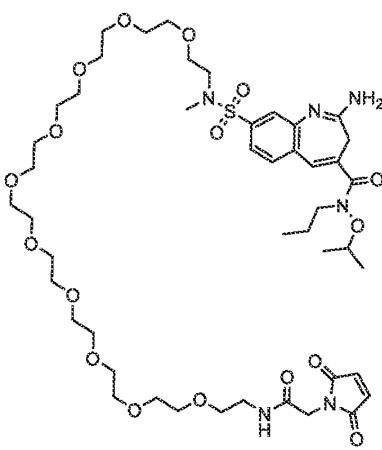
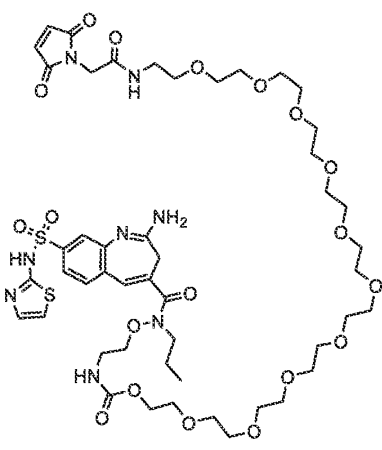
Table 2 8-Sulfonyl-Benzazepine-Linker (8SO2BzL) compounds

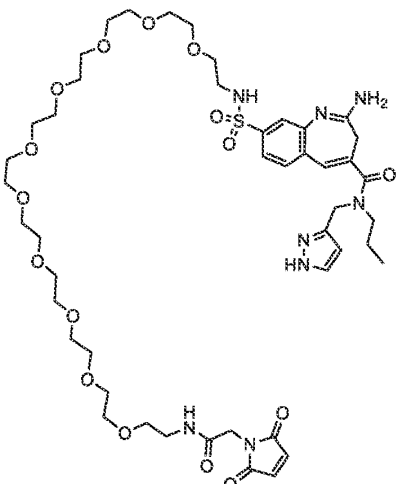
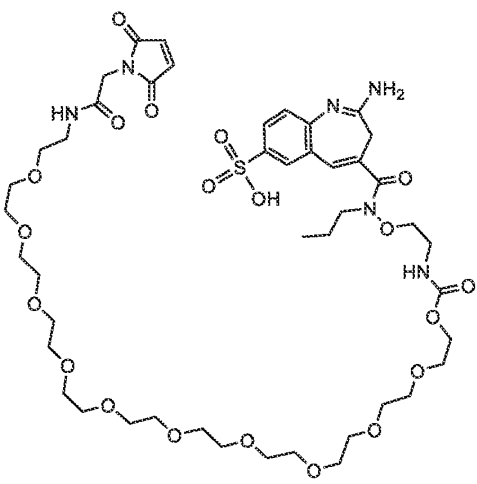
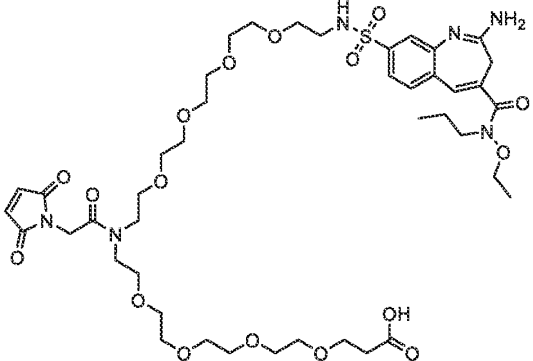
8SO2BzL No.	Structure	MW
8SO2BzL-1	 The structure of 8SO2BzL-1 features a central 8-sulfonylbenzazepine core. The benzazepine system consists of a benzene ring fused to a seven-membered azepine ring. At the 8-position of the benzene ring, there is a sulfonyl group (-SO₂-) connected to a phenyl ring. This phenyl ring is further substituted with an amide group (-NH-C(=O)-) and a long, flexible polyether linker chain. The linker chain is composed of multiple repeating units of -CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CH₂-O-. The other end of the linker chain is connected to a 2,4,6-trifluorophenyl group, which also bears a sulfonate group (-SO₃H) and an ester linkage (-O-C(=O)-). The benzazepine core is also substituted with an amino group (-NH₂) and a carbonyl group (-C(=O)-) linked to a side chain containing an ether and a tertiary amine.	1211.3
8SO2BzL-2	 The structure of 8SO2BzL-2 features a central 8-sulfonylbenzazepine core. The benzazepine system consists of a benzene ring fused to a seven-membered azepine ring. At the 8-position of the benzene ring, there is a sulfonyl group (-SO₂-) connected to a phenyl ring. This phenyl ring is further substituted with a sulfonate group (-SO₃H) and an amide group (-NH-C(=O)-) linked to a long, flexible polyether linker chain. The linker chain is composed of multiple repeating units of -CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CH₂-O-. The other end of the linker chain is connected to a side chain containing an amide group (-NH-C(=O)-) and a tertiary amine. The benzazepine core is also substituted with an amino group (-NH₂) and a carbonyl group (-C(=O)-) linked to a side chain containing an ether and a tertiary amine.	1047.1

8SO2BzL-3		1001.2
8SO2BzL-4		987.1
8SO2BzL-5		1046.2

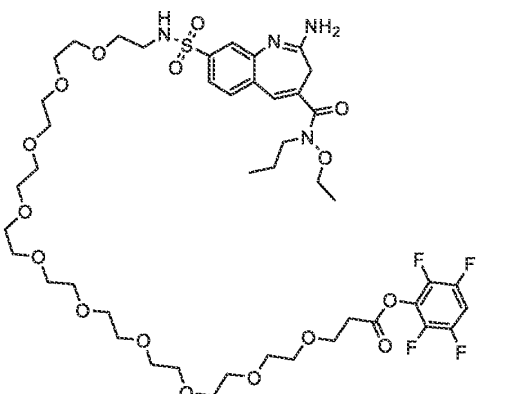
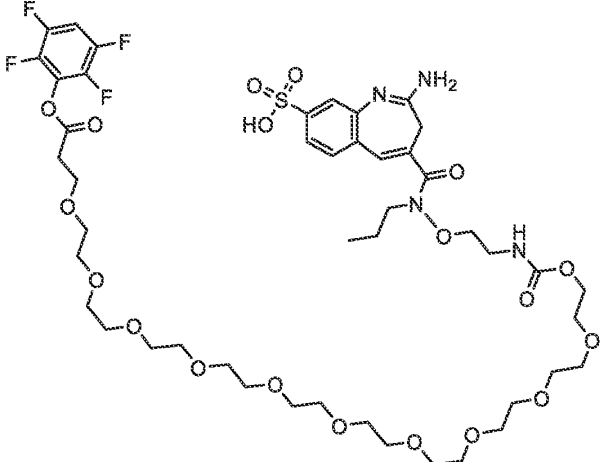
8SO2BzL-6	 Chemical structure of 8SO2BzL-6. It features a long, flexible polyether chain (poly(2-ethyl-2-oxazoline)) with a terminal amide group. The amide is linked to a side chain containing a 4-amino-2-methyl-5-sulfinyl-1H-benzimidazole moiety.	1074.2
8SO2BzL-7	 Chemical structure of 8SO2BzL-7. It features a long, flexible polyether chain (poly(2-ethyl-2-oxazoline)) with a terminal amide group. The amide is linked to a side chain containing a 4-amino-2-methyl-5-sulfinyl-1H-benzimidazole moiety.	1044.2
8SO2BzL-8	 Chemical structure of 8SO2BzL-8. It features a long, flexible polyether chain (poly(2-ethyl-2-oxazoline)) with a terminal amide group. The amide is linked to a side chain containing a 4-amino-2-methyl-5-sulfinyl-1H-benzimidazole moiety.	1045.2

8SO2BzL-9		985.2
8SO2BzL-10		999.2
8SO2BzL-11		1001.2
8SO2BzL-12		1101.3

8SO2BzL-13		1060.2
8SO2BzL-14		1015.2
8SO2BzL-15		1129.3

8SO2BzL-16		1023.2
8SO2BzL-17		1047.1
8SO2BzL-18		971.1

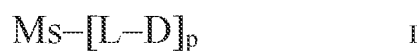
8SO2BzL-19		998.1
8SO2BzL-20		1058.2
8SO2BzL-21		1041.1

8SO2BzL-22		1027.1
8SO2BzL-23		1087.1

MACROMOLECULE-SUPPORTED COMPOUNDS

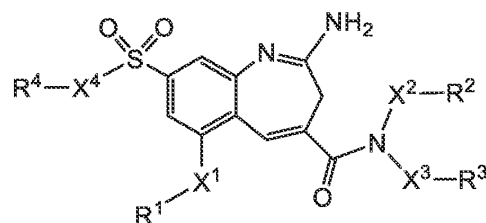
Immune-stimulating antibody conjugates, i.e. immunoconjugates, direct TLR7/8 agonists into tumors to activate tumor-infiltrating myeloid cells and initiate a broad innate and adaptive anti-tumor immune response (Ackerman, et al., (2021) *Nature Cancer* 2:18-33).

Exemplary embodiments of macromolecule-supported compounds comprise a macromolecular support covalently attached to one or more 8-sulfonyl-2-aminobenzazepine moieties by a linker, and having Formula I:



- 10 or a pharmaceutically acceptable salt thereof,
 wherein:
 Ms is the macromolecular support;
 p is an integer from 1 to 50;

D is the 8-sulfonyl-2-aminobenzazepine moiety having the formula:



R^1 , R^2 , R^3 , and R^4 are independently selected from the group consisting of H, C₁-C₁₂ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₁₂ carbocyclyl, C₆-C₂₀ aryl, C₂-C₉ heterocyclyl, and C₁-C₂₀ heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

- (C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₁-C₁₂ alkylidyl)—OR⁵;
- (C₃-C₁₂ carbocyclyl);
- (C₃-C₁₂ carbocyclyl)—*;
- (C₃-C₁₂ carbocyclyl)—(C₁-C₁₂ alkylidyl)—NR⁵—*;
- (C₃-C₁₂ carbocyclyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₃-C₁₂ carbocyclyl)—NR⁵—C(=NR⁵)NR⁵—*;
- (C₆-C₂₀ aryl);
- (C₆-C₂₀ arylidyl)—*;
- (C₆-C₂₀ arylidyl)—N(R⁵)—*;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—(C₂-C₂₀ heterocyclidyl)—*;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—NR⁵—C(=NR^{5a})N(R⁵)—*;
- (C₂-C₂₀ heterocyclyl);
- (C₂-C₂₀ heterocyclyl)—*;
- (C₂-C₉ heterocyclyl)—(C₁-C₁₂ alkylidyl)—NR⁵—*;
- (C₂-C₉ heterocyclyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)₂;
- (C₂-C₉ heterocyclyl)—C(=O)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₂-C₉ heterocyclyl)—NR⁵—C(=NR^{5a})NR⁵—*;
- (C₂-C₉ heterocyclyl)—NR⁵—(C₆-C₂₀ arylidyl)—(C₁-C₁₂ alkylidyl)—N(R⁵)—*;
- (C₂-C₉ heterocyclyl)—(C₆-C₂₀ arylidyl)—*;

- (C₁-C₂₀ heteroaryl);
 --(C₁-C₂₀ heteroaryl)--*;
 --(C₁-C₂₀ heteroaryl)--(C₁-C₁₂ alkylldiyl)--N(R⁵)--*;
 --(C₁-C₂₀ heteroaryl)--(C₁-C₁₂ alkylldiyl)--N(R⁵)₂;
 5 --(C₁-C₂₀ heteroaryl)--NR⁵--C(=NR^{5a})N(R⁵)--*;
 --(C₁-C₂₀ heteroaryl)--N(R⁵)C(=O)--(C₁-C₁₂ alkylldiyl)--N(R⁵)--*;
 --C(=O)--*;
 --C(=O)--(C₁-C₁₂ alkylldiyl)--N(R⁵)--*;
 --C(=O)--(C₂-C₂₀ heterocyclldiyl)--*;
 10 --C(=O)N(R⁵)₂;
 --C(=O)N(R⁵)--*;
 --C(=O)N(R⁵)--(C₁-C₁₂ alkylldiyl)--N(R⁵)C(=O)R⁵;
 --C(=O)N(R⁵)--(C₁-C₁₂ alkylldiyl)--N(R⁵)C(=O)N(R⁵)₂;
 --C(=O)NR⁵--(C₁-C₁₂ alkylldiyl)--N(R⁵)CO₂R⁵;
 15 --C(=O)NR⁵--(C₁-C₁₂ alkylldiyl)--N(R⁵)C(=NR^{5a})N(R⁵)₂;
 --C(=O)NR⁵--(C₁-C₁₂ alkylldiyl)--NR⁵C(=NR^{5a})R⁵;
 --C(=O)NR⁵--(C₁-C₈ alkylldiyl)--NR⁵(C₂-C₅ heteroaryl);
 --C(=O)NR⁵--(C₁-C₂₀ heteroarylldiyl)--N(R⁵)--*;
 --C(=O)NR⁵--(C₁-C₂₀ heteroarylldiyl)--*;
 20 --C(=O)NR⁵--(C₁-C₂₀ heteroarylldiyl)--(C₁-C₁₂ alkylldiyl)--N(R⁵)₂;
 --C(=O)NR⁵--(C₁-C₂₀ heteroarylldiyl)--(C₂-C₂₀ heterocyclldiyl)--C(=O)NR⁵--(C₁-C₁₂
 alkylldiyl)--NR⁵--*;
 --N(R⁵)₂;
 --N(R⁵)--*;
 25 --N(R⁵)C(=O)R⁵;
 --N(R⁵)C(=O)--*;
 --N(R⁵)C(=O)N(R⁵)₂;
 --N(R⁵)C(=O)N(R⁵)--*;
 --N(R⁵)CO₂R⁵;
 30 --NR⁵C(=NR^{5a})N(R⁵)₂;
 --NR⁵C(=NR^{5a})N(R⁵)--*;
 --NR⁵C(=NR^{5a})R⁵;
 --N(R⁵)C(=O)--(C₁-C₁₂ alkylldiyl)--N(R⁵)--*;

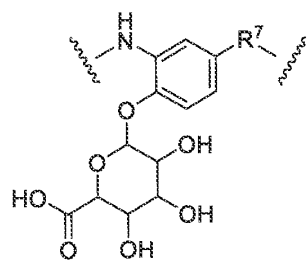
- $-\text{N}(\text{R}^5)-(\text{C}_2-\text{C}_5 \text{ heteroaryl});$
 $-\text{N}(\text{R}^5)-\text{S}(=\text{O})_2-(\text{C}_1-\text{C}_{12} \text{ alkyl});$
 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl});$
 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{N}(\text{R}^5)_2;$
5 $-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{N}(\text{R}^5)-*;$
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)_2;$
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)-*;$
 $-\text{O}-(\text{R}^5)-*;$
 $-\text{OR}^5;$
10 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclidyl})-*;$
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclidyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{N}(\text{R}^5)_2;$
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclidyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{NR}^5-*;$ and
 $-\text{S}(=\text{O})_2-(\text{C}_2-\text{C}_{20} \text{ heterocyclidyl})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{OH};$
or R^2 and R^3 together form a 5- or 6-membered heterocyclidyl ring;
15 $\text{X}^1, \text{X}^2, \text{X}^3$, and X^4 are independently selected from the group consisting of a bond,
 $\text{C}(=\text{O}), \text{C}(=\text{O})\text{N}(\text{R}^5), \text{O}, \text{N}(\text{R}^5), \text{S}, \text{S}(\text{O})_2$, and $\text{S}(\text{O})_2\text{N}(\text{R}^5);$
 R^5 is independently selected from the group consisting of H, C_6-C_{20} aryl, C_3-C_{12}
carbocyclidyl, C_6-C_{20} arylidyl, C_1-C_{12} alkyl, and C_1-C_{12} alkylidyl, or two R^5 groups together form
a 5- or 6-membered heterocyclidyl ring;
20 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 $\text{R}^1, \text{R}^2, \text{R}^3$ and
 R^4 is attached to L;
L is the linker selected from the group consisting of:
 $-\text{C}(=\text{O})-\text{PEG}-;$
25 $-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-\text{Gluc}-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{O}-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{O}-\text{C}(=\text{O})-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-;$
30 $-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-\text{C}(=\text{O})-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-;$
 $-\text{C}(=\text{O})-\text{PEG}-\text{N}^+(\text{R}^6)_2-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-;$

- $-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-$;
 $-\text{C}(=\text{O})-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})\text{N}(\text{R}^6)\text{C}(=\text{O})-(\text{C}_2-\text{C}_5$
 $\text{monoheterocyclyldiyl})-$;
 $-\text{C}(=\text{O})-\text{PEG}-\text{SS}-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{OC}(=\text{O})-$;
5 $-\text{C}(=\text{O})-\text{PEG}-\text{SS}-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-$;
 $-\text{C}(=\text{O})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-\text{PEP}-$;
 $-\text{C}(=\text{O})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-$;
 $-\text{C}(=\text{O})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{N}(\text{R}^5)-\text{C}(=\text{O})$;
 $-\text{C}(=\text{O})-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{N}(\text{R}^6)\text{C}(=\text{O})-$
10 $(\text{C}_2-\text{C}_5 \text{ monoheterocyclyldiyl})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12}$
 $\text{alkylidyl})-\text{C}(=\text{O})-\text{Gluc}-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{O}-$;
15 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{O}-\text{C}(=\text{O})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{N}(\text{R}^5)-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{N}(\text{PEG}-\text{CO}_2\text{H})-\text{PEG}-\text{N}(\text{R}^5)-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{PEG}-\text{CO}_2\text{H})-\text{PEG}-\text{N}(\text{R}^5)-$;
20 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{N}(\text{R}^5)-\text{C}(=\text{O})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{N}(\text{PEG}-\text{CO}_2\text{H})-\text{PEG}-\text{C}(=\text{O})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})\text{N}(\text{PEG}-\text{CO}_2\text{H})-\text{PEG}-\text{C}(=\text{O})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{C}(=\text{O})-\text{PEP}-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})\text{N}(\text{R}^6)-\text{PEG}-\text{SS}-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-\text{OC}(=\text{O})-$;
25 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})-$;
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})\text{N}(\text{R}^6)\text{C}(=\text{O})-$; and
 $-\text{succinimidyl}-(\text{CH}_2)_m-\text{C}(=\text{O})-\text{PEP}-\text{N}(\text{R}^6)-(\text{C}_1-\text{C}_{12} \text{ alkylidyl})\text{N}(\text{R}^6)\text{C}(=\text{O})-(\text{C}_2-\text{C}_5$
 $\text{monoheterocyclyldiyl})-$;

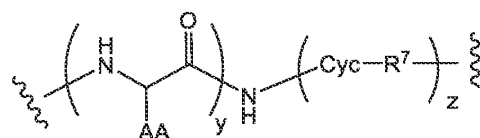
R^6 is independently H or C_1-C_6 alkyl;

- 30 PEG has the formula: $-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_m-$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:



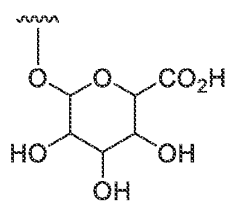
PEP has the formula:



where AA is independently selected from a natural or unnatural amino acid side chain, or

5 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 C₆-C₂₀ arylidyl and C₁-C₂₀ heteroarylidyl, optionally substituted with one or more groups selected from F, Cl, NO₂, -OH, -OCH₃, and a glucuronic acid having the structure:



10

R⁷ is selected from the group consisting of $-\text{CH}(\text{R}^8)\text{O}-$, $-\text{CH}_2-$, $-\text{CH}_2\text{N}(\text{R}^8)-$, and $-\text{CH}(\text{R}^8)\text{O}-\text{C}(=\text{O})-$, where R⁸ is selected from H, C₁-C₆ alkyl, C(=O)-C₁-C₆ alkyl, and $-\text{C}(=\text{O})\text{N}(\text{R}^9)_2$, where R⁹ is independently selected from the group consisting of H, C₁-C₁₂ alkyl, and $-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_m-\text{OH}$, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R⁹ 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, alkylidyl, 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, -CH₃, -CH₂CH₃, -CH=CH₂, -C≡CH, -C≡CCH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -CH₂OH, -CH₂OCH₃, -CH₂CH₂OH, -C(CH₃)₂OH, -CH(OH)CH(CH₃)₂, -C(CH₃)₂CH₂OH, -CH₂CH₂SO₂CH₃, -CH₂OP(O)(OH)₂, -CH₂F, -CHF₂, -CF₃, -CH₂CF₃, -

CH₂CHF₂, -CH(CH₃)CN, -C(CH₃)₂CN, -CH₂CN, -CH₂NH₂, -CH₂NHSO₂CH₃, -CH₂NHCH₃,
 -CH₂N(CH₃)₂, -CO₂H, -COCH₃, -CO₂CH₃, -CO₂C(CH₃)₃, -COCH(OH)CH₃, -CONH₂, -
 CONHCH₃, -CON(CH₃)₂, -C(CH₃)₂CONH₂, -NH₂, -NHCH₃, -N(CH₃)₂, -NHCOCH₃, -
 N(CH₃)COCH₃, -NHS(O)₂CH₃, -N(CH₃)C(CH₃)₂CONH₂, -N(CH₃)CH₂CH₂S(O)₂CH₃, -
 5 NHC(=NH)H, -NHC(=NH)CH₃, -NHC(=NH)NH₂, -NHC(=O)NH₂, -NO₂, =O, -OH, -OCH₃,
 -OCH₂CH₃, -OCH₂CH₂OCH₃, -OCH₂CH₂OH, -OCH₂CH₂N(CH₃)₂, -O(CH₂CH₂O)_n-
 (CH₂)_mCO₂H, -O(CH₂CH₂O)_nH, -OCH₂F, -OCHF₂, -OCF₃, -OP(O)(OH)₂, -S(O)₂N(CH₃)₂, -
 SCH₃, -S(O)₂CH₃, and -S(O)₃H.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 10 includes wherein X¹ is a bond, and R¹ is H.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein X² is a bond, and R² is C₁-C₈ alkyl.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein X² and X³ are each a bond, and R² and R³ are independently selected from C₁-
 15 C₈ alkyl, -O-(C₁-C₁₂ alkyl), -(C₁-C₁₂ alkyl)diyl-OR⁵, -(C₁-C₈ alkyl)diyl-N(R⁵)CO₂R⁵, -(C₁-
 C₁₂ alkyl)-OC(O)N(R⁵)₂, -O-(C₁-C₁₂ alkyl)-N(R⁵)CO₂R⁵, and -O-(C₁-C₁₂ alkyl)-
 OC(O)N(R⁵)₂.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein subscript p is an integer from 1 to 25.

20 An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein subscript p is an integer from 1 to 6.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein the macromolecular support is selected from a peptide, a nucleotide, a
 carbohydrate, a lipid, an antibody construct, a biopolymer, a nanoparticle, and an immune
 25 checkpoint inhibitor.

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein R² is C₁-C₈ alkyl and R³ is -(C₁-C₈ alkyl)diyl-N(R⁵)CO₂R⁴.

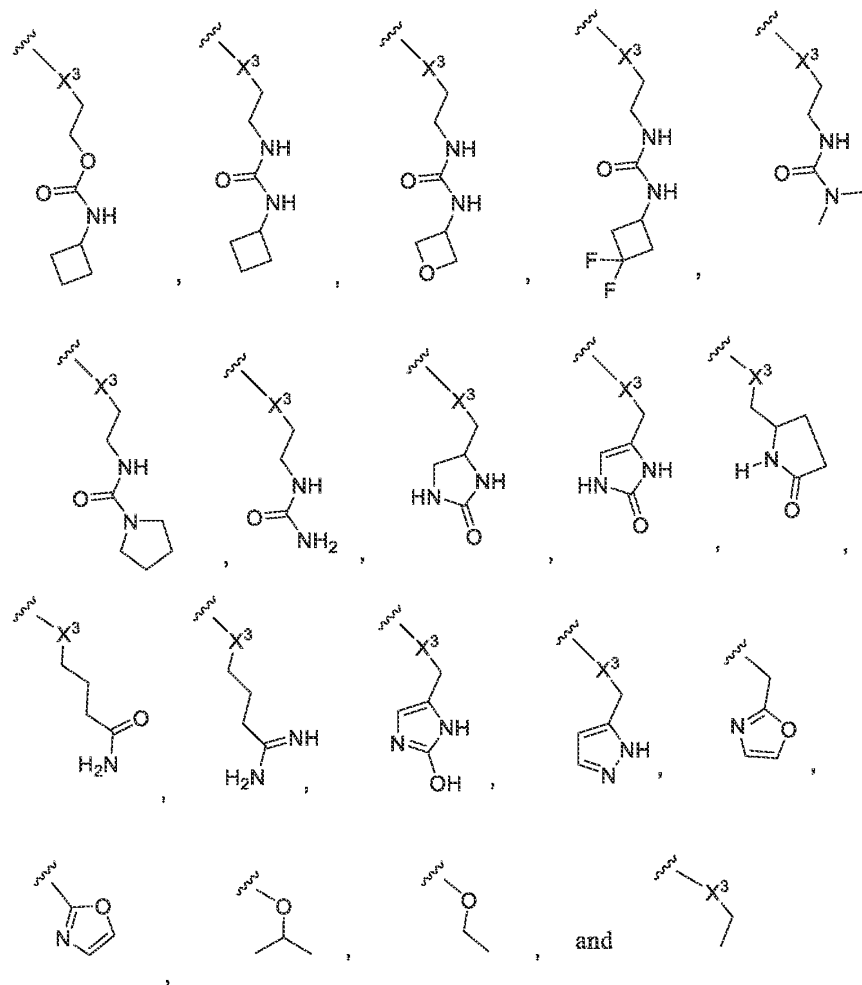
An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein R² is -CH₂CH₂CH₃ and R³ is selected from -CH₂CH₂CH₂NHCO₂(t-Bu), -
 30 OCH₂CH₂NHCO₂(cyclobutyl), and -CH₂CH₂CH₂NHCO₂(cyclobutyl).

An exemplary embodiment of the macromolecule-supported compound of Formula I
 includes wherein R² and R³ are each independently selected from -CH₂CH₂CH₃, -OCH₂CH₃, -
 OCH₂CF₃, -CH₂CH₂CF₃, -OCH₂CH₂OH, and -CH₂CH₂CH₂OH.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein R^2 and R^3 are each $-\text{CH}_2\text{CH}_2\text{CH}_3$.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein R^2 is $-\text{CH}_2\text{CH}_2\text{CH}_3$ and R^3 is $-\text{OCH}_2\text{CH}_3$.

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

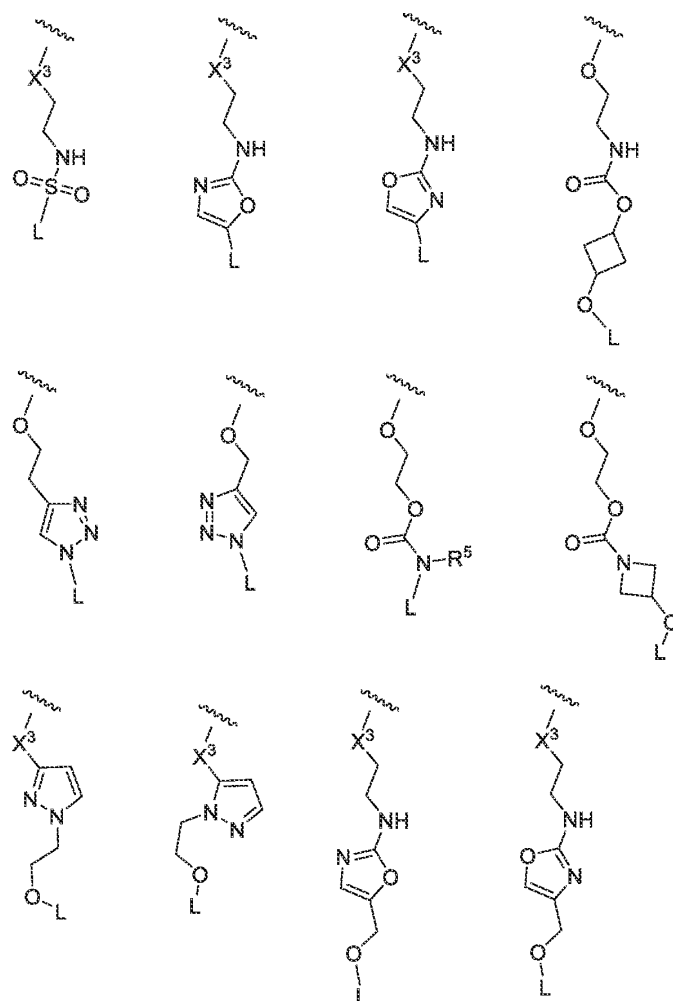


- 10 An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein X^4 is a bond, and R^4 is H.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein R^1 is attached to L.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein R^2 or R^3 is attached to L.

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



5

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

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

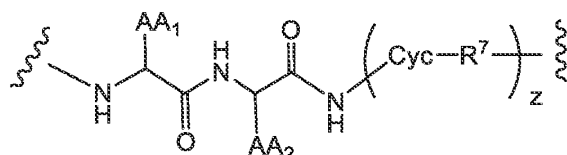
10 An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein R^4 is $-(C_1-C_{12} \text{ alkyldiyl})-N(R^5)-*$; where the asterisk * indicates the attachment site of L.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L is $-C(=O)-PEG-$ or $-C(=O)-PEG-C(=O)-$.

15 An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L is attached to a cysteine thiol of the antibody.

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

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L comprises PEP and PEP is a dipeptide and has the formula:



5

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein AA₁ and AA₂ are independently selected from H, -CH₃, -CH(CH₃)₂, -CH₂(C₆H₅), -CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, -CHCH(CH₃)CH₃, -CH₂SO₃H, and -CH₂CH₂CH₂NHC(O)NH₂; or AA₁ and AA₂ form a 5-membered ring proline amino acid.

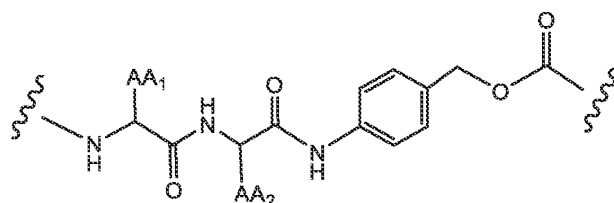
10

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein AA₁ is -CH(CH₃)₂, and AA₂ is -CH₂CH₂CH₂NHC(O)NH₂.

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein AA₁ and AA₂ are independently selected from GlcNAc aspartic acid, -CH₂SO₃H, and -CH₂OPO₃H.

15

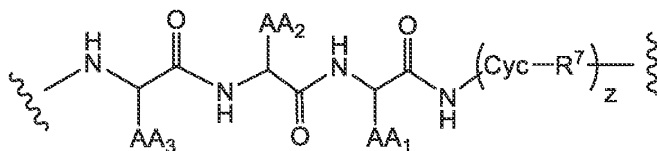
An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein PEP has the formula:



wherein AA₁ and AA₂ are independently selected from a side chain of a naturally-occurring amino acid.

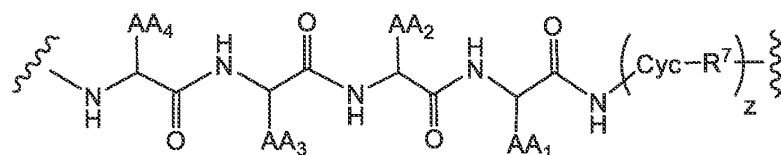
20

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L comprises PEP and PEP is a tripeptide and has the formula:



An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L comprises PEP and PEP is a tetrapeptide and has the formula:

25



An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein:

AA₁ is selected from the group consisting of Abu, Ala, and Val;

5 AA₂ is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

AA₃ is selected from the group consisting of Ala and Met(O)₂; and

AA₄ is selected from the group consisting of Oic, Arg(NO₂), Bpa, and Nle(O-Bzl).

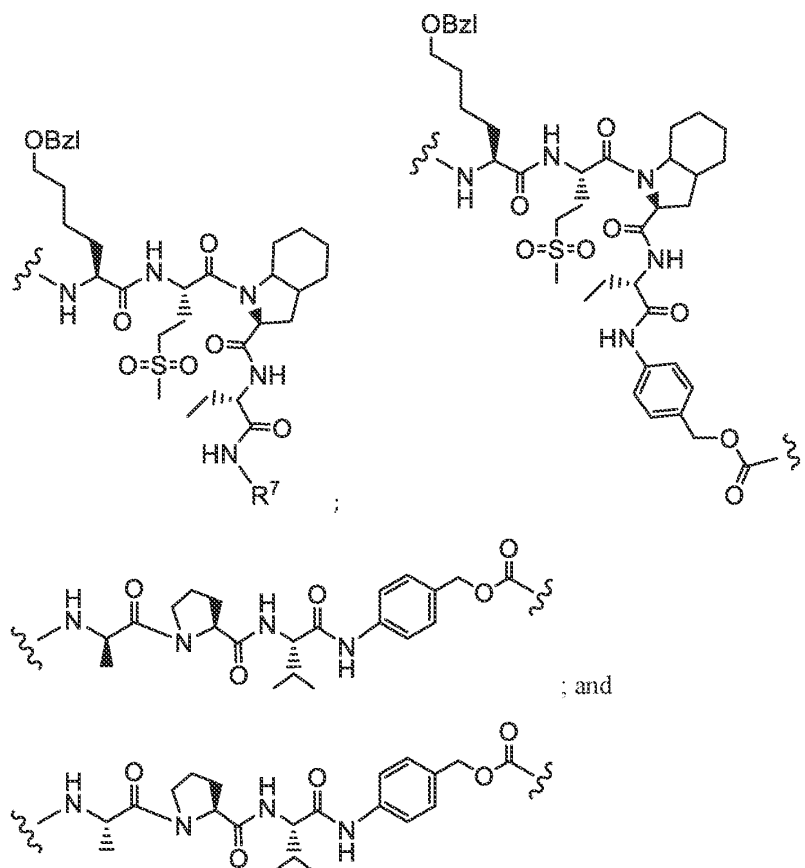
An exemplary embodiment of the macromolecule-supported compound of Formula I

includes wherein L comprises PEP and PEP is selected from the group consisting of Ala-Pro-

10 Val, Asn-Pro-Val, Ala-Ala-Val, Ala-Ala-Pro-Ala, Ala-Ala-Pro-Val, and Ala-Ala-Pro-Nva.

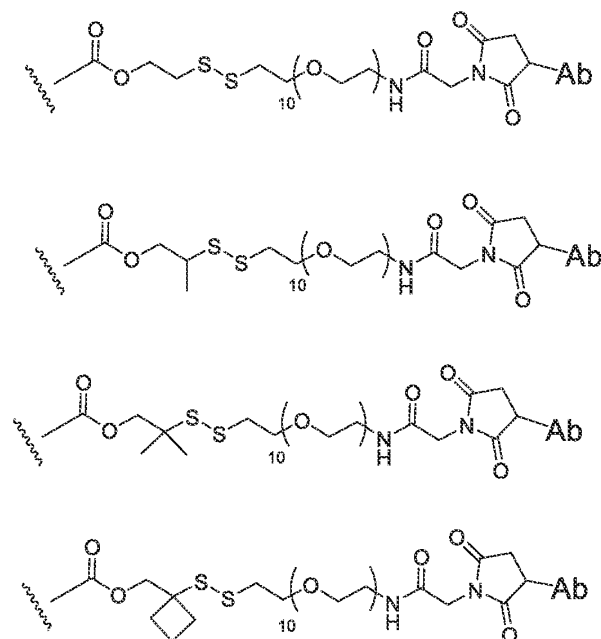
An exemplary embodiment of the macromolecule-supported compound of Formula I

includes wherein L comprises PEP and PEP is selected from the structures:



15

An exemplary embodiment of the macromolecule-supported compound of Formula I includes wherein L is selected from the structures:



where the wavy line indicates the attachment to R⁵.

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

In certain embodiments, the Macromolecule-supported compounds of the invention include those with biological activity. The Macromolecule-supported compounds (MSC) of the invention selectively deliver an effective dose of a 8-sulfonyl-2-aminobenzazepine (8SO2Bz) drug to a mammal, whereby greater selectivity (*i.e.*, a lower efficacious dose) may be achieved while increasing the therapeutic index (“therapeutic window”) relative to unconjugated 8SO2Bz.

Each MSC of Table 3 was prepared, purified by HPLC, and characterized by mass spectroscopy according to the methods of Example 201. Exemplary MSC 1-4 are conjugates of bezlotoxumab. Bezlotoxumab (ZINPLAVA®, Merck & Co.) is an anti-toxin B monoclonal antibody, 145 kDa MW, shown to be effective in treating *Clostridium difficile* infection (Lowy I, et al (2010) *N. Engl. J. Med.* 362(3):197–205; Orth P, et al (2014) *Journal of Biological Chemistry* 289(26):18008–18021; US8257709; US9181632). The antibody sequences of US8257709 and US9181632 are incorporated by reference herein. Exemplary MSC 5-9 are conjugates of BSA monomer, 66 kDa. Bovine serum albumin (BSA) is a globular protein of about 66 kDa MW, used in numerous biochemical applications due to its stability and lack of interference with biological reactions. The BSA structure is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates.

Table 3 8-Sulfonyl-2-Aminobenzazepine Macromolecule-supported Compounds (MSC)

MSC No.	8SO2BzL Table 2	Macromolecule	DAR
MSC-1	8SO2BzL-3	bezlotoxumab	3.9
MSC-2	8SO2BzL-4	bezlotoxumab	3.9
MSC-3	8SO2BzL-5	bezlotoxumab	3.7
MSC-4	8SO2BzL-2	bezlotoxumab	3.7
MSC-5	8SO2BzL-4	BSA monomer	2.2
MSC-6	8SO2BzL-2	BSA monomer	2.1
MSC-7	8SO2BzL-5	BSA monomer	2.2
MSC-8	8SO2BzL-10	BSA monomer	2.0
MSC-9	8SO2BzL-16	BSA monomer	2.1

COMPOSITIONS OF MACROMOLECULE-SUPPORTED COMPOUNDS

The invention provides a composition, e.g., a pharmaceutically or pharmacologically acceptable composition or formulation, comprising a plurality of macromolecule-supported compounds as described herein and optionally a carrier therefor, e.g., a pharmaceutically or pharmacologically acceptable carrier. The macromolecule-supported compounds can be the same or different in the composition, i.e., the composition can comprise macromolecule-supported compounds that have the same number of adjuvants linked to the same positions on the macromolecule and/or macromolecule-supported compounds that have the same number of 8SO2Bz 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, an MSC composition comprises a mixture of the MSC, wherein the average drug (8SO2Bz) loading per macromolecule support in the mixture of MSC is about 2 to about 5.

A composition of MSC of the invention can have an average adjuvant to macromolecule support ratio of about 0.4 to about 10. A skilled artisan will recognize that the number of 8-sulfonyl-2-aminobenzazepine adjuvants conjugated to the macromolecule support may vary in a heterogeneous composition comprising multiple MSC of the invention and thus the adjuvant to macromolecule support ratio can be measured as an average which may be referred to as the drug to macromolecule support ratio. The adjuvant to macromolecule support ratio can be

assessed by any suitable means, many of which are known in the art, including conventional means such as mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of MSC in a composition in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous MSC where p is a certain value from MSC
5 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 MSC of the invention can be formulated for parenteral administration, such as IV administration or administration into a body
10 cavity or lumen of an organ. Alternatively, the MSC can be injected intra-tumorally. Compositions for injection will commonly comprise a solution of the MSC 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
15 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
20 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 MSC. The concentration of the MSC in the composition can vary widely, and will be selected primarily based on fluid
25 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 MSC in a solution formulation for injection will range from about 0.1% (w/w) to about 10% (w/w).

METHOD OF TREATING CANCER WITH MACROMOLECULE-SUPPORTED 8- 30 SULFONYL-BENZAZEPINE COMPOUNDS

The invention provides a method for treating cancer. The method includes administering a therapeutically effective amount of an MSC 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.

It is contemplated that the MSC 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.

5 In another aspect, an MSC for use as a medicament is provided. In certain embodiments, the invention provides an MSC for use in a method of treating an individual comprising administering to the individual an effective amount of the MSC. 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.

10 In a further aspect, the invention provides for the use of an MSC 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
15 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,
20 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
25 cell lung carcinoma; and the like. Carcinomas may be found in prostate, 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 MSC containing an antibody construct that is capable of binding a tumor-associated antigen.

Soft tissue tumors are a highly diverse group of rare tumors that are derived from
30 connective tissue. Examples of soft tissue tumors include, but are not limited to, alveolar soft part sarcoma; angiomatoid fibrous histiocytoma; chondromyxoid fibroma; skeletal chondrosarcoma; extraskeletal myxoid chondrosarcoma; clear cell sarcoma; desmoplastic small round-cell tumor; dermatofibrosarcoma protuberans; endometrial stromal tumor; Ewing's sarcoma; fibromatosis (Desmoid); infantile fibrosarcoma; gastrointestinal stromal tumor; bone
35 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 histiocyoma; high-grade malignant fibrous histiocyoma; 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.

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).

MSC of the invention can be used either alone or in combination with other agents in a therapy. For instance, an MSC 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 MSC can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. MSC can also be used in combination with radiation therapy.

The MSC of the invention (and any additional therapeutic agent) can be administered by any suitable means, including oral, 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.

The MSC 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 labetuzumab, biosimilars thereof, and biobetters thereof. For example, the methods can include administering the MSC to provide a dose of from about 100 ng/kg to about 50 mg/kg to the subject. The MSC 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 MSC dose can be about 100, 200, 300, 400, or 500 µg/kg. The MSC dose can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. The MSC 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 MSC is administered from about once per month to about five times per week. In some embodiments, the MSC 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 MSC (*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.

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

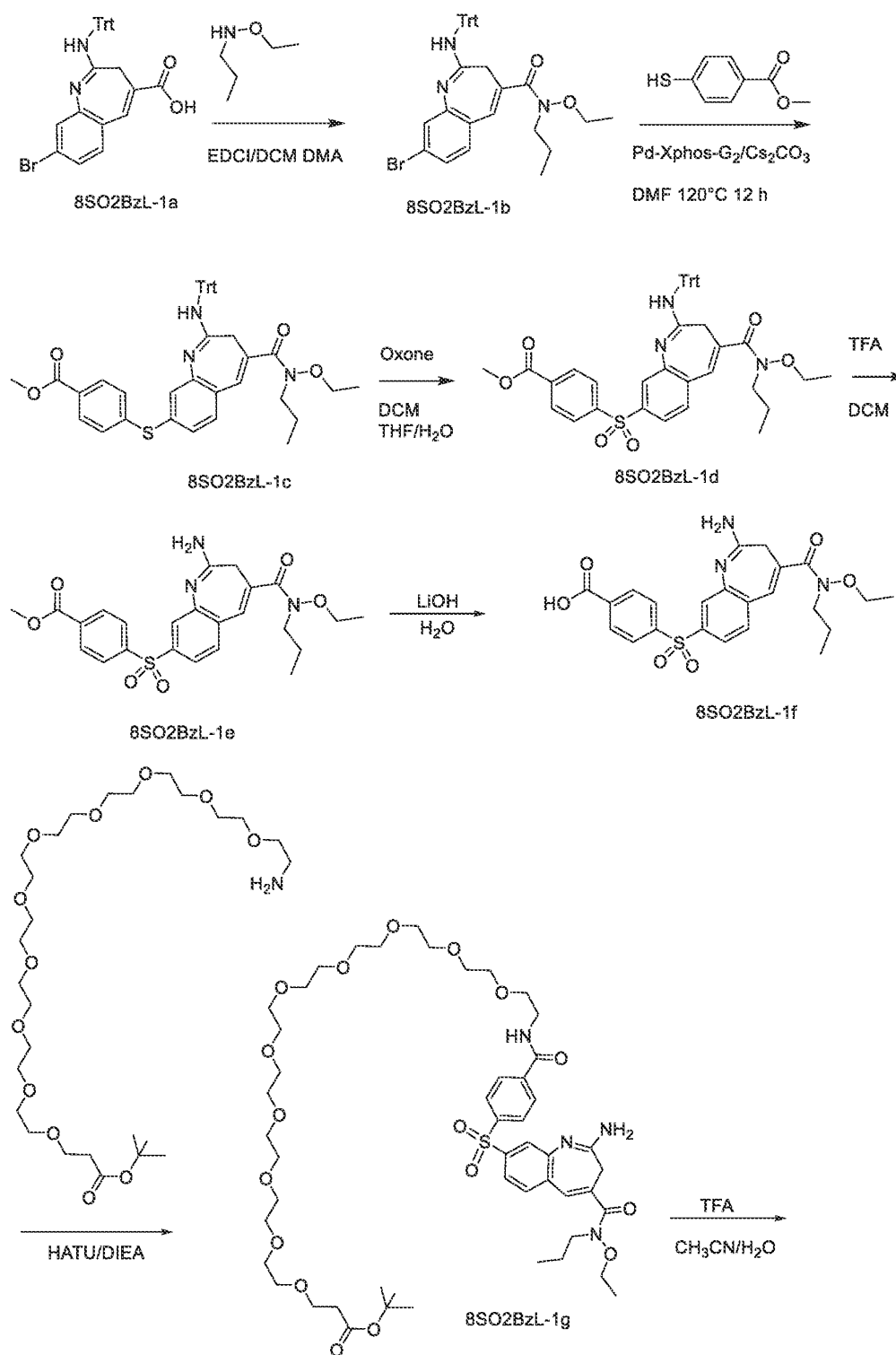
the breast, and a number of different types of breast cancer have been characterized. For example, the MSC of the invention can be used for treating ductal carcinoma *in situ*; invasive ductal carcinoma (e.g., tubular carcinoma; medullary carcinoma; mucinous carcinoma; papillary carcinoma; or cribriform 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 MSC containing cell binding agent that is capable of binding a tumor-associated antigen (TAA), or tumors over-expressing a TAA.

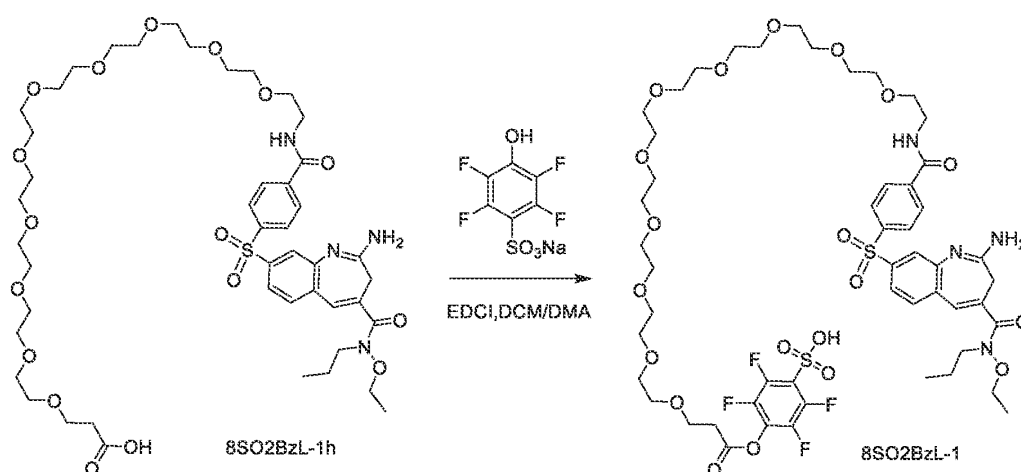
10 In some embodiments, the cancer is susceptible to a pro-inflammatory response induced by TLR7 and/or TLR8.

In some embodiments, a therapeutically effective amount of an MSC 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

20 Example L-1 Synthesis of 4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[4-[2-amino-4-
[ethoxy(propyl) carbamoyl]-3H-1-benzazepin-8-
yl]sulfonyl]benzoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]
ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyloxy]-2,3,5,6-tetrafluoro-benzenesulfonic
acid., 8SO2BzL-1





Preparation of 8-bromo-N-ethoxy-N-propyl-2-(tritylamino)-3H-1-benzazepine-4-carboxamide, 8SO2BzL-1b

To a mixture of 8-bromo-2-(tritylamino)-3H-1-benzazepine-4-carboxylic acid, 8SO2BzL-1a (1 g, 1.91 mmol, 1 *eq*) and N-ethoxypropan-1-amine (320 mg, 2.29 mmol, 1.2 *eq*, HCl) in DCM (15 mL) and DMA (5 mL) was added EDCI (1.10 g, 5.73 mmol, 3.0 *eq*) in one portion at 25°C, and then stirred at 25°C for 0.5 h. The mixture was concentrated to remove DCM. Then the residue was diluted with aq. NaHCO₃ until the pH to between 8 and 9. The mixture was extracted with EtOAc (30 mL x 3). The organic layer was washed with brine, dried over Na₂SO₄, filtered and 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=1/0,3/1) to afford 8SO2BzL-1b (0.7 g, 1.15 mmol, 60.21% yield) as white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.38-7.15 (m, 15H), 7.06-6.94 (m, 2H), 6.72 (s, 1H), 6.16 (s, 1H), 4.03-3.83 (m, 2H), 3.73 (t, J=7.2 Hz, 2H), 2.78 (s, 2H), 1.89-1.64 (m, 2H), 1.25 (t, J=7.2 Hz, 3H), 0.99 (t, J=7.2 Hz, 3H). LC/MS [M+H]⁺ 608.2 (calculated); LC/MS [M+H]⁺ 608.2 (observed).

Preparation of methyl 4-[[4-[ethoxy(propyl)carbamoyl]-2-(tritylamino)-3H-1-benzazepin-8-yl]sulfanyl]benzoate, 8SO2BzL-1c

To a mixture of 8SO2BzL-1b (0.35 g, 575 μmol, 1.0 *eq*) and methyl 4-sulfanylbenzoate (116 mg, 690 μmol, 1.2 *eq*) in DMF (4 mL) was added dicyclohexyl[2',4',6'-tris(propan-2-yl)[1,1'-biphenyl]phosphane, XPhos, CAS Reg. No. 564483-18-7, Huang, X., et al (2003) *J. Am. Chem. Soc.* 125(22):6653-6655; Bruno, N.C. et al, (2013) *Chemical Science*, 4(3):916-920, (82.3 mg, 173 μmol, 0.3 *eq*), Cs₂CO₃ (375 mg, 1.15 mmol, 2.0 *eq*) and [2-(2-aminophenyl)phenyl]-chloro-palladium;dicyclohexyl-[3-(2,4,6-triisopropylphenyl)phenyl]phosphane, Pd-Xphos-G2, CAS Reg. No. 1310584-14-5 (226 mg, 288 μmol, 0.5 *eq*) in one portion at 25 °C under N₂, and then stirred at 120 °C for 12 h. The mixture was

diluted water (20 mL) and extracted with EtOAc (10 mL x 3). The organic layer was washed with brine, dried over Na₂SO₄, filtered and 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, 3/1) to afford 8SO2BzL-1c (0.25 g, 359.26 umol, 62.47% yield). LC/MS [M+H]⁺ 696.3 (calculated); LC/MS [M+H]⁺ 696.2 (observed).

Preparation of methyl 4-[[4-[ethoxy(propyl)carbamoyl]-2-(tritylamino)-3H-1-benzazepin-8-yl]sulfonyl]benzoate, 8SO2BzL-1d

To a mixture of 8SO2BzL-1c (0.25 g, 359 umol, 1.0 eq) in DCM (2 mL), THF (2 mL) and H₂O (2 mL) was added potassium peroxydisulfate, KHSO₅, Oxone (663 mg, 1.08 mmol, 3.0 eq) in one portion at 25°C, and then stirred at 25 °C for 12 h. The mixture was diluted with water and extracted with EtOAc (20 mL x 3). The organic layer was washed with brine, dried over Na₂SO₄, filtered and 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 8SO2BzL-1d (0.2 g, 274.78 umol, 76.48% yield) as yellow oil.

LC/MS [M+H]⁺ 728.27 (calculated); LC/MS [M+H]⁺ 728.2 (observed).

Preparation of methyl 4-[[2-amino-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepin-8-yl]sulfonyl]benzoate, 8SO2BzL-1e

To a mixture of 8SO2BzL-1d (0.1 g, 137 umol, 1.0 eq) in DCM (4 mL) was added TFA (313 mg, 2.75 mmol, 203 uL, 20.0 eq) in one portion at 25°C, and then stirred at 50 °C for 12 h.

The mixture was concentrated in vacuum to give a residue, the residue was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3um;mobile phase: [water(0.1%TFA)-acetonitrile, ACN];B%: 15%-35%,8min) to give 8SO2BzL-1e (0.046 g, 94.74 umol, 68.96% yield) as white solid. ¹H NMR (CDCl₃, 400 MHz) δ 8.21 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 8.04 (s, 1H), 7.95-7.87 (m, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 6.97 (d, J = 1.2 Hz, 1H), 3.95 (s, 3H), 3.93-3.82 (m, 2H), 3.70 (t, J = 7.2 Hz, 2H), 3.23 (s, 2H), 1.82-1.66 (m, 2H), 1.21 (t, J = 7.2 Hz, 3H), 0.96 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 485.16 (calculated); LC/MS [M+H]⁺ 486.1 (observed).

Preparation of 4-[[2-amino-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepin-8-yl]sulfonyl]benzoic acid, 8SO2BzL-1f

To a mixture of 8SO2BzL-1e (0.24 g, 494 umol, 1.0 eq) in MeOH (2 mL), H₂O (2 mL) and THF (2 mL) was added LiOH.H₂O (62.2 mg, 1.48 mmol, 3.0 eq) in one portion at 25°C, and then stirred at 25°C for 2 h. The mixture was quenched with HCl (1 M) to adjust pH to between 6 and 7 and the aqueous phase was extracted with EtOAc (10 mL x 3). The combined organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated to give 8SO2BzL-1f (0.23 g, crude) as yellow oil. ¹H NMR (DMSO, 400 MHz) δ 8.16-8.06 (m, 4H),

7.61-7.54 (m, 2H), 7.49 (dd, $J = 1.6, 8.0$ Hz, 1H), 7.11 (s, 1H), 3.82 (q, $J = 7.2$ Hz, 2H), 3.59 (t, $J = 7.2$ Hz, 2H), 2.89 (s, 2H), 1.70-1.55 (m, 2H), 1.01 (t, $J = 7.2$ Hz, 3H), 0.89 (t, $J = 7.2$ Hz, 3H).
LC/MS [M+H]⁺ 472.1 (calculated); LC/MS [M+H]⁺ 472.1 (observed).

Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[4-[[2-amino-4-[ethoxy(propyl) 5 carbamoyl]-3H-1-benzazepin-8-yl]sulfonyl]benzoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, 8SO2BzL-1g

To a mixture of 8SO2BzL-1f (0.2 g, 424 umol, 1.0 eq) in DMF (4 mL) was added tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] propanoate (248 mg, 424 umol, 1.0 eq), DIEA (164 mg, 1.27 mmol, 222 uL, 3.0 eq), and Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium, HATU, CAS Reg. No. 148893-10-1 (177 mg, 467 umol, 1.1 eq) in one portion at 25°C, and then stirred at 25°C for 0.5 h. The mixture was diluted with water and extracted with EtOAc (30 mL x 3). The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated to give 8SO2BzL-1g (0.5 g, crude) as yellow oil. LC/MS [M+H]⁺ 1039.5 (calculated); LC/MS [M+H]⁺ 1039.5(observed).

[illegible]

To a mixture of 8SO2BzL-1g (0.5 g, 481 μ mol, 1.0 *eq*) in CH₃CN (1 mL) and H₂O (3 mL) was added TFA (439 mg, 3.85 mmol, 285 μ L, 8.0 *eq*) in one portion at 25°C, and then stirred at 80 °C for 1 hours. The mixture was concentrated to give a residue. The residue was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3 μ m; mobile phase: [water(0.1%TFA)-ACN];B%: 10%-35%,8min) to give 8SO2BzL-1h (0.15 g, 152.57 μ mol, 31.71% yield) as yellow oil. ¹H NMR (MeOD, 400 MHz) δ 8.14-8.08 (m, 2H), 8.06-7.95 (m, 4H), 7.78 (d, J = 8.4 Hz, 1H), 7.40 (s, 1H), 3.94 (q, J = 6.8 Hz, 2H), 3.75-3.70 (m, 4H), 3.65-3.50 (m, 40H), 3.40 (s, 2H), 2.53 (t, J = 6.4 Hz, 2H), 1.82-1.69 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 982.45 (calculated); LC/MS [M+H]⁺ 983.4 (observed).

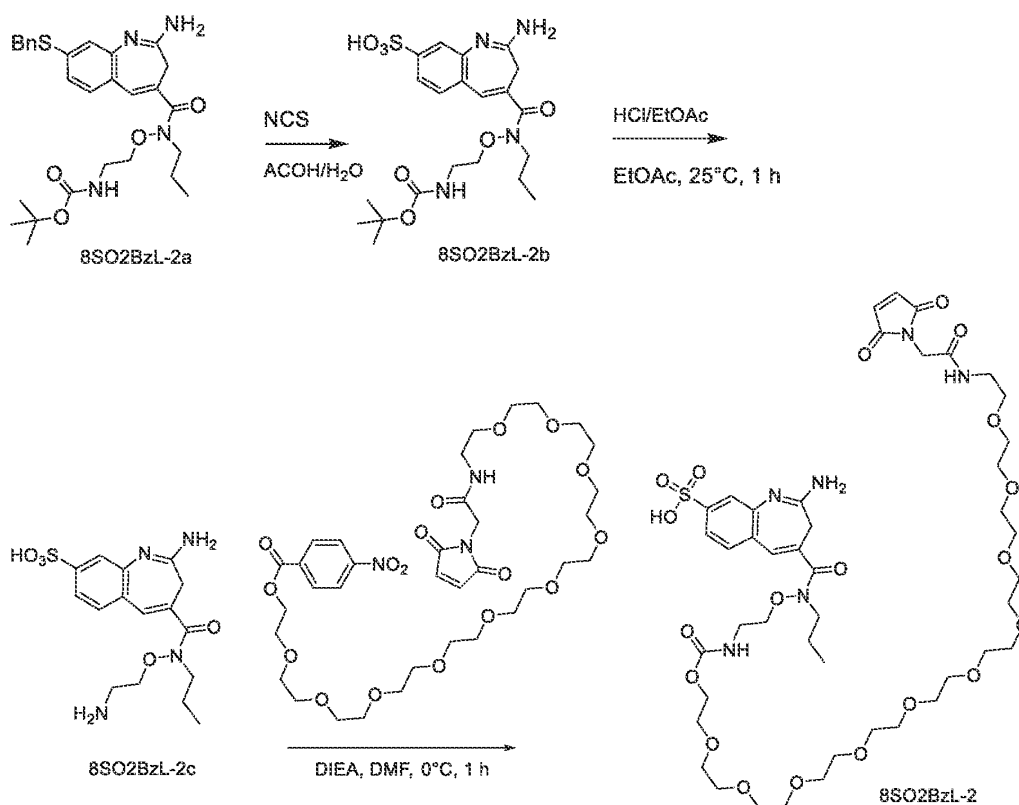
Preparation of 8SO2BzL-1

To a mixture of 8SO2BzL-1h (0.15 g, 152.57 μ mol, 1.0 *eq*) in DCM (3 mL) and DMA (0.5 mL) was added sodium;2,3,5,6-tetrafluoro-4-hydroxy-benzenesulfonate (164 mg, 610 μ mol, 4.0 *eq*) and EDCI (146 mg, 763 μ mol, 5.0 *eq*) in one portion at 25°C, and then stirred at 25°C for 0.5 h. The mixture was concentrated to give a residue. The residue was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3 μ m;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-40%,8min) to give 8SO2BzL-1 (58.9 mg, 48.63 μ mol, 31.87% yield) as light yellow solid.

¹H NMR (MeOD, 400 MHz) δ 8.11-8.06 (m, 2H), 8.05-7.99 (m, 3H), 7.95 (dd, J = 2.0, 8.4 Hz,

1H), 7.77 (d, J = 8.4 Hz, 1H), 7.39 (s, 1H), 3.93 (q, J = 7.2 Hz, 2H), 3.86 (t, J = 6.0 Hz, 2H), 3.71 (t, J = 7.2 Hz, 2H), 3.67-3.53 (m, 34H), 3.53-3.48 (m, 6H), 3.42 (s, 2H), 2.97 (t, J = 6.0 Hz, 2H), 1.83-1.65 (m, 2H), 1.15 (t, J = 7.2 Hz, 3H), 0.97 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 1211.4 (calculated); LC/MS [M+H]⁺ 1211.3 (observed).

- 5 Example L-2 Synthesis of 2-amino-4-[2-[2-[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]ethoxy]ethoxycarbonylamino]propyl-carbamoyl]-3H-1-benzazepine-8-sulfonic acid, 8SO2BzL-2



10

Preparation of 2-amino-4-[2-(tert-butoxycarbonylamino)ethoxy-propyl-carbamoyl]-3H-1-benzazepine-8-sulfonic acid, 8SO2BzL-2b

- To a mixture of tert-butyl N-[2-[(2-amino-8-benzylsulfany]-3H-1-benzazepine-4-carbonyl)-propyl-amino]oxyethyl]carbamate, 8SO2BzL-2a (0.15 g, 286 μmol, 1.0 eq) in H₂O (0.15 mL) and AcOH (0.5 mL) was added N-chlorosuccinimide, NCS (153 mg, 1.14 mmol, 4.0 eq) in one portion at 25 °C, and then stirred at 25 °C for 1 h. The mixture was diluted with aq. NaHCO₃ to adjust pH between 7 and 8. Then the mixture was extracted with EtOAc (10 mL x 3). The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated. The residue was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3μm;mobile

phase: [water(TFA)-ACN];B%: 5%-35%,8min) to give 8SO2BzL-2b (11.5 mg, 23.83 μ mol, 8.34% yield) as yellow solid. ^1H NMR (DMSO, 400 MHz) δ 11.87 (s, 1H), 9.78 (s, 1H), 8.81 (s, 1H), 7.66 (s, 1H), 7.55 (s, 2H), 7.34-7.27 (m, 1H), 3.92-3.78 (m, 2H), 3.63 (t, J = 7.2 Hz, 2H), 3.31 (s, 2H), 3.16-3.01 (m, 2H), 1.70-1.60 (m, 2H), 1.36 (s, 9H), 0.89(t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 483.2(calculated); LC/MS [M+H]⁺ 483.1 (observed).

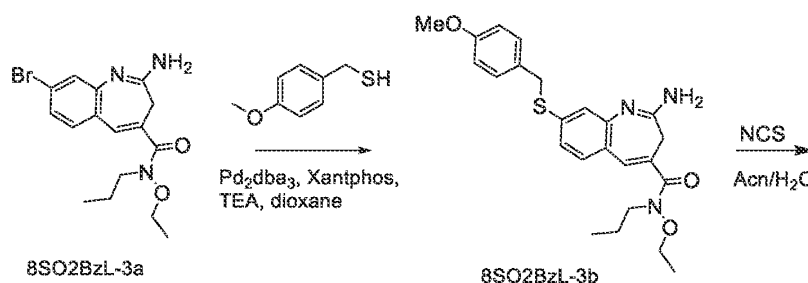
Preparation of 2-amino-4-[2-aminoethoxy(propyl)carbamoyl]-3H-1- benzazepine-8-sulfonic acid, 8SO2BzL-2c

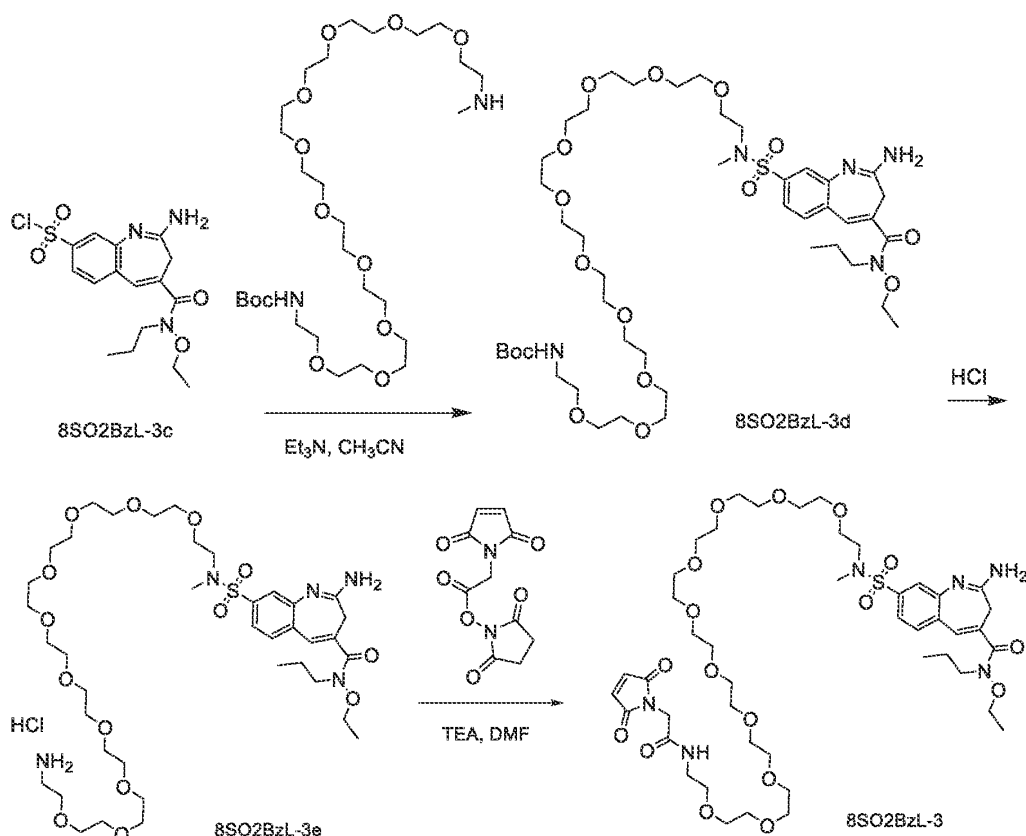
To a solution of 8SO2BzL-2b (150 mg, 310.85 μ mol, 1 eq) in EtOAc (10.0 mL) was added HCl/EtOAc (4 M, 20.0 mL, 257 eq), and then stirred at 25 °C for 1 h. The mixture was concentrated to give 8SO2BzL-2c (200 mg, crude) as white solid. LC/MS [M+H]⁺ 383.1(calculated); LC/MS [M+H]⁺ 383.2 (observed).

Preparation of 8SO2BzL-2

To a solution of 8SO2BzL-2c (70.0 mg, 167.11 μ mol, 1 eq, HCl) in DMF (1.00 mL) was added DIEA (90.0 mg, 668 μ mol, 120 μ L, 4 eq) and 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]ethyl (4-nitrophenyl) carbonate (70.0 mg, 83.5 μ mol, 0.5 eq), and then stirred at 0 °C for 1 h. The mixture was filtered and purified by prep-HPLC (column: Phenomenex Luna 80*30mm*3 μ m;mobile phase: [water(TFA)-ACN];B%: 1%-30%,8min) to give 8SO2BzL-2 (15 mg, 12.92 μ mol, 7.73% yield, TFA) as light yellow oil. ^1H NMR (MeOD, 400 MHz) δ 7.89-7.77 (m, 2H), 7.66 (d, J = 8.0 Hz, 1H), 7.42 (s, 1H), 6.89 (s, 2H), 4.17 (s, 2H), 3.97 (br t, J = 4.8 Hz, 2H), 3.86-3.79 (m, 2H), 3.75 (t, J = 7.2 Hz, 2H), 3.66-3.58 (m, 38H), 3.56-3.49 (m, 4H), 3.42-3.35 (m, 4H), 1.81-1.73 (m, 2H), 1.00 (t, J = 7.6 Hz, 3H). LC/MS [M+H]⁺ 1047.4(calculated); LC/MS [M+H]⁺ 1047.7 (observed).

Example L-3 Synthesis of 2-amino-8-(N-(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)-N-methylsulfamoyl)-N-ethoxy-N-propyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-3





Preparation of 2-amino-8-((4-methoxybenzyl)thio)-N-propyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-3b

- 5 To a mixture of 2-amino-8-bromo-N-ethoxy-N-propyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-3a (0.96 g, 1.99 mmol) and 4-methoxy- α -toluenethiol (0.37 g, 2.39 mmol) in dioxane (10 mL), was added 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene, Xantphos, CAS Reg. No. 161265-03-8 (0.06 g, 0.10 mmol), $\text{Pd}_2(\text{dba})_3$ (0.05 g, 0.05 mmol), and then triethylamine (0.56 mL, 3.99 mmol). The mixture was heated to reflux for 1 h then cooled.
- 10 The solvent was removed by evaporation and the crude product was purified by silica gel chromatography using a gradient of 1-10% MeOH/DCM over 12 column volumes to give 8SO2BzL-3b (0.72 g, 82%).

Preparation of 2-amino-4-(ethoxy(propyl)carbamoyl)-3H-benzo[b]azepine-8-sulfonyl chloride, 8SO2BzL-3c

- 15 To a solution of 8SO2BzL-3b (0.72 g, 1.64 mmol) in acetonitrile/water (9:1), 10 mL at 10 deg C was added in portions N-chlorosuccinimide (0.66 g, 4.91 mmol). After addition was complete, stirred for an additional 20 min to give 8SO2BzL-3c crude product solution, used as is in the next step.

Preparation of *tert*-butyl (2-((2-amino-4-(ethoxy(propyl)carbamoyl)-3H-benzo[b]azepin-8-yl)sulfonyl)-5,8,11,14,17,20,23,26,29,32-decaoxa-2-azatetatriacontan-34-yl)carbamate, 8SO2BzL-3d

An aliquot of 8SO2BzL-3c (1.00 mL, 0.16 mmol) was added dropwise to a stirring mixture of *tert*-butyl (5,8,11,14,17,20,23,26,29,32-decaoxa-2-azatetatriacontan-34-yl)carbamate (0.12 g, 0.19 mmol) and triethylamine (0.09 mL, 0.64 mmol) in acetonitrile (3 mL). After 15 min the reaction was concentrated and purified by reverse phase chromatography using a gradient of 10-90% ACN/water (+0.1% TFA) over 10 min to obtain 8SO2BzL-3d (0.08 g, 51%).

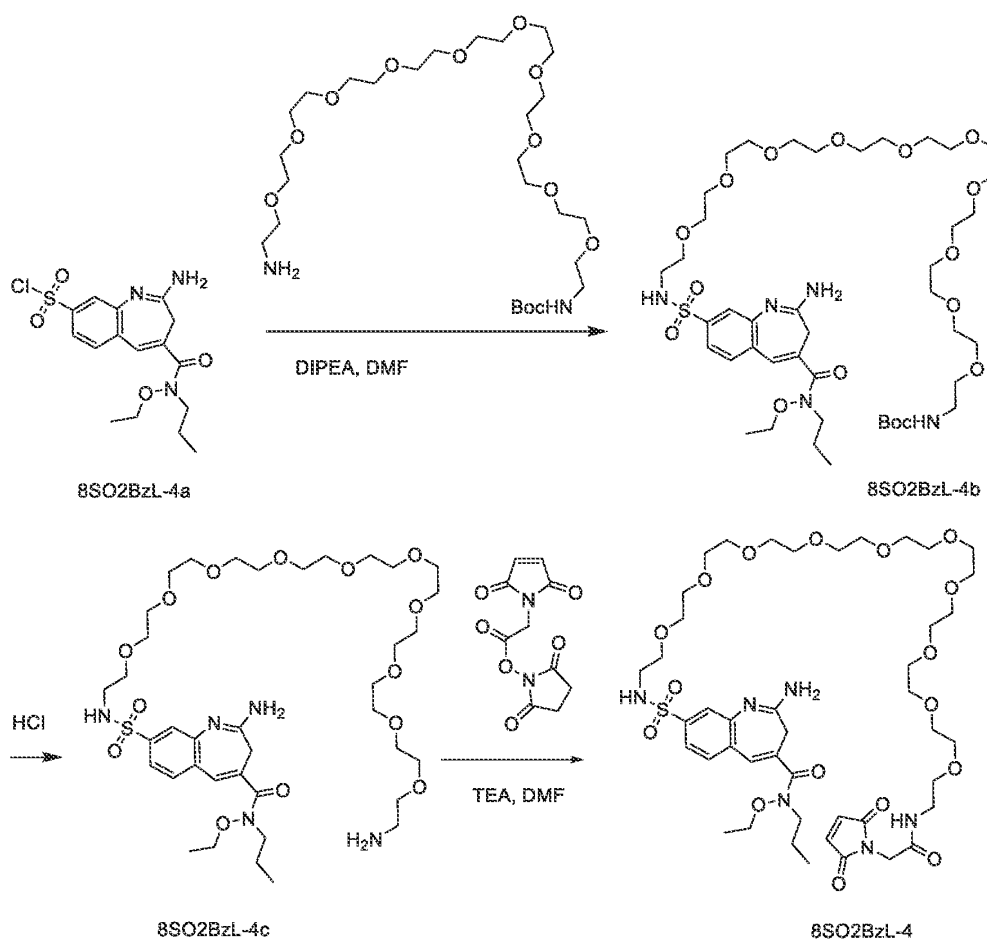
Preparation of 2-amino-8-(N-(32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-N-methylsulfamoyl)-N-ethoxy-N-propyl-3H-benzo[b]azepine-4-carboxamide hydrogen chloride, 8SO2BzL-3e

To a solution of 8SO2BzL-3d (0.08 g, 0.08 mmol) in ACN (3 mL) was added aq. HCl (6M, 3 mL) and the mixture was stirred at room temp. for 45 min. The solvent was removed and the isolated syrup was azeotroped with ACN (3 mL) to provide the HCl salt of 8SO2BzL-3e (0.06 g, 85%) as a hazy, white film.

Preparation of 8SO2BzL-3

To a solution of 8SO2BzL-3e HCl (0.06 g, 0.07 mmol) in DMF (3 mL) was added triethylamine (0.04 mL, 0.28 mmol). 2,5-Dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (0.02 g, 0.08 mmol) was added in parts. After completion of addition, acetic acid (9 μ L) was added and the solvent was removed by vacuum. After purification by reverse phase HPLC, 8SO2BzL-3 (0.03 g, 48%) was obtained as a clear oil after evaporation of solvent. LC/MS [M+H]⁺ 1001.48 (calculated); LC/MS [M+H]⁺ 1074.88 (observed).

Example L-4 Synthesis of 2-amino-8-(N-(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)sulfamoyl)-N-ethoxy-N-propyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-4



Preparation of tert-butyl (32-((2-amino-4-(ethoxy(propyl)carbamoyl)-3H-benzo[b]azepine)-8-sulfonamido)-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)carbamate, 8SO2BzL-4b

To a solution of tert-butyl (32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)carbamate, Boc-amino-PEG10-amine (0.10 g, 0.16 mmol) and DIPEA (0.14 mL, 0.80 mmol) in DMF (4 mL) was added a solution of 2-amino-4-(ethoxy(propyl)carbamoyl)-3H-benzo[b]azepine-8-sulfonyl chloride, 8SO2BzL-4a (0.16 M, 1.00 mL, 0.16 mmol) in DMF. After 20 min the reaction was concentrated and then purified on reverse phase HPLC using a gradient of 10-90% ACN/water over 10 min to give 8SO2BzL-4b (0.07 g, 47%) after removal of solvent.

Preparation of 2-amino-8-(N-(32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)sulfamoyl)-N-ethoxy-N-propyl-3H-benzo[b]azepine-4-carboxamide, SO2BzL-4c

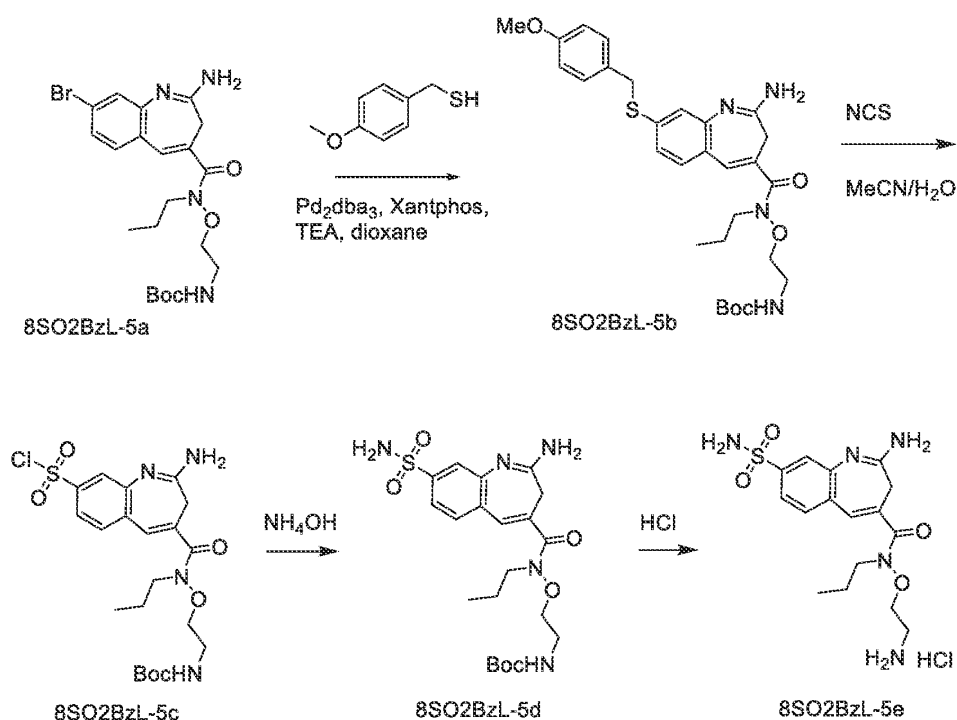
To a solution of 8SO2BzL-4b (0.07 g, 0.07 mmol) in acetonitrile (3 mL) was added aq. HCl (6M, 3 mL) and the mixture was stirred at RT for 45 min. The solvent was removed and the isolated syrup was azeotroped with ACN (3 mL) to provide 8SO2BzL-4c HCl salt (0.06 g, 82%) as a hazy, white film.

5 Preparation of 8SO2BzL-4

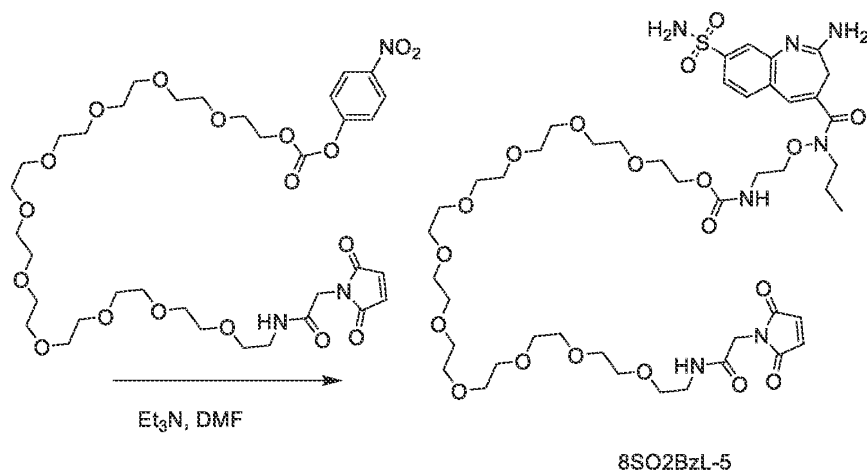
To a solution of 8SO2BzL-4c HCl (0.06 g, 0.06 mmol) in DMF (3 mL) was added triethylamine (0.03 mL, 0.25 mmol). 2,5-Dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (0.02 g, 0.07 mmol) was added in parts. After completion of addition, acetic acid (9 μ L) was added and the solvent was removed by vacuum. After purification by reverse
 10 phase HPLC, 8SO2BzL-4 (0.04 g, 69%) was obtained after solvent removal. LC/MS [M+H]⁺ 987.45 (calculated); LC/MS [M+H]⁺ 987.86 (observed).

Example L-5

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 2-((2-amino-N-propyl-8-sulfamoyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-5



15



Preparation of tert-butyl (2-((2-amino-8-((4-methoxybenzyl)thio)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-b

To a mixture of tert-butyl (2-((2-amino-8-bromo-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-5a (0.96 g, 1.99 mmol) and 4-methoxy- α -toluenethiol (0.37 g, 2.39 mmol) in dioxane (10 mL), added Xantphos (0.06 g, 0.10 mmol), Pd₂(dba)₃ (0.05 g, 0.05 mmol), then triethylamine (0.56 mL, 3.99 mmol). The mixture was heated to reflux for 1 h then cooled. The solvent was removed by evaporation and the crude product was purified by silica gel chromatography using a gradient of 1-10% MeOH/DCM over 12 column volume to give 8SO2BzL-5b (0.78 g, 71%) as a yellow solid.

Preparation of tert-butyl (2-((2-amino-8-(chlorosulfonyl)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-5c

To a solution of 8SO2BzL-5b (0.78 g, 1.41 mmol) in acetonitrile/water (9:1, 10 mL), at 0 deg C was added in three equal portions N-chlorosuccinimide (0.56 g, 4.22 mmol). After the addition was complete, the product 8SO2BzL-5c was used as is without further purification.

Preparation of tert-butyl (2-((2-amino-N-propyl-8-sulfamoyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-5d

To a solution of 8SO2BzL-5c (2.00 mL, 0.28 mmol) in acetonitrile/water (9:1) at 0 deg was added ammonium hydroxide solution (0.20 mL, 1.66 mmol). After 10 minutes the solvent was removed, and the crude product was purified by reverse phase HPLC using a gradient of 10-90% acetonitrile/water to give 8SO2BzL-5d (0.06 g, 41%) as a yellow film after evaporation of solvent.

Preparation of 8SO2BzL-5e

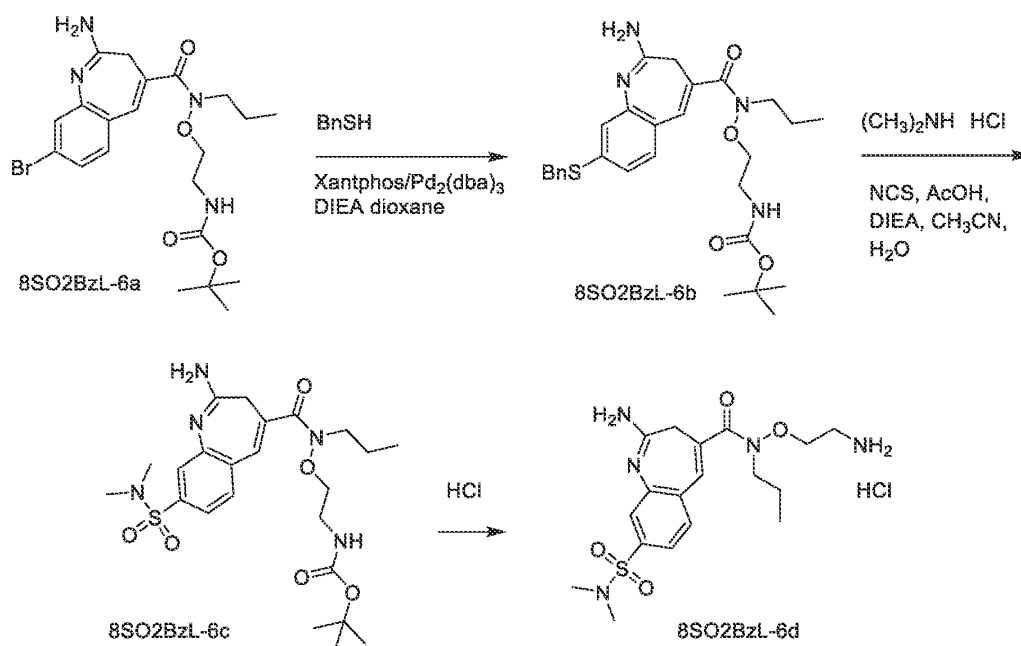
A solution of 8SO2BzL-5e (0.06 g, 0.11 mmol) in acetonitrile (2 mL) and 6 N HCl (2.00 mL, 12.00 mmol) was stirred at room temperature for 45 minutes. The solvent was removed by vacuum to give 8SO2BzL-5e as the HCl salt (0.05 g, 101%).

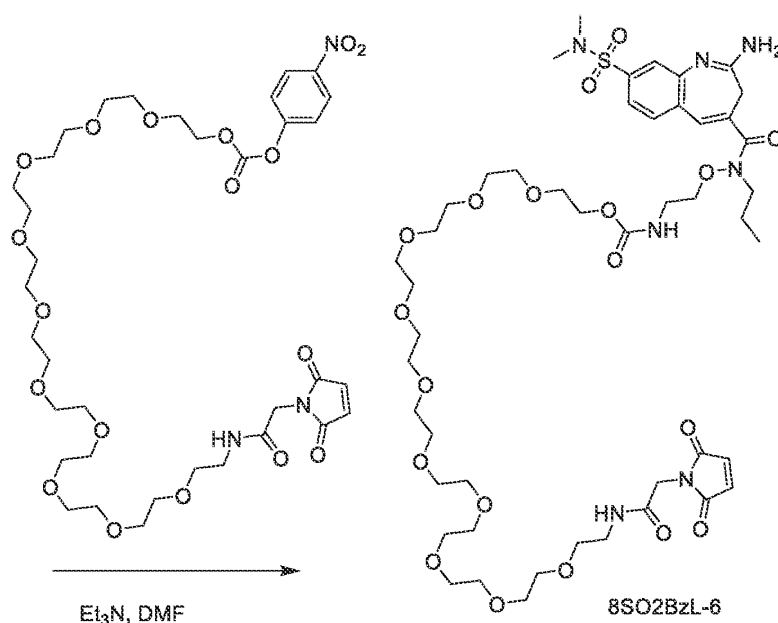
Preparation of 8SO2BzL-5

- 5 To a solution of 8SO2BzL-5e (0.04 g, 0.10 mmol) in DMF (4 mL) at room temperature was added triethylamine (0.06 mL, 0.40 mmol). To this mixture was added a solution 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, PNPC-PEG10-Mal (0.08 g, 0.10 mmol) in DMF (2 mL). After 20 min acetic acid (6 uL) was added and reaction was concentrated under vacuum and purified by reverse phase HPLC using a gradient of 10-90% ACN/water (+0.1% TFA) over 10 min to give 8SO2BzL-5 (0.04 g, 37%) after concentration of pure fractions. LC/MS [M+H]⁺ 1046.45 (calculated); LC/MS [M+H]⁺ 1046.88 (observed).

Example L-6

- 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 (2-((2-amino-8-(N,N-dimethylsulfamoyl)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-6





Preparation of tert-butyl N-[2-[(2-amino-8-benzylsulfanyl-3H-1-benzazepine-4-carbonyl)-propyl-amino]oxyethyl]carbamate, 8SO2BzL-6b

To a mixture of tert-butyl N-[2-[(2-amino-8-bromo-3H-1-benzazepine-4-carbonyl)-propyl-amino]oxyethyl]carbamate, 8SO2BzL-6a (0.5 g, 1.04 mmol, 1.0 *eq*) and benzylthiol, benzylmercaptan, phenylmethanethiol, BnSH, CAS Reg. No. 100-53-8 (155 mg, 1.25 mmol, 146.05 μ L, 1.2 *eq*) in dioxane (15 mL) was added 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene, Xantphos, CAS Reg. No. 161265-03-8 (120 mg, 208 μ mol, 0.2 *eq*) tris(dibenzylideneacetone)dipalladium, Pd₂(dba)₃, CAS Reg. No. 51364-51-3 (190 mg, 208 μ mol, 0.2 *eq*) and diisopropylethylamine, DIEA (268 mg, 2.08 mmol, 362 μ L, 2.0 *eq*) in one portion at 25 °C under N₂, and then stirred at 110°C for 2 h. The mixture was diluted with water (20 mL) and extracted with EtOAc (10 mL x 3). The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated. The mixture was further purification by prep-HPLC (column: Phenomenex Luna 80*30mm*3 μ m; mobile phase: [water(TFA)-ACN]; B%: 25%-55%, 8min) to give 8SO2BzL-6b (0.5 g, 952.97 μ mol, 91.75% yield) as yellow solid. ¹H NMR (MeOD, 400 MHz) δ 7.49 (d, J = 8.4 Hz, 1H), 7.43-7.38 (m, 3H), 7.36-7.22 (m, 5H), 4.30 (s, 2H), 3.91 (t, J = 5.2 Hz, 2H), 3.73 (t, J = 7.2 Hz, 2H), 3.32 (s, 2H), 3.24 (t, J = 5.2 Hz, 2H), 1.81-1.70 (m, 2H), 1.34 (s, 9H), 0.98 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 525.2 (calculated); LC/MS [M+H] 525.2 (observed).

Preparation of tert-butyl N-[2-[[2-amino-8-(dimethylsulfamoyl)-3H-1-benzazepine-4-carbonyl]-propyl-amino]oxyethyl]carbamate, 8SO2BzL-6c

To a solution of 8SO2BzL-6b (50.0 mg, 95.30 μ mol, 1 eq) in CH₃CN (1.00 mL) and H₂O (0.10 mL) was added AcOH (60.0 mg, 953 μ mol, 50.0 μ L, 10 eq), N-chlorosuccinimide, NCS (50.0 mg, 381 μ mol, 4 eq) at 25°C, and then stirred at this temperature for 10 min, then N-methylmethanamine;hydrochloride, dimethylamine HCl (80.0 mg, 953 μ mol, 10 eq) and DIEA (250 mg, 1.91 mmol, 330 μ L, 20 eq) was added. The mixture was stirred at 0 °C for another 1h. The mixture was filtered and purified by prep-HPLC (column: Phenomenex Luna 80*30mm*3 μ m;mobile phase: [water(TFA)-ACN];B%: 10%-40%,8min) to give 8SO2BzL-6c (12 mg, 23.55 μ mol, 24.71% yield) as white solid. ¹H NMR (MeOD, 400 MHz) δ 7.92-7.72 (m, 3H), 7.50 (s, 1H), 3.94 (t, J = 5.2 Hz, 2H), 3.75 (t, J = 7.2 Hz, 2H), 3.44 (s, 2H), 3.26 (br t, J = 5.2 Hz, 2H), 2.77 (s, 6H), 1.77 (sxt, J = 7.2 Hz, 2H), 1.37 (s, 9H), 0.99 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 510.2 (calculated); LC/MS [M+H] 510.3 (observed).

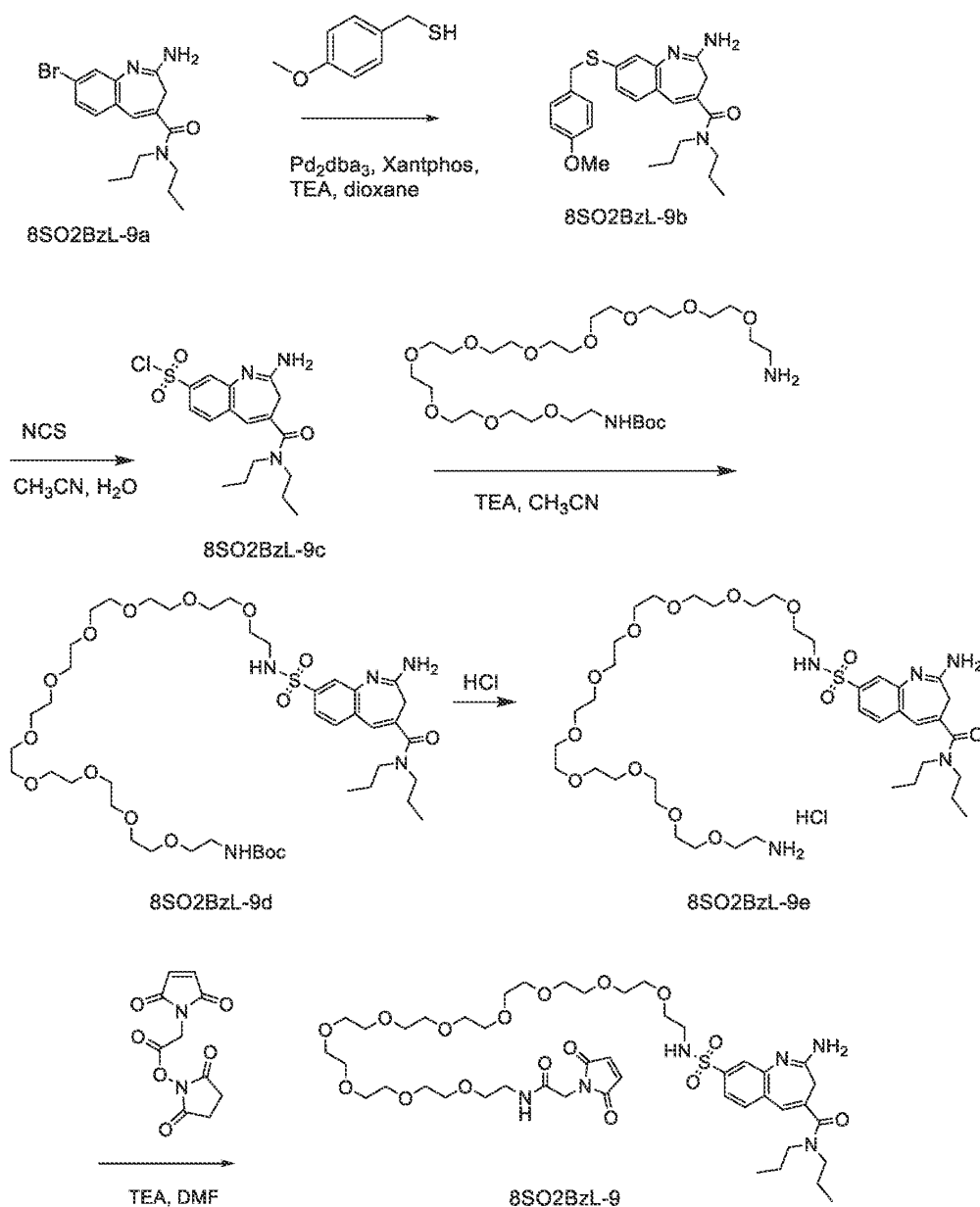
Preparation of 2-amino-N-(2-aminoethoxy)-8-(N,N-dimethylsulfamoyl)-N-propyl-3H-benzo[b]azepine-4-carboxamide hydrogen chloride, 8SO2BzL-6d

A solution of 8SO2BzL-6c (0.04 g, 0.08 mmol) in acetonitrile (2 mL) and 6 N HCl (1.41 mL, 8.46 mmol) was stirred at room temperature for 45 minutes. The solvent was removed by vacuum to give 8SO2BzL-6d (0.04 g, 100%).

Preparation of 8SO2BzL-6

To a solution of 8SO2BzL-6d (0.04 g, 0.10 mmol) in DMF (4 mL) at room temperature added triethylamine (0.04 mL, 0.28 mmol). To this mixture was added a solution 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 (0.06 g, 0.07 mmol) in DMF (2 mL). After 20 min acetic acid (6 μ L) was added and reaction was concentrated under vacuum and purified by reverse phase HPLC using a gradient of 10-90% ACB/water (+0.1% TFA) over 10 min to give 8SO2BzL-6 (0.04 g, 51%) after concentration of pure fractions. LC/MS [M+H] 1074.48 (calculated); LC/MS [M+H] 1074.90 (observed).

Example L-9 Synthesis of 2-amino-8-(N-(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)sulfamoyl)-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-9



Preparation of 2-amino-8-((4-methoxybenzyl)thio)-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-9b

- 5 To a mixture of 2-amino-8-bromo-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-9a (0.96 g, 1.99 mmol) and 4-methoxy- α -toluenethiol (0.37 g, 2.39 mmol) in dioxane (10 mL), added Xantphos (0.06 g, 0.10 mmol), $\text{Pd}_2(\text{dba})_3$ (0.05 g, 0.05 mmol), then triethylamine (0.56 mL, 3.99 mmol). The mixture was heated to reflux for 1 h then cooled. The solvent was removed by evaporation and the crude product was purified by silica gel

chromatography using a gradient of 1-10% MeOH/DCM over 12 column volumes to give 8SO2BzL-9b (0.69 g, 79%).

Preparation of 2-amino-4-(dipropylcarbamoyl)-3H-benzo[b]azepine-8-sulfonyl chloride, 8SO2BzL-9c

- 5 To a solution of 8SO2BzL-9b (0.68 g, 1.55 mmol) in acetonitrile/water (9:1), 10 mL at 10 deg C was added N-chlorosuccinimide, NCS in thirds (0.62 g, 4.66 mmol). After addition was complete, stirred for an additional 20 min. to give 8SO2BzL-9c.

Preparation of tert-butyl (32-((2-amino-4-(dipropylcarbamoyl)-3H-benzo[b]azepine)-8-sulfonamido)-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)carbamate, 8SO2BzL-9d

- 10 An aliquot of 8SO2BzL-9c (1.00 mL, 0.15 mmol) obtained previously was added drop-wise to a stirring mixture of *tert*-butyl (32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)carbamate (0.11 g, 0.18 mmol) and triethylamine (0.08 mL, 0.60 mmol) in acetonitrile (3 mL). After 15 min the reaction was concentrated and purified by reverse phase chromatography using a gradient of 10-90% ACN/water (+0.1% TFA) over 10 min to 15 8SO2BzL-9d (0.07 g, 51%).

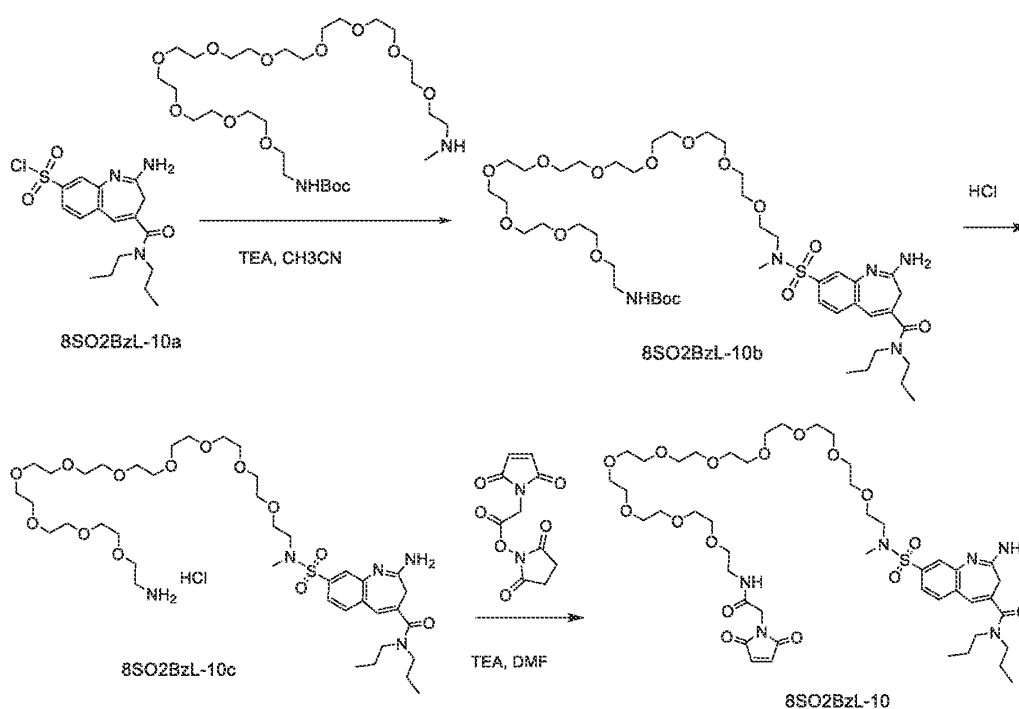
Preparation of 2-amino-8-(N-(32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)sulfamoyl)-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-9e

- 20 To a solution of 8SO2BzL-9d (0.07 g, 0.08 mmol) in acetonitrile (3 mL) was added aq. HCl (6M, 3 mL) and the mixture was stirred at RT for 45 min. The solvent was removed and the isolated syrup was azeotroped with acetonitrile (3 mL) to provide 8SO2BzL-9e as the HCl salt (0.06 g, 86%) as a hazy, white film.

Preparation of 8SO2BzL-9

- 25 To a solution of 8SO2BzL-9e HCl (0.06 g, 0.06 mmol) in DMF (3 mL) was added triethylamine (0.03 mL, 0.25 mmol). 2,5-Dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (0.02 g, 0.07 mmol) was added in parts. After completion of addition, acetic acid (9 uL) was added and the solvent was removed by vacuum. After purification by reverse phase HPLC to give 8SO2BzL-9 (0.03 g, 43%) after evaporation. LC/MS [M+H] 985.47 (calculated); LC/MS [M+H] =985.88 (observed).

- 30 Example L-10 Synthesis of 2-amino-8-(N-(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)-N-methylsulfamoyl)-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-10



Preparation of tert-butyl (2-((2-amino-4-(dipropylcarbamoyl)-3H-benzo[b]azepin-8-yl)sulfonyl)-5,8,11,14,17,20,23,26,29,32-decaoxa-2-azatetratriacontan-34-yl)carbamate,

5 8SO2BzL-10b

An solution of 2-amino-4-(dipropylcarbamoyl)-3H-benzo[b]azepine-8-sulfonyl chloride, 8SO2BzL-10a (1.00 mL, 0.15 mmol) in acetonitrile was added dropwise to a stirring mixture of tert-butyl (5,8,11,14,17,20,23,26,29,32-decaoxa-2-azatetratriacontan-34-yl)carbamate (0.11 g, 0.18 mmol) and triethylamine (0.08 mL, 0.60 mmol) in acetonitrile (3 mL). After 15 min the reaction was concentrated and purified by reverse phase chromatography using a gradient of 10-90% ACN/water (+0.1% TFA) over 10 min to obtain 8SO2BzL-10b (0.05 g, 37%).

Preparation of 2-amino-8-(N-(32-amino-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-N-methylsulfamoyl)-N,N-dipropyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-10c

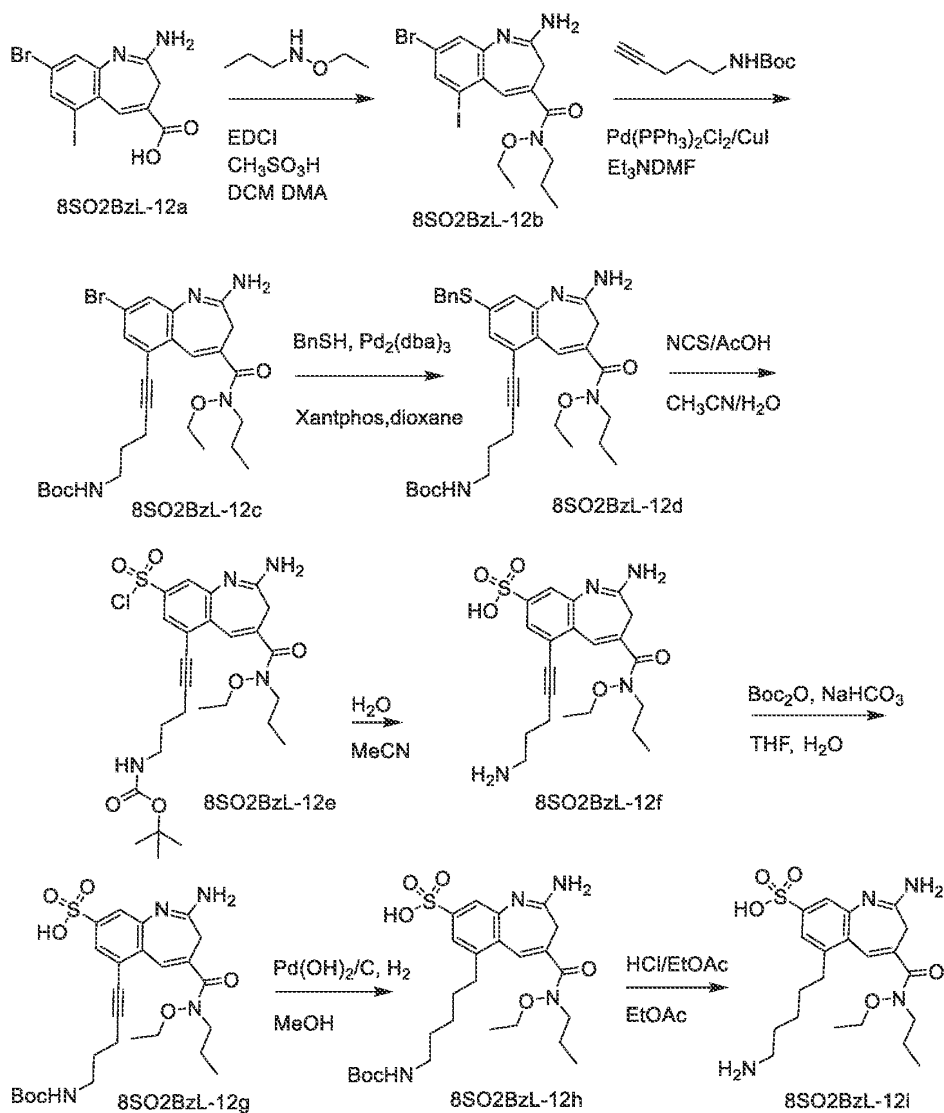
To a solution of 8SO2BzL-10b (0.05 g, 0.05 mmol) in acetonitrile (3 mL) was added aq. HCl (6M, 3 mL) and the mixture was stirred at room temperature, RT for 45 min. The solvent was removed and the isolated syrup was azeotroped with ACN (3 mL) to provide 8SO2BzL-10c as the HCl salt (0.04 g, 91%) as a hazy, white film.

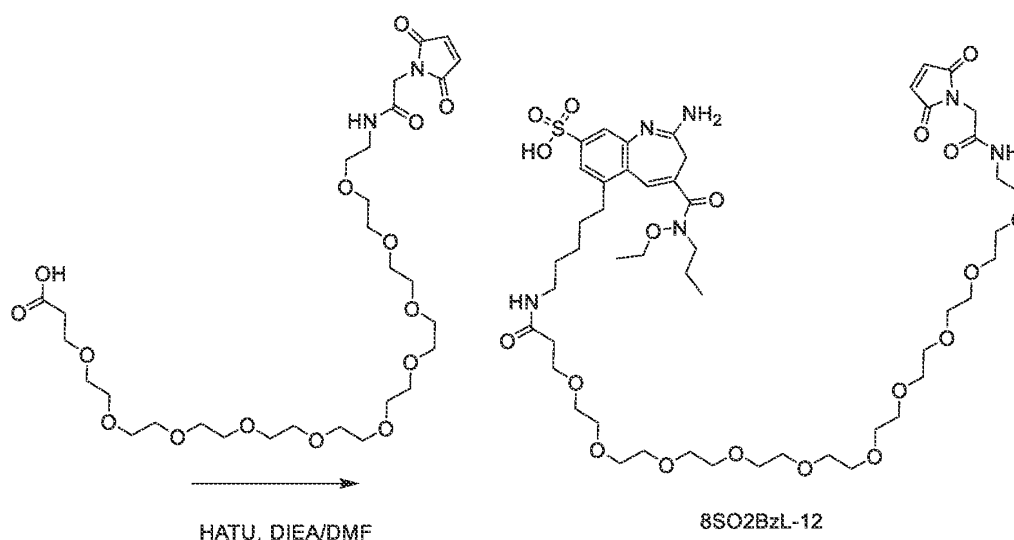
Preparation of 8SO2BzL-10

To a solution of 8SO2BzL-10c HCl (0.04 g, 0.05 mmol) in DMF (3 mL) was added triethylamine (0.03 mL, 0.20 mmol). 2,5-Dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)acetate (0.02 g, 0.06 mmol) was added in parts. After completion of addition, acetic acid (9 μ L) was added and the solvent was removed by vacuum. After purification by reverse phase HPLC, 8SO₂BzL-10 (0.02 g, 42%) was obtained after evaporation. LC/MS [M+H]⁺ 999.49 (calculated); LC/MS [M+H]⁺ 999.92 (observed).

Example L-12 Synthesis of 2-amino-6-[5-{3-[2-{2-[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}ethoxy]propionylamino]pentyl]-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepine-8-sulfonic acid,
8SO₂BzL-12





Preparation of 2-amino-8-bromo-N-ethoxy-6-iodo-N-propyl-3H-1-benzazepine-4-carboxamide, 8SO2BzL-12b

- To a solution of 2-amino-8-bromo-6-iodo-3H-1-benzazepine-4-carboxylic acid, 8SO2BzL-12a (2.0 g, 4.91 mmol, 1.0 eq) in DCM (20 mL) and DMA (10 mL) was added methanesulfonic acid, $\text{CH}_3\text{SO}_3\text{H}$ (472 mg, 4.91 mmol, 350 μL , 1.0 eq), N-ethoxypropan-1-amine (823 mg, 5.90 mmol, 1.2 eq, HCl) and EDCI (3.77 g, 19.7 mmol, 4 eq). The mixture was stirred at 25°C for 2 hrs. The pH of the reaction mixture was adjusted to ~9 with sat. Na_2CO_3 . The aqueous phase was extracted with ethyl acetate (50 mL x 3). The combined organic phase was washed with brine (20 mL x 2), dried with anhydrous Na_2SO_4 , filtered and concentrated in vacuum. The crude product was triturated with EtOAc at 25 °C for 10 min to afford 8SO2BzL-12b (1.24 g, 2.52 mmol, 51.3% yield) as yellow solid. LC/MS $[\text{M}+\text{H}]$ 491.97 (calculated); LC/MS $[\text{M}+\text{H}]$ 491.9 (observed).

- Preparation of tert-butyl N-[5-[2-amino-8-bromo-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepin-6-yl]pent-4-ynyl]carbamate, 8SO2BzL-12c

- A mixture of 8SO2BzL-12b (800 mg, 1.63 mmol, 1.0 eq), tert-butyl N-pent-4-ynylcarbamate (328 mg, 1.79 mmol, 1.1 eq), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (114 mg, 163 μmol , 0.1 eq), cuprous iodide, CuI (61.9 mg, 325 μmol , 0.2 eq) in DMF (16 mL) and Et_3N (6 mL) was degassed and purged with N_2 for 3 times, then stirred at 80 °C for 2 hrs under N_2 atmosphere. The mixture was poured into ice-water (w/w = 1/1) (20 mL) and stirred for 10 min. The aqueous phase was extracted with ethyl acetate (30 mL x 3). The combined organic phase was washed with brine (20 mL x 2), 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 8SO2BzL-12c (690 mg, 1.26

mmol, 77.5% yield) as yellow oil. ¹H NMR (MeOD, 400MHz) δ 7.53 (s, 1H), 7.27 (s, 2H), 3.93 (q, J = 7.2 Hz, 2H), 3.74 (t, J = 7.2 Hz, 2H), 3.19 (t, J = 7.2 Hz, 2H), 2.90-2.83 (m, 2H), 2.51 (t, J = 7.2 Hz, 2H), 1.82-1.70 (m, 4H), 1.43 (s, 9H), 1.17 (t, J = 7.2 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 547.2 (calculated); LC/MS [M+H] 547.2 (observed).

- 5 Preparation of tert-butyl N-[5-[2-amino-8-benzylsulfanyl-4-[ethoxy(propyl) carbamoyl]-3H-1-benzazepin-6-yl]pent-4-ynyl]carbamate, 8SO2BzL-12d

A mixture of 8SO2BzL-12c (350 mg, 639 μmol, 1.0 eq), phenylmethanethiol, BnSH (0.25 g, 2.01 mmol, 236 μL, 3.15 eq), DIEA (165 mg, 1.28 mmol, 223 μL, 2.0 eq), Xantphos (74.0 mg, 128 μmol, 0.2 eq) and Pd₂(dba)₃ (117 mg, 128 μmol, 0.2 eq) in dioxane (10 mL) was degassed and purged with N₂ for 3 times, then stirred at 110 °C for 1 hr under N₂ atmosphere. The residue was poured into ice-water (w/w = 1/1) (10 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (10 mL x 3). The combined organic phase was washed with brine (10mL x 1), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 15 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=1/0, 0/1) to afford 8SO2BzL-12d (300 mg, 508 μmol, 79.4% yield) as yellow solid. ¹H NMR (MeOD, 400MHz) δ 7.56 (s, 1H), 7.37-7.34 (m, 2H), 7.30-7.19 (m, 3H), 7.09 (d, J = 1.6 Hz, 1H), 7.03 (d, J = 1.6 Hz, 1H), 4.20 (s, 2H), 3.93 (q, J = 7.2 Hz, 2H), 3.73 (t, J = 7.2 Hz, 2H), 3.30 (s, 2H), 3.19 (t, J = 6.8 Hz, 2H), 2.49 (t, J = 7.2 Hz, 2H), 1.81-1.72 (m, 4H), 1.43 (s, 9H), 1.16 (t, J = 7.2 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H). 20 LC/MS [M+H] 591.3 (calculated); LC/MS [M+H] 591.3 (observed).

Preparation of tert-butyl N-[5-[2-amino-8-chlorosulfonyl-4-[ethoxy(propyl) carbamoyl]-3H-1-benzazepin-6-yl]pent-4-ynyl]carbamate, 8SO2BzL-12e

To a solution of 8SO2BzL-12d (300 mg, 508 μmol, 1.0 eq) in MeCN (6 mL) and H₂O (0.6 mL) was added AcOH (305 mg, 5.08 mmol, 290 μL, 10 eq) and NCS (271 mg, 2.03 mmol, 25 4.0 eq), and then stirred at 25 °C for 1 hr. The reaction mixture was poured into ice-water (w/w = 1/1) (10 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (10 mL x 3), the combined organic phase was dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum to give the crude product 8SO2BzL-12e (250 mg, 441 μmol, 86.8% yield) as yellow oil which was used in the next step without further purification. LC/MS [M+H] 567.2(calculated); 30 LC/MS [M+H] 567.3 (observed).

Preparation of 2-amino-6-(5-aminopent-1-ynyl)-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepine-8-sulfonic acid, 8SO2BzL-12f

A solution of 8SO2BzL-12e (250 mg, 441 μmol, 1.0 eq) in MeCN (2.5 mL) and H₂O (13 mL) was stirred at 100 °C for 1 hr. The mixture was concentrated in vacuum. The crude 35 product 8SO2BzL-12f (200 mg, 412 μmol, 93.5% yield, HCl) as yellow solid was used in the

next step without further purification. LC/MS [M+H]⁺ 449.18(calculated); LC/MS [M+H]⁺ 449.1 (observed).

Preparation of 2-amino-6-[5-(tert-butoxycarbonylamino)pent-1-ynyl]-4-
[ethoxy(propyl)carbamoyl]-3H-1-benzazepine-8-sulfonic acid, 8SO2BzL-12g

To a solution of 8SO2BzL-12f (200 mg, 446 μ mol, 1.0 eq) in THF (5 mL) and H₂O (5 mL) was added NaHCO₃ (112 mg, 1.34 mmol, 52 μ L, 3.0 eq) and Boc₂O (146 mg, 669 μ mol, 154 μ L, 1.5 eq), and then stirred at 25 °C for 1 hr. The mixture was concentrated under reduced pressure at 30°C. The residue was purified by prep-HPLC(column: Phenomenex Luna 80*30mm*3 μ m;mobile phase: [water(TFA)-ACN].B%: 10%-40%,8min) to afford 8SO2BzL-12g (100 mg, 182 μ mol, 40.9% yield) as yellow solid. ¹H NMR (MeOD, 400MHz) δ 7.84 (d, J = 1.2 Hz, 1H), 7.71 (d, J = 1.2 Hz, 1H), 7.63 (s, 1H), 3.98 (d, J = 7.2 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 3.38 (s, 2H), 3.18 (t, J = 6.8 Hz, 2H), 2.54 (t, J = 7.2 Hz, 2H), 1.81-1.76 (m, 4H), 1.43 (s, 9H), 1.20 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 549.2 (calculated); LC/MS [M+H]⁺ 549.3 (observed).

15 Preparation of 2-amino-6-[5-(tert-butoxycarbonylamino)pentyl]-4-[ethoxy(propyl)carbonyl]-3H-1-benzazepine-8-sulfonic acid. 8SO2BzL-12h

A mixture of 8SO2BzL-12g (100 mg, 182 μ mol, 1.0 eq), Pd(OH)₂/C (64.0 mg, 91.1 μ mol, 20% purity, 0.5 eq) in MeOH (10 mL) was degassed and purged with H₂ (367 μ g, 182 μ mol, 1 eq) for 3 times, and then stirred at 25°C for 1 hr under H₂ (30 psi) atmosphere. The mixture was filtered. The residue was purified by prep-HPLC (column: Phenomenex Luna 80*30mm*3 μ m; mobile phase: [water(TFA)-ACN].B%: 5%-55%, 8min) to afford 8SO2BzL-12h (72 mg, 130 μ mol, 71.5% yield) as white solid. ¹H NMR (MeOD, 400MHz) δ 7.70 (d, J = 1.2 Hz, 1H), 7.65 (d, J = 1.2 Hz, 1H), 7.46 (s, 1H), 3.98 (q, J = 7.2 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 3.36 (s, 2H), 3.02 (t, J = 6.8 Hz, 2H), 2.84 (t, J = 8.0 Hz, 2H), 1.83-1.74 (m, 2H), 1.71-1.59 (m, 2H), 1.52-1.34 (m, 13H), 1.20 (t, J = 7.2 Hz, 3H), 1.01 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 553.26 (calculated); LC/MS [M+H] 553.2 (observed).

Preparation of 2-amino-6-(5-aminopentyl)-4-[ethoxy(propyl)carbamoyl]-3H-1-benzazepine-8-sulfonic acid, 8SO2BzL-12i

To a solution of 8SO2BzL-12h (60 mg, 108 μ mol, 1 eq) in EtOAc (2 mL) was added
30 HCl/EtOAc (4 M, 1 mL, 37 eq), and then stirred at 20 °C for 1 hr. The mixture was
concentrated in vacuum to afford 8SO2BzL-12i (50 mg, 102 μ mol, 94.2% yield, HCl) as white
solid. LC/MS [M+H] 453.2 (calculated); LC/MS [M+H] 453.2 (observed).

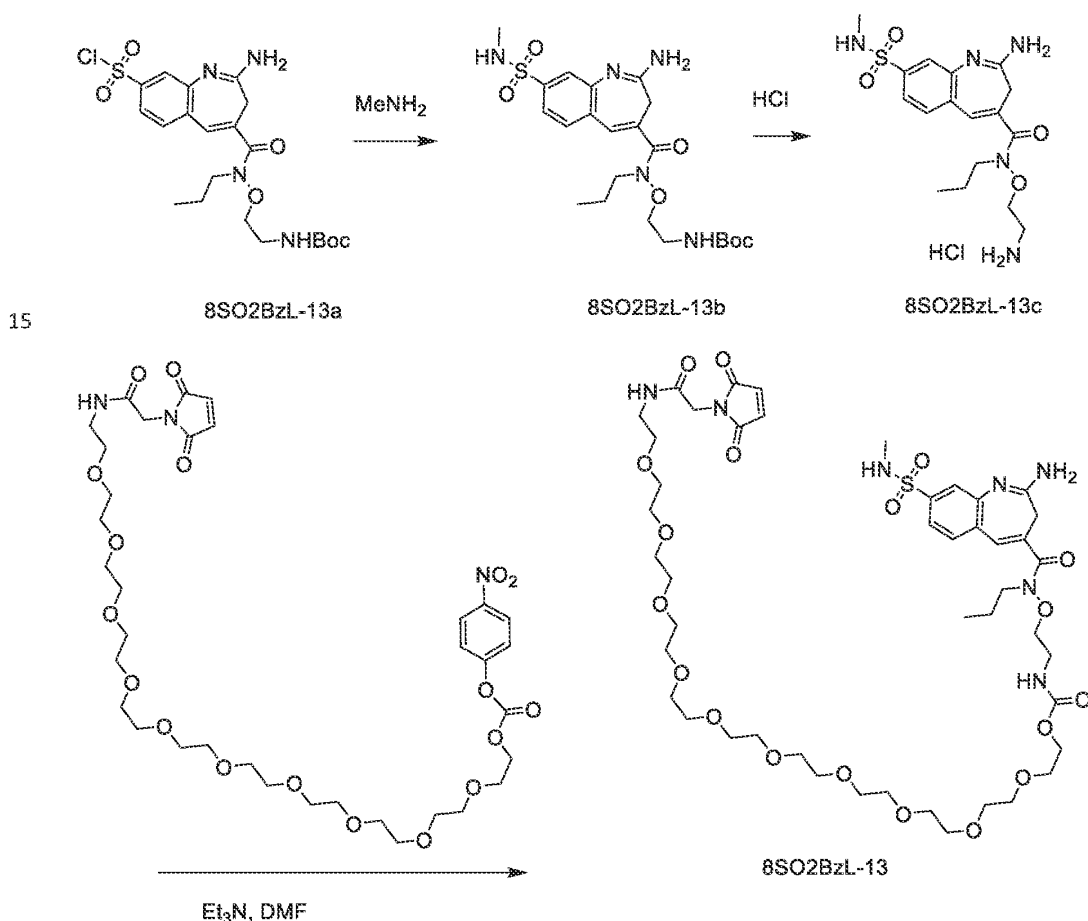
Preparation of 8SO2BzL-12

To a solution of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl]

35 amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic

acid (68.2 mg, 102 μ mol, 1.0 eq) in DMF (1 mL) was added DIEA (66.1 mg, 511 μ mol, 89 μ L, 5.0 eq), 8SO2BzL-12i (50 mg, 102 μ mol, 1 eq, HCl) and HATU (38.9 mg, 102 μ mol, 1.0 eq). And it was stirred at 25°C for 0.5 hr. The mixture was filtered and purified by prep-HPLC (column: Phenomenex Luna 80*30mm*3 μ m; mobile phase: [water(TFA)-ACN]; B%: 5%-35%, 8min) to afford 8SO2BzL-12 (14 mg, 12.7 μ mol, 12.43% yield) as yellow oil. ¹H NMR (MeOD, 400MHz) δ 7.72 (s, 1H), 7.68 (s, 1H), 7.48 (s, 1H), 6.91 (s, 2H), 4.19 (s, 2H), 4.01 (q, J = 7.2 Hz, 2H), 3.80-3.55 (m, 42H), 3.43-3.35 (m, 4H), 3.19 (t, J = 6.8 Hz, 2H), 2.88 (t, J = 8.0 Hz, 2H), 2.42 (t, J = 6.4 Hz, 2H), 1.80 (q, J = 7.2 Hz, 2H), 1.74-1.64 (m, 2H), 1.60-1.51 (m, 2H), 1.45-1.43 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H), 1.02 (t, J = 7.2 Hz, 3H). LC/MS [M+H] 1101.5 (calculated); LC/MS [M+H] 1101.9 (observed).

Example L-13 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 2-((2-amino-8-(N-methylsulfamoyl)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-13



Preparation of tert-butyl (2-((2-amino-8-(N-methylsulfamoyl)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-13b

To a solution of tert-butyl (2-((2-amino-8-(chlorosulfonyl)-N-propyl-3H-benzo[b]azepine-4-carboxamido)oxy)ethyl)carbamate, 8SO2BzL-13a (0.14 M, 2.00 mL, 0.28 mmol) in acetonitrile/water (9:1) at 0 deg was added a methylamine solution (2 M in THF, 0.70 mL, 1.40 mmol). After 10 minutes the solvent was removed and the crude product was purified by reverse phase HPLC using a gradient of 10-90% acetonitrile/water to give 8SO2BzL-13b (0.09 g, 68%) as a yellow film after evaporation of solvent.

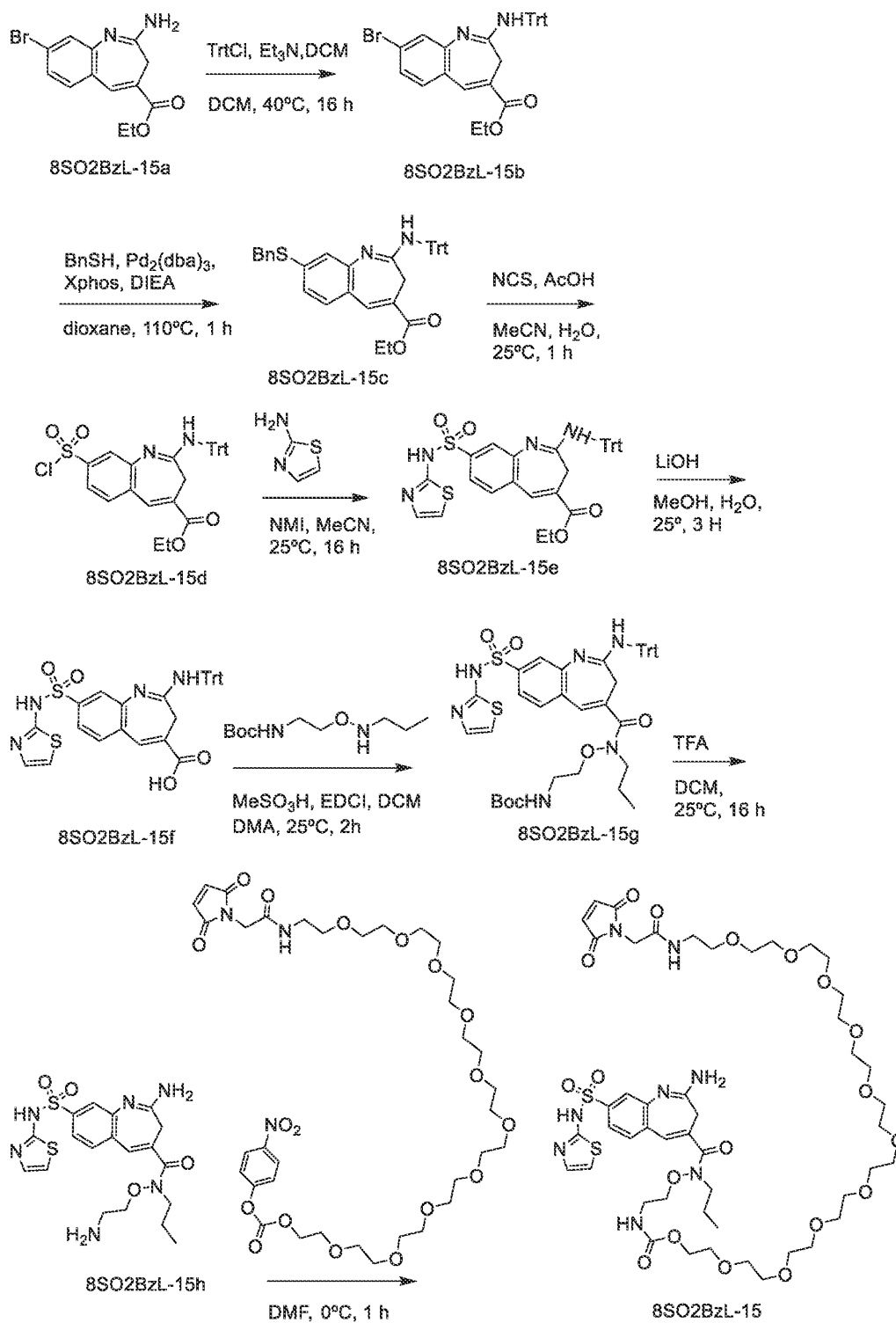
Preparation of 2-amino-N-(2-aminoethoxy)-8-(N-methylsulfamoyl)-N-propyl-3H-benzo[b]azepine-4-carboxamide, 8SO2BzL-13c

A solution of 8SO2BzL-13b (0.09 g, 0.19 mmol) in acetonitrile (2 mL) and 6 N HCl (2.00 mL, 12.00 mmol) was stirred at room temperature for 45 minutes. The solvent was removed by vacuum to give 8SO2BzL-13c as the HCl salt (0.08 g, 99%).

Preparation of 8SO2BzL-13

To a solution of 8SO2BzL-13c HCl (0.03 g, 0.06 mmol) in DMF (4 mL) at room temperature added triethylamine (0.04 mL, 0.26 mmol). To this mixture was added a solution 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, PNPC-PEG10-mal (0.05 g, 0.06 mmol) in DMF (2 mL). After 20 min., acetic acid (6 uL) was added and reaction was concentrated under vacuum and purified by reverse phase HPLC using a gradient of 10-90% ACN/water (+0.1% TFA) over 10 min. to give 8SO2BzL-13 (0.04 g, 52%) after concentration of pure fractions. LC/MS [M+H] 1060.47 (calculated); LC/MS [M+H] 1060.89 (observed).

Example L-15 Synthesis of 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]ethoxy] N-[2-[2-amino-8-(thiazol-2-ylsulfamoyl)-3H-1-benzazepine-4-carbonyl]-propyl-amino]oxyethyl]carbamate, 8SO2BzL-15



Preparation of ethyl 8-bromo-2-(tritylamino)-3H-1-benzazepine-4-carboxylate,

5 8SO2BzL-15b

A mixture of ethyl 2-amino-8-bromo-3H-1-benzazepine-4-carboxylate, 8SO2BzL-15a (5 g, 16.1 mmol, 1 *eq*), TrtCl (6.76 g, 24.2 mmol, 1.5 *eq*), TEA (4.91 g, 48.5 mmol, 6.75 mL, 3 *eq*) and DMAP (395 mg, 3.23 mmol, 0.2 *eq*) in DCM (50 mL) was degassed and purged with N₂ for 3 times, and then stirred at 40°C for 16 h under N₂ atmosphere. The reaction mixture was quenched by addition H₂O (50 mL) and extracted with EtOAc (100 mL). The combined organic layers were washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether:Ethyl acetate=100:1 to 0:1) to give 8SO2BzL-15b (7.8 g, 14.1 mmol, 87.4% yield) as a yellow oil. LC/MS [M+H]⁺ 551.1 (calculated); LC/MS [M+H]⁺ 551.1 (observed).

Preparation of ethyl 8-benzylsulfanyl-2-(tritylamino)-3H-1-benzazepine-4-carboxylate, 8SO2BzL-15c

A mixture of 8SO2BzL-15b (3 g, 5.44 mmol, 1 *eq*), DIEA (1.41 g, 10.8 mmol, 1.90 mL, 2 *eq*), (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one;palladium, Pd₂(dba)₃, CAS Reg. No. 51364-51-3 (996 mg, 1.09 mmol, 0.2 *eq*) and (5-diphenylphosphanyl-9,9-dimethyl-xanthen-4-yl)-diphenyl-phosphane, Xphos (629 mg, 1.09 mmol, 0.2 *eq*) in dioxane (30 mL) was degassed and purged with N₂ for 3 times, then phenylmethanethiol, BnSH (1.35 g, 10.8 mmol, 1.27 mL, 2 *eq*) was added, the mixture was stirred at 110°C for 1 h under N₂ atmosphere. The reaction mixture was quenched by addition H₂O (50 mL) and extracted with EtOAc (100 mL x 2). The combined organic layers were washed with brine (100 mL) dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether:Ethyl acetate=100:1 to 0:18) to give 8SO2BzL-15c (2.3 g, 3.87 mmol, 71.1% yield) as a yellow oil.

Preparation of ethyl 8-chlorosulfonyl-2-(tritylamino)-3H-1-benzazepine-4-carboxylate, 8SO2BzL-15d

A mixture of 8SO2BzL-15c (3 g, 5.04 mmol, 1 *eq*), NCS (2.69 g, 20.2 mmol, 4 *eq*), AcOH (3.03 g, 50.4 mmol, 2.88 mL, 10 *eq*) in MeCN (30 mL) and H₂O (3 mL) was stirred at 25°C for 1 h. The reaction mixture was quenched by addition H₂O (50 mL), and extracted with EtOAc (50 mL). The combined organic layers were wash with brine (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether:Ethyl acetate=99:1 to 0:1) to give 8SO2BzL-15d (2 g, 3.50 mmol, 69.4% yield) as a yellow solid. LC/MS [M+H]⁺ 571.1 (calculated); LC/MS [M+H]⁺ 571.2 (observed).

Preparation of ethyl 8-(thiazol-2-ylsulfamoyl)-2-(tritylamino)-3H-1-benzazepine-4-carboxylate, 8SO2BzL-15e

A mixture of 8SO2BzL-15d (1.5 g, 2.63 mmol, 1 *eq*) and 1-methylimidazole (258 mg, 3.15 mmol, 251 μ L, 1.2 *eq*) in MeCN (30 mL) was degassed and purged with N₂ for 3 times and then stirred at 25°C for 2 h under N₂ atmosphere. Then 4,5-dihydrothiazol-2-amine (1.07 g, 10.5 mmol, 4 *eq*) was added and the result mixture was stirred at 25°C for another 16 h under N₂ atmosphere. The reaction mixture was quenched by addition H₂O (50 mL) and extracted with EtOAc (50 mL). The combined organic layers were washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether:Ethyl acetate=100:1 to 0:1) to give 8SO2BzL-15e (0.5 g, 787 μ mol, 29.9% yield) as a yellow solid. ¹H NMR (MeOD, 400 MHz) δ 7.71 (s, 1H), 7.23 (m, 20H), 6.75 (d, J = 4.8 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 2.98 (s, 2H), 1.38 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 635.17 (calculated); LC/MS [M+H]⁺ 635.1 (observed).

Preparation of 8-(N-(thiazol-2-yl)sulfamoyl)-2-(tritylamino)-3H-benzo[b]azepine-4-carboxylic acid, 8SO2BzL-15f

A mixture of 8SO2BzL-15e (0.5 g, 787.69 μ mol, 1 *eq*), LiOH.H₂O (264 mg, 6.30 mmol, 8 *eq*) in H₂O (4 mL) and THF (4 mL) was stirred at 25 °C for 3 hr. The reaction solution was quenched by added 2M HCl to adjust pH = ~6, then filtered to give 8SO2BzL-15f (0.45 g, 699.66 μ mol, 88.82% yield, HCl) as a white solid. LC/MS [M+H]⁺ 607.1 (calculated); LC/MS [M+H]⁺ 607.2 (observed).

Preparation of tert-butyl N-[2-[propyl-[8-(thiazol-2-yl)sulfamoyl)-2-(tritylamino)-3H-1-benzazepine-4-carbonyl]amino]oxyethyl]carbamate, 8SO2BzL-15g

A mixture of 8SO2BzL-15f (0.42 g, 692 μ mol, 1 *eq*), *tert*-butyl N-[2-(propylamino)oxyethyl]carbamate (181 mg, 830 μ mol, 1.2 *eq*), methanesulfonic acid (133 mg, 1.38 mmol, 98.5 μ L, 2 *eq*), EDCI (663 mg, 3.46 mmol, 5 *eq*) in DMA (5 mL) and DCM (5 mL) was degassed and purged with N₂ for 3 times, and then stirred at 25°C for 2 hr under N₂ atmosphere. The reaction mixture was quenched by addition Na₂HCO₃ (3 mL) until pH about 7, and extracted with EtOAc (5 mL*3). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄ filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether:Ethyl acetate=99:1 to 20:80) to give 8SO2BzL-15g (0.5 g, 619 μ mol, 89.5% yield) as a yellow solid. LC/MS [M+H]⁺ 807.3 (calculated); LC/MS [M+H]⁺ 807.3 (observed).

Preparation of 2-amino-N-(2-aminoethoxy)-N-propyl-8-(thiazol-2-yl)sulfamoyl)-3H-1-benzazepine-4-carboxamide, 8SO2BzL-15h

A mixture of 8SO2BzL-15g (0.5 g, 619 μ mol, 1 *eq*), TFA (5.65 g, 49.5 mmol, 3.67 mL, 80 *eq*) in DCM (10 mL) was stirred at 25 °C for 16 h. The reaction mixture was quenched by addition H₂O (5 mL), and extracted with MTBE (10 mL) (5 mL x 2) to remove the excess TFA.

The combined water layers was concentrated under reduced pressure to give 8SO2BzL-15h (0.25 g, 432 μ mol, 69.7% yield, TFA) as a white solid. LC/MS [M+H] 465.1 (calculated); LC/MS [M+H] 465.1 (observed).

Preparation of 8SO2BzL-15

5 A mixture of 8SO2BzL-15h (0.2 g, 288 μ mol, 1 *eq*, 2TFA), 2-[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]ethoxy]ethyl (4-nitrophenyl)carbonate (232 mg, 288 μ mol, 1 *eq*), DIEA (111 mg, 866 μ mol, 150 μ L, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N₂ for 3 times, and then the mixture was stirred
10 at 0°C for 1 h under N₂ atmosphere. The reaction solution was quenched with TFA until pH = ~6. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30mm x 3 μ m; mobile phase: [water (TFA)-ACN]; B%:15%-40%, 8min) to give 8SO2BzL-15 (15.0 mg, 13.2 μ mol, 4.6% yield) as a white solid. ¹H NMR (MeOD-d₄, 400 MHz) δ 7.97 (s, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 2.0 Hz, 1H), 7.39 (s, 1H), 7.18 (d, J = 4.8 Hz, 1H), 6.90 (s, 2H), 6.81
15 (d, J = 4.8 Hz, 1H), 4.17 (s, 2H), 3.74 (m, 2H), 3.67 (m, 2H), 3.59 (m, 42H), 3.38 (m, 6H), 1.80-1.72 (m, 2H) 1.00 (t, J = 7.2 Hz, 3H). LC/MS [M+H]⁺ 1129.4 (calculated); LC/MS [M+H]⁺ 1129.5 (observed).

Example 201 Preparation of Macromolecule-supported compounds (MSC)

To prepare a lysine-conjugated MSC, a macromolecule 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 SEPHADEXTM desalting columns (Sigma-Aldrich, St. Louis, MO) or ZebaTM Spin Desalting Columns (Thermo Fisher Scientific). The eluates are then each adjusted to a concentration of about 1-10 mg/ml using the buffer and then sterile filtered. The macromolecule is pre-warmed to 20-30 °C and rapidly mixed with 2-20 (e.g., 7-10) molar equivalents of a tetrafluorophenyl (TFP) or sulfonic tetrafluorophenyl (sulfoTFP) ester, 8-sulfonyl-2-aminobenzazepine-linker (8SO2Bz-L) compound of Formula II dissolved in dimethylsulfoxide (DMSO) or dimethylacetamide (DMA) to a concentration of 5 to 20 mM. The reaction is allowed to proceed for about 16 hours at 30 °C and the MSC is separated from reactants by running over two successive G-25 desalting columns or ZebaTM Spin Desalting Columns equilibrated in phosphate buffered saline (PBS) at pH 7.2 to provide the MSC. Adjuvant-MSC ratio is determined by liquid chromatography mass spectrometry analysis using a C4 reverse phase column on an ACQUITYTM UPLC H-class (Waters Corporation, Milford, MA) connected to a XEVOTM G2-XS TOF mass spectrometer (Waters Corporation).

To prepare a cysteine-conjugated MSC, a macromolecule is buffer exchanged into a
35 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 about 30 min to 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
5 macromolecule was adjusted to approximately 5 to 20 mg/ml using the conjugation buffer and sterile-filtered. The maleimide-8SO₂Bz-L compound is either dissolved in dimethylsulfoxide (DMSO) or dimethylacetamide (DMA) to a concentration of 5 to 20 mM. For conjugation, the macromolecule is mixed with 10 to 20 molar equivalents of maleimide-8SO₂Bz-L. In some instances, additional DMA or DMSO up to 20% (v/v), was added to improve the solubility of
10 the maleimide-8SO₂Bz-L in the conjugation buffer. The reaction is allowed to proceed for approximately 30 min to 4 hours at 20 °C. The resulting conjugated MSC is purified away from the unreacted maleimide-8SO₂Bz-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
15 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 macromolecule may be dissolved in an aqueous buffer system known in the art that will not adversely impact the stability or antigen-binding specificity of the macromolecule. Phosphate buffered saline may be used. The 8SO₂Bz-L compound is dissolved
20 in a solvent system comprising at least one polar aprotic solvent as described elsewhere herein. In some such aspects, 8SO₂Bz-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 8-sulfonyl-2-aminobenzazepine-linker intermediate is dissolved
25 in DMSO (dimethylsulfoxide), DMA (dimethylacetamide), acetonitrile, or another suitable dipolar aprotic solvent.

Alternatively in the conjugation reaction, an equivalent excess of 8SO₂Bz-L solution may be diluted and combined with macromolecule solution. The 8SO₂Bz-L solution may suitably be diluted with at least one polar aprotic solvent and at least one polar protic solvent,
30 examples of which include water, methanol, ethanol, n-propanol, and acetic acid. The molar equivalents of 8SO₂Bz-L to macromolecule 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
35 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 8-sulfonyl-2-aminobenzazepine-linker intermediate, unreacted antibody thiol groups may be reacted with a capping reagent. An example of a suitable capping reagent is ethylmaleimide.

Following conjugation, the MSC 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 MSC, 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.

LCMS method: Adjuvant to antibody ratio (DAR) is estimated by liquid chromatography mass spectrometry analysis on an ACQUITY™ UPLC H-class (Waters Corporation, Milford, MA) connected to a XEVO™ G2-XS TOF mass spectrometer (Waters Corporation) using a C4 reverse phase column (ACQUITY™ UPLC Protein BEH C4, 300 Å, 1.7 µm, 2.1 mm x 50 mm). A gradient was used at a flow rate of 0.4 mL/min with water + 0.1% formic acid (Eluent A) and acetonitrile + 0.1% formic acid (Eluent B) starting at 1% B for 1 minute, changing to 90% B over 6 minutes, holding at 90% B for 0.5 min, changing to 1% B over 0.5 min, and holding at 1% B for 0.5 min. A mass spectrum was extracted from the TIC and deconvoluted using MaxENT1 algorithm to identify masses for DAR estimation.

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% CO₂ in DMEM supplemented with 10% FBS, Zeocin, and Blasticidin. Cells were then seeded in 96-well flat plates at 4x10⁴ cells/well with substrate containing HEK detection medium and immunostimulatory molecules (MSC). Activity was measured using a plate reader at 620-655 nm wavelength.

Example 203 Assessment of Macromolecule-supported compound (MSC) Activity *In Vitro*

This example shows that Macromolecule-supported compounds (MSC) of the invention are effective at eliciting immune activation, and therefore are useful for the treatment of cancer.

- a) Isolation of Human Antigen Presenting Cells: Human myeloid antigen presenting cells (APCs) were negatively selected from human peripheral blood obtained from healthy blood donors (Stanford Blood Center, Palo Alto, California) by density gradient centrifugation using a ROSETTESEP™ Human Monocyte Enrichment Cocktail (Stem Cell Technologies, Vancouver, Canada) containing monoclonal antibodies against CD14, CD16, CD40, CD86, CD123, and HLA-DR. Immature APCs were subsequently purified to >90% purity via negative selection using an EASYSEP™ Human Monocyte Enrichment Kit (Stem Cell Technologies) without CD16 depletion containing monoclonal antibodies against CD14, CD16, CD40, CD86, CD123, and HLA-DR.
- b) Myeloid APC Activation Assay: 2×10^5 APCs are incubated in 96-well plates (Corning, Corning, NY) containing Iscove's modified Dulbecco's medium, IMDM (Lonza) supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL (micrograms per milliliter) streptomycin, 2 mM L-glutamine, sodium pyruvate, non-essential amino acids, and where indicated, various concentrations of MSC of the invention (as prepared according to the Example above). Cell-free supernatants are analyzed after 18 hours via ELISA to measure TNFα secretion as a readout of a proinflammatory response.
- c) PBMC Activation Assay: Human peripheral blood mononuclear cells were isolated from human peripheral blood obtained from healthy blood donors (Stanford Blood Center, Palo Alto, California) by density gradient centrifugation. PBMCs were incubated in 96-well plates (Corning, Corning, NY) in a co-culture with CEA-expressing tumor cells (e.g. MKN-45, HPAF-II) at a 10:1 effector to target cell ratio. Cells were stimulated with various concentrations of MSC of the invention (as prepared according to the Example above). Cell-free supernatants were analyzed by cytokine bead array using a LegendPlex™ kit according to manufacturer's guidelines (BioLegend®, San Diego, CA).
- d) 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).

e) 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 MSC 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 TNF α) using a BioLegend LEGENDPLEX cytokine bead array.

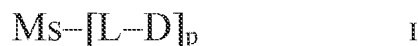
Activation of myeloid cell types can be measured using various screen assays in addition to the assay described in which different myeloid populations are utilized. These may include the following: monocytes isolated from healthy donor blood, M-CSF differentiated Macrophages, GM-CSF differentiated Macrophages, 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 M Φ (IL4/IL13), M2c M Φ (IL10/TGF β), 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 MSC may bind to. 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. A macromolecule-supported compound comprising a macromolecular support covalently attached to one or more 8-sulfonyl-2-aminobenzazepine moieties by a linker, and having Formula I:



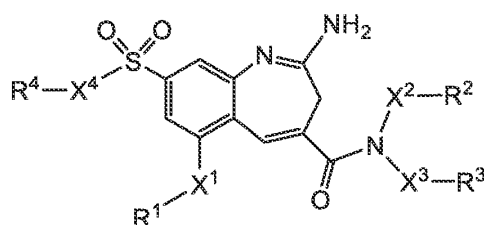
or a pharmaceutically acceptable salt thereof,

wherein:

Ms is the macromolecular support;

p is an integer from 1 to 50;

D is the 8-sulfonyl-2-aminobenzazepine moiety having the formula:



R^1 , R^2 , R^3 , and R^4 are independently selected from the group consisting of H, C₁-C₁₂ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₁₂ carbocyclyl, C₆-C₂₀ aryl, C₂-C₉ heterocyclyl, and C₁-C₂₀ heteroaryl, where alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, and heteroaryl are independently and optionally substituted with one or more groups selected from:

- (C₁-C₁₂ alkylidyl)-N(R⁵)-*;
- (C₁-C₁₂ alkylidyl)-N(R⁵)₂;
- (C₁-C₁₂ alkylidyl)-OR⁵;
- (C₃-C₁₂ carbocyclyl);
- (C₃-C₁₂ carbocyclyl)-*;
- (C₃-C₁₂ carbocyclyl)-(C₁-C₁₂ alkylidyl)-NR⁵-*;
- (C₃-C₁₂ carbocyclyl)-(C₁-C₁₂ alkylidyl)-N(R⁵)₂;
- (C₃-C₁₂ carbocyclyl)-NR⁵-C(=NR⁵)NR⁵-*;
- (C₆-C₂₀ aryl);
- (C₆-C₂₀ arylidyl)-*;
- (C₆-C₂₀ arylidyl)-N(R⁵)-*;
- (C₆-C₂₀ arylidyl)-(C₁-C₁₂ alkylidyl)-N(R⁵)-*;
- (C₆-C₂₀ arylidyl)-(C₁-C₁₂ alkylidyl)-(C₂-C₂₀ heterocyclidyl)-*;

$-(C_6-C_{20} \text{ arylldiyl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)_2$;
 $-(C_6-C_{20} \text{ arylldiyl})-(C_1-C_{12} \text{ alkylldiyl})-NR^5-C(=NR^{5a})N(R^5)-*$;
 $-(C_2-C_{20} \text{ heterocyclyl})$;
 $-(C_2-C_{20} \text{ heterocyclyl})-*$;
 $-(C_2-C_9 \text{ heterocyclyl})-(C_1-C_{12} \text{ alkylldiyl})-NR^5-*$;
 $-(C_2-C_9 \text{ heterocyclyl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)_2$;
 $-(C_2-C_9 \text{ heterocyclyl})-C(=O)-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)-*$;
 $-(C_2-C_9 \text{ heterocyclyl})-NR^5-C(=NR^{5a})NR^5-*$;
 $-(C_2-C_9 \text{ heterocyclyl})-NR^5-(C_6-C_{20} \text{ arylldiyl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)-*$;
 $-(C_2-C_9 \text{ heterocyclyl})-(C_6-C_{20} \text{ arylldiyl})-*$;
 $-(C_1-C_{20} \text{ heteroaryl})$;
 $-(C_1-C_{20} \text{ heteroaryl})-*$;
 $-(C_1-C_{20} \text{ heteroaryl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)-*$;
 $-(C_1-C_{20} \text{ heteroaryl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)_2$;
 $-(C_1-C_{20} \text{ heteroaryl})-NR^5-C(=NR^{5a})N(R^5)-*$;
 $-(C_1-C_{20} \text{ heteroaryl})-N(R^5)C(=O)-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)-*$;
 $-C(=O)-*$;
 $-C(=O)-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)-*$;
 $-C(=O)-(C_2-C_{20} \text{ heterocyclldiyl})-*$;
 $-C(=O)N(R^5)_2$;
 $-C(=O)N(R^5)-*$;
 $-C(=O)N(R^5)-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)C(=O)R^5$;
 $-C(=O)N(R^5)-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)C(=O)N(R^5)_2$;
 $-C(=O)NR^5-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)CO_2R^5$;
 $-C(=O)NR^5-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)C(=NR^{5a})N(R^5)_2$;
 $-C(=O)NR^5-(C_1-C_{12} \text{ alkylldiyl})-NR^5C(=NR^{5a})R^5$;
 $-C(=O)NR^5-(C_1-C_8 \text{ alkylldiyl})-NR^5(C_2-C_5 \text{ heteroaryl})$;
 $-C(=O)NR^5-(C_1-C_{20} \text{ heteroarylldiyl})-N(R^5)-*$;
 $-C(=O)NR^5-(C_1-C_{20} \text{ heteroarylldiyl})-*$;
 $-C(=O)NR^5-(C_1-C_{20} \text{ heteroarylldiyl})-(C_1-C_{12} \text{ alkylldiyl})-N(R^5)_2$;
 $-C(=O)NR^5-(C_1-C_{20} \text{ heteroarylldiyl})-(C_2-C_{20} \text{ heterocyclldiyl})-C(=O)NR^5-(C_1-C_{12} \text{ alkylldiyl})-NR^5-*$;
 $-N(R^5)_2$;

$-\text{N}(\text{R}^5)-*$;
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{R}^5$;
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})-*$;
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{N}(\text{R}^5)_2$;
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})\text{N}(\text{R}^5)-*$;
 $-\text{N}(\text{R}^5)\text{CO}_2\text{R}^5$;
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{N}(\text{R}^5)_2$;
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{N}(\text{R}^5)-*$;
 $-\text{NR}^5\text{C}(=\text{NR}^{5a})\text{R}^5$;
 $-\text{N}(\text{R}^5)\text{C}(=\text{O})-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{N}(\text{R}^5)-*$;
 $-\text{N}(\text{R}^5)-(\text{C}_2\text{-C}_5\text{ heteroaryl})$;
 $-\text{N}(\text{R}^5)-\text{S}(=\text{O})_2-(\text{C}_1\text{-C}_{12}\text{ alkyl})$;
 $-\text{O}-(\text{C}_1\text{-C}_{12}\text{ alkyl})$;
 $-\text{O}-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{N}(\text{R}^5)_2$;
 $-\text{O}-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{N}(\text{R}^5)-*$;
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)_2$;
 $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^5)-*$;
 $-\text{O}-(\text{R}^5)-*$;
 $-\text{OR}^5$;
 $-\text{S}(=\text{O})_2-(\text{C}_2\text{-C}_{20}\text{ heterocyclidyl})-*$;
 $-\text{S}(=\text{O})_2-(\text{C}_2\text{-C}_{20}\text{ heterocyclidyl})-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{N}(\text{R}^5)_2$;
 $-\text{S}(=\text{O})_2-(\text{C}_2\text{-C}_{20}\text{ heterocyclidyl})-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{NR}^5-*$; and
 $-\text{S}(=\text{O})_2-(\text{C}_2\text{-C}_{20}\text{ heterocyclidyl})-(\text{C}_1\text{-C}_{12}\text{ alkylidyl})-\text{OH}$;
 or R^2 and R^3 together form a 5- or 6-membered heterocyclidyl ring;

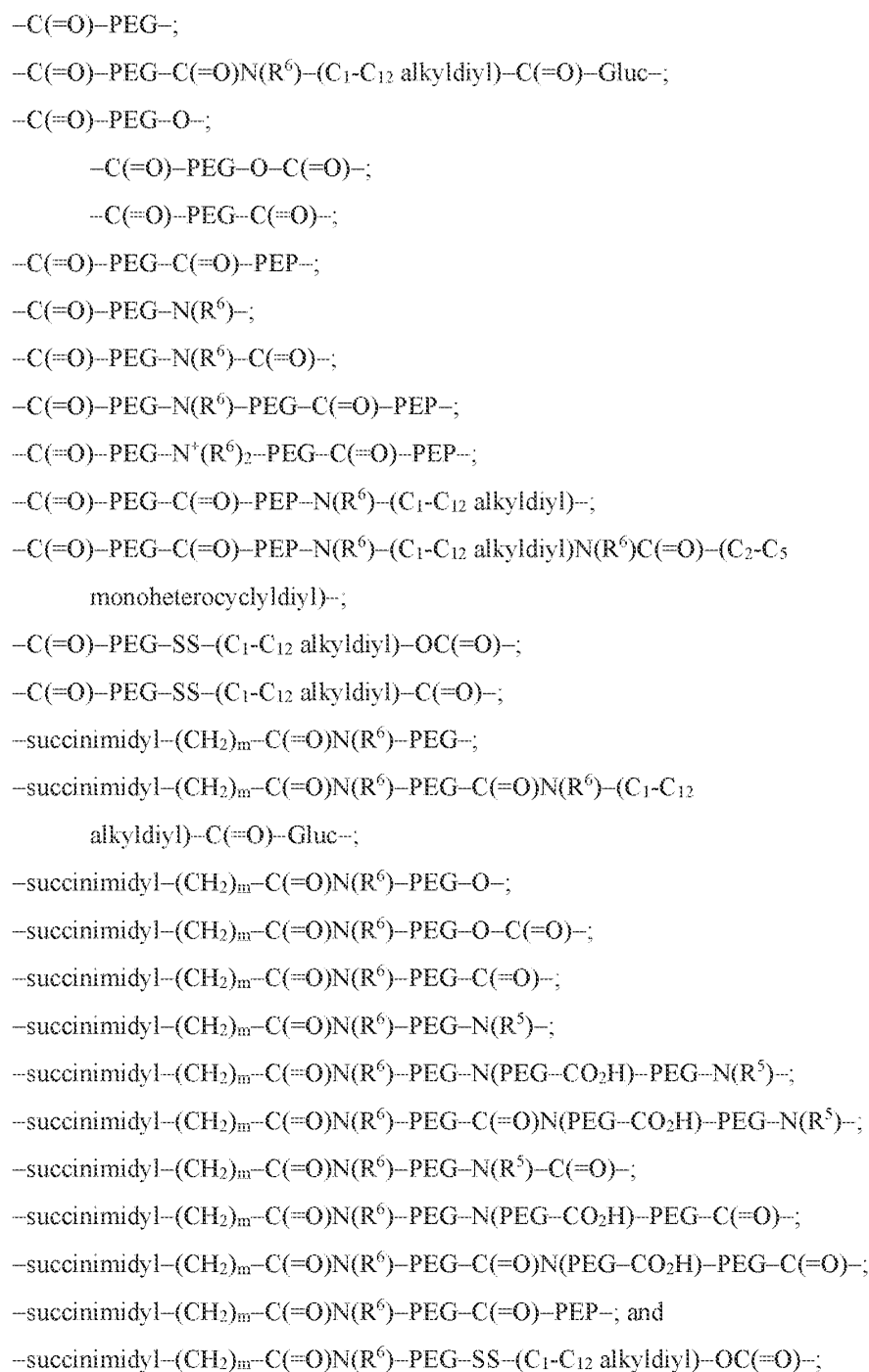
X^1 , X^2 , X^3 , and X^4 are independently selected from the group consisting of a bond, $\text{C}(=\text{O})$, $\text{C}(=\text{O})\text{N}(\text{R}^5)$, O , $\text{N}(\text{R}^5)$, S , $\text{S}(\text{O})_2$, and $\text{S}(\text{O})_2\text{N}(\text{R}^5)$;

R^5 is independently selected from the group consisting of H , $\text{C}_6\text{-C}_{20}$ aryl, $\text{C}_3\text{-C}_{12}$ carbocyclidyl, $\text{C}_6\text{-C}_{20}$ arylidyl, $\text{C}_1\text{-C}_{12}$ alkyl, and $\text{C}_1\text{-C}_{12}$ alkylidyl, or two R^5 groups together form a 5- or 6-membered heterocyclidyl ring;

R^{5a} is selected from the group consisting of $\text{C}_6\text{-C}_{20}$ aryl and $\text{C}_1\text{-C}_{20}$ 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 ;

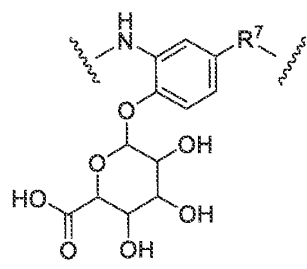
L is the linker selected from the group consisting of:



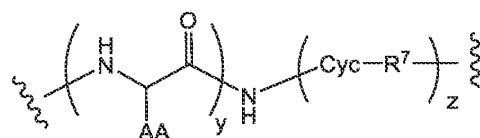
R^6 is independently H or C_1-C_6 alkyl;

PEG has the formula: $-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_m-$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

Gluc has the formula:

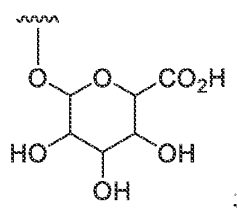


PEP has the formula:



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 C₆-C₂₀ arylidyl and C₁-C₂₀ heteroarylidyl, optionally substituted with one or more groups selected from F, Cl, NO₂, -OH, -OCH₃, and a glucuronic acid having the structure:



R⁷ is selected from the group consisting of $-\text{CH}(\text{R}^8)\text{O}-$, $-\text{CH}_2-$, $-\text{CH}_2\text{N}(\text{R}^8)-$, and $-\text{CH}(\text{R}^8)\text{O}-\text{C}(=\text{O})-$, where R⁸ is selected from H, C₁-C₆ alkyl, C(=O)-C₁-C₆ alkyl, and $-\text{C}(=\text{O})\text{N}(\text{R}^9)_2$, where R⁹ is independently selected from the group consisting of H, C₁-C₁₂ alkyl, and $-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_m-\text{OH}$, where m is an integer from 1 to 5, and n is an integer from 2 to 50, or two R⁹ groups together form a 5- or 6-membered heterocyclic ring;

y is an integer from 2 to 12;

z is 0 or 1; and

alkyl, alkylidyl, 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, -CH₃, -CH₂CH₃, -CH=CH₂, -C≡CH, -C≡CCH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -CH₂OH, -CH₂OCH₃, -CH₂CH₂OH, -C(CH₃)₂OH, -CH(OH)CH(CH₃)₂, -C(CH₃)₂CH₂OH, -CH₂CH₂SO₂CH₃, -CH₂OP(O)(OH)₂, -CH₂F, -CHF₂, -CF₃, -CH₂CF₃, -

CH₂CHF₂, -CH(CH₃)CN, -C(CH₃)₂CN, -CH₂CN, -CH₂NH₂, -CH₂NHSO₂CH₃, -CH₂NHCH₃, -CH₂N(CH₃)₂, -CO₂H, -COCH₃, -CO₂CH₃, -CO₂C(CH₃)₃, -COCH(OH)CH₃, -CONH₂, -CONHCH₃, -CON(CH₃)₂, -C(CH₃)₂CONH₂, -NH₂, -NHCH₃, -N(CH₃)₂, -NHCOCH₃, -N(CH₃)COCH₃, -NHS(O)₂CH₃, -N(CH₃)C(CH₃)₂CONH₂, -N(CH₃)CH₂CH₂S(O)₂CH₃, -NHC(=NH)H, -NHC(=NH)CH₃, -NHC(=NH)NH₂, -NHC(=O)NH₂, -NO₂, =O, -OH, -OCH₃, -OCH₂CH₃, -OCH₂CH₂OCH₃, -OCH₂CH₂OH, -OCH₂CH₂N(CH₃)₂, -O(CH₂CH₂O)_n-(CH₂)_mCO₂H, -O(CH₂CH₂O)_nH, -OCH₂F, -OCHF₂, -OCF₃, -OP(O)(OH)₂, -S(O)₂N(CH₃)₂, -SCH₃, -S(O)₂CH₃, and -S(O)₃H.

2. The macromolecule-supported compound of claim 1, wherein subscript p is an integer from 1 to 25.

3. The macromolecule-supported compound of claim 2, wherein subscript p is an integer from 1 to 6.

4. The macromolecule-supported compound of claim 1, wherein the macromolecular support is selected from a peptide, a nucleotide, a carbohydrate, a lipid, an antibody construct, a biopolymer, a nanoparticle, and an immune checkpoint inhibitor.

5. The macromolecule-supported compound of any one of claims 1 to 4 wherein X¹ is a bond, and R¹ is H.

6. The macromolecule-supported compound of any one of claims 1 to 4 wherein X² is a bond, and R² is C₁-C₈ alkyl.

7. The macromolecule-supported compound of any one of claims 1 to 4 wherein X² and X³ are each a bond, and R² and R³ are independently selected from C₁-C₈ alkyl, -O-(C₁-C₁₂ alkyl), -(C₁-C₁₂ alkyldiyl)-OR⁵, -(C₁-C₈ alkyldiyl)-N(R⁵)CO₂R⁵, -(C₁-C₁₂ alkyl)-OC(O)N(R⁵)₂, -O-(C₁-C₁₂ alkyl)-N(R⁵)CO₂R⁵, and -O-(C₁-C₁₂ alkyl)-OC(O)N(R⁵)₂.

8. The macromolecule-supported compound of claim 7 wherein R² is C₁-C₈ alkyl and R³ is -(C₁-C₈ alkyldiyl)-N(R⁵)CO₂R⁴.

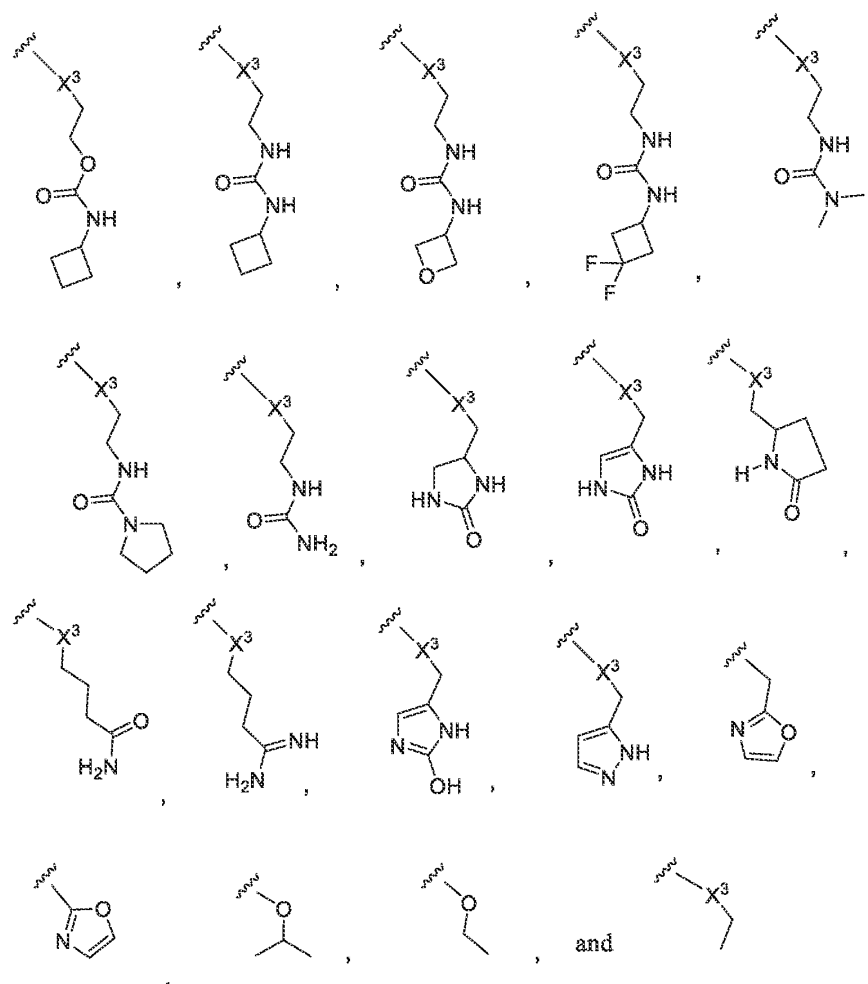
9. The macromolecule-supported compound of claim 7 wherein R² is -CH₂CH₂CH₃ and R³ is selected from -CH₂CH₂CH₂NHCO₂(t-Bu), -OCH₂CH₂NHCO₂(cyclobutyl), and -CH₂CH₂CH₂NHCO₂(cyclobutyl).

10. The macromolecule-supported compound of claim 7 wherein R² and R³ are each independently selected from -CH₂CH₂CH₃, -OCH₂CH₃, -OCH₂CF₃, -CH₂CH₂CF₃, -OCH₂CH₂OH, and -CH₂CH₂CH₂OH.

11. The macromolecule-supported compound of claim 7 wherein R² and R³ are each -CH₂CH₂CH₃.

12. The macromolecule-supported compound of claim 7 wherein R^2 is $-\text{CH}_2\text{CH}_2\text{CH}_3$ and R^3 is $-\text{OCH}_2\text{CH}_3$.

13. The macromolecule-supported compound of any one of claims 1 to 4 wherein X^3 - R^3 is selected from the group consisting of:

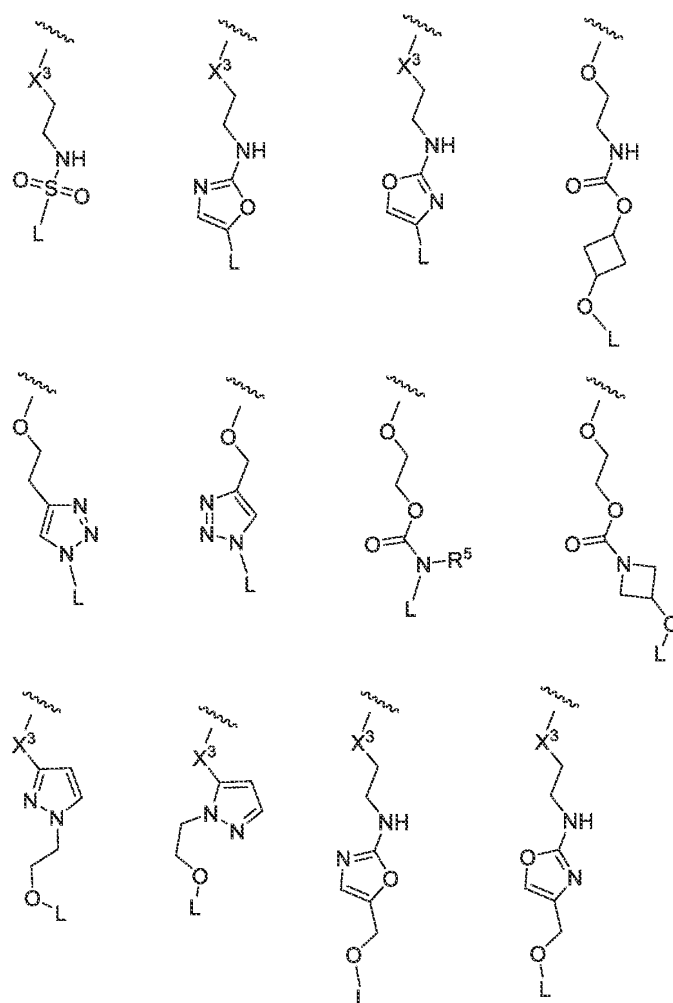


14. The macromolecule-supported compound of any one of claims 1 to 4 wherein X^4 is a bond, and R^4 is H.

15. The macromolecule-supported compound of any one of claims 1 to 4 where R^1 is attached to L.

16. The macromolecule-supported compound of any one of claims 1 to 4 where R^2 or R^3 is attached to L.

17. The macromolecule-supported compound of claim 16 wherein X^3 - R^3 -L is selected from the group consisting of:



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

18. The macromolecule-supported compound of any one of claims 1 to 4 wherein R^4 is C_1 - C_{12} alkyl.

19. The macromolecule-supported compound of any one of claims 1 to 4 wherein R^4 is $-(C_1-C_{12} \text{ alkyldiyl})-N(R^5)-*$; where the asterisk * indicates the attachment site of L.

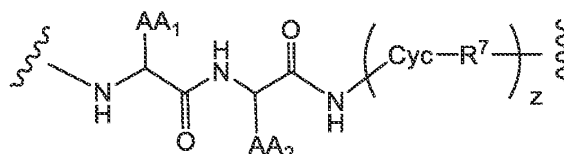
20. The macromolecule-supported compound of any one of claims 1 to 4 wherein L is $-C(=O)-\text{PEG}-$ or $-C(=O)-\text{PEG}-C(=O)-$.

21. The macromolecule-supported compound of any one of claims 1 to 4 wherein L is attached to a cysteine thiol of the antibody.

22. The macromolecule-supported compound of any one of claims 1 to 4 wherein for the PEG, m is 1 or 2, and n is an integer from 2 to 10.

23. The macromolecule-supported compound of claim 22 wherein n is 10.

24. The macromolecule-supported compound of any one of claims 1 to 4 wherein L comprises PEP and PEP is a dipeptide and has the formula:

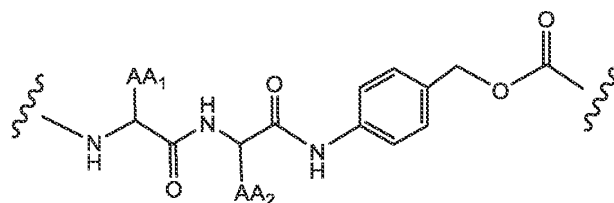


25. The macromolecule-supported compound of claim 24 wherein AA₁ and AA₂ are independently selected from H, -CH₃, -CH(CH₃)₂, -CH₂(C₆H₅), -CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, -CHCH(CH₃)CH₃, -CH₂SO₃H, and -CH₂CH₂CH₂NHC(O)NH₂; or AA₁ and AA₂ form a 5-membered ring proline amino acid.

26. The macromolecule-supported compound of claim 24 wherein AA₁ is -CH(CH₃)₂, and AA₂ is -CH₂CH₂CH₂NHC(O)NH₂.

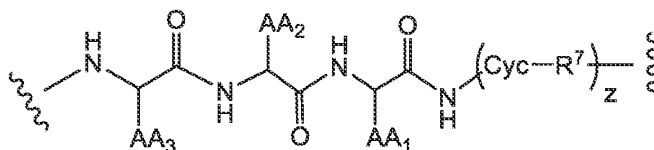
27. The macromolecule-supported compound of claim 24 wherein AA₁ and AA₂ are independently selected from GlcNAc aspartic acid, -CH₂SO₃H, and -CH₂OPO₃H.

28. The macromolecule-supported compound of claim 24 wherein PEP has the formula:

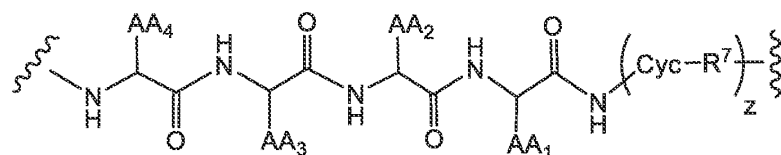


wherein AA₁ and AA₂ are independently selected from a side chain of a naturally-occurring amino acid.

29. The macromolecule-supported compound of any one of claims 1 to 4 wherein L comprises PEP and PEP is a tripeptide and has the formula:



30. The macromolecule-supported compound of any one of claims 1 to 4 wherein L comprises PEP and PEP is a tetrapeptide and has the formula:



31. The macromolecule-supported compound of claim 30 wherein

AA₁ is selected from the group consisting of Abu, Ala, and Val;

AA₂ is selected from the group consisting of Nle(O-Bzl), Oic and Pro;

AA₃ is selected from the group consisting of Ala and Met(O)₂; and

AA₄ is selected from the group consisting of Oic, Arg(NO₂), Bpa, and Nle(O-Bzl).

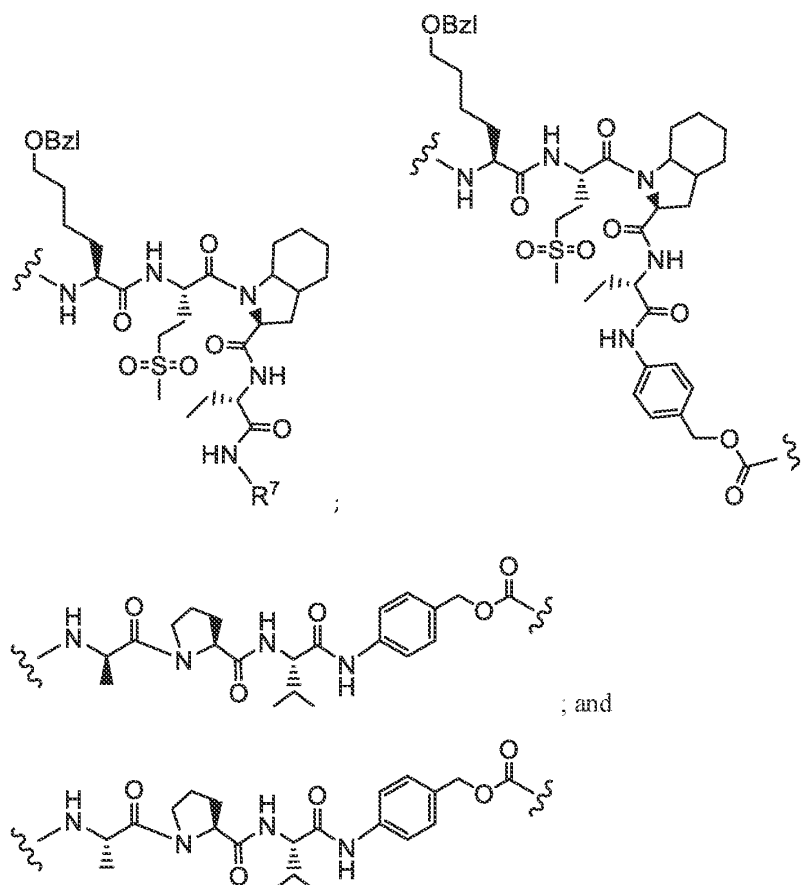
32. The macromolecule-supported compound of any one of claims 1 to 4 wherein L

comprises PEP and PEP is selected from the group consisting of Ala-Pro-Val, Asn-Pro-Val,

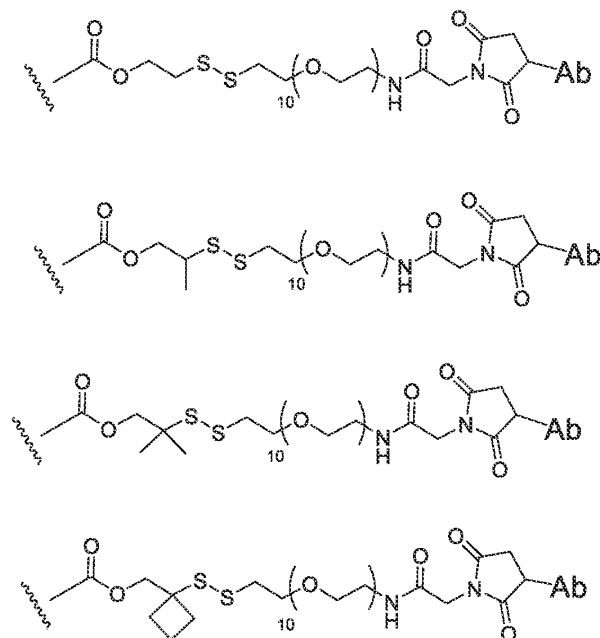
Ala-Ala-Val, Ala-Ala-Pro-Ala, Ala-Ala-Pro-Val, and Ala-Ala-Pro-Nva.

33. The macromolecule-supported compound of any one of claims 1 to 4 wherein L

comprises PEP and PEP is selected from the structures:



34. The macromolecule-supported compound of any one of claims 1 to 4 wherein L is selected from the structures:



where the wavy line indicates the attachment to R⁵.

35. An 8-sulfonyl-2-aminobenzazepine-linker compound selected from Table 2.

36. A macromolecule-supported compound prepared by conjugation of a macromolecule with an 8-sulfonyl-2-aminobenzazepine-linker compound selected from Table 2.

37. A pharmaceutical composition comprising a therapeutically effective amount of a macromolecule-supported compound according to any one of claims 1 to 34, and one or more pharmaceutically acceptable diluent, vehicle, carrier or excipient.

38. A method for treating cancer comprising administering a therapeutically effective amount of a macromolecule-supported compound according to any one of claims 1 to 34 to a patient in need thereof, 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.

39. The method of claim 38, wherein the cancer is susceptible to a pro-inflammatory response induced by TLR7 and/or TLR8 agonism.

40. The method of claim 38, wherein the cancer is selected from triple-negative breast cancer, metastatic Merkel cell carcinoma, and gastroesophageal junction adenocarcinoma.

41. The method of claim 38, wherein the macromolecule-supported compound is administered to the patient intravenously, intratumorally, or subcutaneously.

42. The method of claim 38, wherein the macromolecule-supported compound is administered to the patient at a dose of about 0.01 to 20 mg per kg of body weight.

43. Use of a macromolecule-supported compound according to any one of claims 1 to 34 for treating cancer, 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.

44. A method of preparing a macromolecule-supported compound of Formula I of claim 1 wherein the 8-sulfonyl-2-amino-thienoazepine-linker compound of claim 35 is conjugated with the macromolecule.