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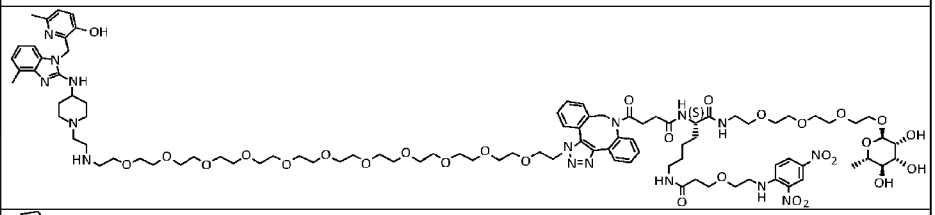
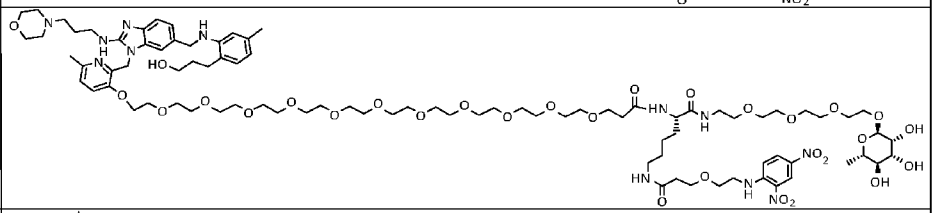
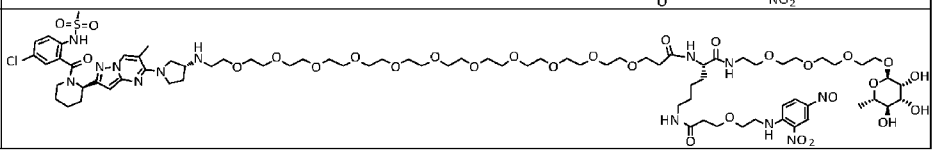
Compound No.	Structure
25	
26	
27	

FIG. 1

(57) Abstract: A conjugate compound comprising (i) a. ligand, which targets a protein of RSV or an RSV-infected cell, (ii) a. linker or linkers, and (iii) two or more haptens; a pharmaceutical composition comprising the conjugate compound; and a method of delivering the agent to RSV or an RSV-infected cell for therapy are described.

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[Continued on next page]

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CONJUGATES, COMPOSITIONS AND METHODS FOR TREATING RSV INFECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from Appl. No. 63/590,991, filed October 17, 2023, and Appl. No. 63/700,417, filed September 27, 2024, each of which is incorporated by reference as if fully set forth herein.

TECHNICAL FIELD

[0002] The present disclosure relates to a conjugate comprising (i) a ligand, which targets a protein on the surface of a virus or a virus-infected cell, (ii) a linker or linkers, and (iii) one or more haptens and the use of the conjugate, such as a composition comprising the conjugate, to deliver the agent to a human in need thereof for RSV treatment.

BACKGROUND

[0003] Respiratory syncytial virus (RSV) is a respiratory virus that causes cold-like symptoms and can lead to serious lung infections, particularly in pediatric and geriatric patients and immunocompromised adults.

[0004] The F glycoprotein ("F protein") is a class I fusion protein that mediates viral entry of RSV into a cell by fusing the viral envelope with a host cell membrane. The F protein consists of two disulfide-linked subunits – F1 and F2. The F1 subunit contains a hydrophobic fusion peptide at its N terminus followed by two heptad repeats – HR1 and HR2, which are separated by an intervening region of about 300 amino acids. The G glycoprotein ("G protein") exists in both membrane-bound and secreted forms, and is primarily responsible for viral attachment to host cells.

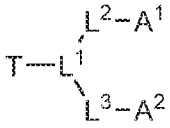
[0005] High-risk pediatric patients can be treated prophylactically with antibodies, such as the monoclonal antibody palivizumab, to reduce the incidence and severity of RSV infection. While ribavirin is approved for the treatment of severe RSV infection, its clinical efficacy is suboptimal.

[0006] Here we provide a non-antibody, small-molecule approach to treat RSV deploying conjugates comprising a ligand targeting the F protein, one or more linkers, and two haptens for the recruitment of antibodies.

[0007] This and other objects, as well as inventive features, will be apparent from the detailed description provided herein.

SUMMARY

[0008] Described herein are compounds of Formula I:



Formula I

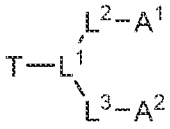
and pharmaceutically acceptable salts and solvates thereof, wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0009] Described herein are compositions comprising one or more compounds of Formula I:



Formula I

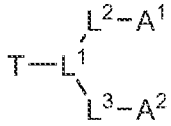
and pharmaceutically acceptable salts and solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof; wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0010] Described herein are compositions for use in treating RSV infection, the compositions comprising one or more compounds of Formula I:



Formula I

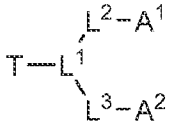
and pharmaceutically acceptable salts and solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof; wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0011] Described herein are unit doses for use in treating RSV infection, the unit doses comprising a therapeutically effective amount of one or more compounds of Formula I:



Formula I

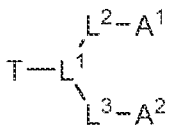
and pharmaceutically acceptable salts and solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof; wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0012] Described herein are methods for treating RSV infection, the methods comprising administering a therapeutically effective amount of one or more compounds of Formula I:



Formula I

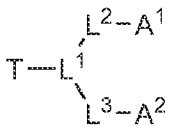
and pharmaceutically acceptable salts and solvates thereof, compositions thereof optionally including one or more diluents, carriers, or excipients, or combinations thereof, or unit doses thereof to a host to a host animal having a RSV infection; wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0013] Described herein are uses of one or more compounds of Formula I:



Formula I

and pharmaceutically acceptable salts and solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof in the manufacture of a medicament for use in treating a RSV infection in a host animal; wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

[0014] In a first illustrative embodiment, T is a radical of a RSV fusion (F) glycoprotein. In a second illustrative embodiment, T is a radical of a RSV attachment glycoprotein (G).

[0015] In another illustrative embodiment, the haptens recruit endogenous antibodies present in the host animal.

[0016] In another illustrative embodiment, compounds, compositions, and unit doses described herein are used in methods for treating RSV infection in a host animal, where the compounds, compositions, and unit doses are administered to the host animal, and after administration A^1 and A^2, or any epitope of the foregoing are each bound by one or more antibodies.

[0017] In another illustrative embodiment, compounds, compositions, and unit doses described herein are used in methods for treating RSV infection in a host animal, where the compounds, compositions, and unit doses are administered to the host animal, and after administration A^1 and A^2, or any epitope of the foregoing are each bound by a different antibody.

[0018] In another illustrative embodiment, each of L^1, L^2, and L^3 is independently selected, and each may comprise a single divalent atom, or a chain of atoms. In another illustrative embodiment, L^2 may be a bond attaching L^1 to A^1. In another illustrative embodiment,

each of L^3 may be a bond attaching L^1 to A^2.

[0019] The compounds, compositions, unit doses, methods, and uses described herein are useful in treating RSV infection. It is to be understood that such treatment includes prophylactic and preventative treatment of host animals, including host animals at risk of RSV infection, or host animals exposed to RSV infection, including when symptoms of RSV infection have not been observed. For example, the compounds, compositions, unit doses, methods, and uses described herein are useful in preventing the onset of RSV infection, and/or decreasing the severity of later arising symptoms associated with RSV infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1** shows example compounds.

[0021] **FIG. 2** shows a reaction scheme for preparing Compound 47.

[0022] **FIG. 3** shows an immunofluorescence assay of Compound 47 in Hep2 cells.

[0023] **FIG. 4** shows the reduction of the virus cytopathic effect by Compound 47 in Hep2 cells. Higher absorbance (Y axis) denotes greater cell viability and a reduction in a virus induced cytopathic effect.

[0024] **FIG. 5** shows the induction of ADCC by Compound 47 in human cells infected with RSV.

[0025] **FIG. 6** shows the in vivo efficacy of Compound 47 against RSV in rats following a single dose compared to ALS-008176 and vehicle.

[0026] **FIG. 7** shows the in vivo efficacy of Compound 26 against RSV in rats following a single dose compared to ALS-008176 and vehicle.

[0027] **FIG. 8** shows the ADCP Assay of Compound 47 against RSV-infected cells.

[0028] **FIG. 9** shows the CDC Assay of Compound 47 against RSV-infected cells.

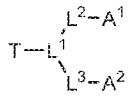
[0029] **FIG. 10** shows the in vivo efficacy of Compound 27 against RSV in rats following a single dose compared to vehicle.

DETAILED DESCRIPTION

[0030] The following enumerated clauses serve to further illustrate the invention described herein. The clauses describe various features and aspects of the ligands, linkers, and

haptens, and various combinations of those features and aspects in the many embodiments the invention. The following enumerated clauses illustrate many embodiments of the invention, and are not to be construed as limiting the scope of the invention in any way.

1. A compound of the formula



Formula I

and pharmaceutically acceptable salts and solvates thereof, wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

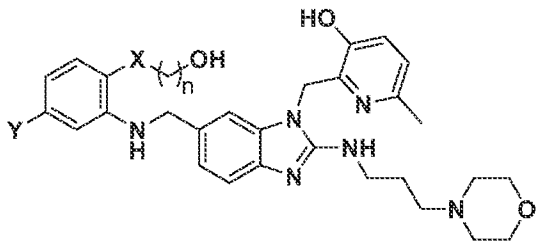
2. The compound of clause 1 wherein the target protein is a RSV fusion (F) glycoprotein.

3. The compound of clause 1 wherein the target protein is a RSV attachment glycoprotein (G).

4. The compound of clause 1 wherein the ligand is an inhibitor of RSV fusion (F) glycoprotein.

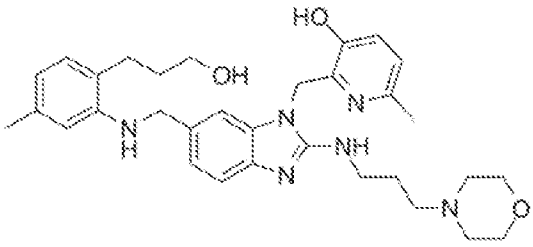
5. The compound of clause 1 wherein the ligand is an inhibitor of RSV attachment glycoprotein (G).

6. The compound of clause 1 wherein the ligand is of the formula



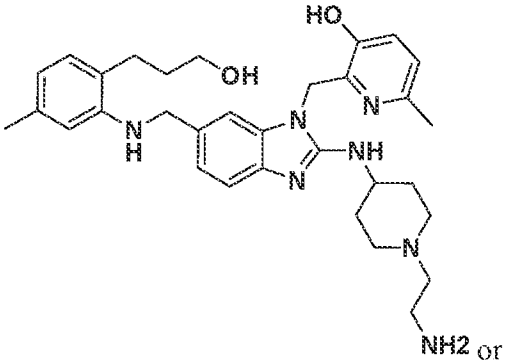
where X is CH_2, O, S, or SO_2; n is 1 or 2 with the proviso that if X is not CH_2, n is 2; and Y is alkyl, halo, CN, alkoxy, alkyl-OH, or H.

7. The compound of clause 1 wherein the ligand is TMC353121

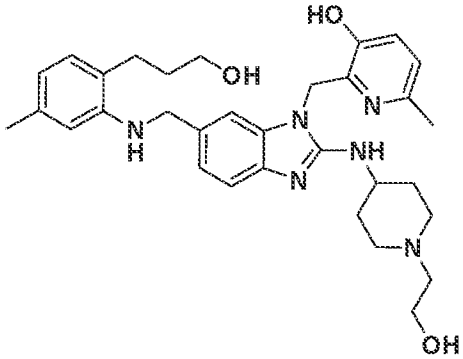


or an analog or derivative thereof.

8. The compound of clause 1 wherein the ligand is

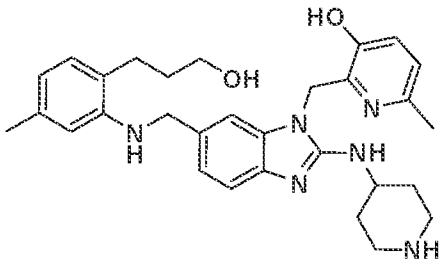


or



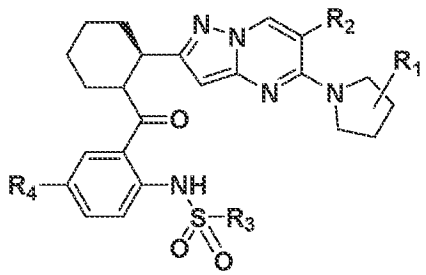
or an analog or derivative thereof.

9. The compound of clause 1 wherein the ligand is



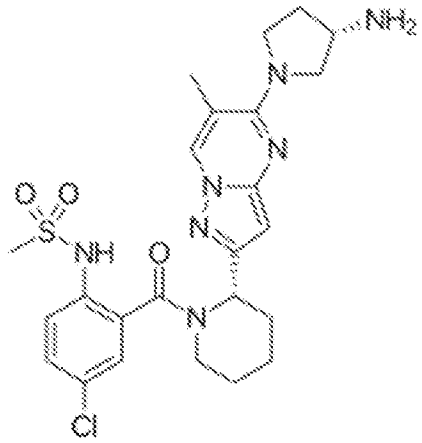
or an analog or derivative thereof.

10. The compound of clause 1 wherein the ligand is of the formula



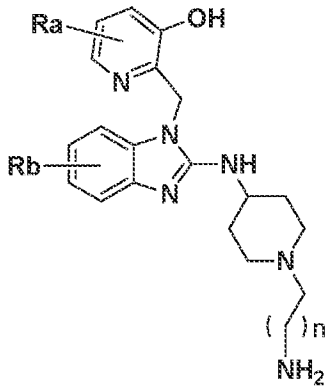
where R_1 is NH_2, NHRn, where Rn is alky, amino, hydroxyalkyl, OR, or OH; R_2 is alkyl (C-1 to C-4), halo, CN, N_3, OR; R_3 is alkyl (C-1 to C-4); and R_4 is alkyl (C-1 to C-4), halo, CN, N_3, OR.

11. The compound of clause 1 wherein the ligand is presatovir



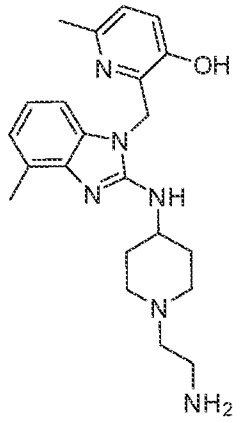
or an analog or derivative thereof.

12. The compound of clause 1 wherein the ligand is of the formula



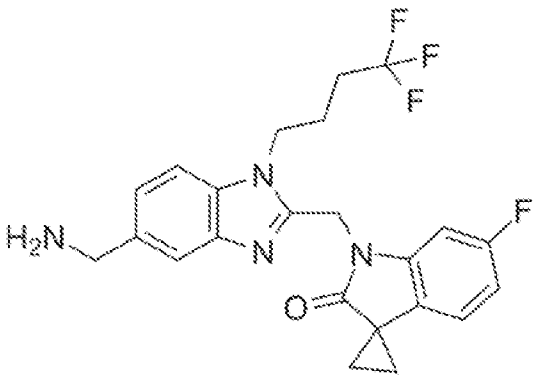
wherein Ra and Rb are independently selected from the group alkyl (C1- to C4), alkoxyl (C1- to C4), halo, cyano, and azido; and n is 1, 2, or 3.

13. The compound of clause 1 wherein the ligand is



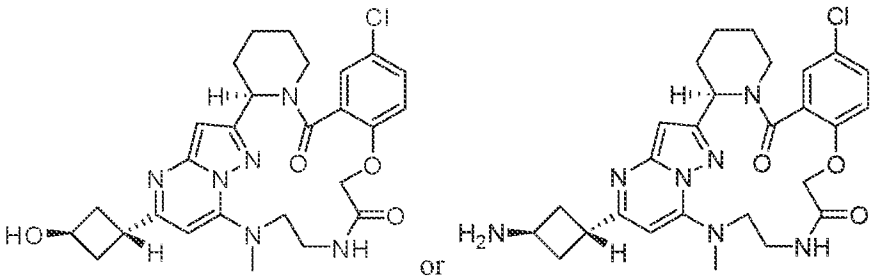
or an analog or derivative thereof.

14. The compound of clause 1 wherein the ligand is susinatovir



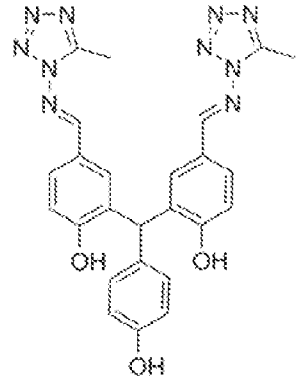
or an analog or derivative thereof.

15. The compound of clause 1 wherein the ligand is



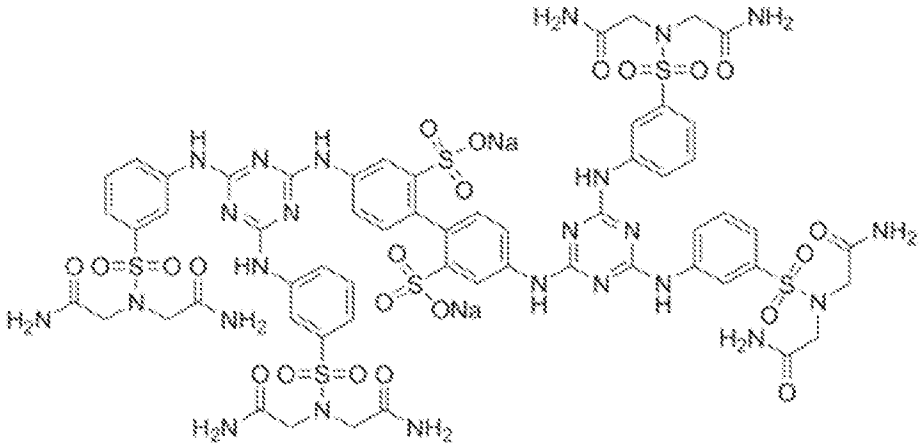
or an analog or derivative thereof.

16. The compound of clause 1 wherein the ligand is VP-14637



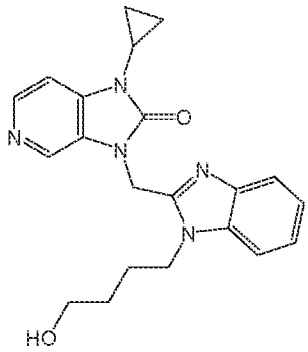
or an analog or derivative thereof.

17. The compound of clause 1 wherein the ligand is of RFI-641



or an analog or derivative thereof.

18. The compound of clause 1 wherein the ligand is BMS-433771



risetovir, rilematovir, ziresovir, , RSV-IN-1, RSV-IN-5, YM 53403, or BTA-9981, or an analog or derivative of the foregoing.

19. The compound of clause 1 wherein the ligand is a peptide having SEQ ID NO: 1, or a fragment thereof.

20. The compound of clause 1 wherein the ligand is a peptide having SEQ ID NO: 18, or a fragment thereof.

21. The compound of clause 1 wherein the ligand is SEQ ID NO: 16 (T118).

22. The compound of clause 1 wherein the ligand is SEQ ID NO: 34 (T205).

23. The compound of clause 1 wherein the ligand is SEQ ID NO: 40 (VIQKI), or a substitution isomer selected from VIQKI-I456F (SEQ ID NO: 41)0 and VIQKI-I454F/I456F SEQ ID NO: 42).

24. The compound of clause 1 wherein the ligand is not a folate.

25. The compound of any preceding clause wherein A^1 and A^2 are the same hapten.

26. The compound of preceding clause wherein A^1 and A^2 are different haptens.

27. The compound of any preceding clause wherein the hapten is selected from a rhamnose, a nitrophenyl, a nitrophenol, a nitroaniline, a dinitrophenyl (DNP), a dinitrophenol, a dinitroaniline, a trinitrophenyl (TNP), a trinitrophenol, a trinitroaniline, chloronitrophenyl, a chloronitrophenol, a chloronitroaniline, an iodonitrophenyl, an iodonitrophenol, an iodonitroaniline, a nitrotyrosine, an hydroxynitrotyrosine, an aminonitrotyrosine, 4-hydroxy-3-nitrophenyl acetic acid, an α-galactosyl moiety, a sulfated Gal, a phosphorylcholine, a bacterial antigen, a viral antigen,
A^1 and A^2 are each an independently selected radical of a rhamnose, α-galactosyl moiety, dinitrobenzene, dinitroaniline, trinitrobenzene, and dinitroaniline.

28. The compound of any preceding clause wherein A^1 and/or A^2 is a radical of a dinitrophenyl (DNP), a dinitrophenol, or a dinitroaniline.

29. The compound of any preceding clause wherein A^1 and/or A^2 is a radical of a rhamnose, including L-rhamnose.

30. The compound of any preceding clause wherein one of A^1 and A^2 is a radical of L-rhamnose; and the other of A^1 and A^2 is a radical of a dinitroaniline.

31. The compound of any preceding clause wherein the hapten has an epitope that has an endogenous Ab, B or T cell.

32. The compound of any preceding clause wherein the hapten is not FITC.

33. The compound of any preceding clause wherein L^2 and L^3 are attached to the same atom of L^1.

34. The compound of any preceding clause wherein L^2 and L^3 are bound to the same atom on L^1.

35. The compound of any preceding clause wherein L^2 and L^3 are bound to the same non-carbon atom on L^1.

36. The compound of any preceding clause wherein L^2 and L^3 are bound to the same nitrogen atom on L^1.

37. The compound of any preceding clause wherein L^2 and L^3 are attached to different atoms of L^1.

38. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 is a single divalent atom selected from N, O, P, and S, where N and P are optionally substituted.

39. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 is a chain of atoms, where the length of each chain is independently selected and in the range of about 2 to about 60.

40. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes ethoxy, ethylamino, ethylene glycol, aza-ethylene glycol, $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is in the range from 2 to about 36.

41. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is 2-36.

42. The compound of any preceding clause wherein each of L^1, L^2, and L^3 includes or also includes ethoxy, ethylamino, ethylene glycol, aza-ethylene glycol, $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is 2-36.

43. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes O-alkyl-O, N-alkyl-N, C(O)-alkyl-C(O), or NC(O)-alkyl-C(O)N, or a combination thereof.

44. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes O-alkyl-C(O), N-alkyl-C(O), O-alkyl-N-alkyl-C(O), N-alkyl-O-alkyl-C(O), or C(O)alkyl-C(O), or a combination thereof.

45. The compound of any preceding clause wherein L^1 includes or also includes O-alkyl-C(O)N-diyl, O-alkyl-O-alkyl-C(O)N-diyl, or N-alkyl-O-alkyl-O-alkyl-C(O)N-diyl.

46. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes one or more amino acids.

47. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes one or more hydrophilic amino acids selected from Arg, Asn, Asp, Cys, Glu, Gln, His, Lys, Met, Orn, Ser, or Thr, including the naturally occurring L-enantiomers of each of the foregoing.

48. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes one or more hydrophilic amino acids selected from β-NH_2-Ala, Arg, Asn, Asp, Cys, Glu, Gln, His, Lys, Met, Orn, Ser, or Thr, including the naturally occurring L-enantiomers of each of the foregoing.

49. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes one or more amino acids selected from glycine, serine, proline, ornithine, and lysine.

50. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes an ornithine or lysine, including L-ornithine and L-lysine.

51. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a lysine, including L-lysine.

52. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a proline, including L-proline.

53. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_n$, including $(L\text{-}Pro)_n$, where n is 1-6.

54. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_3$, including $(L\text{-}Pro)_3$.

55. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_3$-Lys, including $(L\text{-}Pro)_3$-(L-Lys).

56. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_6$, including $(L\text{-}Pro)_6$.

57. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_6$-Lys, including $(L\text{-}Pro)_6$-(L-Lys).

58. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes glycine and serine, including L-serine.

59. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(Gly\text{-}Ser)_n$, including $[Gly\text{-}(L\text{-}Ser)]_n$, where n is 1-3.

60. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(Gly\text{-}Ser)_2$, including $[Gly\text{-}(L\text{-}Ser)]_2$.

61. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(Gly\text{-}Ser)_2$-Lys, including $[Gly\text{-}(L\text{-}Ser)]_2$-(L-Lys).

62. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(Gly\text{-}Ser)_3$, including $[Gly\text{-}(L\text{-}Ser)]_3$.

63. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes $(Gly\text{-}Ser)_3$-Lys, including $[Gly\text{-}(L\text{-}Ser)]_3$-(L-Lys).

64. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a C(O), C(O)O, C(O)NH, OC(O)NH, or NHC(O)NH group.

65. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes 1,2,3-triazol-1,4-diyl, 1,2,3-triazol-1,4-diyl, or a combination thereof.

66. The compound of any preceding clause wherein one or more of L^1, L^2, and L^3 includes or also includes a maleimid-diyl or thiomaleimid-N,S-diyl.

67. The compound of any preceding clause wherein L^2 and/or L^3 are hydrophilic.

68. The compound of any preceding clause wherein L^1 includes a region that is capable of forming an α-helical conformation.

69. The compound of any preceding clause wherein the extended conformation of L^2 and/or L^3 is at least 8 Å, 9 Å, 10 Å, 11 Å, 12 Å, 13 Å, 14 Å, 15 Å, 20 Å, 25 Å, 30 Å, 35 Å, or 40 Å, in length.

70. The compound of any preceding clause wherein L^2 and/or L^3 includes or also includes $(PEG)_n$ or aza-$(PEG)_n$, where n is in the range from about 3 to about 36, from about 4 to about 36, from about 5 to about 36, from about 6 to about 36, or from about 7 to about 36.

71. The compound of any preceding clause wherein L^2 and/or L^3 includes or also includes one or more divalent cycloalkyl, including adamantyl, heterocyclyl, including maleimidyl, aryl, heteroaryl including triazolyl, , stilbene, oligoproline, or oligopiperidine groups..

72. The compound of any preceding clause wherein the extended conformation of T-L^1 is at least 10 Å, 12 Å, 15 Å, 20 Å, 25 Å, 30 Å, 35 Å, 40 Å, 45 Å, or 50 Å in length.

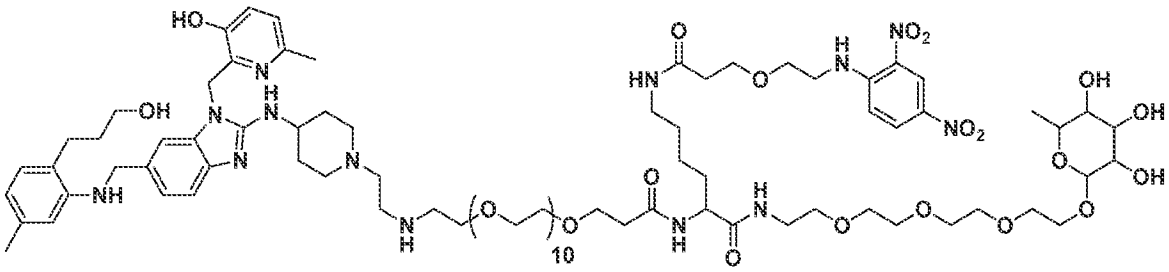
73. The compound of any preceding clause wherein L^1 includes or also includes a linear, unbranched chain of atoms having an extended conformation of at least 5 Å, 6 Å, 7 Å, 8 Å, 9 Å, 10 Å, 11 Å, 12 Å, 13 Å, 14 Å, or 15 Å in length.

74. The compound of any preceding clause wherein L^1 includes or also includes $(PEG)_n$ or aza-$(PEG)_n$, where n is in the range from about 8 to about 36, from about 9 to about 36, from about 10 to about 36, from about 12 to about 36, from about 12 to about 36.

75. The compound of any preceding clause wherein L^1 includes or also includes an hydrophobic region attached to T, and having an extended conformation of at least 3 Å, 4 Å, 5 Å, 6 Å, 7 Å, 8 Å, 9 Å, 10 Å, 11 Å, 12 Å, or 13 Å in length.

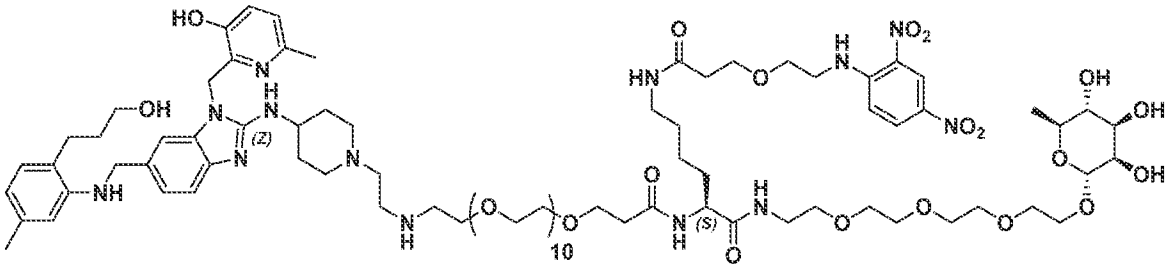
76. A compound of any formula shown in FIG 1 or a pharmaceutically acceptable salt or solvate thereof.

77. A compound of the formula



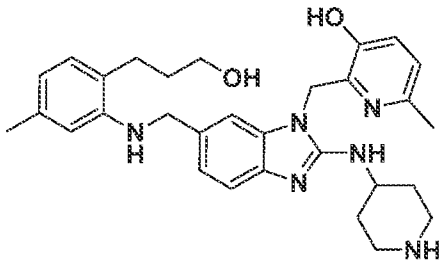
or a pharmaceutically acceptable salt or solvate thereof.

78. A compound of the formula



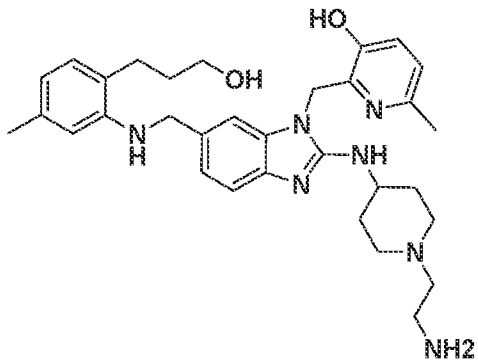
or a pharmaceutically acceptable salt or solvate thereof.

79. A compound of the formula



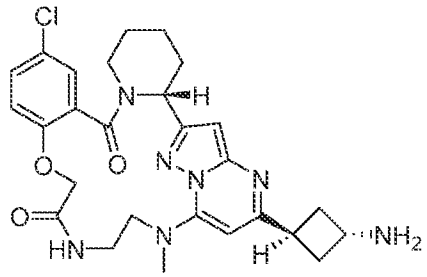
or a pharmaceutically acceptable salt or solvate thereof.

80. A compound of the formula



or a pharmaceutically acceptable salt or solvate thereof.

81. A compound of the formula



or a pharmaceutically acceptable salt or solvate thereof.

[0031] The foregoing illustrative compounds, as defined by clauses 1-81 may be included in any of the compositions, unit doses, uses, or methods described herein.

CONJUGATES

[0032] The conjugate compounds described herein may be formed from a ligand that is capable of targeting a protein of a RSV-infected cell. In many embodiments, the protein is on the surface of a RSV-infected cell, or is otherwise accessible from the surface of a RSV-infected cell. The conjugates described herein may also be formed from two or more haptens, each comprising an immune system responsive epitope or antigen. The conjugates described herein may also be formed from a polyvalent linker that covalently attaches the ligand to the two or more haptens. Described herein are numerous selections for each of the ligand, the haptens, and the linker. Each of the ligands and haptens described herein may be proteinaceous or small molecules. Each of the linkers described herein may be proteinaceous or small molecules, or combinations thereof. It is to be understood that any ligand, any haptens, and any linker described herein may be combined to form the conjugates. In addition, the linkers themselves are formed from various building blocks, including single atoms, functional groups, and chemical fragments. It is to be understood that every combination of building blocks is described herein for forming the linkers included in the conjugates.

[0033] In some embodiments, the conjugates have a dual mechanism of action, where the conjugate inhibits the virus, and also labels or decorates virus-infected cells for intervention by the immune system of the host animal, including humans. The conjugates

elicit host animal immune response against the virus or virus-infected cell by recruiting antibodies in the host animal. Illustratively, the antibodies are endogenous.

[0034] Without being bound by theory, it is believed that once recruited, the anti-hapten antibodies bind to the hapten and activate the innate immune system against the target virus and virus-infected cells. The haptens may be the same or different. In the conjugates where the haptens are the same, the immune system of the host animal may be multiplied. In the conjugates where the haptens are different, the immune system of the host animal may respond in multiple ways. The conjugates described herein provide a therapeutic delivery system for selectively or specifically delivering haptens to target virus and virus-infected cells. In certain embodiments, the haptens are selected to activate the innate immune system of the subject to recruit immune cells and/or otherwise leverage the subject's own immune system against the virus. The targeting ligand can selectively or specifically recognize a target protein or receptor, such as an envelope protein of a virus, which can be highly or exclusively expressed on the virus, the surface of an infected cell, or accessible from the surface of an infected cell.

[0035] In other embodiments, the conjugate compound of Formula I can have fragments L^2-A^1 and L^3-A^2 bound to the same atom on L^1. In some embodiments, the same atom is not a carbon atom. In some embodiments, the same atom is a nitrogen atom. Alternatively, the conjugate of Formula I can have fragments L^2-A^1 and L^3-A^2 bound to a different atom on L^1.

[0036] The compounds, compositions, unit doses, and methods described herein are useful in treating RSV infections, including all strains of RSV, including resistant strains of RSV including, for example, RSV-A and RSV-B.

[0037] Without being bound by theory, it is believed that the method described herein can elicit an immune response leading to clearance of an antibody (Ab)-coated virus or an Ab-coated-virally infected cell via Ab-dependent cellular phagocytosis (ADCP), Ab-dependent cellular cytotoxicity (ADCC), and/or complement-dependent cytotoxicity (CDC) which works in conjunction with the inhibition of viral budding by neuraminidase inhibition leading to viral eradication. The method for activating an immune response can further

comprise administering to the subject autologous antibodies, allogeneic IgG antibodies, or human IVIG. In these and other embodiments, the subject may be further treated with anti-hapten antibodies.

[0038] The present disclosure includes conjugates comprising small-molecule inhibitors of F glycoprotein (F protein) of respiratory syncytial virus (RSV) and their use in the treatment of RSV. The small-molecule inhibitors of F protein are used as ligands to target a conjugated agent to F protein present on the surface of RSV and RSV-infected cells. When the conjugate binds to F protein on the virus, it prevents the virus from binding to nucleolin on the surface of a cell and entering the cell.

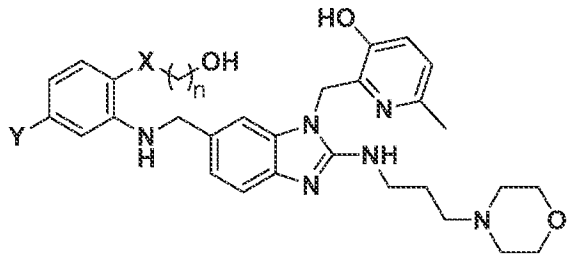
[0039] In one example, the RSV targeting ligand is a RSV fusion (F) glycoprotein ligand, or a compound capable of binding to a fusion (F) glycoprotein.

[0040] In one example, the RSV targeting ligand is a RSV attachment glycoprotein (G) ligand, or a compound capable of binding to a RSV attachment glycoprotein (G).

[0041] In many embodiments, illustrative ligands include compounds that target RSV fusion (F) glycoprotein. Illustrative ligands also include compounds that target RSV attachment glycoprotein (G).

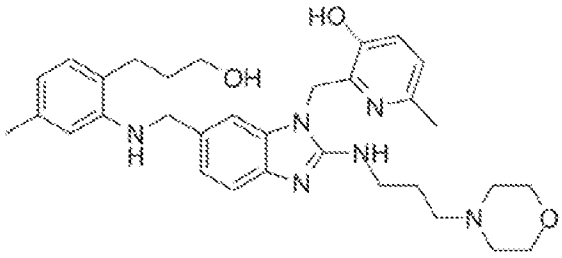
[0042] In many embodiments, illustrative ligands include compounds that are inhibitors of RSV fusion (F) glycoprotein, including RSV fusion inhibitors and compounds that inhibit the six-helix bundle formation. Illustrative ligands include compounds that are inhibitors of RSV attachment glycoprotein (G).

[0043] In many embodiments, the ligand is of the formula



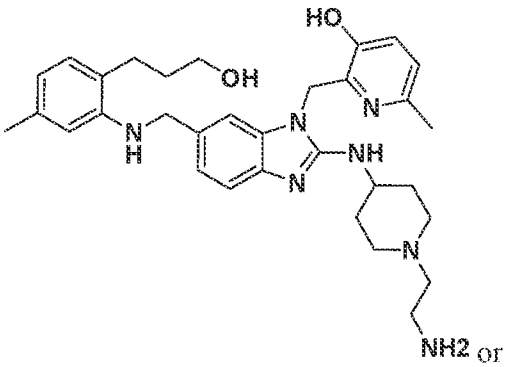
where X is CH_2, O, S, or SO_2; n is 1 or 2 with the proviso that if X is not CH_2, n is 2; and Y is alkyl, halo, CN, alkoxy, alkyl-OH, or H.

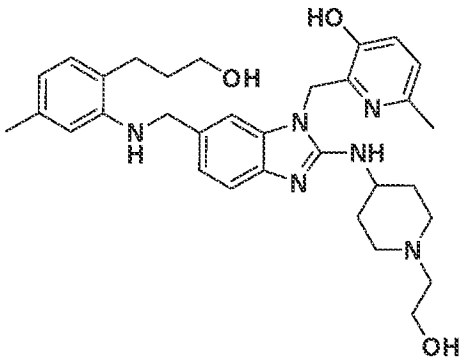
[0044] In many embodiments, the ligand is TMC353121



or an analog or derivative thereof, such as compounds disclosed in WO 2005/058869, WO 2005/058871, WO 20025/058874, and the like.

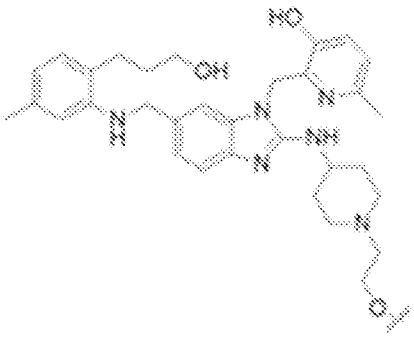
[0045] In many embodiments, the ligand is of the formula

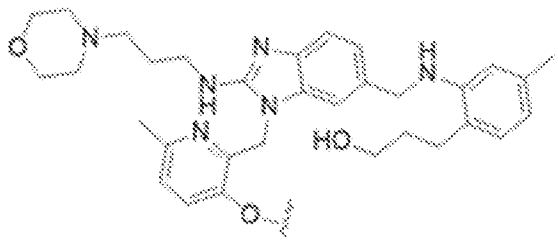




or analogs or derivatives thereof, such as compounds disclosed in Roymans et al. PNAS 107(1):208-13 (2010), such as compounds disclosed in WO 2005/058869, WO 2005/058871, WO 20025/058874, and the like and the like.

[0046] In many embodiments, T is a radical of the formula

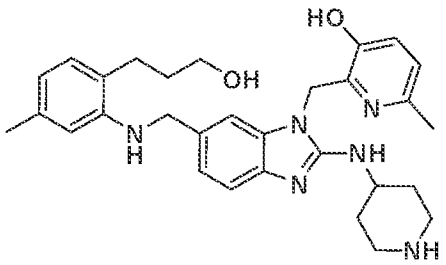




or

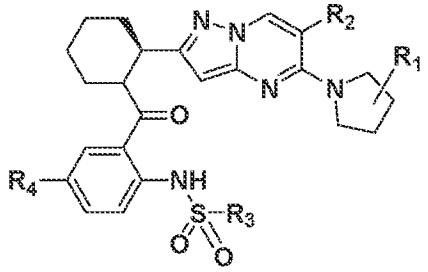
or an analog or derivative thereof.

[0047] In many embodiments, the ligand is of the formula



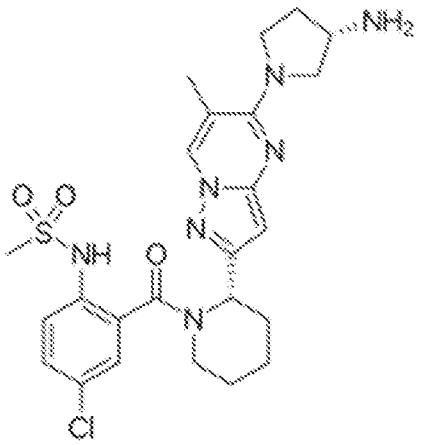
or an analog or derivative thereof.

[0048] In many embodiments, the ligand is of the formula



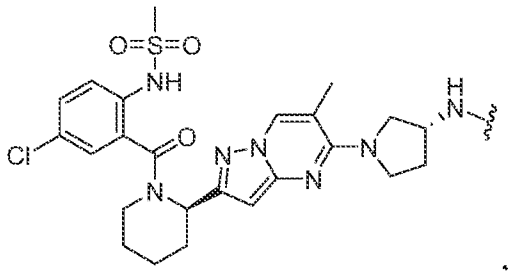
where R_1 is NH_2, NHRn, where Rn is alky, amino, hydroxyalkyl, OR, or OH; R_2 is alkyl (C-1 to C-4), halo, CN, N_3, OR; R_3 is alkyl (C-1 to C-4); and R_4 is alkyl (C-1 to C-4), halo, CN, N_3, OR.

[0049] In many embodiments, the ligand is presatovir

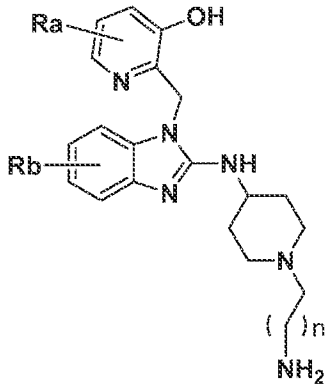


or an analog or derivative thereof, such as compounds disclosed in Mackman et al. J Med Chem 58:1630-43 (2015), WO 2011/163518, and the like.

[0050] In many embodiments, T is a radical of the formula

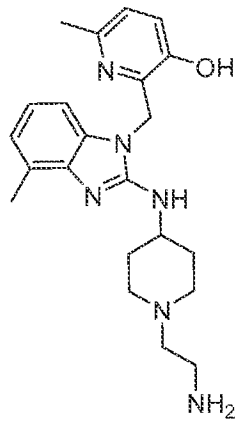


[0051] In many embodiments, the ligand is of the formula



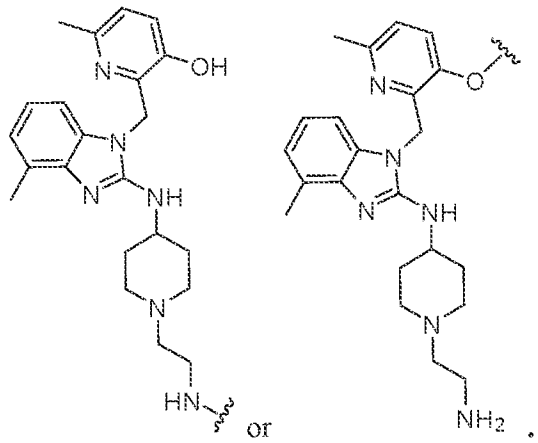
wherein Ra and Rb are independently selected from the group alkyl (C1- to C4), alkoxyl (C1- to C4), halo, cyano, and azido; and n is 1, 2, or 3.

[0052] In many embodiments, the ligand is of the formula

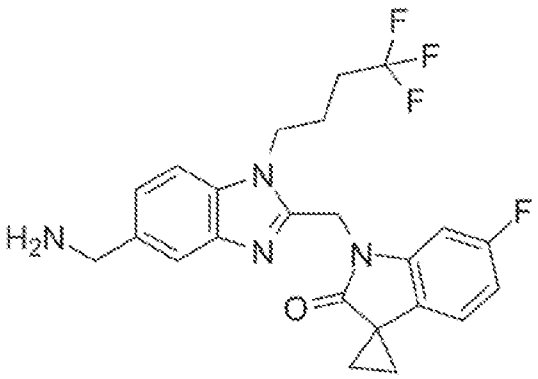


or an analog or derivative thereof, such as compounds disclosed in Bonfanti et al. J Med Chem 51:875-96 (2008), Andries at al. J Antiviral Res 60:209-19 (2003), WO 2001/000611, and the like.

[0053] In many embodiments, T is a radical of the formula

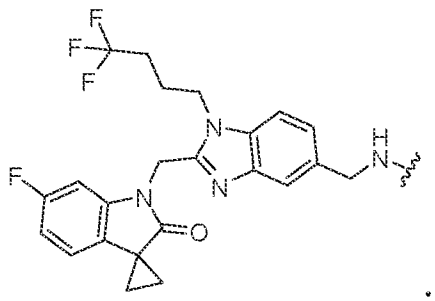


[0054] In many embodiments, the ligand is susinatovir

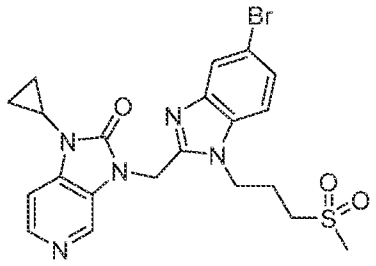


or an analog or derivative thereof, such as compounds disclosed in Cockerill et al. J Med Chem 64:3658-76 (2021), and the like.

[0055] In many embodiments, T is a radical of the formula

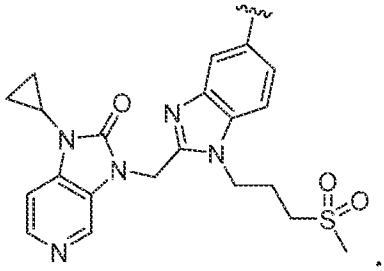


[0056] In many embodiments, the ligand is JNJ-49153390

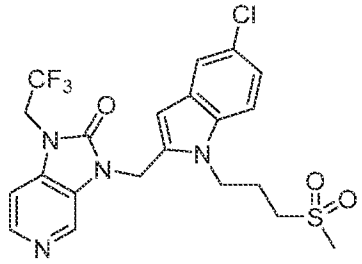


or an analog or derivative thereof.

[0057] In many embodiments, T is a radical of the formula

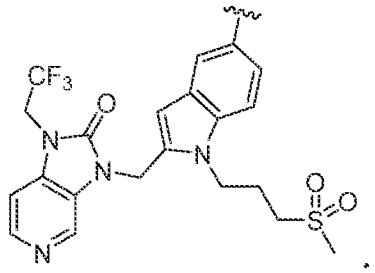


[0058] In many embodiments, the ligand is of the formula

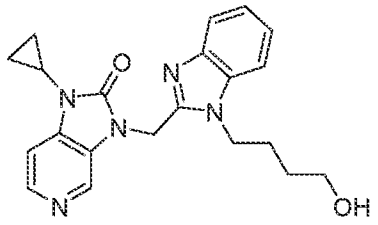


or an analog or derivative thereof.

[0059] In many embodiments, T is a radical of the formula

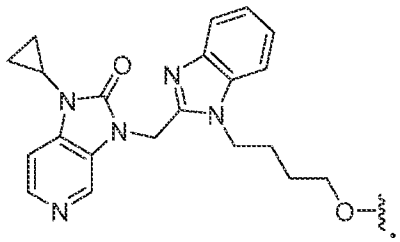


[0060] In many embodiments, the ligand is BMS-433771

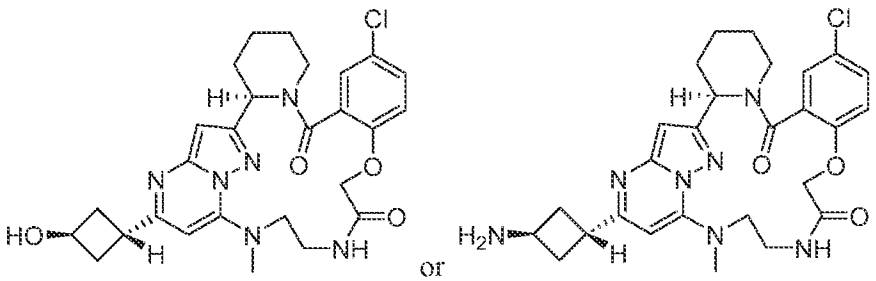


or an analog or derivative thereof.

[0061] In many embodiments, T is a radical of the formula

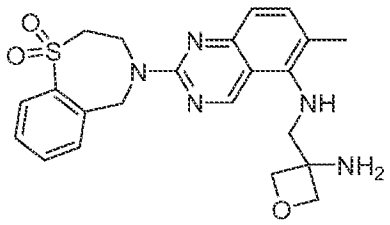


[0062] In many embodiments, the ligand is of the formula



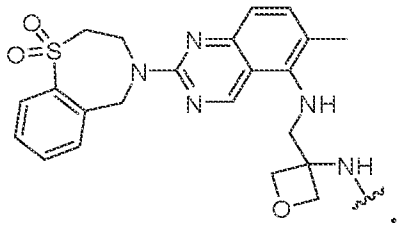
or an analog or derivative thereof, such as compounds disclosed in Yamaguchi-Sasaki et al. ACS Med Chem Lett 11:1145-51 (2020), and the like.

[0063] In many embodiments, the ligand is Ziresovir

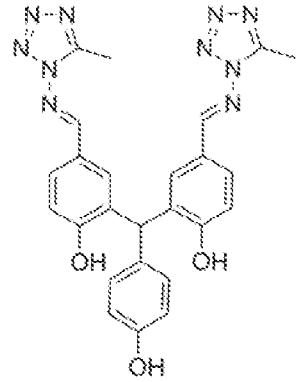


or an analog or derivative thereof.

[0064] In many embodiments, T is a radical of the formula

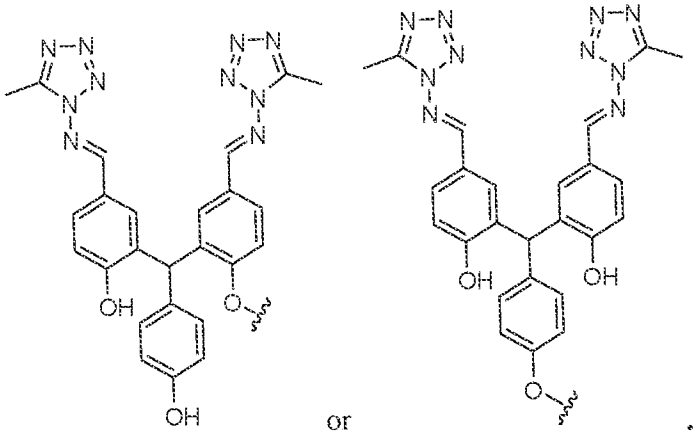


[0065] In many embodiments, the ligand is VP-14637

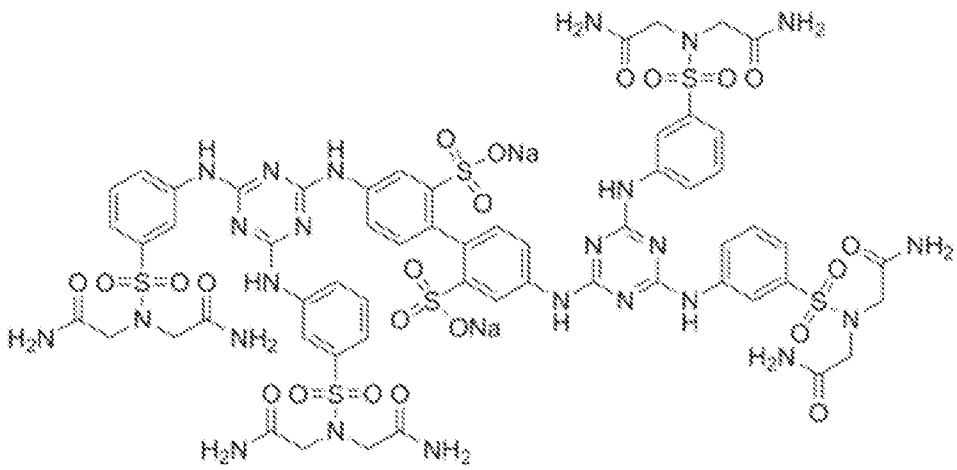


or an analog or derivative thereof.

[0066] In many embodiments, T is a radical of the formula

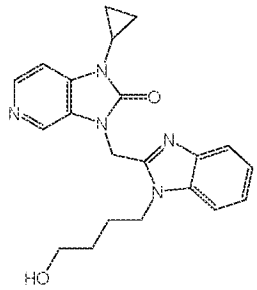


[0067] In many embodiments, the ligand is RFI-641



or an analog or derivative thereof.

[0068] In many embodiments, the ligand is risetovir



or an analog or derivative thereof.

[0069] In many embodiments, the ligand is selected from risetovir, BMS-433771, rilematovir, ziresovir, or an analog or derivative of the foregoing, such as compounds disclosed in Zheng et al. J Med Chem 62:6003-14 (2019), and the like, RSV-IN-1, RSV-IN-5, or an analog or derivative of the foregoing, such as compounds disclosed in Lundin et al. Antiviral Res 88:317-24 (2010), and the like, YM 53403, or BTA-9981, and analogs and derivatives thereof.

[0070] It is to be understood that a radical may be formed on any atom of any of the foregoing ligands to form the radical T.

[0071] In many embodiments, the ligand is not a folate.

[0072] In many embodiments, the ligand is a peptide, such as a peptide having SEQ ID NO: 1, or a fragment thereof, including compounds described in Lambert et al. PNAS 93:2186-91 (1996); or a peptide having SEQ ID NO: 18, or a fragment thereof, including compounds

described in Outlaw et al. JACS 142:2140-44 (2020).

[0073] In many embodiments, the ligand is SEQ ID NO: 16 (T118); SEQ ID NO: 34 (T205), SEQ ID NO: 40 (VIQKI), or a substitution isomer selected from VIQKI-I456F (SEQ ID NO: 41) and VIQKI-I454F/I456F SEQ ID NO: 42).

[0074] In many embodiments, the ligand is VALDPIDISIVLNKIKSQLEESKEWIRRSNKILDSI (VIQKI) (SEQ ID NO: 40), VALDPIDFSIVLNKIKSQLEESKEWIRRSNKILDSI (VIQKI-I456F) (SEQ ID NO: 41), or VALDPIDFSFVLNKIKSQLEESKEWIRRSNKILDSI (VIQKI-I454F/I456F) (SEQ ID NO: 42), and analogs and derivatives thereof.

[0075] Illustrative peptide ligands also include GEPIINFYOPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTT (SEQ ID NO: 1), and fragments thereof, including but not limit to

IINFYDPLVFPSDEFDASISQVNEKINQSLAFIRK (SEQ ID NO: 2)
INFYDPLVFPSDEFDASISQVNEKINQSLAFIRKS (SEQ ID NO: 3)
NFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSD (SEQ ID NO: 4)
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE (SEQ ID NO: 5)
YDPLVFPSDEFDASISQVNEKINQSLAFIRKSDEL (SEQ ID NO: 6)
DPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL (SEQ ID NO: 7)
PLVFPSDEFDASISQVNEKINQSLAFIRKSDELLH (SEQ ID NO: 8)
LVFPSDEFDASISQVNEKINQSLAFIRKSDELLHN (SEQ ID NO: 9)
VFPSDEFDASISQVNEKINQSLAFIRKSDELLHNV (SEQ ID NO: 10)
FPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN (SEQ ID NO: 11)
PSDEFDASISQVNEKINQSLAFIRKSDELLHNVNA (SEQ ID NO: 12)
SDEFDASISQVNEKINQSLAFIRKSDELLHNVNAG (SEQ ID NO: 13)
DEFDASISQVNEKINQSLAFIRKSDELLHNVNAGK (SEQ ID NO: 14)
EFDASISQVNEKINQSLAFIRKSDELLHNVNAGKS (SEQ ID NO: 15)
FDASISQVNEKINQSLAFIRKSDELLHNVNAOKST (SEQ ID NO: 16)
DASISQVNEKINQSLAFIRKSDELLHNVNAGKSTT (SEQ ID NO: 17)

[0076] Illustrative peptide ligands also include

YTPND0ITLNNSVALDPIDISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT (SEQ ID NO: 18)

and fragments thereof, including but not limit to

YTPNDITLNNSVALDPIDISIELNKAKSDLEESKE (SEQ ID NO: 19)

TPNDITLNNSVALDPIDISIELNKAKSDLEESKEW (SEQ ID NO: 20)

PNDITLNNSVALDPIDISIELNKAKSDLEESKEWI (SEQ ID NO: 21)

NDITLNNSVALDPIDISIELNKAKSDLEESKEWIR (SEQ ID NO: 22)

DITLNNSVALDPIDISIELNKAKSDLEESKEWIRR (SEQ ID NO: 23)

ITLNNSVALDPIDISIELNKAKSDLEESKEWIRRS (SEQ ID NO: 24)

TLNNSVALDPIDISIELNKAKSDLEESKEWIRRSN (SEQ ID NO: 25)

LNNSVALDPIDISIELNKAKSDLEESKEWIRRSNQ (SEQ ID NO: 26)

NSVALDPIDISIELNKAKSDLEESKEWIRRSNQKL (SEQ ID NO: 27)

SVALDPIDISIELNKAKSDLEESKEWIRRSNQKLD (SEQ ID NO: 28)

VALDPIDISIELNKAKSDLEESKEWIRRSNQKLDS (SEQ ID NO: 29)

ALDPIDISIELNKAKSDLEESKEWIRRSNQKLDSI (SEQ ID NO: 30)

LDPIDISIELNKAKSDLEESKEWIRRSNQKLDSIG (SEQ ID NO: 31)

DPIDISIELNKAKSDLEESKEWIRRSNQKLDSIGN (SEQ ID NO: 32)

PIDISIELNKAKSDLEESKEWIRRSNQKLDSIGNW (SEQ ID NO: 33)

IDISIELNKAKSDLEESKEWIRRSNQKLDSIGNWH (SEQ ID NO: 34)

DISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQ (SEQ ID NO: 35)

ISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQS (SEQ ID NO: 36)

SIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSS (SEQ ID NO: 37)

IELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSST (SEQ ID NO: 38)

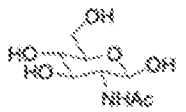
ELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT (SEQ ID NO: 39)

[0077] It is to be understood that radicals may be formed at the N-terminus, the C-terminus, or on an interior atom on the peptide chain to form the radical T. It is also to be understood that other radicals may be formed on a side chain of an amino acid to form the radical T.

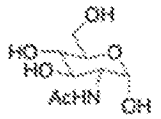
[0078] The haptens described herein can each be bound by an antibody. After administration to a subject, each hapten (A^1 and A^2) can be bound by an antibody. In

certain embodiments, the two haptens, A^1 and A^2 can each be bound by a different antibody after administration to a subject. It is to be understood that any hapten, including peptide sequences, that elicit an immunological response in the host animal may be used to form the conjugates described herein. It is to be further understood an immunological response in the host animal may be elicited or supplemented via vaccination as a co-therapy to the methods and uses described herein.

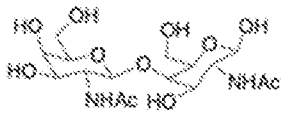
[0079] In another embodiment, A^1 and A^2 are each an independently selected radical of an antigen where for each antigen there is an endogenous antibody present in the host animal. Illustrative haptens include, but are not limited to, a rhamnose, including L-rhamnose, a nitrophenyl, a nitrophenol, a nitroaniline, a dinitrophenyl (DNP), a dinitrophenol, a dinitroaniline, a trinitrophenyl (TNP), a trinitrophenol, a trinitroaniline, chloronitrophenyl, a chloronitrophenol, a chloronitroaniline, an iodonitrophenyl, an iodonitrophenol, an iodonitroaniline, a nitrotyrosine, an hydroxynitrotyrosine, an aminonitrotyrosine, 4-hydroxy-3-nitrophenyl acetic acid, an α-galactosyl moiety, a sulfated Gal, compounds of the formulae



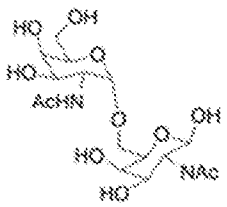
β-GlcNac



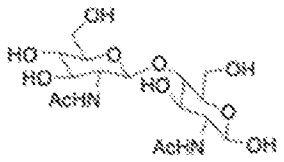
α-GlcNAc



GalNAc β(1-4) GalNAc



GalNAcα(1-3)GalNAc



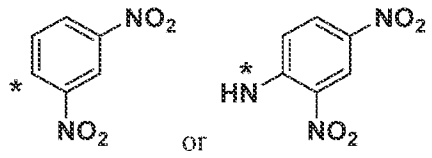
GlcNAc β(1-4) GlcNAc

,

a phosphorylcholine, a bacterial antigen, a viral antigen, and the like, and any combination of two or more of the foregoing.

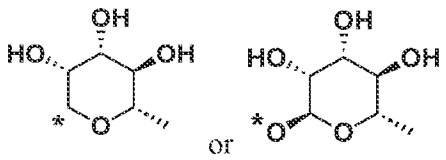
[0080] In many embodiments, the hapten is selected from a dinitrophenyl (DNP), a dinitrophenol, and a dinitroaniline. In many embodiments, A^1 and/or A^2 is a radical of a

dinitrophenyl (DNP), a dinitrophenol, or a dinitroaniline, including radicals of the formulae



where L^2 or L^3 is attached at (*).

[0081] In many embodiments, the hapten is selected from a rhamnose, including L-rhamnose. In many embodiments, A^1 and/or A^2 is a radical of a rhamnose, including L-rhamnose, and including a radicals of the formulae



where L^2 or L^3 is attached at (*).

[0082] In many embodiments, A^1 is a DNP radical and A^2 is a rhamnose radical, or vice versa. In many embodiments, A^1 is a DNP radical and A^2 is an L-rhamnose radical, or vice versa.

[0083] In another embodiment, neither A^1 nor A^2 comprises a fluorescein, such as FITC, and the like.

[0084] As used herein, the term "linker" generally refers to a chain of atoms that connects two or more functional parts of a molecule to form a conjugate. Illustratively, the chain of atoms is selected from C, N, O, S, Si, and P, or C, N, O, S, and P, or C, N, O, and S. The chain of atoms covalently connects different functional capabilities of the conjugate. The linker may have a wide variety of lengths, such as in the range from about 2 to about 100 atoms in the contiguous backbone. The atoms used in forming the linker may be combined in all chemically relevant ways, such as chains of carbon atoms forming alkylene, alkenylene, and alkynylene groups, and the like; chains of carbon and oxygen atoms forming ethers, polyoxyalkylene groups, or when combined with carbonyl groups forming esters and carbonates, and the like; chains of carbon and nitrogen atoms forming amines, imines, polyamines, hydrazines, hydrazones, or when combined with carbonyl groups forming amides, ureas, semicarbazides, carbazides, and the like; chains of carbon,

nitrogen, and oxygen atoms forming alkoxyamines, alkoxylamines, or when combined with carbonyl groups forming urethanes, amino acids, acyloxylamines, hydroxamic acids, and the like; and many others. In addition, it is to be understood that the atoms forming the chain in each of the foregoing illustrative embodiments may be either saturated or unsaturated, thus forming single, double, or triple bonds, such that for example, alkanes, alkenes, alkynes, imines, and the like may be radicals that are included in the linker. In addition, it is to be understood that the atoms forming the linker may also be cyclized upon each other or be part of cyclic structure to form divalent cyclic structures that form the linker, including cycloalkanes, cyclic ethers, cyclic amines, and other heterocycles, arylenes, heteroarylenes, and the like in the linker. In this latter arrangement, it is to be understood that the linker length may be defined by any pathway through the one or more cyclic structures. Illustratively, the linker length is defined by the shortest pathway through the each one of the cyclic structures. It is to be understood that the linkers may be optionally substituted at any one or more of the open valences along the chain of atoms, such as optional substituents on any of the carbon, nitrogen, silicon, or phosphorus atoms. It is also to be understood that the linker may connect the two or more functional parts of a molecule to form a conjugate at any open valence, and it is not necessary that any of the two or more functional parts of a molecule forming the conjugate are attached at any apparent end of the linker.

[0085] In many embodiments, L^1, L^2, and L^3 each, independently comprises a chain of atoms from 3 atoms to about 60 atoms in length. In many embodiments, L^1, L^2, and L^3 each, independently comprises a chain of atoms from about 4 Å to about 72 Å in length. The chain of atoms are part of the backbone of the conjugate of Formula I. As used herein, the term "backbone" of the linker L refers to the shortest chain of contiguous atoms forming a covalently bonded connection between T and L^2, between T and L^3, between A^1 and L^1, or between A^2 and L^1.

[0086] In many embodiments, L^1, L^2, or L^3 each, independently, comprise a chain of atoms at least 3 atoms in length, at least 7 atoms in length, at least 10 atoms in length, at least 14 atoms in length, or at least 20 atoms in length. In many embodiments, L^1,

L^2, or L^3 each, independently, have a chain of between 3 and 7 atoms in length, between 7 and 10 atoms in length, between 10 and 14 atoms in length, between 14 and 20 atoms in length, between 20 and 30 atoms in length, between 30 and 40 atoms in length, between 40 and 50 atoms in length, or between 50 and 60 atoms in length. In many embodiments, L^1, L^2, or L^3 each, independently, comprise a chain of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 , 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 atoms in length. As used herein, the term "between" is inclusive of the endpoints meaning that between 3 and 5 atoms in a chain length includes 3 atoms, 4 atoms, and 5 atoms.

[0087] It is to be understood that while both L^2 and L^3 are covalently attached to L^1, both L^2 and L^3 may be attached to the same atom of L^1, or L^2 and L^3 may each be attached to different atoms of L^1. In another embodiment, L^2 and L^3 are attached to the same atom on L^1 where that atom is not a carbon atom.

[0088] In many embodiments, L^1, L^2, or L^3 can each, independently, comprise one or more amino acid or peptide residues.

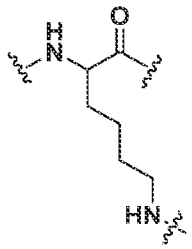
[0089] As used herein, the term "amino acid" refers generally to beta, gamma, and longer amino acids, and including cyclic groups, that have both an amino group and an acid group from each of which a radical can be formed. Illustrative acyclic amino acids include the formula

$$-N(R)-(CR'R'')_q-C(O)-$$

where R is hydrogen, alkyl, acyl, or a suitable nitrogen protecting group, R′ and R″ are hydrogen or a substituent, each of which is independently selected in each occurrence, and q is an integer such as 1, 2, 3, 4, or 5. Illustratively, R′ and/or R″ independently correspond to, but are not limited to, hydrogen or the side chains present on naturally occurring amino acids, such as methyl, benzyl, hydroxymethyl, thiomethyl, carboxyl, carboxylmethyl, guanidinopropyl, and the like, and derivatives and protected derivatives thereof. The above described formula includes all stereoisomeric variations. For example, the amino acid may be selected from asparagine, aspartic acid, cysteine, glutamic acid,

lysine, glutamine, arginine, serine, ornithine, threonine, and the like.

[0090] For example, L^1, L^2, or L^3 can each, independently, comprise one or more lysine residues, each of which is independently optionally substituted. For example, L^1 can comprise a lysine residue of the formula



wherein A^1 or A^2 is attached to the lysine residue via a linker. In another embodiment, the lysine residue is L-Lys. In one example, L^1 comprises at least one lysine residue, such as L-Lys.

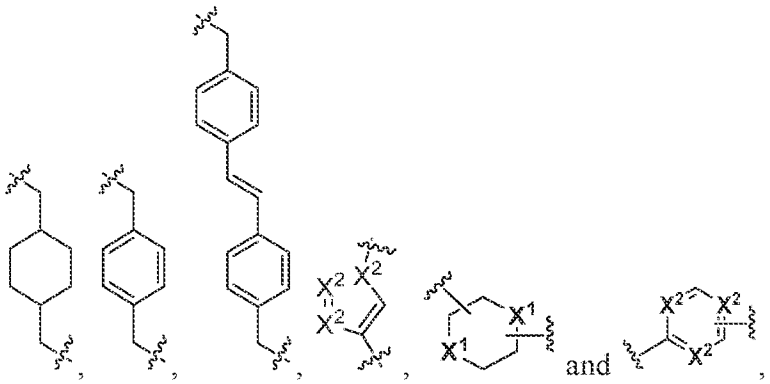
[0091] In many embodiments, L^1, L^2, or L^3 can each, independently, comprise a polyethylene glycol$_n$ (PEG_n) moiety, wherein n = 1-36. In many embodiments, a linker comprises one or more PEG moieties where all carbon and oxygen atoms of the one or more PEG moieties are part of the backbone of the linker.

[0092] In many embodiments, L^1, L^2, or L^3 can each, independently, comprise one or more alkyl groups.

[0093] In many embodiments, L^1, L^2, or L^3 can each, independently, comprise one or more or sugar moieties, glycan residues, or peptidoglycan residues.

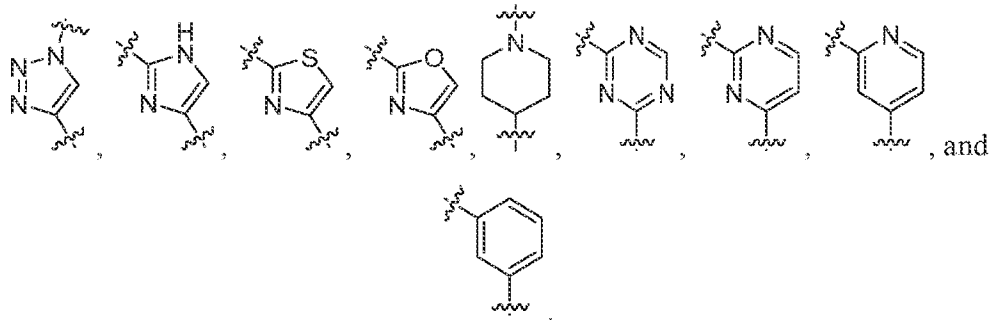
[0094] It is to be understood that the bonds connecting atoms in the chain can be either saturated or unsaturated, such that for example, alkanes, alkenes, alkynes, cycloalkanes, arylenes, imides, and the like can be divalent radicals that are included in L. In each of the foregoing , the atoms of L in the chain can be substituted or unsubstituted.

[0095] In addition, it is to be understood that the atoms forming the linker may also be cyclized to form saturated or unsaturated divalent cyclic radicals in the linker, such as radicals of the formulae:



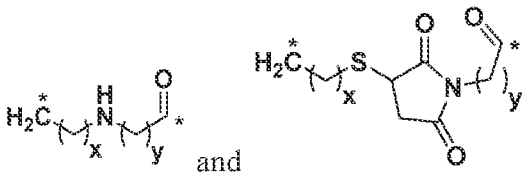
wherein each X^1 is independently CH_2, NR', or O wherein R' is alkyl or hydrogen and each X^2 is independently S, O, N, NH, CR" wherein R" is alkyl or hydrogen.

Examples of such radicals include:



[0096] In some embodiments, L^1, L^2, or L^3 comprises suitable substituents that change the hydrophobicity or hydrophilicity of the linker. Illustrative hydrophobic groups include alkyl, cycloalkyl, aryl, and arylalkyl, each of which is optionally substituted.

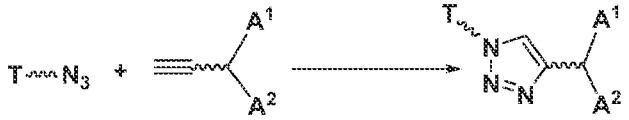
[0097] L^1, L^2, or L^3 can each, independently, comprise alkylene-amino-alkylenecarbonyl, alkylene-thio-(carbonylalkylsuccinimid-3-yl) moieties, including the following formulae:



wherein x and y are each independently 1, 2, 3, 4, or 5, where the asterisk identifies points of attachment either to other linker fragments of to T or A^1 or A^2.

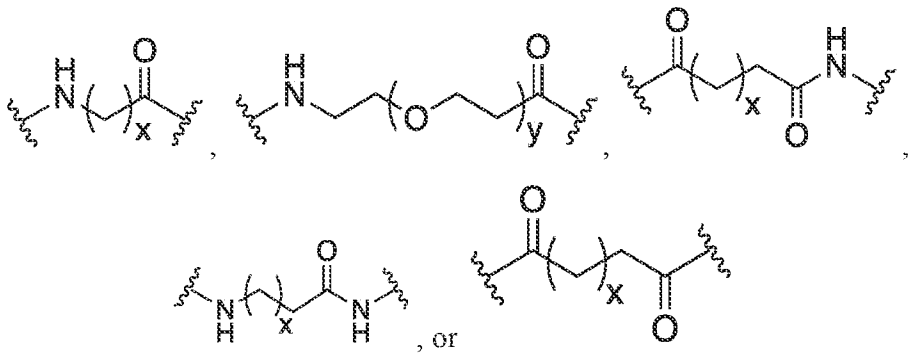
[0098] Additional examples of groups which may be present in the linkers include 1-alkylsuccinimid-3-yl, carbonyl, thionocarbonyl, alkyl, cycloalkyl, alkylcycloalkyl, alkylcarbonyl, cycloalkylcarbonyl, carbonylalkylcarbonyl, 1-alkylsuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl, alkylsulfoxyl, sulfonylalkyl, alkylsulfoxylalkyl, alkylsulfonylalkyl, carbonyltetrahydro-2H-pyranyl, carbonyltetrahydrofuranyl, 1-(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl, and 1-(carbonyltetrahydrofuranyl)succinimid-3-yl, wherein each group can be substituted or unsubstituted. In some embodiments, one or more of the aforementioned groups can be used in combination (or more than once) (e.g., -alkyl-C(O)-alkyl) and may further comprise an additional nitrogen (e.g., alkyl-C(O)-NH-, -NH-alkyl-C(O)- or -NH-alkyl-), oxygen (e.g., -alkyl-O-alkyl-) or sulfur (e.g., -alkyl-S-alkyl-). Examples include alkylcarbonyl, cycloalkylcarbonyl, carbonylalkylcarbonyl, 1-(carbonylalkyl)succinimid-3-yl, and succinimid-3-ylthiol, wherein each group can be substituted or unsubstituted.

[0099] In some embodiments, L^1, L^2, or L^3 can be formed via click chemistry or be click chemistry-derived. For example, L^1, L^2, or L^3 can be derived from copper-catalyzed azide-alkyne cycloaddition (CuAAC), strain promoted azide-alkyne cycloaddition (SPAAC), inverse electron demand Diels-Alder reaction (IEDDA), and Staudinger ligation (SL). For example, T can be a moiety of the formula T-N_3. T-N_3 can then be reacted with an alkyne as shown in the following Scheme:



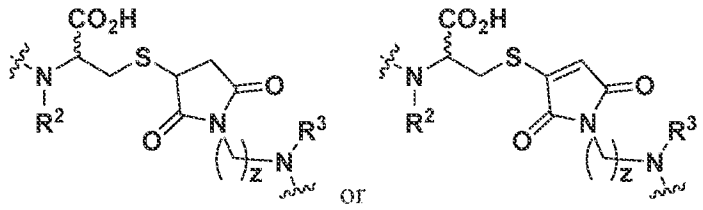
where the wavy line connected to T and to A^1/A^2 represents a linker between T and A^1/A^2 and the groups to which they are attached.

[0100] In other embodiments, L^1, L^2, or L^3 can include



where x is an integer from 0 to 50 and y is an integer from 0 to 50

[0101] In other embodiments, L^1, L^2, or L^3 can include



wherein each of R^2 and R^3 is independently H or C_{1-6}alkyl; and z is an integer from 1 to 8.

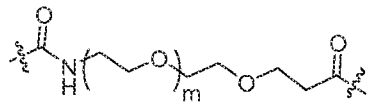
[0102] In other embodiments, L^1, L^2, or L^3 can include an amide, ester, urea, carbonate, carbamate, amino acid, amine, ether, alkyl, alkene, alkyne, heteroalkyl cycloalkyl, aryl, heterocycloalkyl, heteroaryl, carbohydrate, glycan, peptidoglycan, polypeptide, or any combination thereof. In some embodiments, L^1, L^2, or L^3 can include a glycosylated amino acid. In some embodiments, L^1, L^2, or L^3 can include one or more monosaccharide, disaccharide, polysaccharide, glycan, or peptidoglycan. In some embodiments, L^1, L^2, and L^3 do not comprise a glycan. In some embodiments, L^1, L^2, and L^3 do not comprise a sugar.

[0103] In some embodiments, L^1, L^2, or L^3 can include a rigid functionality such as an oligoproline or oligopiperidine.

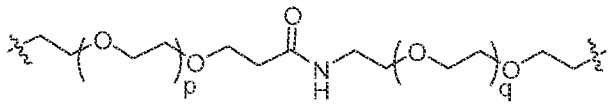
[0104] In some embodiments, an oligoproline or oligopiperidine has about two up to and including about fifty, about two to about forty, about two to about thirty, about two to about twenty, about two to about fifteen, about two to about ten, or about two to about six repeating units (e.g., prolines or piperidines).

[0105] In some embodiments, L^1, L^2, or L^3 can comprise $(-CH_2CH_2-O-)_n$, where n is an integer between and including 1 and 36 (e.g., 1 to 2, 2 to 6, 3 to 8, 6 to 12, and 4 to 10) a peptide, an alkylamido group (e.g., $C(O)N(H)C_2$-C_{18} alkyl- or C_2-C_{18} alkyl-$C(O)N(H)$-), an alkylamidoalkyl group (e.g., a C_2-C_{18} alkyl-$C(O)N(C_2$-C_{18} alkyl$)_2$ or a C_2-C_{18} alkyl-$C(O)N(H)$-C_2-C_{18} alkyl group, such as a $-CH_2CH_2C(O)N(CH_2CH_2)_2$ or a $-CH_2CH_2C(O)N(H)(CH_2CH_2)$- group), or a combination of two or more of the foregoing.

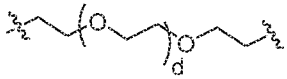
[0106] In some embodiments, L^1, L^2, or L^3 can comprise



wherein m is an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, m can be 0 or 1. Or m can be 5 or 6. Or m can be 7 or 8. In some embodiments, L^1, L^2, or L^3 can comprise

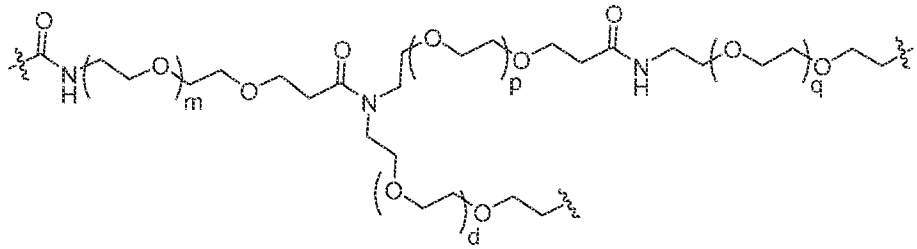


wherein p and q are each, independently, an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, p can be 2 or 3. For example, q can be 2 or 3. In some embodiments, L^1, L^2, or L^3 can comprise

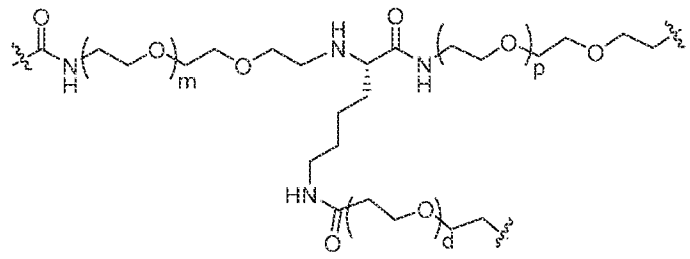


wherein d is an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, d can be 1, 2 or 3.

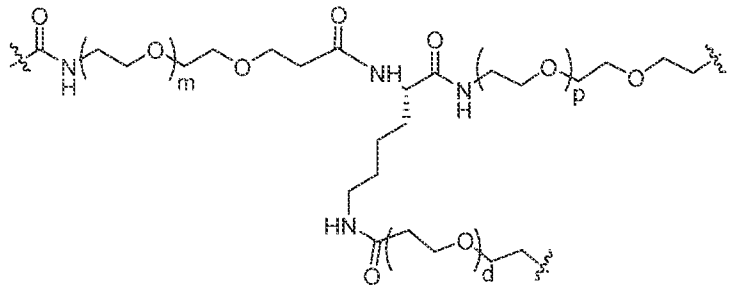
[0107] In some embodiments, L^1, L^2, and L^3 taken together can comprise



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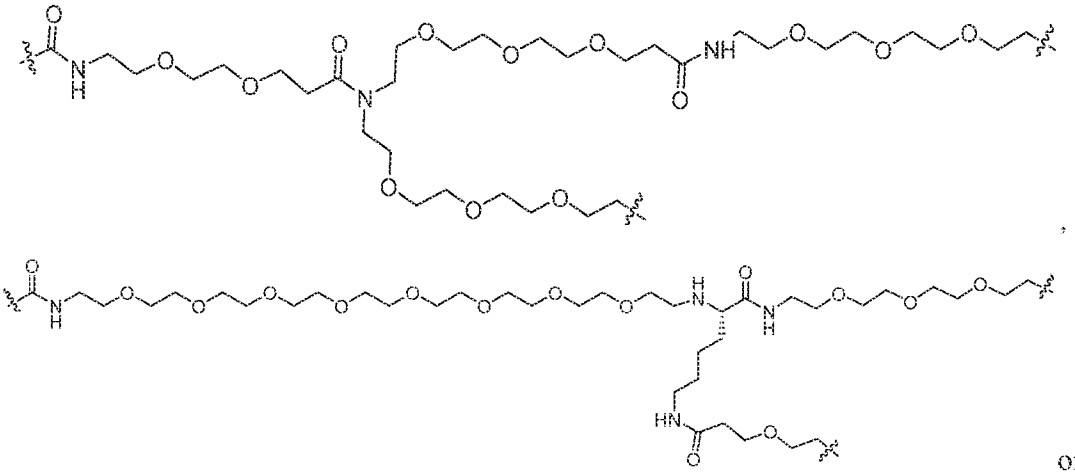
, or



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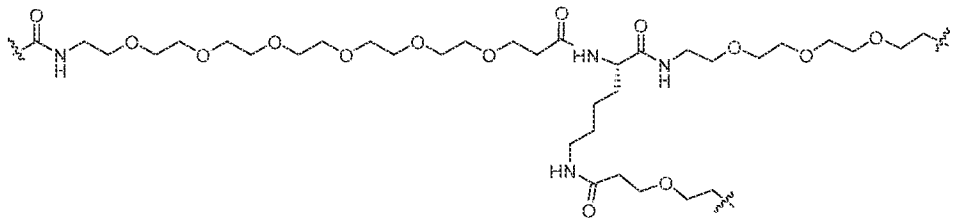
such as wherein m, p, d, and q are each, independently, an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, m can be 0 or 1. Or m can be 5 or 6. Or m can be 7 or 8. For example, p can be 2 or 3. For example, q can be 2 or 3. For example, d can be 1, 2 or 3.

[0108] In some embodiments, L^1, L^2, and L^3 taken together can comprise



,

or



[0109] L^1, L^2, and/or L^3 can increase the water-solubility of the conjugate. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise one or more of an amino acid residue, a polyethylene glycol (PEG) monomer group, a PEG oligomer group, a PEG polymer group, or a combination of any of the foregoing. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise an oligomer of peptidoglycan groups, glycan groups, or a combination of any of the foregoing. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise at least one 2,3-diaminopropionic acid group, at least one glutamic acid group, at least one cysteine group, or a combination of two or more of the foregoing.

[0110] In many embodiments, one or more of L^1, L^2, and L^3 independently comprise one or more rigid or conformationally restricting linkers. Examples of rigid and conformationally restricting linkers include oligoproline, oligoalkyne, oligophenyl and oligopiperidine groups. In many embodiments, one or more of L^1, L^2, and L^3 independently comprise one or more of an oligoethylene group, a PEG group, an alkyl, oligopeptide group, polypeptide group, a peptidoglycan group, an oligoproline group, an oligopiperidine group or any combination thereof. In these and other embodiments, one or more of L^1, L^2, and L^3 independently comprise an oligoethylene glycol group or a PEG group. In some embodiments, one or more of L^1, L^2, and L^3 independently comprise an oligoethylene glycol group. In some embodiments, one or more of L^1, L^2, and L^3 independently comprise a PEG group.

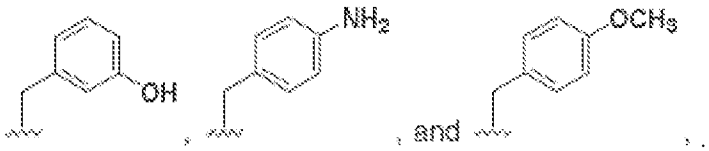
[0111] In some embodiments, when any of L^1, L^2, and L^3 is an oligoproline or oligopiperidine group, then such oligoproline or oligopiperidine group has between (and including the end points of the ranges) 2 and 50 , 2 to 40, or 2 to 30, or 2 to 20, or 2 to 15, to 2 to 10, or 2 to 6 repeating units. In many embodiments such repeating units are prolines (for oligoprolines) and piperedines (for oligopiperidine).

[0112] In some embodiments, when any of L^1, L^2, and L^3 is an oligoproline or oligopiperidine group, then such oligoproline or oligopiperidine group has between (and including the end points of the ranges) 2 and 50 , 2 to 40, or 2 to 30, or 2 to 20, or 2 to 15, to 2 to 10, or 2 to 6 repeating units. In many embodiments such repeating units are prolines (for oligoprolines) and piperedines (for oligopiperidine).

[0113] In many embodiments, one or more of L^1, L^2, and L^3 may independently comprise a moiety comprising an ester, a phosphate, an oxime, an acetal, a pyrophosphate, a polyphosphate, a sulfate, a hydrazide, an imine, a carbonate, a carbamate which may, in turn, be used to bind A^1 or A^2 to L^3 and L^2, respectively.

[0114] In many embodiments, one or more of L^1, L^2, and L^3 may independently comprise a moiety comprising an ester, a phosphate, an oxime, an acetal, a pyrophosphate, a polyphosphate, a sulfate, a hydrazide, an imine, a carbonate, a carbamate which may, in turn, be used to bind A^1 or A^2 to L^3 and L^2, respectively. Thus, for example, A^1 can be bound to L^3 by a moiety comprising an ester, a phosphate, an oxime, an acetal, a pyrophosphate, a polyphosphate, a sulfate, a hydrazide, an imine, a carbonate, a carbamate; and A^2 can be bound to L^2 by a moiety comprising an ester, a phosphate, an oxime, an acetal, a pyrophosphate, a polyphosphate, a sulfate, a hydrazide, an imine, a carbonate, a carbamate.

[0115] In many embodiments, L^1, L^2, and/or L^3 may be substituted so as to alter the affect the hydrophobicity or hydrophilicity of L^1, L^2, and/or L^3. Thus, for example L^1, L^2, and/or L^3 may have a hydrophobic side chain group, such as an alkyl, cycloalkyl, aryl, arylalkyl, or like group, each of which is optionally substituted. In these and other embodiments, L^1, L^2, and/or L^3 comprise or further comprise one or more amino acids with side chains, such as phenylalanine (Phe) and tyrosine (Tyr), including substituted variants thereof, and analogs and derivatives of such side chains. Examples of amino acid side chains include:



[0116] In some embodiments, L^1, L^2, and/or L^3 comprise portions that are neutral under physiological conditions. In some embodiments, L^1, L^2, and/or L^3 comprise portions that can

be protonated or deprotonated to carry one or more positive or one or more negative charges, respectively. In some embodiments, L^1, L^2, and/or L^3 comprises neutral portions and portions that may be protonated to carry one or more positive charges. Examples of neutral portions include polyhydroxyl groups, such as sugars, carbohydrates, saccharides, inositols, and the like, and/or polyether groups, such as polyoxyalkylene groups, including polyoxyethylene, polyoxypropylene, and the like. Examples of portions that can be protonated to carry one or more positive charges include amino groups, such as polyaminoalkylenes, including ethylene diamines, propylene diamines, butylene diamines and the like, and/or heterocycles, including pyrrolidines, piperidines, piperazines, and other amino groups, each of which can be optionally substituted. Examples of portions that can be deprotonated to carry one or more negative charges include carboxylic acids, such as aspartic acid, glutamic acid, and longer chain carboxylic acid groups, and sulfuric acid esters, such as alkyl esters of sulfuric acid.

[0117] The conjugate can be water-soluble. L^1, L^2, and/or L^3 can increase the water-solubility of the conjugate. In one example, the total combined length of $L^1 + L^2 + L^3$ is between about 17 and 100 Å or between about 17 to about 75 Å. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise one or more of an amino acid residue, a polyethylene glycol (PEG) monomer group, a PEG oligomer group, a PEG polymer group, or a combination of any of the foregoing. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise an oligomer of peptidoglycan groups, glycan groups, or a combination of any of the foregoing. In many embodiments, L^1, L^2, and L^3 can each, independently, comprise at least one 2,3-diaminopropionic acid group, at least one glutamic acid group, at least one cysteine group, or a combination of two or more of the foregoing.

[0118] In many embodiments, one or more of L^1, L^2, and L^3 independently comprise carbonyl, thionocarbonyl, alkylene, cycloalkylene, alkylenecycloalkyl, alkylenecarbonyl, cycloalkylenecarbonyl, carbonylalkylcarbonyl, 1-alkylenesuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl, alkylenesulfoxyl, sulfonylalkyl, alkylenesulfoxylalkyl, alkylenesulfonylalkyl, carbonyltetrahydro-2H-pyranyl, carbonyltetrahydrofuranyl, 1-

(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl or 1-(carbonyltetrahydrofuranyl)succinimid-3-yl, each of which is optionally substituted.

[0119] In these and other embodiments, one or more of L^1, L^2, and L^3 independently comprise or further comprise a nitrogen such that one or more of L, L^1, and L^2 comprise alkylenecarbonyl, cycloalkylenecarbonyl, carbonylalkylcarbonyl or 1-(carbonylalkyl)succinimid-3-yl groups, each of which is optionally substituted, bonded to said nitrogen to form an amide. In these and other embodiments, one or more of L^1, L^2, and L^3 independently comprise or further comprise a sulfur atom and alkylene or cycloalkylene groups, each of which is optionally substituted with carboxy, and is bonded to the sulfur to form a thiol. In some embodiments, L^1, L^2, and L^3 independently comprise or further comprise a sulfur atom and 1-alkylenesuccinimid-3-yl and 1-(carbonylalkyl)succinimid-3-yl groups bonded to the sulfur to form a succinimid-3-ylthiol.

[0120] In many embodiments, one or more of L^1, L^2, and/or L^3 can each, independently, comprise an amino acid such as an amino acid selected from Lys, Asn, Thr, Ser, Ile, Met, Pro, His, Gln, Arg, Gly, Asp, Glu, Ala, Val, Phe, Leu, Tyr, Cys, and Trp.

[0121] The compounds described herein may contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. It is to be understood that in one embodiment, the invention described herein is not limited to any particular stereochemical requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be optically pure, or may be any of a variety of stereoisomeric mixtures, including racemic and other mixtures of enantiomers, other mixtures of diastereomers, and the like. It is also to be understood that such mixtures of stereoisomers may include a single stereochemical configuration at one or more chiral centers, while including mixtures of stereochemical configuration at one or more other chiral centers.

[0122] Similarly, the compounds described herein may include geometric centers, such as cis, trans, E, and Z double bonds, or spatial arrangements, such as cis, trans, syn, and anti, relative configurations on a ring. It is to be understood that in another embodiment, the invention described herein is not limited to any particular geometric isomer requirement,

and that the compounds, and compositions, methods, uses, and medicaments that include them may be pure, or may be any of a variety of geometric isomer mixtures. It is also to be understood that such mixtures of geometric isomers may include a single configuration at one or more double bonds, while including mixtures of geometry at one or more other double bonds.

[0123] The conjugates hereof can be "deuterated," meaning one or more hydrogen atoms can be replaced with deuterium. As deuterium and hydrogen have nearly the same physical properties, deuterium substitution is the smallest structural change that can be made. Deuteration is well known to those of ordinary skill in the art.

[0124] As used herein, the term "solvates" refers to compounds described herein complexed with a solvent molecule. It is appreciated that compounds described herein may form such complexes with solvents by simply mixing the compounds with a solvent, or dissolving the compounds in a solvent. It is appreciated that where the compounds are to be used as pharmaceuticals, such solvents are pharmaceutically acceptable solvents. It is further appreciated that where the compounds are to be used as pharmaceuticals, the relative amount of solvent that forms the solvate should be less than established guidelines for such pharmaceutical uses, such as less than International Conference on Harmonization (ICH) Guidelines. It is to be understood that the solvates may be isolated from excess solvent by evaporation, precipitation, and/or crystallization. In some embodiments, the solvates are amorphous, and in other embodiments, the solvates are crystalline. When the solvent is water, the solvate is termed a hydrate.

[0125] As used herein, the term "composition" generally refers to any product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is appreciated that certain functional groups, such as the hydroxy, amino, and like groups form complexes and/or coordination compounds with water and/or various solvents, in the various physical forms of the compounds. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other forms of the compounds described

herein. It is also to be understood that the compositions may be prepared from various amorphous, non-amorphous, partially crystalline, crystalline, and/or other solid forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds described herein. In addition, it is to be understood that the compositions may be prepared from various co-crystals of the compounds described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various solid forms and/or solvate or hydrate forms of the compounds described herein.

[0126] Pharmaceutical compositions can be prepared by combining one or more conjugates with one or more pharmaceutically acceptable excipients, carriers, or diluents, or any combination thereof, and, optionally, one or more additional pharmaceutically active agents

DEFINITIONS

[0127] In this document, the terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. In addition, it is to be understood that the phraseology or terminology employed herein, and not otherwise defined, is for the purpose of description only and not of limitation.

[0128] It is to be understood that in every instance disclosed herein, the recitation of a range of integers for any variable describes the recited range, every individual member in the range, and every possible subrange for that variable. For example, the recitation that n is an integer from 0 to 8, describes that range, the individual and selectable values of 0, 1, 2, 3, 4, 5, 6, 7, and 8, such as n is 0, or n is 1, or n is 2, etc. In addition, the recitation that n is an integer from 0 to 8 also describes each and every subrange, each of which may for the basis of a further embodiment, such as n is an integer from 1 to 8, from 1 to 7, from 1 to 6, from 2 to 8, from 2 to 7, from 1 to 3, from 2 to 4, etc.

[0129] It is also to be understood that unless otherwise indicated the recitation of a numerical value necessarily reflects the relative precision of the numerical value. For

example, the recitation of a number with a specified precision based on significant figures necessarily includes a range of values that would match that number after appropriate rounding. For example, the recitation of the number 1 with a single significant figure is understood to properly refer to a range of values from 0.5 to 1.4. Similarly, the recitation of the number 1.0 with two significant figures is understood to properly refer to a range of values from 0.95 to 1.04. The relative precision of the numerical value can be further indicated by modifying with the term "about" to indicate that the modified number has lower precision.

[0130] As used herein, the term "about" when used with numerical values or limits generally means that the number is approximate and that, as recited, it is understood to include a range of values. For example, a real number that is recited with a single significant figure, would by definition include a so-called rounding range; the number about 5 would at the very least include the range 4.5-5.4, as each of those values rounds to 5. The same is to be understood for real numbers expressed with additional significant figures, where the corresponding rounding range applies to the last significant figure. Integers are to be understood to at least include the values ±1 for single-digit numbers, ±10 for two-digit numbers, etc. Depending upon the context and the variable recited, the term "about" is also interpreted to contemplate a range based on a percentage of the recited number, such as about 5 construed to include 5 ±10% or 5 ±20%. Notwithstanding the foregoing, it is understood that the range of values, unless otherwise indicated, should not be interpreted to include a negative range for a positively recited number, and vice-versa. In addition, depending up on the context, the recited number, unless otherwise indicated, should not be interpreted to include a value of zero when used in conjunction with an added component.

[0131] The statement "about X to Y" has the same meaning as "about X to about Y," unless indicated otherwise. Likewise, the statement "about X, Y, or about Z" has the same meaning as "about X, about Y, or about Z," unless indicated otherwise.

[0132] Values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also

to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range were explicitly recited. For example, a range of "about 0.1% to about 5%" or "about 0.1% to 5%" should be interpreted to include not just about 0.1% to about 5%, but also the individual values (*e.g.*, 1%, 2%, 3%, and 4%) and the sub-ranges (*e.g.*, 0.1% to 0.5%, 1.1% to 2.2%, 3.3% to 4.4%) within the indicated range.

[0133] As used herein the term "radical" with reference to, for example, ligand for a target protein or hapten, refers to a ligand for a target protein or hapten, respectively, as described herein, where one or more atoms or groups, such as a hydrogen atom, or an alkyl group on a heteroatom, and the like, is removed to provide a radical for covalent linking or conjugation to the polyvalent linkers L^1, L^2, and L^3. When the one or more groups are replaced with a different one or more groups then the resulting compound is an analog of the ligand. Illustrative analogs include, but are not limited to, those compounds that share functional and in some cases structural similarity to those compounds described hereinIt is to be understood that such radicals can also be formed on acid, ester, or amide groups, such as carboxy, phosphoryl, and sulfuryl acids, by removing the OH, ester, or amide group. It is also to be understood that such radicals can be formed by removing other fragments, such as halo, alkoxy, amino, heterocyclyl, or heteroaryl groups.

[0134] An "alkyl group" is a saturated, partially saturated, or unsaturated straight chain or branched non-cyclic hydrocarbon having from 1 to 10 carbon atoms (C_1-C_{10} alkyl), from 1 to 8 carbons (C_1-C_8 alkyl), from 1 to 6 (C_1-C_6 alkyl), 1 to 4 (C_1-C_4 alkyl), 1 to 3 (C_1-C_3 alkyl), or 2 to 6 (C_2-C_6 alkyl) carbon atoms. In some embodiments, the alkyl group has monovalency. Examples of alkyl groups with monovalency include $-CH_3$, $-CH_2CH_3$, and the like. Monovalent alkyls may be found on substitutions in the chain of linker, L, for example. In some embodiments, the alkyl group has bivalency, such as when found in the chain of the linker, L. Examples of alkyl groups with bivalency include, but are not limited to, $-CH_2-$, $-CH_2CH_2-$, and the like. In some embodiments, the alkyl group is a saturated alkyl group. In some embodiments, an alkyl group is an unsaturated alkyl group, also termed an alkenyl group or an alkynyl group.

[0135] The term "heteroalkyl" by itself or in combination with another term means, unless otherwise stated, a stable straight or branched chain, or combination(s) thereof, consisting of at least one carbon atom and at least one heteroatom selected from the group consisting of O, N, P, Si, and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quarternized. The heteroatom(s) O, N, P, S, and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, without limitation, —CH_2—CH_2—O—CH_3, —CH_2—CH_2—NH—CH_3, —CH_2—CH_2—N(CH_3)—CH_3, —CH_2—S—CH_2—CH_3, —CH_2—CH_2—S(O)—CH_3, —CH_2—CH_2—S(O)$_2$—CH_3, —CH_2=CH—O—CH_3, —Si(CH_3)$_3$, —CH_2—CH=N—OCH_3, —CH=CH— N(CH_3)—CH_3, —O— CH_3, —O—CH_2— CH_3, and —CN. Up to two heteroatoms may be consecutive, such as, for example, —CH_2—NH—OCH_3.

[0136] As used herein, the term "aryl" includes monocyclic and polycyclic aromatic carbocyclic groups, each of which may be optionally substituted. Illustrative aromatic carbocyclic groups described herein include, but are not limited to, phenyl, naphthyl, and the like. As used herein, the term "heteroaryl" includes aromatic heterocyclic groups, each of which may be optionally substituted. Illustrative aromatic heterocyclic groups include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, quinazolinyl, quinoxalinyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl, and the like.

[0137] The term "optionally substituted" as used herein includes the replacement of hydrogen atoms with other functional groups on the radical that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, and/or sulfonic acid is optionally substituted.

[0138] Illustrative substituents include, but are not limited to, a radical -$(CH_2)_xZ^X$, where x is an integer from 0-6 and Z^X is selected from halogen, hydroxy, alkanoyloxy, including C_1-C_6 alkanoyloxy, optionally substituted aroyloxy, alkyl, including C_1-C_6 alkyl, alkoxy, including C_1-C_6 alkoxy, cycloalkyl, including C_3-C_8 cycloalkyl, cycloalkoxy, including C_3-C_8 cycloalkoxy, alkenyl, including C_2-C_6 alkenyl, alkynyl, including C_2-C_6 alkynyl, haloalkyl, including C_1-C_6 haloalkyl, haloalkoxy, including C_1-C_6 haloalkoxy, halocycloalkyl, including C_3-C_8 halocycloalkyl, halocycloalkoxy, including C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, alkylcarbonylamino, N-(C_1-C_6 alkyl)alkylcarbonylamino, aminoalkyl, C_1-C_6 alkylaminoalkyl, (C_1-C_6 alkyl)(C_1-C_6 alkyl)aminoalkyl, alkylcarbonylaminoalkyl, N-(C_1-C_6 alkyl)alkylcarbonylaminoalkyl, cyano, and nitro; or Z^X is selected from -CO_2R^4 and -$CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected in each occurrence from hydrogen, C_1-C_6 alkyl, aryl-C_1-C_6 alkyl, and heteroaryl-C_1-C_6 alkyl.

[0139] The compounds described herein can be used for both human clinical medicine and veterinary applications. Thus, the host animal treated with the compounds described herein can be human or, in the case of veterinary applications, can be a laboratory, agricultural, domestic, or wild animal. The present invention can be applied to host animals including, but not limited to, humans, laboratory animals such rodents (e.g., mice, rats, hamsters, etc.), rabbits, monkeys, chimpanzees, domestic animals such as dogs, cats, and rabbits, agricultural animals such as cows, horses, pigs, sheep, goats, and wild animals in captivity such as bears, pandas, lions, tigers, leopards, elephants, zebras, giraffes, gorillas, dolphins, and whales.

[0140] The term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that

the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient: the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

[0141] The conjugates and compositions may be delivered by suitable methods of delivery including, for example but not limited to, intranasally, orally, and intravenously. The method can further comprise administering autologous antibodies or allogeneic Immunoglobulin G (IgG) antibodies.

[0142] In each of the foregoing and each of the following embodiments, unless otherwise indicated, it is also to be understood that the transitional phrase "consisting essentially of" means that the scope of the corresponding composition, unit dose, method or use is understood to encompass the specified compounds or recited steps, and those that do not materially affect the basic and novel characteristics of the invention described herein. For example, a method described herein that consists essentially of a single compound, or genus of compounds, is understood to represent a monotherapy for the recited disease. Though the monotherapy may include co-administration of one or more carriers, vehicles, diluents, adjuvants, excipients, and the like, and combinations thereof, and/or include co-administration of one or more additional active pharmaceutical ingredients, those latter additional active pharmaceutical ingredients are to be understood to be for treating diseases and/or symptoms distinct from treating the underlying conditions described herein, such as the treatment of the viral infection itself. Illustrative additional active pharmaceutical ingredients may include, for example, active ingredients for treating pain, inflammation, cough, congestion, and the like.

EXAMPLES

[0143] The following examples serve to further illustrate the invention described herein. The examples are illustrative of the many embodiments of the invention and are not to be construed as limiting the scope of the invention in any way.

LIST OF ABBREVIATIONS

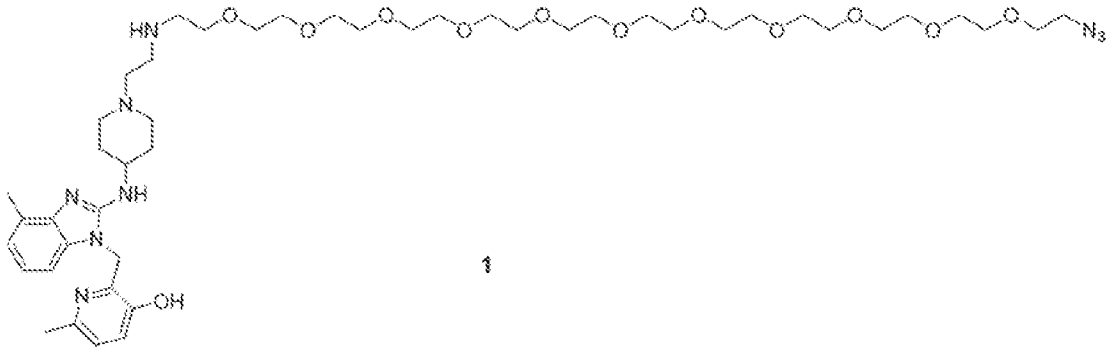
MeOH	Methanol
EtOH	Ethanol
EtOAc	Ethyl acetate
DCM	Dichloromethane
Ac	-C(=O)-CH_3
ACN	Acetonitrile
THF	Tetrahydrofuran
DMSO	Dimethylsulfoxide
TFA	Trifluoroacetic acid
TIPS	Triisopropylsilane
Py	Pyridine
TEA	Triethylamine
DEA	Diethylamine
DIPEA	*N,N*-Diisopropylethylamine
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
PyBOP	Benzotriazole-1-yloxytrispyrrolidinophosphonium hexafluorophosphate
NHS	*N*-hydroxysuccinimide
r.t	Room temperature
M	Molar
DMAP	N,N-Dimethyl aminopyridine
PPh_3	Triphenyl phosphine
PEG	Polyethylene glycol
tBu	*tert*-Butyl
NH_4OAc	Ammonium acetate
Eq.	Equivalents
Anhy.	Anhydrous
aq.	Aqueous
min	Minutes
h	Hour(s)
g	Gram(s)
mg	Milligram(s)
mL	Milliliter(s)
mmol	Millimole(s)
Qty	Quantity
LC-MS	Liquid chromatography-mass spectrometry

RP-HPLC	Reverse phase-high performance liquid chromatography
UPLC	Ultra performance liquid chromatography
ESI	Electron spray ionization
m/z	Mass/charge ratio
pTSOH	p-Toluene sulfonic acid
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulfate
NaOMe	Sodium methoxide
Rha	Rhamnose
L-Rha	L-Rhamnose
DNP	Dinitrophenyl
Fmoc	Fluorenylmethoxycarbonyl
Boc	*tert*-Butyloxycarbonyl

COMPOUND EXAMPLES

[0144] General. Unless otherwise indicated, all starting compounds and reagents are commercially available. Unless otherwise indicated, reactions are performed under an ambient atmosphere, and at ambient temperature and pressure, and reaction progress is monitored by TLC, LCMS, or both. Unless otherwise indicated, solutions are concentrated and compounds are isolated by rotary evaporation under reduced pressure, under high vacuum, or both.

[0145] Example 1, Compound 1

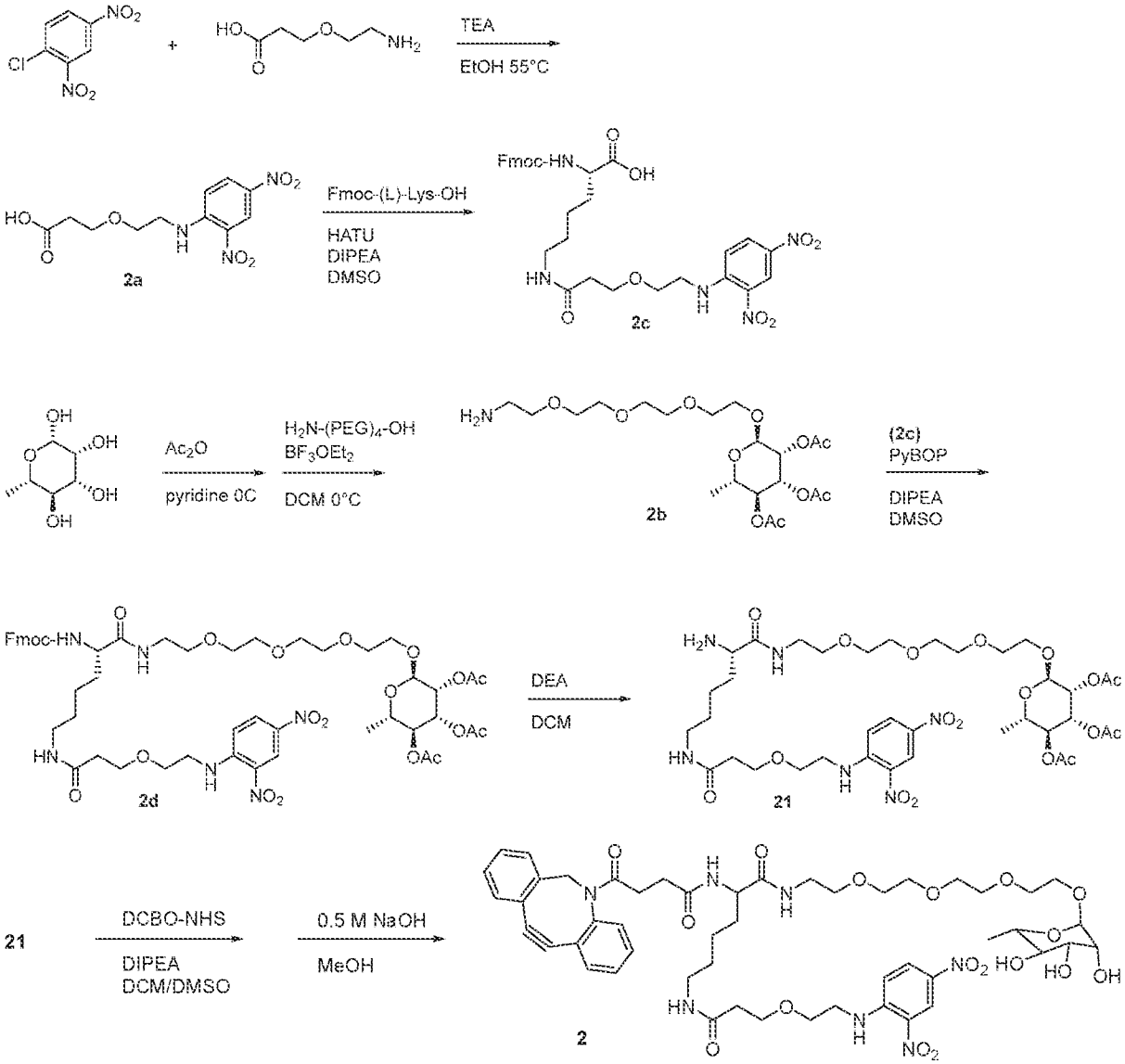


1

[0146] To commercially available JNJ2408068 (10.0 mg, 0.0253 mmol) and N,N-Diisopropylamine (30 uL, 0.253 mmol) in DMSO (0.2 mL) was added commercially available Tosyl-PEG12-Azide 36.8 mg, 0.0507 mmol) in DMSO (0.2 mL). The reaction mixture was heated to 60°C and stirred for 2h. The reaction mixture was cooled to ambient

temperature and charged with MeOH (2mL) and purified by RP-HPLC (30%-95% ACN in 10 mM aq. Ammonium Acetate), then lyophilized to give compound 1 as a white solid (3.0 mg, 13%).

[0147] Example 2, Compound 2



[0148] To a solution of commercially available 1-chloro-2,4-dinitrobenzene (0.5 g, 2.47 mmol) and 3-(2-aminoethoxy)propanoic acid, 0.33 g, 2.47 mmol) dissolved in EtOH (25 mL) was added TEA (1.38 mL, 9.87 mmol). The reaction mixture was heated to 55°C for 16h. After reaction completion, the reaction mixture was cooled and concentrated, and the

remainder was purified by flash column chromatography on Teledyne CombiFlash Rf+ Lumen (silica-gel column, 0-20% MeOH in DCM) to yield 3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanoic acid (2a) as a yellow solid (90% yield. LC-MS [M + H]+ =299.98.

[0149] Commercially available α-L-Rhamnose monohydrate (1.0 g, 5.49 mmol) was dissolved in 9.2 mL of anhydrous pyridine. The solution was stirred in an ice bath and purged with nitrogen prior to the dropwise addition of acetic anhydride (4.15 mL, 43.92 mmol, 8.0 eq.). The reaction mixture was allowed to ambient temperature slowly, and reaction was complete after 20h. The reaction mixture was poured into EtOAc and extracted twice with 1.0 M HCl. The organic layer was washed with saturated Na_2CO_3 solution, water and brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting oil (0.50 g, 1.50 mmol) was dissolved in DCM (DCM) (7.5 mL) prior to addition of commercially available amino-PEG_4-OH , (0.35 g, 1.81 mmol, 1.2 eq.) under N_2 atmosphere. The reaction flask was placed in an ice bath, and boron trifluoride diethyletherate (0.56 mL, 4.51 mmol, 3.0 eq.) was added dropwise over 30 min. The reaction mixture was stirred for 2h, then allowed to reach ambient temperature. After 16h, reaction was complete, and the reaction mixture was poured into ice water and extracted with DCM. The combine organic layers were washed twice with saturated sodium bicarbonate solution, water and brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The remainder was purified by silica gel column chromatography (Hexane/EtOAc, 20:80) to give L-Rha$(OAc)_3$-PEG_4-NH_2 (2b, 90% yield).

[0150] To 3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanoic acid (2a, 0.1 g, 0.33 mmol) in DMSO (2 mL) was added HATU (0.11 g, 0.28 mmol, 0.85 eq.) followed by DIPEA (0.29 mL, 1.67 mmol, 5.0 eq.) under argon atmosphere and stirred for 10 min at room temperature. Fmoc-(L)-Lys-OH·HCl (0.11 g, 0.27 mmol, 0.8 eq.) was added to the reaction mixture and stirred for 2-3h. After reaction completion, the reaction mixture was quenched by adding water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The remainder was purified by silica gel column chromatography (Hexane/EtOAc, 20:80) to give compound 2c 70% yield).

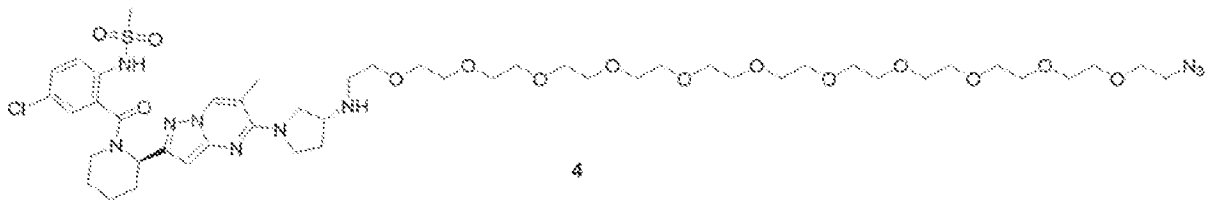
[0151] To compound 2c (0.06 g, 0.09 mmol) and L-Rha(OAc)$_3$-PEG$_4$-NH$_2$ (2b, 0.04 g, 0.09 mmol, 1.0 eq.) in DMSO (1.5 mL) was added PyBOP (0.05 g, 0.10 mmol, 1.1 eq.) followed by DIPEA (0.081 mL, 0.46 mmol, 5.0 eq.) under argon atmosphere. After reaction completion, the reaction mixture was quenched by adding water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The remainder was purified by silica gel column chromatography (0-10% MeOH in DCM) to give compound 2d (93% yield).

[0152] To compound 2d (0.01 g, 0.01 mmol) in dry DCM (0.2 ml) was added DEA (100 uL) under argon. The solution was stirred for 1 h. Upon reaction completion, DEA was evaporated and the remainder was precipitated in diethyl ether to give compound 21 as a yellow solid (quantitative yield). LC-MS [M + H]+ = 876.1.

[0153] To compound 21 (0.024 g, 0.027 mmol, 1.0 eq.) and DBCO-NHS (0.012 g, 0.029 mmol, 1.05 eq.) dissolved in DCM:DMSO (10:1, 0.6 mL) was added DIPEA (0.048 µl, 0.27 mmol, 10 eq.) dropwise under argon atmosphere. The reaction mixture was stirred for 2-3 h. After reaction completion, solvent evaporated and the remainder was purified by silica-gel column chromatography on Teledyne CombiFlash Rf+ Lumen (0-10% MeOH in DCM) to give compound 2f as a yellowish solid (93% yield). LC-MS [M + H]+ = 1163.2.

[0154] Compound 2f (0.025 g, 0.022 mmol) was dissolved in anhydrous MeOH (1 mL) and treated dropwise with 0.5 M NaOH (25 µL). The reaction mixture was stirred for 1h. After reaction completion, the mixture was neutralized by adding Dowex® 50WX8 (H+) resin, filtered, and concentrated to compound 2 as a yellowish solid, which can be used without further purification. LCMS [M + H]+ = 1037.3.

[0155] Example 3, Compound 4

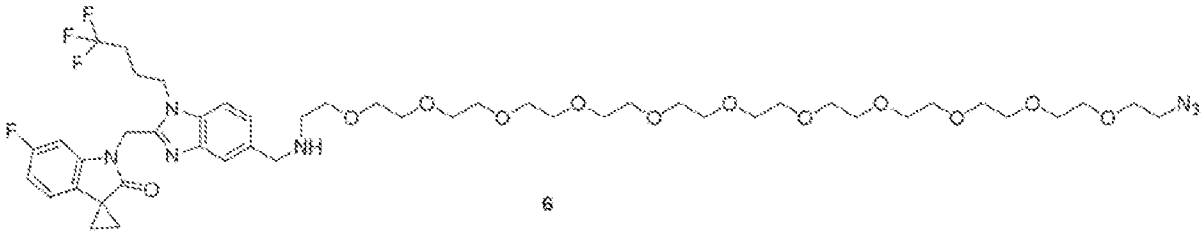


4

[0156] To a solution of commercially available Presatovir (10.0 mg, 0.0188mmol) and N,N-Diisopropylamine (33 uL, 0.188 mmol) in DMSO (0.2 mL) was added commercially

available Tosyl-PEG12-Azide (27.0mg, 0.0376 mmol) in DMSO (0.2 mL). The reaction mixture was heated to 60°C and stirred for 2h. The reaction mixture was cooled to ambient temperature and charged with water (2mL) and purified by RP-HPLC (30%-95% ACN in 10mM aq. Ammonium Acetate), then lyophilized to give compound 4 as a white solid (8.0 mg, 39% yield).

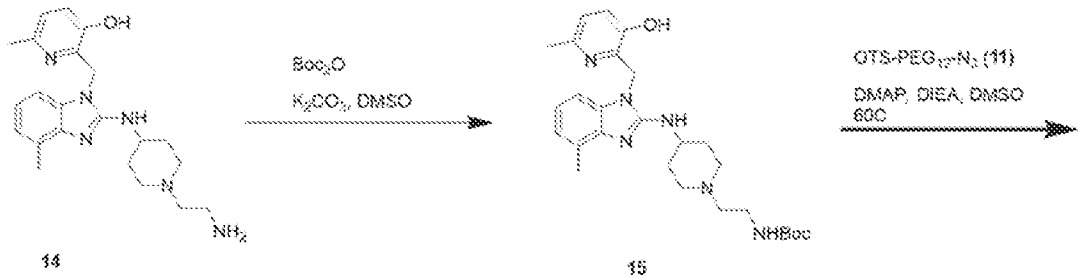
[0157] Example 4, Compound 6



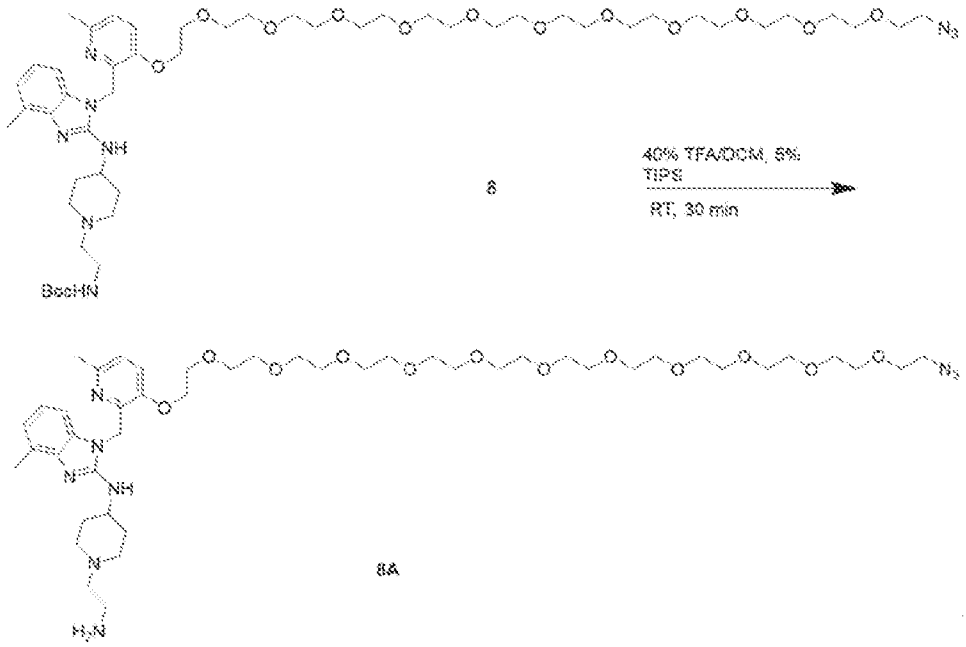
6

[0158] To a solution of commercially available Sisunatovir (10.0 mg, 0.0224 mmol) and K_2CO_3 (17.9 mg, 0.448 mmol) in DMSO (0.2mL) was added Tosyl-PEG12-Azide (17.9mg, 0.0246 mmol) in DMSO (0.2mL). The reaction mixture was heated to 60°C and stirred for 2h. The reaction mixture was cooled to ambient temperature and charged with water (2mL) forming a solid precipitate. The supernatant was removed and the remaining solid was dissolved in DMF and water (50:50, 10mg/mL) and purified by RP-HPLC (30%-95% ACN in 10mM aq. Ammonium Acetate), then lyophilized to give compound 6 as a white solid (6.4 mg, 29% yield).

[0159] Example 5, Compound 8A



14 15



[0160] To a solution of commercially available JNJ2408068 (Compound 14, 10.0 mg, 0.0253 mmol) and Boc Anhydride (11.0 mg, 0.0507 mmol) in DMF (1 mL) was added Potassium carbonate (13.9 mg, 0.101 mmol). The reaction mixture was stirred for 2h. Potassium hydroxide was added (4.2 mg, 0.0759 mmol) and the reaction was stirred for an additional 1h. The reaction mixture was diluted 1:10 with water and purified by RP-HPLC (30%-95% ACN in 10mM aq. Ammonium Acetate), then lyophilized to give compound 15 as a white solid (12.5 mg, 99% yield).

[0161] To compound 15 and N,N-Diisopropylamine (30 uL, 0.253 mmol) in DMF (0.2 mL) was added commercially available Tosyl-PEG12-Azide (18.3 mg, 0.0253 mmol) in DMSO (0.2mL). DMAP (3.0 mg, 0.025 mmol) was added. The reaction mixture was heated to 60°C and stirred for 16h. The reaction mixture was cooled to ambient temperature, charged with MeOH (2mL), and purified by RP-HPLC (30%-95% ACN in 10 mM aq. Ammonium Acetate), then lyophilized to give Compound 8 as a white solid (8.3 mg, 31% yield).

[0162] Compound 8 was dissolved in 50:50 TFA/DCM and stirred for 3h. Volatiles were evaporated to compound 8A.

[0163] Example 6, Compound 47 – FIG. 2. N-(25-((2,4-dinitrophenyl)amino)-13,20-dioxo-1-(((2R,3R,4R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-

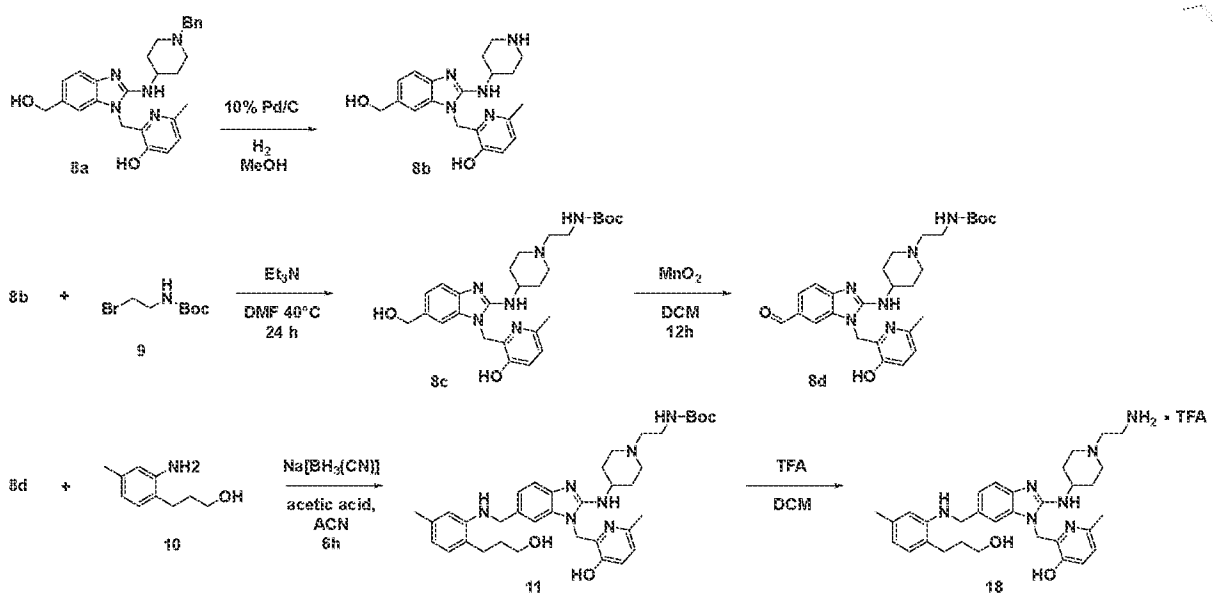
3,6,9,23-tetraoxa-12,19-diazapentacosan-14-yl)-1-(4-((1-((3-hydroxy-6-methylpyridin-2-yl)methyl)-6-(((2-(3-hydroxypropyl)-5-methylphenyl)amino)methyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-6,9,12,15,18,21,24,27,30,33,36-undecaoxa-3-azanonatriacontan-39-amide (Compound 47).

[0164] Compound 17. To tert-butyl 36-hydroxy-4,7,10,13,16,19,22,25,28,31,34-undecaoxahexatriacontanoate ([[commercial source]], 16, 0.2 g, 0.317 mmol; BLD Pharma) in DCM (6 mL), 2,2,6,6-tetramethyl-1-oxypiperidine (0.010 g, 0.063 mmol) was added and the reaction mixture was stirred for 5 min. Acetoxyphenyliodo acetate (0.327 g, 1.01 mmol) was added. The reaction mixture was stirred for 30 min. After reaction completion, the mixture was purified by column chromatography using 2-5% MeOH/DCM as eluent to give aldehyde (17) as viscous colourless liquid (0.150 g, 75 % yield). ^{1}H NMR ($CDCl_3$, d_6); 9.72 (s, 0.57 H), 5.29 (s, 1H), 4.15 (m, 1H), 3.73-3.42 (m, 41H), 2.49 (t, J = 6.8 Hz, 2H), 1.43 (s, 9H).

[0165] Compound 19. To 2-({2-[1-(2-aminoethyl)-4-piperidylamino]-6-{[2-(3-hydroxypropyl)-5-toluidino]methyl}-1H-1,3-benzimidazol-1-yl}methyl)-6-methyl-3-pyridinol ([[commercial source]], 18, 0.030 g, 0.0344 mmol) in MeOH (2 mL), compound 17 (0.026 g, 0.031 mmol) and acetic acid (cat.) was added and the reaction mixture was stirred for 2 h. Sodium cyanoborohydride (0.004 mg, 0.068 mmol) was added at 0°C, cooling was removed and the reaction mixture was stirred for 1 h. After reaction completion, the reaction mixture was filtered, concentrated and dried.. The remainder was purified by Sephadex LH20 column chromatography using MeOH as eluent. Fractions containing compound 19 were concentrated to give tert-butyl 1-(4-((1-((3-hydroxy-6-methylpyridin-2-yl)methyl)-6-(((2-(3-hydroxypropyl)-5-methylphenyl)amino)methyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-6,9,12,15,18,21,24,27,30,33,36-undecaoxa-3-azanonatriacontan-39-oate (19) as colorless liquid (0.030 g, 70 % yield); LC-MS (ESI) m/z 1168.10 $[M-1]^+$.

[0166] Compound 20. To compound 19 (0.030 g, 0.0172 mmol) in DCM (3 mL), TFA (0.5 mL) was added at 0° C. Cooling was removed and the reaction mixture was stirred for 2 h. After reaction completion, the reaction mixture was concentrated to give 1-(4-((1-((3-

hydroxy-6-methylpyridin-2-yl)methyl)-6-(((2-(3-hydroxypropyl)-5-methylphenyl)amino)methyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-6,9,12,15,18,21,24,27,30,33,36-undecaoxa-3-azanonatriacontan-39-oic acid (20) as a colorless liquid (0.020 g), which was used without further pruification; LC-MS (ESI) m/z 1114.25 $[M+1]^+$.



[0167] Compound 21. Compound 8a is prepared according to the process described in Bonfanti et al. J Med Chem 51:875-96 (2008).

[0168] A solution of 8a (0.910 g) in MeOH is treated with 10% Pd/C under H_2 atmosphere. Compound 8b is isolated (600 mg, 62% yield).

[0169] A solution of compound 8b (600 mg) in DMF is treated with Br-$(CH_2)_2$-NH-Boc (9) and TEA at 40°C for 24 to give a mixture of compound 8c and an O,N-dialkylated product. Compound 8c is isolated (0.310 g, 27% yield).

[0170] A solution of compound 8c (0.310 g) in DCM is treated with MnO_2 for 12h. Compound 8d is isolated (230 mg, 63% yield).

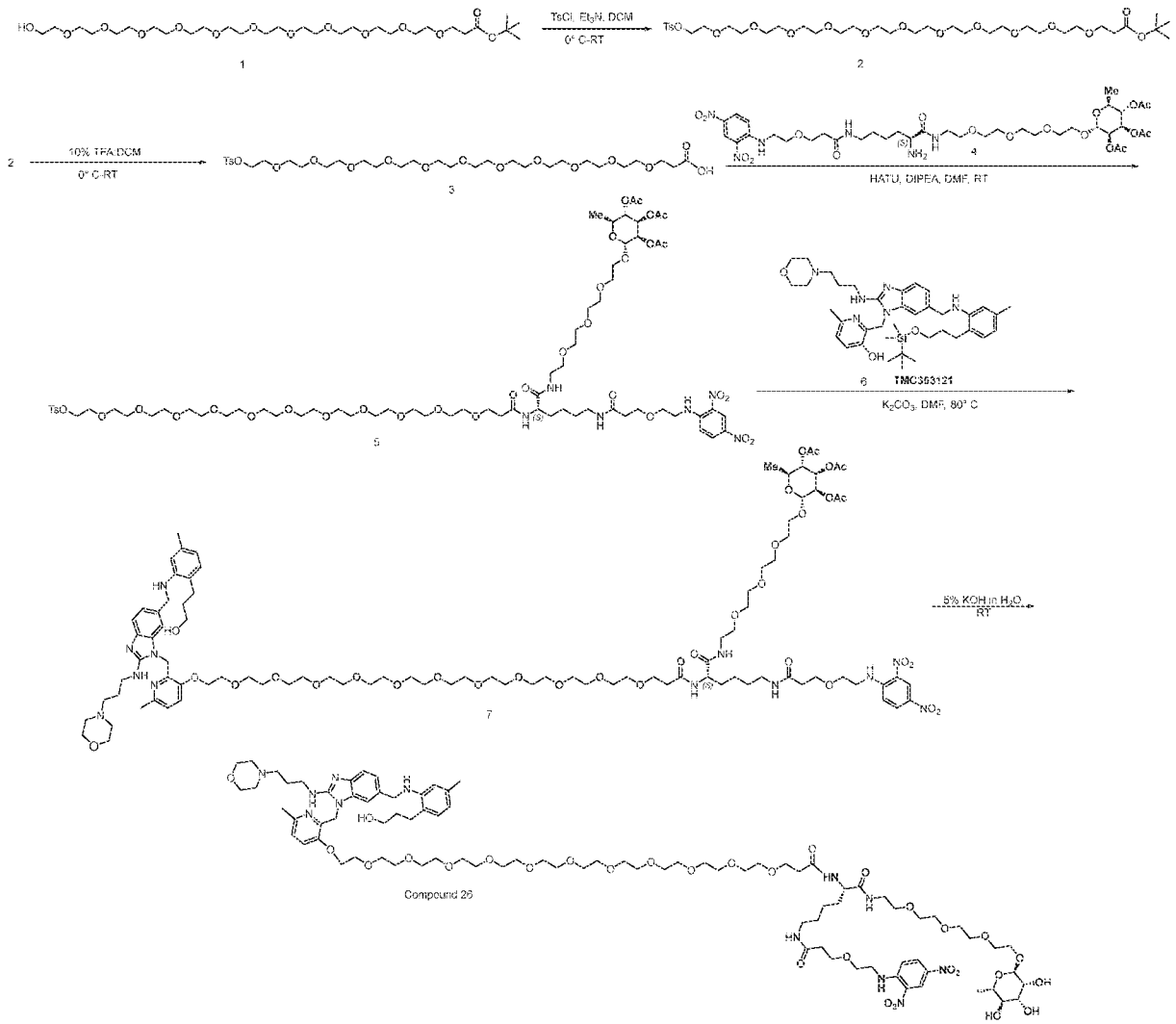
[0171] A solution of compound 8d (230 mg) in ACN is treated with 3-(2-amino-4-methylphen-1-yl)propanol (10) and sodium cyanoborohydride and acetic acid for 6h. Compound 11 is isolated (140 mg, 41% yield).

[0172] A solution of compound 11 (140 mg) in DCM is treated with TFA. Compound 18 is isolated as a TFA salt (70 mg, 84% yield). The free amine of compound 18 is isolated by HPLC.

[0173] Compound 22. To compound 20 (0.020 g, 0.0179 mmol) and compound 21 (0.016 g, 0.0179 mmol) in DMF (1 mL), PyBOP (0.012 g, 0.0215 mmol) and DIPEA (10 µL, 0.054 mmol) were added at 0° C. Cooling was removed, and the reaction mixture was stirred for 2 h. After reaction completion, solvent was concentrated under high vacuum, and the remainder was purified by Sephadex LH20 column chromatography using MeOH as elutant. Fractions containing compound 22 were concentrated to give compound 22 as pale yellow solid (0.025 g, 70% yield). LC-MS (ESI) m/z 986.10 $[M/2+1]^+$.

[0174] Compound 47. To compound 22 (0.024 g, 0.0135 mmol) in water (1.0 mL), 5% KOH aq. (10 µL) was added dropwise to pH=11. The reaction mixture was stirred for 1h. After reaction completion, the reaction mixture was purified by Prep-HPLC purification using ACN:H_2O with 0.1% Ammonium bicarbonate. Fractions containing compound 47 were lyophilized to give compound 47) as yellow solid (5.0 mg; 26% yield). LC-HRMS (ESI) m/z 1846.01 $[M+1]^+$. ^1H NMR (400 MHz, DMSO -d_6 with D_2O): δ 8.85 (d, J = 2.56 Hz, 1H), 8.25-8.22 (m, 1H), 7.47 (s, 1H), 7.24 (d, J = 9.68 Hz, 1H), 7.03 (d, J = 7.84 Hz, 1H), 6.90 (d, J = 7.64 Hz, 1H), 6.79 (d, J = 6.8 Hz, 1H), 6.69-6.61 (m, 2H), 6.33 (s, 1H), 6.28 (d, J = 6.88 Hz, 1H), 4.86 (bs, 2H), 4.54 (s, 1H), 4.29 (s, 1H), 4.17-4.13 (m, 1H), 3.63-3.42 (m, 12H), 3.21-3.15 (m, 3H), 2.96-2.95 (m, 2H), 2.81-2.79 (m, 2H), 2.62-2.56 (m, 4H), 2.36-2.27 (m, 6H), 2.15 (s, 3H), 2.07 (s, 3H), 2.03-2.00 (m, 2H), 1.92(m, 2H), 1.69 (t, J = 7.64 Hz, 2H), 1.57-1.52 (m, 3H), 1.45-1.42(m, 1H), 1.31-1.28 (m, 2H), 1.21-1.19 (m, 2H), 1.12 (d, J = 6.2 Hz, 3H).

[0175] Example 7. N-((S)-25-((2,4-dinitrophenyl)amino)-13,20-dioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,23-tetraoxa-12,19-diazapentacosan-14-yl)-1-((2-((6-(((2-(3-hydroxypropyl)-5-methylphenyl)amino)methyl)-2-((3-morpholinopropyl)amino)-1H-benzo[d]imidazol-1-yl)methyl)-6-methylpyridin-3-yl)oxy)-3,6,9,12,15,18,21,24,27,30,33-undecaoxahexatriacontan-36-amide (Compound 26).



[0176] Compound 2. To a 0 °C solution of compound 1 (BLD Pharma, 0.5 g, 0.793 mmol) in DCM (DCM, 5 mL), TEA (0.252 mL, 1.74 mmol) and p-(chlorosulfonyl)toluene (0.166 g, 0.872 mmol) were added sequentially while maintaining 0° C. Cooling was discontinued and the reaction mixture was stirred for 12 h. After reaction completion, the mixture was diluted with water and extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated, and the remainder was purified by column chromatography using 2-5% MeOH/DCM as eluent to afford compound 2 as a viscous colorless liquid. Yield: 0.480 g, 77 %. LC-MS: 97.6% (ESI) m/z 802.60 $[M+18]^+$.

[0177] Compound 3. To a solution of compound 2 (0.250 g, 0.318 mmol) in DCM (5 mL), TFA (0.5 mL) was added and stirred for 4 h. After reaction completion, solvent was

evaporated to afford compound 3 as a viscous colorless liquid. Yield: 0.20 g, 86 %.; ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.80 (d, J = 8.24 Hz, 2H), 7.35 (d, J = 8.08 Hz, 2H), 4.63 (bs, 14H), 4.15 (t, J = 4.52 Hz, 2H), 3.78 (t, J = 6.0 Hz, 2H), 3.79-3.59 (m, 48H), 2.62 (t, J = 5.84 Hz, 2H), 2.44 (s, 3H).

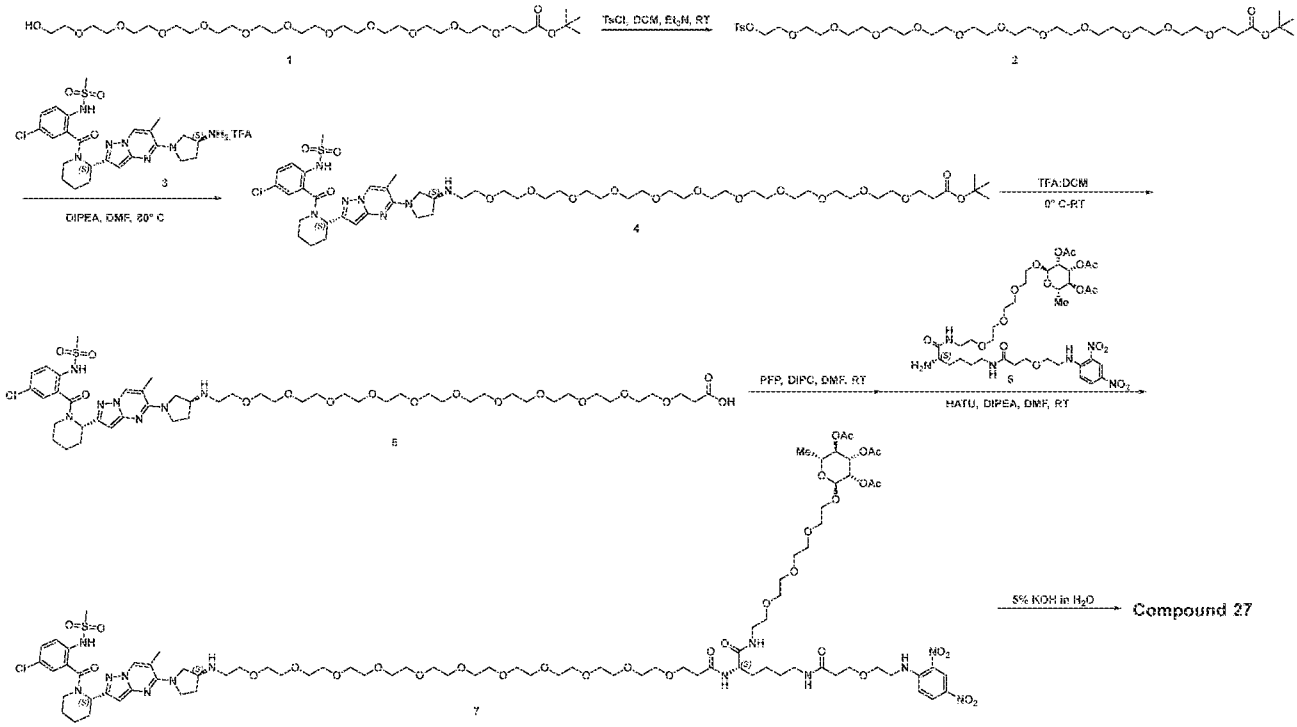
[0178] Compound 5. To a solution of compound 3 (0.2 g, 0.274 mmol) in DMF (3 mL) were sequentially added compound 4 (0.192 g, 0.220 mmol), HATU (0.157 mg, 0.412 mmol), and DIPEA (0.082 mL, 0.412 mmol) at 0°C. Cooling was discontinued and the reaction mixture was stirred for 12 h. After reaction completion, the reaction mixture was diluted with ice water and extracted with EtOAc, the organic layer was dried over Na_2SO_4 and concentrated. The remainder was purified by column chromatography using 5-7.5% MeOH/DCM as eluent to afford compound 5 as a yellow viscous liquid. Yield: 0.130 g, 30 %. LC-MS (ESI) m/z 1585.90 $[M+1]^+$.

[0179] Compound 6 was prepared as described in J Med Chem, 2008, Vol. 51, No. 4, 875-896.

[0180] Compound 7. To a solution of compound 6 (0.070 g, 0.104 mmol) and compound 5 (0.130 g, 0.083 mmol) in DMF (2 mL), dipotassium carbonate (0.043 g, 0.312 mmol) was added, and the reaction mixture was stirred at 100° C for 12 h. After reaction completion, the reaction mixture was filtered through celite pad and filtrate was evaporated under high vacuum, and the remainder was purified by Prep-HPLC using an ACN:H2O gradient with 0.1% TFA. Fractions containing compound 7 were lyophilized to afford compound 7 as a yellow solid. Yield: 0.010 g; 5%. LC-MS (ESI) m/z 1973.38 $[M+1]^+$.

[0181] Compound 26. To a solution of compound 7 (0.010 g, 2.28 µmol) in water (0.5 mL), 5% aq. KOH (20 µL) was added dropwise tol pH 11. The reaction mixture was stirred for 1h. After reaction completion, the reaction mixture was purified by reverse phase purification using C18-silica gel 2g column with ACN:H2O gradient. Compound 26 eluted approximately in 50-60% ACN:H2O. Fractions containing compound 26 were lyophilized to afford Compound 26) as a yellow solid. Yield: 1.6 mg; 16%. LC-HRMS (ESI) m/z 1847.01 $[M+1]^+$.

[0182] Example 8. 1-(((S)-1-(2-((S)-1-(5-chloro-2-(methylsulfonamido)benzoyl)piperidin-2-yl)-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl)amino)-N-(25-((2,4-dinitrophenyl)amino)-13,20-dioxo-1-(((2R,3R,4R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,23-tetraoxa-12,19-diazapentacosan-14-yl)-3,6,9,12,15,18,21,24,27,30,33-undecaoxahexatriacontan-36-amide (Compound 27).



[0183] Compound 2. Compound 2 was prepared using the same procedure used to make compound 2 in Example 7. Compound 2 was prepared from compound 1 (BLD Pharma, 0.5 g, 0.793 mmol) in DCM (5 mL), and TEA (0.252 mL, 1.74 mmol) with p-(chlorosulfonyl)toluene (0.166 g, 0.872 mmol), and was purified by column chromatography using 2-5% MeOH/DCM as eluent to afford compound 2 as a viscous colourless liquid. Yield: 0.480 g, 77 %. LC-MS (ESI) m/z 802.60 $[M+18]^+$. ^1H NMR (400 MHz, $CDCl3$-d_6): δ 7.79 (d, J = 8.24 Hz, 2H), 7.33 (d, J = 8.08 Hz, 2H), 4.15 (t, J = 4.72 and 4.92 Hz, 2H), 3.71-3.58 (m, 41H), 2.50 (t, J = 6.6 Hz, 2H), 2.44 (s, 3H), 1.8 (bs, 3H), 1.44 (s, 9H).

[0184] Compound 4. To a solution of the TFA salt of compound 3 (commercially available presatovir, 0.469 mg, 0.597 mmol) and compound 2 (0.250 g, 0.398 mmol) in DMF

(4 mL), TEA (0.288 mL, 1.99 mmol) was added. The reaction mixture was stirred at 80 °C for 12 h. After reaction completion, the reaction mixture was concentrated under high vacuum, and the remainder was purified by Prep-TLC purification using 0-5% MeOH in DCM to afford compound 4 as a colorless liquid. Yield: 0.080 g, 18 %. LC-MS (ESI) m/z 1145.1 $[M+1]^+$.

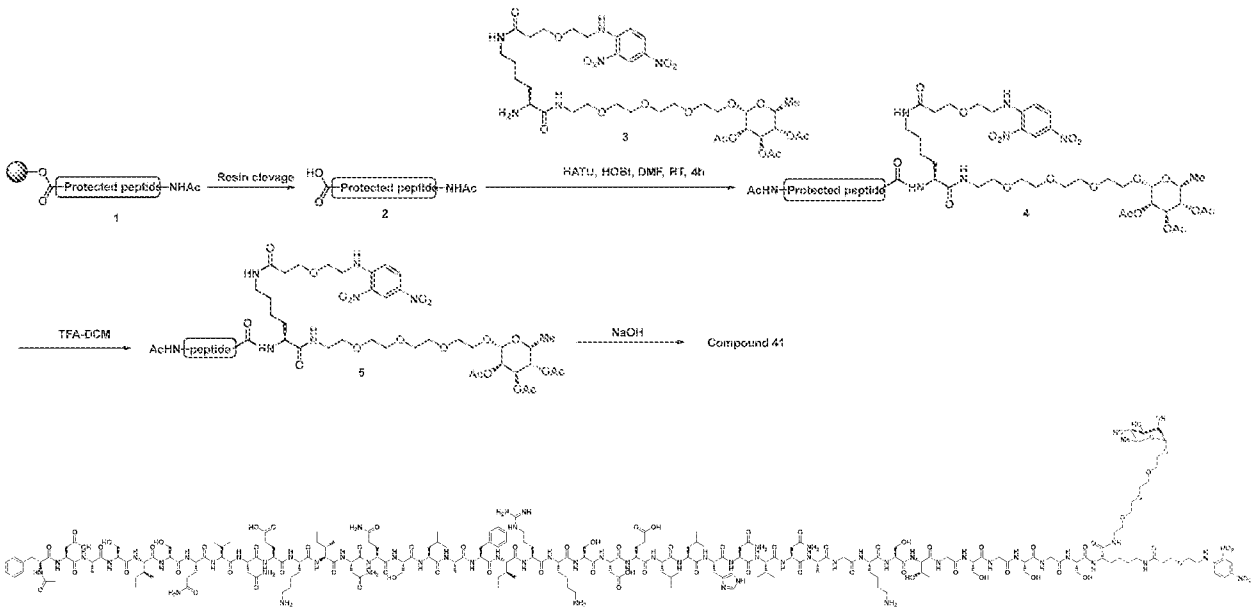
[0185] Compound 5. To a solution of compound 4 (60 mg, 0.0524 mmol) in DCM (1 mL), TFA (0.2 mL) was added at 0° C. Cooling was discontinued and the reaction mixture was stirred for 4 h. After reaction completion, the reaction mixture was evaporated under high vacuum to afford compound 5 as a colourless liquid. Yield: 0.050 g, 87 %. LC-MS (ESI) m/z 1088.20 $[M+1]^+$.

[0186] Compound 7. To a solution of compound 5 (0.050 mg, 0.0551 mmol) in DMF(1 mL), 2-[(isopropylimino)methyleneamino]propane (0.014 g, 0.110 mmol) and 2,3,4,5,6-pentafluorophenol (0.015 g, 0.083 mmol) were added. The reaction mixture was stirred for 6 h. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture (60 mg, 47.8 µmol) was combined with compound 6 (57.0 mg, 0.049 mmol, see Example 6, compound 21) in additional DMF (1 mL), HATU (21.8 mg, 1.2 eq., 57.4 µmol), 1H-1,2,3-benzotriazol-4-ol (0.008 g, 0.0574 mmol) and DIPEA (0.013 mL, 0.0717 mmol) were added. The reaction mixture was stirred for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was evaporated under high vacuum, and the remainder was purified by reverse phase column chromatography using C18 column with 10-100% H_2O:ACN gradient. Fractions compound 7 containing were lyophilized to afford compound 7 as a pale yellow solid. Yield: 0.015 g, 21%. LC-MS (ESI) m/z 973.1 $[M/2+1]^+$.

[0187] Compound 27. To a solution of compound 7 (0.015 g, 0.00771 mmol) in water (1 mL), 5% aq. KOH (0.1 mL) was added to pH=12. The reaction mixture was stirred for 1 h. Progress of the reaction was monitored by LCMS. After reaction completion, the reaction mixture was purified by Prep-HPLC using an ACN:H2O gradient with 0.1% AcOH to afford Compound 27 as a yellow solid. Yield: 0.010 g, 71%. LC-MS (ESI) m/z 1819.2 $[M+1]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (d, J = 11.68 Hz, 2H), 8.40 (t, J =

12.4 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.07-7.79 (m, 3H), 7.27 (d, J = 9.28 Hz, 1H), 7.21-7.12 (m, 1H), 6.99-6.86 (m, 2H), 5.85 (bs, 1H), 5.75 (m, 1H), 4.80 (bs, 2H), 4.60-4.43 (m, 3H), 4.18-4.15 (m, 1H), 3.78-3.76 (m, 1H), 3.64-3.55 (m, 12H), 3.49 (m, 64H), 3.41-3.38 (m, 8H), 3.18-3.14 (m, 4H), 3.96 (bs, 3H), 2.68-2.62 (m, 2H), 2.58-2.50 (m, 3H), 2.37 (d, J = 6.36 Hz, 2H), 2.31-2.29 (m, 8H), 2.18 (d, J = 11.6 Hz, 2H), 2.01 (t, J = 6 Hz, 2H), 1.71 (bs, 3H), 1.56 (s, 10H), 1.44-1.33 (m, 7H), 1.23-1.20 (m, 3H), 1.12 (d, J = 6.2Hz, 2H), 1.05 (m, 1H).

[0188] Example 9. (2R,3R,4R,5R,6S)-Acetyl-NH-Phe-Asp-Ala-Ser-Ile-Ser-Gln-Val-Asn-Glu-Lys-Ile-Asn-Gln-Ser-Leu-Ala-Phe-Ile-Arg-Lys-Ser-Asp-Glu-Leu-Leu-His-Asn-Val-Asn-Ala-Gly-Lys-Ser-Thr-Gly-Ser-Gly-Ser-Gly-Ser-Lys-(N'-2,4-dinitrophenylaminoethoxypropionamide)-N-(3,4,5-trihydroxy-6-methyltetrahydro-(2H)-pyran-2-yloxyethoxyethoxyethoxyethyl)amide (Compound 41).



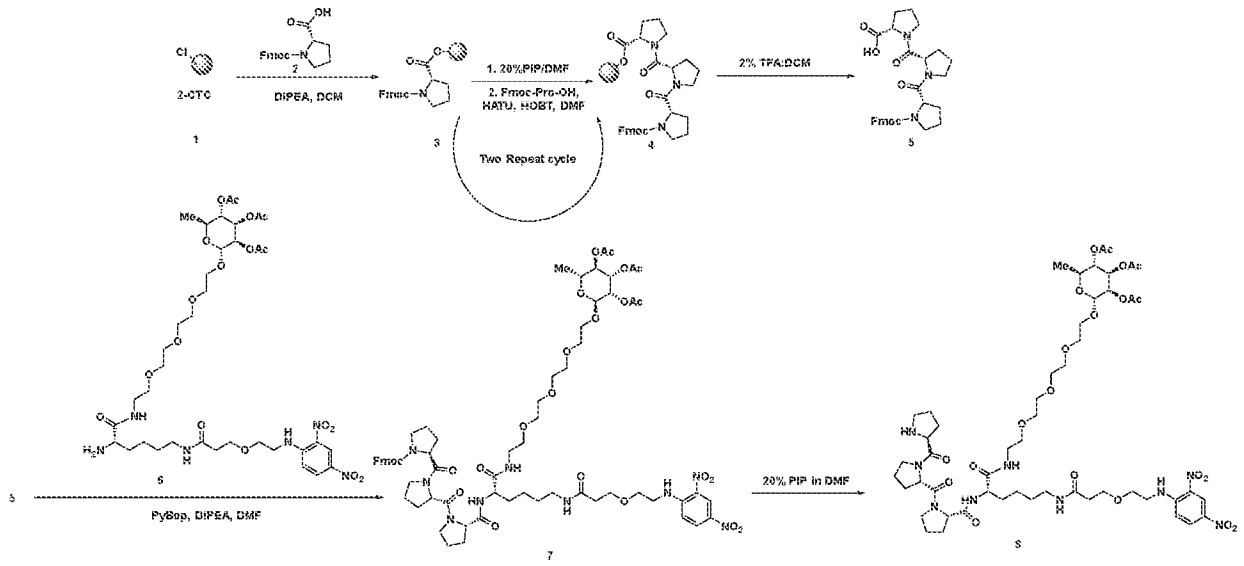
[0189] Compound 1. The 41-mer protected peptide (1) was synthesized on 2-CTC support resin using a conventional automatic peptide synthesizer and Fmoc/OtBu protocol.

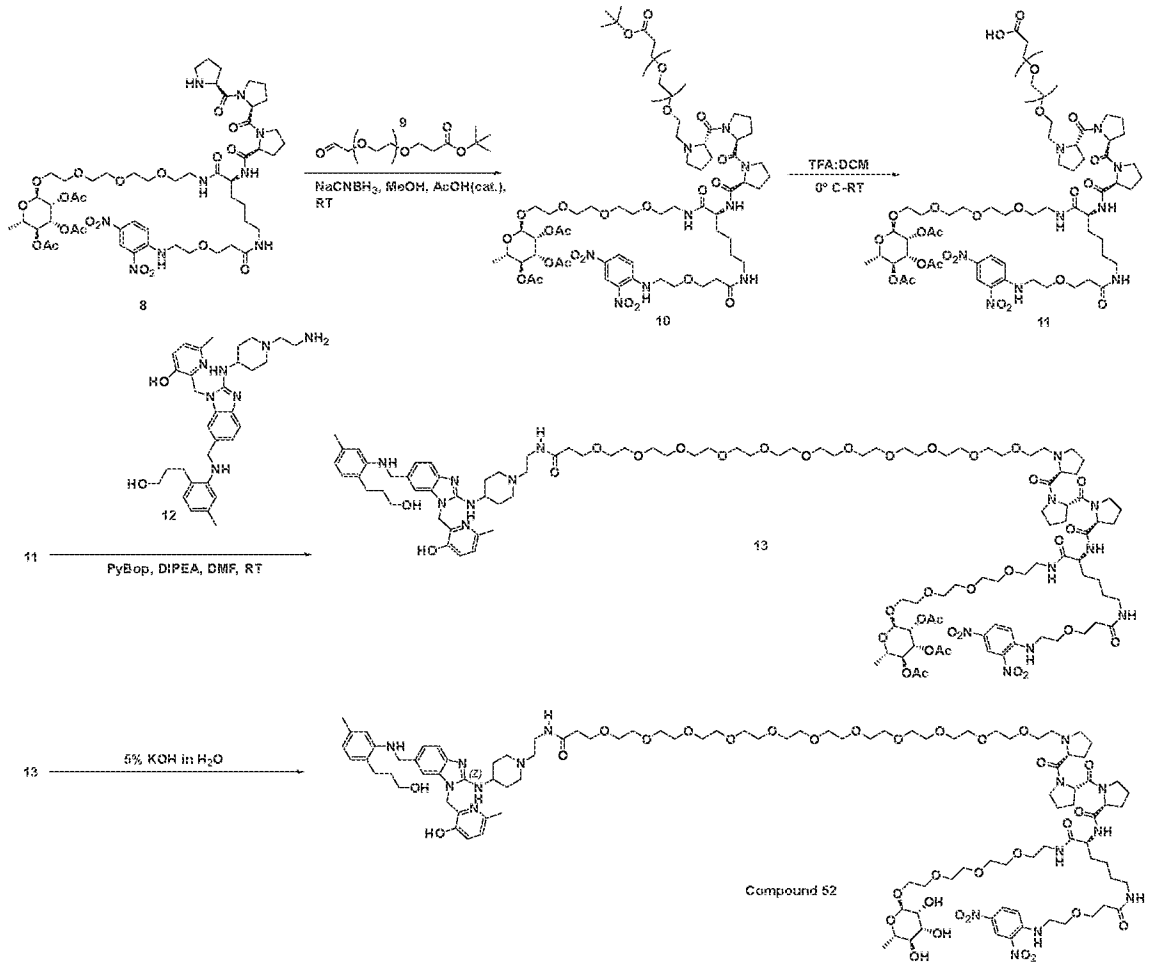
[0190] Compound 2. 2CTC resin loaded with protected peptide 1 (350 mg) was suspended in 20% 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in DCM (20 mL) in a 50 mL peptide reaction vessel. The reaction vessel was shaken for 30 min. The resin was filtered and washed three times with 10 mL portions of 20% HFIP in DCM. Chilled diethyl ether was added to the combined filtrates to give peptide free acid 2; yield, 80 mg.

[0191] Compound 4. To a solution of peptide free acid 2 (80 mg, 0.0109 mmol) in dry DMF (2 mL), HATU (4.3 mg, 1.2 eq.), HOBt (1.1 mg, 1.1 eq.), DIPEA (0.010 mL, 3.0 eq.), and compound 3 were added at 0 °C. Cooling was discontinued, and the reaction mixture was stirred for 4 h. Progress of the reaction was monitor by TLC. The reaction mixture was concentrated to isolate peptide conjugate 4, to which 90% TFA in DCM (10 ml) was added and stirred for 4 h. Progress of the reaction was monitor by LC-HRMS. After reaction completion, the reaction mixture was evaporated to peptide conjugate 5. yield, 120 mg.

[0192] Compound 5. To a solution of peptide conjugate 5 (120 mg) in 2 mL H_2O, 5% aq. sodium hydroxide (0.012 mL) was added to pH 11, and the reaction mixture was stirred for 1 h. Reaction progress was monitored by LC-HRMS. After reaction completion, the reaction mixture was purified by prep-HPLC using an ACN:H_2O gradient with 0.1% TFA to afford Compound 41. Yield: 0.010 g, LC-MS (ESI) m/z 5079.8 $[M+1]^+$.

[0193] Example 10. (S)-N-((S)-25-((2,4-dinitrophenyl)amino)-13,20-dioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,23-tetraoxa-12,19-diazapentacosan-14-yl)-1-((39-(4-((1-((3-hydroxy-6-methylpyridin-2-yl)methyl)-6-(((2-(3-hydroxypropyl)-5-methylphenyl)amino)methyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-36-oxo-3,6,9,12,15,18,21,24,27,30,33-undecaoxa-37-azanonatriacontyl)-L-prolyl-L-prolyl)pyrrolidine-2-carboxamide (Compound 52).


2-CTC
1
Fmoc-Pro-OH
2
DIPEA, DCM
3
1. 20%PIP/DMF
2. Fmoc-Pro-OH,
HATU, HOBT, DMF
Two Repeat cycle
4
2% TFA:DCM
5
6
PyBop, DIPEA, DMF
7
20% PIP in DMF
8



[0194] Compound 5. Solid support peptide synthesis of compound 5 was performed manually in a peptide reaction vessel using a 2CTC resin and a conventional Fmoc/Ot-Bu protocol.

[0195] General procedure for:

[0196] Resin loading: In a peptide vessel 2CTC resin (2.0 g, 2.8 mmol/g) was swollen in DCM for 30 min. A solution of Fmoc-Pro-OH (2.0 eq.) in DIPEA (3.0 eq.) was added and the vessel was shaken for 12 h. After the reaction, the free sites of the resin were capped with MeOH and the resin was washed with DCM (2 X 20 mL).

[0197] Fmoc De-protection: To a peptide reaction vessel containing swollen peptidyl resin in DMF was added 20% piperidine in DMF. The reaction vessel was shaken for 0.5 h. The resin was filtered and was washed with DMF (3×30 mL) and DCM (3X20 mL).

[0198] Amide coupling: To the reaction vessel containing swollen 2CTC resin is added activated amino acid solution (2.0 eq.) and coupling agent (HATU, HOBT; 2.5 eq.) in DMF (20 mL). Add the activated amino acid solution and DIPEA (3.0 eq.) to the reaction vessel containing swollen resin. The reaction vessel was shaken for 2 h. The Fmoc deprotection-Amide coupling cycle was repeated two times to give resin loaded protected peptide 4.

[0199] Resin Cleavage: 2CTC resin loaded with protected peptide 4 was suspended in 2% TFA in DCM (20 mL) in the peptide reaction vessel. The reaction vessel was shaken for 30 min. The resin was filtered and washed three times with portions of 2% TFA in DCM. Chilled diethyl ether was added to the combined filtrates to give peptide free acid 5, which was purified by Prep-HPLC using C-18 reverse phase (ACN/water gradient with 0.1% TFA) to give 0.30 g. LC-MS (ESI) m/z 532.07 $[M+1]^+$.

[0200] Compound 7. To a solution of compound 5 (0.2 g, 0.376 mmol) and compound 6 (0.296 g, 0.339 mmol, see Example 6, compound 21) in DMF (4 mL), HATU (143 mg, 376 µmol) and DIPEA (0.197 mL, 1.13 mmol) were added. The reaction mixture was stirred for 2 h, and the progress of the reaction was monitored by TLC. After reaction completion, the reaction mixture was evaporated under high vacuum, and the remainder was purified by Sephadex LH20 column chromatography using MeOH. Fractions containing compound 7 were evaporated to afford compound 7 as pale yellow solid. Yield: 0.160 g, 26.05%. LC-MS (ESI) m/z 1388.35 $[M+1]^+$.

[0201] Compound 8. To the solution of compound 7 (0.160 g, 0.049 mmol) in DMF (5 ml), piperidine (0.75 ml) was added dropwise, and the reaction mixture was stirred at 25 °C for 1 h. Progress of the reaction was monitored by TLC. After reaction completion, the reaction mixture was concentrated under high vacuum, and the remainder was purified over silica gel chromatography using 15% MeOH/DCM to afford compound 8 as a yellow viscous liquid. Yield: 0.080 g, 59%. LC-MS (ESI) m/z 1166.2 $[M+1]^+$.

[0202] Compound 10. To the solution of compound 8 (80 mg, 0.065 mmol) and compound 9 (commercially available, 0.062 g, 0.098 mmol) in MeOH (4 mL), acetic acid (cat.) was added. The reaction mixture was stirred for 4 h. After reaction completion,

sodium cyanoborohydride (0.020 g, 0.331 mmol) was added portion wise. Progress of the reaction was monitored by TLC. After reaction completion, the reaction mixture was quenched with water and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated, and the remainder was purified by size exclusion chromatography (Sephadex-LH20) using MeOH to afford compound 10 as a yellow solid. Yield: 0.068 g, 58%. LC-MS (ESI) m/z 1778.40 $[M+1]^+$.

[0203] Compound 11. To the solution of compound 10 (0.058 g, 0.033 mmol) in DCM (3 mL), TFA (0.6 mL) was added at 0 °C. The reaction mixture was stirred for 1 h. Progress of the reaction was monitored by TLC. After reaction completion, the reaction mixture was evaporated under high vacuum to afford compound 11 as a yellow sticky solid. Yield: 0.050 g, 89%. LC-MS (ESI) m/z 1720.15 $[M-1]^-$

[0204] **Compound 13.** To the solution of compound 11 (0.050 g, 0.029 mmol) and compound 12 (0.040 g, 0.034 mmol) in DMF (0.5 mL), PyBop (0.027 g, 0.043 mmol) and DIPEA (21.3 µL, 0.116 mmol) was added at 0° C. Cooling was discontinued and the reaction mixture was stirred for 2 h, Progress of the reaction was monitored by TLC. After reaction completion, the reaction mixture was evaporated under vacuum, and the remainder was purified by Prep-HPLC using an ACN:H2O gradient with 0.1% TFA. Fractions containing compound 13 were lyophilized to afford compound 13 as a yellow sticky solid. Yield: 0.010 g, 15%. LC-MS (ESI) m/z 2263.19 $[M+1]^+$.

[0205] Compound 52. To a solution of compound 13 (0.008 g, 0.0025 mmol) in water (0.8 mL), 5% aq. KOH (10 µL) was added dropwise to pH=11. The reaction mixture was stirred for 1 h. After reaction completion, the reaction mixture was purified by reverse phase C18 column chromatography using an ACN:H2O gradient with 0.1% Ammonium bicarbonate. Compound 52 eluted approximately in 40-50% ACN:H2O. Fractions containing compound 52 were lyophilized to afford Compound 52 as a yellow solid. Yield: 0.001 g; 19%. LC-HRMS (ESI) m/z 2136.15 $[M+1]^+$.

[0206] Example 11. 1-(((1S,3r)-3-((22S,E)-45-chloro-11-methyl-3,7-dioxo-5-oxa-8,11-diaza-1(2,7)-pyrazolo[1,5-a]pyrimidina-2(2,1)-piperidina-4(1,2)-benzenacycloundecaphane-15-yl)cyclobutyl)amino)-N-((S)-25-((2,4-dinitrophenyl)amino)-

13,20-dioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,23-tetraoxa-12,19-diazapentacosan-14-yl)-3,6,9,12,15,18,21,24,27,30,33-undecaoxahexatriacontan-36-amide (Compound 53)



[0207] Compound 1 was prepared as described in Bioorg. Med. Chem. 2020, 28, 115818.

[0208] Compound 2. To the stirred solution of compound 1 (0.41 g, 0.72 mmol) in dry DCM (10 mL), triethylamine (0.28 mL, 3.60 mmol) was added dropwise and the mixture was stirred for 10 min. To this mixture, p-(chlorosulfonyl)toluene (0.21 g, 0.72 mmol) was added and the resulting mixture was stirred for 12 h. Progress of the reaction was monitored

by TLC. After reaction completion, the reaction mixture was evaporated to afford compound 2 as a brown solid which was used without further purification. Yield: 0.55 g (Crude); LCMS (ESI) m/z 693.00 $[M+1]^+$.

[0209] Compound 3. To the stirred solution of compound 2 (0.50 g, 0.72 mmol) in dry DMF (8 mL), sodium azide (0.47 g, 7.21 mmol) was added and the mixture was sealed under N_2 atmosphere and stirred at 90°C for 16h. After reaction completion (TLC), the mixture was cooled to ambient temperature, diluted with EtOAc, washed sequentially with ice-cold water and brine. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The remainder was purified by column chromatography using 5% MeOH/DCM as eluents to obtain compound 3 as a brown solid. Yield: 0.26 g, 63.91 %; LCMS (ESI) m/z 564.10 $[M+1]^+$.

[0210] Compound 4. To the stirred solution of compound 3 (0.31 g, 0.49 mmol) in THF (20 mL) was added trimethylphosphine (0.38 g, 4.95 mmol) dropwise. After stirring for 2h, water (5 mL) was added and the resulting mixture was stirred for 6 h. Upon reaction completion, the reaction mixture was evaporated, and the remainder was purified by trituration using diethyl ether-pentane, dried under vacuum to afford compound 4 as a yellowish solid. Yield: 0.15 g, 40.86 %; LCMS (ESI) m/z 538.15 $[M+1]^+$.

[0211] Compound 6. To the stirred solution of compound 5 (BLD Pharma, 0.20 g, 0.317 mmol) in DCM (6 mL), 2,2,6,6-tetramethyl-1-oxypiperidine (9.91 mg, 0.063 mmol) was added. The reaction mixture was stirred for 5 min, then acetoxyphenyliodo acetate (0.33 g, 1.01 mmol) was added. The reaction mixture was stirred for 30 min After reaction completion (TLC), the reaction mixture was purified by column chromatography using 3-4% MeOH/DCM to afford compound 6 as an orange colored liquid. Yield: 0.13 g, 65%; ^1H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 4.18 (s, 1H), 3.77 - 3.50 (m, 41H), 3.45 (s, 2H), 2.52 (t, J = 4.8 Hz, 2H), 1.46 (s, 9H), 1.27 (m, 1 H).

[0212] Compound 7. To the stirred solution of compound 6 (0.13 g, 0.195 mmol) and compound 4 (0.10 g, 0.195 mmol) in MeOH (5.0 mL), acetic acid (0.1 mL) was added dropwise. The reaction mixture was stirred for 1 h. After reaction completion (TLC), sodium borohydride (58.4 mg, 0.98 mmol) was added portion wise. After reaction completion, the

reaction mixture was quenched with water (1 mL) and extracted with DCM (3x50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under high vacuum. The remainder was a mixture of compound 7 and the corresponding di-alkylated side-product. Purification by size exclusion chromatography (LH20) using MeOH gave compound 7 as a light yellow viscous liquid, Yield: (0.08 g, 35.62%); LCMS m/z 1150.15 $[M+1]^+$.

[0213] Compound 8. To a stirred solution of compound 7 (0.10 g, 0.05 mmol), in anhydrous DCM (3.0 mL), TFA (1.0 mL) was added at 0°C. Cooling was discontinued and the reaction mixture was stirred for 3 h. Progress of the reaction was monitored by LCMS. After reaction completion, the mixture was evaporated to afford compound 8 as a light yellow liquid which was used without further purification. Yield: 0.08 g, Crude; LCMS (ESI) m/z 1094.20 $[M+1]^+$.

[0214] Compound 10. Compound 10 was prepared using the same procedure used to make compound 7 in Example 8. Compound 10 was prepared from compound 8 (0.15 g, 0.13 mmol) and compound 9 (0.12 g, 0.13 mmol, see Example 6, compound 21) in DMF (5.0 mL) with HATU (0.057 g, 0.15 mmol) and DIPEA (0.12 mL, 0.68 mmol), and was purified by silica gel chromatography using 8-10% MeOH/DCM as eluent to afford compound 10 as a yellowish liquid. Yield: 0.09 g, 33.66 %; LCMS (ESI) m/z 1954.45 $[M+1]^+$.

[0215] Compound 53. To a stirred solution of compound 10 (0.05 g, 0.025 mmol) in MeOH (10.0 mL) was added sodium methoxide (0.14 mg, 0.002 mmol) at 0 °C. Cooling was discontinued and the reaction mixture was stirred for 2 h. The progress of the reaction was monitored by LCMS. After reaction completion, the reaction mixture was treated with acidic (Dowex 50, H^+) to pH~7 and the suspension was filtered through sintered funnel with MeOH washing. The filtrate was evaporated, and the remainder was purified by prep HPLC to afford Compound 53 as a yellowish color solid. Yield: 25 mg, 53.45 %; LCMS (ESI) m/z 1826.10 $[M+1]^+$, LC-HRMS (ESI) m/z 1824.87 $[M+1]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 8.86 (d, J = 5.20 Hz, 1H), 8.82 (brs, 3H), 8.66 (d, J = 7.48 Hz, 1H), 8.24 (dd, J = 9.6, 2.52 Hz, 1H), 7.90 (qt, J = 8.08 Hz, 2H), 7.77 (t, J = 5.44 Hz, 1H), 7.55 - 7.52 (m, 1H), 7.46 - 7.41 (m, 1H), 7.28 - 7.22 (m, 1H), 7.20 - 6.96 (m, 1H), 6.36 (s, 1H), 6.20 (s, 1H), 5.01 - 4.85 (m, 2H), 4.67 - 4.46 (m, 4H), 4.19 - 3.91 (m, 8H), 3.76 - 3.37 (m, 78 H), 3.20 - 2.96 (m,

14H), 2.58 - 2.53 (m, 3H), 2.40 (qt, J = 7.84 Hz, 2H), 2.36 (qt, J = 6.05 Hz, 2H), 2.28 - 2.16 (m, 1H), 2.10 - 1.89 (m, 1H), 1.80 - 1.65 (m, 1H), 1.62 - 1.50 (m, 3H), 1.49 - 1.43 (m, 2H), 1.36 - 1.29 (m, 2H), 1.23 - 1.19 (m, 2H), 1.13 (d, J = 7.8 Hz, 3 H).

[0216] METHOD EXAMPLE 1. Immunofluorescence Assay (IFA) – **FIG. 3**

[0217] Hep2 cells were seeded into 12 well plates at a density of 0.1 x 10^6 cells per well 48 hours prior to the assay and were incubated at 37°C overnight. The next day selected wells were infected with RSV Long Strain (ATCC – VR-26) at a multiplicity of infection (MOI) of 3 in 250 µl of complete media, consisting of 2% Low IgG FBS Serum (Corning – MT35073CV), 1x EMEM (ATCC – 30-2003) and 1x PenStrep (Fisher – 15-140-148) for 2 hours at 37°C with rocking. After the incubation, complete media was added to the cells to bring the final volume to 1.25 ml. The infection was allowed to progress for 24 hours, after which the media was removed, and cells were washed twice with 1x PBS (phosphate-buffered saline) (Corning – MT21040CV). Cells were fixed with 1ml of 4% Formaldehyde per well at -20°C for 20 minutes. The fixative was removed, and cells were washed twice with 1x PBS. 1ml of blocking buffer (1x PBS, 2% fetal bovine serum (FBS) [Fisher – 10-082-147], 0.05% Tween 20 [Fisher – AAJ20605AP]) was added to each well and plates were incubated at 4°C overnight.

[0218] Cells were washed twice, and treated with Compound 47 in blocking buffer, 250 µL of: 1 µM Compound 47, 10 µM Compound 47, or 1 µM Compound 47 + 1 mM of TMC353121 (MedChemExpress – HY-11097), was added to specific wells and allowed to incubate overnight at 4°C. The next day, cells were washed twice with 1x PBS and 250 µl of primary antibodies against RSV F protein (Palivizumab: Human anti-RSV F protein – Invitrogen – MA5-41718) and DNP (CF® 750 Rabbit Anti-DNP – Biotium – 20869) were added to each well at a dilution of 1:500 in blocking buffer and plates were incubated at RT for 1 hour in the dark. Primary antibodies were removed, and the cells were washed twice. Secondary fluorescent antibodies (Alexa Fluor 488 Goat-anti-Human IgG – Invitrogen – A11013) were then added at a dilution of 1:500 to target the anti-RSV F Primary antibodies at RT for 1 hour in the dark. After washing the cells 3 times, 500 µl of 4′,6-diamidino-2-phenylindole (DAPI), a fluorescent dye that binds to DNA, solution in blocking buffer (1

μg/ml working concentration [Invitrogen – 62248]) was added to each well. Cells were imaged using an Invitrogen™ EVOS™ M5000 Imaging System (Fisher – 12-563-631) and overlays were created using Image J.

[0219] Referring to FIG. 3, the row labelled RSV F Protein in each image shows the signal generated from the anti F protein antibody showing where RSV F protein is located in green. The row labelled Compound 47 shows the signal generated from anti DNP antibody binding to Compound 47 showing where compound 47 is bound in red. The row labelled DAPI Signal shows where DNA is present staining the nucleus of healthy cells in blue to show where cells are present. The row labelled Overlay shows all the of the signals combined to show colocalization and where the drug is specifically binding. Compound 47 was found to only localize to infected cells at both 1 μM and 10 μM concentration and not to uninfected cells. The overlay row shows that Compound 47 colocalized with F protein expression. FIG. 3 also shows that upon addition of 100-fold TMC353121 (the free targeting ligand) to compete with compound 47 at the same binding site as compound 47, the interaction of compound 47 with the binding site is specific and completable with excess free targeting ligand, suggesting that the signal seen in the absence of ligand competition is due to a specific interaction not due to nonspecific binding. Compounds 47 was also evaluated in absence of the anti-DNP antibody as indicated by the column labeled Infection Only compound 47, which shows that compound 47 did not activate the immune system in the absence of the anti-hapten antibodies, and that the signal seen in the other columns is due to anti-DNP binding to compound 47 which is bound to the infected cells and not due to extraneous binding of the secondary antibody to compound 47. The final column labelled No Infection shows a situation in which compound 47 and anti-DNP antibody are present but virus has not been added. The data indicate that compound 47 does not bind or accumulate to healthy non-infected cells. Compound 47 is shown to very specifically bind to infected cells and trigger immunological mediated clearance of those cells. Without being bound by theory, it is believed herein that the specific attachment of compound 47 to RSV F protein on infected cells allows the immune system to only clear the infected cells with high selectivity.

[0220] METHOD EXAMPLE 2. Cytopathic Effect (CPE) Assay – **FIG. 4**

[0221] Hep2 cells were seeded into 96 well plates at a density of 0.2 x 10^5 cells per well 24 hours prior to the assay. The next day, serial 5-fold dilutions of each compound being tested were produced in complete media from 200 µM down to 202.4 pM. Cells were washed twice with 1x PBS, and 50 µl of compound containing media was added to the cells. Then, 50 µl of complete media containing RSV Long strain at a MOI of 0.02 was added to each well, resulting in the halving of the final compound concentration and MOI. Plates were placed at 37°C for 5 days. Media was then removed, cells were washed once with 1x PBS, and 100 µl of fresh complete media was added back onto the cells. Then, 20 µl of MTS reagent (Promega – G5430) was added to the cells, and the plates were incubated at 37°C for 2 hours. After the incubation, absorbance was measured via SpectraMax Plus 384 Microplate Reader (Molecular Devices) at 490 nm. The IC_{50} curves were calculated using GraphPad Prism (version 10.3.1). Higher Absorption at 490 nm is indicative of more live cells. Low absorption is indicative of more dead cells. As seen by the virus only group and cell only group the introduction of the virus to the cells causes cellular death. Compound 47 and TMC353121 are able to prevent cellular death with an IC_{50} of 169 nM, indicating that conjugation to the TMC353121 compound had minimal impact on its ability to bind to RSV F protein. Compound 47 was found to retain the same antiviral activity as the parent targeting ligand TMC353121 after conjugation to the linker and the dual haptens.

[0222] As shown in the following table, compounds 20, 22, 24, 36, 27, 47, 52, 53, and 54 were all found to maintain antiviral activity from the parent targeting ligand after conjugation to the linker and the dual haptens.

compound No	Antiviral Acitivty (IC50) µM
20	0.786
22	0.657
24	1.310
26	1.580
27	1.980

41	no effect
47	0.169
52	0.168
53	0.707
54	1.120

[0223] METHOD EXAMPLE 3. Antibody Dependent Cellular Cytotoxicity (ADCC) Assay – **FIG. 5**

[0224] For ADCC assays using Hep2 cells, Hep2 cells were seeded into 96 well white-walled plates at a density of 0.2 x 10^5 cells per well 48 hours prior to the assay and were infected as described in the IFA. After 24 hours of infection. Media was removed and cells were washed twice with 1x PBS and the ADCC assay was performed on the infected cells according to the manufacture's suggestions (Promega – G7015). Briefly, serial 5-fold dilutions of test compounds or test compounds + 100x free TMC353121 ligand (TMC353121) were made in RPMI (Roswell Park Memorial Institute) media ranging from 400 µM down to 32 nM. After washing the cells, 25 µl of RMPI media was added to the cells, and then 25 µl of RPMI media containing compounds was added to selected wells, and plates were incubated at 37°C for 30 minutes. Next, 25 µl of anti-DNP IgG antibodies (Acro Biosystems – DNP-M2-25mg) were added to selected wells at a concentration of 33.3 µg/ml in RPMI media and plates were incubated again at 37°C for 30 minutes. Finally, 25 µl of ADCC effector cells were then added to the infected cells, and the plates were incubated at 37°C for 6 hours.

[0225] For ADCC assays using clarified RSV (long strain) virions, 25 µl of RPMI media containing 2x10^5 $TCID_{50}$/ml of clarified RSV Long strain virus was added to each well in place of infected cells. The rest of the assay was performed as described for infected cells. After the 6-hour incubation, 70 µl of the luminescent reporter reagent was added to each well and the plates were read using a Spark® 10M Multimode Microplate Reader (Tecan). The EC_{50} curves were calculated using GraphPad Prism (version 10.3.1).

[0226] As shown in FIG. 5, compound 47 was found to activate natural killer effector cells when bound to RSV infected cells and RSV virus particles in the presence of anti-hapten antibodies. This interaction was found to be specific and completable with the free targeting ligand TMC353121. No activation was found in the absence of infection or virus or in the absence of antibody suggesting Compounds 47, 52, and 54 were found to recruit the immune system to RSV infected cells initiating their destruction. Compounds 26, 27 and 47 were found to specifically recruit the innate immune system to RSV virus particles initiating their destruction. The ADCC assay is run with virus only to similarly show that Compound 47 specifically recruits the innate immune system to RSV virus particles initiating their destruction.

[0227] The following data were observed for compounds in the ADCC assay against RSV viral particles

compound No	Antiviral Acitivty (EC50) μM
26	0.928
27	4.980
41	no effect
47	4.680
53	no effect

[0228] The following data were observed for compounds in the ADCC assay against RSV infected cells.

compound No	Antiviral Acitivty (EC50) nM
47	197
52	493.4
54	1540

[0229] The following data were observed for compounds in the ADCC assay against RSV infected cells.

[0230] METHOD EXAMPLE 4. Antibody Dependent Cellular Phagocytosis (ADCP) Assay

[0231] Hep2 cells were seeded into 96 well white-walled plates at a density of 0.2×10^5 cells per well 48 hours prior to the assay and were infected as described in the Immunofluorescence Assay. After 24 hours of infection. Media was removed and cells were washed twice with 1x PBS (Corning – MT21040CV) and the ADCP assay was performed on the infected cells according to the manufacture's suggestions (Promega – GA1341). Briefly, serial 5-fold dilutions of Compound 47 or Compound 47 + 100x free ligand (TMC353121) were made in ADCP media ranging from 400 μM down to 32 nM. After washing the cells, 25 μl of ADCP media was added to the cells, and then 25 μl of ADCP media containing compounds was added to selected wells, and plates were incubated at 37°C for 30 minutes. Next, 25 μl of anti-DNP IgG antibodies (Acro Biosystems – DNP-M2-25mg) were added to selected wells at a concentration of 33.3 μg/ml in ADCC media and plates were incubated again at 37°C for 30 minutes. Finally, 25 μl of ADCP effector cells were then added to the infected cells, and the plates were incubated at 37°C for 6 hours. After the 6-hour incubation, 70 μl of the luminescent reporter reagent was added to each well and the plates were read using a Spark® 10M Multimode Microplate Reader (Tecan). The EC_{50} curves were calculated using GraphPad Prism (version 10.3.1).

[0232] As shown in FIG. 8, compound 47 was found to activate macrophage effector cells upon binding to RSV infected cells in the presence of anti-hapten antibodies. This interaction was found to be specific and capable of being out competed with free ligand TMC353121. No activation was identified in infection alone without Compound 47, both in the presence and absence of antibody, suggesting Compound 47 functions to bridge the effector cells to RSV infected cells, facilitating the uptake of the infected cells by the innate immune system.

[0233] As shown in FIG. 8 Compound 47 was found to have an EC_{50} of 2.52 μM for the activation of Macrophages.

[0234] METHOD EXAMPLE 5. Compliment Dependent Cytotoxicity (CDC) Assay

[0235] Hep2 cells were seeded into 96 well white-walled plates at a density of 0.2×10^5 cells per well 48 hours prior to the assay and were infected as described in the Immunofluorescence Assay. In addition, Hep2 cells were serially diluted 2-fold from 20,000 cells per well down to 312.5 cells per well in triplicate to produce a standard curve from which to determine % cell death upon the completion of the assay. After 24 hours of infection. Media was removed and cells were washed twice with 1x PBS. After washing, 25 µl of serum-free RPMI 1640 media (Promega – G708A) was added to the cells. Serial 5-fold dilutions of Compound 47 or Compound 47 + 100x free ligand (TMC353121) were made in serum-free RPMI 1640 media ranging from 400 µM down to 32 nM. A total of 25 µl of serum-free RPMI 1640 media containing compounds was added to selected wells. For the standard curve wells, 25 µl of serum-free RPMI 1640 media containing no compounds was added to each well. Three negative control wells (complete cell death) were produced by adding 25 µl of 70% ethanol to each well. The plates were places on a plate shaker (Fisher - 88-861-023) for 2 minutes at room temperature. Then, the plates were incubated at 37°C for 30 minutes. Next, 50 µl of serum-free RPMI 1640 media containing 20 % rabbit complement serum (Sigma – S7764-5ML) supplemented with or without 2% anti-DNP antibody (Invitrogen – A6430) was added to specific wells and the plates were incubated at 37°C for 4-6 hours. After the incubation, cells were allowed to equilibrate to room temperature for 30 minutes. Then, 100 µl of Cell Titer Glo 2.0 reagent (Fisher - PRG9242) was added to all wells, and the amount of luminescence was determined using a Spark® 10M Multimode Microplate Reader (Tecan). The % cell death of compound treated cells was determined using the amount of luminescence from the cell dilution standard curve and the ethanol treated cells were used as the baseline of 100% cell death. The EC_{50} curves were calculated using GraphPad Prism (version 10.3.1).

[0236] As shown in FIG. 9, compound 47 was found to enable complement dependent cell death upon binding to RSV infected cells in the presence of anti-hapten antibodies. This interaction was found to be specific. No activation was identified in infection alone without Compound 47, both in the presence and absence of antibody, suggesting Compound 47 functions to bridge the complement proteins to RSV infected cells, facilitating the death of

infected cells. Compound 47 was found to activate complement Cascade with an EC_{50} of 282.1nM.

[0237] METHOD EXAMPLE 6. Binding affinity of F protein ligands with RSV virus particles

The affinity of the conjugates was measured using microscale thermophoresis. A monolith Nano temper label free instrument from Nano temper was used with monolith NT.label free premium capillaries. A 16 point serial dilution of each compound was incubated with 500 nM clarified RSV long strain virus particles. Compounds were diluted with an MST buffer with 0.1% pluronics. Excitation was set to 60% with medium MST power and a 2.5 second on time. The normalized fluorescence was plotted for each concentration and plotted relative to the concentration of the compound. Then the disassociation constant for each ligand was calculated with nano tempers binding software.

Compound	Kd
26	990nM
27	116nM
47	217nM
TMC353121	96.5nM

[0238] The disassociation constants observed for the conjugated described herein show that the targeting ligand retains good affinity for the RSV virus particle after the addition of linkers and haptens. The f protein does have a different confirmation in infected cells

[0239] METHOD EXAMPLE 7. In Vivo Efficacy – **FIG. 6 & FIG. 7**

[0240] Six week old Female cotton rats from Inotiv were intranasally inoculated with $1x10^6$ $TCID_{50}$ RSV Long strain. Cotton rats were randomly assigned to one of 3 treatment groups with 4 cotton rats assigned per group. 24 hours post infection cotton rats were treated with Vehicle(20 mg/ml Avicel 491(sigma), 6 mg/ml n-dodecyl β-D-maltoside(sigma), 1x Phosphate buffered saline(gibco)), or Compound 47 1.5μmol/kg, or ALS-008176 (lumicitabine, medchem express) 100umol/kg. Vehicle and Compound 47 were

administered intranasally to cotton rats which had been anesthetized via isoflurane inhalation with a dose volume of 0.2ml/kg. ALS-008176 was dissolved in phosphate buffered saline and 2% Sucrose and administered orally with a rodent feeding tube. 48 hours post infection the animals were euthanized and the lungs were weighed and removed in a sterile environment. 1 lung was placed in a triazol solution for RNA extraction. The other was placed in to PBS and flash frozen with liquid nitrogen.

[0241] The lung tissue was automatically homogenized using a Fisherbrand Bead Mill 4 Mini Homogenizer. Viral RNA was extracted following the product protocol forymo ran extraction kit . RNA was then quantified with qPCR with primers specific to RSV f protein and GAPDH using Bio-Rad's iTaq Universal SYBR Green SuperMix and a Biorad CFX thermocyler. Relative amounts of Viral RNA were standardized to the amount of the housekeeping gene GAPDH and compared. Compound 47 was found to significantly decrease the viral RNA. The other lobe was subjected to a quantification of live virus in via TCID50 quantification. Lung tissue designated for TCID50 was placed into 1 ml of complete media and flash frozen in liquid nitrogen. Frozen samples were thawed and homogenized using a Fisherbrand™ Bead Mill 4 Mini Homogenizer (Fisher – 15-340-164) for 2 minutes at level 5 speed. The homogenate was clarified using a microcentrifuge at 10,000 x g for 10 minutes. The clarified homogenate was collected in a fresh microcentrifuge tube for TCID50 analysis. The night before, Hep2 cells were seeded into 96 well plates at a density of 0.2 x 105 cells per well and were roughly 85% confluent at the start of the assay. In separate 96 well plates, serial 10-fold dilutions were made of the clarified lung homogenate samples into complete media. The media was removed from the Hep2 cells, and the cells were washed twice with 1x PBS. Next, 25 µl of the homogenate dilutions or control groups were added onto the cells and the infection was allowed to progress for 2 hours at 37°C with rocking. After the incubation period, 75 µl of complete media was added to each well, and the assay was allowed to progress for 5 days. The media and inoculum was removed after 120 hours post infection, and 100 µl of fresh complete media was added to all wells. Then, 20 ml of MTS reagent (Promega – G5430) was added to the cells, and the plates were incubated at 37°C for 2 hours. After the incubation, absorbance was measured via SpectraMax Plus 384

Microplate Reader (Molecular Devices) at 490 nm. The TCID50 curves were calculated using GraphPad Prism (version 10.3.1), and the final titer values were produced by normalizing the TCID50 output to the weight of total lung tissue homogenized (TCID50/mg) and the dilution factors used during infection.[TCID50 value produced in Prism / Total weight of lung tissue (mg)] x [dilution factor of homogenate volume (40)].

[0242] As shown in FIG. 6, compound 47 performed better than ALS-008176, and was found to drop viral titer by more than 100 fold after just 24 hours and 1 dose, a statistically significant improvement over vehicle control.

[0243] Compound 26 was found to drop viral titer by more than 4 fold after just 24 hours and 1 dose.

[0244] METHOD EXAMPLE 8. In Vivo Efficacy – **FIG. 10.** Six-week-old Female cotton rats from Inotiv were intranasally inoculated with $1x10^6$ $TCID_{50}$ RSV Long strain. Cotton rats were randomly assigned to one of 3 treatment groups with 4 cotton rats assigned per group. After 24 hours post infection, cotton rats were treated with Vehicle (20mg/ml Avicel 491(Sigma - 11363), 6mg/ml n-dodecyl β-D-maltoside (Sigma - 69227-93-6), 1x Phosphate buffered saline (Corning – MT21040CV)), or Compound 26 1.5μmol/kg, or ALS-008176(MecChem Express) 100umol/kg. Vehicle and Compound 26 were administered intranasally to cotton rats which had been anesthetized via isoflurane inhalation with a dose volume of 0.2ml/kg. ALS-008176 was dissolved in phosphate buffered saline and 2% Sucrose and administered orally with a rodent feeding tube. Cotton Rats were euthanized 24 hours post treatment with compounds, and their lungs were harvested under sterile conditions. After removal, the lungs were rinsed in 1x PBS containing 1x PenStrep to remove any surface exposed bacteria and were weighed to the nearest mg. Lung tissue designated for $TCID_{50}$ was placed into 1 ml of complete media and flash frozen in liquid nitrogen. The lung tissue was subjected to a quantification of live virus in via TCID50 quantification. Lung tissue designated for TCID50 was placed into 1 ml of complete media and flash frozen in liquid nitrogen. Frozen samples were thawed and homogenized using a Fisherbrand™ Bead Mill 4 Mini Homogenizer for 2 minutes at level 5 speed. The homogenate was clarified using a microcentrifuge at 10,000 x g for 10 minutes. The clarified

homogenate was collected in a fresh microcentrifuge tube for TCID50 analysis. The night before, Hep2 cells were seeded into 96 well plates at a density of 0.2 x 105 cells per well and were roughly 85% confluent at the start of the assay. In separate 96 well plates, serial 10-fold dilutions were made of the clarified lung homogenate samples into complete media. The media was removed from the Hep2 cells, and the cells were washed twice with 1x PBS. Next, 25 µl of the homogenate dilutions or control groups were added onto the cells and the infection was allowed to progress for 2 hours at 37°C with rocking. After the incubation period, 75 µl of complete media was added to each well, and the assay was allowed to progress for 5 days. The media and inoculum was removed after 120 hours post infection, and 100 µl of fresh complete media was added to all wells. Then, 20 ml of MTS reagent (Promega – G5430) was added to the cells, and the plates were incubated at 37°C for 2 hours. After the incubation, absorbance was measured via SpectraMax Plus 384 Microplate Reader (Molecular Devices) at 490 nm. The TCID50 curves were calculated using GraphPad Prism (version 10.3.1), and the final titer values were produced by normalizing the TCID50 output to the weight of total lung tissue homogenized (TCID50/mg) and the dilution factors used during infection.

[TCID50 value produced in Prism / Total weight of lung tissue (mg)] x [dilution factor of homogenate volume (40)].

[0245] Six-week-old male cotton rats from Inotiv were intranasally inoculated with $1x10^4$ $TCID_{50}$ RSV A2 strain. Cotton rats were randomly assigned to one of 2 treatment groups with 3 cotton rats assigned per group. After 24 hours post infection, cotton rats were treated with Vehicle (20mg/ml Avicel 491(Sigma - 11363), 6mg/ml n-dodecyl β-D-maltoside (Sigma - 69227-93-6), 1x Phosphate buffered saline (Corning – MT21040CV)), or Compound 26 1.5µmol/kg,. Vehicle and Compound 26 were administered intranasally to cotton rats which had been anesthetized via isoflurane inhalation with a dose volume of 0.2ml/kg. Cotton Rats were euthanized 4 days post infection and their lungs were harvested under sterile conditions. Lungs were flash frozen in liquid nitrogen immediately upon collection. The lung tissue for RNA analysis was automatically homogenized using a Fisherbrand Bead Mill 4 Mini Homogenizer (Fisher – 15-340-164). Viral RNA was

extracted following the product protocol Zymo RNA extraction kit. RNA was then quantified with qPCR with primers specific to RSV F protein and GAPDH using Bio-Rad's iTaq Universal SYBR Green SuperMix and a Biorad CFX Thermocyler. Relative amounts of Viral RNA were standardized to the amount of the housekeeping gene GAPDH and compared. Compound 26 was found to significantly decrease the viral RNA.

[0246] Compound 27 was also found to significantly decrease the viral RNA.

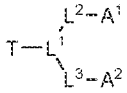
[0247] METHOD EXAMPLE 9. Cytokine release assay.

[0248] A cytokine release assay was performed on compound 47 to assess the risk of causing a cytokine storm in humans. For this assay, human PBMCs pooled from at least 4 donors were plated at $2.5x10^5$ cells/well. 1 μM of compound 47, 1 μM TLR7 agonist, or RPMI was added to the wells. All groups were supplemented with 10mg/mL Immune Globulin Injection (IVIG) to provide anti-DNP and anti-rhamnose antibodies. The PBMCs were then incubated at 37 C, 5% CO_2 for 24 hours. Cells were centrifuged at 450xg for 10 min and supernatant was collected. Cytokines in the supernatant were measured using a LEGENDplex™ Human Inflammation Panel 1 (biolegend, cat. #740809) following manufacturers protocol. The 9 different proteins measured were IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1 (CCL2), IL-10, IL-12p70, IL-18, IL-23, and IL-33. Fluorescence was measured using an Attune NxT flow cytometer (Fisher), and the concentration of each protein was quantified using LEGENDplex™ software (biolegend). Data was graphed using GraphPad prism.. An unpaired t test was performed to determine the significance of the difference in cytokines levels between media and compound 47. There was no significant difference for any of the cytokines measured. This suggests that compound 47 does not have any inherent immunogenicity while in contact with immune cells and antibodies, and it is unlikely to cause a cytokine storm after administration to humans in the absence of binding to it its target of RSV infected cells.

[0249] Each publication cited herein is incorporated herein by reference, in its entirety.

WHAT IS CLAIMED IS:

1. A compound of the formula



Formula I

and pharmaceutically acceptable salts and solvates thereof, wherein:

T is a radical of a ligand for a target protein of a RSV or a RSV-infected cell;

L^1, L^2, and L^3 are each, an independently selected linker; and

A^1 and A^2 are each, a radical of an independently selected hapten.

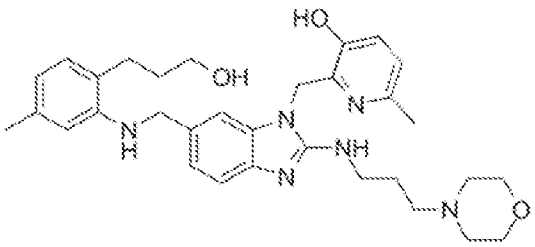
2. The compound of claim 1 wherein the target protein is a RSV fusion (F) glycoprotein.

3. The compound of claim 1 wherein the target protein is a RSV attachment glycoprotein (G).

4. The compound of claim 1 wherein the ligand is an inhibitor of RSV fusion (F) glycoprotein.

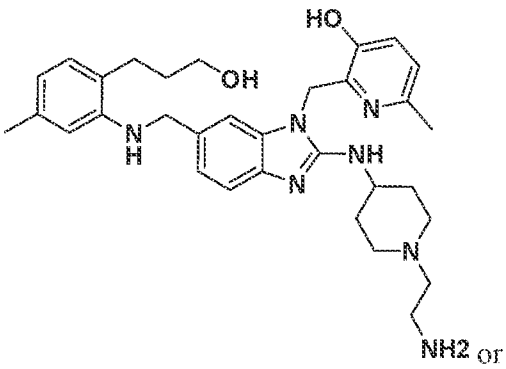
5. The compound of claim 1 wherein the ligand is an inhibitor of RSV attachment glycoprotein (G).

6. The compound of claim 1 wherein the ligand is

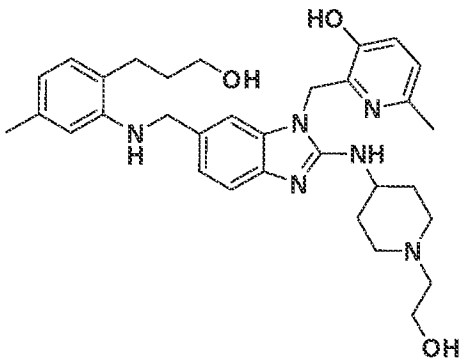


or an analog or derivative thereof.

7. The compound of claim 1 wherein the ligand is

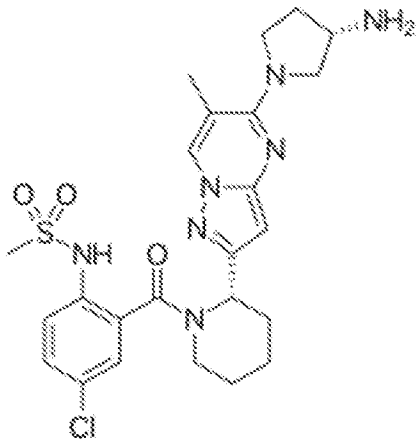


or



or an analog or derivative thereof.

8. The compound of claim 1 wherein the ligand is presatovir



or an analog or derivative thereof.

9. The compound of claim 1 wherein the ligand is SEQ ID NO: 16 (T118).

10. The compound of claim 1 wherein the ligand is SEQ ID NO: 40 (VIQKI), or a substitution isomer selected from VIQKI-I456F (SEQ ID NO: 41)0 and VIQKI-I454F/I456F SEQ ID NO: 42).

11. The compound of any one of claims 1-10 wherein the hapten has an epitope that has an endogenous Ab, B or T cell.

12. The compound of any one of claims 1-10 wherein the hapten is selected from a rhamnose, a nitrophenyl, a nitrophenol, a nitroaniline, a dinitrophenyl (DNP), a dinitrophenol, a dinitroaniline, a trinitrophenyl (TNP), a trinitrophenol, a trinitroaniline, chloronitrophenyl, a chloronitrophenol, a chloronitroaniline, an iodonitrophenyl, an

iodonitrophenol, an iodonitroaniline, a nitrotyrosine, an hydroxynitrotyrosine, an aminonitrotyrosine, 4-hydroxy-3-nitrophenyl acetic acid, an α-galactosyl moiety, a sulfated Gal, a phosphorylcholine, a bacterial antigen, a viral antigen,

13. The compound of any one of claims 1-10 wherein one of A^1 and A^2 is a radical of L-rhamnose; and the other of A^1 and A^2 is a radical of a dinitroaniline.

14. The compound of any one of claims 1-13 wherein one or more of L^1, L^2, and L^3 is a chain of atoms, where the length of each chain is independently selected and in the range of about 2 to about 60.

15. The compound of any one of claims 1-14 wherein one or more of L^1, L^2, and L^3 includes or also includes ethoxy, ethylamino, ethylene glycol, aza-ethylene glycol, $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is in the range from 2 to about 36.

16. The compound of any one of claims 1-15 wherein one or more of L^1, L^2, and L^3 includes or also includes $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is 2-36.

17. The compound of any one of claims 1-16 wherein each of L^1, L^2, and L^3 includes or also includes ethoxy, ethylamino, ethylene glycol, aza-ethylene glycol, $(PEG)_n$, or aza-$(PEG)_n$, or a combination thereof, where n is 2-36.

18. The compound of any one of claims 1-17 wherein one or more of L^1, L^2, and L^3 includes or also includes O-alkyl-O, N-alkyl-N, C(O)-alkyl-C(O), or NC(O)-alkyl-C(O)N, or a combination thereof.

19. The compound of any one of claims 1-18 wherein one or more of L^1, L^2, and L^3 includes or also includes O-alkyl-C(O), N-alkyl-C(O), O-alkyl-N-alkyl-C(O), N-alkyl-O-alkyl-C(O), or C(O)alkyl-C(O), or a combination thereof.

20. The compound of any one of claims 1-19 wherein L^1 includes or also includes O-alkyl-C(O)N-diyl, O-alkyl-O-alkyl-C(O)N-diyl, or N-alkyl-O-alkyl-O-alkyl-C(O)N-diyl.

21. The compound of any one of claims 1-20 wherein one or more of L^1, L^2, and L^3 includes or also includes one or more amino acids.

22. The compound of any one of claims 1-21 wherein one or more of L^1, L^2, and L^3 includes or also includes one or more hydrophilic amino acids selected from β-NH_2-Ala,

Arg, Asn, Asp, Cys, Glu, Gln, His, Lys, Met, Orn, Ser, or Thr, including the naturally occurring L-enantiomers of each of the foregoing.

23. The compound of any one of claims 1-22 wherein one or more of L^1, L^2, and L^3 includes or also includes one or more amino acids selected from glycine, serine, proline, ornithine, and lysine.

24. The compound of any one of claims 1-23 wherein one or more of L^1, L^2, and L^3 includes or also includes an ornithine or lysine.

25. The compound of any one of claims 1-24 wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_n$, including $(L\text{-}Pro)_n$, where n is 1-6.

26. The compound of any one of claims 1-25 wherein one or more of L^1, L^2, and L^3 includes or also includes a $(Pro)_3$-Lys.

27. The compound of any one of claims 1-26 wherein one or more of L^1, L^2, and L^3 includes or also includes a C(O), C(O)O, C(O)NH, OC(O)NH, or NHC(O)NH group.

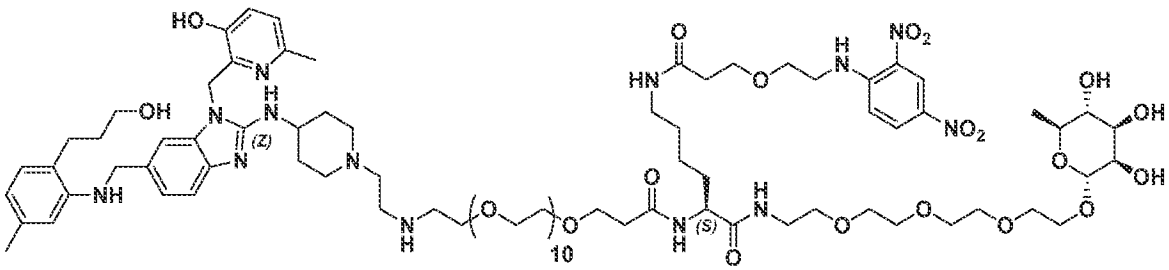
28. The compound of any one of claims 1-27 wherein L^2 and/or L^3 are hydrophilic.

29. The compound of any one of claims 1-28 wherein L^1 includes a region that is capable of forming an α-helical conformation.

30. The compound of any one of claims 1-29 wherein the extended conformation of L^2 and/or L^3 is at least 15 Å in length.

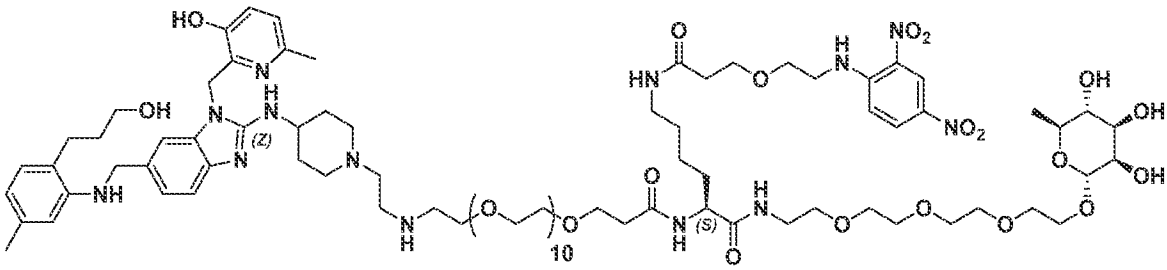
31. A compound of any formula shown in FIG 1 or a pharmaceutically acceptable salt or solvate thereof.

32. A compound of the formula



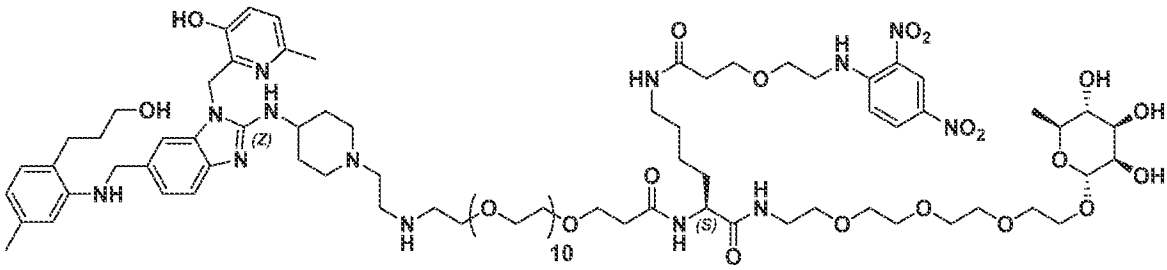
or a pharmaceutically acceptable salt or solvate thereof.

33. A composition comprising a compound of the formula



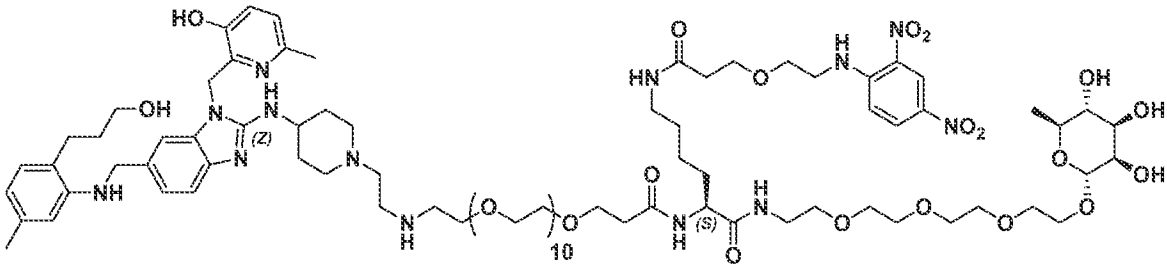
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

34. A composition for use in treating RSV infection, the composition comprising a compound of the formula



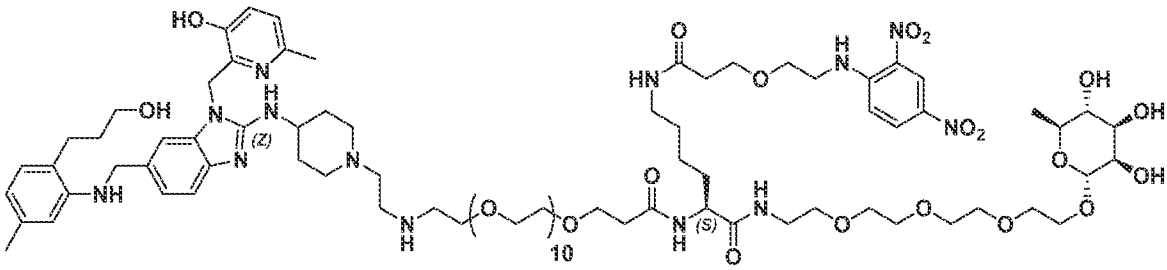
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

35. A unit dose for use in treating RSV infection, the unit dose comprising a compound of the formula



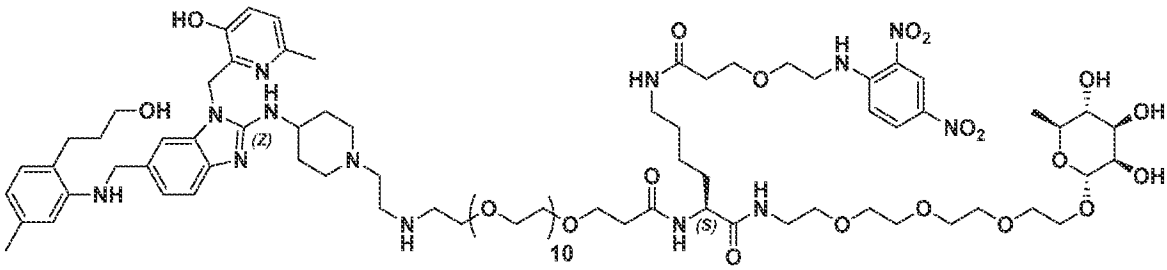
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

36. A method for treating RSV infection in a host animal, the method comprising administering a therapeutically effective amount of a compound of the formula



or a pharmaceutically acceptable salt or solvate thereof, or composition or unit dose comprising the compound.

37. Use of a compound of the formula



or a pharmaceutically acceptable salt or solvate thereof, or composition or unit dose comprising the compound, in the manufacture of a medicament for use in treating RSV infection in a host animal.

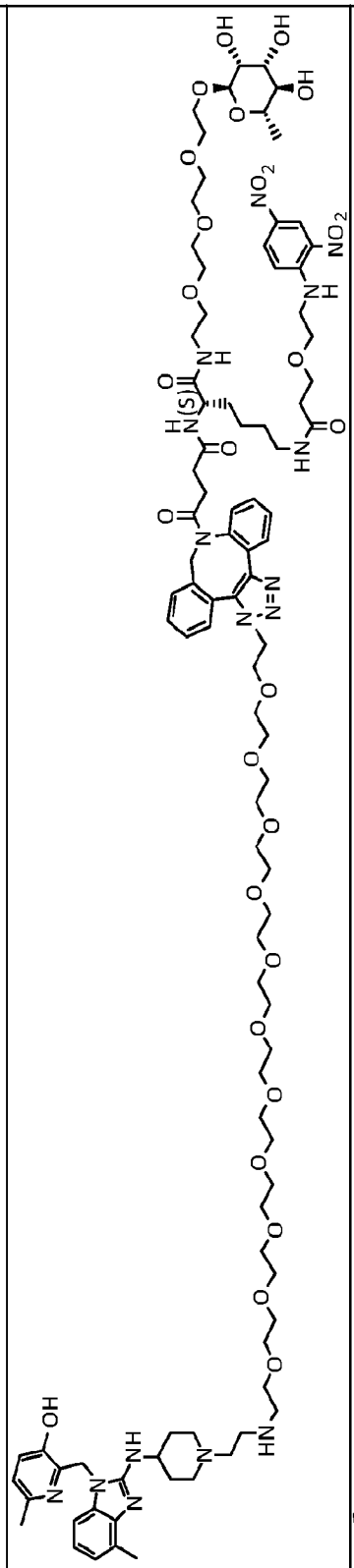
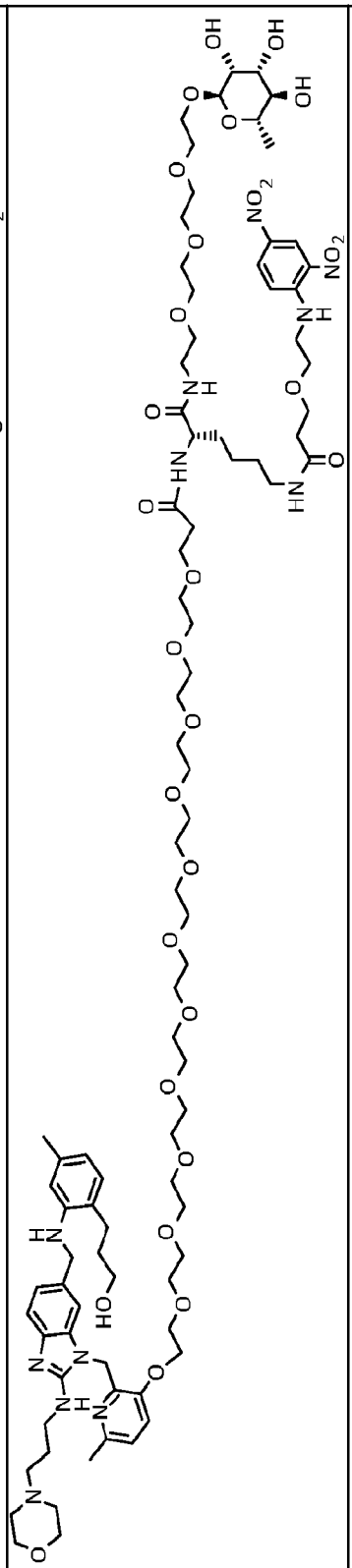
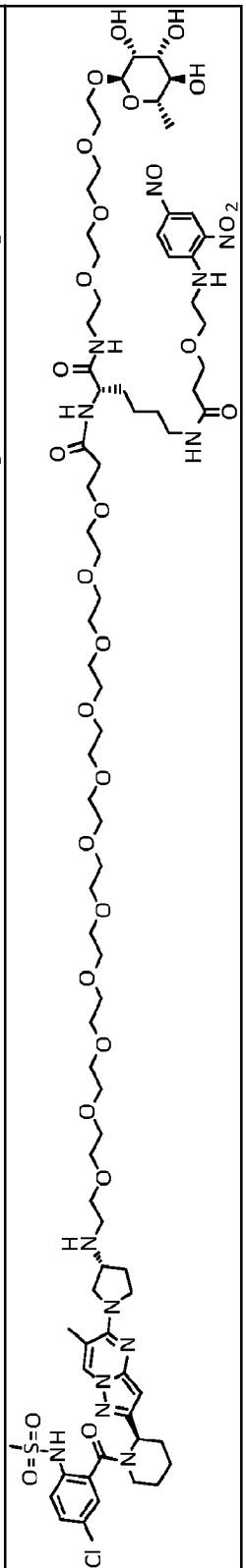
Compound No.	Structure
25	
26	
27	

FIG. 1

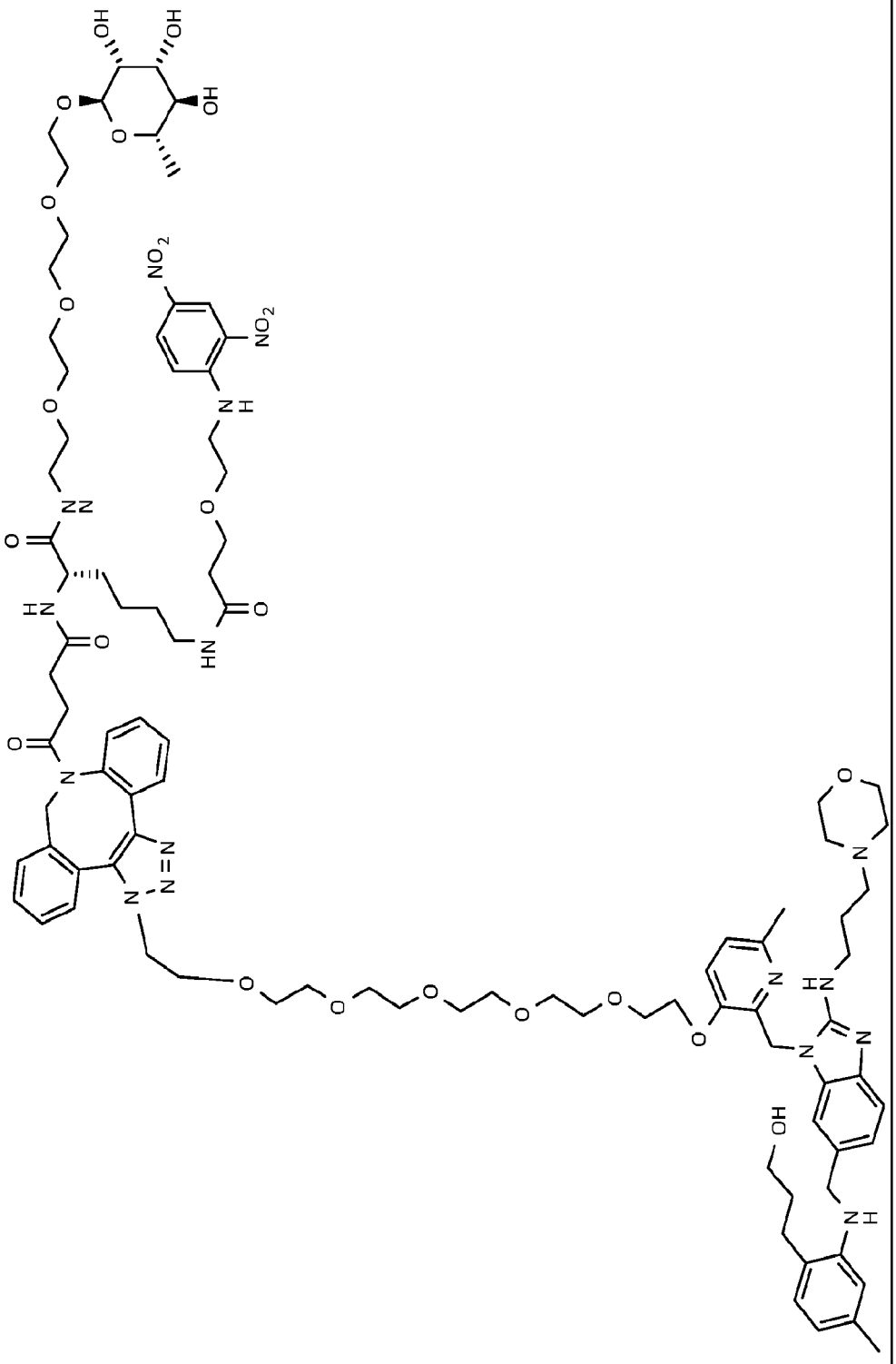
Compound No.	Structure
28	

FIG. 1 (continued)

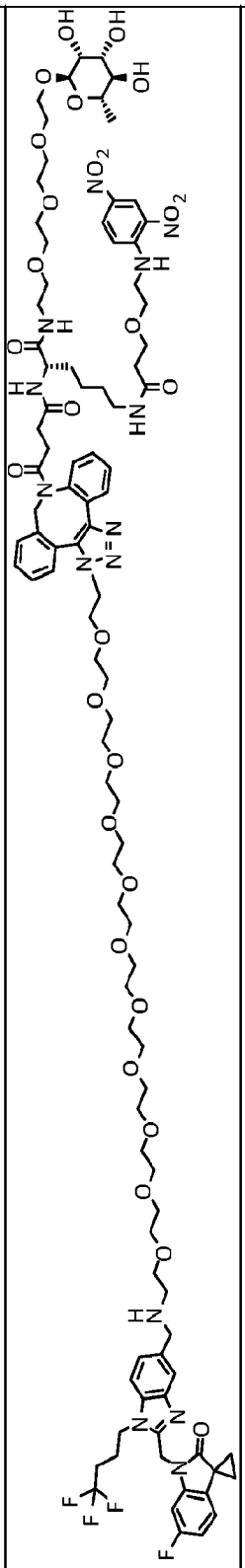
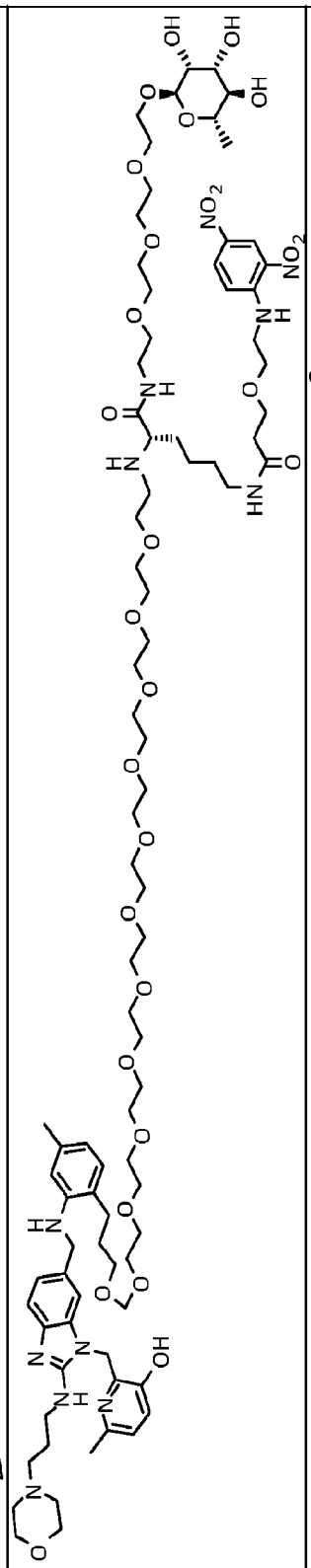
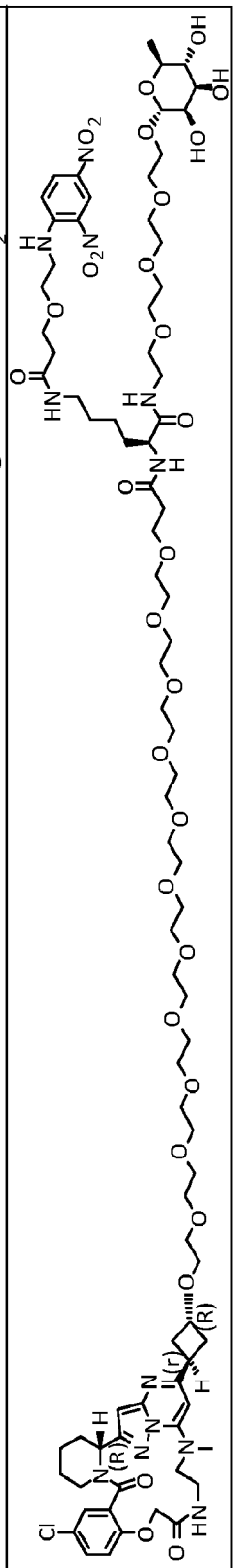
Compound No.	Structure
29	
31	
36	

FIG. 1 (continued)

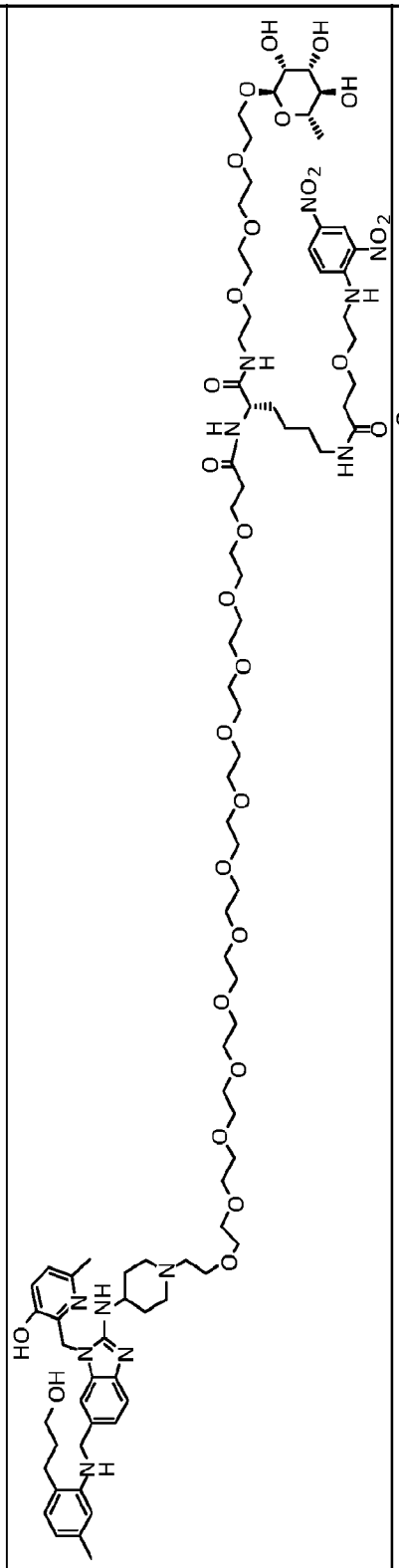
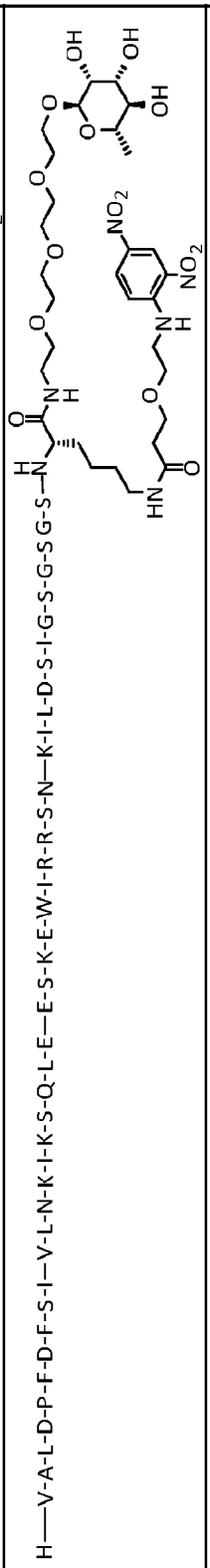
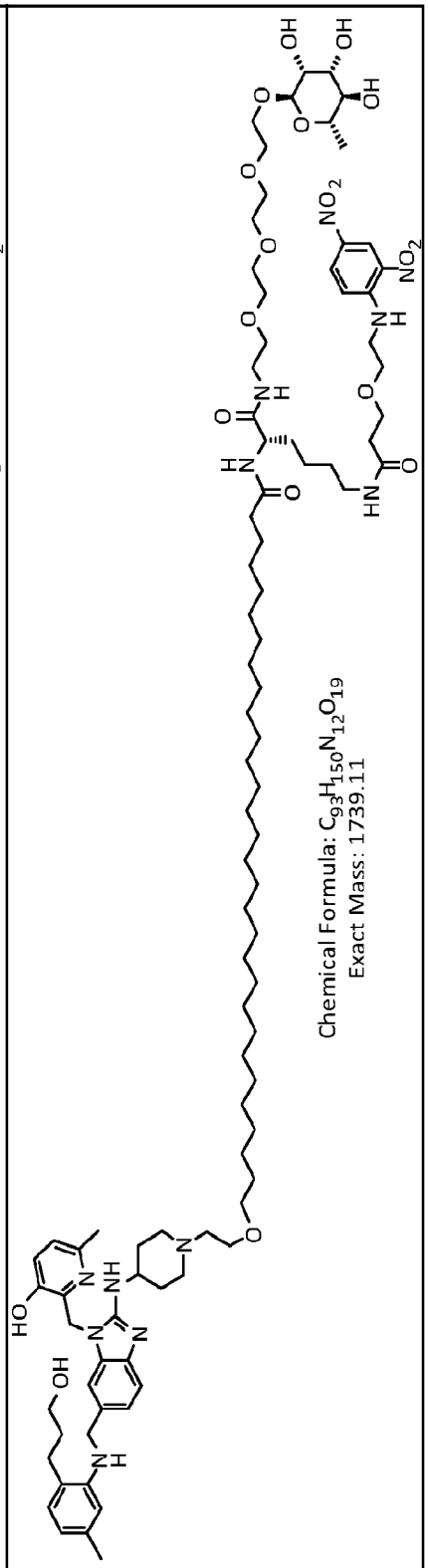
Compound No.	
37	
38	
39	

FIG. 1 (continued)

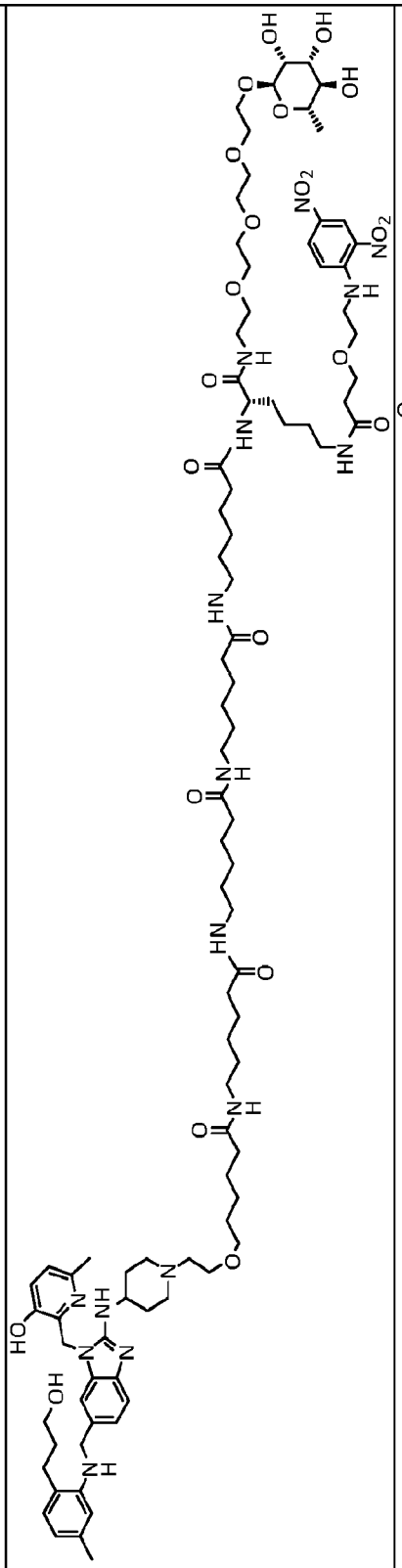
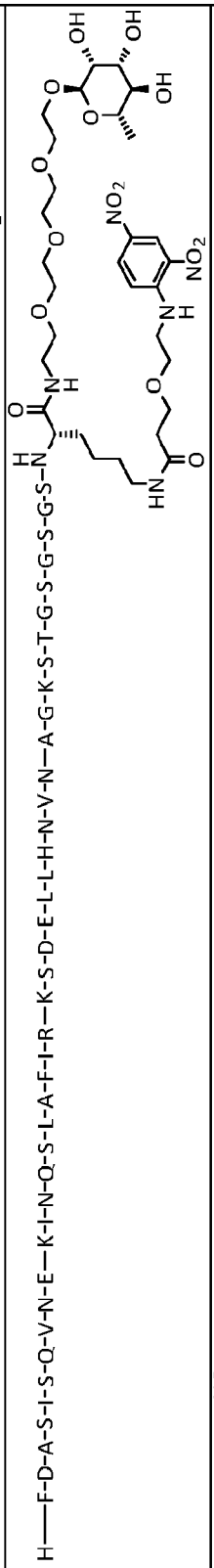
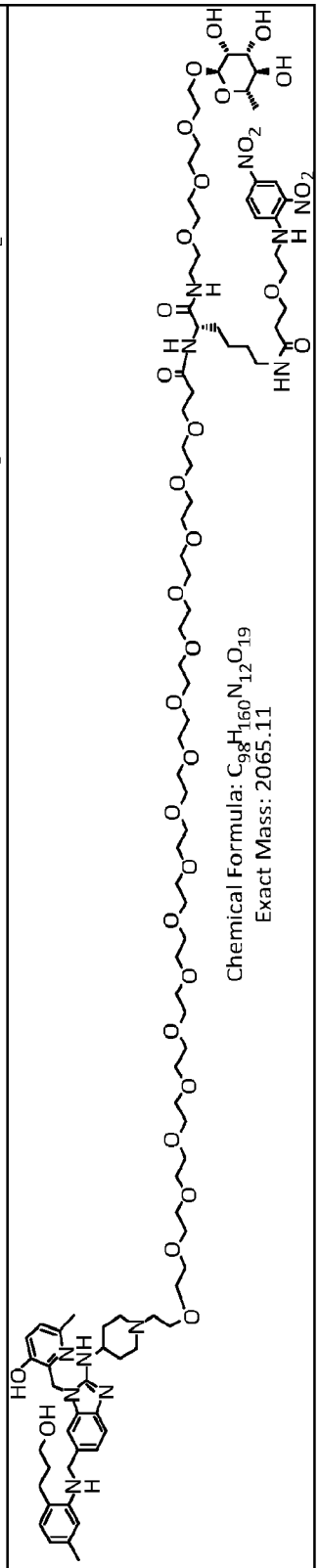
Compound No.	Structure
40	
41	 H——F-D-A-S-I-S-Q-V-N-E——K-I-N-Q-S-L-A-F-I-R——K-S-D-E-L-L-H-N-V-N——A-G-K-S-T-G-S-G-S-G-S-S—N
42	 Chemical Formula: $C_{98}H_{160}N_{12}O_{19}$ Exact Mass: 2065.11

FIG. 1 (continued)

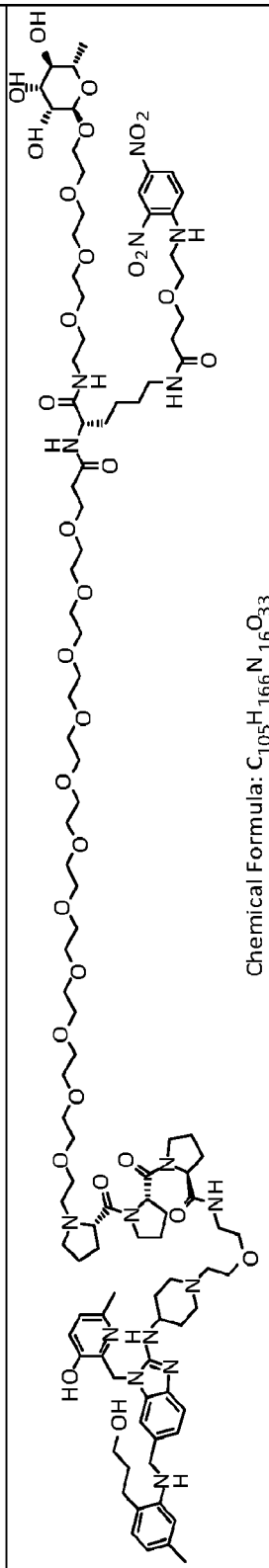
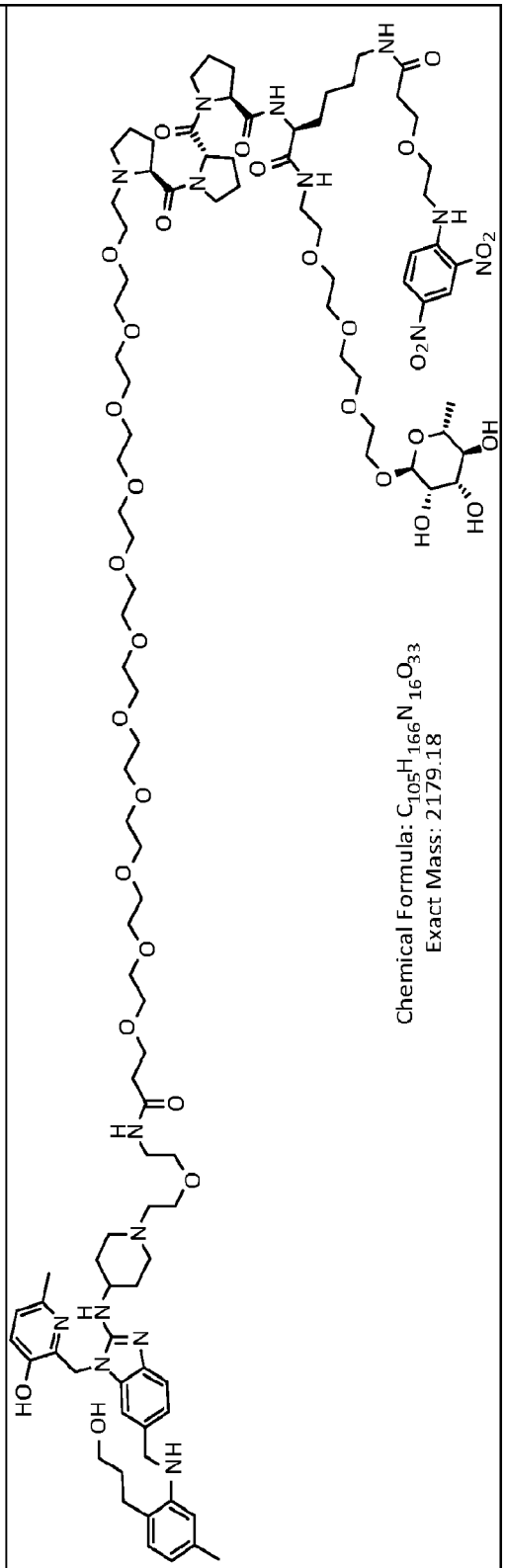
Compound No.	Structure
43	 Chemical Formula: $C_{105}H_{166}N_{16}O_{33}$ Exact Mass: 2179.18
44	 Chemical Formula: $C_{105}H_{166}N_{16}O_{33}$ Exact Mass: 2179.18

FIG. 1 (continued)

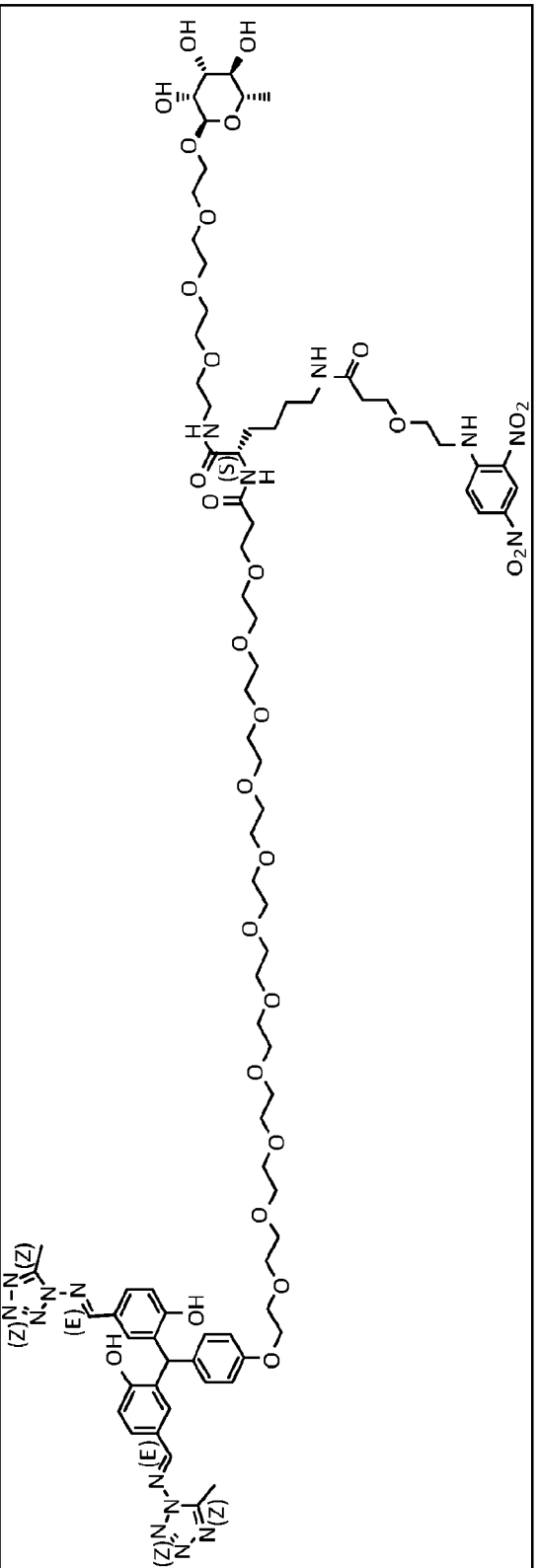
Compound No.	Structure
45	

FIG. 1 (continued)

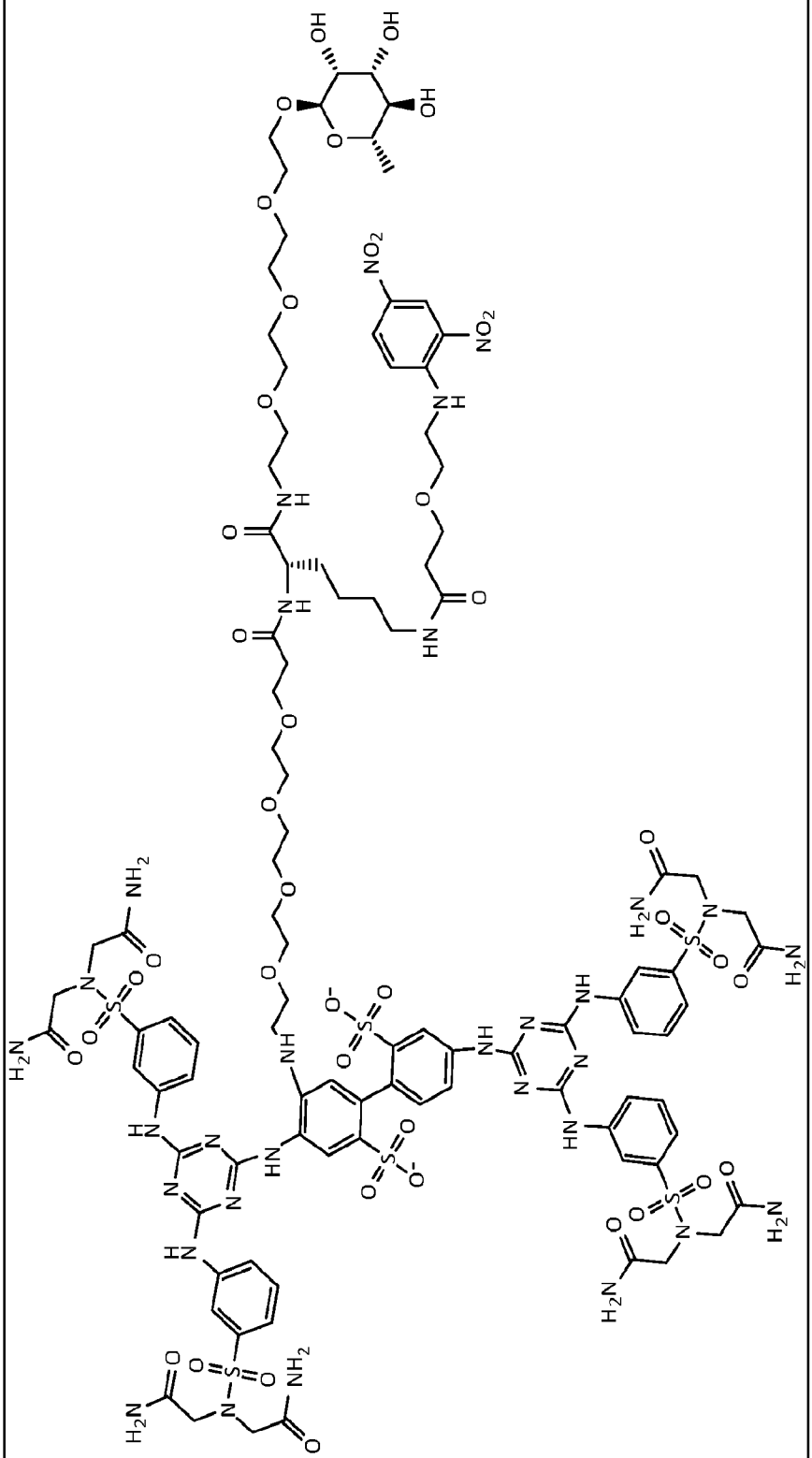
Compound No.	Structure
46	

FIG. 1 (continued)

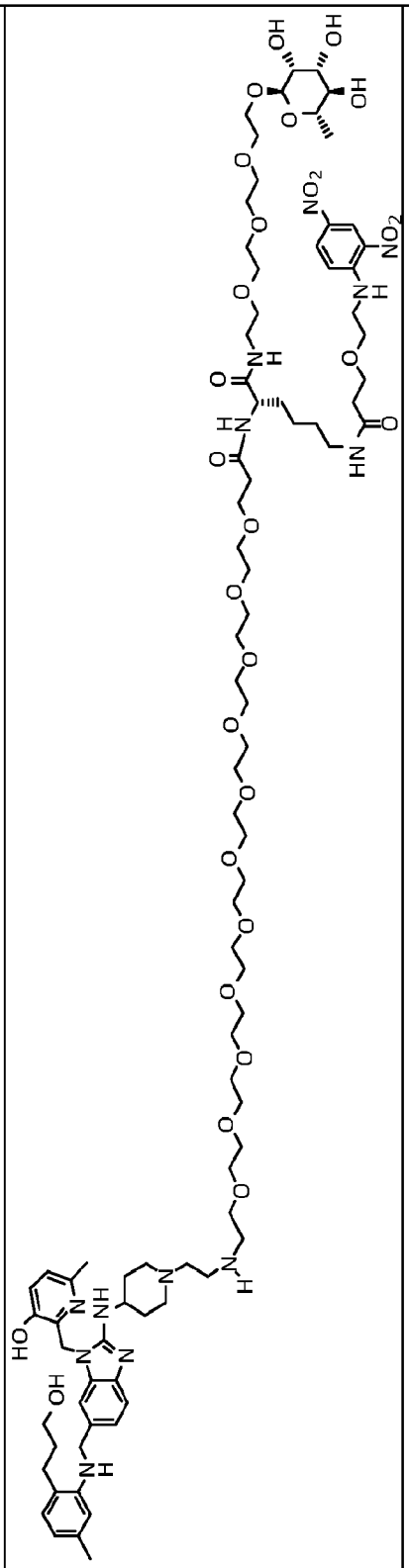
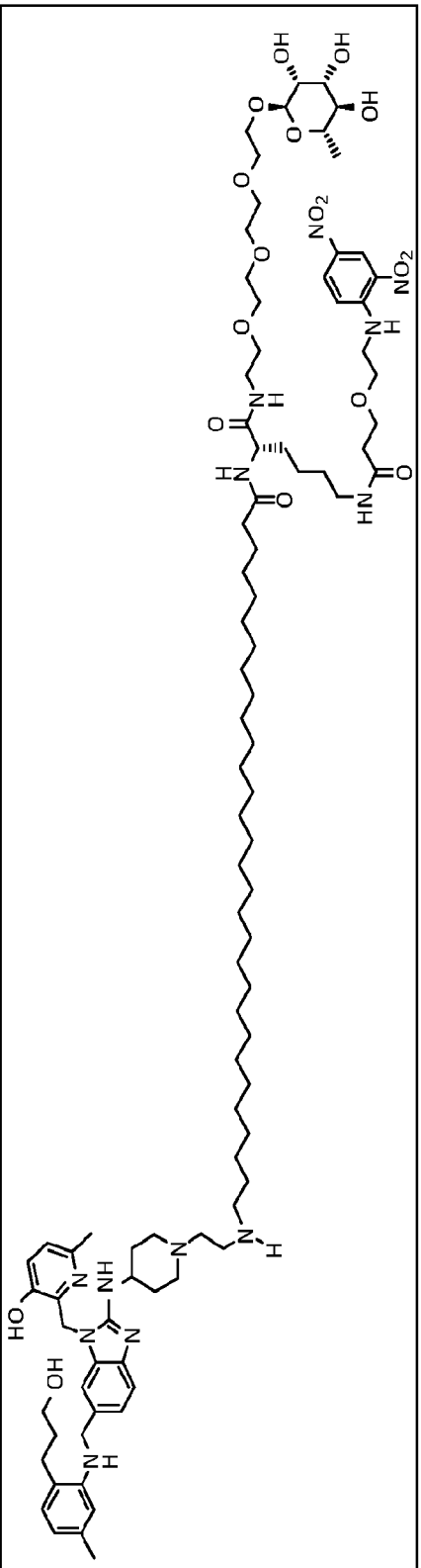
Compound No.	Structure
47	
48	

FIG. 1 (continued)

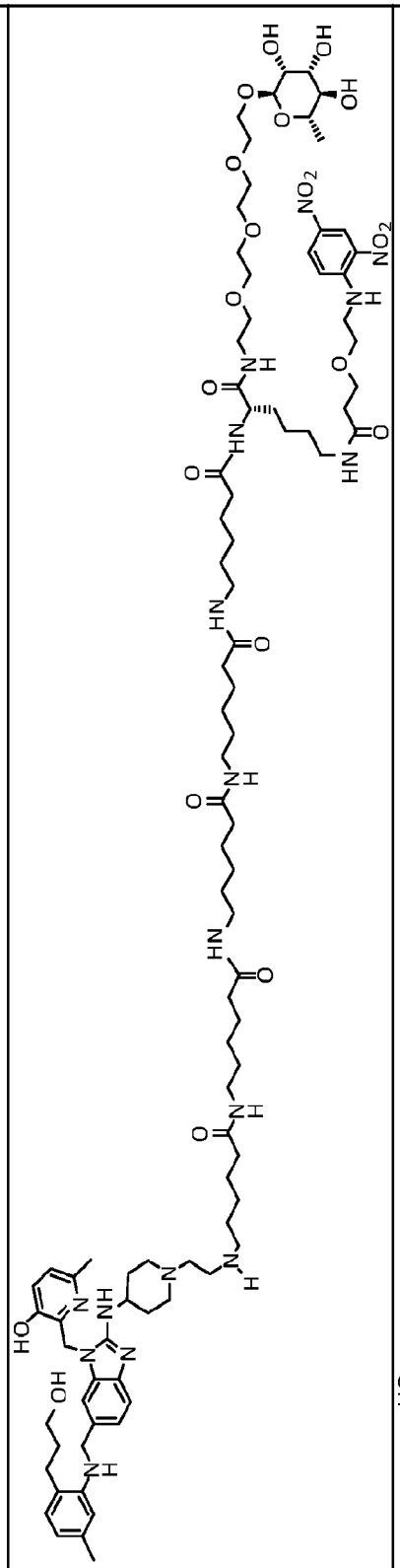
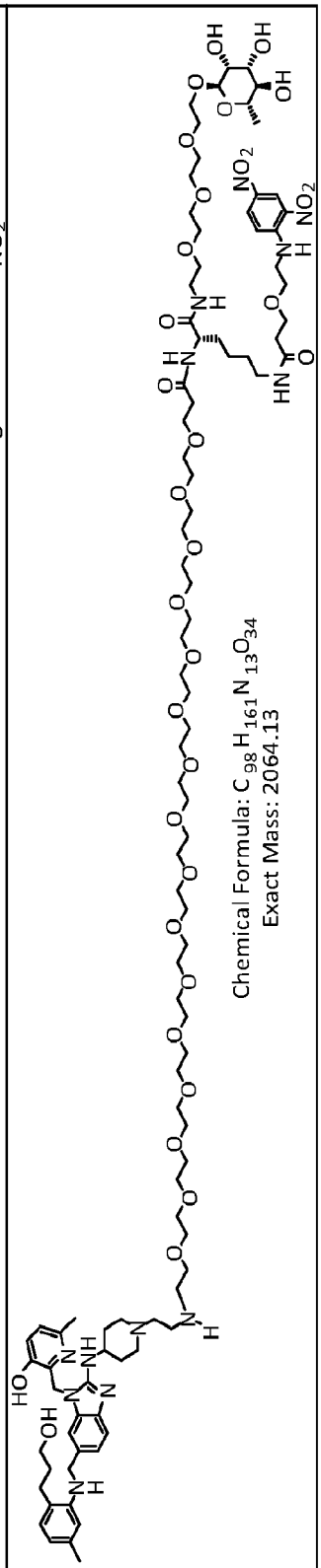
Compound No.	Structure
49	
50	 Chemical Formula: $C_{98}H_{161}N_{13}O_{34}$ Exact Mass: 2064.13

FIG. 1 (continued)

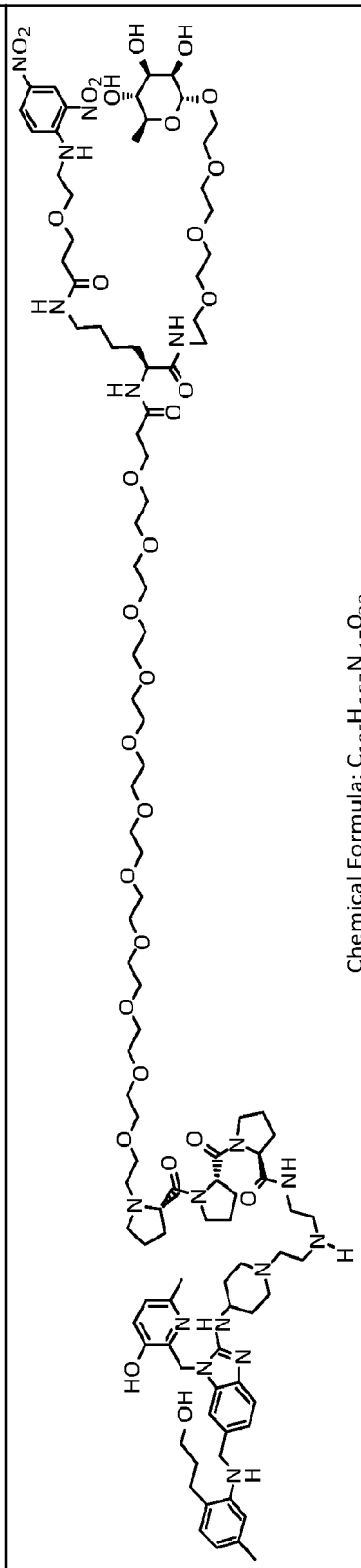
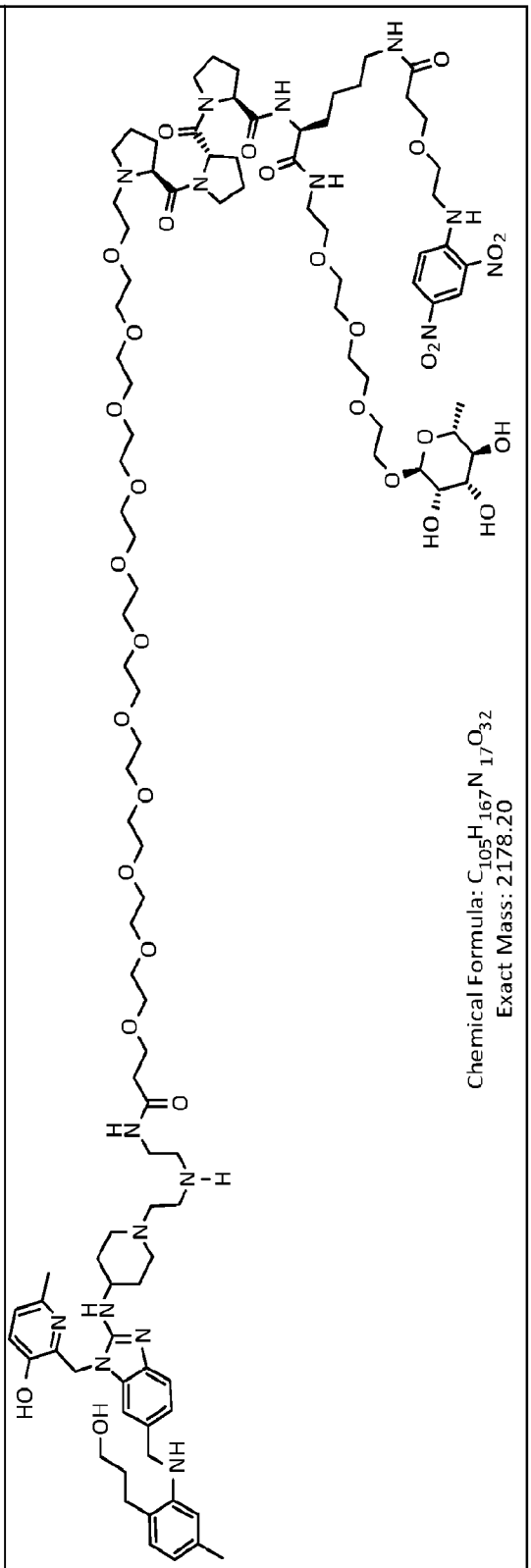
Compound No.	Structure
51	 Chemical Formula: $C_{105}H_{167}N_{17}O_{32}$ Exact Mass: 2178.20
52	 Chemical Formula: $C_{105}H_{167}N_{17}O_{32}$ Exact Mass: 2178.20

FIG. 1 (continued)

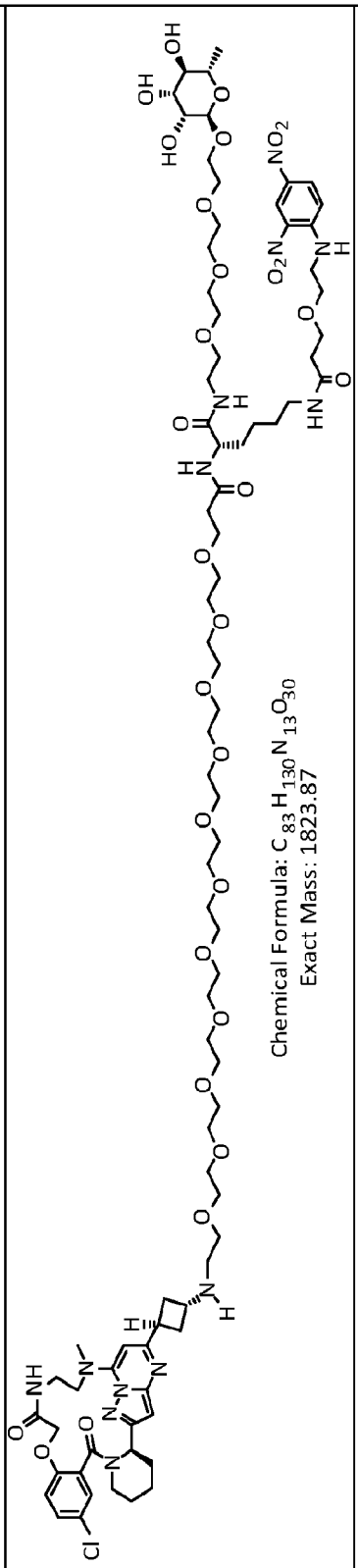
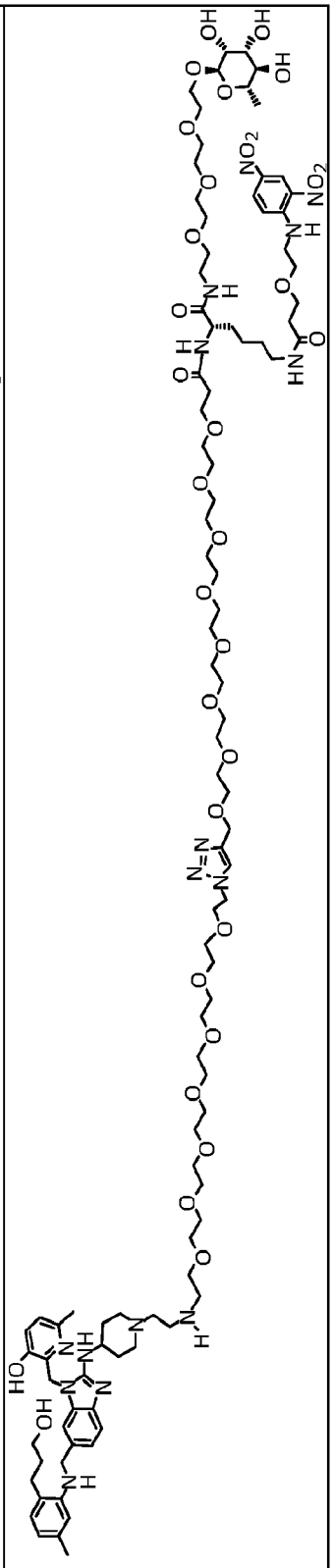
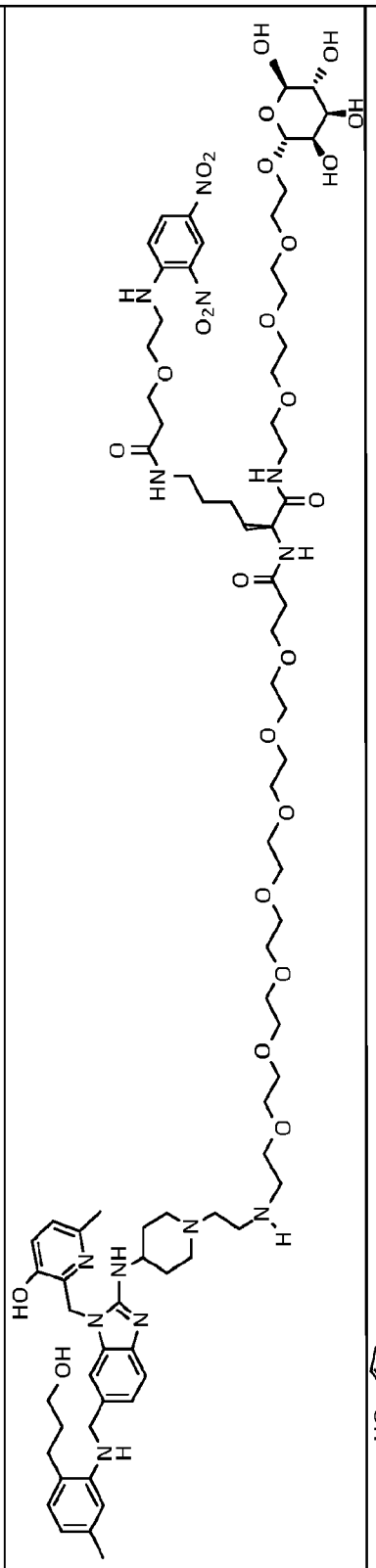
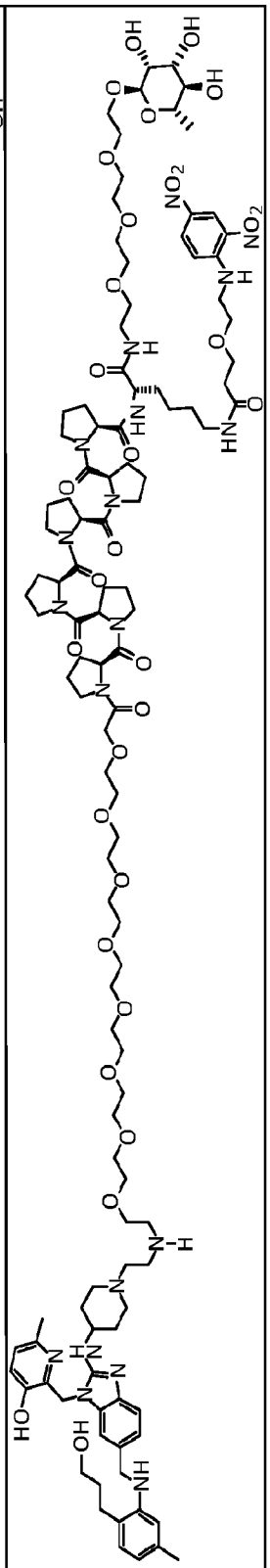
Compound No.	Structure
53	 Chemical Formula: $C_{83}H_{130}N_{13}O_{30}$ Exact Mass: 1823.87
54	

FIG. 1 (continued)

Compound No.	Structure
55	
56	

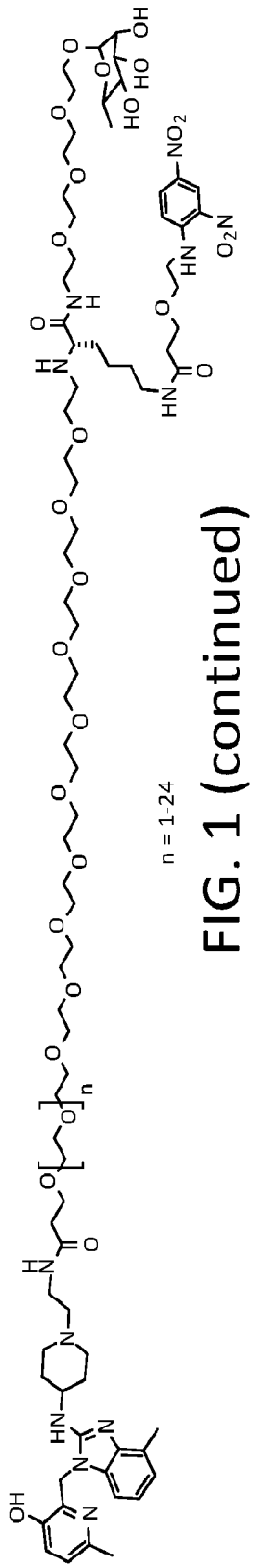


FIG. 1 (continued)

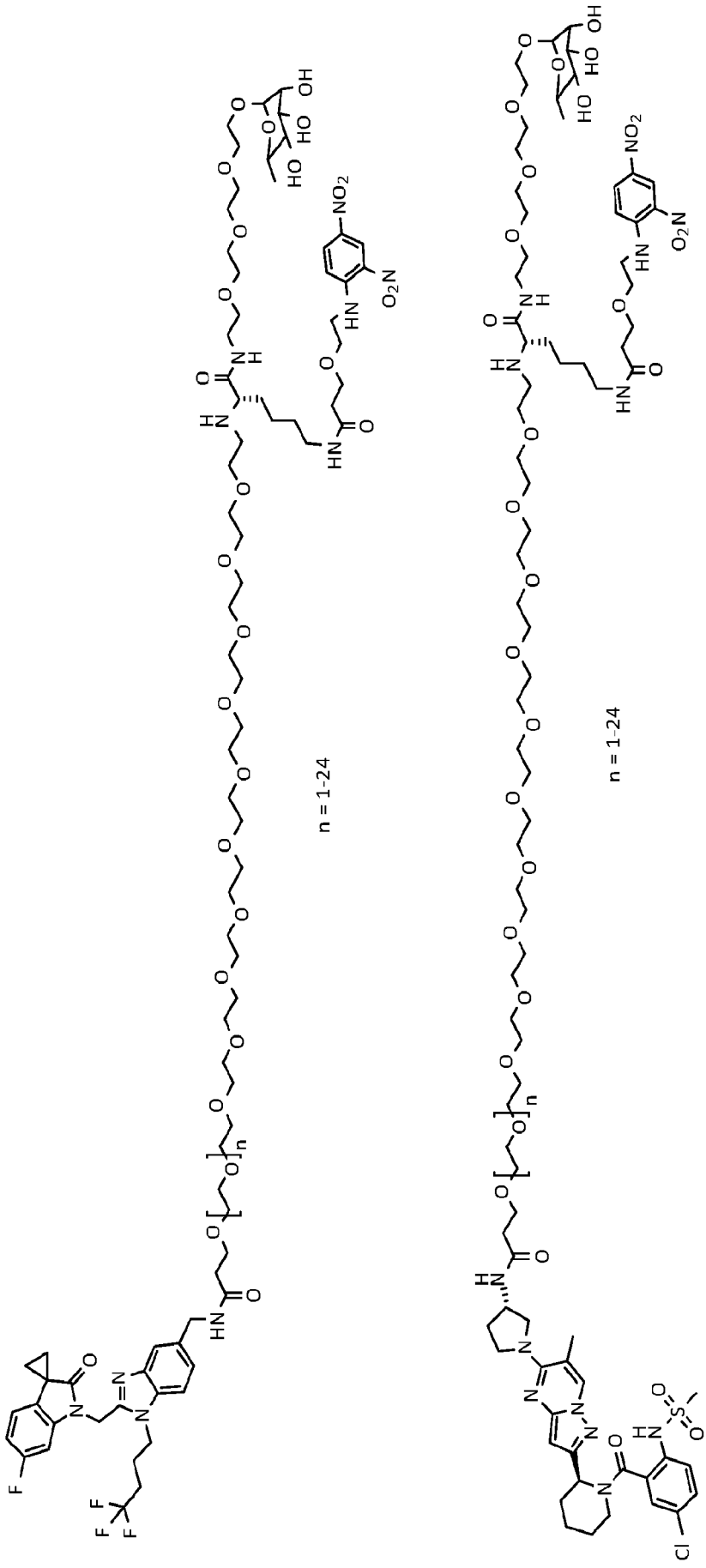


FIG. 1 (continued)

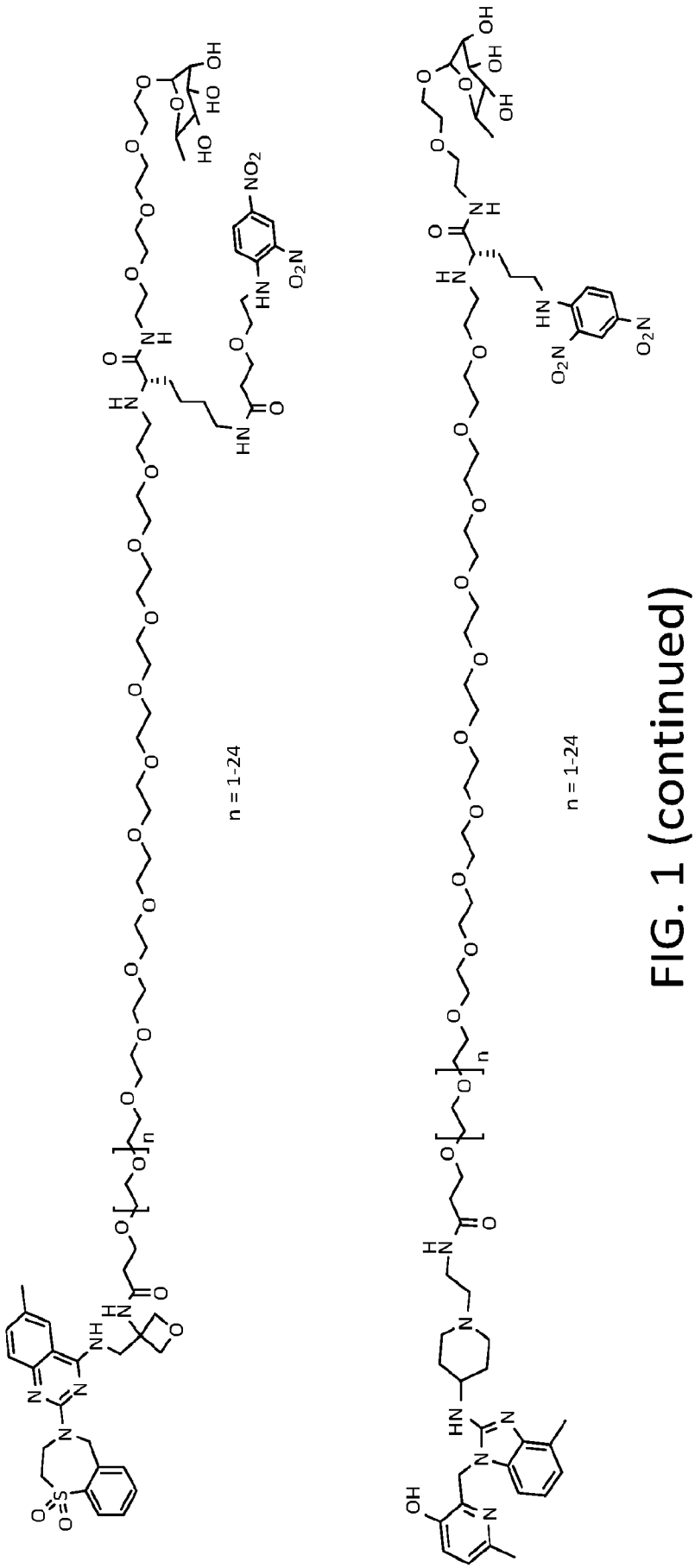


FIG. 1 (continued)

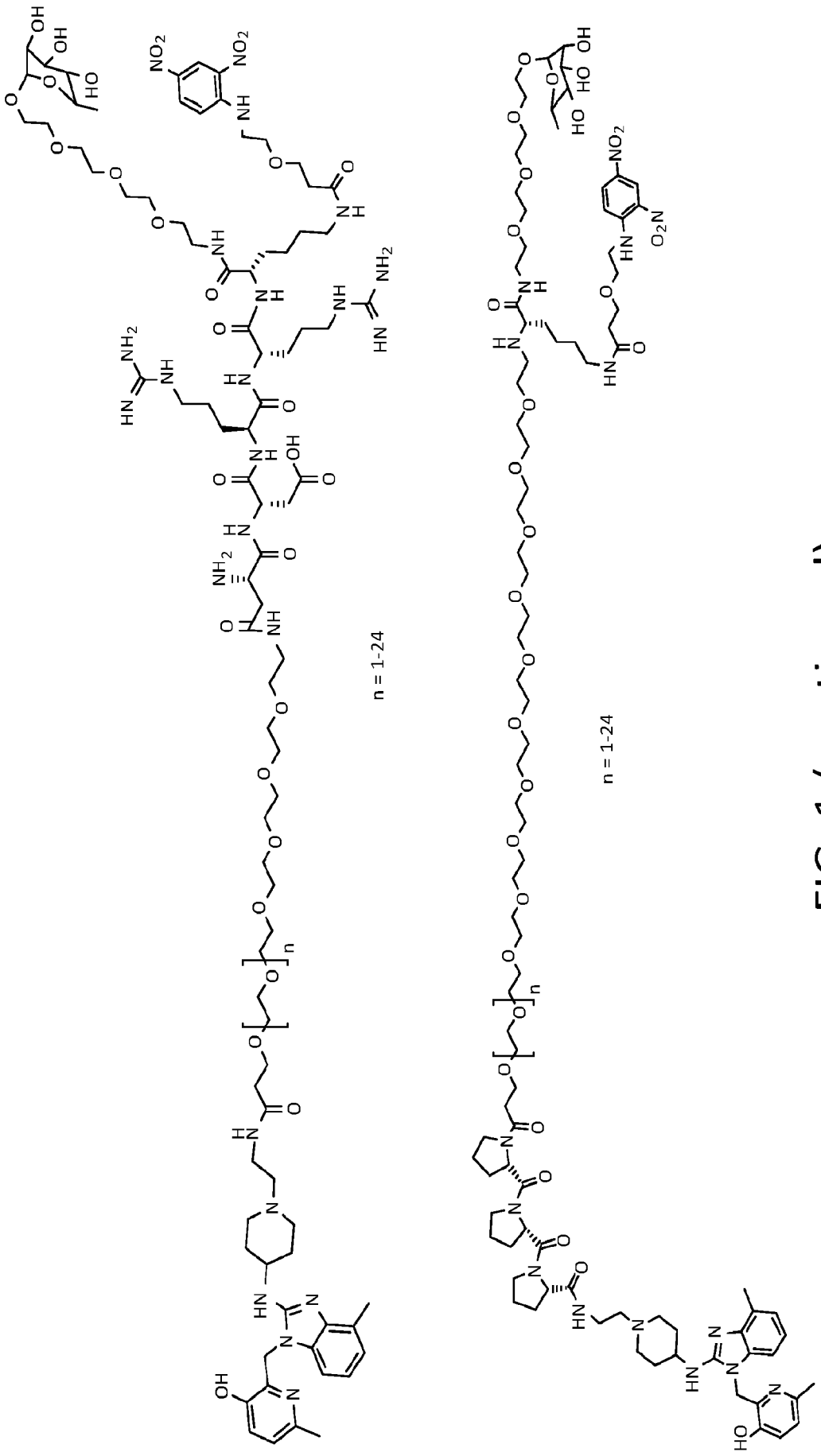


FIG. 1 (continued)

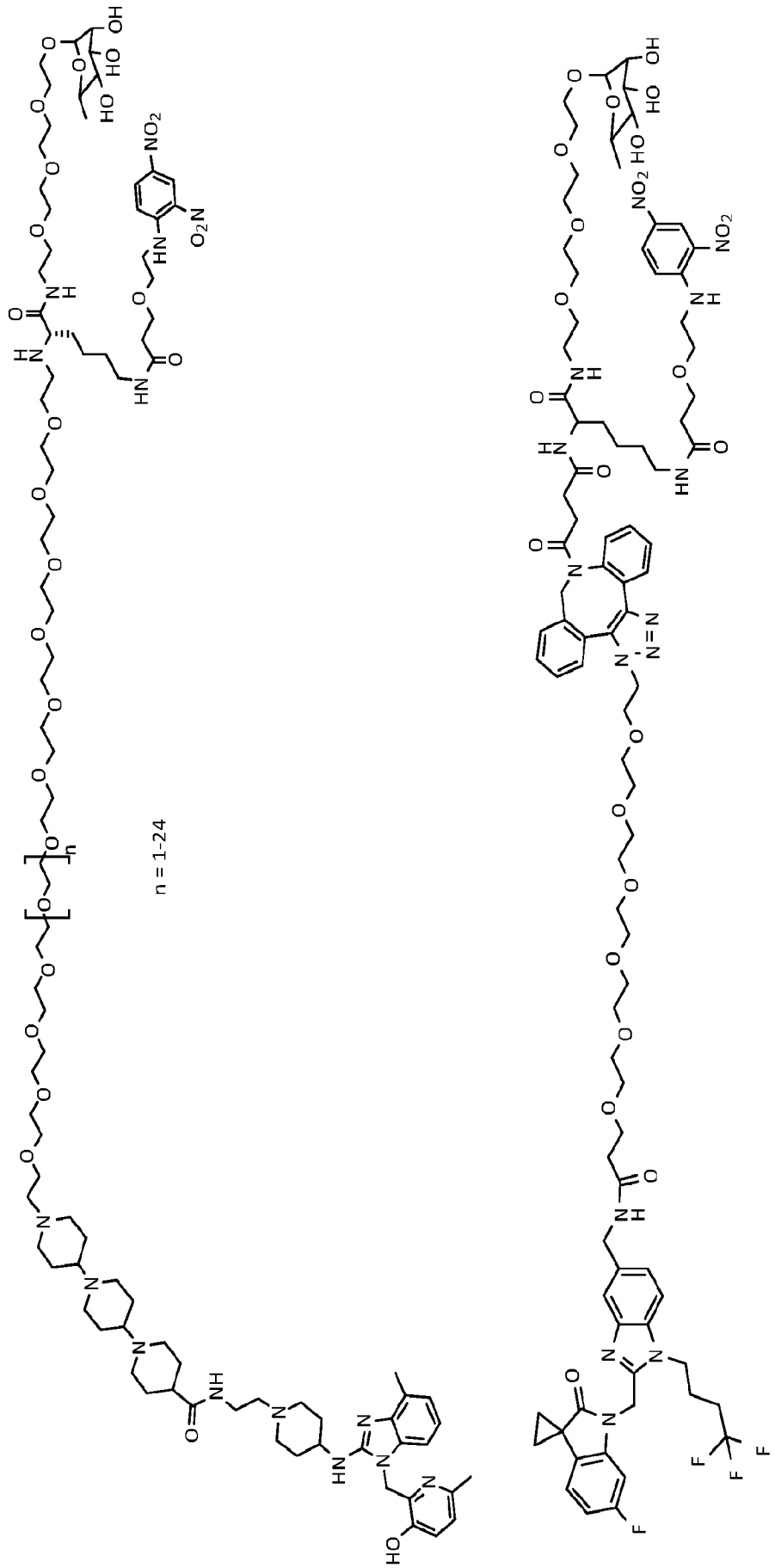


FIG. 1 (continued)

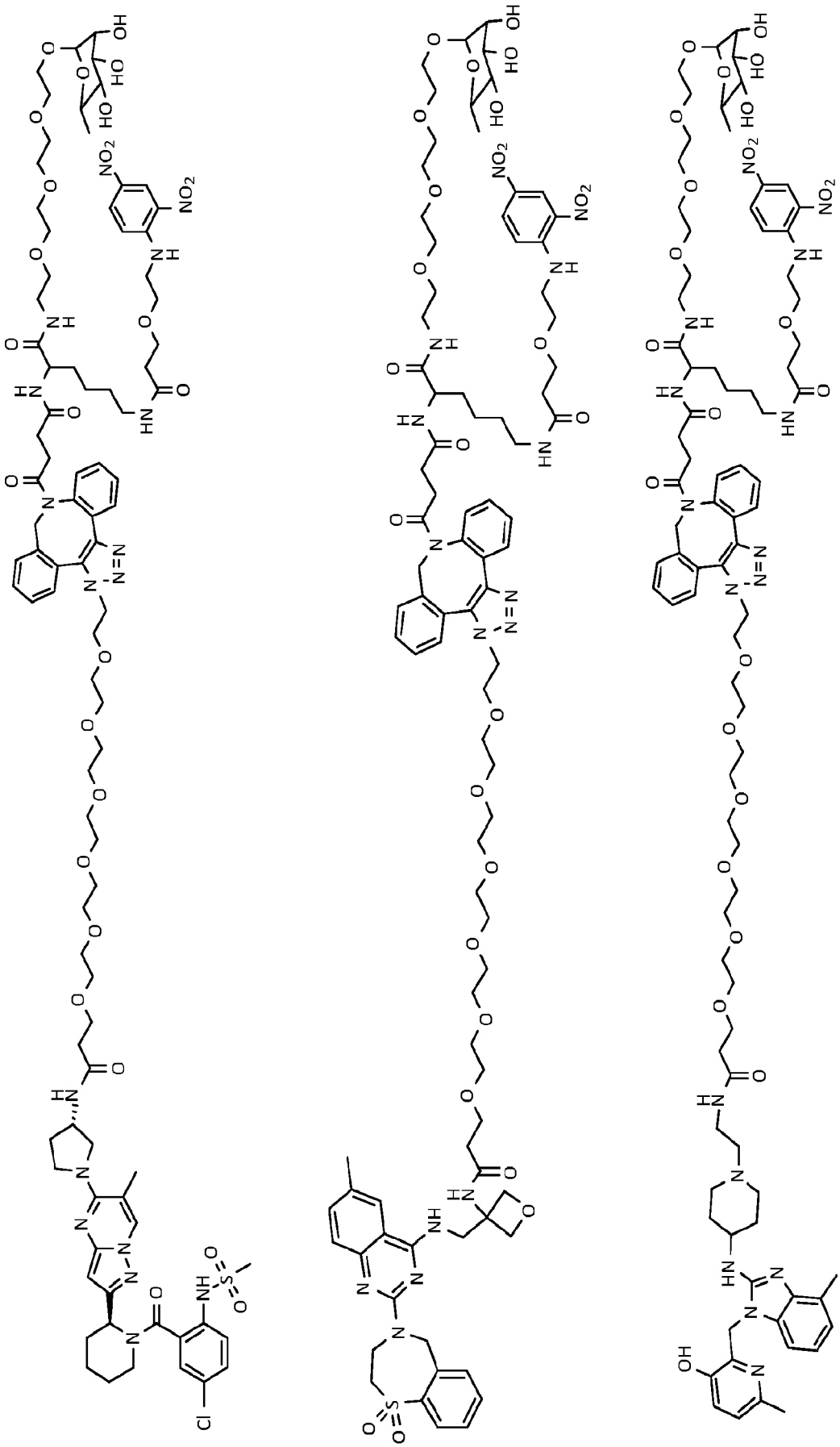


FIG. 1 (continued)

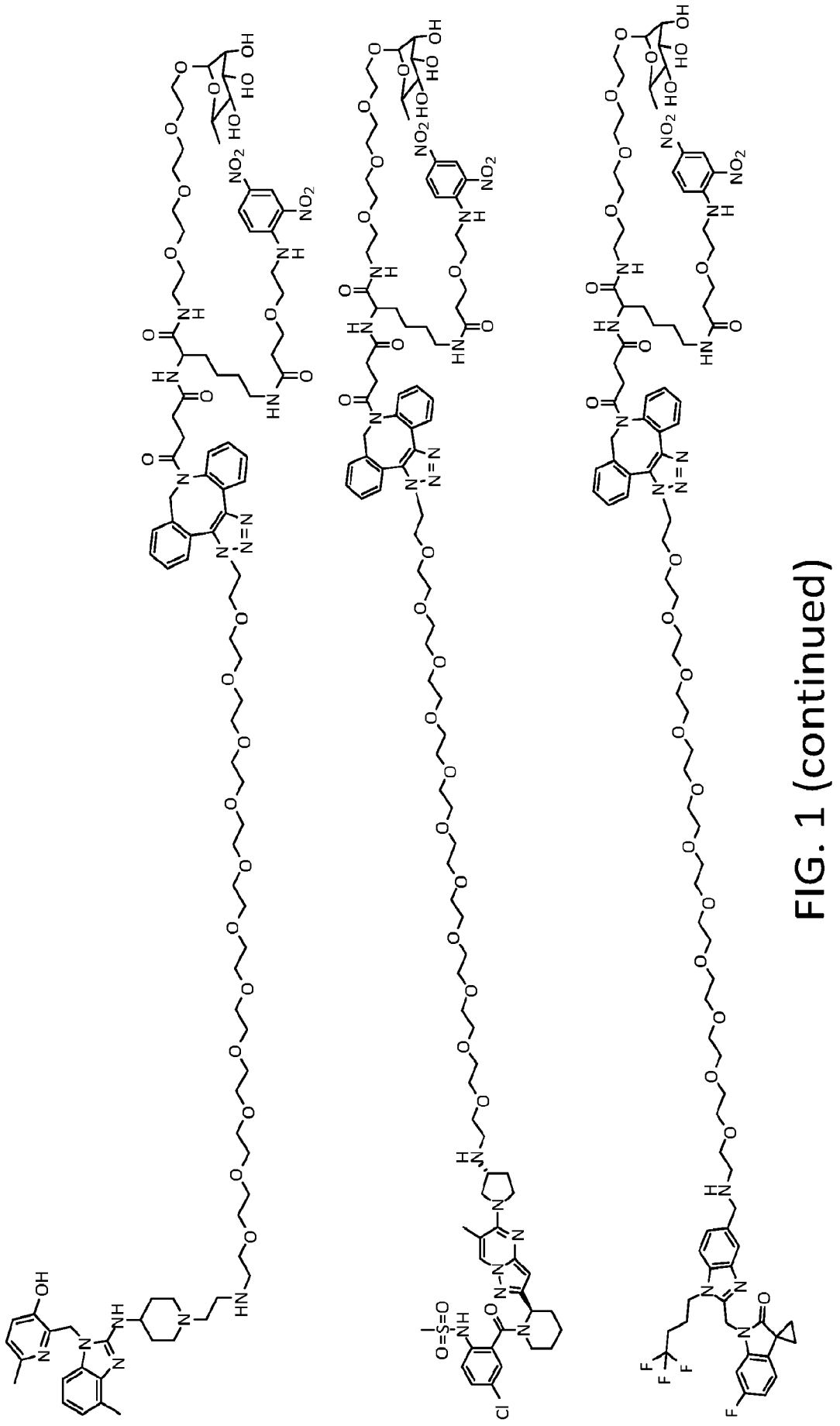
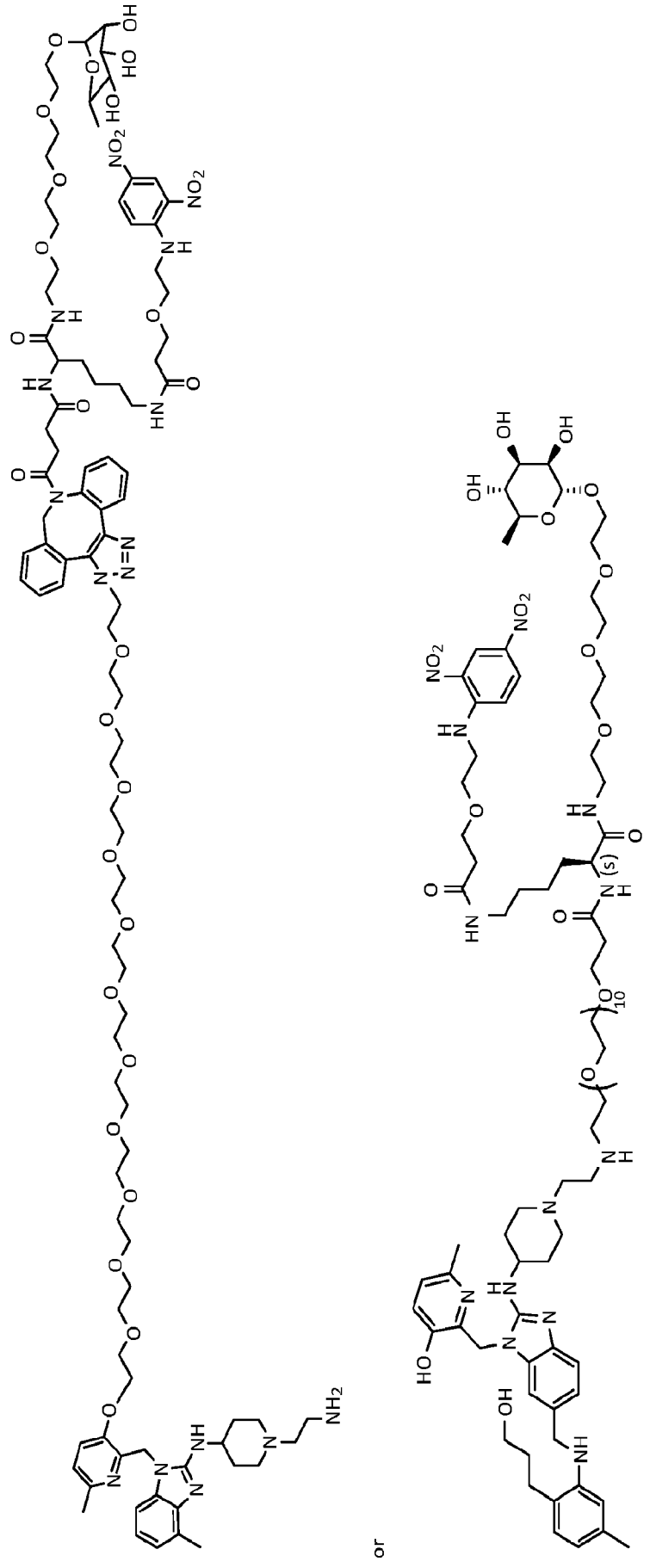


FIG. 1 (continued)



or

wherein n, where applicable, can be an integer from 1-24, 1-2, 1-5, 1-8, 2-10, 5-20, 6-18, 10-15, 10-12, 8-23, 5-15, or 6-20

FIG. 1 (continued)

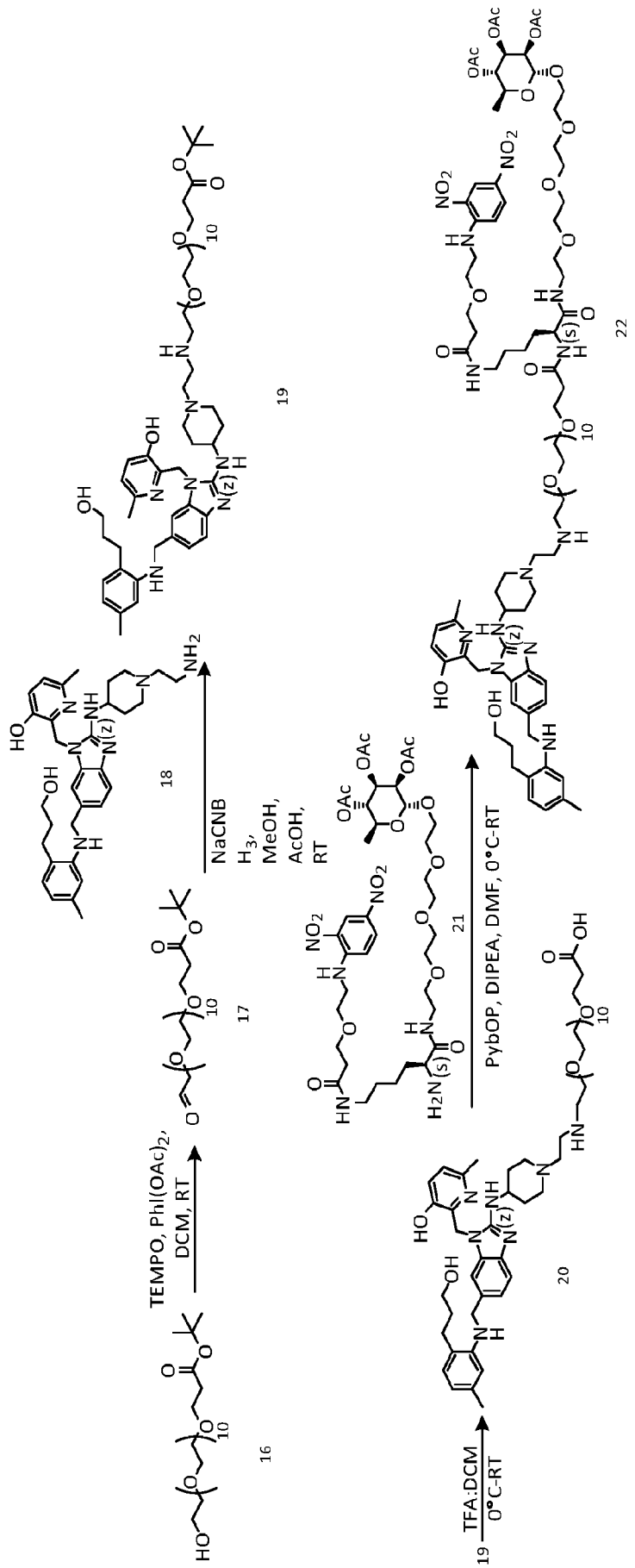

TEMPO, PhI(OAc)2, DCM, RT
NaCNB H3, MeOH, AcOH, RT
PybOP, DIPEA, DMF, 0°C-RT
TFA:DCM 0°C-RT
5% KOH in H2O
16
17
18
19
20
21
22
47

FIG. 2

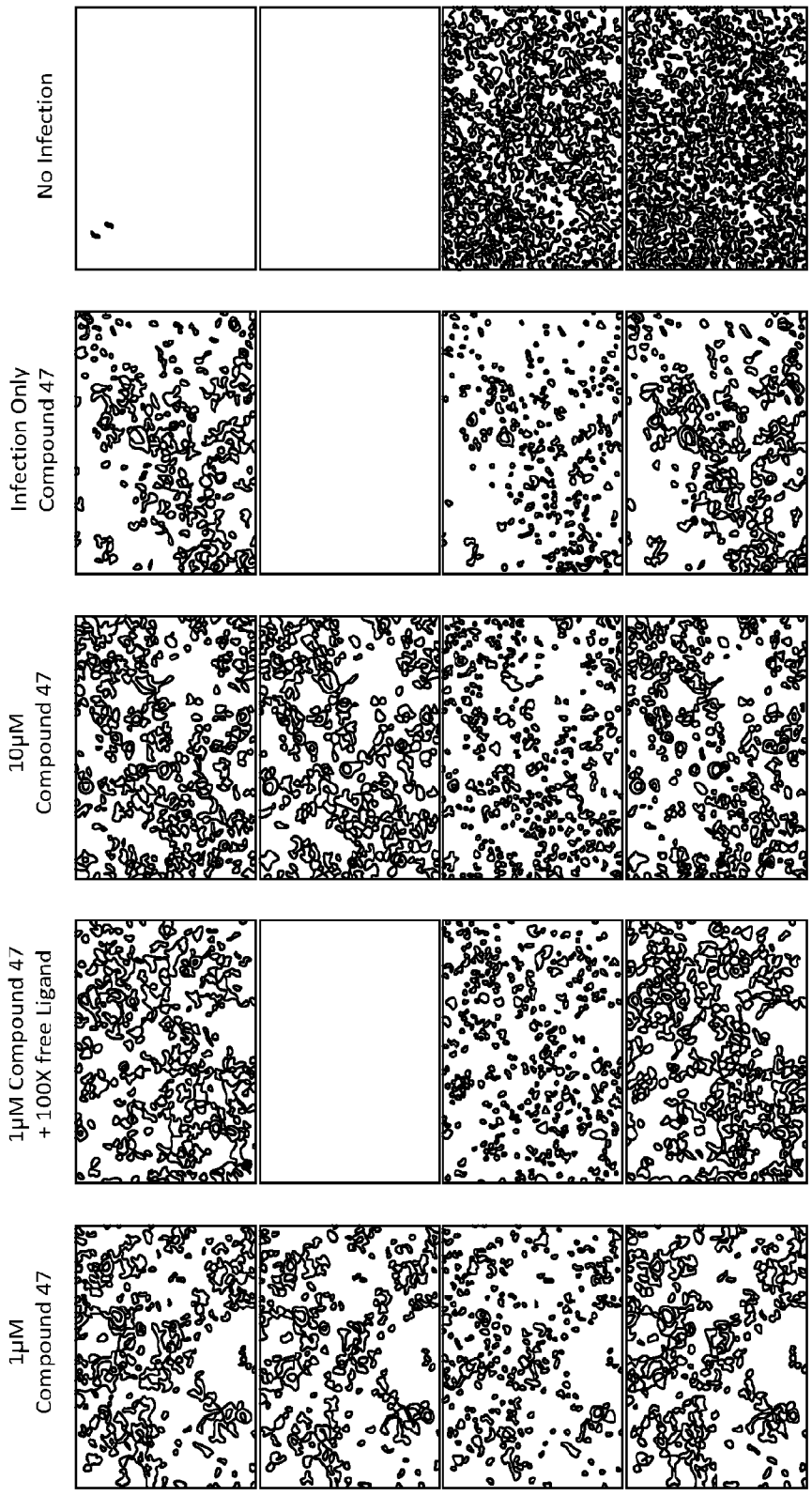

No Infection
Infection Only
Compound 47
10μM
Compound 47
1μM Compound 47
+ 100X free Ligand
1μM
Compound 47
RSV F
Protein
Compound 47
DAPI
Overlay

FIG. 3

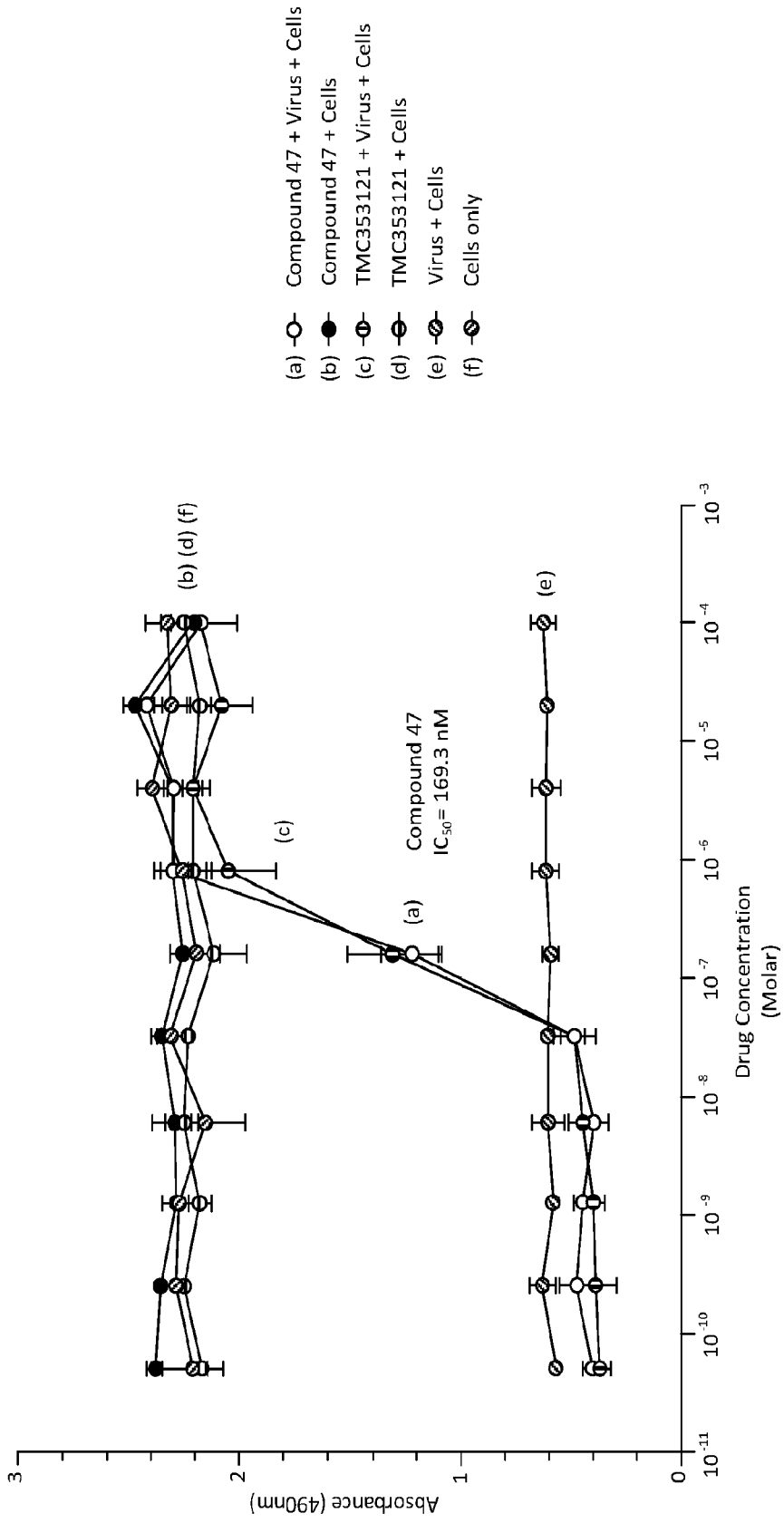

(a) Compound 47 + Virus + Cells
(b) Compound 47 + Cells
(c) TMC353121 + Virus + Cells
(d) TMC353121 + Cells
(e) Virus + Cells
(f) Cells only
(b) (d) (f)
(c)
(a)
Compound 47
IC50= 169.3 nM
(e)
Absorbance (490nm)
Drug Concentration (Molar)

FIG. 4

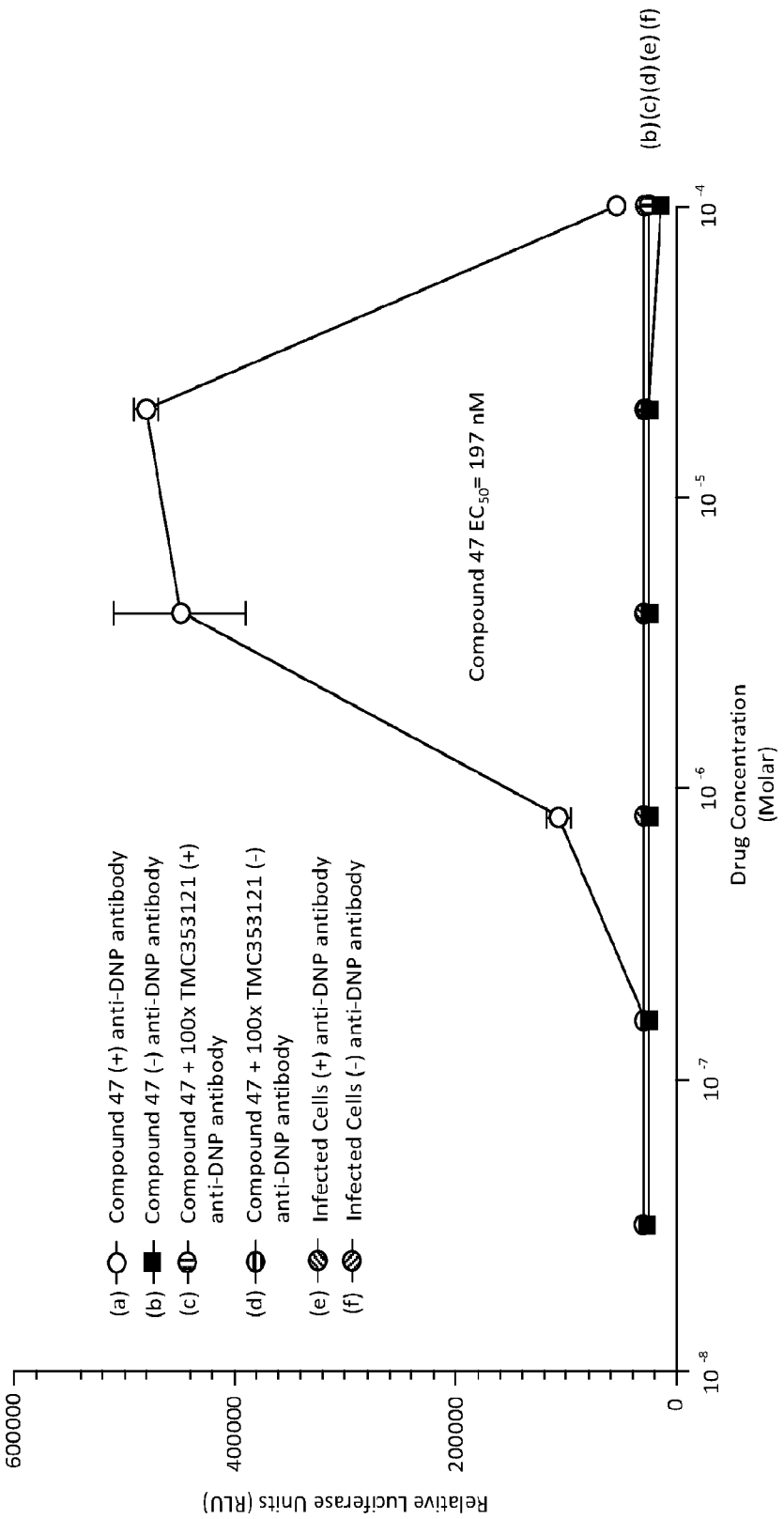

Relative Luciferase Units (RLU)
600000
400000
200000
0
10 -8
10 -7
10 -6
10 -5
10 -4
Drug Concentration (Molar)
Compound 47 EC50= 197 nM
(a) Compound 47 (+) anti-DNP antibody
(b) Compound 47 (-) anti-DNP antibody
(c) Compound 47 + 100x TMC353121 (+) anti-DNP antibody
(d) Compound 47 + 100x TMC353121 (-) anti-DNP antibody
(e) Infected Cells (+) anti-DNP antibody
(f) Infected Cells (-) anti-DNP antibody
(b)(c)(d)(e)(f)

FIG. 5

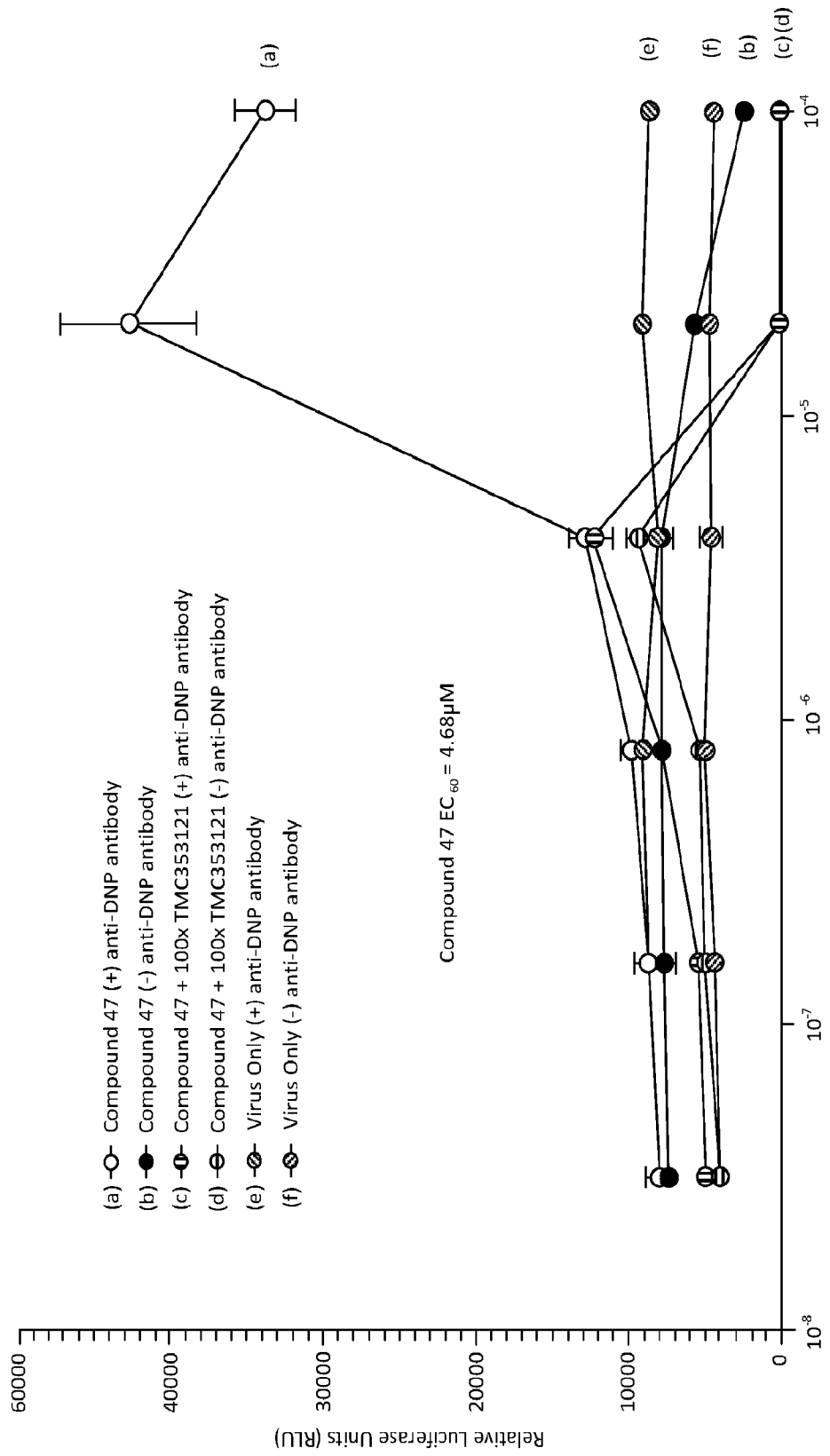


FIG. 5 (continued)

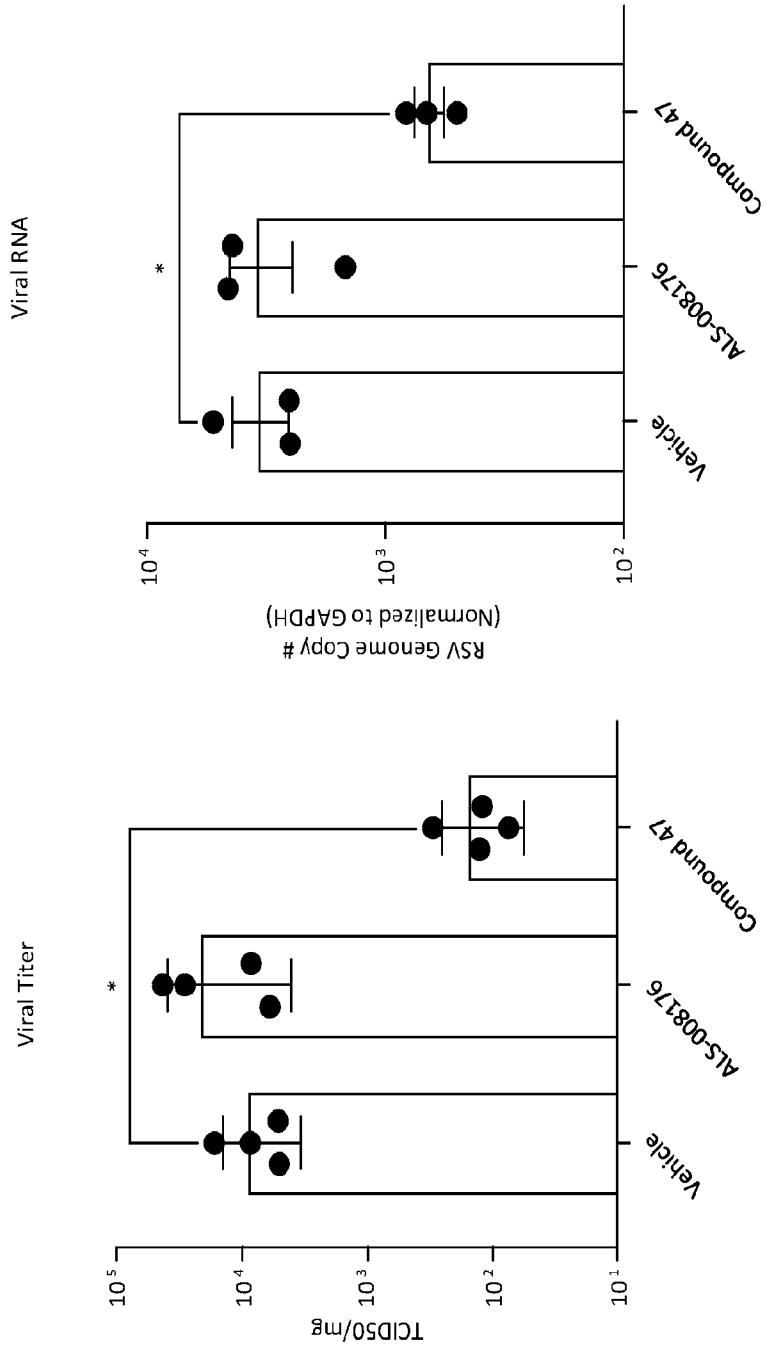

Viral Titer
TCID50/mg
10^5
10^4
10^3
10^2
10^1
*
Vehicle
ALS-008176
Compound 47
Viral RNA
RSV Genome Copy #
(Normalized to GAPDH)
10^4
10^3
10^2
*
Vehicle
ALS-008176
Compound 47

FIG. 6

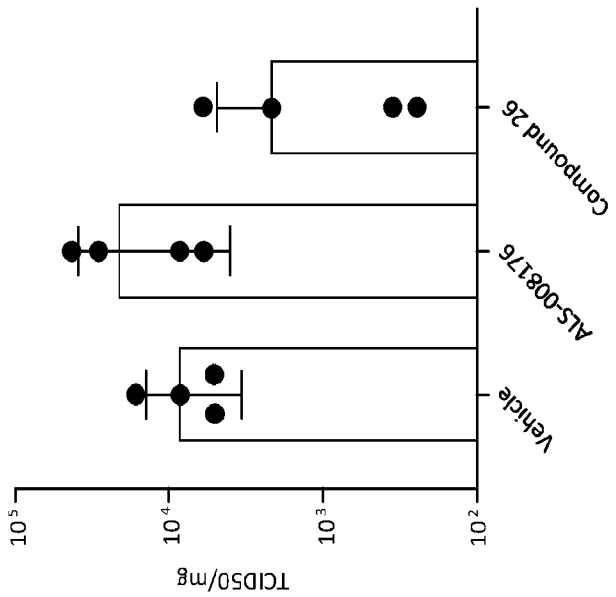


FIG. 7

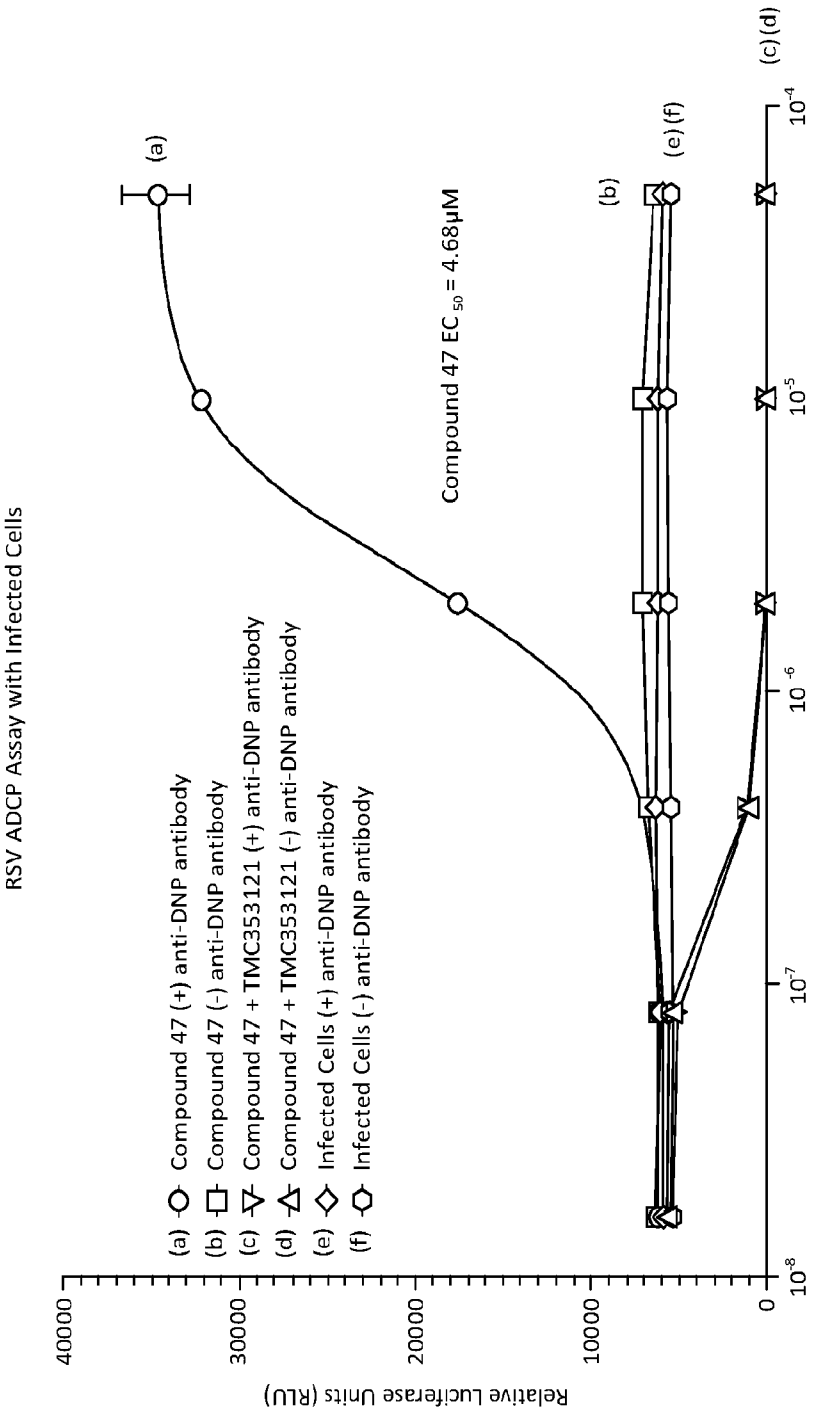

RSV ADCP Assay with Infected Cells
(a) Compound 47 (+) anti-DNP antibody
(b) Compound 47 (-) anti-DNP antibody
(c) Compound 47 + TMC353121 (+) anti-DNP antibody
(d) Compound 47 + TMC353121 (-) anti-DNP antibody
(e) Infected Cells (+) anti-DNP antibody
(f) Infected Cells (-) anti-DNP antibody
Compound 47 EC50 = 4.68µM
Relative Luciferase Units (RLU)
Drug Concentration (Molar)
40000
30000
20000
10000
0
10-8
10-7
10-6
10-5
10-4
(a)
(b)
(e)(f)
(c)(d)

FIG. 8

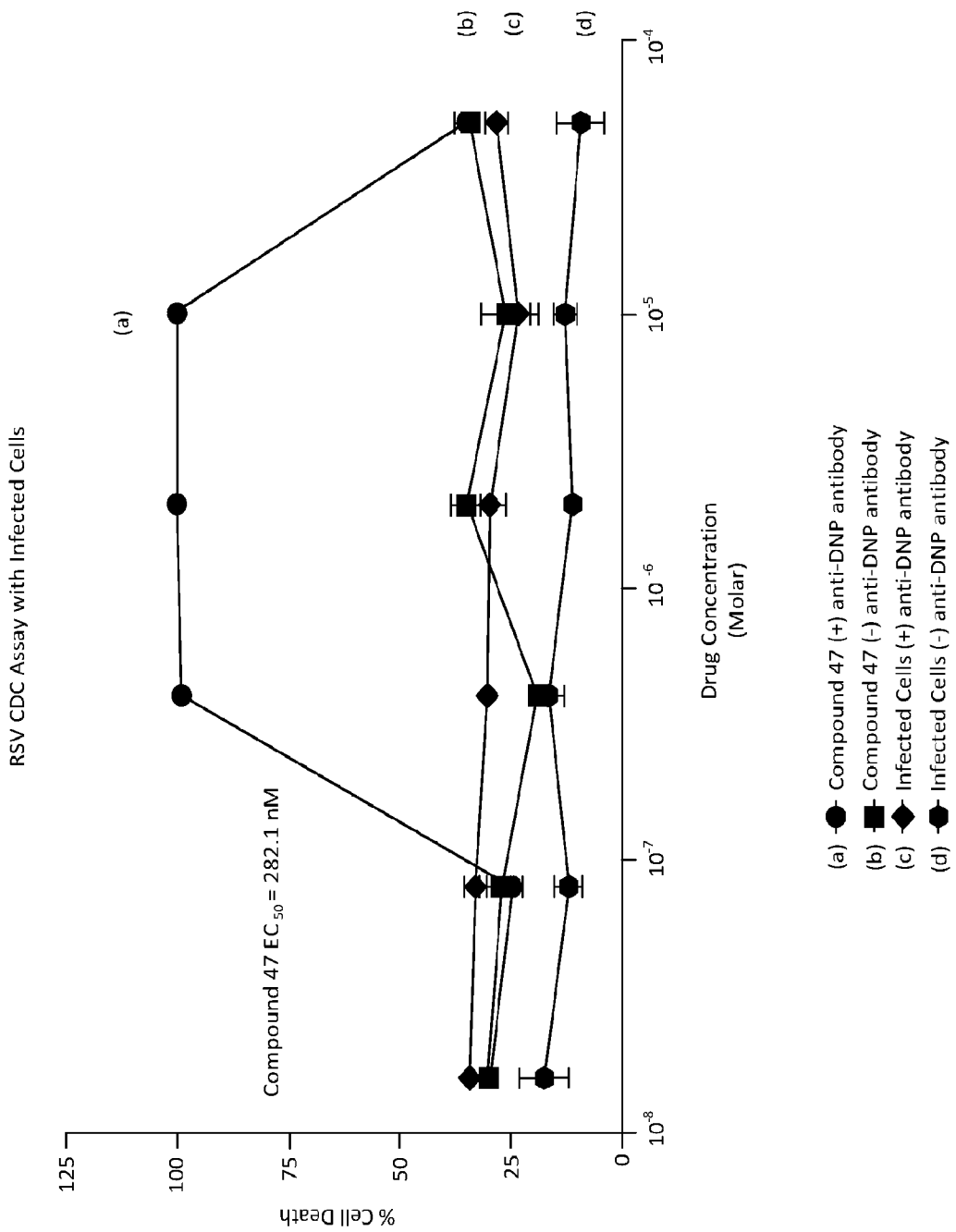

RSV CDC Assay with Infected Cells
% Cell Death
125
100
75
50
25
0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Drug Concentration (Molar)
(a)
(b)
(c)
(d)
Compound 47 EC_{50} = 282.1 nM
(a) Compound 47 (+) anti-DNP antibody
(b) Compound 47 (-) anti-DNP antibody
(c) Infected Cells (+) anti-DNP antibody
(d) Infected Cells (-) anti-DNP antibody

FIG. 9

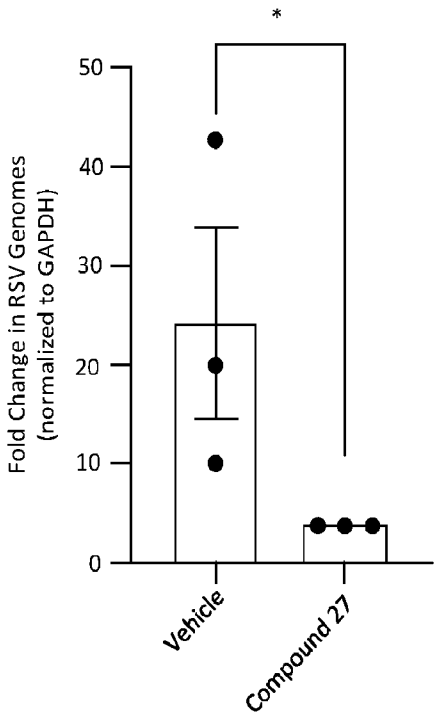

*
50
40
30
20
10
0
Fold Change in RSV Genomes
(normalized to GAPDH)
Vehicle
Compound 27

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/051835

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☐ forming part of the international application as filed.
   b. ☑ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☑ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/051835

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **14-30**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Groups I+: Claims 1-13 and 31-36, directed to a compound of the formula I and a method for treating RSV infection. The composition/method will be searched to the extent that the compound targeting the first named target protein, F glycoprotein (see claim 2) and encompasses the first named ligand, TMC353121 (see claim 6), and the first named hapten, rhamnose (see claim 12). This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-2, 4, 6, (11-12)(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass F glycoprotein, TMC353121 and rhamnose. Additional compound(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected compound(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a compound targeting G glycoprotein and encompassing a ligand that is the first compound listed in claim 7, and the haptens are rhamnose and dinitroaniline (1, 3, 5, 7, (11-13)(in part), 31 (compound 47 in Fig. 1), 32-37).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/051835

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1, 2, 4, 6, (11-12)/(1,2,4,6) limited to F glycoprotein, MC353121 and rhamnose**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/051835

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 39/385*** (2025.01); ***A61K 47/68*** (2025.01); ***C07K 16/30*** (2025.01); ***C07D 401/14*** (2025.01); *C07D 401/06* (2025.01); *C07D 471/04* (2025.01)

CPC: ***A61K 39/385; C07K 16/42; A61K 47/6889; C07K 16/1027;*** *C07D 487/04; A61P 31/14; A61K 2039/505*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2023/141500 A2 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 27 July 2023 (27.07.2023) pg 2, ln 20-23, pg 16, ln 27-34, pg 20, ln 1-18, pg 42, ln 15 to pg 43, ln 5	1, 2, 4, 6, (11-12)/(1,2,4,6)
Y	WO 2021/050612 A1 (CIDARA THERAPEUTICS, INC.) 18 March 2021 (18.03.2021) pg 335, ln 4-40	6, (11-12)/6
Y	LIET et al. "Multifunctional Glycoconjugates for Recruiting Natural Antibodies against Cancer Cells" Chem. Eur. J. 2019, Vol. 25, pp. 15508–15515 abstract, pg 15511, col 1, para 2	1, 2, 4, 6, (11-12)/(1,2,4,6)

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2025 (10.03.2025)	**01 April 2025 (01.04.2025)**

Name and mailing address of the ISA/US	Authorized officer
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