

BIFUNCTIONAL DEGRADERS OF IgG4 IMMUNOGLOBULINS

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, and particularly to bifunctional molecules which have a circulating protein-binding moiety linked through a linker group to a cellular receptor-binding moiety for the treatment of an IgG4-related disease.

BACKGROUND OF THE INVENTION

[002] IgG4-related disease (IgG4-RD), formerly known as IgG4-related systemic disease, is a chronic inflammatory condition characterized by tissue infiltration with lymphocytes and IgG4-secreting plasma cells. In most people with this disease, serum IgG4 concentrations are elevated during an acute phase. Kamisawa et al., *The Lancet*, 385 (9976), 1460–1471 (April 11, 2015).

[003] An international panel of experts developed recommendations for the management of IgG4-related disease. Khosroshahi et al., *Arthritis & Rheumatology*, 67(7), 1688–1699 (July 2015); Walsh, *MedPage Today* (April 1, 2015). The panel concluded that treatment is required for all cases of symptomatic, active IgG4-related disease. Treatment is advised for some organ manifestations, such as aortitis, retroperitoneal fibrosis, proximal biliary strictures, tubulointerstitial nephritis, pachymeningitis, pancreatic enlargement and pericarditis. Some cases with asymptomatic IgG4-related disease also require treatment because some organs tend to not cause symptoms until the late stages of disease.

[004] The recommended first-line agent for induction of remission is glucocorticoids unless contraindications exist, but this treatment does not address the disease cause. The goal of glucocorticoid treatment is the induction and maintenance of remission to prevent progression of fibrosis and organ destruction in affected organs. Recurrences during or after tapering of glucocorticoids are frequent. Thus, there remains a need in the biomedical art for new medicines capable of treating or slowing down progression of IgG4-related disease.

SUMMARY OF THE INVENTION

[005] The invention is directed to bifunctional compositions of matter (agents) capable of binding, removing from blood circulation, and degrading IgG4 immunoglobulins.

[006] In one embodiment, the invention provides a composition of matter (an agent) comprising:
an IgG4-antibody-binding moiety,

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

a linker moiety connecting the IgG4-binding moiety and the cellular receptor-binding moiety, wherein the linker moiety can be a peptide bond or a larger linker moiety.

[007] In some embodiments, the composition of matter (agent) has the structure:



wherein [CPBM] is a IgG4-antibody-binding moiety;

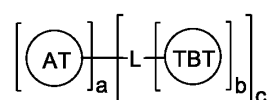
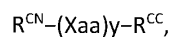
[CRBM] is a cellular receptor-binding moiety;

each [CON] is an optional connector chemical moiety which, when present, connects directly to [CPBM] or to [CRBM] or connects the [LINKER] to [CPBM] or to [CRBM], and

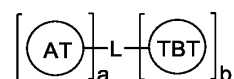
[LINKER] is a chemical moiety that covalently attaches to one or more [CRBM] or [CPBM] groups, or

a pharmaceutically acceptable salt, stereoisomer, solvate, or polymorph thereof.

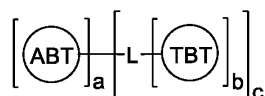
[008] In some embodiments, the composition of matter (agent) has the structure of:



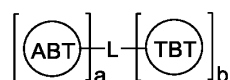
[AGN101],



[AGN102],



[AGN103],



[AGN104], or a salt thereof.

[009] In some specific and nonlimiting embodiments, the composition of matter is selected from the group in TABLE 1.

TABLE 1			
ID NO.	IgG4-binding moiety	Cellular receptor binding moiety	Linkage
AGN401	VHH w/o hinge region; ABT306	β GN3, TBT103	thiosuccinimide
AGN402	VHH-Fc fusion, ABT310	β GN3 via MATE, TBT105	MATE conjugation
AGN403	VHH with hinge region	β GN3, TBT106	sortase
AGN404	VHH w/o hinge region	β GN3, TBT106	sortase
AGN501	VHH-G ₄ S-Cys, ABT502	β GN3, TBT103	thiosuccinimide
AGN502	VHH-G ₃ ECS-Cys, ABT508	β GN3, TBT103	thiosuccinimide
AGN508	VHH-G ₄ S-his6-G ₄ S-Cys ABT501	β GN3, TBT103	thiosuccinimide
AGN509	ABT504	β GN3, TBT103	thiosuccinimide

[0010] **AGN402** has a lysine-based conjugation. In the IgG4-binding moiety, two same single chains are covalently linked by disulfide bonds (**ABT310**, Fc-fusion). To make the composition of matter, **TBT105** is conjugated to lysine amines in the nanobody fusion. During the synthesis of **AGN402**, the leaving group is eliminated when the payload is conjugated to amines.

[0011] **AGN508** has a cysteine-based conjugation. The IgG4-binding moiety has one chain (**ABT501**, nanobody). To make the composition of matter, the maleimide-containing **TBT103** is conjugated to C-terminal cysteine of **ABT501** via a Michael-addition reaction.

[0012] **AGN501** has a cysteine-based conjugation.. The IgG4-binding moiety has one chain (**ABT502**, nanobody). To make the composition of matter, the maleimide-containing **TBT103** is conjugated to C-terminal cysteine of **ABT502** via a Michael-addition reaction.

[0013] **AGN502** has a cysteine-based conjugation. The IgG4-binding moiety has one chain (**ABT503**, nanobody). To make the composition of matter, the maleimide-containing **TBT103** is conjugated to C-terminal cysteine of **ABT503** via a Michael-addition reaction.

[0014] **AGN509** has a cysteine-based conjugation. The IgG4-binding moiety has one chain (**ABT504**, nanobody). To make the composition of matter, the maleimide-containing **TBT103** is conjugated to C-terminal cysteine of **ABT504** via a Michael-addition reaction.

[0015] **AGN401** has a cysteine-based conjugation. The IgG4-binding moiety has one chain (**ABT306**, nanobody). To make the composition of matter, the maleimide-containing **TBT103** is conjugated to C-terminal cysteine of **ABT306** via a Michael-addition reaction.

[0016] **AGN403** has a sortase-ligation based conjugation. The IgG4-binding moiety has one chain (**ABT304**). To make the composition of matter, the pentaglycine-modified **TBT106** is conjugated to a C-terminal pentapeptide motif LPETG of **ABT304**, via a sortase-catalyzed ligation.

[0017] **AGN404** has a sortase-ligation based conjugation. The IgG4-binding moiety has one chain (**ABT305**). To make the composition of matter, the pentaglycine-modified **TBT106** is conjugated to C-terminal pentapeptide motif LPETG of **ABT305**, via a sortase-catalyzed ligation.

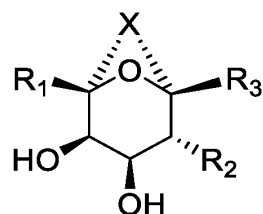
[0018] In another embodiment, the IgG4-antibody-binding moiety is an antibody and antibody-derived binding protein. In a particular and nonlimiting embodiment, the complementary determining regions of the IgG4-antibody-binding moiety include the regions according to the Chothia numbering scheme having the structures of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

[0019] In another embodiment, the IgG4-antibody-binding moiety is a VHH nanobody.

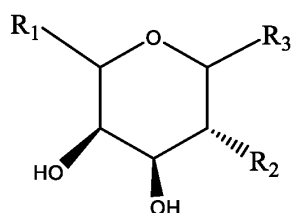
[0020] In some specific and nonlimiting embodiments, the IgG4 binding moiety is selected from the group in TABLE 2.

TABLE 2		
ID NO.	SEQ ID NO.	Protein structure
ABT301	SEQ ID NO: 1	VHH nanobody (122 AAs)
ABT304	SEQ ID NO: 9	VHH nanobody (122 AAs), G4S linker, C-term Sortase site, His6 tag
ABT305	SEQ ID NO: 10	VHH nanobody with truncation of hinge, G4S linker, C-term Sortase site, His6 tag
ABT306	SEQ ID NO: 11	VHH nanobody with truncation of hinge, G4S linker, His6 tag, rigid linker-Cys
ABT310	SEQ ID NO: 12	VHH nanobody with truncation of hinge, G4S linker, hlgG1 hinge (C220A)-human IgG1 Fc-L253A/L254A/P329A
ABT311	SEQ ID NO: 32	VHH nanobody (122 AAs), G4S linker, His6 tag
ABT312	SEQ ID NO: 33	VHH nanobody with truncation of hinge, G4S linker, His6 tag
ABT313	SEQ ID NO: 34	VHH nanobody with truncation of hinge, G4S linker, His6 tag, G2S linker, AviTag
ABT501	SEQ ID NO: 59	VHH nanobody-G4S-his6-G4S-Cys
ABT502	SEQ ID NO: 64	VHH nanobody-G4S-Cys
ABT503	SEQ ID NO: 63	VHH nanobody-G3ECS-Cys
ABT504	SEQ ID NO: 65	VHH nanobody-G4S-his6-Cys

[0021] In another embodiment, the cellular receptor-binding moiety comprises an ASGPR-binding group according to the chemical structure:



[TBT101], or

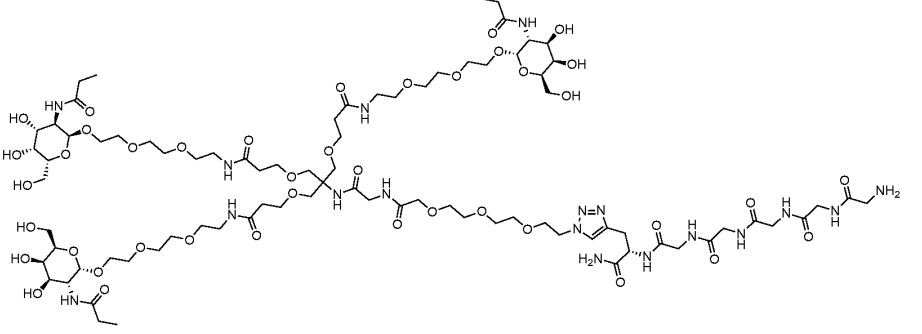
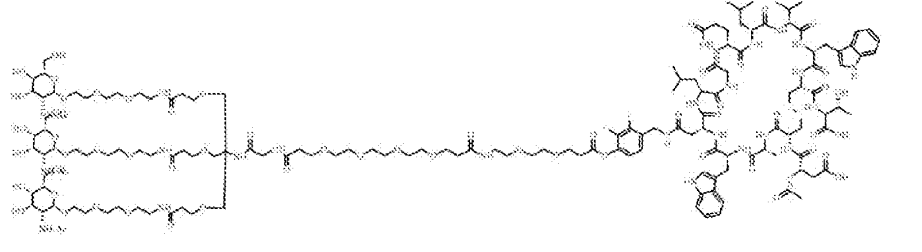
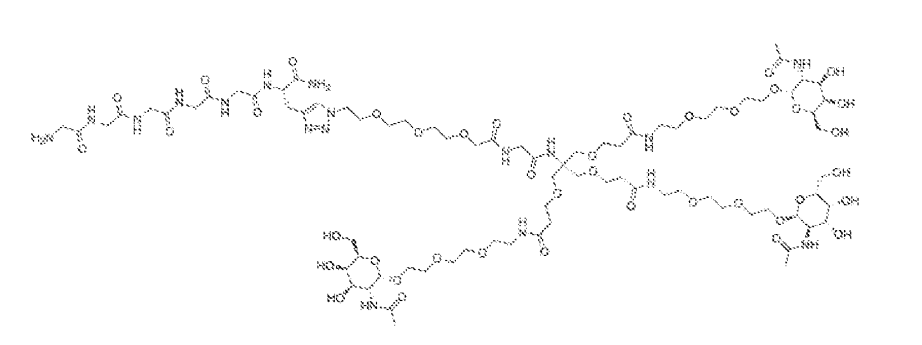


[TBT102],

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

[0022] 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	

TABLE 3	
ID NO.	Chemical Structure
TBT104	
TBT105	
TBT106	

[0023] In another embodiment, the invention provides the composition of matter (agent) for use as a medicine.

[0024] In another embodiment, the invention provides the composition of matter (agent) for use in treating or preventing a disease state or condition associated with the upregulation of IgG4 in a patient or subject.

[0025] In one aspect, the administered composition of matter binds to an IgG4 antibody, especially although not exclusively an IgG4 autoantibody, in the circulation of a subject or patient to form an agent-antibody complex. After this agent-antibody complex is endocytosed by a cell, especially although

not exclusively by a hepatocyte, it is 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. Endocytosed proteins are trafficked to endosomes, which then fuse with lysosomes. Lysosomal proteases then degrade endocytosed proteins, permanently removing them from circulation.

[0026] In another embodiment, the invention provides a pharmaceutical composition including the composition of matter and at least one pharmaceutically acceptable excipient.

[0027] In another embodiment, the invention provides a method of making the composition of matter wherein the method comprises a conjugation step, wherein the conjugation step results in an IgG4-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.

[0028] In another embodiment, the invention provides a method of making a molecule wherein the IgG4-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.

[0029] In another embodiment, the invention provides a method of removing IgG4 in a patient or subject by administering an effective amount of the agent to the patient or subject.

[0030] In another embodiment, the invention provides a method of treating or preventing a disease state or condition associated with the upregulation of IgG4 in a patient or subject by administering to the patient or subject a therapeutically effective amount of the agent. The disease may be selected from the Markush group consisting of pemphigus vulgaris, pemphigus foliaceus, anti-LGI1 encephalitis, and MuSK myasthenia gravis.

[0031] In another embodiment, the invention provides a composition including the agent as a first compound and a second compound (an at least one additional agent) comprising a moiety capable of conjugating to the IgG4-binding moiety of the first compound to form the first compound.

[0032] 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 accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] 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.

[0034] FIG. 1 shows the structure of one embodiment of the bifunctional composition of matter (agent).

[0035] FIG. 2 is a comparison of sequences of the constant heavy chains of human IgG1 (SEQ ID NO: 2), IgG2 (SEQ ID NO: 3), and IgG4 (SEQ ID NO: 4). Sequence differences in IgG2 and IgG4 from IgG1 are noted. The hinge and lower hinge regions, with the N-glycosylation site, N297, are noted. Numbering is according to EU numbering scheme. Sequences important for FcγR-binding, C1q, and FcRn are noted by shading. Some increased ADCC mutants are shown above or below the sequences, as noted: Xencor's S239D, A330L, I332E; Genentech's S298A, E333A, K334A; and Eli Lilly/AME's P247I, A339D/Q. Examples of FcRn-binding mutants for prolongation of half-life are also shown, as noted: MedImmune's YTE mutant (M252Y, S254T, T256E), PDL's T250Q, M428L mutant, Sally Ward's H433K mutant, N434Y mutant, and Genentech's N434A mutant are shown in boxes.

[0036] FIG. 3 is a table of R-groups (TABLE 4) for MATE reagents and bifunctional MoDE final compounds.

[0037] FIG 4 is a set of schematic drawings for four of the composition of matter embodiments, specifically **AGT401**, **AGT402**, **AGT403**, and **AGT404**.

[0038] FIG. 5 is set of chemical structures for some of the composition of matter embodiments, specifically **AGT402** in FIG. 5A, **AGT508** and **AGT501** in FIG. 5B, **AGT502**, **AGT509**, and **AGT401** in FIG. 5C, and **AGT403** and **AGT404** in FIG. 5D.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The following detailed description is provided to aid persons having ordinary skill in the biomedical art. Exemplary embodiments are described in detail. However, these embodiments are only exemplary. This disclosure is not limited thereto but rather 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

[0040] The invention provides a medically useful composition of matter (agent) for treating or slowing down the progression of diseases that are manifestations of IgG4-related diseases (IgG4-RD). IgG4-mediated autoimmune diseases (IgG4-AIDs) establish IgG4 as pharmacological target for degradation their diagnosis, based on clinical symptoms and serum antigen-specific IgG4 autoantibodies. These diseases include pemphigus vulgaris, pemphigus foliaceus, LG1 encephalitis, MuSK (IgG4 myasthenia gravis), type 1 autoimmune pancreatitis, interstitial nephritis, Riedel's thyroiditis, Mikulicz's disease, Küttner's tumor, inflammatory pseudotumors in several sites of the body, mediastinal fibrosis, and some cases of retroperitoneal fibrosis. Khosroshahi & Stone Current Opinion in Rheumatology. 23 (1): 57–66 ((January 2011); Stone, Zen, & Deshpande, The New England Journal of Medicine. 366(6), 539–51 (February 2012); Konecny et al., Frontiers in Immunology. 11, 605214 (January 29, 2021). Alternative IgG4 Indications include membranous nephropathy, thrombotic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, and IgG4 related disease.

[0041] Whatever area of the body is involved, the hallmark histopathological features of IgG4-related disease are (1) a dense infiltrate of lymphocytes and IgG4-positive plasma cells; (2) fibrosis arranged in a storiform pattern, and phlebitis, where the venous channels are obliterated by the dense infiltrate within both the venous walls and the lumen. See Deshpande et al., Modern Pathology. 25(9), 1181–1192 (May 18, 2012).

TABLE 5
IgG4 indication overview.

<u>Criteria</u>	<u>Pemphigus vulgaris</u>	<u>Pemphigus foliaceus</u>	<u>Anti-LGI1 encephalitis</u>	<u>MuSK myasthenia gravis</u>
Patient Population	about 30K patients. Incidence: 1-10/ 1 million	1-9/100,000. Incidence: 5/1 million	about 2,300 (USA). Incidence: 0.8/1 million	Affecting 5–8% of all MG patients. about 4,800 patients in USA

TABLE 5

IgG4 indication overview.

Criteria	<u>Pemphigus vulgaris</u>	<u>Pemphigus foliaceus</u>	<u>Anti-LGI1 encephalitis</u>	<u>MuSK myasthenia gravis</u>
Disease Severity	Acute and chronic; Autoimmune blistering disorder affecting mucous membrane and the skin	Skin blistering primarily in more superficial layers of epidermis (less severe than pemphigus vulgaris)	Acute and chronic disease: Seizures + cognitive impairment	Acute and chronic; usually has an acute onset affecting mainly the facial-bulbar muscles. Rapid progression of symptoms
Unmet Need	High. Current standard of care rituximab has a long onset of act. Prolonged use of steroids has a multitude of severe side effects	Moderate	High. Current standard of care relies on steroid use.	Moderately high. A severe form of the disease has bulbar impairment usually associated with rapid deterioration, frequently leading to respiratory crisis.
Pathophysiology	Antigenic Target: Desmoglein 3, blockade of desmoglein resulting in loss of cell-cell interactions leading to skin blistering.	Antigenic Target: Desmoglein 1. blockade of desmoglein resulting in loss of cell-cell interactions leading to skin blistering.	Antigenic Target: LGI1. LGI1-IgG (a component of the trans-synaptic complex) associated with limbic encephalitis, seizures and cognitive impairment.	Antigenic Target: MuSK. Characterized by antibodies targeting the muscle-specific kinase (MusK) resulting in various neurologic symptoms.
Scientific Rationale	Proven Pathogenicity of IgG4	Proven Pathogenicity of IgG4. Passive transfer of IgG4 antibodies could reproduce the disease in animals	Proven Pathogenicity of LGI1 autoantibodies	Proven Pathogenicity of IgG4. Antibodies disrupt normal signaling in NMJ in murine models

[0042] Pemphigus vulgaris is an autoimmune blistering disorder affecting mucous membrane and the skin where positive functional outcomes can be followed by a visual observation of the improved

skin condition follows the depletion of IgG4 antibodies. There is a relatively large patient population, a chronic patient population. Keratinocytes of the skin and mucous membranes are targeted by IgG4 autoantibodies against Dsg1 or Dsg3. These IgG4 autoantibodies have a direct blocking function and lead to a loss of cellular adhesion. Biomedical data show that the decreases in autoantibody titer are associated with clinical response.

TABLE 6 Target product profile for pemphigus.			
	<u>Acceptable case</u>	<u>Better acceptable case</u>	<u>Aspirational case</u>
Mechanism of action	ASGPR-mediated lysosomal degradation of circulating IgG4 antibodies including those that induce pathogenicity in IgG4-autoimmune diseases (IgG4-AID), Pemphigus vulgaris or foliaceus.		
Technology approach	<u>Molecular degraders of extracellular proteins</u> : Bispecific molecule composed of ASGPR targeting molecule linked to either an anti-IgG4 VHH or IgG4-binding peptide.		
Indication/patient population	<u>Indicated for chronic treatment of IgG4 autoimmune diseases</u> . The initial indication is patients eighteen years and older with confirmed diagnosis of pemphigus vulgaris or foliaceus with anti-Dsg1 or Dsg3 IgG4 antibodies. Broader indications are patients eighteen years and older with IgG4 AID (MuSK-MG, CIDP, anti-LGI1 encephalitis)		
Clinical efficacy profile	<u>Endpoints</u> : Low number of adverse events and no serious adverse events. Reduction in skin lesions. Reduction in pemphigus disease area index (PDAI). Autoimmune blister quality of life (ABQOL score). Time to complete clinical remission (CR).		
Biomarker measurements	Serum levels of total IgG4 Abs and anti-Dsg1/3 IgG4 Abs reduced by >50%. Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.	Serum levels of total IgG4 Abs and anti-Dsg1/3 IgG4 Abs reduced by >70%. Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.	Serum levels of total IgG4 Abs and anti-Dsg1/3 IgG4 Abs reduced by 85-95%. Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.
Safety & tolerability profile	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity. Discontinuations due to AEs <15%.	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity. Discontinuations due to AEs <10%.	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity. Discontinuations due to AEs <5%.

TABLE 6 Target product profile for pemphigus.			
	<u>Acceptable case</u>	<u>Better acceptable case</u>	<u>Aspirational case</u>
Route of administration/ dosing regimen	Intravenous injection, biweekly.	Subcutaneous or IV injection, biweekly.	Subcutaneous or IV injection, monthly, top-up therapy as indicated.

[0043] Muscle-specific kinase (MuSK)-positive myasthenia gravis a distinct and often more severe form of myasthenia gravis often characterized by bulbar features, and which has a neurological indication. There is a correlation between autoantibody titer and clinical outcomes. IgG4 antibodies but not IgG1-3 from inhibit agrin-dependent binding between MuSK and Lrp4. IgG4 antibodies from MuSK MG patients cause disease without requiring complement. IgG4 autoantibodies to MuSK block its interaction with LRP4, thereby obstructing a key trophic signaling cascade at the neuromuscular junction and resulting in inhibited clustering of acetylcholine receptors leading to muscle weakness.

[0044] MuSK myasthenia gravis (MuSK MG) is an indication for the therapeutic targeting of IgG4 autoantibodies, with the objective of achieving clinical remission in patients. The pathogenic mechanism in MuSK myasthenia gravis involves IgG4 autoantibodies with differing rates of re-synthesis compared to antigenic IgG4. This slower recovery of the pathogenic process underscores the need for sustained and targeted therapeutic interventions.

TABLE 7 Target product profile for MuSK-myasthenia gravis.			
	<u>Acceptable case</u>	<u>Better acceptable case</u>	<u>Aspirational case</u>
Mechanism of action	ASGPR-mediated lysosomal degradation of circulating IgG4 antibodies, including those that induce pathogenicity in Muscle-Specific Kinase Myasthenia Gravis (MuSK-MG).		
Technology approach	<u>Molecular degraders of extracellular proteins</u> : Bispecific molecule composed of ASGPR targeting molecule linked to an anti-IgG4 VHH nanobody.		
Indication/ patient population	<u>Indicated for chronic treatment of IgG4 autoimmune diseases</u> . Initial indication is with patients eighteen years and older with confirmed diagnosis of refractory MuSK-MG. Positive IgG4 anti-MuSK antibody test at screening. Broader indications are patients eighteen years and older with IgG4 AID (pemphigus vulgaris, CIDP, anti-LGI1 encephalitis)		
Clinical efficacy profile	<u>Endpoints</u> . Reduction in clinical symptoms using Qualitative MG (QMG), and MG Activities of Daily Living (MG-ADL), MG Composite (MGC) and MG Quality of Life (MG-QoL-15r).		

TABLE 7 Target product profile for MuSK-myasthenia gravis.			
	<u>Acceptable case</u>	<u>Better acceptable case</u>	<u>Aspirational case</u>
Key biomarker measures	Serum levels of total IgG4 Abs and anti-MuSK IgG4 Abs reduced by >50%. Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.	Serum levels total IgG4 Abs and anti-MuSK IgG4 Abs reduced by >70% Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.	Serum total IgG4 Abs and anti-MuSK IgG4 Abs reduced by 85-95% Serum levels of IgG subtypes 1, 2 and 3 remain unchanged.
Safety & tolerability profile	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity Discontinuations due to adverse events <15%.	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity Discontinuations due to adverse events <10%.	Well tolerated with favorable safety profile. No drug hypersensitivity or immunogenicity Discontinuations due to adverse events <5%.
Route of administration/ dosing regimen	Intravenous injection, biweekly.	Subcutaneous or IV injection, biweekly.	Subcutaneous or IV injection, monthly – top-up therapy as indicated.

[0045] Anti-LGI-1 encephalitis is a rare acute IgG4-related disease with a fast readout, including seizures and cognitive impairment. This disease is treated with rituximab, but this medicine takes a relatively long time to act, so there is a need for an earlier treatment.

[0046] Patients with anti-leucine-rich glioma inactivated 1 (LGI1) encephalitis develop memory disturbances, confusion, and faciobrachial dystonic seizures. LGI1 autoantibodies block the binding of LGI1 to ADAM22, reducing AMPA receptor (AMPA) levels postsynaptically, resulting in neuronal hyperexcitability. Ariño et al., *Neurology*, 87(8), 759-65 (August 23, 2016) showed that of fifty-seven LGI1 patients with sera available for antibody subclass analysis, all showed IgG4 Abs.

TABLE 8 Target product profile for diseases.				
<u>Criteria</u>	<u>CIDP</u>	<u>IgG4 Related Disease</u>	<u>TTP</u>	<u>Membranous Nephropathy</u>
Patient population	Prevalence 1 to 9/100,000, about 16,000 (USA). Incidence: 0.33/100,000	Prevalence about 60 / 1 million; about 19,000 (USA). Incidence 0.3-1.1 per 100,000	Prevalence: 10/1 million; about 3,000 (USA). Incidence: 1 case/million	about 23,000 Cases USA. Incidence: about 10/1 million Globally

TABLE 8 Target product profile for diseases.				
Criteria	CIDP	IgG4 Related Disease	TTP	Membranous Nephropathy
Disease severity	Mild to severe; characterized by muscle weakness, fatigue, motor function loss, sensory changes	Chronic, relapsing remitting disease often with systemic multiorgan involvement; life expectancy after diagnosis is about 20 years	Often considered acute; some patients require chronic treatment Microangiopathic hemolytic anemia; risk of neurocognitive deficits and cardiovascular events	Chronic disease: Can cause significant proteinuria and nephrotic syndrome. Can progress to kidney failure in severe cases.
Unmet need	Moderate	High	Mild-moderate	High
Patho-physiology	Antigenic Target: CNTN1, NF155. Blockade of NF155–CNTN1 interaction, resulting in loss of connectivity between cells and impaired neuroconduction.	Systemic fibrosing characterized by dense infiltration of IgG4-positive plasma cells in tissue with or without elevation in plasma	Antigenic Target: ADAMTS13. Blockade of binding and cleavage of vWF, resulting in multimers that cause microthrombi.	Antigenic Target: PLA2R, THSD7A. Antibodies against the phospholipase A2 receptor (PLA2R) on podocytes.
Scientific rationale	Proven Pathogenicity of IgG4 in passive transfer models. The mechanism still not fully understood. IgG3 also likely contributory	IgG4 autoantibodies may not be directly pathogenic	Proven Pathogenicity of IgG4	Likely Pathogenicity of IgG4. Challenging animal models because rodent podocytes do not express PLA2R

TABLE 8 Target product profile for diseases.				
Criteria	CIDP	IgG4 Related Disease	TTP	Membranous Nephropathy
Clinical endpoints	Risk of relapse. INCAT, I-RODS and grip strength.	Primary – Time to flare through week 52. Annualized flare rate. Proportion with flare-free remission over time	Platelet count recovery. Resolution of hemolysis and relapse rates.	Proteinuria reduction, renal function

Previously recommended treatments for IgG4-related diseases.

[0047] 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 two–four weeks, then gradually tapered over three to six months, unless contraindications exist. Glucocorticoids characteristically result in a rapid improvement in clinical features and often a resolution of radiographic features. When advanced fibrotic lesions have resulted in irreversible damage, the response to glucocorticoids and other current treatment options may be poor or even absent.

Definitions

[0048] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are listed below. As used in this application, except as otherwise provided in this specification, each of these terms 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. These definitions aid in describing embodiments but are not intended to limit the claimed invention.

[0049] 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.

[0050] The term "ABT" has the biomedical art-recognized meaning of an antibody-binding moiety. In some embodiments of this specification, the ABT binds to an IgG4 antibody.

[0051] 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.

[0052] The term “ADCC” has the biomedical art-recognized meaning of antibody-dependent cell-mediated cytotoxicity. ADCC 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] The term “anti-IgG4 antibody” has the biomedical art-recognized meaning of an antibody that selectively binds an IgG4 antibody.

[0060] 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 consisting of 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.

[0061] 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 of this specification, an ASGPR-binding group is a component of a bifunctional agent as a cellular receptor-binding moiety which is covalently bound to the IgG4-binding moiety through a linker group or directly. It is through this ASGPR moiety that bifunctional agents complexed with a circulating protein, e.g., IgG4 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.

[0062] 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.

[0063] 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.

[0064] The term “AT” has the biomedical art-recognized meaning of an antibody moiety. In some embodiments of this specification, the AT binds to IgG4.

[0065] The term “bafilomycin” (bafilomycin A1) has the biomedical art-recognized meaning of a macrolide antibiotic that inhibits vacuolar H⁺-ATPase (V-ATPase). It's used in cell biological assays to block autophagy and reduce cell growth in cancer cell lines.

[0066] The term “cellular receptor-binding moiety” has the biomedical art-recognized meaning. In several embodiments of this specification, the cellular receptor-binding moiety is an asialoglycoprotein receptor (ASGPR) binding group.

[0067] 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.

[0068] The term “chemistry, manufacturing, and controls” (pharmaceutical quality, CMC) has the biomedical art-recognized meaning of the several procedures used to assess the physical and chemical characteristics of drug products, to ensure their quality and consistency during manufacturing, and to prepare data for submissions to regulatory authorities.

[0069] 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 retain the foreign antibody’s antigen specificity and affinity.

[0070] 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 the combination of 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 so long as a beneficial effect from the co-action of the combination of the therapeutic agents 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.

[0071] The term “complementarity determining region (CDR)” has the biomedical art-recognized meaning of a polypeptide region of an antibody heavy chain 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.

[0072] The term "comprises," the term "comprising," the term "includes," and the term "including" specify the presence of stated features, regions, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, regions, integers, steps, operations, elements, components, and/or groups thereof.

[0073] The term "desmoglein 3" (Dsg3) has the biomedical art-recognized meaning of a gene that encodes a member of the cadherin family of proteins. Desmoglein 3 multiprotein complexes are involved in cell adhesion, cytoskeletal structural organization, and cell signaling. Mice lacking Dsg3 exhibit loss of keratinocyte cell adhesion, which results in a blistering phenotype observed in pemphigus vulgaris patients. Binding of antibodies against Dsg3 leads to loss of intracellular adhesion and results in intraepithelial blister formation. The overall homology of Dsg3 between mice and humans are 85.6%. See Buxton et al., The Journal of Cell Biology, 121(3), 481-3 (May 1, 1993), Ishikawa et al., Experimental Dermatology, 9(4), 229-39 (August 2000); and Eming et al., J. Immunol., 193(9), 4391-9 (2022). Binding of monoclonal antibodies against Dsg3 on epidermal keratinocytes results in a loss of intercellular adhesion and formation of intraepithelial blisters (symptoms of pemphigus vulgaris).

[0074] The term "DMPK" has the biomedical art-recognized meaning of drug metabolism and pharmacokinetics, which is a discipline that helps identify drugs that are likely to be suitable for further development.

[0075] The term "drug product" has the United States Food & Drug Administration definition described in 21 C.F.R. § 314.3 of a finished dosage form, e.g., tablet, capsule, or solution, that contains a drug substance, generally, but not necessarily, in association with one or more other ingredients.

[0076] The term "drug substance" has the United States Food & Drug Administration definition described in 21 C.F.R. § 314.3 of an active ingredient that is intended to furnish 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 the human body but does not include intermediates used in the synthesis of such ingredient.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] The term “first,” “second,” “third,” etc. have the plain meaning of describing several elements, components, regions, layers, and/or sections, these elements, components, regions, layers, and/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.

[0082] The term “Good Laboratory Practice” has the United States Food & Drug Administration definition described in 21 C.F.R. Part 58, Good Laboratory Practice for Nonclinical Studies.

[0083] 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.

[0084] 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, thus retaining 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 or completely 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.

[0085] The term “IC₅₀” 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.

[0086] The term “IgG” antibody has the biomedical art-recognized meaning. Each IgG molecule consists of 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.

[0087] 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.

[0088] 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.

[0089] The term “IgG4-binding moiety” has the biomedical art-recognized meaning a moiety on a binding protein, e.g., and IgG antibody or a fragment thereof, that binds to an IgG4 antibody.

[0090] 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.

[0091] The term “IVIG” has the biomedical art-recognized meaning of the administration of intravenous immunoglobulin (IVIG).

[0092] 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.

[0093] The term “LG11” has the biomedical art-recognized meaning of a neuronal antigen involved in synaptic function as it is part of the voltage-gated potassium channel complex and is involved in linking transmembrane proteins across the synaptic cleft.

[0094] 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-IgG4 IgG antibody moiety to a cellular receptor-binding moiety,

[0095] The term “MoDE” has the proprietary meaning of molecular degraders. See Intl. Pat. Publ. WO 2019/199634 (Yale University) and Intl. Pat. Publ. WO 2019/199621 (Yale University).

[0096] 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, e.g., a target agent, a peptide agent, an antibody agent, etc. retains one or more or all desirable structural features, properties, functions, and/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 target agent moiety maintains one or more desired structural features, properties, functions, and/or properties comparable to its corresponding target agent; in some embodiments, an antibody agent moiety maintains one or more desired structural features, properties, functions, and/or properties, e.g., 3-dimension structure, antigen specificity, antigen-binding capacity, and/or immunological functions, etc. comparable to its corresponding antibody agent. In some embodiments, a moiety of an agent, e.g., a target agent moiety, a peptide agent moiety, an antibody agent moiety, etc. is a monovalent (for a monovalent moiety), bivalent (for a bivalent moiety), or polyvalent (for a polyvalent moiety) radical of an agent, e.g., a target agent (for a target agent moiety), a peptide agent (for a peptide agent moiety), an antibody agent (for an antibody agent moiety), etc. In some embodiments, a monovalent radical is formed by removing a monovalent part, e.g., hydrogen, halogen, another monovalent group like alkyl, aryl, etc. from a compound/agent. In some embodiments, a bivalent or polyvalent radical is formed by removing one or more monovalent, e.g., hydrogen, halogen, monovalent groups like alkyl, aryl, etc., bivalent and/or polyvalent parts from a compound/agent. In some embodiments, radicals are formed by removing hydrogen atoms. In some embodiments, a moiety is monovalent. In some embodiments, a moiety is bivalent. In some embodiments, a moiety is polyvalent.

[0097] The term “monotherapy” has the biomedical art-recognized meaning of the administration of a single active or therapeutic compound to a subject. Monotherapy usually is the administration of a therapeutically effective amount of an active composition.

[0098] The term “Multimodal Antibody Therapy Enhancers” (MATE or MATES) has the proprietary meaning. See Intl. Pat. Publ. WO 2021/102052 (Kleo Pharmaceuticals).

[0099] The term “nanobody” has the biomedical art-recognized meaning.

[00100] The term “antibody” refers to immunoglobulin molecules and immunologically active parts of immunoglobulin molecules, i.e., molecules that contain 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 as well as a variety of forms including full length antibodies and antigen-binding portions thereof; including, e.g., 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, a diabody, a nanobody, a multi-specific (e.g., trispecific) antibody, a dual specific antibody, a bispecific antibody, an anti-idiotypic antibody, a functionally active epitope-binding portion thereof, and/or bifunctional hybrid antibodies.

[00101] The term "on" has the plain meaning. When an element is referred to as 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 referred to as being "directly on" another element, there are no intervening elements present.

[00102] The term "or" as used in this specification means "and/or." The term "and/or" as used in this specification includes any and all combinations of one or more of the associated listed items.

[00103] 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.

[00104] 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, thus retaining 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 or completely humanized.

[00105] 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 or 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).

[00106] The term "pharmaceutically acceptable" has the biomedical art-recognized meaning of those compounds, anions, cations, materials, compositions, carriers, and/or dosage forms that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00107] 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.

[00108] The term "rlgG" has the biomedical art-recognized meaning of recombinant human IgG.

[00109] The term “ROC” has the biomedical art-recognized meaning of receiver operating characteristic curve.

[00110] 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.

[00111] The term “TBT” in this specification has the meaning of a target-binding moiety, a cellular receptor-binding moiety. In some embodiments of this specification, the TBT binds to ASGPR.

[00112] 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.

[00113] 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.

[00114] The term “VHH” has the biomedical art-recognized meaning of Variable domain of Heavy chain of Heavy-chain antibody derived from camelid antibodies. VHH has nine beta-sheets forming a cylindric structure. Several regions may be involved in binding. Human IgG is about 150 kilodaltons. Camelid IgG is about 90 kilodaltons. VHH single chain domain is about 15 kilodaltons. The most important regions 4>1, 2 (greatest difference in uptake). The least important: regions 3, 5 (very minor difference in uptake).

[00115] Some embodiments are described below by referring to structures and schemes, to explain aspects of the description.

[00116] 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.

[00117] This specification does not concern a process for cloning humans, methods for modifying the germ line genetic identity of humans, uses of human embryos for industrial or commercial purposes, or procedures for modifying 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 administering the bifunctional degrader to subjects and measuring the outcomes.

[00118] How to identify subjects to whom the bifunctional degrader will be administered. Serum immunoglobulin G4 (IgG4) is often elevated, but this is not always the case. However, IgG4 blood lab levels greater than 135 are considered an evolving diagnostic criterion for disease suspicion.

[00119] IgG4 levels vary among healthy individuals and even within an individual patient. In one retrospective cohort study, baseline concentrations of serum IgG4, IgE, and blood eosinophils are independently predictive of relapse risk following treatment. The higher the baseline values, the greater the relapse risk and the shorter the time to relapse. Wallace et al., *Rheumatology*, 55(6), 1000–1008 (June 6, 2016).

[00120] Definite diagnosis is important because treatments for IgG4 disease and other diseases that mimic it differ. Satou et al., *Pathol. Int.*, 70(7), 391–402 (July 2020). While serum IgG4 levels may be suggestive, a definitive histological diagnosis is necessary to confirm IgG4 disease and rule out similar conditions. Chen et al., *Haematologica*, 104(3), 444 (March 2019). The sensitivity of IgG4 levels in diagnosing IgG4-related disease varies depending on the assay used, the number of affected organs, and possibly the patient's geographic origin. See Acharya et al., *J. Community Hosp. Intern. Med. Perspect.*, 14(3), 113–118 (May 7, 2024).

[00121] How to administer. The best mode of administration depends on where treatment is taking place, whether a hospital or outpatient.

[00122] The delivery of therapeutic agents can be via subcutaneous injection, e.g., by administering a 6 mL subcutaneous infusion or by further optimizing dose concentration and volume for subcutaneous administration. Alternative formulations, such as a VHH-Fc fusion protein, can reduce the amount needed to be administered. In one embodiment, the Non-Fc fusion VHH molecules present lower-molecular, which can be advantageous for subcutaneous formulation, with an estimated target of about 125 mg dose.

[00123] Dividing doses, e.g., administering 125 mg across four doses, can be a method for administering a full 500 mg dose. Alternative formulations could necessitate a loading dose to achieve sufficient IgG4 depletion.

[00124] The inventors performed a set of assays in mouse and rat to provide insights into subcutaneous and intravenous administration routes with repeat dose regimens to show the bioavailability to a subject of the agents administered.

[00125] Methods of measuring the removal of IgG4 antibody from a patient or subject. Depletion of antibodies leads to functional outcomes. The inventors have performed In vivo depletion assays to measure IgG4 depletion. See the EXAMPLES provided in this specification. Other assays to measure IgG4

in the blood circulation and functional outcomes of IgG4 related disease are known to persons having ordinary skill in the biomedical art.

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

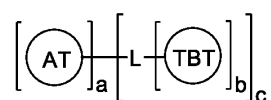
[00126] In one embodiment, the invention provides a composition of matter (agent) comprising:
 an IgG4-binding moiety,
 a cellular receptor-binding moiety which binds to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, and
 a linker moiety linking the antibody moiety and the cellular receptor-binding moiety.

[00127] In some embodiments, the invention provides a composition of matter (an agent), where the composition of matter has the structure:

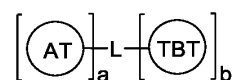


wherein [CPBM] is a IgG4-antibody-binding moiety;
 [CRBM] is a cellular receptor-binding moiety;
 each [CON] is an optional connector chemical moiety which, when present, connects directly to [CPBM] or to [CRBM] or connects the [LINKER] to [CPBM] or to [CRBM], and
 [LINKER] is a chemical moiety that covalently attaches to one or more [CRBM] or [CPBM] groups, or
 a pharmaceutically acceptable salt, stereoisomer, solvate, or polymorph thereof.

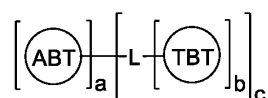
[00128] In some embodiments, the invention provides a composition of matter (an agent) having a structure selected from the Markush group of structures consisting of:



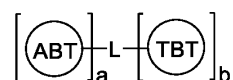
[AGN101],



[AGN102],

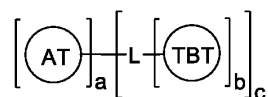


[AGN103], or



[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.

[00129] In some embodiments, the agent has the structure of formula **AGN101**:



[AGN101] or a salt thereof, wherein:

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

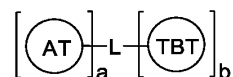
each AT is independently a IgG4-binding moiety;

L is a linker moiety; and

each TBT is independently a cellular receptor-binding moiety,

wherein the IgG4-binding moiety is (1) an anti-IgG4 antibody or an antigen-binding fragment thereof, or (2) anti-IgG4 VHH affinity ligand variant or an antigen-binding fragment thereof.

[00130] In some embodiments, the agent 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 a IgG4-binding moiety;

L is a linker moiety; and

each TBT is independently a cellular receptor-binding moiety,

wherein the IgG4-binding moiety is (1) an anti-IgG4 antibody or an antigen-binding fragment thereof, or (2) anti-IgG4 VHH affinity ligand variant or an antigen-binding fragment thereof.

[00131] In some embodiments, an agent comprises one and no more than one IgG4-binding moiety. In some embodiments, one or no more than one IgG4-binding moiety is bound to a linker moiety. In some embodiments, a is 1. 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 IgG4-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.

[00132] 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 in accordance with this specification. In some embodiments, target-binding moieties are connected to antibody moieties through certain types of groups and/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 particular 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 particular 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 IgG4 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. Persons having ordinary skill in the biomedical art know that an antibody agent may comprise more than one sites, e.g., one on each of the more than one chain, e.g., one on 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 are the same.

[00133] 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.

[00134] In some embodiments, the compositions of matter can be selected from among the following compounds:

TABLE 9			
ID NO.	IgG4-binding moiety	Cellular receptor binding moiety	Linkage
AGN401	VHH w/o hinge region; ABT306	β GN3, TBT103	thiosuccinimide
AGN402	VHH-Fc fusion, ABT310	β GN3 via MATE, TBT105	MATE conjugation
AGN403	VHH with hinge region	β GN3, TBT106	sortase
AGN404	VHH w/o hinge region	β GN3, TBT106	sortase
AGN405	F _c -BP2 (pan-IgG TRAPs)	β GN3, TBT103	thiosuccinimide
AGN406	F _c -BP2 (pan-IgG TRAPs)	PR3	
AGN501	VHH-G ₄ S-Cys, ABT502	β GN3, TBT103	thiosuccinimide
AGN502	VHH-G ₃ ECS-Cys, ABT508	β GN3, TBT103	thiosuccinimide
AGN505	F _c III (pan-IgG TRAPs)	α GN3, TBT104	
AGN506	F _c III (pan-IgG TRAPs)	β GN3, β GN3, TBT103	thiosuccinimide
AGN507	F _c -BP2	α GN3, TBT104	
AGN508	VHH-G ₄ S-his6-G ₄ S-Cys ABT501	β GN3, TBT103	thiosuccinimide
AGN509	ABT504	β GN3, TBT103	thiosuccinimide

The IgG4-binding moiety

[00135] In some embodiments, IgG4-binding moieties are connected to antibody moieties through certain types of groups or amino acid residues. In some embodiments, IgG4-binding moieties are connected to lysine residues optionally through linker moieties. In some embodiments, IgG4-binding moieties are connected to cysteine residues optionally through linker moieties. In some embodiments,

IgG4-binding moieties are connected to unnatural amino acid residues optionally through linker moieties. In some embodiments, the invention provides technologies for selectively linking IgG4-binding moieties to certain amino acid residues optionally through linker moieties. In some embodiments, provided technologies selectively connect IgG4-binding moieties to certain types of amino acid residues, e.g., lysine residues, optionally through linker moieties. In some embodiments, provided technologies selectively connect IgG4-binding moieties to sites of antibody moieties optionally through linker moieties. In some embodiments, provided technologies selectively connect IgG4-binding moieties to certain types of amino acid residues at sites optionally through linker moieties. In some embodiments, IgG4-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, IgG4-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, IgG4-binding moieties are connected to K239 and K241 of a 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.

[00136] Persons having ordinary skill in the biomedical art know that an antibody agent may comprise more than one site, e.g., one on each of the more than one chain, e.g., one on 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 IgG4-binding moieties or both linker moieties are the same.

[00137] In another embodiment, the IgG4-binding moiety is an IgG4-binding antibody or an antigen-binding fragment thereof.

[00138] In another embodiment, the IgG4-binding moiety is a variant of **ABT301** (SEQ ID NO: 1).

[00139] In another embodiment, the IgG4-binding is a full IgG antibody wherein the heavy chain comprises the three CDR regions of the VHH affinity ligand. A full-length human sourced IgG can be native and have variable glycosylation.

[00140] In some embodiments, the IgG4-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.

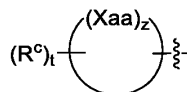
[00141] The IgG4-binding moiety may be or may comprise **ABT101** or a salt form thereof. In some embodiments, **ABT101** is an IgG4-binding moiety. Each IgG4-binding moiety in an agent may be of the same IgG4-binding moiety or a salt thereof. The IgG4-binding moiety can be the anti-IgG4 VHH nanobody or an antigen-binding variant thereof.

[00142] In some embodiments, **ABT101** is a universal antibody-binding moiety. In some embodiments, **ABT101** is a universal antibody-binding moiety which can bind to IgG4 antibodies 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. The VHH nanobody is a universal binder because the IgG4-binding of the VHH nanobody is through the IgG4 Fc.

[00143] In one embodiment, the IgG4-binding moiety is human IgG4 Fc Protein, Tag Free, ACRO Biosystems, Catalog # IG4-H5205, which can be produced by HEK293 expression, with amino acids Glu 99-Lys 327.

[00144] In some embodiments, the IgG4-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.

[00145] The IgG4-binding moiety may be or may comprise:



[ABT101]

or a salt form thereof.

[00146] In some embodiments, **ABT101** is a IgG4-binding moiety. Each IgG4-binding moiety in an agent may be of the same IgG4-binding moiety or a salt thereof.

[00147] Several IgG4-binding moieties can be used in accordance with the teachings of this specification. Some IgG4-binding moieties and technologies for identifying or assessing IgG4-binding moieties are described in Intl. Pat. Publ. WO 2019/023501 and Intl. Pat. Publ. 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 IgG4-binding moieties in accordance with this specification. In some embodiments, an IgG4-binding moiety comprises one or more amino acid residues, each independently natural or unnatural.

[00148] In some embodiments, a IgG4-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 IgG4-binding moiety is or comprises **ABT101** or a salt form thereof, wherein each variable is as described in this specification.

[00149] 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.

[00150] In some embodiments, a IgG4-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 IgG4-binding moiety is or comprises **ABT101** or a salt form thereof, wherein each variable is as described in this specification. In some embodiments, a IgG4-binding moiety is $R^c-(Xaa)_z-$ or **ABT101**, or a salt form thereof, and is or comprises a peptide unit.

[00151] 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.

[00152] In some embodiments, a peptide unit comprises an amino acid residue, e.g., at physiological pH about 7.4, positively charged amino acid residue, Xaa^P). In some embodiments, a peptide unit comprises R. In some embodiments, at least one Xaa is R.

[00153] 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.

[00154] In some embodiments, an IgG4-binding moiety is or comprises an optionally substituted moiety as shown in the table provided in Intl. Pat. Publ. WO 2019/023501, pages 48-60, which table and patent publication are incorporated in this specification by reference.

[00155] In some embodiments, useful technologies for developing or assessing antibody-binding 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), Intl. Pat. Publ. WO 2012/017021, etc., each of which is incorporated in this specification in its entirety by reference.

[00156] In some embodiments, an IgG4-binding moiety is an affinity substance described in Intl. Pat. Publ. WO 2018/199337, the affinity substance of each of which is incorporated in this specification by reference.

[00157] In some embodiments, an IgG4-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 in accordance with this specification, adapter proteins do not require reactive residues, e.g., BPA, to achieve one or more or all advantages.

[00158] In some embodiments, an antibody-binding moiety can be selected from the following compounds:

<u>TABLE 10</u>		
<u>ID NO.</u>	<u>SEQ ID NO.</u>	<u>Protein structure</u>
ABT301	SEQ ID NO: 1	VHH nanobody (122 AAs)
ABT304	SEQ ID NO: 9	VHH nanobody (122 AAs), G4S linker, C-term Sortase site, His6 tag
ABT305	SEQ ID NO: 10	VHH nanobody with truncation of hinge, G4S linker, C-term Sortase site, His6 tag
ABT306	SEQ ID NO: 11	VHH nanobody with truncation of hinge, G4S linker, His6 tag, rigid linker-Cys
ABT310	SEQ ID NO: 12	VHH nanobody with truncation of hinge, G4S linker, hlgG1 hinge (C220A)-human IgG1 Fc-L253A/L254A/P329A
ABT311	SEQ ID NO: 32	VHH nanobody (122 AAs), G4S linker, His6 tag
ABT312	SEQ ID NO: 33	VHH nanobody with truncation of hinge, G4S linker, His6 tag
ABT313	SEQ ID NO: 34	VHH nanobody with truncation of hinge, G4S linker, His6 tag, G2S linker, AviTag
ABT501	SEQ ID NO: 59	VHH nanobody-G4S-his6-G4S-Cys
ABT502	SEQ ID NO: 64	VHH nanobody-G4S-Cys
ABT503	SEQ ID NO: 63	VHH nanobody-G3ECS-Cys
ABT504	SEQ ID NO: 65	VHH nanobody-G4S-his6-Cys

Characterizing antibody-binding moieties.

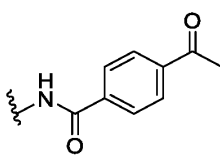
[00159] Many technologies are available for identifying, assessing, or characterizing antibody-binding moieties, e.g., those described in Intl. Pat. Publ. WO 2019/023501, the technologies of which are incorporated in this specification by reference. In some embodiments, an antibody-binding moiety is 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 and/or stimulate ADCC and/or ADCP. In some embodiments, peptide display technologies, e.g., phase display, non-cellular display, etc., can

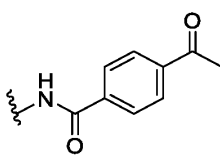
identify antibody-binding moieties. In some embodiments, an antibody-binding moiety is a moiety, e.g., small molecule moiety, peptide moiety, nucleic acid moiety, etc., that can bind to IgG and optionally can compete with known antibody binders, e.g., protein A, protein G, protein L, etc.

[00160] Persons having ordinary skill in the biomedical art know that antibodies of several properties and activities, e.g., antibodies recognizing different antigens, having optional modifications, 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.

[00161] In some embodiments, an IgG4-binding moiety is or comprises a mediotope agent moiety. In some embodiments, a mediotope agent is described in, e.g., U.S. Pat. Publ. 2019/0111149.

[00162] In some embodiments, an IgG4-binding moiety can bind to human IgG. In some embodiments, an IgG4-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 IgG4-binding moiety binds to IgG1, IgG2, and IgG4.



[00163] 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- , $\text{CH}_3\text{C(O)}-$, $\text{CH}_3\text{C(O)NH}-$, $\text{CH}_3\text{C(O)NHCH}_2-$, CH_3CH_2- , $\text{CH}_3\text{CH}_2\text{NH}-$, and $\text{CH}_3\text{CH}_2\text{NHC(O)}-$.

[00164] 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 $1 \text{ mM}^{-1} \text{ pM}$ 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.

[00165] Persons having ordinary skill in the biomedical art know that antibodies of several properties and activities, e.g., antibodies recognizing different antigens, having optional modifications, 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, and/or one or more properties and/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 and/or ADCCP activities toward a virus, e.g., a SARS-CoV-2 virus, and/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 and/or activities of recruited antibodies described in this specification.

Fc mutation strategies to enhanced FcRn-binding.

[00166] There are many publicly available approaches and mutation sets to increase binding of an Fc to FcRn. See TABLE 11. An older summary was provided by Strohl, Optimization of Fc-mediated effector functions of monoclonal antibodies. Current Opinion in Biotechnology, 20(6), 685-691 (2009).

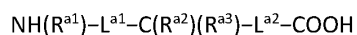
<u>TABLE 11</u> <u>Examples of Fc sequence engineering for modification of half-life</u>		
<u>Function</u>	<u>Company or source</u>	<u>Mutations or changes</u>
Increased half-life	MedImmune	IgG1-M252Y, S254T, T256E
Increased half-life	Protein Design Labs	IgG1- T250Q, M428L
Increased half-life	Genentech	IgG1-N434A
Increased half-life	Sally Ward	IgG1-H4J3K, N434Y
Increased half-life	Deny Roopenian	IgG1-T307A.E380A,N434A
Increased half-life	Xeocor Xtend™ technology	
Lowered endogenous IgG	Sally Ward	IgG1-M252 Y, S254T,T256E, H433K, N434F, 436H
Decreased half-life	Deny Roopenian	IgG1-1253A
Decreased half-life	ElI Lilly	IgG1-P2571, N434H or Q376V, N434H

[00167] For an example of introducing a YTE region in a protein (M252Y/S254T/T256E), see Acqua et al. Increasing the affinity of a human IgG1 for the neonatal Fc receptor: Biological consequences. The Journal of Immunology 169(9), 5171-5180 (2002). For a recent example combining LALA and YTE, see Cobb et al., A combination of two human neutralizing antibodies prevents SARS-CoV-2 infection in rhesus macaques. bioRxiv, 2021-09 (2021). Additional references with details of engineering also available.

[00168] For an example of introducing a LS region in a protein (M428L/N434S). See Zalevsky et al. Nature Biotechnology, 28(2), 157-159 (2010).

Linker moieties and amino acids thereof

[00169] In some embodiments, the linker moiety has the structure of formula **LNK101**:



[LNK101] or a salt thereof, wherein:

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

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 $\text{C}_1\text{-C}_{20}$ aliphatic or $\text{C}_1\text{-C}_{20}$ heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{C}(\text{O})\text{S}-$, or $-\text{C}(\text{O})\text{O}-$;

each $-\text{Cy}-$ 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 $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{CO}_2\text{R}$, or $-\text{SO}_2\text{R}$;

each R is independently $-\text{H}$, 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 together 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 together 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.

[00170] 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.

[00171] In some embodiments, an amino acid analog is a compound in which the amino group and/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 and/or functions of amino acids, are described in the biomedical art and can be used in accordance with 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.

[00172] 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 portions. 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 some embodiments, L^{PM} is a linker moiety described in this specification. In some embodiments, L^{PM} is L.

[00173] Linker moieties of several types and/or for several purposes, e.g., those used in antibody-drug conjugates, etc., may be used in accordance with this specification.

[00174] Linker moieties can be either 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 more than two moieties.

[00175] In some embodiments, a linker moiety, e.g., L^z (wherein z represents superscript text; e.g., L^{PM} , L^{RM} , L^{LG} , L^{LG1} , etc., is or comprises L.

[00176] In some embodiments, L is a covalent bond, or a bivalent or polyvalent optionally substituted, linear or branched C_{1-100} group comprising one or more aliphatic, aryl, heteroaliphatic having 1-20 heteroatoms, heteroaromatic having 1-20 heteroatoms, or any combinations thereof, wherein one or more methylene units of the group are optionally and independently replaced with C_{1-6}

alkylene, C₁₋₆ alkenylene, a bivalent C₁₋₆ heteroaliphatic group having 1-5 heteroatoms, $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, —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 $\text{—}[(\text{—O—C(R')}_2\text{—C(R')}_2\text{—})_n]\text{—}$, wherein n is 1-20. The linker optionally has a cyclic group, Cy, defined below, and a reactive group, RG as defined below. In some embodiments, each amino acid residue is independently a residue of an amino acid having the structure of formula **LNK101** or a salt thereof. In some embodiments, each amino acid residue independently has the structure of $\text{—N(R}^{a1}\text{)—L}^{a1}\text{—C(R}^{a2}\text{)(R}^{a3}\text{)—L}^{a2}\text{—CO—}$ or a salt form thereof.

[00177] In some embodiments, L is bivalent. In some embodiments, L is a covalent bond.

[00178] In some embodiments, L is a bivalent or optionally substituted, linear or branched group selected from C₁₋₁₀₀ aliphatic and C₁₋₁₀₀ heteroaliphatic having 1-50 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with C₁₋₆ alkylene, C₁₋₆ alkenylene, a bivalent C₁₋₆ heteroaliphatic group having 1-5 heteroatoms, $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, —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 $\text{—}[(\text{—O—C(R')}_2\text{—C(R')}_2\text{—})_n]\text{—}$. In some embodiments, L is a bivalent or optionally substituted, linear or branched group selected from C₁₋₂₀ aliphatic and C₁₋₂₀ heteroaliphatic having 1-10 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with C₁₋₆ alkylene, C₁₋₆ alkenylene, a bivalent C₁₋₆ heteroaliphatic group having 1-5 heteroatoms, $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, —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 $\text{—}[(\text{—O—C(R')}_2\text{—C(R')}_2\text{—})_n]\text{—}$. In some embodiments, L is a bivalent or optionally substituted, linear or branched group selected from C₁₋₂₀ aliphatic wherein one or more methylene units of the group are optionally and independently replaced with $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, —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 $\text{—}[(\text{—O—C(R')}_2\text{—C(R')}_2\text{—})_n]\text{—}$. In some embodiments, L is a bivalent or optionally substituted, linear or branched C₁₋₂₀ aliphatic wherein one or

more methylene units of the group are optionally and independently replaced with $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, —C(O)S— , —C(O)O— , an amino acid residue or $\text{—[—O—C(R')}_2\text{—C(R')}_2\text{—}]_n\text{—}$. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-100} aliphatic wherein one or more methylene units of the group are optionally and independently replaced with $\text{—C}\equiv\text{C—}$, —Cy— , $\text{—C(R')}_2\text{—}$, —O— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , $\text{—C(O)C(R')}_2\text{N(R')—}$, —N(R')C(O)N(R')— , —N(R')C(O)O— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, an amino acid residue or $\text{—[—O—C(R')}_2\text{—C(R')}_2\text{—}]_n\text{—}$. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-50} aliphatic wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-40} aliphatic wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-20} aliphatic wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-10} aliphatic wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-100} alkylene wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-50} alkylene wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-40} alkylene wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-20} alkylene wherein one or more methylene units of the group are optionally and independently replaced as described in this specification. In some embodiments, L is a bivalent or optionally substituted, linear or branched group C_{1-10} alkylene wherein one or more methylene units of the group are optionally and independently replaced as described in this specification.

[00179] In some embodiments, a linker moiety, e.g., L, L^{PM} , L^{RM} , etc., comprises an acidic group, e.g., $\text{—S(O)}_2\text{OH}$.

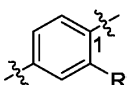
[00180] In some embodiments, L is or comprises $-[(-O-C(R')_2-C(R')_2)_n]-$. In some embodiments, L is or comprises $-[(-O-CH_2-CH_2)_n]-$. In some embodiments, L is $-[(-CH_2-CH_2-O)_6]-CH_2-CH_2-$. In some embodiments, L is $-[(-CH_2-CH_2-O)_8]-CH_2-CH_2-$. In some embodiments, $-CH_2-CH_2-O-$ is bonded to an antibody-binding moiety at a $-CH_2-$. In some embodiments, $-CH_2-CH_2-O-$ is bonded to a cellular receptor-binding moiety at a $-CH_2-$. In some embodiments, L^{PM} is such L. In some embodiments, L^{RM} is such L.

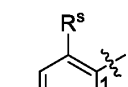
[00181] In some embodiments, a linker moiety, or L, is or comprises $-(CH_2CH_2O)_n-$, wherein each $-CH_2-$ is independently and optionally substituted and n is 1-20. In some embodiments, a linker moiety, or L, is or comprises $-(CH_2)_n-O-(CH_2CH_2O)_n-(CH_2)_n-$, wherein each n is independently 1-10, and each $-CH_2-$ is independently and optionally substituted.

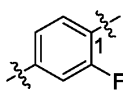
[00182] In some embodiments, a linker moiety is trivalent or polyvalent. In some embodiments, a linker moiety is L, where L is trivalent or polyvalent. In some embodiments, L is trivalent. In some embodiments, L is $-CH_2-N(-CH_2-)-C(O)-$.

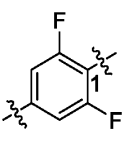
[00183] In some embodiments, a linker moiety, e.g., L, comprises one or more amino acid residues or analogs thereof.

[00184] 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 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 some

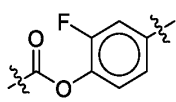
embodiments, an aryl group has the structure of , wherein R^s is halogen, $-NO_2$, $-F$, $-L-R'$, $-C(O)-L-R'$, $-S(O)-L-R'$, $-S(O)_2-L-R'$, or $-P(O)(-L-R')_2$. In some embodiments, an aryl group has the

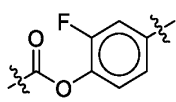
structure of , wherein each R^s is independently halogen, $-NO_2$, $-F$, $-L-R'$, $-C(O)-L-R'$,

$-S(O)-L-R'$, $-S(O)_2-L-R'$, 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 $-O-$.

[00185] In some embodiments, a linker moiety, e.g., 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

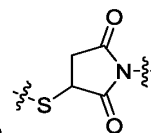
linker. In some embodiments, a reactive group is or comprises , wherein the $-C(O)-$ is connected to a cellular receptor-binding moiety, or a moiety comprising $-(Xaa)y-$, optionally through a linker and the other end of the reactive group is connected to an antibody-binding moiety.

[00186] In some embodiments, L is or comprises a bio-orthogonal or enzymatic reaction product moiety. In some embodiments, L is or comprises an optionally substituted triazole moiety, which is optionally part of a bi- or poly-cyclic ring system. In some embodiments, L is or comprises LPXTG (SEQ ID NO: 74). In some embodiments, L is or comprises LPETG (SEQ ID NO: 75). In some embodiments, L is or comprises LPXT(G) n , wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, L is or comprises LPET(G) n , wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00187] In some embodiments, a linker moiety, e.g., L^{RM} , etc., does not have a reactive group. In some embodiments, a linker moiety, e.g., 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^{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.

[00188] In some embodiments, a linker moiety, e.g., L^{RM} , etc., comprises no $-S-$, wherein none of the two atoms to which the $-S-$ is bonded to is S. In some embodiments, a linker moiety, e.g., 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 $-S-$. In some embodiments, a linker moiety, e.g., L , L^{RM} , etc., comprises no $-S-$.

[00189] In some embodiments, an agent 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. 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.

[00190] 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.

[00191] 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 $-(CH_2CH_2O)_n-$.

[00192] 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.

[00193] In some embodiments, a linker moiety 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. 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.

[00194] 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 some embodiments, one or more methylene units of L are independently replaced with $-NR'-$, wherein R' is as described in this specification.

[00195] 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 $-C(O)-$.

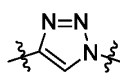
[00196] In some embodiments, a linker moiety, e.g., L, comprises a $-NR'-$ group, which can be used for connections with a moiety. In some embodiments, one or more methylene units of L are independently replaced with $-N(R')-$.

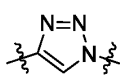
[00197] In some embodiments, a linker moiety, e.g., L, comprises a $-C(O)NR'-$ group, which can be used for connections with a moiety. In some embodiments, one or more methylene units of L are independently replaced with $-C(O)N(R')-$.

[00198] 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 $-COOH$.

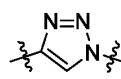
[00199] In some embodiments, a linker moiety is or comprises one or more ring moieties, e.g., one or more methylene units of L are replaced with $-Cy-$. In some embodiments, a linker moiety, e.g., L, comprises an aryl ring. In some embodiments, a linker moiety, e.g., L, comprises a heteroaryl ring. In

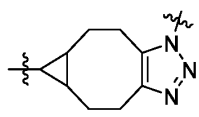
some embodiments, a linker moiety, e.g., L, comprises an aliphatic ring. In some embodiments, a linker moiety, e.g., L, comprises an heterocyclyl ring. In some embodiments, a linker moiety, e.g., L, comprises a polycyclic ring. In some embodiments, a ring in a linker moiety, e.g., L, is 3-20-membered. 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.

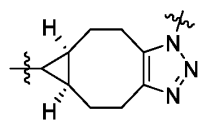
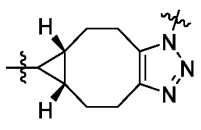
[00200] In some embodiments, a linker moiety, e.g., L) is or comprises . In some

embodiments, a methylene unit of L is replaced with . In some embodiments, a methylene unit of L is replaced with -Cy-. In some embodiments, -Cy- is.

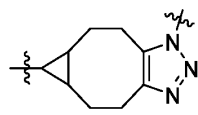
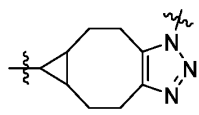
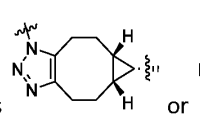
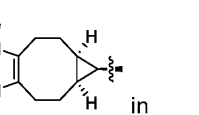
[00201] In some embodiments, a linker moiety, e.g., L) is or comprises -CO)y-. In some embodiments L is or comprises -[(CH₂)nO]mCy[(CH₂)nO]mNH-, or L is -[(CH₂)nO]mCy[(CH₂)nO]mNHC(O)[(CH₂)nO]mNH-, or L is -[(CH₂)nO]mCy[(CH₂)nO]m[NHC(O)[(CH₂)nO]m]pNH-, where n, m, p, are independently chosen at each occurrence from 1-20, from 1-12, or 2-10. In some embodiments each n is 2, m is independently chosen at each occurrence from an integer from 2-10, or in some embodiments m is independently chosen from

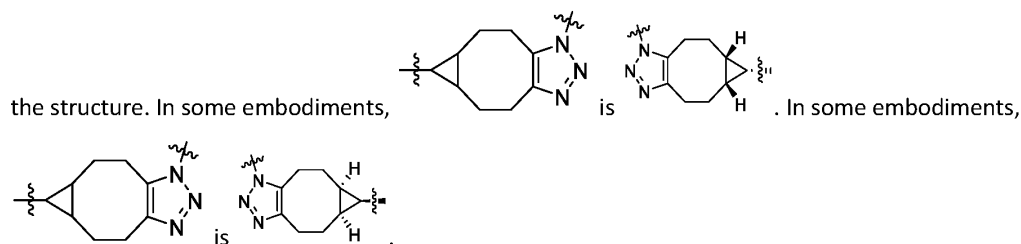
an integer from 2-6 and Cy is . In some embodiments, a methylene unit of L is replaced with

-Cy-. In some embodiments, -Cy- is . In some embodiments, -Cy- is

. In some embodiments, -Cy- is .

[00202] In some embodiments, a linker moiety, e.g., L, in a provided agent, comprises

. In some embodiments,  is  or  in



[00203] In some embodiments, L is L¹. In some embodiments, L is L^b.

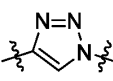
[00204] In some embodiments, L^{RM} is a covalent bond. In some embodiments, L^{RM} is not a covalent bond. In some embodiments, L^{RM} is or comprises $-(CH_2CH_2O)_n-$. In some embodiments, L^{RM} is or comprises $-(CH_2)_n-O-(CH_2CH_2O)_n-(CH_2)_n-$, wherein each n is independently as described in 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.

[00205] 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.

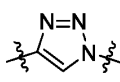
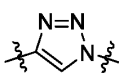
[00206] In some embodiments, L^{PM}, e.g., in a product of a first and a second agents) is or comprises a reaction product moiety formed a first reactive moiety and a second reactive moiety.

[00207] In some embodiments, a linker moiety, e.g., L^{PM} in a product of a first and a second agents is

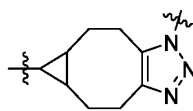
or comprises



. In some embodiments, a methylene unit of a linker moiety, e.g., L or a linker moiety that can be L, e.g., L^{RM}, L^{PM}, etc. is replaced with $-Cy-$. In some embodiments, $-Cy-$ is optionally

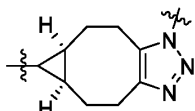
substituted . In some embodiments, -Cy- is . In some embodiments L is –

$[(CH_2)_nO]_mCH_2Cy[(CH_2)_nO]_m$. In some embodiments, -Cy- is

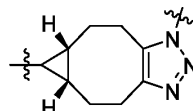


. In some

embodiments, -Cy- is



. In some embodiments, -Cy- is



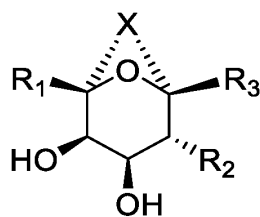
Cellular receptor-binding moieties

[00208] Several receptor-binding moieties, according to embodiments of present invention, are described in Intl. Pat. Publ. WO2019/199621 published October 17, 2019, Intl. Pat. Publ. WO2019/199634 published October 17, 2019, and Intl. Pat. Publ. WO 2021/072246, each of which is incorporated in this specification in its entirety by reference.

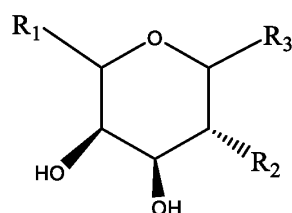
[00209] In an embodiment, the cellular receptor-binding moiety may include an asialoglycoprotein receptor (ASGPR) binding group connected through an amine group to the linker moiety.

[00210] 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.

[00211] 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}),

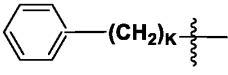
when X is 3 or 4 atoms in length, no more than 2 atoms of X are O, S or N(R^{N1});

wherein R^{N1} is H or a C₁-C₃ alkyl group optionally substituted with from 1-3 halo groups;

R₁ and R₃ are each independently:

H, -(CH₂)_KOH, -(CH₂)_KOC₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, -(CH₂)_K-vinyl, O-(CH₂)_K-vinyl, -(CH₂)_K-alkynyl, -(CH₂)_K-COOH,

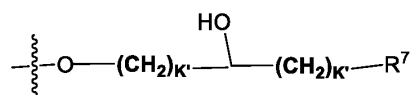
-(CH₂)_KC(O)O-C₁-C₄ alkyl which is optionally substituted with from 1-3 halo groups, O-C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, -C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, or

R₁ and R₃ are each independently a  group, which is optionally

substituted with up to three halo groups, C₁-C₄ 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-C₁-C₄ 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

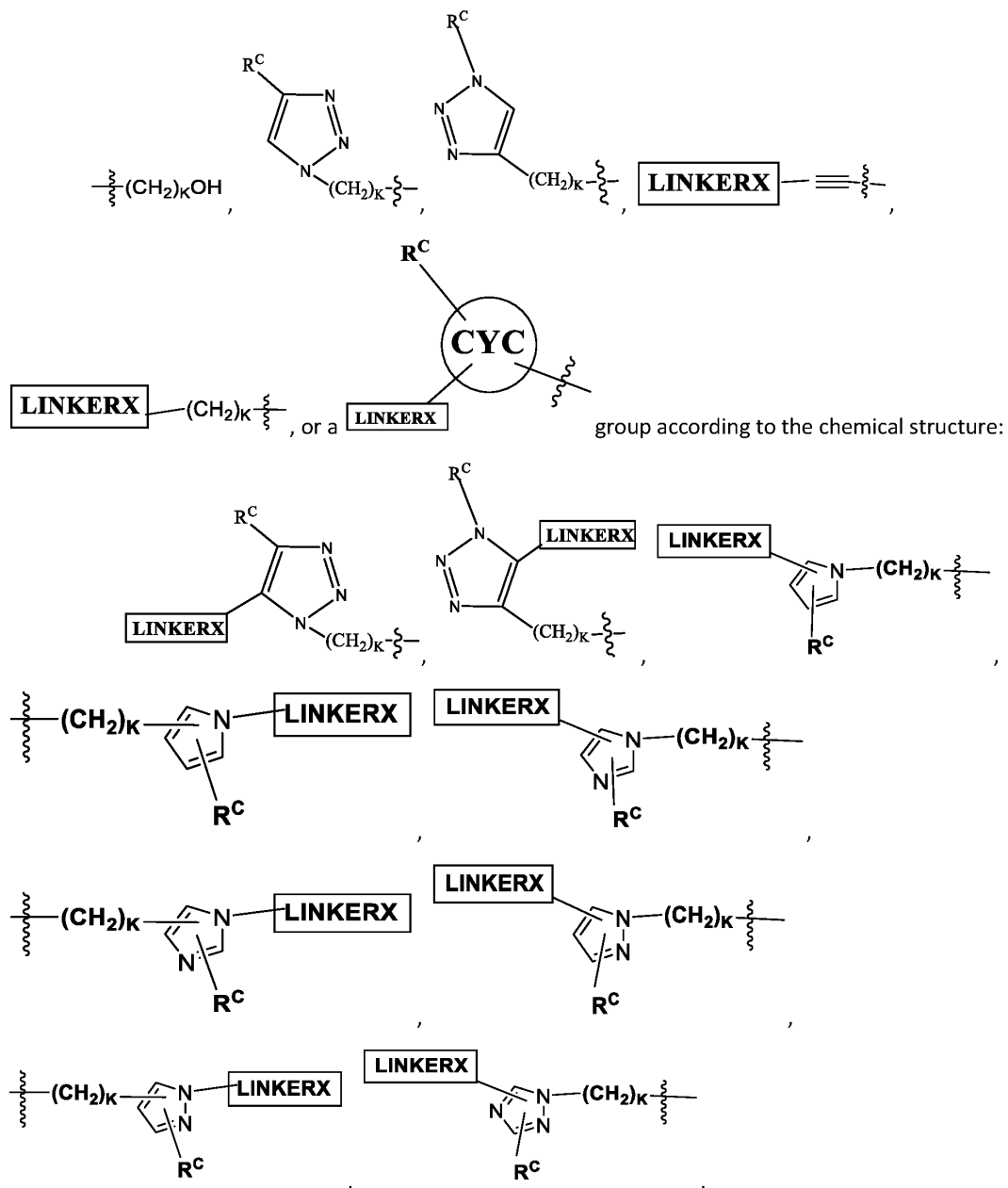
R₁ and R₃ are each independently a group according to the chemical structure:

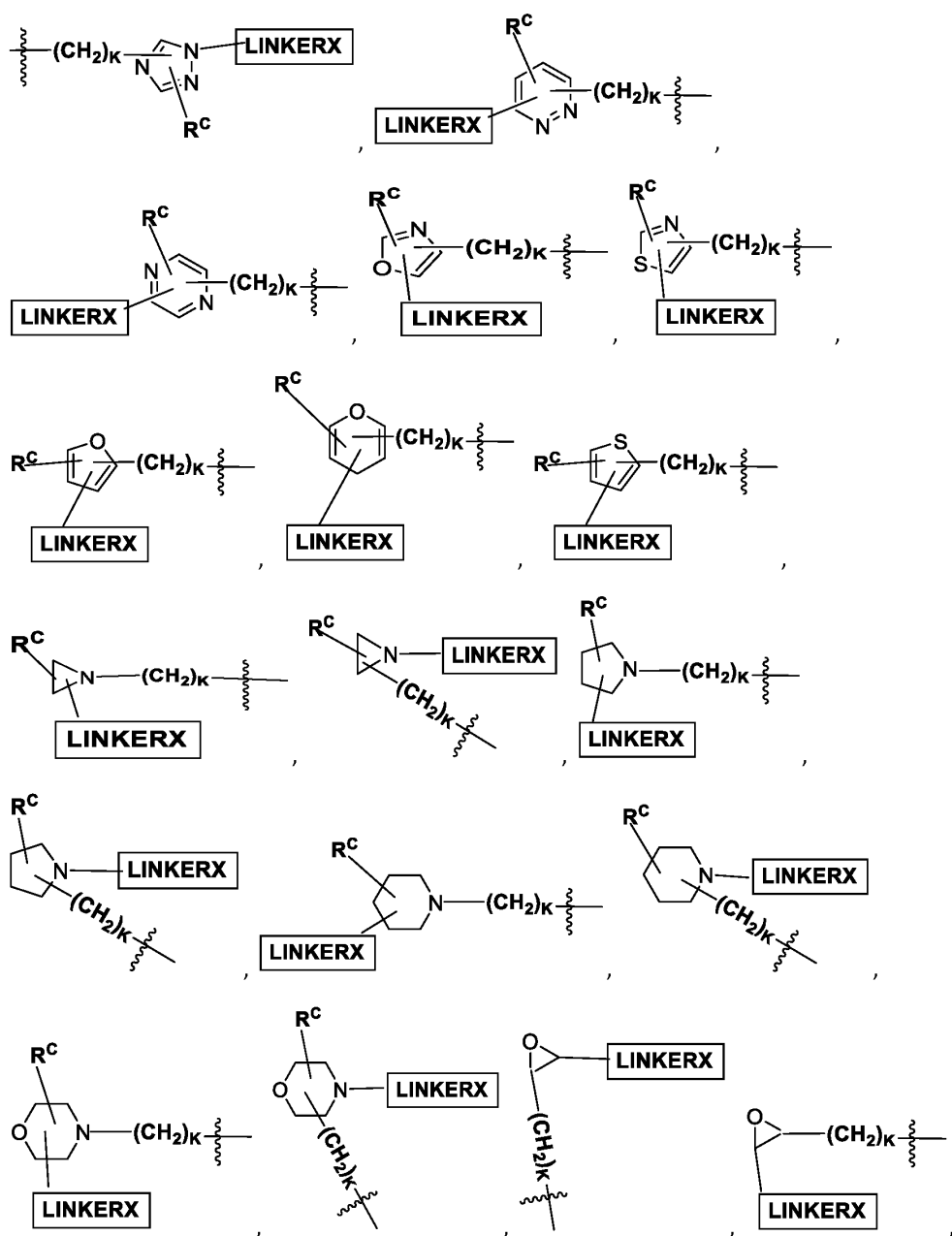


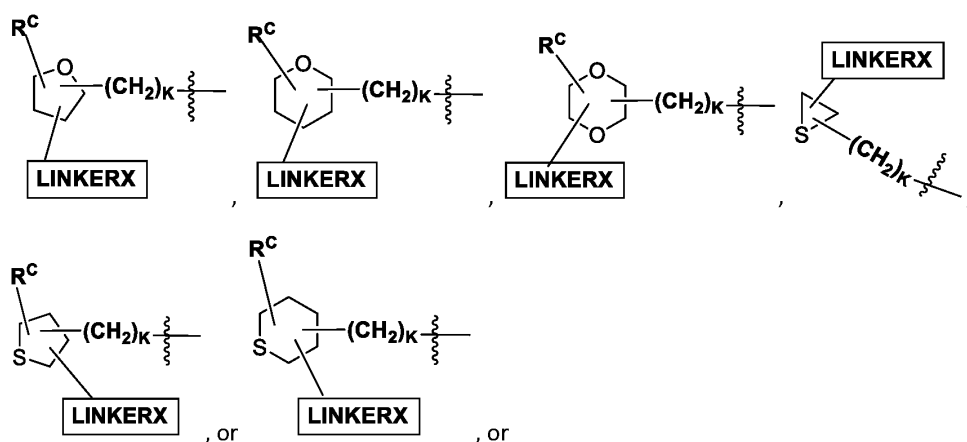
, wherein R⁷ is O-C₁-C₄ alkyl, which is optionally substituted with from 1 to 3 halo groups 1 or 2 hydroxy groups, or R⁷ is a

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

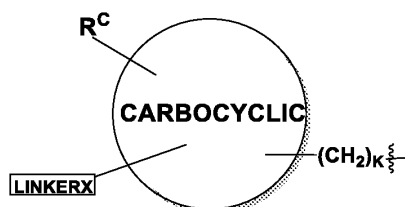
R_1 and R_3 are each independently a group according to the structure:



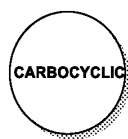




R_1 and R_3 are each independently a

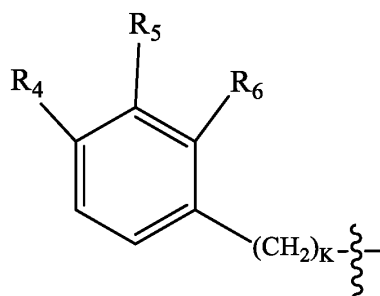


group, where



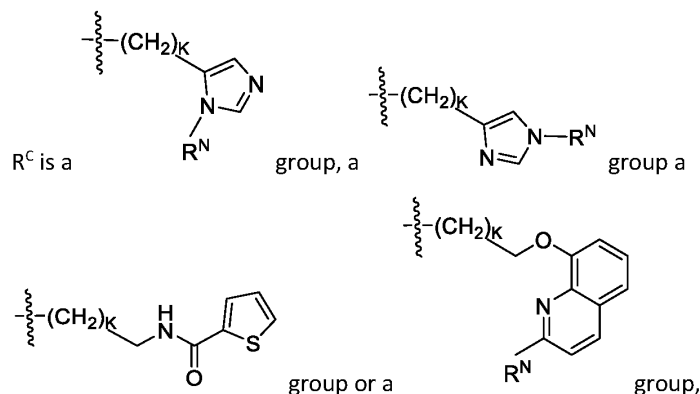
, is a C₃-C₈ saturated carbocyclic group;

R^C is absent, H, C₁-C₄ alkyl which is 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, C₁-C₃ 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 which is optionally substituted with

from 1-3 halo groups, O-C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, -C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo groups, or



wherein R^N , R^{N1} and R^{N2} are each independently H or a C₁-C₃ alkyl group which is optionally substituted with from one to three halo groups or one or two hydroxyl groups;

K is independently an integer of 0 to 4;

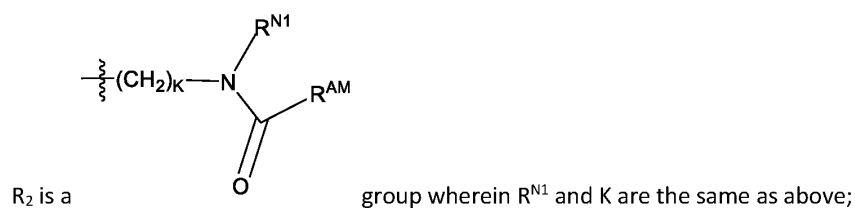
K' is an integer of 1 to 4;

R^{N3} is H, or a C₁-C₃ alkyl group which is optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups; and

R^{N4} is H, a C₁-C₃ alkyl group which is 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 IgG4-binding moiety and links the at least one IgG4-binding moiety to the cellular receptor-binding moiety through the optional linker moiety, or

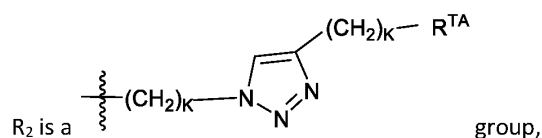
LINKERX is a linker group which has at least one or more functional groups which can be used to covalently bond the linker group to at least one IgG4-binding moiety or optional linker moiety;



R^{AM} is H, a C₁-C₄ alkyl group optionally substituted with up to 3 halo groups and one or two hydroxyl groups, a $-(CH_2)_KCOOH$ group, a $-(CH_2)_KC(O)O-C_1-C_4$ alkyl group which is optionally substituted

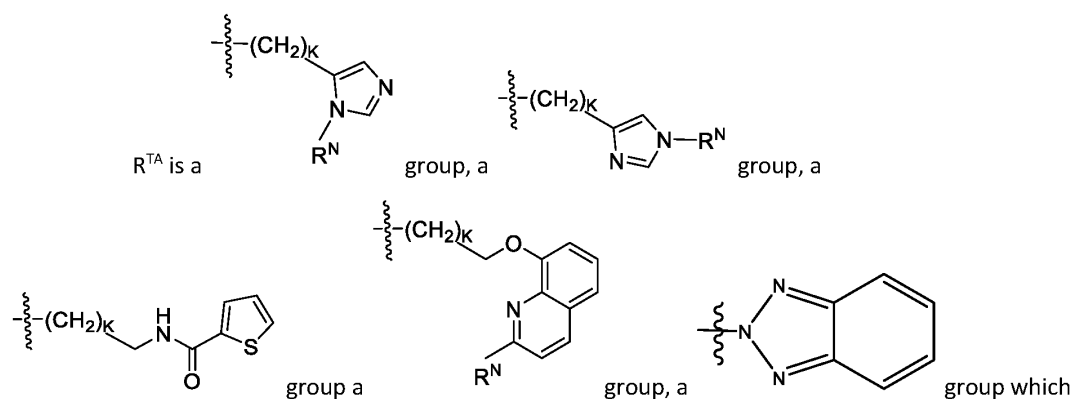
with from 1-3 halo groups, a $\text{O-C(O)-C}_1\text{-C}_4$ alkyl group, which is optionally substituted with from 1-3 halo F groups, a $\text{-C(O)-C}_1\text{-C}_4$ alkyl group, which is optionally substituted with from 1-3 halo groups, a $\text{-(CH}_2\text{)}_k\text{-NR}^{\text{N}3}\text{R}^{\text{N}4}$ group where $\text{R}^{\text{N}3}$ is H, or a $\text{C}_1\text{-C}_3$ alkyl group which is optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups; and

$\text{R}^{\text{N}4}$ is H, a $\text{C}_1\text{-C}_3$ alkyl group which is optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups, or a group, or



wherein R^{TA} is H, CN, $\text{NR}^{\text{N}1}\text{R}^{\text{N}2}$, $\text{-(CH}_2\text{)}_k\text{OH}$, $\text{-(CH}_2\text{)}_k\text{OC}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups, $\text{C}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups, $\text{-(CH}_2\text{)}_k\text{COOH}$, $\text{-(CH}_2\text{)}_k\text{C(O)O-C}_1\text{-C}_4$ alkyl which is optionally substituted with from 1-3 halo groups, $\text{O-C(O)-C}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups, $\text{-C(O)-C}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups, or

R^{TA} is a $\text{C}_3\text{-C}_{10}$ 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, $\text{NR}^{\text{N}1}\text{R}^{\text{N}2}$, $\text{-(CH}_2\text{)}_k\text{OH}$, $\text{-(CH}_2\text{)}_k\text{OC}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups, $\text{C}_1\text{-C}_3$ alkyl, which is optionally substituted with from 1-3 halo groups or 1 or 2 hydroxy groups, $\text{-O-C}_1\text{-C}_3\text{-alkyl}$, which is optionally substituted with from 1-3 halo groups, $\text{-(CH}_2\text{)}_k\text{COOH}$, $\text{-(CH}_2\text{)}_k\text{C(O)O-C}_1\text{-C}_4$ alkyl which is optionally substituted with from 1-3 halo groups, $\text{O-C(O)-C}_1\text{-C}_4$ alkyl, which is optionally substituted with from 1-3 halo groups or $\text{-(CH}_2\text{)}_k\text{C(O)-C}_1\text{-C}_4$ alkyl which is optionally substituted with from 1-3 halo groups, or



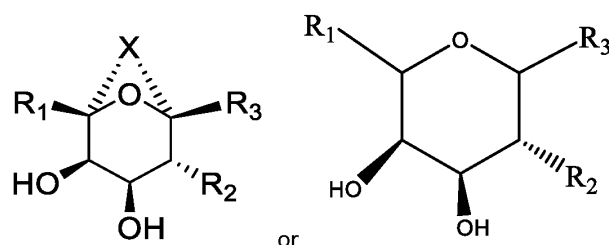
is optionally substituted with up to three C₁-C₃ alkyl groups which are optionally substituted with up to three halo groups, or

R^{TA} is a group,

wherein R^N, R^{N1} and R^{N2} are each independently H or a C₁-C₃ alkyl group which is optionally substituted with from one to three halo groups or one or two hydroxyl groups and each -(CH₂)_K group is optionally substituted with 1-4, preferably 1 or 2, C₁-C₃ alkyl groups which are optionally substituted with from 1-3 fluoro groups or 1-2 hydroxyl groups;

and K is independently 0-4.

[00212] The cellular receptor-binding moiety may include an ASGPR-binding group according to the chemical structure:



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}),

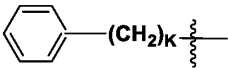
when X is 2 atoms in length, no more than 1 atom of X is O, S or N(R^{N1}),

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

wherein each R^{N1} is independently H or a C₁-C₃ alkyl group optionally substituted with from 1-3 halo groups, preferably F (R^{N1} is preferably H or methyl, more often H);

R₁ and R₃ are each independently H, -(CH₂)_KOH, -(CH₂)_KOC₁-C₄ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, -(CH₂)_Kvinyl, O-(CH₂)_Kvinyl,

-(CH₂)_Kalkynyl, -(CH₂)_KCOOH, -(CH₂)_KC(O)O-C₁-C₄ alkyl which is optionally substituted with from 1-3 halo, preferably F groups, O-C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, -C(O)-C₁-C₄ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, or

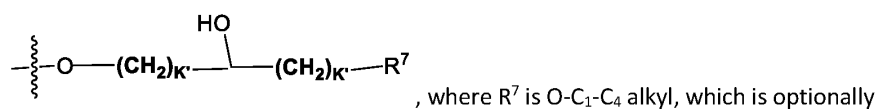
R₁ and R₃ are each independently a  group, which is optionally

substituted with up to three (preferably 1) halo groups (preferably F), C₁-C₄ alkyl groups, each of which is

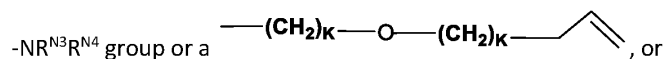
optionally substituted with from one to three halo groups, preferably F, or one or two hydroxyl groups,
or

O-C₁-C₄ alkyl groups, each of which alkyl groups is optionally substituted with from one to three halo groups, preferably F, or one or two hydroxyl groups, and K is independently 0-4 (0, 1, 2, 3 or 4), or

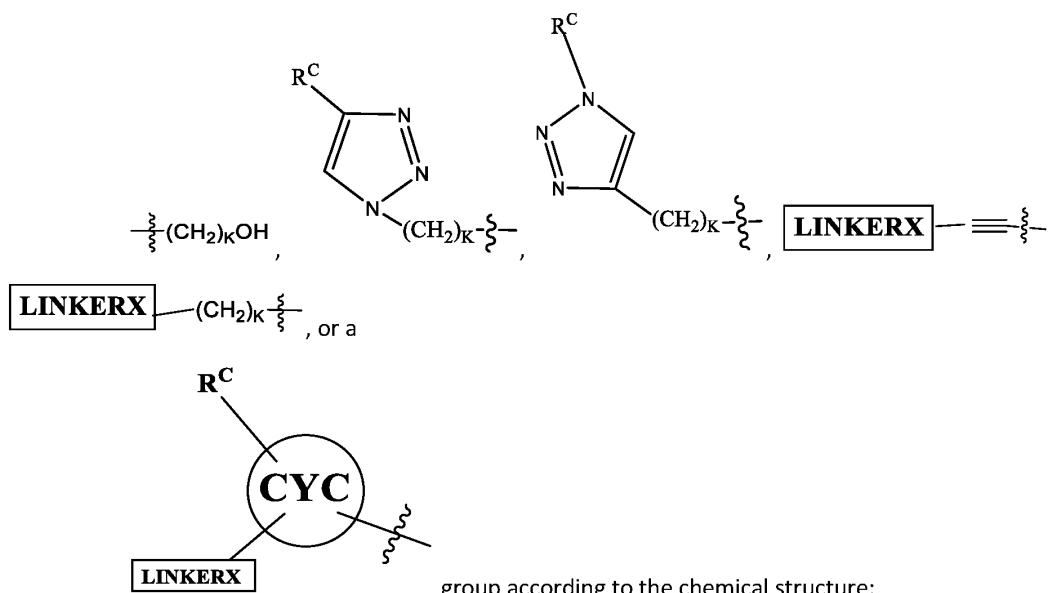
R₁ and R₃ are each independently a group according to the chemical structure:



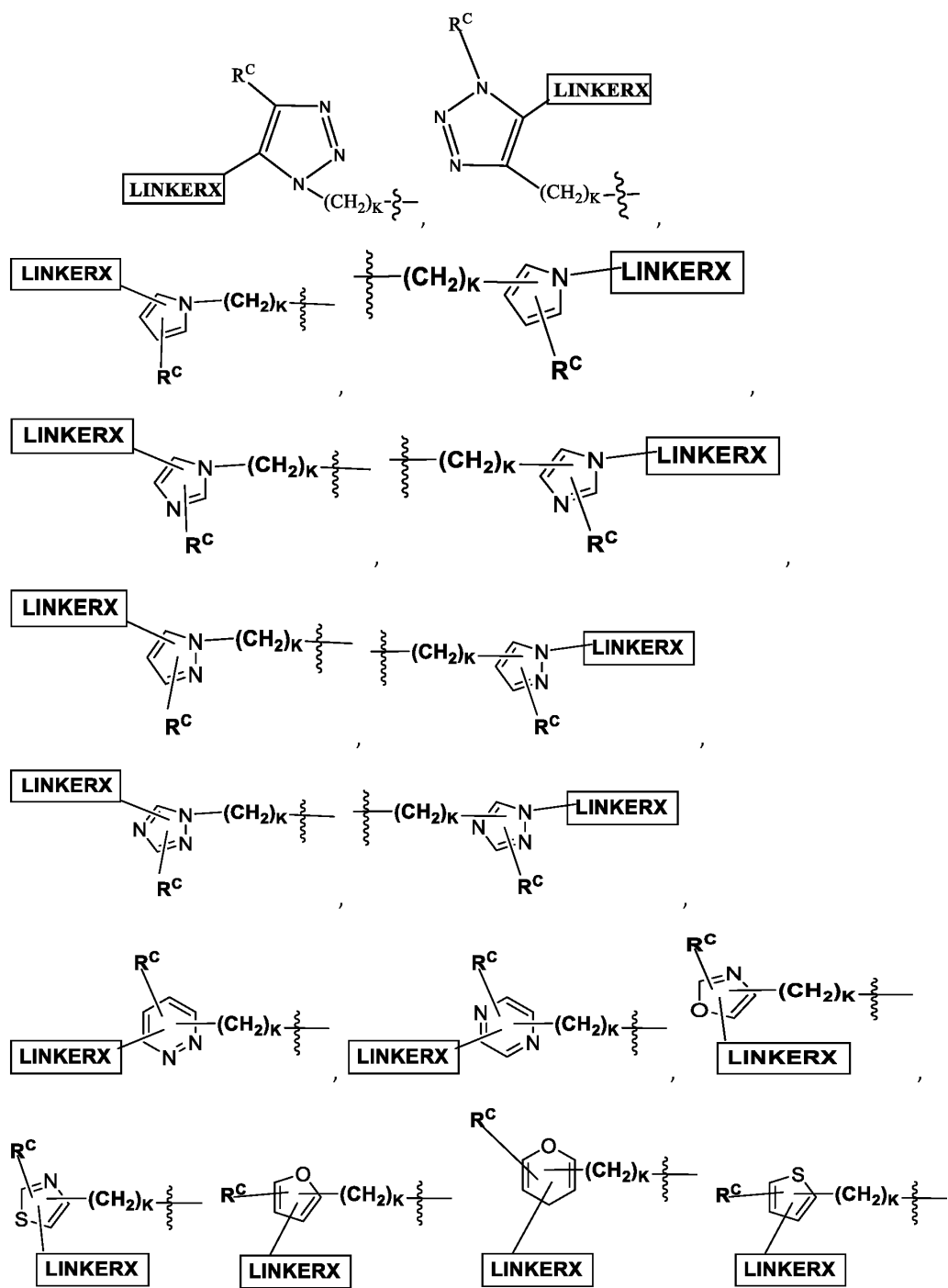
substituted with from 1 to 3 halo groups, preferably F and 1 or 2 hydroxy groups, or R⁷ is a

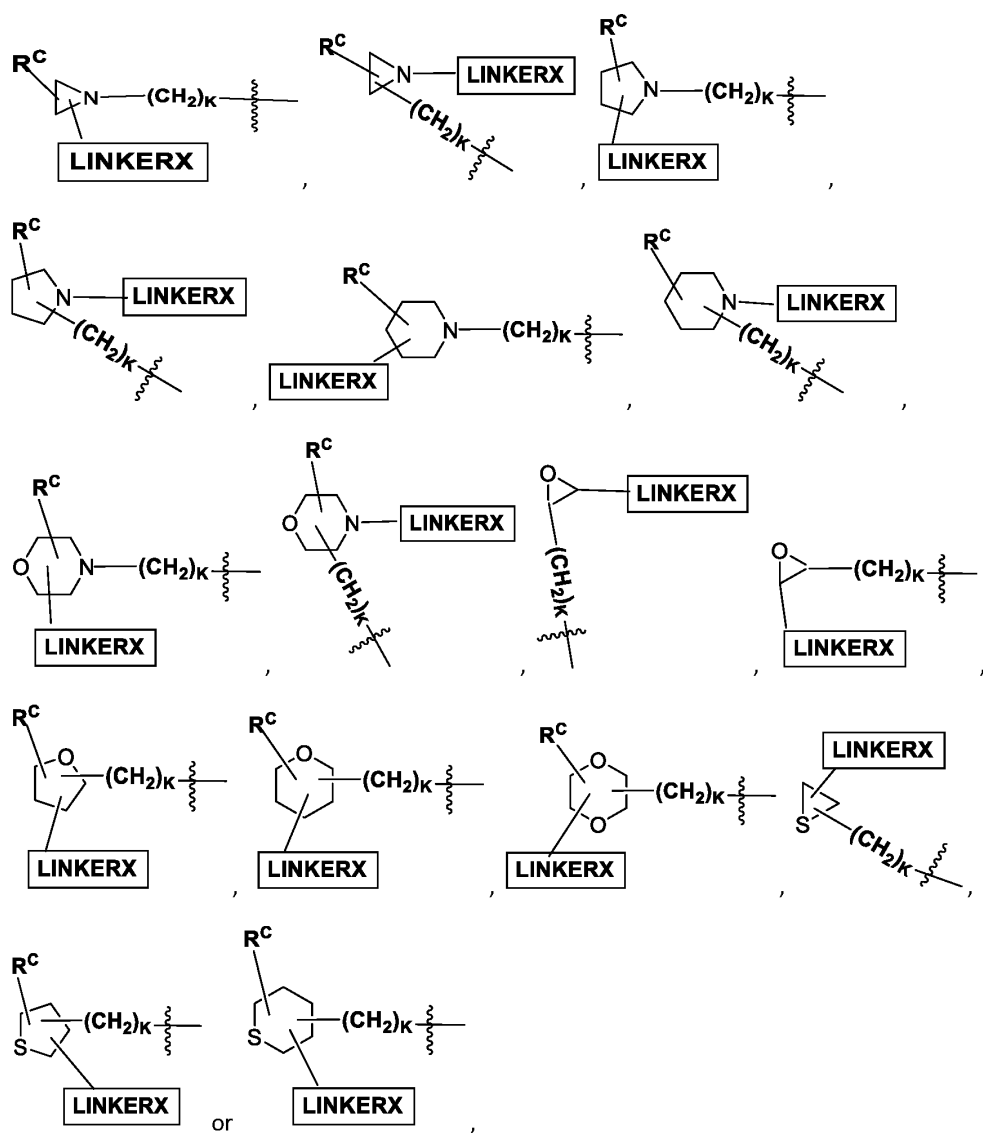


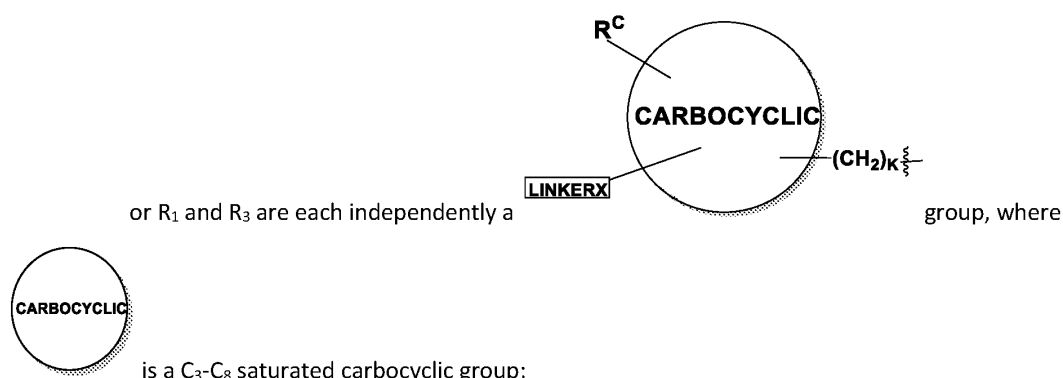
R_1 and R_3 are each independently a group according to the structure:



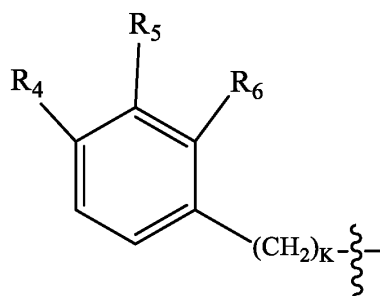
group according to the chemical structure:





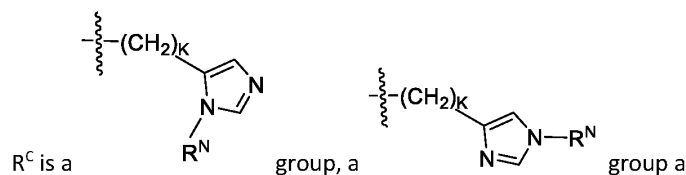


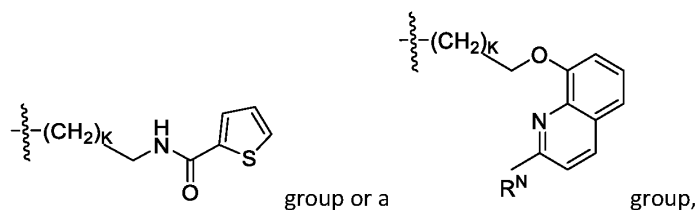
R^C is absent, H, C₁-C₄ alkyl which is optionally substituted with from 1-3 halo (preferably fluoro) groups or 1-2 hydroxyl groups, or a group according to the structure:



where R_4 , R_5 and R_6 are each independently, H, halo (F, Cl, Br, I), CN, $NR^{N1}R^{N2}$,

$-(CH_2)_KOH$, $-(CH_2)_KOC_{1-4}$ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, C₁-C₃ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, $-O-C_{1-3}$ -alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, $-(CH_2)_KCOOH$, $-(CH_2)_KC(O)O-C_{1-4}$ alkyl which is optionally substituted with from 1-3 halo, preferably F groups, $O-C(O)-C_{1-4}$ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, $-C(O)-C_{1-4}$ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, or





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

K is independently 0-4 (0, 1, 2, 3 or 4), preferably 0 or 1;

K' is 1-4, preferably 1;

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

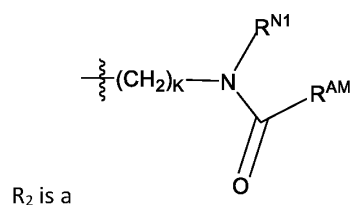
R^{N4} is H, a C_1 - C_3 alkyl group which is optionally substituted with 1-3 halo groups, preferably F or 1 or 2 hydroxy groups, or R^{N4} is a group, where K is preferably 1;

LINKERX

is a linker group which comprises at least one IgG4-binding moiety and links the at least one IgG4-binding moiety to the cellular receptor-binding moiety through the optional linker moiety, or

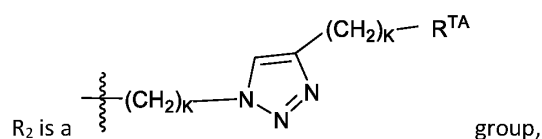
LINKERX

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 IgG4-binding moiety or optional linker moiety;

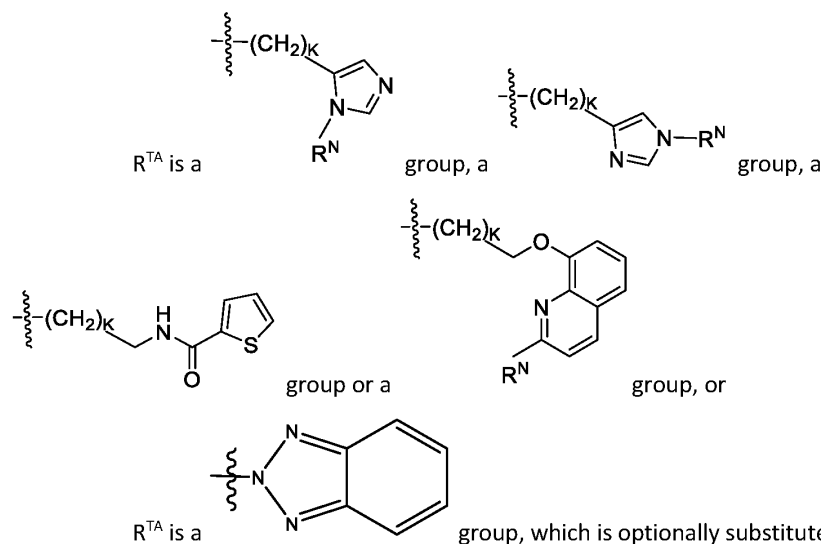


R^{AM} is H, a C_1 - C_4 alkyl group optionally substituted with up to 3 halo groups (preferably F) and one or two hydroxyl groups, a $-(CH_2)_KCOOH$ group, a $-(CH_2)_KC(O)O$ - C_1 - C_4 alkyl group which is optionally substituted with from 1-3 halo, preferably F groups,

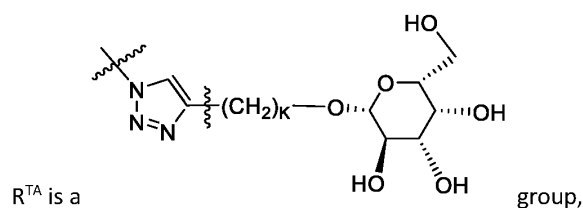
$O-C(O)$ - C_1 - C_4 alkyl group, which is optionally substituted with from 1-3 halo, preferably F groups, a $-C(O)$ - C_1 - C_4 alkyl group, which is optionally substituted with from 1-3 halo, preferably F groups, a $-(CH_2)_K-NR^{N3}R^{N4}$ group where R^{N3} is H, or a C_1 - C_3 alkyl group which is optionally substituted with 1-3 halo groups, preferably F or 1 or 2 hydroxy groups, or



wherein R^{TA} is H, CN, $NR^{N1}R^{N2}$, $-(CH_2)_kOH$, $-(CH_2)_kOC_1-C_4$ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, $-(CH_2)_kCOOH$, $-(CH_2)_kC(O)O-C_1-C_4$ alkyl which is optionally substituted with from 1-3 halo, preferably F groups, $O-C(O)-C_1-C_4$ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, $-C(O)-C_1-C_4$ alkyl, which is optionally substituted with from 1-3 halo, preferably F 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, $NR^{N1}R^{N2}$, $-(CH_2)_kOH$, $-(CH_2)_kOC_1-C_4$ alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups, C_1-C_3 alkyl, which is optionally substituted with from 1-3 halo (F, Cl, Br, I, preferably F) groups or 1 or 2 hydroxy groups, $-O-C_1-C_3$ -alkyl, which is optionally substituted with from 1-3 halo, preferably F groups, $-(CH_2)_kCOOH$, $-(CH_2)_kC(O)O-C_1-C_4$ alkyl which is optionally substituted with from 1-3 halo, preferably F groups, $O-C(O)-C_1-C_4$ alkyl, which is optionally substituted with from 1-3 halo, preferably F groups or $-(CH_2)_kC(O)-C_1-C_4$ alkyl which is optionally substituted with from 1-3 halo, preferably F groups, or



preferably 1 C_1-C_3 alkyl groups which are optionally substituted with up to three halo (preferably F) groups, or



wherein R^N, R^{N1} and R^{N2} are each independently H or a C₁-C₃ alkyl group which is optionally substituted with from one to three halo groups, preferably F, or one or two hydroxyl groups and wherein each -(CH₂)_K group is optionally substituted with 1-4, preferably 1 or 2, C₁-C₃ alkyl groups which are optionally substituted with from 1-3 fluoro groups or 1-2 hydroxyl groups; and K is independently 0-4 (0, 1, 2, 3 or 4), preferably 0 or 1, or a pharmaceutically acceptable salt, stereoisomer, solvate or polymorph thereof.

[00213] 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})-

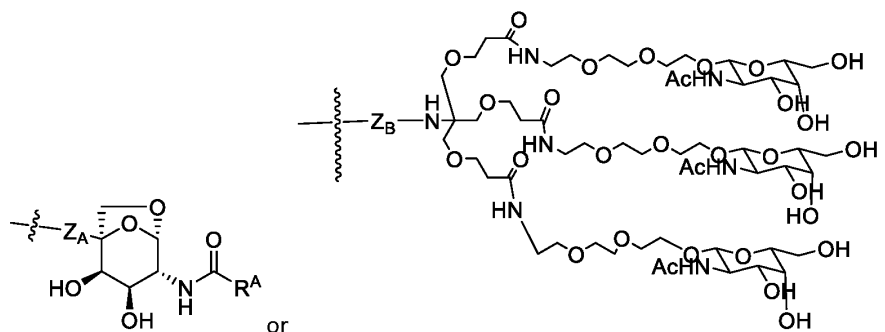
C(R^{N1})(R^{N1})-N(R^{N1})-C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1}), C(R^{N1})(R^{N1})-C(R^{N1})(R^{N1})-N(R^{N1}), N(R^{N1})-C(R^{N1})(R^{N1})-N(R^{N1}) or

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 above.

[00214] In an embodiment, X is OCH₂ or CH₂O and R^{N1} is H.

[00215] The cellular receptor-binding moiety may have the following structure:



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

[00216] In an embodiment, R^A may be a methyl or ethyl group which is optionally substituted with from 1-3 fluoro groups.

[00217] In an embodiment, Z_A may be a PEG group containing from 1 to 4 ethylene glycol residues.

[00218] In an embodiment, the methyl or ethyl group may be substituted with from 1-3 fluoro groups.

[00219] In an embodiment, the ASGPR-binding group may be N-acetyl-D-galactosamine.

[00220] In some embodiments, the cellular receptor-binding moiety can be selected from among the following compounds:

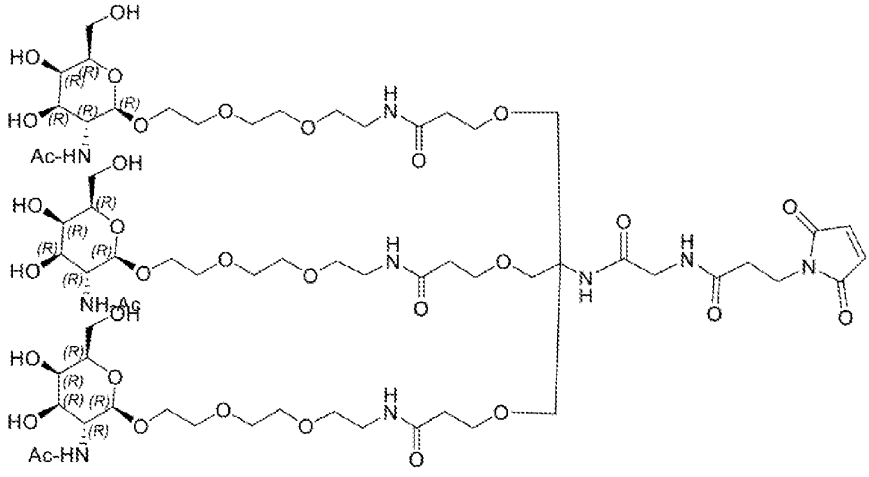
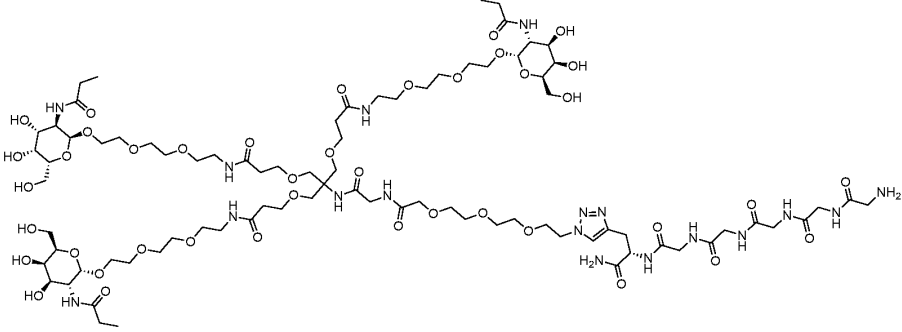
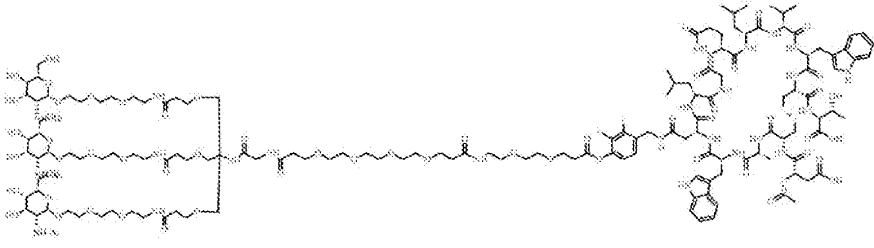
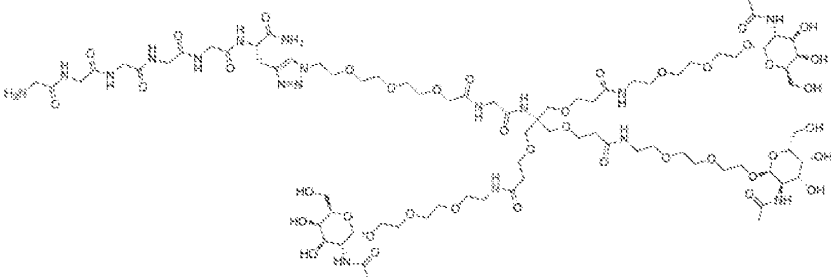
TABLE 12	
ID NO.	Chemical Structure
TBT103	 Chemical structure of TBT103, a complex molecule featuring three sugar units (likely glucose) linked by ether and amide bonds. The structure includes multiple hydroxyl groups, amide linkages, and a terminal amide group.
TBT104	 Chemical structure of TBT104, a complex molecule featuring multiple sugar units (likely glucose) linked by ether and amide bonds. The structure includes multiple hydroxyl groups, amide linkages, and a terminal amide group.
TBT105	 Chemical structure of TBT105, a complex molecule featuring multiple sugar units (likely glucose) linked by ether and amide bonds. The structure includes multiple hydroxyl groups, amide linkages, and a terminal amide group.

TABLE 12	
ID NO.	Chemical Structure
TBT106	

Pharmaceutical composition; pharmaceutically acceptable excipients

[00221] 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 methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[00222] 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 adjustment of 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 making the agents.

[00223] Agents of this specification may be prepared or isolated by synthetic and/or semi-synthetic methods or recombinant methods in accordance with 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 Intl. Pat. Publ. WO 2019/023501, which is incorporated in this specification in its entirety by reference.

[00224] 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 with respect to amino acid residue sites, and product compositions usually have several different types of agents which may differ from each other regarding number of target-binding moieties conjugated and/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.

[00225] 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.

[00226] 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.

[00227] 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.

[00228] In some embodiments, L^{LG2} is $-N(R')-$. In some embodiments, L^{LG2} is $-N(R)-$. In some embodiments, L^{LG2} is $-NH-$.

[00229] 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 $-CH_2NH-C(O)-CH_2-$.

[00230] 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 in accordance with this specification. In some embodiments, an electron-withdrawing group is halogen. In some embodiments, an electron-withdrawing group is $-F$. In some embodiments, it is $-Cl$. In some embodiments, it is $-Br$. In some embodiments, it is $-I$. In some 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 some embodiments, Y is S. In some embodiments, X is C. In some embodiments, X is N. In some embodiments, X is P. In some embodiments, X is S. In some embodiments, $X=Y$ is $C=O$. In some embodiments, $X=Y$ is $N=O$. In some embodiments, $X=Y$ is $S=O$. In some embodiments, $X=Y$ is $P=O$. In some 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 $-P(O)[N(R')_2]_2$.

[00231] 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} .

[00232] 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.

[00233] In some embodiments, a first group, an antibody-binding moiety, and/or LG is released as part of a compound having the structure of $R^{LG}-L^{LG1}-L^{LG2}-H$ or a salt thereof.

[00234] 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 $-S-$.

[00235] 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.

[00236] 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 $Rc-(Xaa)z-$.

[00237] In some embodiments, target-binding moieties may be conjugated to antibody moieties optionally through linker moieties using technologies described in U.S. Pat. Publ. 2020/0190165.

[00238] 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 hereby incorporated in this specification by reference.

[00239] 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, and the like.

[00240] 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-butyltrimethylsilyl, t-butylphenylsilyl, 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.

[00241] 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, and the like. Examples of such groups include t-butyloxycarbonyl (BOC), ethyloxycarbonyl, methyloxycarbonyl, trichloroethyloxycarbonyl, allyloxycarbonyl (Alloc), benzyloxycarbonyl (CBZ), allyl, phthalimide, benzyl (Bn), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzoyl, and the like.

[00242] Persons having ordinary skill in the biomedical art know that the 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 stereo-enriched or stereopure isomers of those compounds, including but not limited to high performance liquid chromatography, chiral high performance liquid chromatography, 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.

[00243] 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 of the aforementioned techniques, and certain methods for synthesizing compounds of this specification are described below in the Exemplification.

[00244] Persons having ordinary skill in the biomedical art know that 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 in accordance with this specification; certain such conditions, reaction times, assessment, etc. are described in this specification.

[00245] 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.

[00246] In some embodiments, linker moieties or a part thereof connected to target-binding moieties and/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.

[00247] Reaction partners, e.g., compounds of formula **AGN301** or salts thereof, rarely contain 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 and/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, and/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 portions thereof, such as R^{LG} , L^{LG} , L^{LG1} , L^{LG2} , L^{LG3} , L^{LG4} , L^{RG1} , L^{RG2} , L^{RM} , and/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.

[00248] In some embodiments, reactions are performed in buffer systems. In some embodiments, buffer systems of present disclosure maintain structures and/or functions of target agents, cellular receptor-binding moiety, etc. In some embodiments, a buffer is a phosphate buffer. In some embodiments, a buffer is a 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 some 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.

[00249] 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 located 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 and/or in one pot. Thus, in many embodiments, no separate reactions/steps are performed to remove antibody-binding moieties. Persons having ordinary skill in the biomedical art know that by performing connection of cellular receptor-binding moiety and release of antibody-binding moiety in a single reaction/operation, the 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, and/or post-translational modifications, e.g., glycans of antibodies) thereof. In some embodiments, provided technologies can provided improved efficiency, e.g., in terms of reaction rates and/or conversion percentages), increased yield, increased purity/homogeneity, and/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.

[00250] 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, and/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 located between an antibody-binding moiety and a cellular receptor-binding moiety.

[00251] 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 provides compositions of such agents.

[00252] 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).

[00253] GN3-maleimide reagent (maleimide-GN3/linker), with a C-terminal Cys for conjugation.

MATES

[00254] As used herein, the term “MATE” refers to Multimodal Antibody Therapy Enhancer, a next-generation antibody conjugation technology that enables site-directed pairing with therapeutic monoclonal antibodies (mAbs) or therapeutic immunoglobulin (IG) pooled from donors. 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.

[00255] This agent may be called a MATE agent or MATE. The MATE agents are described, for example, in Intl. Pat. Publ. WO 2021/102052, 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.

[00256] In one embodiment, the IgG4-binding moiety is a Thermo Scientific® CaptureSelect® Biotin Anti-IgG4 (Human) Conjugate (Catalog number 7102902500 or Catalog Number 7102902100). CaptureSelect® Biotin anti-IgG4 (Hu) Conjugate has a 12 kDa recombinant single domain antibody fragment (VHH affinity ligand) that specifically binds to the Fc part of the human IgG4 subclass. The inventors bound the 12 kDa recombinant antibody fragment by a biotin conjugation. The VHH affinity ligand comprises three complementarity-determining regions (CDRs) that form the antigen-binding domain. The affinity ligand is chemically conjugated to biotin via an appropriate spacer that retains the binding reactivity of the ligand when used in combination with streptavidin-based conjugates or streptavidin pre-coated surfaces.

Exemplary procedure for conjugation and reaction kinetics analysis.

[00257] Buffer exchange: Five mg protein was buffer-exchanged by Amicon® filters and concentrated to target concentration.

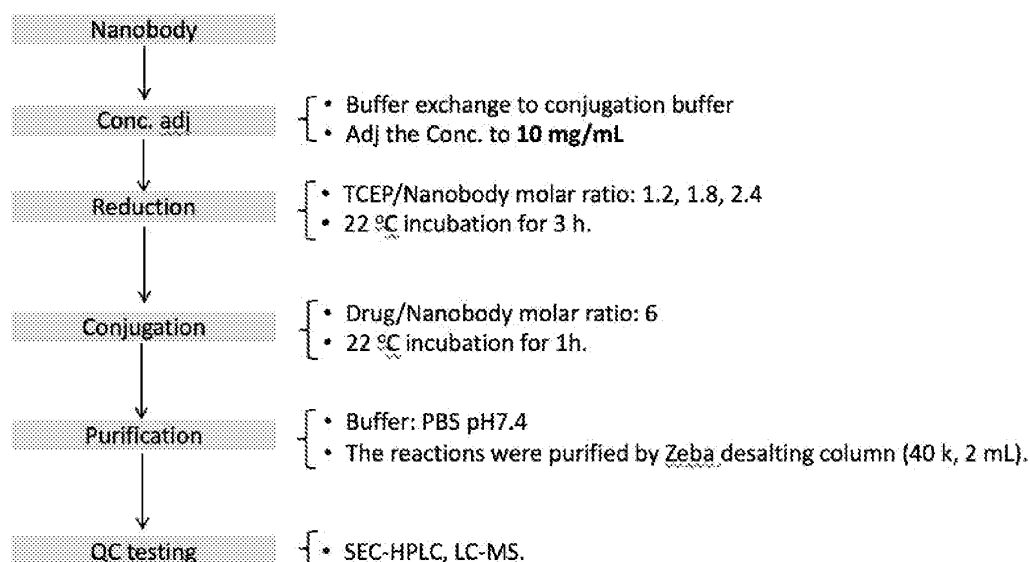
[00258] Conjugation: 1.5 mg of concentrated antibody was added to a 1.5 mL tube, reagent (10 mM in dimethyl sulfoxide) was added. The total dimethyl sulfoxide concentration in the reaction mixture was 3.74%. The concentration of the reaction is 10 mg/mL.

[00259] Incubation: The tube was allowed to approach ambient temperature and then incubated at 25°C.

[00260] Sampling and Analysis: At each of the seven reaction times (zero hours, one hour, two hours, three hours, four hours, five hours, and six hours), 10 µL of sample was quenched with four equivalents propylamine and then frozen at -80°C. After the last sample was quenched and frozen at -80°C for a few minutes, the samples were thawed one by one and analyzed by intact liquid chromatography-mass spectrometry (LC-MS) to obtain MS-DAR. Size exclusion chromatography- high performance liquid chromatography was also acquired to determine purity.

[00261] Using 50 mM HEPES, pH 7.0 as the reaction buffer, the ADC reacting dramatically at the first two hours, and 3x reagent was close to DAR6 in five hours. Measurement by liquid chromatography-mass spectrometry.

[00262] Different degrees of aggregation occurred while using 50 mM HEPES, pH 7.0 as the reaction buffer and quenching with four equivalents propylamine. Measurement by size exclusion chromatography.

[00263] Conjugation process protocol.Plurality of agents. Second agent.

[00264] 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.

[00265] 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 modified. 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.

[00266] 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 some embodiments, a second reactive group is a function group of an amino acid residue, e.g., $-NH_2$ of Lys, $-SH$ of Cys, etc. In some embodiments, a second reactive group is $-NH_2$ 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 an IgG4 heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00267] 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 locations of an antibody moiety, e.g., locations 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 IgG4 heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00268] 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 some 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 $-NH_2$ of a Lys residue of an antibody protein. In some embodiments, provided technologies can provide improved conjugation efficiency, high selectivity,

and/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.

[00269] 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.

[00270] 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.

[00271] 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., modified 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 IgG4 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.

[00272] In some embodiments, antibody heavy chains are selectively conjugated/labeled over light chains.

[00273] This specification can provide controlled cellular receptor-binding moiety, e.g., cellular receptor-binding moiety/antibody moiety ratios, e.g., for cellular receptor-binding moiety being peptide cellular receptor-binding moiety, peptide cellular receptor-binding moiety/antibody ratio (PAR). In some embodiments, a ratio is about 0.1-10, 0.5-6, etc., e.g., about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 to about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6, 7, 8, 9, 10, about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6, 7, 8, 9, 10, etc. In some embodiments, a ratio is of moieties of interest conjugated to antibody moiety and antibody moieties conjugated to moieties of interest, e.g., when a ratio is in the context of a ratio of an agent. In some embodiments, a ratio is of moieties of interest conjugated to antibody moieties and all antibodies in a composition, e.g., when a ratio is in the context of a ratio of a composition. In some embodiments, a ratio is about 0.1-6. In some embodiments, a ratio is about 0.5-2.5. In some embodiments, a ratio is about 0.5-2. In some embodiments, a ratio is about 1-2. In some embodiments, a ratio is about 1.5-2. In some embodiments, a ratio is about 1.5-2 for IgG1, IgG2, and/or IgG4 antibodies or fragments thereof. In some embodiments, for a composition, e.g., target-binding moieties conjugated to IVIG, a ratio is about 1.5-2.5. In some embodiments, a ratio is about 0.1. In some embodiments, a ratio is about 0.2. In some embodiments, a ratio is about 0.3. In some embodiments, a ratio is about 0.4. In some embodiments, a ratio is about 0.5. In some embodiments, a ratio is about 0.6. In some embodiments, a ratio is about 0.7. In some embodiments, a ratio is about 0.8. In some embodiments, a ratio is about 0.9. In some embodiments, a ratio is about 1. In some embodiments, a ratio is about 1.1. In some embodiments, a ratio is about 1.2. In some embodiments, a ratio is about 1.3. In some embodiments, a ratio is about 1.4. In some embodiments, a ratio is about 1.5. In some embodiments, a ratio is about 1.6. In some embodiments, a ratio is about 1.7. In some embodiments, a ratio is about 1.8. In some embodiments, a ratio is about 1.9. In some embodiments, a ratio is about 2. In some embodiments, a ratio is about 2.1. In some embodiments, a ratio is about 2.2. In some embodiments, a ratio is about 2.3. In some embodiments, a ratio is about 2.4. In some embodiments, a ratio is about 2.5. In some embodiments, a ratio is about 1.8 for a composition wherein antibody moieties of a plurality of agents are those of an IVIG preparation. In some embodiments, a ratio of target-binding moieties and antibody moieties is about 1.5-2 wherein antibody

moieties of a plurality of agents are those of IgG1. In some embodiments, a ratio of target-binding moieties and antibody moieties is about 1.5-2 wherein antibody moieties of a plurality of agents are those of IgG2. In some embodiments, a ratio of target-binding moieties and antibody moieties is about 1.5-2.0 wherein antibody moieties of a plurality of agents are those of IgG4. In some embodiments, a ratio is about 1.9-2.0.

[00274] 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 modifications, e.g., all share the same moieties of interest optionally connected through the same linker moieties. In some embodiments, no conjugation sites bear different modifications, e.g., different moieties of interest and/or no moieties of interest and/or different linker moieties.

[00275] 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 an IgG4 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, and/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%.

[00276] 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 modifications, 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 modifications, e.g., different moieties of interest and/or no moieties of interest and/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 modifications, e.g., different moieties of interest and/or no moieties of interest and/or different linker moieties. In some embodiments, agents that share the same (or substantially the same) antibody moieties but different modifications, e.g., different moieties of interest and/or no moieties of interest and/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.

[00277] 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 IgG4 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.

[00278] 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.

[00279] 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.

[00280] 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.

[00281] 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.

[00282] 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 some 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%.

[00283] 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.

[00284] 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 IgG4 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 IgG4 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.

[00285] 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%.

[00286] 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%.

[00287] In some embodiments, antibody moiety of agents of a plurality comprises a common Fc region or a fragment thereof.

[00288] In some embodiments, moieties of interest of agents of a plurality are at particular 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 IgG4 heavy chain and amino acid residues corresponding thereto.

[00289] 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.

[00290] In some embodiments, agents share a common modification at least one common amino acid residue. In some embodiments, agents of a plurality share a common modification 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.

[00291] 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., about or 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 IgG4 heavy chain and amino acid residues corresponding thereto.

[00292] 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%.

[00293] 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.

[00294] In some embodiments, provided agents, compounds, etc., e.g., those of formula **AGN301**, **AGN101**, **AGN102**, etc. and salts thereof have high purity. In some embodiments, a percentage is about 5%-100%. In some embodiments, a percentage is about 10%-100%. In some embodiments, a percentage is about 20%-100%. In some embodiments, a percentage is about 25%-100%. In some embodiments, a percentage is about 30%-100%. In some embodiments, a percentage is about 40%-100%. In some embodiments, a percentage is about 50%-100%. In some embodiments, it is about 5%. In some embodiments, it is about 10%. In some embodiments, it is about 20%. In some embodiments, it is about 25%. In some embodiments, it is about 30%. In some embodiments, it is about 40%. In some embodiments, it is about 50%. In some embodiments, it is about 60%. In some embodiments, it is about 70%. In some embodiments, it is about 80%. In some embodiments, it is about 90%. In some embodiments, it is about 91%. In some embodiments, it is about 92%. In some embodiments, it is about 93%. In some embodiments, it is about 94%. In some embodiments, it is about 95%. In some embodiments, it is about 96%. In some embodiments, it is about 97%. In some embodiments, it is about 98%. In some embodiments, it is about 99%. In some embodiments, it is about 100%. In some embodiments, it is at least about 5%. In some embodiments, it is at least about 10%. In some embodiments, it is at least about 20%. In some embodiments, it is at least about 25%. In some embodiments, it is at least about 30%. In some embodiments, it is at least about 40%. In some embodiments, it is at least about 50%. In some embodiments, it is at least about 60%. In some embodiments, it is at least about 70%. In some embodiments, it is at least about 80%. In some

embodiments, it is at least about 90%. In some embodiments, it is at least about 91%. In some embodiments, it is at least about 50%. In some embodiments, it is at least about 92%. In some embodiments, it is at least about 93%. In some embodiments, it is at least about 94%. In some embodiments, it is at least about 95%. In some embodiments, it is at least about 96%. In some embodiments, it is at least about 97%. In some embodiments, it is at least about 98%. In some embodiments, it is at least about 99%.

[00295] 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 and/or formed product agents. Several technologies are available to separate released antibody-binding moieties from antibody moieties in accordance with 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

[00296] 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 located 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.

[00297] 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 demonstrated 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, and/or improve reaction selectivity, e.g., in terms of target sites wherein conjugation of moieties of interest with target agents occurs.

[00298] 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., $-\text{NH}_2$ 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, K 253, etc. for IgG2, K239, K241 for IgG4, etc. In some embodiments, a site is K246 or K248 of an antibody heavy chain. In some embodiments, sites are K246 and/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 and/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 and/or K241 of an IgG4 heavy chain. In some embodiments, a site is K239 of an IgG4 heavy chain. In some embodiments, a site is K241 of an IgG4 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.

[00299] 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.

[00300] In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{RG1}}-\text{L}^{\text{RG2}}-$, wherein each of L^{RG1} and L^{RG2} is independently L. In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{LG4}}-\text{L}^{\text{RG1}}-\text{L}^{\text{RG2}}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{LG3}}-\text{L}^{\text{LG4}}-\text{L}^{\text{RG1}}-\text{L}^{\text{RG2}}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{LG2}}-\text{L}^{\text{LG3}}-\text{L}^{\text{LG4}}-\text{L}^{\text{RG1}}-\text{L}^{\text{RG2}}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{LG4}}-\text{L}^{\text{RG2}}-$, wherein each variable is as described in this specification. In some embodiments, a reactive group, e.g., RG, is or comprises $-\text{L}^{\text{LG3}}-\text{L}^{\text{LG4}}-\text{L}^{\text{RG2}}-$, 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.

[00301] In some embodiments, L^{LG4} is $-O-$. In some embodiments, L^{LG4} is $-N(R)-$. In some embodiments, L^{LG4} is $-NH-$.

[00302] 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.

[00303] 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 $-S(O)_2-$.

[00304] 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.

[00305] 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 some 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} .

[00306] In some embodiments, R^{RG1} is $-H$. In some embodiments, R^{RG3} is $-H$.

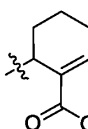
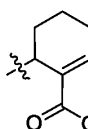
[00307] In some embodiments, L^{RG2} is optionally substituted $-L^{RG3}-C(=CHR^{RG2})-CHR^{RG4}-$, wherein each variable is as described in this specification.

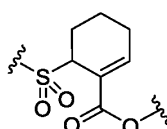
[00308] In some embodiments, R^{RG2} and R^{RG4} are taken together with their intervening atoms to form an optionally substituted ring as described in this specification. In some embodiments, a formed ring is an optionally substituted 3-10-membered monocyclic or bicyclic ring having 0-5 heteroatoms. 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 $C(=CR^{RG1}R^{RG2})$.

[00309] 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.

[00310] 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 $-S-C(O)-$.

[00311] 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.

[00312] 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 $-OP(O(N(R)_2))-$.

[00313] 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 $-OP(O(N(R)_2))-$.

[00314] 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.

[00315] 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 $-NH_2$.

[00316] In some embodiments, reactive groups, e.g., a first reactive group, a second reactive group, etc., are located 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.

[00317] In some embodiments, the invention provides methods for preparing a composition comprising a plurality of agents, wherein each agent independently comprises:

an antibody moiety,

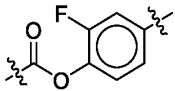
a cellular receptor-binding moiety, and

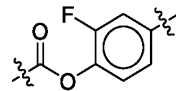
optionally a linker moiety linking an antibody moiety and a cellular receptor-binding moiety;

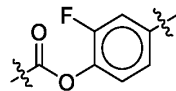
which method comprise:

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

[00318] 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 and/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.

[00319] 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 a variety of 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 in accordance with this specification.

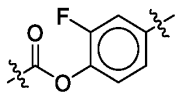
[00320] In some embodiments, antibody-binding moieties bind to Fc regions of antibodies. In some embodiments, reactions occur at residues at Fc regions. In some embodiments, target-binding moieties are conjugated to residues of Fc regions, optionally through linker moieties. In some embodiments, a residue 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.

[00321] 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.

[00322] 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

and/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., residence 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 and/or prolonged half-life. In some embodiments, provided technologies are useful for modifying blood-derived IgG products as provided technologies are suitable for and can use all IgG subclasses.

[00323] In some embodiments, a provided method comprises one of the steps 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.

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

[00324] Protein sequencing. In one method of protein sequencing, eight digestions were prepared using five different enzymes (pepsin, Lys C, trypsin, chymotrypsin, Asp N). The digestions for the sample were 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 were characterized from LC-MS/MS data using de nova peptide sequencing and then assembled into antibody sequences.

Affinity chromatography

[00325] Affinity capture by MabSelect SuRe™ resin for ABT310. Purify at room temperature. Choose the column based on titer, recommended load density (about 20 mg/ml resin). Equilibrate, five column volumes, with the buffer of 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH 7.5. Load sample.

[00326] EQ wash: Wash with the buffer of 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH 7.5 for three-five column volumes.

[00327] Triton wash: Wash with 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH7.5, 0.05% Triton100, 0.05% Triton 114 for five-ten column volumes.

[00328] EQ wash. Wash with 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH 7.5 buffer for five-ten column volumes. Elute with elution buffer 0.1 M histidine-acetate. Neutralize immediately with 1 M arginine, 400 mM succinate, pH9.0.

[00329] Testing for affinity capture eluates: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) non-reducing and reducing, and size exclusion chromatography-high performance liquid chromatography.

[00330] Affinity capture by Amsphere™ A3 resin for **ABT502** and **ABT503**. Purify at room temperature. Choose the column based on titer, recommended load density (5-10 mg/ml resin). Equilibrate, five column volumes, with the buffer of 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH 7.5. Sample Load.

[00331] EQ wash: Wash with the buffer of 25 mM Tris-HCl, 150 mM sodium chloride, 5 mM EDTA, pH 7.5 for two-five column volumes. Elute with elution buffer 50 mM sodium citrate, 150 mM sodium chloride, pH3.0. Neutralize immediately with 1 M Tris-HCl, pH 9.0.

[00332] Testing for affinity capture eluates: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) non-reducing and reducing, and size exclusion chromatography-high performance liquid chromatography.

[00333] Results: VHH-Cys (**ABT502** and **ABT503**) and VHH-Fc fusion (**ABT310**) purification. Mixed culture of cells was collected on the 7th day or when viability <60%. Cell culture fluid was clarified via centrifugation at 10,000 g for 0.5 h and sterile filtered. **ABT310** was purified via affinity capture chromatography by MabSelect SuRe™ resin (Cytiva), and **ABT502** and **ABT503** via affinity capture chromatography by Amsphere A3 Resin (JSR Life Sciences). MabSelect SuRe™ and Amsphere A3 columns were washed extensively (25 mM Tris-HCl, pH 7.5, 150 mM sodium chloride, 5 mM EDTA), and eluted via 100 mM 50 histidine-acetate, and 50 mM sodium citrate, pH 3.0, 150 mM sodium chloride, respectively. MabSelect SuRe™ eluate was neutralized in 1 M arginine, 400 mM succinate, pH 9.0, and Amsphere A3 resin, 1 M Tris-HCl, pH 9.0.

Size exclusive chromatography

[00334] Purify at room temperature. Choose the column based on protein amount and molecule weight.(Superdex 200 pg for **ABT310**, Superdex 75 pg for **ABT502** and **ABT503**). Equilibrate with a buffer

of 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5 for one column volume. Sample load. Elution with a buffer of 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5 for one column volume. Collect target protein with desirable purity.

[00335] Testing for size exclusion chromatography eluates: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) non-reducing and reducing, and size exclusion chromatography-high performance liquid chromatography.

[00336] Results: VHH-Cys (ABT502 and ABT503) and VHH-Fc fusion (ABT310) purification. **ABT310** and **ABT502** or **ABT503** samples were applied to Superdex 200 pg (Cytiva) or Superdex 75 pg (Cytiva), respectively, and eluted in 20 mM histidine-acetate, pH 5.5, 150 mM sodium chloride. Fractions were then resolved and pooled for application to cation exchange column Poros® XS (Thermo Fisher), pre-equilibrated with 20 mM sodium acetate, pH 5.5 (**ABT310**), or 20 mM sodium acetate, pH 5.0 (**ABT502** or **ABT503**). Columns were washed extensively and eluted over fifty columns gradient to 100% 20 mM sodium acetate, pH 5.0/5.5, 1 M sodium chloride. Fractions were resolved and analyzed by size exclusion chromatography-high performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis reducing and non-reducing, endotoxin level testing, and liquid chromatography-mass spectrometry to resolve molecular mass.

Cation exchange chromatography

[00337] Purify at room temperature. Use Poros® XS column. Choose the column based on protein amount, recommended load density (10-20 mg/ml resin). For **ABT310**, the cation exchange chromatography pH is 5.5. For **ABT502** and **ABT503**, the cation exchange chromatography pH is 5.0. Dilute the sample below 5 mS/cm with 20 mM sodium acetate, pH 5.5/5.0. Equilibrate, five column volumes, with the buffer of 20 mM sodium acetate, pH 5.5/5.0. Sample Load.

[00338] EQ wash. Wash with the buffer of 20 mM sodium acetate, pH 5.5/5.0 for 1-5 column volumes. Elute with gradient 0-100% fifty column volumes 20 mM sodium acetate, 1 M sodium chloride, pH 5.5/5.0 (The scale-up gradient can be adjusted based on the pilot run). Strip with 20 mM sodium acetate, 1 M sodium chloride, pH 5.5/5.0 for one-three column volumes. Equilibrate, one column volume, with the buffer of 20 mM sodium acetate, pH 5.5/5.0. Sanitize with 0.5 M sodium hydroxide for thirty minutes. Equilibrate, three-five column volumes, with the buffer of 20 mM sodium acetate, pH 5.5/5.0.

[00339] Testing for cation exchange chromatography eluates: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) non-reducing and reducing, and size exclusion chromatography-high performance liquid chromatography.

SEC-HPLC

[00340] The inventors performed 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). The mobile phase consists of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8. Inject 25 µg of sample per run. Perform isocratic elution for fifteen minutes at a flow rate of 1.0 mL/minute. 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

[00341] The inventors performed non-reducing and reducing Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis using precast NuPAGE™ 4-12% bis-Tris Gel from Thermo Scientific. Sample loading buffer (4x lithium dodecyl sulfate) is from Invitrogen. Gel running buffer (20x MES) is from GenScript. Treat non-reducing samples with 30 mM iodoacetamide and heat at 95°C for five minutes before analysis. Treat reducing samples with 50 mM dithiothreitol 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.

Molecular mass analysis by LC-MS

[00342] Conduct liquid chromatography-mass spectrometry analysis using Agilent 6230 AdvanceBio LC/TOF system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502).

[00343] 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 dithiothreitol (Sigma/D0632) in a total volume of 50 µL. Samples were incubated at 37°C for twenty minutes, then subjected to LC/MS. The inventors analyze the mass spectroscopy data using Bioconfirm 10.0 software and identify molecules based on molecular masses.

Endotoxin level testing:

[00344] Dilute purified proteins based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110). Load samples into Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS11F) and detect them using Charles River Laboratories Endosafe nexgen-MCS™ instrument.

Method of assessing agent for administration

[00345] Persons having ordinary skill in the biomedical art can analyze an agent formulation for appearance, color, and achromicity using the criteria described by the United States Pharmacopeia–National Formulary in USP <631> or by the European Pharmacopoeia in Ph. Eur. 2.2.2.

[00346] Persons having ordinary skill in the biomedical art can analyze an agent formulation for appearance and clarity using the criteria described by the United States Pharmacopeia–National Formulary in USP <1061> or by the European Pharmacopoeia in Ph. Eur. 2.2.2.

[00347] Persons having ordinary skill in the biomedical art can analyze an agent formulation for pH using the criteria described by the United States Pharmacopeia–National Formulary in USP < <791> or by the European Pharmacopoeia in Ph. Eur. 2.2.3. A generally accepted criterion is ± 0.5 pH of the targeted pH.

[00348] Persons having ordinary skill in the biomedical art can analyze the drug product for osmolality using the criteria described by the United States Pharmacopeia–National Formulary in USP <785> or by the European Pharmacopoeia in Ph. Eur. 2.2.35.

[00349] Persons having ordinary skill in the biomedical art can quantify the concentration of an agent by UV-VIS spectroscopy, e.g., at A₂₈₀ (280 nm). A generally accepted criterion is $\pm 10\%$ of the targeted concentration.

[00350] Persons having ordinary skill in the biomedical art can confirm the Identity of an agent by the charge variant peak retention time or pI as measured by imaged capillary isoelectric focusing (iCIEF), a high-resolution technique that separates proteins into groups based on their isoelectric point (pI).

[00351] Persons having ordinary skill in the biomedical art can confirm the purity of the agent by the hydrophobic interaction chromatography-high performance liquid chromatography method. For example, the persons having ordinary skill in the biomedical art can confirm the DAR of the agent. A generally accepted criterion is $\pm 10\%$ of the targeted concentration.

[00352] Persons having ordinary skill in the biomedical art can confirm the purity of an agent formulation by the size exclusion chromatography-high performance liquid chromatography method. For example, the persons having ordinary skill in the biomedical art can confirm the percentage of a monomer, high molecular weight, and low molecular weight. A generally accepted criterion is a result of $\geq 90\%$ or $\leq 5\%$.

[00353] Persons having ordinary skill in the biomedical art can confirm the purity of an agent formulation by the CE-SDS-NR method. For example, the persons having ordinary skill in the biomedical

art can confirm the percentage of a main peak and fragment peaks. A generally accepted criterion is a result of $\geq 85\%$.

[00354] Persons having ordinary skill in the biomedical art can confirm the purity of an agent formulation by the CE-SDS-R method. For example, the persons having ordinary skill in the biomedical art can confirm the percentage of monomer and Minor species peaks. A generally accepted criterion is a result of $\geq 85\%$.

[00355] Persons having ordinary skill in the biomedical art can confirm the purity of an agent formulation by the imaged capillary isoelectric focusing method. For example, the persons having ordinary skill in the biomedical art can confirm the percentage of charge variants.

[00356] Persons having ordinary skill in the biomedical art can confirm the potency of an agent for binding by the ELISA method. A generally accepted criterion is $\pm$ a defined percentage as compared to a reference standard.

[00357] Persons having ordinary skill in the biomedical art can detect a process impurity (HCP) in an agent formulation by the ELISA method. A generally accepted criterion is ≤ 100 ng/mg.

[00358] Persons having ordinary skill in the biomedical art can detect a process impurity (rProtein A) in an agent formulation by the ELISA method. A generally accepted criterion is ≤ 50 ng/mg.

[00359] Persons having ordinary skill in the biomedical art can detect an rDNA impurity in an agent formulation by the reverse transcriptase PCR method. A generally accepted criterion is < 10 ng per dose.

[00360] Persons having ordinary skill in the biomedical art can confirm the purity of an agent formulation by the RP-high performance liquid chromatography method. For example, the persons having ordinary skill in the biomedical art can confirm the presence of residual free drug & free protein intermediates.

[00361] Persons having ordinary skill in the biomedical art can analyze an agent formulation for the presence of endotoxins using the criteria described by the United States Pharmacopeia–National Formulary in USP <85> or by the European Pharmacopoeia in Ph. 2.6.14. A generally accepted criterion is dose dependent based on < 5 EU/kg.

[00362] Persons having ordinary skill in the biomedical art can analyze a drug substance for its bioburden using the criteria described by the United States Pharmacopeia–National Formulary in USP <61> or by the European Pharmacopoeia in Ph. 2.6.12. A generally accepted criterion is ≤ 1 CFU/10 mL.

[00363] Persons having ordinary skill in the biomedical art can analyze a drug product for its sterility using the criteria described by the United States Pharmacopeia–National Formulary in USP <71> or by the European Pharmacopoeia in Ph. 2.6.1. A generally accepted criterion is no growth.

[00364] Persons having ordinary skill in the biomedical art can analyze a drug product for the presence of excipients using the HPLC-ELSD method. For example, the persons having ordinary skill in the biomedical art can measure the percentage of surfactants. A generally accepted criterion is $\pm 50\%$ of the targeted value.

[00365] Persons having ordinary skill in the biomedical art can analyze a drug product for the subvisible particles in a container using the criteria described by the United States Pharmacopeia–National Formulary in USP <787> or by the European Pharmacopoeia in Ph. 2.6.19. Several accepted criterion are known to persons having ordinary skill in the biomedical art.

[00366] Persons having ordinary skill in the biomedical art can analyze a drug product for the extractable volume using the criteria described by the United States Pharmacopeia–National Formulary in USP <697> or by the European Pharmacopoeia in Ph. 2.6.17.

[00367] Persons having ordinary skill in the biomedical art can analyze a drug product for Container Closure Integrity Test (CCIT), using the criteria described by the United States Pharmacopeia–National Formulary in USP <1207>. A bioburden test is usually performed at the final stability time only. A CCIT test is usually performed at repeated time intervals, e.g., annually.

Statistical analyses.

[00368] Normal distribution quantitative variables can be expressed as means and SDs and compared by an independent-samples t test, as was done by Zhang et al. (October 2019). For non-normally distributed variables, we used median and interquartile range and analyzed with the Mann-Whitney U test. Categorical data was summarized by percentages. A two-sided p value <0.05 was considered statistically significant. All statistical tests were performed using SPSS version 16.0.

[00369] $\% \text{ mol/mol} = C(\text{free drug}) / [C(\text{free drug}) + \text{BAR} \times C(\text{protein})] \times 100$, where:

BAR: drug to antibody ratio

$C(\text{free drug})$: concentration of free drug in ADC (μM)

$C(\text{protein})$: concentration of protein in 1 mg/mL ADC (μM)

$C(\text{protein}) = 1 / [MW(\text{protein})]$

EXAMPLES

[00370] The invention is further illustrated by non-limited EXAMPLES.

EXAMPLE 1Transient expression of IgG4-binding moieties in mammalian cells

[00371] The inventors seeded and passaged Chinese Hamster Ovary K1 host cells in advance. On the day of transfection, the inventors centrifuged host cells to specific cell density for transfection, then added DNA and transfection reagent (polyethylenimine) into host cell for transfection. Incubated the transfected culture by shaking at 150 rpm.

[00372] Shifted temperature from 36.5°C to 33°C at twenty-four hours post transfection. Perform feeding on day 0 and day 4, while harvest cell culture on day 7 or when VIA<60%.

[00373] Harvest and Clarification: Subject the cell culture fluid to centrifugation at 10000 g for thirty minutes and then pass through a 0.22 µm sterile filter.

[00374] Results: VHH-Cys (ABT502 and ABT503) and VHH-Fc fusion (ABT310) expression. Chinese Hamster Ovary K1 cells were cultured to target density in cell culture medium, and heavy chain and light chain vectors with transfection reagent (polyethylenimine) were transiently co-transfected. Transfected cells were cultured in a shaking flask at 150 rpm in an incubator at 36.5°C for twenty-four hours post-transfection, followed by incubation at 33°C for remainder of culture time. Feeding was performed on day 0 and day 4.

EXAMPLE 2Material generation

[00375] Determination of assay screening conditions. To optimize ligand concentrations and cell lines for screening, HEK-293T and QT6 cells were transfected with plasmids encoding known ligand targets, Protein A (binds antibody Fc; positive control), or vector alone (pUC used as negative control) in 384-well cell-culture plates. After incubation, four four-fold dilutions of each ligand starting at 20 µg/ml were added in quadruplicate to transfected cells, and bound ligand was detected using a single dilution of a fluorescently-labeled secondary antibody in a high-throughput immunofluorescence flow cytometry assay.

[00376] Average mean fluorescence intensity (MFI) values were determined for each test ligand dilution in each cell line using ForeCyt® Software (Intellicyt) and plotted using Excel® (Microsoft). The high background rate for each assayed condition was calculated as the percentage of positive events above a defined fluorescence threshold in cells transfected with negative control. Optimal screening concentrations and cell line for each test ligand were determined by the background signal (MFI), and high background rate in the vector control.

[00377] *Specific agents. Larger agents.* VHH-Fc fusion + ASGPR ligand conjugated to the Fc region via MATE. Advantages of AGT401 include the use the MATE conjugation platform, unconjugated Fc fusion is more stable, with superior depth and rate of reduction versus nanobody (bivalent). Advantages of AGT402 include low immunogenicity with well-established conjugation chemistry.

[00378] *Specific agents. Smaller agents.* VHH + ASGPR ligand conjugated via sortase mediated-ligation or C-terminal cysteine. Advantages of AGT403 and AGT404 include that they are smaller agents. VHH + ASGPR ligand conjugated via sortase mediated-ligation or C-terminal cysteine.

[00379] Non-Fc fusion degraders show IgG4-depletion. Fc fusion degrader shows IgG4-depletion at eight hours. Fc fusion degrader shows IgG4-depletion and rebound effect at twenty-four hours.

[00380] VHH production has a good titer with Chinese Hamster Ovary as a host 500 mg/L in Chinese Hamster Ovary cell. Doing 500 ml scale is useful because recovery is about 50-60% after purification 500 ml, to get 100 mg protein. The manufacturing risks dimer/aggregation during the purification.

TABLE 13
Material generation of non-Fc fusion maleimide-based degraders

<u>Molecule</u>	<u>Expected yield (mg)</u>
ABT501	about 300
ABT502	about 400
ABT503	about 400
ABT504	about 300

TABLE 14
Purification: Single step Ni-affinity (Ni Excel-SEC 75) or Protein A (MSS-SEC200) chromatography

<u>Agent</u>	<u>Concentration (mg/mL)</u>	<u>Volume (mL)</u>	<u>Amount (mg)</u>	<u>Yield (mg/L)</u>	<u>SEC-HPLC Purity (%)</u>	<u>Endotoxin (EU/mg)</u>
ABT311 (Ni Excel-SEC 75)	6.01	140.77	846.03	1692.06	100.00	<0.0126
ABT312 (Ni Excel-SEC 75)	5.19	142.17	737.86	1475.72	100.00	<0.0118
ABT304 (Ni Excel-SEC 75)	6.30	138.07	869.84	1739.68	100.00	<0.0095
ABT305 (Ni Excel-SEC 75)	5.03	139.98	704.10	1408.20	100.00	<0.0128
ABT306 (Ni Excel-SEC 75)	3.59	78.29	281.06	562.12	99.79	<0.0167

<u>TABLE 14</u> <u>Purification: Single step Ni-affinity (Ni Excel-SEC 75) or Protein A (MSS-SEC200) chromatography</u>						
<u>Agent</u>	<u>Concentration</u> <u>(mg/mL)</u>	<u>Volume</u> <u>(mL)</u>	<u>Amount</u> <u>(mg)</u>	<u>Yield</u> <u>(mg/L)</u>	<u>SEC-HPLC</u> <u>Purity (%)</u>	<u>Endotoxin</u> <u>(EU/mg)</u>
ABT313 (Ni Excel-SEC 75)	6.45	120.83	779.35	1558.70	100.00	0.0114
ABT310 (MSS-SEC200)	5.42	90.56	490.84	981.68	100.00	<0.0074
Expression (purification volume): 0.5 L transient Chinese Hamster Ovary K1						

EXAMPLE 3

Specific mutation strategies to reduce FcγR and C1q binding/effector function, and to enhance FcRn binding/exposure of antibodies.

[00381] The inventors mutated VHH affinity ligand variant to insert a LALA mutation using biomedical-art recognized methods first described by the Winter group in the 1990s. In this EXAMPLE, LALA = L234A/L235A.

[00382] The inventors mutated VHH affinity ligand variant to insert a LALA mutation using biomedical-art recognized methods first described by the Winter group in the 1990s, then inserted P mutations, such as by the biomedical-art recognized methods introduced by Roche team in 2016, such as the technology for adding P329G and P329G combined with LALA. In this EXAMPLE, LALA/PA = L234A/L235A/P329A. LALA/PG = L234A/L235A/P329G. See Tilman Schlothauer et al., Protein Engineering, Design and Selection, Volume 29, Issue 10, October 2016, Pages 457–466. This paper shows that even LALA itself abolishes C1q-binding. P329A alone was tested, and abolishes C1q-binding, and reduces FcγR-binding. This paper did not test P329A/LALA but show that P329G/LALA further reduces FcγR-binding beyond LALA alone.

[00383] The inventors also mutated VHH affinity ligand variant in other regions. N297A/Q removes natural N-linked glycosylation site in the hinge region.

<u>TABLE 15</u> <u>Agents and binding moieties</u>	<u>Molecular</u> <u>weight kDa</u>
ABT304 (SEQ ID NO: 9), VHH Org Sort His	14.7
ABT305 (SEQ ID NO: 10), VHH Sort His	14.2
ABT306 (SEQ ID NO: 11), VHH His Cys	15.1

<u>TABLE 15</u> <u>Agents and binding moieties</u>	<u>Molecular weight kDa</u>
ABT310 (SEQ ID NO. 31). VHH nanobody, truncated hinge, Fc fusion, unconjugated.	77.3
ABT311 (SEQ ID NO: 32). VHH Org His, unconjugated.	14.2
ABT312 (SEQ ID NO: 33). VHH His, truncated hinge, unconjugated.	13.7
ABT313 (SEQ ID NO: 34), VHH His AviTag	15.7
AGT401 . VHH w/o hinge region. β GN3 via maleimide.	19.6
AGT402 . VHH-Fc fusion. β GN3 via MATE conjugation. is more stable for developability.	83.8
AGT403 . VHH w/hinge region. β GN3 via sortase.	18.8
AGT404 . VHH w/o hinge region. β GN3 via sortase.	15.3
AGT501 . Non-Fc fusion VHH molecules present lower-molecular weights, which are advantageous for subcutaneous formulation. Estimated target about 125 mg dose.	14.5
AGT502 . Non-Fc fusion VHH molecules present lower-molecular weights, which are advantageous for subcutaneous formulation. Estimated target about 125 mg dose.	14.6

EXAMPLE 4VHH epitope on IgG4-Fc

[00384] The VHH epitope on IgG4-Fc binding site on IgG4 was determined to be in two parts. Amino acids 47-57 is DVSQEDPEVQFN (SEQ ID NO: 14). Amino acids 106-114 is SNKGPSSI (SEQ ID NO: 15). For the structure of IgG4, see Davies et al., Molecular Immunology, Volume 62, Issue 1, pages 46-53 (November 2014). The inventors overlaid the structure of hIgG1-Fc/Fc γ RI complex onto the IgG4-Fc model, using the Fc regions. See Kiyoshi et al., Nature Communications, 6, 6866 (2015). The conclusion was that binding of the VHH domain is likely to compete with binding to Fc γ RI. IgG4 has little or no affinity to other Fc γ Rs or to C1q.

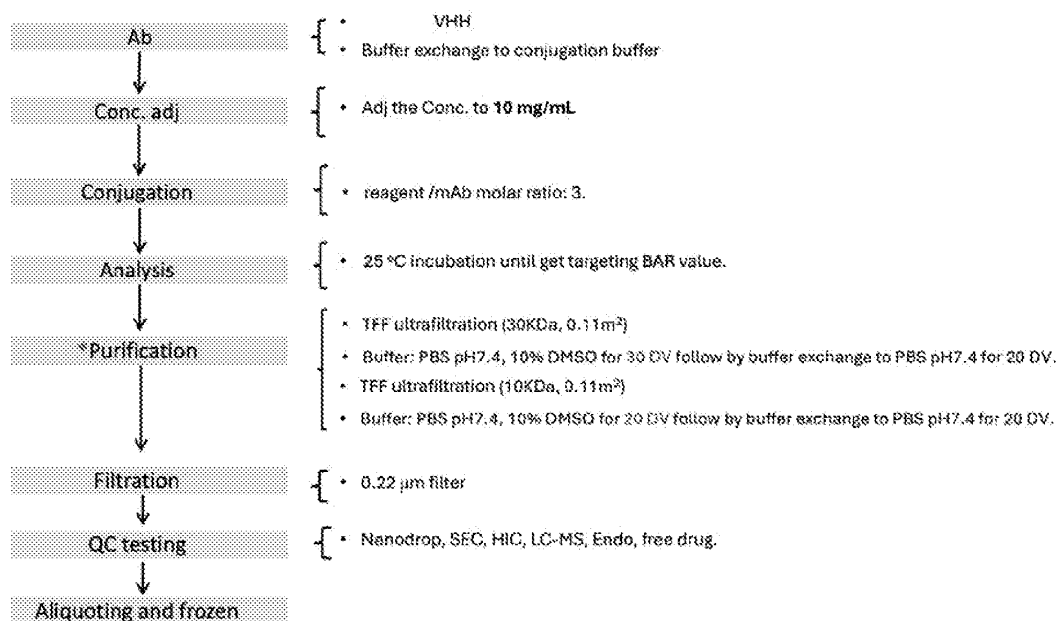
[00385] The inventors also overlaid the structure of hIgG1-FcIII peptide complex onto the IgG4-Fc model, using the Fc regions. See Delano et al., Science, 287, 1279-1283 (2000). The conclusion was that the binding of the VHH domain is not likely to compete with binding to the FcIII peptide, or to FcRn (binds in the same region).

EXAMPLE 5Procedure for preparation of AGN402

[00386] **AGN402 (ABT310-TBT105).**

[00387] *BAR2 chemical formula (amino acid):* C3570H5554N950O1146S24.

[00388] *Modification:* pE(Q)*2,-K*2,G0F,G1F,BAR2.



[00389] **AGN402** was prepared using a lysine-specific conjugation.

[00390] (1) **ABT310** in the original buffer (20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was buffer exchanged to 20 mM PB, pH 6.5 by UFDF (Pellicon® 3, 10 kDa, 0.11 m²) for 14 DV. Concentration was tested with 16.47 mg/mL by Nanodrop.

[00391] (2) 4000 mg of antibody was transferred to 1L bottle (Corning, 430518) and placed in ice bath; then added with conjugation buffer (20 mM PB, pH 6.5) and 10 mM **TBT105** solution (three equivalents). The total dimethyl sulfoxide concentration in the reaction mixture was 3.74%; the concentration of the reaction is 10 mg/mL.

[00392] (3) The reaction vessel was allowed to approach ambient temperature and then incubated at 25°C. The reaction was monitored with liquid chromatography-mass spectrometry and BAR value reached 2.00 after ten hours. Then reaction vessel was placed at 4°C overnight (seventeen hours).

[00393] (4) The crude conjugation was then purified with UFDF (Pellicon® 3, 10 kDa, 2*0.11 m²) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031), 15% dimethyl sulfoxide (Sigma, D4540) for 160

DV, followed by phosphate-buffered saline, pH 7.4 for 20 DV. Two batches of products were generated, to afford 3776.88 mg of **AGN402** (001) (77.30 mg/mL) and 22.44 mg of **AGN402** (002) (11.22 mg/mL).

[00394] (5) The purified sample was filtered with 0.22 µm filter.

[00395] (6) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 83.9 kDa.

[00396] The major conjugation site was also confirmed with K154, identified by peptide mapping (Thermo/Q-Exactive Orbitrap).

TABLE 16 Residual reagents			
	Residual ABT (% mol/mol of mAb)	Residual reagent (% mol/mol of total reagent)	Residual payload (% mol/mol of total payload)
AGN402 (001)	3.86	1.97	<0.39
AGN402 (002)	1.86	1.02	<0.39

EXAMPLE 6

Procedure for preparation of AGN508

[00397] **AGN508 (ABT501-TBT103).**

[00398] BAR1 chemical formula (amino acid). C671H1042N193O229S5.

[00399] Modification. pE(Q),Cys,BAR1.

[00400] **AGN508** was prepared using a Michael-addition reaction.

[00401] (1) Reduction. To the 1L bottle (Corning, 430518) containing 2000 mg of **ABT501** in original buffer (7.26 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 10% (v/v) of 1 M Tris buffer, pH 7.5 to adjust conjugation buffer pH to 6-7, 5 mM Tris(2-carboxyethyl) phosphine hydrochloridesolution (1.5 equivalents) and 200 mM DTPA solution. The protein concentration in the reaction was 5.1 mg/mL. The DTPA concentration in the reaction was 4 mM. The reactions were mixed well and incubated at 22°C for one hour.

[00402] (2) Conjugation. Added **TBT103** (10 mM in dimethyl sulfoxide) (three equivalents) into the reactions, mixed well and incubated at 22°C for another one hour.

[00403] (3) The crude conjugation was then purified with UFDF (Pellicon® 3, 3 kDa, 2*0.11 m²) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) for 70 DV, to afford 1949 mg of **AGN508**.

[00404] (4) The purified sample was filtered with 0.22 µm filter.

[00405] (5) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 15.6 kDa.

EXAMPLE 7

Procedure for preparation of **AGN501**

[00406] **AGN501 (ABT502-TBT103)**.

[00407] BAR1 chemical formula (amino acid). C624H983N170O217S5.

[00408] Modification. pE(Q),Cys,BAR1.

[00409] **AGN501** was prepared using Michael-addition reaction.

[00410] (1) Reduction. To the 1L bottle (Corning, 430518) containing 2000 mg of **ABT502** in original buffer (7.65 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 10% (v/v) of 1 M Tris buffer, 7.5 to adjust conjugation buffer pH to 6-7, 5 mM Tris(2-carboxyethyl)phosphine hydrochloride solution (1.5 equivalents) and 200 mM DTPA solution. The protein concentration in the reaction was 5.15 mg/mL. The DTPA concentration in the reaction was 4 mM. The reactions were mixed well and incubated at 22°C for one hour.

[00411] (2) Conjugation. Added **TBT103** (10 mM in dimethyl sulfoxide) (three equivalents) into the reactions, mixed well and incubated at 22°C for another one hour.

[00412] (3) The crude conjugation was then purified with UFDF (Pellicon® 3, 3 kDa, 2*0.11 m²) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) for 70 DV, to afford 1717.8 mg of **AGN501**.

[00413] (4) The purified sample was filtered with 0.22 µm filter.

[00414] (5) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 14.5 kDa.

EXAMPLE 8Procedure for preparation of AGN502

[00415] **AGN502 (ABT503-TBT103).**

[00416] BAR1 chemical formula (amino acid). C627H987N170O219S5.

[00417] Modification. pE(Q),Cys,BAR1.

[00418] **AGN502** was prepared using Michael-addition reaction.

[00419] (1) Reduction. To the 1L bottle (Corning, 430518) containing 2000 mg of **ABT503** in original buffer (7.41 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 10% (v/v) of 1 M Tris buffer, 7.5 to adjust conjugation buffer pH to 6-7, 5 mM Tris(2-carboxyethyl)phosphine hydrochloride solution (1.5 equivalents) and 200 mM DTPA solution. The protein concentration in the reaction was 5.04 mg/mL. The DTPA concentration in the reaction was 4 mM. The reactions were mixed well and incubated at 22°C for one hour.

[00420] (2) Conjugation. Added **TBT103** (10 mM in dimethyl sulfoxide) (three equivalents) into the reactions, mixed well and incubated at 22°C for another one hour.

[00421] (3) The crude conjugation was then purified with UFDF (Pellicon® 3, 3 kDa, 2*0.11 m²) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) for 70 DV, to afford 2008 mg of AGN502.

[00422] (4) The purified sample was filtered with 0.22 µm filter.

[00423] (5) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 14.6 kDa.

EXAMPLE 9Procedure for preparation of AGN509

[00424] **AGN509 (ABT504-TBT103)**

[00425] BAR1 chemical formula (amino acid). C660H1025N188O223S5

[00426] Modification. pE(Q),Cys,BAR1.

[00427] **AGN509** was prepared using a Michael-addition reaction.

[00428] (1) Reduction. To the 1L bottle (Corning, 430518) containing 1900 bmg of **ABT504** in original buffer (7.78 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 10% (v/v) of 1 M Tris buffer, 7.5 to adjust conjugation buffer pH to 6-7, 5 mM Tris(2-carboxyethyl)phosphine

hydrochloride solution (1.5 equivalents) and 200 mM DTPA solution. The protein concentration in the reaction was 5.30 mg/mL. The DTPA concentration in the reaction was 4 mM. The reactions were mixed well and incubated at 22°C for one hour.

[00429] (2) Conjugation. Added **TBT103** (10 mM in dimethyl sulfoxide) (three equivalents) into the reactions, mixed well and incubated at 22°C for another one hour.

[00430] (3) The crude conjugation was then purified with UFDF (Pellicon® 3, 3 kDa, 2*0.11 m²) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031) for 70 DV, to afford 1896.35 mg of AGN509.

[00431] (4) The purified sample was filtered with 0.22 µm filter.

[00432] (5) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 15.3 kDa.

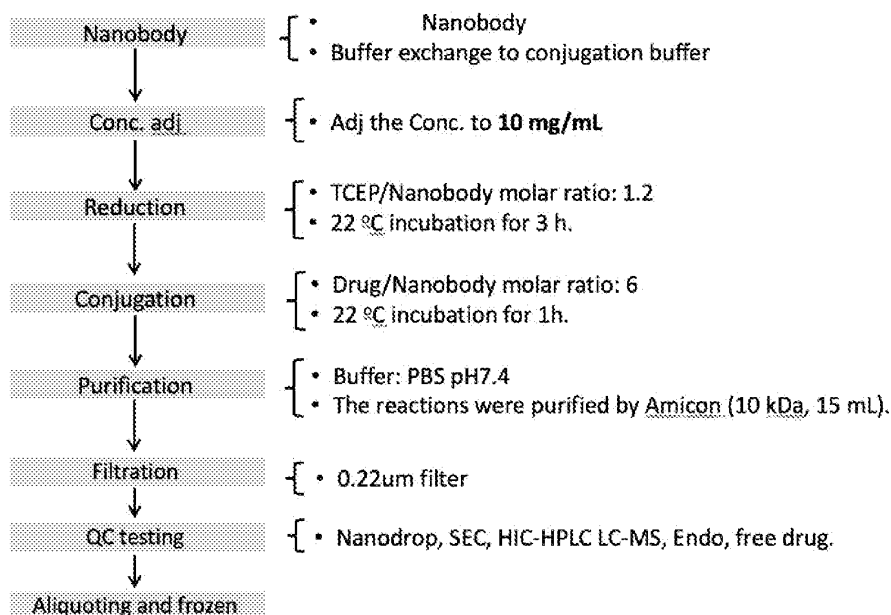
EXAMPLE 10

Procedure for preparation of AGN401

[00433] **AGN401 (ABT306-TBT103).**

[00434] BAR1 chemical formula (amino acid). C719H1115N202O244S5.

[00435] Modification. pE(Q),Cys, O-Glycan, one payload.



[00436] **AGN401** was prepared using a Michael-addition reaction.

[00437] (1) Reduction. To the 150 mL bottle (Corning, 430281) containing 150 mg of **ABT306** in original buffer (3.59 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 10% (v/v) of 1 M Tris buffer, 7.5 to adjust conjugation buffer pH to 6-7, 5 mM Tris(2-carboxyethyl)phosphine hydrochloride solution (1.2 equivalents) and 200 mM EDTA solution. The protein concentration in the reaction was 2.94 mg/mL. The EDTA concentration in the reaction was 2 mM. The reactions were mixed well and incubated at 22°C for three hours.

[00438] (2) Conjugation. Added **TBT103** (10 mM in dimethyl sulfoxide) (three equivalents) into the reactions, mixed well and incubated at 22°C for another one hour.

[00439] (3) The crude conjugation was then purified with Amicon® (Millipore, 10 kDa, 15 mL) with phosphate-buffered saline, pH 7.4 (Gibco, 10010031), to afford 135.24 mg of **AGN401**.

[00440] (4) The purified sample was filtered with 0.22 µm filter.

[00441] (5) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 19.6 kDa.

EXAMPLE 11Procedure for preparation of AGN403

[00442] **AGN403 (ABT304-TBT106).**

[00443] BAR1 chemical formula. C676H1068N188O235S6.

[00444] Modification. pE(Q), BAR1.

[00445] **AGN403** was prepared using a sortase-catalyzed ligation.

[00446] (1) To the 150 mL bottle (Corning, 430281) containing 300 mg of **ABT304** in original buffer (6.30 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 5.08 mg/mL Sortase A (PT-0008, 0.01 equivalents) solution, conjugation buffer (50 mM HEPES, 150 mM sodium chloride, 10% glycerol (V/V), pH 7.5), **TBT106** (40 mM in dimethyl sulfoxide) (eight equivalents) into the reactions, mixed well and incubated at 4°C for overnight (seventeen hours). The **ABT304** concentration in the reaction was 3 mg/mL.

[00447] (2) The crude conjugation was purified with AKTA pure chromatography (column: HisTrap excel, 1 mL; equilibration buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 10 mM imidazole pH 7.4; elution buffer: 0.2 M sodium hydroxide; flow rate is one mL/minute). The conjugate was flowed through during the loading process. The sortase with His-tag and unconjugated peptide with His-tag were eluted by 0.2 M sodium hydroxide. The inventors pooled the flow through together and performed buffer exchange to phosphate-buffered saline, 2 mM EDTA, pH 7.4 with Amicon® (Milipore, 10 kDa, 15 mL) for 30 DV.

[00448] (3) The purified sample was filtered with 0.22 µm filter.

[00449] (4) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, hydrophobic interaction chromatography-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 15.8 kDa.

EXAMPLE 12Procedure for Preparation of AGN404

[00450] **AGN404 (ABT305-TBT106).**

[00451] BAR1 chemical formula. C657H1040N182O229S4.

[00452] Modification. pE(Q), BAR1.

[00453] **AGN404** was prepared using Sortase-catalyzed ligation.

[00454] (1) To the 150 mL bottle (Corning, 430281) containing 300 mg of **ABT305** in original buffer (5.03 mg/mL in 20 mM histidine-acetate, 150 mM sodium chloride, pH 5.5) was added with 5.08 mg/mL Sortase A (PT-0008, 0.01 equivalents) solution, conjugation buffer (50 mM HEPES, 150 mM sodium chloride, 10% glycerol (V/V), pH 7.5), **TBT106** (40 mM in dimethyl sulfoxide) (eight equivalents) into the reactions, mixed well and incubated at 4°C for overnight (seventeen hours). The **ABT305** concentration in the reaction was 3 mg/mL.

[00455] (2) The crude conjugation was purified with AKTA pure chromatography (column: HisTrap excel, 1 mL; equilibration buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 10 mM imidazole pH 7.4; elution buffer: 0.2 M sodium hydroxide; flow rate is 1 mL/minutes). The conjugate was flowed through during the loading process. The sortase with His-tag and unconjugated peptide with His-tag were eluted by 0.2 M sodium hydroxide. Pooled flow through together and performed buffer exchange to phosphate-buffered saline, 2 mM EDTA, pH 7.4 with Amicon® (Milipore, 10 kDa, 15 mL) for 30 DV.

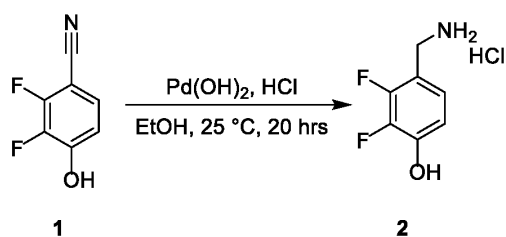
[00456] (3) The purified sample was filtered with 0.22 µm filter.

[00457] (4) The quality control tests were performed, including concentration, size exclusion chromatography-high performance liquid chromatography, HIC-high performance liquid chromatography, liquid chromatography-mass spectrometry, free drug levels, and endotoxin levels. All the quality control tests produced acceptable and expected results. The measured mass was 15.2 kDa.

EXAMPLE 13

Procedure for Preparation of **Building block #1**

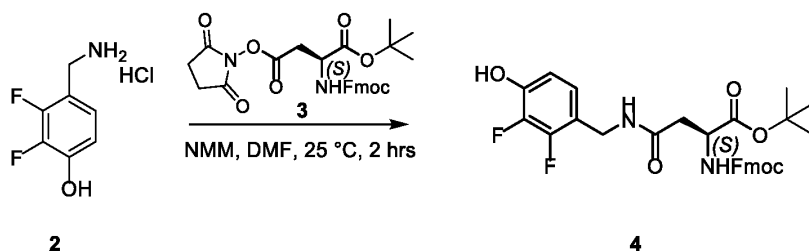
Preparation of **Intermediate 2**



[00458] A mixture of **Reactant 1** (20.0 g, 128 mmol, 1.00 equivalent) in ethanol (400 mL) was added Pd(OH)_2 (8.00 g, 56.9 mmol, 0.44 equivalents) and hydrochloric acid (2 M, 64.4 mL, 1.00 equivalent) under nitrogen gas atmosphere. The mixture was stirred at 25 °C for twenty hours under hydrogen gas atmosphere (50 psi). Thin-layer chromatography (DCM: methanol =10: 1) showed the **Reactant 1** was consumed and one main spot ($R_f = 0.5$) was formed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with ethyl acetate (100 mL)

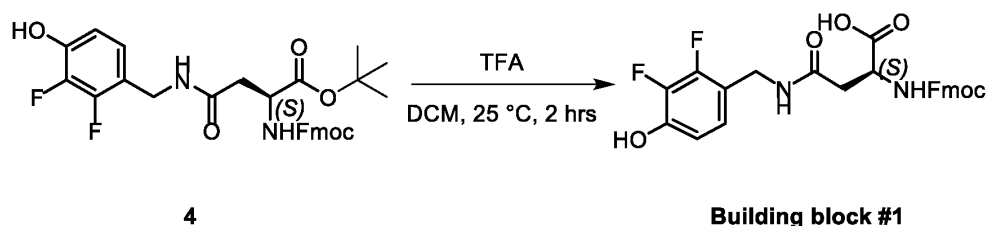
at 25°C for two hours to give **Intermediate 2** (18.0 g, 92.0 mmol, 71.3% yield, 100% purity, HCl salt) was obtained as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ = 8.51 – 7.71 (m, 3H), 7.21 – 7.17 (m, 1H), 6.93 – 6.88 (m, 1H), 3.94 (s, 2H).

Preparation of **Intermediate 4**



[00459] To a solution of **Reactant 3** (40.0 g, 78.6 mmol, 1.00 equivalent) in dimethylformamide (200 mL) was added NMM (15.9 g, 157 mmol, 17.3 mL, 2.00 eq) and **Intermediate 2** (13.1 g, 67.2 mmol, 0.85 equivalents, HCl salt). The mixture was stirred at 25°C for two hours. The liquid chromatography-mass spectrometry results showed **Intermediate 2** was consumed completely. One main peak (retention time = 0.48 minutes) with desired mass (MS calculated was 552.2, MS observed: $[\text{M} - \text{tBu} + \text{H}]^+ = 497.1$) was detected. The residue was diluted with water (800 mL) and extracted with ethyl acetate (1500 mL) (500 mL * 3). The combined organic layers were washed with brine 500 mL, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with ethyl acetate (1000 mL) at 25°C for four hours. **Intermediate 4** (19.0 g, 33.1 mmol, 42.1% yield, 96.4% purity) was obtained as a yellow solid. Liquid chromatography-mass spectrometry was retention time = 0.48 minutes, MS calculated was 552.2. MS found was $[\text{M} - \text{tBu} + \text{H}]^+ = 497.1$.

Preparation of **Building block #1**



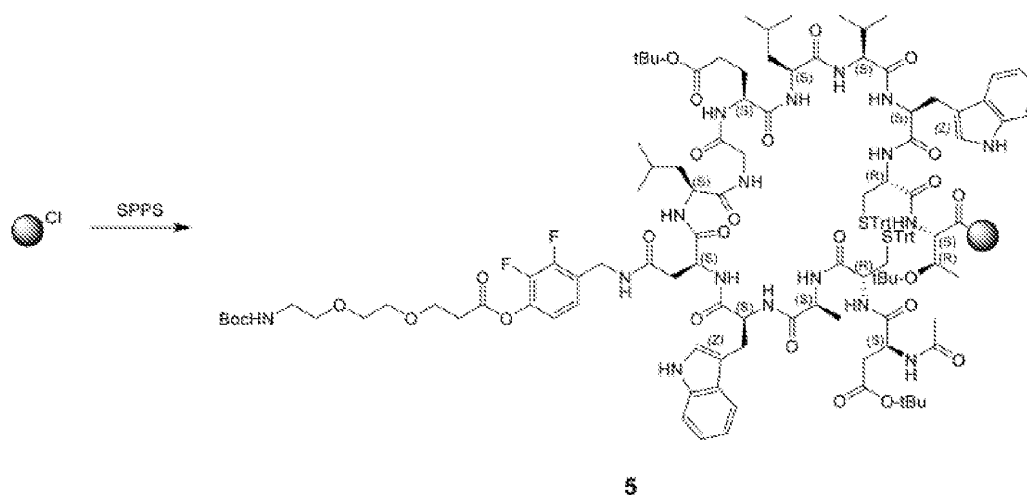
[00460] To a solution of **Intermediate 4** (19.0 g, 34.3 mmol, 1.00 equivalents) in DCM (100 mL) was added trifluoroacetic acid (154 g, 1.35 mol, 100 mL, 39.2 equivalents). The mixture was stirred at 25°C for two hours. Liquid chromatography-mass spectrometry results showed **Intermediate 4** was consumed completely and one main peak (RT = 0.41 minutes) with desired mass (MS calculated was 496.1, MS observed was $[\text{M} + \text{H}]^+ = 497.1$) was detected. The mixture solution was concentrated under reduced

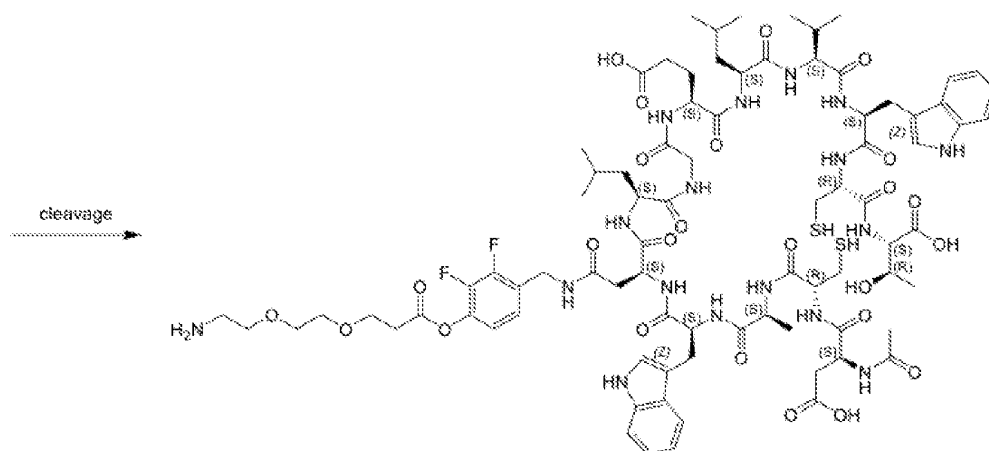
pressure to give a residue. The residue was purified by reversed-phase HPLC (trifluoroacetic acid condition), to give **Building block #1** (9.10 g, 17.9 mmol, 52.1% yield, 97.9% purity) as a white solid. Liquid chromatography-mass spectrometry was retention time = 0.41 minutes, MS calculated was 496.1, MS found was $[M + Na]^+ = 519.1$. HPLC was retention time = 2.95 minutes, purity was 97.9%, SFC was EC6536-156-P1C, retention time = 1.58 minutes. Value was 97.8%. 1H NMR (400 MHz, DMSO- d_6) δ = 13.60-11.82 (m, 1H), 10.26 (s, 1H), 8.34 (t, J = 5.6 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.70 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.48-7.37 (m, 2H), 7.36-7.27 (m, 2H), 6.92 (t, J = 7.6 Hz, 1H), 6.70 (t, J = 7.6 Hz, 1H), 4.46-4.33 (m, 1H), 4.29-4.19 (m, 5H), 2.69-2.61 (m, 1H), 2.52 (s, 1H).

EXAMPLE 14

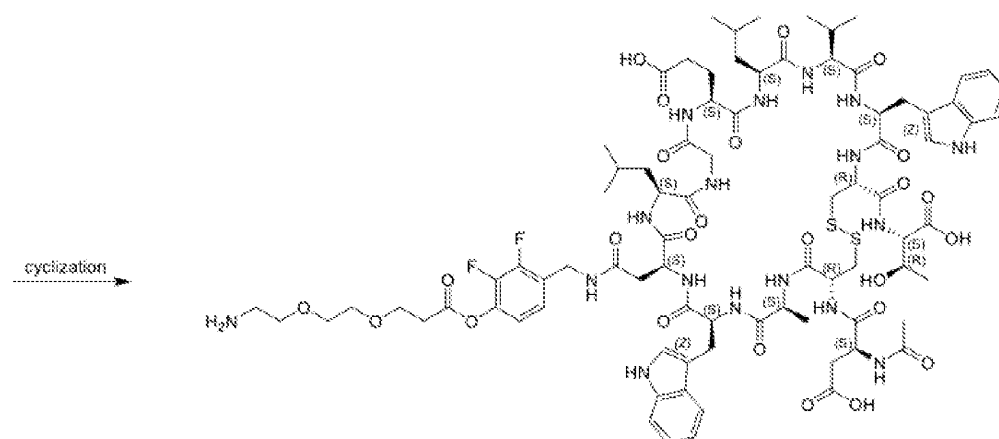
Procedure for preparation of **TBT105**

Preparation of **Intermediate 7**





6



7

[00461] This chemical compound was synthesized using standard Fmoc chemistry (CTC resin).

[00462] (1) *Resin preparation.* To the vessel containing CTC resin (22.7 g, 10.0 mmol, 0.55 mmol/g) and Fmoc-Thr(tBu)-OH (3.97 g, 10.0 mmol, 1.00 equivalent) in DCM (50 mL) was added N,N-diisopropylethylamine (4.00 equivalents) dropwise and mixed for two hours with nitrogen gas bubbling at 25°C. Then methanol (22.7 mL) was added and bubbled with nitrogen gas for another thirty minutes. The resin was washed with dimethylformamide (100 mL) * 5, followed by the addition of 20% piperidine in dimethylformamide (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 dimethylformamide (100 mL) * 5 before proceeding to next step.

[00463] (2) Coupling. A solution of Fmoc-Cys(Trt)-OH (17.5 g, 30.0 mmol, 3.00 equivalents), HBTU (10.8 g, 28.5 mmol, 2.85 equivalents) in dimethylformamide (500 mL) was added to the resin with nitrogen gas bubbling. Then N,N-diisopropylethylamine (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 dimethylformamide (300 mL) * 5.

[00464] (3) Deprotection. 20% piperidine in dimethylformamide (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 dimethylformamide (100 mL) * 5.

[00465] (4) Steps 2 and 3 were repeated for the following amino acids elongation. See numbers 3-15, TABLE 17.

[00466] (5) After all the steps were completed, the resin was washed with dimethylformamide (100 mL) * 5, methanol (100 mL) * 5, then dried under reduced pressure to afford resin-bound peptide **Intermediate 5** (CTC resin, 10.0 mmol).

TABLE 17

The list of amino acids and the corresponding reagents used on SPPS

#	Materials	Coupling reagents
1	Fmoc-Thr(tBu)-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(Boc)-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(OtBu)-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(Boc)-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(OtBu)-OH (3.00 equivalents)	DIC (3.00 equiv.) and HOBt (3.00 equiv.)

TABLE 17 The list of amino acids and the corresponding reagents used on SPPS		
#	Materials	Coupling reagents
14	Ac	Ac ₂ O:NMM:DMF=10:5:85
15	Boc-PEG ₂ -OH (6.00 equivalents)	DIC:HOBt:DMAP=6:6:6

Peptide cleavage and cyclization.

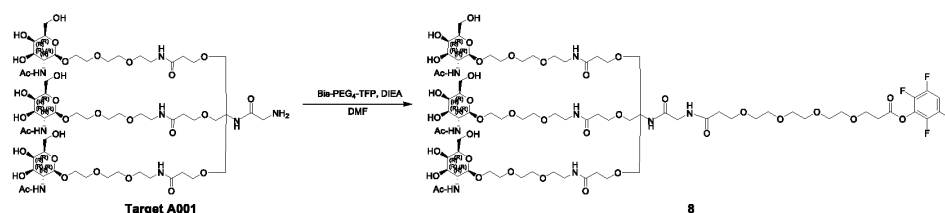
[00467] (1) Cleavage solution (TFA/TIS/water, 95/2.5/2.5, v/v/v, 420 mL) was added to the flask containing the side-chain protected resin-bound peptide (CTC resin, 42 g, 10.0 mmol) at 25°C and stirred for two hours.

[00468] (2) After filtration, the filtrate was collected.

[00469] (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 6** (16.5 g, crude) as a white solid.

[00470] To the mixture of **Intermediate 6** (16.5 g, crude) in HOAc/MeCN/water (4/3/3, v/v/v, 8 L) was added 0.1 M I₂/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. Na₂S₂O₃ dropwise until the yellow color disappeared. After filtration, the filtrate was purified by prep-high performance liquid chromatography (A: 0.075% TFA/water, B: MeCN), followed by lyophilization to afford **Intermediate 7** (6 g, 90.0% purity, 32.43% yield) as a white solid. Liquid chromatography-mass spectrometry: retention time = 0.960 minutes, MS calculated: M_{av} = 1851.01, mass observed: [M + 2H]²⁺ = 925.90, [M + H]⁺ = 1852.01.

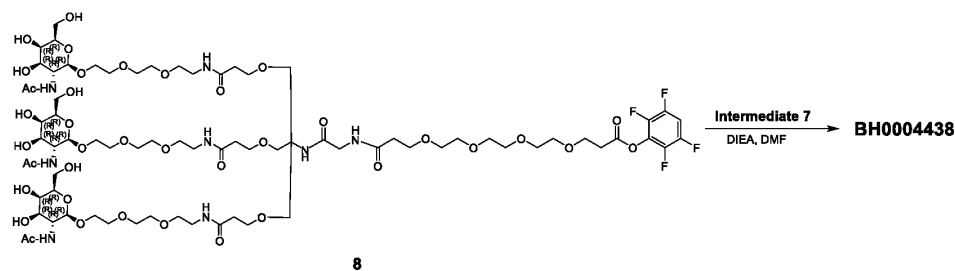
Preparation of Intermediate 8



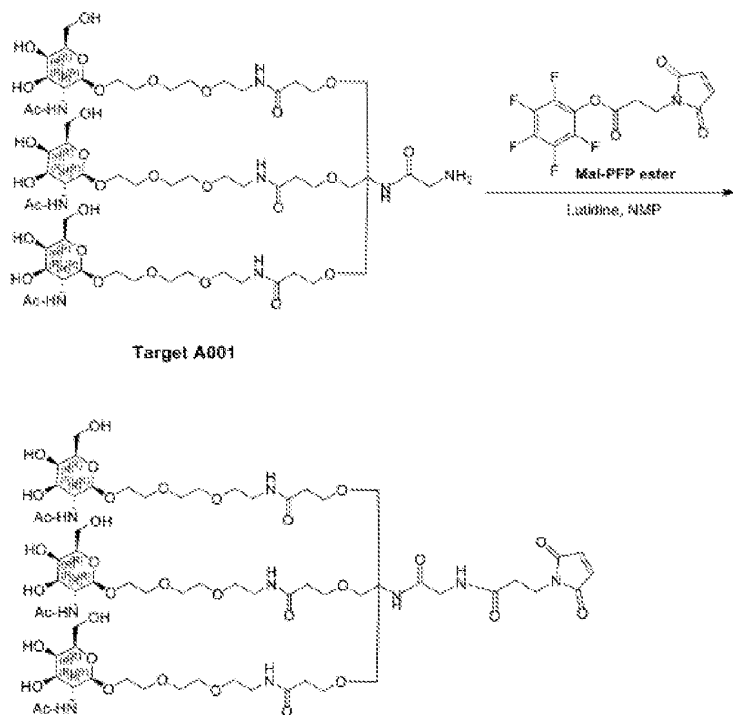
[00471] To a solution of Bis-PEG₄-TFP (5.18 g, 3.00 equivalents) in dimethylformamide (25 mL) was added a mixture of **Target A001** (4.09 g, 1.00 equivalent) and N,N-diisopropylethylamine (1.283 g, 1.307 mL, three equivalents) in 50 mL dimethylformamide at 0°C. The resulting reaction was stirred for five minutes at 0°C. After completion monitored by liquid chromatography-mass spectrometry, the mixture was directly injected into the reverse column, purified by prep-high performance liquid chromatography

(A: 0.075% TFA/water, B: MeCN), followed by lyophilization to afford **Intermediate 8** (2.9 g, 95.0% purity, 50.7% yield) as colorless oil. Liquid chromatography-mass spectrometry was retention time = 0.709 minutes, MS calculated: $M_{av} = 1821.81$, mass observed: $[M+16 + 2H]^{2+} = 911.60$.

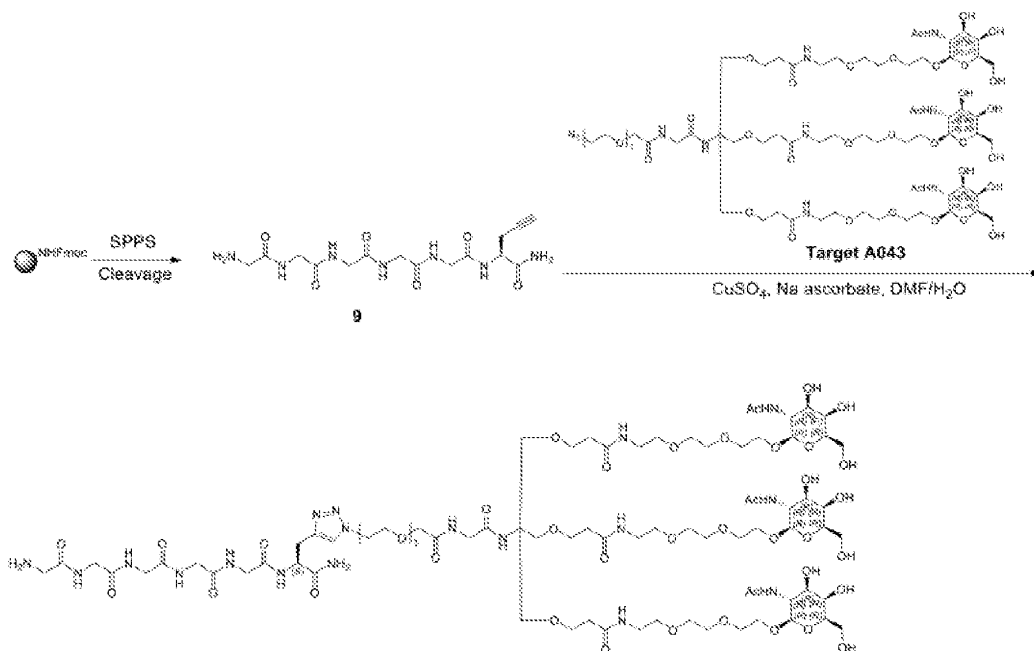
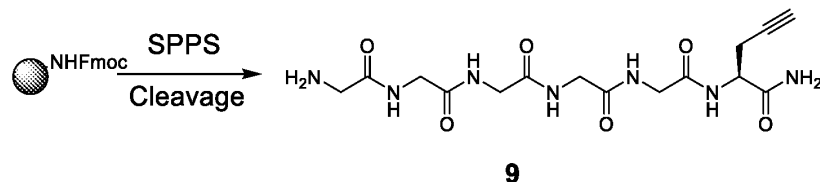
Preparation of **TBT105**



[00472] To a solution of **Intermediate 8** (3.34 g, 1.80 mmol, 1.30 equivalents) and **Intermediate 7** (2.53 g, 1.38 mmol, 1.00 equivalent) in dimethylformamide (35 mL) was added N,N-diisopropylethylamine (358.73 mg, 2.77 mmol, 459.92 μ L, 2.00 equivalents). The mixture was stirred at 20°C for one hour. After completion monitored by liquid chromatography-mass spectrometry, the mixture was acidified by 1 M hydrochloric acid to pH = 5. The mixture was purified by prep-high performance liquid chromatography (A: 0.075% TFA/water, B: MeCN) directly, followed by lyophilization to afford **TBT105** (1.52 g, 96.0% purity, 29.98% yield.) as a white solid. Liquid chromatography-mass spectrometry was retention time = 0.817 minutes, MS calculated: $M_{av} = 3505.75$, mass observed: $[M + 3H]^{3+} = 1169.20$.

EXAMPLE 15**Procedure for preparation of TBT103**

[00473] To a solution of **Target A001** (25 g, 15.74 mmol, 1.0 eq) in NMP (125 mL, 5 V) was added 2,6-dimethylpyridine (3.37 g, 3.8 mL, 31.49 mmol, 2.0 equivalents). Then, then Mal-PFP ester (5.3 g, 15.81 mmol, 1.0 equivalent) was added. The solution was stirred at 25°C for one hour. After completion monitored by liquid chromatography-mass spectrometry, trifluoroacetic acid was added to quench the reaction until pH=6. Dropped the mixture into methyl tert-butyl ether (3 L) and pour out the supernatant and get residue. The residue was purification by prep-high performance liquid chromatography twice (column: Phenomenex luna c18 250 mm*100 mm* 15um; mobile phase: [A: water (0.2%FA); B: ACN]; B%: 0.00%-20.00%, 21.00 minutes), followed by lyophilization to afford **TBT103** (9.5 g, 98.1% purity, 34% yield) as a white solid. Liquid chromatography-mass spectrometry was retention time = 17.129 minutes, MS calculated: $M_{av} = 1548.59$, mass observed: $[M + H]^+ = 1549.50$.

EXAMPLE 16**Procedure for preparation of TBT106****Preparation of Intermediate 9**

[00474] The peptide was synthesized using standard Fmoc chemistry.

[00475] (1) Resin preparation: Dimethylformamide (100.0 mL) was added to the vessel containing Rink Amide MBHA resin (sub: 0.27 mmol/g, 2.00 mmol, 7.5 g).

[00476] (2) De-protection: 20% piperidine/dimethylformamide (200.0 mL) was added and mixed at room temperature (25°C) for thirty minutes. The de-protection reaction was monitored by ninhydrin test, if it showed blue or other brownish red, the reaction was completed. The resin was then washed with dimethylformamide (200.0 mL) *5.

[00477] (3) Coupling: Fmoc-amino acid solution in dimethylformamide (100.0 mL) was added to resin with nitrogen gas bubbling, then the activation buffer was added to the resin with nitrogen gas bubbling

at room temperature (25°C) for one hour. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with dimethylformamide (200.0 mL) *5.

[00478] (4) Step 2 to 3 was repeated for all other amino acids: (numbers 1-3 in TABLE 18). At the last step, the resin was washed with methanol (200.0 mL) * 3 and dried by nitrogen gas bubbling for three hours.

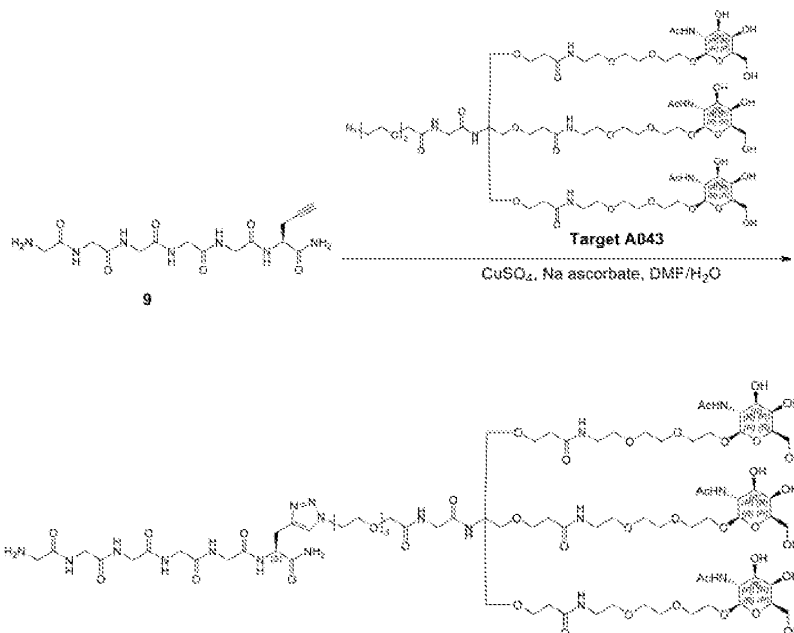
TABLE 18 The list of amino acids and the corresponding reagents used on SPPS		
#	Materials	Coupling reagents
1	Fmoc-Pra-OH (1.50 equivalents)	HBTU (1.425 equiv.) and DIEA (3.00 equiv.)
2	Fmoc-G-G-OH (2.00 equivalents)	HBTU (1.9 equiv.) and DIEA (4.00 equiv.)
3	Fmoc-G-G-G-OH (2.00 equivalents)	HBTU (1.9 equiv.) and DIEA (4.00 equiv.)

Peptide cleavage and cyclization.

[00479] (1) Cleavage solution (TFA/TIS/water, 95/2.5/2.5, v/v/v, 90 mL) was added to the flask containing the side-chain protected resin-bound peptide (Rink Amide MBHA Resin, 9 g, 2.0 mmol) at 25°C and stirred for two hours.

[00480] (2) After filtration, the filtrate was collected.

[00481] (3) The filtrate was precipitated with cold isopropyl ether (450 mL). After filtration, the solid was washed with isopropyl ether (450 mL) twice, and the crude peptide was dried under reduced pressure for two hours to afford Intermediate 1 (1 g, crude) as a white solid. The crude peptide was purified by prep-high performance liquid chromatography (A: 0.1% TFA/water, B: MeCN), followed by lyophilization to afford Intermediate 9 (520 mg, 58.23% yield, 89% purity) as a white solid.

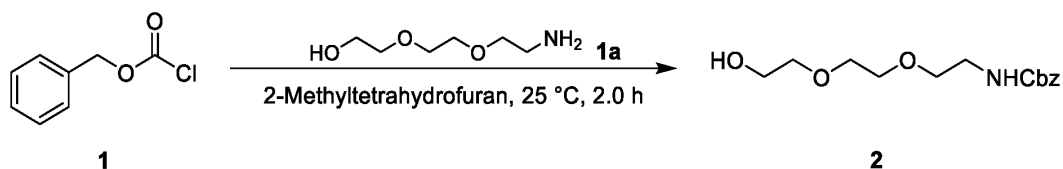
Preparation of TBT106

[00482] To a solution of Na ascorbate (399.32 mg, 2.02 mmol, three equivalents) in water (5 mL) was added CuSO₄ (0.4 M, 1.68 mL, 1 eq) dropwise at 25°C under nitrogen gas. The mixture was turned brown, and then THPTA (291.94 mg, 671.89 μmol, one equivalent) was added to get a clear solution A. To a solution B of **Target A043** (1.084 g, 671.892 μmol, one equivalent) and Intermediate 9 (300 mg, 671.892 μmol, one equivalent) in water (10 mL) at 25°C under nitrogen gas was added a solution A. The mixture was stirred at 25°C for 0.5 hours. After completion monitored by liquid chromatography-mass spectrometry, the crude solution was purified by prep-high performance liquid chromatography (A: 0.1% TFA/water, B: MeCN) to get a crude product. It was further purified by prep-high performance liquid chromatography (A: 0.5% AcOH/water, B: MeCN), followed by lyophilization to afford TBT106 (590 mg, 96.5% purity, 36% yield) as a white solid. Liquid chromatography-mass spectrometry was retention time = 0.55 minutes, MS calculated: M_{av} = 2010.06, mass observed: [M + H]⁺ = 2011.1.

EXAMPLE 17

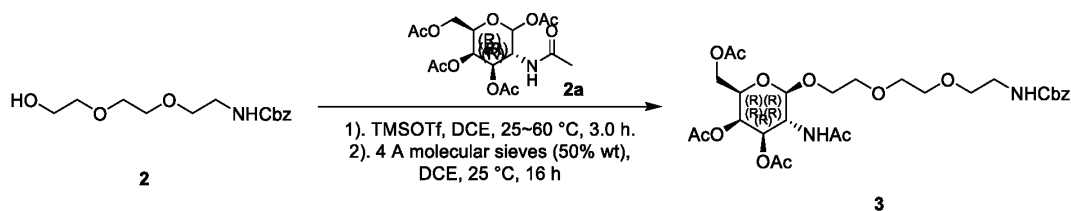
Chemical synthesis. Procedure for Preparation of TBT103 intermediate A001A.

Preparation of Intermediate 2



[00483] 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. Thin-layer chromatography (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. Liquid chromatography-mass spectrometry retention time = 0.797 minutes, MS calculated: 283.14, mass observed: $[M + Na]^+ = 306.1$. 1H 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).

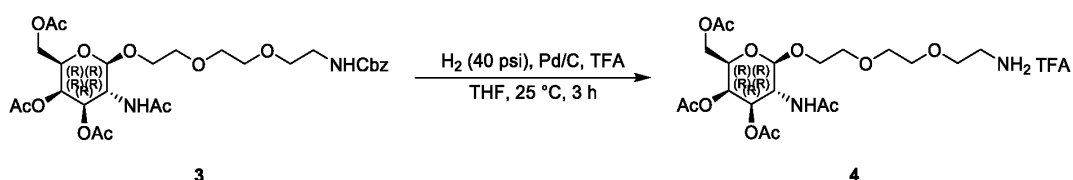
Preparation of Intermediate 3



[00484] To a solution of **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 N₂ 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 N₂ atmosphere. Thin-layer chromatography

(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. Liquid chromatography-mass spectrometry was room temperature = 0.860 minutes, MS calculated: 612.25, mass observed: $[\text{M} + \text{H}]^+ = 613.2$. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 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).

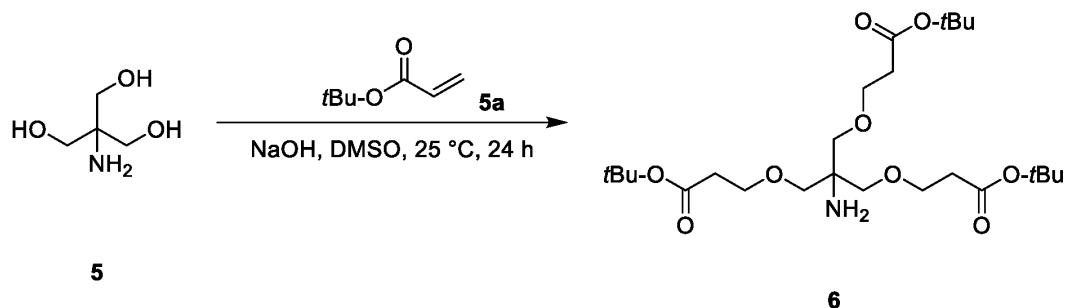
Preparation of **Intermediate 4**



[00485] Pd/C (9.00 g, 10% purity) in reaction bottle (purged with argon gas for three times) was added tetrahydrofuran (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 tetrahydrofuran (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). Thin-layer chromatography (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 tetrahydrofuran (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with tetrahydrofuran (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. Liquid chromatography-mass spectrometry was retention time = 0.484 minutes, MS calculated: 478.22, mass observed: $[\text{M} + \text{H}]^+ = 478.9$. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 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,

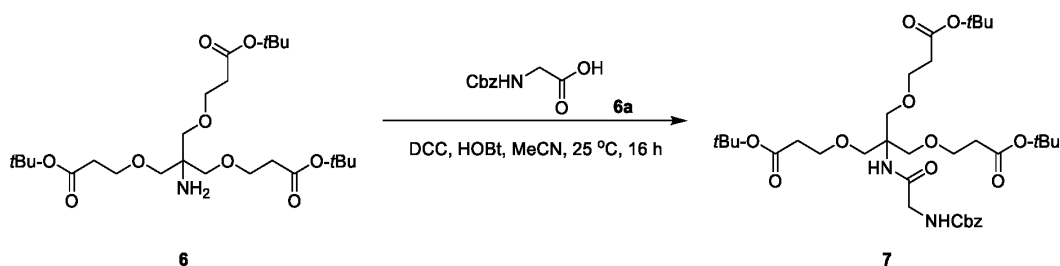
1 H), 3.75-3.84 (m, 1 H), 3.59 (br t, $J = 5.14$ Hz, 3 H), 3.50-3.56 (m, 6 H), 2.98 (br s, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

Preparation of Intermediate 6



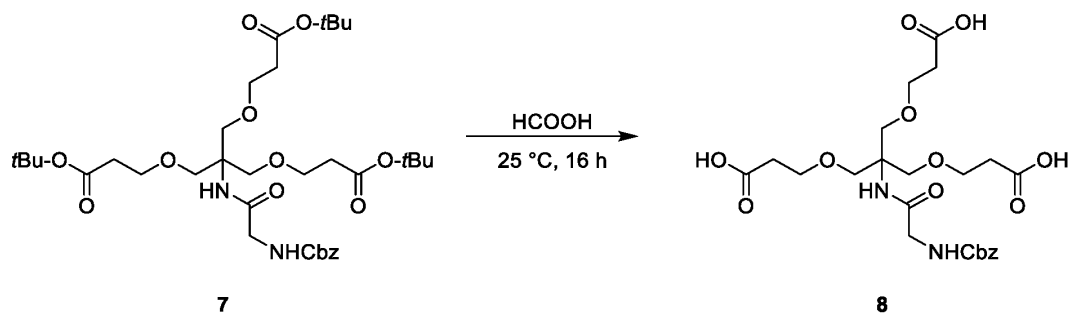
[00486] To a mixture of **5** (60.0 g, 495.0 mmol, 1.00 equivalent) in dimethyl sulfoxide (166 mL) was added aqueous sodium hydroxide (5.0 M, 9.91 mL, 0.10 equivalents) dropwise at 0-15°C for over five minutes. After addition, the mixture was stirred at 0-15°C for 5 minutes, then **5a** (254.0 g, 1.98 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. Thin-layer chromatography (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. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 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



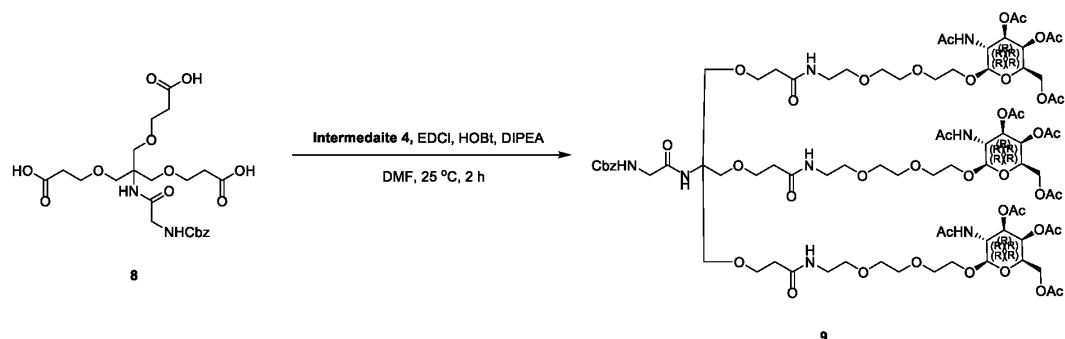
[00487] To a solution of **Intermediate 6** (40.0 g, 79.1 mmol, 1.00 equivalent) in MeCN (400 mL) was added HOBt (10.7 g, 79.1 mmol, 1.00 equivalent). Then **6a** (16.5 g, 79.1 mmol, 1.00 equivalent) and DCC (16.3 g, 79.1 mmol, 1.00 equivalent) were added. The reaction was stirred at 25°C for sixteen 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 (SiO₂, 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. Liquid chromatography-mass spectrometry was 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**



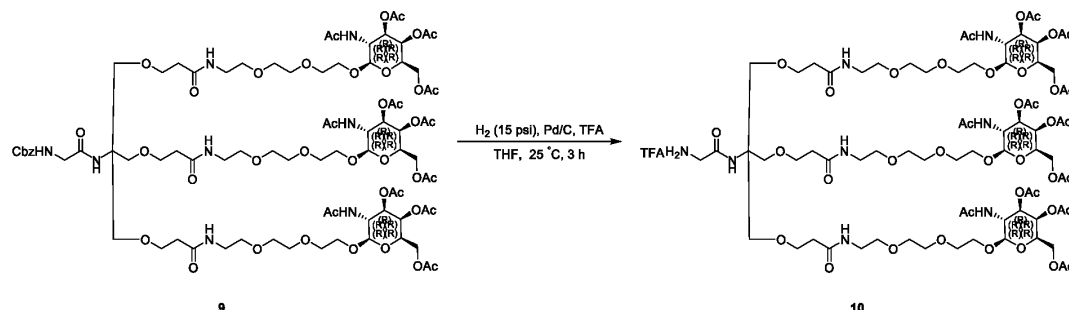
[00488] A solution of **Intermediate 7** (30.0 g, 43.0 mmol, 1.00 equivalent) in HCOOH (300 mL) was stirred at 25°C for sixteen hours. Thin-layer chromatography (PE: EA = 1: 1, R_f = 0.04) indicated **Intermediate 7** was consumed completely, and one major new spot with larger polarity was detected. 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). Liquid chromatography-mass spectrometry was retention time = 0.790 minutes, MS calculated: 528.20, mass observed: $[M + H]^+ = 529.2$.

Preparation of Intermediate 9.

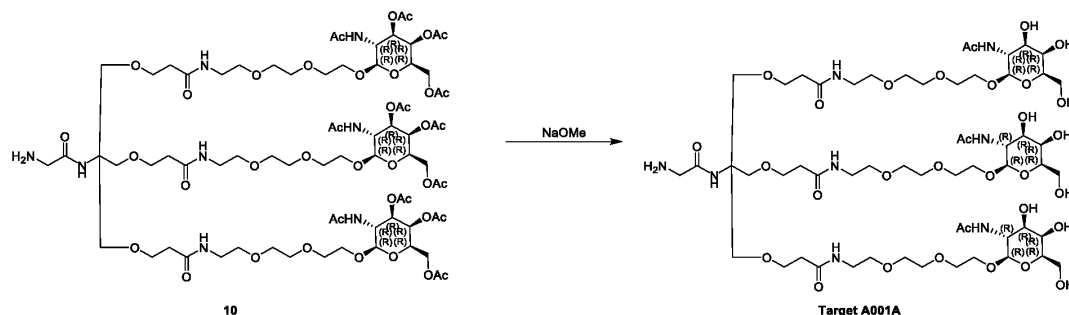


[00489] To a stirring solution of **Intermediate 8** (20.0 g, 37.8 mmol, 1.00 equivalent) and **Intermediate 4** (78.5 g, 132 mmol, 3.50 equivalents, TFA salt) in dimethylformamide (400 mL) was added HOBT (20.4 g, 151 mmol, 4.00 equivalents), EDCI (29.0 g, 151 mmol, 4.00 equivalents) and N,N-diisopropylethylamine (22.0 g, 170 mmol, 4.50 equivalents) successively. The reaction was stirred at 25°C for two hours. Thin-layer chromatography (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 hydrochloric acid 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% NaHCO₃ (450 mL), dried over Na₂SO₄, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO₂, 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. ¹H 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). Liquid chromatography-mass spectrometry was retention time = 3.455 minutes, MS calculated: 1908.81, mass observed: $[M + 2H]^{2+}$ = 955.7.

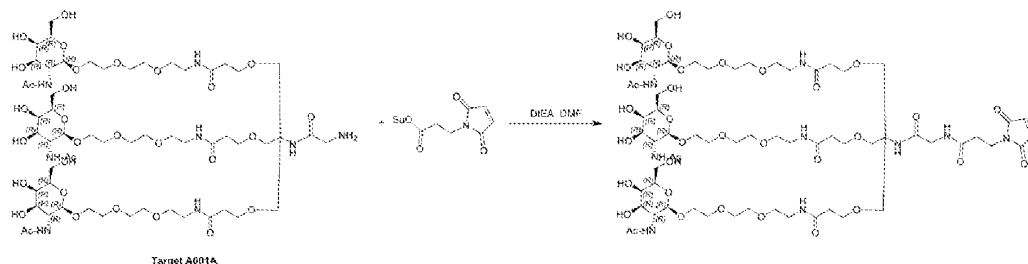
Preparation of Intermediate 10.



[00490] The 500 mL round-bottom flask was purged with argon gas for three times and added dry Pd/C (1.50 g, 1.41 mmol, 10% purity, 1.00 equivalent) carefully. Then tetrahydrofuran (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 μ L, 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. Then the mixture was stirred at 25°C for three hours under hydrogen gas atmosphere (15 psi). The reaction was monitored by liquid chromatography-mass spectrometry, which 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 tetrahydrofuran (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. ^1H 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). Liquid chromatography-mass spectrometry was retention time = 1.333 minutes, MS calculated: 1774.7, found: $[\text{M} + 2\text{H}]^{2+}$ = 888.6.

Preparation of intermediate Target A001A

[00491] 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 hours. The reaction was monitored by liquid chromatography-mass spectrometry, which showed the desired mass (one main peak with desired mass was detected.). The reaction mixture was added with 1.0 M hydrochloric acid 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. ^1H 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). Liquid chromatography-mass spectrometry was retention time = 0.966 minutes, MS calculated: 1396.6, found: $[M + 2H]^{2+}$ = 699.1.

EXAMPLE 18Chemical synthesis. Procedure for preparation of TBT103

[00492] 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

dimethylformamide (5 mL) was added N,N-diisopropylethylamine (46.2 mg, 357 μ mol, 59.1 μ L, 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. Liquid chromatography-mass spectrometry 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 **TBT103** (251.2 mg, 156 μ mol, 43.7% yield, 96.5% purity) as a colorless solid. Liquid chromatography-mass spectrometry was retention time = 1.382 minutes, MS calculated: M_{av} = 1548.59, $[M + H]^+$ = 1548.9, $[M + 2H]^{2+}$ = 775.1.

TABLE 19

Assay specification for TBT103

<u>Test</u>	<u>Analytical Method</u>	<u>Acceptance Criteria</u>	
Appearance ¹	Visual inspection		
Identification by FTIR	FTIR, USP <197A>	The infrared absorption spectrum of sample conforms to reference standard.	
Identification by HPLC Assay	HPLC	The retention time of the main peak of sample solution conforms to that in the reference standard solution $\geq 95.0\%$ (On anhydrous basis and solvent-free basis)	
Related substances	HPLC	Individual impurity:	$\leq 1.2\%$
		Unspecified impurities:	$\leq 5.0\%$
Residual solvents	HS-GC		
Water	KF, USP <921>	$\leq 10\%$	

EXAMPLE 19Membrane Proteome Array

[00493] Antibody deconvolution and specificity profiling. The Membrane Proteome Array (MPA) is a platform for profiling the specificity of antibodies and other ligands that target human membrane proteins. The MPA was used to determine antibody target specificity and deconvolute orphan antibody/ligand targets. Membrane Proteome Array platform uses flow cytometry to directly detect antibody binding to membrane proteins expressed in unfixed cells. The target proteins have native conformations and appropriate post-translational modifications.

[00494] Ligands were tested for reactivity against our library of over 6,000 human membrane proteins, including 94% of all single-pass, multi-pass, and GPI-anchored proteins, including GPCRs, ion channels, and transporters. Identified targets were validated in secondary screens to confirm reactivity.

[00495] Materials and methods. Membrane Proteome Array (MPA) screening was conducted at Integral Molecular, Inc. The MPA is a protein library composed of 6,000 distinct human membrane protein clones, each overexpressed in live cells from expression plasmids. Each clone was individually transfected in separate wells of a 384-well plate followed by a twenty-four hour incubation. See Tucker et al., Proc. Natl. Acad. Sci. USA. 115,E4990- E4999 (2018). Cells expressing each individual MPA protein clone were arrayed in duplicate in a matrix format for high-throughput screening. Before screening on the Membrane Proteome Array, the test ligand concentration for screening was determined on cells expressing positive (membrane- tethered Protein A) and negative (mock-transfected) binding controls, followed by detection by flow cytometry using a fluorescently-labeled secondary antibody. Each test ligand was added to the MPA at the predetermined concentration, and binding across the protein library was measured on an Intellicyt iQue using a fluorescently labeled secondary antibody. Each array plate contains both positive (Fc- binding) and negative (empty vector) controls to ensure plate-by-plate reproducibility. Test ligand interactions with any targets identified by MPA screening were confirmed in a second flow cytometry experiment using serial dilutions of the test antibody, and the target identity was re-verified by sequencing.

[00496] The final screen results are summarized in the following Tables. The project was carried out in three phases:

TABLE 20	
<u>Membrane Proteome Array carried out in three phases</u>	
I. Determination of assay screening conditions. Conditions appropriate for detecting test ligand binding by high-throughput flow cytometry were determined, including the optimal ligand concentration and cell type for screening.	
II. Membrane Proteome Array screen. Binding targets were identified by screening test ligands on the Membrane Proteome Array.	
III. Validation of ligand targets. Newly identified targets were validated in a follow-up titration experiment using flow cytometry.	

TABLE 21						
<u>Test ligands with newly identified targets, results</u>						
Test Ligand Name	Screening Cell Line	Screening Concentration, µg/mL	Known Targets		Newly Identified Targets	
			HGNC	Uniprot	HGNC	Uniprot
AGT402	HEK-293T	20	ASGR1	<u>P07306</u>	GP1R1	<u>Q99527</u>
					ADRA1B	<u>P35368</u>

[00497] Optimization of ligand concentrations for screening. Serial dilutions of each test ligand were assayed by incubation with known targets, and target binding was measured by flow cytometry. The optimal ligand concentration for screening was chosen based on a joint assessment of binding strength and background signal (left) and rate of high background events.

[00498] Membrane Proteome Array screen. To identify ligand binding targets, about 6,000 different membrane proteins were each expressed in individual wells of HEK-293T cells arrayed in 384-well plates. The cells were then matrixed by pooling individual columns and rows of each plate, so that each protein was represented in a unique combination of two different wells of the matrix plate.

[00499] Targets were identified by detecting ligand binding (using previously optimized conditions) to overlapping column and row pools, thereby allowing specific deconvolution. Each individual membrane protein target was assigned values corresponding to the binding values of their unique row and column pools, and targets demonstrating binding signal >3 standard deviations above background in both wells were selected for downstream validation experiments. The resulting paired binding values were subsequently normalized and transformed to give a single numerical value for binding of the test ligand against each target protein. Non-specific fluorescence was determined to be any value below three standard deviations of the mean background value.

[00500] Membrane Proteome Array screen results. Each ligand was tested for binding by flow cytometry against the MPA at the previously determined optimal concentration. Binding interactions confirmed in downstream validation studies are displayed in blue and any proteins that did not pass validation were removed.

[00501] Target validation. To validate any off-target interaction identified, cells were transfected with plasmids encoding the identified targets, protein A, or vector alone in 384-well format. After incubation four four-fold dilutions of each test ligand, starting at 20 µg/ml, were added to transfected cells followed by detection of ligand binding using a high-throughput immunofluorescence flow cytometry assay.

[00502] Average mean fluorescence intensity (MFI) values were determined for each ligand dilution. Only validated targets demonstrating a dose response and mean fluorescence intensity ≥2-fold above background at two consecutive concentrations were tested.

[00503] Validation results for newly identified ligand targets. Serial ligand dilutions were incubated with newly identified targets and binding was measured by flow cytometry. A target was validated if it demonstrated binding signal ≥2-fold above background at two consecutive concentrations tested.

EXAMPLE 20

Surface plasmon resonance. In vitro evaluation to characterize on-mechanism target-engagement.

[00504] Surface plasmon resonance assay. Surface plasmon resonance (SPR) assay is a biophysical approach that provides a label-free and real-time method for measuring biomolecular interactions, including binding affinity, kinetics, and specificity. The assay is performed on a biosensor platform where the binding interaction occurs between a ligand immobilized on a sensor chip and an analyte flowing in solution. Resonance signal (RU) plotted against analyte concentration derives a K_D via a “steady-state” fit.

[00505] The use of surface plasmon resonance (Biacore™ and IBIS-MX96 systems) and bio-layer interferometry (ForteBio™ Octet™ systems) can measure the binding ability of the IgG4-binding moiety. The assay can be performed on a Biacore S200 instrument, which has high sensitivity and low-medium throughput, e.g., using the manufacturer’s instructions. The IgG4-binding can be coupled to surface of a sensor chip as the 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.

IgG4 Fc surface plasmon resonance protocol

[00506] Background: The objective of this assay was to determine binding of bifunctional conjugates to IgG4 Fc using surface plasmon resonance.

[00507] This protocol was developed to confirm on-target engagement of protein-based degraders and pre-conjugated intermediate (monofunctional binders) with IgG4 Fc protein. The experimental setup uses a Biacore S200 instrument but can be adapted to other Biacore instrumentation such as the 8K or 8K+.

[00508] Preparation: Reconstitute lyophilized IgG4 Fc protein in diH₂O according to manufacturer instructions. For 200 µg pack size Human IgG4 Fc protein from ACRO Biosystems, reconstitute lyophilized material with 400 µL diH₂O and gently mix at room temperature for thirty minutes. The mg/mL reconstituted IgG4 Fc should be aliquoted to greater than 10 µg material per vial and stored at -80°C. The inventors avoided repeated freeze-thaw cycles.

[00509] IgG4 Fc was immobilized to target 550 RU on CM5 chip using amine coupling before this assay. hIgA1 was used as reference surface.

[00510] The inventors equilibrated CM5 sensor chip and sodium acetate buffer to room temperature by leaving on bench for approximately twenty minutes.

[00511] The inventors prepared 1x HBS-P running buffer by mixing 100 mL 10x HBS-P with 900 mL ultrapure water. Filter contents through a 0.22 µm PES membrane (Corning #431174). Fresh running buffer should be made up before every experiment.

[00512] Surface immobilization: Dilute IgG4 Fc protein and hlgA1 reference surface antibody to 20 µg/mL each in 10 mM sodium acetate buffer pH 5.0. Prepare reagents from amine coupling kit using volumes and rack orientation instructed by the prompts on the instrument protocol. Set target immobilization level to 550 RU.

Surface plasmon resonance binding assay (single cycle kinetics)

[00513] (1) With CM5 chip in the instrument, prime three times with 1x HBS-P running buffer.

[00514] (2) Dilute test molecules to top concentration of 1 µM in 1x HBS-P. A minimum volume of 200 µL is recommended. Always use the same 1x HBS-P running buffer as the preparation primed through the system for dilution of test molecules.

[00515] (3) In 96-well plate, dilute top concentration of test article 4-fold across six points. This dilution series can be achieved by aspirating 50 µL from the top concentration dose and mixing into 150 µL 1x HBS-P across and to the last (lowest concentration) point.

[00516] (4) Add startup or blank conditions to appropriate wells as required by instrument protocol (1x HBS-P buffer only).

[00517] (5) Prepare 50% dimethyl sulfoxide regeneration solution by mixing equal volumes of dimethyl sulfoxide and 1x HBS-P.

[00518] (6) Allow protocol to run and fit affinity from single cycle kinetics trace.

TABLE 22
Reagents and consumables

<u>Item</u>	<u>Vendor</u>	<u>Cat No</u>
Plastic vials 7 mm, 1000 pcs	Cytiva	BR100212
Rubber caps, type 3 (for 7 mm vials)	Cytiva	BR100502
Plastic vials, 15 mm	Cytiva	29266981
Rubber caps, type 5 (for 15 mm vials)	Cytiva	BR100655
Microplate Foils, 96 Well	Cytiva	28975816
10 mM acetate pH 5.0	Cytiva	BR100351
Amine coupling kit	Cytiva	BR100050
Series S Sensor Chip CM5	Cytiva	29149603
PP Masterblock, 384 well (deep well plate)	Greiner	781270

<u>TABLE 22</u> <u>Reagents and consumables</u>		
<u>Item</u>	<u>Vendor</u>	<u>Cat No</u>
1N sodium hydroxide	Fisher Scientific	SS277
HBS-P+ buffer, 10x	Cytiva	BR100671
Human IgG4 Fc protein, Tag Free (MALS & SPR verified)	ACRO Biosystems	IG4-H5205
Recombinant human IgA1 kappa	BioRad	HCA189
1L bottle top filter, 0.22 µm PES	Corning	431174
96W Polypropylene v bottom plate	Axygen	P-96-450V-C

[00519] *Binding confirmation by surface plasmon resonance.* The inventors performed surface plasmon resonance assays to confirm IgG4 Fc nanobody-binding. The inventors immobilized IgG4 Fc to a CM5 sensor chip by amine coupling right before the assays. The anti-IgG4 fragment was dosed 1 nM to 1 µM and characterized by single cycle kinetics with a contact time of sixty seconds, dissociation time of sixty seconds, and flow rate of 30 µL/minute.

[00520] The inventors obtained IgG4, full length and fragment domains, labelled with, e.g., biotin, producing, e.g., a 12 kDa recombinant antibody fragment with biotin conjugation. The inventors also labelled IgG4 with AF647 (3.5-6 dye molecules/protein) for use in the surface plasmon resonance assays.

[00521] The inventors tested unconjugated material in the surface plasmon resonance assays to make sure chemistry is not lowering the binding of the immunoglobulin region.

<u>TABLE 23</u> <u>Components of the IgG4 assay</u>		
<u>Item</u>	<u>Supplier</u>	<u>Description</u>
CaptureSelect® Biotin anti-IgG4 (Human) Conjugate	Thermo Fisher Catalog # 7102902500	12 kDa recombinant antibody fragment w/biotin conjugation
Human IgG4 Fc protein, Tag Free	ACRO Biosystems Catalog # IG4-H5205	HEK293 expression AA Glu 99-Lys 327
Thermo nanobody, a commercial biotin-VHH fragment, is used as a positive control.		

[00522] The results of the surface plasmon resonance IgG4-degrader target-engagement assays confirmed binding.

[00523] The inventors confirmed commercial anti-IgG4 nanobody binding to human IgG4 Fc. In n=2 characterization, K_D of about 20 nM was obtained. No binding response was observed for full-length IgG1-3 and IgG1-3 Fc regions at up to 1 µM concentrations.

TABLE 24 Single cycle kinetics	
Run	Steady State K_D (nM)
First run, single cycle kinetics, steady state K_D (nM).	22
Second run, single cycle kinetics, steady state K_D (nM).	24
Nanobody (anti-IgG4) binding by surface plasmon resonance showed a K_D of 20nM. This result validates robust binding of the VHH nanobody to IgG4.	

[00524] The steady state affinity results for this surface plasmon resonance assay are consistent with the obtained single cycle kinetic runs.

TABLE 25 Target engagement by surface plasmon resonance, single cycle kinetics.				
Degrader	K_D (nM)	K_D (nM)	SPR K_D (nM) $n=1$	SPR K_D (nM) $n=2$
ABT310 (SEQ ID NO: 31). VHH nanobody, truncated hinge, Fc fusion, unconjugated.			17	19
ABT311 (SEQ ID NO: 32). VHH nanobody, unconjugated.	13 ± 1	19 ± 1	10	10
ABT312 (SEQ ID NO: 33). VHH nanobody, truncated hinge, unconjugated.			8.3	10
AGT401 . VHH w/o hinge region. β GN3 via maleimide.	35 ± 10	20 ± 2	44	25
AGT402 . VHH-Fc fusion. β GN3 via MATE.	52 ± 13		64	39
AGT403 . VHH with hinge region. β GN3 via sortase.	32 ± 3		34	29
AGT404 . VHH w/o hinge region. β GN3 via sortase.	19 ± 5		24	14
Degradation engage IgG4 with potent binding affinity surface plasmon resonance validates IgG4-degrader target-engagement. IgG4 degrader molecules are IgG subclass-selective, and do not engage IgG in any toxicology assay species.				

[00525] With this surface plasmon resonance method, the inventors demonstrated anti-IgG4 Fc nanobody **ABT301** binding towards IgG4 Fc. Anti-IgG4 Fc nanobody **ABT301** presents mid-nM binding affinity for IgG4 Fc.

Additional assay information

[00526] Preparation. The inventors prepared running buffer - 1X HBS-P was made by diluting 100 mL 10x HBS-P in 900 mL Milli-Q water. Buffer was filtered through a Corning 431174 0.22 µm PES membrane.

[00527] Sample preparation. Prepared 1 µM conjugate samples in HBS-P (same freshly made buffer to be used as running buffer in assay:

[00528] Prepared 1000 µL of samples at a concentration of 1 µM in 1X HBS-P buffer using attached protocol calculations (also annotated above). Made up 200 µL for **AGN402** to conserve material.

[00529] (1) Added 150 µL 1X HBS-P to wells A1:F5 using multichannel pipette.

[00530] (2) Added 200 µL 1 µM samples to appropriate wells.

[00531] (3) Using multichannel pipette, transferred 50 µL from top dilution series and mixed in the following row to create a 1:4 serial dilution with 6 points from right to left.

[00532] (4) Added 200 µL 1X HBS-P to well G1 as buffer control.

[00533] (5) Mixed 1 mL DMSO with 1 mL 1X HBS-P to use as 50% DMSO regeneration buffer. Regen buffer was added to a 4 mL plastic vial and placed at position A1 of reagent rack 2, per above configuration.

[00534] In Biacore S200 control software: Before screen, running buffer (1X HBS-P) primed three times by selecting "multi prime" in the tool menu. These selections were continued through template/wizard prompts to set up the protocol in Biacore S200 software.

TABLE 26
Materials

	<u>Vendor</u>	<u>Catalogue #</u>
Echo 650	Beckman Coulter	
Biacore S200	GE Healthcare	
Rubber caps, type 3 (for 7 mm vials)	Cytiva	BR100502
Plastic vials, 15 mm	Cytiva	29266981
Rubber caps, type 5 (for 15 mm vials)	Cytiva	BR100655
Microplate Foils, 96 Well	Cytiva	28975816
Microplate Foils, 384 well	Cytiva	BR100577
HBS-N buffer, 10X	Cytiva	BR100670
Tween 20, 10%	Roche	73922900
10 mM acetate pH 4.0	Cytiva	BR100349

TABLE 26 Materials		
	Vendor	Catalogue #
10 mM acetate pH 4.5	Cytiva	BR100350
10 mM acetate pH 5.0	Cytiva	BR100351
10 mM acetate pH 5.5	Cytiva	BR100352
Amine coupling kit	Cytiva	BR100050
Series S Sensor Chip CM5	Cytiva	29149603
PP Masterblock, 384 well (deep well plate)	Greiner	781270
1N sodium hydroxide	Fisher Scientific	SS277
HBS-P+ buffer, 10x	Cytiva	BR100671
Human IgG4 Fc protein, Tag Free (MALS & SPR verified)	ACRO Biosystems	IG4-H5205
Normal human IgG control	R&D Systems	1-001-A
1L bottle top filter, 0.22 µm PES	Corning	431174
384 well PP 2.0 Microplate, Echo qualified	Labcyte	PP-0200
96W Polypropylene v bottom plate	Axygen	P-96-450V-C

EXAMPLE XSubtype selectivity

[00535] Anti-IgG4 Fc nanobody **ABT301** does not show response to full-length IgG1, full-length IgG2, full-length IgG3, IgG1 Fc, IgG2 Fc, or IgG3 Fc at up to 1 µM concentrations.

TABLE 27 Human isotype selectivity comparisons, as measured by surface plasmon resonance K_D , µM									
Compound	Surface plasmon resonance K_D (nM)								
	Native hIgG1	Native hIgG2	Native hIgG3	Native hIgG4	hIgG1 Fc	hIgG2 Fc	hIgG3 Fc	hIgG4 Fc	hIgG4 Fc
AGT401	>1 µM	>1 µM	>1 µM	36	>1 µM	>1 µM	>1 µM	35 ± 10	0.025
AGT402	>1 µM	>1 µM	>1 µM	15	>1 µM	>1 µM	>1 µM	52 ± 13	0.001 7
AGT403	>1 µM	>1 µM	>1 µM	33	>1 µM	>1 µM	>1 µM	32 ± 3	0.027

TABLE 27 Human isotype selectivity comparisons, as measured by surface plasmon resonance K_D, μM									
Compound	Surface plasmon resonance K_D (nM)								
AGT404	>1 μ M	>1 μ M	>1 μ M	43	>1 μ M	>1 μ M	>1 μ M	19 $\pm$ 5	0.052
For native (FL) and Fc regions of IgG1, IgG2, and IgG3 subclasses, no binding response witnessed at 1 μ M. K_D s $\pm$ std. dev. reported from two independent surface plasmon resonance experiments, unless otherwise indicated.									

TABLE 28 Nonhuman isotype selectivity comparisons, as measured by surface plasmon resonance K_D, μM. IgG4 degraders do not demonstrate cross-species reactivity.							
Agent	Recombinant cynomolgus monkey IgG1	Rhesus monkey IgG2 Fc	Cynomolgus monkey IgG3 Fc	Rhesus monkey IgG4 Fc	Rat IgG, whole molecule	Mouse IgG, whole molecule	Rabbit IgG Fc
AGT401	>1 μ M	>1 μ M	>1 μ M	23	>1 μ M	>1 μ M	>1 μ M
AGT402	>1 μ M	>1 μ M	>1 μ M	6.4	>1 μ M	>1 μ M	>1 μ M
AGT403	>1 μ M	>1 μ M	>1 μ M	23	>1 μ M	>1 μ M	>1 μ M
AGT404	>1 μ M	>1 μ M	>1 μ M	22	>1 μ M	>1 μ M	>1 μ M
Note: no binding response was observed for any IgG species (other than rhesus monkey IgG4 Fc) at 1 μ M. Subsequent follow-up experiments for rhesus monkey IgG4 Fc were performed starting from 25 μ M.							

[00536] The inventors demonstrated little cross species reactivity. The results demonstrated no engagement for non-human species, not even the rhesus monkey. Sequence alignment showed that IgG3 and IgG4 are similar in the binding site for the cynomolgus monkey sequence.

EXAMPLE 21

Ternary complex assay to characterize on-mechanism pharmacological mode of activity.

[00537] The inventors performed cell-based TR-FRET assays to assess ternary complex on cell surface. The objective of this assay was to screen IgG4 degrader conjugates in the FRET-based ternary complex formation assay previously developed using AlexaFluor647 labeled anti-hIgG4 as the FRET acceptor fluorophore.

[00538] The TR-FRET principle is as follows: The donor fluorophore is excited with laser or flash lamp and emits light. The acceptor fluorophore excited by energy from donor if in proximity. The resulting signal emission from acceptor is proportional to level of interaction. See Li et. al., J. Biomol. Screen.,

20(7), 869-75 (August 2015) and Wen et al., AAPS J., 22(3), 68 (April 16, 2020). The TR-FRET assay is an In vitro evaluation to characterize pharmacological mechanism of action. The TR-FRET assay measures the induced proximity of the bifunctional degrader moieties to validate degrader-induced ASGPR-IgG4 proximity.

[00539] The inventors used secondary antibody AF647 labeled anti-hIgG4 as the acceptor fluorophore in the TR-FRET assay. The condition for the assay were: 6.3 nM hIgG4 (Abcam), 25 nM ASGPR1, 2.5 nM streptavidin-europium cryptate, 20 nM AF-647 labeled anti-hIgG4, in 18 μ L reaction, with 0.1% dimethyl sulfoxide.

[00540] Assay: This assay was developed to confirm three-body ternary complex formation between ASGPR1 and IgG4 induced by a heterobifunctional degrader. An Echo acoustic liquid handler is used to dispense dimethyl sulfoxide negative control and **AGN406** positive control, with handheld multichannel pipettes used to dispense the aqueous dilutions of test molecules.

[00541] Assay preparation: The inventors slow thawed rASGPR from -80°C storage on ice.

[00542] The inventors prepared **AGN406** at a concentration of 12.5 μ M in 100% dimethyl sulfoxide (100x final top concentration). The inventors added **AGN406** and dimethyl sulfoxide to an Echo-qualified microplate for dispensing of positive and negative assay controls.

[00543] The inventors diluted **AGN406** two-fold, vertically (over sixteen points) in a 384-well v bottom plate by mixing 25 μ L in 25 μ L 1x buffer, from a top concentration of 250 μ M in an Echo-qualified source plate.

[00544] Assay steps: (1) The inventors prepared an appropriate volume of 1x assay buffer: 25 mM HEPES pH 7.5, 150 mM sodium chloride, 5 mM calcium chloride, 1 mM dithiothreitol, 0.01% TWEEN-20.

TABLE 29

Example calculation for 40 mL of 1x buffer

<u>Component</u>	<u>Stock</u>	<u>Final</u>	<u>Add</u>
HEPES pH 7.5	1000 mM	25 mM	1000 μ L
Sodium chloride	5000 mM	150 mM	1200 μ L
Calcium chloride	1000 mM	5 mM	200 μ L
Tween-20	10%	0.01 %	40 μ L
Dithiothreitol	1000 mM	1 mM	40 μ L
MilliQ water			37520 μ L
		<u>Total:</u>	40000 μ L

[00545] (2) The inventors prepared a 50 μL degrader test article in 1x assay buffer at concentration of 30 μM as top dose in dilution series.

[00546] (3) Using Echo liquid handler, the inventors dispensed 180 nL dimethyl sulfoxide (negative control), 180 nL 12.5 μM **AGN406** (positive assay control), and 180 nL **AGN406** dosed control to appropriate wells of a 384 well OptiPlate. Backfill these wells with 6 μL 1x assay buffer for volume scaling.

[00547] (4) The inventors added 6 μL degrader dilutions to appropriate wells of assay plate using electronic multichannel repeating pipette. The plates were sealed and set aside.

[00548] (5) The inventors prepared a 3x proteins mixture, which contains 75 nM ASGPR1 and 18.75 nM IgG4 in 1x assay buffer.

<u>TABLE 30</u> <u>Example calculation</u>				
<u>Protein</u>	<u>Stock</u>	<u>Final</u>	<u>3x</u>	<u>Add</u>
hIgG4	13.7 μM	0.00625 μM	0.01875 μM	6.84 μL
rASGPR1-8	510 μM	0.025 μM	0.075 μM	0.74 μL
1x Buffer				4992.42 μL
			<u>Total</u>	5000.00 μL

[00549] (6) Using Multidrop liquid handler, the inventors dispensed 6 μL proteins mixture to all wells of assay plate containing degrader dilutions and controls.

[00550] (7) The inventors sealed the plate, centrifuged at 1000 RPM for one minute, and incubated for sixty minutes at room temperature.

[00551] (8) The inventors prepared a 3x detection mix in 1x assay buffer: 7.5 nM streptavidin-Europium cryptate and 60 nM mouse anti-human IgG4 Fc-AlexaFluor647.

<u>TABLE 31</u> <u>Example calculation for 5 mL solution</u>				
<u>Item</u>	<u>Stock</u>	<u>Final</u>	<u>3x</u>	<u>Add</u>
Streptavidin Eu cryptate	733.3 nM	2.5 nM	7.5 nM	51.1 μL
anti-hIgG4 AF647	3333 nM	20 nM	60 nM	90.0 μL
1x Assay Buffer				4858.9 μL
			<u>Total</u>	5000.0 μL

[00552] (9) Using a Multidrop Combi liquid handler, the inventors dispensed 6 μL detection mix to all wells of assay plate.

[00553] (10) The inventors sealed the plate, centrifuged at 1000 RPM for one minute, and incubated for sixty minutes at room temperature.

[00554] (11) The inventors recorded FRET using excitation at 320 nm and emission and 665 nm and 615 nm on an Envision multilabel reader. Data were outputted as a .csv file with FRET ratio data reduction already performed on reader. TR-FRET values were calculated as $[(665/615)*1000]$ and plotted as the response at each test concentration of degrader. Assay signal to background was calculated as the mean signal of fixed concentration positive control (**AGN406**) divided by the mean signal of negative control (dimethyl sulfoxide) wells. Calculations for s/b and Z' were made. Dose-response data were formatted to plot 2 replicate values side by side.

TABLE 32			
Materials			
<u>Item</u>	<u>Vendor</u>	<u>Catalog Number</u>	<u>Lot (if applicable)</u>
Echo 650	Beckman Coulter	E6XX-22020	
Envision 2105	PerkinElmer	1050241	
Mouse anti-human IgG4 Fc-Alexa Fluor647	Southern Biotech	9200-31	E1723-QB54B
Streptavidin-Eu cryptate 20k points	Revvity	610SA KLB	37RA
TWEEN-20	Roche	73922900	11332465001
Calcium chloride 1 M solution	Boston BioProducts	MT-140	B01P105
Sodium chloride 5 M solution	Boston BioProducts	BM-244	K070112
DL-Dithiothreitol	Sigma Aldrich	D9779-5G	SLCM3385
Dimethyl sulfoxide	Sigma Aldrich	D5879	
Native Human IgG4 Protein	Abcam	AB183266	1075014-14
HEPES buffer, pH 7.5 1 M solution	Boston BioProducts	BBH-75	H100101
N-Avi-ASGPR1 (148-291) construct 8 (ASGPR1-8)	Viva	Custom	2023.06.21
384 well PP 2.0 Microplate, Echo qualified	Labcyte/Beckman	PP-0200	
ProxiPlate 384	PerkinElmer	6007290	

[00555] Reagents for protein biotinylation for purification, immobilization or sensitive protein detection are commercially available for the AviTag™ Technology (GeneCopoeia, Rockville MD, USA).

[00556] The inventors confirmed the In vitro activity of degrader-induced ASGPR-IgG4 proximity as measured by formation of a ternary complex by TR-FRET assays. Induced proximity between ASGPR and IgG4 generates a specific energy transfer signal. The TR-FRET signal is dependent on MoDE-induced proximity of ASGPR and IgG4. The TR-FRET signal of VHH-based degrader molecules validates the bifunctional mechanism of action.

TABLE 33

In vitro characterization: TR-FRET validates degrader-induced IgG4-ASGPR proximity

<u>Binding moiety</u>	<u>Ternary complex. TCF observed or not.</u>
ABT310 (SEQ ID NO. 31). VHH nanobody, truncated hinge, Fc fusion, unconjugated.	Not tested
ABT311 (SEQ ID NO: 32). VHH nanobody, unconjugated.	No (negative control)
ABT312 (SEQ ID NO: 33). VHH nanobody, truncated hinge, unconjugated.	Not tested
AGT401 . VHH w/o hinge region. β GN3 via maleimide.	Yes
AGT402 . VHH-Fc fusion. β GN3 via MATE.	Yes
AGT403 . VHH w/hinge region. β GN3 via sortase.	Yes
AGT404 . VHH w/o hinge region. β GN3 via sortase.	Yes

[00557] The inventors titrated **AGT406** across sixteen points in triplicate from 10 μ M to 0.3 nM.

[00558] The inventors used HEK293 fluorescent cell-based assays for assessing ternary complex formation. HEK293 fluorescent cell-based assays allow persons having ordinary skill in the biomedical art to monitor the kinetics of ternary complex formation in cells.

[00559] The inventors also used other assays to assess event-driven mechanism of action. See, e.g., Pettersson & Crews, Drug Discovery Today Technologies, 31, 15-27 (Elsevier BV, 2019). This assay allows persons having ordinary skills in the biomedical art to monitor the kinetics of ternary complex formation in cells.

[00560] The inventors also used NanoBRET, a bioluminescence resonance energy transfer (BRET) assay that measures protein-protein interactions (PPIs) in live cells. NanoBRET uses a small, bright luciferase called Nanoluc to tag intracellular proteins, and a long-wavelength fluorophore called HaloTag to act as an energy acceptor. When the two proteins are in close proximity, energy is transferred from

Nanoluc to HaloTag, resulting in fluorescence emission at a specific wavelength. NanoBRET can be used to analyze binding events, signaling pathways, and receptor trafficking.

EXAMPLE 23

Endocytosis assay for IgG4 compounds

[00561] The inventors used IgG4 from Sydlabs (recombinant and already conjugated to fluorophore IF488).

[00562] For routine culture of HEK-ASGPR1 cells, the inventors used DMEM high glucose, with 10% fetal bovine serum, 2 mM glutamine, and 1% P/S+200ug/ml geneticin.

<u>TABLE 34</u> <u>Reagents for endocytosis assay</u>		
	<u>Vendor</u>	<u>Cat # (and Lot)</u>
DMEM high glucose with phenol	Thermo Fisher (Gibco)	11960044
DMEM high glucose without phenol	Thermo Fisher (Gibco)	31053028
Opti-MEM Reduced serum medium	Thermo Fisher (Gibco)	31985062
Geneticin 50 mg/ml	Thermo Fisher (Gibco)	10131-035
Fetal bovine serum, 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
Human IgG4 S228P isotype control- we asked them to conjugate this product to IF-488	Sydlabs	PA007128 (conjugated to IF-488)

TABLE 35			
<u>Agents tested</u>			
	<u>mg/ml</u>	<u>Molecular weight (Da)</u>	<u>Calculated molarity (μM)</u>
AGN508	8.81	15636.9	563.41
AGN501	8.69	14498.8	599.36
AGN502	8.98	14570.8	616.30
AGN509	8.81	15321.6	575.01
AGN402	8.16	83779.5	97.40

[00563] The inventors used Dulbecco's phosphate-buffered saline without calcium/magnesium to rinse the cells once.

[00564] Then, the inventors harvested HEK-ASGPR1 cells as follows: 5 ml accutase at T175 flasks 37°C for five minutes. Add 10 ml media and pipet up-down. Spin down 1000 rpm for five minutes. The inventors seeded 30k/well of a 96-well plate, 100 μl/well based on the plate map, using DMEM high glucose without phenol (31053028) +10% fetal bovine serum +2 mM glutamine +1% P/S+200 μg/ml geneticin.

[00565] The inventors used poly-D lysine 96-well plates (Corning # 3842).

[00566] The cells grew overnight in an incubator. The next day, the inventors added 50nM IgG4 in the appropriate media (Opti-MEM+ 200 μg/ml G418 and compound).The inventors made an eight-point dose response with 500nM top concentration and two-fold dilution. For the last point, the dilution was ten-fold from the previous.

[00567] The inventors incubated the assay overnight using an Incucyte instrument reading @ zero hours, four hours, six hours, ten hours, twelve hours, fourteen hours, sixteen hours, twenty hours, and twenty-four hours.

[00568] At twenty-four hours, the inventors did a wash out with Dulbecco's phosphate-buffered saline with no calcium/magnesium to reduce the background. Then the inventors added 100 μl/well of media (Opti-MEM+ 200 μg/ml geneticin) and scanned again at Incucyte.

EXAMPLE 24

In vitro degradation assay.

[00569] This on-mechanism degradation assay is by a direct, low-throughput Western blot measurement). Western assays can be used to measure the degradation in HEK293-ASGPR1 cells.

Assay workflow

[00570] Day 0. The inventors seeded cells at 0.5×10^6 /well 6-well plates.

[00571] Day 1. 100nM IgG4-AF488+60nM compound treatment overnight.

[00572] Day 2. The inventors performed three washes with DPBS. The cells were lysed at T_0 hour (max) and other time points. The inventors added bafilomycin, which is a lysosome inhibitor, to some wells.

[00573] Day 3. Lysed at the twenty-four hour point.

TABLE 36 In vitro degradation assay results for AGT402 (Fc- fusion)		
Time point after wash	% degradation (mean)	
	- bafilomycin	+ bafilomycin
One hour	55%	2%
Two hours	79%	11%
Five hours	94%	16%
Twenty-four hours	98%	51%

[00574] AGT402 can lead to almost complete IgG4 degradation by five hours post-wash. Bafilomycin inhibits degradation of IgG4 (degradation is lysosome-dependent). The ASGPR-dependent mechanism of Molecular Degradation of Extracellular targets (MoDE)-induced degradation is described by Caianiello, et al., Nature Chemical Biology (2021). Bafilomycin inhibits degradation of AF-488 conjugated IgG4 because the degradation is lysosome-dependent.

EXAMPLE 25LysolqBiT assay

[00575] The LysolqBiT assay is a tool that can be used for studying the internalization and degradation of proteins of interest (POIs) in real time. By combining lysosome-specific signaling with protein labeling strategies, this assay delivers both precision and adaptability, making it suitable for a broad range of applications. A LqBiT Expression Vector and Stable Cell Line is commercially available from Promega (Catalog number N2681). See U.S. Pat. No. 9,797,889 (Dixon et al.).

[00576] Features of the LysolqBiT assay include:

[00577] LAMP1-LqBiT fusion protein, a lysosomal-specific fusion protein provides a robust and precise readout for the localization and degradation of HiBiT-tagged agents.

[00578] The HiBiT tag, an 11-amino-acid peptide, can be appended to an agent using a simple and efficient copper-free click-chemistry method. This approach minimizes interference with the agent's function and simplifies assay preparation. Fluorogenic protease substrates enable specificity by incorporating customizable linker sequences.

[00579] The fusion of ASGPR1 HiBiT/NanoLuc proteins in HEK293 cells enables real-time monitoring of protein internalization kinetics. Luminescence reduction serves as a direct indicator of internalization and subsequent lysosomal degradation.

[00580] Fluorophore/quencher pairs, such as DyLight 650 and IRDye QC-1, allow the assessment of protein degradation, as described in established protocols. See Li et al., A cell-based internalization and degradation assay with an activatable fluorescence–quencher probe as a tool for functional antibody screening. *Journal of Biomolecular Screening*, 20(7), 869-75 (August 2015).

[00581] The Incucyte assay provides a streamlined workflow with cost-effective protein labeling, optimized for high-throughput imaging. One pH sensitive dye, Incucyte FabFluor, is a Fab fragment conjugated to pH dependent fluorophore binds to Fc The dye is optimized for Incucyte optics and analysis software.

[00582] Flow Cytometry offers high sensitivity with reduced background noise, achieved by gating on labeled cell populations.

[00583] By appending the HiBiT tag, the LysolgBiT assay can be applied to any agent. The HEK293 HiBiT/NanoLuc Fusion often needs no modification to a specific agent and has the potential for throughput scaling and broad utility, real time.

EXAMPLE 26

In vitro toxicology studies

[00584] Among the off-target assays that the inventors performed were measurements of agent cytotoxicity using an In vitro toxicology safety panel. The inventors performed the toxicology evaluation using HEPG2 cells, e.g., in a CellTiter-Glo assay.

[00585] Also, metabolite identification (MetID) is a crucial and integral part of drug discovery and development. In this context, metabolism describes the breakdown and conversion of xenobiotics into molecules that can be excreted from the body. The inventors assessed MetID both in vitro and in vivo (blood, urine and liver) in mice.

[00586] These results were sufficient to proceed with drug development.

[00587] The inventors are also performing cell based screening assays to measure cytokine release.

EXAMPLE 27In vivo toxicology studies

[00588] The inventors are performing toxicology assays to measure intravenous compatibility.

[00589] Choice of toxicology species. The inventors are performing toxicology assays in several mammalian model species.

[00590] Rat can be chosen as a second toxicology species. The inventors have experience with rat toxicology using testing by intravenous bolus and measuring cytokine reaction.

[00591] Humanized IgG4 minipig can be chosen as a second toxicology species. The inventors can assess pharmacodynamics in the live minipig and collect minipig plasma to confirm binding to IgG4.

[00592] A monkey species can be chosen as a second toxicology species because of similarity of immune system to humans. Rabbit only has one IgG isotype, so it is not as valuable.

EXAMPLE 28In vivo tolerability assays

[00593] The inventors performed tolerability assays, starting with in vivo mouse assays (100 mg/kg), and then with in vivo rat assays (up to 1000 mg/kg).

TABLE 37Both Fc and non-Fc degraders well-tolerated in mouse at up to 100 mpk

<u>Agent</u>	<u>Tolerability at 100 mpk (w/o target)</u>
AGT402	Well-tolerated
AGT501	Well-tolerated
AGT502	Well-tolerated

TABLE 38IgG4 + Agent (Fc-Fusion and Non-Fc-Fusion) Tolerability

<u>IgG4 (IV) dose</u>	<u>Agent dose</u>	<u>Agent route of administration</u>	<u>Ratio (Agent/IgG4)</u>	<u># of mice</u>	<u>Clinical signs</u>	<u>Blood collection</u>
2 mg/kg	Fc-fusion (AGT402) 4.5 mg/kg	intravenous and subcutaneous	4:1	3	Mice appeared healthy 96 hours of injection	1-2 submandibular bleeds
2 mg/kg	Fc-fusion (AGT402) 1.125 mg/kg	intravenous	1:1	3	Mice appeared healthy 96 hours of injection	1-2 submandibular bleeds

TABLE 38 IgG4 + Agent (Fc-Fusion and Non-Fc-Fusion) Tolerability						
<u>IgG4 (IV) dose</u>	<u>Agent dose</u>	<u>Agent route of administration</u>	<u>Ratio (Agent/ IgG4)</u>	<u># of mice</u>	<u>Clinical signs</u>	<u>Blood collection</u>
2 mg/kg	Non-fc-fusion (AGT501, AGT502) 0.77 mg/kg	intravenous and subcutaneous	4:1	3	Mice appeared healthy 96 hours of injection	1-2 submandibular bleeds
2 or 4 mg/kg	-	-	-	3	Mice appeared healthy 96 hours of injection	1-2 submandibular bleeds
-	Fc-fusion (AGT402) up to 100 mg/kg	intravenous	-	2	Mice appeared healthy	1-2 submandibular bleeds
-	Non-fc-fusion (AGT501, AGT502) up to 100 mg/kg	intravenous	-	2	Mice appeared healthy	1-2 submandibular bleeds
2 mg/kg	Fc-fusion (AGT402) 1.125 mg/kg	intravenous	1:1	3	Mice appeared healthy 96 hours of injection	2 submandibular bleeds
4 mg/kg	Fc-fusion (AGT402) 2.2 mg/kg	intravenous and subcutaneous	1:1	3	about 72 hours following injections (2nd submandibular bleed), mice were in poor health, low motility, about 20% loss of body weight	2 submandibular bleeds
4 mg/kg	Fc-fusion (AGT402) 2.2 mg/kg	intravenous	1:1	5	Mice appeared healthy and did not lose weight about 100 hours after injection	None
4 mg/kg	Fc-fusion (AGT402) 8.9 mg/kg	intravenous	4:1	5	Mice appeared healthy and did not lose weight about 100 hours after injection	None

TABLE 38 IgG4 + Agent (Fc-Fusion and Non-Fc-Fusion) Tolerability						
<u>IgG4 (IV) dose</u>	<u>Agent dose</u>	<u>Agent route of administration</u>	<u>Ratio (Agent/IgG4)</u>	<u># of mice</u>	<u>Clinical signs</u>	<u>Blood collection</u>
4 mg/kg	Non-fc-fusion (AGT501, AGT502) 0.39 mg/kg	intravenous	1:1	5	Mice appeared healthy and did not lose weight about 100 hours after injection	None
4 mg/kg	Non-Fc-fusion (AGT501, AGT502) 1.55 mg/kg	intravenous	4:1	5	Mice appeared healthy and did not lose weight about 100 hours after injection	None
4 mg/kg	Fc-fusion (AGT402) 2.2 mg/kg	intravenous	1:1	3	Mice did not lose weight 24 , 48, 72, or 96 hours after injection	Three submandibular bleeds did not affect motility or health of the mice

EXAMPLE 29**Physical stability**

[00594] VHH-Fc fusion does not present issues in stability at 90 mg/mL in off-the-shelf biologics formulation (20 mM Histidine, pH 6.0, 8% sucrose).

[00595] The inventors also confirmed in plasma stability assays.

TABLE 39 Hold time study for protein intermediate		
	<u>Conditions</u>	<u>Time points for pemphigus vulgaris batches</u>
Long-term	-20±5°C	T ₀ , 0.5 M, 1 M
Hold time tests for protein intermediate include Appearance, pH, Concentration, ELISA Binding, size exclusion chromatography, CE SDS (reducing and non-reducing), imaged capillary isoelectric focusing		

TABLE 40 Stability study for bioconjugate drug substance		
Stability study	Conditions	Time points for pemphigus vulgaris batches
Long-term	-20±5°C	T ₀ , 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 36 months
Accelerated	5±3°C, upright	T ₀ , 1 month, 3 months, 6 months
	5±3°C, inverted	T ₀ , 1 month, 3 months, 6 months
Stress	25±2°C / 60±5% RH, upright	T ₀ , 1 week, 2 weeks, 1 month, 3 months
Stability tests for agent include appearance, pH, concentration, subvisible particles, ELISA, cell based potency, size exclusion chromatography, CE SDS (R/NR), imaged capillary isoelectric focusing, free drug levels, and excipient (e.g. PS-80)		

EXAMPLE 30***Other in vivo assays using mouse models.***

[00596] *Pemphigus vulgaris model efficacy assay.* Inject BVMAB786 mice subcutaneously. Day 1: Administer anti-Dsg3 IgG4 AutoAb, shortly followed by IgG4 degrader. Day 2: Administer ETA. Symptom onset after 18-72 hours. In presence of degrader, ETA should have no effect. In absence of degrader, ETA should induce pemphigus vulgaris symptoms.

TABLE 41 Mouse models				
	Origin, subclass	In vitro	In vivo	Reference
PVB28	Generated from untreated patient, IgG4	Dsg3 specific, Keratinocyte dissociation assay	Neonates dosed at 300 mg/mouse. + 2 mg ETA. Symptom onset at eighteen hours. Qualitative analysis of cutaneous disease and histology	Di Zenzo, J. Clin. Invest. (2012) (in vitro/vivo)
MAB786 (AKA F706)	Generated from untreated patient, IgG4	Dsg3 specific, Acantholysis induced <i>in vitro</i> organ culture	Neonates dosed at 0.6 mg/g. Symptom onset at eighteen hours. Cutaneous disease score and histology	Yeh, Clin. Imm. (2006)

TABLE 42 Pemphigus vulgaris model efficacy assay.					
<u>Body weight (g)</u>	<u>Antibody dose (mg/g)</u>	<u>Subcutaneous volume to inject (ml/kg)</u>	<u>Volume to inject/mouse (ml)</u>	<u>Amount/mouse (mg)</u>	<u>Dose solution conc (mg/ml)</u>
25	0.6	10	0.25	15	60

[00597] Testing for antidrug antibodies (ADA) is done in mouse models. Many of the antibody drug antibodies are IgG4.

[00598] Other assays using mice. In vivo biology to validate on-mechanism activity (pharmacokinetics/pharmacodynamics in ASGPR knock-out mouse). Pharmacokinetics and pharmacodynamics parameters are derived from intravenous and subcutaneous mouse studies.

EXAMPLE 31

In vivo depletion assay

[00599] In vivo characterization validated IgG4 depletion by VHH-ASGPR ligand bifunctional degraders (non-Fc fusion). Non-Fc fusion degraders also show IgG4-depletion.

TABLE 43 <u>Side-by-side comparison of pharmacodynamics profiles of Fc and non-Fc degraders versus unconjugated at twenty-four hours.</u>	
<u>Agent</u>	<u>%-depletion of IgG4</u>
AGT401	50%
AGT402	60%
ABT503	52%
ABT504	63%
In vivo characterization in mice validates IgG4-depletion.	

TABLE 44 <u>% of depletion of antibodies in mice in vivo at eight hours and twenty-four hours, compared to IgG4 only</u>		
<u>Agent</u>	<u>% Depletion (8 hours)</u>	<u>% Depletion (24 hours)</u>
AGT401, conjugated	50.3%	52.6%
AGT403, conjugated	52.2%	46.1%
AGT404, conjugated	63.3%	58.4%

TABLE 44

% of depletion of antibodies in mice in vivo at eight hours and twenty-four hours, compared to IgG4 only

<u>Agent</u>	<u>% Depletion (8 hours)</u>	<u>% Depletion (24 hours)</u>
ABT306 (SEQ ID NO: 11), unconjugated	Negative-no difference	3.7%
ABT304 (SEQ ID NO: 9), unconjugated	Negative-no difference	Negative-no difference
ABT305 (SEQ ID NO: 10), unconjugated	Negative-no difference	Negative-no difference

TABLE 45

Summary of Results: AGT402 (Fc-fusion)

	<u>Human IgG4 (mg/kg)</u>	<u>Agent route of administration</u>	<u>Agent (mg/kg)</u>	<u>Ratio (Agent/IgG4)</u>	<u>% Depletion relative to hlgG4 AUC</u>				
					<u>AUC 8 hours</u>	<u>AUC 24 hours</u>	<u>AUC 48 hours</u>	<u>AUC 72 hours</u>	<u>AUC 96 hours</u>
PD1	2 mg/kg	IV	4.5	4:1	87.3	60.4	N/A	N/A	N/A
PD2	2 mg/kg	IV	4.5	4:1	89.9	90.2	N/A	90.2	90.8
PD2	2 mg/kg	subQ	4.5	4:1	4.2	27.3	N/A	59.0	65.4
PD3	2 mg/kg	IV	4.5	4:1	90.6	91.8	92.6		
PD3	2 mg/kg	IV	1.125	1:1	88.9	85.5	86.3		
PD4	4 mg/kg	IV	2.2	1:1	89	89.4	88	87.5	87.8
PD4	4 mg/kg	subQ	2.2	1:1	23.9	49.9	62	68.5	72

Intravenous administration of AGT402 depletes IgG4 levels by about 90% regardless of IgG4 dose or AGT402:IgG4 ratio. Subcutaneous administration of AGT402 depletes IgG4 levels by about 69%. Pharmacokinetics studies demonstrate about 50% subcutaneous bioavailability in mice. Pharmacodynamics and pharmacokinetics for non-Fc-fusion compounds.

TABLE 46

In vivo characterization: degrader-dependent depletion of IgG4 (Fc fusion).

<u>Agent</u>	<u>% depletion at 8 hours</u>	<u>% depletion at 24 hours</u>
AGT402	87.3%	60.4%
ABT310	Negative-no difference	Negative-no difference

Fc fusion degrader mediates IgG4-depletion at eight hours. Fc fusion degrader shows IgG4-depletion and rebound effect at twenty-four hours

<u>TABLE 47</u> <u>%-depletion of IgG4</u>		
<u>Agent</u>	<u>Route of administration</u>	<u>%-depletion IgG4</u>
AGT402	subcutaneous	65%
AGT402	intravenous	91%
AGT501	intravenous	49%
AGT502	intravenous	63%
In vivo characterization validates IgG4-depletion by non-Fc-fusion (IV). In vivo characterization validates IgG4-depletion by Fc-fusion in mouse (intravenous and subcutaneous). In vivo characterization validates IgG4-depletion by non-Fc-fusion in mouse (intravenous)		

<u>TABLE 48</u> <u>Summary of Results: AGT501, AGT502</u>					
<u>Agent</u>	<u>Route of administration</u>	<u>% IgG4 Depletion (Run1)</u>	<u>% IgG4 Depletion (Run2)</u>	<u>Pharmacokinetics (Run 1)</u>	<u>Pharmacokinetics (Run 2)</u>
AGT501	intravenous	49%	61%	Signal detected two hours post-dose	Signal detected two hours post-dose
AGT501	subcutaneous	-	72%	-	Signal detected two hours post-dose
AGT502	intravenous	63%	71%	Signal detected two hours post-dose	Signal detected two hours post-dose
AGT502	subcutaneous	-	87%	-	Signals detected two hours post-dose

<u>TABLE 49</u> <u>%-depletion of IgG4 by VHH-Fc</u>			
<u>Route of administration</u>	<u>Dose Frequency</u>	<u>Dose (g)</u>	<u>Depletion (24 hour)</u>
2 mL subcutaneous	Q1W	0.2	17%
2 mL subcutaneous	BIW	0.2	50% (after 3 weeks)
6 mL subcutaneous	Q1W	0.6	51%
intravenous	Q1W	0.4	68%

EXAMPLE 32In vivo bioavailability assays

[00600] The inventors performed a set of assays in mouse and rat to provide insights into subcutaneous and intravenous administration routes with repeat dose regimens to show the bioavailability to a subject of the agents administered.

[00601] Subcutaneous bioavailability: **AGT402** Fc-fusion bifunctional degrader demonstrated approximately 50% bioavailability in a pharmacokinetics study in mice.

[00602] Similarly, subcutaneous administration in rats has been used to measure the bioavailability of Fc and non-Fc agents, revealing potential correlations and distinctions in cross-species pharmacokinetics.

[00603] Intravenous vs. subcutaneous administration: Comparative studies demonstrated the differences in bioavailability between intravenous and subcutaneous administration.

[00604] The data for the four agents, including analysis of the rebound effect, demonstrate critical metrics for understanding dose-dependent behavior.

[00605] Ongoing investigations are refining bioavailability data with additional time points collected beyond twenty-four and forty-eight hours to provide a comprehensive understanding of long-term systemic circulation.

EXAMPLE 33Analytical size exclusion chromatography assays

[00606] The inventors used analytical size exclusion chromatography (SEC) assays to characterize and quantify the size distribution of agents within a drug substance sample. This assay detects agent aggregates and fragments. This information is used for quality control during development and manufacturing processes. All the analytical size exclusion chromatography assay results currently available look good. Developability data discovery indicates only a small decrease in size exclusion chromatography purity (<95%), shift towards acidic peaks by imaged capillary isoelectric focusing under thermal and pH-stressed conditions for VHH-Cys molecules. For VHH-Fc fusion molecules, the effects are less pronounced and limited to thermal stressed conditions. The data indicate Fc fusion confers stability advantage.

EXAMPLE 34**Selecting human subjects for agent administration**

[00607] Outpatient studies can be performed for MuSK myasthenia gravis patients. Subcutaneous administration of the treatment, designed for at-home use, enhances patient convenience and compliance. The quantitative myasthenia gravis score offers a reliable metric to assess disease severity and therapeutic outcomes. The disease severity in MuSK myasthenia gravis correlates more closely with IgG4 autoantibody levels. MuSK myasthenia gravis can show a stronger clinical improvement response compared to classic myasthenia gravis.

[00608] MuSK-specific IgG4 autoantibodies are a robust biomarker, providing a precise means of identifying eligible patients. This precision can assist with recruitment for clinical trials.

<u>TABLE 50</u> <u>Summary</u>			
	<u>Fc Fusion AGT402</u>	<u>VHH-Cys AGT501</u>	<u>VHH-Cys AGT502</u>
Molecular weight (kDa)	83.8	14.5	14.6
Valency	2	1	1
Human stoichiometry dose estimate (mg)	300-600	100	100
Chemistry	MATE conjugation	Maleimide thiosuccinimide	Maleimide thiosuccinimide
Linker	N/A	G4S-Cys	G3E-Cys-S
Intravenous pharmacodynamics: 96 hours % IgG4 depletion	91%	49%	63%
Subcutaneous pharmacodynamics: 96 hours % IgG4 depletion	65		
Mouse tolerability w/o target (mkg)	>100	>100	>100
pI	7.76	8.85	6.76
Surface plasmon resonance K_D (nM)	20	19	20
ASPGR Proximity	Validated	Validated	Validated
Endocytosis	Validated	Validated	Validated
In cell degradation	Validated		

<u>TABLE 50</u> <u>Summary</u>			
	<u>Fc Fusion AGT402</u>	<u>VHH-Cys AGT501</u>	<u>VHH-Cys AGT502</u>
Passive transfer % depletion (subcutaneous)	69% (n=2)	72% (n=1)	87% (n = 1)
Passive transfer % Depletion (intravenous)	90% (n=5)	55% (n=2)	67% (n = 2)
Mouse bioavailability (subcutaneous)	50%	Completed	Completed
Mouse tolerability (100 mg/kg)	Well tolerated	Well tolerated	Well tolerated
Integrated molecular (off target protein binding). Validation completed for Fc fusion,	Validation completed		
Temperature/pH stability	90% size exclusion chromatography purity at 37°C	90% size exclusion chromatography purity at 37°C	90% size exclusion chromatography purity at 37°C
iCIEF (freeze thaw/temperature and pH)	No change, only changes at 37°C	No change, change only in peaks under stressed conditions	No change, change only in peaks under stressed conditions
DLS-kD (aggregation)	Indeterminate	Low aggregation propensity	Low aggregation propensity
Concentratability (Targeted 75 mg/ml), in non-optimized formulation	>75 mg/ml	>75 mg/ml	>75 mg/ml

EXAMPLE 35Human dose determination

[00609] The inventors are developing a stoichiometric human dose determination. The inventors used the following premises for their first human dose determination: (1) IgG4 levels: 40 mg/dL; (2) IgG4 synthesis rate: 23 mg/day VHH: about 15 kDa; (3) VHH-Fc: about 80 kDa; (4) the maximum drug concentration is 100 mg/mL subcutaneous 50% BA; and (5) the maximum drug concentration is intravenous 100% BA. The inventors are refining human pharmacokinetics and pharmacodynamics parameters based on the rat pharmacokinetics/pharmacodynamics intravenous and subcutaneous assay results from the assays that are being performed. The inventors are also refining the human pharmacokinetics and pharmacodynamics parameters by comparing the human dose parameters with

literature data. The inventors are confirming this methodology by estimating ASGPR levels and kinetic parameters

EXAMPLE 36

Agent effectiveness

[00610] Persons having ordinary skills in the biomedical art can assess agent effectiveness using several criteria known to persons having ordinary skill in the biomedical art.

Agent effectiveness can be determined by use of the Rey Auditory Verbal Learning Test (RAVLT).

[00611] Agent effectiveness can be determined by use of the Montreal Overall Cognitive Assessment (MOCA).

[00612] Agent effectiveness can be determined by the time to seizure freedom or cessation of status epilepticus without use of rescue therapy.

EQUIVALENTS

[00613] 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 the following claims. For example, pharmaceutically acceptable salts other than those specifically disclosed 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 such a combination. A person of ordinary skill in the art will further recognize that the present inventive concept encompasses hydrated open-chain versions of the claimed compounds containing succinimide moieties that result from the ring-opening reactions of the succinimide moieties with water (i.e., hydrolysis). Such ring-opened structures and their alternative representations can be readily ascertained by a person of ordinary skill in the art. See, for example, Kai Zheng et al. Characterization of Ring-Opening Reaction of Succinimide Linkers in ADCs. *Pharmaceutical Biotechnology* 108 (1), 133-141 (January 2019). Scheme 1.

[00614] Some embodiments of the invention can be practiced according to the following numbered paragraphs:

[00615] 1. A composition of matter comprising: an IgG4-binding moiety, a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors

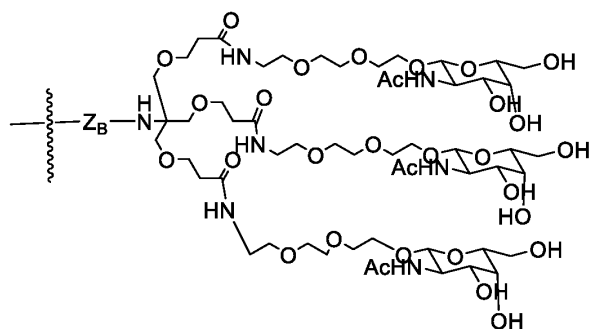
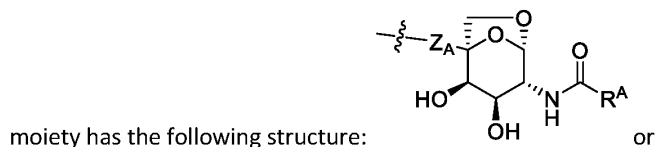
(ASGPR) of hepatocytes or other cell receptors which are on surface degrading cells, and a linker moiety (optionally a single peptide linkage) connecting the IgG4-binding moiety and the cellular receptor-binding moiety.

[00616] 2. The composition of matter of embodiment 1, wherein the IgG4-binding moiety is a published VHH affinity ligand variant antibody, a VHH affinity ligand variant, or an antigen-binding fragment thereof.

[00617] 3. The composition of matter of embodiment 1, wherein the IgG4-binding moiety is a polypeptide having complementary determining regions (CDRs) of the anti-IgG4 VHH affinity ligand variant.

[00618] 4. The composition of matter of embodiment 1, wherein the IgG4-binding moiety comprises three regions according to the Chothia numbering scheme having the structures of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

[00622] 8. The composition of matter of embodiment 1, wherein the cellular receptor-binding



; 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.

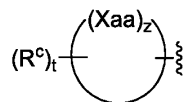
[00623] 9. A pharmaceutical composition comprising composition of matter of the preceding claims and a pharmaceutically acceptable excipient.

[00624] 10. The composition of matter of embodiment 1, wherein the IgG4-binding moiety is a VHH affinity ligand variant or an antigen-binding fragment thereof.

[00625] 11. The composition of matter of embodiment 1, wherein the agent has the structure of

formula $\left[\begin{array}{c} \text{AT} \\ \text{a} \end{array} \right]_a - L - \left[\begin{array}{c} \text{TBT} \\ \text{b} \end{array} \right]_b$, [AGN102] or a pharmaceutically acceptable salt thereof, wherein: each of a and b is independently an integer of 1 or greater; each AT is a IgG4-binding moiety; 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.

[00626] 12. The composition of matter of embodiment 12, wherein the IgG4-binding moiety that



binds to a specific amino acid residue of the antibody comprises

[ABT101].

[00627] 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.

[00628] 14. The composition of embodiment 13, wherein the first compound has the structure of



formula **[AGN102]** wherein AT, L, TBT, a and b are the same as those described in embodiment 2.

[00629] 15. The composition of embodiment 13, wherein the at least one additional agent has the structure of formula: LG-RG-L^{RM}(-TBT)_b, **[AGN302]**; LG-RG-H **[MAT302]**., or a combination thereof.

[00630] 16. A pharmaceutical composition comprising the agent of any of embodiments 1-12.

[00631] 17. A method of removing IgG4 in a patient or subject in need comprising administering to the subject or patient the agent of any of the embodiments 1-12.

[00632] 18. A method of treating a disease state and/or condition associated with the upregulation of IgG4 in a patient or subject in need comprising administering to the subject or patient an effective amount of the agent of any of the embodiments 1-12.

[00633] 19. A method of treating an IgG4-related disease (IgG4-RD) in a subject or patient in need comprising administering to the subject or patient an effective amount of the agent of any of the embodiments 1-12.

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[00634] 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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- [00467] 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 hereby 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 therein as of the date of the invention described and claimed in this specification. However, the citation of a reference in this specification should not be construed as an acknowledgement that this reference is prior art to the present invention.
- [00468] All patents and publications cited throughout this specification are incorporated by reference to disclose 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 given 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 provided 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.

SEQUENCE LISTING

Sequence Number (ID): 1

Length: 122

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..122

> mol_type, protein

> note, ABT301. VHH nanobody

> organism, synthetic construct

- REGION, 26..32

> note, CDR-H1 Chothia scheme

- REGION, 52..56

> note, CDR-H2 Chothia scheme

- REGION, 98..105

> note, CDR-H3 Chothia scheme

- SITE, 121

> note, Cysteine for linkage

- SITE, 118

> note, Cysteine for linkage

- REGION, 113..116

> note, TVSS marks the end of the VH domain

- REGION, 118..122

> note, Hinge region

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELAV	ITTDGSTNYA	60
DSVKGRTIS	RDNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKWYWGQGT	QVTVSSACPG	120
CA						122

Sequence Number (ID): 2

Length: 7

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..7

> mol_type, protein

> organism, Homo sapiens

- REGION, 1..7

> note, CDR-H1 of ABT301, amino acids 26-32

Residues:

GSIFSIS

7

Sequence Number (ID): 3

Length: 5

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..5

> mol_type, protein

> organism, Homo sapiens

- REGION, 1..5

> note, CDR-H2 of ABT301 amino acids 52-56

Residues:

TTDGS

5

Sequence Number (ID): 4

Length: 8

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..8

> mol_type, protein

> organism, Homo sapiens

- REGION, 1..8

> note, CDR-H3 of ABT301 amino acids 98-105

Residues:

SPRALKWY

8

Sequence Number (ID): 5

Length: 22

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..22

> mol_type, protein

> note, C-term sortase tag

> organism, synthetic construct

Residues:

GGGSGGGGS LPETGGHHHH HH

22

Sequence Number (ID): 6

Length: 128

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..128

> mol_type, protein

> note, ABT311. VHH without hinge, (G4S)1-his6-C-term Cys, rigid C-term linker for the unpaired Cys for conjugation (4x Cys)

> organism, synthetic construct

- REGION, 98..105

> note, Complementarity-determining region 3 (Chothia)

- REGION, 52..56

> note, Complementarity-determining region 2 (Chothia)

- REGION, 26..32

> note, Complementarity-determining region 1 (Chothia)

- REGION, 117..121

> note, G4S region for linkage

- REGION, 122..127

> note, His6 region for linkage

- SITE, 128

> note, Terminal cysteine for linkage

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELAV	ITTDGSTNYA	60
DSVKGREFTIS	RDNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKWYWGQGT	QVTVSSGGGG	120
SHHHHHHC						128

Sequence Number (ID): 7

Length: 154

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..154

> mol_type, protein

> note, ABT312. VHH nanobody-G4S-his6

> organism, synthetic construct

- SIGNAL, 1..21

> note, Mouse Ig Kappa signal

```

- REGION, 47..53
> note, CDR-H1 Chothia scheme
- REGION, 73..77
> note, CDR-H2 Chothia scheme
- REGION, 119..126
> note, CDR-H3 Chothia scheme
- REGION, 124..127
> note, G4S linker
- REGION, 129..134
> note, His6 tag

```

Residues:

METDTLLLWV	LLLWVPGSTG	DQVQLQDSGG	GLVQAGGSLR	LSCEASGSIF	SISAMGWYRQ	60
APGKQRELVA	VITTDGSTNY	ADSVKGRFTI	SRDNAKNTVY	LQMNSLKPED	TDVYYCGASP	120
RALKWYWGQG	TQVTVSSACP	GCAGGGGSHH	HHHH			154

Sequence Number (ID): 8

Length: 148

Molecule Type: AA

Features Location/Qualifiers:

```

- source, 1..148
> mol_type, protein
> organism, synthetic construct
- SIGNAL, 1..21
> note, Mouse Ig Kappa signal
- REGION, 47..53
> note, CDR-H1 Chothia scheme
- REGION, 73..77
> note, CDR-H2 Chothia scheme
- REGION, 119..126
> note, CDR-H3 Chothia scheme
- DOMAIN, 118..122
> note, G4S linker
- DOMAIN, 123..138
> note, His6 tag

```

Residues:

METDTLLLWV	LLLWVPGSTG	DQVQLQDSGG	GLVQAGGSLR	LSCEASGSIF	SISAMGWYRQ	60
APGKQRELVA	VITTDGSTNY	ADSVKGRFTI	SRDNAKNTVY	LQMNSLKPED	TDVYYCGASP	120

RALKWYWGQG TQVTVSSGGG GSHHHHHH

148

Sequence Number (ID): 9

Length: 160

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..160
- > mol_type, protein
- > note, ABT304. VHH nanobody, G4S linker, with C-term Sortase site, His6 tag
- > organism, synthetic construct
- SIGNAL, 1..21
- > note, Mouse Ig Kappa signal
- REGION, 47..53
- > note, CDR-H1 Chothia scheme
- REGION, 73..77
- > note, CDR-H2 Chothia scheme
- REGION, 119..126
- > note, CDR-H3 Chothia scheme
- DOMAIN, 124..127
- > note, G4S linker
- DOMAIN, 134..139
- > note, His6 tag
- DOMAIN, 128..133
- > note, C-term Sortase site

Residues:

METDTLLLWV	LLLWVPGSTG	DQVQLQDSGG	GLVQAGGSLR	LSCEASGSIF	SISAMGWYRQ	60
APGKQRELVA	VITTDGSTNY	ADSVKGRFTI	SRDNAKNTVY	LQMNSLKPED	TDVYYCGASP	120
RALKWYWGQG	TQVTVSSACP	GCAGGGGSLP	ETGGHHHHHH			160

Sequence Number (ID): 10

Length: 154

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..154
- > mol_type, protein
- > note, ABT305. VHH nanobody with truncation of hinge-G4S-his6-C-term sortase
- > organism, synthetic construct

```

- SIGNAL, 1..21
> note, Mouse Ig Kappa signal
- REGION, 47..53
> note, CDR-H1 Chothia scheme
- REGION, 73..77
> note, CDR-H2 Chothia scheme
- REGION, 119..126
> note, CDR-H3 Chothia scheme
Residues:
METDTLLLWV LLLWVPGSTG DQVQLQDSGG GLVQAGGSLR LSCEASGSIF SISAMGWYRQ 60
APGKQRELVA VITTDGSTNY ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TDVYYCGASP 120
RALKWYWGQG TQVTVSSGGG GSLPETGGHH HHHH 154

```

Sequence Number (ID): 11

Length: 163

Molecule Type: AA

Features Location/Qualifiers:

```

- source, 1..163
> mol_type, protein
> note, ABT306. VHH nanobody with truncation of hinge region-G4S-his6-rigid
linker-Cys
> organism, synthetic construct
- SIGNAL, 1..21
> note, Mouse Ig Kappa signal
- REGION, 47..53
> note, CDR-H1 Chothia scheme
- REGION, 73..77
> note, CDR-H2 Chothia scheme
- REGION, 119..126
> note, CDR-H3 Chothia scheme
- DOMAIN, 118..122
> note, G4S linker
- DOMAIN, 123..128
> note, His6 tag
- DOMAIN, 129..143
> note, rigid linker + Cys

```

```

Residues:
METDTLLLWV LLLWVPGSTG DQVQLQDSGG GLVQAGGSLR LSCEASGSIF SISAMGWYRQ 60

```

APGKQRELVA	VITTDGSTNY	ADSVKGRFTI	SRDNAKNTVY	LQMNSLKPED	TDVYYCGASP	120
RALKWYWGG	TQVTVSSGGG	GSHHHHHHSP	STPPTPSPST	PPC		163

Sequence Number (ID): 12

Length: 374

Molecule Type: AA

Features Location/Qualifiers:

```

- source, 1..374
> mol_type, protein
> note, ABT310. VHH without hinge, (G3ECS)1-Cys
> organism, Homo sapiens
- SIGNAL, 1..21
> note, Mouse Ig Kappa signal
- REGION, 47..53
> note, CDR-H1 Chothia scheme
- REGION, 73..77
> note, CDR-H2 Chothia scheme
- REGION, 119..126
> note, CDR-H3 Chothia scheme
- DOMAIN, 118..122
> note, G4S linker
- DOMAIN, 123..374
> note, hIgG1 hinge (C>A)-Fc-LALA/PA

```

Residues:

METDTLLLV	WV	LLLWVPGSTG	DQVQLQDSGG	GLVQAGGSLR	LSCEASGSIF	SISAMGWYRQ	60
APGKQRELVA	VITTDGSTNY	ADSVKGRFTI	SRDNAKNTVY	LQMNSLKPED	TDVYYCGASP		120
RALKWYWGG	TQVTVSSGGG	GSEPKSADKT	HTCPPCPAPE	AAGGPSVFLF	PPKPKDTLMI		180
SRTPEVTCVV	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW		240
LNGKEYKCKV	SNKALAAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV	SLTCLVKGFY		300
PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTVD	KSRWQQGNVF	SCSVMEALH		360
NHYTQKSLSL	SPGK						374

Sequence Number (ID): 13

Length: 120

Molecule Type: AA

Features Location/Qualifiers:

```

- source, 1..120

```

```
> mol_type, protein
> organism, Mus musculus
- REGION, 117..120
> note, TVSS marks the end of the VH domain
Residues:
DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWRQA PEKGLEWVAY ISSGSSTLHY 60
ADTVKGRFTI SRDNPKNLTF LQMTSLRSED TGMYYCARWG NYPYYAMDYW GQGTSTVTVSS 120

Sequence Number (ID): 14
Length: 13
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..13
> mol_type, protein
> organism, Homo sapiens
- BINDING, 1..13
> note, VHH epitope on IgG4-Fc. The binding site on IgG4 was determined to be
in two parts: Amino acids 47-57.
Residues:
DVSEQEDPEV QFN 13

Sequence Number (ID): 15
Length: 8
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..8
> mol_type, protein
> organism, Homo sapiens
- BINDING, 1..8
> note, VHH epitope on IgG4-Fc. The binding site on IgG4 was determined to be
in two parts: Amino acids 106-114.
Residues:
SNKGPSSI 8

Sequence Number (ID): 16
Length: 6
Molecule Type: AA
Features Location/Qualifiers:
```

- source, 1..6
> mol_type, protein
> organism, Homo sapiens
Residues:
ISAMGW

6

Sequence Number (ID): 17
Length: 14
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..14
> mol_type, protein
> organism, Homo sapiens
Residues:
YRQAPGKQRE LVAV

14

Sequence Number (ID): 18
Length: 11
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..11
> mol_type, protein
> organism, Homo sapiens
Residues:
ISRDNKNTV Y

11

Sequence Number (ID): 19
Length: 13
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..13
> mol_type, protein
> organism, Homo sapiens
Residues:
YCGASPRALK WYW

13

Sequence Number (ID): 20

Length: 9

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..9

> mol_type, protein

> organism, Homo sapiens

Residues:

VTVSSACPG

9

Sequence Number (ID): 21

Length: 21

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..21

> mol_type, protein

> organism, Homo sapiens

Residues:

DVYYXGASPR ALKQYWGQGT Q

21

Sequence Number (ID): 22

Length: 14

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..14

> mol_type, protein

> organism, Homo sapiens

- BINDING, 1..14

> note, VHH binding region 1: Both Regions 1 and 2 likely involved in VHH binding.

Residues:

SIFSISAMGW YRQA

14

Sequence Number (ID): 23

Length: 22

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..22

> mol_type, protein

```
> organism, Homo sapiens
- BINDING, 1..22
> note, VHH binding region 2: Both Regions 1 and 2 likely involved in VHH
binding.
Residues:
AMGWYRQAPG KQRELVAVIT TD 22

Sequence Number (ID): 24
Length: 28
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..28
> mol_type, protein
> organism, Homo sapiens
- BINDING, 1..28
> note, Qualifier ValueVHH binding region 1+2
Residues:
SIFSISAMGW YRQAPGKQRE LVAVITTD 28

Sequence Number (ID): 25
Length: 20
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..20
> mol_type, protein
> organism, Homo sapiens
- BINDING, 1..20
> note, VHH binding region 4: Ac-TDVYYCGASPRALKWYWGQGT-NH2
Residues:
DVYYCGASPR ALKWYWGQGT 20

Sequence Number (ID): 26
Length: 330
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..330
> mol_type, protein
> organism, Homo sapiens
```

Residues:

ASTKGPSVFP	LAPSSKSTSG	GTAALGCLVK	DYFPBPVTVS	WNSGALTSGV	HTFPAVLQSS	60
GLYSLSSVVT	VPSSSLGTQT	YICNVNHKPS	NTKVDKKVEP	KSCDKTHTCP	PCPAPELLGG	120
PSVFLFPKPK	KDTLMISRTP	EVTCTVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	180
STYRVVSVLT	VLHQDWINGK	EYKCRVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSRDE	240
LTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	300
QQGNVFSCSV	MHEATHNHYT	QKSLSLSPGK				330

Sequence Number (ID): 27

Length: 326

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..326

> mol_type, protein

> organism, Homo sapiens

Residues:

ASTKGPSVFP	LAPCSRSTSE	STAALGCLVK	DYFPBPVTVS	WNSGALTSGV	HTFPAVLQSS	60
GLYSLSSVVT	VPSSNFGTQT	YTCNVDHKPS	NTKVDKTVER	KCCVECPPCP	APPVAGPSVF	120
LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVQFNWYVDG	VEVHNAKTKP	REBQFNSTFR	180
VVSVLTVLHQ	DWLNGKEYKC	KVSNKGLPAP	IEKTISKTKG	OPREPQVYTL	PPSREEMTKN	240
QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTPPMLDSD	GSFFLYSKLT	VDKSRWQQGN	300
VFSCSVMHEA	THNHYTQKSL	SLSPGK				326

Sequence Number (ID): 28

Length: 327

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..327

> mol_type, protein

> organism, Homo sapiens

Residues:

ASTKGPSVFP	LAPCSRSTSE	STAALGCLVK	DYFPBPVTVS	WNSGALTSGV	HTFPAVLQSS	60
GLYSLSSVVT	VPSSSLGTKT	YTCNVDHKPS	NTKVDKRVES	KTGPCCPSCP	APEFLGGPSV	120
FLFPKPKKDT	LMISRTPEVT	CVVVDVSQED	PEVQFNWYVD	GVEVHNAKTK	PREEQFNSTY	180
RVVSVLTVLH	QDWINGKEYK	CKVSNKGLPS	SIEKTISKAK	GOPREPQVYT	LPQSQEEMTK	240
NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTPPVLDS	DGSFFLYSRL	TVDKSRWQEG	300
NVFSCSVMHE	ATHNHYTQKS	LSLSLGK				327

Sequence Number (ID): 29

Length: 133

Molecule Type: AA

Features Location/Qualifiers:

```
- source, 1..133
> mol_type, protein
> note, ABT505. VHH w/o hinge, (G4S)1-his6-(G4S)1-Cys
> organism, synthetic construct
- SITE, 133
> note, Terminal cysteine for linkage
- REGION, 128..132
> note, G4S region for linkage
- REGION, 123..127
> note, His6 region for linkage
- REGION, 117..121
> note, G4S region for linkage
- REGION, 26..32
> note, Complementarity-determining region 1 (Chothia)
- REGION, 52..56
> note, Complementarity-determining region 2 (Chothia)
- REGION, 98..105
> note, Complementarity-determining region 3 (Chothia)
```

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELVAV	ITTDGSTNYA	60
DSVKGRFTIS	RDNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKWYWGQGT	QVTVSSGGGG	120
SHHHHHHGGG	GSC					133

Sequence Number (ID): 30

Length: 122

Molecule Type: AA

Features Location/Qualifiers:

```
- source, 1..122
> mol_type, protein
> organism, synthetic construct
- SITE, 122
> note, Terminal cysteine for linkage
```

```

- REGION, 117..121
> note, G4S region for linkage
- REGION, 26..32
> note, Complementarity-determining region 1 (Chothia)
- REGION, 52..56
> note, Complementarity-determining region 2 (Chothia)
- REGION, 98..105
> note, Complementarity-determining region 3 (Chothia)
Residues:
QVQLQDSGGG LVQAGGSLRL SCEASGSIFS ISAMGWYRQA PGKQRELVAV ITTDGSTNYA 60
DSVKGRFTIS RDNANKTVYL QMNSLKPEDT DVYYCGASPR ALKQYWGQGT QVTVSSGGGG 120
SC 122

```

Sequence Number (ID): 31

Length: 122

Molecule Type: AA

Features Location/Qualifiers:

```

- source, 1..122
> mol_type, protein
> organism, synthetic construct
- REGION, 117..122
> note, G3ECS region for linkage
- SITE, 121
> note, Cysteine for linkage
- REGION, 26..32
> note, Complementarity-determining region 1 (Chothia)
- REGION, 52..56
> note, Complementarity-determining region 2 (Chothia)
- REGION, 98..105
> note, Complementarity-determining region 3 (Chothia)

```

```

Residues:
QVQLQDSGGG LVQAGGSLRL SCEASGSIFS ISAMGWYRQA PGKQRELVAV ITTDGSTNYA 60
DSVKGRFTIS RDNANKTVYL QMNSLKPEDT DVYYCGASPR ALKQYWGQGT QVTVSSGGGE 120
CS 122

```

Sequence Number (ID): 32

Length: 127

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..127

> mol_type, protein

> note, ABT504. VHH nanobody w/o hinge region-G4S-his6-Cys

> organism, synthetic construct

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELVAV	ITTDGSTNYA	60
DSVKGREFTIS	RDNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKWYWGQGT	QVTVSSGGGG	120
SHHHHHH						127

Sequence Number (ID): 33

Length: 324

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..324

> mol_type, protein

> note, Cynomolgus monkey IgG1

> organism, Macaca fascicularis

Residues:

SSASTKGPSV	FPLAPSSRST	SESTAALGCL	VKDYFPEPVT	VSWNSGSLTS	GVHTFFAVLQ	60
SSGLYSLSV	VTVPSSSLGT	QTYVCNVNHK	PSNTKVDKRV	EIKTCGGGSK	PPTCPPCPAP	120
ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	VVDVSQEDPD	VKENWYVNGA	EVHHAQTKPR	180
ETQYNSTYRV	VSVLTVTHQD	WLNGKEYTCK	VSNKALPAPI	QKTISKDKGO	PREPQVYTLF	240
PSREELTKNQ	VSLTCLVKGF	YPSDIVVEWE	SSGOPENTYK	TTPPVLDSDG	SYFLYSKLTV	300
DKSRWGNVFS	CSVMHEALHN	HYTQ				324

Sequence Number (ID): 34

Length: 319

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..319

> mol_type, protein

> note, Cynomolgus monkey IgG2

> organism, Macaca fascicularis

Residues:

SSASTKGPSV	FPLASCSRST	SQSTAALGCL	VKDYFPEPVT	VSWNSGALTS	GVHTFFAVLQ	60
------------	------------	------------	------------	------------	------------	----

SSGLYSLSSV	VTVPSSSLGT	QTYVCNVVHE	PSNTKVDKTV	GLPCRSTCPP	CPAELLGGPS	120
VFLFPPKPKD	TLMISRTPEV	TCVVVDVSQE	EPDVKFNWYV	DGVEVHNAQT	KPREEQFNST	180
YRVVSVLTVT	HQDWLNGKEY	TCKVSNKALP	APRQKTVSKT	KGQPREPQVY	TLPPPREELT	240
KNQVSLTCLI	KGFYPSDIVV	EWASNGQPEN	TYKTTTPVLD	SDGSYFLYSK	LTVDKSRWQQ	300
GNTFSCSVMH	EALHNHYTQ					319

Sequence Number (ID): 35

Length: 325

Molecule Type: AA

Features Location/Qualifiers:

```
- source, 1..325
> mol_type, protein
> note, Cynomolgus monkey IgG3
> organism, Macaca fascicularis
```

Residues:

SSASTKGPSV	FPLVSCSRST	SESTAALGCL	VKDYFPEPVT	VSWNSGALTS	GVHTFPAVLQ	60
SSGLYSLSSV	VTVPSSSLGT	QTYVCNVVHE	PSNTKVDKRV	EFTRPCDDT	PPCPPCPAPE	120
LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSQEDPEV	QFNWYVDGVE	VHNAQTKPRE	180
RQFNSTYRVV	SVLTVTHQDW	LNGKEYTCKV	SNKGLPAPIE	KTISKAKGQP	REPQVYILPP	240
PQEELTKNQV	SLTCLVTGFY	PSDIAVEWES	NGQPENTYKT	TPPVLDSDGS	YFLYSKLTVD	300
KSRWQQGNVF	SCSVMHEALH	NHYTQ				325

Sequence Number (ID): 36

Length: 317

Molecule Type: AA

Features Location/Qualifiers:

```
- source, 1..317
> mol_type, protein
> note, Cynomolgus monkey IgG4
> organism, Macaca fascicularis
```

Residues:

SSASTKGPSV	FPLASSSRST	SESTVALGCL	VKDYFPEPVT	VSWNSGALTS	GVHTFPAVLQ	60
SSGLYSLSGV	VTVPSSSLGT	QTYVCNVVHQ	PSNTKVDKRV	EFTPPCPPCP	APEVLGGPSV	120
FLFPPKPKDT	LMISRIPEVT	CVVVDVSEDP	EVQFNWYVDG	VEVHNAQTKP	RERQFNSTYR	180
VVSVLTVTHQ	DWLNGKEYTC	KVSNKGLPAP	IEKTISKAKG	QPREPQVYIL	PPPQEELTKN	240
QVSLTCLVTG	FYPDSIAVEW	ESNGQPENTY	KTTPPMLDSD	GSYLLYSKLT	VNKSRRWQPGN	300
IFTCSVMHEA	LHNHYTQ					317

Sequence Number (ID): 37

Length: 227

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..227

> mol_type, protein

> note, Rhesus monkey IgG1

> organism, Macaca mulatta

Residues:

TKVDKRVEIK	TCGGGSKPPT	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	60
VSQEDPDVKF	NWYVNGAEVH	HAQTKPIYNS	TYRVVSVLTV	THQDWLNGKE	YTCKVSNKAL	120
PAPIQKTISK	DKGQPREPQV	YTLPPSREEL	TKNQVSLTCL	VKGFYPSDIV	VEWEQPEPTY	180
KTTTPVLDS	GSYFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTQ		227

Sequence Number (ID): 38

Length: 219

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..219

> mol_type, protein

> note, Rhesus monkey IgG2

> organism, Macaca mulatta

Residues:

TKVDKTVGLP	CRSTCPPCPA	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	VVDVSQEEPD	60
VKFNWYVDGV	EVHNAQTKPF	NSTYRVVSVL	TVTHQDWLNG	KEYTCKVSNK	ALPAPRQKTV	120
SKTKGQPREP	QVYTLPPSRE	ELTKNQVSLT	CLVKGFYPSD	IVVEWAQPEN	TYKTTPPVLD	180
SDGSYFLYSK	LTVDKSRWQQ	GNTFSCSVMH	EALHNHYTQ			219

Sequence Number (ID): 39

Length: 225

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..225

> mol_type, protein

> note, Rhesus monkey IgG3

> organism, Macaca mulatta

Residues:

TKVDKRVEFT	PPCGDTTPPC	PPCPAPELLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	60
SQEDPEVQFN	WYVDGAEVHH	AQTKPFNSTY	RVVSVLTVTH	QDWLNGKEYT	CKVSNKGLPA	120
PIEKTISKAK	GQPREPQVYI	LPPPQEELTK	NQVSLTCLVT	GFYPSDIAVE	WEQPENTYKT	180
TPPVLDSDGS	YFLYSKLTVD	KSRWQQGNTF	SCSVMHREALH	NHYTQ		225

Sequence Number (ID): 40

Length: 218

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..218

> mol_type, protein

> note, Rhesus monkey IgG1

> organism, Macaca mulatta

Residues:

TKVDKRVEFT	PPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSQEDPEV	60
QFNWYVDGAE	VHHAQTKPFN	STYRVVSVLT	VTHQDWLNGK	EYTCCKVSNKG	LPAPIEKTIS	120
KAKGQPREPQ	VYILPPPQEE	LTKNQVSLTC	LVTGFYPSDI	AVEWEQPENT	YKTTTPPVLD	180
DGSYFLYSKL	TVDKSRWQPG	NIFTCSVMHE	ALHNHYTQ			218

Sequence Number (ID): 41

Length: 314

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..314

> mol_type, protein

> organism, Mus musculus

Residues:

LAPGSAAQTN	SMVTLGCLVK	GYFPEPVTVT	WNSGSLSSGV	HTFPAVLQSD	LYTLSSSVTV	60
PSSTWPSQTV	TCNVAHPASS	TKVDKKIVPR	DCGCKPCICT	VPEVSSVFIF	PPKPKDVLTI	120
TLTPKVTCVV	VDISKDDPEV	QFSWFVDDVE	VHTAQTKPRE	EQINSTFRSV	SELPIMHQDW	180
LNGKEFKCRV	NSAAFPAPTE	KTISKTKGRP	KAPQVYTIPP	PKEQMAKDKV	SLTCMITNFF	240
PEDITVEWQW	NGQPAENYKN	TQPIMDTDGS	YFVYSKLNQV	KSNWEAGNTF	TCSVLHEGLH	300
NHHTEKSLSH	SPGK					314

Sequence Number (ID): 42

Length: 320

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..320

> mol_type, protein

> organism, Mus musculus

Residues:

LAPVCGDTTG	SSVTLGCLVK	GYFPEPVTLT	WNSGSLSSGV	HTFPAVLQSD	LYTLSSSVTV	60
TSSTWPSQSI	TCNVAHPASS	TKVDKKIEPR	GPTIKPCPPC	KCPAPNLLGG	PSVFIFPPKI	120
KDVLMIISLP	IVTCVVVDVS	EDDPDVQISW	FVNNVEVHTA	QTQTHREDYN	STLRVVSALP	180
IQHGDWMSGK	EFKCKVNNKD	LPAPIERTIS	KPKGSVRAPQ	VYVLPPPEEE	MTKKQVTLTC	240
MVTDFMPEDI	YVEWTNNGKT	ELNYKNTEPV	LDSDGSYFMY	SKLRVEKKNW	VERNSYSCSV	300
VHEGLHNHHT	TKSFSRTPGK					320

Sequence Number (ID): 43

Length: 259

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..259

> mol_type, protein

> organism, Mus musculus

Residues:

LAPGCGDTTG	SSVTLGCLVK	GYFPESVTVT	WNSGSLSSSV	HTFPALLQSG	LYTMSSSVTV	60
PSSTWPSQTV	TCSVAHPASS	TTVDKKLEPS	GPISTINPCP	PCKECHKCPA	PNLEGGPSVF	120
IFPPNIKDVL	MISLTPKVTC	VVVDVSEDDP	DVQISWVFN	VEVHTAQTTQ	HRDYNSTIR	180
VVSTLPIQHQ	DWMSGKEFKC	KVNNKDLSP	IERTISKIKG	LVRAPQVYIL	PPPAEQLSRK	240
DVSLTCLVVG	FNPGDISVE					259

Sequence Number (ID): 44

Length: 253

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..253

> mol_type, protein

> organism, Mus musculus

Residues:

LVPGCGDTSG	SSVTLGCLVK	GYFPEPVTVK	WNYGALSSGV	RTVSSVLQSG	FYSLSSLVTV	60
PSSTWPSQTV	ICNVAHPASK	TELIKRIEPR	IPKPSTPPGS	SCPPGNILGG	PSVFIFPPKP	120

KDALMISLTP	KVTCVVVDVS	EDDPDVHVS	FVDNKEVHTA	WTOPREAQYN	STFRVVSALP	180
IQHQDWMRGK	EFKCKVNNKA	LPAPIERTIS	KPKGRAQTPQ	VYTIPPPREO	MSKKKVSLTC	240
LVTNFFSEAI	SVE					253

Sequence Number (ID): 45

Length: 316

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..316

> mol_type, protein

> organism, Rattus norvegicus

Residues:

LAPGTALKSN	SMVTLGCLVK	GYFPEPVTVT	WNSGALSSGV	HTFPAVLQSG	LYTLTSSVTV	60
PSSTWSSQAV	TCNVAHPASS	TKVDKKIVPR	NCGGDCCKPCI	CTGSEVSSVF	IFPPKPKDVL	120
TITLTPKVTC	VVVDISQDDP	EVHFSWFVDD	VEVHTAQTRP	PEEQFNSTFR	SVSELPILHQ	180
DWLNGRTFRC	KVTSAAFPSP	IEKTISKPEG	RTQVPHVYTM	SPTKEEMIQN	EVSITCMVKG	240
FYPFDIYVEW	QMNGQPQENY	KNTPPTMDTD	GSYFLYSKLN	VKKEKWQQGN	TFTCSVLHEG	300
LHNHHTKSL	SHSPGK					316

Sequence Number (ID): 46

Length: 312

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..312

> mol_type, protein

> organism, Rattus norvegicus

Residues:

LAPGTALKSN	SMVTLGCLVK	GYFPEPVTVT	WNSGALSSGV	HTFPAVLQSG	LYTLTSSVTV	60
PSSTWSSQAV	TCNVAHPASS	TKVDKKIVPR	ECNPGCTGS	EVSSVFIFPP	KTKDVLITL	120
TPKVTCVVVD	ISQNDPEVRF	SWFIDDVEVH	TAQTHAPEKQ	SNSTLRVSE	LPIVHRDNL	180
GKTFKCKVNS	GAFFAPIEKS	ISKPEGTFRG	PQVYTMAPPK	EEMTQSQVSI	TCMVKGFYPP	240
DIYTEWKMNG	QPQENYKNTP	PTMDTDGSYF	LYSKLNVKKE	TWQQGNFTFC	SVLHEGLHNN	300
HTEKSLSHSP	GK					312

Sequence Number (ID): 47

Length: 326

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..326
 > mol_type, protein
 > organism, Rattus norvegicus

Residues:

LAPGCGDTTS	STVTLGCLVK	GYFPEPVTVT	WNSGPLSSDV	HTFPAVLQSG	LYTLTSSSVTV	60
PSSTWPSQTV	TCNVAHPASS	TKVDKKIERR	NGGIEPKPIP	TCPTCHKCPA	PELLGGPSVF	120
IFPPKPKDIL	LISQNAKVTC	VVVDVSEEEP	DVQFSWFVNN	VEVHTAQTQP	REEQYNSTFR	180
VVSALPIQHQ	DWMSGKEFKC	KVNNKALPSP	IEKTISKPKG	LVRKPQVYVM	GPPEQLTEK	240
AVILTCLITD	FLPNNIGVEW	TSNGRIEKNY	KNTEPVMDS	GSFFMYSKLN	VERSRWDSRA	300
SFVCSVVHEG	LHNRHVEKSI	SRPPGK				326

Sequence Number (ID): 48

Length: 319

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..319
 > mol_type, protein
 > organism, Rattus norvegicus

Residues:

LVPGCSGTSG	SLVTLGCLVK	GYFPEPVTVK	WNYGALSSGV	HTFPAVLQSG	LYTLSSSVTV	60
PSSTWSSQTV	TCSVAHPATK	SDLIKRIEPR	RPKPRPPTDI	CSCDDNLGRP	SVFIFPPKPK	120
DTLMITLTPK	VTCVVVDVSE	EEPVDQFSWF	VDNIRVFATAQ	TQPHEEQLNG	TFRVVS TLHI	180
QHQDWMSGKE	FKCKVNNKDL	PSPIEKTISK	PRGKARTPQV	YTIPPPREQM	SKNKVSLTCM	240
VTSFYPA SIS	VEWERNGELE	ODYKNTLPVL	DSDES YFLYS	KLSVDTDSWM	RGDIYTCSVV	300
HEALHNHHTQ	KNLSRSPGK					319

Sequence Number (ID): 49

Length: 202

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..202
 > mol_type, protein
 > organism, Canis lupus

Residues:

DILRITRTPE	VTCVVL DLGR	EDPEVQISWF	VDGKEVHTAK	TQSREQQFNG	TYRVVSVLPI	60
EHQDWLTGKE	FKCRVNHIDL	PSPIERTISK	ARGRAHKPSV	YVLPPSPKEL	SSSDTVSITC	120

LIKDFYPPDI	DVEWQSNQQ	EPERKHRMTP	PQLDEGGSYF	LYSKLSVDKS	RWQQGDPFTC	180
AVMHETLQNH	YTDLSLSHSP	GK				202

Sequence Number (ID): 50

Length: 201

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..201

> mol_type, protein

> organism, Canis lupus

Residues:

DTLLIARTPE	VTCVVVDLDP	EDPEVQISWF	VDGKQMOTAK	TQPREEQFNG	TYRVVSVLPI	60
GHQDWLKGKQ	FTCKVNNKAL	PSPIERTISK	ARGQAHQPSV	YVLPPSREEL	SKNTVSLTCL	120
IKDFFPPDID	VEWQSNQQE	PESKYRTTPP	OLDEGGSYFL	YSKLSVDKSR	WORGDTFICA	180
VMHEALHNHY	TOESLSHSPG	K				201

Sequence Number (ID): 51

Length: 201

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..201

> mol_type, protein

> organism, Canis lupus

Residues:

DILVTARTPT	VTCVVVDLDP	ENPEVQISWF	VDSKQVQTAN	TQPREEQSNG	TYRVVSVLPI	60
GHQDWLSGKQ	FKCKVNNKAL	PSPIEEIISK	TPGQAHQPNV	YVLPPSRDEM	SKNTVTLTCL	120
VKDFFPPEID	VEWQSNQQE	PESKYRMTTP	OLDEGGSYFL	YSKLSVDKSR	WORGDTFICA	180
VMHEALHNHY	TOISLSHSPG	K				201

Sequence Number (ID): 52

Length: 202

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..202

> mol_type, protein

> organism, Canis lupus

Residues:

DILRITRTPE	ITCVVLDLGR	EDPEVQISWE	VDGKEVHTAK	TOPREQQFNS	TYRVVSVLPI	60
EHQDWLTGKE	FKCRVNHIGL	PSPIERTISK	ARGQAHQPSV	YVLPPSPKEL	SSSDTVTLTC	120
LIKDFFPPEI	DVEWQSNQGP	EPESKYHTTA	PQLDEDGSYF	LYSKLSVDKS	RWQQGDTFTC	180
AVMHEALQNH	YTDLSLSHSP	GK				202

Sequence Number (ID): 53

Length: 227

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..227

> mol_type, protein

> organism, Sus scrofa

Residues:

TKPPCPICPG	CEVAGPSVFI	FPPKPKDTLM	ISQTPEVTCV	VVDVSKEHAE	VQFSWYVDGV	60
EVHTAETRPK	EEQFNSTYRV	VSVLPIQHGD	WLKGKEFKCK	VNNVDLPAPI	TRTISKAIGQ	120
SREPQVYTLF	PPAEELSRSK	VTVTCLVIGF	YPPDIHVEWK	SNGQPEPEGN	YRTTPPQQDV	180
DGTFFLYSKL	AVDKARWDHG	ETFECAMMHE	ALHNHYTQKS	ISKTPQGK		227

Sequence Number (ID): 54

Length: 227

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..227

> mol_type, protein

> organism, Sus scrofa

Residues:

TKPPCPICPA	CEGPGPSAFI	FPPKPKDTLM	ISRTPKVTCV	VVDVSQENPE	VQFSWYVDGV	60
EVHTAQTRPK	EEQFNSTYRV	VSVLPIQHGD	WLNGKEFKCK	VNNKDLPAPI	TRIISKAKGQ	120
TREPQVYTLF	PPTEELSRSK	VTTLCLVTGF	YPPDIDVEWQ	RNGQPEPEGN	YRTTPPQQDV	180
DGTFFLYSKL	AVDKASWQRG	DTFQCAVMHE	ALHNHYTQKS	IFKTPGK		227

Sequence Number (ID): 55

Length: 233

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..233

> mol_type, protein

> organism, Sus scrofa

Residues:

EPPTPICPEI	CSCPAAEVLG	APSVFLFPPK	PKDILMISRT	PKVTCVVVDV	SQEEAEVQFS	60
WYVDGVQLYT	AQTRPMEEQF	NSTYRVVSVL	PIQHQDWLKG	KEFKCKVNNK	DLLSPITRTI	120
SKATGPSRVP	QVYTLPPAWE	ELSKSKVSIT	CLVTGFYPPD	IDVEWQSNQ	QEPEGNYRTT	180
PPQQDVGTY	FLYSKLAVDK	VRWQRGDLFQ	CAVMHEALHN	HYTQKSVSQT	PGK	233

Sequence Number (ID): 56

Length: 227

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..227

> mol_type, protein

> organism, Sus scrofa

Residues:

TKPPPCICPA	CEGPGPSAFI	FPPKPKDTLM	ISRTPKVTCV	VVDVSQENPE	VQFSWYVDGV	60
EVHTAQTRPK	EEQFNSTYRV	VSVLPIQHGD	WLNGKEFKCK	VNNKDLPAPI	TRIISKAKGQ	120
TREPQVYTLF	PPTEELSRSK	VTLTCLVTGF	YPPDIDVEWQ	RNGQPEPEGN	YRTTPPQQDV	180
DGTYFLYSKL	AVDKASWQRG	DTFQCAVMHE	ALHNHYTQKS	IFKTPGK		227

Sequence Number (ID): 57

Length: 223

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..223

> mol_type, protein

> organism, Sus scrofa

Residues:

PCPICPGCEV	AGPSVFIFPP	KPKDILMISR	TPEVTCVVVD	VSKEHAEVQF	SWYVDGVEVH	60
TAETRPKEEQ	FNSTYRVVSV	LPIQHEDWLK	GKEFECKVNN	EDLPGPITRT	ISKAKGVVRS	120
PEVYTLPPPA	EELSKSKVTL	TCLVKSFFPP	FIHVGWKING	QLEPEGNYHT	TPPQEDEDGT	180
YFLYSKLAVD	KARLQSGGIH	CAVMHEALHN	HYTEKSVSLT	PGK		223

Sequence Number (ID): 58

Length: 224

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..224
 > mol_type, protein
 > organism, Sus scrofa

Residues:

PCPICPACEG	PGPSAFIFPP	KPKDTLMISR	TPKVTCVVVD	VSQENPEVQF	SWYVDGVEVH	60
TAQTRPKEEQ	FNSTYRVVSV	LPIQHQDWLN	GKEFKCKVNN	KDLPAPITRI	ISKAKGQTRE	120
PQVYTLPPPT	EELSRSKLSV	TCLITGFYPP	DIDVEWQRNG	QPEPEGNYRT	TPPQQDVDGT	180
YFLYSKLAVD	KASWQRGDPF	QCAVMHEALH	NHYTQKSIFK	TPGN		224

Sequence Number (ID): 59

Length: 133

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..133
 > mol_type, protein
 > note, ABT501. VHH nanobody w/o hinge region-G4S-his6-G4S-Cys
 > organism, synthetic construct
 - SITE, 133
 > note, Terminal cysteine for linkage
 - REGION, 128..132
 > note, G4S region for linkage
 - REGION, 125..131
 > note, His6 region for linkage
 - REGION, 115..120
 > note, G4S region for linkage

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELVAV	ITTDGSTNYA	60
DSVKGRFTIS	RDNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKWYWGQGT	QVTVSSGGGG	120
SHHHHHHGGG	GSC					133

Sequence Number (ID): 60

Length: 154

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..154
 > mol_type, protein
 > note, ABT507. Full sequence for sortase linkage with shortened leader sequence.

> organism, synthetic construct
 - REGION, 1..17
 > note, shortened leader sequence
 - REGION, 149..154
 > note, his6 for conjugation

Residues:

MGWSCILFLV	ATATGHSQVQ	LQDSGGGLVQ	AGGSLRLSCE	ASGSIFSISA	MGWYRQAPGK	60
ORELVAVITT	DGSTNYADSK	GFTISRDNAL	NTVYLQMNSL	KPEDTDVYYC	GASPRALKWY	120
WGQGTQVTVS	SACPGCAGGG	GSLPETGGHH	HHHH			154

Sequence Number (ID): 61

Length: 151

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..151
 > mol_type, protein
 > note, ABT508. Truncated sequence for sortase linkage with shortened leader sequence
 > organism, synthetic construct
 - REGION, 1..19
 > note, shortened leader sequence
 - REGION, 146..151
 > note, his6 for conjugation

Residues:

MGWSCILFLV	VATATGVHSQ	VQLQDSGGGL	VQAGGSLRLS	CEASGSIFSI	SAMGWYROAP	60
GKORELVAVI	TTDGSTNYAD	SKGREFTISRD	NAKNTVYLQM	NSLKPEDTDV	YYCGASPRAL	120
KWYWGQGTQV	TVSSGGGGSL	PETGGHHHHH	H			151

Sequence Number (ID): 62

Length: 161

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..161
 > mol_type, protein
 > note, ABT509. Truncated sequence for maleimide beta-GN3 lineage with shortened leader sequence
 > organism, synthetic construct
 - REGION, 1..19

> note, shortened leader sequence
 - REGION, 141..146
 > note, his6 for conjugation
 - SITE, 161
 > note, terminal Cys for conjugation

Residues:

MGWSCIIIFL	VATATGVHSQ	VQLQDSGGGL	VQAGGSLRLS	CEASGSIFS	SAMGWYRQAP	60
GKQRELVAVI	TTDGSTNYAD	SVKGRFTISR	DNAKNTVYLQ	MNSLKPEDTD	VYYCGASPR	120
LKQYWGQGTQ	VTVSSGGGGS	HHHHHHSPST	PPTPSPSTPP	C		161

Sequence Number (ID): 63

Length: 122

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..122
 > mol_type, protein
 > note, ABT503. VHH nanobody with truncation of hinge region-G3E-Cys-S
 > organism, synthetic construct

Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELVAV	ITTDGSTNYA	60
DSVKGRFTIS	RNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKQYWGQGT	QVTVSSGGGE	120
CS						122

Sequence Number (ID): 64

Length: 122

Molecule Type: AA

Features Location/Qualifiers:

- source, 1..122
 > mol_type, protein
 > note, ABT502. VHH nanobody variant
 > organism, synthetic construct

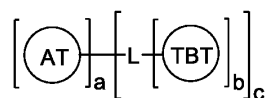
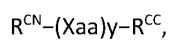
Residues:

QVQLQDSGGG	LVQAGGSLRL	SCEASGSIFS	ISAMGWYRQA	PGKQRELVAV	ITTDGSTNYA	60
DSVKGRFTIS	RNAKNTVYL	QMNSLKPEDT	DVYYCGASPR	ALKQYWGQGT	QVTVSSGGGG	120
SC						122

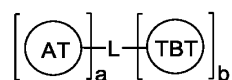
CLAIMS

1. A composition of matter comprising:
an IgG4-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 IgG4-binding moiety and the cellular receptor-binding moiety.

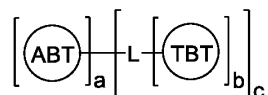
2. The composition of matter of claim 1, having a structure of:



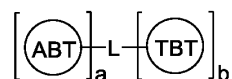
[AGN101],



[AGN102],



[AGN103], or

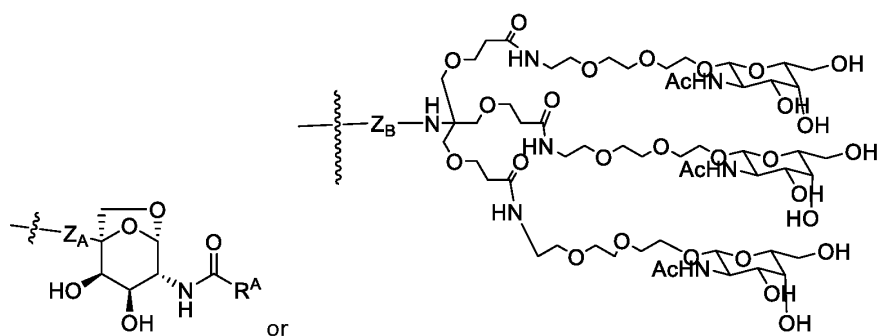


[AGN104]

or a salt thereof.

3. The composition of matter of claim 1, wherein the composition of matter is selected from the group consisting of **AGN401**, **AGN402**, **AGN403**, **AGN404**, **AGN501**, **AGN502**, **AGN508**, and **AGN509**.

4. The composition of matter of claim 1, wherein the IgG4-binding moiety is an anti-IgG4 antibody or an antigen-binding fragment thereof.
5. The composition of matter of claim 1, wherein the IgG4-binding moiety is a VHH affinity ligand variant antibody, a VHH affinity ligand variant, or an antigen-binding fragment thereof.
6. The composition of matter of claim 1, wherein the IgG4-binding moiety comprises three regions having the structures of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.
7. The composition of matter of claim 1, wherein the IgG4-binding moiety is selected from the group consisting of **ABT301**, **ABT304**, **ABT305**, **ABT306**, **ABT310**, **ABT311**, **ABT312**, **ABT313**, **ABT501**, **ABT502**, **ABT503**, and **ABT504**.
8. The composition of matter of claim 1, wherein the cellular receptor-binding moiety has the structure:



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 $C(O)-(CH_2)_{IM}-NR_M$.

9. The composition of matter of claim 1, wherein the cellular receptor-binding moiety is selected from the group consisting of **TBT103**, **TBT104**, **TBT105**, and **TBT106**.

10. The composition of matter of claim 1, for use as a medicament.
11. The composition of matter of claim 1, for use in removing IgG4 from a subject.
12. The composition of matter of claim 1, for use in treating a disease state or condition associated with the upregulation of IgG4 in a subject or patient.
13. The composition of matter of claim 1, for use in treating an IgG4-related disease in a subject or patient.
14. A method of making the composition of matter of claims 1-9, wherein the method comprises a conjugation step to link an IgG4-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.
15. A pharmaceutical composition comprising the composition of matter of claims 1-9 and a pharmaceutically acceptable excipient.
16. A method of removing IgG4 from a subject or patient comprising administering to the subject or patient the composition of matter of any of claims 1-9.
17. A method of treating a disease state or condition associated with the upregulation of IgG4 in a subject or patient comprising administering to the subject or patient an effective amount of the composition of matter of any of claims 1-9.
18. A method of treating an IgG4-related disease in a subject or patient comprising administering to the subject or patient an effective amount of the composition of matter of any of claims 1-9.

19. A composition comprising:
- a first composition of matter comprising:
 - an IgG4-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 IgG4-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 IgG4-binding moiety and the cellular receptor-binding moiety,
20. The composition of claim 19, 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.