

CONJUGATES, COMPOSITIONS AND METHODS FOR TREATING INFLUENZA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to Appl. No. 63/590,999, filed October 17, 2023; and Appl. No. 63/700,420, filed September 27, 2024, each of which is incorporated by reference as if fully set forth herein.

TECHNICAL FIELD

[0002] The present disclosure includes conjugates comprising multiple haptens linked to a targeting ligand for a target protein on a virus or a virus-infected cell, as well as compositions, such as pharmaceutical compositions, comprising such conjugates and processes for making such conjugates. Additionally, the present disclosure further includes methods of treating viral infections. One such viral infection is influenza.

BACKGROUND

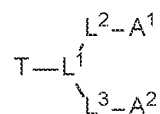
[0003] Influenza is one of the most life-threatening disseminated diseases resulting in about 3 to 5 million yearly cases of severe illness and about 250,000 to 500,000 yearly deaths. In addition to causing high morbidity and mortality, influenza imposes a substantial social economic burden arising from the productivity lost and medical prevention and treatment.

[0004] Because influenza virus constantly changes via antigen shift and drift, vaccines often become ineffective against mutating strains. Chemotherapeutic applications presently available are also limited. Approved anti influenza drugs are either M2 ion channel inhibitor and neuraminidase inhibitors. M2 ion channel inhibitors include amantadine and rimantadine. The mechanism of action of these drugs results from blocking the acid-activated viral M2 ion channel, and as a consequence inhibiting the release of viral ribonucleoprotein from virion to host cytosol. However, both H1N1 and H3N2 viruses currently circulating in humans are resistant to these inhibitors. Thus, for example, Centers for Disease Control and Prevention (CDC) advises against their use due to the rapid emergence of drug resistance.

[0005] The commonly used neuraminidase inhibitors include oseltamivir and zanamivir and act as competitive inhibitors competing with sialic acid to bind to the active site of neuraminidase. While these inhibitors are effective against both influenza A and influenza B viruses, they have two major limitations. First, only small benefits have been observed for neuraminidase inhibitors in terms of symptom severity alleviation and sickness duration reduction (0.6-0.7 day out of 7 days). Second, this class of antivirals also suffer from the drug resistant problem. An increase in the number of oseltamivir- resistant strains has been noted since 2007 to 2008 season. In light of the limitations of the current anti-influenza chemotherapies, there is an urgent need to develop new anti- influenza drugs with novel mechanisms of action.

SUMMARY

[0006] Described herein are compounds of Formula I:



Formula I

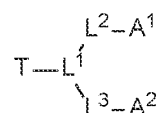
5 and pharmaceutically acceptable salts or solvates thereof, wherein:

T is a radical of a ligand for a target protein of an Influenza virus or a virus-infected cell;

L¹, L², and L³ are each, an independently selected linker; and

A¹ and A² are each, a radical of an independently selected hapten.

[0007] Described herein are compositions comprising one or more compounds of Formula I:



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Formula I

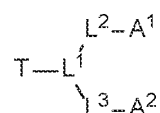
and pharmaceutically acceptable salts or solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof, wherein:

T is a radical of a ligand for a target protein of an Influenza virus or a virus-infected cell;

15 L¹, L², and L³ are each, an independently selected linker; and

A¹ and A² are each, a radical of an independently selected hapten.

[0008] Described herein are compositions for use in treating Influenza and Influenza infection, the compositions comprising one or more compounds of Formula I:



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Formula I

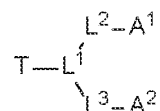
and pharmaceutically acceptable salts or solvates thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof, wherein:

T is a radical of a ligand for a target protein of an Influenza virus or a virus-infected cell;

L¹, L², and L³ are each, an independently selected linker; and

25 A¹ and A² are each, a radical of an independently selected hapten.

[0009] Described herein are unit doses for use in treating Influenza and Influenza infection, the unit doses comprising a therapeutically effective amount of one or more compounds of Formula I:

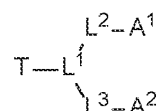


Formula I

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

- 5 T is a radical of a ligand for a target protein of an Influenza virus or a virus-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 methods for treating Influenza and Influenza infection in a host animal, the methods comprising administering a therapeutically effective amount of one or more
 10 compounds of Formula I:

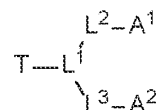


Formula I

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

- T is a radical of a ligand for a target protein of an Influenza virus or a virus-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 uses of one or more compounds of Formula I:



20

Formula I

and pharmaceutically acceptable salts or 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 Influenza and Influenza infection in a host animal; wherein:

- 25 T is a radical of a ligand for a target protein of an Influenza virus or a virus-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] In a first illustrative embodiment, T is a radical of a neuraminidase inhibitor.

[0013] In a second illustrative embodiment, T is a radical of a hemagglutinin inhibitor.

[0014] In another illustrative embodiment, the haptens recruit endogenous antibodies present in the host animal.

[0015] In another illustrative embodiment, compounds, compositions, and unit doses described herein are used in methods for treating Influenza and Influenza infection in a host animal, where the compounds, compositions, and unit doses are administered to the host animal, and after administration A¹ and A², or any epitope of the foregoing are each bound by one or more antibodies.

[0016] In another illustrative embodiment, compounds, compositions, and unit doses described herein are used in methods for treating Influenza and Influenza infection in a host animal, where the compounds, compositions, and unit doses are administered to the host animal, and after administration A¹ and A², or any epitope of the foregoing are each bound by a different antibody.

[0017] In another illustrative embodiment, each of L¹, L², and L³ is independently selected, and each may comprise a single divalent atom, or a chain of atoms. In another illustrative embodiment, L² may be a bond attaching L¹ to A¹. In another illustrative embodiment, each of L³ may be a bond attaching L¹ to A².

[0018] The compounds, compositions, unit doses, methods, and uses described herein are useful in treating Influenza and Influenza infection. It is to be understood that such treatment includes prophylactic and preventative treatment of host animals, including host animals at risk of Influenza or Influenza infection, or host animals exposed to Influenza or Influenza infection, including when symptoms of Influenza or Influenza infection have not been observed. For example, the compounds, compositions, unit doses, methods, and uses described herein are useful in preventing the onset of Influenza or Influenza infection, and/or decreasing the severity of later arising symptoms associated with Influenza and Influenza infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The disclosed embodiments and other features, advantages, and aspects contained herein, and the matter of attaining them, will become apparent in light of the following detailed description of various exemplary embodiments of the present disclosure. Such detailed description will be better understood when taken in conjunction with the accompanying drawings.

[0020] FIG. 1 shows example compounds of the invention.

[0021] FIG. 2A shows plots of effectiveness of Compound 24 and other compounds with various routes of administration.

[0022] FIG. 2B shows plots of effectiveness of Compound 24 and other compounds with various routes of administration.

[0023] FIG. 3 is a synthetic scheme for compound 11, which is used in the synthesis of compound 24.

[0024] FIG. 4 is a synthetic scheme for compound 24.

5 [0025] FIG. 5 is a plot of fluorescence intensity as a function of zanamivir-rhodamine. It shows that zanamivir-rhodamine binds to neuraminidase with a binding affinity of 8.253 nM.

[0026] FIG. 6 are plots of fluorescence intensity as a function of the log[concentration] (nM) for compound 24 and zanamivir. FIG. 6 shows the competitive binding of compound 24 (left panel) and zanamivir (right panel) to neuraminidase-expressing HEK cells. It shows retained affinity for neuraminidase after incorporation into the conjugate compound 24.

10 [0027] FIG. 7 is from the study in Method Example 2 is a plot of TCID₅₀/mL (Tissue Culture Infectious Dose) as a function of dose (mg/kg). FIG. 7 shows the viral titer measured by hemagglutination 24 h after treatment with Tamiflu (oseltamivir phosphate), vehicle (phosphate-buffered saline; "PBS"), or increasing concentrations of compound 24 (the four measurements shown with a "mg/kg" value reported). Mice treated with compound 24 have a 100-fold
15 reduction in infectious virus, as compared to the vehicle, and a greater than 10-fold reduction in infectious virus as compared to the standard of care.

[0028] FIG. 8 shows the cytokine response to various treatments, including compound 24, in human PBMC (Method Example 6).

20 [0029] FIG. 9 shows a plot of normalized fluorescence (RFU) as a function of the log of the concentration of compound 24. FIG. 9 shows neuraminidase inhibition activity of compound 24 against multiple influenza strains.

[0030] FIG. 10 shows a plot of normalized fluorescence (RFU) as a function of the log of the concentration of Compound 41. FIG. 10 shows neuraminidase inhibition activity of compound 41 against multiple influenza strains (Method Example 7).

25 [0031] FIG. 11 from Method Example 5 is a graph of days after infection vs. survival (%) and days after infection vs. body weight (%) for mice (n = 5/group) infected with 10 LD-50 of influenza A H3N2/Wisconsin/15/2009, intraperitoneal administration of human IgG (IVIg (GAMUNEX®-C) at 24 hpi, and administration of compound 24 at 48 hpi.

30 [0032] FIG. 12 from Method Example 5 and shows graphs of cytokine and chemokine levels measured in the lungs of mice from treatment (Compound 24 or Tamiflu) or control groups.

[0033] FIG. 13 from Method Example 5 and graphs of cytokine and chemokine levels measured in the serum of mice from treatment (Compound 24 or Tamiflu) or control groups.

[0034] FIG. 14A shows the in vivo efficacy of Compound 24 against seasonal flu strains A/California/07/2009(H1N1) pdm09, B/Brisbane/60/2008, and A/H3N2/Wisconsin/15/2009 in

mice infected with 10x LD₅₀ titers compared to oseltamivir phosphate and vehicle control (N=5/cohort).

[0035] FIG. 14B shows the viral titer measured by hemagglutination 24 h after treatment with vehicle (PBS), oseltamivir phosphate, or compound 24 in vivo against seasonal flu strains

5 A/California/07/2009(H1N1) pdm09, A/H3N2/Wisconsin/15/2009, and B/Brisbane/60/2008 in mice infected with 10x LD₅₀ titers (N=5/cohort). Compound 24 demonstrates a statistically significant improvement over both vehicle control and oseltamivir phosphate positive control.

[0036] FIG 15A shows the in vivo efficacy of Compound 24 against A/Hong Kong/2369/2009 (H1N1pdm09), an Tamiflu resistant Influenza A, in mice infected with 10x LD₅₀ titers compared
10 to oseltamivir phosphate and vehicle control (N=5/cohort).

[0037] FIG. 15B shows the body weight % (B) following administration of compound 24, oseltamivir phosphate, or vehicle treated mice infected with Influenza A/Hong Kong/2369/2009 (H1N1pdm09, oseltamivir phosphate (Tamiflu) Resistant).

[0038] FIG. 15C shows the viral titers present in the lungs of mice treated with compound 24, oseltamivir phosphate, or vehicle 24 h after drug administration and 48 h after infection with
15 10xLD50 of Influenza A strain – A/Hong Kong/2369/2009 (H1N1pdm09, oseltamivir phosphate (Tamiflu) Resistant).

[0039] FIG. 16 shows the prevention of influenza transmission in co-housed guinea pigs by Compound 24.

20 [0040] FIG. 17A the change in efficacy of compound 24 compared to oseltamivir phosphate after 5 cycles of in vivo mutation as measured by neuraminidase inhibition.

[0041] FIG. 17B shows the change in virulence of influenza virus A/PR8/34 (H1N1) after 5 cycles of in vivo mutation using compound 24 compared to oseltamivir phosphate as measured by LD50 measured in vivo.

25 [0042] FIG. 18A shows IVIS imaging of Group 1 (mock) and Group 2 (infected and vehicle treated mice).

[0043] FIG. 18B shows IVIS imaging of Group 3 (infected and oseltamivir phosphate treated mice) and Group 4 (infected and compound 24 treated mice) from Method Example 12.

[0044] FIG. 19A shows antibody-dependent cellular cytotoxicity (ADCC) induced killing of
30 virus-infected HEK293 cells by Compound 24.

[0045] FIG. 19B shows antibody-dependent cellular phagocytosis (ADCP) induced killing of virus-infected HEK293 cells by Compound 24.

[0046] FIG. 19C shows complement dependent cytotoxicity (CDC) induced killing of virus-

infected HEK293 cells by Compound 24.

[0047] FIG. 20 shows the neuraminidase inhibition activity of Compound 24 intranasally ("IN") against seasonal and drug-resistant Influenza A and B strains.

[0048] FIG. 21 shows the in vivo efficacy of Compound 24 against A/Illinois/37/2018 (H1N1,

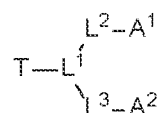
5 Baloxavir Resistant) - Survival over Time.

DETAILED DESCRIPTION

[0049] The following enumerated clauses serve to further illustrate the invention described herein. The clauses describe various features and aspects of the invention including ligands, linkers, and haptens, and various combinations of those features and aspects in the many

10 embodiments the invention. The following enumerated clauses are understood to 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



15 or a pharmaceutically acceptable salts or solvates thereof, wherein

T is a radical of a ligand for a target protein of an Influenza virus or a virus-infected cell;

L¹, L², and L³ are each, an independently selected linker; and

A¹ and A² are each, a radical of an independently selected hapten.

2. The compound of clause 1 wherein the target protein is a neuraminidase.

20 3. The compound of clause 1 wherein the ligand is a neuraminidase inhibitor.

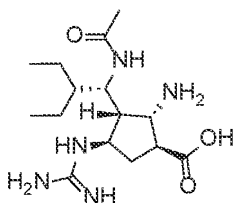
4. The compound of clause 1 wherein the ligand is selected from sialic acid and analogs thereof.

5. The compound of clause 1 wherein the ligand is selected from zanamivir, peramivir, laninamivir, oseltamivir, and 2,3-dehydro-2-deoxy-n-acetylneuraminic acid, and analogs and
25 derivatives of the foregoing.

6. The compound of clause 1 wherein the ligand is zanamivir, or analog or derivative thereof.

7. The compound of clause 1 wherein the ligand is peramivir, or an analog or derivative thereof.

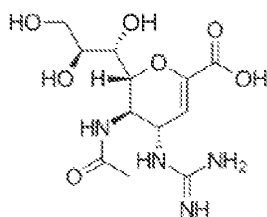
30 8. The compound of clause 1 wherein the ligand is a compound of the formula



or an analog or derivative thereof.

9. The compound of clause 1 wherein the ligand is laninamivir, or an analog or derivative thereof.

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



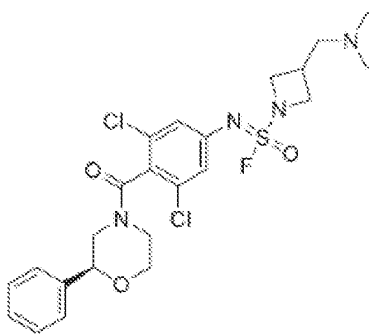
or an analog or derivative thereof.

11. The compound of clause 1 wherein the ligand is oseltamivir, or an analog or derivative thereof.

10 12. The compound of clause 1 wherein the target protein is a hemagglutinin.

13. The compound of clause 1 wherein the ligand is a hemagglutinin inhibitor.

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



and analogs and derivatives thereof.

15 15. The compound of clause 1 wherein the ligand is umifenovir, arbidol, tert-butyl hydroquinone, flufirvitide 3 (VEDTKIDLWSYNAELL (SEQ ID NO: 1)), cyclo(Ac-YWHKNKYVLTYSC)LFAAG-CONH₂ (SEQ ID NO: 2), cyclo(Ac-YRWVWTSFFSEPYFVVC)G-CONH₂ (SEQ ID NO: 3), cyclo(Ac-YLKIYWSKIHGLVSEWC)G- CONH₂ (SEQ ID NO: 4), or cyclo(Ac-YVLFWRWDHGTATHWVC)G-CONH₂ (SEQ ID NO: 5).

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

17. The compound of any preceding clause wherein A^1 and A^2 are the same hapten.
18. The compound of preceding clause wherein A^1 and A^2 are different haptens.
19. 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.
20. 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.
21. The compound of any preceding clause wherein A^1 and/or A^2 is a radical of a rhamnose, including L-rhamnose.
22. 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.
23. The compound of any preceding clause wherein the hapten has an epitope that has an endogenous Ab, B or T cell.
24. The compound of any preceding clause wherein the hapten is not FITC.
25. The compound of any preceding clause wherein L^2 and L^3 are attached to the same atom of L^1 .
26. The compound of any preceding clause wherein L^2 and L^3 are bound to the same atom on L^1 .
27. The compound of any preceding clause wherein L^2 and L^3 are bound to the same non-carbon atom on L^1 .
28. The compound of any preceding clause wherein L^2 and L^3 are bound to the same nitrogen atom on L^1 .
29. The compound of any preceding clause wherein L^2 and L^3 are attached to different atoms of L^1 .
30. 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.
31. 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.
32. 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.

33. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes (PEG)_n, or aza-(PEG)_n, or a combination thereof, where n is 2-36.

5 34. The compound of any preceding clause wherein each of L¹, L², and L³ 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.

35. The compound of any preceding clause wherein one or more of L¹, L², and L³ 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
10 thereof.

36. The compound of any preceding clause wherein one or more of L¹, L², and L³ 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.

37. The compound of any preceding clause wherein L¹ includes or also includes O-alkyl-
15 C(O)N-diyl, O-alkyl-O-alkyl-C(O)N-diyl, or N-alkyl-O-alkyl-O-alkyl-C(O)N-diyl.

38. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes one or more amino acids.

39. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes one or more hydrophilic amino acids selected from Arg, Asn, Asp, Cys, Glu, Gln,
20 His, Lys, Met, Orn, Ser, or Thr, including the naturally occurring L-enantiomers of each of the foregoing.

40. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes one or more hydrophilic amino acids selected from β-NH₂-Ala, Arg, Asn, Asp, Cys, Glu, Gln, His, Lys, Met, Orn, Ser, or Thr, including the naturally occurring L-enantiomers of each
25 of the foregoing.

41. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes one or more amino acids selected from glycine, serine, proline, ornithine, and lysine.

42. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes an ornithine or lysine, including L-ornithine and L-lysine.

30 43. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes a lysine, including L-lysine.

44. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes or also includes a proline, including L-proline.

45. The compound of any preceding clause wherein one or more of L¹, L², and L³ includes
35 or also includes a (Pro)_n, including (L-Pro)_n, where n is 1-6.

46. 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-Pro)₃.
47. 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-Pro)₃-(L-Lys).
- 5 48. 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-Pro)₆.
49. 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-Pro)₆-(L-Lys).
50. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes
10 or also includes glycine and serine, including L-serine.
51. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes or also includes (Gly-Ser)_n, including [Gly-(L-Ser)]_n, where n is 1-3.
52. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes or also includes (Gly-Ser)₂, including [Gly-(L-Ser)]₂.
- 15 53. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes or also includes (Gly-Ser)₂-Lys, including [Gly-(L-Ser)]₂-(L-Lys).
54. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes or also includes (Gly-Ser)₃, including [Gly-(L-Ser)]₃.
55. The compound of any preceding clause wherein one or more of L^1 , L^2 , and L^3 includes
20 or also includes (Gly-Ser)₃-Lys, including [Gly-(L-Ser)]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 C(O), C(O)O, C(O)NH, OC(O)NH, or NHC(O)NH group.
57. 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.
- 25 58. 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.
59. The compound of any preceding clause wherein L^2 and/or L^3 are hydrophilic.
60. The compound of any preceding clause wherein L^1 includes a region that is capable of forming an α -helical conformation.
- 30 61. 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.
62. 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
35 36, from about 5 to about 36, from about 6 to about 36, or from about 7 to about 36.

63. 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..

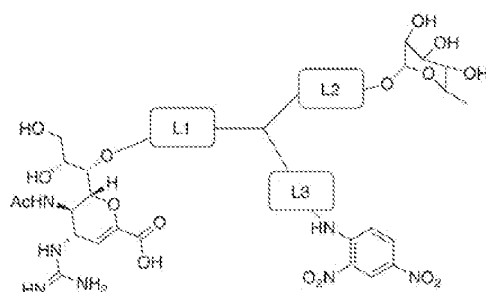
64. The compound of any preceding clause wherein the extended conformation of $T-L^1$ is at least 5 Å, 6 Å, 7 Å, 8 Å, 9 Å, 10 Å, 11 Å, or 12 Å in length.

65. 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 3 to about 36, from about 4 to about 36, from about 5 to about 36, or from about 6 to about 36.

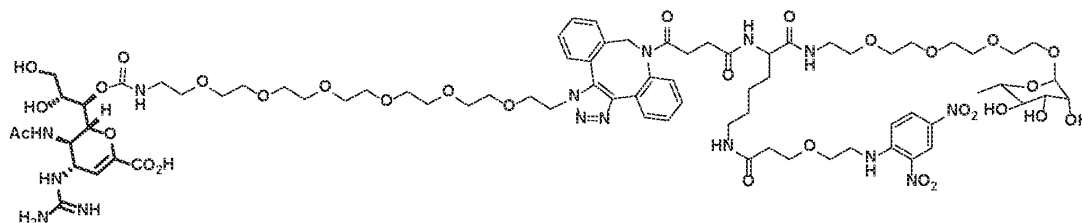
66. The compound of any preceding clause wherein L^1 does not include L-lysine.

67. The compound of any preceding clause wherein L^1 does not include DBCO.

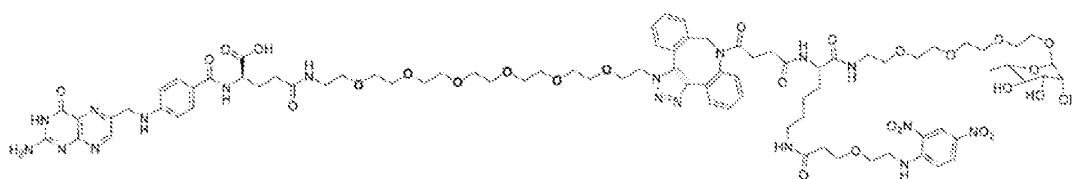
68. The compound of any preceding clause wherein the conjugate is not



where $L1$, $L2$, and $L3$ are each, independently linkers; or

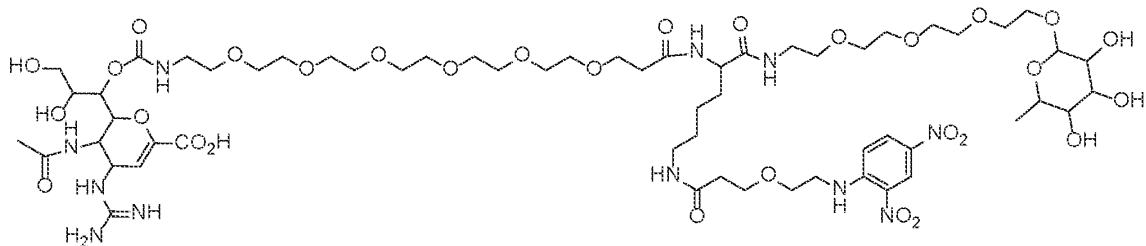


15 or



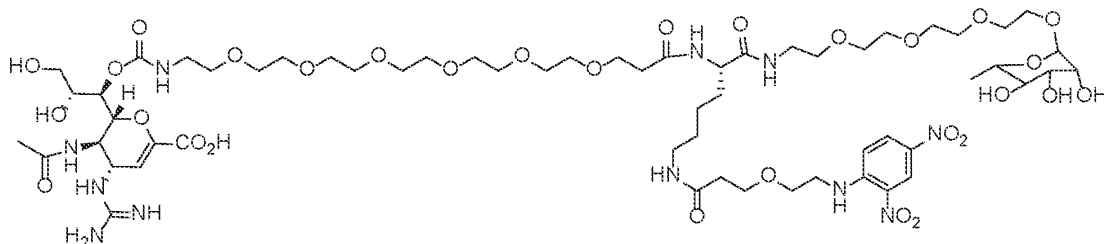
69. A compound of any formula shown in FIG. 1, or a pharmaceutically acceptable salt or solvate thereof.

70. A compound of the formula



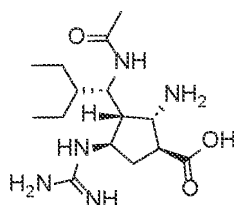
or a pharmaceutically acceptable salt or solvate thereof.

71. A compound of the formula



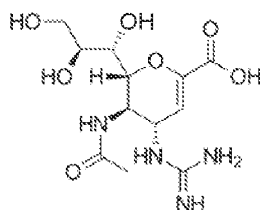
5 or a pharmaceutically acceptable salt or solvate thereof.

72. A compound of the formula



or a pharmaceutically acceptable salt or solvate thereof.

73. A compound of the formula



10

or a pharmaceutically acceptable salt or solvate thereof.

The foregoing illustrative compounds, as defined by clauses 1-73 may be included in any of the compositions, unit doses, uses, or methods described herein.

CONJUGATES

- 15 **[0050]** The conjugate compounds described herein may be formed from a ligand that is capable of targeting a protein of an Influenza or virus-infected cell. In many embodiments, said is protein on the surface of an Influenza or virus-infected cell, or is otherwise accessible from the surface of an Influenza or virus-infected cell. The conjugates described herein may also formed

from two or more haptens, each comprising an immune system responsive epitope or antigen. The conjugates described herein may also 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
5 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
10 of building blocks is described herein for forming the linkers included in the conjugates.

[0051] 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
15 animal. Illustratively, the antibodies are endogenous.

[0052] 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
20 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
25 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.

[0053] 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
30 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 .

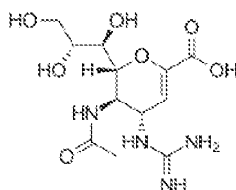
[0054] The compounds, compositions, unit doses, and methods described herein are useful in treating Influenza A, Influenza B, and Avian Influenza. Illustrative strains of Influenza that are treatable using the compounds, compositions, unit doses, and methods described herein include,
35 but are not limited to, H1N1, H1N1pdm09, H3N2, and avian strains H5N1 and H7N9, and

resistant strains, such as H1N1pdm09, oseltamivir-resistant, and H1N1pdm09, baloxavir-resistant.

[0055] Without being bound by theory, it is believed the method 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.

[0056] In many illustrative embodiments, T is a radical of a neuraminidase inhibitor. Illustrative neuraminidase inhibitors include, but are not limited to, sialic acid and analogs and derivatives thereof, zanamivir, peramivir, laninamivir, oseltamivir, 2,3-dehydro-2-deoxy-n-acetylneuraminic acid, and analogs and derivatives of the foregoing, such as compounds disclosed in .US 6340702, WO 1991/016320, WO 1996/026933, WO 1999/033781, and the like.

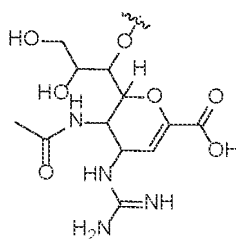
[0057] In many embodiments, the ligand is zanamivir



or an analog or derivative thereof.

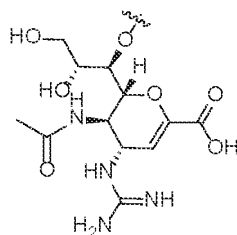
[0058] It is to be understood that a radical may be formed on any atom of the foregoing to form the radical T.

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

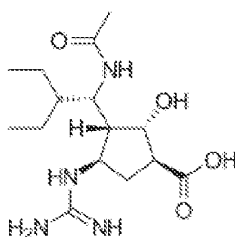


having any specific stereochemical configuration, or a having a mixture of two or more stereochemical configurations.

[0060] In many embodiments, T is a radical of the formula

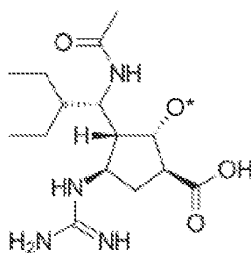


[0061] In many embodiments, the ligand is peramivir

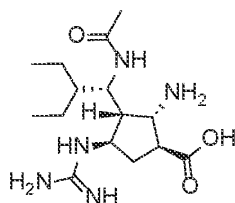


or an analog or derivative thereof, including compounds described in Chand et al. J Med Chem
 5 44(25):4379-92 (2001) Bai et al. Viruses 13(624):1-13 (2021), and the like.

[0062] In many embodiments, T is a radical of the formula

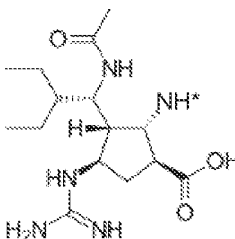


[0063] In many embodiments, the ligand is an amino analog of peramivir

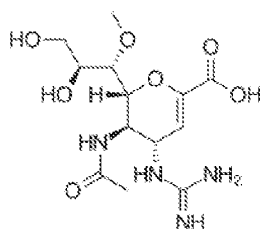


10 or an analog or derivative thereof.

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

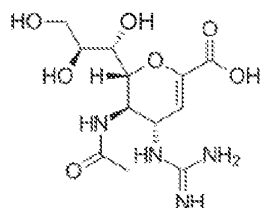


[0065] In many embodiments, the ligand is laninamivir



or an analog or derivative thereof.

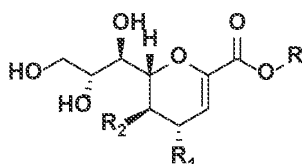
[0066] In many embodiments, the ligand is a compound of the formula



5 or an analog or derivative thereof.

[0067] In many embodiments, the ligand is oseltamivir

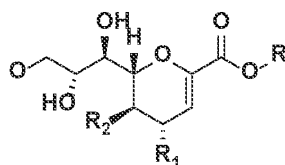
[0068] In many embodiments, the ligand is of the formula



wherein R is alkyl, H, or salt; R₁ is NH₂, NHCH₂CH₂OH, azido, guanidino, NHallyl, N(allyl)₂,

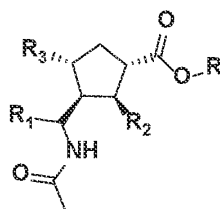
10 or a heterocyclyl group; and R₂ is NH₂ or acetamido.

[0069] In many embodiments, the ligand is of the formula



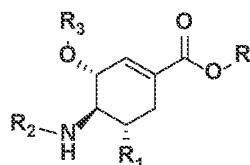
wherein R is Me, H, or salt; R₁ is NH₂ or, guanidino; R₂ is NH₂ or acetamido; and R₄ is alkyl (C-4 to C-14), and ether, acyl, and carbamoyl derivatives thereof.

15 [0070] In many embodiments, the ligand is of the formula



where R is Me or Et; R₂ is H or OH; R₁ is alkyl (primary and secondary, C-3 to C-10); and R₃ is NH₂ or optionally substituted guanidino.

[0071] In many embodiments, the ligand is of the formula

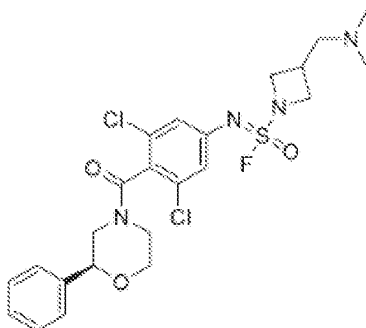


wherein R is H, Me, Et or a salt; R₁ is NH₂, azido, or guanidino; R₂ is C(O)CH₃, C(O)CF₃, or SO₂Me; and R₃ is alkyl (primary or secondary, C-1 to C-5), or CH₂OMe.

5 [0072] In another illustrative embodiment, T is a radical of a hemagglutinin inhibitor.

Illustrative hemagglutinin inhibitors include, but are not limited to, flufirvitide 3, umifenovir, arbidol, tert-butyl hydroquinone, and the like.

[0073] In many embodiments, the ligand is of the formula



10 and analogs and derivatives thereof, including compounds described in Kitamura et al. PNAS 121(22):1-9 (2024), Bai et al. Viruses 13(624):1-13 (2021), and the like.

[0074] 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.

[0075] In many embodiments, T is a radical of flufirvitide 3 of the formula

15 VEDTKIDLWSYNAELL (SEQ ID NO: 1).

20. The compound of clause 1 wherein the ligand is cyclo(Ac-YWHKNKYVLTYSCLFAAG-CONH₂) (SEQ ID NO: 2), cyclo(Ac-YRWVWTSFFSEPYFVVC)G-CONH₂ (SEQ ID NO: 3), cyclo(Ac-YLKIYWSKIHGLVSEWC)G-CONH₂ (SEQ ID NO: 4), or cyclo(Ac-YVLFWRWDHGTATHWVC)G-CONH₂ (SEQ ID NO: 5), including compounds described in
20 Pascha et al. ACS Chem Biol 17:2425-36 (2022).

[0076] 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.

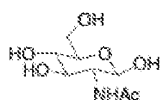
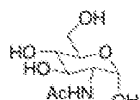
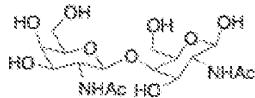
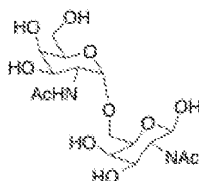
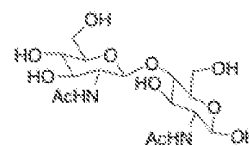
[0077] The haptens described herein can each be bound by an antibody. After administration to
25 a subject, each hapten (A¹ and A²) can be bound by an antibody. In certain embodiments, the

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

5 supplemented via vaccination as a co-therapy to the methods and uses described herein.

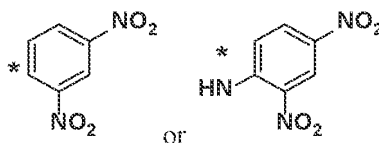
[0078] In another embodiment, A^1 and A^2 are each an independently selected radical of an antigen where for each antigen there is an endogenous antibody present in the host animal. Illustrative haptens include, but are not limited to, a rhamnose, including L-rhamnose, a nitrophenyl, a nitrophenol, a nitroaniline, a dinitrophenyl (DNP), a dinitrophenol, a dinitroaniline, a

10 trinitrophenyl (TNP), a trinitrophenol, a trinitroaniline, chloronitrophenyl, a chloronitrophenol, a chloronitroaniline, an idonitrophenyl, an idonitrophenol, an idonitroaniline, 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 -GlcNAcGalNAc β (1-4) GalNAcGalNAc(α 1-3)GalNAcGlcNAc β (1-4) GlcNAc

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

[0079] 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

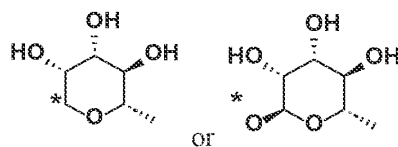


20

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

[0080] 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 (*).

[0081] In many embodiments, A^1 is a DNP radical and A^2 is a rhamnose radical, or vice versa.

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

[0082] In another embodiment, neither A^1 nor A^2 comprises a fluorescein, such as FITC, and the like.

[0083] 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, alkoxyamines, or when combined with carbonyl groups forming urethanes, amino acids, acyloxyamines, 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.

- 5 [0084] 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
- 10 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 .

- [0085] 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,
- 15 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 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.

- [0086] 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.
- 25

[0087] In many embodiments, L^1 , L^2 , or L^3 can each, independently, comprise one or more amino acid or peptide residues.

- 30 [0088] 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:

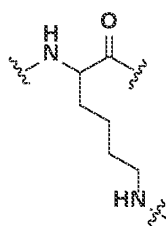


- where R is hydrogen, alkyl, acyl, or a suitable nitrogen protecting group, R' and R'' are hydrogen
- 35 or a substituent, each of which is independently selected in each occurrence, and q is an integer

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, carboxymethyl, 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.

[0089] For example, L¹, L², or L³ can each, independently, comprise one or more lysine residues, each of which is independently optionally substituted. For example, L¹ can

comprise a lysine residue of the formula:



wherein A¹ or A² is attached to the lysine residue via a linker. In another embodiment, the lysine residue is L-Lys. In one example, L¹ comprises at least one lysine residue, such as L-Lys.

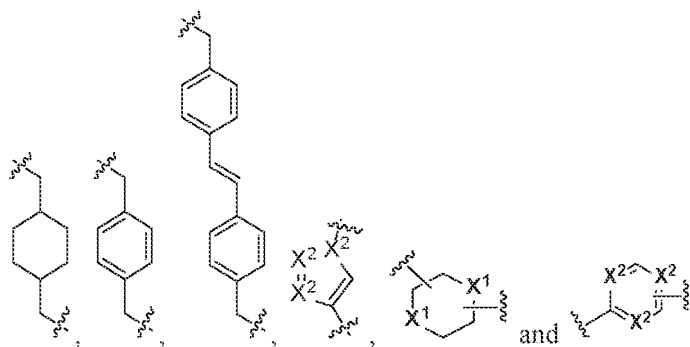
[0090] In many embodiments, L¹, L², or L³ can each, independently, comprise a polyethylene glycol_n (PEG_n) moiety, wherein n = 1-36. In many embodiments, a linker comprises one or more PEG moieties where all carbon and oxygen atoms of the one or more PEG moieties are part of the backbone of the linker.

[0091] In many embodiments, L¹, L², or L³ can each, independently, comprise one or more alkyl groups.

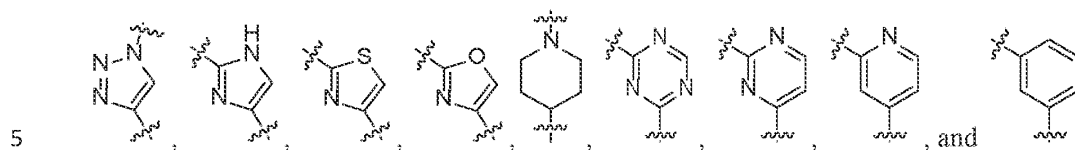
[0092] In many embodiments, L¹, L², or L³ can each, independently, comprise one or more or sugar moieties, glycan residues, or peptidoglycan residues.

[0093] 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.

[0094] 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:

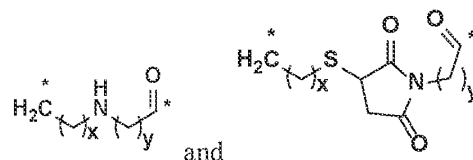


wherein each X^1 is independently CH_2 , NR' , or O wherein R' is alkyl or hydrogen and each X^2 is independently S , O , N , NH , CR'' wherein R'' is alkyl or hydrogen. Examples of such radicals include:



[0095] 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.

[0096] L^1 , L^2 , or L^3 can each, independently, comprise alkylene-amino-alkylenecarbonyl, 10 alkylene-thio-(carbonylalkylsuccinimid-3-yl) moieties, including the following formulae:

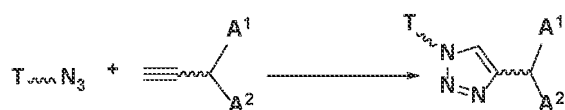


wherein x and y are each independently 1, 2, 3, 4, or 5, where the asterisk identifies points of attachment either to other linker fragments of to T or A^1 or A^2 .

[0097] Additional examples of groups which may be present in the linkers include 1-
15 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, carbonyltetrahydrofuranlyl, 1-(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl, and 1-
20 (carbonyltetrahydrofuranlyl)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., -

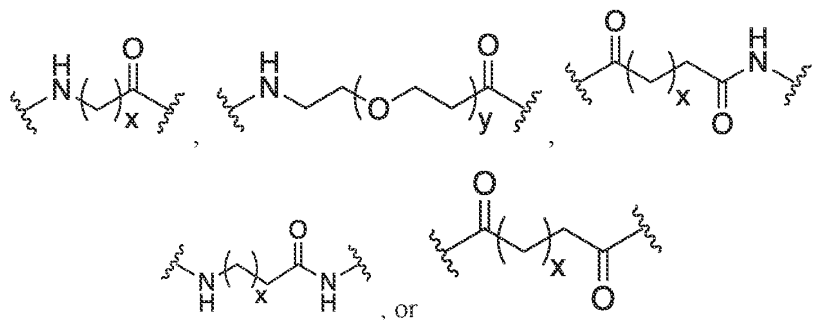
alkyl-O-alkyl-) or sulfur (e.g., -alkyl-S-alkyl-). Examples include alkylcarbonyl, cycloalkylcarbonyl, carbonylalkylcarbonyl, 1-(carbonylalkyl)succinimid-3-yl, and succinimid-3-ylthiol, wherein each group can be substituted or unsubstituted.

- [0098] 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:



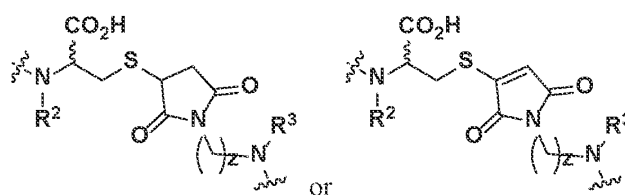
where the wavy line connected to T and to A^1/A^2 represents a linker between T and A^1/A^2 and the groups to which they are attached.

[0099] 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

[0100] 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.

- [0101] 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,

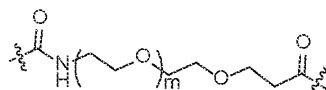
disaccharide, polysaccharide, glycan, or peptidoglycan. In some embodiments, L^1 , L^2 , and L^3 do not comprise a glycan. In some embodiments, L^1 , L^2 , and L^3 do not comprise a sugar.

[0102] In some embodiments, L^1 , L^2 , or L^3 can include a rigid functionality such as an oligoproline or oligopiperidine.

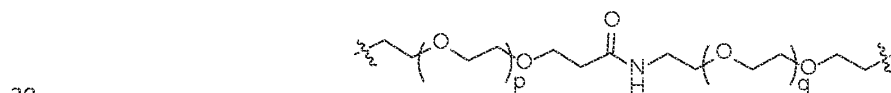
- 5 [0103] In some embodiments, an oligoproline or oligopiperidine has about two up to and including about fifty, about two to about forty, about two to about thirty, about two to about twenty, about two to about fifteen, about two to about ten, or about two to about six repeating units (e.g., prolines or piperidines).

- [0104] In some embodiments, L^1 , L^2 , or L^3 can comprise $(-CH_2CH_2-O-)_n$, where n is an integer
 10 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.

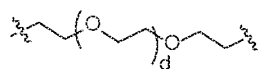
- 15 [0105] In some embodiments, L^1 , L^2 , or L^3 can comprise



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

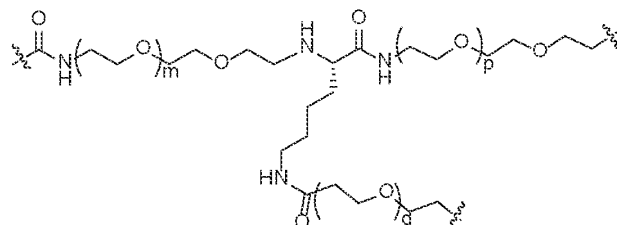
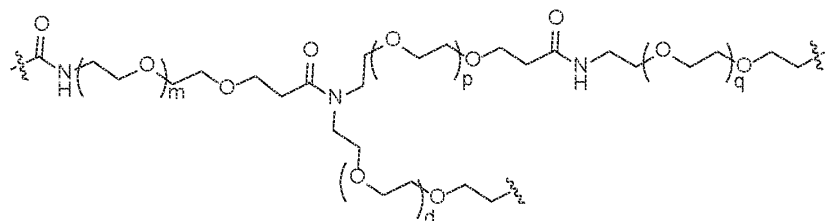


wherein p and q are each, independently, an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, p can be 2 or 3. For example, q can be 2 or 3. In some embodiments, L^1 , L^2 , or L^3 can comprise

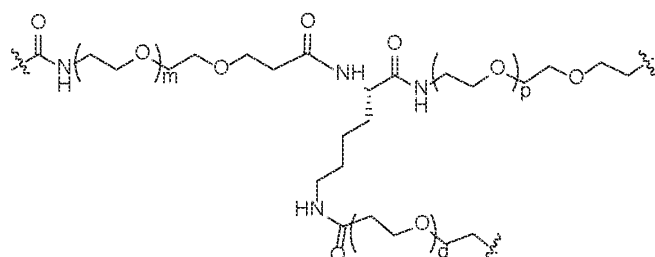


- 25 wherein d is an integer from 0 to 20, such as from 1 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, d can be 1, 2 or 3.

[0106] In some embodiments, L^1 , L^2 , and L^3 taken together can comprise

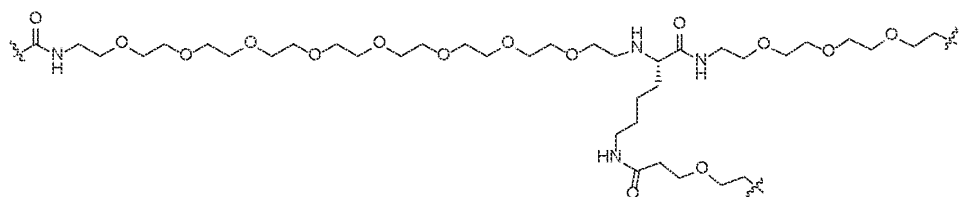
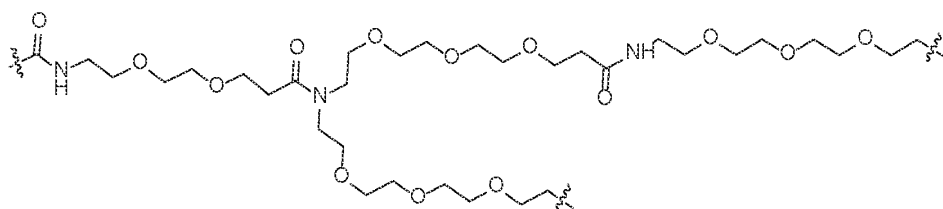


, or



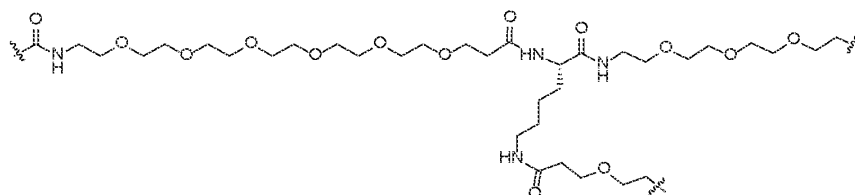
such as wherein m, p, d, and q are each, independently, an integer from 0 to 20, such as from 1
 5 to 20, 0 to 15, 1 to 10, 2 to 10, 2 to 8 or 3 to 9. For example, m can be 0 or 1. Or m can be 5 or 6.
 Or m can be 7 or 8. For example, p can be 2 or 3. For example, q can be 2 or 3. For example, d
 can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

[0107] In some embodiments, L¹, L², and L³ taken together can comprise

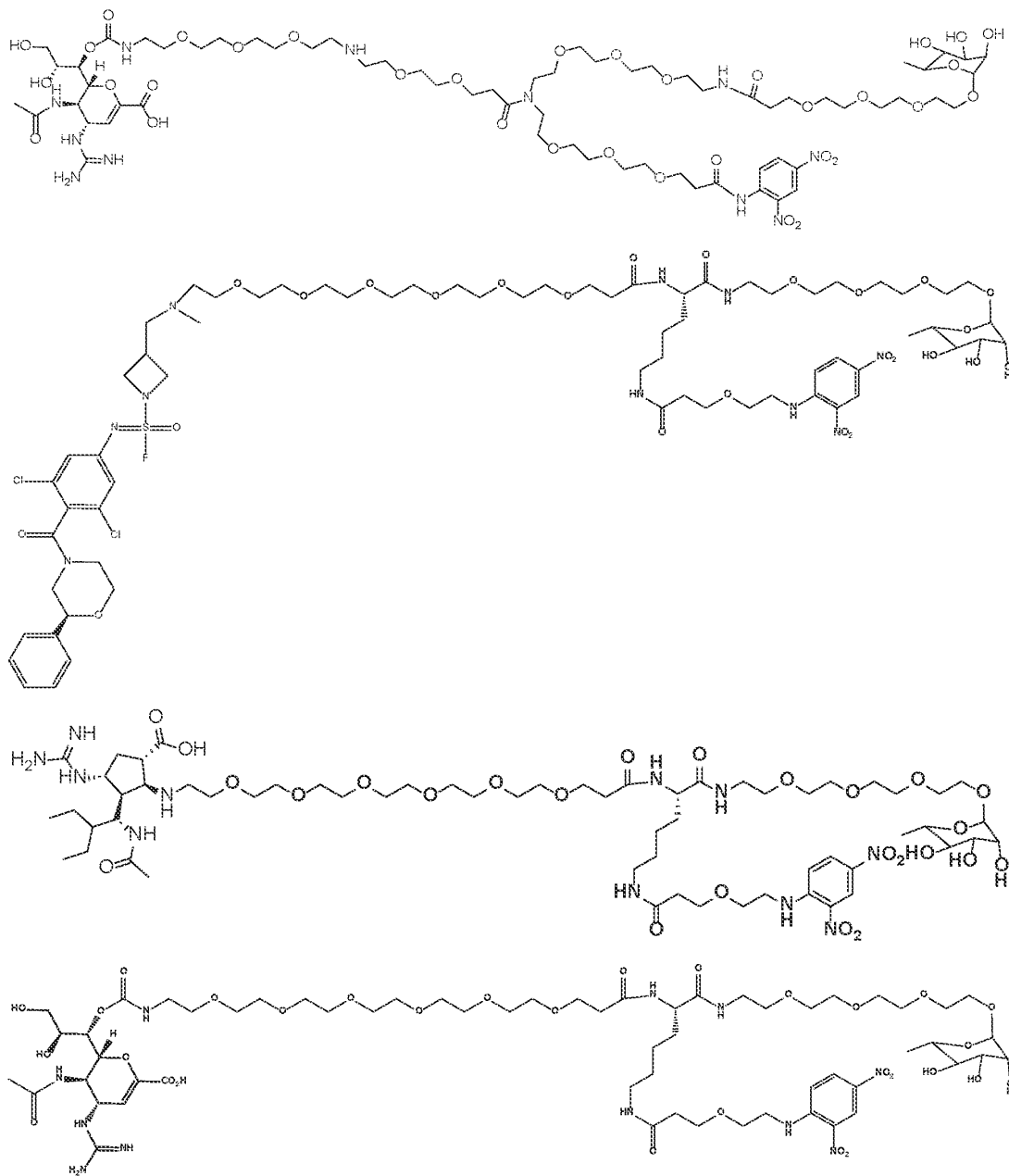


10

or



[0108] In another embodiment, the following compounds are described



5

[0109] In many embodiments, the target protein can be an envelope protein of an influenza or an influenza envelope protein on the surface of a virus-infected cell. In many embodiments, the target protein can be influenza neuraminidase or influenza hemagglutinin.

[0110] 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

10

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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] 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

5 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
10 of the compounds described herein.

[0115] 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

15 [0116] 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.

20 [0117] 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
25 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.

[0118] 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
30 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
35 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.

[0119] 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 $5 \pm 10\%$ or $5 \pm 20\%$. 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.

[0120] 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.

[0121] 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.

[0122] 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 herein. It is to be understood that such radicals can also be formed on acid, ester, or amide groups, such as carboxy, phosphoryl, and sulfonyl

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.

[0123] An “alkyl group” is a saturated, partially saturated, or unsaturated straight chain or

5 branched non-cyclic hydrocarbon having from 1 to 10 carbon atoms (C₁-C₁₀ alkyl), from 1 to 8 carbons (C₁-C₈ alkyl), from 1 to 6 (C₁-C₆ alkyl), 1 to 4 (C₁-C₄ alkyl), 1 to 3 (C₁-C₃ alkyl), or 2 to 6 (C₂-C₆ alkyl) carbon atoms. In some embodiments, the alkyl group has monovalency.

Examples of alkyl groups with monovalency include -CH₃, -CH₂CH₃, and the like. Monovalent alkyls may be found on substitutions in the chain of linker, L, for example. In some

10 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, -CH₂-, -CH₂CH₂-, 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.

15 **[0124]** 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
20 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, —CH₂—CH₂—O—CH₃, —CH₂—CH₂—NH—CH₃, —CH₂—CH₂—N(CH₃)—CH₃, —CH₂—S—CH₂—CH₃, —CH₂—CH₂—S(O)—CH₃, —CH₂—CH₂—S(O)₂—CH₃, —CH₂=CH—O—CH₃, —Si(CH₃)₃, —CH₂—CH=N—OCH₃, —CH=CH—N(CH₃)—CH₃, —O—CH₃, —O—CH₂—
25 CH₃, and —CN. Up to two heteroatoms may be consecutive, such as, for example, —CH₂—NH—OCH₃.

[0125] 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,

30 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
35 like.

[0126] 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.

[0127] 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 C₁-C₆ alkanoyloxy, optionally substituted aroyloxy, alkyl, including C₁-C₆ alkyl, alkoxy, including C₁-C₆ alkoxy, cycloalkyl, including C₃-C₈ cycloalkyl, cycloalkoxy, including C₃-C₈ cycloalkoxy, alkenyl, including C₂-C₆ alkenyl, alkynyl, including C₂-C₆ alkynyl, haloalkyl, including C₁-C₆ haloalkyl, haloalkoxy, including C₁-C₆ haloalkoxy, halocycloalkyl, including C₃-C₈ halocycloalkyl, halocycloalkoxy, including C₃-C₈ halocycloalkoxy, amino, C₁-C₆ alkylamino, (C₁-C₆ alkyl)(C₁-C₆ alkyl)amino, alkylcarbonylamino, N-(C₁-C₆ alkyl)alkylcarbonylamino, aminoalkyl, C₁-C₆ alkylaminoalkyl, (C₁-C₆ alkyl)(C₁-C₆ alkyl)aminoalkyl, alkylcarbonylaminoalkyl, N-(C₁-C₆ 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, C₁-C₆ alkyl, aryl-C₁-C₆ alkyl, and heteroaryl-C₁-C₆ alkyl.

[0128] 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.

[0129] 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.

10 [0130] 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.

[0131] 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

30 [0132] 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	Acetyl, C(O)CH ₃
ACN	Acetonitrile
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
DMSO	Dimethylsulfoxide
TIPS	Triisopropylsilane
Py	Pyridine
TEA	Triethylamine
DEA	Diethylamine
DIPEA	<i>N,N</i> -Diisopropylethylamine
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
PyBOP	BenzotriazoleBenzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate
NHS	<i>N</i> -hydroxysuccinimide
r.t	Room temperature
M	Molar
DMAP	<i>N,N</i> -Dimethyl aminopyridine
PPh ₃	Triphenyl phosphine
PEG	Polyethylene glycol
<i>t</i> Bu	<i>tert</i> -Butyl
NH ₄ OAc	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
pTSH	p-Toluene sulfonic acid
Na ₂ CO ₃	Sodium carbonate
Na ₂ SO ₄	Sodium sulfate
NaOMe	Sodium methoxide
Rha	Rhamnose
L-Rha	L-Rhamnose
DNP	Dinitrophenyl
Fmoc	Fluorenylmethoxycarbonyl
Boc	<i>tert</i> -Butyloxycarbonyl

[0133] 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

5 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.

[0134] EXAMPLE 1. (3S,4R)-3-acetamido-2-((14S,40S,41R)-14-(4-(3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanamido)butyl)-41,42-dihydroxy-13,16,38-trioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,19,22,25,28,31,34,39-decaoxa-12,15,37-triazadotetracontan-40-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound 24) (FIGS. 3-4)

[0135] Compound 3: To compounds **1** (Sigma-Aldrich, 0.5 g, 2.47 mmol) and **2** (BroadPharm, 0.33 g, 2.47 mmol) dissolved in ethyl alcohol (25 mL) was added TEA (1.38 mL, 9.87 mmol). The reaction mixture was heated to 55 °C for 16 h. After reaction completion, the reaction
 15 mixture was cooled and concentrated, and the remainder was purified by flash column chromatography on a Teledyne CombiFlash Rf[®] Lumen (silica-gel, 12 g column, 0-20% MeOH in DCM) to yield compound, **3** as a yellow solid (90% yield). LC-MS [M+H]⁺ = 300.24. Chemistry - An Asian Journal (2012), 7, (2), 272-276.

[0136] **Compound 5:** To compound **3** (0.1 g, 0.33 mmol) in dimethyl sulfoxide (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. Compound, **4** (Chem-Impex International, 0.11 g, 0.27 mmol, 0.8 eq.) was added to the reaction mixture and stirred for 2-3 h. After reaction completion, the reaction mixture was quenched by adding water and extracting with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The remainder was purified by silica-gel (4 g) column chromatography on Teledyne CombiFlash Rf+ Lumen (0-10% MeOH in DCM) and fractions were analyzed by LC-MS to give compound **5** (70% yield). LC-MS [M+H]⁺ = 650.67.

[0137] **Compound 7:** α -L-rhamnose monohydrate (**6**, Sigma-Aldrich, 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 gas prior to the dropwise addition of acetic anhydride (4.15 mL, 43.92 mmol, 8.0 eq.) for 15 min, maintaining the internal temperature below 10 °C. The reaction was slowly warmed to ambient temperature over 2 h. After 20 h, the reaction mixture was poured into EtOAc and extracted twice with 1.0 M HCl. The combined organic layers were washed with saturated sodium carbonate solution, water and brine and dried over anhydrous Na₂SO₄, and concentrated to give compound **7** (98% yield), which was used without further purification. LC-MS [M+H]⁺ = 333.32 and/or LC-MS [M+H₂O]⁺ = 350.32.

[0138] **Compound 9:** To compound **7** (0.50 g, 1.50 mmol) in DCM (7.5 mL) was added H₂N-PEG₄-OH (**8**, BroadPharm, 0.35 g, 1.81 mmol, 1.2 eq.) under an inert atmosphere. The reaction flask was placed in an ice bath, and boron trifluoride diethyletherate (Sigma-Aldrich, 0.56 mL, 4.51 mmol, 3.0 eq.) was added dropwise over 30 min at 4 °C. The reaction mixture was stirred for 2 h, after which cooling was removed. After complete reaction, the reaction mixture was poured into ice water and extracted with DCM (3x10 mL). The combined organic layers were washed twice with saturated sodium bicarbonate solution, water, brine and dried over anhydrous Na₂SO₄ and concentrated. The remainder was purified by silica-gel (12 g) column chromatography on Teledyne CombiFlash Rf+ Lumen (0-10% MeOH in DCM) to give compound **9** (90% yield). LC-MS [M+H]⁺ = 466.51. Biomacromolecules (2020), 21, 793–802

[0139] **Compound 10:** To compounds **5** (0.06 g, 0.09 mmol) and **9** (0.04 g, 0.09 mmol, 1.0 eq.) in dimethyl sulfoxide (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 quenched by adding water and extracting with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The remainder was purified by silica gel (4 g) column chromatography on Teledyne CombiFlash Rf+ Lumen (0-10% MeOH in DCM) to give **10** (93% yield). LC-MS [M+H]⁺ = 1098.15.

[0140] **Compound 11:** To compound **10** (0.01 g, 0.01 mmol) in dry DCM (0.2 ml) was added DEA (100 μ L) under argon. The solution stirred for 1 h. Upon reaction completion, the DEA was evaporated and the remainder was precipitated in diethyl ether to give compound **11** as a yellow solid (quantitative yield), which was used without further purification. LC-MS $[M + H]^+ =$

875.91.

[0141] **Compound 13:** To compound **12** (Alfa Chemistry, 5g, 11.0 mmol) in THF (40mL) was added triphenylphosphine (3.67 g, 14 mmol, 1.27 eq.) and the resulting solution was stirred for 12 h. Subsequently, water (10 mL) was added, and the solution was stirred for another 2-4 h. The reaction mixture was diluted and extracted with EtOAc (2x30 mL), followed by DCM (3x25 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated. The remainder was purified by silica-gel (24 g) flash column chromatography on a Teledyne CombiFlash Rf+ Lumen (0-100% EtOAc in hexanes) to give 2.89 g **13** as a yellow powder (61% yield). LC-MS $[M+H]^+ = 431.42$.

[0142] **Compound 15:** To compound **13** (2.74 g, 6.37 mmol) and N,N'-bis(Boc)-1H-pyrazole-1-carboxamide (**14**, Sigma-Aldrich, 2.57 g, 8.28 mmol, 1.30 eq.) dissolved in THF (20 mL) was added TEA (1.5 mL). The reaction mixture was stirred overnight. After reaction completion, the reaction mixture was quenched with water, diluted and extracted with EtOAc (3x20 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and evaporated. The remainder was purified by flash column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 24 g column, 0-100% EtOAc in hexanes) to give **15** as a white solid (4.14 g, 97% yield). LC-MS $[M+H]^+ = 673.70$.

[0143] **Compound 16:** 0.5 M sodium methoxide in MeOH (2.9 mL, 0.5M, 1.414 mmol) was added to a stirring solution of compound **15** (4.225 g, 6.281 mmol) in anhydrous MeOH (70 mL). The reaction mixture was stirred for 1 h. Dowex 50XW8 (H⁺) resin was added to the reaction mixture to lower the pH ~3 and filtered, washed with MeOH and concentrated to give compound **16**, which was used without further purification. LC-MS $[M+H]^+ = 547.58$.

[0144] **Compound 17:** To compound **16** in anhydrous acetone (70 mL) was added 2,2-dimethoxypropane (7.7 mL, 6.54 g, 62.81 mmol, 10 eq.), followed by p-toluenesulfonic acid (120 mg, 0.628 mmol, 0.1 eq.) and the resulting mixture was stirred overnight. The reaction mixture was concentrated, and the remainder was purified by flash column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 12 g column, 0-100% EtOAc in hexanes) to give **17** as a white solid (2.4 g, 65% yield). LC-MS $[M+H]^+ = 587.64$.

[0145] **Compound 19:** To compound **17** (1.46 g, 2.49 mmol) and **18** (Sigma-Aldrich, 3.51 g, 17.43 mmol) in pyridine (30 mL) was added DMAP (2.13 g, 17.43 mmol). The reaction mixture was stirred overnight. After reaction completion, the reaction mixture was diluted and extracted

with EtOAc (3x 25 mL). The combined organic layer was washed with water, brine and dried over anhydrous Na₂SO₄, concentrated, and the remainder was purified by flash column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 24 g column, 0-100% EtOAc in hexanes) to give **19** as a white solid (1.62 g, 87% yield). LC-MS [M+H]⁺ = 752.45.

- 5 **[0146] Compound 21:** To compound **19** (0.168 g, 0.223 mmol), in THF (1.1 mL) was added **20** (BroadPharm, 0.079 g, 0.223 mmol, 1.0 eq.) followed by DIPEA (0.1 mL, 0.56 mmol, 2.5 eq.) under argon and stirred for 12 h. After reaction completion, the reaction mixture was diluted and extracted with EtOAc (3x 25 mL). The combined organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, and concentrated. The remainder was purified by flash
10 column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 12 g column, 0-10% MeOH in DCM) to give **21** as a white solid (0.205 g, 90% yield). LC-MS [M+H]⁺ = 967.06.

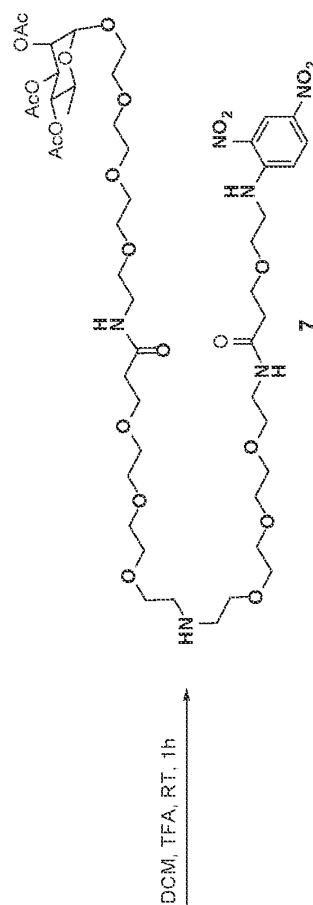
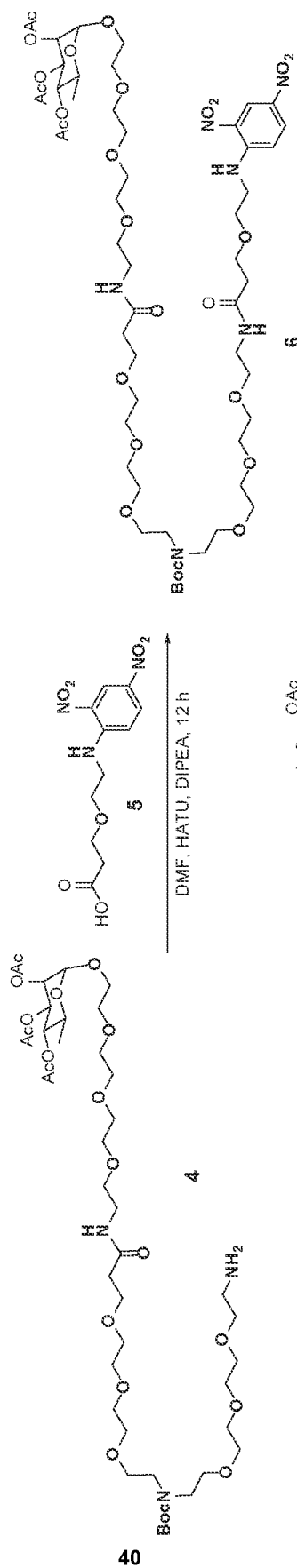
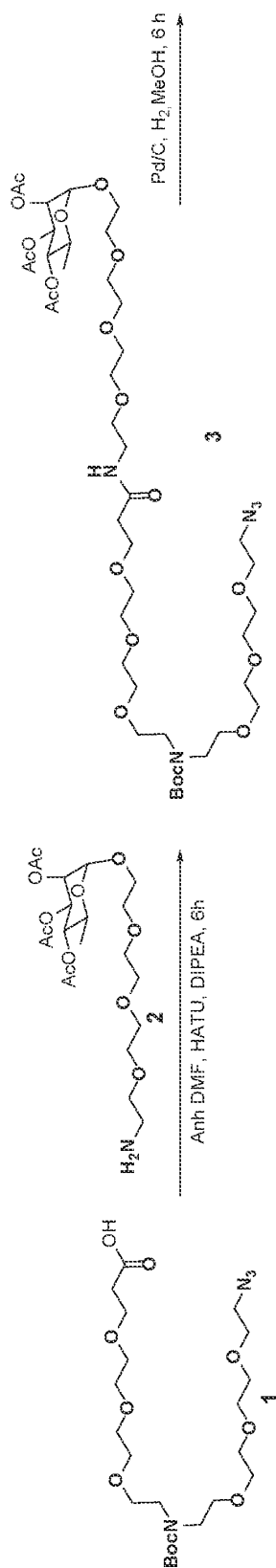
- [0147] Compound 22:** To compound **21** (0.025 g, 0.026 mmol) in DCM:dimethyl sulfoxide (10:1, 0.3 mL) was added HATU (0.01g, 0.026 mmol, 1.0 eq.) followed by DIPEA (0.012 μL, 0.065 mmol, 2.5 eq.) under argon and stirred for 3-5 min. Compound **11** (0.023 g, 0.026 mmol,
15 1.0 eq.) in DCM (0.2 mL) was added to the reaction mixture and stirred for 2-3 h. After reaction completion, the reaction mixture was evaporated, and the remainder was purified by silica-gel (4 g) column chromatography on Teledyne CombiFlash Rf+ Lumen (0-10% MeOH in DCM) and fractions were analyzed by LC-MS. The combined fractions containing compound **22** were evaporated to give compound **22** (61% yield). LC-MS [M+H]⁺ = 1822.94.

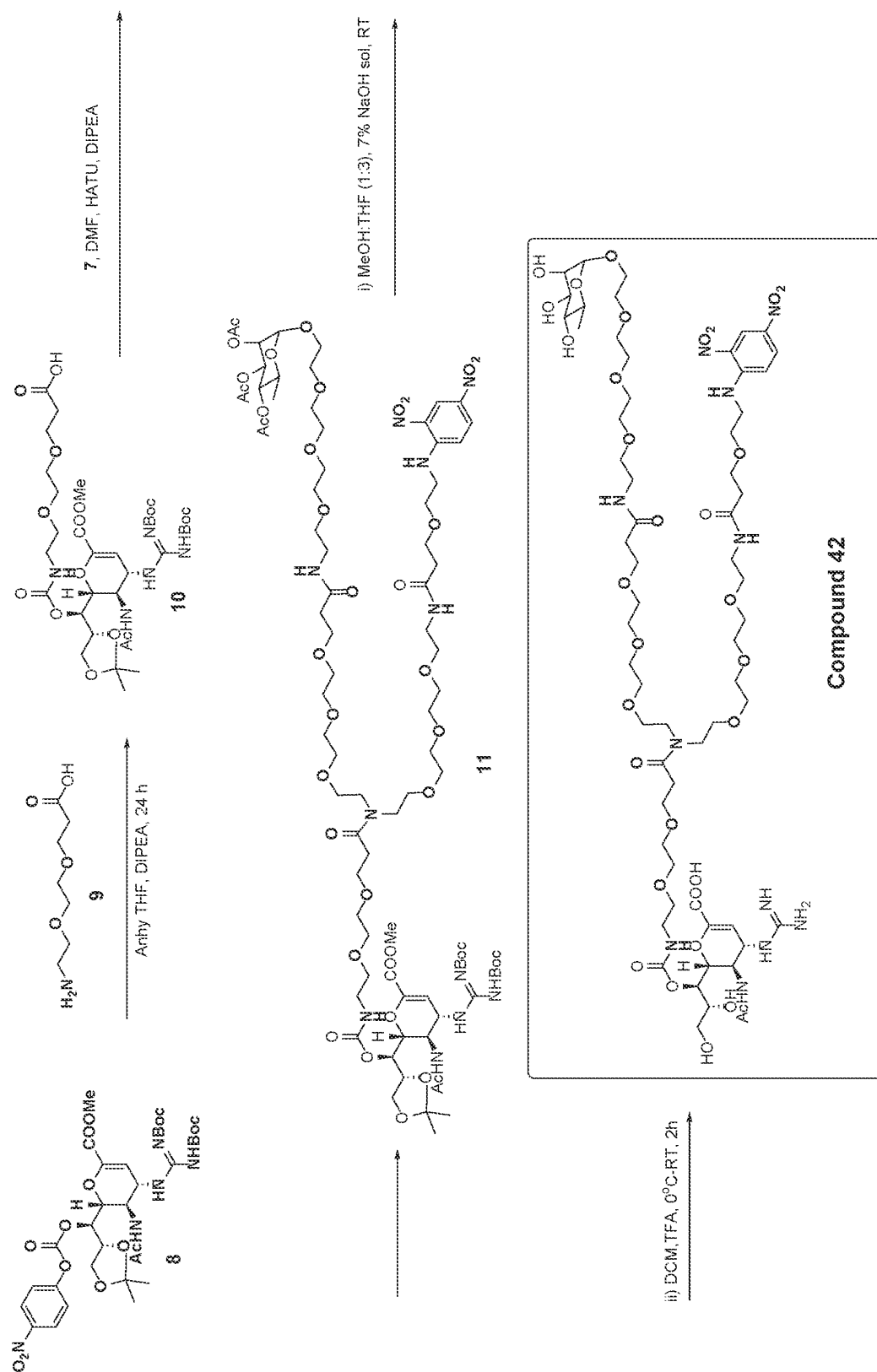
- 20 **[0148] Compound 24:** Compound **24** is prepared by deprotection of compound **22**. It is understood that the deprotection of compound **22** may be accomplished by initial removal of the Boc and ketal protecting groups using TFA in DCM, followed by deprotection of the methyl ester and acetyl ester protecting groups using LiOH·H₂O in MeOH. Alternatively, the deprotection of compound **22** may be accomplished by initial removal of the methyl ester and
25 acetyl ester protecting groups using NaOMe in MeOH, followed by deprotection of the Boc and ketal protecting groups using TFA in DCM, as follows. Compound **22** (38 mg, 0.021 mmol) was dissolved in MeOH (0.5 mL) and treated dropwise with 0.5 M sodium methoxide in MeOH (190 μL). The reaction mixture was stirred for 1 h. Upon reaction completion, the reaction mixture was neutralized by adding Dowex® 50WX8 (H⁺) resin, filtered, and concentrated to
30 give compound **23**, which was used without further purification. LC-MS analysis indicated that the unpurified compound **23** also contained compounds arising from loss of the acetonide protection group, and the Boc protection group.

- [0149] Compound 24:** To compound **23** in DCM (0.8 mL), TFA (0.8 mL) was added at ice-cold temperature. Cooling was removed, and the reaction mixture was stirred for 15 min. TFA was
35 evaporated and the remainder was washed with cold diethyl ether, and purified by prep-HPLC

(UPLC) on a C18 column (5-95% B over 60 min, flow 7 mL/min; B: ACN; A: 20 mM NH₄OAc, pH 7 buffer, UV @ 360 nm and 220 nm) to give compound **24** (42% yield). LC-MS [M + H]⁺ = 1443.83 (95% purity).

- [0150] **EXAMPLE 2.** (2R,3R,4S)-3-acetamido-2-((38R,39R)-25-(18-((2,4-dinitrophenyl)amino)-13-oxo-3,6,9,16-tetraoxa-12-azaoctadecyl)-39,40-dihydroxy-13,26,36-trioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,16,19,22,29,32,37-nonaoxa-12,25,35-triazatetracontan-38-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound 42).





[0151] Compound 3. To a stirred solution of compound 1 (Angene, 1.0 g, 1.91 mmol) and compound 2 (1.07 g, 2.03 mmol) in DMF (15.0 mL) HATU (0.034 g, 0.09 mmol) was added at 0 °C. After 10 min ethylbis(propan-2-yl)amine (GLR, 1.77 mL, 9.57 mmol) was added dropwise to the reaction mixture at 0 °C. Cooling was discontinued and the mixture was stirred for 12 h. After reaction completion, the reaction mixture was concentrated, and the remainder was purified by silica gel column chromatography eluting in 5% MeOH/DCM to afford compound 3 as a yellowish liquid. Yield: 1.20 g, 64.65 %; LCMS (ELSD) m/z 970.60 [M+1]⁺.

[0152] Compound 4. To a solution of compound 3 (1.0 g, 1.03 mmol) in MeOH (50.0 mL), 10% Palladium on carbon (Johnson Matthey, 1.00 g) was added and stirred under an hydrogen atmosphere for 6 h. After reaction completion, the reaction mixture was filtered through a celite bed, washed with MeOH and the filtrate was evaporated to dryness. The remainder was triturated with diethyl ether to afford tert-butyl compound 4 as a pale yellow viscous liquid. Yield: (0.80 g, 82.2 %); LCMS (ELSD) m/z 944.65 [M+1]⁺.

[0153] Compound 6. Compound 6 was prepared using the same procedure to make compound 3 in this Example. Compound 6 was made from compound 5 (0.11 g, 0.37 mmol) and compound 4 (0.35 g, 0.37 mmol) in DMF (10.0 mL), and HATU (0.15 g, 0.40 mmol) and ethylbis(propan-2-yl)amine (0.34 mL, 1.85 mmol). After reaction completion, the reaction mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound 6 as a yellowish liquid. Yield: 0.20 g, 44.03 %; LCMS (ESI) m/z 1225.30 [M+1]⁺.

[0154] Compound 7. To a stirred solution of compound 6 (0.31 g, 0.25 mmol), in anhydrous DCM (5.0 mL), TFA (3.0 mL) was added at 0 °C. Cooling was discontinued and the reaction mixture was stirred for 2 h. After reaction completion, the reaction mixture was evaporated to compound 7 as a light yellow liquid which was used without further purification. Yield: 0.22 g; LCMS (ESI) m/z 1125.10 [M+1]⁺.

[0155] Compound (8) was prepared as described in Carbohydrate Research, 342 (2007) 1636-1650).

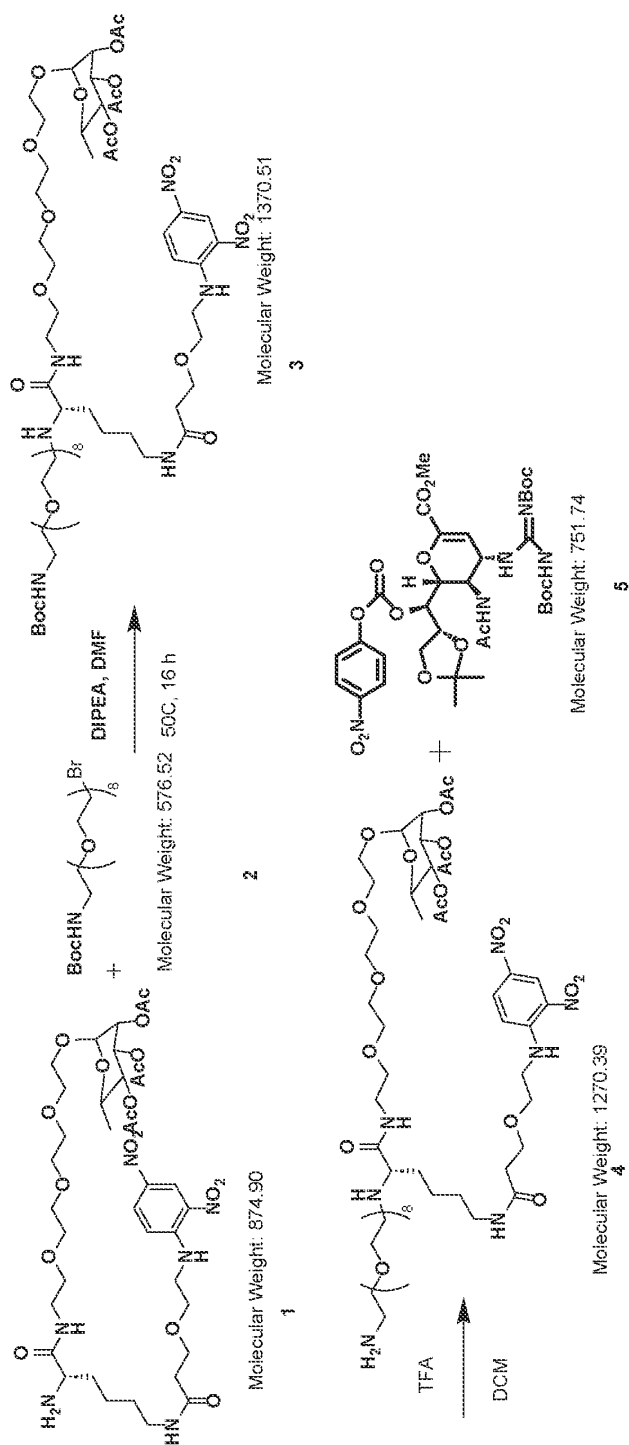
[0156] Compound 10. To a flask containing compound 8 (0.21 g, 0.28 mmol) in dry THF (5 ml) was added DIPEA (GLR, 0.26 ml, 1.44 mmol) dropwise at 25-30°C under a N₂ atmosphere. After 5 mins, compound 9 (Angene, 56 mg, 0.31 mmol) in dry THF (2 ml) was added to the reaction mixture and the resulting suspension was stirred for another 24 h at 25-30°C. Progress of the reaction was monitored by TLC and LCMS. After reaction completion, the reaction mixture was concentrated and the remainder was purified by column chromatography eluting in 10%

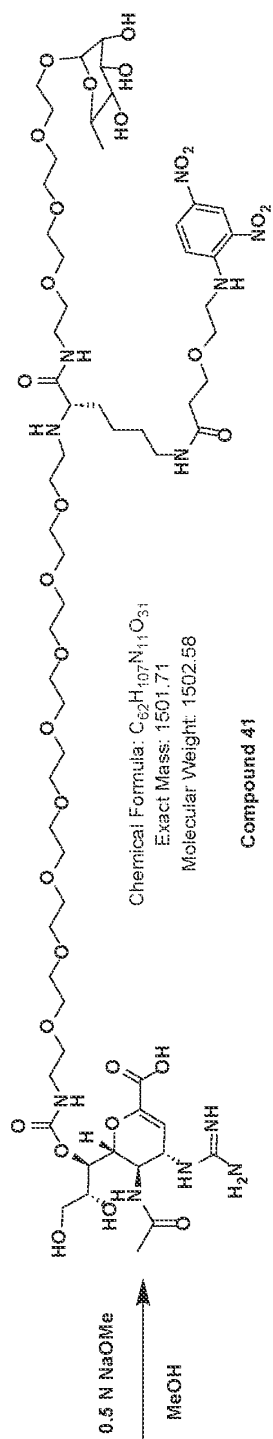
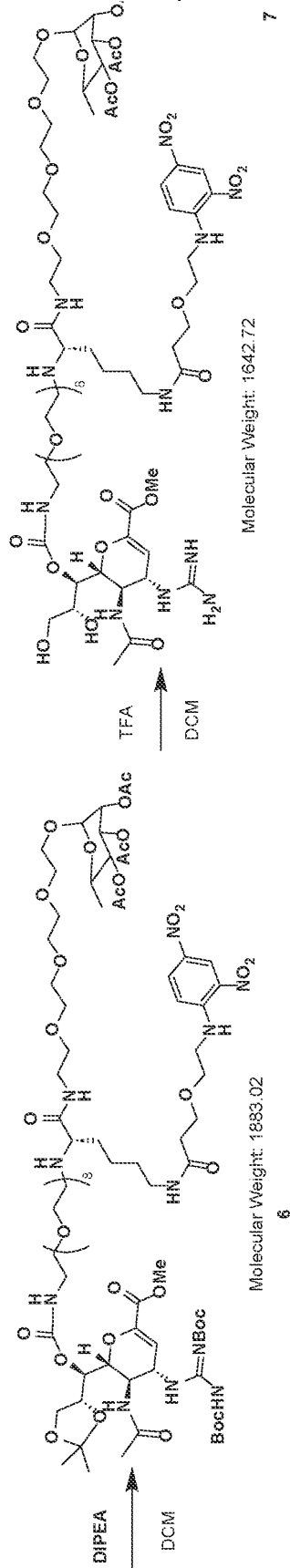
MeOH/DCM to afford compound (**10**) as off white solid. Yield: 0.16 g, 70.05 %; LCMS (ESI) m/z 790.10 [M+1]⁺.

[0157] Compound **11**. Compound **11** was prepared using the same procedure to make compound **3** in this Example. Compound **11** was made from compound **10** (0.11 g, 0.14 mmol) and compound **7** (0.17 g, 0.15 mmol) in DMF (5.0 mL), and HATU (0.08 g, 0.15mmol) and ethylbis(propan-2-yl)amine (GLR, 0.13 mL, 0.70 mmol). After reaction completion, the reaction mixture was concentrated, and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound **11** as a yellowish liquid. Yield: 0.12 g, 45.42 %; LCMS (ESI) m/z 1896.00 [M+1]⁺.

[0158] Compound **42** To a stirred solution of compound **5** (0.11 g, 0.06 mmol) in THF (3 mL) and MeOH (1 mL) was added a 1.0 M solution of sodium hydroxide (0.1 mL) at 25-30 °C over 10 mins. An homogeneous solution was observed and stirred for 4 h at 25-30 °C. Completion of the reaction was confirmed by TLC and LCMS and the reaction mixture pH was slowly adjusted to 7, by using Dowex 50XW8 (H⁺) resin. The reaction mixture was filtered through celite-bed and evaporated. To the stirred solution of the remainder in anhydrous DCM (3.0 mL), TFA (1.0 mL) was added at 0 °C and the reaction mixture was stirred at 15 °C for 2 h. After reaction completion, the solvent was evaporated and the remainder was purified by prep HPLC using water/ACN in 0.1% TFA to afford Compound **42** as a yellowish solid. Yield: 0.015 g, 37.07 %; LCMS (ESI) m/z 1516.35 [M+1]⁺. ¹H NMR (400 MHz, DMSO-d₆): 8.86 (d, J = 2.8 Hz, 1H), 8.82 (brs, 1H), 8.25 (dd, J = 9.2 & 2.4 Hz, 1H), 7.93 (m, 2H), 7.86 (d, J = 8.80 Hz, 1H), 7.35 (brs, 1H), 7.27 (d, J = 9.60 Hz, 1H), 7.18 (brs, 3H), 5.27 (s, 1H), 5.13 - 4.84 (m, 5H), 4.72 (brs, 1H), 4.56 (s, 1H), 4.17 (d, J = 10.40 Hz, 1H), 3.93 - 3.85 (m, 2H), 3.67 - 3.35 (m, 60H), 3.21 - 3.14 (m, 6H), 3.02 (d, J = 5.20 Hz, 1H), 2.59 (t, J = 8.40 Hz, 1H), 2.54 - 2.51 (m, 16H), 2.36 - 2.29 (m, 4H), 1.77 (s, 3H), 1.11 (d, J = 6.0 Hz, 3H).

[0159] EXAMPLE 3. (2R,3R,4S)-3-acetamido-2-((14S,45R,46R)-14-(4-(3-(2-((2,4-dinitrophenyl)amino)ethoxy) propanamido)butyl)-46,47-dihydroxy-13,43-dioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,18,21,24,27,30,33,36,39,44-dodecaoxa-12,15,42-triazaheptatetracontan-45-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound **41**)





[0160] Compound 1 was prepared as described in WO 2023/205669 A2)

[0161] Compound 3: To compound 1 (0.0758 g, 0.087 mmol) and N-Boc-PEG8-bromide, (2, Broad Pharm, 0.05 g, 0.087 mmol, 1.0 eq.) in DMF (0.87 mL) were added DIPEA (0.038 μ L, 0.216 mmol, 2.5 eq.) followed by potassium iodide (0.014 g, 0.087 mmol, 1.0 eq.) under argon atmosphere and stirred for 1-2 h. The reaction temperature was raised to 50°C using oil bath and stirred for 16 h. After reaction completion, the reaction mixture was quenched with water and extracted with EtOAc (3x 5 mL). The combined organic layers were washed with brine and dried over anhydrous Na₂SO₄ and concentrated. The remainder was purified by column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 4 g column, 5-10% MeOH in DCM) to give 34 as a yellow solid (0.083 g, 65 %). LCMS [M+H]⁺ = 1371.52

[0162] Compound 4: To compound 3 (0.083 g, 0.061 mmol) in DCM (2.0 mL) was added TFA (0.6 mL) at ice-cold temperature. Cooling was removed and stirring continued for 1h. After reaction completion, TFA was evaporated, and the remainder was washed with cold diethyl ether (3x5 mL) and hexane (2x3 mL) and dried under vacuum to give compound 4 (quantitative yield), which was used without further purification. LCMS [M+H]⁺ = 1271.41.

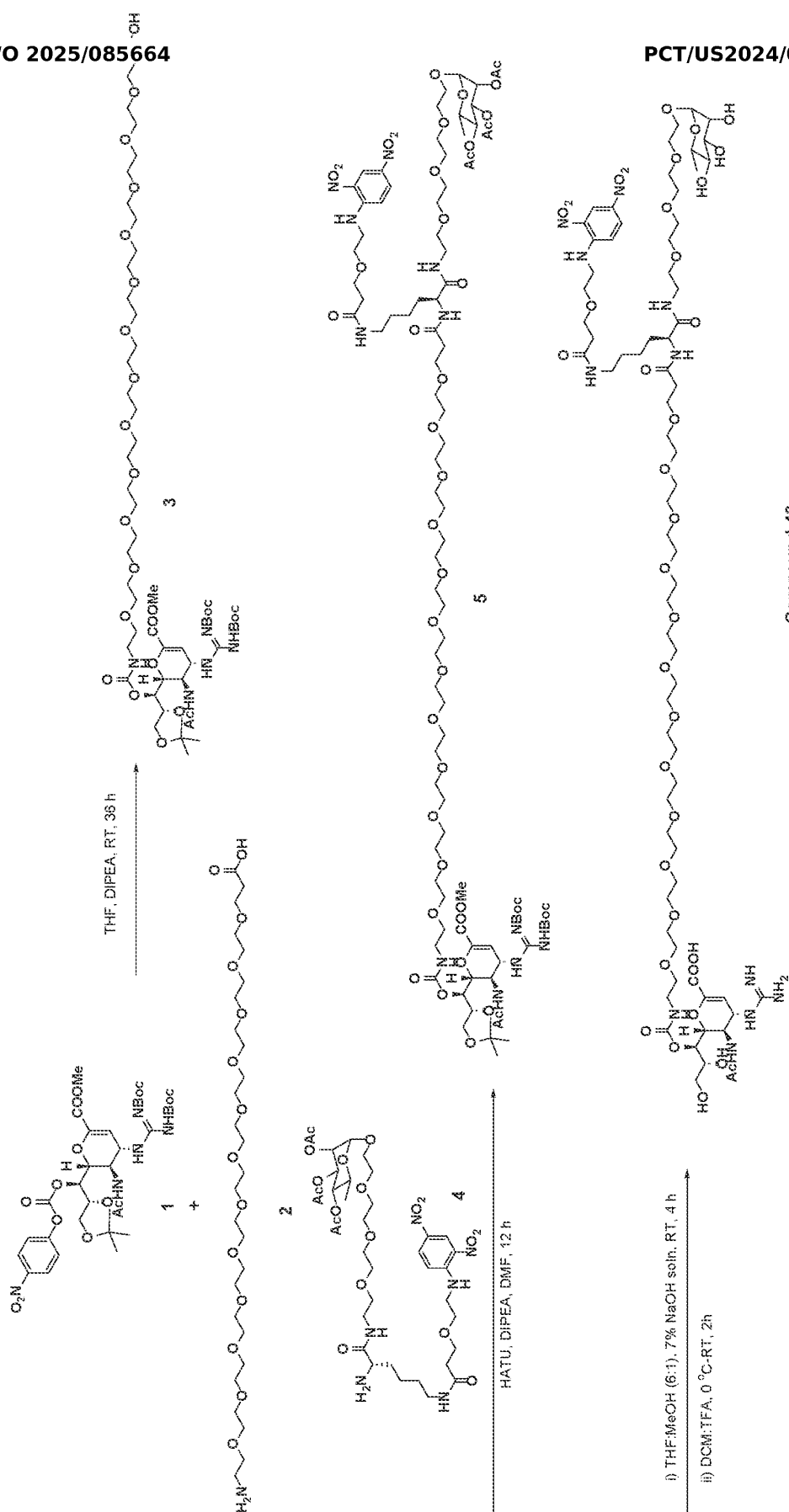
[0163] Compound 6: To compound 5 (0.046 g, 0.061 mmol) in DCM (0.6 mL) was added compound 4 (0.077 g, 0.061 mmol, 1.0 eq.), followed by TEA (0.025 μ L, 0.182 mmol, 3.0 eq.) under argon atmosphere and stirred for 2-6 h. After reaction completion, the reaction mixture diluted with water and extracted with EtOAc (3x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated. The remainder was purified by flash column chromatography on a Teledyne CombiFlash Rf+ Lumen (silica gel, 12 g column, 5-10% MeOH in DCM) to give 6 as a yellow amorphous solid (0.09 g, 79 %). LCMS [M+H]⁺ = 1884.14.

[0164] Compound 7: To compound 6 (0.09 g, 0.048 mmol) dissolved in DCM (2.1 mL), TFA (0.6 mL) was added at ice-cold temperature. Cooling was removed and stirring continued for 1h. After reaction completion, TFA was evaporated and the remainder was washed with cold diethyl ether (3x5 mL) and hexane (2x3 mL) to give 7 (0.079 g, quantitative yield), which was used without further purification. LCMS [M+H]⁺ = 1643.74.

[0165] Compound 41: Compound 7 (0.079 g, 0.048 mmol) was dissolved in MeOH (1.2 mL) and treated dropwise with 0.5 M sodium methoxide in MeOH (0.5 mL, 3.5 eq.) and stirred 1h. After reaction completion, the reaction mixture was neutralized by adding Dowex® 50WX8 (H⁺) resin, filtered, washed with MeOH and concentrated. The remainder was purified by prep-UPLC on a C18 column 5-95% gradient B over 45 min, flow 15 mL/min (A: 20 mM NH₄OAc, pH 7 buffer and B: ACN; UV @ 360 nm and 240 nm) to give Compound 41 (3.3 mg, 50% yield). Purity 89% @360 nm and 84% @ 240 nm. LCMS [M + H]⁺ = 1503.62.

[0166] EXAMPLE 4. (2R,3R,4S)-3-acetamido-2-(((14R,58R,59R)-14-(4-(3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanamido)butyl)-59,60-dihydroxy-13,16,56-trioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,19,22,25,28,31,34,37,40,43,46,49,52,57-hexadecaoxa-12,15,55-triazahexacontan-58-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound 43

5



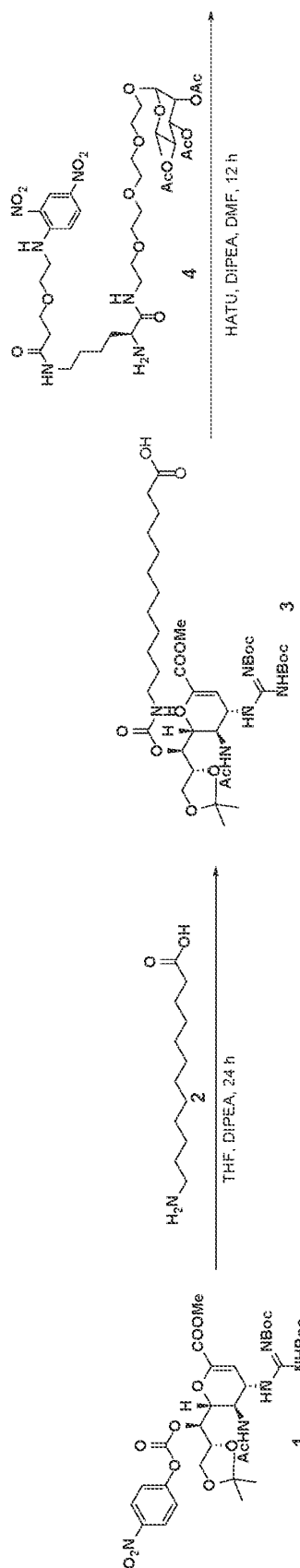
[0167] Compound (1) was prepared as described in Carbohydrate Research, 342 (2007) 1636–1650.

[0168] Compound 3. Compound 3 was prepared using the same procedure to make compound 10 in Example 2. Compound 3 was made from compound 1 (0.10 g, 0.13 mmol) in dry THF (5 ml), DIPEA (0.12 ml, 0.66 mmol), and compound 2 (Havtech, 82.2 mg, 0.13 mmol) in dry THF (2 ml). After reaction completion, the mixture was concentrated and the remainder was purified by column chromatography eluting in 10% MeOH/DCM to afford compound 3 as off white solid. Yield: 0.08 g, 48.88 %; LCMS (ESI) m/z 1230.15 [M+1]⁺.

[0169] Compound 5. Compound 5 was prepared using the same procedure to make compound 3 in Example 2. Compound 5 was made from compound 3 (0.10 g, 0.08 mmol) and compound 4 (78.2 mg, 0.12 mmol) in DMF (3.0 mL), and HATU (0.034 g, 0.09 mmol) and ethylbis(propan-2-yl)amine (GLR, 0.08 mL, 0.41 mmol). After reaction completion, the reaction mixture was concentrated, and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound 5 as a yellowish liquid. Yield: 0.09 g, 53.05 %, LCMS (ESI) M/2 1043.85 [M+1]⁺.

[0170] Compound 43. Compound 43 was prepared using the same procedure to make compound 42 in Example 2. Compound 43 was made from compound 5 (0.09 g, 0.07 mmol) in THF (3 mL), MeOH (1 mL) and 1.0 M aqueous sodium hydroxide. After reaction completion, the mixture was concentrated and dried, and the remainder was purified by prep HPLC purification using water/ACN in 0.1% TFA to afford Compound 43 as a yellowish solid. Yield: 0.016 g, 21.74 %; LCMS (ESI) m/z 1706.80 [M+1]⁺. ¹H NMR (400 MHz, DMSO-d₆): δ 8.86 (d, J = 2.72 Hz, 1H), 8.82 (brs, 1H), 8.26 - 8.23 (m, 2H), 7.99 (d, J = 8.12 Hz, 1H), 7.94 (t, J = 5.48 Hz, 1H), 7.88 (d, J = 9.12 Hz, 1H), 7.79 (t, J = 5.24 Hz, 1H), 7.40 (d, J = 7.0 Hz, 1H), 7.27 (d, J = 9.72 Hz, 1H), 7.18 - 7.04 (m, 2H), 5.34 (brs, 1H), 4.86 - 4.79 (m, 5H), 4.55 (s, 1H), 4.22 - 4.17 (m, 3H), 3.94 - 3.83 (m, 2H), 3.66 - 3.60 (m, 6H), 3.59 - 3.56 (m, 4H), 3.53 - 3.40 (m, 56H), 3.39 - 3.32 (m, 8H), 3.23 - 3.15 (m, 4H), 3.04 - 3.01 (m, 2H), 2.97 - 2.95 (m, 2H), 2.91 - 2.76 (m, 1H), 2.61 - 2.55 (m, 5H), 2.37 (q, J = 6.52 Hz, 2H), 2.33 - 2.99 (m, 2H), 1.90 (s, 1H), 1.77 - 1.76 (m, 3H), 1.58 - 1.56 (m, 1H), 1.49 - 1.43 (m, 1H), 1.35 - 1.29 (m, 2H), 1.28 - 1.19 (m, 3H), 1.16 - 1.11 (m, 5H), 0.99 (t, J = 7.16 Hz, 8H), 0.92 - 0.90 (m, 1H).

[0171] EXAMPLE 5. (2R,3R,4S)-3-acetamido-2-((14R,31R,32R)-14-(4-(3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanamido)butyl)-32,33-dihydroxy-13,16,29-trioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,30-tetraoxa-12,15,28-triazatritriacontan-31-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound 44)



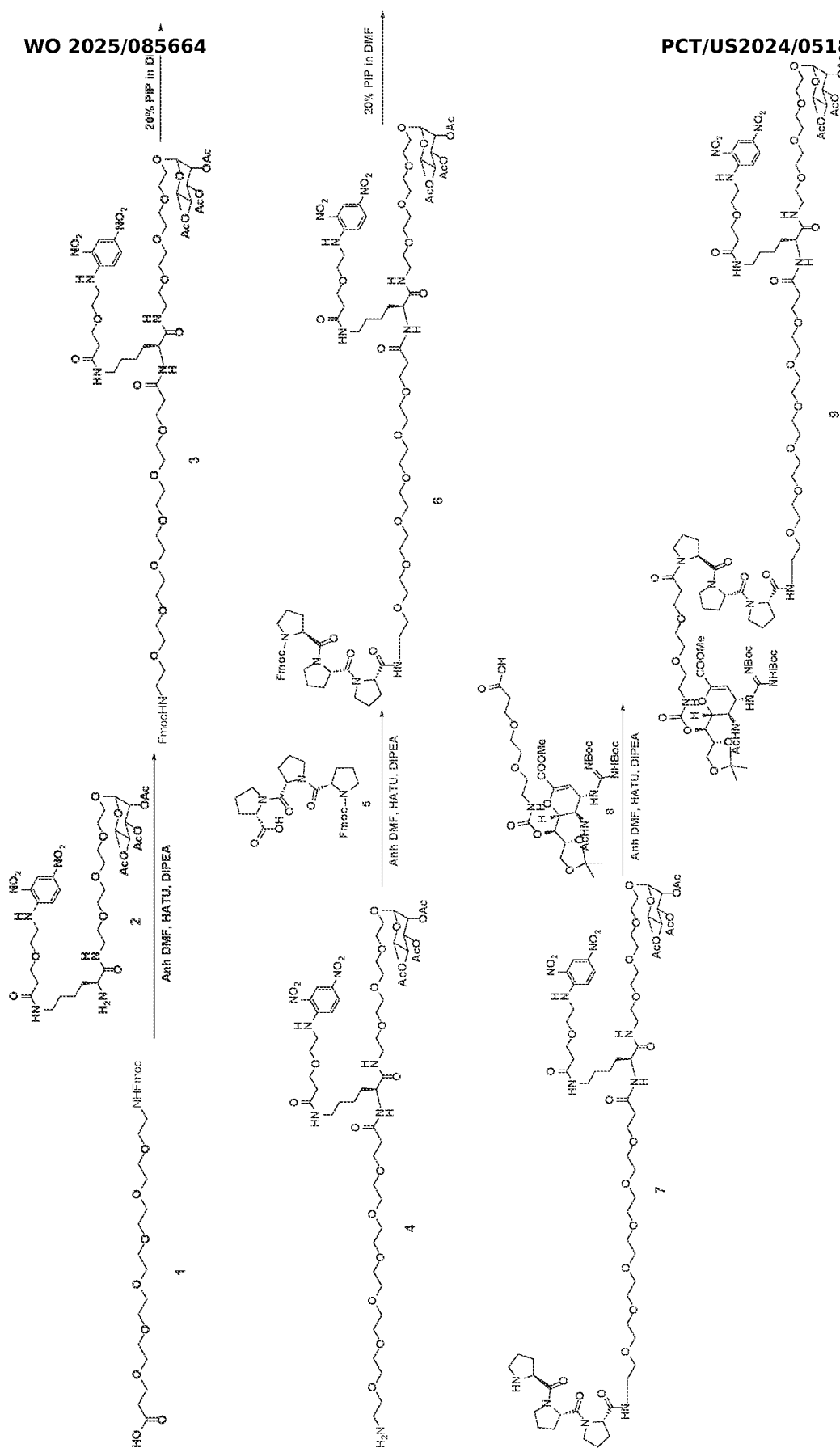
[0172] Compound **1** was prepared as described in Carbohydrate Research, 342 (2007) 1636–1650.

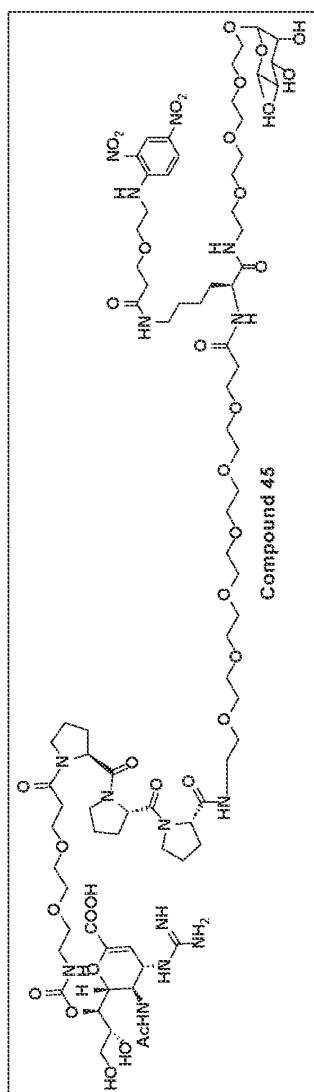
[0173] Compound **3**. Compound **3** was prepared using the same procedure to make compound **10** in Example 2. Compound **10** was made from compound **1** (0.20 g, 0.27 mmol) in dry
5 THF (5 ml), DIPEA (0.25 ml, 1.33 mmol), and 12-aminododecanoic acid (**2**, 53.7 mg, 0.27 mmol) in dry THF (2 ml). After reaction completion, the mixture was concentrated and the remainder was purified by column chromatography eluting in 10% MeOH/DCM to afford compound **3** as off white solid. Yield: 0.09 g, 40.86 %; LCMS (ESI) m/z 828.20 $[M+1]^+$.

[0174] Compound **5**. Compound **5** was prepared using the same procedure as described in
10 Example 2 to make compound **1**. Compound **5** was made from compound **3** (0.09 g, 0.11 mmol) and compound **4** (0.10 mL, 0.12 mmol) in DMF (3.0 mL) with HATU (0.045 g, 0.12 mmol) and ethylbis(propan-2-yl)amine (GLR, 0.09 mL, 0.54 mmol). After reaction completion, the reaction mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound **5** as a yellowish liquid. Yield: 0.08 g, 43.68 %;
15 LCMS (ESI) m/z 1684.30 $[M+1]^+$.

[0175] Compound **44**. Compound **44** was prepared using the same procedure to make compound **42** from Example 2. Compound **44** was made from compound **5** (0.09 g, 0.05 mmol) in THF (3 mL) MeOH (1 mL), and 1.0 M aqueous NaOH. After reaction completion, the mixture was concentrated and dried, and the remainder was purified by prep HPLC purification using
20 water/ACN in 0.1% TFA to afford Compound **44** as a yellowish solid. Yield: 0.024 g, 34.44 %; LCMS (ESI) m/z 1304.9 $[M+1]^+$. ^1H NMR (400 MHz, DMSO- d_6): δ 13.15 (brs, 1H), 8.86 (d, J = 2.8 Hz, 1 H), 8.82 (brs, 1H), 8.25 (dd, J = 9.28, 2.32 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.87 (t, J = 1.6 Hz, 1H), 7.78 (q, J = 8.36 Hz, 2H), 7.53 (d, J = 8.72 Hz, 1H), 7.26 (d, J = 9.68 Hz, 2H), 7.16 - 7.08 (m, 4H), 5.67 (s, 1H), 4.91 (d, J = 1.8 Hz, 1H), 5.15 (d, J = 1.2 Hz, 1H), 4.85 (dd, J = 3.4,
25 1.0 Hz, 1H), 4.71 (brs, 2H), 4.56 - 4.55 (m, 2H), 4.52 - 4.12 (m, 6H), 4.01 (q, J = 2.3 Hz, 2H), 3.85 (brs, 2H), 3.66-3.31 (m, 26H), 3.19 - 3.17 (m, 4H), 2.90 - 2.82 (m, 5H), 2.29 (t, J = 6.36 Hz, 2H), 2.09 - 2.08 (m, 2H), 1.78 (s, 3H), 1.45 - 1.21 (m, 24H), 1.12 (d, J = 6.24 Hz, 3H).

[0177] EXAMPLE 6. (2R,3R,4S)-3-acetamido-2-((2R,3R)-15-((S)-2-((S)-2-(((R)-23-(4-(3-(2-((2,4-dinitrophenyl)amino)ethoxy)propanamido)butyl)-21,24-dioxo-36-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,12,15,18,28,31,34-nona-22,25-diazahexatriacontyl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidin-1-yl)-1,2-dihydroxy-5,15-dioxo-4,9,12-trioxa-6-azapentadecan-3-yl)-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Compound 45)





i) THF:MeOH (3:1), 7% NaOH sol,
RT
ii) DCM, TFA, 0°C-RT

[0178] Compound **3**. Compound **3** was prepared using the same procedure to make compound **3** in Example 2. Compound **3** was made from compound **1** (0.24 g, 0.42 mmol, Habotech) and compound **2** (0.40 g, 0.42 mmol) in DMF (8.0 mL) with HATU (0.17 g, 0.46 mmol) and ethylbis(propan-2-yl)amine (0.22 mL, 1.25 mmol). After reaction completion, the mixture was concentrated, and the remainder was purified by silica gel column chromatography eluting in 5% MeOH/DCM to afford compound **3** as a yellowish liquid. Yield: 0.35 g, 58.6 %; LCMS (ESI) m/z 1449.20 [M+18]⁺.

[0179] Compound **4**. To a stirred solution of compound **3** (0.30 g, 0.21 mmol), in anhydrous dimethyl formamide (3.0 mL), piperidine (0.6 mL) was added and the reaction mixture was stirred for 30 min. After reaction completion, the solvent was evaporated and the remainder was triturated with diethyl ether to afford compound **4** as a light yellow liquid which was used without further purification. Yield: 0.16 g, 63.13%; LCMS (ESI) m/z 1210.20 [M+1]⁺.

[0180] Compound **6**. Compound **6** was prepared using the same procedure to make compound **3** from Example 2. Compound **6** was made from compound **4** (0.20 g, 0.16 mmol) and compound **5** (0.09 g, 0.16 mmol) in DMF (5.0 mL) with HATU (0.07 g, 0.18mmol) and ethylbis(propan-2-yl)amine (0.09 mL, 0.48 mmol). After reaction completion, the mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound **6** as a yellowish liquid. Yield: 0.11 g, 38.61 %; LCMS (ESI) M/Z 1741.48 [M+1]⁺.

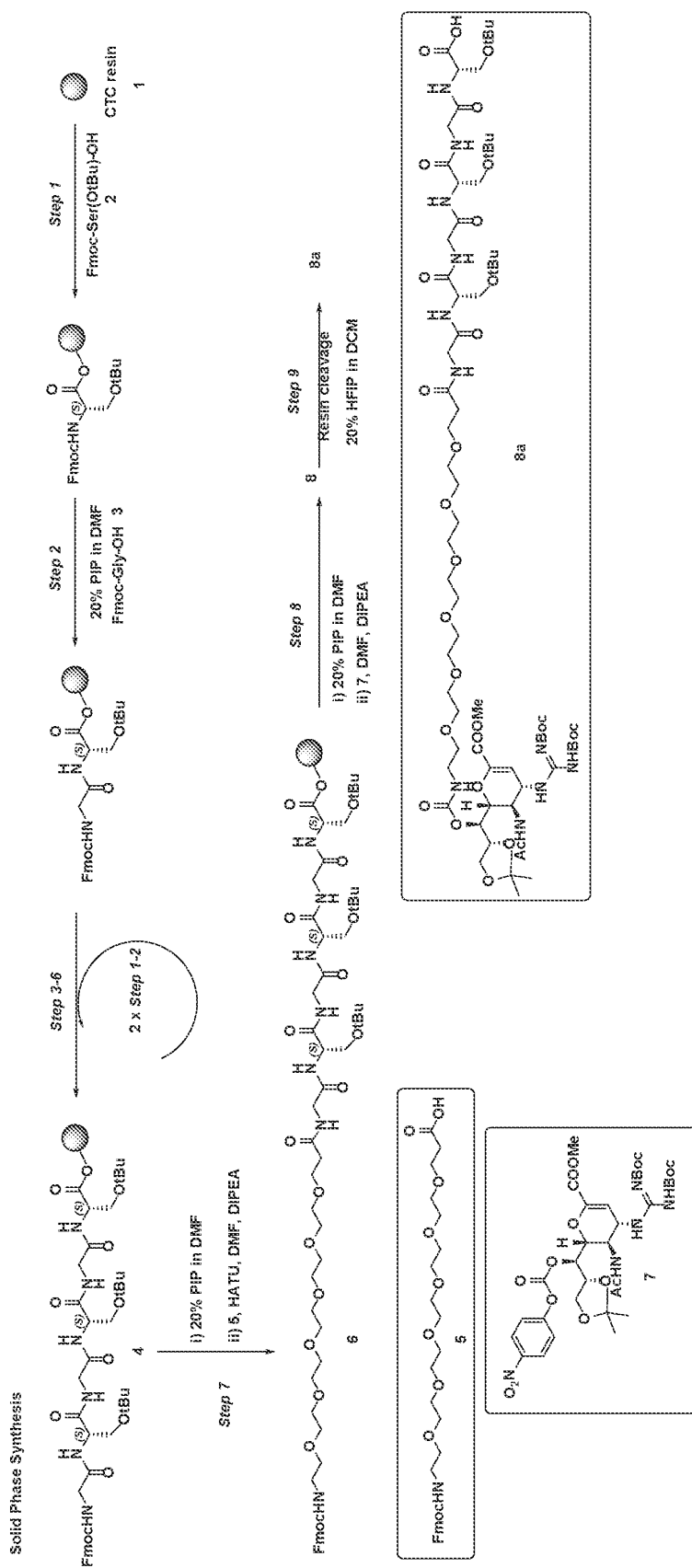
[0181] Compound **7**. To a stirred solution of compound **6** (0.11 g, 0.17 mmol) in anhydrous dimethyl formamide (2.0 mL) piperidine (0.4 mL) was added and the reaction mixture was stirred for 30 min. After reaction completion, the solvent was evaporated and the remainder was triturated with diethyl ether to afford compound **7** as a light yellow liquid which was used without further purification. Yield: 0.085 g, 88.71%; LCMS (ESI) m/z 1500.90 [M+1]⁺.

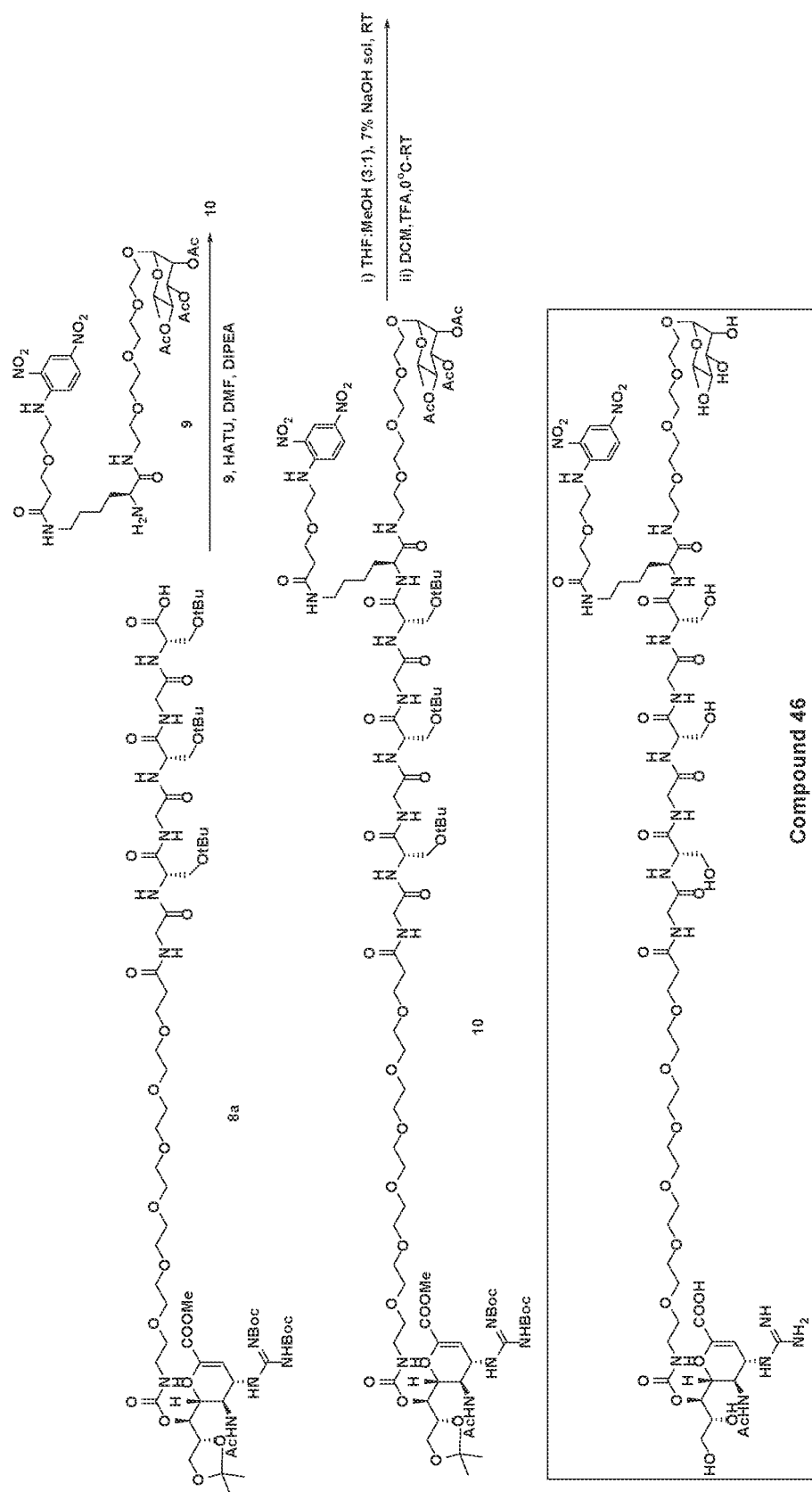
[0182] Compound **9**. Compound **9** was prepared using the same procedure to make compound **3** in Example 2. Compound **3** was made from compound **7** (0.08 g, 0.05 mmol) and compound **8** (0.04 g, 0.05 mmol) in DMF (4.0 mL) with HATU (0.02 g, 0.06 mmol) and ethylbis(propan-2-yl)amine (0.03 mL, 0.15 mmol). After reaction completion, the mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound **9** as a yellowish liquid. Yield: 0.09 g, 74.86 %; LCMS (ESI) m/z 1137.60 [M/2+1]⁺.

[0183] Compound **45**. Compound **45** was prepared using the same procedure to make compound **42** in Example 2. Compound **45** was made from compound **9** (0.09 g, 0.04 mmol) in THF (3 mL) and MeOH (1 mL). After reaction completion, the mixture was concentrated and dried, and the remainder was purified by prep HPLC purification using water/ACN in 0.1% TFA to afford

Compound 45 as a yellowish color solid. Yield: 20 mg, 26.69 %; HRMS (ESI) m/z 1892.91 $[M+1]^+$. 1H NMR (400 MHz, DMSO- d_6): 8.86 (d, J = 2.80 Hz, 1H), 8.83 (brs, 1H), 8.26 (dd, J = 9.2 & 2.4 Hz, 1H), 8.00 - 7.90 (m, 3H), 7.83 - 7.78 (m, 2H), 7.53 (brs, 1H), 7.27 (d, J = 9.6 Hz, 2H), 7.22 - 7.20 (m, 1H), 5.66 (brs, 1H), 5.09 (brs, 1H), 4.84 (dd, J = 10.8 & 2.0 Hz, 1H), 4.76 - 4.73 (m, 2H), 4.61 - 4.51 (m, 4H), 4.36 - 4.28 (m, 1H), 4.26 - 4.19 (m, 3H), 3.99 (qt, J = 8.8 Hz, 1H), 3.81 (brs, 1H), 3.65 - 3.58 (m, 10H), 3.53 - 3.24 (m, 64H), 2.49 - 2.33 (m, 4H), 2.25 - 2.1.83 (m, 7H), 1.80 (s, 3H), 1.62 - 1.1.55 (m, 1H), 1.45 - 1.30 (m, 3H), 1.25 - 1.18 (m, 1H), 1.11 (d, J = 6.4 Hz, 3H).

[0184] **EXAMPLE 7. (2R,3R,4S)-3-acetamido-2-((14R,17R,23R,29R,58R,59R)-14-(4-(3-(2-
10 ((2,4-dinitrophenyl)amino)ethoxy)propanamido)butyl)-59,60-dihydroxy-17,23,29-
tris(hydroxymethyl)-13,16,19,22,25,28,31,34,56-nonaoxo-1-(((2R,3R,4R,5R,6S)-3,4,5-
trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,37,40,43,46,49,52,57-decaoxa-
12,15,18,21,24,27,30,33,55-nonaazahexacontan-58-yl)-4-guanidino-3,4-dihydro-2H-pyran-
6-carboxylic acid (Compound 46)**





[0185] Compound **8a**. Solid phase peptide synthesis (SPS) of compound **8** was performed manually in a peptide reaction vessel using 2-CTC resin under standard peptide coupling and deprotection protocol using Fmoc and tBu protection groups.

[0186] **General Procedure for Amide coupling:** To the reaction vessel containing swollen

- 5 2CTC resin is added activated amino acid solution, prepared by weighing out an appropriate amount of Fmoc-Gly-OH (Chemscene, 2.0 eq) or Fmoc-Ser(OtBu)-OH (BLD pharma, 2.0 eq.) or 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22-hepta-4-azapentacosan-25-oic acid (Habotech; 2.0 eq) in DMF, and HATU (2.5 eq.) and DIPEA (3.0 eq.). The reaction vessel is shaken for 2 h.

- 10 [0187] **General Procedure for Fmoc De-protection:** Reaction vessel containing resins in 20% piperidine in DMF is shaken at for 0.5 h to de-protect Fmoc groups.

[0188] **Compound 8:** To the reaction vessel containing swollen 2CTC resin loaded compound **6** was added a solution of compound **7** (2.0 eq.) in dry THF followed by DIPEA (GLR, 3 eq.) dropwise. The reaction vessel was shaken for 24 h.

- 15 [0189] **Compound 8a (Resin Cleavage):** 2CTC resin loaded with compound **8** was suspended in 20% 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in DCM in a 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. To the combined filtrates was added chilled diethyl ether to give **8a** as a white solid. Yield: 250 mg. LC-MS (ESI) m/z 1567.45 $[M+1]^+$.

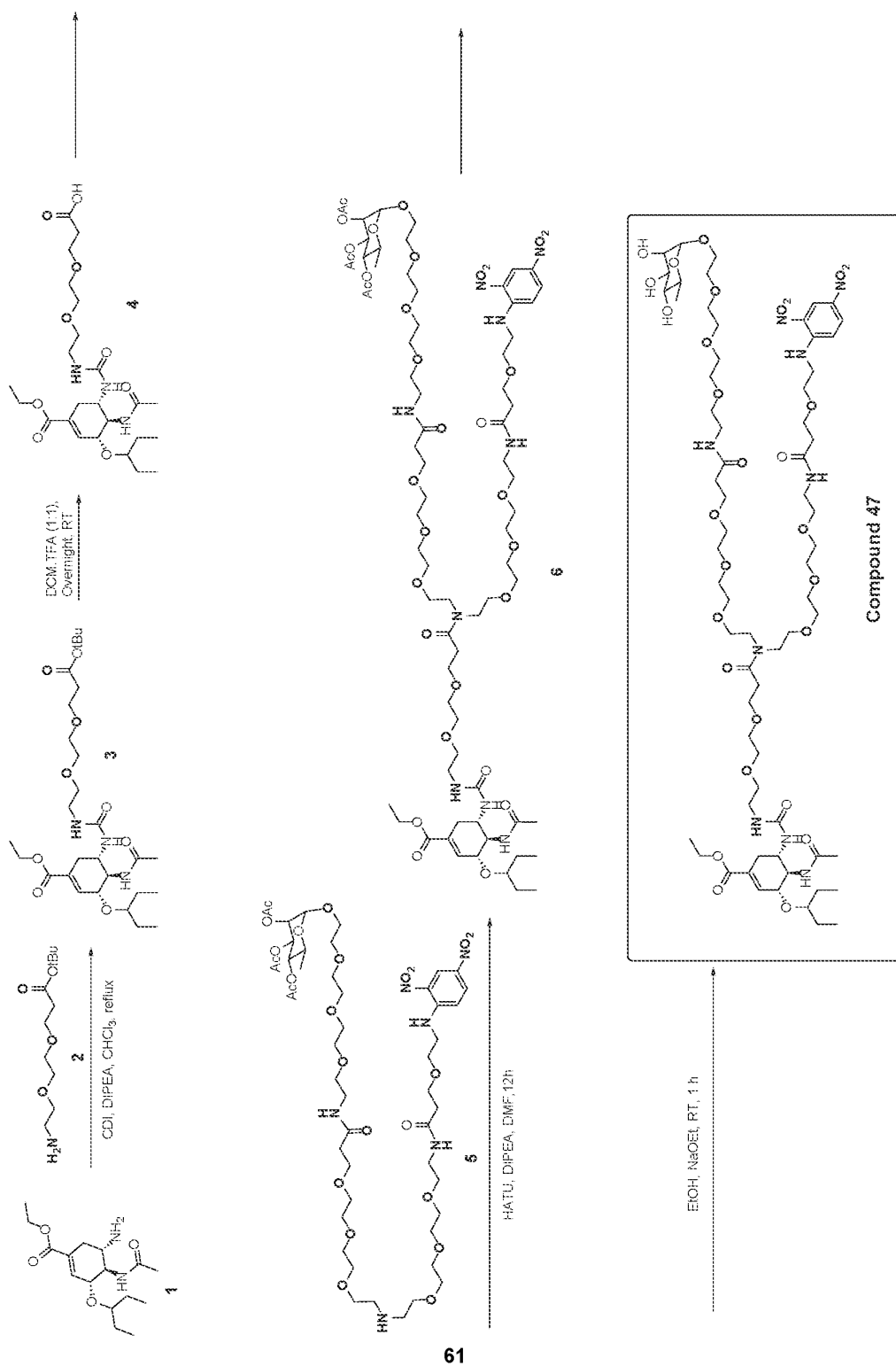
- 20 [0190] **Compound 10.** Compound **10** was prepared using the same procedure to make compound **3** in Example 2. Compound **10** was made from compound **8** (0.25 g, 0.10 mmol) and compound **9** (0.11 g, 0.11 mmol) in DMF (6.0 mL) with HATU (Chempure, 0.043 g, 0.33 mmol) and ethylbis(propan-2-yl)amine (GLR, 0.08 mL, 0.11 mmol). After reaction completion, the mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in
25 5% MeOH/DCM to afford compound **10** as a yellowish liquid. Yield: 0.16 g, 63.65 %; LCMS m/z 1211.85 $[M/2+1]^+$.

- [0191] **Compound 46.** Compound **46** was prepared using the same procedure to make compound **42** in Example 2. Compound **46** was made from compound **10** (0.12 g, 0.05 mmol) in THF (3 mL) and MeOH (1 mL). After reaction completion, the mixture was concentrated and dried, and
30 the remainder was purified by prep HPLC purification using water/ACN in 0.1% TFA to afford **Compound 46** as a yellowish solid. Yield: 0.019 g, 20.47 %; HRMS (ESI) m/z 1874.82 $[M+1]^+$.

- ¹H NMR (400 MHz, DMSO-*d*₆): 8.86 (d, *J* = 2.80 Hz, 1H), 8.83 (brs, 1H), 8.27 - 8.24 (m, 3H), 8.14 (brs, 1H), 8.02 - 7.92 (m, 4H), 7.84 - 7.79 (m, 2H), 7.51 (brs, 1H), 7.27 (d, *J* = 10.6 Hz, 2H), 7.07 - 7.01 (m, 3H), 5.60 (brs, 1H), 5.16 - 5.06 (m, 3H), 4.83 (d, *J* = 10.6 Hz, 1H), 4.74 - 4.73 (m,
35 2H), 4.56 (s, 2H), 4.48 (brs, 1H), 4.33 - 4.20 (m, 5H), 4.15 - 4.14 (m, 1H), 3.96 (qt, *J* = 9.2 Hz,

1H), 3.84 - 3.72 (m, 7H), 3.66 - 3.55 (m, 15H), 3.54 - 3.45 (m, 30 H), 3.44 - 3.36 (m, 7H), 3.23 - 3.16 (m, 4H), 3.05 - 2.5 (4H), 2.38 (t, J = 8.4 Hz, 1H), 2.30 (t, J = 8.4 Hz, 1H), 1.78 (s, 3H), 1.70 - 1.60 (m, 1H), 1.55 - 1.45 (m, 1H), 1.38 - 1.23 (m, 4H), 1.12 (d, J = 6.4 Hz, 3H).

- [0192] **EXAMPLE 8.** Ethyl (3R,4R,5S)-4-acetamido-5-(3-(25-(18-((2,4-dinitrophenyl)amino)-13-oxo-3,6,9,16-tetraoxa-12-azaoctadecyl)-13,26-dioxo-1-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3,6,9,16,19,22,29,32-octaoxa-12,25-diazatetratricontan-34-yl)ureido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (Compound 47)



[0193] Compound **3**. Compound (**3**) was prepared as described in Eur. J. Med. Chem., 2020, 200, 112423 using commercially available Oseltamivir (1, BLD) and Amino-Peg2-t-butyl ester (2, BLD).

[0194] Compound **4**. To a stirred solution of compound **3** (0.10 g, 0.17 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 12 h. After reaction completion, the solvent was evaporated to compound **4** as a light yellow liquid which was used for the next reaction. Yield: 0.08 g; LCMS (ESI) m/z 516.20 [M+1]⁺.

[0195] Compound **6**. Compound **6** was prepared using the same procedure to make compound **3** in Example 2. Compound **6** was made from compound **4** (0.15 g, 0.29 mmol) and compound **5** (0.36 g, 0.32 mmol) in DMF (8.0 mL) with HATU (0.17 g, 0.32 mmol) and ethylbis(propan-2-yl)amine (GLR, 0.27 mL, 1.45 mmol). After reaction completion, the mixture was concentrated and the remainder was purified by silica gel column chromatography eluting in 10% MeOH/DCM to afford compound **6** as a yellowish liquid. Yield: 0.095 g, 22.05 %; LCMS (ESI) m/z 1622.65 [M+1]⁺.

[0196] Compound **47**. To a stirred solution of compound **6** (0.10 g, 0.061 mmol) in ethanol (5.0 mL) was added sodium ethoxide (0.41 mg, 0.002 mmol) at 0 °C. Cooling was discontinued, and the reaction mixture was stirred for 1 h. After reaction completion, the reaction mixture was treated with acidic (Dowex 50, H⁺) to pH~7 and the suspension was filtered through a sintered funnel with MeOH wash. The filtrate was concentrated and the remainder was purified by prep HPLC to afford Compound **47** as a yellowish color solid. Yield: 25 mg, 27.11 %; LCMS (ESI) m/z 1496.80 [M+1]⁺. ¹H NMR (400 MHz, DMSO-d₆): δ 8.86 (d, J = 2.80 Hz, 1H), 8.83 (brs, 1H), 8.25 (dd, J = 9.2 & 2.4 Hz, 1H), 7.90 (m, 2H), 7.80 (d, J = 8.8 Hz, 1H), 7.27 ((d, J = 9.6 Hz, 1H), 6.10 (brs, 1H), 5.77 (d, J = 7.2 Hz, 1H), 4.55 (d, J = 1.6 Hz, 1H), 4.16 - 4.10 (m, 2H), 4.08 - 4.03 (m, 1H), 3.76 - 3.55 (m, 22H), 3.53 - 3.33 (m, 51H), 3.20 - 3.13 (m, 8H), 2.61 - 2.57 (m, 4H), 2.34 - 2.29 (m, 5H), 1.78 (s, 3H), 1.47 - 1.32 (m, 4H), 1.21 (d, J = 7.2 Hz, 3H), 1.11 (d, J = 6.0 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H), 0.76 (t, J = 7.2 Hz, 3H).

[0197] METHOD EXAMPLE 1: Binding affinity to HEK expressing neuraminidase. The K_i of Compound **24** when competed against zanamivir-rhodamine (*Nature Communications 11*, Article number: 5597 (2020)) was determined using a binding and competition assay. First, the K_d of zanamivir-rhodamine binding to neuraminidase is determined. Human Embryonic Kidney cells (HEK239) transfected with influenza viral neuraminidase are suspended in staining buffer (2% fetal bovine serum in phosphate buffered saline) at a density of 2 million cells/mL, and then plated in triplicate (50 µL/well) in a u-bottom plate. Next, two sets of serial dilutions of zanamivir-rhodamine are made in staining buffer. In one set, 100x concentration of zanamivir is

included to determine non-specific binding. The diluted compounds are added to the cells (50 μ L/well) and incubated on a shaker at room temperature for 1 h. Cells are washed twice with 200 μ L of phosphate buffered saline and resuspended in 150 μ L of staining buffer. Fluorescence is measured using an Attune NxT flow cytometer (Fisher), and K_d is calculated using Graph Pad

5 Prism, One site – total and non-specific binding.

[0198] Next the K_i of compound **24** and zanamivir can be determined. Cells are plated as described above. One set of serial dilutions is made for compound **24** and a separate set is made for zanamivir. Each dilution is mixed with an equal volume of 80 nM zanamivir-rhodamine. The compound mixture is added to the cells (50 μ L/well) and incubated on a shaker at room

10 temperature for 1 h. Cells are washed and resuspended as described above. Fluorescence is measured using an Attune NxT flow cytometer (fisher). Using the K_d calculated above and a final concentration of 20 nM zanamivir-rhodamine, the K_i can be calculated using Graph Pad Prism, One site – Fit K_i .

[0199] **FIG. 5** shows that zanamivir-rhodamine binds to neuraminidase with a binding affinity of 8.253 nM. It is well established in the literature that zanamivir binds to neuraminidase, and excess zanamivir blocking fluorescence shows that zanamivir-rhodamine is binding to neuraminidase. This same rhodamine conjugate is used to quantify inhibitor constants for compound **24** and zanamivir (**FIG. 6**). Zanamivir has a K_i of 0.496 nM. The K_i of compound **24** is in the low nanomolar range at 7.152 nM.

20 [0200] **METHOD EXAMPLE 2: Dose range finding study – Viral Titers.** A group of 6-8 weeks old female BALB/c mice ($n = 5$ /group) were infected with 10 LD₅₀ of Influenza virus H3N2/Wisconsin/15/2009 on day 0 of the experiment. Mice were given an intraperitoneal injection of 6 g/kg human IVIg at 24 hpi to achieve humanized titer of anti-DNP and anti-Rhamnose antibodies at the time of test article administration. Mice were treated with test
25 articles at 48 hpi. Compound **24** was administered as a single intranasal dose of 2.2 mg/kg, 0.72 mg/kg, 0.24 mg/kg or, 0.081 mg/kg in the designated cohorts. Mice in the two control groups received Tamiflu (oseltamivir phosphate) as a positive control (5 mg/kg b.i.d. for 5 days) and Vehicle (PBS) as placebo. Viral titers in mouse lungs were measured in 2 ways: live, infectious virus is measured using a hemagglutination assay, and total viral RNA is measured using reverse
30 transcription polymerase chain reaction (rt-PCR). However, the rt-PCR method cannot distinguish between live and dead viruses so is not valuable in determining efficacy.

[0201] First the mouse lungs are homogenized in 5 μ L of cold phosphate buffered saline per milligram of tissue. 100 μ L of lysate is saved for RNA isolation, and the remaining lysate is used for hemagglutination. All samples are stored at -80 °C.

35 [0202] For the hemagglutination assay, MDCK-London (Madin-Darby canine kidney) cells are

plated in a 96-well plate and incubated till confluent. On the day of the assay, lung lysate is serially diluted 3-fold in DMEM/F12 (1:1) supplemented with 0.3% bovine serum albumin. MDCK-London cells are washed and 50 uL of diluted lysate is added to each well, so that each dilution has 4 replicates. Cells are incubated for 1 h at 37 °C, 5% CO₂, and then 200 uL of DMEM/F12 (1:1) supplemented with 0.3% bovine serum albumin and 1 µg/ml of trypsin are added to each well. Cells are incubated 48 h at 37 °C and 5% CO₂. Each well is tested for hemagglutination by incubating 50 uL of tissue culture supernatant with 50 uL of 0.5% turkey red blood cells in phosphate buffered saline in a v-bottom plate. After 30 min at room temperature, the plate is tilted 45-degrees and wells are counted as negative or positive. Negative wells have a red dot, which runs when the plate is tilted, resembling the negative control. Positive wells will appear hazy and will not have a red dot at the bottom of the well. TCID₅₀ is calculated for each lung sample using the Reed-Muench method, and values are plotted using Graph pad prism.

[0203] FIG. 7 shows that much of the live, infectious virus is cleared after treatment with compound 24. This is supported by the high survival rates and quick weight recovery of mice treated with compound 24 as seen in Method Example 5 below and the corresponding figures.

[0204] METHOD EXAMPLE 3: Neuraminidase inhibition assay: To evaluate the neuraminidase inhibition activity of compound 24, a standard neuraminidase inhibition assay was performed using the NA-Fluor™ Influenza Neuraminidase Assay Kit (Catalog no. 4457091, Invitrogen™). The assay was performed according to the manufacturer's protocol. In brief, a standard curve was generated first using the 4-methylumbelliferone sodium salt (4-MU(SS)) to determine the linear range of substrate turnover detection on the Synergy Neo2 HTS Multi-Mode Microplate Reader (Biotek). A Relative Fluorescence Unit (RFU) value was identified within the linear range of fluorescence detection on the instrument. For each viral strain, the dilution factor to be used in the assay was decided that best corresponds to the chosen Relative Fluorescence Unit (RFU) value from the 4-methylumbelliferone sodium salt (4-MU(SS)) standard curve. Then the virus stock solutions were titrated by a neuraminidase activity assay. For this assay, serial dilutions of the virus stock solutions were prepared in a black, 96-well, flat bottom plate. Then 50 µL of 200uM of the NA-Fluor™ substrate (MUNANA, 4-(methylumbelliferyl)-N-acetylneuraminic acid) was added to the designated wells. The plate was incubated at 37°C for 60 min, protected from light. The plate was placed on a shaker inside the incubator. Then the reaction was terminated by adding 100 µL of NA-fluor™ stop solution (0.2 M sodium carbonate) to each well. Then the fluorescence intensities were measured using an excitation wavelength of 350 nm and emission wavelength of 440 nm. The Relative Fluorescence Unit (RFU) values were plotted against the respective virus dilutions and for each

virus strain, the dilution factor that yields the RFU chosen from the 4-methylumbelliferone sodium salt (4-MU(SS)) standard curve was selected to use for neuraminidase activity normalization in the neuraminidase inhibition assay. Finally, for the neuraminidase inhibition assay, serial tenfold dilutions of the test compounds were prepared in NA-Fluor™ assay buffer (66.6 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer, 8 mM CaCl₂, pH 6.5). Then 25 μL of the 4X compound 24 dilution series was added to all rows of a 96-well plate followed by addition of 25 μL of 1X assay buffer to the no virus control wells. 25 μL of diluted virus samples were added to the designated wells and mixed with the compound 24 dilutions. The plate was incubated for 30 min at 37°C with shaking. Then 50 μL of the diluted 200 uM NA-Fluor™ substrate was added to each well and incubated for 60 min at 37°C protected from light. The reaction was terminated by adding 100 μL of NA-Fluor™ stop solution to all wells. The plate was read for measuring fluorescence intensities using an excitation wavelength of 350 nm and emission wavelength of 440 nm. The data was plotted using GraphPad Prism 10.0.0 software to generate the sigmoid dose-response curve. Then the graph was analyzed using a built-in nonlinear regression curve-fitting program to determine the half-maximal inhibitory concentration (IC₅₀). Lower IC₅₀ values indicate higher potency of the compound.

[0205] FIG. 9 demonstrates the sigmoidal dose-response curves for each viral strain and the table therein denotes their respective half-maximal inhibitory concentration (IC₅₀) values. These data illustrate that the zanamivir targeting ligand moiety in compound 24 is not only able to engage the target enzyme but also inhibit its function and act as a direct acting antiviral across many strains. Neuraminidase inhibition suppresses the budding of virus particles from infected cells and limits the spread of the infection to neighboring healthy cells.

METHOD EXAMPLE 4. A group of 6-8 weeks old female BALB/c mice (n = 5/group) were infected with 10 LD₅₀ of Influenza virus A/H1N1/PR8/1934 on day 0 of the experiment. Mice were given an intraperitoneal injection of 6 g/kg human IVIg 24 h before drug administration to achieve humanized titer of anti-DNP and anti-Rhamnose antibodies at the time of test article administration. Mice were treated with test articles at 48 hpi (FIG. 2A) or, 96 hpi (FIG. 2B). Compound 24 was administered as a single intranasal dose of 1.5 μmol/kg, or single intravenous dose of 1.5 μmol/kg, or single oral dose of 13.5 μmol/kg in the designated cohorts. Mice in the three control groups received Tamiflu (oseltamivir phosphate), or Xofluza (baloxavir marboxil) or, PBS as placebo.

[0206] For evaluation of drug efficacy, mice were weighed and monitored daily for 14 days post-infection and counted as dead when they lost 25% of their body weight or were diagnosed as moribund.

Results of this experiment are depicted body weight plots, which graph days after infection vs.

survival (%) and days after infection vs. body weight (%) for mice (n = 5/group).

[0207] At the early-stage treatment (48hpi) mice treated with 1.5 μ mol/kg single intranasal dose of Compound 24 resulted in 100% survival, 1.5 μ mol/kg single intravenous dose of Compound 24 resulted in 100% survival and the cohort treated with single oral dose resulted in 80%

5 survival. Mice treated with Tamiflu with 5 mg/kg dosage, dosed twice daily for five days resulted in 60% survival and mice treated with Xofluza with 10 mg/kg single oral dose resulted in 80% survival. On the other hand, At the late-stage treatment (96 hpi) mice treated with 1.5 μ mol/kg single intranasal dose of Compound 24 resulted in 100% survival, 1.5 μ mol/kg single intravenous dose of Compound 24 resulted in 80% survival and the cohort treated with single
10 oral dose resulted in 60% survival. Mice treated with Tamiflu with 5 mg/kg dosage, dosed twice daily for five days resulted in 20% survival and mice treated with Xofluza with 10 mg/kg single oral dose resulted in 60% survival.

[0208] It indicates the superior efficacy of Compound 24 over Tamiflu and Xofluza at both early and late stages of infection and demonstrates that all the three routes of administration are

15 effective with intranasal being the best route of administration at 1.5 μ mol/kg dose.

[0209] METHOD EXAMPLE 5: Dose range finding study – Efficacy/Toxicity. In the study of Method Example 2, survival, and cytokine response were also measured. With respect to weight gain, mice were weighed and monitored daily for 14 days post-infection and counted as dead when they lost 25% of their body weight or were diagnosed as moribund.

20 [0210] Results of this experiment are depicted in FIG. 11. FIG. 11 is a graph of days after infection vs. survival (%) and days after infection vs. body weight (%) for mice (n = 5/group) infected with 10 LD50 of influenza A H3N2/Wisconsin/15/2009, intraperitoneal administration of human IgG (IVIg (GAMUNEX®-C) at 24 hpi, and administration of conjugate at 48 hpi.

[0211] Mice treated with 2.2 mg/kg, 0.72 mg/kg and 0.24 mg/kg single intranasal dose of
25 compound 24 resulted in 100% survival and the cohort treated with 0.081 mg/kg single intranasal dose resulted in 60% survival. Mice treated with Tamiflu with 5 mg/kg dosage, dosed twice daily for five days also resulted in 60% survival. It indicates the superior efficacy of compound 24 over Tamiflu and demonstrates that 0.24 mg/kg dosage can be considered as the minimal effective dose of compound 24 to achieve a 100% survival in mice. Mice in all cohorts
30 had a gradual loss in body weight but the ones that survived could recover the weight loss induced by viral infection. This data shows that compound 24 is 100% effective and even at 9-fold lower dose, a single dose of compound 24 is comparable to Tamiflu dosed 10 times in a period of five days.

[0212] The cytokines from each of the treatment and control groups were measured in lung

tissue samples (n=3/group) via BioLegend's LEGENDplex™ bead-based immunoassay using standard protocol provided by the manufacturer (BioLegend, San Diego, CA).

[0213] FIGS. 12 (lungs) and 13 (serum) show graphs of specific cytokine expression levels in the lungs and serum respectively, measured for each group at 24 h after drug administration.

5 Compound 24, administered intranasally, did not induce a cytokine storm in the lungs or serum. A dose-dependent decrease in IFN- γ was observed in the lungs. The level of TNF- α and 12 other inflammatory cytokines not reported here but included in BioLegend's LEGENDplex kit was unaltered in both lungs and serum. With respect to IL-6, the data shown were not statistically significant. By comparison, the drop in IFN- γ in lungs is indicative of a reduction
10 in systemic inflammation due to faster clearance of the infection upon treatment with compound 24. In serum, the IFN- γ showed an increase, but with large error bars which are not statistically significant. These data indicate that compound 24 does not induce a cytokine storm.

[0214] METHOD EXAMPLE 6. A cytokine release assay was performed on Compound 24 (results in FIG. 8) to assess the risk of causing a cytokine storm in humans. For this assay,
15 human PBMCs pooled from at least 4 donors were washed and plated at 5×10^5 cells/well. 1000 nM of Compound 24 was added to the PBMCs with and without 10 mg/mL IVIG to reach a final volume of 200 μ L. 1000 nM of a TLR7 agonist was used as a positive control, and media was used as a negative control. The PBMCs were incubated with compound at 37 C, 5% CO₂, and samples of supernatant were collected at 2, 6, and 24 h. Supernatant was centrifuged at
20 450xg for 10 min to remove cells, and stored at -80 C.

[0215] Cytokines in the supernatant were measured using a Human Anti-Virus Response Panel (biolegend). This kit measures 13 different proteins: IL-1 β , IL-6, IL-8, IL-10, IL-12p70, IFN- α 2, IFN- β , IFN- λ 1, IFN- λ 2/3, IFN- γ , TNF- α , IP-10, GM-CSF, using fluorescently coded beads and fluorescently labeled antibodies. Fluorescence was measured using an Attune NxT flow
25 cytometer (Fisher), and the concentration of each protein is quantified using LEGENDplex™ software (biolegend). Finally, data was graphed with GraphPad prism.

[0216] In all the cytokines tested, Compound 24 had similar levels to the negative control, indicating no inherent immunogenicity from the molecule by itself. Because Compound 24 could potentially bind 2 antibodies, IVIG was included to determine the risk of forming an
30 aggregate, which would trigger an immune response. IVIG was at a physiologically relevant concentration of 10 mg/mL, which caused a higher background in some of the proteins tested. Considering this, Compound 24 again had similar levels to the negative control, indicating no immunogenicity from the molecule when bound to antibodies. This suggests that Compound 24 would not recruit immune cells in circulation and is unlikely to induce cytokine release if not
35 bound to neuraminidase expressing cells or virus

[0217] METHOD EXAMPLE 7. To evaluate the neuraminidase inhibition activity of Compound 41, a standard neuraminidase inhibition assay was performed using the NA-Fluor™ Influenza Neuraminidase Assay Kit (Catalog no. 4457091, Invitrogen™). The assay was performed according to the manufacturer's protocol. In brief, a standard curve was generated first using the 4-methylumbelliferone sodium salt (4-MU(SS)) to determine the linear range of substrate turnover detection on the Synergy Neo2 HTS Multi-Mode Microplate Reader (Biotek). A Relative Fluorescence Unit (RFU) value was identified within the linear range of fluorescence detection on the instrument. For each viral strain, the dilution factor to be used in the assay was decided that best corresponds to the chosen Relative Fluorescence Unit (RFU) value from the 4-methylumbelliferone sodium salt (4-MU(SS)) standard curve. Then the virus stock solutions were titrated by a neuraminidase activity assay. For this assay, serial dilutions of the virus stock solutions were prepared in a black, 96-well, flat bottom plate. Then 50uL of 200uM of the NA-Fluor™ substrate (MUNANA, 4-(methylumbelliferyl)-N-acetylneuraminic acid) was added to the designated wells. The plate was incubated at 37°C for 60 min, protected from light. The plate was placed on a shaker inside the incubator. Then the reaction was terminated by adding 100uL of NA-fluor™ stop solution (0.2 M sodium carbonate) to each well. Then the fluorescence intensities were measured using an excitation wavelength of 350 nm and emission wavelength of 440 nm. The Relative Fluorescence Unit (RFU) values were plotted against the respective virus dilutions and for each virus strain, the dilution factor that yields the RFU chosen from the 4-methylumbelliferone sodium salt (4-MU(SS)) standard curve was selected to use for neuraminidase activity normalization in the neuraminidase inhibition assay. Finally, for the neuraminidase inhibition assay, serial tenfold dilutions of the test compounds were prepared in NA-Fluor™ assay buffer (66.6 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer, 8 mM CaCl₂, pH 6.5). Then 25uL of the 4X Compound 41 dilution series was added to all rows of a 96-well plate followed by addition of 25uL of 1X assay buffer to the no virus control wells. 25uL of diluted virus samples were added to the designated wells and mixed with the Compound 41 dilutions. The plate was incubated for 30 min at 37°C with shaking. Then 50uL of the diluted 200uM NA-Fluor™ substrate was added to each well and incubated for 60 min at 37°C protected from light. The reaction was terminated by adding 100uL of NA-Fluor™ stop solution to all wells. The plate was read for measuring fluorescence intensities using an excitation wavelength of 350 nm and emission wavelength of 440nm. The data was plotted using GraphPad Prism 10.0.0 software to generate the sigmoid dose-response curve. Then the graph was analyzed using a built-in nonlinear regression curve-fitting program to determine the half-maximal inhibitory concentration (IC₅₀). Lower IC₅₀ values indicate higher potency of the compound.

[0218] FIG. 10 demonstrates the sigmoidal dose-response curves for each viral strain and the table therein denotes their respective half-maximal inhibitory concentration (IC₅₀) values related to Compound 41. This data illustrates that the zanamivir targeting ligand moiety in Compound 41 is not only able to engage the target enzyme but also inhibit its function and act as a direct acting antiviral across many strains. Neuraminidase inhibition suppresses the budding of virus particles from infected cells and limits the spread of the infection to neighboring healthy cells.

[0219] METHOD EXAMPLE 8. Efficacy of Compound 24 IN against Multiple Influenza Strains (FIG. 14A and FIG. 14B). Efficacy of an intranasal formulation of Compound 24 (0.6% n-dodecyl β-D-maltoside, “DDM” and 2% Avicel 591 in PBS), referred to as “Compound 24 IN”) was evaluated in 3 seasonal Influenza strains: A/H3N2/Wisconsin/15/2009,

A/California/07/2009(H1N1) pdm09, a pandemic strain of H1N1 that represents a large portion of the seasonal flu burden every year, and B/Brisbane/60/2008). In FIG. 14A and FIG. 14B, Compound 24 IN was compared with oseltamivir phosphate (oral) and vehicle control (0.6% DDM and 2% Avicel 591 in PBS and intranasal). In each study, mice were infected with 10x LD50 of 1 of 3 seasonal Influenza strains and given either 2.2 mg/kg Compound 24 IN, oseltamivir phosphate orally, or a vehicle control at 48 h post-infection intranasally. Sub-cohorts were sacrificed 24 h post-infection to detect viral titers in the lungs. The rest of the group was observed for the following 14 days as part of a survival study with body weight recorded daily.

[0220] As shown in FIG. 14A, Compound 24 IN against A/H3N2/Wisconsin/15/2009 showed 100% survival in contrast to the group that received oseltamivir phosphate (60% survival) and the vehicle control (0% survival). A Mantel-Cox Log-rank test shows the compound 24 survival curve to be significant with a p-value of 0.0013 for Influenza A/Wisconsin/15/2009 (H3N2). Compound 24 IN against A/California/07/2009(H1N1) pdm09 showed 100% survival in contrast to 40% of the oseltamivir phosphate group and 0% in the vehicle control group. A Mantel-Cox log-rank test shows the compound 24 survival curve to be significant with a p-value of 0.0006 for A/California/07/2009 (H1N1pdm09) strain Compound 24 against influenza B showed 100% survival in contrast to 0% in the vehicle control group. In the oseltamivir phosphate-treated group, 40% were euthanized before the study ended as they lost more than 25% of their initial weight during the two-week study period. A Mantel-Cox log-rank test shows the compound 24 survival curve to be significant with a p-value of 0.0065 for the B/Brisbane/60/2008 Strain.

[0221] In addition, Compound 24 IN appeared to provide a better quality of life for infected mice as no large changes in body weight were detected. Weight loss was observed in all other treatment groups. FIG. 14A shows the corresponding viral lung titers.

[0222] METHOD EXAMPLE 9. Efficacy against Influenza A/Hong Kong/2369/2009

(H1N1pdm09, Tamiflu Resistant) (**FIG. 15A** and **FIG. 15B**). The efficacy of Compound 24 IN was tested in a mouse model against a representative Influenza A strain – A/Hong Kong/2369/2009 (H1N1pdm09, Oseltamivir [Tamiflu] Resistant), a pandemic strain of H1N1 that represents a large portion of the seasonal flu burden every year that is also resistant to Tamiflu. All mice were first infected with 10x LD₅₀ of the A/Hong Kong/2369/2009 (H1N1pdm09, Oseltamivir [Tamiflu] Resistant) strain of influenza virus. At infection IVIG (6 g/kg) was administered to the Compound 24 IN and Vehicle groups via intraperitoneal injection (IP) to simulate human levels of anti-DNP and anti-rhamnose antibody titers.

[0223] Treatment began at 24 hpi (1 dpi). Mice receiving either 2.2 mg/kg of Compound 24 IN or a vehicle control were first anesthetized with isoflurane. Each mouse then received 0.25 mL/kg of vehicle intranasally or 0.25 mL/kg of Compound 24 IN to each nostril, then released back into their cage. Mice receiving Tamiflu (oseltamivir phosphate) were administered 5 mg/kg via oral gavage, then released back to their cage.

[0224] At 24 h after treatment, 3 mice from each group were selected, euthanized, and the lungs were collected and snap frozen in liquid nitrogen. Lungs were homogenized and a TCID₅₀ assay was performed in triplicate on MDCK cells looking for cytopathic effects and the virus titer were calculated using the Reed-Muench method.

[0225] The remaining mice were observed and weighed daily during the two-week study period, at which point they were sacrificed. Mice that lost 25% of their initial weight at any timepoint during the study were euthanized according to IACUC protocol.

[0226] As shown in **FIG. 15A**, Compound 24 IN was shown to provide protection against Influenza A strain – A/Hong Kong/2369/2009 (H1N1pdm09, Oseltamivir Resistant) by the complete elimination of detectable virus titer and the survival of all mice out to study completion (Day 14). The oseltamivir and vehicle-treated mice were found to contain comparable high viral titers in collected lung samples 24 h post-drug administration (~2x10⁶ TCID₅₀) as shown in **FIG. 15C**, and all mice in these groups (but not in the Compound 24 IN group) were sacrificed by Day 7 due to a body weight loss of >25%, as shown in **FIG. 15B**. A Mantel-Cox log-rank shows the Compound 24 survival curve to be significant with a p-value of 0.0009 for the Influenza A strain – A/Hong Kong/2369/2009 (H1N1pdm09, Oseltamivir Resistant).

[0227] **METHOD EXAMPLE 10. Compound 24 Prevention of Transmission of H1N1 (FIG. 16).** Compound 24 IN was evaluated for its ability to prevent transmission of influenza. It was found that a single dose of Compound 24 IN outperformed vehicle control. For this study, guinea pigs were infected with human H1N1 influenza and received 4g/kg IVIG intraperitoneally. Then the guinea pigs were treated 24 h post infection with vehicle, or 4 mg/kg

intranasally of Compound 24 IN. Twenty-four h after treatment, infected guinea pigs were cohoused with naïve guinea pigs for 14 days. Nasal swabs were taken every other day, and viral RNA was measured using PCR. At the end of the study, anti-neuraminidase and anti-hemagglutinin antibody titers were measured to assess influenza exposure.

5 [0228] The results are shown in FIG. 16. In the group treated with vehicle, all naïve co-housed animals became infected by day 4, and infection was confirmed by increased antibody titers. In the Compound 24 IN treatment group only 1 of the 10 naïve co-housed guinea pigs became infected, and not until day 8, which was confirmed by seropositivity of anti-hemagglutinin and anti-neuraminidase. The data suggest that not only did Compound 24 IN protect 90% of the
10 guinea pigs in this prolonged exposure transmission model, the transmission was also delayed in the one instance of transmission relative to vehicle transmission.

[0229] Mitigating viral shedding through reduced transmission is believed equally important to symptom management in public health interventions. While symptomatic relief can improve patient well-being, it may inadvertently lead to a resumption of normal activities during the
15 infectious phase. These data suggest that a single dose treatment with compound 24 reduces secondary infection rates of human H1N1 by 90%.

[0230] METHOD EXAMPLE 11. Compound 24 Mutation Emergence (FIG. 17A and FIG. 17B). Compound 24 IN was evaluated for its ability to produce drug resistant mutants of influenza A virus (IAV) after serial passaging the virus in the presence of compound 24.
20 Traditionally, drug resistance emergence studies are performed in vitro to streamline experimental design and quickly identify mutations. However, due to the immunological dependent nature of compound 24, the mutation emergence study described here was performed in vivo to more rigorously test whether compound 24 produces drug resistant IAV in conjunction with a functioning immune system. Furthermore, due to the potency of compound
25 24 in eliminating nearly all traces of IAV, a sub-optimal dose was necessarily used to maintain measurable viral load and encourage mutation during passaging. It was found that an 81 µg/kg dose of compound 24 could reduce, but not eliminate, IAV infection in mice and was therefore chosen as the sub-optimal dose.

[0231] Three groups of five mice each were used for each passage cycle. All groups received 10
30 LD₅₀ influenza virus A/PR8/34 (H1N1). Group 1 was administered a single dose of 81 µg/kg of Compound 24 intranasally 48 h post-infection. Group 2 was administered vehicle (20mg/ml Avicel, 6mg/ml DDM, 1x PBS, pH 7.4) once intranasally, 48h post infection. Group 3 was administered 5 mg/kg of oseltamivir phosphate twice daily orally for 2 days starting 48h post infection. Twenty-four h post infection IVIG (6 g/kg) was administered to the Compound 24 IN
35 and vehicle groups via intraperitoneal injection (IP) to simulate human levels of anti-DNP and

anti-rhamnose antibody titers. Body weight and survival monitoring was recorded each day, and each group was sacrificed 4 days after drug administration for viral titer determination by TCID₅₀ and RNA extraction from lung tissue. After titer determination to maintain consistent 10 LD₅₀ infections between passages, the process was repeated for each consecutive cycle.

- 5 [0232] It was found that Compound 24 IN does not produce viable drug resistant mutants after 5 passages and was shown to maintain efficacy with a modest ~1.5-fold decrease in IC₅₀ from ~12 nM to 20 nM as seen in neuraminidase inhibition assays (NIA), as shown in **FIG. 17B**. In contrast, oseltamivir phosphate treatment during IAV passaging resulted in a >1,500-fold decrease in NAI efficacy, from ~2 nM (Hong, B. T., et al. Oseltamivir hydroxamate and acyl
- 10 sulfonamide derivatives as influenza neuraminidase inhibitors. *Bioorganic & medicinal chemistry* 2014, 22(23), 6647–6654) to ~3,000 nM. These results suggest that compound 24 does not encourage the emergence of drug resistant mutations in vivo, even at sub-optimal doses like oseltamivir does. Moreover, compound 24 was shown to maintain NIA efficacy during the duration of the study, while the efficacy of the industry standard oseltamivir was significantly
- 15 impaired. Additionally when an LD50 study was run on the mutants after 5 cycles it was found the LD50 of the Tamiflu treated mice showed a 4000 fold increase in virulence compared to a 4-fold increase in compound 24 treated, mice, as shown in **FIG. 17A**. Altogether, this study suggests that extended use of compound 24 would not encourage the emergence of drug resistant IAV mutants.
- 20 [0233] METHOD EXAMPLE 12. Compound 24 IN Avian Influenza efficacy (**FIG. 18A** and **FIG. 18B**). Three groups of mice (n=4/cohort) were infected with a labeled version of the avian flu currently spreading in Texas - rH5N1 Nluc A/Texas/37/2024 with 10E2 PFU/mouse. A fourth group was mock infected with viral infection media containing no virus as a negative control. Mice were treated with 2mg/kg of Compound 24 IN intranasally, or 5 mg/kg BID for 5
- 25 days of Tamiflu orally or compound 24 vehicle intranasally. Mice were serially imaged with an IVIS imager to track the luminescence of the virus to quantify the viral load. At Day 6, Compound 24 IN treated mice had almost no virus left in their body after a single dose while Tamiflu had much higher amounts. The mice were sacrificed and the viral load was quantified in each via TCID₅₀ assay. The viral load in the lungs of the Tamiflu treated mice was found to be
- 30 significantly higher than in the Compound 24 IN treated mice, suggesting that even at very low doses, compound 24 is effective at clearing avian influenza infections, including high pathogenic avian influenza infections. **FIG. 18A** shows the images of Groups 1 and 2 and **FIG. 18B** of Groups 3 and 4.

- [0234] METHOD EXAMPLE 13. Pharmacological Mechanism of Action of Compound 24. The mechanism of action of Compound 24 is evaluated by the ability to recruit sufficient naturally
- 35

occurring anti-hapten antibodies to mediate antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement dependent cytotoxicity (CDC) induced killing of virus-infected cells. Human embryonic kidney cells (HEK293) are modified to express influenza neuraminidase (HEK293-NA), mimicking influenza-infected cells. Compound 24 is evaluated for the ability to activate specific immune system components (FcγRIIIa, FcγRIIa) in effector cells and the complement system. If activated, these components are expected to trigger ADCC, ADCP, and CDC, ultimately killing the HEK293-NA cells. A control group using unmodified HEK293 cells (HEK293-WT) is used for comparison. The HEK293-N1-neuraminidase cell cultures are also supplemented with or without human anti-DNP antibody and incubated with serial dilutions of Compound 24.

[0235] As shown in the figures of **FIG. 19**, Compound 24 is effective in mediating ADCC (**FIG. 19A**), and ADCP (**FIG. 19B**) of the neuraminidase-transduced HEK293 cells, displaying the anticipated bell-shaped dependence on Compound 24 concentration as detected through changes in luminescence because of activation of the human FcγRIIIa expressing effector cells using a Promega ADCC kit. Cell death can be blocked by either deletion of IVIG or the absence of neuraminidase on the target cell surface, suggesting that ADCC-mediated and ADCP-mediated killing rely on two key elements: 1) the presence of anti-hapten antibodies and 2) cell surface expression of influenza neuraminidase. The dependence upon cell surface expression of influenza neuraminidase is an important safety attribute, suggesting that uninfected cells should not be damaged by Compound 24.

[0236] As shown in **FIG. 19C**, Compound 24 is also found to promote CDC-mediated killing of neuraminidase expressing target cells. Cell death is blocked by introducing a 100-fold excess of free zanamivir, suggesting that Compound 24-mediated activation of CDC-mediated killing should also be limited to cells/virions that express viral neuraminidase.

[0237] The data in the figures of **FIG. 19** validate the immune activity of Compound 24 and demonstrate that Compound 24 can recruit naturally occurring anti-hapten antibodies to activate ADCC-mediated (Panel A), ADCP-mediated (Panel B), and CDC-mediated (Panel C) killing of influenza-infected cells. Compound 24-mediated killing is dependent on the presence of both anti-hapten antibodies and viral neuraminidase on the target cell surface, which is a desirable safety feature.

[0238] METHOD EXAMPLE 14. Neuraminidase Inhibition Activity of Compound 24 IN Against Seasonal and Drug-resistant Influenza A and B Strains (FIG. 20).

[0239] The neuraminidase inhibition activity of Compound 24 IN is evaluated against 4 seasonal influenza strains (A/PR/8/1934 (H1N1), A/California/07/2009 (H1N1pdm09),

5 A/Wisconsin/15/2009 (H3N2), and B/Brisbane/60/2008 (Victoria Lineage)) and 2 drug-resistant influenza virus strains (A/Hong Kong/2369/2009 (H1N1pdm09, oseltamivir-resistant), and A/Illinois/37/2018 (H1N1pdm09, baloxavir-resistant). Neuraminidase inhibition activity is measured based on the IC₅₀ in relative fluorescence unit (RFU) of the enzymatic product resulting from neuraminidase activity of the virus particles.

10 [0240] The IC₅₀ against all six strains are shown in FIG. 20. Compound 24 is found to inhibit neuraminidase activity of the three seasonal influenza A strains with IC₅₀ ranging from 12.7-16.4 nM. Compound 24 also inhibits neuraminidase activity of the seasonal influenza B strain (Victoria Lineage) with an IC₅₀ of 34.5 nM. Compound 24 is found to effectively inhibit the neuraminidase activity of oseltamivir-resistant and baloxavir-resistant flu strains with an IC₅₀ of
15 17.9 and 27.2 nM, respectively).

[0241] METHOD EXAMPLE 15. Neuraminidase Inhibition Activity Against Influenza A/PR/8/1934 (H1N1). A 10-fold serial dilution of test compounds were prepared in 1X NA-Fluor™ Assay Buffer. The 4x dilution series and diluted virus samples were added into the corresponding wells in a black, 96-well, clear flat bottom plate. The plate was incubated on a
20 plate shaker for 30 min at 37°C. 200 µM NA-Fluor™ Substrate working solution was added and incubated on a plate shaker for an additional 1 hr at 37°C to detect the viral neuraminidase activity. The reaction was terminated by a stop solution (60% NA-Fluor™ Stop Solution/40% ethanol). The fluorescence signal was detected with an excitation wavelength of 350 nm and an emission wavelength of 440 nm by BioTek Synergy Neo2 HTS Multi-Mode Microplate Reader.
25 Data were analyzed using GraphPad Prism 10 to determine the half-maximal inhibitory concentration (IC₅₀) of the test compound. Activity in this assay demonstrates that the tested conjugates retained the direct antiviral activity of the ligand after being conjugated to the linker and two haptens. The IC₅₀ values for the tested compounds are shown in the following table.

Test Compound	IC ₅₀ (nM)
24	12.7
41	38.6
42	24.2
43	36.6

44	69.5
45	17.4
46	12.9
47	Not active

[0242] METHOD EXAMPLE 16. Viral Cytopathic Effect Inhibition Activity of Compound 24.

In the presence of viral infections, host cells undergo changes at the cellular level in response to infection. This phenomenon is known as the cytopathic effect (CPE). The effectiveness of Compound 24 in preventing CPE caused by an influenza virus infection and its associated cytotoxicity was determined through a viral CPE-inhibition assay. Virus stock titers were measured by performing a TCID₅₀ to determine the viral inoculation required for the CPE inhibition assay.

[0243] Cell viability from the viral CPE inhibition assay was quantified with neutral red staining to calculate the EC₅₀ of Compound 24 against 4 wild-type (A/PR/8/1934 (H1N1),

A/Wisconsin/629-D00015/2009 (H1N1pdm09), A/Hong Kong/H090-756-V1(0)/2009 (H3N2), and B/Brisbane/60/2008 (Victoria Lineage)) and 1 drug-resistant influenza strains (A/Hong Kong/2369/2009 (H1N1pdm09, oseltamivir-resistant).

[0244] The summary of Viral CPE Inhibition of Compound 24 Against Wild-Type and Drug Resistant Strains is shown in the following table.

Influenza Strains	EC ₅₀ (μM)
A/PR/8/1934 (H1N1)	0.9
A/Wisconsin/696-D00015/2009 (H1N1pdm09)	7.3
A/Hong Kong/H090-756- V1(0)/2009 (H3N2)	3.1
B/Brisbane/60/2008 (Victoria Lineage)	2.1
A/Hong Kong/2369/2009 (H1N1pdm09, Oseltamivir- Resistant)	0.2

[0245] Compound 24 effectively prevented CPE induced by H1N1 and the oseltamivir-resistant

strain ($EC_{50} < 0.9 \mu\text{M}$). Compound 24 also inhibited CPE of H1N1pdm09, H3N2, and influenza B with $EC_{50} < 8 \mu\text{M}$. It is important to note that these experiments only consider the activity of the zanamivir moiety, and Compound 24 is expected to have greater than 1,000-fold activity when the immune system can be recruited *in vivo*.

- 5 [0246] METHOD EXAMPLE 17. Efficacy against Avian Influenza. The efficacy of Compound 24 against the A/Duck/MN/1525/81 (H5N1), A/HK/61/2016 (H7N9), and A/Vietnam/1203/2004 (H5N1) strains of avian influenza was evaluated *in vitro*. Cells were cultured and then exposed to varying dilutions of Compound 24 and an Avian strain. Cytopathic effects were observed microscopically, measured with a red dye and EC_{50} (concentration of 50% CPE inhibition) values were calculated, as shown in the following table.
- 10

Influenza Strains	EC_{50} (μM)
A/Duck/MN/1525/81 (H5N1)	1.3
A/HK/61/2016 (H7N9)	6.2
A/Vietnam/1203/2004 (H5N1)	2.5

- It was found that Compound 24 inhibits H5N1 and H7N9-induced cytopathic effect and production of nascent virions *in vitro* with single digit micromolar potency. Additionally, the results confirm the direct-acting antiviral property of Compound 24 against the avian influenza strains. The direct antiviral action of compound 24 was also validated *in vivo*, where very low doses of compound 24 were 100% protective against the Texas 2024 isolated H5N1.
- 15

[0247] METHOD EXAMPLE 18. Efficacy against A/Illinois/37/2018 (H1N1, Baloxavir Resistant). Baloxavir treatment has very high mutation emergence rate and presents a clinical issue for baloxavir use. Mice were infected with 10LD₅₀ of mouse adapted A/Illinois/37/2018 (H1N1pdm09, baloxavir resistant) in a pilot efficacy study against baloxavir mutants. As shown in FIG. 21, mice that were treated with Compound 24 had 100% survival. The vehicle control and baloxavir treatment groups had similar survival rates suggesting no efficacy from baloxavir. Compound 24 may be useful in treatments against baloxovir resistant strains of influenza.

[0248] METHOD EXAMPLE 19. Compliment Dependent Cytotoxicity (CDC) Assay. Neuraminidase expressing HEK293 cells (HEK293-NA) were plated at 10,000 cells per well in a 96-well poly-D lysine coated, white-walled plate. The cells were allowed to incubate overnight at 37°C, aiming for 50% confluency on the day of the assay. Serial dilutions of compound were prepared in DMEM, adjusted to be 4x higher than the target concentration. The cells were washed with 200 µL of PBS, followed by the addition of 25 µL of DMEM per well. 25 µL of the serially diluted compounds were added to each well, and the plates were then incubated at 37°C for 30 minutes. Rabbit anti-DNP (Invitrogen, cat: A6430) or rabbit anti alpha-gal(Enzo m86) was diluted to 40 µg/mL and rabbit complement (Sigma Aldrich, cat: S7764) was diluted to 20%. 50 µL of this mixture, was added to the cells for a final concentration of 20 µg/mL rabbit anti-DNP and 10% rabbit complement. The cells were incubated at 37°C for 4 hours. After the incubation period, the cells were washed twice with 200 µL PBS. Subsequently, 200 µL of Cell Titer-Glo reagent (Promega, cat: G7570) in PBS was added to each well. Luminescence was measured using a plate reader equipped with glow-type luminescence read capabilities. The percent cell death was calculated and plotted, giving a bell-shaped curve. In the lower concentration range, the compound stimulates the complement proteins to kill cells. In the higher concentration range, the compound blocks complement activity by saturating the receptors on to the target protein and the antibodies. Activity in this assay demonstrates the effective ability of the full conjugate to bind to the target viral protein and recruit the immune system with the other domain of the molecule. EC50 values for both the low concentration range and the high concentration ranges for the tested compounds in the following table.

Compound	EC ₅₀ 1 (nM)	EC ₅₀ 2 (µM)
24	13.6	138
42	48.66	0.4804
43	8.095	0.2027
44	0.56	5

45	10.77	0.6773
46	5.940	1.230
47	Not active	Not active

[0249] METHOD EXAMPLE 20. Summary of Nonclinical Pharmacokinetics and Metabolism. The pharmacokinetics (PK) of Compound 24 were characterized in BALB/c mice across four routes of administration: intravenous (IV), subcutaneous (SC), oral, and IN. While IV and SC have the highest level of drug absorbed, IN was chosen as a preferred route of administration (ROA) for ease of administration in human patients. PK studies were also conducted in mice to determine the minimum pharmacologically active dose (PAD).

[0250] After IN administration, Compound 24 demonstrates rapid absorption across species with a time to maximum plasma concentration (T_{max}) ranging from approximately 0.42 to 1.10 hours in preclinical PK studies. The bioavailability (F) of Compound 24 is dose- and formulation-dependent and was found to be highest at clinically relevant dose levels in rodents. At similar doses and across formulations, mean F was moderate to high in mouse (21% - ~100%), low to moderate in rat (~20% - ~50%), and low (4~20%) in dogs. In each instance, the higher F was a concentration that was $\geq 10\%$ the maximum feasible dose (MFD) and the lower range F is the MFD. The mean elimination half-life ($t_{1/2}$) ranged from 0.603 to 6.81 hours (0.603 to 4.2 hours excluding a single outlier) with escalating IN doses in rats. These data suggested that absorption had become rate-limiting in the *in vivo* elimination of Compound 24 (i.e., flip-flop kinetics). In dogs, the mean $t_{1/2}$ estimate was ~1 hr after IV and IN administration at doses up to ~20 mg/kg. The Day 14/1 ratios for C_{max} and AUC_{0-24} were close to 1 in rats and dogs, indicating there is no accumulation following once daily administration. Compound 24 was found to have low cellular permeability and is not a substrate of human P-glycoprotein (P-gp).

[0251] Compound 24 showed low to moderate plasma protein binding. The percentage of unbound Compound 24 ranged from 74.0% to 89.4% across mice, rats, dogs, monkeys, and humans with human plasma showing the highest binding. Partitioning work indicated that Compound 24 had blood to plasma ratios less than or equal to 0.72, suggesting limited partitioning into red blood cells. Compound 24 was metabolically stable across various species *in vitro*. Profiling pooled rat plasma detected an apparent minor circulating metabolite (0.71%) resulting from amide hydrolysis (M1160), with intact Compound 24 appearing to be the predominant circulating entity.

[0252] Compound 24 did not directly (reversibly) inhibit the major human cytochrome P450s (CYPs) 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. In addition, coincubation with Compound

24 did not result in time dependent inhibition (TDI) of these same CYPs. Thus, Compound 24 is not expected to perpetrate a CYP-mediated drug-drug interaction.

[0253] METHOD EXAMPLE 21. Summary of Nonclinical Safety Pharmacology and Toxicology. Safety pharmacology studies were conducted to identify any unintended effects on organ systems acutely critical for life including the central nervous system (CNS), cardiovascular, and respiratory systems. Compound 24 had no effects on these organ systems. Additionally, there were no changes in electrocardiogram (ECG) parameters in dogs administered Compound 24 for 14 days in a GLP toxicology study.

[0254] Compound 24 was not mutagenic in a bacterial reverse mutation (Ames) test or an *in vitro* micronucleus assay *in vitro*. Compound 24 absorbs light between 290 and 700 nm, with an absorption maximum at 360 nm.

[0255] Non-GLP dose tolerability studies at the MFD were conducted in rats and dogs to support dose selection for 14-Day GLP toxicology studies. In rats, the MFD was 100 mg/kg by the IN route, the intended route of clinical administration. A dose of 100 mg/kg was well tolerated in the single dose tolerability phase. The 100 mg/kg dose was selected for further evaluation across a 3-day tolerability study with daily dosing. There were no effects of treatment on standard in-life and post-life assessments which supported the selection of 100 mg/kg as the high dose for a 14-day GLP study. In the 14-day study, rats were administered vehicle or doses up to 100 mg/kg/day by the IN route. There was no morbidity or mortality, no effect of treatment on any in-life assessments including clinical observations, body weight, food consumption, ophthalmology, CNS (Functional observational battery (FOB)), or clinical pathology parameters (hematology, coagulation, chemistry, urinalysis) and no effect on post-life assessments including necropsy, organ weights and microscopic examination of a standard list of tissues. Accordingly, the NOAEL was 100 mg/kg/day in male and female rats dosed daily by the IN route for 14 days.

[0256] In dogs, the MFD by the IN route was 10 mg/kg. Consequently, to evaluate the potential systemic toxicity of Compound 24, exposures were increased by supplementing the IN dose with a 10 mg/kg SC dose administered simultaneously. A combined dose of 20 mg/kg was well tolerated in the single dose tolerability phase. The combined 20 mg/kg dose was selected for further evaluation across a 3-day tolerability study with daily dosing. There were no effects of treatment on standard in-life and post-life assessments which supported the selection of 20 mg/kg as the high dose for a 14-day GLP study. In the 14-day study, dogs were administered vehicle or doses up to 20 mg/kg/day. There was no morbidity or mortality, no effect of treatment on any in-life assessments including clinical observations, body weight, food consumption, ophthalmology, ECGs, or clinical pathology parameters (hematology, coagulation, chemistry,

urinalysis) and no effect on post-life assessments including necropsy, organ weights and microscopic examination of a standard list of tissues. Accordingly, the NOAEL was 20 mg/kg/day in male and female dogs dosed daily by the combined IN and SC routes for 14 days.

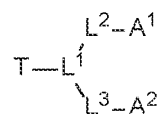
[0257] METHOD EXAMPLE 22. Summary of Chemistry, Manufacturing, and Controls.

- 5 Compound 24 is a small molecule neuraminidase inhibitor covalently conjugated to dinitrophenyl (DNP) and α -L-rhamnose with PEG-based linkers. Compound 24 is manufactured in a four-step process from key starting materials and has been manufactured in batches up to 100g. The structure, including the absolute stereochemistry of 11 chiral centers is confirmed through analysis by infrared spectroscopy (IR), Ultraviolet-Visible Absorbance Spectroscopy,
- 10 High-resolution Mass Spectrometry (HRMS), Elemental Analysis, and 1-D/2-D Nuclear Magnetic Resonance (NMR) spectroscopy. Compound 24 is packaged in HDPE plastic bottles protected from light, and stored at $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ for up to 12 months.

[0258] Each publication cited herein is incorporated herein by reference, in its entirety.

CLAIMS

1. A compound of the formula



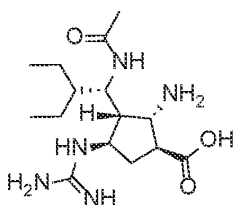
or a pharmaceutically acceptable salts or solvates thereof, wherein

T is a radical of a ligand for a target protein of an Influenza virus or a virus-infected cell;

L¹, L², and L³ are each, an independently selected linker, and

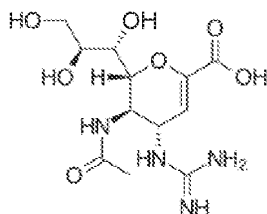
A¹ and A² are each, a radical of an independently selected hapten.

2. The compound of claim 1 wherein the ligand is a neuraminidase inhibitor.
3. The compound of claim 1 wherein the ligand is selected from sialic acid and analogs thereof.
4. The compound of claim 1 wherein the ligand is selected from zanamivir, peramivir, laninamivir, oseltamivir, and 2,3-dehydro-2-deoxy-n-acetylneuraminic acid, and analogs and derivatives of the foregoing.
5. The compound of claim 1 wherein the ligand is zanamivir, or analog or derivative thereof.
6. The compound of claim 1 wherein the ligand is peramivir, or an analog or derivative thereof.
7. The compound of claim 1 wherein the ligand is a compound of the formula



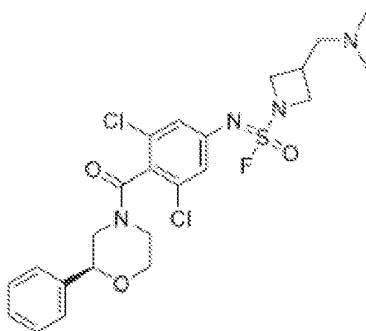
or an analog or derivative thereof.

8. The compound of claim 1 wherein the ligand is laninamivir, or an analog or derivative thereof.
9. The compound of claim 1 wherein the ligand is of the formula



or an analog or derivative thereof.

10. The compound of claim 1 wherein the ligand is oseltamivir, or an analog or derivative thereof.
11. The compound of claim 1 wherein the ligand is a hemagglutinin inhibitor.
12. The compound of claim 1 wherein the ligand is a



and analogs and derivatives thereof.

13. The compound of any one of claims 1-12 wherein the hapten has an epitope that has an endogenous Ab, B or T cell.
14. The compound of any one of claims 1-12 wherein each hapten is independently 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,
15. The compound of any one of claims 1-12 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 dinitrophenyl.
16. The compound of any one of claims 1-12 wherein L^2 and L^3 are bound to the same non-carbon atom on L^1 .
17. The compound of any one of claims 1-12 wherein L^2 and L^3 are bound to the same nitrogen atom on L^1 .
18. The compound of any one of claims 1-12 wherein L^2 and L^3 are attached to different atoms of L^1 .
19. The compound of any one of claims 1-18 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.
20. The compound of any one of claims 1-19 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.

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 $(\text{PEG})_n$, or aza- $(\text{PEG})_n$, or a combination thereof, where n is 2-36.

22. The compound of any one of claims 1-21 wherein each of L^1 , L^2 , and L^3 includes or also includes ethoxy, ethylamino, ethylene glycol, aza-ethylene glycol, $(\text{PEG})_n$, or aza- $(\text{PEG})_n$, or a combination thereof, where n is 2-36.

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 O-alkyl-O, N-alkyl-N, C(O)-alkyl-C(O), or NC(O)-alkyl-C(O)N, or a combination thereof.

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 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.

25. The compound of any one of claims 1-24 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.

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 one or more amino acids.

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 one or more hydrophilic amino acids selected from β -NH₂-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.

28. The compound of any one of claims 1-27 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.

29. The compound of any one of claims 1-28 wherein one or more of L^1 , L^2 , and L^3 includes or also includes an ornithine or lysine.

30. The compound of any one of claims 1-29 wherein one or more of L^1 , L^2 , and L^3 includes or also includes a $(\text{Pro})_n$, where n is 1-6.

31. The compound of any one of claims 1-30 wherein one or more of L^1 , L^2 , and L^3 includes or also includes a $(\text{Pro})_3$ -Lys.

32. The compound of any one of claims 1-31 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.

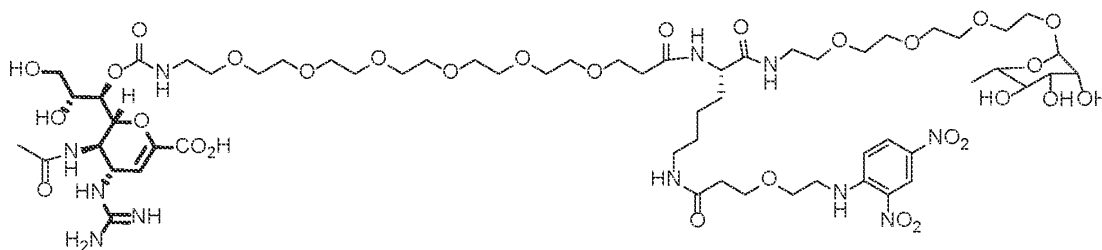
33. The compound of any one of claims 1-32 wherein L^2 and/or L^3 are hydrophilic.

34. The compound of any one of claims 1-18 wherein L^1 includes a region that is capable of forming an α -helical conformation.

35. The compound of any one of claims 1-34 wherein the extended conformation of L^2 and/or L^3 is at least 15 Å in length.

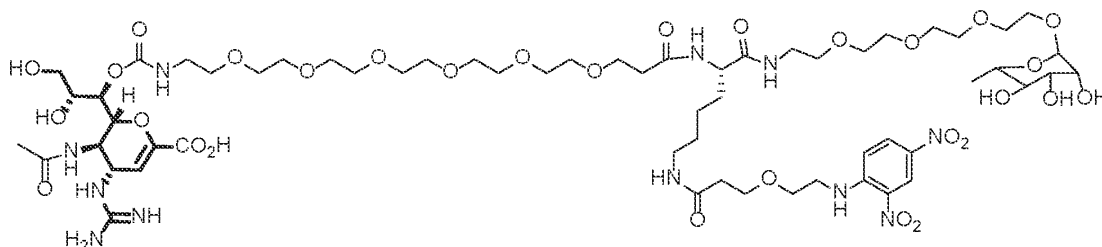
36. The compound of any one of claims 1-35 wherein L^1 does not include DBCO.

37. A compound of the formula



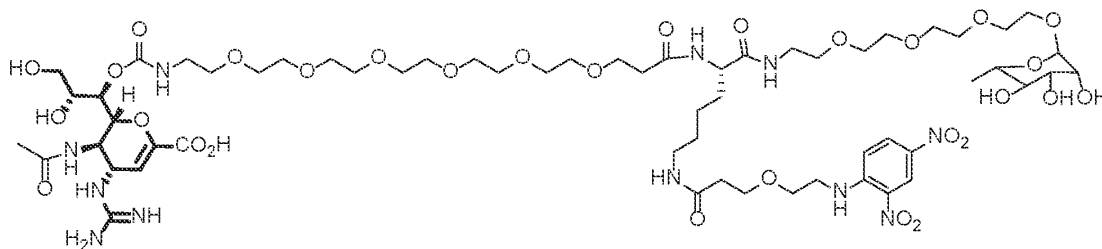
or a pharmaceutically acceptable salt or solvate thereof.

38. A composition comprising a compound of the formula



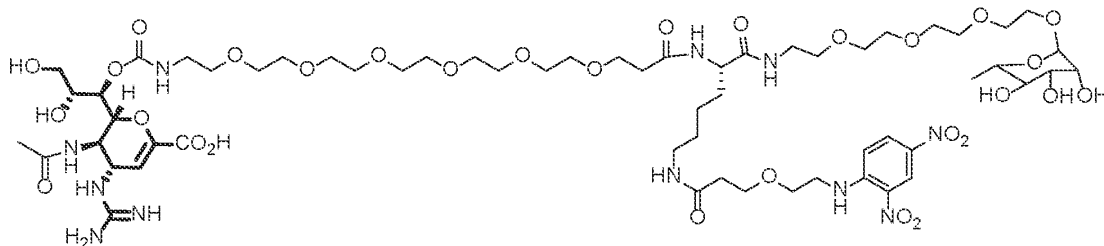
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

39. A composition for use in treating Influenza and Influenza infection, the composition comprising a compound of the formula



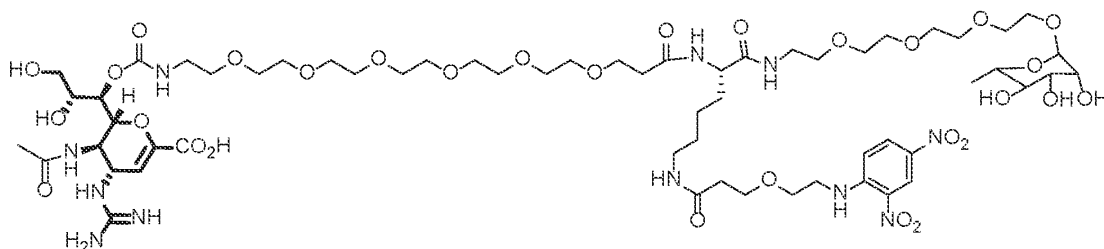
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

40. A unit dose for use in treating Influenza and Influenza infection, the unit dose comprising a compound of the formula



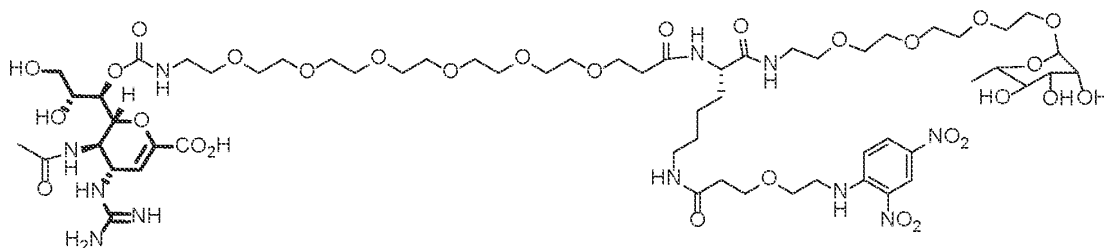
or a pharmaceutically acceptable salt or solvate thereof, and optionally including one or more diluents, carriers, or excipients, or combinations thereof.

41. A method for treating Influenza and Influenza 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.

42. 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 Influenza and Influenza infection in a host animal.