Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Novel hemagglutinin-binding sulfated oligosaccharides and their effect on influenza virus infection

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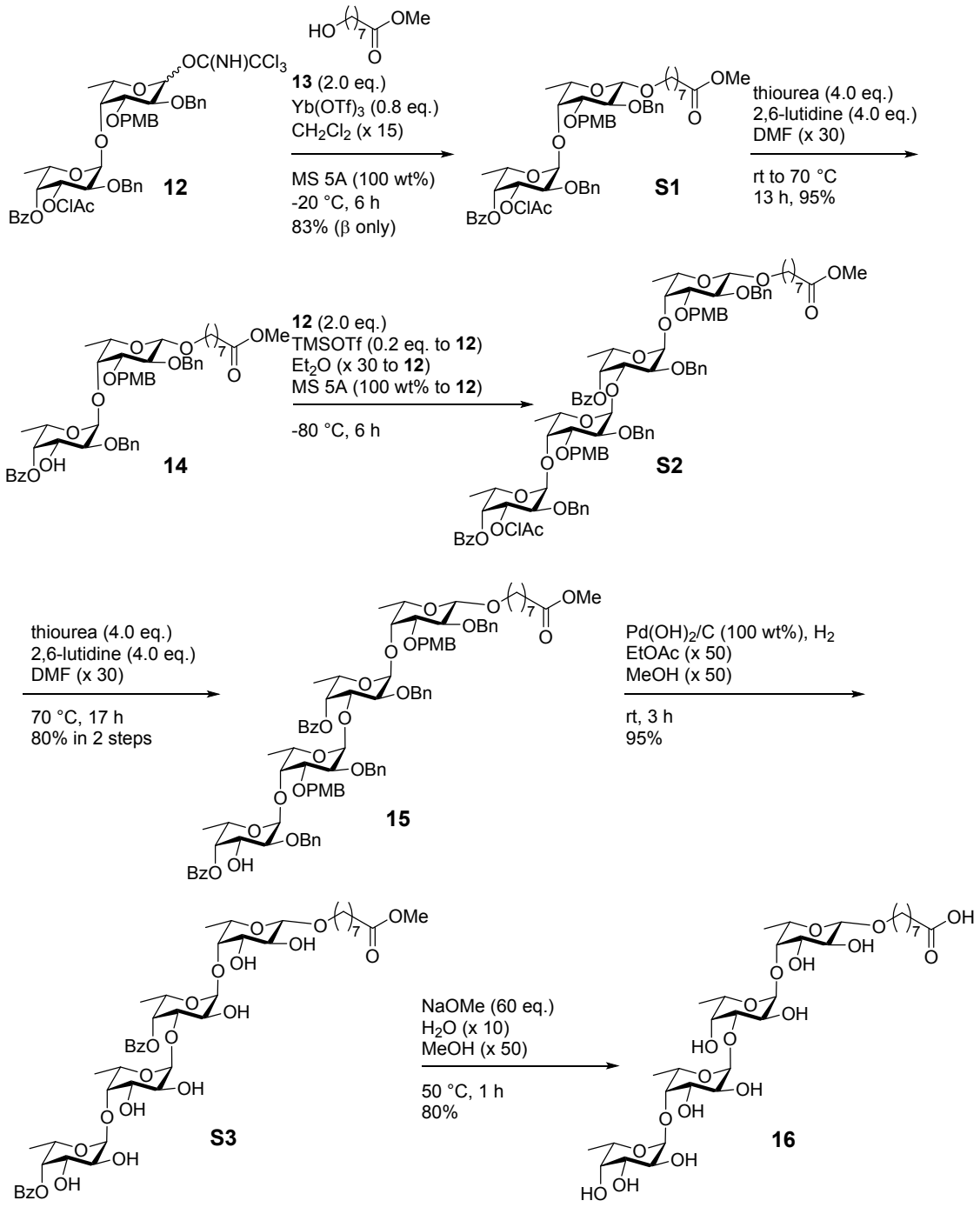
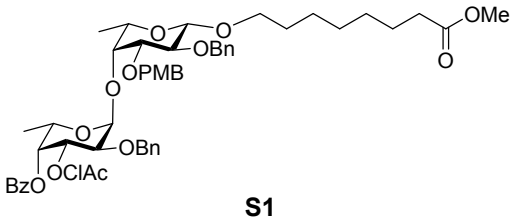
General methods for chemical synthesis.

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer. ^{1}H NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from tetramethylsilane (δ 0.00), D_2O (δ 4.79), CD_3OD (δ 3.31) or $CDCl_3$ (δ 7.26), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet) and coupling constants (Hz). ^{13}C chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (δ 77.2), CD_3OD (δ 49.0) or acetone (δ 30.9). ESI-TOF Mass spectra were measured on a Waters LCT premier XE. Melting points were determined on a micro hot-stage (Yanako MP-S3) and were uncorrected. Optical rotations were measured on a JASCO P-2200 polarimeter. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) and Silica Gel 60 N (spherical, neutral, 63-210 μm) (Kanto Chemical Co., Inc.), respectively. Gel filtration chromatography separations were performed using Sephadex LH-20 (GE Healthcare). Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

Synthesis of tetrafucoside 16.



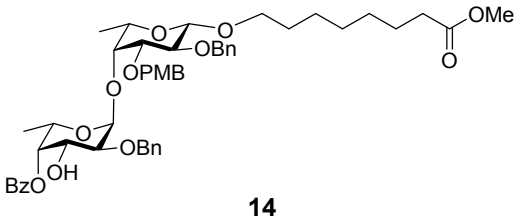
Compound S1



S1

To a solution of **12** (3.00 g, 3.21 mmol) in CH_2Cl_2 (45.0 mL) were added methyl 8-hydroxyoctanoate **13** (1.68 g, 9.64 mmol) and MS 5A (3.00 g, 100 wt% to **12**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −20 °C, and then $Yb(OTf)_3$ (1.59 g, 2.56 mmol) was added to the reaction mixture. After the reaction mixture was stirred for 6 h at the same temperature, the reaction was quenched with triethylamine (4.00 mL, 28.7 mmol). The resultant mixture was filtered through Celite. And then, water was added to the filtrate. The resultant mixture was extracted with $CHCl_3$ (50 mL×3), and then the extracts were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 *n*-hexane/EtOAc) to give **S1** (2.52 g, 2.66 mmol, 83% yield). Colorless syrup; R_f 0.45 (2/1 *n*-hexane/EtOAc); $[\alpha]^{25}_D$ −42.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.01-7.99 (2H, m), 7.62-7.59 (1H, m), 7.48-7.23 (14H, m), 6.84-6.80 (2H, m), 5.53-5.49 (2H, m), 4.99 (1H, d, *J* = 3.5 Hz), 4.94 and 4.80 (2H, ABq, *J* = 11.2 Hz), 4.78 and 4.66 (2H, ABq, *J* = 12.0 Hz), 4.66 (2H, m), 4.60 (1H, br-q, *J* = 6.5 Hz), 4.30 (1H, d, *J* = 7.8 Hz), 4.06 (1H, dd, *J* = 3.5 Hz, *J* = 10.1 Hz), 3.99-3.91 (3H, m), 3.77 (3H, s), 3.70-3.63 (5H, m), 3.51-3.43 (2H, m), 3.37 (1H, dd, *J* = 9.9 Hz, *J* = 2.7 Hz), 2.29 (2H, t, *J* = 7.7 Hz), 1.69-1.59 (4H, m), 1.42-1.24 (9H, m), 0.88 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 166.6, 166.4, 163.5, 159.2, 138.7, 137.9, 133.4, 130.7, 129.9, 129.7, 129.3, 128.7, 128.6×2, 128.5×2, 128.0, 127.7, 113.8, 104.1, 100.0, 79.7, 78.6, 78.5, 74.9, 73.3, 73.1, 72.8, 72.7, 72.4, 70.6, 70.1, 65.2, 55.3, 51.6, 40.8, 34.2, 29.8, 29.2×2, 26.1, 25.0, 16.8, 15.9; HRMS (ESI-TOF) *m/z* 971.3940 (971.3961 calcd. for $C_{52}H_{65}O_{14}NaCl$, $[M+Na]^+$).

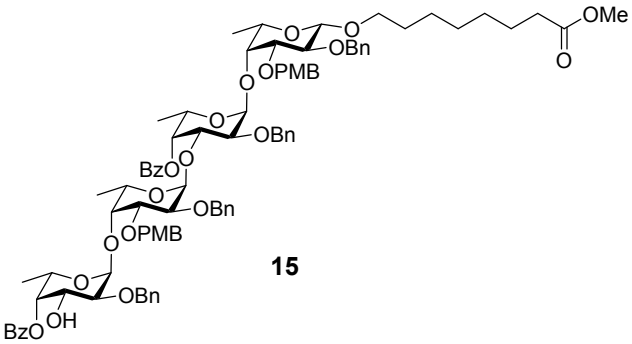
Compound 14



14

To a solution of **S1** (4.49 g, 4.73 mmol) in DMF (120 mL) were added 2,6-lutidine (2.19 mL, 18.9 mmol) and thiourea (1.44 g, 18.9 mmol) at room temperature, and then the reaction mixture was stirred for 13 h at 70 °C. After cooling to room temperature, the reaction mixture was poured into water at room temperature. The resultant mixture was extracted with a mixed solvent of *n*-hexane/EtOAc (1/1, v/v, 100 mL×3), and then the extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (8/1 PhMe/acetone) to give **14** (3.90 g, 4.47 mmol, 95% yield). Colorless syrup; R_f 0.47 (8/1 PhMe/acetone); $[\alpha]^{28}_D$ −112.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-8.01 (2H, m), 7.59-7.26 (15H, m), 6.82-6.81 (2H, m), 5.41 (1H, br-d, *J* = 2.0 Hz), 5.09 (1H, d, *J* = 3.5 Hz), 4.94-4.65 (6H, m), 4.54 (1H, br-q, *J* = 6.0 Hz), 4.41-4.38 (1H, m), 4.30 (1H, d, *J* = 7.5 Hz), 3.96-3.91 (1H, m), 3.87 (1H, dd, *J* = 3.5 Hz, *J* = 10.1 Hz), 3.78 (3H, s), 3.70-3.60 (5H, m), 3.51-3.43 (2H, m), 3.38 (1H, dd, *J* = 9.5 Hz, *J* = 2.0 Hz), 2.29 (2H, t, *J* = 7.5 Hz), 2.20 (1H, s), 1.68-1.58 (4H, m), 1.44-1.26 (9H, m), 0.93 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 166.7, 159.2, 138.8, 138.0, 133.2, 130.7, 130.1, 130.0, 129.3, 128.6, 128.5, 128.4, 128.0, 127.7, 113.8, 104.1, 99.5, 80.0, 78.5, 78.4, 76.8, 74.8, 74.5, 72.7, 70.6, 70.0, 67.9, 65.8, 55.4, 51.6, 34.2, 29.8, 29.2, 26.1, 25.0, 16.9, 16.2; HRMS (ESI-TOF) *m/z* 895.4234 (895.4245 calcd. for $C_{50}H_{63}O_{13}Na$, $[M+Na]^+$).

Compound 15



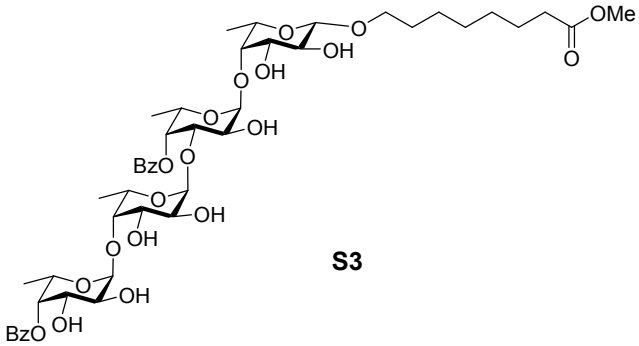
15

To a solution of **12** (859 mg, 0.918 mmol) and **14** (400 mg, 0.459 mmol) in Et_2O (26 mL) was added MS 5A (859 mg, 100 wt% to **12**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −80 °C, and then TMSOTf (17.8 μL, 0.092 mmol) was added to the reaction mixture. After the reaction mixture was stirred for 6 h at the same temperature, the reaction was quenched with triethylamine (0.80 mL, 5.74 mmol). The resultant mixture was filtered through Celite. And then, water was added to the filtrate. The resultant mixture was extracted with EtOAc (30 mL×3), and then the extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was passed through silica gel column chromatography (4/1 PhMe/EtOAc) to give the crude product **S2**.

To a solution of the above crude product in DMF (24 mL) were added 2,6-lutidine (1.73 mL, 3.90 mmol) and thiourea (296 mg, 0.630 mmol) at room temperature, and then the reaction mixture was stirred for 17 h at 70 °C. After cooling to room temperature, the reaction mixture was poured into water at room temperature. The resultant mixture was extracted with a mixed solvent of *n*-hexane/EtOAc (1/1, v/v, 20 mL×3), and then the extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (8/1 PhMe/acetone) to give **15** (577 mg, 0.368 mmol, 80% yield in 2 steps). White foam; R_f 0.36 (8/1 PhMe/acetone); $[\alpha]^{27}_D$ −143.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99-7.94 (4H, m), 7.57-7.16 (30H, m), 6.83-6.80 (2H, m), 6.69- 6.65 (2H, m), 5.58 (1H, br-d, J = 1.5 Hz), 5.39 (1H, d, J = 3.0 Hz), 5.33 (1H, br-d, J = 3.0 Hz), 4.99 (1H, d, J = 3.5 Hz), 4.96 (1H, d, J = 3.0 Hz), 4.92-4.59 (6H, m), 4.56-4.41 (9H, m), 4.30 (1H, d, J = 7.5 Hz), 4.20 (1H, br-dd, J = 10.5 Hz, J = 3.0 Hz), 4.05 (1H, br-q, J = 6.5 Hz), 3.98-3.91 (2H, m), 3.89-3.80 (2H, m), 3.77-3.74 (4H, m), 3.67 (1H, br-d, J = 3.0 Hz), 3.65-3.57 (8H, m), 3.49 (1H, m), 3.43 (1H, br-q, J = 6.5 Hz),

3.36 (1H, dd, J = 10.0 Hz, J = 3.0 Hz), 2.26 (2H, t, J = 7.5 Hz), 2.13 (1H, br-s), 1.70-1.55 (4H, m), 1.43-1.25 (12H, m), 0.89 (3H, d, J = 6.0 Hz), 0.86 (3H, d, J = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 166.6, 166.3, 159.1, 158.9, 138.7, 138.6, 138.5, 137.8, 133.0×2, 130.8, 130.7, 130.0×2, 129.9, 129.1, 128.6, 128.5×2, 128.4×2, 128.3×2, 128.0×2, 127.9, 127.6×2, 127.3, 113.7, 113.6, 103.9, 99.8, 99.1, 92.0, 80.0, 79.0, 78.7, 77.6, 77.4, 77.2, 76.5, 75.6, 74.9, 74.4, 74.0, 73.3, 73.0, 72.5, 72.0, 71.5, 70.6, 70.4, 69.8, 67.9, 66.6, 65.8, 65.6, 55.3, 55.0, 51.5, 34.0, 29.8, 29.2×2, 26.1, 25.0, 16.9, 16.3, 16.1; HRMS (ESI-TOF) *m/z* 1589.7042 (1589.7023 calcd. for $C_{91}H_{106}O_{23}Na$, $[M+Na]^+$).

Compound S3

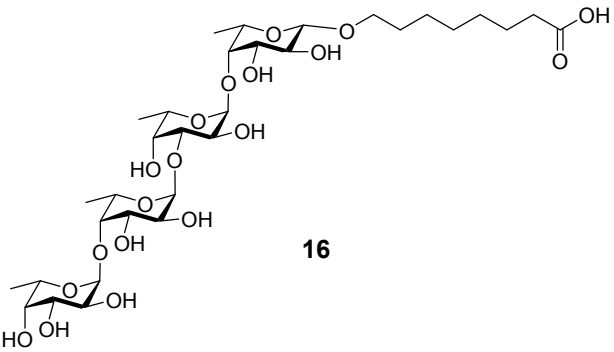


S3

To a solution of **15** (250 mg, 160 μmol) in MeOH/EtOAc (1/1, v/v, 25 mL) was added $Pd(OH)_2/C$ (250 mg, 100 wt% to **15**) under H_2 atmosphere at room temperature. After being stirred for 3 h, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (8/1 $CHCl_3$/MeOH) to give **S3** (136 mg, 152 μmol, 95% yield). White solid; R_f 0.40 (8/1 $CHCl_3$/MeOH); m.p. 120-121 °C; $[\alpha]^{23}_D$ −132.7° (c 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-8.08 (4H, m, Ar-H), 7.64-7.57 (2H, m, Ar-H), 7.50-7.44 (4H, m), 5.50 (1H, br-d), 5.47 (1H, br-d, J = 3.0 Hz), 5.13 (1H, d, J = 3.5 Hz), 5.09 (1H, d, J = 4.0 Hz), 5.03 (1H, d, J = 4.0 Hz), 4.65 (1H, br-q, J = 6.5 Hz), 4.60 (1H, br-q, J = 6.5 Hz), 4.24 (1H, d, J = 7.5 Hz), 4.14-4.10 (3H, m), 4.00-3.91 (3H, m), 3.80 (1H, br-d, J = 2.5 Hz), 3.77-3.67 (6H, m), 3.64-3.60 (1H, m), 3.57-3.47 (3H, m), 3.02-2.94 (3H, m), 2.55 (1H, br-s), 2.46 (1H, br-s), 2.31 (2H, t, J = 7.5 Hz), 2.24-2.22 (1H, m), 1.67-1.62 (4H, m), 1.41 (3H, d, J = 6.5 Hz), 1.37-1.30 (6H, m), 1.28 (3H, d, J = 7.0 Hz), 1.24 (3H, d, J = 7.0 Hz), 1.18 (3H, d, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 167.9, 166.8, 133.7, 133.2, 130.0, 129.5, 128.7, 128.5, 103.5, 100.9, 74.8, 74.2, 73.2, 71.6, 71.3, 70.6, 70.3, 70.1, 69.5, 69.0, 68.8, 67.9, 66.2, 65.8, 51.6, 34.0, 29.8, 29.4,

29.2, 29.1, 25.7, 24.9, 17.0, 16.4, 16.2, 16.1; HRMS (ESI-TOF) *m/z* 989.4032 (989.3994 calcd. for $C_{47}H_{66}O_{21}Na$, $[M+Na]^+$).

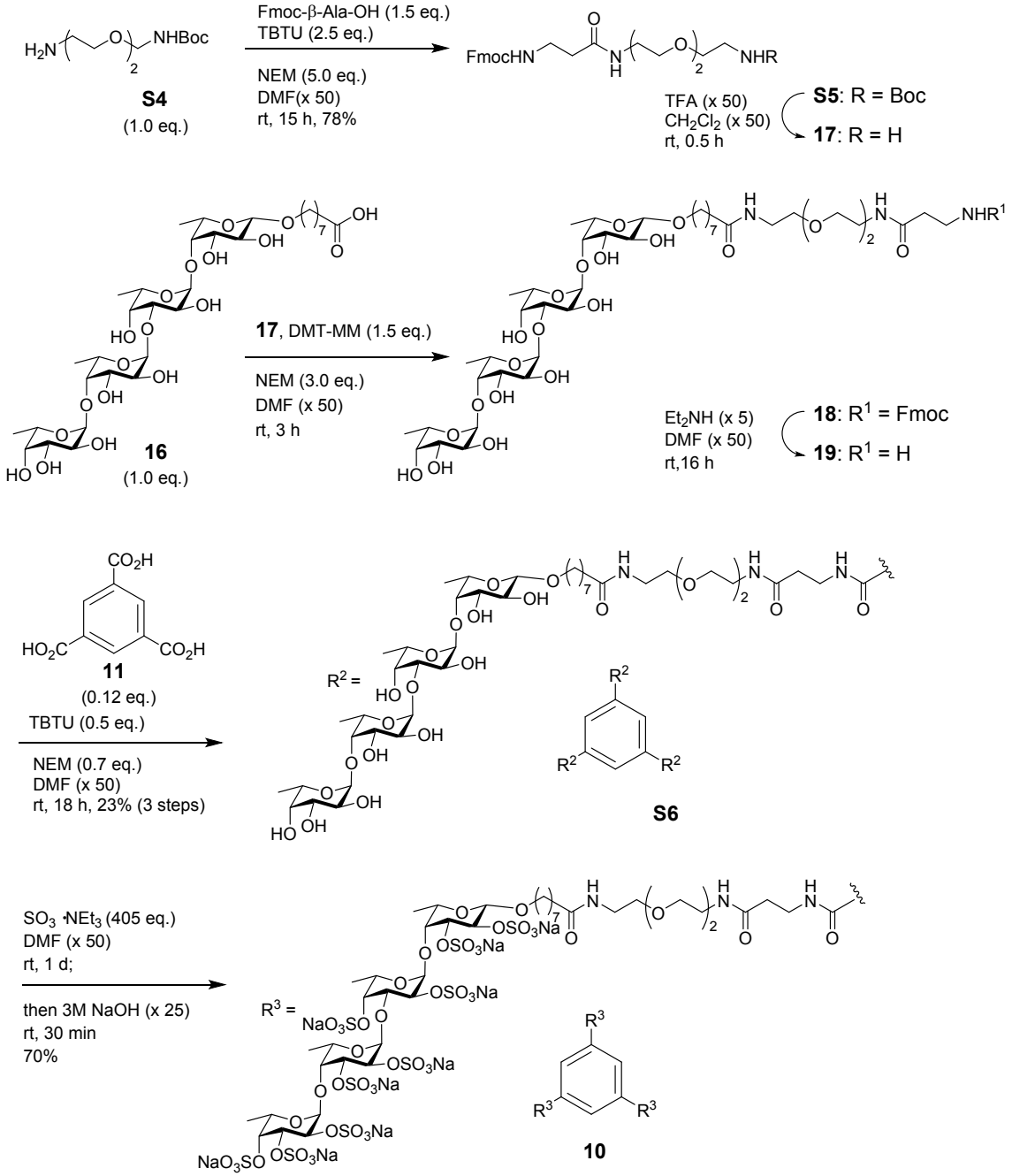
Compound 16



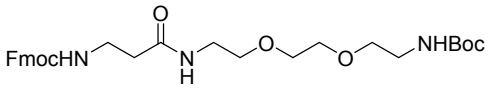
16

To a solution of **S3** (115 mg, 128 μmol) in MeOH/H_2O (1/1, v/v, 6.60 mL) was added 28% NaOMe in MeOH (1.48 mL, 7.70 mmol), and then the resultant mixture was stirred at 50 °C for 1 h. After cooling to room temperature, the reaction was quenched with Amberlite® IR 120 H^+ form. The resultant suspension was filtered, and then the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (10/10/1 $CHCl_3$/MeOH/H_2O) to give **16** (67.7 mg, 103 μmol, 80% yield). White solid; R_f 0.32 (10/10/1 $CHCl_3$/MeOH/H_2O); m.p. 148-149 °C; $[\alpha]^{24}_D$ −95.1° (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.07 (1H, d, J = 4.0 Hz), 4.96 (1H, d, J = 4.0 Hz), 4.94 (1H, d, J = 4.0 Hz), 4.54-4.50 (2H, m), 4.39 (1H, d, J = 8.0 Hz), 4.34 (1H, br-q, J = 7.0 Hz), 4.03 (1H, dd, J = 10.5 Hz, J = 3.0 Hz), 3.99 (1H, br-d, J = 3.0 Hz), 3.95-3.89 (2H, m), 3.88-3.76 (8H, m), 3.71 (1H, dd, J = 10.0 Hz, J = 3.0 Hz), 3.66-3.62 (1H, m), 3.48 (1H, dd, J = 8.0 Hz, J = 10.0 Hz), 2.34 (2H, t, J = 7.0 Hz), 1.62-1.57 (4H, m), 1.36-1.27 (12H, m), 1.17-1.15 (6H, m); ^{13}C NMR (125 MHz, D_2O) δ 180.1, 103.3, 101.1, 97.3, 80.9, 79.8, 76.7, 73.0, 72.6, 71.5, 71.1, 71.0, 70.1, 69.8, 69.6, 69.4, 68.7, 68.3, 67.8, 67.6, 67.0, 34.6, 29.5, 28.8, 28.7, 25.6, 24.9, 16.0, 15.9×2; HRMS (ESI-TOF) *m/z* 743.3347 (743.3338 calcd. for $C_{32}H_{55}O_{19}$, $[M-H]^-$).

Synthesis of trimer 10.



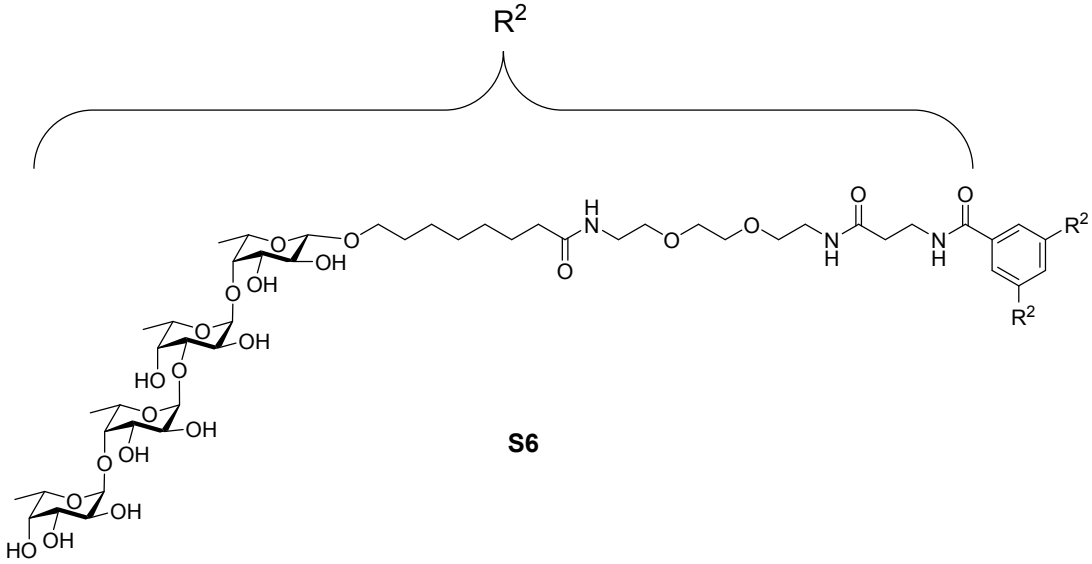
Compound S5



S5

To a solution of Fmoc-β-Ala-OH (3.14 g, 10.1 mmol) in DMF (84 mL) were added TBTU (4.32 g, 13.5 mmol), NEM (4.26 mL, 33.7 mmol) and **S4** (1.67 g, 6.73 mmol) at room temperature. The mixture was stirred for 15 h at the same temperature. The reaction was poured into water. The resultant mixture was extracted with EtOAc (80 mL×3), and then the extracts were washed with brine (250 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (5/2 $CHCl_3$/acetone) to give **S5** (2.84 g, 5.25 mmol, 78% yield). White solid; R_f 0.23 (5/1 $CHCl_3$/acetone); m.p. 104-105 °C ; ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (2H, d, J = 7.5 Hz), 7.63 (2H, d, J = 7.5 Hz), 7.39 (2H, t, J = 7.5 Hz), 7.31 (2H, t, J = 7.5 Hz), 7.07 (1H, s), 6.54 (1H, s), 4.34 (2H, d, J = 7.0 Hz), 4.19 (1H, t, J = 7.0 Hz), 3.55 (4H, m), 3.51 (2H, t, J = 5.5 Hz), 3.46 (2H, t, J = 5.5 Hz), 3.37 (4H, m), 3.19 (2H, t, J = 5.5 Hz), 2.40 (2H, t, J = 6.5 Hz), 1.44 (9H, s); ^{13}C NMR (125 MHz, CD_3OD) δ 173.9, 158.7, 158.4, 145.3, 142.6, 128.8, 128.2, 126.2, 120.9, 80.1, 71.2, 71.1, 70.6, 67.7, 41.3, 41.1, 40.3, 38.5, 37.2, 28.8; HRMS (ESI-TOF) *m/z* 564.2702 (564.2686 calcd. for $C_{29}H_{39}N_3O_7Na$, $[M+Na]^+$).

Compound S6



S6

To a solution of **S5** (429 mg, 0.799 mmol) in CH_2Cl_2 (22 mL) was added TFA (22

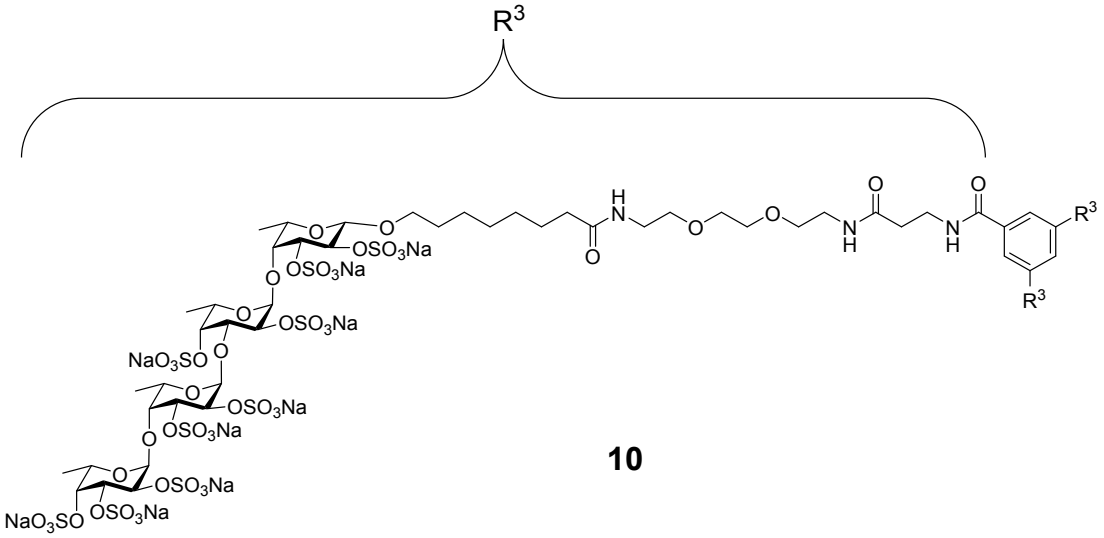
mL) at room temperature. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (5/1 $CHCl_3$/MeOH) to give crude product **17** (342.4 mg).

To a solution of **16** (84.8 mg, 128 μmol) in DMF (4.3 mL) were added DMT-MM (56.7 mg, 193 μmol), NEM (48.7 μL, 385 μmol) and the above **17** (85.0 mg) at room temperature. The mixture was stirred for 3 h at the same temperature. The reaction was subjected to silica gel chromatography (3/1 $CHCl_3$/MeOH) and gel filtration to give crude product **18** (123.0 mg).

To a solution of the above **18** (123.0 mg) in DMF (7.5 mL) was added diethylamine (750 μL) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 16 h at room temperature, the reaction mixture was concentrated in *vacuo* and subjected to reverse phase silica gel column chromatography (100/0 to 50/50 H_2O/MeOH) to give crude product **19** (87.4 mg).

To a solution of benzene-1,3,5-tricarboxylic acid (**11**) (0.77 mg, 3.66 μmol) in DMF (1 mL) were added TBTU (4.26 mg, 16.5 μmol), NEM (2.78 μL, 22.0 μmol) and **19** (20.8 mg) at room temperature. The mixture was stirred for 16 h at the same temperature. The reaction was poured into water. The resultant mixture was subjected to reverse phase silica gel chromatography (100/0 to 40/60 H_2O/MeOH) and gel filtration to give **S6** (7.12 mg, 2.38 μmol, 23% yield in 3 steps). White solid; R_f 0.40 (10/10/1 $CHCl_3$/MeOH/H_2O); m.p. 141-142 °C; ^{1}H NMR (500 MHz, D_2O) δ 8.33 (1H, s), 5.08 (1H, d, J = 4.0 Hz), 4.97 (1H, d, J = 4.0 Hz), 4.95 (1H, d, J = 4.0 Hz), 4.56-4.50 (2H, m), 4.39-4.33 (2H, m), 4.04 (1H, br-dd, J = 11.0 Hz, J = 3.0 Hz), 3.99 (1H, br-d), 3.97-3.90 (2H, m), 3.89-3.79 (8H, m), 3.73-3.69 (3H, m), 3.63-3.47 (10H, m), 3.39 (2H, t, J = 5.5 Hz), 3.32 (2H, t, J = 5.5 Hz), 2.61 (2H, t, J = 6.5 Hz), 2.20 (2H, t, J = 6.5 Hz), 1.60-1.50 (4H, m), 1.37-1.26 (12H, m), 1.18 (6H, m); ^{13}C NMR (125 MHz, D_2O) δ 177.8, 174.5, 168.7, 135.5, 129.7, 103.4, 101.1, 97.4, 80.9, 79.7, 76.7, 73.0, 72.6, 71.5, 71.1, 71.0, 70.0, 69.8, 69.5×2, 69.3, 69.1, 68.7, 68.2, 67.8, 67.6, 67.0, 39.6, 39.4, 37.4, 36.3, 36.0, 29.5, 28.7, 26.0, 25.9, 25.6, 20.7, 16.1, 16.0, 15.9×2; HRMS (ESI-TOF) *m/z* 1519.2152 (1519.2155 calcd. for $C_{132}H_{225}N_9O_{66}Na_2$, $[M+2Na]^{2+}$).

Compound **10**



10

To a solution of **S6** (5.0 mg, 1.67 μmol) in DMF (250 μL) was added SO_3•NEt_3 (123 mg, 677 μmol) at room temperature. After the reaction mixture was stirred for 1 d, 3 M NaOH aq. (449 μL, 1.35 mmol) was added to the reaction mixture and the mixture was stirred for 30 min. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (100/0 to 0/100 H_2O/MeOH) and gel filtration chromatography to give **10** (6.7 mg, 1.17 μmol, 70% yield). White solid; R_f 0.05 (10/10/3 $CHCl_3$/MeOH/H_2O); m.p. >300 °C; ^{1}H NMR (500 MHz, D_2O) δ 8.30 (1H, s), 5.44-5.30 (3H, m), 4.97-4.85 (4H, m), 4.72-4.53 (6H, m), 4.44-4.36 (3H, m), 4.30-4.11 (3H, m), 3.90-3.82 (2H, m) 3.70-3.50 (10H, m), 3.40-3.30 (4H, m), 3.19 (1H, br-q, J = 7.0 Hz), 2.60 (2H, t, J = 6.5 Hz), 2.20 (2H, t, J = 7.5 Hz), 1.60-1.50 (4H, m), 1.42-1.21 (18H, m); ^{13}C NMR (125 MHz, D_2O) δ 178.1, 174.7, 135.5, 129.7, 101.7, 99.3×2, 95.5, 80.5, 80.4, 80.3, 79.0, 78.3, 76.5, 74.6, 74.4, 74.2, 74.1, 73.3, 73.2, 73.1, 73.0, 71.5, 71.1, 70.2, 70.1, 69.5, 68.4, 68.3, 68.1, 68.0, 67.8, 67.6, 47.3, 39.7, 39.5, 37.4, 36.4, 36.2, 36.1, 29.3, 28.8, 26.0, 25.5×2, 19.9, 16.7, 16.5, 16.4, 16.3, 16.1, 16.0; HRMS (ESI-TOF) *m/z* 2871.3877 (2871.3923 calcd. for $C_{132}H_{196}N_9Na_{27}O_{147}S_{27}$, $[M-2H]^{2-}$).

Materials and methods for biological assay.

The HAs prepared from influenza viruses A/New Caledonia/20/99 (H1N1) and A/New York/55/2004 (H3N2) were kindly provided by Dr. Yujiro Suzuki (the Kitasato Institute, Japan).[1] Fetal bovine serum (FBS) was purchased from Biosera. Fucoidan isolated from *F. vesiculosus* (ca 600 kDa) was purchased from Elicityl (Crolles, France). Biotinyl fetuin was purchased from GALAB Technologies. Chicken red blood cells

(cRBCs) were purchased from Cosmo Bio Co., LTD. Horseradish peroxidase (HRP)-conjugated streptavidin was purchased from Thermo Fisher Scientific K. K. (Tokyo, Japan). Madin-Darby canine kidney (MDCK) cells were obtained from Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). Penicillin-Streptomycin Mixed Solution was purchased from Nacalai Tesque (Kyoto, Japan). 2'-(4-Methylumbelliferyl) -α-D-*N*-acetylneuraminic acid, sodium salt hydrate was purchased from Sigma-Aldrich Co. LLC. (Tokyo, Japan). Recombinant influenza virus H1N1 (A/California/04/2009) neuraminidase (wild type and H274Y subtype) was purchased from Sino Biological Inc.

Hemagglutination inhibition (HAI) assay.

Compounds were serially diluted (2-fold) with 25 μL of PBS in 96-well plate. 25 μL of PBS containing H1 HA (influenza virus A/New Caledonia/20/99, 128 HA units) or H3 HA (influenza virus A/New York/55/2004, 16 HA units) was added to the wells. After incubation at room temperature for 1 h, 50 μL of chicken RBCs (0.5%, v/v) was added to each well, mixed, and allowed to settle at room temperature for 30 min. HA titres were expressed as the maximum dilution required to give complete inhibition of hemagglutination.

Docking studies.

Target compounds were docked into the crystal structure of H3 HA (A/Aichi/2/68) (PDB ID: 1HGG). Docking was carried out using Glide protocol. The complex was subjected to preparation steps using the Protein Preparation Wizard in Maestro (version 10.5, Schrödinger) using the default settings. First, the waters beyond 5 Å from the ligands, the cofactors and the ions were removed, bond orders were assigned and hydrogens were added. Next, the orientation of amide (Asn and Gln), hydroxyl (Ser, Thr and Tyr) and thiol groups (Cys), and the protonation and tautomeric state of the His residues were optimized. In the final step, the energy minimization of the structure was carried out using Impref module of the Schrödinger suite with a cut of Root Mean Square Deviation (RMSD) of 0.30 Å. OPLS3 force field was used in the energy minimization phase. Then, a grid box of 17 x 17 x 17 $Å^3$ for the receptor was generated with a default inner box, which was centered on the corresponding ligand.

The ligand molecule was prepared using the LigPrep module of the Schrödinger suite. The LigPrep module generated different conformers of the ligand molecule, with low

energy, based on its ionic states. Partial atomic charges were calculated by using OPLS3 force field. Only one low energy conformer was generated per ligand, with a pH of 7.0 ± 2.0. The structures with higher energy, ionization and tautomeric state were filtered out. The generated lowest energy conformers were used for the docking studies.

A docking procedure was then applied to the compound. The default implementation of Glide module in Standard Precision (SP) protocol was set. The docking affinity was presented in terms of docking scores (kJ/mol). The more negative the docking score, the better binder.

Avidin-biotin-peroxidase complex (ABC) method.

After blocking with 1% BSA/PBS, 10 μL of biotinyl fetuin in PBS (0.161-20.7 μM) was added to HA-coated wells,[2] and the plates were incubated for 1 h. After washing with PBS, 100 μL of 1:2500 (v/v) dilution of HRP-conjugated streptavidin in 1% BSA/PBS was added to each well, followed by a 30-min incubation. Color was developed using *o*-phenylenediamine (OPD), and absorbance (ΔA) at 492 nm was measured. Each experiment was performed in triplicate.

When the ΔA value was plotted against the biotinyl fetuin concentration, simple saturation curves indicating Langmuir adsorption were obtained (Fig. S1). To obtain the K_d value, a reciprocal plot between [biotinyl fetuin]/ΔA and [biotinyl fetuin] gave straight lines according to the following equation: [biotinyl fetuin]/ΔA = [biotinyl fetuin]/ΔA_{max} + $K_d/\Delta A_{max}$, where ΔA_{max} is the maximum amount of biotinyl fetuin that can bind to HA, and K_d is the dissociation constant.

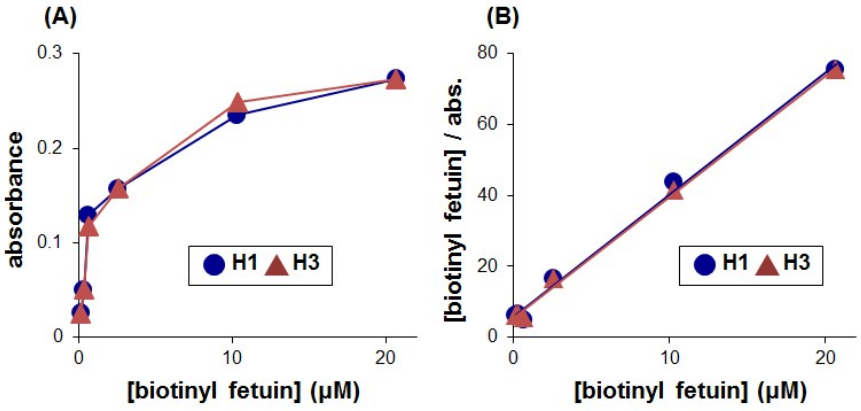


Fig. S1 Binding of the biotinyl fetuin to H1 HA or H3 HA determined by ABC method. Dependence of fetuin concentrations on the amount of the biotinyl fetuin that bound to

HAs. (A) The biotinyl fetuin (0.161-20.7 μM) was incubated in HA-coated wells, and the amount of the fetuin that bound to HA was detected with HRP-conjugated streptavidin. (B) A Langmuir plot indicated that the K_d value was 1.74 μM for H1 HA (blue circle) and 1.76 μM for H3 HA (red triangle), respectively.

Inhibition of the fetuin-HA interaction by fucoidan derivatives.

The amount of the fetuin that bound to HA-coated plates[2] was determined by the ABC method. 10 μL of biotinyl fetuin (10 μM) and 10 μL of inhibitors at the indicated concentration were mixed in advance, and the mixed solution was added to the wells. The plates were incubated for 1 h, and the amount of the biotinyl fetuin was determined as described above. The IC_{50} value was calculated by nonlinear regression analysis using Graph Pad Prism software, and the K_i value was calculated based on the IC_{50} values according to the Cheng-Prosuff equation.

Neuraminidase inhibition assay.

5.0 μL of enzyme solution (25 U/mL in 50 mM tris buffer (containing 5 mM $CaCl_2$ and 200 mM NaCl, pH 7.5)) and 80.0 μL of 500 μM for **5** or 20 μM for oseltamivir carboxylate were added to 0.5 mL micro test tube (Eppendorf Co., Ltd.). Following pre-incubation at 25.0 °C for 60 min, 5.0 μL of the substrate solution (3.88 mM in the same buffer) was then added to the test tube. Following further incubation at 25.0 °C for 10 min, the enzyme reaction was terminated by the addition of 10 μL of 10 wt% NaOH aq. and each sample was moved to a 96-well plate. 4-Methylumbelliferone was immediately quantified by SpectraMax i3 (Molecular Devices) micro plate reader (λ_{ex} = 365 nm, λ_{em} = 450 nm). As a result, NA enzymatic activity was strongly inhibited with oseltamivir carboxylate showing >99% inhibition at 2.0 μM. In contrast, **5** displayed only 4.9 ± 3.9% inhibitory activity against NA at 500 μM.

LC/MS analysis.

11.5 μL of enzyme solution (25 U/mL in 50 mM tris buffer (containing 5 mM $CaCl_2$ and 200 mM NaCl, pH 7.5)) and 184 μL of 100 μM for **5** were added to 0.5 mL micro test tube. Following incubation at 25.0 °C for 60 min, the stability of **5** to NA was evaluated by LS/MS analysis (ACQUITY Ultra Performance LC (Waters); ACQUITY UPLC® BEH C18 1.7 μm, 2.1 x 50 mm (waters); 20 °C; detection by UV (215 nm);

H_2O 15 min; flow rate 0.2 mL min^{-1}).

Cell culture.

Madin-Darby canine kidney (MDCK) cells were culutured in Eagle's Minimun Essential Medium (MEM) supplemented with 10% FBS and Penicillin (10,000 U/mL)-Streptomycin (10 mg/mL) Mixed Solution. MDCK cells were grown at 37 °C under 5% CO_2.

Plaque assay.

The infection of MDCK cells by influenza virus was evaluated using a plaque assay. Briefly, the inhibitors were diluted with PBS at the indicated concentration and mixed with influenza A/Puerto Rico/8/34 (H1N1) or A/Aichi/2/68 (H3N2) virus solution containing 50-200 plaque-forming units (pfu).[3] After 30 min at room temperature, the mixture was incubated with a MDCK monolayer at 37 °C for 30 min under 5% CO_2. After washing, MDCK cells were incubated for 2 days and the number of plaques was counted using crystal violet. The fraction of infection was calculated using the equation N/N_0, where N and N_0 are the number of plaques in the presence and in the absence of the inhibitors, respectively. The IC_{50} values (50% inhibitory concentration) of the inhibitors were obtained from a logit-log plot.

References.

[1] Y. Asashi, T. Yoshikawa, I. Watanabe, T. Iwasaki, H. Hasegawa, Y. Sato, S. Shimada, M. Nanno, Y. Matsuoka, M. Ohwaki, Y. Iwakura, Y. Suzuki, C. Aizawa, T. Sata, T. Kurata, S. Tamura, *J. Immunol.* **2002**, *168*, 2930.

[2] T. Matsubara, A. Onishi, D. Yamaguchi, T. Sato, *Bioorg. Med. Chem.* **2016**, *24*, 1106-1114.

[3] T. Matsubara, A. Onishi, T. Saito, A. Shimada, H. Inoue, T. Taki, K. Nagata, Y. Okahata, T. Sato, *J. Med. Chem.* **2010**, *53*, 4441-4449.

^{1}H and ^{13}C NMR spectrum charts

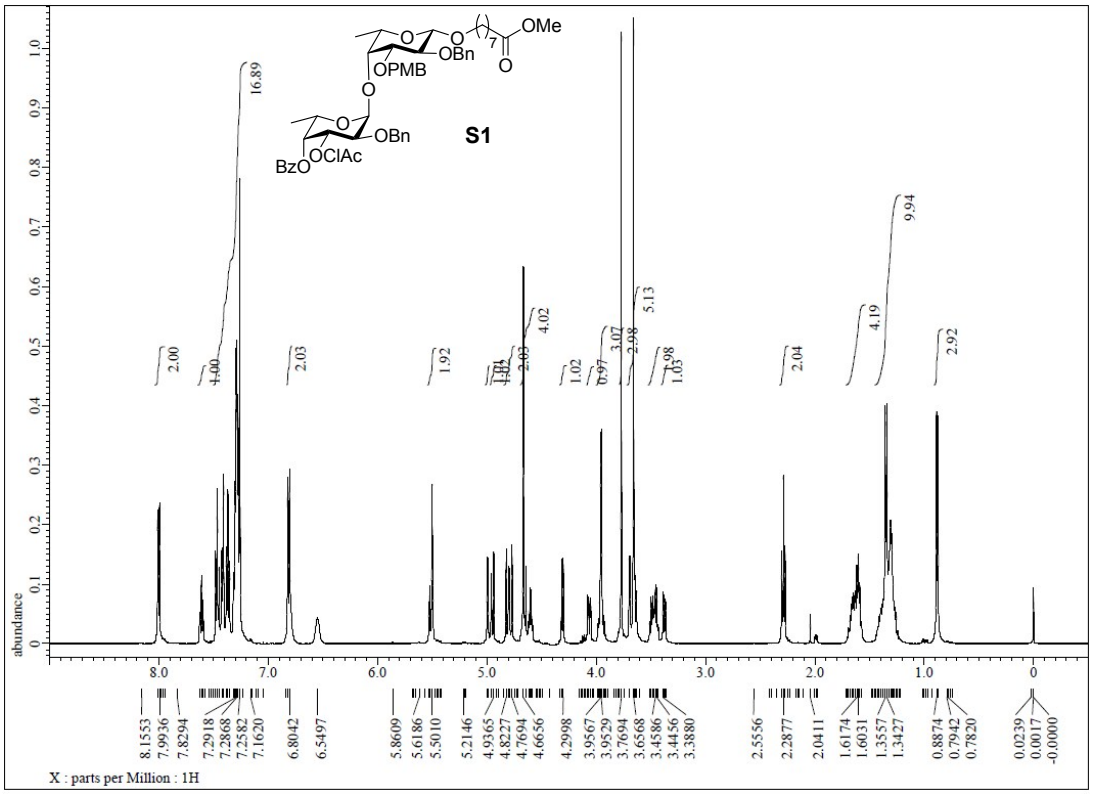


Fig. S2 ^{1}H NMR spectrum of **S1**

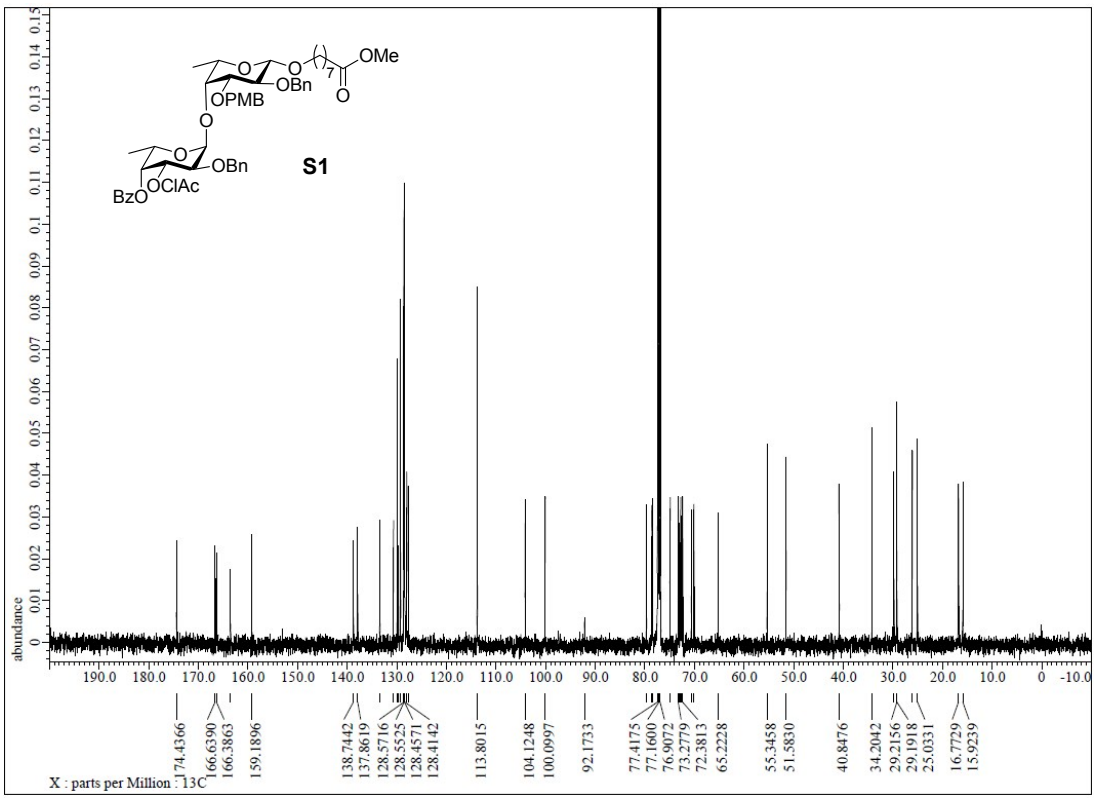


Fig. S3 ^{13}C NMR spectrum of **S1**

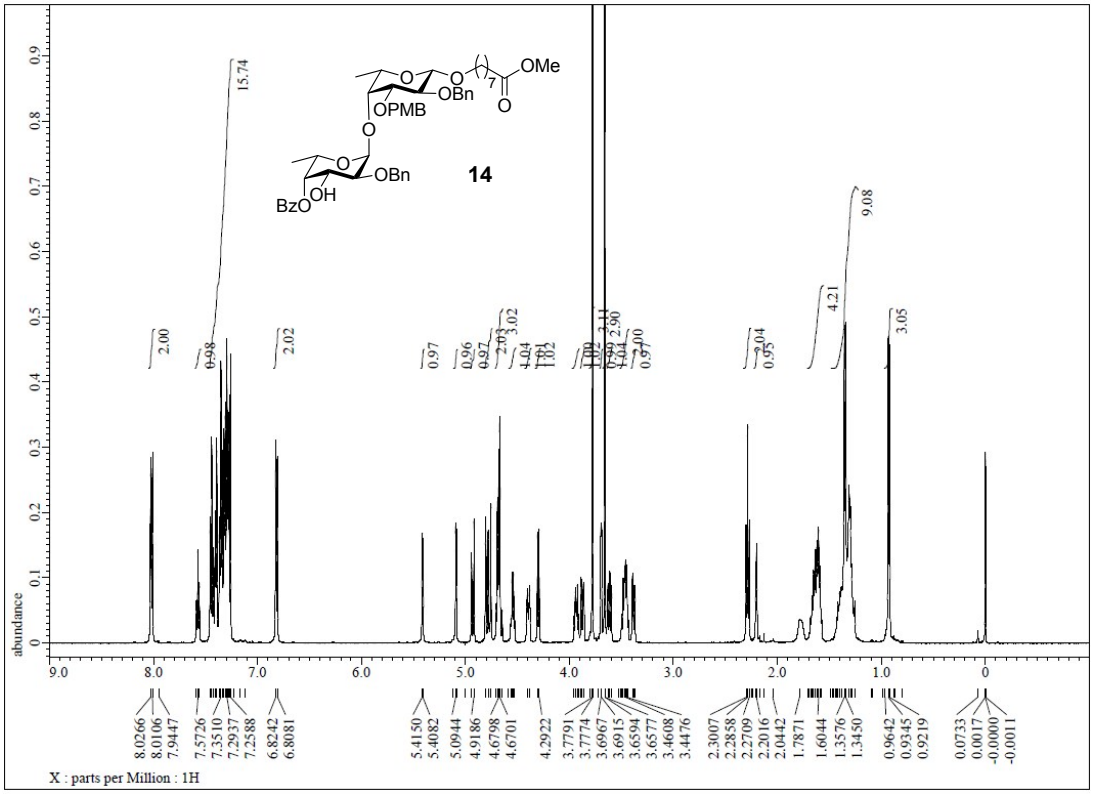


Fig. S4 ^{1}H NMR spectrum of **14**

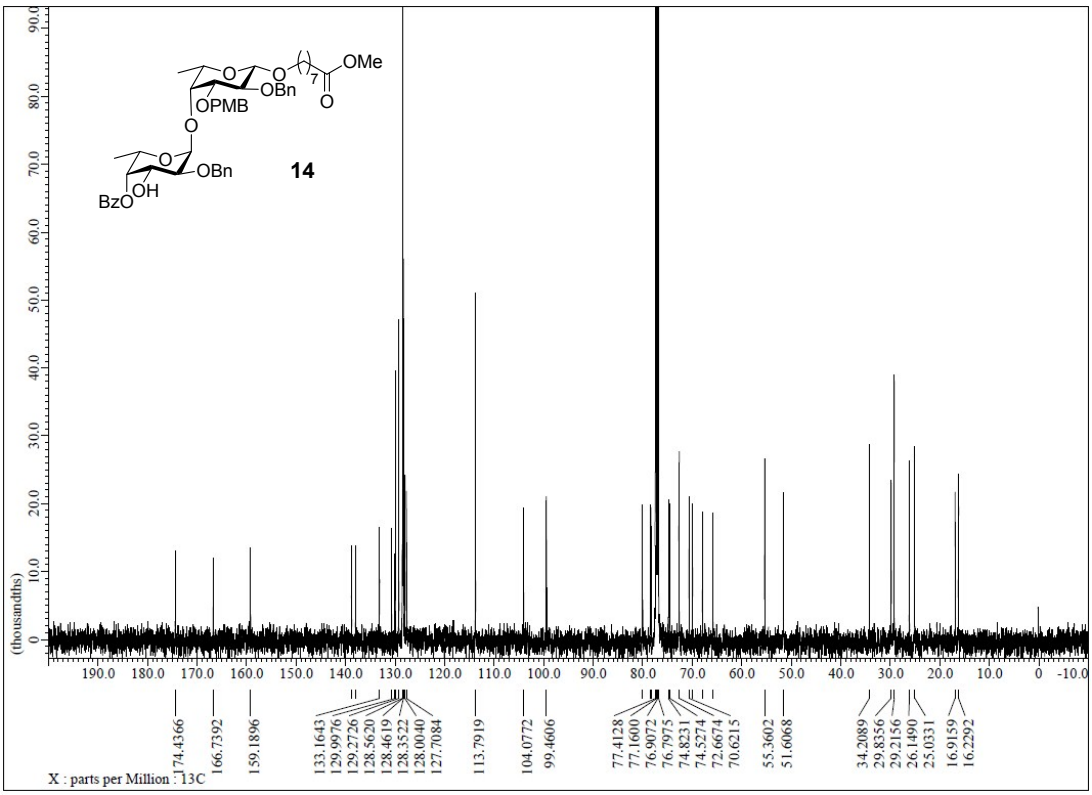


Fig. S5 ^{13}C NMR spectrum of **14**

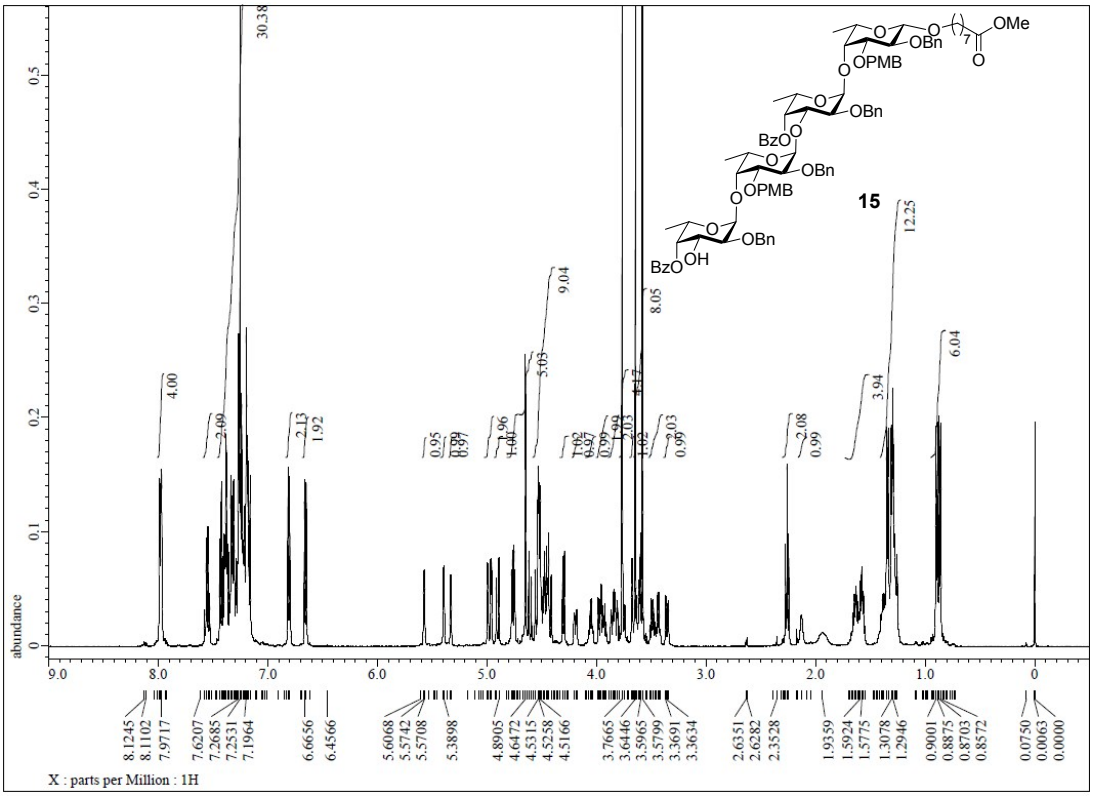


Fig. S6 ^{1}H NMR spectrum of **15**

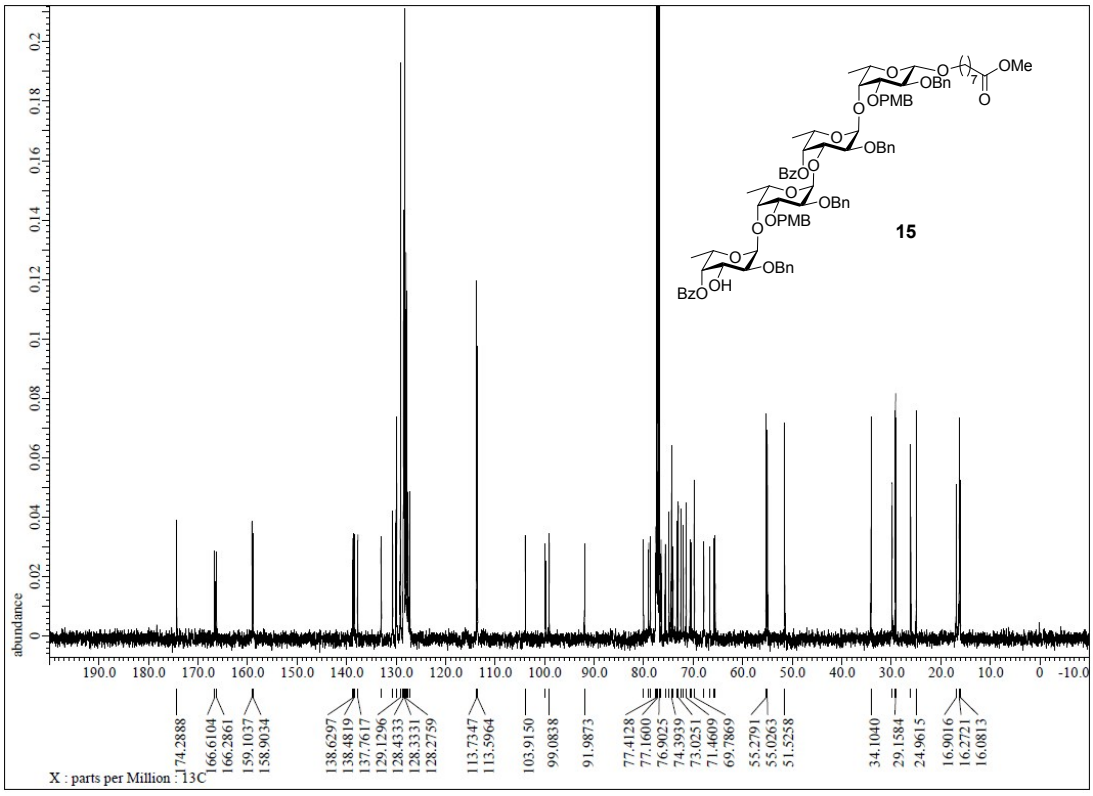


Fig. S7 ^{13}C NMR spectrum of **15**

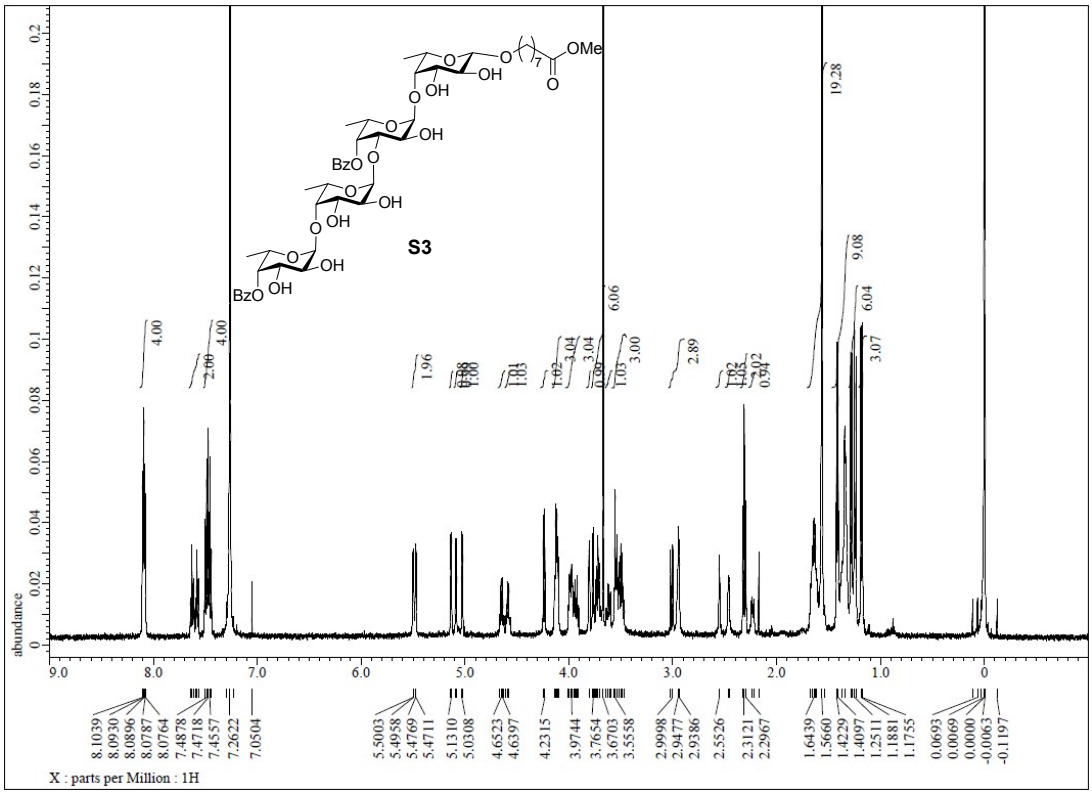


Fig. S8 ^{1}H NMR spectrum of **S3**

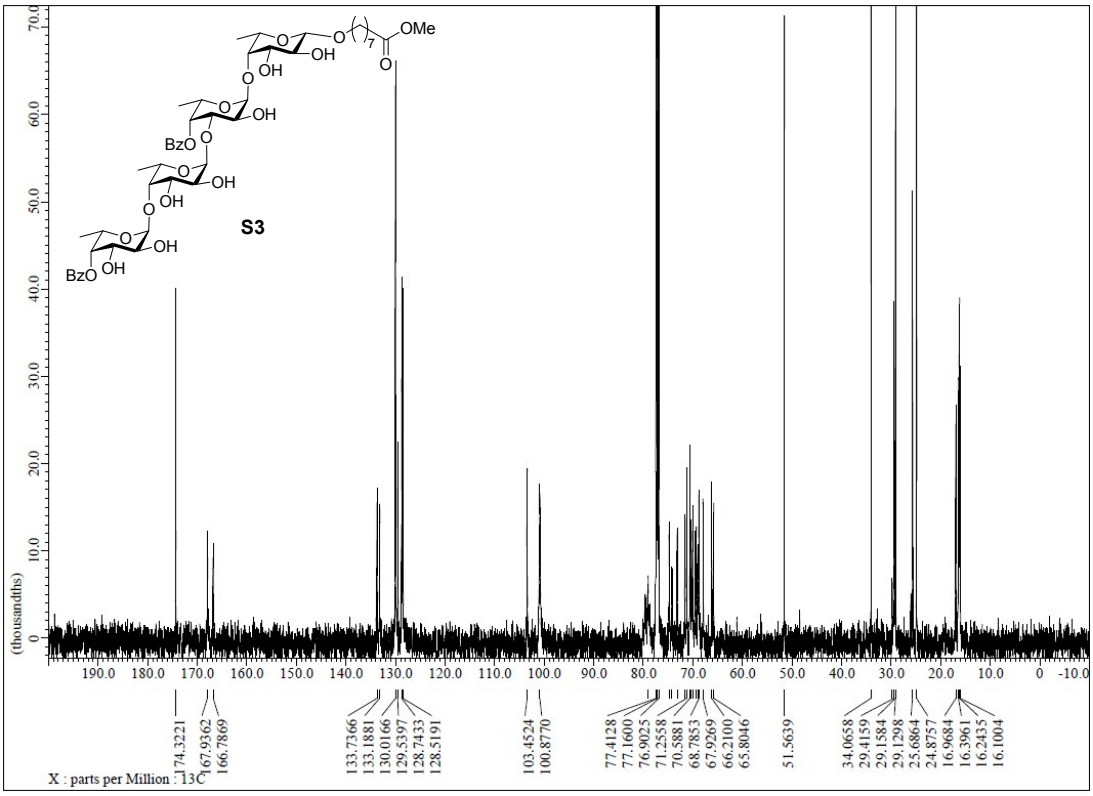


Fig. S9 ^{13}C NMR spectrum of **S3**

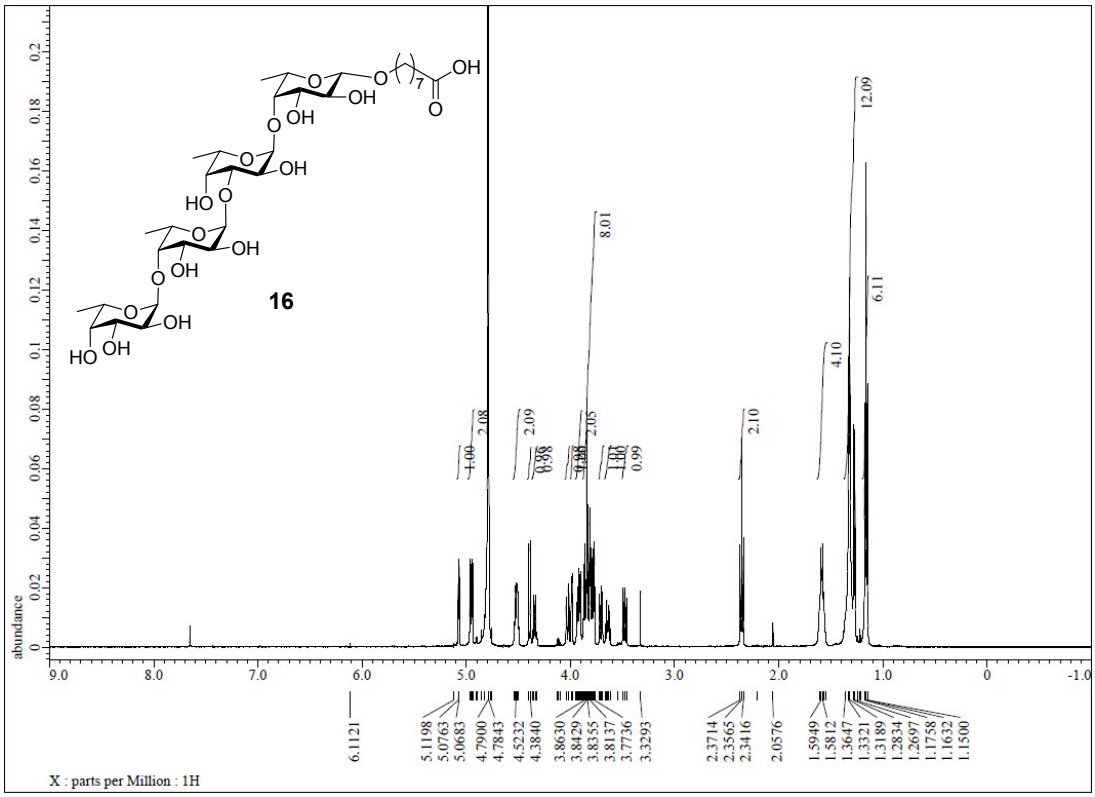


Fig. S10 ^{1}H NMR spectrum of **16**

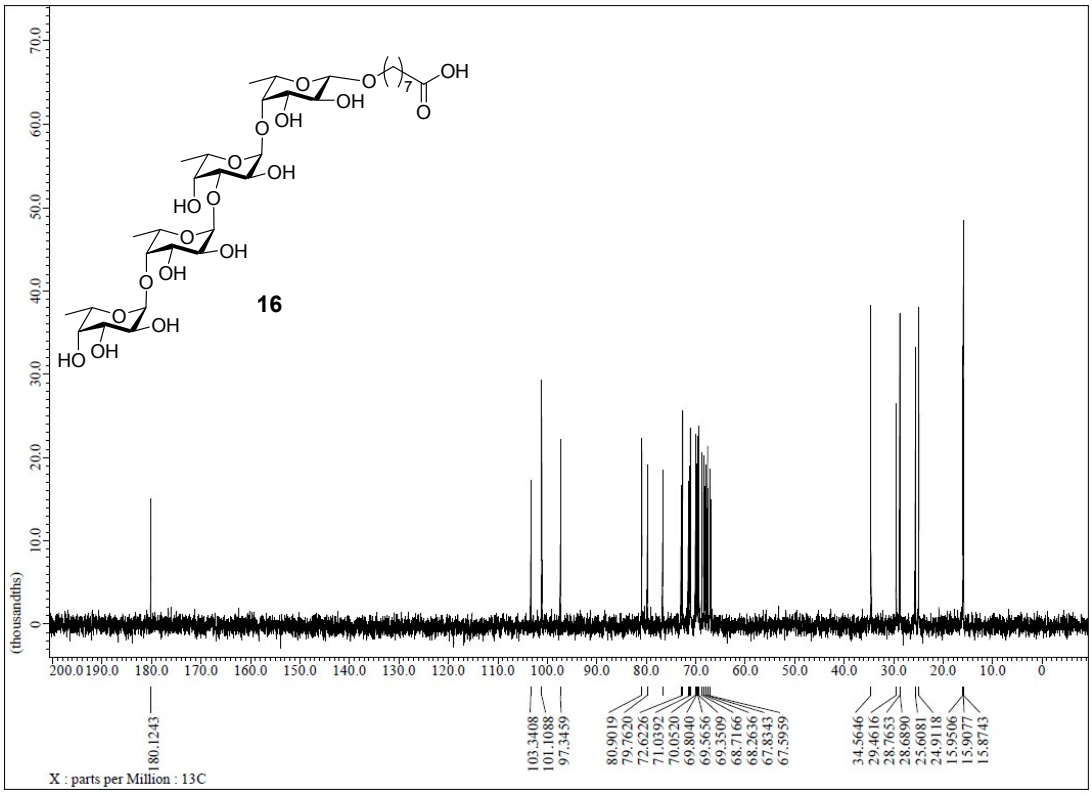


Fig. S11 ^{13}C NMR spectrum of **16**

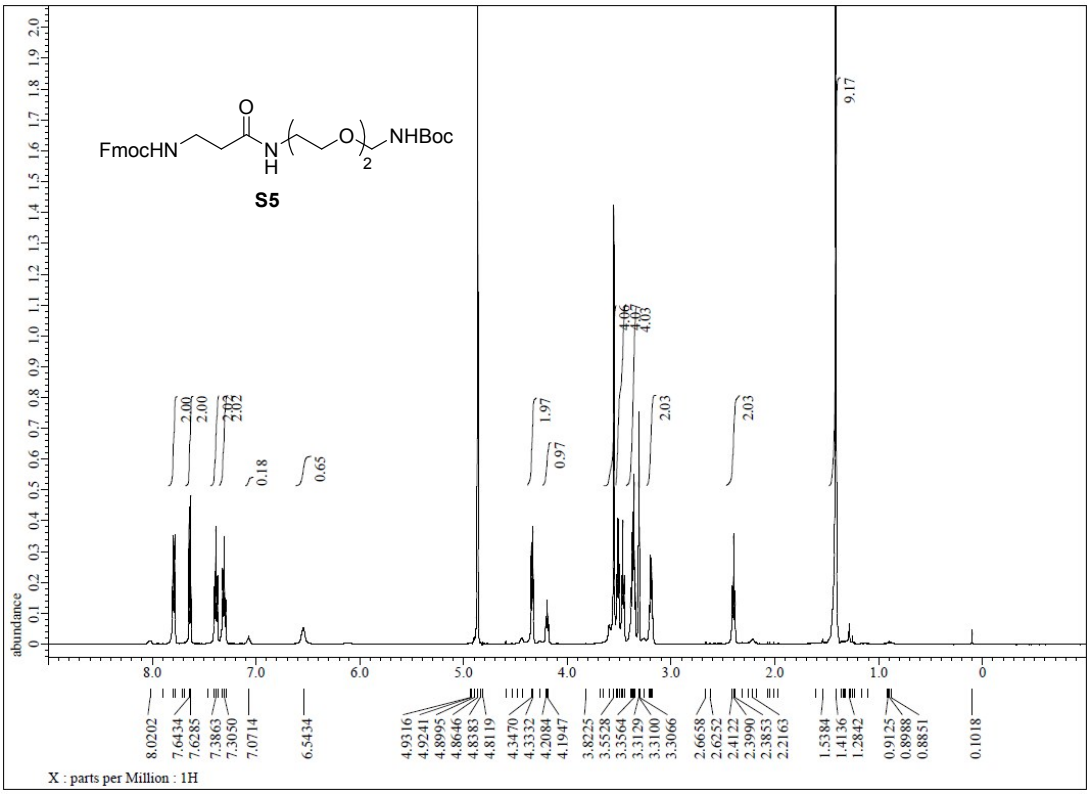


Fig. S12 ^{1}H NMR spectrum of **S5**

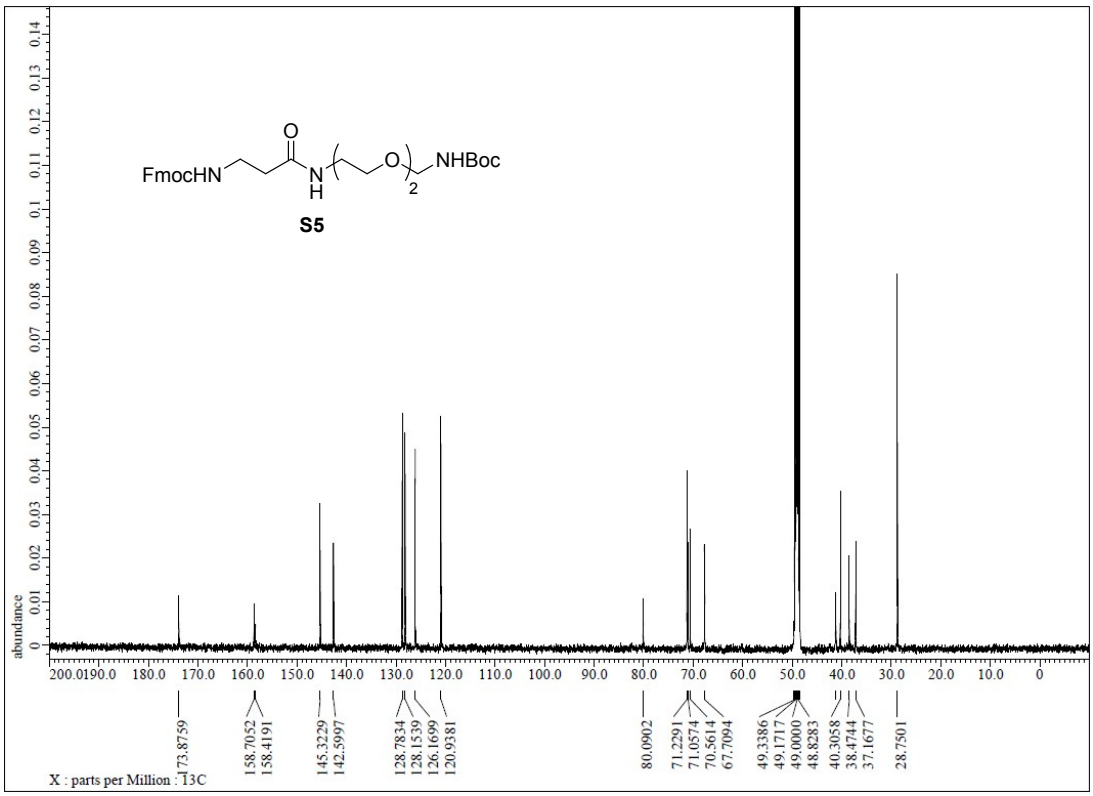


Fig. S13 ^{13}C NMR spectrum of **S5**

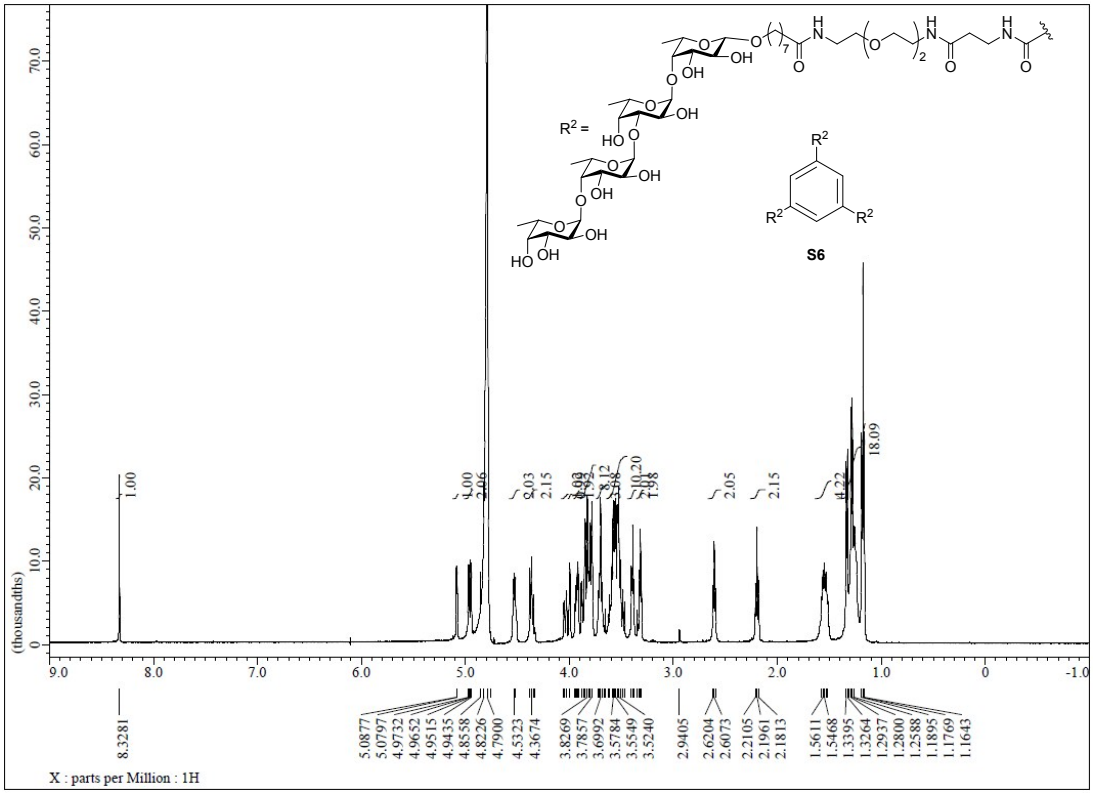


Fig. S14 ^{1}H NMR spectrum of **S6**

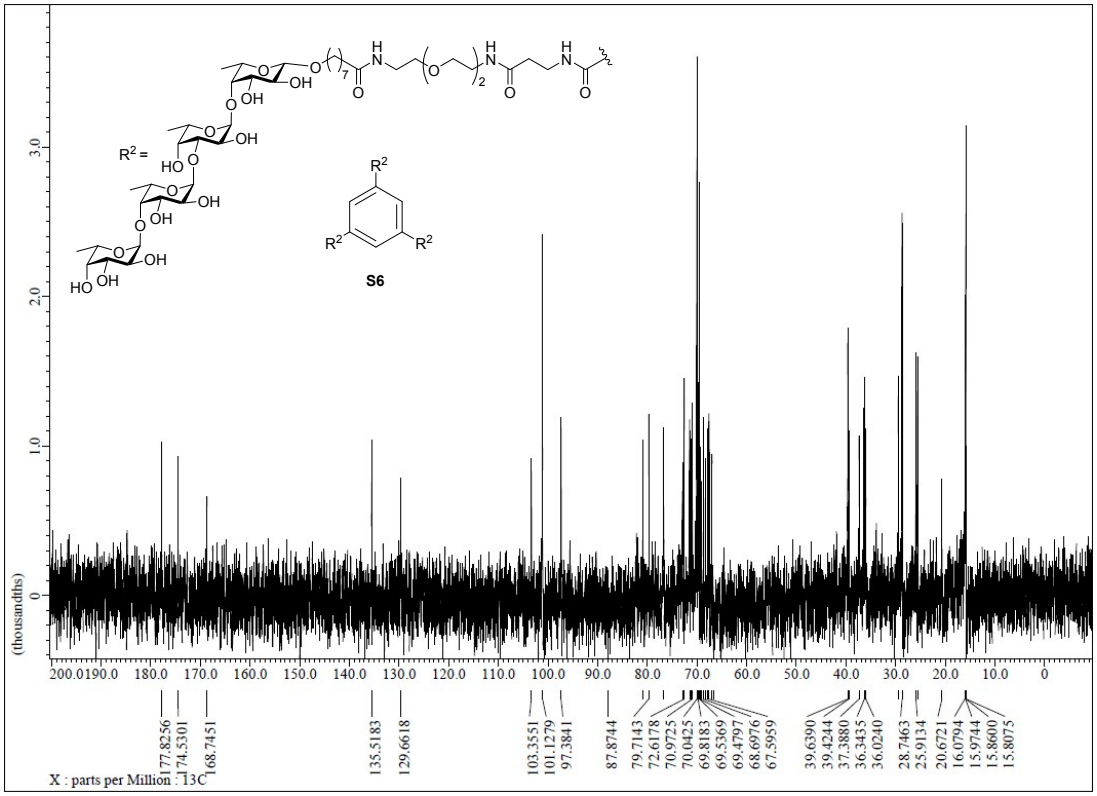


Fig. S15 ^{13}C NMR spectrum of **S6**

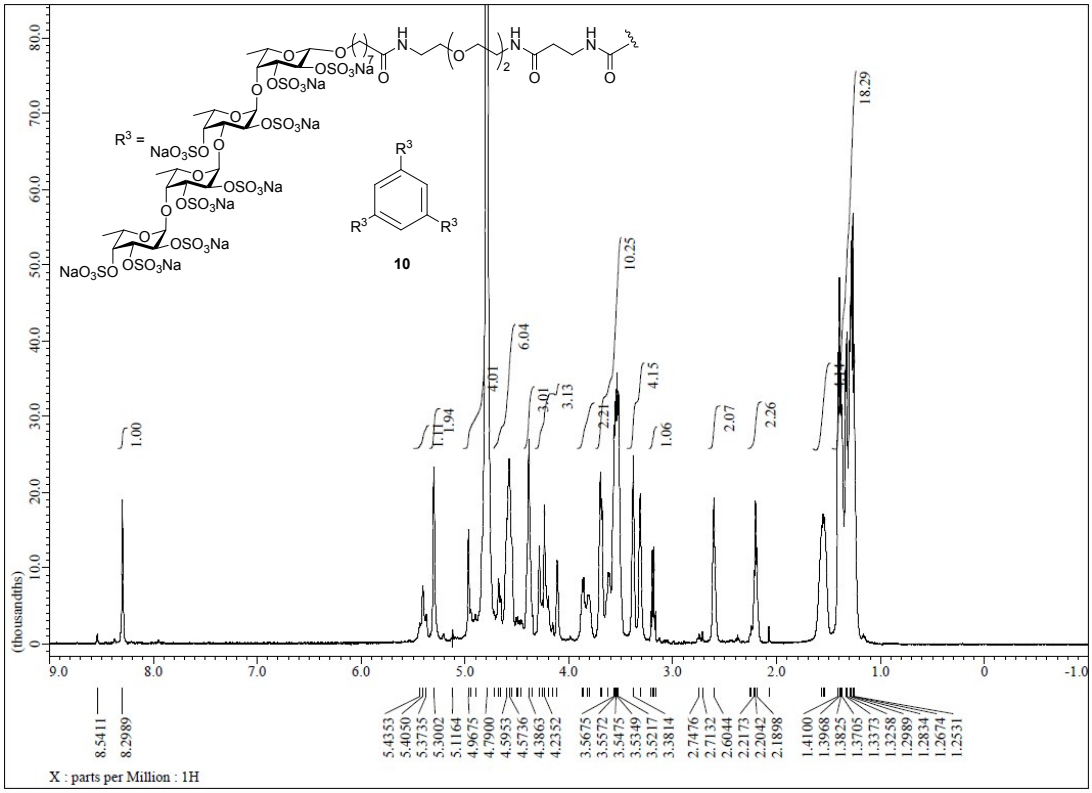


Fig. S16 ^{1}H NMR spectrum of **10**

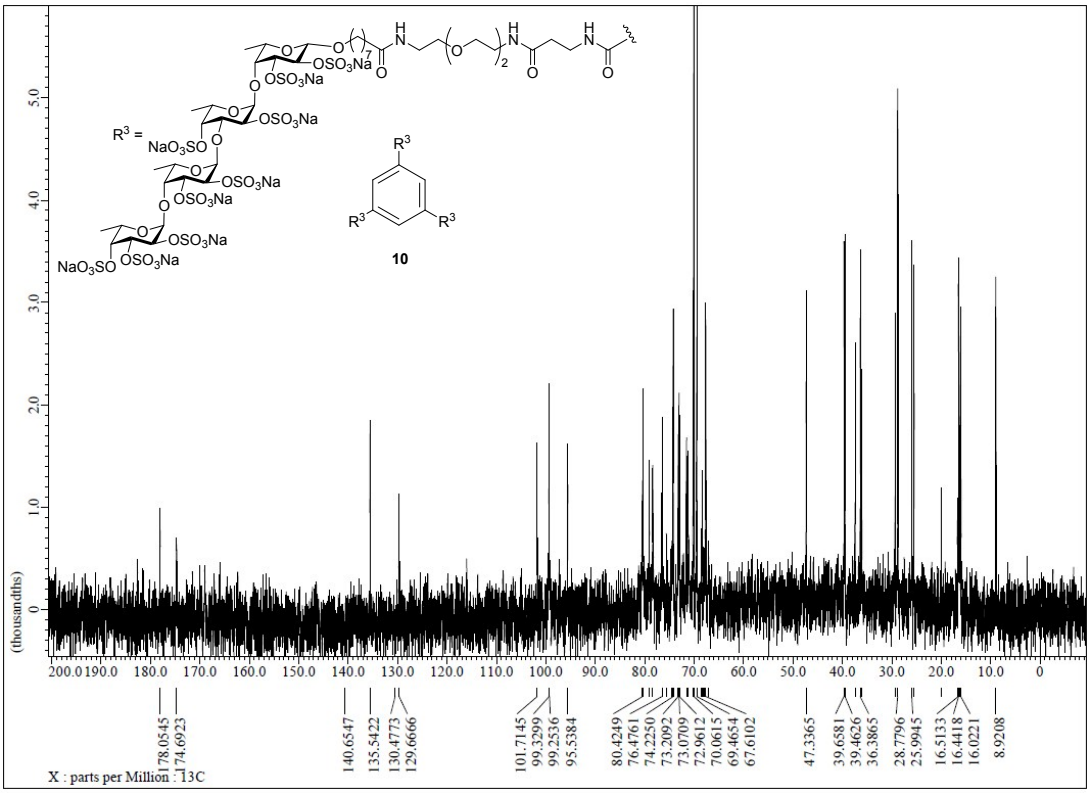


Fig. S17 ^{13}C NMR spectrum of **10**