Supplementary Information

Chitosan-saccharide conjugates for eradication of *Pseudomonas aeruginosa* biofilms

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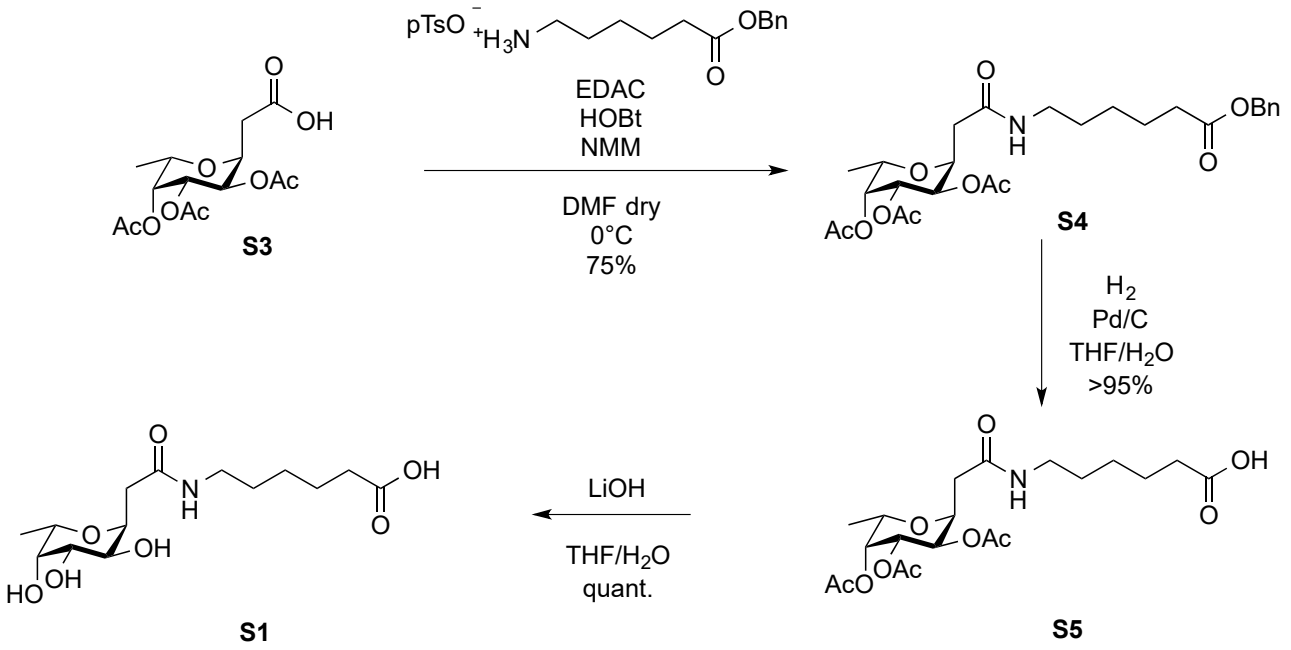
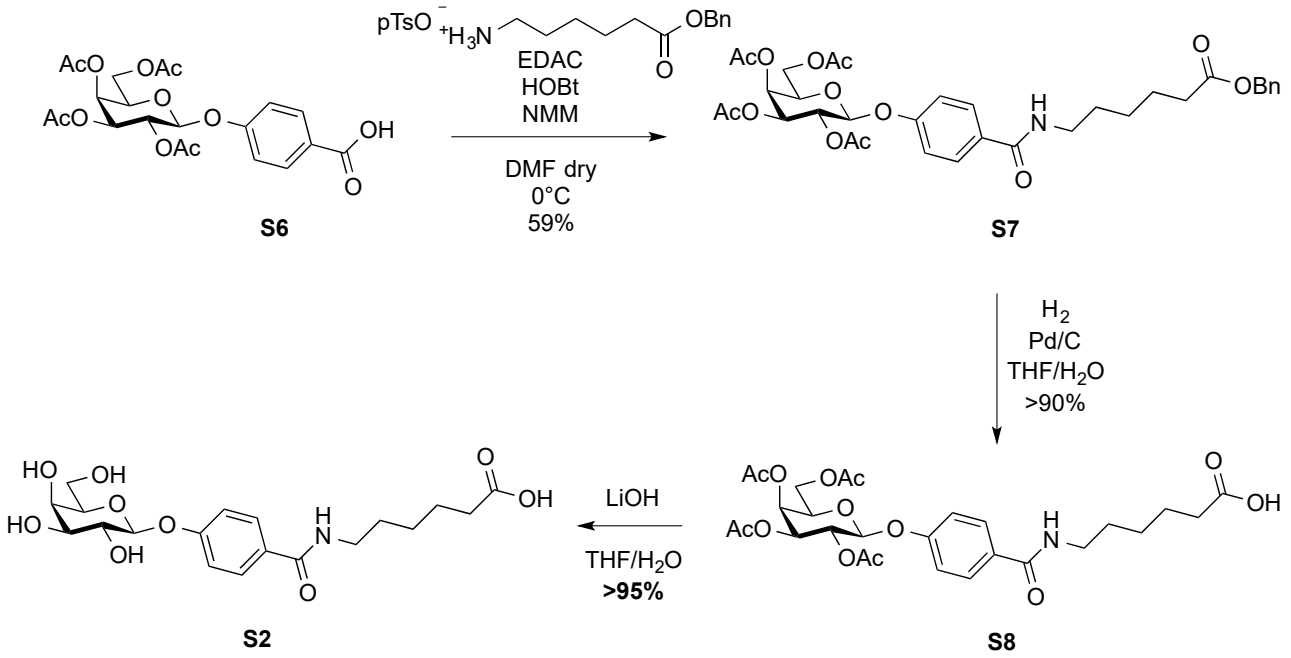
1. Materials

Analytical grade solvents and commercially available reagents were used without further purification. Silica gel flash column chromatography purifications were performed using Geduran® Si 60 (0.040-0.063 mm). TLC analyses were performed on glass Merck silica gel 60 F_{254} plates. ^{1}H NMR, ^{13}C NMR and 2D-NMR spectra were recorded on a 500 MHz Bruker AVANCE II at 298 K. The assignments of resonances, including those for the mixtures of the compounds, were made on the basis of 2D NMR experiments. Multiplicity abbreviations: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m =multiplet, were used.

2. General synthesis of saccharides S1 and S2



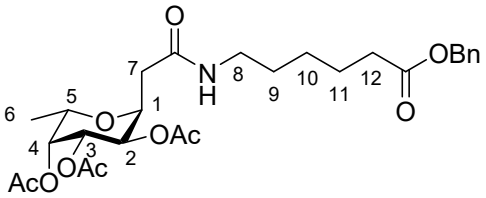
Scheme S4. Synthesis of compound **S1**



Scheme S5. Synthesis of compound **S2**

3. Synthetic procedure for saccharides S1 and S2

Synthesis of compound S4



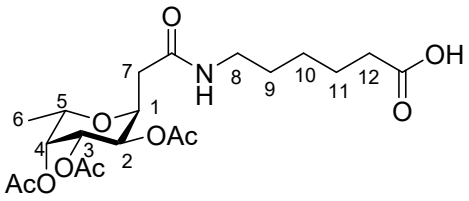
To a solution of **S3**[1] (670 mg, 2.10 mmol) in dry DMF (12 mL), EDAC (686 mg, 3.58 mmol), HOBt (548 mg, 3.58 mmol), NMM (745 mg, 7.37 mmol) and 6-(benzyloxy)-6-oxohexan-1-aminium p-toluenesulphonate linker (649 mg, 2.53 mmol) were added subsequentially. The resulting mixture was stirred at room temperature. After 1 h, the mixture was diluted with EtOAc and washed with HCl 1M (x3) and $NaHCO_3$ s.s. (x2). The organic layer was dried over Na_2SO_4 and concentrated under

vacuum. The crude was purified by flash chromatography (Petroleum ether/EtOAc 7:3) to give 812 mg of **S4** as white solid (75%).

ESI-MS: *m/z* (%) 558.33 (100) $[M-Na]^+$

^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.41-7.32 (m, 5H), 6.12-6.06 (m, 1H, NH), 5.33-5.28 (m, 2H), 5.16 (dd, 1H), 5.13 (s, 2H), 4.66 (dt, H-1), 4.08 (m, H-5), 3.32-3.19 (m, 2H), 2.63-2.55 (m, 1H), 2.46-2.40 (m, 1H), 2.38 (t, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.67 (m, 2H), 1.52 (m, 2H), 1.40-1.32 (m, 2H), 1.21 (d, 3H).

Synthesis of compound S5



Compound **S4** (787 mg, 1.47 mmol) was solubilized in a THF/H_2O 4:1 mixture (5 mL), Pd/C (80 mg) was added and the mixture was stirred at room temperature under hydrogen atmosphere. After 1 h, the reaction was filtered through a pad of Celite® and solvents were evaporated under reduced pressure to give 626 mg of compound **S5** (>95% yield).

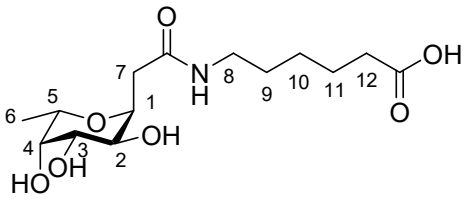
ESI-MS: *m/z* (%): 452.25 (100) $[M-Li]^+$, 468. 33 (28) $[M-Na]^+$

$[\alpha]_D^{21}$ = - 0.113 (c = 0.002 in MeOH)

^{1}H NMR: (500 MHz, CD_3OD) δ 5.32-5.27 (m, 2H, H-2 + H-4), 5.23 (dd, J_{3-2} = 10.3 Hz, J_{3-4} = 3.4 Hz, 1H, H-3), 4.64 (dt, J_{1-2} = 5.4 Hz, J_{1-7} = 9.5 Hz, 1H, H-1), 4.15 (qd, J_{5-4} = 1.8 Hz, J_{5-6} = 6.5 Hz, 1H, H-5), 3.31-3.23 (m, 1H, H-8), 3.22-3.12 (m, 1H, H-8), 2.71-2.64 (m, 1H, CH_2-7), 2.54-2.48 (m, 1H, CH_2-7), 2.29 (t, J_{12-11} = 7.4 Hz, 2H, H-12), 2.16 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.68-1.60 (m, 2H, H-11), 1.58-1.50 (m, 2H, H-9), 1.44-1.38 (m, 2H, H-10), 1.14 (d, J_{6-5} = 6.5 Hz, 3H, H-6).

^{13}C NMR: (125 MHz, CD_3OD) δ 176.5 (C_q, COO), 171.1 (C_q, NCO), 170.8 (COO, OAc), 170.2 (COO, OAc), 170.0 (COO, OAc), 70. 7 (CH, C-3), 70.2 (CH, C-4), 68.5 (CH, C-2), 67.5 (CH, C-1), 66.4 (CH, C-5), 39.0 (CH_2, C-8), 33.8 (CH_2, C-12), 33.4 (CH_2, C-7), 28.7 (CH_2, C-9), 26.1 (CH_2, C-10), 24.5 (CH_2, C-11), 19.3 (CH_3, OAc), 19.2 (CH_3, OAc), 19.1(CH_3, OAc) 15.0 (CH_3, C-6).

Synthesis of compound S1



Compound **S5** (200 mg, 0.45 mmol) was solubilized in a THF/H_2O 1:1 mixture (4 mL), solid LiOH (50 mg, 2.12 mmol) was added and the mixture was stirred at room temperature. After 16 h, the reaction was quenched by Dowex™ 50WX2-100 addition. The resin was eliminated by filtration and solvents were evaporated under reduced pressure to give pure derivative **S1** in a quantitative yield.

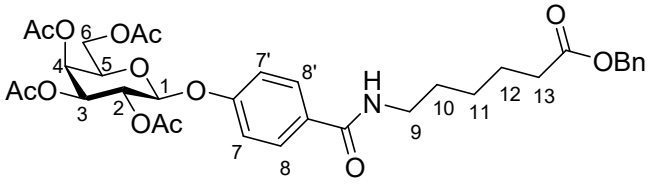
ESI-MS: *m/z* (%) 326. 17 (100) $[M-Li]^+$, 342.25 (26) $[M-Na]^+$

$[\alpha]_D^{21}$ = - 0.032 (c = 0.003 in MeOH)

^{1}H NMR: (500 MHz, CD_3OD) δ 4.42 (ddd, J_{1-7} = 4.2 Hz, $J_{1-7'}$ = 10.6 Hz, J_{1-2} = 5.6 Hz, 1H, H-1), 3.96 (dd, J_{2-1} = 5.8 Hz, J_{2-3} = 9.4 Hz, 1H, H-2), 3.93 (qd, J_{5-4} = 1.8 Hz, J_{5-6} = 6.5 Hz, 1H, H-5), 3.72 (dd, J_{4-5} = 1.8 Hz, J_{4-3} = 3.3 Hz, 1H, H-4), 3.64 (dd, J_{3-4} = 3.3 Hz, J_{3-2} = 9.4 Hz , 1H, H-3), 3.28-3.13 (m, 2H, H-8), 2.65-2.58 (m, 1H, CH_2-7), 2.54-2.48 (m, 1H, CH_2-7), 2,18 (t, J_{12-11} = 7.6 Hz, 2H, H-12), 1.67-1.59 (m, 2H, H-11), 1.59-1.50 (m, 2H, H-9), 1.43- 1.33 (m, 2H, H-10), 1.21 (d, J_{6-5} = 6.5 Hz, 3H, H-6).

^{13}C NMR: (125 MHz, CD_3OD) δ 181.6 (C_q, COO), 179.2 (C_q, NCO), 79. 2 (CH, C-2), 72.8 (CH, C-1), 71.4 (CH, C-4), 70.9 (CH, C-3), 68.0 (CH, C-5), 39.0 (CH_2, C-8), 37.6 (CH_2, C-12), 32.7 (CH_2, C-7), 28.9 (CH_2, C-10), 26.6 (CH_2, C-11), 26.0 (CH_2, C-9), 15.4 (CH_3, C-6).

Synthesis of compound S7

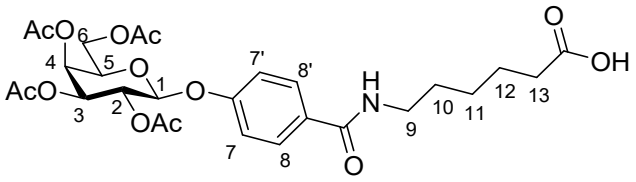


To a solution of **S6**[2] (700 mg, 1.50 mmol), in dry DMF (12 mL), EDAC (487 mg, 2.55 mmol), HOBt (391 mg, 2.55 mmol), NMM (531 mg, 5.25 mmol) and 6-(benzyloxy)-6-oxohexan-1-aminium p-toluenesulphonate linker (462 mg, 1.80 mmol) were added subsequentially. The resulting mixture was stirred at room temperature. After 1 h, the mixture was diluted with EtOAc and washed with HCl 1M (x3) and $NaHCO_3$ s.s. (x2). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by flash chromatography (Petroleum ether/EtOAc 7:3) to give 585 mg of compound **S7** as white solid (59%).

P.M. ($C_{34}H_{41}NO_{13}$) 671.70

^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.77-7.72 (m, 2H), 7.40-7.31 (m, 5H), 7.06-7.02 (m, 2H), 6.14-6.09 (m, 1H), 5.52-5.49 (m, 2H), 5.14-5.11 (m, 4H), 4.27-4.09 (m, 3H), 3.49-3.43 (m, 2H), 2.40 (t, 1H), 2.21 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.76-1.68 (m, 2H,), 1.68-1,60 (m, 2H), 1.47-1.39 (m, 2H).

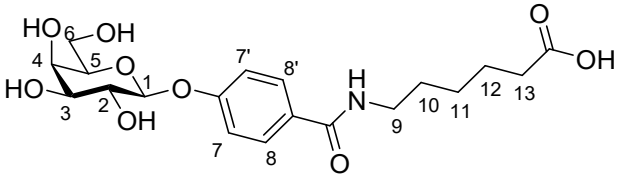
Synthesis of compound S8



Compound **S7** (434 mg, 0.65 mmol) was solubilized in a THF/H_2O 3:1 mixture (8 mL), Pd/C (43 mg) was added and the mixture was stirred at room temperature under hydrogen atmosphere. After 1 h, the reaction was filtered through a pad of Celite® and solvents were evaporated under reduced pressure to give 348 mg of compound **S8** (92% yield).

^{1}H NMR: (500 MHz, CD_3OD) δ 7.84-7.80 (m, 2H), 7.12-7.08 (m, 2H), 5.50-5.48 (dd, J = 3.5 Hz, J = 1.0 Hz, 1H), 5.44-5.37 (m, 2H), 5.29 (ddd, J = 9.6 Hz, 3.4 Hz, J = 0.7 Hz, 1H), 4.38-4.34 (m, 1H), 4.24-4.16 (m, 2H), 3.39 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.5 Hz), 2.20 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.71-1.72 (m, 4H), 1.48 - 1.40 (m, 2H).

Synthesis of compound S2



Compound **S8** (320 mg, 0.78 mmol) was solubilized in a THF/H_2O 1:1 mixture (5 mL), solid LiOH (90 mg, 3.75 mmol) was added and the mixture was stirred at room temperature. After 4 h, the reaction was quenched by Dowex™ 50WX2-100 addition. The resin was eliminated by filtration and solvents were evaporated under reduced pressure to give pure derivative **S2** in a quantitative yield.

ESI-MS: *m/z* (%) 420.25 (100) $[M-Li]^+$, 492.08 (100) $[M-Pd]^+$

$[\alpha]_D^{22}$ = - 0.018 (c = 0.002 in MeOH)

^{1}H NMR: (500 MHz, CD_3OD) δ 7.70-7.60 (m, 2H, H-7 + H-7'), 7.15-7.05 (m, 2H, H-8 + H-8'), 5.05 (d, J_{1-2} = 7.6 Hz, 1H, H-1), 3.93 (ad, J_{4-3} = 3.4 Hz, 1H, H-4), 3.82 (at, J_{5-6} = 6.1 Hz, 1H, H-5), 3.75 (dd, J_{2-1} = 7.6 Hz, J_{2-3} = 10.0 Hz, 1H, H-2), 3.72-3.66 (m, 3H, H-3 + H-6), 3.28 (t, J_{9-10} = 7.0 Hz, 2H, H-9), 2.10 (t, J_{13-12} = 7.4 Hz, 2H, H-13), 1.57-1.45 (m, 4H, H-12 + H-10), 1.33-1.24 (m, 2H, H-11).

^{13}C NMR: (125 MHz, CD_3OD) δ 181.9 (C_q, COO), 179.6 (C_q, NCO), 168.2 (C_q , Ph-O), 160.3 (C_q, Ph-CO), 128.8 (CH, C-7') , 127.9 (CH, C-7), 116.0 (CH, C-8 + C-8'), 100.9 (CH, C-1), 75.4 (CH, C-2), 73.4 (CH, C-3), 70.8 (CH_2, C-6), 68.6 (CH, C-4), 60.8 (CH, C-5), 39.6 (CH_2, C-9), 37.7 (CH_2, C-13), 29.1 (CH_2, C-10), 26.8 (CH_2, C-12), 26.0 (CH_2, C-11).

4. Optimization of amide coupling for synthesis of chitosan-sugar conjugates

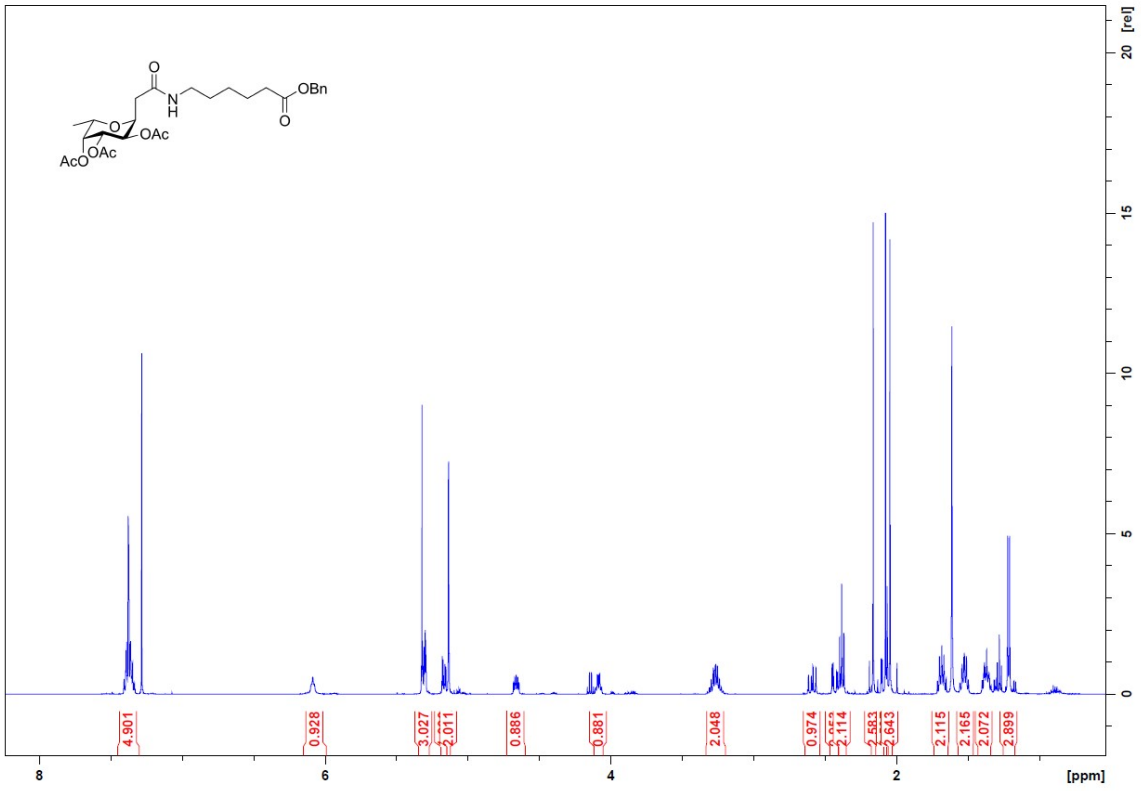
Table S1. Optimization of amide coupling conditions.

Starting material	Reagent	Equivalents of Sugar	Temperature (°C)	Time (h)	DS (%)
TPC/S1	HATU	1	Room temperature	18	40
		1	55	18	45
	EDC/HOBt	1	Room temperature	18	55
		1	55	18	78

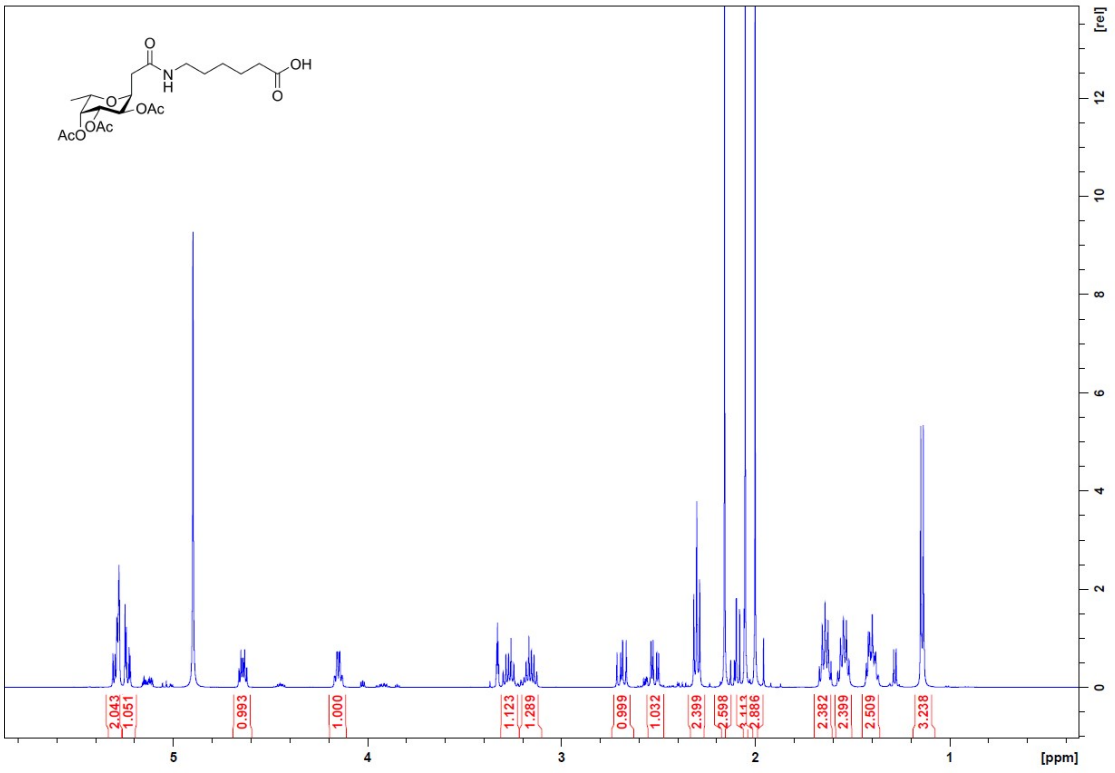
		0.5	55	18	25-27

5. NMR spectra of Chitosan conjugates and precursors:

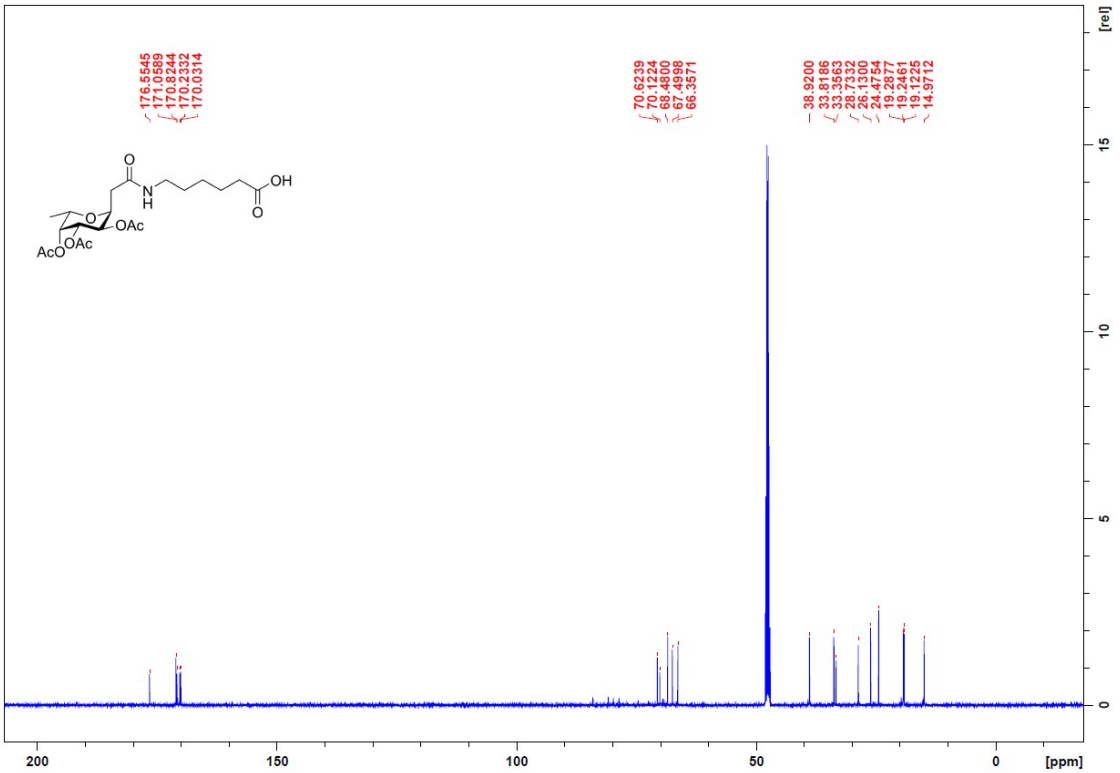
^{1}H NMR of compound S4 in $CDCl_3$



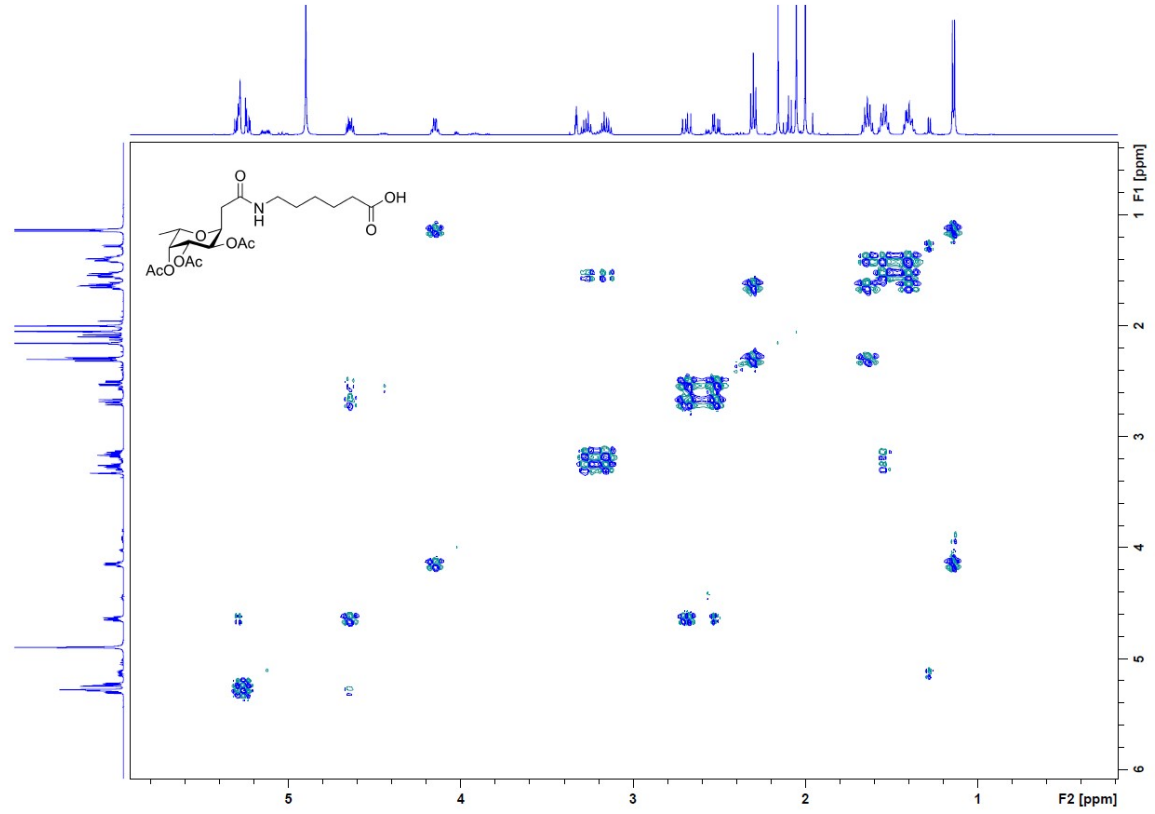
^{1}H NMR of compound S5 in CD_3OD



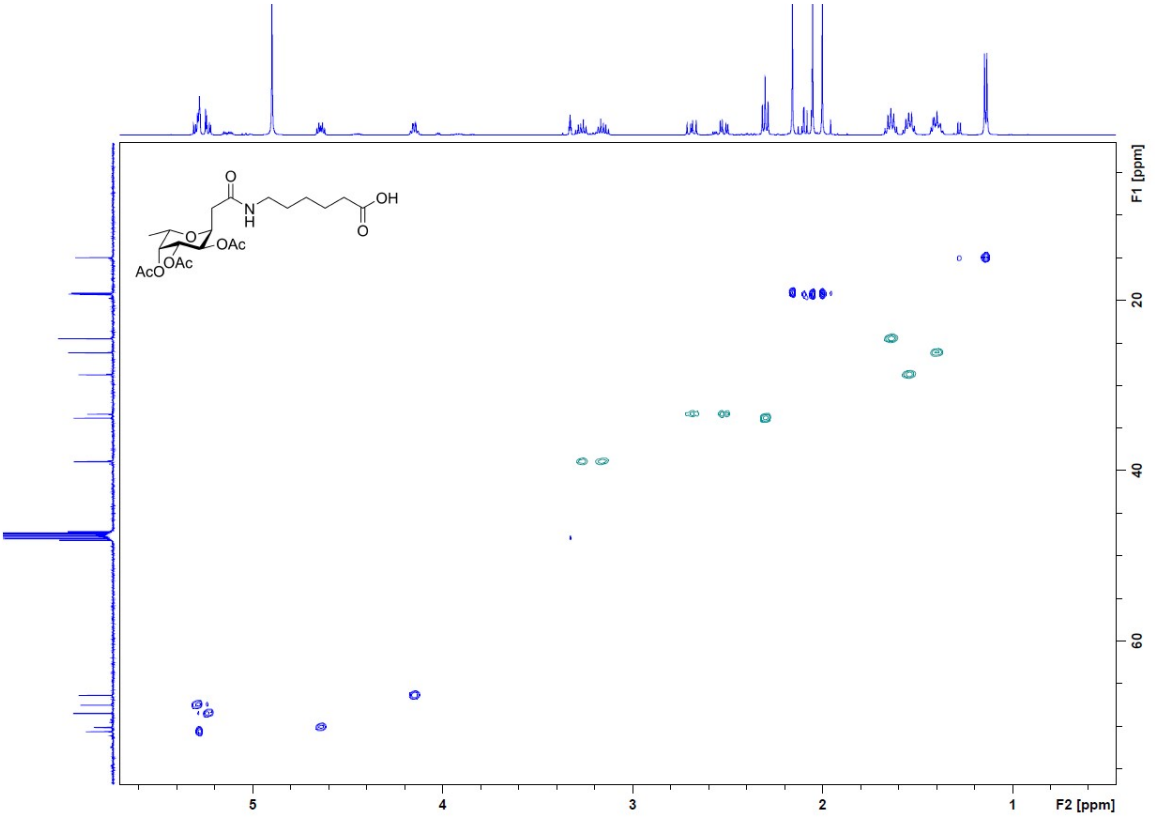
^{13}C NMR of compound S5 in CD_3OD



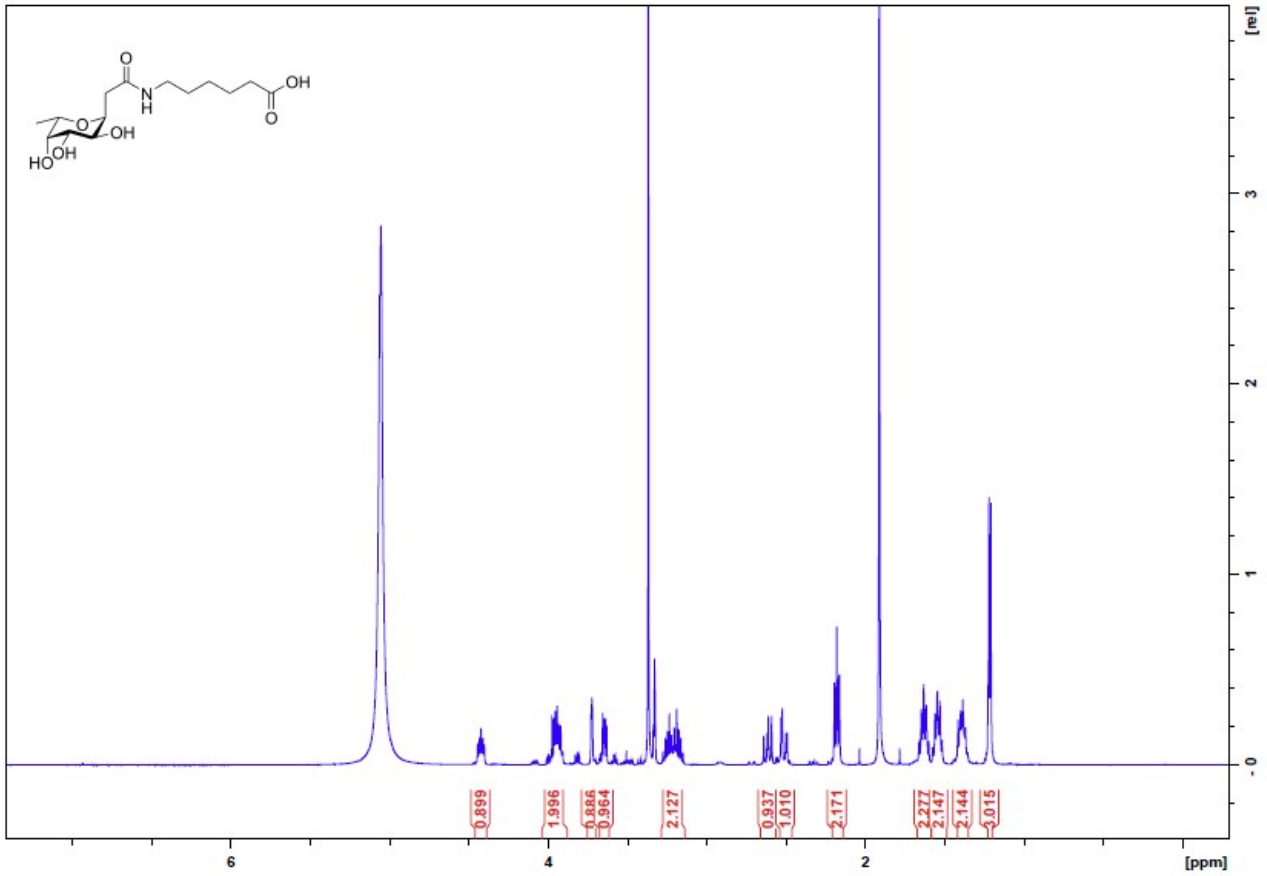
COSY NMR of compound S5 in CD_3OD



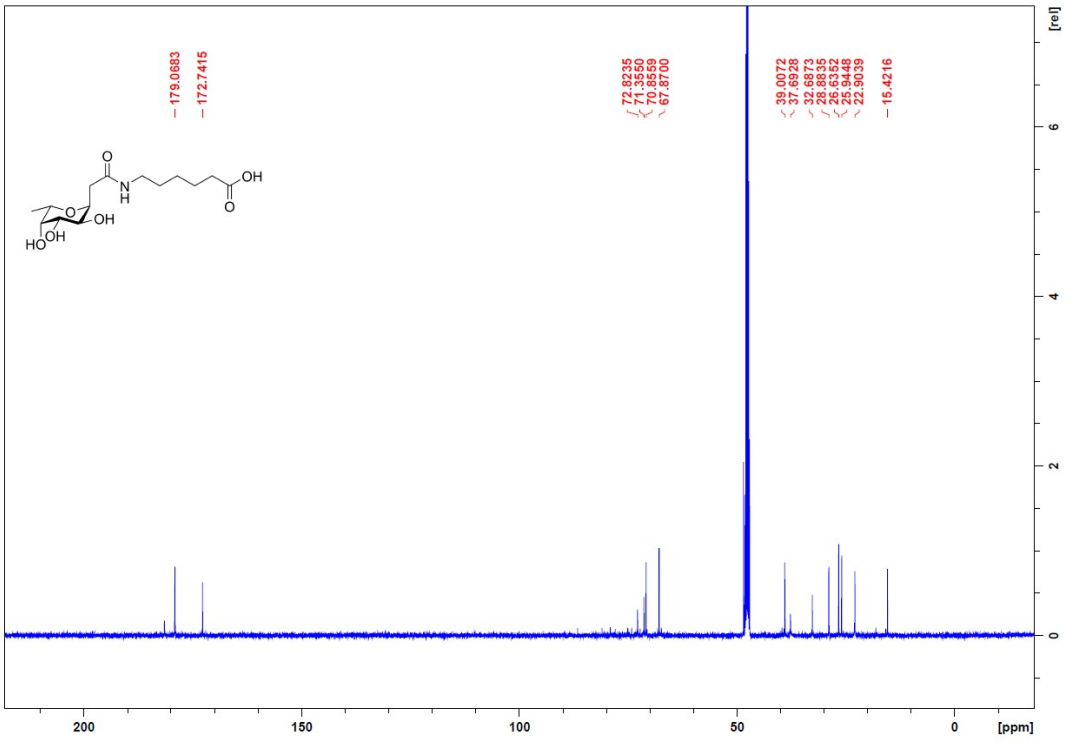
HSQC NMR of compound S5 in CD_3OD



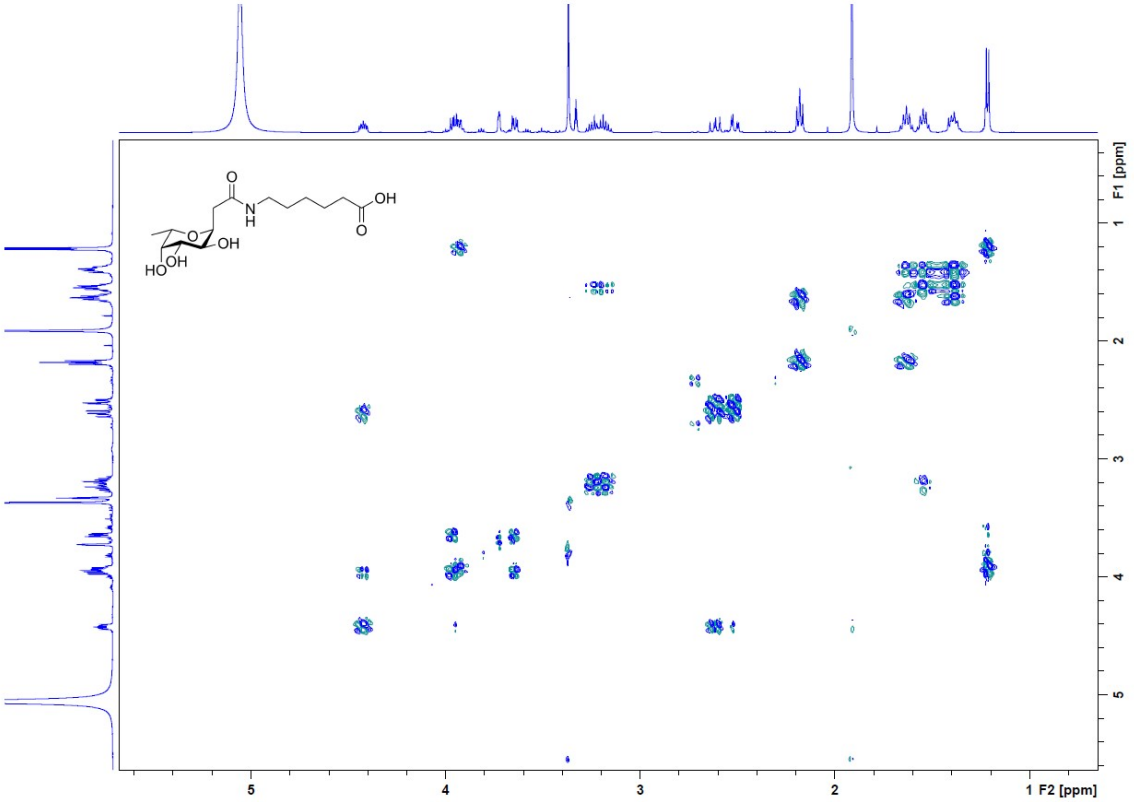
^1H NMR of compound S1 in CD_3OD



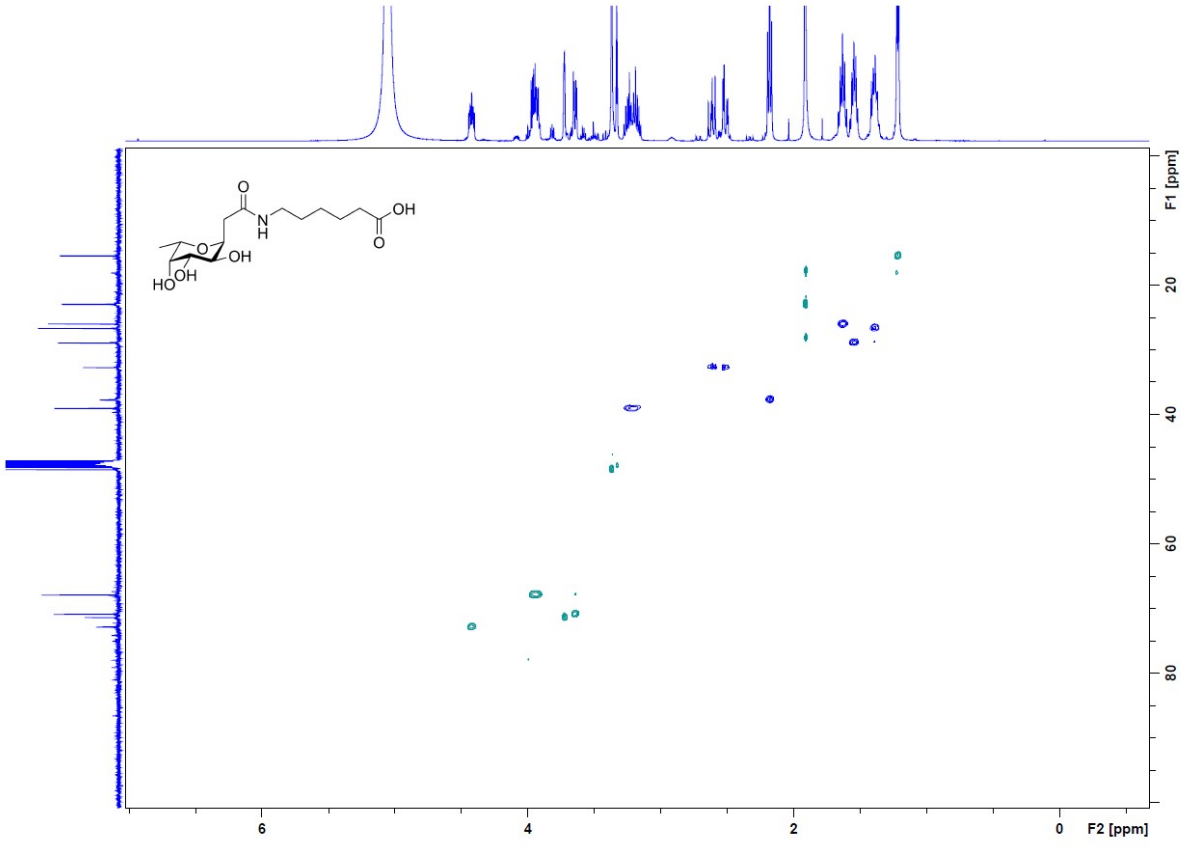
^{13}C NMR of compound S1 in CD_3OD



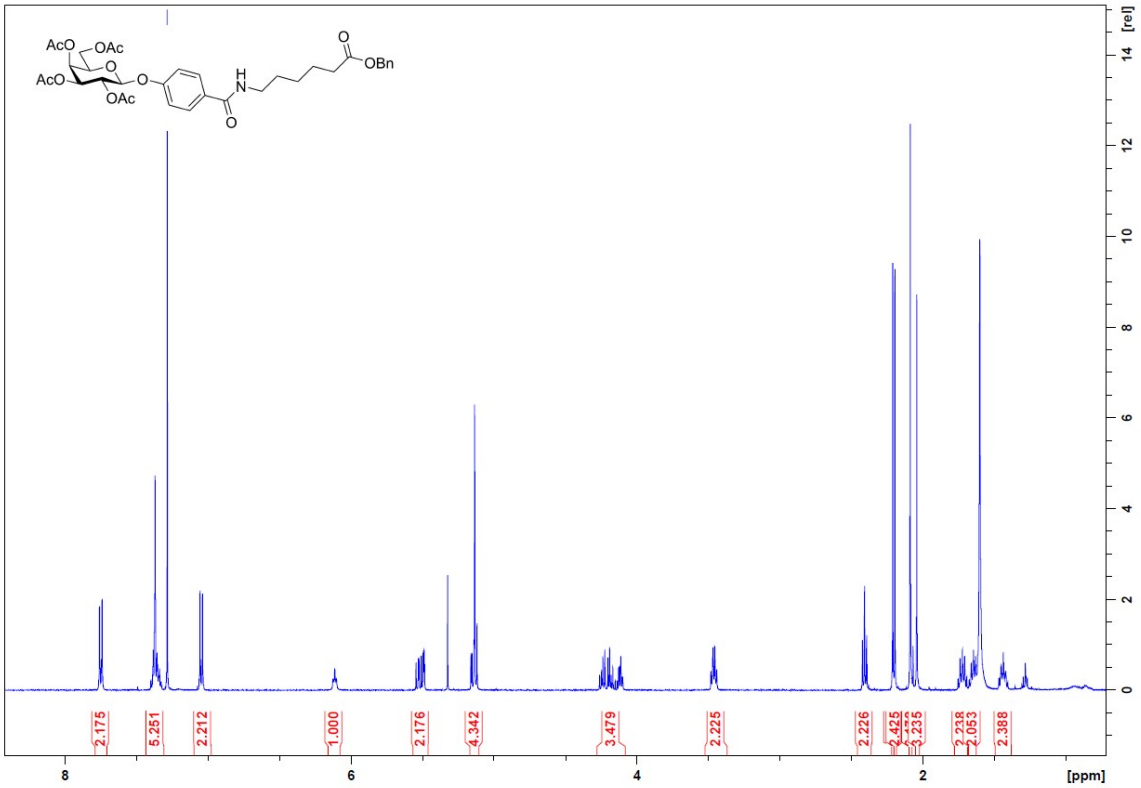
COSY NMR of compound S1 in CD_3OD



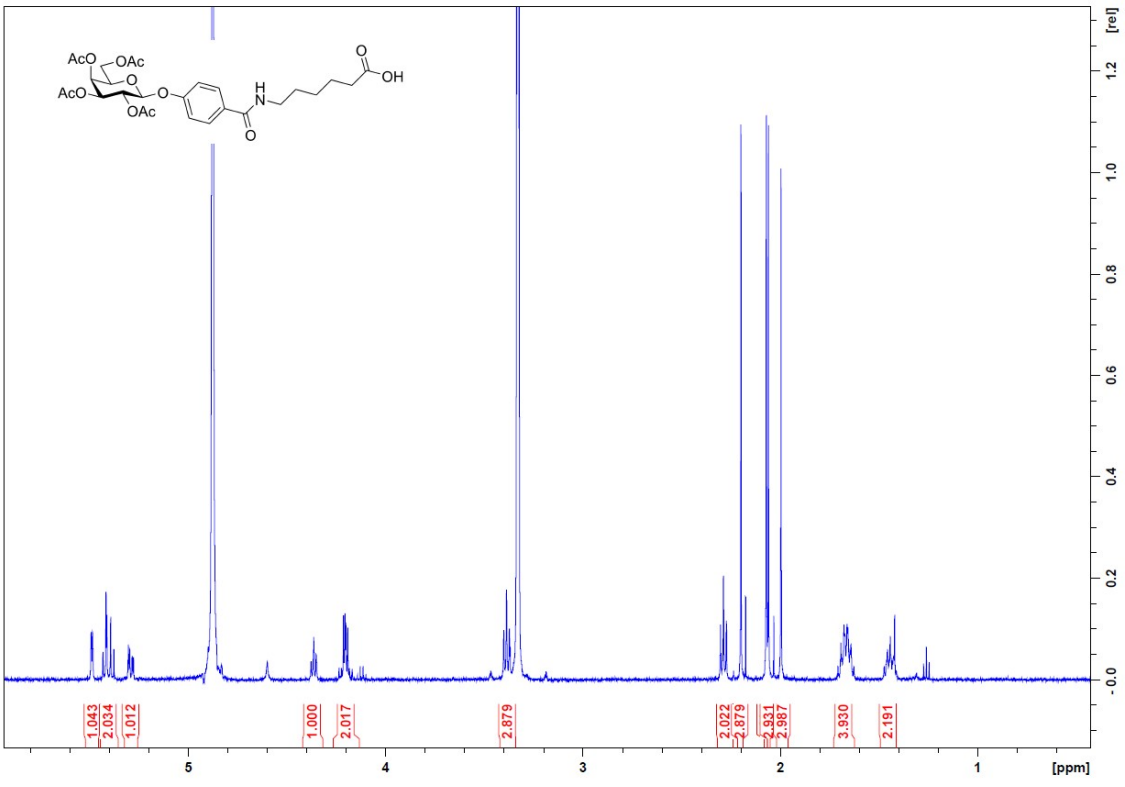
HSQC NMR of compound S1 in CD_3OD



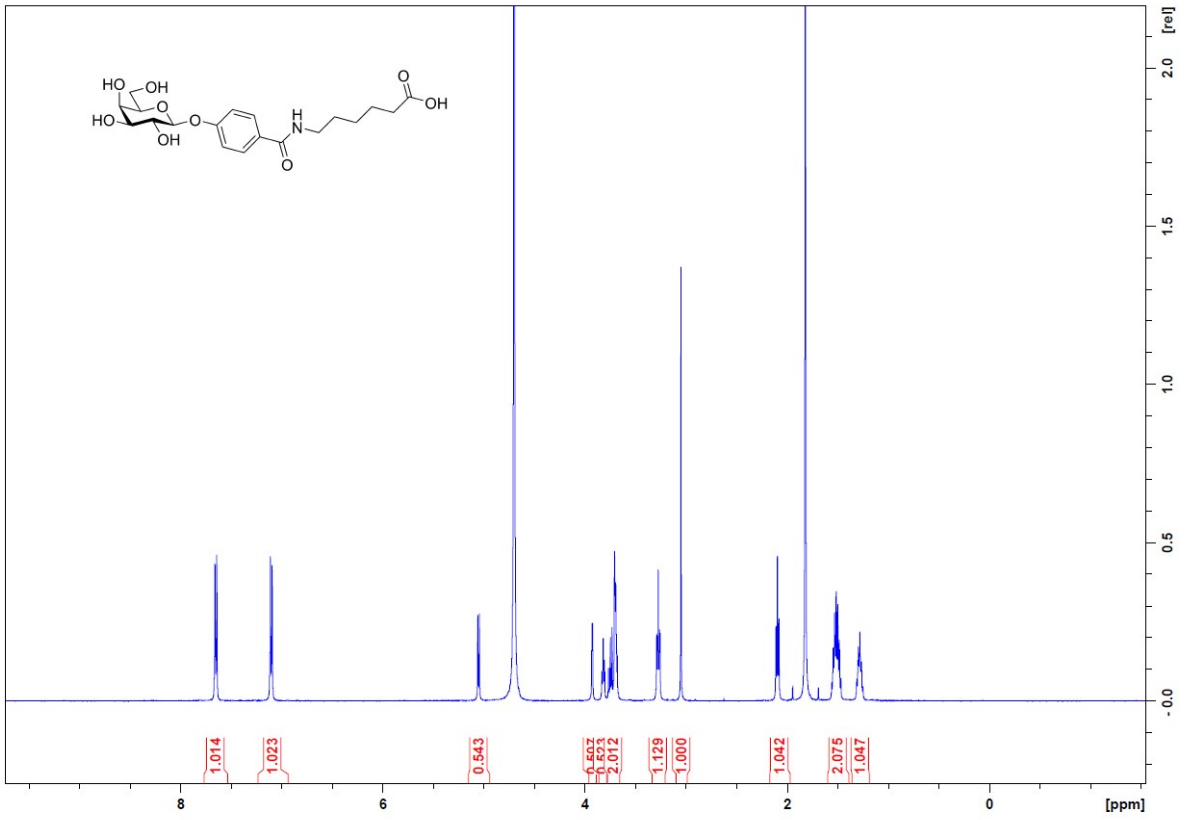
^1H NMR of compound S7 in $CDCl_3$



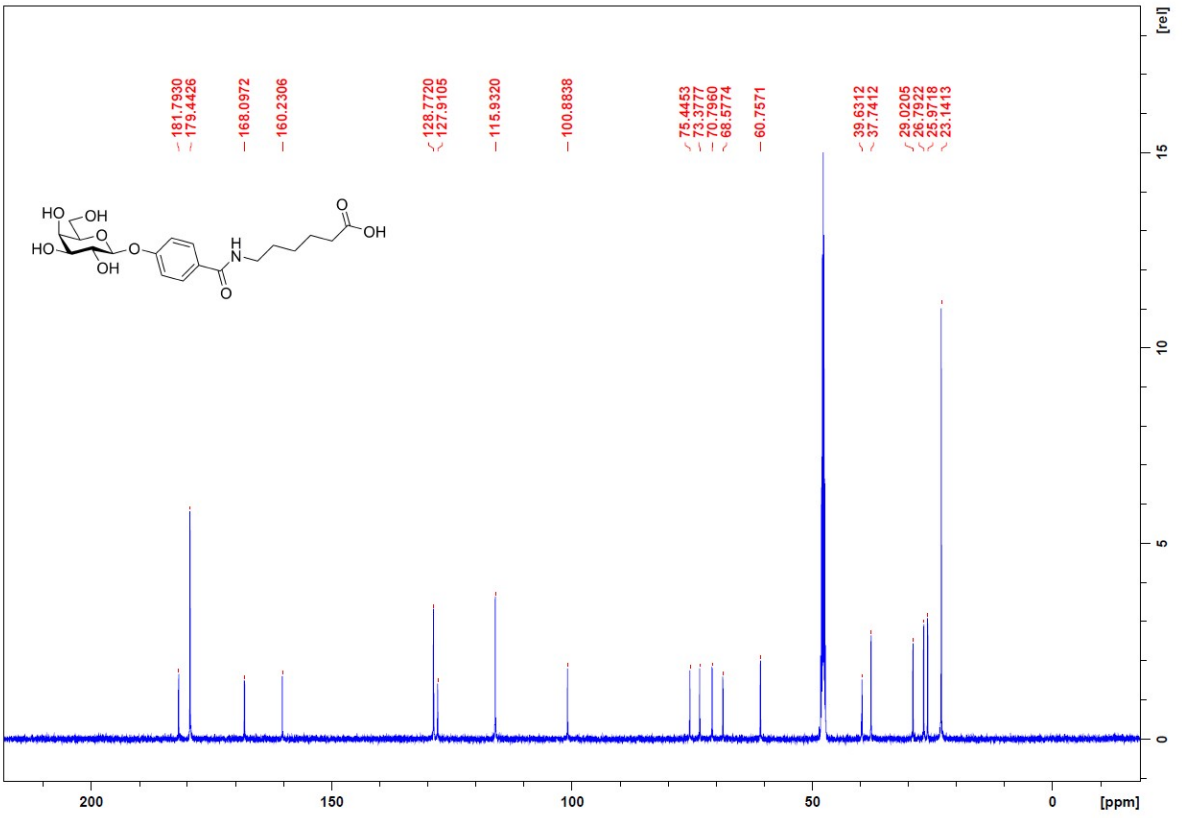
^{1}H NMR of compound S8 in CD_3OD



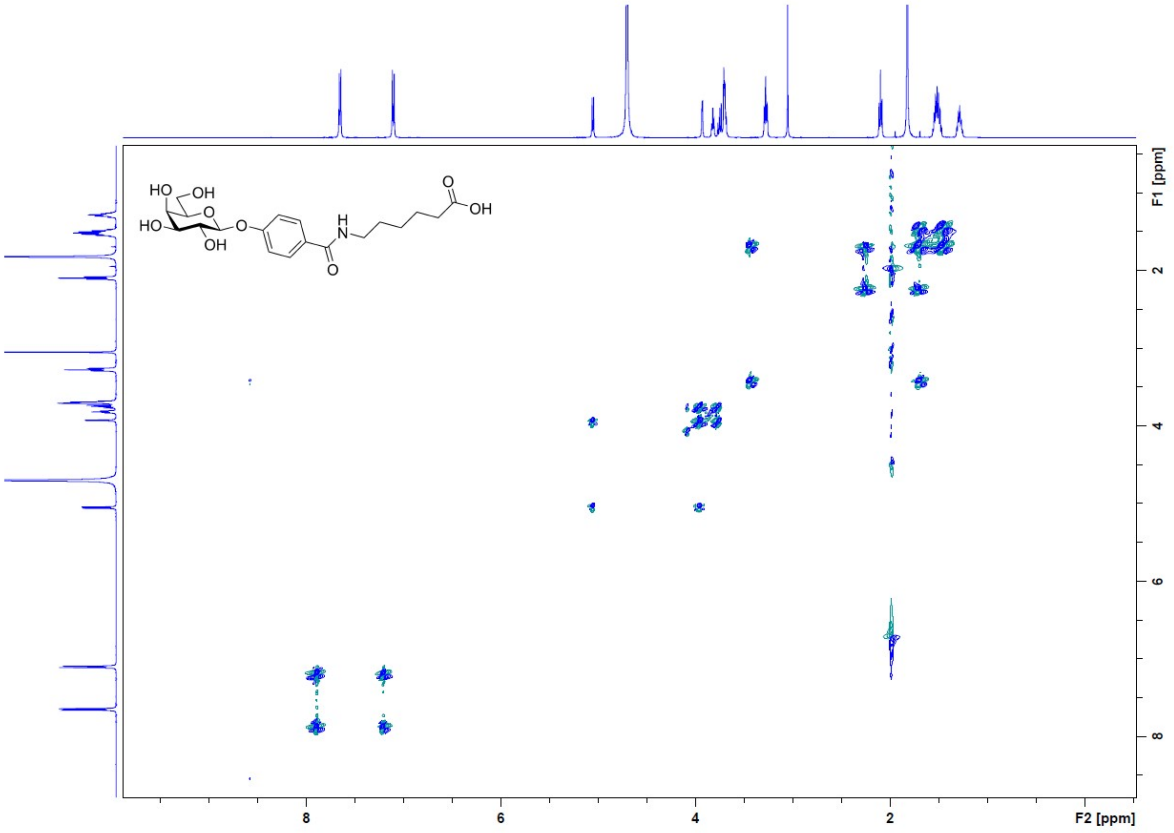
^{1}H NMR of compound S2 in CD_3OD



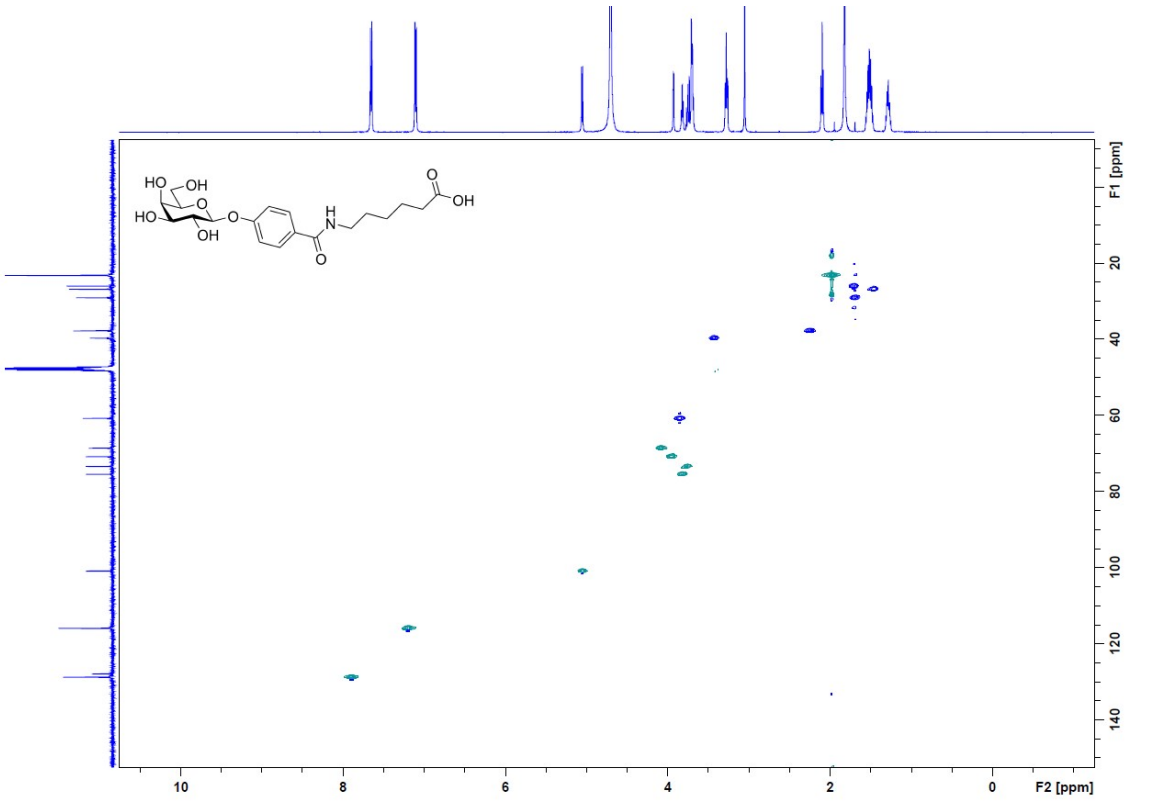
^{13}C NMR of compound S2 in CD_3OD



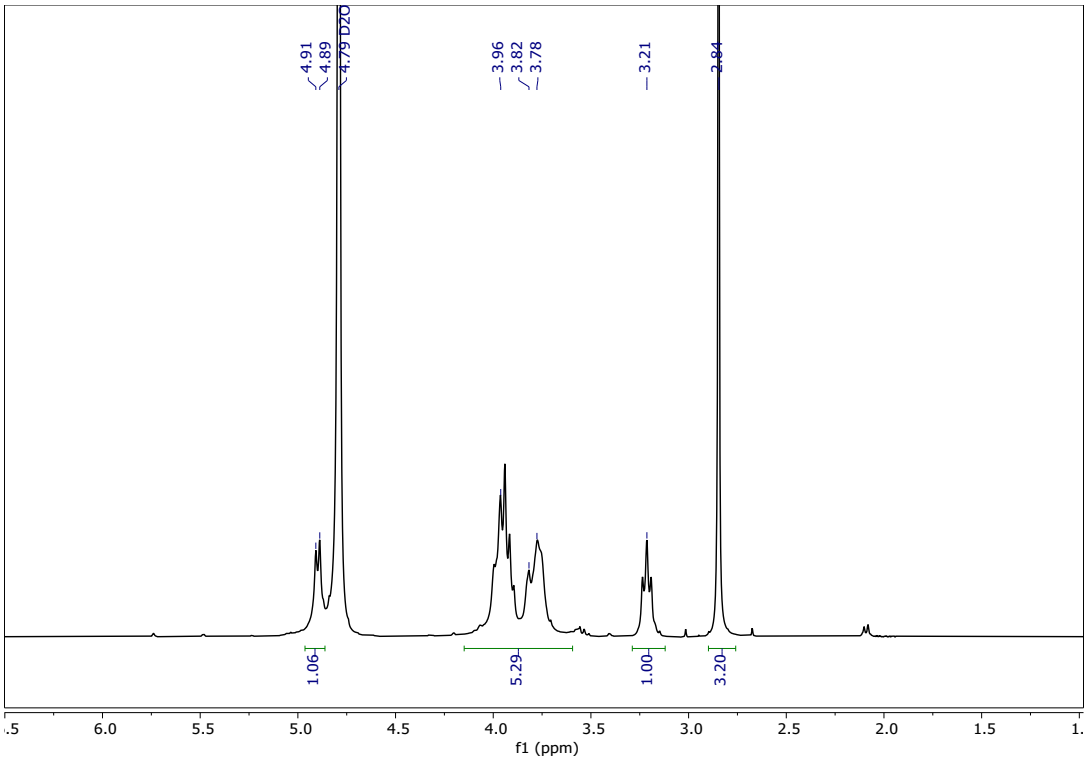
COSY NMR of compound S2 in CD_3OD



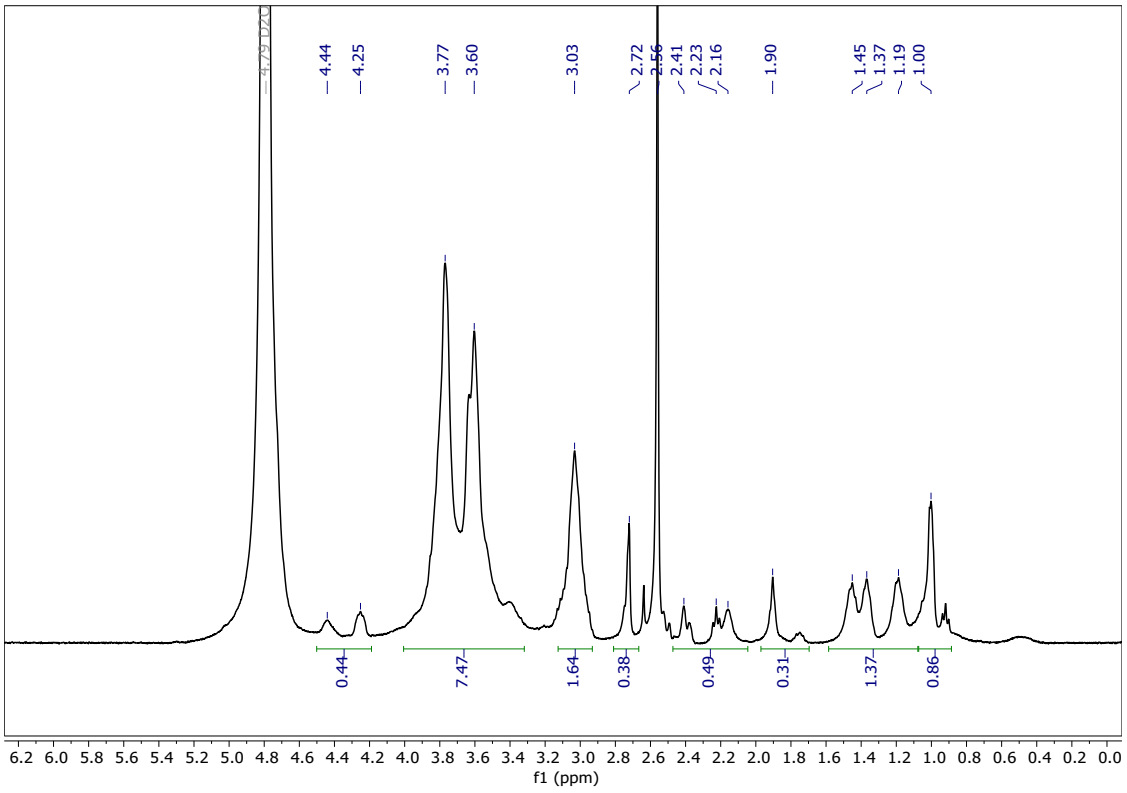
HSQC NMR of compound S2 in CD_3OD



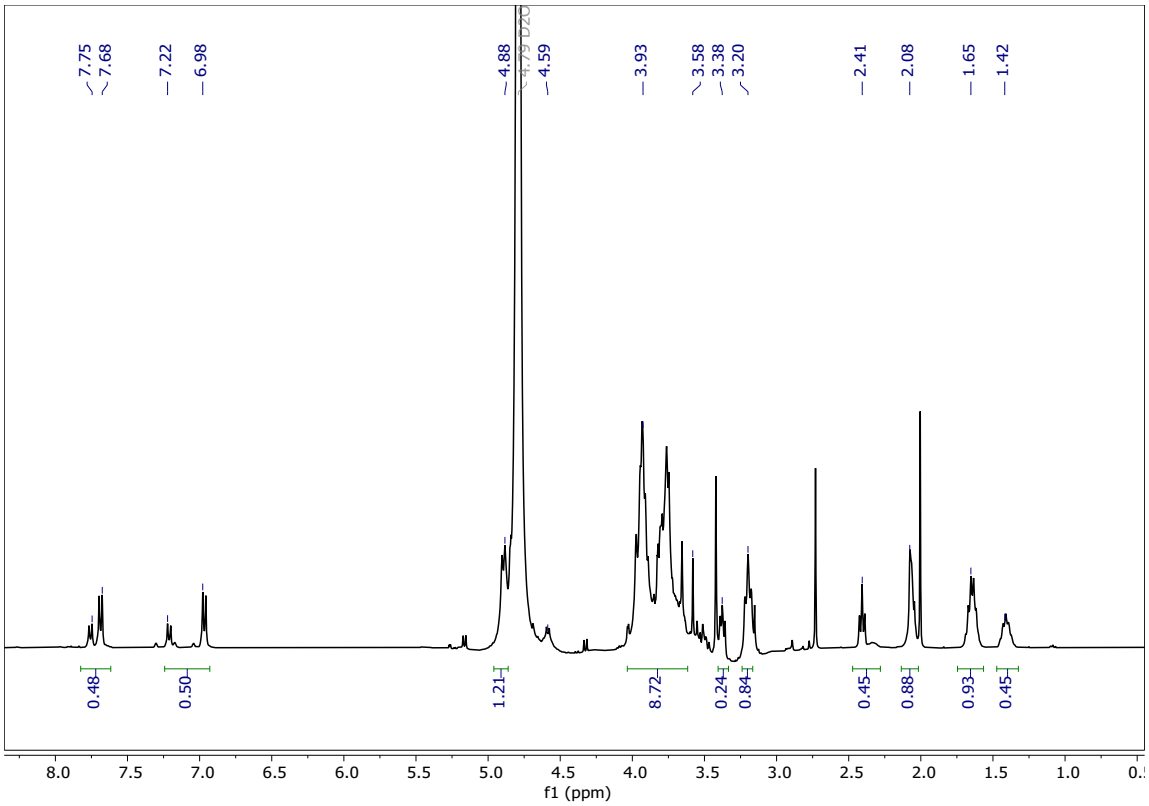
^{1}H-NMR spectra of Chitosan mesylate salt D_2O:



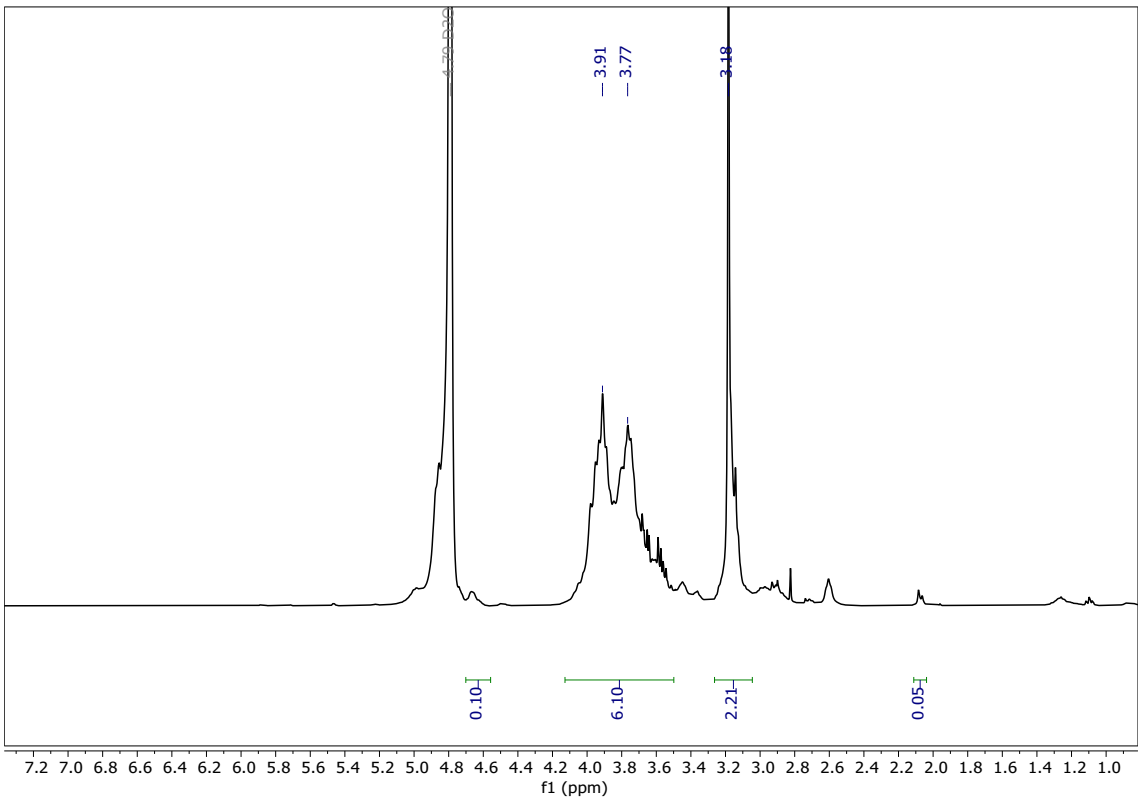
^{1}H-NMR spectra of CS-S1 in D_2O:



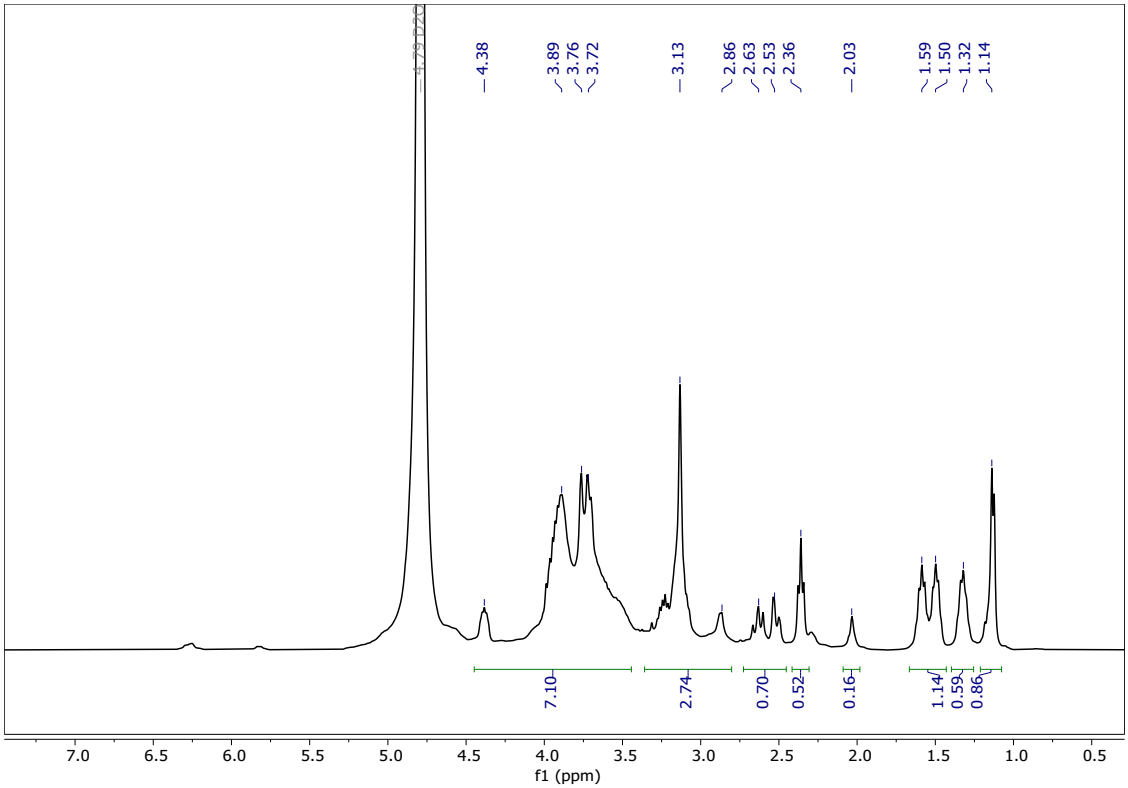
^{1}H-NMR spectra of CS-S2 in D_2O:



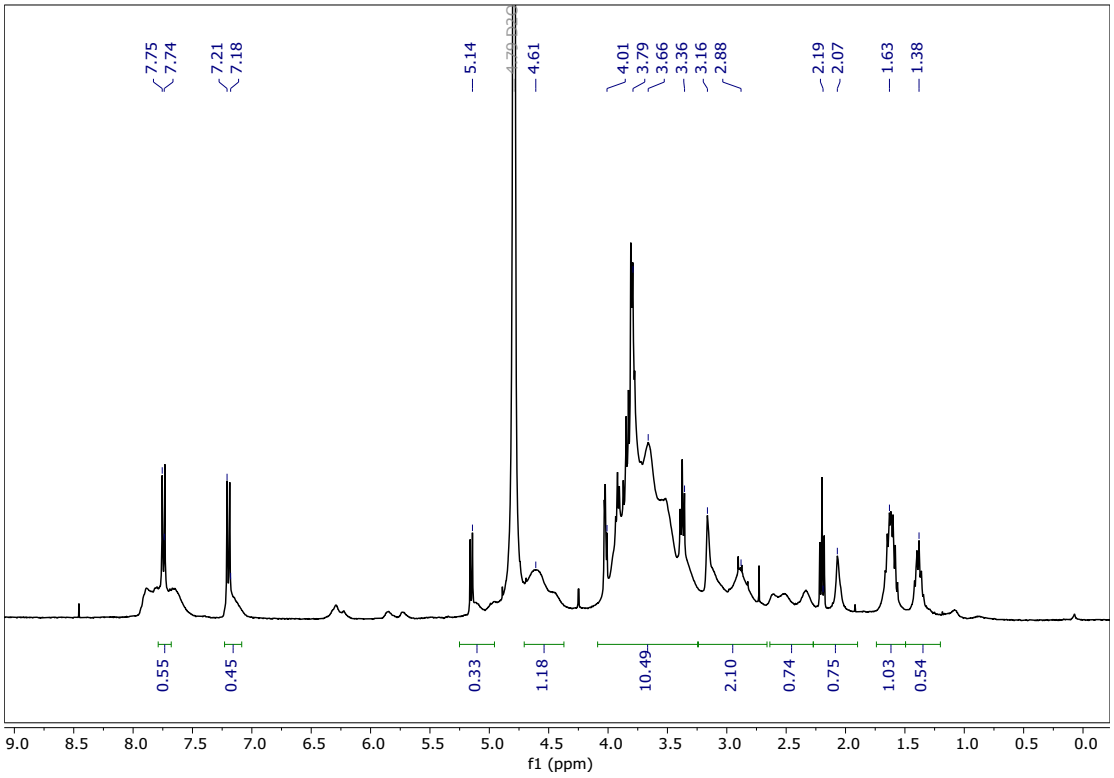
^{1}H-NMR spectra of TMC in D_2O:



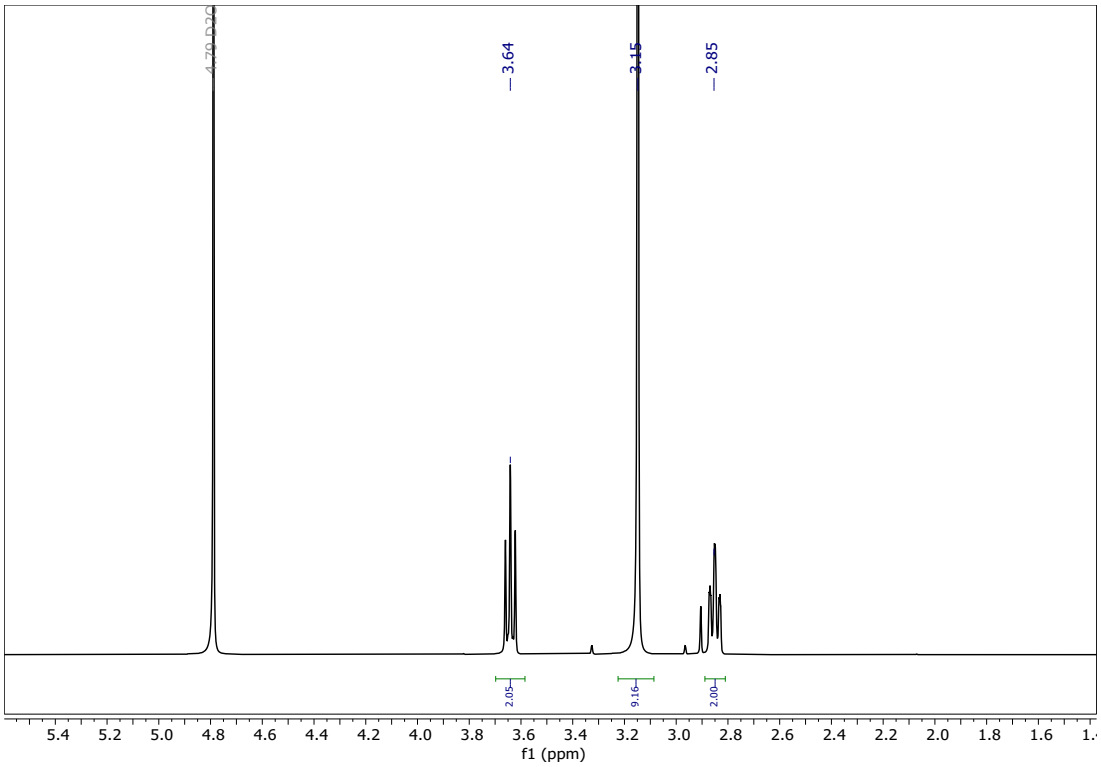
^{1}H-NMR spectra of TMC-S1 in D_2O:



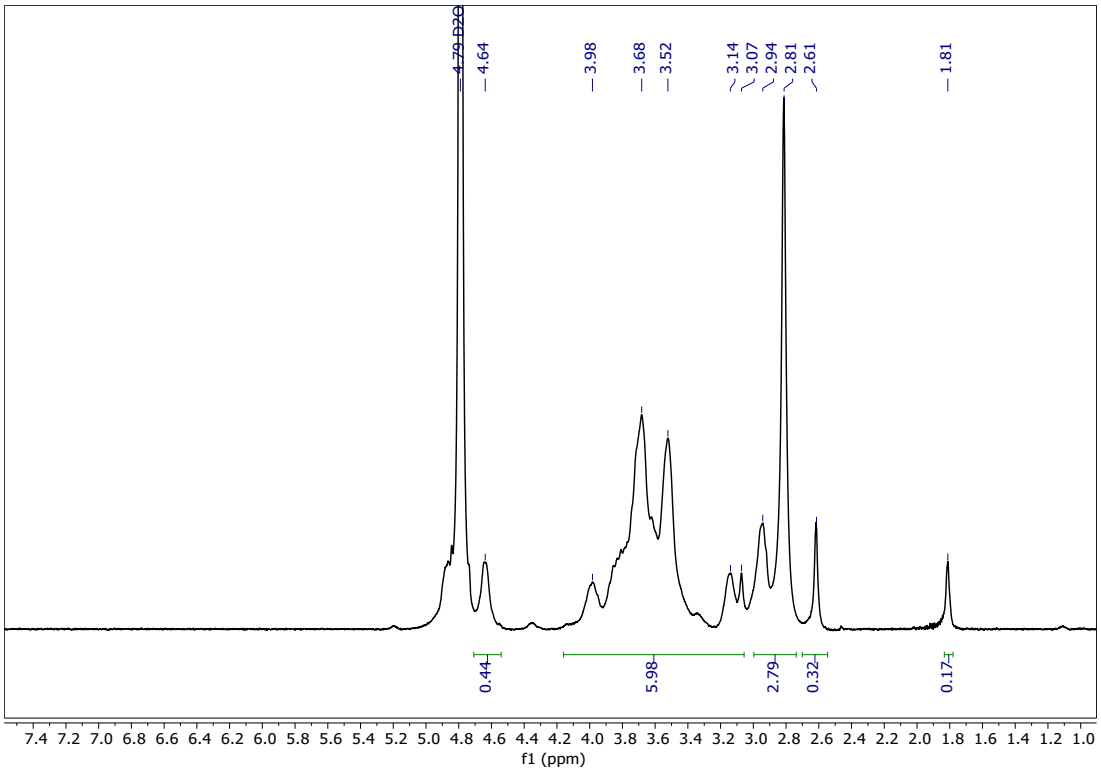
^{1}H-NMR spectra of TMC-S2 in D_2O:



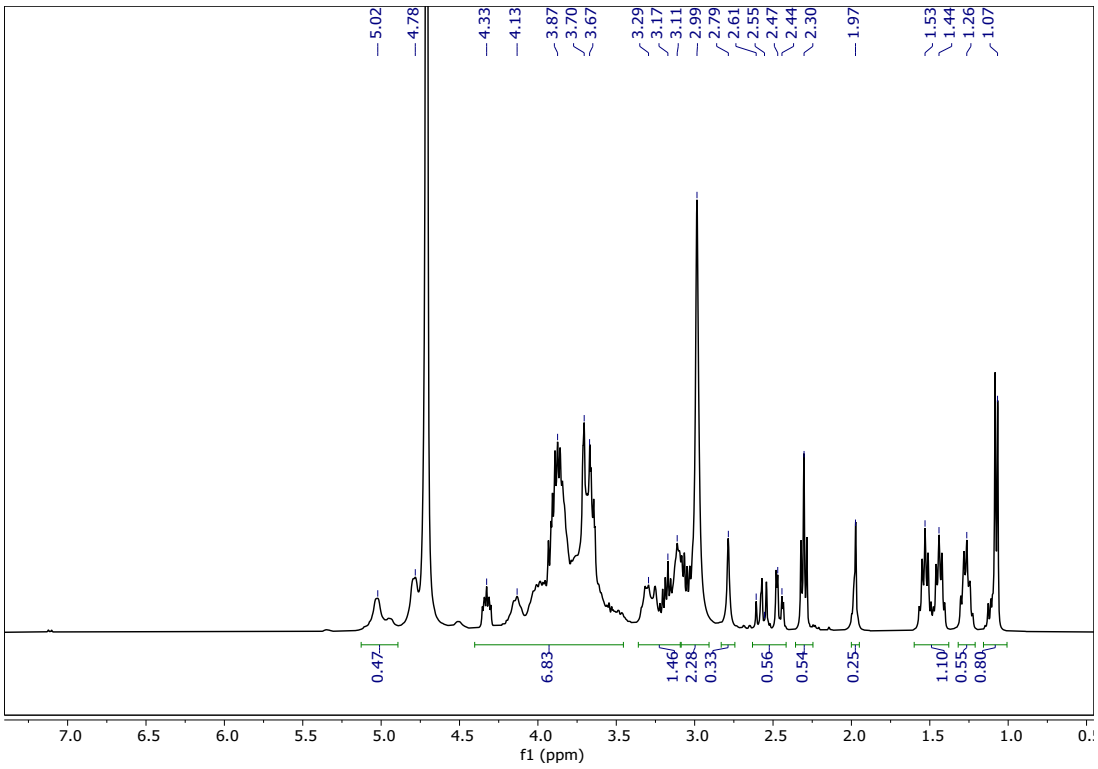
[1]H-NMR spectra of N,N,N-trimethylaminepropanoic acid in D_2O:



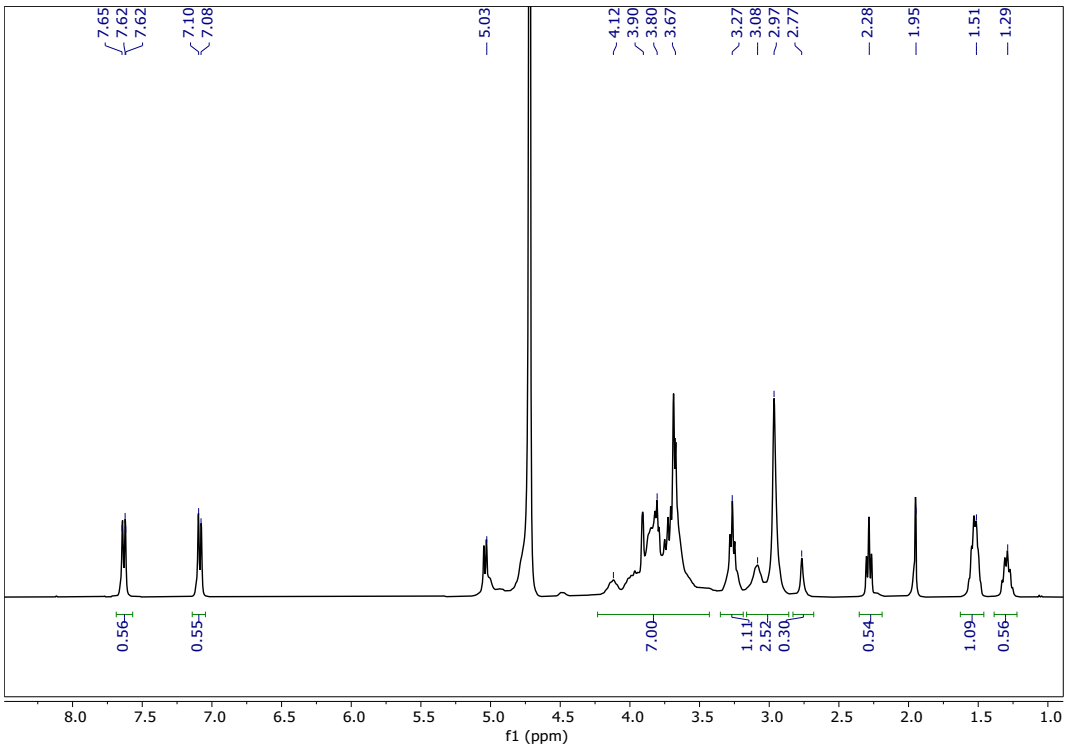
[1]H-NMR spectra of TPC in D_2O:



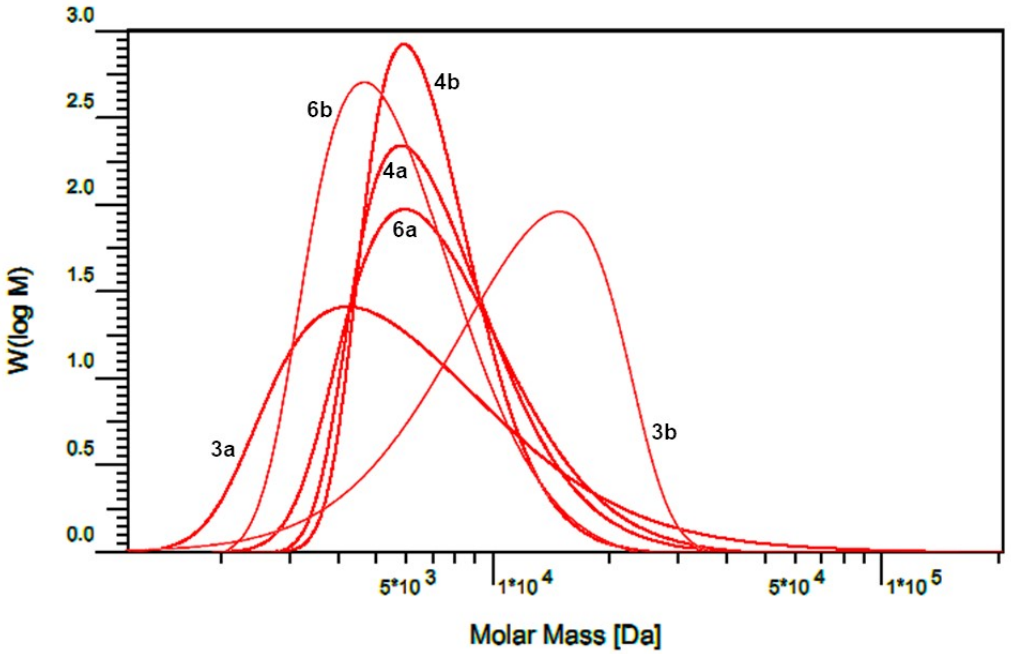
^{1}H-NMR spectra of TPC-S1in D_2O:



^{1}H-NMR spectra of TPC-S2 in D_2O:



6. **GPC chromatograms:** Overlay for CS-S1 (3a), CS-S2 (3b), TMC-S1 (4a), TMC-S2 (4b), TPC-S1 (6a) and TPC-S2 (6b).



7. Confocal Laser Scanning Images

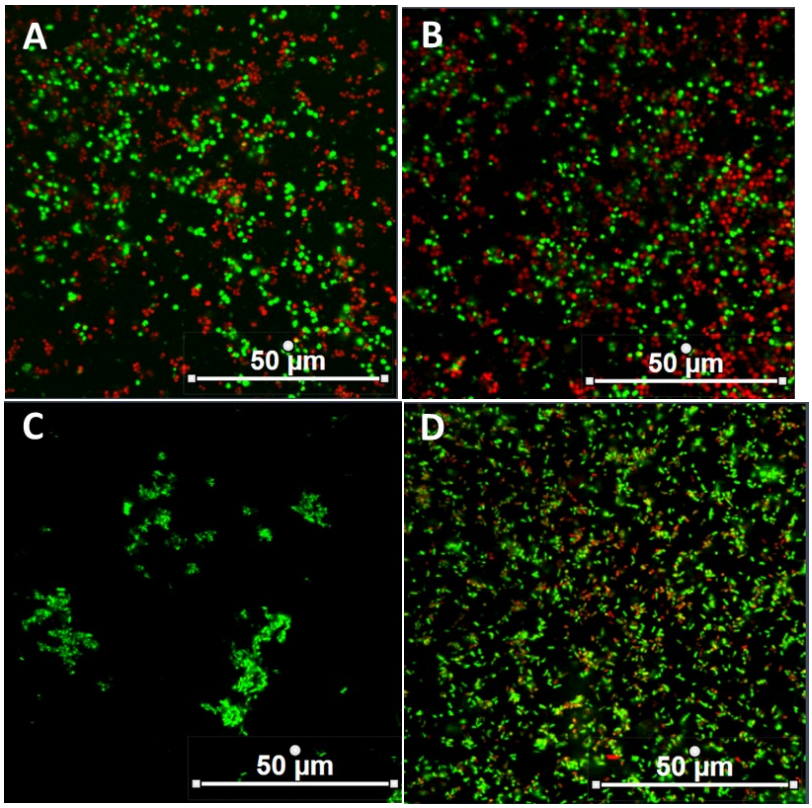


Figure S1. 2D Confocal images of the biofilm population of *P. aeruginosa* treated with A) 0.5 x MBEC of CS-S1; B) 0.5 x MBEC of CS-S2; C) 0.5 x MBEC of TPC-S1, D) 0.5 x MBEC of TPC-S2.

References

1) E.M.V Johansson, *Cell Chemical Biology*, 2008, **15**, 1249-1257

2) R. U. Kadam et al., *Angewandte Chemie International Edition*, 2011, **50**, 10631-10635.