Supporting Information

Design, synthesis and biological activity evaluation of novel conjugated sialic acid and pentacyclic triterpene derivatives as anti-influenza entry inhibitors

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SI Figure 1. Compounds 19a and 20a had no inhibitory effect on the influenza virus neuraminidase activity. Compounds or OSV-P with two-fold serial dilution with PBS were mixed with influenza virus. The substrate solution (4-MUNANA) was mixed with the test compounds/virus mixture and incubated for 30 min at 37 °C. Neuraminidase activity was measured by fluorescence of 4-methylumbelliferone with fluorescence spectrophotometer. Each point represents the mean \pm S.E.M. for three independent experiments.



SI Figure 2. Docking structure of the original ligand Gal-2-Sia binding with a single chain of HA protein (Protein Data Bank: 1RVT) according to docking calculation. (A) Overview of the protein surface. The binding pocket is highlighted in the blue square. Gal-2-Sia from docking data is shown as green spheres and Gal-2-Sia in the X-ray crystal structure PDBid 1RVT is shown as blue spheres. (B) Closer view of the binding pocket. Gal-2-Sia from docking data is shown as green sticks and Gal-2-Sia in the X-ray crystal structure PDBid 1RVT is shown as blue lines.



Figure 1. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 7

Figure 2. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 7





Figure 3. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 8a

Figure 4. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 8a



Figure 5. HR-ESI-MS spectrum of compound 8a

Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-125	C53 H79 N5 O14	C53 H79 N5 Na	1032.5523	1032.551	-0.75
		O14		6	

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Figure 6. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 8b



Figure 7. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 8b



Figure 8. HR-ESI-MS spectrum of compound 8b





Figure 9. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 8c

Figure 10. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 8c



Figure 11. HR-ESI-MS spectrum of compound 8c



Figure 12. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 8d





Figure 13. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 8d

Figure 14. HR-ESI-MS spectrum of compound 8d





Figure 15. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 9a

Figure 16. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 9a





Figure 17. HR-ESI-MS spectrum of compound 9a

Figure 18. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 9b





Figure 19. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 9b

Figure 20. HR-ESI-MS spectrum of compound 9b HIGH RESLUTION MASS SPECTROMETRY REPORT

Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-141	C45 H71 N5 O10	C45 H71 N5 Na	864.5099	864.5093	-0.72
		O10			
x10 \$ 34 34 32- 3- 28- 26- 26- 24- 22- 2- 18- 1.6- 1.4- 1.2- 1- 0.8- 0.6- 0.4- 0.2- 0- 765 770 772	nin) HX3-141.d Subtract (2)	842.5282	864.5093 (M+Na)+ 1.100 855 860 865 870 875	880 885 890 895 90	0 905 910 915 920



Figure 21. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 9c

Figure 22. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 9c







Figure 24. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 9d





Figure 25. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 9d



Figure 26. HR-ESI-MS spectrum of compound 9d





Figure 27. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 10

Figure 28. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 10





Figure 29. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 11

Figure 30. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 11



Figure 31. HR-ESI-MS spectrum of compound 11 HIGH RESLUTION MASS SPECTROMETRY REPORT



Figure 32. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 12



Figure 33. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 12



Figure 34. HR-ESI-MS spectrum of compound 12





Figure 35. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 13a

Figure 36. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 13a



Figure 37. HR-ESI-MS spectrum of compound 13a











Figure 40. HR-ESI-MS spectrum of compound 13b

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Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX2-88	C55 H83 N5 O15	C55 H84 N5 O15	1054.5959	1054.5958	0.42
x10 ⁶ + Scan (7.353 7- 6.5- 6- 5- 5- 4- 3.5- 3- 2.5- 1.5- 1- 0.5- 0- 	7 min) HX2-88-1.d		1054.5959 (M +T)+		
100 15	u 200 250 300 350 400 450 500 55	0 600 650 700 750 800 850 900 Counts vs. Mass-to-Charge	950 1000 1050 1100 1150 1: (m/z)	200 1250 1300 1350 1400 1	450 1500 1550 1600

Figure 41. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 13c



Figure 42. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 13c



Figure 43. HR-ESI-MS spectrum of compound 13c



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Figure 44. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 13d





Figure 45. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 13d

Figure 46. HR-ESI-MS spectrum of compound 13d

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		DI DOINGINDIN	



Figure 47. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 14a



Figure 48. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 14a



Figure 49. HR-ESI-MS spectrum of compound 14a



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Figure 52. HR-ESI-MS spectrum of compound 14b

	HIGH RESLUTION MASS SPECTROMETRY REPORT				
Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX2-90	C47 H75 N5 O11	C47 H76 N5 O11	886.5534	886.5536	0.11
x10 6 + Scan (7.5895 m 3.2- 3- 2.8- 2.6- 2.4- 2.2- 1.8- 1.6- 1.4- 1.2- 1- 0.8- 0.6- 0.4- 0.2- 0 + 830 835	in) H≻2-90.d 840 845 850 855 860 865 870 875 88	896.5554 (M+H)+ 908.5355	20 925 930 935 940 945	950 955 960 965 970	975 980 985 930
	Counts vs. Mass-to-Charge (m/z)				

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Figure 53. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 14c



Figure 54. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 14c



Figure 55. HR-ESI-MS spectrum of compound 14c



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Figure 56. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 14d





Figure 57. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 14d

Figure 58. HR-ESI-MS spectrum of compound 14d

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Figure 60. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 16



Figure 61. HR-ESI-MS spectrum of compound 16



Figure 62. ¹H NMR (400 MHz, D₂O+CD₃OD) spectrum of compound 17



Figure 63. ¹³C NMR (100 MHz, D₂O+ CD₃OD) spectrum of compound 17



Figure 64. HR-ESI-MS spectrum of compound 17

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Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-64	C13 H22 N2 O8	C13 H23 N2 O8	335.1453	335.1449	-1.36



Figure 65. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 18



Figure 66. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 18



Figure 67. HR-ESI-MS spectrum of compound 18



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Figure 68. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 19a



Figure 69. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 19a



Figure 70. HR-ESI-MS spectrum of compound 19a







Figure 71. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 19b

Figure 72. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 19b



Figure 73. HR-ESI-MS spectrum of compound 19b



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Figure 74. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 19c







Figure 76. HR-ESI-MS spectrum of compound 19c HIGH RESLUTION MASS SPECTROMETRY REPORT

Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-80	C52 H79 N O17	C52 H79 N Na O17	1012.5241	1012.5240	0.03
x10 ⁶ + Scan (6.946 3- 2.8- 2.6- 2.4- 2.2- 2- 1.8- 1.6- 1.4- 1.2- 1- 0.8-	1 min) HX3-80.d Subtract 414,1389	1012.5241 [M+Na]+			
0.6- 0.4- 0.2-	474.1601		× .		
100	200 300 400 500 60	0 700 800 900 1000 1100 Counts vs. Mass-to-Charge (m/z)	1200 1300 1400	1500 1600 170	0 1800



Figure 77. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 19d

Figure 78. ¹³C NMR (100 MHz, CDCl₃) spectrum of compound 19d



Figure 79. HR-ESI-MS spectrum of compound 19d



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Figure 80. ¹H NMR (400 MHz, CD₃OD+CDCl₃) spectrum of compound 20a



Figure 81. ¹³C NMR (100 MHz, CD₃OD+CDCl₃) spectrum of compound 20a



Figure 82. HR-ESI-MS spectrum of compound 20a

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Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-84	C44 H71 N O12	C44 H71 N Na O12	828.4866	828.4868	0.3
x10 \$ 3 28 26 26 24 22 2 2 1.8 1.6 1.4 1.2 1. 0.8 0.6 0.4 0.2 0 0 1.1 0.0 0 0 0 1.1 0 0 0 0 0 0 0 0 0	19 min) HX3-84.d Subtract (5)	814.5765	828.4966 (M+Na)+		
720 725	730 735 740 745 750 755 760 765 77	0 775 780 785 790 795 800 805 810 815 Counts vs. Mass-to-Charge (m/z)	820 825 830 835 840 84	45 850 855 860 865 8	70 875 880 885



Figure 83. ¹H NMR (400 MHz, CD₃OD+CDCl₃) spectrum of compound 20b

Figure 84. ¹³C NMR (100 MHz, CD₃OD+CDCl₃) spectrum of compound 20b



Figure 85. HR-ESI-MS spectrum of compound 20b



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Figure 86. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 20c







Figure 88. HR-ESI-MS spectrum of compound 20c



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Figure 89. ¹H NMR (400 MHz, CD₃OD) spectrum of compound 20d

Figure 90. ¹³C NMR (100 MHz, CD₃OD) spectrum of compound 20d



Figure 91. HR-ESI-MS spectrum of compound 20d

Sample No.	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
HX3-75	C44 H71 N O12	C44 H71 N Na O12	828.4873	828.4868	-0.55
x10 ¢ + Scan (7.3786 1- 0.9- 0.8- 0.7- 0.6- 0.5- 0.4- 0.3- 0.2- 0.1- 0- 	min) HX3-75.d Subtract (2)	823 4873 (M+Na)+	ජ්ත පර්ජ පර්ත පර්ත පර්ත පර්ත	र्थत हुईन हरेद हुनेन स्थंत स्थान स	
730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 Counts vs. Mass-to-Charge (m/z)					