BIFUNCTIONAL DEGRADERS

FIELD OF THE INVENTION

[001] The invention relates generally 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 contain a circulating protein binding moiety linked through a linker group to a cellular receptor-binding moiety to treat diabetes and other insulin disorders.

BACKGROUND OF THE INVENTION

[002] Diabetes often has an autoimmune component. Neutralizing antibodies generated against insulin reduce the effectiveness of insulin in diabetic patients. Many β-cell proteins are the targets of autoimmune responses in Type 1 diabetes (T1D). A contribution comes from the protein products of the insulin gene: preproinsulin (PPI), proinsulin (PI), or insulin. Children and most adults with type I diabetes show early generation of antibodies to the proinsulin C-chain and native insulin. Autoantibodies generated against insulin reduce the effectiveness of insulin in diabetic patients.

[003] There remains a need in the biomedical art for new medicines capable of treating or slowing down the progression of diabetes and other insulin disorders. Therapies for removing immunoneutralizing antibodies to insulin and insulin replacement therapies also remain needed.

SUMMARY OF THE INVENTION

[004] The invention provides compositions of matter (agents, TRAPs) that bind to neutralizing antibodies to proinsulin, insulin and insulin replacement therapies. These selective agents significantly reduce or eliminate only the pathogenic species of neutralizing autoantibodies generated by an undesirable immune response while leaving most of the other antibodies unbound in the subject to whom the agents are administered.

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

a target-binding moiety that can bind to anti-insulin antibodies,

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

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

The composition of matter is a bifunctional anti-proinsulin and or anti-insulin autoantibody degrader. This composition of matter, unlike pan-IgG or multi-IgG degraders, avoids the potential for immunosuppression.

[006] In another embodiment, the composition of matter (the agent, the TRAP) has the structure:



wherein [CPBM] is a target-binding moiety that can bind to an anti-proinsulin or anti-insulin antibody;

[CRBM] is a cellular receptor-binding moiety that binds to hepatocytes or other cells through asialoglycoprotein receptors or other receptors on the surface of hepatocytes and other degrading cells;

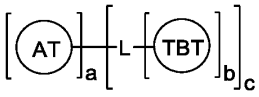
each [CON] is an optional connector chemical moiety which, when present, connects directly to [CPBM] or to [CRBM] or connects the [LINKER] to [CPBM] or to [CRBM] and

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

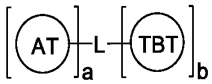
a pharmaceutically acceptable salt, stereoisomer, solvate, or polymorph thereof.

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

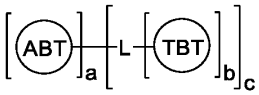
R^{CN}–(Xaa)y–R^{CC},



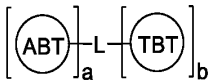
[AGN101],



[AGN102],



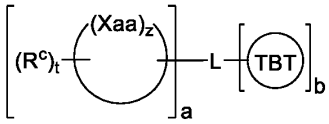
[AGN103], or



[AGN104],

or a salt thereof.

[008] In some embodiments, the composition of matter (the agent, the TRAP) has the structure of formula **AGN105**:



[AGN105]

or a salt thereof, wherein the composition of matter may have additional elements described in this specification.

[009] In another embodiment, the composition of matter is selected from among group consisting of the constructs in the following TABLE.

TABLE 1	
AGN302	Mutant insulin with GN3

TABLE 1	
AGN304	PT0004-**TBT6171**. Native proinsulin construct with C-terminal αGN3 conjugate. Sortase, U2073499G0-8, BAR = 1.
AGN305	PT0004-**TBT6171**. Mutant proinsulin construct with C-terminal αGN3 conjugate. Sortase, U2073499G0-8, BAR = 1.
AGN310	Insulin TRAP. Target binding moiety (ins-004), made by chemical synthesis conjugated to ASGPR ligand αGN3 (triGalNAc). Synthetic insulin MoDE has similar affinity to mAb49. MW: 7424.236. Sequence: GIVEQ[CC*TSIC]SLYQLENYC**N Alpha-GN-triazole-(pent-4-ynoic acid)-FVNQHLC*GSHLVEALYLVC**GERGAFYTPKT (SEQ ID NO: 43). Phenylalanine-to-alanine (F to A) mutation.
AGN4002	α(GalNAc ASGPR binder)3
AGN4003	α(propionic amide-GalNAc ASGPR binder)3
AGN4004	β (propionic amide-GalNAc ASGPR binder)3
AGN4006	α(propionic amide-GalNAc ASGPR binder)3
AGN4007	β(propionic amide-GalNAc ASGPR binder)3
AGN401	Synthetic C-peptide constructs (D-amino acids) with protease αGN3, no binding to either of the C-peptide antibodies.
AGN402	Synthetic C-peptide constructs (D-amino acids) with protease αGN3, no binding to either of the C-peptide antibodies, no binding to either of the C-peptide antibodies.
AGN405	PT0004-**TBT6171**. Native proinsulin made by genetic recombination technology conjugated to αGN3 (triGalNAc) at the C-terminal, conjugation by sortase U2073499G0-8, BAR = 1.
AGN406	C-peptide with βGN3 (L-amino acids). C-peptide with protease site, synthetic C-peptide degrader.
AGN407	C-peptide with protease βGN3 (L-amino acids). C-peptide without protease site, synthetic C-peptide degrader.
AGN408	PT0009-**TBT6170**. Native antibody PT-0009-01 construct with N-terminal αGN3 (**TBT6170**). MW = 13319.04 Da, sortase = U2073499G0-8. BAR = 0.82. IC_{50} = 1.2 nM.
AGN409	PT0009-**TBT6170**. Native proinsulin antibody PT-0009-02, αGN3 (**TBT6170**) conjugate (N-terminal), MW = 13323.34 Da, sortase = U2073499G0-8, BAR = 1.
AGN410	PT0011-**TBT6170**. Native antibody PT-0011-01-αGN3 conjugate (N-terminal), MW = 13242.87 Da, sortase = U2073499G0-8, BAR = 1.
AGN411	PT0011-**TBT6170**. Mutant proinsulin construct with N-terminal αGN3 conjugate (N-terminal), MW = 13247.69 Da, sortase = U2073499G0-8, IC_{50} = 3.1 nM.
AGN412	Synthetic mutant insulin with β-GN3. MW = 7424.24 Da.
AGN413	Fc-insulin-βGN3 conjugate
AGN414	Proinsulin-Fc -fusion G4S linker (**ABT307**) (SEQ ID NO: 17) + βGN3 (**TBT4438**). MW = 77525.74 Da.
AGN415	Proinsulin Fc-fusion 5 x G4S linker (**ABT308**) (SEQ ID NO: 18) + βGN3 (**TBT4438**) MW = 80047.84 Da.

TABLE 1	
AGN416	Synthetic mutant insulin with αGN3 on A chain, MW = 7424.24 Da,
AGN417	**ABT410-TBT403**. Native proinsulin TRAP. Native proinsulin-ASGPR ligand βGN3 (N-terminal). Synonym: GN3-Proinsulin conjugate. MW: 11921.84. **AGN417** shows a 30% reduction in endogenous insulin autoantibodies in 14-week-old NOD mice. **AGN417** shows good subcutaneous bioavailability. The intravenous group exhibited rapid yet strong detection of the degrader, contrasting with the subcutaneous group, where sustained detection was observed between two to eight hours. The subcutaneous group demonstrated 76% bioavailability, indicating effective absorption over time. Both subcutaneous administration (10 mg/kg) and intravenous administration (3 mg/kg) led to a significant reduction in blood glucose levels (BGL) at thirty minutes.
AGN418	**ABT410-TBT405**. Short linker (G4S) with βGN3. MW = 11871.59 Da.
AGN419	**ABT411-TBT404**. Proinsulin with C-terminal βGN3. MW = 12509.77 Da.
AGN420	**ABT412-TBT403**. N-terminal βGN3 long spacer in proinsulin. MW = 12267.07 Da,
AGN421	**ABT7714-TBT403**). Mutant proinsulin TRAP. N-terminal βGN3, with double mutant proinsulin (A-chain V3L and B-chain F25A) with click conjugation between triGalNAc and peptide. MW = 11859.59 Da. In vivo tolerability and blood glucose levels assayed in in NOD mice, aged 0.23 years (12 weeks). No toxic signs observed.
AGN422	**ABT7714-TBT405**). Mutant proinsulin TRAP. N-terminal βGN3-non-click double mutant proinsulin (A-chain V3L and B-chain F25A). Amide between triGalNAc and peptide. MW = 11809.86 Da
AGN423	Insulin-Fc (efsitora alfa)-**TBT4438** (βGN3-MATE) conjugation
AGN6567	**AGN417** with 13 and N15 labels
AGN6601	Mutant proinsulin made by genetic recombination technology conjugated to αGN3 (triGalNAc) at the C-terminal, conjugation by sortase U2073499G0-8, BAR = 1.
AGN7525	Short linker (G4S) with αGN3.
AGN7526	Long linker (G4S x 3) with αGN3.
AGN7849	N-terminal long spacer in proinsulin βGN3 click.
AGN7850	N-terminal long spacer in proinsulin βGN3.
AGN7865	N-terminal spacer in proinsulin βGN3.
AGN8123	This agent has the structure of **AGN421** with a terminal G→A mutation Refold yields may not reach the same level as other insulin analogues due to the B24 F→A mutation (SEQ ID NO: 38)
AGN8138	**ABT410-TBT7988**
AGN8139	**ABT410-TBT7989**
AGN8140	**ABT410-TBT7990**
AGN8141	**ABT410-TBT7991**
AGN2640	β(GalNAc ASGPR binder)3

[0010] In another embodiment, the target-binding moiety is insulin, an insulin variant, or an insulin analogue.

[0011] In another embodiment, the target-binding moiety is proinsulin, a proinsulin variant, or a proinsulin analogue.

[001] In another embodiment, the target-binding moiety is an insulin-IgG1 Fc fusion construct.

[002] In another embodiment, the target-binding moiety is a proinsulin-IgG1 Fc fusion protein. The proinsulin-IgG1 Fc fusion protein can be expressed in and produced from a mammalian cell system because of the Fc domain, which also allows for purification of the expressed protein. The proinsulin-IgG1 Fc fusion protein can then be conjugated to ASGPR-binder via MATE reagent, resulting in a bifunctional degrader molecule. Persons having ordinary skill in the biomedical art can introduce LALA mutations into the Fc region to attenuate immune effector function via abrogation of FcgR. Persons having ordinary skill in the biomedical art can also introduce PA mutations to attenuate immune effector function via abrogation of c1q binding. They can use a DNA sequence encoding a proinsulin-IgG1 Fc fusion protein for recombinant production of proinsulin-IgG1 Fc fusion protein in mammalian systems.

[003] In another embodiment, the target-binding moiety is a proinsulin-IgG1 Fc fusion construct.

[004] In another embodiment, the target-binding moiety is selected from among group consisting of the constructs in the following TABLE:

TABLE 2

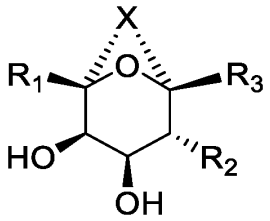
ABT306	Alk-native insulin
ABT307	Proinsulin-Fc-fusion G4S linker, 71006.170 Da, proinsulin-IgG1 Fc fusion, G4S linker, hIgG1 hinge (C > A), LALA-PA (SEQ ID NO: 48).
ABT308	Proinsulin-Fc-fusion 5 x G4S linker, 73528.457 Da, proinsulin-IgG1 Fc fusion, 5x G4S linker, hIgG1 hinge (C > A), LALA-PA (SEQ ID NO: 49).
ABT309	LALA mutation (in Fc domain) does not prevent recognition by **AGN301** (IgG degrader)
ABT311	Homo sapiens sequence 1..18
ABT311	Native proinsulin N-terminal sortase tag (SEQ ID NO: 10). MW = 11127.
ABT312	Mutant proinsulin N-terminal sortase tag (SEQ ID NO: 6). F25A modification. MW = 11051. Contains Homo sapiens sequence 1..18 (SEQ ID NO: 45).
ABT313	Mutant proinsulin N-terminal sortase tag. MW = 11141. Homo sapiens sequence 1..18.
ABT314	Native proinsulin with C-terminal His tag. (SEQ ID NO: 7). MW = 10842.
ABT315	Native proinsulin with C-terminal sortase tag. (SEQ ID NO: 8).
ABT401	LALA mutation (in Fc domain) does not prevent recognition by **AGN301** (IgG degrader)
ABT403	mAb49 (original), Creative Biolabs, FAMAB-0225WJ, with mouse Fab-human IgG3 Fc .

TABLE 2	
ABT404	mAb49 chimeric heavy chain gamma 3, mouse VH/VL (SEQ ID NO: 34) [495 AA], with human IgG1 CH/CL with mAb49 chimeric light chain kappa (SEQ ID NO: 33) [215 AA].
ABT405	Mouse mAb49 VH/VL-human IgG1 CH/CL. Fc regions include "LALA/PA" mutations to effect immune effector function (FcgR-, c1q-binding).
ABT406	Mouse anti-C-peptide antibody (original)
ABT407	Anti-C-peptide antibody with mouse VH/VL-human IgG1 CH/CL
ABT408	Anti-C-peptide antibody, mouse VH/VL-human IgG1 CH/CL. Fc regions include "LALA/PA" mutations to effect immune effector function (FcgR-, c1q-binding).
ABT409	Anti-C-peptide antibody, mouse VH/VL-human IgG3 CH/CL monoclonal antibody.
ABT410	Native proinsulin-N-terminal sortase linker (clean/native) (SEQ ID NO: 44).
ABT411	Proinsulin-C-terminal sortase linker (clean/native).
ABT412	Proinsulin molecule optimization to improve conjugation efficiency
ABT413	Human insulin.
ABT414	Insulin efsitora alfa (insulin-Fc). Synonyms: LY3209590 (SEQ ID NO: 16). MedChemExpress, catalogue # HY-P99665. See Moyers et al., J. Pharmacol. Exp. Ther., 382, 346-355 (2022) (Eli Lilly).
ABT415	Insulin lispro.
ABT416	Mutant proinsulin N-terminal sortase tag (SEQ ID NO: 11)
ABT417	Mutant proinsulin with N-terminal sortase tag (SEQ ID NO: 22). Mutant B-chain insulin F25A.
ABT418	Proinsulin Fc fusion protein – Proinsulin + hIgG1 Fc-LALA/PA**,** Mouse Ig Kappa signal, Proinsulin, G4S linker, hIgG1 hinge(**C>A**)-Fc-LALA/PA. (SEQ ID NO: 30)
ABT419	Proinsulin protein (SEQ ID NO: 4).
ABT420	Proinsulin protein (SEQ ID NO: 5). Creative Biomart, Shirley, NY, USA, Cat. NO: Proinsulin-01H. R&D Systems, Minneapolis, MN, USA,
ABT421	PT0003, native proinsulin C-terminal His Tag (SEQ ID NO: 7). (GenScript U608R952G0-15).
ABT422	PT0004. Native proinsulin C-terminal sortase tag (SEQ ID NO: 8). (GenScript U608R952G0-21).
ABT423	PT0009, Native proinsulin N-terminal His Tag (SEQ ID NO: 9) (GenScript)
ABT424	PT0011, Native proinsulin N-terminal sortase Tag (SEQ ID NO: 10) (GenScript)
ABT6396	SEQ ID NO: 49
ABT7714	Mutant proinsulin (A-chain V3L and B-chain F24A)
ABT17384	Mutant proinsulin + lysine mutation
ABT17385	Mutant proinsulin + methionine

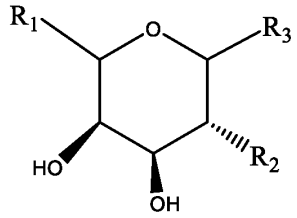
TABLE 2	
ABT17386	Mutant proinsulin + cysteine

[005] In another embodiment, the target-binding moiety further comprises a rigid C-term linker for the unpaired cysteines in the target moiety for conjugation: His6-SPSTPPTPSPSTPPC (SEQ ID NO: 37).

[006] 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.

[007] In another embodiment, the cellular receptor-binding moiety is selected from among group consisting of the constructs listed in the following TABLE.

TABLE 3	
TBT103	Cellular binding moiety maleimide βGN3 for engineered cysteine-based conjugation.
TBT104	αGN3 moiety with polyglycine for sortase-based conjugation.
TBT402	
TBT403	βGN3-GLPETGG-NH2 (SEQ ID NO: 50).
TBT404	GGGGG-Pra(β-GN3)-NH2.
TBT405	PEG3 linkers **TBT7714** to get **AGN422** (Ins-030), glycine to alanine.
TBT6170	For N-terminal modification of proinsulin.
TBT6171	For C-terminal modification of proinsulin.

TABLE 3	
TBT4438	
TBT501	C-terminal sortase tag with His6 tag. GGGGSGGGGSLPETGGHHHHHH (SEQ ID NO: 20).
TBT502	Rigid C-term linker for the unpaired cysteine for conjugation. HHHHHHSPSTPPTPSPSTPPC (SEQ ID NO: 21).
TBT7990	
TBT7991	
TBT7988	
TBT7989	

[008] In another embodiment, the invention provides a method of making the composition of matter.

[009] In another embodiment, the invention provides a method of removing anti-proinsulin and anti-insulin antibodies in a subject or patient by administering the agent to the patient or subject. The administered bifunctional degrader agent removes anti-proinsulin and anti-insulin autoantibodies, whether IgG1, IgG2, or IgG4, from circulation by binding to the autoantibody and to a high capacity ASGPR receptor on hepatocytes.

[0010] In another embodiment, the invention provides a method of treating a disease state or condition associated with the upregulation of anti-proinsulin and anti-insulin antibodies in a subject or patient by administering to the subject or patient an effective amount of the agent.

[0011] In another embodiment, the invention provides a method of treating diabetes in a patient by administering to the patient a therapeutically effective amount of the agent. Methods of following the course treatment for insulin-treated diabetes are known to persons having ordinary skill in the biomedical art. In a particular embodiment, diabetes being treated is Type 1 diabetes. Methods of following the course treatment for insulin-treated Type 1 diabetes are known to persons having ordinary skill in the biomedical art.

[0012] In another particular embodiment, anti-drug, neutralizing antibodies generated against insulin reduce the effectiveness of insulin in diabetic patients. Removal of anti-drug antibodies corrects anti-drug antibody-related insulin resistance in patients. Removal of anti-drug antibodies is useful for treating life-threatening insulin resistance, encephalopathy, or cellular starvation.

[0013] In another particular embodiment, almost all children and most adults with Type I diabetes, show early generation of antibodies to the proinsulin C-chain as well as native insulin. Removal of autoantibodies directed against proinsulin mitigates insulitis, and specifically inflammation directed to

the pancreatic islets of Langerhans, which is in part caused by a secondary antibody-dependent cell-mediated cytotoxicity (ADCC) directed against β-cells of the patient's pancreas. Removal of autoantibodies directed against proinsulin can correct anti-drug antibody-related insulin resistance in patients. Insulin resistance abrogates glucose uptake insulin resistance and IgG-mediated β-cell death. See Liu et al., Diabetes, 71(4), 722-732 (April 1, 2022). Active intervention with these agents can reduce β-cell loss and reduce pancreatic inflammation.

[0014] In another particular embodiment, the agent is useful for treating diabetic animals. Many companion dogs and companion cats in the United States have diabetes mellitus. More than 10% of diabetic dogs in the United States are therapeutically treated with human insulin. These dogs frequently develop immune-neutralizing antibodies which decrease insulin concentrations and antidiabetic efficacy.

[0015] In another embodiment, the invention provides a pharmaceutical composition including the agent and at least one pharmaceutically acceptable excipient.

[0016] In another embodiment, the invention provides a composition including the agent and at least one additional agent comprising a moiety capable of binding to that forms the antibody moiety of the first compound.

[0017] In one aspect, the targeting of anti-insulin autoantibodies or anti-proinsulin autoantibodies with TRAPs has several advantages. In humans Type 1 diabetes, autoantibodies are often found in low concentrations, e.g., ~1000ng/mL (0.01% of total IgG). The target autoantibodies are well-defined and detectable with available clinical diagnostics to track efficacy of lowering titers. A single drug approach can target several Type 1 diabetes-associated factors that induce β cell destruction, including autoantibodies to anti-insulin autoantibodies and anti-proinsulin autoantibodies. The target autoantibodies are primarily of the IgG1 subclass with a half-life of twenty-nine days. Thus, infrequent dosing may be effective.

[0018] Several objects, features, aspects, and advantages of the invention become more apparent from the following detailed description of embodiments of the invention, along with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[001] 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.

[002] FIG. 1 shows two schematics of proinsulin and insulin processing.

[003] FIG. 2 shows the structure of one embodiment of the bifunctional molecules (agents, TRAPs).

[004] FIG. 3 shows a process of preparation of one embodiment of the bifunctional molecules (agents, TRAPS) from a full IgG antibody and a reagent having affinity to the by conjugating the IgG antibody to the reagent.

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

[006] FIG. 5 is a comparison of sequences of the constant heavy chains of human IgG1 (SEQ ID NO: 1), IgG2 (SEQ ID NO: 1), and IgG4 (SEQ ID NO: 3), which support human and humanized therapeutic antibodies. 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 (IMGT). Sequences important for FcgR binding, C1q, and FcRn are noted. Some increased ADCC mutants are shown above or below the sequences, as noted: Xencor's S239D, A330L, I332E [red, above sequences]; 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 (University of Southampton) H433K mutant, N434Y mutant, and Genentech's N434A mutant are shown.

[007] FIG. 6 is a schematic of the chemical reaction conjugating insulin with GN3 to produce an αGN3 wild-type insulin peptide.

[008] FIG. 7 is a schematic of several examples of C-peptide conjugated to GN3. FIG. 7A is a first example of C-peptide conjugated to GN3. FIG. 7B is a second example of C-peptide conjugated to GN3.

[009] FIG. 8 is a schematic of several examples of insulin-peptide conjugated to GN3. FIG. 8A is an example of native insulin with its B chain conjugated to GN3. FIG. 8B is an example of a variant insulin with its B chain conjugated to GN3.

DETAILED DESCRIPTION OF THE INVENTION

[0010] The following detailed description is provided to aid persons having ordinary skill in the biomedical art. Exemplary embodiments are described. However, these embodiments are only exemplary. This disclosure is not limited thereto but is defined by the scope of the appended claims. Persons having ordinary skill in the biomedical art may make modifications and variations in the embodiments described in this specification without departing from the spirit or scope of this disclosure.

Industrial Applicability.

[0011] The invention provides a medically useful composition of matter (agent) for treating or slowing down the progression of diabetes and other insulin disorders. The removal and degradation of circulating anti-insulin and anti-proinsulin antibodies should improve the health of diabetic subjects by increasing the natural levels and availability of the subject's insulin.

[0012] The removal or suppression of pathogenic antibodies previously used general immune-suppressive therapies including TNF inhibitors, Jak inhibitors, FcRn recycling inhibitors, MTOR inhibitors, glucocorticoid receptor antagonists, pan-selective bispecific degraders, cyclophosphamide and other agents resulting in marked susceptibility to microbial, viral, and other infections and an increased incidence of cancer, especially lymphoma, through decreased immune-surveillance. Selective removal of pathogenic antibodies while sparing nonpathogenic antibodies, enables normal humoral immune responses including normal response to vaccination, resistance to infection and other important effector functions of the spared, non-autoreactive antibodies. This approach provides an anti-inflammatory medicine without other unwanted immune-mediated or immunosuppression-mediated consequences. The determination of lowering of immunoneutralizing antibodies to insulin is readily and specifically determined by commercially available diagnostic tests for the detection of these antibodies.

[0013] There are about 300,000 childhood diabetes patients in the United States who are less than twenty years of age. Insulin as an autoantigen in the immune pathogenesis of Type I diabetes.

[0014] Many diabetic adults have autoantibody-associated insulin resistance. In some, this is a life-threatening disease.

[0015] Many domestic animals have autoantibody-associated insulin resistance. About 165,000 dogs and 300,000 cats in the United States have diabetes mellitus. Over 10% are treated with human insulin.

[0016] After the bifunctional degrader and the bound insulin protein are endocytosed, they are released from the ASGPR through depletion of calcium from the endosome and changes in binding site

amino acid protonation changes due to a decrease in pH. The ASGPR is recycled back to the hepatocyte surface. Endocytosed proteins are trafficked to late endosomes, which are fused with lysosomes. Lysosomal proteases then degrade endocytosed proteins, permanently removing them from circulation.

Definitions

[0017] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are listed below. Unless stated otherwise or implicit from context, these terms and phrases shall have the meanings below. These definitions aid in describing embodiments but are not intended to limit the claimed invention.

[0018] As used in this application, except as otherwise provided in this specification, each term shall have the meaning set forth below. Additional definitions are set forth throughout the application. Where a term is not specifically defined in this specification, that term is given a biomedical art-recognized meaning applying that term in context to its use in describing the invention.

[0019] 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.

[34] The term “ABT” has the meaning described in this specification of a binding moiety that is itself an antibody, an antibody variant, or an antigen-binding fragment thereof. In some embodiments of this specification, the ABT binds to insulin.

[0020] 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.

[0021] The term “ADCC” has the biomedical art-recognized meaning of antibody-dependent cell-mediated cytotoxicity, is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system kills a target cell, whose membrane-surface antigens were bound by specific antibodies.

[0055] 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.

[0022] The term “agent” has the biomedical art-recognized meaning of a composition of matter useful for performing a function. Several biomedically useful functions are described in this specification.

[0056] 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.

[0057] 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 depends 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 a clinician. In embodiments, the disease or condition to be treated is tendinopathy.

[0058] The term "analogue" has the biomedical art-recognized meaning of a molecule that is not identical but has analogous functional or structural features. Biochemical modifications could increase the analogue's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. Insulin analogues are known in the biomedical art, such as the fast-working insulins called rapid-acting insulin.

[0059] The term "anti-drug antibody (ADA)" has the biomedical art-recognized meaning. Many ADAs are IgG4 antibodies, such as those in Factor 8 hemophilia.

[0060] The term "anti-insulin antibody" has the biomedical art-recognized meaning and includes insulin autoantibodies (IAA). Anti-C-peptide antibodies are commercially available. See recombinant mouse anti-c-peptide antibody (CB2931), available from Creative Biolabs, Shirley, NY, USA, product number CBMAB-MD1611-LY. See also Mouse Anti-C-Peptide Monoclonal Antibody (CBFYR0644), available from Creative Biolabs, product number CBMAB-R0644-FY. See also human (chimeric) Anti-Insulin Recombinant Antibody (clone mAb49), available from Creative Biolabs, product number FAMAB-0225WJ. The inventors found that synthetic insulin MoDE have similar affinities to mAb49.

[0061] 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.

[0062] 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 antibody binding moiety through a linker group or directly. It is through this ASGPR moiety that bifunctional agents complexed with a circulating protein, e.g., 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.

[0063] The term “asialoglycoprotein receptor” (ASGPR) has the biomedical art-recognized meaning of lectins which bind asialoglycoprotein and glycoproteins from which a sialic acid was 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.

[0064] 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.

[0065] The term “AT” has the meaning described in this specification of an antibody moiety. In some embodiments of this specification, the AT binds to insulin.

[0066] The term “autoantibody” has the biomedical art-recognized meaning.

[0067] The term “binding moiety” has the biomedical art-recognized meaning a moiety on a binding protein, e.g., an antibody, an antibody variant, or an antigen-binding fragment thereof, that binds to insulin.

[0068] 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.

[0069] 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.

[0070] The term “chimerized” has the biomedical art-recognized meaning. Chimeric antibodies are made by fusing variable domains from one species, such as a mouse, with constant domains from another species, such as a human being. With such biotechnical manipulation, chimeric antibodies keep the foreign antibody’s antigen specificity and affinity.

[0071] 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 if a beneficial effect from the co-action of the combination of the therapeutic agents a is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

[0072] The term “complementarity determining region (CDR)” has the biomedical art-recognized meaning of a polypeptide region of an antibody heavy chin or an antibody light chain that is a determinant of the antibody to antigen binding. Each antibody heavy chain contains three CDRs. Each antibody light chain contains three CDRs, usually different from the three CDRs on an antibody heavy chain. Persons having ordinary skill in the biomedical art can calculate the CDR structure using a standardized numbering method known as the Kabat numbering scheme. Other numbering schemes, such as the Chothia, IMGT, Martin (enhanced Chothia), and other standard numbering schemes, are also used by persons having ordinary skill in the biomedical art.

[0073] The term "comprises," the term "comprising," the term "includes," and the term "including" specify 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.

[0074] The term “diabetes mellitus” has the biomedical art-recognized meaning. See Wikipedia, the free encyclopedia, Dipeptidyl peptidase-4 inhibitor (online, accessed July 3, 2024). In type 1 diabetes

mellitus, the destruction of insulin-producing β-pancreatic cells results in an inability to control blood glucose levels since insulin signals glucose uptake into cells. Type 1 diabetes mellitus is a systemic disease that can lead to severe complications. Hyperglycemia is a symptom of diabetes. A human diabetic patient is someone whose blood sugar level is elevated or is expected to be elevated, and therefore someone who desires to inhibit from rising or to lower a blood sugar level.

[0075] The term "diabetic macular edema" (DME) has the biomedical art-recognized meaning of a diabetic retinopathy. See Landry et al., Assay Drug Dev. Technol. 11(5), 326-32 (June 2013). Tiny bulges, called microaneurysms, form in the blood vessels, leaking fluid into the retina.

[0076] The term "EC_{50}" has the biomedical art-recognized meaning of the ligand concentration required to achieve 50% of maximal receptor activation.

[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 "Fc" has the biomedical art-recognized meaning.

[0080] 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.

[0081] 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.

[0082] 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.

[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 keeping the specificity of the rodent antibody. The human origin domain need not originate directly from a human because 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 steps 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 "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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] The term "insulin efsitora alfa" (LY-3209590), means the selective agonist of insulin receptor (IR) that is a fusion protein composed of human IR agonists fused with the crystallizable (Fc) domain of human immunoglobulin G2 (IgG2) fragment, with a molecular weight of 64.1 kDa. Insulin efsitora alfa is being developed as a possible diabetes therapeutic. The protein may be used a tool compound or as a control compound for the insulin-Fc fusion protein and proinsulin FC fusion protein described in this specification.

[0090] The term "insulin growth factor" (IGF) has the biomedical art-recognized meaning. Human IGF-1 means comprising the amino acid sequence corresponding to human IGF native to human tissue. Human IGF-1 may include fusion proteins.

[0091] The term "insulin lispro" has the biomedical art-recognized meaning of a rapid-acting insulin analog, a synthetic version of insulin, used to lower blood sugar levels in people with diabetes, working faster and for a shorter duration than regular human insulin. See Islam, Khanna, Patel, & Zito, Insulin Lispro, In: StatPearls [Internet] (Treasure Island (FL), StatPearls Publishing). In Insulin Lispro (Eli Lilly), the

positions of proline at position B28 and lysine at position B29 in the B chain have been reversed. In insulin lispro, these variations reduce the strength of the interactions that hold insulin dimers together, allowing for faster dissociation of hexamers and hence absorption of monomers. These analogs and formulations result in a quicker onset of action, a quicker time to peak activity, and a shorter duration of action compared to human regular insulin, making them more optimal for mealtime administration.

[0092] The term "insulin receptor" has the biomedical art-recognized meaning. The insulin receptor has two main subunits: α and β. There is a single disulfide bridge between α and β subunits between Cys647 in the insert domain and Cys872 The α subunit contains five main domains, L1 (AA 28–174) CR (AA 182–339), and L2 (AA 340–497), and two fibronectin subunits: FnIII-1 (residue 624–726) and FnIII-2 (757–842). The two α-subunits are linked by four disulfide bonds. The insulin receptor β has different isoforms (IRA and IRB), depending on the gene splicing of exon 11. Insulin receptor B differs from insulin receptor A by including exon 11. The 12-amino acid sequence (residues 745–756) derived from exon 11 is present in the insulin receptor B isoform but absent in the insulin receptor A isoform. The isoforms have functionally different internalization and recycling. Insulin receptor A exhibits a greater internalization and recycling rate than insulin receptor B. Insulin receptor A exhibits a higher affinity for IGFs than IRB. Both isoforms have similar affinity for insulin Binding to these isoforms very different to proinsulin. Because of these differences, insulin receptor B is preferentially associated with metabolic and differentiating signals. Insulin receptor A mainly favors cell growth, proliferation, and survival. See Beneit et al., Cardiovasc. Diabetol., 15, 161 (2016).

[0093] The term "insulin-like growth factor 2" (IGF-2) has the biomedical art-recognized meaning of the well-characterized neutral peptide secreted by the liver to circulate in the blood. IGF-2 has growth-regulating, insulin-like and mitogenic activities.

[0094] The term "insulin" has the biomedical art-recognized meaning. Insulin can be produced by chemical synthesis.

[0095] The term "IVIG" has the biomedical art-recognized meaning of the administration of intravenous immunoglobulin.

[0096] 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.

[0097] The term "MoDE" has the proprietary meaning of molecular degraders. See International Patent Publications WO 2019/199634 (Yale University) and WO 2019/199621 (Yale University).

[0098] 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. In some embodiments, a binding moiety maintains one or more desired structural features, properties, functions, or properties, e.g., 3-dimension structure, antigen specificity, antigen-binding capacity, or immunological functions, etc. comparable to its corresponding binding protein, e.g., an antibody. In some embodiments, a moiety is monovalent. In some embodiments, a moiety is bivalent. In other embodiments, a moiety is polyvalent.

[0099] 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.

[00100] The term “Multimodal Antibody Therapy Enhancers (MATE or MATES)” has the proprietary meaning. See International Patent Publication WO 2021/102052 (Kleo Pharmaceuticals).

[35] The term “nanobody” has the biomedical art-recognized meaning. the term “antibody” refers to immunoglobulin molecules and immunologically active portions 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 and a variety of forms including full length antibodies and antigen-binding portions thereof; including an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab’, a F(ab’)2, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a nanobody, a multi-specific, e.g., tri-specific, antibody, a dual specific antibody, a bispecific antibody, an anti-idiotypic antibody, a functionally active epitope-binding 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 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, as well as on hepatocytes.

[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 keeping the specificity of the rodent antibody. The domain of human origin need not originate directly from a human because 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 "peripheral blood mononuclear cell (PBMC)" has the biomedical art-recognized meaning of any peripheral blood cell having a round nucleus, including lymphocytes (T cells, B cells, NK cells) and monocytes.

[00106] The term "pharmaceutically acceptable excipient" has the biomedical art-recognized meaning of an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use and human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's, Pharmaceutical Sciences 23rd edition (Elsevier, 2020).

[00107] The term "pharmaceutically acceptable" has the biomedical art-recognized meaning of those compounds, anions, cations, materials, compositions, carriers, and/or dosage forms which 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.

[00108] The term "proinsulin" has the biomedical art-recognized meaning, being a precursor to mature insulin. Proinsulin is commercially available from R+D systems. Proinsulin can also be produced by chemical synthesis. The chemically synthesized proinsulin is comparable to commercial proinsulin for the surface plasmon resonance (SPR) assay. Proinsulin does not bind to the insulin receptor.

[00109] 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.

[00110] The term "ROC" has the biomedical art-recognized meaning of receiver operating characteristic curve.

[00111] 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.

[00112] The term "TBT" has the meaning described in this specification of target binding moiety, a cellular receptor-binding moiety. In some embodiments of this specification, the TBT binds to ASGPR.

[00113] The term "TRAP" has the meaning described in this specification of a targeted removal of aberrant protein. A TRAP is a bifunctional degrader.

[00114] 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.

[00115] The term "Type 1 diabetes mellitus" has the biomedical art-recognized meaning of a chronic autoimmune disease that occurs when the immune system attacks the pancreas' insulin-producing cells, resulting in the autoimmune eradication of β-cells in pancreatic islets. Insulin can no longer be synthesized or be secreted into the blood. Other autoantibodies directed to islet cells may also be present in Type 1 diabetics.

[00116] The term "Type 2 diabetes mellitus" has the biomedical art-recognized meaning of the progressive loss of insulin receptors Inducing hyperglycemia. Hyperglycemia induces more production of insulin by β-cells to compensate. The accumulation of amyloid in the pancreatic islets likely disrupts islet anatomy and physiology. Eventually, β-cells wear out leading to decreased insulin over time.

[00117] 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.

[00118] Some embodiments are described below by referring to structures and schemes, to explain aspects of the description.

[00119] 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.

[00120] 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 administration.

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

Methods of measuring the subject's improvement after removal of anti-insulin antibodies.

[00122] Many assays are commercially available to measure a subject's insulin levels. As known by persons having ordinary skill in the biomedical art, a normal and stable insulin level indicates the health and continued health of a subject with diabetes or other insulin disorders.

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

[00123] In an embodiment, the invention provides a composition of matter (agent) comprising:

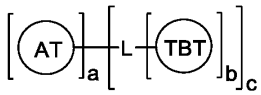
a binding moiety that can bind to anti-insulin antibody,

a cellular receptor-binding moiety that 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

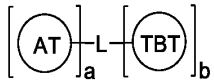
a linker moiety linking the antibody moiety and the cellular receptor-binding moiety.

[00124] In some embodiments, the invention provides a composition of matter (an agent) having a structure selected from the Markush group of structures consisting of:

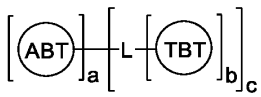
R^{CN}–(Xaa)y–R^{CC},



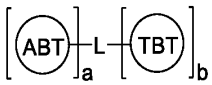
[AGN101],



[AGN102],



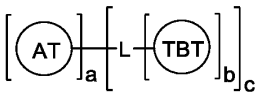
[AGN103], or



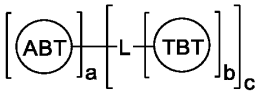
[AGN104]

or a pharmaceutically acceptable 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.

[00125] In some embodiments, the agent has the structure of formula **AGN101 or AGN103**:



[AGN101]



[AGN103], or

or a pharmaceutically acceptable salt thereof, wherein:

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

each AT is independently a binding moiety;

L is a linker moiety; and

each TBT is independently a cellular receptor-binding moiety,

wherein the binding moiety is an antigen-binding protein, an antigen-binding protein variant, or an antigen-binding fragment thereof.

[00126] In some embodiments, an agent comprises one and no more than one binding moiety. In some embodiments, one or no more than one 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

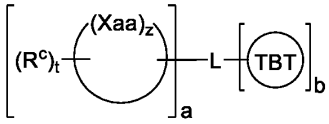
target binding moieties. In some embodiments, b is 2 or more and c is 1. In some embodiments, b is 2 or more and c is 2 or more. In some embodiments, b is 1 and c is 2 or more.

[00127] In some embodiments, each cellular receptor-binding moiety in an agent is the same. In some embodiments, each linker moiety connecting a cellular receptor-binding moiety to an antibody moiety is the same. In some embodiments, the TBT in agents is the same. In some embodiments, the $-L-(TBT)_b$ are the same.

[00128] Persons having ordinary skill in the biomedical art know that several technologies can be used to 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 amino acid residues optionally through linker moieties. In some embodiments, provided technologies selectively connect target binding moieties to certain types of amino acid residues, e.g., lysine residues, optionally through linker moieties. In some embodiments, provided technologies selectively connect target binding moieties to sites of antibody moieties optionally through linker moieties. In some embodiments, provided technologies selectively connect target binding moieties to certain types of amino acid residues at sites optionally through linker moieties. In some embodiments, target binding moieties are connected to K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto optionally through linker moieties. In some embodiments, target binding moieties are connected to K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto optionally through linker moieties. In some embodiments, target binding moieties are connected to K239 and K241 of 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. As known by persons having ordinary skill in the biomedical art, an antibody agent may comprise more than one sites, e.g., one on each of the more than one chain, e.g., one or each heavy chain. In some embodiments, an antibody moiety comprises two heavy chains and one or both amino acid residues or

amino acid residues corresponding thereto are each independently connected to a cellular receptor-binding moiety optionally through a linker. In some embodiments, one and no more than one is connected. In some embodiments, c is 1. In some embodiments, both are connected. In some embodiments, c is 2. In some embodiments, both target binding moieties and/or both linker moieties (if any) are the same.

[00129] In some embodiments, the invention provides a composition of matter (an agent) of formula **AGN105**:



[AGN105]

or a pharmaceutically acceptable salt thereof, wherein:

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

t is 0-50;

z is 1-50;

L is a linker moiety;

TBT is a cellular receptor-binding moiety;

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

each of a and b is independently 1-200;

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

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

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

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur,

phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30 membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30 membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

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

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

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

[00130] Further guidance is provided in International Patent Publication WO 2025/035052 (Biohaven Therapeutics Ltd.), the contents of which are incorporated by reference.

TABLE 5

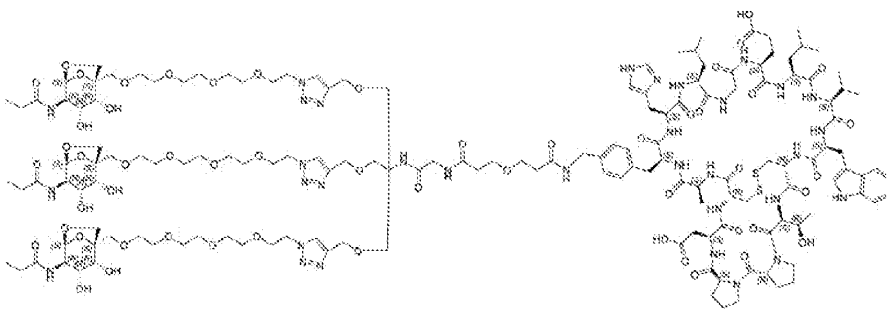
AGN301	IgG degrader (reference molecule) [F_c-BP2-PR3] binds to rabbit antibody. 

TABLE 5

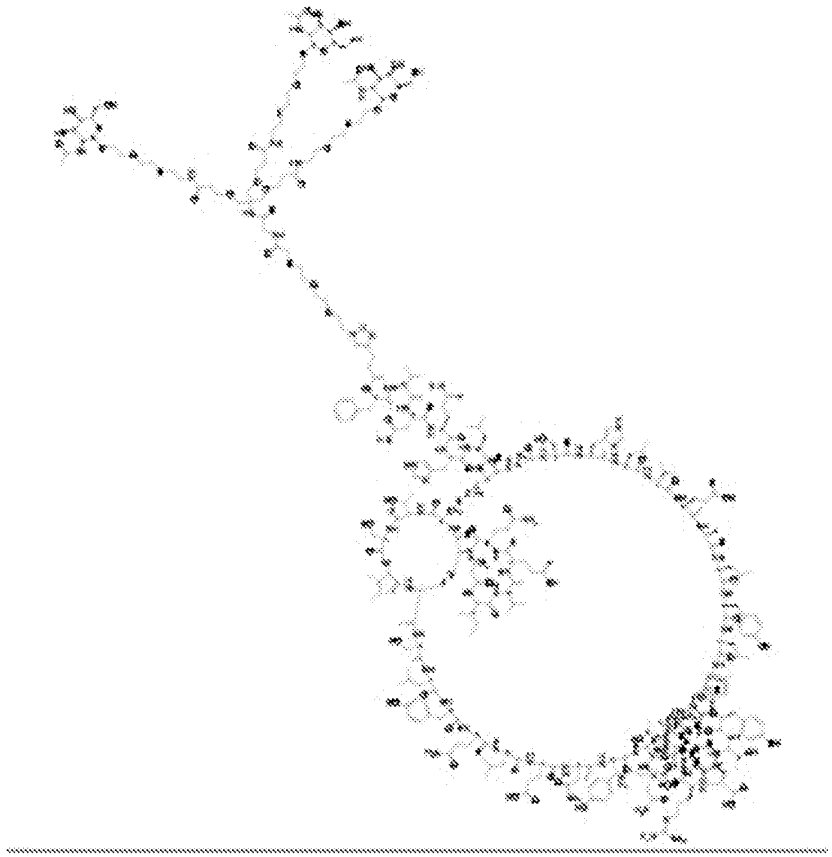
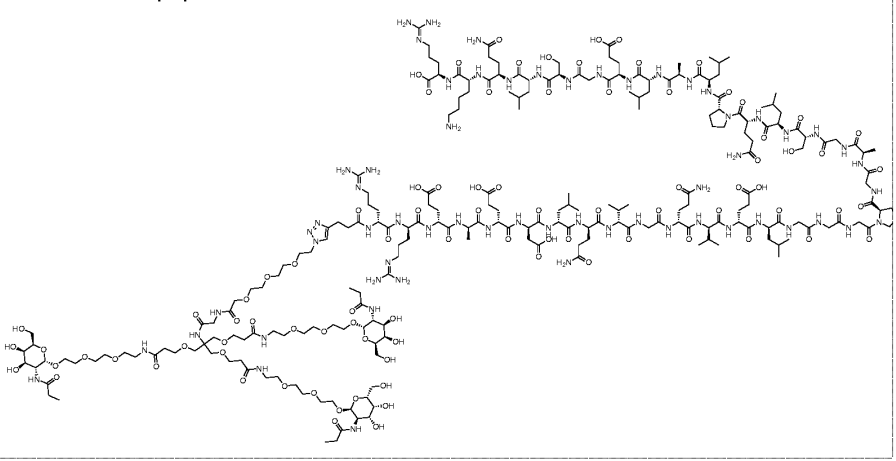
AGN302	Mutant insulin with GN3 
AGN401	Synthetic C-peptide constructs (D-amino acids) with protease αGN3, no binding to either of the C-peptide antibodies. 

TABLE 5

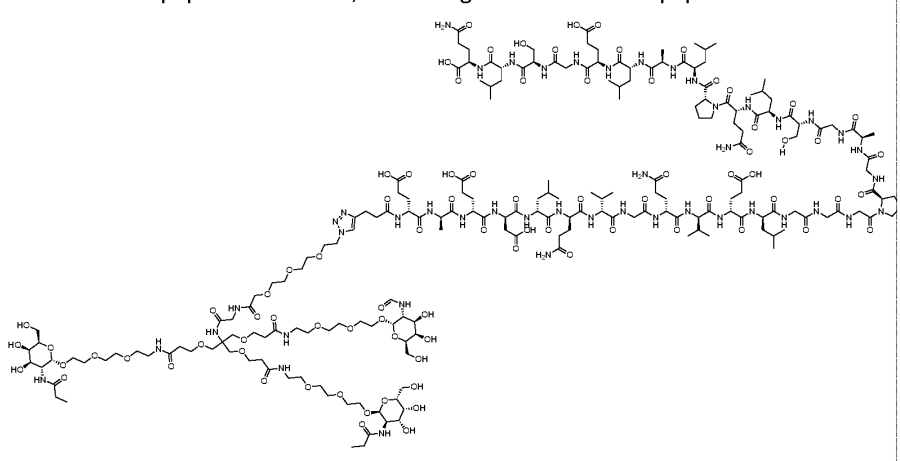
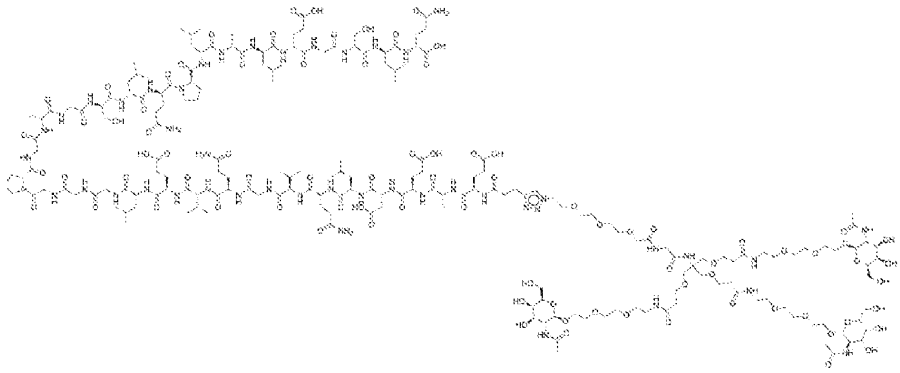
AGN402	Synthetic C-peptide constructs (D-amino acids) with protease αGN3, no binding to either of the C-peptide antibodies, no binding to either of the C-peptide antibodies. 
AGN403	βGN3, synthetic C-peptide constructs (D-amino acids), no binding to either of the C-peptide antibodies. βGN3, synthetic C-peptide constructs (D-amino acids), no binding to either of the C-peptide antibodies. C-peptide with βGN3 (D-amino acids) 

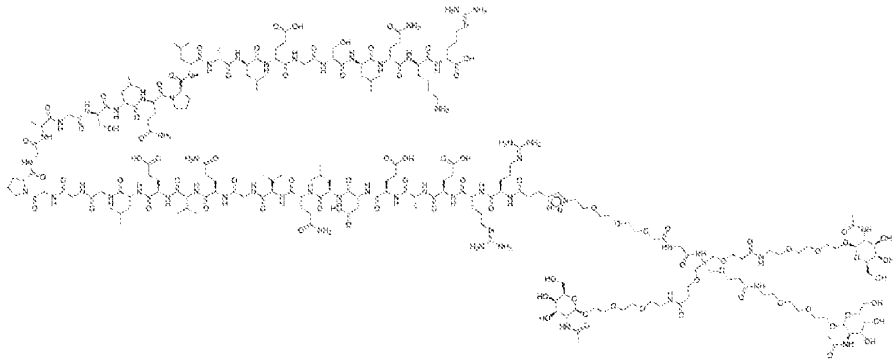
TABLE 5	
AGN404	βGN3, synthetic C-peptide constructs (D-amino acids), no binding to either of the C-peptide antibodies. C-peptide with protease βGN3 (D-amino acids) 

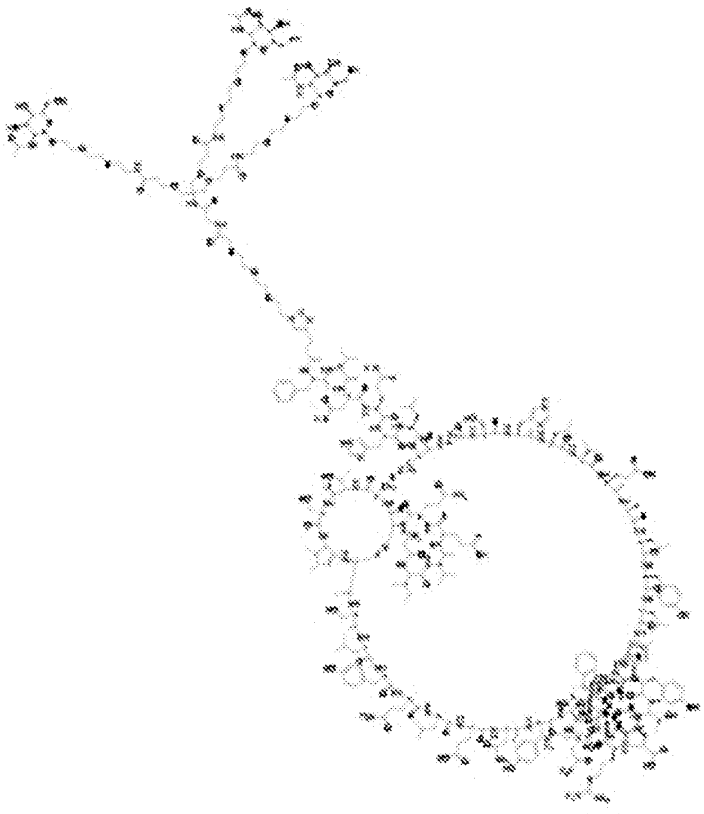
TABLE 5	
AGN303	Native proinsulin, Ins-003, made by chemical synthesis conjugated to GN3 (triGalNAc) (as reference) binds to mouse antibody. Synthetic insulin MoDE has similar affinity to mAb49. 

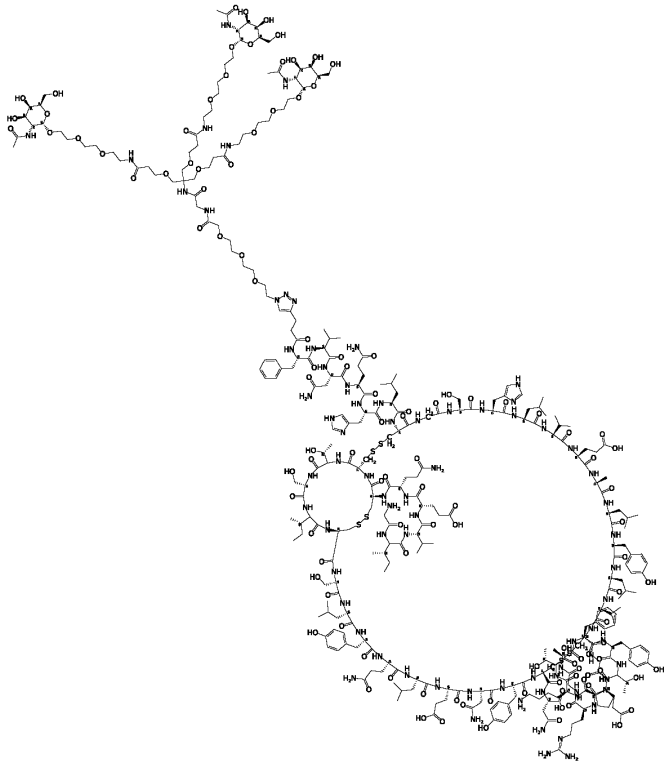
TABLE 5	
AGN310	Insulin TRAP. Target binding moiety (Ins-004), made by chemical synthesis conjugated to ASGPR ligand αGN3 (triGalNAc). Synthetic insulin MoDE has similar affinity to mAb49. MW: 7424.236. Sequence: GIVEQ[CC*TSIC]SLYQLENYC**N Alpha-GN-triazole-(pent-4-ynoic acid)-FVNQHLC*GSHLVEALYLVC**GERGAFYTPKT (SEQ ID NO: 43). Phenylalanine-to-alanine (F to A) mutation. 
AGN405	PT0004-**TBT6171**). Native proinsulin made by genetic recombination technology conjugated to αGN3 (triGalNAc) at the C-terminal, conjugation by sortase U2073499G0-8, BAR = 1.
AGN6601	Mutant proinsulin made by genetic recombination technology conjugated to αGN3 (triGalNAc) at the C-terminal, conjugation by sortase U2073499G0-8, BAR = 1.
AGN304	PT0004-**TBT6171**, Native proinsulin construct with C-terminal αGN3 conjugate. Sortase, U2073499G0-8, BAR = 1.
AGN305	PT0004-**TBT6171**. Mutant proinsulin construct with C-terminal αGN3 conjugate. Sortase, U2073499G0-8, BAR = 1.

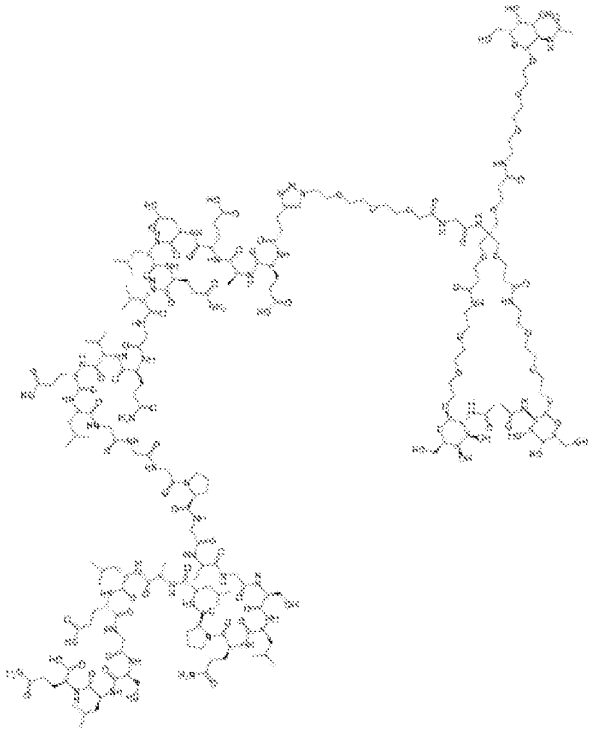
TABLE 5	
AGN406	C-peptide with βGN3 (L-amino acids. C-peptide with protease site, synthetic C-peptide degrader. 

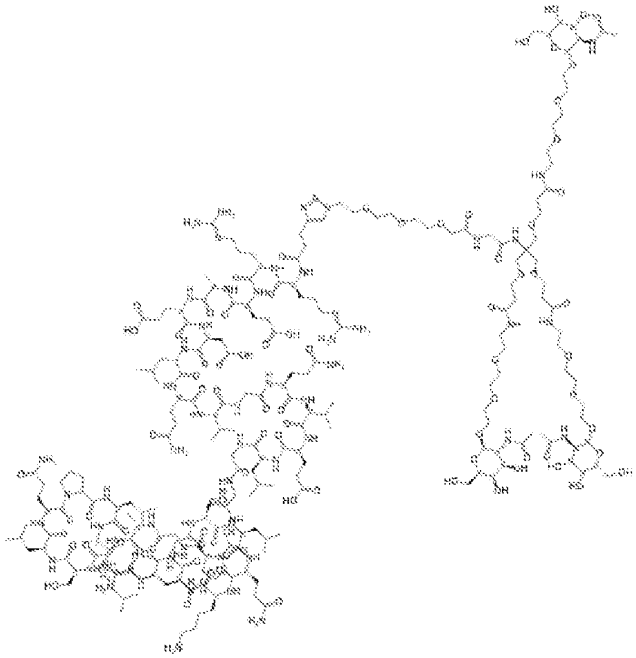
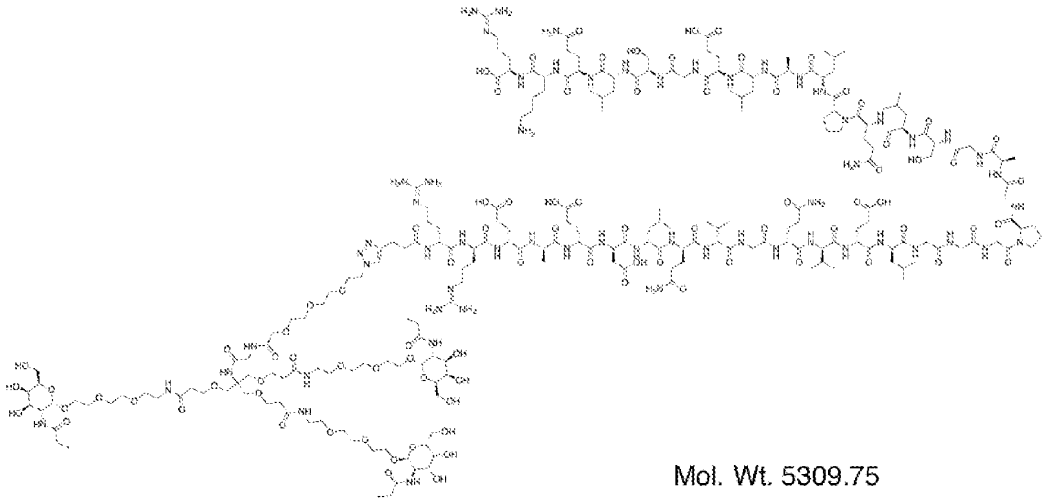
TABLE 5	
AGN407	C-peptide with protease βGN3 (L-amino acids). C-peptide without protease site, synthetic C-peptide degrader. 
AGN408	PT0009-**TBT6170**. Native antibody PT-0009-01 construct with N-terminal αGN3 (**TBT6170**). MW = 13319.04 Da, sortase = U2073499G0-8. BAR = 0.82. IC_{50} = 1.2 nM.
AGN409	PT0009-**TBT6170**. Native proinsulin antibody PT-0009-02, αGN3 (**TBT6170**) conjugate (N-terminal), MW = 13323.34 Da, sortase = U2073499G0-8, BAR = 1.
AGN410	PT0011-**TBT6170**. Native antibody PT-0011-01-αGN3 conjugate (N-terminal), MW = 13242.87 Da, ,MW = 13242.87 Da, sortase = U2073499G0-8, BAR = 1.
AGN411	PT0011-**TBT6170**. Mutant proinsulin construct with N-terminal αGN3 conjugate (N-terminal), MW = 13247.69 Da, sortase = U2073499G0-8, IC_{50} = 3.1 nM.
AGN412	Synthetic mutant insulin with β-GN3. MW = 7424.24 Da.
AGN413	Fc-insulin-βGN3 conjugate
AGN414	Proinsulin-Fc -fusion G4S linker (**ABT307**) (SEQ ID NO: 17) + βGN3 (**TBT4438**). MW = 77525.74 Da.
AGN415	Proinsulin Fc-fusion 5 x G4S linker (**ABT308**) (SEQ ID NO: 18) + βGN3 (**TBT4438**) MW = 80047.84 Da.
AGN416	Synthetic mutant insulin with αGN3 on A chain, MW = 7424.24 Da,

TABLE 5	
AGN417	**ABT410-TBT403**. Native proinsulin TRAP. Native proinsulin-ASGPR ligand βGN3 (N-terminal). Synonym: GN3-Proinsulin conjugate. MW: 11921.84. AGN417 shows a 30% reduction in endogenous insulin autoantibodies in 14-week-old NOD mice. AGN417 shows good subcutaneous bioavailability. The intravenous group exhibited rapid yet strong detection of the degrader, contrasting with the subcutaneous group, where sustained detection was observed between two to eight hours. The subcutaneous group demonstrated 76% bioavailability, indicating effective absorption over time. Both subcutaneous administration (10 mg/kg) and intravenous administration (3 mg/kg) led to a significant reduction in blood glucose levels (BGL) at thirty minutes.
AGN7525	Short linker (G4S) with αGN3.
AGN7526	Long linker (G4S x 3) with αGN3.
AGN418	**ABT410-TBT405**. Short linker (G4S) with βGN3. MW = 11871.59 Da.
AGN419	ABT411-**TBT404**. Proinsulin with C-terminal βGN3. MW = 12509.77 Da.
AGN420	**ABT412-TBT403**. N-terminal βGN3 long spacer in proinsulin. MW = 12267.07 Da,
AGN421	**TBT7714-TBT403**. Mutant proinsulin TRAP. N-terminal βGN3, with double mutant proinsulin (A-chain V3L and B-chain F25A) with click conjugation between triGalNAc and peptide. MW = 11859.59 Da. In vivo tolerability and blood glucose levels assayed in in NOD mice, aged 0.23 years (12 weeks). No toxic signs observed.
AGN422	**TBT7714-TBT405**. Mutant proinsulin TRAP. N-terminal βGN3-non-click double mutant proinsulin (A-chain V3L and B-chain F25A). Amide between triGalNAc and peptide. MW = 11809.86 Da
AGN7849	N-terminal long spacer in proinsulin βGN3 click.
AGN7850	N-terminal long spacer in proinsulin βGN3.
AGN7865	N-terminal spacer in proinsulin βGN3.
AGN423	Insulin-Fc (efsitora alfa)-**TBT4438** (βGN3-MATE) conjugation
AGN8123	Has the structure of AGN421 with a terminal G→A mutation Refold yields may not reach the same level as other insulin analogues due to the B24 F→A mutation (SEQ ID NO: 38)
AGN2640	β (GalNAc ASGPR binder)3
AGN4002	α(GalNAc ASGPR binder)3
AGN4003	α(propionic amide-GalNAc ASGPR binder)3
AGN4004	β (propionic amide-GalNAc ASGPR binder)3
AGN4006	α(propionic amide-GalNAc ASGPR binder)3
AGN4007	β (propionic amide-GalNAc ASGPR binder)3
AGN4586	
AGN3764	
AGN6567	
AGN6567	AGN417 with 13 and N15 labels
AGN8140	**ABT410-TBT7990**
AGN8141	**ABT410-TBT7991**

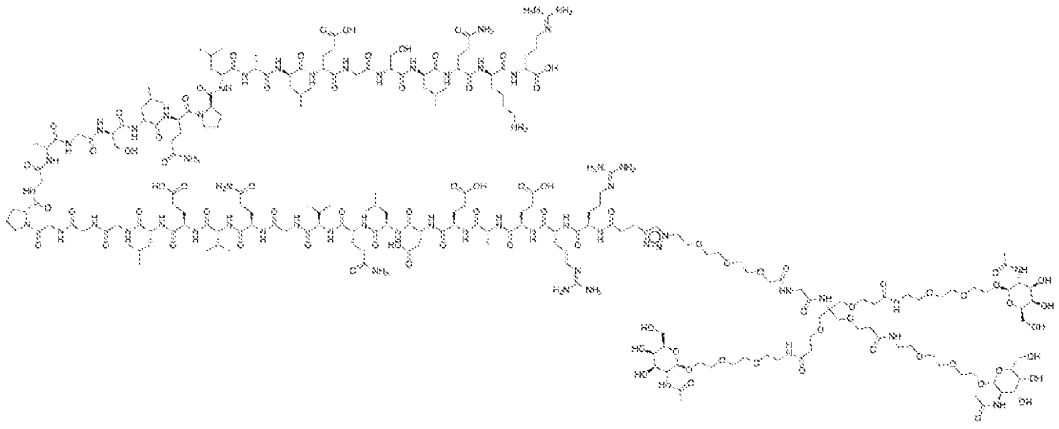
TABLE 5	
AGN8138	**ABT410-TBT7988**
AGN8139	**ABT410-TBT7989**

[00131] **AGN401**, αGN3, synthetic C-peptide constructs (D-amino acids), no binding to either of the C-peptide antibodies.



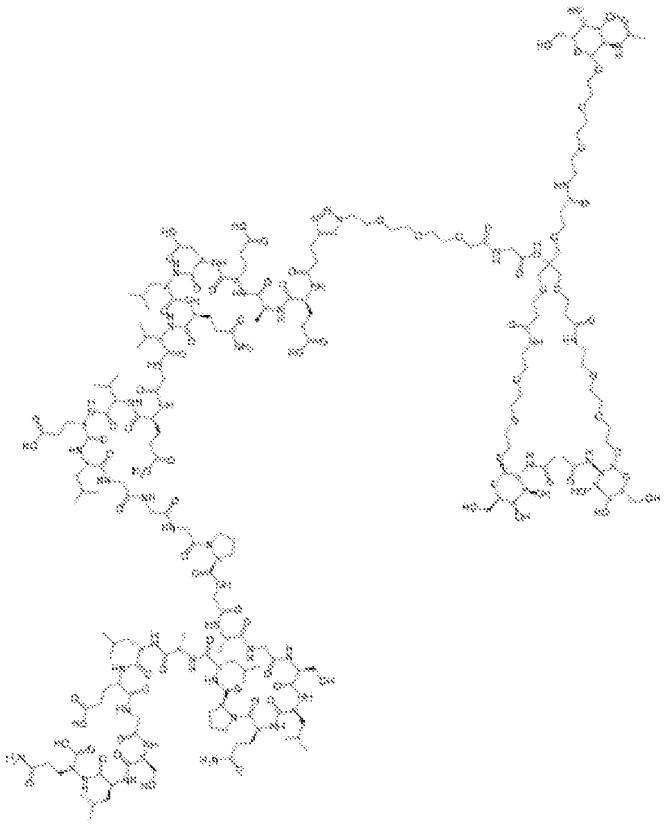
Mol. Wt. 5309.75

[00132] **AGN404**. β-GN3, synthetic C-peptide constructs (D-amino acids), no binding to either of the C-peptide antibodies.



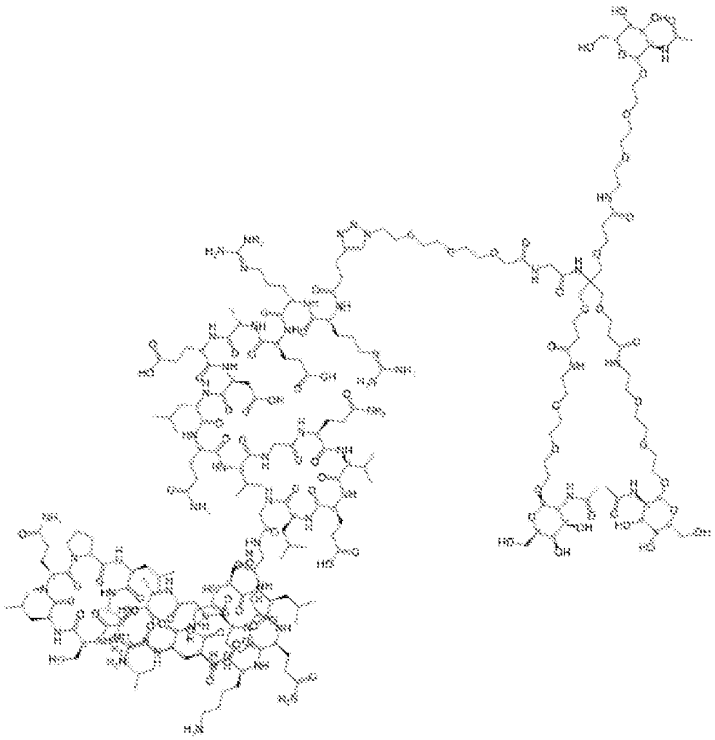
Mol. Wt. 5309.75

[00133] AGN406. β-GN3, synthetic C-peptide constructs (L-amino acids).



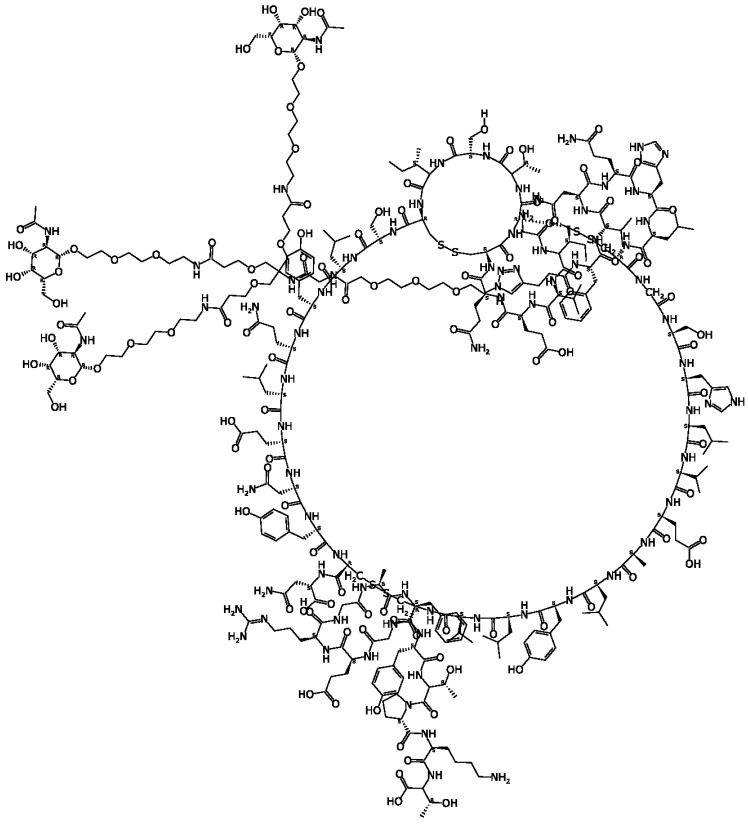
[00134] Mouse anti-C-peptide monoclonal antibody. K_D = 9.7 nM. Recombinant mouse anti-C-peptide. K_D = 15 µM. AGN406 C-peptide internalization IC_{50} = 4.2 nM.

[00135] AGN407. β-GN3 (protease), synthetic C-peptide constructs (L-amino acids).

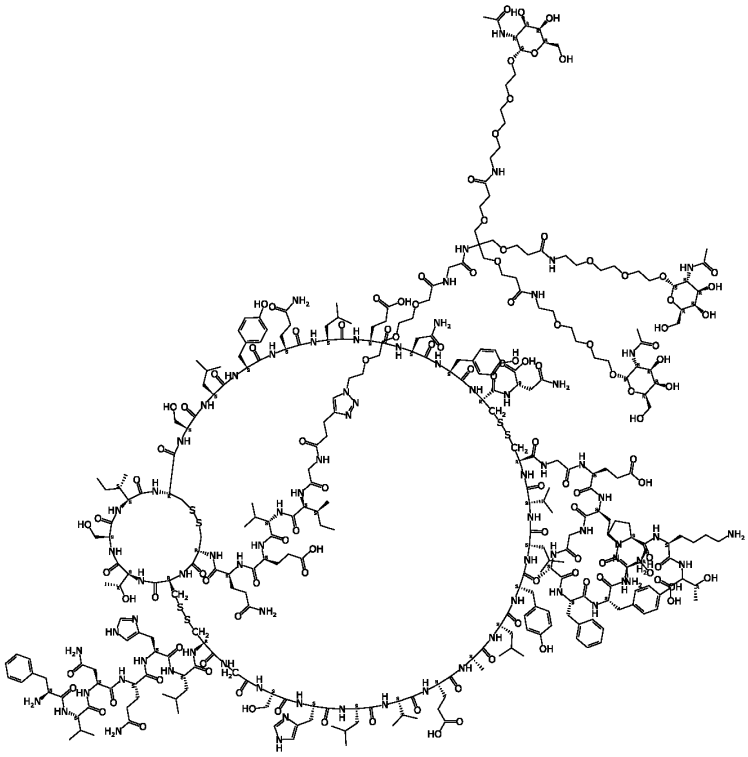


[00136] Mouse anti-C-peptide monoclonal antibody. K_D = 7.5 nM. Recombinant mouse anti-C-peptide. K_D = 7.9 µM. AGN407 C-peptide internalization IC_{50} = 7.9 nM.

[00137] **AGN412.** Sequence: GIVEQ[CC*TSIC]SLYQLENYC**N β-GN-triazole-(pent-4-ynoic acid)-FVNQHLC*GSHLVEALYLVC**GERGAFYTPKT (SEQ ID NO: 43).



[00138] **AGN416.** Sequence: αGN-triazole-(pent-4-ynoic acid)-GIVEQ[CC*TSIC]SLYQLENYC**FVNQHLC*GSHLVEALYLVC**GERGAFYTPKT (SEQ ID NO: 43).



Target-binding moiety.

[00139] In some embodiments, the target 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.

[00140] In some embodiments, each binding moiety in an agent may be of the same binding moiety or a pharmaceutically acceptable salt thereof.

[00141] In some embodiments, the 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.

[00142] Several antibody binding moieties, including universal antibody binding moieties, can be used in accordance with the teachings of this specification. Certain antibody binding moieties and technologies for identifying or assessing antibody binding moieties are described in International Patent Publications WO 2019/023501 (Kleo Pharmaceuticals, Inc.) and WO 2019/136442 (Kleo Pharmaceuticals, Inc.), each of which is incorporated in this specification in its entirety by reference. Persons having ordinary skill in the biomedical art know that additional technologies in the biomedical art may be suitable for identifying or assessing antibody binding moieties in accordance with this specification. In some embodiments, an antibody binding moiety comprises one or more amino acid residues, each independently natural or unnatural.

[00143] In some embodiments, a binding moiety, e.g., a protein binding moiety, e.g., an antibody binding moiety, e.g., a universal antibody binding moiety, has the structure of **ABT101** or a salt form thereof, wherein:

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

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

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

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

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

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

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

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

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

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

L^1 is a trivalent linker moiety; and

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

[00144] In some embodiments, L^1 is an optionally substituted trivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R')–$, –C(O)S–, or –C(O)O–. In some embodiments L^1 is $–(CH_2CH_2O)_{2-4}–$ or $–(CH_2CH_2O)_2–$.

[00145] Several antibody binding moieties, including universal antibody binding moieties, can be used in accordance with the teachings of this specification. Some antibody binding moieties and technologies for identifying or assessing antibody binding moieties are described in International Patent Publications WO 2019/023501 (Kleo Pharmaceuticals, Inc.) and WO 2019/136442 (Kleo Pharmaceuticals, Inc.), each of which is incorporated in this specification in its entirety by reference. Persons having ordinary skill in the biomedical art know that additional technologies in the biomedical art may be suitable for identifying or assessing antibody binding moieties in accordance with this specification. In

some embodiments, an antibody binding moiety comprises one or more amino acid residues, each independently natural or unnatural.

[00146] 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 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.

[00147] In some embodiments, antibody heavy chains are selectively conjugated/labeled over light chains.

[00148] In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target binding moieties, conjugated to antibody moieties of a particular type of antibodies, e.g., IgG1, or fragments thereof are conjugated to one or more particularly sites, typically one or two particularly sites, e.g., K246 and K248 of an IgG1 heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target binding moieties, conjugated to antibody moieties of IgG2 antibodies or fragments thereof are at K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., target binding moieties, conjugated to antibody moieties of IgG2 antibodies or fragments thereof are at K239 and K241 of a heavy chain and amino acid residues corresponding thereto. In some embodiments, about 10%-100% of all, or substantially all, moieties of interest, e.g., for a plurality of agents, for a composition, etc. are conjugated to antibody moieties of IgG1, IgG2, 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%.

[00149] Further guidance is provided in International Patent Publication WO 2025/035052 (Biohaven Therapeutics Ltd.), the contents of which are incorporated by reference.

TABLE 6

ABT401	LALA mutation (in Fc domain) does not prevent recognition by **AGN301** (IgG degrader)
ABT309	LALA mutation (in Fc domain) does not prevent recognition by **AGN301** (IgG degrader)
ABT311	Homo sapiens YNITDHGSLIYSFSECAF 1..18
ABT312	Mutant proinsulin N-terminal sortase tag (SEQ ID NO: 6). F25A modification. MW = 11051. Homo sapiens GSLIYSFSECAFTGPLRP 1..18.
ABT313	Mutant proinsulin N-terminal sortase tag. MW = 11141. Homo sapiens FSECAFTGPLRPFFSPGF 1..18.
ABT314	Native proinsulin with C-terminal His tag. (SEQ ID NO: 7). MW = 10842.
ABT315	Native proinsulin with C-terminal sortase tag. (SEQ ID NO: 8).

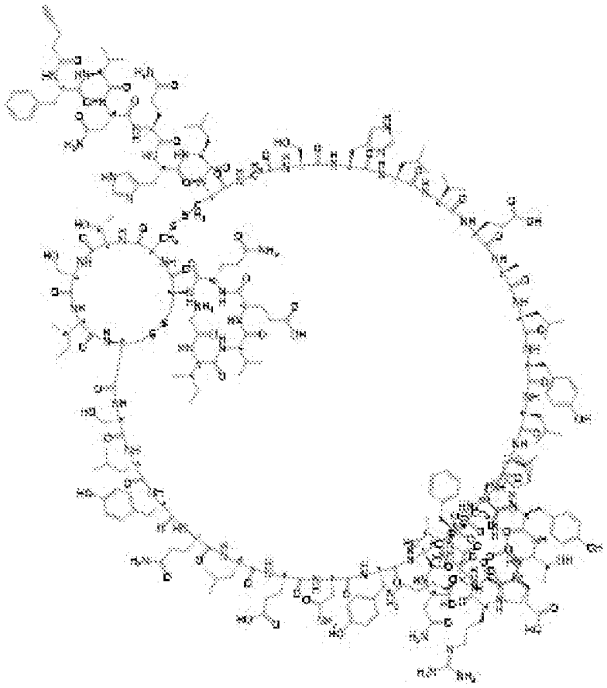
TABLE 6	
ABT306	Alk-native insulin 
ABT307	Proinsulin-Fc-fusion G4S linker, 71006.170 Da, proinsulin-IgG1 Fc fusion, G4S linker, hIgG1 hinge (C > A), LALA-PA (SEQ ID NO: 48).
ABT308	Proinsulin-Fc-fusion 5 x G4S linker, 73528.457 Da, proinsulin-IgG1 Fc fusion, 5x G4S linker, hIgG1 hinge (C > A), LALA-PA (SEQ ID NO: 49).
ABT402	Monoclonal antibody.
ABT403	mAb49 (original), Creative Biolabs, FAMAB-0225WJ, with mouse Fab-human IgG3 Fc
ABT404	mAb49 chimeric heavy chain gamma 3, mouse VH/VL (SEQ ID NO: 34) [495 AA], with human IgG1 CH/CL with mAb49 chimeric light chain kappa (SEQ ID NO: 33) [215 AA].
ABT405	Mouse mAb49 VH/VL-human IgG1 CH/CL. Fc regions include ”LALA/PA” mutations to effect immune effector function (FcgR-, c1q-binding).
ABT406	Mouse anti-C-peptide antibody (original)
ABT407	Anti-C-peptide antibody with mouse VH/VL-human IgG1 CH/CL
ABT408	Anti-C-peptide antibody, mouse VH/VL-human IgG1 CH/CL. Fc regions include ”LALA/PA” mutations to effect immune effector function (FcgR-, c1q-binding).

TABLE 6	
ABT409	Anti-C-peptide antibody, mouse VH/VL-human IgG3 CH/CL monoclonal antibody.
ABT410	Native proinsulin-N-terminal sortase linker (clean/native) (SEQ ID NO: 44).
ABT411	Proinsulin-C-terminal sortase linker (clean/native).
ABT412	Proinsulin molecule optimization to improve conjugation efficiency
ABT413	Human insulin.
ABT414	Insulin efsitora alfa (insulin-Fc). Synonyms: LY3209590 (SEQ ID NO: 16). MedChemExpress, catalogue # HY-P99665. See Moyers et al., J. Pharmacol. Exp. Ther., 382, 346-355 (2022) (Eli Lilly).
ABT415	Insulin lispro.
ABT416	Mutant proinsulin N-terminal sortase tag (SEQ ID NO: 11)
ABT417	Mutant proinsulin with N-terminal sortase tag (SEQ ID NO: 22). Mutant B-chain insulin F25A.
ABT311	Native proinsulin N-terminal sortase tag (SEQ ID NO: 10). MW = 11127.
ABT418	Proinsulin Fc fusion protein – Proinsulin + hIgG1 Fc-LALA/PA**,** Mouse Ig Kappa signal, Proinsulin, G4S linker, hIgG1 hinge(**C>A**)-Fc-LALA/PA. (SEQ ID NO: 30)
ABT419	Proinsulin protein (SEQ ID NO: 4).
ABT420	Proinsulin protein (SEQ ID NO: 5). Creative Biomart, Shirley, NY, USA, Cat. NO: Proinsulin-01H. R&D Systems, Minneapolis, MN, USA,
ABT421	PT0003, native proinsulin C-terminal His Tag (SEQ ID NO: 7). (GenScript U608R952G0-15).
ABT422	PT0004, native proinsulin C-terminal sortase tag (SEQ ID NO: 8). (GenScript U608R952G0-21).
ABT423	PT0009, Native proinsulin N-terminal His Tag (SEQ ID NO: 9) (GenScript)
ABT424	PT0011, Native proinsulin N-terminal sortase Tag (SEQ ID NO: 10) (GenScript)
ABT6396	SEQ ID NO: 49
ABT7714	Mutant proinsulin (A-chain V3L and B-chain F24A)
ABT17384	Mutant proinsulin + lysine mutation
ABT17385	Mutant proinsulin + methionine
ABT17386	Mutant proinsulin + cysteine

Linker Moieties

[00150] 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., L^{G}, 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.

[00151] 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.

[00152] Linker moieties can be bivalent or polyvalent depending on how they are used. In some embodiments, a linker moiety is bivalent. In some embodiments, a linker is polyvalent and connecting more than two moieties.

[00153] In some embodiments, a linker moiety, e.g., L^z, e.g., L^{PM}, L^{RM}, L^{LG}, L^{LG1}, etc. , is or comprises L.

[00154] In some embodiments, the linker moiety has the structure of formula **LNK101**:

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

[LNK101]

or a pharmaceutically acceptable salt thereof, wherein:

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

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

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

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

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

each R is independently –H, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur,

phosphorus and silicon, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30 membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30 membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

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

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

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

[00155] 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.

[00156] Further guidance is provided in International Patent Publication WO 2025/035052 (Biohaven Therapeutics Ltd.), the contents of which are incorporated by reference.

MATES

[00157] 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.

[00158] Such an agent may be called a MATE agent or MATE. The MATE agents are described, for example, in International Patent Publication WO 2021/102052 (Kleo Pharmaceuticals, Inc.), the contents of which are incorporated 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.

Cellular receptor-binding moiety.

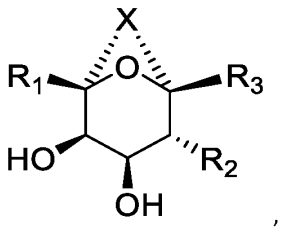
[00159] Several receptor-binding moieties, according to embodiments of present invention, are described in WO 2019/19962 (Bostal Drug Delivery Co., Ltd), WO 2019/199634 (Yale University), WO

2021/072246 (Yale University), and WO 2021/072246 (Yale University), each of which is incorporated in this specification in its entirety by reference.

[00160] 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.

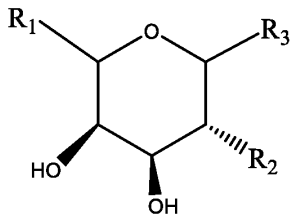
[00161] The amine group may be a primary alkyl amine group or secondary alkyl amine group, each of which is optionally substituted on the amine group with a C_1-C_3 alkyl group.

[00162] The cellular receptor-binding moiety may include an ASGPR binding group according to the chemical structure:



,

[TBT101] or



,

[TBT102]

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

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

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

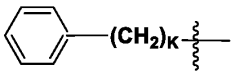
when X is 3 or 4 atoms in length, no more than 2 atoms of X are O, S or $N(R^{N1})$;

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

R_1 and R_3 are each independently:

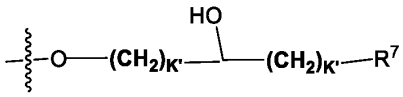
H, $-(CH_2)_KOH$, $-(CH_2)_KOC_1$-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, $-(CH_2)_K$-vinyl, O-$(CH_2)_K$-vinyl, $-(CH_2)_K$-alkynyl, $-(CH_2)_K$-COOH,

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

R_1 and R_3 are each independently a  group, which is optionally substituted with up to three halo groups, C_1-C_4 alkyl groups, each of which alkyl group is optionally substituted with from one to three halo groups or one or two hydroxyl groups, or O-C_1-C_4 alkyl groups, each of which alkyl groups is optionally substituted with from one to three halo groups or one or two hydroxyl groups; and

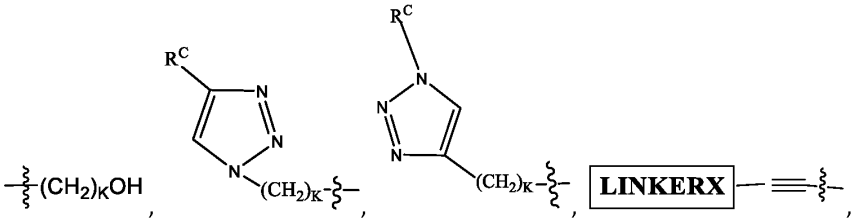
K is independently an integer of 0 to 4, or

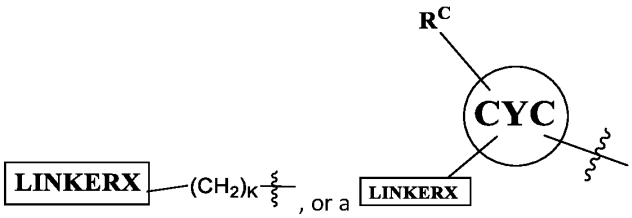
R_1 and R_3 are each independently a group according to the chemical structure:

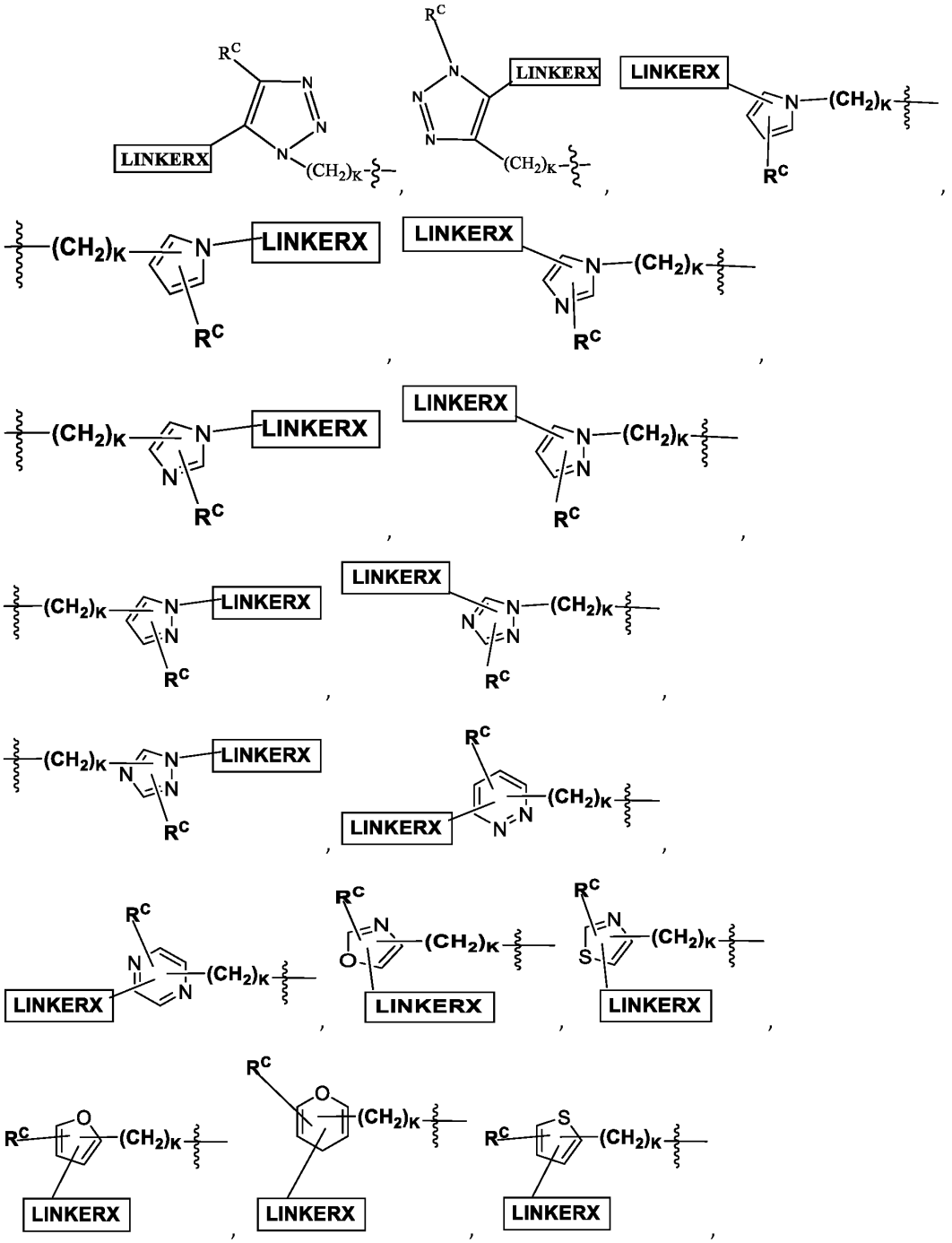
 , wherein R^7 is O-C_1-C_4 alkyl, which is optionally substituted with from 1 to 3 halo groups 1 or 2 hydroxy groups, or R^7 is a

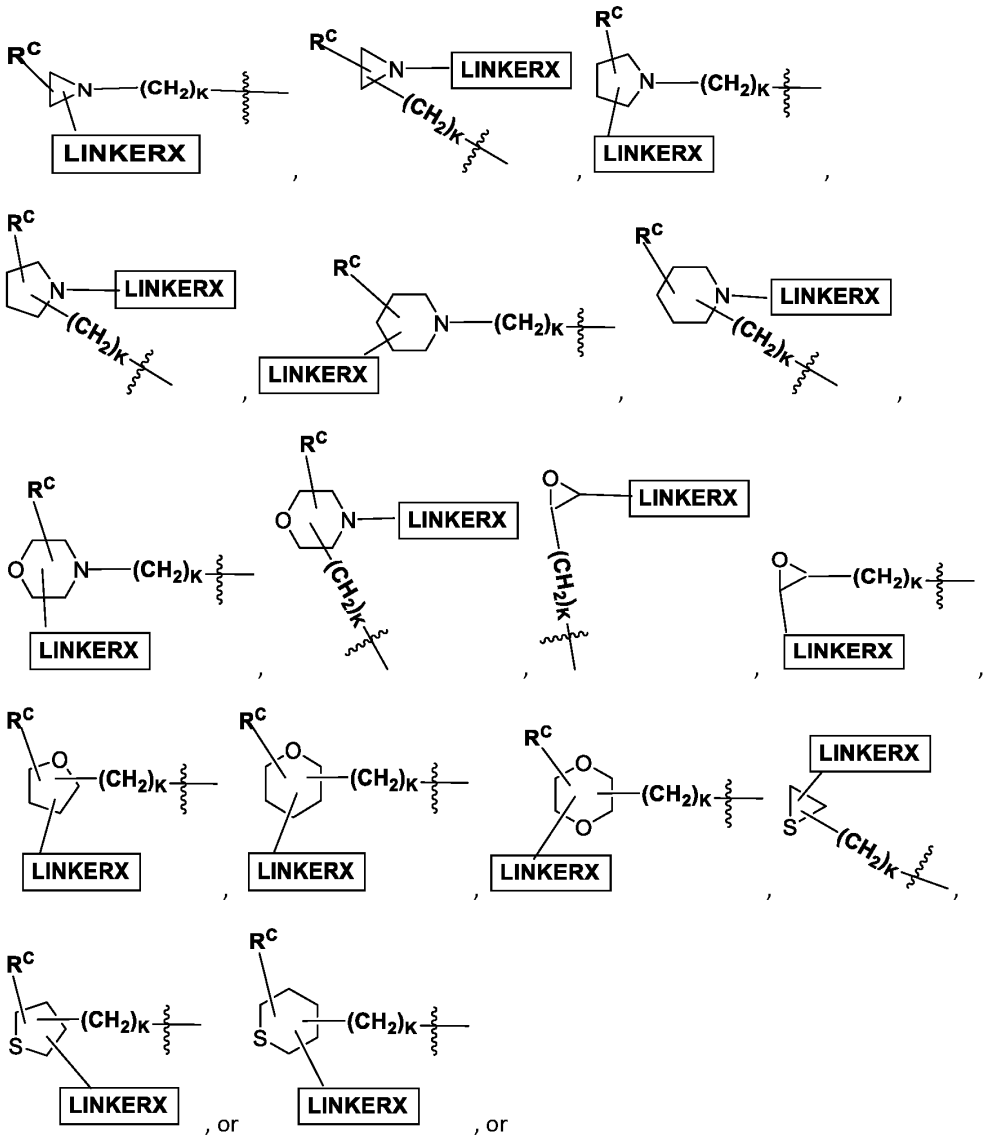
-$NR^{N3}R^{N4}$ group or a; or

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

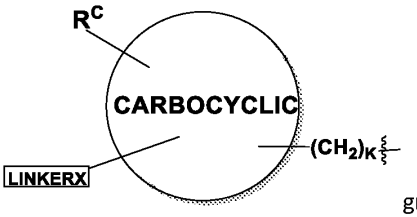
 , , , ,

 , or a group according to the chemical structure:





, or , or

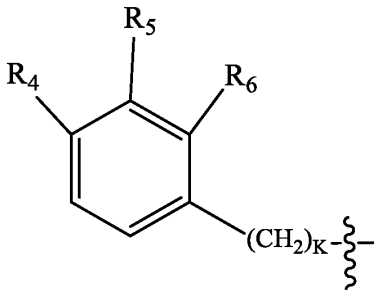
R_1 and R_3 are each independently a 

group, where

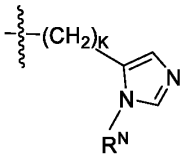


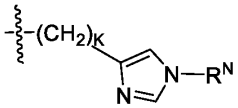
, is a C_3-C_8 saturated carbocyclic group;

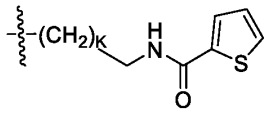
R^C is absent, H, C_1-C_4 alkyl optionally substituted with from 1-3 halo groups or 1-2 hydroxyl groups, or a group according to the structure:

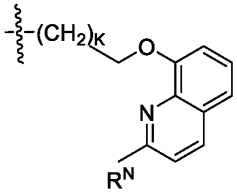


wherein R_4, R_5 and R_6 are each independently, H, halo (F, Cl, Br, I), CN, $NR^{N1}R^{N2}$, -$(CH_2)_K$OH, -$(CH_2)_K$OC_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, C_1-C_3 alkyl, which is optionally substituted with from 1-3 halo groups, -O-C_1-C_3-alkyl, which is optionally substituted with from 1-3 halo groups, -$(CH_2)_K$COOH, -$(CH_2)_K$C(O)O-C_1-C_4 alkyl optionally substituted with from 1-3 halo groups, O-C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, -C(O)-C_1-C_4 alkyl, which is optionally substituted with from 1-3 halo groups, or

R^C is a 

group, a 

group a 

group or a 

group,

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

K is independently an integer of 0 to 4;

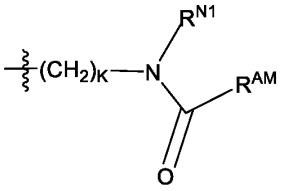
K' is an integer of 1 to 4;

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

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

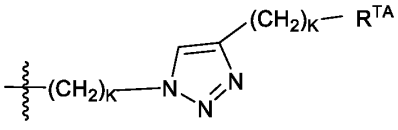
LINKERX is a linker group which comprises at least one binding moiety and links the at least one binding moiety to the cellular receptor-binding moiety through the optional linker moiety, or

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

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

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

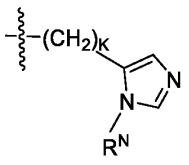
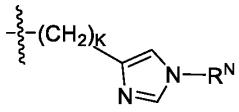
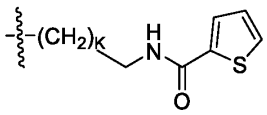
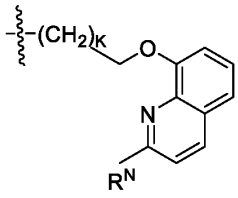
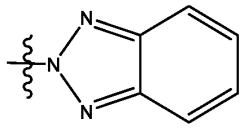
R^{N4} is H, a C_1-C_3 alkyl group optionally substituted with 1-3 halo groups or 1 or 2 hydroxy groups, or a group, or

R_2 is a  group,

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

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

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

R^{TA} is a  group, a  group, a  group a  group, a  group optionally substituted with up to three C_1-C_3 alkyl groups optionally substituted with up to three halo groups, or

R^{TA} is a group,

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

and K is independently 0-4.

[00163] Further guidance is provided in International Patent Publication WO 2025/035052 (Biohaven Therapeutics Ltd.), the contents of which are incorporated by reference.

TABLE 7

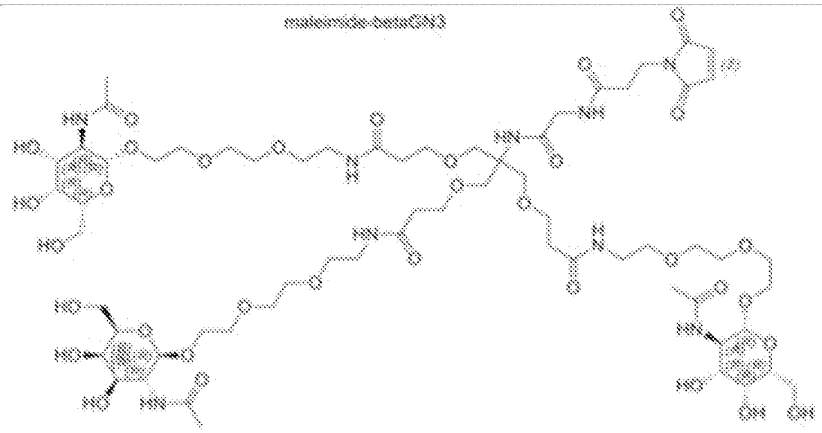
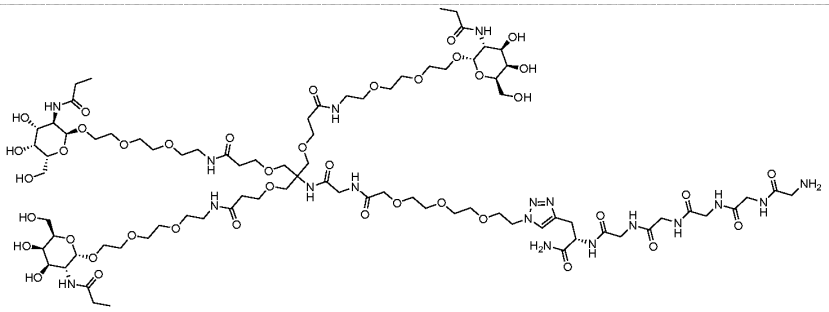
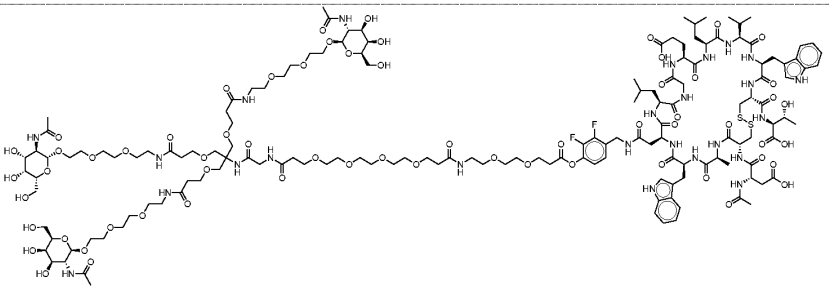
TBT103. Cellular binding moiety maleimide βGN3 for engineered cysteine-based conjugation.	
TBT104. αGN3 moiety with polyglycine for sortase-based conjugation.	
TBT402	

TABLE 7

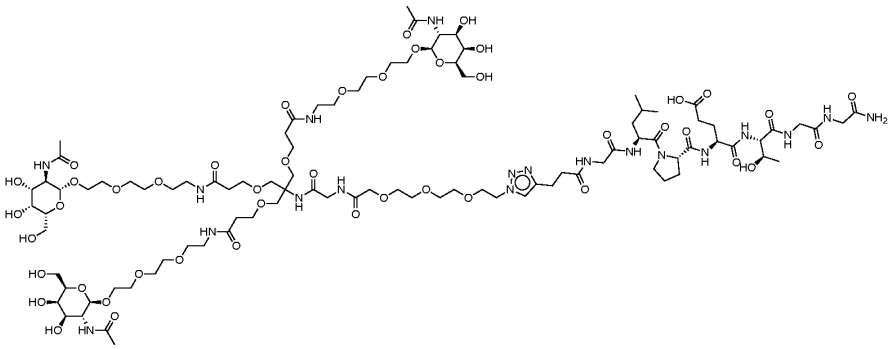
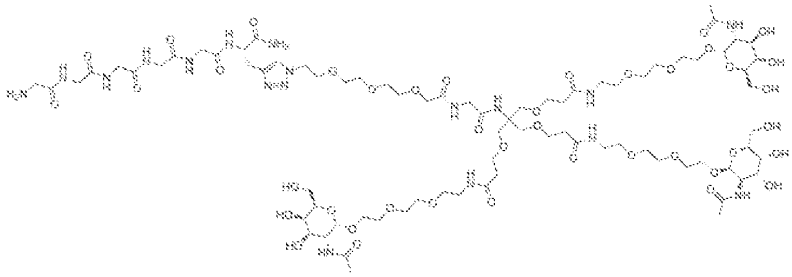
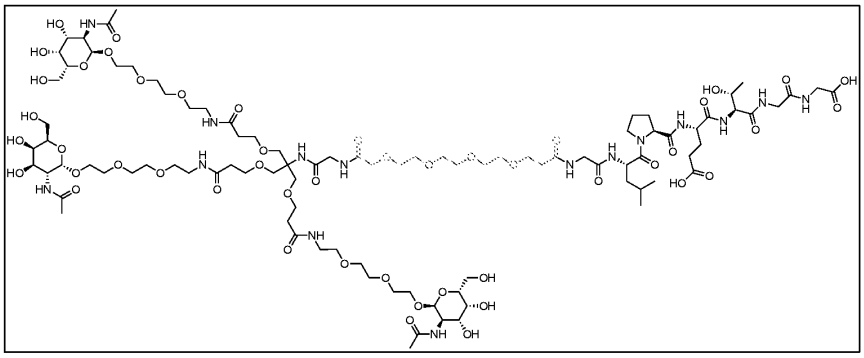
TBT403	βGN3-GLPETGG-NH2. 
TBT404	GGGGG-Pra(β-GN3)-NH2. 
TBT405	Ins-030) PEG3 linkers **TBT7714** to get **AGN422**, glycine to alanine. 

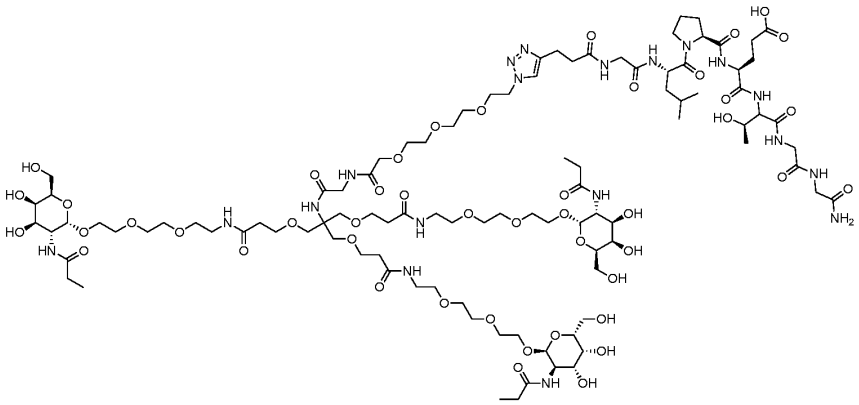
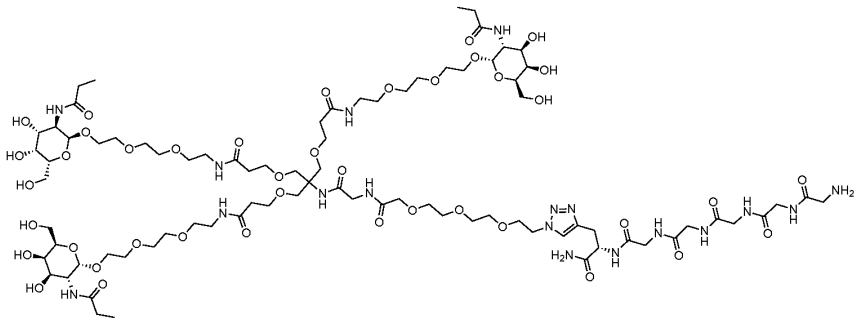
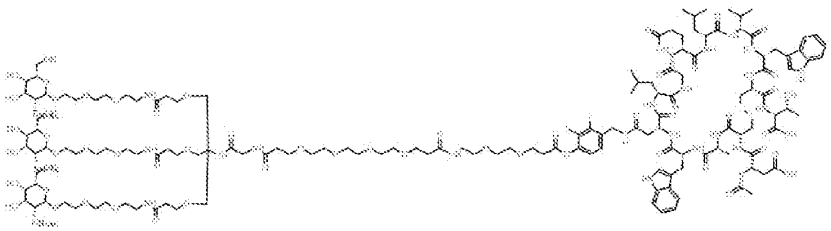
TABLE 7	
TBT6170	N-terminal modification of proinsulin. 
TBT6171	C-terminal modification of proinsulin. 
TBT4438	
TBT501	C-terminal sortase tag with His6 tag. GGGGSGGGGSLPETGGHHHHHH (SEQ ID NO: 20).
TBT502	Rigid C-term linker for the unpaired cysteine for conjugation. HHHHHHSPSTPPTPSPSTPPC (SEQ ID NO: 21).

TABLE 7

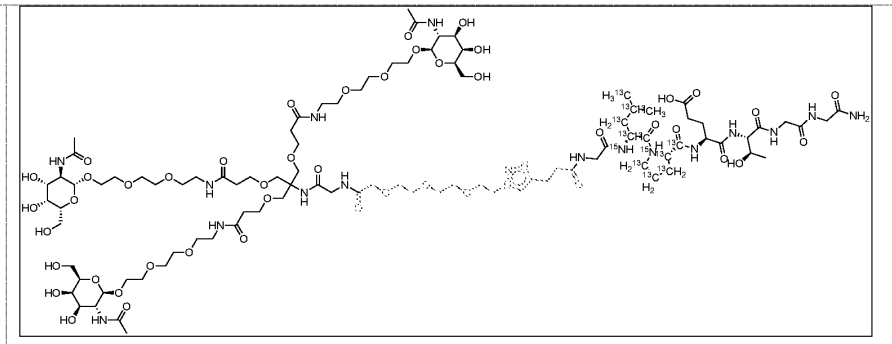
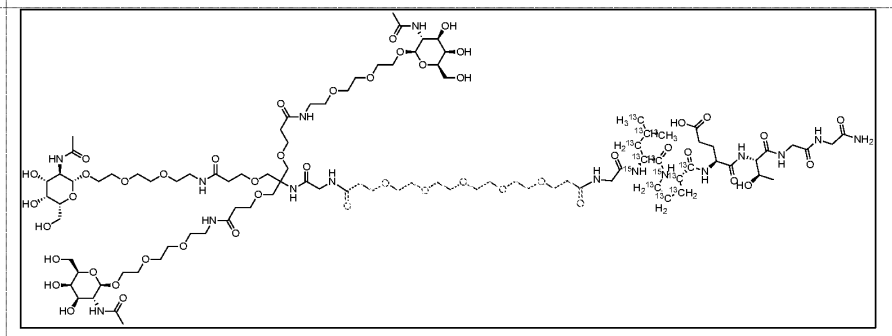
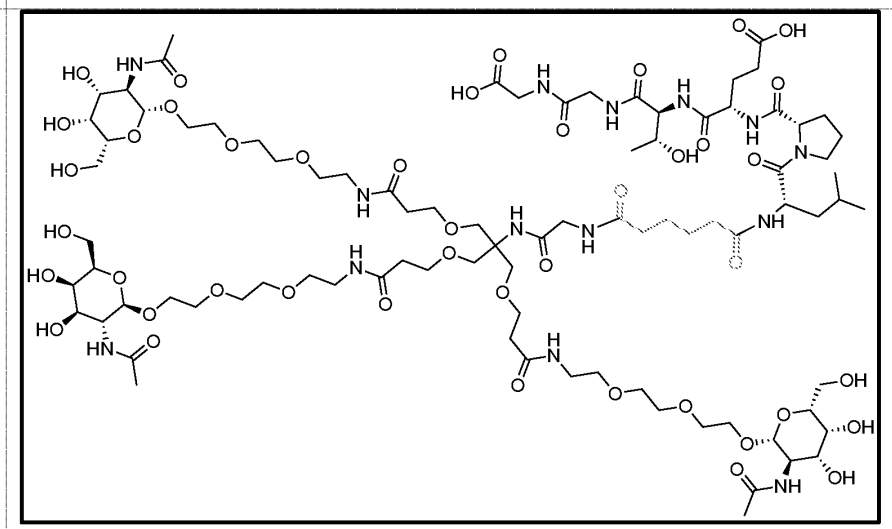
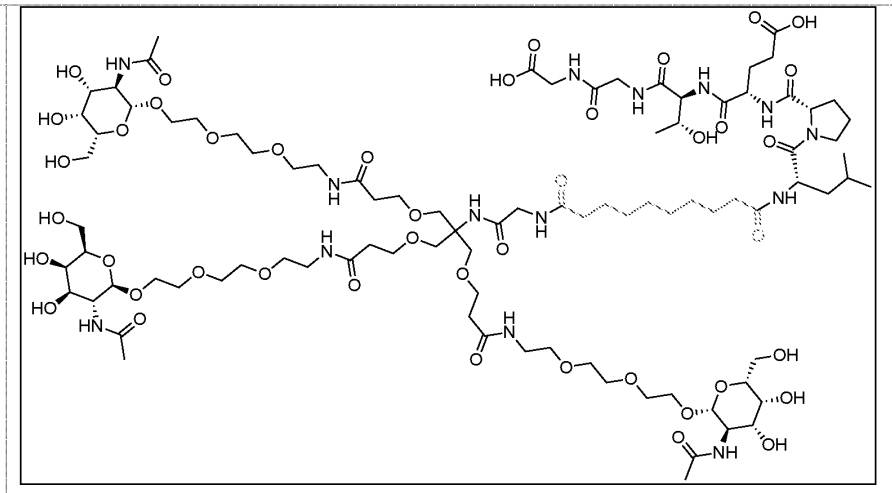
TBT7990	
TBT7991	
TBT7988	

TABLE 7

TBT7989	

Second agent.

[00164] 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.

[00165] 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.

[00166] 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., –NH2 of

lysine, –SH of cysteine, etc. In some embodiments, a second reactive group is –NH2 of a lysine residue, e.g., of a residue selected from K246 and K248 of IgG1 heavy chain amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of a heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00167] In some embodiments, a second reactive group is installed to an antibody moiety optionally through a linker. In some embodiments, a second reactive group is installed to an antibody moiety through a linker. In some embodiments, a second reactive group is selectively linked to certain location(s) of an antibody moiety, e.g., certain location(s) selected from K246 and K248 of IgG1 heavy chain amino acid residues corresponding thereto, K251 and K253 of an IgG2 heavy chain and amino acid residues corresponding thereto, and K239 and K241 of a heavy chain and amino acid residues corresponding thereto. In some embodiments, the invention provides selective reactions at amino acid residues of antibody moieties.

[00168] 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 –NH2 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 **AGN110** or a salt thereof:

LG–RG–LRM–TBT,

[AGN110] 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.

[00169] In some embodiments, L^G is or comprises an antibody binding moiety as described in this specification, and a linker which links an antibody binding moiety and RG.

[00170] In some embodiments, L^G is or comprises R^{LG}–L^{LG}–, wherein R^{LG} is or comprises an antibody binding moiety, and L^{LG} is a linker moiety as described in this specification. In some embodiments, L^G is ABT-L^{LG}–. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–, wherein each of L^{LG1} and L^{LG2} is independently a linker moiety as described in this specification. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–, wherein each of L^{LG1}, L^{LG2} and L^{LG3} is independently as linker moiety described in this specification. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–L^{LG4}–, wherein each of L^{LG1}, L^{LG2}, L^{LG3} and L^{LG4} is independently a linker moiety as described in this specification. In some embodiments, L^{LG1} is bonded to R^{LG}. In some embodiments, L^{LG1} is bonded to cellular receptor-binding moiety. In some embodiments, L^{LG} is –L^{LG1}–, and a reactive group comprises L^{LG2}, L^{LG3} and L^{LG4}. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–, and a reactive group comprises L^{LG3} and L^{LG4}. In some embodiments, L^{LG} is –L^{LG1}–L^{LG2}–L^{LG3}–, and a reactive group comprises L^{LG4}. In some embodiments, each of L^{LG1}, L^{LG2}, L^{LG3} and L^{LG4} is independently L.

[00171] 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–.

[00172] 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.

[00173] 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−.

[00174] In some embodiments, target binding moieties may be conjugated to antibody moieties optionally through linker moieties using technologies described in published U.S. Patent Publication 2020/0190165.

[00175] 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 Greene's Protecting Groups in Organic Synthesis (John Wiley & Sons, 2014), the entirety of which is incorporated in this specification by reference.

[00176] 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.

[00177] 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's Protecting Groups in Organic Synthesis (John Wiley & Sons, 2014), the entirety of which is incorporated in this specification by reference. Examples of suitable hydroxyl protecting groups include, but are not limited to, esters, allyl ethers, ethers, silyl ethers, alkyl ethers, arylalkyl ethers, and alkoxyalkyl ethers. Examples of such esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate (trimethylacetyl), crotonate, 4-methoxy-crotonate, benzoate, p-benylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl. Examples of such silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, β-(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-picolyl and 4-picolyl.

[00178] Amino protecting groups are well known in the biomedical art and include those described in Greene's Protecting Groups in Organic Synthesis (John Wiley & Sons, 2014), 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), benzyloxocarbonyl (CBZ), allyl, phthalimide, benzyl (Bn), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzoyl, and the like.

[00179] Persons having ordinary skill in the biomedical art know that provided agents may have one or more stereocenters and may be present as a racemic or diastereomeric mixture. Persons having ordinary skill in the biomedical art know there are many methods known in the biomedical art for the separation of isomers to obtain stereoenriched or stereopure isomers of those compounds, including but not limited to HPLC, chiral HPLC, fractional crystallization of diastereomeric salts, kinetic enzymatic resolution, e.g. by fungal-derived, bacterial-derived, or animal-derived lipases or esterases), and formation of covalent diastereomeric derivatives using an enantioenriched reagent.

[00180] 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.

[00181] As known by persons having ordinary skill in the biomedical art, reaction partners are generally contacted with each other under conditions and for a time sufficient for production of the desired results, e.g., formation of product agents and compositions thereof to desired extents. Many reaction conditions/reaction times may be assessed and used if they are suitable for desired purposes in accordance with this specification; certain such conditions, reaction times, assessment, etc. are described in the Examples.

[00182] 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.

[00183] 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 **AGN110** 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.

[00184] 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.

[00185] 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.

Pharmaceutically acceptable excipients.

[00186] 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 contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered by intravenous infusion, orally, topically, intraperitoneally, intravesically, or intrathecally. Parenteral administration, oral administration, and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[00187] 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.

Plurality of agents.

[00188] 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.

[00189] 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.

[00190] In some embodiments, the invention provides a composition comprising a plurality of agents each of which independently comprising:

an antibody moiety,

a cellular receptor-binding moiety, and

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

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

[00191] In some embodiments, the invention provides a composition comprising a plurality of agents each of which independently comprising:

an antibody moiety,

a cellular receptor-binding moiety, and

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

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

[00192] Further guidance is provided in International Patent Publication WO 2025/035052 (Biohaven Therapeutics Ltd.), the contents of which are incorporated by reference.

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

[00193] *HDX-MS* measures the exposure of hydrogen molecules on the surface of a protein or protein complex. The first step causes exposed hydrogen molecules to be readily exchanged with deuterium. Hydrogen molecules buried within the structure, for example at the binding site of an antibody-antigen complex, are not exchanged as readily.

[00194] Comparing the deuterium uptake between the free and the complex states of proteins via mass spectrometry provides valuable information about the binding regions.

[00195] HDX-MS is continuing to be performed. The inventors are modeling the antibody Fc-and bifunctional degrader interaction to predict paratope regions in parallel with HDX-MS.

[00196] *Protein sequencing*. In one method of protein sequencing, eight digestions are prepared using five enzymes (pepsin, Lys C, trypsin, chymotrypsin, Asp N). The digestions for the sample are processed with disulfide reduction, cysteine blocking, and then enzyme digestion. Digestions were analyzed by LC-MS/MS using a Thermo-Fisher Orbitrap fusion™ mass spectrometer. Peptides are characterized from LC -MS/MS data using de nova peptide sequencing and then assembled into antibody sequences.

Assays used to determine proof of concept.

[00197] The inventors used these assays to determine proof-of-concept for the anti-insulin/proinsulin autoantibody bifunctional agents of the invention.

[00198] *Biophysical assay. Surface plasmon resonance assay (insulin receptor)*. The inventors produced mutant insulin conjugated with GN3 and is being tested for binding to the insulin receptor by surface plasmon resonance assay. Additional mutant insulins conjugated with GN3 are produced synthetically.

[00199] Insulin receptors are also produced synthetically. qPCR method primer sequence IR-forward: GCAACATCACCCACTACCTGGT (SEQ ID NO: 39). qPCR method primer sequence IR-reverse: GAATGGTGGAGACCAGGTCCTC (SEQ ID NO: 40).

[00200] *Cellular Assay. iLite Insulin reporter assay (DT-40)*. Assay principle: Overexpression of insulin receptor and transfection of a non-canonical STAT5 recognition motif and a STAT5 expression vector. Thus, insulin signaling via the insulin receptor results in STAT5 phosphorylation, dimerization, and transcriptional activation of a promoter leading to luciferase production.

[00201] *Insulin receptor-binding to human insulin via surface plasmon resonance*. Biophysical assessment of insulin/IGF1 binding to their respective receptors was demonstrated. Weak binding of proinsulin constructs to insulin receptor

[00202] *Nonobese diabetic (NOD) mouse model*. The NOD mouse model is described by Jorns et al., Diabetologia, Volume 57, pages 512–521 (December 6, 2014). NOD mice are one of the best-studied mouse models for spontaneous type 1 diabetes. The immunogenic progression of diabetes in NOD mice is similar to that in humans. The disease progression is driven by immune cells (macrophages, dendritic cells, NK cells, B cells, T cells). Insulitis is established at twelve to fourteen weeks. T cells are the

predominant cell infiltrating the islets, causing B cell destruction, and leading to overt diabetes/hyperglycemia.

[00203] In humans, autoantibodies against several β-cell proteins are identified in more than 90% in newly diagnosed type 1 diabetes. The prevalence of most islet autoantibodies is associated with age at diagnosis.

[00204] *Humanized immunodeficient NOD mouse mode of type 1 diabetes*. Several immunodeficient models were described in the scientific literature of the biomedical art. SCID mice reconstituted with PBMC from type 1 diabetic patients can produce autoantibodies against diabetes-associated antigens. See Petersen et al., Diabetes (1994). A genetic modification on the NOD/SCID background enables engraftment by human lymphoid cells.

TABLE 8
Commercially available NOD/SCID mice

Source	Benefit
NOD SCID Mice. NOD.CB17-Prkdcscid/NCrCrl. Congenic immunodeficient.	The "gold standard" in humanized mice. The NOD background results in defective natural killer (NK) cell function. For the SCID mutation, homozygous animals impaired T cell and B cell lymphocyte development.
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ. Strain #:005557. The common name is NSG. Also known as NOD/SCID IL2Rgammanull, NOD/SCID IL2Rgnull, NOD/SCID gamma	The severe immunodeficiency allows the mice to be humanized by engraftment of human CD34+ HSCs or PBMCs. The mutation of IL2 receptor gamma chain (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21) results in severe defects in adaptive and innate immunity (more pronounced immunodeficiency).
NOD.CB17-*Prkdc*^{scid}*Il2rg*^{tm1}/Bcgen. Catalog number 112469. The common name is huPBMC-B-NDG mice	

[00205] Pediatric patients are good pool of autoantibodies. PBMCs from type 1 diabetes patients and controls are commercially available. B cells in vitro generate a polyclonal pool of autoantibodies. The humanized mouse model can show degradation of patient autoantibodies.

[00206] *Canine diabetic patients*. The inventors administer the bifunctional degrader to canine diabetic patients to treat their diabetes. Diabetes dogs are treated therapeutically with human insulin and commonly develop immune-neutralizing antibodies which decrease insulin concentrations and antidiabetic efficacy. The inventors can assay to confirm that the dog polyclonal response to human insulin likely mimics human autoantibody response.

[00207] The inventors can also assay to confirm measurement of hypoglycemia in the dogs.

[00208] *MesoScale Discovery (MSD)-based assay* for detecting low levels of human insulin autoantibodies. Detailed protocols are described in the scientific literature in the biomedical art. See Yu, Methods in Molecular Biology (2016) and Gu et.al., J. Clin. Cellular Immunology (2017). An advantage of using an MSD-based assay is the increased sensitivity and lower sample volume of serum required for the assay. With ELISA-based assays, the antigens are bound to the solid phase, i.e., the ELISA plate. This binding limits the conformational antigen epitope recognition by the antibodies tested. Only one antigen can be recognized per well. MesoScale Discovery-based assays can be developed for up to ten different autoantibodies. MesoScale Discovery-based assays enable a higher throughput capacity.

TABLE 9 Tool antibodies and other biological agents
Abcam, Catalogue # ab254262. Recombinant anti-asialoglycoprotein receptor 1/HL-1 (Anti-ASGPR Ab).
Cell Signaling Technology, catalogue # 14364S. IGF-2 receptor/CI-M6PR (D3V8C) rabbit monoclonal antibody (anti-IGF2 Ab),
Cell Signaling Technology, catalogue # 17174S. IGF-1 receptor α (D3A2W) rabbit monoclonal antibody anti-IGF1 antibody.
Cell Signaling Technology, catalogue # 23413S. Insulin receptor B (E9L5V) XP rabbit monoclonal antibody.
Cell Signaling Technology, catalogue # 23413S. Insulin receptor β (E9L5V) XP® rabbit monoclonal antibody (anti-Insulin B Ab),
Cell Signaling Technology, Catalogue # 74118S. Insulin receptor α (D3U7I), rabbit monoclonal antibody anti-insulin antibody.
Cell Signaling Technology, catalogue # 88175S. FcRn (E6D1S) rabbit monoclonal anti-FcRn antibody.
Cell Signaling Technology, catalogue # 9750S. IGF-1 Receptor β (D23H3) XP® rabbit monoclonal antibody (anti-IGF1 B Ab),
Genscript mouse anti-human insulin monoclonal antibody IgG2a (clone: 6E9F1), GenScript, catalogue # A01715. This antibody detects human Insulin and slightly recognizes proinsulin, with no cross-reactivity with C-peptide. This antibody had better endocytosis signal compared to human mAb49.
LS Bio, Catalogue # LSC331895-200. Polyclonal rabbit anti human insulin antibody (IHC, Western blot).
M6PR antibodies rabbit. CST, catalogue #14364S
mAb13. Creative Biolabs, catalogue # FAMAB-0223WJ (poor binding vis SPR). mAb13 is a human anti-insulin monoclonal antibody derived from IDDM patient treated with human recombinant insulin by EBV transformation and somatic cell hybridization techniques. mAb13 can be used in applications, such as ELISA, Western blot.

TABLE 9 Tool antibodies and other biological agents
mAb48. Creative Biolabs, catalogue # FAMAB-0224WJ-S(P); scFv Fragment (IgG3 subtype) (poor binding in surface plasmon resonance assays). mAb48 is a human anti-insulin monoclonal antibody derived from IDDM patient treated with human recombinant insulin by EBV transformation and somatic cell hybridization techniques. mAb48 can be used in applications, such as ELISA, Western blot.
mAb49 Fab fragment. Creative Biolabs, Catalogue # FAMAB-0225WJ-F(E). This product is a human anti-insulin monoclonal antibody Fab fragment derived from three IDDM patients treated with human recombinant insulin, by EBV transformation and somatic cell hybridization techniques. This antibody Fab fragment can be used in applications, such as ELISA, Western blot.
mAb49. Creative Biolabs, catalogue # FAMAB-0225WJ-F(E)). Surface plasmon resonance results show binding to insulin/proinsulin. Human Anti-Insulin Recombinant Antibody (clone mAb49); Fab Fragment (described as IgG1 subtype) – but confirmed internally as human/chimeric IgG3. mAb49 is a human anti-insulin monoclonal antibody derived from IDDM patient treated with human recombinant insulin by EBV transformation and somatic cell hybridization techniques. mAb49 can be used in applications, such as ELISA, Western blot.
Sino, catalogue # 11038-CR007. Recombinant anti-insulin antibody, rabbit monoclonal.
Sino, catalogue # 11038-T08. Anti-insulin antibody, rabbit polyclonal.
Sino, catalogue # 11038-T24. Anti-insulin antibody, rabbit polyclonal.
ThermoFisher, Catalogue # A20173. Alexa Fluor 647 protein.
Insulin receptor primary sequence. P06213-1|INSR_HUMAN isoform long of insulin receptor OS=Homo sapiens OX=9606 GN=INSR PE=1 SV=4 (SEQ ID NO: 31).
P06213-2, INSR_HUMAN isoform short of Insulin receptor OS=Homo sapiens OX=9606 GN=INSR PE=1 SV=4 (SEQ ID NO: 32).
Mouse anti-C-peptide antibody light chain kappa (SEQ ID NO: 35) [219 AA]
Mouse anti-C-peptide antibody heavy chain gamma 1 (SEQ ID NO: 36) [437 AA]
Mouse Anti-C-peptide monoclonal antibody (CBFYR0644) (CAT#: CBMAB-R0644-FY). This product is mouse antibody that recognizes C-Peptide. The antibody CBFYR0644 can be used for immunoassay techniques such as: radioimmune assay, EIA, IHC.
Mouse anti-C-Peptide monoclonal antibody (CBFYR0645) (CBMAB-R0645-FY).
Mouse anti-C-peptide recombinant antibody (CBCNC-012).
Recombinant mouse Anti-C-peptide antibody (CB2931) (CBMAB-MD1611-LY)
Invitrogen. C-peptide monoclonal antibody (C-PEP-01). Anti-human to C-peptide of Proinsulin (C-PEP-01) Monoclonal antibody (Product # MA1-19159). See also 05-1109 Sigma-Aldrich. Anti-proinsulin C-peptide antibody, clone C-PEP-01. See Hilgert et al., Hybridoma 10(3) 379-386 (1991).

TABLE 10 Reagent kits
ThermoFisher A20173, AF647 labeling kit
ThermoFisher A1023,5AF-488 labeling kit
MedChemExpress HY-14710,2Tri-GalNAc biotin (control reagent)
Abbexa: Human insulin autoantibody (IAA) CLIA Kit
Abbexa: Mouse insulin autoantibody (IAA) CLIA Kit

[00209] Commercial kits are available to measure Insulin, proinsulin, or anti-insulin autoantibodies.

TABLE 11 Detection of insulin/proinsulin			
Vendor	Specificity	Assay	Detection range
MesoScale Discovery, catalogue # K151S5D	Human, non-human primate, canine	Electrochemi-luminescence (ECL)	0.006 - 19.5 µIU/mL
MesoScale Discovery, catalogue # K1516MK	Human proinsulin	Electrochemi-luminescence (ECL)	0.054 - 130 pM
Novus Bio, catalogue # NBP2-60078-1 Kit	Human Insulin	ELISA	

TABLE 12 Detection of insulin/proinsulin		
Specificity	Vendor	ELISA detection range
Human anti-insulin IgG	Eagle Biosciences, catalogue # 3806	0.1 – 20 U/mL
Human anti-insulin	Aviva Systems Biology, catalogue # OKEH00908	3.12-200ng/ml
Mouse anti-insulin	LS Bio, catalogue # LSF74131-1	3.12 - 200 ng/ml
Mouse anti-insulin	Abbexa, catalogue # Abx053161	0.156 - 10 ng/ml
Dog anti-insulin	American Research Products, catalogue # E08I0019	5.0-100ng/mL

Statistical analyses.

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, persons having ordinary skill in the biomedical art can use median and interquartile range and analyze with the Mann-Whitney U test. Categorical data are summarized by percentages. A two-sided p

value <0.05 is considered statistically significant. All statistical tests can be performed using SPSS version 16.0.

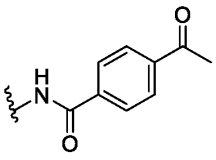
Characterizing antibody binding moieties.

[00210] Many technologies are available for identifying, assessing, or characterizing antibody binding moieties, including protein binding moieties, e.g., antibody binding moieties such as universal antibody binding moieties), and/or their use in provided technologies, e.g., those described in International Patent Publication WO 2019/023501 (Kleo Pharmaceuticals, Inc.), 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.

[00211] 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.

[00212] In some embodiments, an antibody binding moiety, e.g., an antibody binding moiety, is or comprises a Meditope agent moiety. See United States Patent Publication 2019/0111149 (Gardiner et al.).

[00213] In some embodiments, an antibody binding moiety, e.g., an antibody binding moiety, can bind to human IgG. In some embodiments, an antibody binding moiety, e.g., an antibody binding moiety, can bind to an antibody selected from the Markush group of antibodies consisting of rabbit IgG, IgG1, IgG2, IgG3, and IgG4. In some embodiments, an antibody binding moiety, e.g., an antibody binding moiety, binds to IgG1, IgG2, and IgG4.



[00214] In some embodiments, is used in a reference technology as a non-antibody binding moiety. In some embodiments, the chemical group used in a reference technology as a non-antibody binding moiety is selected from the Markush group of chemical groups consisting of CH_3–, $CH_3C(O)$–, $CH_3C(O)NH$–, $CH_3C(O)NHCH_2$–, CH_3CH_2–, CH_3CH_2NH–, and $CH_3CH_2NHC(O)$–.

[00215] In some embodiments, antibody binding moieties, e.g., antibody binding moieties) bind to targets, e.g., antibody agents for antibody binding moieties) with a K_D that is about 1 mM^{-1} pM or less. In some embodiments, a K_D is about 1 mM, 0.5 mM, 0.2 mM, 0.1 mM, 0.05 mM, 0.02 mM, 0.01 mM, 0.005 mM, 0.002 mM, 0.001 mM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.2 nM, 0.1 nM, or less. In some embodiments, K_D is an affinity selected from the Markush group of affinities consisting of about 1 mM or less, about 0.5 mM or less, about 0.1 mM or less, about 0.05 mM or less, about 0.01 mM or less, about 0.005 mM or less, about 0.001 mM or less, about 500 nM or less, about 200 nM or less, about 100 nM or less, about 50 nM or less, about 20 nM or less, about 10 nM or less, about 5 nM or less, about 2 nM or less, and about 1 nM or less. In some embodiments, antibody binding moieties bind to IgG antibody agents with K_D described in this specification.

[00216] 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 human Fcγ receptor IIIA. In some embodiments, recruited antibodies can effectively interact with human Fcγ receptor IIIA on macrophages. In some embodiments, macrophages are recruited to provide ADCC or ADCP 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 human Fcγ receptor IIIA. In some embodiments, recruited antibodies can effectively interact with human Fcγ receptor IIIA 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.

Specific mutation strategies to reduce Fcγ receptor and c1q binding / effector function, and to enhance FcRn binding/exposure of antibodies.

Fc mutation strategies to reduce Fcγ receptor and c1q binding.

[00364] The inventors mutate an antibody, an antibody variant, or an antigen-binding fragment thereof in the Fc region 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.

[00365] The inventors mutate an antibody, an antibody variant, or an antigen-binding fragment thereof in the Fc region 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 et al., Protein Engineering, Design and Selection, Volume 29, Issue 10, pages 457–466 (October 2016), which shows that even LALA itself abolishes c1q binding. P329A alone was tested, and abolishes c1q binding, and reduces FcgR binding. They show that P329G/LALA further reduces Fcγ receptor binding beyond LALA alone.

[00366] The inventors also mutated an antibody, an antibody variant, or an antigen-binding fragment thereof in other regions. N297A/Q removes natural N-linked glycosylation site in the hinge region.

Fc mutation strategies to enhanced FcRn binding to prolong exposure.

[00367] There are many publicly available approaches and mutation sets to increase binding of an Fc to FcRn.

TABLE 13
Examples of Fc sequence engineering for modification of half-life.

Function	Company or source	Mutations or changes
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 M252Y, S254T,T256E, H433K, N434F, 436H
Decreased half-life	Deny Roopenian	IgG1·I253A
Decreased half-life	Eli Lilly	IgG1 P257I, N434H or Q376V, N434H

[00368] An older summary is provided by Strohl, Optimization of Fc-mediated effector functions of monoclonal antibodies. Current Opinion in Biotechnology, 20(6), 685-691 (2009).

[00369] For an example of introducing a YTE region in a protein (M252Y/S254T/T256E), see Acqua et al., Journal of Immunology 169(9), 5171-5180 (2002). For a recent example combining LALA and YTE, see Cobb et al., bioRxiv, 2021-09 (2021). Additional references with details of engineering also available.

[00370] For an example of introducing a LS region in a protein (M428L/N434S), see Zalevsky et al., Nature Biotechnology, 28(2), 157-159 (2010).

Structure of recombinant ligand constructs.

[00371] *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).

[00372] *GN3-maleimide reagent* (maleimide-GN3/linker), with a C-terminal cysteine for conjugation.

[00373] *Biophysical/biochemical potency assay to quantify target-engagement*. A biomolecule coupled to surface of sensor chip as ligand. As analyte is flowed in solution over immobilized ligand, binding to the sensor chip surface induces a change in refractive index proportional to bound mass.

[00374] An assay was done using a Biacore S200 instrument. This machine has a high sensitivity, low-medium throughput. A ligand variant fragment is dosed 1 nM to 1 µM and characterized by single cycle kinetics.

[00375] *ASGPR-dependent uptake assay*. A HEK293 bioluminescent cell-based assay was used to assess ternary complex formation uptake by cells in vitro. This on-mechanism endocytosis assay measures the accumulation of bifunctional degrader in HEK293 cells through the bifunctional degrader mechanism described in this specification. A component of this assay is a determination of the receptor expression on the surfaces of the assay's HEK293 cells.

[00376] *Degradation assay*. This on-mechanism degradation assay is a direct, low-throughput Western blot measurement or a high-throughput assay using an activatable fluorescence-quencher probe.

[00377] Other off-target assays include measurement of cytotoxicity through In vitro toxicity with HepG2 cells, e.g., in a CellTiter-Glo assay, measurement of hemagglutination by red blood cell interactions, measurement of PBMC, and measurement of off-target bindings in a house C-type lectin panel. Signal is boosted in the HEK293 versus HepG2 cells when measuring signal accumulation not degradation. Western assays can measure the degradation in HEK cells and other cell lines.

[00378] Next steps can include a competition assay with FC3 (qualitative).

Manufacturing the agent.

TABLE 14
Purification steps

No.	Steps	Buffer	
1	Lysis	Lysis buffer	50 mM Tris-HCl, 150 mM NaCl, 1 mM TCEP, pH 8.0
2	Wash	Washing buffer, A	Containing 0.5% Triton X-100 and 10 mM EDTA
		Washing buffer, B	ddH_2O
		Washing buffer, C	Containing 2% Triton X-114
3	Denaturation	Denaturation buffer	50 mM Tris-HCl, 7 M Gu-HCl, 50 mM DTT, pH 8.0
4	Refolding	Refolding buffer, A	25 mM Tris-HCl, 6 M Urea, 500 mM NaCl, 50 mM Glycine, 5 mM GSH, 0.5 mM GSSH, pH 9.5
		Refolding buffer, B	25 mM Tris-HCl, 4 M Urea, 500 mM NaCl, 50 mM Glycine, 5 mM GSH, 0.5 mM GSSH, pH 9.5

TABLE 14
Purification steps

No.	Steps	Buffer	
		Refolding buffer, C	25 mM Tris-HCl, 2 M Urea, 500 mM NaCl, 50 mM Glycine, 5 mM GSH, 0.5 mM GSSH, pH 9.5
		Refolding buffer, D	25 mM Tris-HCl, 500 mM NaCl, 50 mM Glycine, 5 mM GSH, 0.5 mM GSSH, pH 9.5
5	Q column	Equilibration buffer	25 mM Tris-HCl, 166 mM NaCl, 10 mM Glycine, pH 9.5
		Elution buffer	Equilibration buffer with 166~500 mM NaCl

[00379] The manufacturing risks dimer/aggregation during the purification.

Methods of making several agents.

[00217] 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 International Patent Publication WO 2019/023501 (Kleo Pharmaceuticals, Inc.), which is incorporated in this specification in its entirety by reference.

[00218] Several technologies, e.g., those for preparing antibody-drug conjugates, may be used in preparation of MATE agents. In many such technologies, conjugation is not selective regarding amino acid residue sites, and product compositions usually contain several 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.

[00219] 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.

[00220] 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.

EXAMPLES

[00221] The invention is further illustrated by non-limited examples.

EXAMPLE 1

Proinsulin and proinsulin-IgG1 Fc fusion protein sequences.

[00380] The inventors produced His tagged and sortase tagged proteins. See SEQUENCE LISTING.

[00381] The Fc fusion construct expression in the E. coli system is improved by using a fusion protein. The Fc fusion construct expression enables a highly purified material with a good yield. Conjugation of ASPGR via MATE reagent to yield degrader molecule. LALA and PA mutations to produce distinct chemical entities can attenuate the immune effector function. Quality control can address any issues with disulfide interactions, where insulin might misfold in with the Fc receptor and the cystines in the Fc to aggregates. Such misfolding can be detected by native protein gel assays and size exclusion assays.

[00382] Persons having ordinary skill in the biomedical art understand how to make proinsulin-Fc fusion proteins. The prior art proinsulin-Fc fusion proteins can be tool compound and reference compounds. Persons having ordinary skill in the biomedical art can also make the insulin-Fc fusion proteins.

TABLE 15		
Protein	Concentration	Buffer
PT0003, native proinsulin C-terminal His tag	0.25 mg/mL	25 mM Tris-HCL, 500 mM NaCl, 50 mM glycine, pH 9.5
PT0003, native proinsulin C-terminal His tag	0.71 mg/mL	50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, pH 8.0
PT0004, native proinsulin C-terminal sortase tag	0.75 mg/mL	50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, pH 8.0
PT0004, native proinsulin C-terminal sortase tag	0.36 mg/mL	25 mM Tris-HCL, 500 mM NaCl, 50 mM glycine, pH 9.5

[00383] *Validation of tool Abs binding to proinsulin Fc protein*. Both rabbit and mouse anti-insulin antibodies bind or recognize Fc fusion protein via the proinsulin moiety). LALA mutation (in Fc domain) does not prevent recognition by anti-insulin antibodies.

[00384] Both rabbit and mouse anti-insulin bind or recognize Fc fusion protein via the proinsulin moiety)

EXAMPLE 2

Surface plasmon resonance assay – first results.

[00385] binding ability of the binding moiety can be measured by surface plasmon resonance (Biacore™ and IBIS-MX96 systems) and bio-layer interferometry (ForteBio™ Octet™ systems).

[00386] The screening cascade plans include surface plasmon resonance target engagement, ternary complex formation, the cell-based assays, and endocytosis assays. Additional bridging assays to confirm degradation. SPR/TR-FRET (ASGPR) → TR-FRET (ternary complex, biophysical) → Uptake → TR-FRET in cells (degradation).

[00387] *Binding confirmation by surface plasmon resonance*. Fc is immobilized to CM5 chip via amine coupling immediately before the assays. binding moiety was dosed 1 nM to 1 µM and characterized by single cycle kinetics. First run, single cycle kinetics, steady state K_D (nM): 22. Second run, single cycle kinetics, steady state K_D (nM): 24. The inventors are validating commercial antibody that binds to human Fc of an . In n=2 characterization, K_D of about20 nM was obtained. No binding response was observed for full-length IgG1-3 and IgG1-3 Fc regions at up to 1 µM concentrations. The theoretical R_{max} indicates active surface (data QC metric and indicator of viable immobilization method).

TABLE 16		
Assay reagent	MW	Note
Insulin (human)	5807.57 Da	MCE
Insulin lispro	5807.63 Da	MCE
Biotinylated Human IGF-1 receptor/CD221 protein, His, Avitag™	106600 Da	IGR-H82E3
Human IGF-1 receptor/CD221 Protein, Fc Tag		IGR-H5253
Human IGF-1 Protein, His tag, premium grade		IG1-H5245
Biotinylated Human insulin receptor/CD220 (28-944) Protein, His, Avitag™ (MALS and SPR verified)	108200 Da	INR-H82E6
Human insulin receptor/ CD220 (28-944) Protein, His Tag (MALS and SPR verified)	106400 Da	INR-H52Ha
Anti-insulin receptor α antibody [83-7]		Abcam ab36550

TABLE 17
Surface plasmon resonance assays

Step	Immobilize	Level (RU)	Capture	Level (RU)
1	Anti-insulin receptor α antibody [83-7] in acetate pH 5.0 buffer	5041.8	Human Insulin R / CD220 (28-944) Protein, His Tag	170
2	Anti-insulin receptor α antibody [83-7] in acetate pH 5.0 buffer	5132.1	Human Insulin R / CD220 (28-944) Protein, His Tag	374.8
3	Anti-insulin receptor α antibody [83-7] in acetate pH 5.0 buffer	5203.8	Human Insulin R / CD220 (28-944) Protein, His Tag	574.1
4	Neutravidin, in acetate pH 5.0 buffer	9965.8	Biotinylated Human Insulin R / CD220 (28-944) Protein, His, Avitag	737.1
5	Neutravidin, in acetate pH 5.0 buffer	9951.1	Biotinylated Human Insulin R / CD220 (28-944) Protein, His, Avitag	1106.3
6	Neutravidin, in acetate pH 5.0 buffer	9616.3	Biotinylated Human Insulin R / CD220 (28-944) Protein, His, Avitag	1472.1
7	Neutravidin, in acetate pH 5.0 buffer	9592.7	Biotinylated Human IGF-1 R / CD221 Protein, His, Avitag	699.6
8	Neutravidin, in acetate pH 5.0 buffer	9563.5	Biotinylated Human IGF-1 R / CD221 Protein, His, Avitag	1389.2

TABLE 18
Summary Table

	Species	Immobilization level	ka (M-1s-1)	K_D (sec-1)	K_D (kinetic) (M)	K_D steady state (M)	R_{max}	1:1 Theoretical R_{max}
Insulin (human)	Human insulin receptor/ CD220,His Tag	170.0	4.88 E+05	1.07 E-01	2.20 E-07	3.34 E-07	9.2	9
		374.8	4.97 E+05	9.17 E-02	1.84 E-07	3.77 E-07	19.5	20
		574.1	5.30 E+05	6.90 E-02	1.30 E-07	3.87 E-07	29.7	31
	Biotinylated human insulin receptor/ CD22	737.1	3.36 E+05	1.04 E-01	3.11 E-07	3.41 E-07	62.1	40
		1106.3	3.12 E+05	9.44 E-02	3.03 E-07	3.66 E-07	98.7	59
		1472.4	1.78 E+06	3.93 E-01	2.21 E-07	3.85 E-07	133.5	79
	Biotinylated human IGF-1 receptor/ CD221	699.6	1.15 E+05	6.35 E-02	5.54 E-07	2.39 E-06	21.0	38
		1389.2	6.43 E+04	6.65 E-02	1.03 E-06	2.78 E-06	50.8	76
Insulin lispro	Human insulin receptor/ CD220,His Tag	170.0	5.14 E+05	1.13 E-01	2.21 E-07	3.38 E-07	8.2	9
		374.8	4.37 E+05	9.51 E-02	2.18 E-07	4.07 E-07	17.1	20
		574.1	3.80 E+05	7.97 E-02	2.10 E-07	4.19 E-07	26.2	31
	Biotinylated human insulin receptor/ CD22	737.1	2.94 E+05	1.09 E-01	3.71 E-07	3.60E-07	56.7	40
		1106.3	1.40 E+06	3.66 E-01	2.62 E-07	3.93E-07	88.9	59
		1472.4	1.33 E+06	3.59 E-01	2.70 E-07	3.99E-07	120.9	79
	Biotinylated human IGF-1 receptor/ CD221	699.6	1.33 E+05	5.80 E-02	4.37 E-07	2.84E-06	19.5	38
		1389.2	8.27 E+04	6.30 E-02	7.63 E-07	3.36E-06	46.9	76

Surface plasmon resonance assays for binding to Insulin

[00388] The inventors tested tree anti-insulin autoantibodies mAb13, mAb48, and mAb49.

Assay 1: (done)

[00389] *Sensor chip*: CM5.

[00390] *Proteins: 1*. Immobilize anti-insulin receptor α antibody [83-7] on a CM5 chip with amine coupling to 5000 RU.

[00391] *Proteins: 2*. Capture human insulin receptor (isoform A) / CD220 (28-944) Protein, His Tag from Acro Biosystems to ~150, 300, and 600 RU. R&D Systems' isoform B, recombinant Human Insulin R/CD220 (aa 28-956) Protein, CF.

[00392] *Analytes*: Human insulin and Insulin lispro, 5 µM top, 2-fold dilution, MCK 1+10+1, 90s on, 180s off.

[00393] *Running buffer*: 10 mM HEPES pH 7.4, 150 mM sodium chloride, 0.005% Tween-20.

Assay 2: (done)

[00394] *Sensor chip*: CM5+Neutravidin.

[00395] *Proteins*: Capture biotinylated human insulin receptor/CD220 (28-944) Protein, His, Avitag from Acro Biosystems on a SA chip at ~750, 1000, and 1500 RU.

[00396] *Analytes*: Human insulin and Insulin lispro, 5 µM top, 2-fold dilution, MCK 1+10+1, 90s on, 180s off.

[00397] *Running buffer*: 10 mM HEPES pH 7.4, 150 mM sodium chloride, 0.005% Tween-20.

Assay 3: (done)

[00398] *Sensor chip*: CM5+Neutravidin.

[00399] *Proteins*: Capture biotinylated human IGF-1 receptor/CD221 Protein, His, Avitag from Acro Biosystems on a SA chip at ~750 and 1250 RU. IGF-1 receptor can be produced synthetically. qPCR method primer sequence IGF1R-forward: CCTGCACAACTCCATCTTCGTG (SEQ ID NO: 41). qPCR method primer sequence IGF1R-reverse: CGGTGATGTTGTAGGTGTCTGC (SEQ ID NO: 42).

[00400] *Analytes*: Human insulin and insulin lispro, 10 µM top, 2-fold dilution, MCK 1+10+1, 90s on, 180s off.

[00401] *Running buffer*: 10 mM HEPES pH 7.4, 150 mM sodium chloride, 0.005% Tween-20.

Assay 4:

[00402] *Sensor chip*: CM5.

[00403] Proteins: Immobilize three anti-insulin autoantibodies on a CM5 chip with amine coupling at ~750 and 1250 RU.

[00404] *Analytes*: Human insulin and Insulin lispro, 5 µM top, 2-fold dilution, MCK 1+10+1, 120s on, 600s off.

[00405] *Running buffer*: 10 mM HEPES pH 7.4, 150 mM sodium chloride, 0.005% Tween-20.

Assay 5:

[00406] *Sensor chip*: protein A.

[00407] *Proteins*: Capture three anti-insulin autoantibodies on a protein A chip to ~750 and 1250 RU.

[00408] *Analytes*: Human insulin and Insulin lispro, 5 µM top, 2-fold dilution, MCK 1+10+1, 120s on, 600s off.

[00409] *Running buffer*: 10 mM HEPES, pH 7.4, 150 mM sodium chloride, 0.005% Tween-20.

EXAMPLE 3

Pharmacodynamics.

[00410] ***AGN310*** *study in non-obese diabetic (NOD) mice*. Four mice per group. An additional two mice per group were excluded because they had very low levels of antibody to begin. The inventors observed 42% depletion by **AGN310**, based on area-under-the curve. There was a 50% depletion if the background signal is accounted for.

[00411] In another analysis, **AGN310** effectively removed 39% of anti-insulin antibodies (2 mg/kg) in nude mice.

[00412] **AGN310** was well-tolerated when administered at 3 mg/kg IV in nude mice, with no observed adverse events and changes in glucose levels when measured at twelve hours-post dose.

EXAMPLE 4

Internalization and ternary complex formation assays.

[00413] *Assay conditions*:

Cell lines: HEK293-ASGPR (clone 3F7)

Culture media: DMEM + 10% FBS + 1% P/S

Antibodies: Constant at 100 nM (all labeled with AF647)

Anti-insulin antibody, rabbit monoclonal, Sino, Cat#11038-CR007

Anti-insulin antibody, mouse monoclonal, Genscript, Cat#A01715 (clone 6E9F1)

Agents: only **AGN301** (constant at 50 nM)

Readout: Ternary complex formation —> flow cytometry (IntelliCyt iQue Screener Plus)

Internalization —> high content imager (Operetta)

[00414] *Molecules tested*:

[00415] **AGN301**: IgG degrader (reference molecule)

[00416] **ABT307**: Proinsulin-IgG1 Fc fusion, G4S linker, hIgG1 hinge (C > A), LALA-PA.

[00417] **ABT308**: Proinsulin-IgG1 Fc fusion, 5x G4S linker, hIgG1 hinge (C > A), LALA-PA.

[00418] **AGN303**: Native insulin with GN3

[00419] **AGN302**: Mutant insulin with GN3

[00420] **ABT306**: Alk-Native Insulin without GN3 (just peptide)

[00421] **ABT304**: Native Proinsulin

[00422] **ABT305**: Mutant Proinsulin

[00423] Testing that anti-insulin antibodies (rabbit versus. mouse) recognize proinsulin Fc fusion proteins.

[00424] *Conclusions*. Anti-insulin agents working. Rabbit monoclonal antibody, but not mouse, can be used as surrogate with IgG degrader (**AGN301**). Non-GN3 peptide (**ABT306**) does not elicit internalization or ternary complex formation. Anti-proinsulin agents (**ABT304**/**ABT305**) do not internalize or complex with anti-insulin antibodies.

EXAMPLE 5

Agents bind to insulin and proinsulin autoantibodies, resulting in uptake, hepatic degradation and correction of glucose homeostasis.

[00425] Anti-insulin autoantibody MoDEs and anti-proinsulin autoantibody MoDEs form ternary complexes, show in vitro uptake and drive in vivo clearance without binding insulin receptor or IGF1 receptor.

[00426] Robust and selective lowering of these autoantibodies was observed in mouse pharmacokinetics/pharmacodynamics assays.

[00427] *Efficacy studies and preliminary toxicology*. Agents deplete naturally-occurring insulin autoantibodies in non-obese diabetic (NOD) mice.

[00428] *Conclusion*: Lowering of neutralizing antibodies in NOD mice suggests potential for restoration of glucose homeostasis in Type 1 diabetes patients.

EXAMPLE 6

Proinsulin production technical report

[00429] *Summary*. ABT410 is a proinsulin molecule with N-terminal H27R leading sequence and TEV recognition site. ABT410 was expressed as inclusion bodies in BL21(DE3) strain. After properly refolding and buffer exchange, target protein was cleaved by TEV protease, then purified by SP HP resins. The purified protein was formulated in 50 mM Tris/HCl, 150 mM sodium chloride, 10% glycerol, pH 7.5 by

UF/DF. The testing panel included protein concentration by Bradford, reducing and non-reducing SDS-PAGE, SEC-HPLC, endotoxin level and LC-MS.

[00430] *Host cell*: E.coli BL21(DE3) competent cell

[00431] *Coding vector name*: pET28a(+).

[00432] *Protein Sequence*:

[00433] **ABT410** (before TEV digestion)
MGMTMITNSPEISHHHHHHHHHHQLISEARENLYFQGGGGGFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED LQVGQVELGGGPGAGSLQPLALEGSL QKRGIVEQCCTSICSLYQLENYCNG (SEQ ID NO: 19).

[00434] ABT410 (after TEV digestion)
GGGGGFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCT SICSLYQLENYCNG.

[00435] *Extinction coefficient*: 0.651 after digestion.

[00436] *Molecular weight*: 9736.98 Da (-SH, after digestion).

[00437] *pI*: 5.20 after digestion.

[00438] *Transformation steps*:

[00439] (1) Add ~100 ng plasmids to 100 µl of BL21(DE3) competent cells.

[00440] (2) Incubate thirty minutes on ice.

[00441] (3) Heat shock forty-five seconds at 42°C water-bath.

[00442] (4) Incubate two minutes on ice.

(5) Add 900 µl of LB media (BD-Cat.244620) and shake forty-five minutes at 37°C, 220rpm.

[00443] (6) Spread the transformation into LB-kanamycin+ agar plate and incubate overnight at 37°C, upside down.

[00444] *Expression*:

[00445] (1) Pick colonies into 100 mL LB media with 50 µg/mL kanamycin + antibiotic.

[00446] (2) Incubate at 30°C, 200 rpm for overnight as a seed.

[00447] (2) Inoculate 10 mL seed into 1 L TB medium (BD-Cat.243820) containing 4 mL glycerol, culture at 37°C, 220 rpm.

[00448] (3) When OD_{600} reaching 1.0~1.2 (~ 2.5 hours), add IPTG to a final concentration of 1 mM, and grow for additional sixteen hours at 18°C.

[00449] (4) In one example, the cells were harvested when OD_{600} reached 8.69. Concentration of solubilized protein was 26.8 mg/mL. Volume of solubilized protein was 90.0 mL. Amount of solubilized protein was 2412.0 mg. The titer was 482.4 mg/L.

[00450] (5) Harvest cells by centrifugation at 8000 g, fifteen minutes.

[00451] (6) Cell pellet can be stored at -80°C.

[00452] *Preparation of inclusion bodies*:

[00453] (1) Thaw the expression pellet at room temperature for ten-twenty minutes.

[00454] (2) Resuspend cells in freshly prepared lysis buffer at a ratio of 1:5 (WITH V).

[00455] (3) In a magnetic stirrer, stir for thirty minutes at room temperature until the cell pellets solution is completely dissolved.

[00456] (4) Homogenizer at 1000 bar for two-three times.

[00457] (5) Centrifuge at 12,000 g for one hour at 4°C.

[00458] (6) Remove supernatant carefully and resuspend the inclusion bodies with wash buffer to its original equal volume. Stir for thirty minutes at room temperature until the cell debris is completely dissolved.

[00459] (7) Centrifuge at 12,000 g for one hour at 4°C.

[00460] (8) Repeat step 6 and step 7 once more.

[00461] (9) Discard the supernatant and the inclusion bodies was resuspended with solubilization buffer at a ratio of 1:10 (WITH V). Stir for overnight 4°C.

[00462] (10) Centrifuge the solubilized inclusion bodies at 40,000 g for one hour at 4°C.

[00463] (11) Collect the supernatant by 0.2 μm filter.

[00464] (12) Determine protein concentration by Pierce Coomassie assay plus protein determination reagent following manufacturer instruction.

[00465] *Lysis buffer*: 50 mM Tris/HCl, pH 8.0, 5mM EDTA, 1% Triton X-100.

[00466] *Wash buffer*: 50 mM Tris/HCl, pH 8.0, 5mM EDTA.

[00467] *Solubilization buffer*: 50 mM Tris/HCl, pH 8.0, 8 M Urea

[00468] In one example, from 1 L pellets, 482.4 mg proteins were obtained.

[00469] *Refolding. For 1 L of refolding*:

[00470] (1) Dilute the denatured sample into 10 mg/mL by solubilization buffer.

[00471] *Refolding buffer*: 20 mM Tris/HCl,10 mM Glycine, 1 mM EDTA,1 mM GSH, 5 mM GSSG, pH 10.0.

[00472] (2) Add DTT to solubilized inclusion bodies to a final concentration of 10 mM and mix completely.

[00473] (3) Incubate thirty minutes at 37°C to break mis-paired disulfide bonds before refolding.

[00474] (4) Drop slowly 50 mL of reduced inclusion bodies (~500 mg protein) in 1 L of freshly made refolding buffer on magnetic stirrer (medium strength).

[00475] (5) Incubate at 4°C for twenty-four hours with slow stirring.

[00476] (6) Run SDS-PAGE and LC-MS to characterize the refolded protein.

[00477] Based on the LC-MS profile, three pairs of disulfide bonds (-6Da/-6H) are formed after overnight refolding. The results also showed that the first 'Met' was successfully removed.

[00478] *Buffer exchange (UF/DF) and TEV digestion*:

[00479] (1) Refolded sample was filtered by 0.45 μm filter firstly to remove visible precipitation before ultrafiltration.

[00480] (2) Sample concentration was concentrated to ~3 mg/mL by UF device.

[00481] (3) Concentrated sample was exchanged into TEV digestion buffer for six-fold volume by DF device.

[00482] *TEV digestion buffer*: 25 mM Tris/HCl, 100 mM sodium chloride, pH 7.5, 10% glycerol.

[00483] *Buffer exchange by UF/DF*: SP HP pool sample can be exchanged into final formulation buffer with six-fold volume by UF/DF device. For small-scale batch production, SEC and desalting column was used to exchange buffer.

[00484] (4) Add in-house produced TEV protease to protein at a ratio of 1:20(WITH W), and the mixtures were incubated at room temperature for overnight.

[00485] (5) Analyze the digestion efficiency by SDS-PAGE.

[00486] In one example, SDS-PAGE gel showed that most of target protein was cleaved by TEV protease.

[00487] *SP HP purification*:

[00488] (1) Add 10% acetic acid to adjust the sample pH to 4.5.

[00489] (2) Dilute the sample with ultra-pure water to make loading conductivity around 7-8 mS/cm.

[00490] (3) Filter the supernatant using 0.2 μm filter.

[00491] (4) Load the protein on SP Sepharose HP (Cytiva) column with dynamic binding density around 20 mg/mL (residence time ~two -five minutes) using AKTA Pure system.

[00492] (5) Wash the SP HP column with five column volumes (CV) buffer A.

[00493] (6) Wash the SP HP column with five column volumes buffer C, assuring contact time 300 minutes.

[00494] (7) Wash the SP HP column with ten column volumes buffer A.

[00495] (8) Elute the protein in a gradient salt concentration. 0-100% buffer B for twenty column volumes.

[00496] (9) Determine protein concentration by Pierce Coomassie plus protein determination reagent following manufacture instruction.

[00497] (10) Run the peak fractions in a non-reduced SDS-PAGE gel and pool the fractions that contain the purified target protein.

[00498] *SP HP buffer A*: 50 mM NaAc-HAc, pH5.0, 10 mM sodium chloride, 10% glycerol.

[00499] *SP HP buffer B*: 50 mM NaAc-HAc, pH5.0, 1M sodium chloride, 10% glycerol.

[00500] *SP HP buffer C*: 50 mM NaAc, pH5.0, 10 mM sodium chloride, 10% glycerol, 0.1% Triton-X114.

[00501] Peak 1 fractions with good purity of SP HP purification were pooled and exchanged into final formulation buffer.

[00502] In one example, the purity of SEC-HPLC was 98.03%.

[00503] *Formulation buffer*: 50 mM Tris/HCl, 150 mM sodium chloride, 10% glycerol, pH 7.5.

[00504] *Product quality analysis*: The purified protein is subjected to quality control testing, including reducing and non-reducing SDS-PAGE, SEC-HPLC, Bradford assay, LC-MS, and endotoxin level.

[00505] *Bradford assay*: The final protein concentration was determined based on Pierce Coomassie plus protein determination reagent following manufacturer instruction. The final concentration was controlled lees than 5 mg/mL. If higher concentration storage is required in the future, stability testing of highly concentrated proteins and screenings of buffer solutions can be conducted.

[00506] *SEC-HPLC*: SEC-HPLC analysis was performed on a Vanquish Flex Duo liquid chromatography instrument using a TSKgel G3000SWxl stainless steel column (7.8 x 300 mm). The mobile phase consisted of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8. Each sample was eluted isocratically for 15 min at a flow rate of 1.0 mL/min. Protein elution was monitored by UV absorbance at 280 nm. The peaks corresponding to aggregates, monomer and low-molecular-weight (LMW) species were integrated to calculate the percentage of each species.

[00507] *SDS-PAGE*. Non-reducing and reducing SDS-PAGE were performed using precast NuPAGE™ 4-12% Bis-Tris gel from ThermoFisher. Sample loading buffer (4X LDS) is from Invitrogen. Gel running buffer (20X MES) is from GenScript. Non-reducing samples were treated with 30 mM iodoacetamide and heated at 95°C for five minutes before analysis. Reducing samples were treated with 50 mM DTT and heated at 95°C for five minutes before analysis. Electrophoresis was carried out at a constant voltage of

180 V for forty minutes. Gels were stained using Coomassie blue for thirty minutes and destained with water for one hour.

[00508] *Endotoxin level*: Purified proteins were subjected to endotoxin test in Limulus Amoebocyte Lysate (LAL) based kinetic turbidimetric test. Purified proteins were diluted based on maximum valid dilution (MVD) in LAL reagent water (Charles River/W110), then sample was loaded in Charles River Laboratories EndoSafe LAL Cartridges (Charles River/ PTS55F) and detected by Charles River Laboratories Endosafe NexGen-MCS™ instrument.

[00509] *LC-MS*: The LC-mass spectroscopy assays were performed with Agilent 6230 AdvanceBio LC/time-of-flight system with a PL1912-1502, PLRP-S 1000Å, 2.1 x 50 mm, 5 µm column (Agilent/ PL1912-1502). The mass spectroscopy data were analyzed by Bioconfirm 10.0 software and identify molecules based on molecular masses. For non-reduced mass spectroscopy, the sample was injected directly. For reduced mass spectroscopy, the sample was treated with 50 mM DTT for thirty minutes at room temperature before injection.

[00510] The average yield of final sample was around 20-30 mg/L.

EXAMPLE 7

AGN417 technical report

[00511] **AGN417** is a sortase-ligation based bifunctional degrader (TRAP). The molecule consists of one single chain (**ABT410**, peptide), which is conjugated to a pentapeptide motif LPETG of **TBT403** by sortase-catalyzed ligation. The targeted antibody to conjugate ratio (BAR) is 1.

[00512] **AGN417** was successfully generated. All the results meet targeting quality. The sample was stable under three freeze-thaw cycles.

[00513] *TRAP purification method by AEX*:

[00514] The TRAP solution was purified by AEX to remove sortase A and linker-payload.

[00515] (1) Instrument was AKTA pure 150 (Pure water generator Milli-Q Reference).

[00516] (2) Column was Capto Q Impres, 500 mL.

[00517] (3) Mobile phase A was 20 mM Tris, pH 8.3. Mobile phase B was 20 mM Tris,1M sodium chloride, pH 8.3.

[00518] (4) Gradient was B from 0% to 45% in four column volumes.

[00519] (5) Flow rate was 40 mL/min.

[00520] (6) Wavelength was 280 nm and 220 nm.

[00521] *Purification method for TRAP by UF/DF*: The UF/DF was preprocessed according to the following procedure:

[00522] (1) Assemble the UF/DF system and install the cassette (Pellicon3 0.11m^2 Cassette, Ultracel 10 kDa).

[00523] (2) Flush the system with water, clean with the 0.1M sodium hydroxide for thirty minutes, and flush with water again.

[00524] (3) Add phosphate-buffered saline, pH7.4 to the feed tank. Start the feed pump. Verify that the pH and conductivity in the system have been equilibrated to the level of the phosphate-buffered saline.

[00525] (4) Add reaction mixture solution to the feed tank. Start the feed pump by partially closing the retentate valve and adjusting the pump speed. Diafilter the product with the phosphate-buffered saline, pH 7.4 for 15 DVs.

[00526] (5) Open the retentate valve fully and collect all the product. Filter the product by 0.22 μm membrane.

[00527] (6) Test the quality and concentration of product.

[00528] *Ultraviolet–visible spectroscopy platform by Nanodrop 2000 (ThermoFisher, 1294881) to determine protein concentration of in-process sample and final TRAP*:

[00529] (1) 750 nm was set up as baseline.

[00530] (2) The ultraviolet absorption at 280 nm and 220 nm were measured respectively

[00531] (3) Calculation method based on Beer-Lambert Law A=E*C*l

[00532] $A_{280}=E^{mAb}{}_{280}*C_{[mAb]}*l$, where E is the molar extinction coefficient; C is the molar concentration; and l is the light path (Nanodrop: 0.1 cm)

[00533] *BAR determination by LC-MS*. LC-MS was performed using a combination of Agilent 1260 series high performance liquid chromatography system and time-of-flight mass spectrometry (Agilent Technologies, HPLC 1260 / 6224 TOF). BAR was calculated based on the peak abundance of the deconvoluted mass.

[00534] *Preparation of sample used in LC-MS analysis*. An antibody-drug conjugate solution was diluted to approximately 1 mg/mL, 30 μL totally with water. The obtained sample was used in an LC-MS analysis.

[00535] *LC-MS/MS method*. A high performance liquid chromatography analysis was carried out under the following measurement conditions. Equipment is HPLC Agilent 1260 (Agilent Technologies, HPLC 1260). Column: Agilent PLRP-S, 50 x 2.1 mm, 8 μm, 1000Å.Detection Wavelength: 280 nm, 214 nm.

Column Oven Temp.: 80°C. Sampler temperature: 2~8°C. Flow Rate: 0.05 mL/min. Injection Amount: 2 µg. Mobile phase: A was 0.025% trifluoroacetic acid+0.1%FA in water. Mobile phase B was 0.025% trifluoroacetic acid + 0.1% formic acid in acetonitrile. Gradient Program is for ten minutes. Time 0.0 minutes at B = 5%; time 0.7 minutes at B = 5%; time five minutes at B = 45%; time six minutes at B = 90%; time seven minutes at B = 90%; time 7.1 minutes at B = 5%; and time 10.0 minutes at B = 5%.

[00536] *Aggregation determination by size exclusion chromatography-high performance liquid chromatography*: Size-exclusion chromatography was performed using an Agilent 1260 series high performance liquid chromatography system with the TSK gel G3000SWXL Size-exclusion chromatography column (7.8×300 mm, 5 µm) at 25°C. The mobile phase was consisted of 78 mM KH_2PO_4, 122 mM K_2HPO_4, 250 mM potassium chloride, 15% isopropanol at pH 7.0±0.1. The flow rate was set at 0.75 mL/min. Sample loading was 40~50 µg per injection. Samples were detected at 280 nm and 370 nm with a UV detector. The retention time of the aggregation peak was recorded based on its relative molecular weight. The aggregation level was determined by the relative area of the peak at 280 nm.

[00537] *Residual free drug determination by LC-MS*. The residual free drug level was determined by LC-MS. After protein precipitation, supernatant was loaded to InfinityLab Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 µm column, and eluted by a gradient of increasing the organic mobile phase. The percentage of residual free drug was quantified via peak area by comparing it to external standard curve.

[00538] (1) *Solvent I preparation*: Weighed 10 g sodium chloride to the pre-mixed organic solvent of 30 mL methanol and 50 mL acetonitrile, mixed and stirred one hour at least, allowed the solution to stand for at least one hour before use. The supernatant was the saturated sodium chloride solution.

[00539] (2) *Preparation of standard curve*: The stock standard linker-drug solution was firstly diluted to 40 µM with dimethyl sulfoxide for standard curve preparation. Then the standard curve and sample were prepared. MW_{mAb} = 9731 Da. TRAP BAR value = 0.91 were used for calculation.

[00540] (3) *Preparation of sample*. The sample were prepared as follows. (TRAP product concentration 22.45 mg/mL was used for calculation). TRAP concentration = 0.50 mg/mL. V_{TRAP} 3.30 µL. Dimethyl sulfoxide volume fraction = 0.1. V_{DMSO} 15.00 µL. V_{H2O} was 31.70 µL. Solvent I was 100.00 µL.

[00541] *Liquid Chromatography parameters for free drug determination*:

[00542] *Column*: Agilent PLRP-S, 50 x 2.1 mm, 8 µm, 1000Å.

[00543] *Detection wavelength*: 280 nm, 220 nM.

[00544] *Column oven temperature*: 80°C.

[00545] *Sampler temperature*: 2~8°C.

[00546] Flow rate was 0.4 mL/min. Stop time was at thirteen minutes. Maximum pressure was 350 bar. Injection Amount was 20 µL.

[00547] *Mobile phases*. Mobile Phase A was 0.025% trifluoroacetic acid + 0.1% formic acid in water. Mobile Phase B was 0.025% trifluoroacetic acid + 0.1% formic acid in acetonitrile. The gradient was as follows: At time 2.00 minutes, A was 95.0%, and B was 5.0%. At time 5.00 minutes, A was 70.0% and B was 30.0%. At time 8.00 minutes, A was 20.0% and B was 80.0%. At time 8.50 minutes, A was 10.0% and B was 90.0%. At time 10.00 minutes, A was 10.0% and B was 90.0%. At time 10.01 minutes, A was 95.0% and B was 5.0%. At time 13.00 minutes, A was 95.0% and B was 5.0%.

[00548] *Quadrupole-time-of-flight parameters for free drug determination*: Equipment was Agilent 6530 QTOF. Polarity was positive. Gas Temp. was 250°C. Drying Gas was 13 L/min. Nebulizer was 45 psig. V_{Cap} was 3500 V. Fragmentor was 125 V. Mass Range was 150 – 8000 m/z. Acquisition Rate was 1 spectra/s.

[00549] *Endotoxin determination*. The endotoxin level was determined by Endosafe® NexGen-PTS™. Diluted TRAP sample with endo-free water, pipetted 25 µL of diluted sample into each of the four reservoirs of the Portable Test System (PTS) Cartridge. The reader drew and mixed the sample with the LAL reagent in the sample channels in addition to the LAL reagent plus positive product control in the spike channels. The sample was combined with the chromogenic substrate then incubated. After mixing, the optical density of the wells was measured and analyzed against an internally archived standard curve.

[00550] *Conjugation buffer and formulation buffer*.

[00551] AGN417 conjugation buffer is 50 mM Tris, 150mM sodium chloride, 10% glycerol, pH 7.5.

[00552] AGN417 formulation buffer is phosphate-buffered saline, pH 7.4.

[00553] *Procedure for bulk conjugation*.

[00554] (1) To two 2000 mL bottle (Corning, 430281) containing 5971.2 mg of **ABT410** in original buffer (50 mM Tris, 150 mM sodium chloride, 10% glycerol, pH 7.5) were added with 5.08 mg/mL sortase A (PT0008, 0.01 equivalents) solution, **TBT404** (40 mM in dimethyl sulfoxide) (eighteen equivalents) into the reactions, mixed well and incubated at 4°C for overnight (seventeen hours). The protein concentration in the reaction was 3.21 mg/mL.

[00555] (2) The crude conjugation was purified with AKTA pure 150 chromatography. The conjugate was bounded to the column in the loading sample process. The sortase A with His-tag and linker-payload

were flowed through. Pooled flow through liquid together and performed buffer exchange to phosphate-buffered saline, pH 7.4 with UF/DF (3 kDa, 2*0.11 m2) for 15 DV.

[00556] (3) The purified sample was filtered with 0.22 μm filter.

[00557] (4) Perform the quality control test (including concentration, size exclusion chromatography-high performance liquid chromatography, HIC-high performance liquid chromatography, LC-MS, free drug, endotoxin level and osmolarity).

[00558] *Results of bulk conjugation*. The reaction condition of bulk conjugation is summarized in the TABLE below. After conjugation, the reaction mixture is purified by AEX and exchange buffer to phosphate-buffered saline, pH 7.4 use UF/DF (3 kDa, 2*0.11 m^2, 10 DV), then filter with 0.22 μm membrane. The molecular information of AGN417 was summarized in the TABLE below. The results of AKTA, LC-MS, free drug, endotoxin level and osmolarity were determined.

[00559] *Reaction conditions for bulk conjugation of **AGN417** (**ABT410-TBT403**)*. Sortase A/monoclonal antibody ratio was 0.01. monoclonal antibody/conjugate ratio was 18. Conjugation concentration was 3.21 mg/mL. Conjugation temperature and time was 4°C, overnight. Conjugation pH was 7-7.5.

[00560] *Molecular information about **AGN417***. In one example, for AGN417 (**ABT410-TBT403**), the BAR1 molecular weight (amino acid,) was 11921.21 Da. Modification was BAR = 1. Measured mass was 11921.92 Da.

[00561] *Results for bulk conjugation*. In one example, for AGN417 (**ABT410-TBT403**): Concentration measured by ultraviolet spectroscopy was 22.45 mg/mL, amount was 5948.45 mg, MS-BAR was 0.91. Size-exclusion chromatography -purity was 97.40%, Free drug was <1.0%; endotoxin was <0.089 EU/mg; and osmolarity was 303 mOsm/kg.

[00562] *Results and discussion of stability test*s. Store the sample under -70°C for one hour to ensure that material is frozen, then the material is allowed to thaw by letting the vial to sit at room temperature for sufficient amount of time until completed thawed. Collect the analytical characterization under different freeze-thaw cycles (1, 2, 3). The resulting ratios were BAR = 0.89-0.91.

TABLE 19

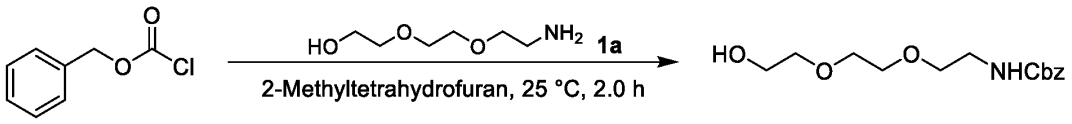
Material name	Material information
Column for LC-MS	Agilent Technologies, PLRP-S, 1000A 8 μm 50*2.1 mm, PL1912-1802
Size Exclusion Chromatography column	TOSOH, TSKgel G3000SWXL, 7.8×300 mm, 5 μm, 08541

TABLE 19	
Material name	Material information
C18 column	Agilent Technologies, InfinityLab Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 µm, 685975-902
Millex-GP Syringe Filter Unit, 0.22 µm	millipore-SLGPR33RB
Acetonitrile	Merck, Supelco-1.00030.4008
Phosphate-buffered saline, Ph7.4	Gibco, 10010031
Trifluoroacetic acid	jkchemical-134753
Na_2HPO_4-$7H_2O$	Sigma-S2429-500G
NaH_2PO_4	Sigma-S5011-500G
$(NH_4)_2SO_4$	Sigma-A4418-1KG
2-propanol	Sigma-34863
Sodium chloride	Domestic

EXAMPLE 8

*Procedure for preparation of **TBT403** (Ins-011)*

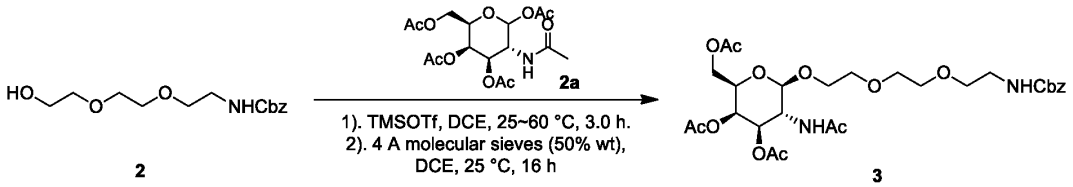
[00563] *Preparation of **intermediate 2**:*



1 **2**

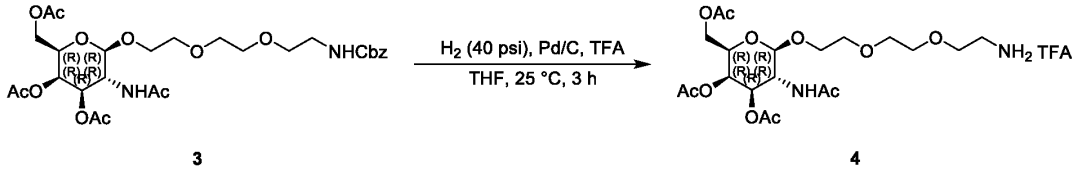
[00564] To a solution of **intermediate 1a** (60.0 g, 400 mmol, 2.0 equivalents) in 2-methyltetrahydrofuran (450 mL) was added 1 (34.2 g, 200 mmol, 1.0 equivalent) in 2-methyltetrahydrofuran (160 mL) at 0°C. The mixture was stirred at 25°C for two hours. Thin layer chromatography (DCM: methanol = 20: 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. 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. LC-MS: 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).

[00565] *Preparation of **intermediate 3**:*



[00566] To a solution of **intermediate 2a** (100.0 g, 257 mmol, 1.0 equivalent) in DCE (500 mL) was added TMSOTf (85.6 g, 385 mmol, 1.5 equivalents) and stirred at 60°C for 2 h. The reaction was then cooled to room temperature (25°C) and stirred for another one hour. A mixture of **Intermediate 2** (80.0 g, 282 mmol, 1.1 equivalents) and 4 Å powder molecular sieves (50.0 g) in DCE (500 mL) was added to the reaction. The resulting mixture was stirred for thirty minutes under nitrogen gas atmosphere. Then a solution of **intermediate 2a** (100.0 g, 257 mmol, 1.0 equivalent) in DCE was added dropwise to the mixture at 0°C. The mixture was stirred for sixteen hours at 25°C under nitrogen gas atmosphere. Thin layer chromatography (DCM: 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. LC-MS: retention time = 0.860 minutes, MS calculated: 612.25, mass observed: $[M + H]^+$ = 613.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.80 (d, J = 9.03 Hz, 1 H), 7.24 - 7.39 (m, 6 H), 5.22 (d, J = 3.51 Hz, 1 H), 4.95 - 5.05 (m, 3 H), 4.53 - 4.59 (m, 1 H), 3.99 - 4.06 (m, 3 H), 3.84 - 3.92 (m, 1 H), 3.73 - 3.82 (m, 1 H), 3.55 - 3.61 (m, 1 H), 3.45 - 3.53 (m, 7 H), 3.41 (t, J = 5.90 Hz, 2 H), 3.11 - 3.18 (m, 3 H), 2.10 (s, 3 H), 1.99 (s, 3 H), 1.89 (s, 3 H), 1.77 (s, 3 H).

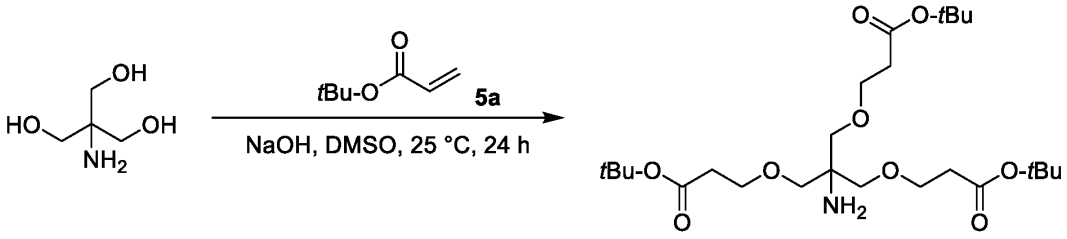
[00567] *Preparation of **intermediate 4**:*



[00568] Pd/C (9.00 g, 10% purity) in reaction bottle (purged with argon gas for three times) was added THF (180 mL) slowly, then a solution of trifluoracetic acid (16.7 g, 147 mmol, 1.0 equivalent) and **intermediate 3** (90.0 g, 147.0 mmol, 1.0 equivalent) in THF (720 mL) was added to the reaction slowly

under nitrogen gas. The reaction was degassed and purged with nitrogen gas and hydrogen gas for three times, then stirred at 25°C for three hours under hydrogen gas atmosphere (40 psi). 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 THF (100 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with THF (100 mL * 2), and the filtrate was concentrated under reduced pressure to get the residue. The residue was diluted with water (1000 mL), washed with DCM (300 mL * 3), the aqueous layer was lyophilized to afford **intermediate 4** (80.0 g, 139.0 mmol, 95.1% purity, 91.8% yield, trifluoracetic acid salt) as a white solid. LC-MS: retention time = 0.484 minutes, MS calculated: 478.22, mass observed: $[M + H]^+$ = 478.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (br t, J = 9.03 Hz, 4 H), 5.21 (d, J = 3.26 Hz, 1 H), 4.96 (dd, J = 11.17, 3.39 Hz, 1 H), 4.54 (d, J = 8.53 Hz, 1 H), 3.98 - 4.08 (m, 3 H), 3.85 - 3.93 (m, 1 H), 3.75 - 3.84 (m, 1 H), 3.59 (br t, J = 5.14 Hz, 3 H), 3.50 - 3.56 (m, 6 H), 2.98 (br s, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.78 (s, 3 H).

[00569] *Preparation of **intermediate 6**:*

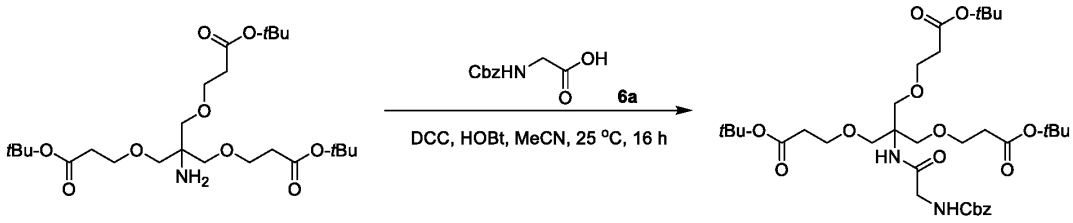


5 **6**

[00570] To a mixture of **intermediate 5** (60.0 g, 495.0 mmol, 1.0 equivalent) in DMSO (166 mL) was added aqueous NaOH (5.0 M, 9.91 mL, 0.1 equivalents) dropwise at 0-15°C for over five minutes. After addition, the mixture was stirred at 0-15°C for five minutes, then **intermediate 5a** (254.0 g, 1.98 mol, 287 mL, 4.0 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. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.51 - 3.61 (m, 7 H), 3.17 (s, 5 H), 2.39 (t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

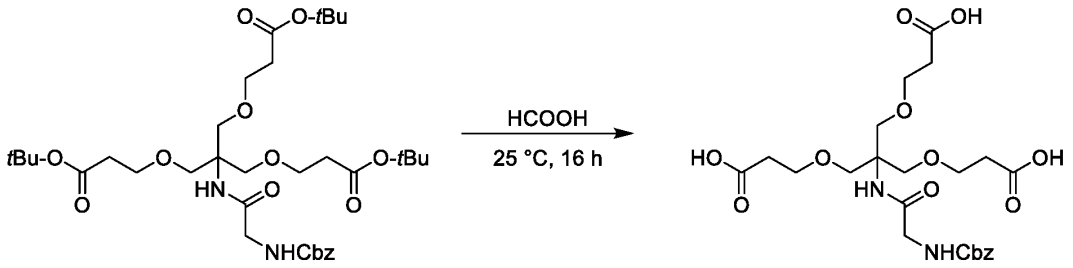
[00571] *Preparation of **intermediate 7**:*



6 7

[00572] To a solution of **intermediate 6** (40.0 g, 79.1 mmol, 1.0 equivalent) in acetonitrile (400 mL) was added hydroxybenzotriazole (10.7 g, 79.1 mmol, 1.0 equivalent). Then 6a (16.5 g, 79.1 mmol, 1.0 equivalent) and DCC (16.3 g, 79.1 mmol, 1.0 equivalent) were added. The reaction was stirred at 25°C for sixteen hours. Thin layer chromatography (PE: EA = 1: 1, R_f = 0.80) indicated **intermediate 6** was consumed completely. One major new spot with lower polarity was detected. Acetonitrile was evaporated to get the residue. The residue was purified by column chromatography (SiO_2, PE: EA = 10: 1 to 1: 1) to afford **intermediate 7** (40.0 g, 57.4 mmol, 82.9% purity, 72.5% yield) as a white solid. LC-MS: retention time = 1.151 minutes, MS calculated: 696.38, mass observed: $[M + H]^+$ = 697.3, $[M + Na]^+$ = 719.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26 - 7.40 (m, 6 H), 7.06 (s, 1 H), 5.03 (s, 2 H), 3.49 - 3.61 (m, 14 H), 2.39 (br t, J = 6.02 Hz, 6 H), 1.40 (s, 27 H).

[00573] *Preparation of **intermediate 8**:*

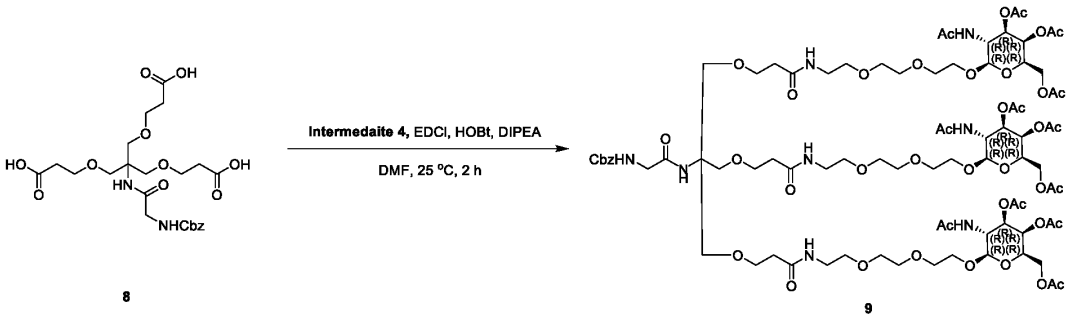


7 8

[00574] A solution of **intermediate 7** (30.0 g, 43.0 mmol, 1.0 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-HPLC (A: 0.1% trifluoracetic acid/water, B: acetonitrile) to afford **intermediate 8** (20.0 g, 37.8 mmol, 98.2% purity, 87.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.17 (br s, 3 H), 7.26 - 7.43 (m, 6 H), 7.06 (s, 1 H), 5.02 (s, 2 H), 3.49 - 3.65 (m, 14 H), 2.42 (br t, J = 6.27 Hz, 6 H). LC-MS: retention time = 0.790 minutes, MS calculated: 528.20, mass observed: $[M + H]^+$ = 529.2.

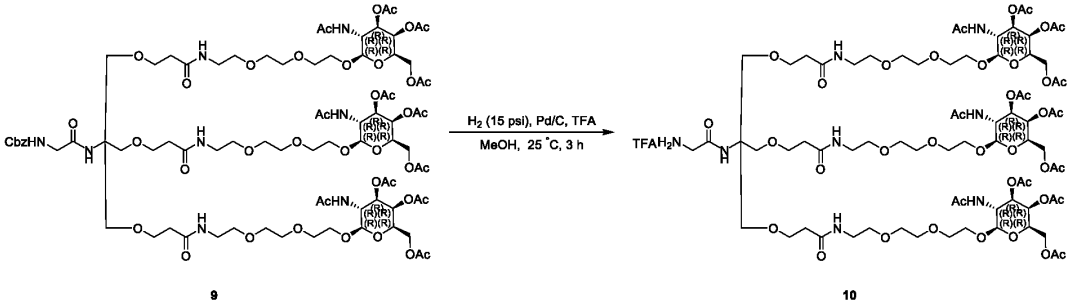
[00575] *Preparation of **intermediate 9**:*



8 9

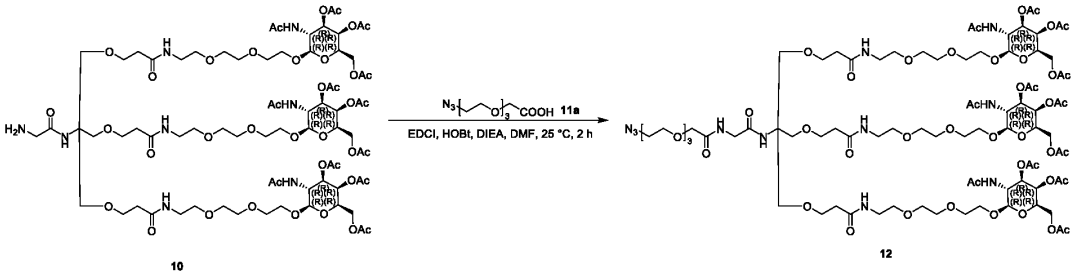
[00576] To a stirring solution of **intermediate 8** (20.0 g, 37.8 mmol, 1.0 equivalent) and **intermediate 4** (78.5 g, 132 mmol, 3.5 equivalents, trifluoracetic acid salt) in DMF (400 mL) was added hydroxybenzotriazole (20.4 g, 151 mmol, 4.0 equivalents), EDCI (29.0 g, 151 mmol, 4.0 equivalents) and DIEA (22.0 g, 170 mmol, 4.5 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% $NaHCO_3$ (450 mL), dried over Na_2SO_4, and concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM: methanol = 100: 1 to 5: 1) to afford **intermediate 9** (58.0 g, 30.4 mmol, 82.7% purity, 80.3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 (br t, J = 5.14 Hz, 3 H), 7.81 (d, J = 9.03 Hz, 3 H), 7.28 - 7.39 (m, 6 H), 7.13 (s, 1 H), 5.21 (d, J = 3.26 Hz, 3 H), 5.02 (s, 2 H), 4.97 (dd, J = 11.17, 3.39 Hz, 3 H), 4.54 (d, J = 8.53 Hz, 3 H), 4.03 (s, 9 H), 3.84 - 3.92 (m, 3 H), 3.75 - 3.81 (m, 3 H), 3.45 - 3.61 (m, 37 H), 3.39 (br s, 3 H), 3.18 - 3.23 (m, 6 H), 2.30 (br t, J=6.15 Hz, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H). LC-MS: retention time = 3.455 minutes, MS calculated: 1908.81, mass observed: $[M + 2H]^{2+}$ = 955.7.

[00577] *Preparation of **intermediate 10**:*



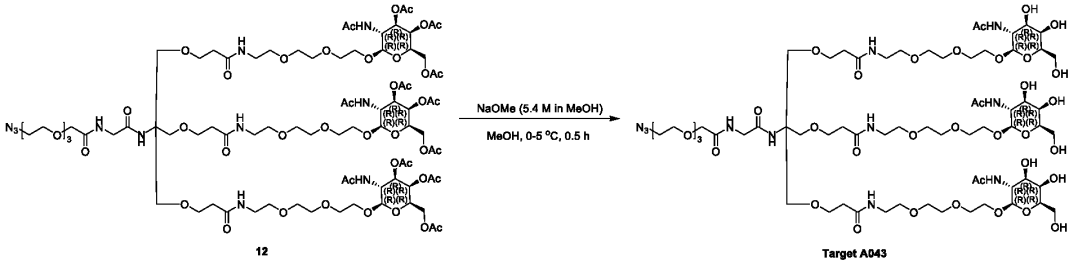
[00578] Pd/C (4.6 g, 25.13 mmol, 10% purity, 1.0 equivalent) in reaction bottle (purged with nitrogen gas for three times) was added methanol (230 mL) slowly, then a solution of trifluoracetic acid (2.75 g, 24.1 mmol, 1.0 equivalent) and **intermediate 9** (46.0 g, 24.1 mmol, 1.0 equivalent) in methanol (230 mL) was added to the reaction slowly under nitrogen gas atmosphere. The reaction was degassed and purged with nitrogen gas and hydrogen gas for three times, stirred at 25°C for two hours under hydrogen gas atmosphere (15 psi). Thin layer chromatography (DCM: methanol = 10: 1, R_f = 0.3) indicated **intermediate 9** was consumed completely, and one major new spot with larger polarity was detected. The reaction mixture was dissolved in methanol (250 mL), filtered carefully through siliceous earth under nitrogen gas atmosphere, the cake was washed with methanol (250 mL * 2), and the filtrate was concentrated under reduced pressure to afford **intermediate 10** (crude, 42.0 g, 22.4 mmol, 94.1% purity, 92.2% yield, trifluoracetic acid salt) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.97 (br t, J = 5.44 Hz, 6 H), 7.81 - 7.88 (m, 3 H), 7.73 - 7.77 (m, 1 H), 5.19 - 5.26 (m, 3 H), 4.97 (dd, J = 11.13, 3.38 Hz, 3 H), 4.52 - 4.61 (m, 3 H), 3.99 - 4.06 (m, 10 H), 3.84 - 3.93 (m, 4 H), 3.74 - 3.82 (m, 4 H), 3.46 - 3.61 (m, 42 H), 3.38 - 3.42 (m, 8 H), 3.19 - 3.24 (m, 6 H), 2.28 - 2.34 (m, 6 H), 2.08 - 2.12 (m, 9 H), 1.98 - 2.02 (m, 9 H), 1.87 - 1.90 (m, 9 H), 1.75 - 1.80 (m, 9 H). LC-MS: retention time = 1.585 minutes, MS calculated: 1774.78, mass observed: $[M + H]^+$ = 1775.7, $[M + 2H]^{2+}$ = 888.7.

[00579] *Preparation of **intermediate 12**:*



[00580] DIEA (8.62 g, 66.6 mmol, 3.0 equivalents), EDCI (6.69 g, 33.3 mmol, 1.5 equivalents) and hydroxybenzotriazole (6.38 g, 33.3 mmol, 1.5 equivalents) were added to the solution of **intermediate 10** (42.0 g, 22.2 mmol, 1.0 equivalent, trifluoracetic acid salt) and 11a (7.77 g, 33.3 mmol, 1.5 equivalents) in DMF (420 mL). The mixture was stirred at 25°C for 2.0 hours. LC-MS indicated **intermediate 10** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was slowly poured into a stirring 0.5 mol/L hydrochloric acid solution (cold, 500 mL). The aqueous phase was extracted with DCM (500 mL * 3). The combined organic layers were washed with 5% $NaHCO_3$ (500 mL), dried over Na_2SO_4, then concentrated under reduced pressure to get a residue. The residue was purified by column chromatography (SiO_2, DCM: methanol = 100: 1 to 5: 1) to afford **intermediate 12** (34.0 g, 17.1 mmol, 93.3% purity, 77.0% yield) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.88 - 7.94 (m, 3 H), 7.78 - 7.83 (m, 3 H), 7.71 - 7.76 (m, 1 H), 7.21 - 7.26 (m, 1 H), 5.19 - 5.23 (m, 3 H), 4.94 - 5.02 (m, 3 H), 4.51 - 4.59 (m, 3 H), 4.00 - 4.06 (m, 9 H), 3.91 - 3.93 (m, 2 H), 3.84 - 3.90 (m, 3 H), 3.72 - 3.81 (m, 5 H), 3.46 - 3.63 (m, 46 H), 3.37 - 3.43 (m, 9 H), 3.18 - 3.23 (m, 6 H), 2.27 - 2.34 (m, 6 H), 2.09 - 2.12 (m, 9 H), 1.98 - 2.01 (m, 9 H), 1.88 - 1.90 (m, 9 H), 1.75 - 1.79 (m, 9 H). LC-MS: retention time = 0.501 minutes, MS calculated: 1989.87, mass observed: $[M + 2H]^{2+}$ = 996.3.

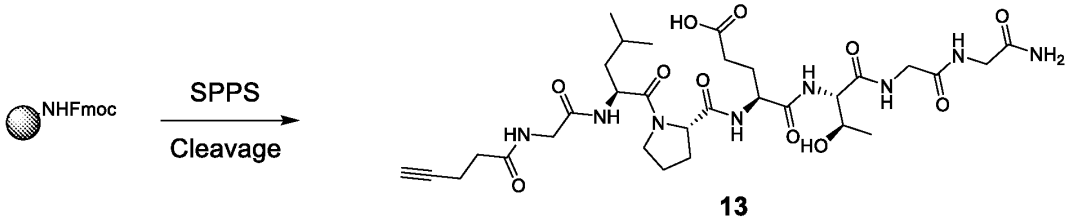
[00581] *Preparation of **Target A043**:*



[00582] To a solution of **intermediate 12** (34.0 g, 12.6 mmol, 1.0 equivalent) in methanol (400 mL) was added NaOMe (5.4 M in methanol, 9.91 mL, 4.2 equivalents) at 0°C. Then the solution was stirred at 0°C for 0.5 h. LC-MS indicated **intermediate 12** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was adjusted pH to 6 with 1.0 M hydrochloric acid solution at 0°C and washed with DCM (250 mL * 3) for three times, the aqueous layer was lyophilized to afford **Target A043** (crude, containing sodium chloride, 20.0 g, 12.4 mmol, 96.3% purity) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 - 7.98 (m, 3 H), 7.73 - 7.77 (m, 1 H), 7.61 - 7.65 (m, 3 H), 7.22 - 7.27 (m, 1 H), 4.59 - 4.64 (m, 3 H), 4.55 - 4.58 (m, 3 H), 4.49 - 4.52 (m, 3 H), 4.25 - 4.31 (m, 3 H), 3.90 - 3.94 (m, 2 H), 3.68 - 3.81 (m, 9 H), 3.63 - 3.67 (m, 4 H), 3.59 - 3.62 (m, 6 H),

3.58 - 3.62 (m, 7 H), 3.38 - 3.41 (m, 13 H), 3.17 - 3.24 (m, 7 H), 2.28 - 2.34 (m, 7 H), 1.78 - 1.82 (m, 9 H). LC-MS: retention time = 0.501 minutes, MS calculated: 1611.77, mass observed: $[M + H]^+$ = 1612.8, $[M + 2H]^{2+}$ = 807.2.

[00583] *Preparation of **intermediate 13**:*



13

[00584] The peptide was synthesized using standard Fmoc chemistry.

[00585] (1) *Resin preparation*: To the vessel containing Rink amide MBHA Resin (sub: 0.27 mmol/g, 5.00 mmol, 18.5 g) and DMF (150 mL) was bubbled with nitrogen gas for two hours at 25°C. Then 20% piperidine in DMF (200 mL) was added and the mixture was bubbled with nitrogen gas for thirty minutes at 25oC. The mixture was filtered and washed with DMF (200 mL) * 5 before proceeding to next step.

[00586] (2) *Coupling*: A solution of Fmoc-G-G-OH (3.54 g, 10.00 mmol, 2.00 equivalents), HBTU (7.2 g, 9.5 mmol, 1.90 equivalents), DIEA (5.16 g, 40.00 mmol, 4.00 equivalents) in DMF (100 mL) was added to the resin with nitrogen gas bubbling for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test. The resin was then washed with DMF (200 mL) * 5.

[00587] (3) *De-protection*: 20% piperidine/DMF (300.0 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 DMF (300.0 mL) *5.

[00588] (4) Step 2 to 3 was repeated for all other amino acids: (#7 in TABLE below). At the last step, the resin was washed with methanol (300.0 mL) * 3 and dried by nitrogen gas bubbling for three hours.

TABLE 20
The list of amino acids and the corresponding reagents used on SPPS.

#	Materials	Coupling reagents
1	Fmoc-Gly-Gly-OH (2.00 equivalents)	HBTU (1.90 equiv.) and DIEA (4.00 equiv.)
2	Fmoc-Thr-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Glu-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
4	Fmoc-Pro-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.)

TABLE 20
The list of amino acids and the corresponding reagents used on SPPS.

#	Materials	Coupling reagents
6	Fmoc-Gly-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
7	4-pentynoic acid (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)

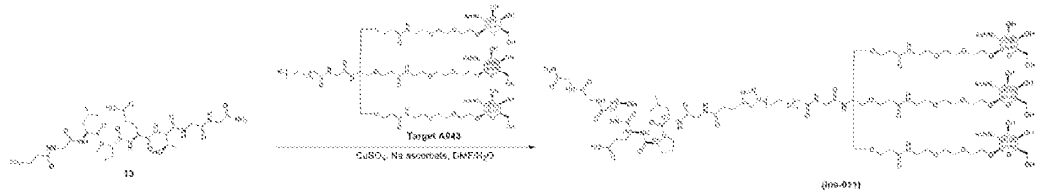
[00589] *Peptide cleavage and cyclization.*

[00590] (1) Cleavage solution (TFA/TIS/water, 95/2.5/2.5, v/v/v, 220 mL) was added to the flask containing the side-chain protected resin-bound peptide (RINK AMIDE MBHA Resin, 22 g, 5.0 mmol) at 25°C and stirred for two hours.

[00591] (2) After filtration, the filtrate was collected.

[00592] (3) The filtrate was precipitated with cold isopropyl ether (1000 mL). After filtration, the solid was washed with isopropyl ether (500 mL) twice, and the crude peptide was dried under reduced pressure for 2 h to afford **intermediate 1** (5 g, crude) as a white solid. The crude peptide was purified by prep-HPLC (A: 0.1% trifluoracetic acid/water, B: acetonitrile), followed by lyophilization to afford **intermediate 13** (1.5 g, 90% purity, 42.3% yield) as a white solid.

[00593] *Preparation of **TBT403** (Ins-011):*



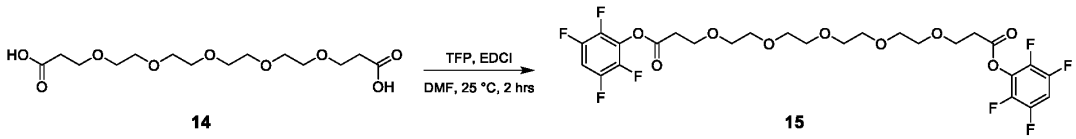
[00594] To a solution of **Target A043** (2.184 g, 1.354 mmol, 1.2 equivalents) and **Intermediate 13** (0.8 g, 1.129 mmol, 1.0 equivalent) in DMF (1 mL) and water (10 mL) was added a solution of sodium ascorbate (670.827 mg, 3.386 mmol, 3.0 equivalents) and TBTA (Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine, 490.430 mg, 1.129 mmol, one equivalent) and $CuSO_4$-$5H_2O$ (0.4 M, 2.822 mL, 1.0 equivalent) in water (3 mL) at 0°C. Then the mixture was stirred at 25°C for one hour. LC-MS indicated **Intermediate 13** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The mixture was purified by prep-HPLC (A: 0.5% acetic acid/water, B: acetonitrile) followed by lyophilization to afford **TBT403** (Ins-011) (1.42 g, 94% purity, 50.9% yield) as a white solid. LC-MS: retention time =0.15 minutes, MS calculated: M_{av} =2321.4, mass observed: $[M + H]^+$ =2321.9.

EXAMPLE 9

*Procedure for reparation of **TBT405** (Ins-030)*

[00595] To a solution of Intermediate 15 (99.0 g, 52.4 mmol, 1.00 equivalent, trifluoracetic acid) in methanol (1.00 L) was added NaOMe (11.3 g, 210 mmol, 4.00 equivalents). The mixture was stirred at 25°C for one-half hour. LC-MS showed **intermediate 15** was consumed completely and one main peak with desired mass. The reaction mixture was concentrated under reduced pressure to remove methanol. The residue was diluted with water (500 mL) and washed with DCM (500 mL *3), the aqueous layer was lyophilized to give a crude product. The crude product was purified by prep-HPLC (A: 0.5% acetic acid/water, B: acetonitrile) to afford **Target A001A** (36.0 g, 25.7 mmol, 49.1% yield, 97.5% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ ppm 7.95 (br t, *J* = 5.38 Hz, 3 H), 7.63 (br d, *J* = 8.88 Hz, 3 H), 7.57 - 7.50 (m, 1 H), 4.28 (d, *J* = 8.51 Hz, 3 H), 3.84 - 3.70 (m, 6 H), 3.70 - 3.60 (m, 6 H), 3.59 - 3.46 (m, 43 H), 3.32 - 3.29 (m, 4 H), 3.25 - 3.19 (m, 6 H), 3.05 (s, 2 H), 2.31 (br t, *J* = 6.25 Hz, 6 H), 1.81 (s, 9 H). LC-MS: retention time = 1.103 minutes, MS calculated:1397.5, found: $[M + H]^+$ = 1397.5, $[(M + 2H)/2]^{2+}$ = 699.6.

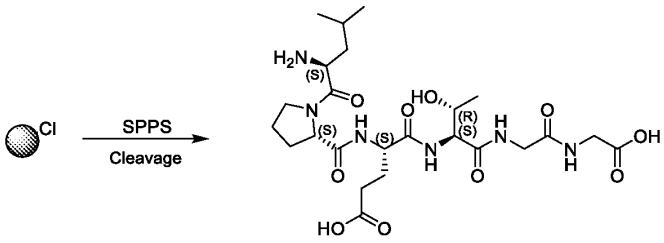
[00596] *Preparation of **intermediate 15**:*



14 **15**

[00597] To a solution of **intermediate 14** (15 g, 44.333 mmol, 1.0 equivalent) in DMF (50 mL), was added a solution of 2,3,5,6-tetrafluorophenol (18.406 g, 110.832 mmol, 2.5 equivalents) in DMF (50 mL) at 0°C. Then a solution of EDCI (21.247 g, 110.832 mmol, 2.5 equivalents) in DMF (50 mL) was added to the mixture and it was stirred at 25°C for two hours. LC-MS indicated **intermediate 14** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The crude solution was purified by prep-HPLC (A: 0.1% TFA/water, B: acetonitrile) followed by lyophilization to afford **intermediate 15** (22 g, 97% purity, 75.8% yield) as a colorless oil. LC-MS: retention time = 0.724 minutes, MS calculated: M_{av} = 634.5, found: $[M +H]^+$ = 635.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.93 (tt, *J*=10.92, 7.40 Hz, 2 H), 3.77 (t, *J*=5.90 Hz, 4 H), 3.58 - 3.47 (m, 16 H), 3.02 (t, *J*=5.90 Hz, 4 H).

[00598] *Preparation of **intermediate 16**:*



16

[00599] Peptide was synthesized using standard Fmoc chemistry (CTC resin).

[00600] (1) *Resin preparation*: To the vessel containing CTC Resin (sub: 0.55 mmol/g, 15.00 mmol, 27.3 g) and Fmoc-Gly-Gly-OH (5.3 g, 15 mmol, 1.0 equivalent) in DCM (200 mL) was added DIEA (4.0 equivalents) dropwise and mixed for 2 h with nitrogen gas bubbling at 25°C. Then methanol (15.0 mL) was added and bubbled with nitrogen gas for another thirty minutes. The resin was washed with DMF (100 mL) * 5, followed by the addition of 20% piperidine in DMF (200 mL) and bubbled with nitrogen gas for thirty minutes at 25°C for Fmoc deprotection. The mixture was filtered. The resin was washed with DMF (100 mL) * 5 before proceeding to next step.

[00601] (2) *Coupling*: A solution of Fmoc-Thr-OH (17.9 g, 45 mmol, 3.0 equivalents), HBTU (16.3 g, 42.75 mmol, 2.85 equivalents) in DMF (100 mL) was added to the resin with nitrogen gas bubbling. Then DIEA (6.0 equivalents) was added to the mixture dropwise and bubbled with nitrogen gas for thirty minutes at 25°C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (200 mL) * 5.

[00602] (3) *Deprotection*: 20% piperidine in DMF (200 mL) was added to the resin and the mixture was bubbled with nitrogen gas for thirty minutes at 25°C. The resin was then washed with DMF (200 mL) * 5.

[00603] (4) Step 2 to 3 was repeated for all other amino acids: (#3-5 in TABLE below). At the last step, the resin was washed with methanol (200.0 mL) * 3 and dried by nitrogen gas bubbling for three hours.

TABLE 21
The list of amino acids and the corresponding reagents used on SPPS.

#	Materials	Coupling reagents
1	Fmoc-Gly-Gly-OH (1.00 equivalent)	DIEA (4.00 equivalents)
2	Fmoc-Thr-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)
3	Fmoc-Glu-OH (3.00 equivalents)	HBTU (2.85 equiv.) and DIEA (6.00 equiv.)

TABLE 21 The list of amino acids and the corresponding reagents used on SPPS.		
#	Materials	Coupling reagents
4	Fmoc-Pro-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.)

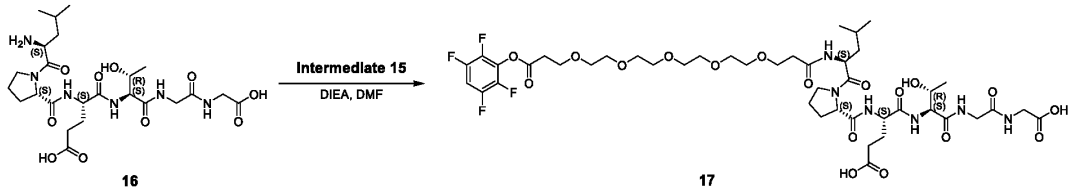
[00604] *Peptide cleavage and cyclization*.

[00605] (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 (CTC Resin, 30 g, 15.0 mmol) at 25°C and stirred for 2 h.

[00606] (2) After filtration, the filtrate was collected.

[00607] (3) The filtrate was precipitated with cold isopropyl ether (1500 mL). After filtration, the solid was washed with isopropyl ether (500 mL) twice, and the crude peptide was dried under reduced pressure for two hours to afford **Intermediate 1** (10 g, crude) as a white solid. The crude peptide was purified by prep-HPLC (A: 0.1% TFA/water, B: acetonitrile) followed by lyophilization to afford in**termediate 16** (6.0 g, 93% purity, 69% yield) as a white solid. LC-MS: retention time = 0.304 minutes, MS calculated: M_{av} =572.6, found: [M +H]+ = 573.4.

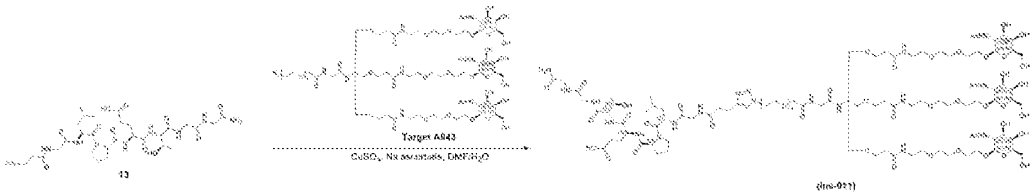
[00608] *Preparation of **intermediate 17**:*



16 17

[00609] To a solution of **intermediate 15** (13.296 g, 20.957 mmol, 2.0 equivalents) and DIEA (2.709 g, 20.957 mmol, 3.464 mL, 2.0 equivalents) in DMF (15 mL) was added **intermediate 16** (6 g, 10.478 mmol, 1.0 equivalent) in DMF (15 mL) slowly at 25°C under nitrogen gas. The mixture was stirred at 25°C for one hour. LC-MS indicated **intermediate 16** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The mixture was adjusted to pH 5-6 with acetic acid. The crude solution was purified by prep-HPLC (A: 0.5% acetic acid/water, B: acetonitrile) followed by lyophilization to afford **intermediate 17** (3.1 g, 95% purity, 28.4% yield) as a white solid. LC-MS: retention time = 0.491 minutes, MS calculated: M_{av} =1040.9, found: $[M+H]^+$ = 1041.7.

[00610] *Preparation of **TBT405** (Ins-030):*



[00611] To a solution of **Target A001A** (700 mg, 500.906 µmol, 1.0 equivalent) and **intermediate 17** (548.89 mg, 500.906 µmol, 1.0 equivalent) in DMF (10 mL) was added DIEA (129.477 mg, 1.002 mmol, 2.0 equivalents) slowly at 0°C under nitrogen gas. The mixture was stirred at 25°C for one hour. LC-MS indicated **intermediate 17** was consumed completely, several new peaks were shown on LC-MS and desired compound was detected. The mixture was adjusted to pH 5-6 with 1M hydrochloric acid at 0°C. The crude solution was purified by prep-HPLC (A: 0.5% acetic acid/water, B: acetonitrile) followed by lyophilization to afford **TBT405** (Ins-030) (495.3 mg, 99.5% purity, 42.9% yield) as a white solid. LC-MS: retention time =0.328 minutes, MS calculated: M_{av} =2272.4, mass observed: $[M + H]^+/2$ =1136.7.

EXAMPLE 10

Biophysical assays. Surface plasmon resonance – second results.

[00612] *Surface plasmon resonance for insulin growth factor 1 receptor assay conditions*:

[00613] *Buffer*: HSB-N with 0.005% Tween-20, pH: 7.4 90 seconds on, 180 seconds off.

[00614] Capture human insulin growth factor receptor (His-Avitag) from Acro on a SA chip at ~750, 1000, and 1500 RU.

[00615] Assess binding of insulin to the receptor (10 µM top, 2-fold dilution).

[00616] *Surface plasmon resonance for insulin growth factor 1 receptor*: Avi-tagged receptor captured on an SA chip. The inventors assayed insulin and insulin lispro.

[00617] Surface shows better activity at higher density (50% versus 65% active)

[00618] Traces were sufficient for a selectivity assay.

[00619] *Conclusion*: A robust surface plasmon resonance assay was developed for insulin growth factor 1 receptor. Mutant insulin has significantly reduced insulin growth factor 1 receptor binding

TABLE 22
Surface plasmon resonance

	IGF-1 receptor
AGN303 (native proinsulin)	K_D = 909 nM
AGN310 (mutant proinsulin)	K_D = 10.6 µM
Insulin	K_D = 1.2 µM

TABLE 23
Surface plasmon resonance updates

Sample	Sample info	IR-B K_D	IGF-1R K_D (µM)	mAb49 K_D (µM)	C-peptide Ab K_D (µM)	ASGPR IC_{50} (µM)
Insulin	Control	0.34 µM	1.24	0.11	-	-
AGN417-03	Proinsulin degrader	8.22 µM	18	0.80	0.20	9.8
AGN417-06	Proinsulin degrader	6.82 µM	22	0.84	0.09	
AGN421-03	Mutant proinsulin degrader	22.5 µM	NB	1.20	0.23	13.2
AGN8138-01	AGN417 with 13 and N15 labels	6.99 µM	17	0.91	0.16	4
AGN8139-01	AGN417 with 13 and N15 labels	6.48 µM	17	0.99	0.14	4.5
ABT17384-01	Mutant proinsulin + lysine mutation	6.34 µM	NB	0.24	0.07	-
ABT17385-01	Mutant proinsulin + methionine	4.54 µM	NB	0.22	0.08	-
ABT17386-01	Mutant proinsulin + cysteine	>5.0 µM	22	3.13	0.07	-

EXAMPLE 11
*Determination of total conjugate of **AGN6567** in Rat K2EDTA plasma by LC-MS/MS*

[00620] *Purpose*. An LC-MS/MS method was developed for the determination of total antibody from **AGN6567** in rat K_2EDTA Plasma using SIL TTPP as the internal standards (IS). This method is used for non-GLP studies. **AGN6567** is too large for direct analysis by LC-MS. Its signature peptide ELVAVITTDGSTNYADSVK (SEQ ID NO: 51) is used for the quantitation of **AGN6567** after the immunoprecipitation and digestion step in rat plasma.

TABLEX Method Summary		
Assay Type	UPLC-MS/MS	
Compounds	**AGN6567**	Solid: No
Internal Standards	SIL ELVA	Solid: Yes
Species/Matrix	Rat Plasma	
Elution	Gradient	
Sample Treatment	Immunocapture and Tryptic digestion	
Sample Vol Requirement	10 µL	
Run Time	10.0 min	
Assay Range (µg/mL) in Rat K2EDTA Plasma	0.1~20	
Solution Storage Temp (°C)	-70°C	
QC Storage Temp (°C)	-70°C	
Injection Volume	15 µL	
Special Conditions Required	N/A	

[00621] *Equipment* (as listed or equivalent). The following equipment, or its equivalent, is utilized during the execution of the method:

TABLE 23	
Equipment	Vendor/Model
LC-MS/MS	Sciex API 6500+ with Shimadzu HPLC pumps and autosampler
Valve	From system
Centrifuge	Beckman GS-6R
Incubation Plate Shaker	VWR
Incubator	Heated Microplate Vortexer
KingFisher Purification System	KingFisher Flex

TABLE 23	
Equipment	Vendor/Model
Analytical Balance	Sartorius CP225D, Capable of weighing 0.00001g
Micro Balance	Mettle Toledo, Capable of weighing 0.000001g
Water System	Sys# 56-2
PureProteome™ Magnetic Stand, 15 mL	Millipore
96 Well plate-1	2 mL KingFisher Deepwell 96 Plate, V-bottom, polypropylene
96 tip comb	KingFisher 96 tip comb for DW magnets
96 well microplate	KingFisher 96 KF microplate (200 µL)

[00622] *Reagents and materials* (as listed or equivalent). The following reagents and materials, or the equivalent, are utilized during the execution of the method:

TABLE 24	
Reagent	Grade
Methanol	HPLC
Acetonitrile	HPLC
Water	Purified
Formic acid	GR
Rat K2EDTA plasma	N/A
Dynabeads M-280 streptavidin from Invitrogen (catalogue No: 60210) or equivalent	N/A
SBA, biotinylated	WOVUS27560 (Vector Laboratories)
RapiGest	N/A
Phosphate-buffered saline with Tween 20X	GR
Phosphate-buffered saline 10X	GR
1M Tris HCl pH 8.0	GR
Trifluoroacetic acid	GR
Trypsin from Promega (V5113)	Sequencing grade
Tween-20	GR

[00623] *HPLC conditions for Sciex 6500+*: The instrument conditions may be adjusted to optimize the response.

TABLE 25

Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
Autosampler wash	Methanol
Autosampler temperature	4°C
Separation type	Gradient
Flow rate	0.5 mL/min
Gradient	Time (min) / Module / Events / Parameter 1.0 / Pumps / Pump B / Conc. 15 4.00 / Pumps / Pump B / Conc. 30 4.10 / Pumps / Pump B / Conc. 95 5.0 / Pumps / Pump B / Conc. 95 5.01 / Pumps / Pump B / Conc. 15 6.00 / Controller / Stop /
Column	Waters Acquity, UPLC BEH Phenyl C18, 2.1 × 50 mm, 1.7 µm, part No. 186002884
Column temperature	40°C
Injection volume	15 µL (may be adjusted based on the sensitivity and platform)
Retention time (minutes)	ELVA ~1.3 SIL ELVA ~1.3
*Switching valve	Event / Time (min) T1 / 1.0 T2 / 4.0
Data acquisition time	Eight minutes

[00624] *MS/MS Conditions*: The instrument conditions may be adjusted to optimize the response. SIL ELVA peptide: EL*VAVITTDGSTNYADSVK (L*=Leu^{13}C6,^{15}N)

TABLE 26

Mass Spectrometer	Sciex API 6500+ or 7500
Interface	ESI
Ionization Mode	Positive

Detection Mode	MRM		
Analyte MS Transitions	ELVA	SIL ELVA	
	992.0→ 1257.6	995.8→ 1257.6	
	MW = 1983 Da	MW = 1990 Da	
Data acquisition time	Four minutes		
Settings: General	Voltage	5500	
	Temp	550	
	GS1	50	
	GS2	50	
	CUR	20	
	CAD	8	
Settings: Compound dependent	Conj ELVA	DP: 80 CE: 48 CXP: 10 EP: 10	
	SIL ELVA	DP: 80 CE: 48 CXP: 10 EP: 10	

[00625] *Preparations*. One full 96-well plate will need 4 mL M-280 beads and four tubes of the SBA, Biotinylated Antibody™. The preparations described below may be scaled up or down to provide larger or smaller amounts of the reagents, mobile phases, etc.

[00626] 1. phosphate-buffered saline buffer (wash buffer). Add 200 mL of phosphate-buffered saline buffer 10× to 1800 mL of purified water. Refrigerate the buffer.

[00627] 2. 1× phosphate-buffered saline with Tween (blocking buffer). Mix 50 mL of 20× phosphate-buffered saline with Tween solution with 950 mL of purified water. Mix well and store at room temperature.

[00628] 3. Immobilize capturing reagent to Dynabeads M-280 Streptavidin beads.

[00629] 4. Coat antibody to Dynabeads M-280 Streptavidin beads

[00630] 5. Vortex and thoroughly re-suspend magnetic beads.

[00631] 6. Transfer 5000 µL (equal to 10 mg of Dynabeads M-280 streptavidin beads) of Dynabeads M-280 streptavidin beads into a 5 mL Protein LoBind® tube.

[00632] 7. Apply magnet to engage beads to the side of the tube and discard the storage buffer.

[00633] 8. Wash beads by adding 4000 µL of phosphate-buffered saline with Tween and vortex to re-suspend the beads

[00634] 9. Apply magnet to engage beads to the side of the tube and remove the supernatant.

[00635] 10. Repeat wash again.

[00636] 11. Add 4000 µL of phosphate-buffered saline with Tween and 100 µL of SBA, Biotinylated Antibody (5 mg/mL) to the washed beads.

[00637] 12. Mix thoroughly and incubate at room temperature while rotating for 60 minutes. Apply magnet and remove supernatant.

[00638] 13. Wash beads two more times by phosphate-buffered saline with Tween and re-suspend beads with 5000 µL phosphate-buffered saline with Tween to a final concentration of 10 mg/mL in phosphate-buffered saline with Tween.

[00639] 14. Beads can be kept in phosphate-buffered saline with Tween at 4°C.

[00640] 15. 10% acetonitrile in water (diluent). Add 10 mL acetonitrile to 90 mL purified water. Mix well. Store at room temperature.

[00641] 16. 300 mM calcium chloride (assay buffer). Dissolve 333 mg of calcium chloride in 10 mL of purified water. Mix well. Store at room temperature. Aliquot appropriately and store at -70°C.

[00642] 17. 50 mM ammonium bicarbonate (base buffer). Dissolve 1.98 g of ammonium bicarbonate with 500 mL purified water. Store at room temperature for one month.

[00643] 18. RapiGest Solution 1mg/mL (denaturation solution).

[00644] 19. 50 mg (pre-weighed) of RapiGest powder is dissolved in 40 mL of 50 mM ammonia bicarbonate and 10 mL of acetonitrile. Make small portions into aliquots and store at -70°C for up to three months.

[00645] 20. 100 mM DTT (Reduction solution). Dissolve 154 mg of DTT (MW 154) in 10 mL of purified water. Aliquot appropriately and store at -70°C.

[00646] 21. 100 mM iodoacetamide (alkylation buffer). Dissolve 185 mg of iodoacetamide in 10 mL of 50 mM ammonium bicarbonate solution. Aliquot appropriately and store at -70°C.

[00647] 22. 10 % trifluoroacetic acid (acid buffer). Add 5 mL of trifluoroacetic acid to 45 mL of purified water. Vortex to mix well. Store at room temperature.

[00648] 23. Mobile Phase A: 0.1% formic acid in water (Mobile Phase A). Add 2 mL formic acid in 2000 mL of purified water. Mix well. Store at room temperature.

[00649] 24. Mobile Phase B: 0.1% formic acid in acetonitrile (Mobile Phase B). Add 2 mL formic acid in 2000 mL of acetonitrile. Mix well. Store at room temperature.

[00650] 25. Needle Wash: 0.1% formic acid in isopropanol/water 50/50 (Needle Wash). Add 2 mL formic acid in 1000 mL of isopropanol and 1000 mL of purified water into a glass bottle. Store at room temperature for six months.

[00651] 26. Preparation of Standard Stock Solutions

[00652] Standard (STD) Stock 1: Freshly pipet 49 µL (8.16 mg/mL) of **AGN6567** to 351 µL rat K_2EDTA Plasma to give a final concentration of 1 mg/mL for total antibody in rat K_2EDTA Plasma.

[00653] Standard (STD) Stock 2: Pipet 100 µL STD stock 1 to 900 µL rat K_2EDTA Plasma to give a final concentration of 100 µg/mL for total antibody in rat K_2EDTA Plasma.

[00654] Standard (STD) Stock 3: Pipet 100 µL STD stock 2 to 900 µL rat K_2EDTA Plasma to give a final concentration of 10 µg/mL for total antibody in rat K_2EDTA Plasma.

[00655] Standard (STD) Stock 4: Pipet 100 µL STD stock 3 to 900 µL rat K_2EDTA Plasma to give a final concentration of 1 µg/mL for total antibody in rat K_2EDTA Plasma.

[00656] The exact pipetting volume for stock solution should be calculated based on the actual antibody concentration of Reference Standard solutions.

[00657] STD stock 1 and 2 are prepared freshly before use and discarded after use.

[00658] Prepare the working standards in rat K_2EDTA Plasma as detailed in the following TABLE:

TABLE 27
Volume of source solution used (µL)

Working standard solution ID	Source solution ID	Volume of source solution used (µL)	Add volume (µL) of Rat K2EDTA Plasma	Total volume (µL)	Concentration of total antibody in Rat K2EDTA Plasma (µg/mL)
STD8	STD stock 2	80	320	400	20
STD7	STD stock 2	64	336	400	16
STD6	STD stock 2	48	352	400	12
STD5	STD stock 2	32	368	400	8
STD4	STD stock 3	80	320	400	2
STD3	STD stock 3	16	384	400	0.4
STD2	STD stock 4	80	320	400	0.2
STD1	STD stock 4	40	360	400	0.1
SST	STD stock 4	40	360	400	0.1

[00659] *Preparation of Quality Control Stock Solutions*: The exact pipetting volume for stock solution should be calculated based on the actual antibody concentration of Reference Standard solutions.

[00660] *Quality Control (QC) Stock 1*: Freshly pipet 49 µL (8.16 mg/mL) of **AGN6567** to 351 µL rat K_2EDTA Plasma to give a final concentration of 1 mg/mL for total antibody in rat K_2EDTA Plasma.

[00661] *Quality Control (QC) Stock 2*: Pipet 100 µL STD stock 1 to 900 µL rat K2EDTA Plasma to give a final concentration of 100 µg/mL for total antibody in rat K_2EDTA Plasma.

[00662] *Quality Control (QC) Stock 3*: Pipet 100 µL STD stock 2 to 900 µL rat K2EDTA Plasma to give a final concentration of 10 µg/mL for total antibody in rat K_2EDTA Plasma.

[00663] *Quality Control (QC) Stock 4*: Pipet 100 µL STD stock 3 to 900 µL rat K2EDTA Plasma to give a final concentration of 1 µg/mL for total antibody in rat K_2EDTA Plasma.

[00664] Preparation of Quality Control Samples. Quality control (QC) samples are prepared by spiking individual QC stock solutions into blank rat K_2EDTA Plasma described in the TABLE below:

TABLE 28
Volume of source solution used (µL)

Working standard solution ID	Source solution ID	Volume of source solution used (µL)	Add volume (µL) of rat K2EDTA Plasma	Total volume (µL)	Concentration of total antibody in rat K2EDTA Plasma (µg/mL)
AQL QC	QC stock 1	300	100	100	750
HQC	QC stock 2	60	340	400	15
MQC	QC stock 2	24	376	400	6
LQC	QC stock 3	12	388	400	0.3
LLOQ	QC stock 4	40	360	400	0.1
LLOQ samples were only used for validation. After preparation, each QC sample is aliquoted into individual tubes. QC samples are stored at -70°C. Always store the QC samples under the same conditions as study samples.					

Preparation of Internal Standard Solution

[00665] *SIL ELVA Stock*: Dissolve 1 mg of SIL ELVA into 1 mL of diluent to give a concentration of 1 mg/mL for SIL ELVA and store at -70°C.

[00666] *IS Stock 1*: Pipet 20 µL of SIL ELVA stock to 1980 µL diluent to give a final concentration of 10 µg/mL SIL ELVA. Store at -70°C.

[00667] *IS Spike*: Pipet 50 µL of IS stock 2 toa total of 10 mL diluent to give a final concentration of 50 ng/mL SIL ELVA. Store at -20°C.

[00668] *Extraction procedures*: Equilibrate the LC-MS/MS system with mobile phases for at least 60 minutes. Thaw calibration standard samples, QC samples, unknown samples and blank samples at ice-water bath. Vortex-mix the samples thoroughly.

[00669] 1. Add 10 µL of each sample (SST and Standards) into a 96-well 2 mL plate. Add 10 µL of each sample (double blank, blank, QC and unknown samples).

[00670] 2. Add 35 µL of 10 mg/mL of Anti-ID SBA-biotin coated beads and 15 µL 300mM CaCl2 solution to each well.

[00671] 3. Add 150 µL of phosphate-buffered saline with Tween into each well. Cover the plate, centrifuge for about one minute at 500 rpm and vortex the plate for thirty seconds.

[00672] 4. Incubate all samples at room temperature for sixty minutes in an incubation plate vortexer with a speed at 1000 RPM.

[00673] 5. Process the plate with a KingFisher™ Flex Magnetic Particle Processor, wash beads two times with 300 µL of phosphate-buffered saline. Then transfer the beads to the "digestion plate" containing 100 µL of Rapi-Gest solution.

[00674] 6. Add 10 µL 100 mM DTT to the digestion plate. Centrifuge for one minute at 1000 rpm and vortex for 20 s.

[00675] 7. Cover the digestion plate with adhesive sealing film and incubate it on a shaker at 60°C, 900 rpm for one hour. Centrifuge the plate briefly at 800 rpm.

[00676] 8. Add 12 µL of 100 mM iodoacetamide to each well. Cover the plate with an adhesive sealing film. Incubate the plate on a shaker in dark at room temperature for thirty minutes at 1000 rpm.

[00677] 9. To prepare 0.1 mg/mL trypsin, thaw the required number of sequencing grade modified trypsin tubes. Add 150 µL of 50 mM ammonium to each tube (20 µg trypsin) and mix well. Combine the contents of all the tubes and mix well. This solution is prepared freshly. Approximately ten tubes of sequencing grade modified trypsin are required for a full 96-well plate. Add 20 µL of 0.1 mg/mL Trypsin to each well, Centrifuge for one minute at 1500 rpm.

[00678] 10. Centrifuge the plate at 4000 rpm for three minutes. Stop the reaction by adding 10 µL of 10% trifluoroacetic acid. Centrifuge the plate at 1000 rpm for one minute, then shake at 1000 rpm for one minute.

[00679] 11. Add 20 µL of the IS spike in each vial except for double blank. Add 20 µL diluent to double blank. Shake the plate at 1000 rpm for one minute, then centrifuge at 1000 rpm for one minute.

[00680] 12. Inject 15 µL of total antibody digest into LC-MS/MS.

[00681] Data evaluation. Retention time and peak area are determined by Analyst Data Acquisition/Processing software. Analyte concentrations are obtained from a calibration curve constructed by plotting peak area ratio versus concentration. Concentrations were calculated using linear regression according to the following equation: y = ax + b

[00682] Where: y = peak area ratio of analyte/internal standard

[00683] a = slope of the corresponding standard curve

[00684] x = concentration of analyte (µg/mL)

[00685] b = intercept of the corresponding standard curve (with $1/x^2$ weighting).

EXAMPLE 12

Antibodies were labeled using an AF647 protein labeling kit

[00686] *Results of CR007 labeling*. The yield is 61.5% = (0.14 ml*4.39 mg/ml)/1 mg. CR007 concentration = 4.39/150,000 = 29.3 µM. AF647 = 79.8 µM. Ratio of labeling = 79.8 µM/29.3 µM = 2.72.

[00687] *Results of 6E9F1 labeling*. The yield is 60.6% = (0.13 ml*4.66 mg/ml)/1 mg. 6E9F1 concentration = 4.66/150,000 = 31.1 µM. AF647=78.0 µM. Ratio of labeling=78.0 µM /31.1 µM = 2.50.

[00688] *Results of **ABT404** labeling*. The yield is 60.6%=(0.13 ml*4.66 mg/ml)/1 mg. 6E9F1 concentration = 4.66/150,000 = 31.1 µM. AF647=78.0 µM. Ratio of labeling=78.0 µM/31.1 µM = 2.50.

[00689] *Results of **ABT403** labeling*: The yield was 58% = (0.25 ml*2.32 mg/ml)/1 mg. **ABT403** concentration = 2.32/155,031 = 15.0 µM. AF647 = 31.0 µM. Ratio of labeling = 31.0 µM/15.0 µM = 2.1.

[00690] *Results of **ABT404** labeling*: The yield is 59.6% = (0.2 ml*2.98 mg/ml)/1 mg. **ABT404** concentration = 2.98/144,685 = 20.6 µM. AF647 = 40.5 µM. Ratio of labeling = 40.5 µM/20.6 µM = 1.97.

[00691] *Results of **ABT405** labeling*: The yield is 62.2% = (0.2 ml*3.11 mg/ml)/1 mg. **ABT405** concentration = 3.11/144,464 = 21.5 µM. AF647 = 35.8 µM. Ratio of labeling = 35.8 µM/21.5 µM = 1.67

[00692] *Results of **ABT410** labeling*: The yield is 55.2% = (0.12 ml*4.60 mg/ml)/1 mg. **ABT410** concentration = 4.60/144,680 = 31.8 µM. AF647 = 30.9 µM. Ratio of labeling = 30.9 µM/31.8 µM = 0.97.

[00693] *Results of **ABT407** labeling*: The yield is 63.1% = (0.19 ml*3.32 mg/ml)/1 mg. **ABT407** concentration = 3.32/145,075 = 22.9 µM. AF647 = 27.4 µM. Ratio of labeling = 27.4 µM/22.9 µM = 1.2

[00694] *Results of **ABT408** labeling*: The yield is 56.9% = (0.18 ml*3.16 mg/ml)/1 mg. **ABT408** concentration = 3.16/144,855 = 21.8 µM. AF647 = 27.3 µM. Ratio of labeling = 88.1 µM/23.9 µM = 1.25.

[00695] *Results of **ABT409** labeling*: The yield is 58.5% = (0.25 ml*2.34 mg/ml)/1 mg. **ABT409** concentration = 2.34/155,421 = 15.1 µM. AF647 = 16.5 µM. Ratio of labeling = 16.5 µM/15.1 µM = 1.09.

[00696] *Results of 6E9F1 labeling*: The yield is 65.1% = (0.37 ml*1.76 mg/ml)/1 mg. Mouse-6E9F1 concentration = 1.76/150,000 = 11.7 µM. AF647 = 48.6 µM. Ratio of labeling = 48.6 µM/11.7 µM = 4.15.

[00697] *Results of LS-C331895 labeling*. The yield is 20%=(0.21 ml*0.95 mg/ml)/1 mg. **ABT403** concentration = 0.95/150,000=6.3 µM. AF647=58.3 µM. Ratio of labeling = 58.3 µM/6.3 µM = 9.25. Compared to other antibodies, labeled yield is only 20% (compared to ~56-65% for other antibodies). Ratio of labeling is 9.25 (compared to ~0.97-4.15 for other antibodies).

EXAMPLE 13

Biophysical assays. Surface plasmon resonance – third results.

[00698] IGF1 receptor binding to IGF1 via surface plasmon resonance assay (Acro Biosystems)

[00699] Biophysical assessment of insulin/IGF1 binding to their respective receptors has been demonstrated.

EXAMPLE 14

Biophysical assays. Surface plasmon resonance – fourth results.

[00700] Surface plasmon resonance assay can be used to measure anti-insulin autoantibodies and anti-proinsulin autoantibodies. Binding affinities of two agents to insulin receptors and human insulin antibody was assayed.

[00701] Two TRAP agents were assayed in a TR-FRET/SPR assay (ASGPR1). **AGN303** (native proinsulin) gave a result of IC_{50} = 1.2 mM. **AGN310** (mutant proinsulin) gave a result of IC_{50} = 1.2 mM.

TABLE 29
Surface plasmon resonance

	mAb49
AGN303 (native proinsulin)	K_D = 131 nM
AGN310 (mutant proinsulin)	K_D = 174 nM
Insulin	K_D = 134 nM
AGN310	K_D = 558 nM
AGN412	Not detected
AGN416	K_D = 1.1 µM

[00702] Mutant insulin maintains high mAb49 binding.

[00703] *Cell-based FRET assay (AF488-labeled or AF647-labeled target)* to assess ternary complex on cell surface. The inventors performed a FRET-based uptake/degradation of POI (DL650 and QC-1 labeled antibody).

TABLE 30	
	TR-FRET
AGN303 (native proinsulin)	IC_{50} = 1.2 µM
AGN310 (mutant proinsulin)	IC_{50} = 1.2 µM

EXAMPLE 15

Biophysical assays. Surface plasmon resonance – fifth results.

[00704] *Insulin receptor selectivity assays*. Insulin receptor binding to human insulin can be measured via surface plasmon resonance assay.

[00705] Native proinsulin was found to have lower affinity to insulin receptors but maintains similar affinity to insulin antibodies.

[00706] *Insulin receptor isoforms*. Insulin receptor B differs from insulin receptor A by the inclusion of exon 11. Both isoforms have similar affinity for insulin. The insulin receptor isoforms show different functional features. Insulin receptor A exhibits a higher affinity for IGFs and a greater internalization and recycling rate than Insulin receptor B. Because of these differences, Insulin receptor B is preferentially associated with metabolic and differentiating signals. Insulin receptor A mainly favors cell growth, proliferation, and survival.

TABLE 31 Surface plasmon resonance		
	Insulin receptor A	Insulin receptor B
AGN303 (native proinsulin)	K_D = 437 nM	K_D = 226 nM
AGN310 (mutant proinsulin)	K_D = 9.4 µM	K_D = 2.8 µM
Insulin		K_D = 2.8 µM
AGN310		K_D = 3.7 µM
AGN412		Not detected
AGN416		K_D = 7.4 µM

[00707] Mutant insulin has significantly reduced insulin receptor A and insulin receptor B binding.

EXAMPLE 16

Biophysical assays. Surface plasmon resonance.

[00708] Two TRAP agents were also assayed for binding affinities of TRAP agents to C-peptide antibodies (SPR). AGN406 (C-peptide with protease site, synthetic C-peptide degrader gave a result of EC_{50} = 9.8 nM. AGN407 (C-peptide without protease site, synthetic C-peptide degrader) gave a result of EC_{50} = 12.5 nM.

EXAMPLE 17

Cellular In vitro internalization assays – first results

[00709] The inventors performed cellular internalization of insulin antibodies in HEK293 cells.

[00710] mAb49: Human anti-Insulin antibody.

[00711] 6E9F1: Mouse anti-Insulin antibody.

TABLE 32
Biochemistry selectivity (anti-insulin autoantibodies)

	AGN303	**AGN310**
mAb49	EC_{50} = 39.68 nM	EC_{50} = 67.21 nM
6E9F1	EC_{50} = 39.13 nM	EC_{50} = 37.61 nM

mAb49: Human anti-Insulin antibody

6E9F1: Mouse anti-Insulin antibody

[00712] Insulin antibody internalization is ASGPR-dependent and independent of insulin receptors.

[00713] This finding provides flexibility with anti-human Insulin antibodies from different species in designing assays.

[00714] Agents (TRAPs) assayed:

[00715] **AGN301**: IgG degrader (reference IgG degrader control). Results: Continued good positive control for internalization with HEK293-ASGPR (clone 3F7) cells + rabbit anti-human insulin antibody.

[00716] **AGN303**: Native insulin with GN3 Reference anti-insulin degrader control). Continued good positive control for internalization with HEK293-ASGPR (clone 3F7) cells + mouse anti-human insulin an.

[00717] AGN411 (mutant construct) > AGN408 (native construct) for internalization, although minimal signal with either. Neither AGN411 (mutant construct) or AGN408 (native construct) induced internalization.

[00718] AGN304: Proinsulin construct with C-terminal GN3 (Native)

[00719] **AGN305**: Proinsulin construct with C-terminal GN3 (Mutant)

[00720] **ABT306**: Alk-native insulin without GN3 (peptide only).

[00721] **ABT307**: Proinsulin-IgG1 Fc fusion, G4S linker, hIgG1 hinge (C > A), LALA-PA.

[00722] ABT308: Proinsulin-IgG1 Fc fusion, 5x G4S linker, hIgG1 hinge (C > A), LALA-PA.

[00723] AGN411: Proinsulin construct with N-terminal αGN3 (mutant)

[00724] **AGN412**: Synthetic Mutant insulin with βGN3. By comparison, **AGN310** has αGN3.

[00725] AGN413: (proinsulin Fc GN3) also low with no internalization.

[00726] **AGN414**: Proinsulin-Fc conjugate (**ABT307**-**TBT4438**)

[00727] **AGN415**: Proinsulin-Fc conjugate (ABT308-**TBT4438**)

[00728] **AGN416**: Synthetic Mutant insulin with αGN3 on insulin A chain. By comparison, other synthetic insulin-GN3 compounds synthesized have GN3 attached to insulin B chain.

[00729] AGN417: Native proinsulin with βGN3 on N-terminal.

TABLE 33

AGN414 dose, 1 µM as top concentration, 9 points, 3 fold dilution in duplicate	DMSO	50 nM AF647 labeled rabbit Ab
AGN415 dose, 1 µM as top concentration, 9 points, 3 fold dilution in duplicate	370 nM **AGN301**	50 nM AF647 labeled rabbit Ab
AGN414 dose, 1 µM as top concentration, 9 points, 3 fold dilution in duplicate	DMSO	50 nM AF647 labeled mouse Ab
AGN415 dose, 1 µM as top concentration, 9 points, 3 fold dilution in duplicate	370 nM **AGN301**	50 nM AF647 labeled mouse Ab

[00730] Rationale for more assaying. Test whether **ABT307** and ABT308 (proinsulin portion) can be recognized by either rabbit or mouse anti-insulin antibodies.

[00731] (1) **AGN301** + labeled rabbit Ab (CR007) should internalize (therefore positive signal) If add Proinsulin Fc protein, will compete with **AGN301**, and therefore have less signal

[00732] (2) **AGN301** + labeled mouse 6E9F1 Ab should internalize (therefore no signal). If add proinsulin Fc fusion protein, should get signal, since **AGN301** binds to Fc portion of Fc fusion protein, and labeled mouse Ab will bind to proinsulin portion

[00733] (3) LALA mutation on proinsulin Fc fusion protein should not interfere with IgG degrader binding, so the LALA mutation should not alter the results.

[00734] Dose range with **ABT307** and ABT308.

[00735] Use both anti-insulin mouse antibody (clone 6E9F1) and rabbit antibody (clone CR007) at constant concentration.

[00736] Test in HEK293-ASGPR (3F7) cells.

[00737] Internalization assay conditions:

[00738] Cell lines: HEPG2 and HEK293-ASGPR (clone 3F7, 3C6, 1D4).

[00739] Culture media: DMEM + 10% fetal bovine serum + 1% P/S.

[00740] Cell density: Constant at 7.5K cells/well.

[00741] Antibodies: Constant at 100 nM (all labeled with AF647). Also tested different concentrations at final 25nM, 50nM, 100nM. Anti-insulin antibody, rabbit monoclonal, Sino, catalogue #11038-CR007. Anti-insulin antibody, mouse monoclonal, Genscript, catalogue #A01715 (clone 6E9F1) at ratio of labeling = about 2.9.

[00742] Agents: **AGN301** (constant at 50 nM). Based on results, antibody concentration at 50 nM is sufficient for internalization assay. Also tested **AGN301** (constant at 370 nM).

[00743] Incubation: Incubate at 37°C for eighteen hours.

[00744] The inventors have also done incubation at 37°C for twenty-four hours.

[00745] Readout: Internalization on High Content Imager (Operetta)

[00746] When rabbit anti-insulin antibody (Sino, catalogue #11038-CR007) is used: Rabbit monoclonal antibody + **AGN301** (IgG degrader) for internalization. **AGN301** competes with IgG Fc-fusion protein in presence of rabbit monoclonal antibody. **ABT307** and ABT308 compete with **AGN301** at reduction in signal.

[00747] When mouse anti-insulin antibody (Genscript, catalogue #A01715 (clone 6E9F1)) is used: Mouse monoclonal antibody + **AGN301** (IgG degrader) for low internalization. **AGN301** allows for complex formation of IgG Fc-fusion protein in presence of mouse monoclonal antibody. **ABT307** and ABT308 binds to mouse antibody via proinsulin protein moiety. Then, **AGN301** binds to Fc moiety of Fc protein at including in signal.

[00748] Conclusion: Both rabbit and mouse anti-insulin antibodies recognize Fc fusion protein (via proinsulin moiety).

[00749] Internalization assay: HEK293-ASGPR (clone 3F7) cells. Repeated four compounds employing AF647-labeled rabbit anti human insulin antibody on HEK293-ASGPR 3F7.

[00750] **AGN303** and **AGN310** using AF647-labeled rabbit anti-human insulin antibody show great effect on HEK293-ASGPR 3F7. The results can be repeated compared to n = 1.

[00751] Labeled Ab (100 nM). **AGN301** (IgG degrader at 50 nM).

TABLE 34 Internalization assay		
370 nM **AGN301**	0.1% DMSO	2.50 E+04
370 nM **AGN301**	0.1% DMSO	5.00 E+04
370 nM **AGN301**	0.1% DMSO	1.00 E+05
370 nM **AGN301**	0.1% DMSO	2.00 E+05

[00752] Cells used: HEK293-ASGPR 3F7+100nM CR007 HEK293+100nM CR007, HEK293-ASGPR 3C6+100nM CR007 HepG2+100nM CR007, HEK293-ASGPR 1D4+100nM CR007 HEK293, and HepG2.

TABLE 35 Internalization assay		
370 nM **AGN301**	0.1% DMSO	2.5K
370 nM **AGN301**	0.1% DMSO	5K
370 nM **AGN301**	0.1% DMSO	7.5K
370 nM **AGN301**	0.1% DMSO	10K

[00753] Cells used: HEK293-ASGPR 3F7 HEK293, HEK293-ASGPR 3C6, HepG2, and HEK293-ASGPR 1D4.

[00754] Conclusions: Anti-insulin agents results were as expected. Rabbit antibody (not mouse) can be used as surrogate with IgG control agent **AGN301**. Non-GN3 peptide (**ABT306**) does not elicit internalization. Anti-proinsulin agents (AGN304, **AGN305**) do not internalize.

[00755] Subsequent actions: Assay anti-C-peptide monoclonal antibody.

[00756] Time-course results. Clear internalization signal was observed for HEK293-ASGPR 3F7, 3C6, and HepG2 cells S/B for HEK293-ASGPR 3F7 and 3C6 peaked at six hours and persisted until eighteen hours.

[00757] Based on the results, AGN418, AGN419, AGN420, and **AGN7865** all show good effects on HEK293-ASGPR 3F7 employing CR007 and 6E9F1 but show some effects when using **ABT404**.

[00758] Internalization: **AGN310**> **AGN303** > **AGN416**. Weak signal with proinsulin constructs: **AGN414**, **AGN415**, AGN408, and AGN411. **AGN310** induced strongest internalization. **AGN301** did not induce internalization.

[00759] Good signal with: AGN417 (proinsulin βGN3) and insulin constructs with GN3 (**AGN303**, **AGN310**, and **AGN416**). Weak or no signal with proinsulin N-terminal alpha constructs: AGN408 and AGN411. Weak or no signal βGN3 mutant insulin constructs: **AGN412**.

TABLE 36
Internalization assay results

Antibody	Description	**AGN310**	AGN417
ABT403	mAb49 (original). Creative Biolabs (FAMAB-0225WJ)	+	+
ABT404	mAb49	+	+
ABT405	mAb49	+	+
ABT406	Anti-C-peptide (original)	-	+
ABT407	Anti-C-peptide	-	+
ABT408	Anti-C-peptide	-	+
ABT409	Anti-C-peptide	-	+
Polyclonal rabbit Ab (LS- C331895)	LSBio (LS- C331895)	-	+
mAb rabbit (CR007)	Sino (11038-CR007)	+++	++
mAb mouse (6E9F1)	Genescript (A01715)	++	++

EXAMPLE 18

Cellular In vitro internalization assays – second results

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN421-002	CR007	2622665	26	758	1800	1797	HEK293T/ASGPR 3F7
AGN421-003	CR007	3109184	20	706	2140	2149	HEK293T/ASGPR 3F7
AGN422-002	CR007	1716017	35	555	1663	1659	HEK293T/ASGPR 3F7
AGN7849-001	CR007	1393821	21	617	2148	2134	HEK293T/ASGPR 3F7
AGN7850-001	CR007	1130832	26	453	2177	2157	HEK293T/ASGPR 3F7
AGN421-002	6E9F1	780072	31	812	526	522	HEK293T/ASGPR 3F7
AGN421-003	6E9F1	791142	27	758	571	566	HEK293T/ASGPR 3F7
AGN422-002	6E9F1	915700	39	826	616	609	HEK293T/ASGPR 3F7
AGN7849-001	6E9F1	553940	48	864	767	761	HEK293T/ASGPR 3F7
AGN7850-001	6E9F1	444341	34	549	788	772	HEK293T/ASGPR 3F7
AGN421-002	**ABT404**	395313	10	1470	147	148	HEK293T/ASGPR 3F7
AGN421-003	**ABT404**	399679	14	551	240	243	HEK293T/ASGPR 3F7
AGN422-002	**ABT404**	567260	27	920	368	367	HEK293T/ASGPR 3F7
AGN7849-001	**ABT404**	108002	16	704	171	171	HEK293T/ASGPR 3F7
AGN7850-001	**ABT404**	109995	14	367	240	240	HEK293T/ASGPR 3F7
AGN421-002	CR007	2953484	33	992	1556	1542	HEK293T/ASGPR 3F7
AGN421-003	CR007	2950178	32	943	1621	1605	HEK293T/ASGPR 3F7
AGN422-002	CR007	1714877	31	625	1594	1583	HEK293T/ASGPR 3F7
AGN7849-001	CR007	982078	27	789	1478	1451	HEK293T/ASGPR 3F7

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN7850-001	CR007	881298	17	584	1467	1456	HEK293T/ASGPR 3F7
AGN421-002	6E9F1	1146907	31	869	790	783	HEK293T/ASGPR 3F7
AGN421-003	6E9F1	1124227	30	855	798	790	HEK293T/ASGPR 3F7
AGN422-002	6E9F1	953794	31	787	711	704	HEK293T/ASGPR 3F7
AGN7849-001	6E9F1	849739	37	816	1241	1208	HEK293T/ASGPR 3F7
AGN7850-001	6E9F1	695318	23	552	1197	1180	HEK293T/ASGPR 3F7
AGN421-002	**ABT404**	316924	21	713	212	212	HEK293T/ASGPR 3F7
AGN421-003	**ABT404**	314685	19	871	207	206	HEK293T/ASGPR 3F7
AGN422-002	**ABT404**	335870	17	987	213	212	HEK293T/ASGPR 3F7
AGN7849-001	**ABT404**	103418	20	474	194	193	HEK293T/ASGPR 3F7
AGN7850-001	**ABT404**	102430	14	367	214	214	HEK293T/ASGPR 3F7
AGN418	CR007	1107853	35	665	1851	1820	HEK293T/ASGPR 3F7
AGN419	CR007	524322	38	375	1157	1142	HEK293T/ASGPR 3F7
AGN420	CR007	891180	36	669	1474	1446	HEK293T/ASGPR 3F7
AGN7865	CR007	984673	29	674	1565	1541	HEK293T/ASGPR 3F7
AGN418	6E9F1	645547	32	608	1083	1067	HEK293T/ASGPR 3F7
AGN419	6E9F1	740253	31	510	1359	1334	HEK293T/ASGPR 3F7
AGN420	6E9F1	662335	35	812	962	943	HEK293T/ASGPR 3F7
AGN7865	6E9F1	578833	30	806	843	824	HEK293T/ASGPR 3F7
AGN418	**ABT404**	45559	23	336	111	111	HEK293T/ASGPR 3F7
AGN419	**ABT404**	22181	20	299	65	65	HEK293T/ASGPR 3F7
AGN420	**ABT404**	43700	23	329	109	109	HEK293T/ASGPR 3F7
AGN7865	**ABT404**	45586	19	312	117	117	HEK293T/ASGPR 3F7
AGN418	CR007	1215721	24	709	1855	1836	HEK293T/ASGPR 3F7
AGN419	CR007	625856	34	336	1492	1470	HEK293T/ASGPR 3F7
AGN420	CR007	1157693	32	585	2012	1980	HEK293T/ASGPR 3F7
AGN7865	CR007	1131165	29	626	1905	1868	HEK293T/ASGPR 3F7
AGN418	6E9F1	655344	30	602	1109	1089	HEK293T/ASGPR 3F7
AGN419	6E9F1	712479	27	504	1296	1281	HEK293T/ASGPR 3F7
AGN420	6E9F1	630028	35	682	1009	990	HEK293T/ASGPR 3F7
AGN7865	6E9F1	692893	28	738	1041	1023	HEK293T/ASGPR 3F7
AGN418	**ABT404**	53266	20	305	129	129	HEK293T/ASGPR 3F7
AGN419	**ABT404**	26822	13	288	70	70	HEK293T/ASGPR 3F7
AGN420	**ABT404**	52443	17	321	120	121	HEK293T/ASGPR 3F7
AGN7865	**ABT404**	48139	13	289	119	119	HEK293T/ASGPR 3F7
AGN414	CR007	385583	13	316	917	896	HEK293T/ASGPR 3F7
AGN415	CR007	238771	14	412	486	473	HEK293T/ASGPR 3F7
AGN414	**ABT404**	44267	3.9	195	134	134	HEK293T/ASGPR 3F7
AGN415	**ABT404**	43848	4.8	228	119	118	HEK293T/ASGPR 3F7
AGN7525	CR007	501233	7.6	241	1463	1425	HEK293T/ASGPR 3F7
AGN7526	CR007	358780	5.9	295	870	857	HEK293T/ASGPR 3F7
AGN7525	**ABT404**	79582	4.0	98	354	353	HEK293T/ASGPR 3F7
AGN7526	**ABT404**	75581	3.8	110	327	325	HEK293T/ASGPR 3F7
AGN417-003	**ABT403**	214317	21	321	270	270	HEK293T/ASGPR 3F7
AGN414	CR007	246693	13	276	729	707	HEK293T/ASGPR 3F7
AGN415	CR007	159414	11	315	375	368	HEK293T/ASGPR 3F7

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN414	**ABT404**	38012	2.9	204	109	109	HEK293T/ASGPR 3F7
AGN415	**ABT404**	38428	3.4	277	95	94	HEK293T/ASGPR 3F7
AGN7525	CR007	335865	6	237	999	978	HEK293T/ASGPR 3F7
AGN7526	CR007	256983	5	264	681	671	HEK293T/ASGPR 3F7
AGN7525	**ABT404**	55657	2.6	113	233	231	HEK293T/ASGPR 3F7
AGN7526	**ABT404**	52579	2.6	94	251	251	HEK293T/ASGPR 3F7
AGN417-003	**ABT403**	68865	30	343	93	94	HEK293T/ASGPR 3F7
AGN417-003	6E9F1	1566311	33	987	959	950	HEK293T/ASGPR 3F7
AGN310	**ABT403**	361271	18	635	293	293	HEK293T/ASGPR 3F7
AGN417	**ABT403**	194965	25	378	288	289	HEK293T/ASGPR 3F7
AGN310	**ABT404**	820058	15	1039	362	363	HEK293T/ASGPR 3F7
AGN417	**ABT404**	338043	18	486	303	306	HEK293T/ASGPR 3F7
AGN310	CR007	10854609	20	1797	3300	3271	HEK293T/ASGPR 3F7
AGN417	CR007	2944204	29	825	1896	1888	HEK293T/ASGPR 3F7
AGN310	6E9F1	3129671	13	573	2482	2481	HEK293T/ASGPR 3F7
AGN417	6E9F1	1878996	26	878	1252	1242	HEK293T/ASGPR 3F7
AGN310	**ABT405**	186011	17	508	162	163	HEK293T/ASGPR 3F7
AGN417	**ABT405**	119172	21	377	149	151	HEK293T/ASGPR 3F7
AGN310	**ABT406**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT406**	188477	21	367	255	257	HEK293T/ASGPR 3F7
AGN310	**ABT407**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT407**	231673	31	347	410	409	HEK293T/ASGPR 3F7
AGN310	**ABT408**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT408**	247710	27	342	437	436	HEK293T/ASGPR 3F7
AGN310	**ABT409**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT409**	113737	29	324	232	232	HEK293T/ASGPR 3F7
AGN310	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	LSC331895	339797	17	749	253	250	HEK293T/ASGPR 3F7
AGN310	**ABT405**	200141	19	604	155	156	HEK293T/ASGPR 3F7
AGN417	**ABT405**	119762	21	394	153	155	HEK293T/ASGPR 3F7
AGN310	**ABT406**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT406**	186130	21	393	248	249	HEK293T/ASGPR 3F7
AGN310	**ABT407**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT407**	193310	30	361	330	330	HEK293T/ASGPR 3F7
AGN310	**ABT408**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT408**	226991	32	331	431	428	HEK293T/ASGPR 3F7
AGN310	**ABT409**	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	**ABT409**	132062	28	343	245	245	HEK293T/ASGPR 3F7
AGN310	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	LSC331895	278697	16	593	250	248	HEK293T/ASGPR 3F7
AGN310	**ABT403**	632102	24	615	528	525	HEK293T/ASGPR 3F7
AGN417	**ABT403**	296613	22	365	452	454	HEK293T/ASGPR 3F7
AGN310	**ABT404**	10314457	19	741	366	368	HEK293T/ASGPR 3F7
AGN417	**ABT404**	3263784	16	560	334	336	HEK293T/ASGPR 3F7
AGN310	CR007	10314457	24	1747	3226	3190	HEK293T/ASGPR 3F7
AGN417	CR007	3263784	27	790	2188	2178	HEK293T/ASGPR 3F7

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN310	6E9F1	3194185	15	651	2378	2374	HEK293T/ASGPR 3F7
AGN417	6E9F1	2247463	26	837	1491	1482	HEK293T/ASGPR 3F7
AGN412	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	FAMAB-0225WJ	358633	24	410	513	516	HEK293T/ASGPR 3F7
AGN408	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN412	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN417	FAMAB-0225WJ	212698	21	339	392	393	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ	262910	11	340	482	481	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ	571517	21	581	554	553	HEK293T/ASGPR 3F7
AGN416	FAMAB-0225WJ	364773	22	546	413	411	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ	213580	15	350	362	350	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ	447435	24	632	407	405	HEK293T/ASGPR 3F7
AGN416	FAMAB-0225WJ	281288	26	534	327	325	HEK293T/ASGPR 3F7
AGN414	FAMAB-0225WJ	52049	3.4	190	177	175	HEK293T/ASGPR 3F7
AGN415	FAMAB-0225WJ	66152	4.6	177	236	232	HEK293T/ASGPR 3F7
AGN408	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN412	CR007	1273315	142	4434	239	238	HEK293T/ASGPR 3F7
AGN416	CR007	5257312	28	673	3323	3308	HEK293T/ASGPR 3F7
AGN412	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN416	6E9F1	3634412	16	491	3177	3196	HEK293T/ASGPR 3F7
AGN412	CR007	2536472	145	4085	505	500	HEK293T/ASGPR 3F7
AGN416	CR007	9316249	18	1058	4455	4409	HEK293T/ASGPR 3F7
AGN412	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN416	6E9F1	6625680	15	564	5535	5543	HEK293T/ASGPR 3F7
AGN414	CR007	422068	6.0	283	1069	1050	HEK293T/ASGPR 3F7

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN415	CR007	457281	9.2	289	1164	1136	HEK293T/ASGPR 3F7
AGN414	6E9F1	535352	4.0	269	1538	1517	HEK293T/ASGPR 3F7
AGN415	6E9F1	667341	6.7	277	1819	1784	HEK293T/ASGPR 3F7
AGN414	LSC331895	72589	1.3	59	518	520	HEK293T/ASGPR 3F7
AGN415	LSC331895	76247	1.4	70	505	506	HEK293T/ASGPR 3F7
AGN303	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	LSC331895	4398965	8.1	759	2565	2552	HEK293T/ASGPR 3F7
AGN310	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN304	LSC331895	1990188	34	939	2754	2690	HEK293T/ASGPR 3F7
AGN305	LSC331895	1083287	18	494	1980	1957	HEK293T/ASGPR 3F7
AGN301	LSC331895	4210248	9.4	650	2742	2756	HEK293T/ASGPR 3F7
AGN408	LSC331895	957957	34	760	1430	1405	HEK293T/ASGPR 3F7
AGN411	LSC331895	-	130	-	905	-	HEK293T/ASGPR 3F7
AGN413	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	LSC331895	3574449	8.3	640	2247	2251	HEK293T/ASGPR 3F7
AGN414	FAMAB-0225WJ-F(E)	58283	4.3	433	109	108	HEK293T/ASGPR 3F7
AGN415	FAMAB-0225WJ-F(E)	65880	5.2	359	138	137	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ-F(E)	307229	10	655	200	200	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ-F(E)	214123	11	507	166	167	HEK293T/ASGPR 3F7
AGN304	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN305	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN408	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN413	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN414	CR007	219652	8.8	253	626	611	HEK293T/ASGPR 3F7
AGN415	CR007	254387	9.9	308	619	605	HEK293T/ASGPR 3F7
AGN414	6E9F1	434915	4.2	267	1139	1105	HEK293T/ASGPR 3F7
AGN415	6E9F1	478977	8.5	280	1250	1202	HEK293T/ASGPR 3F7
AGN301	CR007	7465550	17	690	4786	4785	HEK293T/ASGPR 3F7
AGN408	CR007	-	70	-	98	-	HEK293T/ASGPR 3F7
AGN411	CR007	-	127	-	425	-	HEK293T/ASGPR 3F7
AGN413	CR007	-	45	-	38	-	HEK293T/ASGPR 3F7

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN301	6E9F1	1687786	12	422	1828	1827	HEK293T/ASGPR 3F7
AGN408	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN413	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
ABT307	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
ABT308	CR007	-	-	2.6	-	1210	HEK293T/ASGPR 3F7
ABT307	6E9F1	818081	0.34	213	1976	1972	HEK293T/ASGPR 3F7
ABT308	6E9F1	948577	0.61	137	2704	2704	HEK293T/ASGPR 3F7
AGN303	CR007	17025436	38	1451	6582	6547	HEK293T/ASGPR 3F7
AGN310	CR007	16219273	22	1478	5796	5717	HEK293T/ASGPR 3F7
AGN304	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN305	CR007	1383077	42	2030	541	526	HEK293T/ASGPR 3F7
AGN303	CR007	183786	17	167	64	64	HepG2
AGN310	CR007	228091	53	375	86	86	HepG2
AGN304	CR007	-	-	-	-	-	HepG2
AGN305	CR007	263578	27	1178	55	57	HepG2
AGN301	6E9F1	-	-	-	-	-	HepG2
AGN303	6E9F1	2208088	51	705	197	197	HepG2
AGN310	6E9F1	2442025	48	554	249	250	HepG2
ABT306	6E9F1	-	-	-	-	-	HepG2
AGN304	6E9F1	-	-	-	-	-	HepG2
AGN305	6E9F1	-	-	-	-	-	HepG2
AGN301	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	6E9F1	5582416	22	784	5067	4993	HEK293T/ASGPR 3F7
AGN310	6E9F1	2900143	16	593	5825	5819	HEK293T/ASGPR 3F7
ABT306	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN304	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN305	6E9F1	1363009	8.2	1818	358	350	HEK293T/ASGPR 3F7
AGN402	CR007	-	-	-	-	-	HepG2
AGN401	CR007	-	-	-	-	-	HepG2
AGN403	CR007	-	-	-	-	-	HepG2
AGN404	CR007	-	-	-	-	-	HepG2
AGN303	CR007	-	-	-	-	-	HepG2
AGN310	CR007	139533	53	364	56	56	HepG2
AGN304	CR007	-	-	-	-	-	HepG2
AGN305	CR007	-	-	-	-	-	HepG2
AGN402	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN401	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN403	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN404	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	CR007	22761749	32	1599	8595	8482	HEK293T/ASGPR 3F7
AGN310	CR007	25196241	16	1868	7869	7824	HEK293T/ASGPR 3F7
AGN304	CR007	-	71	-	137	-	HEK293T/ASGPR 3F7
AGN305	CR007	1730240	28	2526	511	502	HEK293T/ASGPR 3F7
AGN4006	CR007	1488243	83	1341	796	798	HepG2
AGN301	CR007	4004648	74	1266	2613	2619	HepG2

TABLE 37
Internalization assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN4006	T24	2179897	25	859	30	30	HepG2
AGN301	T24	2004469	39	818	138	138	HepG2
Cell seeding density is 7500 cells/well. Incubation for eighteen hours.							

EXAMPLE 19

Ternary complex formation assay -first results.

[00760] Ternary complex formation assay (Hep2)

[00761] 1. Labeled two antibodies with AF647. Labelled antibody at fixed 100 nM.

[00762] Anti-insulin antibody (IgG), Rabbit antibody to "T24". Ratio of labeling = 0.49.

[00763] Anti-insulin antibody (IgG), Rabbit monoclonal antibody was "CR007". Ratio of labeling = 2.7.

[00764] 2. For Ternary complex formation assay for assay development and validation, two IgG agents (**AGN4006**, **AGN301**) were tested as reference compounds with two labeled rabbit antibodies.

[00765] Agents (TRAPs) tested:

[00766] **AGN301**: IgG degrader (reference molecule). **AGN301** + CR007 and **AGN301** + T24.

[00767] **AGN4006**: IgG degrader (reference molecule). **AGN4006** + CR007 and **AGN4006** + T24.

[00768] **AGN302**: Mutant insulin with GN3.

[00769] **AGN303**: Native insulin with GN3

[00770] AGN304: Native proinsulin

[00771] **AGN305**: Mutant proinsulin

[00772] **ABT306**: Alk-native insulin without GN3 (peptide only).

[00773] Rationale. Test whether **ABT307** and ABT308 (proinsulin portion) can be recognized by either rabbit or mouse anti-insulin antibodies

[00774] (1) **AGN301** + labeled rabbit Ab (CR007) form ternary complex formation, therefore positive signal. If add proinsulin Fc protein, will compete with **AGN301**, and therefore have less signal

[00775] (2) **AGN301** + labeled mouse 6E9F1 Ab will not form ternary complex formation, therefore no signal. If add proinsulin Fc fusion protein with above, should get signal, since **AGN301** binds to Fc portion of Fc fusion protein, and labeled mouse Ab binds to proinsulin portion

[00776] (3) LALA mutation on proinsulin Fc fusion protein should not interfere with IgG degrader binding, so the LALA mutation should not alter the results.

[00777] *Ternary Complex Formation assay conditions*:

[00778] *Cell lines*: HepG2 or HEK293-ASGPR (clone 3F7).

[00779] *Culture media*: DMEM + 10% fetal bovine serum + 1% P/S.

[00780] *Antibodies*: (all labeled with AF647).

[00781] Anti-insulin antibody, rabbit monoclonal, Sino, Cat#11038-CR007. Ratio of labeling = 2.8

[00782] Anti-insulin antibody, mouse monoclonal, Genscript, Cat#A01715 (Clone 6E9F1). Ratio of labeling = about 2.9.

[00783] Agents assayed: 10 µM high concentration, 3-fold dilutions, 9-point curve. In some assays, **AGN301** was assayed, constant at 50 nM.

[00784] Readout: Flow cytometry (IntelliCyt iQue Screener Plus).

[00785] Results: **AGN301** + CR007 worked well.

[00786] 100 nM **AGN301** with labeled CR007 is to be used as control in further assays

[00787] Anti-insulin agents working as expected.

[00788] Rabbit monoclonal antibody but not mouse monoclonal antibody can be used as surrogate with IgG control agent **AGN301.**

[00789] Non-GN3 peptide (**ABT306**) does not elicit ternary complex formation or complex with anti-insulin antibodies.

[00790] Rabbit monoclonal antibody + **AGN301** (IgG degrader) showed strong ternary complex formation. **ABT307** and ABT308 compete with **AGN301** at reduction in signal.

[00791] Mouse monoclonal antibody + **AGN301** (IgG degrader) showed weak ternary complex formation. **ABT307** and ABT308 binds to mouse Ab via proinsulin protein moiety. **AGN301** then binds to Fc moiety of Fc protein

[00792] Conclusion: Both rabbit and mouse anti-insulin antibody recognize Fc fusion protein via the proinsulin moiety.

TABLE 38 Ternary Complex Formation: HepG2 cells
AGN301 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN303 (10 µM top, 3 fold, 9 points). 370 nM **AGN301**
AGN310 (10 µM top, 3 fold, 9 points)
ABT306 (10 µM top, 3 fold, 9 points)
AGN304 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN305 (10 µM top, 3 fold, 9 points). 370 nM **AGN301**

TABLE 39 Ternary Complex Formation: HEK293-ASGPR (clone 3F7)
AGN301 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN303 (10 µM top, 3 fold, 9 points). 370 nM **AGN301**
AGN310 (10 µM top, 3 fold, 9 points)
ABT306 (10 µM top, 3 fold, 9 points)
AGN304 (10 µM top, 3 fold, 9 points). 1% DMSO

[00793] **AGN303** and **AGN310** plus mouse anti-human Insulin antibody show a little effect on HepG2.

[00794] **AGN301** had effect with rabbit anti-human Insulin antibody but no effect with mouse anti-human insulin antibody on HepG2.

[00795] **AGN301** plus rabbit anti human insulin antibody had good ternary complex formation.

[00796] **AGN303** and **AGN310** plus rabbit anti human insulin antibody had poor ternary complex formation.

[00797] **AGN303** and **AGN310** plus mouse anti-human insulin antibody had good ternary complex formation.

[00798] **AGN301** plus mouse anti-human insulin antibody had poor ternary complex formation, which is consistent with mouse Ab and IgG degrader.

[00799] The data were consistent with the results of internalization assay.

[00800] **AGN3764**, **AGN303**, and **AGN310** plus rabbit anti-human Insulin antibody had good ternary complex formation.

[00801] The data consistent with the results of internalization assay.

[00802] Of the compounds tested, none of the compounds showed effect using human or rabbit antibody on HEK293-ASGPR 3F7. **AGN301** showed effect using rabbit antibody.

[00803] Rabbit monoclonal antibody: **AGN415** > **AGN414** in ternary complex formation. Mouse monoclonal antibody: **AGN415** > **AGN414** in ternary complex formation. Both rabbit and mouse monoclonal antibodies work well with proinsulin-Fc conjugates.

[00804] **AGN414** and **AGN415** show good effects in HEK293-ASGPR 3F7 using rabbit (CR007) and mouse antibody, which is consistent with the results of internalization assay.

EXAMPLE 20

Ternary complex formation assays – second results.

[00805] *Biophysical assays*. Ternary complex formation of TRAP agents was assayed using a method described above in this specification using HepG2 or HEK293 stable cells.

TABLE 40
Ternary complex formation assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN421-002	CR007	293996623	11	481	250851	250929	HEK293T/ASGPR 3F7
AGN422-002	CR007	149504588	11	343	218392	217739	HEK293T/ASGPR 3F7
AGN7849-001	CR007	92134277	9.9	294	225017	222258	HEK293T/ASGPR 3F7
AGN7850-001	CR007	63233426	6.7	185	215949	213209	HEK293T/ASGPR 3F7
AGN421-002	6E9F1	62015311	9.5	283	113461	112331	HEK293T/ASGPR 3F7
AGN422-002	6E9F1	51986270	7.7	233	114977	114728	HEK293T/ASGPR 3F7
AGN7849-001	6E9F1	44497667	7.2	210	138543	137802	HEK293T/ASGPR 3F7
AGN7850-001	6E9F1	266432227	4	137	104729	105083	HEK293T/ASGPR 3F7
AGN421-002	**ABT404**	21775507	7.5	418	18396	18244	HEK293T/ASGPR 3F7
AGN422-002	**ABT404**	11922865	5.7	196	13935	13969	HEK293T/ASGPR 3F7
AGN7849-001	**ABT404**	2319845	5.9	93	12523	12560	HEK293T/ASGPR 3F7
AGN7850-001	**ABT404**	1718671	3.4	125	8749	8721	HEK293T/ASGPR 3F7
AGN421-002	CR007	414532586	16	584	338604	339211	HEK293T/ASGPR 3F7
AGN422-002	CR007	250067753	13	377	301596	302996	HEK293T/ASGPR 3F7
AGN7849-001	CR007	146091629	13	393	294267	292373	HEK293T/ASGPR 3F7
AGN7850-001	CR007	101565767	8.5	246	276637	272258	HEK293T/ASGPR 3F7
AGN421-002	6E9F1	124714692	11	332	195257	194754	HEK293T/ASGPR 3F7
AGN422-002	6E9F1	87333279	9.7	238	185075	183965	HEK293T/ASGPR 3F7
AGN7849-001	6E9F1	85719115	9.8	292	211700	208538	HEK293T/ASGPR 3F7
AGN7850-001	6E9F1	52298223	6.9	162	185971	184974	HEK293T/ASGPR 3F7
AGN421-002	**ABT404**	37288078	10	347	35562	35772	HEK293T/ASGPR 3F7
AGN422-002	**ABT404**	21324694	5.3	184	25364	25846	HEK293T/ASGPR 3F7
AGN7849-001	**ABT404**	7404549	5.5	178	22456	22673	HEK293T/ASGPR 3F7
AGN7850-001	**ABT404**	5021407	2.8	115	18175	18427	HEK293T/ASGPR 3F7
AGN418	CR007	74026938	11	332	163550	161947	HEK293T/ASGPR 3F7
AGN419	CR007	36504926	13	235	112447	110712	HEK293T/ASGPR 3F7
AGN420	CR007	87104306	13	376	180377	178875	HEK293T/ASGPR 3F7
AGN7865	CR007	76049321	11	331	168367	166821	HEK293T/ASGPR 3F7
AGN418	6E9F1	31526124	10	218	99502	98099	HEK293T/ASGPR 3F7
AGN419	6E9F1	42435459	9.5	212	136707	134692	HEK293T/ASGPR 3F7
AGN420	6E9F1	43164894	9.8	252	116346	114812	HEK293T/ASGPR 3F7
AGN7865	6E9F1	35874436	9.4	223	107247	106164	HEK293T/ASGPR 3F7
AGN418	**ABT404**	2399627	5.7	139	8471	8512	HEK293T/ASGPR 3F7
AGN419	**ABT404**	1891383	6.1	140	8370	8391	HEK293T/ASGPR 3F7
AGN420	**ABT404**	3600963	6.7	188	10072	10202	HEK293T/ASGPR 3F7
AGN7865	**ABT404**	2622499	5.2	150	8911	8990	HEK293T/ASGPR 3F7
AGN418	CR007	65604385	14	345	142558	141972	HEK293T/ASGPR 3F7
AGN419	CR007	34318466	15	281	89466	88856	HEK293T/ASGPR 3F7
AGN420	CR007	69601410	15	366	146427	146045	HEK293T/ASGPR 3F7
AGN7865	CR007	62018359	13	337	135526	134793	HEK293T/ASGPR 3F7
AGN418	6E9F1	26236485	11	255	73464	72453	HEK293T/ASGPR 3F7

TABLE 40
Ternary complex formation assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN419	6E9F1	33897945	11	224	104556	103109	HEK293T/ASGPR 3F7
AGN420	6E9F1	27844605	11	240	80480	79383	HEK293T/ASGPR 3F7
AGN7865	6E9F1	26883707	9.9	245	76034	74952	HEK293T/ASGPR 3F7
AGN418	**ABT404**	1903693	5.2	167	6112	5991	HEK293T/ASGPR 3F7
AGN419	**ABT404**	1476232	4.6	155	5595	5489	HEK293T/ASGPR 3F7
AGN420	**ABT404**	2365149	4.7	236	6094	6040	HEK293T/ASGPR 3F7
AGN7865	**ABT404**	2601915	5.2	228	6659	6625	HEK293T/ASGPR 3F7
AGN414	CR007	34940126	3.0	291	83778	82557	HEK293T/ASGPR 3F7
AGN415	CR007	24104416	2.2	336	51457	51301	HEK293T/ASGPR 3F7
AGN414	**ABT404**	5981406	0.9	183	16468	16449	HEK293T/ASGPR 3F7
AGN415	**ABT404**	4642357	1.1	209	12323	12245	HEK293T/ASGPR 3F7
AGN7525	CR007	31988735	1.9	229	90757	90120	HEK293T/ASGPR 3F7
AGN7526	CR007	27207269	1.3	297	62720	62751	HEK293T/ASGPR 3F7
AGN7525	**ABT404**	5845906	0.8	77	21629	21970	HEK293T/ASGPR 3F7
AGN7526	**ABT404**	6052835	0.8	121	18515	18648	HEK293T/ASGPR 3F7
AGN414	CR007	24873069	2.9	310	56451	56172	HEK293T/ASGPR 3F7
AGN415	CR007	17761448	2.7	382	35264	35328	HEK293T/ASGPR 3F7
AGN414	**ABT404**	3740636	1.0	159	11297	11332	HEK293T/ASGPR 3F7
AGN415	**ABT404**	2995554	1.0	222	7688	7681	HEK293T/ASGPR 3F7
AGN7525	CR007	27194361	2.1	288	64259	64051	HEK293T/ASGPR 3F7
AGN7526	CR007	20910871	1.6	320	46139	46187	HEK293T/ASGPR 3F7
AGN7525	**ABT404**	3519935	0.86	77	13917	14103	HEK293T/ASGPR 3F7
AGN7526	**ABT404**	3481239	0.80	108	11846	11923	HEK293T/ASGPR 3F7
AGN417-003	**ABT403**	24533574	6.5	145	15511	15856	HEK293T/ASGPR 3F7
AGN417-003	CR007	106446754	14	317	149981	149579	HEK293T/ASGPR 3F7
AGN310	CR007	180595640	3.8	536	129400	130336	HEK293T/ASGPR 3F7
AGN417	CR007	44541924	16	348	76014	76235	HEK293T/ASGPR 3F7
AGN310	6E9F1	34158932	6.7	161	109076	109330	HEK293T/ASGPR 3F7
AGN417	6E9F1	24504831	13	201	43090	42951	HEK293T/ASGPR 3F7
AGN310	**ABT405**	3572460	2.1	93	4665	4750	HEK293T/ASGPR 3F7
AGN417	**ABT405**	7233146	5.9	84	1652	1648	HEK293T/ASGPR 3F7
AGN417	**ABT406**	6329386	11	139	17209	17293	HEK293T/ASGPR 3F7
AGN417	**ABT407**	6523747	13	148	21660	21737	HEK293T/ASGPR 3F7
AGN417	**ABT408**	5787950	12	154	17345	17392	HEK293T/ASGPR 3F7
AGN417	**ABT409**	3009320	13	135	9896	9934	HEK293T/ASGPR 3F7
AGN417	LSC331895	15734490	3.9	131	14373	14374	HEK293T/ASGPR 3F7
AGN310	**ABT403**	18730287	8.1	262	30999	30978	HEK293T/ASGPR 3F7
AGN417	**ABT403**	5119973	12	160	11473	11396	HEK293T/ASGPR 3F7
AGN310	**ABT404**	17434080	5.9	278	28143	28278	HEK293T/ASGPR 3F7
AGN417	**ABT404**	6188478	7.6	160	9838	9926	HEK293T/ASGPR 3F7
AGN310	CR007	375880365	4.4	870	18436	181504	HEK293T/ASGPR 3F7
AGN417	CR007	63662337	14	339	101617	101846	HEK293T/ASGPR 3F7
AGN310	6E9F1	75696622	8.3	193	178103	178137	HEK293T/ASGPR 3F7
AGN417	6E9F1	33647655	12	213	65729	65562	HEK293T/ASGPR 3F7
AGN310	**ABT405**	4865364	3.1	204	6226	6262	HEK293T/ASGPR 3F7
AGN417	**ABT405**	5047547	5.1	125	2522	2567	HEK293T/ASGPR 3F7

TABLE 40
Ternary complex formation assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN310	**ABT406**	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	**ABT406**	7281303	9.9	124	17333	17446	HEK293T/ASGPR 3F7
AGN310	**ABT407**	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	**ABT407**	13365856	15	172	36832	36918	HEK293T/ASGPR 3F7
AGN310	**ABT408**	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	**ABT408**	11176078	15	169	35586	35681	HEK293T/ASGPR 3F7
AGN310	**ABT409**	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	**ABT409**	8134806	14	163	21271	21320	HEK293T/ASGPR 3F7
AGN310	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	LSC331895	39720375	6.7	134	24728	24844	HEK293T/ASGPR 3F7
AGN412	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	FAMAB-0225WJ	14178555	8.9	142	9221	9300	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ	22511961	33	1475	9696	9509	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ	17307458	20	1013	10553	10347	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ	38255407	30	1018	19458	19560	HEK293T/ASGPR 3F7
AGN416	FAMAB-0225WJ	19267427	40	961	12692	12493	HEK293T/ASGPR 3F7
~~AGN408~~	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN411~~	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN412	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN417~~	FAMAB-0225WJ	9618328	14	317	7363	7331	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ	39925358	5.8	954	19051	19023	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ	27369755	4.6	544	23801	23822	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ	60405203	10	682	47435	47110	HEK293T/ASGPR 3F7
AGN416	FAMAB-0225WJ	29836325	14	507	32582	32404	HEK293T/ASGPR 3F7
AGN414	FAMAB-0225WJ	5683938	0.22	513	9922	9868	HEK293T/ASGPR 3F7
AGN415	FAMAB-0225WJ	4942184	0.49	277	11567	11276	HEK293T/ASGPR 3F7
~~AGN408~~	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN411~~	FAMAB-0225WJ	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN412	CR007	76458748	34	2819	18373	18387	HEK293T/ASGPR 3F7

TABLE 40
Ternary complex formation assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN416	CR007	440252448	8.6	743	247344	248164	HEK293T/ASGPR 3F7
AGN412	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN416	6E9F1	260400828	11	365	310324	309723	HEK293T/ASGPR 3F7
AGN412	CR007	132707886	26	2116	38640	38786	HEK293T/ASGPR 3F7
AGN416	CR007	539139182	7.6	556	395230	397595	HEK293T/ASGPR 3F7
AGN412	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN416	6E9F1	358272147	9.9	309	467454	466336	HEK293T/ASGPR 3F7
AGN414	CR007	27222580	3.0	361	56188	55819	HEK293T/ASGPR 3F7
AGN415	CR007	33152013	3.4	394	65080	65165	HEK293T/ASGPR 3F7
AGN414	6E9F1	37571635	5.0	279	97248	95351	HEK293T/ASGPR 3F7
AGN415	6E9F1	41465544	5.7	278	103211	101093	HEK293T/ASGPR 3F7
AGN414	CR007	7865113	3.8	302	17937	18141	HEK293T/ASGPR 3F7
AGN415	CR007	7499782	4.1	265	20679	20480	HEK293T/ASGPR 3F7
AGN414	6E9F1	9225413	6.7	268	28064	27745	HEK293T/ASGPR 3F7
AGN415	6E9F1	13349047	4.4	274	35099	34485	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN310	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN304~~	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN305	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN408~~	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN411~~	FAMAB-0225WJ-F(E)	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	LSC331895	116665345	7.1	548	97872	97904	HEK293T/ASGPR 3F7
AGN303	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN310	LSC331895	-	-	-	-	-	HEK293T/ASGPR 3F7
~~AGN304~~	LSC331895	8520959	17	712	13725	13308	HEK293T/ASGPR 3F7
AGN301	LSC331895	97854424	7.2	508	92885	93125	HEK293T/ASGPR 3F7
AGN305	LSC331895	3431920	9.1	331	9395	9050	HEK293T/ASGPR 3F7
~~AGN408~~	LSC331895	2198792	14	202	7794	7676	HEK293T/ASGPR 3F7
~~AGN411~~	LSC331895	2319326	10	473	4309	4142	HEK293T/ASGPR 3F7

TABLE 40
Ternary complex formation assay

Compound	Antibody	AUC	EC_{50}-1 (nM)	EC_{50}-2 (nM)	TOP-1	TOP-2	Cell Line
AGN301	CR007	25207429	15	389	30858	31101	HEK293T/ASGPR 3F7
AGN408	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN413	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	6E9F1	25974715	21	294	17723	17742	HEK293T/ASGPR 3F7
AGN408	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN411	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN413	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
ABT307	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
ABT308	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
ABT307	6E9F1	19761430	0.67	254	41572	41010	HEK293T/ASGPR 3F7
ABT308	6E9F1	18890080	0.78	273	39377	38167	HEK293T/ASGPR 3F7
AGN301	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN303	6E9F1	55967412	5.1	315	72773	72744	HEK293T/ASGPR 3F7
AGN310	6E9F1	46065125	2.8	218	71196	71822	HEK293T/ASGPR 3F7
ABT306	6E9F1	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN304	6E9F1	-	662	-	5219	-	HEK293T/ASGPR 3F7
AGN305	6E9F1	-	1528	-	2743	-	HEK293T/ASGPR 3F7
AGN301	CR007	244120632	2.8	640	161946	162065	HEK293T/ASGPR 3F7
AGN303	CR007	215072656	6.1	791	123175	123321	HEK293T/ASGPR 3F7
AGN310	CR007	231564328	6.3	864	121609	121724	HEK293T/ASGPR 3F7
ABT306	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN304	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN305	CR007	-	-	-	-	-	HEK293T/ASGPR 3F7
AGN301	6E9F1	-	-	-	-	-	HepG2
AGN303	6E9F1	-	-	-	-	-	HepG2
AGN310	6E9F1	-	-	-	-	-	HepG2
ABT306	6E9F1	-	-	-	-	-	HepG2
AGN304	6E9F1	-	-	-	-	-	HepG2
AGN305	6E9F1	-	-	-	-	-	HepG2
AGN301	CR007	45590517	11	851	23690	23796	HepG2
AGN303	CR007	-	-	-	-	-	HepG2
AGN310	CR007	-	-	-	-	-	HepG2
ABT306	CR007	-	-	-	-	-	HepG2
AGN304	CR007	-	-	-	-	-	HepG2
AGN305	CR007	-	-	-	-	-	HepG2
AGN4006	CR007	18229209	47	991	10680	10743	HepG2
AGN301	CR007	69481903	33	932	51654	51262	HepG2
AGN4006	T24	9916702	20	540	987	1001	HepG2
AGN301	T24	8600744	17	490	6653	6709	HepG2

Cell Seeding Density at 100000 cells/well. Incubation for three hours.

EXAMPLE 21

Cellular In vitro internalization assays – second results

[00806] *Assay conditions*:

[00807] *Cell lines*: HepG2 vs. HEK293-ASGPR-high (Clone 3F7).

[00808] *Culture media*: DMEM + 10% FBS + 1% P/S.

[00809] *Antibody*: Antibodies all labeled with AF647. Anti-insulin antibody, rabbit monoclonal, Sino, Cat#11038-CR007 (labeled with AF647). Ratio of labeling = 2.8. Anti-insulin antibody, mouse monoclonal, Genscript, catalogue #A01715 (clone 6E9F1). Ratio of labeling = about 2.9.

[00810] *Agents*: 10 µM high conc., 3-fold dilutions, 9-point curve

[00811] *Readout*: High Content Imager (Operetta)

[00812] *Agents tested*:

[00813] **AGN301**: IgG degrader (reference molecule).

[00814] **AGN303**: Native insulin with GN3.

[00815] **AGN302**: Mutant insulin with GN3.

[00816] **ABT306**: Alk-native Insulin without GN3 (just peptide).

[00817] AGN304: Native proinsulin.

[00818] **AGN305**: Mutant proinsulin.

TABLE 41
AGN301 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN401 (10 µM top, 3 fold, 9 points). C-peptide with αGN3. 1% DMSO
AGN402 (10 µM top, 3 fold, 9 points). C-peptide with αGN3. 1% DMSO
AGN403 (10 µM top, 3 fold, 9 points). C-peptide with βGN3. 370 nM **AGN301**
AGN404 (10 µM top, 3 fold, 9 points). C-peptide with βGN3. 370 nM **AGN301**
AGN303 (10 µM top, 3 fold, 9 points). 1% DMSO (HepG2 cells)
AGN303 (10 µM top, 3 fold, 9 points). 370 nM **AGN301**
AGN303 (10 µM top, 3 fold, 9 points). Native insulin with GN3. 1% DMSO
AGN310 (10 µM top, 3 fold, 9 points). 1% DMSO (HepG2 cells)
AGN310 (10 µM top, 3 fold, 9 points). Mutant insulin with GN3. 1% DMSO
AGN310 (10 µM top, 3 fold, 9 points). Native Proinsulin. 370 nM **AGN301**
AGN304 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN304 (10 µM top, 3 fold, 9 points). 370 nM **AGN301** (HepG2 cells)
AGN304 (10 µM top, 3 fold, 9 points). Native Proinsulin. 370 nM **AGN301**

TABLE 41
AGN305 (10 µM top, 3 fold, 9 points). 1% DMSO
AGN305 (10 µM top, 3 fold, 9 points). 370 nM **AGN301** (HepG2 cells)
AGN305 (10 µM top, 3 fold, 9 points). Mutant proinsulin. 370 nM **AGN301**
ABT306 (10 µM top, 3 fold, 9 points). 1% DMSO
ABT306 (10 µM top, 3 fold, 9 points). Native proinsulin. 370 nM **AGN301**

[00819] *Conclusions*: Anti-insulin agents (**AGN303**, **AGN310**) show robust internalization signal in ASGPR-transfected HEK293 cells, but not in HEPG2 cells.

[00820] Anti-proinsulin degrader (**AGN305**) induces some internalization of anti-insulin antibody. Weak internalization with AGN304.

[00821] C-peptide agents were assayed. No internalization of anti-insulin antibody, as expected, but this results shows the specificity of anti-insulin agents.

[00822] **AGN303** and **AGN310** accompanied by mouse anti-human insulin antibody had an effect on HepG2, but these two compounds with rabbit anti-human insulin antibody have no obvious inhibitory effect on HepG2.

[00823] **AGN303** and **AGN310** had a significant effects on HEK293-ASGPR 3F7 cells treated with mouse anti-human insulin antibody. Other data showed that rabbit anti-human insulin antibody has same effects.

[00824] The effect of **AGN305** with mouse anti-human insulin antibody show some weak effect as that of rabbit anti-human insulin antibody on HEK293-ASGPR 3F7.

[00825] Internalization (although weak) of mouse anti-human insulin antibody was seen with **AGN303** and **AGN310** in HepG2 cells.

[00826] Moderate internalization of mouse anti-human insulin antibody was seen with **AGN303** and **AGN310** in HEK293-ASGPR cells.

[00827] *Summary*: Five compounds were tested in internalization assay employing AF647 labeled mouse anti-human insulin antibody.

[00828] **AGN303** and **AGN310** show great effect on both HepG2 and HEK293-ASGPR 3F7.

[00829] **AGN301** plus rabbit anti-human insulin antibody showed great effect on HepG2.

[00830] The compounds and proteins behave as follows:

TABLE 42		
Rabbit Ab	HepG2	HEK293-ASGPR 3F7
AGN303 and **AGN310**	-	+
AGN301	+	+

TABLE 43		
Mouse Ab	HepG2	HEK293-ASGPR 3F7
AGN303 and **AGN310**	+	+
AGN301	-	-

EXAMPLE 22

Agents tested in other assays

[00831] High-Content Screening (HCS) is a high-throughput method used in drug discovery and cell biology that combines automated microscopy and image analysis to extract detailed cellular information.

[00832] Fluorescence-Activated Cell Sorting (FACS) is a flow cytometry technique for sorting cells based on fluorescence.

TABLE 44		
mAb49(F AMAB- 0225WJ)	Effects in HCS	Effects in FACS
AGN301	-	++
AGN303	+++	++
AGN310	+++	+++
AGN414	++	+
AGN415	++	+
AGN304		
AGN305		
AGN408	-	-
AGN411	-	-
AGN412-003	-	-
AGN416-002	++	++
AGN417	++	++

TABLE 44		
mAb49(F AMAB- 0225WJ)	Effects in HCS	Effects in FACS
AGN310	+++	+++
AGN417	++	++

EXAMPLE 23

Pharmacokinetics – first results.

[00833] **AGN303** (native proinsulin), synthetic Insulin degrader. Synthetic insulin MoDE has similar affinity to mAb49. **AGN310** (mutant proinsulin), synthetic Insulin degrader. Synthetic insulin MoDE has similar affinity to mAb49.

[00834] Insulin agents can be captured with GalNAc-binding lectin VVL and detected with an anti-insulin antibody. Proinsulin agents can be captured with an anti-insulin antibody and detected with anti-C-peptide antibody (provides higher sensitivity than VVL).

[00835] *Pharmacokinetics profile **AGN310** in non-obese diabetic (NOD) mice (subcutaneous vs intravenous)*.

[00836] 0.4 mg/kg **AGN310** injection (~700 nM).

[00837] Background is ~30 nM. Compound is cleared within one hour.

[00838] Intravenous area under the curve = 170.

[00839] Subcutaneous area under the curve = 131.

TABLE 45
Study design for NOD mice treated with anti-insulin agents

Groups		Test articles	#	Route	Dosage (mg/kg)	Regimen	Duration (week of age)
Control	1	Vehicle	30	i.v.	TBD	Once weekly or every 4-5 days	6-24
Treatment	2	Anti-insulin degrader (Mutant)	30	i.v.	TBD dose #1	Once weekly or every 4-5 days	6-24
Treatment	3		30	i.v.	TBD dose #2	Once weekly or every 4-5 days	6-24

Methods: Frequency of blood/tissue samples harvesting.

[00840] Urine glucose (optional)

[00841] Blood glucose (once weekly)

[00842] HbA1c. Keep whole blood.

[00843] *Pharmacodynamics*: MesoScale Discovery/ELISA (autoantibody). Obtain serum.

[00844] Blood draw for Insulin levels (MesoScale Discovery/ELISA, or LC/MS). Obtain the serum. Bank the serum to determine timepoints and readout.

[00845] Blood draw for cytokines (MesoScale Discovery). Obtain the serum. Bank the serum to determine timepoints and readout.

[00846] Full Biochemistry (baseline; after first dose; terminal) Obtain the serum.

[00847] Full Hematology (baseline; after first dose; terminal). Keep the whole blood.

[00848] Pancreatic islets (hematoxylin & eosin staining assay from formalin-fixed paraffin-embedded blocks). Save formalin-fixed paraffin-embedded blocks for follow-up staining.

[00849] *Pharmacokinetics*: Multiple dose or single dose done.

EXAMPLE 24

Pharmacokinetics – second results.

[00850] Several agents (TARPs) were tested.

[00851] **AGN2640**-P009

[00852] **AGN301**-P013

[00853] **AGN4586**-P007

Nonclinical protocol template.

[00854] Pharmacokinetic evaluation of AGN417 in CD1 versus NOD mice: 1 mg/kg dosing via Intravenous or mg/kg subcutaneous.

[00855] The objective was to compare the pharmacokinetic profiles of AGN417 administered at a dose of 1 mg/kg in two different mouse strains (CD1 or NOD), using intravenous or subcutaneous (SC) routes of administration. This study aims to evaluate any strain-based differences in absorption, distribution, metabolism, and elimination of AGN417, providing insights into the optimal mouse model and route of administration for future pharmacokinetic and pharmacodynamic studies.

[00856] *NOD mouse*: NOD/ShiLtJ Strain Jackson #: 1976.

[00857] *CD1 female mouse*: CD1 Strain CRL#: Crl:CD1(ICR).

[00858] Age and weight range at start of study: Animals aged six-eight weeks with body weight of approximately 25-30 g.

[00859] The mouse is a well characterized system for drug efficacy evaluation.

[00860] Administration of agents

[00861] Dose: 1 mg/kg or 8.8 mg/mL.

[00862] Concentration dosing solution: 0.1 mg/ml

[00863] Purity: AGN417-003 is 97.91%.

[00864] Routes of administration: Intravenous or subcutaneous.

[00865] Justification for route of administration: Intravenous and subcutaneous administration are acceptable routes of agent administration in small animals.

[00866] Frequency and duration of dosing: See Tables 1 & 2

[00867] Administered doses: 1mg/kg intravenous or subcutaneous.

[00868] Administered volume(s): 10 ml/kg.

[00869] *Assay design*: Upon arrival at the facility, animals are acclimated for 7 days. On day 0, animals is weighed and randomly assigned to groups. All groups of animals receive their treatment of 1 mg/kg AGN417, as described in the TABLE below, either intravenously or subcutaneously. After a defined period, three animals from each group will undergo blood collection at two time points: the first time point involves collecting around 100 µl of blood from the submandibular vein, and the second will involve cardiac exsanguination following carbon dioxide asphyxiation. Blood samples from individual animals is collected in K3EDTA tubes, and plasma is separated. All samples are stored at -80°C until further analysis.

[00870] Non-fasting blood glucose levels is monitored by collecting a small blood drop from a tail nick at the tip of the tail. The blood is applied to a One-Touch test strip and measured using a One-Touch Ultra 2 Glucometer in three mice per group at the following time points: pre-dose, five minutes, ten minutes, fifteen minutes, thirty minutes, one hour, and two hours post-dose.

TABLE 46

Genotype	Number of Animals	Treatment	Plasma Collection
NOD	12	IV AGN417 (1mg/kg)	0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 24 hr
NOD	12	SC AGN417 (1mg/kg)	0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 24 hr
CD1	12	IV AGN417 (1mg/kg)	0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 24 hr
CD1	12	SC AGN417 (1mg/kg)	0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 24 hr

[00871] *Body weights*. Animal body weights is measured and recorded at randomization on Day 0. After dosing, weights is recorded daily.

[00872] *Tissue harvesting and sample analysis*. Blood is harvested into K3EDTA containing tubes. Blood in plasma separator tubes is separated by spinning samples at 9 G at 4□C for 15 minutes. Plasma is collected and analyzed with our in-house MSD. Blood glucose levels is measured after a tail nick using One-Touch Ultra 2 Glucometer.

[00873] *Clinical observations/signs*. During quarantine and the study period, a minimum of once daily cage-side or detailed observations and morbidity/mortality checks is performed on all animals by study personnel or a member of the animal care staff. Observations include but are not limited to the following: reactivity to general stimuli and description of any abnormal behaviors, lesions, or appearances, if applicable.

[00874] *Data analysis and reporting*. Plasma levels of AGN417 are determined by in-house developed MSD. Data are presented in tabular form. A standard pharmacokinetic analysis is to be performed for each dose group.

EXAMPLE 25

Pharmacodynamics.

[00875] *Study design*: 13.3 nmol/kg anti-insulin autoantibodies – 6E9F1 intravenously (2 mg/kg). 133 nmol/kg **AGN310** intravenously – 1:10 ratio.

[00876] An assay in mice showed a 39% depletion by **AGN310**, based on area under the curve.

[00877] The inventors performed a pharmacodynamics evaluation of **AGN310** in nude mice. Nude mouse plasma causes no interference in antibody detection.

[00878] Degrader interference could be prevented by diluting samples to below 10 ng/mL antibody.

EXAMPLE 26

Pharmacodynamics. NOD mouse insulin inhibition.

TABLE 47
Rodent models for Type 1 diabetes

Model of Type 1 diabetes	Onset	Age/time of induction	Immune cell involvement?	Useful to understand pathogenesis?
Nonobese diabetic (NOD) mice	Spontaneous autoimmune	12–30 weeks	Yes	Yes
Biobreeding (BB) rats	Spontaneous autoimmune	8–16 weeks	Yes	Yes

TABLE 47
Rodent models for Type 1 diabetes

Model of Type 1 diabetes	Onset	Age/time of induction	Immune cell involvement?	Useful to understand pathogenesis?
Lew.1AR1-iddm rats	Spontaneous autoimmune	~8 weeks	Yes	Yes
Komeda diabetic-prone (KDP) rats	Spontaneous	~8 weeks	Yes	Yes
Akita mice	Spontaneous / Genetically induced	~4 weeks	No	Some aspects
Low dose streptozotocin	Chemical induced	Within days	Yes	Some aspects. A model of induced insulitis
High dose streptozotocin	Chemical induced	Within days	No	
Alloxan	Chemical induced	Within days	No	

[00879] Non-obese diabetic (NOD) mice were assayed to understand the effect on glucose levels. The assay used female seventeen weeks old NOD mice. The mice were checked for glucose level (>115 mg/dL). The assayed groups each group had five mice with glucose levels = 300 – 500 mg/dL and five mice with glucose levels = 115 – 300 mg/dL. The mice were regularly checked at day 0 and day 5.

[00880] *TRAP agents deplete naturally occurring insulin autoantibodies in non-obese diabetic (NOD) mice*. Two TRAP agents were administered to NOD mice to assay NOD mouse insulin inhibition in vivo. **AGN310** and AGN417 were administered, each both intravenously and subcutaneously. Along with this administration, NOD mice were assayed for body weight and glucose levels (PharmaLegacy). Body weight taken at each dosing, every five days. Non-fasting blood glucose taken every two weeks via tail vein bleed (9-10 AM).

TABLE 48
NOD mice IgG calculated based on background subtraction

	Undiluted
Eight weeks M2	9.2 ng/mL
Eight weeks M3	16.5 ng/mL
Eight weeks M4	39.7 ng/mL
Six weeks M2	5.0 ng/mL

TABLE 48 NOD mice IgG calculated based on background subtraction	
	Undiluted
Six weeks M3	0.6 ng/mL
Six weeks M7	27.5 ng/mL

[00881] The inventors observed small increase in insulin antibodies in NOD mice as they age.

EXAMPLE 27

Chemical synthesis of proinsulin degrader

[00882] *Peptide 1*. (1) Soft cleavage (leave protecting groups) except cysteine (trt). (2) Perform disulfide bond formation. (3) Orthogonal cysteine protection.

[00883] *Peptide 2*. (1) Soft cleavage.(2) N-terminal Fmoc. (3) Fragment condensation (peptide 1 + peptide 2).

[00884] *Peptide 3*. (1) Soft cleavage. (2) N-terminal Fmoc during condensation (peptide 1/2 + peptide 3). (3) Fmoc-deprotection and global deprotection, except Cys-Acm.

[00885] *Peptide 4*. (1) C-terminal thioetser (Dbz resin). (2) In-solution. Coupling of sugar might be needed. (3) Deprotected except cysteine (Acm). (4) Perform NCL.

[00886] See Dawson et al., Science, 266, 776-779 (1994).

EXAMPLE 28

Tolerability in vivo in NOD mice.

[00887] Fit for purpose tolerability assays were run with single intravenous 3 mg/kg dose to match concentration used in the PharmaLegacy assay.

[00888] **AGN303** (native proinsulin) and **AGN310** (mutant proinsulin) Insulin agents were well-tolerated when administered at 3 mg/kg intravenously in pre-diabetic NOD mice, with no observed adverse events and no changes in glycemia when measured at twelve hours-post dose.

EXAMPLE 29

***AGN310** extended tolerability in NOD female mice.*

[00889] The objective of this EXAMPLE was to investigate the longer-term effects of multi-dosing IV **AGN310** on NOD female mice.

TABLE 49
Assay design

Parameter	Details
Animal Model	NOD Female Mice
Number of Mice	20
Age	6 weeks
Source	Taconic

TABLE 50

Group	Number	Treatment	Dose	Administration	Body weight	Volume of Dosing Solution
Control Group	5	Vehicle	N/A	Intravenous (IV), every 4 days	0.025 kg	0.25 ml
Treatment Group 1	5	**AGN310**	0.1 mg/kg	Intravenous (IV), every 4 days	0.025 kg	0.25 ml
Treatment Group 2	5	**AGN310**	0.5 mg/kg	Intravenous (IV), every 4 days	0.025 kg	0.25 ml
Treatment Group 3	5	**AGN310**	5 mg/kg	Intravenous (IV), every 4 days	0.025kg	0.25 ml

TABLE 51
Timeline

Week	Activity
Week 0	Arrival and acclimatization
Week 1	Baseline measurements: body weight, blood glucose and urine glucose at same time of day pre-dose and post-dose, etc. First treatment administration. Monitor for 20 minutes post injection – clinical chemistry and serology. Second treatment administration. Monitor for 20 minutes post injection. Body Weight, Blood Glucose and Urine glucose at same time of day pre- and post-dose, etc.

TABLE 51 Timeline	
Week	Activity
Weeks 2	Third treatment administration. Monitor for 20 minutes post injection: body weight, blood glucose and urine glucose at same time of day pre-dose and post-dose, etc. Sacrifice and tissue collection: livers, pancreas, kidneys, heart, spleen,

Measurements and data collection

[00890] *Body weight*: Weekly

[00891] *Blood glucose and urine glucose*: Weekly pre-dose and post-dose.

[00892] *Behavioral assessments*: Weekly for 20 minutes and side check at end of week.

[00893] *Biochemical assays*: Blood glucose, Insulin.

[00894] *Histological analysis*: Of tissues collected.

EXAMPLE 30

Tolerability assay.

[00895] Also assaying blood glucose levels (BGL). Intravenous administration resulted in a rapid and pronounced change in blood glucose levels, with an initial sharp decrease followed by a peak, indicating that intravenous administration may cause more dramatic blood glucose fluctuations due to faster absorption and clearance. Subcutaneous administration led to more stable blood glucose levels with less extreme fluctuations compared to intravenous administration.

TABLE 52

Dose	Mouse	BW	BGL Pre-dose	BGL 5m	BGL 15m	BGL 30m	BGL 1h	BGL 1:30h	BGL 2h
0.5 mpk	1	18	103	158	150	141	151	130	136
	2	24.5	128	166	173	174	171	154	149
	Average		115.5	162	161.5	157.5	161	142	142.5
1 mpk	1	22	150	171	169	189	199	176	159
	2	21	120	148	145	131	135	103	110
	Average		135	159.5	157	160	167	139.5	134.5
3 mpk	1	25	152	163	150	180	188	173	146
	2	23	130	130	153	142	183	153	139
	Average		141	146.5	151.5	161	185.5	163	142.5
5 mpk	1	25	130	146	149	137	137	135	157
	2	23	138	149	156	144	136	120	130

TABLE 52

Dose	Mouse	BW	BGL Pre-dose	BGL 5m	BGL 15m	BGL 30m	BGL 1h	BGL 1:30h	BGL 2h
	Average		134	147.5	152.5	140.5	136.5	127.5	143.5
10 mpk	1	26	133	126	153	143	153	147	150
	2	23	127	153	148	157	152	146	137
	Average		130	139.5	150.5	150	152.5	146.5	143.5
Control	1	22	122	147	153	162	174	158	148
	2	22	119	160	147	138	118	109	113
	3	25	123	149	142	151	144	146	136
		25	130	149	147	139	136	149	150
	Average		124	153	145	143	133	135	133

EXAMPLE 31

*One-week assay of **AGN417** by subcutaneous Injection and intravenous infusion in Wistar Han IGS rats*

[00896] The objective of this EXAMPLE is to determine the potential toxicity of AGN417 given every once every 3 days via subcutaneous injection or intravenous injection for one week to Wistar Han IGS rats (available from Charles River Laboratories). The toxicokinetic characteristics of AGN417 are to be determined.

[00897] The dose levels can be selected based on information provided in the specification above. Tolerability data associated with AGN417 is limited. In a previous pharmacokinetic study mice given AGN417, intravenous and subcutaneous doses up to 3 mg/kg were well-tolerated, but exposure correlated with transient reduction in blood glucose levels of approximately 50 mg/dL. At no point during pharmacokinetic studies did blood glucose fall below reference range intervals. Blood glucose levels returned to vehicle control animal levels when food was provided. The current study will evaluate the dose-response relationship of AGN417 in rats. The low dose of 15 mg/kg is a low multiple of previous doses in mice, and the mid and high doses of 50 and 150 mg/kg study will explore tolerability and attempt to build a dose-response relationship. The subcutaneous dose of 50 mg/kg is intended to understand the subcutaneous bioavailability and pharmacokinetic profile relative to intravenous administration.

[00898] The Wistar Han IGS rat was chosen as the animal model for this assay because it is an accepted rodent species for nonclinical toxicity testing by regulatory agencies.

[00899] Assays in laboratory animals provide a basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models that do not use live animals currently do not exist.

TABLE 53
Preparation Details

Dose formulation	Frequency of preparation	Storage conditions
Vehicle/Control article	Daily (or prepared day prior, to be used for dosing within 24 hours of completion of preparation)	Temperature set to maintain at 5°C.
Test article	Daily (or prepared day prior, to be used for dosing within 24 hours of completion of preparation)	Temperature set to maintain at 5°C.

[00900] Preparation details for control and test materials. Dosing formulations are prepared at appropriate concentrations to meet dose level requirements. The assay includes testing formulation pH and osmolality to ensure matched and at physiologically appropriate levels.

[00901] All dosing samples are stored on wet ice at the testing facility, before dosing. Collected samples stored at a temperature set to maintain -60-90°C. Dosing samples are evaluated for pH and osmolarity prior to dosing, All dose solutions are normalized to physiological osmolarity where possible, using sodium chloride.

[00902] *Animal Identification*. Subcutaneously implanted electronic identification chip or other approved identification method such as indelible ink where required.

[00903] Environmental Acclimation. A minimum acclimation period of four days is allowed between animal arrival and the start of treatment to accustom the animals to the laboratory environment.

[00904] *Selection and assignment of animals*: Animals are randomly assigned to groups. Males and females is randomized separately. Animals in poor health or at extremes of body weight range will not be assigned to groups. Substitutions may be made after initial group assignment based on evaluation of data from individual animals collected before dosing.

[00905] *Replacement of animals*: Before the initiation of dosing, any assigned animals considered unsuitable for use in the study are replaced by alternate animals. After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-test article-related health issues, or similar circumstances. The alternate animals may be used as replacements on the study within one to three days.

[00906] The housing set-up is as specified in the United States Department of Agriculture Animal Welfare Act (9 C.F.R., Parts 1, 2 and 3) and as described in the Guide for the Care and Use of Laboratory Animals (National Research Council, Current edition; Office of Laboratory Animal Welfare, Current edition). Animals are separated during designated procedures/activities or separated as required for monitoring and/or health purposes, as deemed appropriate by Study Director and/or staff veterinarian.

Animals may be individually housed due to incompatible behavior with a cage mates. Cages are arranged in group order. Control group animals are housed on a separate rack from the test article-treated animals.

TABLE 54
Experimental design

Group No.	Test Article	Dose Level (mg/kg/dose)	Administration Route	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Males
1	Vehicle Control	0	IV	10	0	6
2	AGN417	15	IV	10	1.5	6
3	AGN417	50	IV	10	5	6
4	AGN417	150	IV	10	15	6
5	AGN417	50	SC	10	5	6

SC = subcutaneous injection; IV = intravenous infusion (~3 minutes). Dose Volume is based on the most recent body weight measurement.

TABLE 55
General in-life assessments

Parameter	Populations	Frequency (minimum required)	Comments
Mortality	All animals	Twice daily (morning and afternoon) beginning upon arrival through termination.	Animals are observed within their cage unless necessary for identification or confirmation of possible findings.
Cage side Observations	All main animals	Once daily, from at least Week -1. Not required on days when detailed clinical observations are scheduled.	Animals are observed within their cage unless necessary for identification or confirmation of possible findings.
Post-dose Observations	All main animals	Groups 1-4 , immediately after dosing, SC group 5 approximately 2 hours post-dose. Not required on days when detailed clinical observations are scheduled.	Animals are observed within their cage unless necessary for identification or confirmation of possible findings.

TABLE 55
General in-life assessments

Parameter	Populations	Frequency (minimum required)	Comments
Detailed Clinical Observations	All main animals	Once weekly, from at least Week -1 and throughout the study	Animals are removed from the cage.
Individual Body Weights	All main animals	Once weekly, from at least Week 1 and throughout the study	Not collected from animals found dead or unscheduled euthanized.
Food Consumption	All main animals	Once daily, from at least Week -1 and throughout the study	Quantitatively measured

TABLE 56
Clinical pathology sample collection

Group Numbers	Time Point (Day)	Hematology	Coagulation	Clinical chemistry	Urinalysis
1 to 5	6	-	-	-	X
1 to 5	7 (at termination)	X	X	X	-
Unscheduled euthanasia (when possible)		X	X	X	-
Fasting:		-	-	-	5 to 6 hours
Method/Comments:		Abdominal aorta [a]			Metabolism cages [b]
Target Volume (mL)[a]:		0.5 mL	1.2 mL	1.0 mL	As available
Anticoagulant:		K_3EDTA	Sodium citrate	-	-
Special Requirements:		-	-	Serum separator tube	Ambient
Processing:		None	Plasma	Serum	-

TABLE 57
Hematology parameters

White Blood Cell count Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large Unstained Cells (absolute) Other Cells (as appropriate)	Red Blood Cell count Hemoglobin concentration Hematocrit Mean Corpuscular volume Mean Corpuscular Hemoglobin Mean Corpuscular Hemoglobin concentration Gated Red Blood Cell Distribution Width Platelet count Reticulocyte count (absolute)

TABLE 58
Coagulation parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time Sample quality

TABLE 59
Clinical chemistry parameters

Aspartate Aminotransferase Alanine Aminotransferase Alkaline Phosphatase Gamma-Glutamyltransferase Creatine Kinase Total Bilirubin Urea Nitrogen Creatinine Glucose Cholesterol Triglycerides	Total Protein Albumin Globulin (calculated) Albumin/Globulin Ratio Calcium Phosphorus Sodium Potassium Chloride Sample Quality

TABLE 60
Urinalysis parameters

Color Appearance/Clarity Specific gravity pH Volume	Protein Glucose Bilirubin Ketones Blood

[00907] Bone marrow smear evaluation. Bone marrow smears are collected and prepared. Evaluation of stained smears may be performed.

TABLE 61
Bioanalytical Sample Collection, Target Time Post-dose on Days 1 and 7

Group No.	Subgroup	No. of Animals (M/F)	0 hr [a]	0.5 hr	1 hr	2 hr	6 hr	24 hr	168 hr
1	A	3	X	-	X	-	X	-	X
	B	3	-	X	-	X	-	X	
2	A	3	X	-	X	-	X	-	X
	B	3	-	X	-	X	-	X	-
3	A	3	X	-	X	-	X	-	X
	B	3	-	X	-	X	-	X	-
4	A	3	X	-	X	-	X	-	X
	B	3	-	X	-	X	-	X	-
5	A	3	X	-	X	-	X	-	X
	B	3	-	X	-	X	-	X	-
Method/Comments:			Venipuncture [b]						
Target Volume:			0.3 mL						
Anticoagulant:			K2EDTA						

[00908] *Bioanalytical sample analysis*. Samples are analyzed for concentration of AGN417. Statistical analyses including regression analysis and descriptive statistics including arithmetic means and standard deviations, accuracy, and precision are performed.

[00909] *Toxicokinetic ev*aluation. TK parameters are generated using the concentration units provided by the bioanalytical lab. Concentration values below the limit of quantitation are treated as zero for the purposes of TK data analysis.

TABLE 62
Parameters to be estimated

Parameter	Description of Parameter
t_{max}	The time at which the maximum concentration was observed.
C_0	The theoretical concentration at time zero after intravenous bolus dosing only.
C_0/Dose	The C_0 divided by the dose administered.
C_{max}	The maximum observed concentration measured.

TABLE 62
Parameters to be estimated

Parameter	Description of Parameter
C_{max}/Dose	The C_{max} divided by the dose administered.
AUC_{tlast}	The area under the concentration versus time curve from the start of dose administration to the last observed quantifiable concentration calculated using the linear trapezoidal linear interpolation method.
AUC_{tlast}/Dose	The AUC_{tlast} divided by the dose administered.
AUC_{0-t}	The area under the concentration time curve from time zero to t, where t denotes a specific sampling time following dosing calculated using the linear trapezoidal linear interpolation method.
AUC_{0-t}/Dose	The $AUC_{(0-t)}$ normalized for dose.
t_{last}	The time after dosing at which the last quantifiable concentration was observed.

TABLE 63
Secondary parameter abbreviations

Parameter	Description of parameter
$t_{1/2}$	Terminal half-life.
AUC_{0-inf}	Area under the concentration-time curve from time zero extrapolated to infinity. Only determined from single dose data, if appropriate.
AUC_{0-inf}/Dose	The AUC_{0-inf} normalized for dose.
Cl	Total body clearance of parent test article.
V_z	Volume of distribution of parent test article based on the terminal elimination phase.
Vss	Volume of distribution at steady state (IV samples)
Cl/F	Total body clearance of parent test article divided by the fraction of dose absorbed.
V_z/F	Volume of distribution of parent test article based on the terminal elimination phase divided by the fraction of dose absorbed.

[00910] Partial AUCs (between two defined sample times), and corresponding dose-normalized values, may be derived and reported to aid interpretation. Descriptive statistics (e.g., number, arithmetic mean, median, standard deviation, standard error, coefficient of variation) are reported as deemed appropriate. Ratios for appropriate grouping and sorting variables may be generated when appropriate.

TABLE 64

Ratio	Description of Ratio
F:M AUC	The area under the curve for females divided by the area under the curve for males
F:M C_{max}	The C_{max} for females divided by the C_{max} for males
R_{AUC}	The area under the curve from T1 to T2 after repeat dosing divided by the area under the curve from T1 to T2 during the initial dosing interval.

[00911] *Unscheduled euthanasia*. Main animals that are euthanized for humane reasons will have appropriate samples obtained. Main animals are euthanized by exsanguination under deep isoflurane anesthesia (preferred) or by carbon dioxide asphyxiation. Main animals will undergo necropsy, and specified tissues are retained. If necessary, any animal found dead or unscheduled euthanasia the animal is refrigerated until necropsy to minimize autolysis.

[00912] *Scheduled euthanasia*. Main animals surviving until scheduled euthanasia will have terminal body weights taken prior to euthanasia. At time of termination, appropriate samples are collected from the abdominal aorta under isoflurane anesthesia. Animals will then be euthanized by exsanguination under deep isoflurane anesthesia. When possible, the animals are euthanized rotating across dose groups such that similar numbers of animals from each group, including controls, are necropsied throughout the day.

[00913] *Necropsy*. Animals are subjected to a complete necropsy examination, which includes evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

[00914] *Organ weights*. Organs are weighed at necropsy. Paired organs are weighed together. In the event of gross abnormalities, in addition to the combined weight, the weight of each organ of a pair may be taken and entered as a tissue comment. Organ weight as a percent of body weight (using the terminal body weight) and organ weight as a percent of brain weight is calculated.

[00915] *Tissue collection and preservation*. Representative samples of tissues are collected and preserved in 10% neutral buffered formalin, except for tissues requiring alternate fixatives as defined by standard operating procedures.

[00916] *Histology*. Tissues are processed. Glass slides may be scanned at 20x/40x.

[00917] *Microscopic evaluation*. Tissues are evaluated histopathologically by light microscopy or digitally using whole slide images (WSI). Special stains may be used at the discretion of the pathologist

to further characterize lesions and changes identified during routine evaluation of individual animals. Any special stains are documented in the individual animal data. Any additional stains or evaluations, if deemed necessary by the pathologist, may be added.

[00918] *Statistical analysis*. Any data collected during the pre-dose period are tabulated, summarized, or statistically analyzed. All statistical analyses are performed within the respective study phase, unless otherwise noted. Numerical data collected on scheduled occasions are summarized and statistically analyzed as indicated below according to sex and occasion.

[00919] *Descriptive statistical analyses*. Means, standard deviations (or % coefficient of variation or standard error, when deemed appropriate), ratio, percentages, numbers, or incidences are reported as appropriate by dataset.

[00920] *Inferential statistical methods*. All statistical tests are conducted at the 5% significance level. All pairwise comparisons are conducted using two sided tests and reported at the 1% and 5% levels, unless otherwise noted. Equivalent tests may be reported depending on the terminology defined in the software used in the statistical analysis.

TABLE 65
Statistical matrix

Variables for Inferential Analysis	Statistical method. Parametric/non-parametric
Body Weight	X
Clinical Chemistry Variables	X
Coagulation Variables	X
Hematology Variables	X
Organ Weights	X
Organ Weight relative to Body Weight	X
Organ Weight relative to Brain Weight	X
Urinalysis Variables	X

[00921] *Parametric/non-parametric*. Levene's test is used to assess the homogeneity of group variances. The groups are compared using an overall one-way ANOVA F-test if Levene's test is not significant or the Kruskal-Wallis test if it is significant. If the overall F-test or Kruskal-Wallis test is found to be significant, then pairwise comparisons are conducted using Dunnett's or Dunn's test, respectively.

[00922] *Non-parametric*. The groups are compared using an overall Kruskal-Wallis test. If the overall Kruskal-Wallis test is found to be significant, then the pairwise comparisons are conducted using Dunn's test (equivalent to Wilcoxon Rank-Sum test in Nevis 2012 tables).

[00923] *Incidence*. A Fisher's exact test is used to conduct pairwise group comparisons of interest.

[00924] This assay should comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the Public Health Service Policy on Humane Care and Use of Laboratory Animals from the Office of Laboratory Animal Welfare, and the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.

[00925] If any animal is determined to be in overt pain/distress or appears moribund and is beyond the point where recovery appears reasonable, the animal is euthanized for humane reasons in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

EXAMPLE 32

Primate assay

[00926] Ageing chimpanzees develop aging-related illnesses and thus provide a good model for aging-related diseases associated with increased anti-insulin autoantibodies or ant-proinsulin autoantibodies.

EXAMPLE 33

Guidance for starting dose and human dose.

[00927] The inventors' rationale for starting dose is guided by the United States Food & Drug Administration Guidance for Industry: Estimating the Maximum Safe Starting Dose (2005) and any subsequent updates. The maximum recommended starting dose (MRSD) for first-in-human (FIH) clinical trials of new molecular entities in adult healthy volunteers based on human equivalent dose (HED) based on the no observed effect level (NOEAL) in the most sensitive species.

[00928] The inventors' rationale for starting dose is guided by the EMA Guideline on strategies to identify and mitigate risks for first-in-humans (FIH) and early clinical trials (2017) and any subsequent updates.

[00929] The starting dose is expected to be the lower of the following values: (1) NOAEL in the most sensitive species adjusted for HED, (2) minimal anticipated biological effect level (MABEL), or (3) pharmacologically active dose (PAD).

[00930] The human dose projections for the insulin TRAP proinsulin degrader or the proinsulin TRAP degrader program is expected to further depend on the preclinical data, the in-vitro data, and the

understanding of the pharmacokinetics/pharmacodynamics relationship, some of which is disclosed in the EXAMPLES above.

[00931] *Model independent HED-type of approach to determine MRSD and scale effective animal model exposures/doses to human*. Scaling using body weight-based approach known to persons having ordinary skill in the biomedical arts from preclinical species using standard allometric principles.

[00932] *Model dependent approach*. Leverage animal pharmacokinetics models that can be scaled to humans and use information about pharmacokinetics/pharmacodynamics relationship to understand effective exposure and perform translational simulation.

[00933] Given the potential agonistic activity, i.e., hypoglycemia, a pharmacologically active dose (PAD) approach can be informed by either dose-response, exposure-response and/or pharmacokinetics/pharmacodynamics modeling using mouse, rat, or non-human primate data.

[00934] Quantitative systems pharmacology approaches can also be evaluated and may be beneficial for further clinical development.

EQUIVALENTS

[00935] Persons having ordinary skill in the biomedical art will recognize or be able to determine using no more than routine experimentation many equivalents to the specific procedures described in this specification. Such equivalents are within the scope of this invention and are covered by these claims. For example, pharmaceutically acceptable salts other than those specifically 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 there is a specific disclosure in this specification identifying this combination.

[00936] Some embodiments of the invention can be practiced according to the following numbered paragraphs:

[00937] 1. A composition of matter comprising: a binding moiety, a cellular receptor binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on surface degrading cells, and a linker moiety (optionally a single peptide linkage) connecting the binding moiety and the cellular receptor binding moiety.

[00938] 2. The composition of matter of Embodiment 1, wherein the binding moiety is an antigen-binding protein, an antigen-binding protein variant, or an antigen-binding fragment thereof.

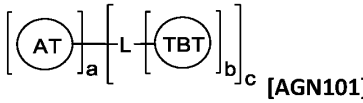
[00939] 3. The composition of matter of Embodiment 1, wherein the binding moiety is an insulin, an insulin variant, an insulin analogue, or an insulin fragment thereof.

[00940] 4. The composition of matter of Embodiment 1, wherein the binding moiety is a proinsulin, a proinsulin variant, a proinsulin analogue, or a proinsulin fragment thereof.

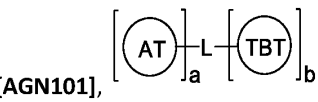
[00941] 5. The composition of matter of Embodiment 1, wherein the binding moiety is an insulin Fc fusion protein.

[00942] 6. The composition of matter of Embodiment 1, wherein the binding moiety is a proinsulin Fc fusion protein.

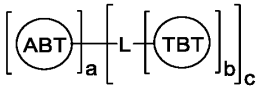
[00943] 7. The composition of matter of Embodiment 1, having a structure of: R^{CN}–(Xaa)y–R^{CC},



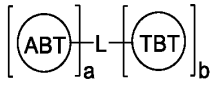
[AGN101],



[AGN102],

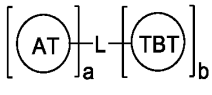


[AGN103], or



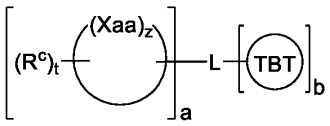
[AGN104] or a pharmaceutically acceptable salt thereof.

[00944] 8. The composition of matter of Embodiment 1, wherein the agent has the structure of formula [**AGN102**]:

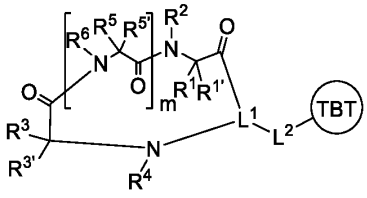


, or a pharmaceutically acceptable salt thereof, wherein: each of a and b is independently an integer of 1 or greater; each AT is a binding moiety or a fragment thereof; L is a linker moiety; and each TBT is independently a cellular receptor binding moiety which binds to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on the surface degrading cells in a patient or subject, wherein the binding moiety is a ligand variant or an antigen-binding fragment thereof.

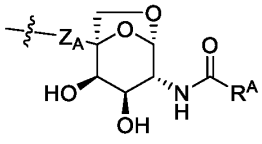
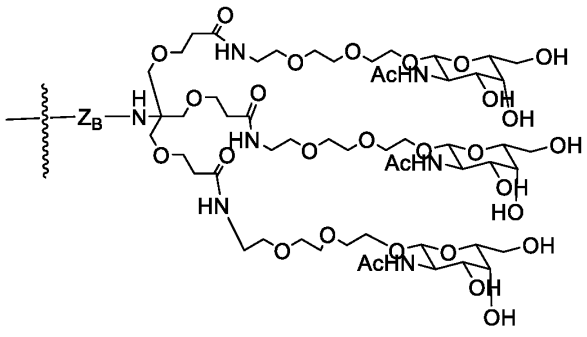
[00945] 9. The composition of matter of Embodiment 1, wherein the agent has the structure of formula **AGN105**:



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

[00946] 10. The composition of matter of Embodiment 1, wherein the agent has the structure of formula **AGN106**:  or a salt thereof, wherein the composition of matter has additional elements described in this specification.

[00947] 16. The composition of matter of Embodiment 15, wherein the cellular receptor binding moiety has the following structure:

[00948]  or ;

[00949] 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.

[00950] 17. The composition of matter of Embodiment 16, wherein R^A is a methyl or ethyl group optionally substituted with from 1-3 fluoro groups.

[00951] 18. The composition of matter of Embodiment 17, wherein Z_A is a PEG group containing from 1 to 4 ethylene glycol residues.

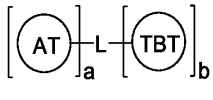
[00952] 19. The composition of matter of Embodiment 18, wherein the methyl or ethyl group is substituted with from 1-3 fluoro groups.

[00953] 20. The composition of matter of Embodiment 17, wherein the ASGPR binding group is N-acetyl-D-galactosamine.

[00954] 21. The composition of matter of Embodiment 1, wherein the cellular receptor binding moiety is a low-density lipoprotein receptor-related protein 1 (LRP1), a low-density lipoprotein receptor (LDLR), a Fcγ receptor I-binding group, a FcRn binding group, a transferrin receptor binding group, or a macrophage scavenger receptor binding group.

[00955] 22. A pharmaceutical composition comprising composition of matter of any of the preceding claims and a pharmaceutically acceptable excipient.

[00956] 23. The composition of matter of Embodiment 1, wherein the binding moiety is a ligand variant or an antigen-binding fragment thereof.

[00957] 24. The composition of matter of Embodiment 1, wherein the agent has the structure of formula [**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 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.

[00958] 25. The composition of matter of Embodiment 24, wherein a is 1 and b is 3.

[00959] 26. The composition of matter of Embodiment 25, wherein binding moiety comprises a peptide moiety that binds to a specific amino acid residue of the glycan-specific IgG antibody.

[00960] 27. 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.

[00961] 28. The composition of Embodiment 27, wherein the at least one additional agent has the structure of formula **AGN302, MAT302**, or a combination thereof: LG–RG–L^{RM}(–TBT)$_b$, **[AGN302]**; and LG–RG–H **[MAT302]**.

[00962] 29. The composition of Embodiment 43, wherein AT is an anti-autoantibody moiety, TBT is N-acetylgalactosamine (GalNAc), a is 1, and b is 3.

[00963] 30. A method of removing in a patient or subject in need comprising administering to the mammal the agent of any of Embodiment 1-29.

[00964] 31. A method of treating a disease state and/or condition associated with the upregulation of in a patient or subject in need administering to the mammal an effective amount of the agent of any of Embodiment 1-29.

[00965] 32. A method of treating IMN in a patient in need comprising administering to the mammal an effective amount of the agent of any of Embodiment 1-29.

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[00966] 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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[001081] Throughout this application, several publications are referenced by author name and date, or by patent number or patent publication number. The disclosures of these publications are incorporated in their entireties by reference into this application to describe the state of the art more fully as known to persons having ordinary skill in the art 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.

[001082] 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 such disclosure under prior invention or for any other reason. If there is an apparent discrepancy between a prior patent or publication and the description provided 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 constitute 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: 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
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 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): 2
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 KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN 300
VFSCSVMHEA THNHYTQKSL SLSPGK 326

Sequence Number (ID): 3
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 KTGPPCPSCP APEFLGGPSV 120
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 180
RVVSVLTVLH QDWINGKEYK CKVSNKGLPS SIEKTISKAK GOPREPQVYT LPPSQEEMTK 240
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG 300
NVFSCSVMHE ATHNHYTQKS LSLSLGK 327

Sequence Number (ID): 4
Length: 110
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..110
> mol_type, protein
> organism, Homo sapiens
- PROPEP, 1..24
> note, Proinsulin leader sequence
- REGION, 25..54
> note, B chain of insulin
- REGION, 55..89
> note, Connecting peptide
- REGION, 90..110
> note, A chain of insulin
Residues:
MALWMRILPL LAILALWGPD PAAAFVNQHI CGSHLVEALY LVCGERGFFY TPKTRREAED 60
LQVGQVEIGG GPGAGSLQPL ALEGSLQKRG IVEQCCTSIC SLYQLENYCN 110

Sequence Number (ID): 5
Length: 98
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..98
> mol_type, protein
> organism, Homo sapiens
- PROPEP, 1..12
> note, Proinsulin leader sequence
- REGION, 13..42

> note, Insulin B-chain
- REGION, 43..77
> note, Connecting peptide
- REGION, 78..98
> note, Insulin A chain
Residues:
MNHKVHHHHH HMFVNQHLCG SHLVEALYLV CGERGFFYTP KTRREAEDLQ VGEVELGGGP 60
GAGSLQPLAL EGSLQKRGIV EQCCTSICSL YQLENYCN 98

Sequence Number (ID): 6
Length: 106
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..106
> mol_type, protein
> organism, Homo sapiens
- REGION, 6..35
> note, Insulin B chain
- REGION, 36..70
> note, Connecting peptide
- REGION, 71..91
> note, Insulin A chain
- BINDING, 92..100
> note, Sortase tag
- BINDING, 101..106
> note, His tag
- VARIANT, 30
> note, F25A modification
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFA YTPKTRREAE DLQVGEVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGS HHHHHH 106

Sequence Number (ID): 7
Length: 101
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..101
> mol_type, protein

> organism, Homo sapiens
- REGION, 96..101
> note, His6 tag
- REGION, 92..95
> note, GGGS linker
- REGION, 87..91
> note, GGGGS
- PEPTIDE, 1..101
> note, Native proinsulin with C-terminal His tag
- VARIANT, 25
> note, F sequence
- VARIANT, 31..32
> note, RR sequence
- VARIANT, 64..65
> note, KR sequence
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG EVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNGGGG SGGGSHHHHH H 101

Sequence Number (ID): 8
Length: 107
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..107
> mol_type, protein
> organism, synthetic construct
- REGION, 102..107
> note, His6 tag
- REGION, 96..101
> note, Sortase tag
- REGION, 92..95
> note, GGGS linker
- REGION, 87..91
> note, GGGGS linker
- PEPTIDE, 1..107
> note, Native proinsulin with C-terminal sortase
- VARIANT, 25
> note, F sequence

- VARIANT, 31..32
> note, RR sequence
- VARIANT, 64..65
> note, KR sequence
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG EVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNGGGG SGGGSLPETG GHHHHHH 107

Sequence Number (ID): 9
Length: 102
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..102
> mol_type, protein
> organism, synthetic construct
- REGION, 1..6
> note, His6 tag
- REGION, 7..11
> note, SGGGG linker
- REGION, 12..16
> note, SGGGG linker
- REGION, 17..46
> note, B chain
- VARIANT, 47..48
> note, RR sequence
- REGION, 47..81
> note, C-peptide in the middle
- VARIANT, 80..81
> note, KR sequence
- REGION, 82..102
> note, A chain
Residues:
HHHHHHSGGG GSGGGGFVNQ HLCGSHLVEA LYLVCGERGF FYTPKTRREA EDLQVGQVEL 60
GGGPGAGSLQ PLALEGSLQK RGIVEQCCTS ICSLYQLENY CN 102

Sequence Number (ID): 10
Length: 107
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..107
> mol_type, protein
> organism, synthetic construct
- REGION, 1..5
> note, Sortase tag
- REGION, 102..107
> note, His6 tag
- REGION, 97..101
> note, GGGGS linker
- REGION, 92..96
> note, GGGGS linker
- REGION, 71..91
> note, A chain
- VARIANT, 69..70
> note, KR sequence
- REGION, 6..35
> note, B chain
- REGION, 36..70
> note, C-peptide in the middle
- VARIANT, 36..37
> note, RR sequence
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFF YTPKTRREAE DLQVGQVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGG SHHHHHH 107

Sequence Number (ID): 11
Length: 107
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..107
> mol_type, protein
> organism, Homo sapiens
- REGION, 1..5
> note, Sortase Tag
- REGION, 6..35
> note, B chain
- VARIANT, 30

> note, A sequence
- REGION, 36..70
> note, C-peptide in the middle
- VARIANT, 69..70
> note, KR sequence
- REGION, 71..91
> note, A chain
- REGION, 102..107
> note, His6 tag
- REGION, 97..101
> note, GGGGS linker
- REGION, 92..96
> note, GGGGS linker
- VARIANT, 36..37
> note, RR sequence
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFA YTPKTRREAE DLQVGQVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGG SHHHHHH 107

Sequence Number (ID): 12
Length: 107
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..107
> mol_type, protein
> organism, Homo sapiens
- VARIANT, 47
> note, I sequence
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFF YTPKTRREAE DLQVGQIELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGG SHHHHHH 107

Sequence Number (ID): 13
Length: 107
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..107
> mol_type, protein

> organism, Homo sapiens
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFA YTPKTRREAE DLQVGQVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGG SHHHHHH 107

Sequence Number (ID): 14
Length: 107
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..107
> mol_type, protein
> organism, Homo sapiens
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFF YTPKTRREAE DLQVGQVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGG SHHHHHH 107

Sequence Number (ID): 15
Length: 106
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..106
> mol_type, protein
> organism, synthetic construct
- REGION, 43..77
> note, Connecting peptide
- REGION, 13..42
> note, B-chain
- REGION, 78..98
> note, A chain
- REGION, 101..106
> note, His6 tag
- REGION, 92..100
> note, GGGGSGGGS linker
- VARIANT, 36..37
> note, RR sequence
- VARIANT, 69..70
> note, KR sequence
Residues:

GGGGGFVNQH LCGSHLVEAL YLVCGERGFA YTPKTRREAE DLQVGEVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGS HHHHHH 106

Sequence Number (ID): 16
Length: 298
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..298
> mol_type, protein
> organism, Homo sapiens
- REGION, 1..26
> note, B chain of insulin
- REGION, 78..298
> note, IgG Fc region
- REGION, 39..47
> note, A chain of insulin
Residues:
FVNQHLCGSH LVEALELVCG ERGFHYGGGG GGSGGGGGLV EQCCTSTCSL DQLENYCGGG 60
GGQGGGGQGG GGQGGGGGEC PPCPAPPVAG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 120
HEDPEVQFNW YVDGVEVHNA KTKPREEQFN STFRVVSVLT WHQDWLNGKE YKCKVSNKGL 180
PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE 240
NNYKTTPPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG 298

Sequence Number (ID): 17
Length: 323
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..323
> mol_type, protein
> organism, synthetic construct
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG QVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNGGGG SEPKSADKTH TCPPCPAPEA AGGPSVFLFP 120
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS 180
VLTVLHQDWL NGKEYKCKVS NKALAAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS 240
LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS 300
CSVMHEALHN HYTQKSLSLS PGK 323

Sequence Number (ID): 18
Length: 343
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..343
> mol_type, protein
> organism, synthetic construct
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG QVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNGGGG SGGGGSGGGG SGGGGSGGGG SEPKSADKTH 120
TCPPCPAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV 180
HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALAAPIEK TISKAKGQPR 240
EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 300
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 343

Sequence Number (ID): 19
Length: 92
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..92
> mol_type, protein
> organism, synthetic construct
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFF YTPKTRREAE DLQVGQVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NG 92

Sequence Number (ID): 20
Length: 22
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..22
> mol_type, protein
> organism, synthetic construct
- PEPTIDE, 1..22
> note, C-terminal sortase tag with His6 tag
- REGION, 17..22
> note, His6 tag
Residues:

GGGGSGGGGS LPETGGHHHH HH 22

Sequence Number (ID): 21
Length: 21
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..21
> mol_type, protein
> organism, synthetic construct
Residues:
HHHHHHSPST PPTPSPSTPP C 21

Sequence Number (ID): 22
Length: 106
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..106
> mol_type, protein
> organism, synthetic construct
- REGION, 1..5
> note, Sortase tag
- VARIANT, 30
> note, A sequence
- REGION, 5..35
> note, B chain
- VARIANT, 36..37
> note, RR sequence
- REGION, 36..70
> note, C-peptide in the middle
- VARIANT, 69..70
> note, KR sequence
- REGION, 71..81
> note, A chain
- REGION, 82..86
> note, GGGGS linker
- REGION, 87..90
> note, GGGS linker
- REGION, 91..96

> note, HHHHHH
Residues:
GGGGGFVNQH LCGSHLVEAL YLVCGERGFA YTPKTRREAE DLQVGEVELG GGPGAGSLQP 60
LALEGSLQKR GIVEQCCTSI CSLYQLENYC NGGGGSGGGS HHHHHH 106

Sequence Number (ID): 23
Length: 299
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..299
> mol_type, protein
> organism, synthetic construct
- REGION, 1..26
> note, B chain
- VARIANT, 16
> note, E sequence
- REGION, 38..58
> note, A chain
- VARIANT, 47
> note, T sequence
- VARIANT, 51
> note, D sequence
- VARIANT, 58
> note, G sequence
- REGION, 79..299
> note, Fc region
Residues:
FVNQHLCGSH LVEALELVCG ERGFHYGGGG GGSGGGGGIV EQCCTSTCSL DQLENYCGGG 60
GGQGGGGQGG GGQGGGGGEC PPCPAPPVAG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 120
HEDPEVQFNW YVDGVEVHNA KTKPREEQFN STFRVVSVLT VVHQDWLNGK EYKCKVSNKG 180
LPAPIEKTIS KTKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP 240
ENNYKTTPPM LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG 299

Sequence Number (ID): 24
Length: 86
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..86

> mol_type, protein
> organism, Canis lupus
Residues:
PVNQHLCGSH LVEALYLVCG ERGFFYTFKA RREVEDLQVR DVZLAGAPGE GGLQPLALEG 60
ALQKRGIVEQ CCTSICSLYQ LENYCN 86

Sequence Number (ID): 25
Length: 84
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..84
> mol_type, protein
> organism, Sus scrofa
Residues:
PVNQHLCGSH LVEALYLVCG ERGFFYTFKA RREAENPPAG AVELGQGLGG LQALALEGPP 60
QKRGIVEQCC TSICSLYQLE NYCN 84

Sequence Number (ID): 26
Length: 86
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..86
> mol_type, protein
> organism, Mus musculus
Residues:
FVKQHLCGSH LVEALYLVCG ERGFFYTFMS RREVEDPDVA QLELGSGPGA GDLQTLALEV 60
AQDKRGIVDQ CCTSICSLYQ LENXCN 86

Sequence Number (ID): 27
Length: 84
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..84
> mol_type, protein
> organism, Mus musculus
Residues:
FVKDHLCGPH LVEALYLVCG ERGFFYTFKS RREVEDPQVE QLELGSSPGD LQTLALEVAR 60
DKRGIVDQCC TSICSLYQLE NXCN 84

Sequence Number (ID): 28
Length: 86
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..86
> mol_type, protein
> organism, Rattus norvegicus
Residues:
FVKQHLCGSH LVEALYLVCG ERGFFYTFMS RREVEDPQVA QLSLGSGPGA GDLQTLALEV 60
ARQKRGIVDQ CCTSICSLYQ LENYCN 86

Sequence Number (ID): 29
Length: 86
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..86
> mol_type, protein
> organism, Rattus norvegicus
Residues:
PVKQHLCCPH LVEALYLVCG ERGFPYTFKS RREVEDPQVP QLELGSGPEA GDLQTLALEV 60
AROKRGIVDQ CCTSICSLYQ LENYCN 86

Sequence Number (ID): 30
Length: 344
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..344
> mol_type, protein
> organism, synthetic construct
- PROPEP, 1..21
> note, Mouse Ig kappa signal
- VARIANT, 117
> note, C>A
- REGION, 108..112
> note, G4S linker
- VARIANT, 131..132
> note, LALA modification

- VARIANT, 226
> note, PA modification
Residues:

```
METDTLLLWV  LLLWVPGSTG  DFVNQHLCGS  HLVEALYLVC  GERGFFYTPK  TRREAEDLQV  60
GQVELGGGPG  AGSLQPLALE  GSLQKRGIVE  QCCTSICSLY  QLENYCNGGG  GSEPKSADKT  120
HTCPPCPAPE  AAGGPSVFLF  PPKPKDTLMI  SRTPEVTCVV  VDVSHEDPEV  KFNWYVDGVE  180
VHNAKTKPRE  EQYNSTYRVV  SVLTVLHQDW  LNGKEYKCKV  SNKALAAPIE  KTISKAKGQP  240
REPQVYTLPP  SRDELTKNQV  SLTCLVKGFY  PSDIAVEWES  NGQPENNYKT  TPPVLDSDGS  300
FFLYSKLTVD  KSRWQQGNVF  SCSVMHEALH  NHYTQKSLSL  SPGK                    344
```

Sequence Number (ID): 31
Length: 1382
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1382
> mol_type, protein
> organism, Homo sapiens
Residues:

```
MATGGRRGAA  AAPLLVAVAA  LLLGAAGHLY  PGEVCPGMDI  RNNLTRLHEL  ENCSVIEGHL  60
QILLMFKTRP  EDFRDLSFPK  LIMITDYLLL  FRVYGLESLK  DLFPNLTVIR  GSRLFFNYAL  120
VIFEMVHLKE  LGLYNLMNIT  RGSVRIEKNN  ELCYLATIDW  SRILDSVEDN  YIVLNKDDNE  180
ECGDICPGTA  KGKTNCPATV  INGQFVERCW  THSHCQKVCP  TICKSHGCTA  EGLCCHSECL  240
GNCSQPDDPT  KCVACRNFYL  DGRCVETCPP  PYYHFQDWRC  VNFSFCQDLH  HKCKNSRRQG  300
CHQYVIHNNK  CIPECPSGYT  MNSSNLLCTP  CLGPCPKVCH  LLEGEKTIDS  VTSAQELRGC  360
TVINGSLIIN  IRGGNNLAAE  LEANLGLIEE  ISGYLKIRRS  YALVSLSFFR  KLRLIRGETL  420
EIGNYSFYAL  DNQNLRQLWD  WSKHNLTITQ  GKLFFHYNPK  LCLSEIHKME  EVSGTKGRQE  480
RNDIALKTNG  DQASCENELL  KFSYIRTSFD  KILLRWEPYW  PPDFRDLLGF  MLFYKEAPYQ  540
NVTEFDGQDA  CGSNSWTVVD  IDPPLRSNDP  KSQNHPGWLM  RGLKPWTQYA  IFVKTLVTFS  600
DERRTYGAKS  DIIYVQTDAT  NPSVPLDPIS  VSNSSSQIIL  KWKPPSDPNG  NITHYLVFWE  660
RQAEDSELFE  LDYCLKGLKL  PSRTWSPPFE  SEDSQKHNQS  EYEDSAGECC  SCPKTDSQIL  720
KELEESSFRK  TFEDYLHNVV  FVPRKTSSGT  GAEDPRPSRK  RRSLGDVGNV  TVAVPTVAAF  780
PNTSSTSVPT  SPEEHRPFEK  VVNKESLVIS  GLRHFTGYRI  ELQACNQDTP  EERCSVAAYV  840
SARTMPEAKA  DDIVGPVTHE  IFENNVVHLM  WQEPKEPNGL  IVLYEVSYRR  YGDEELHLCV  900
SRKHFALERG  CRLRGLSPGN  YSVRIRATSL  AGNGSWTEPT  YFYVTDYLDV  PSNIAKIIIG  960
PLIFVFLFSV  VIGSIYLFLR  KRQPDGPLGP  LYASSNPEYL  SASDVFPCSV  YVPDEWEVSR  1020
EKITLLRELG  QGSFGMVYEG  NARDIIKGEA  ETRVAVKTVN  ESASLRERIE  FLNEASVMKG  1080
FTCHHVVRLL  GVVSKGQPTL  VVMELMAHGD  LKSYLRSLRP  EAENNPGRPP  PTLQEMIQMA  1140
AEIADGMAYL  NAKKFVHRDL  AARNCMVAHD  FTVKIGDFGM  TRDIYETDYY  RKGGKGLLPV  1200
```

RWMAPESLKD GVFTTSSDMW SFGVVLWEIT SLAEQPYQGL SNEQVLKFVM DGGYLDQPDN 1260
CPERVTDLMR MCWQFNPKMR PTFLEIVNLL KDDLHPSFPE VSFFHSEENK APESEELEME 1320
FEDMENVPLD RSSHCQREEA GGRDGGSSLG FKRSYEEHIP YTHMNGGKKN GRILTLPRSN 1380
PS 1382

Sequence Number (ID): 32
Length: 1370
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..1370
> mol_type, protein
> organism, Homo sapiens
Residues:
MATGGRRGAA AAPLLVAVAA LLLGAAGHLY PGEVCPGMDI RNNLTRLHEL ENCSVIEGHL 60
QILLMFKTRP EDFRDLSFPK LIMITDYLLL FRVYGLESLK DLFPNLTVIR GSRLFFNYAL 120
VIFEMVHLKE LGLYNLMNIT RGSVRIEKNN ELCYLATIDW SRILDSVEDN YIVLNKDDNE 180
ECGDICPGTA KGKTNCPATV INGQFVERCW THSHCQKVCP TICKSHGCTA EGLCCHSECL 240
GNCSQPDDPT KCVACRNFYL DGRCVETCPP PYYHFQDWRC VNFSFCQDLH HKCKNSRRQG 300
CHQYVIHNNK CIPECPSGYT MNSSNLLCTP CLGPCPKVCH LLEGEKTIDS VTSAQELRGC 360
TVINGSLIIN IRGGNNLAAE LEANLGLIEE ISGYLKIRRS YALVSLSFFR KLRLIRGETL 420
EIGNYSFYAL DNQNLRQLWD WSKHNLTITQ GKLFFHYNPK LCLSEIHKME EVSGTKGRQE 480
RNDIALKTNG DQASCENELL KFSYIRTSFD KILLRWEPYW PPDFRDLLGF MLFYKEAPYQ 540
NVTEFDGQDA CGSNSWTVVD IDPPLRSNDP KSQNHPGWLM RGLKPWTQYA IFVKTLVTFS 600
DERRTYGAKS DIIYVQTDAT NPSVPLDPIS VSNSSSQIIL KWKPPSDPNG NITHYLVFWE 660
RQAEDSELFE LDYCLKGLKL PSRTWSPPFE SEDSQKHNQS EYEDSAGECC SCPKTDSQIL 720
KELEESSFRK TFEDYLHNVV FVPRPSRKRR SLGDVGNVTV AVPTVAAFPN TSSTSVPTSP 780
EEHRPFEKVV NKESLVISGL RHFTGYRIEL QACNQDTPEE RCSVAAYVSA RTMPEAKADD 840
IVGPVTHEIF ENNVVHLMWQ EPKEPNGLIV LYEVSYRRYG DEELHLCVSR KHFALERGCR 900
LRGLSPGNYS VRIRATSLAG NGSWTEPTYF YVTDYLDVPS NIAKIIIGPL IFVFLFSVVI 960
GSIYLFLRKR QPDGPLGPLY ASSNPEYLSA SDVFPCSVYV PDEWEVSREK ITLLRELGQG 1020
SFGMVYEGNA RDIIKGEAET RVAVKTVNES ASLRERIEFL NEASVMKGFT CHHVVRLLGV 1080
VSKGQPTLVV MELMAHGDLK SYLRSLRPEA ENNPGRPPPT LQEMIQMAAE IADGMAYLNA 1140
KKFVHRDLAA RNCMVAHDFT VKIGDFGMTR DIYETDYYRK GGKGLLPVRW MAPESLKDGV 1200
FTTSSDMWSF GVVLWEITSL AEQPYQGLSN EQVLKFVMDG GYLDQPDNCP ERVTDLMRMC 1260
WQFNPKMRPT FLEIVNLLKD DLHPSFPEVS FFHSEENKAP ESEELEMEFE DMENVPLDRS 1320
SHCQREEAGG RDGGSSLGFK RSYEEHIPYT HMNGGKKNGR ILTLPRSNPS 1370

Sequence Number (ID): 33

Length: 215
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..215
> mol_type, protein
> organism, Homo sapiens
Residues:
QIVLTQSPTI MSASLGERVT MTCTASSSVS SSYLHWYQQK PGSSPKLWIY STSNLASGVP 60
ARFSGSGSGT SYSLTISSME AEDAATYYCH QYHRSPPTFG AGTKLELKRT VAAPSVFIFP 120
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL 180
TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC 215

Sequence Number (ID): 34
Length: 492
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..492
> mol_type, protein
> organism, Homo sapiens
- MOD_RES, 345
> note, N-glycosylation
- MOD_RES, 440
> note, N-glycosylation
Residues:
QIQLVQSGPE LKKPGETVKI SCKASGYTFT DYSMHWVKQA PGKGLKWMDW INTETGVPTY 60
ADDFKGRFAF SLETSASTAY LQINDLKNED TATYFCTRGY GKGYFDVWGA GTTVTVSSAS 120
TKGPSVFPLA PCSRSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 180
YSLSSVTVPS SSLGTQTYTC NVNHKPSNTK VDKRVELKTP LGDTTHTCPR CPEPKSCDTP 240
PPCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPAPE LLGGPSFLFP PKPKDTLMIS 300
RTPEVTCVVV DVSHEDPEVQ FKWYVDGVEV HNAKTKPREE QYNSTFRVVS VLTVLHQDWL 360
NGKEYKCKVS KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS 420
DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS RWQQGNIFSC SVMHEALHNR 480
FTQKSLSLSP GK 492

Sequence Number (ID): 35
Length: 218
Molecule Type: AA
Features Location/Qualifiers:

- source, 1..218
> mol_type, protein
> organism, Mus musculus
Residues:
DVVMTQTPLS LPVSLGDQAS ISCRSSQSLV HNNGNTFLQW YLQKPGQSPK LLIYKLSNRF 60
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHEP YTFGGGTKLE IKRADAAPTV 120
SIFPPSSEQL TSGGASWCFL NNFYPKDINV KWKIDGSERQ NGVLNSWTDQ DSKDSTYSMS 180
STLTLTKDEY ERHNSYTCEA THKTSTSPIV KSFNRNEC 218

Sequence Number (ID): 36
Length: 434
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..434
> mol_type, protein
> organism, Mus musculus
- MOD_RES, 55
> note, N-linked glycosylation
- MOD_RES, 287
> note, N-linked glycosylation
Residues:
EVQLQQSGPE LVRPGASVKI SCKASGYTFA DYVMHWVKQS HGKSLEWIGY FYPHNDSFGY 60
NQKFKSKATL TVDTSSSTAY MELRSTFEDS AVYYCTSGLH SWGQGTTLTV SSAKTTPPSV 120
YPLAPGSAAQ TNSMVTLGCL VKGYFPEPVT VWNSGSLSSG VHTFPAVLQS DLYTLSSSVT 180
VPSSTWPSET VTCNVAHPAS STKVDKKIVP RDCGCKPCIC TVPEVSSVFI FPPKPKDVLT 240
ITLTPKVTCV VDISKDDPEV QFSWFVDDVE VHTAQTQPRE EQFNSTFRSV SELPIMHQDW 300
LNGKEFKCRV NSAAFPAPIE KTISKTKGRP KAPQVYTIPP PKEQMAKDKV SLTCMITDFF 360
PEDITVEWQW NGQPAENYKN TQPIMDTDGS YFVYSKLNVQ KSNWEAGNTF TCSVLHEGLH 420
NHHTEKSLSH SPGK 434

Sequence Number (ID): 37
Length: 21
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..21
> mol_type, protein
> organism, synthetic construct
Residues:

HHHHHHSPST PPTPSPSTPP C 21

Sequence Number (ID): 38
Length: 96
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..96
> mol_type, protein
> organism, synthetic construct
- REGION, 1..5
> note, Sortase tag
- REGION, 10..38
> note, B chain
- VARIANT, 33
> note, B24 F to A mutation
- PEPTIDE, 40..72
> note, C peptide
- REGION, 74..95
> note, A chain
Residues:
LPETGGGGGF VNQHLCGSHL VEALYLVCGE RGAFYTPKTR REAEDLQVGQ VELGGGPGAG 60
SLQPLALEGS LQKRGILEQC CTSICSLYQL ENYCNA 96

Sequence Number (ID): 39
Length: 22
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..22
> mol_type, protein
> organism, Homo sapiens
Residues:
GCAACATCAC CCACTACCTG GT 22

Sequence Number (ID): 40
Length: 22
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..22

> mol_type, protein
> organism, Homo sapiens
Residues:
GAATGGTGGA GACCAGGTCC TC 22

Sequence Number (ID): 41
Length: 22
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..22
> mol_type, protein
> organism, Homo sapiens
Residues:
CCTGCACAAC TCCATCTTCG TG 22

Sequence Number (ID): 42
Length: 22
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..22
> mol_type, protein
> organism, Homo sapiens
Residues:
CGGTGATGTT GTAGGTGTCT GC 22

Sequence Number (ID): 43
Length: 50
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..50
> mol_type, protein
> organism, synthetic construct
Residues:
GIVEQCCTSI CSLYQLENYC FVNQHLCGSH LVEALYLVCG ERGAFYTPKT 50

Sequence Number (ID): 44
Length: 87
Molecule Type: AA

Features Location/Qualifiers:
- source, 1..87
> mol_type, protein
> organism, Homo sapiens
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG QVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNG 87

Sequence Number (ID): 45
Length: 18
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..18
> mol_type, protein
> organism, Homo sapiens
Residues:
GSLIYSFSEC AFTGPLRP 18

Sequence Number (ID): 46
Length: 18
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..18
> mol_type, protein
> organism, Homo sapiens
Residues:
YNITDHGSLI YSFSECAF 18

Sequence Number (ID): 47
Length: 18
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..18
> mol_type, protein
> organism, Homo sapiens
Residues:
FSECAFTGPL RPFFSPGF 18

Sequence Number (ID): 48
Length: 322
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..322
> mol_type, protein
> organism, synthetic construct
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG QVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNGGGG SEPKSADKTH TCPPCPAPEA AGGPSVFLFP 120
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS 180
VLTVLHQDWL NGKEYKCKVS NKALAAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS 240
LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS 300
CSVMHEALHN HYTQKSLSLS PG 322

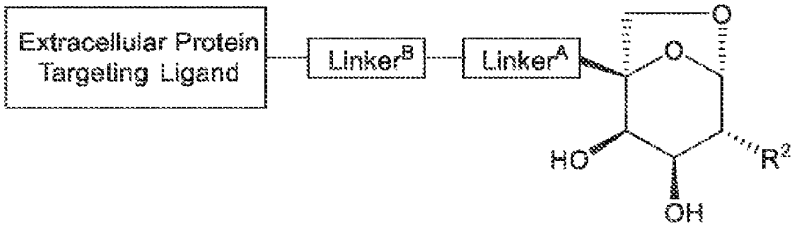
Sequence Number (ID): 49
Length: 87
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..87
> mol_type, protein
> organism, Homo sapiens
Residues:
FVNQHLCGSH LVEALYLVCG ERGFFYTPKT RREAEDLQVG QVELGGGPGA GSLQPLALEG 60
SLQKRGIVEQ CCTSICSLYQ LENYCNG 87

Sequence Number (ID): 50
Length: 7
Molecule Type: AA
Features Location/Qualifiers:
- source, 1..7
> mol_type, protein
> organism, synthetic construct
Residues:
GLPETGG 7

Sequence Number (ID): 51
Length: 19

Molecule Type: AA
Features Location/Qualifiers:
- source, 1..19
> mol_type, protein
> organism, synthetic construct
Residues:
ELVAVITTDG STNYADSVK 19

In another embodiment, the composition of matter (the agent, the TRAP) has one of the structures:



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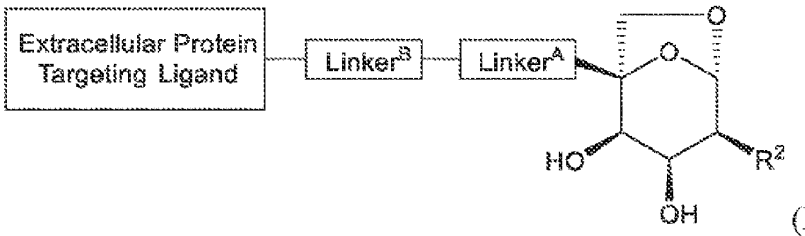

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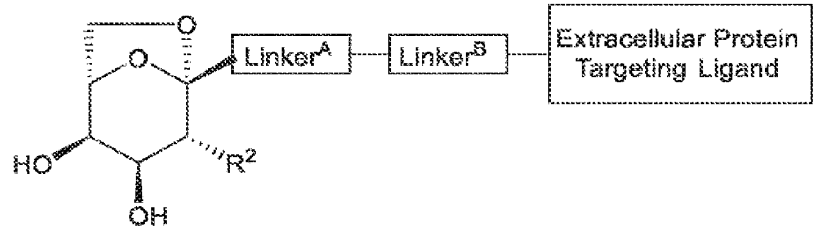

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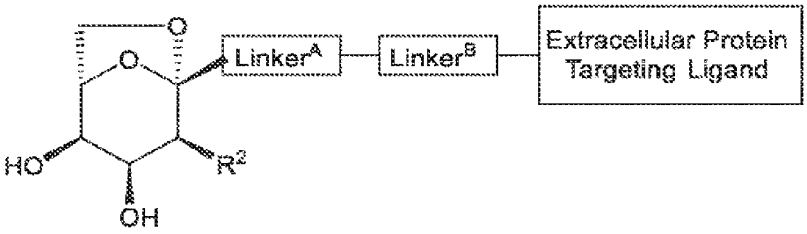

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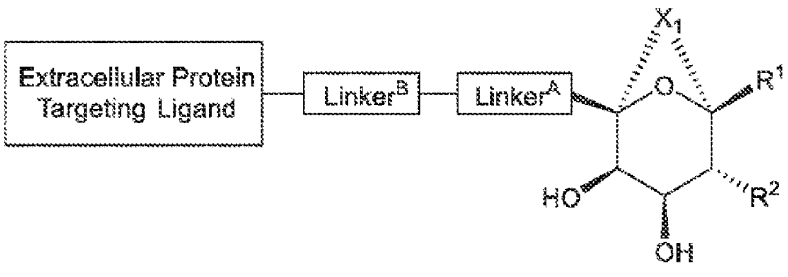
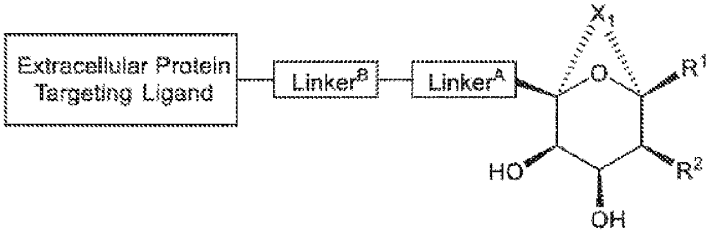


(III),



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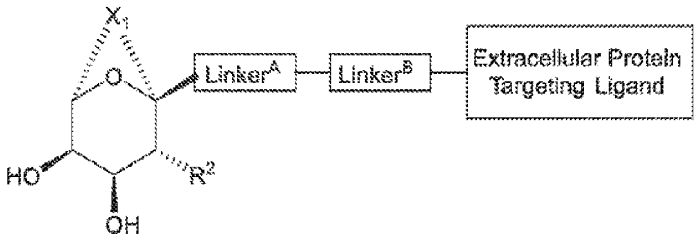

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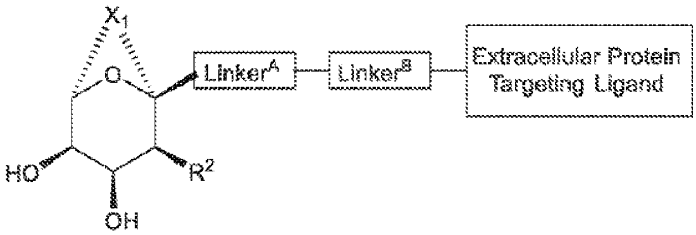

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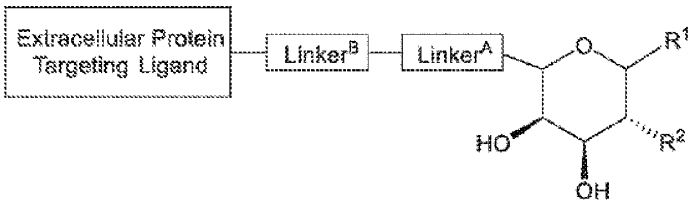

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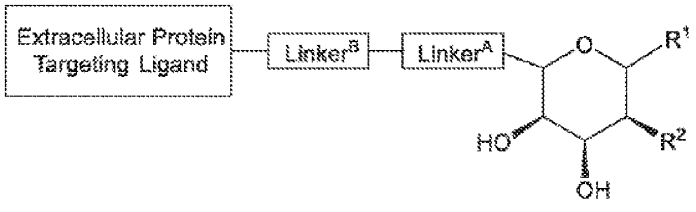

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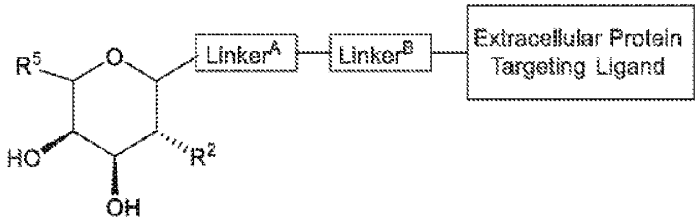

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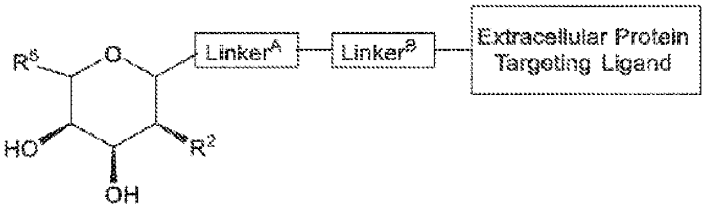

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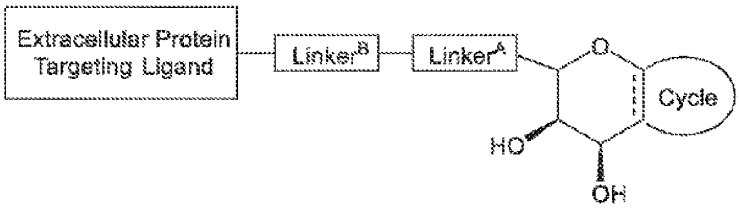

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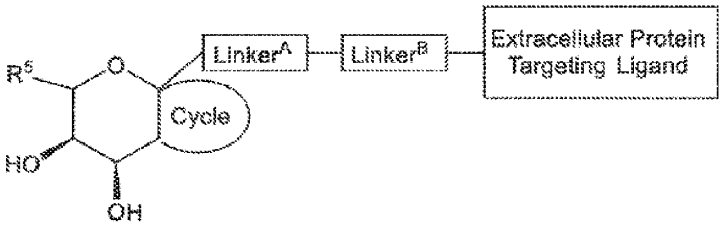

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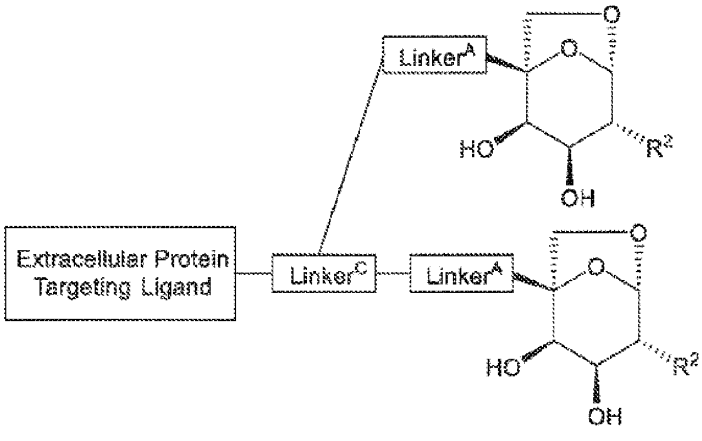

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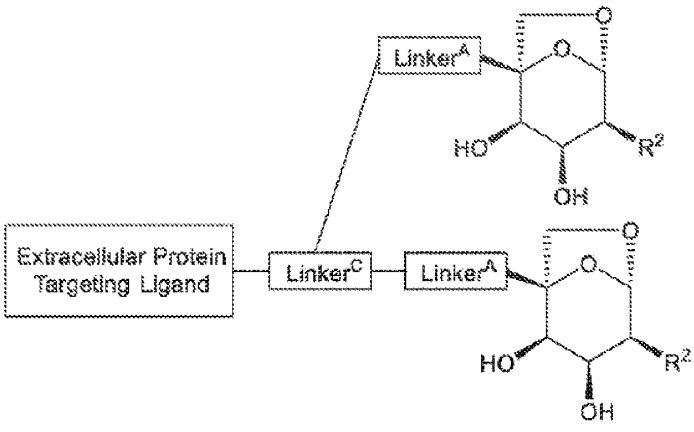

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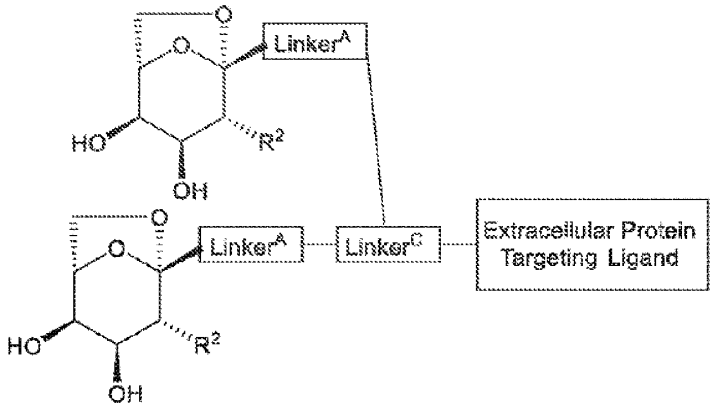

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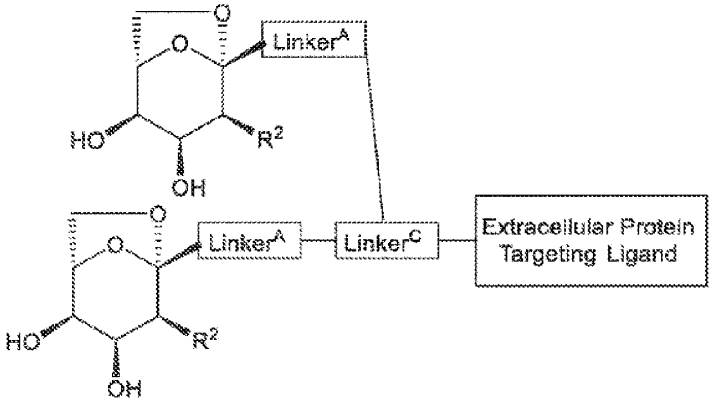

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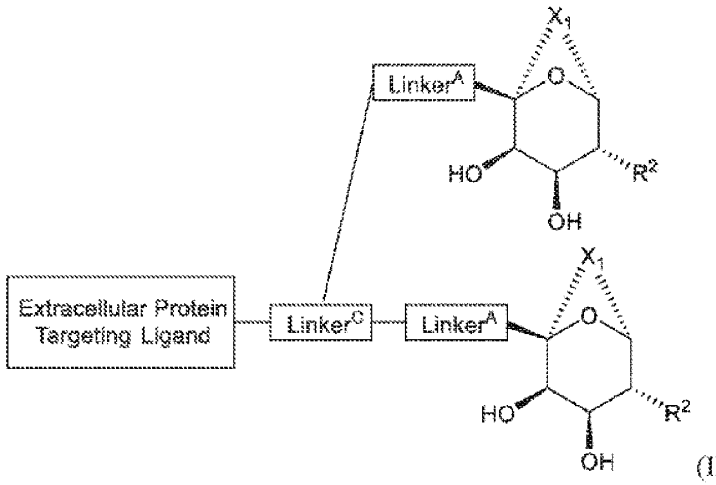

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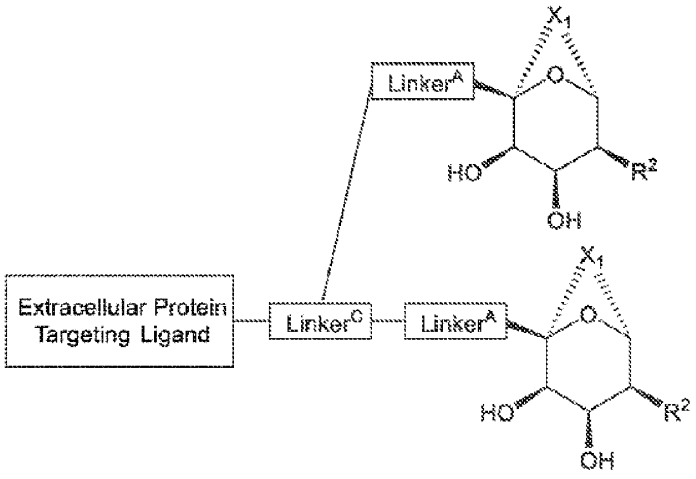

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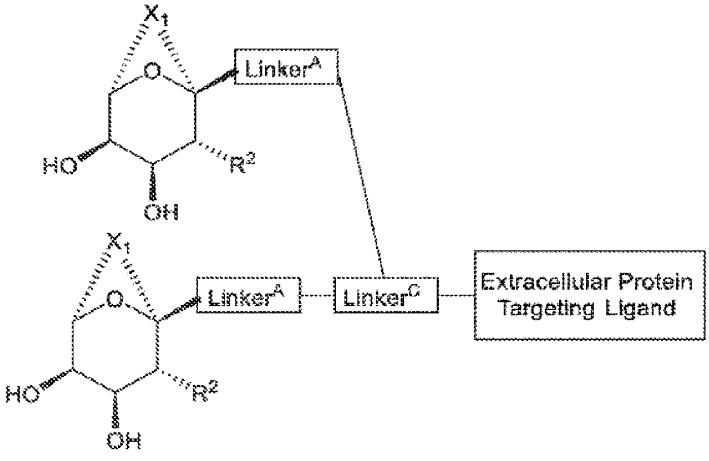

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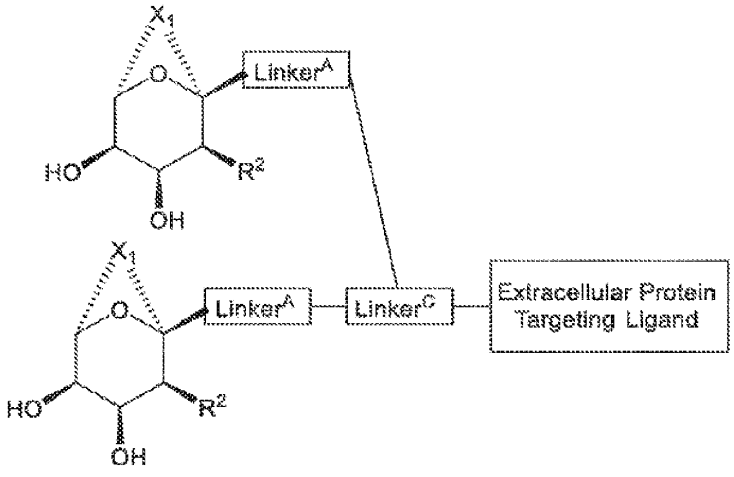

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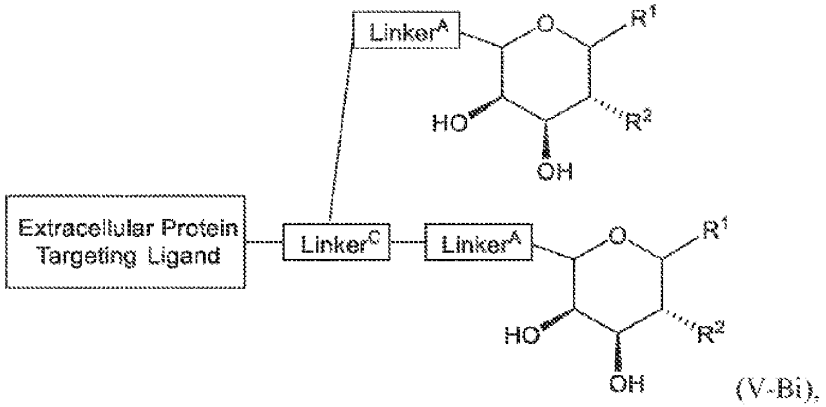

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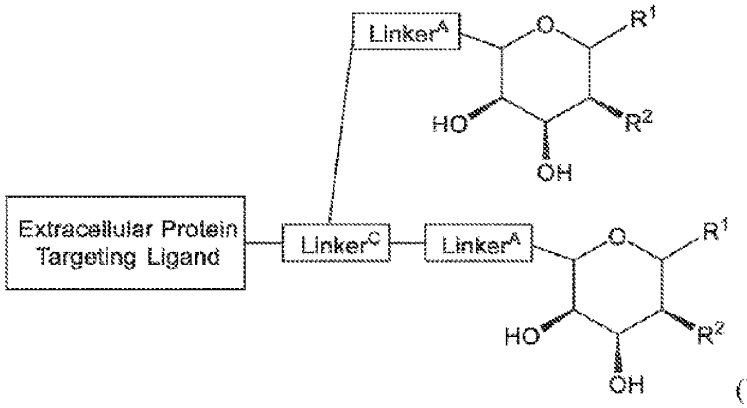

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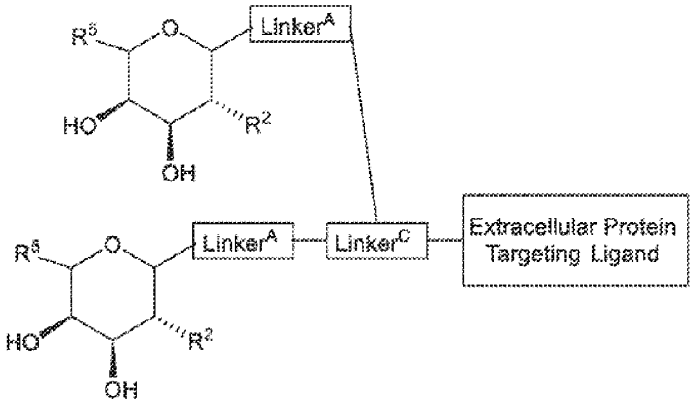

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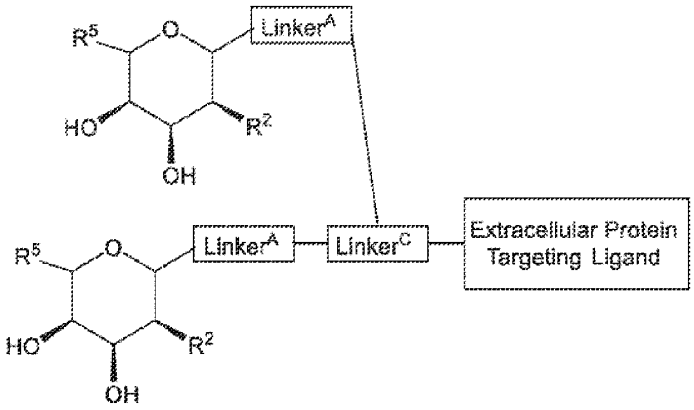

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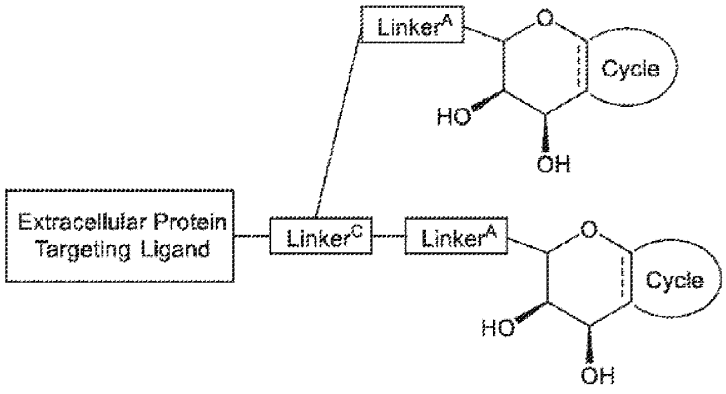

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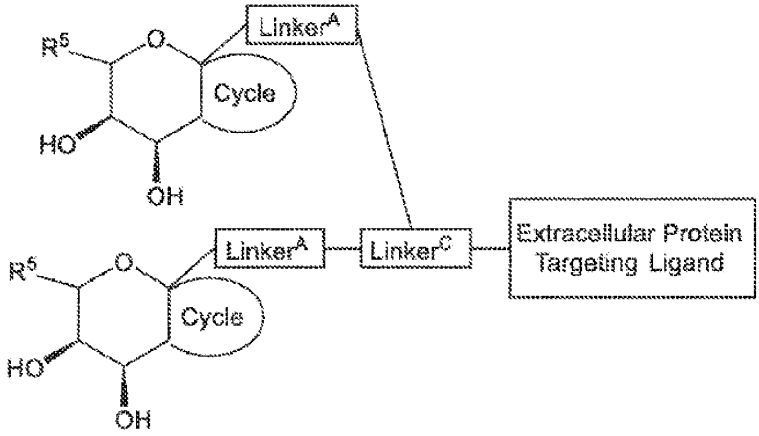
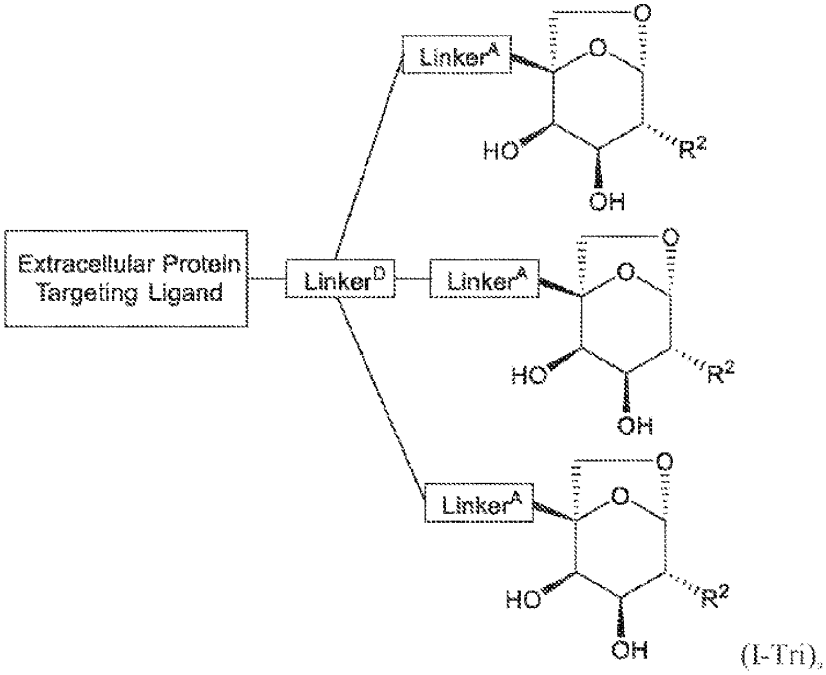

R5
O
Linker^A
Cycle
HO
OH
Linker^A
Linker^C
Extracellular Protein Targeting Ligand
R5
O
Cycle
HO
OH

(VIII-Bi),


Extracellular Protein Targeting Ligand
Linker^D
Linker^A
Linker^A
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R2
OH
O
Linker^A
HO
R2
OH
O
Linker^A
HO
R2
OH
O

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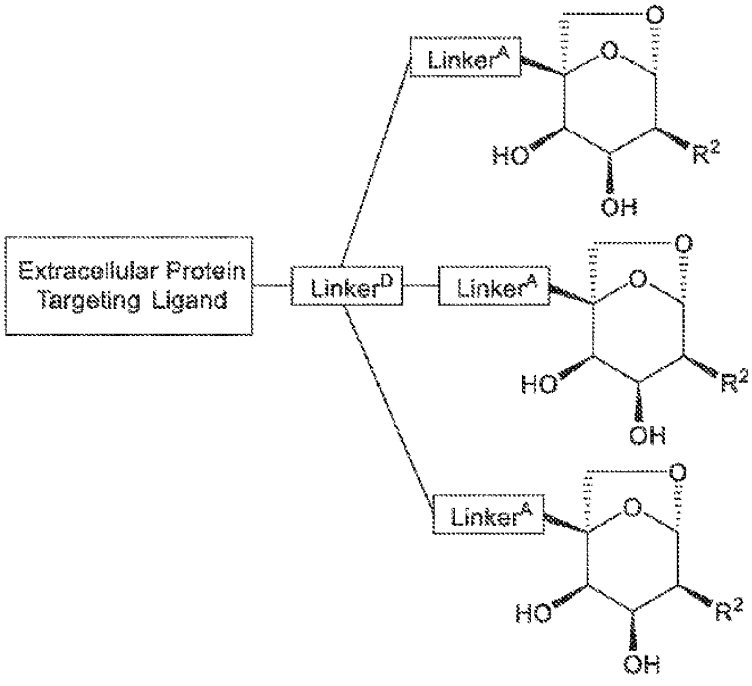
Extracellular Protein Targeting Ligand
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Linker^A
Linker^A
Linker^A
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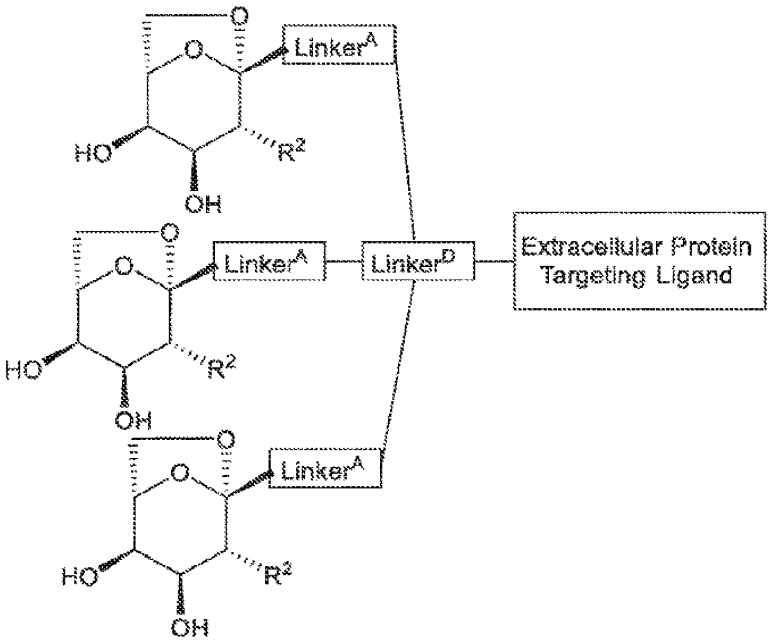
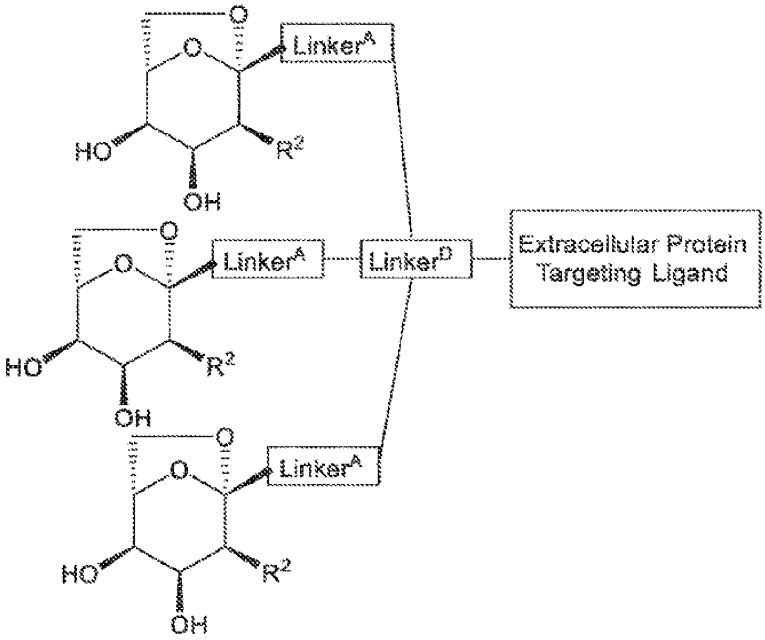
R^2
OH
O

(I-d-Tri),


Linker^A
Linker^A
Linker^A
Linker^D
Extracellular Protein Targeting Ligand
HO
R^2
OH
O

(II-Tri),



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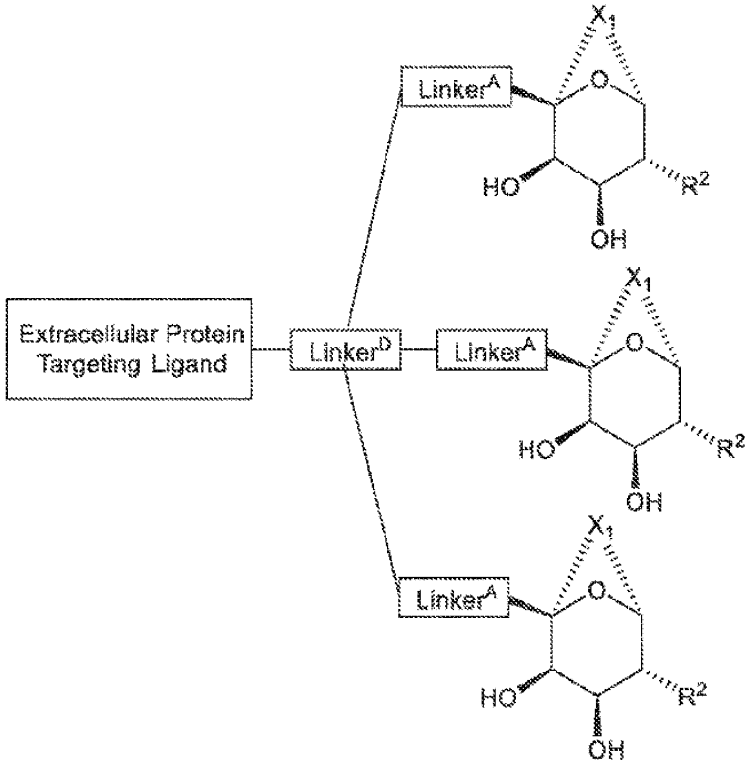

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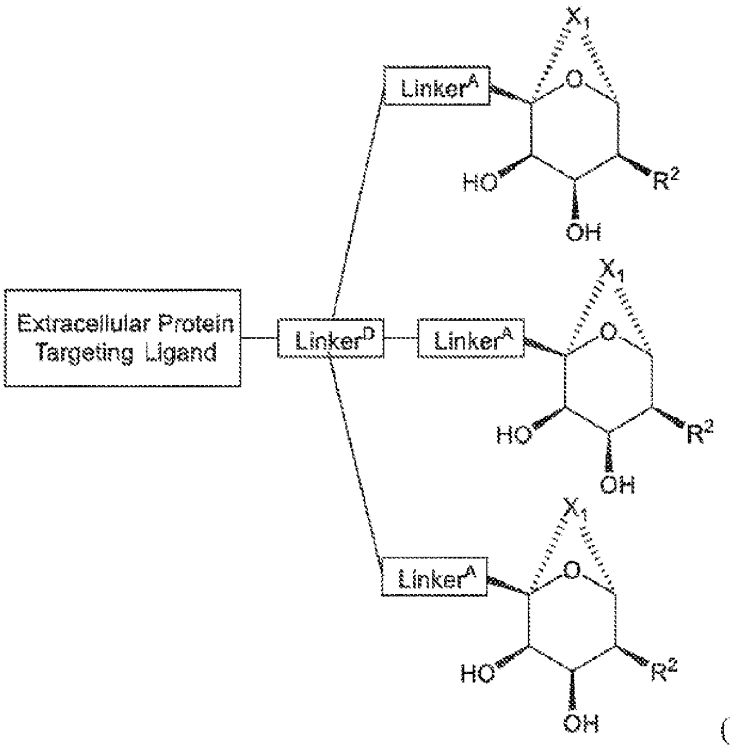

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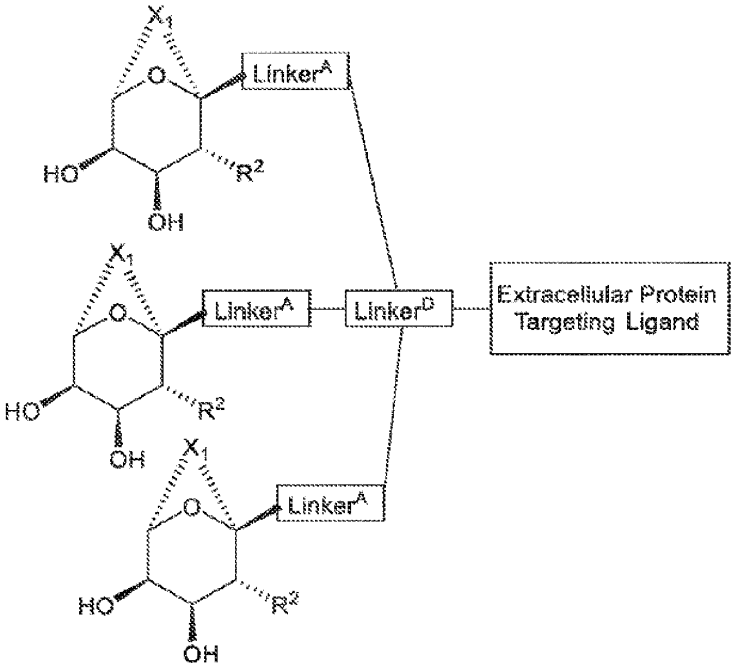

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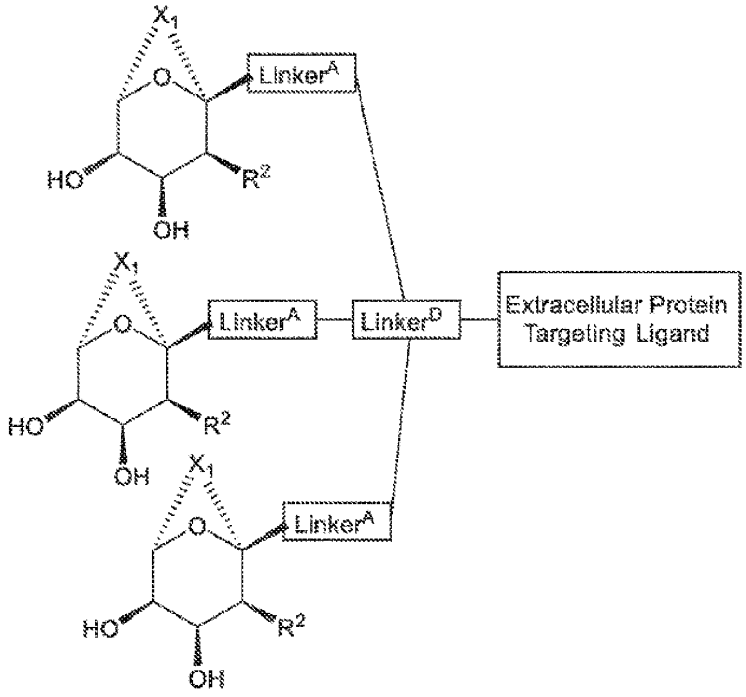
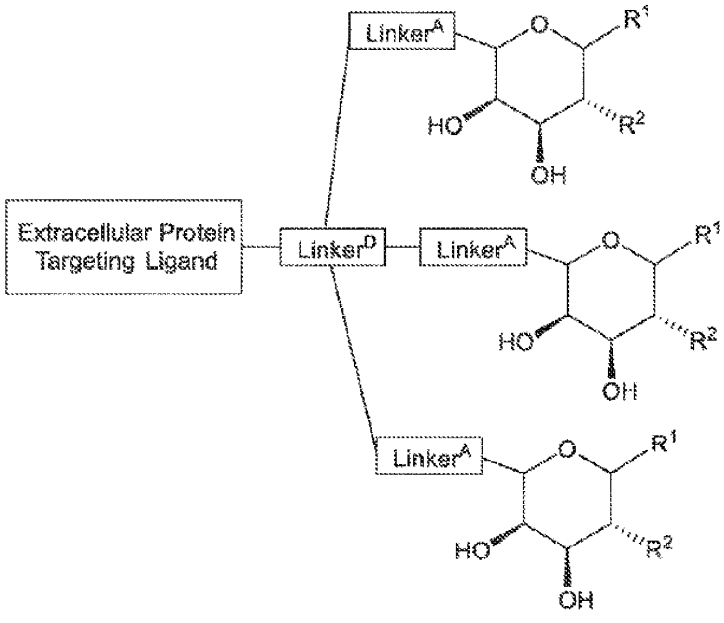
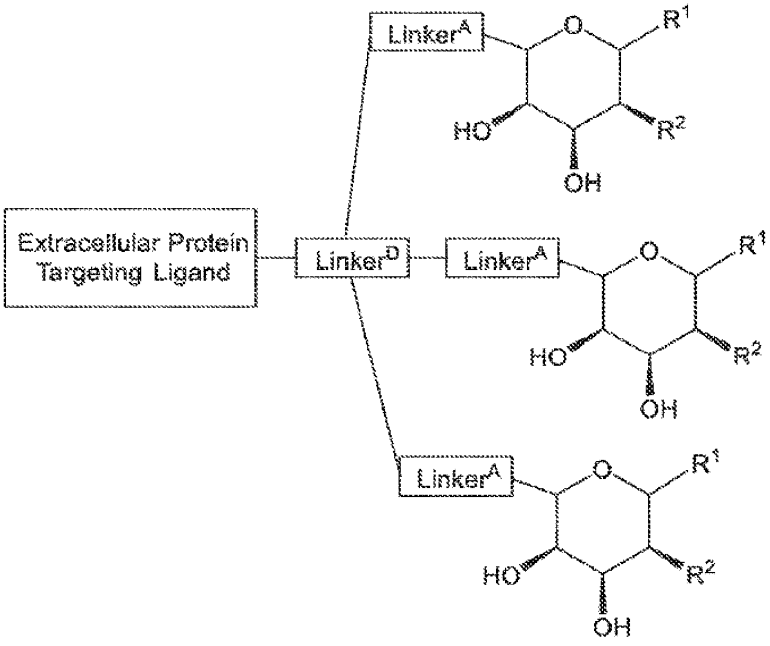

X1
O
Linker^A
HO
R^2
OH
X1
O
Linker^A
Linker^D
Extracellular Protein Targeting Ligand
HO
R^2
OH
X1
O
Linker^A
HO
R^2
OH

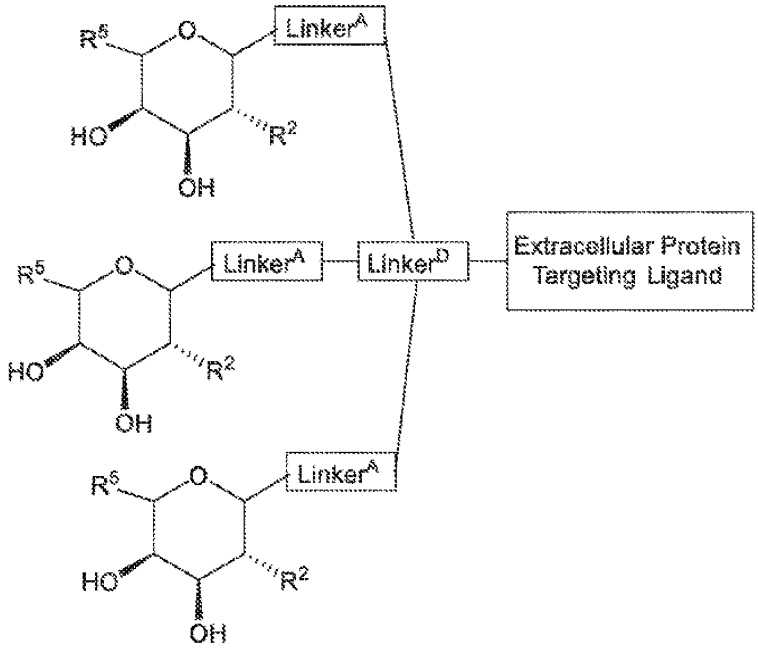
(IV-d-Tri),


Linker^A
O
R^1
HO
R^2
OH
Extracellular Protein Targeting Ligand
Linker^D
Linker^A
O
R^1
HO
R^2
OH
Linker^A
O
R^1
HO
R^2
OH

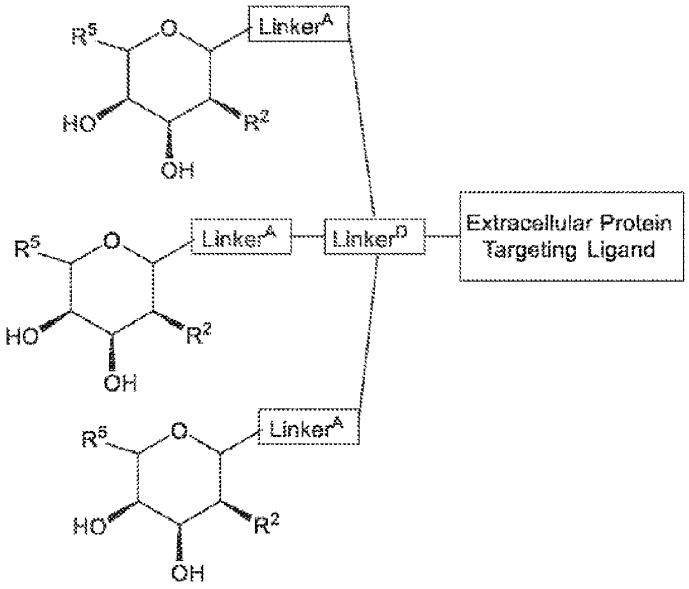
(V-Tri),


Extracellular Protein Targeting Ligand
Linker^D
Linker^A
Linker^A
Linker^A
O
R^1
R^2
HO
OH

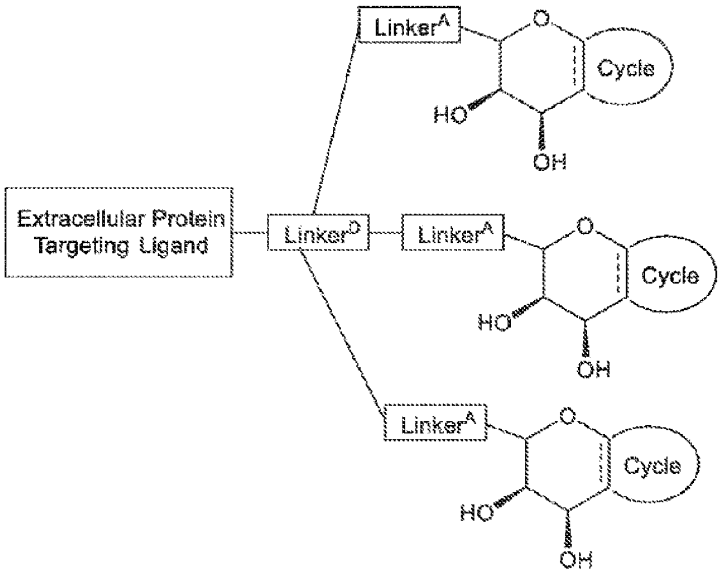
(V-d-Tri),


Linker^A
Linker^A
Linker^A
Linker^D
Extracellular Protein Targeting Ligand
R^5
O
R^2
HO
OH

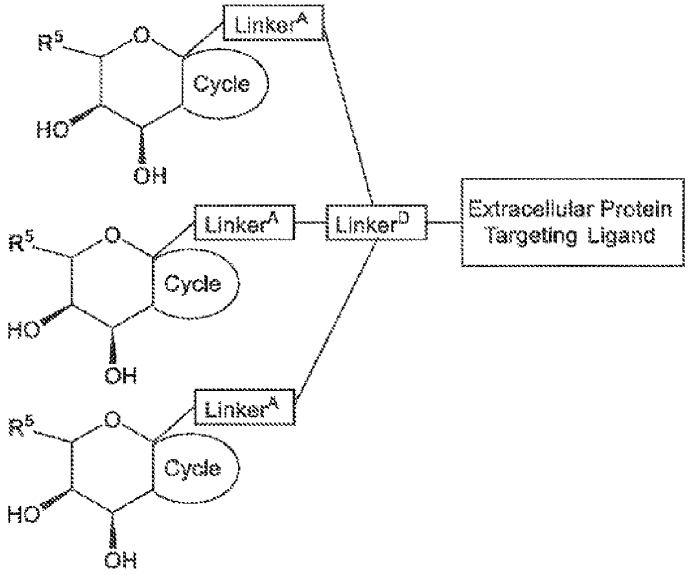
(VI-Tri),



(VI-d-Tri),



(VII-Tri), or



(VIII-Tri),

or a pharmaceutically acceptable salt thereof

wherein:

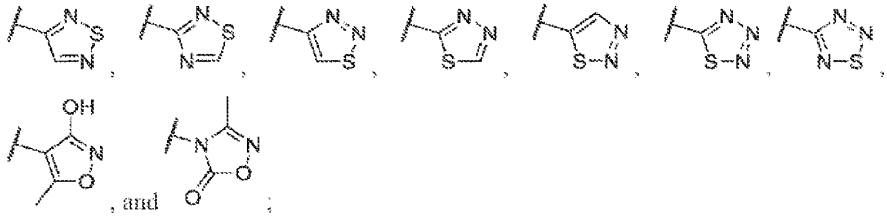
In the above formulae, “Extracellular Protein Targeting Ligand” refers to a target-binding moiety that can bind to an anti-proinsulin or anti-insulin antibody.

In the above formulae,

X^1 is 1 to 5 groups independently selected from O, S, $N(R^6)$, and $C(R^4)(R^4)$, wherein if X^1 is 1 group then X^1 is O, S, $N(R^6)$, or $C(R^4)(R^4)$, if X^1 is 2 groups then no more than 1 group of X^1 is O, S, or $N(R^6)$, if X^1 is 3, 4, or 5 groups then no more than 2 groups of X^1 are O, S, or $N(R^6)$;

R^3 is selected from

(i) aryl, heterocycle, and heteroaryl containing 1 or 2 heteroatoms independently selected from N, O, and S, each of which aryl, heterocycle, and heteroaryl is optionally substituted with 1, 2, 3, or 4 substituents;

(ii) , and ;

(iii) $-NR^8-S(O)-R^3$, $-NR^8-C(S)-R^3$, $-NR^8-S(O)(NR^6)-R^3$, $-N=S(O)(R^3)_2$, $-NR^8C(O)NR^9S(O)_2R^3$, $-NR^8-S(O)_2-R^{10}$, and $-NR^8-C(NR^6)-R^3$ each of which is optionally substituted with 1, 2, 3, or 4 substituents; and

(iv) hydrogen, R^{10}, alkyl-C(O)-R^3, -C(O)-R^3, alkyl, haloalkyl, -OC(O)R^3, and -NR^8-C(O)R^{10};

R^{10} is selected from aryl, alkyl-NR^8-C(O)-R^3, alkyl-aryl, alkyl-heteroaryl with 1, 2, or 4 heteroatoms, alkyl-cyano, alkyl-OR^6, alkyl-NR^6R^8, NR^8-NR^6-C(O)R^3, NR^8-$S(O)_2$-R^3, alkenyl, allyl, alkynyl, -NR^6-alkenyl, -O-alkenyl, -NR^6-alkynyl, -NR^6-heteroaryl, -NR^6-aryl, -O-heteroaryl, -O-aryl, and -O-alkynyl, each of which R^{10} is optionally substituted with 1, 2, 3, or 4 substituents;

R^1 and R^5 are independently selected from hydrogen, heteroalkyl, C_0-C_6alkyl-cyano, alkyl, alkenyl, alkynyl, haloalkyl, F, Cl, Br, I, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycle, heterocycloalkyl, haloalkoxy, -O-alkenyl, -O-alkynyl, C_0-C_6alkyl-OR^6, C_0-C_6alkyl-SR^6, C_0-C_6alkyl-NR^6R^7, C_0-C_6alkyl-C(O)R^3, C_0-C_6alkyl-S(O)R^3, C_0-C_6alkyl-C(S)R^3, C_0-C_6alkyl-$S(O)_2R^3$, C_0-C_6alkyl-$N(R^8)$-C(O)R^3, C_0-C_6alkyl-$N(R^8)$-S(O)R^3, C_0-C_6alkyl-$N(R^8)$-C(S)R^3, C_0-C_6alkyl-$N(R^8)$-$S(O)_2R^3$ C_0-C_6alkyl-O-C(O)R^3, C_0-C_6alkyl-O-S(O)R^3, C_0-C_6alkyl-O-C(S)R^3, -N=S(O)$(R^3)_2$, C_0-C_6alkylN_3, and C_0-C_6alkyl-O-$S(O)_2R^3$, each of which is optionally substituted with 1, 2, 3, or 4 substituents;

R^3 at each occurrence is independently selected from hydrogen, alkyl, heteroalkyl, haloalkyl (including -CF_3, -CHF_2, -CH_2F, -CH_2CF_3, -CH_2CH_2F, and -CF_2CF_3), arylalkyl, heteroarylalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocycle, -OR^8, and -NR^8R^9;

R^4 is independently selected at each occurrence from hydrogen, heteroalkyl, alkyl, haloalkyl, arylalkyl, heteroarylalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocycle, -OR^6, -NR^6R^7, C(O)R^3, S(O)R^3, C(S)R^3, and $S(O)_2R^3$;

R^6 and R^7 are independently selected at each occurrence from hydrogen, heteroalkyl, alkyl, arylalkyl, heteroarylalkyl, alkenyl, alkynyl, aryl, haloalkyl, heteroaryl, heterocycle, -alkyl-OR^8, -alkyl-NR^8R^9, C(O)R^3, S(O)R^3, C(S)R^3, and $S(O)_2R^3$;

R^8 and R^9 are independently selected at each occurrence from hydrogen, heteroalkyl, alkyl, arylalkyl, heteroarylalkyl, alkenyl, alkynyl, aryl, heteroaryl, and heterocycle;

Cycle is a 3-8 membered fused cyclic group optionally substituted with 1, 2, 3, or 4 substituents;

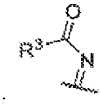
each $Linker^A$ is a bond or a moiety that covalently links the ASGPR ligand to $Linker^B$;

$Linker^B$ is a bond or a moiety that covalently links $Linker^A$ to an Extracellular Protein Targeting Ligand;

Linker^{C} is a chemical group that links each Linker^{A} to the Extracellular Protein Targeting Ligand; and

Linker^{D} is a chemical group that links each Linker^{A} to the Extracellular Protein Targeting Ligand; and

wherein, when R^2 is NR^6-alkenyl, -NR^6-alkynyl,-NR^8-$C(O)R^{10}$, -NR^8-$S(O)_2$-alkenyl, -NR^8-$S(O)_2$-alkynyl, -NR^6-heteroaryl, or -NR^6-aryl, then Extracellular Protein Targeting Ligand does not comprise an oligonucleotide, and

the optional substituents are selected from alkyl, alkenyl, alkynyl, haloalkyl, -OR^6, F, Cl, Br, I, -NR^6R^7, heteroalkyl, cyano, nitro, $C(O)R^3$, , , , and , as allowed by valence such that a stable compound results.

Various moieties [Linker^{A}], [Linker^{B}], [Linker^{C}], [LinkeD^{C}], [Cycle], and X^1 are known in the art and described, for example, in International Publication No. WO 2021/155317 published August 5, 2021 and International Publication No. WO 2022/235699 published November 10, 2022, the contents of which publications are incorporated herein in their entireties by reference.

Throughout this application, various 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 in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be 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 herein 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 herein identifying such a combination.

CLAIMS

1. A composition of matter (agent) comprising:

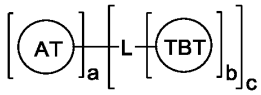
a target-binding moiety that binds to an anti-insulin antibody,

a cellular receptor-binding moiety capable of binding to hepatocytes or other degrading cells through asialoglycoprotein receptors (ASGPR) of hepatocytes or other cell receptors on surface degrading cells, and

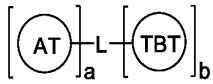
a linker moiety connecting the target-binding moiety and the cellular receptor-binding moiety.

2. The composition of matter of Claim 1, comprising a compound having a structure of:

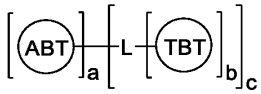
R^{CN}–(Xaa)y–R^{CC},



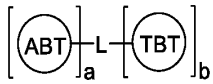
[AGN101],



[AGN102],



[AGN103], or



[AGN104]

or a pharmaceutically acceptable salt thereof.

3. The composition of matter of Claim 1, wherein the composition of matter is selected from the group consisting of **AGN302, AGN304, AGN305, AGN310, AGN4002, AGN4003, AGN4004, AGN4006, AGN4007, AGN401, AGN402, AGN405, AGN406, AGN407, AGN408, AGN409, AGN410, AGN411, AGN412, AGN413, AGN414, AGN415, AGN416, AGN417, AGN418, AGN419, AGN420, AGN421, AGN422, AGN423, AGN6567, AGN6601, AGN7525, AGN7526, AGN7849, AGN7850, AGN7865, AGN8123, AGN8138, AGN8139, AGN8140, AGN8141,** and **AGN2640.**

4. The composition of matter of Claim 1, wherein the target-binding moiety is an anti-insulin antibody-binding protein, an anti-insulin antibody-binding protein variant, or an anti-insulin antibody-binding fragment thereof.

4. The composition of matter of Claim 1, wherein the target-binding moiety is an insulin, an insulin variant, an insulin analogue, or an insulin fragment thereof.

6. The composition of matter of Claim 1, wherein the target-binding moiety is a proinsulin, a proinsulin variant, a proinsulin analogue, or a proinsulin fragment thereof.

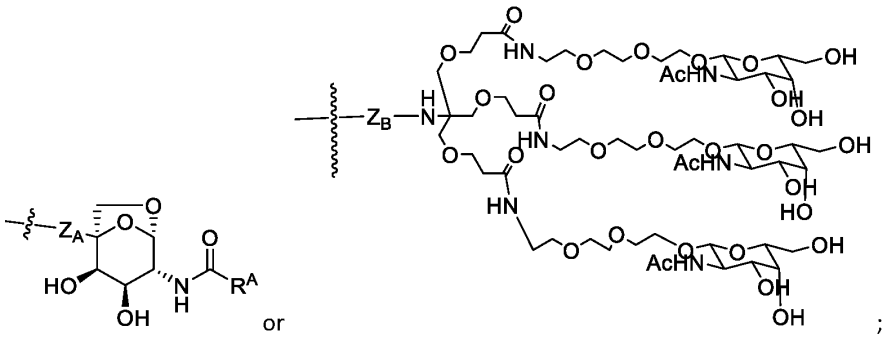
7. The composition of matter of Claim 1, wherein the target-binding moiety is made by chemical synthesis.

8. The composition of matter of Claim 1, wherein the target-binding moiety is made by genetic engineering technology.

9. The composition of matter of Claim 1, wherein the target-binding moiety is an insulin-Fc fusion protein.

10. The composition of matter of Claim 1, wherein the target-binding moiety is selected from the group consisting of **ABT306, ABT307, ABT308, ABT309, ABT311, ABT311, ABT312, ABT313, ABT314, ABT315, ABT401, ABT403, ABT404, ABT405, ABT406, ABT407, ABT408, ABT409, ABT410, ABT411, ABT412, ABT413, ABT414, ABT415, ABT416, ABT417, ABT418, ABT419, ABT420, ABT421, ABT422, ABT423, ABT424, ABT6396, ABT7714, ABT17384, ABT17385,** and **ABT17386**.

11. The composition of matter of Claim 1, wherein the cellular receptor-binding moiety comprises a compound having a structure selected from the group consisting of:



or ;

where R^A is a C_1-C_3 alkyl group optionally substituted with 1-5 halo (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 $C(O)-(CH_2)_{IM}-NR_M$.

12. The composition of matter of Claim 1, wherein the cellular receptor-binding moiety comprises N-acetyl-D-galactosamine.

13. The composition of matter of Claim 1, wherein the cellular receptor-binding moiety is selected from the group consisting of **TBT103, TBT104, TBT402, TBT403, TBT404, TBT405 , TBT6170, TBT6171, TBT4438, TBT501, TBT502, TBT7990, TBT7991, TBT7988,** and **TBT7989**.

14. The composition of matter of Claim 1 for use as a medicament.

15. The composition of matter of Claim 1 for use in removing an anti-insulin antibody from a subject.

16. The composition of matter of Claim 1 for use in treating a disease state or condition associated with the upregulation of an anti-insulin antibody in a patient.

17. The composition of matter of Claim 1 for use in treating Type 1 diabetes.

18. A pharmaceutical composition comprising a composition of matter of any of Claims 1-13 and a pharmaceutically acceptable excipient.

19. A method of making composition of matter of any of Claims 1-13.

20. A method of removing an anti-insulin antibody from a subject comprising the step of administering to the subject the agent of any of Claims 1-13.

21. A method of treating a disease state or condition associated with the upregulation of an anti-insulin antibody in a patient comprising the step of administering to the subject an effective amount of the agent of any of Claims 1-13.

22. A method of treating Type 1 diabetes in a patient comprising administering to the patient an effective amount of the agent of any of Claims 1-13.

23. A composition comprising:

a first composition of matter comprising:

a target-binding moiety that binds to an anti-insulin antibody,

a cellular receptor-binding moiety that 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.