

BICYCLIC PEPTIDE LIGANDS SPECIFIC FOR TRANSFERRIN RECEPTOR 1 (TfR1)**FIELD OF THE INVENTION**

The present invention relates to polypeptides which are covalently bound to molecular
5 scaffolds such that two or more peptide loops are subtended between attachment points to
the scaffold. In particular, the invention describes peptides which bind to TfR1. The invention
also relates to multimeric binding complexes which comprise at least two of said bicyclic
peptide ligands. The invention also includes pharmaceutical compositions comprising said
peptide ligands and multimeric binding complexes and the use of said peptide ligands, and
10 multimeric binding complexes and pharmaceutical compositions in preventing, suppressing or
treating a disease or disorder through TfR1 mediated delivery of a therapeutic agent.

BACKGROUND OF THE INVENTION

Cyclic peptides are able to bind with high affinity and specificity to protein targets and hence
15 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
20 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 Å²; Wu *et al.* (2007), Science 330, 1066-71), a cyclic peptide with the
Arg-Gly-Asp motif binding to integrin αVβ3 (355 Å²) (Xiong *et al.* (2002), Science 296(5565),
151-5) or the cyclic peptide inhibitor upain-1 binding to urokinase-type plasminogen activator
25 (603 Å²; 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
30 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 favourable binding properties achieved through macrocyclization are even more
pronounced in multicyclic peptides having more than one peptide ring as for example in
35 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
5 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 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) (Heinis *et al.* (2014) Angewandte Chemie, International Edition 53(6) 1602-1606).

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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-
15 Cys) were displayed on phage and cyclised by covalently linking the cysteine side chains to a small molecule scaffold.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a peptide ligand specific for
20 transferrin receptor 1 (TfR1) comprising 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.

25 According to a further aspect of the invention, there is provided a multimeric binding complex which comprises at least two bicyclic peptide ligands, wherein said peptide ligands may be the same or different, each of which comprises a peptide ligand specific for transferrin receptor 1 (TfR1) comprising a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent
30 bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

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

According to a further aspect of the invention, there is provided a peptide ligand, or multimeric binding complex or pharmaceutical composition as defined herein for use in preventing, suppressing or treating a disease or disorder through TfR1 mediated delivery of a therapeutic agent.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Results of transcytosis assay with BCY17986 in primary cultures of human proximal convoluted cells.

Figure 2: Results of transcytosis assay with BCY17988 in primary cultures of human proximal convoluted cells.

Figure 3: Results of transcytosis assay with BCY17989 in primary cultures of human proximal convoluted cells.

Figure 4: Results of transcytosis assay with BCY17994 in primary cultures of human proximal convoluted cells.

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DETAILED DESCRIPTION OF THE INVENTION

It will be appreciated that the present invention relates to both “monomeric” bicyclic peptides, i.e. those which contain a single (monomeric) bicyclic peptide ligand and “multimeric” bicyclic peptides, i.e. those which contain more than one bicyclic peptide (such as 2, 3 or 4) conjugated via one or more linkers.

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Monomeric Bicyclic Peptide Ligands

According to a first aspect of the invention, there is provided a peptide ligand specific for transferrin receptor 1 (TfR1) comprising 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.

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In one embodiment, said reactive groups comprise cysteine residues.

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It will be appreciated that the term “specific for TfR1” refers to the ability of the peptide ligand to bind to transferrin receptor 1 (TfR1). It will also be appreciated that the peptide ligand will have a differing affect upon TfR1 depending on the precise epitope of binding. For example, the affect will either be inhibitory (i.e. the peptide ligand impedes/inhibits the binding of transferrin to TfR1) or non-inhibitory (i.e. the peptide ligand does not impede/inhibit the binding of transferrin to TfR1).

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Inhibitory Peptide Ligands

In one embodiment, the peptide ligand is specific for TfR1 and binds to TfR1 in a manner which impedes/inhibits the binding of transferrin to TfR1.

5 In a further embodiment, said loop sequences comprise 2, 3, 6, 8 or 9 amino acids.

In one embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 2 amino acids and the second of which consists of 9 amino acids.

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In one embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences both of which consist of 6 amino acids.

15 In one embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 3 amino acids and the second of which consists of 8 amino acids.

In one embodiment, the peptide ligand comprises an amino acid sequence of:

20 C_iALC_{ii}NDWTLPW_{HH}C_{iii} (SEQ ID NO: 1);
C_iREFFDTC_{ii}GLAFIEC_{iii} (SEQ ID NO: 2); and
C_iLEAC_{ii}YDGVY_{WY}SC_{iii} (SEQ ID NO: 3);

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

25 In a further embodiment, the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises N- and/or C-terminal additions and is selected from:

30 A-(SEQ ID NO: 1)-A (herein referred to as BCY12455);
A-(SEQ ID NO: 1)-A-[Sar₆]-[K-FI] (herein referred to as BCY12652);
A-(SEQ ID NO: 2)-A (herein referred to as BCY12452);
A-(SEQ ID NO: 2)-A-[Sar₆]-[K-FI] (herein referred to as BCY12650);
A-(SEQ ID NO: 3)-A (herein referred to as BCY12454); and
A-(SEQ ID NO: 3)-A-[Sar₆]-[K-FI] (herein referred to as BCY12651).

wherein Sar represents sarcosine and FI represents fluorescein.

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For the purpose of this description, inhibitory bicyclic peptides are assumed to be cyclised with TATA

and yielding a tri-substituted structure. However, as will be clear from the descriptions of the invention presented herein, cyclisation may be performed with any suitable molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed. Cyclisation occurs on C_i, C_{ii}, and C_{iii}.

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Non-Inhibitory Peptide Ligands

In one embodiment, the peptide ligand is specific for TfR1 and binds to TfR1 in a manner which does not inhibit/impede the binding of transferrin to TfR1.

10 In a further embodiment, said loop sequences comprise 3 or 7 amino acids.

In one embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 7 amino acids and the second of which consists of 3 amino acids.

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In one embodiment, the peptide ligand comprises an amino acid sequence of:

- C_iSADDWLGC_{ii}ISWC_{iii} (SEQ ID NO: 4);
- C_iSSDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 5);
- C_iPPDAHLGC_{ii}ISWC_{iii} (SEQ ID NO: 6);
- 20 C_iPQDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 7);
- C_iPPDSWQGC_{ii}ISYC_{iii} (SEQ ID NO: 8);
- C_iSPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 9) (herein referred to as BCY15935);
- C_iPGDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 10);
- C_iPPDSHLGC_{ii}ISYC_{iii} (SEQ ID NO: 11);
- 25 C_iSADDWLGC_{ii}ISYC_{iii} (SEQ ID NO: 12);
- C_iP[HyP]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 13);
- C_iP[HyP]DAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 14);
- C_iS[HyP]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 15);
- C_iP[Aib]DAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 16);
- 30 C_iPPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 17);
- C_iP[Aib]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 18);
- C_iSADAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 19);
- C_iS[Aib]DAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 20);
- C_iSPDAHLGC_{ii}[EPA]SYC_{iii} (SEQ ID NO: 21);
- 35 C_iPPDAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 22);
- C_iS[Aib]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 23);
- C_iAPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 24);

- C_iP[Aib]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 25);
 C_iSPDAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 26);
 C_iSPDAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 27);
 C_iPND AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 28);
 5 C_iPID AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 29);
 C_iSPDAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 30);
 C_iPPDAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 31);
 C_iS[Aib]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 32);
 C_iSPDAHLGC_{ii}[Chg]SYC_{iii} (SEQ ID NO: 33);
 10 C_iAPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 34);
 C_iYLPDW[tBuAla]C_{ii}GDEYC_{iii} (SEQ ID NO: 35);
 C_iSPDAHLGC_{ii}S[2Na]C_{iii} (SEQ ID NO: 36);
 C_iSPDAHLGC_{ii}S[3tBuTyr]C_{iii} (SEQ ID NO: 37);
 C_iSPD[Aib]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 38);
 15 C_iSPDAHLGC_{ii}S[1Na]C_{iii} (SEQ ID NO: 39);
 C_iSPDAH[tBuAla]GC_{ii}ISYC_{iii} (SEQ ID NO: 40);
 C_iSPDAH[Cba]GC_{ii}ISYC_{iii} (SEQ ID NO: 41);
 C_iSPDAHLGC_{ii}ISWC_{iii} (SEQ ID NO: 42);
 C_iSPD[Abu]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 43);
 20 C_iS[Aze]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 44);
 C_iSPDDHLGC_{ii}ISYC_{iii} (SEQ ID NO: 45);
 C_iSPDSHLGC_{ii}ISYC_{iii} (SEQ ID NO: 46);
 C_iSPDAH[Abu]GC_{ii}ISYC_{iii} (SEQ ID NO: 47);
 C_iSPDAHLGC_{ii}S[4Pal]C_{iii} (SEQ ID NO: 48);
 25 C_iP[dA]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 49);
 C_iSPDAYLGC_{ii}[tBuAla]SYC_{iii} (SEQ ID NO: 50);
 C_iSPDAHLGC_{ii}[C5g]SYC_{iii} (SEQ ID NO: 51);
 C_iSPDAHLGC_{ii}[Cbg]SYC_{iii} (SEQ ID NO: 52);
 C_iSPDAHL[dA]C_{ii}ISYC_{iii} (SEQ ID NO: 53);
 30 C_iSPDAH[Aib]GC_{ii}ISYC_{iii} (SEQ ID NO: 54);
 C_iSPDAHLGC_{ii}[Cpg]SYC_{iii} (SEQ ID NO: 55);
 C_iSPDAHLGC_{ii}[B-Melle]SYC_{iii} (SEQ ID NO: 56);
 C_iSAD AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 57);
 C_iSPAAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 58);
 35 C_iSPDAALGC_{ii}ISYC_{iii} (SEQ ID NO: 59);
 C_iSPDAHAGC_{ii}ISYC_{iii} (SEQ ID NO: 60);
 C_iSPDAHLAC_{ii}ISYC_{iii} (SEQ ID NO: 61);

- C_iSPDAHLGC_{ii}ASYC_{iii} (SEQ ID NO: 62);
 C_iSPDAHLGC_{ii}IAYC_{iii} (SEQ ID NO: 63);
 C_iSPDAHLGC_{ii}ISAC_{iii} (SEQ ID NO: 64);
 C_i[K(N₃)]PDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 65);
 5 C_iS[K(N₃)]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 66); and
 C_iSPD[K(N₃)]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 67);

wherein Abu represents aminobutyric acid, Aib represents aminoisobutyric acid, Aze represents azetidine, B-Melle represents beta-methyl isoleucine, C5g represents cyclopentyl glycine, Cba represents β-cyclobutylalanine, Cbg represents cyclobutyl glycine, Chg
 10 represents cyclohexyl glycine, Cpg represents cyclopropyl glycine, EPA represents 2-amino-3-ethyl-pentanoic acid, HyP represents trans-4-hydroxy-L-proline, [K(N₃)] represents 6-azido lysine, 1Nal represents 1-naphthylalanine, 2Nal represents 2-naphthylalanine, 4Pal represents 4-pyridylalanine, tBuAla represents t-butyl-alanine, tBuGly represents t-butyl-glycine, 3tBuTyr represents 3-t-Butyl-Tyrosine, and C_i, C_{ii} and C_{iii} represent first, second and
 15 third cysteine residues, respectively, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the peptide ligand comprises an amino acid sequence of:

- C_iSADDWLGC_{ii}ISWC_{iii} (SEQ ID NO: 4);
 C_iSSDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 5);
 20 C_iPPDAHLGC_{ii}ISWC_{iii} (SEQ ID NO: 6);
 C_iPQDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 7);
 C_iPPDSWQGC_{ii}ISYC_{iii} (SEQ ID NO: 8);
 C_iSPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 9);
 C_iPGDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 10);
 25 C_iPPDSHLGC_{ii}ISYC_{iii} (SEQ ID NO: 11); and
 C_iSADDWLGC_{ii}ISYC_{iii} (SEQ ID NO: 12);

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

- 30 In a further embodiment, the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)tris(2-bromoethanone) (TATB) and the peptide ligand comprises N- and/or C-terminal additions and is selected from:

- A-(SEQ ID NO: 4)-A (herein referred to as BCY13983);
 A-(SEQ ID NO: 4)-A-[Sar₆]-[K-FI] (herein referred to as BCY14474);
 35 A-(SEQ ID NO: 5)-A (herein referred to as BCY13986);
 A-(SEQ ID NO: 5)-A-[Sar₆]-[K-FI] (herein referred to as BCY14475);
 A-(SEQ ID NO: 6)-A (herein referred to as BCY15466);

Ac-(SEQ ID NO: 6) (herein referred to as BCY15889);
 A-(SEQ ID NO: 7)-A (herein referred to as BCY15467);
 Ac-(SEQ ID NO: 7) (herein referred to as BCY15890);
 A-(SEQ ID NO: 8)-A (herein referred to as BCY13989);
 5 A-(SEQ ID NO: 8)-A-[Sar₆]-[K-FI] (herein referred to as BCY14476);
 A-(SEQ ID NO: 9)-A (herein referred to as BCY15468);
 A-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15768);
 (SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15934);
 Ac-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15937);
 10 Ac-(SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15938);
 [FI]G[Sar₅]-A-(SEQ ID NO: 9)-A (herein referred to as BCY15940);
 N[1Nal]N-(SEQ ID NO: 9) (herein referred to as BCY18030);
 Ac-(SEQ ID NO: 9)-E[Pip]W (herein referred to as BCY18039);
 Ac-(SEQ ID NO: 9)-EPW (herein referred to as BCY17994);
 15 NWN-(SEQ ID NO: 9) (herein referred to as BCY18029);
 NWN-(SEQ ID NO: 9)-A (herein referred to as BCY17109);
 Ac-(SEQ ID NO: 9)-E[Aze]W (herein referred to as BCY18037);
 Ac-NWN-(SEQ ID NO: 9) (herein referred to as BCY17992);
 Ac-(SEQ ID NO: 9)-E[dP]W (herein referred to as BCY18038);
 20 Ac-N[1Nal]N-(SEQ ID NO: 9) (herein referred to as BCY18034);
 N[dW]N-(SEQ ID NO: 9) (herein referred to as BCY18031);
 Ac-N[dW]N-(SEQ ID NO: 9) (herein referred to as BCY18035);
 HWM-(SEQ ID NO: 9)-A (herein referred to as BCY17110);
 A-(SEQ ID NO: 9)-PHP (herein referred to as BCY17115);
 25 A-(SEQ ID NO: 9)-EPW (herein referred to as BCY17114);
 NEV-(SEQ ID NO: 9)-A (herein referred to as BCY17112);
 A-(SEQ ID NO: 9)-PIVH (herein referred to as BCY17120);
 Ac-(SEQ ID NO: 9) (herein referred to as BCY15891);
 HTS-(SEQ ID NO: 9)-A (herein referred to as BCY17111);
 30 Ac-N[NMeTrp]N-(SEQ ID NO: 9) (herein referred to as BCY18036);
 N[NMeTrp]N-(SEQ ID NO: 9) (herein referred to as BCY18032);
 Ac-A-(SEQ ID NO: 9)-A (herein referred to as BCY15939);
 A-(SEQ ID NO: 9)-EHQE (herein referred to as BCY17119);
 ESF-(SEQ ID NO: 9)-A (herein referred to as BCY17113);
 35 NWN-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17870);
 Ac-NWN-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17871);
 [AzPro]-NWN-(SEQ ID NO: 9) (herein referred to as BCY17872);

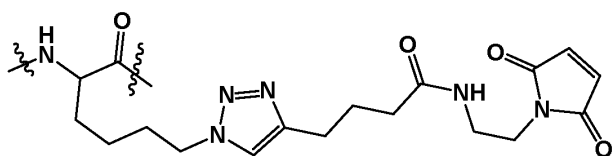
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 [AzPro]-(SEQ ID NO: 9)-EPW (herein referred to as BCY17874);
 Ac-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17868);
 [AzPro]-(SEQ ID NO: 9) (herein referred to as BCY17869);
 5 Ac-N[dY]N-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17882);
 Ac-(SEQ ID NO: 9)-E-[dP]-W-[K(N₃)] (herein referred to as BCY17890);
 Ac-(SEQ ID NO: 9)-E-[Aze]-W-[K(N₃)] (herein referred to as BCY17892);
 Ac-(SEQ ID NO: 9)-E-[Pip]-W-[K(N₃)] (herein referred to as BCY17894);
 Ac-(SEQ ID NO: 9)-[K(N₃)](PYA-maleimide) (herein referred to as BCY17906);
 10 Ac-(SEQ ID NO: 9)-EPW-[Peg₁₀]-[K(N₃)] (herein referred to as BCY19405);
 Ac-(SEQ ID NO: 9)-EPW-[Peg₂₄]-[K(N₃)] (herein referred to as BCY19406);
 Ac-(SEQ ID NO: 9)-EPWGGSGGS-[K(N₃)] (herein referred to as BCY19407);
 A-(SEQ ID NO: 10)-A (herein referred to as BCY15469);
 Ac-(SEQ ID NO: 10) (herein referred to as BCY15892);
 15 A-(SEQ ID NO: 11)-A (herein referred to as BCY15470);
 Ac-(SEQ ID NO: 11) (herein referred to as BCY15893);
 A-(SEQ ID NO: 12)-A (herein referred to as BCY15471);
 Ac-(SEQ ID NO: 12) (herein referred to as BCY15894);
 Ac-(SEQ ID NO: 13) (herein referred to as BCY17991);
 20 Ac-(SEQ ID NO: 13)-EPW (herein referred to as BCY17995);
 Ac-NWN-(SEQ ID NO: 13) (herein referred to as BCY17993);
 NWN-(SEQ ID NO: 13) (herein referred to as BCY18033);
 A-(SEQ ID NO: 13)-A (herein referred to as BCY16754);
 Ac-(SEQ ID NO: 13)-[K(N₃)] (herein referred to as BCY17896);
 25 Ac-NWN-(SEQ ID NO: 13)-[K(N₃)] (herein referred to as BCY17899);
 Ac-(SEQ ID NO: 13)-EPW-[K(N₃)] (herein referred to as BCY17901);
 Ac-(SEQ ID NO: 14) (herein referred to as BCY17990);
 Ac-(SEQ ID NO: 14)-[K(N₃)] (herein referred to as BCY17875);
 [AzPro]-(SEQ ID NO: 14) (herein referred to as BCY17876);
 30 Ac-(SEQ ID NO: 15) (herein referred to as BCY17989);
 A-(SEQ ID NO: 15)-A (herein referred to as BCY16047);
 Ac-(SEQ ID NO: 15)-[K(N₃)] (herein referred to as BCY17877);
 [AzPro]-(SEQ ID NO: 15) (herein referred to as BCY17878);
 A-(SEQ ID NO: 16)-A (herein referred to as BCY16962);
 35 TYMN-(SEQ ID NO: 17)-A (herein referred to as BCY17117);
 A-(SEQ ID NO: 17)-A (herein referred to as BCY16048);
 A-(SEQ ID NO: 18)-A (herein referred to as BCY16963);

Ac-(SEQ ID NO: 19) (herein referred to as BCY17987);
A-(SEQ ID NO: 20)-A (herein referred to as BCY16753);
A-(SEQ ID NO: 21)-A (herein referred to as BCY16046);
A-(SEQ ID NO: 22)-A (herein referred to as BCY16964);
5 A-(SEQ ID NO: 23)-A (herein referred to as BCY16965);
Ac-(SEQ ID NO: 24) (herein referred to as BCY17986);
A-(SEQ ID NO: 25)-A (herein referred to as BCY16550);
A-(SEQ ID NO: 26)-A (herein referred to as BCY16966);
A-(SEQ ID NO: 27)-A (herein referred to as BCY16051);
10 IDSN-(SEQ ID NO: 28)-A (herein referred to as BCY17118);
WGKS-(SEQ ID NO: 29)-A (herein referred to as BCY17116);
A-(SEQ ID NO: 30)-A (herein referred to as BCY16053);
A-(SEQ ID NO: 31)-A (herein referred to as BCY16557);
A-(SEQ ID NO: 32)-A (herein referred to as BCY16035);
15 A-(SEQ ID NO: 33)-A (herein referred to as BCY16043);
A-(SEQ ID NO: 34)-A-[Sar₆]-[K-Fl] (herein referred to as BCY15769);
A-(SEQ ID NO: 35)-A (herein referred to as BCY15648);
A-(SEQ ID NO: 36)-A (herein referred to as BCY16031);
A-(SEQ ID NO: 37)-A (herein referred to as BCY16079);
20 A-(SEQ ID NO: 38)-A (herein referred to as BCY16036);
A-(SEQ ID NO: 39)-A (herein referred to as BCY16029);
A-(SEQ ID NO: 40)-A (herein referred to as BCY16089);
A-(SEQ ID NO: 41)-A (herein referred to as BCY16088);
A-(SEQ ID NO: 42)-A (herein referred to as BCY16052);
25 A-(SEQ ID NO: 43)-A (herein referred to as BCY16033);
A-(SEQ ID NO: 44)-A (herein referred to as BCY16039);
Ac-(SEQ ID NO: 44) (herein referred to as BCY17988);
Ac-(SEQ ID NO: 44)-[K(N₃)] (herein referred to as BCY17879);
[AzPro]-(SEQ ID NO: 44) (herein referred to as BCY17880);
30 A-(SEQ ID NO: 45)-A (herein referred to as BCY16038);
A-(SEQ ID NO: 46)-A (herein referred to as BCY16050);
A-(SEQ ID NO: 47)-A (herein referred to as BCY16034);
A-(SEQ ID NO: 48)-A (herein referred to as BCY16032);
A-(SEQ ID NO: 49)-A (herein referred to as BCY16049);
35 A-(SEQ ID NO: 50)-A (herein referred to as BCY16558);
A-(SEQ ID NO: 51)-A (herein referred to as BCY16041);
A-(SEQ ID NO: 52)-A (herein referred to as BCY16042);

- A-(SEQ ID NO: 53)-A (herein referred to as BCY16045);
 A-(SEQ ID NO: 54)-A (herein referred to as BCY16037);
 A-(SEQ ID NO: 55)-A (herein referred to as BCY16044);
 A-(SEQ ID NO: 56)-A (herein referred to as BCY16040);
 5 A-(SEQ ID NO: 57)-A-[Sar₆]-[K-FI] (herein referred to as BCY15771);
 A-(SEQ ID NO: 58)-A-[Sar₆]-[K-FI] (herein referred to as BCY15772);
 A-(SEQ ID NO: 59)-A-[Sar₆]-[K-FI] (herein referred to as BCY15773);
 A-(SEQ ID NO: 60)-A-[Sar₆]-[K-FI] (herein referred to as BCY15774);
 A-(SEQ ID NO: 61)-A-[Sar₆]-[K-FI] (herein referred to as BCY15775);
 10 A-(SEQ ID NO: 62)-A-[Sar₆]-[K-FI] (herein referred to as BCY15776);
 A-(SEQ ID NO: 63)-A-[Sar₆]-[K-FI] (herein referred to as BCY15777);
 A-(SEQ ID NO: 64)-A-[Sar₆]-[K-FI] (herein referred to as BCY15770);
 Ac-(SEQ ID NO: 65) (herein referred to as BCY17903);
 Ac-(SEQ ID NO: 66) (herein referred to as BCY17904); and
 15 Ac-(SEQ ID NO: 67) (herein referred to as BCY17905);

wherein AzPro represents azidopropyl, Aze represents azetidine, 1Nal represents 1-naphthylalanine, NMeTrp represents N-methyl-tryptophan, [K(N₃)] represents 6-azido lysine, Peg represents polyethylene glycol, Pip represents pipecolic acid, Sar represents sarcosine, FI represents fluorescein and [K(N₃)(PYA-Maleimide)] represents a modified lysine having

20 the following structure:



In a yet further embodiment, the molecular scaffold is TATB and the peptide ligand comprises N- and/or C-terminal additions and is selected from:

- A-(SEQ ID NO: 4)-A (herein referred to as BCY13983);
 25 A-(SEQ ID NO: 4)-A-[Sar₆]-[K-FI] (herein referred to as BCY14474);
 A-(SEQ ID NO: 5)-A (herein referred to as BCY13986);
 A-(SEQ ID NO: 5)-A-[Sar₆]-[K-FI] (herein referred to as BCY14475);
 A-(SEQ ID NO: 6)-A (herein referred to as BCY15466);
 A-(SEQ ID NO: 7)-A (herein referred to as BCY15467);
 30 A-(SEQ ID NO: 8)-A (herein referred to as BCY13989);
 A-(SEQ ID NO: 8)-A-[Sar₆]-[K-FI] (herein referred to as BCY14476);
 A-(SEQ ID NO: 9)-A (herein referred to as BCY15468);
 A-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15768);
 (SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15934);

Ac-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15937);
Ac-(SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15938);
[FI]G[Sar₅]-A-(SEQ ID NO: 9)-A (herein referred to as BCY15940);
A-(SEQ ID NO: 10)-A (herein referred to as BCY15469);
5 A-(SEQ ID NO: 11)-A (herein referred to as BCY15470); and
A-(SEQ ID NO: 12)-A (herein referred to as BCY15471);
wherein Sar represents sarcosine and FI represents fluorescein.

In an alternative embodiment, the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-
10 triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises N- and/or C-terminal
additions and is:

Ac-(SEQ ID NO: 13) (herein referred to as BCY20546).

For the purpose of this description, non-inhibitory bicyclic peptides are assumed to be cyclised
15 with TATA or TATB and yielding a tri-substituted structure. However, as will be clear from the
descriptions of the invention presented herein, cyclisation may be performed with any suitable
molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide
such that at least two polypeptide loops are formed. Cyclisation occurs on C_i, C_{ii}, and C_{iii}.

20 In a further embodiment, the pharmaceutically acceptable salt is selected from the free acid
or the sodium, potassium, calcium or ammonium salt.

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
25 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.

30

Multimeric Bicyclic Peptide Ligands

According to a further aspect of the invention, there is provided a multimeric binding complex
which comprises at least two bicyclic peptide ligands, wherein said peptide ligands may be
the same or different, each of which comprises a peptide ligand specific for transferrin receptor
35 1 (TfR1) comprising 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.

Thus, in this aspect of the invention the multimeric binding complex comprises at least two
5 (i.e. 2, 3 or 4) of any of the monomeric bicyclic peptide ligands as defined herein.

This aspect of the invention describes a series of multimerized bicyclic peptides with various chemical linkers and hinges of various lengths and rigidity using different sites of attachments within said bicyclic peptide which bind and activate TfR1 with a wide range of potency and
10 efficacy.

It will be appreciated by the skilled person that this aspect of the invention presents multiply arranged (multimeric) bicyclic peptides which provide a synergistic benefit by virtue of the resultant properties of said multimeric binding complexes compared to the corresponding
15 monomeric binding complexes which contain a single bicyclic peptide. For example, the multimeric binding complexes of this aspect of the invention typically have greater levels of binding potency or avidity (as measured herein by K_d values) than their monomeric counterparts. Furthermore, the multimeric binding complexes of the invention are designed to be sufficiently small enough to be cleared by the kidneys.

Without being bound by theory it is believed that multimerized bicyclic peptides are able to activate receptors by homo-crosslinking more than one of the same receptor. Thus, in one embodiment, said bicyclic peptide ligands are specific for the same target within TfR1. In a further embodiment, the multimeric binding complex comprises at least two identical bicyclic
20 peptide ligands. By "identical" it is meant bicyclic peptides having the same amino acid sequence, most critically the same amino acid sequence refers to the binding portion of said bicyclic peptide (for example, the sequence may vary in attachment position). In this embodiment, each of the bicyclic peptides within the multimeric binding complex will bind exactly the same epitope upon the same target of TfR1— the resultant target bound complex
25 will therefore create a homodimer (if the multimeric complex comprises two identical bicyclic peptides), homotrimer (if the multimeric complex comprises three identical bicyclic peptides) or homotetramer (if the multimeric complex comprises four identical bicyclic peptides), etc.

In an alternative embodiment, the multimeric binding complex comprises at least two differing
35 bicyclic peptide ligands. By "differing" it is meant bicyclic peptides having a different amino acid sequence. In this embodiment, the differing bicyclic peptide ligands within the multimeric binding complex will bind to different epitopes on TfR1 - the resultant target bound complex

will therefore create a biparatopic (if the multimeric complex comprises two differing bicyclic peptides), triparatopic (if the multimeric complex comprises three differing bicyclic peptides) or tetraparatopic (if the multimeric complex comprises four differing bicyclic peptides), etc.

- 5 Without being bound by theory it is believed that multimerized bicyclic peptides are able to activate receptors by hetero-crosslinking differing targets, such as differing target sites on TfR1. Thus, in one embodiment, said bicyclic peptide ligands are specific for different targets on TfR1. It will be appreciated that in this embodiment, the multimeric binding complex comprises at least two differing bicyclic peptide ligands (i.e. bicyclic peptide ligands having
- 10 differing amino acid sequences). In this embodiment, each of the bicyclic peptides within the multimeric binding complex will bind a differing epitope upon TfR1— the resultant target bound complex will therefore create a bispecific multimeric binding complex (if the multimeric complex comprises two differing bicyclic peptides), trispecific multimeric binding complex (if the multimeric complex comprises three differing bicyclic peptides), tetraspecific multimeric
- 15 binding complex (if the multimeric complex comprises four differing bicyclic peptides), etc.

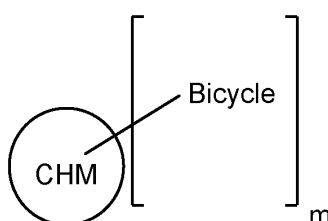
It will be appreciated that the multimeric binding complexes of the invention may be designed to be capable of binding to a range of different targets on TfR1.

- 20 The bicyclic peptides within the multimeric binding complexes of the invention may be assembled via a number of differing options. For example, there may be a central hinge or branching moiety with spacer or arm elements radiating from said hinge or branch point each of which will contain a bicyclic peptide. Alternatively, it could be envisaged that a circular support member may hold a number of inwardly or outwardly projecting bicyclic peptides.

25

In one embodiment, each bicyclic peptide ligand is connected to a central hinge moiety by a spacer group.

- It will be appreciated that the spacer group may be linear and connect a single bicyclic peptide
- 30 with the central hinge moiety. Thus, in one embodiment, the multimeric binding complex comprises a compound of formula (I):



(I)

wherein CHM represents a central hinge moiety;

Bicycle represents a bicyclic peptide ligand as defined herein; and

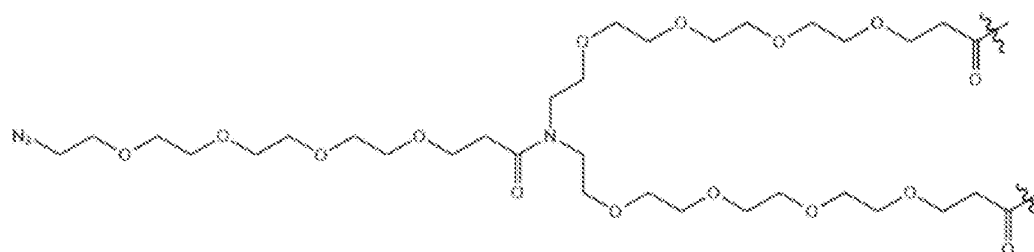
m represents an integer selected from 2 to 10.

5

In one embodiment, m represents an integer selected from 2, 3 or 4.

In a further embodiment, m represents 2.

- 10 When m represents 2, it will be appreciated that the central hinge moiety will require 2 points of attachment. Thus, in one embodiment, m represents 2 and CHM is a motif of formula (A):



(A).

15 Dimers

In one embodiment, the multimeric binding complex comprises two identical bicyclic peptides and comprises a dimeric binding complex described in the following Table A:

Table A: Exemplified Dimeric Binding Complexes of the Invention

Multimer Compound Number	Corresponding Monomer	Number of Monomers	Central Hinge Moiety	Attachment Point
BCY19409	BCY17994	2	A	C-terminus

20

Numbering

When referring to amino acid residue positions within the peptides 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 the peptides of the invention is referred to as below:

25

$-C_i-A_1-L_2-C_{ii}-N_3-D_4-W_5-T_6-L_7-P_8-W_9-H_{10}-H_{11}-C_{iii}-$ (SEQ ID NO: 1).

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 biotin-G-Sar₅ tail would be denoted as:

5 [Biot]-G-[Sar₅]-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.

10 For example, the sequence is reversed (i.e. N-terminus become C-terminus and *vice versa*) and their stereochemistry is likewise also reversed (i.e. D-amino acids become L-amino acids and *vice versa*).

Peptide Ligand Definition

15 A peptide ligand, as referred to herein, refers to a peptide, peptidic or peptidomimetic covalently bound to a molecular scaffold. Typically, such peptides, peptidics or peptidomimetics comprise a peptide having natural or non-natural amino acids, 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
20 loop sequence, since it forms a loop when the peptide, peptidic or peptidomimetic is bound to the scaffold. In the present case, the peptides, peptidics or peptidomimetics comprise at least three cysteine residues (referred to herein as C_i, C_{ii} and C_{iii}), and form at least two loops on the scaffold.

Advantages of the Peptide Ligands

Certain bicyclic peptides 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
30 and pharmacokinetic evaluation;
- Protease stability. Bicyclic peptide ligands should in most circumstances 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 bicyclic peptide lead
35 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; and

- An optimal plasma half-life in the circulation. Depending upon the clinical indication and treatment regimen, it may be required to develop a bicyclic peptide with short or prolonged
- 5 *in vivo* exposure times for the management of either chronic or acute disease states. The optimal exposure time will be governed by the requirement for sustained exposure (for maximal therapeutic efficiency) versus the requirement for short exposure times to minimise toxicological effects arising from sustained exposure to the agent.

10 ***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

15 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.

20

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,

25 butanoic, (+) camphoric, camphor-sulfonic, (+)-(1S)-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,

30 ($\pm$)-DL-lactic), lactobionic, maleic, malic, (-)-L-malic, malonic, ($\pm$)-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

35 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.

- 5 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
- 10 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^{2+} . 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,
- 15 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 $\text{N}(\text{CH}_3)_4^+$.

- Where the peptides of the invention contain an amine function, these may form quaternary
- 20 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 peptides of the invention.

Modified Derivatives

- 25 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
- 30 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 one or more replacement amino acids, such as an alanine, replacement of one or more L-
- 35 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
5 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-
10 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.

15 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 residue 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
20 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.
25

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 residue is synthesized as an amide during peptide synthesis leading to a molecule which is C-terminally amidated. This embodiment provides the
30 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
35 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 α -disubstituted derivatives (for example, aminoisobutyric acid, Aib), and cyclo amino acids, a
5 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}).

10

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
15 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
20 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
25 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).

30

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 β -turn conformations (Tugyi *et al.* (2005) PNAS, 102(2), 413–418).

35

In one embodiment, the modified derivative comprises removal of any amino acid residues and substitution with alanines, such as D-alanines. This embodiment provides the advantage

of identifying key binding residues and 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
5 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).

Isotopic Variations

The present invention includes all pharmaceutically acceptable (radio)isotope-labelled 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,
25 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 ^2H (D) and ^3H (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 , sulphur, such as 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 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.* ^3H (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.
- 5
- 10 Substitution with heavier isotopes such as deuterium, *i.e.* ^2H (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.
- 15 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-labelled 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-labelled reagent in place of the non-labelled reagent previously employed.

20

Molecular Scaffold

In one embodiment, the molecular scaffold comprises a non-aromatic molecular scaffold. References herein to "non-aromatic molecular scaffold" refers to any molecular scaffold as defined herein which does not contain an aromatic (*i.e.* unsaturated) carbocyclic or heterocyclic ring system.

25

Suitable examples of non-aromatic molecular scaffolds are described in Heinis *et al.* (2014) *Angewandte Chemie, International Edition* 53(6) 1602-1606.

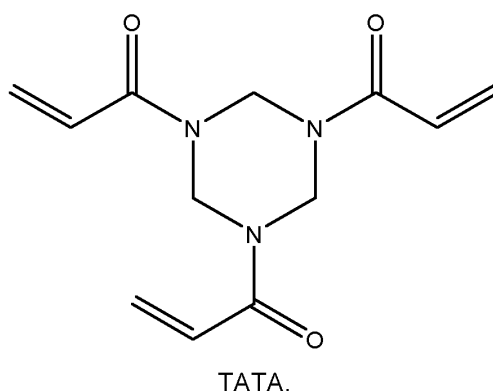
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As noted in the foregoing documents, the molecular scaffold may be a small molecule, such as a small organic molecule.

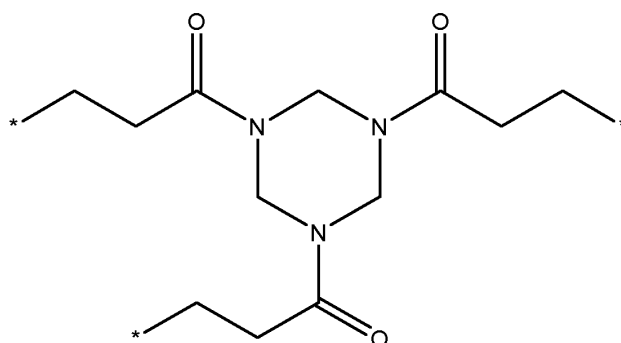
35 In one embodiment the molecular scaffold may be a macromolecule. In one embodiment the molecular scaffold is a macromolecule composed of amino acids, nucleotides or carbohydrates.

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.

- 5 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.
- 10 In one embodiment, the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (also known as triacryloylhexahydro-s-triazine (TATA):



- 15 Thus, following cyclisation with the bicyclic peptides of the invention on the C_i, C_{ii}, and C_{iii} cysteine residues, the molecular scaffold forms a tri-substituted 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)tripropan-1-one derivative of TATA having the following structure:

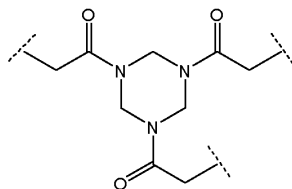


wherein * denotes the point of attachment of the three cysteine residues.

20

In an alternative embodiment, the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl) tris(2-bromoethanone) (TATB).

Thus, following cyclisation with the bicyclic peptides of the invention on the C_i, C_{ii}, and C_{iii} cysteine residues, the molecular scaffold forms a tri-substituted derivative of TATB having the following structure:



TATB.

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 the 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
5 disulphide bonds. This has the additional advantage of allowing the first and second peptide
to dissociate from each other once within the reducing environment of the cell. In this case,
the molecular scaffold (e.g. TATA or TATB) 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
10 reacted with a free cysteine or thiol of the second peptide, forming a disulphide-linked bicyclic
peptide-peptide conjugate.

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
15 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
20 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
25 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
30 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
35 (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 cyclosporine, methotrexate, adriamycin or cisplatin and immunotoxins. Further examples of other agents which may
5 be administered separately or in conjunction with the peptide ligands of the invention include cytokines, lymphokines, other hematopoietic factors, thrombolytic and anti-thrombotic factors. 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
10 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
15 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 intravenously. The dosage and
20 frequency of administration will depend on the age, sex and condition of the patient, concurrent administration of other drugs, contraindications 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
25 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.

30 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
35 depend upon the severity of the disease, 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.

5 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
10 the undesired cells are killed or otherwise removed from the blood for return to the mammal in accordance with standard techniques.

Therapeutic Uses

The bicyclic peptides of the invention have specific utility as transferrin receptor 1 (TfR1)
15 binding agents. According to a further aspect of the invention, there is provided a peptide ligand or pharmaceutical composition as defined herein for use in preventing, suppressing or treating a disease or disorder through TfR1 mediated delivery of a therapeutic agent.

Transferrins are glycoproteins found in vertebrates which bind to and consequently mediate
20 the transport of Iron (Fe) through blood plasma. It is produced in the liver and contains binding sites for two Fe³⁺ atoms. Human transferrin is encoded by the *TF* gene and produced as a 76 kDa glycoprotein.

Transferrin glycoproteins bind iron tightly, but reversibly. Although iron bound to transferrin is
25 less than 0.1% (4 mg) of total body iron, it forms the most vital iron pool with the highest rate of turnover (25 mg/24 h). Transferrin has a molecular weight of around 80 kDa and contains two specific high-affinity Fe(III) binding sites. The affinity of transferrin for Fe(III) is extremely high (association constant is 10²⁰ M⁻¹ at pH 7.4) but decreases progressively with decreasing pH below neutrality. Transferrins are not limited to only binding to iron but also to
30 different metal ions. These glycoproteins are located in various bodily fluids of vertebrates. When not bound to iron, transferrin is known as "apotransferrin".

In one embodiment, the transferrin is mammalian transferrin. In a further embodiment, the mammalian transferrin is human transferrin. In one embodiment, the human transferrin is
35 human transferrin receptor 1 (TfR1; also known as CD71).

It will be appreciated that TfR1 binding peptides may be useful in the treatment of neurological disorders. Examples of such neurological disorders include but are not limited to:

a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease,
5 ischemia, a behavioural disorder, and CNS inflammation.

In one embodiment, the neurological disorder is in a human subject. It will be appreciated that the dose amount and/or frequency of administration is modulated to reduce the concentration of peptide ligand to which the red blood cells are exposed. In a further embodiment, the
10 treatment further comprises the step of monitoring the human subject for depletion of red blood cells.

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
15 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
20 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.

Transferrin receptor 1 (TfR1) is an extensively studied model receptor-ligand system and has
25 provided considerable insight into the cellular properties and mechanisms of nutrient/scavenger receptor cargo internalization and endocytic sorting (Qian *et al* (2002) Pharmacological Reviews 54(4), 561-587). TfR1 is known to undergo constitutive endocytosis and recycling to the plasma membrane and possesses pH-dependent ligand binding to enable proper sorting of endocytosed cargo. Anti-TfR1 antibodies have previously been believed to
30 be the primary agents for TfR1 targeting of oligonucleotide therapeutics, however, the present TfR1 binding peptide ligands of the invention have the potential for demonstrating efficient and profound knockdown of gene expression in skeletal and cardiac muscle via systemically delivered TfR1-Bicyclic Peptide-siRNA conjugates.

Thus, in light of this mechanism it is believed that the peptide ligands of the invention may find
35 utility as tissue delivery complexes, such as delivery of the TfR1-peptide ligand-payload (i.e. siRNA) complex to tissue cells, in particular muscle cells.

Thus, according to a further aspect of the invention there is provided a tissue delivery complex which comprises a peptide ligand of the invention bound to TfR1 in combination with a payload, such as another peptide, small molecule drug or oligonucleotide, in particular siRNA.

5

Said tissue delivery complexes therefore find utility in the treatment of musculoskeletal disorders. Examples of suitable musculoskeletal disorders include, but are not limited, to:

12q14 microdeletion syndrome

2q37 deletion syndrome

10 3M syndrome

Absence of Tibia

Absence of tibia with polydactyly

Absent patella

Acheiropody

15 Achondrogenesis type 1A - See Achondrogenesis

Achondrogenesis type 1B - See Achondrogenesis

Achondrogenesis type 2 - See Achondrogenesis

Achondroplasia

Acro-pectoro-renal field defect

20 Acrocallosal syndrome, Schinzel type

Acrocapitofemoral dysplasia

Acrocephalopolydactyly

Acrodysostosis

Acrodysplasia scoliosis

25 Acrofacial dysostosis Catania type

Acrofacial dysostosis Palagonia type

Acrofacial dysostosis Rodriguez type

Acrofrontofacionasal dysostosis syndrome

Acromelic frontonasal dysostosis

30 Acromesomelic dysplasia

Acromesomelic dysplasia Hunter Thompson type

Acromesomelic dysplasia Maroteaux type

Acromicric dysplasia

Acroosteolysis dominant type

35 Acropectoral syndrome

Acropectorovertebral dysplasia F form

Acute febrile neutrophilic dermatosis

- Adactylia unilateral
- Adams-Oliver syndrome
- Adenosine Deaminase 2 deficiency
- ADULT syndrome
- 5 Adult-onset Still's disease
- Aicardi-Goutieres syndrome
- Al Gazali Sabrinathan Nair syndrome
- Allain-Babin-Demarquez syndrome
- Alpha-mannosidosis
- 10 Amyotrophy, neurogenic scapulooperoneal, New England type
- Anauxetic dysplasia
- Angel shaped phalangoepiphyseal dysplasia
- Ankyloblepharon-ectodermal defects-cleft lip/palate syndrome
- Ankylosing spondylitis - Not a rare disease
- 15 Ankylosing vertebral hyperostosis with tylosis
- Anonychia-onychodystrophy with hypoplasia or absence of distal phalanges
- Antley Bixler syndrome
- Apert syndrome
- Arthrogryposis multiplex congenita
- 20 Arts syndrome
- Aspartylglycosaminuria
- Atelosteogenesis type 1
- Atelosteogenesis type 2
- Atelosteogenesis type 3
- 25 Auralcephalosyndactyly
- Auriculo-condylar syndrome
- Auriculoosteodysplasia
- Autosomal dominant spondyloepiphyseal dysplasia tarda
- Autosomal recessive early-onset inflammatory bowel disease
- 30 Autosomal recessive protein C deficiency
- Axial osteomalacia
- Axial spondylometaphyseal dysplasia
- Baby rattle pelvic dysplasia
- Baller-Gerold syndrome
- 35 Banki syndrome
- Beare-Stevenson cutis gyrata syndrome
- Behçet disease

- Benallegue Lacete syndrome
- Bethlem myopathy
- Beukes familial hip dysplasia
- Blau syndrome
- 5 Blount disease
- BOD syndrome
- Bone dysplasia Azouz type
- Bone dysplasia lethal Holmgren type
- Boomerang dysplasia
- 10 Bowing of legs, anterior with dwarfism
- Brachycephalofrontonasal dysplasia
- Brachydactylous dwarfism Mseleni type
- Brachydactyly elbow wrist dysplasia
- Brachydactyly long thumb type
- 15 Brachydactyly Mononen type
- Brachydactyly type A1
- Brachydactyly type A2
- Brachydactyly type A4
- Brachydactyly type A5
- 20 Brachydactyly type A6
- Brachydactyly type A7
- Brachydactyly type B
- Brachydactyly type C
- Brachydactyly type E
- 25 Brachydactyly types B and E combined
- Brachyolmia type 3
- Branchial arch syndrome X-linked
- Brody myopathy
- Bruck syndrome 1
- 30 Buschke-Ollendorff syndrome
- C syndrome
- Caffey disease
- Campomelia Cumming type
- Campomelic dysplasia
- 35 Camptobrachydactyly
- Camptodactyly arthropathy coxa vara pericarditis syndrome
- Camptodactyly syndrome Guadalajara type 2

- Camptodactyly, tall stature, and hearing loss syndrome
- Camurati-Engelmann disease
- Cantu syndrome
- Carpenter syndrome
- 5 Carpotarsal osteochondromatosis
- Cartilage-hair hypoplasia
- Catel Manzke syndrome
- Cerebellar hypoplasia with endosteal sclerosis
- Cerebro-costo-mandibular syndrome
- 10 Cervical dystonia
- Charlie M syndrome
- Cherubism
- CHILD syndrome
- Childhood hypophosphatasia
- 15 Chondrocalcinosis 2
- Chondrodysplasia Blomstrand type
- Chondrodysplasia punctata 1, X-linked recessive
- Chondrodysplasia punctata Sheffield type
- Chondrodysplasia with joint dislocations, GPAPP type
- 20 Chondrodysplasia, Grebe type
- Chondrosarcoma
- Chordoma
- Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
- Chronic recurrent multifocal osteomyelitis
- 25 Cleft hand absent tibia
- Cleidocranial dysplasia
- Cleidocranial dysplasia recessive form
- Cleidorhizomelic syndrome
- CLOVES syndrome
- 30 Coccygodynia
- CODAS syndrome
- Coffin-Siris syndrome
- COG1-CDG (CDG-IIg)
- Cole Carpenter syndrome
- 35 Collagenopathy type 2 alpha 1
- Condensing osteitis of the clavicle
- Congenital adrenal hyperplasia due to cytochrome P450 oxidoreductase deficiency

- Congenital contractural arachnodactyly
- Congenital femoral deficiency
- Congenital primary aphakia
- Congenital radioulnar synostosis
- 5 Cornelia de Lange syndrome
 - Cousin syndrome
 - Craniodiaphyseal dysplasia
 - Cranioectodermal dysplasia
 - Craniofacial dysostosis with diaphyseal hyperplasia
- 10 Craniofacial dyssynostosis
 - Craniofrontonasal dysplasia
 - Cranio metaphyseal dysplasia, autosomal dominant
 - Cranio metaphyseal dysplasia, autosomal recessive type
 - Craniosynostosis, anal anomalies, and porokeratosis
- 15 Craniotelencephalic dysplasia
 - Crouzon syndrome
 - Culler-Jones syndrome
 - Currarino triad
 - Curry Jones syndrome
- 20 Czech dysplasia metatarsal type
 - Dandy-Walker malformation with postaxial polydactyly
 - Dandy-Walker malformation with sagittal craniosynostosis and hydrocephalus
 - Deficiency of interleukin-1 receptor antagonist
 - Delayed membranous cranial ossification
- 25 Dentatorubral-pallidoluysian atrophy
 - Desbuquois syndrome
 - Desmosterolosis
 - Diaphyseal medullary stenosis with malignant fibrous histiocytoma
 - Diastrophic dysplasia
- 30 Dihydropyrimidine dehydrogenase deficiency - Not a rare disease
 - Dyggve-Melchior-Clausen syndrome
 - Dyschondrosteosis nephritis
 - Dysferlinopathy
 - Dysosteosclerosis
- 35 Dysplasia epiphysealis hemimelica
 - Dyssegmental dysplasia Rolland-Desbuquois type
 - Dyssegmental dysplasia Silverman-Handmaker type

- DYT-GNAL
EEC syndrome
EEM syndrome
Ellis-Van Creveld syndrome
- 5 Enthesitis-related juvenile idiopathic arthritis
Epidermolysa bullosa simplex with muscular dystrophy
Epiphyseal dysplasia multiple with early-onset diabetes mellitus
Erdheim-Chester disease
Ewing sarcoma
- 10 Familial avascular necrosis of the femoral head
Familial cold autoinflammatory syndrome
Familial hypocalciuric hypercalcemia type 1
Familial hypocalciuric hypercalcemia type 2
Familial hypocalciuric hypercalcemia type 3
- 15 Familial Mediterranean fever
Familial osteochondritis dissecans
Familial tumoral calcinosis
Fanconi anemia
Feingold syndrome
- 20 Felty's syndrome
Femoral facial syndrome
Femur bifid with monodactylous ectrodactyly
Femur fibula ulna syndrome
Fetal thalidomide syndrome
- 25 Fibrochondrogenesis
Fibrodysplasia ossificans progressiva
Fibular aplasia ectrodactyly
Fibular aplasia, tibial campomelia, and oligosyndactyly syndrome
Fibular hemimelia
- 30 Fibular hypoplasia and complex brachydactyly
Filippi syndrome
Fitzsimmons-Guilbert syndrome
Focal segmental glomerulosclerosis
Frank Ter Haar syndrome
- 35 Freiberg's disease
Frontofacionasal dysplasia
Frontometaphyseal dysplasia

- Frontonasal dysplasia
Frontonasal dysplasia with alopecia and genital anomaly - See Frontonasal dysplasia
Frontonasal dysplasia-severe microphthalmia-severe facial clefting syndrome - See Frontonasal dysplasia
- 5 Frontorhiny - See Frontonasal dysplasia
Fryns Hofkens Fabry syndrome
Fucosidosis
Fuhrmann syndrome
Galactosialidosis
- 10 Gaucher disease type 1
Gaucher disease type 3
Geleophysic dwarfism
Genitopatellar syndrome
Genoa syndrome
- 15 Genochondromatosis
Geroderma osteodysplastica
Ghosal hematodiaphyseal dysplasia syndrome
Giant cell tumor of bone
GM1 gangliosidosis type 1
- 20 GM1 gangliosidosis type 2
GM1 gangliosidosis type 3
Goldenhar disease
Gorham's disease
Gracile bone dysplasia
- 25 Grant syndrome
Greenberg dysplasia
Greig cephalopolysyndactyly syndrome
Gurrieri syndrome
Hallermann-Streiff syndrome
- 30 Hand foot uterus syndrome
Hanhart syndrome
Heart-hand syndrome, Slovenian type
Heart-hand syndrome, Spanish type
Hemifacial microsomia
- 35 Hemifacial myohyperplasia
Hereditary antithrombin deficiency
Hereditary multiple osteochondromas

- Holt-Oram syndrome
- Hunter-McAlpine syndrome
- Hurler syndrome
- Hurler–Scheie syndrome
- 5 Hyaline fibromatosis syndrome
- Hyper-IgD syndrome
- Hyperostosis corticalis generalisata
- Hyperphosphatemic familial tumoral calcinosis
- Hypochondroplasia
- 10 Hypophosphatasia
- Hypophosphatemic rickets
- I cell disease
- IMAGe syndrome
- Imperforate oropharynx-costo vertebral anomalies
- 15 Inclusion body myopathy 3
- Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia
- Inclusion body myositis
- Intellectual disability-spasticity-ectrodactyly syndrome
- Iridogoniodysgenesis type 1
- 20 IVIC syndrome
- Jackson-Weiss syndrome
- Jansen type metaphyseal chondrodysplasia
- Jeune syndrome
- Johnson Munson syndrome
- 25 Juvenile dermatomyositis
- Juvenile osteoporosis
- Juvenile Paget disease
- Kaplan Plauchu Fitch syndrome
- Kenny-Caffey syndrome type 1
- 30 Kenny-Caffey syndrome type 2
- Keutel syndrome
- Kienbock's disease
- Kleiner Holmes syndrome
- Klippel Feil syndrome
- 35 Klippel-Trenaunay syndrome
- Kniest dysplasia
- Kniest like dysplasia lethal

- Kohler disease
- Kyphomelic dysplasia
- Lacrimo-auriculo-dento-digital syndrome
- Lambdoid synostosis
- 5 Lambert Eaton myasthenic syndrome
- Langer mesomelic dysplasia
- Larsen syndrome
- Lateral meningocele syndrome
- Laurin-Sandrow syndrome
- 10 Legg-Calve-Perthes disease
- Lenz Majewski hyperostotic dwarfism
- Leri pleonosteosis
- Leri Weill dyschondrosteosis
- Lethal chondrodysplasia Moerman type
- 15 Lethal chondrodysplasia Seller type
- Levator syndrome
- Limb-girdle muscular dystrophy type 1A
- Limb-girdle muscular dystrophy type 2A
- Limb-girdle muscular dystrophy type 2B
- 20 Limb-girdle muscular dystrophy type 2E
- Limb-girdle muscular dystrophy type 2F
- Limb-girdle muscular dystrophy type 2H
- Limb-girdle muscular dystrophy, type 2C
- Limb-girdle muscular dystrophy, type 2D
- 25 Limb-mammary syndrome
- Loeys-Dietz syndrome
- Lowry Maclean syndrome
- Lowry Wood syndrome
- Macrophagic myofasciitis
- 30 Maffucci syndrome
- MAGIC syndrome
- Majeed syndrome
- Mandibuloacral dysplasia with type A lipodystrophy
- Mandibuloacral dysplasia with type B lipodystrophy
- 35 Mandibulofacial dysostosis with microcephaly
- Mannosidosis, beta A, lysosomal
- Marshall syndrome

- Marshall-Smith syndrome
- McCune-Albright syndrome
- Meckel syndrome
- Median cleft of upper lip with polyps of facial skin and nasal mucosa
- 5 Meier-Gorlin syndrome
- Melnick-Needles syndrome
- Melorheostosis
- Melorheostosis with osteopoikilosis
- Mesomelia-synostoses syndrome
- 10 Mesomelic dwarfism cleft palate camptodactyly
- Mesomelic dysplasia Kantaputra type
- Mesomelic dysplasia Savarirayan type
- Metacarpals 4 and 5 fusion
- Metachondromatosis
- 15 Metaphyseal acroscyphodysplasia
- Metaphyseal chondrodysplasia Schmid type
- Metaphyseal chondrodysplasia Spahr type
- Metaphyseal dysostosis-intellectual disability-conductive deafness syndrome
- Metaphyseal dysplasia maxillary hypoplasia brachydactyly
- 20 Metaphyseal dysplasia without hypotrichosis
- Metatropic dysplasia
- Mevalonic aciduria
- Microcephalic osteodysplastic primordial dwarfism type 1
- Microcephalic osteodysplastic primordial dwarfism type 2
- 25 Microcephalic primordial dwarfism Toriello type
- Microsomia hemifacial radial defects
- Miller syndrome
- Minicore myopathy with external ophthalmoplegia
- Monomelic amyotrophy
- 30 Muckle-Wells syndrome
- Mucopolidosis III alpha/beta
- Mucopolidosis type 4
- Mucopolysaccharidosis type III
- Mucopolysaccharidosis type IIIA
- 35 Mucopolysaccharidosis type IIIB
- Mucopolysaccharidosis type IIIC
- Mucopolysaccharidosis type IIID

- Mucopolysaccharidosis type IV
- Mucopolysaccharidosis type IVA
- Mucopolysaccharidosis type VII
- Muenke Syndrome
- 5 Multicentric carpotarsal osteolysis syndrome
- Multiple epiphyseal dysplasia
- Multiple epiphyseal dysplasia 2
- Multiple sulfatase deficiency
- Multiple synostoses syndrome 1
- 10 Multiple system atrophy
- Muscular dystrophy
- Muscular dystrophy, congenital, megaconial type
- MYH7-related scapuloperoneal myopathy
- Myhre syndrome
- 15 Myosinopathies
- Myostatin-related muscle hypertrophy
- Myotonic dystrophy
- Myotonic dystrophy type 2
- Nager acrofacial dysostosis
- 20 Nail-patella syndrome
- Nakajo Nishimura syndrome
- Neonatal Onset Multisystem Inflammatory disease
- Neonatal severe hyperparathyroidism
- Nestor-guillermo progeria syndrome
- 25 Neurofibromatosis type 1
- Nievergelt syndrome
- Normophosphatemic familial tumoral calcinosis
- Occipital horn syndrome
- Oculoauriculofrontonasal syndrome
- 30 Oculodentodigital dysplasia
- Oculomaxillofacial dysostosis
- Oculopharyngeal muscular dystrophy
- Oliver syndrome
- Ollier disease
- 35 Omodysplasia 1
- Omodysplasia 2
- Opsismodysplasia

- Orofaciodigital syndrome 1
- Orofaciodigital syndrome 10
- Orofaciodigital syndrome 11
- Orofaciodigital syndrome 2
- 5 Orofaciodigital syndrome 3
- Orofaciodigital syndrome 4
- Orofaciodigital syndrome 5
- Orofaciodigital syndrome 6
- Orofaciodigital syndrome 8
- 10 Orofaciodigital syndrome 9
- Oslam syndrome
- OSMED Syndrome
- Ossification of the posterior longitudinal ligament of the spine - Not a rare disease
- Osteoarthropathy of fingers familial
- 15 Osteochondritis dissecans
- Osteodysplasia familial Anderson type
- Osteodysplasty precocious of Danks Mayne and Kozlowski
- Osteofibrous dysplasia
- Osteogenesis imperfecta type I
- 20 Osteogenesis imperfecta type II
- Osteogenesis imperfecta type III
- Osteogenesis imperfecta type IV
- Osteogenesis imperfecta type V
- Osteogenesis imperfecta type VI
- 25 Osteoglophonic dysplasia
- Osteomesopyknosis
- Osteopathia striata with cranial sclerosis
- Osteopenia and sparse hair
- Osteopetrosis autosomal dominant type 1
- 30 Osteopetrosis autosomal dominant type 2
- Osteopetrosis autosomal recessive 3
- Osteopetrosis autosomal recessive 4
- Osteopetrosis autosomal recessive 7
- Osteopoikilosis and dacryocystitis
- 35 Osteoporosis oculocutaneous hypopigmentation syndrome
- Osteoporosis-pseudoglioma syndrome
- Osteosarcoma

- Oto-palato-digital syndrome type 1
- Oto-palato-digital syndrome type 2
- Pachydermoperiostosis
- Pacman dysplasia
- 5 Pallister-Hall syndrome
- Paramyotonia congenita
- Parastremmatic dwarfism
- PARC syndrome
- Parkes Weber syndrome
- 10 Patterson-Stevenson-Fontaine syndrome
- Pelvic dysplasia arthrogryposis of lower limbs
- Periodic fever, aphthous stomatitis, pharyngitis and adenitis
- Pfeiffer-type cardiocranial syndrome
- Phocomelia ectrodactyly deafness sinus arrhythmia
- 15 Pigmented villonodular synovitis
- Piriformis syndrome
- Platyspondylic lethal skeletal dysplasia Torrance type
- Pleconial myopathy with salt craving
- Poland syndrome
- 20 Polycystic bone disease
- Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy
- Polydactyly myopia syndrome
- Polyostotic osteolytic dysplasia, hereditary expansile
- Potassium aggravated myotonia
- 25 Preaxial deficiency, postaxial polydactyly and hypospadias
- Preaxial polydactyly type 1
- Preaxial polydactyly type 2
- Preaxial polydactyly type 3
- Preaxial polydactyly type 4
- 30 Progeria
- Progressive osseous heteroplasia
- Progressive pseudorheumatoid dysplasia
- Protein C deficiency - Not a rare disease
- Proteus syndrome
- 35 Proximal symphalangism
- Pseudoachondroplasia
- Pseudoaminopterin syndrome

- Pseudodiastrophic dysplasia
- Pseudohypoparathyroidism type 1A
- Pseudohypoparathyroidism type 1C
- Pseudopseudohypoparathyroidism
- 5 Psoriatic juvenile idiopathic arthritis
- Pycnodysostosis
- Pyknoachondrogenesis
- Pyle disease
- Pyoderma gangrenosum
- 10 Pyogenic arthritis, pyoderma gangrenosum and acne
- Radio-ulnar synostosis type 1 - See Congenital radioulnar synostosis
- Radio-ulnar synostosis type 2 - See Congenital radioulnar synostosis
- Radioulnar synostosis-microcephaly-scoliosis syndrome
- Raine syndrome
- 15 Ramon Syndrome
- Rapadilino syndrome
- Reactive arthritis
- Renal dysplasia, retinal pigmentary dystrophy, cerebellar ataxia and skeletal dysplasia
- Retinal vasculopathy with cerebral leukodystrophy with systemic manifestations
- 20 Rhizomelic chondrodysplasia punctata type 1
- Rhizomelic dysplasia Patterson Lowry type
- Rhizomelic syndrome
- Richieri Costa Da Silva syndrome
- Rigid spine syndrome
- 25 Roberts syndrome
- Saethre-Chotzen syndrome
- Salla disease - See Free sialic acid storage disease
- SAPHO syndrome
- Sarcoidosis - Not a rare disease
- 30 Say Meyer syndrome
- Say-Field-Coldwell syndrome
- Scalp defects postaxial polydactyly
- SCARF syndrome
- Scheie syndrome
- 35 Scheuermann disease
- Schimke immunoosseous dysplasia
- Schinzel Giedion syndrome

- Schinzel type phocomelia
- Schneckenbecken dysplasia
- Schnitzler syndrome
- Schwartz Jampel syndrome
- 5 Sclerosteosis
- Seckel syndrome
- Sepiapterin reductase deficiency
- Short rib-polydactyly syndrome type 3
- Short rib-polydactyly syndrome type 1
- 10 Short rib-polydactyly syndrome type 4
- Short rib-polydactyly syndrome, Majewski type
- Short stature syndrome, Brussels type
- Shprintzen-Goldberg craniosynostosis syndrome
- Shwachman-Diamond syndrome
- 15 Sickle beta thalassemia
- Sickle cell anemia
- Sillence syndrome
- Singleton-Merten syndrome
- Slipped capital femoral epiphysis - Not a rare disease
- 20 Small patella syndrome
- Smith McCort dysplasia
- Smith-Lemli-Opitz syndrome
- Sotos syndrome
- Spheroid body myopathy
- 25 Spinal muscular atrophy Ryukyuan type
- Spinal muscular atrophy type 1 with congenital bone fractures
- Spinal muscular atrophy type 3
- Spinal muscular atrophy type 4
- Spinal muscular atrophy with respiratory distress 1
- 30 Splenogonadal fusion limb defects micrognathia
- Split hand foot malformation
- Split hand split foot nystagmus
- Spondylocamptodactyly
- Spondylocarpotarsal synostosis syndrome
- 35 Spondylocostal dysostosis 1 - See Spondylocostal dysostosis
- Spondylocostal dysostosis 2 - See Spondylocostal dysostosis
- Spondylocostal dysostosis 3 - See Spondylocostal dysostosis

- Spondylocostal dysostosis 4 - See Spondylocostal dysostosis
- Spondylocostal dysostosis 5 - See Spondylocostal dysostosis
- Spondylocostal dysostosis 6 - See Spondylocostal dysostosis
- Spondylodysplastic Ehlers-Danlos syndrome
- 5 Spondyloenchondrodysplasia with immune dysregulation
- Spondyloepimetaphyseal dysplasia Genevieve type
- Spondyloepimetaphyseal dysplasia joint laxity
- Spondyloepimetaphyseal dysplasia Matrilin-3 related
- Spondyloepimetaphyseal dysplasia Missouri type
- 10 Spondyloepimetaphyseal dysplasia Shohat type
- Spondyloepimetaphyseal dysplasia Sponastrime type
- Spondyloepimetaphyseal dysplasia Strudwick type
- Spondyloepimetaphyseal dysplasia with hypotrichosis
- Spondyloepimetaphyseal dysplasia with multiple dislocations
- 15 Spondyloepimetaphyseal dysplasia X-linked
- Spondyloepimetaphyseal dysplasia, Aggreacan type
- Spondyloepiphyseal dysplasia congenita
- Spondyloepiphyseal dysplasia Maroteaux type
- Spondyloepiphyseal dysplasia tarda X-linked
- 20 Spondyloepiphyseal dysplasia-brachydactyly and distinctive speech
- Spondylometaphyseal dysplasia short limb-hand type
- Spondylometaphyseal dysplasia Algerian type
- Spondylometaphyseal dysplasia corner fracture type
- Spondylometaphyseal dysplasia Sedaghatian type
- 25 Spondylometaphyseal dysplasia type A4
- Spondylometaphyseal dysplasia with cone-rod dystrophy
- Spondylometaphyseal dysplasia with dentinogenesis imperfecta
- Spondylometaphyseal dysplasia X-linked
- Spondylometaphyseal dysplasia, Kozlowski type
- 30 Spondyloperipheral dysplasia
- Spondylothoracic dysostosis
- Sprengel deformity
- STAR syndrome
- Stiff person syndrome
- 35 Stuve-Wiedemann syndrome
- Symphalangism with multiple anomalies of hands and feet
- Syndactyly Cenani Lenz type

- Syndactyly type 3
- Syndactyly type 5
- Syndactyly type 9
- Syndactyly-polydactyly-earlobe syndrome
- 5 Syngnathia multiple anomalies
- Synovial Chondromatosis
- Systemic onset juvenile idiopathic arthritis
- TAR syndrome
- TARP syndrome
- 10 Tarsal carpal coalition syndrome
- Tarsal tunnel syndrome
- Tetra-amelia syndrome
- Tetraamelia-multiple malformations syndrome
- Tetramelic monodactyly
- 15 Thanatophoric dysplasia type 1
- Thanatophoric dysplasia type 2
- Thoracic dysplasia hydrocephalus syndrome
- Thoracolumbar pelvic dysplasia
- Tibia absent polydactyly arachnoid cyst
- 20 Tietze syndrome
- TMEM165-CDG (CDG-IIk)
- Townes-Brocks syndrome
- Treacher Collins syndrome
- Tricho-dento-osseous syndrome
- 25 Trichohepatoenteric syndrome
- Trichorhinophalangeal syndrome type 1
- Trichorhinophalangeal syndrome type 2
- Trichorhinophalangeal syndrome type 3
- Trigonobrachycephaly, bulbous bifid nose, micrognathia, and abnormalities of the hands and
- 30 feet
- Triphalangeal thumbs brachyectrodactyly
- Trochlea of the humerus aplasia of
- Trochlear dysplasia
- Troyer syndrome
- 35 Tubular aggregate myopathy
- Tumor necrosis factor receptor-associated periodic syndrome
- Ulna and fibula, hypoplasia of

- Ulna hypoplasia-intellectual disability syndrome
- Ulna metaphyseal dysplasia syndrome
- Ulnar hypoplasia lobster claw deformity of feet
- Ulnar-mammary syndrome
- 5 Undifferentiated pleomorphic sarcoma
- Upington disease
- Verloes Bourguignon syndrome
- Viljoen Kallis Voges syndrome
- Warman Mulliken Hayward syndrome
- 10 Weaver syndrome
- Weill-Marchesani syndrome
- Weissenbacher-Zweymuller syndrome
- Weyers acrofacial dysostosis
- Wildervanck syndrome
- 15 Worth type autosomal dominant osteosclerosis
- Wrinkly skin syndrome
- X-linked dominant chondrodysplasia punctata 2
- X-linked dominant scapuloperoneal myopathy
- X-linked hypophosphatemia
- 20 X-linked intellectual disability-plagiocephaly syndrome
- X-linked skeletal dysplasia-intellectual disability syndrome
- Yunis-Varon syndrome

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

25

EXAMPLES

Materials and Methods

Preparation of Bicyclic Peptide Ligands (General Method)

- 30 Bicyclic peptides were synthesized on Rink amide resin using standard Fmoc (9-fluorenylmethyloxycarbonyl) solid-phase peptide synthesis, either by manual coupling (for large scale) or using a Biotage Syroil automated peptide synthesizer (for small scale). Following TFA-based cleavage from the resin, peptides were precipitated with diethyl ether and dissolved in 50:50 acetonitrile/water. The crude peptides (at ~1 mM concentration) were
- 35 then cyclized with 1.3 equiv. of the scaffold, using ammonium bicarbonate (100 mM) as a base. Completion of cyclization was determined by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) or LC-MS. Once complete, the cyclization reaction was quenched

using N-acetyl cysteine (10 equiv. with respect to the peptide), and the solutions were lyophilized. The residue was dissolved in an appropriate solvent and purified by RP-HPLC. Peptide fractions of sufficient purity and the correct molecular weight (verified by either MALDI-TOF and HPLC or LC-MS) were pooled and lyophilized. Concentrations were determined by

5 UV absorption using the extinction coefficient at 280 nm, which was based on Trp/Tyr content.

All amino acids, unless noted otherwise, were used in the L-configurations.

BIOLOGICAL DATA

10 1. TfR1 Direct Binding Assay

Affinity of the peptides of the invention for human or cynomolgus TfR1 (Kd) was determined using a fluorescence polarisation assay, in accordance with the following method. Peptides of the invention were labelled with a fluorescent tag (fluorescein) and diluted to 2.5nM in 25mM HEPES with 100mM NaCl, 4mM CaCl₂ and 0.005% P20, pH 7.4. TfR1 protein (Human: R&D

15 Systems, 2474-TR or Acro Biosystems, CD1-H5243; Cyno: Acro Biosystems, TFR-C524a) was titrated starting at 1-5µM in the same assay buffer as the peptide to assay 1nM peptide in a total volume of 25µL in black walled and bottomed low bind low volume 384 well plates. The assay was typically set up by adding 5µL assay buffer, 10µL TfR1 protein then 10µL fluorescent peptide. The concentrations of TfR1 protein were 1 in 2 serial dilutions to give 12

20 different concentrations starting at 1-5µM. Measurements were conducted on a BMG PHERAstar FS equipped with an FP 485 520 520 optic module at 25°C with 200 flashes per well and a positioning delay of 0.1 second. Each well was measured every 5 minutes for 60 minutes. The gain used for analysis was determined for each tracer at the end of the 60 minutes where there was no protein in the well. The mP were fit to a standard 1:1 binding

25 model with a quadratic equation to generate a Kd value. Selected peptides of the invention were tested in the above mentioned assay and the results are shown in Table 1:

Table 1: FP Direct Binding of Selected Peptide Ligands of the Invention

Peptide Ligand	Geomean Kd (µM) Human TfR1	Standard Deviation (SD)	Geomean Kd (µM) Cyno TfR1	Standard Deviation (SD)
BCY12652	0.298	0.215 (n = 9)	0.098	0.035 (n = 3)
BCY12650	0.862	0.118 (n = 3)	nd	nd
BCY12651	0.971	0.633 (n = 5)	nd	nd
BCY14474	0.751	2.1419 (n= 2)	nd	nd

BCY14475	0.078	0.0664 (n= 2)	2.617	0 (n = 1)
BCY14476	0.087	0.0327 (n= 8)	3.6549	0 (n = 1)
BCY15768	0.047	0.0171 (n= 6)	0.70684	0 (n = 1)
BCY15934	0.064	0.0067 (n= 2)	nd	nd
BCY15937	0.034	0.0000 (n= 1)	nd	nd
BCY15938	0.060	0.0014 (n= 2)	nd	nd
BCY15940	0.054	0.0002 (n= 2)	nd	nd

nd = not determined

2. TfR1SPR Binding Assay

Biacore experiments were performed to determine k_a ($M^{-1}s^{-1}$), k_d (s^{-1}), K_D (nM) values of various peptides binding to TfR1.

Recombinant human and cynomolgus TfR1 were received from Bicycle as His₆-tagged TfR1 (a.a. 89-760) (ACRO Biosystems, CD1-H5243 and TFR-C524a).

For analysis of TfR1 peptide binding, a Biacore T200 or S200 instrument was used utilising a capture/coupling approach with a Cytiva NTA chip at 25°C with 25mM HEPES, 0.1M NaCl, 0.05% Tween 20 pH 7.4 as the running buffer. Immobilisation was carried out as follows. The chip was pre-equilibrated with an injection of 500mM EDTA (pH 8), before activation with 5mM NiSO₄. The surface was then activated using standard amine-coupling chemistry. Briefly, the carboxymethyl dextran surface was activated with a 1:1 ratio of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/0.1 M *N*-hydroxy succinimide (NHS). The TfR1 protein (human or cynomolgus) was then captured onto the activated surface after dilution into running buffer to 200nM and 250nM respectively. Residual activated groups were blocked with a 7 min injection of 1 M ethanolamine (pH 8.5):HBS-N (1:1). Reference surfaces were activated and blocked as above with no TfR1 protein capture. Capture levels were in the range of 1,500-5,000 RU dependent upon the individual study Buffer was changed to 25mM HEPES, 0.1M NaCl, 0.05% Tween 20 pH 7.4 1% DMSO.

A dilution series of test peptides was prepared in this buffer with a top peptide concentration of 5μM and 6 further 2-fold dilutions. The SPR analysis was run at 25°C at a flow rate of 30μl/min with 160 seconds association and 700-800 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.

Selected peptides of the invention were tested in the above mentioned assay and the results

5 are shown in Table 2:

Table 2: SPR Binding of Selected Peptide Ligands of the Invention

Peptide Ligand	Geomean Kd (μ M) Human TfR1	Standard Deviation (SD)	Geomean Kd (μ M) Cyno TfR1	Standard Deviation (SD)
BCY12455	65.600	72.8	42.384	34.0
BCY13983	450.0	0 (n = 1)	4580	0 (n = 1)
BCY13986	132.0	148 (n = 4)	2590	0 (n = 1)
BCY15466	32.1	6.7 (n = 4)	1394.3	465.6 (n = 4)
BCY15467	29.5	29.5 (n = 5)	1580.8	516.6 (n = 3)
BCY13989	376.3	44.5 (n = 4)	3440.5	2347.6 (n = 2)
BCY15468	37.8	28.4 (n = 5)	1717.6	2143.6 (n = 4)
BCY15469	54.3	29.9 (n = 4)	2117.7	1347.0 (n = 4)
BCY15470	36.4	16.1 (n = 4)	1615.1	1036.7 (n = 4)
BCY15471	262.9	109.8 (n = 5)	4660	0 (n = 1)

10 Further selected peptides of the invention were tested in the above mentioned assay and the results are shown in Table 3:

Table 3: SPR Binding of Selected Peptide Ligands of the Invention

Peptide Ligand	Human TfR1 Kd (nM)	Cynomolgus TfR1 Kd (nM)
BCY13989	nd	~11000
BCY 15768	nd	3800
BCY 15769	70	2200
BCY 15771	65	4000
BCY 15772	nd	99000
BCY 15773	178	2600
BCY 15774	nd	41000
BCY 15775	nd	NB

BCY 15776	nd	NB
BCY 15777	nd	NB
BCY 15770	nd	>100000
BCY 15891	46	3990
BCY 17992	42	535
BCY 17993	9	235
BCY 18033	nd	589
BCY 18034	25	2080
BCY 18035	77	10300
BCY 18036	422	7310
BCY 17994	6.6, 22	688
BCY 17995	3.4	nd
BCY 18037	11	1080
BCY 18038	9.7	1650
BCY 18039	8.6	819
BCY 17109	3.2	281*/535
BCY 17114	48	845
BCY 17110	15	232
BCY 17111	8.3	907
BCY 17112	4.6	2604
BCY 17113	308	1664
BCY 17115	60	1242
BCY 17116	1	474
BCY 17117	4.2	805
BCY 17118	120	1959
BCY 17119	212	5653
BCY 17120	32	1257
BCY 15468	29	4015
BCY 16048	28	1280
BCY 16049	175	28000
BCY 16035	37	4600
BCY 16047	49	4828
BCY 16039	89	10234
BCY 16036	83	5902
BCY 16033	82	6664

BCY 16038	210	~10000
BCY 16050	210	25800
BCY 16053	33	3120
BCY 16089	63	7466
BCY 16088	67	5878
BCY 16034	238	25000
BCY 16045	nd	10000
BCY 16046	32	2089
BCY 16051	25	1820
BCY 16031	68	6481
BCY 16079	60	5665
BCY 16029	47	5483
BCY 16052	73	7478
BCY 16032	305	30000
BCY 16550	18	682
BCY 16753	12	1700
BCY 16962	6.1	599
BCY 16963	nd	501
BCY 16964	6.6	514
BCY 16966	11	1900
BCY 16557	20	1180
BCY 16558	20000	50000
BCY 17986	52	4660
BCY 17987	50	5140
BCY 17988	649	nd
BCY 17991	3.4	35
BCY 20546	1660	nd
BCY 17986	52	4660
BCY 17988	649	nd
BCY 17994	6.6, 22	688

nd = not determined

NB = no binding

3. TfR1 Inhibition Assay

- 5 TfR1 inhibitory activity of peptides of the invention (IC_{50}) was determined using Alpha assay, in accordance with the following method. Proteins, peptides and Alpha reagents were

prepared to 5X concentration and 5µl of each reagent added to 25µl total volume in white 384-well Optiplate to make 1X final concentration. Fluorescently labelled human transferrin (Invitrogen, T2871) was diluted to 2.5nM in 25mM HEPES with 100mM NaCl, 4mM CaCl₂, 0.5% BSA and 0.05% P20, pH 7.4. Human or cynomolgus TfR1 protein was diluted to 50nM and unlabelled human transferrin (R&D Systems, 2914-HT) was diluted to 500nM in the same assay buffer. Non-labelled peptides from DMSO stock were diluted 20-fold in the same assay buffer, followed by 1 in 3 serial dilution in assay buffer containing 5% DMSO to give 11-different concentrations. 5µl fluorescently labelled transferrin, 5µl human or cynomolgus TfR1, 5µl non-labelled peptide or unlabelled human transferrin (R&D Systems, 2914-HT) were added to white 384-well Optiplate and incubated for 30 min. Anti-FITC Acceptor (PerkinElmer, AL127) was diluted 50-fold in assay buffer, 5µl added to assay plate and incubated for 30 min. Nickel Chelate Donor (PerkinElmer, AS101) was diluted 50-fold in assay buffer, 5µl added to assay plate and incubated for 180 min. Luminescence measurements were conducted on a BMG PHERAstar FS or FSX equipped with an AlphaScreen 520-620 module at 25°C following excitation at 680nm. Raw data was normalized to 100nM unlabelled transferrin and buffer. Data was standardized to 100nM unlabelled transferrin and buffer controls and fit to standard 4 parameter fit to generate IC₅₀ value.

Selected peptides of the invention were tested in the above mentioned assay and the results are shown in Table 4:

Table 4: Transferrin Inhibition Assay for Selected Peptide Ligands of the Invention

Peptide Ligand	Geomean IC ₅₀ (µM) Human TfR1	Standard Deviation (SD)	Geomean IC ₅₀ (µM) Cyno TfR1	Standard Deviation (SD)
BCY12455	0.673	0.147 (n = 4)	nd	nd
BCY12452	1.215	0.564 (n = 2)	nd	nd
BCY12454	0.893	0.356 (n = 2)	nd	nd

nd = not determined

4. TfR1 Competition Binding Assay

Peptides without a fluorescent tag were tested in competition with 1nM of a peptide with a fluorescent tag and a known K_d (BCY15768). Peptides were first diluted 100% DMSO then diluted to an appropriate concentration in assay buffer as described in the direct binding assay with a maximum of 2.5% DMSO, then serially diluted 1 in 2. Ten µL of diluted peptide

was added to the plate followed by 10 μ L of human TfR1 as described in direct binding assay at a fixed concentration (200nM). Then 5 μ L fluorescent peptide added. Measurements were conducted as for the direct binding assay, however the gain was determined prior to the first measurement. Data analysis was in Dotmatics where equation was fit to Cheng-Prusoff.

5

Selected peptides of the invention were tested in the above mentioned assay and the results are shown in Table 5:

Table 5: TfR1 Competition Binding Assay for Selected Peptide Ligands of the Invention

10

Peptide Ligand	Geomean Ki (μ M)	Standard Deviation (SD)
BCY17991	0.002	0.001
BCY17995	0.002	0.003
BCY17993	0.003	0.002
BCY18033	0.004	0.002
BCY18030	0.005	0.003
BCY18039	0.008	n=1
BCY17994	0.008	0.010
BCY18029	0.008	0.004
BCY17109	0.009	0.006
BCY18037	0.011	0.000
BCY17990	0.011	0.010
BCY17992	0.012	0.007
BCY18038	0.012	0.001
BCY18034	0.014	0.006
BCY18031	0.020	0.006
BCY18035	0.020	0.005
BCY17110	0.022	0.005
BCY17115	0.030	0.012
BCY17989	0.030	0.019
BCY16962	0.036	0.025
BCY17117	0.037	0.003
BCY16963	0.039	0.030
BCY15889	0.040	0.005
BCY17114	0.041	0.012
BCY17987	0.042	0.015

BCY15893	0.044	0.006
BCY16754	0.044	0.024
BCY17112	0.047	n=1
BCY15890	0.047	0.014
BCY16753	0.048	0.021
BCY16046	0.049	0.008
BCY15466	0.050	0.013
BCY17120	0.052	0.002
BCY16964	0.052	0.008
BCY16965	0.052	0.018
BCY17986	0.054	0.021
BCY15470	0.055	0.007
BCY16550	0.056	0.029
BCY16966	0.063	0.017
BCY15892	0.063	0.005
BCY16051	0.066	0.018
BCY15891	0.067	0.027
BCY17118	0.070	0.017
BCY16048	0.071	0.016
BCY17116	0.075	0.016
BCY16053	0.075	0.021
BCY16557	0.078	0.058
BCY17111	0.086	0.010
BCY18036	0.090	0.012
BCY16035	0.091	0.007
BCY17113	0.103	0.028
BCY18032	0.105	0.046
BCY15648	0.107	0.028
BCY15469	0.119	0.032
BCY16031	0.119	0.014
BCY16079	0.123	0.012
BCY15939	0.125	0.028
BCY16036	0.127	0.031
BCY16029	0.131	0.009
BCY16047	0.133	0.039

BCY15467	0.133	0.036
BCY16089	0.136	0.018
BCY17119	0.160	0.012
BCY16088	0.161	0.026
BCY16052	0.169	0.034
BCY16033	0.180	0.010
BCY16039	0.219	0.021
BCY16038	0.221	0.030
BCY17988	0.272	0.099
BCY15935	0.300	0.333
BCY15894	0.392	0.202
BCY15471	0.434	0.124
BCY16050	0.473	0.091
BCY16034	0.510	0.202
BCY13989	0.565	0.550
BCY16032	0.654	0.042
BCY16049	0.805	0.728
BCY16558	1.623	n=1
BCY16041	2.189	0.629
BCY16042	3.451	0.783
BCY16045	6.866	7.594
BCY16037	9.220	8.922
BCY16044	13.900	0.000
BCY16040	20.000	0.000
BCY16043	23.600	0.000

Selected peptides of the invention were tested in the above mentioned assay using human and/or cynomolgus TfR1 and the results are shown in Table 6:

Table 6: TfR1 Competition Binding Assay for Selected Peptide Ligands of the Invention

5

Peptide Ligand	Human TfR1 Ki (nM)	Cynomolgus TfR1 Ki (nM)
BCY 13989	565	3440
BCY 14476	79	nd
BCY 15469	119	2117
BCY 15892	63	nd
BCY 15470	55	1615
BCY 15893	44	nd
BCY 15471	434	4660
BCY 15894	392	nd
BCY 15468	103	1230
BCY 15768	44	1075
BCY 15769	32	1120
BCY 15771	32	1065
BCY 15772	926	7084
BCY 15773	83	2301
BCY 15774	269	3471
BCY 15775	6342	>250 μ M
BCY 15776	1334	>90 μ M
BCY 15777	2652	2910
BCY 15770	651	4042
BCY 15935	300	nd
BCY 15891	94	nd
BCY 15939	125	nd
BCY 15934	63	nd
BCY 15938	60	nd
BCY 15937	34	nd
BCY 15940	54	nd
BCY 17870	9	nd
BCY 17871	9	nd

BCY 17872	14	nd
BCY 17992	11	nd
BCY 17993	5	nd
BCY 18029	6	nd
BCY 18030	7	nd
BCY 18031	17	nd
BCY 18032	77	nd
BCY 18033	6	nd
BCY 18034	10	nd
BCY 18035	17	nd
BCY 18036	82	nd
BCY 17873	13	nd
BCY 17874	13	nd
BCY 17994	8	nd
BCY 17995	5	nd
BCY 18037	11	nd
BCY 18038	11	nd
BCY 18039	8	nd
BCY 17868	23	nd
BCY 17869	30	nd
BCY 17875	13	nd
BCY 17876	16	nd
BCY 17877	29	nd
BCY 17878	28	nd
BCY 17879	32	nd
BCY 17880	29	nd
BCY 17109	11	nd
BCY 17114	32	nd
BCY 17110	22	nd
BCY 17111	86	nd
BCY 17112	47	nd
BCY 17113	103	nd
BCY 17115	30	nd
BCY 17116	75	nd
BCY 17117	37	nd

BCY 17118	70	nd
BCY 17119	160	nd
BCY 17120	52	nd
BCY 16048	71	621
BCY 16049	805	nd
BCY 16035	91	nd
BCY 16047	42	nd
BCY 16039	72	nd
BCY 16036	127	nd
BCY 16033	180	nd
BCY 16038	221	nd
BCY 16050	473	nd
BCY 16053	75	nd
BCY 16089	136	nd
BCY 16088	161	nd
BCY 16034	510	nd
BCY 16037	9220	nd
BCY 16045	6866	nd
BCY 16046	49	746
BCY 16051	66	482
BCY 16041	2189	nd
BCY 16042	3451	nd
BCY 16031	119	nd
BCY 16079	123	nd
BCY 16029	131	nd
BCY 16052	169	nd
BCY 16032	654	nd
BCY 16550	56	nd
BCY 16753	48	nd
BCY 16754	18	nd
BCY 16962	36	nd
BCY 16963	39	nd
BCY 16964	52	nd
BCY 16965	52	nd
BCY 16966	63	nd

BCY 16557	37	nd
BCY 16558	1623	nd
BCY 17986	33	nd
BCY 17987	383 (n = 2)	nd
BCY 17988	213	nd
BCY 17989	24	nd
BCY 17990	8	nd
BCY 17991	3	nd
BCY 17986	33	nd
BCY 17988	213	nd
BCY 17989	24	nd
BCY 17994	8	nd

nd = not determined

5. Transcytosis assays with TfR1 binding bicyclic peptides in primary cultures of human proximal convoluted cells

In order to understand the handling of the TfR1 binding bicyclic peptides, transepithelial fluxes were measured across polarised monolayers of human proximal tubule cell monolayers. Two fluxes JAB (flux in the absorptive direction) and JBA (flux in the secretory direction) were measured over a flux period of 180 minutes. From these fluxes, the net direction (absorption or secretion) and magnitude of TA flux was determined. The experimental details are outlined below:

- The absorptive flux (JAB) and secretory flux (JBA) flux of the TA was determined by applying the compound to either the apical or basolateral side of the confluent monolayer and monitoring the time-resolved distribution of the substrate between the two compartments. From these the net flux (Jnet) was calculated. Bicycle peptides were tested at three concentrations 0.1, 1 and 10 μ M.
- Confluent monolayers were paired so that monolayers used for measurement of absorptive flux (JAB) and secretory flux (JBA) had similar TEER values.
- Culture media was aspirated from the insert wells before sequential transfer of the inserts into three beakers of around 100 ml warm modified-Krebs buffer.
- The inserts, with human proximal tubule cell monolayers, were placed in new 24-well plates, each well containing 800 μ l warm modified-Krebs buffer of pH 7.4, and 200 μ l modified-Krebs buffer of pH 7.4 was added to the insert's upper chamber (apical chamber). The temperature of the experiment was kept at 37 °C.

- Prior to the initiation of flux of the Test Articles, monolayers were pre-incubated with Krebs buffer only or Krebs buffer plus vehicle. Monolayers were incubated with Krebs at pH7.4 at either the apical or basolateral membrane as appropriate.
- Flux was initiated when the modified-Krebs buffer was aspirated from the apical or
5 basolateral chambers and replaced with equal volume of the required test concentration of the bicyclic peptide at the appropriate pH.
- This chamber is referred to as the donor chamber. In addition to the bicyclic peptides, Lucifer Yellow with the same concentration of bicyclic peptide was also co-administered to determine the paracellular flux.
- 10 • Sampling of 50 µl from the contralateral chamber (referred to as the receiver chamber) at predetermined time points after experiment initiation was then carried out. Samples were collected after gentle pipetting twice to mix the buffer.
- After each sampling, equal amount of fresh Krebs with the appropriate pH and substrate was replaced. At the last sampling, the reaction was terminated by sequentially
15 transferring the inserts into three beakers of ice-cold Krebs buffer and left to dry.
- The 50 µl samples was stored in 96-well PCR plates and spiked with 5.6 µl of 0.1 % trifluoroacetic acid (TFA) to give final concentration of 0.01% TFA, before being snap frozen in dry ice for storage.
- Monolayers were lysed with 50 µl of 0.01 % TFA to determined intracellular amount of
20 bicyclic peptides, and snap frozen as described above.
- All samples was stored at -80 °C. Samples were submitted for LC-MS/MS determination of bicyclic peptide concentration.

6. Bicyclic Peptide Detection by LC-MS/MS

- 25 A total of 648 samples were received for LC-MS/MS analysis.

BCY17986, BCY17988, BCY17989 and BCY17994 were provided individually as 1 mg/mL solutions in DMSO. These were further diluted in acetonitrile/DMSO (50/50, v/v) to make working solutions.

30

Bulk calibration standards for BCY17986, BCY17988, BCY17989 and BCY17994 in transporter media (modified Krebs buffer), with matrix concentrations ranging from 1.00 – 1000 nmol/L, were prepared by fortifying transporter media with appropriate amounts of BCY17986, BCY17988, BCY17989 and BCY17994 working solution.

- 35 The donor chamber, receiver chamber and lysed kidney cell samples were all quantified using bulk calibration standards and QC samples prepared in transporter media. Any samples which

were anticipated to be above the ULOQ on initial analysis were diluted up to 20-fold prior to re-analysis. BCY17986, BCY17988, BCY17989 and BCY17994 were detected in transporter media and lysed kidney cell samples from all test item-dosed *in vitro* kidney monolayers after dose administration.

5

Total bicyclic peptide content for each chamber was calculated from the analysed concentrations and corrected for paracellular leak, using the percentage leak of lucifer yellow, to derive true net flux in each direction at each bicyclic peptide concentration. Net fluxes were expressed as pmol/cm² and plotted against time for apical to basolateral (A-B) and basolateral to apical (B-A) directions.

10

The results of the analysis in sections 5 and 6 above are shown in Figures 1 to 4 where it can be seen that all four tested bicyclic peptides showed concentration and time dependent transcytosis in both A-B and B-A direction. This is in agreement with parallel studies which showed binding of FITC transferrin to TfR1 localised on both membranes. Generally Basolateral to Apical flux was greater than Apical to Basolateral. Previous studies have shown internalisation of these bicyclic peptides. This data shows transcytosis of TfR1 binding bicyclic peptides in a human primary culture expressing TfR1, with passage across a polarized cell, indicative of likely transport across endothelial cells of the peripheral and cerebral vasculature.

15

20

CLAIMS

1. A peptide ligand specific for transferrin receptor 1 (TfR1) comprising a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a
5 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 peptide ligand as defined in claim 1, wherein said reactive groups comprise cysteine residues.
- 10 3. The peptide ligand as defined in claim 1 or claim 2, wherein the peptide ligand inhibits the binding of transferrin to TfR1.
4. The peptide ligand as defined in any one of claims 1 to 3, wherein said loop sequences
15 comprise 2, 3, 6, 8 or 9 amino acids.
5. The peptide ligand as defined in claim 4, wherein said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 2 amino acids and the second of which consists of 9 amino acids.
- 20 6. The peptide ligand as defined in claim 4, wherein said loop sequences comprise three cysteine residues separated by two loop sequences both of which consist of 6 amino acids.
7. The peptide ligand as defined in claim 4, wherein said loop sequences comprise three
25 cysteine residues separated by two loop sequences the first of which consists of 3 amino acids and the second of which consists of 8 amino acids.
8. The peptide ligand as defined in claim 4, wherein said peptide ligand comprises an amino acid sequence of:
30 C_iALC_{ii}NDWTLPW_{HH}C_{iii} (SEQ ID NO: 1);
C_iREFFDTC_{ii}GLAFIEC_{iii} (SEQ ID NO: 2); and
C_iLEAC_{ii}YDGVY_{WY}SC_{iii} (SEQ ID NO: 3);
wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, or a pharmaceutically acceptable salt thereof, such as wherein the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises N-
35 and/or C-terminal additions and is selected from:
A-(SEQ ID NO: 1)-A (herein referred to as BCY12455);

- A-(SEQ ID NO: 1)-A-[Sar₆]-[K-FI] (herein referred to as BCY12652);
 A-(SEQ ID NO: 2)-A (herein referred to as BCY12452);
 A-(SEQ ID NO: 2)-A-[Sar₆]-[K-FI] (herein referred to as BCY12650);
 A-(SEQ ID NO: 3)-A (herein referred to as BCY12454); and
 5 A-(SEQ ID NO: 3)-A-[Sar₆]-[K-FI] (herein referred to as BCY12651).

wherein Sar represents sarcosine and FI represents fluorescein.

9. The peptide ligand as defined in claim 1 or claim 2, wherein the peptide ligand does not inhibit the binding of transferrin to TfR1.
10. The peptide ligand as defined in claim 9, wherein said loop sequences comprise 3 or 7 amino acids.
11. The peptide ligand as defined in claim 10, wherein said loop sequences comprise three
 15 cysteine residues separated by two loop sequences the first of which consists of 7 amino acids and the second of which consists of 3 amino acids.
12. The peptide ligand as defined in any one of claims 9 to 11, wherein said peptide ligand comprises an amino acid sequence of:
- 20 C_iSADDWLGC_{ii}ISWC_{iii} (SEQ ID NO: 4);
 C_iSSDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 5);
 C_iPPDAHLGC_{ii}ISWC_{iii} (SEQ ID NO: 6);
 C_iPQDAYLGC_{ii}ISWC_{iii} (SEQ ID NO: 7);
 C_iPPDSWQGC_{ii}ISYC_{iii} (SEQ ID NO: 8);
 25 C_iSPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 9) (herein referred to as BCY15935);
 C_iPGDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 10);
 C_iPPDSHLGC_{ii}ISYC_{iii} (SEQ ID NO: 11);
 C_iSADDWLGC_{ii}ISYC_{iii} (SEQ ID NO: 12);
 C_iP[HyP]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 13);
 30 C_iP[HyP]DAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 14);
 C_iS[HyP]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 15);
 C_iP[Aib]DAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 16);
 C_iPPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 17);
 C_iP[Aib]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 18);
 35 C_iSADAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 19);
 C_iS[Aib]DAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 20);
 C_iSPDAHLGC_{ii}[EPA]SYC_{iii} (SEQ ID NO: 21);

- C_iPPDAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 22);
 C_iS[Aib]DAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 23);
 C_iAPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 24);
 C_iP[Aib]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 25);
 5 C_iSPDAYLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 26);
 C_iSPDAHLGC_{ii}[tBuGly]SYC_{iii} (SEQ ID NO: 27);
 C_iPND AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 28);
 C_iPID AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 29);
 C_iSPDAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 30);
 10 C_iPPDAYLGC_{ii}ISYC_{iii} (SEQ ID NO: 31);
 C_iS[Aib]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 32);
 C_iSPDAHLGC_{ii}[Chg]SYC_{iii} (SEQ ID NO: 33);
 C_iAPDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 34);
 C_iYLPDW[tBuAla]C_{ii}GDEYC_{iii} (SEQ ID NO: 35);
 15 C_iSPDAHLGC_{ii}S[2Na]C_{iii} (SEQ ID NO: 36);
 C_iSPDAHLGC_{ii}S[3tBuTyr]C_{iii} (SEQ ID NO: 37);
 C_iSPD[Aib]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 38);
 C_iSPDAHLGC_{ii}S[1Na]C_{iii} (SEQ ID NO: 39);
 C_iSPDAH[tBuAla]GC_{ii}ISYC_{iii} (SEQ ID NO: 40);
 20 C_iSPDAH[Cba]GC_{ii}ISYC_{iii} (SEQ ID NO: 41);
 C_iSPDAHLGC_{ii}ISWC_{iii} (SEQ ID NO: 42);
 C_iSPD[Abu]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 43);
 C_iS[Aze]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 44);
 C_iSPDDHLGC_{ii}ISYC_{iii} (SEQ ID NO: 45);
 25 C_iSPDSHLGC_{ii}ISYC_{iii} (SEQ ID NO: 46);
 C_iSPDAH[Abu]GC_{ii}ISYC_{iii} (SEQ ID NO: 47);
 C_iSPDAHLGC_{ii}S[4Pal]C_{iii} (SEQ ID NO: 48);
 C_iP[dA]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 49);
 C_iSPDAYLGC_{ii}[tBuAla]SYC_{iii} (SEQ ID NO: 50);
 30 C_iSPDAHLGC_{ii}[C5g]SYC_{iii} (SEQ ID NO: 51);
 C_iSPDAHLGC_{ii}[Cbg]SYC_{iii} (SEQ ID NO: 52);
 C_iSPDAHL[dA]C_{ii}ISYC_{iii} (SEQ ID NO: 53);
 C_iSPDAH[Aib]GC_{ii}ISYC_{iii} (SEQ ID NO: 54);
 C_iSPDAHLGC_{ii}[Cpg]SYC_{iii} (SEQ ID NO: 55);
 35 C_iSPDAHLGC_{ii}[B-Melle]SYC_{iii} (SEQ ID NO: 56);
 C_iSAD AHLGC_{ii}ISYC_{iii} (SEQ ID NO: 57);
 C_iSPAAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 58);

- C_iSPDAALGC_{ii}ISYC_{iii} (SEQ ID NO: 59);
 C_iSPDAHAGC_{ii}ISYC_{iii} (SEQ ID NO: 60);
 C_iSPDAHLAC_{ii}ISYC_{iii} (SEQ ID NO: 61);
 C_iSPDAHLGC_{ii}ASYC_{iii} (SEQ ID NO: 62);
 5 C_iSPDAHLGC_{ii}IAYC_{iii} (SEQ ID NO: 63);
 C_iSPDAHLGC_{ii}SAC_{iii} (SEQ ID NO: 64);
 C_i[K(N₃)]PDAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 65);
 C_iS[K(N₃)]DAHLGC_{ii}ISYC_{iii} (SEQ ID NO: 66); and
 C_iSPD[K(N₃)]HLGC_{ii}ISYC_{iii} (SEQ ID NO: 67);
- 10 wherein Abu represents aminobutyric acid, Aib represents aminoisobutyric acid, Aze represents azetidine, B-Melle represents beta-methyl isoleucine, C5g represents cyclopentyl glycine, Cba represents β-cyclobutylalanine, Cbg represents cyclobutyl glycine, Chg represents cyclohexyl glycine, Cpg represents cyclopropyl glycine, EPA represents 2-amino-3-ethyl-pentanoic acid, HyP represents trans-4-hydroxy-L-proline, [K(N₃)] represents 6-azido
- 15 lysine, 1Nal represents 1-naphthylalanine, 2Nal represents 2-naphthylalanine, 4Pal represents 4-pyridylalanine, tBuAla represents t-butyl-alanine, tBuGly represents t-butyl-glycine, 3tBuTyr represents 3-t-Butyl-Tyrosine, and C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, or a pharmaceutically acceptable salt thereof,
- 20 such as wherein the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)tris(2-bromoethanone) (TATB) and the peptide ligand comprises N- and/or C-terminal additions and is selected from:
- A-(SEQ ID NO: 4)-A (herein referred to as BCY13983);
 A-(SEQ ID NO: 4)-A-[Sar₆]-[K-FI] (herein referred to as BCY14474);
 25 A-(SEQ ID NO: 5)-A (herein referred to as BCY13986);
 A-(SEQ ID NO: 5)-A-[Sar₆]-[K-FI] (herein referred to as BCY14475);
 A-(SEQ ID NO: 6)-A (herein referred to as BCY15466);
 Ac-(SEQ ID NO: 6) (herein referred to as BCY15889);
 A-(SEQ ID NO: 7)-A (herein referred to as BCY15467);
 30 Ac-(SEQ ID NO: 7) (herein referred to as BCY15890);
 A-(SEQ ID NO: 8)-A (herein referred to as BCY13989);
 A-(SEQ ID NO: 8)-A-[Sar₆]-[K-FI] (herein referred to as BCY14476);
 A-(SEQ ID NO: 9)-A (herein referred to as BCY15468);
 A-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15768);
 35 (SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15934);
 Ac-(SEQ ID NO: 9)-A-[Sar₆]-[K-FI] (herein referred to as BCY15937);
 Ac-(SEQ ID NO: 9)-[Sar₆]-[K-FI] (herein referred to as BCY15938);

[FI]G[Sar₅]-A-(SEQ ID NO: 9)-A (herein referred to as BCY15940);
 N[1NaI]N-(SEQ ID NO: 9) (herein referred to as BCY18030);
 Ac-(SEQ ID NO: 9)-E[Pip]W (herein referred to as BCY18039);
 Ac-(SEQ ID NO: 9)-EPW (herein referred to as BCY17994);
 5 NWN-(SEQ ID NO: 9) (herein referred to as BCY18029);
 NWN-(SEQ ID NO: 9)-A (herein referred to as BCY17109);
 Ac-(SEQ ID NO: 9)-E[Aze]W (herein referred to as BCY18037);
 Ac-NWN-(SEQ ID NO: 9) (herein referred to as BCY17992);
 Ac-(SEQ ID NO: 9)-E[dP]W (herein referred to as BCY18038);
 10 Ac-N[1NaI]N-(SEQ ID NO: 9) (herein referred to as BCY18034);
 N[dW]N-(SEQ ID NO: 9) (herein referred to as BCY18031);
 Ac-N[dW]N-(SEQ ID NO: 9) (herein referred to as BCY18035);
 HWM-(SEQ ID NO: 9)-A (herein referred to as BCY17110);
 A-(SEQ ID NO: 9)-PHP (herein referred to as BCY17115);
 15 A-(SEQ ID NO: 9)-EPW (herein referred to as BCY17114);
 NEV-(SEQ ID NO: 9)-A (herein referred to as BCY17112);
 A-(SEQ ID NO: 9)-PIVH (herein referred to as BCY17120);
 Ac-(SEQ ID NO: 9) (herein referred to as BCY15891);
 HTS-(SEQ ID NO: 9)-A (herein referred to as BCY17111);
 20 Ac-N[NMeTrp]N-(SEQ ID NO: 9) (herein referred to as BCY18036);
 N[NMeTrp]N-(SEQ ID NO: 9) (herein referred to as BCY18032);
 Ac-A-(SEQ ID NO: 9)-A (herein referred to as BCY15939);
 A-(SEQ ID NO: 9)-EHQE (herein referred to as BCY17119);
 ESF-(SEQ ID NO: 9)-A (herein referred to as BCY17113);
 25 NWN-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17870);
 Ac-NWN-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17871);
 [AzPro]-NWN-(SEQ ID NO: 9) (herein referred to as BCY17872);
 Ac-(SEQ ID NO: 9)-EPW-[K(N₃)] (herein referred to as BCY17873);
 [AzPro]-(SEQ ID NO: 9)-EPW (herein referred to as BCY17874);
 30 Ac-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17868);
 [AzPro]-(SEQ ID NO: 9) (herein referred to as BCY17869);
 Ac-N[dY]N-(SEQ ID NO: 9)-[K(N₃)] (herein referred to as BCY17882);
 Ac-(SEQ ID NO: 9)-E-[dP]-W-[K(N₃)] (herein referred to as BCY17890);
 Ac-(SEQ ID NO: 9)-E-[Aze]-W-[K(N₃)] (herein referred to as BCY17892);
 35 Ac-(SEQ ID NO: 9)-E-[Pip]-W-[K(N₃)] (herein referred to as BCY17894);
 Ac-(SEQ ID NO: 9)-[K(N₃)](PYA-maleimide) (herein referred to as BCY17906);
 Ac-(SEQ ID NO: 9)-EPW-[Peg₁₀]-[K(N₃)] (herein referred to as BCY19405);

Ac-(SEQ ID NO: 9)-EPW-[Peg₂₄]-[K(N₃)] (herein referred to as BCY19406);
 Ac-(SEQ ID NO: 9)-EPWGSGGS-[K(N₃)] (herein referred to as BCY19407);
 A-(SEQ ID NO: 10)-A (herein referred to as BCY15469);
 Ac-(SEQ ID NO: 10) (herein referred to as BCY15892);
 5 A-(SEQ ID NO: 11)-A (herein referred to as BCY15470);
 Ac-(SEQ ID NO: 11) (herein referred to as BCY15893);
 A-(SEQ ID NO: 12)-A (herein referred to as BCY15471);
 Ac-(SEQ ID NO: 12) (herein referred to as BCY15894);
 Ac-(SEQ ID NO: 13) (herein referred to as BCY17991);
 10 Ac-(SEQ ID NO: 13)-EPW (herein referred to as BCY17995);
 Ac-NWN-(SEQ ID NO: 13) (herein referred to as BCY17993);
 NWN-(SEQ ID NO: 13) (herein referred to as BCY18033);
 A-(SEQ ID NO: 13)-A (herein referred to as BCY16754);
 Ac-(SEQ ID NO: 13)-[K(N₃)] (herein referred to as BCY17896);
 15 Ac-NWN-(SEQ ID NO: 13)-[K(N₃)] (herein referred to as BCY17899);
 Ac-(SEQ ID NO: 13)-EPW-[K(N₃)] (herein referred to as BCY17901);
 Ac-(SEQ ID NO: 14) (herein referred to as BCY17990);
 Ac-(SEQ ID NO: 14)-[K(N₃)] (herein referred to as BCY17875);
 [AzPro]-(SEQ ID NO: 14) (herein referred to as BCY17876);
 20 Ac-(SEQ ID NO: 15) (herein referred to as BCY17989);
 A-(SEQ ID NO: 15)-A (herein referred to as BCY16047);
 Ac-(SEQ ID NO: 15)-[K(N₃)] (herein referred to as BCY17877);
 [AzPro]-(SEQ ID NO: 15) (herein referred to as BCY17878);
 A-(SEQ ID NO: 16)-A (herein referred to as BCY16962);
 25 TYMN-(SEQ ID NO: 17)-A (herein referred to as BCY17117);
 A-(SEQ ID NO: 17)-A (herein referred to as BCY16048);
 A-(SEQ ID NO: 18)-A (herein referred to as BCY16963);
 Ac-(SEQ ID NO: 19) (herein referred to as BCY17987);
 A-(SEQ ID NO: 20)-A (herein referred to as BCY16753);
 30 A-(SEQ ID NO: 21)-A (herein referred to as BCY16046);
 A-(SEQ ID NO: 22)-A (herein referred to as BCY16964);
 A-(SEQ ID NO: 23)-A (herein referred to as BCY16965);
 Ac-(SEQ ID NO: 24) (herein referred to as BCY17986);
 A-(SEQ ID NO: 25)-A (herein referred to as BCY16550);
 35 A-(SEQ ID NO: 26)-A (herein referred to as BCY16966);
 A-(SEQ ID NO: 27)-A (herein referred to as BCY16051);
 IDSN-(SEQ ID NO: 28)-A (herein referred to as BCY17118);

WGKS-(SEQ ID NO: 29)-A (herein referred to as BCY17116);
A-(SEQ ID NO: 30)-A (herein referred to as BCY16053);
A-(SEQ ID NO: 31)-A (herein referred to as BCY16557);
A-(SEQ ID NO: 32)-A (herein referred to as BCY16035);
5 A-(SEQ ID NO: 33)-A (herein referred to as BCY16043);
A-(SEQ ID NO: 34)-A-[Sar₆]-[K-FI] (herein referred to as BCY15769);
A-(SEQ ID NO: 35)-A (herein referred to as BCY15648);
A-(SEQ ID NO: 36)-A (herein referred to as BCY16031);
A-(SEQ ID NO: 37)-A (herein referred to as BCY16079);
10 A-(SEQ ID NO: 38)-A (herein referred to as BCY16036);
A-(SEQ ID NO: 39)-A (herein referred to as BCY16029);
A-(SEQ ID NO: 40)-A (herein referred to as BCY16089);
A-(SEQ ID NO: 41)-A (herein referred to as BCY16088);
A-(SEQ ID NO: 42)-A (herein referred to as BCY16052);
15 A-(SEQ ID NO: 43)-A (herein referred to as BCY16033);
A-(SEQ ID NO: 44)-A (herein referred to as BCY16039);
Ac-(SEQ ID NO: 44) (herein referred to as BCY17988);
Ac-(SEQ ID NO: 44)-[K(N₃)] (herein referred to as BCY17879);
[AzPro]-(SEQ ID NO: 44) (herein referred to as BCY17880);
20 A-(SEQ ID NO: 45)-A (herein referred to as BCY16038);
A-(SEQ ID NO: 46)-A (herein referred to as BCY16050);
A-(SEQ ID NO: 47)-A (herein referred to as BCY16034);
A-(SEQ ID NO: 48)-A (herein referred to as BCY16032);
A-(SEQ ID NO: 49)-A (herein referred to as BCY16049);
25 A-(SEQ ID NO: 50)-A (herein referred to as BCY16558);
A-(SEQ ID NO: 51)-A (herein referred to as BCY16041);
A-(SEQ ID NO: 52)-A (herein referred to as BCY16042);
A-(SEQ ID NO: 53)-A (herein referred to as BCY16045);
A-(SEQ ID NO: 54)-A (herein referred to as BCY16037);
30 A-(SEQ ID NO: 55)-A (herein referred to as BCY16044);
A-(SEQ ID NO: 56)-A (herein referred to as BCY16040);
A-(SEQ ID NO: 57)-A-[Sar₆]-[K-FI] (herein referred to as BCY15771);
A-(SEQ ID NO: 58)-A-[Sar₆]-[K-FI] (herein referred to as BCY15772);
A-(SEQ ID NO: 59)-A-[Sar₆]-[K-FI] (herein referred to as BCY15773);
35 A-(SEQ ID NO: 60)-A-[Sar₆]-[K-FI] (herein referred to as BCY15774);
A-(SEQ ID NO: 61)-A-[Sar₆]-[K-FI] (herein referred to as BCY15775);
A-(SEQ ID NO: 62)-A-[Sar₆]-[K-FI] (herein referred to as BCY15776);

A-(SEQ ID NO: 63)-A-[Sar₆]-[K-FI] (herein referred to as BCY15777);

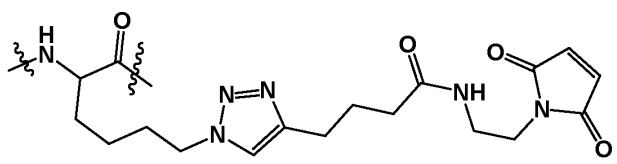
A-(SEQ ID NO: 64)-A-[Sar₆]-[K-FI] (herein referred to as BCY15770);

Ac-(SEQ ID NO: 65) (herein referred to as BCY17903);

Ac-(SEQ ID NO: 66) (herein referred to as BCY17904); and

5 Ac-(SEQ ID NO: 67) (herein referred to as BCY17905);

wherein AzPro represents azidopropyl, Aze represents azetidine, 1Nal represents 1-naphthylalanine, NMeTrp represents N-methyl-tryptophan, [K(N₃)] represents 6-azido lysine, Peg represents polyethylene glycol, Pip represents pipecolic acid, Sar represents sarcosine, FI represents fluorescein and [K(N₃)(PYA-Maleimide)] represents a modified lysine having



10 the following structure:

or wherein the molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises N- and/or C-terminal additions and is:

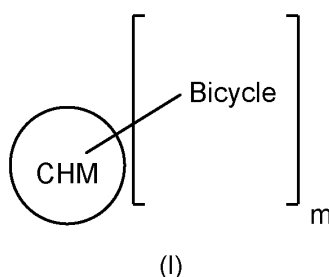
15 Ac-(SEQ ID NO: 13) (herein referred to as BCY20546).

13. The peptide ligand as defined in any one of claims 1 to 12, wherein the pharmaceutically acceptable salt is selected from the free acid or the sodium, potassium, calcium or ammonium salt.

20

14. A multimeric binding complex which comprises at least two of the peptide ligands according to any one of claims 1 to 13.

15. The multimeric binding complex according to claim 14, which comprises a compound
25 of formula (I):

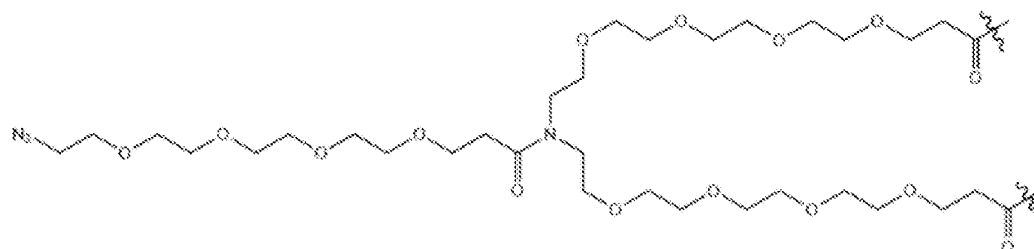


wherein CHM represents a central hinge moiety;

Bicycle represents a bicyclic peptide ligand according to any one of claims 1 to 13; and

m represents an integer selected from 2 to 10, such as 2, 3 or 4.

16. The multimeric binding complex according to claim 15 wherein m represents 2 and CHM is a motif of formula (A):



5

(A),

such as BCY19409.

17. A pharmaceutical composition which comprises the peptide ligand as defined in any one of claims 1 to 13 or the multimeric binding complex as defined in any one of claims 14 to 16, in combination with one or more pharmaceutically acceptable excipients.

18. The peptide ligand as defined in any one of claims 1 to 13, or the multimeric binding complex as defined in any one of claims 14 to 16, or the pharmaceutical composition of claim 17, for use in preventing, suppressing or treating a disease or disorder through TfR1 mediated delivery of a therapeutic agent.

19. A tissue delivery complex which comprises a peptide ligand as defined in any one of claims 1 to 13 or the multimeric binding complex as defined in any one of claims 14 to 16, bound to TfR1 in combination with a payload, such as an oligonucleotide, in particular siRNA.

20. The tissue delivery complex as defined in claim 19, which is a muscle tissue delivery complex.

21. The tissue delivery complex as defined in claim 19 or claim 20, for use in the treatment of a musculoskeletal disorder.