Supplementary Information

Raman-encoded, multivalent glycan-nanoconjugates for traceable specific binding and killing of bacteria

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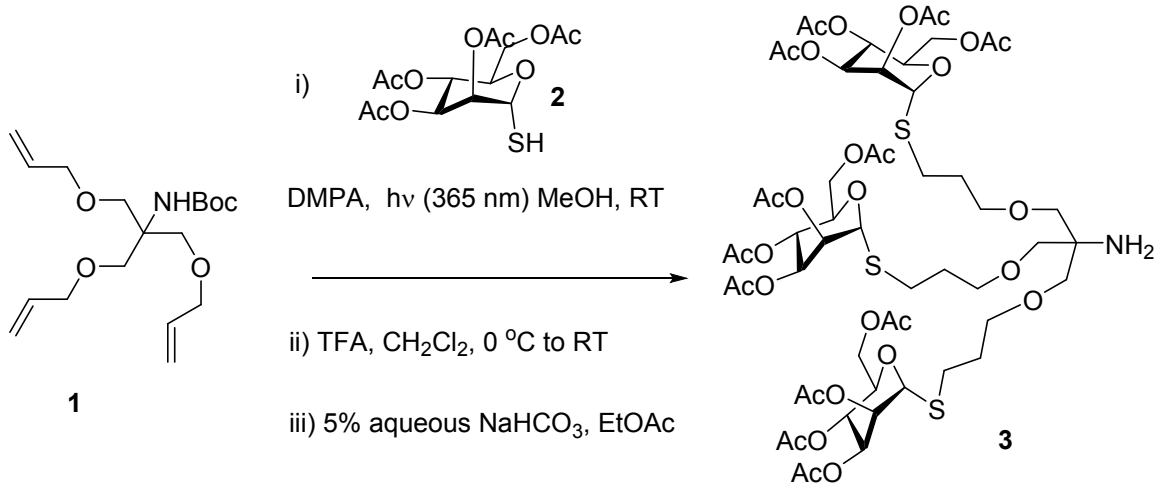


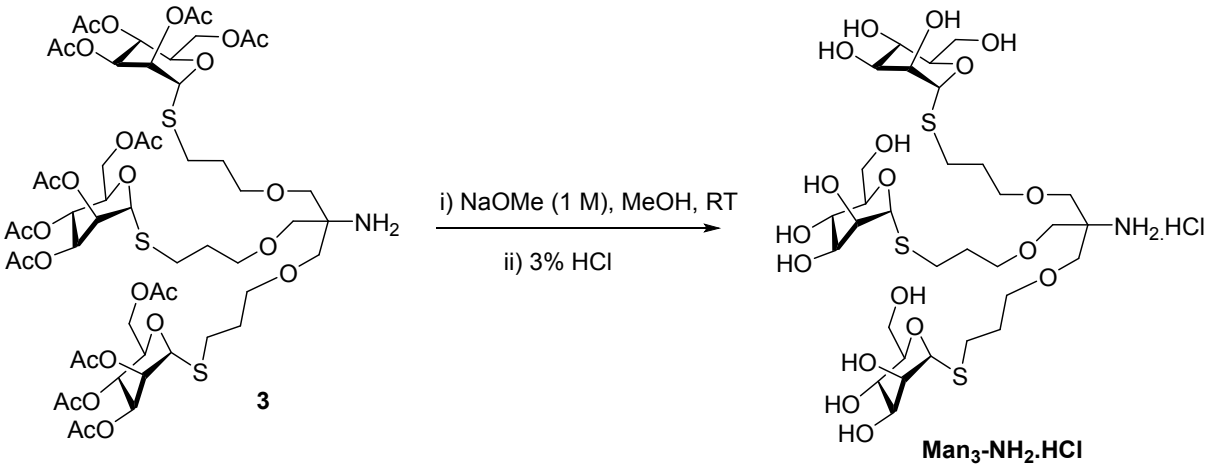
Scheme S1:

Synthesis of tri-thiomannoside cluster amine 3 from tri-O-allyl derivative[1] 1: A solution of **1** (1.78 g, 5.21 mmol), mannoside thiol[2] **2** (9.5 g, 26.07 mmol), 2,2-dimethoxy-2-phenylacetophenone (668 mg, 2.60 mmol) in anhydrous MeOH (12 mL) was bubbled with argon for 40 mins. Then reaction mixture was stirred and exposed to UV light of wavelength 365 nm for 4 h (^{1}H NMR for crude reaction mixture shows complete conversion of **1**). Then volatiles of reaction mixture were evaporated by rotary evaporator and resultant mixture was dried for 2 h under vacuum. The obtained crude was dissolved in anhydrous CH_2Cl_2 (90 mL) and TFA (10 mL) was added dropwise to reaction mixture at 0 ^{o}C, and then reaction mixture was warmed to room temperature. After stirring the reaction mixture for 4 h, the volatiles were evaporated by vacuum and crude was purified over silica gel column with eluent 100% CH_2Cl_2 to 20% MeOH in CH_2Cl_2 (v/v) to afford Boc deprotected TFA salt (7.40 g). The obtained TFA salt was dissolved in EtOAc (300 mL), washed with 5% aqueous $NaHCO_3$ (3x100 mL), washed once with brine solution (120 mL), organic layer was separated, dried with anhydrous Na_2SO_4 and volatiles were evaporated under reduced pressure to afford tri-thiomannoside cluster amine **3** (6.32 g, 4.74 mmol, overall yield 92% from **1**).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.01-5.23 (m, 9 H), 3.87-4.29 (m, 11 H), 3.43 (br s, 10 H), 3.22 (s, 6H), 2.55-2.72 (m, 6H), 2.07 (s, 9H), 2.00 (s, 9H), 1.96 (s, 9H), 1.89 (s, 9H), 1.80 (br t, J = 6.1 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 170.46, 169.88, 169.69, 169.63, 82.55, 72.21, 71.04, 69.36, 68.91, 66.18, 62.32, 56.10, 29.41, 28.18, 20.82, 20.64, 20.60, 20.53.

HRMS (ESI): m/z calcd for $C_{55}H_{84}NO_{30}S_3$ $[M+H]^+$ 1334.4240, found 1334.4141.



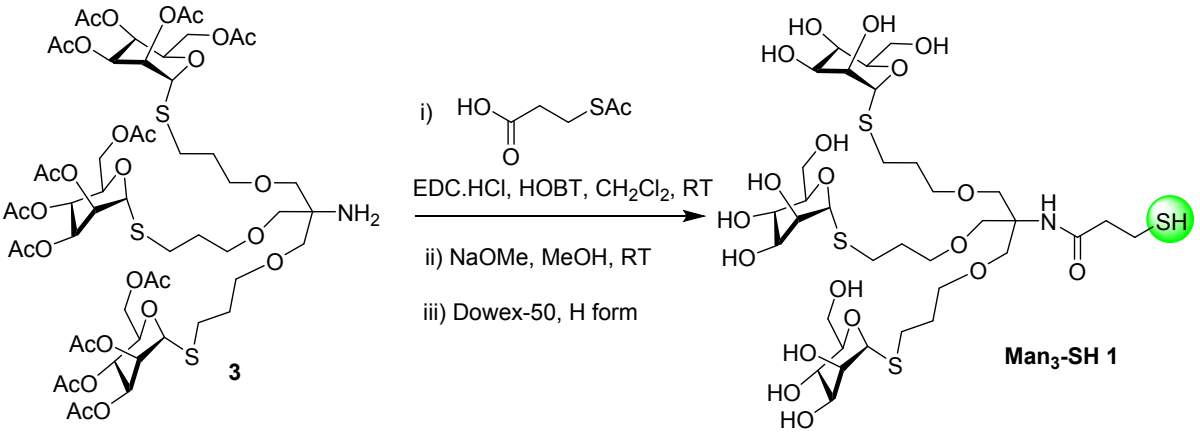
Scheme S2:

Synthesis of Man_3-NH_2.HCl from 3: To a stirred solution of compound **3** (1.1 g, 0.824 mmol) in anhydrous MeOH (40 mL) was added 1 M NaOMe in MeOH (3 mL) dropwise, and the reaction mixture was further allowed to stir for 48 h. Then the reaction mixture was acidified up to pH ≈ 4 by using 3% aqueous HCl solution. Then volatiles were evaporated under vacuum, further dried under vacuum to afford **Man_3-NH_2.HCl** (700 mg, 0.807 mmol, yield = 98%).

^{1}H NMR (300 MHz, D_2O): δ 5.30 (d, J = 1.3 Hz, 3H), 4.05 (dd, J = 3.2, 1.5 Hz, 3H), 3.96-4.01 (m, 3H), 3.68-3.90 (m, 15 H), 3.61-3.65 (br m, 15H), 2.67-2.85 (m, 6H), 1.89-1.97 (m, 6H).

^{13}C NMR (75 MHz, D_2O): δ 84.8, 73.1, 71.7, 71.0, 69.9, 67.9, 67.0, 60.8, 59.6, 28.5, 27.4.

HRMS (ESI): m/z calcd for $C_{31}H_{60}NO_{18}S_3$ $[M]^+$ 830.2973, found 830.2945.



Scheme S3:

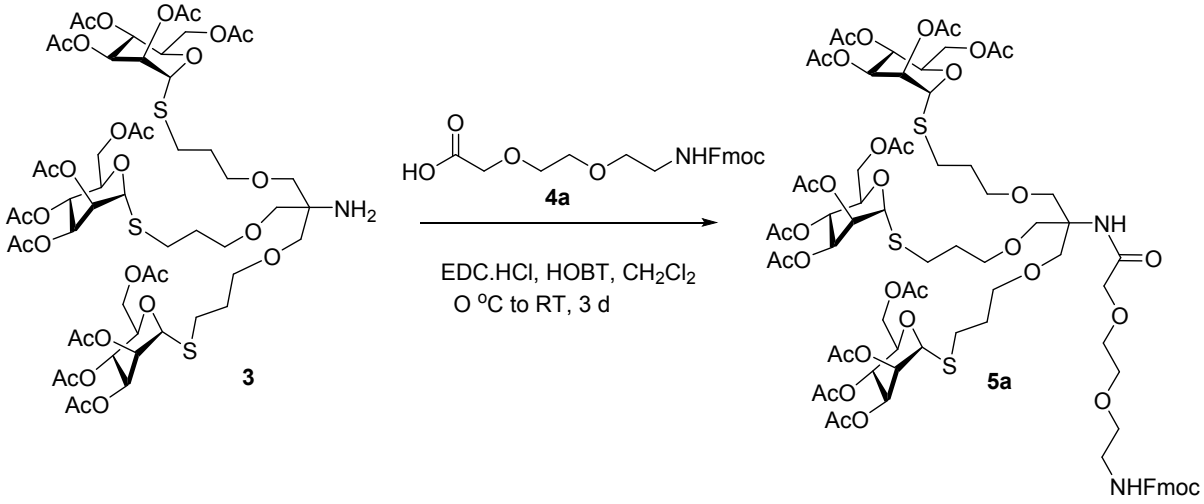
Synthesis of Man_3-SH 1 from mannoside cluster 3: To a stirred solution of 3-(acetylthio)propanoic acid (1.21 g, 8.16 mmol) in anhydrous CH_2Cl_2 (16 mL), was added

EDC.HCl (1.87 g, 9.75 mmol) and HOBT (1.36 g, 10.06 mmol) at 0 ^{o}C and reaction mixture was stirred for 30 mins. Then a solution of amine **3** (2.18 g in 14 mL of anhydrous CH_2Cl_2, 1.63 mmol) was added to reaction mixture and further allowed to stir at room temperature. After 3 days, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (80 mL), washed twice with water (2x 120 mL) and brine solution (140 mL). The obtained crude was purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAC to afford coupled amide (2.46 g). The obtained amide (2.46 g, mmol) was dissolved in anhydrous MeOH (50 mL) and bubbled with argon for 20 mins, to this stirred solution was added argon bubbled methanolic NaOMe solution (1 M, 8 mL) dropwise and further allowed to stir for 3 days under argon atmosphere. Then reaction mixture was acidified up to pH ≈ 4 with Dowex® 50WX8 hydrogen form, filtered to remove resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford a thiol **Man$_3$-SH 1** (1.38 g. 1.50 mmol, overall yield = 93% from **3**).

^{1}H NMR (300 MHz, DMSO-D6): δ 8.29 (s, 1H), 5.08 (s, 3H), 4.45-4.94 (m, 14H), 3.54-3.68 (m, many H), 3.41 (br s, many H), 2.50-2.90 (m, many H, overlap with solvent residual peak), 1.73-1.80 (m, 6H), 1.22 (s, 1H).

^{13}C NMR (75 MHz, CD_3OD): δ 174.13, 86.44, 74.79, 73.63, 73.08, 70.70, 69.59, 68.68, 62.63, 61.77, 35.45, 30.65, 28.74, 27.67.

HRMS (ESI): m/z calcd for $C_{34}H_{64}NO_{19}S_4$ $[M+H]^+$ 918.2955, found 918.3000.



Scheme S4:

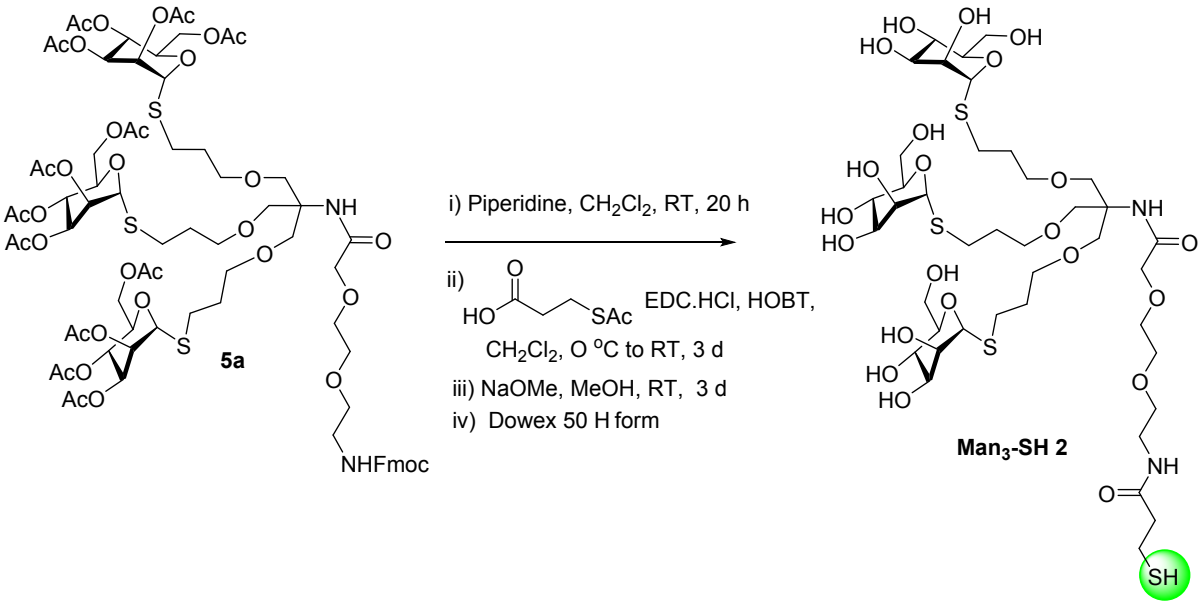
Synthesis of tri-thiomannoside cluster 5a from amine 3: To a stirred solution of acid **4a** (1.11 g, 2.89 mmol) in anhydrous CH_2Cl_2 (12 mL), was added EDC.HCl (2.43 g, 12.71 mmol) and HOBT (1.79 g, 13.29 mmol) at 0 ^{o}C and reaction mixture was stirred for 20 mins. Then a solution of amine **3** (1.93 g in 10 mL of CH_2Cl_2, 1.44 mmol) was added to reaction mixture and further allowed to stir at room temperature. After 3 days, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (70 mL), and further washed twice with water (2x 100 mL) and brine solution (120 mL). The obtained crude was

purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAC to afford coupled product **5a** (1.19 g, 0.699 mmol, yield = 49%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 7.3 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 7.25 (t, J = 7.4 Hz, 2H), 6.74 (br s, 1H), 5.17-5.29 (m, 12H), 4.15-4.35 (m, 10H), 3.36-4.09 (m, many H), 2.54-2.71 (m, 6H), 2.10 (s, 9H), 2.03 (s, 9H), 1.99 (s, 9H), 1.93 (s, 9H), 1.82 (br t, J = 5.9 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 170.43, 169.86, 169.68, 169.60, 156.45, 143.88, 141.17, 127.57, 126.94, 124.97, 119.86, 82.57, 71.03, 70.80, 70.02, 69.34, 69.08, 68.92, 66.49, 66.15, 62.30, 59.42, 47.14, 40.75, 29.38, 28.13, 20.78, 20.60, 20.56, 20.49.

HRMS (ESI): m/z calcd for $C_{76}H_{105}N_2O_{35}S_3$ $[M+H]^+$ 1701.5660, found 1701.5741.



Scheme S5:

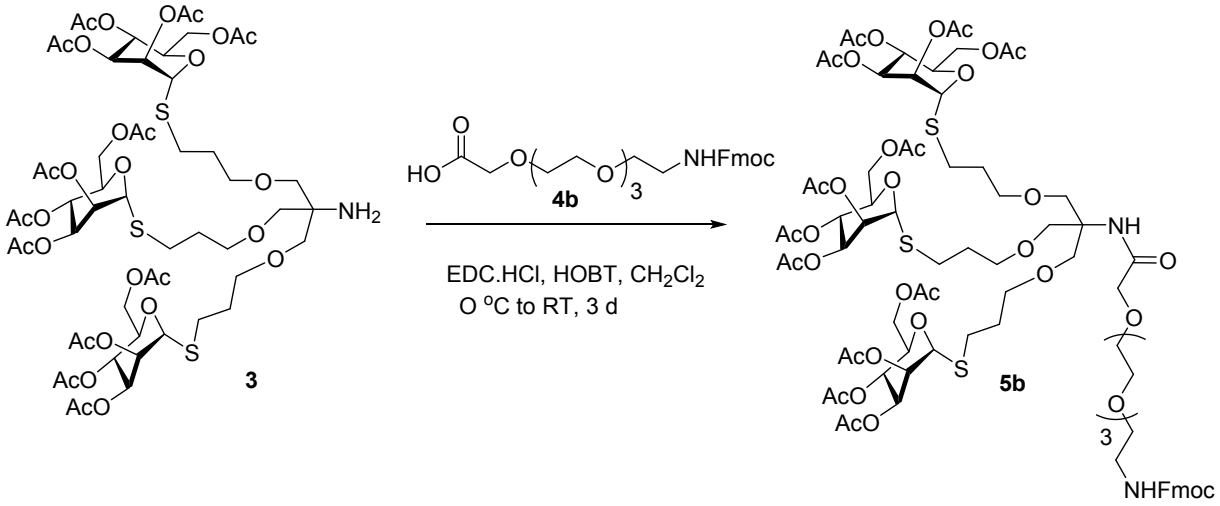
Synthesis of Man$_3$-SH 2 from compound 5a: To a stirred solution of **5a** (1.06 g, 0.62 mmol) in anhydrous CH_2Cl_2 (30 mL) was added piperidine (1.2 mL) at RT and allowed to stir further for 20 h. Then volatiles were evaporated under reduced pressure, toluene was added to the crude and then volatiles were further evaporated under reduced pressure, dried under vacuum and the obtained crude amine used for next step without further purification. To a stirred solution of 3-(acetylthio)propanoic acid (460 mg, 3.1 mmol), was added EDC.HCl (2.61 g, 13.61 mmol) and HOBT (1.92g, 14.21 mmol) at 0 °C and reaction mixture was stirred for 30 mins. Then a solution of obtained amine in CH_2Cl_2 (12 mL), was added to reaction mixture and further allowed to stir at room temperature. After 3 d, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (80 mL), washed twice with water (2x 120 mL) and brine solution (120 mL). The obtained crude was purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAC and finally eluted with 10% MeOH in EtOAc (v/v) to afford coupled amide (500 mg). The obtained amide (500 mg) was dissolved in anhydrous MeOH (20 mL), bubbled with argon for 20 mins, and to this stirred solution was added argon bubbled solution of 1 M NaOMe in MeOH

(4 mL) at RT and allowed to stir for 3 d under argon atmosphere. Then reaction mixture was acidified up to pH ≈ 4 with Dowex® 50WX8 hydrogen form, filtered to remove the resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford a thiol **Man$_3$-SH 2** (280 mg, 0.263 mmol, overall yield = 42% from **5a**).

^{1}H NMR (300 MHz, CD_3OD): δ 5.20 (br s, 3H), 3.77-3.94 (m, 14 H), 3.64-3.74 (m, 11H), 3.51 (br s, 11H), 3.38 (br s), 3.27 (br s, 2H), 2.93 (br s, 2H), 2.61-2.74 (m, 8H), 1.85 (br t, J = 5.1 Hz), 1.24 (s, 1H).

^{13}C NMR (75 MHz, D_2O): δ 173.87, 172.05, 86.49, 74.91, 73.67, 73.12, 71.94, 71.48, 71.16, 70.73, 70.56, 70.01, 68.74, 62.68, 61.17, 40.41, 36.42, 35.02, 30.65, 28.72.

HRMS (ESI): m/z calcd for $C_{40}H_{75}N_2O_{22}S_4$ $[M+H]^+$ 1063.3694, found 1063.3732.



Scheme S6:

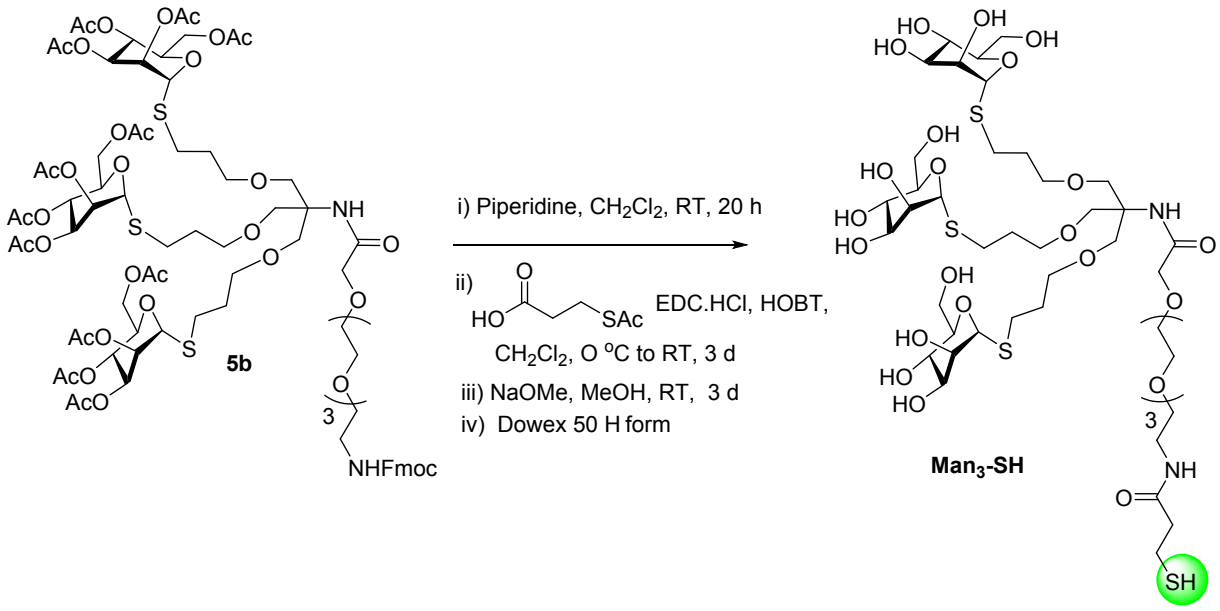
Synthesis of tri-thiomannoside cluster 5b from amine 3: To a stirred solution of acid **4b** (1.13 g, 2.38 mmol) in anhydrous CH_2Cl_2 (15 mL), was added EDC.HCl (2.00 g, 10.47 mmol) and HOBT (1.48 g, 10.94 mmol) at 0 ^{o}C and reaction mixture was stirred for 30 mins. Then a solution of amine **3** (1.60 g in 10 mL of CH_2Cl_2, 1.20 mmol) was added to reaction mixture and further allowed to stir at room temperature. After 3 days, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (60 mL), and further washed twice with water (2x 100 mL) and brine solution (120 mL). The obtained crude was purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAC to afford coupled product **5b** (500 mg, 0.279 mmol, yield = 23%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, J = 7.4 Hz, 2H), 7.52 (d, J = 7.3 Hz, 2H), 7.30 (t, J = 7.3 Hz), 7.22 (t, J = 7.1 Hz, 2H), 6.70 (br s, 1H), 5.13-5.25 (m, 12H), 3.87-4.31 (m, 15H), 3.80 (s, 2H), 3.54 (s, 14H), 3.42 (t, J = 5.5 Hz, 6H), 3.47-3.63 (m, 9H), 3.30 (br s, 2H), 2.51-2.68 (m, 6H), 2.07 (s, 9H), 2.00 (s, 9H), 1.96 (s, 9H), 1.89 (s, 9H), 1.74-1.82 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 170.25, 169.69, 169.51, 169.46, 156.33, 143.79, 141.04, 127.43, 126.81, 124.87, 119.72, 82.45, 70.88, 70.65, 70.49, 70.29, 70.15, 70.04, 69.80, 69.22,

68.92, 68.80, 67.84, 66.03, 62.18, 59.29, 47.03, 40.68, 29.28, 28.02, 20.64, 20.47, 20.44, 20.37.

HRMS (ESI): m/z calcd for $C_{80}H_{113}N_2O_{37}S_3$ $[M+H]^+$ 1789.6184, found 1789.6185.



Scheme S7:

Synthesis of Man_3-SH from compound 5b: To a stirred solution of **5b** (500 mg, 0.279 mmol) in anhydrous CH_2Cl_2 (14 mL) was added piperidine (0.5 mL) at RT and allowed to stir further for 20 h. Then volatiles were evaporated under reduced pressure and toluene was added to the crude and then volatiles were further evaporated under reduced pressure and then dried under vacuum. The obtained crude amine was used for next step without further purification. Then, to a stirred solution of 3-(acetylthio)propanoic acid (248 mg, 1.67 mmol), was added EDC.HCl (1.40 g, 7.34 mmol) and HOBT (1.04 g, 7.70 mmol) at 0 °C and reaction mixture was stirred for 30 mins. Then a solution of obtained amine in CH_2Cl_2 (12 mL), was added to reaction mixture and further allowed to stir at room temperature. After 3 days, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (40 mL), washed twice with water (2x70 mL) and brine solution (80 mL). The obtained crude was purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAC and finally eluted with 10% MeOH in EtOAc (v/v) to afford coupled amide (260 mg). The obtained amide (260 mg) was dissolved in anhydrous MeOH (20 mL), bubbled with argon for 20 mins, and to this stirred solution was added argon bubbled solution of 1 M NaOMe in MeOH (2.0 mL) at RT and allowed to stir for 3 days under argon atmosphere. Then reaction mixture was acidified up to pH ≈ 4 with Dowex® 50WX8 hydrogen form, filtered to remove

the resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford a thiol **Man_3-SH** (165 mg, 0.143 mmol, overall yield = 51% from compound **5b**).

^{1}H NMR (300 MHz, D_2O): δ 5.25 (s, 3H), 3.87-4.08 (m, 10H), 3.56-3.84 (m, many H), 3.37 (br t, J = 4.9 Hz, 2H), 2.93 (t, J = 6.7 Hz, 2H), 2.64-2.78 (m, 8H), 1.87 (br t, J = 5.79 Hz, 6H).

^{13}C NMR (75 MHz, D_2O): δ 173.84, 171.64, 84.90, 73.20, 72.30, 71.87, 71.22, 70.42, 69.83, 69.66, 68.87, 68.44, 66.98, 66.60, 60.82, 60.02, 39.02, 35.02, 33.37, 28.69, 27.55.

HRMS (ESI): m/z calcd for $C_{44}H_{83}N_2O_{24}S_4$ $[M+H]^+$ 1151.4219, found 1151.4183.

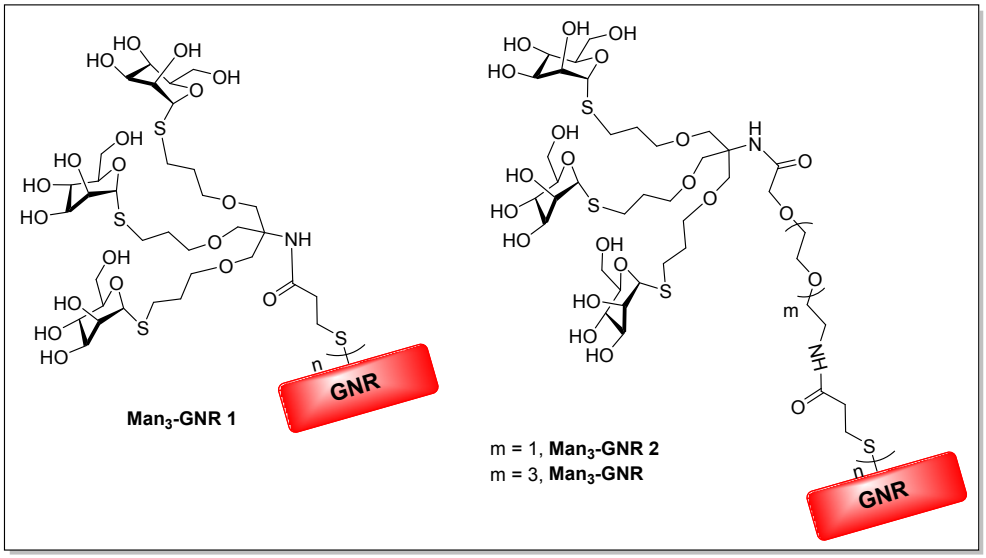


Fig. S1: Structures of mannoside clusters immobilized GNR with varied length of linkers.

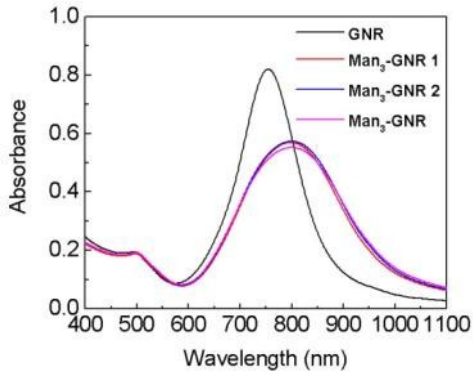
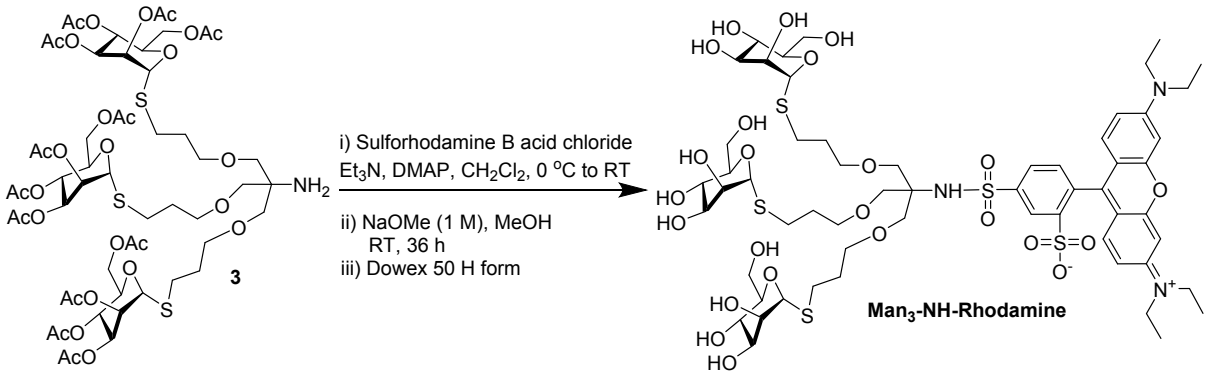


Fig. S2: NIR range absorption spectra GNRs and GNRs immobilized with mannoside clusters of varied length of thiol terminated linkers and broadening absorption band is due to aggregation of nanorods through ConA recognition with covalently attached tri-thiomannosides.



Scheme S8:

Synthesis of Man$_3$-NH-Rhodamine from amine 3: To a stirred solution of amine **3** (220 mg, 0.164 mmol), Et_3N (0.6 mL) in anhydrous CH_2Cl_2 (5 mL) was added sulforhodamine B acid chloride (115 mg, 0.197 mmol) and followed by DMAP (5 mg) at 0 ^oC. After stirring the reaction mixture for 3 days at RT, volatiles were evaporated under reduced pressure and the obtained crude was purified over SiO_2 column with the eluent CH_2Cl_2 to 10% MeOH in CH_2Cl_2 to afford the sulfonamide product (160 mg). The product (160 mg) was dissolved in anhydrous MeOH (10 mL), to this stirred solution was added 1 M NaOMe in MeOH (1 mL) at RT. After stirring the reaction mixture for 36 h, the reaction mixture was acidified up to pH ≈ 4 with Dowex® 50WX8 hydrogen form, filtered to remove the resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford a compound **Man$_3$-NH-Rhodamine** (65 mg, 0.047 mmol, overall yield = 28% from amine **3**).

^{1}H NMR (300 MHz, D_2O): δ 8.47 (d, J = 1.6 Hz, 1H), 8.06-8.17 (m, 1H), 7.86-7.90 (m, 1H), 7.77 (dd, J = 5.7, 3.4 Hz, 1H), 7.66 (dd, J = 5.8, 3.2 Hz, 1H), 7.50 (d, J = 7.9 Hz, 1H), 6.97 (d, J = 9.6 Hz, 1H), 6.84 (dd, J = 9.9, 1.7 Hz, 1H), 6.68 (br s, 1H), 5.27 (d, J = 1.1 Hz, 3H), 4.15 (dd, J = 3.3, 1.7 Hz, 1H), 3.37-4.02 (many H), 2.65-2.78 (m, 6H), 1.84-1.93 (m, 6H), 1.03-1.33 (m, 12H).

HRMS (ESI): m/z calcd for $C_{58}H_{88}N_3O_{24}S_5$ $[M+H]^+$ 1370.4361, found 1370.4309.

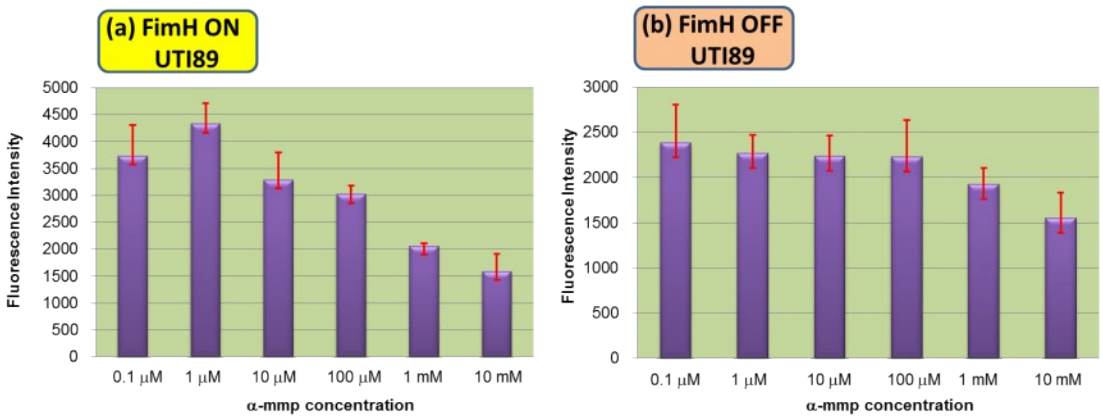


Fig. S3: Competitive fluorescence intensity of bacteria challenged with the dye attached cluster **Man_3-NH-Rhodamine** in the presence of varying **α-mmp** concentrations using FimH ON UTI89 (a) and FimH OFF UTI89 (b) *E.coli* strains. **Man_3-NH-Rhodamine** of 100 μM mannose molar concentration was used to perform the competitive binding assay of glycocluster using **α-mmp** with two UTI89 gene strains (a-b).

Experimental procedure for competitive binding assay of Man_3-NH-Rhodamine with *E.coli* UTI89 strains

UTI89 bacterial strains were cultured as UT189 FimH over expressed strain and FimH off conditions in a LB medium at 37 ^{0}C. Dilute the overnight culture 1:100 into fresh medium and grow to O.D 1 for competitive assay. The bacterial culture was added methyl α-D-mannopyranoside **(α-mmp**) at concentration 10 mM, 1 mM, 100 μM, 10 μM, 1 μM and 0.1 μM, then concentration 100 μM **Man_3-NH-Rhodamine** was added. After incubated for 1 hr, the bacterial samples were washed by PBS 3 times. The bacterial culture was suspended in 100 μL PBS and loaded in 96 well plate. The fluorescence intensity was measured by Tecan microplate reader. Biological repeats in triplets were used for statistics analysis.

Quantitative estimation of number of covalently connected tri-thiomannosides to GNR by using Ellman's reagent.

Solutions of **$(Man)_3$-SH** (50 μL, 1 mM) and GNRs (950 μL, 5 nM) were mixed and incubated for 2 h. GNRs were removed by centrifugation (6000 rcf, 30 min). The supernatant was collected for determination of **Man_3-SH** concentration.

For quantification of **$(Man)_3$-SH** concentration, Ellman's reagent (DTNB) was used. DTNB (160 μL, 0.4 mM) in sodium phosphate buffer (pH 8, 0.1 M) was added into 800 μL of the supernatant and a series of standard **Man_3-SH** solution (0, 40, 50, 60, and 70 μM). UV-vis absorption spectra were measured for the determination of sugar concentration after incubation for 15 min.

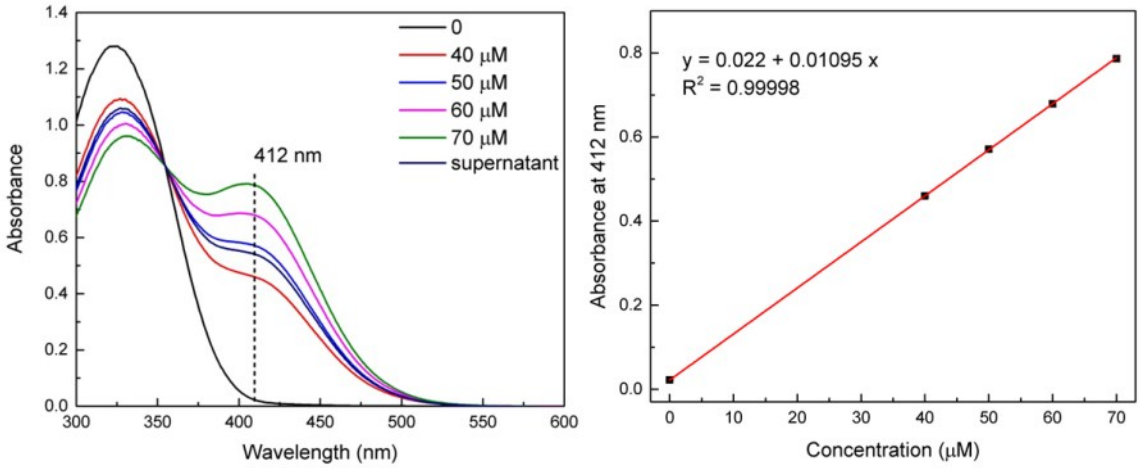


Fig. S4: UV-vis absorption spectra of solutions after treatment of **Man_3-SH** with Ellman's reagent (DTNB) and the standard concentration dependent absorbance plot at 412 nm. The absorbance at 412 nm for the supernatant is 0.540 ± 0.006, which corresponds to 47.34 μM according to the standard curve. It can be calculated that there are 533 units of tri-thiomannosides attached to one nanorod.

Determination of binding constant of Man_3-GNR with ConA by isothermal calorimetric titration (ITC)

When a solution of two ligands **α-mmp** (ligand 1, L1) and **Man_3-GNR** (ligand 2, L2) was titrated into ConA (receptor, A), two binding reactions occur:

$L_1 + A \rightarrow L_1A$ $\quad K_1 = [L_1A] / [L_1][A]$ and $\Delta G_1 = -RT \ln K_1$

$L_2 + A \rightarrow L_2A$ $\quad K_2 = [L_2R] / [L_2][A]$ and $\Delta G_2 = -RT \ln K_2$

ΔG_1 and ΔG_2 are the changes of Gibbs free energy for 1 mole of each reaction.

Accordingly, ΔG for the titration is

$\Delta G = [L_1A]\ \Delta G1 + [L_2A]\ \Delta G_2 = -RT\ ([L_1A] \ln K_1 + [L_2A] \ln K_2)$

$= -RT\ (K_1\ [L_1][A] \ln K_1 + K_2\ [L_2][A] \ln K_2)$ (1)

Where K_1 and K_{mix} are the binding constants determined by ITC for the titration of **α-mmp**, and a mixture of **α-mmp** and **Man$_3$-GNR** into ConA respectively. K_2 is the binding constant of **Man$_3$-GNR** with ConA which is need to estimate.

K_{mix} is the apparent binding constant when L_1 and L_2 are taken as indistinguishable (denoted A).

$L + A \rightarrow LA$ $\quad\quad$ $Kmix = [LA] / [L][A]$ and $\Delta Gmix = -RT \ln K_{mix}$

So, we have

$$\Delta G = [LA]\,\Delta G_{mix} = -RT\,K_{mix}\,([L_1]+[L_2])[A]\,\ln K_{mix} \quad (2)$$

Compare Eq.1 and Eq.2, we have

$$[L_1]\,K_1 \ln K_1 + [L_2]\,K_2 \ln K_2 = ([L_1] + [L_2])\,K_{mix} \ln K_{mix} \quad (3)$$

Although $[L_1]$ and $[L_2]$ increases as the ligand solution is added fractions, the ratio of $[L_1]$ and $[L_2]$ is a constant: 2000:48. According to ITC results, $K_1 = 7.5 \times 10^3\ M^{-1}$ (**α-mmp** titrated into ConA) and $Kmix = 2.347 \times 10^4\ M^{-1}$ (**Man$_3$-GNR** and **α-mmp** titrated into ConA). Taken these values into Eq. 3, we have

Binding constant of **Man$_3$-GNR** with ConA $K_2 = 5.51 \times 10^5\ M^{-1}$.

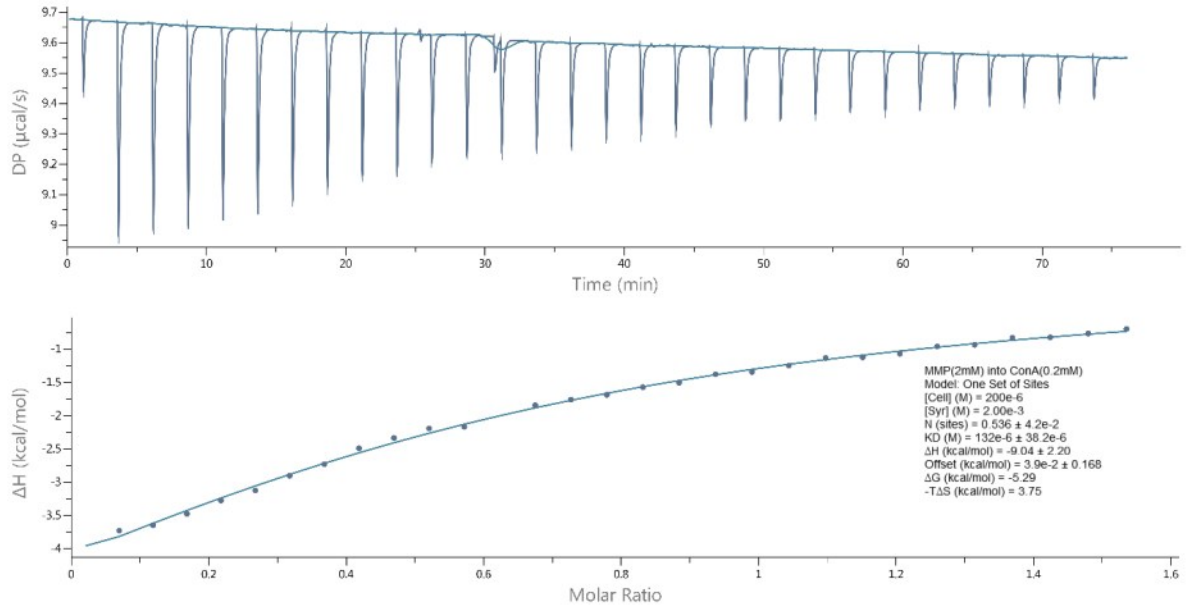


Fig. S5: Chromatogram of isothermal calorimetric titration (ITC) of ConA (0.2 mM) with **α-mmp** (2 mM)

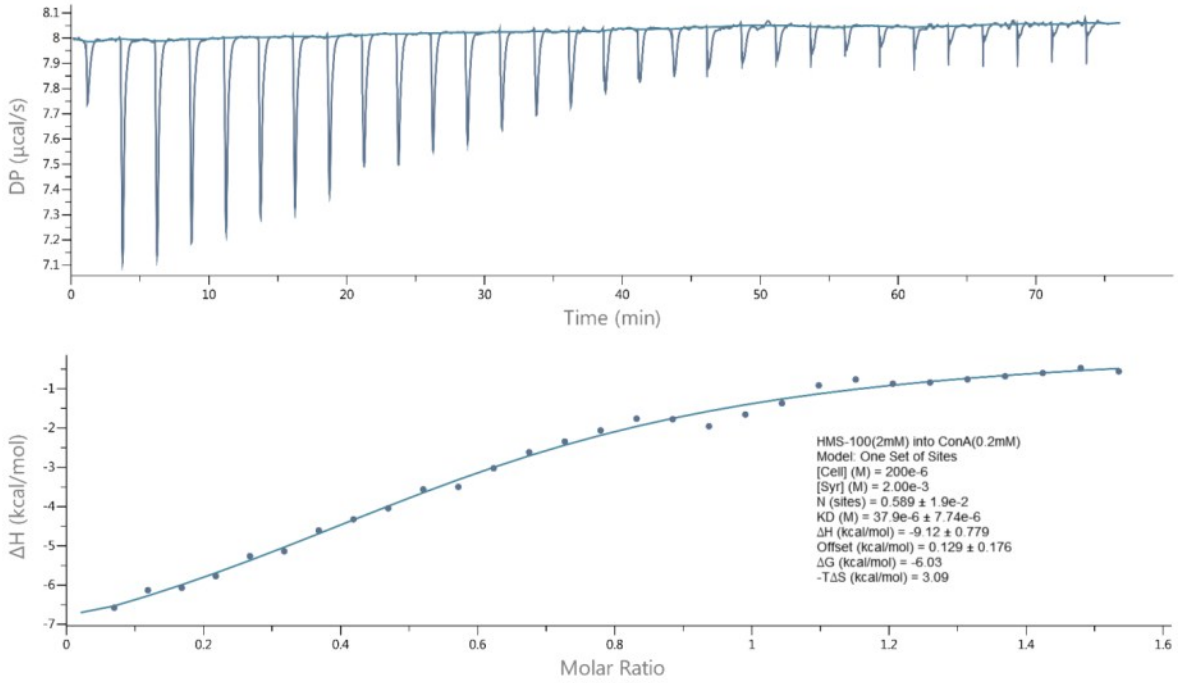


Fig. S6: Chromatogram of isothermal calorimetric titration (ITC) of ConA (0.2 mM) with **Man_3-SH** (2 mM, molar concentration).

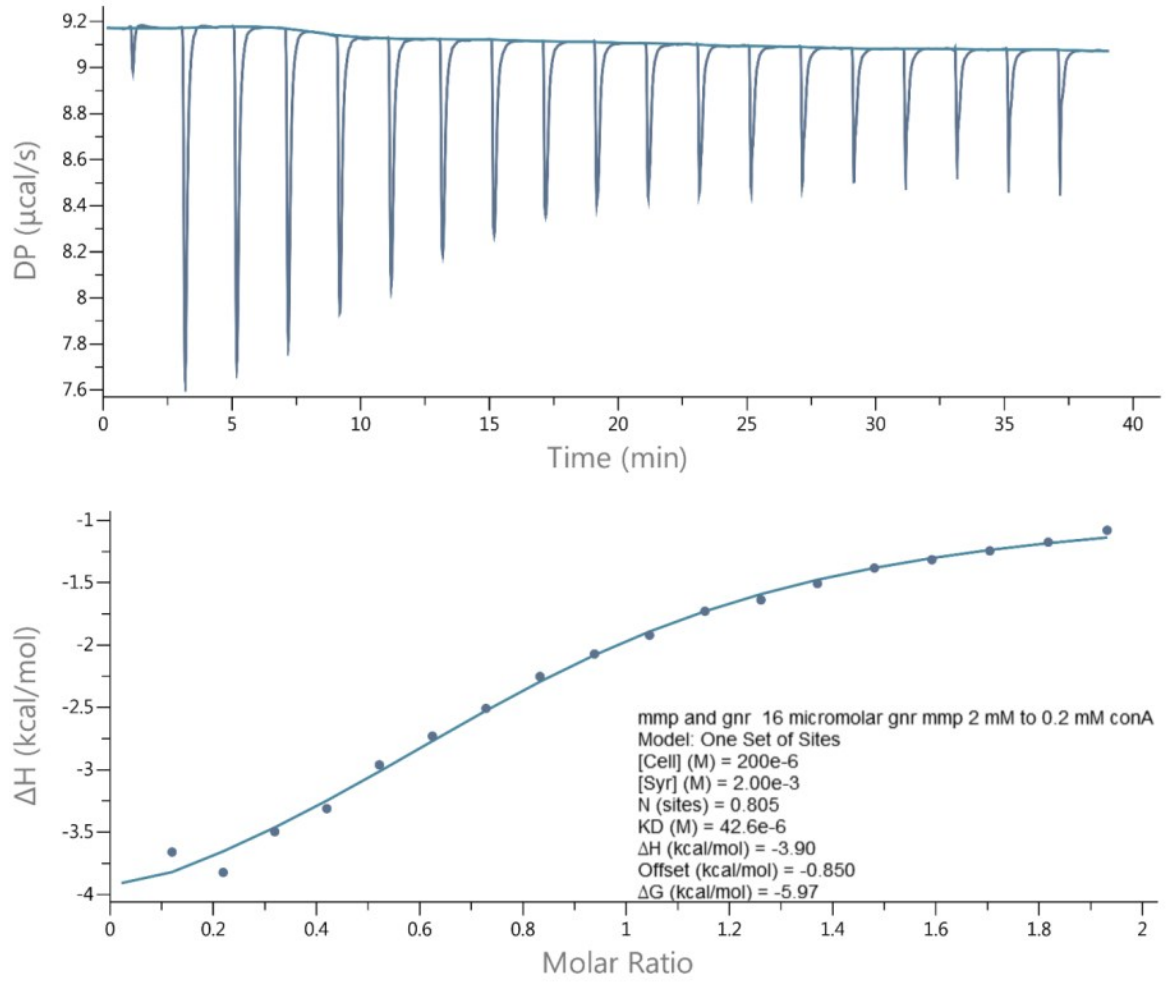
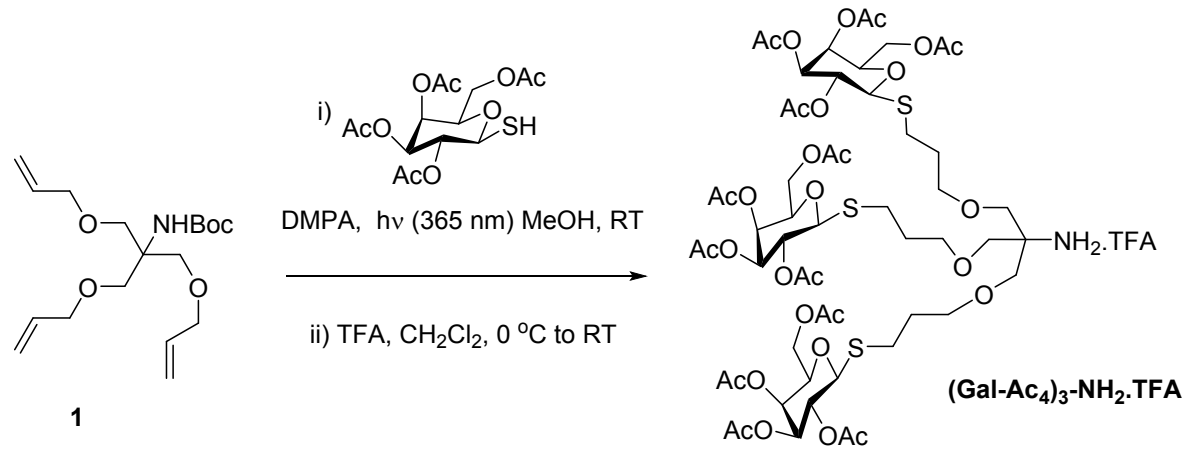


Fig. S7: Chromatogram of isothermal calorimetric titration (ITC) of ConA (0.2 mM) with two ligands **Man$_3$GNR** (48 µM, mannose basis) and **α–mmp** (2 mM).

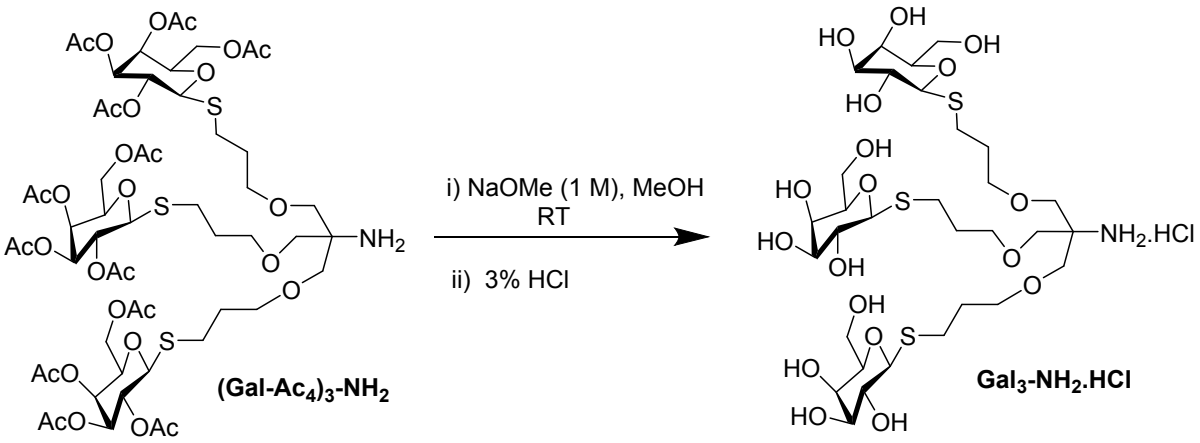


Scheme S9:

Synthesis of (Gal-Ac$_4$)$_3$-NH$_2$.TFA from tri-O-allyl derivative 1: A solution of **1** (1.32 g, 3.86 mmol), (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-mercaptotetrahydro-2H-pyran-3,4,5-triyl triacetate[2] (8.45 g, 23.19 mmol), 2,2-dimethoxy-2-phenylacetophenone (595 mg, 1.93 mmol) in anhydrous MeOH (25 mL) was bubbled with argon for 40 mins. Then reaction mixture was stirred and exposed to UV light of wavelength 365 nm for 4.5 h (^{1}H NMR for crude reaction mixture shows complete conversion of **1**). Then volatiles of reaction mixture were evaporated by rotary evaporator and resultant mixture was dried for 2.5 h under vacuum. The obtained crude was dissolved in anhydrous CH_2Cl_2 (60 mL) and TFA (10 mL) was added dropwise to the reaction mixture at 0 ^{o}C, and then reaction mixture was warmed to room temperature. After stirring the reaction mixture for 4 h at RT, the volatiles were evaporated by vacuum and crude was purified over silica gel column with eluent 100% CH_2Cl_2 to 20% MeOH in CH_2Cl_2 (v/v) to afford **(Gal-Ac$_4$)$_3$-NH$_2$.TFA** (7.30 g, 3.58 mmol, overall yield from **1** = 93%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (br s, 2H), 5.39 (d, J = 2.9 Hz, 3H), 5.16 (t, J = 9.9 Hz, 3H), 5.02 (dd, J = 10.0, 3.0 Hz, 3H), 4.47 (d, J = 9.8 Hz, 3H), 4.00-4.15 (m, 6H), 3.92 (t, J = 6.4 Hz, 3H), 3.56 (s, 6H), 3.52 (t, J = 5.4 Hz, 6H), 2.65-2.80 (m, 6H), 2.11 (s, 9H), 2.02 (s, 9H), 2.00 (s, 9H), 1.94 (s, 9H), 1.78-1.90 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 170.63, 170.27, 170.15, 169.94, 84.18, 74.37, 71.83, 69.98, 68.24, 67.31, 67.25, 61.42, 59.87, 29.41, 26.83, 20.83, 20.70 (2C), 20.62.



Scheme S10:

Synthesis of Gal_3-NH_2.HCl from (Gal-Ac_4)$_3$-NH_2: To a stirred solution of compound **(Gal-Ac_4)$_3$-NH_2** (amine was prepared similar to compound **3** from corresponding TFA salt, 1.15 g, 0.861 mmol) was dissolved in anhydrous MeOH (30 mL) and to this stirred solution 1 M NaOMe in MeOH (3 mL) was added dropwise, and the reaction mixture was further allowed to stir for 36 h. Then the reaction mixture was acidified up to pH ≈ 4 by using 3% aqueous HCl solution. Then volatiles were evaporated under vacuum, then further dried under vacuum to afford **Gal_3-NH_2.HCl** (730 mg, 0.842 mmol, yield = 97%).

^{1}H NMR (300 MHz, D_2O): δ 4.47 (d, J = 9.6 Hz, 3H), 3.97 (d, J = 3.2 Hz, 3H), 3.51-3.75 (m, 26H), 2.74-2.88 (m, 6H), 1.89-1.97 (m, 6H).

^{13}C NMR (75 MHz, D_2O): δ 85.88, 78.90, 73.90, 70.10, 69.60, 68.78, 68.07, 61.07, 59.71, 29.11, 26.73.

HRMS (ESI): m/z calcd for $C_{31}H_{60}NO_{18}S_3$ $[M]^+$ 830.2973, found 830.2902.

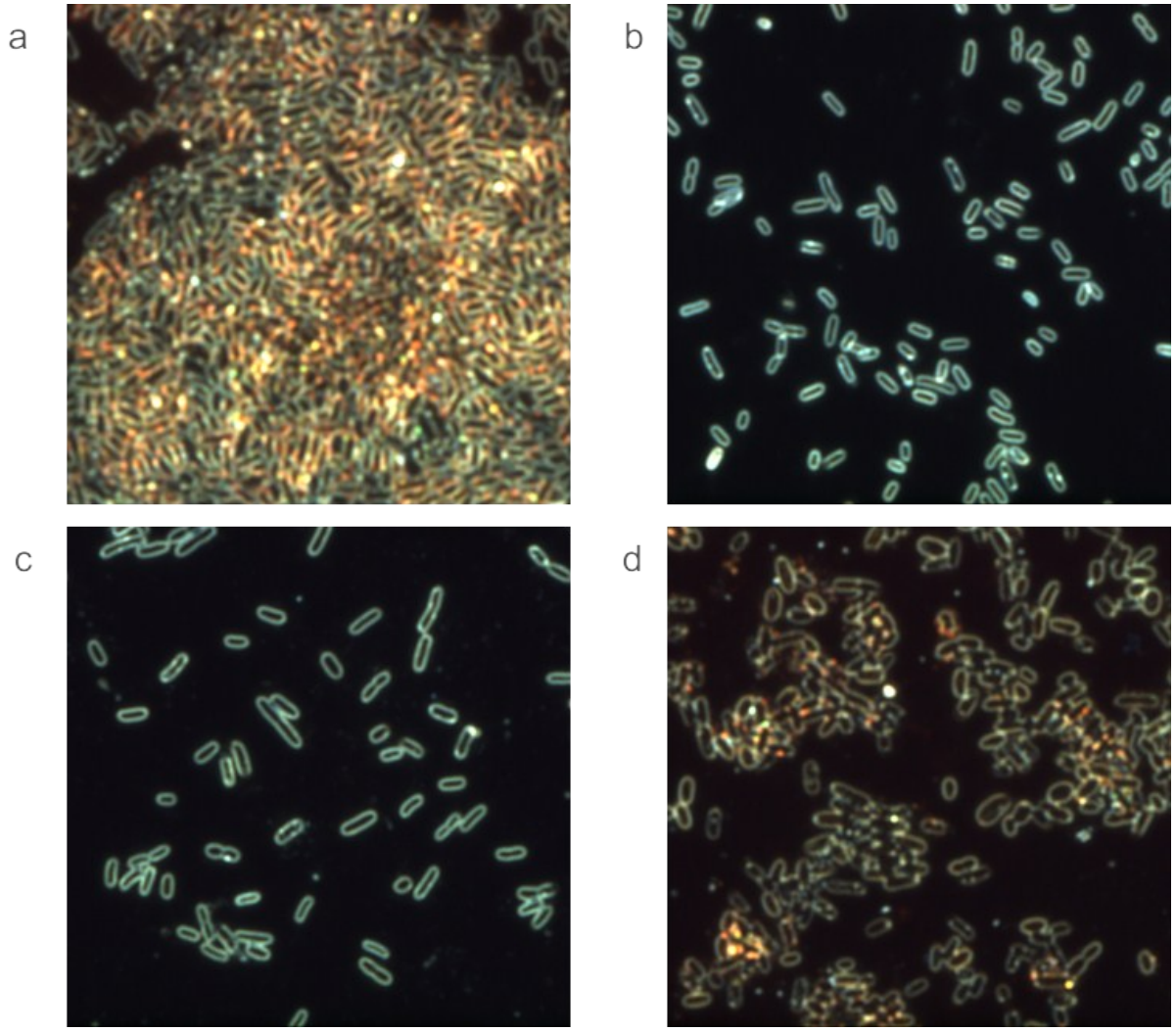


Fig. S8: Dark-field images of (a) FimH ON *E. coli* treated with **Man_3-GNR**. (b) FimH ON *E. coli* treated with gold nanorods which are initially modified with HS-PEG-COOH (Mol weight 2000 g/mol) and functionalized with **Gal_3-NH_2** cluster using NHS coupling agent. (c) FimH OFF *E. coli* UTI89 treated with **Man_3-GNR**. (d) FimH ON *E. coli* first treated with 50 mM D-mannose and then **Man_3-GNR**.

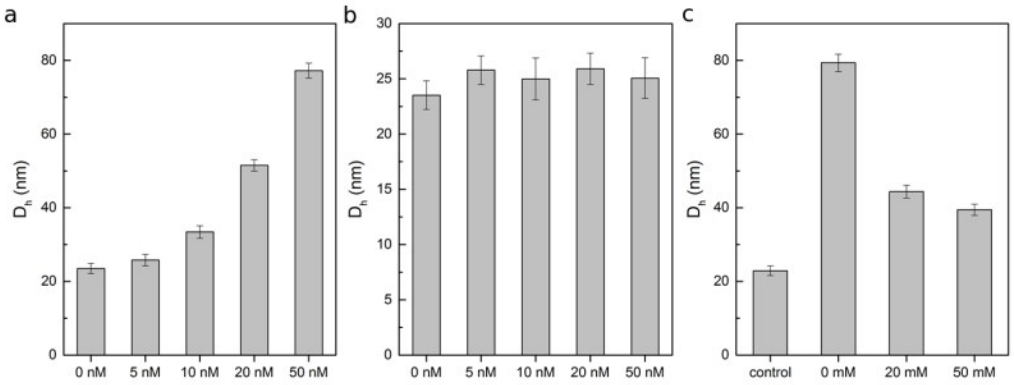


Fig. S9: Hydrodynamic diameters of Man_3-GNR with increasing concentrations of ConA (a), Gal_3-GNR with increasing concentrations of ConA (b), and Man_3-GNR with different concentrations of mannose after the addition of 50 nM ConA (c).

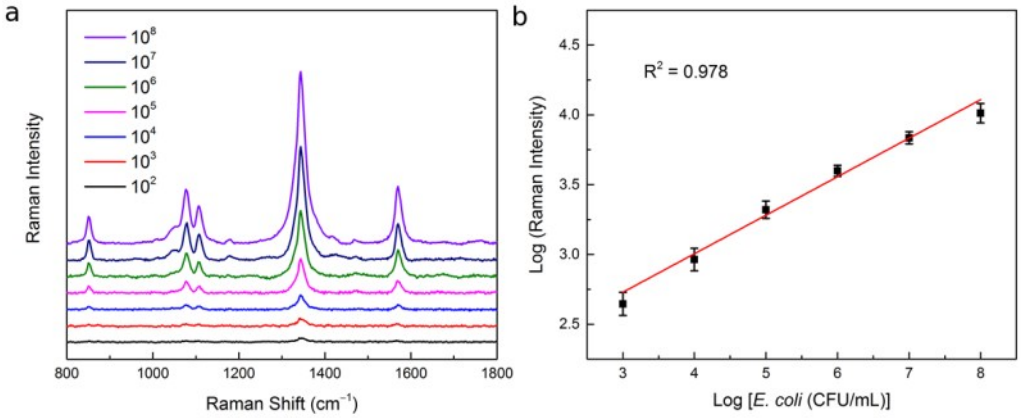
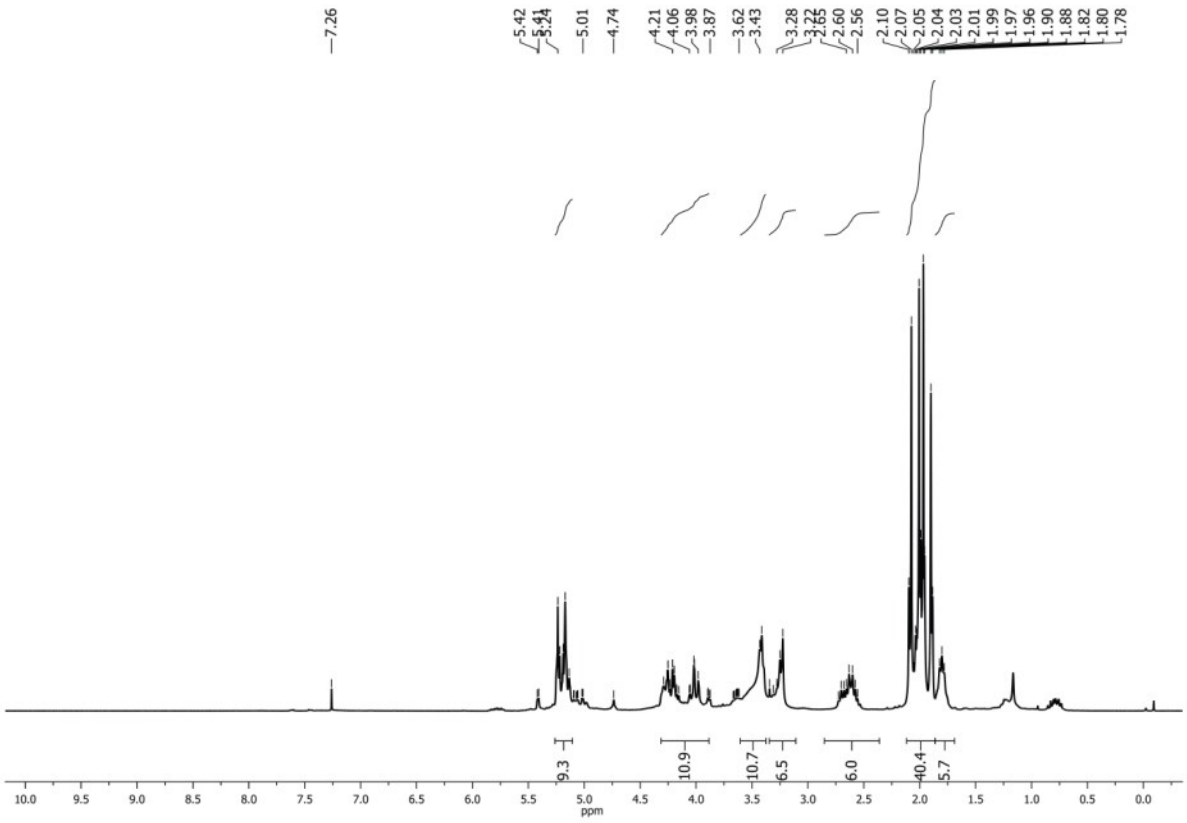


Fig. S10: (a) Raman spectra of Man_3-GNR treated FimH ON bacteria with concentrations from 10^2 to 10^8. (b) Raman intensity vs. bacterium concentration plot.

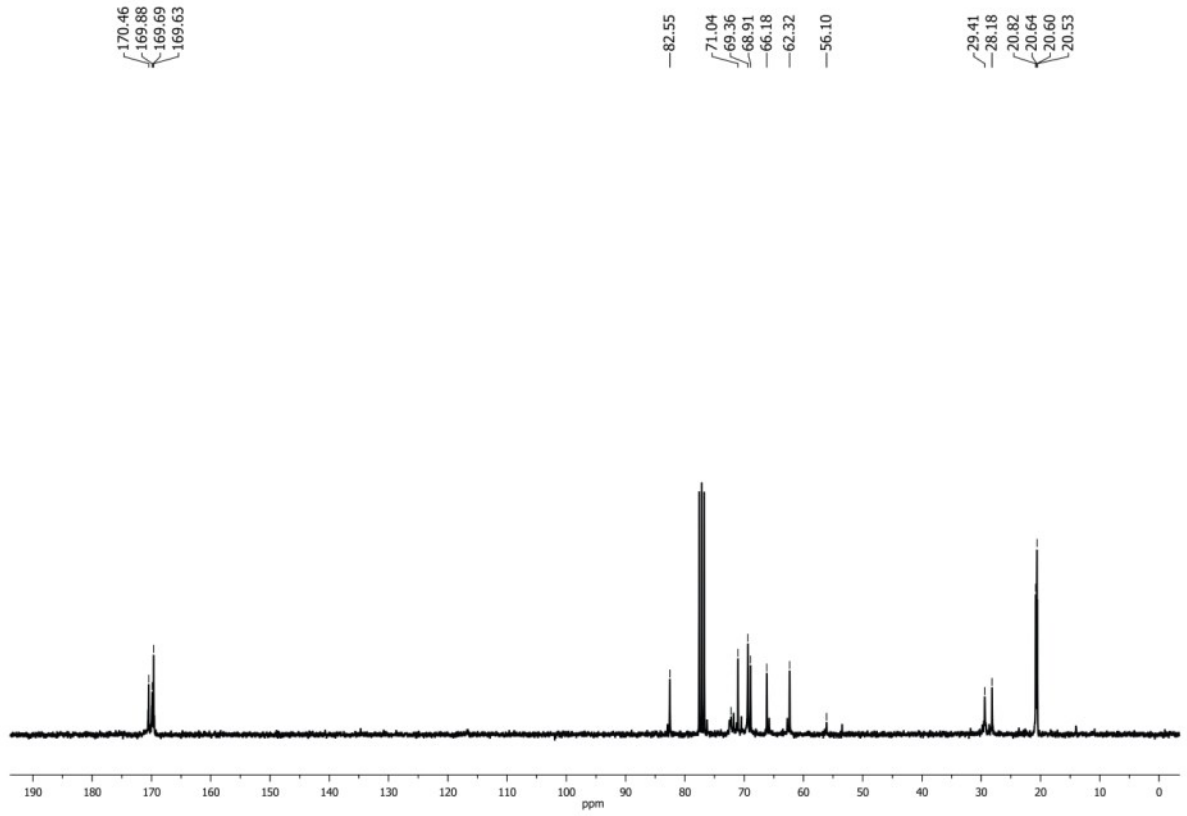
References:

1. Compound **1** was synthesized by following the literature procedure: M. Segura, F. Sansone, A. Casnati and R. Ungaro, *Synthesis,* 2001, **14**, 2105–2112.

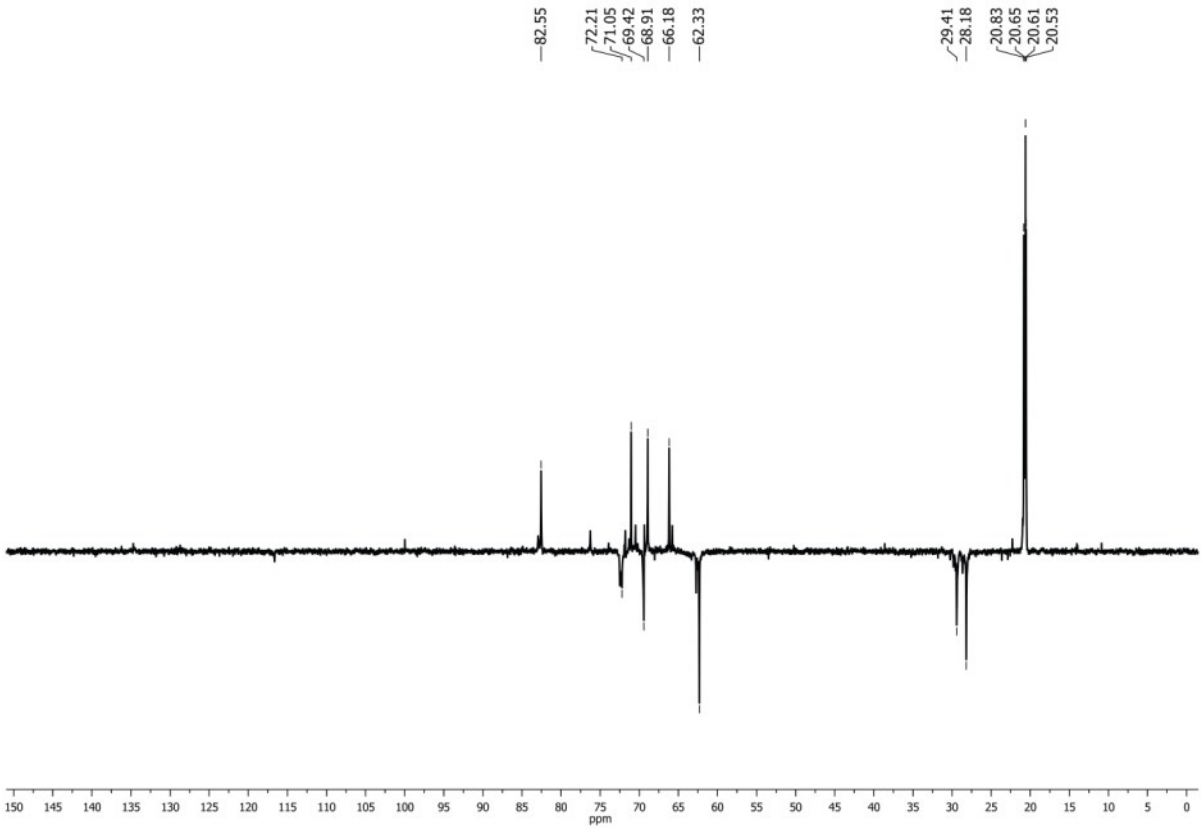
2. The 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl thiol (**2**) and 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl thiol were synthesized using these literature procedures: (a) Q. V. Vo, C. Trenerry, S. Rochfort and A. B. Hughes, *Tetrahedron*, 2013, **69**, 8731-8737. (b) N. Floyd, B. Vijayakrishnan, J. R. Koeppe and B. G. Davis, *Angew. Chem. Int. Ed.* 2009, **48**, 7798 –7802.



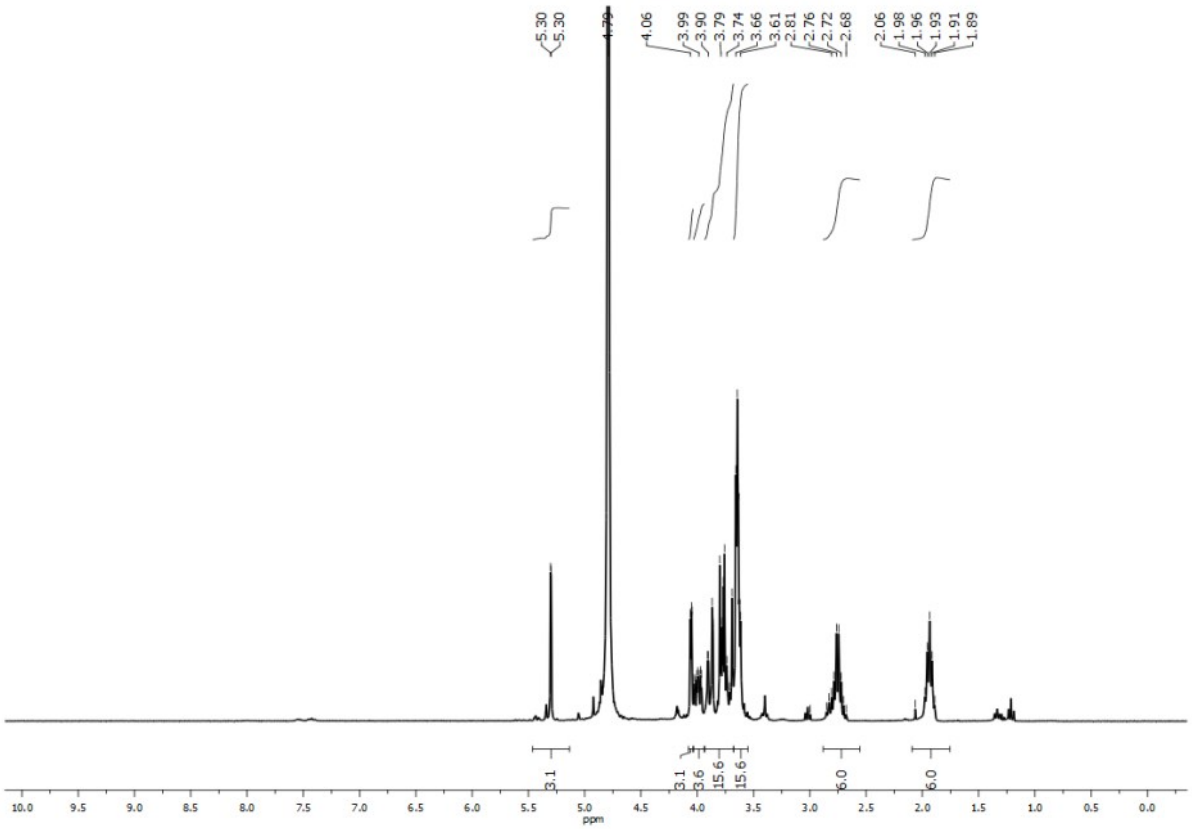
^{1}H NMR spectra of compound **3** (300 MHz, $CDCl_3$)



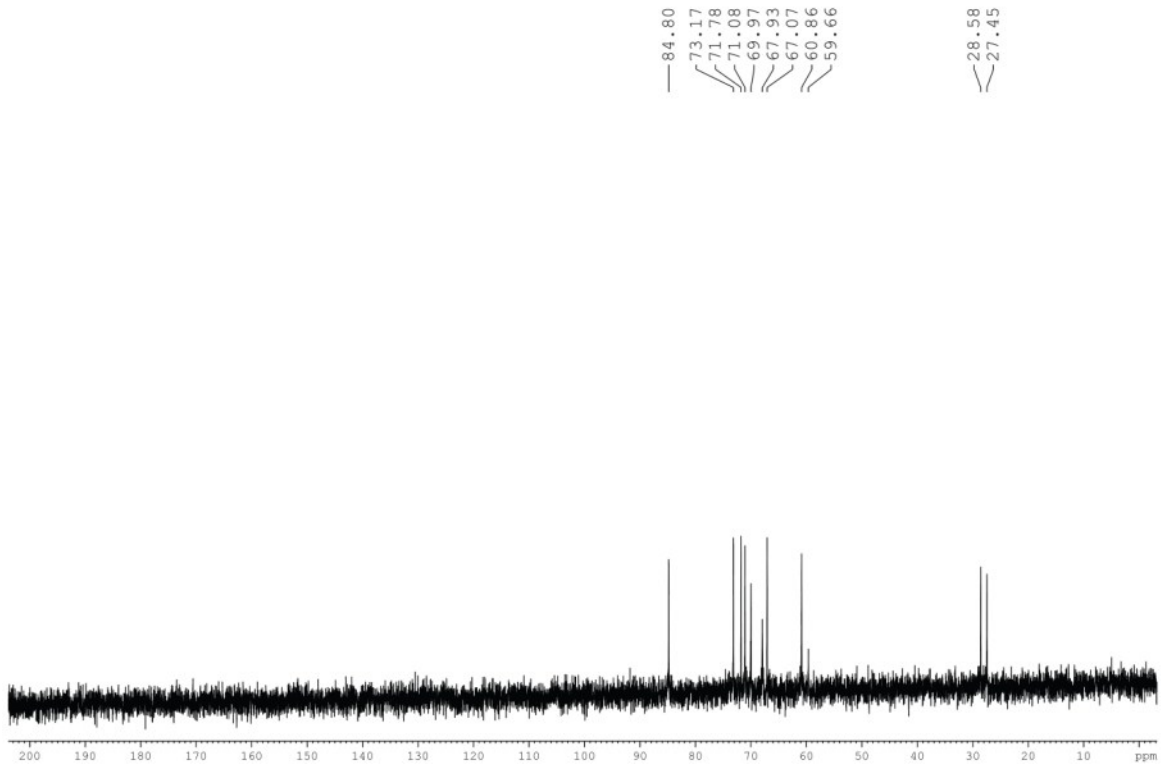
^{13}C NMR spectra of compound **3** (75 M Hz, $CDCl_3$)



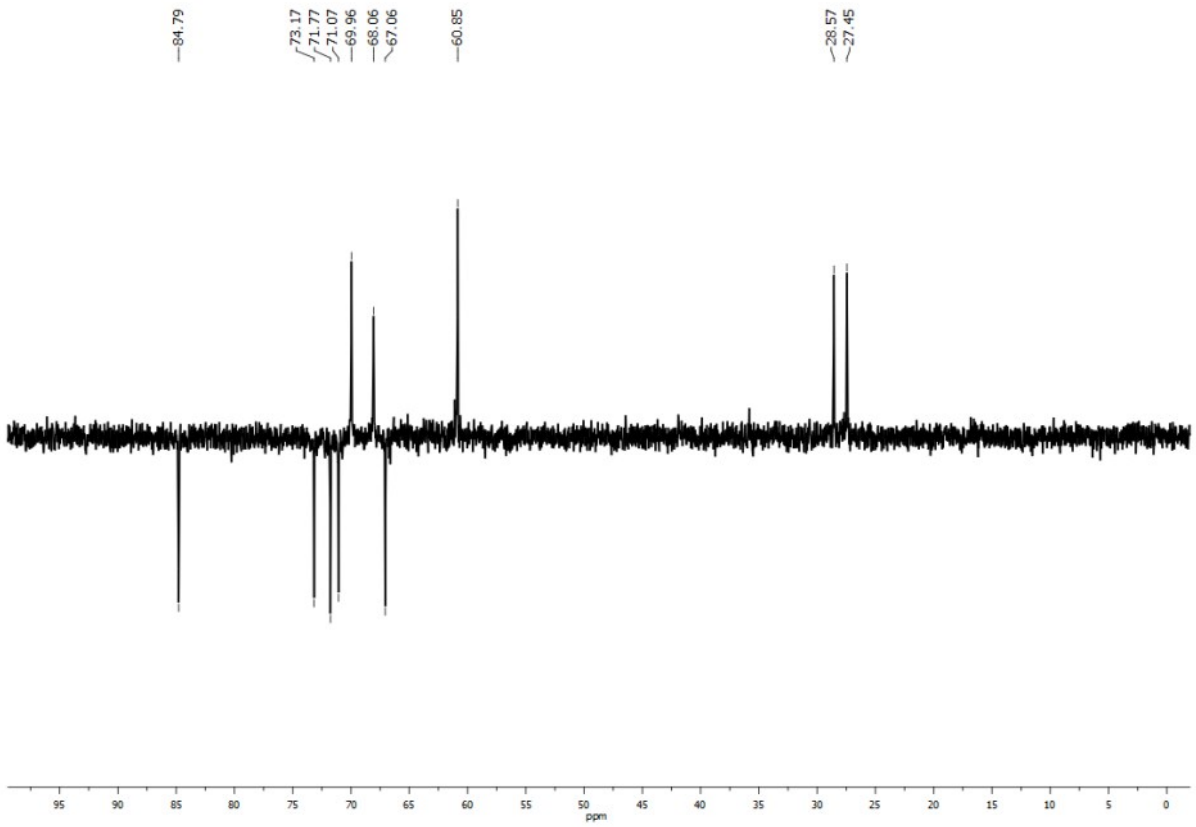
DEPT-135 spectra of compound **3** (75 M Hz, $CDCl_3$)



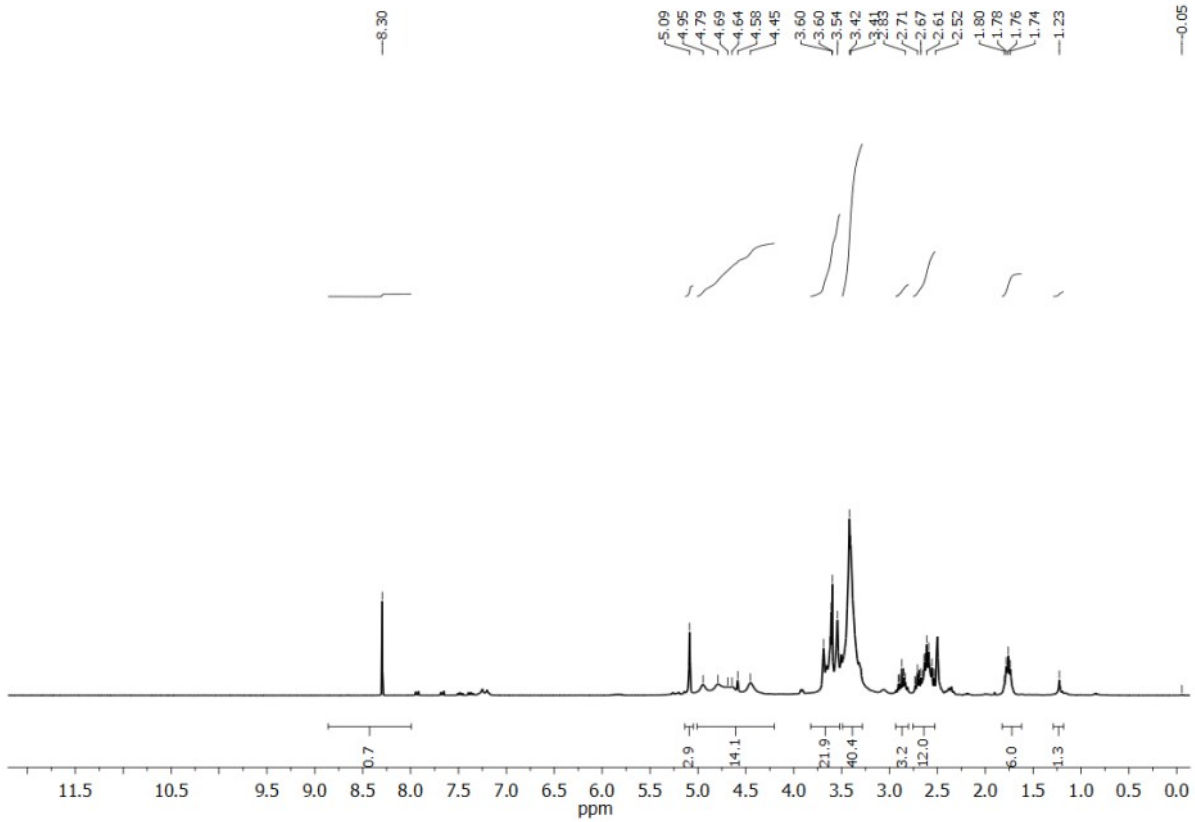
^{1}H NMR of compound **Man_3-NH_2.HCl** (300 MHz, D_2O)



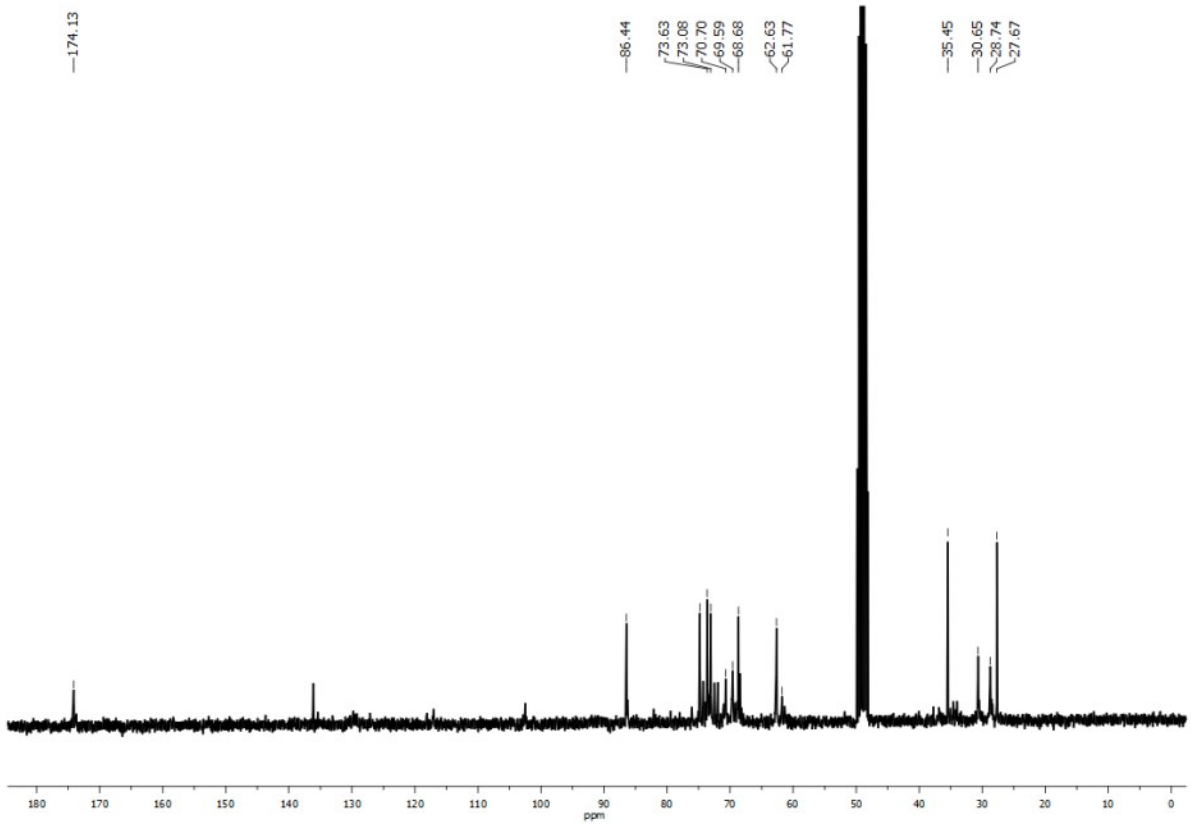
^{13}C NMR spectra of **Man_3-NH_2.HCl** (75 MHz, D_2O)



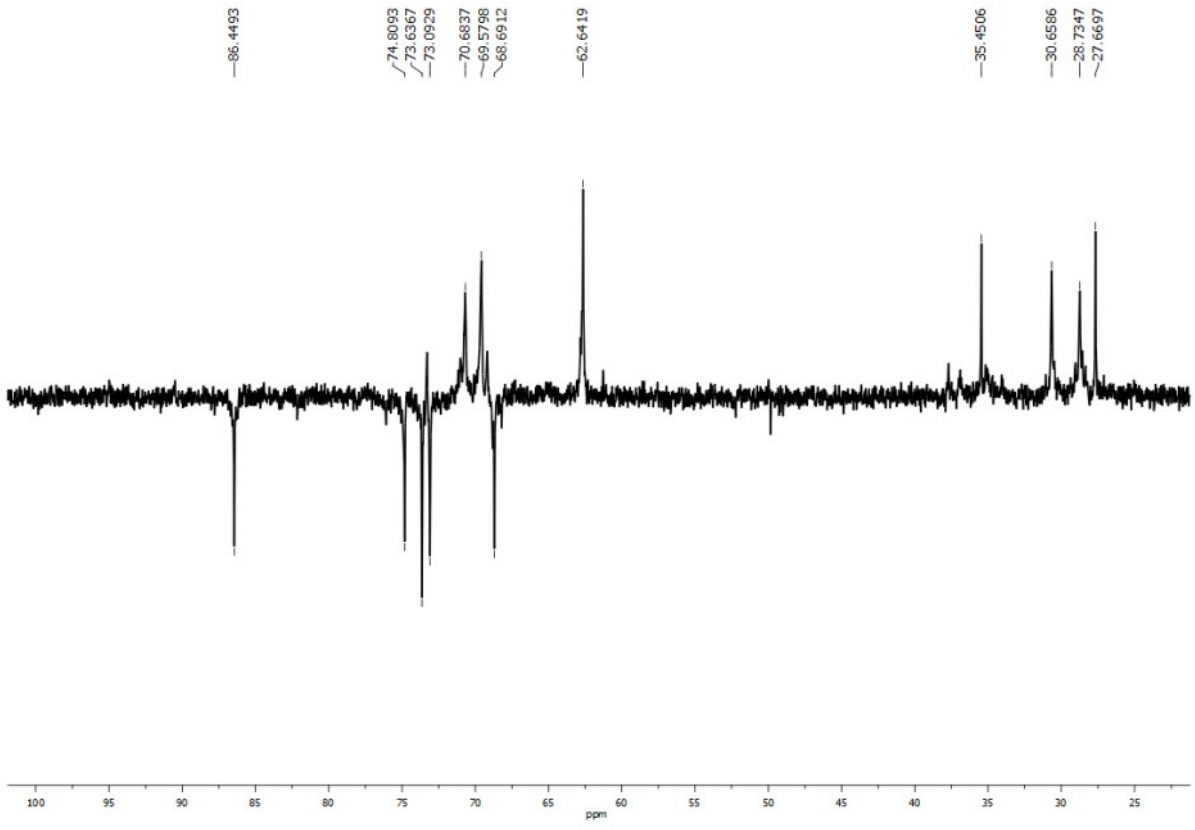
DEPT-135 spectra of **Man_3-NH_2.HCl** (75 MHz, D_2O)



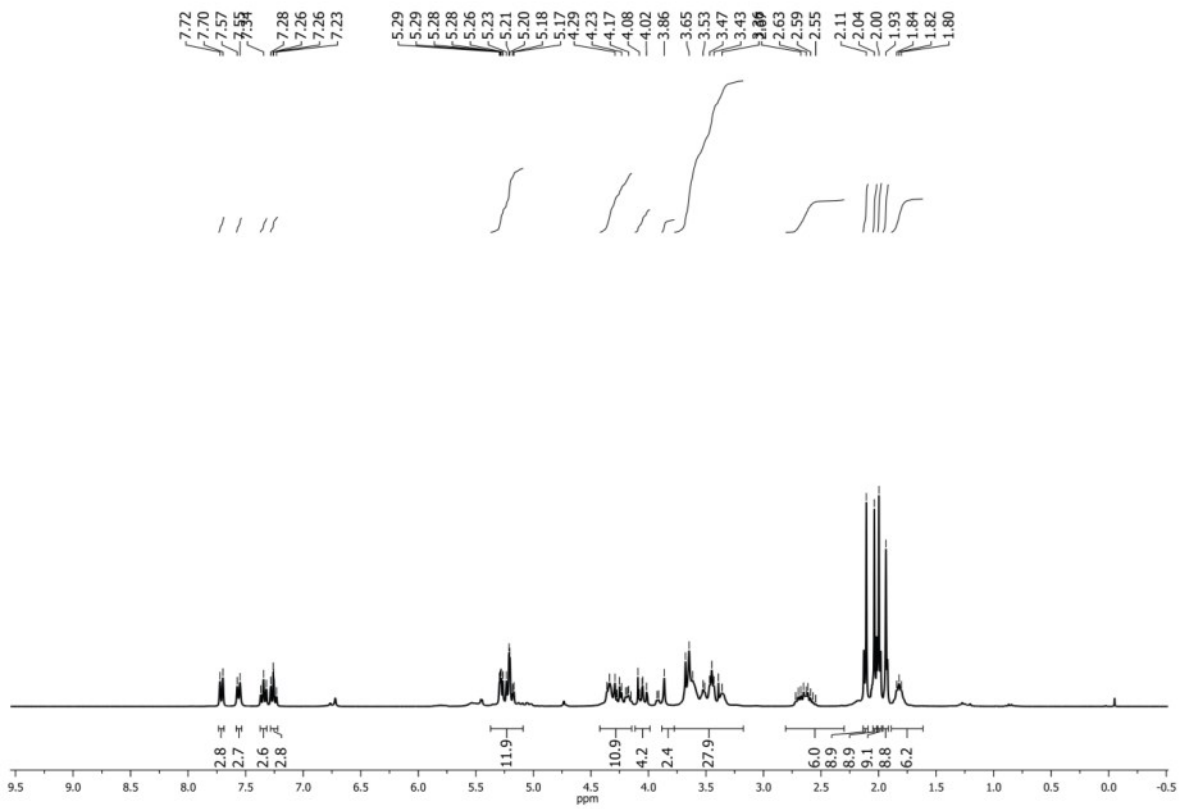
^{1}H NMR Spectra (in DMSO-D6, 300 MHz) of thiol **Man_3-SH 1**



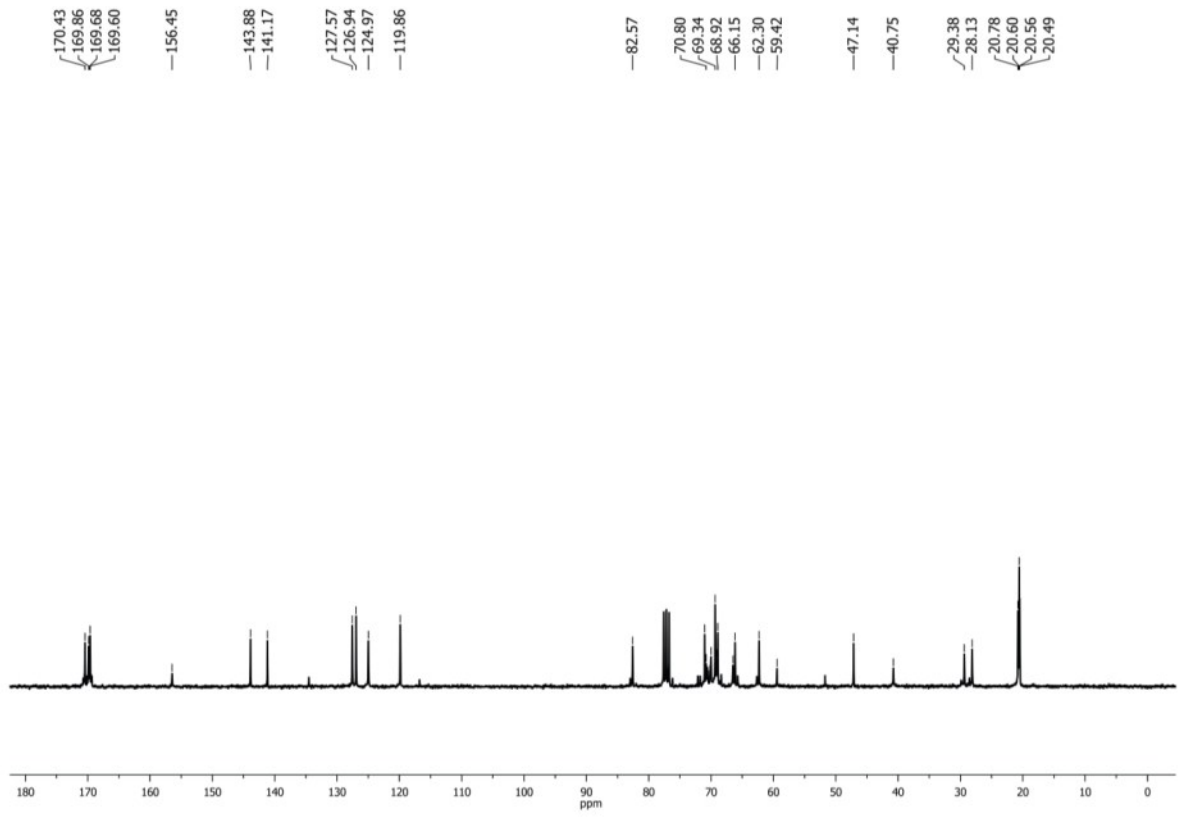
^{13}C NMR Spectra (in CD_3OD, 75 MHz) of thiol **Man_3-SH 1**



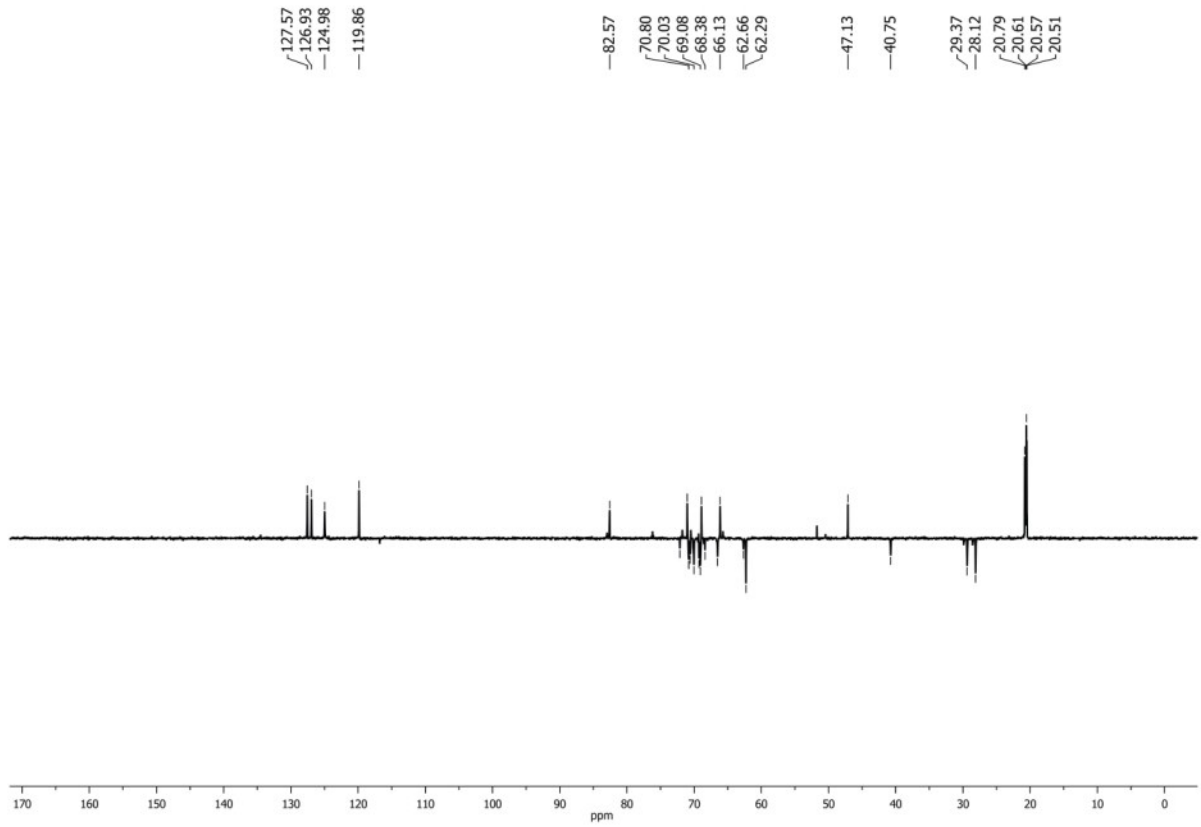
DEPT-135 Spectra (in CD_3OD, 75 MHz) of thiol **Man_3-SH 1**



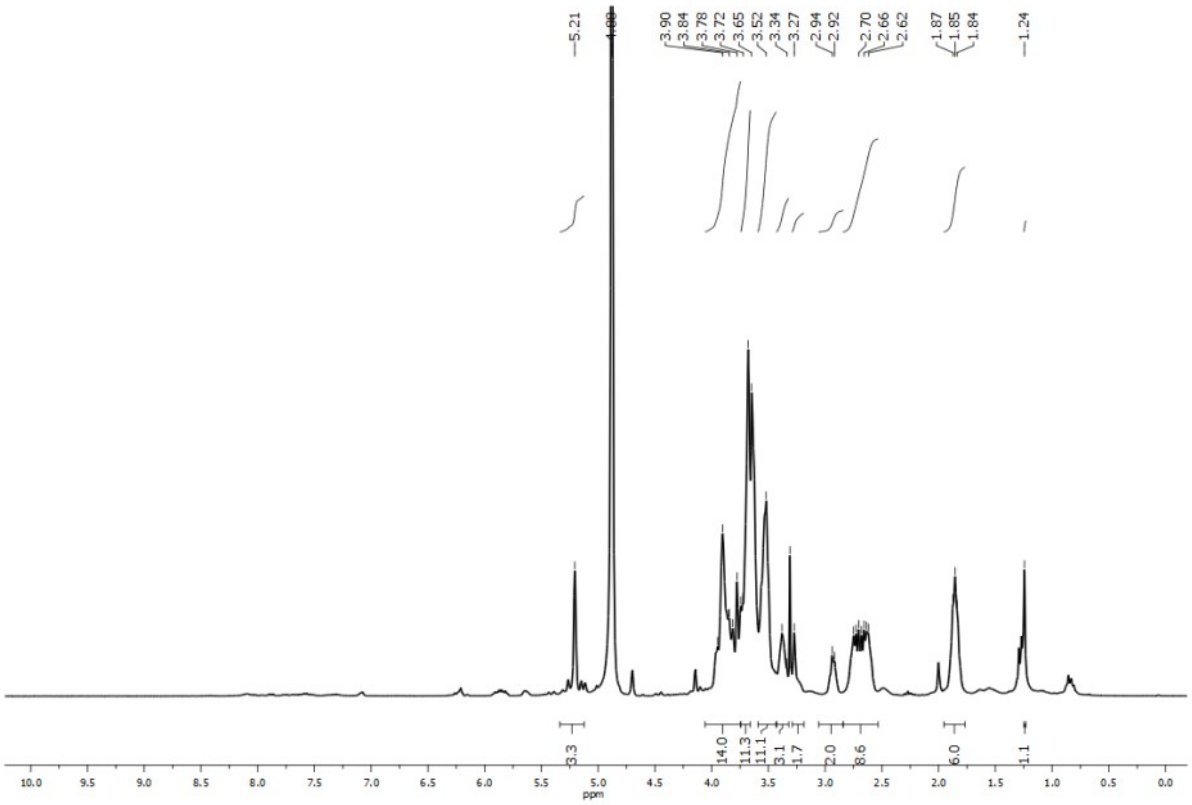
^{1}H NMR spectra of compound **5a** (300 M Hz, $CDCl_3$)



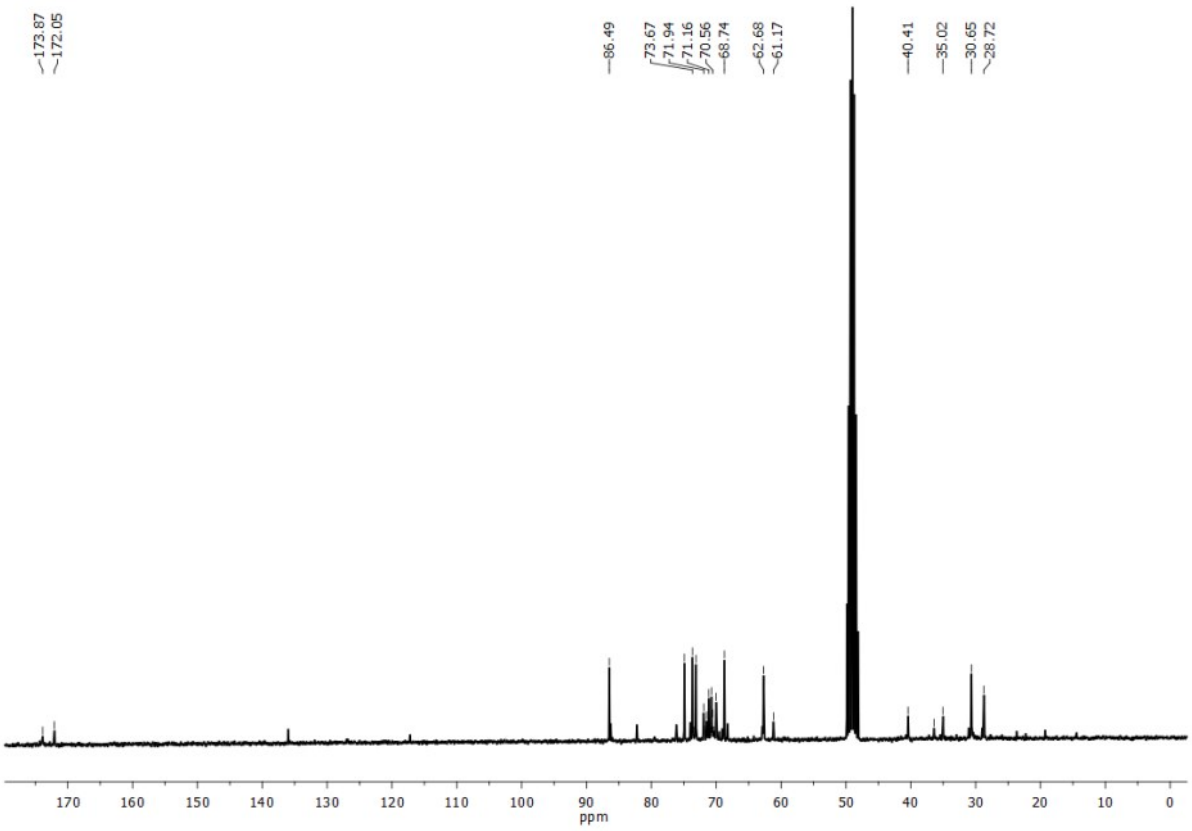
^{13}C NMR spectra of compound **5a** (75 M Hz, $CDCl_3$)



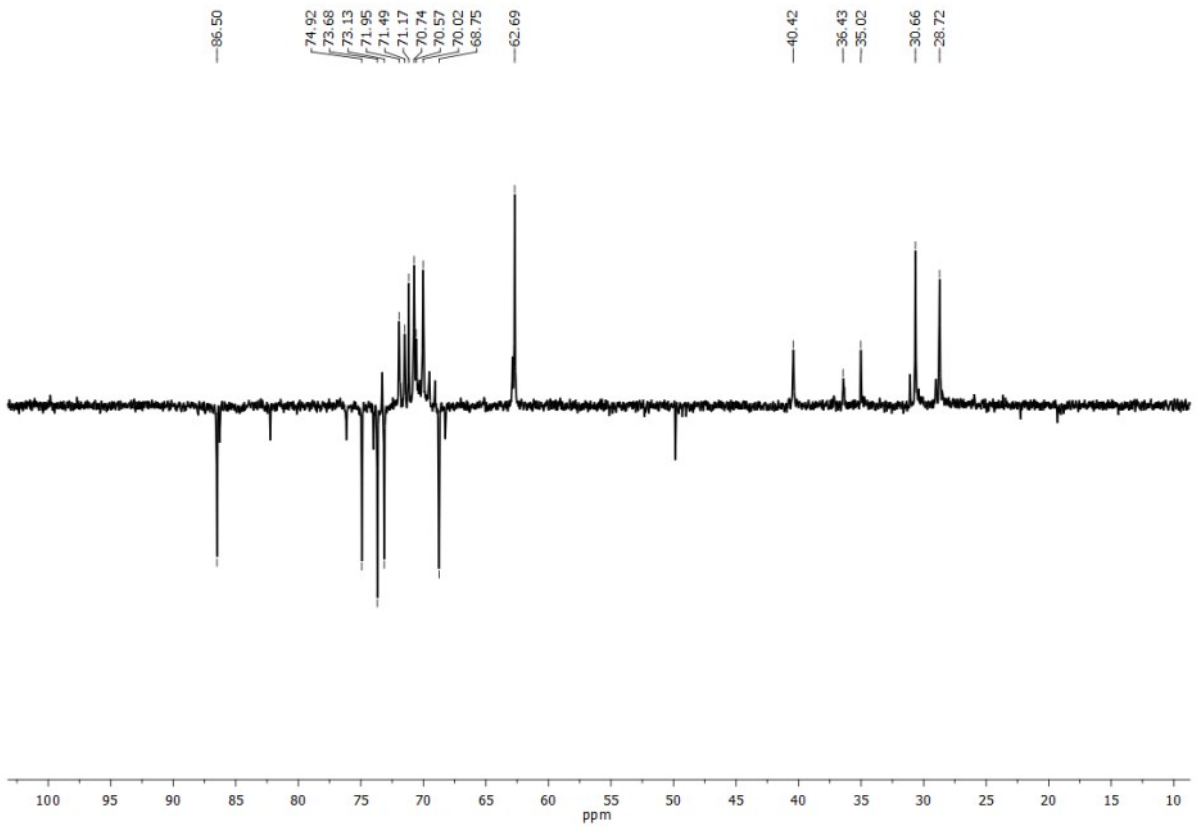
DEPT-135 spectra of compound **5a** (75 MHz, $CDCl_3$)



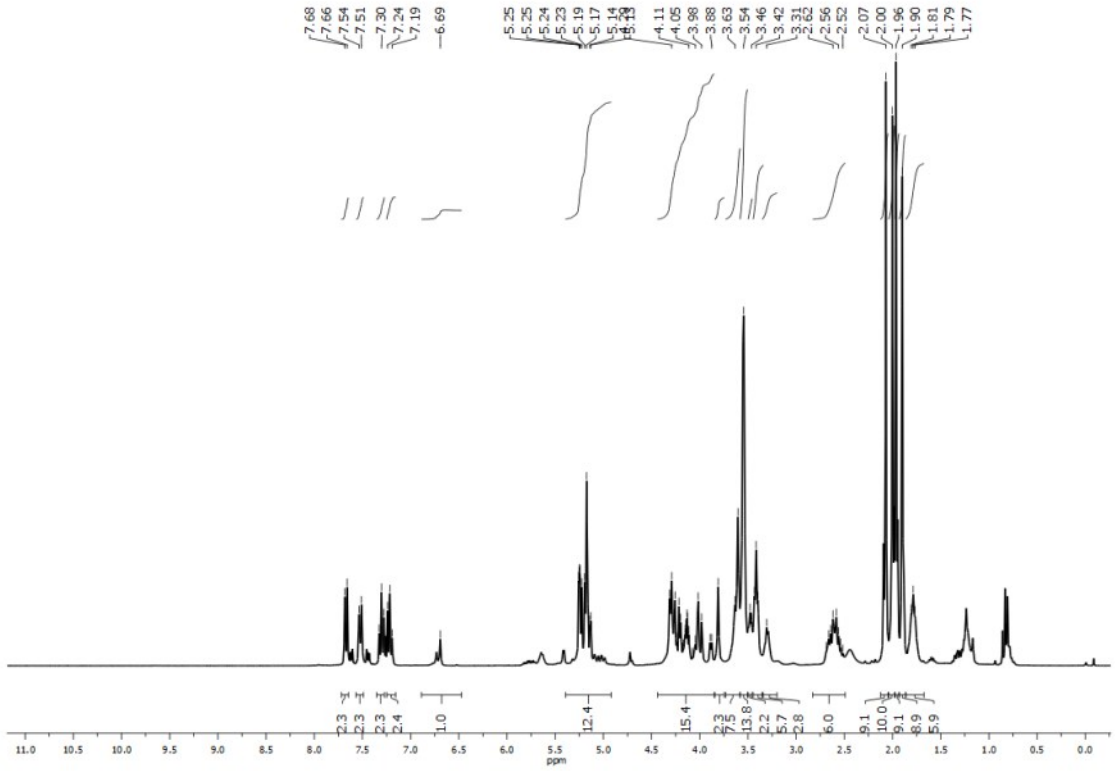
^{1}H NMR Spectra (in CD_3OD, 300 MHz) of thiol **Man_3-SH 2**



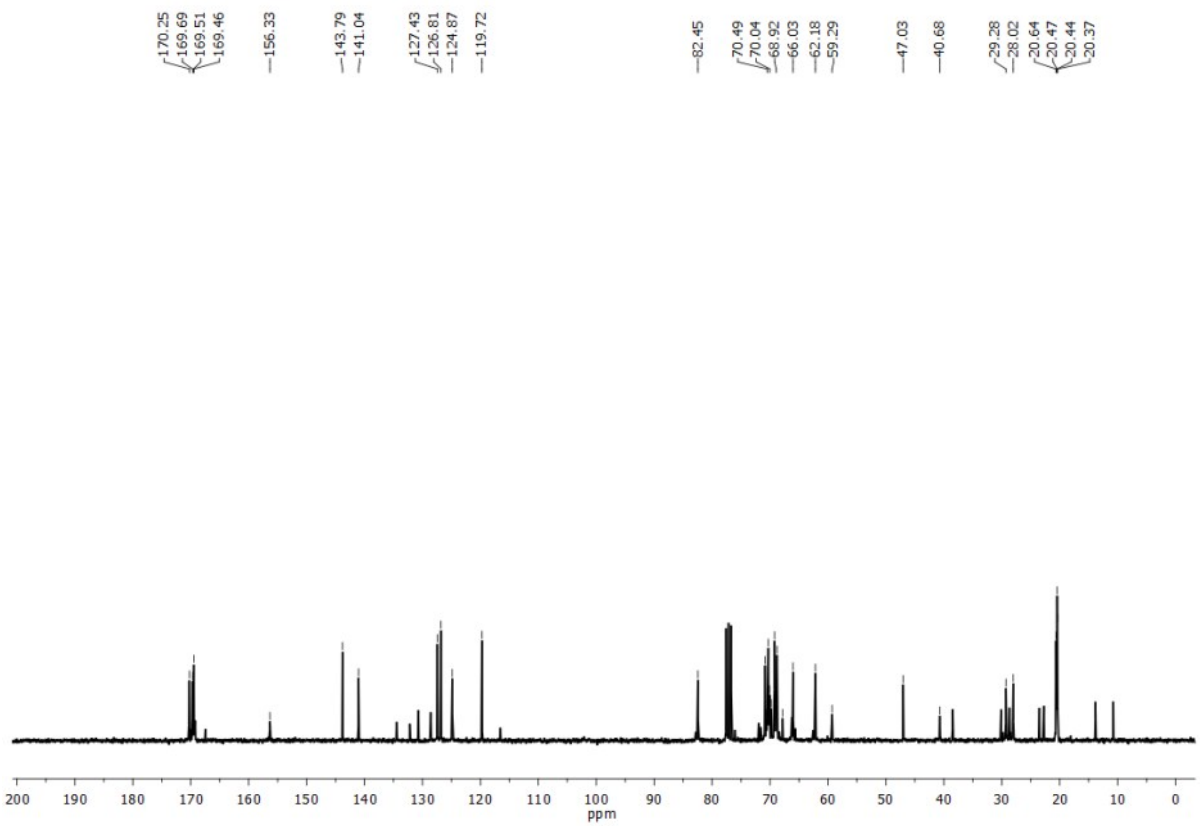
^{13}C NMR Spectra (in CD_3OD, 75 MHz) of thiol **Man_3-SH 2**



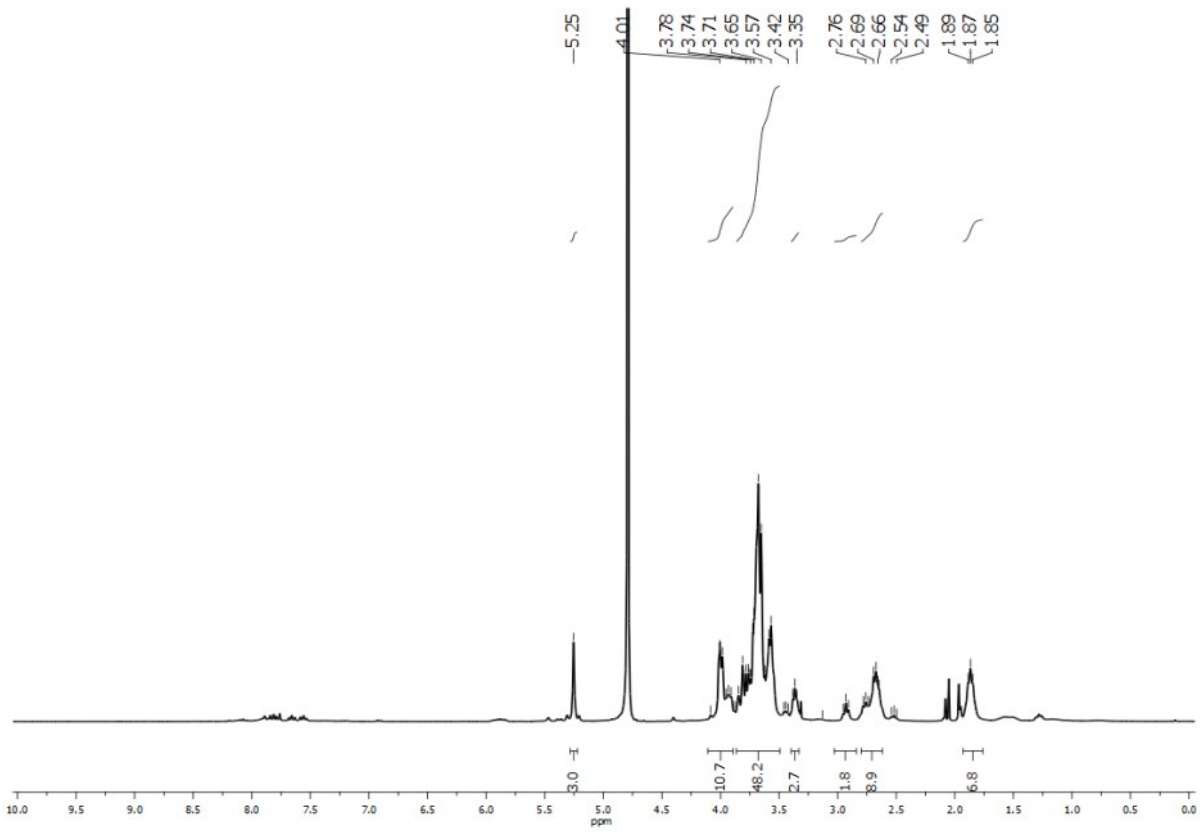
DEPT-135 Spectra (in CD_3OD, 75 MHz) of thiol **Man_3-SH 2**.



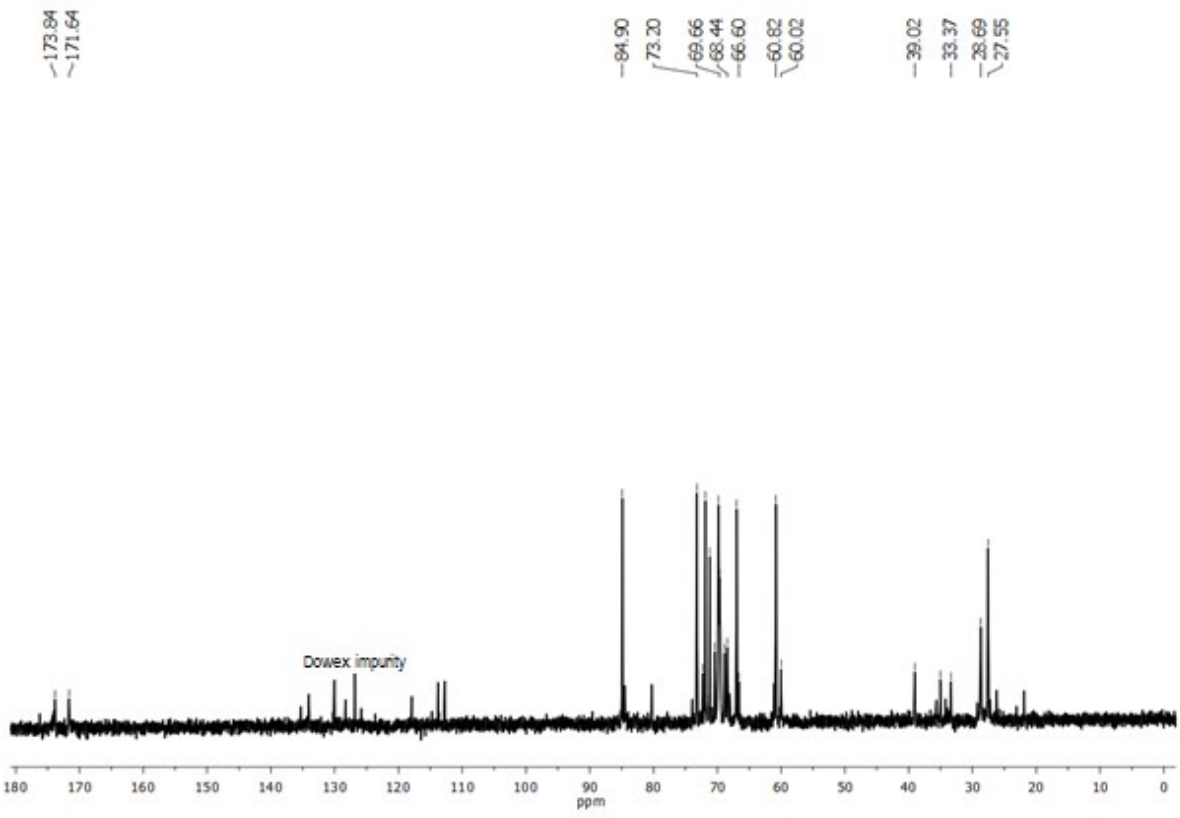
^{1}H NMR spectra of compound **5b** (300 M Hz, $CDCl_3$)



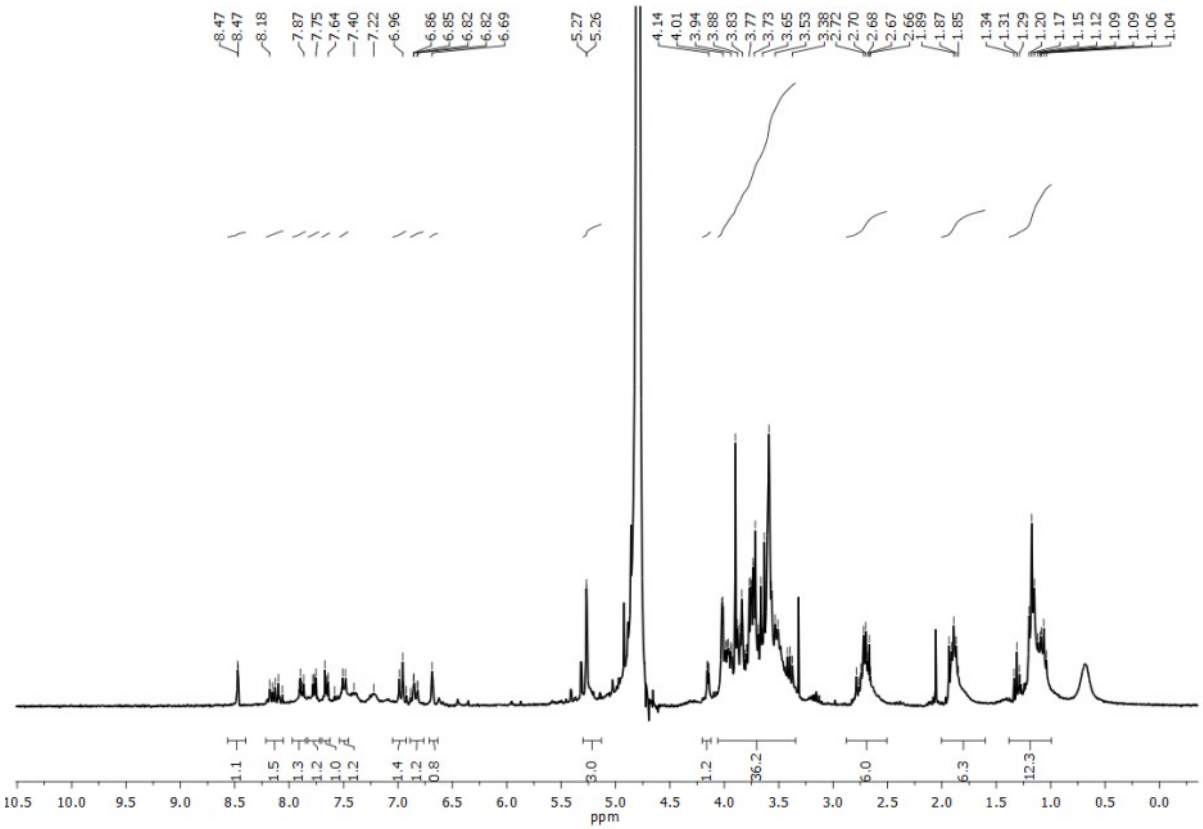
^{13}C NMR spectra of compound **5b** (75 M Hz, $CDCl_3$)



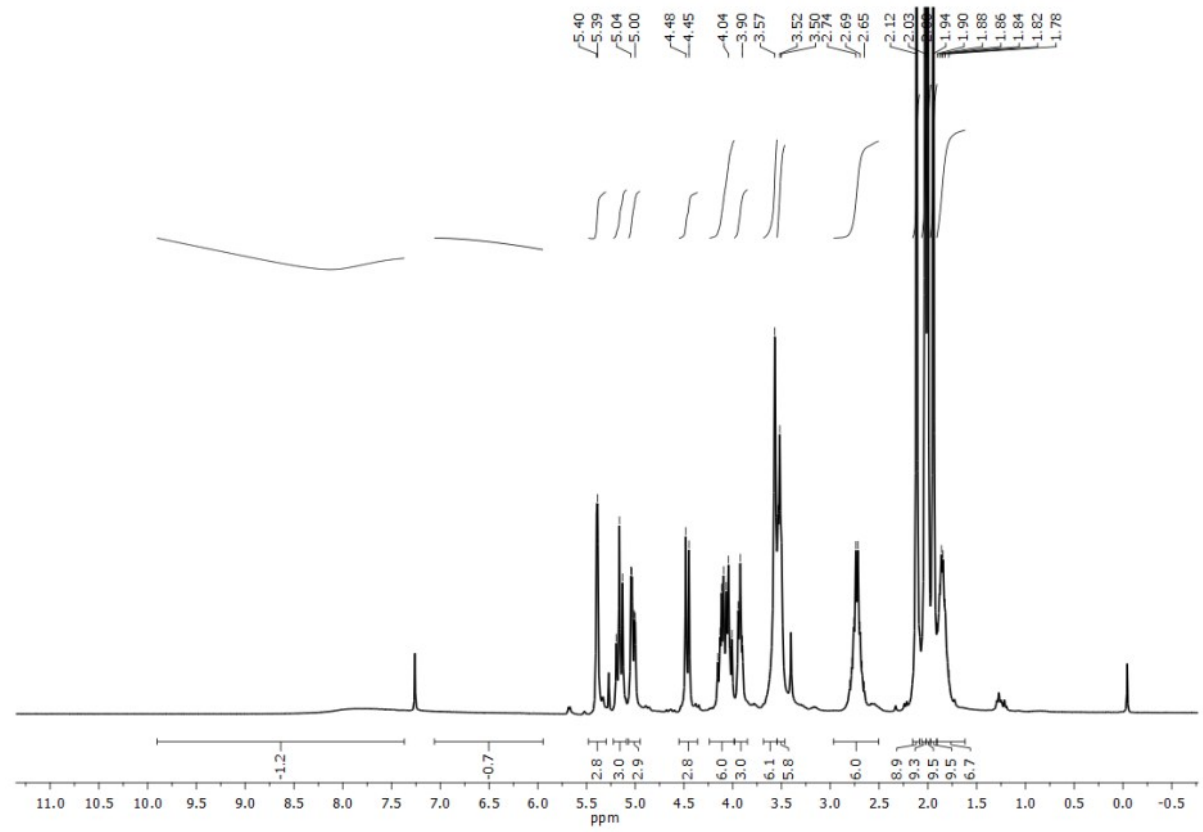
^{1}H NMR Spectra (in D_2O, 300 MHz) of thiol **Man$_3$-SH**



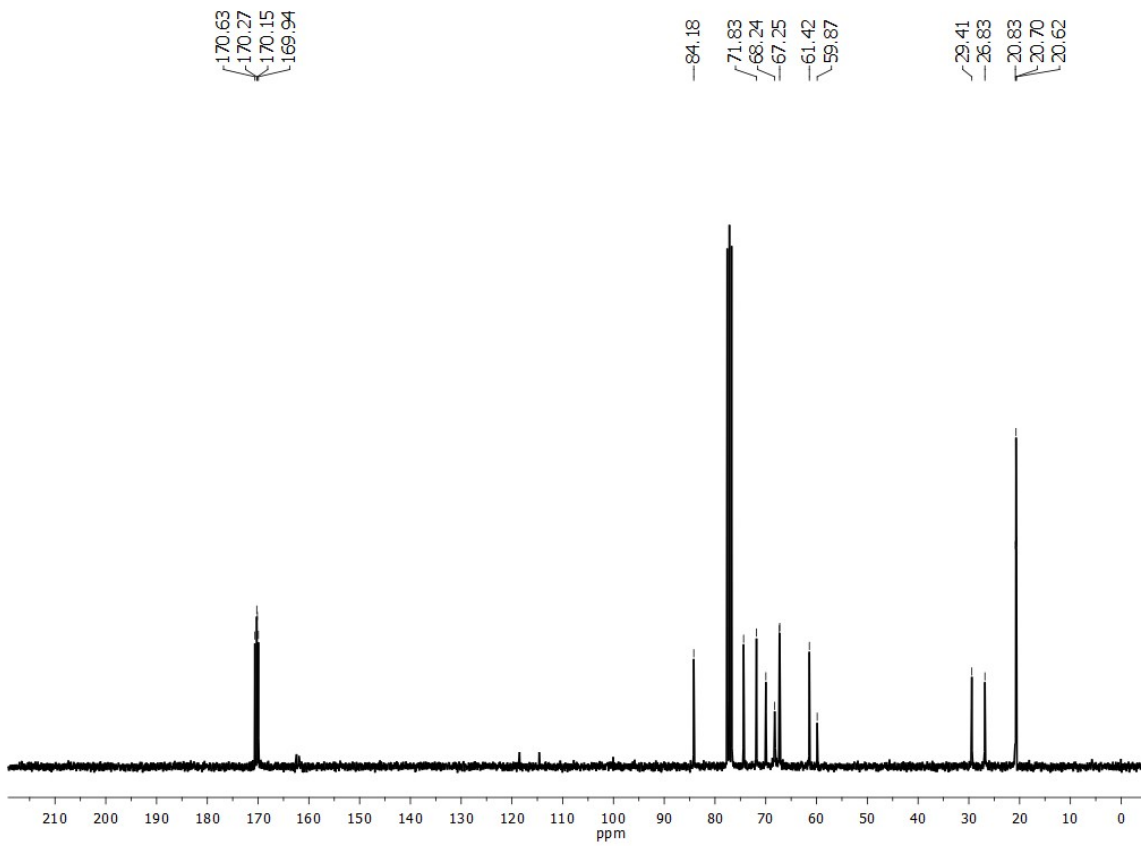
^{13}C NMR Spectra (in D_2O, 75 MHz) of thiol **Man_3-SH**



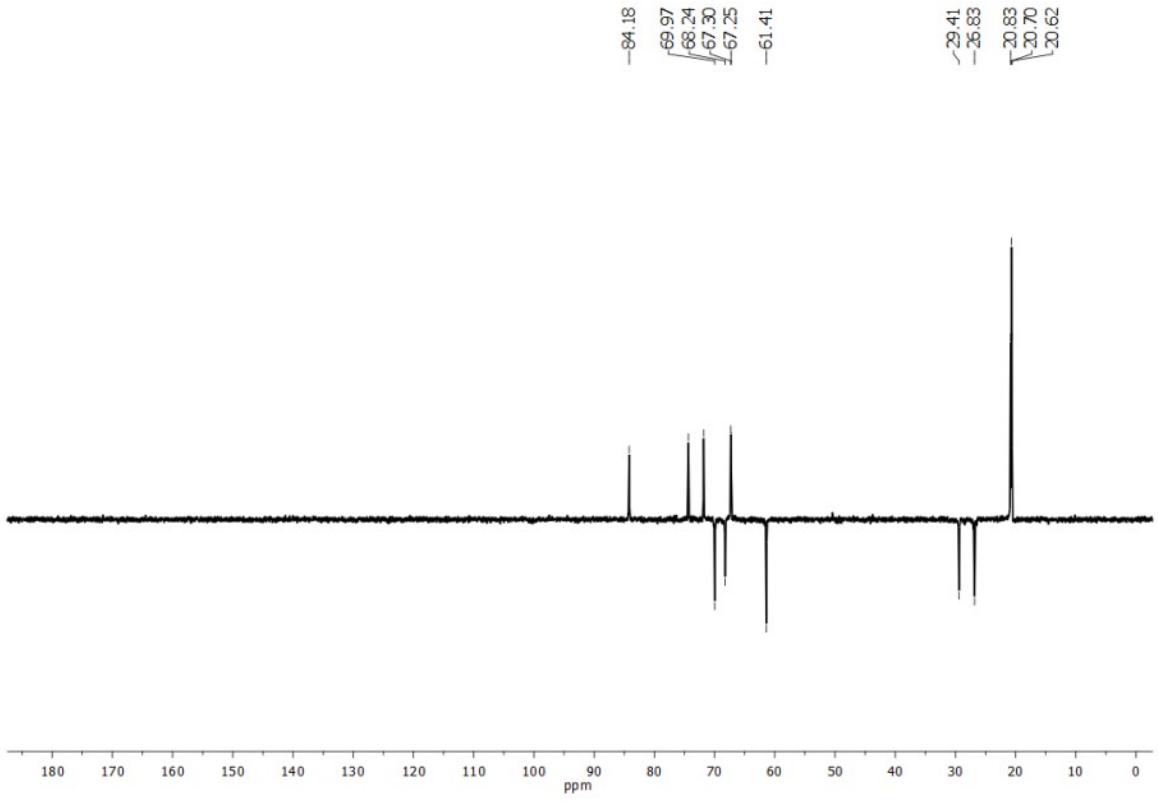
^{1}H NMR Spectra (in D_2O, 300 MHz) of **Man_3-NH-Rhodamine**.



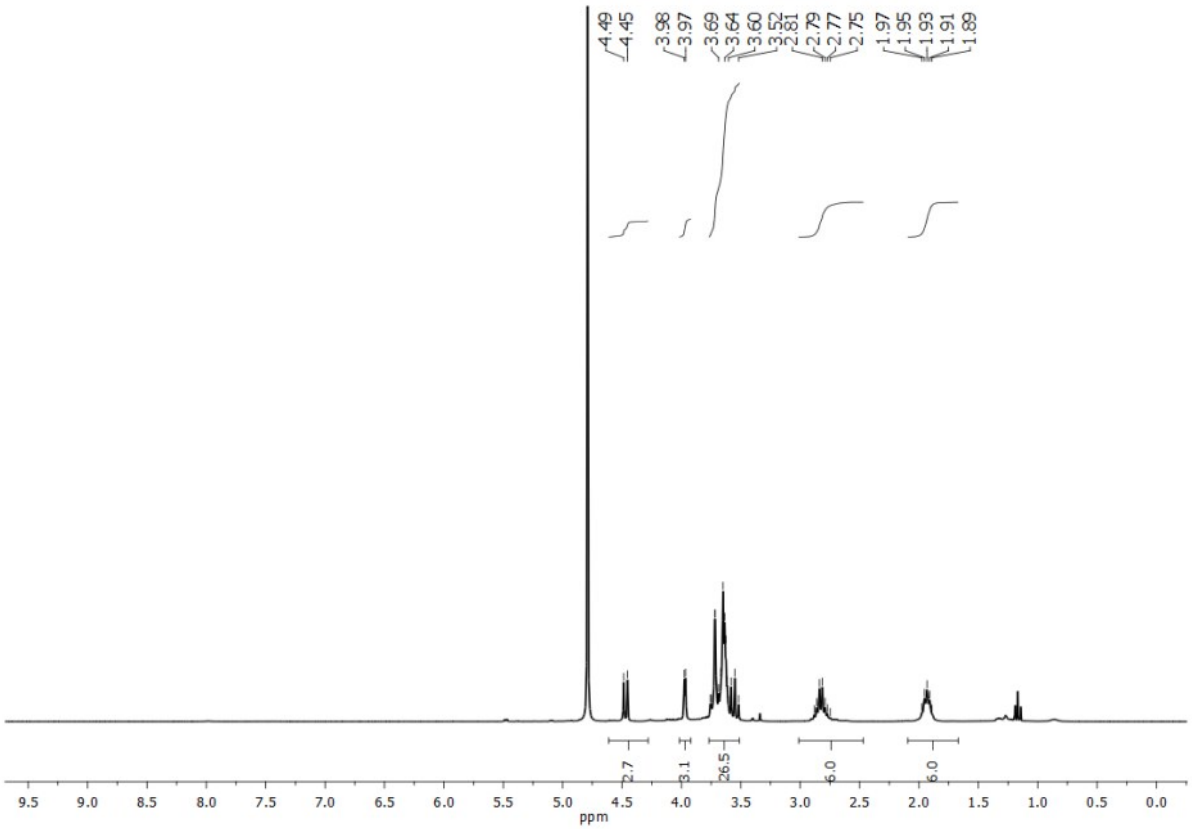
^{1}H NMR Spectra (in $CDCl_3$, 300 MHz) of **Gal_3-NH_2.TFA**



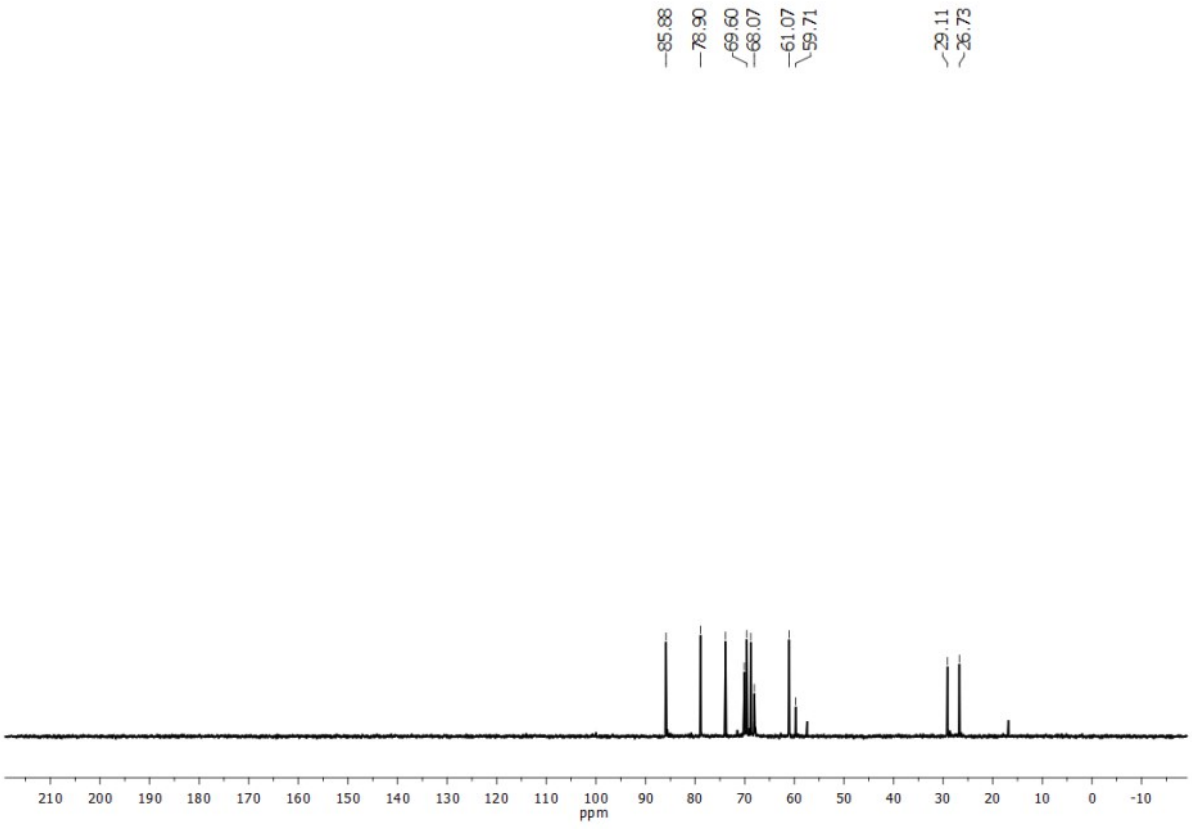
^{13}C NMR Spectra (in $CDCl_3$, 300 MHz) of **Gal_3-NH_2.TFA**



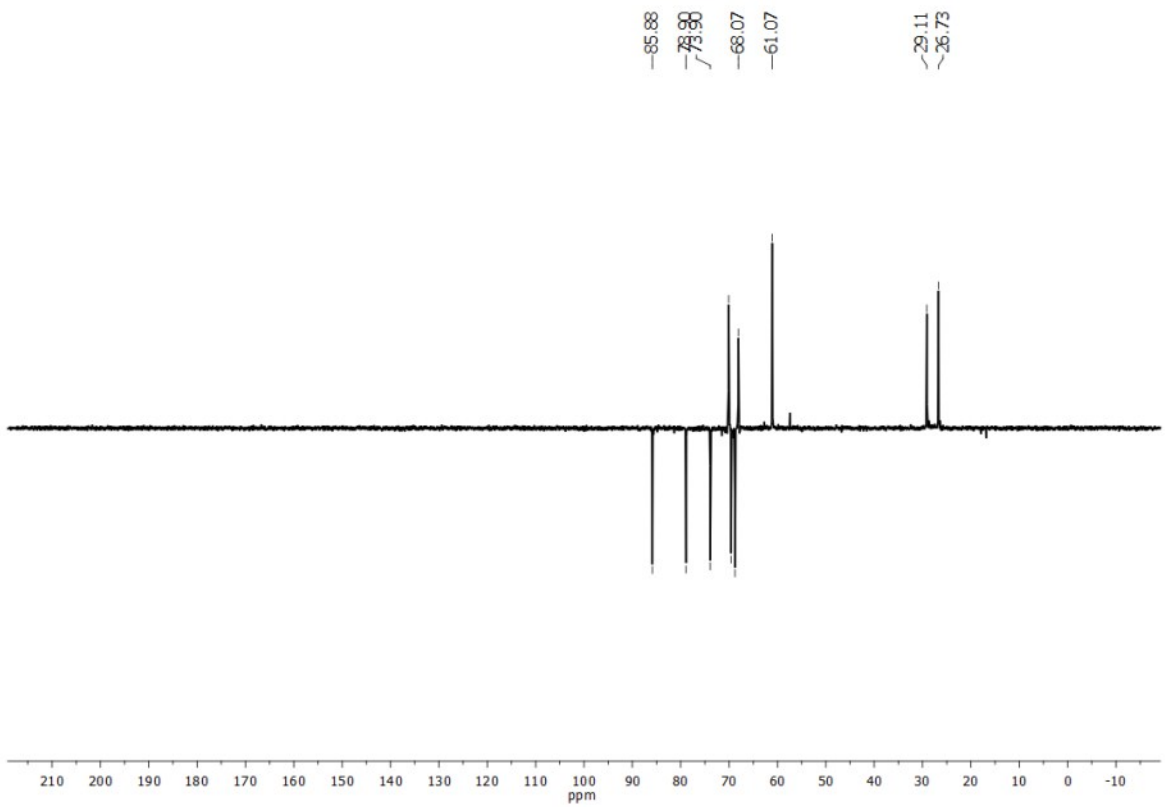
DEPT-135 Spectra (in $CDCl_3$, 75 MHz) of **Gal_3-NH_2.TFA**



^{1}H NMR spectra of **Gal_3-NH_2.HCl** (300 M Hz, D_2O)



^{13}C NMR Spectra of **Gal_3-NH_2.HCl** (in D_2O, 75 MHz)



D

EPT-135 NMR spectra of **Gal_3-NH_2.HCl** (in D_2O, 75 MHz)