BIFUNCTIONAL DEGRADERS OF ANTI-PLA2R ANTIBODY

FIELD OF THE INVENTION

[001] The invention generally relates to medicinal preparations characterized by the non-active ingredients used, e.g., carriers or inert additives, targeting or modifying agents chemically bound to the active ingredient, the non-active ingredient being chemically bound to the active ingredient, e.g., polymer-drug conjugates the non-active ingredient being a modifying agent the modifying agent being an antibody, an immunoglobulin or a fragment thereof, e.g., an Fc-fragment the modifying agent being an antibody or an immunoglobulin bearing at least one antigen-binding site the antibody targeting a receptor, a cell surface antigen or a cell surface determinant. Specifically, the invention relates to bifunctional molecules containing a circulating protein-binding moiety that binds to anti-PLA2R antibody for the treatment of membranous nephropathy.

BACKGROUND OF THE INVENTION

[002] Membranous nephropathy (primary membranous nephropathy, pMN) is an autoimmune disease characterized by the accumulation of immune complexes in the renal glomeruli. Idiopathic membranous nephropathy (IMN) is a glomerular disease, causing fatigue, swelling, and high cholesterol. The current standard of care is an immunosuppressive therapy. High-risk patients receive immunosuppressive therapy. Cyclophosphamide is used in patients with severe decline in kidney function.

[003] The phospholipase A2 receptor 1 (PLA2R1) is an autoantigen in membranous nephropathy patients. A mouse model of phospholipase A2 receptor 1-associated membranous nephropathy mimics podocyte injury in patients a transgenic mouse line expressing murine full-length PLA2R1 in podocytes. Meyer-Schwesinger et al., Kidney International, 97(5), 913-919 (2020).

[004] No specific therapy exists for Idiopathic membranous nephropathy. About one-third of patients eventually develop kidney failure or end-stage renal disease (ESRD) within five-fifteen years despite standard-of-care immunosuppressive treatment, requiring kidney allograft. Idiopathic membranous nephropathy recurs in 50% of all kidney allograft recipients.

[005] There remains a need in the biomedical art for new medicines capable of treating or slowing down progression of idiopathic membranous nephropathy.

SUMMARY OF THE INVENTION

[006] The invention is directed to bifunctional molecules (agents, TRAPs) capable of binding and degrading anti-PLA2R antibody. In one embodiment, the invention provides an anti-PLA2R autoantibody degrader to treat idiopathic membranous nephropathy. An anti-PLA2R degrader could be a safe and effective treatment for idiopathic membranous nephropathy, with a side effect profile better than the current standard of care.

[007] In another embodiment, the invention provides a composition of matter (an agent, a TRAP) comprising:

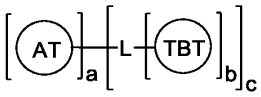
an anti-PLA2R antibody-binding moiety,

a cellular receptor-binding moiety that can bind to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, and

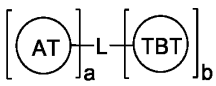
a linker moiety connecting the anti-PLA2R antibody moiety and the cellular receptor-binding moiety, wherein the linker moiety can be a single peptide bond or a larger linker moiety.

[008] In some embodiments, the invention provides a composition of matter (an agent, a TRAP) having a structure of:

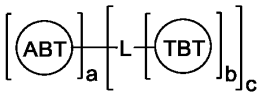
R^{CN}–(Xaa)y–R^{CC},



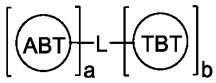
[AGN101],



[AGN102],

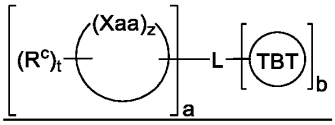


[AGN103], or



[AGN104], or a salt thereof.

[009] In some embodiments, the invention provides a composition of matter (an agent, a TRAP) of formula **AGN105**:



[AGN105] or a salt thereof, wherein the composition of matter has additional elements described in this specification.

[010] In more particular embodiments, the invention provides a composition of matter (an agent, a TRAP) selected from the group consisting of the compounds in TABLE 1.

TABLE 1	
#	Characteristics
AGN302	31mer (bicyclic)-**TBT103** MW 5240.9 Da
AGN303	31mer (bicyclic)-**TBT104**, MW 5240.9 Da.
AGN304	31mer (bicyclic)-CF3 pyrazine, MW 4080.61 Da.
AGN306	31mer-**TBT103,** MW 5258.92 Da.
AGN307	31mer-**TBT104,** MW 5258.92 Da.
AGN308	31mer-CF3 pyrazine, MW 4098.62 Da
AGN309	31mer- PR1, MW 4036.68 Da.
AGN310	31mer- GN2 MW 4690.36
AGN362	**ABT816-TBT307**
AGN363	**ABT816-TBT443**
AGN364	**ABT817** sortase conjugated with **TBT544). MW** 22436.86 Da.
AGN365	**ABT819** sortase conjugated with **TBT544** MW 44914.95 Da.

TABLE 1	
#	Characteristics
AGN579	**ABT818 MATE** conjugated with **TBT307**). The product exists as a dimer (Fc). GN3 is conjugated at K248 of each Fc unit. MW 140601 Da.
AGN580	**ABT818-TBT443**
AGN167	**ABT740** maleimide conjugated with **TBT506.** GN3 conjugated to the C-terminal cysteine residue via maleimide conjugation

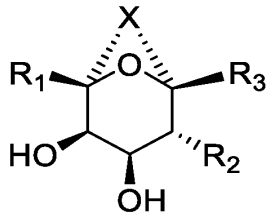
[011] In some embodiments, the anti-PLA2R antibody binding moiety is a peptide selected from secretory phospholipase A2 receptor peptides disclosed by Fresquet et al., J. Am. Soc. Nephrol. 26, 302–313 (2015). See SEQ ID NO: 60-145. See corresponding peptides SEQ ID NO: 636-721 from International Patent Application PCT/IB2025/050867, filed January 26, 2025.

[012] In more particular embodiments, the anti-PLA2R antibody-binding moiety selected from the group consisting of the moieties in TABLE 2.

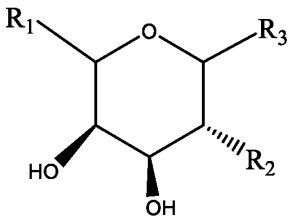
TABLE 2
#
ABT301, unconjugated 31mer (bicyclic), 3475.05 Da.
ABT305. unconjugated (31-mer) WQDKGIFVIQSESLKK(CIQAGKSVLTLENC)K, 3493.11 Da.
ABT309, 24mer VIQSESLKK(CIQAGKSVLTLENC)K, 2618.08 Da.
ABT310 (SEQ ID NO: 10) [VIQSES], 661.702 Da.
ABT311 (SEQ ID NO: 11) [SVLTLENCK], 1006.175 Da.
ABT312, 31mer (biotinylated), 4094.87 Da.
ABT407 (SEQ ID NO: 146). CysR-CTLD1, GGGGS-His6-GGGGS-Cys.
ABT408 (SEQ ID NO: 147). CysR-CTLD1, GGGGS-His6-Cys. Number of amino acids: 359, Molecular weight: 41072.01 Da, Theoretical pI: 5.76.
ABT409 (SEQ ID NO: 148). CysR-CTLD1, GGGGS-His6-GGGE-Cys-S, Number of amino acids: 364, Molecular weight: 41459.36 Da, Theoretical pI: 5.70.
ABT427 (FnII-CTLD1-his)
ABT530 (SEQ ID NO: 14), heavy chain sequence for hIgG1 LALA/PA sequence.
ABT603, SEQ ID NO: 19. Light chain, Molecular weight: 22904.35Da, Theoretical pI: 4.72
ABT606, Abcam anti-rabbit PLA2R mAb, combined Light chain + Heavy chain
ABT816 (SEQ ID NO: 15). CysR domain + GGGGS linker, hIgG1 Fc (C→A)-LALA/PA. Number of amino acids: 389, Molecular weight: 43224.10 Da, Theoretical pI: 8.91.

TABLE 2
#
ABT817 (SEQ ID NO: 16). CysR_Sortase, GGGGS linker, Sortase site, His6 tag. Number of amino acids: 169, Molecular weight: 18669.24 Da, Theoretical pI: 9.46. N-glycoform is present.
ABT818 (SEQ ID NO: 17). CysR-CTLD1_Fc-fusion, GGGGS linker, hIgG1 Fc (C→A)-LALA/PA. Number of amino acids: 584, Molecular weight: 66078.34 Da, Theoretical pI: 6.23. 2 N-glycoforms are present on each chain.
ABT819 (SEQ ID NO: 18). CysR-CTLD1 + Sortase, GGGGS linker, sortase site, His6 tag. Number of amino acids: 364, Molecular weight: 41523.47 Da, Theoretical pI: 6.33. N-glycoform is present.
ABT976, CysR-CTLD1 no tag. CysR-CTLD1 (21-367) + Thrombin recognition sequence + His10 tag, Before thrombin cleavage: Number of amino acids: 363, Molecular weight: 41811.88 Da, Theoretical pI: 5.95. After thrombin cleavage: Number of amino acids: 351, Molecular weight: 40296.34 Da, Theoretical pI: 5.55.
ABT983 (final mAb product with **ABT603**, SEQ ID NO: 19 + **ABT982**, SEQ ID NO: 20)
ABT985 (final mAb product with **ABT603**, SEQ ID NO: 19 + **ABT984**, SEQ ID NO: 21)

[013] In another embodiment, the cellular receptor-binding moiety comprises an ASGPR-binding group according to the chemical structure:



[TBT101], or



[TBT102],

wherein the cellular receptor-binding moiety has additional elements described in this specification.

[014] In some specific and nonlimiting embodiments, the cellular receptor-binding moiety is selected from the group in TABLE 3.

TABLE 3

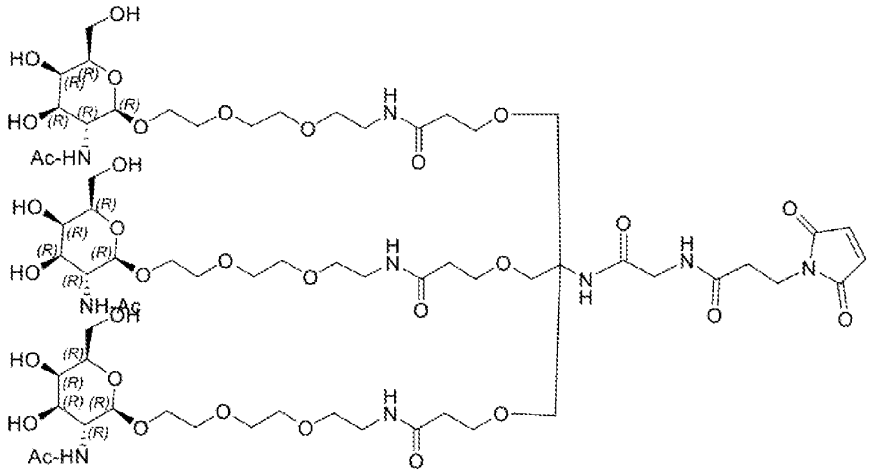
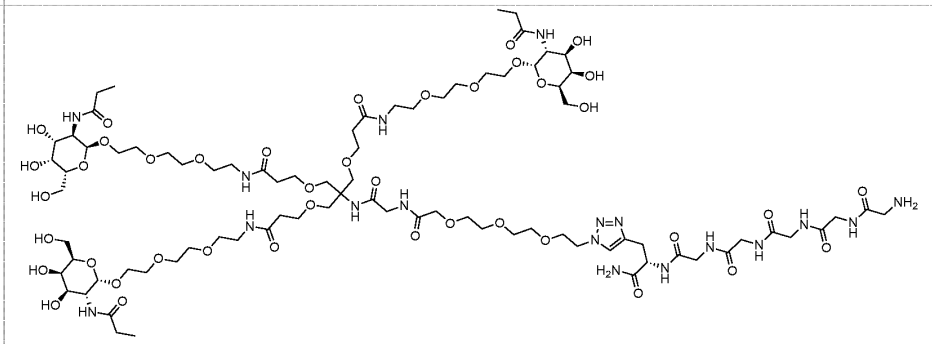
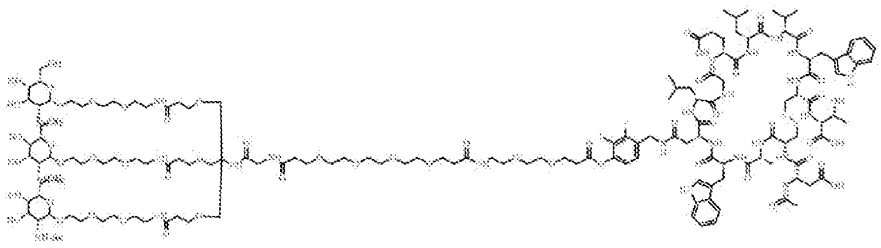
ID NO.	Chemical Structure
TBT103	
TBT104	
TBT105	

TABLE 3

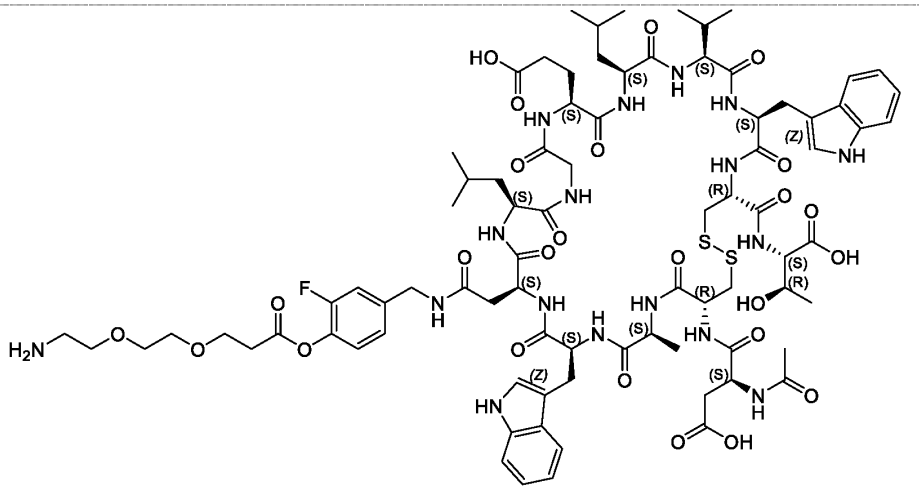
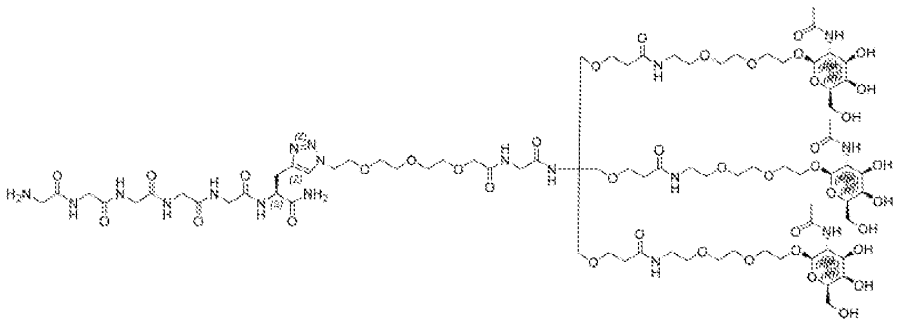
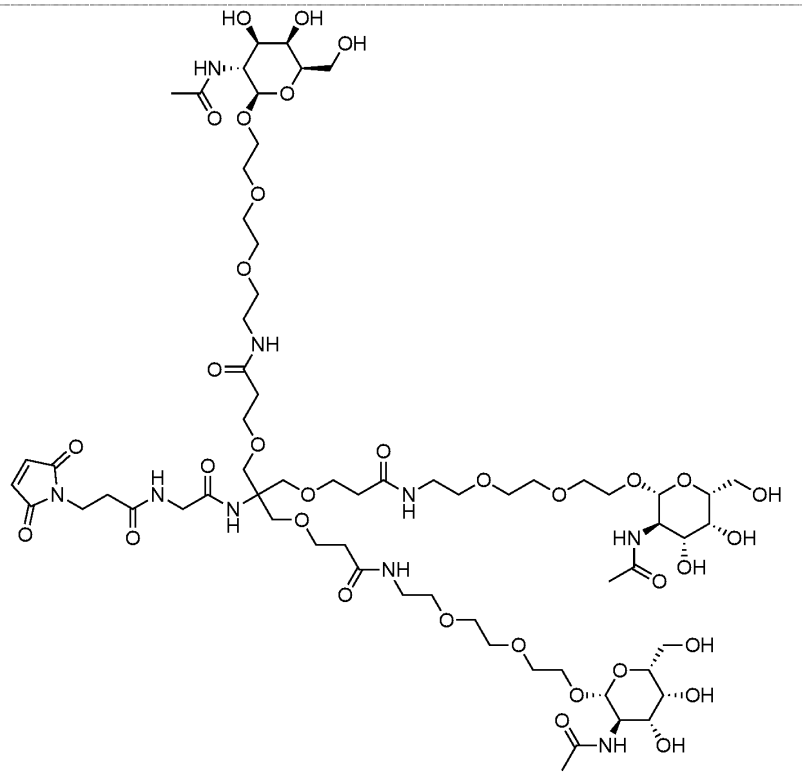
ID NO.	Chemical Structure
TBT307	 Molecular Weight: 1833.02 **Intermediate 30**
TBT544	

TABLE 3

ID NO.	Chemical Structure
TBT506	

[015] In another embodiment, the invention provides the composition of matter (agent, TRAP) for use as a medicine.

[016] In another embodiment, the invention provides the composition of matter (agent), TRAP for use in treating or preventing a disease state or condition associated with the presence of anti-PLA2R autoantibody in a subject or patient.

[017] In another embodiment, the invention provides the composition of matter (agent, TRAP) for use in treating a disease membranous nephropathy.

[018] In another embodiment, the invention provides a pharmaceutical composition including the composition of matter and at least one pharmaceutically acceptable excipient.

[019] In another embodiment, the invention provides a method of making a composition of matter wherein the method comprises a conjugation step, wherein the conjugation step results in an anti-PLA2R antibody-binding moiety being linked to a cellular receptor-binding moiety. In another embodiment, the method of making the composition of matter comprises a conjugation step selected

from the Markush group comprising a MATE conjugation step, a maleimide conjugation step, and a sortase conjugation step.

[020] In another embodiment, the invention provides a method of making a molecule wherein the anti-PLA2R antibody-binding moiety is linked to another molecule or a device, wherein the method comprises a conjugation step selected from the Markush group comprising a MATE conjugation step, a maleimide conjugation step, a sortase conjugation step, and an AviTag™ conjugation step.

[021] In another embodiment, the invention provides a method of removing anti-PLA2R antibody in a patient or subject in need thereof by administering a composition of matter described herein to the patient or subject.

[022] In another embodiment, the invention provides a method of treating or condition associated with the upregulation of anti-PLA2R autoantibody in a patient or subject in need by administering to the patient or subject an effective amount of a composition of matter described herein.

[023] In another embodiment, the invention provides a composition including a composition of matter described herein and at least one additional molecule comprising a moiety capable of binding to an anti-PLA2R antibody that forms the antibody moiety of the first compound.

[024] Several objects, features, aspects, and advantages of the invention will become more apparent from the following detailed description of embodiments of the invention, along with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[025] For illustration, some embodiments of the invention are shown in the drawings described below. Like numerals in the drawings indicate like elements throughout. The invention is not limited to the precise arrangements, dimensions, and instruments shown.

[026] FIG. 1 is a chemical drawing showing that azidohomoalanine can be incorporated into a synthetic 31-mer to help with click to ASGPR ligand. CryoEM and epitope mapping of PLA2R have shown that the 31-mer peptide mimics key epitope CysR region. FIG. 1 highlights key amino acids in the binding: V, I, and E. Two key antigenic regions, SVLTLENCK (**ABT311**, SEQ ID NO: 11) and VIQSES (**ABT310**; SEQ ID NO: 10) were determined by peptide microarray. See Fresquet, Lockhart-Cairns, Rhoden, & Lennon, Proc. Natl. Acad. Sci, U.S.A., 119 (29), e2202209119) (July 11, 2022). The linker attachment unlikely to interfere with antibody-binding.

[027] FIG. 2 is TABLE 4, showing R-Groups for MATE reagents and bifunctional MoDE final compounds.

[028] FIG. 3 is a flow chart showing the decision-making for the prior art standard-of-care treatment for idiopathic membranous nephropathy. One-third of patients experience spontaneous remission. According to the KDIGO guidelines, "Changes in anti-PLA2R antibodies levels during follow-up likely add to risk estimation. Disappearance of anti-PLA2R antibodies precedes clinical remission."

[029] FIG. 4 shows the structure of several anti-PLA2R-degrader compounds.

[030] FIG. 5 shows a visual representation of anti-PLA2R degrader conjugation methods.

[031] FIG. 6 shows a visual representation of PLA2R degraders-sortase conjugated.

[032] FIG. 7 shows a visual representation of PLA2R degraders-maleimide conjugated.

[033] FIG. 8 shows a visual representation of PLA2R degraders- MATE conjugated.

[034] FIG. 9 is a chart showing anti-PLA2R binding in ischemic monomelic neuropathy (IMN) samples with AFN364 and AGN365. Anti-PLA2R degraders AGN364 and AGN365 captured the majority of anti-PLA2R antibodies from IMN patient samples via competitive EuroImmun clinical anti-PLA2R ELISA. Most samples brought below diagnostic cut-off for anti-PLA2R positivity (14 RU/ml).

DETAILED DESCRIPTION OF THE INVENTION

[035] The following detailed description is provided to aid persons having ordinary skill in the biomedical art. Exemplary embodiments are described. However, these embodiments are only exemplary. This disclosure is not limited thereto but is defined by the scope of the appended claims. Persons having ordinary skill in the biomedical art may make modifications and variations in the embodiments described in this specification without departing from the spirit or scope of this disclosure.

Industrial Applicability

[036] The invention provides a medically useful composition of matter (agent, TRAP) for the treating or slowing down progression of diseases that are manifestations of anti-PLA2R antibody disfunction, such as membranous nephropathy.

[037] The Kidney Disease Improving Global Outcome (KDIGO) organization issues guidelines for treatment for physicians. KDIGO recommends using anti-PLA2R titers for treatment guidance. Eighty % of idiopathic membranous nephropathy patients have anti-PLA2R antibodies. High anti-PLA2R titers are associated with poor prognosis, worsened disease, and lower chance of spontaneous remission. Depletion of anti-PLA2R by immunosuppressive therapy is predictive of disease remission. See KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney international, Vol.

100, Issue 45, Supplement (October 2021). Changes in anti-PLA2R antibodies levels during follow-up can help risk estimation and patient stratification. Persistent anti-PLA2R titers is predictive of poor outcome.

[038] About 80% of idiopathic membranous nephropathy patients are anti-PLA2R antibody-positive. Despite treatment, titers persist in 30-40% of patients. These patients typically develop end-stage renal disease and require kidney transplant. Idiopathic membranous nephropathy recurs in 50% of allograft recipients.

[039] Chronic membranous nephropathy can cause significant proteinuria and nephrotic syndrome and can progress to kidney failure and nephrotic kidneys in severe cases. Autoantibodies bind to phospholipase A2 receptor (PLA2R) on podocytes in the Bowman's capsule, injuring glomerular basement membranes. Subepithelial immune complex deposits leads to podocyte injury and thickening of basement membrane, which leads further to proteinuria and hyperlipidemia, resulting in kidney failure. Patients rendered anti-PLA2r negative by immunosuppression have greater disease remission. Lu et al., Medicine (Baltimore), 98(18), e15303 (May 2019).

[040] The standard of care is supportive care includes rituximab, cyclophosphamide, calcineurin INH, glucocorticoids, B-cell therapies, and anticoagulants, as well as therapies that address non-autoantibody-targeted systems including the renin-angiotensin-aldosterone system (RAAS), angiotensin-converting enzyme (ACE), angiotensin receptors, and sodium-glucose cotransporter 2 (SGLT2). Rituximab or cyclophosphamide + glucocorticoids are first-line therapies but have undesirable side effects. Combination of plasmapheresis with standard of care shows more favorable outcomes. See Rovin et al. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int (2021) 100(4, Supplement):S1–276, and Bennani et al., J. Pers. Med., 14(3), 249 (2024).

[041] The inventors conceived of an anti-PLA2R degrader that is a safe and effective treatment to address this unmet need. Disease modifying therapies that stop progression to end-stage renal disease. PLA2R antigen-specific MoDEs rapidly remove pathogenic autoantibodies. Deep reductions in anti-PLA2R autoantibodies will prevent further glomerular injury.

Definitions

[0035] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are listed below. Unless stated otherwise or implicit from context, these terms and phrases shall have the meanings below. These definitions aid in describing embodiments but are not intended to limit the claimed invention.

[0036] As used in this application, except as otherwise provided in this specification, each term shall have the meaning set forth below. Additional definitions are set forth throughout the application. Where a term is not specifically defined in this specification, that term is given a biomedical art-recognized meaning applying that term in context to its use in describing the invention.

[0037] The articles "a" and "an" have the plain meaning of one or to more than one, *i.e.*, at least one, of the grammatical object of the article unless the context indicates otherwise. For example, "an element" means one element or more than one element.

[0038] The term “ABT” has the biomedical art-recognized meaning of a binding moiety that is itself an antibody, an antibody variant, or an -binding fragment thereof. In some embodiments, the ABT binds to anti-PLA2R antibody.

[0039] The term “active Ingredient” has the United States Food & Drug Administration-provided meaning of any component that provides pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of a human body or an animal body.

[0042] The term “ADCC” has the biomedical art-recognized meaning of antibody-dependent cell-mediated cytotoxicity, is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system kills a target cell, whose membrane-surface antigens have been bound by specific antibodies.

[0043] The term “ADCP” has the biomedical art-recognized meaning of antibody-dependent cell-mediated phagocytosis, an immunological mechanism of elimination whereby tumor cells are targeted with antibodies to promote their clearance from the body by phagocytic immune cells.

[0040] The term “AF488” has the biomedical art-recognized meaning of Alexa Fluor 488, a bright, green-fluorescent dye with excitation suited for the 488 nm laser line and is used for imaging and flow cytometry.

[0041] The term “AF647” has the biomedical art-recognized meaning of the far-red dye Alexa Fluor 647, which has an excitation peak at 650 nm and an emission peak at 665 nm. Alexa 647 is useful for flow cytometry, microscopy, and super-resolution microscopy.

[0044] The term "agent" has the biomedical art-recognized meaning of a composition of matter useful for performing a function. Several useful biomedically functions are described in this specification.

[0045] The term "alleviate" has the biomedical art-recognized meaning of a process by which the severity of a sign or symptom of a disorder is reduced. A sign or symptom can be alleviated without being eliminated. The administration of compositions or pharmaceutical compositions of the invention may or can lead to the elimination of a sign or symptom, however, elimination is not required. Effective dosages should be expected to decrease the severity of a sign or symptom.

[0046] The term "an effective amount" and the term "a therapeutically effective amount" has the biomedical art-recognized meaning of an amount effective to achieve its intended purpose. The effect can be detected by any assay method known in the art. The precise effective amount for a subject will depend on the subject's body weight, size, and health; the nature and extent of the condition; and the therapeutic or combination of therapeutics selected for administration. Therapeutically effective amounts for a situation can be determined by routine experimentation that is within the skill and judgment of the clinician. In embodiments, the disease or condition to be treated is tendinopathy.

[0047] The term "anti-PLA2R antibody-binding moiety" has the biomedical art-recognized meaning a moiety on a binding protein, e.g., an antibody, an antibody variant, or an -binding fragment thereof, that binds to an anti-PLA2R antibody.

[0042] The term "anti-PLA2R antibody" has the biomedical art-recognized meaning. Anti-PLA2R antibodies can be used for the diagnosis idiopathic membranous nephropathy without a kidney biopsy. Anti-PLA2R antibodies are found in ~80% of idiopathic membranous nephropathy patients. Clinically approved anti-PLA2R tests are commercially available. See, e.g., Mayo Clinic Laboratories Test ID: PLA2M ELISA assay, CPT code information: 83520. Anti-PLA2R antibodies are commercially available. A commercial monoclonal antibody from Abcam Limited specific for the PLA2R epitope being planned for the anti-PLA2R degrader program [fragment corresponding to Human PLA2R aa 1-700]: Mouse monoclonal anti-PLA2R antibody [12-6-5] (ab211490).

[0048] The term "antigen-binding fragment thereof" has the biomedical art-recognized meaning of (1) a fragment of an intact antibody that binds to the same antigen recognized by the full-length antibody, such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, sFv, or other fragments including the variable regions, or (2) any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. The term antigen-binding portion of an antibody encompasses single chain antibodies.

[0049] The term “asialoglycoprotein receptor (ASGPR) binding group” has the biomedical art-recognized meaning of a binding group which binds to a hepatocyte asialoglycoprotein receptor. The ASGPR-binding group selectively binds to hepatocyte asialoglycoprotein receptor on the surface of hepatocytes. In several embodiments, an ASGPR-binding group is a component of a bifunctional agent as a cellular receptor-binding moiety which is covalently bound to the antibody-binding moiety through a linker group or directly. It is through this ASGPR moiety that bifunctional agents complexed with a circulating protein, e.g., anti-PLA2R antibody, bind to hepatocytes. After the bifunctional agent complexed with a circulating protein is bound to a hepatocyte or other cell, the circulating protein is taken into the hepatocyte or other cell via a phagocytosis mechanism, wherein the circulating protein is degraded through lysosomal degradation.

[0050] The term “asialoglycoprotein receptor (ASGPR) has the biomedical art-recognized meaning of lectins which bind asialoglycoprotein and glycoproteins from which a sialic acid has been removed to expose galactose residues. These cellular receptors are located on mammalian hepatocytes and other cells, such as glandular cells of the gallbladder and the stomach. ASGPR remove target glycoproteins from circulation.

[0051] The term "at least one of," when preceding a list of elements, modifies the entire list of elements and does not modify the individual elements of the list.

[0052] The term “AT” has the biomedical art-recognized meaning of an antibody moiety. In some embodiments, the AT binds to anti-PLA2R antibody.

[0053] The term “cellular receptor-binding moiety” has the biomedical art-recognized meaning. In several embodiments, the cellular receptor-binding moiety is an asialoglycoprotein receptor (ASGPR) binding group.

[0054] The term “cellular receptor” has the biomedical art-recognized meaning of a protein on the surface of a cell that binds to a compound, e.g., a ligand, e.g., a protein, in solution or on another cell. Generally, ligand-receptor binding induces one or more biological responses. In this specification, an asialoglycoprotein receptor (ASGPR) is a cellular receptor on the surface of hepatocytes or other cells that binds to an asialoglycoprotein or a derivative thereof.

[0055] The term “chimerized” has the biomedical art-recognized meaning. Chimeric antibodies are made by fusing variable domains from one species, such as a mouse, with constant domains from another species, such as a human being. With such biotechnical manipulation, chimeric antibodies keep the foreign antibody’s antigen specificity and affinity.

[0056] The term “combination therapy” and the “co-therapy” has the biomedical art-recognized meaning of the administration of a composition described in this specification and at least a second agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination may include, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time, usually minutes, hours, days, or weeks depending on the combination selected. The term combination therapy” includes the administration of the therapeutic agents as described above in further combination with other biologically active ingredients and non-drug therapies, e.g., surgery or radiation treatment. Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time if a beneficial effect from the co-action of the therapeutic agents a is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

[0057] The term “complementarity determining region (CDR)” has the biomedical art-recognized meaning of a polypeptide region of an antibody heavy chin or an antibody light chain that is a determinant of the antibody to antigen-binding. Each antibody heavy chain has three CDRs. Each antibody light chain has three CDRs, usually different from the three CDRs on an antibody heavy chain. persons having ordinary skill in the biomedical art calculate the using a standardized numbering method known as the Kabat numbering scheme. Kabat et al, (1991) Sequences of Proteins of Immunological Interest, 5th Ed Public Health Service, National Institutes of Health, Bethesda, MD., USA), although other numbering schemes such as Chothia and IMGT are also used by persons having ordinary skill in the biomedical art.

[0058] The term "comprises," the term "comprising," the term "includes," and the term "including" specify stated features, regions, integers, steps, operations, elements, or components, but do not prevent the presence or addition of one or more other features, regions, integers, steps, operations, elements, components, or groups thereof.

[0059] The term “Fc-III-4c” has the biomedical art-recognized meaning of a polypeptide region in the fragment crystallizable region (Fc region), the tail region, of an antibody.

[0060] The term “Fc-M” has the biomedical art-recognized meaning of a polypeptide region in the fragment crystallizable region (Fc region), the tail region, of an antibody.

[0061] The term "FcB-1" has the biomedical art-recognized meaning of a polypeptide region in the fragment crystallizable region (Fc region), the tail region, of an antibody.

[0062] The term "FcB-2" has the biomedical art-recognized meaning of a polypeptide region in the fragment crystallizable region (Fc region), the tail region, of an antibody.

[0063] The term "first," "second," "third," etc. have the plain meaning of describing several elements, components, regions, layers, or sections, these elements, components, regions, layers, or sections should not be limited by these terms. These terms are only used to distinguish one element, component, region, layer, or section from another element, component, region, layer, or section. A first element, component, region, layer, or section could be called a second element, component, region, layer, or section without departing from the teachings of the present embodiments.

[0064] The term "hepatocyte" has the biomedical art-recognized meaning of a cell of the main parenchymal tissue of the liver. Hepatocytes make up 55-65% of the liver's mass.

[0065] The term "humanized" has the biomedical art-recognized meaning a protein, e.g., an antibody, is genetically engineered so it closely resembles the polypeptide structure of the human homologue. A variable domain of an antibody of rodent origin can be fused to a constant domain of human origin, keeping the specificity of the rodent antibody. The human origin domain need not originate directly from a human in that it is first synthesized in a human. Instead, human domains can be generated in rodents whose genome incorporates human immunoglobulin genes. The antibody can be partially humanized. In one approach, there are four general steps used to humanize a monoclonal antibody, These are (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains; (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process; (3) the actual humanizing methodologies/techniques; and (4) the transfection and expression of the humanized antibody.

[0066] The term "IC_{50}" has the biomedical art-recognized meaning of an amount, concentration, or dosage of a particular test compound that achieves a 50% inhibition of a maximal response in an assay that measures such response.

[0067] The term "idiopathic membranous nephropathy (IMN)" has the biomedical art-recognized meaning. Idiopathic membranous nephropathy is caused by autoimmunity to podocytes. Disease severity is characterized by proteinuria, decrease in glomerular filtration rate (eGFR), and anti-PLA2R antibody titers. M-type phospholipase A2 receptor (PLA2R) is the most often targeted antigen, with antibodies being present in about 80% of idiopathic membranous nephropathy patients. Idiopathic

membranous nephropathy is driven by autoimmunity to podocytes, causing increased thickness of the glomerular membrane and changes in podocyte morphology.

[0068] The term "IgG" antibody has the biomedical art-recognized meaning. Each IgG molecule includes the basic four-chain immunoglobulin structure—two γ (gamma) heavy chains and two identical light chains (either kappa or lambda)—and carries two identical antigen-binding sites. There are four subclasses of IgG, each with minor differences in its H chains but with distinct biological properties.

[0069] The term "IgG1" antibody has the biomedical art-recognized meaning of an IgG antibody where the Ig gamma-1 chain C region is a protein that in humans is encoded by the IGHG1 gene.

[0070] The term "IgG2" antibody has the biomedical art-recognized meaning of an IgG antibody where the Ig gamma-2 chain C region is a protein that in humans is encoded by the IGHG2 gene.

[0071] The term "IgG4" antibody has the biomedical art-recognized meaning of an IgG antibody where the Ig gamma-4 chain C region is a protein that in humans is encoded by the IGHG4 gene. IgG4 has little effector function. IgG4 cannot fix complement.

[0072] The term "IVIG" has the biomedical art-recognized meaning of the administration of intravenous immunoglobulin (IVIG).

[0042] The term "K_D" has the biomedical art-recognized meaning of the measured equilibrium dissociation constant between a compound or ligand and a protein or binding domain of a protein.

[0043] The term "lipoprotein-associated phospholipase A2" has the biomedical art-recognized meaning. See Blackie et al., Bioorg. Med. Chem. Lett., 13(6), 1067-70 (March 24, 2003). The phospholipase A2 receptor 1 (PLA2R1) is the major autoantigen in patients suffering from membranous nephropathy. Meyer-Schwesinger et al., Kidney International, 97 (5), 913-919 (2020).

[0044] The term "linker moiety" has the biomedical art-recognized meaning of a moiety of a chemical compound that links one moiety of the chemical compound to another moiety of the same compound. In several embodiments of this invention, the linker moiety connects an anti-anti-PLA2R antibody IgG antibody moiety to a cellular receptor-binding moiety.

[0045] The term "MoDE" has the proprietary meaning of molecular degraders. See International Pat. Publ. WO 2019/199634 (Yale University) and WO 2019/199621 (Yale University).

[0046] The term "membranous neuropathy" (primary membranous nephropathy, pMN) has the biomedical art-recognized meaning of an autoimmune disease characterized by the accumulation of immune complexes in the renal glomeruli. It is the most common cause of nephrotic syndrome in adults.

[0047] The term "moiety" has the biomedical meaning of a defined chemical group or entity with a particular structure or activity. A moiety generally refers to a part of a molecule, e.g., in an ester

RCOOR', the alcohol moiety is RO−. In some embodiments, a moiety of an agent keeps one or more or all desirable structural features, properties, functions, or activities of a compound. In some embodiments, a cellular receptor-binding moiety can bind to a target, optionally in a comparable fashion, as its corresponding target-binding agent. In some embodiments, a moiety is monovalent. In some embodiments, a moiety is bivalent. In some embodiments, a moiety is polyvalent.

[0048] The term "monotherapy" has the biomedical art-recognized meaning of the administration of a single active or therapeutic compound to a subject in need thereof. Monotherapy usually is the administration of a therapeutically effective amount of an active composition.

[0049] The term "Multimodal Antibody Therapy Enhancers (MATE or MATES)" has the proprietary meaning. See International Pat. Publ. WO 2021/102052 (Kleo Pharmaceuticals).

[0043] The term "nanobody" has the biomedical art-recognized meaning. the term "antibody" refers to immunoglobulin molecules and immunologically active parts of immunoglobulin molecules, i.e., molecules that have an antigen-binding site that specifically binds an antigen. The term also refers to antibodies comprised of two immunoglobulin heavy chains and two immunoglobulin light chains and many forms including full length antibodies and antigen-binding parts thereof; including an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')2, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, a nanobody, a multi-specific (e.g., tri-specific) antibody, a dual specific antibody, a bispecific antibody, an anti-idiotypic antibody, a functionally active epitope-binding part thereof, or bifunctional hybrid antibodies.

[0050] The term "on" has the plain meaning. When an element is called being on another element, it can be directly in contact with the other element or intervening elements may be present therebetween. When an element is called being "directly on" another element, there are no intervening elements present.

[0051] The term "or" as used in this specification means "or." The term "or" as used in this specification includes all combinations of one or more of the associated listed items.

[0052] The term "other degrading cells" has the biomedical art-recognized meaning. Asialoglycoprotein receptors (ASGPRs) are on the glandular cells of the gallbladder and the stomach.

[0053] The term "partially humanized" has the biomedical art-recognized meaning a protein, e.g., an antibody, is genetically engineered so it more closely resembles the polypeptide structure of the human homologue. A variable domain of an antibody of rodent origin can be fused to a constant domain of human origin, keeping the specificity of the rodent antibody. The domain of human origin need not

originate directly from a human in that it is first synthesized in a human. Instead, human domains can be generated in rodents whose genome incorporates human immunoglobulin genes. The antibody can be partially humanized.

[0054] The term "pharmaceutically acceptable excipient" has the biomedical art-recognized meaning of an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use and human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's, Pharmaceutical Sciences 23rd edition (Elsevier, 2020).

[0055] The term "pharmaceutically acceptable" has the biomedical art-recognized meaning of those compounds, anions, cations, materials, compositions, carriers, or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of humans and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0056] The term "protein-binding moiety" has the biomedical art-recognized meaning of a region of a chemical composition, e.g., a polypeptide region of a chemical composition, that specifically binds to a protein, e.g., a specific protein.

[0057] The term "ROC" has the biomedical art-recognized meaning of receiver operating typical curve.

[0058] The term "subject" and the term "patient" have the biomedical art-recognized meanings. The term patient includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0059] The term "TBT" has the biomedical art-recognized meaning of a target-binding moiety, a cellular receptor-binding moiety. In some embodiments, the TBT binds to ASGPR.

[0060] The term "treating" and the "treat" has the biomedical art-recognized meaning of the management and care of a patient for combating a disease, condition, or disorder and includes the administration of a composition described in this specification to alleviate the symptoms or complications of a disease, condition, or disorder, or to eliminate the disease, condition, or disorder.

[0061] The term "universal antibody-binding moiety" has the biomedical art-recognized meaning of a polypeptide region of an antibody-binding protein that binds a class of antibodies, rather than a specific set of antibodies.

[0062] The term "VHH" has the biomedical art-recognized meaning. VHH has 9 beta-sheets forming a cylindric structure. Several regions may bind. The most important regions 4>1, 2 (greatest difference in uptake). The least important: regions 3, 5 (very minor difference in uptake).

[0063] Some embodiments are described below by referring to structures and schemes, to explain parts of the description.

[0064] Unless otherwise defined, all technical and scientific terms used in this specification have the same meaning as commonly understood by persons having ordinary skill in the biomedical art.

[0065] This specification does not concern a process for cloning humans, methods for changing the germ line genetic identity of humans, uses of human embryos for industrial or commercial purposes, or procedures for changing the genetic identity of animals likely to cause them suffering with no substantial medical benefit to humans or animals resulting from such processes.

Methods of selecting subjects for administration of a composition of matter.

[042] The presence of anti-PLA2R antibodies can diagnose idiopathic membranous nephropathy without a kidney biopsy. Anti-PLA2R antibodies are highly specific to idiopathic membranous nephropathy (~100%) and found in ~80% of patients.

[043] The synthetic 31-mer peptide recognizes the majority of anti-PLA2R antibodies in idiopathic membranous nephropathy patients. The 31-mer peptide can be used in assays to detect anti-PLA2R antibodies in idiopathic membranous nephropathy patients.

[044] Laboratory testing for autoimmune disease uses well validated assays to establish the presence of autoantibodies. For membranous nephropathy, autoantibodies to the M-type phospholipase A2 receptor 1 (PLA2R) were first described in 2009. Beck et al., N. Engl. J. Med. 361, 11–21 (2009). The use of serological testing extends beyond diagnosis to encompass prognosis and assessment of disease activity and treatment response. Quantitative assessment of autoantibody production can provide a biomarker for clinical decision-making, as shown by the antibody response to PLA2R in membranous nephropathy. van de Logt et al., Kidney Int. 93, 1016–1017 (2018); and Wu et al., Medicine 97, e11018 (2018).

Methods of measuring the removal of anti-PLA2R antibody from a patient or subject.

[0066] Clinically approved anti-PLA2R tests are commercially available. See, e.g., Mayo Clinic Laboratories Test ID: PLA2M ELISA assay, CPT code information: 83520.

Methods of treatment by administering composition of matter.

[0067] The ideal mode of administration depends on where treatment is taking place, whether a hospital or outpatient.

[0068] In untreated patients with active disease, the previously recommended first-line agent for induction of remission is glucocorticoids, e.g., prednisolone 30–40 mg per day for 2–4 weeks, then gradually tapered over 3 to 6 months, unless contraindications exist. Glucocorticoids characteristically result in a rapid improvement in clinical features and often a resolution of radiographic features. However, where advanced fibrotic lesions have resulted in irreversible damage, the response to glucocorticoids and other current treatment options may be poor or even absent.

TABLE 5
Target product profile for a bifunctional degrader of anti-PLA2R in idiopathic membranous nephropathy.

	Short-term profile	Long-term profile
Objectives	Improvement in kidney function and improved side effect profile	Complete remission of disease, including in non-responsive patients. Improved side effect profile
Mechanism of action	Clearance of anti-PLA2R to reduce immune complex formation in glomerular membrane/podocytes	
Indication/Patient population	Idiopathic membranous nephropathy	Idiopathic membranous nephropathy
Route of administration/ Dosing regimen	Intravenous or subcutaneous, once-a-week	Subcutaneous, twice-a-week
Clinical efficacy profile	Equivalent to standard of care in primary outcome measures. Reduction in anti-PLA2R titer. Reduction in proteinuria. Improvement in eGFR	Superior to standard of care in primary outcome measures and effective in non-responsive patients. Anti-PLA2R below LOD. Proteinuria <3.5 g/d. Normal eGFR
Safety & tolerability profile	Improved side effect profile relative to standard of care. Well tolerated for chronic treatment.	

[045] Idiopathic membranous nephropathy patients are to be stratified after immunosuppressive treatment. 80% of idiopathic membranous nephropathy patients are anti-PLA2R+, and despite treatment, titers persist in 30-40% of patients. These patients typically develop end-stage renal disease (ESRD) and require kidney transplant. Idiopathic membranous nephropathy recurs in 50% of allograft

recipients. The efficacy of immunosuppressive therapy can be predicted by its effect on anti-PLA2R titers.

[046] Reduction of anti-PLA2R levels is followed by partial or complete remission in most patients. Conversely, persistent anti-PLA2R levels are associated with no remission and poor outcome. Faster depletion in anti-PLA2R levels in patients is also associated with earlier reduction in proteinuria and improved nephroprotection.

[047] After the bifunctional degrader of the invention and the bound anti-PLA2R antibody are endocytosed, they are released from the ASGPR through depletion of calcium from the endosome and changes in binding site amino acid protonation changes due to a decrease in pH. The ASGPR is recycled back to the hepatocyte surface. Endocytosed proteins are trafficked to late endosomes, which are fused with lysosomes. Lysosomal proteases then degrade endocytosed proteins, permanently removing them from circulation.

The chemical structure of the composition of matter (agent, TRAP).

[0069] In an embodiment, the invention composes matter (agent, TRAP) comprising:

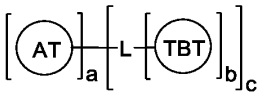
an anti-PLA2R antibody-binding moiety,

a cellular receptor-binding moiety which binds to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, and

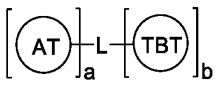
a linker moiety linking the antibody moiety and the cellular receptor-binding moiety.

[0070] In some embodiments, the invention composes matter (an agent, a TRAP) having a structure selected from the Markush group of structures including:

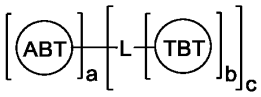
R^{CN}–(Xaa)y–R^{CC},



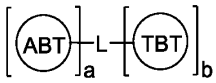
[AGN101],



[AGN102],

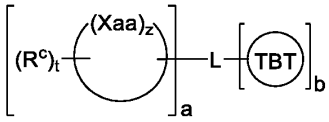


[AGN103], or



[AGN104] or a salt thereof. In these structures, a, b, and c may independently be an integer of 1 or greater. In some embodiments, each cellular receptor-binding moiety independently has the structure of $-(R^{CN}-(Xaa)y-R^{CC})$ or salt form thereof.

[0071] In some embodiments, the invention composes matter (an agent, a TRAP) of formula **AGN105**:



[AGN105] or a salt thereof, wherein:

each Xaa is independently a residue of an amino acid or an amino acid analog;

t is 0-50;

z is 1-50;

L is a linker moiety;

TBT is a cellular receptor-binding moiety;

each R^c is independently $-L^a-R'$;

each of a and b is independently 1-200;

each L^a is independently a covalent bond, or an optionally substituted bivalent group selected from a C_1-C_{20} aliphatic group or a C_1-C_{20} heteroaliphatic group having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$;

each $-Cy-$ is independently an optionally substituted bivalent monocyclic, bicyclic, or polycyclic group wherein each monocyclic ring is independently selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20-membered heteroaryl ring having 1-10 heteroatoms independently selected from

oxygen, nitrogen, sulfur, phosphorus, and silicon, and a 3-20-membered heterocyclyl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon;

each R' is independently –R, –C(O)R, $-CO_2R$, or $-SO_2R$;

each R is independently –H, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

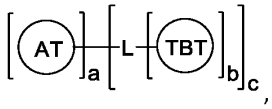
two R groups are optionally and independently taken together to form a covalent bond, or:

two or more R groups on the same atom are optionally and independently taken with the atom to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the atom, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; or

two or more R groups on two or more atoms are optionally and independently taken with their intervening atoms to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon.

[0072] In some embodiments, a is 1. In some embodiments, b is 3. In some embodiments, a is 1 and b is 3.

[0073] In some embodiments, the agent (TRAP) has the structure of formula **AGN101**:



[AGN101] or a salt thereof, wherein:

each of a, b and c is independently 1-200;

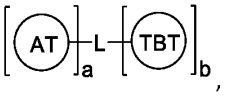
each AT is independently an anti-PLA2R antibody-binding moiety;

L is a linker moiety; and

each TBT is independently a cellular receptor-binding moiety,

wherein the anti-PLA2R antibody-binding moiety is an anti-PLA2R antibody or an antigen-binding fragment thereof.

[0074] In some embodiments, the agent (TRAP) has the structure of formula **AGN102**:



[AGN102] or a salt thereof, wherein:

each of a and b is independently 1-200;

each AT is independently an anti-PLA2R antibody-binding moiety;

L is a linker moiety; and

each TBT is independently a cellular receptor-binding moiety,

wherein the anti-PLA2R antibody-binding moiety is an anti-PLA2R antibody or an antigen-binding fragment thereof.

[0075] In some embodiments, an agent (TRAP) comprises one and no more than one anti-PLA2R antibody-binding moiety. In some embodiments, one or no more than one anti-PLA2R antibody-binding moiety is bound to a linker moiety. In some embodiments, a is 1. In some embodiments, an agent comprises two or more anti-PLA2R antibody moieties. In some embodiments, two or more anti-PLA2R antibody moieties are bound to a single linker moiety. In some embodiments, a is 2 or more. In some embodiments, one and no more than one cellular receptor-binding moiety is bonded to a linker moiety. In some embodiments, b is 1. In some embodiments, two or more cellular receptor-binding moiety is bonded to a single linker moiety. In some embodiments, b is 2 or more. In some embodiments, an agent comprises one and no more than one cellular receptor-binding moiety. In some embodiments, c is 1. In some embodiments, b is 1 and c is 1. In some embodiments, a is 1, b is 1 and c is 1. In some embodiments, an agent comprises two or more target-binding moieties. In some embodiments, b is 2 or more and c is 1. In some embodiments, b is 2 or more and c is 2 or more. In some embodiments, b is 1 and c is 2 or more.

[0076] In some embodiments, c is 1-20, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, c is selected from the Markush group of size ranges, where c is 1-15, c is 1-10, c is 1-9, c is 1-8, c is 1-7, c is 1-6, c is 1-5, c is 1-4, c is 1-3, and c is 1-2. In some embodiments, c is a size selected from the Markush group of sizes consisting of 1, 2, 3, 4, 5, 6, 7, 8, and 9.

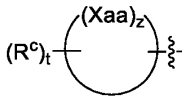
[0077] In some embodiments, each cellular receptor-binding moiety in an agent is the same. In some embodiments, each linker moiety connecting a cellular receptor-binding moiety to an antibody moiety is the same. In some embodiments, the TBT in agents is the same. In some embodiments, $-L-(TBT)_b$ is the same.

[0078] In some embodiments, b is 1. In some embodiments, c is 1. In some embodiments, c is two or more. In some embodiments, c is 2. Persons having ordinary skill in the biomedical art know that several technologies can conjugate antibody moieties with target-binding moieties, e.g., certain technologies used for preparing antibody-drug conjugates under this specification. In some embodiments, target-binding moieties are connected to antibody moieties through certain types of groups or amino acid residues. In some embodiments, target-binding moieties are connected to lysine residues optionally through linker moieties. In some embodiments, target-binding moieties are connected to cysteine residues optionally through linker moieties. In some embodiments, target-binding moieties are connected to unnatural amino acid residues optionally through linker moieties. In some embodiments, the invention provides technologies for selectively linking target-binding moieties to certain amino acid residues optionally through linker moieties. In some embodiments, provided technologies selectively connect target-binding moieties to certain types of amino acid residues, e.g., lysine residues, optionally through linker moieties. In some embodiments, provided technologies selectively connect target-binding moieties to sites of antibody moieties optionally through linker moieties. In some embodiments, provided technologies selectively connect target-binding moieties to certain types of amino acid residues at sites optionally through linker moieties. In some embodiments, target-binding moieties are connected to K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto optionally through linker moieties. In some embodiments, target-binding moieties are connected to K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto optionally through linker moieties. In some embodiments, target-binding moieties are connected to K239 and K241 of an anti-PLA2R antibody heavy chain and amino acid residues corresponding thereto optionally through linker moieties. In some embodiments, a cellular receptor-binding moiety is connected to a particular amino acid residue or site optionally through a linker. In some embodiments, each cellular receptor-binding moiety is independently connected to a particular amino acid residue or site optionally through a linker. As known by persons having ordinary skill in the biomedical art, an antibody agent may comprise more than one sites, e.g., one on each of the more than one chain, e.g., one or each heavy chain. In some embodiments, an antibody moiety comprises two heavy chains and one or both amino acid residues or amino acid residues corresponding thereto are each independently connected to a cellular receptor-binding moiety optionally through a linker. In some embodiments, one and no more than one is connected. In some embodiments, c is 1. In some embodiments, both are connected. In some embodiments, c is 2. In some embodiments, both target-binding moieties or both linker moieties (if any) are the same.

The anti-PLA2R antibody-binding moiety

[0079] In some embodiments, the anti-PLA2R antibody-binding moiety comprises a moiety selected from the Markush group consisting of one or more amino acid residues, a peptide moiety, a cyclic peptide moiety, a peptide comprising one or more natural amino acid residues, and a peptide comprising one or more unnatural natural amino acid residues.

[0080] The anti-PLA2R antibody-binding moiety may be or may comprise:



[ABT101]

or a salt form thereof.

[0081] In some embodiments, the anti-PLA2R antibody-binding moiety comprises a moiety selected from the Markush group consisting of one or more amino acid residues, a peptide moiety, a cyclic peptide moiety, a peptide comprising one or more natural amino acid residues, and a peptide comprising one or more unnatural natural amino acid residues.

[0082] In some embodiments, **ABT101** is a universal antibody-binding moiety. In some embodiments, **ABT101** is a universal antibody-binding moiety which can bind to anti-PLA2R antibody having different Fab regions. In some embodiments, **ABT101** is a universal antibody-binding moiety that binds to a Fc region, e.g., the Fc region that binds to an Fc receptor.

[0083] In some embodiments, the anti-PLA2R antibody-binding moiety comprises a universal antibody-binding moiety which recruit antibodies of diverse specificities, and no more than 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% percent of recruited antibodies are toward the same antigen, protein, lipid, carbohydrate, etc. An advantage of the invention is that provided technologies comprising universal antibody-binding moieties can use diverse pools of antibodies such as those present in serum. In some embodiments, universal antibody-binding moieties of this specification, e.g., those in ARMs, are contacted with a plurality of antibodies, wherein no more than 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% percent of the plurality of antibodies are toward the same antigen, protein, lipid, carbohydrate, etc.

[0084] In some embodiments, the antibody-binding moiety is a universal antibody-binding moiety. In some embodiments, an antibody-binding moiety, e.g., a protein-binding moiety, e.g., an antibody-

binding moiety, e.g., a universal antibody-binding moiety, comprises a peptide unit that can be connected to a linker moiety through the C-terminus of the peptide unit. In some embodiments, the antibody-binding moiety is connected to a linker moiety through the N-terminus of the peptide unit. In some embodiments, the antibody-binding moiety is connected to a linker through a side chain group of the peptide unit. In some embodiments, an antibody-binding moiety, e.g., a universal antibody-binding moiety comprises a peptide unit and is connected to an antibody-binding moiety optionally through a linker moiety through the C-terminus of the peptide unit. In some embodiments, the antibody-binding moiety, e.g., a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, comprises a peptide unit connected optionally through a linker moiety through the N-terminus of the peptide unit. In some embodiments, an antibody-binding moiety, e.g., a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, comprises a peptide unit connected optionally through a linker moiety through a side chain of the peptide unit.

[0085] In some embodiments, the antibody-binding moiety comprises a universal antibody-binding moiety which can bind to anti-PLA2R antibody antibodies having different Fab regions. In some embodiments, the universal antibody-binding moiety binds to a Fc region, e.g., the Fc region that binds to an Fc receptor.

[0086] Several antibody-binding moieties, including universal antibody-binding moieties, can be used under the teachings of this specification. Some antibody-binding moieties and technologies for identifying or assessing antibody-binding moieties are described in WO 2019/023501 and WO 2019/136442, each of which is incorporated in this specification in its entirety by reference. Persons having ordinary skill in the biomedical art know that additional technologies in the biomedical art may be suitable for identifying or assessing antibody-binding moieties under this specification. In some embodiments, an antibody-binding moiety comprises one or more amino acid residues, each independently natural or unnatural.

[0087] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g. a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, has the structure of or a salt form thereof, wherein:

each of R^7 is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8-membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10-membered bicyclic aromatic carbocyclic ring, a 4-8-membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6-membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10-membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or:

an R^7 group and the $R^{7'}$ group attached to the same carbon atom are optionally taken with their intervening carbon atom to form a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each of $R^{7'}$ is independently hydrogen or optionally substituted C_{1-3} aliphatic;

each of R^8 is independently hydrogen, or optionally substituted C_{1-4} aliphatic, or:

an R^8 group and its adjacent R^7 group are optionally taken with their intervening atoms to form a 4-8-membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

R^9 is hydrogen, optionally substituted C_{1-3} aliphatic, or –C(O)–.

[0088] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a universal antibody-binding moiety is or comprises a peptide moiety, e.g., a moiety having the structure of R^c–(Xaa)z– or a salt form thereof, wherein each of R^c, z and Xaa is independently as described in this specification. One or more Xaa may be independently an unnatural amino acid residue. Side chains of two or more amino acid residues may be linked to form bridges. Side chains of two cysteine residues may form a disulfide bridge comprising –S–S–, which, as in many proteins, can be formed by two –SH groups.

[0089] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g. a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, is or comprises a cyclic peptide moiety, e.g., a moiety having the structure of or a salt form thereof, wherein:

each Xaa is independently a residue of an amino acid or an amino acid analog;

t is 0-50;

z is 1-50;

each R^c is independently $-L^a-R'$;

each L^a is independently a covalent bond, or an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$;

each $-Cy-$ is independently an optionally substituted bivalent monocyclic, bicyclic, or polycyclic group wherein each monocyclic ring is independently selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20-membered heteroaryl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon, and a 3-20-membered heterocyclyl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-SO_2R$;

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

two R groups are optionally and independently taken together to form a covalent bond, or:

two or more R groups on the same atom are optionally and independently taken with the atom to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the atom, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; or

two or more R groups on two or more atoms are optionally and independently taken with their intervening atoms to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[0090] In some embodiments, the heteroatom is independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon.

[0091] Several antibody-binding moieties, including universal antibody-binding moieties, can be used under the teachings of this specification. Certain antibody-binding moieties and technologies for identifying or assessing antibody-binding moieties are described in WO2019/023501 and WO2019/136442, each of which is incorporated in this specification in its entirety by reference. Persons having ordinary skill in the biomedical art know that additional technologies in the biomedical art may be suitable for identifying or assessing antibody-binding moieties under this specification. In some embodiments, an antibody-binding moiety comprises one or more amino acid residues, each independently natural or unnatural.

[0092] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, has the structure of or a salt form thereof, wherein:

each of R^1, R^3 and R^5 is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8-membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10-membered bicyclic aromatic carbocyclic ring, a 4-8-membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6-membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10-membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or:

R^1 and $R^{1'}$ are optionally taken with their intervening carbon atom to form a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8-membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

R^3 and $R^{3'}$ are optionally taken with their intervening carbon atom to form a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8-membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

an R^5 group and the $R^{5'}$ group attached to the same carbon atom are optionally taken with their intervening carbon atom to form a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8-membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or

two R^5 groups are optionally taken with their intervening atoms to form a C_{1-10} optionally substituted bivalent straight or branched saturated or unsaturated hydrocarbon chain wherein 1-3 methylene units of the chain are independently and optionally replaced with –S–, –SS–, –N(R)–, –O–, –C(O)–, –OC(O)–, –C(O)O–, –C(O)N(R)–, –N(R)C(O)–, –S(O)–, $–S(O)_2–$, or $–Cy^1–$, wherein each $–Cy^1–$ is independently a 5-6-membered heteroarylenyl with 1-4 heteroatoms independently selected from nitrogen, oxygen or sulfur;

each of $R^{1'}$, $R^{3'}$ and $R^{5'}$ is independently hydrogen or optionally substituted C_{1-3} aliphatic;

each of R^2, R^4 and R^6 is independently hydrogen, or optionally substituted C_{1-4} aliphatic, or:

R^2 and R^1 are optionally taken with their intervening atoms to form a 4-8-membered, optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

R^4 and R^3 are optionally taken with their intervening atoms to form a 4-8-membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or

an R^6 group and its adjacent R^5 group are optionally taken with their intervening atoms to form a 4-8-membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a trivalent linker moiety; and

each of m and n is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0093] In some embodiments, L^1 is an optionally substituted trivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, or –C(O)O–. In some embodiments L^1 is $–(CH_2CH_2O)_{2-4}–$ or $–(CH_2CH_2O)_2–$.

[0094] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g. a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, has the structure of or a salt form thereof, wherein:

each of R^7 is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8-membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10-membered bicyclic aromatic carbocyclic ring, a 4-8-membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6-membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10-membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or:

an R^7 group and the $R^{7'}$ group attached to the same carbon atom are optionally taken with their intervening carbon atom to form a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8-membered optionally substituted saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each of $R^{7'}$ is independently hydrogen or optionally substituted C_{1-3} aliphatic;

each of R^8 is independently hydrogen, or optionally substituted C_{1-4} aliphatic, or:

an R^8 group and its adjacent R^7 group are optionally taken with their intervening atoms to form a 4-8-membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

R^9 is hydrogen, optionally substituted C_{1-3} aliphatic, or –C(O)–.

[0095] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a universal antibody-binding moiety is or comprises a peptide moiety, e.g., a moiety having the structure of R^c–(Xaa)z– or a salt form thereof, wherein each of R^c, z and Xaa is independently as described in this specification. One or more Xaa may be independently an unnatural amino acid residue. Side chains of two or more amino acid residues may be linked to form bridges. Side chains of two cysteine residues may form a disulfide bridge comprising –S–S– (which, as in many proteins, can be formed by two –SH groups).

[0096] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g. a protein-binding moiety, e.g., an antibody-binding moiety, e.g., a universal antibody-binding moiety, is or comprises a cyclic peptide moiety, e.g., a moiety having the structure of or a salt form thereof, wherein:

each Xaa is independently a residue of an amino acid or an amino acid analog;

t is 0-50;

z is 1-50;

each R^c is independently $-L^a-R'$;

each L^a is independently a covalent bond, or an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$;

each $-Cy-$ is independently an optionally substituted bivalent monocyclic, bicyclic, or polycyclic group wherein each monocyclic ring is independently selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20-membered heteroaryl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon, and a 3-20-membered heterocyclyl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-SO_2R$;

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

two R groups are optionally and independently taken together to form a covalent bond, or:

two or more R groups on the same atom are optionally and independently taken with the atom to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the atom, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; or

two or more R groups on two or more atoms are optionally and independently taken with their intervening atoms to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[0097] In some embodiments, the heteroatom is independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon.

[0098] In some embodiments, an anti-PLA2R antibody-binding moiety is or comprises $R^c-(Xaa)z-$ or a salt form thereof, wherein each variable is as described in this specification.

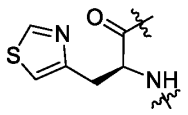
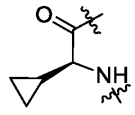
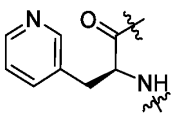
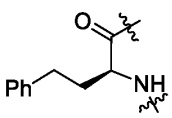
[0099] In some embodiments, a protein-binding moiety is or comprises R^c–(Xaa)z– or a salt form thereof, wherein each variable is as described in this specification. In some embodiments, a protein-binding moiety is or comprises **ABT101** or a salt form thereof, wherein each variable is as described in this specification.

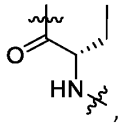
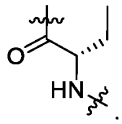
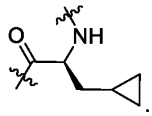
[00100] In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a universal antibody-binding moiety, is or comprises R^c–(Xaa)z– or a salt form thereof, wherein each variable is as described in this specification. In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a universal antibody-binding moiety, is or comprises **ABT101** or a salt form thereof, wherein each variable is as described in this specification. In some embodiments, an anti-PLA2R antibody-binding moiety, e.g., a universal antibody-binding moiety is R^c–(Xaa)z– or **ABT101** , or a salt form thereof, and is or comprises a peptide unit.

[00101] In some embodiments, –(Xaa)z– is or comprises a peptide unit.

[00102] In some embodiments, amino acid residues may form bridges, e.g., connections formed by side chains optionally through linker moieties, e.g., L); for example, as in many polypeptides, cysteine residues may form disulfide bridges.

[00103] In some embodiments, a peptide unit is or comprises a cyclic peptide unit. In some embodiments, a cyclic peptide unit comprises amide group formed by an amino group of a side chain and the C-terminus (–COOH). Persons having ordinary skill in the biomedical art know that in several embodiments, when a peptide unit is connected to another moiety, an amino acid residue of a peptide unit may be connected through several positions, e.g., its backbone, its side chain, etc. In embodiments, an amino acid residue is changed for connection.

[00104] In some embodiments, X^5 is Xaa^A or Xaa^P. In some embodiments, X^5 is Xaa^A. In some embodiments, X^5 is Xaa^P. In some embodiments, X^5 is an amino acid residue whose side chain comprises an optionally substituted saturated, partially saturated, or aromatic ring. In some embodiments, X^5 is  or . In some embodiments, X^6 is selected from the Markush group of amino acids consisting of Xaa^A, Xaa^P, and His. In some embodiments, X^{12} is selected from the Markush group of amino acids consisting of Xaa^A, Xaa^P,  , and . In some embodiments, X^9 is selected from the Markush group of amino acids consisting of Asp and Glu. In some

embodiments, each of X^7, X^{10}, and X^{11} is independently an amino acid residue with a hydrophobic side chain (hydrophobic amino acid residue, Xaa^H). In some embodiments, X^7 is selected from the Markush group of amino acids consisting of Xaa^H, , and Val. In some embodiments, X^{10} is selected from the Markush group of amino acids consisting of Xaa^H, Met, and . In some embodiments, X^{11} is selected from the Markush group of amino acids including Xaa^H and . In some embodiments, X^8 is Gly. In some embodiments, X^4 is Pro. In some embodiments, X^3 is Lys. In some embodiments, the −COOH of X^{12} forms an amide bond with the side chain amino group of Lys (X^3), and the other amino group of the Lys (X^3) is connected to a linker moiety and then an anti-PLA2R antibody-binding moiety.

[00105] In some embodiments, −(Xaa)z− is or comprises $-X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}-$, wherein:

each of X^3, X^4, X^5, X^6, X^7, X^8, X^9, X^{10}, X^{11}, and X^{12} is independently an amino acid residue;

at least two amino acid residues are connected through one or more linkages L^b;

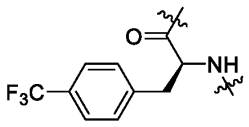
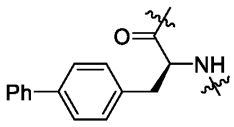
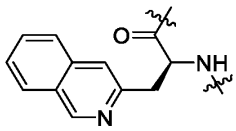
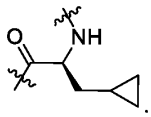
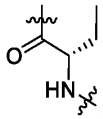
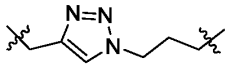
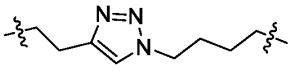
L^b is an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, −Cy−, −O−, −S−, −S−S−, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$, wherein L^b is bonded to a backbone atom of one amino acid residue and a backbone atom of another amino acid residue, and comprises no backbone atoms;

X^6 is Xaa^A or Xaa^P;

X^9 is Xaa^N; and

X^{12} is Xaa^A or Xaa^P.

[00106] In some embodiments, each of X^3, X^4, X^5, X^6, X^7, X^8, X^9, X^{10}, X^{11}, and X^{12} is independently an amino acid residue of an amino acid of formula **LNK101**. In some embodiments, two non-neighboring amino acid residues are connected by L^b. In some embodiments, X^5 and X^{10} are connected by L^b. In some embodiments, there is one linkage L^b. In some embodiments, X^6 is an amino acid selected from the Markush group of amino acids consisting of Xaa^A, Xaa^P, and His. In some embodiments, X^9 is Asp or Glu.

In some embodiments, X^{12} is an amino acid selected from the Markush group of amino acids consisting of Xaa^A, , , and . In some embodiments, each of X^4, X^7, and X^{11} is independently Xaa^H. In some embodiments, X^4 is Xaa^H or Ala. In embodiments, X^7 is Xaa^H. In some embodiments, X^7 is . In some embodiments, X^{11} is Xaa^H or . In some embodiments, X^8 is Gly. In some embodiments, X^3 is Lys. In some embodiments, the −COOH of X^{12} forms an amide bond with the side chain amino group of Lys (X^3), and the other amino group of the Lys (X^3) is connected to a linker moiety and then an anti-PLA2R antibody-binding moiety. In some embodiments, L^b is . In some embodiments, L^b is . In some embodiments, L^b connects two alpha-carbon atoms of two amino acid residues. In some embodiments, both X^5 and X^{10} are Cys, and the two −SH groups of their side chains form −S−S− (L^b is $-CH_2-S-S-CH_2-$).

[00107] In some embodiments, −(Xaa)z− is or comprises $-X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}-$, wherein:

each of X^2, X^3, X^4, X^5, X^6, X^7, X^8, X^9, X^{10}, X^{11}, and X^{12} is independently an amino acid residue;

at least two amino acid residues are connected through one or more linkages L^b;

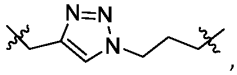
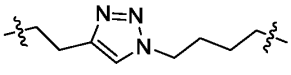
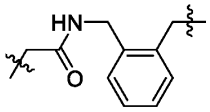
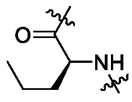
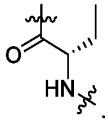
L^b is an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, −Cy−, −O−, −S−, −S−S−, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$, wherein L^b is bonded to a backbone atom of one amino acid residue and a backbone atom of another amino acid residue, and comprises no backbone atoms;

X^4 is Xaa^A;

X^5 is Xaa^A or Xaa^P;

X^8 is Xaa^N; and

X^{11} is Xaa^A.

[00108] In some embodiments, each of X^2, X^3, X^4, X^5, X^6, X^7, X^8, X^9, X^{10}, X^{11}, and X^{12} is independently an amino acid residue of an amino acid of formula **LNK101**. In some embodiments, two non-neighboring amino acid residues are connected by L^b. In some embodiments, there is one linkage L^b. In some embodiments, X^2 and X^{12} are connected by L^b. In some embodiments, L^b is a linker selected from the Markush group of linkers consisting of $-CH_2-S-S-CH_2-$, $-CH_2-CH_2-S-CH_2-$, , , and $-CH_2CH_2CO-N(R')-CH_2CH_2-$. In some embodiments, R' are taken with an R group on the backbone atom that $-N(R')-CH_2CH_2-$ is bonded to form a ring, e.g., as in A-34. In some embodiments, a formed ring is 3-, 4-, 5-, 6-, 7- or 8-membered. In some embodiments, a formed ring is monocyclic. In some embodiments, a formed ring is saturated. In some embodiments, L^b is . In some embodiments, L^b connects two alpha-carbon atoms of two amino acid residues. In some embodiments, X^4 is Xaa^A or Tyr. In some embodiments, X^5 is an amino acid selected from the Markush group of amino acids consisting of Xaa^A, Xaa^P, and His. In some embodiments, X^8 is Asp or Glu. X^{11} is Tyr. In some embodiments, both X^2 and X^{12} are Cys, and the two –SH groups of their side chains form –S–S– (L^b is $-CH_2-S-S-CH_2-$). In some embodiments, each of X^3, X^6, X^9, and X^{10} is independently Xaa^H. In some embodiments, X^3 is Xaa^H or Ala. In embodiments, X^6 is Xaa^H or Leu. In some embodiments, X^9 is an amino acid selected from the Markush group of amino acids consisting of Xaa^H, Leu, and . In some embodiments, X^{10} is an amino acid selected from the Markush group of amino acids including Xaa^H, Val, . In some embodiments, X^7 is Gly. In some embodiments, p1 is 1. In some embodiments, X^1 is Asp. In some embodiments, p13 is 1. In some embodiments, p14, p15 and p16 are 0. In some embodiments, X^{13} is an amino acid residue comprising a polar uncharged side chain, e.g., at physiological pH, polar uncharged amino acid residue, Xaa^L). In some embodiments, X^{13} is Thr. In some embodiments, X^{13} is Val. In some embodiments, p13 is 0. In some embodiments, R^c is $-NHCH_2CH(OH)CH_3$. In some embodiments, R^c is (*R*)$-NHCH_2CH(OH)CH_3$. In some embodiments, R^c is (*S*)$-NHCH_2CH(OH)CH_3$.

[00109] In some embodiments, –(Xaa)z– is or comprises $-X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}-$, wherein:

each of X^2, X^3, X^4, X^5, X^6, X^7, X^8, X^9, X^{10}, X^{11}, and X^{12} is independently an amino acid residue;

at least two amino acid residues are connected through one or more linkages L^b;

L^b is an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$, wherein L^b is bonded to a backbone atom of one amino acid residue and a backbone atom of another amino acid residue, and comprises no backbone atoms;

X^5 is Xaa^A or Xaa^P;

X^8 is Xaa^N; and

X^{11} is Xaa^A.

[00110] Persons having ordinary skill in the biomedical art know that an amino acid residue may be replaced by another amino acid residue having similar properties, e.g., one Xaa^H, e.g., Val, Leu, etc. may be replaced with another Xaa^H, e.g., Leu, Ile, Ala, etc. , one Xaa^A may be replaced with another Xaa^A, one Xaa^P may be replaced with another Xaa^P, one Xaa^N may be replaced with another Xaa^N, one Xaa^L may be replaced with another Xaa^L, etc.

[00111] In some embodiments, the anti-PLA2R antibody-binding moiety is or comprises optionally substituted moiety disclosed in TABLE 2 of the published patent application WO 2024/228935 (Biohaven Therapeutics Ltd.).

[00112] In some embodiments, an antibody-binding moiety is an antibody-binding moiety described in this specification. In some embodiments, a protein-binding moiety is an antibody-binding moiety described in this specification. In some embodiments, –COOH or amino groups of amino acid residues, e.g., those at the C-terminus or N-terminus, is optionally capped. In some embodiments, a –COOH group, e.g., a C-terminus –COOH) is amidated, e.g., converted into $-CON(R')_2$, e.g., $-C(O)NHR$, e.g., $-C(O)NH_2$, and in some embodiments, an amino group, e.g. $-NH_2$, e.g., a N-terminus $-NH_2$) is capped with R'– or R'C(O)–, e.g., in some embodiments, by conversion $-NH_2$ into –NHR', e.g., $-NHC(O)R$, e.g., $-NHC(O)CH_3$).

[00113] In some embodiments, an antibody-binding moiety is or comprises (X_{1-3})-C-(X_2)-H-(Xaa1)-G-(Xaa2)-L-V-W-C-(X_{1-3}), wherein each of X and Xaa is independently an amino acid residue and optionally not a cysteine residue. In some embodiments, Xaa1 is R, L, L, D, E, a 2-amino suberic acid residue, or a diaminopropionic acid residue. In some embodiments, Xaa1 is an arginine residue or a leucine residue. In some embodiments, Xaa2 is L, D, E, N, or Q. In embodiments, Xaa2 is a glutamic acid residue or an

aspartic acid residue. In some embodiments, Xaa2 is a lysine residue, a glutamine residue, or an aspartic acid residue. In some embodiments, this antibody-binding moieties are antibody-binding moieties.

[00114] In some embodiments, an antibody-binding moiety is or comprises (X1-3)-C-(Xaa3)-(xaa4)-H-(Xaa1)-G-(Xaa2)-L-V-W-C-(Xaa5)-(Xaa6)-(Xaa7), wherein each of X and Xaa is independently an amino acid residue and optionally not a cysteine residue. In some embodiments, Xaa3 is an alanine residue or a lysine residue. In some embodiments, Xaa4 is a tryptophan residue or a tyrosine residue. In some embodiments, Xaa1 is an arginine residue, a leucine residue, a lysine residue, an aspartic acid residue, a glutamic acid residue, a 2-amino suberic acid residue, or a diaminopropionic acid residue. In some embodiments, Xaa2 is a lysine residue, a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue. In some embodiments, Xaa5 is a threonine residue or a lysine residue. In some embodiments, Xaa6 is a tyrosine residue, a lysine residue, or absent. In some embodiments, Xaa7 is a histidine residue, a lysine residue, or absent. In some embodiments, this antibody-binding moieties are antibody-binding moieties.

[00115] In some embodiments, an antibody-binding moiety is or comprises D-C-(Xaa3)-(Xaa4)-H-(Xaa1)-G-(Xaa2)-L-V-W-C-(Xaa5)-(Xaa6)-(Xaa7), wherein each of X and Xaa is independently an amino acid residue and optionally not a cysteine residue. In some embodiments, Xaa3 is an alanine residue or a lysine residue. In some embodiments, Xaa4 is a tryptophan residue or a tyrosine residue. In some embodiments, Xaa1 is an arginine residue, a leucine residue, a lysine residue, an aspartic acid residue, a glutamic acid residue, a 2-amino suberic acid residue, or a diaminopropionic acid residue. In some embodiments, Xaa2 is a lysine residue, a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue. In some embodiments, Xaa5 is a threonine residue or a lysine residue. In some embodiments, Xaa6 is a tyrosine residue, a lysine residue, or absent. In some embodiments, Xaa7 is a histidine residue, a lysine residue, or absent. In some embodiments, this antibody-binding moieties are antibody-binding moieties.

[00116] In some embodiments, an antibody-binding moiety is or comprises D-C-(Xaa3)-(Xaa4)-H-(Xaa1)-G-(Xaa2)-L-V-W-C-T, wherein each of X and Xaa is independently an amino acid residue and optionally not a cysteine residue. In some embodiments, Xaa3 is an alanine residue or a lysine residue. In some embodiments, Xaa4 is a tryptophan residue or a tyrosine residue. In some embodiments, Xaa1 is an arginine residue, a leucine residue, a lysine residue, an aspartic acid residue, a glutamic acid residue, a 2-amino suberic acid residue, or a diaminopropionic acid residue. In some embodiments, Xaa2 is a lysine residue, a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue. In some embodiments, this antibody-binding moieties are antibody-binding moieties.

[00117] In some embodiments, an antibody-binding moiety is or comprises R-G-N-C-(Xaa3)-(Xaa4)-H-(Xaa1)-G-(Xaa2)-L-V-W-C-(Xaa5)- (Xaa6)-(Xaa7), wherein each of X and Xaa is independently an amino acid residue and optionally not a cysteine residue. In some embodiments, Xaa3 is an alanine residue or a lysine residue. In some embodiments, Xaa4 is a tryptophan residue or a tyrosine residue. In some embodiments, Xaa1 is an arginine residue, a leucine residue, a lysine residue, an aspartic acid residue, a glutamic acid residue, a 2-amino suberic acid residue, or a diaminopropionic acid residue. In some embodiments, Xaa2 is a lysine residue, a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue. In some embodiments, Xaa5 is a threonine residue or a lysine residue. In some embodiments, Xaa6 is a tyrosine residue, a lysine residue, or absent. In some embodiments, Xaa7 is a histidine residue, a lysine residue, or absent. In some embodiments, this antibody-binding moieties are antibody-binding moieties.

[00118] In some embodiments, antibody-binding moieties, e.g., several antibody-binding moieties described above, are protein-binding moieties. In some embodiments, antibody-binding moieties are antibody-binding moieties. In some embodiments, LG is or comprises this antibody-binding moiety. In some embodiments, LG is or comprises a protein-binding moiety. In some embodiments, LG is or comprises an antibody-binding moiety.

[00119] In some embodiments, antibody-binding moieties, e.g., antibody-binding moieties, and useful technologies for developing or assessing such moieties are described in, e.g., Alves, Langmuir, 28, 9640–9648 (2012), Choe et al., Materials, 9, 994 (2016), Gupta et al., Nature Biomedical Engineering, 3, 917–929 (2019), Muguruma et al., ACS Omega, 4, 14390−14397 (2019), Yamada et al., Angew Chem. Int., Ed Engl.; 58(17), 5592-5597 (April 16, 2019), Kruljec et al., Bioconjugate Chem., 28(8): 2009-2030 (2017), e.g., Fabsorbent, triazines, etc.; Kruljec et al., Bioconjugate Chem., 29(8), 2763-2775 (2018), WO2012017021A2, etc., the-binding moieties, e.g., antibody-binding moieties) of each of which is incorporated in this specification in its entirety by reference.

[00120] In some embodiments, an antibody-binding moiety, e.g., a protein-binding moiety, e.g., an antibody-binding moiety), is an affinity substance described in AU 2018259856 or WO 2018199337, the affinity substance of each of which is incorporated in this specification by reference.

[00121] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, is or comprises an adapter protein agent, e.g., as described in Hui et al., Bioconjugate Chem., 26, 1456−1460 (2015). In some embodiments, when used under this specification, adapter proteins do not require reactive residues, e.g., BPA, to achieve one or more or all advantages.

[00122] In some embodiments, antibody-binding moiety, e.g., an antibody-binding moiety is or comprises a triazine moiety, e.g., one described in US 2009/0286693. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is of such a structure that its corresponding compound is a compound described in US 2009/0286693, the compounds of which are independently incorporated in this specification by reference. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, is ABT. In some embodiments, ABT is of such a structure that H–ABT is a compound described in US 2009/0286693, the compounds of which are independently incorporated in this specification by reference. In some embodiments, this compound can bind to an antibody. In some embodiments, this compound can bind to Fc region of an antibody.

[00123] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is or comprises a triazine moiety, e.g., one described in Teng et al., J. Mol. Recognit., 12, 67–75 (1999). In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is of such a structure that its corresponding compound is a compound described in Teng, the compounds of which are independently incorporated in this specification by reference. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, ABT is of such a structure that H–ABT is a compound described in Teng, the compounds of which are independently incorporated in this specification by reference. In some embodiments, this compound can bind to an antibody. In some embodiments, this compound can bind to Fc region of an antibody.

[00124] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is a triazine moiety, e.g., one described in Uttamchandani, et al., J. Comb. Chem., 6(6), 862-8 (November-December 2004). In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is of such a structure that its corresponding compound is a compound described in Uttamchandani, the compounds of which are independently incorporated in this specification by reference. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, ABT is of such a structure that H–ABT is a compound described in Uttamchandani, the compounds of which are independently incorporated in this specification by reference. In some embodiments, this compound can bind to an antibody. In some embodiments, this compound can bind to Fc region of an antibody.

[00125] In some embodiments, an antibody-binding moiety binds to one or more-binding sites of a protein selected from the Markush group of proteins consisting of protein A, protein G, protein L, protein Z, protein LG, protein LA, and protein AG. In some embodiments, an antibody-binding moiety is described in Choe, Durgannavar, & Chung, Materials, 9(12) (2016).

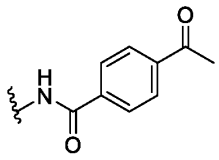
[00126] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety can bind to a nucleotide-binding site. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety is a small molecule moiety that can bind to a nucleotide-binding site. In some embodiments, a small molecule is tryptamine. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, ABT is of such a structure that H−ABT is tryptamine. Certain useful technologies were described in Mustafaoglu et al., Analyst, 141(24), 6571–6582 (November 28, 2016).

[00127] Many technologies are available for identifying, assessing, or characterizing antibody-binding moieties, including protein-binding moieties, e.g., antibody-binding moieties such as universal antibody-binding moieties), or their use in provided technologies, e.g., those described in Intl. Pat. Publ. WO 2019/023501, the technologies of which are incorporated in this specification by reference. In some embodiments, an antibody-binding moiety is a moiety, e.g., small molecule moiety, peptide moiety, nucleic acid moiety, etc., that can selectively bind to IgG, and when used in provided technologies can provide or stimulate ADCC or ADCP. In some embodiments, peptide display technologies, e.g., phase display, non-cellular display, etc., can identify antibody-binding moieties. In some embodiments, an antibody-binding moiety is a moiety, e.g., small molecule moiety, peptide moiety, nucleic acid moiety, etc., that can bind to IgG and optionally can compete with known antibody binders, e.g., protein A, protein G, protein L, etc.

[00128] Persons having ordinary skill in the biomedical art know that antibodies of several properties and activities, e.g., antibodies recognizing different antigens, having optional changes, etc., may be targeted by antibody-binding moieties described in this specification. In some embodiments, such antibodies include antibodies administered to a subject, e.g., for therapeutic purposes. In some embodiments, antibody-binding moieties described in this specification may bind antibodies toward different antigens and are useful for conjugating moieties of interest with several antibodies.

[00129] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, is or comprises a meditope agent moiety. In some embodiments, a meditope agent is described in, e.g., US 2019/0111149.

[00130] In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, can bind to human IgG. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, can bind to an antibody selected from the Markush group of antibodies consisting of rabbit IgG, IgG1, IgG2, IgG3, and IgG4. In some embodiments, an antibody-binding moiety, e.g., an antibody-binding moiety, binds to IgG1, IgG2, and IgG4.



[00131] In some embodiments, is used in a reference technology as a non-antibody-binding moiety. In some embodiments, the chemical group used in a reference technology as a non-antibody-binding moiety is selected from the Markush group of chemical groups consisting of CH_3–, $CH_3C(O)$–, $CH_3C(O)NH$–, $CH_3C(O)NHCH_2$–, CH_3CH_2–, CH_3CH_2NH–, and $CH_3CH_2NHC(O)$–.

[00132] In some embodiments, antibody-binding moieties, e.g., antibody-binding moieties) bind to targets, e.g., antibody agents for antibody-binding moieties) with a K_D that is about 1 mM^{-1} pM or less. In some embodiments, a K_D is about 1 mM, 0.5 mM, 0.2 mM, 0.1 mM, 0.05 mM, 0.02 mM, 0.01 mM, 0.005 mM, 0.002 mM, 0.001 mM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.2 nM, 0.1 nM, or less. In some embodiments, K_D is an affinity selected from the Markush group of affinities consisting of about 1 mM or less, about 0.5 mM or less, about 0.1 mM or less, about 0.05 mM or less, about 0.01 mM or less, about 0.005 mM or less, about 0.001 mM or less, about 500 nM or less, about 200 nM or less, about 100 nM or less, about 50 nM or less, about 20 nM or less, about 10 nM or less, about 5 nM or less, about 2 nM or less, and about 1 nM or less. In some embodiments, antibody-binding moieties bind to IgG antibody agents with K_D described in this specification.

[00133] Persons having ordinary skill in the biomedical art know that antibodies of several properties and activities, e.g., antibodies recognizing different antigens, having optional changes, etc., may be recruited by antibody-binding moieties described in this specification. In some embodiments, such antibodies include antibodies administered to a subject, e.g., for therapeutic purposes. In some embodiments, antibodies recruited by antibody-binding moieties comprise antibodies toward different antigens. In some embodiments, antibodies recruited by antibody-binding moieties comprise antibodies whose antigens are not present on the surface or cell membrane of target cells. In some embodiments, antibodies recruited by antibody-binding moieties comprise antibodies not targeting antigens present on surface or cell membrane of targets. In some embodiments, antigens on surface of target cells may interfere with the structure, conformation, or one or more properties or activities of recruited antibodies which bind such antigens. In some embodiments, recruited antibodies are those in IVIG. In some embodiments, IVIG may be administered before, concurrently with or subsequently to an agent or composition. Antibodies of several types of immunoglobulin structures may be recruited. In some embodiments, one or more subclasses of IgG are recruited. In some embodiments, recruited antibodies are selected from the Markush group of antibody classes consisting of IgG1, IgG2, IgG3, and IgG4. In

some embodiments, recruited antibodies are or comprise IgG1 and IgG2. In some embodiments, recruited antibodies are or comprise IgG1, IgG2 and IgG4. In some embodiments, recruited antibodies are or comprise IgG1, IgG2, IgG3 and IgG4. Recruited antibodies may interact several types of receptors, e.g., those expressed by several types of immune cells. In some embodiments, recruited antibodies can effectively interact several types of Fc receptors and provide desired immune activities. In some embodiments, recruited antibodies can recruit immune cells. In some embodiments, recruited antibodies can effectively interact with hFcγRIIIA. In some embodiments, recruited antibodies can effectively interact with hFcγRIIIA on macrophages. In some embodiments, macrophages are recruited to provide ADCC or ADCP activities toward a virus, e.g., a SARS-CoV-2 virus, or cells infected thereby. In some embodiments, NK cells are recruited to provide immune activities. In some embodiments, recruited antibodies can effectively interact with hFcγRIIA. In some embodiments, recruited antibodies can effectively interact with hFcγRIIA on dendritic cells. In some embodiments, antibody moieties in agents of this specification comprise one or more properties, structures or activities of recruited antibodies described in this specification.

[00134] The ricin domain of PLA2R (rather than the fibronectin type II domain or the C-type lectin domains) has the major epitope on a PLA2R molecule. Peptide screening by competitive ELISA determined two peptides (fragment 1 and fragment 2) of the ricin domain bind the majority of anti-PLA2R antibodies.

[00135] These two fragments were combined into one anti-PLA2R ligand. This 31-mer peptide from the ricin domain inhibits the binding of 80-85% of human anti-PLA2R to PLA2R. Anti-PLA2R antibodies can be effectively targeted by a single peptide. Other fragments (fragment 3, fragment 4, fragment 5, fragment 6, fragment 7) were less effective. One fragment (fragment 8) was ineffective.

Fragment 1. WQDKGIFVIQSESLKKC (SEQ ID NO 1)(major epitope).

Fragment 2. WSVLTENCK (SEQ ID NO 2) (major epitope).

Fragment 3. TREGREDDLLWCATTSR (SEQ ID NO 3).

Fragment 4. YLNHIQHEIVEKDAWK (SEQ ID NO 4).

Fragment 5. YYATHCEPGWNPYNR (SEQ ID NO 5).

Fragment 6. KEEKTWHEARLRSC (SEQ ID NO 6).

Fragment 7. AGHVLSOAESGCQEGWER (SEQ ID NO 7).

Fragment 8. PRYSGGCVAMRGRHP (SEQ ID NO 8).

Synthetic 31-mer. WQDKGIFVIQSESLKKCIQAGKSVLTLENCK (SEQ ID NO 9).

[00136] Most anti-PLA2R antibodies and ~90% of patient anti-PLA2R antibodies bind to the N-C3 domain of the PLA2R. Immunoreactive tryptic fragments of PLA2R were identified by mass spectrometry. Peptide screening by competitive ELISA determined two peptides of the ricin domain of PLA2R bind to most anti-PLA2R antibodies.

[00137] These two fragments were combined into one anti-PLA2R ligand (31-mer). See SEQ ID NO: 9. The synthetic 31-mer binds anti-PLA2R antibodies strongly, K_D = 500 pM.

TABLE 6
Biochemical selectivity. Binding Affinity (nM)

#	Anti-PLA2R
ABT301	0.3
AGN302	10±3
AGN304	3±0.3
AGN303	7±1
AGN306	23±1
AGN307	7

Linker moiety

[00138] In some embodiments, an amino acid has the structure of formula **LNK101**:

$NH(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–COOH$,

[LNK101] or a salt thereof, wherein:

each of R^{a1}, R^{a2}, R^{a3} is independently $–L^a–R'$;

each of L^{a1} and L^{a2} is independently L^a;

each L^a is independently a covalent bond, or an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, or –C(O)O–;

each –Cy– is independently an optionally substituted bivalent monocyclic, bicyclic, or polycyclic group wherein each monocyclic ring is independently selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20-membered heteroaryl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon, and a 3-20-membered heterocyclyl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon;

each R' is independently –R, –C(O)R, $–CO_2R$, or $–SO_2R$;

each R is independently –H, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

two R groups are optionally and independently taken together to form a covalent bond, or:

two or more R groups on the same atom are optionally and independently taken with the atom to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the atom, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; or

two or more R groups on two or more atoms are optionally and independently taken with their intervening atoms to form an optionally substituted, 3-30-membered, monocyclic, bicyclic, or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon.

[00139] In some embodiments, an amino acid residue has the structure of $–N(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–COO–$ or a salt form thereof.

[00140] In some embodiments, an amino acid analog is a compound in which the amino group or carboxylic acid group are independently replaced with an optionally substituted aliphatic or heteroaliphatic moiety. As persons having ordinary skill in the biomedical art know, many amino acid analogs, which mimics structures, properties or functions of amino acids, are described in the biomedical art and can be used under this specification, e.g., in several moieties. In some embodiments, one or more peptide groups are optionally and independently replaced with non-peptide groups. In some embodiments, an amino acid moiety in a polypeptide or peptide is replaced with an amino acid analog moiety.

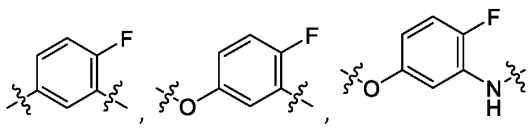
[00141] In some embodiments, the invention provides a derivative of an amino acid of formula **LNK101** or a salt thereof. In some embodiments, a derivative is an ester. In some embodiments, the invention composes matter of formula $NH(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–COOR^{CT}$ or salt thereof, wherein R^{CT} is R' and each other variable is independently as described in this specification. In some embodiments, R^{CT} is R. In embodiments, R^{CT} is optionally substituted aliphatic. In some embodiments, R^{CT} is t-butyl.

[00142] In some embodiments, L^{a1} is a covalent bond. In some embodiments, a composition of matter of formula **LNK101** is of the structure $NH(R^{a1})–C(R^{a2})(R^{a3})–L^{a2}–COOH$. In some embodiments, L^{a2} is $–CH_2SCH_2–$.

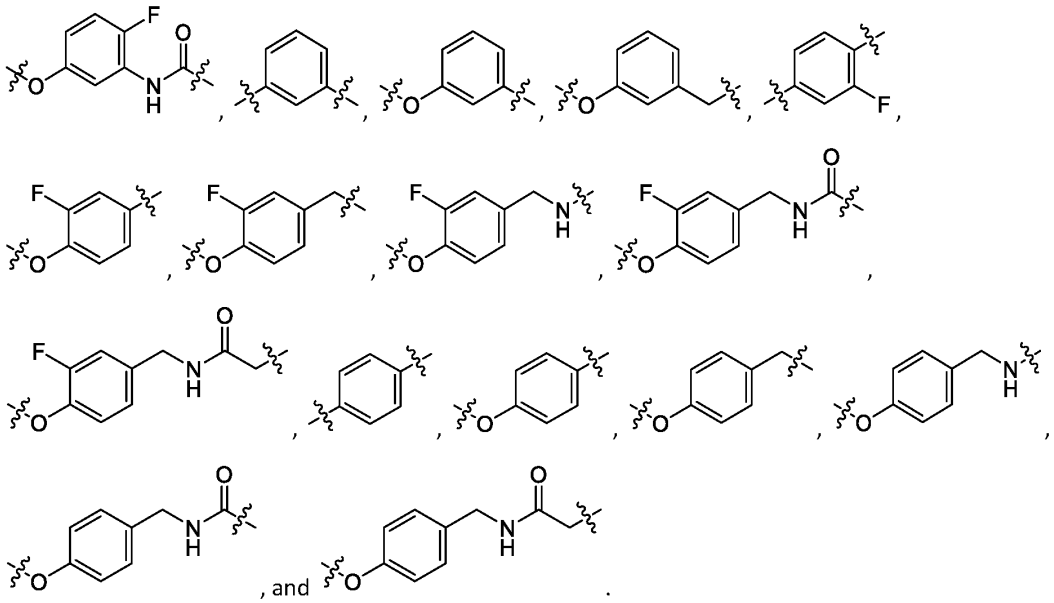
[00143] In some embodiments, L^{a2} is a covalent bond. In some embodiments, a composition of matter of formula **LNK101** is of the structure $NH(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–COOH$. In some embodiments, an amino acid residue has the structure of $–N(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–CO–$. In some embodiments, L^{a1} is $–CH_2CH_2S–$. In some embodiments, L^{a1} is $–CH_2CH_2S–$, wherein the CH_2 is bonded to $NH(R^{a1})$.

[00144] In some embodiments, L^{a1} is a covalent bond and L^{a2} is a covalent bond. In some embodiments, a composition of matter of formula **LNK101** is of the structure $NH(R^{a1})–C(R^{a2})(R^{a3})–COOH$. In some embodiments, a composition of matter of formula **LNK101** is of the structure $NH(R^{a1})–CH(R^{a2})–COOH$. In some embodiments, a composition of matter of formula **LNK101** has a structure selected from the Markush group of peptides consisting of $NH(R^{a1})–CH(R^{a3})–COOH$, $NH_2–CH(R^{a2})–COOH$, $NH_2–CH(R^{a3})–COOH$, $–N(R^{a1})–C(R^{a2})(R^{a3})–CO–$, $–N(R^{a1})–CH(R^{a2})–CO–$, $–N(R^{a1})–CH(R^{a3})–CO–$, $–NH–CH(R^{a2})–CO–$, and $–NH–CH(R^{a3})–CO–$.

[00145] In some embodiments, L^a is a covalent bond. In some embodiments, L^a is optionally substituted C_{1-6} bivalent aliphatic. In some embodiments, L^a is optionally substituted C_{1-6} alkylene. In some embodiments, L^a is $–CH_2–$. In some embodiments, L^a is $–CH_2CH_2–$. In some embodiments, L^a is $–CH_2CH_2CH_2–$.

[00146] In some embodiments, L^a is bivalent optionally substituted C_{1-20} aliphatic, wherein one or more methylene units are independently replaced with $–C(O)–$, $–N(R')–$, $–Cy–$, or $–O–$. In some embodiments, L^a is bivalent optionally substituted C_{1-20} aliphatic, wherein one or more methylene units are independently replaced with $–C(O)N(R')–$, $–Cy–$, and $–O–$. In some embodiments, L^a is bivalent optionally substituted C_{1-20} aliphatic, wherein two or more methylene units are independently replaced with $–C(O)N(R')–$, and $–Cy–$ in addition to other optional replacements. In some embodiments, $–Cy–$ is optionally substituted. In some embodiments, $–Cy–$ is optionally substituted with an electron-withdrawing group as described in this specification. In some embodiments, $–Cy–$ is substituted with one or more $–F$. In embodiments, $–Cy–$ is optionally substituted 1,3-phenylene. In some embodiments, $–Cy–$ is optionally substituted 1,4-phenylene. In some embodiments, L^a is or comprises a chemical group selected from the Markush group consisting of 

,



[00147] In some embodiments, R' is R. In embodiments, R^{a1} is R, wherein R is as described in this specification. In some embodiments, R^{a1} is R, wherein R methyl. In some embodiments, R^{a2} is R, wherein R is as described in this specification. In some embodiments, R^{a3} is R, wherein R is as described in this specification. In some embodiments, each of R^{a1}, R^{a2}, and R^{a3} is independently R, wherein R is as described in this specification.

[00148] In some embodiments, R^{a1} is hydrogen. In some embodiments, R^{a1} is a protective group. In some embodiments, R^{a1} is –Fmoc. In some embodiments, R^{a1} is –Dde.

[00149] In some embodiments, each of R^{a1}, R^{a2} and R^{a3} is independently $-L^a-R'$.

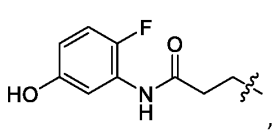
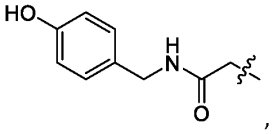
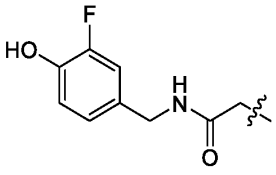
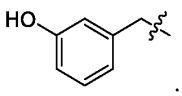
[00150] In some embodiments, R^{a2} is hydrogen. In some embodiments, R^{a3} is hydrogen. In some embodiments, R^{a1} is hydrogen, and at least one of R^{a2} and R^{a3} is hydrogen. In some embodiments, R^{a1} is hydrogen, one of R^{a2} and R^{a3} is hydrogen, and the other is not hydrogen. In some embodiments, R^{a2} is $-L^a-R$ and R^{a3} is –H. In embodiments, R^{a3} is $-L^a-R$ and R^{a2} is –H. In embodiments, R^{a2} is $-CH_2-R$ and R^{a3} is –H. In embodiments, R^{a3} is $-CH_2-R$ and R^{a2} is –H. In embodiments, R^{a2} is R and R^{a3} is –H. In embodiments, R^{a3} is R and R^{a2} is –H.

[00151] In some embodiments, R^{a2} is $-L^a-R$, wherein R is as described in this specification. In some embodiments, R^{a2} is $-L^a-R$, wherein R is an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{5-30} aryl, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon. In some

embodiments, R^{a2} is $-L^a-R$, wherein R is an optionally substituted group selected from C_{6-30} aryl and 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon. In some embodiments, R^{a2} is a side chain of an amino acid. In some embodiments, R^{a2} is a side chain of a standard amino acid.

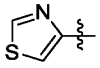
[00152] In some embodiments, R^{a3} is $-L^a-R$, wherein R is as described in this specification. In some embodiments, R^{a3} is $-L^a-R$, wherein R is an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{5-30} aryl, 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon, and 3-30-membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon. In some embodiments, R^{a3} is $-L^a-R$, wherein R is an optionally substituted group selected from C_{6-30} aryl and 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon. In some embodiments, R^{a3} is a side chain of an amino acid. In some embodiments, R^{a3} is a side chain of a standard amino acid.

[00153] In some embodiments, one or R^{a2} and R^{a3} is $-H$. In embodiments, one or R^{a2} and R^{a3} is $-L^a-R$, wherein L^a is as described in this specification. In some embodiments, L^a is not a covalent bond. In some embodiments, one or more methylene units of L^a are independently and optionally replaced as described in this specification, e.g., with $-C(O)-$, $-N(R')-$, $-O-$, $-C(O)-N(R')-$ or $-Cy-$, etc. In embodiments, L^a is or comprises $-C(O)-$, $-N(R')-$ and $-Cy-$. In some embodiments, L^a is or comprises $-C(O)N(R')-$ and $-Cy-$. In some embodiments, $-Cy-$ is substituted and one or more substituents are independently an electron-withdrawing group.

[00154] In some embodiments, an amino acid side chain is R^{a2} or R^{a3}. In some embodiments, an amino acid side chain is or comprises $-L^{LG1}-L^{LG2}-L^{LG3}-L^{LG4}-H$. In embodiments, an amino acid side chain is or comprises $-L^{LG2}-L^{LG3}-L^{LG4}-H$. In embodiments, an amino acid side chain is or comprises $-L^{LG3}-L^{LG4}-H$. In embodiments, an amino acid side chain is or comprises $-L^{LG4}-H$. In embodiments, this side chain selected from the Markush group consisting of , , , and .

[00155] In some embodiments, R is an optionally a group selected from the Markush group consisting of substituted C_{1-6} aliphatic, substituted C_{1-6} alkyl, $-CH_3$, pentyl, and n-pentyl.

[00156] In some embodiments, R is a cyclic group. In some embodiments, R is an optionally substituted C_{3-30} cycloaliphatic group. In some embodiments, R is cyclopropyl.

[00157] In some embodiments, R is an optionally substituted aromatic group, and an amino acid residue of an amino acid of formula **LNK101** is a Xaa^A. In some embodiments, R^{a2} or R^{a3} is $-CH_2-R$, wherein R is an optionally substituted aryl or heteroaryl group. In some embodiments, R is an amino acid selected from the Markush group of amino acids consisting of optionally substituted phenyl, phenyl, 4-trifluoromethylphenyl, and 4-phenylphenyl. In some embodiments, R is optionally substituted 5-30-membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, and silicon. In some embodiments, R is optionally substituted 5-14-membered heteroaryl having 1-5 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, R is . In some embodiments, R is optionally substituted pyridinyl. In some embodiments, R is 1- pyridinyl. In some embodiments, R is 2- pyridinyl. In some embodiments, R is 3-pyridinyl. In some embodiments, R is.

[00158] In some embodiments, R' is–COOH. In some embodiments, a compound of and an amino acid residue of an amino acid of formula **LNK101** is a Xaa^N.

[00159] In some embodiments, R' is$-NH_2$. In some embodiments, a compound of an amino acid residue of an amino acid of formula **LNK101** is a Xaa^P.

[00160] In some embodiments, R^{a2} or R^{a3} is R, wherein R is C_{1-20} aliphatic. In some embodiments, a compound of an amino acid residue of an amino acid of formula **LNK101** is a Xaa^H. In some embodiments, R is $-CH_3$. In some embodiments, R is ethyl. In some embodiments, R is propyl. In some embodiments, R is n-propyl. In some embodiments, R is butyl. In some embodiments, R is n-butyl. In some embodiments, R is pentyl. In some embodiments, R is n-pentyl. In some embodiments, R is cyclopropyl.

[00161] In some embodiments, two or more of R^{a1}, R^{a2}, and R^{a3} are R and are taken together to form an optionally substituted ring as described in this specification.

[00162] In some embodiments, R^{a1} and one of R^{a2} and R^{a3} are R and are taken together to form an optionally substituted 3-6-membered ring having no additional ring heteroatom other than the nitrogen atom to which R^{a1} is bonded to. In some embodiments, a formed ring is a 5-membered ring as in proline.

[00163] In some embodiments, R^{a2} and R^{a3} are R and are taken together to form an optionally substituted 3-6-membered ring. In some embodiments, R^{a2} and R^{a3} are R and are taken together to form an optionally substituted 3-6-membered ring having one or more nitrogen ring atom. In some embodiments, R^{a2} and R^{a3} are R and are taken together to form an optionally substituted 3-6-membered ring having one and no more than one ring heteroatom which is a nitrogen atom. In some embodiments, a ring is a saturated ring.

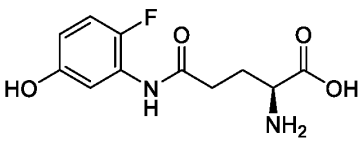
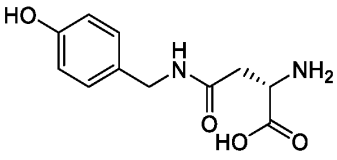
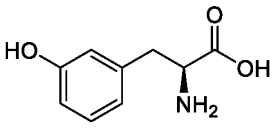
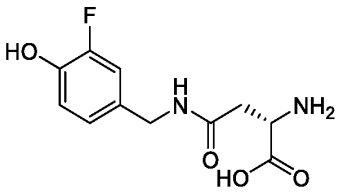
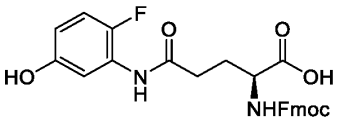
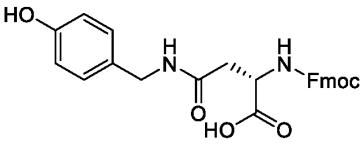
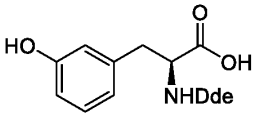
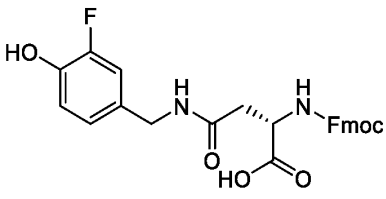
[00164] In some embodiments, an amino acid is an amino acid selected from the Markush group of amino acids consisting of a natural amino acid, an unnatural amino acid, an alpha-amino acid, and a beta-amino acid. In some embodiments, a composition of matter of formula **LNK101** is a natural amino acid or unnatural amino acid.

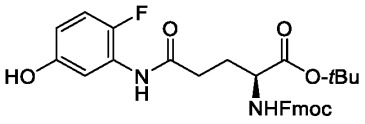
[00165] In some embodiments, an amino acid comprises a hydrophobic side chain. In some embodiments, an amino acid with a hydrophobic side chain is A, V, I, L, M, F, Y or W. In embodiments, an amino acid with a hydrophobic side chain is A, V, I, L, M, or F. In embodiments, an amino acid with a hydrophobic side chain is A, V, I, L, or M. In embodiments, an amino acid with a hydrophobic side chain is A, V, I, or L. In embodiments, a hydrophobic side chain is R wherein R is $C_{1\text{-}10}$ aliphatic. In some embodiments, R is $C_{1\text{-}10}$ alkyl. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, R is propyl. In some embodiments, R is butyl. In some embodiments, R is pentyl. In some embodiments, R is n-pentyl. In some embodiments, an amino acid with a hydrophobic side chain is $NH_2CH(CH_2CH_2CH_2CH_2CH_3)COOH$. In some embodiments, an amino acid with a hydrophobic side chain is (*S*)-$NH_2CH(CH_2CH_2CH_2CH_2CH_3)COOH$. In some embodiments, an amino acid with a hydrophobic side chain is (*R*)-$NH_2CH(CH_2CH_2CH_2CH_2CH_3)COOH$. In some embodiments, a hydrophobic side chain is $-CH_2R$ wherein R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is phenyl substituted with one or more hydrocarbon group. In some embodiments, R is 4-phenylphenyl. In some embodiments, an amino acid with a hydrophobic side chain is $NH_2CH(CH_2$–4-phenylphenyl)COOH. In some embodiments, an amino acid with a hydrophobic side chain is (*S*)-$NH_2CH(CH_2$–4-phenylphenyl)COOH. In some embodiments, an amino acid with a hydrophobic side chain is (*R*)-$NH_2CH(CH_2$–4-phenylphenyl)COOH.

[00166] In some embodiments, an amino acid comprises a positively charged side chain, e.g., at physiological pH. In some embodiments, this amino acid comprises a basic nitrogen in its side chain. In some embodiments, this amino acid is Arg, His or Lys. In some embodiments, this amino acid is Arg. In some embodiments, this amino acid is His. In some embodiments, this amino acid is Lys.

[00167] In some embodiments, an amino acid comprises a negatively charged side chain, e.g., at physiological pH. In some embodiments, this amino acid comprises a –COOH in its side chain. In some embodiments, this amino acid is Asp. In some embodiments, this amino acid is Glu.

[00168] In some embodiments, an amino acid comprises a side chain comprising an aromatic group. In some embodiments, this amino acid is Phe, Tyr, Trp, or His. In some embodiments, this amino acid is Phe. In some embodiments, this amino acid is Tyr. In some embodiments, this amino acid is Trp. In some embodiments, this amino acid is His. In some embodiments, this amino acid is NH_2–CH(CH_2–4-phenylphenyl)–COOH. In some embodiments, this amino acid is (*S*)-NH_2–CH(CH_2–4-phenylphenyl)–COOH. In some embodiments, this amino acid is (*R*)-NH_2–CH(CH_2–4-phenylphenyl)–COOH.

[00169] In some embodiments, an amino acid is an amino acid selected from the Markush group of amino acids consisting of  or a salt thereof,  or a salt thereof,  or a salt thereof,  or a salt thereof,  or a salt thereof,  or a salt thereof,  or a salt thereof, and  or a salt thereof. In some embodiments, a provided compound is



. In some embodiments, the invention provides polypeptide agents comprising one or more amino acid residues described in this specification.

Amino acids

[00170] In some embodiments, each residue is independently a residue of an amino acid or an amino acid analog, e.g., Xaa, wherein the amino acid or the amino acid analog has the structure of $H–L^{a1}–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–L^{a2}–H$ or a salt thereof. In some embodiments, an amino acid has the structure of $NH(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–COOH$ or a salt thereof. In some embodiments, an amino acid analog has the structure of $H–L^{a1}–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–L^{a2}–H$ or a salt thereof. In some embodiments, in this amino acid analog, the first $–L^{a1}–$ (bonded to –H in the formula) is not $– N(R^{a1})–$, e.g., is optionally substituted bivalent C_{1-6} aliphatic. In some embodiments, in $H–L^{a1}–L^{a1}–$, $–L^{a1}–L^{a1}–$ bonds to the –H through an atom that is not nitrogen. In some embodiments, in $–L^{a2}–L^{a2}–H$, $–L^{a2}–L^{a2}–$ is not bonded to the –H through –C(O)O–.

[00171] In some embodiments, each Xaa independently has the structure of $–L^{a1}–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–L^{a2}–$. In some embodiments, each Xaa independently has the structure of $–L^{aX1}–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–L^{aX2}–$, wherein L^{aX1} is optionally substituted –NH–, optionally substituted $–CH_2–$, $– N(R^{a1})–$, or –S–, L^{aX2} is optionally substituted –NH–, optionally substituted $–CH_2–$, $– N(R^{a1})–$, or –S–, and each other variable is independently as described in this specification. In some embodiments, L^{aX1} is optionally substituted –NH–, or $– N(R^{a1})–$. In some embodiments, L^{aX1} is optionally substituted $–CH_2–$, or –S–. In some embodiments, L^{aX2} is optionally substituted –NH–, optionally substituted $–CH_2–$, $– N(R^{a1})–$, or –S–. In some embodiments, optionally substituted $–CH_2–$ is –C(O)–. In some embodiments, optionally substituted $–CH_2–$ is not –C(O)–. In some embodiments, L^{aX2} is –C(O)–. In some embodiments, each Xaa independently has the structure of $–N(R^{a1})–L^{a1}–C(R^{a2})(R^{a3})–L^{a2}–CO–$.

[00172] In some embodiments, two or more residues, e.g., two or more Xaa residues, are linked such that one or more cyclic structures are formed. Residues can be linked, optionally through a linker, e.g., L^T) at any suitable positions. A linkage between two residues can connect each residue independently at its N-terminus, C-terminus, a point on the backbone, or a point on a side chain, etc. In embodiments, two or more side chains of residues, e.g., in compounds of formula **AGN105**, e.g., R^{a2} or R^{a3} of one amino acid residue with R^{a2} or R^{a3} of another amino acid residue, are optionally take together

to form a bridge, e.g., in some embodiments, two cysteine residues form a –S–S– bridge as typically observed in natural proteins.

[00173] In some embodiments, an amino acid residue has the structure of $-N(R^{a1})-L^{a1}-C(R^{a2})(R^{a3})-L^{a2}-COO-$ or a salt form thereof.

[00174] In some embodiments, an amino acid analog is a compound in which the amino group or carboxylic acid group are independently replaced with an optionally substituted aliphatic or heteroaliphatic moiety. As persons having ordinary skill in the biomedical art know, many amino acid analogs, which mimics structures, properties or functions of amino acids, are described in the biomedical art and can be used under this specification, e.g., in several moieties. In some embodiments, one or more peptide groups are optionally and independently replaced with non-peptide groups. In some embodiments, an amino acid moiety in a polypeptide or peptide is replaced with an amino acid analog moiety.

[00175] In some embodiments, moieties are optionally connected to each other through linker moieties. In some embodiments, a reactive group, e.g., RG, is connected to a cellular receptor-binding moiety, e.g., TBT, through a linker, e.g., L^{RM}. In some embodiments, a moiety, e.g., LG, may also comprise one or more linkers, e.g., L^{LG1}, L^{LG2}, L^{LG3}, L^{LG4}, etc., to link several parts. In some embodiments, L^{LG} is a linker moiety described in this specification. In some embodiments, L^{LG1} is a linker moiety described in this specification. In some embodiments, L^{LG2} is a linker moiety described in this specification. In some embodiments, L^{LG3} is a linker moiety described in this specification. In some embodiments, L^{LG4} is a linker moiety described in this specification. In some embodiments, L^{RM} is a linker moiety described in this specification. In some embodiments, L^{PM} is L. In embodiments, L^{PM} is a linker moiety described in this specification. In some embodiments, L^{PM} is L.

[00176] Linker moieties of several types or for several purposes, e.g., those used in antibody-drug conjugates, etc., may be used under this specification.

[00177] Linker moieties can be bivalent or polyvalent depending on how they are used. In some embodiments, a linker moiety is bivalent. In some embodiments, a linker is polyvalent and connecting over two moieties.

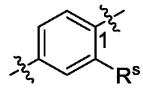
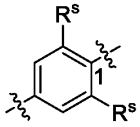
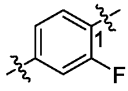
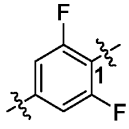
[00178] In some embodiments, L is bivalent. In some embodiments, L is a covalent bond.

[00179] In some embodiments, a linker moiety, or L, is or comprises $-(CH_2CH_2O)n-$, wherein each $-CH_2-$ is independently and optionally substituted and n is 1-20. In some embodiments, a linker moiety,

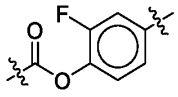
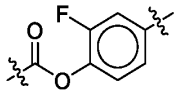
or L, is or comprises −(CH_2)n−O−(CH_2CH_2O)n−(CH_2)n−, wherein each n is independently 1-10, and each −CH_2− is independently and optionally substituted.

[00180] In some embodiments, a linker moiety is trivalent or polyvalent. In some embodiments, a linker moiety is L, where L is trivalent or polyvalent. In some embodiments, L is trivalent. In some embodiments, L is −CH_2−N(−CH_2−)−C(O)−.

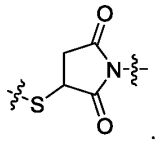
[00181] In some embodiments, a linker moiety, e.g., L, comprises one or more amino acid residues or analogs thereof.

[00182] In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., is or comprises a reactive group as described in this specification. In some embodiments, an agent (a TRAP) comprises an antibody-binding moiety and a cellular receptor-binding moiety linked through a linker which is or comprises a reactive group. In some embodiments, a reactive group can react with a lysine residue of an antibody in an aqueous buffer. In some embodiments, a reactive group is or comprises −C(O)−O−. In some embodiments, a reactive group is or comprises −C(O)−O−, wherein −O− is bonded to an optionally substituted aryl group. In some embodiments, a reactive group is or comprises −C(O)−O−, wherein −O− is bonded to an aryl group substituted with one or more electron-withdrawing groups. In some embodiments, one or more or each electron-withdrawing group is independently selected from $-NO_2$ and −F. In embodiments, an aryl group has the structure of , wherein R^s is halogen, $-NO_2$, -F, −L−R', −C(O)−L−R', −S(O)−L−R', $-S(O)_2$−L−R', or −P(O)(−L−R')$_2$. In some embodiments, an aryl group has the structure of , wherein each R^s is independently halogen, $-NO_2$, -F, −L−R', −C(O)−L−R', −S(O)−L−R', $-S(O)_2$−L−R', or −P(O)(−L−R')$_2$. In some embodiments, an aryl group is . In some embodiments, an aryl group is . In some embodiments, C1 is bound to the −O− of −C(O)−O−. In some embodiments, a cellular receptor-binding moiety is at the side of −C(O)− and an antibody-binding moiety is at the side of −O−.

[00183] In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises a reactive group, wherein upon contact with an antibody, the reactive group reacts with a group of the antibody and conjugates a

cellular receptor-binding moiety, or a moiety comprising –(Xaa)y–, to the antibody optionally through a linker. In some embodiments, a reactive group is or comprises , wherein the –C(O)– is connected to a cellular receptor-binding moiety, or a moiety comprising –(Xaa)y–, optionally through a linker. In some embodiments, a reactive group is or comprises , wherein the –C(O)– is connected to a cellular receptor-binding moiety, or a moiety comprising –(Xaa)y–, optionally through a linker and the other end of the reactive group is connected to an antibody-binding moiety.

[00184] In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., does not have a reactive group. In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., does not have a reactive group that readily reacts with proteins under aqueous conditions with pH about 6-9, e.g., physiological conditions. In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., does not have a reactive group that readily reacts with natural amino acid residues under aqueous conditions with pH about 6-9, e.g., physiological conditions.

[00185] In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises no –S–, wherein none of the two atoms to which the –S– is bonded to is S. In embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises no –S–S–. In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises no –S– bonded to a beta carbon of a carbonyl group or a double or triple bond conjugated to a carbonyl group. In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises no . In some embodiments, a linker moiety, e.g., L, L^{RM}, etc., comprises no –S–.

[00186] In some embodiments, an agent (a TRAP) comprises no cleavable groups whose cleavage can release LG except one or more optionally in RG. In some embodiments, an agent comprises no –S–S–, acetal or imine groups except in RG or TBT. In some embodiments, an agent comprises no –S–S–, acetal or imine groups except that the agent may have –S–S– formed by two amino acid residues. In some embodiments, an agent comprises no –S–S–, acetal or imine groups except that the agent may have –S–S– formed by cysteine residues. In some embodiments, an agent comprises no –S–S–, acetal or imine groups.

[00187] In some embodiments, L is a covalent bond. In some embodiments, L is a bivalent optionally substituted, linear or branched C_{1-100} aliphatic group wherein one or more methylene units of the group

are optionally and independently replaced. In some embodiments, L is a bivalent optionally substituted, linear or branched C_{6-100} arylaliphatic group wherein one or more methylene units of the group are optionally and independently replaced. In some embodiments, L is a bivalent optionally substituted, linear or branched C_{5-100} heteroarylaliphatic group having 1-20 heteroatoms wherein one or more methylene units of the group are optionally and independently replaced. In some embodiments, L is a bivalent optionally substituted, linear or branched C_{1-100} heteroaliphatic group having 1-20 heteroatoms wherein one or more methylene units of the group are optionally and independently replaced.

[00188] In some embodiments, a linker moiety, e.g., L) is or comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) polyethylene glycol units. In some embodiments, a linker moiety is or comprises $-(CH_2CH_2O)_n-$, wherein n is as described in this specification. In some embodiments, one or more methylene units of L are independently replaced with $-(CH_2CH_2O)_n-$.

[00189] In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6. In some embodiments, n is 7. In some embodiments, n is 8. In some embodiments, n is 9. In some embodiments, n is 10. In some embodiments, n is 11. In some embodiments, n is 12. In some embodiments, n is 13. In some embodiments, n is 14. In some embodiments, n is 15. In some embodiments, n is 16. In some embodiments, n is 17. In some embodiments, n is 18. In some embodiments, n is 19. In some embodiments, n is 20.

[00190] In some embodiments, a linker moiety, e.g., L) is or comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acid residues. As used in this specification, "one or more" can be 1-100, 1-50, 1-40, 1-30, 1-20, 1-10, 1-5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more. In some embodiments, one or more methylene units of L are independently replaced with an amino acid residue. In some embodiments, one or more methylene units of L are independently replaced with an amino acid residue, wherein the amino acid residue is of an amino acid of formula **LNK101** or a salt thereof. In some embodiments, one or more methylene units of L are independently replaced with an amino acid residue, wherein each amino acid residue independently has the structure of $-N(R^{a1})-L^{a1}-C(R^{a2})(R^{a3})-L^{a2}-CO-$ or a salt form thereof.

[00191] In some embodiments, a linker moiety comprises one or more moieties, e.g., amino, carbonyl, etc., that can be used for connection with other moieties. In some embodiments, a linker moiety comprises one or more $-NR'-$, wherein R' is as described in this specification. In some embodiments, $-NR'-$ improves solubility. In some embodiments, $-NR'-$ serves as connection points to

another moiety. In some embodiments, R' is −H. In embodiments, one or more methylene units of L are independently replaced with −NR'−, wherein R' is as described in this specification.

[00192] In some embodiments, a linker moiety, e.g., L, comprises a −C(O)− group, which can be used for connections with a moiety. In some embodiments, one or more methylene units of L are independently replaced with −C(O)−.

[00193] In some embodiments, a linker moiety, e.g., L, comprises a −NR'− group, which can be used for connections with a moiety. In some embodiments, one or more methylene units of L are independently replaced with −N(R')−.

[00194] In some embodiments, a linker moiety, e.g., L, comprises a −C(O)NR'− group, which can be used for connections with a moiety. In some embodiments, one or more methylene units of L are independently replaced with −C(O)N(R')−.

[00195] In some embodiments, a linker moiety, e.g., L, comprises a $-C(R')_2-$ group. In some embodiments, one or more methylene units of L are independently replaced with $-C(R')_2-$. In some embodiments, $-C(R')_2-$ is −CHR'−. In some embodiments, R' is $-(CH_2)_2C(O)NH(CH_2)_{11}COOH$. In some embodiments, R' is $-(CH_2)_2COOH$. In some embodiments, R' is −COOH.

[00196] In some embodiments, a linker moiety is or comprises one or more ring moieties, e.g., one or more methylene units of L are replaced with −Cy−. In some embodiments, a linker moiety, e.g., L, comprises an aryl ring. In some embodiments, a linker moiety, e.g., L, comprises an heteroaryl ring. In some embodiments, a linker moiety, e.g., L, comprises an aliphatic ring. In some embodiments, a linker moiety, e.g., L, comprises an heterocyclyl ring. In some embodiments, a linker moiety, e.g., L, comprises a polycyclic ring. In some embodiments, a ring in a linker moiety, e.g., L, is 3-20-membered. In some embodiments, a ring is 5-membered. In some embodiments, a ring is 6-membered. In some embodiments, a ring in a linker is product of a cycloaddition reaction, e.g., click chemistry, and variants thereof) used to link different moieties.

[00197] In some embodiments, L is L^1. In some embodiments, L is L^b.

[00198] In some embodiments, L^{RM} is a covalent bond. In some embodiments, L^{RM} is not a covalent bond. In some embodiments, L^{RM} is or comprises $-(CH_2CH_2O)n-$. In some embodiments, L^{RM} is or comprises $-(CH_2)n-O-(CH_2CH_2O)n-(CH_2)n-$, wherein each n is independently as described in this specification, and each $-CH_2-$ is independently optionally substituted. In some embodiments, L^{RM} is $-(CH_2)n-O-(CH_2CH_2O)n-(CH_2)n-$, wherein each n is independently as described in this specification, and each $-CH_2-$ is independently optionally substituted. In some embodiments, L^{RM} is $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification, and each $-CH_2-$ is

independently optionally substituted. In some embodiments, L^{RM} is $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification.

[00199] In some embodiments, L^{PM} is a covalent bond. In some embodiments, L^{PM} is not a covalent bond. In some embodiments, L^{PM} is or comprises $-(CH_2CH_2O)n-$. In some embodiments, L^{PM} is or comprises $-(CH_2)n-O-(CH_2CH_2O)n-(CH_2)n-$, wherein each n is independently as described in this specification, and each $-CH_2-$ is independently optionally substituted. In some embodiments, L^{PM} is $-(CH_2)n-O-(CH_2CH_2O)n-(CH_2)n-$, wherein each n is independently as described in this specification, and each $-CH_2-$ is independently optionally substituted. In some embodiments, L^{PM} is $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification, and each $-CH_2-$ is independently optionally substituted. In some embodiments, L^{PM} is $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification.

[00200] In some embodiments, L^{PM}, e.g., in a product of a first and a second agents) is or comprises a reaction product moiety formed a first reactive moiety and a second reactive moiety.

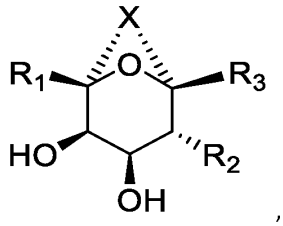
Cellular receptor-binding moiety

[00201] Several receptor-binding moieties, according to embodiments of present invention, are described in WO2019/199621A1 published October 17, 2019, WO2019/199634 published October 17, 2019, International Application No. PCT/US2020/055053 filed October 9, 2020, and International Application No. PCT/US2020/055053 filed October 9, 2020, each of which is incorporated in this specification in its entirety by reference.

[00202] In an embodiment, the cellular receptor-binding moiety may include an asialoglycoprotein receptor (ASGPR) binding group connected through an amine group to the linker moiety.

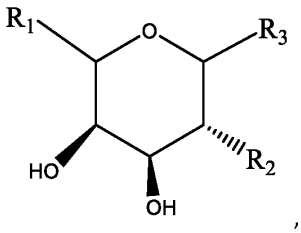
[00203] The amine group may be a primary alkyl amine group or secondary alkyl amine group, each of which is optionally substituted on the amine group with a C_1-C_3 alkyl group.

[00204] The cellular receptor-binding moiety may include an ASGPR-binding group according to the chemical structure:



,

[TBT101] or



,

[TBT102]

wherein X is 1-4 atoms in length and comprises O, S, N(R^{N1}) or C(R^{N1})(R^{N1}) groups such that:

when X is 1 atom in length, X is O, S, N(R^{N1}) or C(R^{N1})(R^{N1}),

when X is 2 atoms in length, no more than 1 atom of X is O, S or N(R^{N1}),

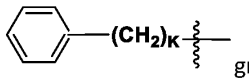
when X is 3 or 4 atoms in length, no more than 2 atoms of X are O, S or N(R^{N1});

wherein R^{N1} is H or a C_1-C_3 alkyl group optionally substituted with from 1-3 halo groups;

R_1 and R_3 are each independently:

H, -$(CH_2)_K$OH, -$(CH_2)_K$OC_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, -$(CH_2)_K$-vinyl, O-$(CH_2)_K$-vinyl, -$(CH_2)_K$-alkynyl, -$(CH_2)_K$-COOH,

-$(CH_2)_K$C(O)O-C_1-C_4 alkyl optionally substituted with from 1-3 halo groups, O-C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, -C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, or

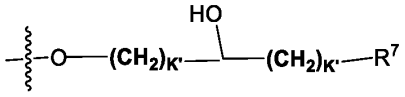
R_1 and R_3 are each independently a 

group, which is optionally substituted with up to three halo groups, C_1-C_4 alkyl groups, each of which alkyl group is optionally substituted with from one to three halo groups or one or two hydroxyl groups, or O-C_1-C_4 alkyl groups,

each of which alkyl groups is optionally substituted with from one to three halo groups or one or two hydroxyl groups; and

K is independently an integer of 0 to 4, or

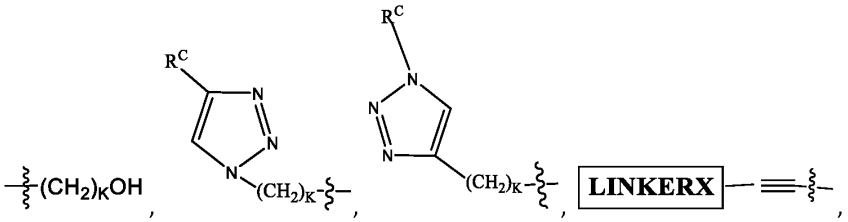
R_1 and R_3 are each independently a group according to the chemical structure:



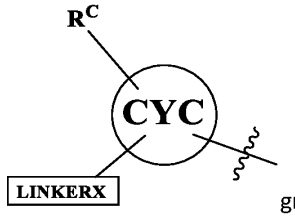
, wherein R^7 is O-C_1-C_4 alkyl, which is optionally substituted with from 1 to 3 halo groups 1 or 2 hydroxy groups, or R^7 is a

$-NR^{N3}R^{N4}$ group or a; or

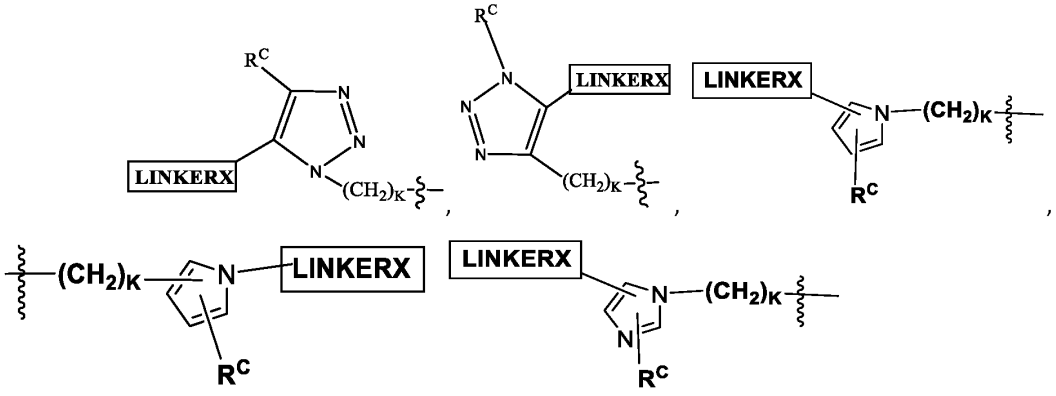
R_1 and R_3 are each independently a group according to the structure:

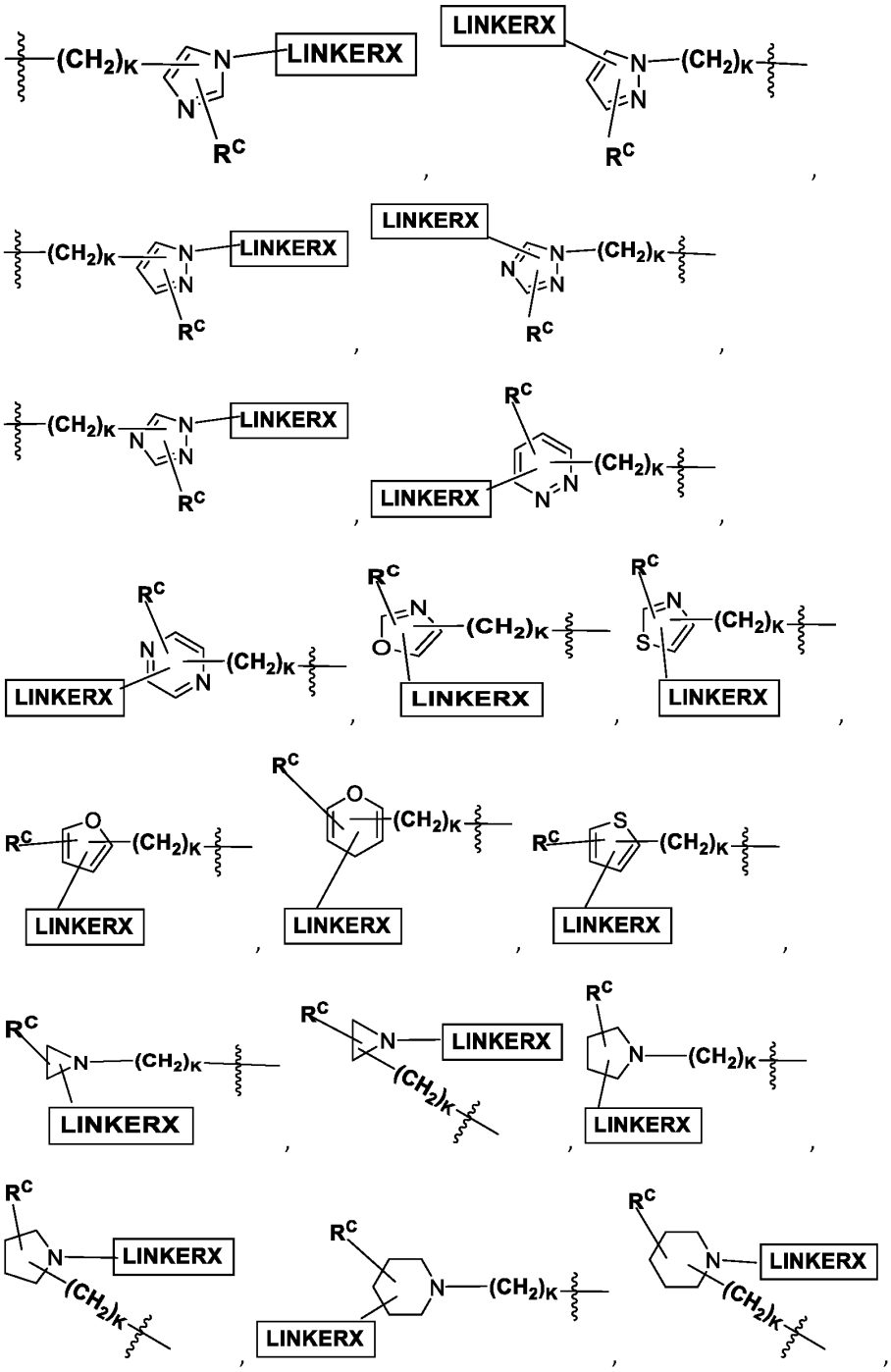


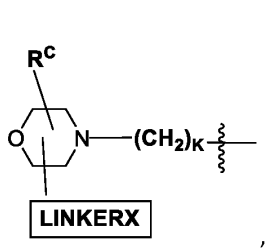
, or a

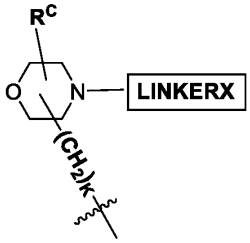


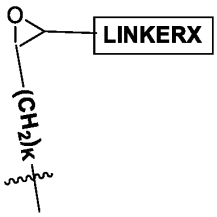
group according to the chemical structure:

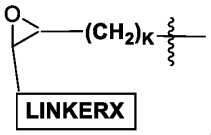


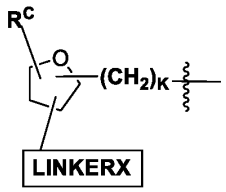

LINKERX
(CH2)K
RC
N
O
S

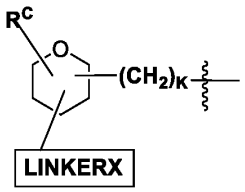


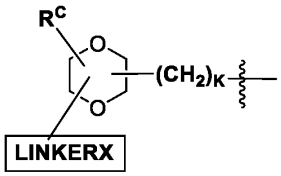
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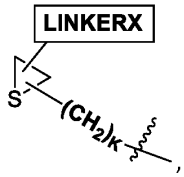
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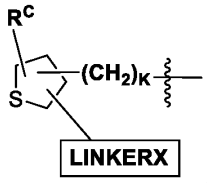
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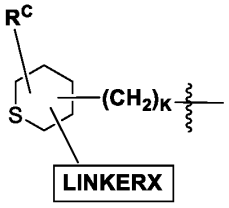
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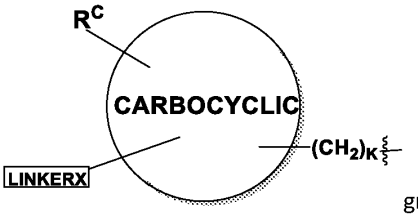
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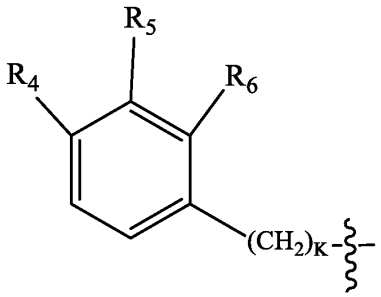
, or


R_1 and R_3 are each independently a group, where

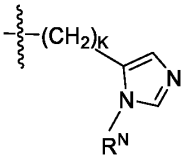
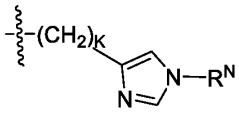
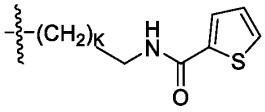
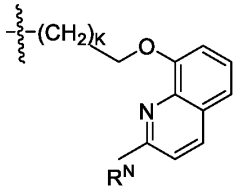


, is a C_3-C_8 saturated carbocyclic group;

R^C is absent, H, C_1-C_4 alkyl optionally substituted with from 1-3 halo groups or 1-2 hydroxyl groups, or a group according to the structure:



wherein R_4, R_5 and R_6 are each independently, H, halo (F, Cl, Br, I), CN, $NR^{N1}R^{N2}$, $-(CH_2)_KOH$, $-(CH_2)_KOC_1$-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_3 alkyl, which is optionally substituted with from 1-3 halo groups, $-O-C_1$-C_3-alkyl, which is optionally substituted with from 1-3 halo groups, $-(CH_2)_KCOOH$, $-(CH_2)_KC(O)O-C_1$-C_4 alkyl optionally substituted with from 1-3 halo groups, $O-C(O)-C_1$-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, $-C(O)-C_1$-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, or

R^C is a  group, a  group a  group or a  group,

wherein R^N, R^{N1} and R^{N2} are each independently H or a C_1-C_3 alkyl group optionally substituted with from one to three halo groups or one or two hydroxyl groups;

K is independently an integer of 0 to 4;

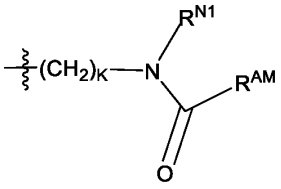
K' is an integer of 1 to 4;

R^{N3} is H, or a C_1-C_3 alkyl group optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups; and

R^{N4} is H, a C_1-C_3 alkyl group optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups, or R^{N4} is a group, where K is preferably 1;

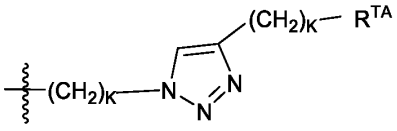
LINKERX is a linker group which comprises at least one anti-PLA2R antibody-binding moiety and links the at least one anti-PLA2R antibody-binding moiety to the cellular receptor-binding moiety through the optional linker moiety, or

LINKERX is a linker group which has at least one or more functional groups which can be used to covalently bond the linker group to at least one anti-PLA2R antibody-binding moiety or optional linker moiety;

R_2 is a  group wherein R^{N1} and K are the same as above;

R^{AM} is H, a C_1-C_4 alkyl group optionally substituted with up to 3 halo groups and one or two hydroxyl groups, a -$(CH_2)_K$COOH group, a -$(CH_2)_K$C(O)O-C_1-C_4 alkyl group optionally substituted with from 1-3 halo groups, a O-C(O)-C_1-C_4 alkyl group, which is optionally substituted with from 1-3 halo F groups, a -C(O)-C_1-C_4 alkyl group, which is optionally substituted with from 1-3 halo groups, a -$(CH_2)_K$-$NR^{N3}R^{N4}$ group where R^{N3} is H, or a C_1-C_3 alkyl group optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups; and

R^{N4} is H, a C_1-C_3 alkyl group optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups, or a group, or

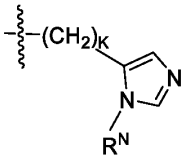
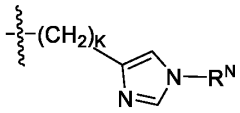
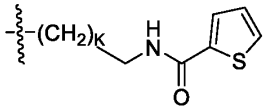
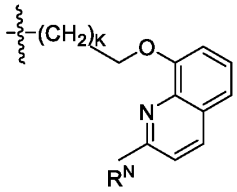
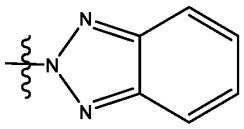
R_2 is a  group,

wherein R^{TA} is H, CN, $NR^{N1}R^{N2}$, -$(CH_2)_K$OH, -$(CH_2)_K$OC_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups,

-$(CH_2)_K$COOH, -$(CH_2)_K$C(O)O-C_1-C_4 alkyl optionally substituted with from 1-3 halo groups, O-C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, -C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, or

R^{TA} is a C_3-C_{10} aryl or a three- to ten-membered heteroaryl group containing up to 5 heteroaryl atoms, each of said aryl or heteroaryl groups being optionally substituted with up to three (preferably 1) CN, $NR^{N1}R^{N2}$, -$(CH_2)_K$OH, -$(CH_2)_K$OC_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_3 alkyl, which is optionally substituted with from 1-3 halo groups or 1 or 2 hydroxy

groups, -O-C_1-C_3-alkyl, which is optionally substituted with from 1-3 halo groups, -$(CH_2)_K$COOH, -$(CH_2)_K$C(O)O-C_1-C_4 alkyl optionally substituted with from 1-3 halo groups, O-C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups or -$(CH_2)_K$C(O)-C_1-C_4 alkyl optionally substituted with from 1-3 halo groups, or

R^{TA} is a  group, a  group, a  group a  group, a  group

optionally substituted with up to three C_1-C_3 alkyl groups optionally substituted with up to three halo groups, or

R^{TA} is a group,

wherein R^N, R^{N1} and R^{N2} are each independently H or a C_1-C_3 alkyl group optionally substituted with from one to three halo groups or one or two hydroxyl groups and each -$(CH_2)_K$ group is optionally substituted with 1-4, preferably 1 or 2, C_1-C_3 alkyl groups optionally substituted with from 1-3 fluoro groups or 1-2 hydroxyl groups;

and K is independently 0-4.

[00205] In an embodiment,

X is -O-C(R^{N1})(R^{N1}),

C(R^{N1})(R^{N1})-O-, -S-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-S-, N(R^{N1})-C(R^{N1})(R^{N1}),

C(R^{N1})(R^{N1})-N(R^{N1}) or C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}) when X is 2 atoms in length,

X is -O-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-O-C(R^{N1})(R^{N1})-,

-O-C(R^{N1}) (R^{N1})-O-, -O-C(R^{N1}) (R^{N1})-S-, -O-C(R^{N1}) (R^{N1})-N(R^{N1})-,

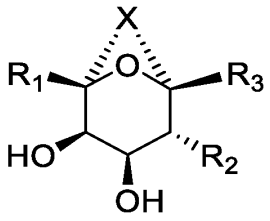
-S-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-S-C(R^{N1})(R^{N1})-, C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})-S, -S-C(R^{N1})(R^{N1})-S-, -S-C(R^{N1})(R^{N1})-O-, -S-C(R^{N1})(R^{N1})-N(R^{N1})-, N(R^{N1})-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-N(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- N(R^{N1}), N(R^{N1})-C(R^{N1})(R^{N1})-N(R^{N1}) or C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- C(R^{N1})(R^{N1}) when X is 3 atoms in length, and

X is-O-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-O-C(R^{N1})(R^{N1})-(R^{N1})(R^{N1})-, -O-C(R^{N1})(R^{N1})-O-C(R^{N1})(R^{N1})-, -S-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- C(R^{N1})(R^{N1})-, C(R^{N1})(R^{N1})-S-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})-, C(R^{N1})(R^{N1})-(R^{N1})(R^{N1})-S-C(R^{N1})(R^{N1})-, -S-C(R^{N1})(R^{N1})-S-C(R^{N1})(R^{N1})-, N(R^{N1})-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- C(R^{N1})(R^{N1})-, C(R^{N1})(R^{N1})-N(R^{N1})-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- N(R^{N1}), N(R^{N1})-C(R^{N1})(R^{N1})-N(R^{N1}) or C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})- C(R^{N1})(R^{N1}) when X is 4 atoms in length,

wherein R^{N1} is the same as stated in claim 4 or 6 above.

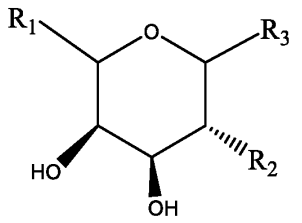
[00206] In an embodiment, X is OCH_2 or CH_2O and R^{N1} is H.

[00207] The cellular receptor-binding moiety may include an ASGPR-binding group according to the chemical structure:



,

[TBT101] or

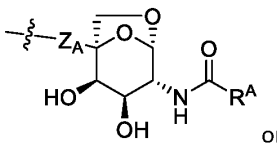


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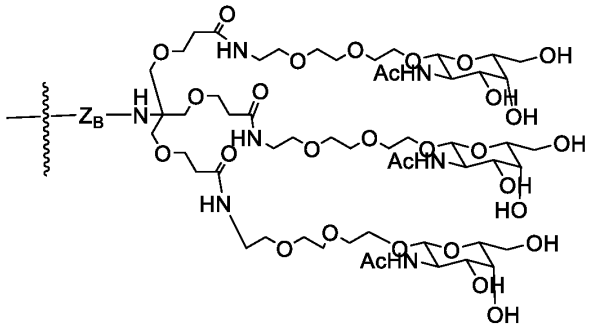
[TBT102]

where R_1, R_2 and R_3 are the same as in Claim 9, or a pharmaceutically acceptable salt, stereoisomer, solvate or polymorph thereof.

[00208] The cellular receptor-binding moiety may have the following structure:



or



;

where R^A is a C_1-C_3 alkyl group optionally substituted with 1-5 halo (preferably fluoro) groups (preferably R^A is a methyl or ethyl group optionally substituted with from 1-3 fluoro groups);

Z_A is $-(CH_2)_{IM}$, $-O-(CH_2)_{IM}$, $S-(CH_2)_{IM}$, $NR_M-(CH_2)_{IM}$, $C(O)-(CH_2)_{IM}-$, a PEG group containing from 1 to 8 preferably 1-4 ethylene glycol residues or a $-C(O)(CH_2)_{IM}NR_M$ group (preferably a PEG containing group comprising from 1 to 8 ethylene glycol, preferably 2-4 ethylene glycol residues) where IM and R_M are the same as above; and

Z_B is absent, $(CH_2)_{IM}$, $C(O)-(CH_2)_{IM}-$ or $C(O)-(CH_2)_{IM}-NR_M$, where IM and R_M are the same as above.

[00209] In an embodiment, R^A may be a methyl or ethyl group optionally substituted with from 1-3 fluoro groups. In an embodiment, Z_A may be a PEG group containing from 1 to 4 ethylene glycol residues. In an embodiment, the methyl or ethyl group may be substituted with from 1-3 fluoro groups. In an embodiment, the ASGPR-binding group may be N-acetyl-D-galactosamine. In an embodiment, the cellular receptor-binding moiety may be a low-density lipoprotein receptor-related protein 1 (LRP1), a low-density lipoprotein receptor (LDLR), a FcγRI-binding group, a FcRN-binding group, a transferrin receptor-binding group, or a macrophage scavenger receptor-binding group.

Methods of making several agents.

[00210] Agents of this specification may be prepared or isolated by synthetic or semi-synthetic methods or recombinant methods under this specification. In some embodiments, polypeptide agents, e.g., cellular receptor-binding moiety peptide agents, maybe be prepared using biological expression systems. In some embodiments, provided agents are prepared synthetically. In some embodiments, provided agents are prepared using certain technologies described in WO2019/023501, which is incorporated in this specification in its entirety by reference.

[00211] Several technologies, e.g., those for preparing antibody-drug conjugates, may be used in preparation of MATE agents. In many such technologies, conjugation is not selective regarding amino acid residue sites, and product compositions usually have several types of agents which may differ from each other regarding number of target-binding moieties conjugated or conjugation sites. In some embodiments, the invention provides technologies that can be used for selective conjugation of target-binding moieties at amino acid residue sites.

[00212] In some embodiments, the invention provides a method of synthesis, comprising the steps of:

contacting a first agent comprising a cellular receptor-binding moiety linked to a first reactive group optionally through a first linker with a second agent comprising an antibody moiety linked to a second reactive group optionally through a second linker, wherein the first reactive group reacts with a second reactive group, and

forming a product agent comprising a cellular receptor-binding moiety and an antibody-binding moiety optionally through a linker.

[00213] In some embodiments, the invention provides a method of synthesis, comprising the steps of:

contacting a first composition comprising a plurality of first agents each independently comprising a cellular receptor-binding moiety linked to a first reactive group optionally through a first linker moiety with a second composition comprising a plurality of second agents each independently comprising an antibody moiety optionally linked to a second reactive group optionally through a second linker moiety,

wherein a product composition comprising a plurality of product agents each independently comprising a cellular receptor-binding moiety and an antibody-binding moiety optionally through a linker is formed.

MATES

[00214] Persons having ordinary skill in the biomedical art can use MATES materials and methods as guidance to predictable results when making and using the invention.

[00215] In some embodiments, the invention provides an agent comprising:

an antibody moiety,

a cellular receptor-binding moiety, and

a linker moiety (optionally a single peptide linkage) linking an antibody moiety and a cellular receptor-binding moiety.

[00216] This agent may be called a MATE agent or MATE. The MATE agents are described, for example, in International Application No. PCT/US2020/061127 filed November 18, 2020, the content of which is incorporated in this specification in its entirety by reference. In some embodiments, an agent comprises an antibody moiety, a cellular receptor-binding moiety, and a linker moiety linking an antibody moiety and a cellular receptor-binding moiety.

[00217] In another embodiment, the anti-PLA2R antibody-binding moiety is an antibody, an antibody variant, or an antigen-binding fragment thereof.

[00218] In another embodiment, the anti-PLA2R antibody-binding moiety is a full IgG antibody or nanobody wherein the heavy chain comprises the three CDR regions of the anti-PLA2R antibody-binding moiety. A full-length human sourced IgG can be native and have variable glycosylation.

Plurality of agents. Second agent.

[00219] In another embodiment, the invention provides a composition including the agent and at least one additional agent comprising a moiety capable of-binding to the antibody that forms the antibody moiety of the first compound.

[00220] In some embodiments, a first composition is a composition comprising a first agent as described in this specification. In some embodiments, second agents independently comprise second reactive groups. In some embodiments, a second composition is a composition comprising a plurality of agents as described in this specification, wherein each cellular receptor-binding moiety is independently a reactive group as described in this specification. In some embodiments, a second composition is an antibody composition, wherein antibodies in the composition are not chemically changed. In some embodiments, a second composition is an IVIG preparation. In some embodiments, a product composition is a composition comprising a plurality of agents as described in this specification, wherein each cellular receptor-binding moiety is independently a cellular receptor-binding moiety as described in this specification.

[00221] In some embodiments, a cellular receptor-binding moiety in a product agent is a cellular receptor-binding moiety in a first agent. In some embodiments, an antibody moiety in a product agent is an antibody moiety in a second agent. In some embodiments, a second agent is an antibody agent, e.g., a monoclonal antibody, an antibody in a polyclonal antibody, an antibody in an IVIG preparation, etc. In embodiments, a second reactive group is a function group of an amino acid residue, e.g., −NH2 of Lys, −SH of Cys, etc. In embodiments, a second reactive group is −NH2 of a Lys residue, e.g., of a residue selected from K246 and K248 of IgG1 heavy chain amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of a heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00222] In some embodiments, a second reactive group is installed to an antibody moiety optionally through a linker. In some embodiments, a second reactive group is installed to an antibody moiety

through a linker. In some embodiments, a second reactive group is selectively linked to certain location(s) of an antibody moiety, e.g., certain location(s) selected from K246 and K248 of IgG1 heavy chain amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of an anti-PLA2R antibody heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00223] In some embodiments, the invention provides agents each independently comprising an antibody-binding moiety that binds to an antibody agent, a reactive group, a cellular receptor-binding moiety, and optionally one or more linker moieties linking such groups/moieties. In some embodiments, such agents are useful as reaction partners, e.g., first agents) for conjugating moieties of interest, e.g., target-binding moieties, reactive groups, e.g., second reactive groups) to agents comprising antibody moieties, e.g., second agents). In some embodiments, the invention provides agents for conjugating moieties of interest to antibody moieties in several agents or antibody agents, e.g., monoclonal antibody agents, polyclonal antibody agents, antibody agents of IVIG preparations, etc. In embodiments, provided agents each comprise a cellular receptor-binding moiety, a reactive group, an antibody-binding moiety, and optionally one or more linker moieties (linkers) linking such moieties. In some embodiments, an antibody-binding moiety is part of a leaving group released after contacting this agent, e.g., a first agent, with an antibody moiety, e.g., of a second agent, and reacting a reactive group of this agent, e.g., a first reactive group of a first agent, with a reactive group of an antibody moiety, e.g., a second reactive group of a second agent, such as –NH2 of a Lys residue of an antibody protein. In some embodiments, provided technologies can provide improved conjugation efficiency, high selectivity, or fewer steps (sometimes, single step) to conjugation product agents. In some embodiments, a provided agent, e.g., a first agent, is a composition of matter of formula **AGN301** or a salt thereof:

LG–RG–LRM–TBT,

[AGN301] or a salt thereof, wherein:

LG is a group comprising an antibody-binding moiety;

RG is a reactive group;

LRM is a linker; and

TBT is a cellular receptor-binding moiety.

[00224] In some embodiments, LG is or comprises an antibody-binding moiety as described in this specification, and a linker which links an antibody-binding moiety and RG.

[00225] In some embodiments, LG is or comprises R^{LG}–L^{LG}–, wherein R^{LG} is or comprises an antibody-binding moiety, and L^{LG} is a linker moiety as described in this specification. In some embodiments, LG is ABT-L^{LG}–. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–, wherein each of L^{LG1} and L^{LG2} is independently a linker moiety as described in this specification. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–, wherein each of L^{LG1}, L^{LG2} and L^{LG3} is independently as linker moiety described in this specification. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–, wherein each of L^{LG1}, L^{LG2}, L^{LG3} and L^{LG4} is independently a linker moiety as described in this specification. In some embodiments, L^{LG1} is bonded to R^{LG}. In some embodiments, L^{LG1} is bonded to cellular receptor-binding moiety. In some embodiments, L^{LG} is –L^{LG1}–, and a reactive group comprises L^{LG2}, L^{LG3} and L^{LG4}. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–, and a reactive group comprises L^{LG3} and L^{LG4}. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–, and a reactive group comprises L^{LG4}. In some embodiments, each of L^{LG1}, L^{LG2}, L^{LG3} and L^{LG4} is independently L.

[00226] In some embodiments, antibody-binding moieties, LG, etc. are released after reactions, e.g., after first agents, e.g., wherein MOIs are target-binding moieties) react with second agents, e.g., which are antibody agents comprising reactive amino acid residues such as amino groups as second reactive groups or second agents comprising second reactive groups introduced to antibody agents), or after first agents, e.g., wherein MOIs are reactive groups such as second reactive groups) react with second agents which are antibody agents. In some embodiments, an antibody-binding moiety is released after a reaction. In some embodiments, LG is released after a reaction. In some embodiments, a leaving group is released as part of a compound having the structure of LG−H or a salt thereof. In some embodiments, an antibody-binding moiety is released as part of a compound having the structure of LG−H or a salt thereof. In some embodiments, LG is released as part of a compound having the structure of LG−H or a salt thereof. In some embodiments, a released compound has the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof. In some embodiments, an antibody-binding moiety is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof. In some embodiments, an antibody-binding moiety is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof, wherein R^{LG} is or comprises an antibody-binding moiety. In some embodiments, LG is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof, wherein LG is R^{LG}–L^{LG}, and L^{LG} is –L^{LG1}–, –L^{LG1}–L^{LG2}–, –L^{LG1}–L^{LG2}–L^{LG3}–, or –L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–. In some embodiments, LG is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof, wherein LG is R^{LG}–L^{LG1}–. In some embodiments, LG is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof, wherein LG is R^{LG}–L^{LG1}–L^{LG2}. In some embodiments, LG is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or

a salt thereof, wherein LG is R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}. In some embodiments, LG is released as part of a compound having the structure of R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–H or a salt thereof, wherein LG is R^{LG}–L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}.

[00227] In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_{1-100} group comprising one or more aliphatic moieties, aryl moieties, heteroaliphatic moieties each independently having 1-20 heteroatoms, heteroaromatic moieties each independently having 1-20 heteroatoms, or any combinations of any one or more of such moieties, wherein one or more methylene units of the group are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, a bivalent C_{1-6} heteroaliphatic group having 1-5 heteroatoms, —C≡C—, –Cy–, $–C(R')_2–$, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, $–C(O)C(R')_2N(R')–$, $–N(R')C(O)N(R')–$, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, –C(O)O–, –P(O)(OR')–, –P(O)(SR')–, –P(O)(R')–, –P(O)(NR')–, –P(S)(OR')–, –P(S)(SR')–, –P(S)(R')–, –P(S)(NR')–, –P(R')–, –P(OR')–, –P(SR')–, –P(NR')–, an amino acid residue, or $–[(–O–C(R')_2–C(R')_2–)_n]–$, wherein n is 1-20. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_{1-100} aliphatic or heteroaliphatic group 1-20 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with —C≡C—, –Cy–, $–C(R')_2–$, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, $–C(O)C(R')_2N(R')–$, $–N(R')C(O)N(R')–$, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, –C(O)O–, –P(O)(OR')–, –P(O)(SR')–, –P(O)(R')–, –P(O)(NR')–, –P(S)(OR')–, –P(S)(SR')–, –P(S)(R')–, –P(S)(NR')–, –P(R')–, –P(OR')–, –P(SR')–, –P(NR')–, or $–[(–O–C(R')_2–C(R')_2–)_n]–$, wherein n is 1-20. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_1, C_2, C_3, C_4, C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, C_{40}, C_{50}, C_{60}, C_{1-2}, C_{1-5}, C_{1-10}, C_{1-15}, C_{1-20}, C_{1-30}, C_{1-40}, C_{1-50}, C_{1-60}, C_{1-70}, C_{1-80}, or C_{1-90} aliphatic or heteroaliphatic group 1-10 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with —C≡C—, –Cy–, $–C(R')_2–$, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, $–C(O)C(R')_2N(R')–$, $–N(R')C(O)N(R')–$, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, –C(O)O–, –P(O)(OR')–, –P(O)(SR')–, –P(O)(R')–, –P(O)(NR')–, –P(S)(OR')–, –P(S)(SR')–, –P(S)(R')–, –P(S)(NR')–, –P(R')–, –P(OR')–, –P(SR')–, –P(NR')–, amino acid residues, or $–[(–O–C(R')_2–C(R')_2–)_n]–$, wherein n is 1-20. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_1, C_2, C_3, C_4, C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, C_{40}, C_{50}, C_{60}, C_{1-2}, C_{1-5}, C_{1-10}, C_{1-15}, C_{1-20}, C_{1-30}, C_{1-40}, C_{1-50}, C_{1-60}, C_{1-70}, C_{1-80}, or C_{1-90} aliphatic or heteroaliphatic group 1-10 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with –C≡C–, –Cy–, $–C(R')_2–$, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, $–C(O)C(R')_2N(R')–$, $–N(R')C(O)N(R')–$, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, –C(O)O–,

amino acid residues, or −[(−O−C(R')$_2$−C(R')$_2$−)$_n$]−, wherein n is 1-10. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_1, C_2, C_3, C_4, C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, C_{40}, C_{50}, C_{60}, C_{1-2}, C_{1-5}, C_{1-10}, C_{1-15}, C_{1-20}, C_{1-30}, C_{1-40}, C_{1-50}, C_{1-60}, C_{1-70}, C_{1-80}, or C_{1-90} aliphatic group, wherein one or more methylene units of the group are optionally and independently replaced with −O−, −N(R')−, −C(O)−, −C(O)N(R')−, −C(O)C(R')$_2$N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, amino acid residues, or −[(−O−C(R')$_2$−C(R')$_2$−)$_n$]−, wherein n is 1-10. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_1, C_2, C_3, C_4, C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, C_{40}, C_{50}, C_{60}, C_{1-2}, C_{1-5}, C_{1-10}, C_{1-15}, C_{1-20}, C_{1-30}, C_{1-40}, C_{1-50}, C_{1-60}, C_{1-70}, C_{1-80}, or C_{1-90} aliphatic group, wherein one or more methylene units of the group are optionally and independently replaced with −O−, −N(R')−, −C(O)−, −C(O)N(R')−, −C(O)C(R')$_2$N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, or −[(−O−C(R')$_2$−C(R')$_2$−)$_n$]−, wherein n is 1-10. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_{1-10} aliphatic group, wherein one or more methylene units of the group are optionally and independently replaced with −O−, −N(R')−, −C(O)−, −C(O)N(R')−, −C(O)C(R')$_2$N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, −Cy−, or −[(−O−C(R')$_2$−C(R')$_2$−)$_n$]−, wherein n is 1-10. In some embodiments, L is a covalent bond, or a bivalent optionally substituted, linear or branched C_{1-10} aliphatic group, wherein one or more methylene units of the group are optionally and independently replaced with −O−, −N(R')−, −C(O)−, −C(O)N(R')−, −C(O)C(R')$_2$N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, or −[(−O−C(R')$_2$−C(R')$_2$−)$_n$]−, wherein n is 1-10. In some embodiments, L comprises no −C(O)O−. In some embodiments, L comprises no −C(O)−N(R')−. In some embodiments, L comprises no −S−. In some embodiments, L comprises no −S−Cy−. In some embodiments, L comprises no −S−S−. In some embodiments, L does not have one or more or any of −C(O)O−, −C(O)−N(R')−, −S−, and −S−S−. In some embodiments, L does not have one or more or any of −C(O)O−, −C(O)−N(R')−, −S−Cy−, and −S−S−. In some embodiments, L does not have one or more or any of −C(O)O−, −S−, and −S−S−. In some embodiments, L does not have one or more or any of −C(O)O−, −S−Cy−, and −S−S−. In some embodiments, L has none of −C(O)O−, −S−, and −S−S−. In some embodiments, L has none of −C(O)O−, −S−Cy−, and −S−S−. In some embodiments, L has none of −C(O)O− and −S−S−.

[00228] In some embodiments, L is a covalent bond. In some embodiments, L is not a covalent bond.

[00229] In some embodiments, L^{LG1} is a covalent bond. In some embodiments, L^{LG1} is not a covalent bond. In some embodiments, L^{LG1} is or comprises −(CH_2CH_2O)n−. In some embodiments, L^{LG1} is or comprises a moiety selected from the Markush group of moieties consisting of −(CH_2)n−O−(CH_2CH_2O)n−(CH_2)n−, wherein each n is independently as described in this specification, and

each $-CH_2-$ is independently optionally substituted, $-(CH_2)n-O-(CH_2CH_2O)n-(CH_2)n-$, wherein each n is independently as described in this specification, and each $-CH_2-$ is independently optionally substituted, $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification, and each $-CH_2-$ is independently optionally substituted, and $-(CH_2)_2-O-(CH_2CH_2O)n-(CH_2)_2-$, wherein n is as described in this specification.

[00230] In some embodiments, L^{LG1} is a moiety selected from the Markush group of moieties consisting of L^{LG1} is $-CH_2-$, $-(CH_2)_2-$, $-(CH_2)_2-C(O)-$, $-(CH_2)_2-C(O)-NH-$, $-(CH_2)_3-$, $-(CH_2)_3NH-$, $-(CH_2)_3NH-C(O)-$, $-C(O)-(CH_2)_3NH-C(O)-$, $-C(O)-(CH_2)_3-$, $-NH-C(O)-(CH_2)_3-$, and $-NHC(O)-(CH_2)_3NH-C(O)-$. In some embodiments, a $-CH_2-$ is bonded to an antibody-binding moiety.

[00231] In some embodiments, L^{LG1} is a moiety selected from the Markush group of moieties consisting of $-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-$, $-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(O)-$, $-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(O)NH-$, and $-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-C(O)NH-CH_2-$. In some embodiments, $-CH_2CH_2-$ is bonded to an antibody-binding moiety.

[00232] In some embodiments, L^{LG1} is $-(CH_2CH_2O)n-$. In some embodiments, L^{LG1} is $-(CH_2CH_2O)n-CH_2-CH_2-$. In some embodiments, L^{LG1} is $-(CH_2CH_2O)n-CH_2-CH_2-C(O)-$. In some embodiments, L^{LG1} is $-(CH_2CH_2O)_2-CH_2-CH_2-C(O)-$. In some embodiments, L^{LG1} is $-(CH_2CH_2O)_4-CH_2-CH_2-C(O)-$. In some embodiments, L^{LG1} is $-(CH_2CH_2O)_8-CH_2-CH_2-C(O)-$. In some embodiments, $-C(O)-$ is bonded to an antibody-binding moiety.

[00233] In some embodiments, L^{LG1} is $-N(R')-$. In some embodiments, L^{LG1} is $-NH-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]n-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]n-CH_2CH_2-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]n-CH_2CH_2-NH-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]n-CH_2CH_2-NH-C(O)-$. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, L^{LG1} is $-NH-CH_2CH_2-O-$. In some embodiments, L^{LG1} is $-NH-CH_2CH_2-O- CH_2CH_2-$. In some embodiments, L^{LG1} is $-NH-CH_2CH_2-O- CH_2CH_2-NH-$. In some embodiments, L^{LG1} is $-NH-CH_2CH_2-O- CH_2CH_2-NH-C(O)-$.

[00234] In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_2-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_2-CH_2CH_2-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_2-CH_2CH_2-NH-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_2-CH_2CH_2-NH-C(O)-$.

[00235] In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_3-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_3-CH_2CH_2-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_3-CH_2CH_2-NH-$. In some embodiments, L^{LG1} is $-NH-[(-CH_2CH_2-O-)]_3-CH_2CH_2-NH-C(O)-$. In some embodiments, L^{LG1} is

−NH−[(−CH_2CH_2−O−)]$_4$−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_4$−CH_2CH_2−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_4$−CH_2CH_2−NH−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_4$−CH_2CH_2−NH−C(O)−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_5$−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_5$−CH_2CH_2−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_5$−CH_2CH_2−NH−. In some embodiments, L^{LG1} is −NH−[(−CH_2CH_2−O−)]$_5$−CH_2CH_2−NH−C(O)−. In some embodiments, −NH− is bonded to an antibody-binding moiety.

[00236] In some embodiments, L^{LG1} is −CH_2−. In some embodiments, L^{LG1} is −CH_2CH_2−. In some embodiments, L^{LG1} is −CH_2CH_2NH−. In some embodiments, L^{LG1} is −CH_2CH_2NH−(CO)−. In some embodiments, −CH_2− is bonded to an antibody-binding moiety.

[00237] In some embodiments, L^{LG1} is −CH_2−. In some embodiments, L^{LG1} is −CH_2C(O)−. In some embodiments, L^{LG1} is −CH_2C(O)NH−. In some embodiments, L^{LG1} is −CH_2(CO)NHCH_2−. In some embodiments, −CH_2−C(O)− is bonded to an antibody-binding moiety at −CH_2−.

[00238] In some embodiments, L^{LG2} is a covalent bond. In some embodiments, L^{LG2} is not a covalent bond. In some embodiments, L^{LG2} is −N(R')C(O)−. In some embodiments, L^{LG2} is −NHC(O)−. In some embodiments, L^{LG2} is −(CH_2)n−N(R')C(O)−, wherein −(CH_2)n− is optionally substituted. In some embodiments, L^{LG2} is −(CH_2)n−OC(O)−, wherein −(CH_2)n− is optionally substituted. In some embodiments, L^{LG2} is −(CH_2)n−OC(O)N(R')−, wherein −(CH_2)n− is optionally substituted. In some embodiments, L^{LG2} is −(CH_2)n−OC(O)NH−, wherein −(CH_2)n− is optionally substituted. In some embodiments, n is 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, −(CH_2)n− is substituted. In some embodiments, −(CH_2)n− is unsubstituted. In some embodiments, L^{LG2} is −CH_2N($CH_2CH_2CH_2$S(O)$_2$OH)−C(O)−. In some embodiments, L^{LG2} is −C(O)−NHCH_2−. In some embodiments, L^{LG2} is −C(O)−NHCH_2CH_2−. In some embodiments, L^{LG2} is −C(O)O−CH_2−. In some embodiments, L^{LG2} is −NH−C(O)O−CH_2−. In some embodiments, −C(O)− is bonded to L^{LG3}. In some embodiments, −N(R')−, −NH−, or an optionally substituted −CH_2− unit (of optionally substituted −(CH_2)n−) is bonded to L^{LG3}. In some embodiments L^{LG2} is −NH−, −NHC(O)−,−(CH_2)n−NHC(O)−, −(CH_2)n−OC(O)−, −(CH_2)n−OC(O)NH−, −C(O)−NHCH_2−, −C(O)−NHCH_2CH_2−, −C(O)O−CH_2−, or −NH−C(O)O−CH_2−.

[00239] In some embodiments, L^{LG2} is −N(R')−. In some embodiments, L^{LG2} is −N(R)−. In some embodiments, L^{LG2} is −NH−.

[00240] In some embodiments, L^{LG2} is optionally substituted bivalent C_{1-6} aliphatic. In some embodiments, L^{LG2} is $-CH_2-$. In some embodiments, L^{LG2} is $-CH_2NH-$. In some embodiments, L^{LG2} is $-CH_2NH-C(O)-$. In some embodiments, L^{LG2} is $-CH_2NH-C(O)-CH_2-$.

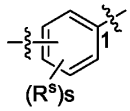
[00241] In some embodiments, L^{LG3} is or comprises an optionally substituted aryl ring. In some embodiments, L^{LG3} is or comprises an optionally substituted phenyl ring. In some embodiments, L^{LG3} is a phenyl ring substituted with one or more electron-withdrawing groups. As understood by persons having ordinary skill in the biomedical art, several electron-withdrawing groups are known in the biomedical art and may be used under this specification. In some embodiments, an electron-withdrawing group is halogen. In some embodiments, an electron-withdrawing group is −F. In embodiments, it is −Cl. In some embodiments, it is −Br. In embodiments, it is −I. In embodiments, an electron-withdrawing group comprises an X=Y double bond, wherein X is bonded to the group to which the electron-withdrawing group is a substituent, and at least one of X and Y is a heteroatom. In some embodiments, X is a heteroatom. In some embodiments, Y is a heteroatom. In some embodiments, each of X and Y is independently a heteroatom. In some embodiments, Y is O. In embodiments, Y is S. In embodiments, X is C. In embodiments, X is N. In embodiments, X is P. In embodiments, X is S. In embodiments, X=Y is C=O. In embodiments, X=Y is N=O. In embodiments, X=Y is S=O. In embodiments, X=Y is P=O. In embodiments, an electron-withdrawing group is $-C(O)-L-R'$. In some embodiments, an electron-withdrawing group is $-C(O)-R'$. In some embodiments, it is $-NO_2$. In some embodiments, it is $-S(O)-L-R'$. In some embodiments, it is $-S(O)-R'$. In some embodiments, it is $-S(O)_2-L-R'$. In some embodiments, it is $-S(O)_2-O-R'$. In some embodiments, it is $-S(O)_2-N(R')_2$. In some embodiments, it is $-P(O)(-L-R')_2$. In some embodiments, it is $-P(O)(R')_2$. In some embodiments, it is $-P(O)(OR')_2$. In some embodiments, it is $-P(O)[N(R')_2]_2$.

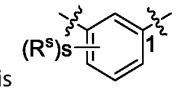
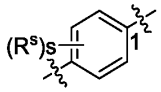
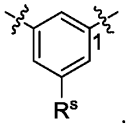
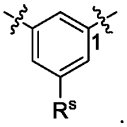
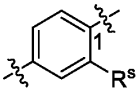
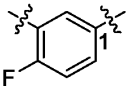
[00242] In some embodiments, L^{LG3} is $-L^{LG3a}-L^{LG3b}-$, wherein L^{LG3a} is a covalent bond or $-C(O)O-CH_2-$, wherein $-CH_2-$ is optionally substituted, and L^{LG3b} is an optionally substituted aryl ring. In some embodiments, L^{LG3a} is bonded to L^{LG2}, and L^{LG3b} is bonded to L^{LG4}.

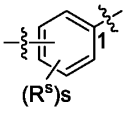
[00243] In some embodiments, L^{LG3a} is a covalent bond. In some embodiments, L^{LG3a} is $-C(O)O-CH_2-$, wherein $-CH_2-$ is optionally substituted. In some embodiments, L^{LG3a} is $-C(O)O-CH_2-$, wherein $-CH_2-$ is substituted. In some embodiments, L^{LG3a} is $-C(O)O-CH_2-$, wherein $-CH_2-$ is unsubstituted.

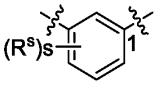
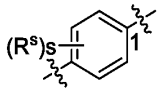
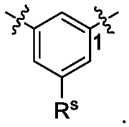
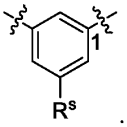
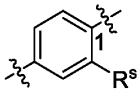
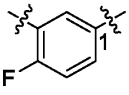
[00244] In some embodiments, a first group, an antibody-binding moiety, or LG is released as part of a compound having the structure of $R^{LG}-L^{LG1}-L^{LG2}-H$ or a salt thereof.

[00245] In some embodiments, L^{LG3b} is an optionally substituted phenyl ring. In some embodiments, at least one substituent is an electron-withdrawing group as described in this specification.



[00246] In some embodiments, L^{LG3} is , wherein s is 0-4, each R^s is independently halogen, $-NO_2$, −L−R', −C(O)−L−R', −S(O)−L−R', $-S(O)_2$−L−R', or $-P(O)(-L-R')_2$. In some embodiments, C1 is bonded to L^{LG4}. In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is .

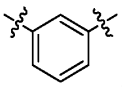
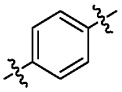
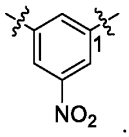
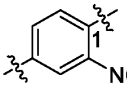
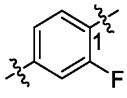
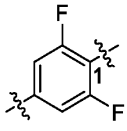
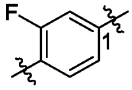
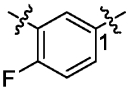


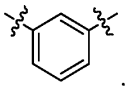
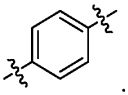
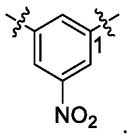
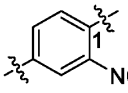
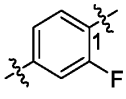
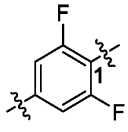
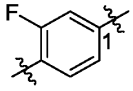
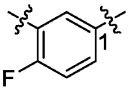
[00247] In some embodiments, L^{LG3b} is , wherein s is 0-4, each R^s is independently halogen, $-NO_2$, −L−R', −C(O)−L−R', −S(O)−L−R', $-S(O)_2$−L−R', or $-P(O)(-L-R')_2$. In some embodiments, C1 is bonded to L^{LG4}. In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is .

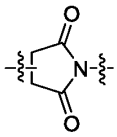
[00248] In some embodiments, s is 0. In some embodiments, s is 1-4. In some embodiments, s is 1. In some embodiments, s is 2. In some embodiments, s is 3. In some embodiments, s is 4.

[00249] In some embodiments, s is 1-4, and at least one R^s is an electron-withdrawing group, e.g., an electron-withdrawing group described above. In some embodiments, at least one R^s is $-NO_2$. In some embodiments, at least one R^s is −F. In embodiments, each R^s is independently an electron-withdrawing group. In some embodiments, each R^s is $-NO_2$. In some embodiments, each R^s is −F.

[00250] In some embodiments, an electron-withdrawing group or R^s is at C2. In some embodiments, an electron-withdrawing group or R^s is at C3. In some embodiments, an electron-withdrawing group or R^s is at C4. In some embodiments, an electron-withdrawing group or R^s is at C2 and C5.

[00251] In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is . In some embodiments, L^{LG3} is .

[00252] In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is . In some embodiments, L^{LG3b} is .

[00253] In some embodiments, L^{LG3b} is optionally substituted . In some embodiments, the nitrogen atom is bound to L^{LG4} which is –O–. In some embodiments, the nitrogen atom is bound to L^{LG4} which is –O–, and $-L^{RG1}-L^{RG2}-$ is –C(O)–.

[00254] In some embodiments, $-L^{LG4}-L^{RG1}-L^{RG2}-$ is $-O-C(O)-$. In some embodiments, $-L^{LG4}-L^{RG1}-L^{RG2}-$ is $-S-C(O)-$.

[00255] In some embodiments, L^{LG4} is a covalent bond. In some embodiments, L^{LG4} is not a covalent bond. In some embodiments, L^{LG4} is $-O-$. In some embodiments, L^{LG4} is $-N(R')-$. In some embodiments, L^{LG4} is $-NH-$. In some embodiments, L^{LG4} is $-N(CH_3)-$. In some embodiments, L^{LG4} is $-N(R')-$, and L^{LG3} is $-O-$. In some embodiments, R' is optionally substituted C_{1-6} alkyl. In some embodiments, L^{LG4} is $-S-$.

[00256] In some embodiments, R^{LG} is or comprises an antibody-binding moiety. In some embodiments, R^{LG} is or comprises a protein-binding moiety. In some embodiments, R^{LG} is or comprises an antibody-binding moiety. In some embodiments, R^{LG} is an antibody-binding moiety. In some embodiments, R^{LG} is a protein-binding moiety. In some embodiments, R^{LG} is an antibody-binding moiety.

[00257] In some embodiments, R^{LG} is **ABT101**, $R^{c}-(Xaa)z-$, a nucleic acid moiety, or a small molecule moiety. In some embodiments, R^{LG} is or comprises ABT101. In some embodiments, R^{LG} is or comprises $Rc-(Xaa)z-$. In some embodiments, R^{LG} is or comprises a small molecule moiety. In some embodiments, R^{LG} is or comprises a peptide agent. In some embodiments, R^{LG} is or comprises a nucleic acid agent. In some embodiments, R^{LG} is or comprises an aptamer agent. In some embodiments, an antibody-binding moiety is or comprises ABT101. In some embodiments, a protein-binding moiety is or comprises ABT101. In some embodiments, an antibody-binding moiety is or comprises ABT101. In some embodiments, an antibody-binding moiety is or comprises $Rc-(Xaa)z-$. In some embodiments, a protein-binding moiety is or comprises $Rc-(Xaa)z-$. In some embodiments, an antibody-binding moiety is or comprises $Rc-(Xaa)z-$.

[00258] In some embodiments, target-binding moieties may be conjugated to antibody moieties optionally through linker moieties using technologies described in US 2020/0190165.

[00259] In some embodiments, where a particular protecting group (PG), leaving group (LG), or transformation condition is depicted, persons having ordinary skill in the biomedical art know that other protecting groups, leaving groups, and transformation conditions are also suitable and are contemplated. Such groups and transformations are described in Smith & March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (John Wiley & Sons, 2001), Larock, Comprehensive Organic Transformations, 2nd edition (John Wiley & Sons, 1999), and Greene & Wuts, Protecting Groups in Organic Synthesis, 3rd edition (John Wiley & Sons, 1999), the entirety of each of which is incorporated in this specification by reference.

[00260] In some embodiments, leaving groups include but are not limited to, halogens, e.g. fluoride, chloride, bromide, iodide, sulfonates, e.g. mesylate, tosylate, benzenesulfonate, brosylate, nosylate, triflate), diazonium.

[00261] In some embodiments, an oxygen protecting group includes carbonyl protecting groups, hydroxyl protecting groups, etc. Hydroxyl protecting groups are well known in the biomedical art and include those described in Greene & Wuts, Protecting Groups in Organic Synthesis, 3rd edition (John Wiley & Sons, 1999), the entirety of which is incorporated in this specification by reference. Examples of suitable hydroxyl protecting groups include, but are not limited to, esters, allyl ethers, ethers, silyl ethers, alkyl ethers, arylalkyl ethers, and alkoxyalkyl ethers. Examples of such esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate (trimethylacetyl), crotonate, 4-methoxy-crotonate, benzoate, p-benylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl. Examples of such silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of arylalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, and 2- and 4-picolyl.

[00262] Amino protecting groups are well known in the biomedical art and include those described in Greene & Wuts, Protecting Groups in Organic Synthesis, 3rd edition (John Wiley & Sons, 1999), the entirety of which is incorporated in this specification by reference. Suitable amino protecting groups include, but are not limited to, aralkylamines, carbamates, cyclic imides, allyl amines, amides. Examples of such groups include t-butyloxycarbonyl (BOC), ethyloxycarbonyl, methyloxycarbonyl, trichloroethyloxycarbonyl, allyloxycarbonyl (Alloc), benzyloxocarbonyl (CBZ), allyl, phthalimide, benzyl (Bn), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzoyl.

[00263] Persons having ordinary skill in the biomedical art know that provided agents may have one or more stereocenters and may be present as a racemic or diastereomeric mixture. Persons having ordinary skill in the biomedical art know there are many methods known in the biomedical art for the

separation of isomers to obtain stereoenriched or stereopure isomers of those compounds, including but not limited to HPLC, chiral HPLC, fractional crystallization of diastereomeric salts, kinetic enzymatic resolution, e.g. by fungal- derived, bacterial- derived, or animal-derived lipases or esterases, and formation of covalent diastereomeric derivatives using an enantioenriched reagent.

[00264] persons having ordinary skill in the biomedical art know that several functional groups present in compounds of this specification such as aliphatic groups, alcohols, carboxylic acids, esters, amides, aldehydes, halogens, and nitriles can be interconverted by techniques well known in the biomedical art including, but not limited to reduction, oxidation, esterification, hydrolysis, partial oxidation, partial reduction, halogenation, dehydration, partial hydration, and hydration. See Smith & March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (John Wiley & Sons, 2001), the entirety of which is incorporated in this specification by reference. Such interconversions may require one or more techniques, and certain methods for synthesizing compounds of this specification are described below in the Exemplification.

[00265] As known by persons having ordinary skill in the biomedical art, reaction partners are generally contacted with each other under conditions and for a time sufficient for production of the desired results, e.g., formation of product agents and compositions thereof to desired extents. Many reaction conditions/reaction times may be assessed and used if they are suitable for desired purposes under this specification; certain such conditions, reaction times, assessment, etc. are described in the Examples.

[00266] In some embodiments, an agent formed, e.g., a product MATE agent, has the structure of formula **AGN101** or **AGN102**, or a salt thereof. In some embodiments, a cellular receptor-binding moiety in a product agent, e.g., a MATE agent, is the same as a cellular receptor-binding moiety in a reaction partner, e.g., a first agent comprising a cellular receptor-binding moiety) used to prepare a product agent. In some embodiments, an antibody moiety in a product agent, e.g., a MATE agent, is the same as an antibody moiety in a reaction partner, e.g., a second agent comprising an antibody moiety) used to prepare a product agent.

[00267] In some embodiments, linker moieties or a part connected to target-binding moieties or antibody moieties may be transferred from reaction partners, e.g., L^{RM} of formula **AGN301** or a salt thereof. In some embodiments, a linker moiety in a product agent (may be called L^{PM}; e.g., L in formula **AGN101** or **AGN102**, is or comprises a linker moiety in a reaction partner, e.g., one between a reactive group and a cellular receptor-binding moiety, e.g., L^{RM}. In some embodiments, L^{PM} is or comprises L^{RM}. In some embodiments, L^{PM} is $-L^{RM}-L^{RG2}-$. In some embodiments, L^{RG2} is $-C(O)-$. In some embodiments, L^{RG2}

is −C(O)−, and is bonded to −NH− of a target agent moiety, e.g., −NH− in a side chain of a lysine residue of a protein moiety, which in some embodiments, is an antibody moiety.

[00268] Reaction partners, e.g., compounds of formula **AGN301** or salts thereof, rarely have moieties that can react with reactive groups under conditions under which reactive groups react with target agents. In some embodiments, to the extent that some moieties in reaction partners may react with reactive groups under conditions under which reactive groups react with target agents, reactions between such moieties and reactive groups are significantly slower or less efficient compared to reactions between reactive groups and target agents. In some embodiments, reactions between such moieties and reactive groups do not significantly reduce, e.g., no more than about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, etc. of reduction, efficiencies, yields, rates, or conversions, etc., of reactions between reactive groups and target agents. In some embodiments, reactive groups, e.g., ester groups, activated carboxylic acid derivatives, etc. react with amino groups, e.g., $-NH_2$ groups, of target agents, e.g., protein agents such as antibody agents. In some embodiments, reaction partners, e.g., compounds of formula **AGN301** or salts thereof, do not have amine groups. In some embodiments, compounds of formula **AGN301** or salts thereof (or parts of it, such as R^{LG}, L^{LG}, L^{LG1}, L^{LG2}, L^{LG3}, L^{LG4}, L^{RG1}, L^{RG2}, L^{RM}, or MOI, do not have amine groups. In some embodiments, they do not have primary amine groups ($-NH_2$). In some embodiments, they do not have $-CH_2NH_2$. In some embodiments, they do not have $-CH_2CH_2NH_2$. In some embodiments, they do not have $-CH_2CH_2CH_2NH_2$. In some embodiments, they do not have $-CH_2CH_2CH_2CH_2NH_2$. In some embodiments, amine groups, e.g., primary amine groups, are capped, e.g., by introduction of acyl groups, e.g., R−C(O)−, e.g., acetyl to form amide groups) to prevent or reduce undesired reactions.

[00269] In some embodiments, reactions are performed in buffer systems. In some embodiments, buffer systems of present disclosure maintain structures or functions of target agents, cellular receptor-binding moiety, etc. In embodiments, a buffer is a phosphate buffer. In some embodiments, a buffer is a phosphate-buffered saline (PBS) buffer. In some embodiments, a buffer is a borate buffer. In some embodiments, buffers of this specification provide and optionally maintain certain pH value or range. In some embodiments, a useful pH is about 7-9, e.g., 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 9.0, etc. In embodiments, a pH is 7.4. In some embodiments, a pH is 7.5. In some embodiments, a pH is 7.8. In some embodiments, a pH is 8.0. In some embodiments, a pH is 8.2. In some embodiments, a pH is 8.3.

[00270] Provided technologies can provide several advantages. In some embodiments, connection of a cellular receptor-binding moiety in a reaction partner, e.g., a compound comprising a reactive group

between an antibody-binding moiety and a cellular receptor-binding moiety, e.g., a composition of matter of formula **AGN301** or a salt thereof to an agent comprising an antibody moiety, e.g., a second agent such as an antibody agent, and release of an antibody-binding moiety in a provided reaction partner can be achieved in one reaction or in one pot. In many embodiments, no separate reactions/steps are performed to remove antibody-binding moieties. As known by persons having ordinary skill in the biomedical art, by performing connection of cellular receptor-binding moiety and release of antibody-binding moiety in a single reaction/operation, provided technologies can avoid separate steps for antibody-binding moiety removal and can improve overall efficiency, e.g., by simplify operations, increasing overall yield, etc. , reduce manufacturing cost, improve product purity, e.g., by avoiding exposure to antibody-binding moiety removal conditions, which typically involve one or more of reduction, oxidation, hydrolysis, e.g., of ester groups), etc., conditions and may damage target agent moieties, e.g., for protein agent moieties, protein amino acid residues, overall structures, or post-translational changes, e.g., glycans of antibodies) thereof. In some embodiments, provided technologies can provided improved efficiency, e.g., in terms of reaction rates or conversion percentages), increased yield, increased purity/homogeneity, or enhanced selectivity, particularly compared to reference technologies wherein a reaction partner containing no antibody-binding moieties is used, without introducing step(s) for antibody-binding moiety removal, e.g., antibody-binding moiety is removed in the same step as cellular receptor-binding moiety conjugation.

[00271] In some embodiments, the invention provides products of provided processes, which have low levels of damage to antibody moieties compared to processes comprising steps performed for antibody-binding moiety removal but not for substantial conjugation of moieties of interest, e.g. target-binding moieties. In some embodiments, provided product agent compositions have high homogeneity, e.g., regarding the number of cellular receptor-binding moiety per antibody moiety, or positions of amino acid residues in antibody moieties conjugated to moieties of interest) compared to reference product compositions, e.g., those from technologies without using antibody-binding moieties, or using extra step(s) for antibody-binding moiety removal, e.g., not using reaction partners described in this specification which comprise a reactive group between an antibody-binding moiety and a cellular receptor-binding moiety.

[00272] In some embodiments, the invention provides a product agent which is an agent comprising an antibody moiety, a cellular receptor-binding moiety and optionally a linker moiety linking an antibody-binding moiety and a cellular receptor-binding moiety. In some embodiments, the invention composes such agents.

[00273] In some embodiments, the invention provides a composition comprising a plurality of agents, wherein each agent independently comprises:

an antibody moiety,

a cellular receptor-binding moiety, and

optionally a linker moiety linking an antibody-binding moiety and a cellular receptor-binding moiety.

[00274] In some embodiments, product agents are MATE agents. In some embodiments, an antibody agent moiety comprises IgG Fc region. In some embodiments, an antibody moiety is connected to a cellular receptor-binding moiety through an amino group optionally through a linker. In some embodiments, it is through a lysine residue wherein the amino group of the side chain is connected to a cellular receptor-binding moiety optionally through a linker, e.g., forming –NH–C(O)– as part of an amide group, a carbamate group, etc.

[00275] In some embodiments, selected locations of antibody moieties are used for conjugation. In some embodiments, K246 or K248 of an antibody agent (EU numbering, or corresponding residues) are conjugation locations. In some embodiments, a conjugation location is K246 of heavy chain (unless otherwise specified, locations in this specification include corresponding residues in, e.g., changed sequence, e.g., longer, shorter, rearranged, etc., sequences. In some embodiments, a location is K248 of heavy chain. In some embodiments, a location is K288 or K290 of heavy chain. In some embodiments, a location is K288 of heavy chain. In some embodiments, a location is K290 of heavy chain. In some embodiments, a location is K317. In some embodiments, an antibody moiety is a moiety of an IgG1 antibody or a fragment thereof. In some embodiments, an antibody moiety is a moiety of an IgG2 antibody or a fragment thereof. In some embodiments, an antibody moiety is a moiety of an antibody or a fragment thereof. In some embodiments, a composition comprises a plurality of MATE agents, wherein antibody moieties of the plurality of MATE agents are independently an antibody moiety of an IgG1, IgG2, or IgG4 antibody, or a fragment thereof.

[00276] In some embodiments, antibody heavy chains are selectively conjugated/labeled over light chains.

[00277] In some embodiments, in provided agents, e.g., agents of formula **AGN101** or **AGN102**, or a salt thereof) substantially all conjugation sites of antibody moieties have the same changes, e.g., all share the same moieties of interest optionally connected through the same linker moieties. In some embodiments, no conjugation sites bear different changes, e.g., different moieties of interest or no moieties of interest or different linker moieties.

[00278] In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target-binding moieties, conjugated to antibody moieties of a particular type of antibodies, e.g., IgG1, or fragments thereof are conjugated to one or more particularly sites, typically one or two particularly sites, e.g., K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target-binding moieties, conjugated to antibody moieties of IgG2 antibodies or fragments thereof are at K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target-binding moieties, conjugated to antibody moieties of IgG2 antibodies or fragments thereof are at K239 and K241 of a heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., for a plurality of agents, for a composition, etc. are conjugated to antibody moieties of IgG1, IgG2, or IgG4 antibodies, or fragments thereof, e.g., for conjugation products with IgG1 antibodies or fragments thereof (antibody moieties being of IgG1 antibodies or fragments thereof), IgG2 antibodies or fragments thereof (antibody moieties being of IgG2 antibodies or fragments thereof), IgG4 antibodies or fragments thereof (antibody moieties being of IgG4 antibodies or fragments thereof), or for conjugation products with IVIG (when certain provided technologies described in this specification are used, selective conjugation with IgG1, IgG2 and IgG4). In some embodiments, a percentage is about 10% or more. In some embodiments, a percentage is about 20% or more. In some embodiments, a percentage is about 25% or more. In some embodiments, a percentage is about 30% or more. In some embodiments, a percentage is about 40% or more. In some embodiments, a percentage is about 50% or more. In some embodiments, a percentage is about 60% or more. In some embodiments, a percentage is about 65% or more. In some embodiments, a percentage is about 70% or more. In some embodiments, a percentage is about 75% or more. In some embodiments, a percentage is about 80% or more. In some embodiments, a percentage is about 85% or more. In some embodiments, a percentage is about 90% or more. In some embodiments, a percentage is about 95% or more. In some embodiments, a percentage is about 100%.

[00279] In some embodiments, a composition comprises a plurality of agents, e.g., MATE agents, agents of formula **AGN101** or **AGN102**, or a salt thereof, each independent comprising a cellular receptor-binding moiety, an antibody moiety, and optionally a linker moiety linking a cellular receptor-binding moiety and an antibody moiety. In some embodiments, substantially all target-binding moieties of a plurality of agents are the same. In some embodiments, substantially all target-binding moieties of a plurality of agents comprise peptide moieties of a common amino acid sequence. In some

embodiments, substantially all target-binding moieties of a plurality of agents are peptide moieties of a common amino acid sequence. In some embodiments, substantially all conjugation sites of antibody moieties in a plurality of agents have the same changes, e.g., all share the same moieties of interest optionally connected through the same linker moieties. In some embodiments, no conjugation sites of a plurality of agents bear different changes, e.g., different moieties of interest or no moieties of interest or different linker moieties. In some embodiments, a plurality of agents does not have agents that share the same (or substantially the same) antibody moieties but different changes, e.g., different moieties of interest or no moieties of interest or different linker moieties. In some embodiments, agents that share the same (or substantially the same) antibody moieties but different changes, e.g., different moieties of interest or no moieties of interest or different linker moieties) are intermediates of multiple-step preparations, e.g., comprising steps for removal of antibody-binding moieties in addition to steps for cellular receptor-binding moiety conjugation) of final product agents.

[00280] In some embodiments, the invention provides a composition comprising a plurality of agents each of which independently comprising:

an antibody moiety,

a cellular receptor-binding moiety, and

optionally a linker moiety linking the antibody moiety and the cellular receptor-binding moiety; wherein antibody moieties of agents of the plurality comprise a common amino acid sequence, and agents of the plurality share a common cellular receptor-binding moiety independently at least one common amino acid residue of the common amino acid sequence; and

wherein about 1%-100% of all agents that comprise an antibody moiety that comprise the common amino acid sequence and the cellular receptor-binding moiety are agents of the plurality.

[00281] In some embodiments, the invention provides a composition comprising a plurality of agents each of which independently comprising:

an antibody moiety,

a cellular receptor-binding moiety, and

optionally a linker moiety linking an antibody moiety and a cellular receptor-binding moiety; wherein agents of the plurality share the same or substantially the same antibody moiety, and a cellular receptor-binding moiety at least one common location; and

wherein about 1%-100% of all agents that comprise the antibody moiety and the cellular receptor-binding moiety are agents of the plurality.

[00282] In some embodiments, an antibody moiety is a moiety of an IgG1 antibody or a fragment thereof. In some embodiments, an antibody moiety is a moiety of an IgG2 antibody or a fragment thereof. In some embodiments, an antibody moiety is a moiety of an IgG3 antibody or a fragment thereof. In some embodiments, an antibody moiety is a moiety of an antibody or a fragment thereof. In some embodiments, about 1-100% of all moieties of interest are at common locations. In some embodiments, a cellular receptor-binding moiety is a cellular receptor-binding moiety as described in this specification. In some embodiments, agents of a plurality are each independently of formula **AGN101** or **AGN102**, or a salt thereof.

[00283] In some embodiments, antibody moieties of agents of a plurality comprise a common amino acid sequence. In some embodiments, antibody moieties of agents of a plurality comprise a common amino acid sequence in a Fc region. In some embodiments, antibody moieties of agents of a plurality comprise a common Fc region. In some embodiments, antibody moieties of agents of a plurality can bind a common antigen specifically. In some embodiments, antibody moieties are monoclonal antibody moieties. In some embodiments, antibody moieties are polyclonal antibody moieties. In some embodiments, antibody moieties bind to two or more different antigens. In some embodiments, antibody moieties bind to two or more different proteins. In some embodiments, antibody moieties are IVIG moieties.

[00284] In some embodiments, a cellular receptor-binding moiety in an agent of a plurality is a cellular receptor-binding moiety. In some embodiments, each cellular receptor-binding moiety is independently a cellular receptor-binding moiety. In some embodiments, a composition comprises a plurality of agents, antibody moieties of agents of the plurality comprise a common amino acid sequence, and agents of a plurality share a common cellular receptor-binding moiety independently linked to a common amino acid residue in the common amino acid sequence, each independently and optionally through a linker; and wherein about 1%-100% of all agents that comprise an antibody moiety that comprises a common amino acid sequence and a common cellular receptor-binding moiety independently comprise a common cellular receptor-binding moiety linked to a common amino acid residue independently and optionally through a linker. In some embodiments, a composition comprises a plurality of agents, antibody moieties of agents of a plurality comprise a common amino acid sequence, and agents of a plurality share a common cellular receptor-binding moiety independently linked to a common amino acid residue in the common amino acid sequence, each independently through a common linker; and wherein about 1%-100% of all agents that comprise an antibody moiety that comprises a common amino acid sequence and a common cellular receptor-binding moiety

independently comprise a common cellular receptor-binding moiety linked to a common amino acid residue independently and through a common linker. In some embodiments, a composition comprises a plurality of agents, antibody moieties of agents of a plurality comprise a common amino acid sequence, and agents of a plurality share a common cellular receptor-binding moiety independently linked to a common amino acid residue in the common amino acid sequence, each independently and optionally through a linker; and wherein about 1%-100% of all agents that comprise an antibody moiety that comprises a common amino acid sequence and a common cellular receptor-binding moiety are agents of a plurality. In some embodiments, a composition comprises a plurality of agents, wherein antibody moieties of agents of a plurality comprise a common amino acid sequence, and agents of a plurality share a common cellular receptor-binding moiety independently linked to a common amino acid residue in the common amino acid sequence, each independently through a common linker; and wherein about 1%-100% of all agents that comprise an antibody moiety that comprises a common amino acid sequence, a common cellular receptor-binding moiety, and a common linker are agents of a plurality.

[00285] In some embodiments, “at least one” or “one or more” is 1-1000, 1-500, 1-200, 1-100, 1-90, 1-80, 1-70, 1-60, 1-50, 1-40, 1-30, 1-20, 1-10, 1-5, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more. In some embodiments, it is one. In some embodiments, it is two or more. In some embodiments, it is about 3. In some embodiments, it is about 4. In some embodiments, it is about 5. In some embodiments, it is about 6. In some embodiments, it is about 7. In some embodiments, it is about 8. In some embodiments, it is about 9. In some embodiments, it is about 10. In some embodiments, it is about 10 or more.

[00286] In some embodiments, a common amino acid sequence comprises 1-1000, 1-500, 1-400, 1-300, 1-200, 1-100, 1-50, 10-1000, 10-500, 10-400, 10-300, 10-200, 10-100, 10-50, 20-1000, 20-500, 20-400, 20-300, 20-200, 20-100, 20-50, 50-1000, 50-500, 50-400, 50-300, 50-200, 50-100, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, 300, 400, 500, 600 or more amino acid residues. In some embodiments, a length is at least 5 amino acid residues. In some embodiments, a length is at least 10 amino acid residues. In some embodiments, a length is at least 50 amino acid residues. In some embodiments, a length is at least 100 amino acid residues. In some embodiments, a length is at least 150 amino acid residues. In some embodiments, a length is at least 200 amino acid residues. In some embodiments, a length is at least 300 amino acid residues. In some embodiments, a length is at least 400 amino acid residues. In some embodiments, a length is at least 500 amino acid residues. In some embodiments, a length is at least 600 amino acid residues.

[00287] In some embodiments, a common amino acid sequence is at least 10%-100%, 50%-100%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of an amino acid sequence of an antibody moiety, a protein agent moiety, etc. In embodiments, it is 10% or more. In some embodiments, it is 20% or more. In some embodiments, it is 30% or more. In some embodiments, it is 40% or more. In some embodiments, it is 50% or more. In some embodiments, it is 60% or more. In some embodiments, it is 70% or more. In some embodiments, it is 80% or more. In some embodiments, it is 90% or more. In some embodiments, it is 100%.

[00288] In some embodiments, in a common amino acid sequence, one and only one amino acid residue is linked to a common cellular receptor-binding moiety, e.g., a common cellular receptor-binding moiety. In some embodiments, in a common amino acid sequence, two and only two amino acid residues are linked to a common cellular receptor-binding moiety, e.g., a common cellular receptor-binding moiety. In some embodiments, in a common amino acid sequence, two or more amino acid residues are linked to a common cellular receptor-binding moiety, e.g., a common cellular receptor-binding moiety. In some embodiments, each common cellular receptor-binding moiety, e.g., a common cellular receptor-binding moiety, is independently linked to an amino acid residue in a common amino acid sequence.

[00289] In some embodiments, a common amino acid sequence comprises one or more amino acid residues selected from K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of an antibody heavy chain and amino acid residues corresponding thereto. In some embodiments, a common amino acid sequence comprises one or more amino acid residues selected from K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto. In some embodiments, a common amino acid sequence comprises one or more amino acid residues selected from K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto. In some embodiments, a common amino acid sequence comprises one or more amino acid residues selected from K239 and K241 of an antibody heavy chain and amino acid residues corresponding thereto. In some embodiments, a cellular receptor-binding moiety is connected to this amino acid residue (unless explicitly noted, optionally through a linker moiety). In some embodiments, each cellular receptor-binding moiety is connected to this amino acid residue each optionally and independently through a linker moiety.

[00290] In some embodiments, antibody moieties share a high percentage of amino acid sequence homology. In some embodiments, the amino acid sequence homology is about 50%-100%. In some

embodiments, the amino acid sequence homology is a percentage selected from the Markush group of percentages consisting of 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%.

[00291] In some embodiments, a percentage used in this specification is a percentage selected from the Markush group of percentages consisting of about 1%-100%, about 10% or more, about 20% or more, about 25% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 100%.

[00292] In some embodiments, antibody moiety of agents of a plurality comprises a common Fc region or a fragment thereof.

[00293] In some embodiments, moieties of interest of agents of a plurality are at specific locations. In some embodiments, all moieties of interest are at amino acid residues of a common amino acid sequence. In some embodiments, all moieties of interest are at common locations of amino acid residues of a common amino acid sequence. In some embodiments, the number of common locations is 1. In some embodiments, it is 2. In some embodiments, it is 3. In some embodiments, it is 4. In some embodiments, antibody moieties comprise two heavy chains or fragments thereof, and the number of common locations is 2 (one on each chain). In some embodiments, common locations are selected from K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of an antibody heavy chain and amino acid residues corresponding thereto.

[00294] In some embodiments, agents of a plurality share a common cellular receptor-binding moiety independently at least one location. In some embodiments, agents of a plurality share a common cellular receptor-binding moiety and linker independently at least one location. In some embodiments, moieties of interest at two or more or all locations comprise a common cellular receptor-binding moiety. In some embodiments, moieties of interest are the same.

[00295] In some embodiments, agents share common changes at least one common amino acid residue. In some embodiments, agents of a plurality share common changes at each location connected to a cellular receptor-binding moiety and optionally a linker. In some embodiments, agents of a plurality the same $-L^{PM}-$TBT at each location connected to a linker moiety.

[00296] In some embodiments, a location is selected from K246, K248, K288, K290, K317 of antibody agents and locations corresponding thereto. In some embodiments, a location is selected from K246 and

K248, and locations corresponding thereto. In some embodiments, a location is selected from K288 and K290, and locations corresponding thereto. In some embodiments, a location is K246 or a location corresponding thereto. In some embodiments, a location is K248 or a location corresponding thereto. In some embodiments, a location is K288 or a location corresponding thereto. In some embodiments, a location is K290 or a location corresponding thereto. In some embodiments, a location is K317 or a location corresponding thereto. In some embodiments, a location is K185 of light chain or a location corresponding thereto. In some embodiments, a location is K187 of light chain or a location corresponding thereto. In some embodiments, a location is K133 of heavy chain or a location corresponding thereto. In some embodiments, a location is K246 or K248 of heavy chain or a location corresponding thereto. In some embodiments, a location is K414 of heavy chain or a location corresponding thereto. In some embodiments, a common sequence is a sequence that is about or at least about 10-100, 20-50, e.g., at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, amino acid residues in length, and comprises one or more of such residues or residues corresponding thereto. In some embodiments, a common sequence is a sequence that is about or at least about 10-100, 20-50, e.g., about or at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, amino acid residues in length, and comprises one, two or more residues selected from K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of an antibody heavy chain and amino acid residues corresponding thereto.

[00297] In some embodiments, about 1%-100% of all agents that comprise an antibody moiety and a cellular receptor-binding moiety are agents of a plurality. In some embodiments, about 1%-100% of all agents that comprise an antibody moiety that comprises a common amino acid sequence and a cellular receptor-binding moiety are agents of a plurality. In some embodiments, about 1%-100% of all agents that comprise an antibody moiety that comprise a common amino acid sequence or can bind to a common antigen and a cellular receptor-binding moiety are agents of a plurality. In some embodiments, about 1%-100% of all agents that comprise an antibody moiety are agents of a plurality. In some embodiments, about 1%-100% of all agents that comprise an antibody moiety that comprise the common amino acid sequence are agents of a plurality. In some embodiments, about 1%-100% of all agents that comprise a protein agent moiety that comprise the common amino acid sequence are agents of a plurality. In some embodiments, a range of all agent agents that comprise an antibody agent moiety that comprise the common amino acid sequence or can bind to the common antigen are selected from a Markush group of ranges where the agents are of a plurality consisting of about 1%-

100%, about 5%-100%, 10%-100%, about 20%-100%, about 25%-100%, about 30%-100%, and about 40%-100%, about 50%-100%. In some embodiments, a range of all agent agents that comprise an antibody agent moiety that comprise the common amino acid sequence or can bind to the common antigen are selected from a Markush group of ranges where the agents are of a plurality consisting of about 5%, about 10%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, and about 100%. In some embodiments, a range of all agent agents that comprise an antibody agent moiety that comprise the common amino acid sequence or can bind to the common antigen are selected from a Markush group of ranges where the agents are of a plurality consisting of at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50 at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

[00298] In some embodiments, each agent of the plurality does not have −S−Cy−, wherein −Cy− is optionally substituted 5-membered monocyclic ring, does not have −S−S− which is not formed by cysteine residues and does not have −SH or salt form thereof that is not of a cysteine residue. In some embodiments, each agent of the plurality does not have $-S-CH_2-CH_2-$. In some embodiments, each agent of the plurality does not have a moiety that can specifically bind to an antibody agent. In some embodiments, a composition is substantially free from a moiety that can specifically bind to an antibody agent.

[00299] In some embodiments, the invention provides product agent compositions comprising product agents, e.g., agents of formula **AGN101** or **AGN102**, or a salt thereof. In some embodiments, a product agent composition, e.g., aa agent composition formed from certain methods, comprises a product agent comprising an antibody moiety and a cellular receptor-binding moiety and optionally a linker, e.g., an agent of formula **AGN101** or **AGN102**, or a salt thereof, a released antibody-binding moiety, e.g., a compound comprising $R^{LG}-(L^{LG1})_{0-1}-(L^{LG2})_{0-1}-(L^{LG3})_{0-1}-(L^{LG4})_{0-1}-$) or a compound comprising a released antibody-binding moiety, e.g., a compound having the structure of $R^{LG}-(L^{LG1})_{0-1}-(L^{LG2})_{0-1}-(L^{LG3})_{0-1}-(L^{LG4})_{0-1}-H$ or a salt thereof, and a reaction partner, e.g., a composition of matter of formula **AGN301** or a salt thereof. In some embodiments, released antibody-binding moieties may bind to antibody moieties in target agents or formed product agents. Several technologies are available to separate released antibody-binding moieties from antibody moieties under this specification, for example, in

some embodiments, contacting a composition with a composition comprising glycine at certain pH. In some embodiments, each agent of a plurality is independently this product agent.

Reactive Group

[00300] In some embodiments, provided agents, compounds, e.g., those useful as reaction partners such as first agents, comprise reactive groups, e.g., RG. In some embodiments, reactive groups, e.g., RG) are between antibody-binding moieties, e.g., ABT) and moieties of interest, e.g., MOI), and are optionally and independently linked to antibody-binding moieties and moieties of interest via linkers. In some embodiments, RG is a reaction group as described in this specification.

[00301] In some embodiments, reactive groups when used in agents that comprise no antibody-binding moieties react slowly and provide low level of, in some embodiments, substantially no conjugation of moieties of interest with target agents. As shown in this specification, combination of reactive groups with antibody-binding moieties in the same agents, e.g., as in compounds of formula **AGN301** or salts thereof, can promote reactions between reactive groups and target agents, enhance reaction efficiency, reduce side reactions, or improve reaction selectivity, e.g., in terms of target sites wherein conjugation of moieties of interest with target agents occurs.

[00302] Reactive groups in agents can react with several types of groups in target agents. In some embodiments, reactive groups in agents selectively react with amino groups of target agents, e.g., $-NH_2$ groups on side chains of lysine residues of proteins. In some embodiments, reactive groups when used in agents, e.g., those of formula **AGN301** or salts thereof, selectively react with particular sites of target agents, e.g., as shown in examples in this specification, one or more of K246, K248, K288, K290, K317, etc. of IgG1, K251, K253, etc. for IgG2, K239, K241 for IgG4 , etc. In embodiments, a site is K246 or K248 of an antibody heavy chain. In some embodiments, sites are K246 or K248 of an antibody heavy chain. In some embodiments, a site is K246 of an antibody heavy chain. In some embodiments, a site is K248 of an antibody heavy chain. In some embodiments, a site is K288 or K290 of an antibody heavy chain. In some embodiments, a site is K288 of an antibody heavy chain. In some embodiments, a site is K290 of an antibody heavy chain. In some embodiments, a site is K317. In some embodiments, a site is K414 of an antibody heavy chain. In some embodiments, a site is K185 of an antibody light chain. In some embodiments, a site is K187 of an antibody light chain. In some embodiments, sites are K251 or K253 of an IgG2 heavy chain. In some embodiments, a site is K251 of an IgG2 heavy chain. In some embodiments, a site is K253 of an IgG2 heavy chain. In some embodiments, sites are K239 or K241 of an antibody heavy chain. In some embodiments, a site is K239 of an antibody heavy chain. In some embodiments, a site is K241 of an antibody heavy chain. In some embodiments, conjugation selectively

occurs at one or more heavy chain sites over light chain sites. In some embodiments, for technologies without antibody-binding moieties, conjugation occurs at light chain sites more than heavy chain sites.

[00303] In some embodiments, a reactive group, e.g., RG, is or comprises an ester group. In some embodiments, a reactive group, e.g., RG, is or comprises an electrophilic group, e.g., a Michael acceptor.

[00304] In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{RG1}-L^{RG2}-$, wherein each of L^{RG1} and L^{RG2} is independently L. In embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG3}-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG2}-L^{LG3}-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG4}-L^{RG2}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG3}-L^{LG4}-L^{RG2}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-L^{LG2}-L^{LG3}-L^{LG4}-L^{RG2}-$, wherein each variable is as described in this specification.

[00305] In some embodiments, L^{LG4} is $-O-$. In some embodiments, L^{LG4} is $-N(R)-$. In some embodiments, L^{LG4} is $-NH-$.

[00306] In some embodiments, L^{LG3} is or comprises an optionally substituted aryl ring. In some embodiments, L^{LG3} is or comprises a phenyl ring. In some embodiments, an aryl or phenyl ring is substituted. In some embodiments, a substituent is an electron-withdrawing group as described in this specification, e.g., $-NO_2$, $-F$, etc.

[00307] In some embodiments, L^{RG1} is a covalent bond. In some embodiments, L^{RG1} is not a covalent bond. In some embodiments, L^{RG1} is $-S(O)_2-$.

[00308] In some embodiments, L^{RG2} is $-C(O)-$. In some embodiments, a reactive group is or comprises $-L^{LG4}-C(O)-$, wherein each variable is as described in this specification. In some embodiments, a reactive group is or comprises $-L^{LG3}-L^{LG4}-C(O)-$, wherein each variable is as described in this specification. In some embodiments, a reactive group is or comprises $-L^{LG2}-L^{LG3}-L^{LG4}-C(O)-$, wherein each variable is as described in this specification.

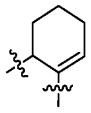
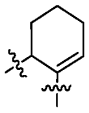
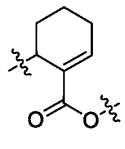
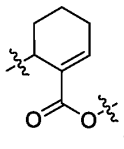
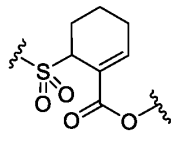
[00309] In some embodiments, L^{RG2} is $-L^{RG3}-C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}-$, wherein each of R^{RG1}, R^{RG2}, R^{RG3} and R^{RG4} is independently $-L-R'$, and L^{RG3} is $-C(O)-$, $-C(O)O-$, $-C(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-P(O)(OR')-$, $-P(O)(SR')-$, or $-P(O)(N(R')_2)-$. In some embodiments, each of R^{RG1}, R^{RG2}, R^{RG3} and R^{RG4} is independently R'. In some embodiments, one or more of R^{RG1}, R^{RG2}, R^{RG3} and R^{RG4} is independently $-H$. In

embodiments, L^{RG3} is –C(O)–. In some embodiments, L^{RG3} is –C(O)O–. In some embodiments, –O–, –N(R')–, etc. of L^{RG3} is bonded to L^{PM}.

[00310] In some embodiments, R^{RG1} is –H. In embodiments, R^{RG3} is –H.

[00311] In some embodiments, L^{RG2} is optionally substituted $-L^{RG3}-C(=CHR^{RG2})-CHR^{RG4}-$, wherein each variable is as described in this specification.

[00312] In some embodiments, R^{RG2} and R^{RG4} are taken with their intervening atoms to form an optionally substituted ring as described in this specification. In some embodiments, a formed ring is an optionally substituted 3-10-membered monocyclic or bicyclic ring having 0-5 heteroatoms. In some embodiments, a formed ring is an optionally substituted 3-10-membered cycloaliphatic ring. In some embodiments, a formed ring is selected from the Markush group consisting of optionally substituted cycloaliphatic rings consisting of a 3-8-membered cycloaliphatic ring, a 5-8-membered cycloaliphatic ring., a 5-membered cycloaliphatic ring, a 6-membered cycloaliphatic ring, and a 7-membered cycloaliphatic ring. In some embodiments, a formed ring is substituted. In some embodiments, a formed ring is not substituted. In some embodiments, a formed ring has no additional unsaturation in addition to the double bond in $C(=CHR^{RG2})$ or $C(=CR^{RG1}R^{RG2})$.

[00313] In some embodiments, $-C(=CHR^{RG2})-CHR^{RG4}$ or $-C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}$ is optionally substituted  or is . In some embodiments, $-[C(=CHR^{RG2})-CHR^{RG4}]-L^{RG3}-$ or $-[C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}]-L^{RG3}-$ is optionally substituted  or is . In some embodiments, $-L^{RG1}-[C(=CHR^{RG2})-CHR^{RG4}]-L^{RG3}-$ or $-L^{RG1}-[C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}]-L^{RG3}-$ is optionally substituted . In some embodiments, $-L^{RG1}-[C(=CHR^{RG2})-CHR^{RG4}]-L^{RG3}-$ or $-L^{RG1}-[C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}]-L^{RG3}-$ is optionally substituted.

[00314] In some embodiments, the reactive group has a structure disclosed in TABLE 3-of the published International patent application WO 2024/228935 (Biohaven Therapeutics Ltd.).

[00315] In some embodiments, $-L^{LG4}-L^{RG2}-$ is –O–C(O)– or –S–C(O)–. In some embodiments, $-L^{LG4}-L^{RG1}-L^{RG2}-$ is –S–C(O)–.

[00316] In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-$, wherein N is a ring atom of an optionally substituted heteroaryl ring. In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-$, wherein N is a ring atom of L^{LG4} which is or comprises an optionally substituted heteroaryl ring. In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-O-$, wherein N is a ring atom of L^{LG4} which is or comprises an optionally substituted heteroaryl ring.

[00317] In some embodiments, L^{RG2} is optionally substituted $-CH_2-C(O)-$, wherein $-CH_2-$ is bonded to an electron-withdrawing group comprising or connected to an antibody-binding moiety. In some embodiments, L^{RG2} is optionally substituted $-CH_2-$ bonded to an electron-withdrawing group comprising or connected to an antibody-binding moiety. In some embodiments, L^{RG1} is an electron-withdrawing group. In some embodiments, L^{RG1} is selected from the Markush group consisting of $-C(O)-$, $-S(O)-$, $-S(O)_2-$, $-P(O(OR)-$, $-P(O(SR)-$, $-P(O(N(R)_2)-$, $-OP(O(OR)-$, $-OP(O(SR)-$, and $-OP(O(N(R)_2)-$.

[00318] In some embodiments, L^{RG2} is optionally substituted $-CH_2-C(O)-$, wherein $-CH_2-$ is bonded to a leaving group comprising or connected to an antibody-binding moiety. In some embodiments, L^{RG2} is optionally substituted $-CH_2-$ bonded to a leaving group comprising or connected to an antibody-binding moiety. In some embodiments, L^{RG1} is selected from the Markush group consisting of $-O-C(O)-$, $-OS(O)_2-$, $-OP(O(OR)-$, $-OP(O(SR)-$, and $-OP(O(N(R)_2)-$.

[00319] In some embodiments, a reactive group reacts with an amino group of a target agent. In some embodiments, an amino group is $-NH_2$ of the side chain of a lysine residue.

[00320] In some embodiments, a target agent is a protein agent. In some embodiments, a target agent is an antibody agent. In some embodiments, a reactive group reacts with an amino acid residue of this protein or antibody agent. In some embodiments, an amino acid residue is a lysine residue. In some embodiments, a reactive group reacts with $-NH_2$ of the side chain of a lysine residue. In some embodiments, a reactive group is or comprises $-C(O)-O-$, it reacts with $-NH_2$, e.g., of the side chain of a lysine residue), and forms an amide group $-C(O)-O-$ with the $-NH_2$.

[00321] In some embodiments, reactive groups, e.g., a first reactive group, a second reactive group, etc., are at terminal locations. In some embodiments, agents such as first agents comprise first reactive groups linked to target-binding moieties optionally through linker moieties, and do not have antibody-binding moieties.

[00322] In some embodiments, the invention provides methods for preparing a composition comprising a plurality of agents, wherein each agent independently comprises:

an antibody moiety,

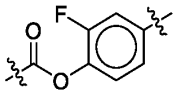
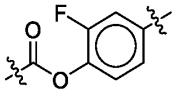
a cellular receptor-binding moiety, and

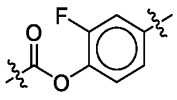
optionally a linker moiety linking an antibody moiety and a cellular receptor-binding moiety;

which method comprise:

contacting a plurality of agents each of which independently comprises a reactive group with a plurality of antibody agents.

[00323] In some embodiments, an agent comprising a reactive group comprises an antibody-binding moiety, a cellular receptor-binding moiety and optionally a linker. In some embodiments, agents comprising a reactive group share the same cellular receptor-binding moiety. In some embodiments, agents comprising a reactive group share the same structure. In some embodiments, antibody molecules are of such structures, properties or activities to provide antibody moieties in agents described in this specification. In some embodiments, a plurality of antibody molecules comprises two or more IgG subclasses. In some embodiments, a plurality of antibody molecules comprises IgG1. In some embodiments, a plurality of antibody molecules comprises IgG2. In some embodiments, a plurality of antibody molecules comprises IgG4. In some embodiments, a plurality of antibody molecules comprises IgG1 and IgG2. In some embodiments, a plurality of antibody molecules comprises IgG1, IgG2 and IgG4. In some embodiments, a plurality of antibody molecules comprises IgG1, IgG2, IgG3 and IgG4. In some embodiments, a plurality of antibody molecules is IVIG antibody molecules.

[00324] In some embodiments, provided agents comprise a reactive group, e.g., . In some embodiments, −C(O)− is connected to a cellular receptor-binding moiety, or a moiety comprising −(Xaa)y−, optionally through a linker and the other end is connected to an antibody-binding moiety. In some embodiments,  reacts with an amino group of another moiety, e.g., an antibody moiety, forming an amide group with the moiety and releasing a moiety which is or comprises antibody-binding moiety. In some embodiments, an amino group is $-NH_2$ of a lysine side chain. In some embodiments, −C(O)− is connected to a cellular receptor-binding moiety, or a moiety comprising −(Xaa)y−, optionally through a linker and the other end is connected to R' or an optional substituent. In

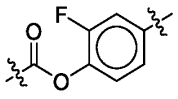
some embodiments, provided agents comprise optionally substituted  . Such reactive groups may be useful for conjugation with detection, diagnosis, or therapeutic agents. Persons having ordinary skill in the biomedical art know that many agents, and many technologies, e.g., click chemistry, reactions based on functional groups such as amino groups, e.g., amide formation), hydroxyl groups, carboxyl groups, etc. can be used for conjugation under this specification.

[00325] In some embodiments, antibody-binding moieties bind to Fc regions of antibodies. In some embodiments, reactions occur at residues at Fc regions. In some embodiments, target-binding moieties are conjugated to residues of Fc regions, optionally through linker moieties. In some embodiments, a residue is a Lys residue. In some embodiments, an antibody is or comprises IgG1. In some embodiments, an antibody is or comprises IgG2. In some embodiments, an antibody is or comprises IgG4. In some embodiments, an antibody composition used in a method comprises IgG1 and IgG2. In some embodiments, an antibody composition used in a method comprises IgG1, IgG2 and IgG4. In some embodiments, an antibody composition used in a method comprises IgG1, IgG2, IgG3 and IgG4.

[00326] In some embodiments, a product is or comprises IgG1. In some embodiments, a product is or comprises IgG2. In some embodiments, a product is or comprises IgG4. In some embodiments, a product composition comprises IgG1 and IgG2. In some embodiments, a product composition comprises IgG1, IgG2 and IgG4. In some embodiments, a product composition comprises IgG1, IgG2, IgG3 and IgG4.

[00327] In some embodiments, provided agents comprising antibody moieties provide one or more or substantially all antibody immune activities, e.g. for recruiting one or more types of immune cells or provide short-term and long-term immune activities. In some embodiments, provided agents comprising antibody moieties do not significantly reduce one or more or substantially all relevant antibody immune activities. In some embodiments, provided agents comprising antibody moieties improve one or more or substantially all relevant antibody immune activities, e.g., compared to antibody moieties by themselves. In some embodiments, provided agents provides comparable or better stability compared to antibody moieties by themselves, e.g., home time in blood. In some embodiments, antibody moieties in provided agents can bind to FcRy of immune cells, e.g., several FcRy of immune effector cells for desired immune activities, typically, at comparable or better levels. In some embodiments, antibody moieties in provided agents have comparable Fab/antigen-binding capabilities. In some embodiments, antibody moieties in provided agents have comparable Fab/antigen-binding capabilities. In some embodiments, antibody moieties in provided agents provide FcRn-binding. In some embodiments,

antibody moieties in provided agents provide FcRn-binding, e.g., for antibody recycle or prolonged half-life. In some embodiments, provided technologies are useful for changing blood-derived IgG products as provided technologies are suitable for and can use all IgG subclasses.

[00328] In some embodiments, a provided method comprises one step described below. In some embodiments,  reacts with an amino group of a lysine side chain to form an amide bond with an antibody molecule, and releases or a salt form thereof.

Pharmaceutically acceptable excipients.

[00329] Formulations suitable for parenteral administration, such as by intraarticular (in the joints), intravenous, intramuscular, intratumoral, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can have antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered by intravenous infusion, orally, topically, intraperitoneally, intravesically, or intrathecally. Parenteral administration, oral administration, and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[00330] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include these components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the change in tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Methods of assessing the chemical structure and function of the agent.

[00331] *HDX-MS* measures the exposure of hydrogen molecules on the surface of a protein or protein complex. The first step causes exposed hydrogen molecules to be readily exchanged with

deuterium. Hydrogen molecules buried within the structure, for example at the binding site of an antibody-antigen complex, are not exchanged as readily.

[00332] Comparing the deuterium uptake between the free and the complex states of proteins via mass spectrometry provides valuable information about the binding regions.

[00333] *Protein sequencing*. In one method of protein sequencing, eight digestions are prepared using five enzymes (Pepsin, Lys C, Trypsin, Chymotrypsin, Asp N). The digestions for the sample are processed with disulfide reduction, cysteine blocking, and then enzyme digestion. Digestions were analyzed by LC-MS/MS using a Thermo-Fisher Orbitrap fusion™ mass spectrometer. Peptides are characterized from LC -MS/MS data using de nova peptide sequencing and then assembled into antibody sequences.

[00334] *Competition ELISA assay protocol*.

[00335] (1) Dilute patient plasma samples at 1:100 in the provided ELISA sample buffer.

[00336] (2) Make the working stock solution for the degraders for a final concentration of 400 nM in 115 µl total reaction volume per well.

[00337] (3) Incubate 100 ul patient plasma sample with 15 ul of degrader for 2 hours at room temperature.

[00338] (4) Transfer 100 ul from this reaction to the EuroImmun anti-PLA2R ELISA plate.

[00339] (5) Also transfer the calibrators, and positive and negative controls to the plate.

[00340] (6) Incubate for 30 minutes at room temperature.

[00341] (7) Empty the wells and wash thrice with 300 ul/well of wash buffer.

[00342] (8) Pipette 100 ul of enzyme conjugate into each well of the ELISA plate.

[00343] (9) Incubate for 30 minutes at room temperature.

[00344] (10) Empty the wells and wash thrice with 300 ul/well of wash buffer.

[00345] (11) Pipette 100 ul of substrate solution into each well of the ELISA plate.

[00346] (12) Incubate for 15 minutes at room temperature.

[00347] (13) Pipette 100 ul of stop solution into each well of the ELISA plate.

[00348] (14) Measure absorbance at 450 nm within 30 minutes of adding the stop solution.

[00349] *ASGPR-mediated endocytosis of anti-PLA2R antibody*.

[00350] For routine culture of HEK-ASGPR cells, use DMEM high glucose with phenol + 10% FBS + 2 mM glutamine + 1% Pen Strep + 200 ug/ml geneticin.

[00351] For seeding of HEK-ASGPR cells in step 2, use DMEM high glucose without phenol + 10% FBS + 2 mM glutamine + 1% Pen Strep + 200 ug/ml geneticin.

[00352] (1) Harvest HEK-ASGPR1 cells using Accutase

[00353] (2) Seed 30,000 cells/well in 100 ul media/well in poly-D-lysine-coated black TC-treated 96-well plates

[00354] (3) The next day, label rabbit anti-PLA2R with anti-rabbit-F(ab)2-AF488 as follows (amounts for 4:1 molar ratio of anti-PLA2R:anti-rabbit-F(ab)2-AF488, with anti-PLA2R at 12.5 nM, for one 96-well plate):

[00355] (a) Add 11.6 ul 6B3-1 anti-PLA2R (@1.63 ug/ul) and 2.3 ul anti-rabbit-F(ab)2-Alexa Fluor488 (@ 1.5 ug/ul) to 10.4 ml Opti-MEM.

[00356] (b) Invert a couple of times to mix.

[00357] (c) Incubate for 30 min at 37 C in the dark, inverting to mix every 10-15 min.

[00358] (4) Prepare a 11-point titration series for the degraders using PBS, starting at 20 uM top concentration with three-fold serial dilutions

[00359] (5) Remove media from the plate by flicking and dispense 90 ul/well of the anti-PLA2R + anti-rabbit-F(ab)2-AlexaFluor488 mix at the slowest dispense speed

[00360] (6) Add 10 ul/well of the degrader titration series to achieve a final top concentration of 2 uM

[00361] (7) Place plates in the IncuCyte and scan at 0, 3, 6, 9, 12, 15, 18, 21 and 24 hours

[00362] (8) Analyze the images to obtain green fluorescent area per image using the following algorithm parameters: Surface fit segmentation, 0.75 threshold, edge split off, 0.5 μm^2 hole fill.

TABLE 7

Reagents	Vendor	Cat #
DMEM high glucose with phenol	Gibco	11960044
DMEM high glucose without phenol	Gibco	31053028
Opti-MEM Reduced serum medium	Thermo Fisher (Gibco)	31985062
Geneticin 50mg/ml	Gibco	10131-035
FBS heat inactivated	R&D systems (Optima)	S12450H
Glutamine	Corning	25-005-CI
Penicillin Streptomycin	Corning	30-002-CI
Accutase	Stemcell technologies	07920
96-well black, flat clear bottom, poly-D lysine coated	Corning	3842
Anti-PLA2R antibody	GenScript	

TABLE 7		
Reagents	Vendor	Cat #
Alexa Fluor® 488 AffiniPure™ Goat Anti-Rabbit IgG, F(ab')$_2$ fragment specific	Jackson ImmunoResearch	111-545-006

Immunoglobulin Sequence Analysis

[00363] *Sequence Analysis Tools*. The following free online tools for sequence analysis of immunoglobulin variable regions: (1) NCBI Nucleotide BLAST, (2) IMGT/V Quest program, and (3) NCBI IgBLAST.

[00364] *Sequence Analysis Materials*. B cells prepared by GenScript; RNA-easy Isolation Reagent (Vazyme, Cat. No. : R701-01-AA); PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Cat. No.: 6110A).

[00365] *Sequence Analysis Methods*. Total RNA was isolated from the B cells following the technical manual of RNA-easy Isolation Reagent. Total RNA was then reverse-transcribed into cDNA using either isotype-specific anti-sense primers or universal primers following the technical manual of PrimeScript™ 1st Strand cDNA Synthesis Kit. Antibody fragments of heavy chain and light chain were amplified using specific primers according to the standard operating procedure (SOP) of GenScript. Amplified antibody fragments were cloned into a standard cloning vector separately. Colony PCR was performed to screen for clones with inserts of correct sizes. The consensus sequence was provided.

[00366] *Phospholipase A2 receptor (PLA2R) antibody, IgG with reflex to titer*. This commercially-available assay aids in the differential diagnosis of membranous glomerulonephritis (MGN) or nephrotic syndrome of unknown etiology. This assay is commercially-available from ARUP Laboratories. Component Test Code* 2011829. Component Chart Name, Phospholipase A2 Receptor, IgG. LOINC. 82991-1. This assay is New York State approved.

[00367] *Patient Preparation*. Instructions patient must follow before/during specimen collection.

[00368] *Collect*. Specimen type to collect. May include collection media, tubes, kits, etc.

[00369] *Serum Separator Tube*. Specimen preparation, instructions for specimen prep before/after collection and prior to transport. Separate serum from cells ASAP or within two hours of collection. Transfer 1 mL serum to an ARUP Standard Transport Tube. (minimum 0.2 mL).

[00370] *Processes used to perform the test*. Semi-Quantitative Cell-Based Indirect Fluorescent Antibody.

[00371] *Additional information related to the* test. If phospholipase A2 receptor antibody, IgG is positive, then a phospholipase receptor A2 antibody, IgG titer can be added.

Statistical analyses.

[00372] Normal distribution quantitative variables can be expressed as means and standard deviations and compared by an independent-samples t test. For non-normally distributed variables, we used median and interquartile range and analyzed with the Mann-Whitney U test. Categorical data is summarized by percentages. A two-sided p value <0.05 is statistically significant. All statistical tests are performed using SPSS version 16.0.

EXAMPLES

[00373] The invention is further illustrated by non-limiting EXAMPLES.

EXAMPLE 1

Bifunctional degrader directed to anti-PLA2R Abs in idiopathic membranous nephropathy.

[00374] Anti-PLA2R degraders were constructed using the reported 31-mer peptide ligand as the antibody-binding moiety. Depleting anti-PLA2R antibodies with a degrader should result in remission of idiopathic membranous nephropathy faster and safer than immunosuppressive therapy. Depleting anti-PLA2R antibodies could treat patients unresponsive to immunosuppressants.

Material generation.

[00375] The inventors constructed PLA2R bifunctional degraders.

TABLE 8

Moiety	aPLA2R-binding moiety	ASGPR-binding moiety	K_D (nM) as assayed by surface plasmon resonance
ABT305	31-mer	N/A	7
AGN306	31-mer	βGN3	23 ± 1
ABT301	31-mer (bicyclic)	N/A	0.3 ± 2
AGN302	31-mer (bicyclic)	βGN3	10 ± 3
AGN304	31-mer (bicyclic)	CF_3 pyrazine	3 ± 0.3
AGN303	31-mer (bicyclic)	αGN3	7 ± 1
AGN308	31-mer	CF_3 pyrazine	9 ± 2

Measure uptake in vitro.

[00376] The inventors have successfully measured the uptake of anti-PLA2R antibodies and biodegraders in HepG2 assays. The inventors now have an assay to measure antibody degradation in vitro. The inventors assaying and analyzing patient plasma in this assay.

[00377] The inventors are demonstrating the biophysical characterization of peptide alone and degrader to monoclonal anti-PLA2R antibody and patient anti-PLA2R antibody. The inventors are proceeding to measure competition of idiopathic membranous nephropathy anti-PLA2R to PLA2R by Meso Scale Discovery.

In vitro toxicity assays.

[00378] The inventors successfully completed a hemagglutination assay using bifunctional degraders.

TABLE 9
PLA2R degrader hemagglutinin activity assay

Agent	PLA2R-binding moiety	ASGPR ligand	50 µM	500 µM
AGN306	WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K (31-mer)	βGN	+	-
AGN307	WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K (31-mer)	αGN3	+	-
AGN308	WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K (31-mer)	CF3 pyrazine	-	+
ABT305	WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K (31-mer)	None	-	+
AGN302	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	βGN	-	-
AGN303	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	αGN3	-	-
AGN304	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	CF3 pyrazine	-	+
ABT301	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	None	-	-
ABT309	VIQSESLKK(CIQAGKSVLTLENKC)K	None	-	-

[00379] Both N-C cyclization and shortened peptide (**ABT309**) show no hemagglutinin activity at 50/500 µM.

[00380] **AGN306** (βGN3) shows hemagglutinin activity in mice, rats, and humans. **AGN302** (βGN3) only shows moderate hemagglutinin activity in rats.

TABLE 10
AGN303 and peptide alone show HA activity at 5 mM

Compound	PLA2R ligand	ASGPR ligand	5 mM	500 µM
AGN303	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	αGN3	+	-
ABT301	[WQDKGIFVIQSESLKK(CIQAGKSVLTLENKC)K](bic 31-mer)	None	+	-
ABT309**	VIQSESLKK(CIQAGKSVLTLENKC)K	None	+	-
ABT310**	VIQSES [SEQ ID NO: 10]	None	+	-
ABT311**	SVLTENCK [SEQ ID NO: 11]	None	+	-
** Insoluble at 5 mM				

[00381] The inventors successfully completed a proliferation and cytotoxicity assay using bifunctional degraders.

[00382] The inventors will be performing assays of PBMC cytokine release using standard assays known to persons having ordinary skill in the art.

EXAMPLE 2

Depletion of anti-PLA2R antibody in vivo.

[00383] The inventors are demonstrating the ability of the degraders to deplete anti-PLA2R antibody *in vivo* using the in vitro-validated anti-PLA2R degraders.

[00384] The inventors measured depletion of anti-PLA2R antibody in mice using *in vitro*-validated anti-PLA2R bifunctional degraders. The inventors have successfully completed assays of murine tolerability of the degraders.

[00385] The inventors are proceeding with assays of mouse pharmacokinetics/ pharmacodynamics. Mouse pharmacokinetics/ pharmacodynamics studies were run using **AGN303** (αGN3) and **AGN302** (βGN3). Mice were dosed at 0.1 mg/kg rabbit anti-PLA2R antibody (commercially available antibody that binds to the 31-mer). Degraders were dosed at 0.3 or 1 mg/kg, intravenous or subcutaneous. Degraders rapidly depleted anti-PLA2R from plasma for all doses tested. An antibody rebound effect was observed at four-eight hours.

EXAMPLE 3

Mouse maximum tolerated dose (MTD).

[00386] **AGN303** (αGN3) was better tolerated intravenously than **AGN302** (βGN3). Clinical observations at 25 and 50 milligram/kilogram were mild and transient. Mice were fully recovered by end of the assay.

[00387] **AGN303** (αGN3)-treated mice showed severe toxic clinical signs at 100 milligram/kilogram (+++) when dosed intravenously: low motility, hunched posture, cyanosis, and coldness.

[00388] **AGN303** (αGN3)-treated mice showed no clinical signs of toxicity up to 100 milligram/kilogram when dosed subcutaneously.

EXAMPLE 4

Assays to characterize activities of anti-PLA2R bifunctional degraders.

[00377] *GN3 sortase reagent*. A basic protocol for sortase conjugation including the ASPGR binder for the sortase. C-terminal sortase tag (LPETGG) for conjugation (GN3/linker).

[00378] *Surface plasmon resonance*. Direct binding of anti-PLA2R antibody binding moiety to anti-PLA2R antibody was determined using Biacore™ instruments and IBIS-MX96 systems. Anti-PLA2R antibody was immobilized by amine coupling to the surface of a sensor chip as the ligand. Anti-PLA2R antibody binding moiety is flowed over in solution as analyte. Binding affinity was determined using single-cycle kinetics, e.g., by bio-layer interferometry (ForteBio™oCtet™ systems).

TABLE 11

Compound	aPLA2R SPR SCK KD (nM)	Theoretical binding ratio	Immobili-zation (RU)	Experimental Rmax (RU)	Theoretical Rmax (RU)	Active Surface
Peptides						
ABT305	6.9					0.96
ABT301	0.26					0.92
ABT309	No binding					
Degraders						
AGN306	22.7					0.80
AGN306	32.7					0.40
AGN306	23.5					0.75
AGN308	51.6					0.27
AGN308	6.72					0.81
AGN308	9.45					0.86
AGN308	15.7					0.26
AGN308	9.64					0.41

TABLE 11

Compound	aPLA2R SPR SCK KD (nM)	Theoretical binding ratio	Immobili-zation (RU)	Experimental Rmax (RU)	Theoretical Rmax (RU)	Active Surface
AGN308	14.4					0.28
AGN307	27.8					0.37
AGN307	41.8					0.27
AGN309	17.8					0.26
AGN310	45.5					0.27
AGN302	11.9					0.81
AGN302	7.86					0.43
AGN304	2.75					
AGN304	3.17					
AGN303	7.69					
AGN303	6.34					
AGN364*	136	2:1	416.8	38.5	124.7	0.31
AGN364*	219	2:1	454	37.2	135.8	0.27
AGN365*	959	2:1	416.8	39.6	249.6	0.16
AGN365*	661	2:1	454	49	271.9	0.18
AGN579*	451	1:1	382	55.5	358	0.16
AGN579*	1240	1:1	429	36.8	402	0.09
AGN579*	1150	1:1	418	41.1	392	0.10
*Ligand: Boster Bio pAb						

[00379] *GN3-maleimide reagent* (maleimide-GN3/linker), with a C-terminal Cys for conjugation.

[00380] *Biophysical/biochemical potency assay to measure target-engagement*. A biomolecule coupled to surface of sensor chip as ligand. As analyte is flowed in solution over immobilized ligand, binding to the sensor chip surface induces a change in refractive index proportional to bound mass.

[00381] *ASGPR-dependent uptake assay*. An HEK293 fluorescent cell-based assay was used to assess antibody uptake by cells in vitro. This on-mechanism endocytosis assay measures the anti-PLA2R antibody accumulation in HEK293 cells.

[00382] Other off-target assays include measurement of cytotoxicity through In vitro toxicity with HEPG2 cells, e.g., in a CellTiter-Glo assay, measurement of hemagglutination by red blood cell interactions, measurement of PMBC, and measurement of off-target bindings in a house C-type lectin panel. Signal is boosted in the HEK293 versus HepG2 cells when measuring signal accumulation not degradation. Western assays can measure the degradation in HEK cells and other cell lines.

[00383] *Ternary complex formation*. Formation of a ternary complex of anti-PLA2R antibody and ASGPR mediated by anti-PLA2R bifunctional molecules was determined.

TABLE 12

Degrader	Ternary Complex HTRF (max signal:noise)	Ternary Complex [degrader] at max signal (nM)	Ternary Complex EC_{50} (nM)
AGN306	9.2	78	5.6
AGN306	6.9	39	2.6
AGN306	5	78	2.3
AGN308	33.4	19	2.8
AGN308	27.6	19	2.3
AGN307	13.5	19	1.3
AGN307	12	39	0.9
AGN309	17.1	39	
AGN309	15.1	78	1.3
AGN310	3.7	156	
AGN310	3.5	156	5.2
AGN302	7.5	2.5	
AGN302	6.1	9	2
AGN304	35.3	5	0.9
AGN304	30	9	1
AGN303	16.9	5	0.7
AGN303	14.3	19	1.3

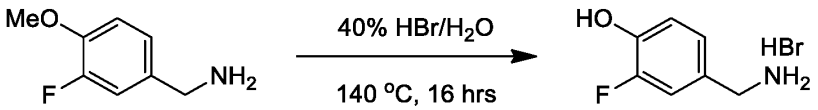
[00384] *Animal models*. Testing for antidrug antibodies (ADA) can be done in mouse models. See Meyer-Schwesinger et al., Kidney International, 97(5), 913-919 (2020). Many of the ADAs are IgG4 , such as those in Factor 8 hemophilia.

EXAMPLE 5

*Preparation of **TBT307** - A*

*Preparation of **Building block #1:***

*Preparation of **Intermediate-26**:*

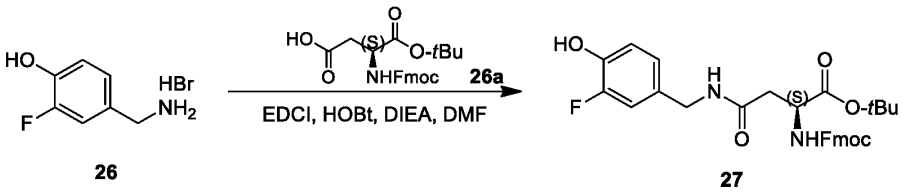


25 **26**

[00385] A mixture of **Intermediate-25** (75.0 g, 64.4 mmol) in HBr/water (40% HBr, 1000 mL in total) was stirred at 140°C for sixteen hours. The solvent was removed at 70°C under reduced pressure. The residue was triturated in MeCN (50 mL) for ten minutes. After filtration, the solid was dried under lyophilization to afford **Intermediate-26** (100.0 g, 427.9 mmol, 88.4% yield, HBr salt) as a brown solid. ^{1}H

NMR (400 MHz, DMSO-d_6) δ ppm 10.03 (s, 1 H) 8.20 (s, 3 H) 7.32 (dd, *J* = 12.17, 1.88 Hz, 1 H) 7.11 (dd, *J* = 8.28, 1.51 Hz, 1 H) 6.95-7.03 (m, 1 H) 3.93 (q, *J* = 5.27 Hz, 2 H).

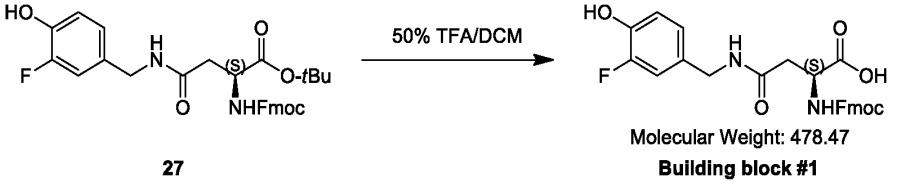
Preparation of ***Intermediate-27***:



26 **27**

[00386] To a mixture of **Intermediate-26** (60.0 g, 270.2 mmol, 1.00 equivalent, HBr salt), **Reactant 26a** (111.1 g, 270.2 mmol, 1.00 equivalent), DIEA (34.9 g, 270.2 mmol, 47.0 mL, 1.00 equivalent) and HOBt (54.7 g, 405.3 mmol, 1.50 equivalents) in DMF (1 L) was added EDCI (56.7 g, 297.2 mmol, 1.10 equivalents) at 20°C. The mixture was stirred at 20°C for three hours. The mixture was precipitated with 0.5 M HCl (cold, 10 L). After filtration, the solid was dissolved in DCM (2 L), washed with 0.5 M HCl (800 mL), water (800 mL), brine (800 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column (DCM/methanol = from 1/0 to 20/1) to afford **Intermediate-27** (120.0 g, 90% purity, containing a little DMF, 83.3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.70 (s, 1 H) 8.34 (t, *J* = 5.77 Hz, 1 H) 7.90 (d, *J* = 7.53 Hz, 2 H) 7.71 (d, *J* = 7.53 Hz, 2 H) 7.61 (d, *J* = 8.28 Hz, 1 H) 7.39-7.47 (m, 2 H) 7.29-7.36 (m, 2 H) 7.02 (d, *J* = 12.30 Hz, 1 H) 6.85-6.92 (m, 2 H) 4.20-4.39 (m, 4 H) 4.11-4.19 (m, 2 H) 1.36 (s, 9 H).

Preparation of ***Building block #1***:



27

Molecular Weight: 478.47

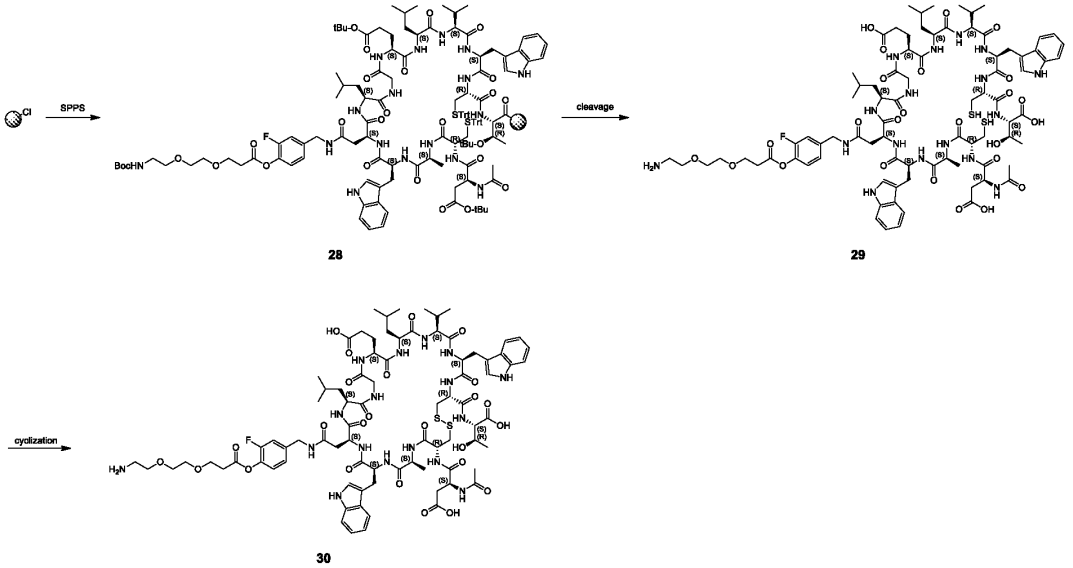
Building block #1

[00387] A mixture of **Intermediate-27** (120.0 g, 224.4 mmol, 1.00 equivalent) in trifluoroacetic acid (600 mL) and DCM (600 mL) was stirred at 20°C for one half hour. The solvent was removed under reduced pressure. The residue was purified by silica gel column (DCM/methanol = from 1/0 to 10/1) to afford **Building block #1** (100.0 g, 93.5% purity, 93.1% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.69 (s, 1 H) 8.34 (t, *J* = 5.90 Hz, 1 H) 7.90 (d, *J* = 7.28 Hz, 2 H) 7.71 (d, *J* = 7.53 Hz, 2 H) 7.54 (d, *J* = 6.53 Hz, 1 H) 7.42 (t, *J* = 7.40 Hz, 2 H) 7.27-7.37 (m, 1 H) 7.27-7.37 (m, 1 H) 7.02 (d, *J* = 12.05 Hz, 1 H) 6.82-6.93 (m, 2 H) 4.35-4.43 (m, 1 H) 4.20-4.31 (m, 3 H) 4.13-4.19 (m, 2 H).

EXAMPLE 6

*Preparation of **TBT307** – B*

*Preparation of **Intermediate-30**:*



[00388] The peptide was synthesized using standard Fmoc chemistry (CTC resin).

[00389] (1) *Resin preparation*: To the vessel containing CTC resin (2.0 g, 2.0 mmol, 1.00 mmol/g) and Fmoc-Thr(tBu)-OH (795 mg, 2.0 mmol, 1.00 equivalent) in DCM (50 mL) was added DIEA (4.00 equivalents) dropwise and mixed for two hours with nitrogen gas bubbling at 25°C. Then methanol (2 mL) was added and bubbled with nitrogen gas for another thirty minutes. The resin was washed with DMF (100 mL) * 5, followed by the addition of 20% piperidine in DMF (100 mL) and bubbled with nitrogen gas for thirty minutes at 25°C for Fmoc deprotection. The mixture was filtered. The resin was washed with DMF (100 mL) * 5 before proceeding to next step.

[00390] (2) *Coupling*: A solution of Fmoc-Cys(Trt)-OH (3.51 g, 6.0 mmol, 3.00 equivalents), HBTU (2.16 g, 5.7 mmol, 2.85 equivalents) in DMF (50 mL) was added to the resin with nitrogen gas bubbling. Then DIEA (6.00 equivalents) was added to the mixture dropwise and bubbled with nitrogen gas for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (100 mL) * 5.

[00391] (3) *Deprotection*: 20% piperidine in DMF (100 mL) was added to the resin and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The resin was then washed with DMF (100 mL) * 5.

[00392] (4) Steps 2 and 3 were repeated for the following amino acids elongation: Number # 3-15, TABLE 13.

[00393] (5) After all the steps were completed, the resin was washed with DMF (100 mL) * 5, methanol (100 mL) * 5, then dried under reduced pressure to afford resin-bound peptide **Intermediate-28** (CTC resin, 2.0 mmol).

TABLE 13
List of amino acids and the corresponding reagents used on solid-phase peptide synthesis

#	Materials	Coupling reagents
1	Fmoc-Thr(*t*Bu)-OH (3.00 equivalents)	DIEA (4.00 equivalents)
2	Fmoc-Cys(Trt)-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Trp-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
4	Fmoc-Val-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
5	Fmoc-Leu-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
6	Fmoc-Glu(O*t*Bu)-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
7	Fmoc-Gly-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
8	Fmoc-Leu-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
9	**Building block #1** (2.00 equivalents)	DIC (2.00 equiv.) and HOBt (2.00 equiv.)
10	Fmoc-Trp-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOBt (3.00 equiv.)
11	Fmoc-Ala-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOBt (3.00 equiv.)
12	Fmoc-Cys(Trt)-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOBt (3.00 equiv.)
13	Fmoc-Asp(O*t*Bu)-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOBt (3.00 equiv.)
14	AC	Ac_2O:NMM:DMF=10:5:85
15	Boc-PEG_2-OH (3.00 equivalents)	DIC:HOBt:DMAP=3:3:3

Peptide cleavage and cyclization:

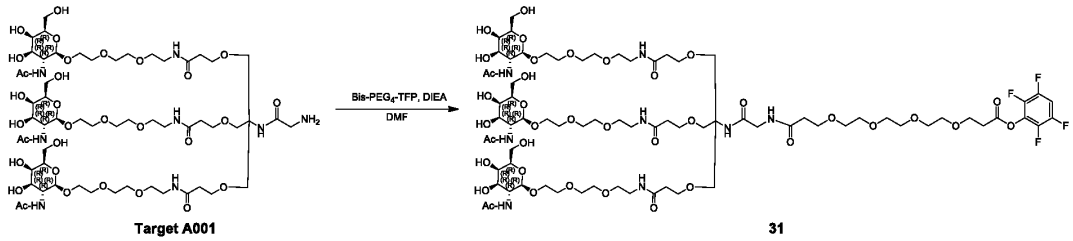
[00394] (1) Cleavage solution (trifluoroacetic acid/TIS/water, 95/2.5/2.5, v/v/v, 500 mL) was added to the flask containing the side-chain protected resin-bound peptide (CTC resin, 5 g, 2.0 mmol) at 25°C and stirred for two hours.

[00395] (2) After filtration, the filtrate was collected.

[00396] (3) The filtrate was precipitated with cold isopropyl ether (2.5 L). After filtration, the solid was washed with isopropyl ether (2.5 L) twice, and the crude peptide was dried under reduced pressure for two hours to afford **Intermediate-29** (3.0 g, crude) as a white solid.

[00397] To the mixture of **Intermediate-29** (3.0 g, crude) in HOAc/MeCN/water (4/3/3, v/v/v, 2 L) was added 0.1 M I_2/AcOH dropwise until a yellow color persisted, then the mixture was stirred at 25°C for five minutes. The mixture was quenched by addition of 0.1 M aq. $Na_2S_2O_3$ dropwise until the yellow color disappeared. After filtration, the filtrate was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN), followed by lyophilization to afford **Intermediate-30** (360 mg, 90.0% purity, 50.8% yield) as a white solid. LCMS: retention time = 0.907=0.960 minutes, MS calculated: M_{av} = 1833.02, mass observed: $[M + 2H]^{2+}$ =917.00, $[M + H]^{+}$ =1832.91.

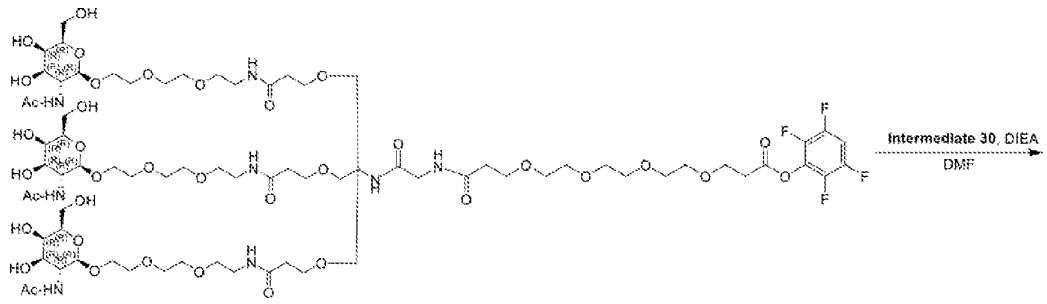
*Preparation of **Intermediate-31***:



Target A001 **31**

[00398] To a solution of **Bis-PEG4-TFP** (2.53 g, 5.00 equivalents) in DMF (25 mL) was added a mixture of **Target A001** (1.2 g, 1.00 equivalent) and DIEA (332.9 mg, 449.9 µL, 3 equivalents) in 10 mL DMF at 0°C. The resulting reaction was stirred for five minutes at 0°C. After completion monitored by LC-MS, the mixture was directly injected into the reverse column, purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN), followed by lyophilization to afford **Intermediate-31** (700 mg, 95.0% purity, 57.5% yield) as colorless oil. LCMS: retention time = 0.709 minutes, MS calculated: M_{av} = 1821.81, mass observed: $[M+16 + 3H]^{3+}$ =613.10.

*Preparation of **TBT307**:*



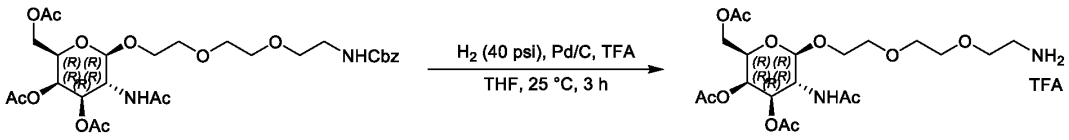
31

[00399] To a solution of **Intermediate-30** (140.86 mg, 76.85 µmol, 1.00 equivalent) and **Intermediate-31** (140 mg, 76.85 µmol, 1.00 equivalent) in DMF (2 mL) was added DIEA (29.80 mg, 230.54 µmol, 40.16 µL, 3.00 equivalents). The mixture was stirred at 20°C for one hour. After completion monitored by LC-MS, the mixture was acidified by 1 M HCl to pH = 5. The mixture was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN) directly, followed by lyophilization to afford **TBT307** (103 mg, 92.8% purity, 35.6% yield.) as a white solid. LCMS: retention time = 1.391 minutes, MS calculated: M_{av} = 3488.76, mass observed: $[M + 3H]^{3+}$ =1163.90.

EXAMPLE 7

*Procedure for Preparation of **Intermediate-27**:*

*Preparation of **Intermediate-2**:*

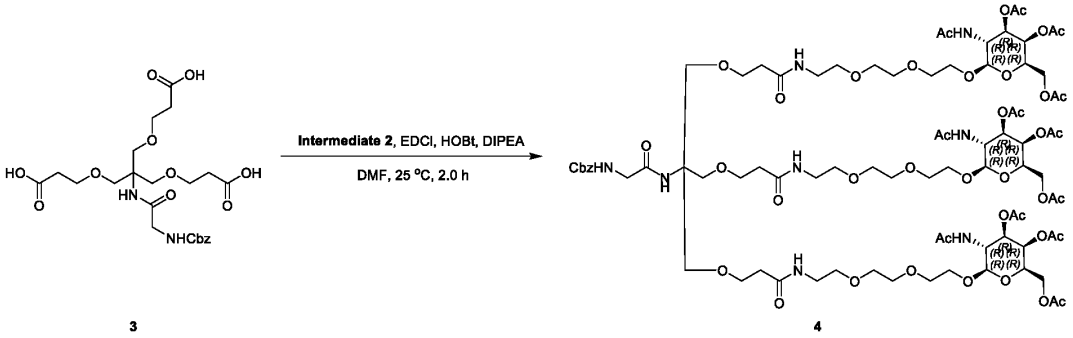


1 2

[00400] Pd/C (4.00 g, 10% purity) in reaction bottle (purged with argon gas for three times) was added THF (40 mL) slowly, then a solution of trifluoroacetic acid (7.44g, 65.2 mmol, 4.86 mL, one equivalent) and **Intermediate-1** (40.0 g, 65.2 mmol, 1.00 equivalent) in THF (360 mL) was added to the reaction slowly under nitrogen gas. The reaction was degassed and purged with argon gas and hydrogen gas for three times, then stirred at 25°C for three hours under hydrogen gas atmosphere (40 psi). TLC (DCM: methanol = 10: 1, R_f = 0.20) indicated **Intermediate-1** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was dissolved in THF (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL

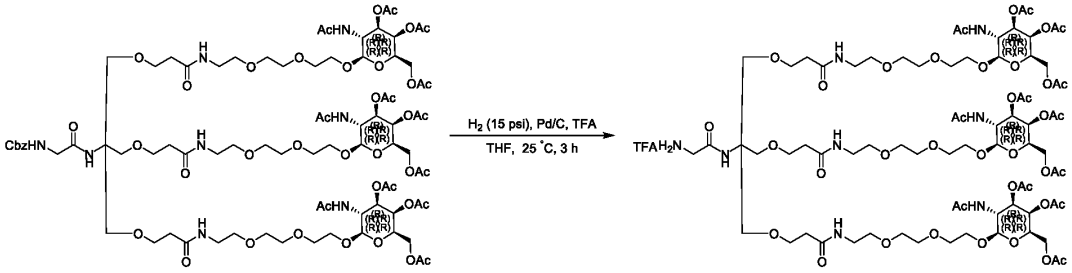
* 2), and the filtrate was concentrated under reduced pressure to get **Intermediate-2** (38.7 g, 38.5 mmol, 59.0% yield, 59.0% purity, trifluoroacetic acid salt) as a white solid. LCMS: retention time = 0.428 minutes, MS calculated: 478.22, mass observed: $[M + Na]^+$ = 501.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.79-7.91 (m, 3 H), 5.16-5.28 (m, 1 H), 4.97 (br dd, *J* = 11.07, 2.81 Hz, 1 H), 4.54 (br d, *J* = 8.50 Hz, 1 H), 4.03 (s, 2 H), 3.77-3.93 (m, 2 H), 3.53-3.62 (m, 8 H), 2.98 (br d, *J* = 5.00 Hz, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

*Preparation of **Intermediate-4***:



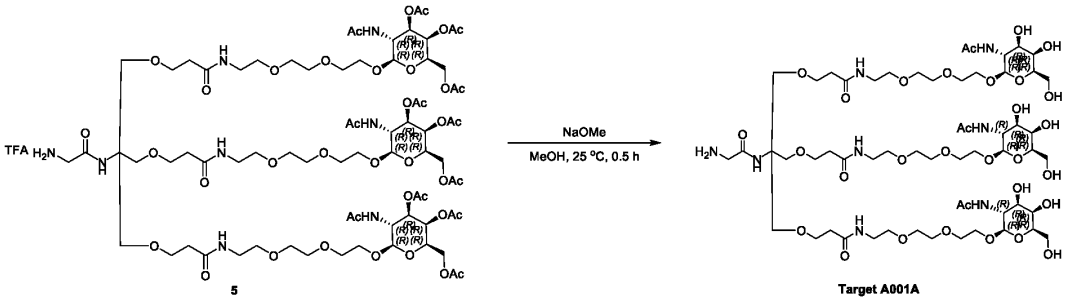
[00401] To a stirring solution of **Intermediate-3** (8.50 g, 16.0 mmol, 1.00 equivalent) and **Intermediate-2** (38.1 g, 64.3 mmol, 4.00 equivalents, trifluoroacetic acid salt) in DMF (17 mL) and DCM (340 mL) was added HOBT (8.69 g, 64.3 mmol, 4.00 equivalents), EDCI (12.3 g, 64.3 mmol, 4.00 equivalents) and DIEA (9.35 g, 72.3 mmol, 4.50 equivalents) successively. The reaction was stirred at 25°C for two hours. TLC (DCM: methanol = 10: 1, R_f = 0.5) indicated **Intermediate-3** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was slowly poured into a stirring cold 1.0 mol/L HCl solution (350 mL) and stirred for ten minutes. A white precipitate formed and was filtered. The aqueous phase was extracted with DCM (350 mL* 2) twice. The combined organic layers were washed with sat. $NaHCO_3$ (350 mL), dried over Na_2SO_4, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM:methanol = 100: 1 to 15: 1) to afford **Intermediate-4** (22.0 g, 10.6 mmol, 66.0% yield, 92.2% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (br t, *J* = 5.32 Hz, 3 H), 7.81 (d, *J* = 9.26 Hz, 3 H), 7.26-7.49 (m, 6 H), 7.12 (s, 1 H), 5.21 (d, *J* = 3.25 Hz, 3 H), 5.02 (s, 2 H), 4.97 (dd, *J* = 11.13, 3.25 Hz, 3 H), 4.55 (d, *J* = 8.38 Hz, 3 H), 4.03 (s, 9 H), 3.84-3.92 (m, 3 H), 3.74-3.82 (m, 3 H), 3.47-3.65 (m, 38 H), 3.39 (br s, 3 H), 3.20 (q, *J* = 5.42 Hz, 6 H), 2.30 (br t, *J* = 6.13 Hz, 6 H), 2.10 (s, 9 H), 1.99 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H). LCMS: retention time = 0.403 minutes, MS calculated: 1908.81, mass observed: $[M + 2H]^{2+}$ = 1910.2.

*Preparation of **Intermediate-5***:



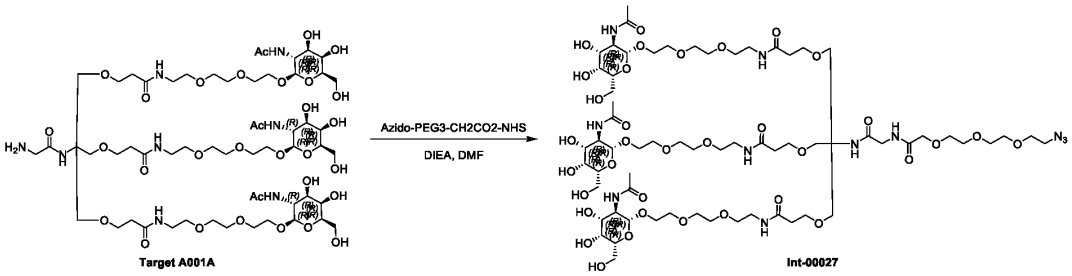
[00402] The 500 mL round-bottom flask was purged with argon gas for three times and added dry Pd/C (1.60 g, 1.50 mmol, 10% purity, 0.17 equivalents) carefully. Then THF (60.0 mL) was added to infiltrate the Pd/C completely, followed by the solution of **Intermediate-4** (16.4 g, 8.59 mmol, 1.00 equivalent) and trifluoroacetic acid (979 mg, 8.59 mmol, 637.8 µL, 1.00 equivalent) in THF (100 mL) slowly under argon gas atmosphere. The resulting mixture was degassed and purged with hydrogen gas for three times, and then the mixture was stirred at 25°C for three hours under hydrogen gas atmosphere (15 psi). TLC (DCM:methanol = 10: 1, Rf = 0.6) indicated **Intermediate-9** was consumed completely, and one major new spot was detected. The reaction mixture was filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (50 mL*2). The filter cake was added water immediately. The organic layer concentrated under reduced pressure to afford **Intermediate-5** (15.7 g, 6.78 mmol, 78.9% yield, 81.6% purity, trifluoroacetic acid salt) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91-7.98 (m, 5 H), 7.82 (br d, *J* = 9.13 Hz, 3 H), 7.74 (s, 1 H), 7.12-7.28 (m, 1 H), 5.22 (br d, J = 2.63 Hz, 3 H), 4.97 (br dd, *J* = 11.13, 2.75 Hz, 3 H), 4.54 (d, *J* = 8.51 Hz, 3 H), 3.84-3.93 (m, 3 H), 3.78 (br dd, *J* = 10.13, 4.88 Hz, 3 H), 3.53-3.62 (m, 22 H), 3.33-3.45 (m, 22 H), 3.18-3.25 (m, 6 H), 2.31 (br t, *J* = 6.13 Hz, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.73-1.81 (m, 14 H). LCMS: retention time = 0.341 minutes, MS calculated: 1774.7, found: $[M + 2H]^{2+}$ = 1776.9.

*Preparation of **Target-A001A**:*



[00403] To a solution of **Intermediate-5** (5.5 g, 2.91 mmol, 1.00 equivalent, trifluoroacetic acid) in methanol (50.0 mL) was added NaOMe (707 mg, 13.1 mmol, 4.50 equivalents) at 0°C. The mixture was stirred at 0°C for 0.5 hour. The reaction was monitored by LCMS, which showed the desired mass (one main peak with desired was detected.). The reaction mixture was added with 1.0 M HCl solution till the pH = 6. The mixture was diluted with water (75.0 mL) and extracted with DCM (120 mL * 3). The mixture was freeze-dried to afford **Target-A001A** (4.1 g, 2.74 mmol, 94.1% yield, 93.4% purity, HCl) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.94 (br t, *J* = 5.50 Hz, 3 H), 7.62 (d, *J* = 9.01 Hz, 3 H), 7.54 (s, 1 H), 4.28 (d, *J* = 8.50 Hz, 3 H), 3.70-3.88 (m, 6 H), 3.61-3.70 (m, 6 H), 3.49-3.57 (m, 31 H), 3.38-3.43 (m, 12 H), 3.31 (br d, *J* = 6.25 Hz, 4 H), 3.17 (s, 6 H), 3.09 (s, 2 H), 2.30 (br t, *J* = 6.32 Hz, 6 H), 1.80 (s, 9 H). LCMS: retention time = 0.22 minutes, MS calculated: 1396.6, found: $[M + H]^+$ = 1397.8.

*Preparation of **Intermediate-27**:*

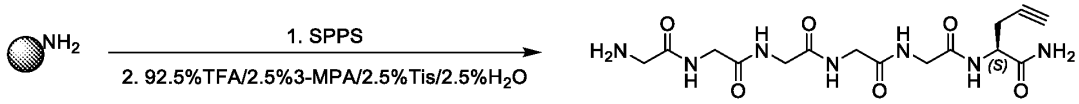


[00404] To a solution of **Target-A001A** (2.00 g, 1.43 mmol, 1.00 equivalent) and **Azido-PEG3-CH2CO2-NHS** (567 mg, 1.71 mmol, 1.20 equivalents) in DMF (20 mL) was added DIEA (554 mg, 4.29 mmol, 709 µL, 3.00 equivalents) at 0°C. The mixture was stirred at 0°C for two hours under nitrogen gas atmosphere. LCMS indicated **Target-A001A** was consumed completely, one main peak with desired MS was detected. The residue was purified by prep-high performance liquid chromatography (AcOH condition) directly to afford **Intermediate-27** (1.75 g, 1.04 mmol, 73.2% yield, 96.5% purity) as a white solid. LCMS: retention time = 0.286 minutes, MS calculated: M_{av} = 1612.68, $[M + 2H]^{2+}$ = 807.1, $[M\text{-sugar} + 2H]^{2+}$ = 705.6.

EXAMPLE 8

*Procedure for Preparation of **TBT544***:

*Preparation of **Intermediate-37***:



Int-00037

[00405] Peptide was synthesized using standard Fmoc chemistry (C resin).

[00406] (1) *Resin preparation*: DMF (100 mL) was added to the vessel containing Rink MBHA-Amide Resin (5.00 mmol, 16.7 g, 0.30 mmol/g) with nitrogen gas bubbling for thirty minutes. The resin was washed with DMF (100 mL * 5), followed by adding 20% piperidine in DMF (50 mL) and bubbled with nitrogen gas for thirty minutes at 25°C for Fmoc deprotection. The mixture was filtered. The resin was washed with DMF (100 mL * 5) before proceeding to next step.

[00407] (2) *Coupling*: A solution of Fmoc-Pra-OH (3.00 equivalents), HATU (2.85 equivalents) in DMF (50 mL) was added to the resin with nitrogen gas bubbling. Then DIEA (6.00 equivalents) was added to the mixture dropwise and bubbled with nitrogen gas for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (100 mL) * 5.

[00408] (3) *Deprotection*: 20% piperidine in DMF (50 mL) was added to the resin and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The resin was then washed with DMF (100 mL) * 5. The deprotection reaction was monitored by ninhydrin test. If it showed blue or brownish red, the reaction was completed.

[00409] (4) Steps 2 and 3 were repeated for the following amino acids elongation: Number # 2-3, in TABLE 14.

[00410] (5) After the last position completed, the resin was then washed with DMF (100 mL) * 5, methanol (100 mL) * 5, and dried under reduced pressure to afford peptide-bound-resin (5 mmol).

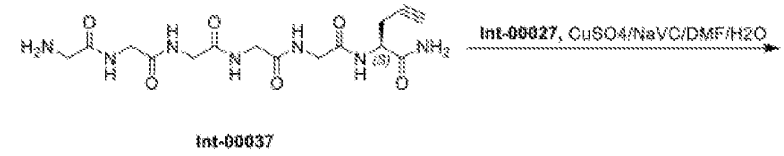
TABLE 14
The list of amino acids and the corresponding reagents used on solid-phase peptide synthesis

#	Materials	Coupling reagents
1	Fmoc-Pra-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
2	Fmoc-Gly-Gly-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Gly-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)

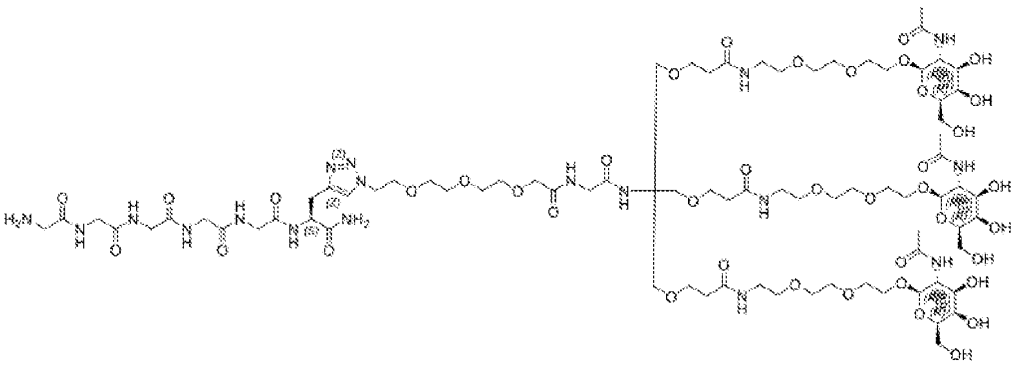
Peptide TFA deprotection:

[00411] *Trifluoroacetic acid deprotection*: peptide-bound-resin was stirred in a solution of trifluoroacetic acid/3-MPA/Tis/water (92.5/2.5/2.5/2.5, v/v/v/v, 200 mL) at 25°C for two hours. The mixture was precipitated with isopropyl ether (cold, 2 L). After filtration, the solid was washed with isopropyl ether (cold, 2 L) for two additional times, and dried under reduced pressure for two hours to afford **Intermediate-37** (620 mg, crude) as a white solid. The crude was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN) directly to afford **Intermediate-37** (1.59 g, 3.77 mmol, 75.5% yield, 94.3% purity) as a white solid. LCMS: retention time = 0.071 minutes, MS calculated: M_{av} = 397.39, mass observed: $[M + H]^+$ = 398.1.

*Preparation of **TBT544***:



Int-00037

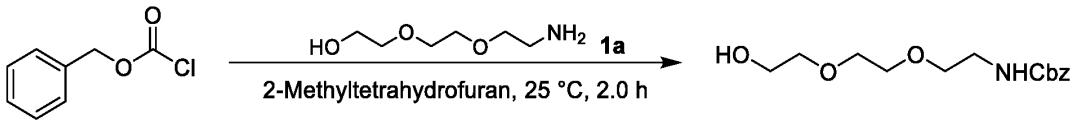


[00412] To a solution of **Intermediate-37** (500 mg, 1.25 mmol, 1.20 equivalents) and **Intermediate-27** (1.69 g, 1.04 mmol, 1.00 equivalent) in DMF (10 mL) was added a solution of $CuSO_4$ (0.4 M, 2.62 mL, 1.00 equivalent), sodium L-ascorbate (0.4 M, 10.4 mL, 4.00 equivalents) at 0°C. The mixture was stirred at 0°C for two hours under nitrogen gas atmosphere. LCMS indicated **Intermediate-27** was consumed completely, one main peak with desired MS was detected. The residue was purified by prep-high performance liquid chromatography (AcOH condition) directly to afford **TBT544** (1.377 g, 666 µmol, 63.5% yield, 97.3% purity) as a white solid. LCMS: retention time = 1.372 minutes, MS calculated: M_{av} = 2010.06, $[M + 2H]^{2+}$ = 1005.7, $[M + 3H]^{3+}$ = 671.0, [M -sugar + 2H]$^{2+}$ = 904.2, [M -2sugar + 2H]$^{2+}$ = 802.6.

EXAMPLE 9

*Procedure for Preparation of **Target-A001A***:

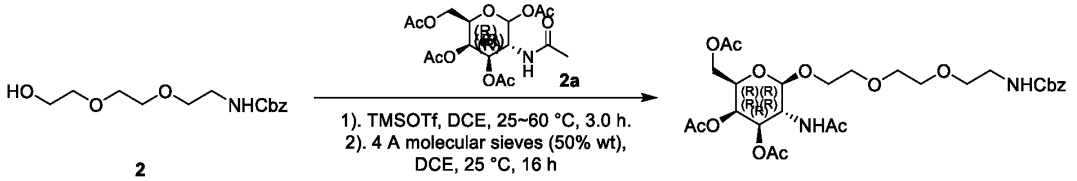
*Preparation of **Intermediate-2***:



1 2

[00413] To a solution of **1a** (60.0 g, 400 mmol, 2.00 equivalents) in 2-Methyltetrahydrofuran (450 mL) was added **1** (34.2 g, 200 mmol, 1.00 equivalent) in 2-Methyltetrahydrofuran (160 mL) at 0°C. The mixture was stirred at 25°C for two hours. TLC (DCM:methanol = 20: 1, R_f = 0.70) showed the reaction was completed, one major new spot with lower polarity was detected. The reaction mixture was added HCl/EA (1 N, 27.0 mL) and stirred for thirty minutes, and the white precipitate was removed by filtration, the filtrate was concentrated under reduced pressure to afford **Intermediate-2** (crude, 105.0 g, 370.6 mmol) as yellow oil. LCMS: retention time = 0.797 minutes, MS calculated: 283.14, mass observed: $[M + Na]^+$ = 306.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.23-7.41 (m, 5 H), 5.01 (s, 2 H), 4.60 (br s, 1 H), 3.45-3.52 (m, 6 H), 3.38-3.43 (m, 5 H), 3.14 (q, J = 5.94 Hz, 2 H), 2.53-2.55 (m, 1 H).

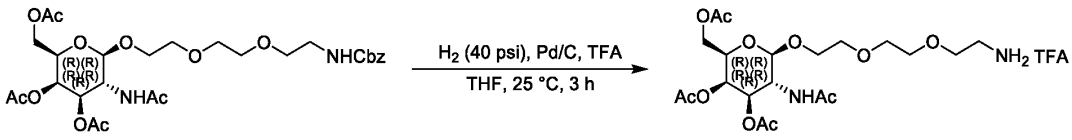
*Preparation of **Intermediate-3***:



[00414] To a solution of **Intermediate-2a** (100.0 g, 257 mmol, 1.00 equivalent) in DCE (500 mL) was added TMSOTf (85.6 g, 385 mmol, 1.50 equivalents) and stirred at 60°C for two hours. The reaction was then cooled to room temperature (25°C) and stirred for another one hour. A mixture of **Intermediate-2** (80.0 g, 282 mmol, 1.10 equivalents) and 4 Å powder molecular sieves (50.0 g) in DCE (500 mL) was added to the reaction. The resulting mixture was stirred for thirty minutes under nitrogen gas atmosphere. Then a solution of **Intermediate-2a** (100.0 g, 257 mmol, 1.00 equivalent) in DCE was added dropwise to the mixture at 0°C. The mixture was stirred for sixteen hours at 25°C under nitrogen gas atmosphere. TLC (DCM:methanol = 10: 1, R_f = 0.42) indicated **Intermediate-2a** was consumed

completely, and one major new spot with larger polarity was detected. The reaction mixture was filtered and washed with sat. $NaHCO_3$ (500 mL), water (500 mL) and brine (500 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, PE: EA = 3: 1 to 1: 6, then DCM:methanol = 20: 1) to afford **Intermediate-3** (90.0 g, 146.9 mmol, 91.6% purity, 57.2% yield) as yellow oil. LCMS: retention time = 0.860 minutes, MS calculated: 612.25, mass observed: $[M + H]^+$ = 613.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.80 (d, J = 9.03 Hz, 1 H), 7.24-7.39 (m, 6 H), 5.22 (d, J = 3.51 Hz, 1 H), 4.95-5.05 (m, 3 H), 4.53-4.59 (m, 1 H), 3.99-4.06 (m, 3 H), 3.84-3.92 (m, 1 H), 3.73-3.82 (m, 1 H), 3.55-3.61 (m, 1 H), 3.45-3.53 (m, 7 H), 3.41 (t, J = 5.90 Hz, 2 H), 3.11-3.18 (m, 3 H), 2.10 (s, 3 H), 1.99 (s, 3 H), 1.89 (s, 3 H), 1.77 (s, 3 H).

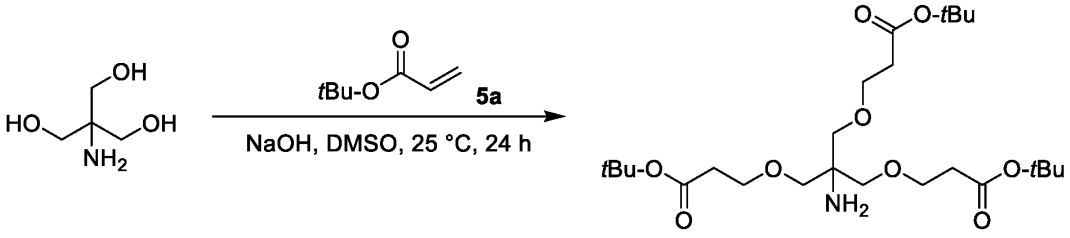
*Preparation of **Intermediate-4***:



3 4

[00415] Pd/C (9.00 g, 10% purity) in reaction bottle (purged with argon gas three times) was added THF (180 mL) slowly, then a solution of trifluoroacetic acid (16.7 g, 147 mmol, 1.00 equivalent) and **Intermediate-3** (90.0 g, 147.0 mmol, 1.00 equivalent) in THF (720 mL) was added to the reaction slowly under nitrogen gas. The reaction was degassed and purged with nitrogen gas and hydrogen gas for three times, then stirred at 25°C for three hours under hydrogen gas atmosphere (40 psi). TLC (DCM:methanol = 10: 1, R_f = 0.20) indicated **Intermediate-3** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was dissolved in THF (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL * 2), and the filtrate was concentrated under reduced pressure to get the residue. The residue was diluted with water (1000 mL), washed with DCM (300 mL * 3), the aqueous layer was lyophilized to afford **Intermediate-4** (80.0 g, 139.0 mmol, 95.1% purity, 91.8% yield, trifluoroacetic acid salt) as a white solid. LCMS: retention time = 0.484 minutes, MS calculated: 478.22, mass observed: $[M + H]^+$ = 478.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (br t, J = 9.03 Hz, 4 H), 5.21 (d, J = 3.26 Hz, 1 H), 4.96 (dd, J = 11.17, 3.39 Hz, 1 H), 4.54 (d, J = 8.53 Hz, 1 H), 3.98-4.08 (m, 3 H), 3.85-3.93 (m, 1 H), 3.75-3.84 (m, 1 H), 3.59 (br t, J = 5.14 Hz, 3 H), 3.50-3.56 (m, 6 H), 2.98 (br s, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

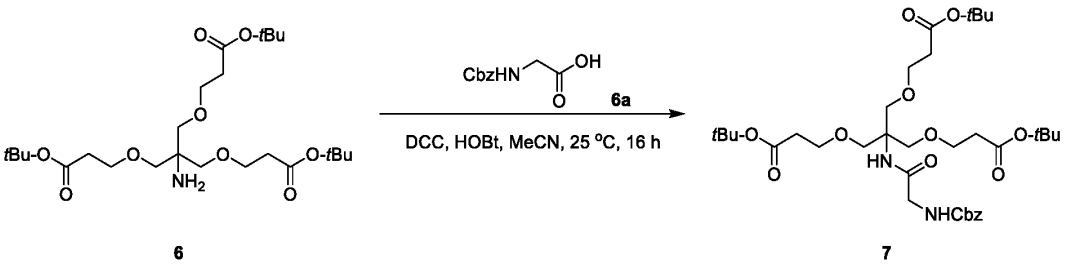
*Preparation of **Intermediate-6***:



5 **6**

[00416] To a mixture of **5** (60.0 g, 495.0 mmol, 1.00 equivalent) in DMSO (166 mL) was added aqueous NaOH (5.0 M, 9.91 mL, 0.10 equivalents) dropwise at 0-15°C for over five minutes. After addition, the mixture was stirred at 0-15°C for five minutes, then **5a** (254.0 g, 1.98 mol, 287 mL, 4.00 equivalents) was added to the reaction mixture dropwise at 20°C. The resulting mixture was stirred at 25°C for sixteen hours. TLC (DCM:methanol = 10: 1, R_f = 0.7) indicated **Intermediate-5** was consumed completely, and one major new spot with lower polarity was detected. The resulting reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in ethyl acetate (400 mL), quenched by addition of water (400 mL), and extracted with ethyl acetate (400 mL * 3). The combined organic layers were washed with brine (300 mL * 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **Intermediate-6** (100.0 g, 197.8 mmol, 96.0% purity, 40.0% yield) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.51-3.61 (m, 7 H), 3.17 (s, 5 H), 2.39 (t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

*Preparation of **Intermediate-7***:

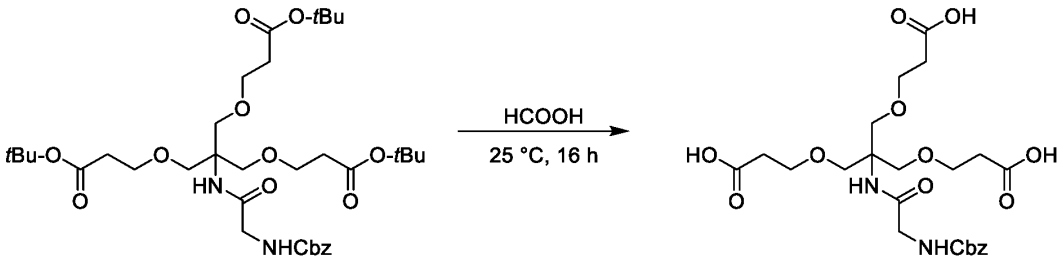


6 **7**

[00417] To a solution of **Intermediate-6** (40.0 g, 79.1 mmol, 1.00 equivalent) in MeCN (400 mL) was added HOBt (10.7 g, 79.1 mmol, 1.00 equivalent). Then **6a** (16.5 g, 79.1 mmol, 1.00 equivalent) and DCC (16.3 g, 79.1 mmol, 1.00 equivalent) were added. The reaction was stirred at 25°C for sixteen hours. TLC (PE: EA = 1: 1, R_f = 0.80) indicated **Intermediate-6** was consumed completely, and one major new spot with lower polarity was detected. MeCN was evaporated to get the residue. The residue was purified by

column chromatography (SiO_2, PE: EA = 10: 1 to 1: 1) to afford **Intermediate-7** (40.0 g, 57.4 mmol, 82.9% purity, 72.5% yield) as a white solid. LCMS: retention time = 1.151 minutes, MS calculated: 696.38, mass observed: $[M + H]^+$ = 697.3, $[M + Na]^+$ = 719.3. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.26-7.40 (m, 6 H), 7.06 (s, 1 H), 5.03 (s, 2 H), 3.49-3.61 (m, 14 H), 2.39 (br t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

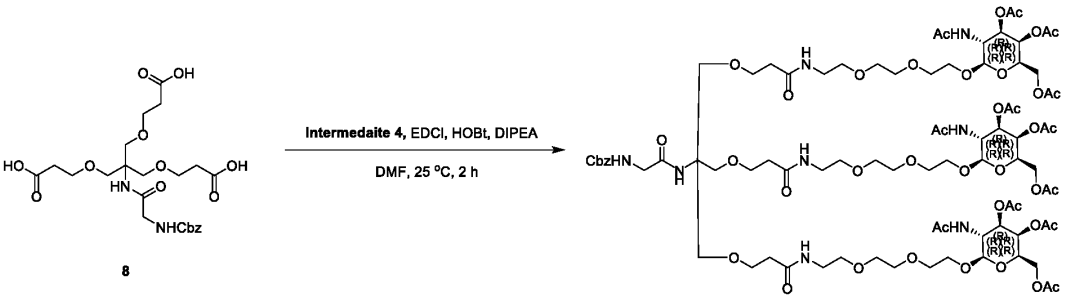
*Preparation of **Intermediate-8***:



7 8

[00418] A solution of **Intermediate-7** (30.0 g, 43.0 mmol, 1.00 equivalent) in HCOOH (300 mL) was stirred at 25°C for sixteen hours. TLC (PE: EA = 1: 1, R_f = 0.04) indicated **Intermediate-7** was consumed completely, and one major new spot with larger polarity was detected. Solvent was evaporated under reduced pressure, then co-evaporated with toluene (50 mL * 3) under reduced pressure and dried under reduced pressure to get the residue. The residue was purified by prep-high performance liquid chromatography (A: 0.1% FA condition/water, B: MeCN) to afford **Intermediate-8** (20.0 g, 37.8 mmol, 98.2% purity, 87.9% yield). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.17 (br s, 3 H), 7.26-7.43 (m, 6 H), 7.06 (s, 1 H), 5.02 (s, 2 H), 3.49-3.65 (m, 14 H), 2.42 (br t, J = 6.27 Hz, 6 H). LCMS: retention time = 0.790 minutes, MS calculated: 528.20, mass observed: $[M + H]^+$ = 529.2.

*Preparation of **Intermediate-9***:

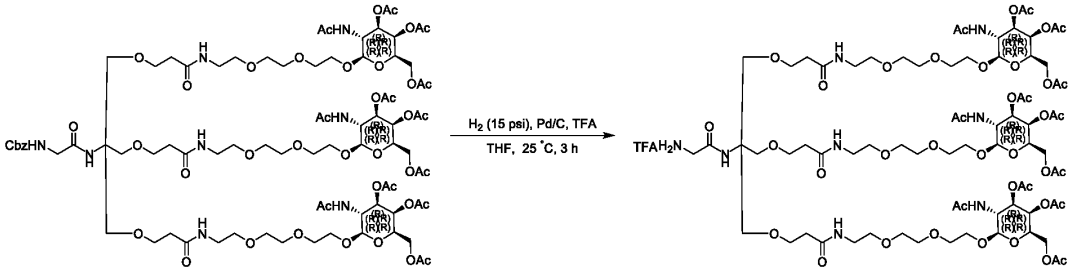


8 9

[00419] To a stirring solution of **Intermediate-8** (20.0 g, 37.8 mmol, 1.00 equivalent) and **Intermediate-4** (78.5 g, 132 mmol, 3.50 equivalents, trifluoroacetic acid salt) in DMF (400 mL) was

added HOBT (20.4 g, 151 mmol, 4.00 equivalents), EDCI (29.0 g, 151 mmol, 4.00 equivalents) and DIEA (22.0 g, 170 mmol, 4.50 equivalents) successively. The reaction was stirred at 25°C for two hours. TLC (DCM:methanol = 10: 1, R_f = 0.4) indicated **Intermediate-8** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was slowly poured into a stirring cold 0.5 mol/L HCl solution (900 mL) and stirred for ten minutes. White precipitate formed and was filtered. The aqueous phase was extracted with DCM (600 mL* 2) twice. The combined organic layers were washed with 5% $NaHCO_3$ (450 mL), dried over Na_2SO_4, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM:methanol = 100: 1 to 5: 1) to afford **Intermediate-9** (58.0 g, 30.4 mmol, 82.7% purity, 80.3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 (br t, J = 5.14 Hz, 3 H), 7.81 (d, J = 9.03 Hz, 3 H), 7.28-7.39 (m, 6 H), 7.13 (s, 1 H), 5.21 (d, J = 3.26 Hz, 3 H), 5.02 (s, 2 H), 4.97 (dd, J = 11.17, 3.39 Hz, 3 H), 4.54 (d, J = 8.53 Hz, 3 H), 4.03 (s, 9 H), 3.84-3.92 (m, 3 H), 3.75-3.81 (m, 3 H), 3.45-3.61 (m, 37 H), 3.39 (br s, 3 H), 3.18-3.23 (m, 6 H), 2.30 (br t, J=6.15 Hz, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H). LCMS: retention time = 3.455 minutes, MS calculated: 1908.81, mass observed: $[M + 2H]^{2+}$ = 955.7.

*Preparation of **Intermediate-10***:

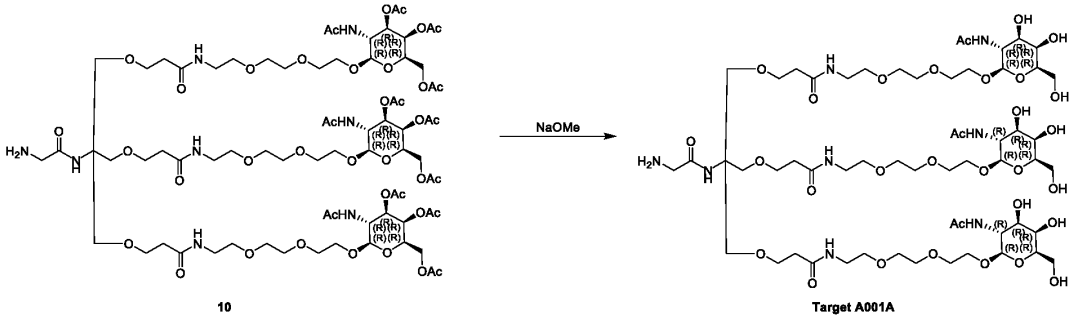


9 10

[00420] The 500 mL round-bottom flask was purged with argon gas three times and added dry Pd/C (1.50 g, 1.41 mmol, 10% purity, 1.00 equivalent) carefully. Then THF (150.0 mL) was added to infiltrate the Pd/C completely, followed by the solution of **Intermediate-9** (15.0 g, 7.85 mmol, 1.00 equivalent) and trifluoroacetic acid (895 mg, 7.85 mmol, 583 µL, 1.00 equivalent) in tetrahydrofuran (75 mL) slowly under argon gas atmosphere. The resulting mixture was degassed and purged with hydrogen gas for three times, and then the mixture was stirred at 25°C for three hours under hydrogen gas atmosphere (15 psi). The reaction was monitored by LCMS. LCMS showed the desired mass. One main peak with desired was detected. The reaction mixture was filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL*2). The filter cake was added water immediately. The organic layer concentrated under reduced pressure to afford **Intermediate-10** (13.0 g,

6.54 mmol, 83.2% yield, 99.7% purity, trifluoroacetic acid salt) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.89-7.99 (m, 5 H), 7.82 (d, *J* = 9.3 Hz, 3 H), 7.74 (s, 1 H), 7.14-7.28 (m, 1 H), 5.22 (d, *J* = 3.3 Hz, 3 H), 4.97 (dd, *J* = 11.3, 3.4 Hz, 3 H), 4.54 (d, *J* = 8.5 Hz, 3 H), 3.84-3.93 (m, 3 H), 3.76-3.82 (m, 3 H), 3.47-3.61 (m, 43 H), 3.21 (q, *J* = 5.8 Hz, 6 H), 2.29-2.34 (m, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 8 H), 1.77 (s, 9 H). LCMS: retention time = 1.333 minutes, MS calculated: 1774.7, found: $[M + 2H]^{2+}$ = 888.6.

Preparation of ***Target-A001A***:



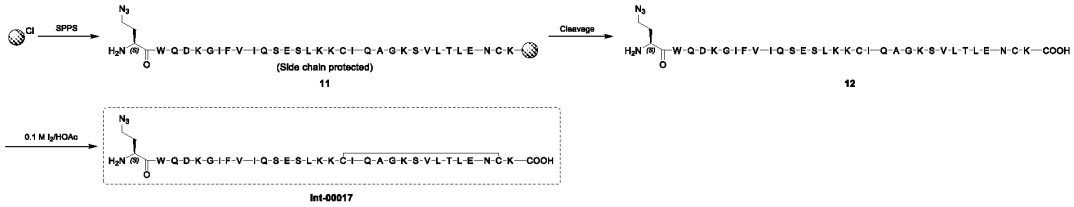
10 Target A001A

[00421] To a solution of **Intermediate-10** (12.0 g, 6.35 mmol, 1.00 equivalent, trifluoroacetic acid) in methanol (120.0 mL) was added NaOMe (5.4 M, 5.01 mL, 4.26 equivalents) at 0°C. The mixture was stirred at 0°C for 0.5 h. The reaction was monitored by LCMS, LCMS showed the desired mass (one main peak with desired was detected.). The reaction mixture was added with 1.0 M HCl solution (10.0 mL) till the pH = 6. The mixture was diluted with water (75.0 mL) and extracted with DCM (120 mL * 3). The mixture was freeze-dried to afford **Target-A001A** (9.0 g, 5.96 mmol, 93.9% yield, >95% purity, HCl) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.96 (br t, *J* = 5.4 Hz, 3 H), 7.67 (d, *J* = 8.9 Hz, 3 H), 7.51 (s, 1 H), 4.27 (d, *J* = 8.4 Hz, 3 H), 3.75-3.80 (m, 6 H), 3.70 (br d, *J* = 10.0 Hz, 6 H), 3.49 (br d, *J* = 4.0 Hz, 31 H), 3.37-3.42 (m, 12 H), 3.30 (br d, *J* = 6.1 Hz, 4 H), 3.20 (br d, *J* = 5.8 Hz, 6 H), 2.99 (s, 2 H), 2.30 (br t, *J* = 6.4 Hz, 6 H), 1.80 (s, 9 H). LCMS: retention time = 0.966 minutes, MS calculated: 1396.6, found: $[M + 2H]^{2+}$ = 699.1.

EXAMPLE 10

*Procedure for Preparation of **AGN306***:

*Preparation of **Intermediate-17***:



[00422] The peptide was synthesized using standard Fmoc chemistry (CTC resin).

[00423] (1) *Resin preparation*: To the vessel containing CTC Resin (4.00 mmol, 20.0 g, 1.00 mmol/g) and Fmoc-Lys(Boc)-OH (1.87 g, 4.00 mmol, 1.00 equivalent) in DCM (100 mL) was added DIEA (4.00 equivalents) dropwise and mix for two hours with nitrogen gas bubbling at 25°C. Methanol (20 mL) was added. The mixture was bubbled with nitrogen gas for another thirty minutes. The resin was washed with DMF (100 mL) * 5. Then 20% piperidine in DMF (50 mL) was added and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The mixture was filtered to obtain the resin. The resin was washed with DMF (100 mL) * 5 before proceeding to next step.

[00424] (2) *Coupling*: A solution of Fmoc-Cys(Trt)-OH (7.02 g, 12.0 mmol, 3.00 equivalents), HATU (4.33 g, 2.85 equivalents) in DMF (50 mL) was added to the resin with nitrogen gas bubbling. DIEA (6.00 equivalents) was added to the mixture dropwise. The mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was washed with DMF (100 mL) * 5.

[00425] (3) *Deprotection*: 20% piperidine in DMF (50 mL) was added to the resin and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The resin was washed with DMF (100 mL) * 5. The deprotection reaction was monitored by ninhydrin test, if it showed blue or brownish red, the reaction was completed.

[00426] (4) Steps 2 and 3 were repeated for the following amino acids elongation: Number # 3-31, TABLE 15.

[00427] (5) After the last position completed, the resin was washed with DMF (100 mL) * 5, methanol (100 mL) * 5, and dried under reduced pressure to afford **Intermediate-11** (peptide-bound-resin, 4.00 mmol, 47.0 g).

TABLE 15
The list of amino acids and the corresponding reagents used on solid-phase peptide synthesis

#	Materials	Coupling reagents
1	Fmoc-Lys(Boc)-OH (1.00 equivalent)	DIEA (4.00 equivalents)
2	Fmoc-Cys(Trt)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Asn(Trt)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
4	Fmoc-Glu(OtBu)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
5	Fmoc-Leu-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
6	Fmoc-Thr(Trt)-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOAt (3.00 equiv.)
7	Fmoc-Leu-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
8	Fmoc-Val-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
9	Fmoc-Lys(Boc)-Ser(psi(Me,Me)pro)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
10	Fmoc-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
11	Fmoc-Ala-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
12	Fmoc-Gln(Trt)-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOAt (3.00 equiv.)
13	Fmoc-Ile-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
14	Fmoc-Cys(Trt)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
15	Fmoc-Lys(Boc)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
16	Fmoc-Lys(Boc)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
17	Fmoc-Leu-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
18	Fmoc-Ser(tBu)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
19	Fmoc-Glu(OtBu)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
20	Fmoc-Ser(tBu)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
21	Fmoc-Gln(Trt)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
22	Fmoc-Ile-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
23	Fmoc-Val-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
24	Fmoc-Phe-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
25	Fmoc-Ile-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
26	Fmoc-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
27	Fmoc-Lys(Boc)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
28	Fmoc-Asp(OtBu)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
29	Fmoc-Gln(Trt)-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)

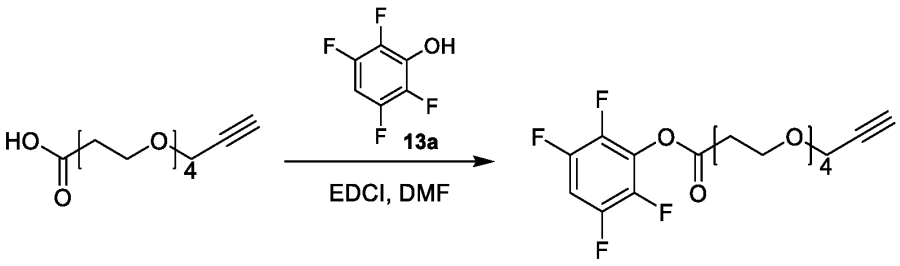
TABLE 15 The list of amino acids and the corresponding reagents used on solid-phase peptide synthesis		
#	Materials	Coupling reagents
30	Fmoc-Trp-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
31	Fmoc-Dab(N_3)-OH (1.50 equivalents)	DIC (1.50 equiv.) and HOAt (1.50 equiv.)

Peptide cleavage and disulfide formation:

[00428] 1) (1) *TFA deprotection*: **Intermediate-11** (47.0 g, resin) was stirred in a solution of trifluoroacetic acid/3-MPA/Tis/water (92.5/2.5/2.5/2.5, v/v/v/v, 500 mL) at 25°C for two hours. After filtration, the filtrate was precipitated with isopropyl ether (cold, 5 L). After filtration, the solid was washed with isopropyl ether (cold, 5 L) for two additional times, and dried under reduced pressure for two hours to afford **Intermediate-12** (14.0 g, crude) as a white solid. LCMS: retention time = 0.474 minutes, MS calculated: Mav = 3621.19, mass observed: $[M + 2H]^{2+}$ = 1811.0 $[M + 3H]^{3+}$ = 1207.7, $[M + 4H]^{4+}$ = 906.1, $[M + 5H]^{5+}$ = 725.0, $[M + 6H]^{6+}$ = 604.4, $[M + 7H]^{7+}$ = 518.3.

[00429] 2) (2) *Disulfide formation*: **Intermediate-12** (14.0 g, crude) was dissolved in water (2.8 L) and MeCN (1.2 L) at 25°C. To the mixture was added 0.1 M I_2/AcOH dropwise until a yellow color persisted. Then the mixture was stirred at 25°C for five minutes. After filtration, the filtrate was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN) directly to afford **Intermediate-17** (1.75 g, 438 μmol, 10.95% yield, 90.26% purity) as a white solid. LCMS: retention time = 0.439 minutes, MS calculated: Mav = 3619.18, mass observed: $[M + 2H]^{2+}$ = 1810.5 $[M + 3H]^{3+}$ = 1207.1, $[M + 4H]^{4+}$ = 905.6, $[M + 5H]^{5+}$ = 724.6.

*Preparation of **Intermediate-14***:

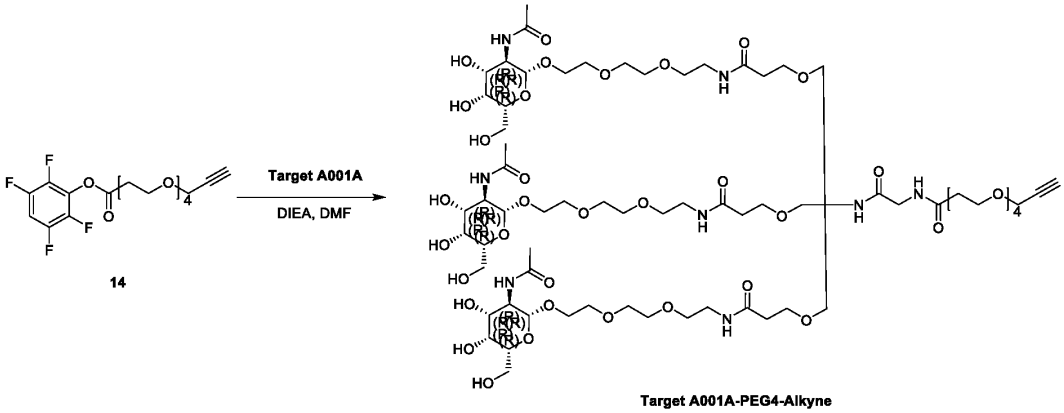


13 **14**

[00430] To a solution of **Intermediate-13** (600 mg, 2.31 mmol, 1.00 equivalent), **Intermediate-13a** (2.30 g, 13.8 mmol, 6.00 equivalents) in DMF (6 mL) was added EDCI (1.33 g, 6.92 mmol, 3.00 equivalents) at 0°C. The mixture was stirred at 0°C for one hour. The mixture was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN) directly to afford

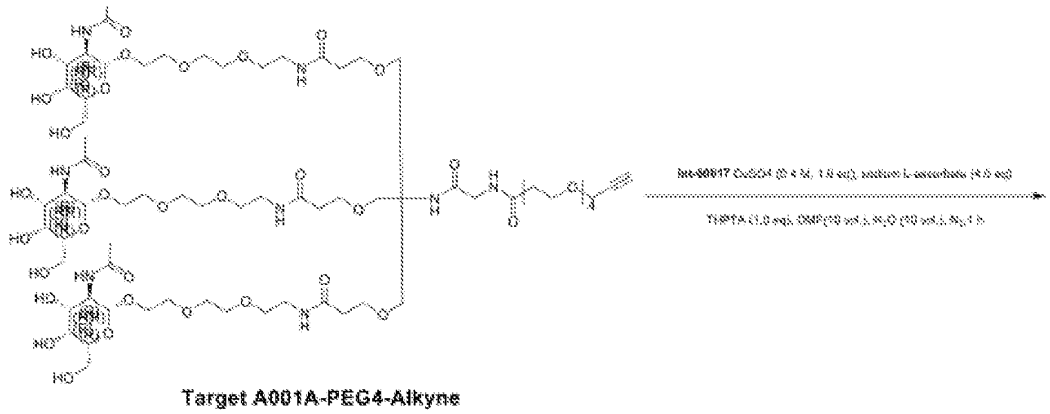
Intermediate-14 (800 mg, 83.4% yield, 98.2 % purity) as yellow oil. LCMS: retention time = 1.4 thirty minutes, MS calculated: Mav = 408.34, mass observed: $[M + Na]^+$ = 431.0.

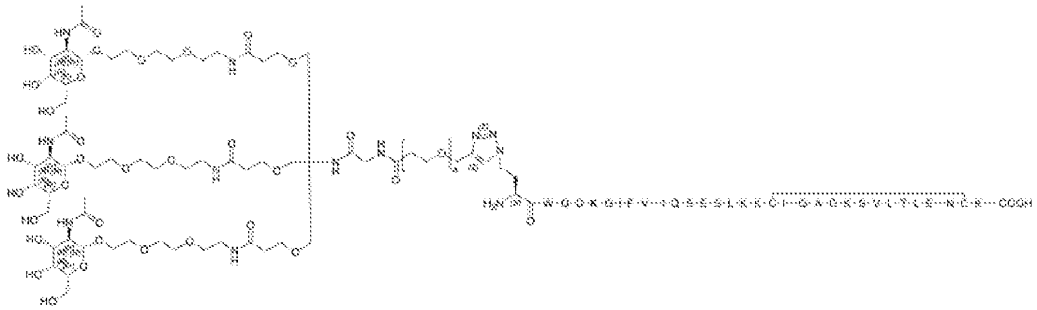
*Preparation of **Target-A001A-PEG4-Alkyne***:



[00431] To a solution of **Intermediate-14** (276 mg, 676 μmol, 1.05 equivalents) and **Target-A001A** (1.00 g, 644 μmol, 1.00 equivalent) in DMF (0.25 mL) was added DIEA (166 mg, 230 μl, 2.00 equivalents). The mixture was stirred at 25°C for twelve hours. The mixture was diluted with MeCN/water (cold, v/v, 3/3, 10 mL), then dried by lyophilization to remove DMF. The residue was purified by prep-high performance liquid chromatography (AcOH condition) directly to afford **Target-A001A-PEG4-Alkyne** (0.60 g, 56.8% yield, 93.5% purity) as colorless oil. LCMS: retention time = 0.368 minutes, MS calculated: Mav = 1639.74, mass observed: $[M + H]^+$ = 1639.8, [M-sugar + H]$^+$ = 1426.6, $[M - 2*sugar + H]^+$ = 1233.6, $[M - 3*sugar + H]^+$ = 1030.3, $[M + 2H]^{2+}$ = 820.6.

*Preparation of **AGN306**:*





[00432] To a solution of **Intermediate-17** (370 mg, 102 µmol, 1.00 equivalent) and **Target-A001A-PEG4-Alkyne** (201 mg, 122 µmol, 1.20 equivalents) in DMF (2 mL) and water (2 mL) was added a fresh solution of $CuSO_4$ (0.4 M, 255 µL, 1.00 equivalent), sodium L-ascorbate (0.4 M, 1.02 mL, 4.00 equivalents) and THPTA (44.4 mg, 102 µmol, 1.00 equivalent) at 0°C. The mixture was stirred at 0°C for one hour under nitrogen gas atmosphere. The residue was purified by prep-high performance liquid chromatography (ACOH condition) directly to afford **AGN306** (144 mg, 26.6 µmol, 26.08% yield, 96.91% purity) as a white solid. LCMS: retention time = 1.639 minutes, MS calculated: M_{av} = 5258.92, $[M + 3H]^{3+}$ = 1754.2, $[M + 4H]^{4+}$ = 1315.9, $[M + 5H]^{5+}$ = 1052.7.

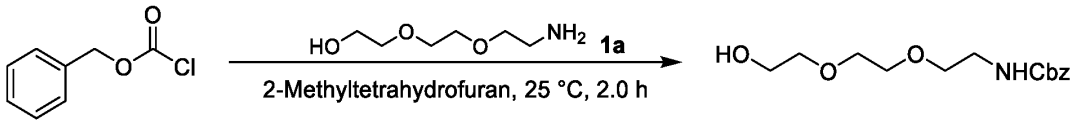
EXAMPLE 11

*Maleimide-beta-GN3(**TBT506**)*

*Procedure for Preparation of **Target-A001A**.*

See EXAMPLE 9 above.

*Preparation of **Intermediate-2**:*

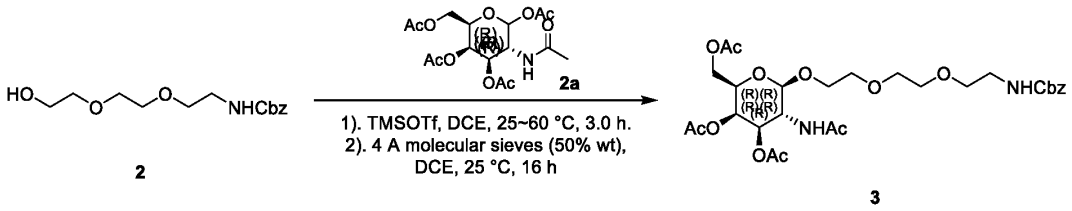


1 **2**

[00433] To a solution of **1a** (60.0 g, 400 mmol, 2.00 equivalents) in 2-Methyltetrahydrofuran (450 mL) was added **1** (34.2 g, 200 mmol, 1.00 equivalent) in 2-methyltetrahydrofuran (160 mL) at 0°C. The mixture was stirred at 25°C for two hours. TLC (DCM: MeOH = 20: 1, R_f = 0.70) showed the reaction was completed, one major new spot with lower polarity was detected. The reaction mixture was added HCl/EA (1 N, 27.0 mL) and stirred for thirty minutes, and the white precipitate was removed by filtration, the filtrate was concentrated under reduced pressure to afford **Intermediate-2** (crude, 105.0 g, 370.6

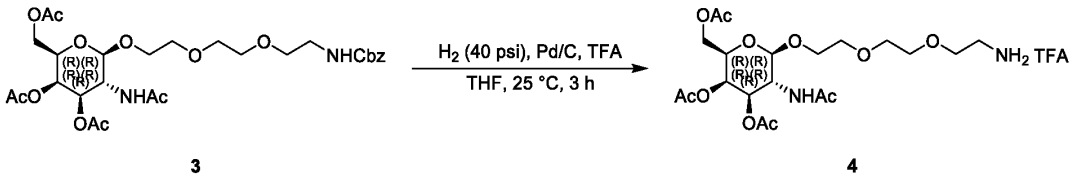
mmol) as yellow oil. LCMS: retention time = 0.797 minutes, MS cal.: 283.14, mass observed: $[M + Na]^+$ = 306.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.23-7.41 (m, 5 H), 5.01 (s, 2 H), 4.60 (br s, 1 H), 3.45-3.52 (m, 6 H), 3.38-3.43 (m, 5 H), 3.14 (q, J = 5.94 Hz, 2 H), 2.53-2.55 (m, 1 H).

*Preparation of **Intermediate-3**:*



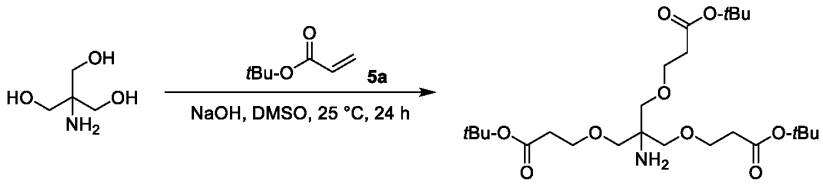
[00434] To a solution of **Intermediate-2a** (100.0 g, 257 mmol, 1.00 equivalent) in DCE (500 mL) was added TMSOTf (85.6 g, 385 mmol, 1.50 equivalents) and stirred at 60°C for 2 h. The reaction was then cooled to room temperature (25°C) and stirred for another one hour. A mixture of **Intermediate-2** (80.0 g, 282 mmol, 1.10 equivalents) and 4 Å powder molecular sieves (50.0 g) in DCE (500 mL) was added to the reaction. The resulting mixture was stirred for thirty minutes under nitrogen gas atmosphere. Then a solution of **Intermediate-2a** (100.0 g, 257 mmol, 1.00 equivalent) in DCE was added dropwise to the mixture at 0°C. The mixture was stirred for sixteen hours at 25°C under nitrogen gas atmosphere. Thin-layer chromatography (DCM: MeOH = 10: 1, R_f = 0.42) indicated **Intermediate-2a** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was filtered and washed with sat. $NaHCO_3$ (500 mL), water (500 mL) and brine (500 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, PE: EA = 3: 1 to 1: 6, then DCM: MeOH = 20: 1) to afford **Intermediate-3** (90.0 g, 146.9 mmol, 91.6% purity, 57.2% yield) as yellow oil. LCMS: retention time = 0.860 minutes, MS cal.: 612.25, mass observed: $[M + H]^+$ = 613.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.80 (d, J = 9.03 Hz, 1 H), 7.24-7.39 (m, 6 H), 5.22 (d, J = 3.51 Hz, 1 H), 4.95-5.05 (m, 3 H), 4.53-4.59 (m, 1 H), 3.99-4.06 (m, 3 H), 3.84-3.92 (m, 1 H), 3.73-3.82 (m, 1 H), 3.55-3.61 (m, 1 H), 3.45-3.53 (m, 7 H), 3.41 (t, J = 5.90 Hz, 2 H), 3.11-3.18 (m, 3 H), 2.10 (s, 3 H), 1.99 (s, 3 H), 1.89 (s, 3 H), 1.77 (s, 3 H).

*Preparation of **Intermediate-4**:*



[00435] Pd/C (9.00 g, 10% purity) in reaction bottle (purged with Ar for three times) was added THF (180 mL) slowly, then a solution of trifluoroacetic acid (16.7 g, 147 mmol, 1.00 equivalent) and **Intermediate-3** (90.0 g, 147.0 mmol, 1.00 equivalent) in THF (720 mL) was added to the reaction slowly under nitrogen gas. The reaction was degassed and purged with nitrogen gas and hydrogen gas for three times, then stirred at 25°C for three hours under H_2 atmosphere (40 psi). TLC (DCM:methanol = 10: 1, R_f = 0.20) indicated **Intermediate-3** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was dissolved in THF (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL * 2), and the filtrate was concentrated under reduced pressure to get the residue. The residue was diluted with water (1000 mL), washed with DCM (300 mL * 3), the aqueous layer was lyophilized to afford **Intermediate-4** (80.0 g, 139.0 mmol, 95.1% purity, 91.8% yield, trifluoroacetic acid salt) as a white solid. LCMS: retention time = 0.484 minutes, MS cal.: 478.22, mass observed: $[M + H]^+$ = 478.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (br t, *J* = 9.03 Hz, 4 H), 5.21 (d, *J* = 3.26 Hz, 1 H), 4.96 (dd, *J* = 11.17, 3.39 Hz, 1 H), 4.54 (d, *J* = 8.53 Hz, 1 H), 3.98-4.08 (m, 3 H), 3.85-3.93 (m, 1 H), 3.75-3.84 (m, 1 H), 3.59 (br t, *J* = 5.14 Hz, 3 H), 3.50-3.56 (m, 6 H), 2.98 (br s, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

*Preparation of **Intermediate-6**:*

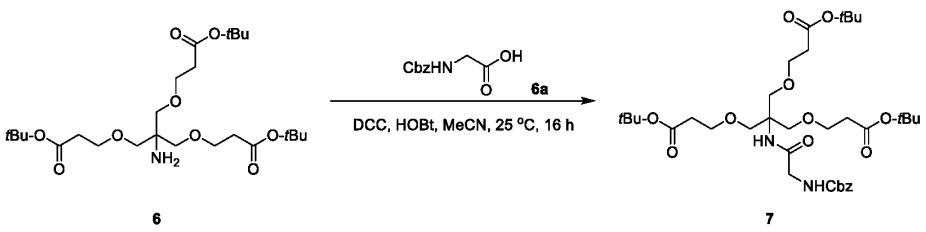


5 **6**

[00436] To a mixture of **Intermediate-5** (60.0 g, 495.0 mmol, 1.00 equivalent) in DMSO (166 mL) was added aqueous NaOH (5.0 M, 9.91 mL, 0.10 equivalents) dropwise at 0-15°C for over five minutes . After addition, the mixture was stirred at 0-15°C for five minutes, then **5a** (254.0 g, 1.98 mol, 287 mL, 4.00 equivalents) was added to the reaction mixture dropwise at 20°C. The resulting mixture was stirred at 25°C for sixteen hours. TLC (DCM: MeOH = 10: 1, R_f = 0.7) indicated **Intermediate-5** was consumed completely, and one major new spot with lower polarity was detected. The resulting reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in ethyl acetate (400 mL), quenched by addition of water (400 mL), and extracted with ethyl acetate (400 mL * 3). The combined organic layers were washed with brine (300 mL * 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **Intermediate-6** (100.0 g, 197.8 mmol, 96.0% purity,

40.0% yield) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.51-3.61 (m, 7 H), 3.17 (s, 5 H), 2.39 (t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

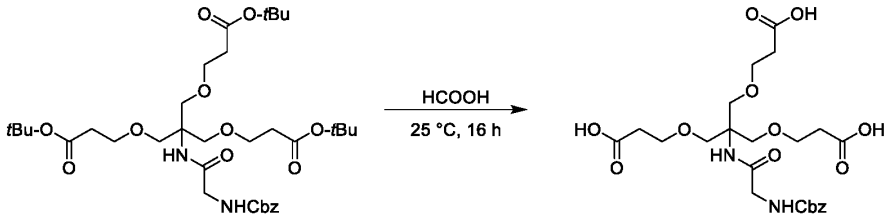
*Preparation of **Intermediate-7**:*



6 7

[00437] To a solution of **Intermediate-6** (40.0 g, 79.1 mmol, 1.00 equivalent) in MeCN (400 mL) was added HOBt (10.7 g, 79.1 mmol, 1.00 equivalent). Then **6a** (16.5 g, 79.1 mmol, 1.00 equivalent) and DCC (16.3 g, 79.1 mmol, 1.00 equivalent) were added. The reaction was stirred at 25°C for sixteen hours. TLC (PE: EA = 1: 1, R_f = 0.80) indicated **Intermediate-6** was consumed completely, and one major new spot with lower polarity was detected. MeCN was evaporated to get the residue. The residue was purified by column chromatography (SiO_2, PE: EA = 10: 1 to 1: 1) to afford **Intermediate-7** (40.0 g, 57.4 mmol, 82.9% purity, 72.5% yield) as a white solid. LCMS: retention time = 1.151 minutes, MS cal.: 696.38, mass observed: $[M + H]^+$ = 697.3, $[M + Na]^+$ = 719.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26-7.40 (m, 6 H), 7.06 (s, 1 H), 5.03 (s, 2 H), 3.49-3.61 (m, 14 H), 2.39 (br t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

*Preparation of **Intermediate 8**:*

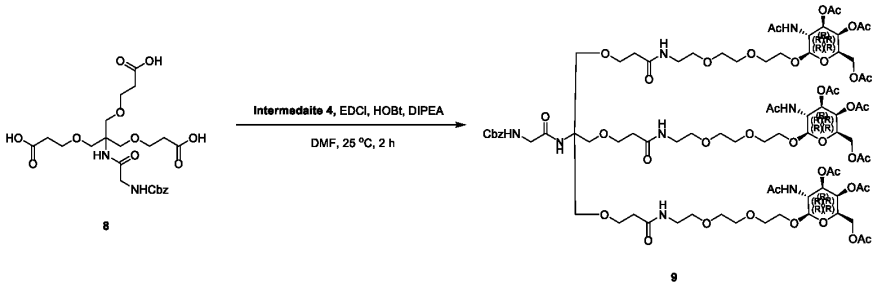


7 8

[00438] A solution of **Intermediate-7** (30.0 g, 43.0 mmol, 1.00 equivalent) in HCOOH (300 mL) was stirred at 25°C for sixteen hours. TLC (PE: EA = 1: 1, R_f = 0.04) indicated **Intermediate-7** was consumed completely, and one major new spot with larger polarity was detected. Solvent was evaporated under reduced pressure, then co-evaporated with toluene (50 mL * 3) under reduced pressure and dried under reduced pressure to get the residue. The residue was purified by prep-high performance liquid chromatography (A: 0.1% FA condition/water, B: MeCN) to afford **Intermediate-8** (20.0 g, 37.8 mmol,

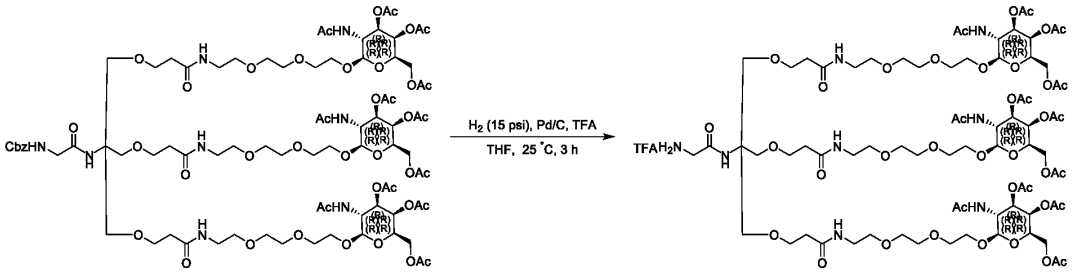
98.2% purity, 87.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.17 (br s, 3 H), 7.26-7.43 (m, 6 H), 7.06 (s, 1 H), 5.02 (s, 2 H), 3.49-3.65 (m, 14 H), 2.42 (br t, J = 6.27 Hz, 6 H). LCMS: retention time = 0.790 minutes, MS cal.: 528.20, mass observed: $[M + H]^+$ = 529.2.

*Preparation of **Intermediate-9**:*



[00439] To a stirring solution of **Intermediate-8** (20.0 g, 37.8 mmol, 1.00 equivalent) and **Intermediate-4** (78.5 g, 132 mmol, 3.50 equivalents, trifluoroacetic acid salt) in DMF (400 mL) was added HOBT (20.4 g, 151 mmol, 4.00 equivalents), EDCI (29.0 g, 151 mmol, 4.00 equivalents) and DIEA (22.0 g, 170 mmol, 4.50 equivalents) successively. The reaction was stirred at 25°C for 2 h. TLC (DCM: MeOH = 10: 1, R_f = 0.4) indicated **Intermediate-8** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was slowly poured into a stirring cold 0.5 mol/L HCl solution (900 mL) and stirred for ten minutes. White precipitate was formed and filtered. The aqueous phase was extracted with DCM (600 mL* 2) twice. The combined organic layers were washed with 5% $NaHCO_3$ (450 mL), dried over Na_2SO_4, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 100: 1 to 5: 1) to afford **Intermediate-9** (58.0 g, 30.4 mmol, 82.7% purity, 80.3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 (br t, J = 5.14 Hz, 3 H), 7.81 (d, J = 9.03 Hz, 3 H), 7.28-7.39 (m, 6 H), 7.13 (s, 1 H), 5.21 (d, J = 3.26 Hz, 3 H), 5.02 (s, 2 H), 4.97 (dd, J = 11.17, 3.39 Hz, 3 H), 4.54 (d, J = 8.53 Hz, 3 H), 4.03 (s, 9 H), 3.84-3.92 (m, 3 H), 3.75-3.81 (m, 3 H), 3.45-3.61 (m, 37 H), 3.39 (br s, 3 H), 3.18-3.23 (m, 6 H), 2.30 (br t, J=6.15 Hz, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H). LCMS: retention time = 3.455 minutes, MS cal.: 1908.81, mass observed: $[M + 2H]^{2+}$ = 955.7.

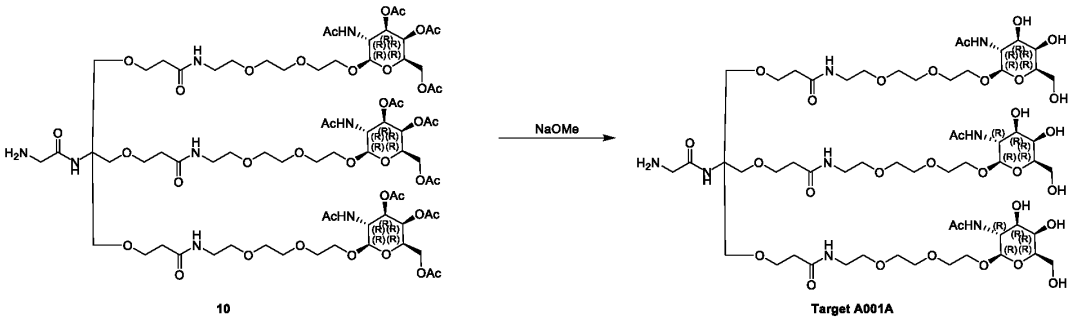
*Preparation of **Intermediate 10**:*



9 10

[00440] The 500 mL round-bottom flask was purged with Ar gas for 3 times and added dry Pd/C (1.50 g, 1.41 mmol, 10% purity, 1.00 equivalent) carefully. Then THF (150.0 mL) was added to infiltrate the Pd/C completely, followed by the solution of **Intermediate-9** (15.0 g, 7.85 mmol, 1.00 equivalent) and trifluoroacetic acid (895 mg, 7.85 mmol, 583 µL, 1.00 equivalent) in THF (75 mL) slowly under Ar atmosphere. The resulting mixture was degassed and purged with H_2 for 3 times, and then the mixture was stirred at 25°C for three hours under H_2 atmosphere (15 psi). The reaction was monitored by LCMS, LCMS showed the desired mass (one main peak with desired was detected.). The reaction mixture was filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL*2). Then, the filter cake was added water immediately. The organic layer concentrated under reduced pressure to afford **Intermediate-10** (13.0 g, 6.54 mmol, 83.2% yield, 99.7% purity, trifluoroacetic acid salt) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.89-7.99 (m, 5 H), 7.82 (d, *J* = 9.3 Hz, 3 H), 7.74 (s, 1 H), 7.14-7.28 (m, 1 H), 5.22 (d, *J* = 3.3 Hz, 3 H), 4.97 (dd, *J* = 11.3, 3.4 Hz, 3 H), 4.54 (d, *J* = 8.5 Hz, 3 H), 3.84-3.93 (m, 3 H), 3.76-3.82 (m, 3 H), 3.47-3.61 (m, 43 H), 3.21 (q, *J* = 5.8 Hz, 6 H), 2.29-2.34 (m, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 8 H), 1.77 (s, 9 H). LCMS: retention time = 1.333 minutes, MS cal.: 1774.7, found: $[M + 2H]^{2+}$ = 888.6.

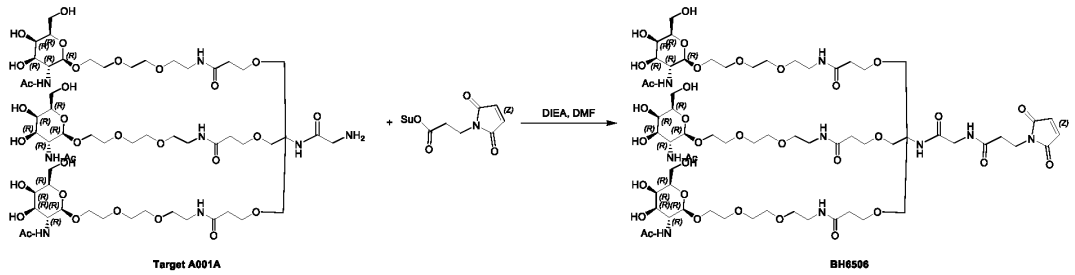
*Preparation of Intermediate **Target-A001A**:*



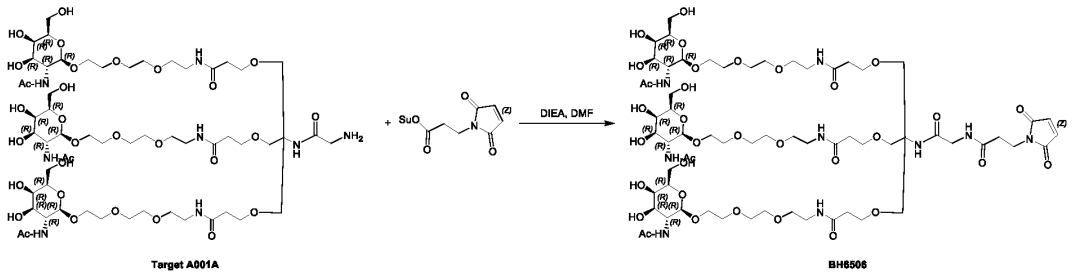
10 Target A001A

[00441] To a solution of **Intermediate-10** (12.0 g, 6.35 mmol, 1.00 equivalent, trifluoroacetic acid) in MeOH (120.0 mL) was added NaOMe (5.4 M, 5.01 mL, 4.26 equivalents) at 0°C. The mixture was stirred at 0°C for 0.5 h. The reaction was monitored by LCMS, LCMS showed the desired mass (one main peak with desired was detected.). The reaction mixture was added with 1.0 M HCl solution (10.0 mL) till the pH = 6. The mixture was diluted with water (75.0 mL) and extracted with DCM (120 mL * 3). The mixture was freeze-dried to afford **Target-A001A** (9.0 g, 5.96 mmol, 93.9% yield, >95% purity, HCl) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.96 (br t, *J* = 5.4 Hz, 3 H), 7.67 (d, *J* = 8.9 Hz, 3 H), 7.51 (s, 1 H), 4.27 (d, *J* = 8.4 Hz, 3 H), 3.75-3.80 (m, 6 H), 3.70 (br d, *J* = 10.0 Hz, 6 H), 3.49 (br d, *J* = 4.0 Hz, 31 H), 3.37-3.42 (m, 12 H), 3.30 (br d, *J* = 6.1 Hz, 4 H), 3.20 (br d, *J* = 5.8 Hz, 6 H), 2.99 (s, 2 H), 2.30 (br t, *J* = 6.4 Hz, 6 H), 1.80 (s, 9 H). LCMS: retention time = 0.966 minutes, MS cal.: 1396.6, found: $[M + 2H]^{2+}$ = 699.1.

*Procedure for Preparation of **TBT506**:*



*Preparation of **TBT506**:*



[00442] To a solution of **Target-A001A** (500 mg, 357 µmol, 1.00 equivalent) and 3-maleimidopropionic acid N-hydroxysuccinimide ester (142.869 mg, 536 µmol, 1.50 equivalents) in DMF (5 mL) was added DIEA (46.2 mg, 357 µmol, 59.1 µL, 1.00 equivalent) degassed and purged with nitrogen gas for three times. The mixture was stirred at 0°C for five minutes under nitrogen gas atmosphere. LCMS showed the desired mass (one main peak with desired was detected.). The reaction mixture was purified by prep-high performance liquid chromatography (AcOH condition) directly to

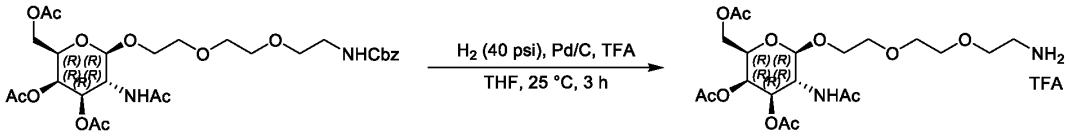
afford **TBT506** (251.2 mg, 156 µmol, 43.7% yield, 96.5% purity) as a colorless solid. LCMS: retention time = 1.382 minutes, MS cal.: M_{av} = 1548.59, $[M + H]^+$ = 1548.9, $[M + 2H]^{2+}$ = 775.1.

EXAMPLE 12

*Preparation of **TBT644** - A*

*Procedure for Preparation of **Intermediate-27**:*

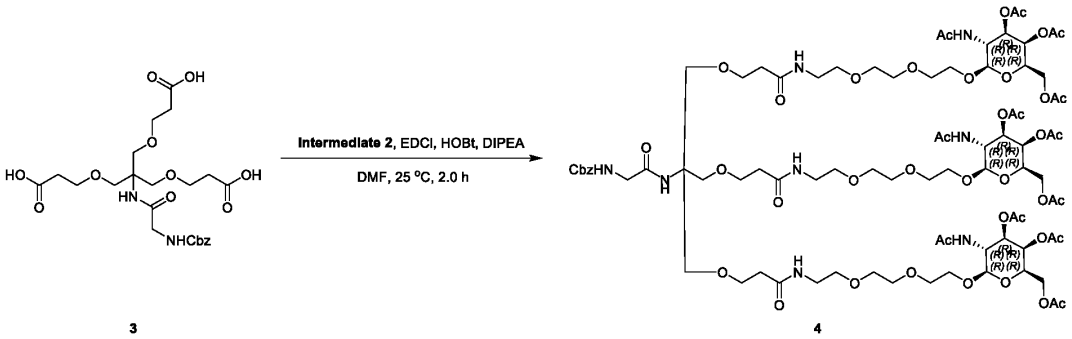
*Preparation of **Intermediate 2**:*



1 2

[00443] Pd/C (4.00 g, 10% purity) in reaction bottle (purged with Ar for three times) was added THF (40 mL) slowly, then a solution of trifluoroacetic acid (7.44g, 65.2 mmol, 4.86 mL, one equivalent) and **Intermediate-1** (40.0 g, 65.2 mmol, 1.00 equivalent) in THF (360 mL) was added to the reaction slowly under nitrogen gas. The reaction was degassed and purged with argon gas and hydrogen gas for three times, then stirred at 25°C for under hydrogen gas atmosphere (40 psi). TLC (DCM: MeOH = 10: 1, R_f = 0.20) indicated **Intermediate-1** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was dissolved in THF (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL * 2), and the filtrate was concentrated under reduced pressure to get **Intermediate-2** (38.7 g, 38.5 mmol, 59.0% yield, 59.0% purity, trifluoroacetic acid salt) as a white solid. LCMS: retention time = 0.428 minutes, MS cal.: 478.22, mass observed: $[M + Na]^+$ = 501.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.79-7.91 (m, 3 H), 5.16-5.28 (m, 1 H), 4.97 (br dd, J = 11.07, 2.81 Hz, 1 H), 4.54 (br d, J = 8.50 Hz, 1 H), 4.03 (s, 2 H), 3.77-3.93 (m, 2 H), 3.53-3.62 (m, 8 H), 2.98 (br d, J = 5.00 Hz, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

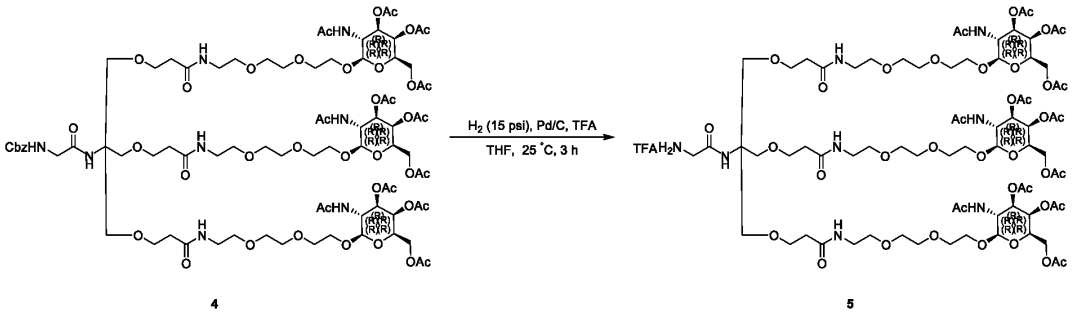
*Preparation of **Intermediate-4**:*



3 4

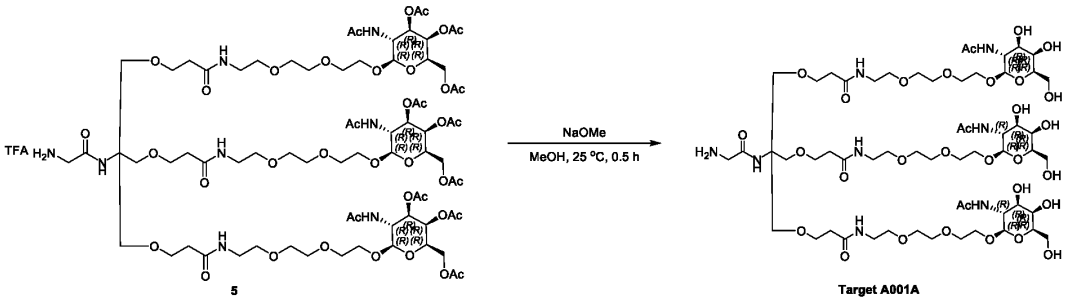
[00444] To a stirring solution of **Intermediate-3** (8.50 g, 16.0 mmol, 1.00 equivalent) and **Intermediate-2** (38.1 g, 64.3 mmol, 4.00 equivalents, trifluoroacetic acid salt) in DMF (17 mL) and DCM (340 mL) was added HOBT (8.69 g, 64.3 mmol, 4.00 equivalents), EDCI (12.3 g, 64.3 mmol, 4.00 equivalents) and DIEA (9.35 g, 72.3 mmol, 4.50 equivalents) successively. The reaction was stirred at 25°C for two hours. TLC (DCM:methanol = 10: 1, R_f = 0.5) indicated **Intermediate 3** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was slowly poured into a stirring cold 1.0 mol/L HCl solution (350 mL) and stirred for ten minutes. White precipitate was formed and filtered. The aqueous phase was extracted with DCM (350 mL* 2) twice. The combined organic layers were washed with saturated $NaHCO_3$ (350 mL), dried over Na_2SO_4, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 100: 1 to 15: 1) to afford **Intermediate 4** (22.0 g, 10.6 mmol, 66.0% yield, 92.2% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (br t, J = 5.32 Hz, 3 H), 7.81 (d, J = 9.26 Hz, 3 H), 7.26-7.49 (m, 6 H), 7.12 (s, 1 H), 5.21 (d, J = 3.25 Hz, 3 H), 5.02 (s, 2 H), 4.97 (dd, J = 11.13, 3.25 Hz, 3 H), 4.55 (d, J = 8.38 Hz, 3 H), 4.03 (s, 9 H), 3.84-3.92 (m, 3 H), 3.74-3.82 (m, 3 H), 3.47-3.65 (m, 38 H), 3.39 (br s, 3 H), 3.20 (q, J = 5.42 Hz, 6 H), 2.30 (br t, J = 6.13 Hz, 6 H), 2.10 (s, 9 H), 1.99 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H). LCMS: retention time = 0.403 minutes, MS cal.: 1908.81, mass observed: $[M + 2H]^{2+}$ = 1910.2.

*Preparation of **Intermediate 5**:*



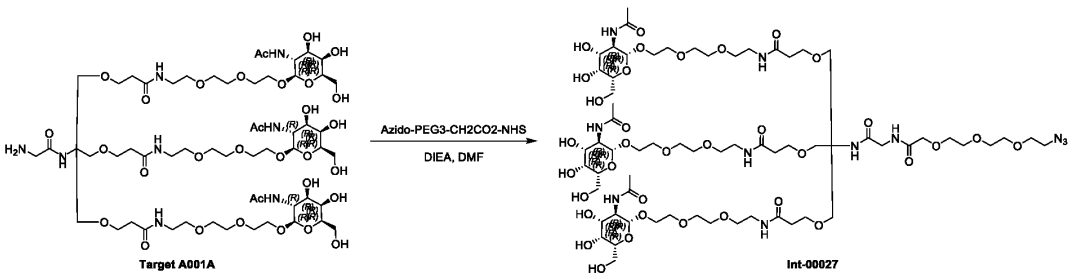
[00445] The 500 mL round-bottom flask was purged with argon gas for three times and added dry Pd/C (1.60 g, 1.50 mmol, 10% purity, 0.17 equivalents) carefully. Then THF (60.0 mL) was added to infiltrate the Pd/C completely, followed by the solution of **Intermediate-4** (16.4 g, 8.59 mmol, 1.00 equivalent) and trifluoroacetic acid (979 mg, 8.59 mmol, 637.8 µL, 1.00 equivalent) in THF (100 mL) slowly under argon gas atmosphere. The resulting mixture was degassed and purged with H_2 for 3 times, and then the mixture was stirred at 25°C for under H_2 atmosphere (15 psi). TLC (DCM: MeOH = 10: 1, Rf = 0.6) indicated Intermediate 9 was consumed completely, and one major new spot was detected. The reaction mixture was filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (50 mL*2). Then, the filter cake was added water immediately. The organic layer concentrated under reduced pressure to afford **Intermediate-5** (15.7 g, 6.78 mmol, 78.9% yield, 81.6% purity, trifluoroacetic acid salt) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91-7.98 (m, 5 H), 7.82 (br d, *J* = 9.13 Hz, 3 H), 7.74 (s, 1 H), 7.12-7.28 (m, 1 H), 5.22 (br d, J = 2.63 Hz, 3 H), 4.97 (br dd, *J* = 11.13, 2.75 Hz, 3 H), 4.54 (d, *J* = 8.51 Hz, 3 H), 3.84-3.93 (m, 3 H), 3.78 (br dd, *J* = 10.13, 4.88 Hz, 3 H), 3.53-3.62 (m, 22 H), 3.33-3.45 (m, 22 H), 3.18-3.25 (m, 6 H), 2.31 (br t, *J* = 6.13 Hz, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.73-1.81 (m, 14 H). LCMS: retention time = 0.341 minutes, MS cal.: 1774.7, found: $[M + 2H]^{2+}$ = 1776.9.

*Preparation of Intermediate **Target-A001A**:*



[00446] To a solution of **Intermediate-5** (5.5 g, 2.91 mmol, 1.00 equivalent, trifluoroacetic acid) in MeOH (50.0 mL) was added NaOMe (707 mg, 13.1 mmol, 4.50 equivalents) at 0°C. The mixture was stirred at 0°C for 0.5 hour. The reaction was monitored by LCMS, LCMS showed the desired mass (one main peak with desired was detected.). The reaction mixture was added with 1.0 M HCl solution till the pH = 6. The mixture was diluted with water (75.0 mL) and extracted with DCM (120 mL * 3). The mixture was freeze-dried to afford **Target-A001A** (4.1 g, 2.74 mmol, 94.1% yield, 93.4% purity, HCl) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.94 (br t, *J* = 5.50 Hz, 3 H), 7.62 (d, *J* = 9.01 Hz, 3 H), 7.54 (s, 1 H), 4.28 (d, *J* = 8.50 Hz, 3 H), 3.70-3.88 (m, 6 H), 3.61-3.70 (m, 6 H), 3.49-3.57 (m, 31 H), 3.38-3.43 (m, 12 H), 3.31 (br d, *J* = 6.25 Hz, 4 H), 3.17 (s, 6 H), 3.09 (s, 2 H), 2.30 (br t, *J* = 6.32 Hz, 6 H), 1.80 (s, 9 H). LCMS: retention time = 0.22 minutes, MS cal.: 1396.6, found: $[M + H]^+$ = 1397.8.

*Preparation of Intermediate **Intermediate-27**:*



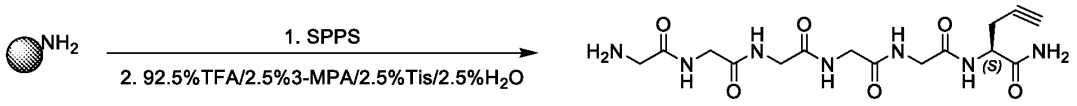
[00447] To a solution of **Target-A001A** (2.00 g, 1.43 mmol, 1.00 equivalent) and Azido-PEG3-CH2CO2-NHS (567 mg, 1.71 mmol, 1.20 equivalents) in DMF (20 mL) was added DIEA (554 mg, 4.29 mmol, 709 µL, 3.00 equivalents) at 0°C. The mixture was stirred at 0°C for two hours under nitrogen gas atmosphere. LCMS indicated **Target-A001A** was consumed completely, one main peak with desired MS was detected. The residue was purified by prep-high performance liquid chromatography (AcOH condition) directly to afford **Intermediate-27** (1.75 g, 1.04 mmol, 73.2% yield, 96.5% purity) as a white

solid. LCMS: retention time = 0.286 minutes, MS cal.: M_{av} = 1612.68, $[M + 2H]^{2+}$ = 807.1, [M - sugar + $2H]^{2+}$ = 705.6.

EXAMPLE 13

*Procedure for Preparation of **TBT644**- B*

*Preparation of **Intermediate-37**:*



Int-00037

Peptide was synthesized using standard Fmoc chemistry (C resin).

[00448] *Resin preparation*: DMF (100 mL) was added to the vessel containing Rink MBHA-Amide Resin (5.00 mmol, 16.7 g, 0.30 mmol/g) with nitrogen gas bubbling for thirty minutes. The resin was washed with DMF (100 mL * 5), followed by adding 20% piperidine in DMF (50 mL) and bubbled with nitrogen gas for thirty minutes at 25°C for Fmoc deprotection. The mixture was filtered. The resin was washed with DMF (100 mL * 5) before proceeding to next step.

[00449] *Coupling*: A solution of Fmoc-Pra-OH (3.00 equivalents), HATU (2.85 equivalents) in DMF (50 mL) was added to the resin with nitrogen gas bubbling. Then DIEA (6.00 equivalents) was added to the mixture dropwise and bubbled with nitrogen gas for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (100 mL) * 5.

[00450] *Deprotection*: 20% piperidine in DMF (50 mL) was added to the resin and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The resin was then washed with DMF (100 mL) * 5. The de-protection reaction was monitored by ninhydrin test, if it showed blue or brownish red, the reaction was completed.

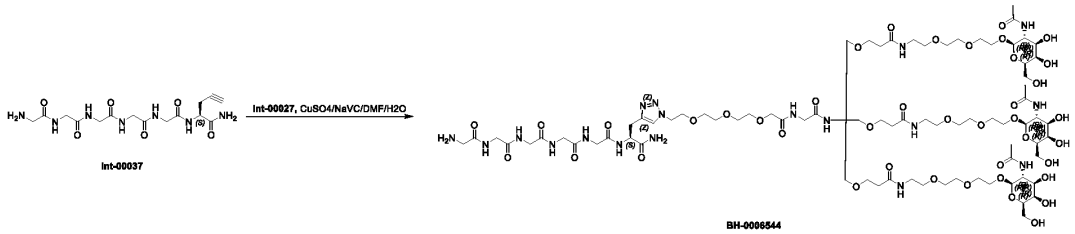
[00451] Steps 2 and 3 were repeated for the following amino acids elongation: Number # 2-3, TABLE 16 below. After the last position completed, the resin was then washed with DMF (100 mL) * 5, MeOH (100 mL) * 5, and dried under reduced pressure to afford peptide-bound-resin (5 mmol).

TABLE 16 The list of amino acids and the corresponding reagents used on SPPS.		
#	Materials	Coupling reagents
1	Fmoc-Pra-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
2	Fmoc-Gly-Gly-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Gly-Gly-OH (3.00 equivalents)	HATU (2.85 equiv.) and DIEA (6.00 equiv.)

Peptide TFA de-protection:

[00452] *Trifluoroacetic acid deprotection*: peptide-bound-resin was stirred in a solution of TFA/3-MPA/Tis/water (92.5/2.5/2.5/2.5, v/v/v/v, 200 mL) at 25°C for two hours. The mixture was precipitated with isopropyl ether (cold, 2 L). After filtration, the solid was washed with isopropyl ether (cold, 2 L) for two additional times, and dried under reduced pressure for two hours to afford **Intermediate-37** (620 mg, crude) as a white solid. The crude was purified by prep-high performance liquid chromatography (A: 0.075% trifluoroacetic acid/water, B: MeCN) directly to afford **Intermediate-37** (1.59 g, 3.77 mmol, 75.5% yield, 94.3% purity) as a white solid. LCMS: retention time = 0.071 minutes, MS calculated: M_{av} = 397.39, mass observed: $[M + H]^+$ = 398.1.

*Preparation of **TBT644**:*



[00453] To a solution of **Intermediate-37** (500 mg, 1.25 mmol, 1.20 equivalents) and **Intermediate-27** (1.69 g, 1.04 mmol, 1.00 equivalent) in DMF (10 mL) was added a solution of $CuSO_4$ (0.4 M, 2.62 mL, 1.00 equivalent), sodium L-ascorbate (0.4 M, 10.4 mL, 4.00 equivalents) at 0°C. The mixture was stirred at 0°C for two hours under nitrogen gas atmosphere. LCMS indicated **Intermediate-27** was consumed completely, one main peak with desired MS was detected. The residue was purified by prep-high performance liquid chromatography (AcOH condition) directly to afford **TBT644** (1.377 g, 666 µmol, 63.5% yield, 97.3% purity) as a white solid. LCMS: retention time = 1.372 minutes, MS cal.: M_{av} = 2010.06, $[M + 2H]^{2+}$ = 1005.7, $[M + 3H]^{3+}$ = 671.0, $[M - sugar + 2H]^{2+}$ = 904.2, $[M - 2sugar + 2H]^{2+}$ = 802.6.

EXAMPLE 14

*Production protocol for **ABT817***

Transient expression:

[00454] Seed and passage CHO K1 host cells in advance.

[00455] On the day of transfection, centrifuge host cells to specific cell density for transfection.

[00456] Add DNA and transfection reagent (PEI) into host cell for transfection.

[00457] Incubate the transfected culture by shaking at 150 rpm.

[00458] Shift temperature from 36.5°C to 33°C for twenty-four hours post transfection.

[00459] Perform feeding on day 0 and day 4, while harvest cell culture on day 7 or when VIA<60%.

Harvest and clarification:

[00460] Subject the cell culture fluid to centrifugation at 10000 g for thirty minutes and then pass through a 0.22 μm sterile filter.

*Affinity capture by Ni-Excel resin for **ABT817** (His tagged recombinant protein)*

[00461] Purify at 4°C.

[00462] Choose the column based on titer, recommended load density (~10 mg/ml resin).

[00463] Equilibrate, 10 column volume, with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole.

[00464] Sample Load.

[00465] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for 2-5 column volume.

[00466] *Triton wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, 0.1% TX114, for thirty minutes.

[00467] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for ten column volume.

[00468] Elute with elution buffer 50 mM PB, 200 mM sodium chloride, 400 mM Imidazole, pH 8.0, for five column volume.

[00469] *Testing for AC eluates*: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

*Size Exclusive Chromatography for **ABT817***

[00470] Purify at room temperature.

[00471] Choose the column based on protein amount and molecule weight. (Superdex 200 pg)

[00472] Equilibrate with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00473] Sample load.

[00474] Elution with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00475] Collect target protein with desirable purity. Testing for size exclusion chromatography eluates: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

Cation Exchange Chromatography ***ABT817***

[00476] Purify at room temperature. Use POROS XS column. Choose the column based on protein amount, recommended load density (10-20 mg/ml resin).

[00477] Dilute the sample below 5 mS/cm with 50 mM Hepes, pH7.2.

[00478] Equilibrate, 5 column volume, with the buffer of 50 mM Hepes, pH7.2.

[00479] Sample Load.

[00480] *EQ wash*. Wash with the buffer of 50 mM Hepes, pH7.2.

[00481] Elute with Gradient 0-100% 50 column volume, 50 mM Hepes, 1 M sodium chloride, pH7.2 (The scale-up gradient can be adjusted based on the pilot run).

[00482] Strip with 50 mM Hepes, 1 M sodium chloride, pH7.2 for 1-3 column volume.

[00483] Equilibrate, one column volume, with the buffer of 50 mM Hepes, pH7.2.

[00484] Sanitize with 0.5M sodium hydroxide for thirty minutes.

[00485] Equilibrate, 3-5 column volume, with the buffer of 50 mM Hepes, pH7.2.

[00486] *Testing for CEX eluates*: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

Size exclusion chromatography-high performance liquid chromatography

[00487] Perform size exclusion chromatography-high performance liquid chromatography analysis on a Vanquish Flex Duo liquid chromatography instrument using a TSKgel G3000SWxl stainless steel column (7.8 x 300 mm, particle size 5µm).

[00488] The mobile phase consists of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8.

[00489] Inject 25 µg of sample per run. Perform isocratic elution for fifteen minutes at a flow rate of 1.0 mL/min. Monitor protein elution by ultraviolet absorbance at 280 nm. Integrate the peaks corresponding to aggregates, monomers and LMW species, and calculate the percentage of each species.

SDS-PAGE

[00490] Perform non-reducing and reducing SDS-PAGE analysis using precast NuPAGE™ 4-12% Bis-Tris Gel from Thermo Scientific. Sample loading buffer (4X LDS) is from Invitrogen. Gel running buffer (20X MES) is from GenScript.

[00491] Treat non-reducing samples with 30 mM iodoacetamide and heat at 95°C for five minutes before analysis. Treat reducing samples with 50 mM DTT and heat at 95°C for five minutes before analysis. Carry out electrophoresis at a constant voltage of 180 V for forty minutes. Stain gels using Coomassie blue for thirty minutes and destain with water for one hour.

Endotoxin Level Testing

[00492] Dilute purified proteins based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110).

[00493] Load samples into Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS11F) and detect them using Charles River Laboratories Endosafe Nexgen-MCS™ instrument.

Molecular mass analysis by LC-MS

[00494] Conduct LC-MS analysis by using Agilent 6230 AdvanceBio LC/time of flight system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502).

[00495] For the non-reduced intact mass analysis, dilute 200 pmol sample by ddH2O, and subject to LC/MS. For the reduced mass analysis, denature and reduce 100 pmol sample using 10mM Urea (Sigma/15568), 30 mM Tris-HCl (Invitrogen/15568), and DTT (Sigma/D0632) in a total volume of 50 µL. Incubate samples at 37℃ for twenty minutes and then subjected them to LC/MS.

[00496] Analyze the mass spectroscopy data using Bioconfirm 10.0 software. Identify molecules based on molecular masses.

EXAMPLE 15

*Production protocol for **ABT818***

Transient expression:

[00497] Seed and passage CHO K1 host cells in advance.

[00498] On the day of transfection, centrifuge host cells to specific cell density for transfection.

[00499] Add DNA and transfection reagent (PEI) into host cell for transfection.

[00500] Incubate the transfected culture by shaking at 150 rpm.

[00501] Shift temperature from 36.5°C to 33°C for twenty-four hours post transfection.

[00502] Perform feeding on day 0 and day 4, while harvest cell culture on day 7 or when VIA<60%.

Harvest and clarification:

[00503] Subject the cell culture fluid to centrifugation at 10000 g for thirty minutes and then pass through a 0.22 µm sterile filter.

*Anion exchange chromatography **ABT818***

[00504] Purify at room temperature.

[00505] Use POROS 50HQ column.

[00506] Choose the column based on protein amount, recommended load density (10-20 mg/ml resin).

[00507] Dilute the sample below 5 mS/cm with 20 mM Tris-HCl, pH 7.5.

[00508] Equilibrate, 5 column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00509] Sample Load.

[00510] EQ wash. Wash with the buffer of 20 mM Tris-HCl, pH 7.5.

[00511] Elute with Gradient 0-100% 50 column volume 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 (The scale-up gradient can be adjusted based on the pilot run).

[00512] Strip with 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 for 1-3 column volume.

[00513] Equilibrate, one column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00514] Sanitize with 0.5 M sodium hydroxide for thirty minutes.

[00515] Equilibrate, 3-5 column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00516] *Testing for CEX eluates*: SDS-PAGE gel NR and R, size exclusion chromatography-high performance liquid chromatography.

*Size exclusive chromatography for **ABT818***

[00517] Purify at room temperature. Choose the column based on protein amount and molecule weight. (Superdex 200 pg)

[00518] Equilibrate with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00519] Sample load.

[00520] Elution with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00521] Collect target protein with desirable purity. Testing for size exclusion chromatography eluates: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

Size exclusion chromatography-high performance liquid chromatography

[00522] Perform size exclusion chromatography-high performance liquid chromatography analysis on a Vanquish Flex Duo liquid chromatography instrument using a TSKgel G3000SWxl stainless steel column (7.8 x 300 mm, particle size 5 µm).

[00523] The mobile phase consists of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8.

[00524] Inject 25 µg of sample per run. Perform isocratic elution for fifteen minutes at a flow rate of 1.0 mL/min. Monitor protein elution by ultraviolet absorbance at 280 nm. Integrate the peaks corresponding to aggregates, monomers and low molecular weight species, and calculate the percentage of each species.

SDS-PAGE

[00525] Perform non-reducing and reducing SDS-PAGE analysis using precast NuPAGE™ 4-12% Bis-Tris Gel from Thermo Scientific. Sample loading buffer (4X LDS) is from Invitrogen. Gel running buffer (20X MES) is from GenScript.

[00526] Treat non-reducing samples with 30 mM iodoacetamide and heat at 95°C for five minutes before analysis. Treat reducing samples with 50 mM DTT and heat at 95°C for five minutes before analysis. Carry out electrophoresis at a constant voltage of 180 V for forty minutes. Stain gels using Coomassie blue for thirty minutes and destain with water for one hour.

Endotoxin level testing

[00527] Dilute purified proteins based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110).

[00528] Load samples into Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS11F) and detect them using Charles River Laboratories Endosafe Nexgen-MCS™ instrument.

Molecular mass analysis by LC-MS

[00529] Conduct LC-MS analysis by using Agilent 6230 AdvanceBio LC/ time of flight system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502).

[00530] For the nonreduced intact mass analysis, dilute 200 pmol sample by ddH2O, and subject to LC/MS. For the reduced mass analysis, denature and reduce 100 pmol sample using 10 mM Urea (Sigma/15568), 30 mM Tris-HCl (Invitrogen/15568), and DTT (Sigma/D0632) in a total volume of 50 µL. Incubate samples at 37°C for 20 min and then subjected them to LC/MS.

[00531] Analyze the mass spec data using Bioconfirm 10.0 software and identify molecules based on molecular masses.

EXAMPLE 16

*Production protocol **ABT819***

Transient expression:

[00532] Seed and passage CHO K1 host cells in advance.

[00533] On the day of transfection, centrifuge host cells to specific cell density for transfection.

[00534] Add DNA and transfection reagent (PEI) into host cell for transfection.

[00535] Incubate the transfected culture by shaking at 150 rpm.

[00536] Shift temperature from 36.5°C to 33°C for twenty-four hours post transfection.

[00537] Perform feeding on day 0 and day 4, while harvest cell culture on day 7 or when VIA<60%.

Harvest and clarification:

[00538] Subject the cell culture fluid to centrifugation at 10000 g for thirty minutes and then pass through a 0.22 μm sterile filter.

*Affinity capture by Ni-Excel Resin for **ABT819** (His tagged recombinant protein)*

[00539] Purify at 4°C.

[00540] Choose the column based on titer, recommended load density (5-10 mg/ml resin).

[00541] Equilibrate, 10 column volume, with the buffer of 50 mM NaPO4, pH 8.0, 200 mM sodium chloride, 10 mM Imidazole.

[00542] Sample Load.

[00543] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for 2-5 column volume.

[00544] *Triton wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride,10 mM Imidazole, 0.1% TX114, for thirty minutes.

[00545] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for 10 column volume.

[00546] Elute with elution buffer 50 mM PB, 200 mM sodium chloride, 400 mM Imidazole, pH 8.0, for five column volumes.

[00547] Testing for AC eluates: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

*Size exclusive chromatography **ABT819***

[00548] Purify at 4°C. Choose the column based on protein amount and molecule weight. (Superdex 200 pg)

[00549] Equilibrate with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00550] Sample load.

[00551] Elution with a buffer of phosphate-buffered saline, pH7.4 for one column volume.

[00552] Collect target protein with desirable purity.

[00553] *Testing for size exclusion chromatography eluates*: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

*Anion exchange chromatography for **ABT819***

[00554] Purify at room temperature. Use POROS 50HQ column.

[00555] Choose the column based on protein amount, recommended load density (10-20 mg/ml resin).

[00556] Dilute the sample below 5 mS/cm with 20 mM Tris-HCl, pH 7.5.

[00557] Equilibrate, 5 column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00558] Sample Load.

[00559] *EQ wash*. Wash with the buffer of 20 mM Tris-HCl, pH 7.5.

[00560] Elute with Gradient 0-100% 50 column volume 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 The scale-up gradient can be adjusted based on the pilot run.

[00561] Strip with 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 for 1-3 column volume.

[00562] Equilibrate, one column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00563] Sanitize with 0.5M sodium hydroxide for thirty minutes.

[00564] Equilibrate, 3-5 column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00565] *Testing for CEX eluates*: SDS-PAGE gel nonreducing and reducing, size exclusion chromatography-high performance liquid chromatography.

Size exclusion chromatography-high performance liquid chromatography

[00566] Perform size exclusion chromatography-high performance liquid chromatography analysis on a Vanquish Flex Duo liquid chromatography instrument using a TSKgel G3000SWxl stainless steel column (7.8 x 300 mm, particle size 5µm).

[00567] The mobile phase consists of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8.

[00568] Inject 25 µg of sample per run. Perform isocratic elution for fifteen minutes at a flow rate of 1.0 mL/min. Monitor protein elution by ultraviolet absorbance at 280 nm. Integrate the peaks corresponding to aggregates, monomers and LMW species, and calculate the percentage of each species.

SDS-PAGE

[00569] Perform Non-reducing and reducing SDS-PAGE analysis using precast NuPAGE™ 4-12% Bis-Tris Gel from Thermo Scientific. Sample loading buffer (4X LDS) is from Invitrogen. Gel running buffer (20X MES) is from GenScript.

[00570] Treat non-reducing samples with 30 mM iodoacetamide and heat at 95°C for five minutes before analysis. Treat reducing samples with 50 mM DTT and heat at 95°C for five minutes before analysis. Carry out electrophoresis at a constant voltage of 180 V for forty minutes. Stain gels using Coomassie blue for thirty minutes and destain with water for one hour.

Endotoxin level testing

[00571] Dilute purified proteins based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110).

[00572] Load samples into Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS11F) and detect them using Charles River Laboratories Endosafe Nexgen-MCS™ instrument.

Molecular mass analysis by LC-MS

[00573] Conduct LC-MS analysis by using Agilent 6230 AdvanceBio LC/time of flight system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502).

[00574] For the nonreduced intact mass analysis, dilute 200 pmol sample by ddH2O, and subject to LC/MS. For the reduced mass analysis, denature and reduce 100 pmol sample using 10mM Urea (Sigma/15568), 30mM Tris-HCl (Invitrogen/15568), and DTT (Sigma/D0632) in a total volume of 50 µL. Incubate samples at 37℃ for 20 min and then subjected them to LC/MS.

[00575] Analyze the mass spec data using Bioconfirm 10.0 software and identify molecules based on molecular masses.

EXAMPLE 17

*Production protocol for **ABT740***

Transient expression:

[00576] Seed and passage CHO K1 host cells in advance.

[00577] On the day of transfection, centrifuge host cells to specific cell density for transfection.

[00578] Add DNA and transfection reagent (PEI) into host cell for transfection.

[00579] Incubate the transfected culture by shaking at 150 rpm.

[00580] Shift temperature from 36.5°C to 33°C for twenty-four hours post-transfection.

[00581] Perform feeding on day 0 and day 4, while harvest cell culture on day 7 or when VIA<60%.

Harvest and clarification:

[00582] Subject the cell culture fluid to centrifugation at 10000 g for thirty minutes and then pass through a 0.22 µm sterile filter.

*Affinity capture by Ni-Excel Resin for **ABT740** (His tagged recombinant protein)*

[00583] Purify at 4°C. Choose the column based on titer, recommended load density (~10 mg/ml resin).

[00584] Equilibrate, ten column volumes, with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole.

[00585] Sample Load.

[00586] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for 2-5 column volume.

[00587] *Triton wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, 0.1% TX114, for thirty minutes.

[00588] *EQ wash*. Wash with the buffer of 50 mM NaPO4, pH8.0, 200 mM sodium chloride, 10 mM Imidazole, for 10 column volume.

[00589] Elute with elution buffer 50 mM PB, 200 mM sodium chloride, 400 mM Imidazole, pH 8.0, for 5 column volume.

[00590] Testing for AC eluates: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

*Anion Exchange Chromatography for **ABT740***

[00591] Purify at room temperature. Use Q-HP column.

[00592] Choose the column based on protein amount, recommended load density (10-20 mg/ml resin).

[00593] Dilute the sample below 5 mS/cm with 20 mM Tris-HCl, pH 7.5.

[00594] Equilibrate, five column volumes, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00595] Sample Load.

[00596] *EQ wash*. Wash with the buffer of 20 mM Tris-HCl, pH 7.5.

[00597] Elute with Gradient 0-100% 50 column volume, 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 (The scale-up gradient can be adjusted based on the pilot run).

[00598] Strip with 20 mM Tris-HCl, 1 M sodium chloride, pH 7.5 for 1-3 column volume.

[00599] Equilibrate, one column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00600] Sanitize with 0.5M sodium hydroxide for thirty minutes.

[00601] Equilibrate, 3-5 column volume, with the buffer of 20 mM Tris-HCl, pH 7.5.

[00602] *Testing for CEX eluates*: SDS-PAGE gel non-reducing and reducing, size exclusion chromatography-high performance liquid chromatography.

Size exclusion chromatography-high performance liquid chromatography

[00603] Perform size exclusion chromatography-high performance liquid chromatography analysis on a Vanquish Flex Duo liquid chromatography instrument using a TSKgel G3000SWxl stainless steel column (7.8 x 300 mm, particle size 5 µm).

[00604] The mobile phase consists of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8.

[00605] Inject 25 µg of sample per run. Perform isocratic elution for fifteen minutes at a flow rate of 1.0 mL/min. Monitor protein elution by ultraviolet absorbance at 280 nm. Integrate the peaks corresponding to aggregates, monomers and low molecular weight species, and calculate the percentage of each species.

SDS-PAGE

[00606] Perform non-reducing and reducing SDS-PAGE analysis using precast NuPAGE™ 4-12% Bis-Tris Gel from Thermo Scientific. Sample loading buffer (4X LDS) is from Invitrogen. Gel running buffer (20X MES) is from GenScript.

[00607] Treat non-reducing samples with 30 mM iodoacetamide and heat at 95°C for five minutes before analysis. Treat reducing samples with 50 mM DTT and heat at 95°C for five minutes before analysis. Carry out electrophoresis at a constant voltage of 180 V for forty minutes. Stain gels using Coomassie blue for thirty minutes and destain with water for one hour.

Endotoxin level testing

[00608] Dilute purified proteins based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110).

[00609] Load samples into Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS11F) and detect them using Charles River Laboratories Endosafe Nexgen-MCS™ instrument.

Molecular mass analysis by LC-MS

[00610] Conduct LC-MS analysis by using Agilent 6230 AdvanceBio LC/time of flight system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502).

[00611] For the non-reduced intact mass analysis, dilute 200 pmol sample by ddH2O, and subject to LC/MS. For the reduced mass analysis, denature and reduce 100 pmol sample using 10 mM Urea

(Sigma/15568), 30 mM Tris-HCl (Invitrogen/15568), and DTT (Sigma/D0632) in a total volume of 50 µL. Incubate samples at 37°C for 20 min and then subjected them to LC/MS.

[00612] Analyze the mass spec data using Bioconfirm 10.0 software and identify molecules based on molecular masses.

EXAMPLE 18

*Technical Report for **AGN167 (ABT740-TBT506**, target BAR = 1)*

[00613] The of **AGN167** molecule is a cysteine-based antibody-conjugate. The molecule consists of one single chain (**ABT740**, His-tagged protein). The maleimide-containing payload (**TBT506**) is conjugated to C-terminal cysteine of **ABT740** via Michael-addition reaction. The targeting BAR is 1

Expected outcome:

[00614] *BAR*: 0.9-1.1.

[00615] *Purity by size exclusion chromatography (high performance liquid chromatography)*: >95%.

[00616] *Free drug residue*: <5% (mol/mol of total drug).

[00617] *Endotoxin*: <0.5 EU/mg.

Methods

[00618] Purification method for antibody-conjugate by Zeba Spin desalting column.

[00619] The Zeba Spin desalting column (40K, 10mL) was preprocessed according to the following procedure:

[00620] (1) Removed the column's bottom closure and centrifuged (700 g, 2 min) to remove the storage solution.

[00621] (2) Added 5 mL of phosphate-buffered saline, pH 7.4 onto the resin, centrifuged (700 g, 2 min) and discarded flow-through. Repeated this step two additional times until the pH of the flow through was same as formulation buffer. The centrifuge time for the last balance was five minutes .

[00622] (3) Transferred the columns to new collection tubes and applied the conjugation mixture on top of the resin.

[00623] (4) Centrifuged (700 g, four minutes) and collected flow-through that contained product.

[00624] *Purification method for antibody-conjugate by Amicon® Ultra Centrifugal Filter Unit*. An ultrafiltration membrane having a molecular weight of 10 kDa is preferable to concentrate the sample. The Amicon Ultra Centrifugal Filter Unit was used according to the following procedure:

[00625] (1) dH_2O wash the centrifugal filter unit.

[00626] (2) Equilibrated the centrifugal filter unit with phosphate-buffered saline, pH 7.4.

[00627] (3) Add up sample to the Amicon® Ultra filter device and centrifuged to concentrate.

[00628] (4) Collected and filtered sample with 0.22 μm membrane.

[00629] Ultraviolet–visible spectroscopy platform by Nanodrop to determine monoclonal antibody concentration.

[00630] (1) The ultraviolet absorption at 280 nm were measured respectively.

[00631] (2) Calculation method based on Beer-Lambert Law A=E*C*l.

[00632] $A_{280}=E^{mAb}_{280}*C_{[mAb]}*l$.

[00633] E: weight extinction coefficient.

[00634] C: weight concentration (mg/mL).

[00635] l: light path (Nanodrop: 0.1 cm).

[00636] (3) $C_{[mAb]}$(mg/mL) = A280 /(E^{mAb}_{280}*l).

[00637] *BAR determination by LC-MS*. LC-MS was performed using a combination of Agilent 1260 series high performance liquid chromatography system and time-of-flight mass spectrometry. BAR was calculated based on the peak abundance of the deconvoluted Mass.

[00638] (1) Preparation of sample used in LC-MS analysis (reduction of antibody-drug conjugate). The preparation of LC-MS analysis samples includes denaturation, deglycosylation and reduction.

[00639] (a) *Denaturation*. Transfer 20 μg of sample into 1.5 mL tube, add conjugation buffer to final 10 μL (2 mg/mL), then incubate this solution at 75°C for five minutes .

[00640] (b) *Deglycosylation*. Transfer 15 μg of denatured samples (7.5 μL) into a 1.5 mL tube, add 3 μL Rapid PNGase F (non-reducing format, 5X) enzyme buffer and 1 μL Rapid PNGase F (non-reducing format) enzyme for de-glycosylation and 3.5 μL water to ensuring the total volume is 15 μL, then incubate this solution at 50°C for fifteen minutes .

[00641] can *Reduction*. 1 μL of 0.1M DTT, 3 μL Tris, pH 8.0 was added to 11 μL deglycosylated sample. The mixture was incubated at 37°C for fifteen minutes to prepare a sample in which the disulfides between the heavy chain and the light chain and between the heavy chain and the heavy chain of the antibody-drug conjugate were cleaved. The obtained sample was used in an LC-MS analysis. LC-MS/MS method. A high performance liquid chromatography analysis was carried out under the following measurement conditions.

TABLE 17 LC parameters for BAR determination		
Equipment	HPLC Agilent 1260	
Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 µm, 1000Å	
Detection Wavelength:	280 nm, 214nm	
Column Oven Temp.:	80°C	
Sampler Temp.:	2~8°C	
Flow Rate:	0.5 mL/min	
Injection Amount:	2 µg	
Mobile Phases:	A:0.025% TFA + 0.1% FA in water B:0.025% TFA + 0.1% FA in CAN	
Gradient Program	Ten minutes	
Gradient	Time (min)	B (%)
	0.0	25
	0.7	34
	5	45
	6	90
	7	90
	7.1	25
	10.0	25

TABLE 18 TOF parameters for BAR determination	
Equipment	Agilent 6224TOF
Polarity	positive
Gas Temp.	350oC
Drying Gas	13 L/min
Nebulizer	45 psig
Vcap	5000 V
Fragmentor	250 V
Mass Range	500 – 8000 m/z
Acquisition Rate	1 spectra/s

[00642] *Aggregation determination by size exclusion chromatography-high performance liquid chromatography*. Size-exclusion chromatography was performed using an Agilent 1260 series high performance liquid chromatography system with the TSK gel G3000SWXL Size-exclusion chromatography column (7.8×300 mm, 5 µm) at 25°C. The mobile phase was consisted of 78 mM KH_2PO_4, 122 mM K_2HPO_4, 250 mM potassium chloride, 15% isopropanol at pH 7.0±0.1. The flow rate was set at 0.75 mL/min. Sample loading was 40~50 µg per injection. Samples were detected at 280 nm and 220 nm with an ultraviolet detector. The retention time of the aggregation peak was recorded based on its relative molecular weight. And the aggregation level was determined by the relative area of the peak at 280 nm.

[00643] *Hydrophobicity determination by hydrophobic interaction chromatography-high performance liquid chromatography*. Hydrophobic Interaction Chromatography was performed using an Agilent 1260 series high performance liquid chromatography system with the TSK gel butyl-nonporous Hydrophobic Interaction chromatography column (4.6×35 mm, 5 µm) at 25°C. The mobile phase A was consisted of 1.5 M $(NH_4)_2SO_4$ and 50 mM $K_2HPO_4{\cdot}3H_2O$, at pH 7.2±0.2, Cond. 190,000±200 us/cm. The mobile phase B was consisted of 50 mM Potassium Phosphate and 25% 2-propanol, at pH 7.4±0.2, Cond. 3400±200 us/cm. The flow rate was set at 0.6 mL/min. Sample loading was 8 µL per injection. Samples were detected at 280 nm with an ultraviolet detector.

TABLE 19
hydrophobic interaction chromatography-high performance liquid chromatography parameters

Column	TOSOH, TSKgel Butyl-NPR, 2.5 µm, 4.6 * 35 mm
Detection	280 nm BW 4
Column Oven Temp.:	25°C
Sampler Temp.:	6 ± 2°C
Flow Rate:	0.6 mL/min
Stop Time:	Twenty minutes
Maximum Pressure:	300 bar
Injection Amount:	8 µL
Mobile Phases:	Mobile Phase A: 1.5 M $(NH_4)_2SO_4$ and 50 mM $K_2HPO_4{\cdot}3H_2O$ Mobile Phase B: 50 mM potassium phosphate and 25% 2-propanol

TABLE 19 hydrophobic interaction chromatography-high performance liquid chromatography parameters			
Column	TOSOH, TSKgel Butyl-NPR, 2.5 µm, 4.6 * 35 mm		
Gradient Program:	Time (min)	A (%)	B (%)
	0	100	0
	2	100	0
	15	0	100
	16	0	100
	17	100	0
	20	100	0

[00644] *Residual free drug determination by Reverse phase-ultra performance liquid chromatography*. The residual free drug level was determined by reverse phase ultra-performance liquid chromatography. After protein precipitation, supernatant was loaded to Luna Omega 1.6 µm Polar C18 100Å column, then eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

Solvent preparation:

[00645] *Solvent I (for protein precipitation)*. Weighed 10 g sodium chloride to the pre-mixed organic solvent of 30 mL methanol and 50 mL CAN, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00646] *Solvent II (diluent)*. Added 3 mL dimethyl sulfoxide to 27 mL formulation buffer and mixed well. Then added 60 mL solvent I and mixed well again.

[00647] *Preparation of standard curve*. The stock standard linker-drug solution was diluted with dimethyl sulfoxide to 1 mM. Added 20 mL of the 1 mM linker-payload solution to 180 mL Solvent II to a final concentration of 100 µM. Then serially diluted the 100 µM standard solution.

[00648]

TABLE 20 Linker-payload standard curve preparation				
Standard Concentration (µM)	Volume of Stock (µL)	Volume of Diluent (µL)	Total Volume (µL)	Final Concentration (µM)
100	150	150	300	50
50	200	300	500	20
20	200	200	400	10

TABLE 20
Linker-payload standard curve preparation

Standard Concentration (µM)	Volume of Stock (µL)	Volume of Diluent (µL)	Total Volume (µL)	Final Concentration (µM)
10	200	200	400	5
5	200	300	500	2
2	200	200	400	1
1	200	200	400	0.5

[00649] *Preparation of samples*. Added 3 µL of dimethyl sulfoxide to 27 µL of antibody-conjugate sample and mixed well. Then added 60 µL solvent I and vortex the mixture for ten minutes on a mixer at room temperature. Centrifuged the mixture for ten minutes at 16,000 rcf at room temperature. And then pipetted 100 µL of the supernatant immediately into a glass high performance liquid chromatography vial for analysis.

TABLE 21
Reverse phase-ultra performance liquid chromatography method for free drug test

Column:	Luna Omega 1.6 µm Polar C18 100Å	
Detection Wavelength:	220nm	
Column Oven Temp.:	30°C	
Sampler Temp.:	2~8°C	
Flow Rate:	0.4 mL/min	
Injection Volume:	40 µL	
Mobile Phases:	A: 0.1% trifluoroacetic acid in water B: 0.1% trifluoroacetic acid in CAN	
Gradient Program	Forty minutes	
Gradient	Time (min)	B (%)
	0.00	2.00
	32.00	50.00
	32.01	90.00
	35.00	90.00
	37.01	2.00
	40.00	2.00

[00650] *Data analysis*: Integrated the stand BAR curve injections. Plotted the peak area (Y) as a function of concentration (X), Y=Kx + b. Calculated the slope (k) and intercept (b).

[00651] Integrated the related drug peak in the sample. Recorded the sum peak area (Y) and interpolated against the standard curve. Calculated the concentration obtained.

[00652] $\mathrm{Cfreedrug} = \left(\frac{\mathrm{Y-b}}{\mathrm{k}}\right) \times \text{dilute factor}$

[00653] $C_{\text{free drug}}$ = concentration of free drug by C18-ultra performance liquid chromatography

[00654] Y = sum peak areas of drug related impurities

[00655] k = slope of standard curve

[00656] b = intercept of standard curve

[00657] Report the relative amount of residual free drug in antibody-conjugate product (%mol/mol) with two decimal place.

[00658] $\%\mathrm{mol/mol} = \frac{\frac{\mathrm{Cfree\ drug}}{\mathrm{Mwdrug}}}{\frac{\mathrm{Cfree\ drug}}{\mathrm{Mwdrug}} + \frac{\mathrm{Cprotein}}{\mathrm{Mwprotein}} * \mathrm{BAR}} * 100$

[00659] $C_{\text{free drug}}$ = concentration of free drug by C18-ultra performance liquid chromatography (mg/mL)

[00660] C_{protein}= concentration of protein (mg/mL)

[00661] BAR = drug to antibody ratio by LC-MS

[00662] $Mw_{\text{Linker payload}}$ = molecular weight of drug

[00663] Mw_{protein} = molecular weight of monoclonal antibody

[00664] The endotoxin level was determined by kinetic turbidimetric assay.

[00665] *Preparation of Endotoxin Standards and quality control*: The CSE (10EU/vial, lyophilized) is reconstituted with 1 mL of water for BET to yield a 10 EU/mL standard solution. Make 10-fold dilution using the water for BET, prepare standard solutions with four concentrations (S1_10 EU/mL, S2_1 EU/mL, S3_0.1 EU/mL, S4_0.01 EU/mL). Dilute CSE with Water for BET to prepare quality control (0.05 EU/mL).

[00666] *Preparation of TAL Reagents*: The TAL reagent is stable when stored at 2~8℃ before expiration. Allow reagents to equilibrate to room temperature prior to use. Added 1.25 mL Water for BET to dissolve one bottle of TAL reagent.

[00667] *Preparation of Samples*: diluted all the sample X/2 folds with Water for BET. Taken half volume of X/2 folds sample and added equal volume of Water for BET to make the finally tested sample with X folds dilution. Added equal volume of S3 in the remaining half of volume of X/2 folds sample to make its PPC sample with endotoxin concentration to quality control.

[00668] Add 100 µL of the standard curve, quality control, sample, PPC and NTC into wells. Add 100 µL of TAL reagent into the 96-well plat wells(Greiner-655185). All standards control and samples need duplicate. Read microplate with a plate reader.

[00669] OD 405 nm was read during incubating at 37°C for 100 min in a microplate reader.

[00670] A standard curve was prepared by plotting the average 405nm measurement for each standard vs. its endotoxin level in EU/mL.

[00671] Standard curve was used to determine the endotoxin level of samples.

Conjugation methods

[00672] *Conjugation buffer and formulation buffer*. **AGN167**, reduction/conjugation buffer: phosphate-buffered saline, pH 7.4, formulation buffer: phosphate-buffered saline, pH 7.4.

[00673] *Procedure of bulk conjugation*

[00674] *Reduction*: To the 5 mL tube (MACRO TUBE 5) containing 12 mg of **ABT740** in original buffer (7.13 mg/mL in phosphate-buffered saline, pH 7.4), 5 mM tris(2-carboxyethyl)phosphine solution (1.5 equivalents), 200 mM diethylenetriamine-pentaacetic acid solution and phosphate-buffered saline, pH 7.4 reduction/conjugation buffer were added. The protein concentration in the reaction was 6.00 mg/mL. The diethylenetriamine-pentaacetic acid concentration in the reaction was 4 mM. The reactions were mixed well and incubated at 22°C for one hour.

[00675] *Conjugation*: Added **TBT506** (10 mM in dimethyl sulfoxide) (3 equivalents) into the reactions, mixed well and incubated at 22°C for another one hour. The crude conjugation was then purified by Zeba Spin desalting column (40K, 10mL) and collected flow-through that contained product.

[00676] The flow-through was then buffer exchanged and concentrated via Amicon® Ultra Centrifugal Filter Unit (10K, 15mL) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) for 30 DV.

[00677] The purified sample was filtered with 0.22 µm filter.

[00678] Perform the quality control test (including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography -high performance liquid chromatography, LC-MS, free drug and endo level).

[00679] Results and discussion of **AGN167**. After conjugation, the reaction mixture is purified by Zeba and buffer exchanged via Amicon® with phosphate-buffered saline, pH 7.4 buffer, then filter with 0.22 µm membrane.

TABLE 22
Reaction condition for bulk conjugation

#		Scale (mg)	TCEP/Protein ratio	Drug/Protein ratio	Organic Solvent	Reaction Temp. & Time
AGN167	**ABT740-TBT506**	12	1.5	3	4.4% DMSO	Reduction: 22°C, one hour, Conj.: 22°C, one hour

TABLE 23
Molecular information of **AGN167**

#	BAR1 Molecular weight (Amino acid, Da)	Modification	Theoretical Mass (Da)	Measured Mass (Da)	Difference (Da)
AGN167	42918.22	BAR1	42934.36	42935.10	0.74

TABLE 24
Quality control summary of **AGN167**

#	Purity %	LC-MS BAR	Percentage of target BAR % (LCMS)	Amount (mg)	Yield (%)	Conc. (mg/mL)	Endo (EU/mg)	Free drug (%)
AGN167	95.48	0.91	84.37	8.13	67.75	7.27	<0.206	<1.03

[00680] Freeze/thaw stability tests. Store the sample under -70°C for one hour to ensure that material is frozen, then the material is allowed to thaw by letting the vial to sit at room temperature for sufficient amount of time until completed thawed. Collect the analytical characterization under different freeze-thaw cycles (1, 2, 3).

TABLE 25
Results for Freeze/thaw tests

Sample ID	Name	SEC purity%	Conc.(mg/mL)	BAR	Percentage of target BAR %
AGN167	Before F/T	95.48	7.27	0.91	84.37
	1st F/T	95.01	7.29	0.89	83.65
	2nd F/T	95.05	6.90	0.90	83.50
	3rd F/T	95.08	7.02	0.89	83.27

[00681] *Summary*. The **AGN167** product was successfully generated. All the results meet targeting quality. Samples are stable under three freeze-thaw cycles.

TABLE 26	
Equipment name	Equipment information
HPLC	Agilent Technologies, HPLC 1260
UHPLC	Agilent Technologies/Agilent 1290
LC-MS	Agilent Technologies, HPLC 1260 / 6224 TOF
Nanodrop 2000	Thermo Scientific, 1294881
Pure water generator	Milli-Q Reference

TABLE 27	
Material name	Material information
Column for LC-MS	Agilent Technologies, PLRP-S, 1000Å 8 μm 50*2.1 mm, PL1912-1802
SEC column	TOSOH, TSKgel G3000SWXL, 7.8×300 mm, 5 μm, 08541
C18 column	Agilent Technologies, InfinityLab Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 μm, 685975-902
HIC column	TOSOH, TSKgel Butyl-NPR, 2.5 μm, 4.6 * 35 mm
Free drug column	Luna Omega 1.6 μm Polar C18 100 Å
Zeba Spin Desalting Column	Thermo Scientific, 40 K MWCO, 10 mL
Amicon, 10 kDa 15 mL	Millipore-UFC901024
Millex-GP Syringe Filter Unit, 0.22 μm	millipore-SLGPR33RB
1X PBS, pH 7.4	Gibco, 10010031
Acetonitrile	Merck, Supelco-1.00030.4008
Trifluoroacetic acid	jkchemical-134753
$Na_2HPO_4.7H_2O$	Sigma-S2429-500G
NaH_2PO_4	Sigma-S5011-500G
$(NH_4)_2SO_4$	Sigma-A4418-1KG
2-propanol	Sigma-34863
K_2HPO_4	SIGMA/ P5655
$KH_2PO_4·3H_2O$	SIGMA/ 900170
Methanol	Merck, 106007
Sodium Chloride	Domestic
Tris(2-carboxyethyl)phosphine	Sigma-C4706

Diethylenetriamine-pentaacetic acid	Sigma-17969-500ML
Dimethyl sulfoxide	Sigma-Aldrich,BCCL4606
Rapid PNGase F (non-reducing format)	BioLabs-P0711S
5X Rapid PNGase F buffer (non-reducing format)	BioLabs-B0717S
L-histidine	Merck/1.04352.1000
L-histidine.HCl	Avantor-4942-06
Sucrose	Pfanstiehl/ S-124-1-MC

EXAMPLE 19

AGN364* (*ABT817-TBT644*, *Target BAR = 1)

[00682] The molecule of **AGN364** is a sortase-ligation based antibody-conjugate. The molecule consists of one single chain (**ABT817**, peptide), which is conjugated to a pentaglycine modified **TBT644** via sortase-catalyzed ligation. The targeting BAR is 1.

Characteristics for the antibody-conjugate product are shown:

[00683] *BAR*: 0.9-1.1

[00684] *Purity by size exclusion chromatography (HPLC)*: >95%

[00685] *Free drug residue*: <5% (mol/mol of total drug)

[00686] *Endotoxin*: <0.5 EU/mg.

Methods. Purification method for antibody conjugate by Ni Excel.

[00687] The solution was purified by Ni Excel to remove sortase A and unconjugated protein.

[00688] *Instrument*: AKTA pure.

[00689] *Column*: HisTrap excel, 5 mL.

[00690] *Equilibration buffer*: 20 mM sodium phosphate, 500 mM sodium chloride, 10 mM imidazole pH 7.4

[00691] *Elution buffer*: 0.2M sodium hydroxide.

[00692] *Flow rate*: 4 mL/min.

[00693] *Wavelength*: 280 nm.

[00694] Purification method by Amicon® Ultra Centrifugal Filter Unit

[00695] An ultrafiltration membrane having a molecular weight of 10 kDa is preferable to concentrate the sample. The Amicon Ultra Centrifugal Filter Unit was used according to the following procedure:

[00696] dH_2O wash the centrifugal filter unit.

[00697] Equilibrated the centrifugal filter unit with phosphate-buffered saline, pH 7.4.

[00698] Add up sample to the Amicon® Ultra filter device and centrifuged to concentrate.

[00699] Collected and filtered sample with 0.22 μm membrane.

[00700] Ultraviolet–visible spectroscopy platform by Nanodrop to determine protein concentration of in-process sample and final product.

[00701] (1) 750 nm was set up as baseline.

[00702] (2) The UV absorption at 280 nm and 220 nm were measured respectively

[00703] (3) Calculation method based on Beer-Lambert Law A=E*C*l

[00704] $A_{280}=E^{mAb}{}_{280}*C_{[mAb]}*l$

[00705] E: molar extinction coefficient;

[00706] C: molar concentration;

[00707] l: light path (Nanodrop: 0.1 cm)

[00708] *BAR determination by LC-MS*. LC-MS was performed using a combination of Agilent 1260 series high performance liquid chromatography system and time-of-flight mass spectrometry. BAR was calculated based on the peak abundance of the deconvoluted mass.

[00709] Preparation of sample used in LC-MS analysis.

[00710] An antibody-drug conjugate solution was diluted to approximately 1 mg/mL, 30uL totally with water. The obtained sample was used in an LC-MS analysis.

[00711] *LC-MS/MS method*. A high performance liquid chromatography analysis was carried out under the following measurement conditions.

TABLE 28
LC parameters for BAR determination

Equipment	HPLC Agilent 1260
Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 μm, 1000Å
Detection Wavelength:	280 nm, 214nm
Column Oven Temp.:	80°C
Sampler Temp.:	2~8°C
Flow Rate:	0.05 mL/min
Injection Amount:	2 μg
Mobile Phases:	A:0.025%TFA+0.1%FA in water B:0.025%TFA+0.1%FA in acetonitrile

TABLE 28 LC parameters for BAR determination		
Gradient Program	ten minutes	
Gradient	Time (min)	B (%)
	0.0	5
	0.7	5
	5	45
	6	90
	7	90
	7.1	5
	10.0	5

TABLE 29 *Time of flight parameters for BAR determination*	
Equipment	Agilent 6230TOF
Polarity	positive
Gas Temp.	350oC
Drying Gas	13 L/min
Nebulizer	45 psig
VCap	5000 V
Fragmentor	350 V
Mass Range	500 – 8000 m/z
Acquisition Rate	1 spectra/s

[00712] Aggregation determination by size exclusion chromatography-high performance liquid chromatography. Size-exclusion chromatography was performed using an Agilent 1260 series high performance liquid chromatography system with the TSK gel G3000SWXL Size-exclusion chromatography column (7.8×300 mm, 5 µm) at 25°C. The mobile phase was consisted of 78 mM KH_2PO_4, 122 mM K_2HPO_4, 250 mM potassium chloride, 15% isopropanol at pH 7.0±0.1. The flow rate was set at 0.75 mL/min. Sample loading was 40~50 µg per injection. Samples were detected at 280 nm and 370 nm with an ultraviolet detector. The retention time of the aggregation peak was recorded based on its relative molecular weight. The aggregation level was determined by the relative area of the peak at 280 nm.

[00713] *Residual free drug determination by LC-MS*. The residual free drug level was determined by LC-MS. After protein precipitation, supernatant was loaded to InfinityLab Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 µm column, and eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

[00714] *Solvent I preparation*: Weighed 10 g sodium chloride to the pre-mixed organic solvent of 30 mL methanol and 50 mL acetonitrile, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00715] *Preparation of standard curve*. The stock standard linker-drug solution was firstly diluted to 40 µM with dimethyl sulfoxide for standard curve preparation. Then the standard curve and sample were prepared (MW_{mAb} 21382 Da, monoclonal antibody concentration 7.87mg/mL and BAR value 1.00 were used for calculation).

TABLE 30
Linker-payload Standard Preparation

Solution ID	Final mAb Conc. (mg/mL)	$V_{protein}$ (µL)	Final Drug Conc. (%mol/mol)	Drug Conc. (µM)	V_{Drug} (µL)	DMSO Vol. frac.	V_{DMSO} (µL)	V_{H2O} (µL)	Solvent I (µL)
STD 5%	1.00	19.06	5.00	2.46	9.23	0.1	5.77	15.9	100.00
STD 2%	1.00	19.06	2.00	0.95	3.58	0.1	11.42	15.9	100.00
STD 1%	1.00	19.06	1.00	0.47	1.77	0.1	13.23	15.9	100.00

[00716] The sample were prepared (product concentration 10.86 mg/mL was used for calculation).

TABLE 31
Sample Preparation

Conc. (mg/mL)	V_{ADC}(µL)	DMSO volume frac.	V_{DMSO}(µL)	V_{H2O}(µL)	Solvent I(µL)
1.00	13.8	0.1	15.00	21.2	100.00

TABLE 32
LC parameters for free drug determination

Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 µm, 1000Å
Detection Wavelength:	280 nm, 220nm
Column Oven Temp.:	80°C
Sampler Temp.:	2~8°C
Flow Rate:	0.4 mL/min

TABLE 32 LC parameters for free drug determination			
Stop Time:	13 minutes		
Maximum Pressure:	350 bar		
Injection Amount:	20 µL		
Mobile Phases:	Mobile Phase A: 0.025% TFA + 0.1% FA in water Mobile Phase B: 0.025% TFA + 0.1% FA in acetonitrile		
Gradient Program	Time(min)	A%	B%
	2.00	95.0	5.0
	5.00	70.0	30.0
	8.00	20.0	80.0
	8.50	10.0	90.0
	10.00	10.0	90.0
	10.01	95.0	5.0
	13.00	95.0	5.0

TABLE 33 Q-TOF parameters for free drug determination	
Equipment	Agilent 6530 QTOF
Polarity	positive
Gas Temp.	250°C
Drying Gas	13 L/min
Nebulizer	45 psig
VCap	3500 V
Fragmentor	125 V
Mass Range	150 – 8000 m/z
Acquisition Rate	spectra/s

Endotoxin determination

[00717] The endotoxin level was determined by Endosafe® Nexgen-PTS™.

[00718] Diluted sample with endo-free water, pipetted 25 µL of diluted sample into each of the four reservoirs of the PTS Cartridge. The reader drew and mixed the sample with the LAL reagent in the sample channels in addition to the LAL reagent plus positive product control in the spike channels. The

sample was combined with the chromogenic substrate then incubated. After mixing, the optical density of the wells was measured and analyzed against an internally archived standard curve.

Conjugation methods:

[00719] *Conjugation and formulation buffer*. AGN364, conjugation buffer: 50mM HEPES, 150mM sodium chloride, 10% glycerol(V/V), pH 7.5, formulation buffer, phosphate-buffered saline, 2mM EDTA, pH 7.4.

Procedure of bulk conjugation

[00720] (1) To 150 mL bottle (Corning, 431175) containing 100 mg of **ABT817** in original buffer (50 mM HEPES, 150 mM sodium chloride, 10% glycerol(V/V), pH 7.5) were added with 5.08 mg/mL sortase A (PT-0008, 0.01 equivalents) solution, **TBT644** (40 mM in dimethyl sulfoxide) (sixteen equivalents) into the reactions, mixed well and incubated at 4°C for overnight. The protein concentration in the reaction was 3 mg/mL.

[00721] (2) The crude conjugation was purified with AKTA pure chromatography. The conjugate and free linker-payload were flowed through. The sortase A and unconjugated protein with His-tag were bounded to the column and eluted by 0.2M NaOH. Pooled flow through liquid together and performed buffer exchange to phosphate-buffered saline, pH7.4, 5% dimethyl sulfoxide for 30 DV follow by phosphate-buffered saline, pH7.4 for 20 DV with Amicon® Ultra Centrifugal Filter Unit (10 kDa, 4*15mL). Add 200mM EDTA to keep the final formulation buffer contained 2mM EDTA.

[00722] (3) The purified sample was filtered with 0.22 μm filter.

[00723] (4) Perform the quality control test (including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, LC-MS, free drug and endotoxin level).

[00724] *Results and discussion of bulk conjugation*. After conjugation, the reaction mixture is purified by Ni Excel and exchange buffer to phosphate-buffered saline, pH 7.4 use with Amicon® Ultra Centrifugal Filter Unit (10 kDa, 4*15mL), supplement 2mM EDTA, then filter with 0.22 μm membrane.

TABLE 34
Reaction condition for bulk conjugation

#		Sortase A/ mAb ratio	Drug/mAb ratio	Conjugation Conc. (mg/mL)	Conjugation temp.& time	Conjugation pH
AGN364	**ABT817-TBT644**	0.01	16	3	4°C, overnight	7-7.5

TABLE 35
Molecular information of **AGN364**

#	BAR1 Molecular weight (Amino acid, Da)	Modification	Theoretical Mass (Da)	Measured Mass (Da)	Difference (Da)
AGN364	19718.04	G3FNS2	22435.5	22436.86	1.36

TABLE 36
Results for bulk conjugation

#	UV Conc. (mg/mL)	Amount (mg)	MS-BAR	SEC-Purity (%)	Free drug (%)	Endo (EU/mg)
AGN364	10.86	69.40	1.00	99.05	<1.0	<0.092

[00725] *Results and discussion of stability tests*. Store the sample under -70°C for one hour to ensure that material is frozen, then the material is allowed to thaw by letting the vial to sit at room temperature for sufficient amount of time until completed thawed. Collect the analytical characterization under different freeze-thaw cycles (1, 2, 3).

TABLE 37
Results for thermal stability tests.

	Time point (cycles)	Conc.(mg/mL)	SEC purity (%)	BAR
AGN364	1	98.65	10.93	1.00
	2	98.65	10.87	1.00
	3	98.68	11.02	1.00

[00726] *Summary*. The **AGN364** product was successfully generated. All the results meet targeting quality. Sample was stable under three freeze-thaw cycles.

EXAMPLE 20

***AGN365 (ABT819-TBT644**, target BAR = 1)*

[00727] The molecule of **AGN365** is a sortase-ligation based antibody-conjugate. The molecule consists of one single chain (**ABT819**, peptide), which is conjugated to a pentaglycine modified **TBT644** via sortase-catalyzed ligation.

Characteristics for the product are shown:

[00728] *BAR*: 0.9-1.1

[00729] *Purity by size exclusion chromatography (HPLC)*: >95%

[00730] *Free drug residue*: <5% (mol/mol of total drug)

[00731] *Endotoxin*: <0.5 EU/mg.

Methods

[00732] Purification method by Ni Excel. The solution was purified by Ni Excel to remove sortase A and unconjugated protein.

[00733] *Instrument*: AKTA pure.

[00734] *Column*: HisTrap excel, 5 mL.

[00735] *Equilibration buffer*: 20 mM sodium phosphate, 500 mM sodium chloride, 10 mM imidazole pH 7.4

[00736] *Elution buffer*: 0.2M NaOH

[00737] *Flow rate*: 4 mL/min.

[00738] *Wavelength*: 280 nm.

[00739] Purification method by Amicon® Ultra Centrifugal Filter Unit

[00740] An ultrafiltration membrane having a molecular weight of 10 kDa is preferable to concentrate the sample. The Amicon Ultra Centrifugal Filter Unit was used according to the following procedure:

[00741] dH_2O wash the centrifugal filter unit.

[00742] Equilibrated the centrifugal filter unit with phosphate-buffered saline, pH 7.4.

[00743] Add up sample to the Amicon® Ultra filter device and centrifuged to concentrate.

[00744] Collected and filtered sample with 0.22 μm membrane.

[00745] Ultraviolet–visible spectroscopy platform by Nanodrop to determine protein concentration of in-process sample and final product.

[00746] (1) 750 nm was set up as baseline.

[00747] (2) The ultraviolet absorption at 280 nm and 220 nm were measured respectively.

[00748] (3) Calculation method based on Beer-Lambert Law A=E*C*l.

[00749] $A_{280}=E^{mAb}{}_{280}*C_{[mAb]}*l$.

[00750] E: molar extinction coefficient.

[00751] C: molar concentration.

[00752] l: light path (Nanodrop: 0.1 cm).

[00753] BAR determination by LC-MS.

[00754] LC-MS was performed using a combination of Agilent 1260 series high performance liquid chromatography system and time-of-flight mass spectrometry. BAR was calculated based on the peak abundance of the deconvoluted Mass.

[00755] *Preparation of sample used in LC-MS analysis*. An antibody-drug conjugate solution was diluted to approximately 1 mg/mL, 30uL totally with water. The obtained sample was used in an LC-MS analysis.

[00756] A high performance liquid chromatography analysis was carried out under the following measurement conditions.

TABLE 38
LC parameters for BAR determination

Equipment	HPLC Agilent 1260	
Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 μm, 1000Å	
Detection Wavelength:	280 nm, 214nm	
Column Oven Temp.:	80°C	
Sampler Temp.:	2~8°C	
Flow Rate:	0.05 mL/min	
Injection Amount:	2 μg	
Mobile Phases:	A:0.025%TFA+0.1%FA in water B:0.025%TFA+0.1%FA in acetonitrile	
Gradient Program	Ten minutes	
Gradient	Time (min)	B (%)
	0.0	5
	0.7	5
	5	45
	6	90
	7	90
	7.1	5
	10.0	5

TABLE 39 Time-of-flight parameters for BAR determination	
Equipment	Agilent 6230TOF
Polarity	positive
Gas Temp.	350°C
Drying Gas	13 L/min
Nebulizer	45 psig
VCap	5000 V
Fragmentor	350 V
Mass Range	500 – 8000 m/z
Acquisition Rate	1 spectra/s

[00757] *Aggregation determination by SEC-HPLC*. Size-exclusion chromatography was performed using an Agilent 1260 series high performance liquid chromatography system with the TSK gel G3000SWXL Size-exclusion chromatography column (7.8×300 mm, 5 μm) at 25°C. The mobile phase was consisted of 78 mM KH_2PO_4, 122 mM K_2HPO_4, 250 mM potassium chloride, 15% isopropanol at pH 7.0±0.1. The flow rate was set at 0.75 mL/min. Sample loading was 40~50 μg per injection. Samples were detected at 280 nm and 370 nm with an ultraviolet detector. The retention time of the aggregation peak was recorded based on its relative molecular weight. And the aggregation level was determined by the relative area of the peak at 280 nm.

[00758] *Residual free drug determination by LC-MS*: The residual free drug level was determined by LC-MS. After protein precipitation, supernatant was loaded to InfinityLab Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 μm column, and eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

[00759] *Solvent I preparation*: Weighed 10g sodium chloride to the pre-mixed organic solvent of 30 mL methanol and 50 mL ACN, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00760] *Preparation of standard curve*. The stock standard linker-drug solution was firstly diluted to 40 μM with dimethyl sulfoxide for standard curve preparation. Then the standard curve and sample were prepared (MW_{mAb} 43861 Da, monoclonal antibody concentration 7.38 mg/mL and BAR value 0.99 were used for calculation).

TABLE 40
Linker-payload Standard Preparation

	Final mAb Conc. (mg/mL)	$V_{protein}$ (µL)	Final Drug Conc. (%mol/mol)	Drug Conc. (µM)	V_{Drug} (µL)	DMSO Volume frac.	V_{DMSO} (µL)	V_{H2O} (µL)	Solvent I (µL)
STD 5%	1.00	20.33	5.00	1.19	4.45	0.1	10.55	14.7	100.00
STD 2%	1.00	20.33	2.00	0.46	1.73	0.1	13.27	14.7	100.00
STD 1%	1.00	20.33	1.00	0.23	0.85	0.1	14.15	14.7	100.00

[00761] The sample were prepared (product concentration 10.41 mg/mL.)

TABLE 41
Sample preparation

Conc. (mg/mL)	V_{ADC}(µL)	DMSO volume frac.	V_{DMSO} (µL)	V_{H2O} (µL)	Solvent I (µL)
1.00	14.4	0.1	15.00	20.6	100.00

TABLE 42
LC parameters for free drug determination

Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 µm, 1000Å
Detection Wavelength:	280 nm, 220nm
Column Oven Temp.:	80°C
Sampler Temp.:	2~8°C
Flow Rate:	0.4 mL/min
Stop Time:	13 min
Maximum Pressure:	350 bar
Injection Amount:	20 µL
Mobile Phases:	Mobile Phase A: 0.025%TFA+0.1%FA in H_2O Mobile Phase B: 0.025%TFA+0.1%FA in ACN

TABLE 42
LC parameters for free drug determination

Gradient Program	Time(min)	A%	B%
	2.00	95.0	5.0
	5.00	70.0	30.0
	8.00	20.0	80.0
	8.50	10.0	90.0
	10.00	10.0	90.0
	10.01	95.0	5.0
	13.00	95.0	5.0

TABLE 43
Q-TOF parameters for free drug determination

Equipment	Agilent 6530 Q-TOF
Polarity	positive
Gas Temp.	250°C
Drying Gas	13 L/min
Nebulizer	45 psig
VCap	3500 V
Fragmentor	125 V
Mass Range	150 – 8000 m/z
Acquisition Rate	1 spectra/s

[00762] *Endotoxin determination*. The endotoxin level was determined by Endosafe® Nexgen-PTS™. Diluted sample with endo-free water, pipetted 25 µL of diluted sample into each of the four reservoirs of the PTS Cartridge. The reader drew and mixed the sample with the LAL reagent in the sample channels in addition to the LAL reagent plus positive product control in the spike channels. The sample was combined with the chromogenic substrate then incubated. After mixing, the optical density of the wells was measured and analyzed against an internally archived standard curve.

Conjugation methods

[00763] *Conjugation buffer and formulation buffer*: **AGN365**, conjugation buffer, 50mM HEPES, 150mM sodium chloride, 10% glycerol(V/V), pH 7.5, formulation buffer, phosphate-buffered saline, 2mM EDTA, pH 7.4.

Procedure of bulk conjugation

[00764] (1) To 150 mL bottle (Corning, 431175) containing 100 mg of **ABT819** in original buffer (50mM HEPES, 150 mM sodium chloride, 10% Glycerol(V/V), pH 7.5) were added with 5.08 mg/mL sortase A (PT-0008, 0.01 equivalents) solution, **TBT644** (40 mM in DMSO) (sixteen equivalents) into the reactions, mixed well and incubated at 4°C for overnight. The protein concentration in the reaction was 3 mg/mL.

[00765] (2) The crude conjugation was purified with AKTA pure chromatography. The conjugate and free linker-payload were flowed through. The sortase A and unconjugated protein with His-tag were bounded to the column and eluted by 0.2M NaOH. Pooled flow through liquid together and performed buffer exchange to phosphate-buffered saline, pH7.4, 5% DMSO for 30 DV follow by phosphate-buffered saline, pH7.4 for 20 DV with Amicon® Ultra Centrifugal Filter Unit (10 kDa, 4*15mL). Add 200 mM EDTA to keep the final formulation buffer contained 2 mM EDTA.

[00766] (3) The purified sample was filtered with 0.22 μm filter.

[00767] (4) Perform the quality control test (including concentration, SEC-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, LC-MS, free drug and endotoxin level).

[00768] *Results and discussion of bulk conjugation*. After conjugation, the reaction mixture is purified by Ni Excel and exchange buffer to phosphate-buffered saline, pH 7.4 use with Amicon® Ultra Centrifugal Filter Unit (10 kDa, 4*15mL), supplement 2 mM EDTA, then filter with 0.22 μm membrane.

TABLE 44
Reaction condition for bulk conjugation

#	Sample ID	Sortase A/mAb ratio	Drug/mAb ratio	Conjugation Conc. (mg/mL)	Conjugation temp.& time	Conjugation pH
AGN365	**ABT819-TBT644**	0.01	16	3	4°C, overnight	7-7.5

TABLE 45
Molecular information of **AGN365**

	BAR1 Molecular weight (Amino acid, Da)	Modification	Theoretical Mass (Da)	Measured Mass (Da)	Difference (Da)
AGN365	42561.91	G2FS2	44914.12	44914.95	0.83

TABLE 46
Results for bulk conjugation

#	UV Conc. (mg/mL)	Amount (mg)	MS-BAR	SEC-Purity (%)	Free drug (%)	Endo (EU/mg)
AGN365	10.41	69.12	0.99	98.79	<1.0	<0.048

[00769] *Results and discussion of stability tests*. Store the sample under -70°C for one hour to ensure that material is frozen, then the material is allowed to thaw by letting the vial to sit at room temperature for sufficient amount of time until completed thawed. Collect the analytical characterization under different freeze-thaw cycles (1, 2, 3).

TABLE 47
Results for thermal stability tests

#	Time point (cycles)	Concentration (mg/mL)	SEC purity (%)	BAR
AGN365	1	98.78	10.10	0.99
	2	98.78	10.20	0.98
	3	98.78	10.09	0.98

[00770] *Summary*. The **AGN365** product was successfully generated. All the results meet targeting quality. Sample was stable under three freeze-thaw cycles.

EXAMPLE 21

***AGN579 (ABT818-TBT-307**, Target BAR = 2)*

[00771] The molecule of **AGN579** is a lysine-based antibody-conjugate. The molecule consists of two same single chains covalently linked by disulfide bonds (**ABT818**, Fc-fusion). The payload (**TBT307**) is conjugated to lysine amines in the antibody. The leaving group will be eliminated when the payload is conjugated to amines.

Characteristics for the product:

[00772] *BAR*: 1.8-2.2

[00773] *Purity by size exclusion chromatography (HPLC)*: >95%

[00774] *Free drug residue*: <5% (mol/mol of total drug)

[00775] *Endotoxin*: <0.5 EU/mg.

[00776] *Purification method by UF/DF*. An ultrafiltration membrane (Pellicon3 0.11m^2 Cassette, Ultracel 30 kDa) having a molecular weight of 30 kDa is preferable to concentrate the sample. The UF/DF was preprocessed according to the following procedure:

[00777] (1) Assemble the UF/DF system and install the cassette (0.11 m^2 membrane area, 30 kDa MWCO).

[00778] (2) Flush the system with water, clean with the 0.1 M sodium hydroxide for 30minutes, and flush with water again.

[00779] (3) Add phosphate-buffered saline, pH 7.4 to the feed tank. Start the feed pump. Verify that the pH and conductivity in the system have been equilibrated to the level of the phosphate-buffered saline.

[00780] (4) Add reaction mixture solution to the feed tank. Start the feed pump by partially closing the retentate valve and adjusting the pump speed. Diafilter the product with the phosphate-buffered saline, pH7.4 10% DMSO for 40 DV, followed by phosphate-buffered saline, pH7.4 for 20 DV.

[00781] (5) Open the retentate valve fully and collect all the product. Filter the product by 0.22µm membrane.

[00782] (6) Test the quality and concentration of product.

[00783] Ultraviolet–visible spectroscopy platform by Nanodrop to determine protein concentration of in-process sample and final product.

[00784] (1) 750 nm was set up as baseline.

[00785] (2) The UV absorption at 280 nm and 220 nm were measured respectively.

[00786] (3) Calculation method based on Beer-Lambert Law A=E*C*l.

[00787] $A_{280}=E^{mAb}{}_{280}*C_{[mAb]}*l$

[00788] E: molar extinction coefficient;

[00789] C: molar concentration;

[00790] l: light path (Nanodrop: 0.1 cm)

[00791] *BAR determination by LC-MS*. LC-MS was performed using a combination of Agilent 1260 series high performance liquid chromatography system and time-of-flight mass spectrometry. BAR was calculated based on the peak abundance of the deconvoluted Mass.

[00792] *Preparation of sample used in LC-MS analysis (Deglycosylation)*. Transfer 15 µg of samples into a 1.5 mL tube, add 3 µL Rapid PNGase F (non-reducing format, 5X) enzyme buffer and 0.5 µL Rapid PNGase F (non-reducing format) enzyme for de-glycosylation and some water to ensuring the total volume is 15 µL, then incubate this solution at 37 °C for two hours or overnight. The obtained sample was used in an LC-MS analysis.

[00793] A high performance liquid chromatography analysis was carried out under the following measurement conditions.

TABLE 48
Liquid chromatography parameters for BAR determination

Equipment	HPLC Agilent 1290	
Column:	Agilent PLRP-S, 50 x 2.1 mm, 8 µm, 1000A	
Detection Wavelength:	280 nm, 214nm	
Column Oven Temp.:	30°C	
Sampler Temp.:	2~8°C	
Flow Rate:	0.05 mL/min	
Injection Amount:	3 µg	
Mobile Phases:	A:0.025%TFA+0.1%FA in water B:0.025%TFA+0.1%FA in ACN	
Gradient Program	ten minutes	
Gradient	Time (min)	B (%)
	0.0	5
	0.3	20
	2	30
	5	50
	7	90
	8	90
	8.1	5
	10.0	5

TABLE 49 Q-TOF parameters for BAR determination	
Equipment	Agilent 6530 Q-TOF
Polarity	positive
Gas Temp.	350°C
Drying Gas	13 L/min
Nebulizer	45 psig
VCap	5000 V
Fragmentor	350 V
Mass Range	500 – 8000 m/z
Acquisition Rate	1 spectra/s

[00794] *Aggregation determination by SEC-HPLC*. Size-exclusion chromatography was performed using an Agilent 1260 series HPLC system with the TSK gel G3000SWXL Size-exclusion chromatography column (7.8×300 mm, 5 µm) at 25°C. The mobile phase was consisted of 78 mM KH_2PO_4, 122 mM K_2HPO_4, 250 mM potassium chloride, 15% isopropanol at pH 7.0±0.1. The flow rate was set at 0.75 mL/min. Sample loading was 40~50 µg per injection. Samples were detected at 280 nm and 370 nm with a UV detector. The retention time of the aggregation peak was recorded based on its relative molecular weight. And the aggregation level was determined by the relative area of the peak at 280 nm.

[00795] *Residual uABT & LP determination by reverse phase-UPLC*. The residual free drug level was determined by reverse phase UPLC. After protein precipitation, supernatant was loaded to Luna Omega 1.6 µm Polar C18 100Å column and eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

[00796] *Solvent preparation*: Solvent I (for protein precipitation)

[00797] Weighed 10 g sodium chloride to the pre-mixed organic solvent of 30 mL methanol and 50 mL ACN, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00798] *Dilution buffer I*. Mix 250 µL of DMSO, 700 µL of phosphate-buffered saline, 1000 µL of 6 mg/mL Herceptin® in phosphate-buffered saline as buffer I.

[00799] *Dilution buffer II*. Mix 300 µL of DMSO, 700 µL of phosphate-buffered saline, 1000µL of 6 mg/mL Herceptin® in phosphate-buffered saline as buffer II.

[00800] *Preparation of standard curve (uABT (cpd1724) /linker payload (**TBT307**))*. The stock standard uABT (cpd1724) /linker payload (**TBT307**) solution was firstly diluted to 1000 µM with DMSO for standard curve preparation. Add 10 µL of the 1000 µM uABT to 390 µL of dilution buffer I for a final concentration of 25 µM. Then the standard curve and sample were prepared. Take 160 µL of each standard samples and add 240 µl solvent buffer I as final standard curve (10 µM, 5 µM, 2 µM, 1 µM, 0.5 µM, 0.2 µM, 0.1 µM). Vortex solution for ten minutes at room temperature. Centrifuge solution for ten minutes at 16,000 rcf at room temperature. Remove the supernatant immediately into a glass vial for analysis.

TABLE 50
uABT/linker Dilution table

Standard Conc. (µM)	Volume of Stock (µL)	Volume of dilution buffer II (µL)	Total Volume (µL)	Final Conc. (µM)
25	200	200	400	12.5
12.5	160	240	400	5
5	200	200	400	2.5
2.5	200	200	400	1.25
1.25	160	240	400	0.5
0.5	120	120	240	0.25

[00801] Preparation of sample. Mix 30 µL sample with 60 µL solvent I (sample: precipitant 1:2 v/v). Vortex solution for ten minutes at room temperature. Centrifuge solution for ten minutes at 16,000 rcf at room temperature. Remove the supernatant immediately into a glass vial for analysis.

TABLE 51
Reverse phase-HPLC parameters for free drug determination

Column:	Luna Omega 1.6 µm Polar C18 100Å
Detection Wavelength:	220nm
Column Oven Temp.:	30°C
Sampler Temp.:	2~8°C
Flow Rate:	0.4 mL/min
Injection Volume:	40 µL
Mobile Phases:	A: 0.1% TFA in water B: 0.1% TFA in ACN
Gradient Program	forty minutes

TABLE 51
Reverse phase-HPLC parameters for free drug determination

Gradient	Time (min)	B (%)
	0.00	2.00
	32.00	50.00
	32.01	90.00
	35.00	90.00
	37.01	2.00
	40.00	2.00

[00802] Data analysis (linker payload)

[00803] Integrated the stand BAR curve injections. Plotted the peak area (Y) as a function of concentration (X), Y=Kx + b. Calculated the slope (k) and intercept (b).

[00804] Integrated the related drug peak in the sample. Recorded the sum peak area (Y) and interpolated against the standard curve. Calculated the concentration obtained.

[00805] $\text{Clinker payload} = \left(\frac{Y-b}{k}\right) \times \text{dilute factor}$

[00806] $C_{\text{linker payload}}$ = concentration of linker payload by C18-UPLC

[00807] Y = sum peak areas of drug related impurities

[00808] k = slope of standard curve

[00809] b = intercept of standard curve

[00810] Report the relative amount of residual linker payload in product (%mol/mol) with two decimal place.

[00811] $\%\text{mol/mol} = \frac{\frac{\text{Clinker paylaod}}{\text{Mwlinker payload}}}{\frac{\text{Clinker payload}}{\text{Mwlinker payload}} + \frac{\text{Cprotein}}{\text{Mwprotein}} * \text{BAR}} * 100$

[00812] $C_{\text{linker payload}}$ = concentration of linker payload by C18-UPLC (mg/mL)

[00813] C_{protein}= concentration of protein (mg/mL)

[00814] BAR = drug to antibody ratio by LC-MS

[00815] $Mw_{\text{Linker payload}}$ = molecular weight of drug

[00816] Mw_{protein} = molecular weight of monoclonal antibody

[00817] Data analysis (uABT)

[00818] Integrated the stand BAR curve injections. Plotted the peak area (Y) as a function of concentration (X), Y=Kx + b. Calculated the slope (k) and intercept (b).

[00819] Integrated the related drug peak in the sample. Recorded the sum peak area (Y) and interpolated against the standard curve. Calculated the concentration obtained.

[00820] $\mathrm{CuABT} = \left(\frac{\mathrm{Y}-\mathrm{b}}{\mathrm{k}}\right) \times \text{dilute factor}$

[00821] C_{uABT} = concentration of uABT by C18-UPLC

[00822] Y = sum peak areas of drug related impurities

[00823] k = slope of standard curve

[00824] b = intercept of standard curve

[00825] Report the relative amount of residual uABT in product (%mol/mol) with two decimal place.

[00826] $\%\mathrm{mol/mol} = \frac{\frac{\mathrm{C\ uABT}}{\mathrm{MwuABT}}}{\frac{\mathrm{Cprotein}}{\mathrm{Mwprotein}}} * 100$

[00827] C_{uABT} = concentration of uABT by C18-UPLC (mg/mL)

[00828] $C_{protein}$= concentration of protein (mg/mL)

[00829] BAR = drug to antibody ratio by LC-MS

[00830] Mw_{uABT} = molecular weight of uABT

[00831] $Mw_{protein}$ = molecular weight of mAb

[00832] *Residual payload determination by RP-UPLC*. The residual free drug level was determined by reverse phase UPLC. After protein precipitation, supernatant was loaded to Luna Omega 1.6 µm Polar C18 100Å column and eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

[00833] *Solvent preparation*: Solvent I (for protein precipitation)

[00834] Weighed 10 g sodium chloride to the pre-mixed organic solvent of 30 mL MeOH and 50 mL ACN, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00835] *Solvent II*. Add 5 mL formulation buffer to 10 mL Solvent I (FB: precipitant 1:2 v/v) and mix well.

[00836] *Preparation of standard curve (payload cpd1777)*. The stock standard payload cpd1777 solution was firstly diluted to 1000 µM with formulation buffer for standard curve preparation. Add 10 µL of the 1000 µM payload cpd1777 to 90 µL of solvent II for a final concentration of 100 µM. Then the standard curve and sample were prepared**Error! Reference source not found.**. Vortex solution for ten minutes at room temperature. Centrifuge solution for ten minutes at 16,000 rcf at room temperature. Remove the supernatant immediately into a glass vial for analysis.

[00837] *Preparation of sample*. Add phosphate-buffered saline and DMSO to sample to 6 mg/mL with 15% (v/v) DMSO. Then mix 30 µL 6 mg/mL sample with 45 µL solvent I (sample: precipitant 1:1.5v/v). Vortex solution for ten minutes at room temperature. Centrifuge solution for ten minutes at 16,000 rcf at room temperature. Remove the supernatant immediately into a glass vial for analysis.

TABLE 52
RP-HPLC parameters for free drug determination

Column:	Luna Omega 1.6 µm Polar C18 100Å	
Detection Wavelength:	196nm	
Column Oven Temp.:	30°C	
Sampler Temp.:	2~8°C	
Flow Rate:	0.4 mL/min	
Injection Volume:	40 µL	
Mobile Phases:	A: 0.1% TFA in water B: 0.1% TFA in ACN	
Gradient Program	Twenty-eight minutes	
Gradient	Time (min)	B (%)
	0.00	5.00
	20.00	35.00
	20.01	90.00
	23.00	90.00
	23.01	5.00
	28.00	5.00

[00838] *Data analysis*. Integrated the stand BAR curve injections. Plotted the peak area (Y) as a function of concentration (X), Y=Kx + b. Calculated the slope (k) and intercept (b).

[00839] Integrated the related drug peak in the sample. Recorded the sum peak area (Y) and interpolated against the standard curve. Calculated the concentration obtained.

[00840] $\text{Cpayload} = \left(\frac{\text{Y}-\text{b}}{\text{k}}\right) \times \text{dilute factor}$

[00841] $C_{payload}$ = concentration of payload by C18-UPLC

[00842] Y = sum peak areas of drug related impurities

[00843] k = slope of standard curve

[00844] b = intercept of standard curve

[00845] Report the relative amount of residual payload in product (%mol/mol) with two decimal place.

[00846] $$\%\mathrm{mol/mol} = \frac{\frac{\mathrm{Cpayload}}{\mathrm{Mwpayload}}}{\frac{\mathrm{Cpayload}}{\mathrm{Mpayload}} + \frac{\mathrm{Cprotein}}{\mathrm{Mwprotein}} * \mathrm{BAR}} * 100$$

[00847] $C_{payload}$ = concentration of payload by C18-UPLC (mg/mL).

[00848] $C_{protein}$= concentration of protein (mg/mL).

[00849] BAR = drug to antibody ratio by LC-MS.

[00850] $Mw_{payload}$ = molecular weight of payload.

[00851] $Mw_{protein}$ = molecular weight of mAb.

[00852] *Hydrophobicity determination by hydrophobic interaction chromatography-HPLC*. Hydrophobic Interaction Chromatography was performed using an Agilent 1260 series HPLC system with the TSK gel butyl-nonporous hydrophobic interaction chromatography column (4.6×35 mm, 5 µm) at 25°C. The mobile phase A was consisted of 1.5 M $(NH_4)_2SO_4$ and 50 mM $K_2HPO_4{\cdot}3H_2O$, at pH 7.2±0.2, Cond. 190,000±200 us/cm. The mobile phase B was consisted of 50 mM potassium phosphate and 25% 2-propanol, at pH 7.4±0.2, Cond. 3400±200 us/cm. The flow rate was set at 0.6 mL/min. Sample loading was 8 µL per injection. Samples were detected at 280 nm with an ultraviolet detector.

TABLE 53
HIC-high performance liquid chromatography parameters

Column	TOSOH, TSKgel butyl-nonporous, 2.5 µm, 4.6 * 35 mm		
Detection	280 nm BW 4		
Column Oven Temp.:	25°C		
Sampler Temp.:	6 ± 2°C		
Flow Rate:	0.6 mL/min		
Stop Time:	Twenty minutes		
Maximum Pressure:	300 bar		
Injection Amount:	8 µL		
Mobile Phases:	Mobile Phase A: 1.5 M $(NH_4)_2SO_4$ and 50 mM $K_2HPO_4{\cdot}3H_2O$ Mobile Phase B: 50 mM potassium phosphate and 25% 2-propanol		
Gradient Program:	Time (min)	A (%)	B (%)
	0	100	0
	2	100	0
	15	0	100
	16	0	100

TABLE 53
HIC-high performance liquid chromatography parameters

	17	100	0
	20	100	0

[00853] *Endotoxin determination*. The endotoxin level was determined by kinetic turbidimetric assay.

[00854] (1) *Preparation of endotoxin standards and quality control*: The CSE (10EU/vial, lyophilized) is reconstituted with 1 mL of Water for BET to yield a 10 EU/mL standard solution. Make 10-fold dilution using the Water for BET, prepare standard solutions with four concentrations (S1_10 EU/mL, S2_1 EU/mL, S3_0.1 EU/mL, S4_0.01 EU/mL). Dilute CSE with Water for BET to prepare quality control (0.05 EU/mL).

[00855] (2) *Preparation of TAL Reagents*: The TAL reagent is stable when stored at 2~8°C before expiration. Allow reagents to equilibrate to room temperature prior to use. Added 1.25 mL Water for BET to dissolve 1 bottle of TAL reagent.

[00856] (3) *Preparation of Samples*: diluted all the sample X/2 folds with Water for BET. Taken half volume of X/2 folds sample and added equal volume of Water for BET to make the finally tested sample with X folds dilution. Added equal volume of S3 in the remaining half of volume of X/2 folds sample to make its PPC sample with endotoxin concentration to quality control.

[00857] (4) Add 100 µL of the standard curve, quality control, sample, PPC and NTC into wells. Add 100 µL of TAL reagent into the 96-well plat wells(Greiner-655185). All standards control and samples need duplicate. Read microplate with a plate reader.

[00858] (5) OD 405 nm was read during incubating at 37°C for 100 min in a microplate reader.

[00859] (6) A standard curve was prepared by plotting the average 405nm measurement for each standard vs. its endotoxin level in EU/mL.

[00860] The standard curve was used to determine the endotoxin level of samples.

Conjugation methods.

[00861] *Conjugation buffer and formulation buffer*. **AGN579**, conjugation buffer 50 mM HEPES, pH7.4, and formulation buffer: phosphate-buffered saline, pH 7.4.

[00862] *Procedure of bulk conjugation*. **ABT818** in original buffer (phosphate-buffered saline, pH7.4) was buffer exchanged to 50 mM HEPES, pH7.4 by Amicon® Ultra Centrifugal Filter Unit. Concentration was tested with 12.36 mg/mL by Nanodrop.

[00863] 140 mg of antibody was transferred to 50mL tube (Greiner, GN227270) and placed in ice bath; then added with conjugation buffer (50 mM HEPES, pH7.4) and 10 mM **TBT307** solution (4.59 equivalents). The total DMSO concentration in the reaction mixture was 3.48%; the concentration of the reaction is 10 mg/mL. The reaction vessel was then incubated at 4°C for five hours followed by 20°C for thirty-nine hours.

[00864] The crude conjugation was purified with UF/DF (Pellicon 3, 30 kDa, 0.11m2) with phosphate-buffered saline, pH7.4 (Gibco, 10010031), 10% DMSO (Sigma, D4540) for 40 DV, followed by phosphate-buffered saline, pH7.4 for 20 DV.

[00865] The purified sample was filtered with 0.22 μm filter.

[00866] Perform the quality control test (including concentration, size exclusion chromatography - high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, LC-MS, free drug, endotoxin level).

[00867] *Results and discussion of bulk conjugation*. The reaction condition of bulk conjugation is summarized below. After conjugation, the reaction mixture is purified with UF/DF (Pellicon 3, 30 kDa, 0.11m2) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) contain 10% DMSO (Sigma-Aldrich) for 40 DV and followed by phosphate-buffered saline, pH7.4 for 20 DV. Then filter with 0.22 μM membrane.

TABLE 54
Reaction condition for bulk conjugation

#		Drug/mAb ratio	Conjugation Conc.(mg/mL)	Conjugation temp.& time	Conjugation pH
AGN579	**ABT818-TGT307**	3	10	4°C, five hours & 20°C, thirty-nine hours	7.4

TABLE 55
Molecular information of AGN579

#	BAR2 Molecular weight (Amino acid, Da)	Modification	Theoretical Mass (Da)	Measured Mass (Da)	Difference (Da)
AGN579	135484.02	-K*2, N-Glycan, BAR2	135492.13	135495.52	3.39

TABLE 56								
#	UV Conc. (mg/mL)	MS-BAR	BAR2% (LCMS)	SEC-Purity (%)	Residual uABT (%)	Residual Reagent (%)	Residual Payload (%)	Endo (EU/mg)
AGN579	8.44	1.88	81.02	98.88	<2.34	<1.23	<0.70	<0.095

[00868] *Results and discussion of stability tests*. Store the sample under -70°C for one hour to ensure that material is frozen, then the material is allowed to thaw by letting the vial to sit at room temperature for sufficient amount of time until completed thawed. Collect the analytical characterization under different freeze-thaw cycles (1, 2, 3).

TABLE 57 Results for freeze-thaw stability tests				
Sample	Time point (cycles)	Conc.(mg/mL)	SEC purity (%)	BAR
AGN579	1	8.63	98.01	1.89
	2	8.64	98.91	1.90
	3	8.73	98.92	1.90

[00869] *Summary*. The **AGN579** was successfully generated. All the results meet targeting quality. Sample was stable under three freeze-thaw cycles.

EXAMPLE 22

Anti-PLA2R TR-FRET assay

Materials:

[00870] *Stock Buffers*: HEPES pH 7.5 (1 M), sodium chloride (5 M), calcium chloride (1 M), Tween-20 (10%), and DTT (1 M).

[00871] *Assay components*:

[00872] Biotin-Avi-ASGPR1 (ASGPR1-10: 148-291) (3.86 mg/mL; 205.8 µM).

[00873] Rabbit anti-PLA2R Abcam AB211573 (1.22 mg/mL; 7.22 µM).

[00874] *Donor*: Streptavidin-Europium cryptate Revvity 610SAKLA (733 nM).

[00875] *Acceptor*: Anti-rabbit IgG d2 Revvity 61PARDAA (250 ug/mL; 1.66 µM).

[00876] 384 well PP 2.0 Microplate Echo qualified (Labcyte PP-0200).

[00877] Proxiplate 384 (Revvity 6007290).

Conditions:

[00878] 20 µL final.

[00879] [Biotin-Avi-ASGPR1] : 25 nM.

[00880] [Rabbit anti-PLA2RR] : 3.1 nM.

[00881] [Europium Donor] : 3 nM.

[00882] [IgG d2 Acceptor] : 10 M.

[00883] *Assay buffer*: 25 mM HEPES pH 7.5, 150 mM sodium chloride, 5 mM calcium chloride, 0.01% Tween-20, 1 mM DTT.

[00884] *Streptavidin Europium cryptate donor Ex/Em*: 320/620 nm.

[00885] *Anti-rabbit IgG d2 acceptor Ex/Em*: 615/665 nm.

Protocol outline

[00886] Make fresh assay buffer.

[00887] Prepare compound dilutions and use Echo to dispense 180 nL fixed positive control, DMSO, and titrated compounds to 384-well ProxiPlate.

[00888] Seal plate.

[00889] Prepare 2x mix of anti-PLA2R and ASGPR1 and dispense 10 µl in respective wells in plate with compounds. Seal plate, spin, and incubate for one hour at room temperature.

[00890] Prepare 2x TR-FRET donor/acceptor pair and dispense 9 µl in all wells of plate. Seal plate, spin, and incubate for one hour at room temperature.

[00891] Read plate.

TABLE 58

Degraders	TR-FRET (ASGPR) IC_{50} (µM)	Notes
AGN306	19.4	
AGN308	0.12	
AGN307	5.8	
AGN309	5.8	
AGN310	60.7	
AGN364	3.4	
AGN364	3.4	
AGN365	0.16	
AGN365	0.12	
AGN579	*1.8*	*Poor fit*
AGN579	>10	Not concentrated enough

EXAMPLE 23

In vivo subcutaneous/intravenous bioavailability study

[00892] *Study objectives*: Subcutaneous bioavailability is highly desired.

[00893] *Three degrader assays*: **AGN364**, **AGN365**, and **AGN579**.

[00894] *Two routes of administration*: intravenous and subcutaneous.

[00895] *Two doses*: 2 mg/kg and 20 mg/kg.

[00896] *Twelve study groups*. N = three per time point.

[00897] *Time points*: one-eighth, one-quarter, one-half, one, two, four, eight, and twenty-four hours.

[00898] Mouse assays: Four bleeds per mouse (using retro-orbital bleeding technique) = six mice per group to achieve n = 3. Seventy-two mice for all twelve groups. 288 samples total.

[00899] *Establishing PLA2R pharmacokinetics assay*: Degrader calibrators and setup optimization.

[00900] Detection of pharmacokinetics assay degrader using soybean agglutinin (SBA) lectin on Strep Avidin Meso Scale Discovery (MSD) plate.

[00901] The assay used soybean agglutinin (SBA) lectin bound to a streptavidin-coated Meso Scale Discovery (MSD) plate to capture the PLA2R degrader. Detection was achieved using an anti-PLA2R antibody, followed by a sulfo-tagged anti-rabbit secondary antibody. The inventors used these results to prepare PLA2R degrader standard curves.

[00902] *Pharmacokinetic Meso Scale Discovery (MSD) assay results*: subcutaneous compared with intravenous.

[00903] *Pharmacokinetic Meso Scale Discovery (MSD) assay results*: 20 mg/kg intravenous

[00904] *Summary*. Proceeding with pharmacodynamics assays: **AGN365** at 20 mg/kg intravenous. **AGN364** 20 mg/kg intravenous.

EXAMPLE 24

Internalization trend of monoclonal antibodies mirrors Meso Scale Discovery (MSD) binding results

[00905] *Assay conditions*: ASGPR-HEK293, 12.5 nM anti-PLA2R, twenty-four hours. ASGPR-HEK293, 25 nM anti-PLA2R, for forty-eight hours.

[00906] All degraders containing CysR were internalized, but not that with the 31-mer or the baits. The degraders containing CysR-CTLD1 were internalized, but not those with CysR alone or the 31-mer or the baits.

[00907] **AGN579** (CysR-CTLD1 Fc fusion) is the most potent degrader.

[00908] Rank order: **AGN579**>>> **AGN364**>> **AGN167** > **AGN365**

EXAMPLE 25

Internalization of anti-PLA2R with next degraders

[00909] Internalization trend of monoclonals mirrors Meso Scale Discovery (MSD) binding results. Internalized by all degraders containing CysR, but not that with the 31-mer or the baits. Only internalized by degraders containing CysR-CTLD1, but not those with CysR alone or the 31-mer or the baits.

[00910] **AGN579** (CysR-CTLD1 Fc fusion) is the most potent degrader.

[00911] *Internalization assay*: Repeat with technical replicates to obtain EC_{50}.

[00912] Compare epitope specificity with patient autoantibodies.

[00913] Idiopathic membranous nephropathy patient antibodies

[00914] Confirm binding to **AGN167** (CysR-CTLD1 maleimide degrader)

[00915] Isolate from serum for possible use in in vitro and in vivo studies.

EXAMPLE 26

Analysis of rabbit Anti-PLA2R monoclonal antibodies

[00916] The inventors evaluated binding of CysR or CTLD1-specific clones against degrader to identify antibodies to be used in mouse pharmacodynamics studies and as a detection reagent for pharmacokinetics analysis.

[00917] Screening mAbs for pharmacokinetics assay with PLA2R degraders on biotinylated soybean agglutinin (SBA)-coated streptavidin Meso Scale Discovery (MSD) plates.

[00918] Following an initial monoclonal antibody screening, a comparative evaluation of five CysR-specific monoclonal antibodies (mAbs) and a polyclonal antibody against PLA2R degraders identified a lead Monoclonal antibody for pharmacokinetics/pharmacodynamics assays. Selection focused on achieving equal or superior binding affinities to the CysR domain and consistent binding across all degraders than the polyclonal antibodies.

[00919] Screening monoclonal antibodies for pharmacokinetics assay with PLA2R degraders on biotinylated soybean agglutinin (SBA)-coated streptavidin Meso Scale Discovery (MSD) plates.

EXAMPLE 27

[00920] *Purpose of assay*: Ternary complexes were assayed using both CysR and CysR-CTLD1 degraders. Some signal for CysR bait protein was assayed for **AGN364**, **AGN365**, **ABT817** (CysR), and **ABT819** (CysR-CTLD1). The inventors compared the three antibodies using **AGN364** or **AGN365** as a

control. The dose-dependent signal was more comparable for both degraders than for the other antibodies.

[00921] *Takeaways*: CysR-CTLD1 degrader forms ternary complex with polyclonal antibodies.

EXAMPLE 28

PLA2R degrader peptide mapping of **AGN364**, **AGN365**, and **AGN579**

[00922] **AGN364** was digested by trypsin and the peptide mapping coverage is 59.6%. The unidentified peptides were either digested into very small sizes (<= 4AA) or very big sizes (64~94). The C-term peptide was not observed possibly due to it low ionization efficiency. The N-glycosylation site possibly at N73, located at a big tryptic peptide, was not identified by mass spectrometry.

TABLE 59

AGN364 PTM sites	Modifications	Peptides
T103, S109, T117, S121	GalNac, GalNac, GalNac, GalNac-6GGn-3G	MITGPLQYSVQVAHDNTVVASR

[00923] **AGN365** was digested by trypsin and the peptide mapping coverage is 60.4% The unidentified peptides were either digested into very small sizes (<= 4AA) or very big sizes (64~94, 216~260, 267~322). Conjugation site was located at C terminal Gly361. The N-glycosylation site possibly at N73, located at a big tryptic peptide, was not identified by mass spectrometry.

TABLE 60

AGN365 PTM sites	Modifications	Peptides
S109, T117, S121	GalNac	MITGPLQYSVQVAHDNTVVASR
T203	GalNac-6S-3SG	WGFCPDPTSAEVGCDTIWEK
G361	triGalNac	YLNHIDHEIVEGGGGSLPETGGGGG

[00924] **AGN579** was digested by trypsin and the peptide mapping coverage is 67.3%. The unidentified peptides are either digested into very small sizes (<= 4AA) or very big sizes (64~94, 216~260, 267~322). The conjugation site is K385. The Fc N-glycosylation site is N434.

TABLE 61

AGN579 PTM sites	Modifications	Peptides
K385	triGalNac	THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR
N434	N-glycoforms	EEQYNSTYR

EXAMPLE 29

TABLE 62
Monoclonal antibody screening (SPR)

Tool mAb	Binding entity	Immobilization level	Rmax (RU)	K_D (nM)	Notes
6A5-8	**ABT817** (CysR-sortase-his)	464.2	12.7	32.7	Low signal and active surface seen for analyte
6A5-8	**ABT976** (CysR-CTLD1 no tag)	464.2	11.4	>1000	Low signal and active surface seen for analyte
6A5-8	**ABT427** (FnII-CTLD1-his)	464.2	12.8	104	Low signal and active surface seen for analyte
6B3-1	**ABT817** (CysR-sortase-his)	455.2	181.6	<0.97	Binds to analyte (preference for CysR)
6B3-1	**ABT976** (CysR-CTLD1 no tag)	455.2	143.6	6.18	Binds to analyte (preference for CysR)
6B3-1	**ABT427** (FnII-CTLD1-his)	455.2	81.0	13.8	Binds to analyte (preference for CysR)
9A12-1	**ABT817** (CysR-sortase-his)	369.7	0	NA	No binding
9A12-1	**ABT976** (CysR-CTLD1 no tag)	369.7	14.0	131	Low active surface
9A12-1	**ABT427** (FnII-CTLD1-his)	369.7	5.7	205	Low active surface (preference for FnII/CTLD1)
9D10-1	**ABT817** (CysR-sortase-his)	438.5	93.8	4.69	Binds to analyte (slight preference for CysR)
9D10-1	**ABT976** (CysR-CTLD1 no tag)	438.5	158.9	12	Binds to analyte
9D10-1	**ABT427** (FnII-CTLD1-his)	438.5	76.4	45.5	Binds to analyte
12A2-1	**ABT817** (CysR-sortase-his)	465.3	0	NA	No binding
12A2-1	**ABT976** (CysR-CTLD1 no tag)	465.3	69.2	278	
12A2-1	**ABT427** (FnII-CTLD1-his)	465.3	22.5	225	Binding preference for FnII/CTLD1

TABLE 62
Monoclonal antibody screening (SPR)

Tool mAb	Binding entity	Immobilization level	Rmax (RU)	K_D (nM)	Notes
20D11-1	**ABT817** (CysR-sortase-his)	476.3	61.1	22.6	Binds to analyte (slight preference for CysR)
20D11-1	**ABT976** (CysR-CTLD1 no tag)	476.3	97.1	72.4	Binds to analyte
20D11-1	**ABT427** (FnII-CTLD1-his)	476.3	53.0	119	Binds to analyte
47E6-1	**ABT817** (CysR-sortase-his)	445.8	100.7	3.81	Binds to analyte (slight preference for CysR)
47E6-1	**ABT976** (CysR-CTLD1 no tag)	445.8	166.7	9.66	Binds to analyte
47E6-1	**ABT427** (FnII-CTLD1-his)	445.8	79.1	38.2	Binds to analyte
47E7-1	**ABT817** (CysR-sortase-his)	449.2	0	NA	No binding
47E7-1	**ABT976** (CysR-CTLD1 no tag)	449.2	144.3	7.19	
47E7-1	**ABT427** (FnII-CTLD1-his)	449.2	78.6	15.5	Binding preference for FnII/CTLD1
2C6-1	**ABT817** (CysR-sortase-his)	467.8	0	NA	No binding
2C6-1	**ABT976** (CysR-CTLD1 no tag)	467.8	1.1	54.3	Low signal and active surface seen
2C6-1	**ABT427** (FnII-CTLD1-his)	467.8	0	NA	No binding
4F6-1	**ABT817** (CysR-sortase-his)	550.1	99.9	31.4	Binds to analyte (preference for CysR).
4F6-1	**ABT976** (CysR-CTLD1 no tag)	550.1	146.7	323	Binds to analyte
4F6-1	**ABT427** (FnII-CTLD1-his)	550.1	86.5	260	Binds to analyte

Approx. Rmax for **ABT817** (CysR) (based on 450 RU immobilized): 126.

Approx. Rmax for **ABT976**: 252

Approx. Rmax for **ABT427**: 144

EXAMPLE 30

Conjugation summaries

Conjugation process protocol for **AGN364 (ABT817-TBT544)** and **AGN365 (ABT819-TBT544)**.

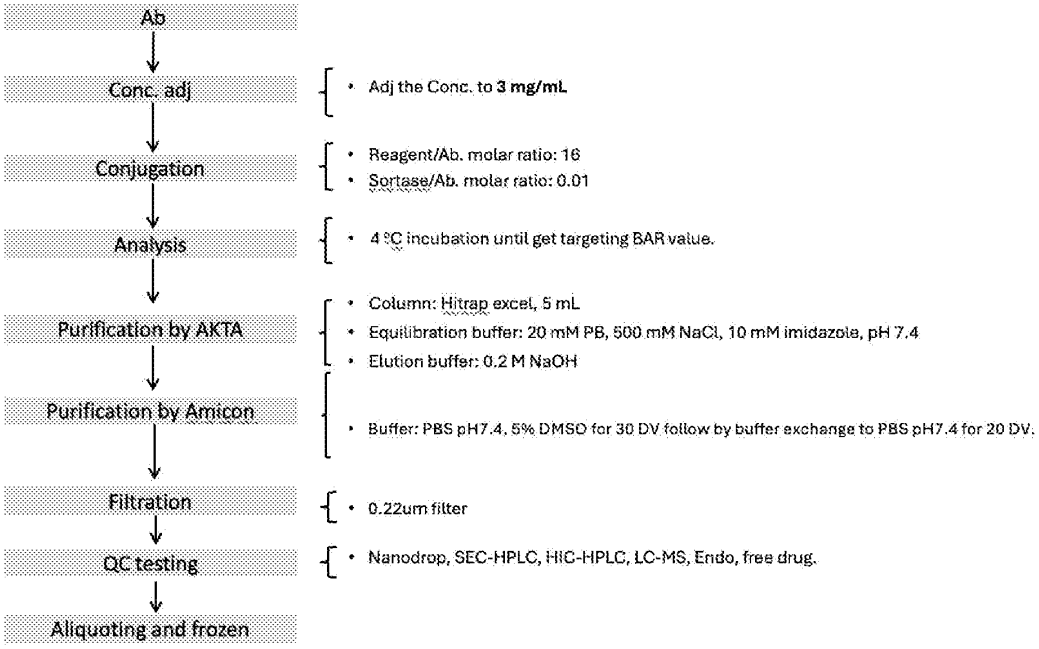


TABLE 63
Conjugation conditions

Antibody	**ABT817** (CysR)	**ABT817** (CysR)	**ABT819** (CysR-CTLD1)	**ABT819** (CysR-CTLD1)
Drug	**TBT544**	**TBT544**	**TBT544**	**TBT544**
Sortase	PT-0008	PT-0008	PT-0008	PT-0008
Scale (mg)	2	2	2	2
Drug eq./Ab	8	16	8	16
Sortase eq./Ab	0.01	0.01	0.01	0.01
Ab Conc. (mg/mL)	7.87	7.87	7.38	7.38
Conj. Conc. (mg/mL)	3	3	3	3
Organic solvents %	3.21% DMSO	6.43% DMSO	1.45% DMSO	2.89% DMSO
Conjugation Buffer	50 mM HEPES, 150 mM sodium chloride,10% glycerol, pH 7.5	50 mM HEPES, 150 mM sodium chloride,10% glycerol, pH 7.5	50 mM HEPES, 150 mM sodium chloride,10% glycerol, pH 7.5	50 mM HEPES, 150 mM sodium chloride,10% glycerol, pH 7.5

TABLE 63 Conjugation conditions				
Temperature and time	4°C, 42 hours	4°C, overnight	4°C, 42 hours	4°C, 42 hours

TABLE 64 Conjugation conditions - Results							
Agent	Conjugate	Sortase	4°C, overnight	4°C, overnight-zeba	4°C, twenty-four hours	4°C, 42 hours	4°C, 42 hours-zeba
AGN	**ABT817-TBT544**	PT-0008	0.92	-	0.94	0.93	0.94
AGN	**ABT817-TBT544**	PT-0008	0.99	0.98	-	-	-
AGN	**ABT819-TBT544**	PT-0008	0.77	-	0.79	0.79	0.79
AGN	**ABT819-TBT544**	PT-0008	0.87	-	0.88	0.87	0.87

[00925] C-terminal ligation was performed for **ABT817-TBT544** and **ABT819-TBT544** with provided conditions. Increasing drug ratio from eight to sixteen increased the final BAR value for two conjugates. **ABT817-TBT544** reached BAR 0.99 after 4°C overnight and didn't change with prolonged time. **ABT819-TBT544** reached slightly lower BAR value 0.87 after 4°C overnight and didn't change with prolonged time. Zeba desalting to phosphate-buffered saline buffer didn't influence BAR value.

[00926] *Size exclusion chromatography results*: **ABT817-TBT544** and **ABT819-TBT544**. Zeba desalting to phosphate-buffered saline buffer didn't influence size exclusion chromatography purity. Both conjugates achieved size exclusion chromatography purity over 95%.

TABLE 65 LC-MS results **ABT817-TBT544**				
Sample ID	Sortase	Condition	BAR value (001)	BAR value (002)
ABT817-TBT544	PT-0008	4°C, overnight	0.92	0.99
ABT817-TBT544	PT-0008	4°C, twenty-four hours	0.94	
ABT817-TBT544	PT-0008	4°C, 42 hours	0.93	
ABT817-TBT544	PT-0008	4°C, 42 hours-zeba	0.94	0.98

TABLE 66
LC-MS results **ABT819-TBT544**

Sample ID	Sortase	Condition	BAR value (001)	BAR value (002)
ABT819-TBT544	PT-0008	4°C, overnight	0.77	0.87
ABT819-TBT544	PT-0008	4°C, twenty-four hours	0.79	0.88
ABT819-TBT544	PT-0008	4°C, 42 hours	0.79	0.87
ABT819-TBT544	PT-0008	4°C, 42 hours-zeba	0.79	0.87

[00927] C-terminal ligation was performed for **ABT817-TBT544** and **ABT819-TBT544** with provided conditions. Increasing drug ratio from eight to sixteen could increase final BAR value for two conjugates. **ABT817-TBT544** reached BAR 0.99 after 4°C overnight and didn't change with prolonged time. **ABT819-TBT544** reached slightly lower BAR value 0.87 after 4°C overnight and did not change with prolonged time.

[00928] Both conjugates achieved size exclusion chromatography purity over 95%. Neither BAR value nor size exclusion chromatography purity were influenced by Zeba desalting to phosphate-buffered saline buffer after conjugation.

TABLE 67
Sortase A project

Agent	Antibody	Mate Reagent	Target
AGN364	**ABT817** (CysR)	**TBT544**	100mg scale, BAR1
AGN365	**ABT819** (CysR-CTLD1)	**TBT544**	100mg scale, BAR1

TABLE 68
Reaction conditions

Antibody	**ABT817** (CysR)	**ABT819** (CysR-CTLD1)
Drug	**TBT544**	**TBT544**
Sortase	PT-0008	PT-0008
Scale (mg)	100	100
Drug eq./Ab	16	16

TABLE 68 Reaction conditions		
Sortase eq./Ab	0.01	0.01
Ab Conc. (mg/mL)	3	3
Solvent	4% DMSO	4% DMSO
Conjugation Buffer	50 mM HEPES, 150 mM sodium chloride, 10% glycerol (V/V), pH 7.5	50 mM HEPES, 150 mM sodium chloride, 10% glycerol (V/V), pH 7.5
Temp. & Time	4°C, overnight	4°C, overnight

TABLE 69
Quality control results

#	Ab Scale (mg)	Conc. (mg/mL)	Amount (mg)	Yield (%)	MS-BAR	SEC Purity(%)
AGN364	100	10.86	69.40	69.40	1.00	99.05
AGN364	100	10.41	69.12	69.12	0.99	98.79

Formulation buffer: phosphate-buffered saline, 2 mM EDTA, pH7.4

[00929] Competitive binding of PLA2R constructs (50 nM) and PLA2R autoantibodies in patient serum against full-length PLA2R.

TABLE 70
Meso Scale Discovery (MSD) analysis: Detection of PLA2R Ab binding against PLA2R constructs

Anti-PLA2R Ab	**ABT817** (CysR)		**ABT819** (CysR-CTDL1)		**ABT305** (31-mer)	
#	Goat Anti-Human	Goat Anti-Rabbit	Goat Anti-Human	Goat Anti-Rabbit	Goat Anti-Human	Goat Anti-Rabbit
ABT985 Rabbit F_{ab}, Human $IgG4_{FC}$	~0.007	~0.009	~0.0150	~0.0140	~0.0045	~0.0065

TABLE 71
Meso Scale Discovery (MSD) analysis: Detection of PLA2R Ab binding against PLA2R Constructs

Anti-PLA2R Ab, EC_{50} (µg/mL)	**ABT817** (CysR)	**ABT819** (CysR-CTDL1)	**ABT305** (31-mer)
E21-Q66 Rabbit Polyclonal Ab	0.0018	~0.0015	~0.0019
EPR20483 Rabbit Monoclonal Ab	~0.0021	~0.0021	~0.002
ABT985 Rabbit Fab, Human IgG4FC	~0.013	~0.015	~0.019
E21-Q66 Rabbit Polyclonal Ab	~0.008	~0.006	~0.015
ABT983 Rabbit Fab, Mouse IgG1FC	~0.020	~0.014	~0.012

EXAMPLE 31

TABLE 72

#	Type	Construct	Amino acid range
ABT606	Antibody	Abcam anti-rabbit PLA2R mAb	
ABT816	Fc-fusion Protein	CysR hIgG1 Fc-fusion	21-172, human IgG1 Fc
ABT817	Protein	CysR Sortase	21-172
ABT818	Fc-fusion Protein	CysR-CTLD1 hIgG1 Fc-fusion	21-367, human IgG1 Fc
ABT819	Protein	CysR-CTLD1 sortase	21-367
ABT976	Protein	CysR-CTLD1 + Thrombin	21-367
ABT6977	Protein	CTLD7	1108-1234
ABT6978	Protein	Full ECD minus CysR	171-1397. The inventors did not choose for further development.
ABT6979	Protein	Full ECD + Thrombin to cleave CysR (166-171) *full length protein*	21-165/thrombin/172-1397
ABT6980	Protein	Full ECD minus CysR-CTLD1	369-1397
ABT6981	Protein	Full ECD + Thrombin to cleave CysR-CTLD1	21-364/thrombin/371-1397
ABT6983	Antibody	Anti-PLA2R Biotinylated Mouse Chimera	Rabbit Fab, Murine IgG1 Fc
ABT6985	Antibody	Anti-PLA2R Human Chimera	Rabbit Fab, Human IgG4 Fc
ABT7300	Protein	Full ECD + Thrombin to cleave CysR (161-166)	21-1397. The inventors did not choose for further development.

TABLE 72

#	Type	Construct	Amino acid range
ABT7301	Protein	Full ECD + Thrombin to cleave CysR (183-188)	21-1397. The inventors did not choose for further development.
AGN364	Degrader	CysR BGN3	21-172
AGN365	Degrader	CysR-CTLD1 BGN3	21-367
ABT427	Protein	CysR-CTLD1 + thrombin to cleave CysR (166-171)	21-165/thrombin/172-367
ABT7428	Protein	CysR-CTLD1 +Thrombin to cleave CysR+FnII (231-236)	21-230/thrombin/237-367 The inventors did not choose for further development.
ABT740	Protein	CysR-CTLD1, GGGGS-His-GGGGS-Cys	21-367
ABT7408	Protein	CysR-CTLD1, GGGGS-His-Cys	21-367
ABT7409	Protein	CysR-CTLD1-GGGGS-His-GGGE-Cys-S	21-367
AGN579	Degrader	CysR-CTLD1-Fc BGN3	21-367
ABT7607	Protein	CysR-CTLD3 Sortase	21-656
ABT7608	Protein	CysR-CTLD3 Fc-fusion	21-656
ABT7609	Protein	CysR-CTLD5 Sortase	21-946
ABT7610	Protein	CysR-CTLD5 Fc-fusion	21-946
ABT7611	Protein	CysR-CTLD7 Sortase	21-1237
ABT7612	Protein	CysR-CTLD7 Fc-fusion	21-1237
ABT7613	Protein	CysR-CTLD8 Sortase	21-1397
ABT7614	Protein	CysR-CTLD8 Fc-fusion	21-1397
ABT7617	Protein	CysR Fc-fusion (K123R, K127R, K144R)	21-172
ABT7688	Protein	CysR, GGGGS-His-GGGGS-Cys	21-172
ABT7689	Protein	CysR, GGGGS-His-Cys	21-172
ABT7690	Protein	CysR, GGGGS-His-GGGE-Cys-S	21-172

TABLE 73

#	Tag	Mutant	Expression system	Notes
ABT606			CHO	
ABT816	N/A	LALA/PA	CHO K1	For conjugation
ABT817	C-term Sortase-His6	wild-type	CHO K1	For conjugation
ABT818	N/A	LALA/PA	CHO K1	For conjugation
ABT819	C-term sortase-His6	wild-type	CHO K1	For conjugation
ABT976	C-term thrombin site-His6	wild-type	HEK293	For immunization (cleave his tag)
ABT6977	C-term Avi-His10	wild-type	HEK293	
ABT6978	C-term Avi-His10	wild-type	HEK293	The inventors did not choose for further development. Did not express well.
ABT6979	C-term Avi-His10	wild-type with engineered thrombin site from 166-171	HEK293	Did not cleave-use as full-length protein
ABT6980	C-term Avi-His10	wild-type	HEK293	
ABT6981	C-term Avi-His10	wild-type	HEK293	
ABT6983	HC Avi		CHO K1	Biotinylated antibody for mouse studies
ABT6985	N/A	S to P mutation in hinge (Fab arm exchange)	CHO K1	
ABT7300	C-term Avi-His10	wild-type with engineered thrombin site from 161-166	HEK293	The inventors did not choose for further development. Did not express well.
ABT7301	C-term Avi-His10	wild-type with engineered thrombin site from 183-188	HEK293	The inventors did not choose for further development. Did not express well.

TABLE 73

#	Tag	Mutant	Expression system	Notes
AGN364	BGN3		CHO K1	Sortase conjugation
AGN365	BGN3		CHO K1	Sortase conjugation
ABT427	C-term His6	wild-type with engineered thrombin site from 166-171	HEK293	
ABT7428	C-term His6	wild-type with engineered thrombin site from 231-236	HEK293	The inventors did not choose for further development. Did not express well.
ABT740	C-term His6 and Cys	wild-type	HEK293	
ABT7408	C-term His6 and Cys	wild-type	HEK293	
ABT7409	C-term His6 and Cys	wild-type	HEK293	
AGN579	N/A			
ABT7607	C-term Sortase site-His6	wild-type	CHO K1	
ABT7608	N/A	wild-type	CHO K1	
ABT7609	C-term Sortase site-His6	wild-type	CHO K1	
ABT7610	N/A	wild-type	CHO K1	
ABT7611	C-term Sortase site-His6	wild-type	CHO K1	
ABT7612	N/A	wild-type	CHO K1	
ABT7613	C-term Sortase site-His6	wild-type	CHO K1	
ABT7614	N/A	wild-type	CHO K1	
ABT7617	N/A	K123R, K127R, K144R	CHO K1	
ABT7688	C-term His6 and Cys	wild-type	CHO K1	

TABLE 73

#	Tag	Mutant	Expression system	Notes
ABT7689	C-term His6 and Cys	wild-type	CHO K1	
ABT7690	C-term His6 and Cys	wild-type	CHO K1	

EQUIVALENTS

[00930] persons having ordinary skill in the biomedical art will recognize or be able to determine using no more than routine experimentation many equivalents to the specific procedures described in this specification. Such equivalents are within the scope of this invention and are covered by these claims. For example, pharmaceutically acceptable salts other than those specifically revealed in the description and Examples in this specification can be employed. Furthermore, it is intended that specific items within lists of items, or subset groups of items within larger groups of items, can be combined with other specific items, subset groups of items or larger groups of items whether or not there is a specific disclosure in this specification identifying this combination.

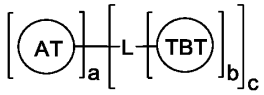
[00931] Some embodiments of the invention can be practiced according to the following numbered paragraphs:

[00932] 1. A composition of matter comprising: an anti-PLA2R antibody-binding moiety, a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on surface degrading cells, and a linker moiety (optionally a single peptide linkage) connecting the anti-PLA2R antibody-binding moiety and the cellular receptor-binding moiety.

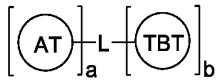
[00933] 2. The composition of matter of embodiment 1, wherein the anti-PLA2R antibody-binding moiety is a published anti-PLA2R antibody ligand variant antibody, an anti-PLA2R antibody ligand variant, or an antigen-binding fragment thereof.

[00934] 3.The composition of matter of embodiment 1, having a structure of:

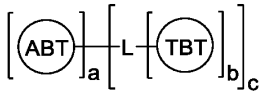
R^{CN}–(Xaa)y–R^{CC},



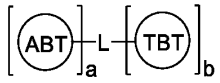
[AGN101],



[AGN102],

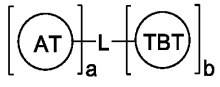


[AGN103],



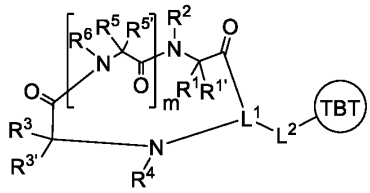
[AGN104] or a salt thereof.

[00935] 4.The composition of matter of embodiment 1, wherein the agent has the structure of formula [**AGN102**]:



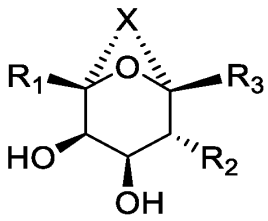
[**AGN102**] or a pharmaceutically acceptable salt thereof, wherein: each of a and b is independently an integer of 1 or greater; each AT is an anti-PLA2R antibody-binding moiety or a fragment thereof; L is a linker moiety; and each TBT is independently a cellular receptor-binding moiety which binds to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, wherein the anti-PLA2R antibody-binding moiety is a ligand variant or an antigen-binding fragment thereof.

[00936] 5. The composition of matter of embodiment 1, wherein the agent has the structure of formula **AGN105**:

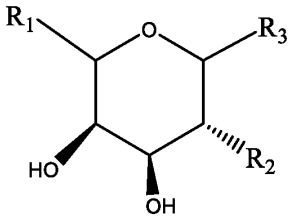


[AGN106] or a salt thereof, wherein the composition of matter has more elements described in this specification.

[00937] 6. The composition of matter of embodiment 1, wherein the cellular receptor-binding moiety comprises an ASGPR-binding group according to the chemical structure:

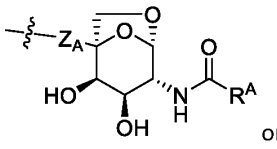


[TBT101], or

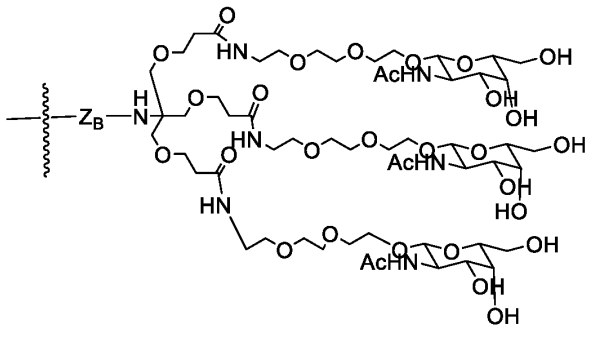


[TBT102], where R_1, R_2 and R_3 are the same as in embodiment 9, or a pharmaceutically acceptable salt, stereoisomer, solvate or polymorph thereof.

[00938] 7. The composition of matter of embodiment 1, wherein the cellular receptor-binding moiety has the following structure:



or



;

where R^A is a C_1-C_3 alkyl group optionally substituted with 1-5 halo (preferably fluoro) groups (preferably R^A is a methyl or ethyl group optionally substituted with from 1-3 fluoro groups); Z_A is -$(CH_2)_{IM}$, -O-$(CH_2)_{IM}$, S-$(CH_2)_{IM}$, NR_M-$(CH_2)_{IM}$, C(O)-$(CH_2)_{IM}$-, a PEG group containing from 1 to 8 preferably 1-4 ethylene glycol residues or a -C(O)$(CH_2)_{IM}NR_M$ group (preferably a PEG containing group comprising from 1 to 8 ethylene glycol, preferably 2-4 ethylene glycol residues); and Z_B is absent, $(CH_2)_{IM}$, C(O)-$(CH_2)_{IM}$- or C(O)-$(CH_2)_{IM}$-NR_M.

[00939] 8. The composition of matter of embodiment 1, wherein the cellular receptor-binding moiety is a low-density lipoprotein receptor-related protein 1 (LRP1), a low-density lipoprotein receptor (LDLR), a FcγRI-binding group, a FcRN-binding group, a transferrin receptor-binding group, or a macrophage scavenger receptor-binding group.

[00940] 9. A pharmaceutical composition comprising a composition of matter of embodiments 1-8 and a pharmaceutically acceptable excipient.

[00941] 10. A method of removing anti-PLA2R antibody in a subject or patient comprising administering to the subject or patient the agent of any of embodiments 1-8.

[00942] 11. A method of treating a disease state or condition associated with the upregulation of anti-PLA2R antibody in a subject or patient administering to the subject or patient an effective amount of the agent of any of embodiments 1-8.

[00943] 12. A method of treating idiopathic membranous nephropathy in a patient comprising administering to the patient an effective amount of the agent of any of embodiments 1-8.

[00944] 13. A composition comprising: a first composition of matter comprising: an antibody moiety, a cellular receptor-binding moiety which binds to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, and a linker moiety linking the antibody moiety and the cellular receptor-binding moiety, and at least one additional composition of matter comprising a moiety capable of binding to the antibody that forms the antibody moiety of the first composition of matter.

REFERENCES

[00945] Persons having ordinary skill in the biomedical art can use these patents, patent applications, and scientific references as guidance to predictable results when making and using the invention.

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[001017] Throughout this application, several publications are referenced by author name and date, or by patent number or patent publication number. The disclosures of these publications are incorporated in their entireties by reference into this application to describe the state of the art more fully as known to persons having ordinary skill in the biomedical art as of the invention described and claimed in this specification. However, the citation of a reference in this specification should not be construed as an acknowledgment that this reference is prior art to the present invention.

[001018] All patents and publications cited throughout this specification are incorporated by reference to reveal and describe the materials and methods that might be used with the technologies described in this specification. The publications discussed are provided only for their disclosure before the filing date. They should not be construed as an admission that the inventors may not antedate this disclosure under past invention or for any other reason. If there is an apparent discrepancy between a past patent or publication and the description in this specification, the specification (including any definitions) and claims shall control. All statements about the date or contents of these documents are based on the information available to the applicants. These statements are no admission to the correctness of the dates or contents of these documents. The publication dates in this specification may differ from the actual publication dates. If there is an apparent discrepancy between a publication date in this specification and the actual publication date supplied by the publisher, the actual publication date shall control.

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LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSXD KTHTCPPCPA PEAAGGPSVF 240
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> note, LALA sequence
- SITE, 271
> note, PA sequence
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- REGION, 360..368
> note, THTCPPCPA
- SITE, 371..372
> note, LALA sequence
- SITE, 466
> note, PA sequence
- REGION, 464..471
> note, ALAAPIEK
- MOD_RES, 357..393
> note, SADKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR (K30+GalNacX1)(5+)
- MOD_RES, 431..439
> note, EEQYNSTYR (N5+A1G0F)(2+)
Residues:

```
EGVAAALTPE  RLLEWQDKGI  FVIQSESLKK  CIQAGKSVLT  LENCKQANKH  MLWKWVSNHG  60
LFNIGGSGCL  GLNFSAPEQP  LSLYECDSTL  VSLRWRCNRK  MITGPLQYSV  QVAHDNTVVA  120
SRKYIHKWIS  YGSGGGDICE  YLHKDLHTIK  GNTHGMPCMF  PFQYNHQWHH  ECTREGREDD  180
LLWCATTSRY  ERDEKWGFCP  DPTSAEVGCD  TIWEKDLNSH  ICYQFNLLSS  LSWSEAHSSC  240
QMQGGTLLSI  TDETEENFIR  EHMSSKTVEV  WMGLNQLDEH  AGWQWSDGTP  LNYLNWSPEV  300
NFEPFVEDHC  GTFSSFMPSA  WRSRDCESTL  PYICKKYLNH  IDHEIVEGGG  GSEPKSADKT  360
HTCPPCPAPE  AAGGPSVFLF  PPKPKDTLMI  SRTPEVTCVV  VDVSHEDPEV  KFNWYVDGVE  420
VHNAKTKPRE  EQYNSTYRVV  SVLTVLHQDW  LNGKEYKCKV  SNKALAAPIE  KTISKAKGQP  480
REPQVYTLPP  SRDELTKNQV  SLTCLVKGFY  PSDIAVEWES  NGQPENNYKT  TPPVLDSDGS  540
FFLYSKLTVD  KSRWQQGNVF  SCSVMHEALH  NHYTQKSLSL  SPGK                    584
```

Sequence Number (ID): 18
Length: 364
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..364
> mol_type, protein
> organism, synthetic construct
- REGION, 359..364

> note, His6 tag
- REGION, 353..358
> note, Sortase site
- REGION, 348..352
> note, GGGGS linker
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEGGG GSLPETGGHH 360
HHHH 364

Sequence Number (ID): 19
Length: 216
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..216
> mol_type, protein
> organism, synthetic construct
Residues:
QVLTQTASPV SAAVGGTVTI NCQASQSLYN NKNLAWYQQK PGQPPKLLIY YTATLASGVS 60
SRFEGSGSGT HFTLTISGVQ CDDAATYYCQ GEFTCESADC YTFGGGTEVL VKGDPVAPTV 120
LIFPPAADQV ATGTVTIVCV ANKYFPDVTV TWEVDGTTQT TGIENSKTPQ NSADCTYNLS 180
STLTLTSTQY NSHKEYTCKV TQGTTSVVQS FNRGDC 216

Sequence Number (ID): 20
Length: 455
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..455
> mol_type, protein
> organism, synthetic construct
- REGION, 441..455
> note, Avi-tag
- REGION, 436..440
> note, GGGGS linker

- REGION, 209..221
> note, VPRDAGCKPCICT
Residues:
QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YAMIWVRQAP GKGLEWIGII YAIGIKYYAN 60
WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGDLWGP GTLVTVSSGQ PKAPSVFPLA 120
PCCGDTPSST VTLGCLVKGY LPEPVTVTWN SGTLTNGVRT FPSVRQSSGL YSLSSVVSVT 180
SSSQPVTCNV AHPATNTKVD KTVAPSTCVP RDAGCKPCIC TVPEVSSVFI FPPKPKDVLT 240
ITLTPKVTCV VVDISKDDPE VQFSWFVDDV EVHTAQTQPR EEQFNSTFRS VSELPIMHQD 300
WLNGKEFKCR VNSAAFPAPI EKTISKTKGR PKAPQVYTIP PPKEQMAKDK VSLTCMITDF 360
FPEDITVEWQ WNGQPAENYK NTQPIMNTNG SYFVYSKLNV QKSNWEAGNT FTCSVLHEGL 420
HNHHTEKSLS HSPGKGGGGS GLNDIFEAQK IEWHE 455

Sequence Number (ID): 21
Length: 437
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..437
> mol_type, protein
> organism, synthetic construct
- REGION, 209..220
> note, ESKYGPPCPPCP
Residues:
QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YAMIWVRQAP GKGLEWIGII YAIGIKYYAN 60
WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGDLWGP GTLVTVSSGQ PKAPSVFPLA 120
PCCGDTPSST VTLGCLVKGY LPEPVTVTWN SGTLTNGVRT FPSVRQSSGL YSLSSVVSVT 180
SSSQPVTCNV AHPATNTKVD KTVAPSTCES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT 240
LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH 300
QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK 360
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE 420
ALHNHYTQKS LSLSLGK 437

Sequence Number (ID): 22
Length: 474
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..474
> mol_type, protein
> organism, synthetic construct

- SIGNAL, 1..19
> note, Signal peptide
- PEPTIDE, 49..53
> note, HCDR1 SYAMI
- PEPTIDE, 68..83
> note, HCDR2 IIYAIGIKYYANWAKG
- PEPTIDE, 460..474
> note, Terminal Avi tag GLNDIFEAQKIEWHE
- PEPTIDE, 114..116
> note, HCDR3 GDL
- SITE, 52
> note, M is an oxidation site
- SITE, 80
> note, W is an oxidation site
Residues:

MGWSCIILFL	VATATGVHSQ	SVEESGGRLV	TPGTPLTLTC	TVSGFSLSSY	AMIWVRQAPG	60
KGLEWIGIIY	AIGIKYYANW	AKGRFTISKT	STTVDLKITS	PTTEDTATYF	CARGDLWGPG	120
TLVTVSSGQP	KAPSVFPLAP	CCGDTPSSTV	TLGCLVKGYL	PEPVTVTWNS	GTLTNGVRTF	180
PSVRQSSGLY	SLSSVVSVTS	SSQPVTCNVA	HPATNTKVDK	TVAPSTCVPR	DAGCKPCICT	240
VPEVSSVFIF	PPKPKDVLTI	TLTPKVTCVV	VDISKDDPEV	QFSWFVDDVE	VHTAQTQPRE	300
EQFNSTFRSV	SELPIMHQDW	LNGKEFKCRV	NSAAFPAPIE	KTISKTKGRP	KAPQVYTIPP	360
PKEQMAKDKV	SLTCMITDFF	PEDITVEWQW	NGQPAENYKN	TQPIMNTNGS	YFVYSKLNVQ	420
KSNWEAGNTF	TCSVLHEGLH	NHHTEKSLSH	SPGKGGGGSG	LNDIFEAQKI	EWHE	474

Sequence Number (ID): 23
Length: 236
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..236
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..20
> note, Signal peptide
- REGION, 43..55
> note, LCDR1 QASQSLYNNKNLA
- REGION, 71..77
> note, LCDR2 YTATLAS
- REGION, 110..122

> note, LCDR3 QGEFTCESADCYT
- REGION, 50..51
> note, NN are deamidation sites
Residues:

```
METDTLLLWV  LLLWVPGSTG  QVLTQTASPV  SAAVGGTVTI  NCQASQSLYN  NKNLAWYQQK  60
PGQPPKLLIY  YTATLASGVS  SRFEGSGSGT  HFTLTISGVQ  CDDAATYYCQ  GEFTCESADC  120
YTFGGGTEVL  VKGDPVAPTV  LIFPPAADQV  ATGTVTIVCV  ANKYFPDVTV  TWEVDGTTQT  180
TGIENSKTPQ  NSADCTYNLS  STLTLTSTQY  NSHKEYTCKV  TQGTTSVVQS  FNRGDC      236
```

Sequence Number (ID): 24
Length: 456
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..456
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
- REGION, 49..53
> note, HCDR1 SYAMI
- REGION, 68..83
> note, HCDR2 IIYAIGIKYYANWAKG
- REGION, 114..116
> note, HCDR3 GDL
- SITE, 80
> note, W is an oxidation site
- SITE, 52
> note, M is an oxidation site
Residues:

```
MGWSCIILFL  VATATGVHSQ  SVEESGGRLV  TPGTPLTLTC  TVSGFSLSSY  AMIWVRQAPG  60
KGLEWIGIIY  AIGIKYYANW  AKGRFTISKT  STTVDLKITS  PTTEDTATYF  CARGDLWGPG  120
TLVTVSSGQP  KAPSVFPLAP  CCGDTPSSTV  TLGCLVKGYL  PEPVTVTWNS  GTLTNGVRTF  180
PSVRQSSGLY  SLSSVVSVTS  SSQPVTCNVA  HPATNTKVDK  TVAPSTCESK  YGPPCPPCPA  240
PEFLGGPSVF  LFPPKPKDTL  MISRTPEVTC  VVVDVSQEDP  EVQFNWYVDG  VEVHNAKTKP  300
REEQFNSTYR  VVSVLTVLHQ  DWLNGKEYKC  KVSNKGLPSS  IEKTISKAKG  QPREPQVYTL  360
PPSQEEMTKN  QVSLTCLVKG  FYPSDIAVEW  ESNGQPENNY  KTTPPVLDSD  GSFFLYSRLT  420
VDKSRWQEGN  VFSCSVMHEA  LHNHYTQKSL  SLSLGK                          456
```

Sequence Number (ID): 25
Length: 455
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..455
> mol_type, protein
> organism, synthetic construct
- REGION, 209..221
> note, VPRDAGCKPCICT
- SITE, 213
> note, A
- REGION, 441..455
> note, Terminal Avi tag GLNDIFEAQKIEWHE
- REGION, 436..440
> note, GGGGS-linker
Residues:

QSVEESGGRL	VTPGTPLTLT	CTVSGFSLSS	YAMIWVRQAP	GKGLEWIGII	YAIGIKYYAN	60
WAKGRFTISK	TSTTVDLKIT	SPTTEDTATY	FCARGDLWGP	GTLVTVSSGQ	PKAPSVFPLA	120
PCCGDTPSST	VTLGCLVKGY	LPEPVTVTWN	SGTLTNGVRT	FPSVRQSSGL	YSLSSVVSVT	180
SSSQPVTCNV	AHPATNTKVD	KTVAPSTCVP	RDAGCKPCIC	TVPEVSSVFI	FPPKPKDVLT	240
ITLTPKVTCV	VVDISKDDPE	VQFSWFVDDV	EVHTAQTQPR	EEQFNSTFRS	VSELPIMHQD	300
WLNGKEFKCR	VNSAAFPAPI	EKTISKTKGR	PKAPQVYTIP	PPKEQMAKDK	VSLTCMITDF	360
FPEDITVEWQ	WNGQPAENYK	NTQPIMNTNG	SYFVYSKLNV	QKSNWEAGNT	FTCSVLHEGL	420
HNHHTEKSLS	HSPGKGGGGS	GLNDIFEAQK	IEWHE			455

Sequence Number (ID): 26
Length: 431
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..431
> mol_type, protein
> organism, synthetic construct
Residues:

QSVEESGGRL	VTPGTPLTLT	CTVSGFSLSS	YAMIWVRQAP	GKGLEWIGII	YAIGIKYYAN	60
WAKGRFTISK	TSTTVDLKIT	SPTTEDTATY	FCARGDLWGP	GTLVTVSSGQ	PKAPSVFPLA	120
PCCGDTPSST	VTLGCLVKGY	LPEPVTVTWN	SGTLTNGVRT	FPSVRQSSGL	YSLSSVVSVT	180
SSSQPVTCNV	AHPATNTKVD	KTVAPSTCSK	PTCPPPELLG	GPSVFIFPPK	PKDTLMISRT	240
PEVTCVVVDV	SQDDPEVQFT	WYINNEQVRT	ARPPLREQQF	NSTIRVVSTL	PIAHQDWLRG	300

KEFKCKVHNK ALPAPIEKTI SKARGQPLEP KVYTMGPPRE ELSSRSVSLT CMINGFYPSD 360
ISVEWEKNGK AEDNYKTTPA VLDSDGSYFL YSKLSVPTSE WQRGDVFTCS VMHEALHNHY 420
TQKSISRSPG K 431

Sequence Number (ID): 27
Length: 447
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..447
> mol_type, protein
> organism, synthetic construct
- REGION, 431..436
> note, GGGGS-linker
- REGION, 437..447
> note, HiBiT sequence
Residues:
QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YAMIWVRQAP GKGLEWIGII YAIGIKYYAN 60
WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGDLWGP GTLVTVSSGQ PKAPSVFPLA 120
PCCGDTPSST VTLGCLVKGY LPEPVTVTWN SGTLTNGVRT FPSVRQSSGL YSLSSVVSVT 180
SSSQPVTCNV AHPATNTKVD KTVAPSTCSK PTCPPPELLG GPSVFIFPPK PKDTLMISRT 240
PEVTCVVVDV SQDDPEVQFT WYINNEQVRT ARPPLREQQF NSTIRVVSTL PIAHQDWLRG 300
KEFKCKVHNK ALPAPIEKTI SKARGQPLEP KVYTMGPPRE ELSSRSVSLT CMINGFYPSD 360
ISVEWEKNGK AEDNYKTTPA VLDSDGSYFL YSKLSVPTSE WQRGDVFTCS VMHEALHNHY 420
TQKSISRSPG KGGGGSVSGW RLFKKIS 447

Sequence Number (ID): 28
Length: 235
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..235
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
Residues:
MGSTAILGLL LAVLQGGRAQ VLTQTASPVS AAVGGTVTIN CQASQSLYNN KNLAWYQQKP 60
GQPPKLLIYY TATLASGVSS RFEGSGSGTH FTLTISGVQC DDAATYYCQG EFTCESADCY 120
TFGGGTEVLV KGDPVAPTVL IFPPAADQVA TGTVTIVCVA NKYFPDVTVT WEVDGTTQTT 180

GIENSKTPQN SADCTYNLSS TLTLTSTQYN SHKEYTCKVT QGTTSVVQSF NRGDC 235

Sequence Number (ID): 29
Length: 450
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..450
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
Residues:
MGSTAILGLL LAVLQGGRAQ SVEESGGRLV TPGTPLTLTC TVSGFSLSSY AMIWVRQAPG 60
KGLEWIGIIY AIGIKYYANW AKGRFTISKT STTVDLKITS PTTEDTATYF CARGDLWGPG 120
TLVTVSSGQP KAPSVFPLAP CCGDTPSSTV TLGCLVKGYL PEPVTVTWNS GTLTNGVRTF 180
PSVRQSSGLY SLSSVVSVTS SSQPVTCNVA HPATNTKVDK TVAPSTCSKP TCPPPELLGG 240
PSVFIFPPKP KDTLMISRTP EVTCVVVDVS QDDPEVQFTW YINNEQVRTA RPPLREQQFN 300
STIRVVSTLP IAHQDWLRGK EFKCKVHNKA LPAPIEKTIS KARGQPLEPK VYTMGPPREE 360
LSSRSVSLTC MINGFYPSDI SVEWEKNGKA EDNYKTTPAV LDSDGSYFLY SKLSVPTSEW 420
QRGDVFTCSV MHEALHNHYT QKSISRSPGK 450

Sequence Number (ID): 30
Length: 466
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..466
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
- REGION, 456..466
> note, HiBiT
- REGION, 451..455
> note, GGGGS-linker
Residues:
MGSTAILGLL LAVLQGGRAQ SVEESGGRLV TPGTPLTLTC TVSGFSLSSY AMIWVRQAPG 60
KGLEWIGIIY AIGIKYYANW AKGRFTISKT STTVDLKITS PTTEDTATYF CARGDLWGPG 120
TLVTVSSGQP KAPSVFPLAP CCGDTPSSTV TLGCLVKGYL PEPVTVTWNS GTLTNGVRTF 180

PSVRQSSGLY SLSSVVSVTS SSQPVTCNVA HPATNTKVDK TVAPSTCSKP TCPPPELLGG 240
PSVFIFPPKP KDTLMISRTP EVTCVVVDVS QDDPEVQFTW YINNEQVRTA RPPLREQQFN 300
STIRVVSTLP IAHQDWLRGK EFKCKVHNKA LPAPIEKTIS KARGQPLEPK VYTMGPPREE 360
LSSRSVSLTC MINGFYPSDI SVEWEKNGKA EDNYKTTPAV LDSDGSYFLY SKLSVPTSEW 420
QRGDVFTCSV MHEALHNHYT QKSISRSPGK GGGGSVSGWR LFKKIS 466

Sequence Number (ID): 31
Length: 414
Molecule Type: DNA
Features Location/Qualifiers:
- source, 1..414
> mol_type, other DNA
> organism, synthetic construct
- sig_peptide, 1..57
> note, Coding for signal peptide
- misc_feature, 145..159
> note, Coding for HCDR1
- misc_feature, 202..249
> note, Coding for HCDR2
- misc_feature, 333..371
> note, Coding for HCDR3
Residues:
atggagactg ggctgcgctg gcttctcctg gtcgctgtgt tcaaaggtgt ccagtgtcag 60
tcgctggagg agtccggggg tcgcctggtc acgcctggga cacccctgac actcacctgc 120
accgtctctg gattctccct cagtagcaat gcaattggct gggtccgcca ggctccaggg 180
aaggggctgg aatacatcgg gatcattggt gaaactggta ggacatacta cgcgacctgg 240
gcgaaaggcc gattcaccat ctccaaaacc tcgtcgacca cggtggatct gaaaatcacc 300
agtccgacaa ccgaggacac ggccacctat ttctgtgcca gaggttatcc tgatactagc 360
gttgccgact actttaatat gtggggccca ggcaccctgg tcaccgtctc ctca 414

Sequence Number (ID): 32
Length: 138
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..138
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19

> note, Signal peptide
- REGION, 49..53
> note, HCDR1 SNAIG
- REGION, 67..83
> note, HCDR2 IIGETGRTYYATWAKG
- REGION, 115..127
> note, HCDR3 GYPDTSVADYFNM
Residues:
METGLRWLLL VAVFKGVQCQ SLEESGGRLV TPGTPLTLTC TVSGFSLSSN AIGWVRQAPG 60
KGLEYIGIIG ETGRTYYATW AKGRFTISKT SSTTVDLKIT SPTTEDTATY FCARGYPDTS 120
VADYFNMWGP GTLVTVSS 138

Sequence Number (ID): 33
Length: 399
Molecule Type: DNA
Features Location/Qualifiers:
- source, 1..399
> mol_type, other DNA
> organism, synthetic construct
- sig_peptide, 1..69
> note, Coding for signal peptide
- misc_feature, 139..171
> note, Coding for LCDR1
- misc_feature, 217..237
> note, Coding for LCDR2
- misc_feature, 334..369
> note, Coding for LCDR3
Residues:
atggacacga gggcccccac tcagctgctg gggctcctgc tgctctggct cccaggtgcc 60
acatttgccc aaattgatat gacccagact ccatcgtccg tgtctgaacc tgtgggaggc 120
acagtcacca tcaattgcca ggccagtcag agtattaata gttggttatc ctggtatcag 180
cagaaaccag ggcagcctcc caagctcctg atctattatg catccactct ggcatctggg 240
gtctcatcgc ggttcaaagg cagtggatct gggacacagt tcactctcac catcagcggc 300
gtgcagtgtg acgatgctgc cacttactac tgtcagcagg cttatagtta tagtgaggct 360
atcaatactt tcggcggagg gaccgaggtg gtggtcaaa 399

Sequence Number (ID): 34
Length: 133

Molecule Type: AA
Features Location/Qualifiers:
- source, 1..133
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..23
> note, Signal peptide
- REGION, 47..57
> note, LCDR1 QASQSINSWLS
- REGION, 73..79
> note, LCDR2 YASTLAS
- REGION, 112..123
> note, QQAYSYSEAINT
Residues:
MDTRAPTQLL GLLLLWLPGA TFAQIDMTQT PSSVSEPVGG TVTINCQASQ SINSWLSWYQ 60
QKPGQPPKLL IYYASTLASG VSSRFKGSGS GTQFTLTISG VQCDDAATYY CQQAYSYSEA 120
INTFGGGTEV VVK 133

Sequence Number (ID): 35
Length: 166
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..166
> mol_type, protein
> organism, synthetic construct
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLI LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEOP LSLYECDSTL VSIRWRCNRK MITGPLOYSV OVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNGGGGSLPE TGGGGG 166

Sequence Number (ID): 36
Length: 235
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..235
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19

> note, Signal number
Residues:
MGWSCIILFL VATATGVHSQ VLTQTASPVS AAVGGTVTIN CQASQSLYNN KNLAWYQQKP 60
GQPPKLLIYY TATLASGVSS RFEGSGSGTH FTLTISGVQC DDAATYYCQG EFTCESADCY 120
TFGGGTEVLV KGDPVAPTVL IFPPAADQVA TGTVTIVCVA NKYFPDVTVT WEVDGTTQTT 180
GIENSKTPQN SADCTYNLSS TLTLTSTQYN SHKEYTCKVT QGTTSVVQSF NRGDC 235

Sequence Number (ID): 37
Length: 450
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..450
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
Residues:
MGWSCIILFL VATATGVHSQ SVEESGGRLV TPGTPLTLTC TVSGFSLSSY AMIWVRQAPG 60
KGLEWIGIIY AIGIKYYANW AKGRFTISKT STTVDLKITS PTTEDTATYF CARGDLWGPG 120
TLVTVSSGQP KAPSVFPLAP CCGDTPSSTV TLGCLVKGYL PEPVTVTWNS GTLTNGVRTF 180
PSVRQSSGLY SLSSVVSVTS SSQPVTCNVA HPATNTKVDK TVAPSTCSKP TCPPPELLGG 240
PSVFIFPPKP KDTLMISRTP EVTCVVVDVS QDDPEVQFTW YINNEQVRTA RPPLREQQFN 300
STIRVVSTLP IAHQDWLRGK EFKCKVHNKA LPAPIEKTIS KARGQPLEPK VYTMGPPREE 360
LSSRSVSLTC MINGFYPSDI SVEWEKNGKA EDNYKTTPAV LDSDGSYFLY SKLSVPTSEW 420
QRGDVFTCSV MHEALHNHYT QKSISRSPGK 450

Sequence Number (ID): 38
Length: 466
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..466
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..19
> note, Signal peptide
- REGION, 456..466
> note, HiBiT
- REGION, 451..455

> note, GGGGS-linker
Residues:
MGWSCIILFL VATATGVHSQ SVEESGGRLV TPGTPLTLTC TVSGFSLSSY AMIWVRQAPG 60
KGLEWIGIIY AIGIKYYANW AKGRFTISKT STTVDLKITS PTTEDTATYF CARGDLWGPG 120
TLVTVSSGQP KAPSVFPLAP CCGDTPSSTV TLGCLVKGYL PEPVTVTWNS GTLTNGVRTF 180
PSVRQSSGLY SLSSVVSVTS SSQPVTCNVA HPATNTKVDK TVAPSTCSKP TCPPPELLGG 240
PSVFIFPPKP KDTLMISRTP EVTCVVVDVS QDDPEVQFTW YINNEQVRTA RPPLREQQFN 300
STIRVVSTLP IAHQDWLRGK EFKCKVHNKA LPAPIEKTIS KARGQPLEPK VYTMGPPREE 360
LSSRSVSLTC MINGFYPSDI SVEWEKNGKA EDNYKTTPAV LDSDGSYFLY SKLSVPTSEW 420
QRGDVFTCSV MHEALHNHYT QKSISRSPGK GGGGSVSGWR LFKKIS 466

Sequence Number (ID): 39
Length: 231
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..231
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..15
> note, Signal peptide
Residues:
MNLLLILTFV AAAVAQVLTQ TASPVSAAVG GTVTINCQAS QSLYNNKNLA WYQQKPGQPP 60
KLLIYYTATL ASGVSSRFEG SGSGTHFTLT ISGVQCDDAA TYYCQGEFTC ESADCYTFGG 120
GTEVLVKGDP VAPTVLIFPP AADQVATGTV TIVCVANKYF PDVTVTWEVD GTTQTTGIEN 180
SKTPQNSADC TYNLSSTLTL TSTQYNSHKE YTCKVTQGTT SVVQSFNRGD C 231

Sequence Number (ID): 40
Length: 446
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..446
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..15
> note, Signal peptide
Residues:
MNLLLILTFV AAAVAQSVEE SGGRLVTPGT PLTLTCTVSG FSLSSYAMIW VRQAPGKGLE 60
WIGIIYAIGI KYYANWAKGR FTISKTSTTV DLKITSPTTE DTATYFCARG DLWGPGTLVT 120

VSSGQPKAPS VFPLAPCCGD TPSSTVTLGC LVKGYLPEPV TVTWNSGTLT NGVRTFPSVR 180
QSSGLYSLSS VVSVTSSSQP VTCNVAHPAT NTKVDKTVAP STCSKPTCPP PELLGGPSVF 240
IFPPKPKDTL MISRTPEVTC VVVDVSQDDP EVQFTWYINN EQVRTARPPL REQQFNSTIR 300
VVSTLPIAHQ DWLRGKEFKC KVHNKALPAP IEKTISKARG QPLEPKVYTM GPPREELSSR 360
SVSLTCMING FYPSDISVEW EKNGKAEDNY KTTPAVLDSD GSYFLYSKLS VPTSEWQRGD 420
VFTCSVMHEA LHNHYTQKSI SRSPGK 446

Sequence Number (ID): 41
Length: 462
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..462
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..15
> note, Signal peptide
- REGION, 452..462
> note, HiBiT VSGWRLFKKIS
- REGION, 447..451
> note, GGGGS-linker
Residues:
MNLLLILTFV AAAVAQSVEE SGGRLVTPGT PLTLTCTVSG FSLSSYAMIW VRQAPGKGLE 60
WIGIIYAIGI KYYANWAKGR FTISKTSTTV DLKITSPTTE DTATYFCARG DLWGPGTLVT 120
VSSGQPKAPS VFPLAPCCGD TPSSTVTLGC LVKGYLPEPV TVTWNSGTLT NGVRTFPSVR 180
QSSGLYSLSS VVSVTSSSQP VTCNVAHPAT NTKVDKTVAP STCSKPTCPP PELLGGPSVF 240
IFPPKPKDTL MISRTPEVTC VVVDVSQDDP EVQFTWYINN EQVRTARPPL REQQFNSTIR 300
VVSTLPIAHQ DWLRGKEFKC KVHNKALPAP IEKTISKARG QPLEPKVYTM GPPREELSSR 360
SVSLTCMING FYPSDISVEW EKNGKAEDNY KTTPAVLDSD GSYFLYSKLS VPTSEWQRGD 420
VFTCSVMHEA LHNHYTQKSI SRSPGKGGGG SVSGWRLFKK IS 462

Sequence Number (ID): 42
Length: 347
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..347
> mol_type, protein
> organism, synthetic construct
Residues:

EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVE 347

Sequence Number (ID): 43
Length: 104
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..104
> mol_type, protein
> organism, Oryctolagus cuniculus
Residues:
GDPVAPTVLI FPPAADQVAT GTVTIVCVAN KYFPDVTVTW EVDGTTQTTG IENSKTPQNS 60
ADCTYNLSST LTLTSTQYNS HKEYTCKVTQ GTTSVVQSFN RGDC 104

Sequence Number (ID): 44
Length: 323
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..323
> mol_type, protein
> organism, Oryctolagus cuniculus
Residues:
GQPKAPSVFP LAPCCGDTPS STVTLGCLVK GYLPEPVTVT WNSGTLTNGV RTFPSVRQSS 60
GLYSLSSVVS VTSSSQPVTC NVAHPATNTK VDKTVAPSTC SKPTCPPPEL LGGPSVFIFP 120
PKPKDTLMIS RTPEVTCVVV DVSQDDPEVQ FTWYINNEQV RTARPPLREQ QFNSTIRVVS 180
TLPITHQDWL RGKEFKCKVH NKALPAPIEK TISKARGQPL EPKVYTMGPP REELSSRSVS 240
LTCMINGFYP SDISVEWEKN GKAEDNYKTT PAVLDSDGSY FLYNKLSVPT SEWQRGDVFT 300
CSVMHEALHN HYTQKSISRS PGK 323

Sequence Number (ID): 45
Length: 214
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..214

> mol_type, protein
> organism, synthetic construct
Residues:
QIDMTQTPSS VSEPVGGTVT INCQASQSIN SWLSWYQQKP GQPPKLLIYY ASTLASGVSS 60
RFKGSGSGTQ FTLTISGVQC DDAATYYCQQ AYSYSEAINT FGGGTEVVVK GDPVAPTVLI 120
FPPAADQVAT GTVTIVCVAN KYFPDVTVTW EVDGTTQTTG IENSKTPQNS ADCTYNLSST 180
LTLTSTQYNS HKEYTCKVTQ GTTSVVQSFN RGDC 214

Sequence Number (ID): 46
Length: 442
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..442
> mol_type, protein
> organism, synthetic construct
Residues:
QSLEESGGRL VTPGTPLTLT CTVSGFSLSS NAIGWVRQAP GKGLEYIGII GETGRTYYAT 60
WAKGRFTISK TSSTTVDLKI TSPTTEDTAT YFCARGYPDT SVADYFNMWG PGTLVTVSSG 120
QPKAPSVFPL APCCGDTPSS TVTLGCLVKG YLPEPVTVTW NSGTLTNGVR TFPSVRQSSG 180
LYSLSSVVSV TSSSQPVTCN VAHPATNTKV DKTVAPSTCS KPTCPPPELL GGPSVFIFPP 240
KPKDTLMISR TPEVTCVVVD VSQDDPEVQF TWYINNEQVR TARPPLREQQ FNSTIRVVST 300
LPITHQDWLR GKEFKCKVHN KALPAPIEKT ISKARGQPLE PKVYTMGPPR EELSSRSVSL 360
TCMINGFYPS DISVEWEKNG KAEDNYKTTP AVLDSDGSYF LYNKLSVPTS EWQRGDVFTC 420
SVMHEALHNH YTQKSISRSP GK 442

Sequence Number (ID): 47
Length: 217
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..217
> mol_type, protein
> organism, synthetic construct
Residues:
AQVLTQTPSP VSAAVGGTVT INCQASQSVY NNENLAWYQQ KLGQPPKLLI YDASTLASGV 60
PLRFSASGSG TQFTLTISDV QCDDAATYYC QGEFSCDSAD CNVFGGGTEV VVKGDPVAPT 120
VLIFPPAADQ VATGTVTIVC VANKYFPDVT VTWEVDGTTQ TTGIENSKTP QNSADCTYNL 180
SSTLTLTSTQ YNSHKEYTCK VTQGTTSVVQ SFNRGDC 217

Sequence Number (ID): 48
Length: 438
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..438
> mol_type, protein
> organism, synthetic construct
Residues:
QSVEESGGRL VTPGTPLTLT CTVSGFSLST YAMNWVRQAP GKGLEWIGVI SAGGTTFYTS 60
WAKGRFTISR TSTTVDLKIT SPTTEDTATY FCVRKNYSIR SFDIWGPGTL VTVSSGQPKA 120
PSVFPLAPCC GDTPSSTVTL GCLVKGYLPE PVTVTWNSGT LTNGVRTFPS VRQSSGLYSL 180
SSVVSVTSSS QPVTCNVAHP ATNTKVDKTV APSTCSKPTC PPPELLGGPS VFIFPPKPKD 240
TLMISRTPEV TCVVVDVSQD DPEVQFTWYI NNEQVRTARP PLREQQFNST IRVVSTLPIT 300
HQDWLRGKEF KCKVHNKALP APIEKTISKA RGQPLEPKVY TMGPPREELS SRSVSLTCMI 360
NGFYPSDISV EWEKNGKAED NYKTTPAVLD SDGSYFLYNK LSVPTSEWQR GDVFTCSVMH 420
EALHNHYTQK SISRSPGK 438

Sequence Number (ID): 49
Length: 217
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..217
> mol_type, protein
> organism, synthetic construct
Residues:
AQVLTQTPSP VSAAVGGTVT INCQASQSVY NNENLAWYQQ KPGQPPKLLI YDASTLASGV 60
PSRFSGSGSG TQFTLTISGV QCEDAATYYC QGEFDCNSAD CHVFGGGTEV VVKGDPVAPT 120
VLIFPPAADQ VATGTVTIVC VANKYFPDVT VTWEVDGTTQ TTGIENSKTP QNSADCTYNL 180
SSTLTLTSTQ YNSHKEYTCK VTQGTTSVVQ SFNRGDC 217

Sequence Number (ID): 50
Length: 438
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..438
> mol_type, protein
> organism, synthetic construct
Residues:

QSMEESGGRL VTPGTPLTLT CTVSGFSLSS FQMNWVRQTP GKGLEWIGGI SAGGSVFYAN 60
WAKGRFTISR TSTTLDLQIT SPTTEDTATY FCARKSYNIA GLDNWGPGTL VTVSSGQPKA 120
PSVFPLAPCC GDTPSSTVTL GCLVKGYLPE PVTVTWNSGT LTNGVRTFPS VRQSSGLYSL 180
SSVVSVTSSS QPVTCNVAHP ATNTKVDKTV APSTCSKPTC PPPELLGGPS VFIFPPKPKD 240
TLMISRTPEV TCVVVDVSQD DPEVQFTWYI NNEQVRTARP PLREQQFNST IRVVSTLPIT 300
HQDWLRGKEF KCKVHNKALP APIEKTISKA RGQPLEPKVY TMGPPREELS SRSVSLTCMI 360
NGFYPSDISV EWEKNGKAED NYKTTPAVLD SDGSYFLYNK LSVPTSEWQR GDVFTCSVMH 420
EALHNHYTQK SISRSPGK 438

Sequence Number (ID): 51
Length: 214
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..214
> mol_type, protein
> organism, synthetic construct
Residues:
EVVMTQTPSS VSGAVGGTVT IKCQASEDIG RLLAWYQQKP GQPPKLLIYG TSILESGVPS 60
RFKGSGAGTQ FTLTISDLEC ADAATYYCQA TYGSSTNGNS FGGGTEVVVK GDPVAPTVLI 120
FPPAADQVAT GTVTIVCVAN KYFPDVTVTW EVDGTTQTTG IENSKTPQNS ADCTYNLSST 180
LTLTSTQYNS HKEYTCKVTQ GTTSVVQSFN RGDC 214

Sequence Number (ID): 52
Length: 435
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..435
> mol_type, protein
> organism, synthetic construct
Residues:
QSVEESGGRL VMPGTPLTLT CTVSGFSLSR YNMAWVRQAP GKGLEYIGII SYSNNTYYAN 60
WAKGRFTISK TSSTTVDLKM TSLTTEDTAT YFCARGVGTN IWGPGTLVTV SSGQPKAPSV 120
FPLAPCCGDT PSSTVTLGCL VKGYLPEPVT VTWNSGTLTN GVRTFPSVRQ SSGLYSLSSV 180
VSVTSSSQPV TCNVAHPATN TKVDKTVAPS TCSKPTCPPP ELLGGPSVFI FPPKPKDTLM 240
ISRTPEVTCV VVDVSQDDPE VQFTWYINNE QVRTARPPLR EQQFNSTIRV VSTLPITHQD 300
WLRGKEFKCK VHNKALPAPI EKTISKARGQ PLEPKVYTMG PPREELSSRS VSLTCMINGF 360
YPSDISVEWE KNGKAEDNYK TTPAVLDSDG SYFLYNKLSV PTSEWQRGDV FTCSVMHEAL 420
HNHYTQKSIS RSPGK 435

Sequence Number (ID): 53
Length: 363
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..363
> mol_type, protein
> organism, synthetic construct
- REGION, 354..363
> note, His10
- REGION, 348..353
> note, Thrombin recognition sequence LVPRGS
Residues:

EGVAAALTPE	RLLEWQDKGI	FVIQSESLKK	CIQAGKSVLT	LENCKQANKH	MLWKWVSNHG	60
LFNIGGSGCL	GLNFSAPEQP	LSLYECDSTL	VSLRWRCNRK	MITGPLQYSV	QVAHDNTVVA	120
SRKYIHKWIS	YGSGGGDICE	YLHKDLHTIK	GNTHGMPCMF	PFQYNHQWHH	ECTREGREDD	180
LLWCATTSRY	ERDEKWGFCP	DPTSAEVGCD	TIWEKDLNSH	ICYQFNLLSS	LSWSEAHSSC	240
QMQGGTLLSI	TDETEENFIR	EHMSSKTVEV	WMGLNQLDEH	AGWQWSDGTP	LNYLNWSPEV	300
NFEPFVEDHC	GTFSSFMPSA	WRSRDCESTL	PYICKKYLNH	IDHEIVELVP	RGSHHHHHHH	360
HHH						363

Sequence Number (ID): 54
Length: 158
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..158
> mol_type, protein
> organism, synthetic construct
- REGION, 149..158
> note, His10
- REGION, 134..147
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 128..133
> note, GGSGGS linker
Residues:

NTSDMYPMPN	TLEYGNRTYK	IINANMTWYA	AIKTCLMHKA	QLVSITDQYH	QSFLTVVLNR	60
LGYAHWIGLF	TTDNGLNFDW	SDGTKSSFTF	WKDEESSLLG	DCVFADSNGR	WHSTACESFL	120
QGAICHVGGS	GGSGLNDIFE	AQKIEWHEHH	HHHHHHHH			158

Sequence Number (ID): 55
Length: 1258
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1258
> mol_type, protein
> organism, synthetic construct
- REGION, 1249..1258
> note, His10
- REGION, 1244..1258
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1238..1243
> note, GGSGGS linker
Residues:

GNTHGMPCMF PFQYNHQWHH ECTREGREDD LLWCATTSRY ERDEKWGFCP DPTSAEVGCD 60
TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC QMQGGTLLSI TDETEENFIR EHMSSKTVEV 120
WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV NFEPFVEDHC GTFSSFMPSA WRSRDCESTL 180
PYICKKYLNH IDHEIVEKDA WKYYATHCEP GWNPYNRNCY KLQKEEKTWH EALRSCQADN 240
SALIDITSLA EVEFLVTLLG DENASETWIG LSSNKIPVSF EWSNDSSVIF TNWHTLEPHI 300
FPNRSQLCVS AEQSEGHWKV KNCEERLFYI CKKAGHVLSD AESGCQEGWE RHGGFCYKID 360
TVLRSFDQAS SGYYCPPALV TITNRFEQAF ITSLISSVVK MKDSYFWIAL QDQNDTGEYT 420
WKPVGQKPEP VQYTHWNTHQ PRYSGGCVAM RGRHPLGRWE VKHCRHFKAM SLCKQPVENQ 480
EKAEYEERWP FHPCYLDWES EPGLASCFKV FHSEKVLMKR TWREAEAFCE EFGAHLASFA 540
HIEEENFVNE LLHSKFNWTE ERQFWIGFNK RNPLNAGSWE WSDRTPVVSS FLDNTYFGED 600
ARNCAVYKAN KTLLPLHCGS KREWICKIPR DVKPKIPFWY QYDVPWLFYQ DAEYLFHTFA 660
SEWLNFEFVC SWLHSDLLTI HSAHEQEFIH SKIKALSKYG ASWWIGLQEE RANDEFRWRD 720
GTPVIYQNWD TGRERTVNNQ SQRCGFISSI TGLWGSEECS VSMPSICKRK KVWLIEKKKD 780
TPKQHGTCPK GWLYFNYKCL LLNIPKDPSS WKNWTHAQHF CAEEGGTLVA IESEVEQAFI 840
TMNLFGQTTS VWIGLQNDDY ETWLNGKPVV YSNWSPFDII NIPSHNTTEV QKHIPLCALL 900
SSNPNFHFTG KWYFEDCGKE GYGFVCEKMQ DTSGHGVNTS DMYPMPNTLE YGNRTYKIIN 960
ANMTWYAAIK TCLMHKAQLV SITDQYHQSF LTVVLNRLGY AHWIGLFTTD NGLNFDWSDG 1020
TKSSFTFWKD EESSLLGDCV FADSNGRWHS TACESFLQGA ICHVPPETRQ SEHPELCSET 1080
SIPWIKFKSN CYSFSTVLDS MSFEAAHEFC KKEGSNLLTI KDEAENAFLL EELFAFGSSV 1140
QMVWLNAQFD GNNETIKWFD GTPTDQSNWG IRKPDTDYFK PHHCVALRIP EGLWQLSPCQ 1200
EKKGFICKME ADIHTAEALP EKGPSHSGGS GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1258

Sequence Number (ID): 56

Length: 1408
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1408
> mol_type, protein
> organism, synthetic construct
- REGION, 1399..1408
> note, His10
- REGION, 1324..1398
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1318..1323
> note, GGSGGS linker
- REGION, 136..141
> note, Thrombin cleavage site engineered between CysR and FnII domains
LVPRGS
Residues:

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EGVAAALTPE  RLLEWQDKGI  FVIQSESLKK  CIQAGKSVLT  LENCKQANKH  MLWKWVSNHG  60
LFNIGGSGCL  GLNFSAPEQP  LSLYECDSTL  VSLRWRCNRK  MITGPLQYSV  QVAHDNTVVA  120
SRKYIHKWIS  YGSGGGDICE  YLHKDLVPRG  SNTHGMPCMF  PFQYNHQWHH  ECTREGREDD  180
LLWCATTSRY  ERDEKWGFCP  DPTSAEVGCD  TIWEKDLNSH  ICYQFNLLSS  LSWSEAHSSC  240
QMQGGTLLSI  TDETEENFIR  EHMSSKTVEV  WMGLNQLDEH  AGWQWSDGTP  LNYLNWSPEV  300
NFEPFVEDHC  GTFSSFMPSA  WRSRDCESTL  PYICKKYLNH  IDHEIVEKDA  WKYYATHCEP  360
GWNPYNRNCY  KLQKEEKTWH  EALRSCQADN  SALIDITSLA  EVEFLVTLLG  DENASETWIG  420
LSSNKIPVSF  EWSNDSSVIF  TNWHTLEPHI  FPNRSQLCVS  AEQSEGHWKV  KNCEERLFYI  480
CKKAGHVLSD  AESGCQEGWE  RHGGFCYKID  TVLRSFDQAS  SGYYCPPALV  TITNRFEQAF  540
ITSLISSVVK  MKDSYFWIAL  QDQNDTGEYT  WKPVGQKPEP  VQYTHWNTHQ  PRYSGGCVAM  600
RGRHPLGRWE  VKHCRHFKAM  SLCKQPVENQ  EKAEYEERWP  FHPCYLDWES  EPGLASCFKV  660
FHSEKVLMKR  TWREAEAFCE  EFGAHLASFA  HIEEENFVNE  LLHSKFNWTE  ERQFWIGFNK  720
RNPLNAGSWE  WSDRTPVVSS  FLDNTYFGED  ARNCAVYKAN  KTLLPLHCGS  KREWICKIPR  780
DVKPKIPFWY  QYDVPWLFYQ  DAEYLFHTFA  SEWLNFEFVC  SWLHSDLLTI  HSAHEQEFIH  840
SKIKALSKYG  ASWWIGLQEE  RANDEFRWRD  GTPVIYQNWD  TGRERTVNNQ  SQRCGFISSI  900
TGLWGSEECS  VSMPSICKRK  KVWLIEKKKD  TPKQHGTCPK  GWLYFNYKCL  LLNIPKDPSS  960
WKNWTHAQHF  CAEEGGTLVA  IESEVEQAFI  TMNLFGQTTS  VWIGLQNDDY  ETWLNGKPVV  1020
YSNWSPFDII  NIPSHNTTEV  QKHIPLCALL  SSNPNFHFTG  KWYFEDCGKE  GYGFVCEKMQ  1080
DTSGHGVNTS  DMYPMPNTLE  YGNRTYKIIN  ANMTWYAAIK  TCLMHKAQLV  SITDQYHQSF  1140
LTVVLNRLGY  AHWIGLFTTD  NGLNFDWSDG  TKSSFTFWKD  EESSLLGDCV  FADSNGRWHS  1200
TACESFLQGA  ICHVPPETRQ  SEHPELCSET  SIPWIKFKSN  CYSFSTVLDS  MSFEAAHEFC  1260
KKEGSNLLTI  KDEAENAFLL  EELFAFGSSV  QMVWLNAQFD  GNNETIKWFD  GTPTDQSNWG  1320
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IRKPDTDYFK PHHCVALRIP EGLWQLSPCQ EKKGFICKME ADIHTAEALP EKGPSHSGGS 1380
GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1408

Sequence Number (ID): 57
Length: 1060
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1060
> mol_type, protein
> organism, synthetic construct
- REGION, 1051..1060
> note, His10
- REGION, 1036..1050
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1030..1035
> note, GGSGGS linker
Residues:
DAWKYYATHC EPGWNPYNRN CYKLQKEEKT WHEALRSCQA DNSALIDITS LAEVEFLVTL 60
LGDENASETW IGLSSNKIPV SFEWSNDSSV IFTNWHTLEP HIFPNRSQLC VSAEQSEGHW 120
KVKNCEERLF YICKKAGHVL SDAESGCQEG WERHGGFCYK IDTVLRSFDQ ASSGYYCPPA 180
LVTITNRFEQ AFITSLISSV VKMKDSYFWI ALQDQNDTGE YTWKPVGQKP EPVQYTHWNT 240
HQPRYSGGCV AMRGRHPLGR WEVKHCRHFK AMSLCKQPVE NQEKAEYEER WPFHPCYLDW 300
ESEPGLASCF KVFHSEKVLM KRTWREAEAF CEEFGAHLAS FAHIEEENFV NELLHSKFNW 360
TEERQFWIGF NKRNPLNAGS WEWSDRTPVV SSFLDNTYFG EDARNCAVYK ANKTLLPLHC 420
GSKREWICKI PRDVKPKIPF WYQYDVPWLF YQDAEYLFHT FASEWLNFEF VCSWLHSDLL 480
TIHSAHEQEF IHSKIKALSK YGASWWIGLQ EERANDEFRW RDGTPVIYQN WDTGRERTVN 540
NQSQRCGFIS SITGLWGSEE CSVSMPSICK RKKVWLIEKK KDTPKQHGTC PKGWLYFNYK 600
CLLLNIPKDP SSWKNWTHAQ HFCAEEGGTL VAIESEVEQA FITMNLFGQT TSVWIGLQND 660
DYETWLNGKP VVYSNWSPFD IINIPSHNTT EVQKHIPLCA LLSSNPNFHF TGKWYFEDCG 720
KEGYGFVCEK MQDTSGHGVN TSDMYPMPNT LEYGNRTYKI INANMTWYAA IKTCLMHKAQ 780
LVSITDQYHQ SFLTVVLNRL GYAHWIGLFT TDNGLNFDWS DGTKSSFTFW KDEESSLLGD 840
CVFADSNGRW HSTACESFLQ GAICHVPPET RQSEHPELCS ETSIPWIKFK SNCYSFSTVL 900
DSMSFEAAHE FCKKEGSNLL TIKDEAENAF LLEELFAFGS SVQMVWLNAQ FDGNNETIKW 960
FDGTPTDQSN WGIRKPDTDY FKPHHCVALR IPEGLWQLSP CQEKKGFICK MEADIHTAEA 1020
LPEKGPSHSG GSGGSGLNDI FEAQKIEWHE HHHHHHHHHH 1060

Sequence Number (ID): 58
Length: 1408

Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1408
> mol_type, protein
> organism, synthetic construct
- REGION, 1399..1408
> note, His6
- REGION, 1384..1398
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1378..1383
> note, GGSGGS linker
- REGION, 325..330
> note, Thrombin cleavage site engineered between CTDL1 and CTDL2 domains
LVPRGS
Residues:

EGVAAALTPE	RLLEWQDKGI	FVIQSESLKK	CIQAGKSVLT	LENCKQANKH	MLWKWVSNHG	60
LFNIGGSGCL	GLNFSAPEQP	LSLYECDSTL	VSLRWRCNRK	MITGPLQYSV	QVAHDNTVVA	120
SRKYIHKWIS	YGSGGGDICE	YLHKDLHTIK	GNTHGMPCMF	PFQYNHQWHH	ECTREGREDD	180
LLWCATTSRY	ERDEKWGFCP	DPTSAEVGCD	TIWEKDLNSH	ICYQFNLLSS	LSWSEAHSSC	240
QMQGGTLLSI	TDETEENFIR	EHMSSKTVEV	WMGLNQLDEH	AGWQWSDGTP	LNYLNWSPEV	300
NFEPFVEDHC	GTFSSFMPSA	WRSRDCESTL	PYICKKYLNH	IDHELVPRGS	WKYYATHCEP	360
GWNPYNRNCY	KLQKEEKTWH	EALRSCQADN	SALIDITSLA	EVEFLVTLLG	DENASETWIG	420
LSSNKIPVSF	EWSNDSSVIF	TNWHTLEPHI	FPNRSQLCVS	AEQSEGHWKV	KNCEERLFYI	480
CKKAGHVLSD	AESGCQEGWE	RHGGFCYKID	TVLRSFDQAS	SGYYCPPALV	TITNRFEQAF	540
ITSLISSVVK	MKDSYFWIAL	QDQNDTGEYT	WKPVGQKPEP	VQYTHWNTHQ	PRYSGGCVAM	600
RGRHPLGRWE	VKHCRHFKAM	SLCKQPVENQ	EKAEYEERWP	FHPCYLDWES	EPGLASCFKV	660
FHSEKVLMKR	TWREAEAFCE	EFGAHLASFA	HIEEENFVNE	LLHSKFNWTE	ERQFWIGFNK	720
RNPLNAGSWE	WSDRTPVVSS	FLDNTYFGED	ARNCAVYKAN	KTLLPLHCGS	KREWICKIPR	780
DVKPKIPFWY	QYDVPWLFYQ	DAEYLFHTFA	SEWLNFEFVC	SWLHSDLLTI	HSAHEQEFIH	840
SKIKALSKYG	ASWWIGLQEE	RANDEFRWRD	GTPVIYQNWD	TGRERTVNNQ	SQRCGFISSI	900
TGLWGSEECS	VSMPSICKRK	KVWLIEKKKD	TPKQHGTCPK	GWLYFNYKCL	LLNIPKDPSS	960
WKNWTHAQHF	CAEEGGTLVA	IESEVEQAFI	TMNLFGQTTS	VWIGLQNDDY	ETWLNGKPVV	1020
YSNWSPFDII	NIPSHNTTEV	QKHIPLCALL	SSNPNFHFTG	KWYFEDCGKE	GYGFVCEKMQ	1080
DTSGHGVNTS	DMYPMPNTLE	YGNRTYKIIN	ANMTWYAAIK	TCLMHKAQLV	SITDQYHQSF	1140
LTVVLNRLGY	AHWIGLFTTD	NGLNFDWSDG	TKSSFTFWKD	EESSLLGDCV	FADSNGRWHS	1200
TACESFLQGA	ICHVPPETRQ	SEHPELCSET	SIPWIKFKSN	CYSFSTVLDS	MSFEAAHEFC	1260
KKEGSNLLTI	KDEAENAFLL	EELFAFGSSV	QMVWLNAQFD	GNNETIKWFD	GTPTDQSNWG	1320
IRKPDTDYFK	PHHCVALRIP	EGLWQLSPCQ	EKKGFICKME	ADIHTAEALP	EKGPSHSGGS	1380

GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1408

Sequence Number (ID): 59
Length: 364
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..364
> mol_type, protein
> organism, synthetic construct
- MOD_RES, 364
> note, GN3 conjugated to the C-terminal cysteine residue via maleimide conjugation
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEGGG GSHHHHHHGG 360
GGSC 364

Sequence Number (ID): 60
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
AEQSEGHWKV KNCEE 15

Sequence Number (ID): 61
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens

Residues:
AESGCQEGWE RHGGF 15

Sequence Number (ID): 62
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
AHEFCKKEGS NLLTI 15

Sequence Number (ID): 63
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
AHSSCQMQGG TLLSI 15

Sequence Number (ID): 64
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
ARNCAVYKAN KTLLP 15

Sequence Number (ID): 65
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15

> mol_type, protein
> organism, Homo sapiens
Residues:
CAEEGGTLVA IESEV 15

Sequence Number (ID): 66
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CEKMQDTSGH GVNTS 15

Sequence Number (ID): 67
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CESTLPYICK KYLNH 15

Sequence Number (ID): 68
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CIQAGKSVLT LENCK 15

Sequence Number (ID): 69
Length: 15
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CKKAGHVLSD AESGC 15

Sequence Number (ID): 70
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CQADNSALID ITSLA 15

Sequence Number (ID): 71
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CYKIDTVLRS FDQAS 15

Sequence Number (ID): 72
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
CYSFSTVLDS MSFEA 15

Sequence Number (ID): 73

Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
DCGKEGYGFV CEKMQ 15

Sequence Number (ID): 74
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
EALRSCQADN SALID 15

Sequence Number (ID): 75
Length: 20
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..20
> mol_type, protein
> organism, Homo sapiens
Residues:
EALRSCQADN SALIDITSLA 20

Sequence Number (ID): 76
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
EESSLLGDCV FADSN 15

Sequence Number (ID): 77
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
EGLWQLSPCQ EKKGF 15

Sequence Number (ID): 78
Length: 25
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..25
> mol_type, protein
> organism, Homo sapiens
Residues:
EGLWQLSPCQ EKKGFICKME ADIHT 25

Sequence Number (ID): 79
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
EKKGFICKME ADIHT 15

Sequence Number (ID): 80
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens

Residues:
EKTWHEALRS CQADN 15

Sequence Number (ID): 81
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
ESLKKCIQAG KSVLT 15

Sequence Number (ID): 82
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
EVGCDTIWEK DLNSH 15

Sequence Number (ID): 83
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
FADSNGRWHS TACES 15

Sequence Number (ID): 84
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15

> mol_type, protein
> organism, Homo sapiens
Residues:
FLQGAICHVP PETRQ 15

Sequence Number (ID): 85
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
FPNRSQLCVS AEQSE 15

Sequence Number (ID): 86
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GCVAMRGRHP LGRWE 15

Sequence Number (ID): 87
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GDICEYLHKD LHTIK 15

Sequence Number (ID): 88
Length: 13
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..13
> mol_type, protein
> organism, Homo sapiens
Residues:
GIFVIQSESL KKC 13

Sequence Number (ID): 89
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GLNFSAPEQP LSLYE 15

Sequence Number (ID): 90
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GNTHGMPCMF PFQYN 15

Sequence Number (ID): 91
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GRWHSTACES FLQGA 15

Sequence Number (ID): 92

Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GSGCLGLNFS APEQP 15

Sequence Number (ID): 93
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
GYGFVCEKMQ DTSGH 15

Sequence Number (ID): 94
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
HAQHFCAEEG GTLVA 15

Sequence Number (ID): 95
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
HFKAMSLCKQ PVENQ 15

Sequence Number (ID): 96
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
HQWHHECTRE GREDD 15

Sequence Number (ID): 97
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
HVLSDAESGC QEGWE 15

Sequence Number (ID): 98
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
ICHVPPETRQ SEHPE 15

Sequence Number (ID): 99
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens

Residues:
ICKMEADIHT AEALP 15

Sequence Number (ID): 100
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
KKEGSNLLTI KDEAE 15

Sequence Number (ID): 101
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
KLQKEEKTWH EALRS 15

Sequence Number (ID): 102
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
KTLLPLHCGS KREWI 15

Sequence Number (ID): 103
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15

> mol_type, protein
> organism, Homo sapiens
Residues:
KYLNHIDHEI VEKDA 15

Sequence Number (ID): 104
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LEPHIFPNRS QLCVS 15

Sequence Number (ID): 105
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LFNIGGSGCL GLNFS 15

Sequence Number (ID): 106
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LGDCVFADSN GRWHS 15

Sequence Number (ID): 107
Length: 15
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LHTIKGNTHG MPCMF 15

Sequence Number (ID): 108
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LLLGAPRGCA EGVAA 15

Sequence Number (ID): 109
Length: 20
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..20
> mol_type, protein
> organism, Homo sapiens
Residues:
LLLGAPRGCA EGVAAALTPE 20

Sequence Number (ID): 110
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LLWCATTSRY ERDEK 15

Sequence Number (ID): 111

Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LSPCQEKKGF ICKME 15

Sequence Number (ID): 112
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
LSWSEAHSSC QMQGG 15

Sequence Number (ID): 113
Length: 20
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..20
> mol_type, protein
> organism, Homo sapiens
Residues:
LSWSEAHSSC QMQGGTLLSI 20

Sequence Number (ID): 114
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
MPCMFPFQYN HQWHH 15

Sequence Number (ID): 115
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
MSFEAAHEFC KKEGS 15

Sequence Number (ID): 116
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
NFEPFVEDHC GTFSS 15

Sequence Number (ID): 117
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
NGLNFDWSDG TKSSF 15

Sequence Number (ID): 118
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens

Residues:
NRNCYKLQKE EKTWH 15

Sequence Number (ID): 119
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
NTTEVQKHIP LCALL 15

Sequence Number (ID): 120
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
PFDIINIPSH NTTEV 15

Sequence Number (ID): 121
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
PRGCAEGVAA ALTPE 15

Sequence Number (ID): 122
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15

> mol_type, protein
> organism, Homo sapiens
Residues:
PRYSGGCVAM RGRHP 15

Sequence Number (ID): 123
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
PYICKKYLNH IDHEI 15

Sequence Number (ID): 124
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
QKHIPLCALL SSNPN 15

Sequence Number (ID): 125
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
QLCVSAEQSE GHWKV 15

Sequence Number (ID): 126
Length: 15
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
RCNRKMITGP LQYSV 15

Sequence Number (ID): 127
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
SEECSVSMPS ICKRK 15

Sequence Number (ID): 128
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
SLCKQPVENQ EKAEY 15

Sequence Number (ID): 129
Length: 9
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..9
> mol_type, protein
> organism, Homo sapiens
Residues:
SVLTLENCK 9

Sequence Number (ID): 130

Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TCLMHKAQLV SITDQ 15

Sequence Number (ID): 131
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TGLWGSEECS VSMPS 15

Sequence Number (ID): 132
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TKSSFTFWKD EESSL 15

Sequence Number (ID): 133
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TLLSITDETE ENFIR 15

Sequence Number (ID): 134
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TTSRYERDEK WGFCP 15

Sequence Number (ID): 135
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
TVNNQSQRCG FISSI 15

Sequence Number (ID): 136
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
VEDHCGTFSS FMPSA 15

Sequence Number (ID): 137
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens

Residues:
VKHCRHFKAM SLCKQ 15

Sequence Number (ID): 138
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
VSLRWRCNRK MITGP 15

Sequence Number (ID): 139
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
VSNHGLFNIG GSGCL 15

Sequence Number (ID): 140
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
WKNWTHAQHF CAEEG 15

Sequence Number (ID): 141
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15

> mol_type, protein
> organism, Homo sapiens
Residues:
WNTHQPRYSG GCVAM 15

Sequence Number (ID): 142
Length: 31
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..31
> mol_type, protein
> organism, Homo sapiens
Residues:
WQDKGIFVIQ SESLKKCIQA GKSVLTLENC K 31

Sequence Number (ID): 143
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
YAAIKTCLMH KAQLV 15

Sequence Number (ID): 144
Length: 15
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
YFGEDARNCA VYKAN 15

Sequence Number (ID): 145
Length: 15
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..15
> mol_type, protein
> organism, Homo sapiens
Residues:
YLHKDLHTIK GNTHG 15

Sequence Number (ID): 146
Length: 364
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..364
> mol_type, protein
> organism, synthetic construct
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEGGG GSHHHHHHGG 360
GGSC 364

Sequence Number (ID): 147
Length: 359
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..359
> mol_type, protein
> organism, synthetic construct
- SITE, 359
> note, Terminal Cys
- REGION, 353..358
> note, His6
- REGION, 348..352
> note, GGGGS-linker
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60

LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEGGG GSHHHHHHC 359

Sequence Number (ID): 148
Length: 364
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..364
> mol_type, protein
> organism, synthetic construct
- SITE, 363
> note, Next to terminal Cys
- REGION, 359..362
> note, GGGE-linker
- REGION, 353..358
> note, His6
- REGION, 348..352
> note, GGGGS-linker
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEGGG GSHHHHHHGG 360
GECS 364

Sequence Number (ID): 149
Length: 1408
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1408
> mol_type, protein
> organism, synthetic construct
- REGION, 1398..1408

> note, His10
- REGION, 1383..1397
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1377..1382
> note, GGSGGS linker
- REGION, 1..141
> note, CysR
- REGION, 153..201
> note, FnII region
Residues:

EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHQWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEKDA WKYYATHCEP 360
GWNPYNRNCY KLQKEEKTWH EALRSCQADN SALIDITSLA EVEFLVTLLG DENASETWIG 420
LSSNKIPVSF EWSNDSSVIF TNWHTLEPHI FPNRSQLCVS AEQSEGHWKV KNCEERLFYI 480
CKKAGHVLSD AESGCQEGWE RHGGFCYKID TVLRSFDQAS SGYYCPPALV TITNRFEQAF 540
ITSLISSVVK MKDSYFWIAL QDQNDTGEYT WKPVGQKPEP VQYTHWNTHQ PRYSGGCVAM 600
RGRHPLGRWE VKHCRHFKAM SLCKQPVENQ EKAEYEERWP FHPCYLDWES EPGLASCFKV 660
FHSEKVLMKR TWREAEAFCE EFGAHLASFA HIEEENFVNE LLHSKFNWTE ERQFWIGFNK 720
RNPLNAGSWE WSDRTPVVSS FLDNTYFGED ARNCAVYKAN KTLLPLHCGS KREWICKIPR 780
DVKPKIPFWY QYDVPWLFYQ DAEYLFHTFA SEWLNFEFVC SWLHSDLLTI HSAHEQEFIH 840
SKIKALSKYG ASWWIGLQEE RANDEFRWRD GTPVIYQNWD TGRERTVNNQ SQRCGFISSI 900
TGLWGSEECS VSMPSICKRK KVWLIEKKKD TPKQHGTCPK GWLYFNYKCL LLNIPKDPSS 960
WKNWTHAQHF CAEEGGTLVA IESEVEQAFI TMNLFGQTTS VWIGLQNDDY ETWLNGKPVV 1020
YSNWSPFDII NIPSHNTTEV QKHIPLCALL SSNPNFHFTG KWYFEDCGKE GYGFVCEKMQ 1080
DTSGHGVNTS DMYPMPNTLE YGNRTYKIIN ANMTWYAAIK TCLMHKAQLV SITDQYHQSF 1140
LTVVLNRLGY AHWIGLFTTD NGLNFDWSDG TKSSFTFWKD EESSLLGDCV FADSNGRWHS 1200
TACESFLQGA ICHVPPETRQ SEHPELCSET SIPWIKFKSN CYSFSTVLDS MSFEAAHEFC 1260
KKEGSNLLTI KDEAENAFLL EELFAFGSSV QMVWLNAQFD GNNETIKWFD GTPTDQSNWG 1320
IRKPDTDYFK PHHCVALRIP EGLWQLSPCQ EKKGFICKME ADIHTAEALP EKGPSHSGGS 1380
GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1408

Sequence Number (ID): 150
Length: 1408
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..1408
> mol_type, protein
> organism, synthetic construct
- REGION, 1398..1408
> note, His10
- REGION, 1383..1397
> note, Avi tag GLNDIFEAQKIEWHE
- REGION, 1377..1382
> note, GGSGGS linker
- REGION, 1..140
> note, Full length ectodomain
- REGION, 141..146
> note, Thrombin cleavage site engineered to cleave off Cys R domain
(thrombin sequence replacing Y161-L166)
Residues:

EGVAAALTPE	RLLEWQDKGI	FVIQSESLKK	CIQAGKSVLT	LENCKQANKH	MLWKWVSNHG	60
LFNIGGSGCL	GLNFSAPEQP	LSLYECDSTL	VSLRWRCNRK	MITGPLQYSV	QVAHDNTVVA	120
SRKYIHKWIS	YGSGGGDICE	LVPRGSHTIK	GNTHGMPCMF	PFQYNHQWHH	ECTREGREDD	180
LLWCATTSRY	ERDEKWGFCP	DPTSAEVGCD	TIWEKDLNSH	ICYQFNLLSS	LSWSEAHSSC	240
QMQGGTLLSI	TDETEENFIR	EHMSSKTVEV	WMGLNQLDEH	AGWQWSDGTP	LNYLNWSPEV	300
NFEPFVEDHC	GTFSSFMPSA	WRSRDCESTL	PYICKKYLNH	IDHEIVEKDA	WKYYATHCEP	360
GWNPYNRNCY	KLQKEEKTWH	EALRSCQADN	SALIDITSLA	EVEFLVTLLG	DENASETWIG	420
LSSNKIPVSF	EWSNDSSVIF	TNWHTLEPHI	FPNRSQLCVS	AEQSEGHWKV	KNCEERLFYI	480
CKKAGHVLSD	AESGCQEGWE	RHGGFCYKID	TVLRSFDQAS	SGYYCPPALV	TITNRFEQAF	540
ITSLISSVVK	MKDSYFWIAL	QDQNDTGEYT	WKPVGQKPEP	VQYTHWNTHQ	PRYSGGCVAM	600
RGRHPLGRWE	VKHCRHFKAM	SLCKQPVENQ	EKAEYEERWP	FHPCYLDWES	EPGLASCFKV	660
FHSEKVLMKR	TWREAEAFCE	EFGAHLASFA	HIEEENFVNE	LLHSKFNWTE	ERQFWIGFNK	720
RNPLNAGSWE	WSDRTPVVSS	FLDNTYFGED	ARNCAVYKAN	KTLLPLHCGS	KREWICKIPR	780
DVKPKIPFWY	QYDVPWLFYQ	DAEYLFHTFA	SEWLNFEFVC	SWLHSDLLTI	HSAHEQEFIH	840
SKIKALSKYG	ASWWIGLQEE	RANDEFRWRD	GTPVIYQNWD	TGRERTVNNQ	SQRCGFISSI	900
TGLWGSEECS	VSMPSICKRK	KVWLIEKKKD	TPKQHGTCPK	GWLYFNYKCL	LLNIPKDPSS	960
WKNWTHAQHF	CAEEGGTLVA	IESEVEQAFI	TMNLFGQTTS	VWIGLQNDDY	ETWLNGKPVV	1020
YSNWSPFDII	NIPSHNTTEV	QKHIPLCALL	SSNPNFHFTG	KWYFEDCGKE	GYGFVCEKMQ	1080
DTSGHGVNTS	DMYPMPNTLE	YGNRTYKIIN	ANMTWYAAIK	TCLMHKAQLV	SITDQYHQSF	1140
LTVVLNRLGY	AHWIGLFTTD	NGLNFDWSDG	TKSSFTFWKD	EESSLLGDCV	FADSNGRWHS	1200
TACESFLQGA	ICHVPPETRQ	SEHPELCSET	SIPWIKFKSN	CYSFSTVLDS	MSFEAAHEFC	1260
KKEGSNLLTI	KDEAENAFLL	EELFAFGSSV	QMVWLNAQFD	GNNETIKWFD	GTPTDQSNWG	1320

IRKPDTDYFK PHHCVALRIP EGLWQLSPCQ EKKGFICKME ADIHTAEALP EKGPSHSGGS 1380
GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1408

Sequence Number (ID): 151
Length: 1408
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1408
> mol_type, protein
> organism, synthetic construct
Residues:
EGVAAALTPE RLLEWQDKGI FVIQSESLKK CIQAGKSVLT LENCKQANKH MLWKWVSNHG 60
LFNIGGSGCL GLNFSAPEQP LSLYECDSTL VSLRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFLVPRGSHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDLNSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNQLDEH AGWQWSDGTP LNYLNWSPEV 300
NFEPFVEDHC GTFSSFMPSA WRSRDCESTL PYICKKYLNH IDHEIVEKDA WKYYATHCEP 360
GWNPYNRNCY KLQKEEKTWH EALRSCQADN SALIDITSLA EVEFLVTLLG DENASETWIG 420
LSSNKIPVSF EWSNDSSVIF TNWHTLEPHI FPNRSQLCVS AEQSEGHWKV KNCEERLFYI 480
CKKAGHVLSD AESGCQEGWE RHGGFCYKID TVLRSFDQAS SGYYCPPALV TITNRFEQAF 540
ITSLISSVVK MKDSYFWIAL QDQNDTGEYT WKPVGQKPEP VQYTHWNTHQ PRYSGGCVAM 600
RGRHPLGRWE VKHCRHFKAM SLCKQPVENQ EKAEYEERWP FHPCYLDWES EPGLASCFKV 660
FHSEKVLMKR TWREAEAFCE EFGAHLASFA HIEEENFVNE LLHSKFNWTE ERQFWIGFNK 720
RNPLNAGSWE WSDRTPVVSS FLDNTYFGED ARNCAVYKAN KTLLPLHCGS KREWICKIPR 780
DVKPKIPFWY QYDVPWLFYQ DAEYLFHTFA SEWLNFEFVC SWLHSDLLTI HSAHEQEFIH 840
SKIKALSKYG ASWWIGLQEE RANDEFRWRD GTPVIYQNWD TGRERTVNNQ SQRCGFISSI 900
TGLWGSEECS VSMPSICKRK KVWLIEKKKD TPKQHGTCPK GWLYFNYKCL LLNIPKDPSS 960
WKNWTHAQHF CAEEGGTLVA IESEVEQAFI TMNLFGQTTS VWIGLQNDDY ETWLNGKPVV 1020
YSNWSPFDII NIPSHNTTEV QKHIPLCALL SSNPNFHFTG KWYFEDCGKE GYGFVCEKMQ 1080
DTSGHGVNTS DMYPMPNTLE YGNRTYKIIN ANMTWYAAIK TCLMHKAQLV SITDQYHQSF 1140
LTVVLNRLGY AHWIGLFTTD NGLNFDWSDG TKSSFTFWKD EESSLLGDCV FADSNGRWHS 1200
TACESFLQGA ICHVPPETRQ SEHPELCSET SIPWIKFKSN CYSFSTVLDS MSFEAAHEFC 1260
KKEGSNLLTI KDEAENAFLL EELFAFGSSV QMVWLNAQFD GNNETIKWFD GTPTDQSNWG 1320
IRKPDTDYFK PHHCVALRIP EGLWQLSPCQ EKKGFICKME ADIHTAEALP EKGPSHSGGS 1380
GGSGLNDIFE AQKIEWHEHH HHHHHHHH 1408

Sequence Number (ID): 152
Length: 362

Molecule Type: AA
Features Location/Qualifiers:
- source, 1..362
> mol_type, protein
> organism, synthetic construct
- MOD_RES, 102..123
> note, ITGPLQYSVQVAHDNTVVASRK (S10+GalNae)(T18+GalNAc.6GGn.3G)(S22+GalNAc-6GGn-3G)(5+)
- MOD_RES, 339..362
> note, NH1DHE1VEGGGGSLPETGGGGG (C26-sortase_2GnlNnc)(4+)
- MOD_RES, 196..205
> note, WGFCPDPTSAEVGCDTIWEK (T8+GalNAc-6S-3SG)(3+)
Residues:

EGVAAALTPE RLLEWQDKGI FVIQSESIKK CIQAGKSVLT LENCKQANKH MIWKWVSNHG 60
LENIGGSGCL GLNFSAPEQP LSLYECDSTL VSIRWRCNRK MITGPLQYSV QVAHDNTVVA 120
SRKYIHKWIS YGSGGGDICE YLHKDLHTIK GNTHGMPCMF PFQYNHOWHH ECTREGREDD 180
LLWCATTSRY ERDEKWGFCP DPTSAEVGCD TIWEKDINSH ICYQFNLLSS LSWSEAHSSC 240
QMQGGTLLSI TDETEENFIR EHMSSKTVEV WMGLNOLDEH AGWOWSDGIP LNYLNWSPEV 300
NFEPFVEDHC GIFSSEMPSA WRSRDCESTL PYICKKYLNH IDHETIVEGG GGSLPETGGG 360
GG 362

CLAIMS

1. A composition of matter comprising:

an anti-PLA2R antibody-binding moiety, and

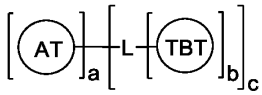
a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on surface degrading cells,

wherein the anti-PLA2R antibody-binding moiety and the cellular receptor-binding moiety are covalently connected,

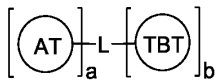
optionally through a linker moiety connecting the anti-PLA2R antibody-binding moiety and the cellular receptor-binding moiety.

2. The composition of matter of Claim 1, having a structure of:

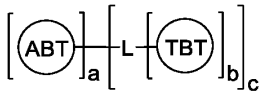
R^{CN}–(Xaa)y–R^{CC},



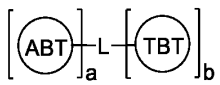
[AGN101],



[AGN102],



[AGN103],



[AGN104]

or a salt thereof.

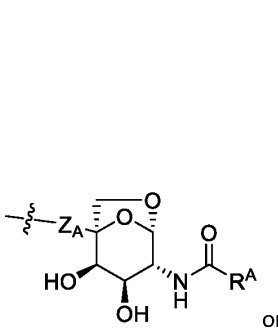
3. The composition of matter of claim 1, wherein the composition of matter is selected from the group consisting of **AGN302**, **AGN303**, **AGN304**, **AGN306**, **AGN307**, **AGN308**, **AGN309**, **AGN310**, **AGN362**, **AGN363**, **AGN364**, **AGN365**, **AGN579**, **AGN580,** and **AGN167**.

4. The composition of matter of claim 1, wherein the anti-PLA2R antibody-binding moiety is selected from the group of compounds listed in TABLE 72.

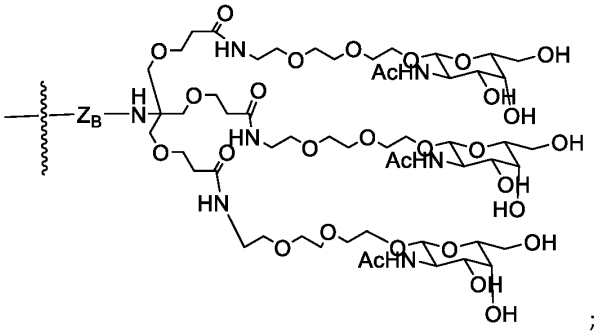
5 The composition of matter of claim 1, wherein the anti-PLA2R antibody-binding moiety is a peptide selected from the groups of secretory phospholipase A2 receptor peptides in SEQ ID NO: 60-145.

6 The composition of matter of claim 1, wherein the anti-PLA2R-autoantibody-binding moiety is selected from the group consisting of **ABT301, ABT305, ABT309, ABT310, ABT311, ABT312, ABT407, ABT408, ABT409, ABT427, ABT530, ABT603, ABT606, ABT816, ABT817, ABT818, ABT819, ABT976, ABT983,** and **ABT985.**

7. The composition of matter of claim 1, wherein the cellular receptor-binding moiety has the structure:



or



;

where R^A is a C_1-C_3 alkyl group optionally substituted with 1-5 halo, preferably fluoro, groups, preferably R^A is a methyl or ethyl group optionally substituted with from 1-3 fluoro groups;

Z_A is $-(CH_2)_{IM}$, $-O-(CH_2)_{IM}$, $S-(CH_2)_{IM}$, $NR_M-(CH_2)_{IM}$, $C(O)-(CH_2)_{IM}-$, a PEG group containing from 1 to 8 preferably 1-4 ethylene glycol residues or a $-C(O)(CH_2)_{IM}NR_M$ group (preferably a PEG containing group comprising from 1 to 8 ethylene glycol, preferably 2-4 ethylene glycol residues); and

Z_B is absent, $(CH_2)_{IM}$, $C(O)-(CH_2)_{IM}-$ or $C(O)-(CH_2)_{IM}-NR_M$.

8. The composition of matter of claim 1, wherein the cellular receptor-binding moiety is selected from the group consisting of **TBT103, TBT104, TBT105, TBT307, TBT506,** and **TBT544**.

9. The composition of matter of claim 1, for use as a medicament.

10. The composition of matter of claim 1, for use in removing anti-PLA2R-autoantibody from a subject.

11. The composition of matter of claim 1, for use in treating a disease state or condition associated with the upregulation of anti-PLA2R-autoantibody in a subject or patient.

12. The composition of matter of claim 1, for use in treating membranous nephropathy.

13. A method of making the composition of matter of claims 1-8, wherein the method comprises a conjugation step to link an anti-PLA2R-autoantibody-binding moiety to a cellular receptor-binding moiety through a linker moiety, wherein the conjugation step is selected from the group consisting of a MATE conjugation step, a maleimide conjugation step, and a sortase conjugation step.

14. A pharmaceutical composition comprising the composition of matter of claims 1-8 and a pharmaceutically acceptable excipient.

15. A method of removing anti-PLA2R-autoantibody from a subject or patient comprising administering to the subject or patient the composition of matter of any of claims 1-8.

16. A method of treating a disease state or condition associated with the upregulation of anti-PLA2R-autoantibody in a subject or patient comprising administering to the subject or patient an effective amount of the composition of matter of any of claims 1-8.

17. A method of treating membranous nephropathy in a subject or patient comprising administering to the subject or patient an effective amount of the composition of matter of any of claims 1-8.

18. A composition comprising:

a first composition of matter comprising:

an anti-PLA2R-autoantibody-binding moiety,

a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors which are on surface degrading cells, and

a linker moiety connecting the anti-PLA2R-autoantibody-binding moiety and the cellular receptor-binding moiety, and

at least one additional composition of matter comprising a moiety comprising:

a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors which are on surface degrading cells, and

a linker moiety connecting the anti-PLA2R-autoantibody-binding moiety and the cellular receptor-binding moiety.

19. The composition of claim 18, wherein the additional agent has a structure selected from the group consisting of formula LG−RG−L^{RM}(−TBT)$_b$,, LG−RG−H, and a combination thereof.