Supporting information

HPLC-assisted automated oligosaccharide synthesis

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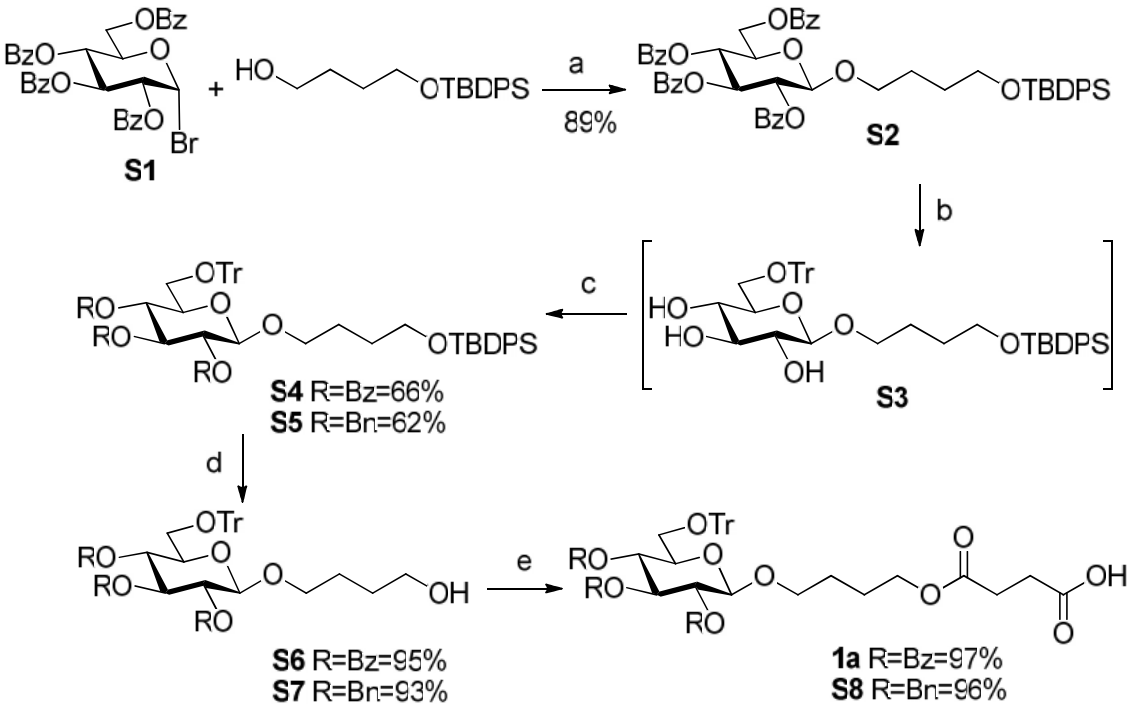
Contents

General remarks

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at < 40 ^{o}C. CH_2Cl_2 and $ClCH_2CH_2Cl$ were distilled from CaH_2 directly prior to application. Anhydrous DMF (EM Science) was used as is. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine and acetonitrile were dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. DOWEX MONOSPHERE 650C (H) was washed three times with MeOH and stored under MeOH. Optical rotations were measured at 'JASCO P-1020' polarimeter. ^{1}H-NMR spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C-NMR spectra were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) unless otherwise noted. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer. VARIAN 9012 Solvent Delivery System and VARIAN 9050 Variable Wavelength UV-Vis Detector were used to build the automated synthesizer set-up.

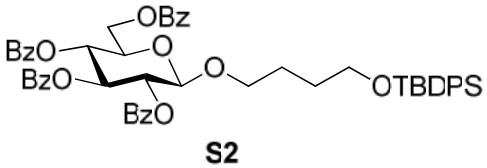
Synthesis of glycosyl acceptors

Scheme 1S. Synthesis of resin bound glycosyl acceptors 1a and S8.



Conditions: a) $Hg(CN)_2$, $HgBr_2$, $ClCH_2CH_2Cl$, 4 Å MS, rt, 36 h; b) i. $NaOCH_3/CH_3OH$, rt, 5 h; ii. TrCl, Py, DMAP, 80 ^oC, 6 h; c). BzCl, Py, rt, 10 h or BnBr, NaH (60%), DMF, 0 ^oC, 5 h; d) TBAF/THF, THF, rt, 3 h; e) Succinic anhydride, Py, DMAP, 65 ^oC, 18 h.

4-tert-Butyldiphenylsilyloxybut-1-yl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside (S2).

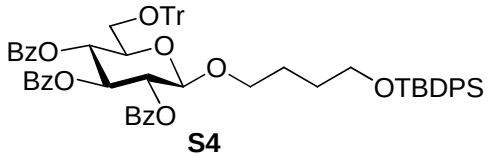


S2

To a suspension of 4-tert-butyldiphenylsilyloxybutan-1-ol (0.995 g, 3.03 mmol.), $Hg(CN)_2$ (0.766 g, 3.03 mmol), $HgBr_2$ (0.546 g, 1.5 mmol) and molecular sieves (4 Å, 2 g) in $ClCH_2CH_2Cl$ (20 mL), a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**S1**)[1] (2.0 g, 3.03 mmol.) in $ClCH_2CH_2Cl$ (20 mL) was added with stirring at room temperature under argon atmosphere. The mixture was stirred for 36 h, filtered through celite, diluted with CH_2Cl_2 and the organic layer was washed with aq. $Na_2S_2O_3$ (10%, 2 x 50 mL), aq. $NaHCO_3$ (5%, 50 mL) water (50 mL) and brine (30 mL). The organic phase was separated, dried over $MgSO_4$, and

concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound (2.44 g, 89%) as a white foam. Analytical data for **S2**: R_f = 0.50 (ethyl acetate - hexane, 1/3, v/v); $[\alpha]_D^{23}$ +9.9° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 0.99 (s, 9H, t-Butyl), 1.46-1.49 (m, 2H, -CH_2-), 1.61-1.65 (m, 2H, -CH_2-), 3.49 (t, 2H, *J* = 6.2 Hz, -CH_2-OSi), 3.53 (dt, 1H, , *J* = 9.7, 6.2 Hz, O-CH_2-), 3.92 (dt, 1H, *J* = 9.7, 6.2 Hz, O-CH_2-), 4.09-4.15 (m, 1H, H-5), 4.50 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6), 4.62 (dd, 1H, *J* = 12.0, 3.5 Hz, H-6'), 4.78 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 5.51 (dd, 1H, $J_{2,3}$ = 9.5, 7.5 Hz, H-2), 5.67 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 5.89 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 7.25-8.02 ppm (m, 30H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 19.2, 25.8, 26.8, 28.6, 63.2, 69.8, 69.9, 71.9, 72.1, 72.9, 101.3, 127.6, 128.3 (x 3), 128.4, 128.8, 129.3, 129.5, 129.6, 129.7 (x 3), 129.8, 133.1 (x 2), 133.2, 133.4, 133.9, 135.5, 165.1, 165.2, 165.8, 166 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{54}O_{11}Si$ Na 929.3333, found 929.3351.

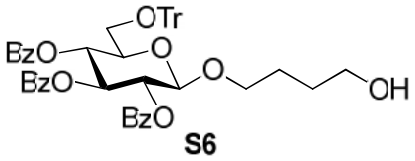
4-tert-Butyldiphenylsilyloxybut-1-yl 2,3,4-Tri-*O*-benzoyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (S4).



S4

To a stirred solution of compound **S2** (2.10 g, 0.035 mmol) in MeOH (15 mL) was added NaOMe until pH ~9. The reaction mixture was stirred under argon for 5 h at room temperature. After that, the reaction was neutralized with Dowex, filtered and the filtrate was concentrated *in vacuo*. To a solution of the dried crude product **S3** obtained above, in pyridine (15 mL) were added trityl chloride (1.94 g, 6.95 mmol) and DMAP (0.057 g, 0.463 mmol) and stirred at 80 °C for 6 h under argon atmosphere. After TLC analysis, the reaction mixture was cooled to 0 °C, benzoyl chloride (0.97 mL, 8.33 mmol) was added and continued stirring for additional 10 h. After TLC analysis, it was diluted with CH_2Cl_2 (50 mL), washed with water (3 x 50 mL) and brine (30 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound (1.596 g, 66%, 3 steps) as a white foam. Analytical data for **S4**: R_f = 0.50 (ethyl acetate - hexane, 1/4, v/v); $[\alpha]_D^{27}$ -1.3° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.00 (s, 9H,-C(CH_3)$_3$), 1.49-1.59 (m, 2H, -CH_2-), 1.66-1.76 (m, 2H, -CH_2-), 3.25 (dd, 1H, *J* = 8.1, 2.0 Hz, H-6), 3.34 (dd, 1H, *J* = 10.6, 1.9 Hz, H-6'), 3.54 (t, 2H, *J* = 6.2 Hz, -CH_2-O-Si), 3.61 (dt, 1H, *J* = 9.7, 6.3 Hz, O-CH_2-), 3.80-3.85 (m, 1H, H-5), 3.99 (dt, 1H, *J* = 9.7, 6.0 Hz, O-CH_2-), 4.75 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.53 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.62 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 5.78 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 7.07-7.95 ppm (m, 40H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 19.2, 25.9, 26.8, 28.8, 62.5, 63.3, 69.5, 69.6, 72.0, 73.3, 73.8, 86.6, 101.2, 126.9, 127.6, 127.7, 128.2, 128.3, 128.6, 128.9, 129.1, 129.4, 129.5, 129.6, 129.7, 129.8, 133.0 (x2), 133.1, 133.9, 135.5, 143.6, 164.8, 165.1, 165.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{66}H_{64}O_{10}Si$ Na 1067.4166, found 1067.4192.

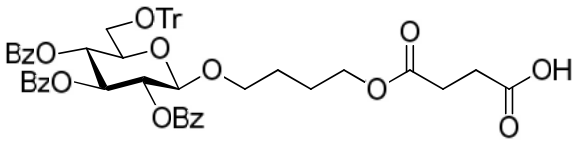
4-Hydroxybut-1-yl 2,3,4-Tri-*O*-benzoyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (S6).



S6

To a stirred solution of compound **S4** (0.806 g, 0.772 mmol) in THF (5 mL) was added TBAF (0.44 mL, 1.544 mmol). The reaction mixture was stirred under argon for 3 h at room temperature. Upon completion, the reaction was diluted with CH_2Cl_2 (30 mL), washed with water (30 mL) and brine (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (0.591 g, 95%) as a white foam. Analytical data for **S6**: R_f = 0.50 (ethyl acetate - toluene, 1/3, v/v); $[\alpha]_D^{23}$ -0.9° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.48 (t, 1H, *J* = 5.2 Hz, -O*H*), 1.52-1.59 (m, 2H, -CH_2-), 1.63-1.76 (m, 2H, -CH_2-), 3.27 (dd, 1H, *J* = 10.5, 4.9 Hz, H-6), 3.35 (dd, 1H, *J* = 10.5, 2.3 Hz, H-6'), 3.55 (dt, 2H, *J* = 6.0, 1.9 Hz, -CH_2-O), 3.66 (dt, 1H, *J* = 9.7, 5.9 Hz, O-CH_2-), 3.83-3.89 (m, 1H, H-5), 4.05 (dt, 1H, *J* = 9.7, 5.9 Hz, O-CH_2-), 4.80 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.55 (dd, 1H, $J_{2,3}$ = 7.9 Hz, H-2), 5.64 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 5.80 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 7.08-7.98 ppm (m, 30H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 25.8, 29.4, 62.2, 62.4, 69.5, 69.7, 72.2, 73.2, 73.9, 86.6, 101.2, 126.9, 127.7, 128.2 (x2), 128.4, 128.6, 128.9, 129.1, 129.4, 129.6, 129.7, 133.0, 133.1, 133.2, 143.6, 164.8, 165.2, 165.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{50}H_{46}O_{10}$ Na 829.2989, found 829.2987.

4-Succinoyloxybut-1-yl 2,3,4-Tri-*O*-benzoyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (1a).

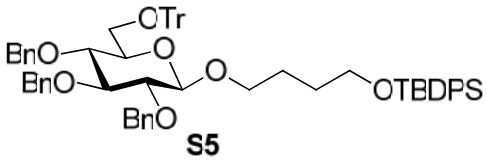


1a

To a solution of compound **S6** (1.280 g, 1.588 mmol) in pyridine (5 mL), were added succinic anhydride (0.191 g, 1.90 mmol) and DMAP (0.039 g, 0.317 mmol) and stirred at 65 °C for 16 h under argon atmosphere. After TLC analysis, the solvents were removed *in vacuo* and the residue was co-evaporated with toluene (3 x 10 mL). The resulting residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (1.40 g, 97%) as a white foam. Analytical data for **1a**: R_f = 0.50 (ethyl acetate - toluene, 1/1, v/v); $[\alpha]_D^{23}$ -3.0° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.60-1.75 (m, 4H, 2x -CH_2-), 2.51-2.58 (m, 2H, -CH_2-CO), 2.59-2.65 (m, 2H, -CH_2-CO), 3.26 (dd, 1H, *J* = 10.6, 4.9 Hz, H-6), 3.35 (dd, 1H, *J* = 10.6, 2.5 Hz, H-6'), 3.65 (dt, 1H, *J* = 9.6, 5.9 Hz, O-CH_2-), 3.84-3.90 (m, 1H, H-5), 3.96-4.06 (m, 3H, -CH_2-O-CO, O-CH_2-), 4.82 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.54 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 5.64 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 5.83 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 7.08-7.97 ppm (m, 30H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 25.1, 25.9, 28.8 (x2), 62.4, 64.3, 68.9, 69.4, 72.1, 73.3, 73.9, 86.6, 100.9, 125.3, 126.9, 127.7, 128.2 (x3), 128.3,

128.6, 128.8, 129.0, 129.1, 129.3, 129.6, 129.8 (x2), 133.0, 133.2 (x2), 143.6, 164.8, 165.2, 166.0, 172.1, 177.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{50}O_{13}$ Na 929.3149, found 929.3132.

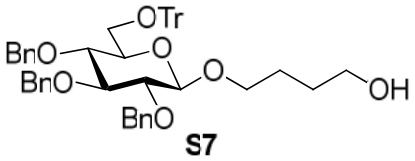
4-tert-Butyldiphenylsilyloxybut-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (S5).



S5

To a stirred solution of compound **S3** (0.320 g, 0.437 mmol) in DMF (3 mL), NaH (60 % dispersion in oil, 0.105 g, 2.62 mmol) was added portion wise at 0 ^oC under argon atmosphere. After 30 min, BnBr (0.19 mL, 1.57 mmol) was added and stirred for additional 5 h at room temperature. The reaction mixture was neutralized with AcOH/MeOH (1:10, v/v), diluted with CH_2Cl_2 (50 mL), washed with water (2 x 50 mL) and brine (30 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound (0.271 g, 62%, 2 steps) as a pale yellow foam. Analytical data for **S5**: R_f = 0.50 (ethyl acetate - hexane, 1/9, v/v); $[\alpha]_D^{23}$ +3.9° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.05 (s, 9H, t-butyl), 1.72-1.79 (m, 2H, -CH_2-), 1.82-1.89 (m, 2H, -CH_2-), 3.24 (dd, 1H, J = 9.8, 3.3 Hz, H-6), 3.36-3.41 (m, 1H, H-5), 3.51-3.65 (m, 4H, H-2, 3, 6', O-CH_2-), 3.73 (t, 2H, J = 6.0 Hz, -CH_2-OSi), 3.82 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 4.04-4.11 (m, 1H, O-CH_2-), 4.43 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 4.54 (dd, 2H, J^2 = 10.2 Hz, CH_2Ph), 4.84 (dd, 2H, J^2 = 10.8 Hz, CH_2Ph), 4.87 (dd, 2H, J^2 = 8.9 Hz, CH_2Ph), 6.86-7.72 ppm (m, 40H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 19.2, 26.5, 26.8, 29.5, 62.4, 63.6, 69.6, 74.5, 74.9, 75.0, 77.9, 82.5, 84.7, 86.3, 103.6, 126.9, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2 (x2), 128.3, 128.4, 128.8, 129.5, 129.6, 133.9, 134.8, 135.5, 137.8, 138.6, 143.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{66}H_{70}O_7Si$ Na 1025.4789, found 1025.4813.

4-Hydroxybut-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (S7).

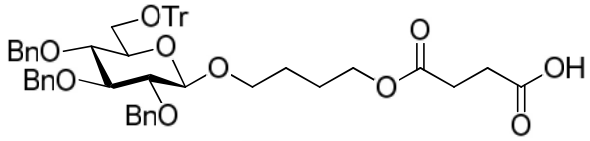


S7

The title compound was obtained from **S5** as a white foam in 93%, as described in the synthesis of compound **S6**. Analytical data for **S7**: R_f = 0.50 (ethyl acetate - hexane, 1/3, v/v); $[\alpha]_D^{23}$ +5.7° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.53 (br s, 1H, -OH), 1.69-1.88 (m, 4H, 2x -CH_2-), 3.24 (dd, 1H, J = 10.0, 4.0 Hz, H-6), 3.39-3.43 (m, 1H, H-5), 3.51-3.61 (m, 3H, H-2, 3, 6'), 3.63-3.65 (m, 1H, O-CH_2-), 3.69 (t, 2H, J = 6.3 Hz, -CH_2-OH), 3.80 (dd, 1H, $J_{4,5}$ = 9.0 Hz,

H-4), 4.09 (dt, 1H, *J* = 6.0, 3.8 Hz, O-CH_2-), 4.45 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.52 (dd, 2H, J^2 = 10.3 Hz, CH_2Ph), 4.85 (dd, 2H, J^2 = 10.8 Hz, CH_2Ph), 4.88 (dd, 2H, J^2 = 10.5 Hz, CH_2Ph), 6.86-7.52 ppm (m, 30H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 26.3, 29.7, 62.4, 62.6, 69.5, 74.6, 74.9, 75.0, 75.9, 77.9, 82.5, 84.7, 86.3, 103.5, 126.9, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 128.8, 137.8, 138.5, 138.6, 143.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{50}H_{52}O_7$ Na 787.3611, found 787.3591.

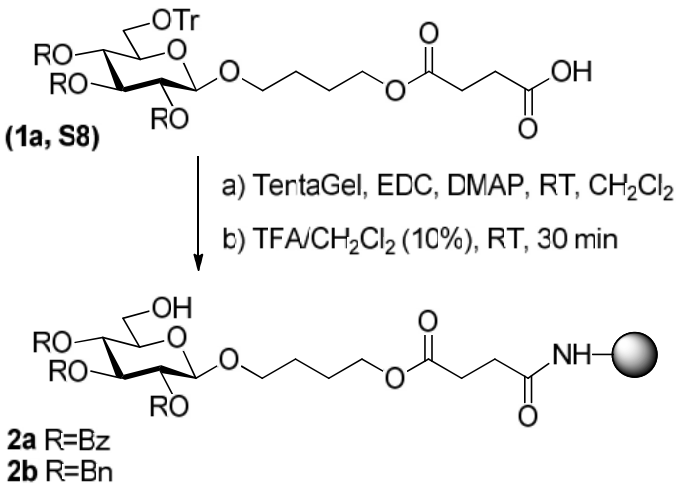
4-Succinoyloxybut-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (S8).



S8

The title compound was obtained from **S7** as a white foam in 96%, as described in the synthesis of compound **1a**. Analytical data for **S8**: R_f = 0.50 (ethyl acetate - toluene, 1/1, v/v); $[\alpha]_D^{23}$ +2.5° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.72 (br s, 4H, 2x -CH_2-), 2.48-2.56 (m, 4H, 2x -CH_2-CO), 3.16 (dd, 1H, *J* = 10.0, 3.9 Hz, H-6), 3.30-3.38 (m, 1H, H-5), 3.44-3.59 (m, 4H, H-2, 3, 6', O-CH_2-), 3.73 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 3.99 (dt, 1H, *J* = 9.8, 6.0 Hz, O-CH_2-), 4.08 (t, 2H, *J* = 5.7 Hz, -CH_2-O-CO), 4.38 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.45 (dd, 2H, J^2 = 10.3 Hz, CH_2Ph), 4.76 (dd, 2H, J^2 = 10.8 Hz, CH_2Ph), 4.80 (dd, 2H, J^2 = 11.0 Hz, CH_2Ph), 6.78-7.44 ppm (m, 30H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 25.6, 26.3, 28.7, 28.8, 62.4, 64.5, 68.9, 74.6, 74.9, 75.0, 75.9, 77.9, 82.5, 84.7, 86.3, 103.4, 126.9, 127.7, 127.8, 127.9, 128.0 (x2), 128.1 (x2), 128.3, 128.4, 128.8, 137.8, 138.4, 138.5, 172.1, 177.0 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{56}O_{10}$ Na 887.3771, found 887.3790.

Synthesis of resin bound acceptors 2a and 2b.

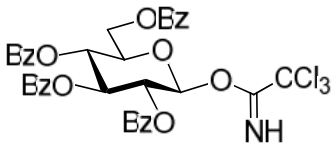


*General procedure for conventional synthesis of glycosyl acceptors **2a** and **2b**.* To a solution of carboxylic acid derived sugar (3.0 mmol), EDC hydrochloride (3.0 mmol), and DMAP (1 mmol) in CH_2Cl_2 (5 mL) was added TentaGel® MB-NH_2 resin (1% cross-linked polystyrene, 1.0 mmol) under argon and agitated for 48 h at room temperature. After Kaiser analysis, the resin was filtered, washed with CH_2Cl_2 (3 x 20 mL) and acetone (2 x 20 mL). A solution of TFA/CH_2Cl_2 (5 mL, 20%) was added drop wise into a flask containing sugar loaded resin in wet CH_2Cl_2 (5 mL) at room temperature and agitated for 30 min. Then it was neutralized with Et_3N, filtered, washed with CH_2Cl_2 (3 x 20 mL), dried to afford glycosyl acceptor conjugated resin **2a** (0.29 mmol/g) and **2b** (0.22 mmol/g).

*General procedure for HPLC-mediated synthesis of glycosyl acceptors **2a** and **2b**.* CH_2Cl_2 (Pump A, flow rate: 2 mL/min) was purged through the column containing swelled TentaGel® MB-NH_2 resin (1% cross-linked polystyrene, 180-200 mg, 0.40 mmol/g theoretical loading capacity) for 2 min. After that, a solution (Pump B, flow rate: 1 mL/min) containing carboxylic acid derived sugar (3 equiv), EDC hydrochloride (3 equiv), and DMAP (1 equiv) in CH_2Cl_2 (3 mL) under argon was circulated (pumped) through the column for 8 h. After that, CH_2Cl_2 (Pump A, flow rate: 2 mL/min) was purged through the column for 10 min. Then, a solution (Pump C, flow rate: 1 mL/min) containing TFA/CH_2Cl_2/ H_2O (3 mL, 1/9/0.1, v/v/v) was circulated through the column containing resin loaded with a tritylated sugar for 30 min. After that, CH_2Cl_2 (Pump A, flow rate: 2 mL/min) was purged through the column for 10 min, afforded required glycosyl acceptor conjugated resin **2a** and **2b**.

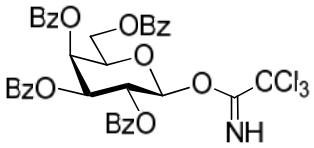
Synthesis of glycosyl donors 3a-3f.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (3a).



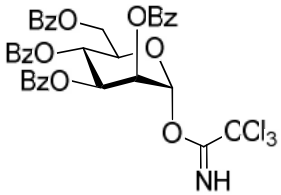
Analytical data for the title compound was essentially the same as previously described.[2-4]

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate (3b).



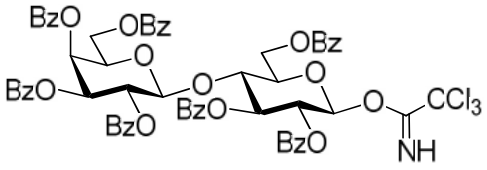
Analytical data for the title compound was essentially the same as previously described.[5]

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (3c).



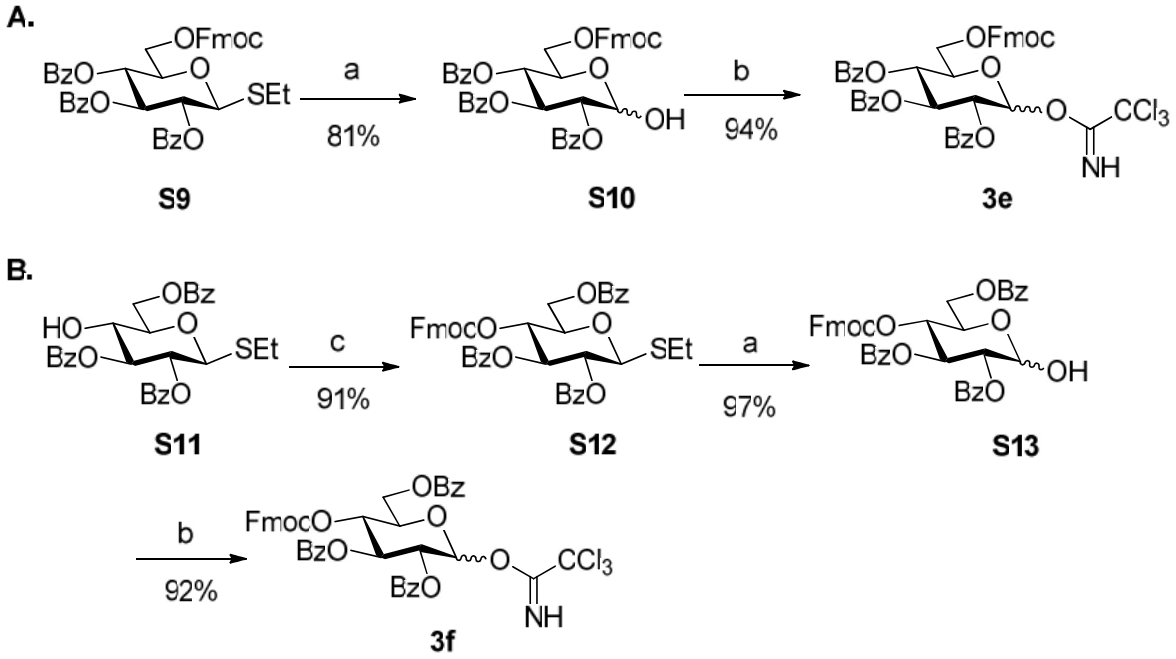
Analytical data for the title compound was essentially the same as previously described.[6]

***O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (3d).**



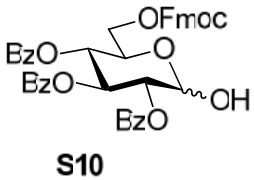
Analytical data for the title compound was essentially the same as previously described.[7]

Scheme 2S. Synthesis of Fmoc protected glycosyl donors 3e and 3f.



Conditions: a) NBS (3 equiv), acetone/H_2O (9/1, v/v), rt, 30 min., b) CCl_3CN (20 equiv.), NaH (60%, 0.1 equiv.), CH_2Cl_2, rt, 10 min., c) FmocCl, DMAP, Py, rt, 3 h

2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranose (S10).

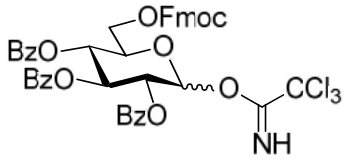


S10

To a stirred solution of ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-glucopyranoside[8] (**S9**, 1.350 g, 1.78 mmol) in acetone-water (9/1, v/v, 10 mL), N-bromosuccinimide (0.950 g 5.34 mmol) was added and stirred for 30 min at room temperature. Upon completion, the reaction was diluted with CH_2Cl_2 (80 mL), washed with aq. $Na_2S_2O_3$ (2 x 30 mL), water (30 mL) and brine (20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (1.029 g, 81 %, α/β = 7.7/1.0) as a white foam. Analytical data for **S10**: R_f = 0.50 (ethyl acetate - toluene, 1/9, v/v); Selected NMR values for major anomer **S10α**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 3.25 (br. s, 1H, -OH), 4.21-4.32 (m, 1H, H-6), 4.33-4.43 (m, 4H, H-6', 3H Fmoc), 4.55-4.65 (m, 1H, H-5), 5.33 (dd, 1H, $J_{2,3}$ = 3.6, 10.3 Hz, H-2), 5.63 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 5.78 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 6.25 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3), 7.30-8.0 ppm (m, 23H, aromatic); ^{13}C-NMR (75 MHz,

$CDCl_3$): δ, 46.7, 66.1, 67.8, 69.2, 69.9, 70.3, 72.1, 90.4, 120.0, 125.3, 127.2 (x2), 127.9, 128.3, 128.5 (x2), 128.8, 128.9, 129.1, 129.7, 129.9 (x2), 133.3, 133.5, 141.3, 143.3, 143.4, 154.9, 165.4, 165.8 (x2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{42}H_{34}O_{11}$ Na 737.1999, found 737.2003.

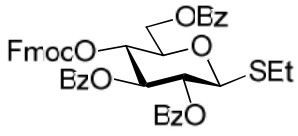
2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (3e).



3e

To a stirred solution of **S10** (0.920 g, 1.28 mmol) in CCl_3CN (2.58 mL, 25.7 mmol)/CH_2Cl_2 (10 mL), NaH (60 % dispersion in oil, 0.005 g, 0.128 mmol) was added at room temperature under argon atmosphere. Upon completion, solvents were evaporated and the residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (1.04 g, 94 %, α/β = 1/1) as an off-white foam. Analytical data for **3e**: R_f = 0.50 (ethyl acetate - toluene, 0.5/9.5, v/v); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 4.28-4.49 (m, 11H, H-5$_\alpha$, H-6$_\alpha$, H-6'$_\alpha$, H-6$_\beta$, H-6'$_\beta$, 6H Fmoc), 4.55-4.59 (m, 1H, H-5$_\beta$), 5.63 (dd, 1H, $J_{2,3}$ = 3.6, 10.2 Hz, H-2$_\alpha$), 5.72-5.86 (m, 3H, H-4$_\alpha$, H-2$_\beta$, H-4$_\beta$), 5.98 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3$_\beta$), 6.24 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1$_\beta$), 6.28 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3$_\alpha$), 6.86 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1$_\alpha$), 7.18-7.99 (m, 46H, aromatic), 8.64 (s, 1H, N-H$_\alpha$), 8.70 ppm (s, 1H, N-H$_\beta$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 46.6, 65.5, 65.8, 68.5, 68.9, 70.0, 70.3 (x2), 70.5, 70.6 (x2), 72.5, 72.9, 90.2, 90.7, 93.1, 95.7, 120.0, 125.3 (x2), 125.4, 127.2, 127.9, 128.2, 128.4 (x2), 128.5 (x2), 128.6, 128.8, 128.9, 129.0, 129.7 (x2), 129.8, 129.9 (x3), 133.3, 133.4, 133.6 (x2), 141.2 (x2), 143.2 (x2), 143.4 (x2), 154.7 (x2), 160.4, 160.9, 164.7, 165.1, 165.2, 165.3, 165.6 (x2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{44}H_{34}Cl_3NO_{11}Na$ 880.1095, found 880.1107.

Ethyl 2,3,6-Tri-*O*-benzoyl-4-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-glucopyranoside (S12).

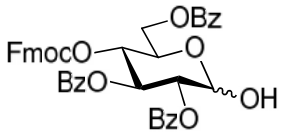


S12

To a stirred solution of ethyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside[9] (**S11**, 1.40 g, 2.63 mmol) in pyridine (15 mL), were added FmocCl (1.35 g, 5.22 mmol) and DMAP (0.160 g, 1.30 mmol) under argon at room temperature. After 3 h, the reaction mixture was co-evaporated with toluene (3 x 15 mL) , dissolved in DCM (70 mL), washed with water (50 mL) and brine (30 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue

was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (1.29 g, 91%) as a white foam. Analytical data for **S12**: R_f = 0.50 (ethyl acetate - toluene, 2/8, v/v); $[\alpha]_D^{23}$ +4.2° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.29 (t, 3H, *J* = 7.4 Hz, -C*H*$_3$), 2.80 (m, 2H, -S-C*H*$_2$-), 3.99 (t, 1H, *J* = 7.3 Hz, 1H Fmoc), 4.14-4.28 (m, 3H, H-5, 2H Fmoc), 4.56 (dd, 1H, *J* = 12.3, 4.8 Hz, H-6), 4.71 (dd, 1H, *J* = 12.3, 2.8 Hz, H-6'), 4.85 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.34 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 5.57 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.89 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 7.16-8.16 (m, 23H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.6, 46.6, 63.1, 70.6 (x2), 73.2, 74.3, 75.9, 84.1, 120.1 (x2), 125.2 (x2), 125.5, 127.3, 127.9 (x2), 128.4 (x2), 128.5, 128.6 (x2), 128.8, 129.2 (x2), 129.8, 129.9, 130.0 (x2), 133.4, 133.5 (x2), 141.2, 141.3, 143.0, 143.2, 154.3, 165.4, 165.8, 166.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{44}H_{38}O_{10}SNa$ 781.2083, found 781.2091.

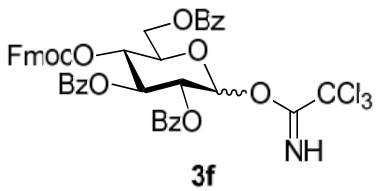
2,3,6-Tri-*O*-benzoyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranose (S13).



S13

The title compound was obtained from **S12** in 97% (α/β = 6.6/1.0) yield as a white foam as described in the synthesis of compound **S10**. Analytical data for **S13**: R_f = 0.50 (ethyl acetate - toluene, 1.5/8.5, v/v); Selected NMR values for major anomer **S13α**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.90 (br s, 1H, -O*H*), 3.88-3.98 (m, 1H, Fmoc), 4.06-4.26 (m, 3H, H-6, 2H Fmoc), 4.43 (dd, 1H, *J* = 3.4, 12.0 Hz, H-6'), 4.57-4.70 (m, 1H, H-5), 5.25 (dd, 1H, $J_{2,3}$ = 7.7 Hz, H-2), 5.34 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 5.71 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 6.18 (dd, 1H, $J_{3,4}$ = 13.0 Hz, H-3), 7.11-8.10 ppm (m, 23H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 46.5, 62.5, 67.2, 69.9, 70.3 (x2), 72.1, 72.9, 73.0, 90.3, 120.0 (x2), 120.9 (x2), 121.2, 125.0, 125.1, 125.2, 125.3, 127.2, 127.6, 127.8, 128.0, 128.2, 128.3, 128.5, 128.9, 129.0, 129.6, 129.9, 130.0, 131.0, 133.3, 133.5, 137.9, 140.0, 140.1, 140.2, 141.1 (x2), 142.5, 142.9, 143.0, 143.1, 144.8, 145.2, 154.2, 154.3, 165.8, 165.9, 166.4 (x2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{42}H_{34}O_{11}Na$ 737.1999, found 737.2001.

2,3,6-Tri-*O*-benzoyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (3f).



3f

The title compound was obtained from **S13** in 92% (α/β = 1.0/2.8) yield as an off-white foam as described in the synthesis of compound **3e**. Analytical data for **7**: R_f = 0.50 (ethyl acetate - toluene, 0.5/9.5, v/v); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 3.90-4.26 (m, 6H, Fmoc), 4.30-4.36 (m, 2H, H-6$_\alpha$, H-5$_\beta$), 4.49-4.73 (m, 4H, H-5$_\alpha$, H-6'$_\alpha$, H-6$_\beta$, H-6'$_\beta$), 5.46 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4$_\alpha$), 5.46 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4$_\beta$), 5.56 (dd, 1H, $J_{2,3}$ = 3.6, 10.2 Hz, H-2$_\alpha$), 5.80 (dd, 1H, $J_{2,3}$ = 8.9 Hz, H-2$_\beta$), 5.90 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-3$_\beta$), 6.19 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1$_\beta$), 6.22 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-3$_\alpha$), 6.81 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1$_\alpha$), 7.11-8.11 ppm (m, 46H, aromatic), 8.63 (s, 1H, N-H$_\alpha$), 8.72 ppm (s, 1H, N-H$_\beta$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 46.4, 62.3, 70.5 (x2), 70.6, 72.4, 72.5, 72.7, 90.2, 95.8, 119.9 (x2), 125.0 (x2), 125.3, 127.1, 127.8, 128.2, 128.3, 128.4 (x2), 128.5 (x2), 129.0, 129.8, 129.9 (x2), 133.2, 133.4, 133.5, 137.8, 141.1 (x2), 142.9, 143.0, 154.0, 160.9, 164.8, 165.4, 165.5, 166.0 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{44}H_{34}Cl_3NO_{11}Na$ 880.1095, found 880.1078.

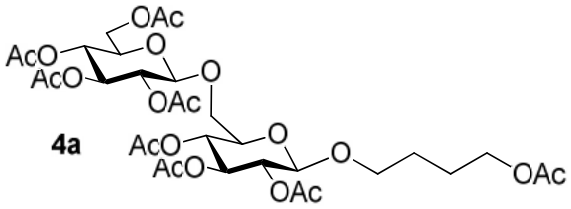
HPLC-mediated synthesis of disaccharides 4a-4c, S15, and trisaccharide 4d.

*General procedure for HPLC-mediated glycosylation of **2a** with glycosyl donors **3a-3f.*** A solution of glycosyl donor (**3a-3f,** Pump B, 39 mM in CH_2Cl_2, percentage flow: 80%), and TMSOTf (Pump C, 0.28 mM in CH_2Cl_2, percentage flow: 20%) were passed through the column containing resin-bound glycosyl acceptor **2a** at the flow rate of 0.3 mL/min for 60 min. Then the flow was switched to Pump A for 10 min. (flow rate: 2 mL/min).

Operation	Action	Flow rate, mL/min	Total volume	Time, min
Glycosylation (acceptor **2a**)	Pump B: Glycosyl donor **(3a-3f)** (39 mM) in CH_2Cl_2 Pump C: TMSOTf (0.28 M) in CH_2Cl_2	0.3 B/C = 4/1	18 mL	60
Washing	Pump A: CH_2Cl_2	2.0	20 mL	10
Cleaving off	Pump C: 0.1 M $NaOCH_3$ in CH_3OH/CH_2Cl_2	1.0	5 mL (recirc.)	60

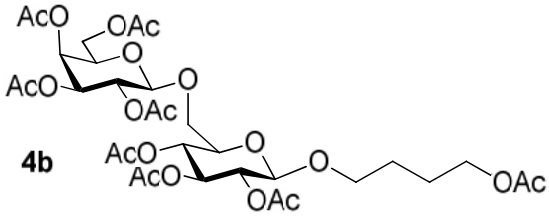
General procedure for HPLC-mediated cleavage of sugar from resin. A solution of $NaOCH_3$ in CH_3OH/CH_2Cl_2 (0.1 M, 5 mL, 1/1, v/v) was circulated through the column for 60 min using Pump C (flow rate: 1 mL/min). The column was then purged with CH_3OH for 10 min using Pump A (flow rate: 2 mL/min). The combined eluate was neutralized with Dowex (H^+) resin, filtered, and concentrated *in vacuo* to afford the corresponding deprotected di- and trisaccharides. The crude product was then acetylated as follows. To a stirred solution of crude di or trisaccharide (0.0482 mmol) in pyridine (2 mL) Ac_2O (73 μL, 0.771 mmol) was added dropwise in the presence of catalytic DMAP. The reaction mixture was stirred under argon for 6 h at room temperature. The reaction mixture was quenched with CH_3OH (1 mL) and the resulting mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL), and washed with 1N HCl (2 x 10 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and water (20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford corresponding disaccharides **4a-4c, S15** (73-98%) or trisaccharide **4d** (67%).

4-Acetyloxybut-1-yl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4a).



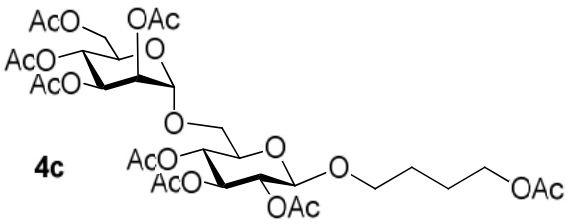
The title compound was synthesized from glycosyl donor **3a** and glycosyl acceptor **2a** in 98% yield. Analytical data for **4a**: R_f = 0.50 (ethyl acetate - toluene, 1/4, v/v); $[\alpha]_D^{23}$ -10.5° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.61-1.70 (m, 4H, 2x -C*H*$_2$-), 1.99 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 2.02 (s, 3H, -COC*H*$_3$), 2.03 (s, 3H, -COC*H*$_3$), 2.04 (s, 9H, 3x -COC*H*$_3$), 2.10 (s, 3H, -COC*H*$_3$), 3.45-3.53 (m, 1H, O-C*H*$_2$-), 3.58-3.73 (m, 3H, H-5a, 6a, 5b), 3.85-3.95 (m, 2H, H-6'a, O-C*H*$_2$-), 4.07 (t, 2H, *J* = 5.9 Hz, -C*H*$_2$-OAc), 4.12 (dd, 1H, *J* = 12.3, 2.1 Hz, H-6b), 4.28 (dd, 1H, *J* = 12.4, 4.8 Hz, H-6'b), 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.59 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.85-5.22 (m, 6H, H-2a, 3a, 4a, 2b, 3b, 4b); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 20.5, 20.6 (x4), 20.7, 20.8, 20.9, 25.1, 25.9, 61.8, 63.9, 68.2, 68.3, 69.1, 69.2, 71.1, 71.3, 71.9, 72.7, 72.8, 73.3, 100.5, 100.8, 169.2, 169.3, 169.4, 169.6, 170.2, 170.3, 170.6, 171.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{32}H_{46}O_{20}$ Na 773.2480, found 773.2455.

4'-Acetyloxybut-1-yl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4b).



The title compound was synthesized from glycosyl donor **3b** and glycosyl acceptor **2a** in 96% yield. Analytical data for **4b**: R_f = 0.50 (ethyl acetate - toluene, 1/4, v/v); $[\alpha]_D^{23}$ -9.5° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.61-1.70 (m, 4H, 2x -C*H*$_2$-), 1.98 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 2.03 (s, 3H, -COC*H*$_3$), 2.04 (s, 3H, -COC*H*$_3$), 2.05 (s, 9H, 3x -COC*H*$_3$), 2.15 (s, 3H, -COC*H*$_3$), 3.49 (dt, 1H, *J* = 9.6, 5.7 Hz, O-C*H*$_2$-), 3.57-3.69 (m, 2H, H-5a, 6a), 3.87-3.93 (m, 3H, H-6'a, 5b, O-C*H*$_2$-), 4.07 (t, 2H, *J* = 6.0 Hz, -C*H*$_2$-OAc), 4.10-4.19 (m, 2H, H-6b, 6'b), 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1a), 4.55 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.85-5.03 (m, 3H, H-2a, 4a, 3b), 5.16-5.24 (m, 2H, H-3a, 2b), 5.39 (d, 1H, $J_{4,5}$ = 3.3 Hz, H-4b); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 20.6, 20.7 (x5), 20.8, 20.9, 25.1, 25.9, 61.2, 63.9, 67.0, 68.3, 68.7, 69.1 (x2), 70.8 (x2), 71.3, 72.7, 73.3, 100.5, 101.2, 169.3 (x2), 169.6, 170.1, 170.2, 170.3, 170.4, 171.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{32}H_{46}O_{20}$ Na 773.2480, found 773.2468.

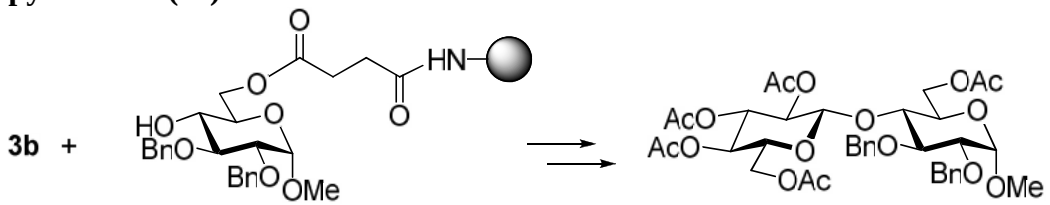
4-Acetyloxybut-1-yl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4c).



4c

The title compound was synthesized from glycosyl donor **3c** and glycosyl acceptor **2a** in 78% yield. Analytical data for **4c**: R_f = 0.50 (ethyl acetate - toluene, 1/4, v/v); $[\alpha]_D^{23}$ +9.0° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.63-1.72 (m, 4H, 2x -CH_2-), 1.99 (s, 3H, -COCH_3), 2.01 (s, 3H, -COCH_3), 2.03 (s, 3H, -COCH_3), 2.04 (s, 3H, -COCH_3), 2.05 (s, 6H, 2x -COCH_3), 2.11 (s, 3H, -COCH_3), 2.16 (s, 3H, -COCH_3), 3.49-3.56 (m, 1H, O-CH_2-), 3.53 (dd, 1H, *J* = 9.7, 1.6 Hz, H-6b), 3.67-3.79 (m, 2H, H-5b, 6'b), 3.91 (dt, 1H, *J* = 9.5, 5.6 Hz, O-CH_2-), 3.98-4.05 (m, 1H, H-5a), 4.06 (t, 2H, *J* = 6.0 Hz, -CH_2-OAc), 4.09 (dd, 1H, *J* = 12.6, 2.2 Hz, H-6a), 4.27 (dd, 1H, *J* = 12.3, 5.2 Hz, H-6'a), 4.51 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1a), 4.81 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1b), 4.92-4.99 (m, 2H, H-2a, 4b), 5.19-5.35 (m, 4H, H-3a, 4a, 2b, 3b); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 20.6 (x5), 20.8, 20.9, 21.0, 25.2, 26.0, 62.3, 64.0, 65.9, 66.6, 68.7, 68.9, 69.4 (x2), 69.5, 71.3, 72.4, 72.7, 97.2, 100.6, 169.4, 169.7 (x2), 169.9, 170.0, 170.3, 170.6, 171.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{32}H_{46}O_{20}$ Na 773.2480, found 773.2507.

Methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranoside (10).

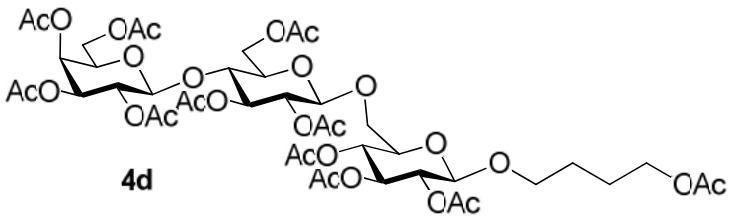


S14 **S15**

Resin-bound glycosyl acceptor **S14** was obtained from methyl 2,3-di-*O*-benzyl-6-*O*-(3-carboxypropanoyl)-α-D-glucopyranoside[10] as described for the synthesis of **2a** and **2b**. The title compound was synthesized using glycosyl donor **3a** and resin bound glycosyl acceptor **S14** in 73% yield. Analytical data for **S15**: R_f = 0.50 (ethyl acetate / toluene, 1/1, v/v); $[\alpha]_D^{23}$ +4.3° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.97 (s, 3H, -COCH_3), 1.98 (s, 3H, -COCH_3), 1.99 (s, 3H, -COCH_3), 2.05 (s, 3H, -COCH_3), 2.10 (s, 3H, -COCH_3), 3.36 (s, 3H, -OCH_3), 3.41-3.46 (m, 1H, H-5b), 3.47 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2a), 3.66 (dd, 1H, $J_{4,5}$ = 8.6 Hz, H-4a), 3.77-3.83 (m, 2H, H-5a, 6a), 3.94 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 4.07-4.15 (m, 2H, H-6'a, 6b), 4.37 (dd, 1H, *J* = 2.1, 11.8 Hz, H-6'b), 4.55 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1a), 4.64 (dd, 2H, J^2 = 12.4 Hz, CH_2Ph), 4.73 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.94 (d, 2H, J^2 = 3.1 Hz, CH_2Ph), 5.03 (d, 1H, $J_{2,3}$ = 8.0 Hz, H-2b), 5.07-5.12 (m, 2H, H-3b, 4b), 7.23-7.34 (m, 10H, aromatic); ^{13}C-NMR: δ, 20.5, 20.6 (x3), 20.8, 55.3, 61.4, 62.6, 67.8, 68.0, 71.8, 72.0, 73.1, 73.4, 74.8, 78.7, 79.4, 79.7, 97.8, 100.8, 126.7,

127.3, 127.9, 128.1, 128.2, 128.4, 129.0, 137.9, 139.2, 169.3 (x2), 170.3, 170.5, 170.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{37}H_{46}O_{16}$ Na 769.2684, found 769.2685.

4-Acetyloxybut-1-yl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4d).



4d

The title compound was synthesized from glycosyl donor **3d** and glycosyl acceptor **2a** in 67% yield. Analytical data for **4d**: R_f = 0.30 (ethyl acetate - toluene, 1/4, v/v); $[\alpha]_D^{23}$ -5.9° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.65-1.71 (m, 4H, 2x -C*H*$_2$-), 1.97 (s, 3H, -COC*H*$_3$), 1.99 (s, 3H, -COC*H*$_3$), 2.03 (s, 3H, -COC*H*$_3$), 2.05 (s, 15H, 5 x -COC*H*$_3$), 2.06 (s, 3H, -COC*H*$_3$), 2.13 (s, 3H, -COC*H*$_3$), 2.15 (s, 3H, -COC*H*$_3$), 3.51 (dt, 1H, *J* = 9.6, 5.4 Hz, O-C*H*$_2$-), 3.58-3.67 (m, 3H, H-5a, 6a, 6b), 3.77-3.93 (m, 4H, H-6'a, H-6'b, 5c, O-C*H*$_2$-), 4.04-4.15 (m, 5H, H-5b, 6c, 6'c, -C*H*$_2$-OAc), 4.44-4.49 (m, 3H, H-1a, 1b, 4b), 4.56 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), 4.86-4.98 (m, 4H, H-2a, 3b, 2c, 3c), 5.08-5.21 (m, 3H, H-3a, 4a, 2b), 5.35 (d, 1H, $J_{4,5}$ = 2.7 Hz, H-4c); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 20.5, 20.6 (x7), 20.8, 20.9, 21.0, 25.1, 25.9, 60.7, 61.9, 63.9, 66.6, 68.1, 69.0 (x2), 69.2, 70.6, 70.9, 71.3, 71.5, 72.7, 72.8 (x2), 73.3, 76.2, 100.4, 100.5, 101.1, 169.1, 169.3, 169.5 (x2), 169.7, 170.1, 170.2, 170.3, 170.4 (x2), 171.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{44}H_{62}O_{28}$ Na 1061.3325, found 1061.3292.

HPLC-mediated synthesis of pentasaccharide 8

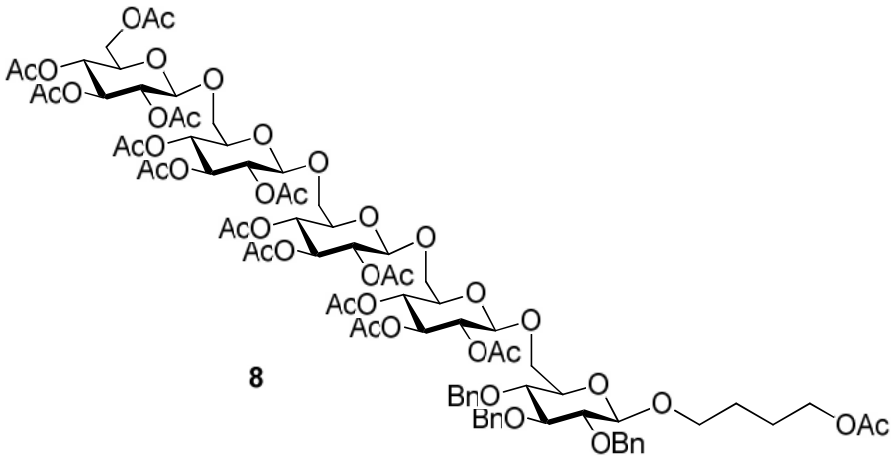
*Synthesis of disaccharide acceptor **5b**:* A solution of glycosyl donor **(3e,** Pump B, 39 mM in CH_2Cl_2, Percentage flow: 80 %), and TMSOTf (Pump C, 0.28 mM in CH_2Cl_2, Percentage flow: 20 %) were passed into column containing glycosyl acceptor bound resin **2b** at the flow rate of 0.3 mL/min for 60 min, maintained under argon atmosphere. Then it was switched to Pump A for 10 min (flow rate: 2 mL/min). A solution of piperidine/DMF (1/5, v/v) from Pump C was passed into column containing resin with Fmoc-protected sugar for 10 min. (flow rate: 0.5 mL/min, λ = 312 nm) and switched to Pump A for 10 min. (flow rate: 2 mL/min).

*Synthesis of trisaccharide acceptor **6**:* A solution of glycosyl donor **(3e,** Pump B, 39 mM in CH_2Cl_2, Percentage flow: 80 %), and TMSOTf (Pump C, 0.28 mM in CH_2Cl_2, Percentage flow: 20 %) were passed into column containing disaccharide acceptor bound resin **5b** at the flow rate of 0.3 mL/min. for 60 min., maintained under argon atmosphere. Then it was switched to Pump A for 10 min (flow rate: 2 mL/min). A solution of piperidine/DMF (1/5, v/v) from Pump C was passed into column containing resin with Fmoc-protected sugar for 10 min. (flow rate: 0.5 mL/min, λ = 312 nm) and switched to Pump A for 10 min (flow rate: 2 mL/min).

*Synthesis of tetrasaccharide acceptor **7**:* A solution of glycosyl donor **(3e,** Pump B, 39 mM in CH_2Cl_2, Percentage flow: 80 %), and TMSOTf (Pump C, 0.28 mM in CH_2Cl_2, Percentage flow: 20 %) were passed into column containing trisaccharide acceptor bound resin **6** at the flow rate of 0.3 mL/min for 60 min, maintained under argon atmosphere. Then it was switched to Pump A for 10 min. (flow rate: 2 mL/min.). A solution of piperidine/DMF (1/5, v/v) from Pump C was passed into column containing resin with Fmoc-protected sugar for 10 min. (flow rate: 0.5 mL/min, λ = 312 nm) and switched to Pump A for 10 min. (flow rate: 2 mL/min.).

*Synthesis of pentasaccharide **8***: A solution of glycosyl donor (**3e,** Pump B, 39 mM in CH_2Cl_2, Percentage flow: 80 %), and TMSOTf (Pump C, 0.28 mM in CH_2Cl_2, Percentage flow: 20 %) were passed into column containing tetrasaccharide acceptor bound resin **7** at the flow rate of 0.3 mL/min. for 60 min., maintained under argon atmosphere. Then it was switched to Pump A for 10 min. (flow rate: 2 mL/min.). A solution of $NaOCH_3$ in CH_3OH/CH_2Cl_2 (0.1 M, 5 mL, 1/1, v/v) from Pump C was circulated into column for 60 min. (flow rate: 1 mL/min) and then the flow was switched through Pump A (containing CH_3OH) for 10 min. (flow rate: 2 mL/min). The obtained solution was neutralized with Dowex resin, filtered, and concentrated in *vacuo* to afford the corresponding oligosaccharide residue. The residue obtained was acetylated as follows. Ac_2O (0.5 mL) was added dropwise into the solution of the residue in pyridine (2 mL) containing catalytic DMAP. The reaction mixture was stirred under argon for 16 h at room temperature. The reaction mixture was quenched with CH_3OH (1 mL) and the resulting mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL), and washed with water (20 mL), 1N HCl (20 mL), water (20 mL), sat. aq. $NaHCO_3$ (2 x 20 mL), and water (3 x 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by size exclusion chromatography on LH-20 to afford pentasaccharide **8** (62%).

4-Acetyloxybut-1-yl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (8).



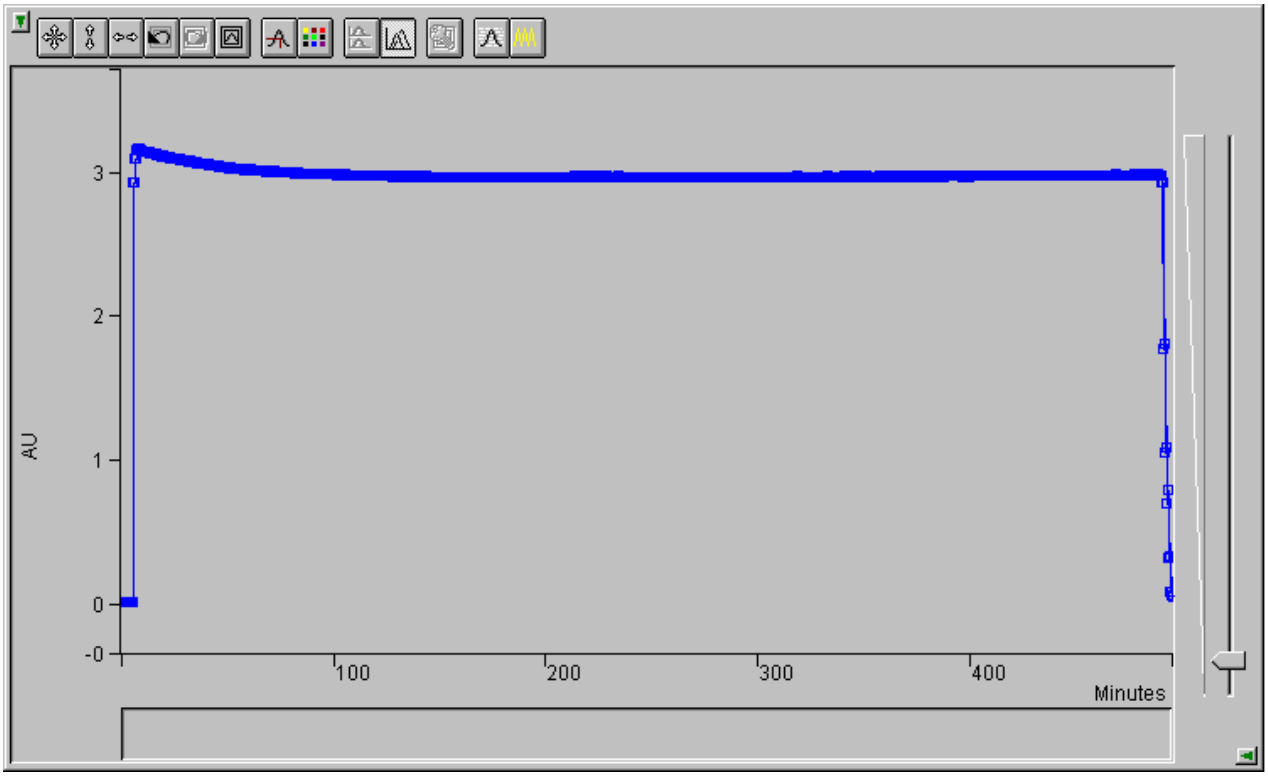
8

The title compound was synthesized from glycosyl donor **3e** and glycosyl acceptor **2b** in 62% yield. Analytical data for **8**: R_f = 0.40 (ethyl acetate - toluene, 1/4, v/v); $[\alpha]_D^{23}$ +10.7° (c = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.69-1.81 (m, 4H, 2x -C*H*$_2$-), 1.97 (s, 3H, -COC*H*$_3$), 1.98 (s, 3H, -COC*H*$_3$), 1.99 (s, 6H, 2 x -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 2.02 (s, 12H, 4 x -COC*H*$_3$), 2.03 (s, 3H,-COC*H*$_3$), 2.04 (s, 3H,-COC*H*$_3$), 2.06 (s, 6H, 2 x -COC*H*$_3$), 2.10 (s, 3H, -COC*H*$_3$), 3.35-3.42 (m, 3H, H-2a, H-3a, H-5a), 3.43-3.48 (m, 1H), 3.52-3.64 (m, 5H), 3.65-3.74 (m, 4H), 3.85-3.95 (m, 3H, H-6b, H-6c, H-6d), 3.96-4.02 (m, 1H, O-C*H*$_2$-), 4.07-4.12 (m, 3H, H-6'a, -C*H*$_2$-OAc), 4.13-4.16 (dd, 1H, *J* = 12.0, 2.2 Hz, H-6e), 4.27 (dd, 1H, *J* = 12.5, 5.0 Hz, H-6'e), 4.36 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1a), 4.52 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1c), 4.55 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1d), 4.57 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1e), 4.64 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.71 (dd, 2H, J^2 = 11.0 Hz, C*H*$_2$Ph), 4.81 (dd, 2H, J^2 = 11.0 Hz, C*H*$_2$Ph), 4.84 (dd, 2H, J^2 = 11.0 Hz, C*H*$_2$Ph), 4.89-5.25 (m, 12H, H-2b, 3b, 4b, 2c, 3c, 4c, 2d, 3d, 4d, 2e, 3e, 4e), 7.25-7.33 ppm (m, 15H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 20.6 (x8), 20.7 (x4), 20.8, 21.0, 25.4, 26.2, 61.8, 64.1, 68.0, 68.1 (x2), 68.3 (x2), 69.0 (x3), 69.2, 69.4, 71.0 (x4), 71.4, 72.0, 72.7 (x2), 72.8, 72.9, 73.0, 73.2, 74.9, 75.1, 75.7, 77.8, 82.1, 84.6, 100.6 (x2), 100.7, 100.9, 103.5, 127.7, 127.8 (x2), 127.9 (x2), 128.0, 128.4, 128.5, 138.0, 138.3, 138.4, 169.0, 169.3 (x2), 169.4, 169.5 (x2), 169.6 (x2), 170.1 (x2), 170.2 (x2), 170.6, 171.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{83}H_{106}O_{41}$ Na 1781.6107, found 1781.6107.

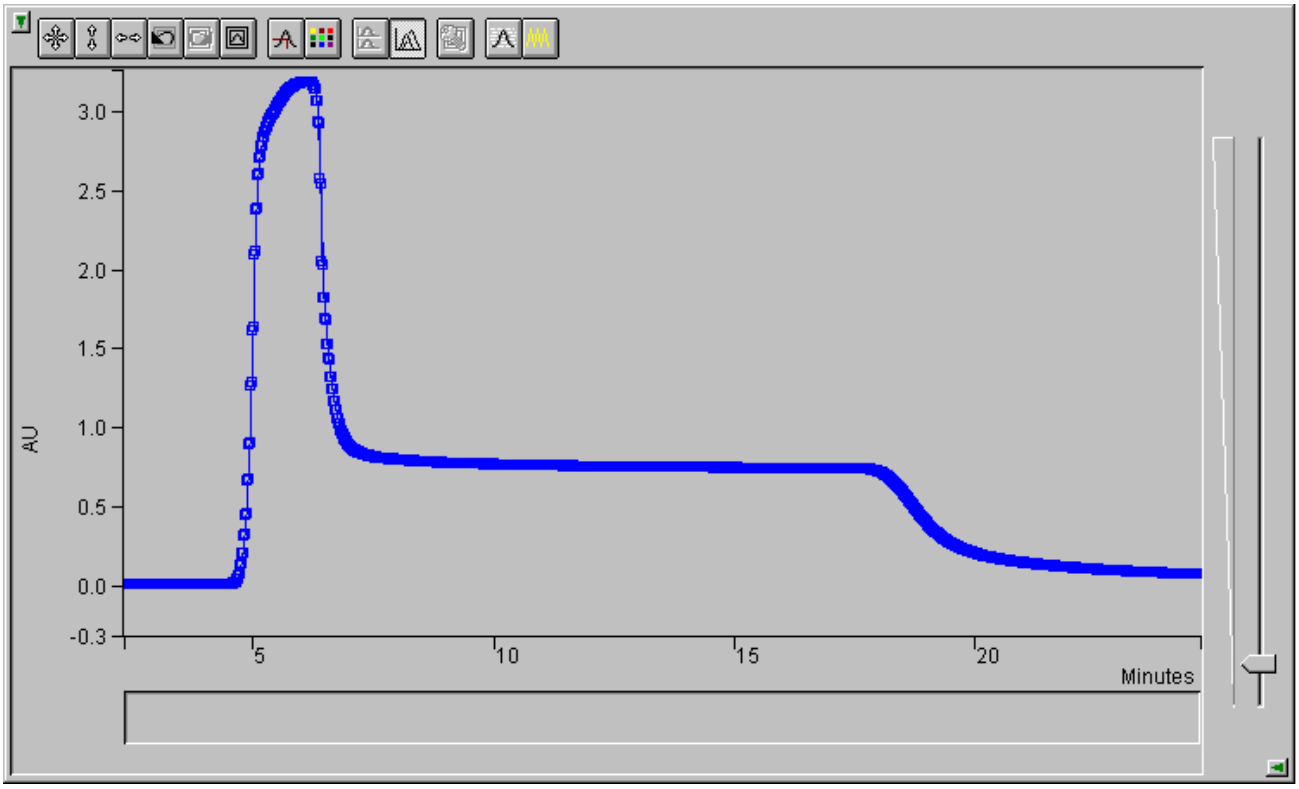
HPLC detector plots

1. Loading of carboxylic acid derived sugar 1a or S8 on resin:

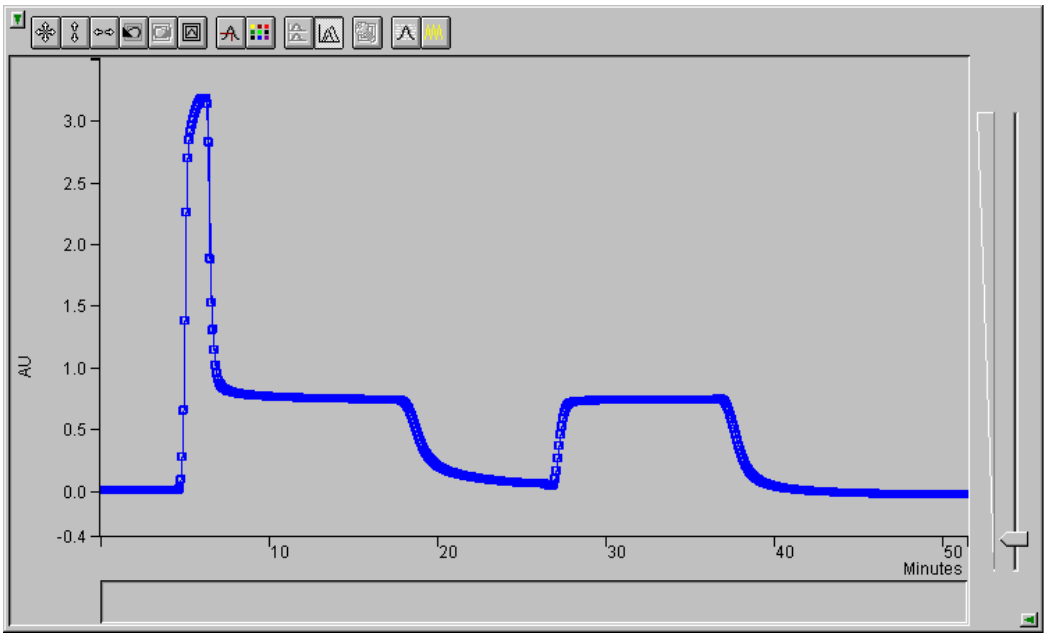
This plot is rather uninformative because it shows a relatively steady absorbance reading during the recirculation of reagents; shown for illustrative purposes only.



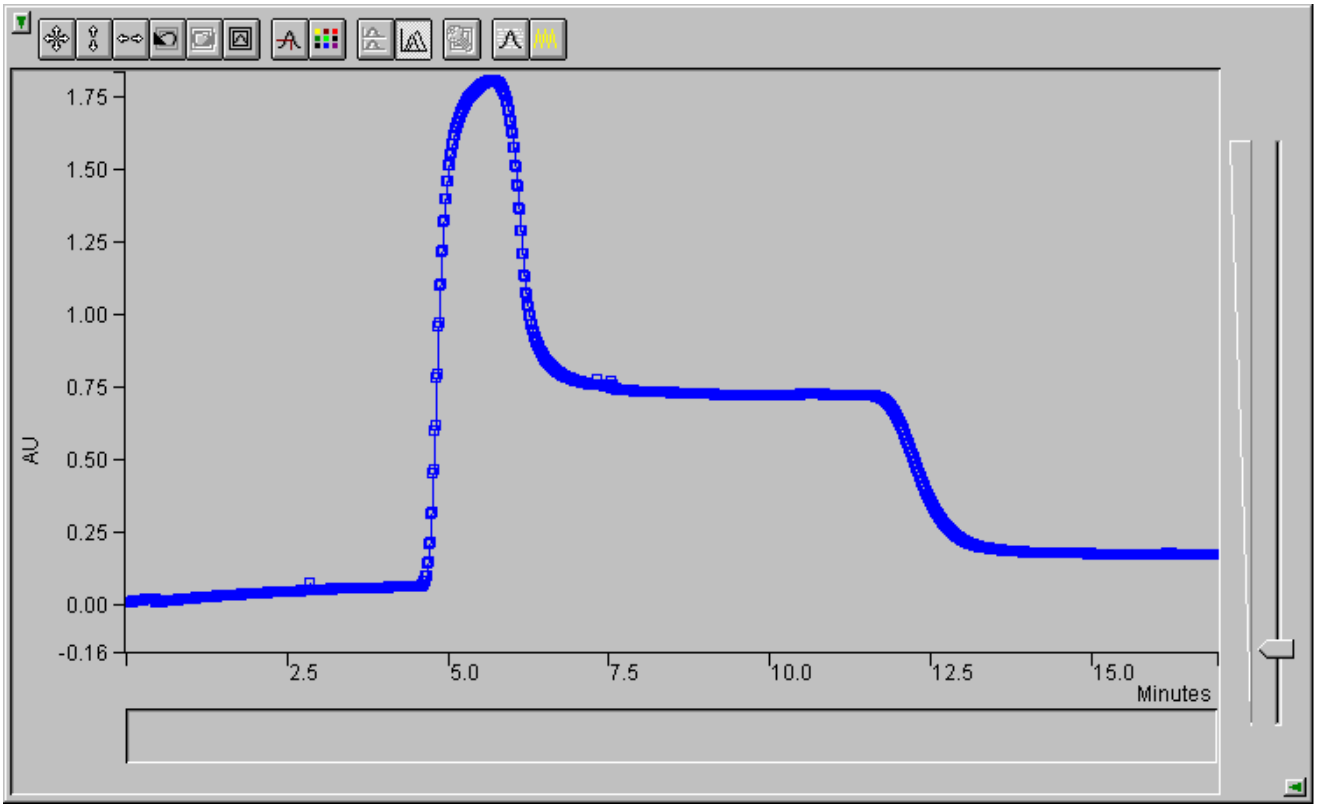
2. Synthesis of glycosyl acceptor bound resin 2a or 2b (trityl group deprotection):



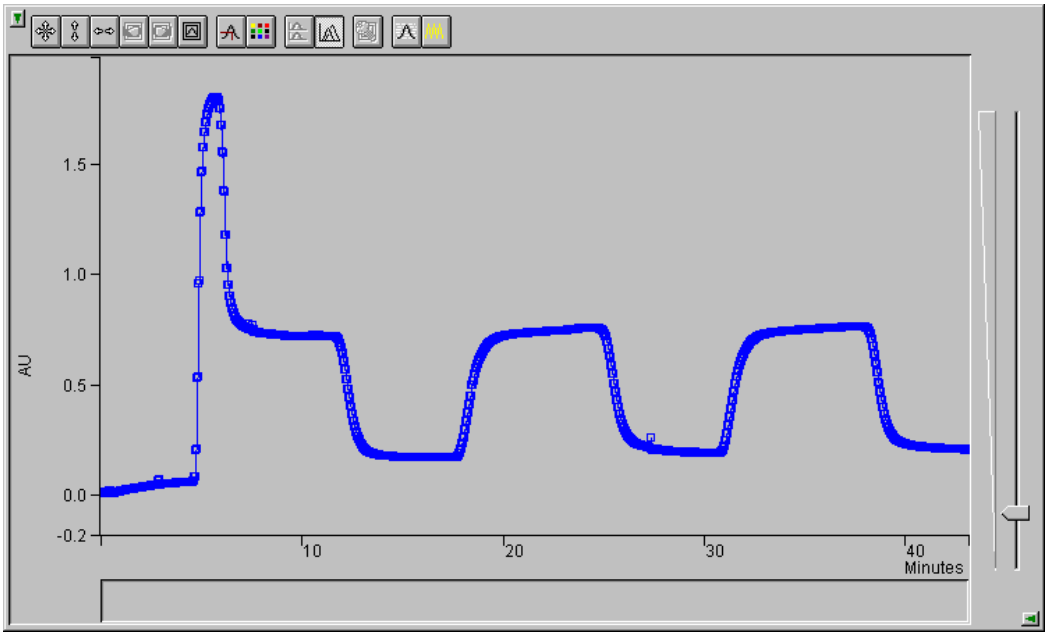
The following supplementary plot shows the second attempt of detritylation (no trityl left after the first treatment)



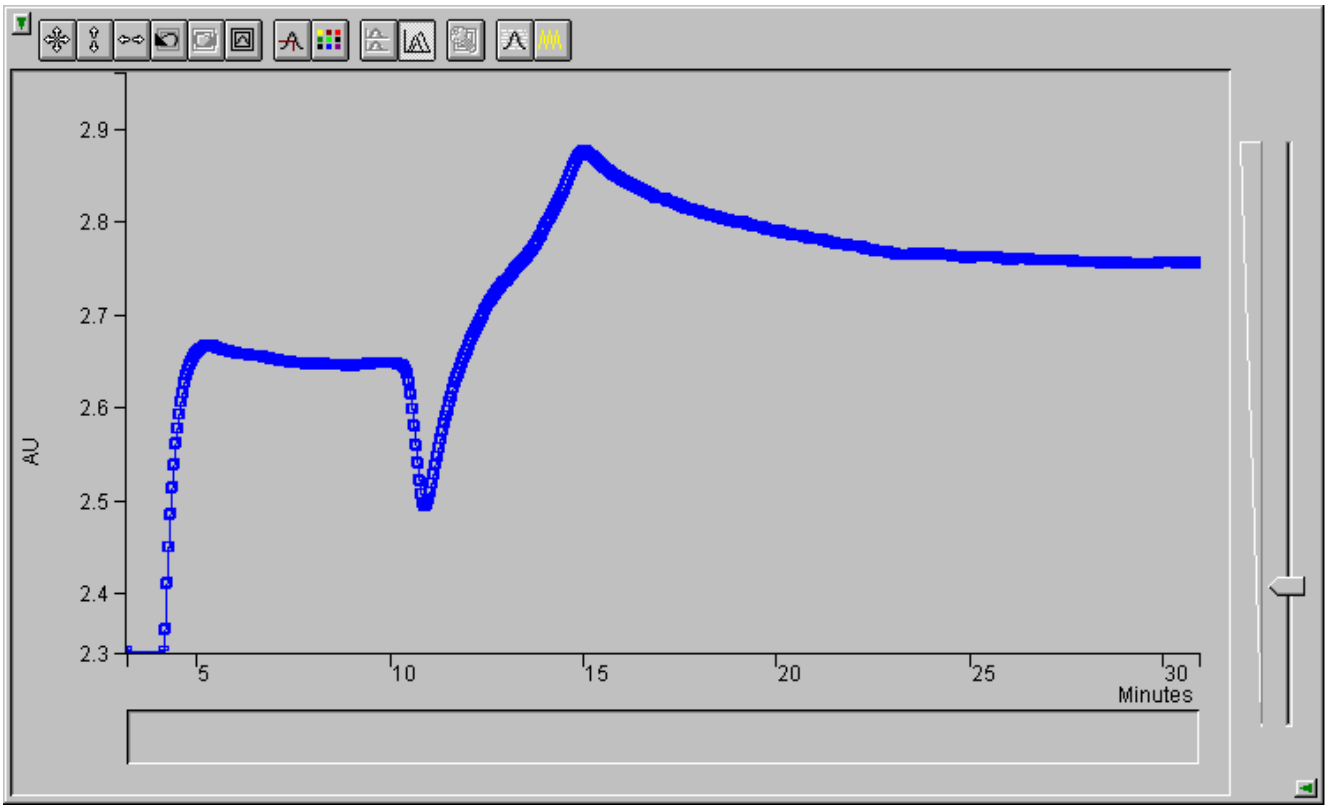
3. Fmoc group deprotection:



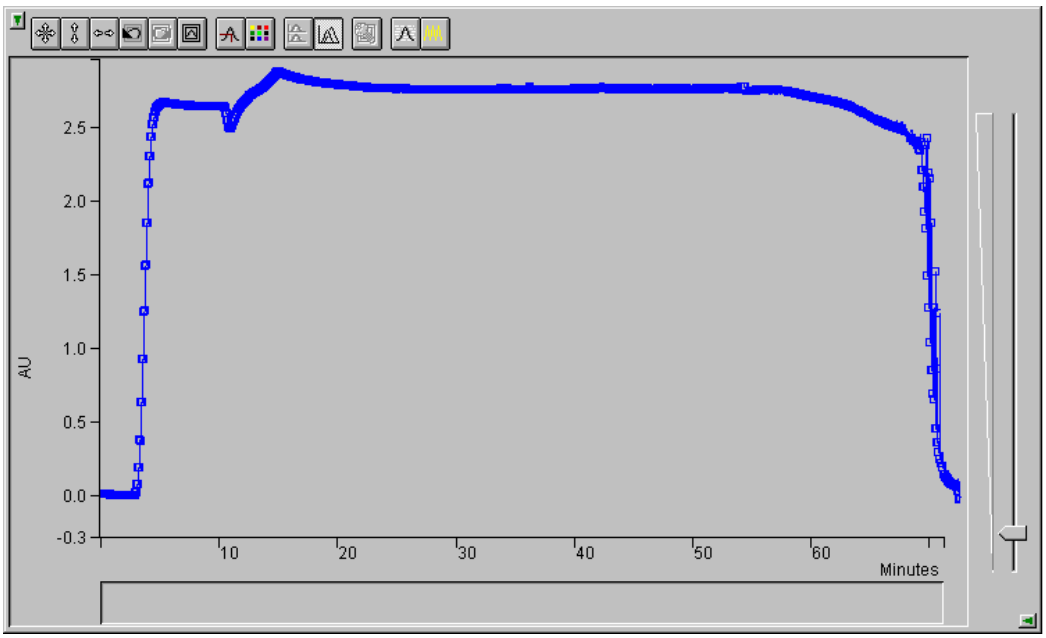
This following supplementary plot shows the second and the third attempts of Fmoc removal (Fmoc is completely removed after the first treatment)



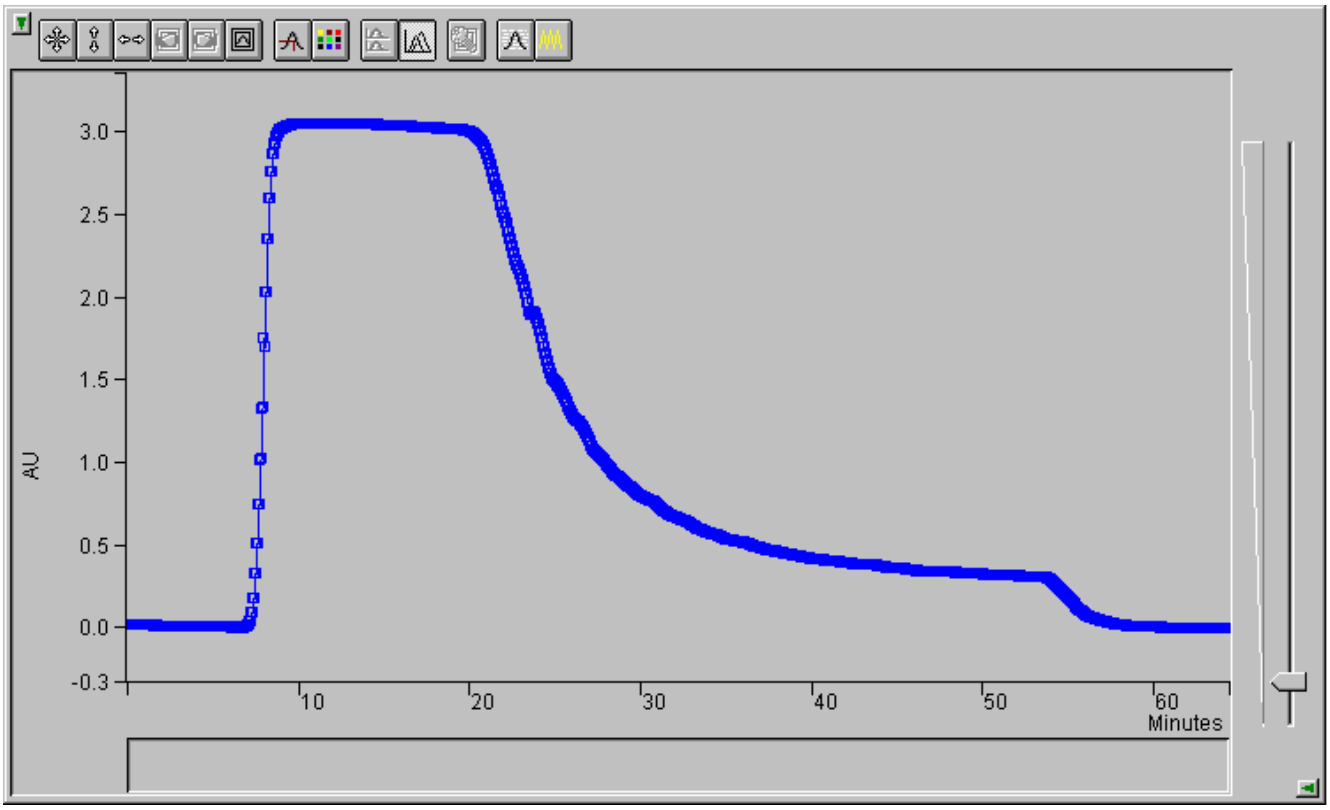
4. A typical plot for glycosylation reactions (obtained consistently using a variety of glycosyl donors and acceptors)



Extended glycosylation plot

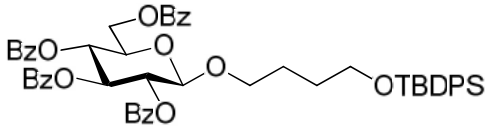


5. Debenzoylation/cleavage of sugar off the resin recorded without recirculation.

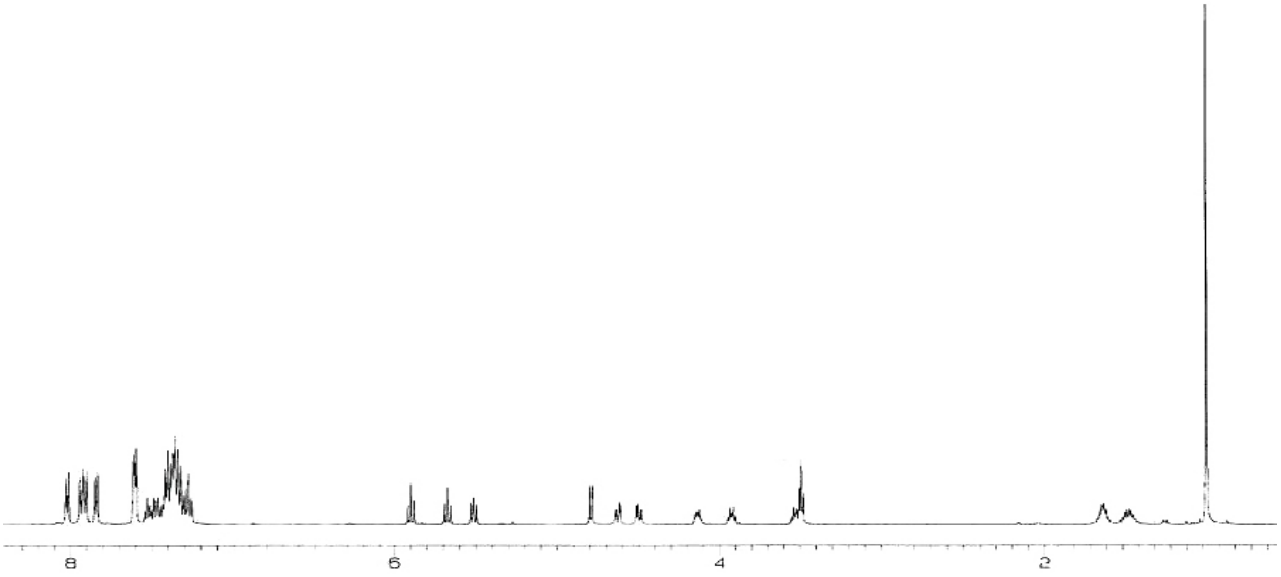


A plot from a typical recirculation-based cleavage is very uninformative and it not shown herein.

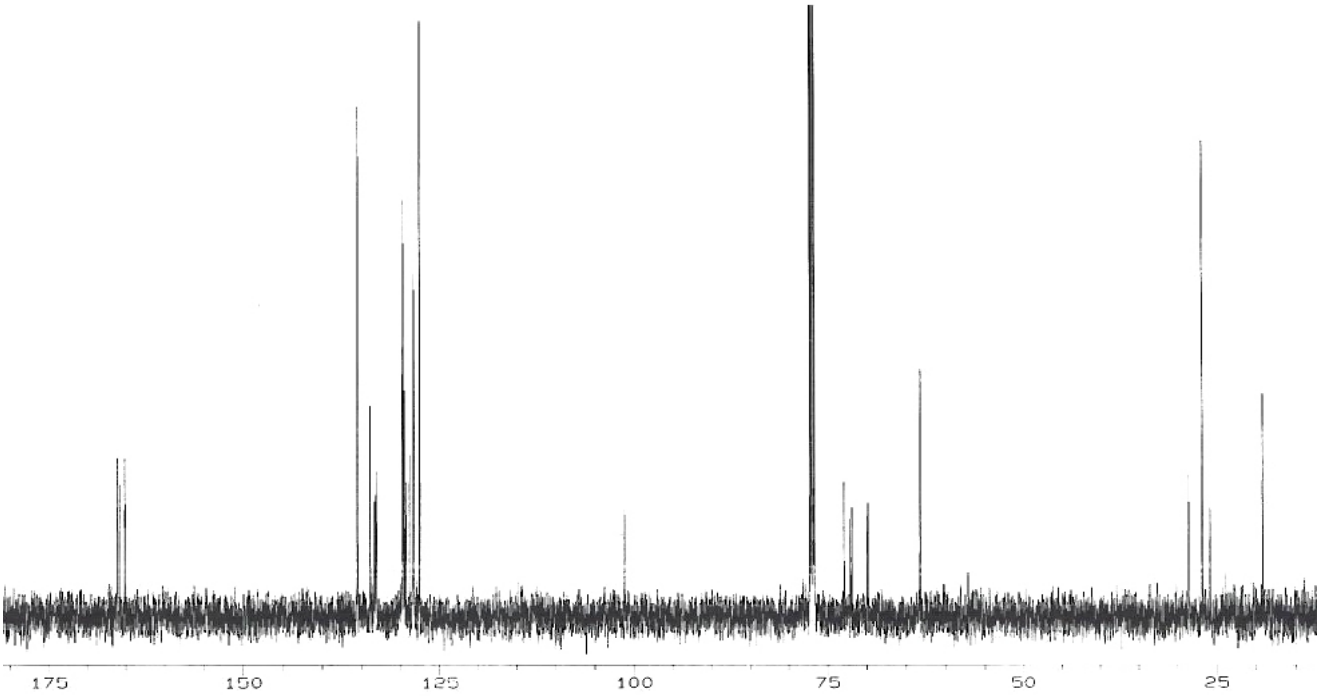
NMR spectra



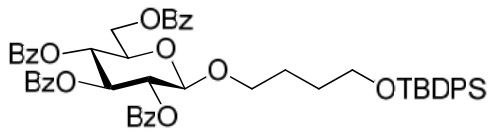
S2



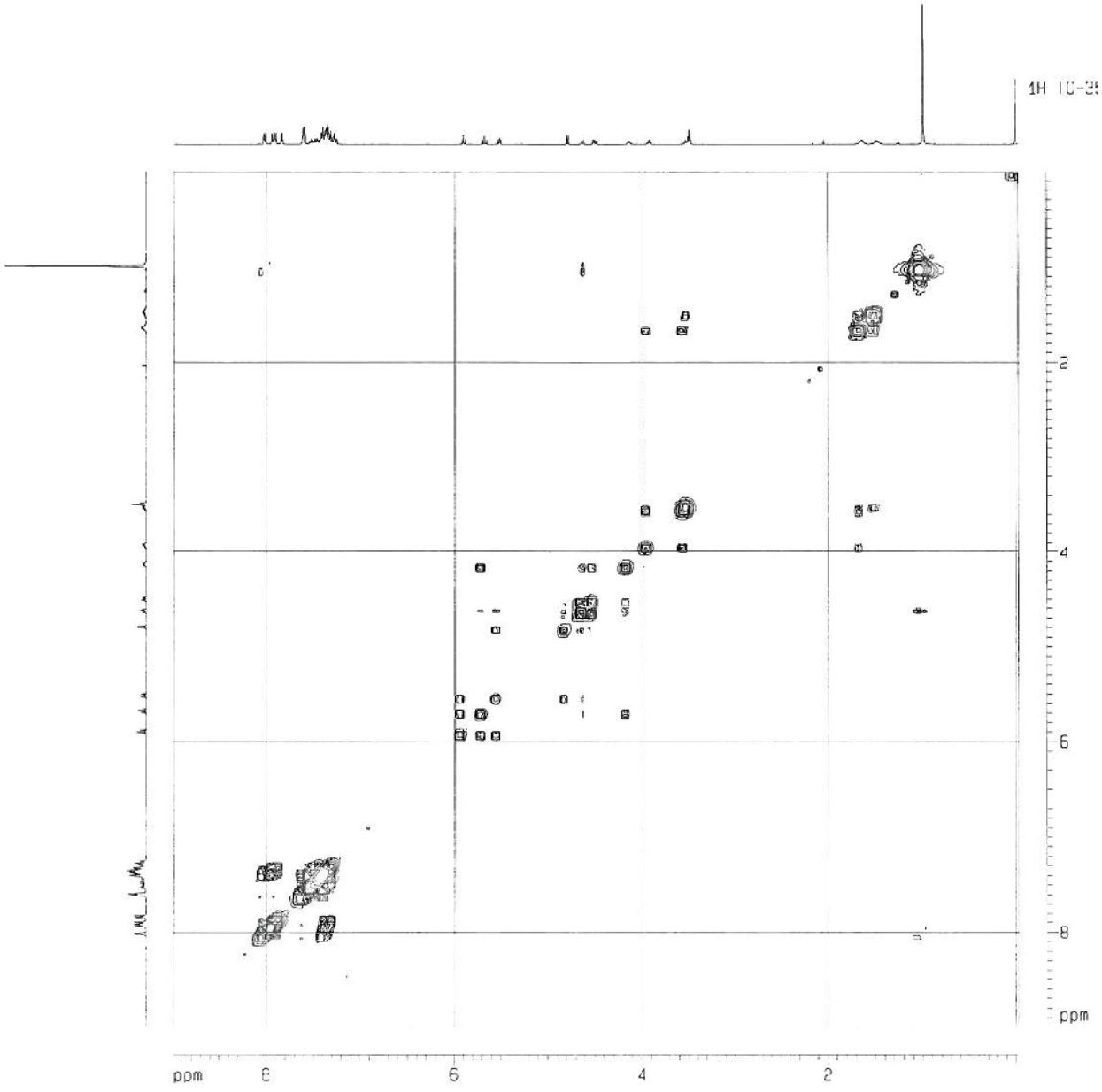
$CDCl_3$ at 500 MHz



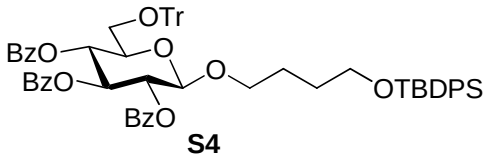
$CDCl_3$ at 125 MHz



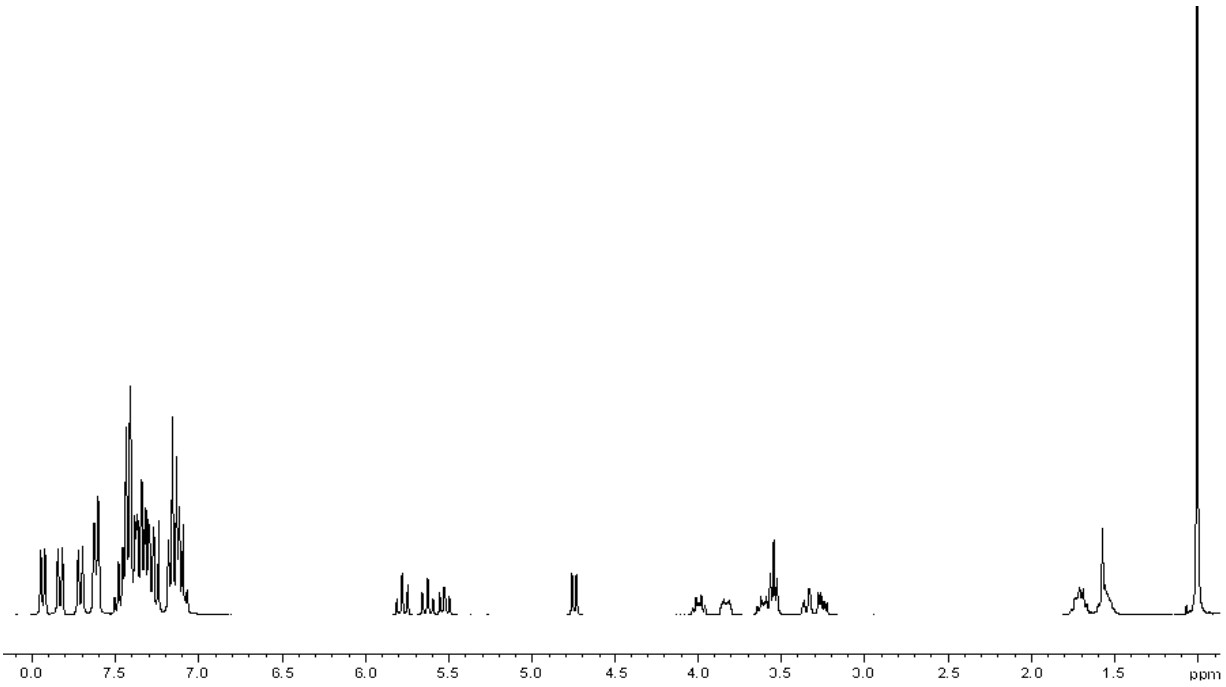
S2



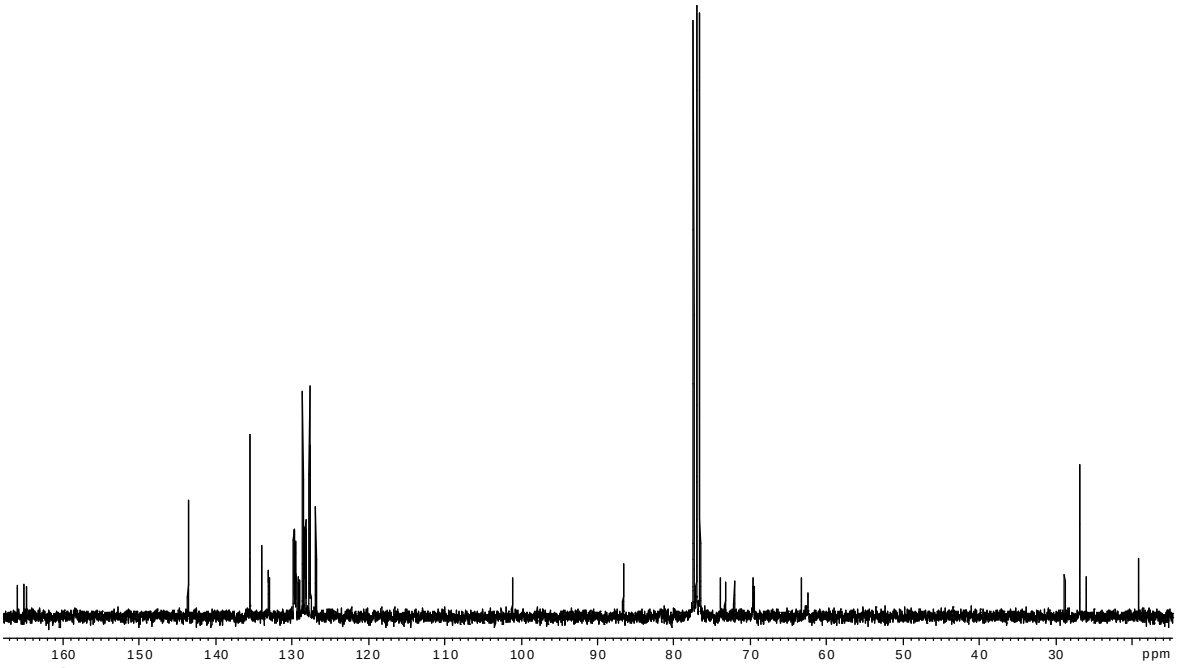
$CDCl_3$ at 500 MHz



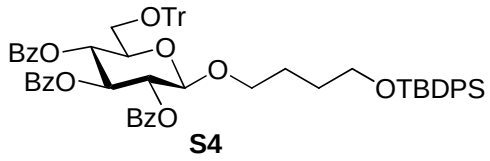
S4



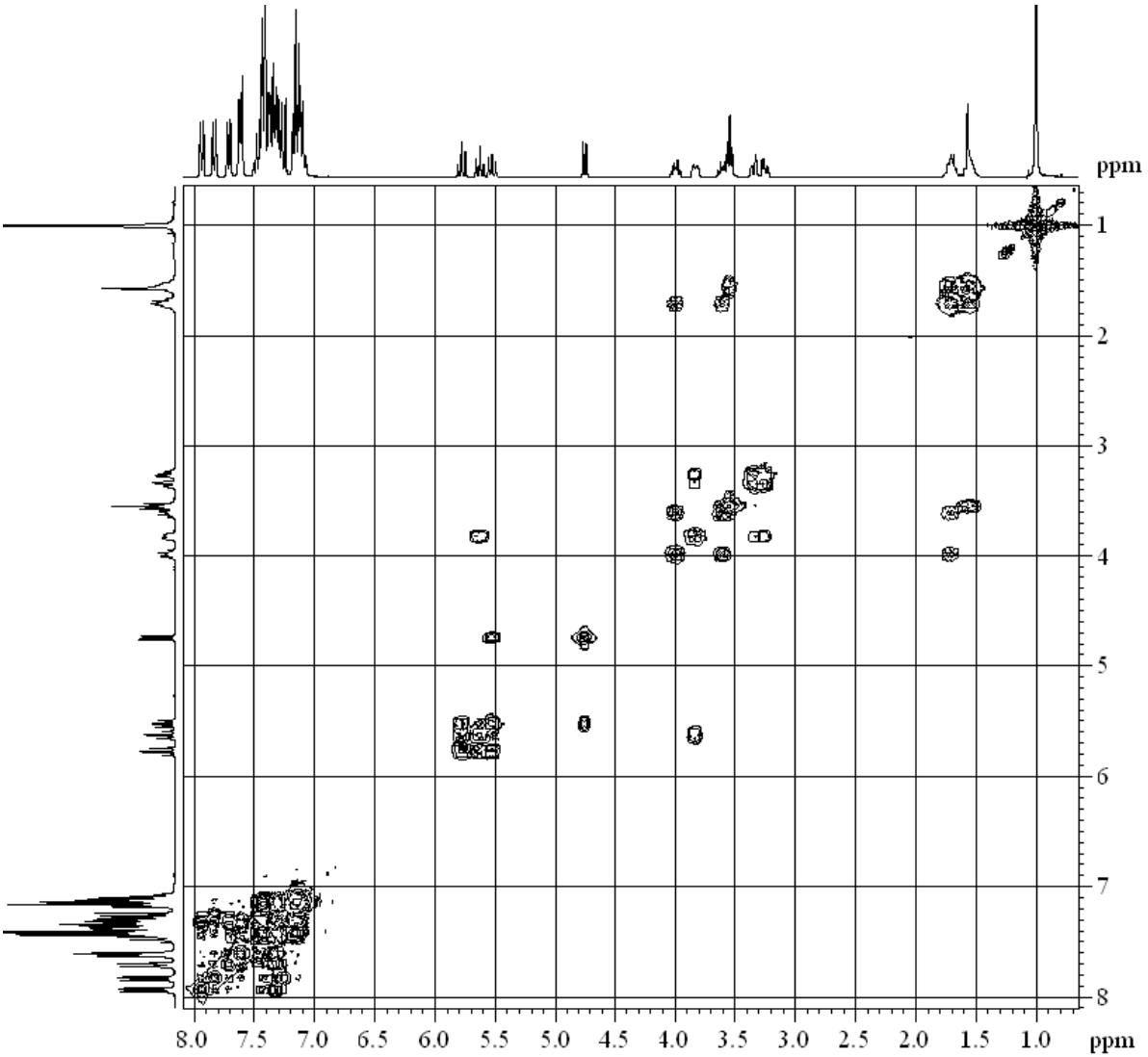
$CDCl_3$ at 300 MHz



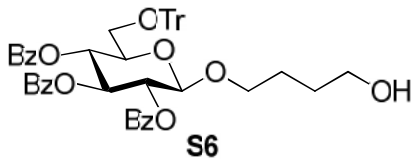
$CDCl_3$ at 75 MHz



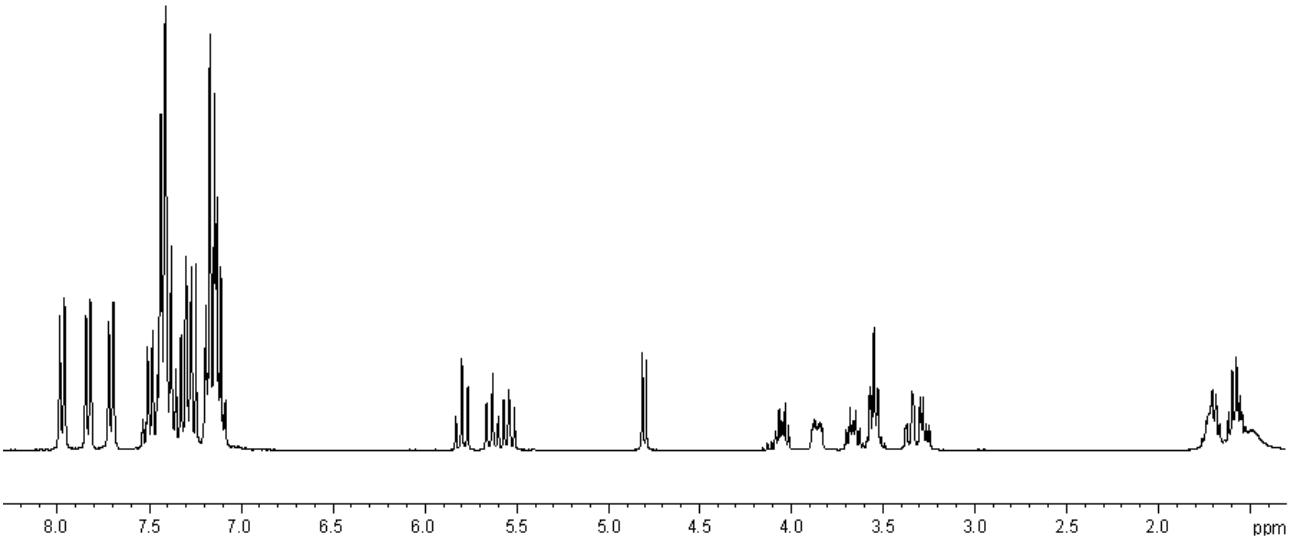
S4



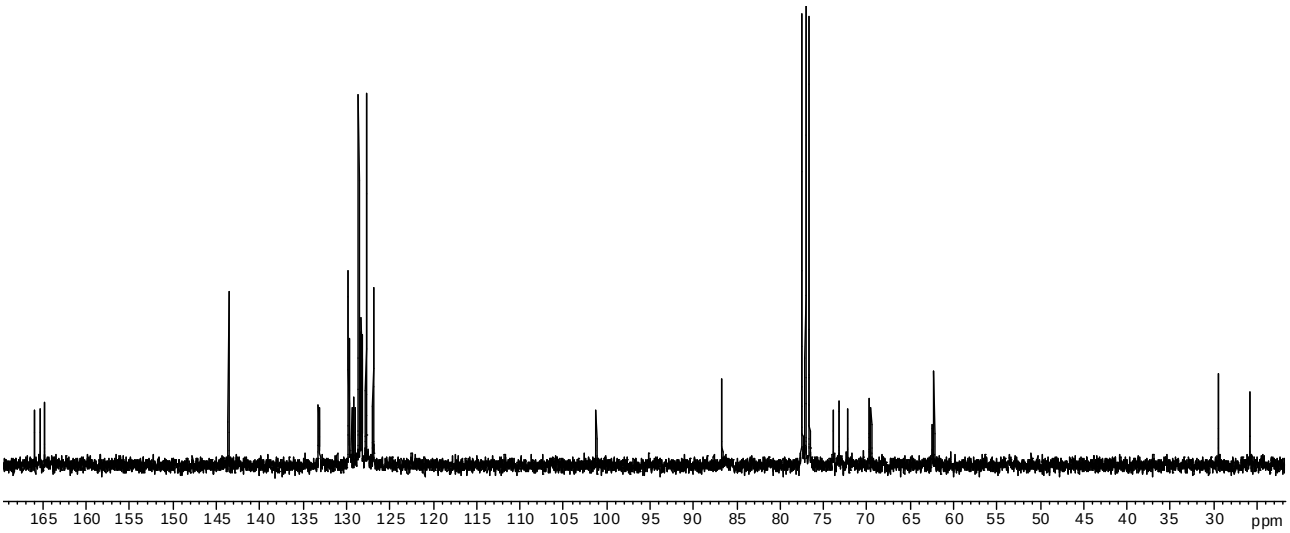
$CDCl_3$ at 300 MHz



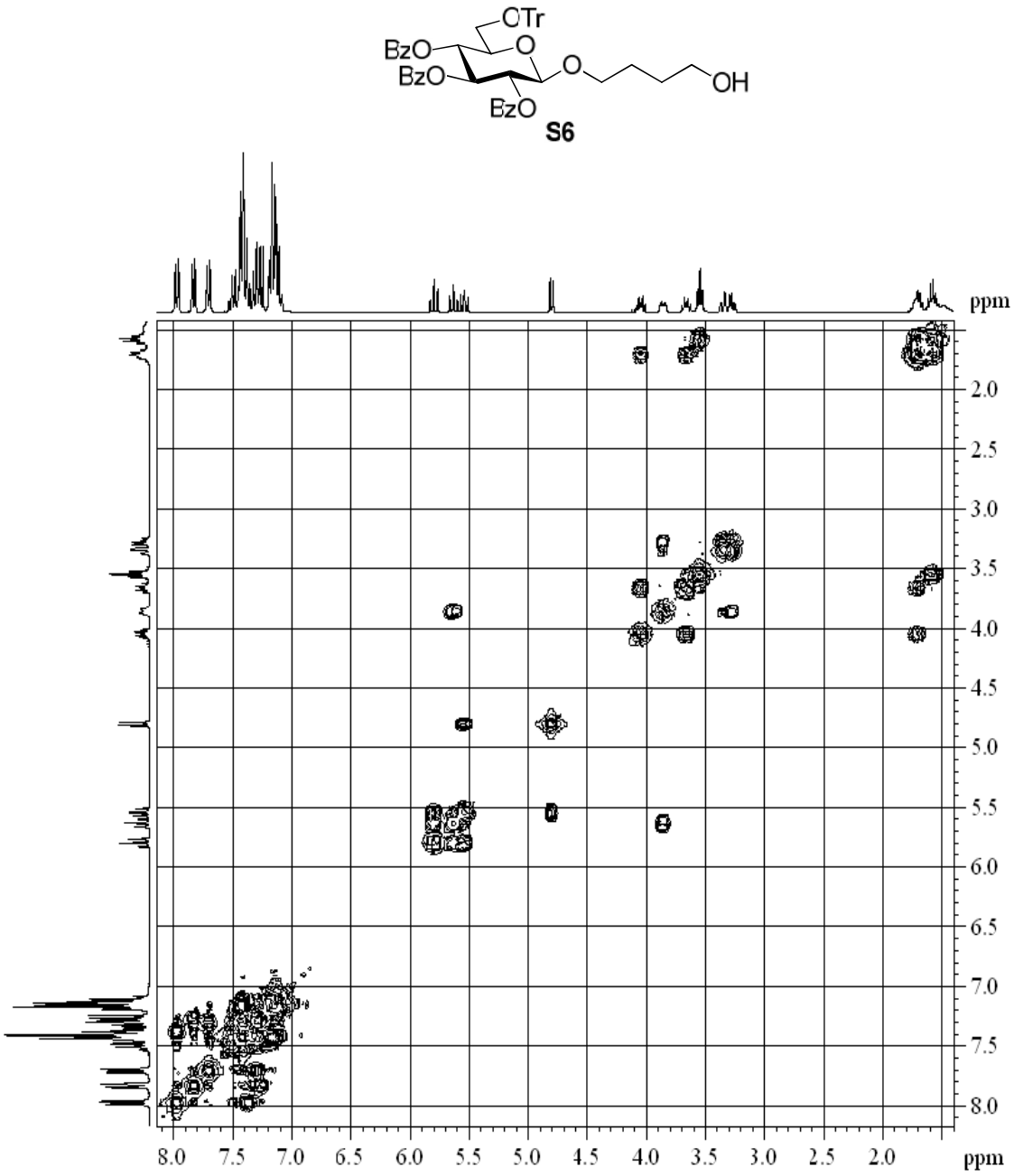
S6



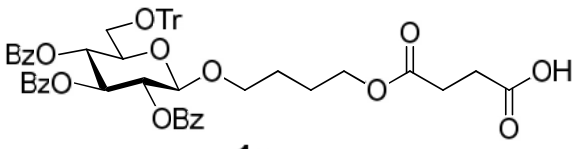
$CDCl_3$ at 300 MHz



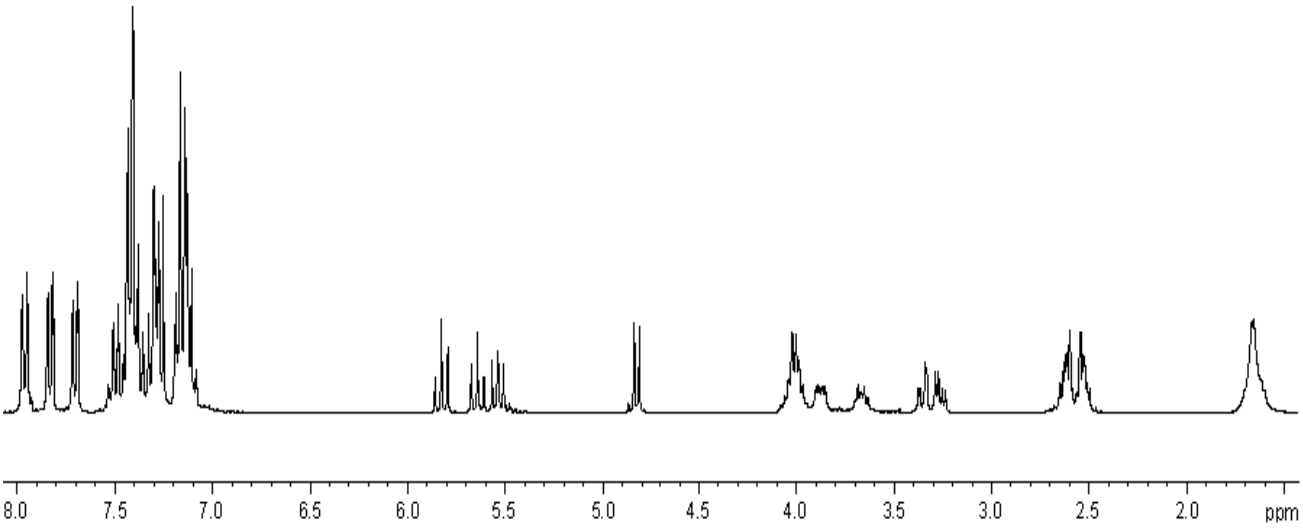
$CDCl_3$ at 75 MHz



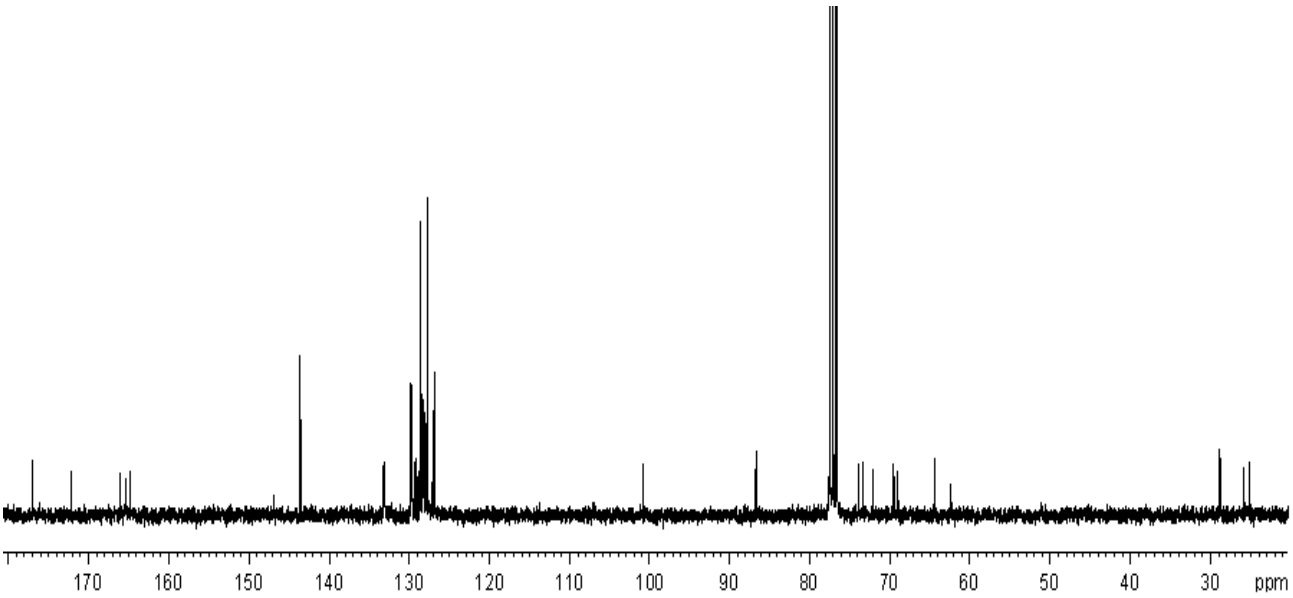
$CDCl_3$ at 300 MHz



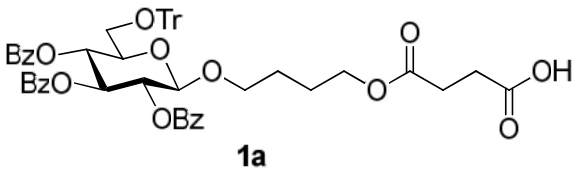
1a



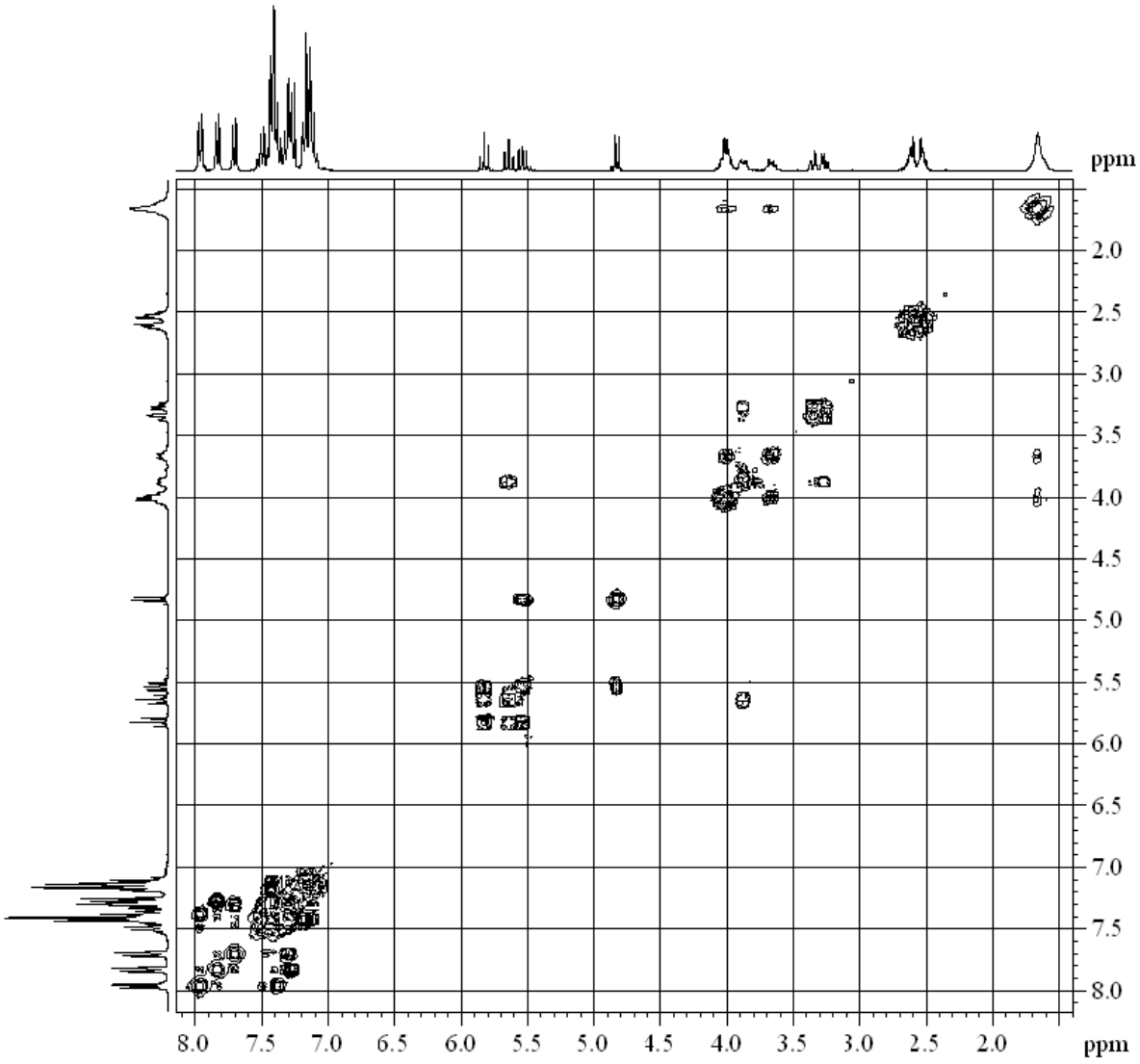
$CDCl_3$ at 300 MHz



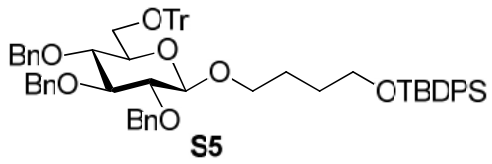
$CDCl_3$ at 75 MHz



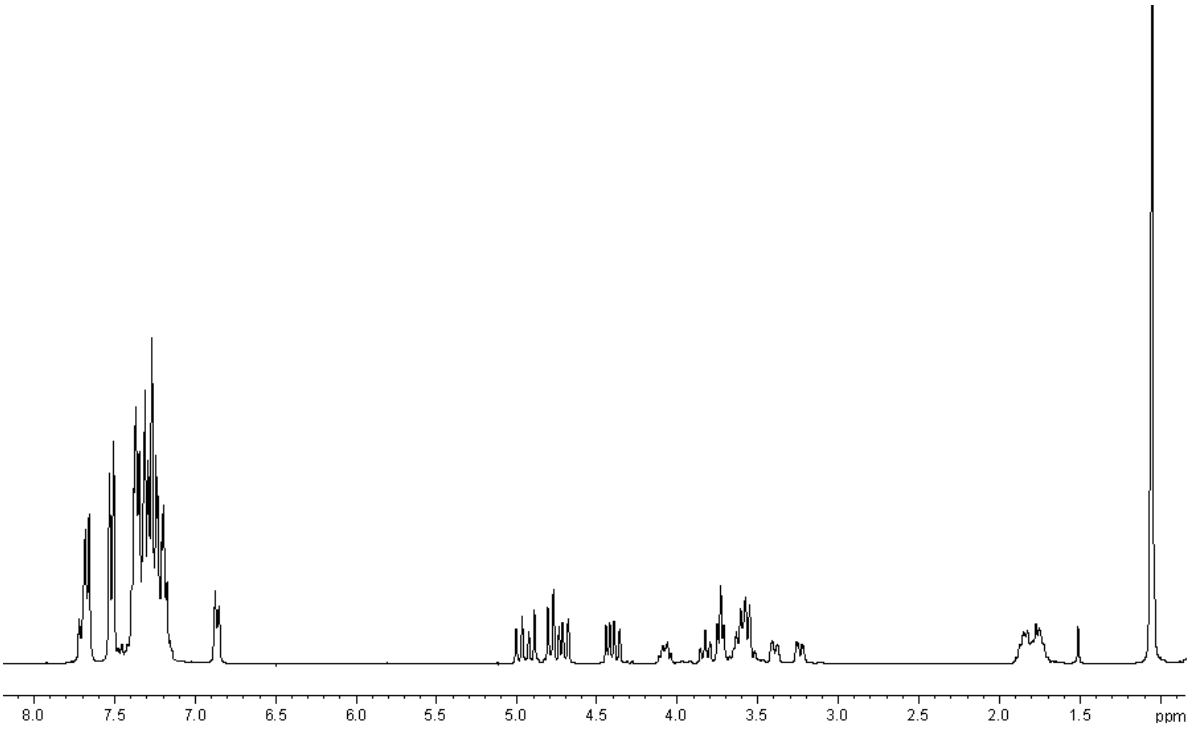
1a



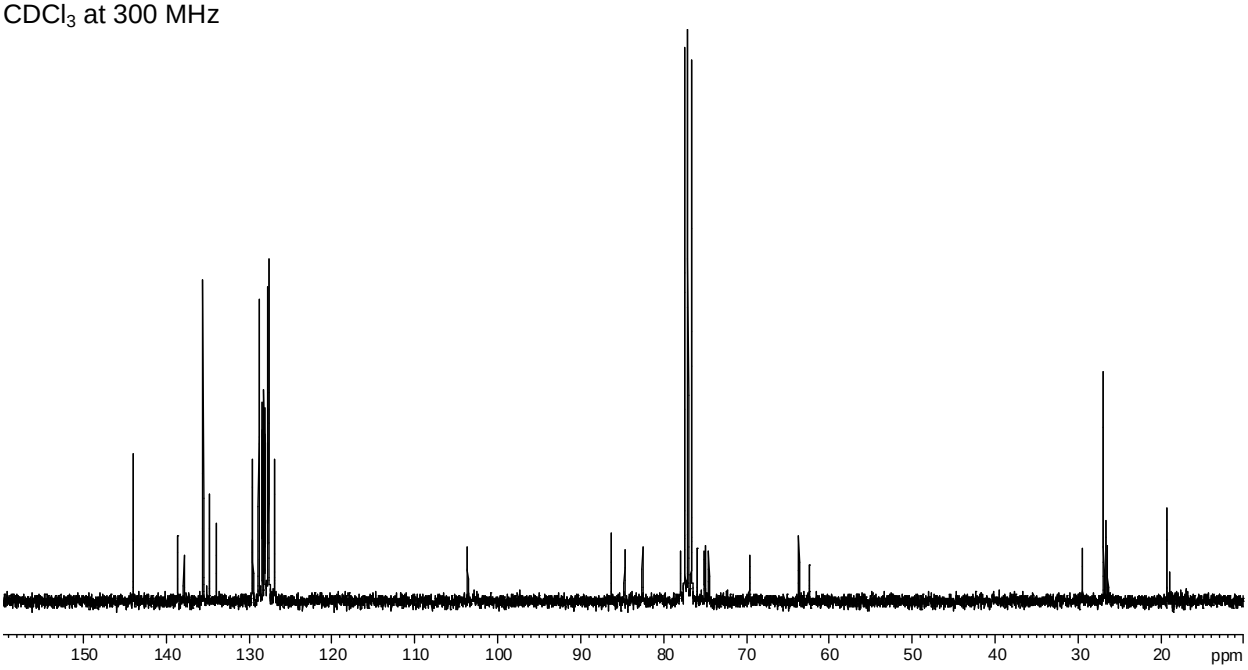
$CDCl_3$ at 300 MHz



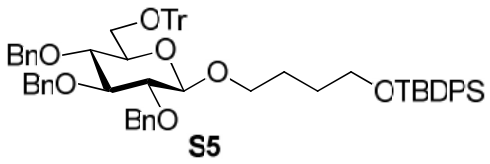
S5



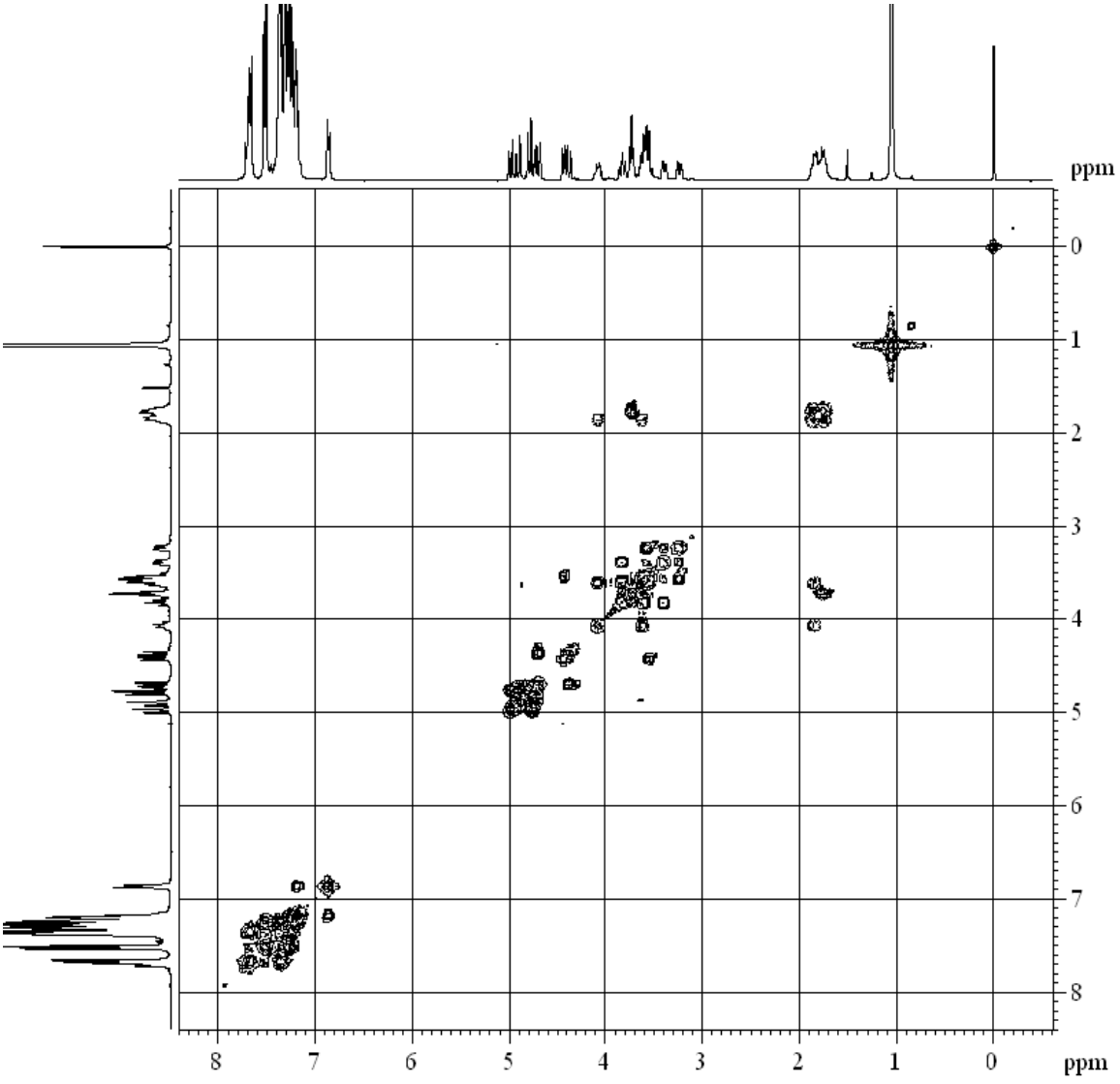
$CDCl_3$ at 300 MHz



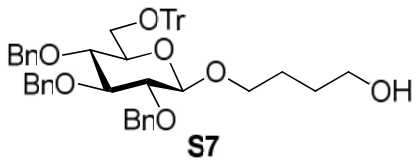
$CDCl_3$ at 75 MHz



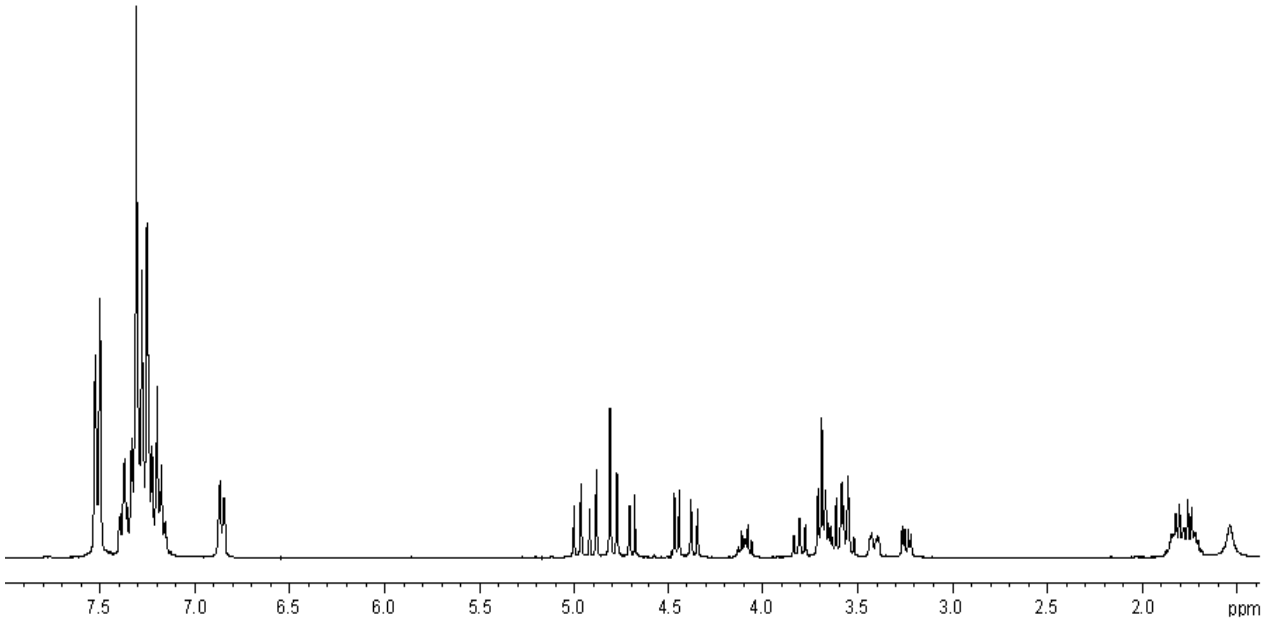
S5



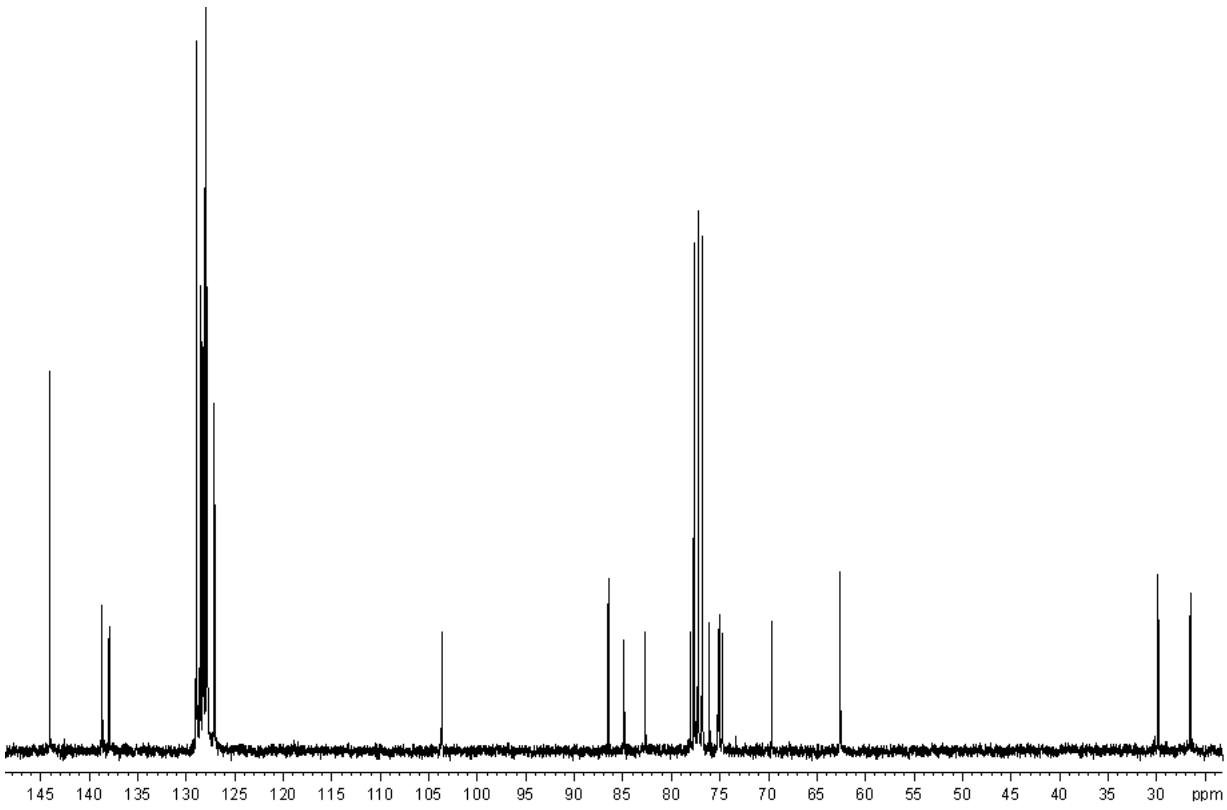
$CDCl_3$ at 300 MHz



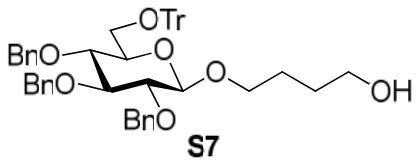
S7



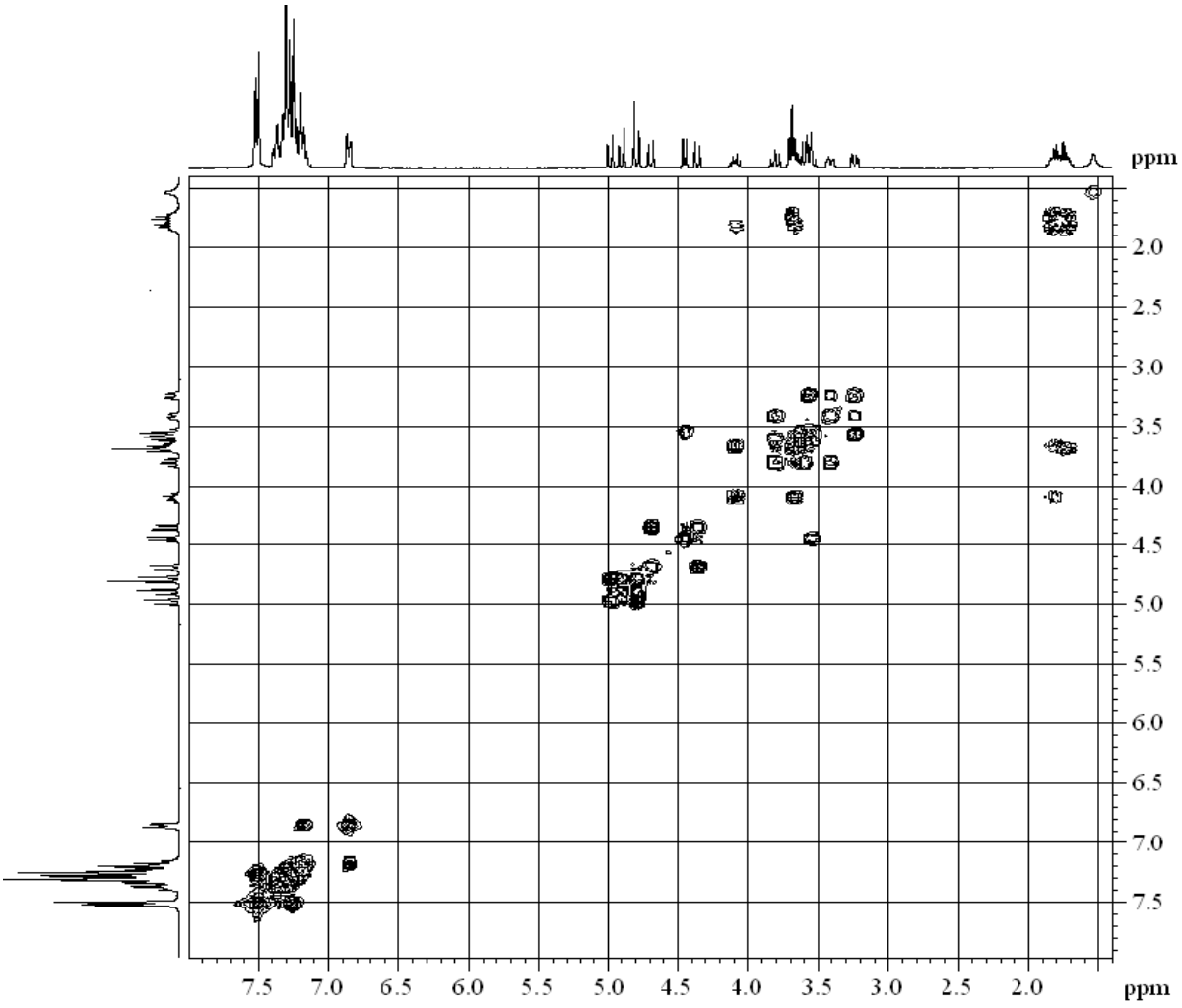
$CDCl_3$ at 300 MHz



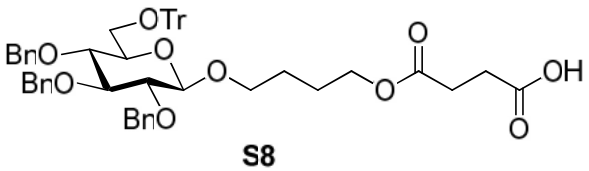
$CDCl_3$ at 75 MHz



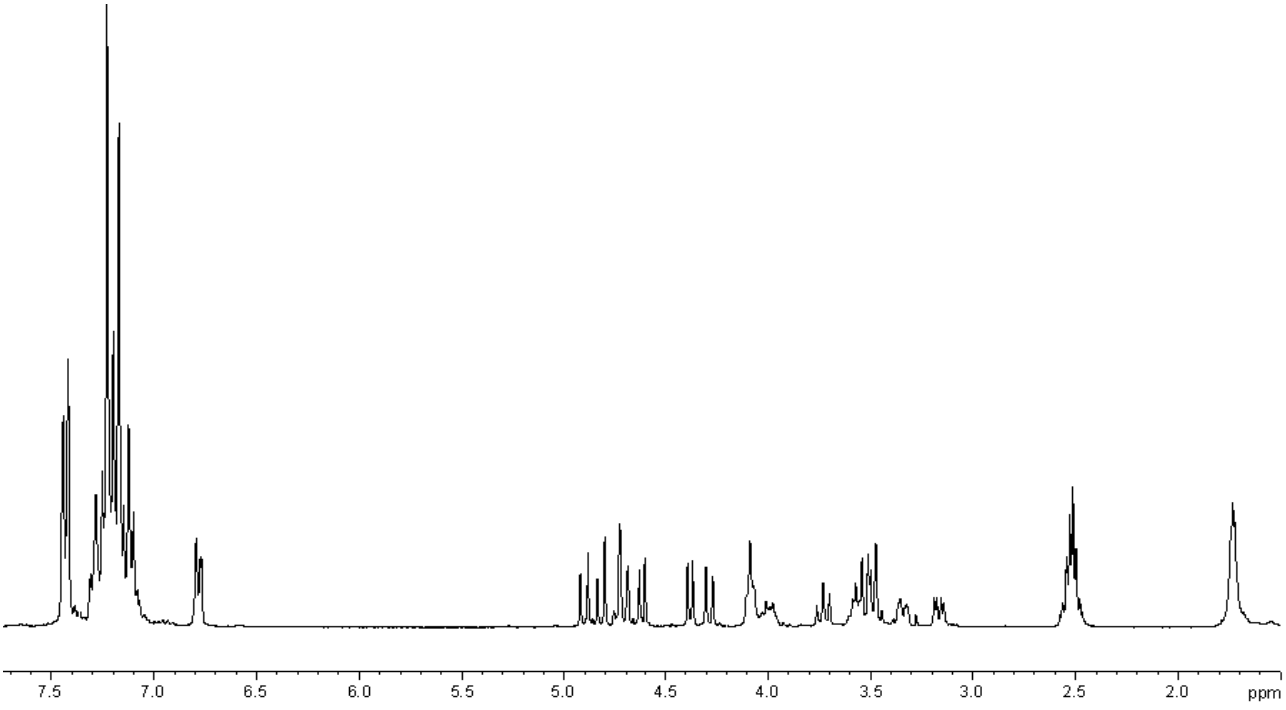
S7



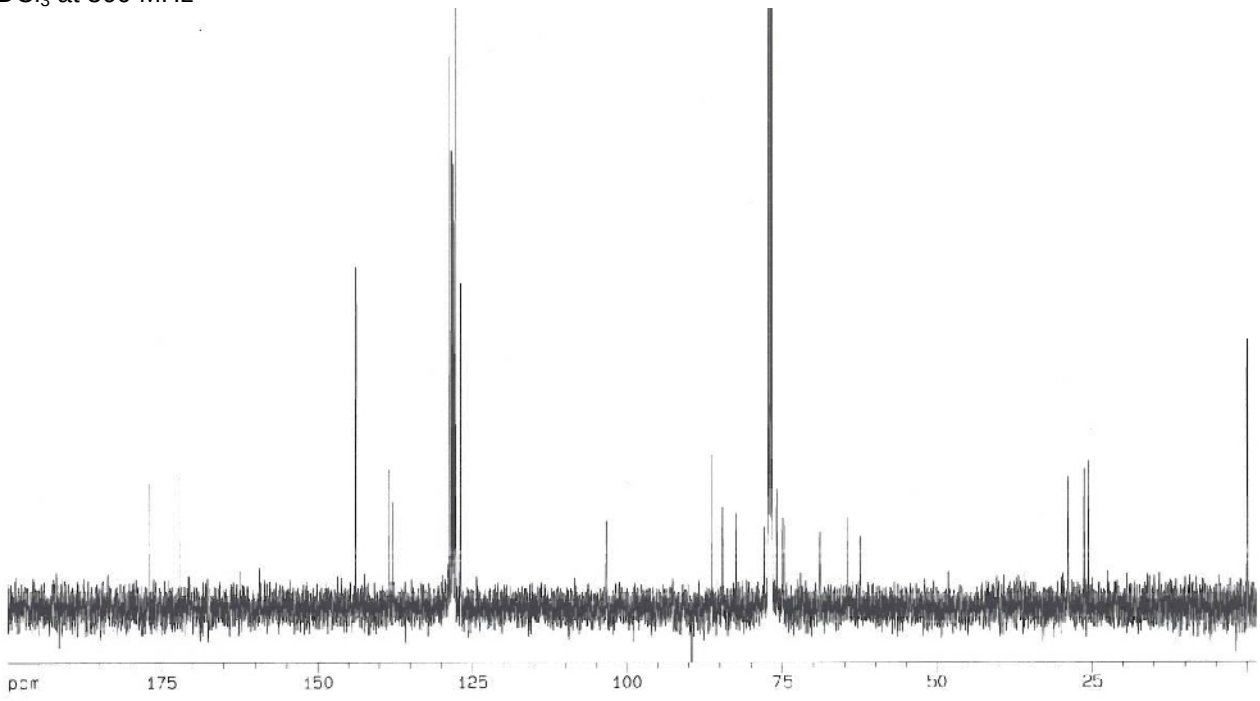
$CDCl_3$ at 300 MHz



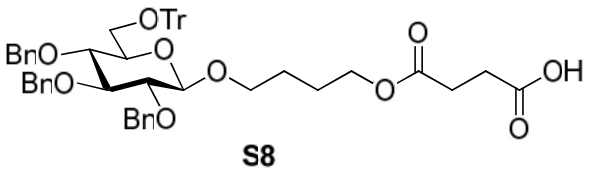
S8



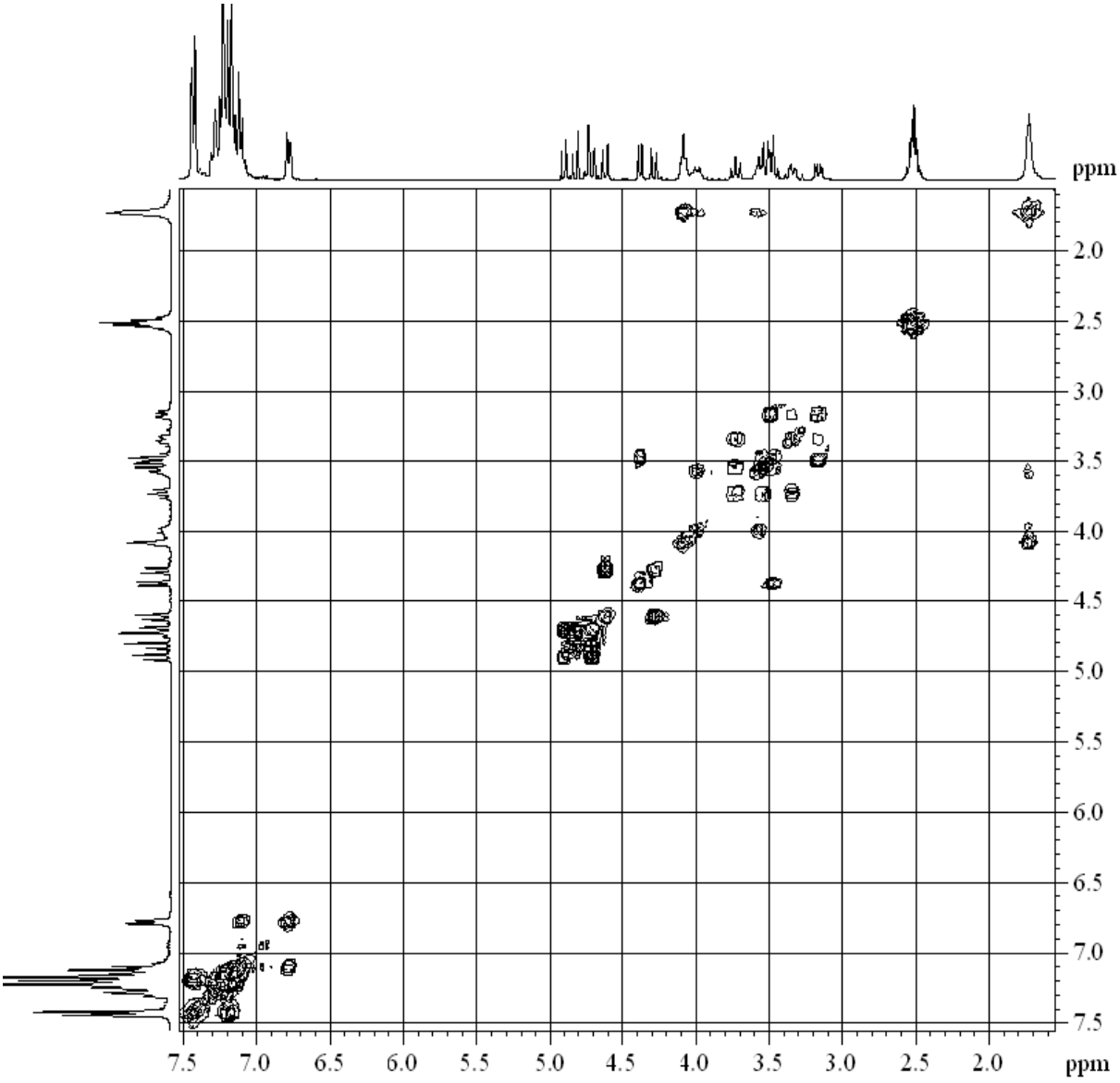
$CDCl_3$ at 300 MHz



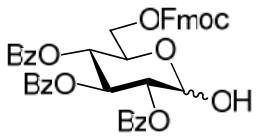
$CDCl_3$ at 75 MHz



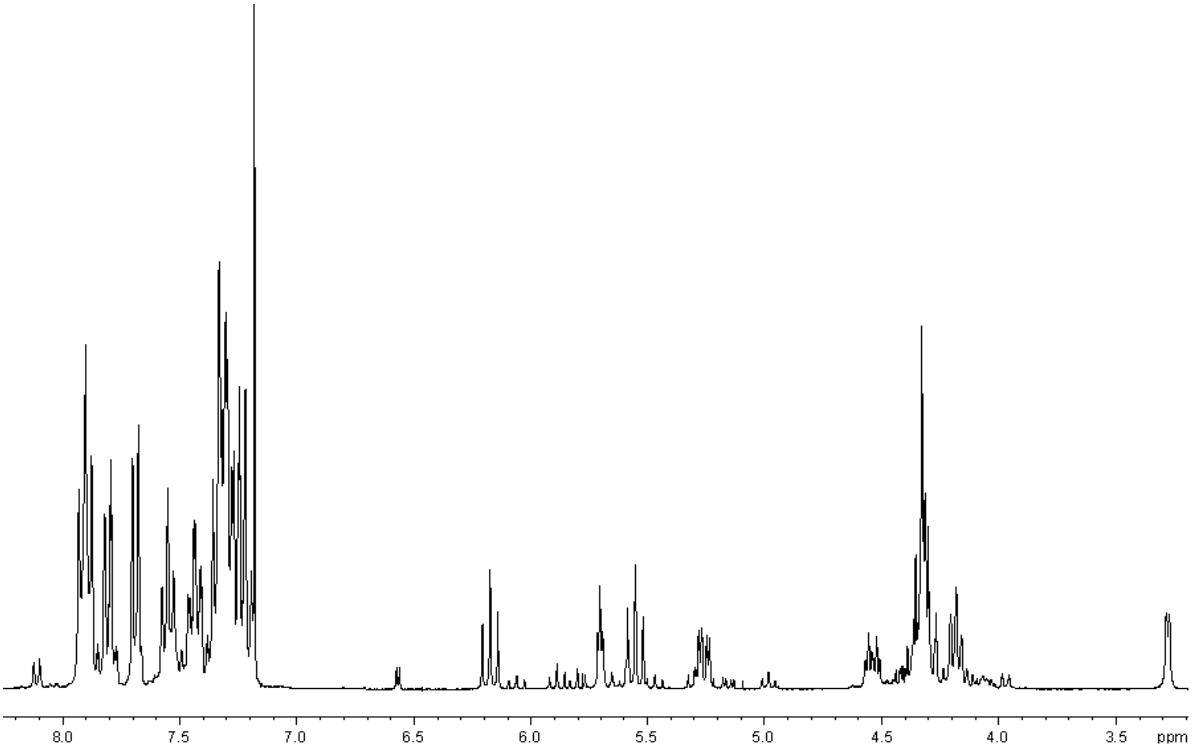
S8



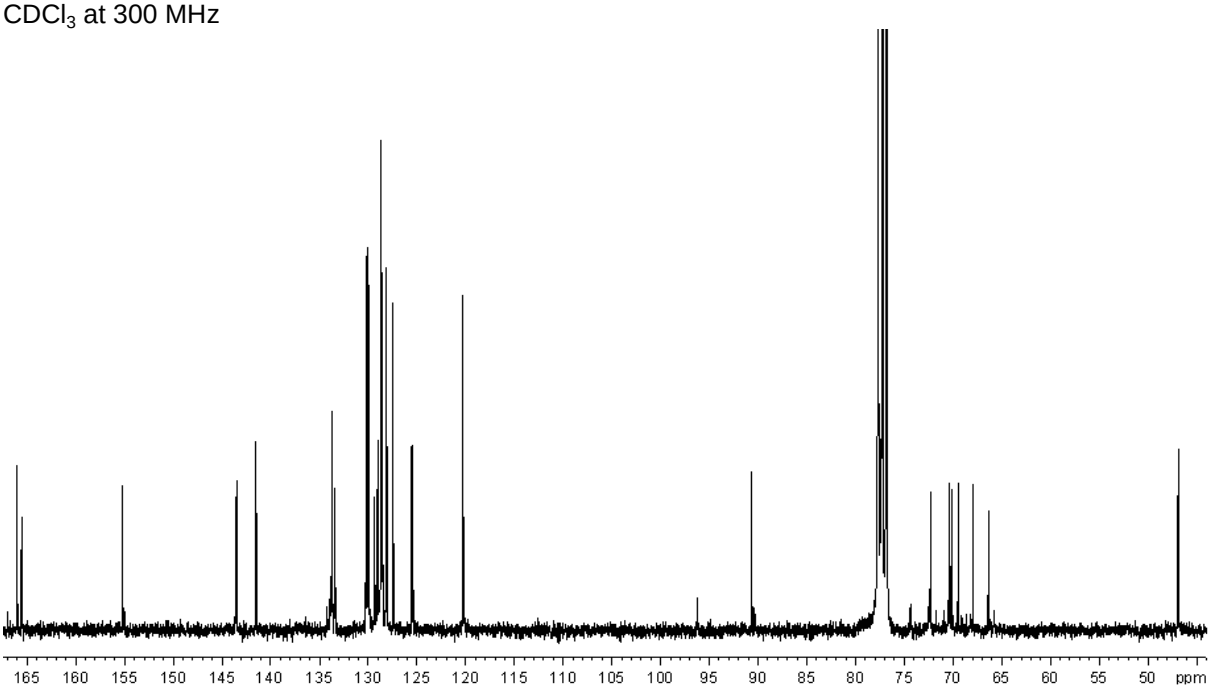
$CDCl_3$ at 300 MHz



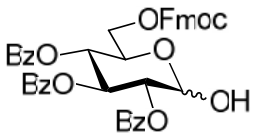
S10



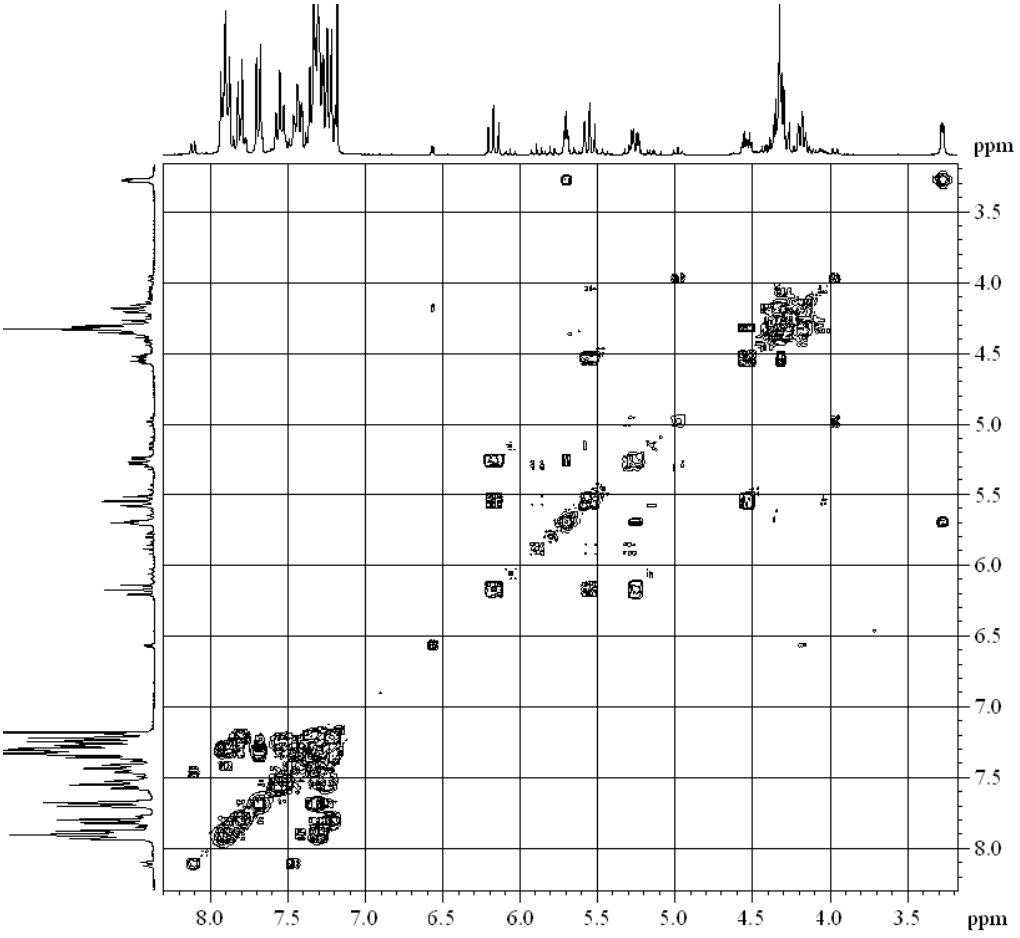
$CDCl_3$ at 300 MHz



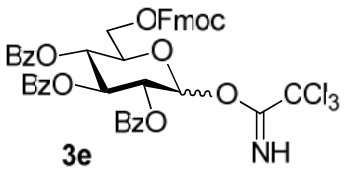
$CDCl_3$ at 75 MHz



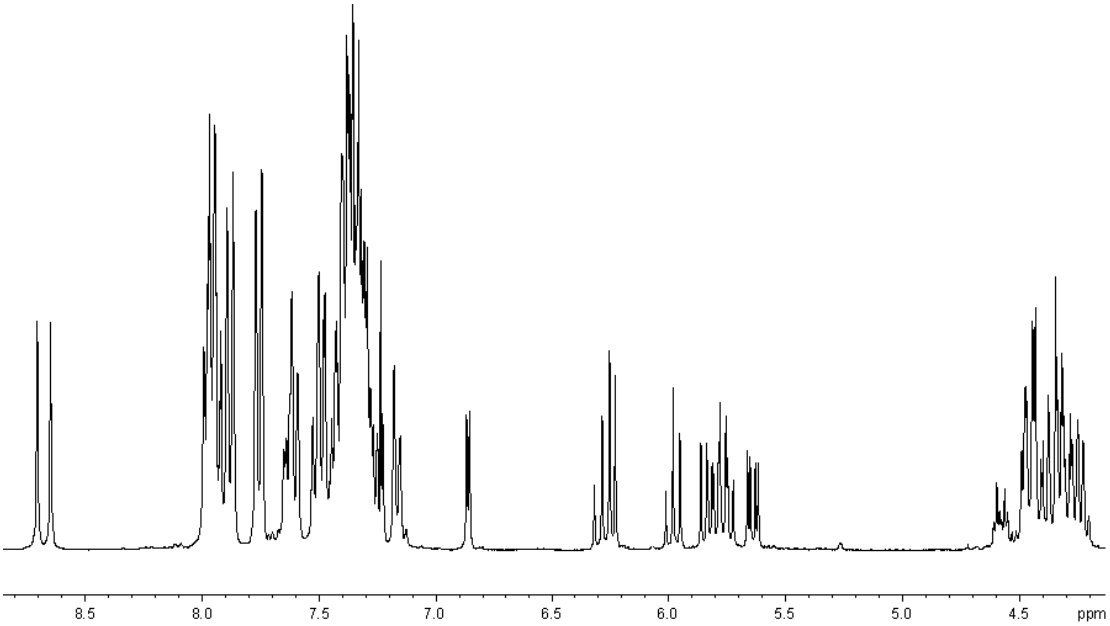
S10



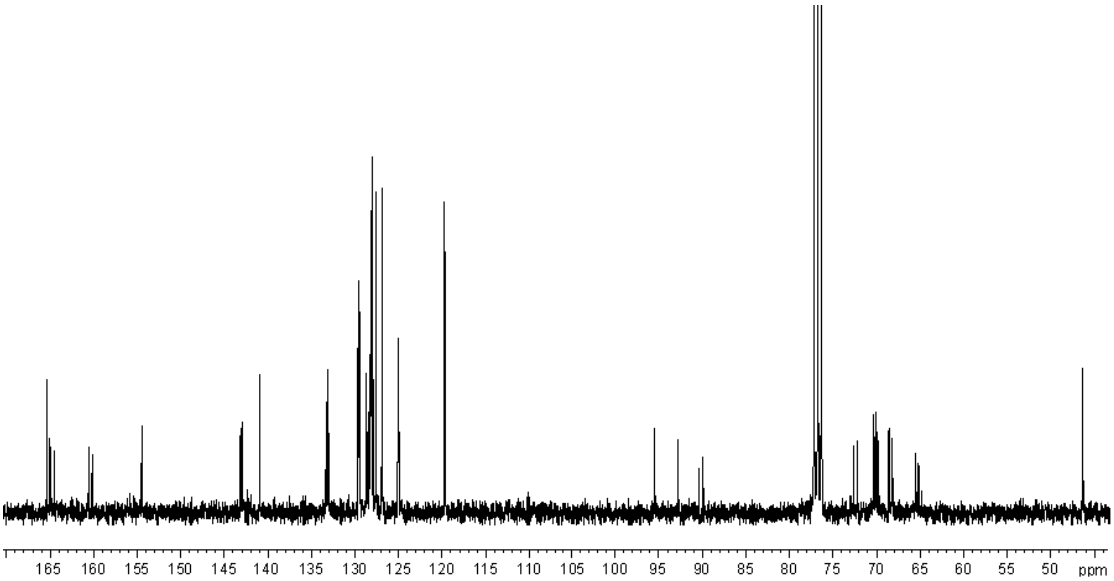
$CDCl_3$ at 300 MHz



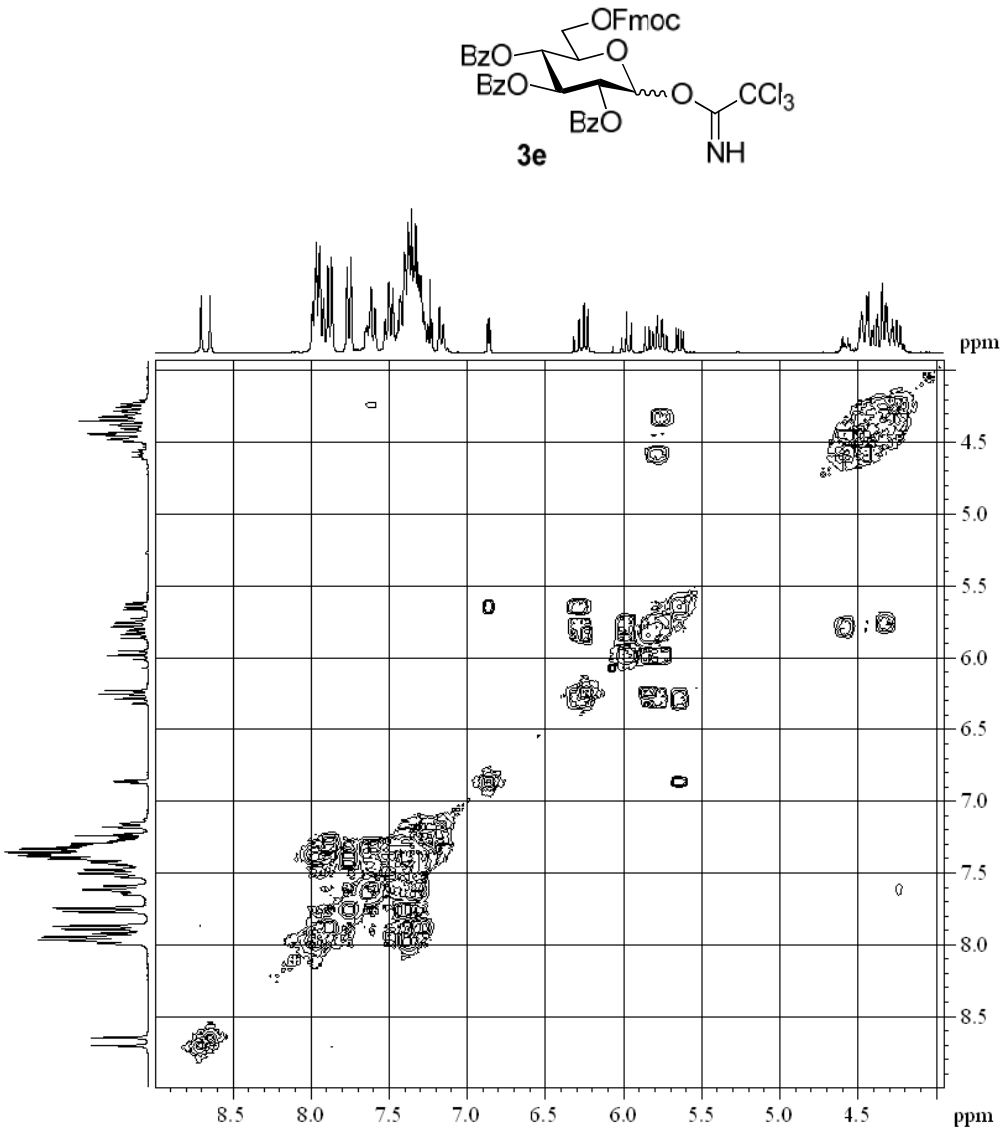
3e



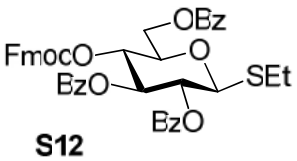
$CDCl_3$ at 300 MHz



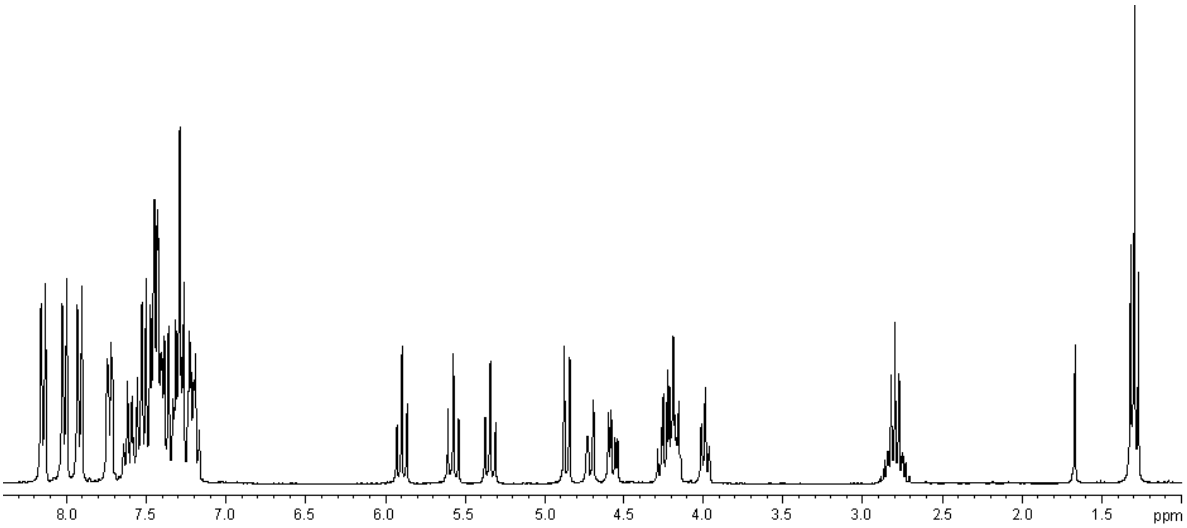
$CDCl_3$ at 75 MHz



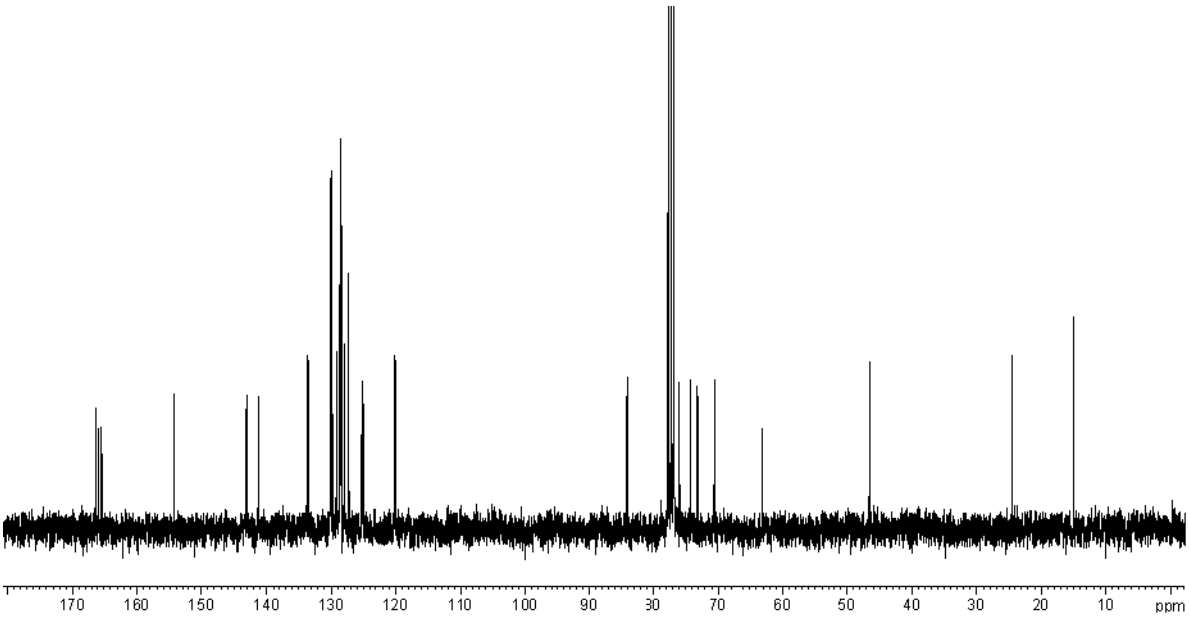
$CDCl_3$ at 300 MHz



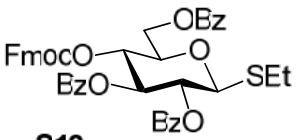
S12



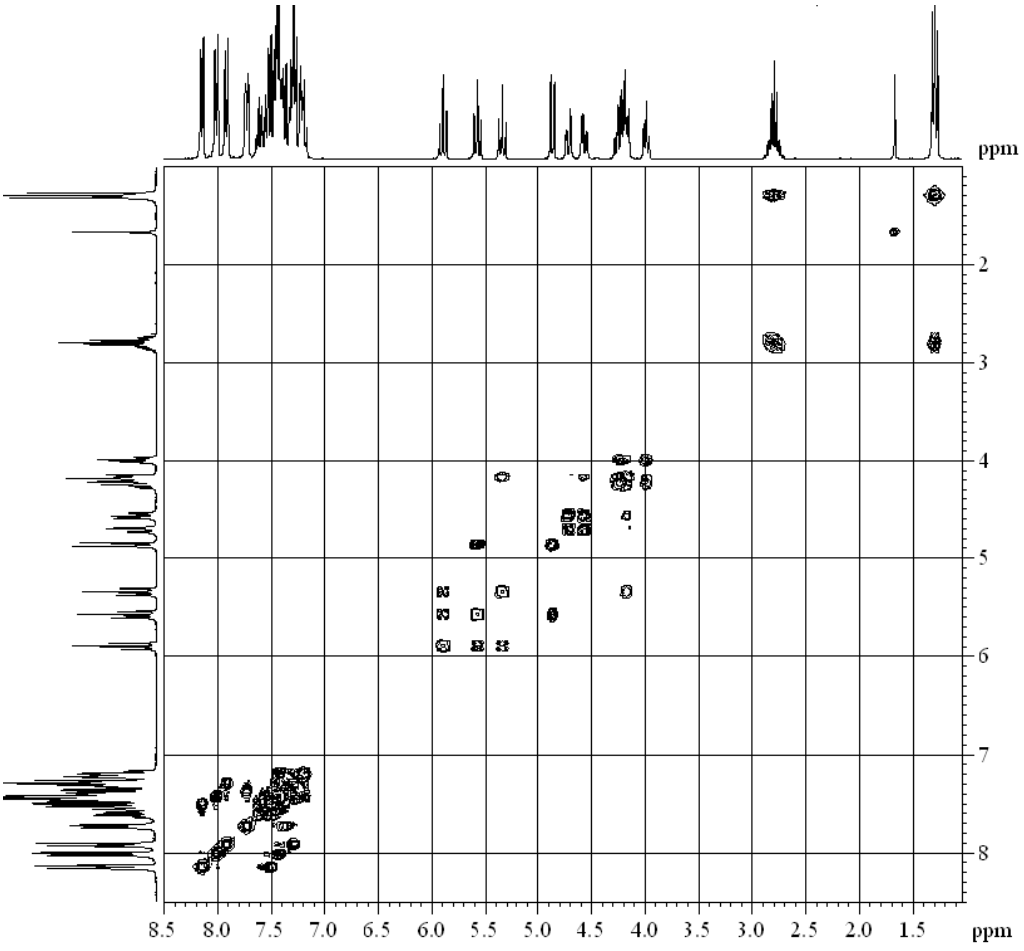
$CDCl_3$ at 300 MHz



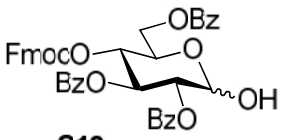
$CDCl_3$ at 75 MHz



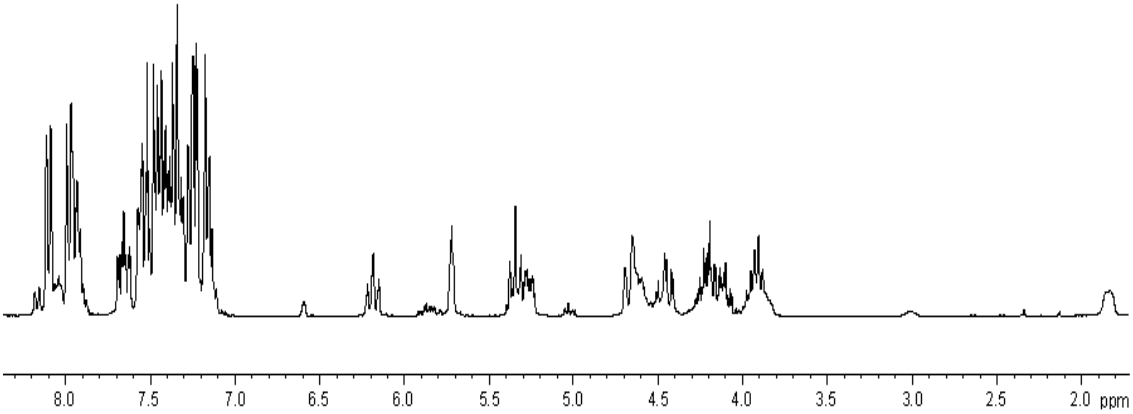
S12



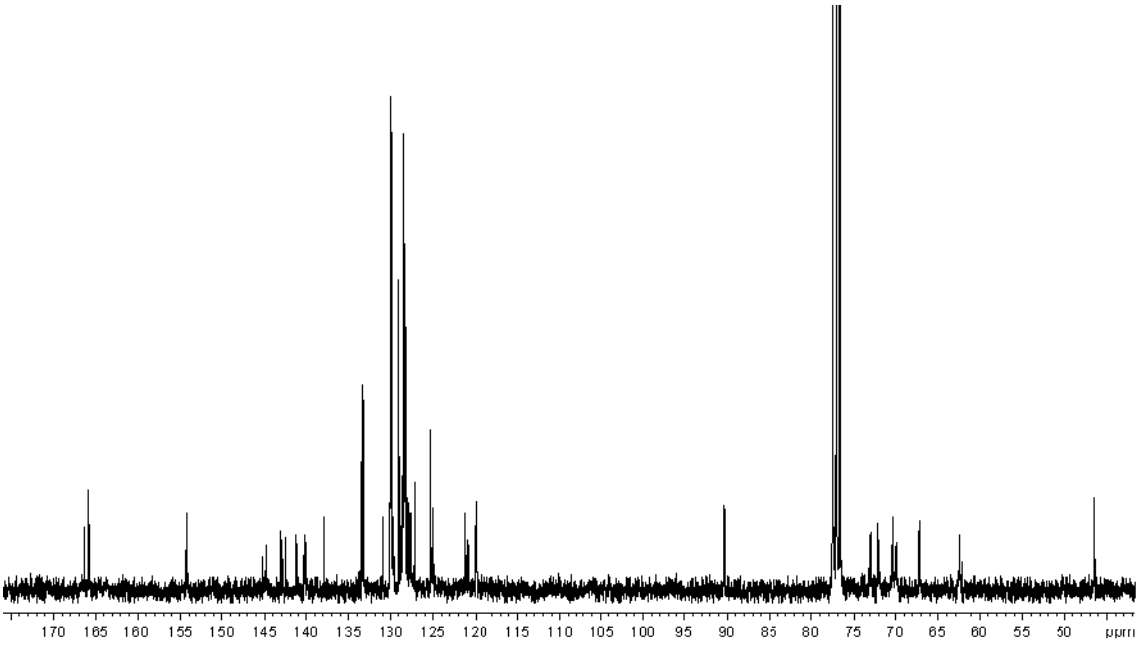
$CDCl_3$ at 300 MHz



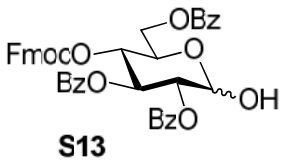
S13



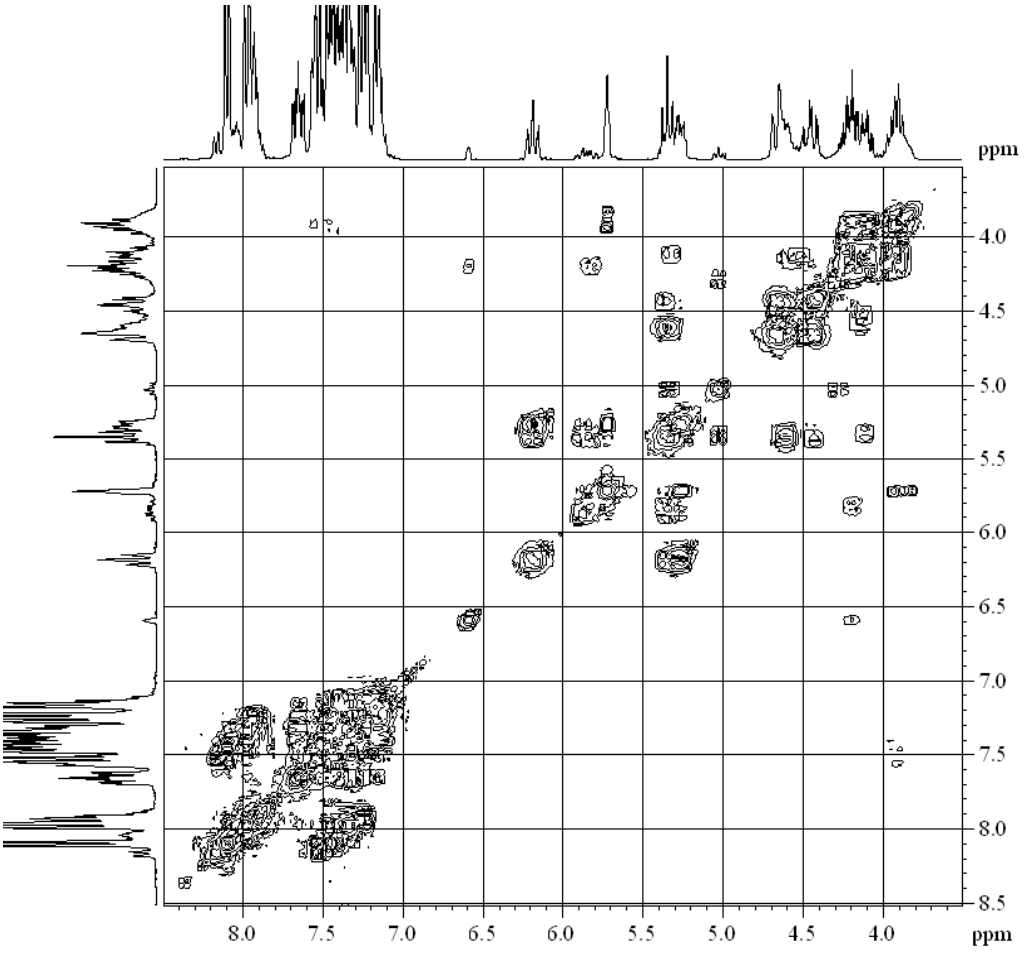
$CDCl_3$ at 300 MHz



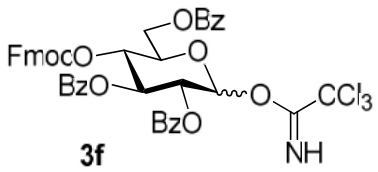
$CDCl_3$ at 75 MHz



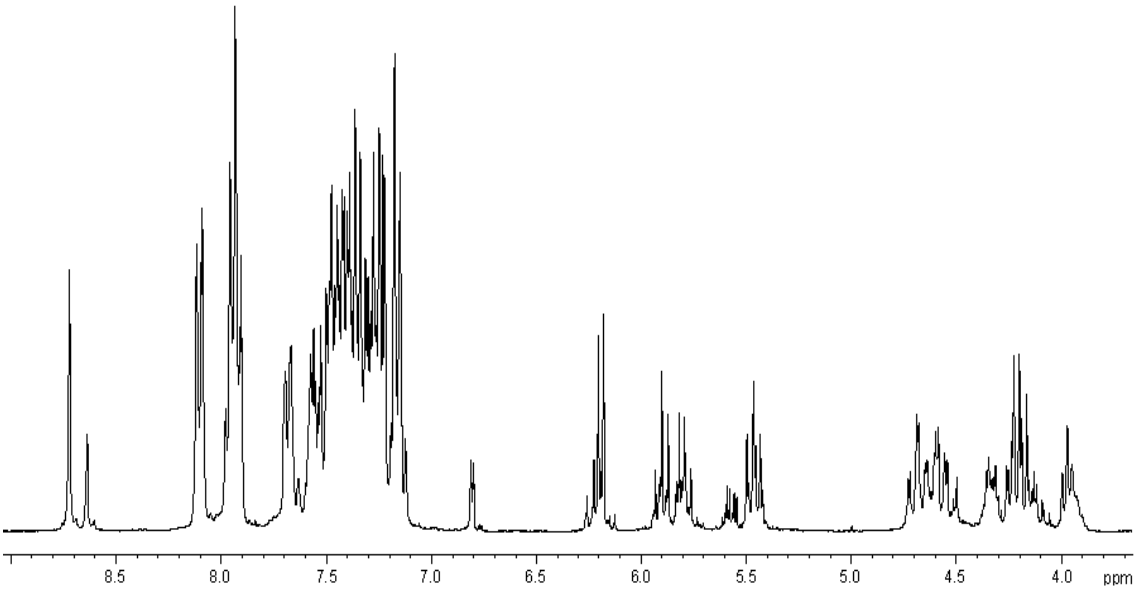
S13



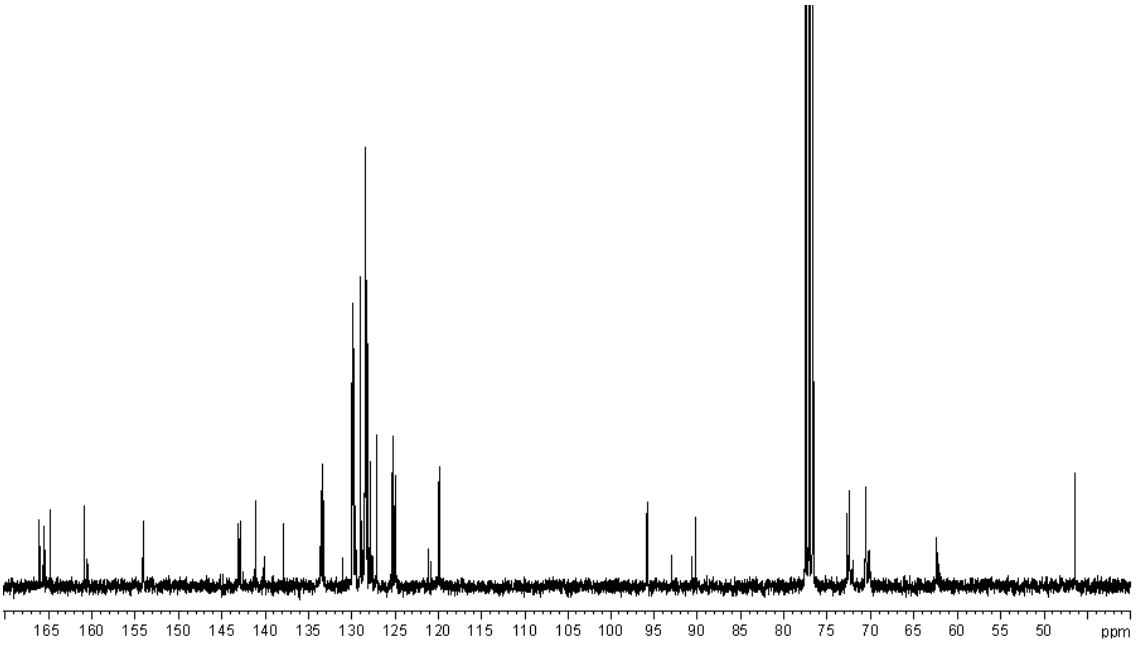
$CDCl_3$ at 300 MHz



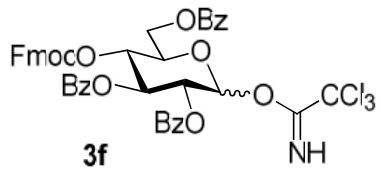
3f



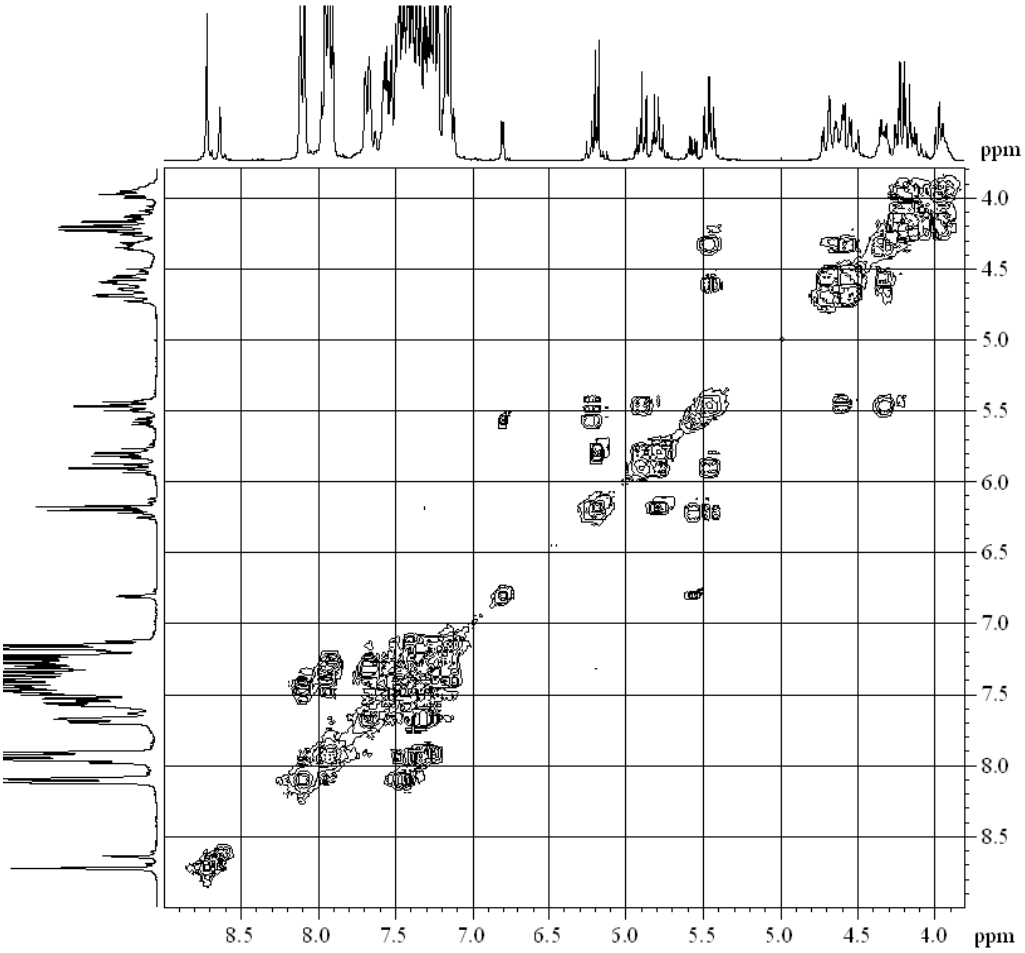
$CDCl_3$ at 300 MHz



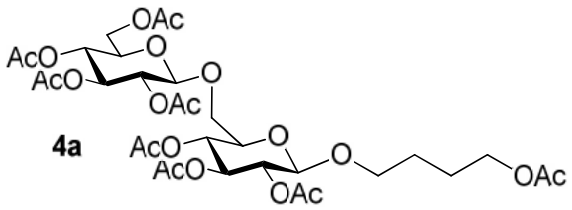
$CDCl_3$ at 75 MHz

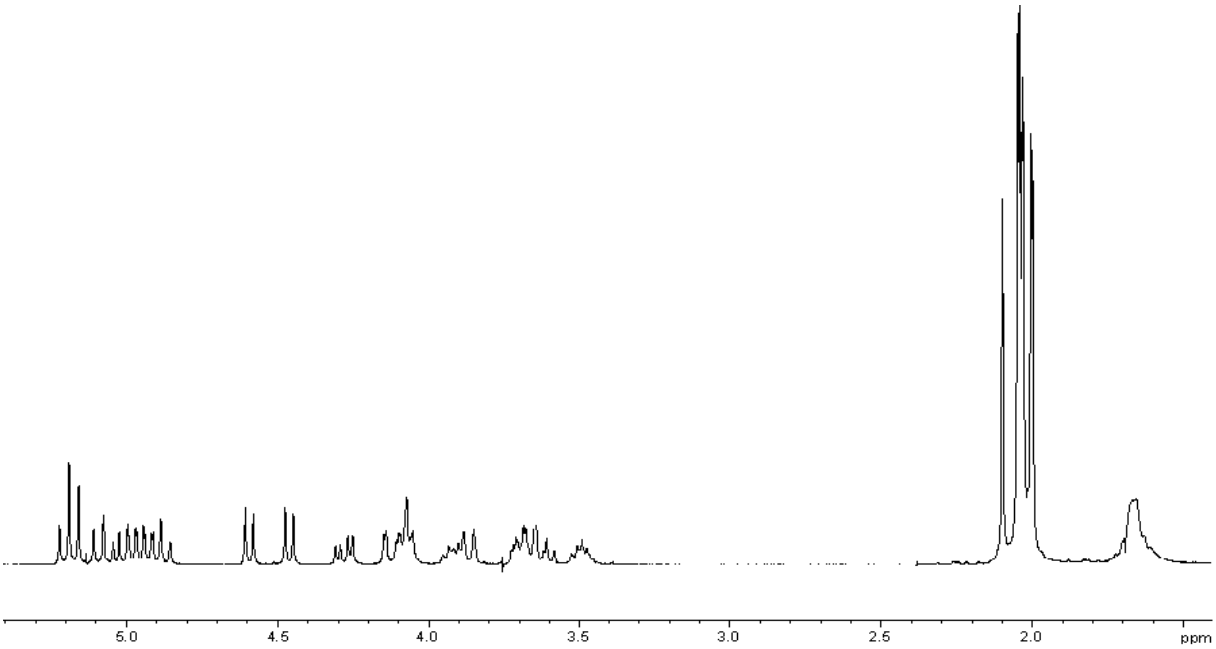


3f

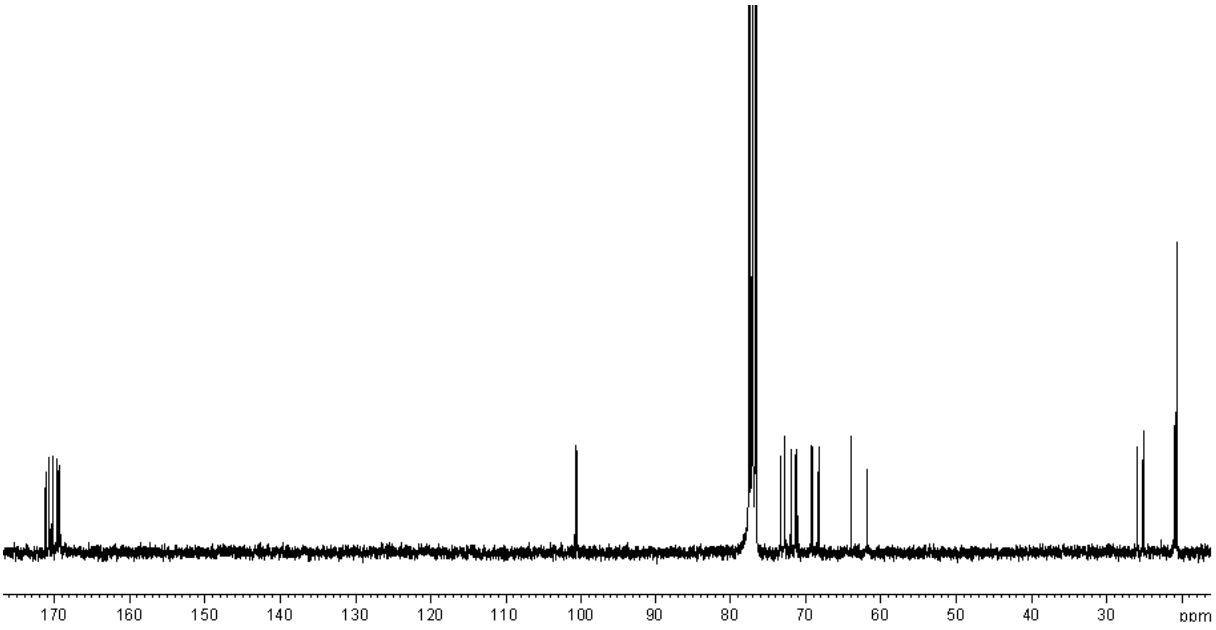


$CDCl_3$ at 300 MHz

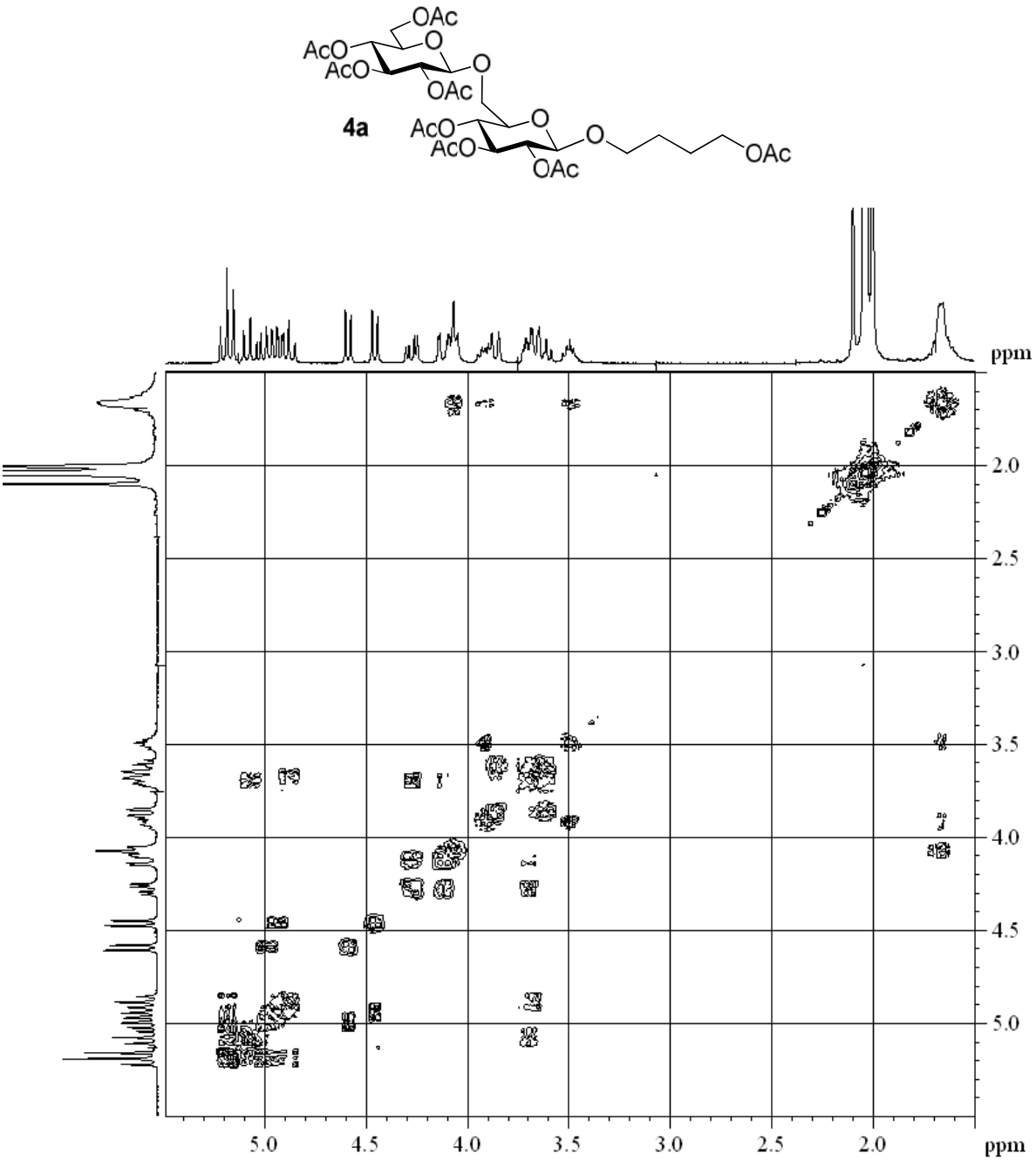




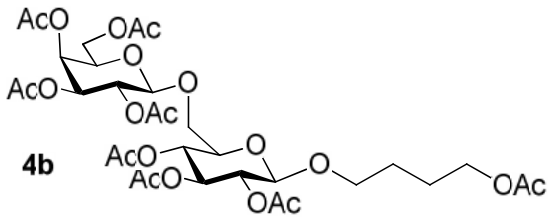
$CDCl_3$ at 300 MHz



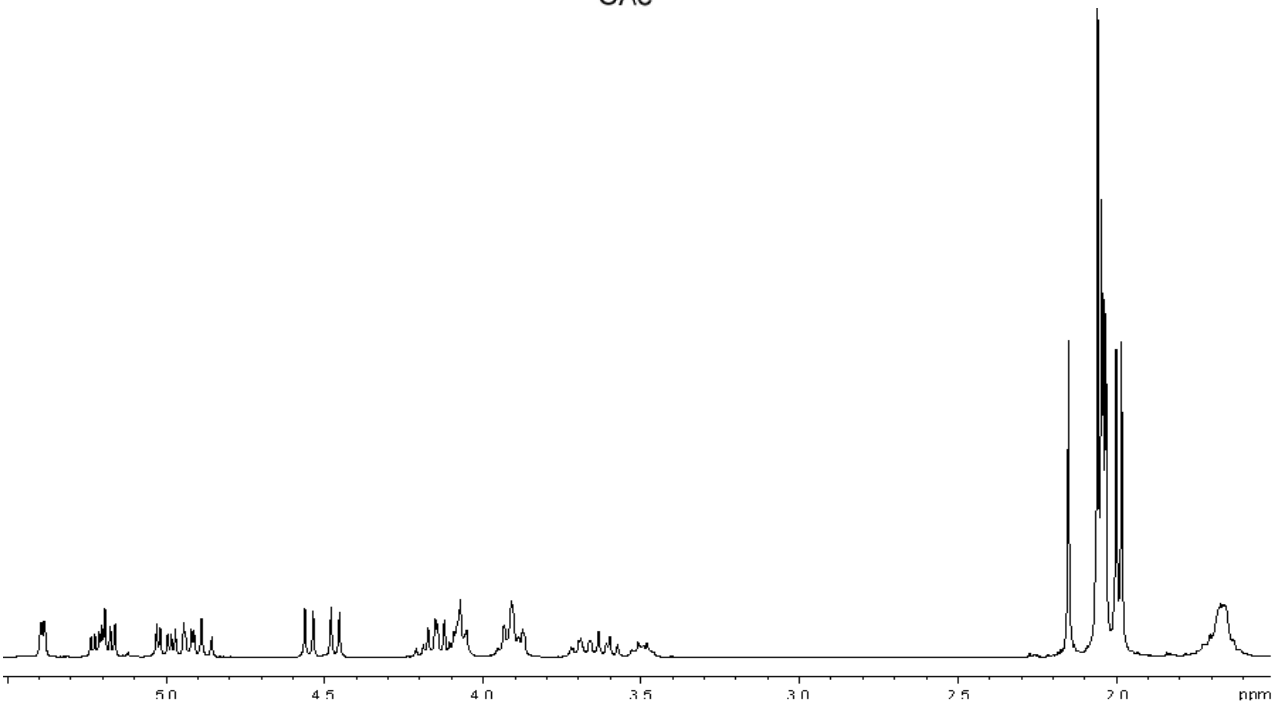
$CDCl_3$ at 75 MHz



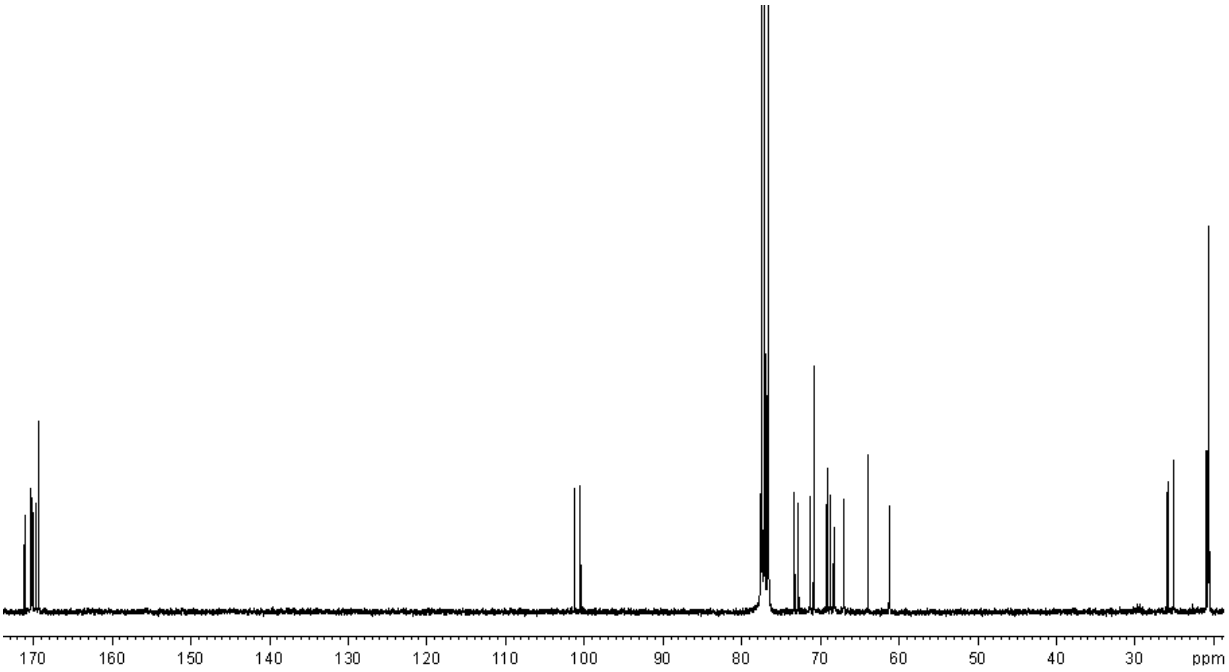
$CDCl_3$ at 300 MHz



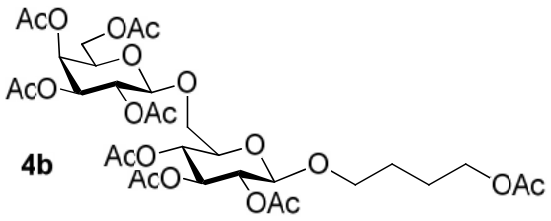
4b



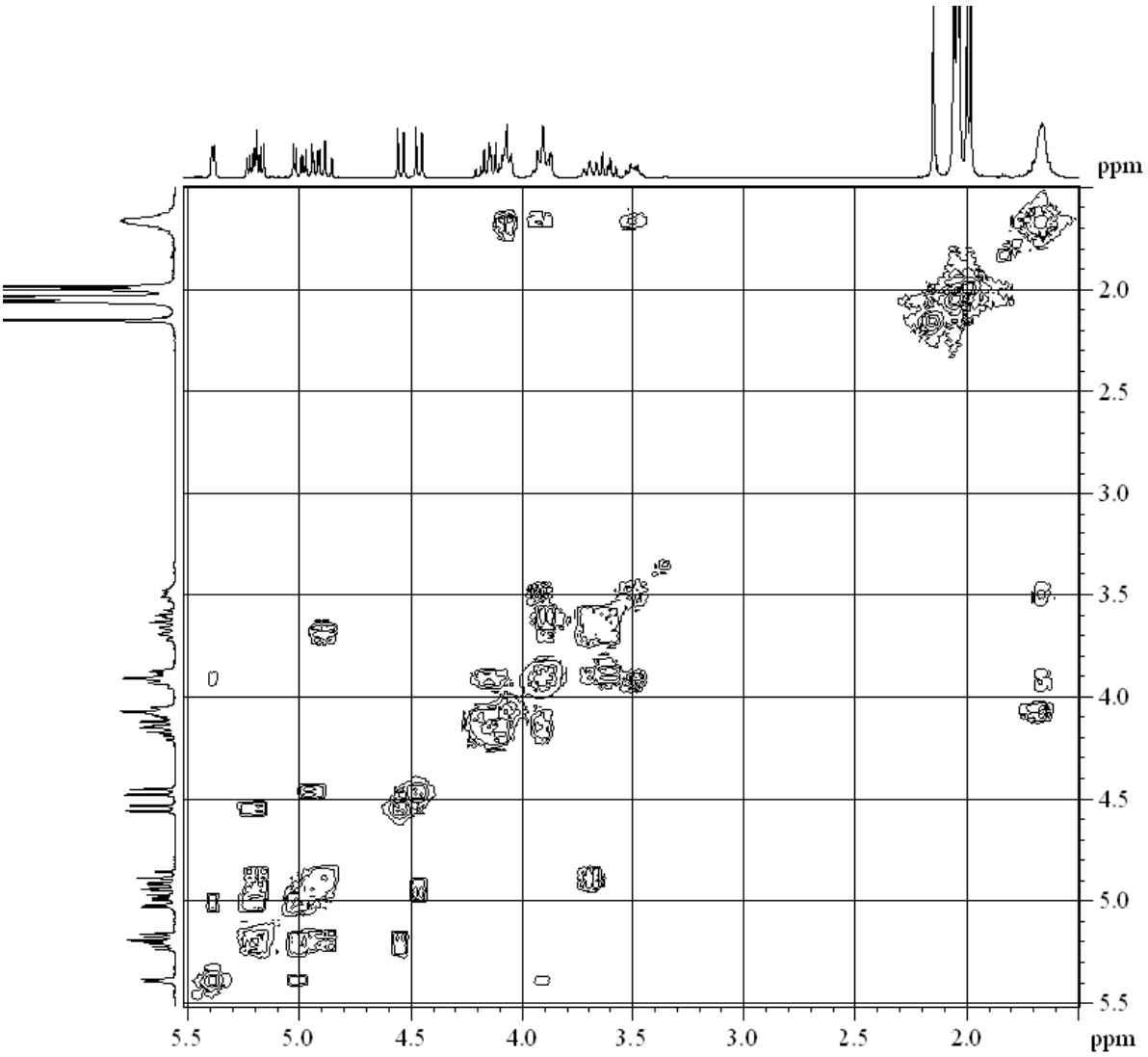
$CDCl_3$ at 300 MHz



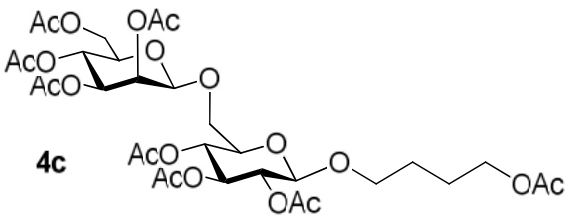
$CDCl_3$ at 75 MHz



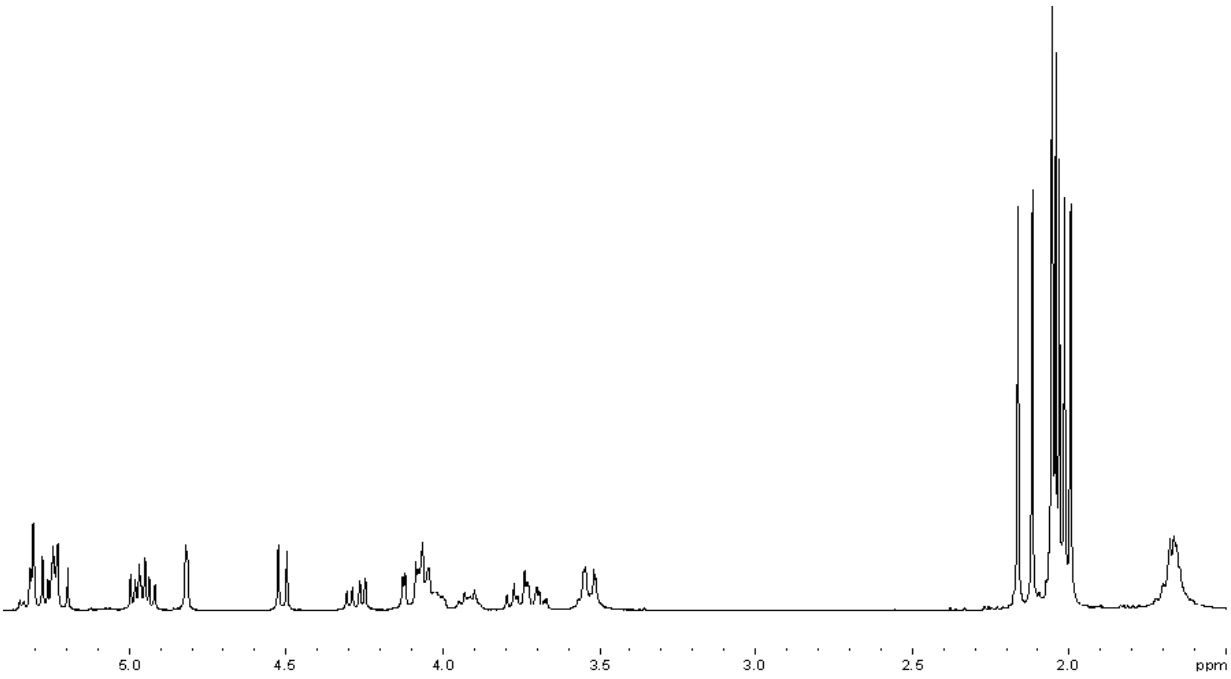
4b



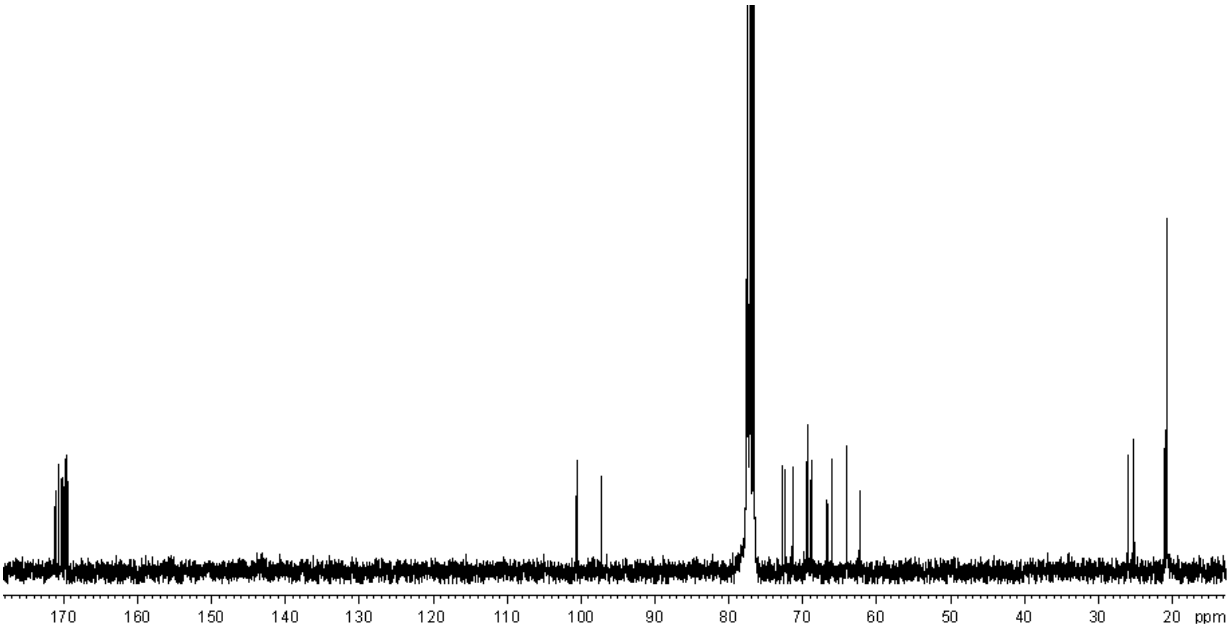
$CDCl_3$ at 300 MHz



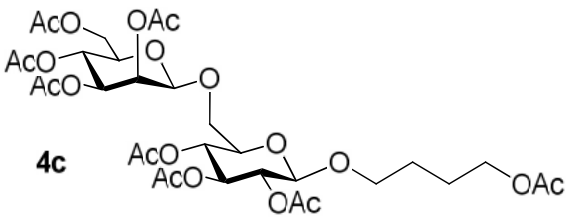
4c

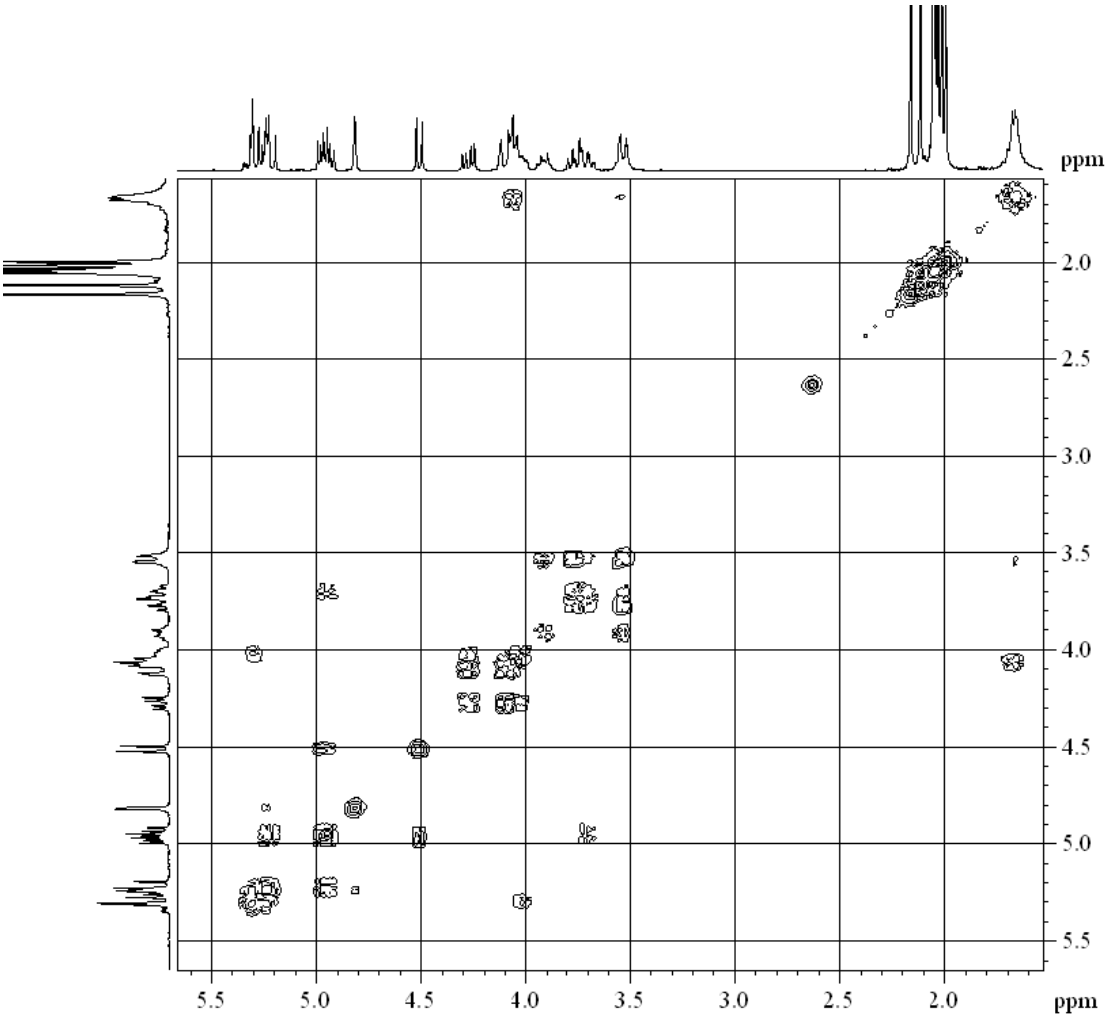


$CDCl_3$ at 300 MHz

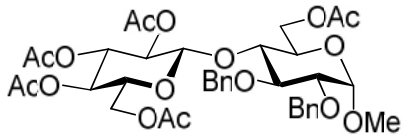


$CDCl_3$ at 75 MHz

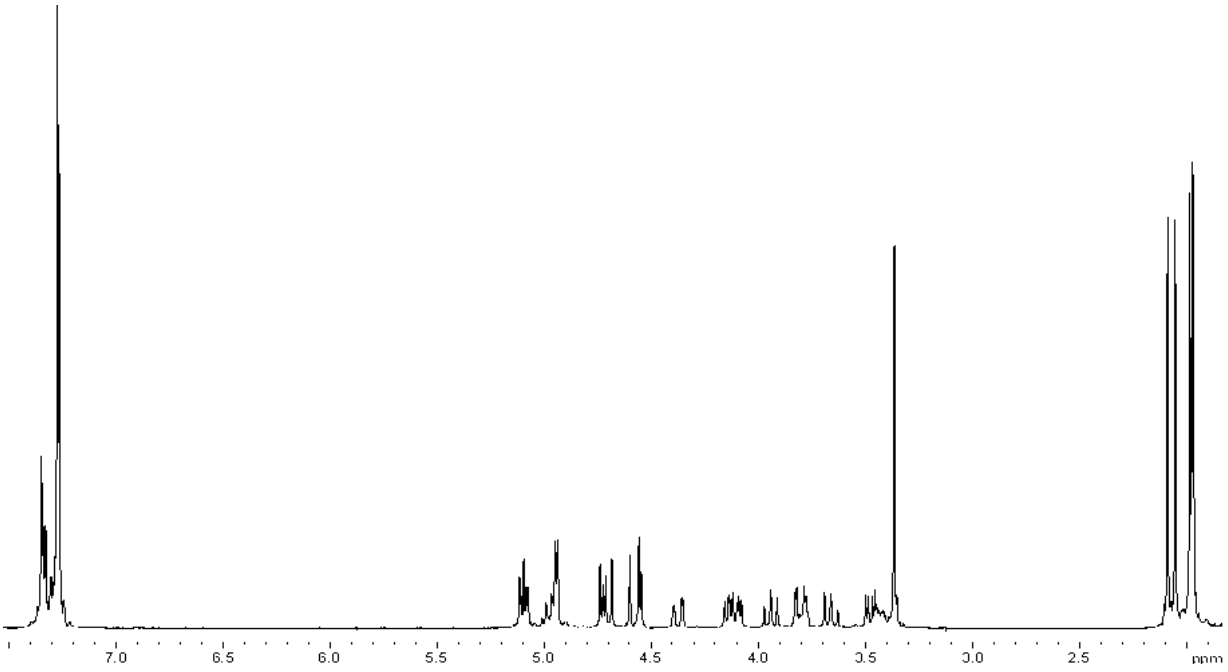




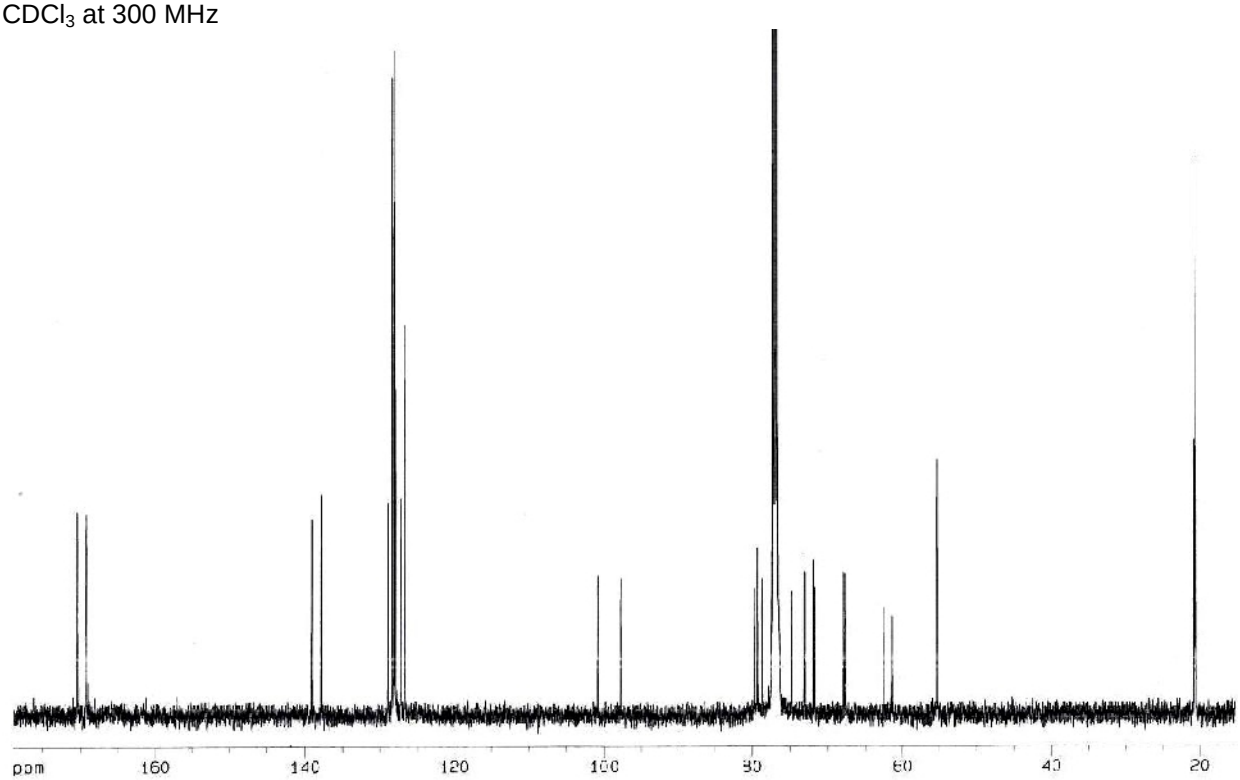
$CDCl_3$ at 300 MHz



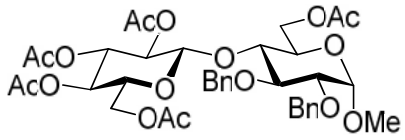
S15



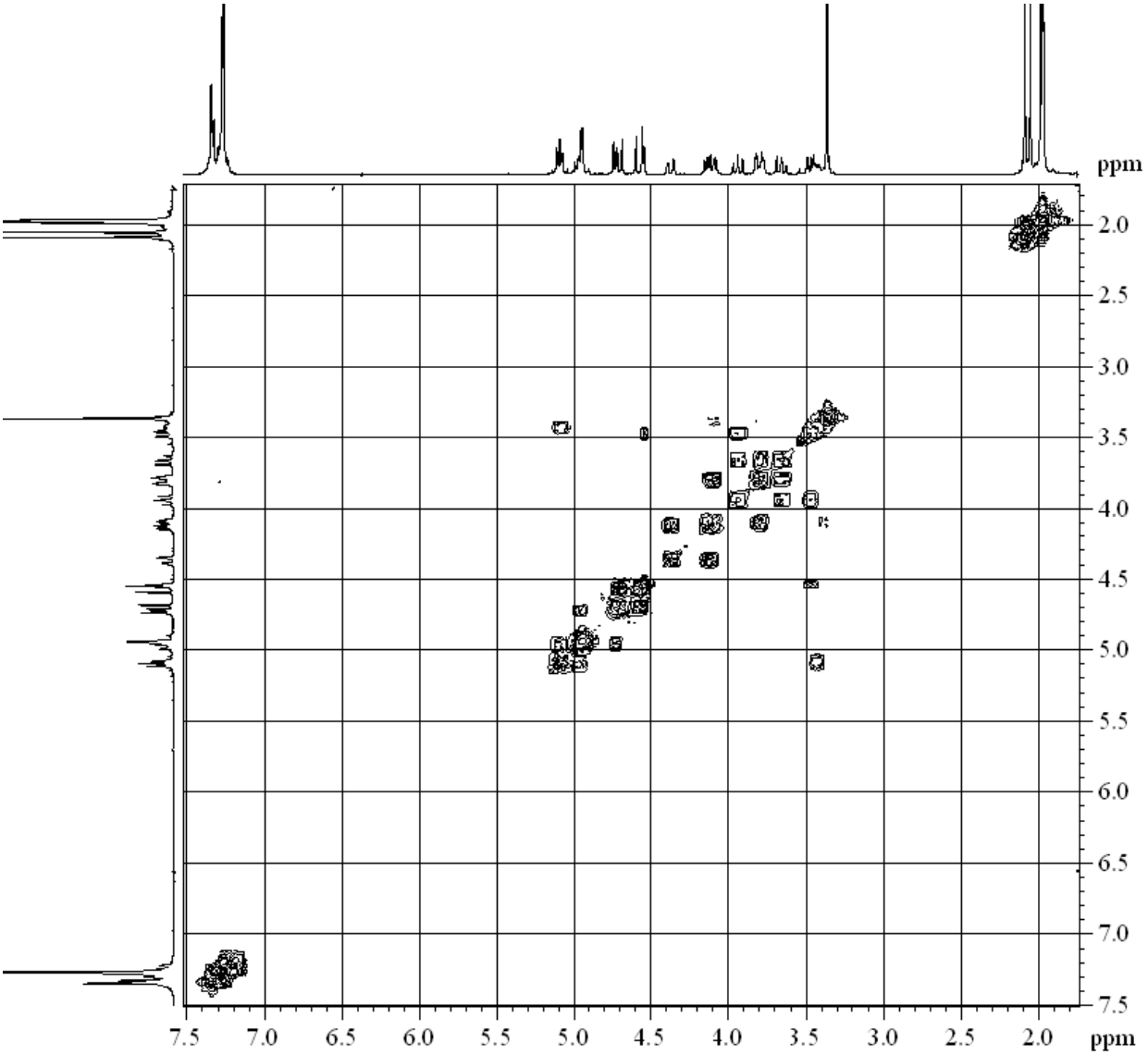
$CDCl_3$ at 300 MHz



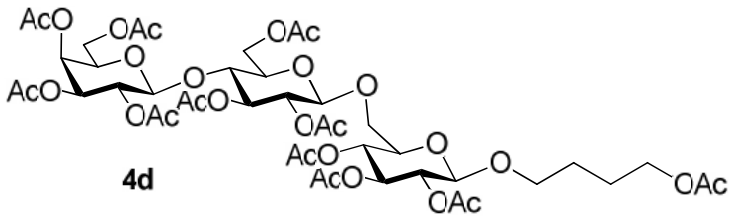
$CDCl_3$ at 125 MHz



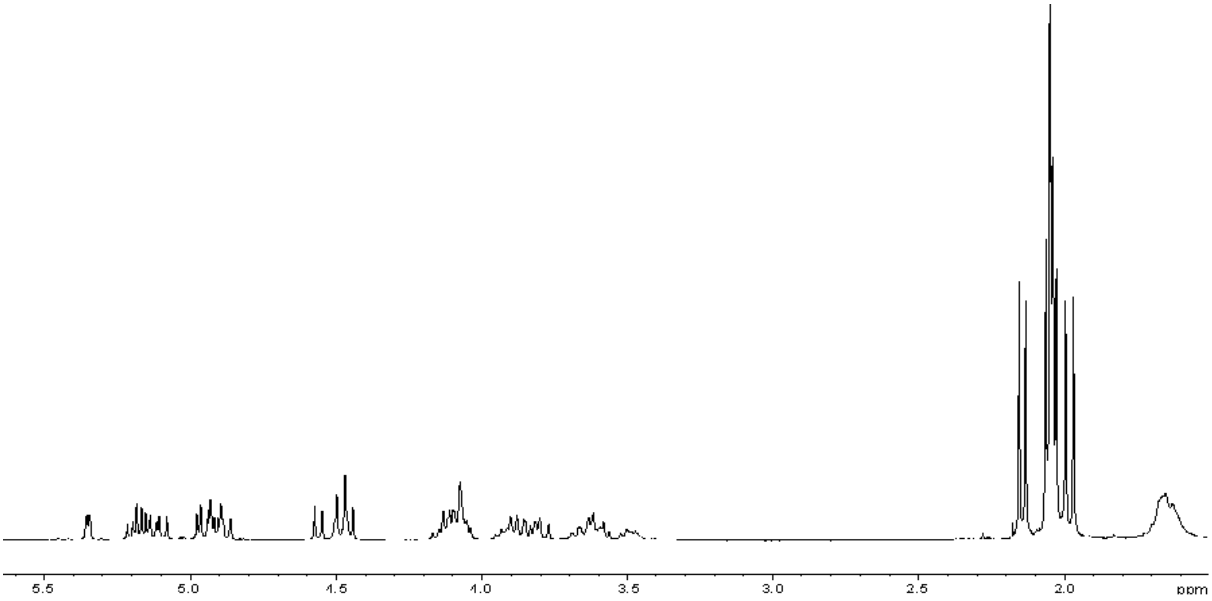
S15



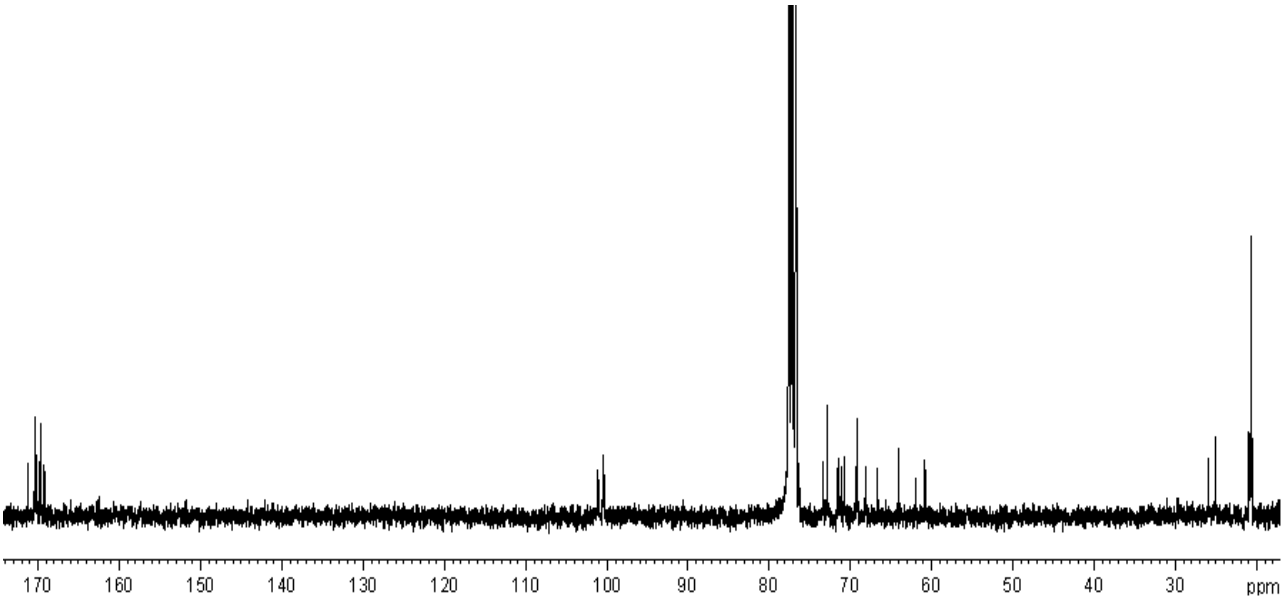
$CDCl_3$ at 300 MHz



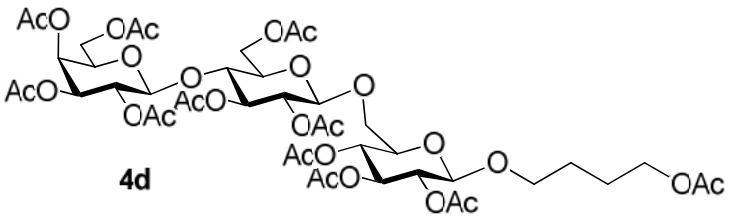
4d



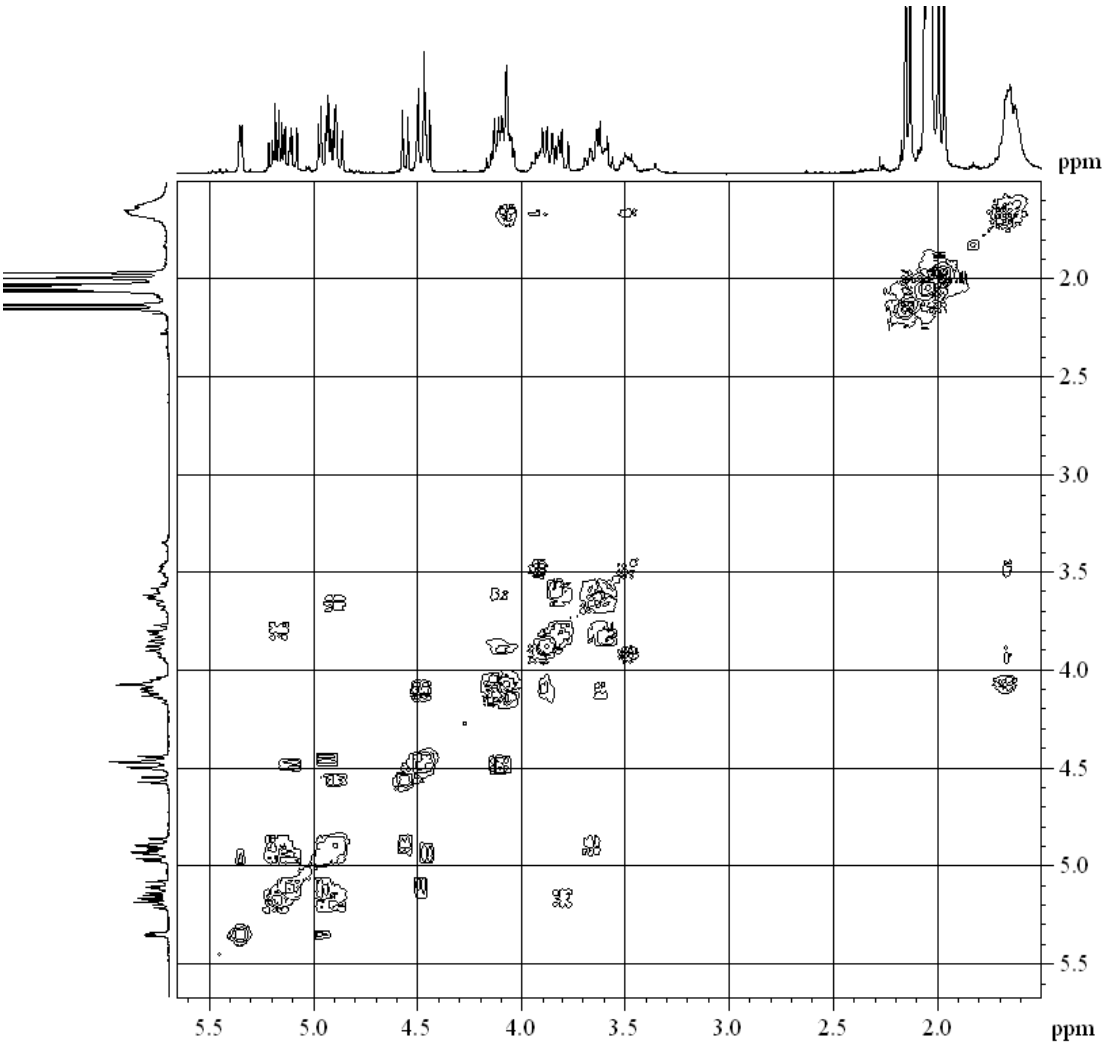
$CDCl_3$ at 300 MHz



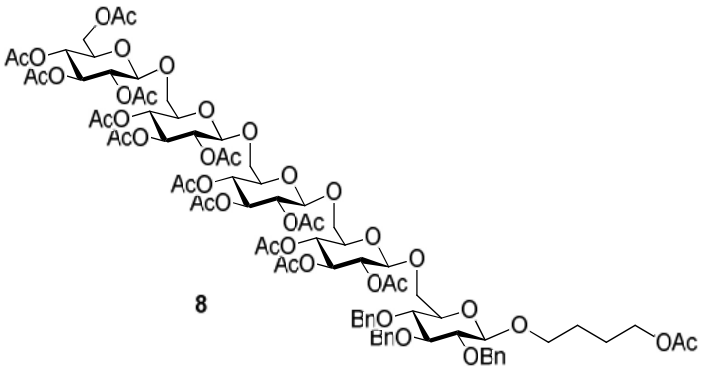
$CDCl_3$ at 75 MHz



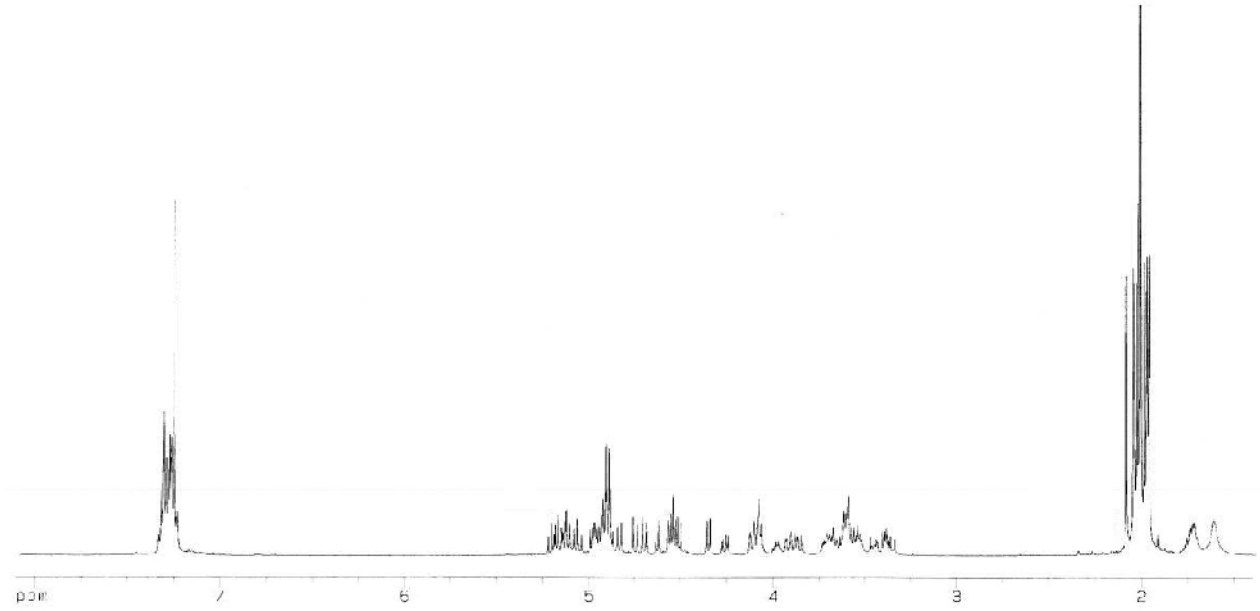
4d



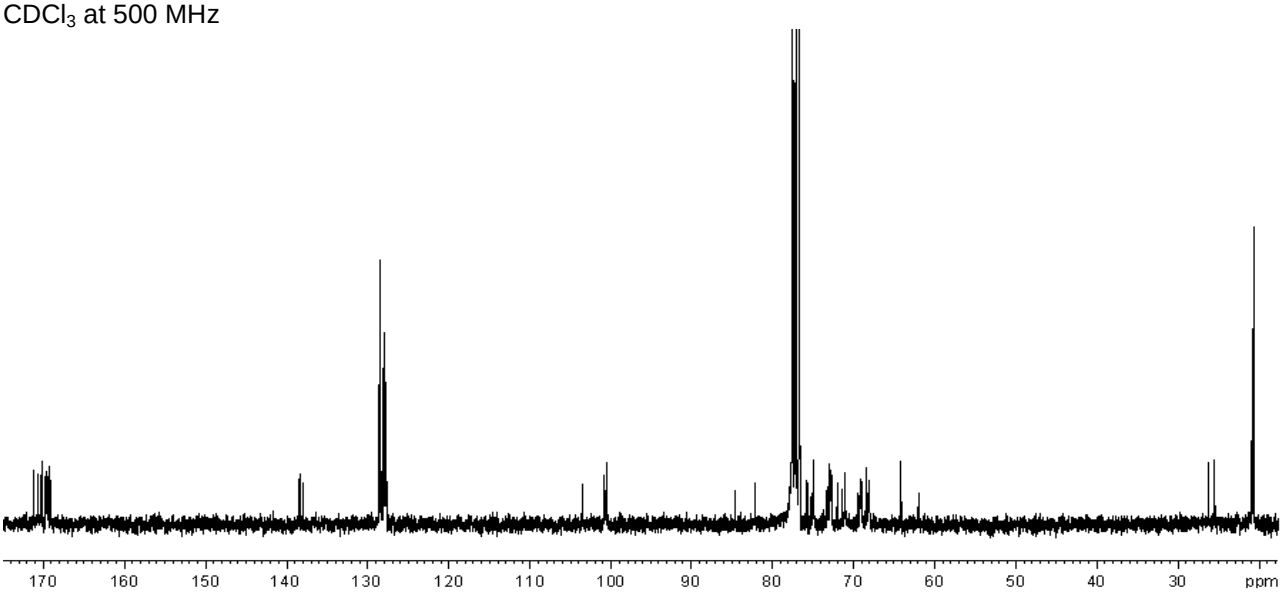
$CDCl_3$ at 300 MHz



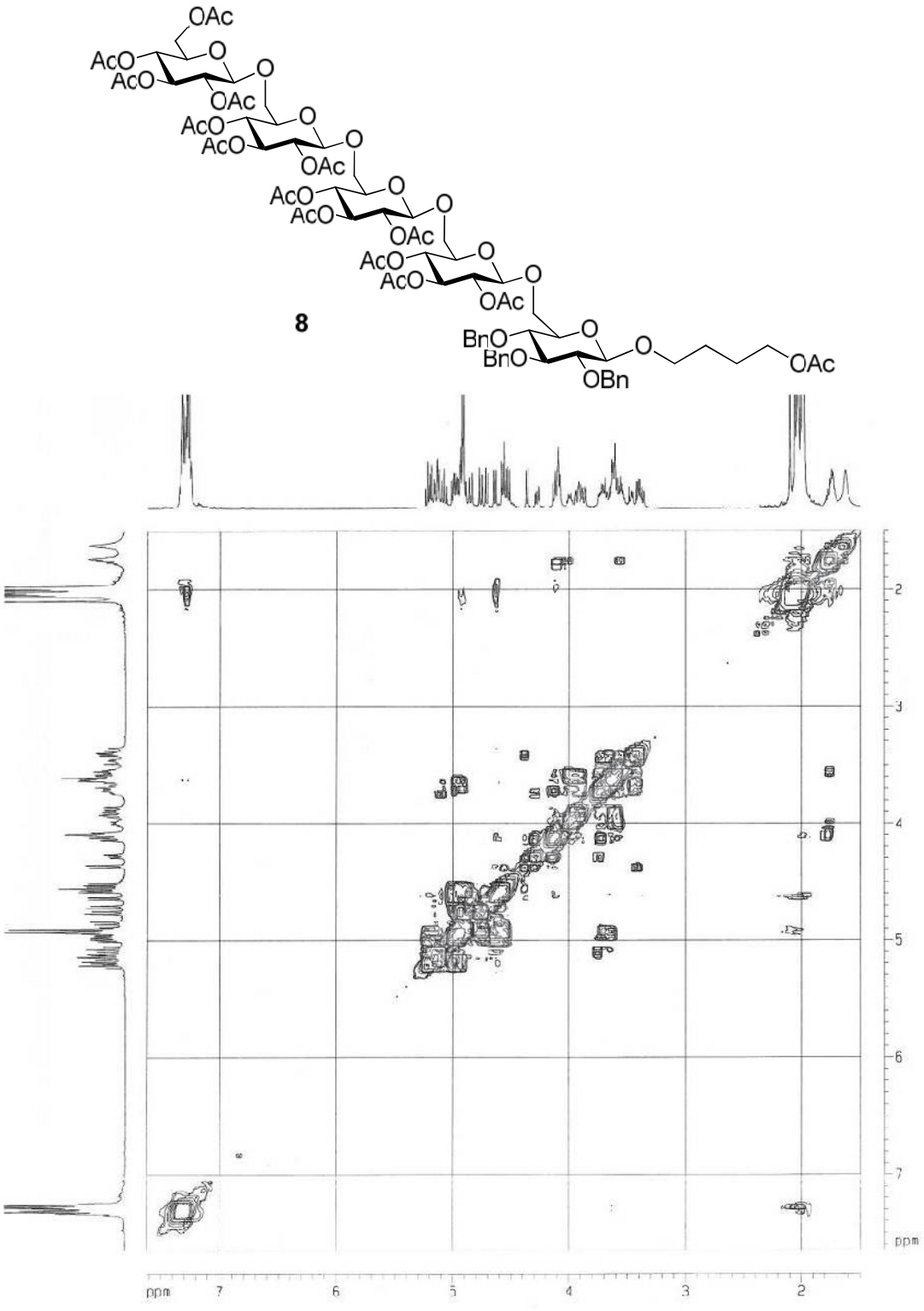
8



$CDCl_3$ at 500 MHz



$CDCl_3$ at 75 MHz



$CDCl_3$ at 500 MHz

References

(1) Lemieux, R. U. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolform, M. L., Eds.; Academic Press Inc.: New York and London, 1963; Vol. 2, p 226-228.
(2) Colonna, B.; Harding, V. D.; Nepogodiev, S. A.; Raymo, F. M.; Spencer, N.; Stoddart, J. F. *Chem. Eur. J.* **1998**, *4*, 1244-1254.
(3) Verduyn, R.; Douwes, M.; van der Klein, P. A. M.; Mösinger, E. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1993**, *49*, 7301-7316.
(4) Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343-.
(5) Rio, S.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1991**, *219*, 71-90.
(6) Bien, F.; Ziegler, T. *Tetrahedron: Asymmetry* **1998**, *9*, 781-790.
(7) Sandbhor, M. S.; Soya, N.; Albohy, A.; Zheng, R. B.; Cartmell, J.; Bundle, D. R.; Klassen, J. S.; Cairo, C. W. *Biochemistry* **2011**, *50*, 6753-6762.
(8) Majumdar, D.; Zhu, T.; Boons, G.-J. *Org. Lett.* **2003**, *5*, 3591-3594.
(9) Lefeber, D. J.; Arévalo, E. A.; Kamerling, J. P.; Vliegenthart, J. F. G. *Can. J. Chem.* **2002**, *80*, 76.
(10) Kaeothip, S.; Paranjape, G.; Terrill, S. E.; Bongat, A. F. G.; Udan, M. L. D.; Kamkhachorn, T.; Johnson, H. L.; Nichols, M. R.; Demchenko, A. V. *RSC Adv.* **2011**, *1*, 83-92.