Supporting information

Enhanced Inhibition of Influenza A virus adhesion by Di- and Trivalent Hemagglutinin Inhibitors

Wenjing Lu,[†] Wenjuan Du,[‡] Victor J. Somovilla Busto,[†] Guangyun Yu,[†] Diksha Haksar,[†] Erik de Vries,[‡] Geert-Jan Boons,[†] Robert P. de Vries,[†] Cornelis A. M. de Haan,[‡*], Roland J. Pieters[†,*]

[†] Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O.Box 80082, NL-3508 TB Utrecht, The Netherlands

[‡] Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

Table of content:

Synthesis

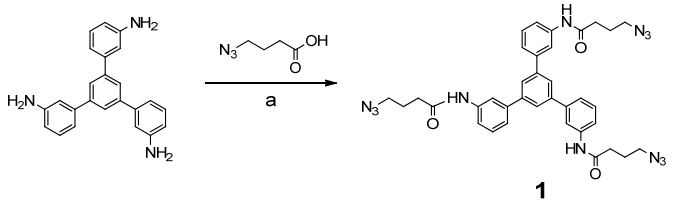
1. General Information

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. Compound **2**, **3**[1] and **4**[2] were prepared as reported in the literature. The solvents were obtained as synthesis grade and stored on molecular sieves (4 Å). Column chromatography was performed using Silica-P Flash silica gel (60 Å, particle size 40-63 µm) from Silicycle. TLC was performed on Merck precoated silica gel 60F254 glass plates and compound spots were visualized with UV light and/or 10 % H_2SO_4 (MeOH). Microwave reaction were carried out in a Biotage Initiator (300 W) reactor. ^{1}H NMR spectra were recorded on a 400 MHz, 500 MHz or 600 MHz spectrometer. ^{13}C NMR analysis was recorded at 101 MHz, 125 MHz or 151 MHz. High resolution mass spectrometry (HRMS) analysis was recorded using an Agilent 6560 Ion Mobility Q-TOF LC/MS instrument.

All enzymatic reactions were performed in aqueous buffered systems with the appropriate pH for each enzyme. Gel filtration chromatography was performed with columns packed with Bio-gel P-2 Fine (Bio-Rad) and eluted with water. Water was purified by a Milli-Q Gradient A10 Water Purification System. Lyophilization was performed on a Christ Alpha 1-2 apparatus. Analytical LC-MS was performed on an Agilent 6560 Ion Mobility Q-TOF LC/MS using a Waters XBridge HILIC column (5 µm, 250×4.6 mm) at a flow rate of 0.6 mL/min. The used buffers were 50 mM formic acid in H_2O (Buffer A, pH 4.4) and CH_3CN (Buffer B). UV-absorption was measured at 254 nm. Purification using preparative HPLC was performed on a Shimadzu 20A HPLC system with a Waters XBridge BEH Prep Amide column (5 µm, 250×10 mm) at a flow rate of 3.6 mL/min. Runs were performed using a standard protocol: 80 – 50 % gradient buffer B in 60 min, with the same buffers as described for the analytical LC-MS.

β-1,3-N-acetylglucosaminyltransferase (*H. Pylori*β3GlcNAcT)[3] (0.7 mg/mL), β-1,4-galactosyltransferase (LgtB)[4] (71.3 mg/mL) and α2,6-sialyltransferase (PmST1 mutant P34H/M144L)[5] (2.3 mg/mL) were made in house by cell lysate extraction (measured by Thermo Scientific™ NanoDrop 2000). Alkaline Phosphatase from calf intestine (1,000 U/mL) was purchased from Invitrogen. Uridine 5'-diphospho-N-acetylglucosamine (UDP-GlcNAc) and Uridine 5'-diphospho-galactose (UDP-Gal) were purchased from Sigma-Aldrich. Cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-NANA) was purchased from Roche Diagnostics GmbH.

2. Synthesis of the scaffolds

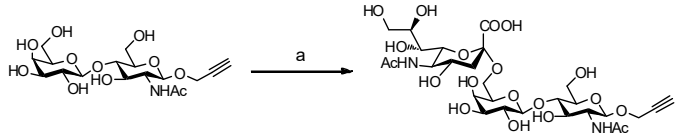


Scheme S1. a) EDC·HCl, DMAP, DCM, 48 h, 40 %.

Compound 1: 1,3,5-tris(3-aminophenyl)benzene (70 mg, 0.2 mmol) was dissolved in DCM (5 mL). 4-azidobutanoic acid (77 mg, 0.6 mmol, 3 equiv) was added, followed by DMAP (7.3 mg, 0.06 mmol, 0.3 equiv), EDC·HCl (190 mg, 1.0 mmol, 5 equiv). The mixture was stirred for 48 h at room temperature. Then the solution was washed with 1 M HCl solution, followed by saturated $NaHCO_3$ solution and by saturated NaCl solution. After drying (Na_2SO_4) the solvent was removed and the residue was purified using column

chromatography over silica gel (eluent DCM/MeOH 75:1 v/v) to give 55 mg (40 %) of an off-white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81(s, 3H, N*H*), 7.72 (s, 3H, ), 7.60 (s, 3H, ), 7.52 (d, 3H, ), 7.35-7.33 (m, 6H, ), 3.43-3.40 (t, *J* = 6.5 Hz, 6H, CH_2N$_3$), 2.52-2.49 (t, *J* = 7.2 Hz, 6H, COCH_2), 2.05-2.00 (m, 6H, CH_2CH$_2$N$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.53 (C=O), 141.81, 141.71, 138.15, 129.51, 125.29, 123.52, 119.24, 118.89, 50.75, 34.16, 24.66. HRMS: *m/z* calcd for $C_{36}H_{36}N_{12}O_3$ $[M+H]^+$ 685.3106, found 685.3107.

3. Synthesis of propargyl-sialyl-LacNAc

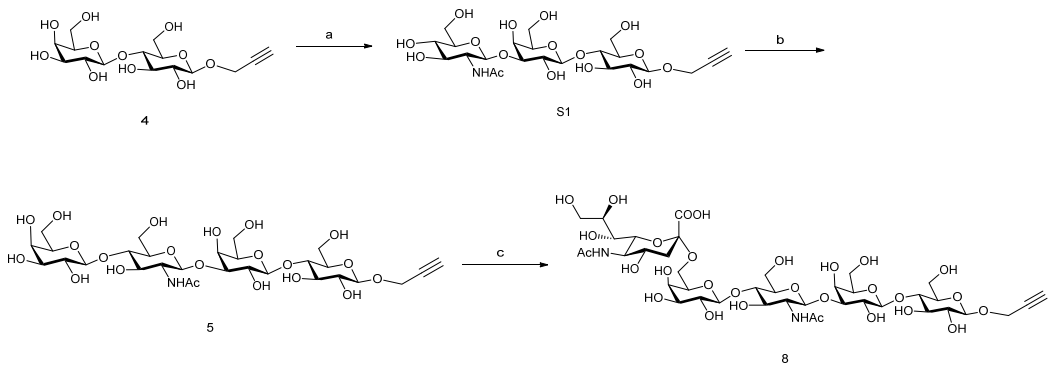


7

Scheme S2. a) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 °C, 14 h, 77 %.

Compound 7: Propargyl-LacNAc (5.0 mg, 0.012 mmol) and CMP-NANA (10.0 mg, 0.015 mmol, 1.2 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 7.5, 500 µL) containing $MgCl_2$ (20 mM). To this, PmST1 mutant P34H/M144L (α2-6 sialyltransferase, 50 µL) was added to the reaction mixture. Then the resulting reaction mixture was incubated at 37 °C for 4 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized to give the respective product as an amorphous white solid (6.5 mg, 77 % yield). ^{1}H NMR (600 MHz, D_2O):δ 4.67 (d, *J* = 7.1 Hz, 1H, H-1^{GlcNAc}), 4.34 (d, *J* = 8.1 Hz, 1H, H-1^{Gal}), 4.33 – 4.30 (m, 2H, CH_2C≡CH), 3.92 – 3.39 (m, 20H), 2.81 (t, *J* = 2.4 Hz, 1H, C≡C*H*), 2.57 (dd, *J* = 12.6, 4.4 Hz, 1H, H-3$_{eq}^{Sia}$), 1.96 (s, 3H, Ac^{Sia}), 1.92 (s, 3H, Ac^{GlcNAc}), 1.61 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}^{Sia}$); ^{13}C NMR (151 MHz, D_2O): δ 174.9 (C=O), 174.7 (C=O), 173.5 (C=O), 103.5, 100.1, 99.1, 80.6, 76.2, 74.6, 73.6, 72.5, 72.4, 72.4, 71.7, 70.7, 68.4, 68.3, 68.2, 63.3, 62.6, 60.2, 56.6, 54.6, 51.9, 40.1 (C-3^{Sia}), 22.3 (*C*H$_3$CO), 22.0 (*C*H$_3$CO). HRMS: *m/z* calcd for $C_{28}H_{44}N_2O_{19}$ $[M+H]^+$ 713.2611, found 713.2618.

4. Synthesis of propargyl-sialyl-LacNAc-Lactoside



Scheme S3. a) UDP-GlcNAc, *H. Pylori* β3GlcNAcT, CIAP, HEPES buffer, 37 ºC, 14 h, 70 %; b) UDP-Gal, LgtB, MES buffer, 37 ºC, 3 h, 81 %; c) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 ºC, 14 h, 27 %.

Compound S1: Propargyl-Lactoside (12 mg, 0.031 mmol) and UDP-GlcNAc (30 mg, 0.046 mmol, 1.5 equiv) were dissolved in HEPES buffer (50 mM, pH 7.3, 2.5 mL) containing KCl (25 mM), $MgCl_2$ (2 mM), and dithiothreitol (1 mM). To this, 20 µL CIAP (10 mU) and 50 µL *H. Pylori* β3GlcNAcT (β1-3GlcNAc Transferase) were added. The resulting reaction mixture was incubated at 37 ºC for 14 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized. Then the crude product was purified by using column chromatography over silica gel (eluent EtOAc/MeOH/H_2O 7:2:1 v/v/v) to give compound **S1** as an amorphous white solid (12.8 mg, 70 % yield). ^{1}H NMR (600 MHz, D_2O): δ 4.58 (d, J = 8.5 Hz, 1H, H-1^{GlcNAc}), 4.57 (d, J = 8.1 Hz, 1H, H-1^{Glc}), 4.37 (d, J = 5.7 Hz, 2H), 4.33 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.05 (d, J = 3.4 Hz, 1H), 3.88 (dd, J = 12.3, 2.1 Hz, 1H), 3.79 (dd, J = 12.4, 2.1 Hz, 1H), 3.70 – 3.33 (m, 14H), 3.28 – 3.20 (m, 1H, H-2^{Glc}), 2.81 (t, J = 2.4 Hz, 1H, C≡C*H*), 1.94 (s, 3H, Ac); ^{13}C NMR (151 MHz, D_2O): δ 175.0 (C=O), 102.9 (C-1^{Gal}), 102.8(C-1^{GlcNAc}), 100.3(C-1^{Glc}), 81.9, 78.2, 75.6, 74.9, 74.8, 74.8, 74.3, 73.5, 72.6, 72.5, 70.0, 69.7, 68.3, 60.9, 60.4, 59.9, 56.6, 55.6, 22.1 (*C*H_3CO). HRMS: *m/z* calcd for $C_{23}H_{37}NO_{16}$ $[M+Na]^+$ 606.2005, found 606.2011.

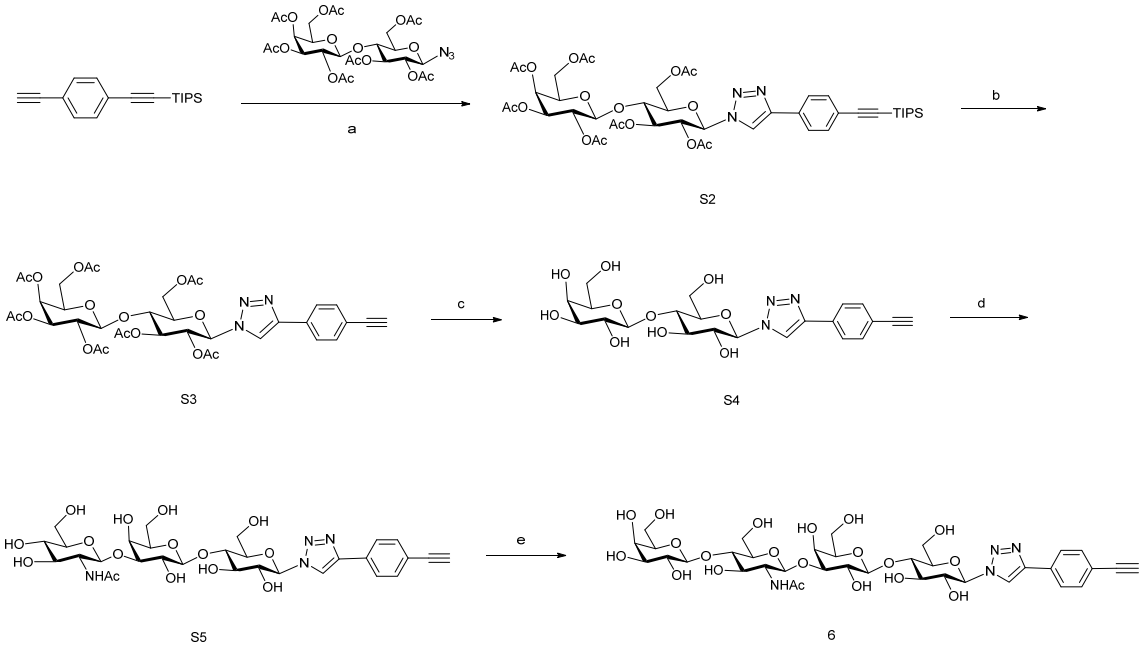
Compound 5: Compound **S1**(10 mg, 0.017 mmol) and UDP-Gal (15.7 mg, 0.026 mmol, 1.5 equiv) were dissolved in MES buffer (100 mM, 500 µL) containing $MnCl_2$ (20 mM). To this, 50µL LgtB (β1-4Gal Transferase) were added. The resulting reaction mixture was incubated at 37 ºC for 3 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized to give the respective product as an amorphous white solid (10.3 mg, 81 % yield). ^{1}H NMR (600 MHz, D_2O):δ 4.60 (d, J = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.57 (d, J = 8.0Hz, 1H, H-1^{Glc}), 4.40 – 4.36 (m, 3H, CH_2C≡CH, H-1$^{Gal'}$), 4.34 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.06 (d, J = 3.2 Hz, 1H), 3.91 – 3.82 (m, 3H), 3.78 – 3.41 (m, 20H), 3.27 – 3.21 (m, 1H, H-2^{Glc}), 2.81 (t, J = 2.4 Hz, 1H, C≡C*H*), 1.93 (s, 3H, Ac); ^{13}C NMR (151 MHz, D_2O): δ 174.9 (C=O), 102.9 (C-1^{Gal}), 102.8 (C-1$^{Gal'}$), 102.7 (C-1^{GlcNAc}), 100.3 (C-1^{Glc}), 82.0, 78.7, 78.2, 78.1, 76.3, 75.3, 74.9, 74.8, 74.5, 74.3, 72.5, 72.5, 72.1, 70.9, 69.9, 68.5, 68.3, 61.0, 60.9, 59.9, 59.8, 56.6, 55.2, 22.1(*C*H_3CO). HRMS: *m/z* calcd for $C_{29}H_{47}NO_{21}$ $[M+Na]^+$ 768.2538, found 768.2547.

Compound 8: Compound **5** (2 mg, 2.7 μmol) and CMP-NANA (2.6 mg, 4.0 μmol, 1.5 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 7.5, 500 μL) containing $MgCl_2$ (20 mM). To this, PmST1 mutant P34H/M144L (α2-6sialyltransferase, 50 μL) was added. The resulting reaction mixture was incubated at 37 ºC for 4 h. The completion of the reaction was analyzed by TLC. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized for further preparative HPLC using a HILIC column (**Table 1**). Then the fractions containing product were combined and lyophilized which gave the respective product as an amorphous white solid (0.75 mg, 27 % yield).^{1}H NMR (600 MHz, D_2O): δ 4.63 (d, *J* = 7.2 Hz, 1H, H-1^{GlcNAc}), 4.58 (d, *J* = 8.3 Hz, 1H, H-1^{Glc}), 4.39 – 4.33 (m, 4H, C*H*$_2$C≡CH, H-1$^{Gal'}$, H-1^{Gal}), 4.06 (d, *J* = 3.2 Hz, 1H), 3.90 – 3.42 (m, 28H), 3.27 – 3.24 (m, 1H, H-2^{Glc}), 2.90 (t, *J* = 2.4 Hz, 1H, C≡C*H*), 2.57 (dd, *J* = 12.2, 4.1 Hz, 1H, H-3$_{eq}^{Sia}$), 1.96 (s, 3H, Ac^{Sia}), 1.93 (s, 3H, Ac ^{GlcNAc}), 1.63 (t, *J* = 12.2 Hz, 1H, H-3$_{ax}^{Sia}$); ^{13}C NMR (151 MHz, D_2O, extracted from HSQC): δ 103.3(C-1^{Gal}), 103.3(C-1$^{Gal'}$), 102.7 (C-1^{GlcNAc}), 100.4(C-1^{Glc}), 82.1, 80.6, 78.4, 75.0, 74.6, 74.5, 73.8, 72.7, 72.5, 72.4, 71.8, 70.8, 70.1, 68.5, 68.5, 68.4, 63.4, 63.4, 62.8, 62.7, 61.0, 60.3, 60.2, 56.6, 55.0, 51.9, 40.1(C-3^{Sia}), 22.4(*C*H$_3$CO), 22.1(*C*H$_3$CO). HRMS: *m/z* calcd for $C_{40}H_{64}N_2O_{29}$ $[M+Na]^+$ 1059.3487, found 1059.3490.

Table 1. Method 1 for preparation HPLC using HILIC column.

Time (min)	Buffer A (%)	Buffer B (%)	Flow rate (mL/min)
0.0	10	90	3.6
5.0	28	72	3.6
7.0	28	72	3.6
10.0	31	69	3.6
14.0	31	69	3.6
14.1	50	50	3.6
18.0	50	50	3.6

5. Synthesis of sialyl-LacNAc-Lactoside-spacer



Scheme S4. a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O (9:1), microwave 80 °C, 30 min, 86 %; b) TBAF, 97 % ; c) NaOMe, MeOH, 14 h, quant.; d) UDP-GlcNAc, *H. Pylori* β3GlcNAcT, CIAP, HEPES buffer, 37 °C, 14 h, 71 %; e) UDP-Gal, LgtB, MES buffer, 37 °C, 3 h, 82 %.

Compound S2: 1-((triisopropylsilyl)ethynyl)-4-ethynylbenzene (0.14 g, 0.5 mmol), Azido-Ac-Lactoside (0.5 g, 0.76 mmol, 1.5 equiv), $CuSO_4 \cdot 5H_2O$ (12.5 mg, 0.05 mmol, 0.1 equiv), sodium L-ascorbate (0.1 g, 0.5 mmol, 1.0 equiv) were dissolved in DMF/H_2O (9:1, 5 mL). The reaction was performed under microwave irradiation at 80 °C for 30 min. Then the mixture was concentrated *in vacuo*. The residue was used in the next step without further purification. HRMS: *m/z* calcd for $C_{45}H_{61}N_3O_{17}Si$ $[M+Na]^+$ 966.3662, found 966.3655.

Compound S3: Compound **S2** (244 mg, 0.26 mmol) was dissolved in THF (10 mL) and to this solution TBAF·$3H_2O$ (122 mg, 0.39 mmol) was added. The obtained reaction mixture was stirred for 2 h until the reaction was complete, based on TLC. H_2O (10 mL) was added to the reaction mixture for quenching. The mixture was extracted with DCM and washed with H_2O and brine, then dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified using column chromatography over silica gel (eluent DCM/MeOH 9:1 v/v) to give the compound **S3** as a white solid (198 mg, 97 % yield).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H, H-triazole), 7.75 (d, J = 8.2 Hz, 2H, H-phenyl), 7.51 (d, J = 8.3 Hz, 2H, H-phenyl), 5.85 (d, J = 8.7 Hz, 1H, H-1^{Glc}), 5.48 – 5.36 (m, 2H, H-2^{Glc}, H-3^{Glc}), 5.33 (d, J = 3.2 Hz, 1H, H-4^{Gal}), 5.10 (dd, J = 10.4, 7.8 Hz, 1H, H-2 ^{Gal}), 4.95 (dd, J = 10.4, 3.4 Hz, 1H, H-3 ^{Gal}), 4.51 (d, J = 7.9 Hz, 1H, H-1^{Gal}), 4.45 (d, J = 12.0 Hz, 1H, H-6a^{Glc}), 4.17 – 4.03 (m, 4H, H-5^{Glc}, 2H-6^{Gal}, H-6b^{Glc}), 3.99 – 3.85 (m, 2H, H-5^{Gal}, H-4^{Glc}), 3.11 (s, 1H, C≡C*H*), 2.13 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 6H, 2Ac), 1.94 (s, 3H, Ac), 1.84 (s, 3H, Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3(*C*=O), 170.2(*C*=O), 170.0(*C*=O), 170.0 (*C*=O), 169.4(*C*=O), 169.2(*C*=O), 169.0(*C*=O), 147.5, 132.6, 132.6 (C-phenyl), 130.2, 125.6, 125.6 (C-phenyl)

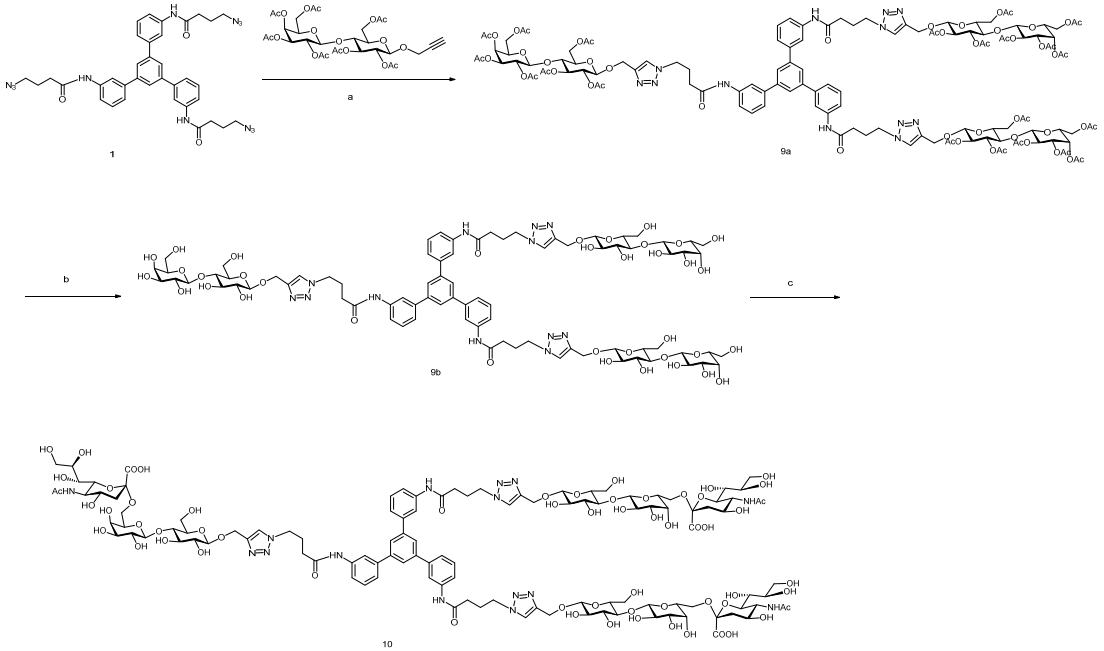
122.1, 118.2(C-triazole), 101.1(C-1^{Gal}), 85.5(C-1^{Glc}), 83.3, 78.2, 75.9, 75.6, 72.6, 70.9, 70.8, 70.4, 69.0, 66.6, 61.8, 60.8, 20.8 (*C*H_3CO), 20.7(*C*H_3CO), 20.6(*C*H_3CO), 20.6 (*C*H_3CO), 20.6(*C*H_3CO), 20.5(*C*H_3CO), 20.2(*C*H_3CO). HRMS: *m/z* calcd for $C_{36}H_{41}N_3O_{17}$ $[M+H]^+$ 788.2509, found 788.2509.

Compound S4: NaOMe (0.69 mg, 0.013 mmol, 0.1 equiv) was added in the solution of compound **S3** (100 mg, 0.13 mmol) in MeOH (10 mL) and the mixture was stirred for 14 h at room temperature. The solution was neutralized with Dowex-H^+ resin to a pH of ca.7 when the resin was removed by filtration. The residue was concentrated *in vacuo*. Compound **S4** was obtained as a white solid (62 mg, quant.). ^{1}H NMR (400 MHz, CD_3OD): δ 8.60 (s, 1H, H-triazole), 7.82 (d, *J* = 8.1 Hz, 2H, H-phenyl), 7.52 (d, *J* = 8.5 Hz, 2H, H-phenyl), 5.68 (d, *J* = 9.2 Hz, 1H, H-1^{Glc}), 4.42 (d, *J* = 7.6 Hz, 1H, H-1^{Gal}), 4.00 (t, *J* = 9.1 Hz, 1H, H-2^{Glc}), 3.90 (d, *J* = 2.8 Hz, 2H), 3.83 – 3.70 (m, 6H), 3.63 – 3.54 (m, 2H), 3.51 – 3.47 (m, 1H), 2.13 (s, 1H, C≡C*H*);. ^{13}C NMR (101 MHz, CD_3OD): δ 146.6, 132.2, 132.2 (C-phenyl), 130.5, 125.2, 125.2 (C-phenyl), 122.3, 120.4(C-triazole), 103.7(C-1^{Gal}), 88.1(C-1^{Glc}), 78.2, 78.2, 75.7, 75.4, 73.4, 72.3, 71.1, 68.9, 61.1, 60.1. HRMS: *m/z* calcd for $C_{22}H_{27}N_3O_{10}$ $[M+H]^+$ 494.1775, found 494.1782.

Compound S5: Compound **S4** (9 mg, 0.018 mmol) and UDP-GlcNAc (17 mg, 0.027 mmol 1.5 equiv) were dissolved in HEPES buffer (50 mM, pH 7.3, 500 µL) containing KCl (25 mM), $MgCl_2$ (2 mM), and dithiothreitol (1 mM). To this, 20 µL CIAP (10 mU) and 50 µL *H. Pylori* β3GlcNAcT (β1-3GlcNAc Transferase) were added. The resulting reaction mixture was incubated at 37 °C for 14 h. The reaction mixture was centrifuged and the supernatant was subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized to give compound **S5** as an amorphous white solid (8.9 mg, 71 % yield). ^{1}H NMR (600 MHz, D_2O): δ 8.50 (s, 1H, H-triazole), 7.73 (d, *J* = 8.4 Hz, 2H, H-phenyl), 7.56 (d, *J* = 8.4 Hz, 2H, H-phenyl), 5.75 (d, J = 9.2 Hz, 1H, H-1^{Glc}), 4.61 (d, J = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.42 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.08 (d, *J* = 3.4 Hz, 1H), 4.01 (t, *J* = 9.0 Hz, 1H, H-2^{Glc}), 3.90 (d, *J* = 12.0 Hz, 1H), 3.84 – 3.64 (m, 11H), 3.56 – 3.52 (m, 1H), 3.50 – 3.46 (m, 1H), 3.41 – 3.34 (m, 2H), 1.98 (s, 1H, C≡C*H*), 1.96 (s, 3H, Ac); ^{13}C NMR (151 MHz, D_2O): δ 175.0(C=O), 147.0, 132.8, 132.8 (C-phenyl), 129.7, 125.8, 125.8 (C-phenyl), 122.5, 121.7(C-triazole), 102.9(C-1^{Gal}), 102.9(C-1^{GlcNAc}), 87.4(C-1^{Glc}), 81.9, 77.7, 77.7, 77.2, 75.6, 74.9, 74.5, 73.5, 72.0, 70.0, 69.7, 69.6, 68.3, 61.0, 60.4, 59.7, 55.6, 22.1(*C*H_3CO). HRMS: *m/z* calcd for $C_{30}H_{40}N_4O_{15}$ $[M+Na]^+$ 719.2382, found 719.2388.

Compound 6: Compound **S5** (5 mg, 0.007 mmol) and UDP-Gal (6.5 mg, 0.01 mmol, 1.5 equiv) were dissolved in MES buffer (100 mM, 300 µL) containing $MnCl_2$ (20 mM). To this, LgtB (β1-4Gal Transferase, 30 µL) was added. The resulting reaction mixture was incubated at 37 °C for 3 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized to give the respective product as an amorphous white solid (4.9 mg, 82 % yield). ^{1}H NMR (600 MHz, D_2O): δ 8.53 (s, 1H, H-triazole), 7.76 (d, *J* = 8.4 Hz, 2H, H-phenyl), 7.58 (d, *J* = 8.0 Hz, 2H, H-phenyl), 5.76 (d, *J* = 9.1 Hz, 1H, H-1^{Glc}), 4.63 (d, *J* = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.42 (d, *J* = 7.8 Hz, 1H, H-1^{Gal}), 4.39 (d, *J* = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.08 (d, *J* = 3.2 Hz, 1H), 4.01 (t, *J* = 8.8 Hz, 1H, H-2^{Glc}), 3.92 – 3.52 (m, 21H), 3.47 – 3.43 (m, 1H), 1.97 (s, 1H, C≡C*H*), 1.95 (s, 3H, Ac); ^{13}C NMR (151 MHz, D_2O, extracted from HSQC): δ 132.9, 125.9, 121.7 (C-triazole), 102.9 (C-1^{Gal}), 102.9 (C-1$^{Gal'}$), 102.8 (C-1^{GlcNAc}), 87.3 (C-1^{Glc}), 82.0, 78.3, 77.6, 75.4, 75.1, 74.6, 74.6, 72.6, 72.3, 72.0, 71.0, 70.0, 69.6, 68.6, 68.4, 61.0, 58.9, 59.9, 55.2, 22.0 (*C*H_3CO). HRMS: *m/z* calcd for $C_{36}H_{50}N_4O_{20}$ $[M+H]^+$ 859.3091, found 859.3090.

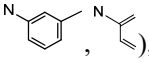
5. Synthesis of compound **10**



Scheme S5. a) propargyl Ac-Lactoside, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O (9:1), microwave 100 ºC, 1.5 h, 97 %; b) NaOMe, MeOH, 14 h, 90 %; c) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 ºC, 14 h, 57 %.

Compound 9a: Compound **1** (12.3 mg, 0.018 mmol), **4** (61 mg, 0.09 mmol, 5 equiv), $CuSO_4 \cdot 5H_2O$ (2.2 mg, 0.009 mmol, 0.5 equiv), sodium L-ascorbate (8.9 mg, 0.045 mmol, 2.5 equiv) were dissolved in DMF/H_2O (9:1, 2 mL). The reaction was performed under microwave irradiation at 100 ºC for 1.5 h. The completion of the reaction was analyzed by TLC (DCM/MeOH 7:1 v/v). Then the mixture was concentrated *in vacuo*. The residue was purified by silica chromatography (DCM/ MeOH 20:1 v/v) which gave the product **9a** as a light yellow solid (47.3 mg, 97 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 3H, H-triazole), 7.80 (s, 3H, N*H*), 7.71 (s, 3H, ), 7.58 (m, 6H,  , ), 7.40 (m, 6H, ), 5.31 (d, *J* = 3.4 Hz, 3H, H-4^{Gal}), 5.15 (t, *J* = 9.3 Hz, 3H, H-3^{Glc}), 5.07 (dd, *J* = 10.4, 7.1 Hz, 3H, H-2^{Gal}), 4.94 – 4.72 (m, 12H, H-3^{Gal}, C*H*$_2$N, H-2^{Glc}), 4.75 (dd, *J* = 12.4, 2.4 Hz, 3H,), 4.59 (d, *J* = 7.8 Hz, 3H, H-1^{Glc}), 4.52 – 4.42 (m, 12H, H-1^{Gal}, H-6a^{Glc}, OC*H*$_2$C), 4.12 – 4.00 (m, 9H, 2H-6^{Gal}, H-6b^{Glc}), 3.82 (t, *J* = 7.0 Hz, 3H, H-5^{Gal}), 3.75 (t, *J* = 9.4 Hz, 3H, H-4^{Glc}), 3.61 – 3.53 (m, 3H, H-5^{Glc}), 2.43 – 2.26 (m, 12H, COC*H*$_2$, CC*H*$_2$C), 2.14 – 2.08 (m, 18H, Ac), 2.03 – 1.92 (m, 45H, Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.5, 170.3, 170.1, 170.1, 170.1, 169.8, 169.7, 169.0, 141.7, 141.5, 138.5, 129.5, 125.1, 123.4, 123.1, 119.2, 118.7, 101.0(C-1^{Gal}), 99.9(C-1^{Glc}), 76.0, 72.8, 72.6, 71.6, 70.9, 70.6, 69.1, 66.6, 63.1, 61.8, 60.7, 49.4, 33.4, 29.7, 25.8, 20.9, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5. HRMS: *m/z* calcd for $C_{123}H_{150}N_{12}O_{57}$ $[M+2H]^{2+}$ 1354.4677, found 1354.4673..

Compound 9b: NaOMe (0.8 mg, 0.001 mmol, 0.1 equiv) was added to a solution of compound **9a** (37 mg, 0.014 mmol) in MeOH (5 mL) and the mixture was stirred for 14 h at room temperature. The solution was

neutralized with Dowex-H^+ resin to a pH of ca.7 when the resin was removed by filtration. The residue was concentrated *in vacuo*. Compound **9b** was obtained as a white solid (23 mg, 90 % yield).^{1}H NMR (500 MHz, D_2O): δ 7.70 (s, 3H, H-triazole), 7.26 (s, 3H, ), 7.12 – 6.42 (m, 12H, ), 4.49 (d, *J* = 9.5 Hz, 3H, H-6b^{Glc}), 4.32 (d, *J* = 7.0 Hz, 3H, H-1^{Glc}), 4.27 (d, *J* = 7.3 Hz, 3H, H-1^{Gal}), 4.20 – 4.00 (m, 6H, C*H*$_2$N), 3.83 – 3.27 (m, 36H), 3.18 (t, *J* = 8.1 Hz, 3H, H-2^{Glc}), 2.09 – 1.79 (m, 12H, COC*H*$_2$, CC*H*$_2$C); ^{13}C NMR (126 MHz, D_2O): δ 171.9, 143.4, 140.4, 137.8, 129.0, 124.8, 123.9, 122.6, 118.9, 118.3, 102.9(C-1^{Gal}), 101.5(C-1^{Glc}), 78.4, 75.28, 74.71, 74.32, 72.66, 72.51, 70.89, 68.49, 61.84, 60.97, 60.05, 49.48, 33.05, 25.37; HRMS: *m/z* calcd. for $C_{81}H_{108}N_{12}O_{36}$ $[M+2H]^{2+}$ 913.3567, found 913.3568.

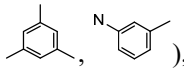
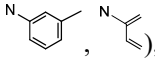
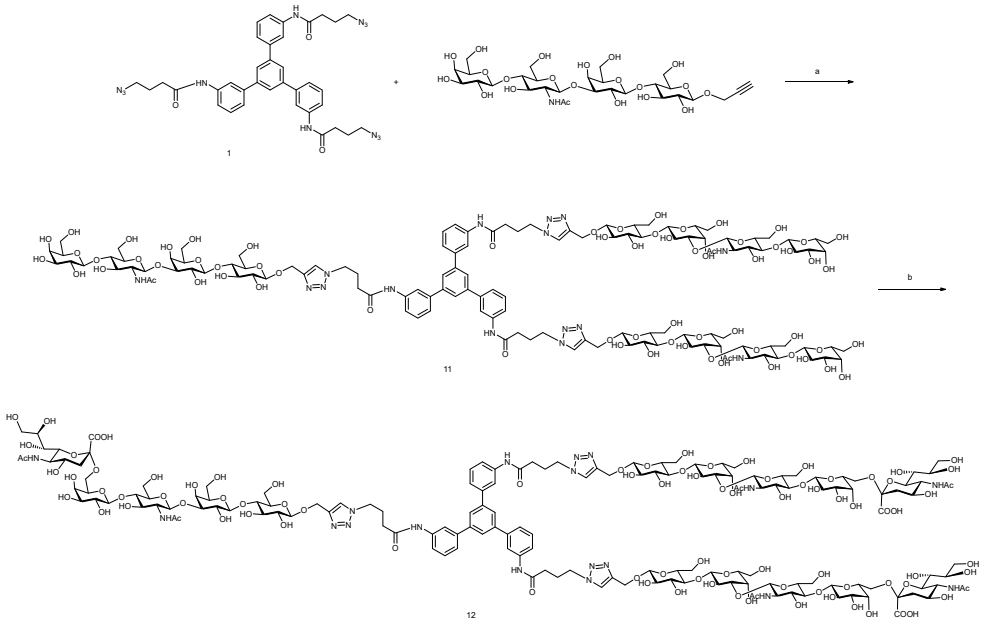
Compound 10: Compound **9b** (1 mg, 0.55 μmol) and CMP-NANA (2.1 mg, 3.3 μmol, 6 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 8.0, 500 μL) containing $MgCl_2$ (20 mM). To this, PmST1 mutant P34H/M144L (α2-6sialyltransferase, 50 μL) was added. The resulting reaction mixture was incubated at 37 °C for 14 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized for further preparation HPLC (HILIC column) using the standard protocol (**Table 2**) which then gave the respective product as an amorphous white solid (0.85 mg, 57 % yield).^{1}H NMR (400 MHz, D_2O): δ 7.84 (s, 3H, H-triazole), 7.63 – 7.49 (m, 6H, ), 7.42 – 7.27 (m, 6H, ), 7.18 – 7.11 (m, 3H, ), 4.60 – 4.54 (d, *J* = 12.6 Hz, 3H, H-6a^{Glc}), 4.44 (d, *J* = 12.6 Hz, 3H, H-6b^{Glc}), 4.38 – 4.27 (m, 6H, C*H*$_2$N), 4.19 (d, *J* = 8.3 Hz, 3H, H-1^{Glc}), 4.06 (d, *J* = 7.8 Hz, 3H, H-1^{Gal}), 3.77 – 3.60 (m, 18H), 3.55 – 3.31 (m, 33H), 3.23 – 3.19 (m, 3H), 3.03 (t, *J* = 8.6 Hz, 3H, H-2^{Glc}), 2.55 (dd, *J* = 12.4, 4.6 Hz, 3H, H-3$_{eq}^{Sia}$), 2.29 (t, *J* = 11.6 Hz, 6H, COC*H*$_2$), 2.12 (t, *J* = 11.3 Hz, 6H, CC*H*$_2$C), 1.85 (s, 9H, Ac^{sia}), 1.55 (t, *J* = 12.0 Hz, 3H, H-3$_{ax}^{Sia}$). HRMS: *m/z* calcd. for $C_{114}H_{159}N_{15}O_{60}$ $[M+2H]^{2+}$ 1349.9999, found 1349.9967.

Table 2. Method 2 for preparation HPLC using HILIC column.

Time (min)	Buffer A (%)	Buffer B (%)	Flow rate (mL/min)
0.0	30	70	3.6
11.0	32	68	3.6
14.0	33	67	3.6
17.0	36	64	3.6
23.0	37	63	3.6
23.1	50	50	3.6
27.0	50	50	3.6

6. Synthesis of compound **12**



Scheme S6. a) THPTA, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O (1:2), microwave 80 °C, 1.5 h, 63 %; b) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 °C, 14 h, 44 %.

Compound 11: Compound **1** (0.82 mg, 1.2 µmol), **5** (4 mg, 5.4 µmol, 4.5 equiv), $CuSO_4 \cdot 5H_2O$ (12.5 µg, 0.05 µmol, 0.04 equiv), sodium L-ascorbate (0.5 mg, 2.5 µmol, 2 equiv) and THPTA (50 µg, 0.1 µmol, 0.08 equiv) were dissolved in DMF/H_2O (1:2, 0.5 mL)[6]. The reaction was performed under microwave irradiation at 80 °C for 1.5 h. Then the mixture was concentrated *in vacuo*. The reaction mixture was dissolved in water (50 µL) and centrifuged. Then the supernatant was subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized without further purification which gave the respective product as an amorphous white solid (2.2 mg, 63 % yield). HRMS: *m/z* calcd. for $C_{123}H_{177}N_{15}O_{66}$ $[M+2H]^{2+}$ 1461.0550, found 1461.0551.

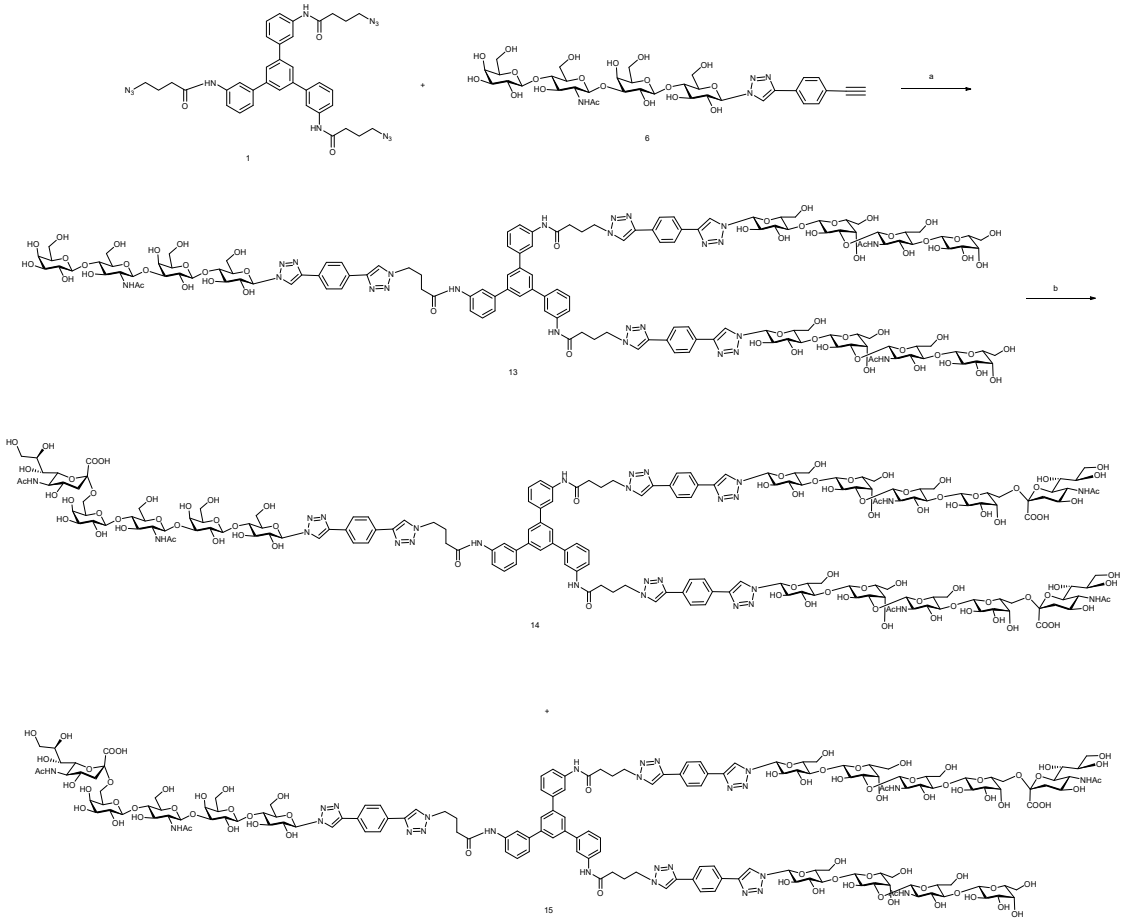
Compound 12: Compound **11**(1 mg, 0.34 µmol) and CMP-NANA (2.2 mg, 3.4 µmol, 10 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 7.5, 200 µL) containing $MgCl_2$ (20 mM). To this, PmST1 mutant P34H/M144L (α2-6sialyltransferase, 20µL) was added. The resulting reaction mixture was incubated at 37 °C for 14 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized for further preparative HPLC (HILIC column) using the standard protocol (**Table 3**) which then gave the respective product as an amorphous white solid (0.57 mg, 44 % yield). ^{1}H NMR (600 MHz, D_2O): δ 7.94 (s, 3H, H-triazole), 7.61 – 7.12 (m, 15H, H-phenyl), 4.70 (d, 3H, H6), 4.58 (d, *J* = 12.2 Hz, 3H, H6), 4.48 – 4.39 (m, 9H, H-1, CH_2N), 4.34 (d, *J* = 7.9 Hz, 3H, H-1), 4.32 (d, *J* = 7.7 Hz, 3H, H-1^{Glc}), 4.05 (d, *J* = 7.7 Hz, 3H, H-1), 3.94 – 3.28 (m, 90H), 3.27 – 3.21 (m, 3H), 3.08 (t, *J* = 17.4, 8.6 Hz, 3H, H-2^{Glc}), 2.57 (dd, *J* = 12.4, 4.7 Hz, 3H, H-3$_{eq}^{Sia}$), 2.39 – 2.26 (m, 6H, COCH_2), 2.22 – 2.16 (m, 6H, CCH_2C), 1.93 (s, 9H, Ac^{sia}),

1.91 (s, 9H, Ac), 1.62 (t, J = 12.2 Hz, 3H, H-3_{ax}^{Sia}); ^{13}C NMR (151 MHz, D_2O, extracted from HSQC): δ 125.3, 120.2, 109.3, 103.5, 103.0, 102.6, 101.7, 82.1, 80.4, 78.7, 74.7, 74.6, 73.8, 72.6, 72.5, 72.5, 71.7, 70.8, 69.7, 68.5, 68.4, 68.3, 68.2, 63.4, 62.8, 62.1, 60.9, 60.8, 60.2, 60.1, 54.9, 51.9, 51.8, 49.9, 40.1, 33.4, 25.3, 22.3, 22.1. HRMS: *m/z* calcd. for $C_{156}H_{228}N_{18}O_{90}$ $[M+2H]^{2+}$ 1897.6982, found 1897.6985.

Table 3. Method 3 for preparation HPLC using HILIC column.

Time (min)	Buffer A (%)	Buffer B (%)	Flow rate (mL/min)
0.0	30	70	3.6
10.0	32	68	3.6
15.0	33	67	3.6
20.0	36	64	3.6
40.0	37	63	3.6
50.0	37	63	3.6
65.0	50	50	3.6
80.0	50	50	3.6

7. Synthesis of compound **14 & 15**



Scheme S7. a) THPTA, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O (1:2), microwave 80 ºC, 1.5 h, 77 %; b) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 ºC, 4 h.

Compound 13: Compound **1** (0.79 mg, 1.0 µmol), **6** (6 mg, 7.0 µmol, 7 equiv), $CuSO_4 \cdot 5H_2O$ (25 µg, 0.1 µmol, 0.1 equiv), sodium L-ascorbate (0.5 mg, 2.5 µmol, 2.5 equiv) and THPTA (50 µg, 0.1 µmol, 0.1 equiv) were dissolved in DMF/H_2O (1:2, 0.2 mL). The reaction was performed under microwave irradiation at 80 ºC for 1.5 h. Then the mixture was concentrated *in vacuo*. The reaction mixture was dissolved in water (50 µL) and centrifuged. Then the supernatant was subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized without further purification which gave the respective product as an amorphous white solid (2.5 mg, 77 % yield). HRMS: *m/z* calcd. for $C_{144}H_{186}N_{24}O_{63}$ $[M+3H]^{3+}$ 1087.4102, found 1087.4070.

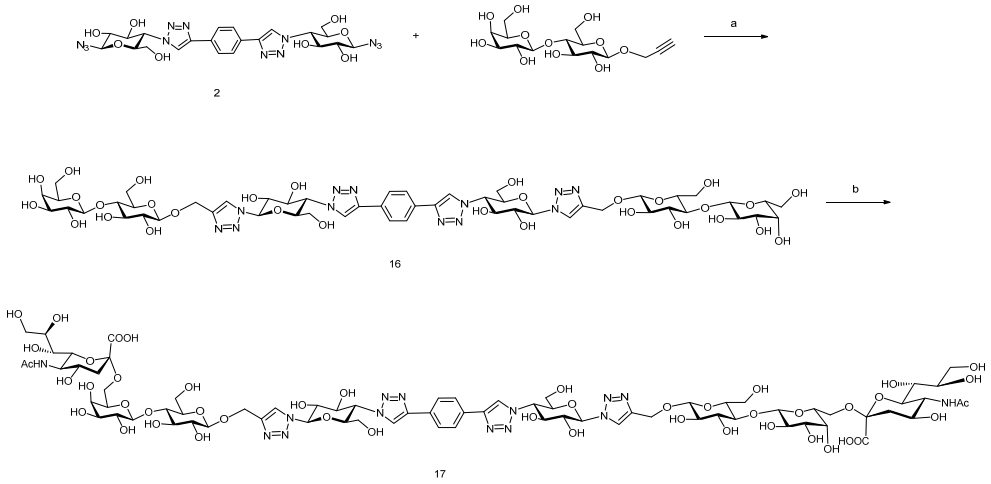
Compound 14&15: Compound **13** (2.5 mg, 0.77 µmol) and CMP-NANA (2.9 mg, 4.6 µmol, 6 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 7.5, 300 µL) containing $MgCl_2$ (20 mM). To this, PmST1 mutant P34H/M144L (α2-6sialyltransferase, 50 µL) was added. The resulting reaction mixture was incubated at 37 ºC for 4 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized for

further preparative HPLC (HILIC column) using the standard protocol (**Table 3**) which then gave the respective products as amorphous white solids.

Compound 14 (0.38 mg, 12 % yield): ^{1}H NMR (600 MHz, D_2O): δ 8.10 (s, 1H, H-triazole), 8.20 – 6.72 (m, 30H, H-triazole, H-phenyl), 5.32 (s, 3H, H-1^{Glc}), 4.42 – 4.36 (m, 9H), 4.14 – 3.41 (m, 99H), 2.65 – 2.32 (m, 9H), 1.98 – 1.94 (m, 24H), 1.66 (t, J = 12.3 Hz, 3H, H-3$_{ax}^{Sia}$). HRMS: *m/z* calcd. for $C_{177}H_{237}N_{27}O_{87}$ $[M+3H]^{3+}$ 1378.5056, found 1378.5057.

Compound 15 (0.86 mg, 27 % yield): ^{1}H NMR (600 MHz, D_2O): δ 4.37 (s, 6H), 4.15 – 3.24 (m, 92H), 2.64 – 2.56 (m, 2H, H-3$_{eq}^{Sia}$), 2.07 – 1.84 (m, 27H), 1.70 – 1.60 (m, 2H, H-3$_{ax}^{Sia}$). HRMS: *m/z* calcd. for $C_{166}H_{220}N_{26}O_{79}$ $[M+3H]^{3+}$ 1281.4738, found 1281.4747.

8. Synthesis of compound **17**



Scheme S8. a) THPTA, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O (1:2), microwave 80 °C, 1.5 h, 79 %; b) CMP-NANA, PmST1 mutant P34H/M144L, Tris-HCl buffer, 37 °C, 4 h, 52 %.

Compound 16: Compound **2**[7] (10 mg, 17 µmol), propargyllactoside (9.7 mg, 25.5 µmol, 1.5 equiv), $CuSO_4 \cdot 5H_2O$ (0.4 mg, 1.7 µmol, 0.1 equiv), sodium L-ascorbate (8.4 mg, 42.5 µmol, 2.5 equiv) and THPTA (0.7 mg, 1.7 µmol, 0.1 equiv) were dissolved in DMF/H_2O (1:2, 0.5 mL). The reaction was performed under microwave irradiation at 80 °C for 1.5 h. The completion of the reaction was analyzed by TLC (DCM/MeOH 4:1 v/v). Then the mixture was concentrated *in vacuo*. The reaction mixture was dissolved in water (50 µL) and centrifuged. Then the supernatant was subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized without further purification which gave the respective product as an amorphous white solid (18 mg, 79 % yield).^{1}H NMR (400 MHz, D_2O): δ 8.38 (s, 2H, H-triazole), 8.24 (s, 2H, H-triazole), 7.74 (s, 4H, H-phenyl), 5.89 (d, J = 9.1 Hz, 2H, H-1^{Glc}), 4.89 (d, J = 12.8 Hz, 2H, CCH_2a), 4.83 – 4.74 (m, 4H, CCH_2b, H-4^{Glc}), 4.46 (d, J = 8.0 Hz, 2H, H-1$^{Glc'}$), 4.35 (t, J = 9.8 Hz, 2H), 4.29 (d, J = 7.8 Hz, 2H, H-1^{Gal}), 4.12 (t, J = 9.2 Hz, 2H, H-2^{Glc}),

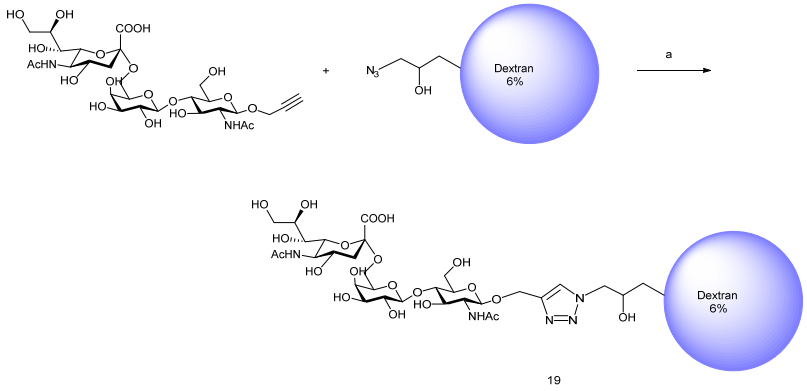
3.81 (d, J = 12.8 Hz, 2H), 3.76 (d, J = 3.4 Hz, 2H), 3.69 – 3.43 (m, 18H), 3.38 (dd, J = 10.0, 7.7 Hz, 2H, H-2^{Gal}), 3.24 – 3.15 (m, 4H, H-$2^{Glc'}$, H6); ^{13}C NMR (101 MHz, D_2O, extracted from HSQC): δ 126.3, 124.7, 122.6, 102.8(C-1^{Gal}), 101.3(C-$1^{Glc'}$), 87.4 (C-1^{Glc}), 78.2, 76.9, 76.9, 75.3, 74.6, 74.3, 73.5, 72.6, 72.5, 72.5, 70.9, 70.9, 68.5, 62.0, 61.9, 61.2, 60.9, 59.9, 59.6. HRMS: *m/z* calcd. for $C_{52}H_{74}N_{12}O_{30}$ $[M+H]^+$ 1347.4707, found 1347.4707.

Compound 17: Compound **16** (3.5 mg, 2.6 μmol) and CMP-NANA (5 mg, 7.8 μmol, 3 equiv) were dissolved in Tris-HCl buffer (100 mM, pH 8.0, 500 μL) containing $MgCl_2$ (20 mM).To this, PmST1 mutant P34H/M144L (α2-6sialyltransferase, 50 μL) was added. The resulting reaction mixture was incubated at 37 °C for 4 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Bio-gel P-2 (eluent H_2O). Fractions containing product were combined and lyophilized for further preparative HPLC (HILIC column) using the standard protocol (**Table 4**) which then gave the respective product as an amorphous white solid (2.6 mg, 52 % yield). ^{1}H NMR (600 MHz, D_2O): δ 8.50 (s, 2H, H-triazole), 8.33 (s, 2H, H-triazole), 7.89 (s, 4H, H-phenol), 5.97 (d, J = 9.2 Hz, 2H, H-1^{Glc}), 4.97 (d, J = 12.9 Hz, 2H, CCH_2a), 4.91 – 4.85 (m, 4H, CCH_2b, H-4^{Glc}), 4.54 (d, J = 8.0 Hz, 2H, H-$1^{Glc'}$), 4.45 – 4.39 (m, 4H), 4.33 (d, J = 7.9 Hz, 2H, H-1^{Gal}), 4.20 (t, J = 9.2 Hz, 2H, H-2^{Glc}), 3.90 – 3.85 (m, 4H), 3.83 (d, J = 3.5 Hz, 2H), 3.80 – 3.70 (m, 10H), 3.62 (dd, J = 10.4, 1.9 Hz, 2H), 3.60 – 3.52 (m, 14H), 3.50 (dd, J = 10.7, 4.0 Hz, 2H), 3.46 (dd, J = 9.1, 1.9 Hz, 2H), 3.43 (dd, J = 10.1, 7.9 Hz, 2H), 3.33 – 3.26 (m, 4H, H-$2^{Glc'}$, H6), 2.61 (dd, J = 12.4, 4.7 Hz, 2H, H-3_{eq}^{Sia}), 1.92 (s, 6H, Ac^{sia}), 1.64 (t, J = 12.2 Hz, 2H, H-3_{ax}^{Sia}); ^{13}C NMR (151 MHz, D_2O, extracted from HSQC): δ 126.5, 124.7, 122.7, 103.2(C-1^{Gal}), 101.3(C-$1^{Glc'}$), 87.4(C-1^{Glc}), 79.5, 77.0, 74.6, 73.7, 73.6, 72.7, 72.6, 72.5, 72.4, 71.8, 70.8, 70.0, 68.5, 68.4, 68.4, 67.0, 63.6, 63.5, 62.7, 62.0, 61.5, 60.2, 59.7, 59.7, 51.8, 51.7, 40.2, 40.1, 22.1(CH$_3$CO). HRMS: *m/z* calcd. for $C_{74}H_{108}N_{14}O_{46}$ $[M+2H]^{2+}$ 965.3344, found 965.3341.

Table 4. Method 4 for preparation HPLC using HILIC column.

Time (min)	Buffer A (%)	Buffer B (%)	Flow rate (mL/min)
0.0	20	80	3.6
10.0	30	70	3.6
45.0	33	67	3.6
60.0	50	50	3.6
75.0	50	50	3.6

10. Synthesis of polymer **18**



Scheme S10. a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, H_2O, microwave 100 ºC, 60 min, 24 %.

Polymer 18: The azido polymer (compound **3**) was dissolved in water followed by the addition of compound **7** (3 mg, 1.3 equiv). $CuSO_4 \cdot 5H_2O$ (0.1 equiv) and sodium L-ascorbate (0.3 equiv) were dissolved in water separately and added to the reaction mixture. The reaction was carried out at 100 ºC with microwave radiation for 60 min. The solvent was evaporated and the crude reaction mixture was purified by dialysis using a cellulose based dialysis cassette (MWCO: 2K) against deionized water for 3-4 days and freeze dried to give a white compound (3 mg, 24 %). The disappearance of the azide stretching peak in the IR spectra of the final compound confirmed that all of the azido groups had reacted.

Virus assays

Recombinant viruses

Influenza virus WU95 and VI75 contain the HA gene of A/Netherlands/178/95 (H3N2) or A/Bilthoven/1761/76 (H3N2), respectively, in the genetic background of A/Puerto Rico/8/34/Mount Sinai H1N1 (PR8; 7+ 1 virus). These viruses were kindly provided by Ron Fouchier (Erasmus Medical Center, the Netherlands). Influenza virus H5N1 contains the HA and NA genes from A/duck/Hunan/795/2002 (H5N1) in the genetic background of PR8 (6+2 virus). Generation of these viruses has been described previously.[8,9] Influenza virus A/Netherlands/602/2009 (H1N1) was characterized previously.[10] Viruses were grown in MDCK-II cells (ATCC) as described previously[11] and stored aliquoted at −80°C until use.

Biolayer interferometry (BLI) binding assay

All BLI experiment were carried out using OctetRed384 (Fortebio) and initial binding rates were determined similarly as described previously.[11] In short, streptavidin sensors were loaded to saturation with biotinylated Lysosomal-associated membrane glycoprotein 1 (LAMP1) in phosphate buffered saline (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.4). The synthesis of this recombinant protein was performed similarly as described previously for fetuin.[11] Subsequently, sensors were moved to wells containing a mix of virus and different concentrations of the indicated compounds, which had been pre-incubated for 4 hours at room temperature, to analyze virus binding. 8 hemagglutinating units of WU95 virus corresponding to 2.5x10^8 particles as determined by nanoparticle tracking analysis (Nanosight NS300; Malvern) were used per well. When indicated Oseltamivir carboxylate (OC; 10 μM end concentration, gift from Roche) was added to this mixture to block NA activity. As a control, the initial binding rate was determined in the absence of inhibitory compounds.

Additional BLI experiments with H1N1 and H5N1 viruses and compound 14

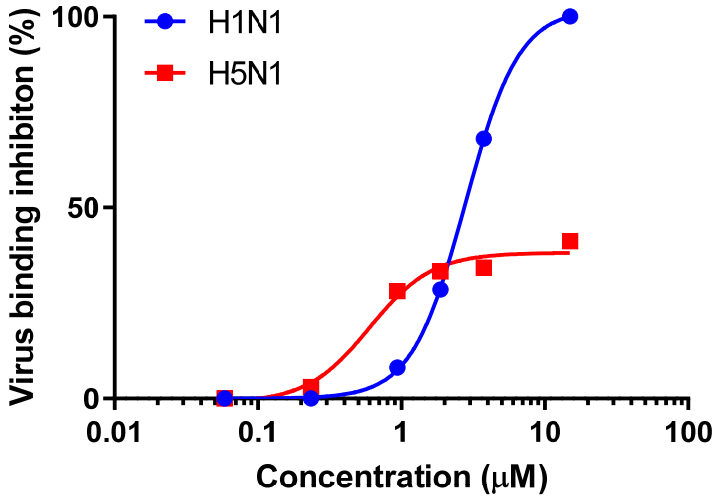


Figure 1. BLI experiments with H1N1 and H5N1 viruses and compound **14** were performed similarly as described above.

Effect of Oseltamivir Carboxylate (OC) in BLI assay

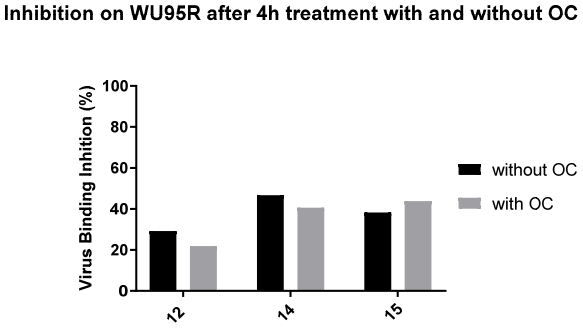


Figure 2. Analysis of compounds **12, 14** and **15** at 3 μM inhibition targeting IAV WU95 by BLI assay performed with/without oseltamivir (10 μM).

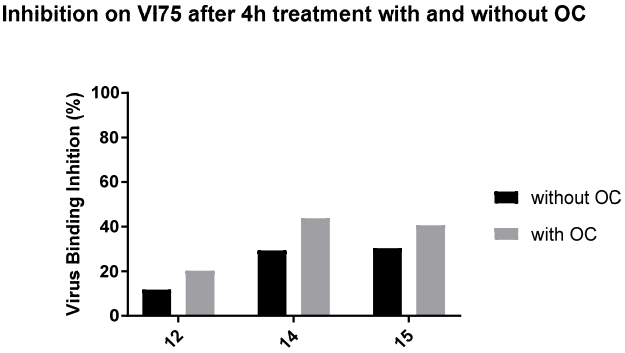


Figure 3. Analysis of compounds **12, 14** and **15** at 3 μM inhibition targeting IAV VI75 by BLI assay performed with/without oseltamivir (10 μM).

Hemagglutination inhibition assay

Four hemagglutination units of influenza virus were preincubated with limiting dilutions of the indicated compounds for 4 hours at room temperature in the presence of OC. Subsequently, 0.5% (v/v) erythrocytes were added and incubated for 2 hours at 4°C. The lowest concentration of compound that inhibited hemagglutination was determined. The hemagglutination inhibition assay was performed twice in duplicate. The mean values of these experiments are shown.

Infection experiments

Prior to infection, viruses (IAV WU95) diluted in Opti-mem (Gibco) were or were not incubated with varying concentration of **12** or **14** for 4 h at room temperature. Wells (96-well plate) fully covered with MDCK-II cells were inoculated in triplicate at a MOI of 0.005. At 7 h post-infection, cells were fixed in methanol at -20℃ for 5 min, after which infected cells were visualized by using antibody HB65 specific for the nucleoprotein and Alexa Fluor 488 -labeled Donkey anti-Mouse IgG (H+L) antibodies (Thermo Fisher Scientific) similarly as described previously.[12] Cells were visualized using the nuclear stain DAPI (Thermo Fischer Scientific) according to the manufacturer's instructions. Monolayers were inspected and total number of infected cells per well were determined (approximately 200 infected cells per well in the absence of inhibitory compound), and pictures were taken using EVOS FL (Thermo Fisher Scientific) (Fig. 4). In other experiments, MDCK-II cells were infected with IAV WU95 similarly as described above except that cells were incubated for 3 days in the presence of 1 μg/ml TPCK-trypsin (Sigma) to allow multiple rounds of infection and to analyze the ability of the compounds to protect against cell killing resulting from ongoing virus replication. Cells were fixed and stained with DAPI as described above prior to visual inspection using EVOS FL (Fig. 5).

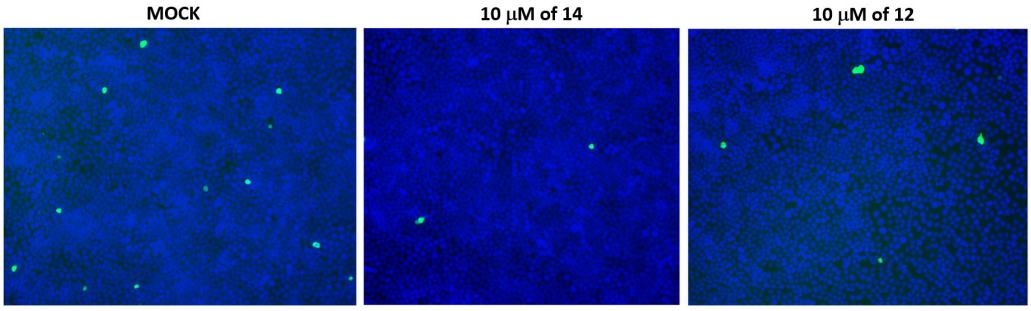


Figure 4. Inhibition of infection by **14** and **12** in the absence of NA inhibitor. Cells fixed at 7 h post-infection were stained using DAPI (blue; nuclei) and for virus infection (green; nucleocapsid protein). Representative images of cells inoculated with IAV WU95 in the presence or absence of 10 μM of the indicated compounds are shown.

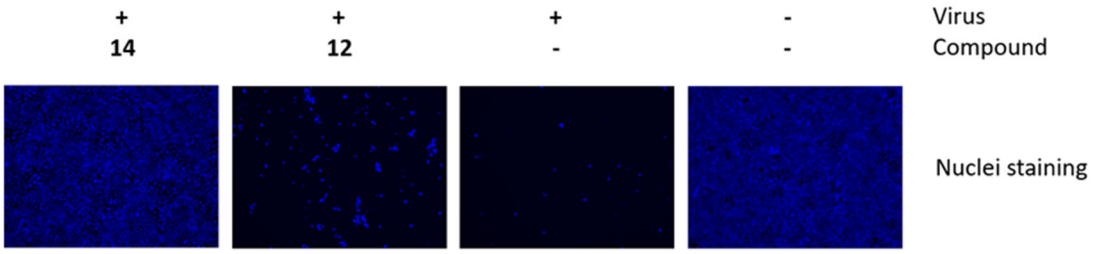


Figure 5. Compounds **12** and **14** show varying protective potencies against cytopathogenic effects of virus infection. Cells (mock) inoculated with IAV WU95 in the absence or presence of the indicated compounds were incubated for 3 days, after which the cells were stained with DAPI. Absence of DAPI-stained cells indicates cell killing.

Toxicity Assay

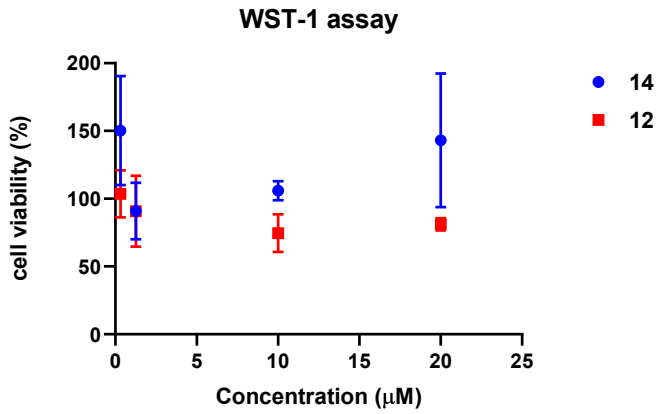


Figure 6. After 24 h incubation of MDCK-II cells with the different compounds at different concentrations in Opti-mem, the cell number and viability was measured by Wst-1 assay according to the manufacturer's protocol (Roche Diagnostics GmbH).

Infection-inhibition experiments, synergy between OC and 15

Prior to infection, viruses (IAV WU95) were or were not incubated with **15 (**15 μM) for 4 h at room temperature in the absence or presence of 10 μM OC (Oseltamivir carboxylate) in Opti-mem. Wells containing a confluent MDCK-II cell layer were incubated for 2 h with a 500-fold dilution of the virus-compound mixture in Opti-mem, resulting in an end concentration of 30 nM of **15** and 20 nM of OC and a MOI of 0.01, after which cells were washed with PBS, and cells were incubated in Opti-mem with bafilomycin (10 nM) overnight at 37 ℃, 5% CO2. Bafilomycin prevents acidification of endosomes and thereby blocks infection by IAVs that have not yet fused during the 2 h inoculation period.[12] Cells were fixed in methanol at -20℃ for 5 min, after which infected cells were visualized and analyzed similarly as described above. Inspection of monolayers by bright field microscopy indicated 100% confluency of the MDCK-II cells.

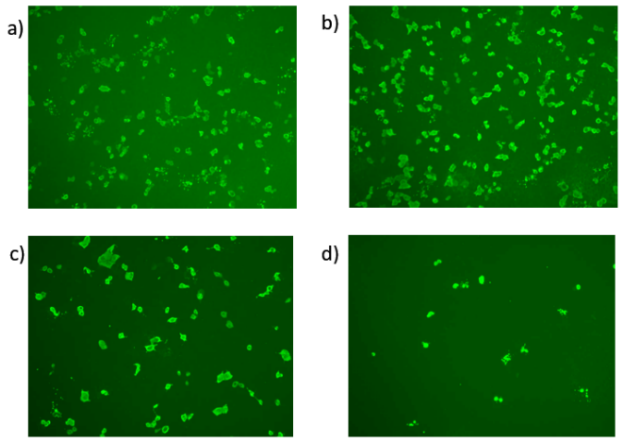
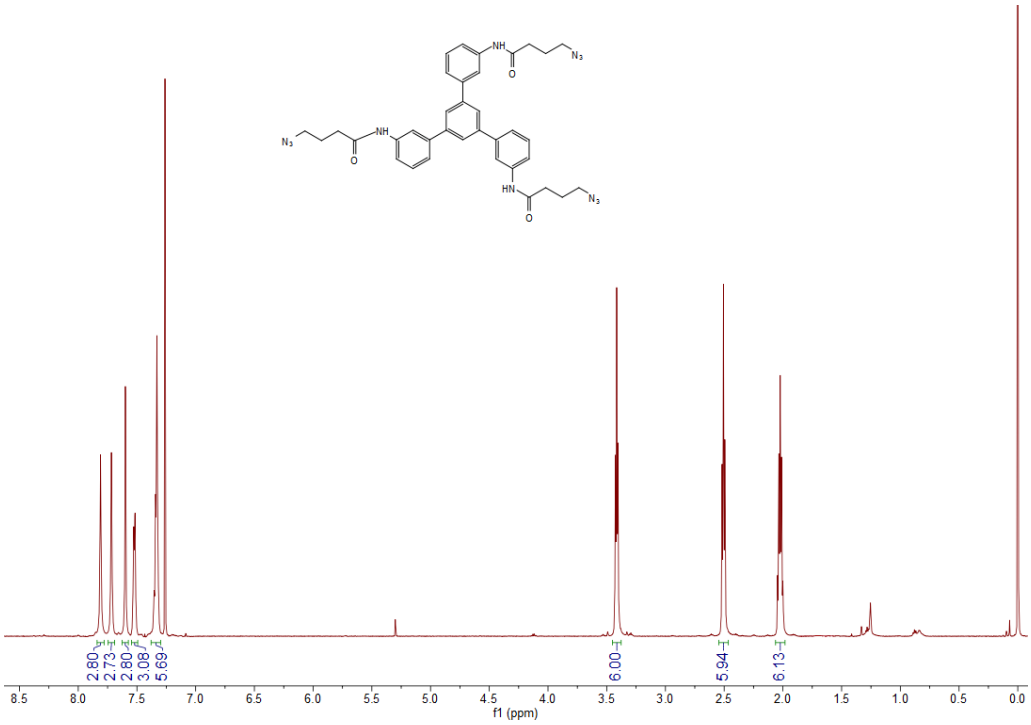
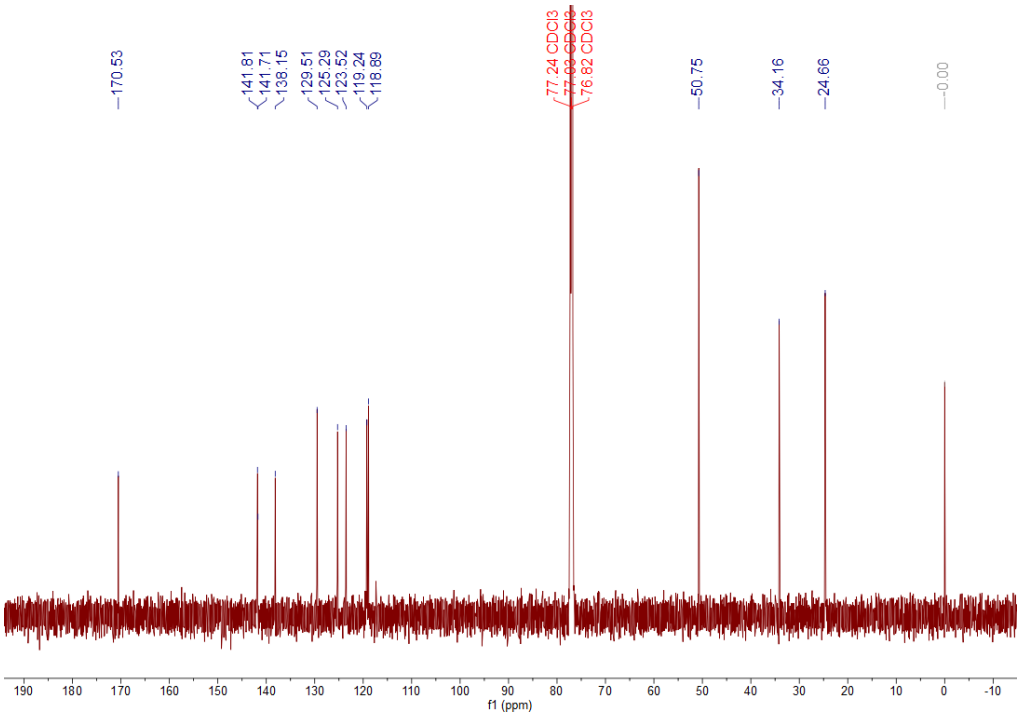


Figure 7. Demonstration of synergy between protective efficacy of oseltamivir carboxylate (OC, 20 nM) and **15** (30 nM**)** against IAV infection. Cells were inoculated with IAV WU95 in the absence or presence of 15 and/or OC. Infected cells were visualized using a nucleoprotein-specific antibody. a) no inhibitors, b) only **15**; c) only OC; d) both **15** and OC.

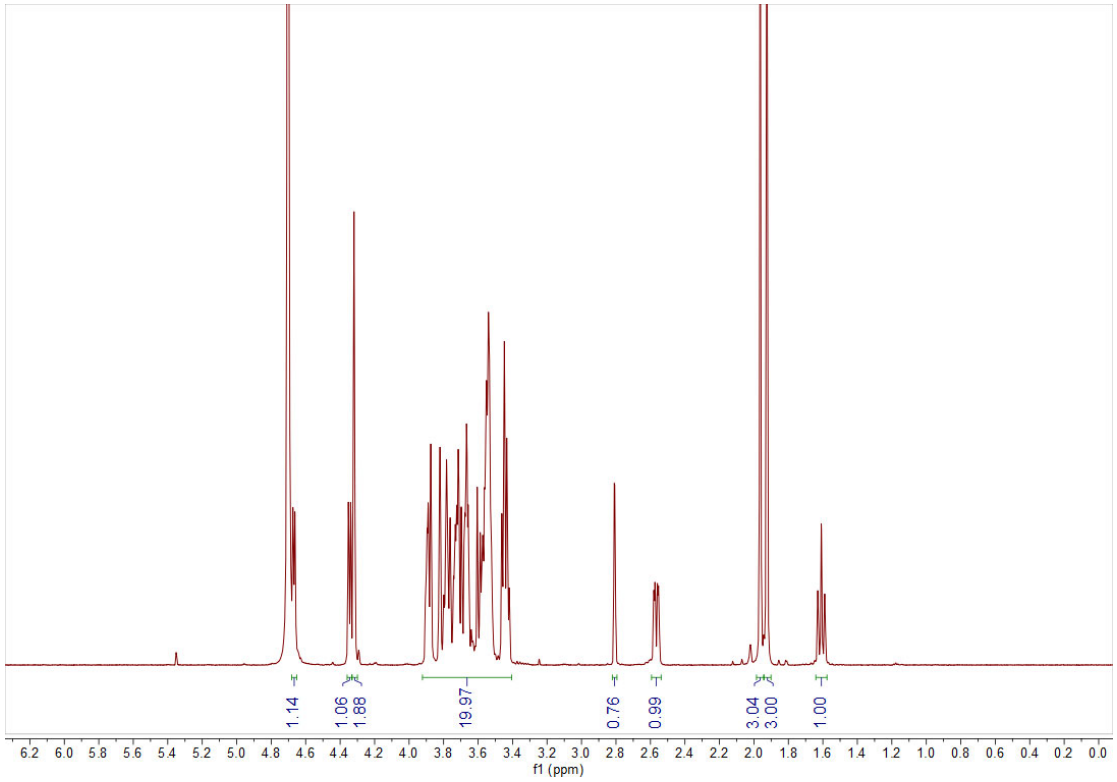
NMR spectra

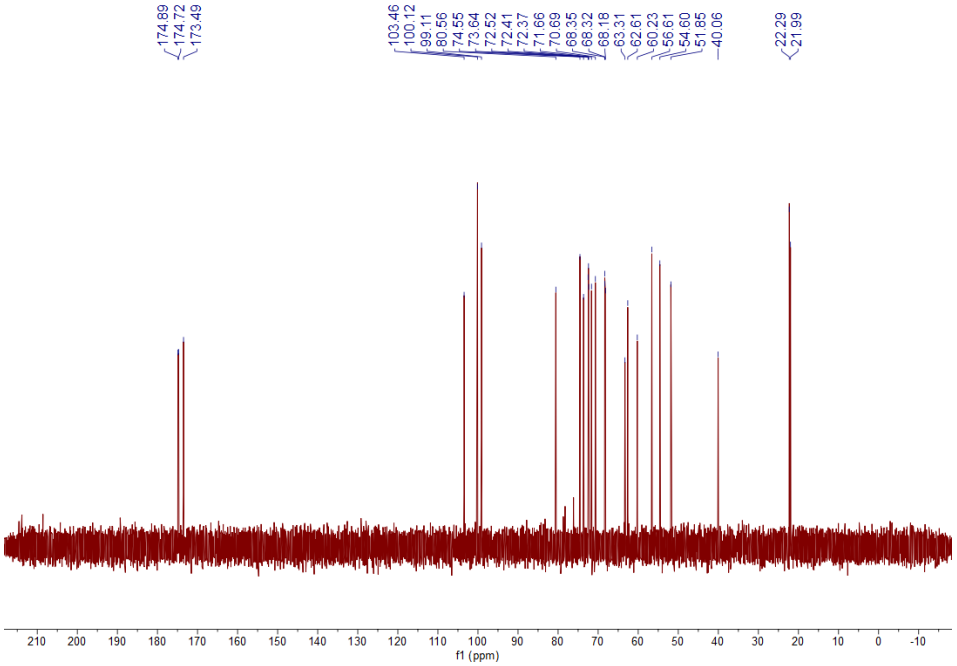
Compound **1**



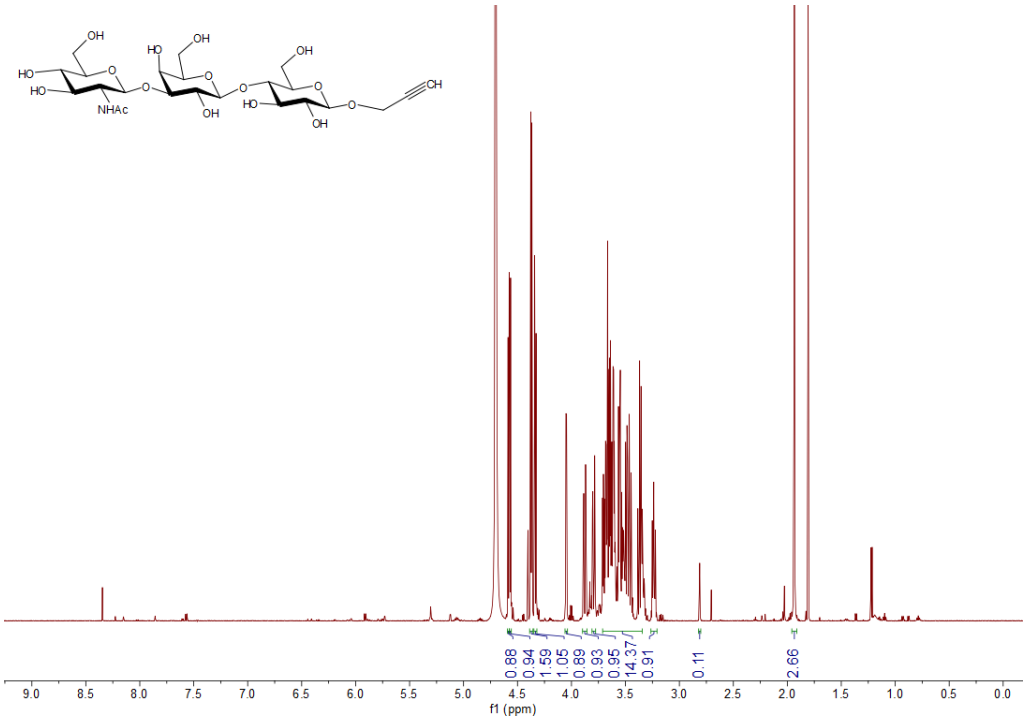


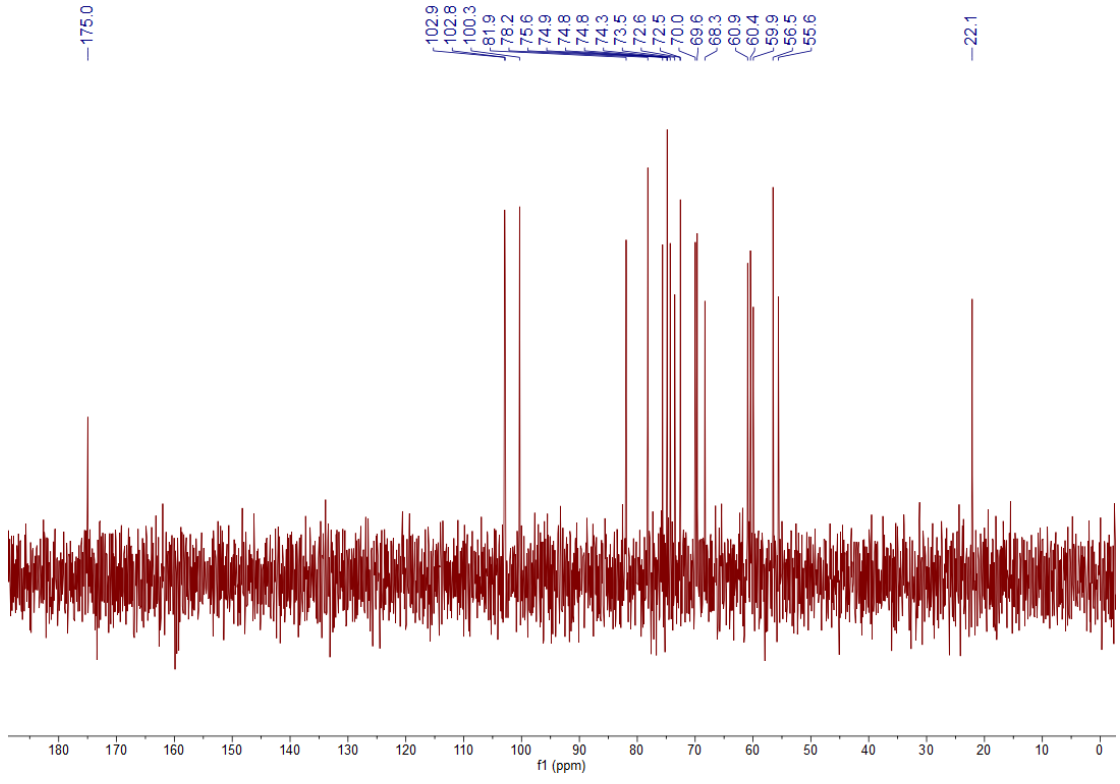
Compound **7**


1.14
1.06
1.88
19.97
0.76
0.99
3.04
3.00
1.00
f1 (ppm)

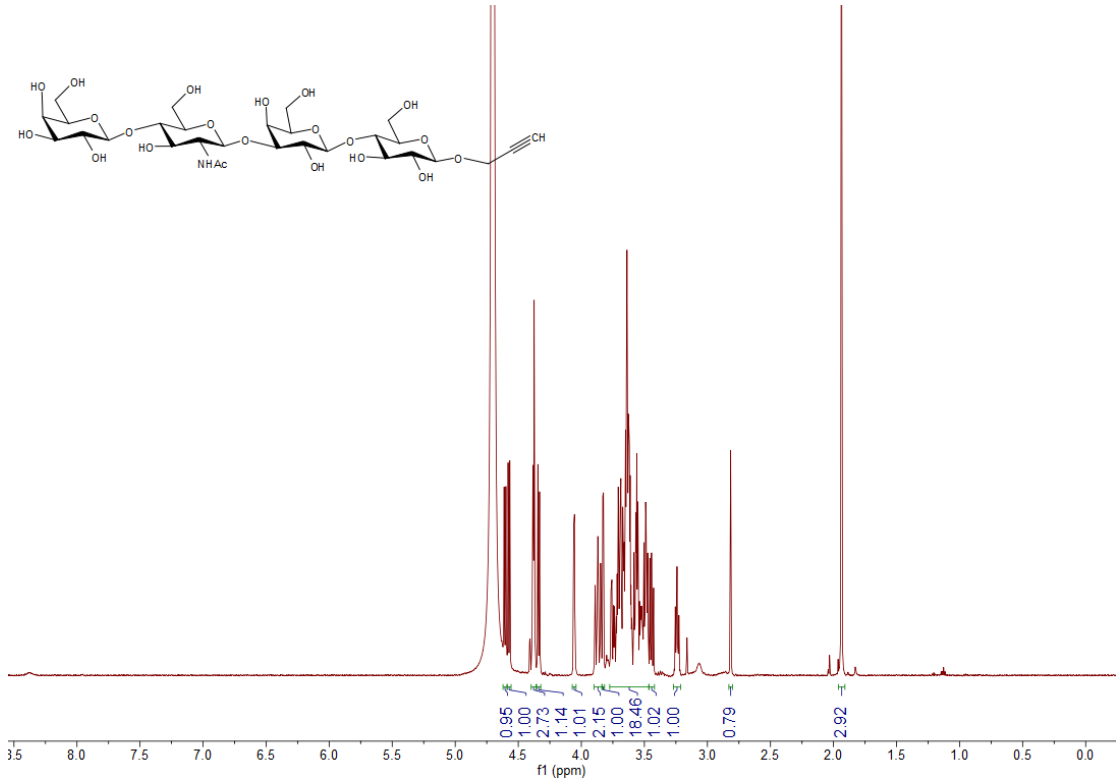

174.89
174.72
173.49
103.46
100.12
99.11
80.56
74.55
73.64
72.52
72.41
72.37
71.66
70.69
68.35
68.32
68.18
63.31
62.61
60.23
56.61
54.60
51.85
40.06
22.29
21.99
f1 (ppm)

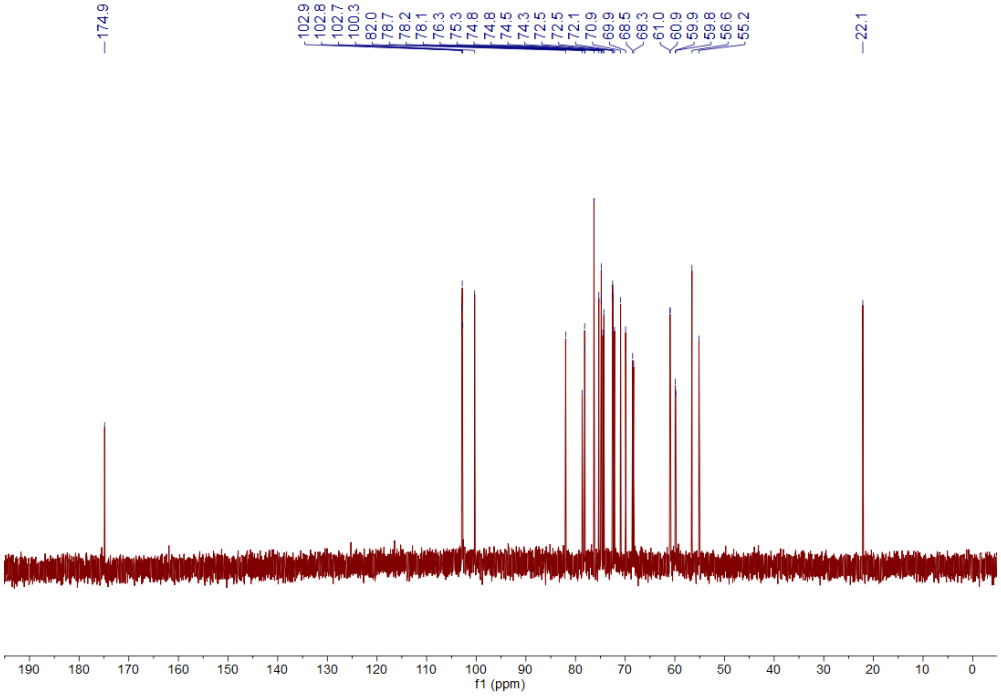
Compound **S1**


OH
HO
O
NHAc
OH
HO
OH
O
O
OH
HO
O
OH
O
CH
0.88
0.94
1.59
1.05
0.89
0.93
0.95
14.37
0.91
0.11
2.66
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

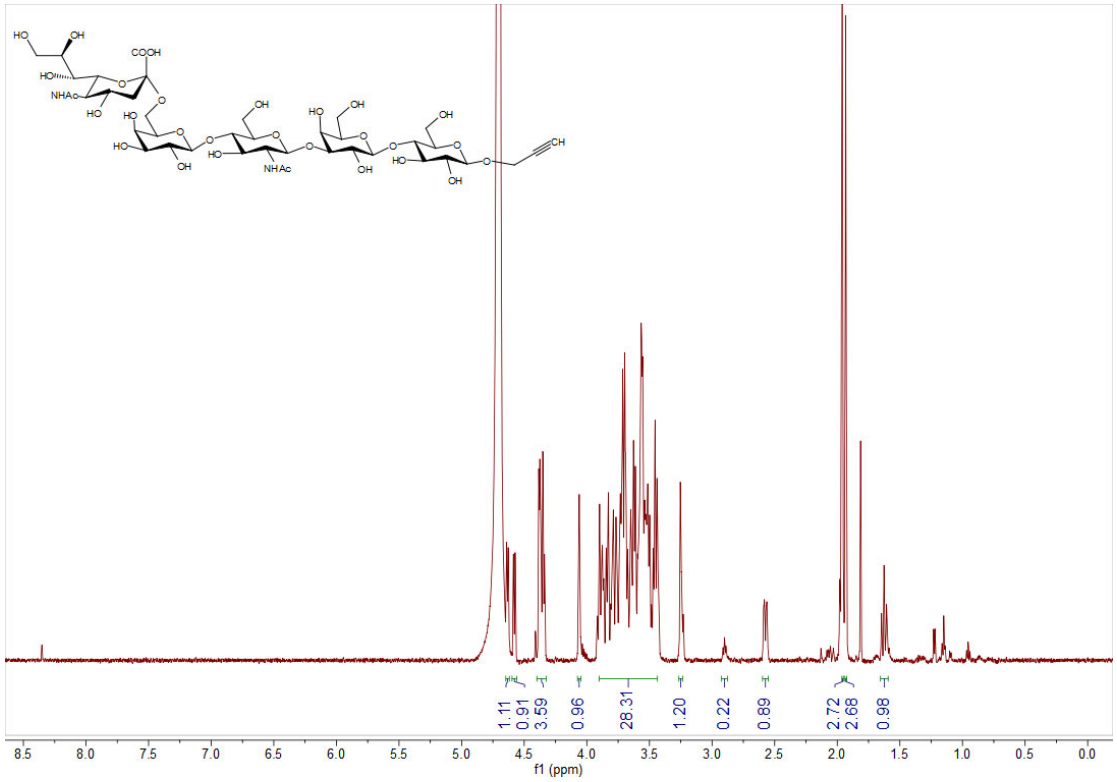

175.0
102.9
102.8
100.3
81.9
78.2
75.6
74.9
74.8
74.8
74.3
73.5
72.6
72.5
70.0
69.6
68.3
60.9
60.4
59.9
56.5
55.6
22.1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

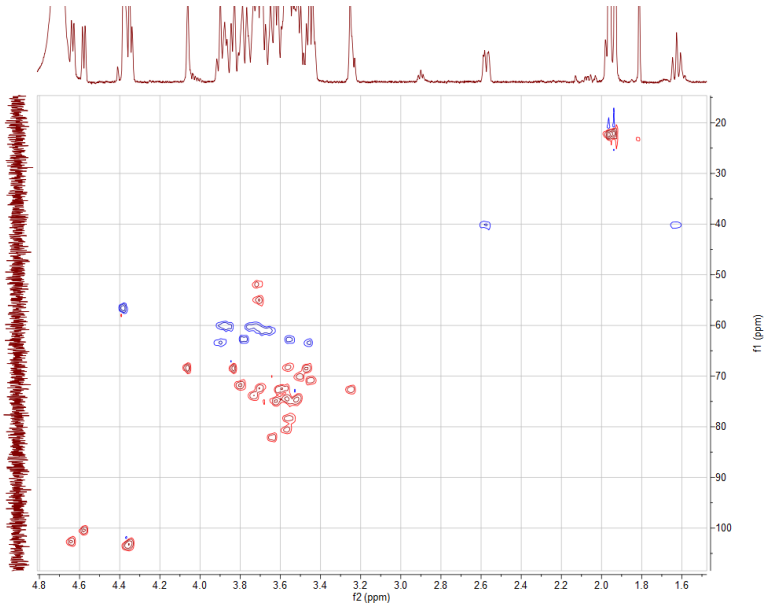
Compound **5**


0.95
1.00
2.73
1.14
1.01
2.15
1.00
18.46
1.02
1.00
0.79
2.92
f1 (ppm)

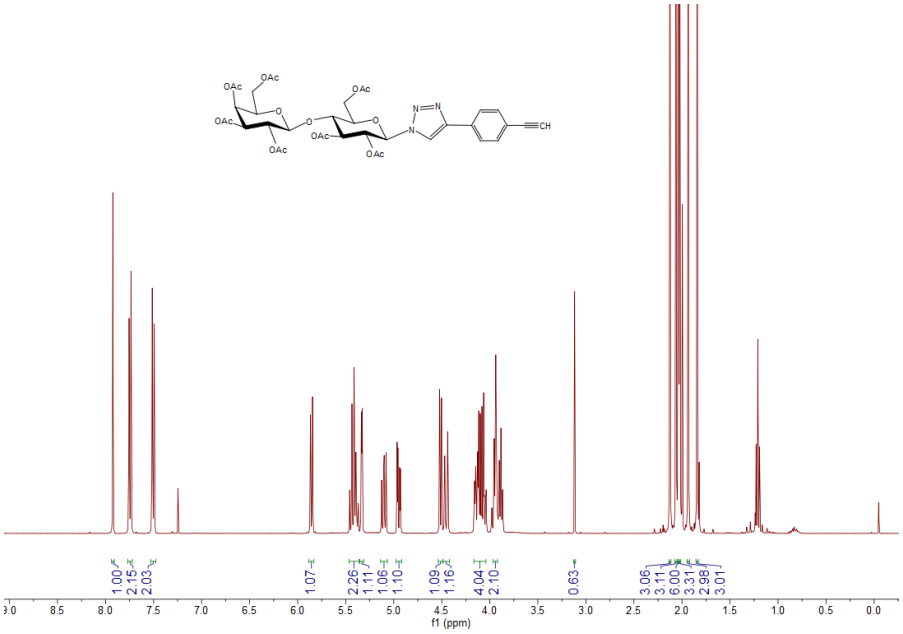

174.9
102.9
102.8
102.7
100.3
82.0
78.7
78.2
78.1
76.3
75.3
74.8
74.8
74.5
74.3
72.5
72.5
72.1
70.9
69.9
68.5
68.3
61.0
60.9
59.9
59.8
56.6
55.2
22.1
f1 (ppm)

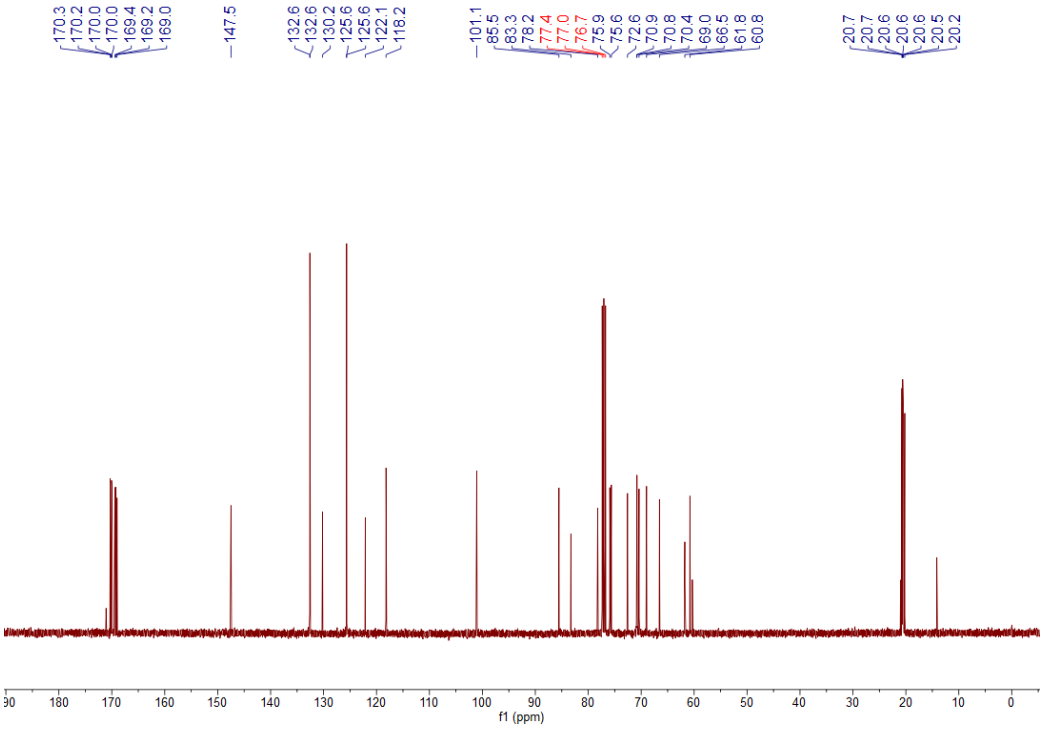
Compound **8**



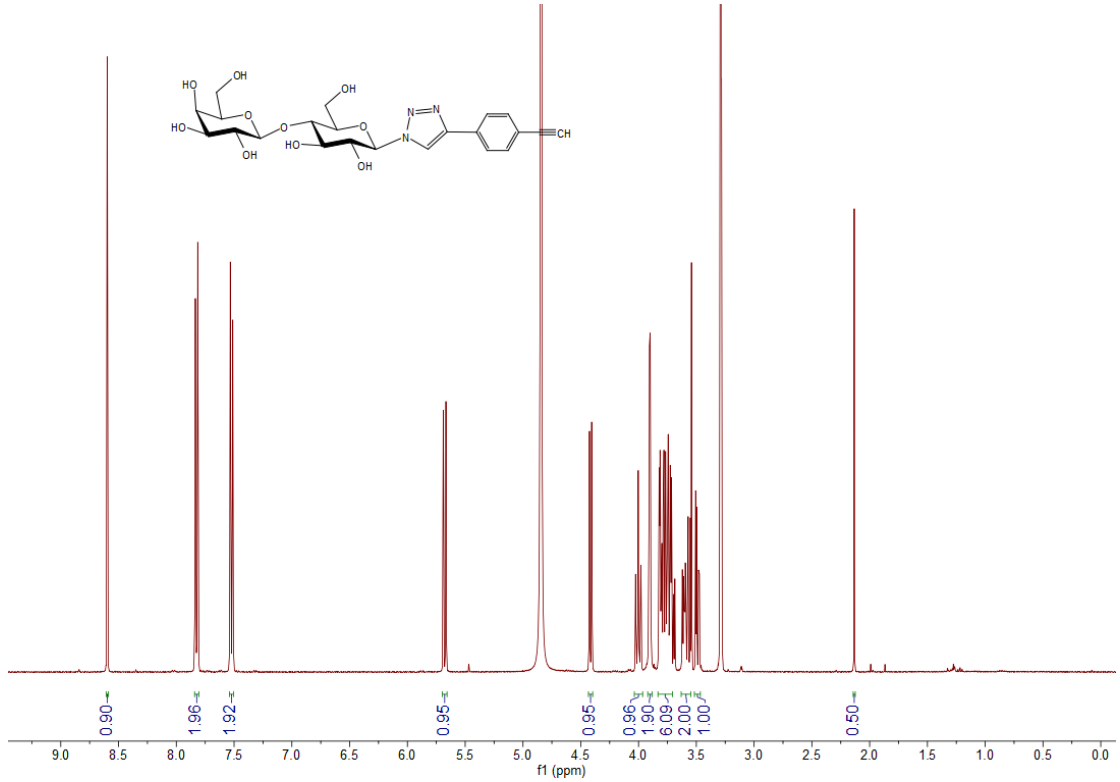


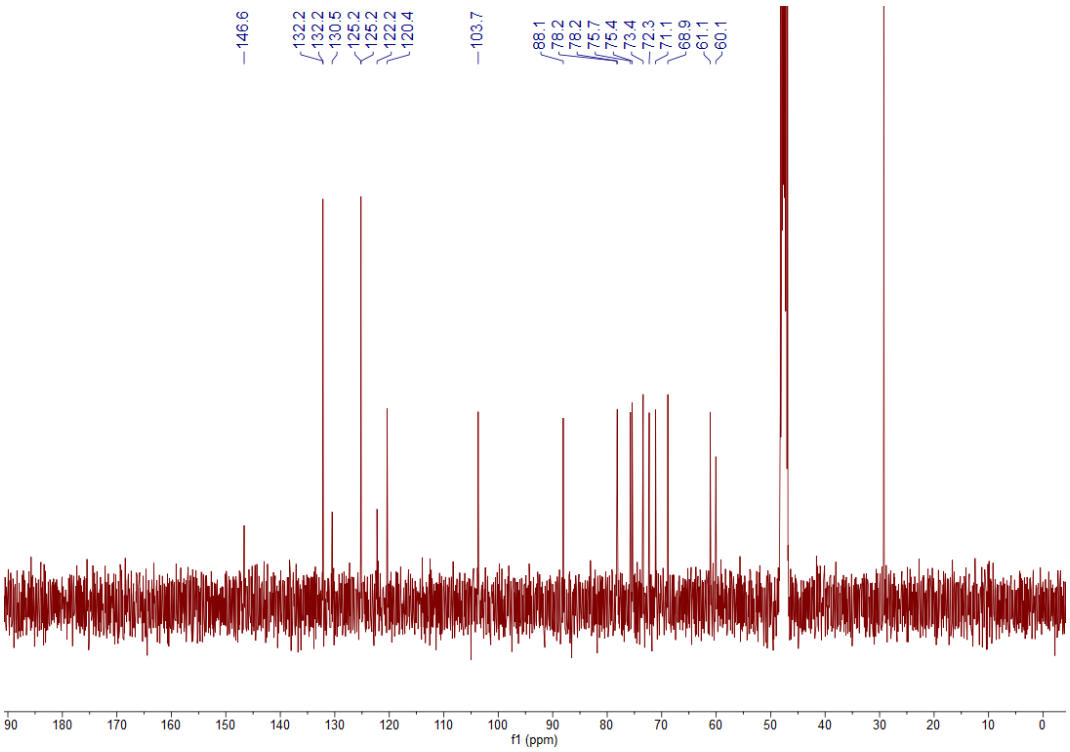
Compound **S3**


OAc
OAc
OAc
OAc
OAc
OAc
OAc
N=N
N
CH
1.00
2.15
2.03
1.07
2.26
1.11
1.06
1.10
1.09
1.16
4.04
2.10
0.63
3.06
3.11
6.00
3.31
2.98
3.01
f1 (ppm)

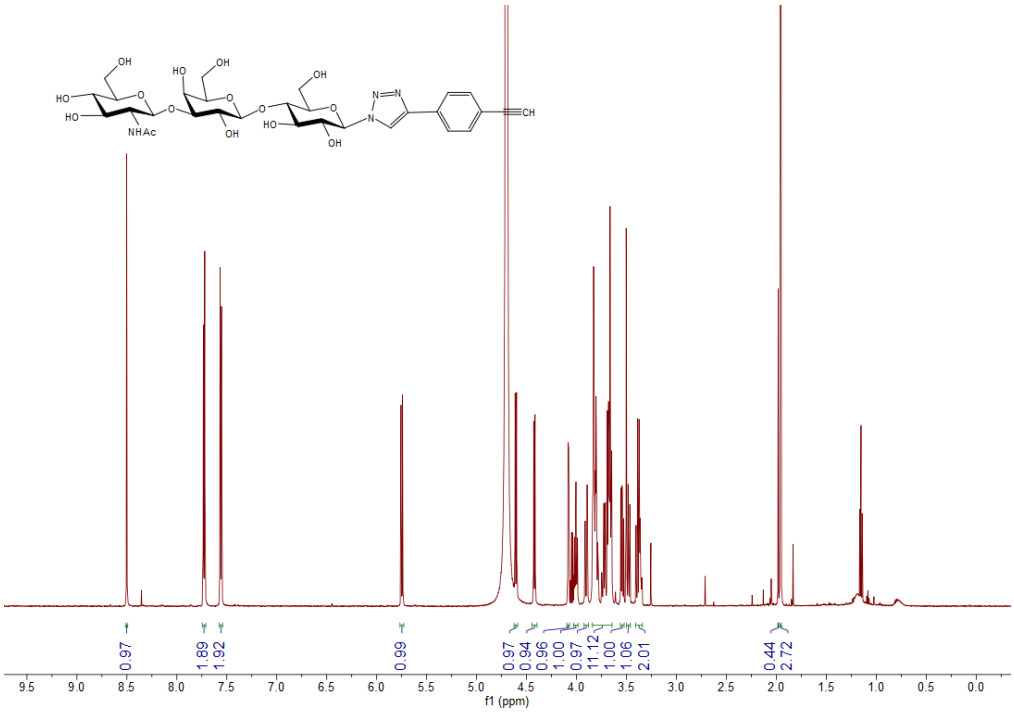

170.3
170.2
170.0
170.0
169.4
169.2
169.0
147.5
132.6
132.6
130.2
125.6
125.6
122.1
118.2
101.1
85.5
83.3
78.2
77.4
77.0
76.7
75.9
75.6
72.6
70.9
70.8
70.4
69.0
66.5
61.8
60.8
20.7
20.7
20.6
20.6
20.6
20.5
20.2
f1 (ppm)

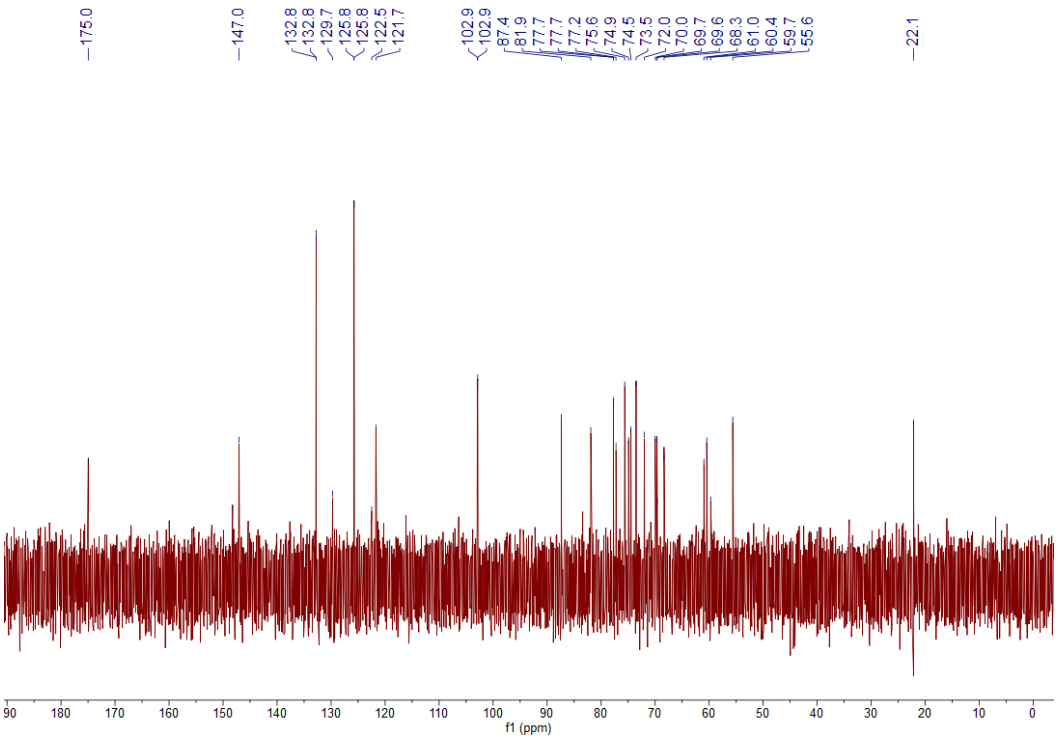
Compound **S4**


HO
OH
O
HO
OH
O
HO
OH
O
N
N
N
CH
0.90
1.96
1.92
0.95
0.95
0.96
1.90
6.09
2.00
1.00
0.50
f1 (ppm)

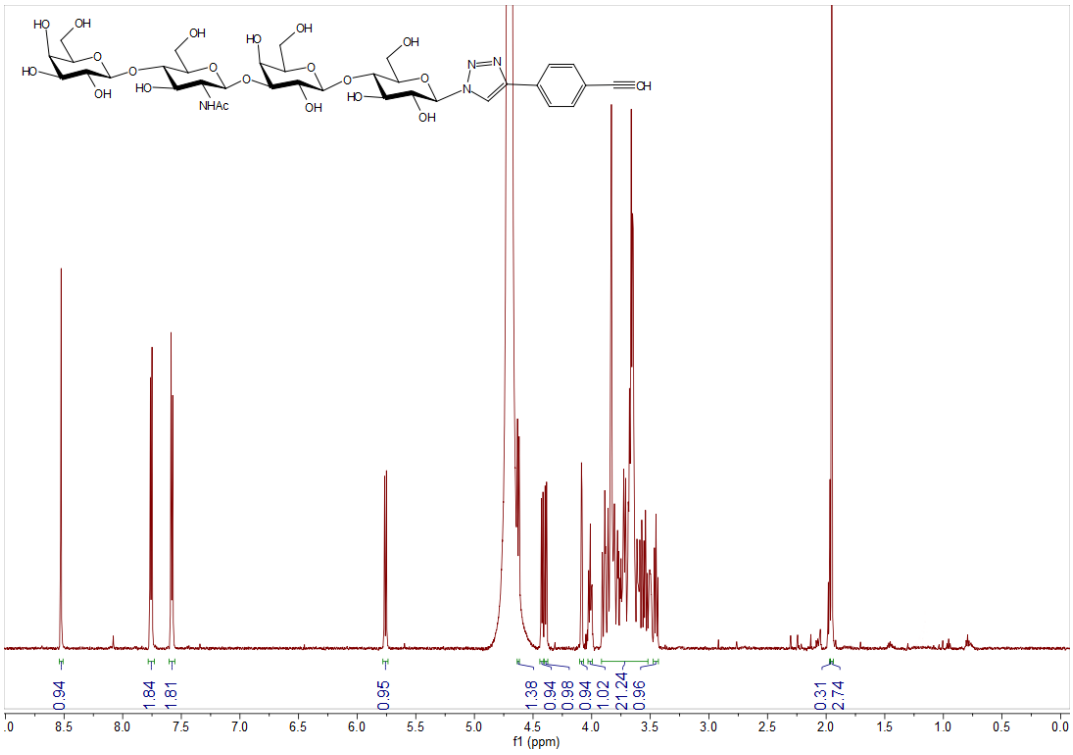

146.6
132.2
132.2
130.5
125.2
125.2
122.2
120.4
103.7
88.1
78.2
78.2
75.7
75.4
73.4
72.3
71.1
68.9
61.1
60.1
f1 (ppm)

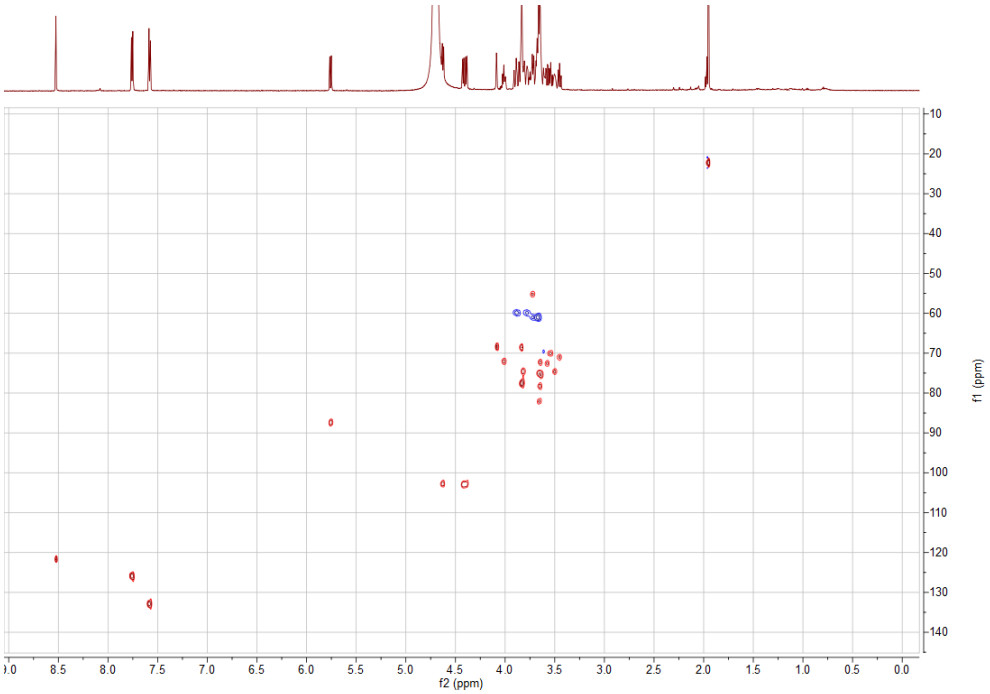
Compound **S5**


0.97
1.89
1.92
0.99
0.97
0.94
0.96
1.00
0.97
11.12
1.00
1.06
2.01
0.44
2.72
f1 (ppm)

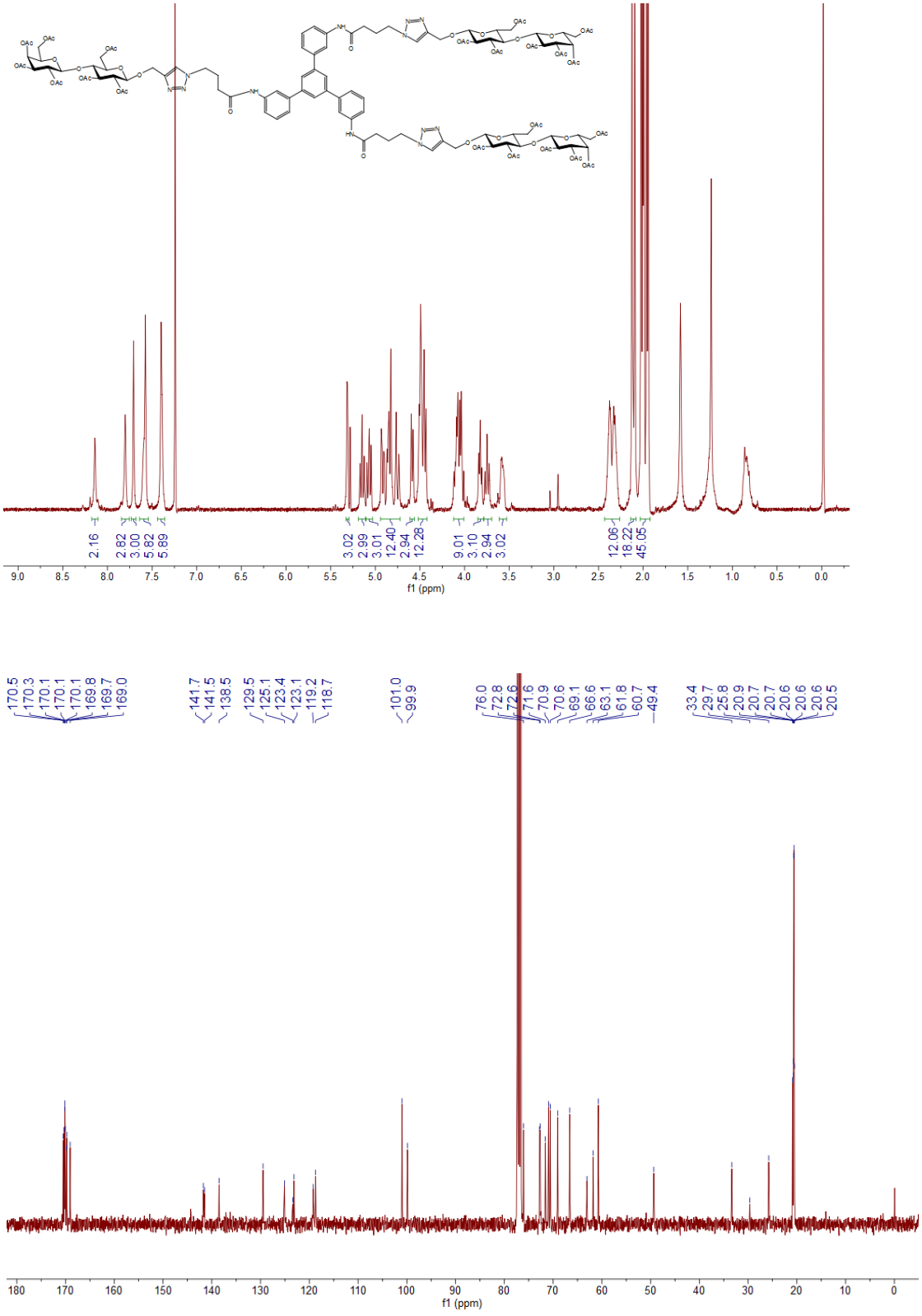

175.0
147.0
132.8
132.8
129.7
125.8
125.8
122.5
121.7
102.9
102.9
87.4
81.9
77.7
77.7
77.2
75.6
74.9
74.5
73.5
72.0
70.0
69.7
69.6
68.3
61.0
60.4
59.7
55.6
22.1
f1 (ppm)

Compound **6**

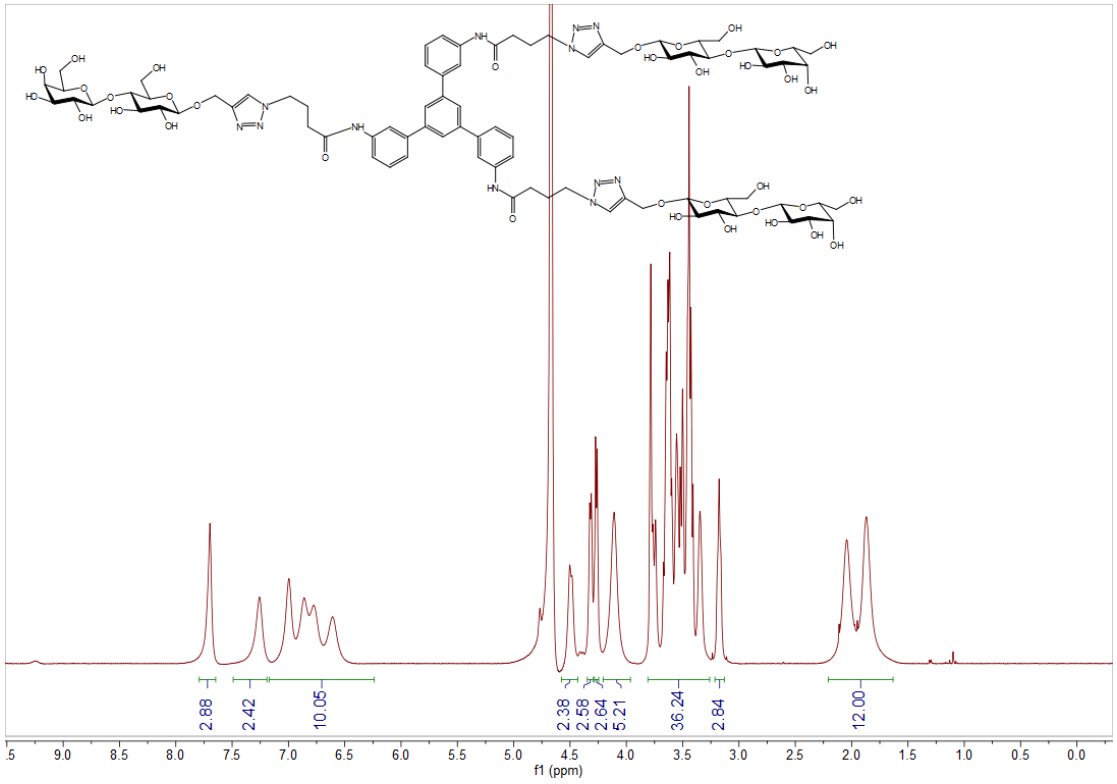

HO
OH
O
HO
OH
NHAc
N=N
N
OH
0.94
1.84
1.81
0.95
1.38
0.94
0.98
0.94
1.02
21.24
0.96
0.31
2.74
f1 (ppm)

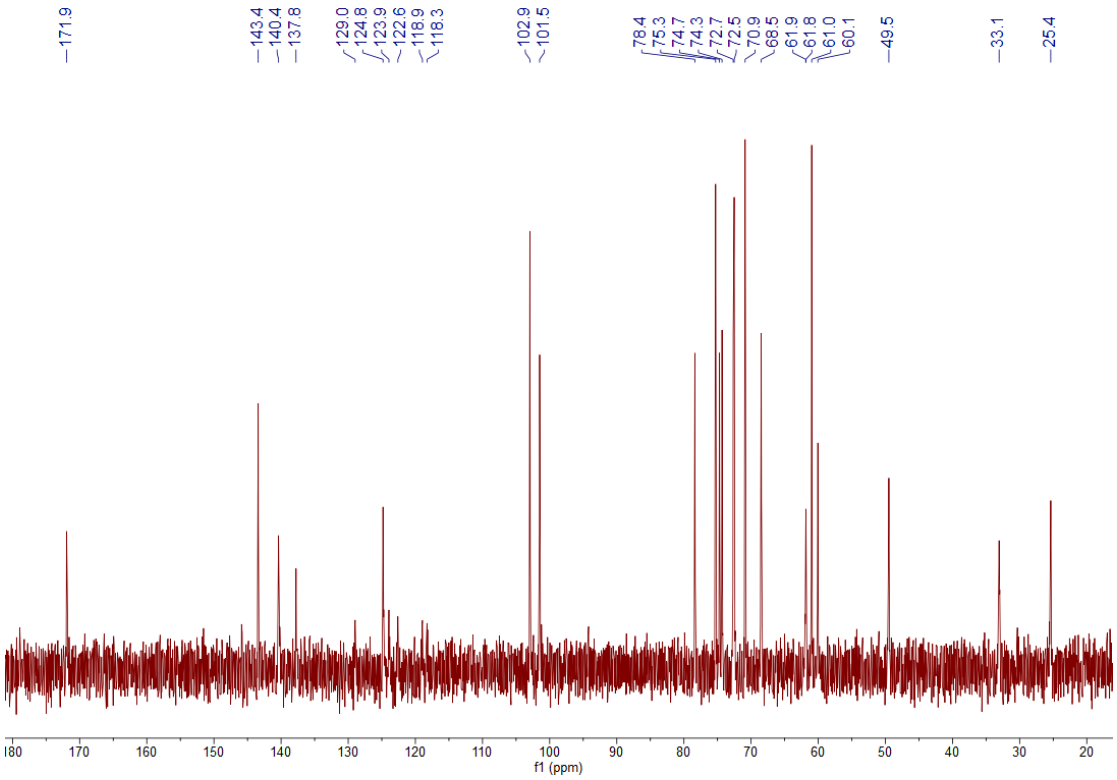

f2 (ppm)
f1 (ppm)

Compound **9a**

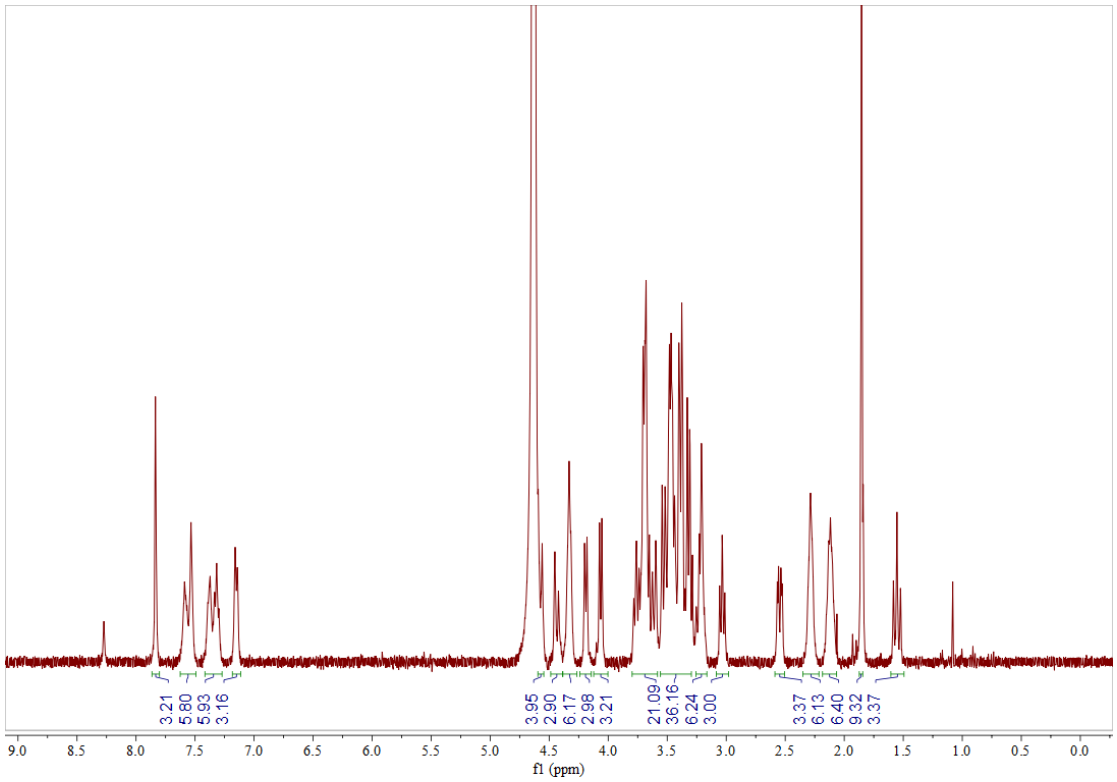

2.16
2.82
3.00
5.82
5.89
3.02
2.99
3.01
12.40
2.94
12.28
9.01
3.10
2.94
3.02
12.06
18.22
45.05
f1 (ppm)
170.5
170.3
170.1
170.1
170.1
169.8
169.7
169.0
141.7
141.5
138.5
129.5
125.1
123.4
123.1
119.2
118.7
101.0
99.9
76.0
72.8
72.6
71.6
70.9
70.6
69.1
66.6
63.1
61.8
60.7
49.4
33.4
29.7
25.8
20.9
20.7
20.7
20.6
20.6
20.6
20.5
f1 (ppm)

Compound **9b**

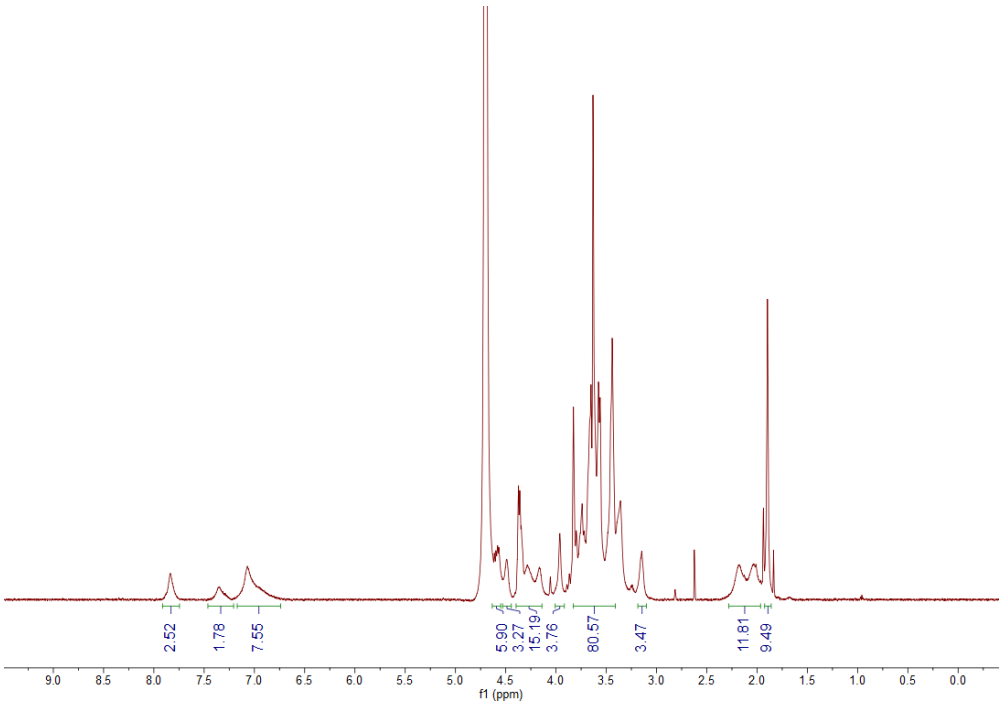

2.88
2.42
10.05
2.38
2.58
2.64
5.21
36.24
2.84
12.00
f1 (ppm)


171.9
143.4
140.4
137.8
129.0
124.8
123.9
122.6
118.9
118.3
102.9
101.5
78.4
75.3
74.7
74.3
72.7
72.5
70.9
68.5
61.9
61.8
61.0
60.1
49.5
33.1
25.4
f1 (ppm)

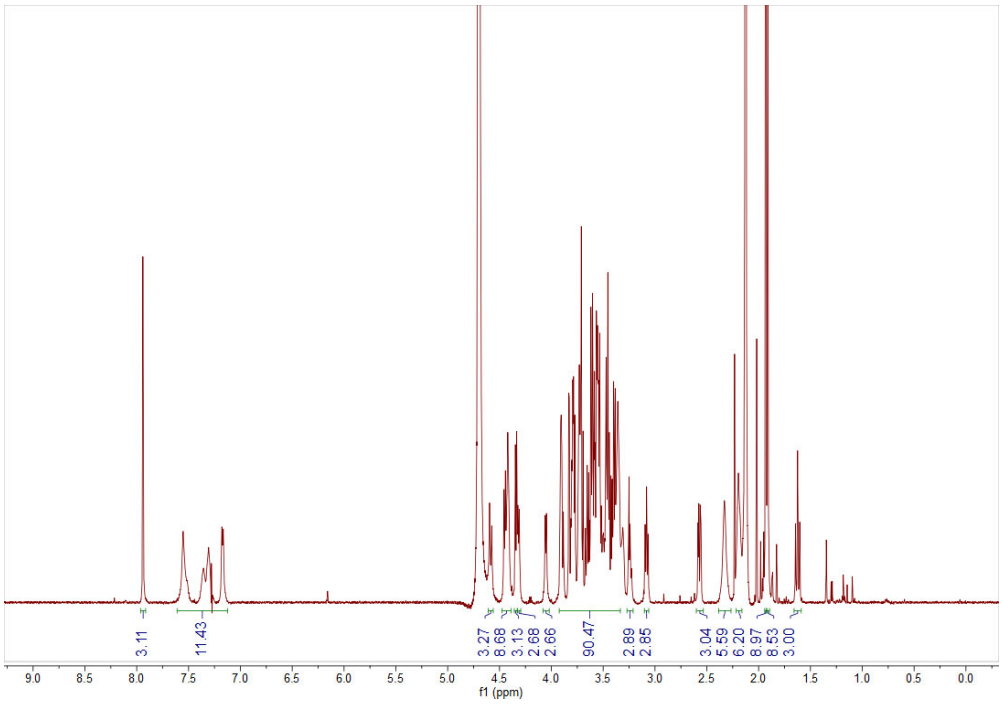
Compound **10**

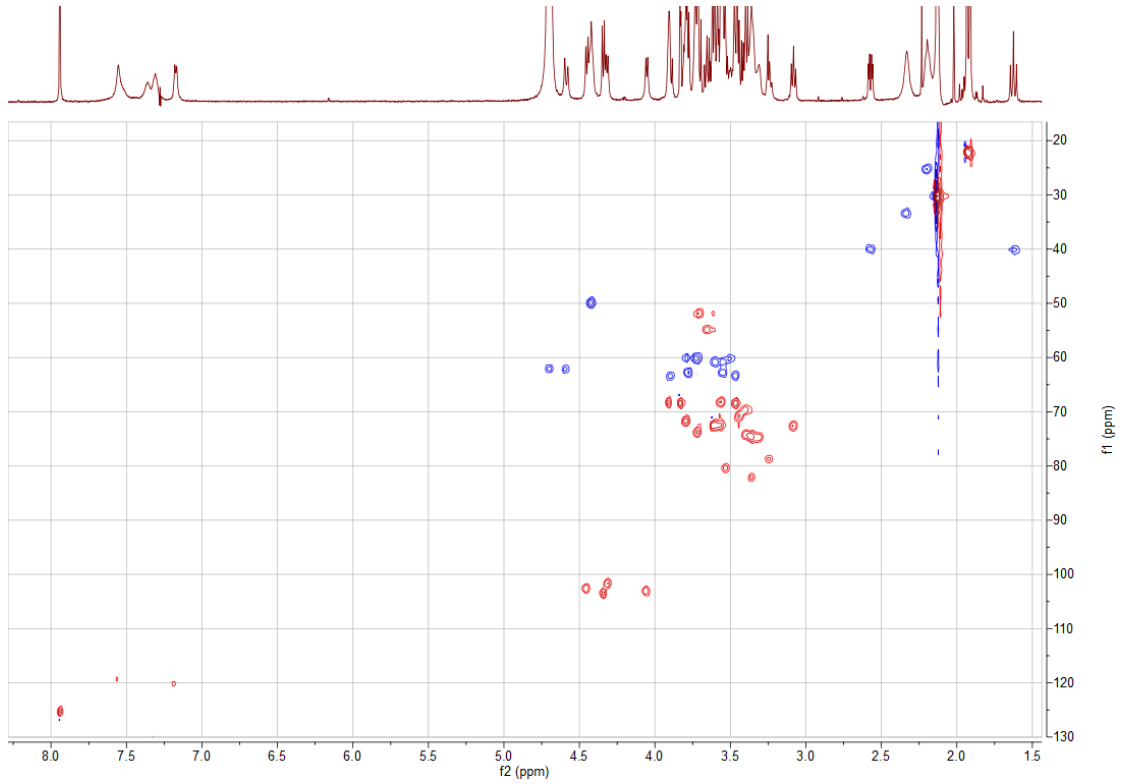


Compound **11**

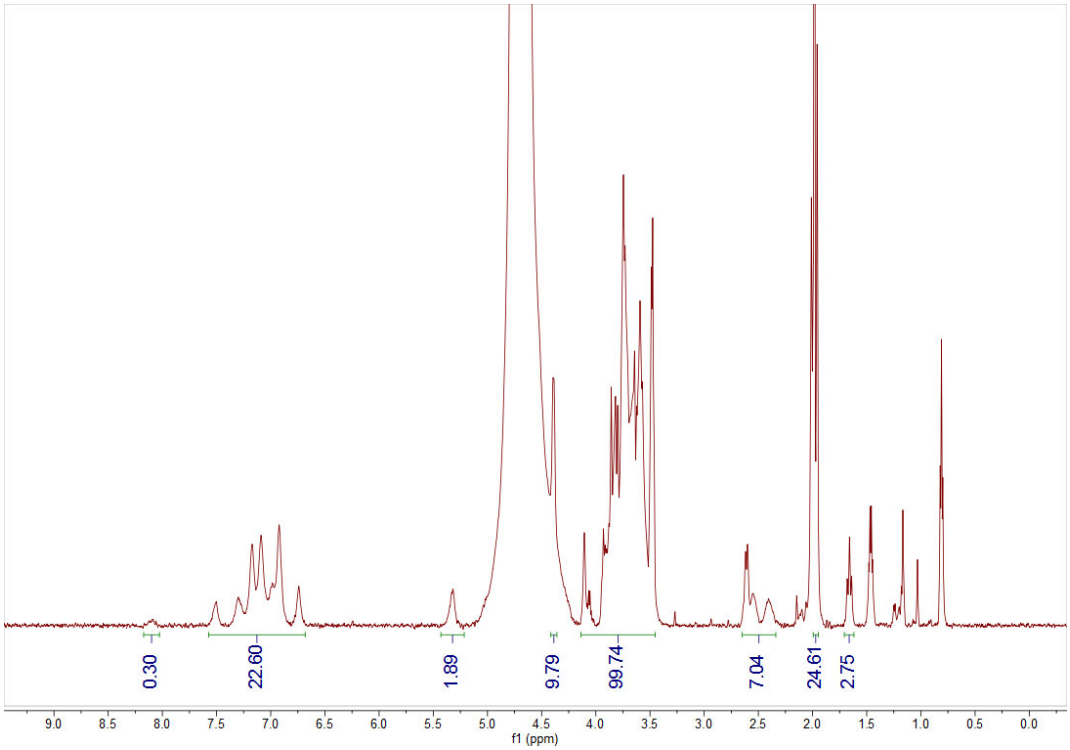


Compound **12**

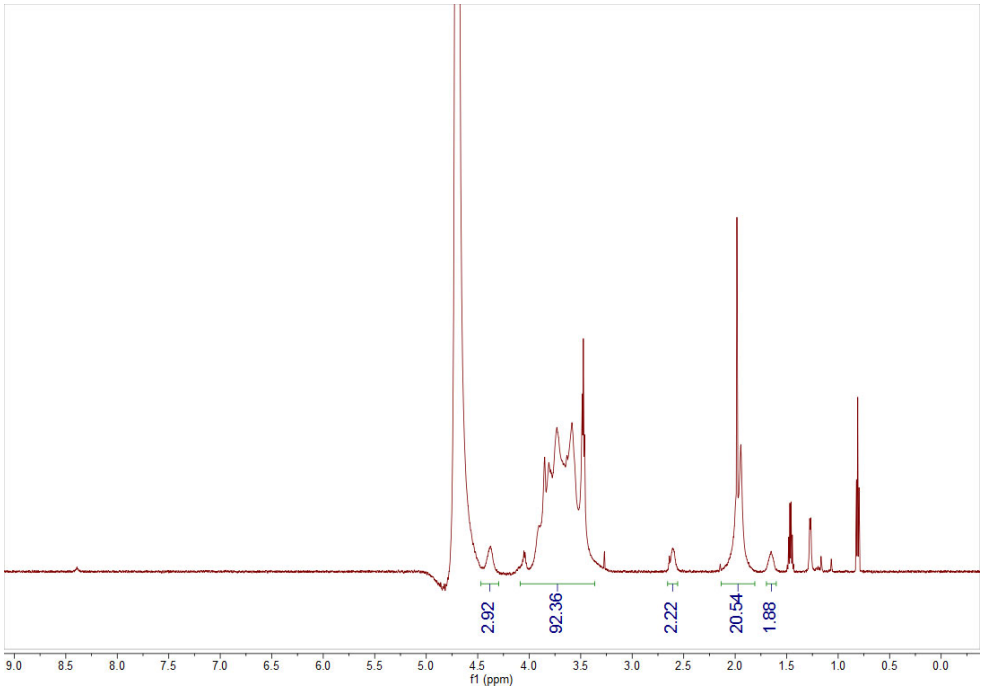

3.11
11.43
3.27
8.68
3.13
2.68
2.66
90.47
2.89
2.85
3.04
5.59
6.20
8.97
8.53
3.00
f1 (ppm)


f2 (ppm)
f1 (ppm)

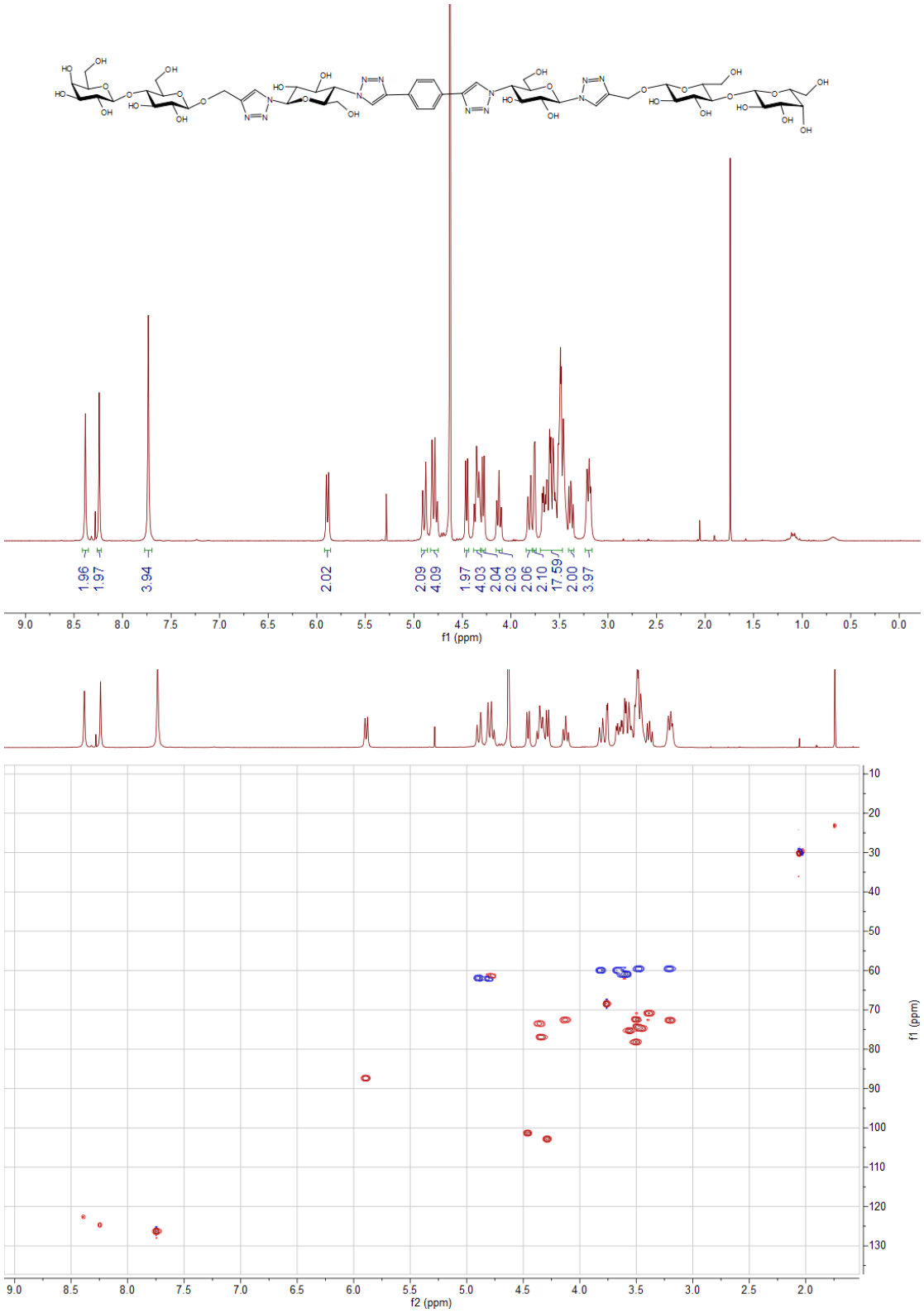
Compound **14**



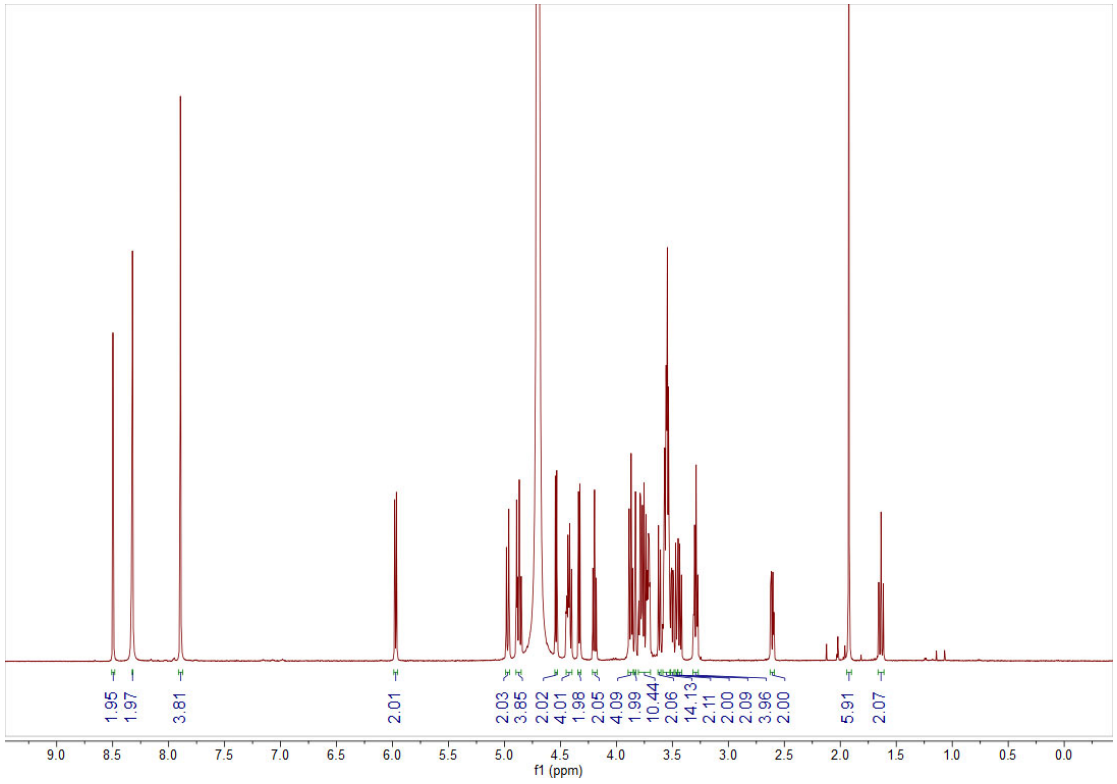
Compound **15**

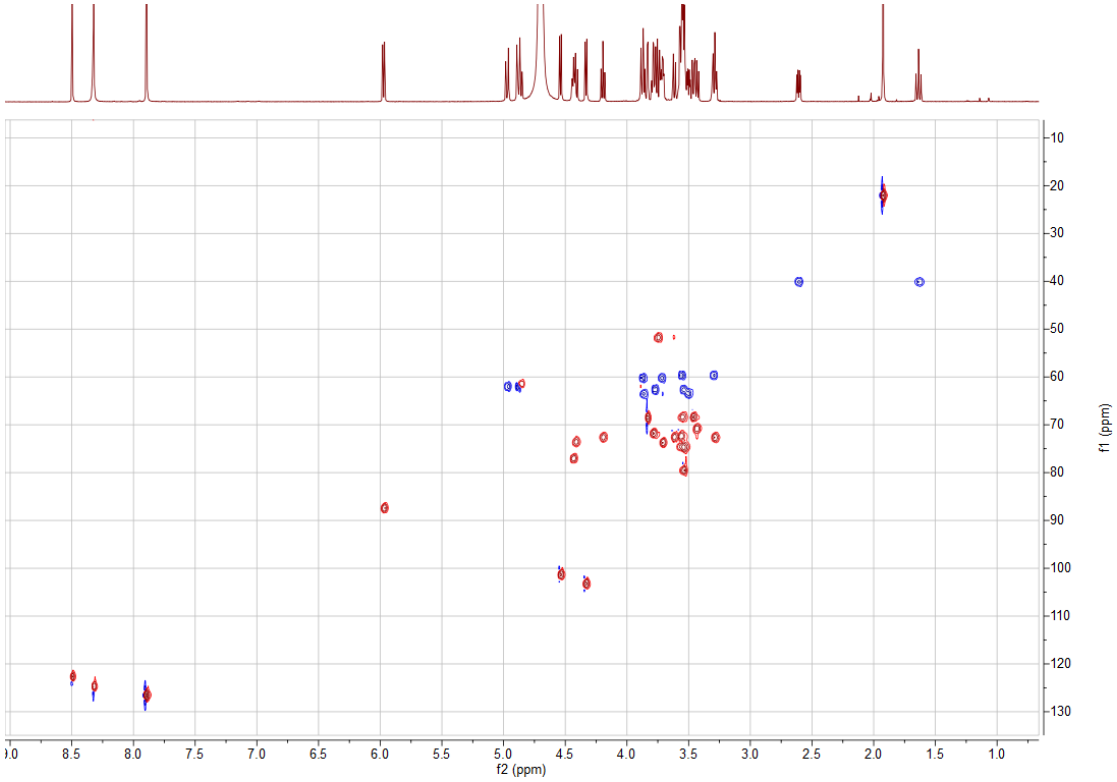


Compound **16**


1.96
1.97
3.94
2.02
2.09
4.09
1.97
4.03
2.04
2.03
2.06
2.10
17.59
2.00
3.97
f1 (ppm)
f2 (ppm)
f1 (ppm)

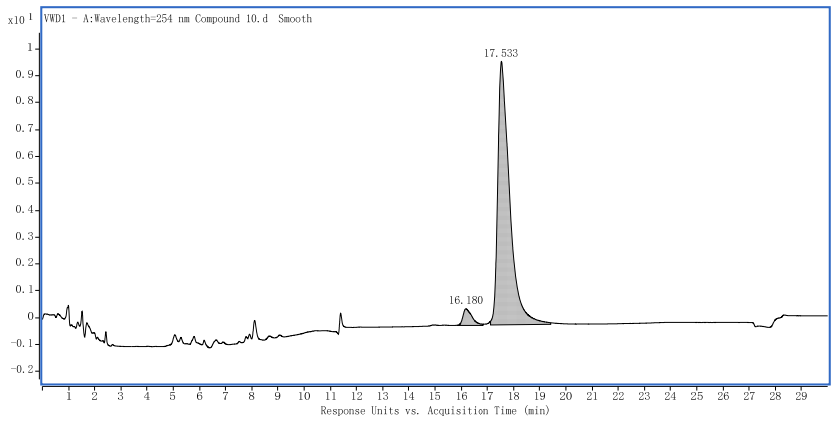
Compound **17**


1.95
1.97
3.81
2.01
2.03
3.85
2.02
4.01
1.98
2.05
4.09
1.99
10.44
2.06
14.13
2.11
2.00
2.09
3.96
2.00
5.91
2.07
f1 (ppm)

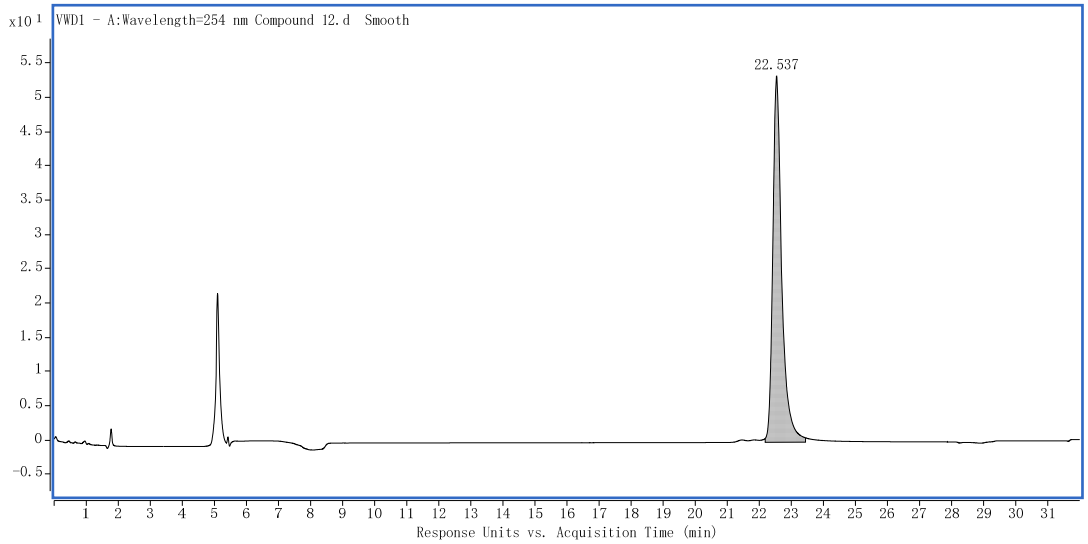

f2 (ppm)
f1 (ppm)

HPLC spectra (tested compounds were .95% pure)

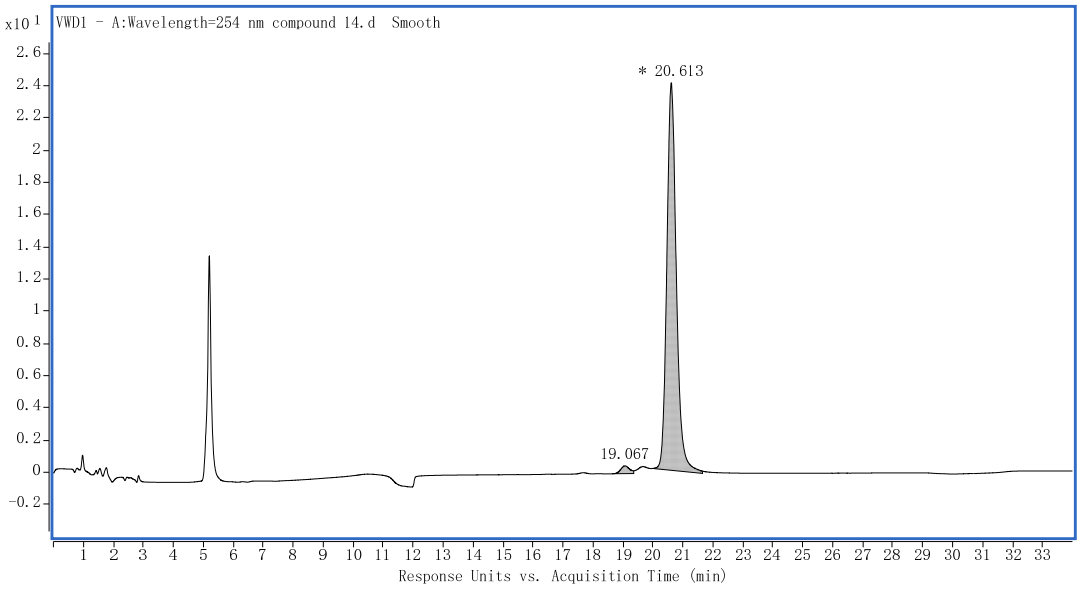
Compound **10** (95.8 %)



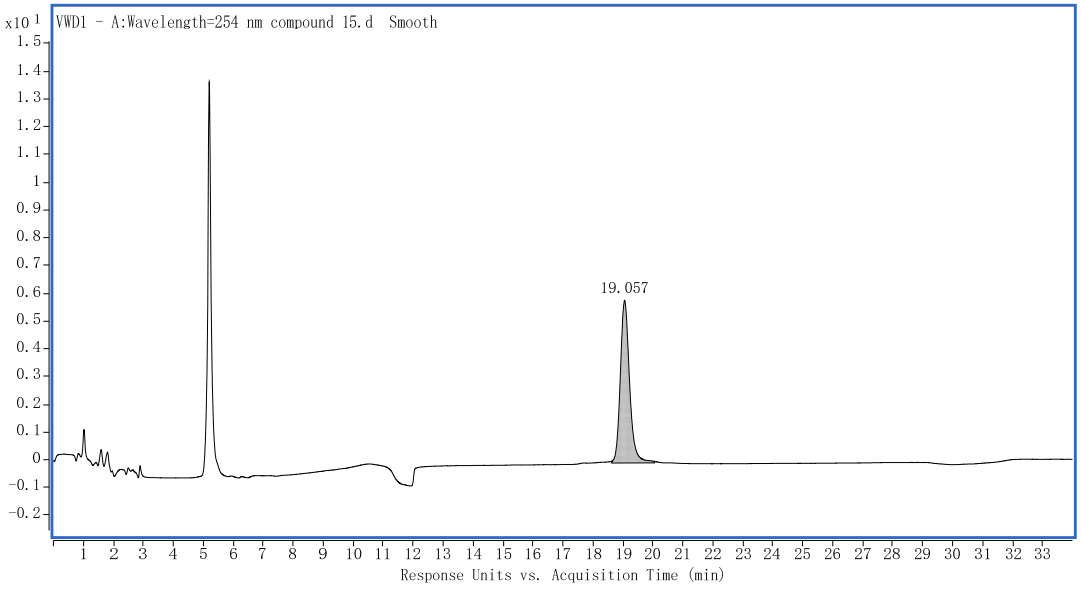
Compound **12** (99.9 %)



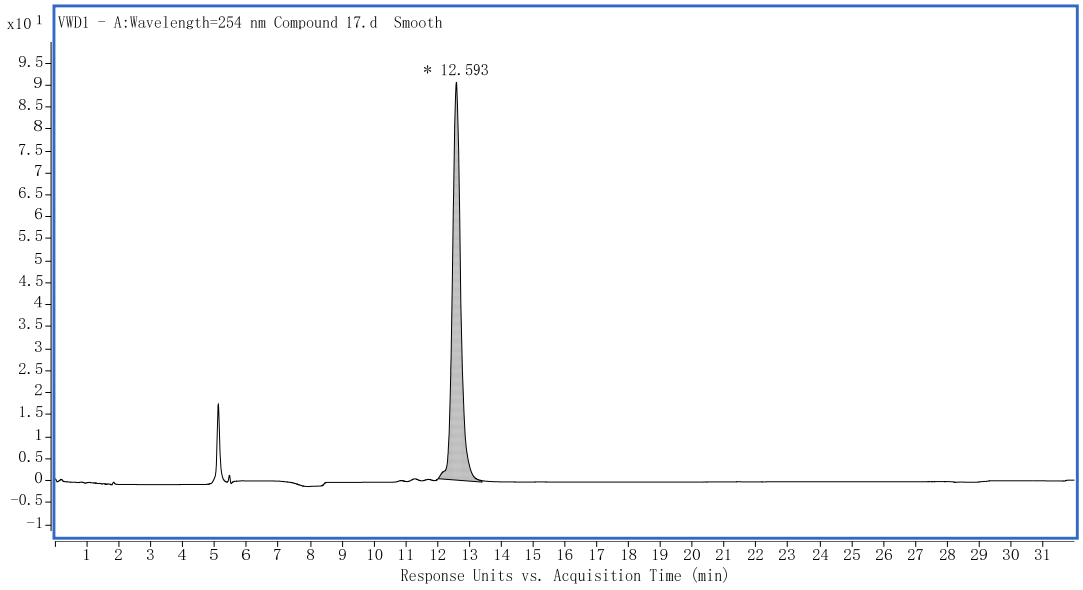
Compound **14** (98.1 %)



Compound **15** (99.9 %)

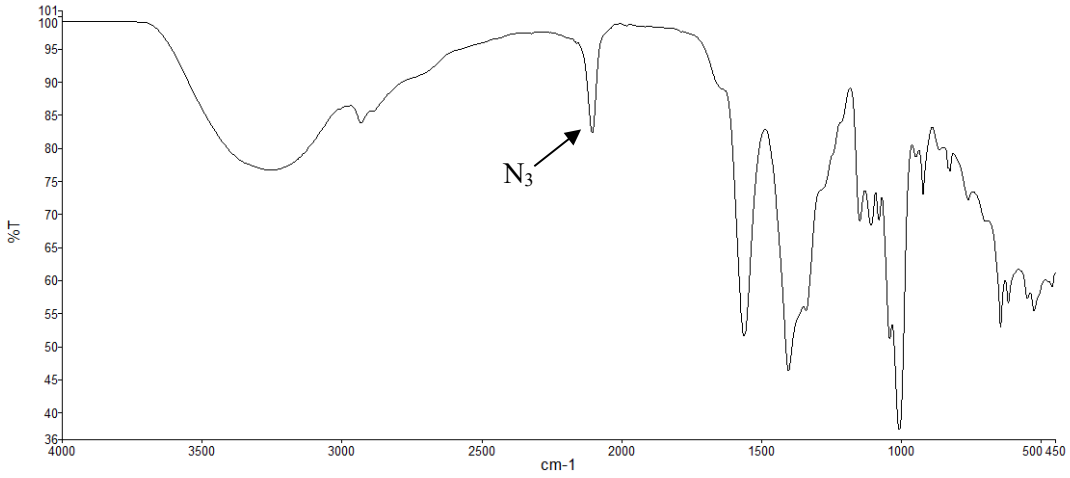


Compound **17** (99.9 %)

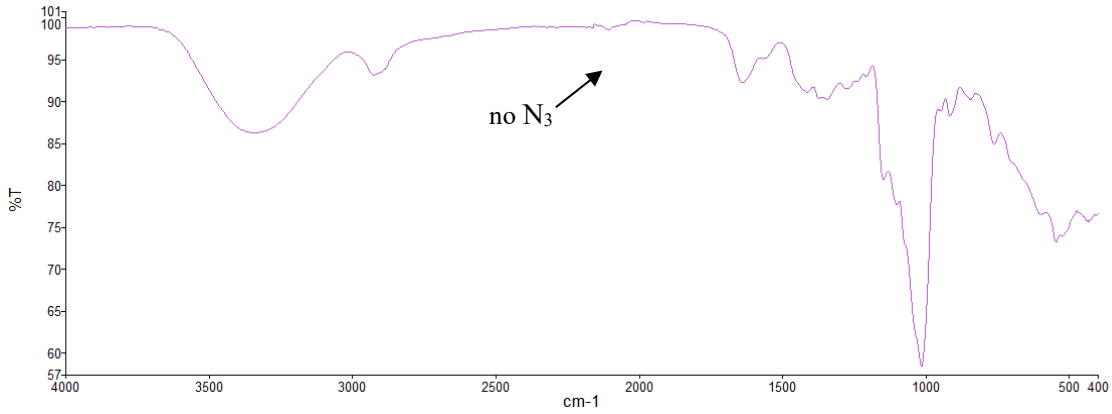


IR spectra

Azido-Dextran **3**



Polymer **18**



References

(1) Pahimanolis, N.; Vesterinen, A. H.; Rich, J.; Seppala, J. Modification of Dextran Using Click-Chemistry Approach in Aqueous Media. *Carbohydr. Polym.* **2010**, *82*, 78–82.

(2) Moni, L.; Ciogli, A.; D'Acquarica, I.; Dondoni, A.; Gasparrini, F.; Marra, A. Synthesis of Sugar-Based Silica Gels by Copper-Catalysed Azide-Alkyne Cycloaddition via a Single-Step Azido-Activated Silica Intermediate and the Use of the Gels in Hydrophilic Interaction Chromatography. *Chem. - A Eur. J.* **2010**, *16*, 5712–5722.

(3) Peng, W.; Pranskevich, J.; Nycholat, C.; Gilbert, M.; Wakarchuk, W.; Paulson, J. C.; Razi, N. Helicobacter Pylori 1,3-N-Acetylglucosaminyltransferase for Versatile Synthesis of Type 1 and Type 2 Poly-LacNAcs on N-Linked, O-Linked and I-Antigen Glycans. *Glycobiology* **2012**, *22*, 1453–1464.

(4) Blixt, O.; Brown, J.; Schur, M. J.; Wakarchuk, W.; Paulson, J. C. Efficient Preparation of Natural and Synthetic Galactosides with a Recombinant β-1,4-Galactosyltransferase-/UDP-4′-Gal Epimerase Fusion Protein. *J. Org. Chem.* **2001**, *66*, 2442–2448.

(5) McArthur, J. B.; Yu, H.; Zeng, J.; Chen, X. Converting Pasteurella Multocida A2-3-Sialyltransferase 1 (PmST1) to a Regioselective A2-6-Sialyltransferase by Saturation Mutagenesis and Regioselective Screening. *Org. Biomol. Chem.* **2017**, *15*, 1700–1709.

(6) Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. Analysis and Optimization of Copper-Catalyzed Azide-Alkyne Cycloaddition for Bioconjugation. *Angew. Chemie - Int. Ed.* **2009**, *48*, 9879–9883.

(7) Yu, G.; Vicini, A. C.; Pieters, R. J. Assembling of Divalent Ligands and Their Effect on Divalent Binding to Pseudomonas Aeruginosa Lectin LecA. *J. Org. Chem.* **2019**, *84*, 2470–2488.

(8) Koel, B.; Burke, D. F.; Bestebroer, T. M.; Vliet, S. Van Der; Zondag, G. C. M.; Vervaet, G.; Skepner, E.; Lewis, N. S.; Spronken, M. I. J.; Russell, C. A.; et al. Substitutions Near the Receptor Binding Site Determine Major Antigenic Change During Influenza Virus Evolution. *Science* **2013**, *342*, 976–980.

(9) Du, W.; Dai, M.; Li, Z.; Boons, G.-J.; Peeters, B.; van Kuppeveld, F. J. M.; de Vries, E.; de Haan, C. A. M. Substrate Binding by the Second Sialic Acid-Binding Site of Influenza A Virus N1 Neuraminidase Contributes to Enzymatic Activity. *J. Virol.* **2018**, *92*, e01243–e01248.

(10) Munster, V. J.; Wit, E. De; Brand, J. M. A. Van Den; Herfst, S.; Schrauwen, E. J. A.; Bestebroer, T. M.; Vijver, D. Van De; Boucher, C. A.; Koopmans, M.; Rimmelzwaan, G. F.; et al. Pathogenesis and Transmission of Swine-Origin 2009 A(H1N1) Influenza Virus in Ferrets. *Science* **2009**, *503*, 481–483.

(11) Guo, H.; Rabouw, H.; Slomp, A.; Dai, M.; van der Vegt, F.; van Lent, J. W. M.; McBride, R.; Paulson, J. C.; de Groot, R. J.; van Kuppeveld, Frank, J. M.; et al. Kinetic Analysis of the Influenza A Virus HA/NA Balance Reveals Contribution OfNA to Virus- Receptor Binding and NA-Dependent Rolling on Receptor-Containing Surfaces. *PLoS Pathog.* **2018**, *14*, e1007233.

(12) de Vries, E.; Tscherne, D. M.; Wienholts, M. J.; Cobos-Jiménez, V.; Scholte, F.; García-Sastre, A.; Rottier, P. J. M.; de Haan, C. A. M. Dissection of the Influenza a Virus Endocytic Routes Reveals Macropinocytosis as an Alternative Entry Pathway. *PLoS Pathog.* **2011**, *7*, e1001329.