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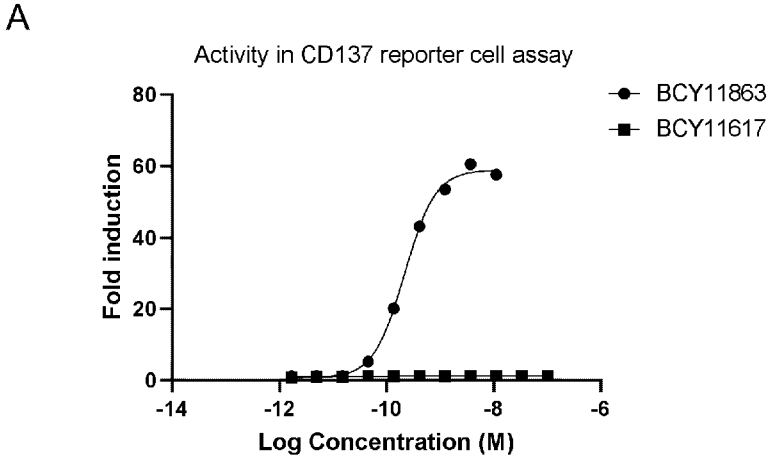

FIGURE 1

(57) Abstract: The present invention relates to heterotandem bicyclic peptide complexes which comprise a first peptide ligand, which binds to a component present on a cancer cell, conjugated via a linker to two or more second peptide ligands, which bind to a component present on an immune cell. The invention also relates to the use of said heterotandem bicyclic peptide complexes in preventing, suppressing or treating cancer.

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HETEROTANDEM BICYCLIC PEPTIDE COMPLEXES

FIELD OF THE INVENTION

The present invention relates to heterotandem bicyclic peptide complexes which comprise a first peptide ligand, which binds to a component present on a cancer cell, conjugated via a linker to two or more second peptide ligands, which bind to a component present on an immune cell. The invention also relates to the use of said heterotandem bicyclic peptide complexes in preventing, suppressing or treating cancer.

BACKGROUND OF THE INVENTION

Cyclic peptides are able to bind with high affinity and target specificity to protein targets and hence are an attractive molecule class for the development of therapeutics. In fact, several cyclic peptides are already successfully used in the clinic, as for example the antibacterial peptide vancomycin, the immunosuppressant drug cyclosporine or the anti-cancer drug octreotide (Driggers *et al.* (2008), Nat Rev Drug Discov 7 (7), 608-24). Good binding properties result from a relatively large interaction surface formed between the peptide and the target as well as the reduced conformational flexibility of the cyclic structures. Typically, macrocycles bind to surfaces of several hundred square angstrom, as for example the cyclic peptide CXCR4 antagonist CVX15 (400 $Å^2$; Wu *et al.* (2007), Science 330, 1066-71), a cyclic peptide with the Arg-Gly-Asp motif binding to integrin αVb3 (355 $Å^2$) (Xiong *et al.* (2002), Science *296* (5565), 151-5) or the cyclic peptide inhibitor upain-1 binding to urokinase-type plasminogen activator (603 $Å^2$; Zhao *et al.* (2007), J Struct Biol 160 (1), 1-10).

Due to their cyclic configuration, peptide macrocycles are less flexible than linear peptides, leading to a smaller loss of entropy upon binding to targets and resulting in a higher binding affinity. The reduced flexibility also leads to locking target-specific conformations, increasing binding specificity compared to linear peptides. This effect has been exemplified by a potent and selective inhibitor of matrix metalloproteinase 8 (MMP-8) which lost its selectivity over other MMPs when its ring was opened (Cherney *et al.* (1998), J Med Chem 41 (11), 1749-51). The favorable binding properties achieved through macrocyclization are even more pronounced in multicyclic peptides having more than one peptide ring as for example in vancomycin, nisin and actinomycin.

Different research teams have previously tethered polypeptides with cysteine residues to a synthetic molecular structure (Kemp and McNamara (1985), J. Org. Chem; Timmerman *et al.* (2005), ChemBioChem). Meloen and co-workers had used tris(bromomethyl)benzene and related molecules for rapid and quantitative cyclisation of multiple peptide loops onto synthetic

scaffolds for structural mimicry of protein surfaces (Timmerman *et al.* (2005), ChemBioChem). Methods for the generation of candidate drug compounds wherein said compounds are generated by linking cysteine containing polypeptides to a molecular scaffold as for example tris(bromomethyl)benzene are disclosed in WO 2004/077062 and WO 2006/078161.

Phage display-based combinatorial approaches have been developed to generate and screen large libraries of bicyclic peptides to targets of interest (Heinis *et al.* (2009), Nat Chem Biol *5* (7), 502-7 and WO 2009/098450). Briefly, combinatorial libraries of linear peptides containing three cysteine residues and two regions of six random amino acids (Cys-$(Xaa)_6$-Cys-$(Xaa)_6$-Cys) were displayed on phage and cyclised by covalently linking the cysteine side chains to a small molecule (tris-(bromomethyl)benzene).

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two or more second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising a heterotandem bicyclic peptide complex as defined herein in combination with one or more pharmaceutically acceptable excipients.

According to a further aspect of the invention, there is provided a heterotandem bicyclic peptide complex as defined herein for use in preventing, suppressing or treating cancer.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: (A) Analysis of the Nectin-4/CD137 heterotandem bicyclic peptide complex in the Promega CD137 luciferase reporter assay in the presence of Nectin-4 expressing H292 cells. BCY11617 is a heterotandem bicyclic peptide complex that binds to Nectin-4 with the same affinity as BCY11863 but that does not bind to CD137. (B) Summary of EC50 (nM) of

BCY11863 in the Promega CD137 luciferase reporter assay in coculture with different cell lines that express Nectin-4 endogenously or are engineered to overexpress Nectin-4.

Figure 2: Nectin-4/CD137 heterotandem bicyclic peptide complexes induce IFN-γ (Figure 2A) and IL-2 (Figure 2B) cytokine secretion in a PBMC-4T1 co-culture assay. 4T1 cells were engineered to express Nectin-4. BCY11617 is a heterotandem bicyclic peptide complex that binds to Nectin-4 with the same affinity as BCY11863 but does not bind to CD137. Figure 2C represents a summary of EC50 (nM) of BCY11863 in the cytokine secretion assay with multiple human PBMC donors and tumor cell lines.

Figure 3: Pharmacokinetics of heterotandem bicyclic peptide complex BCY11863 in SD Rats and Cynomolgus monkey (cyno) dosed IV at 2 mg/kg (n =3) and 1 mg/kg (n=2) respectively.

Figure 4: MC38#13 anti-tumor activity of BCY11863 in a syngeneic Nectin-4 overexpressing MC38 tumor model (MC38#13). Tumor volumes during and after BCY11863 treatment. Number of complete responder (CR) mice on D69 are indicated in parentheses. QD: daily dosing; Q3D: every three days dosing; ip: intraperitoneal administration.

Figure 5: BCY11863 treatment leads to an immunogenic memory to Nectin-4 overexpressing MC38 tumor model. MC38#13 tumor volumes after inoculation to naïve C57BL/6J-hCD137 mice or mice that had complete responses (CR) to BCY11863. Note that none of the CR mice developed tumors by the end of the observation period (22 days).

Figure 6: BCY11863 demonstrates anti-tumor activity in a syngeneic Nectin-4 overexpressing CT26 tumor model (CT26#7). CT26#7 tumor volumes during BCY11863 treatment. Q3D: every three days dosing; ip: intraperitoneal administration.

Figure 7: Total T cells and CD8+ T cells increase in CT26#7 tumor tissue 1h after the last (6th) Q3D dose of BCY11863. Analysis of total T cells, CD8+ T cells, CD4+ T cells, Tregs and CD8+ T cell/Treg -ratio in CT26#7 tumor tissue 1h after last Q3D dose of BCY11863.

Figure 8: Pharmacokinetic profiles of BCY11863 in plasma and tumor tissue of CT26#7 syngeneic tumor bearing animals after a single intravenous (iv) administration of 5 mg/kg of BCY11863.

Figure 9: Anti-tumor activity of BCY12491 in a syngeneic MC38 tumor model. MC38 tumor volumes during and after BCY12491 treatment. Number of complete responder (CR) mice on D73 are indicated in parentheses. QD: daily dosing; Q3D: every three days dosing; ip: intraperitoneal administration.

Figure 10: EphA2/CD137 heterotandem bicyclic peptide complex BCY12491 induces IFN-γ cytokine secretion in an MC38 co-culture assay. BCY12762 is a heterotandem bicyclic peptide complex that binds to EphA2 with the same affinity as BCY12491 but does not bind to CD137. (A) Donor 1 = Patient 228769, EC_{50} = 34pM. (B) Donor 2 = Patient 228711, EC_{50} = 85pM.

Figure 11: Plasma concentration vs time curves of BCY11863 and BCY12491 from a 15 mg/kg intraperitoneal dose in CD-1 mice (n =3) and the terminal plasma half lives for BCY11863 and BCY12491.

Figure 12: Plasma concentration versus time plot for heterotandem bispecific complex BCY12491 after a 15 min IV infusion of 1 mg/kg in cynomolgous monkeys (n=2).

Figure 13: BCY11027 induces target dependent cytokine release in *ex vivo* cultures of primary patient-derived lung tumors. (A) *Ex vivo* patient derived tumor cells form 3D spheroids within 4h in culture, 10X image under light microscope. (B) Flow analysis of Nectin-4 expression in patient derived tumor samples. Table indicates %$CD137^+$ T cells and Nectin-4^+ cells in 3 donor samples. (C) %$CD8^+ki67^+$ T cells in response to treatment with BCY11027 (D) IL-2 Cytokine release (background subtracted) as a function of concentration of BCY11027 (E) Heatmap indicating % change in immune markers (normalized to vehicle) in response to treatment with control/test compounds.

Figure 14: Results of BCY12967 in Promega OX40 cell-activity assay in co-culture with tumor cells in comparison with OX40L and non-binding control peptide BCY12968.

Figure 15: Results of BCY12491 in mouse tumor models. BCY12491 and anti-CD137 treatments increase the (A) cytotoxic cell score, (B) T cell score and (C) macrophage cell score and CD8+ cell infiltration (D) in MC38 syngeneic tumors on D6 after treatment initiation.

Figure 16: Analysis of the EphA2/CD137 heterotandem bicyclic peptide complex BCY13272 in the Promega CD137 luciferase reporter assay in the presence of EphA2 expressing A549, PC-3 and HT29 cells (n = 3). BCY13626 is a heterotandem bicyclic peptide complex similar to BCY13272 but comprises D-amino acids and does not bind to EphA2 or CD137.

Figure 17: Plasma concentration versus time plot of BCY13272 from a 5.5 mg/kg IV dose in CD1 mice (n=3), a 3.6 mg/kg IV infusion (15 min) in SD rats (n =3) and a 8.9 mg/kg IV infusion (15 min) in cynomolgus monkeys (n = 2). The pharmacokinetic profile of BCY13272 has a terminal half-life of 2.9 hours in CD-1 mice, 2.5 hours in SD Rats and 8.9 hours in cyno.

Figure 18: Anti-tumor activity of BCY13272 in a syngeneic MC38 tumor model. (A) MC38 tumor volumes during and after BCY13272 treatment. Number of complete responder (CR) mice on D28 (and that remain CRs on D62) are indicated in parentheses. BIW: twice weekly dosing; IV: intravenous administration. (B) Tumor growth curves of complete responder animals to BCY13272 and naïve age-matched control animals after MC38 tumor cell implantation. CR: complete responder.

Figure 19: BCY13272 induces IFN-γ cytokine secretion in a (A) PBMC/MC38 and a (B) PBMC/HT29 co-culture assay. BCY12762 is a heterotandem bicyclic peptide complex that binds to EphA2 but does not bind to CD137. BCY13692 is a heterotandem bicycle peptide

complex that binds to CD137 but does not bind to EphA2. (C) Plot of EC50 (nM) values of BCY13272 induced IL-2 and IFN-γ secretion in PBMC coculture assay with MC38 (mouse) cell line with 5 PBMC donors and HT1080 (human) cell line with 4 PBMC donors.

Figure 20: Surface plasmon resonance (SPR) binding of BCY13272 to immobilized (A) EphA2 and (B) CD137.

Figure 21: Surface plasmon resonance (SPR) binding study of BCY11863 to immobilized (A) Nectin-4 and (B) CD137. Dual binding SPR assay immobilizing (C) CD137 and (D) Nectin-4 on the SPR chip followed by capturing BCY11863. The affinity of bound BCY11863 to soluble human Nectin-4 (C) or CD137 (D) is measured by flowing the soluble receptor over the chip at different concentrations. (E) Binding of BCY13582 (biotinylated BCY11863) immobilized on streptavidin SPR chip to soluble human CD137.

Figure 22: Retrogenix's cell microarray technology used to explore non-specific off target interactions of BCY13582 (biotinylated BCY11863). Shown here is screening data that shows that 1 μM of BCY13582 added to microarray slides expressing 11 different proteins only binds to CD137 and Nectin-4 (detected using AlexaFluor647 labelled streptavidin). The binding signal is displaced when incubated with BCY11863.

Figure 23: Tumor growth curves of MC38#13 tumors in huCD137 C57Bl/6 mice demonstrate the anti-tumor activity of BCY11863 after different doses and dose intervals. The number of complete responder animals (CR; no palpable tumor) on day 15 after treatment initiation is indicated in parentheses.

Figure 24: Tumor growth curves (mean±SEM) of MC38#13 tumors (n=6/cohort) in huCD137 C57Bl/6 mice demonstrate the anti-tumor activity of BCY11863 at different doses and dose schedules. The number of complete responder animals (CR; no palpable tumor) on day 52 after treatment initiation is indicated in parentheses. (A) Cohorts dosed with vehicle or 3 mg/kg total weekly dose of BCY11863. (B) Cohorts dosed with vehicle or 10 mg/kg total weekly dose of BCY11863. (C) Cohorts dosed with vehicle or 30 mg/kg total weekly dose of BCY11863.

Figure 25: Pharmacokinetics of heterotandem bicyclic peptide complex BCY11863 in SD Rats dosed IV at 100 mg/kg (n =3) and measurement of concentration of BCY11863 and potential metabolites BCY15155 and BCY14602 in plasma.

Figure 26: EphA2/CD137 heterotandem bicyclic peptide complexes induce IFN-γ cytokine secretion in a PBMC-MC38 co-culture assay (A,B,C). BCY12762 and BCY12759 are 1:2 and 1:1 heterotandem complex where CD137 bicycle is replaced with an all D-amino acid non-binding control.

Figure 27: A) Growth curves of MC38 tumors in huCD137 C57Bl/6 mice (n=6/cohort) until day 27 demonstrate the anti-tumor activity of BCY12491 at different doses and dose intervals. B) Individual tumor growth measurements of the MC38 tumors until day 73. The

number of complete responder animals (CR; no palpable tumor) is indicated in parentheses. C) Tumor growth curves (n=5/cohort) from complete responder animals or naïve control animals implanted with MC38 cells. Tumor take rate (% of animals with palpable tumor growth) is indicated in parentheses.

Figure 28: BCY12491 activity is dependent on CD8+ T cells but not NK cells. (A) MC38#13 tumor bearing mice depleted of CD8+ cells and/or NK cells (D-5, D0 and D5) or treated with vehicle or isotype-control antibodies received 4 doses of 15 mg/kg BCY12491 or vehicle BIW. (B) Survival data corresponding to graph (A) where survival event is tumor volume exceeding 2000mm^3. The median survival time after treatment initiation is indicated in parentheses. Undefined survival time means that median survival time has not been reached by day 28 (end of study).

Figure 29: Growth curves of individual MC38 tumors in huCD137 C57Bl/6 mice demonstrate the anti-tumor activity of BCY12491, BCY12730 and BCY12723 at Q3D dosing schedule with 15 mg/kg dose. The number of complete responder animals (CR; no palpable tumor) on day 28 after treatment initiation is indicated in parentheses.

Figure 30: Growth curves of MC38 tumors in huCD137 C57Bl/6 mice (n=6/cohort) demonstrate the anti-tumor activity of BCY12491, BCY13048 and BCY13050 at BIW dosing schedule with 5 mg/kg dose. The number of complete responder animals (CR; no palpable tumor) on day 28 after treatment initiation is indicated in parentheses.

DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two or more second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

According to one aspect of the invention which may be mentioned, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two or more second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three cysteine residues, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the cysteine residues of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

First Peptide Ligands

References herein to the term "cancer cell" includes any cell which is known to be involved in cancer. Cancer cells are created when the genes responsible for regulating cell division are damaged. Carcinogenesis is caused by mutation and epimutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death. This results in uncontrolled cell division and the evolution of those cells by natural selection in the body. The uncontrolled and often rapid proliferation of cells can lead to benign or malignant tumors (cancer). Benign tumors do not spread to other parts of the body or invade other tissues. Malignant tumors can invade other organs, spread to distant locations (metastasis) and become life-threatening.

In one embodiment, the cancer cell is selected from an HT1080, A549, SC-OV-3, PC3, HT1376, NCI-H292, LnCap, MC38, MC38 #13, 4T1-D02, H322, HT29, T47D and RKO tumor cell.

In one embodiment, the component present on a cancer cell is Nectin-4.

Nectin-4 is a surface molecule that belongs to the nectin family of proteins, which comprises 4 members. Nectins are cell adhesion molecules that play a key role in various biological processes such as polarity, proliferation, differentiation and migration, for epithelial, endothelial, immune and neuronal cells, during development and adult life. They are involved in several pathological processes in humans. They are the main receptors for poliovirus, herpes simplex virus and measles virus. Mutations in the genes encoding Nectin-1 (PVRL1) or Nectin-4 (PVRL4) cause ectodermal dysplasia syndromes associated with other abnormalities. Nectin-4 is expressed during foetal development. In adult tissues its expression is more restricted than that of other members of the family. Nectin-4 is a tumor-associated antigen in 50%, 49% and 86% of breast, ovarian and lung carcinomas, respectively, mostly on tumors of bad prognosis. Its expression is not detected in the corresponding normal tissues. In breast tumors, Nectin-4 is expressed mainly in triple-negative and ERBB2+ carcinomas. In the serum of patients with these cancers, the detection of soluble forms of Nectin-4 is

associated with a poor prognosis. Levels of serum Nectin-4 increase during metastatic progression and decrease after treatment. These results suggest that Nectin-4 could be a reliable target for the treatment of cancer. Accordingly, several anti-Nectin-4 antibodies have been described in the prior art. In particular, Enfortumab Vedotin (ASG-22ME) is an antibody-drug conjugate (ADC) targeting Nectin-4 and is currently clinically investigated for the treatment of patients suffering from solid tumors.

In one embodiment, the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand.

Suitable examples of Nectin-4 binding bicyclic peptide ligands are disclosed in WO 2019/243832, the peptides of which are incorporated herein by reference.

In one embodiment, the Nectin-4 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

C_iP[1Nal][dD]C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 1; herein referred to as BCY8116);

C_iP[1Nal][dD]C_{ii}M[HArg]D[dW]STP[HyP][dW]C_{iii} (SEQ ID NO: 2);

C_iP[1Nal][dK](Sar_{10}-(B-Ala))C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 3);

C_iPFGC_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 4; herein referred to as BCY11414);

C_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 14);

[MerPro]$_i$P[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 15; herein referred to as BCY12363);

C_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]W[Cysam]$_{iii}$ (SEQ ID NO: 16);

[MerPro]$_i$P[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]W[Cysam]$_{iii}$ (SEQ ID NO: 17; herein referred to as BCY12365);

C_iP[1Nal][dK]C_{ii}M[HArg]HWSTP[HyP]WC_{iii} (SEQ ID NO: 18);

C_iP[1Nal][dK]C_{ii}M[HArg]EWSTP[HyP]WC_{iii} (SEQ ID NO: 19);

C_iP[1Nal][dE]C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 20; herein referred to as BCY12368);

C_iP[1Nal][dA]C_{ii}M[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 21; herein referred to as BCY12369);

C_iP[1Nal][dE]C_{ii}L[HArg]DWSTP[HyP]WC_{iii} (SEQ ID NO: 22; herein referred to as BCY12370); and

C_iP[1Nal][dE]C_{ii}M[HArg]EWSTP[HyP]WC_{iii} (SEQ ID NO: 23; herein referred to as BCY12384);

wherein $[MerPro]_i$, C_i, C_{ii}, C_{iii} and $[Cysam]_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, 1Nal represents 1-naphthylalanine, HArg represents homoarginine, HyP represents trans-4-hydroxy-L-proline, Sar_{10} represents 10 sarcosine units, B-Ala represents beta-alanine, MerPro represents 3-mercaptopropionic acid and Cysam represents cysteamine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the Nectin-4 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

$C_iP[1Nal][dD]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 1; herein referred to as BCY8116);

$C_iP[1Nal][dK](Sar_{10}\text{-}(B\text{-}Ala))C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 3); and

$C_iPFGC_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 4; herein referred to as BCY11414);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, 1Nal represents 1-naphthylalanine, HArg represents homoarginine, HyP represents trans-4-hydroxy-L-proline, Sar_{10} represents 10 sarcosine units, B-Ala represents beta-alanine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the Nectin-4 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

SEQ ID NO: 1 (herein referred to as BCY8116);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 1) (herein referred to as BCY8846);

[PYA]-(SEQ ID NO: 1) (herein referred to as BCY11015);

[PYA]-[B-Ala]-(SEQ ID NO: 1) (herein referred to as BCY11016);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 2) (herein referred to as BCY11942);

Ac-(SEQ ID NO: 3) (herein referred to as BCY8831);

SEQ ID NO: 4 (herein referred to as BCY11414);

[PYA]-[B-Ala]-(SEQ ID NO: 14) (herein referred to as BCY11143);

Palmitic-yGlu-yGlu-(SEQ ID NO: 14) (herein referred to as BCY12371);

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Ac-(SEQ ID NO: 18) (herein referred to as BCY12366); and

Ac-(SEQ ID NO: 19) (herein referred to as BCY12367);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units, or a pharmaceutically acceptable salt thereof.

In a yet further embodiment, the Nectin-4 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

SEQ ID NO: 1 (herein referred to as BCY8116);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 1) (herein referred to as BCY8846);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 2) (herein referred to as BCY11942);

Ac-(SEQ ID NO: 3) (herein referred to as BCY8831); and

SEQ ID NO: 4 (herein referred to as BCY11414);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units, or a pharmaceutically acceptable salt thereof.

In a yet further embodiment, the Nectin-4 binding bicyclic peptide ligand comprises SEQ ID NO: 1 (herein referred to as BCY8116).

In an alternative embodiment, the component present on a cancer cell is EphA2.

Eph receptor tyrosine kinases (Ephs) belong to a large group of receptor tyrosine kinases (RTKs), kinases that phosphorylate proteins on tyrosine residues. Ephs and their membrane bound ephrin ligands (ephrins) control cell positioning and tissue organization (Poliakov *et al.* (2004) Dev Cell 7, 465-80). Functional and biochemical Eph responses occur at higher ligand oligomerization states (Stein *et al.* (1998) Genes Dev 12, 667-678).

Among other patterning functions, various Ephs and ephrins have been shown to play a role in vascular development. Knockout of EphB4 and ephrin-B2 results in a lack of the ability to remodel capillary beds into blood vessels (Poliakov *et al.*, *supra*) and embryonic lethality. Persistent expression of some Eph receptors and ephrins has also been observed in newly-formed, adult micro-vessels (Brantley-Sieders *et al.* (2004) Curr Pharm Des 10, 3431-42; Adams (2003) J Anat 202, 105-12).

The de-regulated re-emergence of some ephrins and their receptors in adults also has been observed to contribute to tumor invasion, metastasis and neo-angiogenesis (Nakamoto *et al.* (2002) Microsc Res Tech 59, 58-67; Brantley-Sieders *et al.*, *supra*). Furthermore, some Eph family members have been found to be over-expressed on tumor cells from a variety of human tumors (Brantley-Sieders *et al.*, *supra*); Marme (2002) Ann Hematol 81 Suppl 2, S66; Booth *et al.* (2002) Nat Med 8, 1360-1).

EPH receptor A2 (ephrin type-A receptor 2) is a protein that in humans is encoded by the *EPHA2* gene.

EphA2 is upregulated in multiple cancers in man, often correlating with disease progression, metastasis and poor prognosis e.g.: breast (Zelinski *et al* (2001) Cancer Res. 61, 2301–2306; Zhuang *et al* (2010) Cancer Res. 70, 299–308; Brantley-Sieders *et al* (2011) PLoS One 6, e24426), lung (Brannan *et al* (2009) Cancer Prev Res (Phila) 2, 1039–1049; Kinch *et al* (2003) Clin Cancer Res. 9, 613-618; Guo *et al* (2013) J Thorac Oncol. 8, 301-308), gastric (Nakamura *et al* (2005) Cancer Sci. 96, 42-47; Yuan *et al* (2009) Dig Dis Sci 54, 2410-2417), pancreatic (Mudali *et al* (2006) Clin Exp Metastasis 23, 357-365), prostate (Walker-Daniels *et al* (1999) Prostate 41, 275–280), liver (Yang *et al* (2009) Hepatol Res. 39, 1169–1177) and glioblastoma (Wykosky *et al* (2005) Mol Cancer Res. 3, 541–551; Li *et al* (2010) Tumor Biol. 31, 477–488).

The full role of EphA2 in cancer progression is still not defined although there is evidence for interaction at numerous stages of cancer progression including tumor cell growth, survival, invasion and angiogenesis. Downregulation of EphA2 expression suppresses tumor cancer cell propagation (Binda *et al* (2012) Cancer Cell 22, 765-780), whilst EphA2 blockade inhibits VEGF induced cell migration (Hess *et al* (2001) Cancer Res. 61, 3250–3255), sprouting and angiogenesis (Cheng *et al* (2002) Mol Cancer Res. 1, 2–11; Lin *et al* (2007) Cancer 109, 332-40) and metastatic progression (Brantley-Sieders *et al* (2005) FASEB J. 19, 1884–1886).

An antibody drug conjugate to EphA2 has been shown to significantly diminish tumor growth in rat and mouse xenograft models (Jackson *et al* (2008) Cancer Research 68, 9367-9374) and a similar approach has been tried in man although treatment had to be discontinued for treatment related adverse events (Annunziata *et al* (2013) Invest New drugs 31, 77-84).

In one embodiment, the first peptide ligand comprises an EphA2 binding bicyclic peptide ligand.

Suitable examples of EphA2 binding bicyclic peptide ligands are disclosed in WO 2019/122860, WO 2019/122861 and WO 2019/122863, the peptides of which are incorporated herein by reference.

In one embodiment, the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

C_i[HyP]LVNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 24);

C_iLWDPTPC_{ii}ANLHL[HArg]C_{iii} (SEQ ID NO: 25);

C_i[HyP]LVNPLC$_{ii}$L[K(PYA)]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 26);

C_i[HyP][K(PYA)]VNPLC$_{ii}$LHP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 27);

C_i[HyP]LVNPLC$_{ii}$[K(PYA)]HP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 28);

C_i[HyP]LVNPLC$_{ii}$LKP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 29);

C_i[HyP]KVNPLC$_{ii}$LHP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 30);

C_i[HyP]LVNPLC$_{ii}$KHP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 31);

C_i[HyP]LVNPLC$_{ii}$LHP[dE]W[HArg]C$_{iii}$ (SEQ ID NO: 32);

C_i[HyP]LVNPLC$_{ii}$LEP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 33);

C_i[HyP]LVNPLC$_{ii}$LHP[dD]WTC$_{iii}$ (SEQ ID NO: 34);

C_i[HyP]LVNPLC$_{ii}$LEP[dD]WTC$_{iii}$ (SEQ ID NO: 35);

C_i[HyP]LVNPLC$_{ii}$LEP[dA]WTC$_{iii}$ (SEQ ID NO: 36);

C_i[HyP]LVNPLC$_{ii}$L[3,3-DPA]P[dD]WTC$_{iii}$ (SEQ ID NO: 37; herein referred to as BCY12860);

C_i[HyP][Cba]VNPLC$_{ii}$LHP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 38);

C_i[HyP][Cba]VNPLC$_{ii}$LEP[dD]WTC$_{iii}$ (SEQ ID NO: 39);

C_i[HyP][Cba]VNPLC$_{ii}$L[3,3-DPA]P[dD]WTC$_{iii}$ (SEQ ID NO: 40);

C_i[HyP]LVNPLC$_{ii}$L[3,3-DPA]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 41);

C_i[HyP]LVNPLC$_{ii}$LHP[d1Nal]W[HArg]C$_{iii}$ (SEQ ID NO: 42);

C_i[HyP]LVNPLC$_{ii}$L[1Nal]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 43);

C_i[HyP]LVNPLC$_{ii}$LEP[d1Nal]WTC$_{iii}$ (SEQ ID NO: 44);

C_i[HyP]LVNPLC$_{ii}$L[1Nal]P[dD]WTC$_{iii}$ (SEQ ID NO: 45; herein referred to as BCY13119);

C_i[HyP][Cba]VNPLC$_{ii}$LEP[dA]WTC$_{iii}$ (SEQ ID NO: 46);

C_i[HyP][hGlu]VNPLC$_{ii}$LHP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 47);

C_i[HyP]LVNPLC$_{ii}$[hGlu]HP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 48);

C_i[HyP]LVNPLC$_{ii}$L[hGlu]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 49);

C_i[HyP]LVNPLC$_{ii}$LHP[dNle]W[HArg]C$_{iii}$ (SEQ ID NO: 50);

C_i[HyP]LVNPLC$_{ii}$L[Nle]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 51);

[MerPro]$_i$[HyP]LVNPLC$_{ii}$L[3,3-DPA]P[dD]WTC$_{iii}$ (SEQ ID NO: 154);

C_i[HyP]LVNPLC$_{ii}$LHP[dD]W[HArg][Cysam]$_{iii}$ (SEQ ID NO: 155);

C_i[HyP]LVNPLC$_{ii}$L[His3Me]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 156);

C_i[HyP]LVNPLC$_{ii}$L[His1Me]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 157);

C_i[HyP]LVNPLC$_{ii}$L[4ThiAz]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 158);

C_i[HyP]LVNPLC$_{ii}$LFP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 159);

C_i[HyP]LVNPLC$_{ii}$L[Thi]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 160);

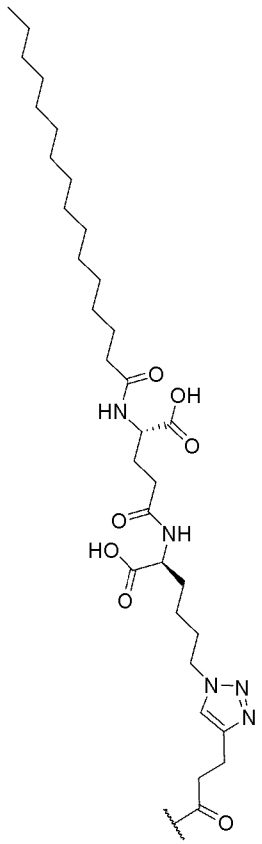
C_i[HyP]LVNPLC$_{ii}$L[3Thi]P[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 161);

C_i[HyP]LVNPLC$_{ii}$LNP[dD]W[HArg]C$_{iii}$ (SEQ ID NO: 162);

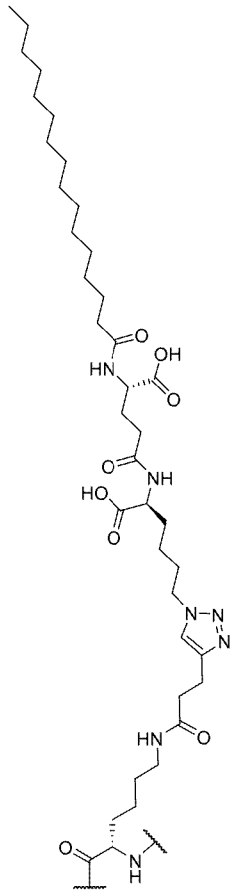
C_i[HyP]LVNPLC_{ii}LQP[dD]W[HArg]C_{iii} (SEQ ID NO: 163); and

C_i[HyP]LVNPLC_{ii}L[K(PYA-(Palmitoyl-Glu-LysN_3)]P[dD]W[HArg]C_{iii} (SEQ ID NO: 164);

wherein $[MerPro]_i$, C_i, C_{ii}, C_{iii} and $[Cysam]_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, HyP represents trans-4-hydroxy-L-proline, HArg represents homoarginine, PYA represents 4-pentynoic acid, 3,3-DPA represents 3,3-diphenylalanine, Cba represents β-cyclobutylalanine, 1Nal represents 1-naphthylalanine, hGlu represents homoglutamic acid, Thi represents 2-thienyl-alanine, 4ThiAz represents beta-(4-thiazolyl)-alanine, His1Me represents N1-methyl-L-histidine, His3Me represents N3-methyl-L-histidine, 3Thi represents 3-thienylalanine, Palmitoyl-Glu-LysN_3[PYA] represents:



(Palmitoyl-Glu-LysN3)[PYA] ,



[K(PYA-(Palmitoyl-Glu-LysN_3)] represents [K(PYA(Palmitoyl-Glu-LysN3))], Nle represents norleucine, MerPro represents 3-mercaptopropionic acid and Cysam represents cysteamine, or a pharmaceutically acceptable salt thereof.

In one particular embodiment, the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence which is:

C_i[HyP]LVNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 24);

wherein C_i, C_{ii}, C_{iii} and represent first (i), second (ii) and third (iii) cysteine groups, HyP represents trans-4-hydroxy-L-proline, HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

In one alternative particular embodiment, the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence which is:

C_i[HyP]LVNPLC_{ii}LEP[d1Nal]WTC_{iii} (SEQ ID NO: 44);

wherein C_i, C_{ii}, C_{iii} and represent first (i), second (ii) and third (iii) cysteine groups, HyP represents trans-4-hydroxy-L-proline, 1Nal represents 1-naphthylalanine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal and/or C-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY9594);

[B-Ala]-[Sar_{10}]-A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY6099);

[PYA]-A-[HArg]-D-(SEQ NO: 24) (herein referred to as BCY11813);

Ac-A-[HArg]-D-(SEQ ID NO: 24)-[K(PYA)] (herein referred to as BCY11814);

Ac-A-[HArg]-D-(SEQ ID NO: 24)-K (herein referred to as BCY12734);

[NMeAla]-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY13121);

[Ac]-(SEQ ID NO: 24)-L[dH]G[dK] (herein referred to as BCY13125);

[PYA]-[B-Ala]-[Sar_{10}]-VGP-(SEQ ID NO: 25) (herein referred to as BCY8941);

Ac-A-[HArg]-D-(SEQ ID NO: 26) (herein referred to as BCY11815);

Ac-A-[HArg]-D-(SEQ ID NO: 27) (herein referred to as BCY11816);

Ac-A-[HArg]-D-(SEQ ID NO: 28) (herein referred to as BCY11817);

Ac-A-[HArg]-D-(SEQ ID NO: 29) (herein referred to as BCY12735);

(Palmitoyl-Glu-LysN_3)[PYA]A[HArg]D-(SEQ ID NO: 29) (hereinafter known as BCY14327);

Ac-A-[HArg]-D-(SEQ ID NO: 30) (herein referred to as BCY12736);

Ac-A-[HArg]-D-(SEQ ID NO: 31) (herein referred to as BCY12737);

A-[HArg]-D-(SEQ ID NO: 32) (herein referred to as BCY12738);

A-[HArg]-E-(SEQ ID NO: 32) (herein referred to as BCY12739);

A-[HArg]-D-(SEQ ID NO: 33) (herein referred to as BCY12854);

A-[HArg]-D-(SEQ ID NO: 34) (herein referred to as BCY12855);

A-[HArg]-D-(SEQ ID NO: 35) (herein referred to as BCY12856);

A-[HArg]-D-(SEQ ID NO: 35)-[dA] (herein referred to as BCY12857);

(SEQ ID NO: 35)-[dA] (herein referred to as BCY12861);

[NMeAla]-[HArg]-D-(SEQ ID NO: 35) (herein referred to as BCY13122);

[dA]-ED-(SEQ ID NO: 35) (herein referred to as BCY13126);

[dA]-[dA]-D-(SEQ ID NO: 35) (herein referred to as BCY13127);

AD-(SEQ ID NO: 35) (herein referred to as BCY13128);

A-[HArg]-D-(SEQ ID NO: 36) (herein referred to as BCY12858);

A-[HArg]-D-(SEQ ID NO: 37) (herein referred to as BCY12859);

Ac-(SEQ ID NO: 37)-[dK] (herein referred to as BCY13120);

A-[HArg]-D-(SEQ ID NO: 38) (herein referred to as BCY12862);

A-[HArg]-D-(SEQ ID NO: 39) (herein referred to as BCY12863);

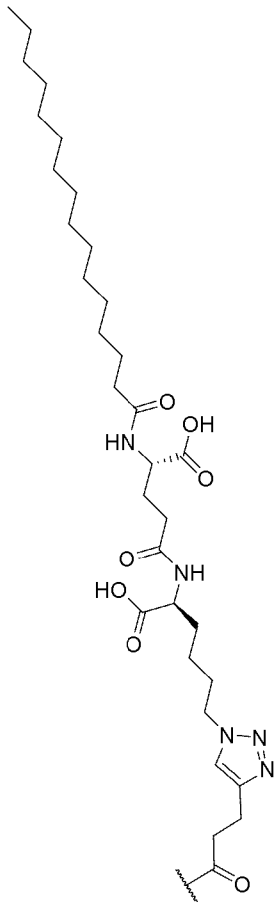
[dA]-[HArg]-D-(SEQ ID NO: 39)-[dA] (herein referred to as BCY12864);

(SEQ ID NO: 40)-[dA] (herein referred to as BCY12865);

A-[HArg]-D-(SEQ ID NO: 41) (herein referred to as BCY12866);

A-[HArg]-D-(SEQ ID NO: 42) (herein referred to as BCY13116);
A-[HArg]-D-(SEQ ID NO: 43) (herein referred to as BCY13117);
A-[HArg]-D-(SEQ ID NO: 44) (herein referred to as BCY13118);
[dA]-[HArg]-D-(SEQ ID NO: 46)-[dA] (herein referred to as BCY13123);
[d1Nal]-[HArg]-D-(SEQ ID NO: 46)-[dA] (herein referred to as BCY13124);
A-[HArg]-D-(SEQ ID NO: 47) (herein referred to as BCY13130);
A-[HArg]-D-(SEQ ID NO: 48) (herein referred to as BCY13131);
A-[HArg]-D-(SEQ ID NO: 49) (herein referred to as BCY13132);
A-[HArg]-D-(SEQ ID NO: 50) (herein referred to as BCY13134);
A-[HArg]-D-(SEQ ID NO: 51) (herein referred to as BCY13135);
(SEQ ID NO: 154)-[dK] (herein referred to as BCY13129);
A[HArg]D-(SEQ ID NO: 155) (herein referred to as BCY13133);
A[HArg]D-(SEQ ID NO: 156) (herein referred to as BCY13917);
A[HArg]D-(SEQ ID NO: 157) (herein referred to as BCY13918);
A[HArg]D-(SEQ ID NO: 158) (herein referred to as BCY13919);
A[HArg]D-(SEQ ID NO: 159) (herein referred to as BCY13920);
A[HArg]D-(SEQ ID NO: 160) (herein referred to as BCY13922);
A[HArg]D-(SEQ ID NO: 161) (herein referred to as BCY13923);
A[HArg]D-(SEQ ID NO: 162) (herein referred to as BCY14047);
A[HArg]D-(SEQ ID NO: 163) (herein referred to as BCY14048); and
A[HArg]D-(SEQ ID NO: 164) (herein referred to as BCY14313);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units, HArg represents homoarginine, NMeAla represents N-methyl-alanine, 1Nal represents 1-naphthylalanine, Palmitoyl-Glu-LysN_3[PYA] represents:



(Palmitoyl-Glu-LysN3)[PYA] , or a pharmaceutically acceptable salt thereof.

In one particular embodiment, the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal and/or C-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY9594);

wherein HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

In one alternative particular embodiment, the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal and/or C-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 44) (herein referred to as BCY13118);

wherein HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

In an alternative embodiment, the component present on a cancer cell is PD-L1.

Programmed cell death 1 ligand 1 (PD-L1) is a 290 amino acid type I transmembrane protein encoded by the CD274 gene on mouse chromosome 19 and human chromosome 9. PD-L1 expression is involved in evasion of immune responses involved in chronic infection, e.g., chronic viral infection (including, for example, HIV, HBV, HCV and HTLV, among others),

chronic bacterial infection (including, for example, Helicobacter pylori, among others), and chronic parasitic infection (including, for example, Schistosoma mansoni). PD-L1 expression has been detected in a number of tissues and cell types including T-cells, B-cells, macrophages, dendritic cells, and nonhaematopoietic cells including endothelial cells, hepatocytes, muscle cells, and placenta.

PD-L1 expression is also involved in suppression of anti-tumor immune activity. Tumors express antigens that can be recognised by host T-cells, but immunologic clearance of tumors is rare. Part of this failure is due to immune suppression by the tumor microenvironment. PD-L1 expression on many tumors is a component of this suppressive milieu and acts in concert with other immunosuppressive signals. PD-L1 expression has been shown *in situ* on a wide variety of solid tumors including breast, lung, colon, ovarian, melanoma, bladder, liver, salivary, stomach, gliomas, thyroid, thymic epithelial, head, and neck (Brown JA *et al.* 2003 Immunol. 170:1257-66; Dong H *et al.* 2002 Nat. Med. 8:793-800; Hamanishi J, *et al.* 2007 Proc. Natl. Acad. Sci. USA 104:3360-65; Strome SE *et al.* 2003 Cancer Res. 63:6501-5; Inman BA *et al.* 2007 Cancer 109:1499-505; Konishi J *et al.* 2004 Clin. Cancer Res. 10:5094-100; Nakanishi J *et al.* 2007 Cancer Immunol. Immunother. 56:1173-82; Nomi T *et al.* 2007 Clin. Cancer Res. 13:2151-57; Thompson RH *et al.* 2004 Proc. Natl. Acad. Sci. USA 101: 17174-79; Wu C *et al.* 2006 Acta Histochem. 108:19-24). In addition, the expression of the receptor for PD-L1, Programmed cell death protein 1 (also known as PD-1 and CD279) is upregulated on tumor infiltrating lymphocytes, and this also contributes to tumor immunosuppression (Blank C *et al.* 2003 Immunol. 171:4574-81). Most importantly, studies relating PD-L1 expression on tumors to disease outcome show that PD-L1 expression strongly correlates with unfavourable prognosis in kidney, ovarian, bladder, breast, gastric, and pancreatic cancer (Hamanishi J *et al.* 2007 Proc. Natl. Acad. Sci. USA 104:3360-65; Inman BA *et al.* 2007 Cancer 109:1499-505; Konishi J *et al.* 2004 Clin. Cancer Res. 10:5094-100; Nakanishi J *et al.* 2007 Cancer Immunol. Immunother. 56:1173-82; Nomi T *et al.* 2007 Clin. Cancer Res. 13:2151-57; Thompson RH *et al.* 2004 Proc. Natl. Acad. Sci. USA 101:17174-79; Wu C *et al.* 2006 Acta Histochem. 108:19-24). In addition, these studies suggest that higher levels of PD-L1 expression on tumors may facilitate advancement of tumor stage and invasion into deeper tissue structures.

The PD-1 pathway can also play a role in haematologic malignancies. PD-L1 is expressed on multiple myeloma cells but not on normal plasma cells (Liu J *et al.* 2007 Blood 110:296-304). PD-L1 is expressed on some primary T-cell lymphomas, particularly anaplastic large cell T lymphomas (Brown JA *et al*, 2003 Immunol. 170:1257-66). PD-1 is highly expressed on the T-cells of angioimmunoblastic lymphomas, and PD-L1 is expressed on the associated

follicular dendritic cell network (Dorfman DM *et al.* 2006 Am. J. Surg. Pathol. 30:802-10). In nodular lymphocyte-predominant Hodgkin lymphoma, the T-cells associated with lymphocytic or histiocytic (L&H) cells express PD-1. Microarray analysis using a readout of genes induced by PD-1 ligation suggests that tumor-associated T-cells are responding to PD-1 signals *in situ* in Hodgkin lymphoma (Chemnitz JM *et al.* 2007 Blood 110:3226-33). PD-1 and PD-L1 are expressed on CD4 T-cells in HTLV-1 -mediated adult T-cell leukaemia and lymphoma (Shimauchi T *et al.* 2007 Int. J. Cancer 121: 2585-90). These tumor cells are hyporesponsive to TCR signals.

Studies in animal models demonstrate that PD-L1 on tumors inhibits T-cell activation and lysis of tumor cells and in some cases leads to increased tumor-specific T-cell death (Dong H *et al.* 2002 Nat. Med. 8:793-800; Hirano F *et al.* 2005 Cancer Res. 65:1089-96). Tumor-associated APCs can also utilise the PD-1:PD-L1 pathway to control antitumor T-cell responses. PD-L1 expression on a population of tumor-associated myeloid DCs is upregulated by tumor environmental factors (Curiel TJ *et al.* 2003 Nat. Med. 9:562-67). Plasmacytoid dendritic cells (DCs) in the tumor-draining lymph node of B16 melanoma express IDO, which strongly activates the suppressive activity of regulatory T-cells. The suppressive activity of IDO-treated regulatory T-cells required cell contact with IDO- expressing DCs (Sharma MD *et al.* 2007 Clin. Invest. 117:2570-82).

In one embodiment, the first peptide ligand comprises a PD-L1 binding bicyclic peptide ligand.

Suitable examples of PD-L1 binding bicyclic peptide ligands are disclosed in WO 2020/128526 and WO 2020/128527, the peptides of which are incorporated herein by reference.

In one embodiment, the PD-L1 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

$C_iSAGWLTMC_{ii}QKLHLC_{iii}$ (SEQ ID NO: 52);

$C_iSAGWLTMC_{ii}Q[K(PYA)]LHLC_{iii}$ (SEQ ID NO: 53);

$C_iSKGWLTMC_{ii}Q[K(Ac)]LHLC_{iii}$ (SEQ ID NO: 54);

$C_iSAGWLTKC_{ii}Q[K(Ac)]LHLC_{iii}$ (SEQ ID NO: 55);

$C_iSAGWLTMC_{ii}K[K(Ac)]LHLC_{iii}$ (SEQ ID NO: 56);

$C_iSAGWLTMC_{ii}Q[K(Ac)]LKLC_{iii}$ (SEQ ID NO: 57);

$C_iSAGWLTMC_{ii}Q[HArg]LHLC_{iii}$ (SEQ ID NO: 58); and

$C_iSAGWLTMC_{ii}[HArg]QLNLC_{iii}$ (SEQ ID NO: 59);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, PYA represents 4-pentynoic acid and HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the PD-L1 binding bicyclic peptide ligand optionally comprises N-terminal and/or C-terminal modifications and comprises:

[PYA]-[B-Ala]-[Sar_{10}]-SDK-(SEQ ID NO: 52) (herein referred to as BCY10043);
Ac-D-[HArg]-(SEQ ID NO: 52)-PSH (herein referred to as BCY11865);
Ac-SDK-(SEQ ID NO: 53) (herein referred to as BCY11013);
Ac-SDK-(SEQ ID NO: 53)-PSH (herein referred to as BCY10861);
Ac-D-[HArg]-(SEQ ID NO: 54)-PSH (herein referred to as BCY11866);
Ac-D-[HArg]-(SEQ ID NO: 55)-PSH (herein referred to as BCY11867);
Ac-D-[HArg]-(SEQ ID NO: 56)-PSH (herein referred to as BCY11868);
Ac-D-[HArg]-(SEQ ID NO: 57)-PSH (herein referred to as BCY11869);
Ac-SD-[HArg]-(SEQ ID NO: 58)-PSHK (herein referred to as BCY12479); and
Ac-SD-[HArg]-(SEQ ID NO: 59)-PSHK (herein referred to as BCY12477);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units and HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

In an alternative embodiment, the component present on a cancer cell is prostate-specific membrane antigen (PSMA).

Prostate-specific membrane antigen (PSMA) (also known as Glutamate carboxypeptidase II (GCPII), N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALADase I) and NAAG peptidase) is an enzyme that in humans is encoded by the *FOLH1* (folate hydrolase 1) gene. Human GCPII contains 750 amino acids and weighs approximately 84 kDa.

Human PSMA is highly expressed in the prostate, roughly a hundred times greater than in most other tissues. In some prostate cancers, PSMA is the second-most upregulated gene product, with an 8- to 12-fold increase over levels in noncancerous prostate cells. Because of this high expression, PSMA is being developed as potential biomarker for therapy and imaging of some cancers. In human prostate cancer, the higher expressing tumors are associated with quicker time to progression and a greater percentage of patients suffering relapse.

In one embodiment, the first peptide ligand comprises a PSMA binding bicyclic peptide ligand.

Suitable examples of PSMA binding bicyclic peptide ligands are disclosed in WO 2019/243455 and WO 2020/120980, the peptides of which are incorporated herein by reference

Second Peptide Ligands

References herein to the term "immune cell" includes any cell within the immune system. Suitable examples include white blood cells, such as lymphocytes (e.g. T lymphocytes or T cells, B cells or natural killer cells). In one embodiment, the T cell is CD8 or CD4. In a further embodiment, the T cell is CD8. Other examples of immune cells include dendritic cells, follicular dendritic cells and granulocytes.

In one embodiment, the component present on an immune cell is CD137.

CD137 is a member of the tumor necrosis factor (TNF) receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 9 (TNFRSF9), 4- IBB and induced by lymphocyte activation (ILA). CD137 can be expressed by activated T cells, but to a larger extent on CD8+ than on CD4+ T cells. In addition, CD137 expression is found on dendritic cells, follicular dendritic cells, natural killer cells, granulocytes and cells of blood vessel walls at sites of inflammation. One characterized activity of CD137 is its costimulatory activity for activated T cells. Crosslinking of CD137 enhances T cell proliferation, IL-2 secretion, survival and cytolytic activity. Further, it can enhance immune activity to eliminate tumors in mice.

CD137 is a T-cell costimulatory receptor induced on TCR activation (Nam et al., Curr. Cancer Drug Targets, 5:357-363 (2005); Waits et al., Annu. Rev, Immunol., 23:23-68 (2005)). In addition to its expression on activated CD4+ and CD8+ T cells, CD137 is also expressed on CD4+CD25+ regulatory T cells, natural killer (NK) and NK-T cells, monocytes, neutrophils, and dendritic cells. Its natural ligand, CD137L, has been described on antigen- presenting cells including B cells, monocyte/macrophages, and dendritic cells (Watts et al. Annu. Rev. Immunol, 23:23-68 (2005)). On interaction with its ligand, CD137 leads to increased TCR-induced T-cell proliferation, cytokine production, functional maturation, and prolonged CD8+ T-cell survival (Nam et al, Curr. Cancer Drug Targets, 5:357-363 (2005), Watts et d - l., Annu. Rev. Immunol, 23:23-68 (2005)).

Signalling through CD137 by either CD137L or agonistic monoclonal antibodies (mAbs) against CD137 leads to increased TCR-induced T cell proliferation, cytokine production and functional maturation, and prolonged CD8+ T cell survival. These effects result from: (1) the activation of the NF-κB, c-Jun NH2-terminal kinase/stress-activated protein kinase

(JNK/SAPK), and p38 mitogen-activated protein kinase (MAPK) signalling pathways, and (2) the control of anti-apoptotic and cell cycle -related gene expression.

Experiments performed in both CD137 and CD137L-deficient mice have additionally demonstrated the importance of CD137 costimulation in the generation of a fully competent T cell response.

IL-2 and IL-15 activated NK cells express CD137, and ligation of CD137 by agonistic mAbs stimulates NK cell proliferation and IFN-γ secretion, but not their cytolytic activity.

Furthermore, CD137-stimulated NK cells promote the expansion of activated T cells *in vitro*.

In accordance with their costimulatory function, agonist mAbs against CD137 have been shown to promote rejection of cardiac and skin allografts, eradicate established tumors, broaden primary antiviral CD8+ T cell responses, and increase T cell cytolytic potential. These studies support the view that CD137 signalling promotes T cell function which may enhance immunity against tumors and infection.

In one embodiment, the two or more second peptide ligands comprise a CD137 binding bicyclic peptide ligand.

Suitable examples of CD137 binding bicyclic peptide ligands are disclosed in WO 2019/025811, the peptides of which are incorporated herein by reference.

In one embodiment, the CD137 binding bicyclic peptide ligand comprises an amino acid sequence:

C_ilEEGQYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 5);

C_i[tBuAla]PE[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 6);

C_ilEEGQYC_{ii}F[D-Ala]DPY[Nle]C_{iii} (SEQ ID NO: 7);

C_i[tBuAla]PK[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 8);

C_i[tBuAla]PE[D-Lys]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 9);

C_i[tBuAla]P[K(PYA)][D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 10);

C_i[tBuAla]PE[D-Lys(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 11);

C_ilEE[D-Lys(PYA)]QYC_{ii}FADPY(Nle)C_{iii} (SEQ ID NO: 12);

[dC_i][dI][dE][dE][K(PYA)][dQ][dY][dC_{ii}][dF][dA][dD][dP][dY][dNle][dC_{iii}] (SEQ ID NO: 13);

C_i[tBuAla]PE[dK]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 60);

C_iIEE[dK(PYA)]QYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 61);

C_i[tBuAla]EE(dK)PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 62);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 63);

C_i[tBuAla]EE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 64);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FANPY[Nle]C_{iii} (SEQ ID NO: 65);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAEPY[Nle]C_{iii} (SEQ ID NO: 66);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FA[Aad]PY[Nle]C_{iii} (SEQ ID NO: 67);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAQPY[Nle]C_{iii} (SEQ ID NO: 68);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle][Cysam]$_{iii}$ (SEQ ID NO: 69);

[MerPro]$_i$[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 70; herein referred to as BCY12353);

[MerPro]$_i$[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle][Cysam]$_{iii}$ (SEQ ID NO: 71; herein referred to as BCY12354);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 72);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 73);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 74; herein referred to as BCY12372);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAD[NMeAla]Y[Nle]C_{iii} (SEQ ID NO: 75);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAD[NMeDAla]Y[Nle]C_{iii} (SEQ ID NO: 76);

C_i[tBuAla]P[K(PYA)][dA]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 77);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 78);

C_i[tBuAla]PE[dK(Me,PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 79);

C_i[tBuAla]PE[dK(Me,PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 80); and

[MerPro]$_i$[tBuAla]EE[dK]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 81; herein referred to as BCY13137);

wherein [MerPro]$_i$, C_i, C_{ii}, C_{iii} and [Cysam]$_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, Nle represents norleucine, tBuAla represents t-butyl-alanine, PYA represents 4-pentynoic acid, Aad represents alpha-L-aminoadipic acid, MerPro represents 3-mercaptopropionic acid and Cysam represents cysteamine, NMeAla represents N-methyl-alanine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the CD137 binding bicyclic peptide ligand comprises an amino acid sequence:

C_iIEEGQYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 5);

C_i[tBuAla]PE[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 6);

C_iIEEGQYC_{ii}F[D-Ala]DPY[Nle]C_{iii} (SEQ ID NO: 7);

C_i[tBuAla]PK[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 8);

C_i[tBuAla]PE[D-Lys]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 9);

C_i[tBuAla]P[K(PYA)][D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 10);

C_i[tBuAla]PE[D-Lys(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 11);

C_iIEE[D-Lys(PYA)]QYC_{ii}FADPY(Nle)C_{iii} (SEQ ID NO: 12); and

[dC_i][dI][dE][dE][K(PYA)][dQ][dY][dC_{ii}][dF][dA][dD][dP][dY][dNle][dC_{iii}] (SEQ ID NO: 13);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, Nle represents norleucine, tBuAla represents t-butyl-alanine, PYA represents 4-pentynoic acid, or a pharmaceutically acceptable salt thereof.

In one embodiment, the bicyclic peptide ligand is other than the amino acid sequence [dC_i][dI][dE][dE][K(PYA)][dQ][dY][dC_{ii}][dF][dA][dD][dP][dY][dNle][dC_{iii}] (SEQ ID NO: 13), which has been demonstrated not to bind to CD137.

In one particular embodiment which may be mentioned, the CD137 binding bicyclic peptide ligand comprises an amino acid sequence:

C_i[tBuAla]PE[D-Lys(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 11);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, tBuAla represents t-butyl-alanine, PYA represents 4-pentynoic acid, Nle represents norleucine, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the CD137 binding bicyclic peptide ligand comprises N- and C-terminal modifications and comprises:

Ac-A-(SEQ ID NO: 5)-Dap (herein referred to as BCY7732);

Ac-A-(SEQ ID NO: 5)-Dap(PYA) (herein referred to as BCY7741);

Ac-(SEQ ID NO: 6)-Dap (herein referred to as BCY9172);

Ac-(SEQ ID NO: 6)-Dap(PYA) (herein referred to as BCY11014);

Ac-A-(SEQ ID NO: 7)-Dap (herein referred to as BCY8045);

Ac-(SEQ ID NO: 8)-A (herein referred to as BCY8919);

Ac-(SEQ ID NO: 9)-A (herein referred to as BCY8920);

Ac-(SEQ ID NO: 10)-A (herein referred to as BCY8927);

Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928);

(SEQ ID NO: 11)-A (herein referred to as BCY14601);

Ac-A-(SEQ ID NO: 12)-A (herein referred to as BCY7744);

Ac-[dA]-(SEQ ID NO: 13)-[dA]-NH_2 (herein referred to as BCY11506);

Ac-(SEQ ID NO: 60)-Dap(PYA) (herein referred to as BCY11144);

Ac-A-(SEQ ID NO: 61)-K (herein referred to as BCY11613);
Ac-(SEQ ID NO: 62)-Dap(PYA) (herein referred to as BCY12023);
Ac-(SEQ ID NO: 63) (herein referred to as BCY12149);
Ac-(SEQ ID NO: 64) (herein referred to as BCY12143);
Ac-(SEQ ID NO: 65) (herein referred to as BCY12147);
Ac-(SEQ ID NO: 66) (herein referred to as BCY12145);
Ac-(SEQ ID NO: 67) (herein referred to as BCY12146);
Ac-(SEQ ID NO: 68) (herein referred to as BCY12150);
Ac-(SEQ ID NO: 69) (herein referred to as BCY12352);
Ac-(SEQ ID NO: 72)-[1,2-diaminoethane] (herein referred to as BCY12358);
[Palmitic Acid]-[yGlu]-[yGlu]-(SEQ ID NO: 73) (herein referred to as BCY12360);
Ac-(SEQ ID NO: 75) (herein referred to as BCY12381);
Ac-(SEQ ID NO: 76) (herein referred to as BCY12382);
Ac-(SEQ ID NO: 77)-K (herein referred to as BCY12357);
Ac-(SEQ ID NO: 78)-[dA] (herein referred to as BCY13095);
[Ac]-(SEQ ID NO: 78)-K (herein referred to as BCY13389);
Ac-(SEQ ID NO: 79)-[dA] (herein referred to as BCY13096); and
Ac-(SEQ ID NO: 80) (herein referred to as BCY13097); wherein Ac represents an acetyl group, Dap represents diaminopropionic acid and PYA represents 4-pentynoic acid, or a pharmaceutically acceptable salt thereof.

In a yet further embodiment, the CD137 binding bicyclic peptide ligand comprises N- and C-terminal modifications and comprises:

Ac-A-(SEQ ID NO: 5)-Dap (herein referred to as BCY7732);
Ac-A-(SEQ ID NO: 5)-Dap(PYA) (herein referred to as BCY7741);
Ac-(SEQ ID NO: 6)-Dap (herein referred to as BCY9172);
Ac-(SEQ ID NO: 6)-Dap(PYA) (herein referred to as BCY11014);
Ac-A-(SEQ ID NO: 7)-Dap (herein referred to as BCY8045);
Ac-(SEQ ID NO: 8)-A (herein referred to as BCY8919);
Ac-(SEQ ID NO: 9)-A (herein referred to as BCY8920);
Ac-(SEQ ID NO: 10)-A (herein referred to as BCY8927);
Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928);
Ac-A-(SEQ ID NO: 12)-A (herein referred to as BCY7744); and
Ac-[dA]-(SEQ ID NO: 13)-[dA]-NH_2 (herein referred to as BCY11506);
wherein Ac represents an acetyl group, Dap represents diaminopropionic acid and PYA represents 4-pentynoic acid, or a pharmaceutically acceptable salt thereof.

In one embodiment, the bicyclic peptide ligand is other than BCY11506, which has been demonstrated not to bind to CD137.

In a further embodiment which may be mentioned, the CD137 binding bicyclic peptide ligand comprises N- and C-terminal modifications and comprises:

Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928);

wherein Ac represents an acetyl group, or a pharmaceutically acceptable salt thereof.

In an alternative embodiment, the component present on an immune cell is OX40.

The OX40 receptor (also known as Tumor necrosis factor receptor superfamily, member 4 (TNFRSF4) and also known as CD134 receptor), is a member of the TNFR-superfamily of receptors which is not constitutively expressed on resting naïve T cells, unlike CD28. OX40 is a secondary co-stimulatory immune checkpoint molecule, expressed after 24 to 72 hours following activation; its ligand, OX40L, is also not expressed on resting antigen presenting cells, but is following their activation. Expression of OX40 is dependent on full activation of the T cell; without CD28, expression of OX40 is delayed and of fourfold lower levels.

OX40 has no effect on the proliferative abilities of CD4+ cells for the first three days, however after this time proliferation begins to slow and cells die at a greater rate, due to an inability to maintain a high level of PKB activity and expression of Bcl-2, Bcl-XL and survivin. OX40L binds to OX40 receptors on T-cells, preventing them from dying and subsequently increasing cytokine production. OX40 has a critical role in the maintenance of an immune response beyond the first few days and onwards to a memory response due to its ability to enhance survival. OX40 also plays a crucial role in both Th1 and Th2 mediated reactions *in vivo*.

OX40 binds TRAF2, 3 and 5 as well as PI3K by an unknown mechanism. TRAF2 is required for survival via NF-κB and memory cell generation whereas TRAF5 seems to have a more negative or modulatory role, as knockouts have higher levels of cytokines and are more susceptible to Th2-mediated inflammation. TRAF3 may play a critical role in OX40-mediated signal transduction. CTLA-4 is down-regulated following OX40 engagement *in vivo* and the OX40-specific TRAF3 DN defect was partially overcome by CTLA-4 blockade *in vivo*. TRAF3 may be linked to OX40-mediated memory T cell expansion and survival, and point to the down-regulation of CTLA-4 as a possible control element to enhance early T cell expansion through OX40 signaling.

In one embodiment, the OX40 is mammalian OX40. In a further embodiment, the mammalian OX40 is human OX40 (hOX40).

OX40 peptides will be primarily (but not exclusively) used to agonistically activate OX40, and consequently immune cells, to prevent, suppress or treat cancer such as early or late stage human malignancies, which includes solid tumors such as Non-Small Cell Lung Carcinomas (NSCLC), breast cancers, including triple negative breast cancers (TNBC), ovarian cancers, prostate cancers, bladder cancers, urothelial carcinomas, colorectal cancers, head and neck cancer, Squamous Cell Carcinoma of the Head and Neck (SCCHN), melanomas, pancreatic cancers, and other advanced solid tumors where immune suppression blocks anti-tumor immunity. Other solid and non-solid malignancies where OX40 peptides will be used as a therapeutic agent includes, but not limited to, B-cell lymphoma including low grade/follicular non-Hodgkin's lymphoma and Acute Myeloid Leukemia (AML).

In one embodiment, the two or more second peptide ligands comprise an OX40 binding bicyclic peptide ligand.

Suitable examples of OX40 binding bicyclic peptide ligands are disclosed in International Patent Application No. PCT/GB2020/051144, the peptides of which are incorporated herein by reference.

In one embodiment, the OX40 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

$C_iILWC_{ii}LPEPHDEC_{iii}$ (SEQ ID NO: 82);
$C_iA^K/_S{}^N/_EC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 83);
$C_iAKNC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 84);
$C_iASEC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 85);
$C_i{}^L/_NYSPC_{ii}WHPLN^D/_KC_{iii}$ (SEQ ID NO: 86);
$C_iLYSPC_{ii}WHPLNDC_{iii}$ (SEQ ID NO: 87);
$C_iNYSPC_{ii}WHPLNKC_{iii}$ (SEQ ID NO: 88);
$C_iWYEYDC_{ii}NNWERC_{iii}$ (SEQ ID NO: 89);
$C_iVIRYSPC_{ii}SHYLNC_{iii}$ (SEQ ID NO: 90);
$C_iDYSPWWHPC_{ii}NHIC_{iii}$ (SEQ ID NO: 91);
$C_iDAC_{ii}LYPDYYVC_{iii}$ (SEQ ID NO: 92);
$C_iRLWC_{ii}IPAPTDDC_{iii}$ (SEQ ID NO: 93);
$C_iTMWC_{ii}IPAKGDWC_{iii}$ (SEQ ID NO: 94);
$C_iMLWC_{ii}LPAPTDEC_{iii}$ (SEQ ID NO: 95);

$C_iILWC_{ii}LPEPPDEC_{iii}$ (SEQ ID NO: 96);
$C_iLLWC_{ii}IPNPDDNC_{iii}$ (SEQ ID NO: 97);
$C_iWLWC_{ii}VPNPDDTC_{iii}$ (SEQ ID NO: 98);
$C_iVLWC_{ii}TPYPGDDC_{iii}$ (SEQ ID NO: 99);
$C_iALWC_{ii}IPDPQDEC_{iii}$ (SEQ ID NO: 100);
$C_iTLWC_{ii}IPDASDSC_{iii}$ (SEQ ID NO: 101);
$C_iQLWC_{ii}IPDADDDC_{iii}$ (SEQ ID NO: 102);
$C_iQLWC_{ii}VPEPGDSC_{iii}$ (SEQ ID NO: 103);
$C_iALWC_{ii}IPEESDDC_{iii}$ (SEQ ID NO: 104);
$C_iVLWC_{ii}IPEPQDKC_{iii}$ (SEQ ID NO: 105);
$C_iTLWC_{ii}IPDPDDSC_{iii}$ (SEQ ID NO: 106);
$C_iRLWC_{ii}VPKAEDYC_{iii}$ (SEQ ID NO: 107);
$C_iTKPC_{ii}IAYYNQSC_{iii}$ (SEQ ID NO: 108);
$C_iMNPC_{ii}IAYYQQEC_{iii}$ (SEQ ID NO: 109);
$C_iTNAC_{ii}VAYYHQAC_{iii}$ (SEQ ID NO: 110);
$C_iSDPC_{ii}ISYYNQAC_{iii}$ (SEQ ID NO: 111);
$C_iDPPC_{ii}DPFWYAFYC_{iii}$ (SEQ ID NO: 112);
$C_iPDDC_{ii}DPFWYNFYC_{iii}$ (SEQ ID NO: 113);
$C_iRYSPC_{ii}YHPHNC_{iii}$ (SEQ ID NO: 114);
$C_iLYSPC_{ii}NHPLNSC_{iii}$ (SEQ ID NO: 115);
$C_iEDNYC_{ii}FMWTPYC_{iii}$ (SEQ ID NO: 116);
$C_iLDSPC_{ii}WHPLNDC_{iii}$ (SEQ ID NO: 117);
$C_iRFSPC_{ii}SHPLNQC_{iii}$ (SEQ ID NO: 118);
$C_iKYSPC_{ii}WHPLNLC_{iii}$ (SEQ ID NO: 119);
$C_iRYSPC_{ii}WHPLNNC_{iii}$ (SEQ ID NO: 120);
$C_iEWISC_{ii}PGEPHRWWC_{iii}$ (SEQ ID NO: 121);
$C_iVWEAC_{ii}PEHPDQWWC_{iii}$ (SEQ ID NO: 122);
$C_iSTWHC_{ii}FWNLQEGKC_{iii}$ (SEQ ID NO: 123);
$C_iEWKAC_{ii}EHDRERWWC_{iii}$ (SEQ ID NO: 124);
$C_iRTWQC_{ii}FYEWQNGHC_{iii}$ (SEQ ID NO: 125);
$C_iKTWDC_{ii}FWASQVSEC_{iii}$ (SEQ ID NO: 126);
$C_iSTWQC_{ii}FYDLQEGHC_{iii}$ (SEQ ID NO: 127);
$C_iTTWEC_{ii}FYDLQEGHC_{iii}$ (SEQ ID NO: 128);
$C_iETWEC_{ii}FWRLQAGEC_{iii}$ (SEQ ID NO: 129);
$C_iRTWQC_{ii}FWDLQEGLC_{iii}$ (SEQ ID NO: 130);
$C_iSTWQC_{ii}FWDSQLGAC_{iii}$ (SEQ ID NO: 131);
$C_iETWEC_{ii}FWEWQVGSC_{iii}$ (SEQ ID NO: 132);

C_iTTWEC$_{ii}$FWDLQEGLC$_{iii}$ (SEQ ID NO: 133);
C_iHTWDC$_{ii}$FYQWQDGHC$_{iii}$ (SEQ ID NO: 134);
C_iTTWEC$_{ii}$FYSLQDGHC$_{iii}$ (SEQ ID NO: 135);
C_iNEDMYC$_{ii}$FMWMEC$_{iii}$ (SEQ ID NO: 136);
C_iLYEYDC$_{ii}$YTWRRC$_{iii}$ (SEQ ID NO: 137);
C_iRYEYDC$_{ii}$HTWQRC$_{iii}$ (SEQ ID NO: 138);
C_iWYEYDC$_{ii}$TTWERC$_{iii}$ (SEQ ID NO: 139);
C_iWYEYDC$_{ii}$RTWTRC$_{iii}$ (SEQ ID NO: 140);
C_iLYEYDC$_{ii}$HTWTRC$_{iii}$ (SEQ ID NO: 141);
C_iWYEYDC$_{ii}$RTWTFC$_{iii}$ (SEQ ID NO: 142);
C_iHGGVWC$_{ii}$IPNINDSC$_{iii}$ (SEQ ID NO: 143);
C_iDSPVRC$_{ii}$YWNTQKGC$_{iii}$ (SEQ ID NO: 144);
C_iGSPVPC$_{ii}$YWNTRKGC$_{iii}$ (SEQ ID NO: 145);
C_iAPFEFNC$_{ii}$YTWRPC$_{iii}$ (SEQ ID NO: 146);
C_iRVLYSPC$_{ii}$YHWLNC$_{iii}$ (SEQ ID NO: 147);
C_iSIMYSPC$_{ii}$EHPHNHC$_{iii}$ (SEQ ID NO: 148);
C_iDKWEPDHLC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 149);
C_iDAWPETHVC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 150);
C_iDEYTPEHLC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 151);
C_iWINYSISPC$_{ii}$YVGEC$_{iii}$ (SEQ ID NO: 152); and
C_iRYEYPEHLC$_{ii}$YTWQC$_{iii}$ (SEQ ID NO: 153);

such as:

C_iLYSPC$_{ii}$WHPLNDC$_{iii}$ (SEQ ID NO: 87);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, or a modified derivative, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the OX40 binding bicyclic peptide ligand additionally comprises N- and/or C-terminal modifications and comprises an amino acid sequence selected from:

A-(SEQ ID NO: 82)-A-[Sar6]-[KBiot] (herein referred to as BCY10551);
A-(SEQ ID NO: 82)-A (herein referred to as BCY10371);
A-(SEQ ID NO: 84)-A-[Sar6]-[KBiot] (herein referred to as BCY10552);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 84)-A (herein referred to as BCY10479);
A-(SEQ ID NO: 84)-A (herein referred to as BCY10378);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 85)-A (herein referred to as BCY11371);
A-(SEQ ID NO: 85)-A (herein referred to as BCY10743);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 87)-A (herein referred to as BCY10482);
A-(SEQ ID NO: 87)-A-[Sar6]-[KBiot] (herein referred to as BCY10549);

A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY11607);
Ac-A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY12708);
A-(SEQ ID NO: 87)-A (herein referred to as BCY10351);
A-(SEQ ID NO: 88)-A-[Sar6]-[KBiot] (herein referred to as BCY11501);
A-(SEQ ID NO: 88)-A (herein referred to as BCY10729);
A-(SEQ ID NO: 89)-A-[Sar6]-[KBiot] (herein referred to as BCY10550);
A-(SEQ ID NO: 89)-A (herein referred to as BCY10361);
A-(SEQ ID NO: 90)-A-[Sar6]-[KBiot] (herein referred to as BCY10794);
A-(SEQ ID NO: 90)-A (herein referred to as BCY10349);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 91)-A (herein referred to as BCY11369);
A-(SEQ ID NO: 91)-A (herein referred to as BCY10331);
A-(SEQ ID NO: 92)-A (herein referred to as BCY10375);
A-(SEQ ID NO: 93)-A (herein referred to as BCY10364);
A-(SEQ ID NO: 94)-A (herein referred to as BCY10365);
A-(SEQ ID NO: 95)-A (herein referred to as BCY10366);
A-(SEQ ID NO: 96)-A (herein referred to as BCY10367);
A-(SEQ ID NO: 97)-A (herein referred to as BCY10368);
A-(SEQ ID NO: 98)-A (herein referred to as BCY10369);
A-(SEQ ID NO: 99)-A (herein referred to as BCY10374);
A-(SEQ ID NO: 100)-A (herein referred to as BCY10376);
A-(SEQ ID NO: 101)-A (herein referred to as BCY10737);
A-(SEQ ID NO: 102)-A (herein referred to as BCY10738);
A-(SEQ ID NO: 103)-A (herein referred to as BCY10739);
A-(SEQ ID NO: 104)-A (herein referred to as BCY10740);
A-(SEQ ID NO: 105)-A (herein referred to as BCY10741);
A-(SEQ ID NO: 106)-A (herein referred to as BCY10742);
A-(SEQ ID NO: 107)-A (herein referred to as BCY10380);
A-(SEQ ID NO: 108)-A (herein referred to as BCY10370);
A-(SEQ ID NO: 109)-A (herein referred to as BCY10372);
A-(SEQ ID NO: 110)-A (herein referred to as BCY10373);
A-(SEQ ID NO: 111)-A (herein referred to as BCY10379);
A-(SEQ ID NO: 112)-A (herein referred to as BCY10377);
A-(SEQ ID NO: 113)-A (herein referred to as BCY10744);
A-(SEQ ID NO: 114)-A (herein referred to as BCY10343);
A-(SEQ ID NO: 115)-A (herein referred to as BCY10350);
A-(SEQ ID NO: 116)-A (herein referred to as BCY10352);
A-(SEQ ID NO: 117)-A (herein referred to as BCY10353);

A-(SEQ ID NO: 118)-A (herein referred to as BCY10354);
A-(SEQ ID NO: 119)-A (herein referred to as BCY10730);
A-(SEQ ID NO: 120)-A (herein referred to as BCY10731);
A-(SEQ ID NO: 121)-A (herein referred to as BCY10339);
A-(SEQ ID NO: 122)-A (herein referred to as BCY10340);
A-(SEQ ID NO: 123)-A (herein referred to as BCY10342);
A-(SEQ ID NO: 124)-A (herein referred to as BCY10345);
A-(SEQ ID NO: 125)-A (herein referred to as BCY10347);
A-(SEQ ID NO: 126)-A (herein referred to as BCY10348);
A-(SEQ ID NO: 127)-A (herein referred to as BCY10720);
A-(SEQ ID NO: 128)-A (herein referred to as BCY10721);
A-(SEQ ID NO: 129)-A (herein referred to as BCY10722);
A-(SEQ ID NO: 130)-A (herein referred to as BCY10723);
A-(SEQ ID NO: 131)-A (herein referred to as BCY10724);
A-(SEQ ID NO: 132)-A (herein referred to as BCY10725);
A-(SEQ ID NO: 133)-A (herein referred to as BCY10726);
A-(SEQ ID NO: 134)-A (herein referred to as BCY10727);
A-(SEQ ID NO: 135)-A (herein referred to as BCY10728);
A-(SEQ ID NO: 136)-A (herein referred to as BCY10360);
A-(SEQ ID NO: 137)-A (herein referred to as BCY10363);
A-(SEQ ID NO: 138)-A (herein referred to as BCY10732);
A-(SEQ ID NO: 139)-A (herein referred to as BCY10733);
A-(SEQ ID NO: 140)-A (herein referred to as BCY10734);
A-(SEQ ID NO: 141)-A (herein referred to as BCY10735);
A-(SEQ ID NO: 142)-A (herein referred to as BCY10736);
A-(SEQ ID NO: 143)-A (herein referred to as BCY10336);
A-(SEQ ID NO: 144)-A (herein referred to as BCY10337);
A-(SEQ ID NO: 145)-A (herein referred to as BCY10338);
A-(SEQ ID NO: 146)-A (herein referred to as BCY10346);
A-(SEQ ID NO: 147)-A (herein referred to as BCY10357);
A-(SEQ ID NO: 148)-A (herein referred to as BCY10362);
A-(SEQ ID NO: 149)-A (herein referred to as BCY10332);
A-(SEQ ID NO: 150)-A (herein referred to as BCY10717);
A-(SEQ ID NO: 151)-A (herein referred to as BCY10718);
A-(SEQ ID NO: 152)-A (herein referred to as BCY10334); and
A-(SEQ ID NO: 153)-A (herein referred to as BCY10719);

such as:

A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY11607);
wherein Pya represents 4-pentynoyl moiety.

In one embodiment, the two or more second peptides are specific for the same immune cell. In a further embodiment, each of said two or more second peptides are specific for the same binding site or target on the same immune cell. In an alternative embodiment, each of said two or more second peptides are specific for a different binding site or target on the same immune cell. In an alternative embodiment, the two or more second peptides are specific for two differing immune cells (i.e. CD137 and OX40). In a further embodiment, each of said two or more second peptides are specific for the same binding site or target on two differing immune cells. In an alternative embodiment, each of said two or more second peptides are specific for a different binding site or target on two differing immune cells.

In one embodiment, each of said two or more second peptides has the same peptide sequence.

In one embodiment, said heterotandem bicyclic peptide complex comprises two second peptide ligands. Thus, according to a further aspect of the invention, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

According to a further aspect of the invention which may be mentioned, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three cysteine residues, separated by at least two loop sequences, and a molecular scaffold which forms

covalent bonds with the cysteine residues of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

In an alternative embodiment, said heterotandem bicyclic peptide complex comprises three second peptide ligands. Thus, according to a further aspect of the invention, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) three second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

According to a further aspect of the invention which may be mentioned, there is provided a heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) three second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three cysteine residues, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the cysteine residues of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

In a further embodiment, each of said two or more second peptides has the same peptide sequence and said peptide sequence comprises Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928), wherein Ac represents an acetyl group, or a pharmaceutically acceptable salt thereof.

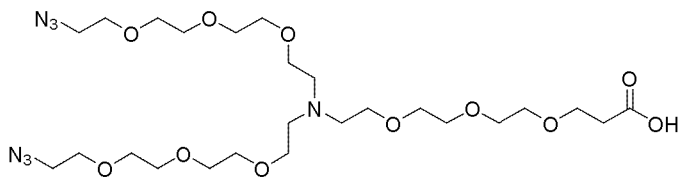
In a yet further embodiment, said heterotandem bicyclic peptide complex comprises two second peptide ligands and both of said two second peptides have the same peptide sequence which comprises Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928), wherein Ac represents an acetyl group, or a pharmaceutically acceptable salt thereof.

Linkers

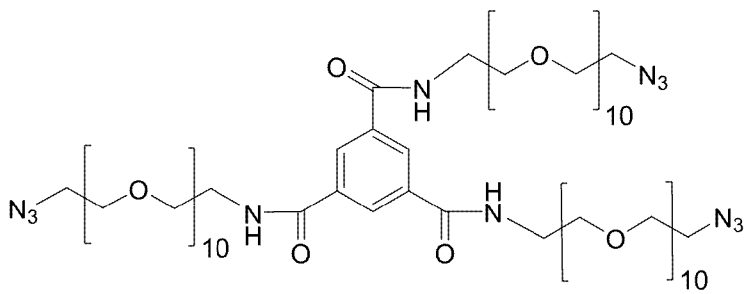
It will be appreciated that the first peptide ligand may be conjugated to the two or more second peptide ligands via any suitable linker. Typically, the design of said linker will be such that the three Bicyclic peptides are presented in such a manner that they can bind unencumbered to their respective targets either alone or while simultaneously binding to both target receptors. Additionally, the linker should permit binding to both targets simultaneously while maintaining an appropriate distance between the target cells that would lead to the desired functional outcome. The properties of the linker may be modulated to increase length, rigidity or solubility to optimise the desired functional outcome. The linker may also be designed to permit the attachment of more than one Bicycle to the same target. Increasing the valency of either binding peptide may serve to increase the affinity of the heterotandem for the target cells or may help to induce oligomerisation of one or both of the target receptors.

In one embodiment, the linker is a branched linker to allow one first peptide at one end and the two or more second peptides at the other end.

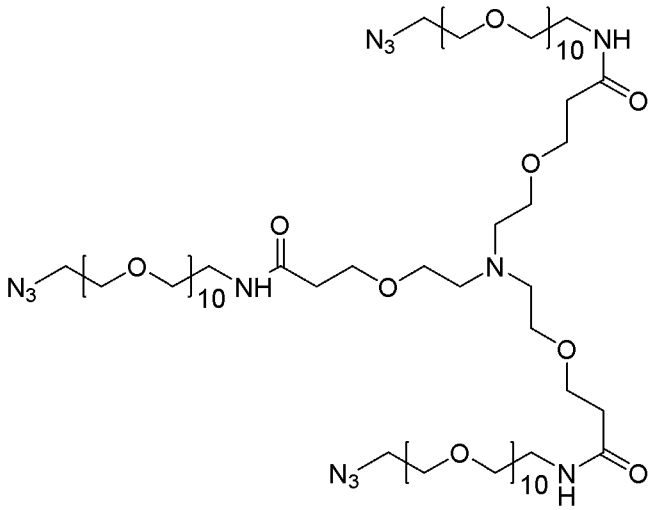
In a further embodiment, the branched linker is selected from:



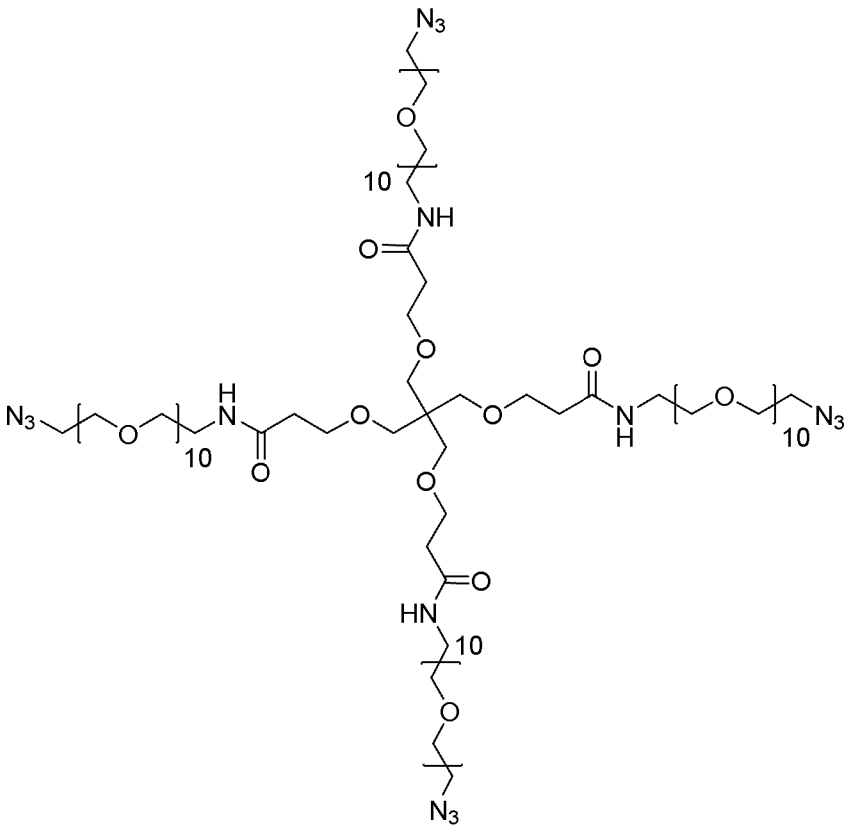
N-(acid-PEG$_3$)-N-bis(PEG$_3$-azide);



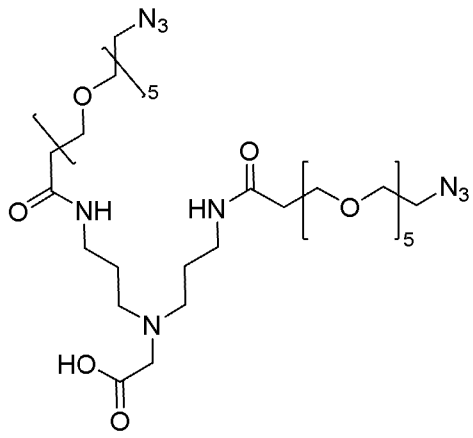
Trimesic-[Peg$_{10}$]$_3$;



TCA-[Peg$_{10}$]$_3$;

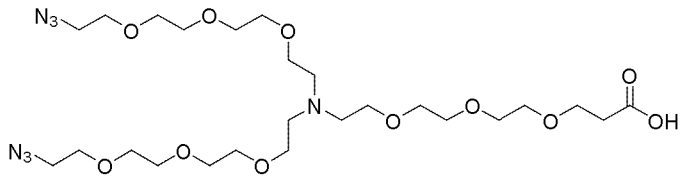


Tet-[Peg$_{10}$]$_4$; and



BAPG-$(Peg_5)_2$.

In on particular embodiment, the branched linker is:



N-(acid-PEG_3)-N-bis(PEG_3-azide).

Heterotandem Complexes

In one specific embodiment, the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand attached to a TATA scaffold, the two or more second peptide ligands comprise two CD137 binding bicyclic peptide ligands attached to a TATA scaffold and said heterotandem complex is selected from the complexes listed in Table A:

Table A (Nectin-4 : CD137; 1:2)

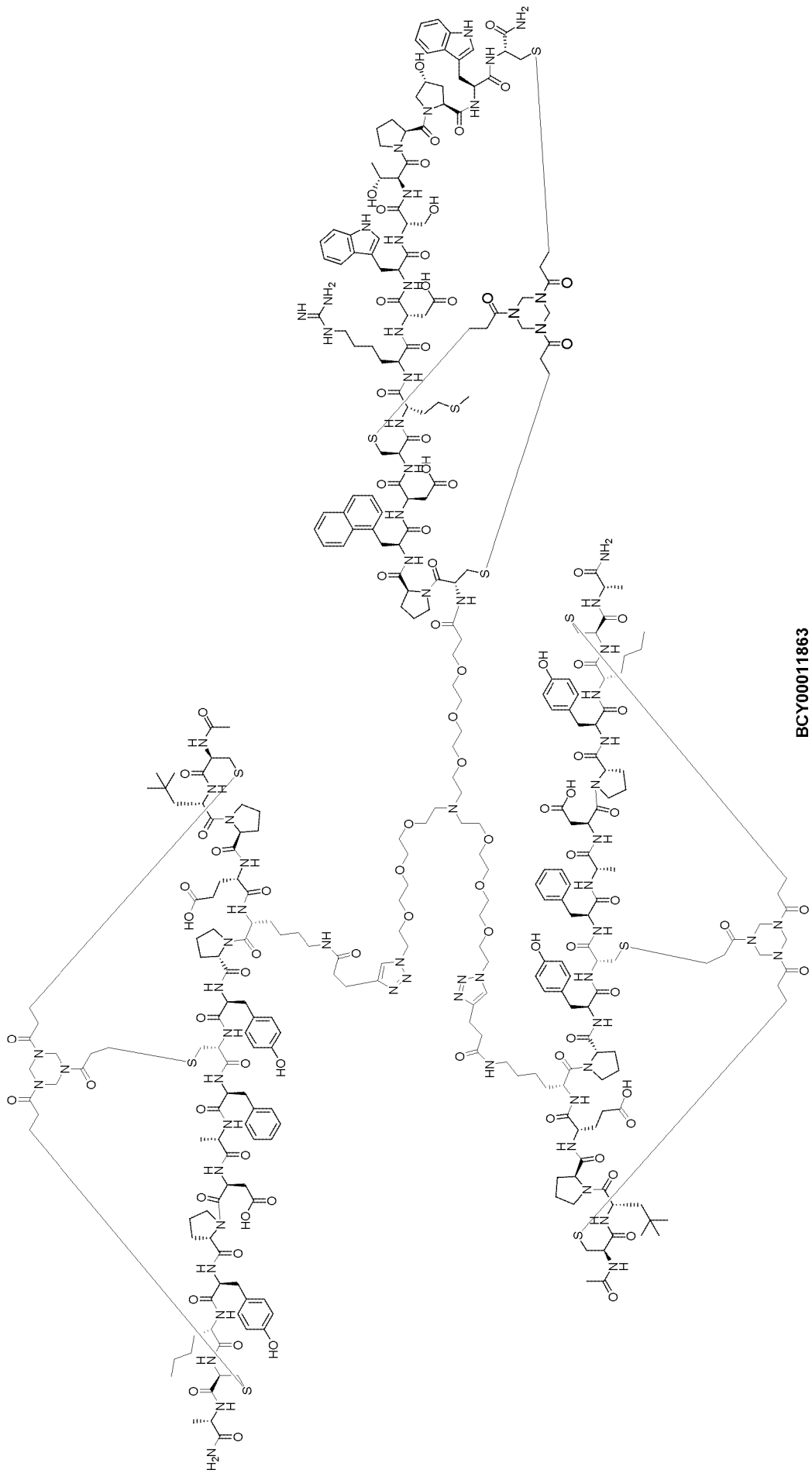
Complex No.	***Nectin-4 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***
BCY11863	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12484	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12143	dLys(PYA)4
BCY10918	BCY11015	N-term PYA	Trimesic-$[Peg_{10}]_3$	BCY8928	dLys(PYA)4

BCY10919	BCY11015	N-term PYA	Trimesic-$[Peg_{10}]_3$	BCY11014	C-term Dap(PYA)
BCY11027	BCY11015	N-term PYA	TCA-$[Peg_{10}]_3$	BCY8928	dLys(PYA)4
BCY11385	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY11014	C-term Dap(PYA)
BCY11864	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY7744	dLys(PYA)4
BCY12485	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12149	dLys(PYA)4
BCY12486	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12147	dLys(PYA)4
BCY12586	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12352	dLys(PYA)4
BCY12487	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12145	dLys(PYA)4
BCY12490	BCY12024	dLys3	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys(PYA)4
BCY12587	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12353	dLys(PYA)4
BCY12588	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12354	dLys(PYA)4
BCY12589	BCY12371	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys(PYA)4
BCY12590	BCY12384	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys(PYA)4

BCY12760	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12381	dLys(PYA)4
BCY12761	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12382	dLys(PYA)4
BCY13390	BCY8116	N terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928 BCY13389	dLys(PYA)4 dLys(PYA)4
BCY14602	BCY8116	N terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY14601	dLys(PYA)4
BCY15155	BCY8116	N terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY14601 BCY8928	dLys(PYA)4 dLys(PYA)4

In one embodiment, the heterotandem bicyclic peptide complex is selected from: BCY11027, BCY11863 and BCY11864. In a further embodiment, the heterotandem bicyclic peptide complex is selected from: BCY11863 and BCY11864.

The heterotandem bicyclic peptide complex BCY11863 consists of a Nectin-4 specific peptide BCY8116 linked to two CD137 specific peptides (both of which are BCY8928) via a N-(acid-PEG_3)-N-bis(PEG_3-azide) linker, shown pictorially as:

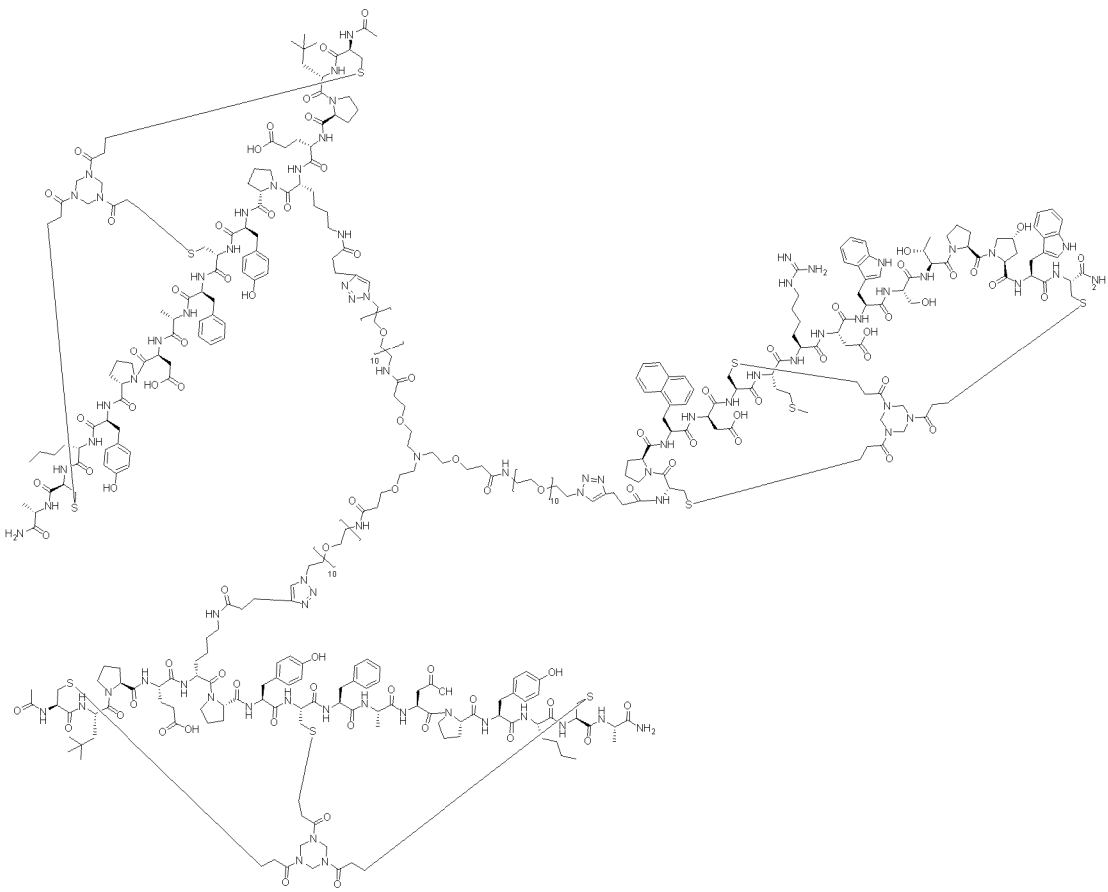


BCY00011863

BIC-C-P2610PCT

CD137 is a homotrimeric protein and the natural ligand CD137L exists as a homotrimer either expressed on immune cells or secreted. The biology of CD137 is highly dependent on multimerization to induce CD137 activity in immune cells. One way to generate CD137 multimerization is through cellular cross-linking of the CD137 specific agonist through interaction with a specific receptor present on another cell. The advantage of the heterotandem complexes of the present invention is that the presence of two or more peptide ligands specific for an immune cell component, such as CD137, provides a more effective clustering of CD137. For example, data is presented herein in Figure 1 and Table 1 which shows that BCY11863 demonstrated strong CD137 activation in a CD137 reporter assay. In addition, data is presented herein in Figure 2 and Table 5 which shows that BCY11863 induces robust IL-2 and IFN-γ cytokine secretion in a PBMC-4T1 co-culture assay. Furthermore, data is presented herein in Figure 3 and Table 7 which shows that BCY11863 demonstrated an excellent PK profile with a terminal half-life of 4.1 hours in SD Rats and 5.3 hours in cyno.

The heterotandem bicyclic peptide complex BCY11027 consists of a Nectin-4 specific peptide BCY11015 linked to two CD137 specific peptides (both of which are BCY8928) via a TCA-[Peg_{10}]$_3$ linker, shown pictorially as:



Data shown in Figure 13 demonstrates that Nectin-4/CD137 heterotandem BCY11027 induces target dependent cytokine release in *ex vivo* cultures of primary patient-derived lung tumors. Treatment with BCY11027 induced Nectin-4 dependent change in several immune markers (normalized to vehicle) and in %CD8 +ki67+ T cells in patient-derived samples that correlated with the level of Nectin-4 expression.

In an alternative specific embodiment, the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand attached to a TATA scaffold, the two or more second peptide ligands comprise three CD137 binding bicyclic peptide ligands attached to a TATA scaffold and said heterotandem complex is selected from the complexes listed in Table B:

Table B (Nectin-4 : CD137; 1:3)

Complex No.	***Nectin-4 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***
BCY11021	BCY11016	N-term PYA	Tet-[Peg_{10}]$_4$	BCY7744	dLys(PYA)4
BCY11022	BCY11016	N-term PYA	Tet-[Peg_{10}]$_4$	BCY8928	dLys(PYA)4

In one specific embodiment, the first peptide ligand comprises an EphA2 binding bicyclic peptide ligand attached to a TATA scaffold, the two or more second peptide ligands comprise two CD137 binding bicyclic peptide ligands attached to a TATA scaffold and said heterotandem complex is selected from the complexes listed in Table C:

Table C (EphA2 : CD137; 1:2)

Complex No.	***EphA2 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***
BCY12491	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12723	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12143	dLys (PYA)4
BCY12724	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12149	dLys (PYA)4

BCY12725	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12147	dLys (PYA)4
BCY12726	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12145	dLys (PYA)4
BCY12728	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12150	dLys (PYA)4
BCY12729	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12352	dLys (PYA)4
BCY12730	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12353	dLys (PYA)4
BCY12731	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12354	dLys (PYA)4
BCY12732	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12360	dLys (PYA)4
BCY12973	BCY12734	C-term Lys	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12974	BCY12735	Lys8	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12975	BCY12736	Lys2	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12976	BCY12737	Lys7	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12977	BCY12738	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4

BCY12978	BCY12739	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY12979	BCY9594	N-terminus	BAPG-$(Peg_5)_2$	BCY8928	dLys (PYA)4
BCY13042	BCY12854	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13043	BCY12855	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13044	BCY12856	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13045	BCY12857	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13046	BCY12858	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13047	BCY12859	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13048	BCY12860	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13049	BCY12861	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13050	BCY12862	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13051	BCY12863	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4

BCY13052	BCY12864	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13053	BCY12865	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13054	BCY12866	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13138	BCY12856	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12353	dLys (PYA)4
BCY13139	BCY9594	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY13137	dLys (PYA)4
BCY13140	BCY12856	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY13137	dLys (PYA)4
BCY13270	BCY13116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13271	BCY13117	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13272	BCY13118	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13273	BCY13119	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13274	BCY13120	C-term dLys	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13275	BCY13121	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4

BCY13276	BCY13122	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13277	BCY13123	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13278	BCY13124	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13280	BCY13126	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13281	BCY13127	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13282	BCY13128	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13284	BCY13130	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13285	BCY13131	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13286	BCY13132	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13288	BCY13134	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13289	BCY13135	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys (PYA)4
BCY13341	BCY12865	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12353	dLys (PYA)4

BCY13343	BCY12860	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12353	dLys (PYA)4
BCY13279	BCY13125	C-term dLys	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY13283	BCY13129	C-term dLys	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY13287	BCY13133	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14049	BCY13917	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14050	BCY13918	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14051	BCY13919	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14052	BCY13920	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14053	BCY13922	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4

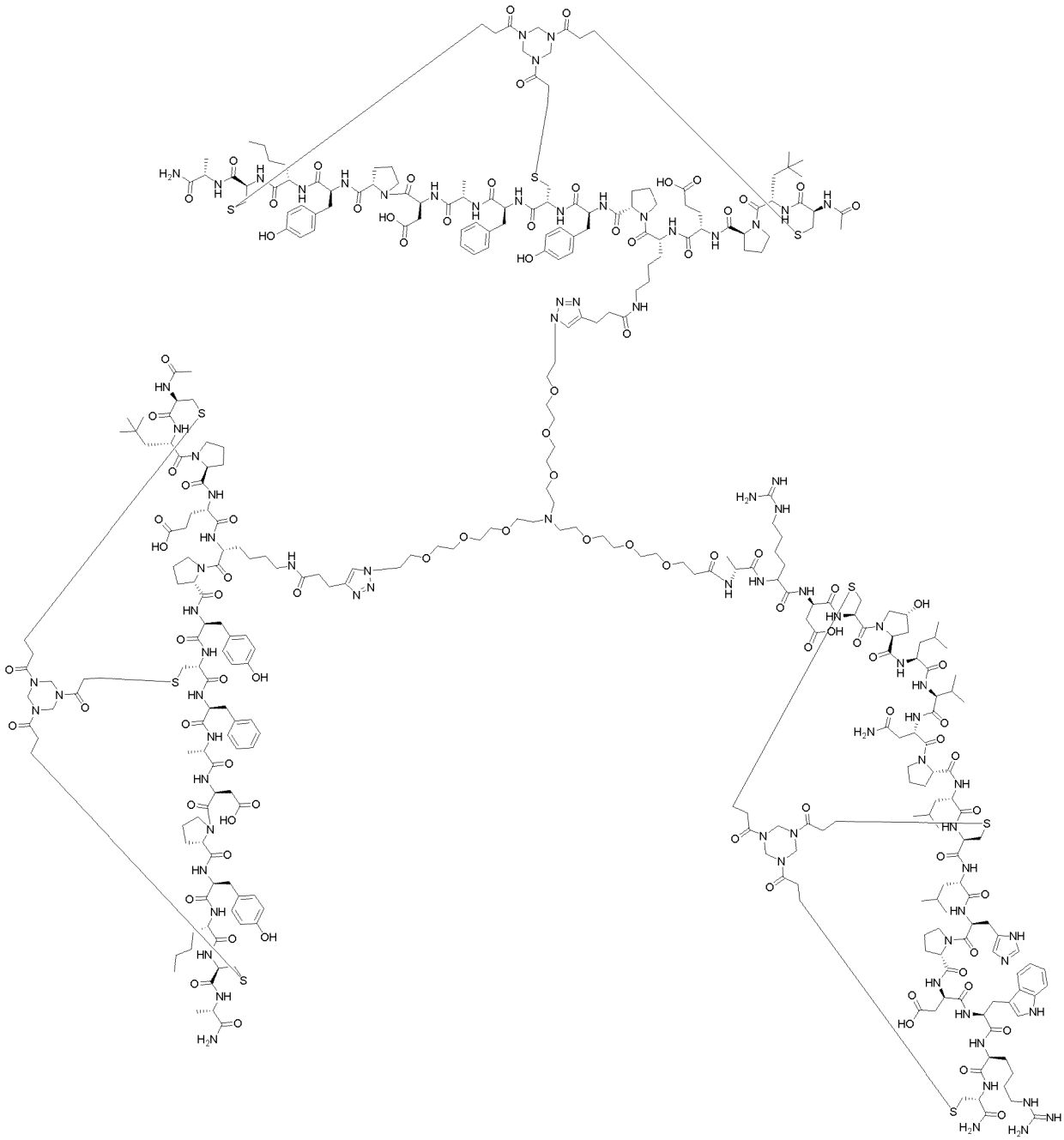
BCY14054	BCY13923	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14055	BCY14047	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14056	BCY14048	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14334	BCY14313	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14335	BCY14327	Lys 8	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928	dLys (PYA)4
BCY14413	BCY9594	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys (PYA)4 dLys (PYA)4
BCY14414	BCY13118	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys(PYA)4 dLys(PYA)4
BCY15217	BCY13118	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY14601 BCY14601	dLys(PYA)4 dLys(PYA)4
BCY15218	BCY13118	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY14601	dLys(PYA)4 dLys(PYA)4

In one embodiment, the heterotandem bicyclic peptide complex is selected from: BCY12491, BCY12730, BCY13048, BCY13050, BCY13053 and BCY13272.

In one embodiment, the heterotandem bicyclic peptide complex is selected from: BCY12491, BCY12730, BCY13048, BCY13050 and BCY13053.

In a further embodiment, the heterotandem bicyclic peptide complex is BCY12491.

The heterotandem bicyclic peptide complex BCY12491 consists of a EphA2 specific peptide BCY9594 linked to two CD137 specific peptides (both of which are BCY8928) via a N-(acid-PEG_3)-N-bis(PEG_3-azide) linker, shown pictorially as:

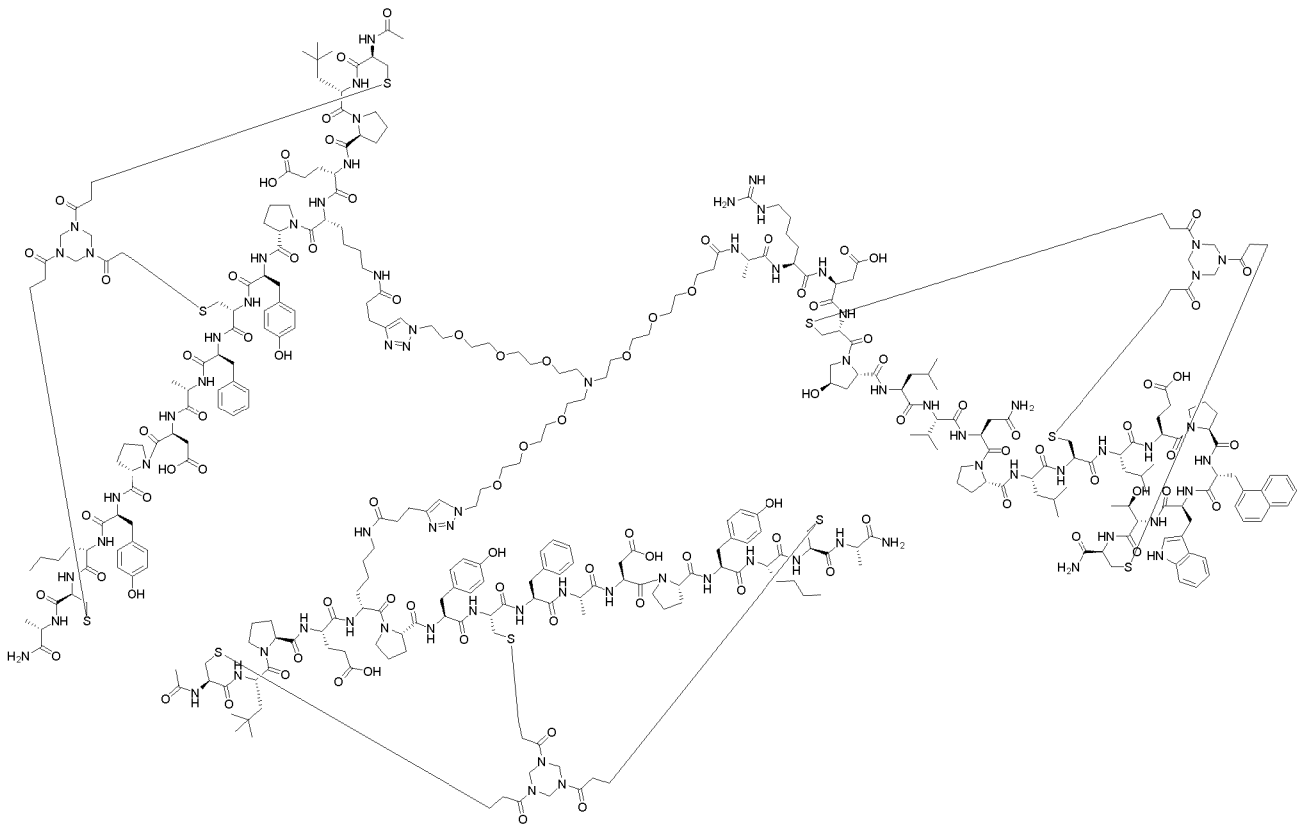


BCY12491

Data is presented here in Figure 9 and Figure 15 which demonstrates that BCY12491 leads to a significant anti-tumor response and modulation (increase) of the tumor infiltrating immune cells and immune response.

In an alternative embodiment, the heterotandem bicyclic peptide complex is BCY13272.

The heterotandem bicyclic peptide complex BCY13272 consists of a EphA2 specific peptide BCY13118 linked to two CD137 specific peptides (both of which are BCY8928) via a N-(acid-PEG_3)-N-bis(PEG_3-azide) linker, shown pictorially as:



BCY13272

Data is presented here in Figure 18 which demonstrates that BCY13272 leads to a significant anti tumor effect in a MC38 tumor model in mice.

In one specific embodiment, the first peptide ligand comprises a PD-L1 binding bicyclic peptide ligand attached to a TATA scaffold, the two or more second peptide ligands comprise two CD137 binding bicyclic peptide ligands attached to a TATA scaffold and said heterotandem complex is selected from the complexes listed in Table D:

Table D (PD-L1 : CD137; 1:2)

Complex No.	***PD-L1 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***
BCY11780	BCY10861	Lys(PYA)9	TCA-[Peg_{10}]$_3$	BCY8928	dLys4

BCY12662	BCY12479	C-term Lys	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys(PYA)4
BCY12722	BCY12477	C-term Lys	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928	dLys(PYA)4

In one specific embodiment, the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand attached to a TATA scaffold, the two or more second peptide ligands comprise two OX40 binding bicyclic peptide ligands attached to a TATA scaffold and said heterotandem complex is the complex listed in Table E:

Table E (Nectin-4 : OX40; 1:2)

Complex No.	***Nectin-4 BCY No.***	***Attachment Point***	***Linker***	***OX40 BCY No.***	***Attachment Point***
BCY12967	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BC11607	C-term Lys(PYA)

In one specific embodiment, the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand attached to a TATA scaffold, one of the two or more second peptide ligands comprises an OX40 binding bicyclic peptide ligand attached to a TATA scaffold and the other of the two or more second peptide ligands comprises a CD137 binding bicyclic peptide ligand attached to a TATA scaffold and said heterotandem complex is the complex listed in Table F:

Table F (Nectin-4 : OX40 : CD137; 1:1:1)

Complex No.	***Nectin-4 BCY No.***	***Attachment Point***	***Linker***	***OX40 BCY No.***	***Attachment Point***	***CD137 BCY No.***	***Attachment Point***
BCY12733	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY12708	C-term dLys	BCY7744	dLys (PYA)4

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art, such as in the arts of

peptide chemistry, cell culture and phage display, nucleic acid chemistry and biochemistry. Standard techniques are used for molecular biology, genetic and biochemical methods (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel *et al.*, Short Protocols in Molecular Biology (1999) 4th ed., John Wiley & Sons, Inc.), which are incorporated herein by reference.

Nomenclature

Numbering

When referring to amino acid residue positions within compounds of the invention, cysteine residues (C_i, C_{ii} and C_{iii}) are omitted from the numbering as they are invariant, therefore, the numbering of amino acid residues within SEQ ID NO: 1 is referred to as below:

C_i-P_1-1Nal$_2$-dD_3-C_{ii}-M_4-HArg$_5$-D_6-W_7-S_8-T_9-P_{10}-HyP_{11}-W_{12}-C_{iii} (SEQ ID NO: 1).

For the purpose of this description, all bicyclic peptides are assumed to be cyclised with TBMB (1,3,5-tris(bromomethyl)benzene) or 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and yielding a tri-substituted structure. Cyclisation with TBMB and TATA occurs on C_i, C_{ii}, and C_{iii}.

Molecular Format

N- or C-terminal extensions to the bicycle core sequence are added to the left or right side of the sequence, separated by a hyphen. For example, an N-terminal βAla-Sar10-Ala tail would be denoted as:

βAla-Sar10-A-(SEQ ID NO: X).

Inversed Peptide Sequences

In light of the disclosure in Nair *et al* (2003) J Immunol 170(3), 1362-1373, it is envisaged that the peptide sequences disclosed herein would also find utility in their retro-inverso form. For example, the sequence is reversed (i.e. N-terminus becomes C-terminus and *vice versa*) and their stereochemistry is likewise also reversed (i.e. D-amino acids become L-amino acids and *vice versa*). For the avoidance of doubt, references to amino acids either as their full name or as their amino acid single or three letter codes are intended to be represented herein as L-amino acids unless otherwise stated. If such an amino acid is intended to be represented as a D-amino acid then the amino acid will be prefaced with a lower case d within square parentheses, for example [dA], [dD], [dE], [dK], [d1Nal], [dNle], etc.

Advantages of the Peptide Ligands

Certain heterotandem bicyclic peptide complexes of the present invention have a number of advantageous properties which enable them to be considered as suitable drug-like molecules for injection, inhalation, nasal, ocular, oral or topical administration. Such advantageous properties include:

- Species cross-reactivity. This is a typical requirement for preclinical pharmacodynamics and pharmacokinetic evaluation;

- Protease stability. Heterotandem bicyclic peptide complexes should ideally demonstrate stability to plasma proteases, epithelial ("membrane-anchored") proteases, gastric and intestinal proteases, lung surface proteases, intracellular proteases and the like. Protease stability should be maintained between different species such that a heterotandem bicyclic peptide lead candidate can be developed in animal models as well as administered with confidence to humans;

- Desirable solubility profile. This is a function of the proportion of charged and hydrophilic versus hydrophobic residues and intra/inter-molecular H-bonding, which is important for formulation and absorption purposes;

- Selectivity. Certain heterotandem bicyclic peptide complexes of the invention demonstrate good selectivity over other targets;

- An optimal plasma half-life in the circulation. Depending upon the clinical indication and treatment regimen, it may be required to develop a heterotandem bicyclic peptide complex for short exposure in an acute illness management setting, or develop a heterotandem bicyclic peptide complex with enhanced retention in the circulation, and is therefore optimal for the management of more chronic disease states. Other factors driving the desirable plasma half-life are requirements of sustained exposure for maximal therapeutic efficiency versus the accompanying toxicology due to sustained exposure of the agent.

Crucially, data is presented herein where selected heterotandem bicyclic peptide complexes demonstrate anti-tumor efficacy when dosed at a frequency that does not maintain plasma concentrations above the *in vitro* EC_{50} of the compound. This is in contrast to larger recombinant biologic (i.e. antibody based) approaches to CD137 agonism or bispecific CD137 agonism (Segal *et al.*, Clin Cancer Res., 23(8):1929-1936

(2017), Claus *et al.*, Sci Trans Med., 11(496): eaav5989, 1-12 (2019), Hinner *et al.*, Clin Cancer Res., 25(19):5878-5889 (2019)). Without being bound by theory, the reason for this observation is thought to be due to the fact that heterotandem bicycle complexes have relatively low molecular weight (typically <15 kDa), they are fully synthetic and they are tumor targeted agonists of CD137. As such, they have relatively short plasma half lives but good tumor penetrance and retention. Data is presented herein which fully supports these advantages. For example, anti-tumor efficacy in syngeneic rodent models in mice with humanized CD137 is demonstrated either daily or every 3rd day. In addition, intraperitoneal pharmacokinetic data shows that the plasma half life is <3 hours, which would predict that the circulating concentration of the complex would consistently drop below the *in vitro* EC_{50} between doses. Furthermore, tumor pharmacokinetic data shows that levels of heterotandem bicycle complex in tumor tissue may be higher and more sustained as compared to plasma levels.

It will be appreciated that this observation forms an important further aspect of the invention. Thus, according to a further aspect of the invention, there is provided a method of treating cancer which comprises administration of a heterotandem bicyclic peptide complex as defined herein at a dosage frequency which does not sustain plasma concentrations of said complex above the *in vitro* EC_{50} of said complex.

- Immune Memory. Coupling the cancer cell binding bicyclic peptide ligand with the immune cell binding bicyclic peptide ligand provides the synergistic advantage of immune memory. Data is presented herein which demonstrates that selected heterotandem bicyclic peptide complexes of the invention not only eradicate tumors but upon readministration of the tumorigenic agent, none of the inoculated complete responder mice developed tumors (see Figure 5). This indicates that treatment with the selected heterotandem bicyclic peptide complexes of the invention has induced immunogenic memory in the complete responder mice. This has a significant clinical advantage in order to prevent recurrence of said tumor once it has been initially controlled and eradicated.

Peptide Ligands

A peptide ligand, as referred to herein, refers to a peptide covalently bound to a molecular scaffold. Typically, such peptides comprise two or more reactive groups (i.e. cysteine residues) which are capable of forming covalent bonds to the scaffold, and a sequence subtended between said reactive groups which is referred to as the loop sequence, since it forms a loop when the peptide is bound to the scaffold. In the present case, the peptides

comprise at least three reactive groups selected from cysteine, 3-mercaptopropionic acid and/or cysteamine and form at least two loops on the scaffold.

Reactive Groups

The molecular scaffold of the invention may be bonded to the polypeptide via functional or reactive groups on the polypeptide. These are typically formed from the side chains of particular amino acids found in the polypeptide polymer. Such reactive groups may be a cysteine side chain, a lysine side chain, or an N-terminal amine group or any other suitable reactive group, such as penicillamine. Details of suitable reactive groups may be found in WO 2009/098450.

Examples of reactive groups of natural amino acids are the thiol group of cysteine, the amino group of lysine, the carboxyl group of aspartate or glutamate, the guanidinium group of arginine, the phenolic group of tyrosine or the hydroxyl group of serine. Non-natural amino acids can provide a wide range of reactive groups including an azide, a keto-carbonyl, an alkyne, a vinyl, or an aryl halide group. The amino and carboxyl group of the termini of the polypeptide can also serve as reactive groups to form covalent bonds to a molecular scaffold/molecular core.

The polypeptides of the invention contain at least three reactive groups. Said polypeptides can also contain four or more reactive groups. The more reactive groups are used, the more loops can be formed in the molecular scaffold.

In a preferred embodiment, polypeptides with three reactive groups are generated. Reaction of said polypeptides with a molecular scaffold/molecular core having a three-fold rotational symmetry generates a single product isomer. The generation of a single product isomer is favourable for several reasons. The nucleic acids of the compound libraries encode only the primary sequences of the polypeptide but not the isomeric state of the molecules that are formed upon reaction of the polypeptide with the molecular core. If only one product isomer can be formed, the assignment of the nucleic acid to the product isomer is clearly defined. If multiple product isomers are formed, the nucleic acid cannot give information about the nature of the product isomer that was isolated in a screening or selection process. The formation of a single product isomer is also advantageous if a specific member of a library of the invention is synthesized. In this case, the chemical reaction of the polypeptide with the molecular scaffold yields a single product isomer rather than a mixture of isomers.

In another embodiment, polypeptides with four reactive groups are generated. Reaction of said polypeptides with a molecular scaffold/molecular core having a tetrahedral symmetry generates two product isomers. Even though the two different product isomers are encoded by one and the same nucleic acid, the isomeric nature of the isolated isomer can be determined by chemically synthesizing both isomers, separating the two isomers and testing both isomers for binding to a target ligand.

In one embodiment of the invention, at least one of the reactive groups of the polypeptides is orthogonal to the remaining reactive groups. The use of orthogonal reactive groups allows the directing of said orthogonal reactive groups to specific sites of the molecular core. Linking strategies involving orthogonal reactive groups may be used to limit the number of product isomers formed. In other words, by choosing distinct or different reactive groups for one or more of the at least three bonds to those chosen for the remainder of the at least three bonds, a particular order of bonding or directing of specific reactive groups of the polypeptide to specific positions on the molecular scaffold may be usefully achieved.

In another embodiment, the reactive groups of the polypeptide of the invention are reacted with molecular linkers wherein said linkers are capable to react with a molecular scaffold so that the linker will intervene between the molecular scaffold and the polypeptide in the final bonded state.

In some embodiments, amino acids of the members of the libraries or sets of polypeptides can be replaced by any natural or non-natural amino acid. Excluded from these exchangeable amino acids are the ones harbouring functional groups for cross-linking the polypeptides to a molecular core, such that the loop sequences alone are exchangeable. The exchangeable polypeptide sequences have either random sequences, constant sequences or sequences with random and constant amino acids. The amino acids with reactive groups are either located in defined positions within the polypeptide, since the position of these amino acids determines loop size.

In one embodiment, a polypeptide with three reactive groups has the sequence $(X)_lY(X)_mY(X)_nY(X)_o$, wherein Y represents an amino acid with a reactive group, X represents a random amino acid, m and n are numbers between 3 and 6 defining the length of intervening polypeptide segments, which may be the same or different, and l and o are numbers between 0 and 20 defining the length of flanking polypeptide segments.

Alternatives to thiol-mediated conjugations can be used to attach the molecular scaffold to the peptide via covalent interactions. Alternatively these techniques may be used in modification or attachment of further moieties (such as small molecules of interest which are distinct from the molecular scaffold) to the polypeptide after they have been selected or isolated according to the present invention – in this embodiment then clearly the attachment need not be covalent and may embrace non-covalent attachment. These methods may be used instead of (or in combination with) the thiol mediated methods by producing phage that display proteins and peptides bearing unnatural amino acids with the requisite chemical reactive groups, in combination small molecules that bear the complementary reactive group, or by incorporating the unnatural amino acids into a chemically or recombinantly synthesised polypeptide when the molecule is being made after the selection/isolation phase. Further details can be found in WO 2009/098450 or Heinis *et al.*, *Nat Chem Biol* **2009,** *5* (7), 502-7.

In one embodiment, the reactive groups are selected from cysteine, 3-mercaptopropionic acid and/or cysteamine residues.

Pharmaceutically Acceptable Salts

It will be appreciated that salt forms are within the scope of this invention, and references to peptide ligands include the salt forms of said ligands.

The salts of the present invention can be synthesized from the parent compound that contains a basic or acidic moiety by conventional chemical methods such as methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two.

Acid addition salts (mono- or di-salts) may be formed with a wide variety of acids, both inorganic and organic. Examples of acid addition salts include mono- or di-salts formed with an acid selected from the group consisting of acetic, 2,2-dichloroacetic, adipic, alginic, ascorbic (e.g. L-ascorbic), L-aspartic, benzenesulfonic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulfonic, (+)-(1*S*)-camphor-10-sulfonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulfuric, ethane-1,2-disulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, formic, fumaric, galactaric, gentisic, glucoheptonic, D-gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α-oxoglutaric, glycolic, hippuric, hydrohalic acids (e.g. hydrobromic, hydrochloric, hydriodic), isethionic, lactic (e.g. (+)-L-lactic,

(±)-DL-lactic), lactobionic, maleic, malic, (-)-L-malic, malonic, (±)-DL-mandelic, methanesulfonic, naphthalene-2-sulfonic, naphthalene-1,5-disulfonic, 1-hydroxy-2-naphthoic, nicotinic, nitric, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, pyruvic, L-pyroglutamic, salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulfuric, tannic, (+)-L-tartaric, thiocyanic, *p*-toluenesulfonic, undecylenic and valeric acids, as well as acylated amino acids and cation exchange resins.

One particular group of salts consists of salts formed from acetic, hydrochloric, hydriodic, phosphoric, nitric, sulfuric, citric, lactic, succinic, maleic, malic, isethionic, fumaric, benzenesulfonic, toluenesulfonic, sulfuric, methanesulfonic (mesylate), ethanesulfonic, naphthalenesulfonic, valeric, propanoic, butanoic, malonic, glucuronic and lactobionic acids. One particular salt is the hydrochloride salt. Another particular salt is the acetate salt.

If the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be $-COO^-$), then a salt may be formed with an organic or inorganic base, generating a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Li^+, Na^+ and K^+, alkaline earth metal cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+} or Zn^+. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: methylamine, ethylamine, diethylamine, propylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

Where the compounds of the invention contain an amine function, these may form quaternary ammonium salts, for example by reaction with an alkylating agent according to methods well known to the skilled person. Such quaternary ammonium compounds are within the scope of the invention.

Modified Derivatives

It will be appreciated that modified derivatives of the peptide ligands as defined herein are within the scope of the present invention. Examples of such suitable modified derivatives include one or more modifications selected from: N-terminal and/or C-terminal modifications; replacement of one or more amino acid residues with one or more non-natural amino acid residues (such as replacement of one or more polar amino acid residues with one or more isosteric or isoelectronic amino acids; replacement of one or more non-polar amino acid

residues with other non-natural isosteric or isoelectronic amino acids); addition of a spacer group; replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues; replacement of one or more amino acid residues with an alanine, replacement of one or more L-amino acid residues with one or more D-amino acid residues; N-alkylation of one or more amide bonds within the bicyclic peptide ligand; replacement of one or more peptide bonds with a surrogate bond; peptide backbone length modification; substitution of the hydrogen on the alpha-carbon of one or more amino acid residues with another chemical group, modification of amino acids such as cysteine, lysine, glutamate/aspartate and tyrosine with suitable amine, thiol, carboxylic acid and phenol-reactive reagents so as to functionalise said amino acids, and introduction or replacement of amino acids that introduce orthogonal reactivities that are suitable for functionalisation, for example azide or alkyne-group bearing amino acids that allow functionalisation with alkyne or azide-bearing moieties, respectively.

In one embodiment, the modified derivative comprises an N-terminal and/or C-terminal modification. In a further embodiment, wherein the modified derivative comprises an N-terminal modification using suitable amino-reactive chemistry, and/or C-terminal modification using suitable carboxy-reactive chemistry. In a further embodiment, said N-terminal or C-terminal modification comprises addition of an effector group, including but not limited to a cytotoxic agent, a radiochelator or a chromophore.

In a further embodiment, the modified derivative comprises an N-terminal modification. In a further embodiment, the N-terminal modification comprises an N-terminal acetyl group. In this embodiment, the N-terminal cysteine group (the group referred to herein as C_i) is capped with acetic anhydride or other appropriate reagents during peptide synthesis leading to a molecule which is N-terminally acetylated. This embodiment provides the advantage of removing a potential recognition point for aminopeptidases and avoids the potential for degradation of the bicyclic peptide.

In an alternative embodiment, the N-terminal modification comprises the addition of a molecular spacer group which facilitates the conjugation of effector groups and retention of potency of the bicyclic peptide to its target.

In a further embodiment, the modified derivative comprises a C-terminal modification. In a further embodiment, the C-terminal modification comprises an amide group. In this embodiment, the C-terminal cysteine group (the group referred to herein as C_{iii}) is synthesized as an amide during peptide synthesis leading to a molecule which is C-terminally amidated.

This embodiment provides the advantage of removing a potential recognition point for carboxypeptidase and reduces the potential for proteolytic degradation of the bicyclic peptide.

In one embodiment, the modified derivative comprises replacement of one or more amino acid residues with one or more non-natural amino acid residues. In this embodiment, non-natural amino acids may be selected having isosteric/isoelectronic side chains which are neither recognised by degradative proteases nor have any adverse effect upon target potency.

Alternatively, non-natural amino acids may be used having constrained amino acid side chains, such that proteolytic hydrolysis of the nearby peptide bond is conformationally and sterically impeded. In particular, these concern proline analogues, bulky sidechains, C□-disubstituted derivatives (for example, aminoisobutyric acid, Aib), and cyclo amino acids, a simple derivative being amino-cyclopropylcarboxylic acid.

In one embodiment, the modified derivative comprises the addition of a spacer group. In a further embodiment, the modified derivative comprises the addition of a spacer group to the N-terminal cysteine (C_i) and/or the C-terminal cysteine (C_{iii}).

In one embodiment, the modified derivative comprises replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues. In a further embodiment, the modified derivative comprises replacement of a tryptophan residue with a naphthylalanine or alanine residue. This embodiment provides the advantage of improving the pharmaceutical stability profile of the resultant bicyclic peptide ligand.

In one embodiment, the modified derivative comprises replacement of one or more charged amino acid residues with one or more hydrophobic amino acid residues. In an alternative embodiment, the modified derivative comprises replacement of one or more hydrophobic amino acid residues with one or more charged amino acid residues. The correct balance of charged versus hydrophobic amino acid residues is an important characteristic of the bicyclic peptide ligands. For example, hydrophobic amino acid residues influence the degree of plasma protein binding and thus the concentration of the free available fraction in plasma, while charged amino acid residues (in particular arginine) may influence the interaction of the peptide with the phospholipid membranes on cell surfaces. The two in combination may influence half-life, volume of distribution and exposure of the peptide drug, and can be tailored according to the clinical endpoint. In addition, the correct combination and number of charged versus hydrophobic amino acid residues may reduce irritation at the injection site (if the peptide drug has been administered subcutaneously).

In one embodiment, the modified derivative comprises replacement of one or more L-amino acid residues with one or more D-amino acid residues. This embodiment is believed to increase proteolytic stability by steric hindrance and by a propensity of D-amino acids to stabilise □-turn conformations (Tugyi *et al* (2005) PNAS, 102(2), 413–418).

In one embodiment, the modified derivative comprises removal of any amino acid residues and substitution with alanines. This embodiment provides the advantage of removing potential proteolytic attack site(s).

It should be noted that each of the above mentioned modifications serve to deliberately improve the potency or stability of the peptide. Further potency improvements based on modifications may be achieved through the following mechanisms:

- Incorporating hydrophobic moieties that exploit the hydrophobic effect and lead to lower off rates, such that higher affinities are achieved;

- Incorporating charged groups that exploit long-range ionic interactions, leading to faster on rates and to higher affinities (see for example Schreiber *et al*, *Rapid, electrostatically assisted association of proteins* (1996), Nature Struct. Biol. 3, 427-31); and

- Incorporating additional constraint into the peptide, by for example constraining side chains of amino acids correctly such that loss in entropy is minimal upon target binding, constraining the torsional angles of the backbone such that loss in entropy is minimal upon target binding and introducing additional cyclisations in the molecule for identical reasons.

(for reviews see Gentilucci *et al*, Curr. Pharmaceutical Design, (2010), 16, 3185-203, and Nestor *et al*, Curr. Medicinal Chem (2009), 16, 4399-418).

Examples of modified heterotandem bicyclic peptide complexes of the invention include those listed in Tables G and H below:

Table G: (EphA2: CD137; 1:2)

Complex No.	***EphA2 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***	***Modifier***

BCY14415	BCY9594	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys (PYA)4 dLys (PYA)4	Peg12-Biotin
BCY14416	BCY9594	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys (PYA)4 dLys (PYA)4	Alexa Fluor® 488
BCY14417	BCY13118	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys(PYA)4 dLys(PYA)4	Peg12-Biotin
BCY14418	BCY13118	N-terminus	N-(acid-PEG3)-N-bis(PEG3-azide)	BCY8928 BCY13389	dLys(PYA)4 dLys(PYA)4	Alexa Fluor® 488

Table H: (Nectin-4:CD137; 1:2)

Complex No.	***Nectin-4 BCY No.***	***Attachment Point***	***Linker***	***CD137 BCY No.***	***Attachment Point***	***Modifier***
BCY13582	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928, BCY13389	dLys(PYA)4 dLys(PYA)4	Biotin-Peg12
BCY13583	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928, BCY13389	dLys(PYA)4 dLys(PYA)4	Alexa Fluor 488
BCY13628	BCY8116	N-terminus	N-(acid-PEG_3)-N-bis(PEG_3-azide)	BCY8928, BCY13389	dLys(PYA)4 dLys(PYA)4	Cyanine 5

Isotopic variations

The present invention includes all pharmaceutically acceptable (radio)isotope-labeled peptide ligands of the invention, wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature, and peptide ligands of the invention, wherein metal chelating groups are attached (termed “effector”) that are capable of holding relevant (radio)isotopes,

and peptide ligands of the invention, wherein certain functional groups are covalently replaced with relevant (radio)isotopes or isotopically labelled functional groups.

Examples of isotopes suitable for inclusion in the peptide ligands of the invention comprise isotopes of hydrogen, such as ^{2}H (D) and ^{3}H (T), carbon, such as ^{11}C, ^{13}C and ^{14}C, chlorine, such as ^{36}Cl, fluorine, such as ^{18}F, iodine, such as ^{123}I, ^{125}I and ^{131}I, nitrogen, such as ^{13}N and ^{15}N, oxygen, such as ^{15}O, ^{17}O and ^{18}O, phosphorus, such as ^{32}P, sulfur, such as ^{35}S, copper, such as ^{64}Cu, gallium, such as ^{67}Ga or ^{68}Ga, yttrium, such as ^{90}Y and lutetium, such as ^{177}Lu, and Bismuth, such as ^{213}Bi.

Certain isotopically-labelled peptide ligands of the invention, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies, and to clinically assess the presence and/or absence of the Nectin-4 target on diseased tissues. The peptide ligands of the invention can further have valuable diagnostic properties in that they can be used for detecting or identifying the formation of a complex between a labelled compound and other molecules, peptides, proteins, enzymes or receptors. The detecting or identifying methods can use compounds that are labelled with labelling agents such as radioisotopes, enzymes, fluorescent substances, luminous substances (for example, luminol, luminol derivatives, luciferin, aequorin and luciferase), etc. The radioactive isotopes tritium, *i.e.* ^{3}H (T), and carbon-14, *i.e.* ^{14}C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, *i.e.* ^{2}H (D), may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

Substitution with positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, can be useful in Positron Emission Topography (PET) studies for examining target occupancy.

Isotopically-labeled compounds of peptide ligands of the invention can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

Molecular scaffold

Molecular scaffolds are described in, for example, WO 2009/098450 and references cited therein, particularly WO 2004/077062 and WO 2006/078161.

As noted in the foregoing documents, the molecular scaffold may be a small molecule, such as a small organic molecule.

In one embodiment, the molecular scaffold may be a macromolecule. In one embodiment, the molecular scaffold is a macromolecule composed of amino acids, nucleotides or carbohydrates.

In one embodiment, the molecular scaffold comprises reactive groups that are capable of reacting with functional group(s) of the polypeptide to form covalent bonds.

The molecular scaffold may comprise chemical groups which form the linkage with a peptide, such as amines, thiols, alcohols, ketones, aldehydes, nitriles, carboxylic acids, esters, alkenes, alkynes, azides, anhydrides, succinimides, maleimides, alkyl halides and acyl halides.

In one embodiment, the molecular scaffold may comprise or may consist of hexahydro-1,3,5-triazine, especially 1,3,5-Triacryloylhexahydro-1,3,5-triazine ('TATA'), or a derivative thereof.

The molecular scaffold of the invention contains chemical groups that allow functional groups of the polypeptide of the encoded library of the invention to form covalent links with the molecular scaffold. Said chemical groups are selected from a wide range of functionalities including amines, thiols, alcohols, ketones, aldehydes, nitriles, carboxylic acids, esters, alkenes, alkynes, anhydrides, succinimides, maleimides, azides, alkyl halides and acyl halides.

Scaffold reactive groups that could be used on the molecular scaffold to react with thiol groups of cysteines are alkyl halides (or also named halogenoalkanes or haloalkanes).

Examples include bromomethylbenzene (the scaffold reactive group exemplified by TBMB) or iodoacetamide. Other scaffold reactive groups that are used to selectively couple compounds to cysteines in proteins are maleimides, α-unsaturated carbonyl containing compounds and α-halomethylcarbonyl containing compounds. Examples of maleimides which may be used as molecular scaffolds in the invention include: tris-(2-maleimidoethyl)amine, tris-(2-maleimidoethyl)benzene, tris-(maleimido)benzene. An example of an αβ unsaturated

carbonyl containing compound is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) (Angewandte Chemie, International Edition (2014), 53(6), 1602-1606). An example of an α-halomethylcarbonyl containing compound is N,N',N''-(benzene-1,3,5-triyl)tris(2-bromoacetamide). Selenocysteine is also a natural amino acid which has a similar reactivity to cysteine and can be used for the same reactions. Thus, wherever cysteine is mentioned, it is typically acceptable to substitute selenocysteine unless the context suggests otherwise.

Synthesis

The peptides of the present invention may be manufactured synthetically by standard techniques followed by reaction with a molecular scaffold *in vitro*. When this is performed, standard chemistry may be used. This enables the rapid large scale preparation of soluble material for further downstream experiments or validation. Such methods could be accomplished using conventional chemistry such as that disclosed in Timmerman *et al* (*supra*).

Thus, the invention also relates to manufacture of polypeptides or conjugates selected as set out herein, wherein the manufacture comprises optional further steps as explained below. In one embodiment, these steps are carried out on the end product polypeptide/conjugate made by chemical synthesis.

Optionally amino acid residues in the polypeptide of interest may be substituted when manufacturing a conjugate or complex.

Peptides can also be extended, to incorporate for example another loop and therefore introduce multiple specificities.

To extend the peptide, it may simply be extended chemically at its N-terminus or C-terminus or within the loops using orthogonally protected lysines (and analogues) using standard solid phase or solution phase chemistry. Standard (bio)conjugation techniques may be used to introduce an activated or activatable N- or C-terminus. Alternatively additions may be made by fragment condensation or native chemical ligation e.g. as described in (Dawson *et al.* 1994. Synthesis of Proteins by Native Chemical Ligation. Science 266:776-779), or by enzymes, for example using subtiligase as described in (Chang *et al.* Proc Natl Acad Sci U S A. 1994 Dec 20; 91(26):12544-8 or in Hikari *et al* Bioorganic & Medicinal Chemistry Letters Volume 18, Issue 22, 15 November 2008, Pages 6000-6003).

Alternatively, the peptides may be extended or modified by further conjugation through disulphide bonds. This has the additional advantage of allowing the first and second peptides to dissociate from each other once within the reducing environment of the cell. In this case, the molecular scaffold (e.g. TATA) could be added during the chemical synthesis of the first peptide so as to react with the three cysteine groups; a further cysteine or thiol could then be appended to the N or C-terminus of the first peptide, so that this cysteine or thiol only reacted with a free cysteine or thiol of the second peptides, forming a disulfide –linked bicyclic peptide-peptide conjugate.

Similar techniques apply equally to the synthesis/coupling of two bicyclic and bispecific macrocycles, potentially creating a tetraspecific molecule.

Furthermore, addition of other functional groups or effector groups may be accomplished in the same manner, using appropriate chemistry, coupling at the N- or C-termini or via side chains. In one embodiment, the coupling is conducted in such a manner that it does not block the activity of either entity.

Pharmaceutical Compositions

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising a peptide ligand as defined herein in combination with one or more pharmaceutically acceptable excipients.

Generally, the present peptide ligands will be utilised in purified form together with pharmacologically appropriate excipients or carriers. Typically, these excipients or carriers include aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and/or buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride and lactated Ringer's. Suitable physiologically-acceptable adjuvants, if necessary to keep a polypeptide complex in suspension, may be chosen from thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin and alginates.

Intravenous vehicles include fluid and nutrient replenishers and electrolyte replenishers, such as those based on Ringer's dextrose. Preservatives and other additives, such as antimicrobials, antioxidants, chelating agents and inert gases, may also be present (Mack (1982) Remington's Pharmaceutical Sciences, 16th Edition).

The peptide ligands of the present invention may be used as separately administered compositions or in conjunction with other agents. These can include antibodies, antibody fragments and various immunotherapeutic drugs, such as cylcosporine, methotrexate, adriamycin or cisplatinum and immunotoxins. Pharmaceutical compositions can include "cocktails" of various cytotoxic or other agents in conjunction with the protein ligands of the present invention, or even combinations of selected polypeptides according to the present invention having different specificities, such as polypeptides selected using different target ligands, whether or not they are pooled prior to administration.

The route of administration of pharmaceutical compositions according to the invention may be any of those commonly known to those of ordinary skill in the art. For therapy, the peptide ligands of the invention can be administered to any patient in accordance with standard techniques. The administration can be by any appropriate mode, including parenterally, intravenously, intramuscularly, intraperitoneally, transdermally, via the pulmonary route, or also, appropriately, by direct infusion with a catheter. Preferably, the pharmaceutical compositions according to the invention will be administered by inhalation. The dosage and frequency of administration will depend on the age, sex and condition of the patient, concurrent administration of other drugs, counterindications and other parameters to be taken into account by the clinician.

The peptide ligands of this invention can be lyophilised for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective and art-known lyophilisation and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of activity loss and that levels may have to be adjusted upward to compensate.

The compositions containing the present peptide ligands or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In certain therapeutic applications, an adequate amount to accomplish at least partial inhibition, suppression, modulation, killing, or some other measurable parameter, of a population of selected cells is defined as a "therapeutically-effective dose". Amounts needed to achieve this dosage will depend upon the severity of the disease and the general state of the patient's own immune system, but generally range from 0.005 to 5.0 mg of selected peptide ligand per kilogram of body weight, with doses of 0.05 to 2.0 mg/kg/dose being more commonly used. For prophylactic applications, compositions containing the present peptide ligands or cocktails thereof may also be administered in similar or slightly lower dosages.

A composition containing a peptide ligand according to the present invention may be utilised in prophylactic and therapeutic settings to aid in the alteration, inactivation, killing or removal of a select target cell population in a mammal. In addition, the peptide ligands described herein may be used extracorporeally or *in vitro* selectively to kill, deplete or otherwise effectively remove a target cell population from a heterogeneous collection of cells. Blood from a mammal may be combined extracorporeally with the selected peptide ligands whereby the undesired cells are killed or otherwise removed from the blood for return to the mammal in accordance with standard techniques.

Therapeutic Uses

According to a further aspect of the invention, there is provided a heterotandem bicyclic peptide complex as defined herein for use in preventing, suppressing or treating cancer.

Examples of cancers (and their benign counterparts) which may be treated (or inhibited) include, but are not limited to tumors of epithelial origin (adenomas and carcinomas of various types including adenocarcinomas, squamous carcinomas, transitional cell carcinomas and other carcinomas) such as carcinomas of the bladder and urinary tract, breast, gastrointestinal tract (including the esophagus, stomach (gastric), small intestine, colon, rectum and anus), liver (hepatocellular carcinoma), gall bladder and biliary system, exocrine pancreas, kidney, lung (for example adenocarcinomas, small cell lung carcinomas, non-small cell lung carcinomas, bronchioalveolar carcinomas and mesotheliomas), head and neck (for example cancers of the tongue, buccal cavity, larynx, pharynx, nasopharynx, tonsil, salivary glands, nasal cavity and paranasal sinuses), ovary, fallopian tubes, peritoneum, vagina, vulva, penis, cervix, myometrium, endometrium, thyroid (for example thyroid follicular carcinoma), adrenal, prostate, skin and adnexae (for example melanoma, basal cell carcinoma, squamous cell carcinoma, keratoacanthoma, dysplastic naevus); haematological malignancies (i.e. leukemias, lymphomas) and premalignant haematological disorders and disorders of borderline malignancy including haematological malignancies and related conditions of lymphoid lineage (for example acute lymphocytic leukemia [ALL], chronic lymphocytic leukemia [CLL], B-cell lymphomas such as diffuse large B-cell lymphoma [DLBCL], follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, T-cell lymphomas and leukaemias, natural killer [NK] cell lymphomas, Hodgkin's lymphomas, hairy cell leukaemia, monoclonal gammopathy of uncertain significance, plasmacytoma, multiple myeloma, and post-transplant lymphoproliferative disorders), and haematological malignancies and related conditions of myeloid lineage (for example acute myelogenousleukemia [AML], chronic myelogenousleukemia [CML], chronic myelomonocyticleukemia [CMML], hypereosinophilic syndrome, myeloproliferative disorders such as polycythaemia vera, essential

thrombocythaemia and primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, and promyelocyticleukemia); tumors of mesenchymal origin, for example sarcomas of soft tissue, bone or cartilage such as osteosarcomas, fibrosarcomas, chondrosarcomas, rhabdomyosarcomas, leiomyosarcomas, liposarcomas, angiosarcomas, Kaposi's sarcoma, Ewing's sarcoma, synovial sarcomas, epithelioid sarcomas, gastrointestinal stromal tumors, benign and malignant histiocytomas, and dermatofibrosarcomaprotuberans; tumors of the central or peripheral nervous system (for example astrocytomas, gliomas and glioblastomas, meningiomas, ependymomas, pineal tumors and schwannomas); endocrine tumors (for example pituitary tumors, adrenal tumors, islet cell tumors, parathyroid tumors, carcinoid tumors and medullary carcinoma of the thyroid); ocular and adnexal tumors (for example retinoblastoma); germ cell and trophoblastic tumors (for example teratomas, seminomas, dysgerminomas, hydatidiform moles and choriocarcinomas); and paediatric and embryonal tumors (for example medulloblastoma, neuroblastoma, Wilms tumor, and primitive neuroectodermal tumors); or syndromes, congenital or otherwise, which leave the patient susceptible to malignancy (for example Xeroderma Pigmentosum).

In a further embodiment, the cancer is selected from a hematopoietic malignancy such as selected from: non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma (BL), multiple myeloma (MM), B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), T cell lymphoma (TCL), acute myeloid leukemia (AML), hairy cell leukemia (HCL), Hodgkin's Lymphoma (HL), and chronic myeloid leukemia (CML).

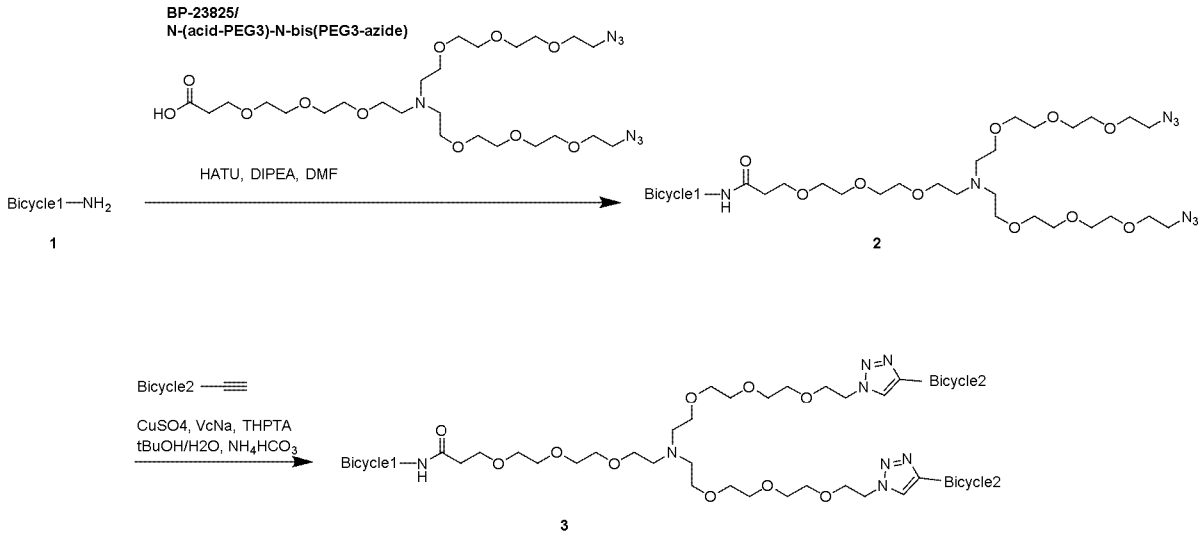
References herein to the term "prevention" involves administration of the protective composition prior to the induction of the disease. "Suppression" refers to administration of the composition after an inductive event, but prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after disease symptoms become manifest.

Animal model systems which can be used to screen the effectiveness of the peptide ligands in protecting against or treating the disease are available. The use of animal model systems is facilitated by the present invention, which allows the development of polypeptide ligands which can cross react with human and animal targets, to allow the use of animal models.

The invention is further described below with reference to the following examples.

EXAMPLES

In general, some of the heterotandem bicyclic peptide complexes of the invention may be prepared in accordance with the following general method:



All solvents are degassed and purged with N_2 3 times. A solution of **BP-23825** (1.0 eq), HATU (1.2 eq) and DIEA (2.0 eq) in DMF is mixed for 5 minutes, then **Bicycle1** (1.2 eq.) is added. The reaction mixture is stirred at 40°C for 16 hr. The reaction mixture is then concentrated under reduced pressure to remove solvent and purified by prep-HPLC to give intermediate **2**.

A mixture of **intermediate 2** (1.0 eq) and **Bicycle2** (2.0 eq) are dissolved in t-BuOH/H_2O (1:1), and then $CuSO_4$ (1.0 eq), VcNa (4.0 eq), and THPTA (2.0 eq) are added. Finally, 0.2 M NH_4HCO_3 is added to adjust pH to 8. The reaction mixture is stirred at 40°C for 16 hr under N_2 atmosphere. The reaction mixture was directly purified by prep-HPLC.

Heterotandem bicyclic peptide complexes which were prepared using this method are listed below:

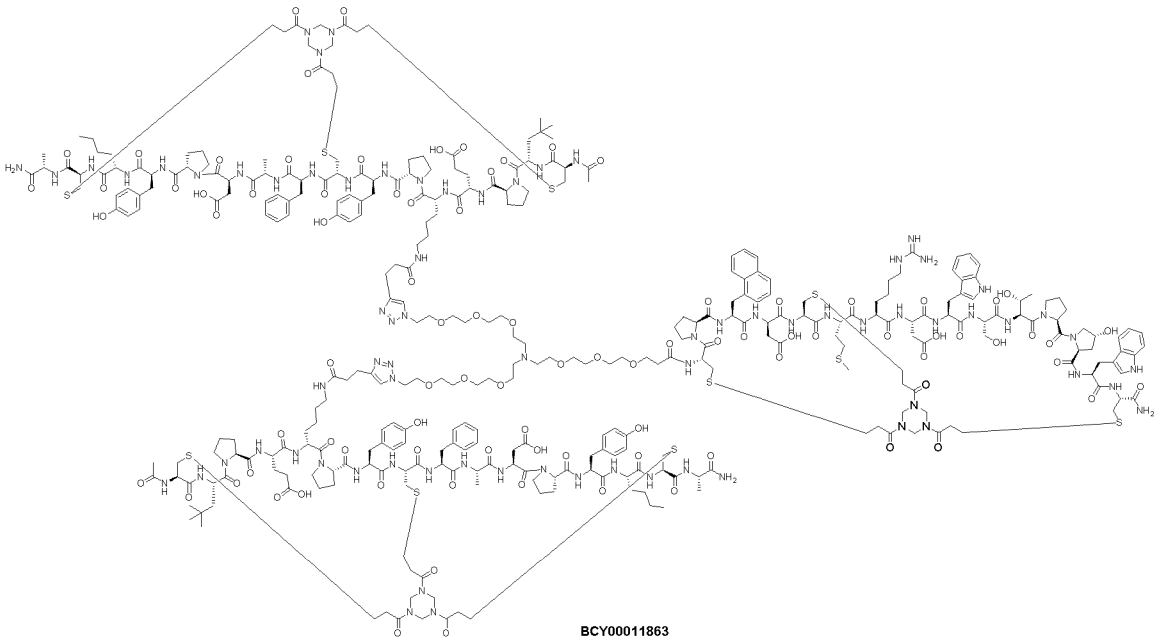
EphA2/CD137	Nectin/CD137	PDL1/CD137
BCY12491	BCY11385	BCY12662
BCY12723	BCY11864	BCY12722
BCY12724	BCY11863	
BCY12725	BCY12484	**OX40**
BCY12726	BCY12485	BCY12967
BCY12728	BCY12486	
BCY12729	BCY12487	
BCY12730	BCY12490	

BCY12731	BCY12586	
BCY12732	BCY12587	
BCY12973	BCY12589	
BCY12974	BCY12590	
BCY12975	BCY12588	
BCY12976	BCY12760	
BCY12977	BCY12761	
BCY12978	BCY14602	
BCY13042		
BCY13043		
BCY13044		
BCY13045		
BCY13046		
BCY13047		
BCY13048		
BCY13049		
BCY13050		
BCY13051		
BCY13052		
BCY13053		
BCY13054		
BCY13138		
BCY13139		
BCY13140		
BCY13270		
BCY13271		
BCY13272		
BCY13273		
BCY13274		
BCY13275		
BCY13276		
BCY13277		
BCY13278		
BCY13279		
BCY13280		

BCY13281		
BCY13282		
BCY13283		
BCY13284		
BCY13285		
BCY13286		
BCY13287		
BCY13288		
BCY13289		
BCY13341		
BCY13343		
BCY14049		
BCY14050		
BCY14051		
BCY14052		
BCY14053		
BCY14054		
BCY14055		
BCY14056		
BCY14334		
BCY14335		
BCY15217		

More detailed experimental for selected heterotandem bicyclic peptide complexes of the invention are provided herein below:

Example 1: Synthesis of BCY11863



Preparation of Compound 2

N-(acid-PEG3)-N-bis(PEG3-azide) + **BCY8116** $\xrightarrow[\text{DMF}]{\text{HATU DIEA}}$ **N-(acid-PEG3)-N-bis(PEG3-azide)-BCY8116**

1 2

A mixture of **N-(acid-PEG3)-N-bis(PEG3-azide)** (70.0 mg, 112.2 μmol, 1.0 eq.), HATU (51.2 mg, 134.7 μmol, 1.2 eq.) and DIEA (29.0 mg, 224.4 μmol, 40 μL, 2.0 eq.) was dissolved in DMF (2 mL), and mixed for 5 min. Then **BCY8116** (294.0 mg, 135.3 μmol, 1.2 eq.) was added. The reaction mixture was stirred at 40°C for 16 hr. LC-MS showed a small fraction of compound **2** remained (MW: 2172.49, observed m/z: 1087.1) and one main peak with desired m/z (MW: 2778.17, observed *m/z*: 1389.3 ([(M/2+H$^+$]), 926.7 ([(M/3+H$^+$])) was detected. The reaction mixture was concentrated under reduced pressure to remove solvent and produced a residue. The residue was then purified by prep-HPLC (neutral condition). **Compound 2** (194.5 mg, 66.02 μmol, 29.41% yield, 94.3% purity) was obtained as a white solid.

Preparation of BCY11863

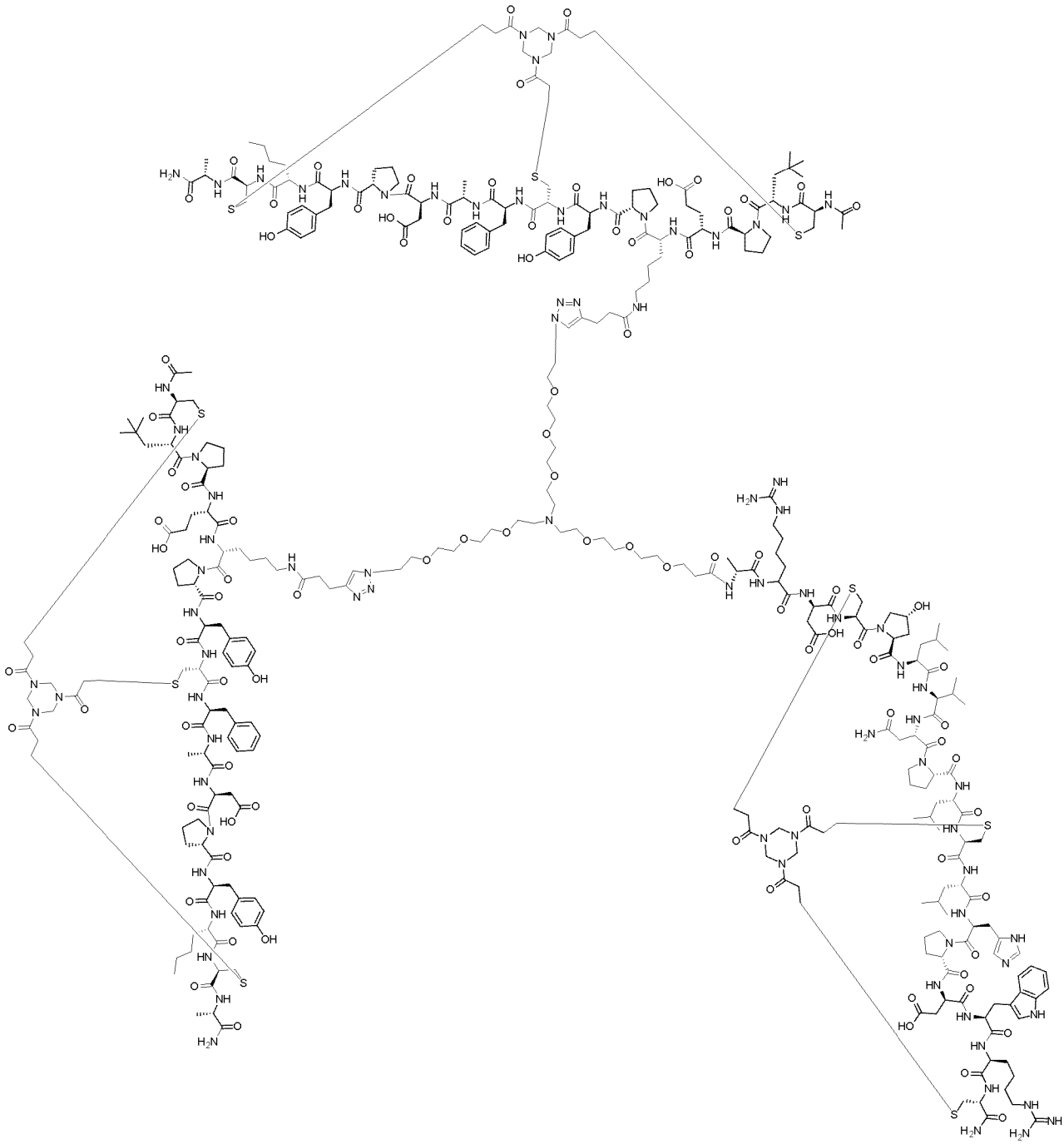
N-(acid-PEG3)-N-bis(PEG3-azide)-BCY8116 + **BCY8928** $\xrightarrow[\text{t-BuOH/H}_2\text{O}]{\text{CuSO}_4\ \text{VcNa}\ \text{THPTA}}$ **BCY11863**

2

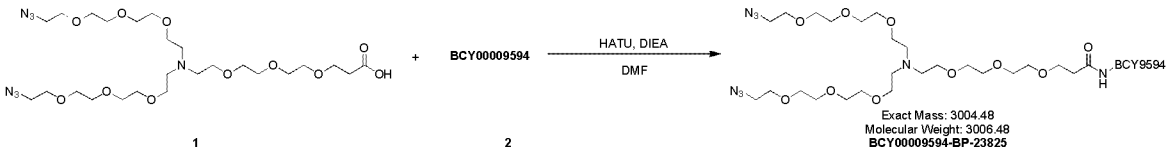
A mixture of Compound **2** (100.0 mg, 36.0 μmol, 1.0 eq), **BCY8928** (160.0 mg, 72.0 μmol, 2.0 eq) were first dissolved in 2 mL of t-BuOH/H_2O (1:1), and then $CuSO_4$ (0.4 M, 180 μL, 1.0 eq) and VcNa (28.5 mg, 143.8 μmol, 4.0 eq), THPTA (31.2 mg, 71.8 μmol, 2.0 eq) were added. Finally, 0.2 M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2 for 3 times. The reaction mixture was stirred at 40°C for 16 hr under N_2 atmosphere. LC-MS showed **BCY8928** remained and desired m/z (calculated MW:

7213.32, observed *m/z*: 1444.0 ([M/5+H]$^+$)) was also detected. The reaction mixture was directly purified by prep-HPLC. First purification resulted in **BCY11863** (117.7 mg, 15.22 µmol, 42.29% yield, 93.29% purity) as TFA salt, while less pure fractions were purified again by prep-HPLC (TFA condition), producing **BCY11863** (33.2 mg, 4.3 µmol, 11.92% yield, 95.55% purity) as TFA salt.

Example 2: Synthesis of BCY12491



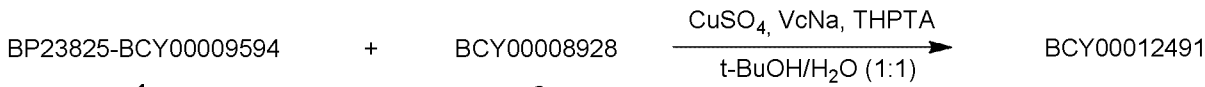
***General procedure for preparation of* BP-23825-BCY9594**



1 2

To a mixture of compound **1** (**BP-23825,** 60.0 mg, 96.2 μmol, 1.0 eq) in DMF (3 mL) was added DIEA (12.4 mg, 96.2 μmol, 16.8 μL, 1.0 eq) and HATU (38.4 mg, 101 μmol, 1.05 eq) and the mixture stirred for 5 min. Then **BCY9594** (243 mg, 101 μmol, 1.05 eq) was added to the mixture and purged with N_2 3 times, then stirred at 40 °C for 16 hr under N_2 atmosphere. LC-MS showed compound **1** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was purified by preparative-HPLC to give **(BP-23825)-BCY9594** (154 mg, 48.1 μmol, 50.0% yield, 94.0% purity) as a white solid. Calculated MW: 3006.48, observed m/z: 1002.8 $[M/3+H]^+$, 1504.4 $[M/2+H]^+$

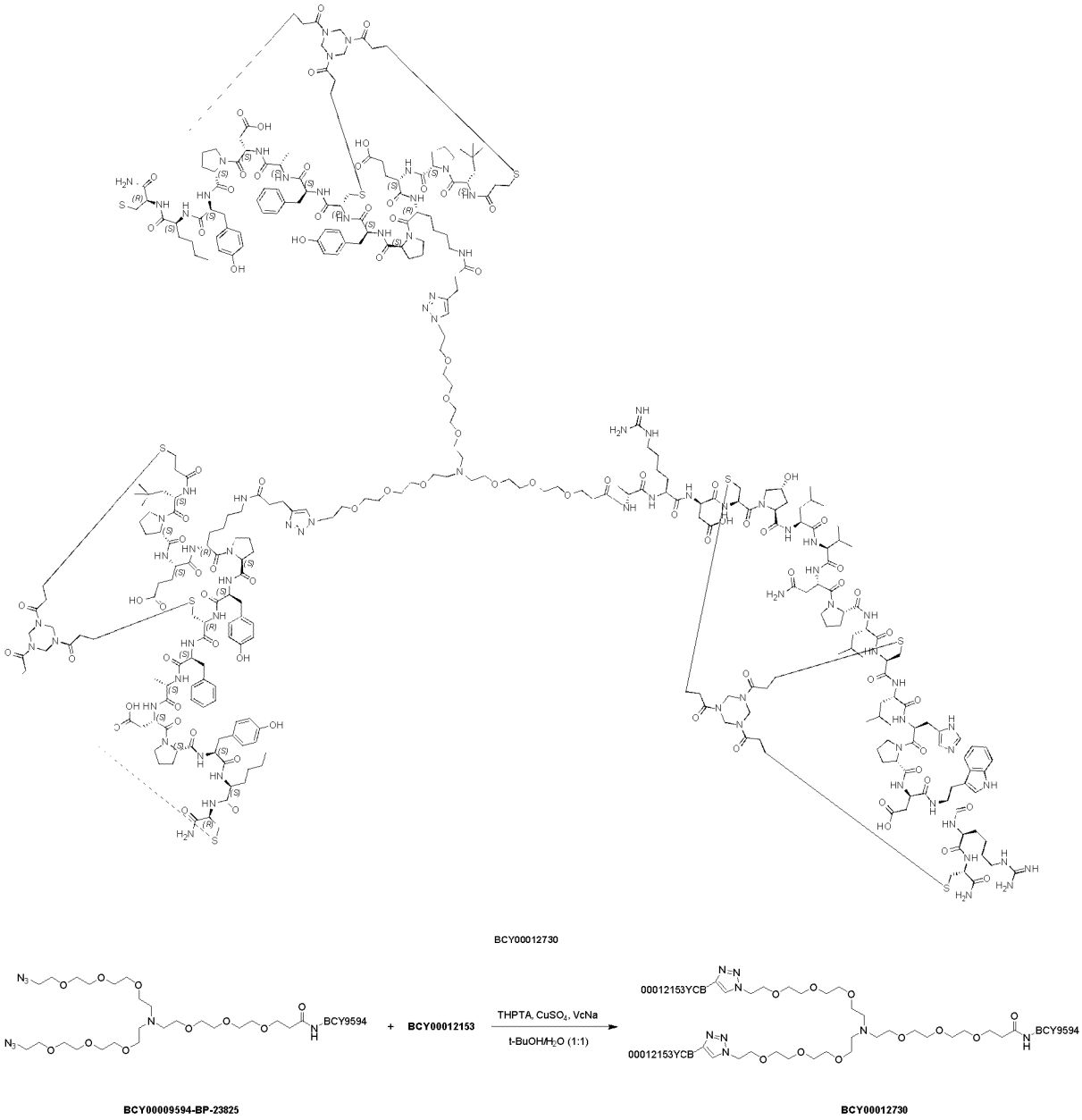
General procedure for preparation of compound BCY12491



1 **2**

A mixture of compound **1** (56.0 mg, 18.6 μmol, 1.0 eq.), **BCY8928** (83.0 mg, 37.2 μmol, 2.0 eq.), and THPTA (17.0 mg, 39.1 μmol, 2.1 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 94.0 μL, 2.0 eq.) and VcNa (15.0 mg, 74.5 μmol, 4.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned to light yellow. The reaction mixture was stirred at 40 °C for 3 hr under N_2 atmosphere. LC-MS showed compound **3** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (TFA condition), and **BCY12491** (59.2 mg, 7.79 μmol, 41.81% yield, 97.9% purity) was obtained as a white solid. Calculated MW: 7441.63, observed *m/z*: 1861.1 ($[M/4+H]^+$), 1489.0 ($[M/5+H]^+$).

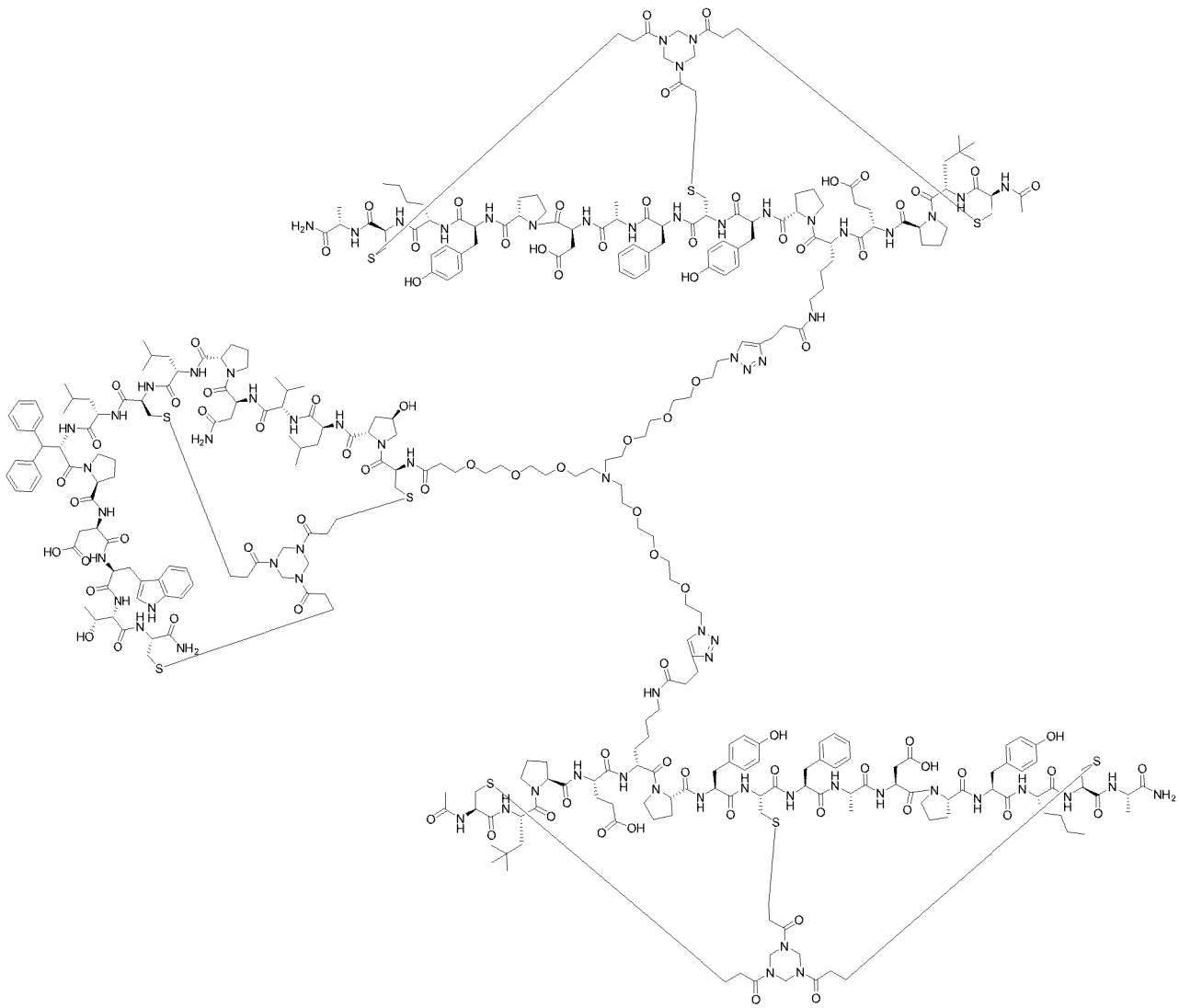
Example 3: Synthesis of BCY12730



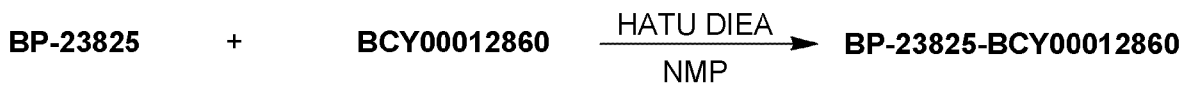
General procedure for preparation of compound BCY12730

A mixture of **(BP-23825)-BCY9594** (20.0 mg, 6.65 μmol, 1.0 eq), **BCY12153** (27.8 mg, 13.3 μmol, 2.0 eq) and THPTA (5.8 mg, 13.3 μmol, 2.0 eq) was dissolved in t-BuOH (0.5 mL) and H_2O (0.5 mL) (all solvents were pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 33.3 μL, 13.3 μmol, 2.0 eq), VcNa (0.4 M, 66.5 μL, 26.6 μmol, 4.0 eq) were added to the mixture under N_2 atmosphere. Then NH_4HCO_3 was added to the mixture until pH is 8. The mixture was stirred at 25 °C for 2 hr under N_2 atmosphere. LC-MS showed one main peak with desired m/z was detected. The reaction mixture was purified twice by prep-HPLC to give compound **BCY12730** (7.50 mg, 0.84 μmol, 12.7% yield, 96.3% purity) as a white solid. Calculated MW: 7185.39, observed m/z: 1197.5 $[M/6+H]^+$, 1438.4 $[M/5+H]^+$.

Example 4: Synthesis of BCY13048



Procedure for preparation of BP-23825-BCY12860

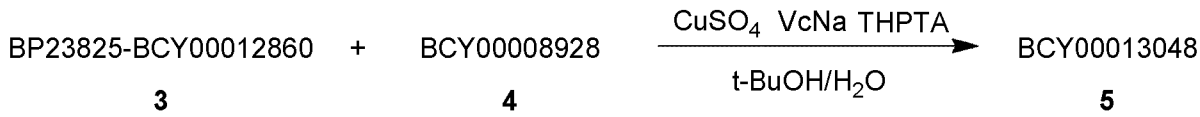


1 **2** **3**

A mixture of **BP-23825** (12.0 mg, 19.24 μmol, 1.2 eq.), and HATU (7.32 mg, 19.24 μmol, 1.2 eq.) was dissolved in NMP (0.3 mL), then the pH of this solution was adjusted to 8 by dropwise addition of DIEA (5.12 mg, 40.26 μmol,7 μL, 2.4 eq.), and then the solution was activated at 40 °C for 5 min. Compound **2** (33.0 mg, 16.03 μmol, 1.0 eq.) was dissolved in NMP (0.5 mL), and then dropped to the activated solution, the pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 40 °C for 0.5 hr. LC-MS showed **BCY12860** was consumed completely and one main peak with desired m/z (MW: 2667.12, observed *m/z*: 1334.2 ([(M/2+H$^+$]), 889.8 ([(M/3+H$^+$])) was detected. The reaction mixture was concentrated under reduced pressure to remove solvent and produced

a residue. The residue was then purified by prep-HPLC (neutral condition). **BP-23825-BCY12860 (**26.5 mg, 7.88 μmol, 49.12% yiled, 79.26% purity) was obtained as a white solid.

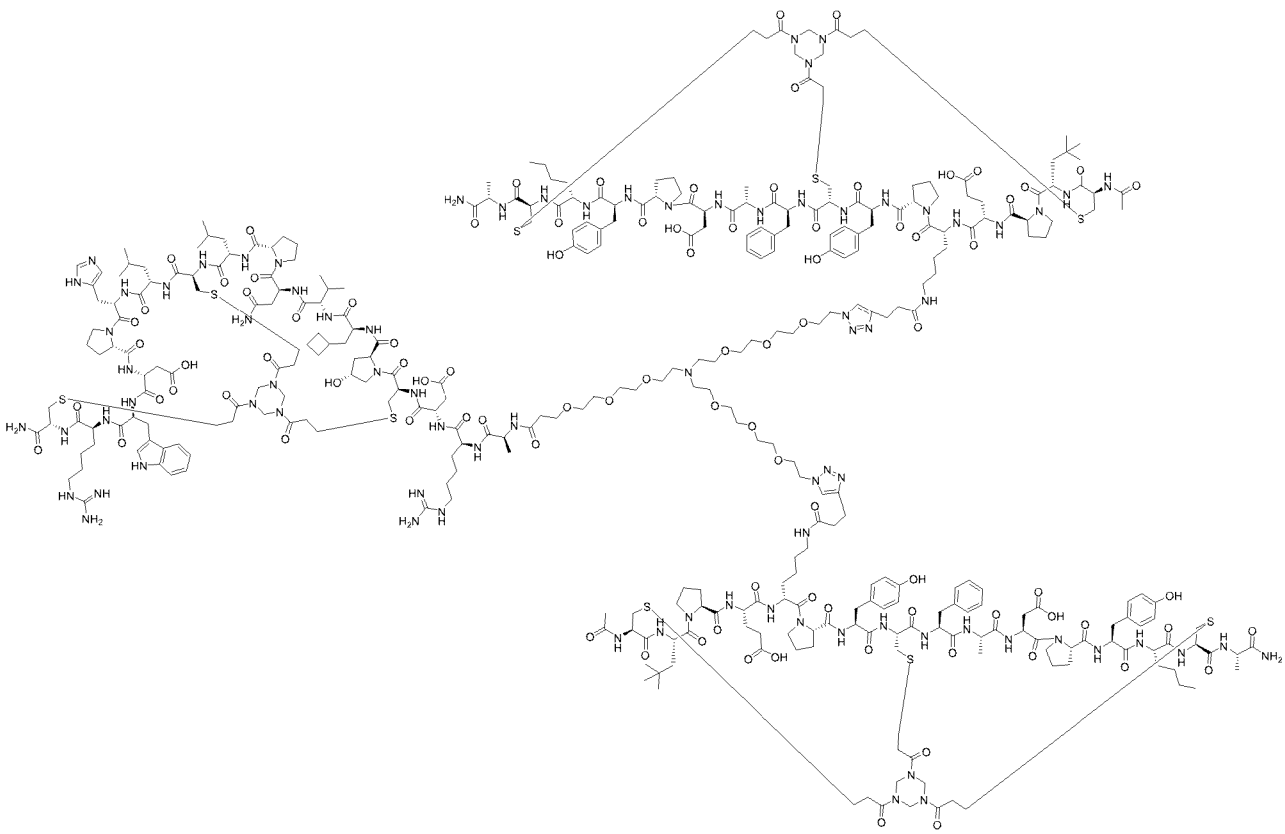
Procedure for preparation of BCY13048



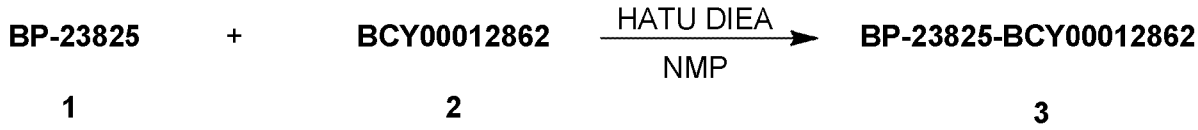
3 **4** **5**

A mixture of compound **3** (26.5 mg, 9.94 μmol, 1.0 eq.), compound **4** (47.0 mg, 20.87 μmol, 2.1 eq.), and THPTA (0.4 M, 58 μL, 2.3 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 58 μL, 2.3 eq.) and VcNa (0.4 M, 115 μL, 4.6 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned to light yellow. The reaction mixture was stirred at 40 °C for 1 hr under N_2 atmosphere. LC-MS showed compound **4** remained and one main peak with desired m/z (calculated MW: 7102.28, observed *m/z*: 1776.4([M/4+H]$^+$), 1421.3([M/3+H]$^+$)) was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (TFA condition), and **BCY13048** (14.1 mg, 1.91 μmol, 19.00% yield, 96.2% purity) was obtained as a white solid.

Example 5: Synthesis of BCY13050

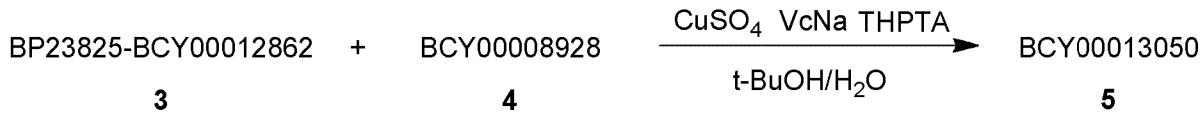


Procedure for preparation of BP-23825-BCY12862



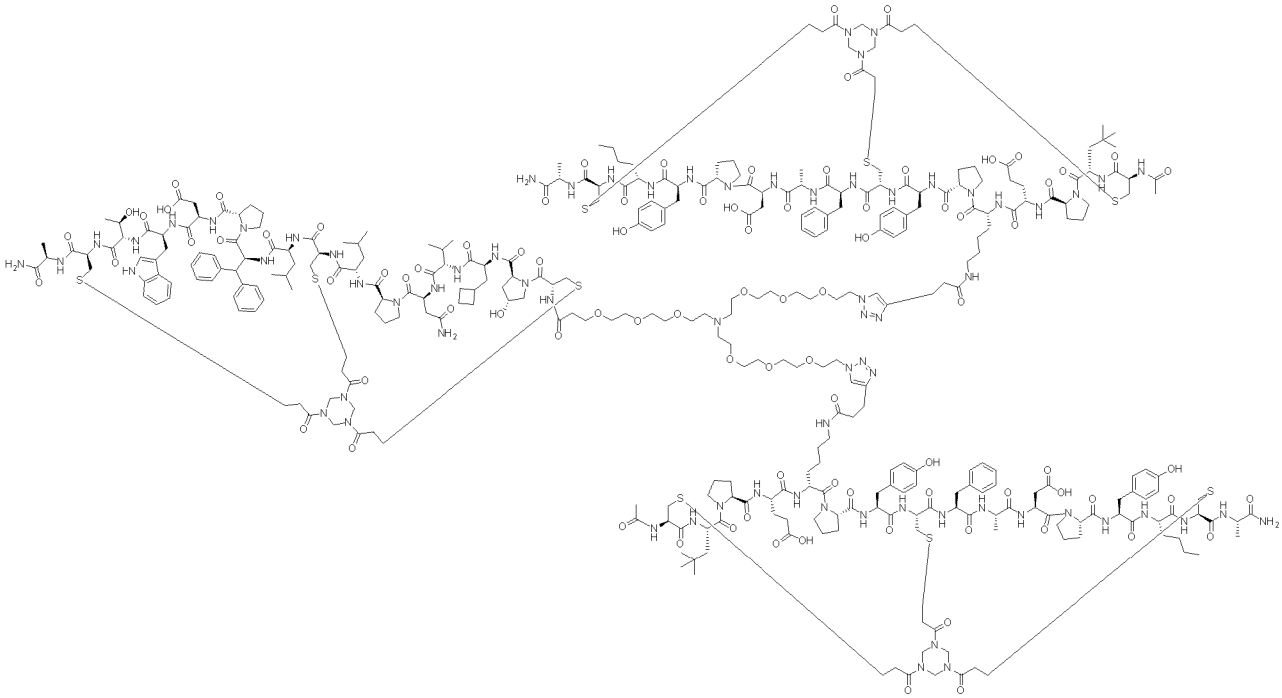
A mixture of **BP-23825** (10.0 mg, 16.03 μmol, 1.2 eq.), and HATU (6.10 mg, 16.03 μmol, 1.2 eq.) was dissolved in NMP (0.3 mL), then the pH of this solution was adjusted to 8 by dropwise addition of DIEA (4.45 mg, 34.37 μmol, 6 μL, 2.6 eq.), and then the solution was stirred at 25 °C for 6 min. Compound **2** (33.0 mg, 13.36 μmol, 1.0 eq.) was dissolved in NMP (0.5 mL), and then added dropwise into the activated solution. The pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 40 °C for 0.5 hr. LC-MS showed **BCY12862** was consumed completely and one main peak with desired m/z (MW: 3018.49, observed *m/z*: 1007.0 ([(M/3+H$^+$])) was detected. The reaction mixture was concentrated under reduced pressure to remove solvent and produced a residue. The residue was then purified by prep-HPLC (neutral condition). **BP-23825-BCY12862** (20.9 mg, 6.92 μmol, 51.82% yield, 94.9% purity) was obtained as a white solid.

Procedure for preparation of BCY13050



A mixture of compound **3** (20.9 mg, 6.92 μmol, 1.0 eq.), compound **4** (32.2 mg, 14.54 μmol, 2.1 eq.), and THPTA (7.0 mg, 15.93 μmol, 2.3 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 39 μL, 2.3 eq.) and VcNa (6.3 mg, 31.85 μmol, 4.6 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned to light yellow. The reaction mixture was stirred at 40 °C for 1 hr under N_2 atmosphere. LC-MS showed compound **4** remained and one main peak with desired m/z (calculated MW: 7453.66, observed *m/z*: 1864.2([M/4+H]$^+$)) was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (TFA condition), and **BCY13050** (6.0 mg, 0.77 μmol, 11.24% yield, 96.7% purity) was obtained as a white solid.

Example 6: Synthesis of BCY13053

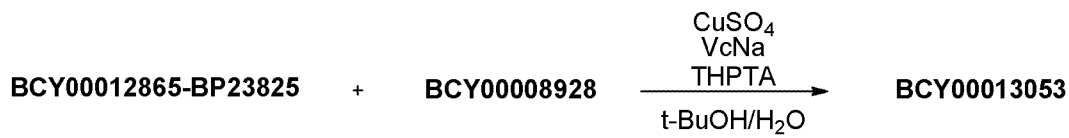


Procedure for preparation of BCY12865-BP23825

1

BP-23825 (14.0 mg, 22.45 μmol, 1.2 eq) and HATU (8.5 mg, 22.35 μmol, 1.2 eq) were first dissolved in 0.5 mL of NMP, then was added DIEA (7.8 μL, 44.77 μmol, 2.4 eq), the mixture was stirred at 25°C for 6 minutes, and then **BCY12865** (40.0 mg, 18.65 μmol, 1.0 eq) was added. The reaction mixture was stirred at 25°C for 0.5 hr. LC-MS showed one peak with desired m/z (calculated MW: 2750.21, observed *m/z*: 1375.5 ($[M/2+H]^+$)). The reaction mixture was purified by prep-HPLC (TFA condition) and compound 1 (15.9 mg, 5.78 μmol, 31.0% yield, 96.69% purity) was obtained as a white solid.

Procedure for preparation of BCY13053

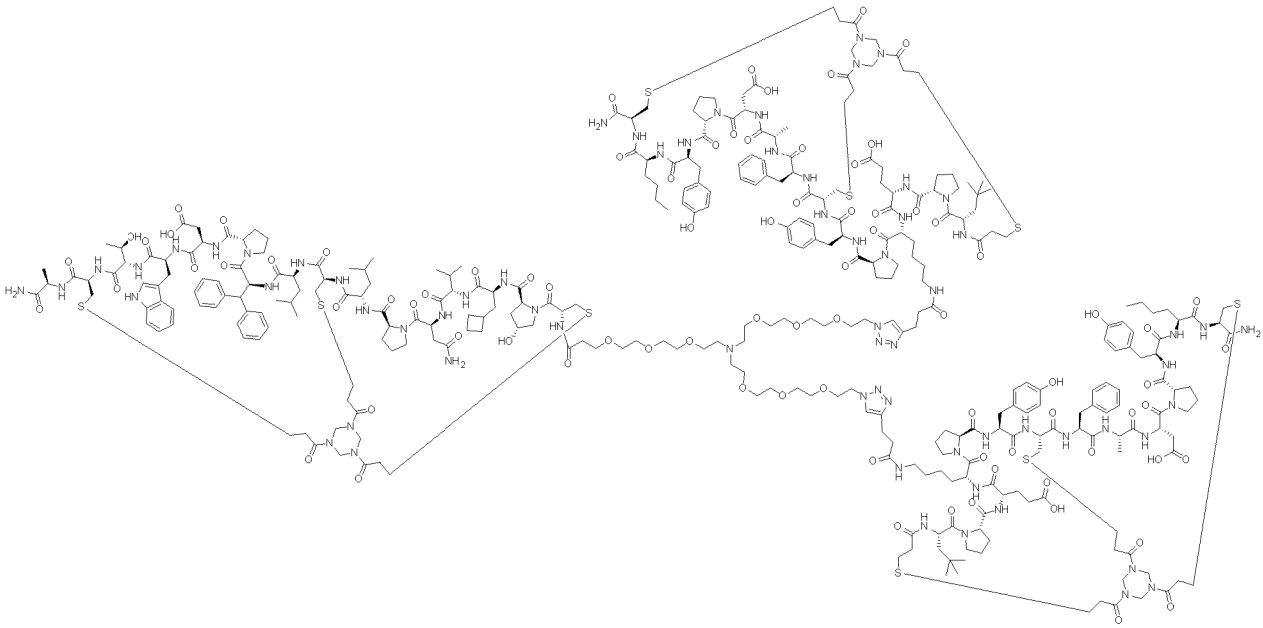


1

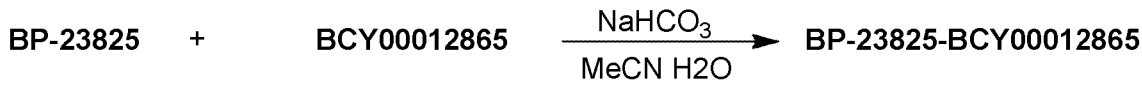
Compound **1** (15.9 mg, 5.78 μmol, 1.0 eq) and **BCY8928** (26.0 mg, 11.72 μmol, 2.1 eq) were first dissolved in 2 mL of t-BuOH/H_2O (1:1), and then $CuSO_4$ (0.4 M, 29.0 μL, 2.0 eq), VcNa (4.6 mg, 23.2 μmol, 4.0 eq) and THPTA (5.1 mg, 11.7 μmol, 2.0 eq) were added.

Finally, 0.2 M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2 3 times. The reaction mixture was stirred at 40°C for 16 hr under N_2 atmosphere. LC-MS showed compound **1** was consumed completely and one main peak with desired m/z (calculated MW: 7185.38, observed *m/z*: 1796.7 ([M/4+H]$^+$)) was detected. The reaction mixture was purified by prep-HPLC (TFA condition) and **BCY13053** (21.8 mg, 3.03 μmol, 52.84% yield, 98.01% purity) was obtained as a white solid.

Example 7: Synthesis of BCY13341



Procedure for preparation of BP-23825-BCY12865



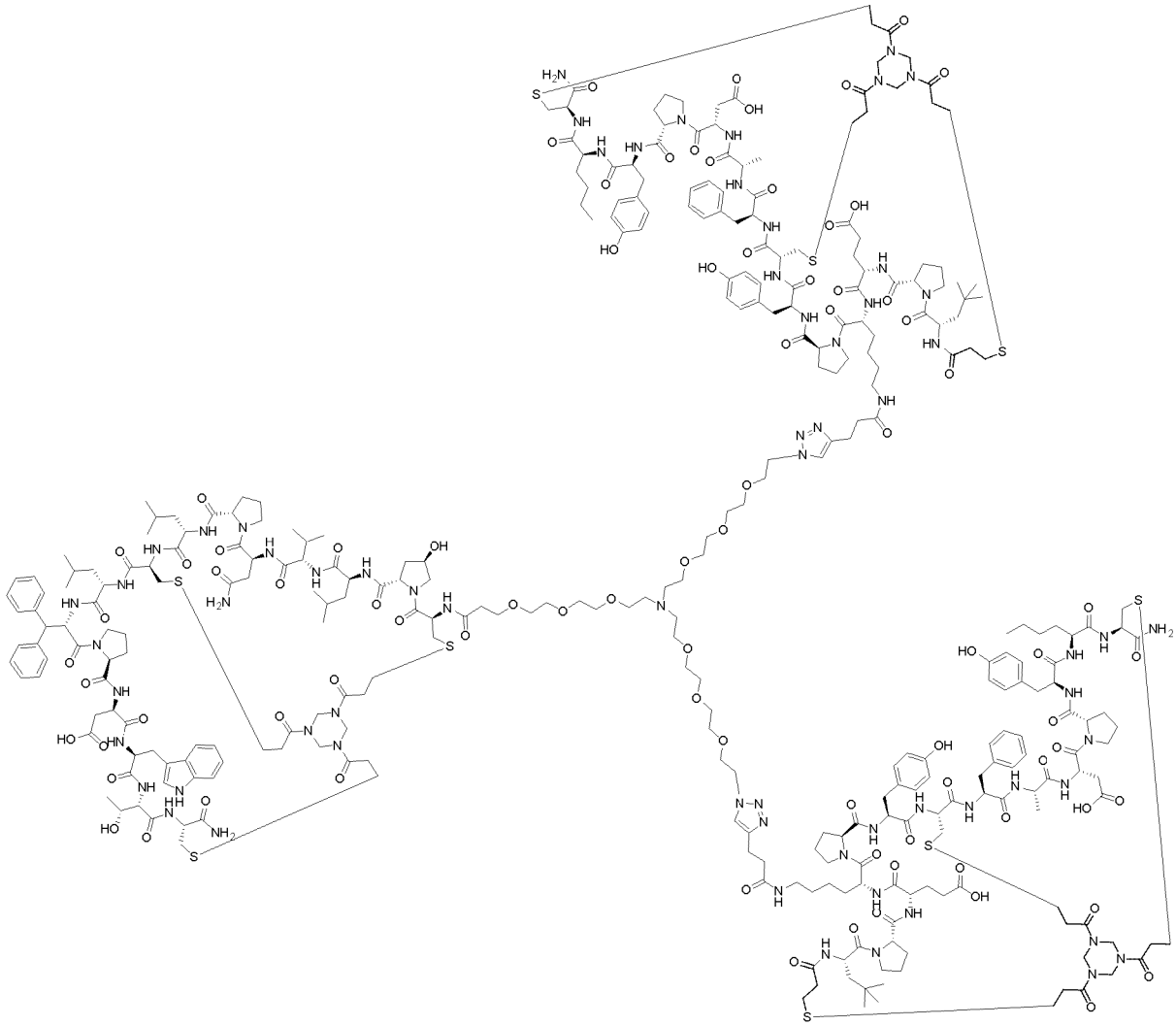
1 **2**

A mixture of **compound 1** (14.0 mg, 22.45 μmol, 1.20 eq.) and HATU (8.5 mg, 22.37 μmol, 1.20 eq.) was dissolved in NMP (0.3 mL), then the pH of this solution was adjusted to 8 by dropwise addition of DIEA (5.8 mg, 44.86 μmol, 7.8 μL, 2.40 eq.), and then the solution was activated at 25 °C for 6 min. **BCY12865** (40.0 mg, 18.65 μmol, 1.00 eq.) was dissolved in NMP (0.2 mL), and then added to the activated solution dropwise. The pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C for 0.5 hr. LC-MS showed **BCY12865** was consumed completely and one main peak with desired m/z (MW: 2750.21, observed *m/z*: 1375.5 ([(M/2+H$^+$]) and 917.3 ([(M/3+H$^+$])) was detected. The reaction mixture was concentrated under reduced pressure to remove solvent and produced a residue. The residue was then purified by prep-HPLC (neutral condition). **Compound 2** (20.6 mg, 7.24 μmol, 38.83% yield, 95.51% purity) was obtained as a white solid.

Procedure for preparation of BCY13341

A mixture of **compound 2** (20.6 mg, 7.49 μmol, 1.00 eq.), **BCY12353** (31.5 mg, 15.08 μmol, 2.01 eq), and THPTA (7.0 mg, 16.11 μmol, 2.15 eq) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 37.5 μL, 2.00 eq) and VcNa (6.0 mg, 30.29 μmol, 4.04 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned to light yellow. The reaction mixture was stirred at 40 °C for 1 hr under N_2 atmosphere. LC-MS showed one main peak with desired m/z (calculated MW: 6929.13, observed *m/z*: 1386.5($[M/5+H]^+$) and 1155.8($[M/6+H]^+$)). The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (first run in TFA condition and second run in AcOH condition), and **BCY13341** (10.3 mg, 1.49 μmol, 19.85% yield, 93.48% purity) was obtained as a white solid.

Example 8: Synthesis of BCY13343



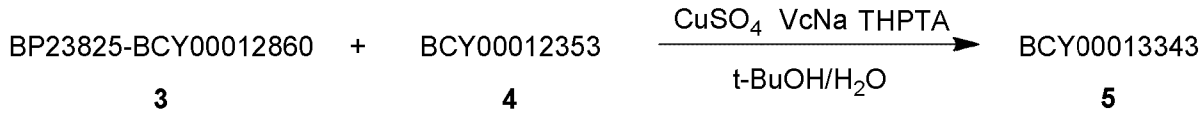
Procedure for preparation of BP-23825-BCY12860

BP-23825 + **BCY00012860** $\xrightarrow[\text{NMP}]{\text{HATU DIEA}}$ **BP-23825-BCY00012860**

1 **2** **3**

A mixture of **BP-23825** (13.0 mg, 20.84 μmol, 1.2 eq.), and HATU (8.0 mg, 20.84 μmol, 1.2 eq.) was dissolved in NMP (0.3 mL), then the pH of this solution was adjusted to 8 by dropwise addition of DIEA (5.4 mg, 41.69 μmol, 7.3 μL, 2.4 eq.), and then the solution was activated at 25 °C for 5 min. Compound **2** (35.8 mg, 17.37 μmol, 1.0 eq.) was dissolved in NMP (0.5 mL), and then dropped to the activated solution, the pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25°C for 0.5 hr. LC-MS showed **BCY12860** was consumed completely and one main peak with desired m/z (MW: 2667.12, observed *m/z*: 1334.4 ([(M/2+H^+])). The reaction mixture was concentrated under reduced pressure to remove solvent and produced a residue. The residue was then purified by prep-HPLC (neutral condition). **BP-23825-BCY12860** (25.2 mg, 9.16 μmol, 52.76% yield, 97.0% purity) was obtained as a white solid.

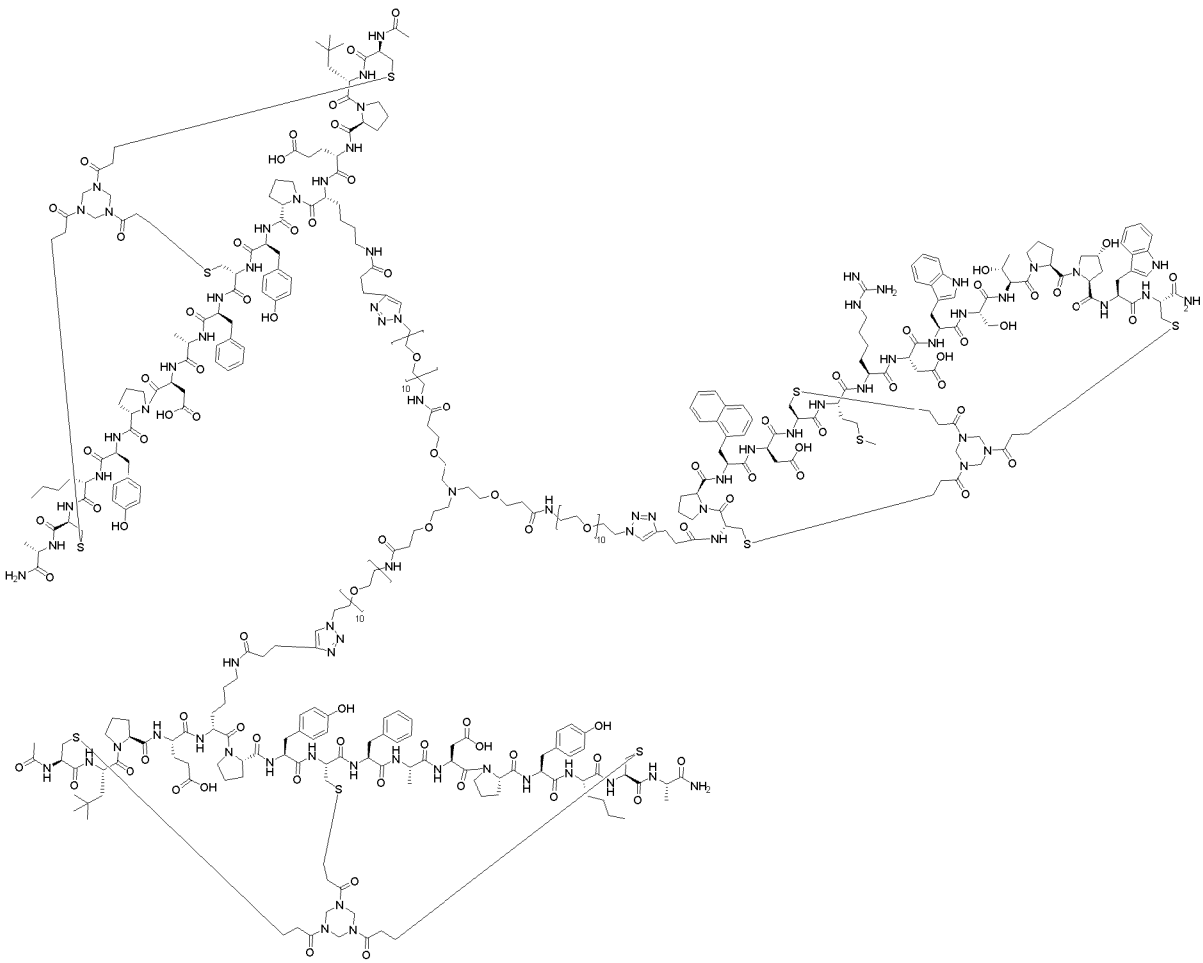
Procedure for preparation of BCY13343



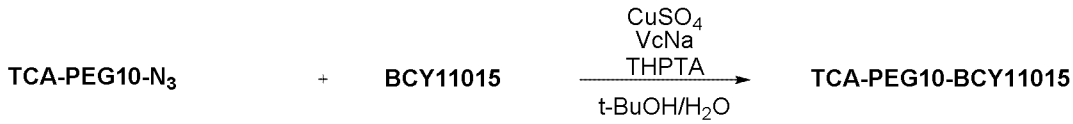
3 **4** **5**

A mixture of compound **3** (25.2 mg, 9.45 μmol, 1.0 eq.), compound **4** (40.4 mg, 19.37 μmol, 2.05 eq.), and THPTA (9.5 mg, 21.73 μmol, 2.3 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2 3 times), and then $CuSO_4$ (0.4 M, 54.3 μL, 2.3 eq.) and VcNa (8.7 mg, 43.51 μmol, 2.5 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 25 °C for 1 hr under N_2 atmosphere. LC-MS showed compound **3** was also consumed completely and one main peak with desired m/z (calculated MW: 6846.04, observed *m/z*: 1370.3 ([M/5+H^+])) was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (TFA condition), and **BCY13343** (28.2 mg, 3.61μmol, 38.23% yield, 87.7% purity) was obtained as a white solid.

Example 9: Synthesis of BCY11027



Procedure for preparation of TCA-PEG10-BCY11015



1 **2**

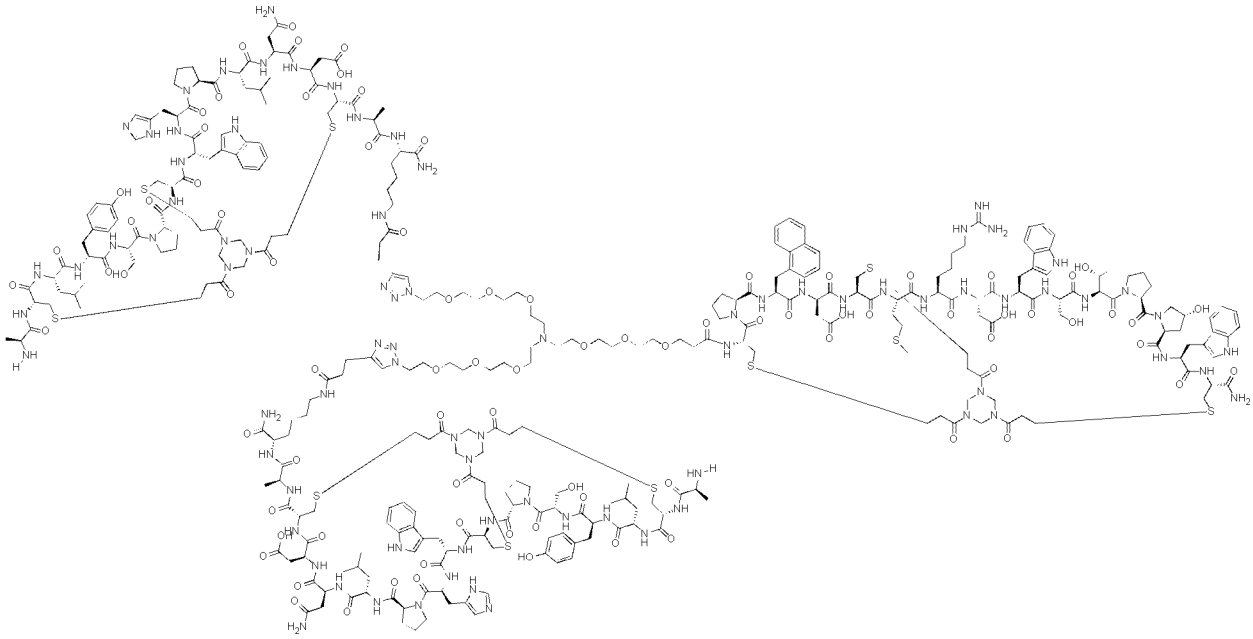
TCA-PEG10-N_3 (22.0 mg, 10.58 μmol, 1.0 eq) and **BCY11015** (26.0 mg, 34.72 μmol, 1.1 eq) were first dissolved in 2 mL of t-BuOH/H_2O (1:1), and then $CuSO_4$ (0.4 M, 26.4 μL, 1.0 eq), VcNa (4.2 mg, 21.2 μmol, 2.0 eq) and THPTA (4.6 mg, 10.58 μmol, 1.0 eq) was added. Finally, 1 M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2 for 3 times. The reaction mixture was stirred at 30°C for 16 hr under N_2 atmosphere. LC-MS showed one main peak with desired m/z (calculated MW: 4143.75, observed *m/z*: 1040.50 ([(M+18]/4+H]$^+$), and 1381.27([M/3+H]$^+$)). The reaction mixture was purified by prep-HPLC (TFA condition) and **TCA-PEG10-BCY11015** (11.0 mg, 2.50 μmol, 23.66% yield, 94.26% purity) was obtained as a white solid.

Procedure for preparation of BCY11027

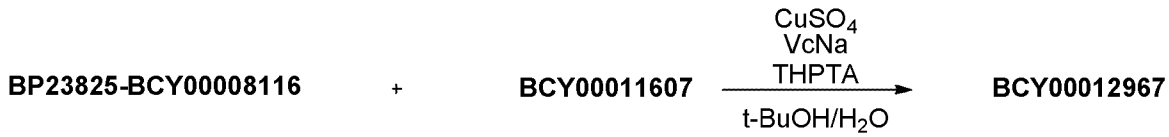
2

Compound 2 (5.5 mg, 1.33 μmol, 1.0 eq) and **BCY8928** (5.9 mg, 2.66 μmol, 2.0 eq) were first dissolved in 2 mL of t-BuOH/H_2O (1:1), and then $CuSO_4$ (0.4 M, 10.0 μL, 3.0 eq), VcNa (1.0 mg, 5.05 μmol, 3.8 eq) and THPTA (1.0 mg, 2.30 μmol, 1.7 eq) were added. Finally, 1 M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2 for 3 times. The reaction mixture was stirred at 35°C for 16 hr under N_2 atmosphere. LC-MS showed **compound 2** was consumed completely and one main peak with desired m/z (calculated MW: 8578.91, observed *m/z*: 1430.6([M/6+H]$^+$)). The reaction mixture was purified by prep-HPLC (TFA condition) and **BCY11027** (2.8 mg, 0.32 μmol, 24.5% yield, 91.71% purity) was obtained as a white solid.

Example 10: Synthesis of BCY12967



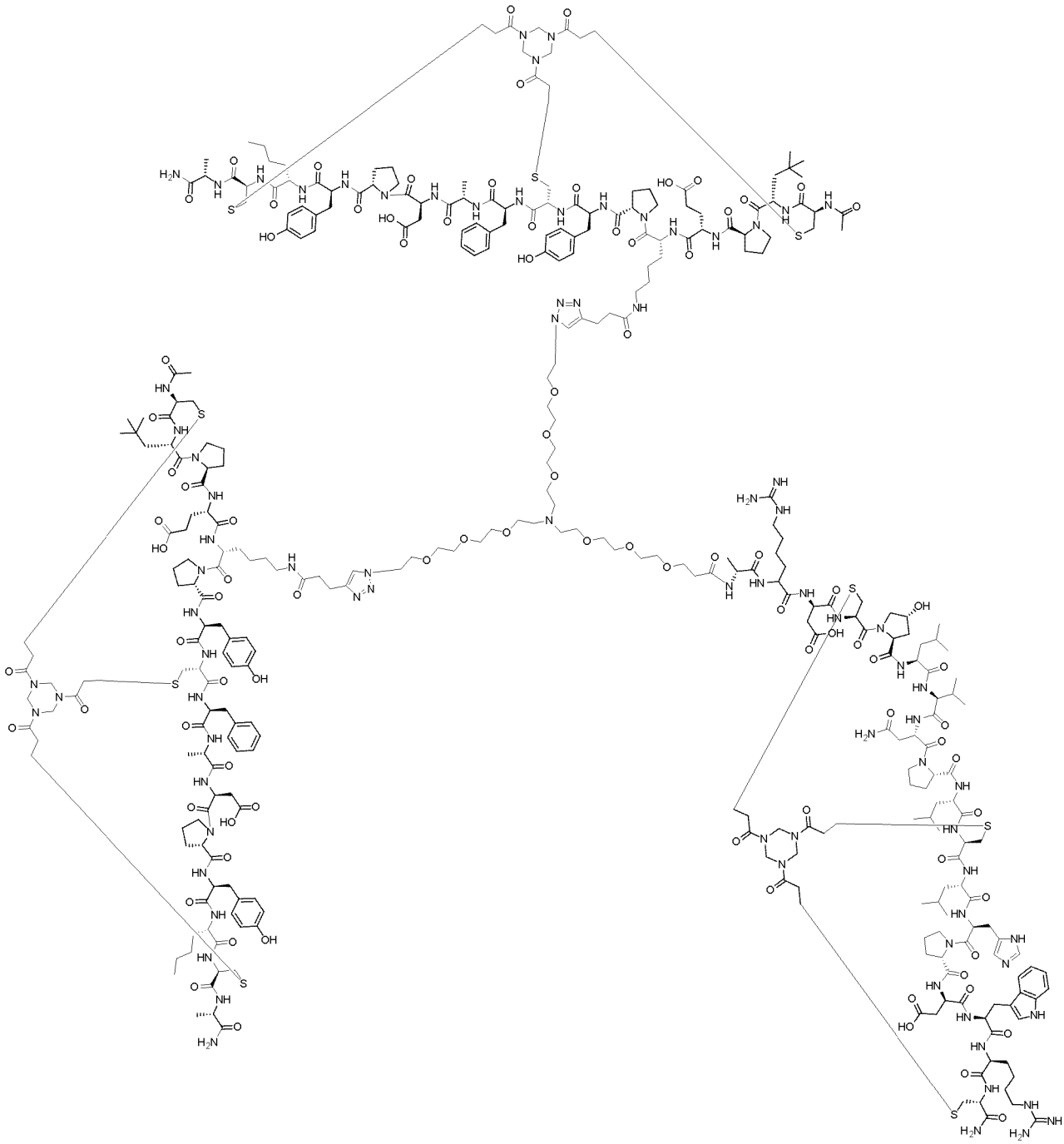
BCY00012967



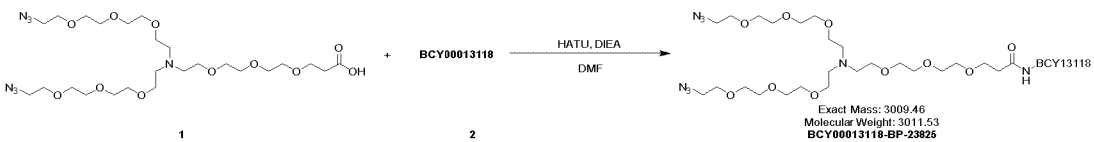
1 **2**

Compound **1** (20.0 mg, 7.20 μmol, 1.0 eq) and **BCY11607** (32.0 mg, 14.9 μmol, 2.1 eq) were first dissolved in 2 mL of t-BuOH/H_2O (1:1), and then $CuSO_4$ (0.4 M, 36.0 μL, 2.0 eq), VcNa (6.0 mg, 30.3 μmol, 4.2 eq) and THPTA (6.4 mg, 14.7 μmol, 2.0 eq) were added. Finally 1 M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2 for 3 times. The reaction mixture was stirred at 40°C for 16 hr under N_2 atmosphere. LC-MS showed compound **2** was consumed completely and one main peak with desired m/z (calculated MW: 7077.7 observed *m/z*: 1416.3 ($[M/5+H]^+$), 1180.4 ($[M/6+H]^+$), 1011.9 ($[M/7+H]^+$)). The reaction mixture was purified by prep-HPLC (TFA condition) and **BCY12967** (20.6 mg, 2.82 μmol, 39.17% yield, 96.82% purity) was obtained as a white solid.

Example 11: Synthesis of BCY13272



***General procedure for preparation of* BP-23825-BCY13118**

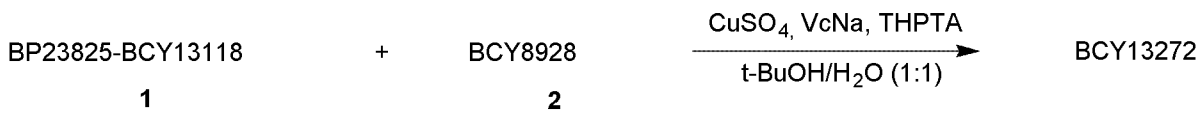


1 2

A mixture of 1 (**BP-23825**, 155.5 mg, 249.40 μmol, 1.2 eq.), and HATU (95.0 mg, 249.92 μmol, 1.2 eq.) was dissolved in NMP (1.0 mL), then the pH of this solution was adjusted to 8 by dropwise addition of DIEA (64.6 mg, 499.83 μmol, 87.0 μL, 2.4 eq.), and then the solution was allowed to stir at 25 °C for 5 min. Compound 2 (**BCY13118**, 500.0 mg, 207.83 μmol, 1.0

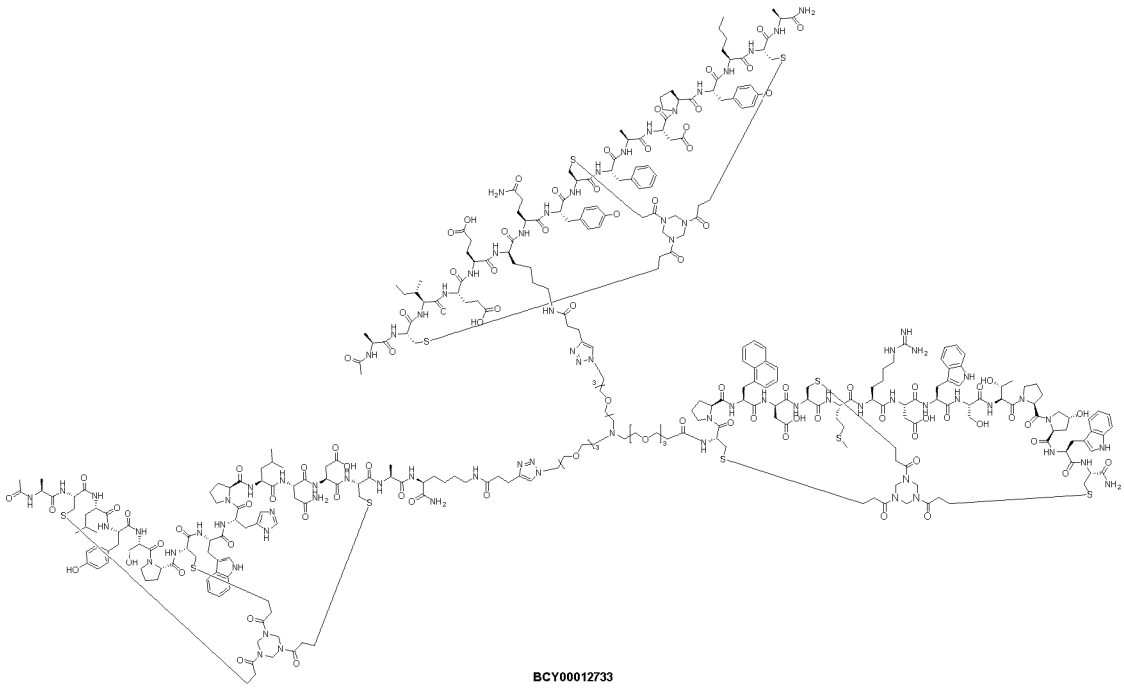
eq.) was dissolved in NMP (5.0 mL), and then added to the reaction solution, the pH of the resulting solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C for 45 min. LC-MS showed **BCY13118** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was concentrated under reduced pressure to remove solvent and produced a residue. The residue was then purified by preparative-HPLC to give **BP-23825-BCY13118** (1.35 g, 403.46 μmol, 64.71% yield, 90% purity) as a white solid. Calculated MW: 3011.53, observed m/z: 1506.8 ([M/2+H]+), 1005.0 ([M/3+H]+.

General procedure for preparation of compound BCY13272

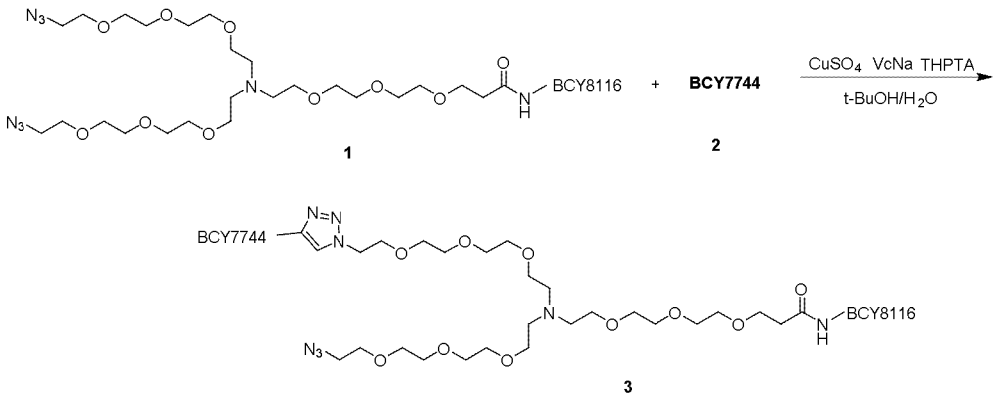


A mixture of **BCY8928** (644.0 mg, 290.55 μmol, 2.5 eq.), THPTA (50.5 mg, 116.22 μmol, 1.0 eq.), $CuSO_4$ (0.4 M, 145.0 μL, 0.5 eq.) and Vc (82.0 mg, 464.89 μmol, 4.0 eq.) were dissolved in t-BuOH/0.2 M NH4HCO3 (1:1, 6.0 mL), The pH of this solution was adjusted to 7.5 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/0.2 M NH_4HCO_3), and then the solution was stirred at 25 °C for 3 min. **BP-23825-BCY13118** (350.0 mg, 116.22 μmol, 1.0 eq.) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1, 11.0 mL), and then dropped into the stirred solution. All solvents here were pre-degassed and purged with N_2 3 times. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/0.2 M NH_4HCO_3), and the solution turned light yellow. The reaction mixture was stirred at 25 °C for 6 hr under N_2 atmosphere. LC-MS showed one main product peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC (TFA condition), and **BCY13272** (1.75 g, 235.01 μmol, 67.40% yield, 94% purity) was obtained as a white solid. Calculated MW: 7446.64, observed m/z: 1242.0 ([M/6+H]+), 1491.0 ([M/5+H]+.

Example 12: Synthesis of BCY12733

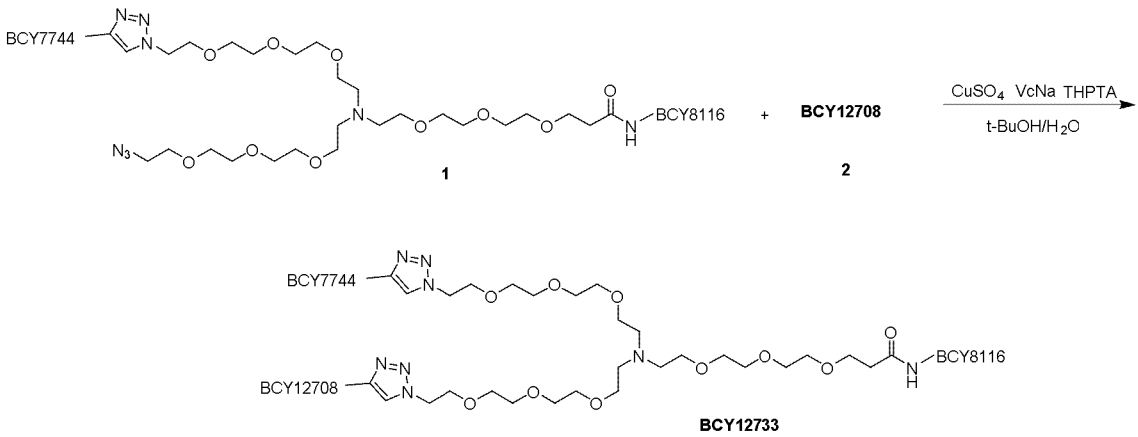


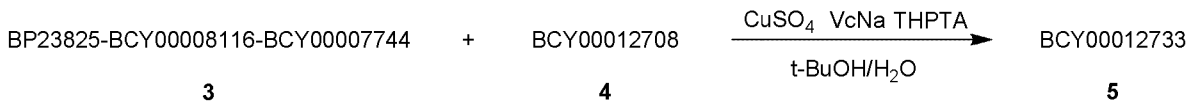
Procedure for preparation of BP23825-BCY8116-BCY7744



A mixture of compound **1** (10.0 mg, 3.60 μmol, 1.0 eq.), compound **2** (6.73 mg, 2.88 μmol, 0.8 eq.), and THPTA (3.0 mg, 6.90 μmol, 2.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 0.5 mL, degassed and purged with N_2), and then aqueous solution of $CuSO_4$ (0.4 M, 9 μL, 1.0 eq.) and VcNa (2.0 mg, 10.10 μmol, 2.8 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 1 hr under N_2 atmosphere. LC-MS showed compound **2** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and compound **3** (5.0 mg, 0.93 μmol, 25.88% yield, 95.33% purity) was obtained as a white solid. Calculated MW: 5115.80, observed *m/z*: 1278.95 ($[M+4H]^{4+}$).

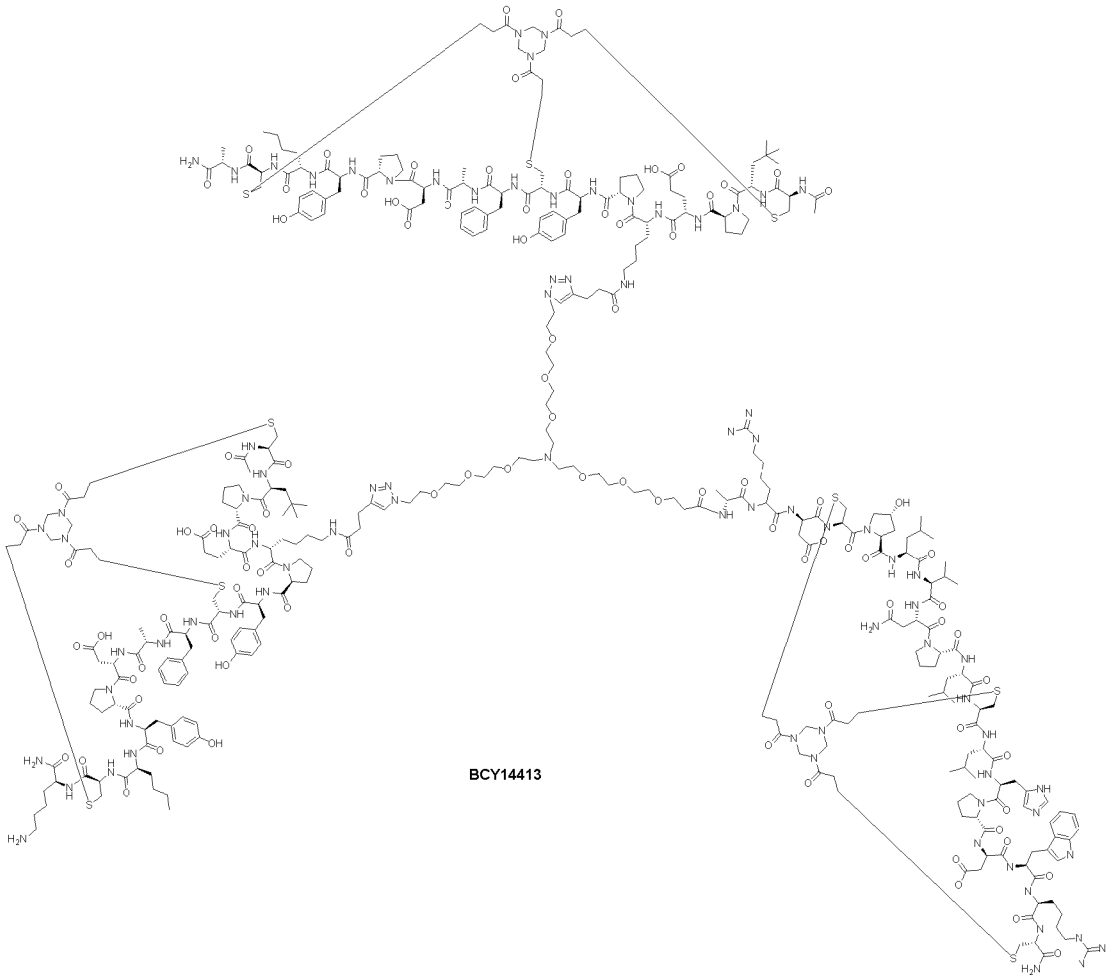
Procedure for preparation of BCY12733



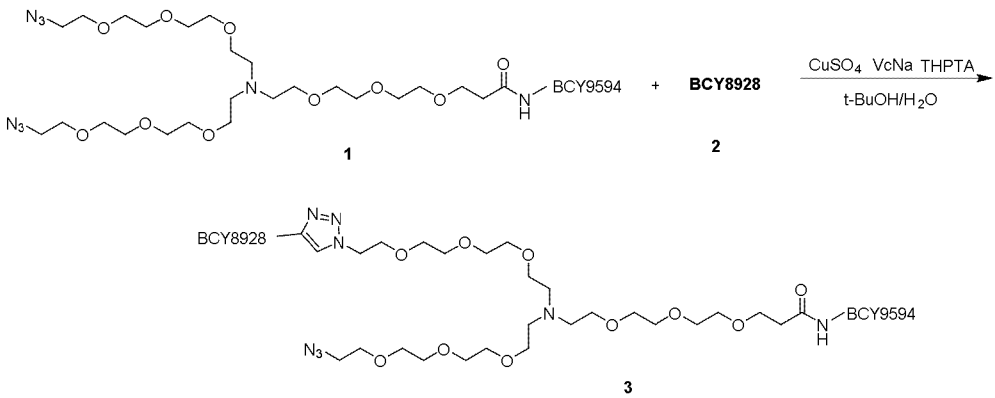


A mixture of compound **1** (5.0 mg, 9.77 μmol, 1.0 eq.), compound **2** (2.4 mg, 1.08 μmol, 1.1 eq.), and THPTA (0.4 M, 3 μL, 1.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 0.5 mL, degassed and purged with N_2), and then aqueous solution of $CuSO_4$ (0.4 M, 3 μL, 1.0 eq.) and VcNa (0.4 M, 3 μL, 1.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 1 hr under N_2 atmosphere. LC-MS showed compound **3** and compound **4** also remained, and desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY12733** (3.3 mg, 0.41 μmol, 42.42% yield, 94.60% purity) was obtained as a white solid. Calculated MW: 7307.33, observed *m/z*: 1827.1 ($[M+4H]^{4+}$), 1462.1 ($[M+5H]^{5+}$).

Example 13: Synthesis of BCY14413



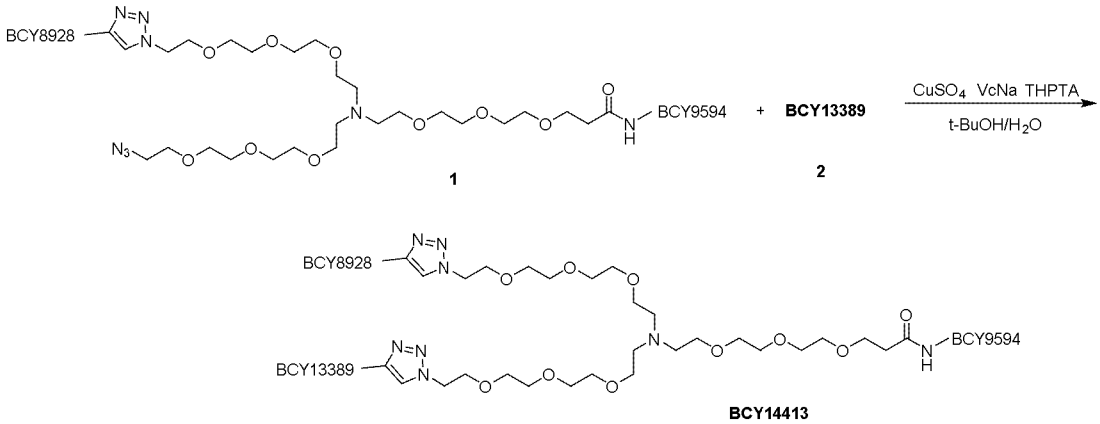
Procedure for preparation of BCY9594-BP-23825-BCY8928



A mixture of compound **1** (50.0 mg, 16.6 μmol, 1.0 eq.), compound **2** (29.5 mg, 13.3 μmol, 0.8 eq.), and THPTA (36.1 mg, 83.1 μmol, 5.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 8 mL, degassed and purged with N_2), and then aqueous solution of $CuSO_4$ (0.4 M, 20.8 μL, 0.5 eq.) and VcNa (65.9 mg, 332.6 μmol, 20.0 eq.) were added under N_2. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.5 mL 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. Then the reaction mixture was stirred at 25 °C for 24 hr under N_2 atmosphere. The reaction was set up for two batches in parallel. LC-MS showed compound **1** and little amount of compound **2** remained, and desired m/z was detected. The

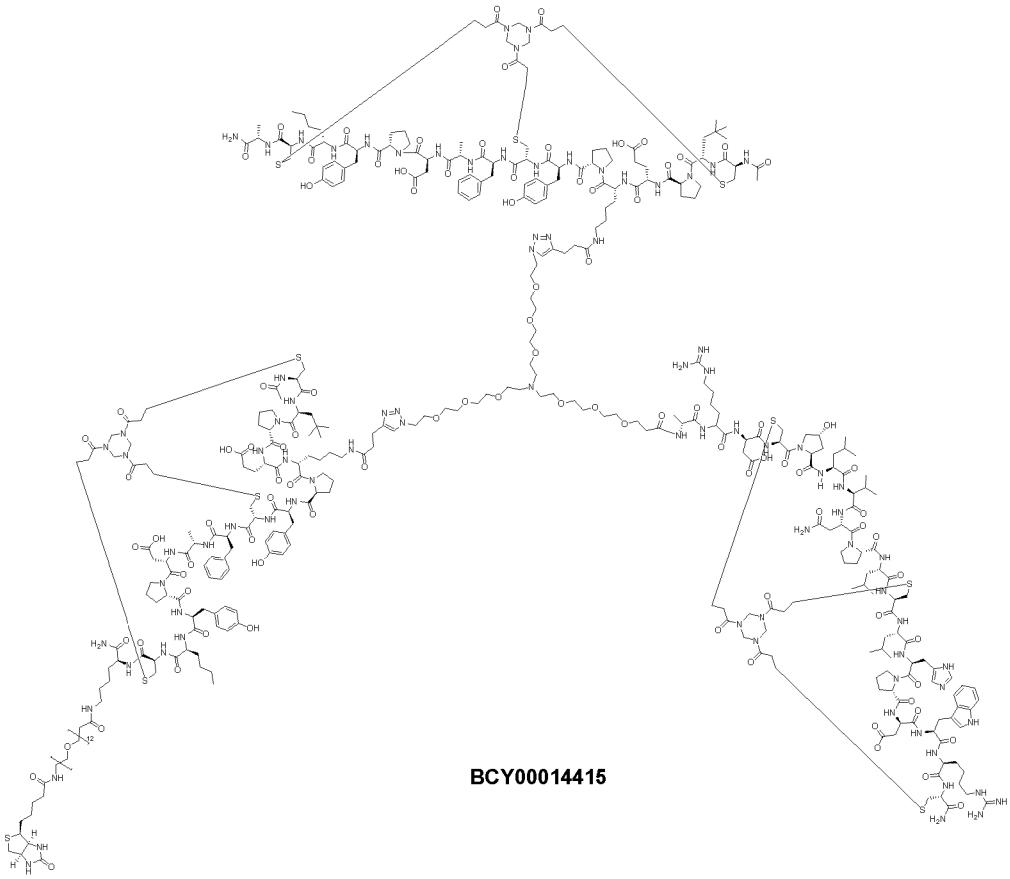
reaction mixture was filtered to remove the insoluble residue. The crude product was purified by preparative HPLC, and compound **3** (31.5 mg, 5.44 μmol, 16.36% yield, 90.22% purity) was obtained as a white solid. Calculated MW: 5224.07, observed m/z: 1306.9 ($[M+4H]^{4+}$), 871.6 ($[M+6H]^{6+}$).

Procedure for preparation of BCY14413

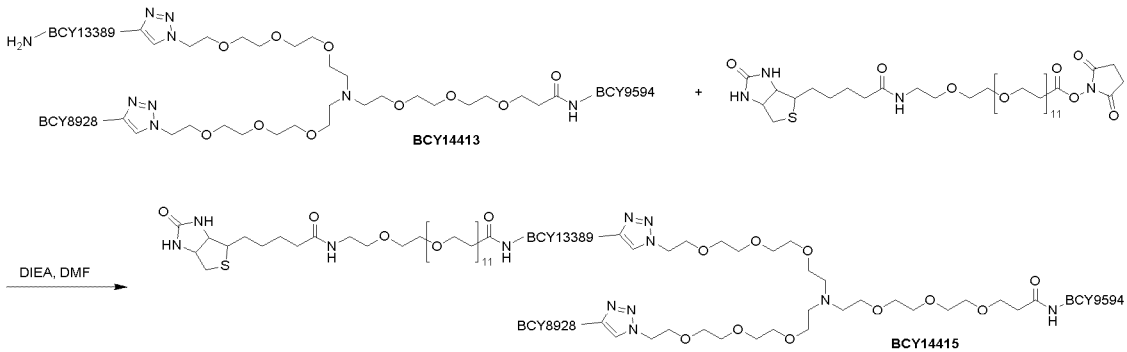


A mixture of compound **1** (31.5 mg, 6.03 μmol, 1.0 eq.), compound **2** (14.4 mg, 6.33 μmol, 1.05 eq.), and THPTA (2.62 mg, 6.03 μmol, 1.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 1.0 mL, degassed and purged with N_2), and then aqueous solution of $CuSO_4$ (0.4 M, 15.07 μL, 1.0 eq.) and VcNa (4.78 mg, 24.12 μmol, 4.0 eq.) were added under N_2. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.5 mL 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. Then the reaction mixture was stirred at 25 °C for 3 hrs under N_2 atmosphere. LC-MS showed little amount of compound **2** remained, compound **1** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered to remove the insoluble residue. The crude product was purified by preparative HPLC, and **BCY14413** (22.5 mg, 3.00 μmol, 43.10% yield, 86.63% purity) was obtained as a white solid. Calculated MW: 7498.75, observed *m/z*: 938.3 ($[M+8H]^{8+}$), 1072.2 ($[M+7H]^{7+}$), 1250.9 ($[M+6H]^{6+}$), 1500.8 ($[M+5H]^{5+}$).

Example 14: Synthesis of BCY14415

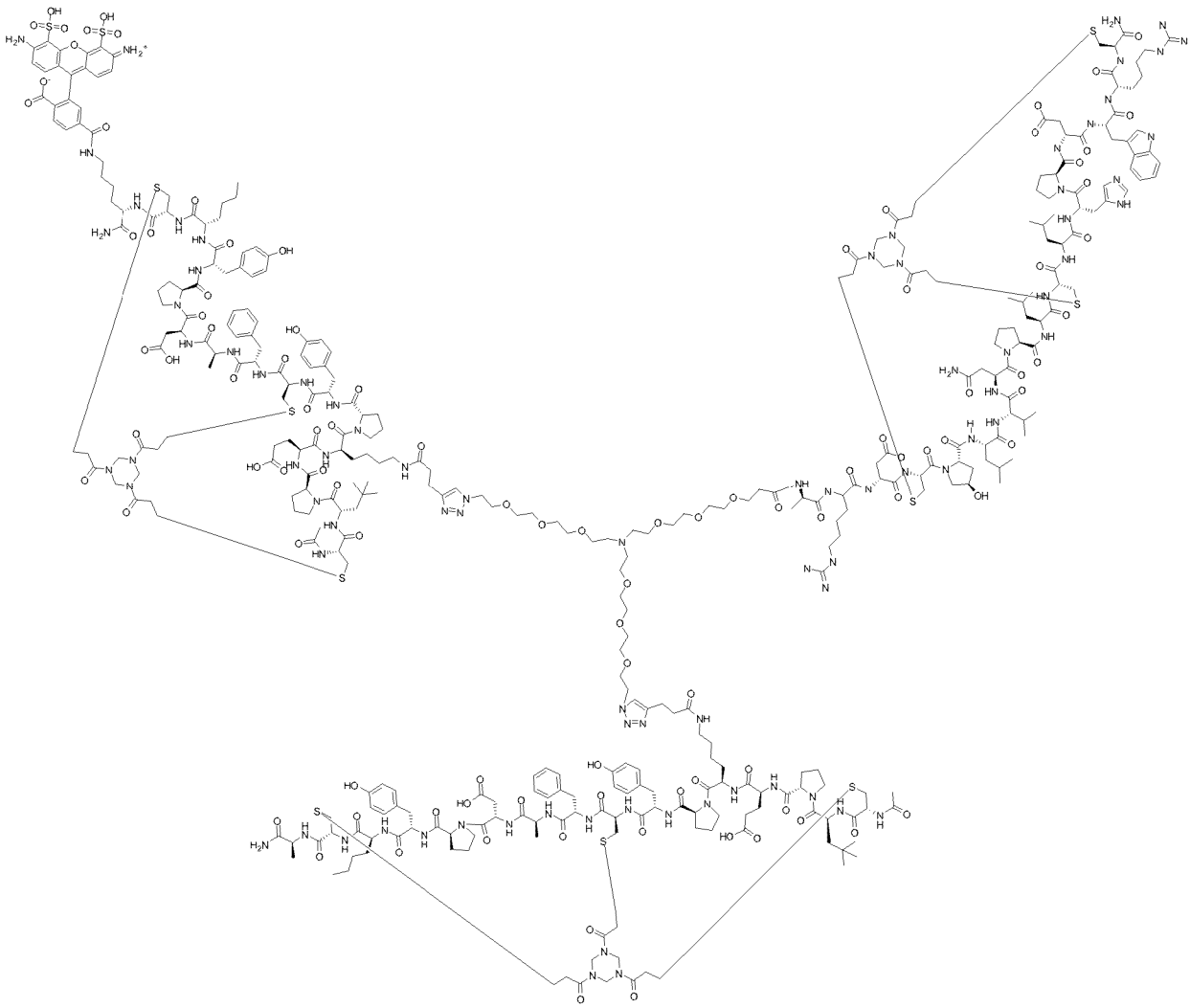


Procedure for preparation of BCY14415



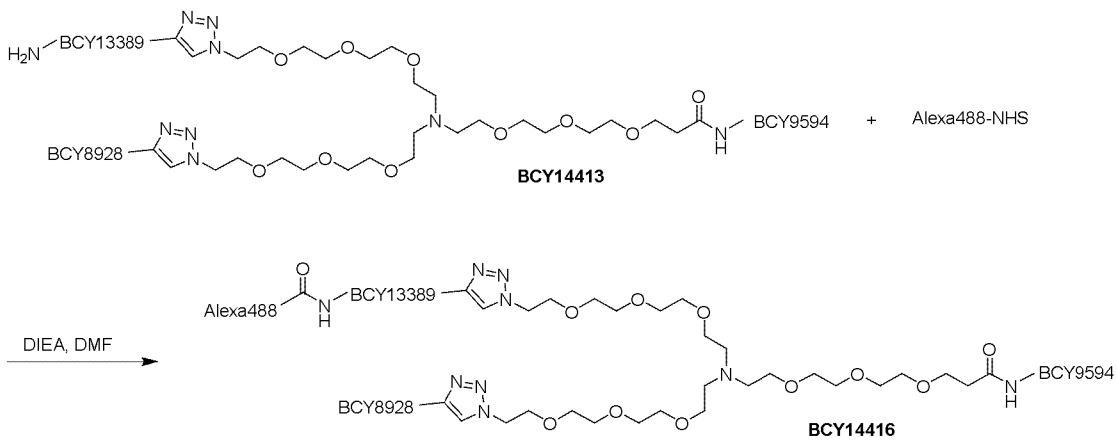
A mixture of **BCY14413** (10.0 mg, 1.33 μmol, 1.0 eq.) and biotin-Peg12-NHS (2.6 mg, 2.80 μmol, 2.6 eq.) was dissolved in DMF (0.3 mL). The pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C for 0.5 hr. LC-MS showed **BCY14413** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY14415** (10 mg, 1.07 μmol, 80.49% yield, 90.2% purity) was obtained as a white solid. Calculated MW: 8324.73, observed *m/z*: 1388.4 ($[M+6H]^{6+}$), 1190.2 ($[M+7H]^{7+}$), 1041.5 ($[M+8H]^{8+}$), 926.0 ($[M+9H]^{9+}$)

Example 15: Synthesis of BCY14416



BCY14416

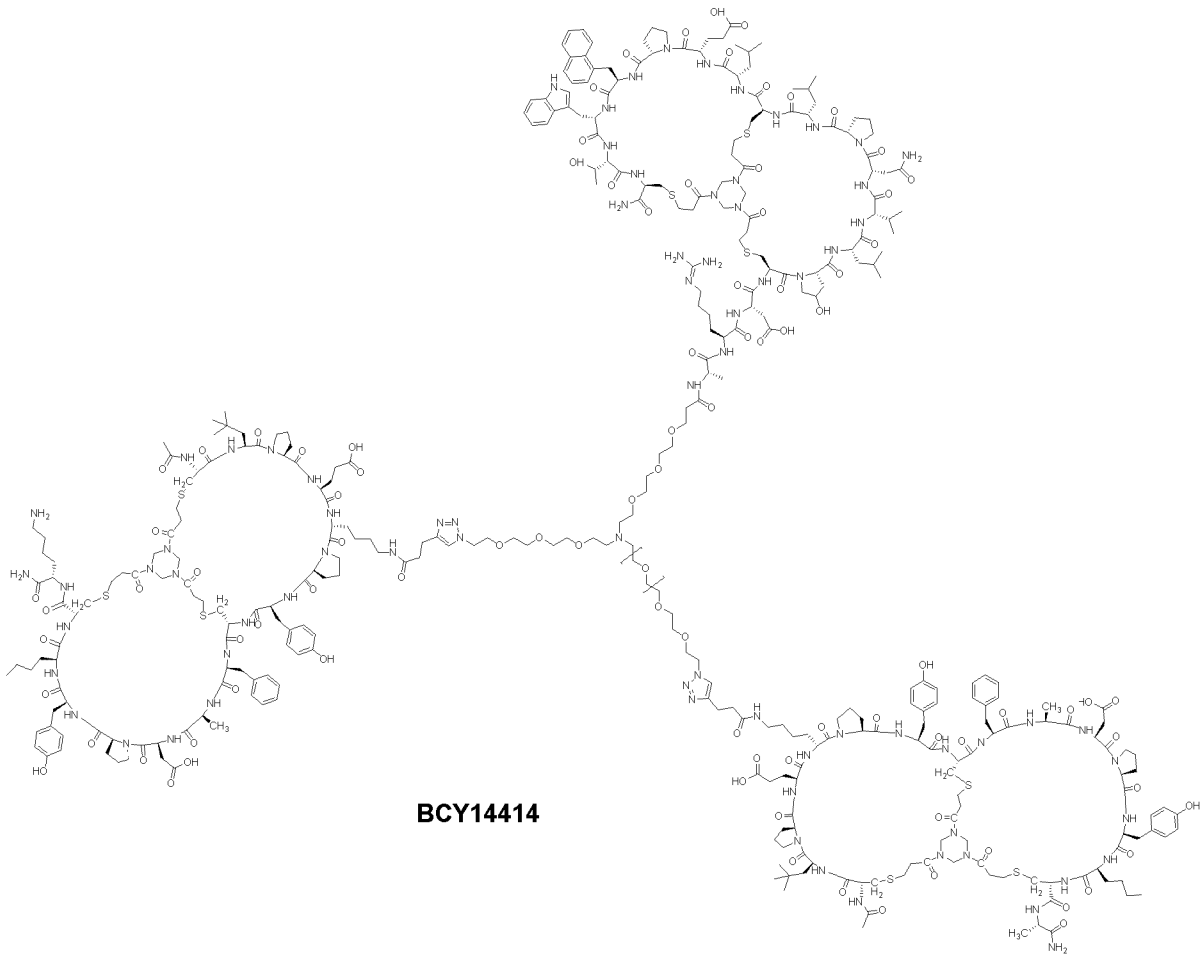
Procedure for preparation of BCY14416



A mixture of compound **BCY14413** (5.1 mg, 0.68 µmol, 1.0 eq.) and Alexa Fluor 488 NHS ester (0.5 mg, 8.16e-1 µmol, 1.2 eq.) was dissolved in DMF (0.3 mL). The pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C

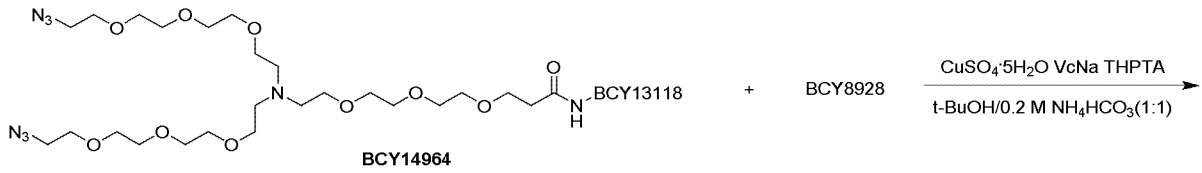
for 0.5 hr. LC-MS showed that some **BCY14413** remained and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and the main peak was collected as two fractions with different purity, and **BCY14416** (0.7 mg, 0.065 μmol, 9.84% yield, 96.4% purity) and (0.5 mg, 0.047 μmol, 7.03% yield, 91.2% purity) were obtained as red solid. Calculated MW: 8015, observed *m/z*: 1336.5 ($[M+7H]^{7+}$).

Example 16: Synthesis of BCY14414

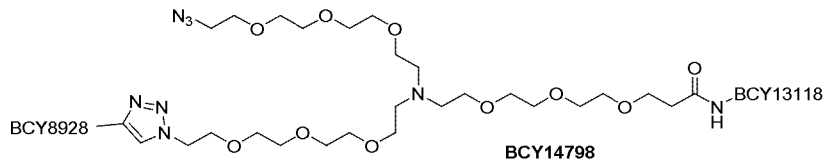


BCY14414

Procedure for preparation of BCY14798



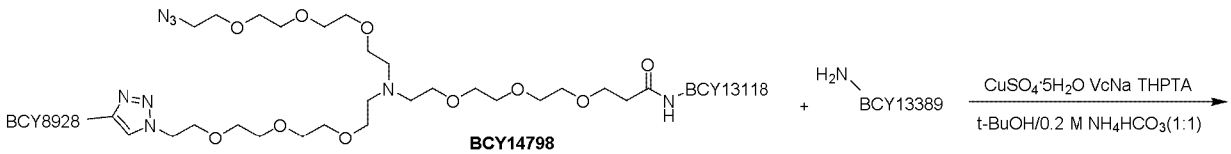
BCY14964



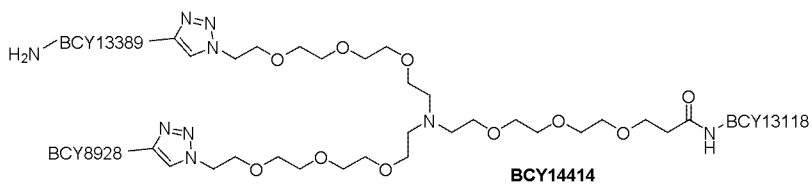
BCY14798

A mixture of **BCY14964** (55.0 mg, 18.26 μmol, 1.0 eq), **BCY8928** (32.4 mg, 14.61 μmol, 0.8 eq), and THPTA (39.8 mg, 91.32 μmol, 5.0 eq) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1, 0.5 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 23.0 μL, 0.5 eq) and sodium ascorbate (72.0 mg, 365.27 μmol, 20.0 eq) were added under N_2. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/0.2 M NH_4HCO_3), and the solution turned to light yellow. The reaction mixture was stirred at 25 °C for 1.5 h under N_2 atmosphere. LC-MS showed **BCY14964** remained, compound **BCY8928** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY14798** (51 mg, 9.17 μmol, 33.37% yield, 94% purity) was obtained as a white solid. Calculated MW: 5229.07, observed *m/z*: 1308.3 ($[M+4H]^{4+}$), 1046.7 ($[M+5H]^{5+}$).

Procedure for preparation of BCY14414



BCY14798

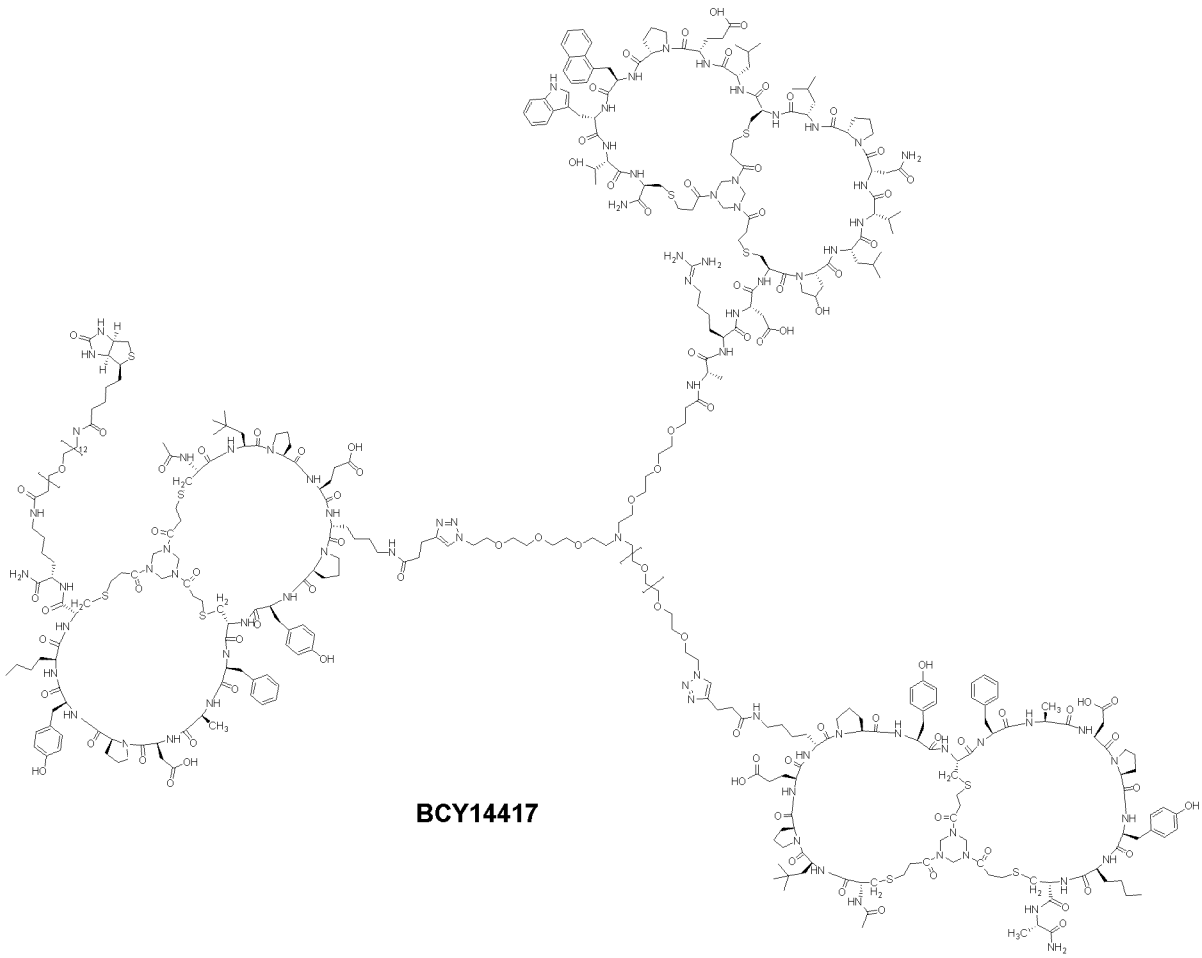


BCY14414

A mixture of **BCY14798** (21.0 mg, 4.02 μmol, 1.0 eq), **BCY13389** (10.0 mg, 4.42 μmol, 1.1 eq), and THPTA (1.8 mg, 4.02 μmol, 1.0 eq) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1, 0.5 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 5.0 μL, 0.5 eq) and sodium ascorbate (2.8 mg, 16.06 μmol, 4.0 eq) were added under N_2. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/0.2 M NH_4HCO_3) and

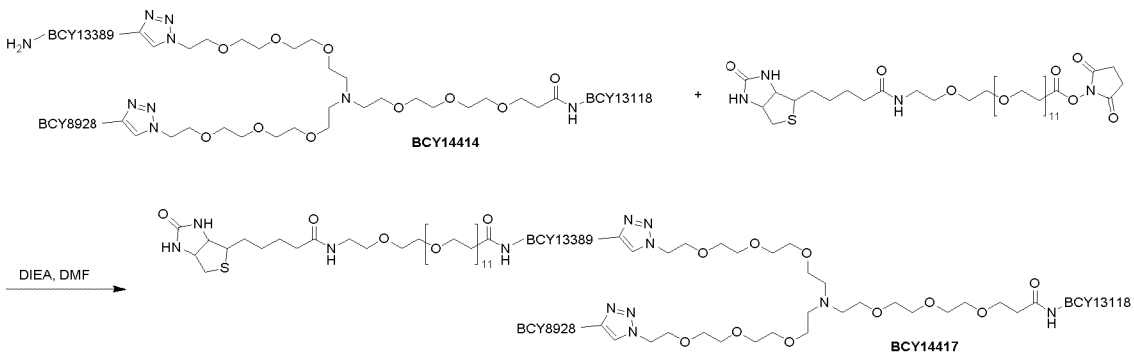
the solution turned to light yellow. The reaction mixture was stirred at 25 °C for 2 hr under N_2 atmosphere. LC-MS showed **BCY14798** was consumed completely, some **BCY13389** remained and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative and **BCY14414** (20 mg, 2.40 μmol, 59.73% yield, 90.9% purity) was obtained as a white solid. Calculated MW: 7503.74, observed *m/z*: 1251.5 ($[M+5H]^{5+}$), 1072.9 ($[M+7H]^{7+}$).

Example 17: Synthesis of BCY14417



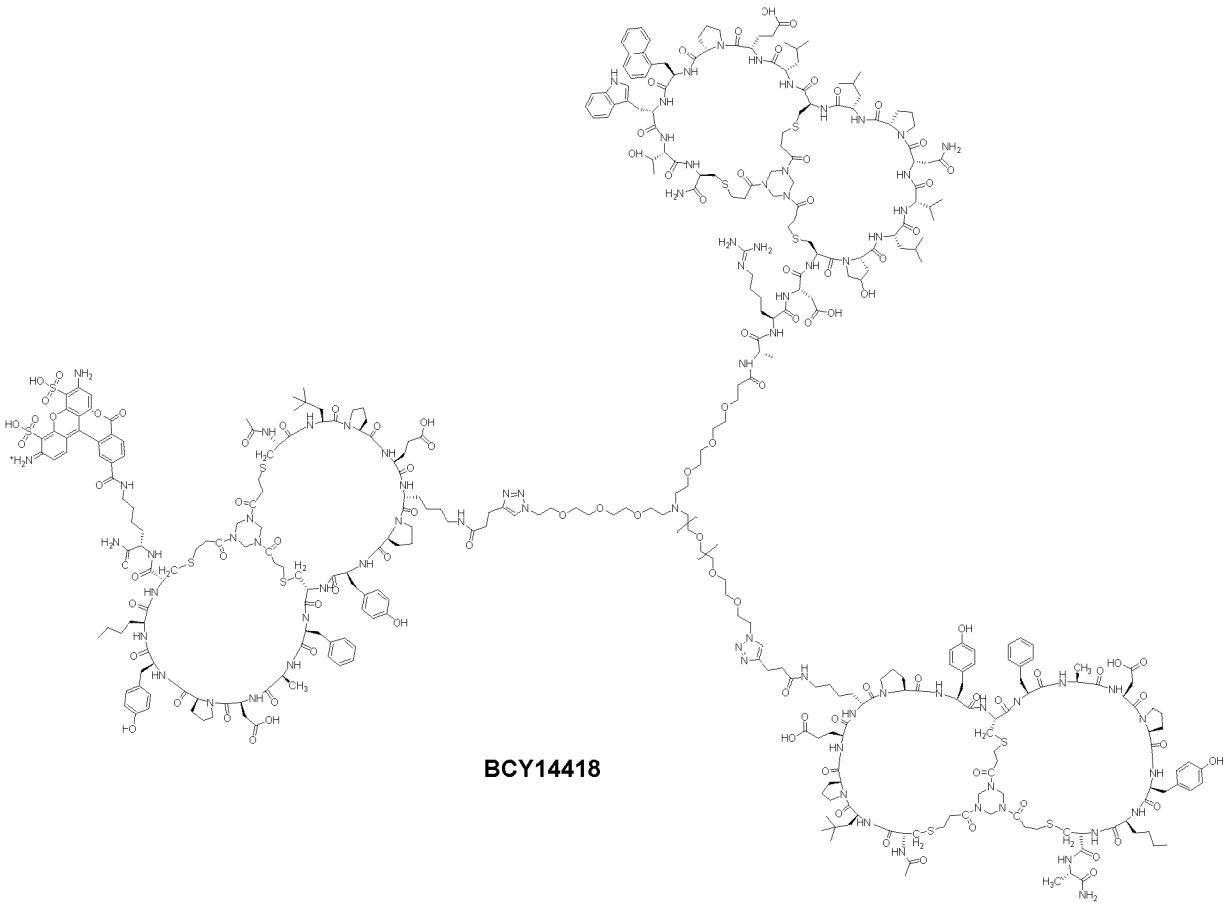
BCY14417

Procedure for preparation of BCY14417



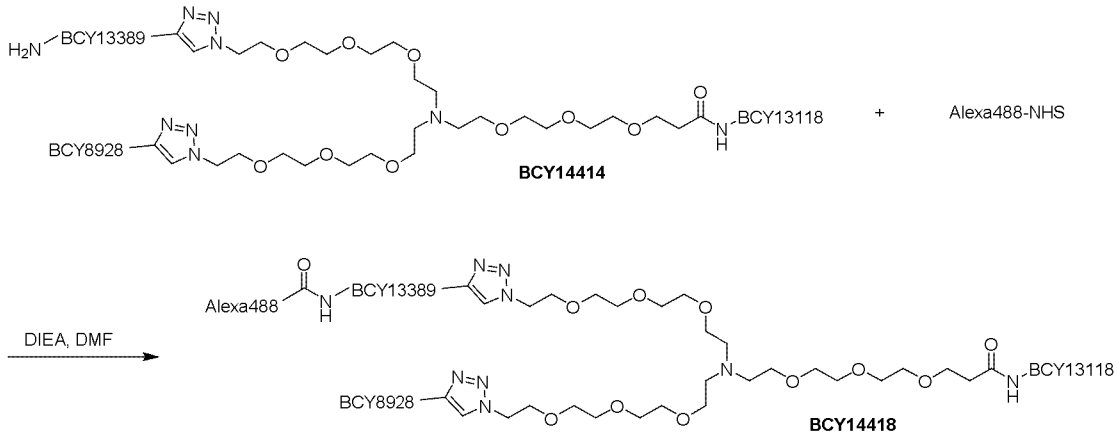
A mixture of **BCY14414** (13.0 mg, 1.73 μmol, 1.0 eq) and biotin-PEG12-NHS ester (CAS 365441-71-0, 4.2 mg, 4.50 μmol, 2.6 eq) was dissolved in DMF (0.5 mL). The pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C for 0.5 hr. LC-MS showed **BCY14414** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC and **BCY14417** (9.0 mg, 1.07 μmol, 80.49% yield, 90.8% purity) was obtained as a white solid. Calculated MW: 8329.74, observed *m/z*: 1389.6 ($[M+6H]^{6+}$), 1191.9 ($[M+7H]^{7+}$).

Example 18: Synthesis of BCY14418



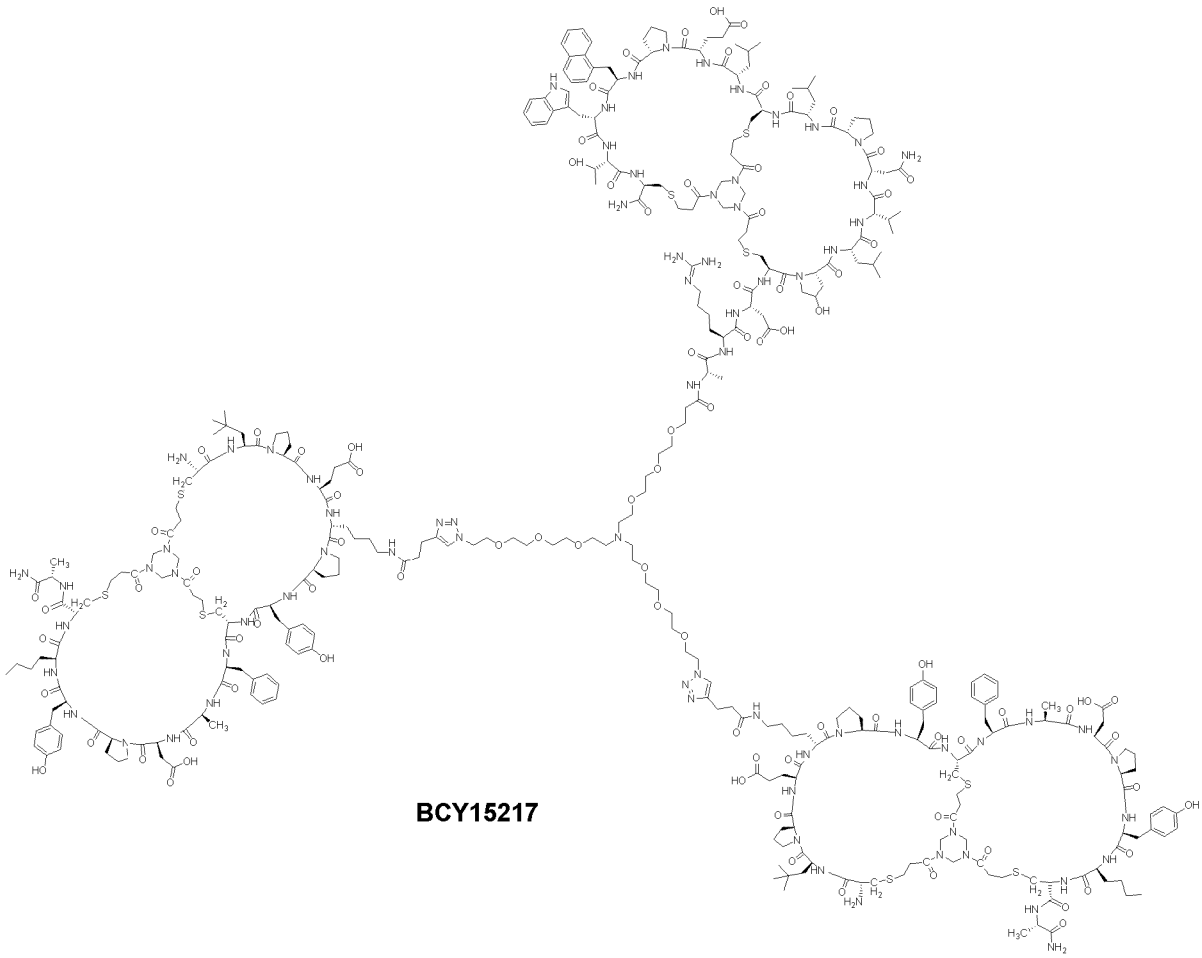
BCY14418

Procedure for preparation of BCY14418



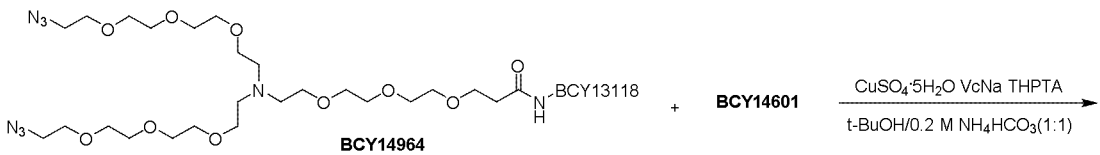
A mixture of **BCY14414** (5.6 mg, 0.75 μmol, 1.0 eq) and Alexa fluor® 488 (0.9 mg, 1.49 μmol, 2.0 eq) was dissolved in DMF (0.3 mL). Then pH of this solution was adjusted to 8 by dropwise addition of DIEA. The reaction mixture was stirred at 25 °C for 1.0 hr. LC-MS showed **BCY14414** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY14418 (**2.3 mg, 0.25 μmol, 32.89% yield, 85.6% purity) was obtained as a red solid. Calculated MW: 8020.19, observed *m/z*: 1337.2 ($[M+6H]^{6+}$).

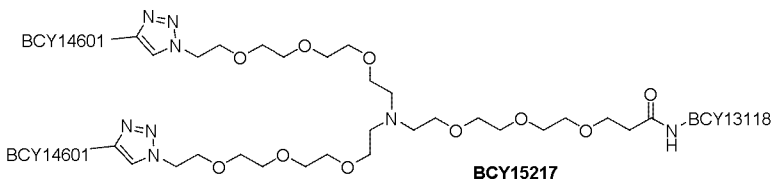
Example 19: Synthesis of BCY15217



BCY15217

Procedure for preparation of BCY15217

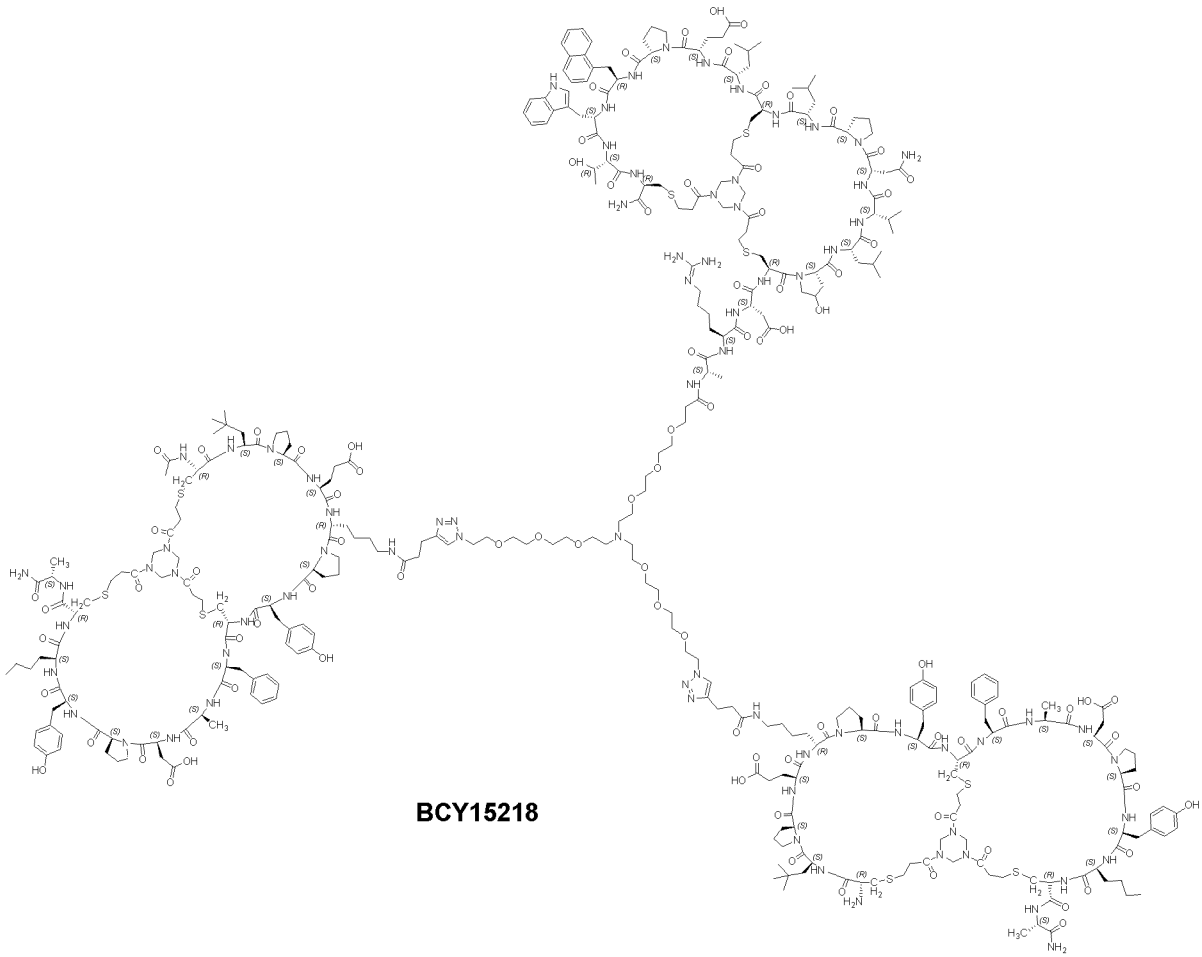




A mixture of **BCY14964** (20.0 mg, 6.64 μmol, 1.0 eq), **BCY14601** (30.5 mg, 13.95 μmol, 2.1 eq), and THPTA (2.9 mg, 6.64 μmol, 1.0 eq) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1, 0.5 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 16.6 μL, 1.0 eq) and sodium ascorbate (4.7 mg, 26.56 μmol, 4.0 eq) were added under N_2. The pH of this solution was adjusted to 8, and the solution turned to light yellow. The reaction mixture was stirred at 25 °C for 2 hr under N_2 atmosphere. LC-MS showed **BCY14964** remained, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and

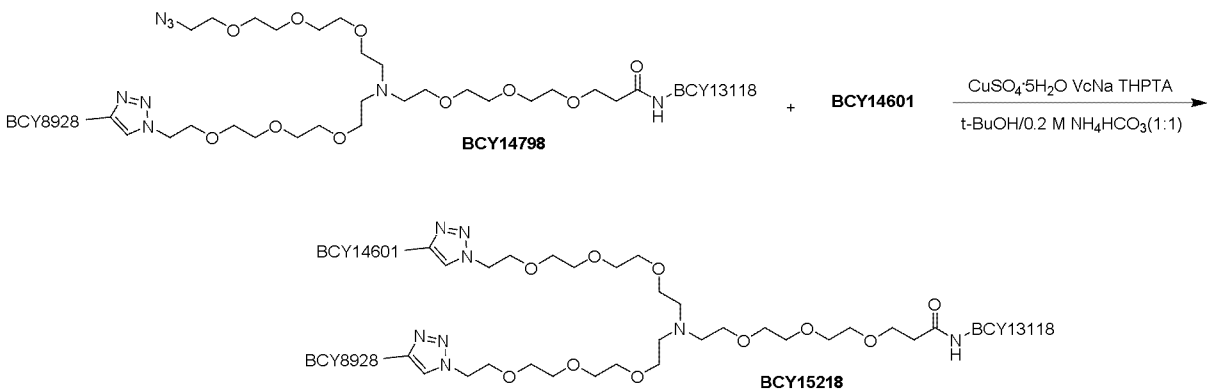
BCY15217 (19.7 mg, 2.41 µmol, 36.26% yield, 96.2% purity) was obtained as a white solid. Calculated MW: 7362.5, observed *m/z*: 1473.5 ($[M+5H]^{5+}$), 1228.2 ($[M+6H]^{6+}$), 1052.8 ($[M+7H]^{7+}$).

Example 20: Synthesis of BCY15218



BCY15218

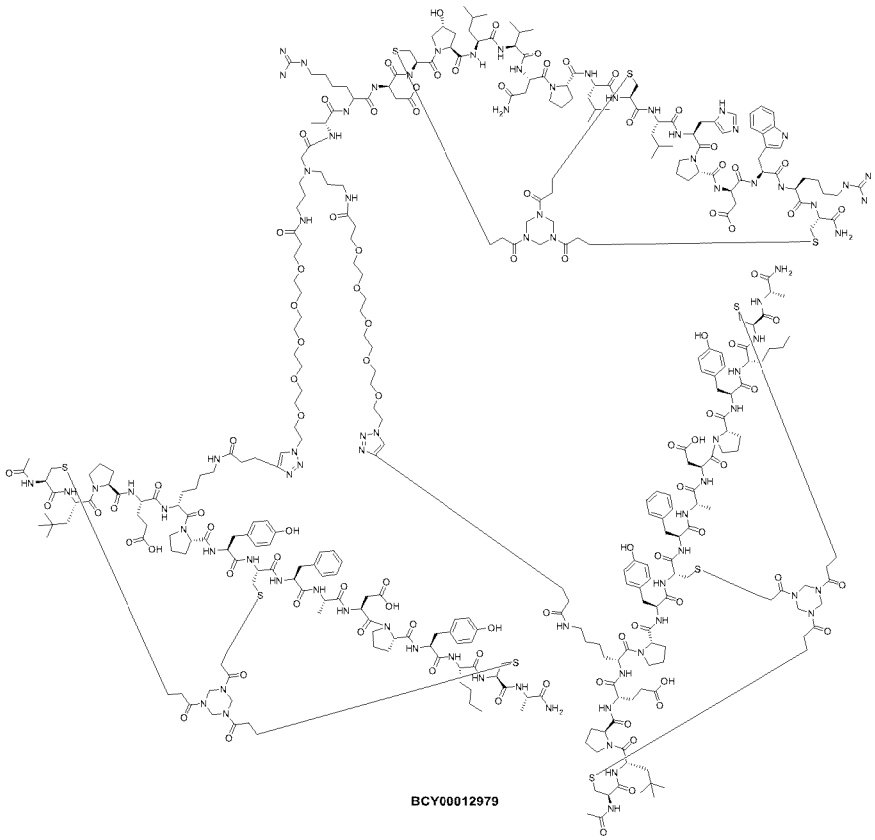
Procedure for preparation of BCY15218



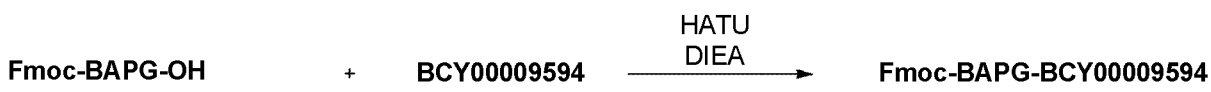
A mixture of **BCY14798** (30.0 mg, 5.74 µmol, 1.0 eq), BCY14601 (15.0 mg, 6.88 µmol, 1.2 eq), and THPTA (2.5 mg, 5.74 µmol, 1.0 eq) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1, 0.5 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 14.0 µL, 1.0 eq) and

sodium ascorbate (4.0 mg, 22.95 μmol, 4.0 eq) were added under N_2. The pH of this solution was adjusted to 7.5 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/0.2 M NH_4HCO_3), and the solution turned light yellow. The reaction mixture was stirred at 25 °C for 2 h under N_2 atmosphere. LC-MS showed **BCY14798** was consumed completely, BCY14601 remained, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY15218** (22 mg, 2.67 μmol, 46.61% yield, 95.0% purity) was obtained as a white solid. Calculated MW: 7404.6, observed *m/z*: 1234.8 ($[M+6H]^{6+}$).

Example 21: Synthesis of BCY12979



Procedure for preparation of Fmoc-BAPG- BCY9594

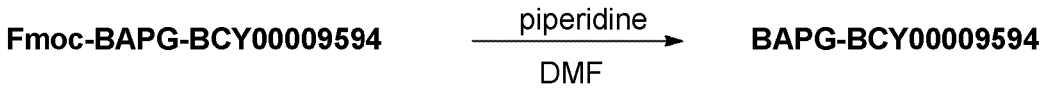


1 **2**

Compound 1 (N,N-Bis[3-(Fmoc-amino)propyl]glycine potassium sulfate, 10.0 mg, 15.78 μmol, 1.0 eq) and HATU (7.2 mg, 18.94 μmol, 1.2 eq) were first dissolved in 1 mL of DMF, then added DIEA (11.0 μL, 63.15 μmol, 4.0 eq). The mixture was stirred at 30°C for 30 minutes, and then **BCY9594** (80.0 mg, 30.09 μmol, 1.0 eq) was added. The reaction mixture was stirred at 25°C for 2 hr. LC-MS showed one main peak with desired m/z (calculated

MW: 3016.51, observed *m/z*: 1006.4 ($[M+3H]^{3+}$). The reaction mixture was used in the next step without purification.

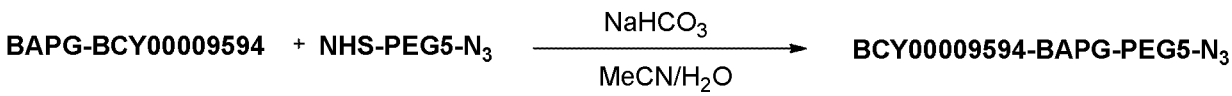
Procedure for preparation of BAPG- BCY9594:



2 **3**

Compound 2 (47.6 mg, 15.78 µmol, 1.0 eq) was first dissolved in 1 mL of DMF, then was added piperidine (0.2 mL, 2.03 mmol, 128.0 eq). The mixture was stirred at 30°C for 30 minutes. LC-MS showed one main peak with desired m/z (calculated MW: 2572.04, observed *m/z*: 1286.8 ($[M+2H]^{2+}$), 858.1 ($[M+3H]^{3+}$). The reaction mixture was purified by preparative HPLC and **compound 3** (24.4 mg, 9.06 µmol, 57% yield, 95% purity) was obtained as a white solid.

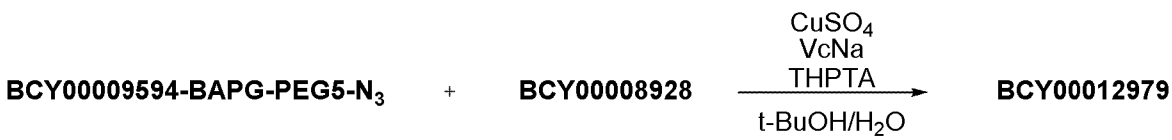
Procedure for preparation of BCY9594-BAPG-PEG5-N_3



3 **4** **5**

Compound 3 (24.4 mg, 9.06 µmol, 1.0 eq) and **compound 4** (10.0 mg, 23.13 µmol, 2.4 eq), were dissolved in 2 mL of $MeCN/H_2O$ (1:1), 1 M $NaHCO_3$ was added to adjust pH to 8. The mixture was stirred at 25°C for 2 hr. LC-MS showed **compound 3** was consumed completely and one main peak with desired m/z (calculated MW: 3206.71, observed *m/z*: 1069.7 ($[M+3H]^{3+}$) was detected. The reaction mixture was purified by preparative HPLC and **compound 5** (12.8 mg, 3.99 µmol, 42.08% yield, 88.62% purity) was obtained as a white solid.

Procedure for preparation of BCY12979

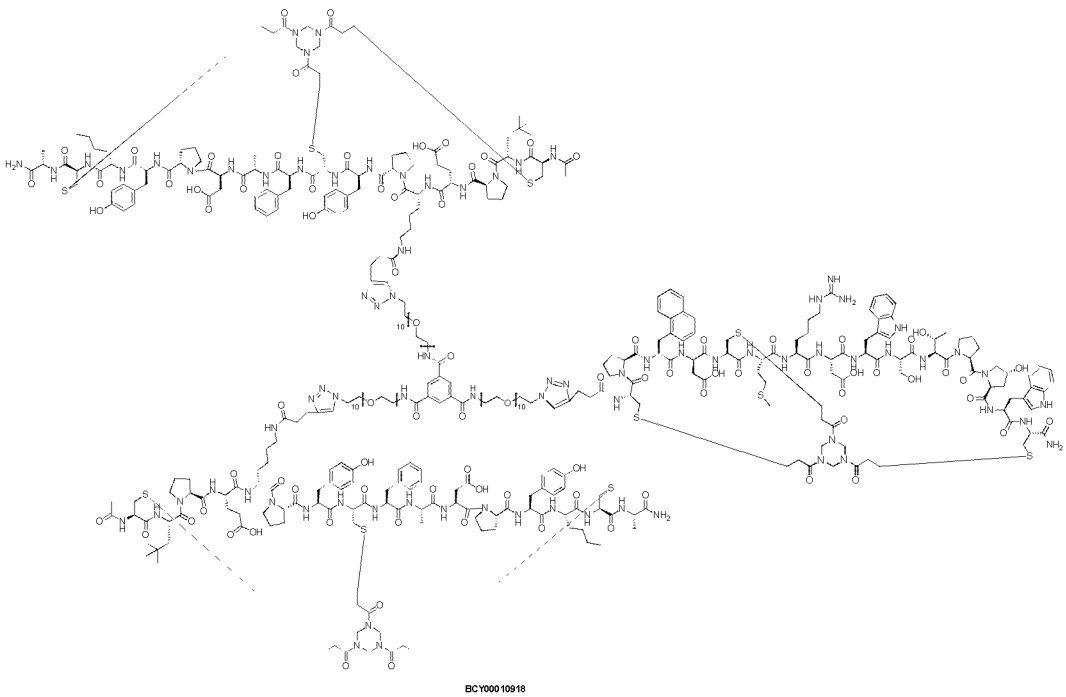


5

Compound 5 (12.8 mg, 3.99 µmol, 1.0 eq) and **BCY8928** (18.0 mg, 8.12 µmol, 2.0 eq) were first dissolved in 2 mL of t-$BuOH/H_2O$ (1:1), and then $CuSO_4$ (0.4 M, 20.0 µL, 2.0 eq), VcNa (3.2 mg, 16.1 µmol, 4.0 eq) and THPTA (3.5 mg, 8.0 µmol, 2.0 eq) was added. Finally, 1M NH_4HCO_3 was added to adjust pH to 8. All solvents here were degassed and purged with N_2. The reaction mixture was stirred at 40°C for 16 hr under N_2 atmosphere. LC-MS showed

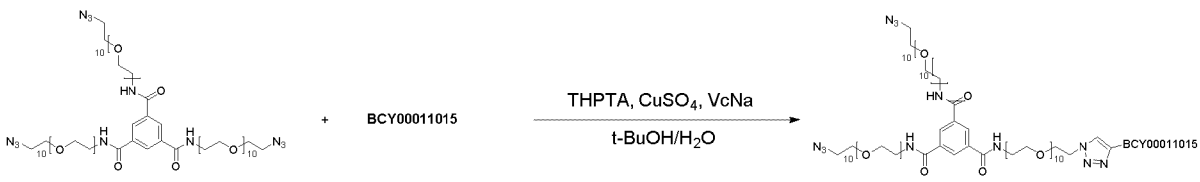
compound 5 was consumed completely and one main peak with desired m/z. The reaction mixture was purified by preparative HPLC and **BCY12979** (16.0 mg, 2.02 μmol, 51% yield, 96.6% purity) was obtained as a white solid. Calculated MW: 7641.87, observed *m/z*: 1911.2 ($[M+4H]^{4+}$), 1528.3 ($[M+5H]^{5+}$), 1247.5 ($[M+6H]^{6+}$), 1092.2 ($[M+7H]^{7+}$).

Example 22: Synthesis of BCY10918



BCY00010918

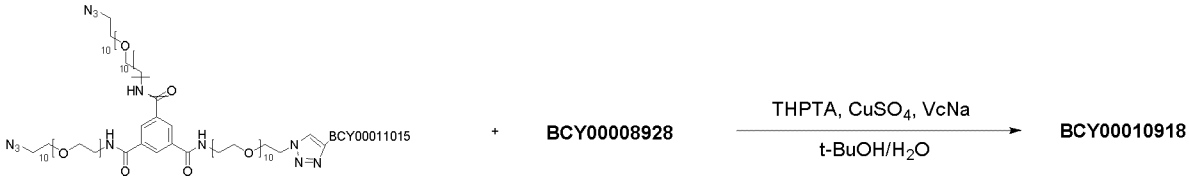
Procedure for preparation of compound 1



COM00000329 1

A mixture of COM00000329 (102 mg, 58.76 μmol, 1.0 eq BCY11015 (92.6 mg, 41.13 μmol, 0.7 eq) and THPTA (0.4 M, 146.9 μL, 1.0 eq) was dissolved in t-BuOH/H_2O (1:1, 2 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 146. 9 μL, 1.0 eq) and VcNa (0.4 M, 293.8 μL, 2.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 25-30 °C for 12 hr under N_2 atmosphere. LC-MS showed COM00000329 was consumed completely and one main peak with desired m/z was detected. The reaction mixture was directly purified by preparative HPLC. **Compound 1** (60 mg, 13.61 μmol, 23.16% yield, 90.45% purity) was obtained as a white solid. Calculated MW: 3988.52, observed *m/z*: 1329.97 ($[M+3H]^{3+}$), 990.56 ($[M+4H]^{4+}$).

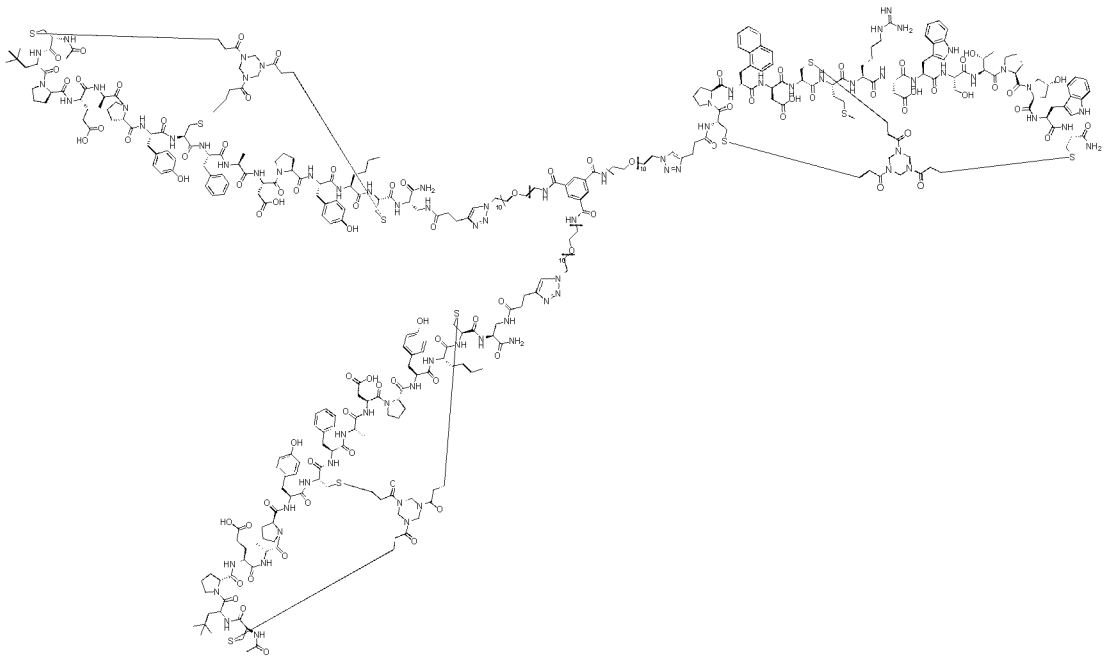
Procedure for preparation of BCY10918



1

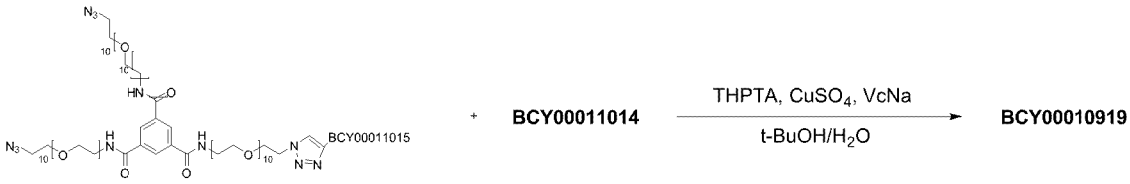
A mixture of Compound **1** (60 mg, 15.04 μmol, 1.0 eq), BCY8928 (72.0 mg, 32.47 μmol, 2.2 eq) and THPTA (0.4 M, 37.6 μL, 1.0 eq) was dissolved in t-BuOH/H_2O (1:1, 2 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 37.6 μL, 1.0 eq) and VcNa (0.4 M, 75.2 μL, 2.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 25-30 °C for 12 hr under N_2 atmosphere. LC-MS showed Compound **1** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was directly purified by preparative HPLC. **BCY10918** (48 mg, 5.47 μmol, 36% yield, 96% purity) was obtained as a white solid. Calculated MW: 8423.67, observed *m/z*: 1404.27 ($[M+6H]^{6+}$), 1203.73 ($[M+7H]^{7+}$).

Example 23: Synthesis of BCY10919



BCY00010919

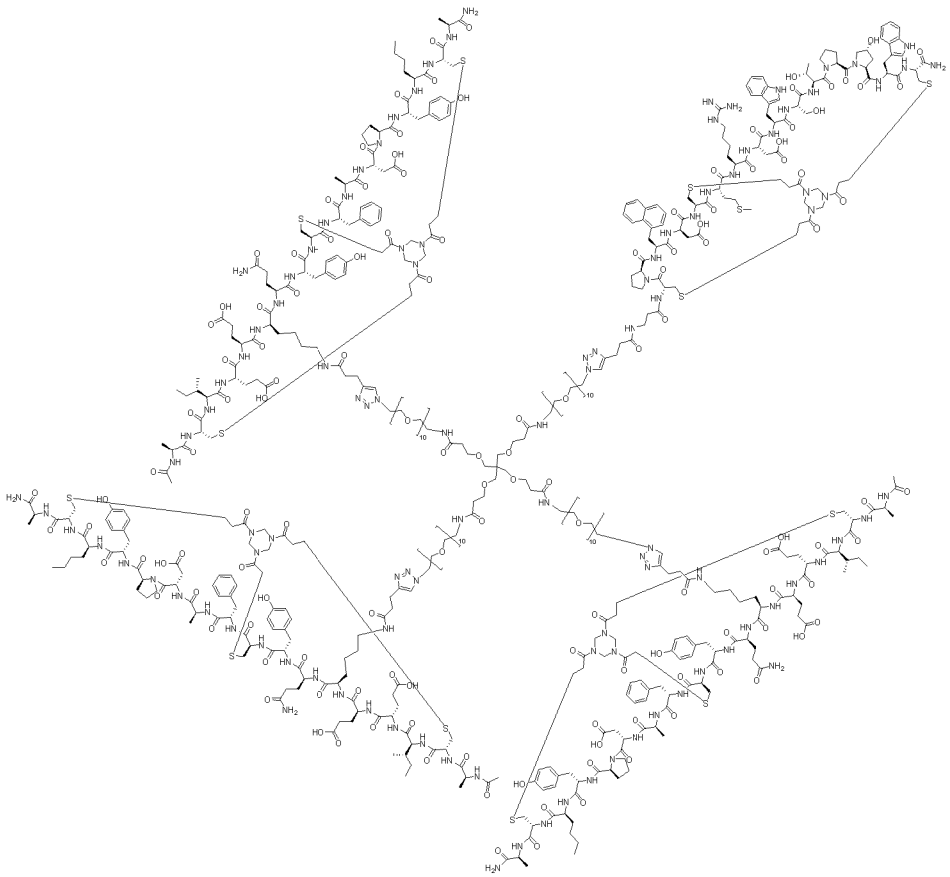
Procedure for preparation of BCY10919



1

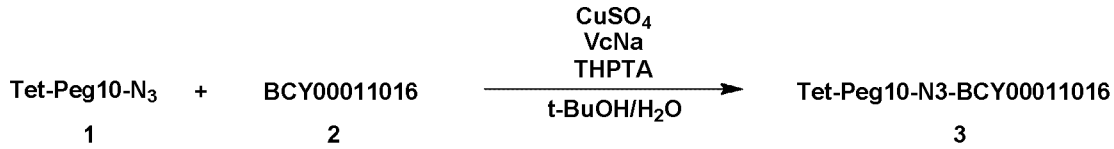
A mixture of Compound **1** (75 mg, 18.8 μmol, 1.0 eq), BCY11014 (93.75 mg, 43.1 μmol, 2.3 eq) and THPTA (0.4 M, 47.0 μL, 1.0 eq) was dissolved in t-BuOH/H_2O (1:1, 2 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 47.0 μL, 1.0 eq) and VcNa (0.4 M, 94.0 μL, 2.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 25-30 °C for 12 hr under N_2 atmosphere. LC-MS showed Compound **1** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was directly purified by preparative HPLC. **BCY10919** (96 mg, 11.39 μmol, 60.59% yield, 96.12% purity) was obtained as a white solid. Calculated MW: 8339.54, observed *m/z*: 1391.3 ($[M+6H]^{6+}$), 1192.5 ($[M+7H]^{7+}$).

Example 24: Synthesis of BCY11021



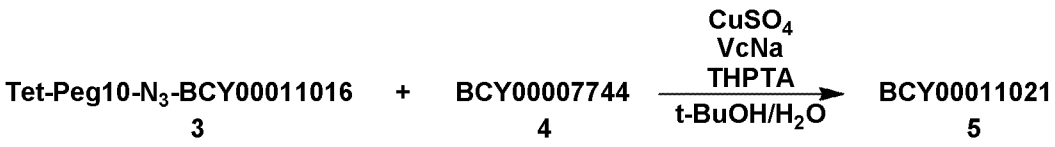
BCY00011021

Procedure for preparation of compound 3



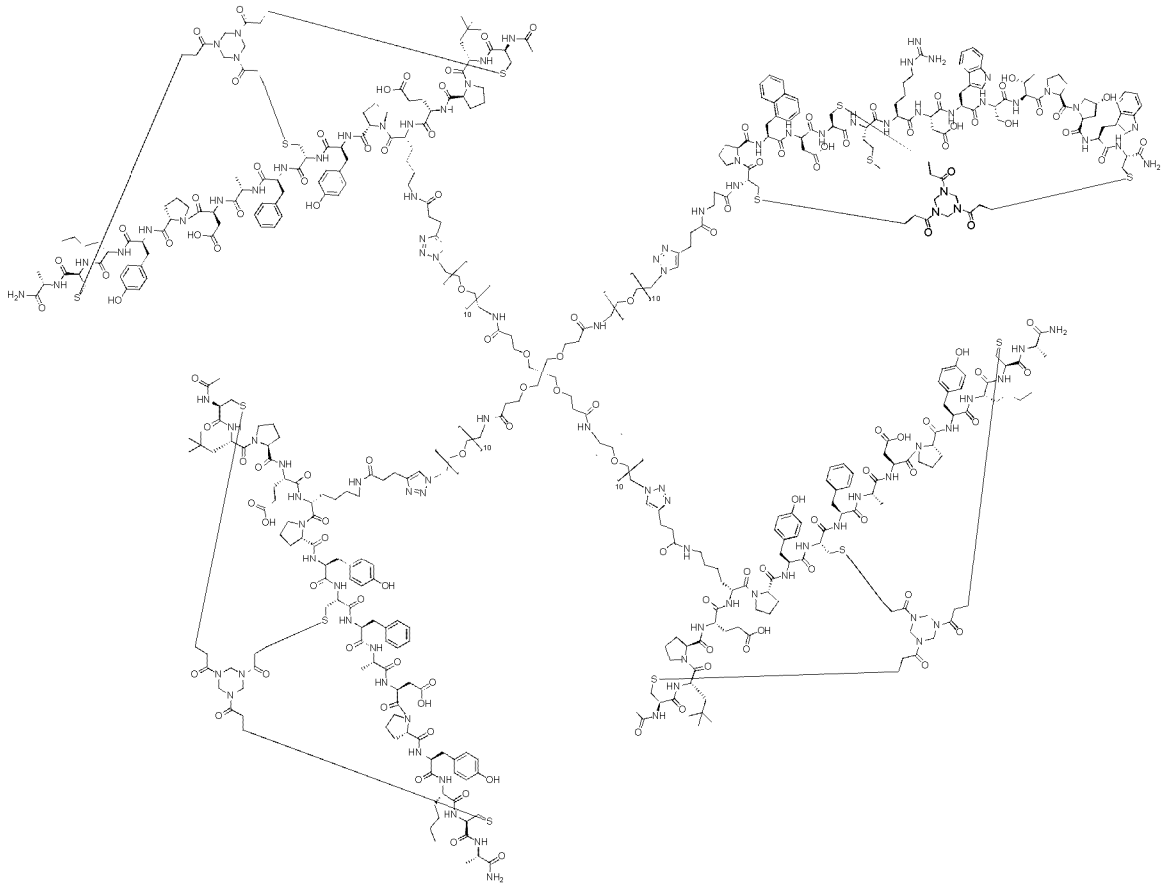
A mixture of compound **1** (15.0 mg, 6.10 μmol, 1.0 eq.), **BCY11016** (18.4 mg, 7.93 μmol, 1.3 eq.), and THPTA (2.65 mg, 6.10 μmol, 1.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (30.0 μL, 0.4M, 2.0 eq.) and VcNa (0.4 M, 30.0 μL, 2.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 4 hr. LC-MS showed compound **1** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was then concentrated under reduced pressure to remove solvent and produced a residue, following by purification by preparative HPLC. Compound **3** (2.89 mg, 0.514 μmol, 8.42% yield, 83.4% purity) was obtained as a white solid. Calculated MW: 4782.46, observed *m/z*: 963.9 ($[M+3H+2H_2O]^{5+}$).

Procedure for preparation of BCY11021



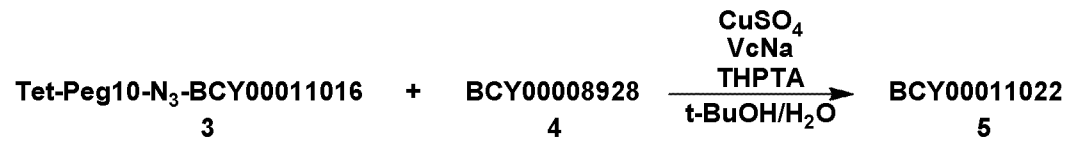
A mixture of compound **3** (2.89 mg, 0.60 μmol, 1.0 eq.), **BCY7744** (4.38 mg, 1.87 μmol, 3.1 eq.), and THPTA (0.9 mg, 2.1 μmol, 3.5 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 3.0 μL, 2.0 eq.) and VcNa (0.4 M, 6.0 μL, 4.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 4 hr under N_2 atmosphere. LC-MS showed compound **3** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY11021** (2.8 mg, 0.229 μmol, 37% yield, 96.4% purity) was obtained as a white solid. Calculated MW: 11795.38, observed *m/z*: 1310.6 ($[M+9H]^{9+}$), 786.6 ($[M+15H]^{15+}$).

Example 25: Synthesis of BCY11022



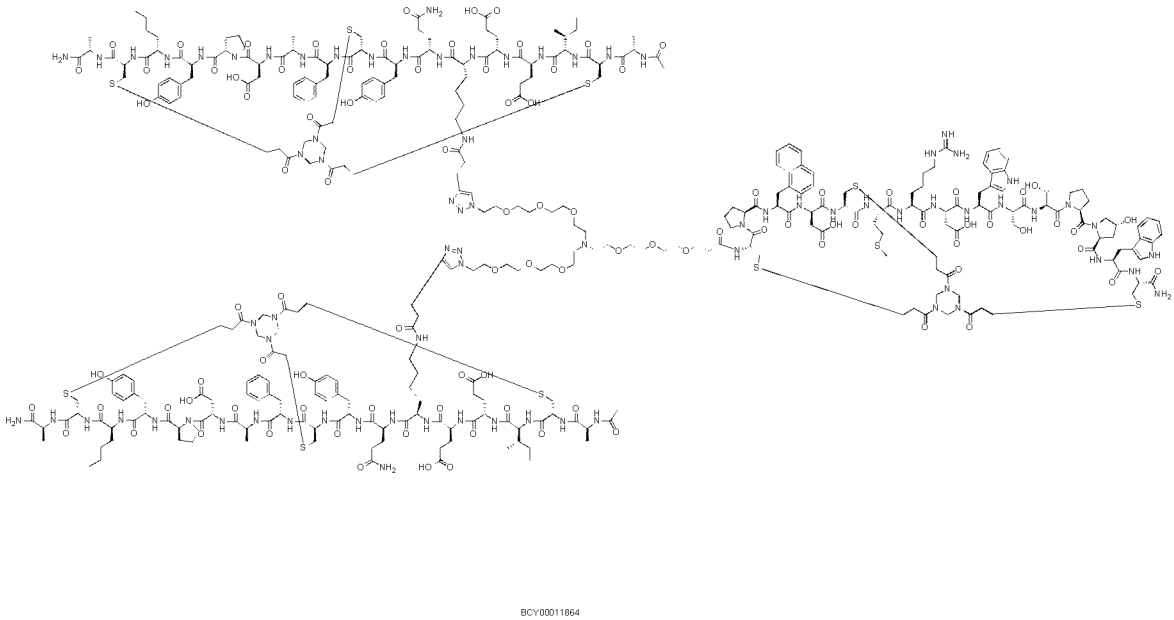
BCY00011022

Procedure for preparation of BCY11022



A mixture of compound **3** (2.7 mg, 0.6 μmol, 1.0 eq.), **BCY8928** (5.3 mg, 2.38 μmol, 4.0 eq.), and THPTA (0.9 mg, 2.1 μmol, 3.5 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 6.0 μL, 4.0 eq.) and VcNa (0.4 M, 6.0 μL, 4.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 4 hr under N_2 atmosphere. LC-MS showed compound **3** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY11022** (1.9 mg, 1.0 μmol, 23.2% yield, 94.6% purity) was obtained as a white solid. Calculated MW: 11435.19, observed *m/z*: 1143.2 ($[M+10H]^{10+}$).

Example 26: Synthesis of BCY11864

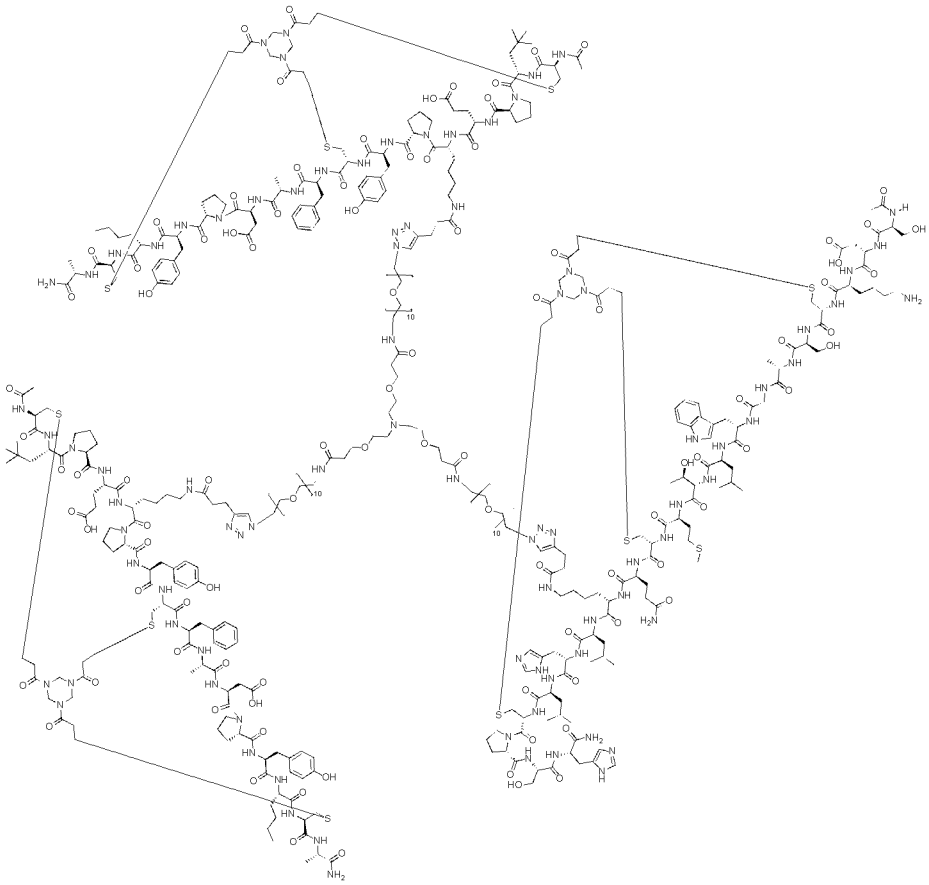


BCY00011864

Procedure for preparation of BCY11864

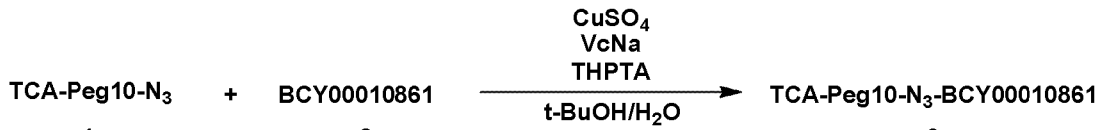
A mixture of compound **2** (5 mg, 1.80 μmol, 1.0 eq), **BCY7744** (9 mg, 3.85 μmol, 2.1 eq), THPTA (0.4 M, 9 μL, 1.0 eq) was dissolved in t-BuOH/H_2O (1:1, 2 mL, pre-degassed and purged with N_2), then $CuSO_4$ (0.4 M, 9 μL, 2.0 eq) and VcNa (0.4 M, 18 μL, 4.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 16 hr under N_2 atmosphere. LC-MS showed **BCY7744** remained and desired m/z was detected. The reaction mixture was directly purified by preparative HPLC. **BCY11864** (5.2 mg, 0.62 μmol, 34% yield, 89% purity) was obtained as a white solid. Calculated MW: 7453.44, observed *m/z*: 1490.70 ($[M+5H]^{5+}$).

Example 27: Synthesis of BCY11780



BCY00011780

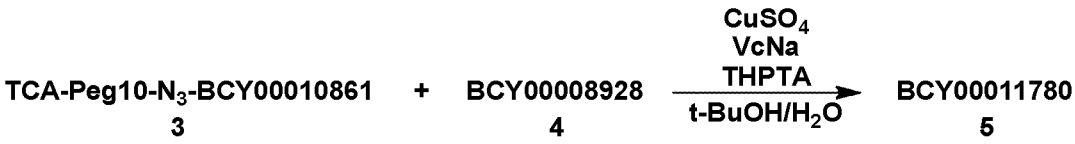
Procedure for preparation of compound 3



1 2 3

A mixture of compound **1** (40.0 mg, 21.15 μmol, 1.0 eq.), compound **2** (43.0 mg, 15.86 μmol, 0.75 eq.), and THPTA (10.0 mg, 21.20 μmol, 1.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (53.0 μL, 0.4M, 1.0 eq.) and VcNa (0.4 M, 53.0 μL, 1.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 4 hr, LC-MS showed compound **2** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was then concentrated under reduced pressure to remove solvent and a residuewas produced. This was purified by preparative HPLC. Compound **3** (11.7 mg, 2.44 μmol, 11% yield, 96.2% purity) was obtained as a white solid. Calculated MW: 4607.33, observed *m/z*: 1152.36 ($[M+4H]^{4+}$).

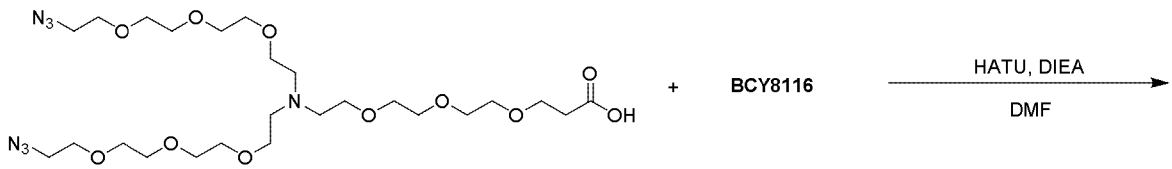
Procedure for preparation of BCY11780



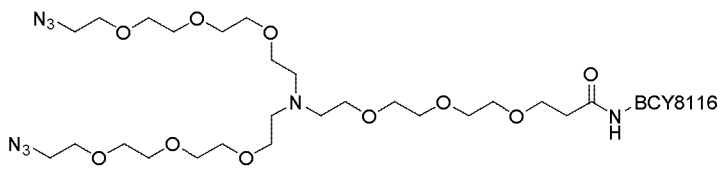
A mixture of compound **3** (11.7 mg, 2.54 μmol, 1.0 eq.), **BCY8928** (11.8 mg, 5.33 μmol, 2.1 eq.), and THPTA (2.3 mg, 5.3 μmol, 2.0 eq.) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 12.7 μL, 2.0 eq.) and VcNa (0.4 M, 25.4 μL, 4.0 eq.) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow. The reaction mixture was stirred at 40 °C for 4 hr under N_2 atmosphere. LC-MS showed compound **3** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY11780** (5.0 mg, 0.509 μmol, 20.03% yield, 92.0% purity) was obtained as a white solid. Calculated MW: 9042.48, observed *m/z*: 1292.8 ($[M+7H]^{7+}$)., 1130.96 ($[M+8H]^{8+}$).

Example 28: Synthesis of BCY13390

Procedure for preparation of BCY12476



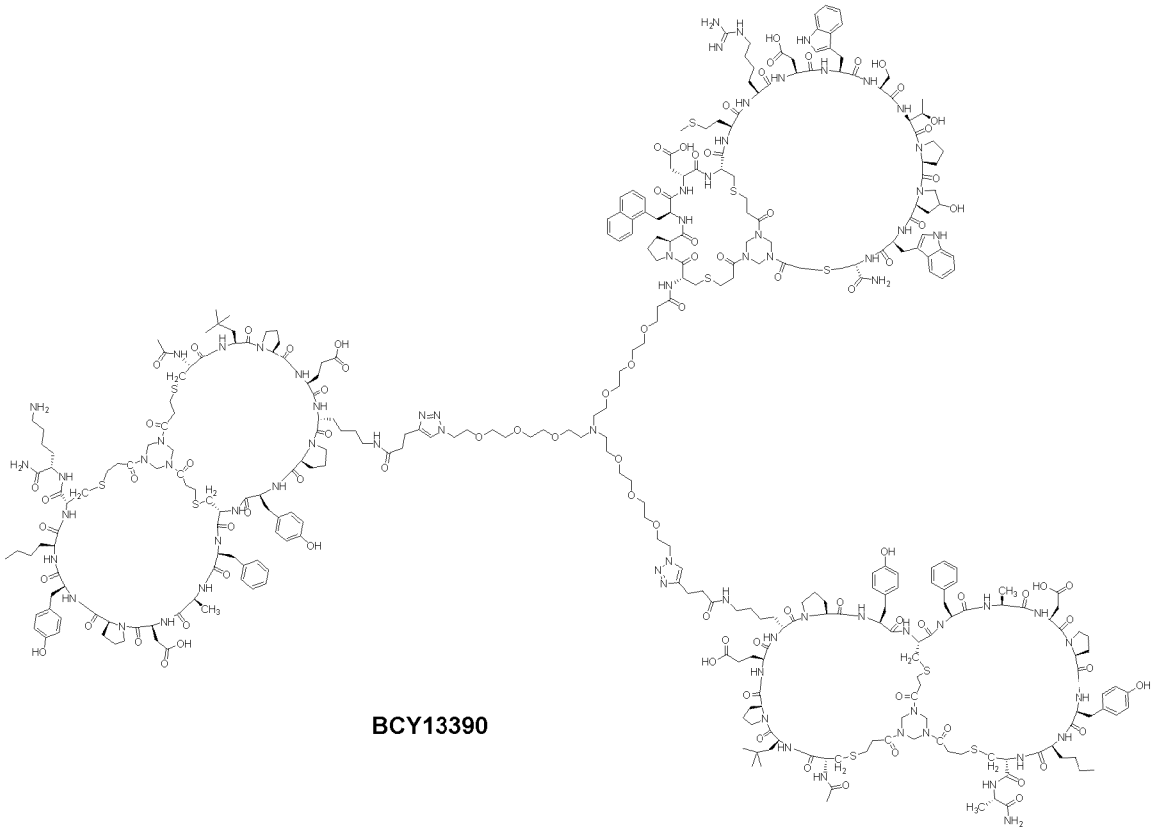
N-(acid-PEG3)-N-bis(PEG3-azide)



BCY12476

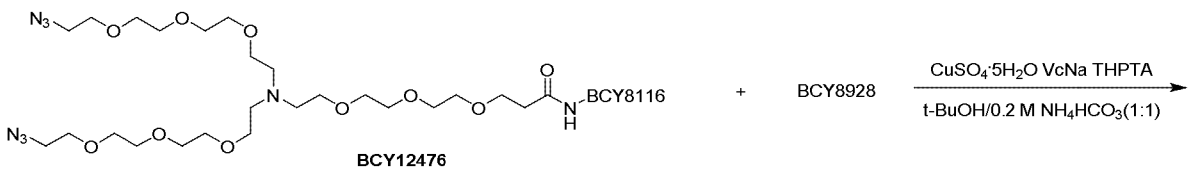
A mixture of **N-(acid-PEG3)-N-bis(PEG3-azide)** (70.0 mg, 112.2 μmol, 1.0 eq), HATU (51.2 mg, 134.7 μmol, 1.2 eq) and DIEA (29.0 mg, 224.4 μmol, 40 μL, 2.0 eq) was dissolved in DMF (2 mL), and mixed for 5 min. Then **BCY8116** (294.0 mg, 135.3 μmol, 1.2 eq) was added. The reaction mixture was stirred at 40°C for 16 hr. LC-MS showed one main peak with desired m/z. The reaction mixture was concentrated under reduced pressure to remove solvent and

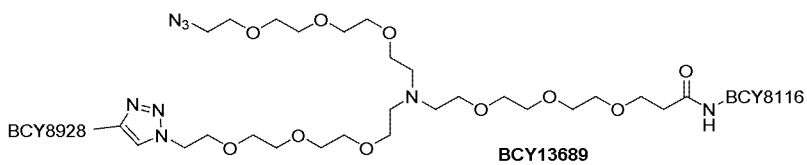
produced a residue. The residue was then purified by preparative HPLC. **BCY12476** (194.5 mg, 66.02 μmol, 29% yield, 94% purity) was obtained as a white solid. Calculated MW: 2778.17, observed m/z: 1389.3 ($[M+2H]^{2+}$), 926.7 ($[M+3H]^{3+}$).



BCY13390

Procedure for preparation of BCY13689

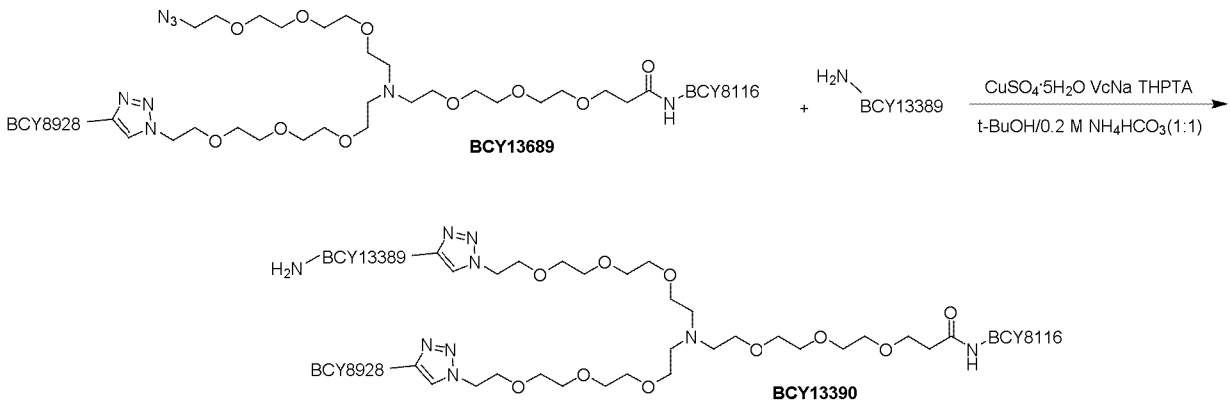




A mixture of **BCY12476** (47.0 mg, 16.91 μmol, 1.0 eq), **BCY8928** (30.0 mg, 13.53 μmol, 0.8 eq), and THPTA (36.7 mg, 84.55 μmol, 5.0 eq) was dissolved in t-BuOH/H_2O (1:1, 8 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 21.0 μL, 0.5 eq) and VcNa (67.0 mg, 338.21 μmol, 20.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned light yellow.

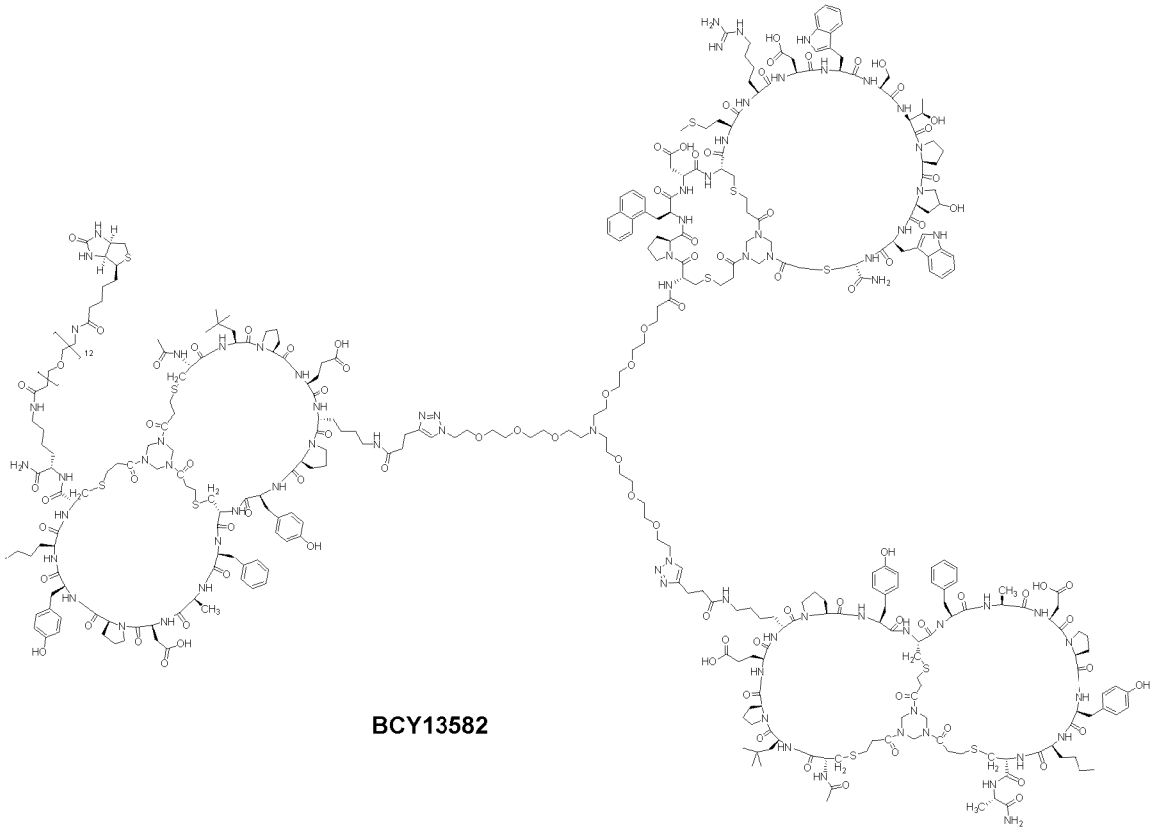
The reaction mixture was stirred at 25 °C for 1.5 h under N_2 atmosphere. LC-MS showed that some **BCY12476** remained, **BCY8928** was consumed completely, and a peak with the desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY13689** (25.3 mg, 4.56 μmol, 27% yield, 90% purity) was obtained as a white solid. Calculated MW: 4995.74, observed *m/z*: 1249.4 ($[M+4H]^{4+}$), 999.9($[M+5H]^{5+}$).

Procedure for preparation of BCY13390



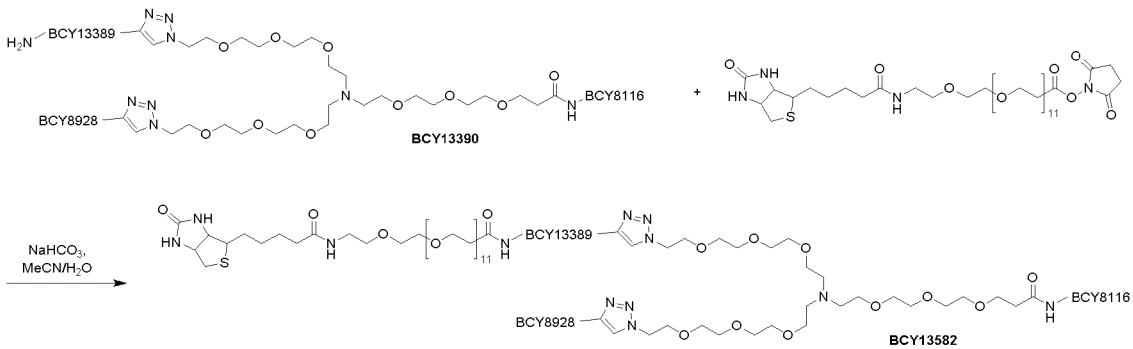
A mixture of **BCY13689** (43.6 mg, 8.73 μmol, 1.0 eq), **BCY13389** (20.8 mg, 9.16 μmol, 1.05 eq), and THPTA (3.8 mg, 8.73 μmol, 1.0 eq) was dissolved in t-BuOH/H_2O (1:1, 1 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 22.0 μL, 1.0 eq) and VcNa (3.5 mg, 17.45 μmol, 2.0 eq) were added under N_2. The pH of this solution was adjusted to 8 by dropwise addition of 0.2 M NH_4HCO_3 (in 1:1 t-BuOH/H_2O), and the solution turned to light yellow. The reaction mixture was stirred at 25 °C for 2 hr under N_2 atmosphere. LC-MS showed a significant peak corresponding to the desired m/z. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY13390** (33.8 mg, 4.21 μmol, 48% yield, 90% purity) was obtained as a white solid. Calculated MW: 7270.41, observed *m/z*: 1454.9($[M+5H]^{5+}$), 1213.2($[M+6H]^{6+}$).

Example 29: Synthesis of BCY13582



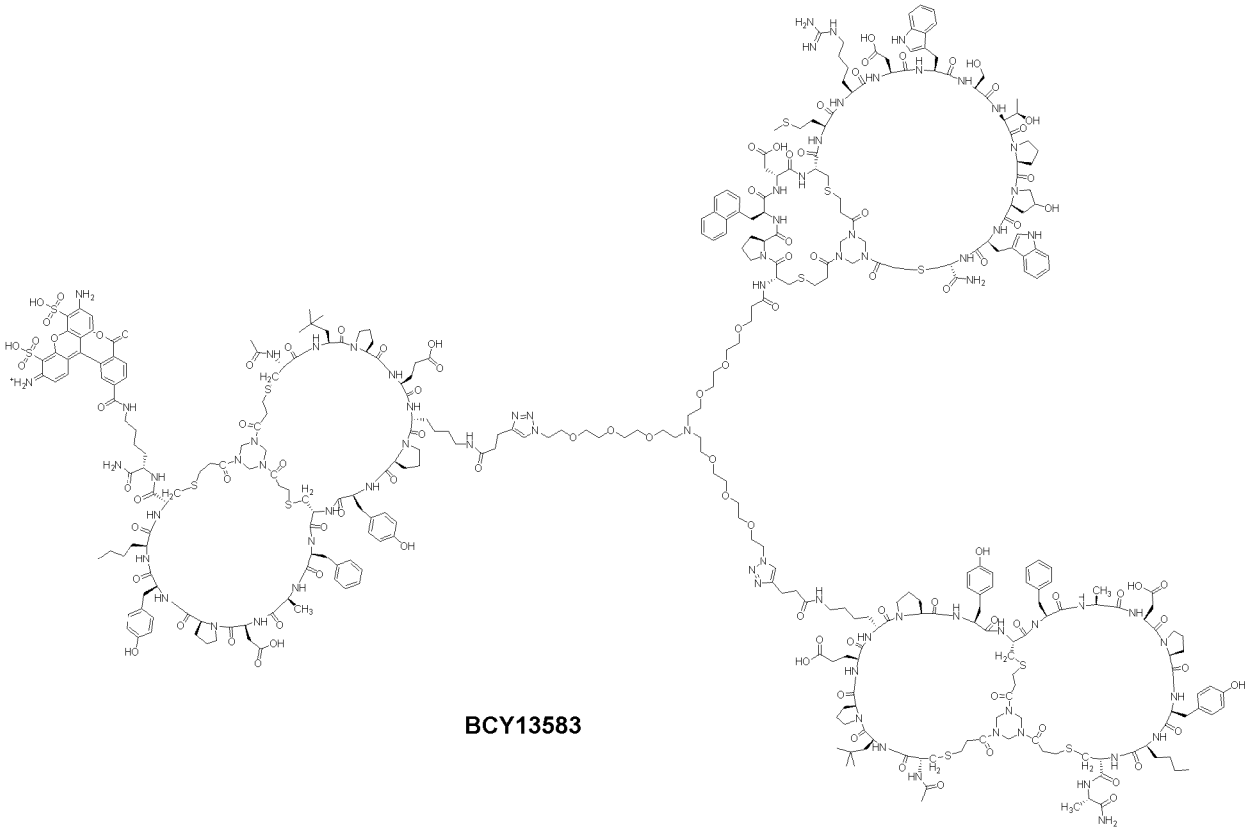
BCY13582

Procedure for preparation of BCY13582



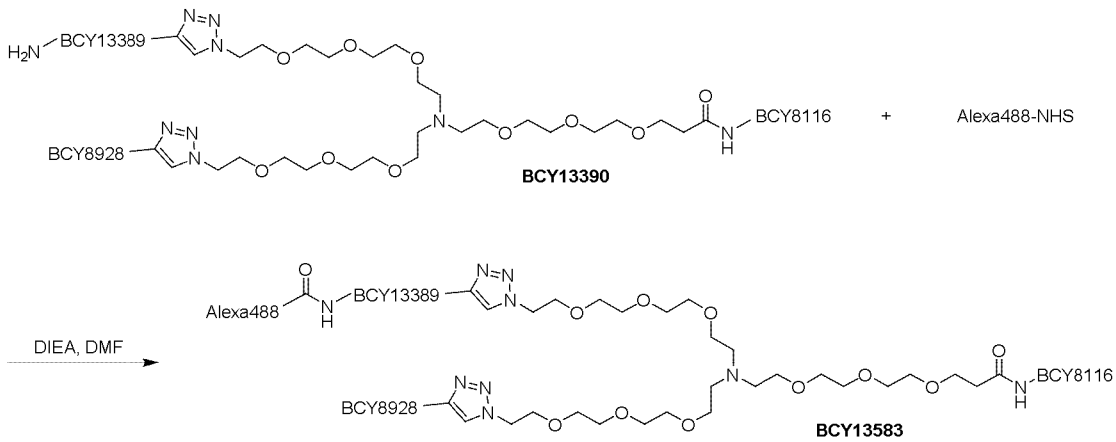
A mixture of **BCY13390** (5.0 mg, 0.6 μmol, 1.0 eq), biotin-PEG12-NHS ester (CAS 365441-71-0, 0.7 mg, 0.72 μmol, 1.1 eq) was dissolved in MeCN/H_2O (1:1, 2 mL). The pH of this solution was adjusted to 8 by dropwise addition of 1.0 M $NaHCO_3$. The reaction mixture was stirred at 25 °C for 0.5 hr. LC-MS showed **BCY13390** was consumed completely, and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY13582** (2.5 mg, 0.30 μmol, 43% yield, 96% purity) was obtained as a white solid. Calculated MW: 8096.43, observed *m/z*: 1351.1 ($[M+6H]^{6+}$), 1158.5 ($[M+7H]^{7+}$).

Example 30: Synthesis of BCY13583



BCY13583

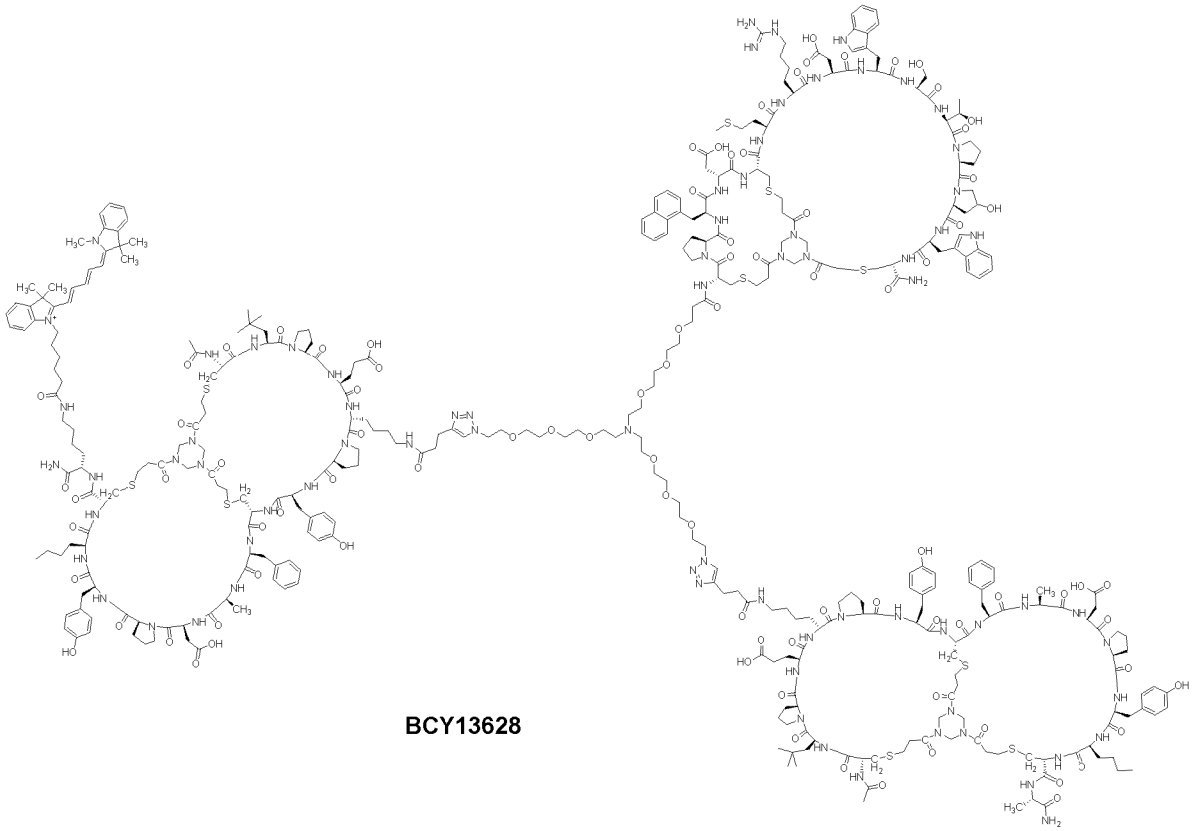
Procedure for preparation of BCY13583



A mixture of **BCY13390** (15.0 mg, 2.06 μmol, 1.0 eq) and Alexa fluor® 488 NHS ester (2.5 mg, 4.12 μmol, 2.0 eq) was dissolved in DMF (0.5 mL). DIEA (2.6 mg, 20.63 μmol, 3.6 μL, 10 eq) was then added dropwise. The reaction mixture was stirred at 25 °C for 1 hr. LC-MS showed **BCY13390** remained, and one main peak with desired m/z was detected. Additional Alexa fluor® 488 NHS ester (2.0 mg, 3.09 μmol, 1.5 eq) was added to the reaction mixture, and the reaction mixture was stirred at 25 °C for one additional hour. HPLC showed **BCY13390** was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY13583** (5 mg, 0.61 μmol, 29% yield, 95% purity) was obtained as a red solid.

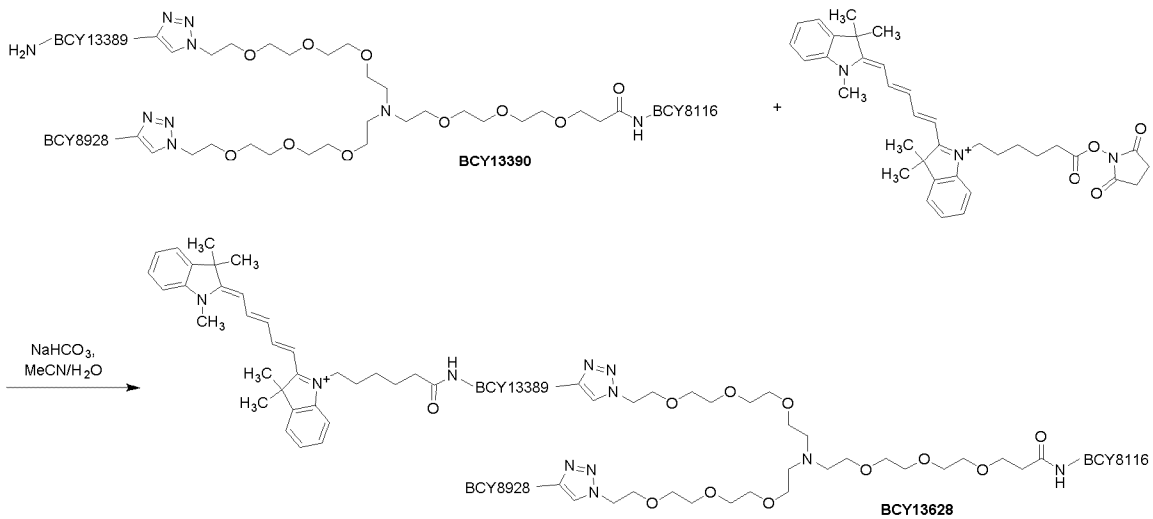
Calculated MW: 7787.9, observed *m/z*: 1948.8 ($[M+4H+H_2O]^{4+}$), 1558.6 ($[M+5H+H_2O]^{5+}$), 1299.1 ($[M+7H+H_2O]^{7+}$).

Example 31: Synthesis of BCY13628



BCY13628

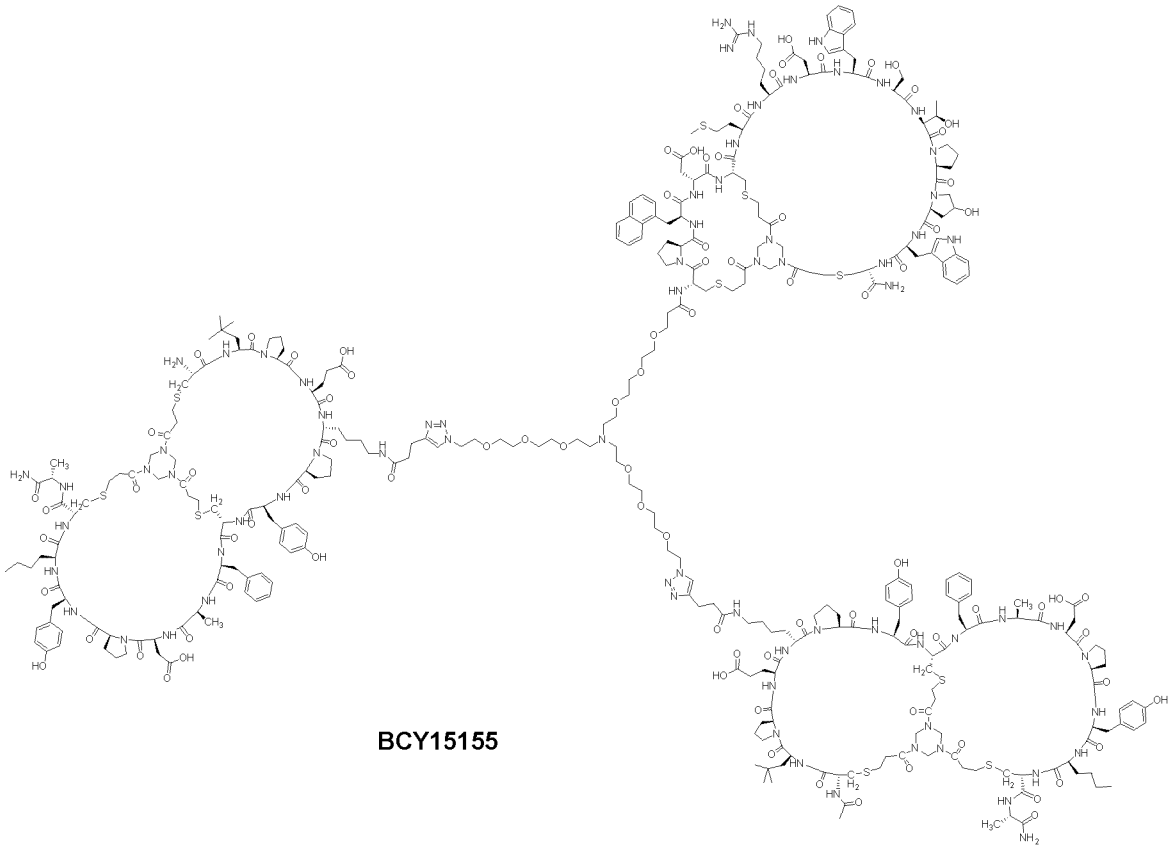
Procedure for preparation of BCY13628



A mixture of **BCY13390** (5.6 mg, 0.77 μmol, 1.0 eq) and cyanine 5 NHS ester (0.5 mg, 0.85 μmol, 1.1 eq) was dissolved in MeCN/H_2O (1:1, 2 mL). The pH of this solution was adjusted to 8 by dropwise addition of 1.0 M $NaHCO_3$. The reaction mixture was stirred at 25 °C for 0.5

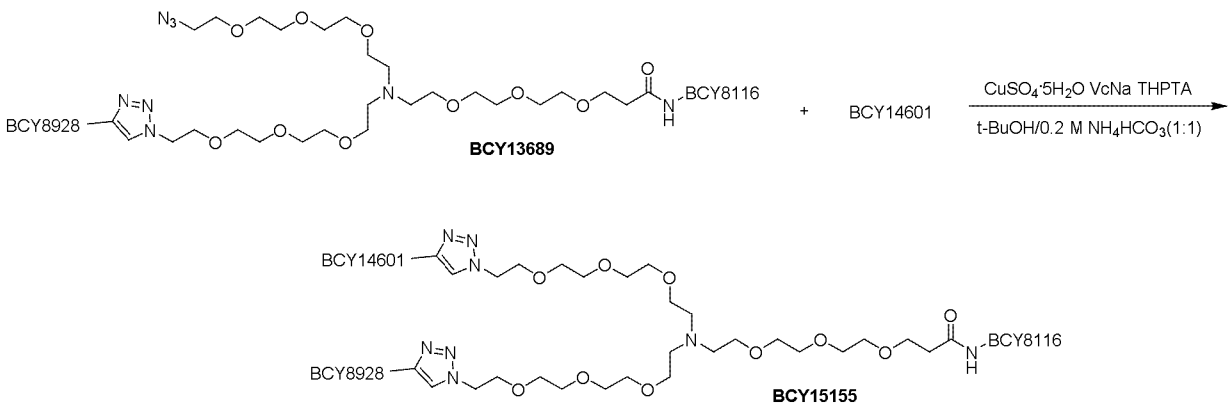
hr. LC-MS showed **BCY13390** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY13628** (2.9 mg, 0.36 μmol, 46% yield, 95% purity) was obtained as a blue solid. Calculated MW: 7736.06, observed *m/z*: 1289.9 ([M+6H]$^{6+}$), 1105.5 ([M+7H]$^{7+}$).

Example 32: Synthesis of BCY15155



BCY15155

Procedure for preparation of BCY15155



A mixture of **BCY13689** (25.0 mg, 5.00 μmol, 1.0 eq), **BCY14601** (13.0 mg, 6.01 μmol, 1.2 eq), and THPTA (2.0 mg, 5.00 μmol, 1.0 eq) was dissolved in t-BuOH/0.2 M NH_4HCO_3 (1:1,

0.5 mL, pre-degassed and purged with N_2), and then $CuSO_4$ (0.4 M, 12.5 µL, 1.0 eq) and Vc (3.5 mg, 20.02 µmol, 4.0 eq) were added under N_2. The pH of this solution was adjusted to 8, and the solution turned light yellow. The reaction mixture was stirred at 25 °C for 2 hr under N_2 atmosphere. LC-MS showed **BCY13689** was consumed completely, some **BCY14601** remained and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by preparative HPLC, and **BCY15155** (19.7 mg, 2.41 µmol, 36% yield, 97% purity) was obtained as a white solid. Calculated MW: 7171.3, observed *m/z*: 1434.7 ($[M+5H]^{5+}$), 1196.2 ($[M+6H]^{6+}$).

ANALYTICAL DATA

The following heterotandem bicyclic peptide complexes of the invention were analysed using mass spectrometry and HPLC. HPLC setup was as follows for analytical method A-C below:

Mobile Phase: A: 0.1%TFA in H_2O B: 0.1%TFA in ACN
Flow: 1.0ml/min
Column: Gemini-NX C18 5um 110A 150*4.6mm
Instrument: Agilent 1200 HPLC-BE(1-614)

HPLC setup was as follows for analytical method D below:

Mobile Phase: A: 0.1%TFA in H_2O B: 0.1%TFA in ACN
Flow: 1.0ml/min
Column: Kintex 1.7um C18 100A 2.1mm*150mm
Instrument: Agilent UPLC 1290

Gradients used are described in the table below:

Analytical Method	Gradient Description
A	25-55% B over 20 minutes
B	30-60% B over 20 minutes
C	45-75% B over 20 minutes
D	30-60% B over 10 minutes

and the data was generated as follows:

Complex ID	***Analytical Data – Mass Spectrometry***	***HPLC Retention Time (min)***	***Analytical Method***
BCY11027	MW: 8578.91, observed m/z: 1430.6 ([M+6H]$^{6+}$)	13.423	A
BCY11863	MW: 7213.32, observed m/z: 1444.0 ([M+5H]$^{5+}$)	10.649	B
BCY12486	calculated MW: 7069.21, observed m/z: 1768.2([M+4H]$^{4+}$), 1415.0([M+5H]$^{5+}$)	15.799	A
BCY12487	calculated MW: 7099.21, observed *m/z*: 1775.8([M+4H]$^{4+}$)	10.936	B
BCY12586	calculated MW: 6985.11, observed m/z: 1746.5([M+4H]$^{4+}$)	11.512	B
BCY12588	calculated MW: 6871.01, observed m/z: 1718.5([M+4H]$^{4+}$)	12.44	B
BCY12491	calculated MW: 7441.63, observed m/z: 1861.1 ([M+4H]$^{4+}$), 1489.0 ([M+5H]$^{5+}$)	12.274	A
BCY12723	Calculated MW: 7363.49, observed m/z: 1473.3 [M+5H]$^{5+}$, 1841.5 [M+4H]$^{4+}$	13.33	A
BCY12724	Calculated MW: 7299.50, observed m/z: 1217.5 [M+6H]$^{6+}$, 1460.8 [M+5H]$^{5+}$, 1825.5 [M+4H]$^{4+}$	12.411	A
BCY12725	Calculated MW: 7295.51, observed m/z: 1460.7 [M+5H]$^{5+}$, 1825.4 [M+4H]$^{4+}$	8.704	B
BCY12726	Calculated MW: 7327.55, observed m/z: 1466.7 [M+5H]$^{5+}$, 1832.2 [M+4H]$^{4+}$	8.679	B
BCY12728	calculated MW: 7325.58, observed m/z: 1466.3 [M+5H]$^{5+}$, 1831.9 [M+4H]$^{4+}$	11.81	A
BCY12729	calculated MW: 7213.44, observed m/z: 1443.4 [M+5H]$^{5+}$, 1803.9 [M+4H]$^{4+}$	13.066	A
BCY12730	Calculated MW: 7185.39, observed m/z: 1197.5 [M+6H]$^{6+}$, 1438.4 [M+5H]$^{5+}$	9.81	B
BCY12731	Calculated MW: 7099.34, observed m/z: 1184.5 [M+6H]$^{6+}$, 1421.3 [M+5H]$^{5+}$	10.583	B
BCY12732	Calculated MW: 8208.70, observed m/z: 1173.4 [M+7H]$^{7+}$	11.117	C

BCY13272	Calculated MW: 7102.28, observed m/z: 1776.4 $[M+4H]^{4+}$, 1421.3 $[M+5H]^{5+}$	7.07	D

Further analytical data was generated as follows:

Complex ID	***Analytical Data – Mass Spectrometry***
BCY13279	MW: 7562.83 observed m/z: 2521.9 ($[M+3H]^{3+}$), 1891.4 ($[M+4H]^{4+}$), 1513.5 ($[M+5H]^{5+}$)
BCY13283	MW: 7215.45 observed m/z: 2406.1 ($[M+3H]^{3+}$), 1804.8 ($[M+4H]^{4+}$), 1444.1 ($[M+5H]^{5+}$)
BCY13287	MW: 7397.64 observed m/z: 2467.1 ($[M+3H]^{3+}$), 1850.6 ($[M+4H]^{4+}$), 1480.7 ($[M+5H]^{5+}$)
BCY14049	MW: 7455.68 observed m/z: 2486.2 ($[M+3H]^{3+}$), 1864.9 ($[M+4H]^{4+}$), 1492.1 ($[M+5H]^{5+}$)
BCY14050	MW: 7455.68 observed m/z: 2486.2 ($[M+3H]^{3+}$), 1864.9 ($[M+4H]^{4+}$), 1492.1 ($[M+5H]^{5+}$)
BCY14051	MW: 7458.7 observed m/z: 2487.2 ($[M+3H]^{3+}$), 1865.6 ($[M+4H]^{4+}$), 1492.7 ($[M+5H]^{5+}$)
BCY14052	MW: 7451.69 observed m/z: 2484.9 ($[M+3H]^{3+}$), 1863.9 ($[M+4H]^{4+}$), 1491.3 ($[M+5H]^{5+}$)
BCY14053	MW: 7457.71 observed m/z: 2486.8 ($[M+3H]^{3+}$), 1865.4 ($[M+4H]^{4+}$), 1492.5 ($[M+5H]^{5+}$)
BCY14054	MW: 7457.71 observed m/z: 2486.8 ($[M+3H]^{3+}$), 1865.4 ($[M+4H]^{4+}$), 1492.5 ($[M+5H]^{5+}$)
BCY14055	MW: 7418.62 observed m/z: 2473.8 ($[M+3H]^{3+}$), 1855.6 ($[M+4H]^{4+}$), 1484.7 ($[M+5H]^{5+}$)
BCY14056	MW: 7432.64 observed m/z: 2478.5 ($[M+3H]^{3+}$), 1859.1 ($[M+4H]^{4+}$), 1487.5 ($[M+5H]^{5+}$)
BCY14334	MW: 8052.48 observed m/z: 1611.4 ($[M+5H]^{5+}$), 1343.0 ($[M+6H]^{6+}$), 1151.2 ($[M+7H]^{7+}$)
BCY14335	MW: 8052.48 observed m/z: 1611.4 ($[M+5H]^{5+}$), 1342.8 ($[M+6H]^{6+}$), 1151.1 ($[M+7H]^{7+}$)
BCY14413	MW: 7498.75 observed m/z: 938.3 ($[M+8H]^{8+}$), 1072.2 ($[M+7H]^{7+}$), 1250.9 ($[M+6H]^{6+}$)
BCY14415	MW: 8324.75 observed m/z: 1388.4 ($[M+6H]^{6+}$), 1190.2 ($[M+7H]^{7+}$), 1041.5 ($[M+8H]^{8+}$)

BCY14416	MW: 8015.2 observed m/z: 1336.5 ([M+6H]$^{6+}$)
BCY12733	MW: 7307.33 observed m/z: 1827.1 ([M+4H]$^{4+}$), 1462.1 ([M+5H]$^{5+}$)
BCY12973	MW: 7611.86 observed m/z: 1269.9 ([M+6H]$^{6+}$)
BCY12974	MW: 7474.70 observed m/z: 1869.3 ([M+4H]$^{4+}$), 1246.1 ([M+5H]$^{5+}$)
BCY12975	MW: 7498.70 observed m/z: 1249.8 ([M+6H]$^{6+}$)
BCY12976	MW: 7498.70 observed m/z: 1249.8 ([M+6H]$^{6+}$)
BCY12977	MW: 7455.68 observed m/z: 1242.7 ([M+6H]$^{6+}$)
BCY12978	MW: 7469.68 observed m/z: 1067.1 ([M+7H]$^{7+}$)
BCY12979	MW: 7641.87 observed m/z: 1911.2 ([M+4H]$^{4+}$), 1528.3 ([M+5H]$^{5+}$), 1247.5 ([M+6H]$^{6+}$)
BCY13042	MW: 7433.62 observed m/z: 1859.8 ([M+4H]$^{4+}$), 1487.1 ([M+5H]$^{5+}$)
BCY13043	MW: 7372.54 observed m/z: 1843.5 ([M+4H]$^{4+}$), 1474.8 ([M+5H]$^{5+}$)
BCY13044	MW: 7364.50 observed m/z: 1842.0 ([M+4H]$^{4+}$)
BCY13045	MW: 7435.60 observed m/z: 1859.4 ([M+4H]$^{4+}$)
BCY13046	MW: 7320.51 observed m/z: 1831.1 ([M+4H]$^{4+}$), 1464.6 ([M+5H]$^{5+}$)
BCY13047	MW: 7458.67 observed m/z: 1865.7 ([M+4H]$^{4+}$)
BCY13049	MW: 7079.22 observed m/z: 1770.9 ([M+4H]$^{4+}$), 1416.7 ([M+5H]$^{5+}$)
BCY13051	MW: 7376.53 observed m/z: 1844.5 ([M+4H]$^{4+}$), 1476.1 ([M+5H]$^{5+}$),
BCY13052	MW: 7447.61 observed m/z: 1862.6 ([M+4H]$^{4+}$), 1490.2 ([M+5H]$^{5+}$)
BCY13054	MW: 7527.78 observed m/z: 1882.6 ([M+4H]$^{4+}$), 1506.7 ([M+5H]$^{5+}$)
BCY13138	MW: 7108.24 observed m/z: 1422.5 ([M+5H]$^{5+}$), 1185.6 ([M+6H]$^{6+}$)
BCY13139	MW: 7249.37 observed m/z: 1449.8 ([M+5H]$^{5+}$)
BCY13140	MW: 7172.24 observed m/z: 1435.4 ([M+5H]$^{5+}$), 1196.2 ([M+6H]$^{6+}$)
BCY13270	MW: 7523.8 observed m/z: 1881.87 ([M+4H]$^{4+}$), 1505.70 ([M+5H]$^{5+}$)
BCY13271	MW: 7501.75 observed m/z: 1876.4 ([M+4H]$^{4+}$), 1501.3 ([M+5H]$^{5+}$)
BCY13273	MW: 7076.26 observed m/z: 1770.1 ([M+4H]$^{4+}$), 1416.2 ([M+5H]$^{5+}$)
BCY13274	MW: 7272.51 observed m/z: 1819.1 ([M+4H]$^{4+}$), 1455.5 ([M+5H]$^{5+}$)
BCY13275	MW: 7455.66 observed m/z: 1865.5 ([M+4H]$^{4+}$), 1492.2 ([M+5H]$^{5+}$), 1243.5 ([M+6H]$^{6+}$)
BCY13276	MW: 7378.52 observed m/z: 1845.7 ([M+4H]$^{4+}$), 1476.7 ([M+5H]$^{5+}$), 1230.6 ([M+6H]$^{6+}$)
BCY13277	MW: 7403.58 observed m/z: 1850.9 ([M+4H]$^{4+}$), 1481.4 ([M+5H]$^{5+}$), 1234.8 ([M+6H]$^{6+}$)
BCY13278	MW: 7529.73 observed m/z: 1506.4 ([M+5H]$^{5+}$), 1255.2 ([M+6H]$^{6+}$)

BCY13280	MW: 7323.4 observed m/z: 1831.0 ([M+4H]$^{4+}$), 1465.4 ([M+5H]$^{5+}$), 1221.1 ([M+6H]$^{6+}$)
BCY13281	MW: 7265.36 observed m/z: 1817.0 ([M+4H]$^{4+}$), 1453.6 ([M+5H]$^{5+}$), 1211.3 ([M+6H]$^{6+}$)
BCY13282	MW: 7194.29 observed m/z: 1439.6 ([M+5H]$^{5+}$), 1199.9 ([M+6H]$^{6+}$)
BCY13284	MW: 7471.61 observed m/z: 1245.5 ([M+6H]$^{6+}$), 1068.1 ([M+7H]$^{7+}$)
BCY13285	MW: 7471.61 observed m/z: 1246.4 ([M+6H]$^{6+}$)
BCY13286	MW: 7447.63 observed m/z: 1241.7 ([M+6H]$^{6+}$)
BCY13288	MW: 7439.7 observed m/z: 1860.8 ([M+4H]$^{4+}$), 1240.6 ([M+6H]$^{6+}$)
BCY13289	MW: 7417.65 observed m/z: 1854.8 ([M+4H]$^{4+}$), 1484.4 ([M+5H]$^{5+}$), 1237.0 ([M+6H]$^{6+}$)
BCY10918	MW: 8423.67 observed m/z: 1404.27 ([M+6H]$^{6+}$), 1203.73 ([M+7H]$^{7+}$)
BCY10919	MW: 8339.54 observed m/z: 1391.3 ([M+6H]$^{6+}$), 1192.5 ([M+7H]$^{7+}$)
BCY11021	MW: 11795.38 observed m/z: 1310.6 ([M+9H]$^{9+}$), 786.6 ([M+15H]$^{15+}$)
BCY11022	MW: 11435.19 observed m/z: 1143.2 ([M+10H]$^{10+}$)
BCY11385	MW: 7129.18 observed m/z: 1782.2 ([M+4H]$^{4+}$), 1426.3 ([M+5H]$^{5+}$), 1188.9 ([M+6H]$^{6+}$)
BCY11864	MW: 7453.44 observed m/z: 1864.31 ([M+4H]$^{4+}$), 1490.70 ([M+5H]$^{5+}$)
BCY12484	MW: 7135.17 observed m/z: 1784.8 ([M+4H]$^{4+}$), 1427.8 ([M+5H]$^{5+}$)
BCY12485	MW: 7071.18 observed m/z: 1768.7 ([M+4H]$^{4+}$), 1416.4 ([M+5H]$^{5+}$)
BCY12490	MW: 7268.46 observed m/z: 1818.0 ([M+4H]$^{4+}$), 1453.9 ([M+5H]$^{5+}$)
BCY12587	MW: 6957.08 observed m/z: 1740.2 ([M+4H]$^{4+}$), 1392.6 ([M+5H]$^{5+}$)
BCY12589	MW: 7724.06 observed m/z: 1931.4 ([M+4H]$^{4+}$), 1545.1 ([M+5H]$^{5+}$), 1288.3 ([M+6H]$^{6+}$)
BCY12590	MW: 7241.39 observed m/z: 1810.7 ([M+4H]$^{4+}$), 1448.6 ([M+5H]$^{5+}$), 1208.4 ([M+6H]$^{6+}$)
BCY11780	MW: 9042.48 observed m/z: 1292.8 ([M+7H]$^{7+}$), 1130.96 ([M+8H]$^{8+}$)
BCY12662	MW: 7889.16 observed m/z: 1578.8 ([M+5H]$^{5+}$), 1315.6 ([M+6H]$^{6+}$), 1128.3 ([M+7H]$^{7+}$)
BCY12722	MW: 7866.12 observed m/z: 1967.0 ([M+4H]$^{4+}$), 1574.0 ([M+5H]$^{5+}$), 1312.0 ([M+6H]$^{6+}$)
BCY12760	MW: 7047.16, observed m/z: 1762.5[M+4H]$^{4+}$
BCY12761	MW: 7047.16, observed m/z: 1411.5[M+5H]$^{5+}$,1762.7[M+4H]$^{4+}$
BCY13390	MW: 7270.41, observed *m/z*: 1454.9([M+5H]$^{5+}$), 1213.2([M+6H]$^{6+}$
BCY13582	MW: 8096.43, observed *m/z*: 1351.1 ([M+6H]$^{6+}$), 1158.5 ([M+7H]$^{7+}$

BCY13583	MW: 7787.9, observed *m/z*: 1948.8 ([M+4H+H_2O]$^{4+}$), 1558.6 ([M+5H+H_2O]$^{5+}$), 1299.1 ([M+7H+H_2O]$^{7+}$
BCY13628	MW: 7736.06, observed *m/z*: 1289.9 ([M+6H]$^{6+}$), 1105.5 ([M+7H]$^{7+}$
BCY14602	MW: 7129.2, observed *m/z*: 1426.6 ([M+5H]$^{5+}$), 1189.1([M+6H]$^{6+}$
BCY15155	MW: 7171.3, observed *m/z*: 1434.7 ([M+5H]$^{5+}$), 1196.2 ([M+6H]$^{6+}$
BCY13048	MW: 7102.28, observed *m/z*: 1776.4([M+4H]$^{4+}$), 1421.3([M+3H]$^{3+}$
BCY13050	MW: 7453.66, observed *m/z*: 1864.2([M+4H]$^{4+}$)
BCY13053	MW: 7185.38, observed *m/z*: 1796.7 ([M+4H]$^{4+}$)
BCY13341	MW: 6929.13, observed *m/z*: 1386.5([M+5H]$^{5+}$) and 1155.8([M+6H]$^{6+}$)
BCY13343	MW: 6846.04, observed *m/z*: 1370.3 ([M+5H^{5+}]
BCY14414	MW: 7503.74, observed *m/z*: 1251.5 ([M+5H]$^{5+}$), 1072.9 ([M+7H]$^{7+}$
BCY14417	MW: 8329.74, observed *m/z*: 1389.6 ([M+6H]$^{6+}$), 1191.9 ([M+7H]$^{7+}$
BCY14418	MW: 8020.19, observed *m/z*: 1337.2 ([M+6H]$^{6+}$
BCY15217	MW: 7362.5, observed *m/z*: 1473.5 ([M+5H]$^{5+}$), 1228.2 ([M+6H]$^{6+}$), 1052.8 ([M+7H]$^{7+}$
BCY15218	MW: 7404.6, observed *m/z*: 1234.8 ([M+6H]$^{6+}$
BCY12967	MW: 7077.7 observed *m/z*: 1416.3 ([M+5H]$^{5+}$), 1180.4 ([M+6H]$^{6+}$), 1011.9 ([M+7H]$^{7+}$

BIOLOGICAL DATA

1. CD137 Reporter Assay Co-Culture with Tumor Cells

Culture medium, referred to as R1 media, is prepared by adding 1% FBS to RPMI-1640 (component of Promega kit CS196005). Serial dilutions of test articles in R1 are prepared in a sterile 96 well-plate. Add 25 µL per well of test articles or R1 (as a background control) to designated wells in a white cell culture plate. Tumor cells* are harvested and resuspended at a concentration of 400,000 cells/mL in R1 media. Twenty five (25) µL/well of tumor cells are added to the white cell culture plate. Jurkat cells (Promega kit CS196005, 0.5 mL) are thawed in the water bath and then added to 5 ml pre-warmed R1 media. Twenty five (25) µL/well of Jurkat cells are then added to the white cell culture plate. Incubate the cells and test articles for 6h at 37°C, 5 % CO_2. At the end of 6h, add 75 µL/well Bio-Glo™ reagent (Promega) and incubate for 10 min before reading luminescence in a plate reader (Clariostar, BMG). The fold change relative to cells alone (Jurkat cells + Cell line used in co-culture) is calculated and plotted in GraphPad Prism as log(agonist) vs response to determine EC_{50} (nM) and Fold Induction over background (Max).

The tumor cell type used in co-culture is NCI-H292 and HT1376 which has been shown to express Nectin-4. The tumor cell types used in co-culture for EphA2 are A549, PC3 and HT29. The tumor cell type used in co-culture for PD-L1 is RKO.

Data presented in Figure 1A shows that the Nectin-4/CD137 heterotandem (BCY11863) induces strong CD137 activation in a CD137 reporter assay and the activation is dependent on the binding of the heterotandem to CD137. BCY11617, a molecule in which CD137 bicyclic peptide is comprised of all D-amino acids which abrogates binding does not induce CD137 agonism.

A summary of the EC_{50} (nM) and Fold Induction induced by heterotandem bicyclic peptide complexes in a CD137 reporter assay in co-culture with a Nectin-4-expressing tumor cell line is reported in Table 1A below:

Table 1A: Fold induction induced by a Nectin-4/CD137 heterotandem bicyclic peptide complex in a CD137 reporter assay

Complex ID	Tumor Target	Cell Line used in Coculture	EC_{50} (nM)	Fold Induction over Background
BCY11863	Nectin-4	NCI-H292	0.168 ± 0.049	81±29

A summary of the EC_{50} (nM) induced by heterotandem bicyclic peptide complexes BCY11863 and close analogues in a CD137 reporter assay in co-culture with a Nectin-4-expressing tumor cell line is reported in Table 1B below and visualized in Figure 1B. This data demonstrates the potential of BCY11863 to induce CD137 agonism in coculture with cell lines that have a range of Nectin-4 expression.

Table 1B: EC50 (nM) of Fold induction over background induced by Nectin-4/CD137 heterotandem bicyclic peptide complexes in a CD137 reporter assay

Complex ID	Tumor cell line Species	Cell Line used in Coculture	Arithmetic mean EC_{50} (nM)
BCY11863	mouse	CT26#7	0.14 ± 0.07
BCY11863	mouse	MC38#13	0.31 ± 0.26
BCY11863	human	NCI-H292	0.28 ± 0.20
BCY11863	human	HT1376	0.52 ± 0.30
BCY11863	human	NCI-H322	0.33 ± 0.21

BCY11863	human	T47D	0.42 ± 0.24
BCY11863	human	MDA-MB-468	0.23 ± 0.01
BCY13582	human	HT1376	0.58 ± 0.27
BCY13582	human	MDA-MB-468	0.34 ± 0.02
BCY13583	human	HT1376	1.7 ± 0.9
BCY13583	human	MDA-MB-468	0.84 ± 0.07

A summary of fold induction induced by Nectin-4/CD137 heterotandem peptides in a CD137 reporter coculture assay with NCI-H292 cells is shown in Table 2 below. All compounds are compared to plate control BCY10000 which has an average EC_{50} of 1.1±0.5 nM and Emax of 28±11 fold over background.

Table 2: Fold induction induced by Nectin-4/CD137 heterotandem bicyclic peptide complexes in a CD137 reporter assay

Complex ID	Fold improvement in EC_{50} over BCY10000 on same plate	Fold improvement in Emax over BCY10000 on same plate
BCY12484	5.46	1.86
BCY11385	9.35	1.28
BCY11863	4.56	2.16
BCY11864	1.54	2.43
BCY12485	3.82	2.18
BCY12486	0.25	1.73
BCY12586	1.9	3.79
BCY12587	4.2	2.90
BCY12588	0.72	3.20
BCY12590	5.5	2.73
BCY11021	9.63	4.42

A summary of fold induction induced by Nectin-4/CD137 heterotandem peptides in a CD137 reporter coculture assay with HT1376 tumor cells is shown in Table 2A below with EC50 (nM) and Emax(fold induction over background) being reported. Most Nectin-4/CD137 heterotandems have EC50 below 1 nM.

Table 2A: Nectin-4 Reporter Assay EC50 and Emax

Complex ID	Nectin-4 cell line	Number of replicates (n)	EC50 (nM)	SD EC50 (nM)	Emax	SD Emax
BCY10918	HT1376	6	0.16	0.08	63	12
BCY10919	HT1376	6	0.19	0.12	62	11
BCY11022	HT1376	10	0.20	0.12	66	28
BCY11027	HT1376	4	0.18	0.08	45	13
BCY12487	HT1376	3	13	6	12	6
BCY12490	HT1376	3	0.23	0.07	70	16
BCY12589	HT1376	6	0.44	0.12	79	6
BCY12760	HT1376	6	1.8	0.4	76	12
BCY12761	HT1376	6	0.37	0.08	76	10
BCY13582	HT1376	3	0.58	0.27	57	16
BCY13583	HT1376	3	1.7	0.9	63	17

Data presented in Figure 16 shows that the EphA2/CD137 heterotandem BCY13272 induces strong CD137 activation in a CD137 reporter assay in the presence of an EphA2 expressing cell line (PC3, A549 and HT29) while a non-binding control molecule (BCY13626) shows no activation of CD137.

A summary of fold induction induced by EphA2/CD137 heterotandem peptides in a CD137 reporter coculture assay with PC3 cells is shown in Table 3A below. All compounds are compared to plate control BCY9173 which has an average EC_{50} of 0.54 nM and Emax of 42 fold over background.

Table 3A: Fold induction induced by EphA2/CD137 heterotandem bicyclic peptide complexes in a CD137 reporter assay

Complex ID	EphA2 cell line	Fold Improvement over BCY9173, EC_{50} (nM)	Fold Improvement over Emax
BCY12723	PC3	2.2	1.8
BCY12724	PC3	1.1	1.9
BCY12725	PC3	0.1	0.7
BCY12726	PC3	0.2	0.9
BCY12729	PC3	0.5	1.6
BCY12731	PC3	0.2	1.5

BCY12732	PC3	0.5	0.4
BCY12491	PC3	2.3	1.8
BCY13279	PC3	4.74	2.08
BCY13283	PC3	1.80	2.06
BCY13287	PC3	3.26	1.98
BCY14049	PC3	2.65	1.82
BCY14050	PC3	2.05	1.91
BCY14051	PC3	3.15	1.88
BCY14052	PC3	4.16	1.89
BCY14053	PC3	4.83	1.84
BCY14054	PC3	2.44	1.88
BCY14055	PC3	3.28	1.87
BCY14056	PC3	4.02	1.80
BCY14334	PC3	7.21	1.62
BCY14335	PC3	7.14	0.94

A summary of the EC_{50} (nM) and Fold Induction induced by BCY13272 in a CD137 reporter assay in co-culture with an EphA2 expressing tumor cell line is reported in Table 3B below:

Table 3B: Activity of EphA2/CD137 heterotandem bicyclic peptide complexes in a CD137 reporter assay

Complex ID	EphA2 cell line	EC50 (nM)	Emax	Geo mean EC50/cell line
BCY13272	PC-3	0.245	44.5	**0.117**
		0.0805	44.2	
		0.0898	53	
	A549	0.1468	25.7	**0.127**
		0.107	23.6	
		0.132	30.2	
	HT-29	0.567	36.5	**0.279**
		0.187	26	
		0.205	36.4	

A summary of fold induction induced by EphA2/CD137 heterotandem peptides in a CD137 reporter coculture assay with PC3 tumor cells is shown in Table 3C below with EC50 (nM) and Emax(fold induction over background) being reported. Most EphA2/CD137 heterotandems have EC50 below 1 nM.

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Table 3C: EphA2 Reporter Assay EC50 and Emax

Complex ID	EphA2-4 cell line	Number of replicates (n)	EC50 (nM)	SD EC50 (nM)	Emax	SD Emax
BCY12730	PC3	4	0.25	0.31	61.73	25.80
BCY12973	PC3	3	0.16	0.01	48.57	6.24
BCY12974	PC3	3	0.18	0.05	59.60	21.23
BCY12975	PC3	3	0.16	0.13	68.80	18.37
BCY12976	PC3	3	0.18	0.16	73.20	23.93
BCY12977	PC3	3	0.08	0.07	67.17	20.03
BCY12978	PC3	3	0.05	0.04	78.10	11.62
BCY12979	PC3	2	0.08		71.60	
BCY13042	PC3	3	0.08	0.03	58.27	12.43
BCY13043	PC3	4	0.11	0.02	52.70	6.04
BCY13044	PC3	3	0.21	0.06	49.53	16.53
BCY13045	PC3	3	0.23	0.09	49.87	15.25
BCY13046	PC3	2	0.17		57.60	
BCY13047	PC3	4	0.07	0.02	46.43	5.56
BCY13048	PC3	2	0.09		48.35	
BCY13049	PC3	3	0.30	0.19	46.03	14.81
BCY13050	PC3	5	0.09	0.01	42.46	3.84
BCY13051	PC3	2	0.21		37.05	
BCY13052	PC3	2	0.21		32.90	
BCY13053	PC3	3	0.10	0.04	40.33	8.56
BCY13054	PC3	3	0.07	0.03	36.53	6.01
BCY13138	PC3	1	0.17		32.60	
BCY13139	PC3	2	0.16		43.75	
BCY13140	PC3	1	0.12		46.70	
BCY13270	PC3	4	0.10	0.04	42.50	3.45
BCY13271	PC3	3	0.09	0.03	44.20	4.61
BCY13273	PC3	3	0.13	0.08	51.27	5.37

BCY13274	PC3	3	0.19	0.10	47.43	7.50
BCY13275	PC3	2	0.08		47.50	
BCY13276	PC3	2	0.10		50.55	
BCY13277	PC3	2	0.08		51.10	
BCY13278	PC3	2	0.14		37.90	
BCY13280	PC3	3	0.19	0.03	38.13	8.29
BCY13281	PC3	3	0.17	0.06	35.60	5.89
BCY13282	PC3	3	0.22	0.05	40.03	11.87
BCY13284	PC3	3	0.25	0.12	34.73	6.18
BCY13285	PC3	3	0.26	0.10	36.53	8.56
BCY13286	PC3	3	0.11	0.02	34.13	12.00
BCY13288	PC3	4	0.09	0.02	43.28	5.69
BCY13289	PC3	4	0.08	0.04	45.78	5.15
BCY13341	PC3	2	0.19		49.15	
BCY13343	PC3	2	0.11		44.00	

A summary of fold induction induced by PD-L1/CD137 heterotandem peptides in CD137 reporter coculture assay with RKO cells is shown in Table 4 below.

Table 4: Fold induction induced by PD-L1/CD137 heterotandem bicyclic peptide complexes in a CD137 reporter assay

Complex ID	EC_{50} (nM)	Fold Induction over background
BCY11780	1.9	13

2. Human PBMC-tumor cell Co-Culture (Cytokine stimulation assay) Assay

Tumor cell lines were cultured according to suppliers recommended protocol. Frozen PBMCs from healthy human donors were thawed and washed one time in room temperature PBS, and then resuspended in R10 medium. 100 µl of PBMCs (1,000,000 PBMCs/ml) and 100 µl of tumor cells (100,000 tumor cells/ml) (Effector: Target cell ratio (E:T) 10:1) were plated in each well of a 96 well flat bottom plate for the co-culture assay. 100 ng/ml of soluble anti-CD3 mAb (clone OKT3) was added to the culture on day 0 to stimulate human PBMCs. Test, control compounds, or vehicle controls were diluted in R10 media and 50 µL was added to respective wells to bring the final volume per well to 250 µL. Plates were covered with a breathable film and incubated in a humidified chamber at 37ºC with 5% CO_2 for three days. Supernatants

were collected 48 hours after stimulation, and human IL-2 and IFN-γ were detected by Luminex. Briefly, the standards and samples were added to black 96 well plate. Microparticle cocktail (provided in Luminex kit, R&D Systems) was added and shaken for 2 hours at room temperature. The plate was washed 3 times using magnetic holder. Biotin cocktail was then added to the plate and shaken for 1 hour at RT. The plate was washed 3 times using magnetic holder. Streptavidin cocktail was added to the plate and shaken for 30 minutes at RT. The plates were washed 3 times using magnetic holder, resuspended in 100 µL of wash buffer, shaken for 2 minutes at RT, and read using the Luminex 2000. Raw data were analyzed using built-in Luminex software to generate standard curves and interpolate protein concentrations, all other data analyses and graphing were performed using Excel and Prism software. Data represents one study with three independent donor PBMCs tested in experimental duplicates.

Data presented in Figures 2A and 2B demonstrate that the Nectin-4/CD137 heterotandem (BCY11863) induces robust IL-2 and IFN-γ cytokine secretion in a PBMC-4T1 co-culture assay. BCY11617 is a negative control that binds Nectin-4 but does not bind CD137.

A summary of the EC_{50} (nM) and maximum IFN-γ cytokine secretion (pg/ml) induced by selected Nectin-4/CD137 heterotandem bicyclic peptide complexes in Human PBMC co-culture (cytokine release) assay is reported in Table 4A below and visualized in Figure 2C. This demonstrates the potential of BCY11863 to induce cytokine secretion in the presence of a number of different tumor cell lines expressing Nectin-4.

Table 4A: EC_{50} of IFN-γ cytokine secretion induced by selected Nectin-4/CD137 heterotandem bicyclic peptide complexes in Human PBMC-4T1 co-culture (cytokine release) assay

Cell Line	IL-2 (nM)	IFNy (nM)	No. of Donors
MC38 # 13 (mouse)	0.25 ± 0.08	0.17 ± 0.11	4
4T1-D02 (mouse)	0.16 ± 0.22	0.04 ± 0.04	4
HT1376 (human)	0.39 ± 0.29	0.23 ± 0.15	5
T-47D (human)	0.20 ± 0.07	0.08 ± 0.06	3
H322 (human)	0.84 ± 0.15	0.85 ± 0.66	3

4T1-Parental(Nectin4-)	No induction up to 100 nM

3. Pharmacokinetics of CD137 Heterotandem Bicyclic Peptide Complexes in SD Rats

Male SD Rats were dosed with each heterotandem Bicycle peptide complex formulated in 25 mM Histidine HCl, 10% sucrose pH 7 by IV bolus or IV infusion (15 minutes). Serial bleeding (about 80 µL blood/time point) was performed via submandibular or saphenous vein at each time point. All blood samples were immediately transferred into prechilled microcentrifuge tubes containing 2 µL K2-EDTA (0.5M) as anti-coagulant and placed on wet ice. Blood samples were immediately processed for plasma by centrifugation at approximately 4°C, 3000g. The precipitant including internal standard was immediately added into the plasma, mixed well and centrifuged at 12,000 rpm, 4°C for 10 minutes. The supernatant was transferred into pre-labeled polypropylene microcentrifuge tubes, and then quick-frozen over dry ice. The samples were stored at 70°C or below as needed until analysis. 7.5 µL of the supernatant samples were directly injected for LC-MS/MS analysis using an Orbitrap Q Exactive in positive ion mode to determine the concentrations of analyte. Plasma concentration versus time data were analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program. C0, Cl, Vdss, T½, AUC(0-last), AUC(0-inf), MRT(0-last), MRT(0-inf) and graphs of plasma concentration versus time profile were reported. The pharmacokinetic parameters from the experiment are as shown in Table 6A:

Table 6A: Pharmacokinetic Parameters in SD Rats

Compound	Dosing Route	T1/2(h)	Vdss (L/kg)	Clp (ml/min/kg)
BCY12491	IV Bolus	1.3	1.6	20
BCY12730	IV Inf	2.0	2.6	18
BCY12724	IV Inf	1.5	1.2	13
BCY13050	IV Inf	3.3	1.4	11
BCY13048	IV Inf	3.8	1.2	11
BCY13272	IV Inf	2.5	1.0	7.4

The pharmacokinetic parameters specifically for BCY11863 are as shown in Table 6B:

Table 6B: Pharmacokinetic Parameters in SD Rats

Compound	Dose (mg/kg)	Dosing Route	T1/2(h)	Vdss (L/kg)	Clp (ml/min/kg)	% F
BCY11863	1.9	IV Bolus	4.1	1.6	7.7	-
	3.2	IV Inf (15 min)	3.1	1.3	9.3	-
	6.3	SC	2.5	-	-	95%

Data in Table 6B above and Figure 5 shows that BCY11863 is a low clearance molecule with volume of distribution larger than plasma volume. In addition, the bioavailability from SC dosing of BCY11863 is high in rats.

Table 6C: Pharmacokinetic Parameters of BCY11863 and potential metabolites in SD Rat PK study following 100 mg/kg dose administered by IV administration

Analytes	Cmax (ng/mL)	AUC (ng.h/mL)	T1/2(h)	Vdss (L/kg)	Clp (ml/min/kg)
BCY11863	279540	129863	5.4	2.3	13
BCY15155	2854	1296	3.1	-	-
BCY14602	-	-	-	-	-

Data in Table 6C and Figure 25 shows that < 1% of BCY11863 gets metabolized to BCY15155 upon IV administration of BCY11863 to SD rats. No significant conversion to BCY14602 is noted during the first 24h of the study.

4. Pharmacokinetics of CD137 Heterotandem Bicyclic Peptide Complexes in Cynomolgus monkey

Non-naïve Cynomolgus Monkeys were dosed via intravenous infusion (15 or 30 min) into the cephalic vein with 1 mg/kg of each Heterotandem Bicycle Peptide Complex formulated in 25 mM Histidine HCl, 10% sucrose pH 7. Serial bleeding (about 1.2 ml blood/time point) was performed from a peripheral vessel from restrained, non-sedated animals at each time point into a commercially available tube containing Potassium (K2) EDTA*$2H_2O$ (0.85-1.15 mg) on wet ice and processed for plasma. Samples were centrifuged (3,000 x g for 10 minutes at 2 to 8°C) immediately after collection. 0.1 mL plasma was transferred into labelled polypropylene micro-centrifuge tubes. 5-fold of the precipitant including internal standard 100 ng/mL Labetalol & 100 ng/mL dexamethasone & 100 ng/mL tolbutamide & 100 ng/mL Verapamil & 100 ng/mL Glyburide & 100 ng/mL Celecoxib in MeOH was immediately added

into the plasma, mixed well and centrifuged at 12,000 rpm for 10 minutes at 2 to 8°C. Samples of supernatant were transferred into the pre-labeled polypropylene microcentrifuge tubes, and frozen over dry ice. The samples were stored at -60℃ or below until LC-MS/MS analysis. An aliquot of 40 µL calibration standard, quality control, single blank and double blank samples were added to the 1.5 mL tube. Each sample (except the double blank) was quenched with 200 µL IS1 respectively (double blank sample was quenched with 200 µL MeOH with 0.5% tritonX-100), and then the mixture was vortex-mixed well (at least 15 s) with vortexer and centrifuged for 15 min at 12000 g, 4°C. A 10 µL supernatant was injected for LC-MS/MS analysis using an Orbitrap Q Exactive in positive ion mode to determine the concentrations of analyte. Plasma concentration versus time data were analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program. C0, Cl, Vdss, T½, AUC(0-last), AUC(0-inf), MRT(0-last), MRT(0-inf) and graphs of plasma concentration versus time profile were reported. The pharmacokinetic parameters for three bispecific compounds are as shown in Table 7.

Table 7: Pharmacokinetic Parameters in cynomolgous monkey

Compound	Route	$T_{1/2}$(h)	Clp (ml/min/kg)	Vdss (L/kg)
BCY11863 (0.93 mg/kg)	IV infusion (30 min)	5.3	3.3	0.62
BCY11863 (0.97 mg/kg)	IV infusion (15 min)	4.5	4.8	0.91
BCY11863 (9.4 mg/kg)	IV infusion (15 min)	8.9	3.9	1.1
BCY12491	IV infusion (15 min)	3.2	3.0	0.36
BCY13272	IV infusion (15 min)	8.9	4.1	0.82

Figure 3 shows the plasma concentration vs time curve of BCY11863 from a 2 mg/kg IV dose in SD Rat (n =3) and 1 mg/kg IV infusion in cynomolgus monkey (n = 2). BCY11863 has a volume of distribution at steady state (Vdss) of 1.6 L/kg and a clearance of 7.7 mL/min/kg in rats which results in a terminal half life of 4.1h. BCY11863 has a volume of

distribution at steady state (Vdss) of 0.62 L/kg and a clearance of 3.3 mL/min/kg in cyno which results in a terminal half life of 5.3 h.

Figure 12 shows the plasma concentration vs time curve of BCY12491 from a 15 minute 1 mg/kg IV infusion in cynomolgus monkey (n = 2).

Figure 17 shows the plasma concentration vs time curve of BCY13272 from a 3.6 mg/kg IV infusion (15 min) in SD Rat (n =3) and a 9.2 mg/kg IV infusion (15 min) in cynomolgus monkey (n = 3). BCY13272 has a volume of distribution at steady state (Vdss) of 1.0 L/kg and a clearance of 7.5 mL/min/kg in rats which results in a terminal half life of 2.9h. BCY13272 has a volume of distribution at steady state (Vdss) of 0.82 L/kg and a clearance of 4.1 mL/min/kg in cyno which results in a terminal half life of 8.9 h.

5. Pharmacokinetics of CD137 Heterotandem Bicyclic Peptide Complexes in CD1 Mice

6 Male CD-1 mice were dosed with 15 mg/kg of each Heterotandem Bicycle Peptide Complex formulated in 25 mM Histidine HCl, 10% sucrose pH 7 via intraperitoneal or intravenous administration. Serial bleeding (about 80 µL blood/time point) was performed via submandibular or saphenous vein at each time point. All blood samples were immediately transferred into prechilled microcentrifuge tubes containing 2 µL K2-EDTA (0.5M) as anti-coagulant and placed on wet ice. Blood samples were immediately processed for plasma by centrifugation at approximately 4°C, 3000g. The precipitant including internal standard was immediately added into the plasma, mixed well and centrifuged at 12,000 rpm, 4°C for 10 minutes. The supernatant was transferred into pre-labeled polypropylene microcentrifuge tubes, and then quick-frozen over dry ice. The samples were stored at 70°C or below as needed until analysis. 7.5 µL of the supernatant samples were directly injected for LC-MS/MS analysis using an Orbitrap Q Exactive in positive ion mode to determine the concentrations of analyte. Plasma concentration versus time data were analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program. C0, Cl, Vdss, T½, AUC(0-last), AUC(0-inf), MRT(0-last) , MRT(0-inf) and graphs of plasma concentration versus time profile were reported.

Figure 11 shows the plasma concentration vs time curves of BCY11863 and BCY12491 from a 15 mg/kg IP dose in CD1 mice (n =3) and the terminal plasma half lives for BCY11863 and BCY12491.

Table 7A: Pharmacokinetic Parameters in CD-1 Mice

Compound	Dose (mg/kg)	Dosing Route	T1/2(h)	Vdss (L/kg)	Clp (ml/min/kg)	% F
BCY11863	5.6	IV Bolus	2.6	1.6	9.7	
	0.96	IV Bolus	1.7	2.9	21	
	12	IV Bolus	2.6	2.5	17	
	32	IV Bolus	2.4	2.1	16	
	15.5	IP	2.5	-	-	100

Data in Figure 11 and Table 7A above shows BCY11863 can be dosed as IV bolus and IP in mice. The bioavailability from IP dosing of BCY11863 is high in mice. The PK parameters from the IV study indicates that this is a low clearance molecule with volume of distribution larger than plasma volume.

Figure 17 shows the plasma concentration vs time curve of BCY13272 from a 5.5 mg/kg IV dose in CD1 mice (n =3); the volume of distribution (Vdss) of BCY13272 is 1.1 L/kg with a Clearance of 7.5 mL/min/kg which results in terminal plasma half life of 2.9 h.

6. Anti-tumor activity of BCY11863 in a syngeneic Nectin-4 overexpressing MC38 tumor model (MC38#13)

6-8 weeks old C57BL/6J-hCD137 female mice were inoculated in the flank with $1x10^6$ syngeneic Nectin-4 overexpressing MC38 cells (MC38#13). When tumors reached $72mm^3$ size on average, mice were randomized to receive vehicle or BCY11863 (intraperitoneal administration). BCY11863 was administered (n=6 mice/treatment cohort) at either 1 mg/kg or 10 mg/kg either daily (QD) or every three days (Q3D). QD dosed mice received 16 doses of BCY11863 and Q3D dosed mice received 10 doses of BCY11863. Tumor growth was monitored by caliper measurements until day 69 after treatment initiation. The results of this experiment may be seen in Figure 4 where significant reduction ($p<0.05$, 2-way ANOVA with Dunnett's test for multiple comparisons) of tumor growth was observed in 2 treatment cohorts by day 7 and by day 14 all treatment groups were significantly different from the vehicle group. By day 48, 22 out of 24 BCY11863 -treated animals had responded to the treatment completely and had no palpable tumors remaining.

Based on the circulating plasma half-life of BCY11863 in mice after IP injection (2.5 h), plasma trough levels will be close to 0 after both BCY11863 doses (1 and 10 mg/kg) and dosing intervals (QD and Q3D) thus demonstrating that less than continuous plasma exposure of BCY11863 from intermittent dosing is sufficient to lead to significant anti-tumor activity leading to durable complete responses.

7. BCY11863 treatment leads to an immunogenic memory to Nectin-4 overexpressing MC38 tumor model

On day 69, 5 animals that had responded completely to BCY11863 treatment were re-inoculated with $1x10^6$ MC38#13 -cells. A cohort of 5 naïve C57BL/6J-hCD137 female mice were inoculated with $1x10^6$ MC38#13 -cells as a control. The results of this experiment may be seen in Figure 5 where all 5 inoculated naïve C57BL/6J-hCD137 female mice grew tumors by day 13 after inoculation whereas none of the inoculated complete responder mice developed tumors. This demonstrates that animals that achieved a complete antitumor response as a result of BCY11863 treatment have developed immunogenic memory.

8. BCY11863 demonstrates anti-tumor activity in a syngeneic Nectin-4 overexpressing CT26 tumor model (CT26#7)

6-8 weeks old BALB/c-hCD137 female mice were inoculated in the flank with $3x10^5$ syngeneic Nectin-4 overexpressing CT26 cells (CT26#7). When tumors reached around $70mm^3$ size on average, mice were randomized to receive vehicle or 5 mg/kg BCY11863 intraperitoneally every three days (6 doses total). Tumor growth was monitored by caliper measurements until day 14 after treatment initiation. The results of this experiment may be seen in Figure 6 where BCY11863 treatment significantly ($p<0.0001$, Student's t-test) reduced the tumor growth from day 7 forward.

Based on the circulating plasma half-life of BCY11863 in mice at IP injection (2.5 h), plasma exposure will not be continuous throughout the dosing period demonstrating that less than continuous plasma exposure of BCY11863 is sufficient to lead to significant anti-tumor activity.

9. Total T cells and CD8+ T cells increase in CT26#7 tumor tissue 1h after the last (6^{th}) Q3D dose of BCY11863

1 hour after the last vehicle or BCY11863 dose the CD26#7 bearing mice were sacrificed and tumors were harvested, processed for single cell suspensions and stained for flow cytometry analysis for total T cells (CD45+CD3+), CD8+ T cells (CD45+CD3+CD8+), CD4+ T cells (CD45+CD3+CD4+) and regulatory T cells (Tregs; CD45+CD3+CD4+Foxp3+). The results of this experiment may be seen in Figure 7 where it can be seen that BCY11863 treatment led to significant increase of total T cells ($p<0.0001$, Student's t-test) and CD8+ T cells ($p<0.0001$, Student's t-test) as well as to a significant increase in the CD8+ T cell/Treg ratio ($p<0.05$, Student's t-test).

This demonstrates that treatment with BCY11863 can lead to an increased level of T-cells locally in the tumor tissue after intermittent dosing.

10. Pharmacokinetic profiles of BCY11863 in plasma and tumor tissue of CT26#7 syngeneic tumor bearing animals after a single intravenous (iv) administration of 5 mg/kg of BCY11863

6-8 weeks old BALB/c female mice were inoculated in the flank with $3x10^5$ syngeneic Nectin-4 overexpressing CT26 cells (CT26#7). When tumors reached around $400mm^3$ size on average, mice were randomized to receive a single intravenous dose of vehicle or 5 mg/kg BCY11863. A cohort of mice (n=3/timepoint) were sacrificed at 0.25, 0.5, 1, 2, 4, 8 and 24h timepoints and harvested plasma and tumor tissue were analyzed for BCY11863. For tumor BCY11863 content analysis, tumor homogenate was prepared by homogenizing tumor tissue with 10 volumes (w:v) of homogenizing solution (MeOH/15 mM PBS (1:2, v:v)). 40 µL of sample was quenched with 200 µL IS1 and the mixture was mixed by vortexing for 10 min at 800 rpm and centrifuged for 15 min at 3220 g at 4 ℃.The supernatant was transfer to another clean 96-well plate and centrifuged for 5 min at 3220 g at 4 ℃, and 10.0 µL of supernatant was then injected for LC-MS/MS analysis using an Orbitrap Q Exactive in positive ion mode to determine the concentrations of analyte. For plasma BCY11863 content analysis, blood samples were collected in K2-EDTA tubes and immediately processed to plasma by centrifugation at approximately 4 ˚C, 3000g. 40 µL of plasma sample was quenched with 200 µL IS1 and the mixture was mixed by vortexing for 10 min at 800 rpm and centrifuged for 15 min at 3220 g at 4 ℃.The supernatant was transfer to another clean 96-well plate and centrifuged for 5 min at 3220 g at 4 ℃, and 10.0 µL of supernatant was then injected for LC-MS/MS analysis using an Orbitrap Q Exactive in positive ion mode to determine the concentrations of analyte.

The results of this experiment may be seen in Figure 8 where it can be seen that BCY11863 was retained in the tumor tissue after the plasma BCY11863 is eliminated from circulation as indicated by the difference of BCY11863 plasma $T_{1/2}$ (1.65h) and tumor $T_{1/2}$ (13.4h).

11. Anti-tumor activity of BCY12491 in a syngeneic MC38 tumor model

6-8 weeks old C57BL/6J-hCD137 female mice were inoculated in the flank with $1x10^6$ syngeneic MC38 cells. When tumors reached $76mm^3$ size on average, mice were randomized to receive vehicle or BCY12491 (intraperitoneal administration). BCY12491 was administered (n=6 mice/treatment cohort) at either 5 mg/kg or 15 mg/kg either daily (QD) or every three days (Q3D). QD dosed mice received 22 doses of BCY12491 and Q3D dosed mice received 8 doses of BCY12491. Tumor growth was monitored by caliper

measurements until day 73 after treatment initiation. The results of this experiment may be seen in Figure 9 where it can be seen that the effect of BCY12491 on tumor growth becomes apparent in the first two weeks of the dosing period reducing the tumor growth and causing reduction of volumes of many of the treated tumors. By day 41, 15 out of 24 BCY12491 treated animals had completely responded to the treatment and had no palpable tumors left.

Based on the circulating plasma half-life BCY12491 in mice mice after IP injection (2.5 h), plasma trough levels will be close to 0 after both BCY12491 doses (5 and 15 mg/kg) and dosing intervals (QD and Q3D) thus demonstrates that less than continuous plasma exposure of BCY12491 from intermittent dosing is sufficient to lead to significant anti-tumor activity and durable complete responses.

12. EphA2/CD137 heterotandem bicyclic peptide complex BCY12491, BCY13272, BCY12723, BCY13050, BCY13048 and BCY13047 induces IFN-γ cytokine secretion in an MC38 co-culture assay

Mouse mammary gland tumor cell line MC38 were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% heat inactivated Fetal Bovine Serum (FBS), 1x Penicillin/Streptomycin, 10 mM HEPES, and 2 mM L-Glutamine (referred to as R10 medium). Frozen PBMCs from healthy human donors were thawed and washed once in room temperature PBS with benzonase, and then resuspended in RPMI supplemented with 10% heat inactivated Fetal Bovine Serum (FBS), 1x Penicillin/Streptomycin, 10 mM HEPES, and 2 mM L-Glutamine (herein referred to as R10 medium). 100 µl of PBMCs (1,000,000 PBMCs/ml) and 100 µl of tumor cells (100,000 tumor cells/ml) (Effector: Target cell ratio (E:T) 10:1) were plated in each well of a 96 well flat bottom plate for the co-culture assay. 100 ng/ml of soluble anti-CD3 mAb (clone OKT3) was added to the culture on day 0 to stimulate human PBMCs. Test, control compounds, or vehicle controls were diluted in R10 media and 50 µL was added to respective wells to bring the final volume per well to 250 µL. Plates were covered with a breathable film and incubated in a humidified chamber at 37ºC with 5% CO_2 for two days. Supernatants were collected 24 and 48 hours after stimulation, and human IFN-γ was detected by Luminex. Briefly, the standards and samples were added to a black 96 well plate. Microparticle cocktail (provided in Luminex kit, R&D Systems) was added and shaken for 2 hours at room temperature. The plate was washed 3 times using a magnetic holder. Biotin cocktail was then added to the plate and shaken for 1 hour at RT. The plate was washed 3 times using a magnetic holder. Streptavidin cocktail was added to the plate and shaken for 30 minutes at RT. The plates were washed 3 times using a magnetic holder, resuspended in 100 µL of wash buffer, shaken for 2 minutes at RT, and

read using the Luminex 2000. Raw data were analyzed using built-in Luminex software to generate standard curves and interpolate protein concentrations, all other data analyses and graphing were performed using Excel and Prism software. Data represents one study with three independent donor PBMCs tested in experimental duplicates.

Data presented in Figure 10 demonstrates that the EphA2/CD137 heterotandem bicyclic peptide complex BCY12491 induces IFN-γ cytokine secretion in an MC38 co-culture assay with an EC_{50} of 34 pM (Figure 10A = donor 228769) or 85 pM (Figure 10B = donor 228711) using PBMCs from two different human donors. BCY12762 is a heterotandem bicyclic peptide complex that binds to EphA2 with the same affinity as BCY12491 but does not bind to CD137.

Similarly, PBMCs from healthy donors were co-cultured with EphA2 expressing cancer cells (MC38 and HT-1080) at a ratio of 5:1 in presence of anti-CD3 and BCY13272. Supernatants were analyzed after 48h by Luminex for cytokines (IL-2 and IFNγ), data is shown in Table 8 and is representative of PBMCs from one donor (from a total of n= 4 or 5 individual experiments).

Table 8: EC50 of IL-2 cytokine secretion induced by EphA2/CD137 heterotandem bicyclic complexes in human PBMC-MC38/HT-1080 co-culture assay

Complex ID	Cell line	EC_{50} (nM)	N =
BCY13272	MC38	0.79± 0.24	5
BCY13272	HT-1080	0.55± 0.47	4

Data presented in Figure 26 and tabulated in Table 8A demonstrates that the EphA2/CD137 heterotandem bicyclic peptide complexes induce IFN-γ cytokine secretion in an MC38 co-culture assay with subnanomolar potency.

Table 8A: EC50 and Emax of IFNγ secretion induced by EphA2/CD137 heterotandem bicyclic complexes in human PBMC-MC38 co-culture assays.

Complex ID	Time of incubation (h)	Donor 1 EC50 (nM)	Donor 2 EC50 (nM)
BCY12491	48	0.034	0.085
BCY12730	48	0.13	0.19
BCY12723	72	0.13	0.095
BCY13050	72	0.38	0.19

BCY13048	48	0.30	0.24
BCY13047	48	0.31	0.30

13. Target dependent cytokine release in *ex vivo* cultures of primary patient-derived lung tumors

Primary patient derived tumor cells from Discovery Life Sciences (DLS) were thawed gently in 10mL pre-warmed wash medium spiked fresh with Benzonase. The 3D spheroid kit from Greiner (cat# 655840) is used to maintain cells in culture for 2 days. Briefly, tumor cells were counted with trypan blue using a haemocytometer. The cells were centrifuged at 1500rpm for 5min to wash, and the pellet is resuspended in 100µL per 1 x 10^6 cells N3D nanoshuttle. To make them magnetic, cells were spun down at 1500rpm for 5 min and resuspended; this process is repeated for a total of 4 times. After the final spin, cells were resuspended in the appropriate amount of fresh Lung DTC medium (DLS) to give 50,000-100,000 cells per well in 100µL/well. Greiner cell-repellent, 96-well plates (cat #655976) were used for this experiment. If there were cell clumps or debris visible, sample is applied to a 70-100µm filter before plating. At least 50,000 cells per sample were reserved for a Day 0 flow cytometry panel, these cells were stained, fixed, and stored at 4°C for later flow analysis. Control/test compound dilutions were prepared in a separate plate at 2x in Lung DTC medium, and 100µL/well of these 2X drug solutions were added to the wells as described by the plate map. The assay plate was then placed onto the 96-well magnetic spheroid drive in a humidified chamber at 37°C, 5% CO_2. At 24h, the magnetic spheroid drive was removed. At 48h, medium was collected for cytokine analysis and cells were collected for a Day 2 flow cytometry panel. Cytokines were quantified using a custom-built cytokine/chemokine panel (IP-10, Granzyme B, IFNγ, IL-2, IL-6, TNFα, IL-8, MIP-1a, MIP-1b, MCP-1, IL-10, MIG) from R&D systems on a Luminex reader. Flow panels: Day 0 = Live/Dead, CD45, EpCAM, Nectin4, CD3, CD4, CD8, CD137; Day 2 = Live/Dead, CD45, EpCAM, Nectin4, CD3, CD8, Ki67, and counting beads. Flow data is analysed with Flowjo software.

Data shown in Figure 13 demonstrate that Nectin-4/CD137 heterotandem BCY11027 induces target dependent cytokine release in *ex vivo* cultures of primary patient-derived lung tumors. Treatment with BCY11027 induced Nectin-4 dependent change in several immune markers (normalized to vehicle) and in %CD8 +ki67+ T cells in patient-derived samples that correlated with the level of Nectin-4 expression.

14. Promega OX40 cell-activity assay in co-culture with tumor cells

Promega have developed an OX40 cell-activity assay that uses NF-κB luciferase luminescence as a read-out of OX40 activation in Jurkat cells (Promega CS197704). On the day of the experiment, prepare medium by thawing FBS and adding 5 % FBS to RPMI-1640. Thaw OX40 Jurkat cells in the water-bath and then add 500 µl cells to 11.5 ml pre-warmed 5 % FBS RPMI-1640 medium. Add 55 µl cells/well to white cell culture plates. Harvest tumor cells from culture. 4T1 is a Nectin-4 negative murine mammary gland epithelial cancer cell and it was genetically modified to express murine Nectin-4 on the cell surface (4T1 Nectin-4 positive; clone 4T1-D02). Tumor cells were cultured to 80% confluency *in vitro* in RPMI1640 medium supplemented with 10% heat-inactivated FBS, 1X Penicillin/Streptomycin, 1X L-Glutamine, 20 mM HEPES and 1X NEAA (RPMI working medium). Tumor cells were trypsinized and washed two times at 1500 rpm for 5 minutes in RPMI1640 working medium prewarmed to 37ºC. Count cells and resuspend at 2,000,000 cells/mL in R5 media (for 10,000 cells/well). Add 5 µL of tumor cells per well.

Proceed to dilute agonists at concentration giving the maximum fold induction and then titrate down the amount in a sterile 96 well-plate. Prepare enough reagent for duplicate samples and then perform 1/3 dilution series or 1/10 dilution series. Include positive control OX40L trimer (AcroBiosystems, R&D systems) and negative control monomeric or non-binding peptides. Add 20 µl of agonist as duplicate samples or 5% FBS RPMI-1640 alone as background control.

Co-incubate cells together with agonists for 6 hours at 37°C, 5 % CO_2. After 6 hours, thaw Bio-Glo™ and develop the assay at room-temperature. Add 80 µl Bio-Glo™ per well and incubate 5-10 min. Read luciferase signal on CLAIROStar plate-reader using the MARS program and normalize the fold induction relative to background (medium alone). Analyse data by transforming the data to x=log (X), then plot log (agonist) vs. response variable slope (4 parameters) to calculate EC_{50} values.

The results of this assay are shown in Table 9 and Figure 14 where it can be seen that the BCY12967 Nectin-4:OX40 compound showed potent OX40 agonism when in co-culture with Nectin-4 positive 4T1-D02 cells as compared to OX40L and non-binding control peptide BCY12968.

Table 9: EC_{50} Values from Promega OX40 cell-activity assay in co-culture with tumor cells

Peptide Number	*EC_{50} (nM)*
BCY12967	0.83

15. Dosing of an EphA2 : CD137 1:2 Heterotandem Complex induces a dramatic immune response in mouse tumor models

6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38 cells. Mice were randomized into treatment groups when average tumor volumes reached around 240 mm^3 and were treated (n=6/treatment cohort) with vehicle (25 mM histidine, 10% sucrose, pH7) intravenously (IV), 15 mg/kg BCY12491 (EphA2 : CD137 1:2 Heterotandem Complex) IV, 15 mg/kg BCY13626 (non-binding control for EphA2) IV or 2 mg/kg Anti-CD137 (urelumab analogue) intraperitoneally. All treatments were given Q3D for three doses and tumor tissues were harvested 1 hour after the last dose. Part of the tumor tissue was used for RNA isolation for transcriptional analysis and a part of the tumor tissue was used for formalin fixed paraffin embedded (FFPE) sample preparation for immunohistochemical (IHC) analysis. RNA was isolated from tumor tissues using RNAeasy kit [Qiagen] and transcriptional analysis was performed using nCounter Mouse PanCancer IO 360 panel (Nanostring) from 100ng RNA/tumor. Data was analysed using the nSolver Analysis Software (Nanostring). CD8+ tumor infiltrating cells were stained in FFPE tissue sections using anti-mouse CD8 antibody (Abcam, # ab217344) and Ventana Discovery OmniMap anti Rabbit-HRP Kit (Ventana #760 4310).

The results of this study are shown in Figures 15A to D where it can be observed that transcriptional analysis revealed a significant increase in immune cell scores such as cytotoxic cell score (Figure 15A), macrophage cell (Figure 15B) and T cell score (Figure 15C) in tumor tissue upon EphA2 BCY12491 treatment when compared to tumors from vehicle treated mice. The anti-CD137 antibody treatment also increased significantly the cytotoxic cell score and T cell score in tumor tissue, although to a lesser extent than BCY12491. No changes were observed in immune cell scores in tumor tissues from non-binding control (BCY13626) treated animals. IHC analysis for CD8+ cells in the tumor tissues demonstrated an intense infiltration of CD8+ cells in the tumors from BCY12491 treated mice when compared to tumors from vehicle or non-binder BCY13626 treated mice (Figure 15D). Some increase in CD8+ cell infiltration was also observed in tumors from anti-CD137 antibody treated mice. These changes in immune cell scores and CD8+ cells in tumor tissue indicate that agonism of CD137 in tumor tissue by the EphA2 : CD137 1:2 Heterotandem Complex BCY12491 leads to a significant modulation (increase) of the tumor infiltrating immune cells and immune response.

16. Anti-tumor activity of BCY13272 in a syngeneic MC38 tumor model

6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38 cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 80 mm^3 and were treated with vehicle (25 mM histidine, 10% sucrose, pH7) intravenously (IV), 8 mg/kg BCY13272, 0.9 mg/kg BCY13272 and 0.1 mg/kg BCY13272 IV. All treatments were given twice a week (BIW) for 6 doses in total. Tumor growth was monitored until Day 28 from treatment initiation. Complete responder animals (n=7) were followed until day 62 after treatment initiation and re-challenged with an implantation of $2x10^6$ MC38 tumor cells and tumor growth was monitored for 28 days. In parallel, naïve age-matched control huCD137 C57Bl/6 mice (n=5) were implanted with $2x10^6$ MC38 tumor cells monitored for 28 days.
The results of this experiment may be seen in Figure 18 where it can be seen that BCY13272 leads to significant anti-tumor activity with complete responses observed at 0.9 (2 out of 6 complete responders) and 8 mg/kg (5 out of 6 complete responders) dose levels (Figure 18A). Unlike in naïve age-matched control huCD137 C57Bl/6 mice (tumor take rate 100%), no tumor regrowth was observed in BCY13272 complete responder animals (Figure 18B). These data indicate that BCY13272 has significant anti-tumor activity and that the BCY13272 treatment can lead into immunogenic memory in the complete responder animals.

17. Binding of BCY13272 to EphA2 and CD137 as measured by SPR

(a) CD137

Biacore experiments were performed to determine k_a ($M^{-1}s^{-1}$), k_d (s^{-1}), K_D (nM) values of heterotandem peptides binding to human CD137 protein. Recombinant human CD137 (R&D systems) was resuspended in PBS and biotinylated using EZ-Link™ Sulfo-NHS-LC-LC-Biotin reagent (Thermo Fisher) as per the manufacturer's suggested protocol. The protein was desalted to remove uncoupled biotin using spin columns into PBS.

For analysis of peptide binding, a Biacore T200 or a Biacore 3000 instrument was used with a XanTec CMD500D chip. Streptavidin was immobilized on the chip using standard amine-coupling chemistry at 25°C with HBS-N (10 mM HEPES, 0.15 M NaCl, pH 7.4) as the running buffer. Briefly, the carboxymethyl dextran surface was activated with a 7 min injection of a 1:1 ratio of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/0.1 M *N*-hydroxy succinimide (NHS) at a flow rate of 10 µl/min. For capture of streptavidin, the protein was diluted to 0.2 mg/ml in 10 mM sodium acetate (pH 4.5) and captured by injecting 120µl of onto the activated chip surface. Residual activated groups were blocked with a 7 min injection of 1 M ethanolamine (pH 8.5) and biotinylated CD137 captured to a level of 270-1500 RU.

Buffer was changed to PBS/0.05% Tween 20 and a dilution series of the peptides was prepared in this buffer with a final DMSO concentration of 0.5%. The top peptide concentration was 500nM with 6 further 2-fold or 3-fold dilutions. The SPR analysis was run at 25°C at a flow rate of 90µl/min with 60 seconds association and 900 seconds dissociation. After each cycle a regeneration step (10µl of 10mM glycine pH 2) was employed. Data were corrected for DMSO excluded volume effects as needed. All data were double-referenced for blank injections and reference surface using standard processing procedures and data processing and kinetic fitting were performed using Scrubber software, version 2.0c (BioLogic Software). Data were fitted using simple 1:1 binding model allowing for mass transport effects where appropriate.

(b) EphA2

Biacore experiments were performed to determine k_a ($M^{-1}s^{-1}$), k_d (s^{-1}), K_D (nM) values of BCY13272 binding to human EphA2 protein.

EphA2 were biotinylated with EZ-Link™ Sulfo-NHS-LC-Biotin for 1 hour in 4mM sodium acetate, 100mM NaCl, pH 5.4 with a 3x molar excess of biotin over protein. The degree of labelling was determined using a Fluorescence Biotin Quantification Kit (Thermo) after dialysis of the reaction mixture into PBS. For analysis of peptide binding, a Biacore T200 instrument was used with a XanTec CMD500D chip. Streptavidin was immobilized on the chip using standard amine-coupling chemistry at 25°C with HBS-N (10 mM HEPES, 0.15 M NaCl, pH 7.4) as the running buffer. Briefly, the carboxymethyl dextran surface was activated with a 7 min injection of a 1:1 ratio of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/0.1 M N-hydroxy succinimide (NHS) at a flow rate of 10 µl/min. For capture of streptavidin, the protein was diluted to 0.2 mg/ml in 10 mM sodium acetate (pH 4.5) and captured by injecting 120µl onto the activated chip surface. Residual activated groups were blocked with a 7 min injection of 1 M ethanolamine (pH 8.5):HBS-N (1:1). Buffer was changed to PBS/0.05% Tween 20 and biotinylated EphA2 was captured to a level of 500-1500 RU using a dilution of protein to 0.2µM in buffer. A dilution series of the peptides was prepared in this buffer with a final DMSO concentration of 0.5% with a top peptide concentration was 50 or 100nM and 6 further 2-fold dilutions. The SPR analysis was run at 25°C at a flow rate of 90µl/min with 60 seconds association and 900-1200 seconds dissociation. Data were corrected for DMSO excluded volume effects. All data were double-referenced for blank injections and reference surface using standard processing procedures and data processing and kinetic fitting were performed using Scrubber software, version 2.0c (BioLogic Software). Data were fitted using simple 1:1 binding model allowing for mass transport effects where appropriate.

Figure 20A shows the sensorgram which demonstrates that BCY13272 binds to EphA2 (human) with an affinity of 2.0 nM. Figure 20B shows the sensorgram that BCY13272 binds to CD137 (human) with high affinity. Due to the presence of 2 CD137 binding bicycles in BCY13272, the off rate from immobilized CD137 protein is very slow and the reported K_D may be an overestimation (Figure 19B).

18. Binding of BCY11863 to Nectin-4 and CD137 across four preclinical species

The binding of BCY11863 to its primary target Nectin-4 and CD137 was characterized using surface plasmon resonance (SPR).

(a) Nectin-4

BCY11863 binds to cyno, rat, mouse and human Nectin-4 with K_D between 5 – 27 nM as measured by direct binding to the extracellular domain that has been biotinylated and captured on a streptavidin sensor chip surface.

Table 10: Binding affinities of BCY11863 to Biotinylated - Nectin-4 extracellular domain: SPR data

SPR K_D (nM)	Assay Type	Human (25 °C)	Human (37 °C)	NHP (25 °C)	Rat (25 °C)	Mouse (25 °C)
BCY11863	Direct Binding	5.0 ± 2.1 n = 7	5.2 ± 1.1 n = 9	27 ± 15 n = 9	15 ± 1 n = 6	4.6 ± 2.1 n = 9

To understand whether the binding of BCY11863 to Nectin-4 was altered in the context of the ternary complex, i.e. when also bound to CD137, a multicomponent SPR binding assay was developed. BCY11863 was first captured to human CD137 immobilized on the SPR chip surface and then Nectin-4 from different species were passed over the chip to determine their affinities to the captured BCY11863 (see Figure 21C). The affinities to Nectin-4 were generally maintained in the presence of CD137 binding as shown below:

Table 11: Binding affinities of BCY11863 to Nectin-4 extracellular domain using biotinylated human CD137 as capture reagent

SPR K_D (nM)	Assay Type	Human	NHP	Rat	Mouse
BCY11863	Sandwich Assay	12 ± 2 n = 4	28 ± 5 n = 3	25 ± 2 n = 3	6.7 ± 1.7 n = 3

(b) CD137

Direct binding of BCY11863 to surface bound CD137 cannot be measured accurately by SPR because of avidity resulting from two CD137 binding bicycles in BCY11863 which leads to extremely slow k_{off} (See Figure 21B). In addition, biotinylation of cyno CD137 abrogates binding of BCY11863, likely due to modification of a lysine on the cyno protein that is important for BCY11863 binding. Hence, a BCY11863 analogue containing a C-terminal biotinylated lysine (BCY13582) was tested in SPR to determine cross species specificity of BCY11863. BCY13582 was captured to the sensor chip using a reversible biotin capture kit and the affinities to Nectin-4 from different species were determined. Both strategies showed that these BCY11863 analogs bound to human and cyno CD137 with K_D < 10 nM and had negligible binding to both mouse and rat CD137.

Table 12: Binding affinities of biotinylated BCY11863 analogues to CD137 extracellular domain: SPR data

SPR K_D (nM)	**Assay Type**	**Human**	**NHP**	**Rat**	**Mouse**
BCY13582	Direct Binding	8.4 ± 4.2 n = 3	4.23 n = 1	NB n = 1	NB n = 1

To understand whether the binding of BCY11863 to CD137 was altered in the context of the ternary complex, i.e. when also bound to Nectin-4, a dual binding SPR binding assay was developed. BCY11863 was first captured to human Nectin-4 immobilized on the SPR chip surface and then soluble CD137 from different species were passed over the chip to determine their affinities to the captured BCY11863 (see Figure 21D). The affinities to CD137 were generally maintained in the presence of Nectin-4 binding as shown below:

Table 13: Binding affinities of BCY11863 to CD137 ECD using biotinylated human Nectin-4 as capture reagent

SPR K_D (nM)	**Assay Type**	**Human**	**NHP**	**Rat**	**Mouse**
BCY11863	Dual Binding	6.3 ± 0.7 n = 4	18 ± 6 n = 3	NB n = 2	NB n = 2

Figure 21A shows one example sensorgram which demonstrates that BCY11863 binds to Nectin-4 (human) with an affinity of 4.1 nM. Figure 21B shows the sensorgram that BCY11863 binds to CD137 (human) with high affinity. Due to the presence of 2 CD137

binding bicycles in BCY11863, the off rate from immobilized CD137 protein is very slow and the reported K_D may be an overestimation (Figure 21B). Figure 21C shows BCY11863 binds to Nectin-4 while the CD137 arms are bound to CD137 protein immobilized on the chip to form a ternary complex. Figure 21D shows BCY11863 binds to CD137 while the Nectin-4 binding arm is bound to Nectin-4 protein immobilized on the chip to form a ternary complex. Figure 21E demonstrates the ability of BCY13582 immobilized on SPR chip to bind human CD137.

19. Selectivity of BCY11863 for Nectin-4 and CD137

Nectin – 4 Paralogue screening: Binding of BCY11863 was assessed using SPR against Nectin-1 (2880-N1, R&D Systems), Nectin-2 (2229-N2, R&D Systems), Nectin-3 (3064-N3, R&D Systems), Nectin-like-1 (3678-S4-050, R&D Systems), Nectin-like-2 (3519-S4-050, R&D Systems), Nectin-like-3 (4290-S4-050, R&D Systems), Nectin-like-4 (4164-S4, R&D Systems) and Nectin-like-5 (2530-CD-050, R&D Systems) by labelling them with biotin and immobilizing them on a streptavidin surface. BCY11863 did not show any binding to these targets up to a concentration of 5000 nM.

CD137 Paralogue screening: Binding of streptavidin captured BCY13582 (biotinylated-BCY11863) was assessed using SPR against soluble TNF family receptors OX40 and CD40. BCY13582 did not bind to these targets up to a concentration of 100 nM.

Retrogenix microarray screening: Retrogenix's cell microarray technology was used to screen for specific off-target binding interactions of a biotinylated BCY11863 known as BCY13582.

Investigation of the levels of binding of the test peptide to fixed, untransfected HEK293 cells, and to cells over-expressing Nectin-4 and CD137 (TNFRSF9), showed 1 μM of the test peptide to be a suitable screening concentration. Under these conditions, the test peptide was screened for binding against human HEK293 cells, individually expressing 5484 full-length human plasma membrane proteins and secreted proteins. This revealed 9 primary hits, including Nectin-4 and CD137.

Each primary hit was re-expressed, along with two control receptors (TGFBR2 and EGFR), and re-tested with 1 μM BCY13582 test peptide, 1 μM BCY13582 test peptide in the presence of 100 μM BCY11863, and other positive and negative control treatments (Figure 4). After removing non-specific, non-reproducible and non-significant hits, there remained

three specific interactions for the test peptide. These were untethered and tethered forms of Nectin-4, and CD137 - the primary targets.

No specific off-target interactions were identified for BCY13582, indicating high specificity for its primary targets.

20. Anti-tumor activity of BCY11863 in a syngeneic Nectin-4 overexpressing MC38 tumor model (MC38#13) on dosing on twice a week at 5mg/kg at 0,24h and 10 mg/kg at 0h

6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38#13 (MC38 cells engineered to overexpress murine Nectin-4) cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 95 mm^3 and were treated with a weekly dose of vehicle (25 mM histidine, 10% sucrose, pH7) or 10 mg/kg BCY11863 with two different dosing schedules for two dosing cycles (5 mg/kg BCY11863 at 0h and 24h on D0 and D7, or 10 mg/kg at 0h on D0 and D7). All treatments were administered intravenously (IV). Tumor growth was monitored until Day 15 from treatment initiation.

BCY11863 leads to significant anti-tumor activity with both dosing schedules, but the dose schedule with 5 mg/kg dosing at 0h and 24h was superior to 10 mg/kg dosing at 0h when complete responses were analyzed on day 15 after treatment initiation (Figure 23). 5 mg/kg BCY11863 at 0h and 24h on D0 and D7 dosing led to 4 out of 6 complete tumor responses whereas 10 mg/kg BCY11863 at 0h on D0 and D7 dosing led to one out of 6 complete tumor responses. These data together with the BCY11863 mouse plasma PK data indicate that maintaining a BCY11863 plasma exposure at the level produced by 5 mg/kg 0h and 24h dosing in a weekly cycle produces close to complete anti-tumor response in the MC38#13 tumor model.

21. Anti-tumor activity of BCY11863 in a syngeneic Nectin-4 overexpressing MC38 tumor model (MC38#13)

At 3 weekly doses of 3, 10 and 30 mg/kg with dose fractionated weekly, biweekly and daily 6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38#13 (MC38 cells engineered to overexpress murine Nectin-4) cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 107 mm^3 and were treated with 21 daily doses of vehicle (25 mM histidine, 10% sucrose, pH7). BCY11863 treatment was done at three different total dose levels (3, 10 and 30 mg/kg total weekly dose) fractionated in three different schedules (QD: daily; BIW: twice a week or QW: weekly). Different BCY11863

treatment cohorts received either 21 daily doses (0.43, 1.4 or 4.3 mg/kg), 6 twice weekly doses (1.5, 5 or 15 mg/kg) or 3 weekly doses (3, 10 or 30 mg/kg). All treatments were administered intravenously (IV). Tumor growth was monitored until tumor reached volumes over 2000 mm^3 or until 31 days after treatment initiation. Complete responders (animals with no palpable tumors) were followed until D52.

BCY11863 leads to significant anti-tumor activity with many of the dosing schedules the BIW dosing schedule being the most efficacious schedule, the 5 mg/kg BIW dose in particular. This is demonstrated by the number of complete responder animals on day 52. On day 52 after treatment initiation, 15/18 mice treated BIW with BCY11863 were complete responders, 12/18 mice treated QD with BCY11863 were complete responders and 6/18 mice treated QW with BCY11863 were complete responders. 5 mg/kg BIW dosing lead to 100% complete response rate with 6/6 CRs (Figure 24). These data together with the BCY11863 mouse plasma PK data indicate that continuous BCY11863 plasma exposure is not needed for anti-tumor response to BCY11863 in the MC38#13 tumor model.

22. *In vivo* efficacy study for EphA2 Heterotandem Bicyclic Complexes

6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38 cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 76 mm^3 and were treated with daily doses of vehicle (25 mM histidine, 10% sucrose, pH7). BCY12491 treatment was conducted at two different dose levels (5 and 15 mg/kg) and two different dosing schedules (QD: daily; Q3D: every three days). Animals received either 22 QD doses or 8 Q3D doses intraperitoneally (ip). Tumor growth was monitored until tumor reached volumes over 2000 mm^3 or until 73 days after treatment initiation. After Day 73, 5 complete responder animals were re-challenged with MC38 tumor cell implantation alongside with 5 naïve C57BL/6J-hCD137 mice. Tumor growth was monitored for 20 days.

BCY12491 led to significant anti-tumor activity with all the doses and dose schedules used in the study. By day 41 after treatment initiation, 2 out of 6 BCY12491 5 mg/kg Q3D treated animals had become complete responders (CRs; no palpable tumor left), 3 out of 6 BCY12491 5 mg/kg QD treated animals became CRs, 4 out of 6 BCY12491 15 mg/kg Q3D treated animals became CRs and all (6/6) BCY12491 15 mg/kg QD treated animals became CRs. These data together with the BCY12491 mouse plasma PK -data indicate that continuous BCY12491 plasma exposure is not needed for maximal anti-tumor response to BCY12491 in the MC38 tumor model. Furthermore, complete responder animals rejected the re-challenge with MC38 tumor cell implantation and did not show any tumor growth whereas

naïve mice implanted simultaneously with the same tumor cells established tumor growth at 100% take rate by day 22 after implantation of tumor cells. This indicates development of immunogenic memory upon BCY12491-treatment leading to complete tumor response (Figure 27).

Dependency of BCY12491 activity of different immune cell populations was determined in treating MC38 tumor bearing C57BL/6J-hCD137 mice that had been depleted of CD8+ T cells or NK 1.1+ NK cells with BCY12491. 6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38#13 cells (clone of MC38 that has been engineered to overexpress Nectin-4). Three days after cell implantation mice received an ip injection of vehicle (PBS), 100 µg of depleting anti-CD8 (Rat IgG2b, clone 2.42) or anti-NK (Mouse IgG2a, clone PK136) antibodies (or their combination) or the corresponding isotype control antibodies (Rat IgG2b isotype control or Mouse IgG2a isotype control). Mice received additional doses of depletion antibodies (or isotype controls) 5 and 10 days after the first dose of antibodies. Cell depletion was verified by flow cytometry 4 and 12 days after the first dose of depletion antibody. When tumor volumes reached around $111mm^3$ (5 days after the first dose of depletion antibodies), mice started receiving vehicle or BCY12491 intravenously (iv) at 15 mg/kg twice weekly (BIW). Mice received a total of 4 doses of BCY12491. Tumor growth was monitored until Day 28 or until tumor volume exceeded $2000mm^3$.

BCY12491 treatment led to significantly decreased tumor growth rate and increased survival in MC38#13 tumor bearing mice that had been treated with vehicle or isotype control antibodies. The benefit of BCY12491 treatment on decreasing tumor growth rate and survival was lost in CD8 –depleted mice. Depletion of NK1.1+ cells did not affect the anti-tumor activity of BCY12491 treatment and subsequent survival benefit. This data demonstrates that the activity of BCY12491 in MC38#13 tumor model is dependent on CD8+ T cells, but not on NK1.1+ NK cells (Figure 28).

Anti-tumor activity of BCY12730 and BCY12723 was demonstrated alongside with BCY12491 activity. 6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38 cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 92 mm^3 and were treated intravenously with Q3D doses of vehicle (25 mM histidine, 10% sucrose, pH7), 15 mg/kg of BCY12730, BCY12723 or BCY12491 (7 Q3D doses). Tumor growth was monitored for 28 days or until tumors exceeded $2000mm^3$. BCY12491, BCY12730 and BCY12723 demonstrated significant anti-tumor activity leading to complete

responses in 4 out of 6 BCY12491 treated animals, 3 out of 6 BCY12730 treated animals and 2 out of 6 BCY12723 treated animals (Figure 29).

Anti-tumor activity of BCY13048 and BCY13050 was demonstrated alongside with BCY12491 activity. 6-8 week old female C57BL/6J-hCD137 mice [B-hTNFRSF9(CD137) mice; Biocytogen] were implanted subcutaneously with $1x10^6$ MC38 cells. Mice were randomized into treatment groups (n=6/cohort) when average tumor volumes reached around 76 mm^3 and were treated intravenously with twice weekly (BIW) doses of vehicle (25 mM histidine, 10% sucrose, pH7), 5 mg/kg of BCY13048, BCY13050, or BCY12491 (6 BIW doses). Tumor growth was monitored for 28 days or until tumors exceeded 2000mm^3. BCY12491, BCY13048 andBCY13050 demonstrated significant anti-tumor activity leading to complete responses in 2 out of 6 BCY12491 treated animals, 5 out of 6 BCY13048 treated animals and 3 out of 6 BCY13050 treated animals (Figure 30).

CLAIMS

1. A heterotandem bicyclic peptide complex comprising:

(a) a first peptide ligand which binds to a component present on a cancer cell; conjugated via a linker to

(b) two or more second peptide ligands which bind to a component present on an immune cell;

wherein each of said peptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

2. The heterotandem bicyclic peptide complex as defined in claim 1, wherein the immune cell is selected from: white blood cells; lymphocytes (e.g. T lymphocytes or T cells, B cells or natural killer cells); CD8 or CD4; CD8; dendritic cells, follicular dendritic cells and granulocytes.

3. The heterotandem bicyclic peptide complex as defined in claim 1 or claim 2, wherein said reactive groups are selected from cysteine, 3-mercaptopropionic acid and/or cysteamine residues.

4. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 3, wherein the component present on an immune cell is CD137.

5. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 4, wherein the two or more second peptide ligands comprise a CD137 binding bicyclic peptide ligand.

6. The heterotandem bicyclic peptide complex as defined in claim 5, wherein the CD137 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

C_iIEEGQYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 5);

C_i[tBuAla]PE[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 6);

C_iIEEGQYC_{ii}F[D-Ala]DPY[Nle]C_{iii} (SEQ ID NO: 7);

C_i[tBuAla]PK[D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 8);

C_i[tBuAla]PE[D-Lys]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 9);

C_i[tBuAla]P[K(PYA)][D-Ala]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 10);

C_i[tBuAla]PE[D-Lys(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 11);

(SEQ ID NO: 11)-A (herein referred to as BCY14601);

C_iIEE[D-Lys(PYA)]QYC_{ii}FADPY(Nle)C_{iii} (SEQ ID NO: 12);

C_i[tBuAla]PE[dK]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 60);

C_iIEE[dK(PYA)]QYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 61);

C_i[tBuAla]EE(dK)PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 62);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 63);

C_i[tBuAla]EE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 64);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FANPY[Nle]C_{iii} (SEQ ID NO: 65);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAEPY[Nle]C_{iii} (SEQ ID NO: 66);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FA[Aad]PY[Nle]C_{iii} (SEQ ID NO: 67);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAQPY[Nle]C_{iii} (SEQ ID NO: 68);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle][Cysam]$_{iii}$ (SEQ ID NO: 69);

[MerPro]$_i$[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 70; herein referred to as BCY12353);

[MerPro]$_i$[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle][Cysam]$_{iii}$ (SEQ ID NO: 71; herein referred to as BCY12354);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 72);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 73);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 74; herein referred to as BCY12372);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAD[NMeAla]Y[Nle]C_{iii} (SEQ ID NO: 75);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FAD[NMeDAla]Y[Nle]C_{iii} (SEQ ID NO: 76);

C_i[tBuAla]P[K(PYA)][dA]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 77);

C_i[tBuAla]PE[dK(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 78);

C_i[tBuAla]PE[dK(Me,PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 79);

C_i[tBuAla]PE[dK(Me,PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 80); and

[MerPro]$_i$[tBuAla]EE[dK]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 81; herein referred to as BCY13137);

wherein [MerPro]$_i$, C_i, C_{ii}, C_{iii} and [Cysam]$_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, Nle represents norleucine, tBuAla represents t-butyl-alanine, PYA represents 4-pentynoic acid, Aad represents alpha-L-aminoadipic acid, MerPro represents 3-mercaptopropionic acid and Cysam represents cysteamine, NMeAla represents N-methyl-alanine, or a pharmaceutically acceptable salt thereof.

7. The heterotandem bicyclic peptide complex as defined in claim 5 or claim 6, wherein the CD137 binding bicyclic peptide ligand comprises an amino acid sequence which is:

C_i[tBuAla]PE[D-Lys(PYA)]PYC_{ii}FADPY[Nle]C_{iii} (SEQ ID NO: 11);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, tBuAla represents t-butyl-alanine, PYA represents 4-pentynoic acid, Nle represents norleucine, or a pharmaceutically acceptable salt thereof.

8. The heterotandem bicyclic peptide complex as defined in claim 6 or claim 7, wherein the CD137 binding bicyclic peptide ligand comprises N- and C-terminal modifications and comprises:

Ac-A-(SEQ ID NO: 5)-Dap (herein referred to as BCY7732);
Ac-A-(SEQ ID NO: 5)-Dap(PYA) (herein referred to as BCY7741);
Ac-(SEQ ID NO: 6)-Dap (herein referred to as BCY9172);
Ac-(SEQ ID NO: 6)-Dap(PYA) (herein referred to as BCY11014);
Ac-A-(SEQ ID NO: 7)-Dap (herein referred to as BCY8045);
Ac-(SEQ ID NO: 8)-A (herein referred to as BCY8919);
Ac-(SEQ ID NO: 9)-A (herein referred to as BCY8920);
Ac-(SEQ ID NO: 10)-A (herein referred to as BCY8927);
Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928);
Ac-A-(SEQ ID NO: 12)-A (herein referred to as BCY7744);
Ac-(SEQ ID NO: 60)-Dap(PYA) (herein referred to as BCY11144);
Ac-A-(SEQ ID NO: 61)-K (herein referred to as BCY11613);
Ac-(SEQ ID NO: 62)-Dap(PYA) (herein referred to as BCY12023);
Ac-(SEQ ID NO: 63) (herein referred to as BCY12149);
Ac-(SEQ ID NO: 64) (herein referred to as BCY12143);
Ac-(SEQ ID NO: 65) (herein referred to as BCY12147);
Ac-(SEQ ID NO: 66) (herein referred to as BCY12145);
Ac-(SEQ ID NO: 67) (herein referred to as BCY12146);
Ac-(SEQ ID NO: 68) (herein referred to as BCY12150);
Ac-(SEQ ID NO: 69) (herein referred to as BCY12352);
Ac-(SEQ ID NO: 72)-[1,2-diaminoethane] (herein referred to as BCY12358);
[Palmitic Acid]-[yGlu]-[yGlu]-(SEQ ID NO: 73) (herein referred to as BCY12360);
Ac-(SEQ ID NO: 75) (herein referred to as BCY12381);
Ac-(SEQ ID NO: 76) (herein referred to as BCY12382);
Ac-(SEQ ID NO: 77)-K (herein referred to as BCY12357);
Ac-(SEQ ID NO: 78)-[dA] (herein referred to as BCY13095);
[Ac]-(SEQ ID NO: 78)-K (herein referred to as BCY13389);
Ac-(SEQ ID NO: 79)-[dA] (herein referred to as BCY13096); and
Ac-(SEQ ID NO: 80) (herein referred to as BCY13097);

wherein Ac represents an acetyl group, Dap represents diaminopropionic acid and PYA represents 4-pentynoic acid, or a pharmaceutically acceptable salt thereof.

9. The heterotandem bicyclic peptide complex as defined in any one of claims 6 to 8, wherein the CD137 binding bicyclic peptide ligand comprises N- and C-terminal modifications and comprises:

Ac-(SEQ ID NO: 11)-A (herein referred to as BCY8928);

wherein Ac represents an acetyl group, or a pharmaceutically acceptable salt thereof.

10. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 3, wherein the component present on an immune cell is OX40.

11. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 3 and 10, wherein the two or more second peptide ligands comprise an OX40 binding bicyclic peptide ligand.

12. The heterotandem bicyclic peptide complex as defined in claim 11, wherein the OX40 binding bicyclic peptide ligand comprises an amino acid sequence:

$C_iILWC_{ii}LPEPHDEC_{iii}$ (SEQ ID NO: 82);

$C_iA^K/_S{}^N/_EC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 83);

$C_iAKNC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 84);

$C_iASEC_{ii}DPFWYQFYC_{iii}$ (SEQ ID NO: 85);

$C_i{}^L/_NYSPC_{ii}WHPLN^D/_KC_{iii}$ (SEQ ID NO: 86);

$C_iLYSPC_{ii}WHPLNDC_{iii}$ (SEQ ID NO: 87);

$C_iNYSPC_{ii}WHPLNKC_{iii}$ (SEQ ID NO: 88);

$C_iWYEYDC_{ii}NNWERC_{iii}$ (SEQ ID NO: 89);

$C_iVIRYSPC_{ii}SHYLNC_{iii}$ (SEQ ID NO: 90);

$C_iDYSPWWHPC_{ii}NHIC_{iii}$ (SEQ ID NO: 91);

$C_iDAC_{ii}LYPDYYVC_{iii}$ (SEQ ID NO: 92);

$C_iRLWC_{ii}IPAPTDDC_{iii}$ (SEQ ID NO: 93);

$C_iTMWC_{ii}IPAKGDWC_{iii}$ (SEQ ID NO: 94);

$C_iMLWC_{ii}LPAPTDEC_{iii}$ (SEQ ID NO: 95);

$C_iILWC_{ii}LPEPPDEC_{iii}$ (SEQ ID NO: 96);

$C_iLLWC_{ii}IPNPDDNC_{iii}$ (SEQ ID NO: 97);

$C_iWLWC_{ii}VPNPDDTC_{iii}$ (SEQ ID NO: 98);

$C_iVLWC_{ii}TPYPGDDC_{iii}$ (SEQ ID NO: 99);

$C_iALWC_{ii}IPDPQDEC_{iii}$ (SEQ ID NO: 100);

$C_iTLWC_{ii}IPDASDSC_{iii}$ (SEQ ID NO: 101);

$C_iQLWC_{ii}IPDADDDC_{iii}$ (SEQ ID NO: 102);

$C_iQLWC_{ii}VPEPGDSC_{iii}$ (SEQ ID NO: 103);

$C_iALWC_{ii}IPEESDDC_{iii}$ (SEQ ID NO: 104);

$C_iVLWC_{ii}IPEPQDKC_{iii}$ (SEQ ID NO: 105);

$C_iTLWC_{ii}IPDPDDSC_{iii}$ (SEQ ID NO: 106);

$C_iRLWC_{ii}VPKAEDYC_{iii}$ (SEQ ID NO: 107);

$C_iTKPC_{ii}IAYYNQSC_{iii}$ (SEQ ID NO: 108);

$C_iMNPC_{ii}IAYYQQEC_{iii}$ (SEQ ID NO: 109);

$C_iTNAC_{ii}VAYYHQAC_{iii}$ (SEQ ID NO: 110);

$C_iSDPC_{ii}ISYYNQAC_{iii}$ (SEQ ID NO: 111);

$C_iDPPC_{ii}DPFWYAFYC_{iii}$ (SEQ ID NO: 112);

$C_iPDDC_{ii}DPFWYNFYC_{iii}$ (SEQ ID NO: 113);

$C_iRYSPC_{ii}YHPHNC_{iii}$ (SEQ ID NO: 114);

$C_iLYSPC_{ii}NHPLNSC_{iii}$ (SEQ ID NO: 115);

$C_iEDNYC_{ii}FMWTPYC_{iii}$ (SEQ ID NO: 116);

$C_iLDSPC_{ii}WHPLNDC_{iii}$ (SEQ ID NO: 117);

$C_iRFSPC_{ii}SHPLNQC_{iii}$ (SEQ ID NO: 118);

$C_iKYSPC_{ii}WHPLNLC_{iii}$ (SEQ ID NO: 119);

$C_iRYSPC_{ii}WHPLNNC_{iii}$ (SEQ ID NO: 120);

$C_iEWISC_{ii}PGEPHRWWC_{iii}$ (SEQ ID NO: 121);

$C_iVWEAC_{ii}PEHPDQWWC_{iii}$ (SEQ ID NO: 122);

$C_iSTWHC_{ii}FWNLQEGKC_{iii}$ (SEQ ID NO: 123);

$C_iEWKAC_{ii}EHDRERWWC_{iii}$ (SEQ ID NO: 124);

$C_iRTWQC_{ii}FYEWQNGHC_{iii}$ (SEQ ID NO: 125);

$C_iKTWDC_{ii}FWASQVSEC_{iii}$ (SEQ ID NO: 126);

$C_iSTWQC_{ii}FYDLQEGHC_{iii}$ (SEQ ID NO: 127);

$C_iTTWEC_{ii}FYDLQEGHC_{iii}$ (SEQ ID NO: 128);

$C_iETWEC_{ii}FWRLQAGEC_{iii}$ (SEQ ID NO: 129);

$C_iRTWQC_{ii}FWDLQEGLC_{iii}$ (SEQ ID NO: 130);

$C_iSTWQC_{ii}FWDSQLGAC_{iii}$ (SEQ ID NO: 131);

$C_iETWEC_{ii}FWEWQVGSC_{iii}$ (SEQ ID NO: 132);

$C_iTTWEC_{ii}FWDLQEGLC_{iii}$ (SEQ ID NO: 133);

$C_iHTWDC_{ii}FYQWQDGHC_{iii}$ (SEQ ID NO: 134);

$C_iTTWEC_{ii}FYSLQDGHC_{iii}$ (SEQ ID NO: 135);

$C_iNEDMYC_{ii}FMWMEC_{iii}$ (SEQ ID NO: 136);

$C_iLYEYDC_{ii}YTWRRC_{iii}$ (SEQ ID NO: 137);

C_iRYEYDC$_{ii}$HTWQRC$_{iii}$ (SEQ ID NO: 138);
C_iWYEYDC$_{ii}$TTWERC$_{iii}$ (SEQ ID NO: 139);
C_iWYEYDC$_{ii}$RTWTRC$_{iii}$ (SEQ ID NO: 140);
C_iLYEYDC$_{ii}$HTWTRC$_{iii}$ (SEQ ID NO: 141);
C_iWYEYDC$_{ii}$RTWTFC$_{iii}$ (SEQ ID NO: 142);
C_iHGGVWC$_{ii}$IPNINDSC$_{iii}$ (SEQ ID NO: 143);
C_iDSPVRC$_{ii}$YWNTQKGC$_{iii}$ (SEQ ID NO: 144);
C_iGSPVPC$_{ii}$YWNTRKGC$_{iii}$ (SEQ ID NO: 145);
C_iAPFEFNC$_{ii}$YTWRPC$_{iii}$ (SEQ ID NO: 146);
C_iRVLYSPC$_{ii}$YHWLNC$_{iii}$ (SEQ ID NO: 147);
C_iSIMYSPC$_{ii}$EHPHNHC$_{iii}$ (SEQ ID NO: 148);
C_iDKWEPDHLC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 149);
C_iDAWPETHVC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 150);
C_iDEYTPEHLC$_{ii}$YWWC$_{iii}$ (SEQ ID NO: 151);
C_iWINYSISPC$_{ii}$YVGEC$_{iii}$ (SEQ ID NO: 152); and
C_iRYEYPEHLC$_{ii}$YTWQC$_{iii}$ (SEQ ID NO: 153);

such as:

C_iLYSPC$_{ii}$WHPLNDC$_{iii}$ (SEQ ID NO: 87);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, or a modified derivative, or a pharmaceutically acceptable salt thereof.

13. The heterotandem bicyclic peptide complex as defined in claim 12, wherein the OX40 binding bicyclic peptide ligand additionally comprises N- and/or C-terminal modifications and comprises an amino acid sequence selected from:

A-(SEQ ID NO: 82)-A-[Sar6]-[KBiot] (herein referred to as BCY10551);
A-(SEQ ID NO: 82)-A (herein referred to as BCY10371);
A-(SEQ ID NO: 84)-A-[Sar6]-[KBiot] (herein referred to as BCY10552);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 84)-A (herein referred to as BCY10479);
A-(SEQ ID NO: 84)-A (herein referred to as BCY10378);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 85)-A (herein referred to as BCY11371);
A-(SEQ ID NO: 85)-A (herein referred to as BCY10743);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 87)-A (herein referred to as BCY10482);
A-(SEQ ID NO: 87)-A-[Sar6]-[KBiot] (herein referred to as BCY10549);
A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY11607);
Ac-A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY12708);
A-(SEQ ID NO: 87)-A (herein referred to as BCY10351);
A-(SEQ ID NO: 88)-A-[Sar6]-[KBiot] (herein referred to as BCY11501);

A-(SEQ ID NO: 88)-A (herein referred to as BCY10729);
A-(SEQ ID NO: 89)-A-[Sar6]-[KBiot] (herein referred to as BCY10550);
A-(SEQ ID NO: 89)-A (herein referred to as BCY10361);
A-(SEQ ID NO: 90)-A-[Sar6]-[KBiot] (herein referred to as BCY10794);
A-(SEQ ID NO: 90)-A (herein referred to as BCY10349);
[Biot]-G-[Sar5]-A-(SEQ ID NO: 91)-A (herein referred to as BCY11369);
A-(SEQ ID NO: 91)-A (herein referred to as BCY10331);
A-(SEQ ID NO: 92)-A (herein referred to as BCY10375);
A-(SEQ ID NO: 93)-A (herein referred to as BCY10364);
A-(SEQ ID NO: 94)-A (herein referred to as BCY10365);
A-(SEQ ID NO: 95)-A (herein referred to as BCY10366);
A-(SEQ ID NO: 96)-A (herein referred to as BCY10367);
A-(SEQ ID NO: 97)-A (herein referred to as BCY10368);
A-(SEQ ID NO: 98)-A (herein referred to as BCY10369);
A-(SEQ ID NO: 99)-A (herein referred to as BCY10374);
A-(SEQ ID NO: 100)-A (herein referred to as BCY10376);
A-(SEQ ID NO: 101)-A (herein referred to as BCY10737);
A-(SEQ ID NO: 102)-A (herein referred to as BCY10738);
A-(SEQ ID NO: 103)-A (herein referred to as BCY10739);
A-(SEQ ID NO: 104)-A (herein referred to as BCY10740);
A-(SEQ ID NO: 105)-A (herein referred to as BCY10741);
A-(SEQ ID NO: 106)-A (herein referred to as BCY10742);
A-(SEQ ID NO: 107)-A (herein referred to as BCY10380);
A-(SEQ ID NO: 108)-A (herein referred to as BCY10370);
A-(SEQ ID NO: 109)-A (herein referred to as BCY10372);
A-(SEQ ID NO: 110)-A (herein referred to as BCY10373);
A-(SEQ ID NO: 111)-A (herein referred to as BCY10379);
A-(SEQ ID NO: 112)-A (herein referred to as BCY10377);
A-(SEQ ID NO: 113)-A (herein referred to as BCY10744);
A-(SEQ ID NO: 114)-A (herein referred to as BCY10343);
A-(SEQ ID NO: 115)-A (herein referred to as BCY10350);
A-(SEQ ID NO: 116)-A (herein referred to as BCY10352);
A-(SEQ ID NO: 117)-A (herein referred to as BCY10353);
A-(SEQ ID NO: 118)-A (herein referred to as BCY10354);
A-(SEQ ID NO: 119)-A (herein referred to as BCY10730);
A-(SEQ ID NO: 120)-A (herein referred to as BCY10731);
A-(SEQ ID NO: 121)-A (herein referred to as BCY10339);

A-(SEQ ID NO: 122)-A (herein referred to as BCY10340);
A-(SEQ ID NO: 123)-A (herein referred to as BCY10342);
A-(SEQ ID NO: 124)-A (herein referred to as BCY10345);
A-(SEQ ID NO: 125)-A (herein referred to as BCY10347);
A-(SEQ ID NO: 126)-A (herein referred to as BCY10348);
A-(SEQ ID NO: 127)-A (herein referred to as BCY10720);
A-(SEQ ID NO: 128)-A (herein referred to as BCY10721);
A-(SEQ ID NO: 129)-A (herein referred to as BCY10722);
A-(SEQ ID NO: 130)-A (herein referred to as BCY10723);
A-(SEQ ID NO: 131)-A (herein referred to as BCY10724);
A-(SEQ ID NO: 132)-A (herein referred to as BCY10725);
A-(SEQ ID NO: 133)-A (herein referred to as BCY10726);
A-(SEQ ID NO: 134)-A (herein referred to as BCY10727);
A-(SEQ ID NO: 135)-A (herein referred to as BCY10728);
A-(SEQ ID NO: 136)-A (herein referred to as BCY10360);
A-(SEQ ID NO: 137)-A (herein referred to as BCY10363);
A-(SEQ ID NO: 138)-A (herein referred to as BCY10732);
A-(SEQ ID NO: 139)-A (herein referred to as BCY10733);
A-(SEQ ID NO: 140)-A (herein referred to as BCY10734);
A-(SEQ ID NO: 141)-A (herein referred to as BCY10735);
A-(SEQ ID NO: 142)-A (herein referred to as BCY10736);
A-(SEQ ID NO: 143)-A (herein referred to as BCY10336);
A-(SEQ ID NO: 144)-A (herein referred to as BCY10337);
A-(SEQ ID NO: 145)-A (herein referred to as BCY10338);
A-(SEQ ID NO: 146)-A (herein referred to as BCY10346);
A-(SEQ ID NO: 147)-A (herein referred to as BCY10357);
A-(SEQ ID NO: 148)-A (herein referred to as BCY10362);
A-(SEQ ID NO: 149)-A (herein referred to as BCY10332);
A-(SEQ ID NO: 150)-A (herein referred to as BCY10717);
A-(SEQ ID NO: 151)-A (herein referred to as BCY10718);
A-(SEQ ID NO: 152)-A (herein referred to as BCY10334); and
A-(SEQ ID NO: 153)-A (herein referred to as BCY10719);

such as:

A-(SEQ ID NO: 87)-A-K(Pya) (herein referred to as BCY11607);

wherein Pya represents 4-pentynoyl moiety.

14. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 13, wherein the cancer cell is selected from an HT1080, A549, SC-OV-3, PC3, HT1376, NCI-H292, LnCap, MC38, MC38 #13, 4T1-D02, H322, HT29, T47D and RKO tumor cell.

15. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 14, wherein the component present on a cancer cell is Nectin-4.

16. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 15, wherein the first peptide ligand comprises a Nectin-4 binding bicyclic peptide ligand.

17. The heterotandem bicyclic peptide complex as defined in claim 16, wherein the Nectin-4 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

$C_iP[1Nal][dD]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 1; herein referred to as BCY8116);

$C_iP[1Nal][dK](Sar_{10}\text{-}(B\text{-}Ala))C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 3);

$C_iPFGC_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 4; herein referred to as BCY11414);

$C_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 14);

$[MerPro]_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 15; herein referred to as BCY12363);

$C_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]W[Cysam]_{iii}$ (SEQ ID NO: 16);

$[MerPro]_iP[1Nal][dK]C_{ii}M[HArg]DWSTP[HyP]W[Cysam]_{iii}$ (SEQ ID NO: 17; herein referred to as BCY12365);

$C_iP[1Nal][dK]C_{ii}M[HArg]HWSTP[HyP]WC_{iii}$ (SEQ ID NO: 18);

$C_iP[1Nal][dK]C_{ii}M[HArg]EWSTP[HyP]WC_{iii}$ (SEQ ID NO: 19);

$C_iP[1Nal][dE]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 20; herein referred to as BCY12368);

$C_iP[1Nal][dA]C_{ii}M[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 21; herein referred to as BCY12369);

$C_iP[1Nal][dE]C_{ii}L[HArg]DWSTP[HyP]WC_{iii}$ (SEQ ID NO: 22; herein referred to as BCY12370); and

$C_iP[1Nal][dE]C_{ii}M[HArg]EWSTP[HyP]WC_{iii}$ (SEQ ID NO: 23; herein referred to as BCY12384);

wherein $[MerPro]_i$, C_i, C_{ii}, C_{iii} and $[Cysam]_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, 1Nal represents 1-naphthylalanine, HArg represents homoarginine, HyP represents trans-4-hydroxy-L-proline, Sar_{10} represents 10 sarcosine units, B-Ala represents beta-alanine, MerPro represents 3-

mercaptopropionic acid and Cysam represents cysteamine, or a pharmaceutically acceptable salt thereof.

18. The heterotandem bicyclic peptide complex as defined in claim 17, wherein the Nectin-4 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

SEQ ID NO: 1 (herein referred to as BCY8116);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 1) (herein referred to as BCY8846);

[PYA]-(SEQ ID NO: 1) (herein referred to as BCY11015);

[PYA]-[B-Ala]-(SEQ ID NO: 1) (herein referred to as BCY11016);

[PYA]-[B-Ala]-[Sar_{10}]-(SEQ ID NO: 2) (herein referred to as BCY11942);

Ac-(SEQ ID NO: 3) (herein referred to as BCY8831);

SEQ ID NO: 4 (herein referred to as BCY11414);

[PYA]-[B-Ala]-(SEQ ID NO: 14) (herein referred to as BCY11143);

Palmitic-yGlu-yGlu-(SEQ ID NO: 14) (herein referred to as BCY12371);

Ac-(SEQ ID NO: 14) (herein referred to as BCY12024);

Ac-(SEQ ID NO: 16) (herein referred to as BCY12364);

Ac-(SEQ ID NO: 18) (herein referred to as BCY12366); and

Ac-(SEQ ID NO: 19) (herein referred to as BCY12367);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units, or a pharmaceutically acceptable salt thereof.

19. The heterotandem bicyclic peptide complex as defined in claim 17 or claim 18, wherein the Nectin-4 binding bicyclic peptide ligand comprises SEQ ID NO: 1 (herein referred to as BCY8116).

20. The heterotandem bicyclic peptide complex as defined in any one of claims 15 to 19, which is selected from any one of the complexes listed in Tables A and B, such as BCY11027, BCY11863 and BCY11864.

21. The heterotandem bicyclic peptide complex as defined in any one of claims 15 to 19, which is selected from any one of the complexes listed in Table E, such as BCY12967.

22. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 14, wherein the component present on a cancer cell is EphA2.

23. The heterotandem bicyclic peptide complex as defined in claim 22, wherein the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

C_i[HyP]LVNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 24);

C_iLWDPTPC_{ii}ANLHL[HArg]C_{iii} (SEQ ID NO: 25);

C_i[HyP]LVNPLC_{ii}L[K(PYA)]P[dD]W[HArg]C_{iii} (SEQ ID NO: 26);

C_i[HyP][K(PYA)]VNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 27);

C_i[HyP]LVNPLC_{ii}[K(PYA)]HP[dD]W[HArg]C_{iii} (SEQ ID NO: 28);

C_i[HyP]LVNPLC_{ii}LKP[dD]W[HArg]C_{iii} (SEQ ID NO: 29);

C_i[HyP]KVNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 30);

C_i[HyP]LVNPLC_{ii}KHP[dD]W[HArg]C_{iii} (SEQ ID NO: 31);

C_i[HyP]LVNPLC_{ii}LHP[dE]W[HArg]C_{iii} (SEQ ID NO: 32);

C_i[HyP]LVNPLC_{ii}LEP[dD]W[HArg]C_{iii} (SEQ ID NO: 33);

C_i[HyP]LVNPLC_{ii}LHP[dD]WTC_{iii} (SEQ ID NO: 34);

C_i[HyP]LVNPLC_{ii}LEP[dD]WTC_{iii} (SEQ ID NO: 35);

C_i[HyP]LVNPLC_{ii}LEP[dA]WTC_{iii} (SEQ ID NO: 36);

C_i[HyP]LVNPLC_{ii}L[3,3-DPA]P[dD]WTC_{iii} (SEQ ID NO: 37; herein referred to as BCY12860);

C_i[HyP][Cba]VNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 38);

C_i[HyP][Cba]VNPLC_{ii}LEP[dD]WTC_{iii} (SEQ ID NO: 39);

C_i[HyP][Cba]VNPLC_{ii}L[3,3-DPA]P[dD]WTC_{iii} (SEQ ID NO: 40);

C_i[HyP]LVNPLC_{ii}L[3,3-DPA]P[dD]W[HArg]C_{iii} (SEQ ID NO: 41);

C_i[HyP]LVNPLC_{ii}LHP[d1Nal]W[HArg]C_{iii} (SEQ ID NO: 42);

C_i[HyP]LVNPLC_{ii}L[1Nal]P[dD]W[HArg]C_{iii} (SEQ ID NO: 43);

C_i[HyP]LVNPLC_{ii}LEP[d1Nal]WTC_{iii} (SEQ ID NO: 44);

C_i[HyP]LVNPLC_{ii}L[1Nal]P[dD]WTC_{iii} (SEQ ID NO: 45; herein referred to as BCY13119);

C_i[HyP][Cba]VNPLC_{ii}LEP[dA]WTC_{iii} (SEQ ID NO: 46);

C_i[HyP][hGlu]VNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 47);

C_i[HyP]LVNPLC_{ii}[hGlu]HP[dD]W[HArg]C_{iii} (SEQ ID NO: 48);

C_i[HyP]LVNPLC_{ii}L[hGlu]P[dD]W[HArg]C_{iii} (SEQ ID NO: 49);

C_i[HyP]LVNPLC_{ii}LHP[dNle]W[HArg]C_{iii} (SEQ ID NO: 50);

C_i[HyP]LVNPLC_{ii}L[Nle]P[dD]W[HArg]C_{iii} (SEQ ID NO: 51);

[MerPro]$_i$[HyP]LVNPLC_{ii}L[3,3-DPA]P[dD]WTC_{iii} (SEQ ID NO: 154);

C_i[HyP]LVNPLC_{ii}LHP[dD]W[HArg][Cysam]$_{iii}$ (SEQ ID NO: 155);

C_i[HyP]LVNPLC_{ii}L[His3Me]P[dD]W[HArg]C_{iii} (SEQ ID NO: 156);

C_i[HyP]LVNPLC_{ii}L[His1Me]P[dD]W[HArg]C_{iii} (SEQ ID NO: 157);

C_i[HyP]LVNPLC_{ii}L[4ThiAz]P[dD]W[HArg]C_{iii} (SEQ ID NO: 158);

C_i[HyP]LVNPLC_{ii}LFP[dD]W[HArg]C_{iii} (SEQ ID NO: 159);

C_i[HyP]LVNPLC_{ii}L[Thi]P[dD]W[HArg]C_{iii} (SEQ ID NO: 160);

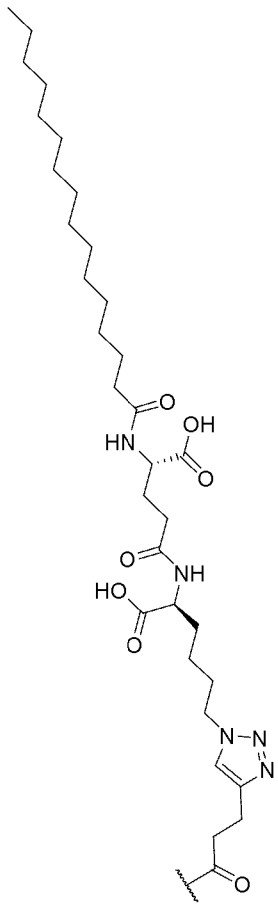
C_i[HyP]LVNPLC_{ii}L[3Thi]P[dD]W[HArg]C_{iii} (SEQ ID NO: 161);

C_i[HyP]LVNPLC_{ii}LNP[dD]W[HArg]C_{iii} (SEQ ID NO: 162);

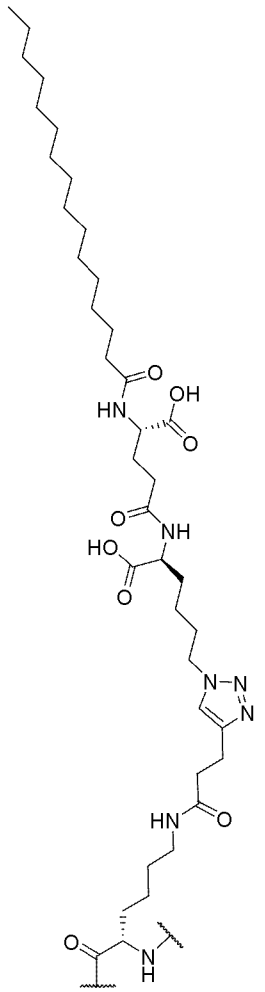
C_i[HyP]LVNPLC_{ii}LQP[dD]W[HArg]C_{iii} (SEQ ID NO: 163); and

C_i[HyP]LVNPLC_{ii}L[K(PYA-(Palmitoyl-Glu-LysN_3)]P[dD]W[HArg]C_{iii} (SEQ ID NO: 164);

wherein $[MerPro]_i$, C_i, C_{ii}, C_{iii} and $[Cysam]_{iii}$ represent first (i), second (ii) and third (iii) reactive groups which are selected from cysteine, MerPro and Cysam, HyP represents trans-4-hydroxy-L-proline, HArg represents homoarginine, PYA represents 4-pentynoic acid, 3,3-DPA represents 3,3-diphenylalanine, Cba represents β-cyclobutylalanine, 1Nal represents 1-naphthylalanine, hGlu represents homoglutamic acid, Thi represents thienyl-alanine, 4ThiAz represents beta-(4-thiazolyl)-alanine, His1Me represents N1-methyl-L-histidine, His3Me represents N3-methyl-L-histidine, 3Thi represents , Palmitoyl-Glu-LysN_3[PYA] represents:



(Palmitoyl-Glu-LysN3)[PYA] , [K(PYA-(Palmitoyl-Glu-LysN_3)] represents:



[K(PYA(Palmitoyl-Glu-LysN3))], Nle represents norleucine, MerPro represents 3-mercaptopropionic acid and Cysam represents cysteamine, or a pharmaceutically acceptable salt thereof.

24. The heterotandem bicyclic peptide complex as defined in claim 23, wherein the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence which is:

C_i[HyP]LVNPLC_{ii}LHP[dD]W[HArg]C_{iii} (SEQ ID NO: 24);

wherein C_i, C_{ii}, C_{iii} and represent first (i), second (ii) and third (iii) cysteine groups, HyP represents trans-4-hydroxy-L-proline, HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

25. The heterotandem bicyclic peptide complex as defined in claim 23, wherein the EphA2 binding bicyclic peptide ligand comprises an amino acid sequence which is:

C_i[HyP]LVNPLC_{ii}LEP[d1Nal]WTC_{iii} (SEQ ID NO: 44);

wherein C_i, C_{ii}, C_{iii} and represent first (i), second (ii) and third (iii) cysteine groups, HyP represents trans-4-hydroxy-L-proline, 1Nal represents 1-naphthylalanine, or a pharmaceutically acceptable salt thereof.

26. The heterotandem bicyclic peptide complex as defined in any one of claims 23 to 25, wherein the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY9594);

[B-Ala]-[Sar_{10}]-A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY6099);

[PYA]-A-[HArg]-D-(SEQ NO: 24) (herein referred to as BCY11813);

Ac-A-[HArg]-D-(SEQ ID NO: 24)-[K(PYA)] (herein referred to as BCY11814);

Ac-A-[HArg]-D-(SEQ ID NO: 24)-K (herein referred to as BCY12734);

[NMeAla]-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY13121);

[Ac]-(SEQ ID NO: 24)-L[dH]G[dK] (herein referred to as BCY13125);

[PYA]-[B-Ala]-[Sar_{10}]-VGP-(SEQ ID NO: 25) (herein referred to as BCY8941);

Ac-A-[HArg]-D-(SEQ ID NO: 26) (herein referred to as BCY11815);

Ac-A-[HArg]-D-(SEQ ID NO: 27) (herein referred to as BCY11816);

Ac-A-[HArg]-D-(SEQ ID NO: 28) (herein referred to as BCY11817);

Ac-A-[HArg]-D-(SEQ ID NO: 29) (herein referred to as BCY12735);

(Palmitoyl-Glu-LysN_3)[PYA]A[HArg]D-(SEQ ID NO: 29) (hereinafter known as BCY14327);

Ac-A-[HArg]-D-(SEQ ID NO: 30) (herein referred to as BCY12736);

Ac-A-[HArg]-D-(SEQ ID NO: 31) (herein referred to as BCY12737);

A-[HArg]-D-(SEQ ID NO: 32) (herein referred to as BCY12738);

A-[HArg]-E-(SEQ ID NO: 32) (herein referred to as BCY12739);

A-[HArg]-D-(SEQ ID NO: 33) (herein referred to as BCY12854);

A-[HArg]-D-(SEQ ID NO: 34) (herein referred to as BCY12855);

A-[HArg]-D-(SEQ ID NO: 35) (herein referred to as BCY12856);

A-[HArg]-D-(SEQ ID NO: 35)-[dA] (herein referred to as BCY12857);

(SEQ ID NO: 35)-[dA] (herein referred to as BCY12861);

[NMeAla]-[HArg]-D-(SEQ ID NO: 35) (herein referred to as BCY13122);

[dA]-ED-(SEQ ID NO: 35) (herein referred to as BCY13126);

[dA]-[dA]-D-(SEQ ID NO: 35) (herein referred to as BCY13127);

AD-(SEQ ID NO: 35) (herein referred to as BCY13128);

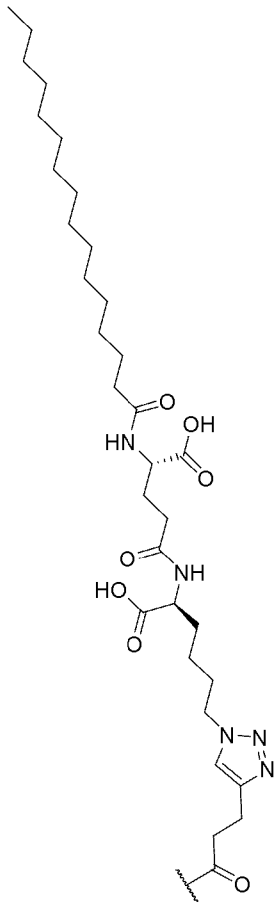
A-[HArg]-D-(SEQ ID NO: 36) (herein referred to as BCY12858);

A-[HArg]-D-(SEQ ID NO: 37) (herein referred to as BCY12859);

Ac-(SEQ ID NO: 37)-[dK] (herein referred to as BCY13120);

A-[HArg]-D-(SEQ ID NO: 38) (herein referred to as BCY12862);
A-[HArg]-D-(SEQ ID NO: 39) (herein referred to as BCY12863);
[dA]-[HArg]-D-(SEQ ID NO: 39)-[dA] (herein referred to as BCY12864);
(SEQ ID NO: 40)-[dA] (herein referred to as BCY12865);
A-[HArg]-D-(SEQ ID NO: 41) (herein referred to as BCY12866);
A-[HArg]-D-(SEQ ID NO: 42) (herein referred to as BCY13116);
A-[HArg]-D-(SEQ ID NO: 43) (herein referred to as BCY13117);
A-[HArg]-D-(SEQ ID NO: 44) (herein referred to as BCY13118);
[dA]-[HArg]-D-(SEQ ID NO: 46)-[dA] (herein referred to as BCY13123);
[d1Nal]-[HArg]-D-(SEQ ID NO: 46)-[dA] (herein referred to as BCY13124);
A-[HArg]-D-(SEQ ID NO: 47) (herein referred to as BCY13130);
A-[HArg]-D-(SEQ ID NO: 48) (herein referred to as BCY13131);
A-[HArg]-D-(SEQ ID NO: 49) (herein referred to as BCY13132);
A-[HArg]-D-(SEQ ID NO: 50) (herein referred to as BCY13134);
A-[HArg]-D-(SEQ ID NO: 51) (herein referred to as BCY13135);
(SEQ ID NO: 154)-[dK] (herein referred to as BCY13129);
A[HArg]D-(SEQ ID NO: 155) (herein referred to as BCY13133);
A[HArg]D-(SEQ ID NO: 156) (herein referred to as BCY13917);
A[HArg]D-(SEQ ID NO: 157) (herein referred to as BCY13918);
A[HArg]D-(SEQ ID NO: 158) (herein referred to as BCY13919);
A[HArg]D-(SEQ ID NO: 159) (herein referred to as BCY13920);
A[HArg]D-(SEQ ID NO: 160) (herein referred to as BCY13922);
A[HArg]D-(SEQ ID NO: 161) (herein referred to as BCY13923);
A[HArg]D-(SEQ ID NO: 162) (herein referred to as BCY14047);
A[HArg]D-(SEQ ID NO: 163) (herein referred to as BCY14048); and
A[HArg]D-(SEQ ID NO: 164) (herein referred to as BCY14313);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units, HArg represents homoarginine, NMeAla represents N-methyl-alanine, 1Nal represents 1-naphthylalanine, Palmitoyl-Glu-$LysN_3$[PYA] represents:



(Palmitoyl-Glu-LysN3)[PYA], or a pharmaceutically acceptable salt thereof.

27. The heterotandem bicyclic peptide complex as defined in any one of claims 23 to 26, wherein the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 24) (herein referred to as BCY9594);

wherein HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

28. The heterotandem bicyclic peptide complex as defined in any one of claims 23 to 26, wherein the EphA2 binding bicyclic peptide ligand optionally comprises N-terminal modifications and comprises:

A-[HArg]-D-(SEQ ID NO: 44) (herein referred to as BCY13118);

wherein HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

29. The heterotandem bicyclic peptide complex as defined in any one of claims 22 to 28, which is selected from any one of the complexes listed in Table C, such as BCY12491, BCY12730, BCY13048, BCY13050, BCY13053 and BCY13272.

30. The heterotandem bicyclic peptide complex as defined in claim 29, which is BCY12491.

31. The heterotandem bicyclic peptide complex as defined in claim 29, which is BCY13272.

32. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 13, wherein the component present on a cancer cell is PD-L1.

33. The heterotandem bicyclic peptide complex as defined in claim 32, wherein the PD-L1 binding bicyclic peptide ligand comprises an amino acid sequence selected from:

C_iSAGWLTMC_{ii}QKLHLC_{iii} (SEQ ID NO: 52);

C_iSAGWLTMC_{ii}Q[K(PYA)]LHLC_{iii} (SEQ ID NO: 53);

C_iSKGWLTMC_{ii}Q[K(Ac)]LHLC_{iii} (SEQ ID NO: 54);

C_iSAGWLTKC_{ii}Q[K(Ac)]LHLC_{iii} (SEQ ID NO: 55);

C_iSAGWLTMC_{ii}K[K(Ac)]LHLC_{iii} (SEQ ID NO: 56);

C_iSAGWLTMC_{ii}Q[K(Ac)]LKLC_{iii} (SEQ ID NO: 57);

C_iSAGWLTMC_{ii}Q[HArg]LHLC_{iii} (SEQ ID NO: 58); and

C_iSAGWLTMC_{ii}[HArg]QLNLC_{iii} (SEQ ID NO: 59);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, PYA represents 4-pentynoic acid and HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

34. The heterotandem bicyclic peptide complex as defined in claim 33, wherein the PD-L1 binding bicyclic peptide ligand optionally comprises N-terminal and/or C-terminal modifications and comprises:

[PYA]-[B-Ala]-[Sar_{10}]-SDK-(SEQ ID NO: 52) (herein referred to as BCY10043);

Ac-D-[HArg]-(SEQ ID NO: 52)-PSH (herein referred to as BCY11865);

Ac-SDK-(SEQ ID NO: 53) (herein referred to as BCY11013);

Ac-SDK-(SEQ ID NO: 53)-PSH (herein referred to as BCY10861);

Ac-D-[HArg]-(SEQ ID NO: 54)-PSH (herein referred to as BCY11866);

Ac-D-[HArg]-(SEQ ID NO: 55)-PSH (herein referred to as BCY11867);

Ac-D-[HArg]-(SEQ ID NO: 56)-PSH (herein referred to as BCY11868);

Ac-D-[HArg]-(SEQ ID NO: 57)-PSH (herein referred to as BCY11869);

Ac-SD-[HArg]-(SEQ ID NO: 58)-PSHK (herein referred to as BCY12479); and

Ac-SD-[HArg]-(SEQ ID NO: 59)-PSHK (herein referred to as BCY12477);

wherein PYA represents 4-pentynoic acid, B-Ala represents beta-alanine, Sar_{10} represents 10 sarcosine units and HArg represents homoarginine, or a pharmaceutically acceptable salt thereof.

35. The heterotandem bicyclic peptide complex as defined in any one of claims 32 to 34, which is selected from any one of the complexes listed in Table D.

36. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 5, wherein the two or more second peptide ligands comprise one CD137 binding bicyclic peptide ligand and one OX40 binding bicyclic peptide.

37. The heterotandem bicyclic peptide complex as defined in claim 36, which is the complex listed in Table F.

38. The heterotandem bicyclic peptide complex as defined in claim 1, which is selected from any one of the complexes listed in Tables G and H.

39. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 38, wherein the molecular scaffold is selected from 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA).

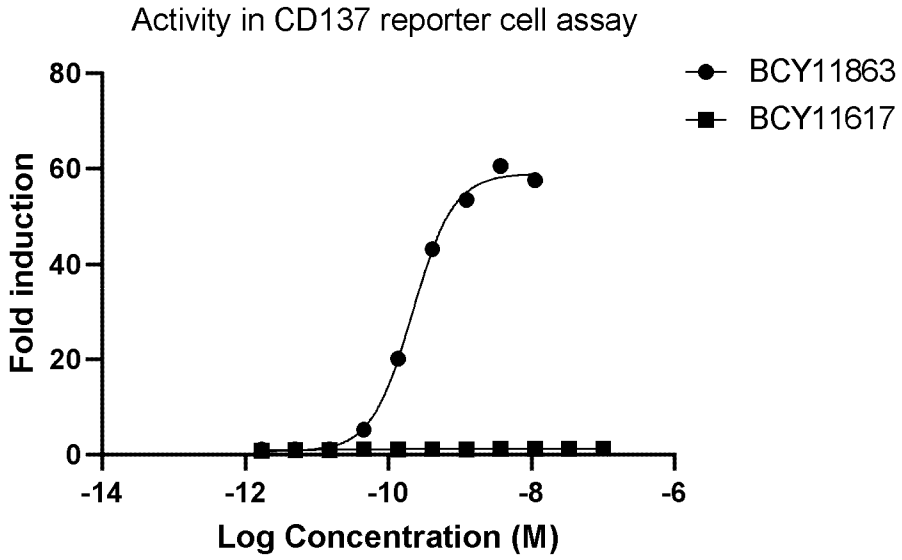
40. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 39, wherein the pharmaceutically acceptable salt is selected from the free acid or the sodium, potassium, calcium, ammonium salt.

41. A pharmaceutical composition which comprises the heterotandem bicyclic peptide complex of any one of claims 1 to 40 in combination with one or more pharmaceutically acceptable excipients.

42. The heterotandem bicyclic peptide complex as defined in any one of claims 1 to 40 for use in preventing, suppressing or treating cancer.

43. A method of treating cancer which comprises administration of a heterotandem bicyclic peptide complex as defined in any one of claims 1 to 40 at a dosage frequency which does not sustain plasma concentrations of said complex above the *in vitro* EC_{50} of said complex.

A


Activity in CD137 reporter cell assay
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BCY11617
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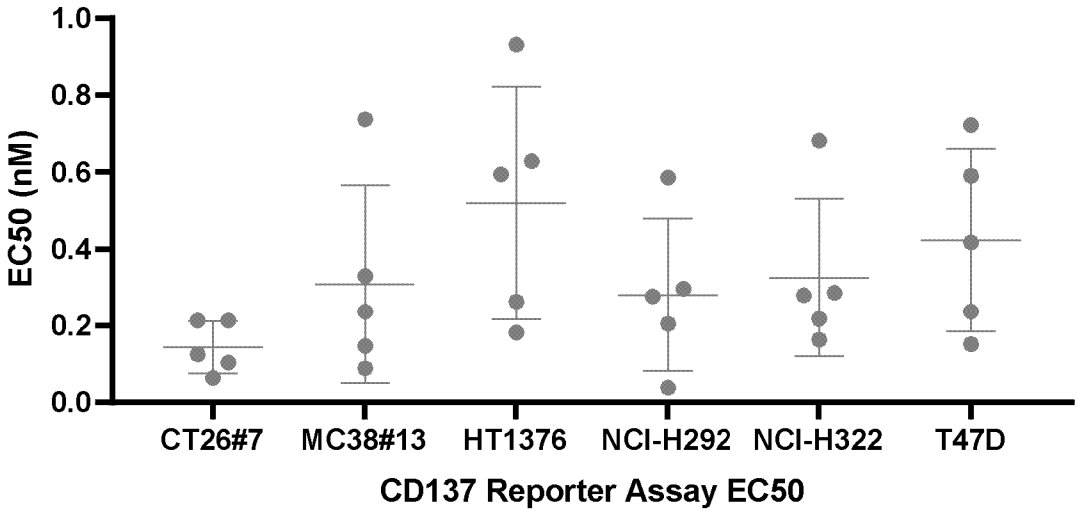
60
40
20
0
-14
-12
-10
-8
-6
Log Concentration (M)

B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0.4
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0.0
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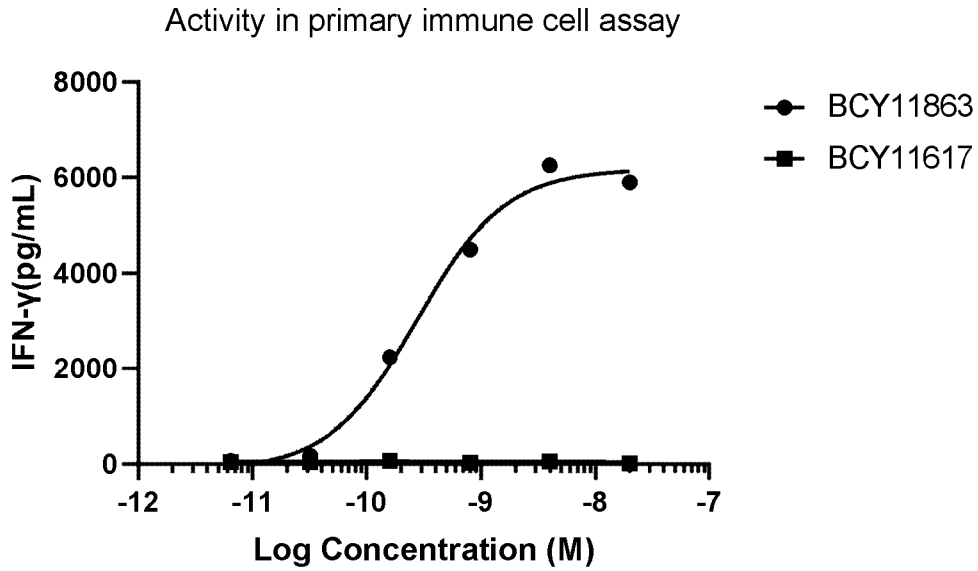
MC38#13
HT1376
NCI-H292
NCI-H322
T47D
CD137 Reporter Assay EC50

FIGURE 1

A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4000
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-12
-11
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-9
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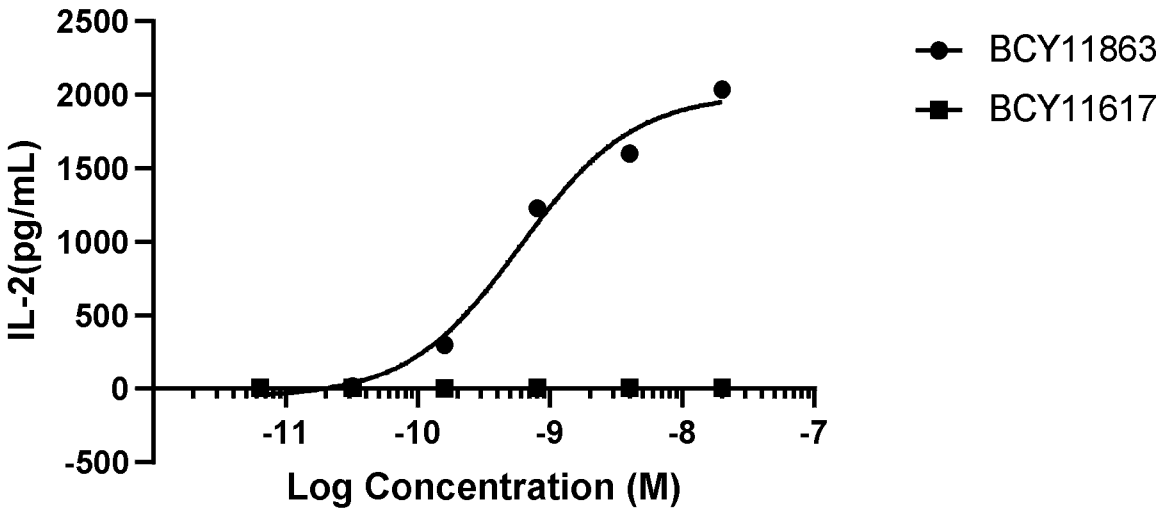
Log Concentration (M)
BCY11863
BCY11617

B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0
-500
-11
-10
-9
-8
-7
Log Concentration (M)
BCY11863
BCY11617

FIGURE 2

C

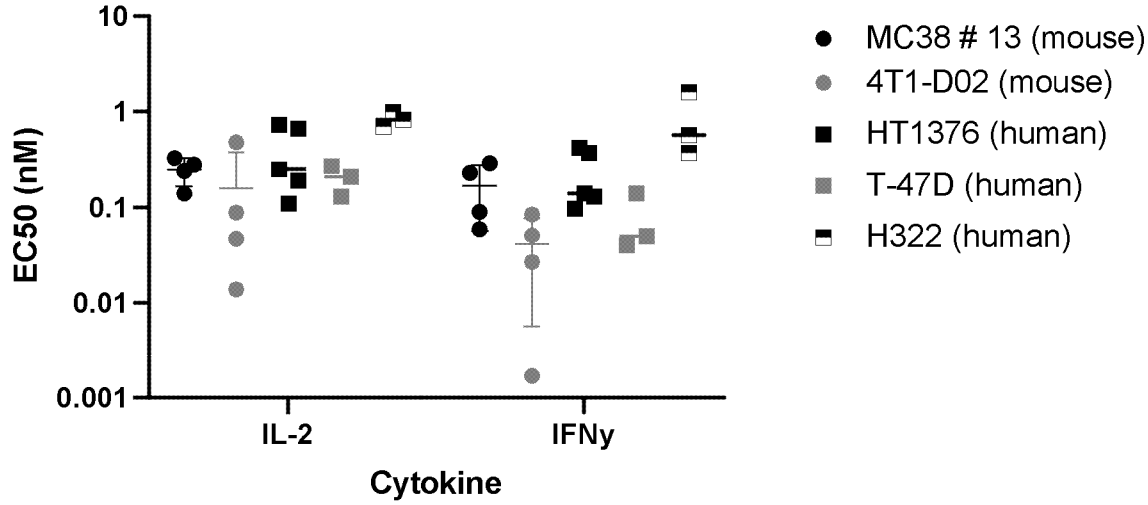


FIGURE 2 (ctd)

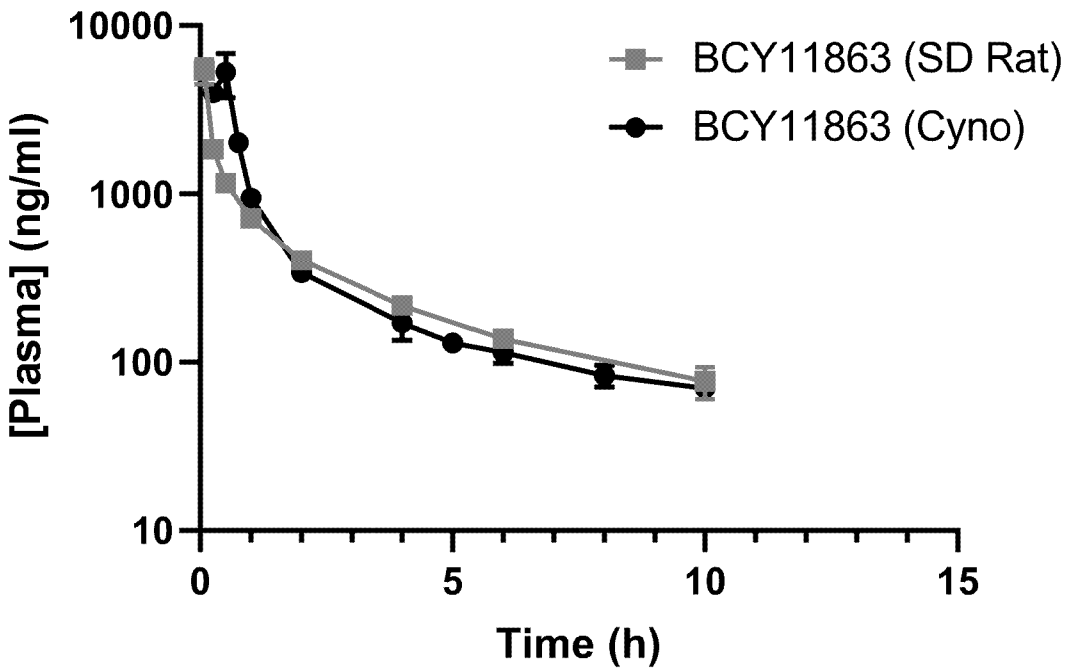


FIGURE 3

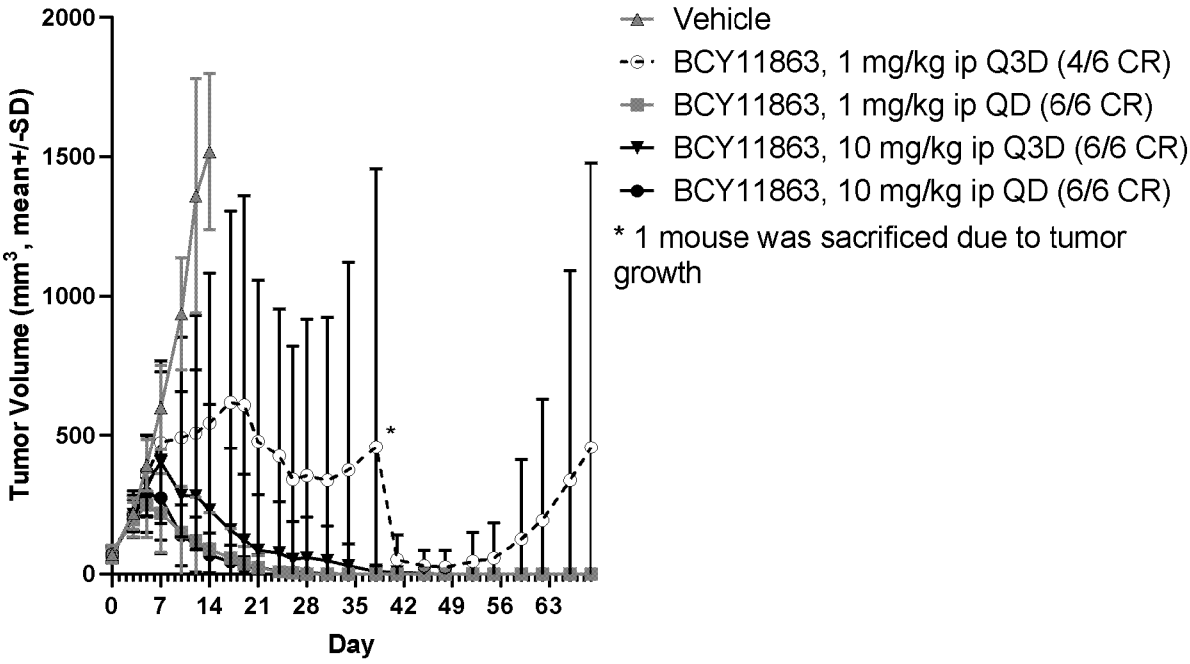


FIGURE 4

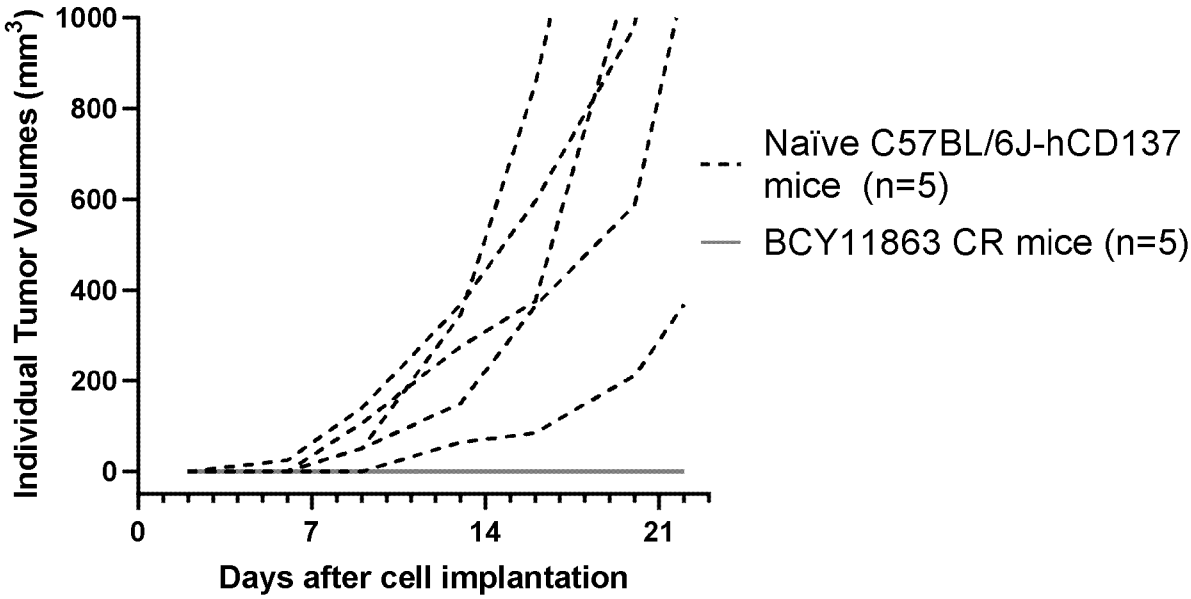


FIGURE 5

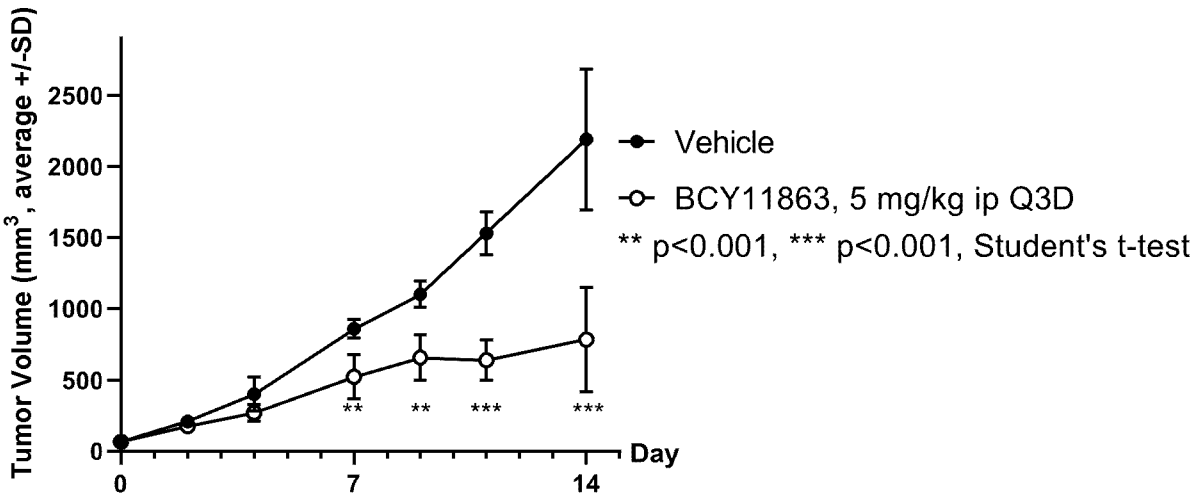
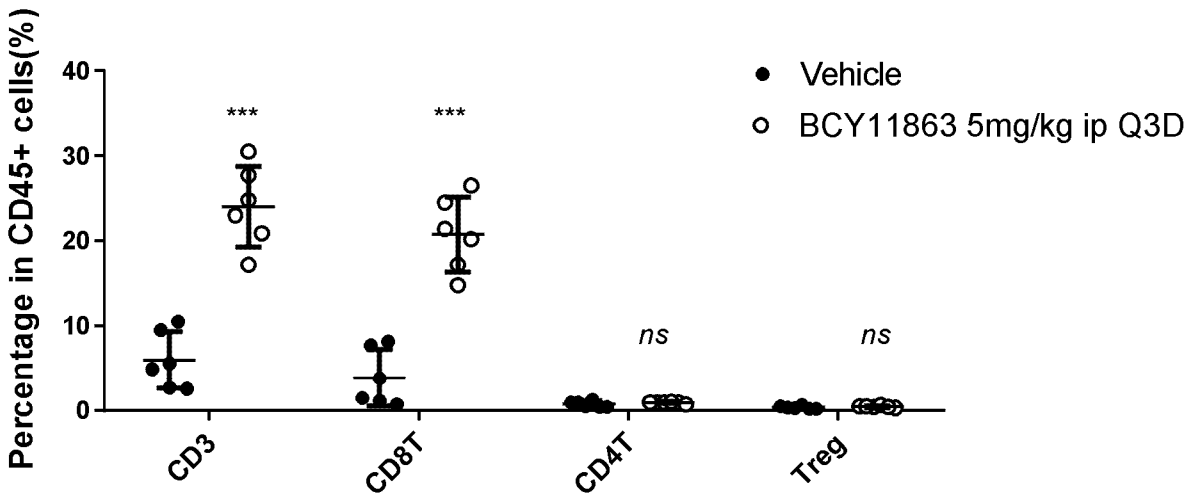

Tumor Volume (mm3, average +/-SD)
2500
2000
1500
1000
500
0
0
7
14
Day
**
**
***
***
Vehicle
BCY11863, 5 mg/kg ip Q3D
** p<0.001, *** p<0.001, Student's t-test

FIGURE 6

A


Percentage in CD45+ cells(%)
40
30
20
10
0
***
***
ns
ns
CD3
CD8T
CD4T
Treg
Vehicle
BCY11863 5mg/kg ip Q3D

B

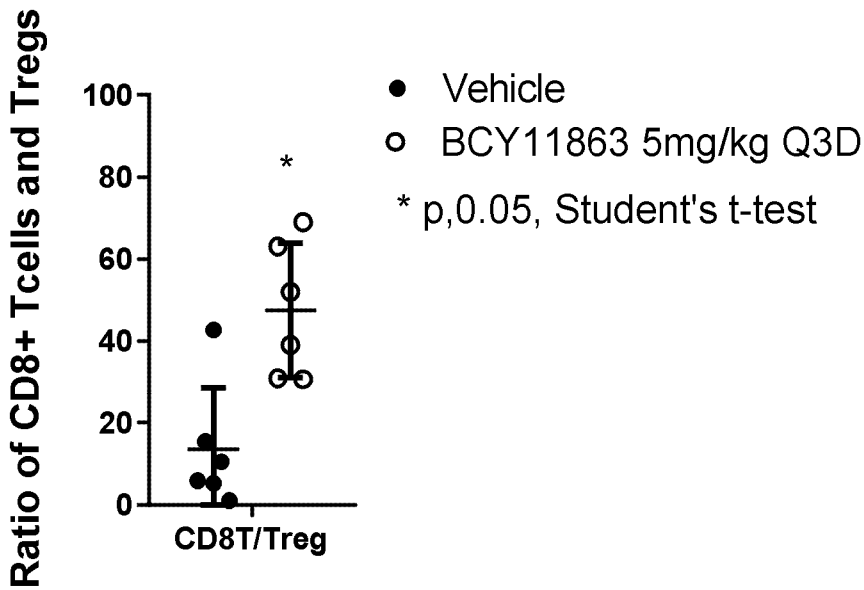

Ratio of CD8+ Tcells and Tregs
100
80
60
40
20
0
*
CD8T/Treg
Vehicle
BCY11863 5mg/kg Q3D
* p,0.05, Student's t-test

FIGURE 7

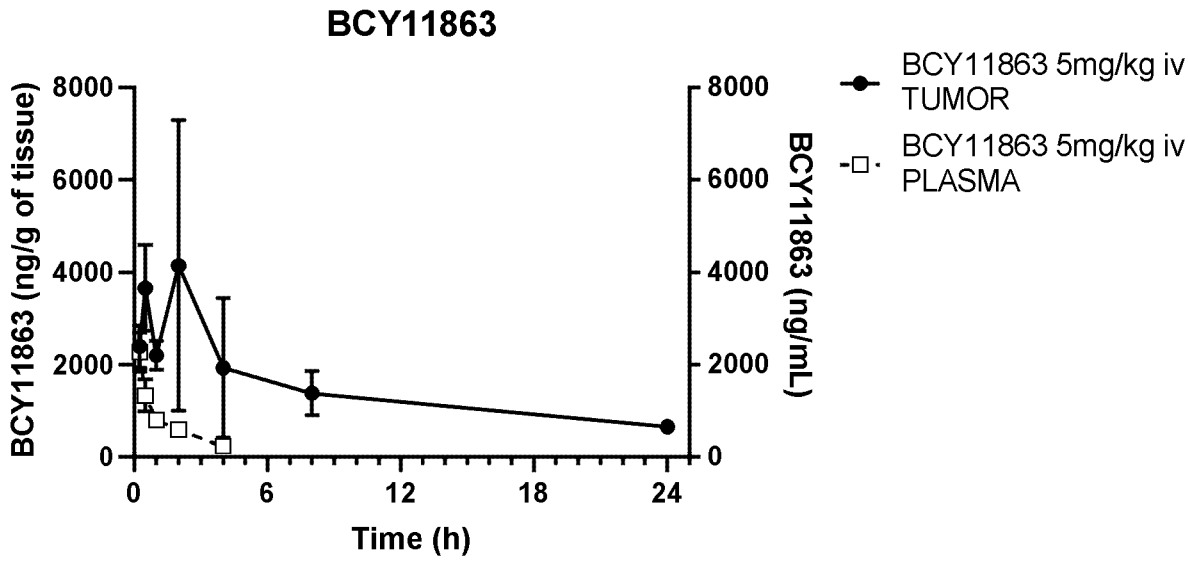


FIGURE 8

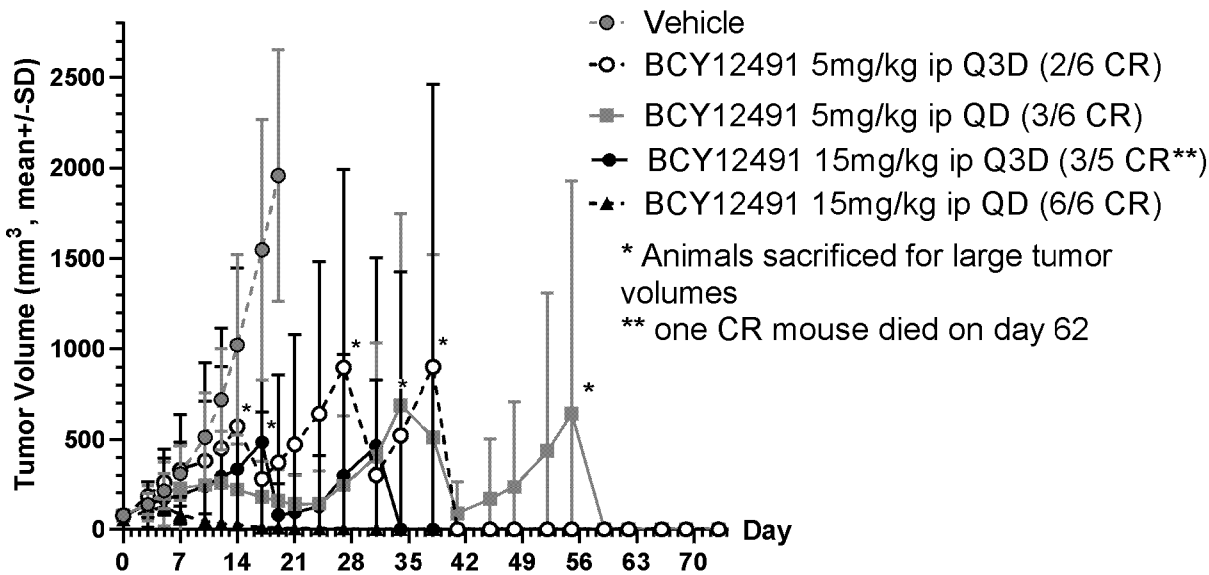
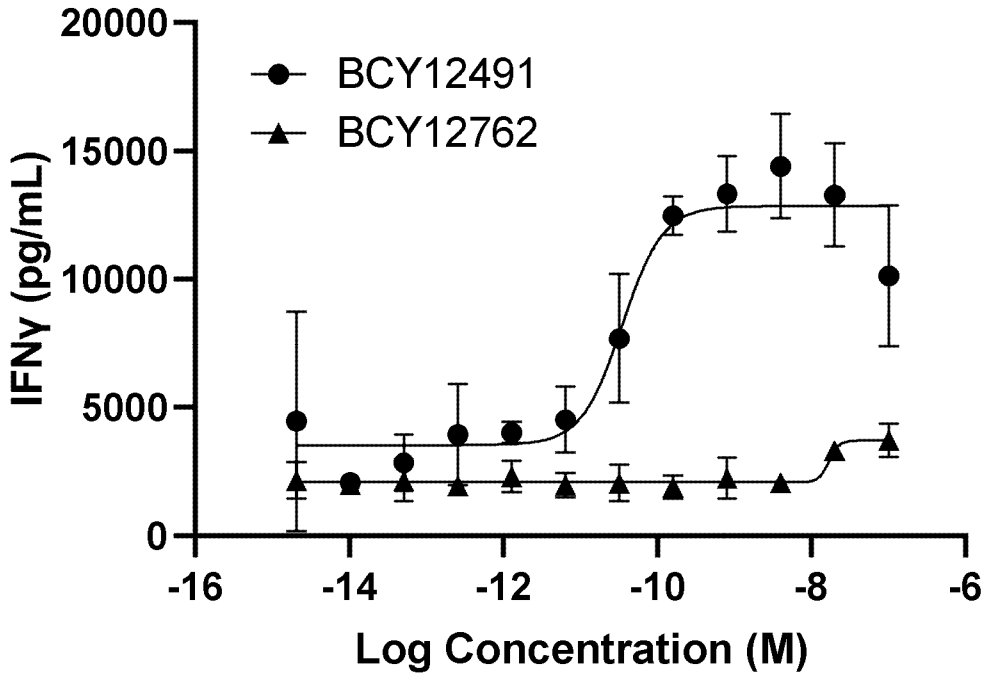


FIGURE 9

A


20000
15000
10000
5000
0
IFNγ (pg/mL)
BCY12491
BCY12762
-16
-14
-12
-10
-8
-6
Log Concentration (M)

B

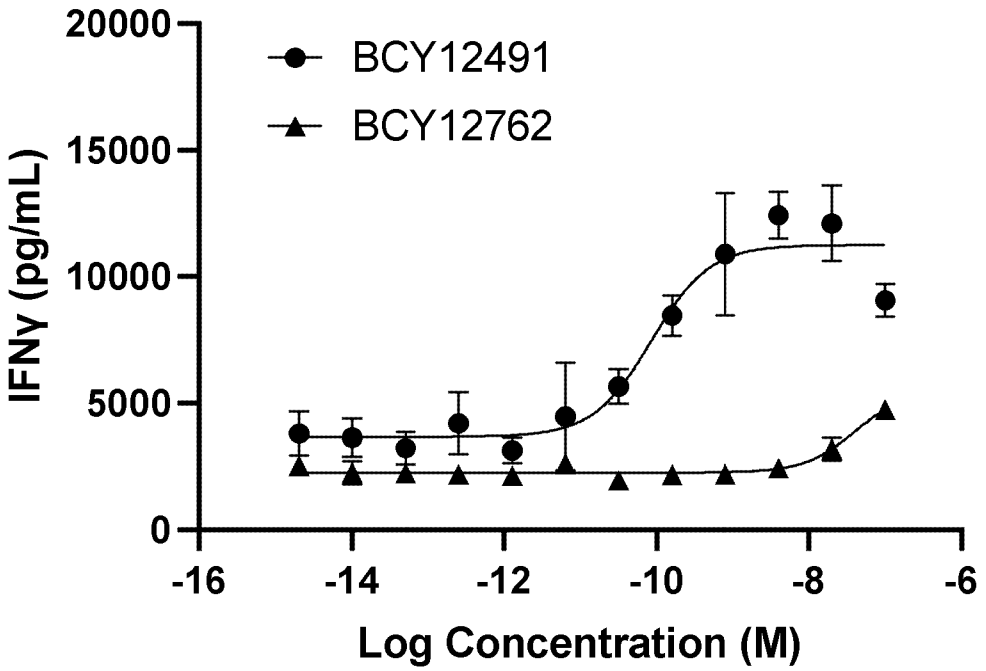
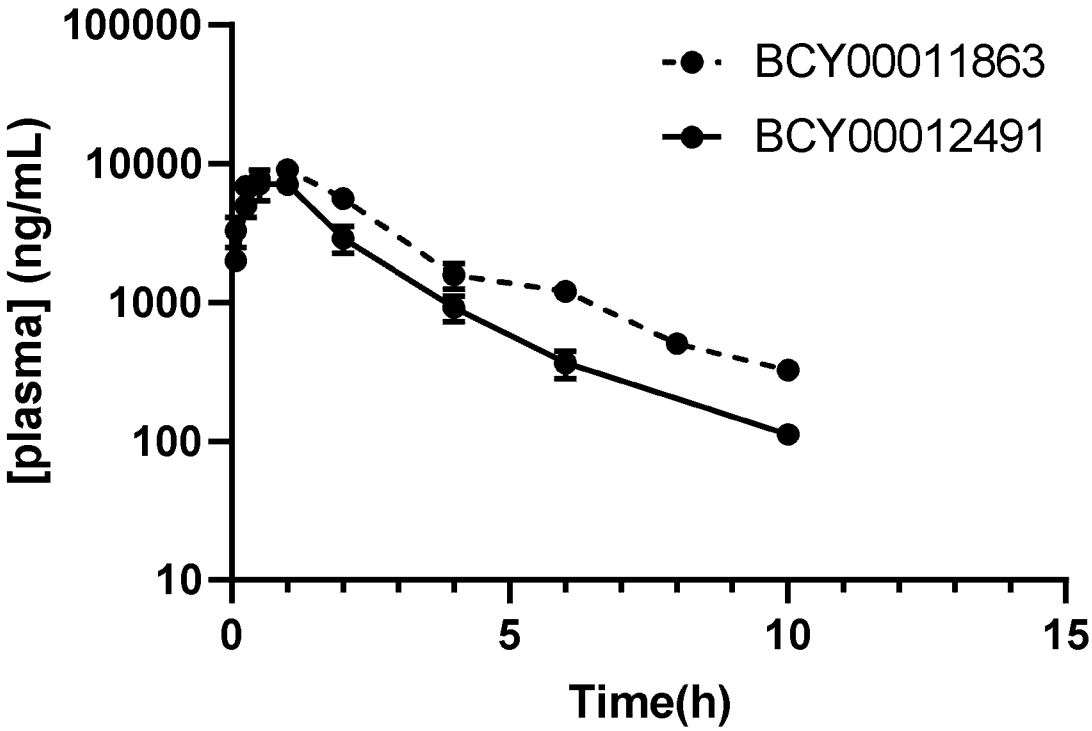

20000
15000
10000
5000
0
IFNγ (pg/mL)
BCY12491
BCY12762
-16
-14
-12
-10
-8
-6
Log Concentration (M)

FIGURE 10



Compound	Terminal half-life, $T_{1/2}$(h)
BCY11863	2.5
BCY12491	2.0

FIGURE 11

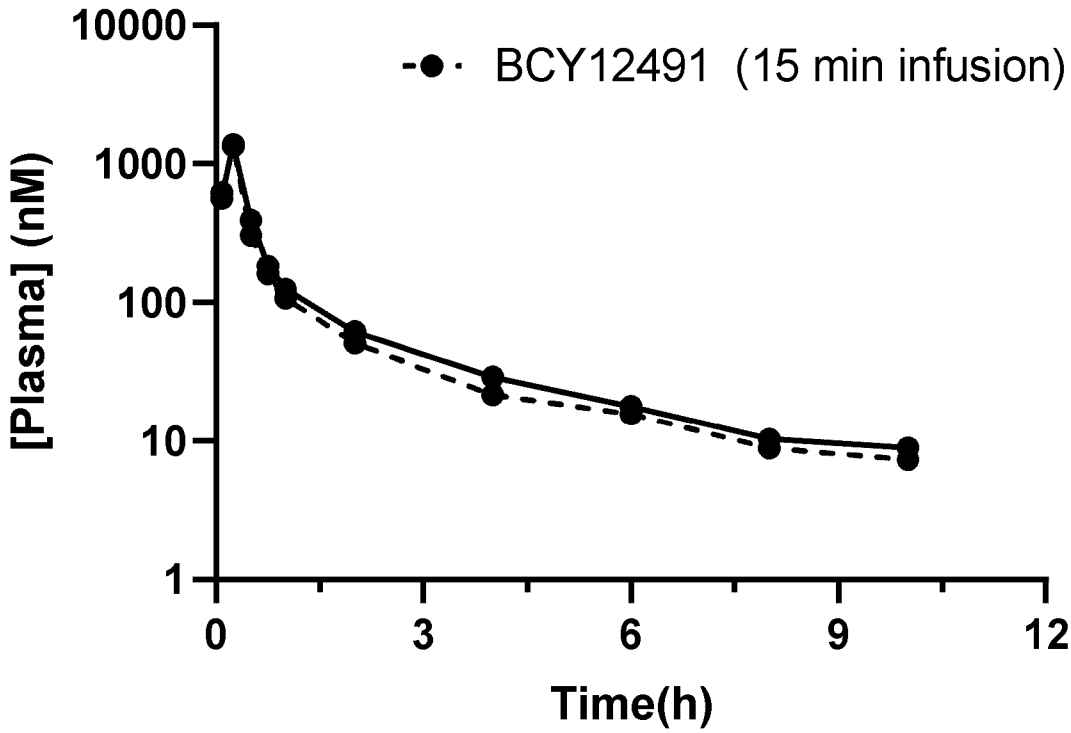
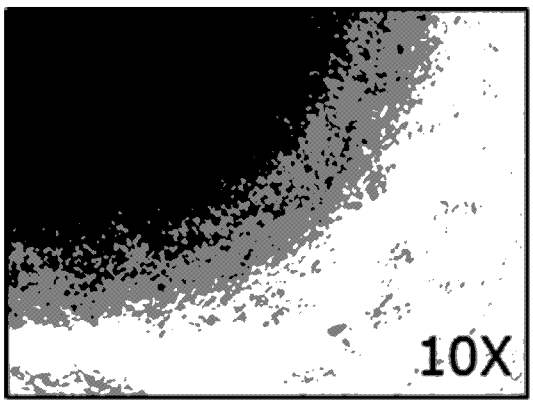

10000
1000
100
10
1
[Plasma] (nM)
BCY12491 (15 min infusion)
0
3
6
9
12
Time(h)

FIGURE 12

A



B

	CD137+ T cells (%)	Nectin-4+ cells (%)
PT1	19.8	4.4
PT2	15.1	25.8
PT3	30.0	15.1

C

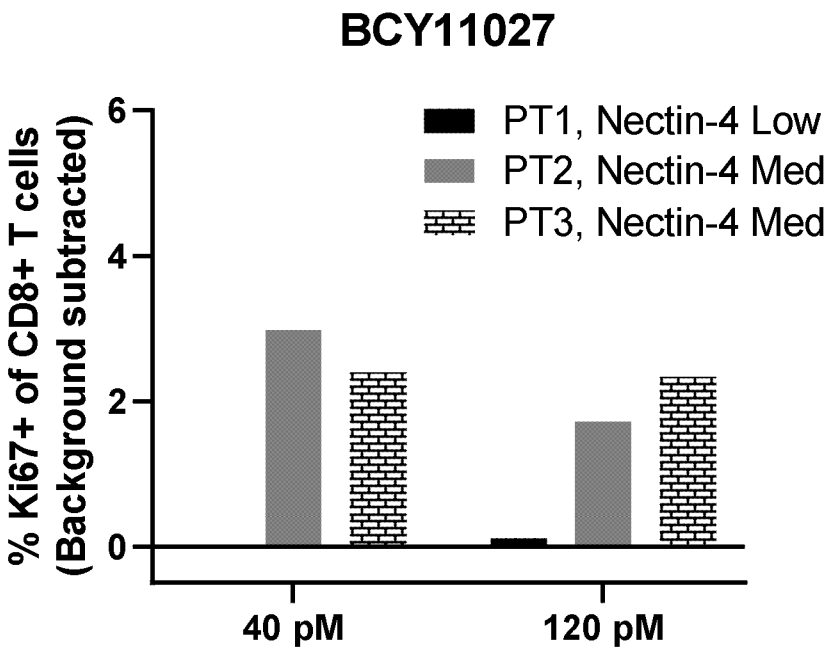
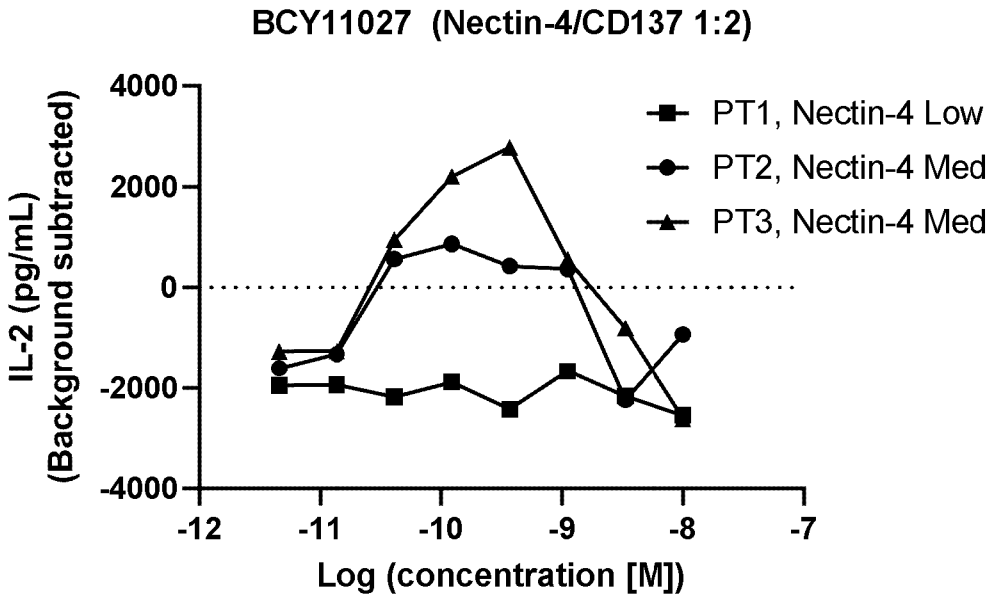


FIGURE 13

D



E

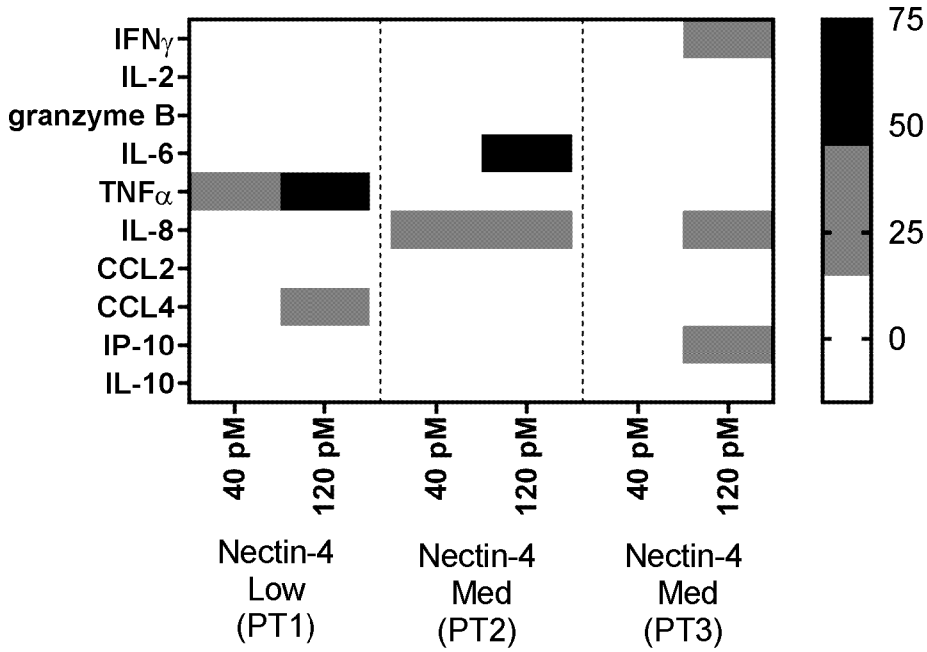


FIGURE 13 (ctd)

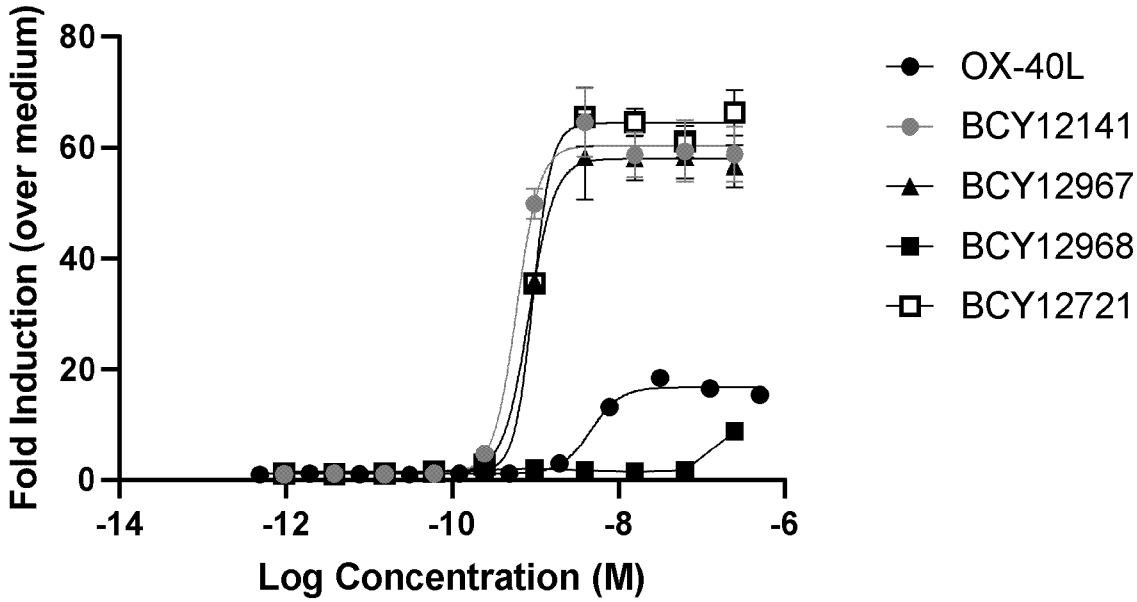

Fold Induction (over medium)
80
60
40
20
0
-14
-12
-10
-8
-6
Log Concentration (M)
OX-40L
BCY12141
BCY12967
BCY12968
BCY12721

FIGURE 14

A

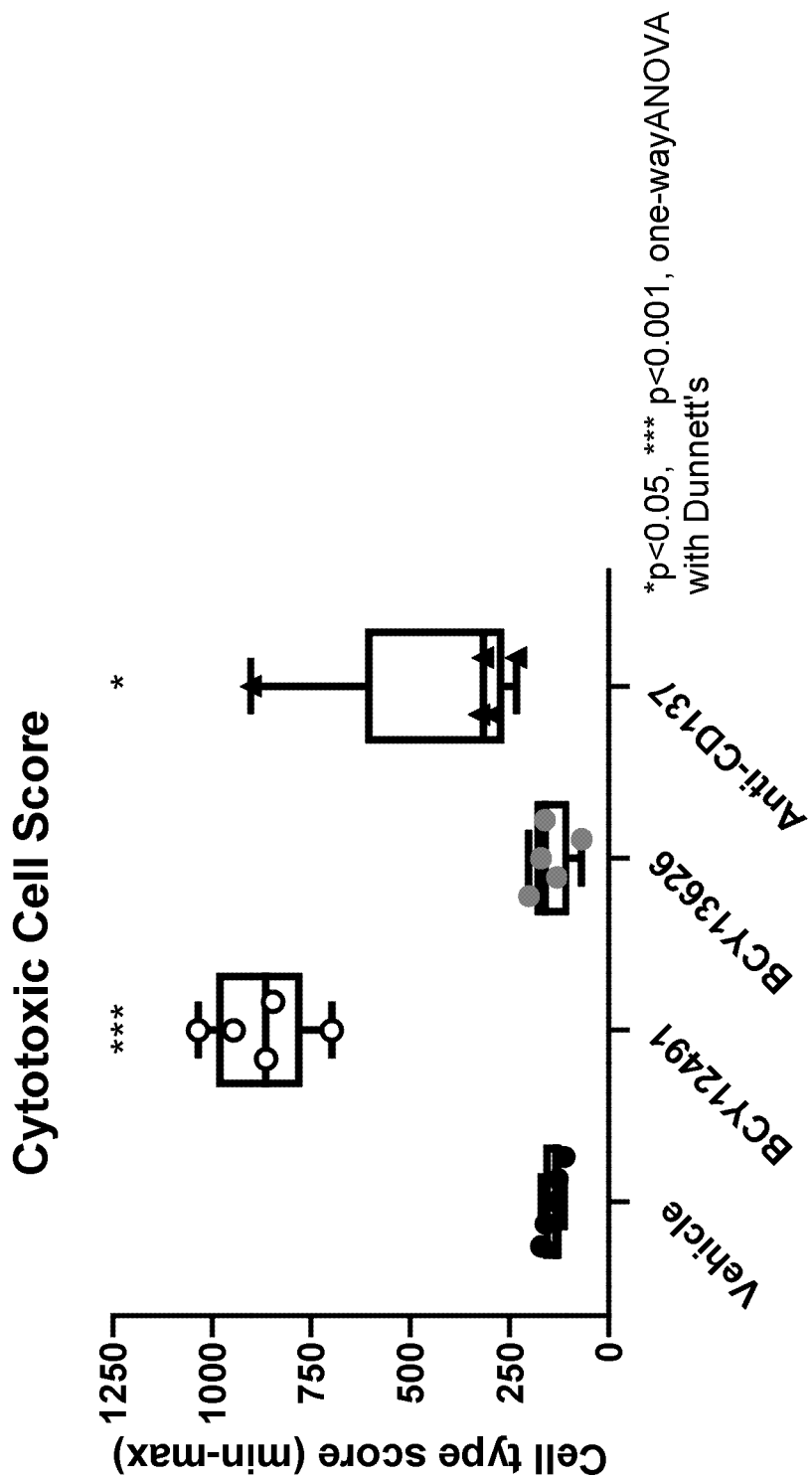

Cytotoxic Cell Score
*p<0.05, *** p<0.001, one-wayANOVA with Dunnett's
Cell type score (min-max)
1250
1000
750
500
250
0
Vehicle
BCY12491
BCY13626
Anti-CD137
***
*

FIGURE 15

B

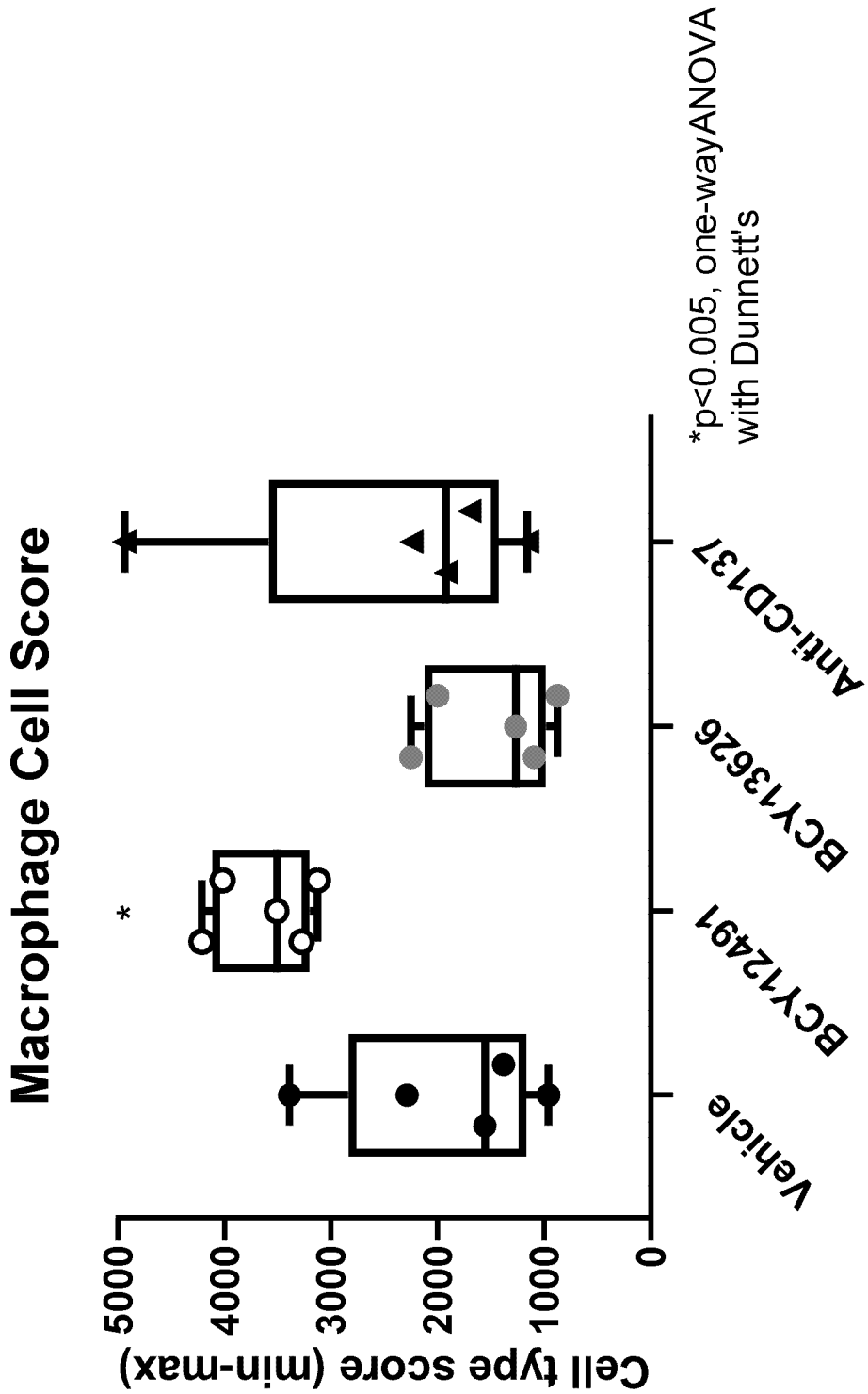


FIGURE 15 (ctd)

C

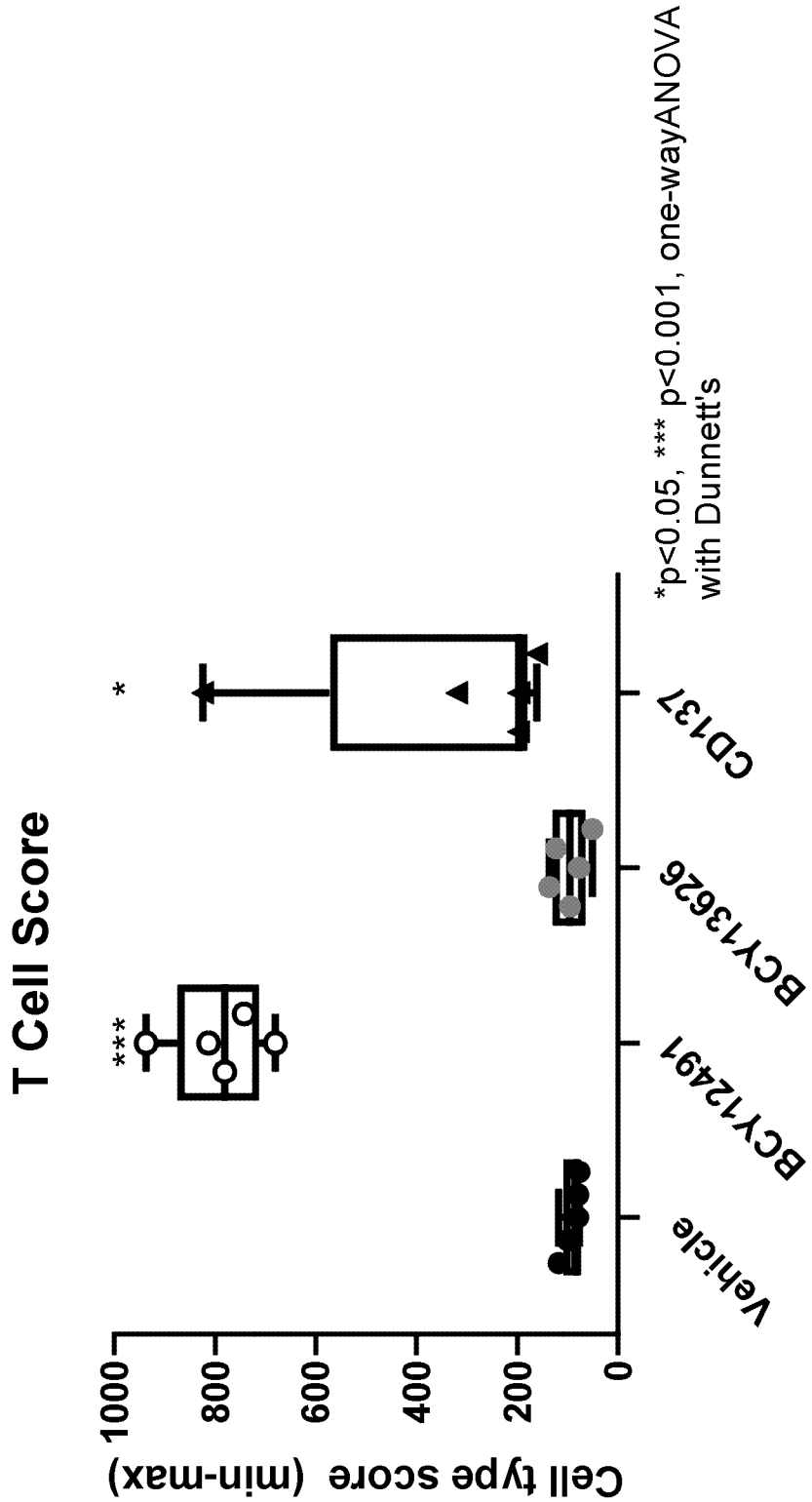


FIGURE 15 (ctd)

D

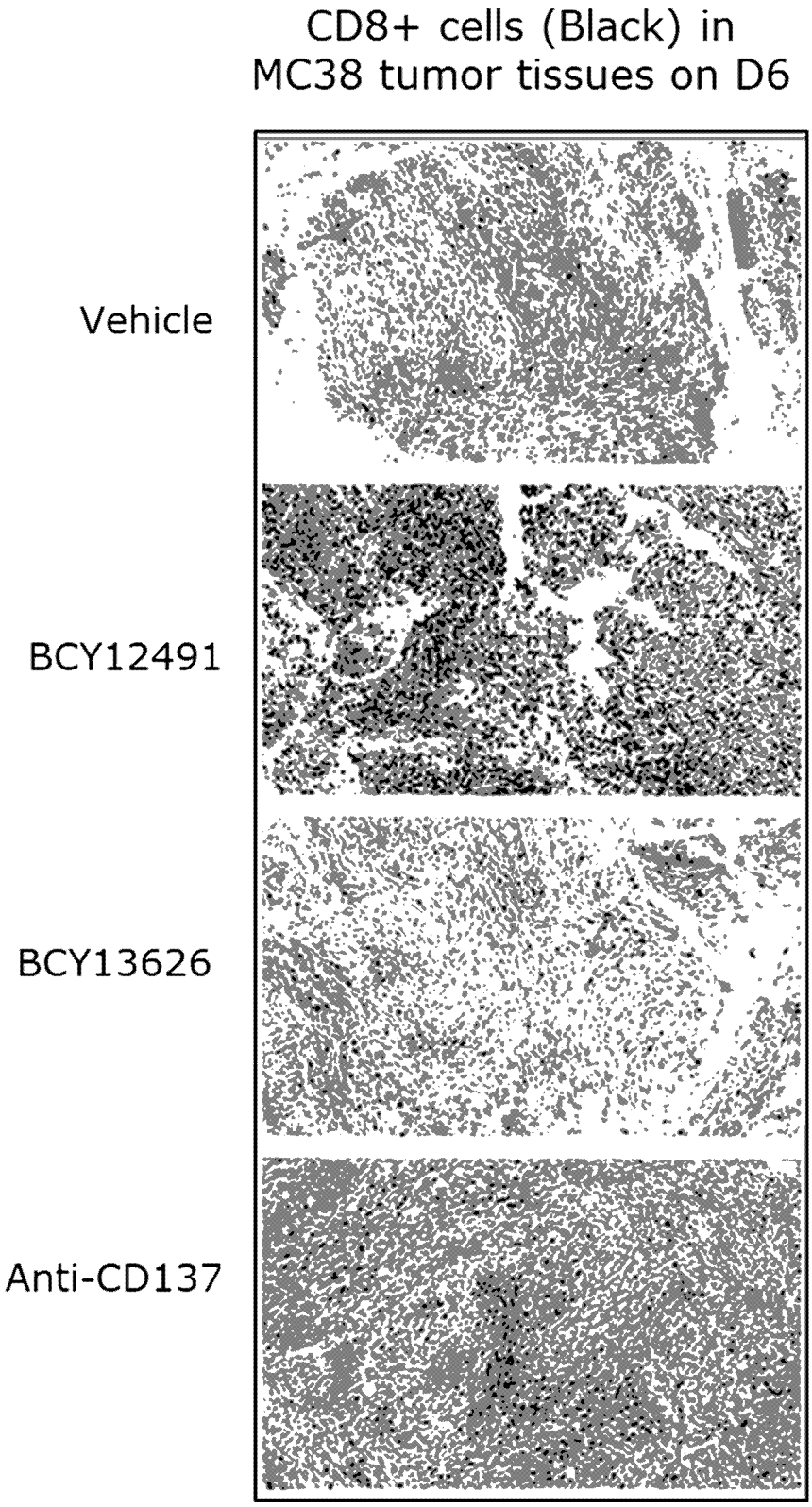


FIGURE 15 (ctd)

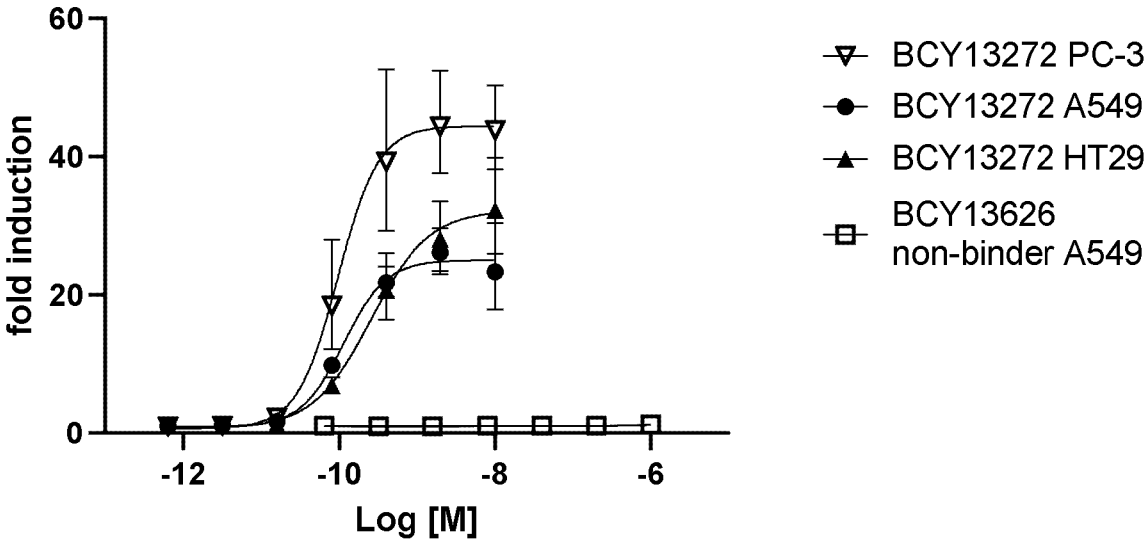


FIGURE 16

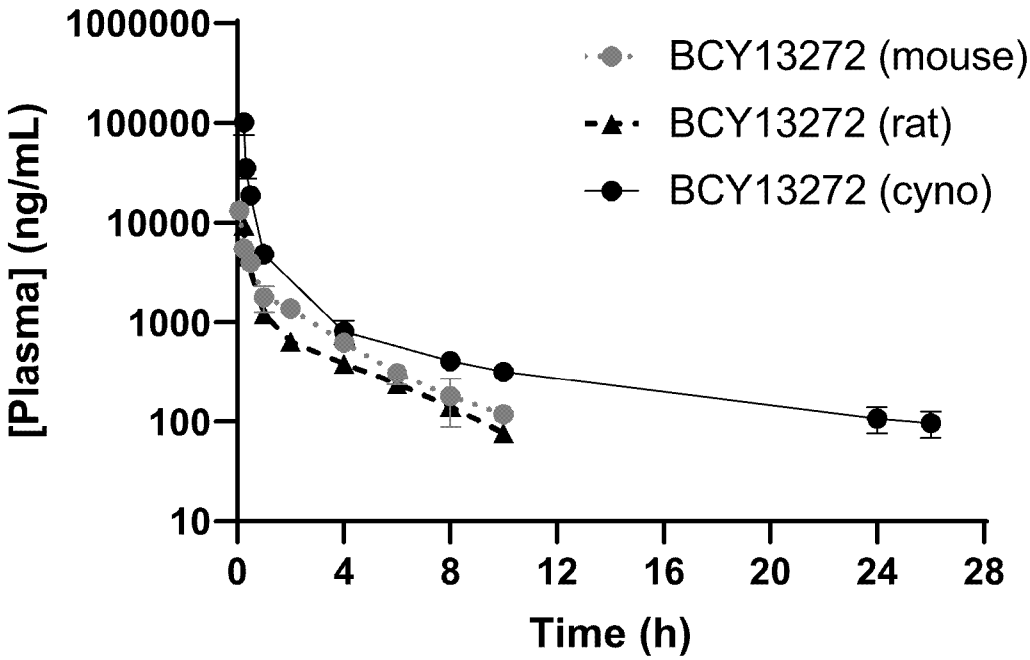


FIGURE 17

A

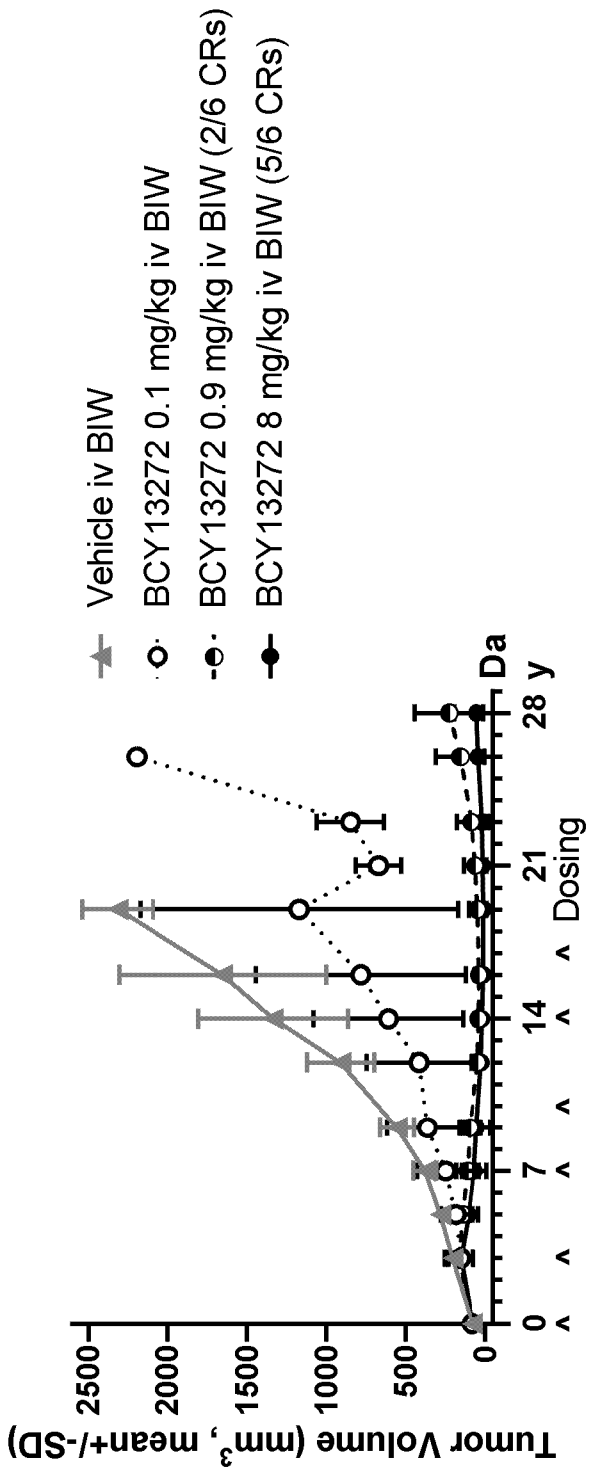

Vehicle iv BIW
BCY13272 0.1 mg/kg iv BIW
BCY13272 0.9 mg/kg iv BIW (2/6 CRs)
BCY13272 8 mg/kg iv BIW (5/6 CRs)
Tumor Volume (mm3, mean+/-SD)
2500
2000
1500
1000
500
0
0
7
14
21
28
Dosing
Da
y

FIGURE 18

B

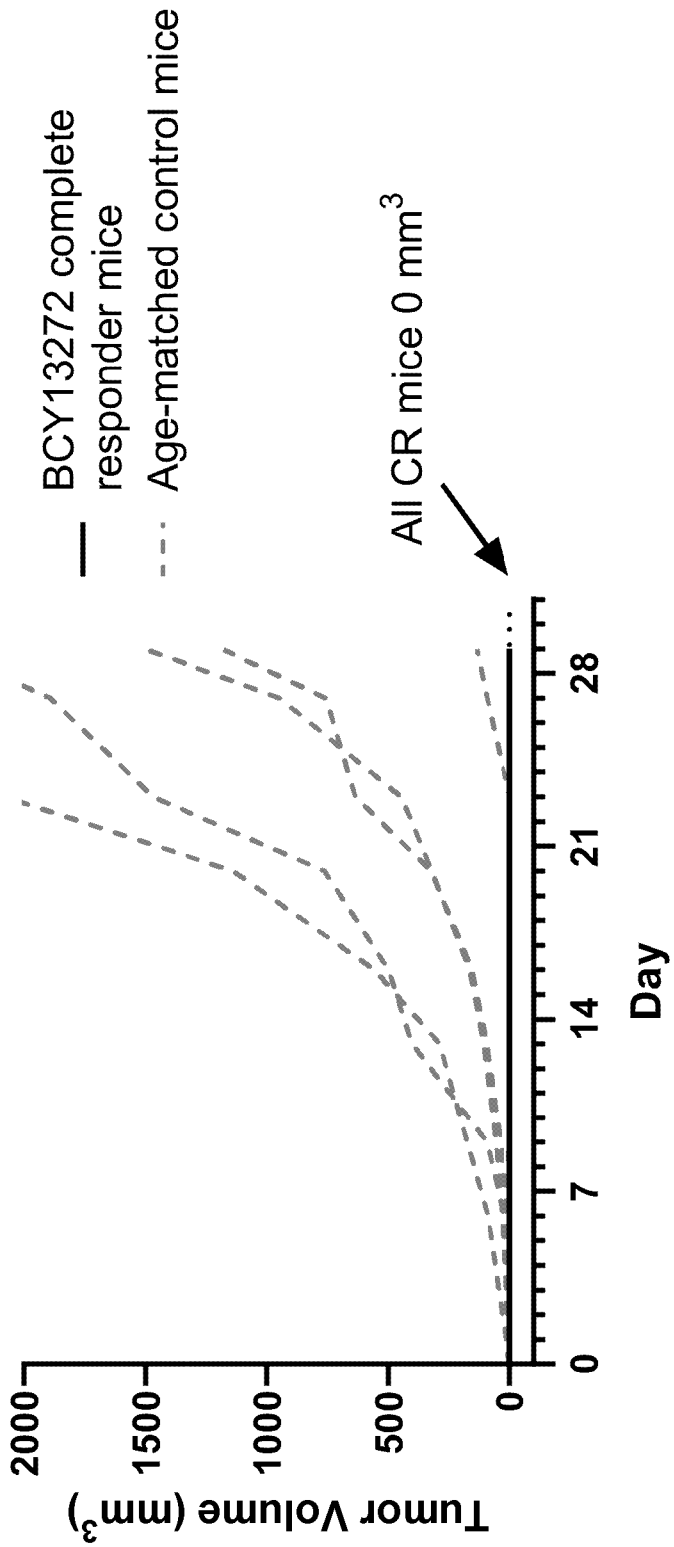
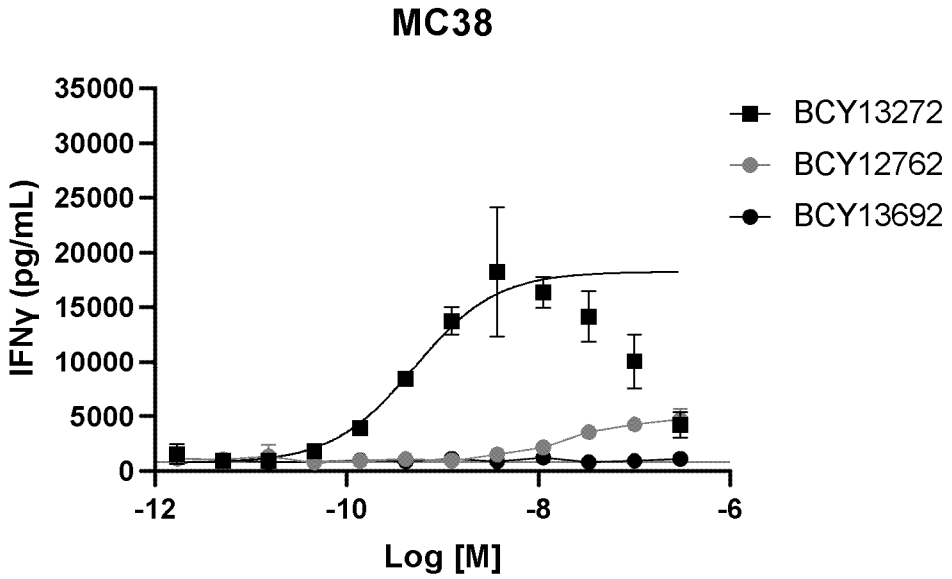


FIGURE 18 (ctd)

A


MC38
IFNγ (pg/mL)
35000
30000
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
Log [M]
BCY13272
BCY12762
BCY13692

B

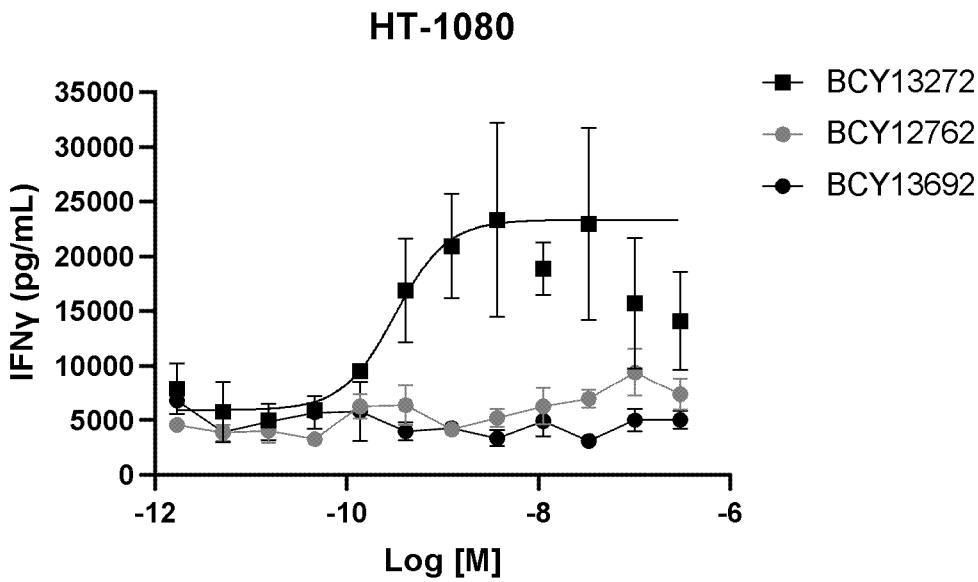

HT-1080
IFNγ (pg/mL)
35000
30000
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
Log [M]
BCY13272
BCY12762
BCY13692

FIGURE 19

C

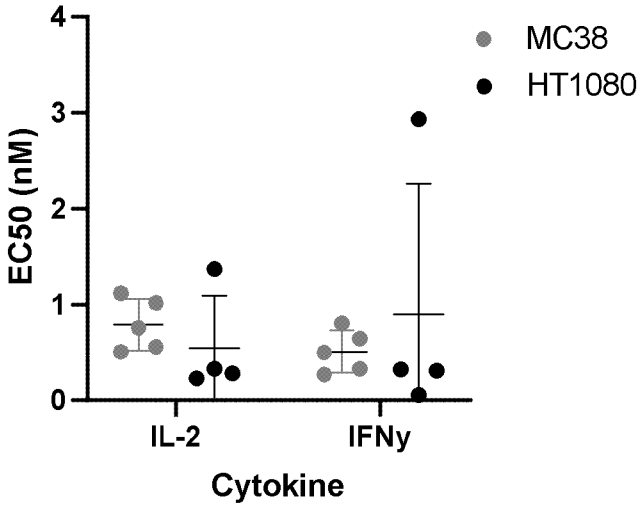
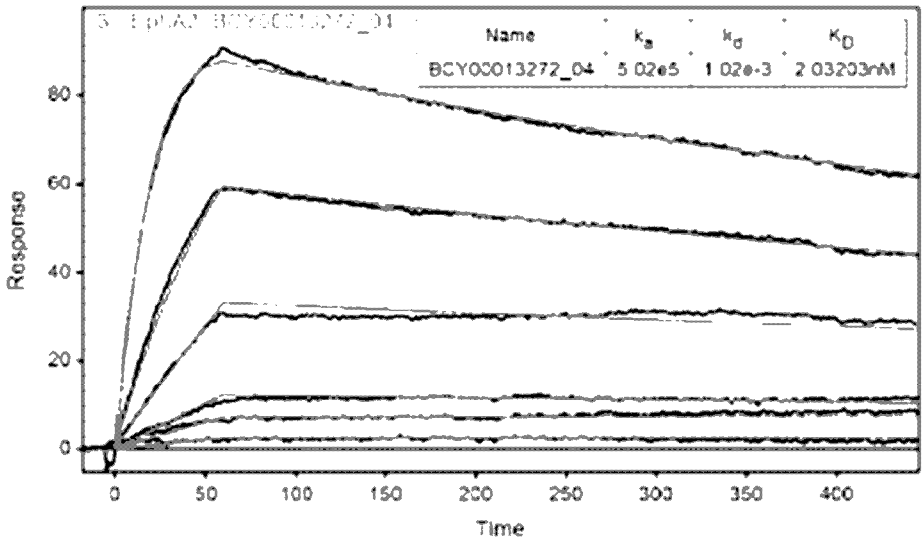


FIGURE 19 (ctd)

A


Name
k_a
k_d
K_D
BCY00013272_04 5.02e5 1.02e-3 2.03203nM
Response
Time
0
20
40
60
80
0
50
100
150
200
250
300
350
400

B

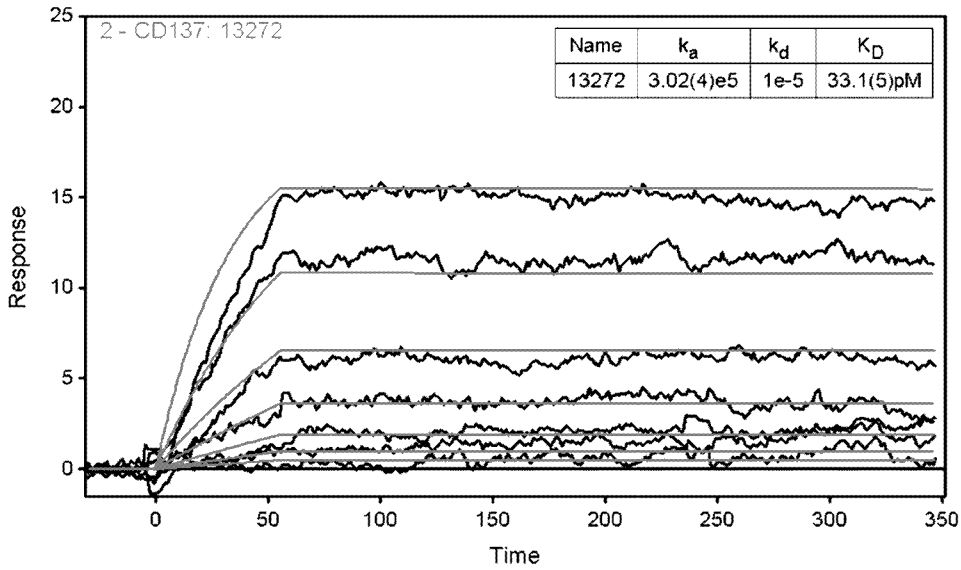
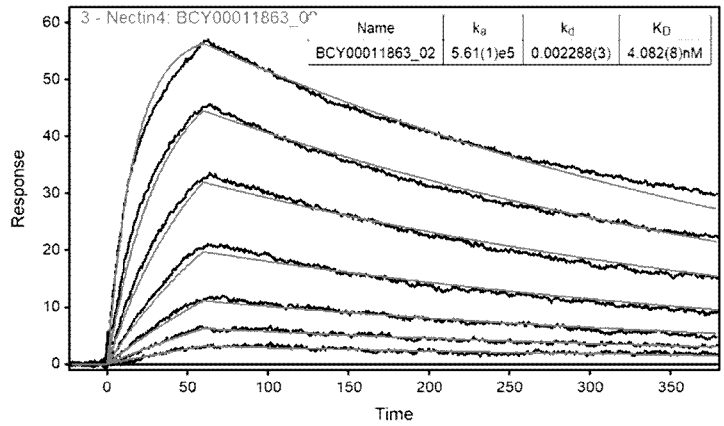
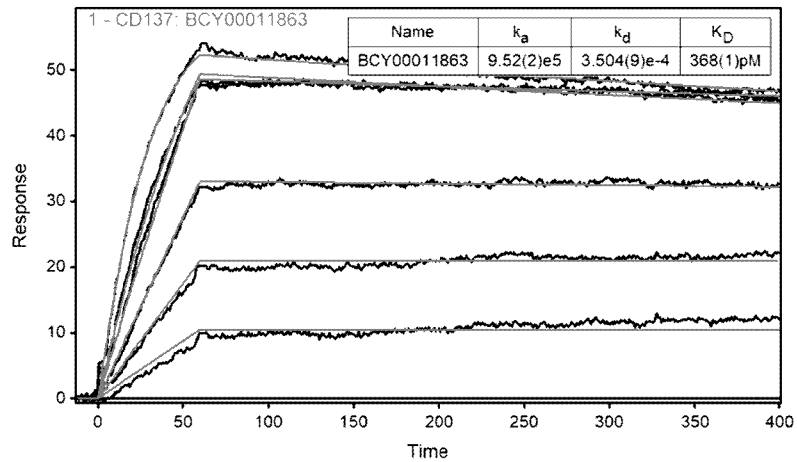

2 - CD137: 13272
Name
k_a
k_d
K_D
13272
3.02(4)e5
1e-5
33.1(5)pM
Response
Time
0
5
10
15
20
25
0
50
100
150
200
250
300
350

FIGURE 20

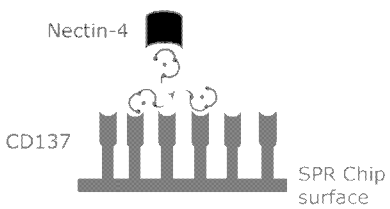
A



B



C



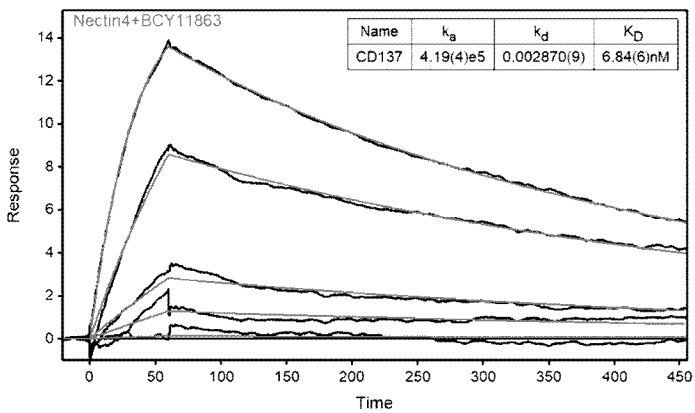
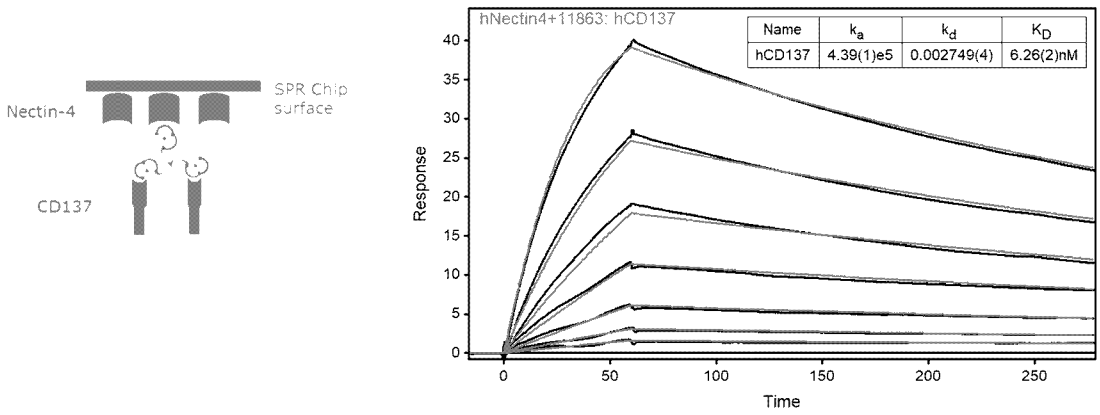


FIGURE 21

D



E

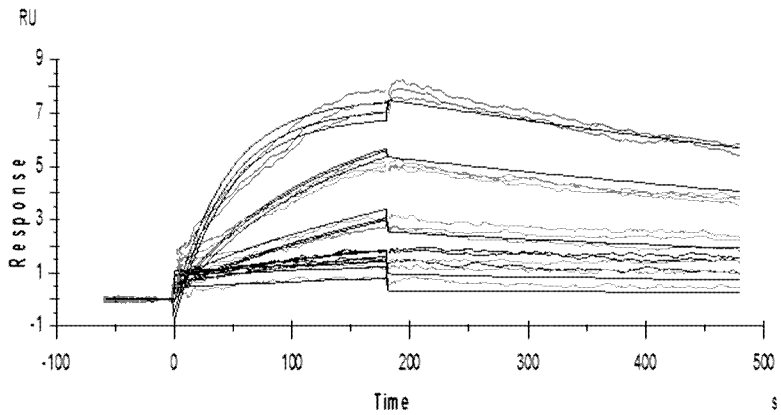


FIGURE 21 (ctd)

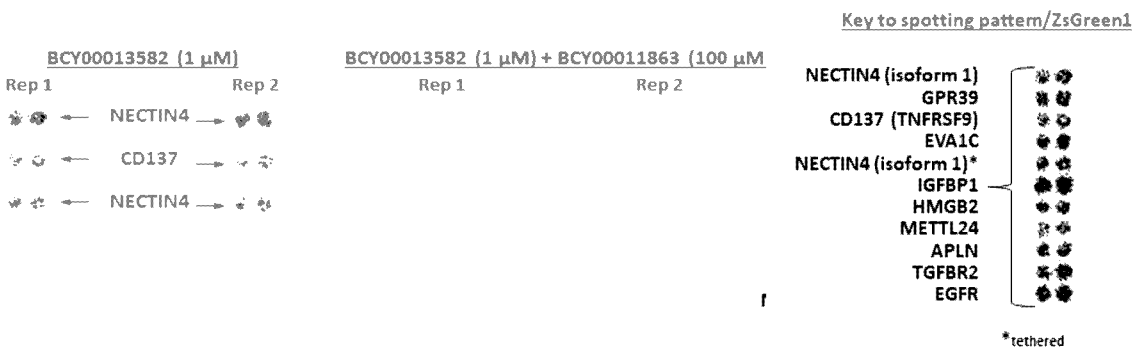


FIGURE 22

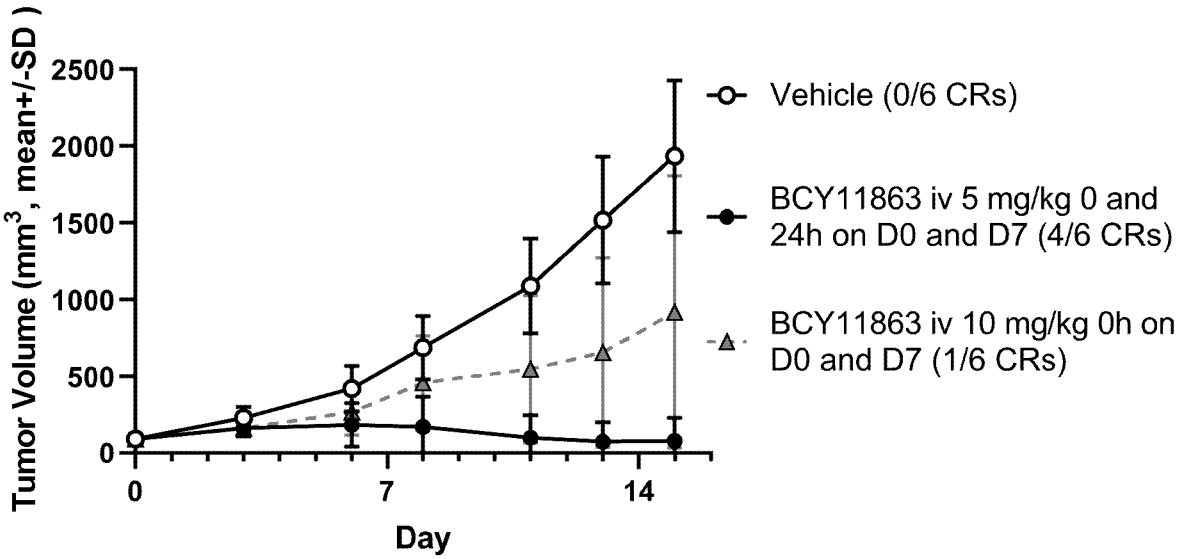
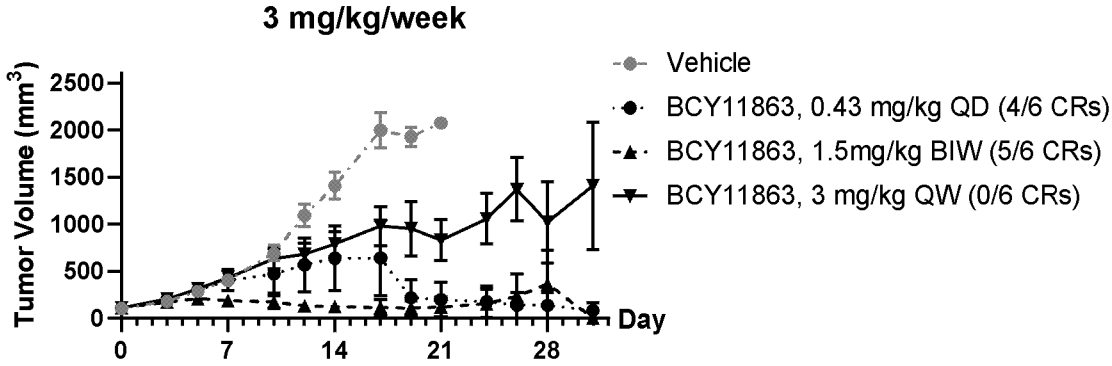
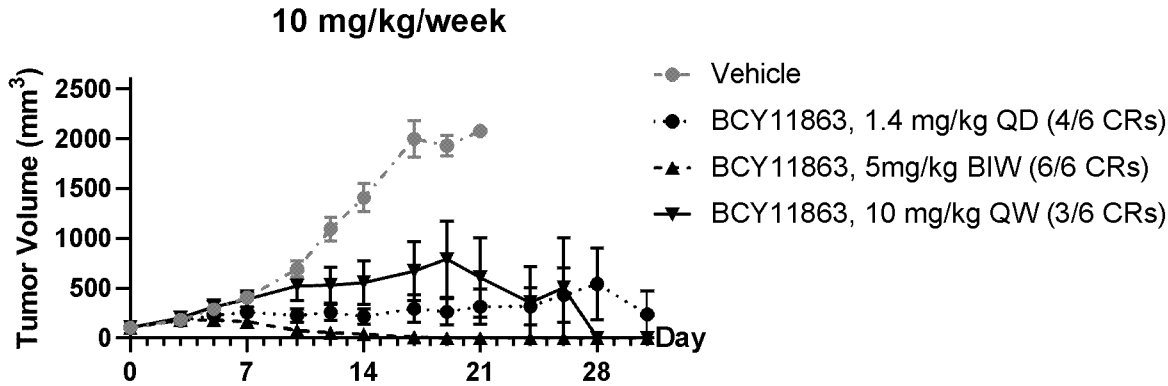

Tumor Volume (mm3, mean+/-SD)
2500
2000
1500
1000
500
0
0
7
14
Day
Vehicle (0/6 CRs)
BCY11863 iv 5 mg/kg 0 and 24h on D0 and D7 (4/6 CRs)
BCY11863 iv 10 mg/kg 0h on D0 and D7 (1/6 CRs)

FIGURE 23

A



B



C

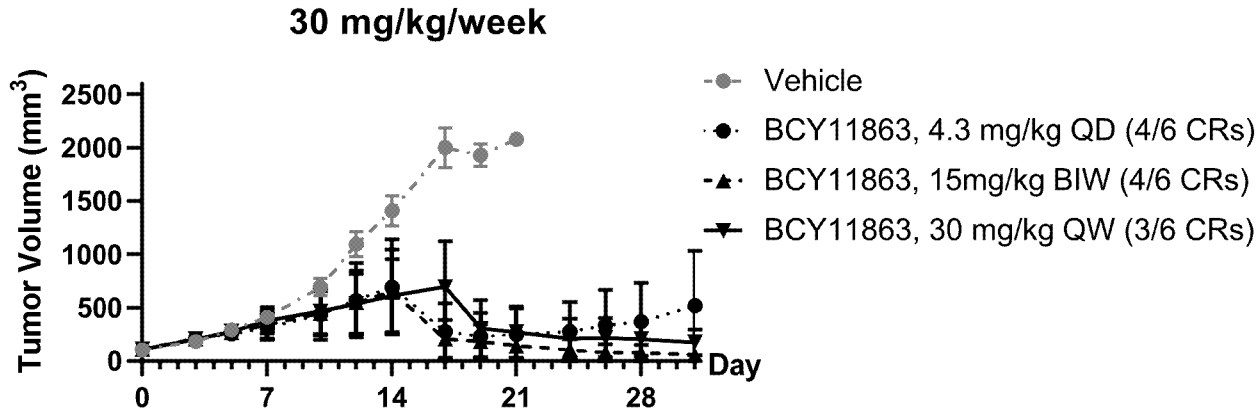


FIGURE 24

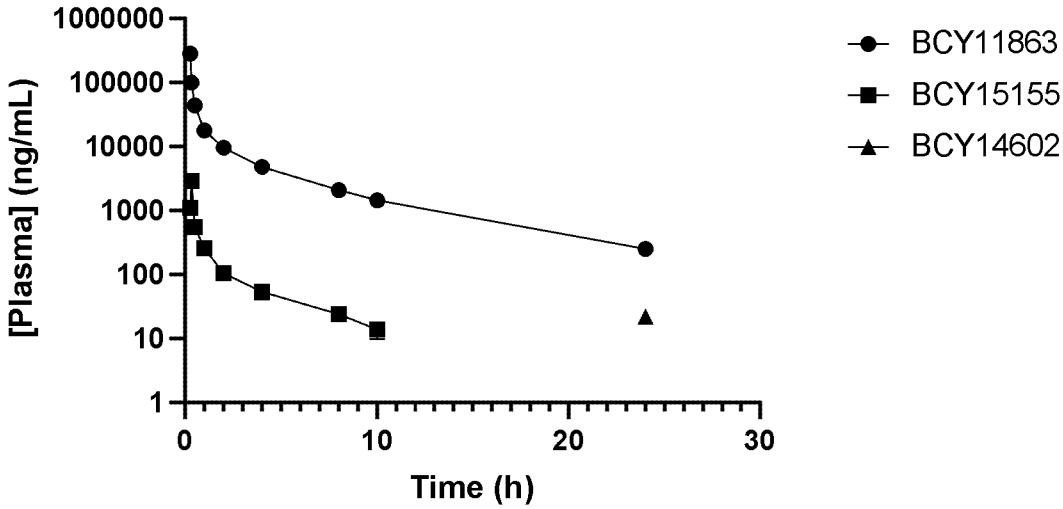
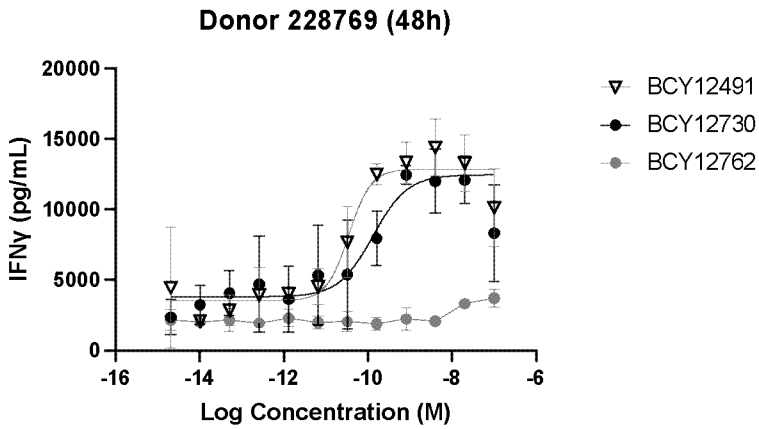
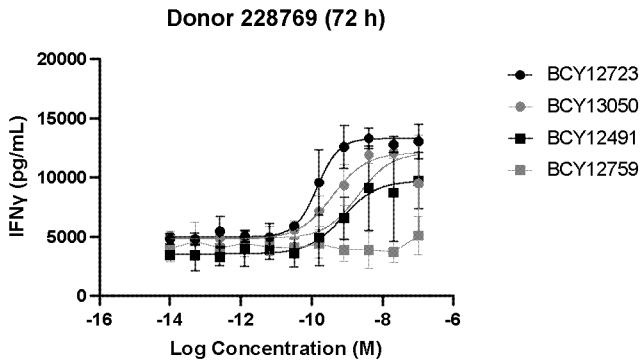

1000000
100000
10000
1000
100
10
1
[Plasma] (ng/mL)
0
10
20
30
Time (h)
BCY11863
BCY15155
BCY14602

FIGURE 25

A



B



C

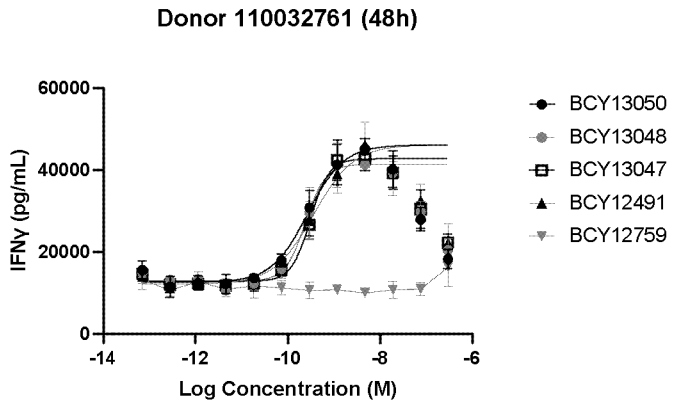
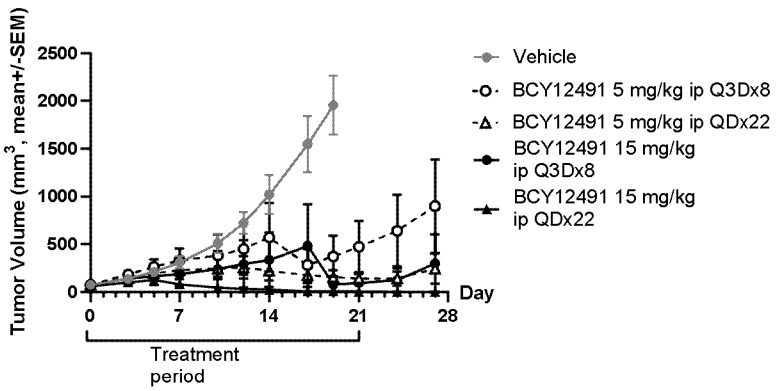
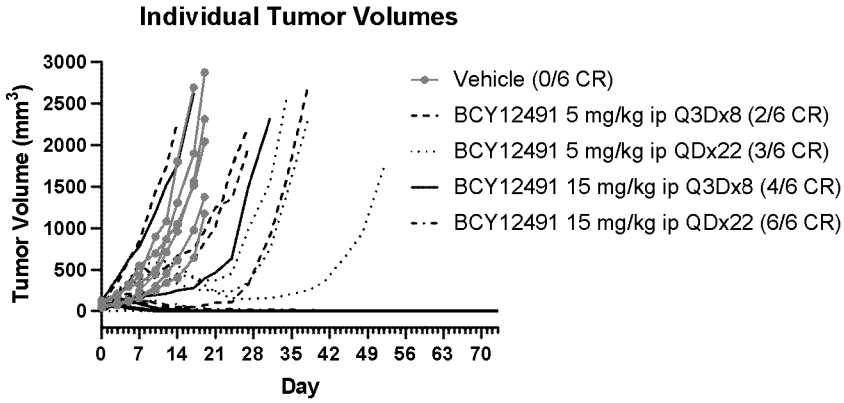


FIGURE 26

A



B



C

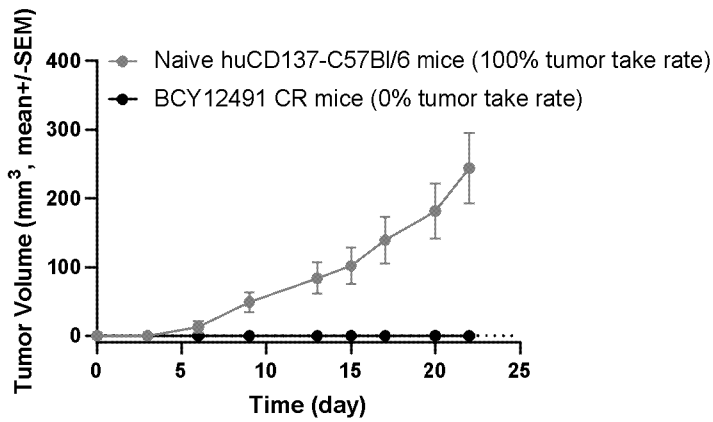
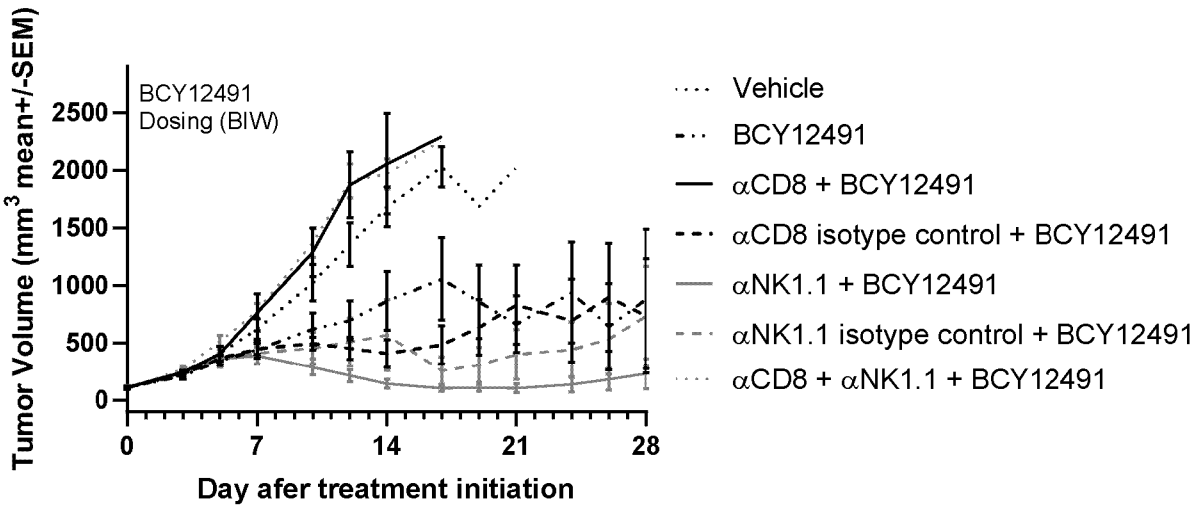


FIGURE 27

A


BCY12491
Dosing (BIW)
Tumor Volume (mm3 mean+/-SEM)
2500
2000
1500
1000
500
0
0
7
14
21
28
Day afer treatment initiation
Vehicle
BCY12491
αCD8 + BCY12491
αCD8 isotype control + BCY12491
αNK1.1 + BCY12491
αNK1.1 isotype control + BCY12491
αCD8 + αNK1.1 + BCY12491

B

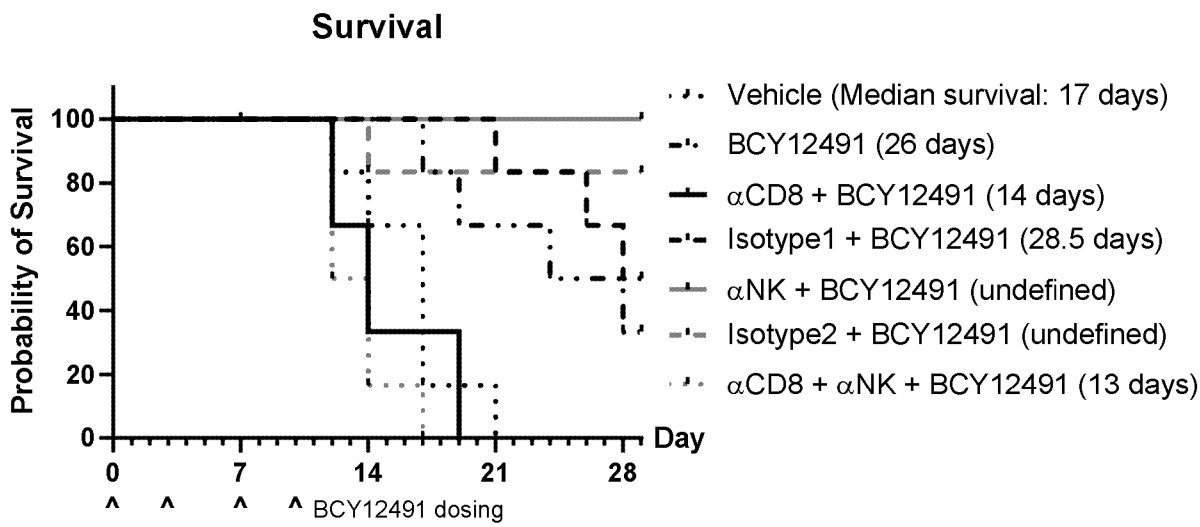

Survival
Probability of Survival
100
80
60
40
20
0
Day
0
7
14
21
28
BCY12491 dosing
Vehicle (Median survival: 17 days)
BCY12491 (26 days)
αCD8 + BCY12491 (14 days)
Isotype1 + BCY12491 (28.5 days)
αNK + BCY12491 (undefined)
Isotype2 + BCY12491 (undefined)
αCD8 + αNK + BCY12491 (13 days)

FIGURE 28

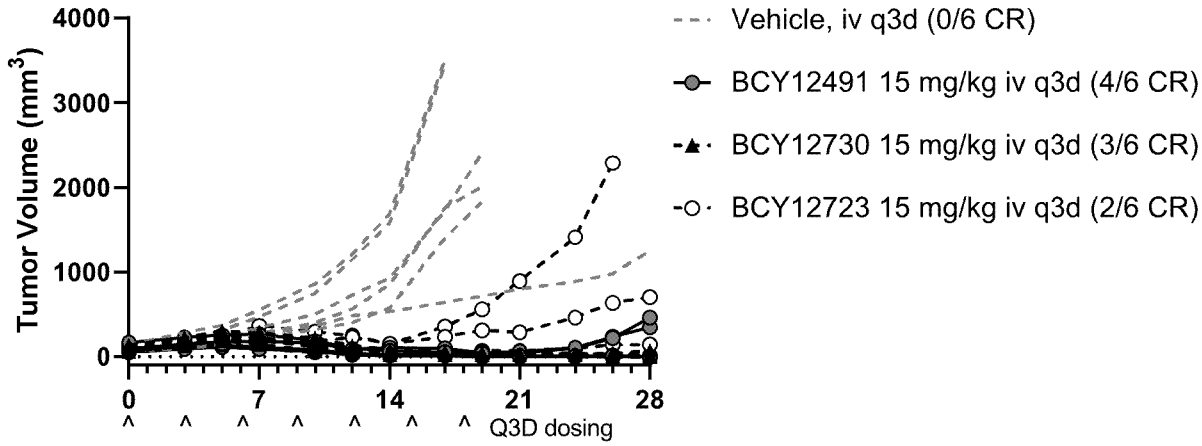


FIGURE 29

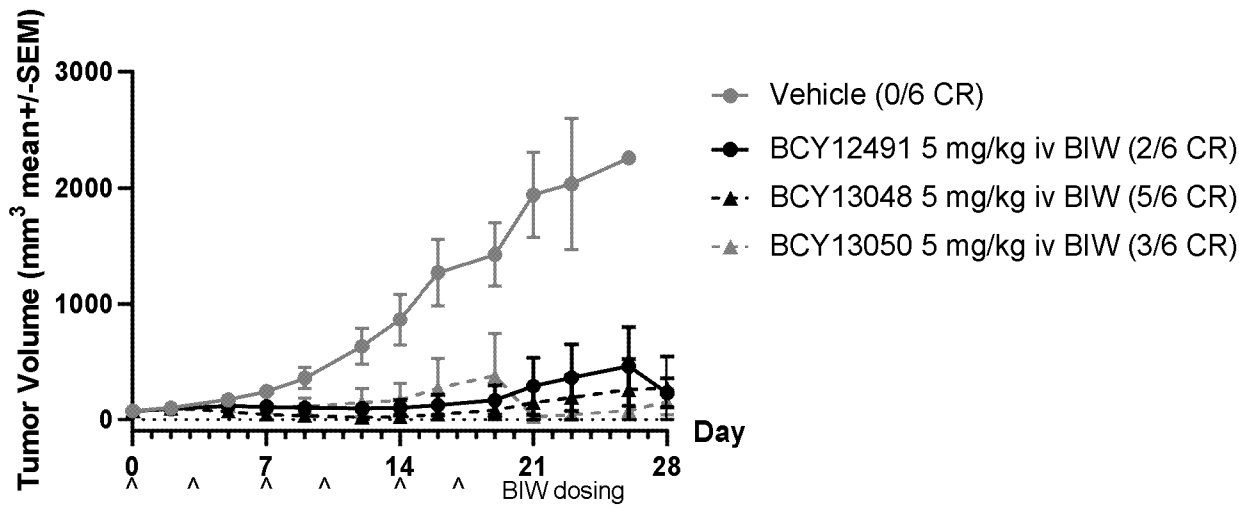


FIGURE 30

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/051831

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K47/50 A61K38/12 C07K7/54
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Kristen Hurov ET AL: "BT7480, a novel fully synthetic tumor-targeted immune cell agonist (TICA(TM)) induces tumor localized CD137 agonism", , 22 June 2020 (2020-06-22), XP055719960, Retrieved from the Internet: URL:https://www.bicycletherapeutics.com/wp-content/uploads/2020-06-16-BT7480-AACR-2020-poster-P5552_Final_CD137-in-title-002.pdf [retrieved on 2020-08-04] abstract ----- -/--	1-6, 14-17

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2020	04/11/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Winger, Rudolf

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/051831

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2019/193328 A1 (BICYCLETX LTD [GB]) 10 October 2019 (2019-10-10) claims 1-28	1-5, 14-16, 22,32, 40-43
X	Punit Upadhyaya: "Activation of CD137 using multivalent and tumour targeted bicyclic peptides", , 25 April 2019 (2019-04-25), XP055669343, Retrieved from the Internet: URL:https://www.bicycletherapeutics.com/wp-content/uploads/PU_2019-Peptide-Congress_publication.pdf [retrieved on 2020-02-17] pages 3,9,15 - page 19	1-5, 14-16, 22,32, 40-43
A	WO 2018/156740 A1 (MACROGENICS INC [US]) 30 August 2018 (2018-08-30) claims	1-43
A	WO 2017/182672 A1 (ALLIGATOR BIOSCIENCE AB [SE]) 26 October 2017 (2017-10-26) claims	1-43

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2020/051831

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 2019193328 A1	10-10-2019
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		EP 3585431 A1	01-01-2020
		JP 2020508334 A	19-03-2020
		KR 20190121802 A	28-10-2019
		SG 11201907753T A	27-09-2019
		TW 201831511 A	01-09-2018
		US 2020062854 A1	27-02-2020
		WO 2018156740 A1	30-08-2018
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		EP 3445788 A1	27-02-2019
		JP 2019523630 A	29-08-2019
		KR 20180135454 A	20-12-2018
		RU 2018139339 A	22-05-2020
		US 2019169308 A1	06-06-2019
		WO 2017182672 A1	26-10-2017