Supplementary information

Synthesis of low-molecular weight fucoidan derivatives and their binding abilities to the SARS-CoV-2 spike proteins

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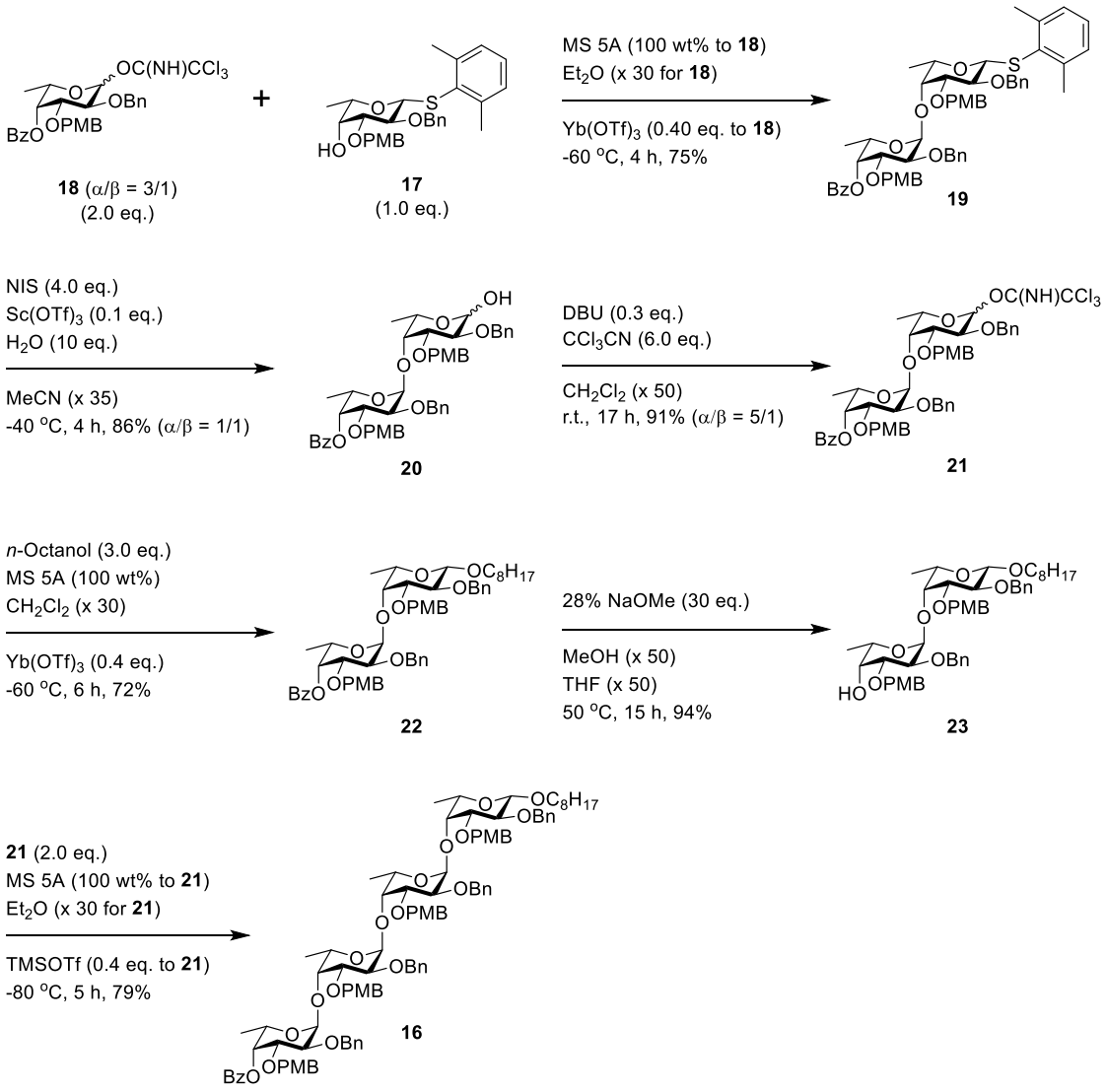
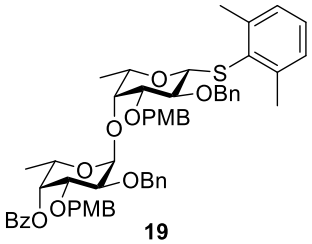
General methods for chemical synthesis

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer or a JEOL Lambda-300 (300 MHz for ^{1}H) spectrometer. ^{1}H-NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from $CDCl_3$ (δ 7.26), CD_3OD (δ 3.31), D_2O (δ 4.79) or tetramethyl silane (δ 0.00), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), and coupling constants (Hz). ^{13}C-NMR chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0) or acetone-d_6 (δ 29.8). ESI-TOF MS 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 was performed on a Merck TLC 60F-254 (0.25 mm). Column chromatography separation was performed on a Silica Gel 60N (spherical, neutral, 63-210 μm or 40-50 μm) (Kanto Chemical Co., Inc.). Reverse phase column chromatography separation was performed on a Wakosil 25C18 (Wako pure chemical industries, Ltd.) or Sep-Pak C18 reversed-phase cartridge (Waters). Gel filtration chromatography separations were performed using a Sephadex™ LH-20 (GE Healthcare). Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware.

Synthesis of tetrafucoside 16



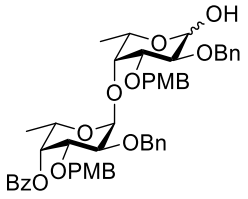
Compound 19



19

To a solution of **18**[1)] (3.70 g, 5.94 mmol) and **17**[2)] (1.47 g, 2.97 mmol) in Et_2O (111 mL) was added MS 5A (3.70 g, 100 wt% to **18**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −60 ºC, and then $Yb(OTf)_3$ (1.47 g, 2.38 mmol) was added to the reaction mixture. After the reaction mixture was stirred for 4 h at the same temperature, the reaction was quenched with triethylamine (4.0 mL). The resultant mixture was filtered through Celite. And then, water was added to the filtrate. The aqueous layer was extracted with EtOAc (100 mL×3), and then the combined 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 (15/1 PhMe/EtOAc) to give **19** (2.14 g, 2.24 mmol, 75% yield). White foam; R_f 0.50 (6/1 PhMe/EtOAc); $[\alpha]^{18}_D$ −116.7° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.07-8.05 (2H, m, Ar-H), 7.59-7.55 (1H, m, Ar-H), 7.46-7.43 (4H, m, Ar-H), 7.36-7.22 (12H, m, Ar-H), 7.16-7.08 (3H, m, Ar-H), 6.82-6.78 (4H, m, Ar-H), 5.60 (1H, br-d, $J_{3',4'}$ = 3.0 Hz, H-4'), 5.02 (1H, d, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.00 and 4.90 (2H, ABq, J = 11.0 Hz, ArCH_2), 4.90 and 4.80 (2H, ABq, J = 11.0 Hz, ArCH_2), 4.69-4.57 (5H, m, ArCH_2×2, H-5 or 5'), 4.30 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.25 (1H, dd, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 3.98 (1H, dd, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe), 3.74 (1H, br-d, $J_{3,4}$ = 3.0 Hz, H-4), 3.67 (1H, t, $J_{1,2}$ = 9.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.38 (1H, dd, $J_{3,4}$ = 2.5 Hz, $J_{2,3}$ = 9.0 Hz, H-3), 3.27 (1H, br-q, J = 6.0 Hz, H-5 or 5'), 2.58 (6H, s, SPhMe_2), 1.24 (3H, d, J = 6.5 Hz, H-6 or 6'), 0.95 (3H, d, J = 6.5 Hz, H-6 or 6'); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 159.0, 144.5, 138.8, 138.5, 132.9, 132.4, 130.4, 130.2, 129.9, 129.7, 129.0, 128.7, 128.3×2, 128.2, 128.1, 127.9×2, 127.5, 127.2, 113.7, 113.6, 100.2, 90.1, 82.3, 78.1, 77.3, 75.5, 75.4, 75.3, 74.5, 73.0, 72.3, 71.9, 71.2. 65.5, 55.2×2, 22.7, 16.9, 16.2; HRMS (ESI-TOF) *m/z* 955.4108 (955.4091 calcd. for $C_{57}H_{63}O_{11}S$, $[M+H]^+$).

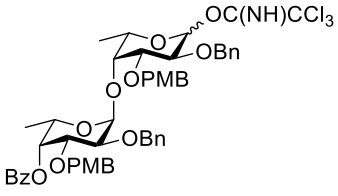
Compound 20



20

To a solution of **19** (1.21 g, 1.27 mmol) in MeCN (42 mL) and H_2O (228 μL) were added NIS (1.14 g, 5.07 mmol) and $Sc(OTf)_3$ (63.0 mg, 128 μmol) at −40 °C. After being stirred at the same temperature for 4 h, the reaction mixture was poured into a solution of saturated aq. $NaHCO_3$ (30 mL) and saturated aq. $Na_2S_2O_3$ (30 mL) at 0 °C. The aqueous layer was extracted with EtOAc (200 mL×3), and then the combined extracts were washed with brine (600 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 PhMe/EtOAc) to give **20** (911 mg, 1.09 mmol, 86% yield, α/β = 1/1). White foam; R_f 0.50 (2/1 PhMe/EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.05-8.01 (2H, m, Ar-H), 7.59-7.56 (1H, m, Ar-H), 7.46-7.19 (16H, m, Ar-H), 6.85-6.77 (4H, m, Ar-H), 5.58 (1H, m), 5.34 (1/2H, m), 4.97-4.90 (3/2H, m), 4.86-4.63 (7H, m), 4.56-4.42 (2H, m), 4.13-4.03 (3/2H, m), 3.94-3.86 (3/2H, m), 3.82-3.75 (15/2H, m), 3.61-3.53 (1H, m), 3.44-3.40 (1/2H, m), 3.23 (1/2H, d, J = 7.5 Hz), 2.80 (1/2H, s, OH-1), 1.37 (3/2H, d, J = 6.5 Hz), 1.32 (3/2H, d, J = 6.5 Hz), 0.98-0.93 (3H, m); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 166.2, 159.1×2, 159.0×2, 138.5, 138.4, 138.3, 138.1, 133.0, 130.5, 130.4×2, 130.3, 130.1, 129.8, 129.6, 129.5, 129.1×2, 128.5, 128.4, 128.3×2, 128.2×2, 128.1, 127.9, 127.7, 127.6, 127.5, 113.7, 113.6, 113.5, 100.3, 100.1, 97.7, 91.6, 80.0, 79.8, 78.4, 76.4, 76.3, 76.0, 75.4, 74.9, 74.7, 74.5, 73.8×2, 73.0, 72.3, 71.6, 71.2, 71.1, 67.3, 65.5, 65.4, 55.2×2, 17.0, 16.5, 16.2×2; HRMS (ESI-TOF) *m/z* 835.3700 (835.3694 calcd. for $C_{49}H_{55}O_{12}$, $[M+H]^+$).

Compound 21

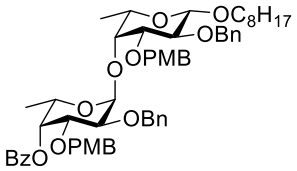


21

To a solution of **20** (2.70 g, 3.23 mmol) in CH_2Cl_2 (135 mL) were added DBU (146 μL, 0.970 mmol) and CCl_3CN (1.95 mL, 19.4 mmol) at room temperature. After being stirred at the same temperature for 17 h, the reaction mixture was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 *n*-Hexane/EtOAc, 2% NEt_3) to give **21** (2.90 g, 2.96 mmol, 91% yield, α/β = 5/1). White foam; R_f 0.60 (α), 0.30 (β) (2/1 *n*-Hexane/EtOAc, 2% NEt_3);

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.63 (1/6H, s, OC(N*H*)CCl_3), 8.50 (5/6H, s, OC(N*H*)CCl_3), 8.06-8.01 (2H, m, Ar-H), 7.60-7.55 (1H, m, Ar-H), 7.47-7.41 (2H, m, Ar-H), 7.39-7.20 (14H, m, Ar-H), 6.83-6.76 (4H, m, Ar-H), 6.60 (5/6H, d, $J_{1,2}$ = 2.7 Hz, H-1), 5.72 (1/6H, d, $J_{1,2}$ = 8.1 Hz, H-1), 5.57 (1H, m, H-4'), 5.01-4.43 (10H, m, H-1', H-5 or 5', Ar*CH*$_2$), 4.15-3.73 (12H, m, H-2, 2', H-3, 3', H-4, H-5 or 5', OMe×2), 1.39 (3/6H, d, J = 6.0 Hz, H-6 or 6'), 1.32 (15/6H, d, J = 6.3 Hz, H-6 or 6'), 0.99-0.93 (3H, m, H-6 or 6'); ^{13}C-NMR (125 MHz, $CDCl_3$) α isomer : δ 166.2, 161.3, 159.1, 159.0, 138.4, 138.3, 133.0, 130.4×2, 130.1, 129.8, 129.5×2, 128.4, 128.3, 128.2, 127.6×2, 127.5, 113.6×2, 100.2, 95.1, 91.5, 78.1, 76.3, 75.2, 74.8, 74.7, 73.9, 72.6, 72.3, 71.6, 71.1, 70.1, 65.5, 55.2×2, 16.5, 16.2; HRMS (ESI-TOF) *m/z* 976.2656 (976.2633 calcd. for $C_{51}H_{53}NO_{12}Cl_3$, $[M-H]^-$).

Compound 22

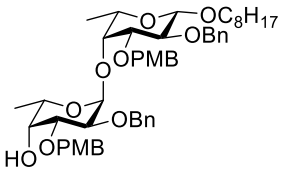


22

To a solution of **21** (2.02 g, 2.06 mmol) in CH_2Cl_2 (61 mL) were added *n*-octanol (979 μL, 6.18 mmol) and MS 5A (2.02 g, 100 wt% to **21**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −60 ºC, and then $Yb(OTf)_3$ (512 mg, 0.824 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 (4.0 mL). The resultant mixture was filtered through Celite. And then, water was added to the filtrate. The aqueous layer was extracted with EtOAc (200 mL×3), and then the combined extracts were washed with brine (600 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (15/1 PhMe/EtOAc) to give **22** (1.41 g, 1.48 mmol, 72% yield). Yellow syrup; R_f 0.40 (15/1 PhMe/EtOAc); $[\alpha]^{22}_D$ −93.7° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.04-8.02 (2H, m, Ar-H), 7.58-7.55 (1H, m, Ar-H), 7.46-7.38 (4H, m, Ar-H), 7.35-7.22 (12H, m, Ar-H), 6.82-6.77 (4H, m, Ar-H), 5.56 (1H, br-d, $J_{3',4'}$ = 2.0 Hz, H-4'), 4.97-4.93 (2H, m, H-1', Ar*CH*$_2$), 4.84-4.62 (6H, m, Ar*CH*$_2$×3), 4.54-4.49 (2H, m, H-5 or 5', Ar*CH*$_2$), 4.29 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.17 (1H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 3.99-3.90 (2H, m, H-2', -O*CH*$_2$CH$_2$-), 3.78 (3H, s, OMe), 3.75 (3H, s, OMe), 3.68 (1H, br-d, $J_{3,4}$ = 2.5 Hz, H-4), 3.62 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.52-3.41 (2H, m, H-5 or 5', -O*CH*$_2$CH$_2$-), 3.37 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 1.73-1.60 (2H, m, -OCH$_2$*CH*$_2$-), 1.48-1.23 (13H, m), 0.95 (3H, d, J = 6.5 Hz, H-6 or 6'), 0.88 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.2, 159.0, 158.9, 138.7, 138.5, 132.9, 130.6, 130.5, 130.2, 129.8, 129.6, 129.1, 128.3×3,

128.1×2, 127.5, 127.4, 113.6, 113.5, 104.0, 100.5, 79.8, 78.3, 78.0, 75.9, 74.9, 74.7, 73.2, 72.4, 71.8, 71.2, 70.7, 70.2, 65.5, 55.1×2, 31.8, 29.8, 29.4, 29.2, 26.1, 22.6, 16.6, 16.2, 14.1; HRMS (ESI-TOF) *m/z* 969.4774 (969.4765 calcd. for $C_{57}H_{70}O_{12}Na$, $[M+Na]^+$).

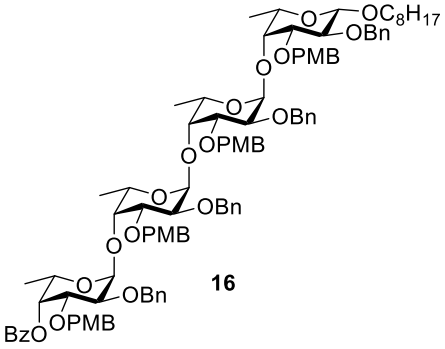
Compound 23



23

To a solution of **22** (1.26 g, 1.33 mmol) in MeOH/THF (1/1, v/v, 76 mL) was added 28% NaOMe in MeOH (7.92 mL, 39.9 mmol) at room temperature, and then the resultant mixture was stirred at 50 °C. After being stirred at the same temperature for 15 h, the reaction mixture 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 (6/1 PhMe/EtOAc) to give **23** (1.06 g, 1.26 mmol, 94% yield). Yellow syrup; R_f 0.50 (6/1 PhMe/EtOAc); $[\alpha]^{24}_D$ −65.1° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.37 (2H, m, Ar-H), 7.34-7.24 (12H, m, Ar-H), 6.90-6.87 (2H, m, Ar-H), 6.82-6.79 (2H, m, Ar-H), 4.93-4.90 (2H, m, H-1', Ar*CH*$_2$), 4.81-4.76 (2H, m, Ar*CH*$_2$), 4.71-4.60 (5H, m, Ar*CH*$_2$), 4.32 (1H, br-q, J = 6.0 Hz, H-5 or 5'), 4.28 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.01 (1H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 3.97-3.92 (1H, m, -O*CH*$_2$CH$_2$-), 3.84-3.78 (8H, m, H-2', H-4', OMe×2), 3.64 (1H, br-d, $J_{3,4}$ = 2.5 Hz, H-4), 3.59 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.51-3.46 (1H, m, -O*CH*$_2$CH$_2$-), 3.42 (1H, br-q, J = 6.0 Hz, H-5 or 5'), 3.35 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 2.40 (1H, br-s, OH-4'), 1.71-1.59 (2H, m, -OCH$_2$*CH*$_2$-), 1.44-1.23 (13H, m), 1.09 (3H, d, J = 7.0 Hz, H-6 or 6'), 0.87 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 159.3, 159.0, 138.8, 138.5, 130.6, 130.4, 129.5, 129.1, 128.2, 128.1, 128.0, 127.4×2, 113.8, 113.6, 103.9, 100.1, 80.1, 78.5, 78.1, 77.5, 75.9, 74.7, 73.4, 72.2, 71.9, 70.7, 70.2×2, 65.8, 55.2×2, 31.8, 29.8, 29.4, 29.2, 26.1, 22.6, 16.7, 16.1, 14.1; HRMS (ESI-TOF) *m/z* 865.4527 (865.4503 calcd. for $C_{50}H_{66}O_{11}Na$, $[M+Na]^+$).

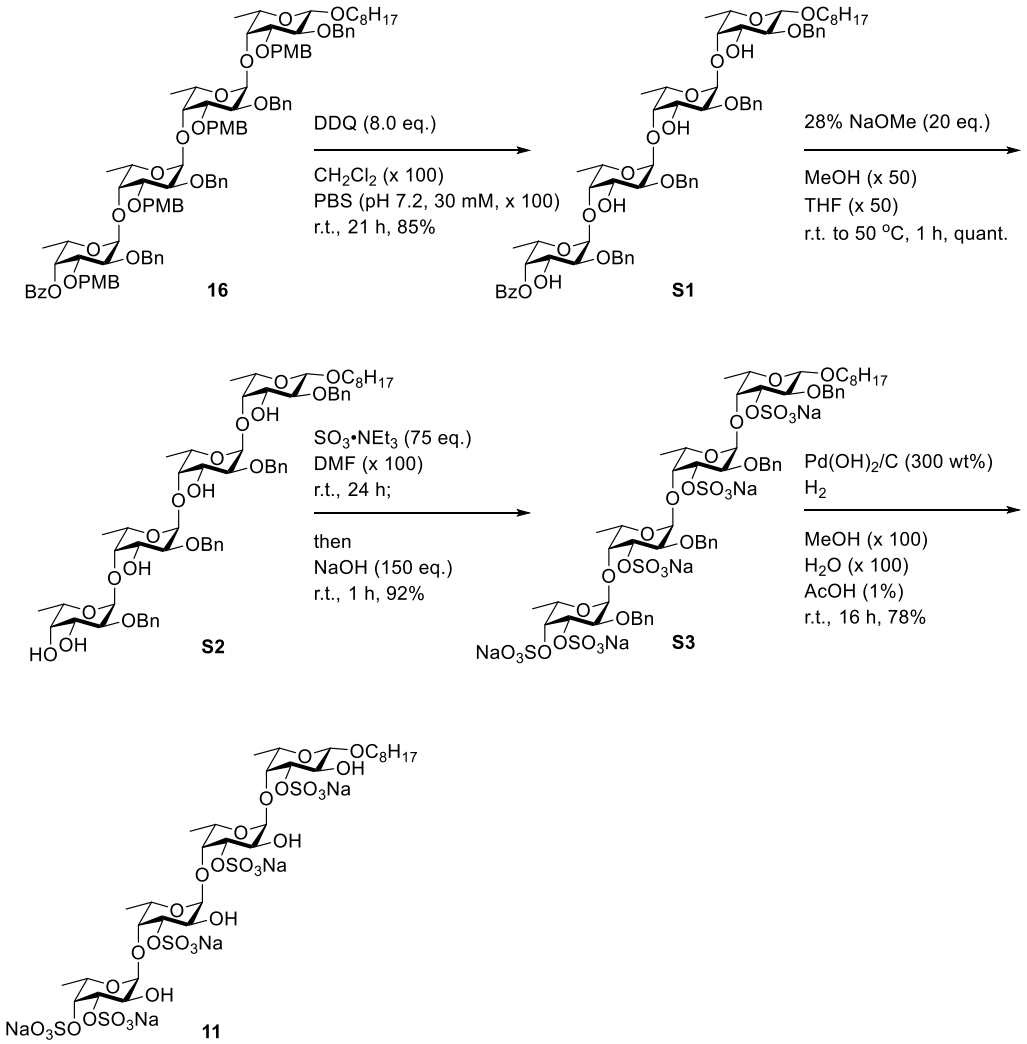
Compound 16



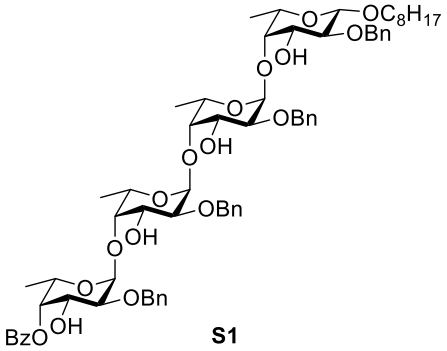
16

To a solution of **21** (26.9 mg, 27.5 μmol) and **23** (11.6 mg, 13.8 μmol) in Et_2O (0.81 mL) was added MS 5A (26.9 mg, 100 wt% to **21**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −80 ºC, and then TMSOTf (2.13 μL, 11.0 μmol) was added to the reaction mixture. After the reaction mixture was stirred for 5 h at the same temperature, the reaction was quenched with triethylamine (20 μL). The resultant mixture was filtered through Celite. And then, water was added to the filtrate. The aqueous layer was extracted with EtOAc (3 mL×3), and then the combined extracts were washed with brine (3 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (6/1 PhMe/EtOAc) to give **16** (18.1 mg, 10.9 μmol, 79% yield). White foam; R_f 0.50 (10/1 PhMe/EtOAc); $[\alpha]^{25}_D$ −72.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.00-7.98 (2H, m, Ar-H), 7.58-7.54 (1H, m, Ar-H), 7.44-7.40 (2H, m, Ar-H), 7.37-7.20 (28H, m, Ar-H), 6.88-6.77 (8H, m, Ar-H), 5.49 (1H, br-d, $J_{3''',4'''}$ = 2.0 Hz, H-4'''), 4.99 (1H, d, J = 3.0 Hz, H-1' or 1'' or 1'''), 4.94 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.92-4.89 (1H, m, ArCH_2), 4.82 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.80-4.59 (14H, m, ArCH_2), 4.42-4.38 (2H, m, H-5 or 5' or 5'' or 5''', ArCH_2), 4.28 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.21 (2H, br-q, J = 6.5 Hz, H-5 or 5' or 5'' or 5'''×2), 3.98-3.67 (22H, m, H-2', 2'', 2''', H-3', 3'', 3''', H-4, 4', 4'', OMe×4, -O$CH_2$$CH_2$-), 3.56 (1H, dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.52-3.46 (1H, m, -O$CH_2$$CH_2$-), 3.41 (1H, br-q, J = 6.0 Hz, H-5 or 5' or 5'' or 5'''), 3.32 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 1.73-1.59 (2H, m, -OCH_2CH_2-), 1.45-1.20 (13H, m), 1.13-1.09 (6H, m, H-6 or 6' or 6'' or 6'''×2), 0.87 (3H, t, J = 7.0 Hz, -$OC_7H_{14}CH_3$), 0.81 (3H, d, J = 6.5 Hz, H-6 or 6' or 6'' or 6'''); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 159.0, 158.9×2, 138.9, 138.7, 138.5×2, 132.9, 131.1×2, 130.7, 130.6, 130.2, 129.8, 129.4, 129.2, 129.0, 128.9, 128.6, 128.5, 128.3, 128.2, 128.1×2, 127.7, 127.5, 127.4×2, 113.6, 113.5, 103.9, 100.3, 99.7, 99.2, 79.8, 79.5, 78.6, 78.5, 76.6, 76.0, 75.6, 74.8, 74.6, 73.7, 72.9, 72.8, 72.5, 72.1×2, 71.8, 71.2, 70.7, 70.1, 67.6, 67.4, 65.4, 55.2×2, 31.8, 29.8, 29.5, 29.3, 26.2, 22.7, 16.8, 16.4, 16.3, 16.0, 14.1; HRMS (ESI-TOF) *m/z* 1659.8180 (1659.8193 calcd. for $C_{99}H_{119}O_{22}$, $[M+H]^+$).

Synthesis of tetrafucoside 11



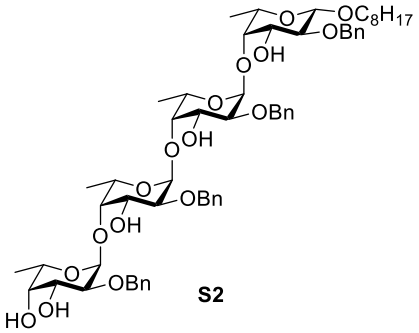
Compound S1



S1

To a solution of **16** (29.5 mg, 17.8 μmol) in CH_2Cl_2/PBS (pH 7.2, 30 mM) (1/1, v/v, 6.0 mL) was added DDQ (32.3 mg, 142 μmol) at room temperature. After being stirred at the same temperature for 21 h, the reaction was quenched with saturated aq. $NaHCO_3$ (6.0 mL). The aqueous layer was extracted with $CHCl_3$ (10 mL×3), and then the combined extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 PhMe/EtOAc) to give **S1** (17.9 mg, 15.1 μmol, 85% yield). White solid; R_f 0.50 (2/1 PhMe/EtOAc); m.p. 130-131 ºC; $[\alpha]^{26}_D$ −196.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.03-8.00 (2H, m, Ar-H), 7.61-7.57 (1H, m, Ar-H), 7.47-7.44 (2H, m, Ar-H), 7.42-7.22 (20H, m, Ar-H), 5.40 (1H, br-d, $J_{3''',4'''}$ = 3.5 Hz, H-4'''), 5.00 (1H, d, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 4.97 (1H, d, J = 3.5 Hz, H-1' or 1''), 4.95 (1H, d, J = 4.0 Hz, H-1' or 1''), 4.89 and 4.78 (2H, ABq, J = 11.5 Hz, Ar*CH*$_2$), 4.73-4.59 (6H, m), 4.31 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.22-4.06 (5H, m, H-3, H-3''', H-5 or 5' or 5'' or 5'''×3), 4.00-3.92 (2H, m, H-3' or 3'', -O*CH*$_2$CH$_2$-), 3.85 (1H, dd, $J_{1''',2'''}$ = 3.5 Hz, $J_{2''',3'''}$ = 10.5 Hz, H-2'''), 3.72-3.54 (8H, m, H-2', 2'', H-4, H-4' or 4'', H-5 or 5' or 5'' or 5''', OH×3), 3.51-3.46 (1H, m, -O*CH*$_2$CH$_2$-), 3.26-3.21 (2H, m, H-2, H-3' or 3''), 2.94 (1H, d, J = 6.5 Hz, H-4' or 4''), 2.18 (1H, d, J = 3.0 Hz, OH), 1.71-1.59 (2H, m, -OCH$_2$*CH*$_2$-), 1.43-1.22 (16H, m), 1.17 (3H, d, J = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 1.07 (3H, d, J = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 0.88 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.5, 138.6, 138.1, 137.7, 137.4, 133.2, 129.8, 129.7, 128.6×2, 128.4, 128.2×2, 128.1×2, 127.8×2, 127.5, 104.0, 100.0, 99.8, 99.7, 83.5, 83.4, 82.6, 79.5, 76.5×2, 75.9, 74.4, 73.9, 73.7, 73.2, 72.8, 72.7, 70.5, 70.3, 69.0, 68.9, 67.9×2, 67.7, 66.2, 31.8, 29.7, 29.4, 29.2, 26.1, 22.6, 16.6, 16.4, 16.2×2, 14.1; HRMS (ESI-TOF) *m/z* 1179.5872 (1179.5892 calcd. for $C_{67}H_{87}O_{18}$, $[M+H]^+$).

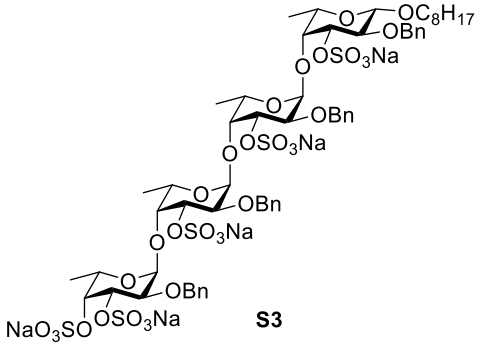
Compound S2



S2

To a solution of **S1** (233 mg, 198 μmol) in MeOH/THF (1/1, v/v, 23 mL) was added 28% NaOMe in MeOH (787 μL, 3.96 mmol) at room temperature, and then the resultant mixture was stirred at 50 ºC. After being stirred at the same temperature for 1 h, 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 (10/1 $CHCl_3$/MeOH) to give **S2** (212.8 mg, 198 μmol, quant.). White solid; R_f 0.60 (7/1 $CHCl_3$/MeOH); m.p. 173-174 ºC; $[\alpha]^{27}_D$ −122.3° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.42-7.37 (8H, m, Ar-H), 7.35-7.22 (12H, m, Ar-H), 4.98 (1H, d, *J* = 3.5 Hz, H-1' or 1'' or 1'''), 4.95 (1H, d, *J* = 3.5 Hz, H-1' or 1'' or 1'''), 4.90 (1H, d, *J* = 4.0 Hz, H-1' or 1'' or 1'''), 4.86-4.66 (8H, m, Ar*CH*$_2$), 4.34 (1H, d, $J_{1,2}$ = 7.0 Hz, H-1), 4.22-4.17 (3H, m, H-5 or 5' or 5'' or 5'''×3), 4.03 (1H, dd, *J* = 10.5 Hz, *J* = 3.0 Hz, H-3' or 3'' or 3'''), 3.93-3.86 (3H, m, H-3' or 3'' or 3'''×2, -O*CH*$_2$CH$_2$-), 3.76-3.63 (8H, m, H-2', 2'', 2''', H-4, 4', 4'', 4''', H-5 or 5' or 5'' or 5'''), 3.57 (1H, dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 3.53-3.48 (1H, m, -O*CH*$_2$CH$_2$-), 3.28 (1H, dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 1.63-1.59 (2H, m, -OCH$_2$*CH*$_2$-), 1.45-1.22 (13H, m), 1.16 (9H, d, *J* = 6.5 Hz, H-6 or 6' or 6'' or 6'''×3), 0.88 (3H, t, *J* = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, CD_3OD) δ 140.1, 140.0, 139.8, 139.7, 129.5, 129.4, 129.3×3, 129.2×2, 128.8, 128.7×2, 128.6, 105.1, 101.3, 101.1, 101.0, 84.1, 83.8, 83.5, 80.4, 78.0, 77.8, 77.5, 75.4, 74.4, 74.0, 73.9, 73.7, 72.0, 70.9, 70.6, 70.3, 70.0, 69.2, 69.1, 68.5, 33.0, 30.9, 30.5, 30.4, 27.4, 23.7, 17.1, 17.0×2, 16.7, 14.4; HRMS (ESI-TOF) *m/z* 1075.5580 (1075.5630 calcd. for $C_{60}H_{83}O_{17}$, $[M+H]^+$).

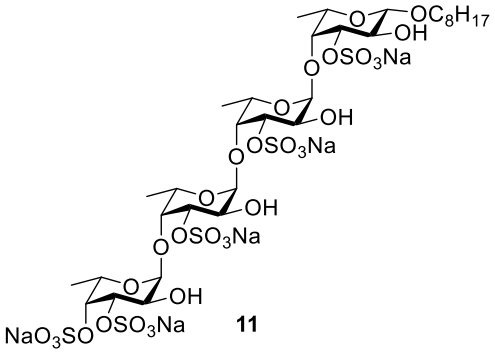
Compound S3



S3

To a solution of **S2** (5.2 mg, 4.84 μmol) in DMF (0.52 mL) was added $SO_3 \cdot NEt_3$ (65.7 mg, 363 μmol) at room temperature. After being stirred at the same temperature for 24 h, 3 M NaOH aq. (240 μL, 725 μmol) was added to the reaction mixture and the mixture was stirred for 1 h. 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 **S3** (7.1 mg, 4.45 μmol, 92% yield). White solid; R_f 0.60 (10/10/1 $CHCl_3$/MeOH/H_2O); m.p. >300 °C; $[\alpha]^{31}_D$ −99.6° (*c* 1.0, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 7.51-7.31 (20H, m, Ar-H), 5.09 (1H, d, J = 4.0 Hz, H-1’ or 1’’ or 1’’’), 5.05 (1H, d, J = 4.0 Hz, H-1’ or 1’’ or 1’’’), 4.95 (1H, d, J = 4.0 Hz, H-1’ or 1’’ or 1’’’), 4.86-4.51 (12H, m, H-1, H-3’ or 3’’, H-3’’’, H-4’’’, ArCH_2), 4.45 (1H, dd, J = 3.0 Hz, J = 11.0 Hz, H-3’ or 3’’), 4.33 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.07 (1H, br-d, $J_{3,4}$ = 3.0 Hz, H-4), 4.04-3.99 (2H, m, H-4’ or 4’’, H-5 or 5’ or 5’’ or 5’’’), 3.96-3.61 (10H, m, H-2, 2’, 2’’, 2’’’, H-4’ or 4’’, H-5 or 5’ or 5’’ or 5’’’×3, -O$CH_2$$CH_2$-), 1.67-1.60 (2H, m, -O$CH_2CH_2$-), 1.44-1.37 (16H, m), 1.22 (3H, d, J = 6.5 Hz, H-6 or 6’ or 6’’ or 6’’’), 1.15 (3H, d, J = 6.5 Hz, H-6 or 6’ or 6’’ or 6’’’), 0.86 (3H, t, J = 7.0 Hz, -$OC_7H_{14}CH_3$); ^{13}C-NMR (125 MHz, D_2O) δ 138.2, 137.8, 137.6×2, 130.6×2, 130.1, 129.8, 129.3, 129.2, 128.9×2, 103.3, 99.8, 99.6, 99.3, 80.5, 80.4, 79.9, 79.3, 78.5, 76.0, 75.7, 75.4, 75.3, 75.0, 74.6, 73.8, 73.5, 72.8, 72.3, 71.6, 71.4, 68.6, 68.5, 67.1, 31.8, 29.6, 29.2, 26.0, 22.7, 16.5, 16.0×2, 15.8, 14.1; HRMS (ESI-TOF) *m/z* 1585.2616 (1585.2568 calcd. for $C_{60}H_{78}O_{32}S_5Na_5$, $[M+H]^+$).

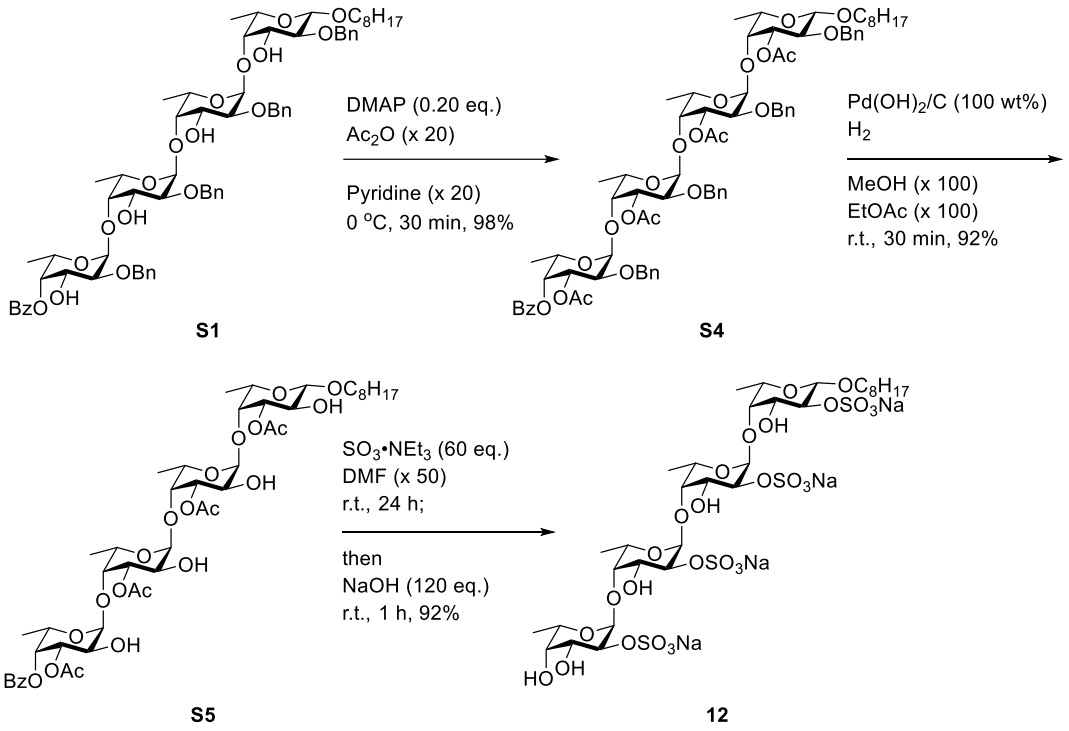
Compound 11



11

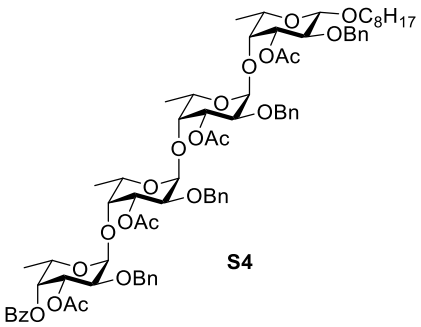
To a solution of **S3** (7.7 mg, 4.86 μmol) in MeOH/H_2O (1/1, v/v, 1.4 mL) were added AcOH (14 μL, 2% to MeOH) and $Pd(OH)_2$/C (23.0 mg, 300wt% to **S3**) under H_2 atmosphere at room temperature. After being stirred for 16 h, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in *vacuo*. The residue was subjected to reverse phase silica gel column chromatography (100/0 to 0/100 H_2O/MeOH) and gel filtration chromatography to give **11** (4.6 mg, 3.79 μmol, 78% yield). White solid; R_f 0.40 (10/10/1 $CHCl_3$/MeOH/H_2O); m.p. >300 °C; $[\alpha]^{28}_D$ −131.7° (*c* 1.0, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 5.16 (1H, d, J = 3.5 Hz, H-1’ or 1’’ or 1’’’), 5.12 (2H, m, H-1’ or 1’’ or 1’’’×2), 4.92 (1H, br-d, J = 2.0 Hz, H-4’ or 4’’ or 4’’’), 4.69-4.61 (4H, m, H-3’, 3’’, 3’’’, H-5 or H-5’ or H-5’’ or H-5’’’), 4.58-4.51 (3H, m, H-1, H-5 or 5’ or 5’’ or 5’’’×2), 4.35 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.27 (1H, br-d, J = 2.0 Hz, H-4’ or H-4’’ or H-4’’’), 4.25 (1H, br-d, J = 2.0 Hz, H-4’ or H-4’’ or H-4’’’), 4.19 (1H, br-d, $J_{3,4}$ = 2.5 Hz, H-4), 4.16-4.10 (2H, m, H-2’ or 2’’ or 2’’’×2), 4.01 (1H, dd, J = 10.5 Hz, J = 3.5 Hz, H-2’ or 2’’ or 2’’’), 3.93-3.87 (2H, m, H-5 or 5’ or 5’’ or 5’’’, -O*CH*$_2$CH$_2$-), 3.76-3.66 (2H, m, H-2, -O*CH*$_2$CH$_2$-), 1.66-1.60 (2H, m, -OCH$_2$*CH*$_2$-), 1.43-1.27 (22H, m), 0.87 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, D_2O) δ 102.7, 100.6, 100.3, 100.1, 79.9, 79.7, 78.2, 78.0, 77.0, 76.6, 76.5, 75.4, 71.3, 71.2, 69.2, 68.5, 68.4, 67.2×2, 67.1, 31.5, 29.2, 28.8, 28.7, 25.4, 22.4, 16.4, 15.9, 13.8; HRMS (ESI-TOF) *m/z* 1225.0746 (1225.0690 calcd. for $C_{32}H_{54}O_{32}S_5Na_5$, $[M+H]^+$).

Synthesis of tetrafucoside 12



S1 **S4** **S5** **12**

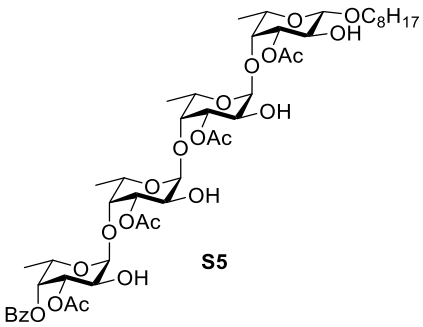
Compound S4



S4

To a solution of **S1** (18.2 mg, 15.4 μmol) in pyridine (0.3 mL) was added DMAP (0.376 mg, 3.08 μmol) at 0 ºC. And then, Ac_2O (0.3 mL) was dropwisely added to the reaction mixture at the same temperature. After being stirred at room temperature for 0.5 h, the reaction was quenched with 1 M HCl aq. (0.5 mL) at 0 ºC. The aqueous layer was extracted with EtOAc (5 mL×3), and then the combined extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (6/1 PhMe/EtOAc) to give **S4** (20.4 mg, 15.1 μmol, 98% yield). White solid; R_f 0.50 (6/1 PhMe/EtOAc); m.p. 135-136 ºC; $[\alpha]^{26}_D$ −150.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.03-8.00 (2H, m, Ar-H), 7.64-7.60 (1H, m, Ar-H), 7.50-7.46 (2H, m, Ar-H), 7.35-7.22 (20H, m, Ar-H), 5.51 (1H, br-d, $J_{3''',4'''}$ = 3.0 Hz, H-4'''), 5.42 (1H, dd, $J_{2''',3'''}$ = 10.5 Hz, $J_{3''',4'''}$ = 3.5 Hz, H-3'''), 5.27-5.21 (2H, m, H-3', 3''), 4.89 (1H, d, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 4.87-4.83 (3H, m, ArCH_2, H-1', 1''), 4.80 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.68-4.55 (7H, m, ArCH_2), 4.37 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.25-4.12 (3H, m, H-5 or 5' or 5''×2, H-5'''), 4.00-3.90 (6H, m, H-2', 2'', 2''', H-4', 4'', -O$CH_2$$CH_2$-), 3.82 (1H, br-d, $J_{3,4}$ = 3.0 Hz, H-4), 3.63-3.57 (2H, m, H-2, H-5 or 5' or 5''), 3.51-3.46 (1H, m, -O$CH_2$$CH_2$-), 2.03 (3H, s, OAc), 2.02 (3H, s, OAc), 1.99 (3H, s, OAc), 1.94 (3H, s, OAc), 1.70-1.58 (2H, m, -OCH$_2$$CH_2$-), 1.43-1.22 (16H, m), 1.17 (3H, d, *J* = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 1.06 (3H, d, *J* = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 0.87 (3H, t, *J* = 7.0 Hz, -$OC_7H_{14}CH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.0, 170.7, 170.2, 166.0, 138.6, 138.2, 137.8, 137.7, 133.3, 129.8, 128.5×2, 128.4×2, 128.3, 128.2×2, 128.0, 127.9, 127.6, 127.5, 103.8, 100.4, 100.0, 99.7, 80.1, 79.5, 78.7, 76.2, 74.6, 74.5, 74.2, 73.6, 73.5, 73.3, 73.0, 72.2, 72.1, 71.9, 70.6, 70.4, 70.1, 66.9, 65.2, 31.8, 29.7, 29.4, 29.3, 26.1, 22.7, 21.5×2, 21.3, 20.9, 16.7, 16.6, 16.3, 14.1; HRMS (ESI-TOF) *m/z* 1347.6273 (1347.6315 calcd. for $C_{75}H_{95}O_{22}$, $[M+H]^+$).

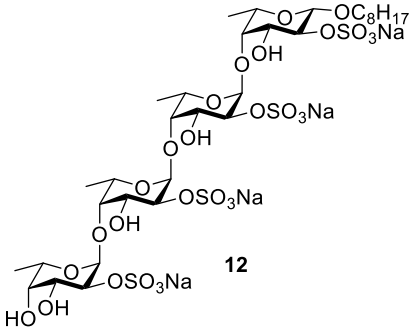
Compound S5



S5

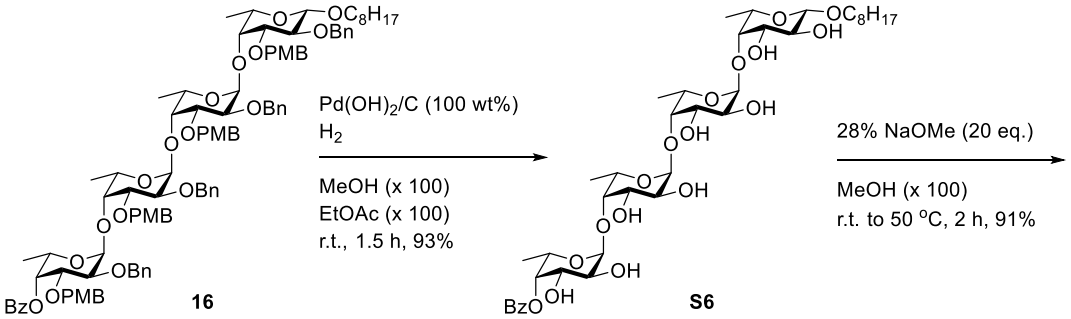
To a solution of **S4** (14.3 mg, 10.6 μmol) in MeOH/EtOAc (1/1, v/v, 2.8 mL) was added $Pd(OH)_2/C$ (14.3 mg, 100 wt% to **S4**) under H_2 atmosphere at room temperature. After being stirred for 0.5 h, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (10/1 $CHCl_3$/MeOH) to give **S5** (9.7 mg, 9.8 μmol, 92% yield). White solid; R_f 0.60 (10/1 $CHCl_3$/MeOH); m.p. 127-128 °C; $[\alpha]^{24}_D$ −193.1° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 8.08-8.06 (2H, m, Ar-H), 7.67-7.63 (1H, m, Ar-H), 7.54-7.51 (2H, m, Ar-H), 5.54 (1H, br-d, $J_{3''',4'''}$ = 1.5 Hz, H-4'''), 5.29-5.21 (3H, m, H-3', 3'', 3'''), 5.07 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 5.02 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.99 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.90 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.67 (1H, q, J = 7.0 Hz, H-5 or 5' or 5'' or 5'''), 4.49-4.42 (2H, m, H-5 or 5' or 5'' or 5'''×2), 4.36 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.15-4.02 (5H, m, H-2', 2'', 2''', H-4', 4''), 3.98-3.97 (1H, m, H-4), 3.90-3.86 (1H, m, -O*CH*$_2$CH$_2$-), 3.81 (1H, q, J = 6.5 Hz, H-5 or 5' or 5'' or 5'''), 3.66 (1H, dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.61-3.56 (1H, m, -O*CH*$_2$CH$_2$-), 2.17 (3H, s, OAc), 2.17 (3H, s, OAc), 2.15 (3H, s, OAc), 1.95 (3H, s, OAc), 1.68-1.62 (2H, m, -OCH$_2$*CH*$_2$-), 1.42-1.27 (19H, m), 1.18 (3H, d, J = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 0.90 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, CD_3OD) δ 172.7, 172.6, 172.4, 172.3, 167.6, 134.7, 130.9, 130.7, 129.8, 104.9, 102.4, 102.2, 102.0, 79.4, 78.6, 77.6, 76.1, 73.6, 73.4, 72.3, 72.2, 71.6, 70.2, 68.7×2, 68.4, 68.2, 68.1, 66.4, 33.0, 30.9, 30.6, 30.4, 27.1, 23.7, 21.5×2, 21.4, 20.8, 17.5, 17.4, 16.9, 14.4 ; HRMS (ESI-TOF) *m/z* 987.4412 (987.4437 calcd. for $C_{47}H_{71}O_{22}$, $[M+H]^+$).

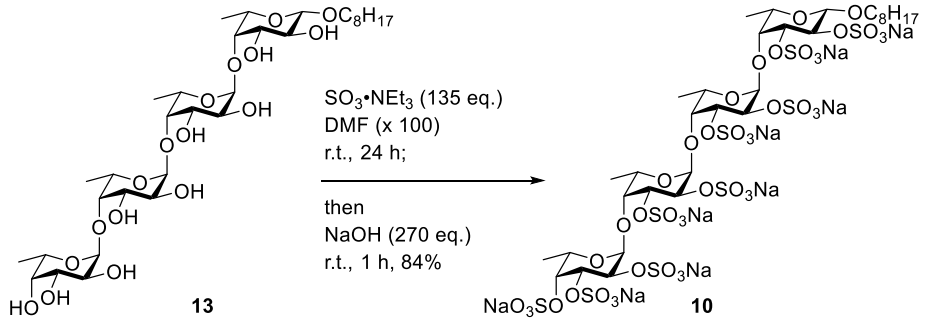
Compound 12



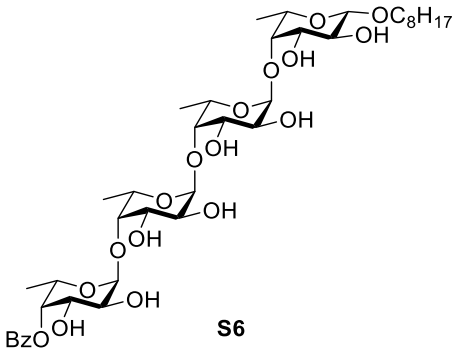
To a solution of **S5** (9.4 mg, 9.52 μmol) in DMF (0.5 mL) was added $SO_3 \cdot NEt_3$ (104 mg, 571 μmol) at room temperature. After being stirred at the same temperature for 24 h, 3 M NaOH aq. (46 μL, 1.14 mmol) was added to the reaction mixture and the mixture was stirred for 1 h. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (100/0 to 0/100 H_2O/MeOH) and gel filtration chromatography to give **12** (9.8 mg, 8.76 μmol, 92% yield). White solid; R_f 0.40 (10/10/1 $CHCl_3$/MeOH/H_2O); m.p. >300 ºC; $[\alpha]^{25}_D$ −127.2° (*c* 0.79, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 5.26-5.24 (3H, m, H-1', 1'', 1'''), 4.57-4.42 (7H, m, H-1, H-2', 2'', 2''', H-5 or 5' or 5'' or 5'''×3), 4.23-4.18 (3H, m, H-2, H-3' or 3'' or 3'''×2), 4.15 (1H, dd, *J* = 10.5 Hz, 3.5 Hz, H-3' or 3'' or 3'''), 4.01-3.98 (2H, m, H-4' or 4'' or 4'''×2), 3.94 (1H, br-d, *J* = 3.0 Hz, H-4' or 4'' or 4'''), 3.88-3.83 (4H, m, H-3, H-4, H-5 or 5' or 5'' or 5''', -O*CH*$_2$CH$_2$-), 3.68-3.61 (1H, m, -O*CH*$_2$CH$_2$-), 1.64-1.58 (2H, m, -OCH$_2$*CH*$_2$-), 1.41-1.26 (19H, m), 1.22 (3H, d, *J* = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 0.86 (3H, t, *J* = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, D_2O) δ 101.0, 99.7×2, 99.4, 83.4, 83.0, 81.6, 79.2, 76.0, 75.9, 72.5, 72.4, 71.3, 71.0, 68.9, 68.8, 68.6, 68.0, 67.6, 67.5, 67.4, 31.5, 29.1, 28.8×2, 25.3, 22.4, 15.9, 15.8×2, 15.6, 13.8; HRMS (ESI-TOF) *m/z* 1145.1085 (1145.1122 calcd. for $C_{32}H_{54}O_{29}S_4Na_5$, $[M+Na]^+$).

Synthesis of tetrafucosides 13 and 10





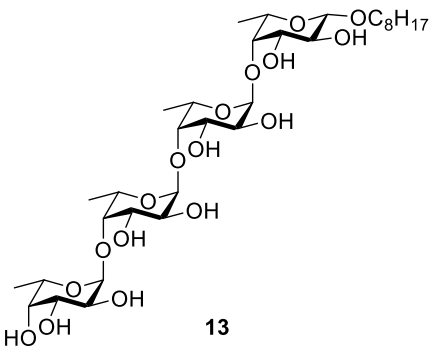
Compound S6



S6

To a solution of **16** (31.0 mg, 18.7 μmol) in MeOH/EtOAc (1/1, v/v, 6.2 mL) was added $Pd(OH)_2/C$ (31.0 mg, 100 wt% to **16**) under H_2 atmosphere at room temperature. After being stirred for 1.5 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 (6/1 $CHCl_3$/MeOH) to give **S6** (14.2 mg, 17.3 μmol, 93% yield). White solid; R_f 0.60 (6/1 $CHCl_3$/MeOH); m.p. 189-190 °C; $[\alpha]^{25}_D$ −96.7° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 8.08-8.06 (2H, m, Ar-H), 7.64-7.60 (1H, m, Ar-H), 7.51-7.47 (2H, m, Ar-H), 5.43 (1H, br-d, $J_{3''',4'''}$ = 3.0 Hz, H-4'''), 4.99 (1H, d, $J_{1''',2'''}$ = 4.0 Hz, H-1'''), 4.92 (1H, d, J = 4.0 Hz, H-1' or 1''), 4.88-4.81 (2H, m, H-1' or 1'', H-5 or 5' or 5'' or 5'''), 4.65-4.58 (2H, m, H-5 or 5' or 5'' or 5'''×2), 4.26 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.10 (1H, dd, $J_{2''',3'''}$ = 11.0 Hz, $J_{3''',4'''}$ = 3.0 Hz, H-3'''), 3.95-3.79 (7H, m, H-2' or 2'' or 2'''×2, H-3', 3'', H-4', 4'', -O$CH_2$$CH_2$-), 3.74-3.70 (3H, m, H-2' or 2'' or 2''', H-4, H-5 or 5' or 5'' or 5'''), 3.60-3.54 (2H, m, H-3, -O$CH_2$$CH_2$-), 3.42 (1H, dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 1.68-1.62 (2H, m, -OCH$_2$$CH_2$-), 1.45-1.27 (19H, m), 1.09 (3H, d, J = 6.0 Hz, H-6 or 6' or 6'' or 6'''), 0.91 (3H, t, J = 6.5 Hz, -O$C_7H_{14}CH_3$); ^{13}C-NMR (125 MHz, CD_3OD) δ 168.0, 134.2, 131.5, 130.7, 129.5, 105.1, 102.7, 102.5×2, 82.1, 81.9, 80.8, 76.4, 74.4, 72.5, 72.3, 71.4, 71.2, 71.0×2, 70.9, 70.8, 69.9, 68.9, 68.8, 67.0, 33.0, 30.9, 30.6, 30.4, 27.1, 23.7, 16.7×2, 16.6, 14.4; HRMS (ESI-TOF) *m/z* 819.3989 (819.4014 calcd. for $C_{39}H_{63}O_{18}$, $[M+H]^+$).

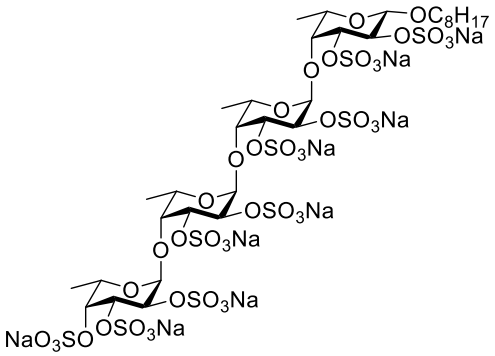
Compound 13



13

To a solution of **S6** (14.2 mg, 17.3 μmol) in MeOH (1.4 mL) was added 28% NaOMe in MeOH (380 μL, 34.6 μmol) at room temperature, and then the resultant mixture was stirred at 50 °C. After being stirred at the same temperature for 2 h, 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 (4/1 $CHCl_3$/MeOH) to give **13** (11.2 mg, 15.7 μmol, 91% yield). White solid; R_f 0.40 (4/1 $CHCl_3$/MeOH); m.p. 142-143 °C; $[\alpha]^{29}_D$ −77.9° (*c* 0.53, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 4.99-4.97 (2H, m, H-1' or 1'' or 1'''x2), 4.95 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.58-4.50 (3H, m, H-5 or 5' or 5'' or 5'''×3), 4.40 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.03-4.00 (2H, m, H-3' or 3'' or 3'''×2), 3.92 (1H, dd, J = 3.0 Hz, 10.5 Hz, H-3' or 3'' or 3'''), 3.88-3.77 (9H, m, H-2', 2'', 2''', H-4, 4', 4'', 4''', H-5 or 5' or 5'' or 5''', -O*CH*$_2$CH$_2$-), 3.71 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.68-3.63 (1H, m, -O*CH*$_2$CH$_2$-), 3.47 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 1.64-1.58 (2H, m, -OCH$_2$*CH*$_2$-), 1.37-1.24 (19H, m), 1.17 (3H, d, J = 6.5 Hz, H-6 or 6' or 6'' or 6'''), 0.86 (3H, t, J = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, D_2O, acetone-d_6) δ 103.1, 100.9, 100.8, 100.7, 80.6, 80.5, 79.6, 72.7, 72.3, 71.1, 70.9, 69.7, 69.2×2, 69.1×2, 69.0, 67.9, 67.8, 67.2, 31.5, 29.3, 28.9, 28.8, 25.5, 22.4, 15.8, 15.6, 15.5, 13.8; HRMS (ESI-TOF) *m/z* 737.3563 (737.3572 calcd. for $C_{32}H_{58}O_{17}Na$, $[M+Na]^+$).

Compound 10



10

To a solution of **13** (2.4 mg, 3.36 μmol) in DMF (0.24 mL) was added SO_3·NEt_3 (82.2 mg, 453 μmol) at room temperature. After being stirred at the same temperature for 24 h, 3 M NaOH aq. (302 μL, 907 μmol) was added to the reaction mixture and the mixture was stirred for 1 h. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (H_2O) and gel filtration chromatography to give **10** (4.6 mg, 2.82 μmol, 84% yield). White solid; R_f 0.30 (10/10/3 $CHCl_3$/MeOH/H_2O); m.p. >300 °C; $[\alpha]^{28}_D$ −89.1° (*c* 1.0, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 5.33-5.31 (2H, m, H-1' or 1'' or 1'''×2), 5.28 (1H, d, J = 3.5 Hz, H-1' or 1'' or 1'''), 4.95 (1H, br-d, $J_{3''',4'''}$ = 3.0 Hz, H-4'''), 4.83-4.77 (3H, m, H-3', 3'', 3'''), 4.70-4.65 (2H, m, H-2' or 2'' or 2''×2), 4.60-4.45 (5H, m, H-1, H-2' or 2'' or 2''', H-5 or 5' or 5'' or 5'''×3), 4.39-4.37

(2H, m, H-2, H-3), 4.31-4.27 (2H, m, H-4', 4''), 4.24-4.22 (1H, m, H-4), 3.88-3.78 (2H, m, H-5 or 5' or 5'' or 5''', -O*CH*$_2$CH$_2$-), 3.66-3.60 (1H, m, -O*CH*$_2$CH$_2$-), 1.62-1.55 (2H, m, -OCH$_2$*CH*$_2$-), 1.44-1.22 (22H, m), 0.83 (3H, t, *J* = 7.0 Hz, -OC$_7$H$_{14}$*CH*$_3$); ^{13}C-NMR (125 MHz, D$_2$O) δ 101.8, 99.4, 80.9, 80.5, 80.3, 79.1, 78.2, 76.5, 74.2, 73.5, 73.4, 73.3, 73.0, 71.6, 71.4, 68.9, 67.6, 31.8, 29.4, 29.2, 29.1, 25.6, 22.7, 16.4×2, 16.1×2, 14.1; HRMS (ESI-TOF) *m/z* 1654.8132 (1654.8060 calcd. for $C_{32}H_{49}O_{44}S_9Na_{10}$, $[M+Na]^+$).

Materials and methods for biological assay

His-tagged SARS-CoV-2 S1+S2 protein was purchased from Sino Biological Inc. (China). Streptavidin-coated biosensor was purchased from Fortebio (U.S.A.). Biolayer interferometry system was measured by BLItz (Fortebio). Biotinylated heparin (11 kDa) was purchased from PG Research (Japan). Bovine serum albumin (BSA) and fondaparinux were purchased from Sigma-Aldrich Co. LLC. (Japan). BIOPHEN^{TM} ANTI-Xa (2 Stages Heparin Assay) kit was purchased from Hyphen BioMed (France).

Expression and purification of recombinant spike proteins

The codon-optimized gene of SARS-CoV-2 S protein (GenBank: QHD43416) was designed for expression in mammalian cells and synthesized from GeneArt DNA Synthesis (Thermo). For expression of recombinant S protein, the sequence encoding the S ectodomain (residues 1 - 1208) with proline substitutions at residues 986 and 987, a "GSAS" substitution at furin cleavage site (residues 682 - 685), and C-terminal foldon trimerization motif followed by an octa-histidine tag was cloned into a pcDNA3.1 expression vector (Invitrogen). Further, S protein mutations occurring in the B.1.1.7 variant (*Δ*69-70, *Δ*144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), B.1.351 variant (D80A, D215G, K417N, E484K, N501Y, D614G, A701V), and P.1 variant (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I) were introduced by inverse PCR method.

Recombinant S proteins were transiently expressed in Expi293f cells (Thermo) maintained in HE400AZ medium (Gmep, Japan). The expression vector was transfected by using Gxpress 293 Transfection Kit (Gmep, Japan) as following manufacturer's protocol. After 5 days post transfection, culture supernatants were harvested, and His-tagged S proteins were purified by Ni^{2+} affinity chromatography using Ni Sepharose 6 Fast Flow (Cytiva), followed by size exclusion chromatography using Superdex 200 Increase 10/300 GL (Cytiva).

Binding affinity of heparin to wild type SARS-CoV-2 S protein

Streptavidin-coated biosensor was hydrated in phosphate buffered saline (PBS, 137 mM NaCl, 8.1 mM Na_2HPO_4, 2.68 mM KCl, 1.47 mM KH_2PO_4, pH 7.4) containing 0.02% BSA for 10 minutes. Next, biotinylated heparin (0, 22.7, 45.5, 90.9, and 181.8 nM in 0.02% BSA/PBS) was loaded on streptavidin biosensors for 120 s placed in the drop holder. The loaded sensor tips were dipped into the solution of SARS-CoV-2 S protein (1 μM in 0.02% BSA/tris buffered saline (TBS, 20 mM Tris, 150 mM NaCl, pH 7.4)) for 300 s from the drop holder position. Finally, biosensor was exposed to 0.02% BSA/TBS for 120 s from the tube position. The dissociation constant (K_d) was generated by fitting 1:1 Langmuir model.

Competitive inhibition assay

Streptavidin-coated biosensor was hydrated in 0.02% BSA/PBS for 10 minutes. Next, biotinylated heparin (181.8 nM in 0.02% BSA/PBS or 0 nM as control) was loaded on streptavidin biosensors for 120 s placed in the drop holder. The loaded sensor tips were dipped into the solution of SARS-CoV-2 S protein (1 μM) and fucoidan derivatives **1**-**13**, or fondaparinux (**24**) (50 μM) in 0.02% BSA/TBS for 300 s from the drop holder position. Finally, biosensor was exposed to 0.02% BSA/TBS for 120 s from the tube position. The inhibition rate was evaluated by the decrease of signal.

Binding affinities of heparin to mutant SARS-CoV-2 S proteins

Streptavidin-coated biosensor was hydrated in 0.02% BSA/PBS for 10 minutes. Next, biotinylated heparin (181.8 nM in 0.02% BSA/PBS or 0 nM as control) was loaded on streptavidin biosensors for 120 s placed in the drop holder. Next, the loaded sensor tips were dipped into the solution of mutant SARS-CoV-2 S protein derived from B.1.1.7, B.1.351 and P.1 (0.125, 0.25, 0.5, and 1 μM) in 0.02% BSA/TBS for 300 s (for P.1) or 120 s (for B.1.1.7 and B.1.351) from the drop holder position. Finally, biosensor was exposed to 0.02% BSA/TBS for 120 s from the tube position. Global fitting of these data to a 1:1 binding model using the BLItz Pro 1.3 software gave dissociation constant (K_d) for heparin to several mutant SARS-CoV-2 S proteins.

Binding affinities of 10 to mutant SARS-CoV-2 S proteins

Streptavidin-coated biosensor was hydrated in 0.02% BSA/PBS for 10 minutes. Next, biotinylated heparin (181.8 nM in 0.02% BSA/PBS or 0 nM as control) was loaded on streptavidin biosensors for 120 s placed in the drop holder. Next, the loaded sensor tips were dipped into the solution of several SARS-CoV-2 S protein derived from wild type, B.1.1.7, B.1.351 and P.1 (1 μM) and **10** (0-500 μM) in 0.02% BSA/TBS for 300 s (for wild type and P.1) or 120 s (for B.1.1.7 and B.1.351) from the drop holder position. Finally, biosensor was exposed to 0.02% BSA/TBS for 120 s. IC_{50} values were calculated based on the decrease of signal using GraphPad Prism software. Inhibition constant (K_i) of **10** to several SARS-CoV-2 S proteins were calculated based on the IC_{50} values according to the Cheng-Prusoff equation[3].

Coagulation (Factor Xa) assay

The anti-factor Xa activity were measured using BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay) kit containing R1, R2 and R3 solutions. 40 μL of R1 solution containing 0.5 IU mL^{-1} antithrombin III and 40 μL of **10** or fondaparinux (**24**) (0.001-10 μg mL^{-1}) in Tris-EDTA buffer (50 mM Tris, 175 mM NaCl, 7.5 mM EDTA, 0.1% PEG, 0.9 g/L NaN_3, pH 8.4) were mixed in 96-well plate and incubated for 2 min at 37 °C. And then, 40 μL of R2 solution containing 4 μg

mL^{-1} factor Xa was added into the plate and incubated for 2 min at 37 °C. After incubation for 2 min, 40 µL of R3 solution containing 0.4 mg mL^{-1} factor Xa specific chromogenic substrate was added and incubated exactly 2 min at 37 °C, and then the reaction was stopped by adding 80 µL of citric acid (20 g L^{-1}). The absorbance was measured at 405 nm using SpectraMax i3 (Molecular Devices) micro plate reader.

References

1) A. Kasai, S. Arafuka, N. Koshiba, D. Takahashi and K. Toshima, *Org. Biomol. Chem*., 2015, **13**, 10556.
2) S. Arafuka, N. Koshiba, D. Takahashi and K. Toshima, *Chem. Commun.*, 2014, **50**, 9831.
3) Y. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099.

NMR
spectrum charts

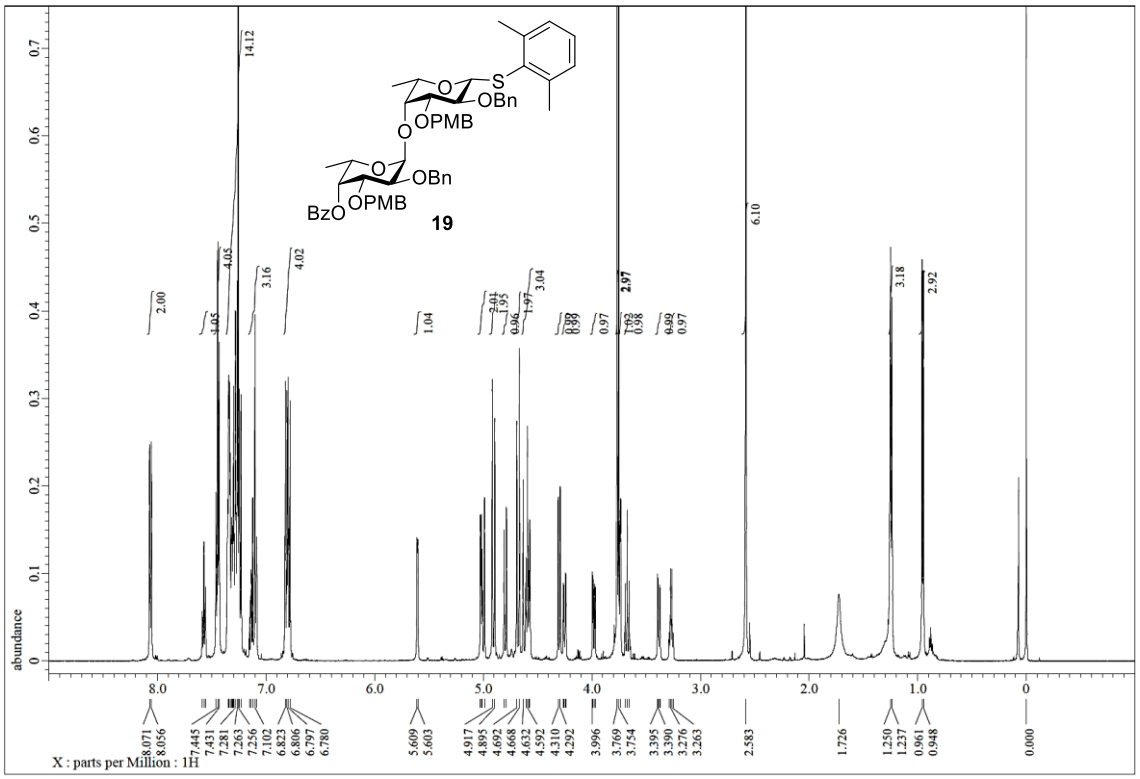


Fig. S1 ^{1}H-NMR spectrum of **19**

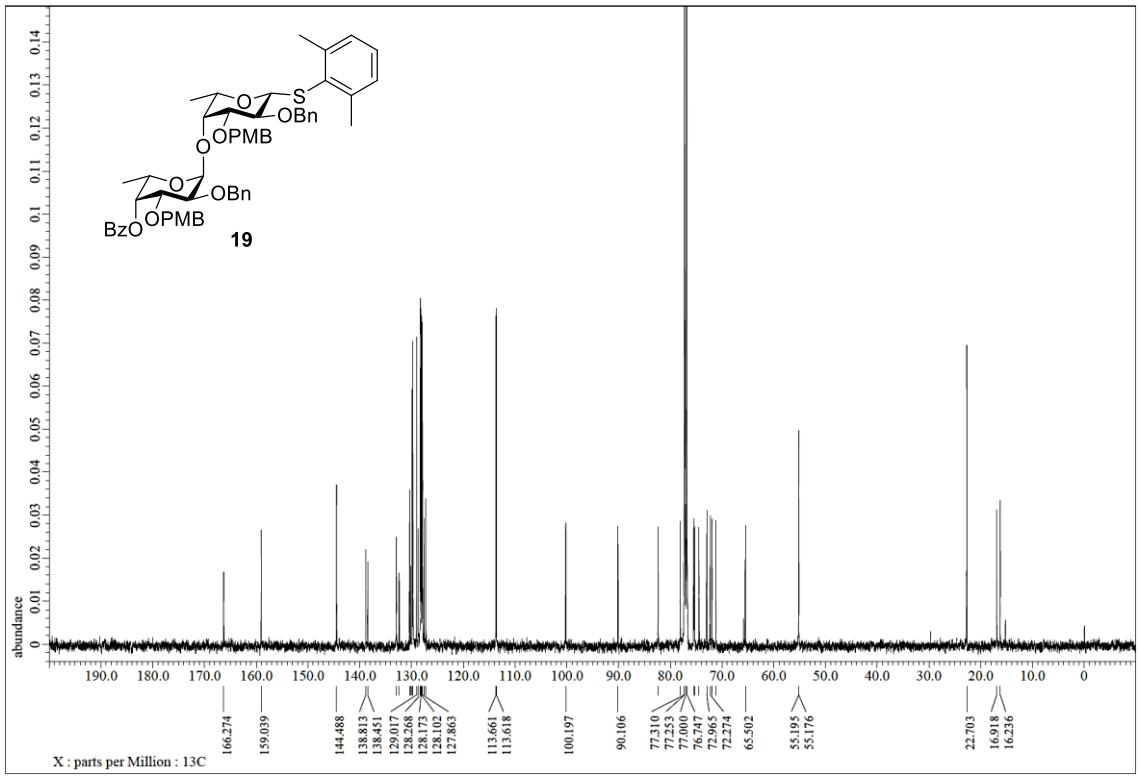


Fig. S2 ^{13}C-NMR spectrum of **19**

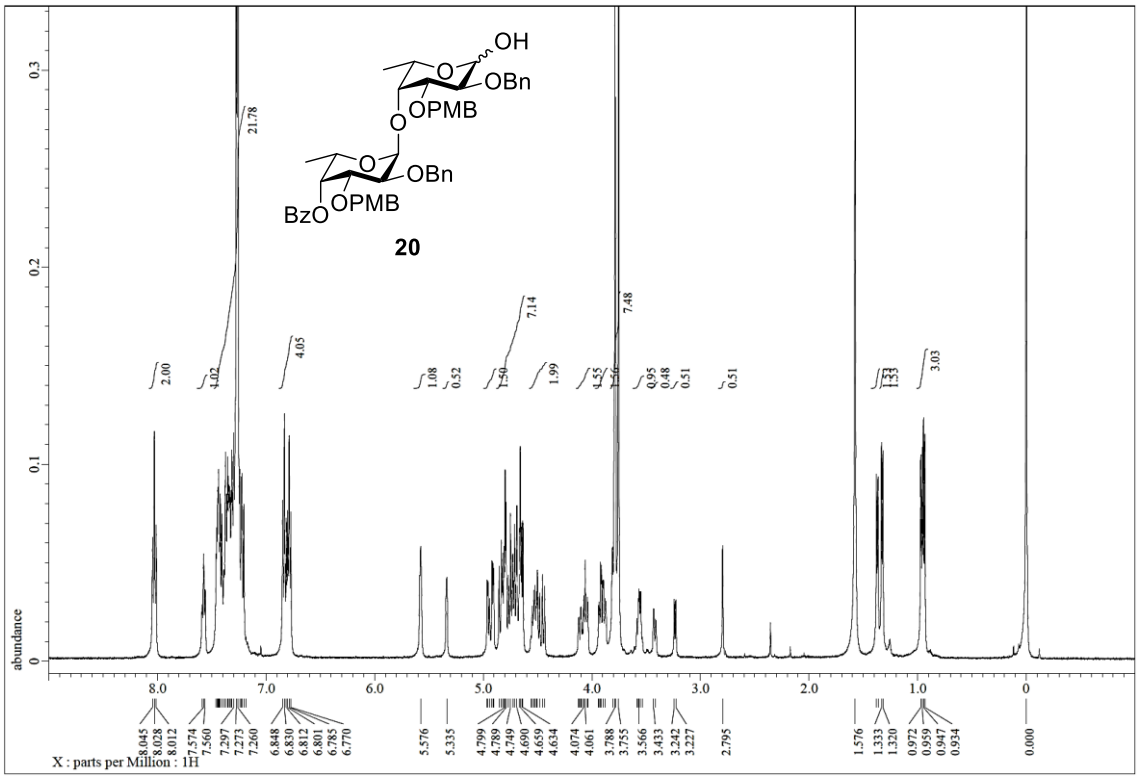


Fig. S3 ^{1}H-NMR spectrum of **20**

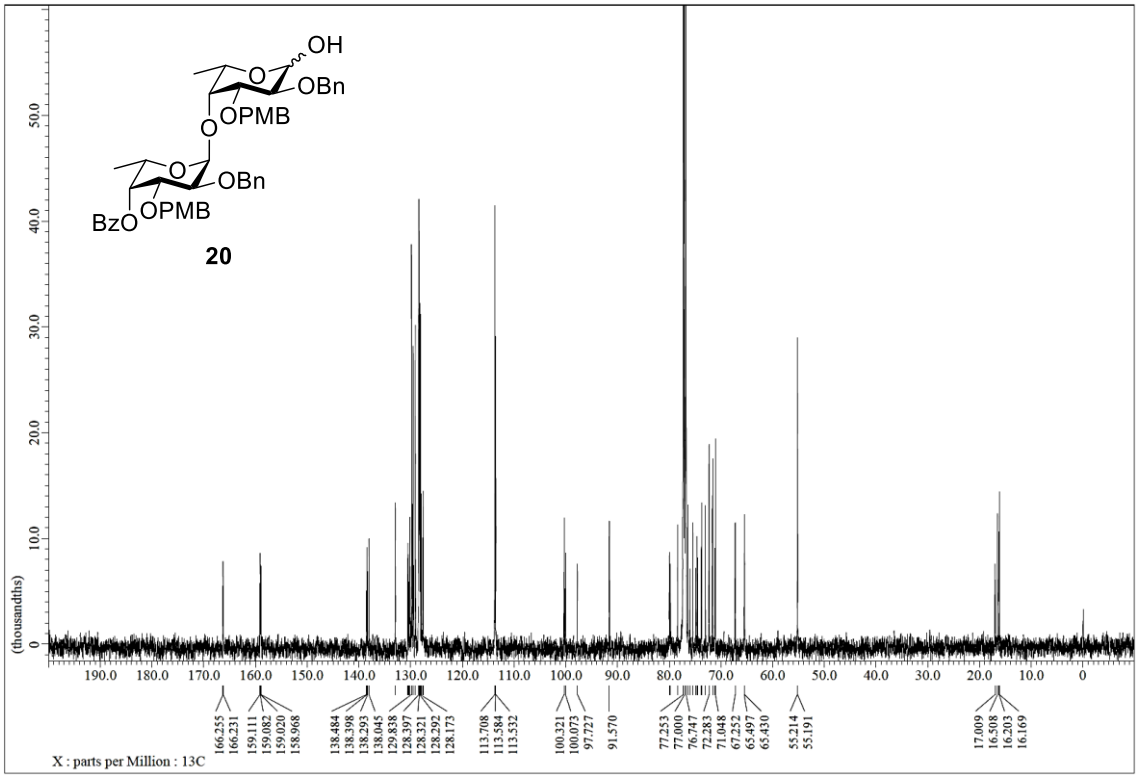


Fig. S4 ^{13}C-NMR spectrum of **20**

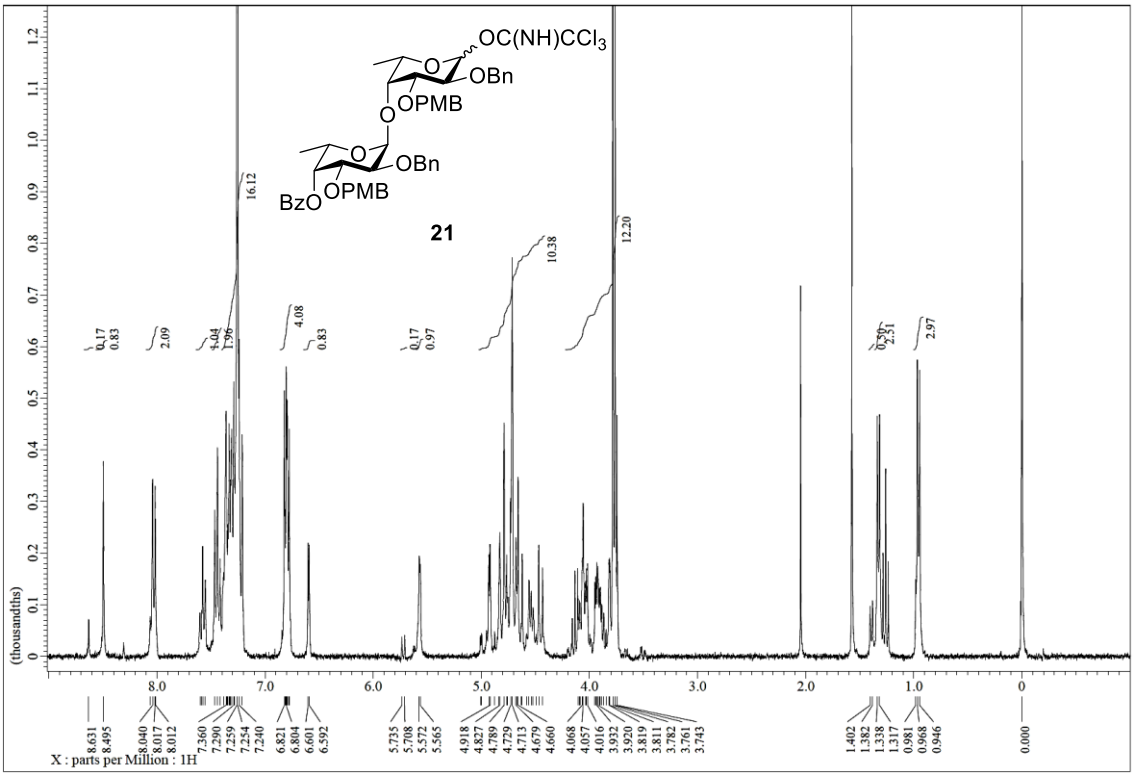


Fig. S5 ^{1}H-NMR spectrum of **21**

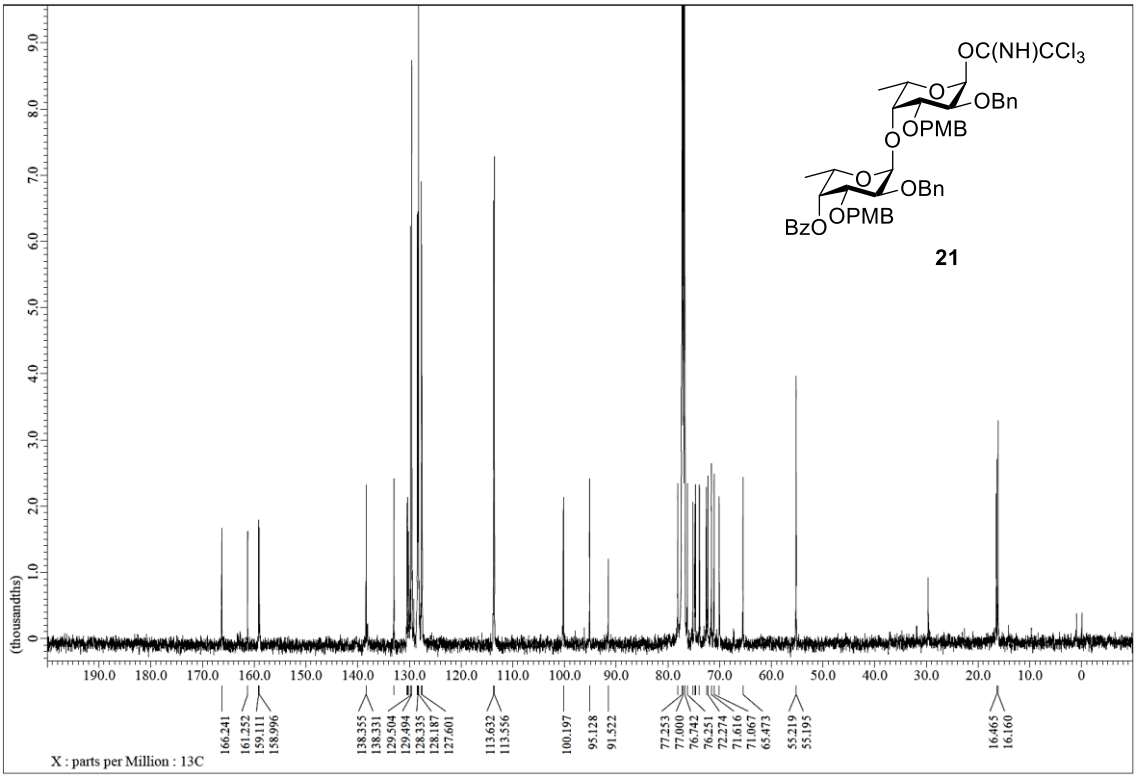


Fig. S6 ^{13}C-NMR spectrum of **21**

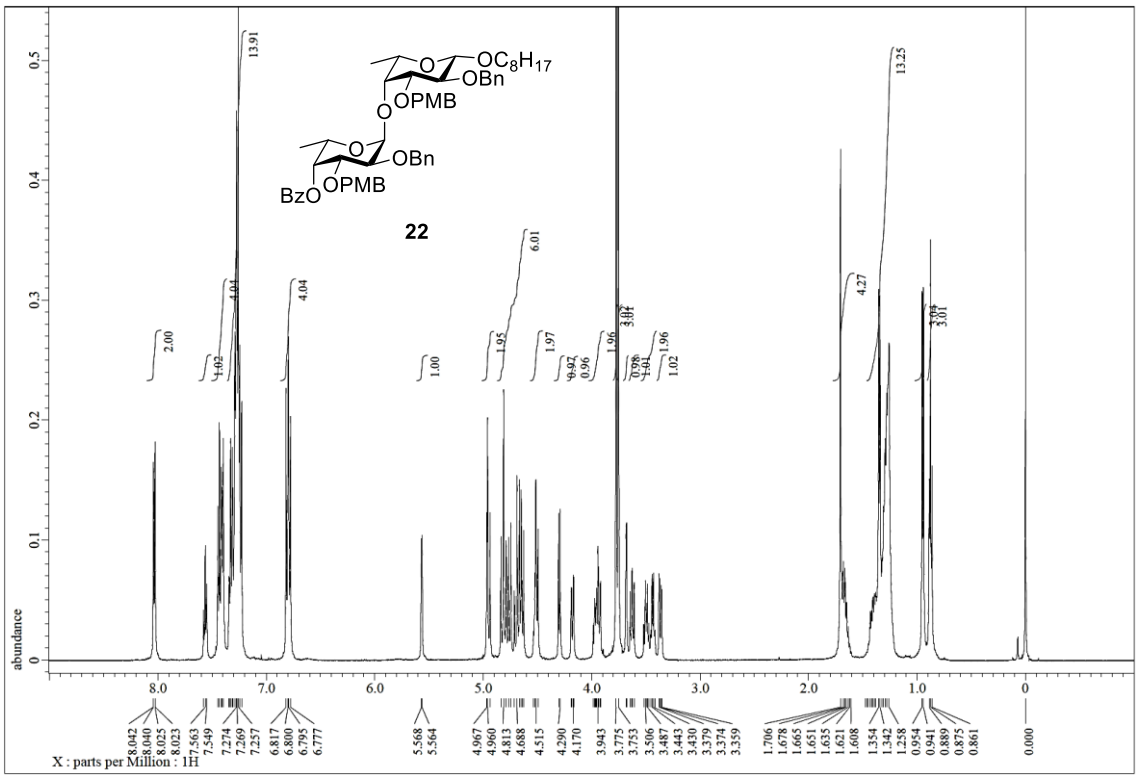


Fig. S7 ^{1}H-NMR spectrum of **22**

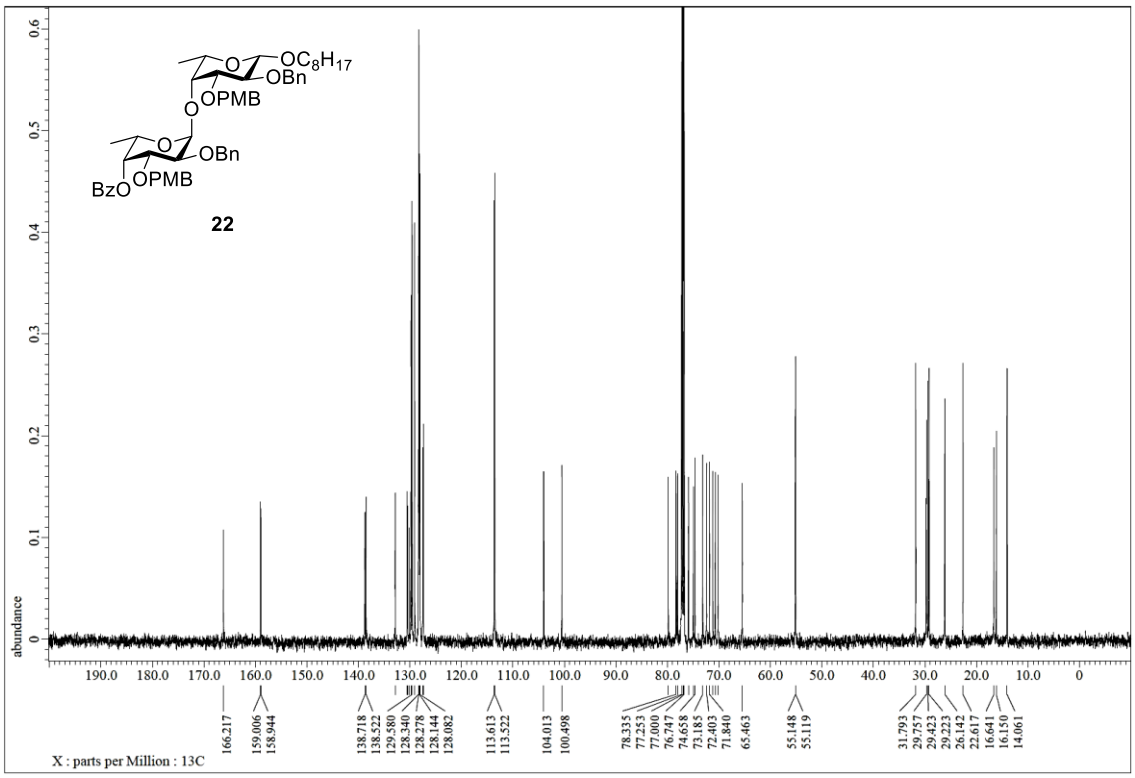


Fig. S8 ^{13}C-NMR spectrum of **22**

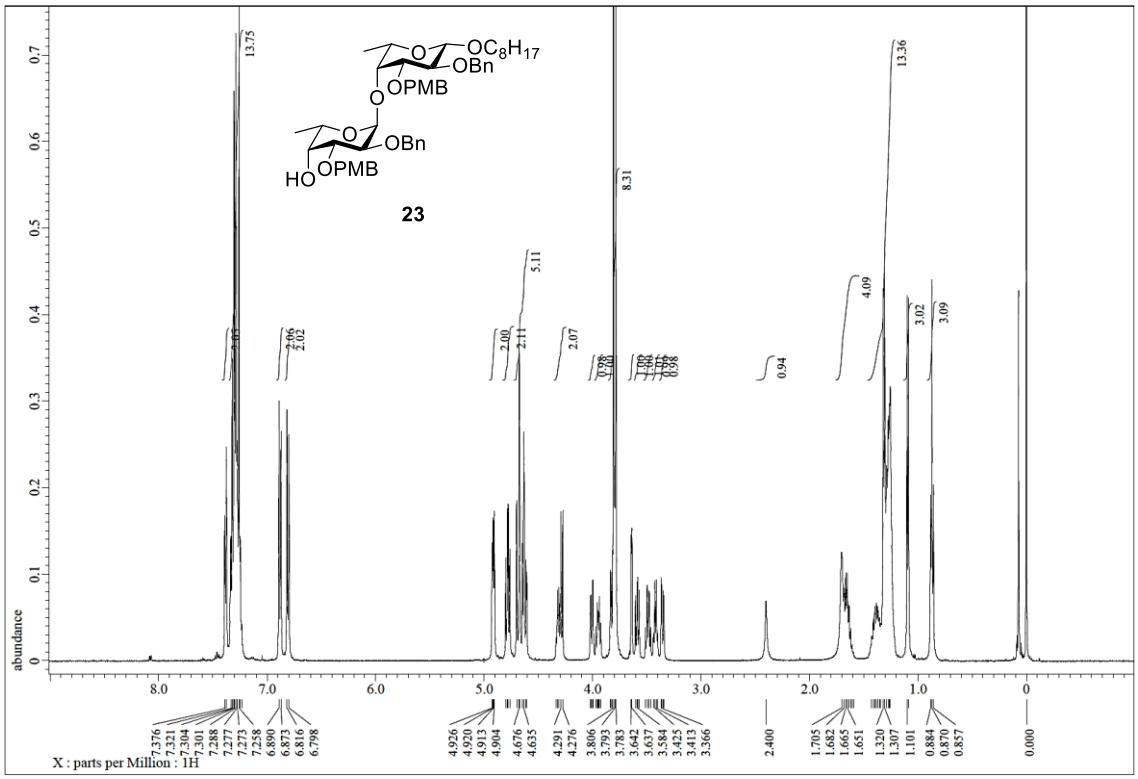


Fig. S9 ^{1}H-NMR spectrum of **23**

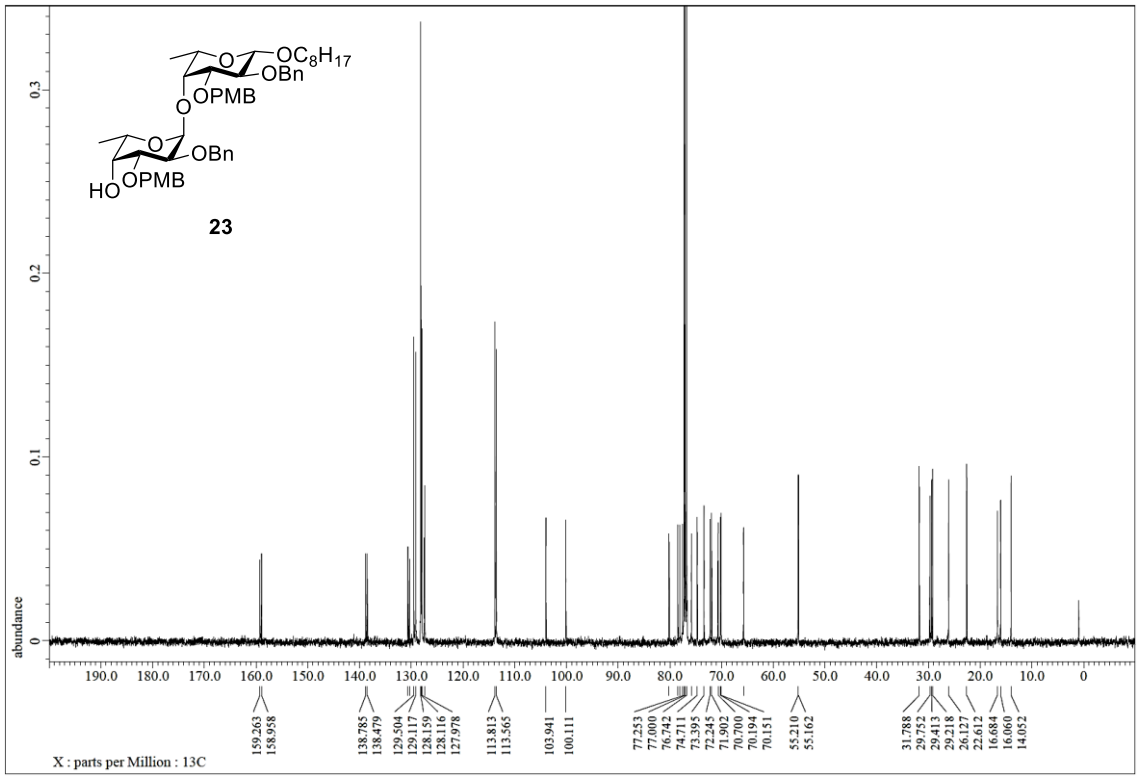


Fig. S10 ^{13}C-NMR spectrum of **23**

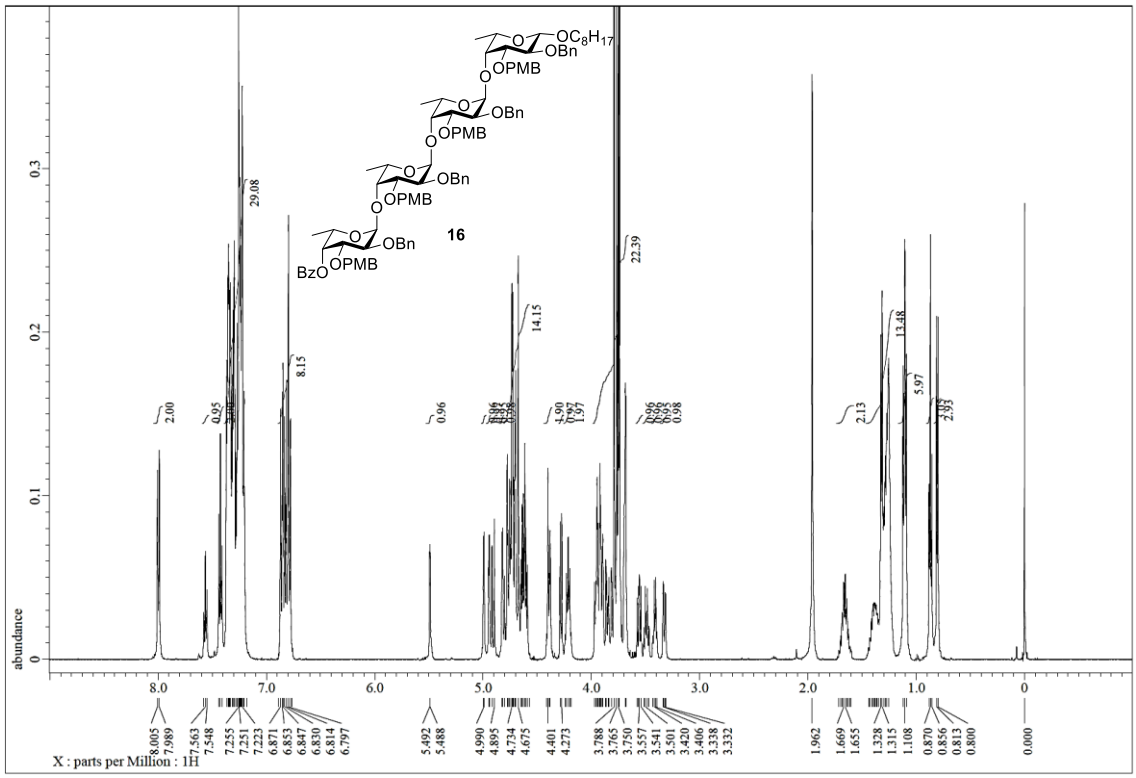


Fig. S11 ^{1}H-NMR spectrum of **16**

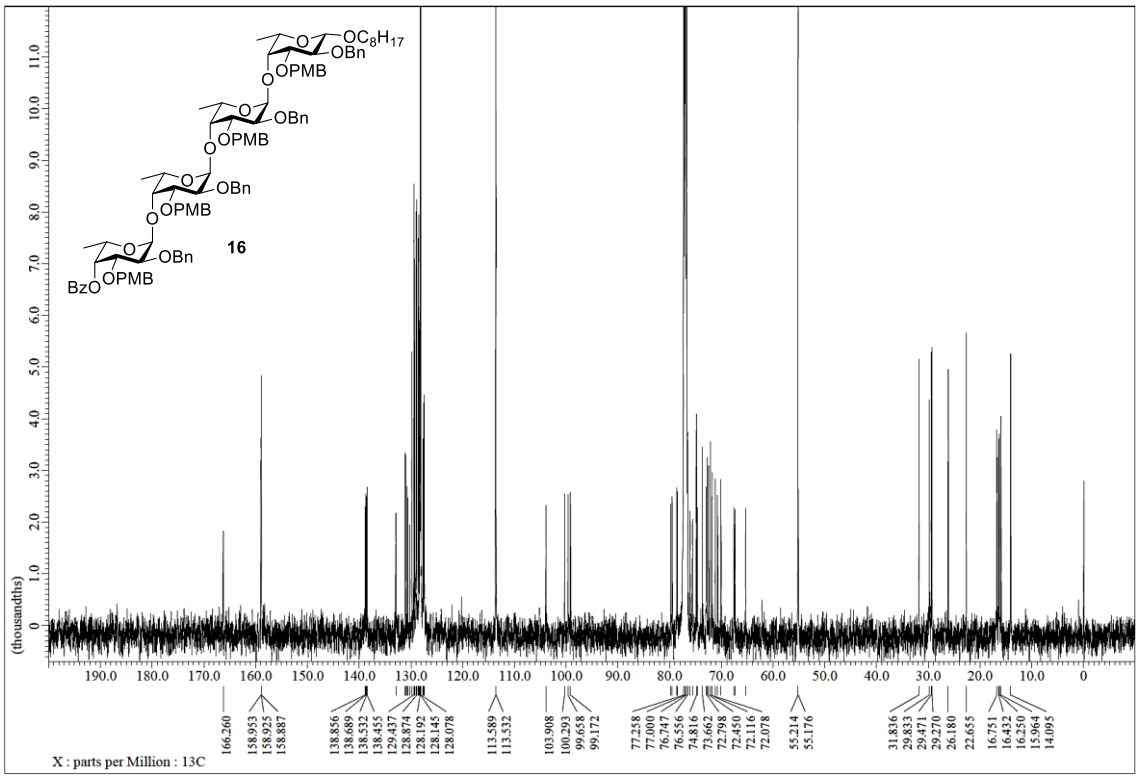


Fig. S12 ^{13}C-NMR spectrum of **16**

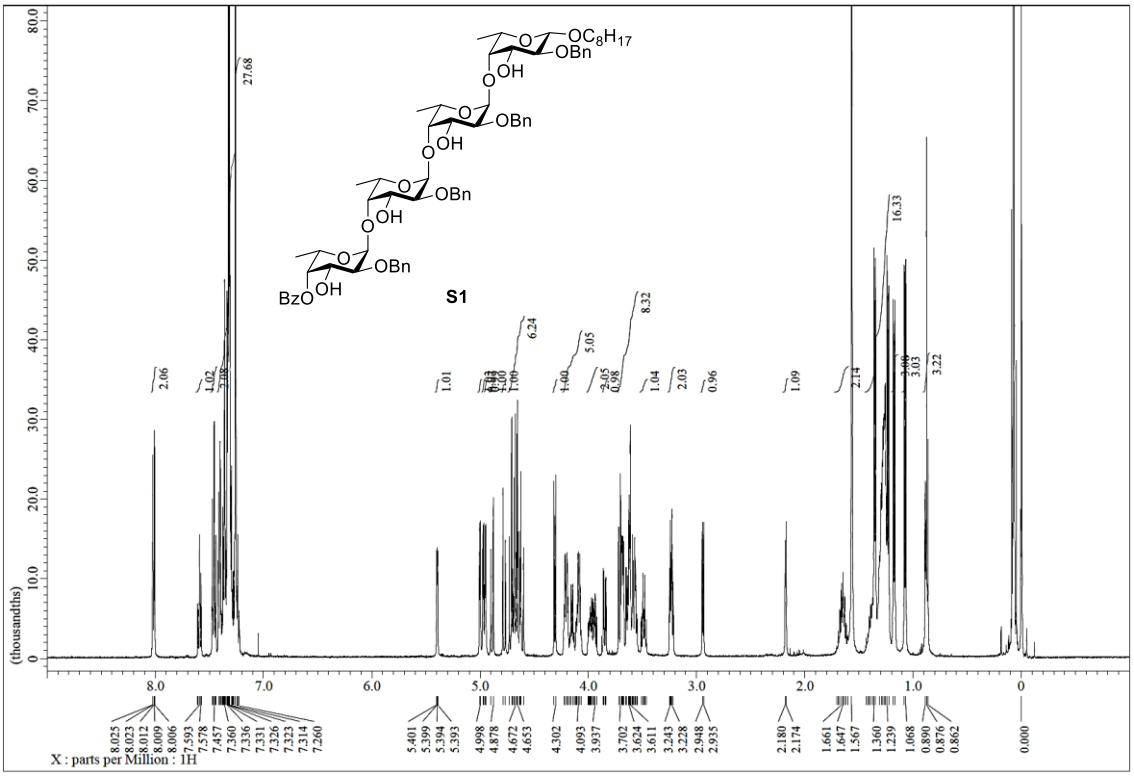


Fig. S13 ^{1}H-NMR spectrum of **S1**

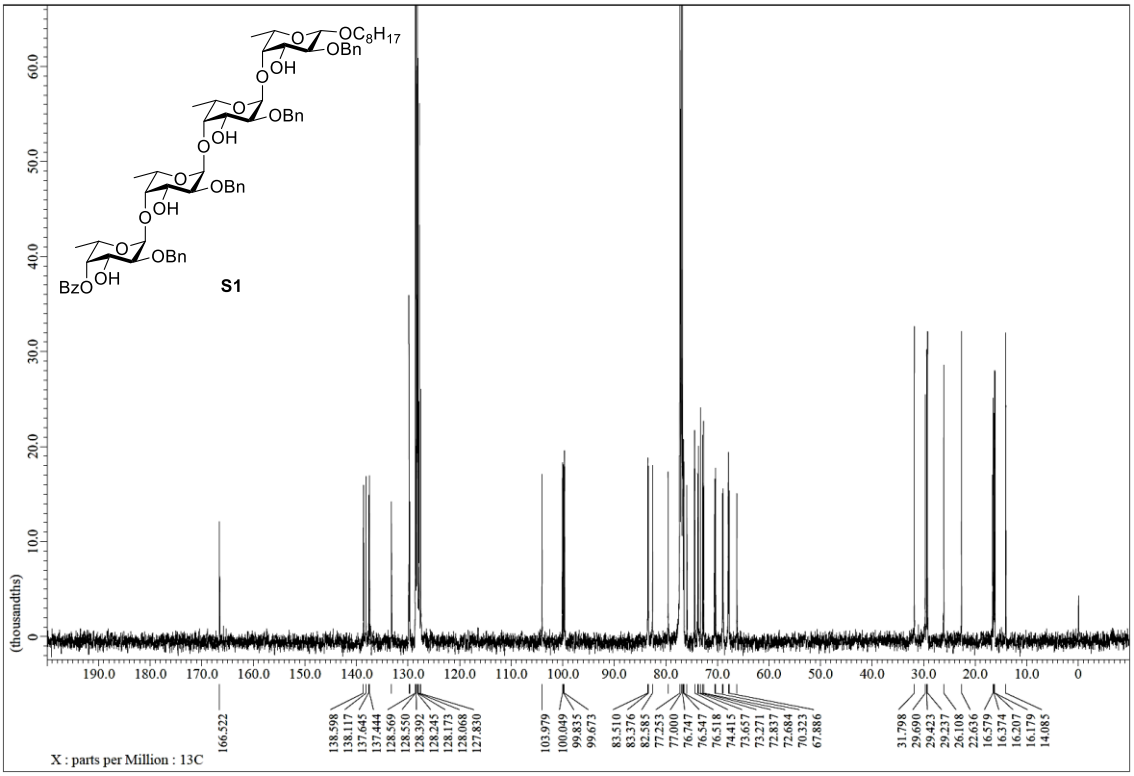


Fig. S14 ^{13}C-NMR spectrum of **S1**

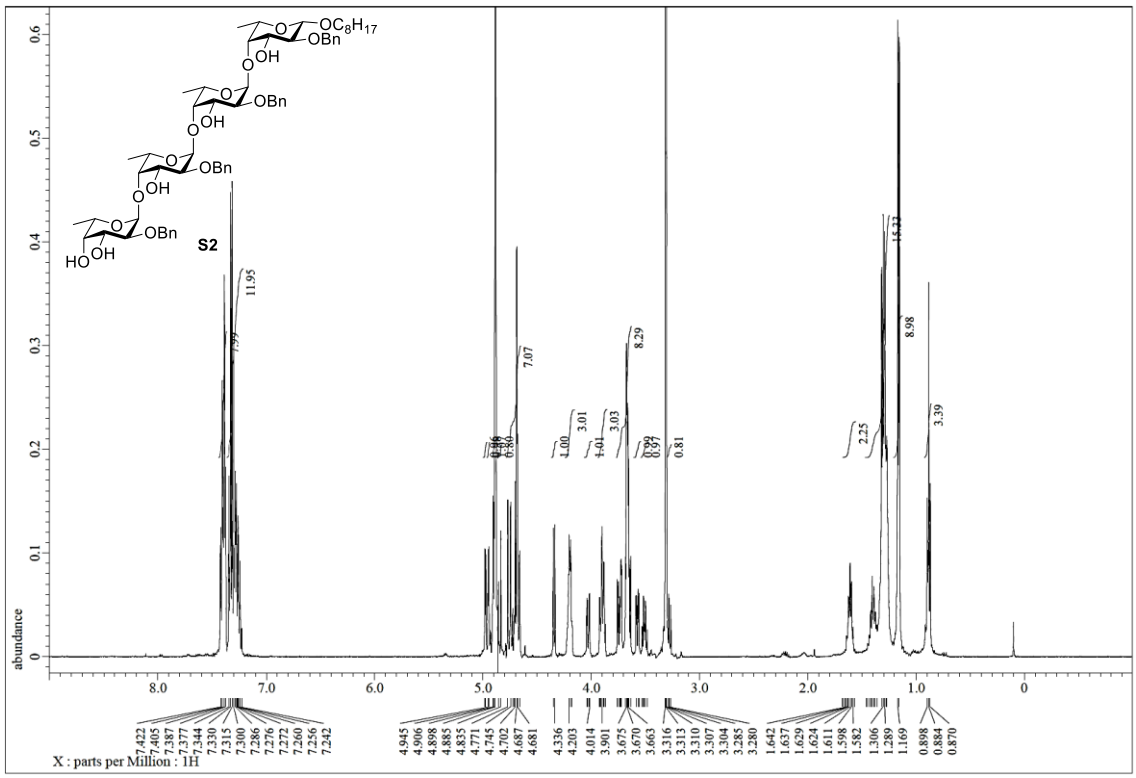


Fig. S15 ^{1}H-NMR spectrum of **S2**

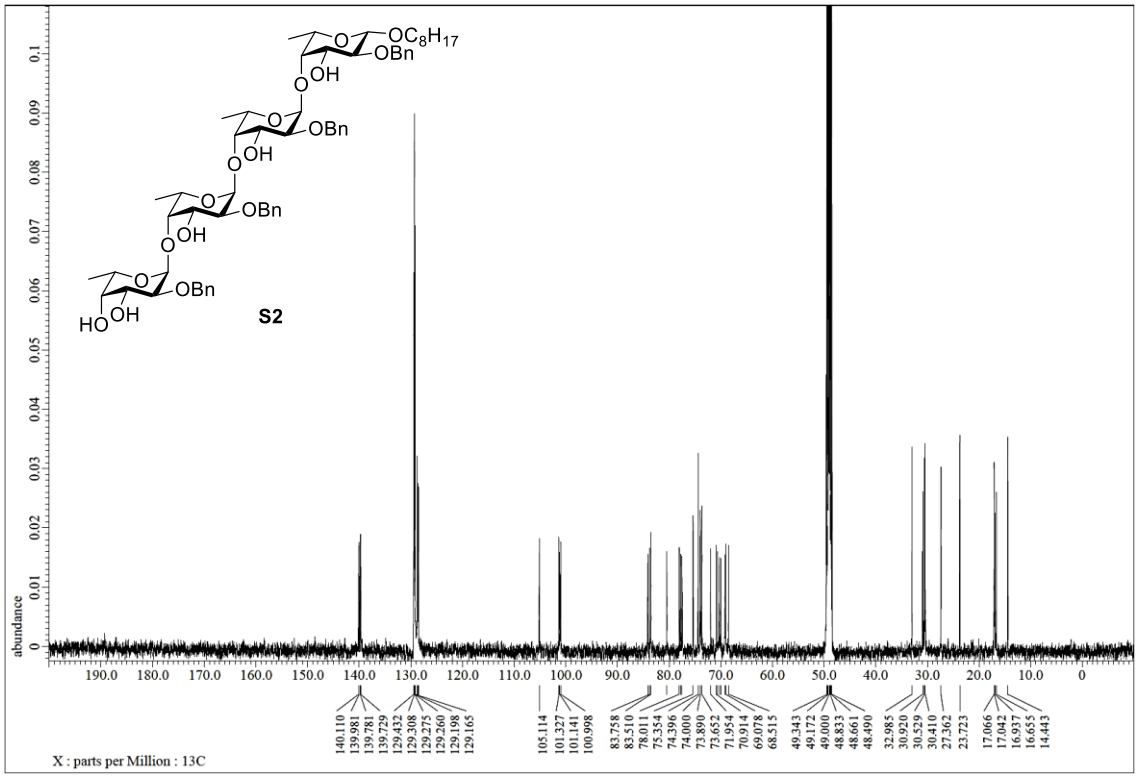


Fig. S16 ^{13}C-NMR spectrum of **S2**

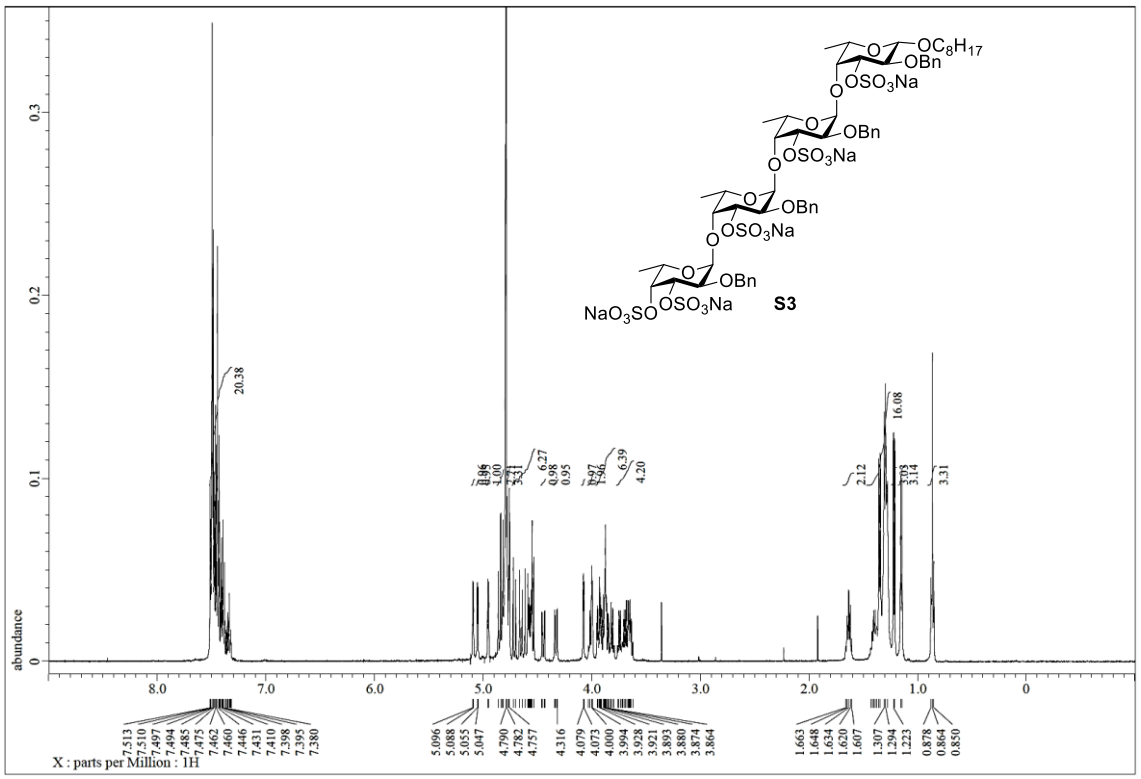


Fig. S17 ^{1}H-NMR spectrum of **S3**

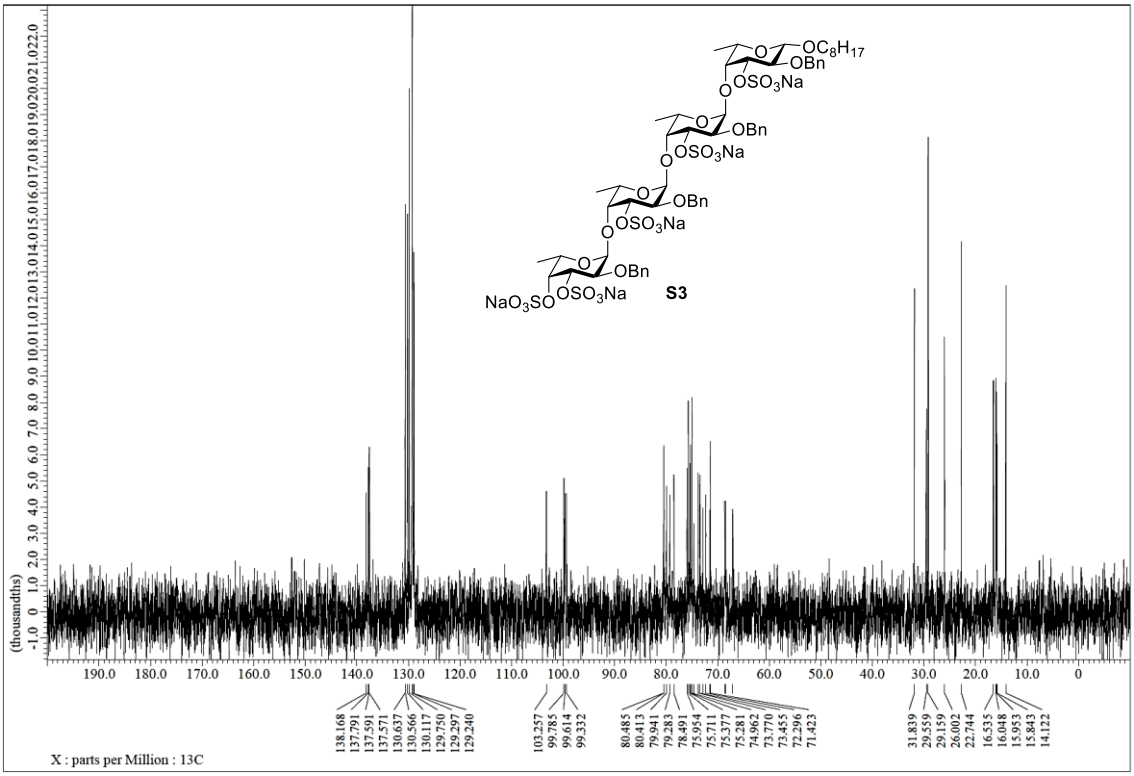


Fig. S18 ^{13}C-NMR spectrum of **S3**

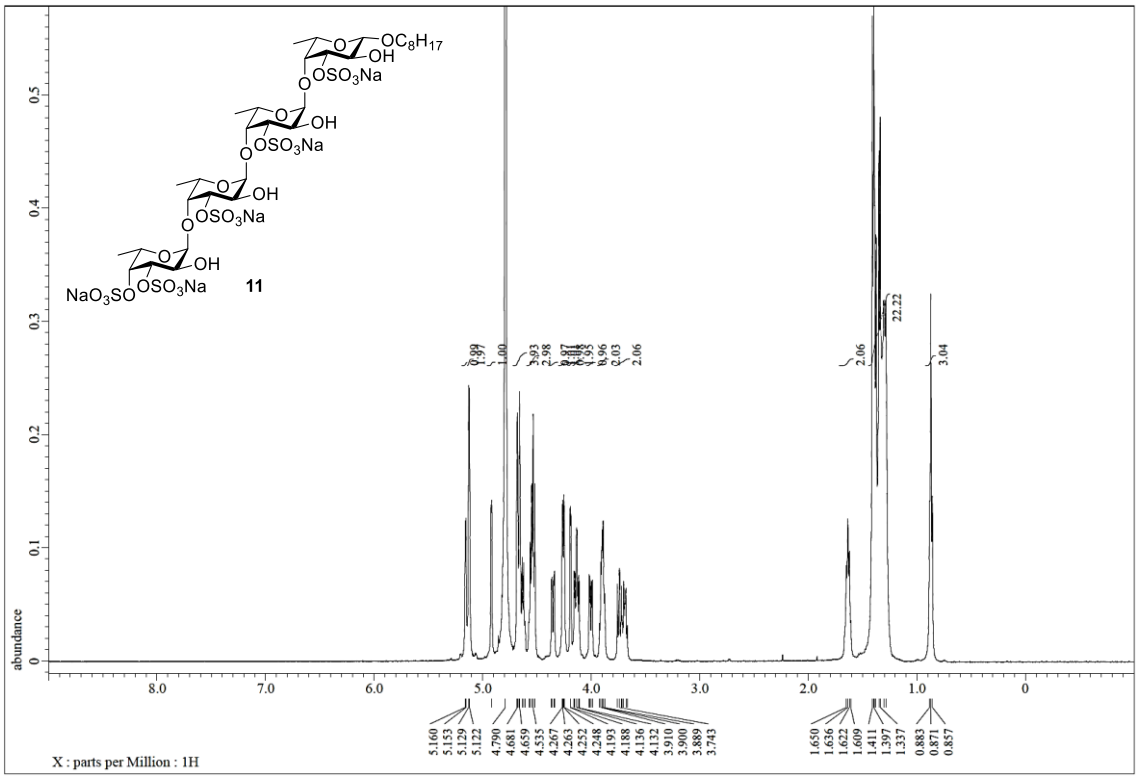


Fig. S19 ^{1}H-NMR spectrum of **11**

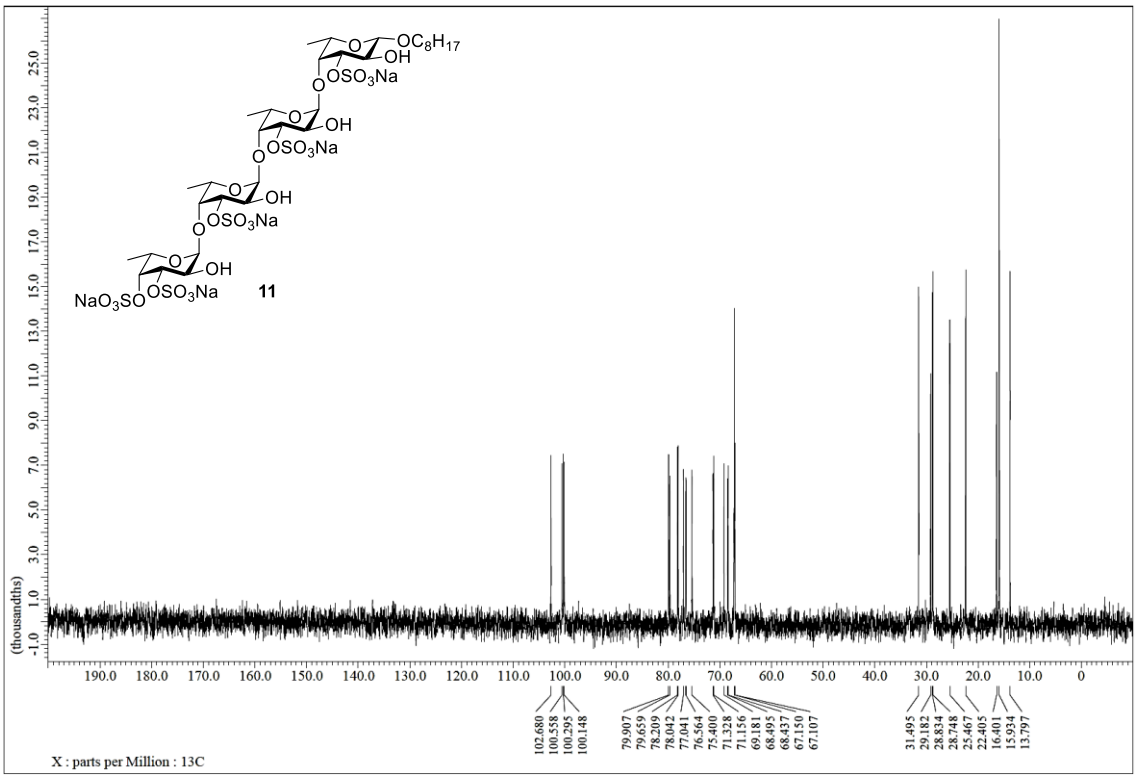


Fig. S20 ^{13}C-NMR spectrum of **11**

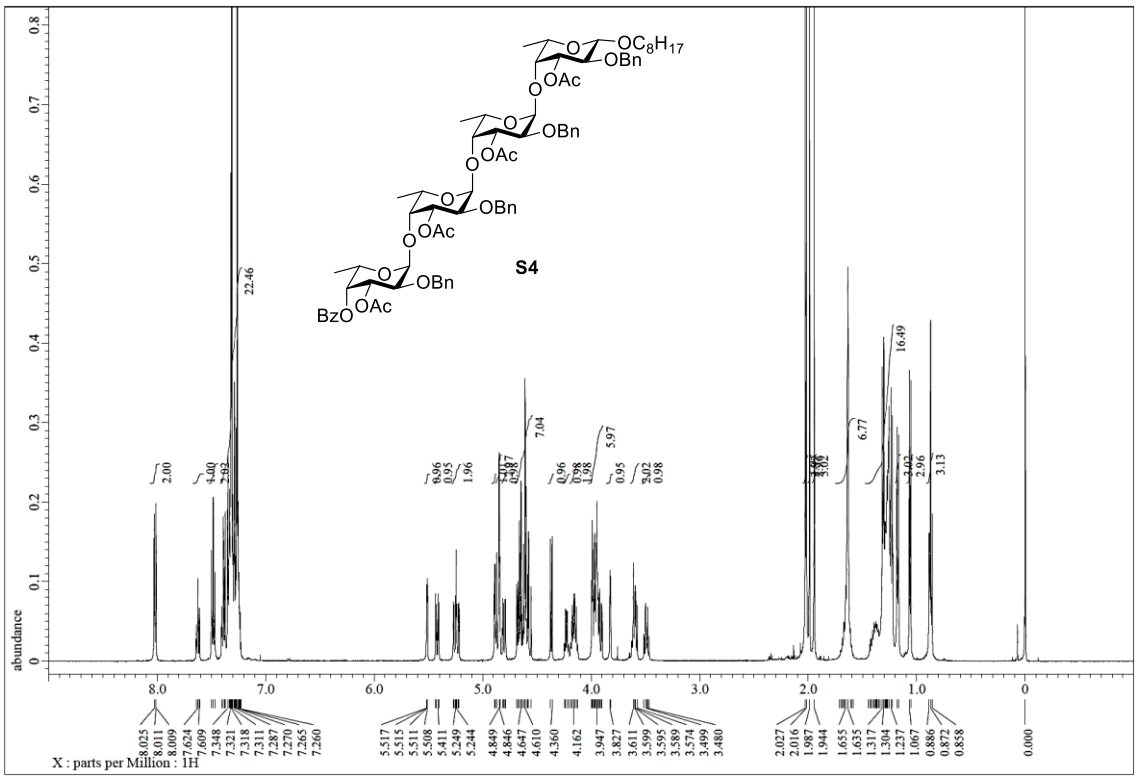


Fig. S21 ^{1}H-NMR spectrum of **S4**

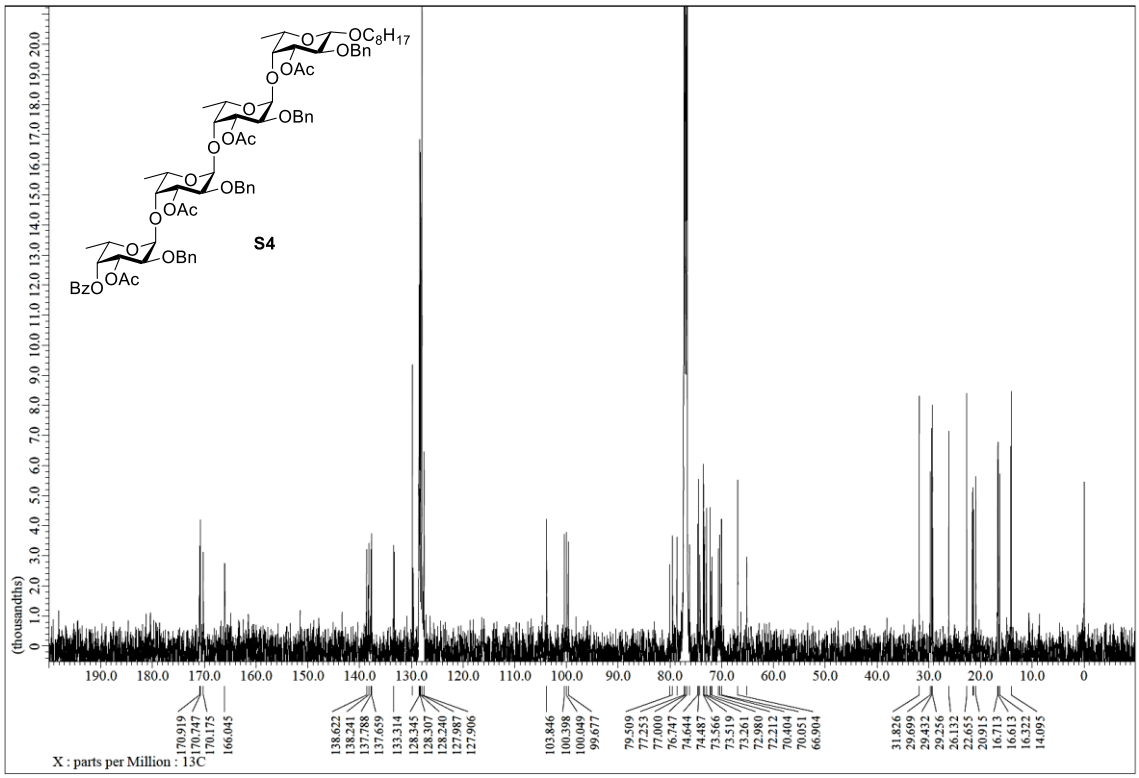


Fig. S22 ^{13}C-NMR spectrum of **S4**

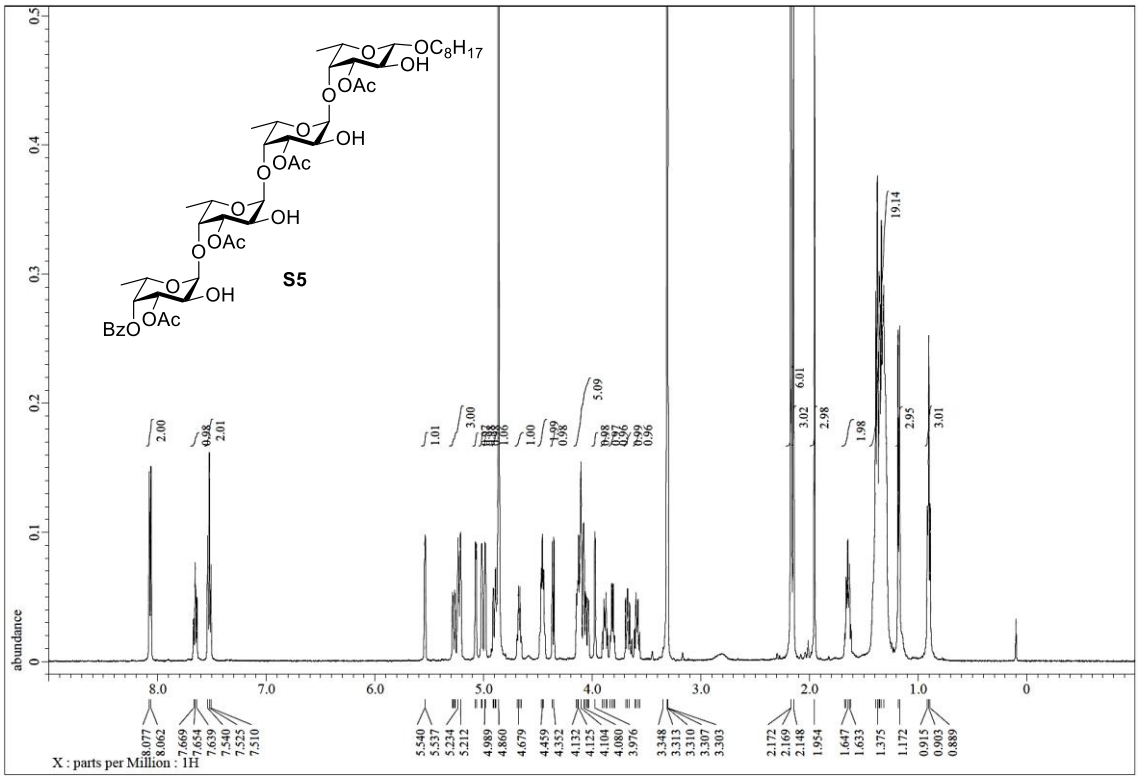


Fig. S23 ^{1}H-NMR spectrum of **S5**

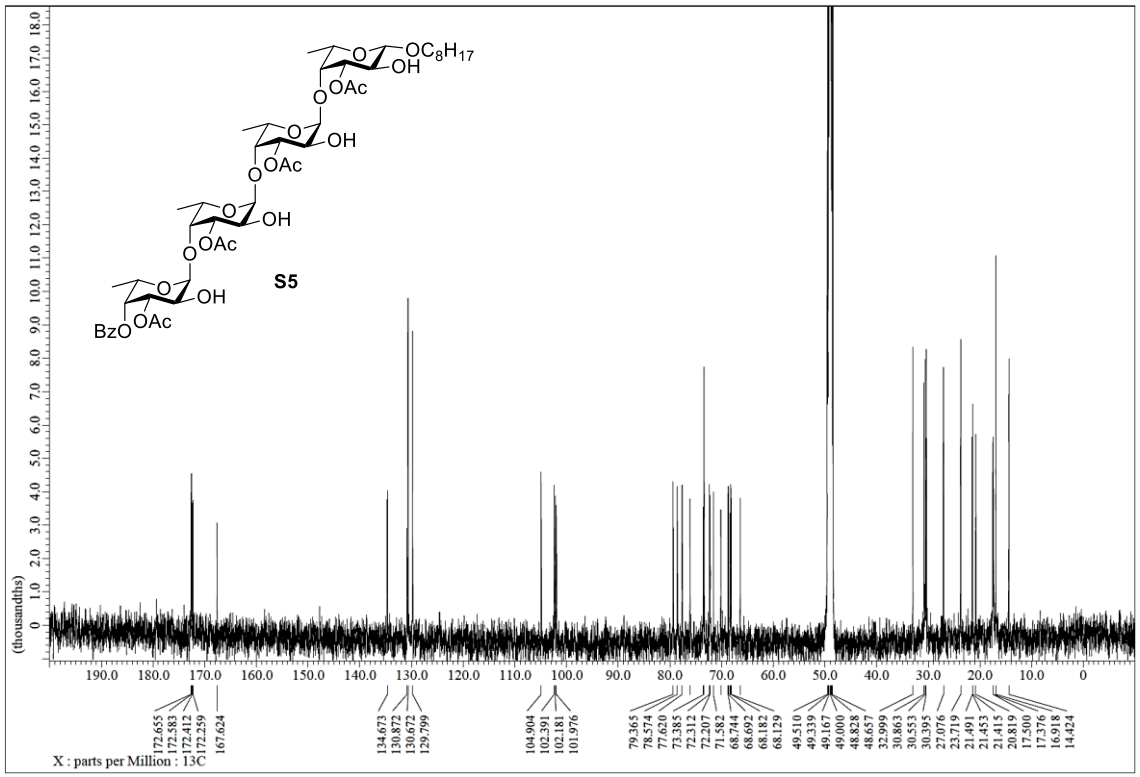


Fig. S24 ^{13}C-NMR spectrum of **S5**

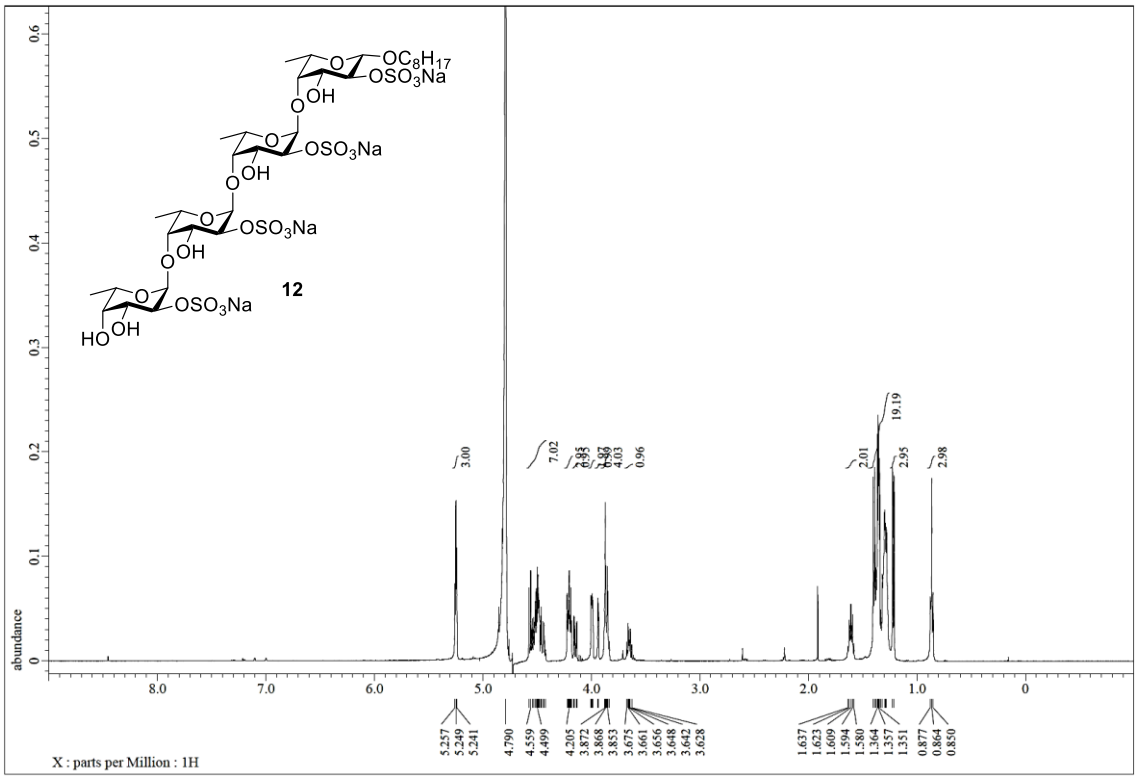


Fig. S25 ^{1}H-NMR spectrum of **12**

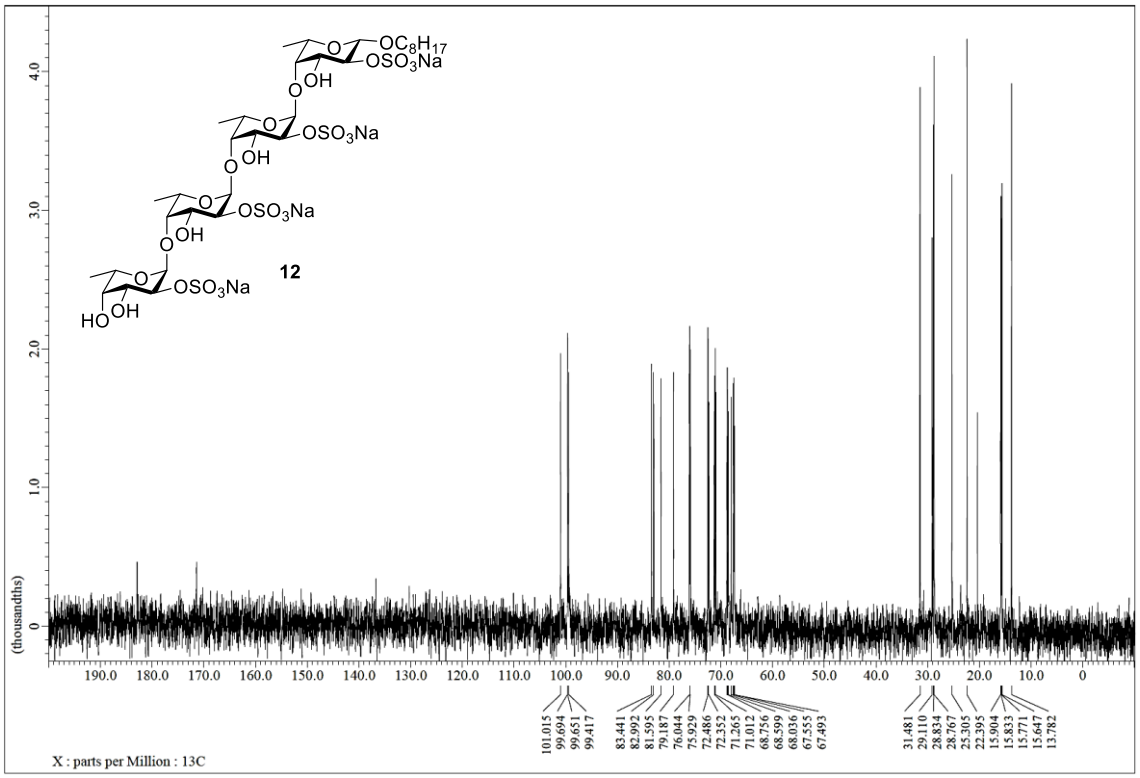


Fig. S26 ^{13}C-NMR spectrum of **12**

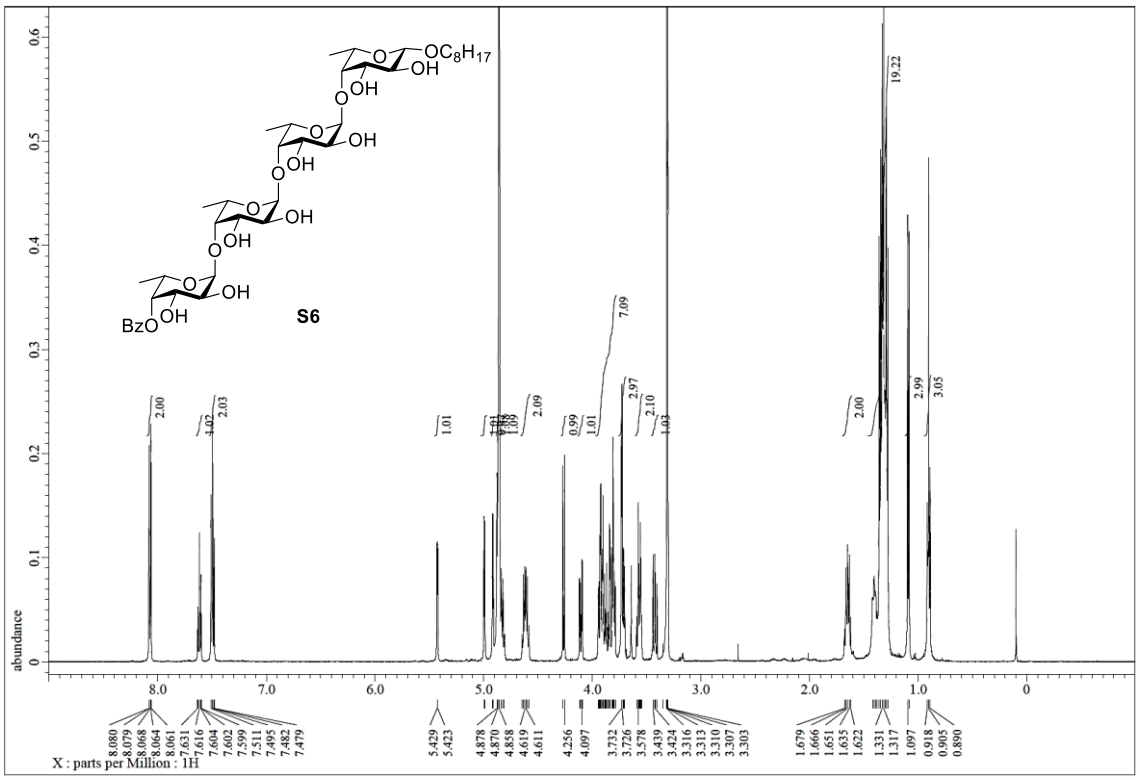


Fig. S27 ^{1}H-NMR spectrum of **S6**

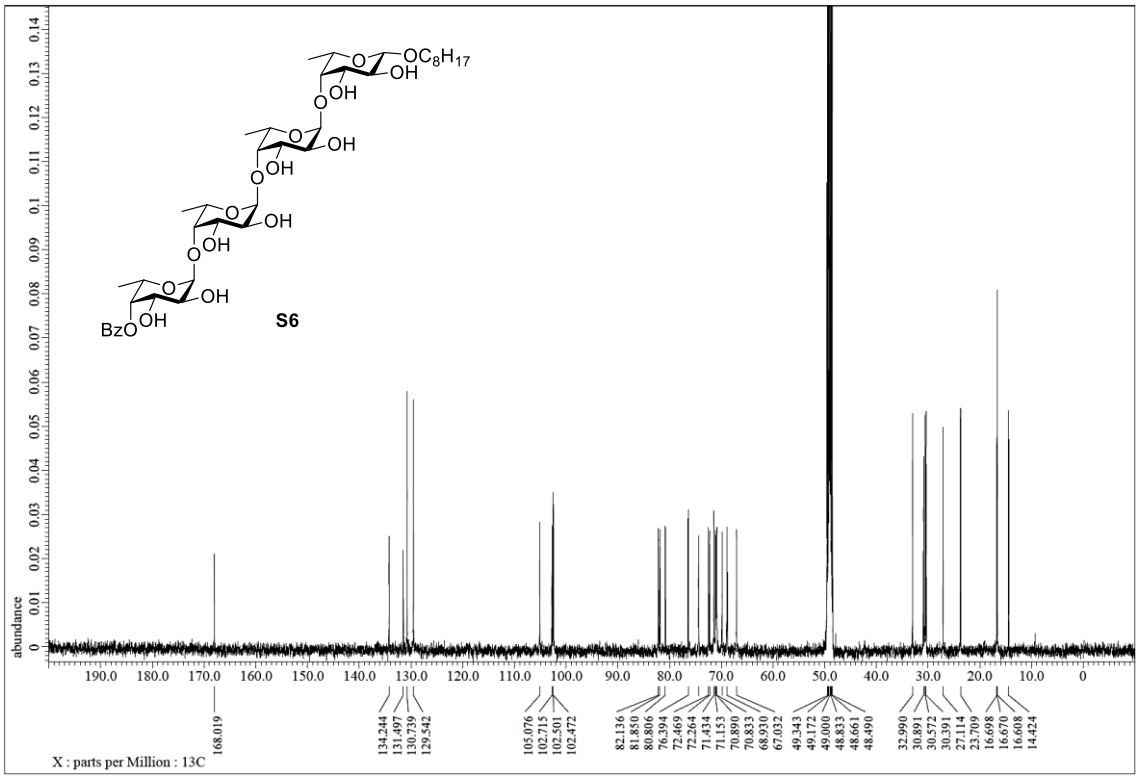


Fig. S28 ^{13}C-NMR spectrum of **S6**

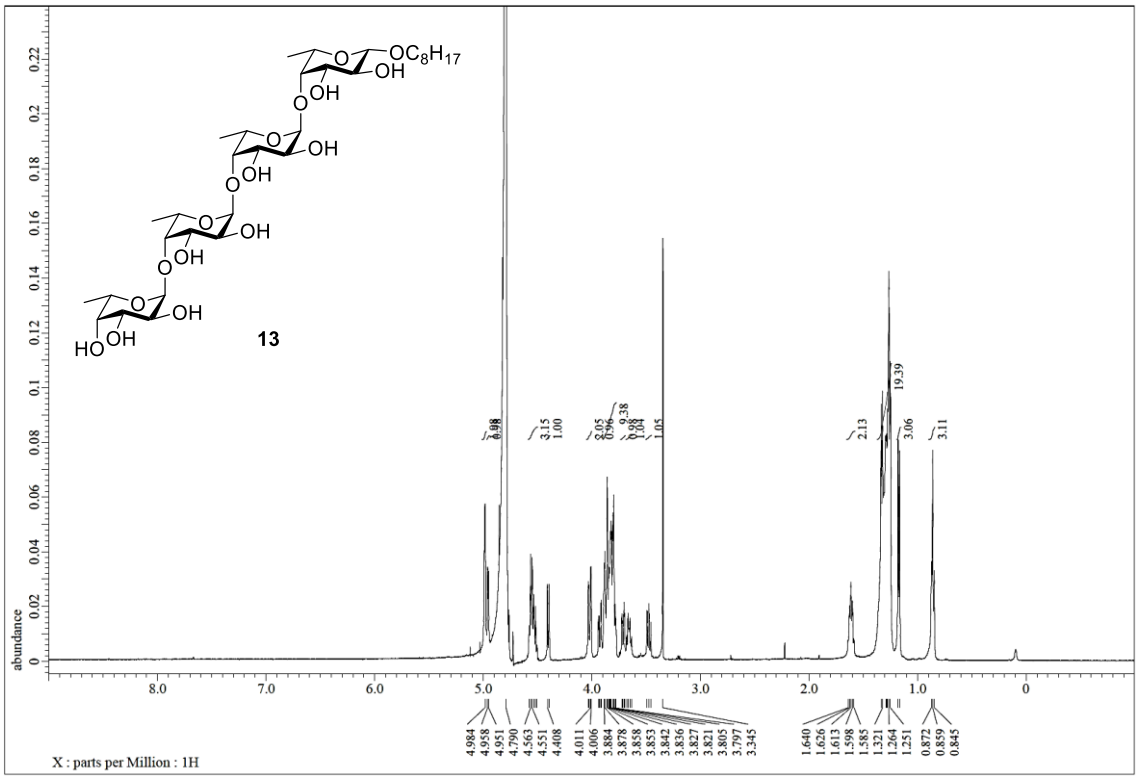


Fig. S29 ^{1}H-NMR spectrum of **13**

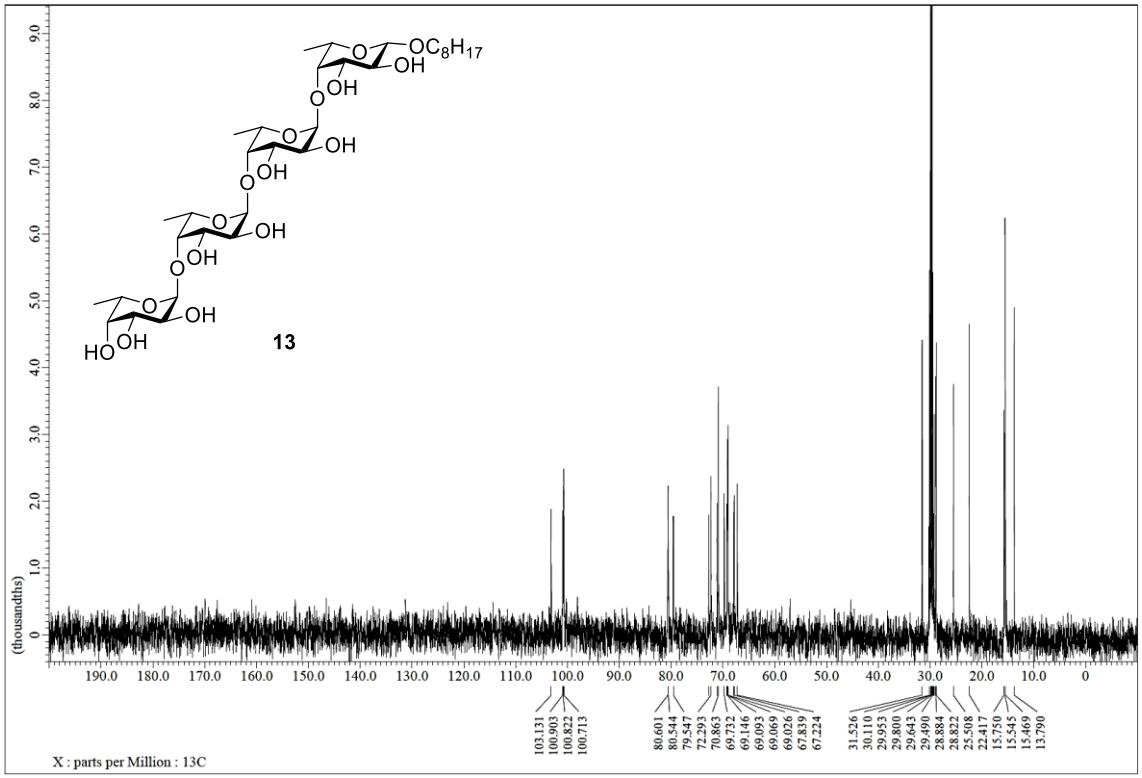


Fig. S30 ^{13}C-NMR spectrum of **13**

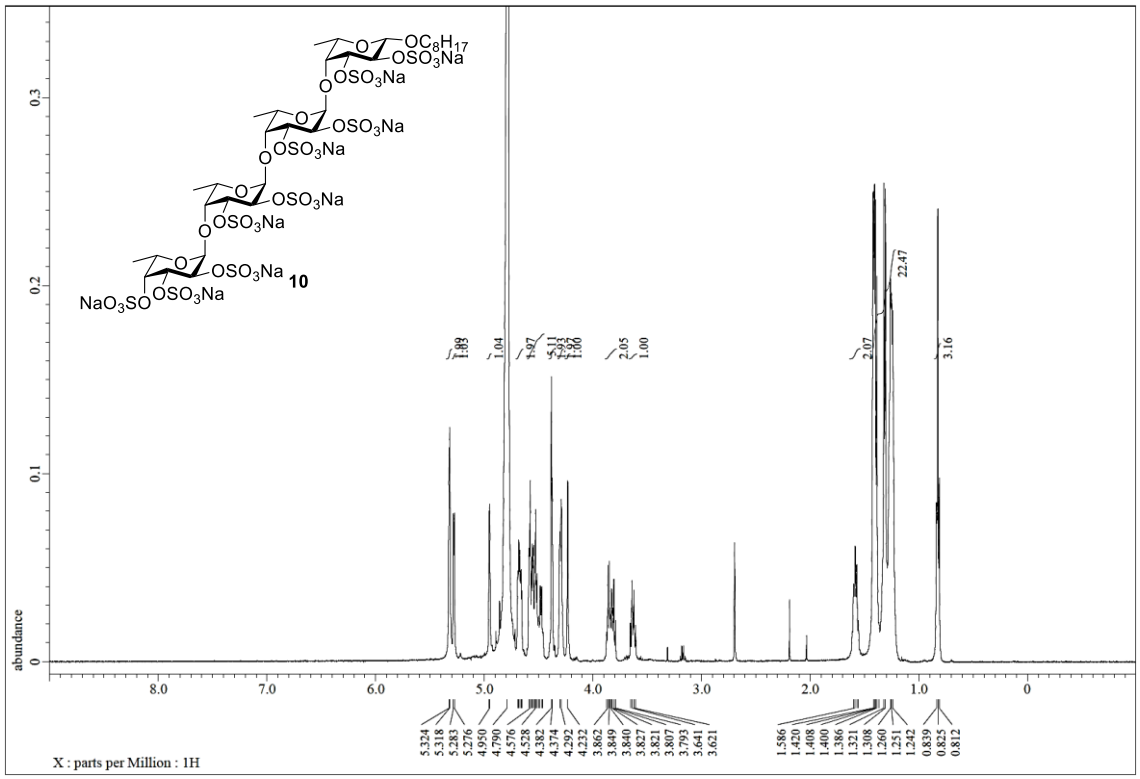


Fig. S31 ^{1}H-NMR spectrum of **10**

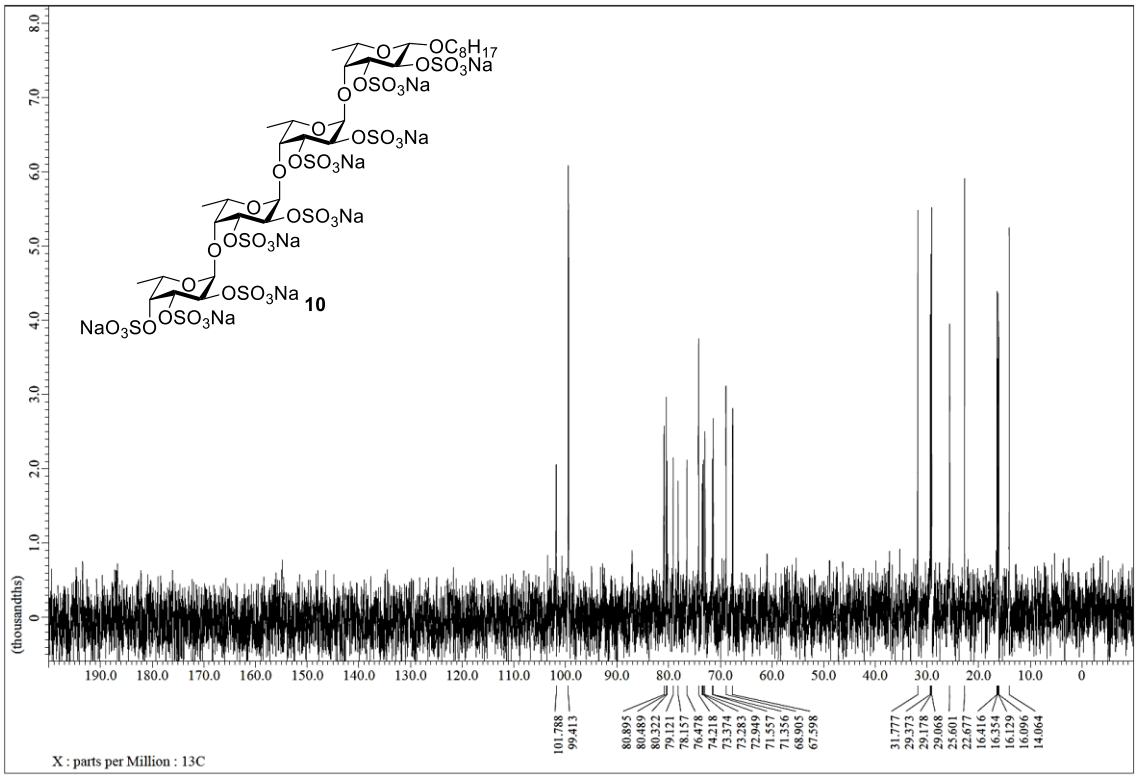


Fig. S32 ^{13}C-NMR spectrum of **10**