Supporting Information

Glycan Encapsulated Gold Nanoparticles Selectively Inhibit Shiga Toxins 1 and 2

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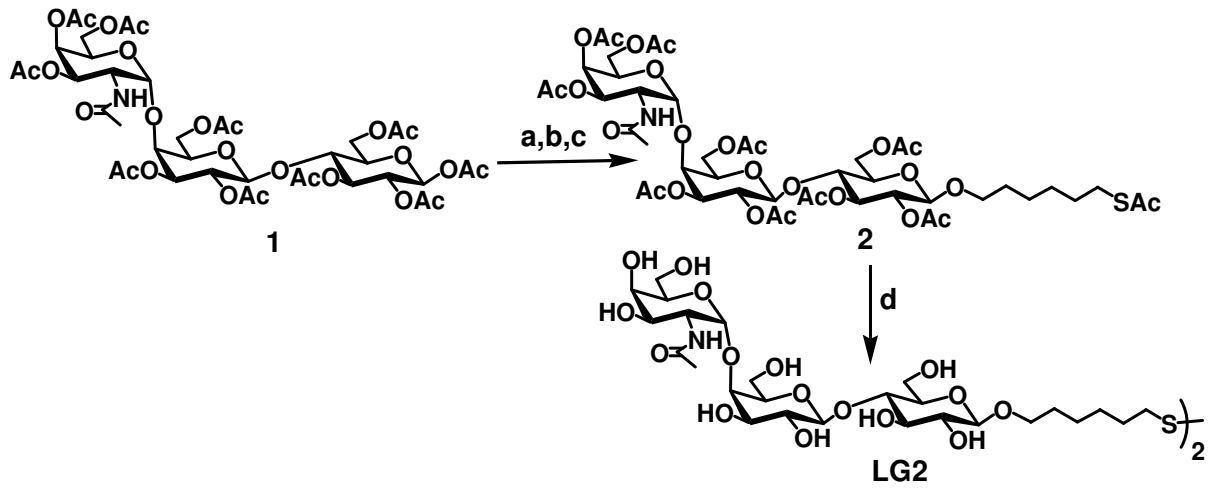
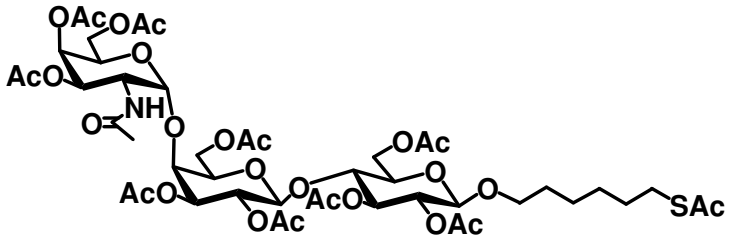
Table of Contents

General:

All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. Acetic anhydride and acetyl chloride were distilled under an inert atmosphere and stored under argon. 4Å Molecular sieves were stored in an oven (>130 ºC) and cooled *in vacuo*. The acidic ion-exchange resin used was Dowex-50 and Amberlite (H^+ form). Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F254 (Merck). Plates were visualized under UV light, and/or by treatment with acidic cerium ammonium molybdate followed by heating. Column chromatography was conducted using silica gel (230-400 mesh) from Qualigens. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400MHz spectrometer. Chemical shifts are reported in δ (ppm) units using ^{13}C and residual ^{1}H signals from deuterated solvents as references. Spectra were analyzed with Mest-Re-C Lite (Mestrelab Research) and/or XWinPlot (Bruker Biospin). Electrospray ionization mass spectra were recorded on a Micromass Q Tof 2 (Waters) and data were analyzed with MassLynx 4.0 (Waters) software.



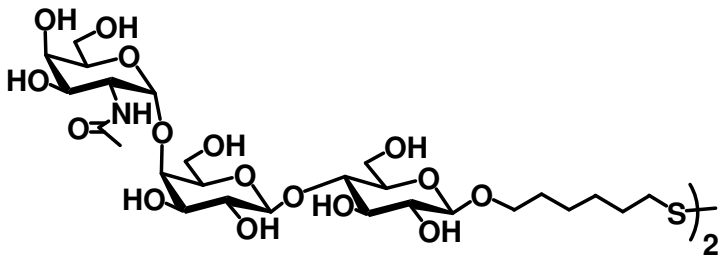
Scheme 1. *Reagents and conditions:* a) NH_2NH_2.HOAc,THF,rt, 85%; b) K_2CO_3, Cl_3CCN, DCM, rt, 80% ; c) S-acyl-6-mercaptohexanol,TMSOTf, DCM, 0^0C, 75% ; d) NaOMe, MeOH, rt, 16h, 96%



1-Thioacetyl-hexyl (2-N-acetamido 2-deoxy 3,4,6-tri-O-acetyl-α-D-galacto pyranosyl) (1 →4) (2,3,6-tri-O-acetyl-β-D-galactopyranosyl) (1 →4) 2,3,6-tri-O-acetyl-β-D-glucopyranoside.

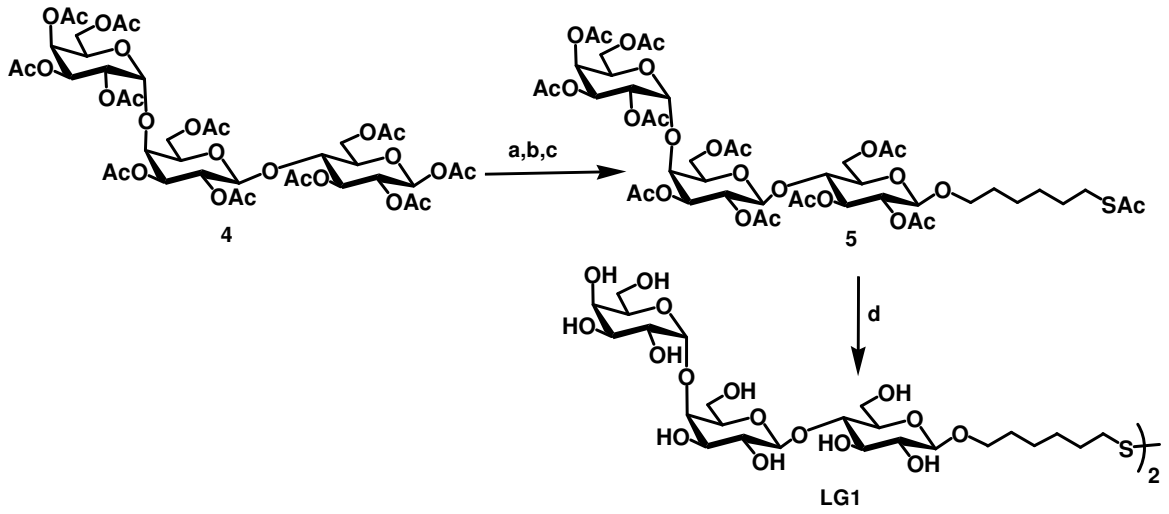
1(*1*) (100 mg, 0.100 mmol) was dissolved in 3 ml of anhydrous THF and NH_2NH_2.HOAc (12 mg, 0.120 mmol) was added to it. The reaction was stirred at rt for 6 h. The reaction mixture was diluted with 5 ml of EtOAc, 5 ml of water was added, and the organic layer was separated and dried in vacuo to give the hemiacetal (80 mg, 85 %), which was directly used in the next step. Anhydrous K_2CO_3 (0.300 g, 2.16 mol) was added to the solution of hemiacetal (80 mg, 0.087 mmol) and trichloroacetonitrile (70 µL, 0.700 mmol) in CH_2Cl_2 (3 ml) at rt. The reaction mixture was stirred at rt for 8 h, washed with water and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by column chromatography, eluting with EtOAc, to give the trichloroimidate as a pale yellow solid (75 mg, 80 %). The trichloroimidate (60 mg, 0.056 mmol) and S-(6-Hydroxy-hexyl)thioacetate(*2*) (15 mg, 0.084 mmol) were dissolved in CH_2Cl_2 (2 ml) and cooled to 0 ^{0}C. A 0.22 M solution of TMSOTf in CH_2Cl_2 (65 µl, 0.014 mmol) was added drop

wise and the resulting solution was stirred for 2 h at 0 ^{0}C. The reaction was quenched by triethylamine solution and the solvent was removed in *vacuo.* The crude product was purified by column chromatography, eluting with 8:2 mixture of EtOAc and hexane, to give **2** as white solid (45 mg, 75%). ^{1}H NMR ($CDCl_3$, 400MHz): δ 6.22 (d, 1H, *J* = 8.8 Hz), 5.50 (m, 1H), 5.25-5.20 (m, 2H), 5.14 (dd , 1H, *J* = 8.0, *J* = 10.4), 5.02 (d, 1H, *J* = 3.6), 4.92-4.85 (m, 2H), 4.63-4.38 (m, 6H), 4.16-4.02 (m, 4H), 3.96 (m, 1H), 3.86-3.74 (m, 3H), 3.65-3.60 (m, 1H), 3.50-3.40 (m, 1H), 2.86 (t, 2H, *J* = 7.2), 2.33 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 2.03 (s, 3H), 2.02 (s, 3H). ^{13}C NMR ($CDCl_3$): 195.92, 170.90, 170.69, 170.59, 170.28, 170.20, 170.15, 169.76, 169.57, 169.03, 100.95, 100.41, 99.10, 73.60, 73.03, 72.56, 72.18, 72.10, 72.05, 69.95, 69.41, 67.72, 67.13, 67.03, 62.45, 60.78, 48.62, 30.64, 29.47, 29.24, 28.99, 28.44, 25.35, 23.10, 20.93, 20.69, 20.84, 20.76, 20.72, 20.67, 20.57, 20.53. HRMS Calculated for $[C_{46}H_{67}NO_{26}S+Na]^+$:1104.3570 Found:1104.3284.

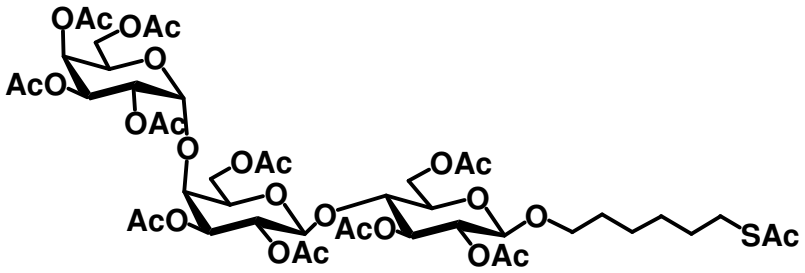


1-Thiohexyl (2-N-acetamido 2-deoxy-α-D-galacto pyranosyl) (1 →4) (β-D-galactopyranosyl) (1 →4)β-D-glucopyranoside.

2 (17 mg, 0.016 mmol) was dissolved in methanol (2 ml) and a solution of NaOMe in MeOH (0.50M, 0.5 ml) was added and the reaction mixture was stirred at rt for 6h. The reaction was neutralized by careful addition of Amberlite-15 H^+ resin and the resin was filtered. The solvent was removed in *vacuo* and the residue was purified by Biogel P-2 gel column chromatography, eluting with methanol, to give the dimerized product **LG2** as a white solid (10 mg, 96%). ^{1}H NMR (CD_3OD, 400MHz): δ 4.88 (bs, 1H), 4.87 (bs, 1H), 4.59 (s, 2H), 4.40-4.33 (m, 4H), 4.26-4.23 (m, 4H), 3.90-3.84(m, 10H), 3.79-3.75 (m, 3H), 3.69-3.65 (m, 6H), 3.61-3.57 (m, 6H), 3.54-3.47 (m, 10H), 3.37-3.34 (m, 2H), 3.22-3.18 (m, 2H), 2.67-2.63 (t, 2H), 2.56-2.53 (t, 2H), 1.98 (s, 6H), 1.67-1.55 (m, 9H), 1.40-1.37 (m, 10H), 1.24 (m, 6H). ^{13}C NMR (CD_3OD): 172.63, 160.08, 103.96, 102.88, 98.94, 79.45, 79.40, 76.43, 75.69, 75.18, 75.05, 73.57, 73.15, 71.11, 70.88, 69.45, 69.01, 68.05, 61.27, 60.57, 59.89, 54.42, 50.31, 38.33, 30.46, 29.61, 29.27, 28.79, 28.26, 27.87, 25.27, 21.39, 21.36. HRMS Calculated for $[2C_{52}H_{92}N_2O_{32}S_2+Na]^+$: 1343.4972. Found: 1343.5111.

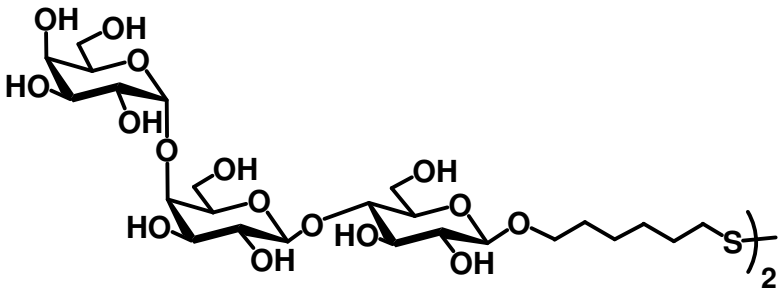


Scheme 2. *Reagents and conditions:* a) NH_2NH_2.HOAc, THF, rt, 85%; b) K_2CO_3, Cl_3CCN, DCM, rt, 80% ; c) S-acyl-6-mercaptohexanol , TMSOTf, DCM, 0°C, 85% ; d) NaOMe, MeOH, rt, 16h, 99%

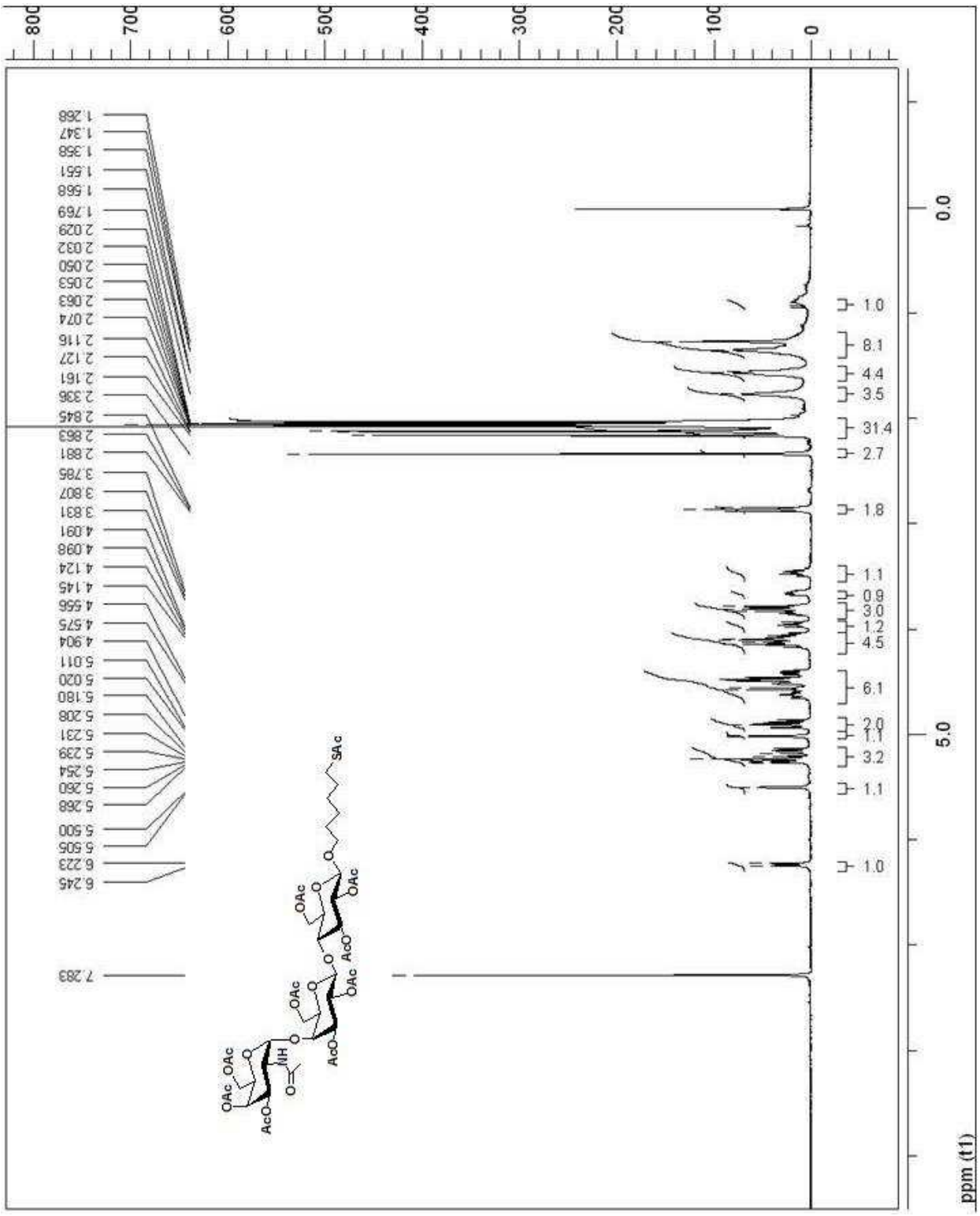


4(*3*) (110 mg, 0.113 mmol) was dissolved in 3 ml of dry THF and NH_2NH_2.HOAc (12.5 mg, 0.136 mmol) was added to it. The reaction was stirred at rt for 6 h. The reaction mixture was diluted with 5 ml of EtOAc and 5 ml of water was added, the organic layer was separated and dried *in vacuo* to give the hemiacetal (89 mg, 85 %), which was directly used in the next step. Anhydrous K_2CO_3 (132 mg, 0.960 mmol) was added to the solution of hemiacetal (89 mg, 0.096 mmol) and trichloroacetonitrile (100 µL, 0.960 mmol) in CH_2Cl_2 (3 ml) at rt. The reaction mixture was stirred at rt for 16 h, washed with water and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with a 1:1 mixture of hexane and EtOAc, to give the trichloroimidate as a pale yellow solid (82 mg, 80 %). The imidate (45 mg, 0.042 mmol) and S-(6-Hydroxy-hexyl)thioacetate(*2*) (11 mg, 0.063 mmol) were dissolved in CH_2Cl_2 (2 ml) and cooled to 0^0C. A 0.22 M solution of TMSOTF in CH_2Cl_2 (42 µl, 0.010 mmol) was added drop wise and the resulting solution was stirred for 2 h at 0^0C. The reaction was quenched by triethylamine solution and the solvent was removed in *vacuo.* The crude product was

purified by column chromatography, eluting with 7:3 mixture of EtOAc and hexane, to give **5** as white solid (39 mg, 85%). ^{1}H NMR ($CDCl_3$, 400MHz): δ 5.6 (d, 1H, *J* = 2.8Hz), 5.39 (dd, 1H, *J* = 3.2 and 11.2 Hz), 5.23-5.17 (m, 2H), 5.11 (dd, 1H, *J* = 8 and 10.8 Hz), 5.00 (d, 1H, *J* = 3.6 Hz), 4.89 (dd, 1H, *J* = 8.0, 9.2 Hz), 4.74 (dd, 1H, *J* = 2.8 and 10.8 Hz), 4.54 - 4.42 (m, 5H), 4.20 – 4.09 (m, 5H), 4.025 (d, 1H, *J* = 2.4 Hz), 3.85 – 3.77 (m, 3H), 3.65 – 3.62 (m, 1H), 3.47 – 3.45 (m, 1H), 2.9 – 2.84 (m, 4H), 2.33 (d, 4H), 2.14 (s, 3H), 2.13 (s, 3H), 2.1 (s, 3H), 2.09 (s, 3H), 2.08 (s, 6H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.61 – 1.55 (m, 4H), 1.41 – 1.27 (m, 4H). ^{13}C NMR ($CDCl_3$): 196.02, 195.93, 170.68, 170.49, 170.45, 170.08, 169.68, 169.53, 168.87, 101.13, 100.54, 99.64, 73.17, 72.85, 72.55, 71.85, 69.95, 69.03, 68.88, 67.94, 67.17, 62.85, 62.27, 62.10, 62.07, 61.37, 61.13, 60.31, 60.09, 60.03, 32.58, 30.65, 29.48, 29.46, 29.25, 29.01, 28.51, 28.44, 25.35, 25.24, 20.95, 20.89, 20.74, 20.69. HRMS Calculated for $[C_{46}H_{66}O_{27}S + Na]^+$: 1105.3409; Found: 1105.3167



Compound **5** (17 mg, 0.016 mmol) was dissolved in methanol (2 ml) and a solution of NaOMe in methanol (0.5M, 0.5 ml) was added and the reaction mixture was stirred at rt for 6h. The reaction was neutralized by careful addition of Amberlite-15 H^+ resin and the resin was filtered. The solvent was removed in *vacuo* and the residue was purified by Biogel P-2 gel column chromatography, eluting with methanol, to give dimerized product **LG1** as a white solid (10 mg, 99%). ^{1}H NMR (CD_3OD, 400MHz): δ 4.92 (d, 1H, *J* = 3.6 Hz), 4.6 (bs, 1H), 4.39 – 4.38 (m, 1H), 4.26 -4.23 (m, 2H), 3.95 (bs, 1H), 3.89-3.65 (m, 12H), 3.52-3.48 (m, 6H), 3.32-3.4 (m, 1H), 3.24-3.17 (m, 1H), 2.67 – 2.55 (m, 3H), 1.6-1.46 (m, 6H), 1.45-1.20 (m, 8H). ^{13}C NMR (CD_3OD): 103.97, 102.83, 101.30, 79.67, 79.64, 78.40, 75.13, 75.08, 73.49, 73.28, 71.47, 71.26, 69.89, 69.68, 69.16, 61.50, 61.32, 60.59, 60.07, 38.35, 38.31, 32.13, 30.46, 30.18, 29.65, 29.61, 29.26, 28.79, 28.34, 28.29, 28.26, 27.87, 25.30, 25.27, 25.16. HRMS Calculated for $[2C_{52}H_{92}N_2O_{32}S_2+Na]^+$: 1262.4372. Found: 1262.4688.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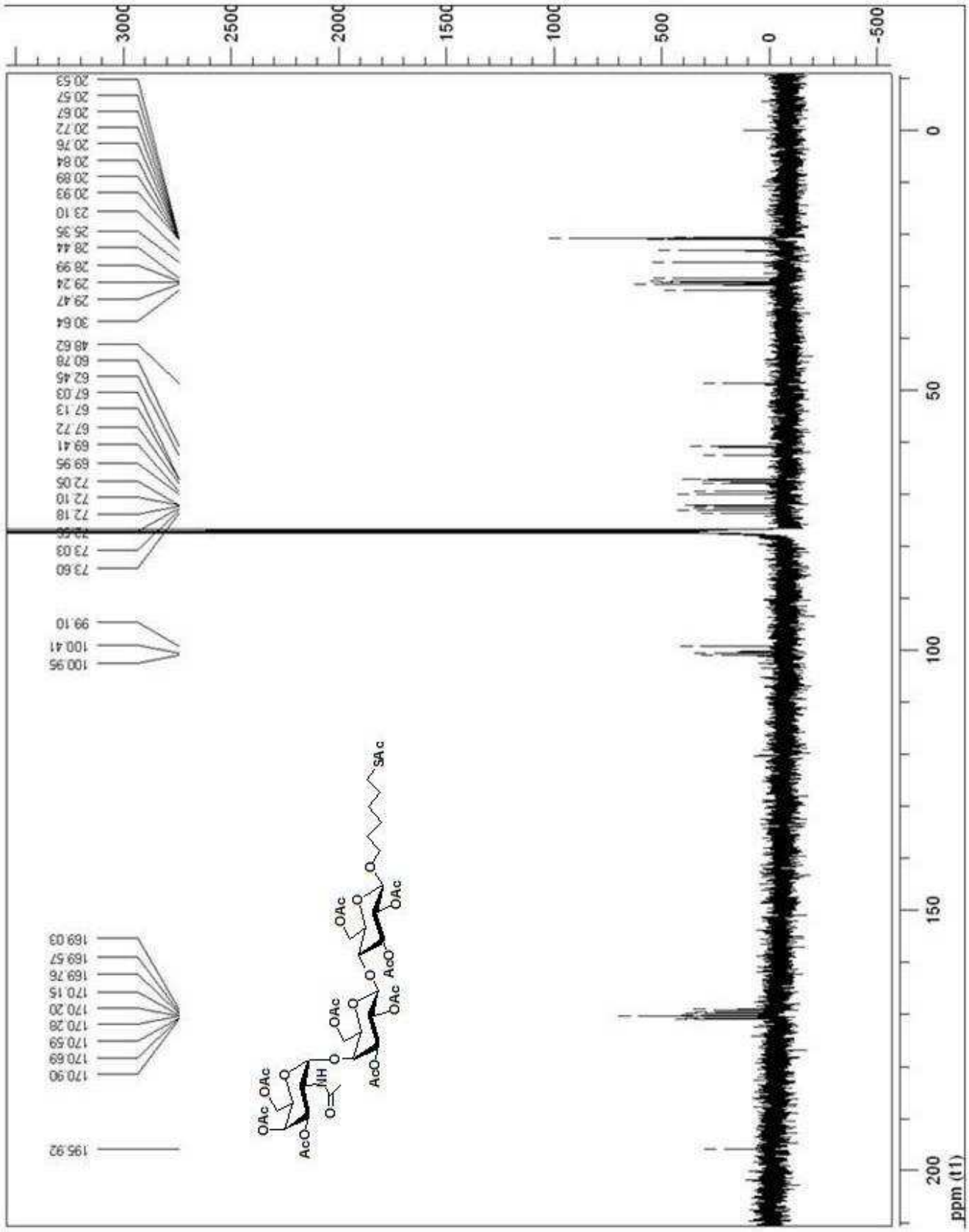
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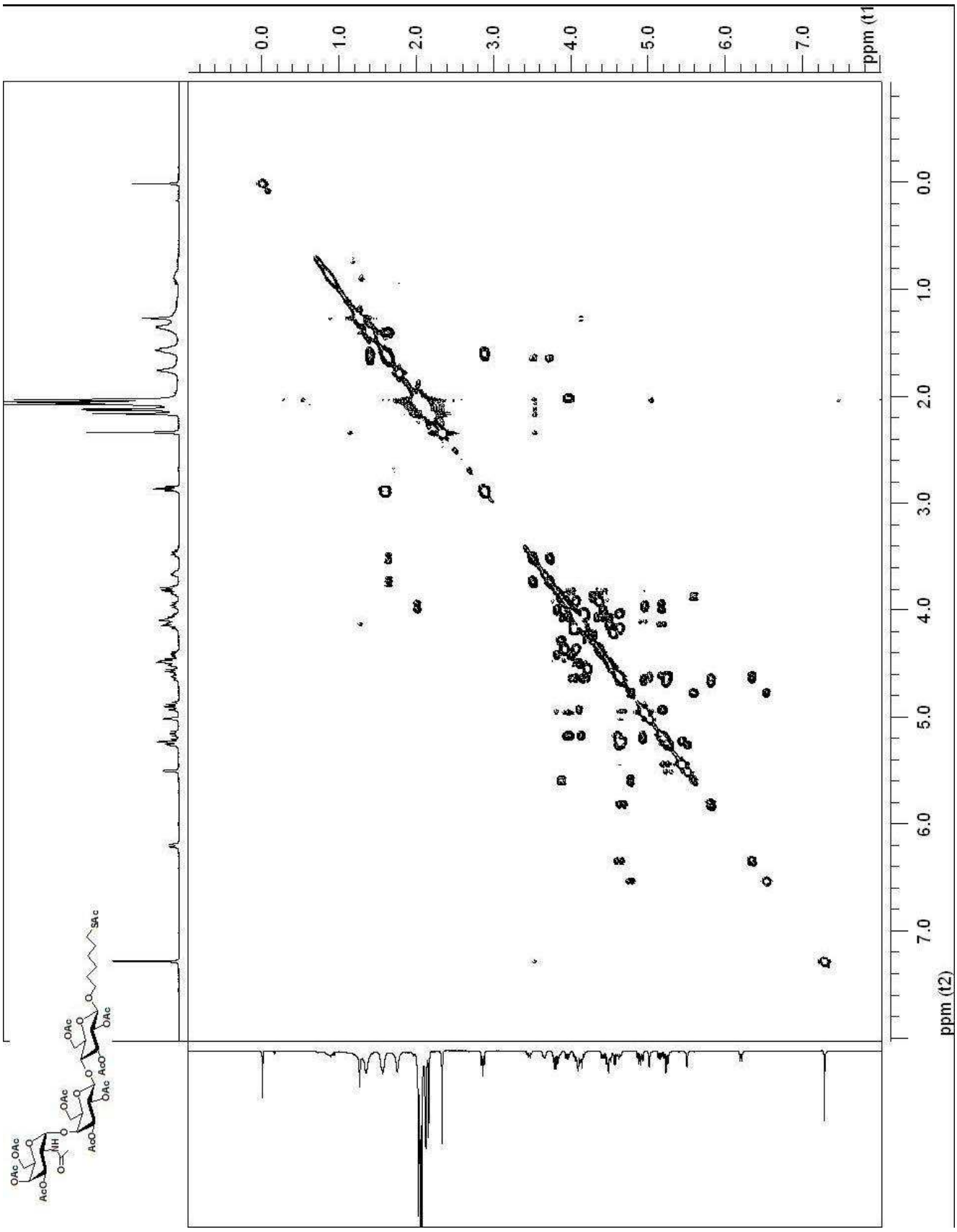
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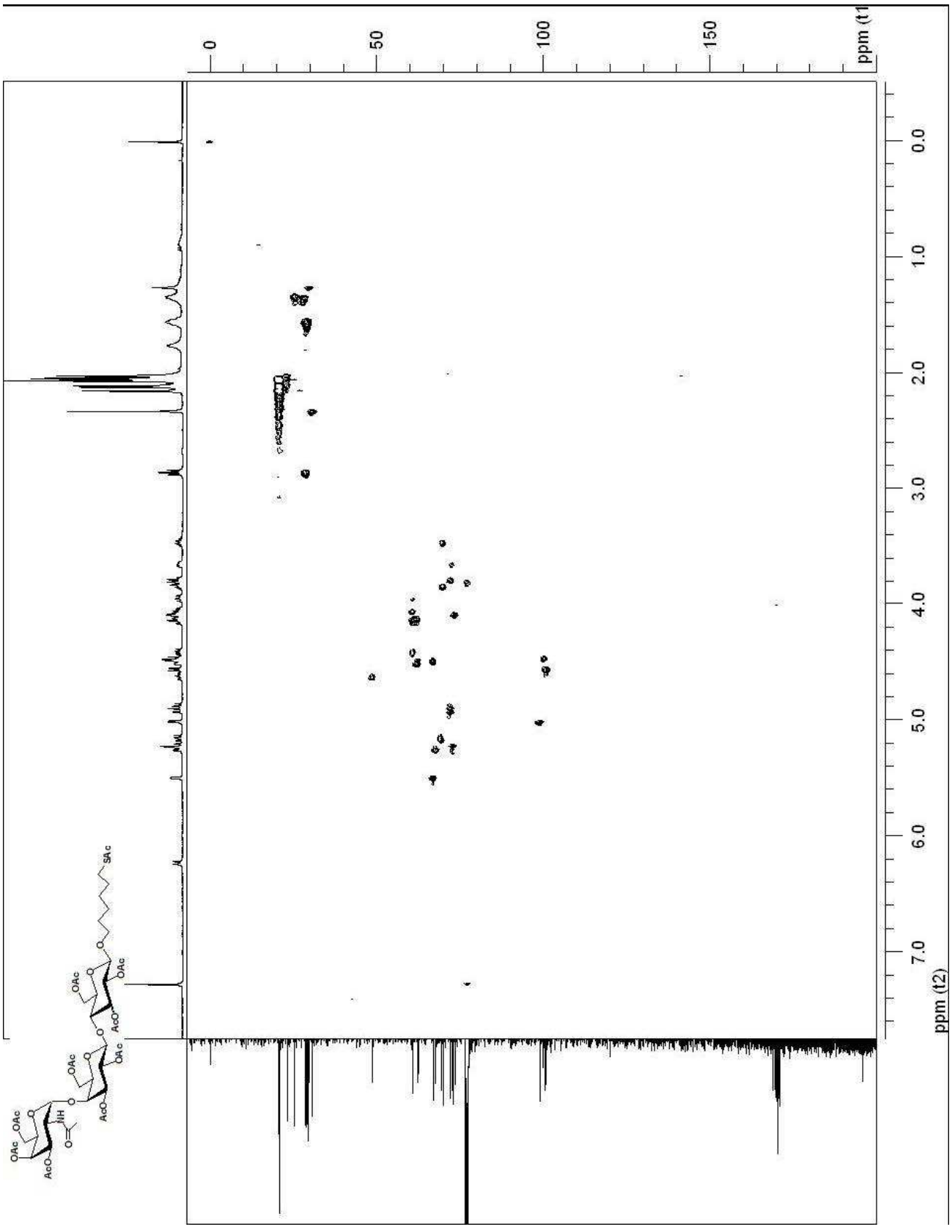
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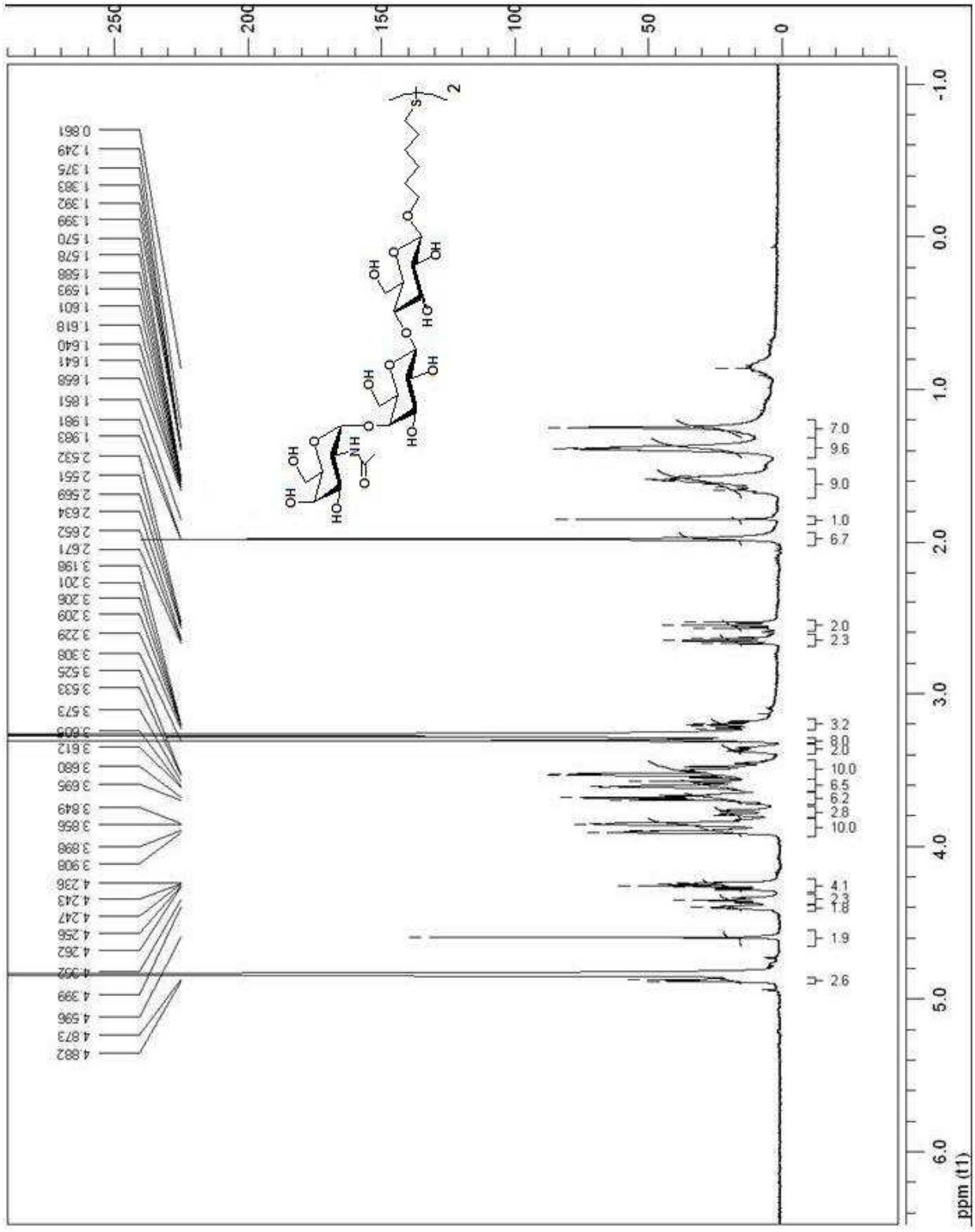
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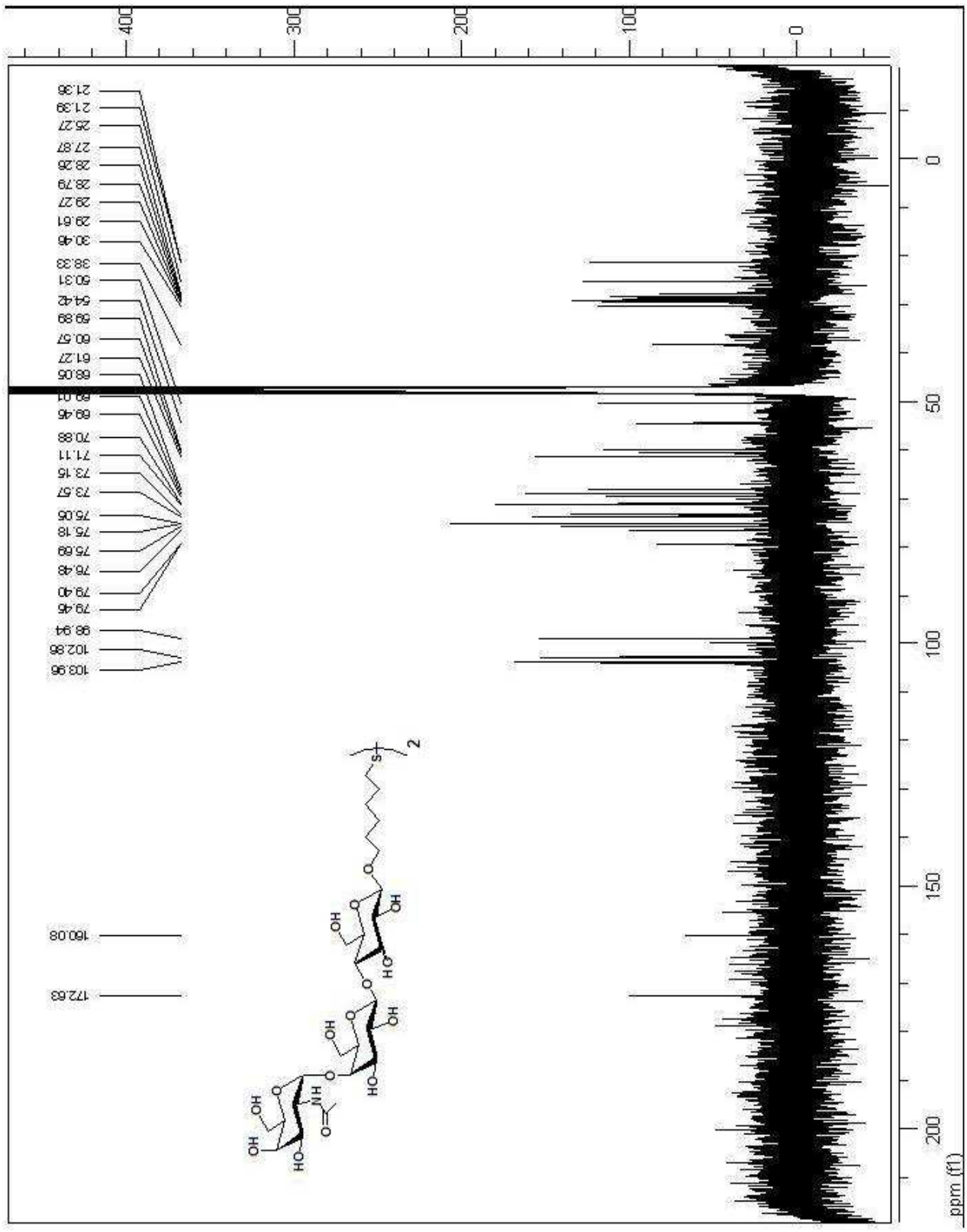
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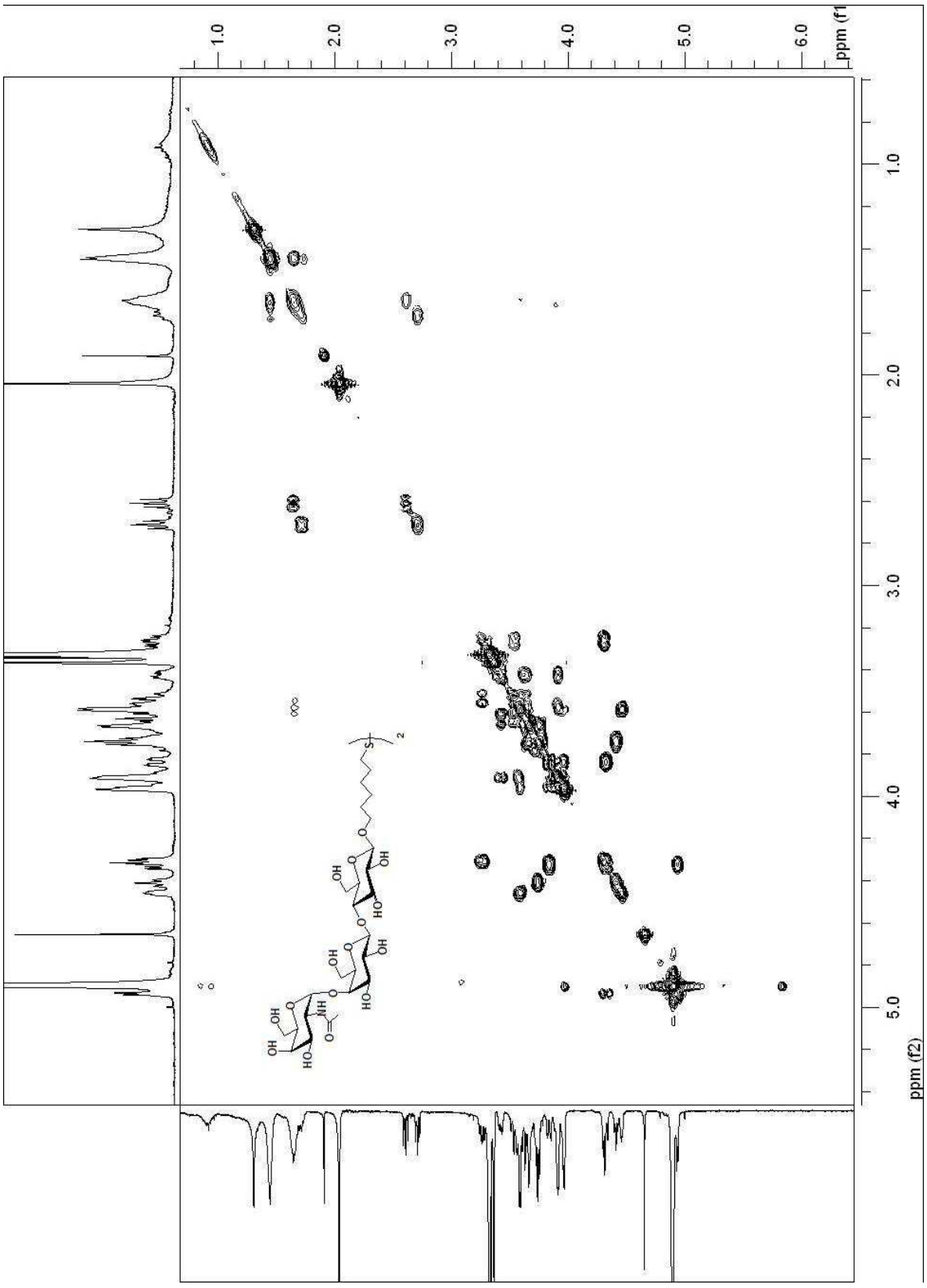
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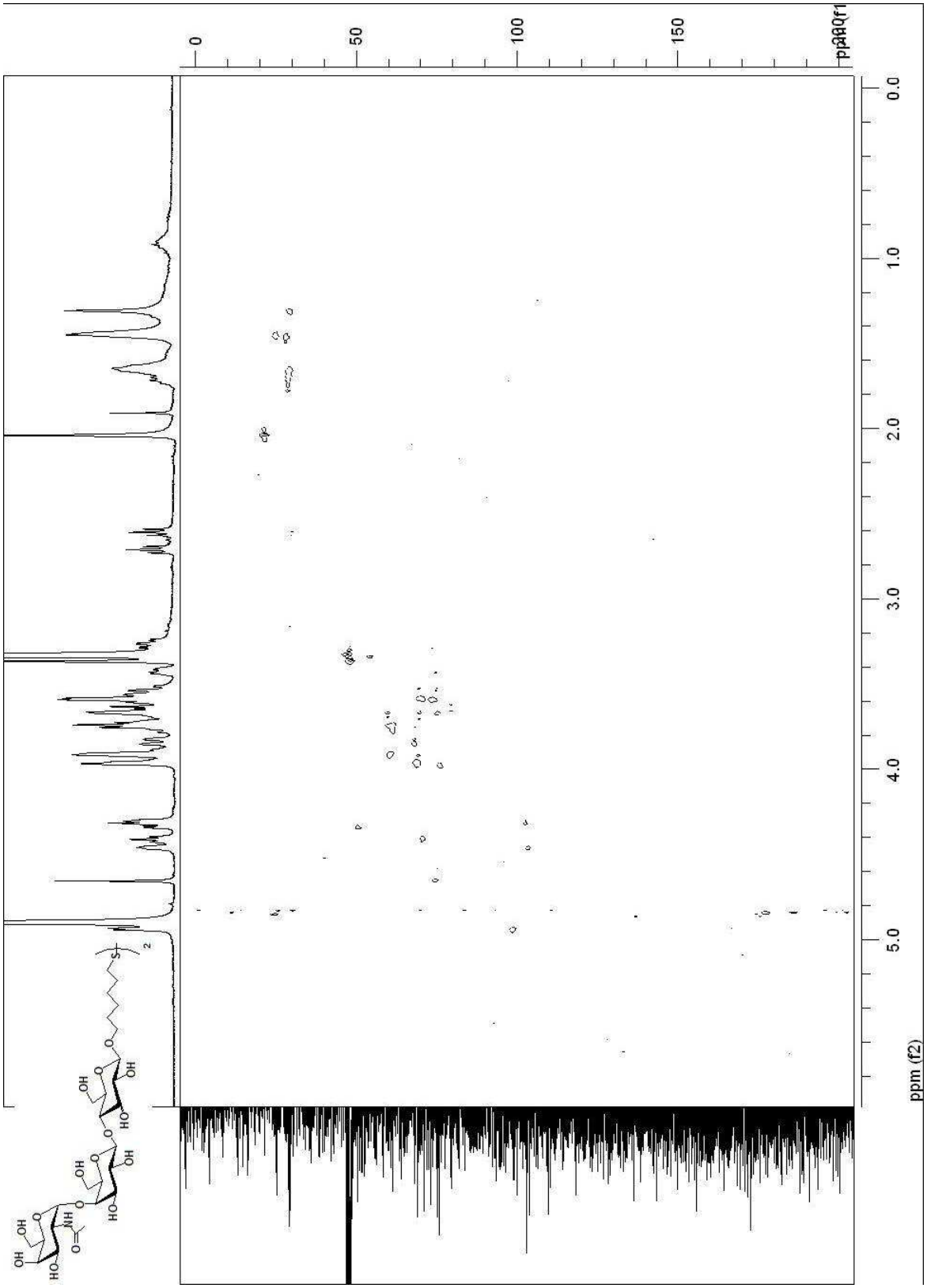
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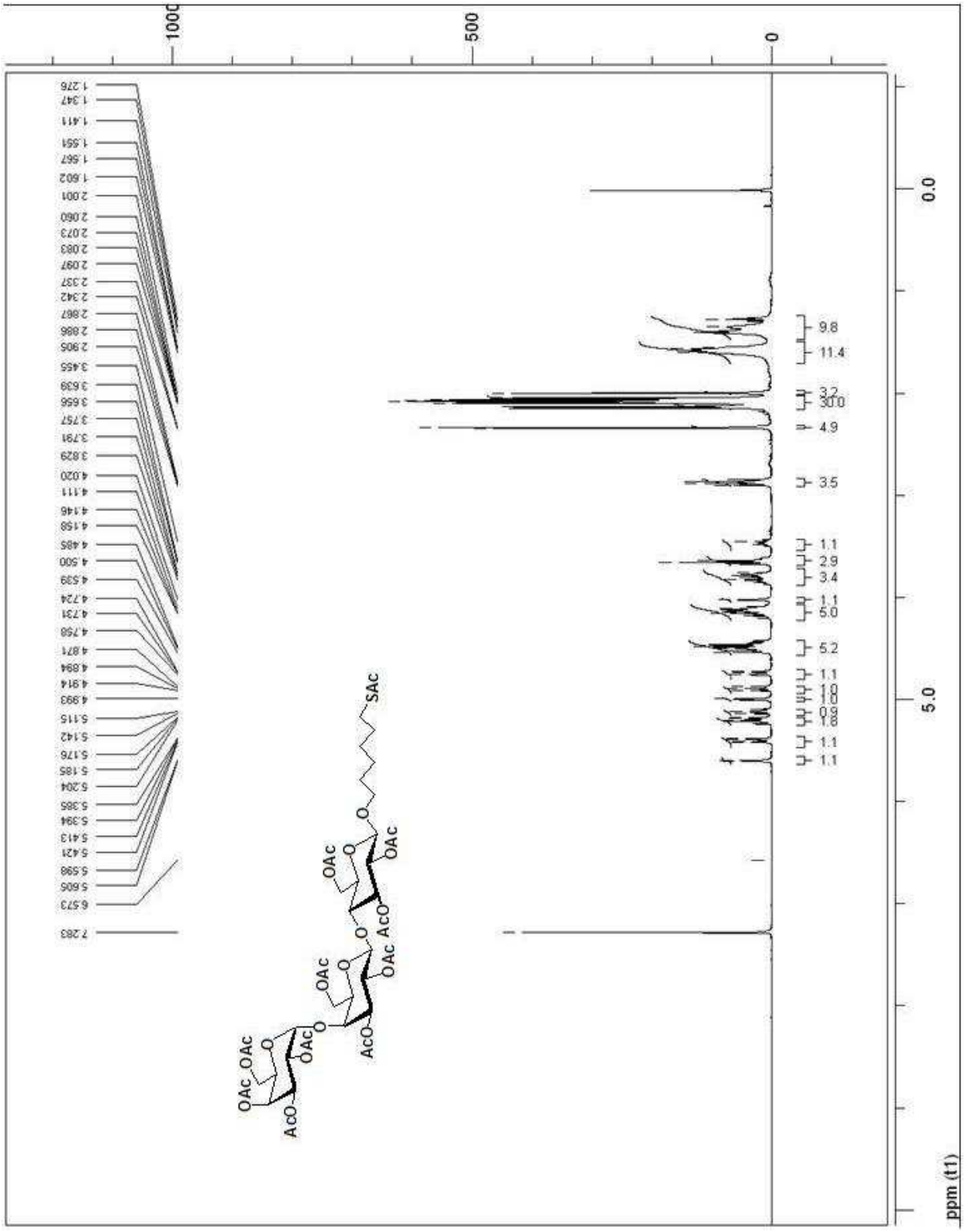
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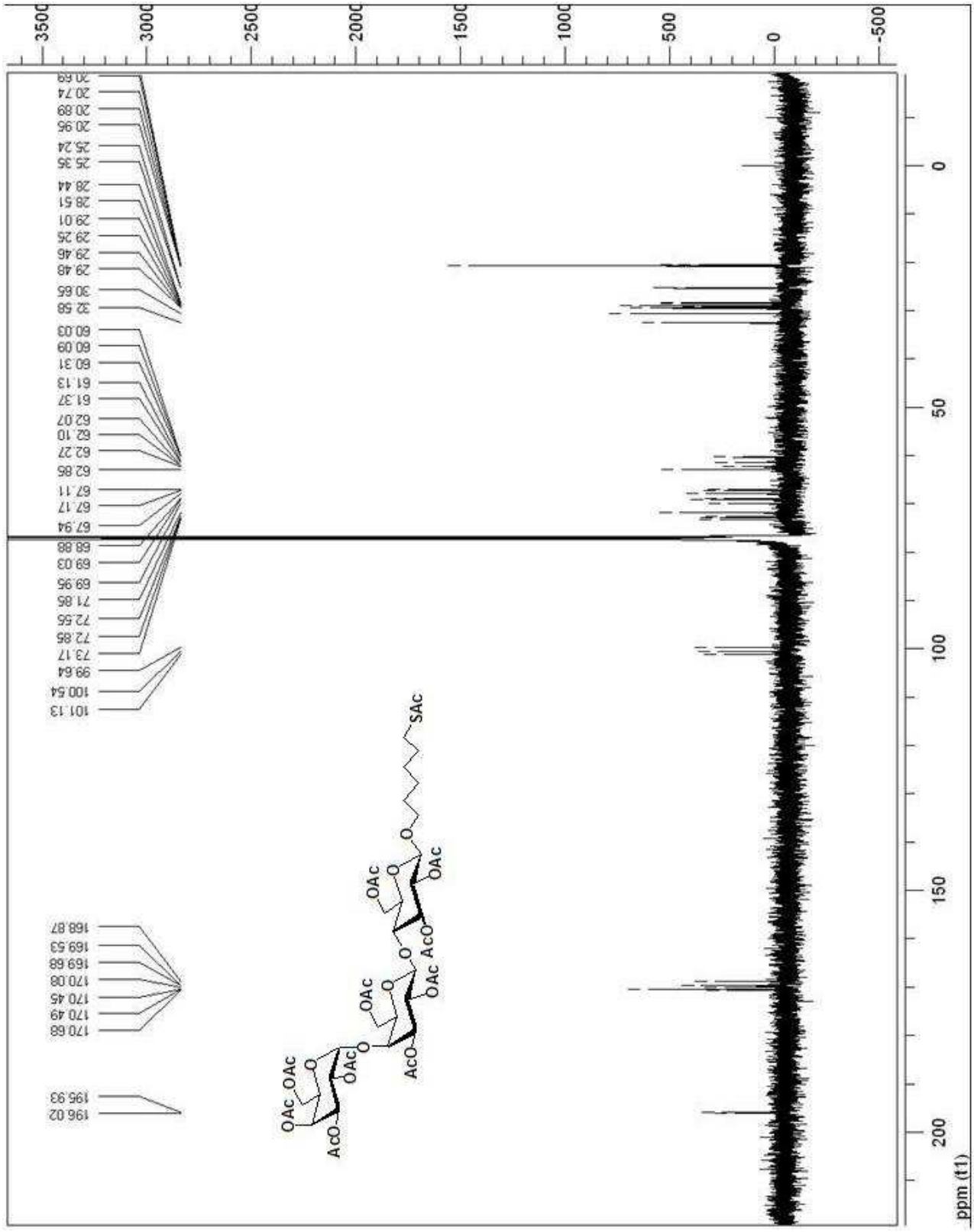
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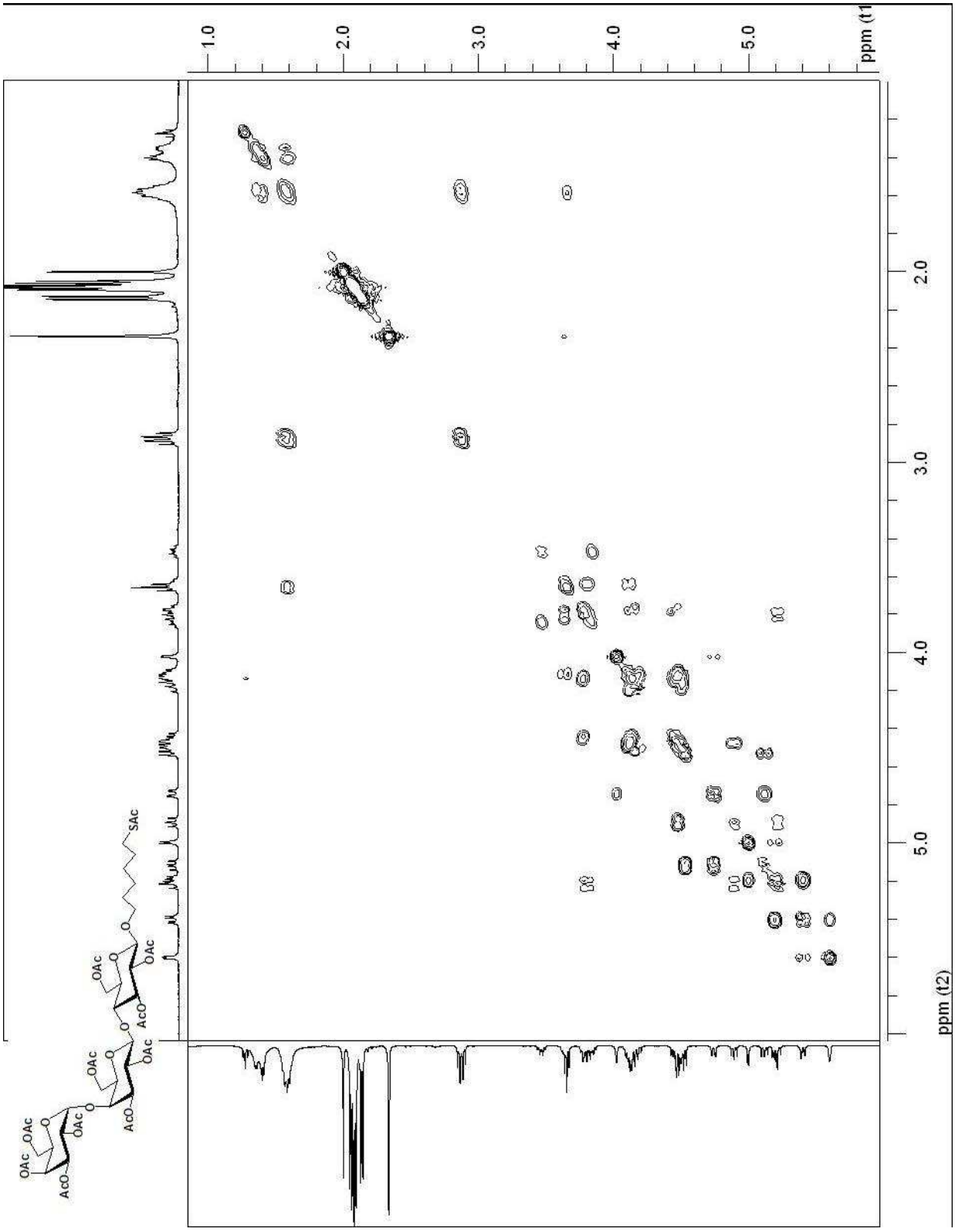
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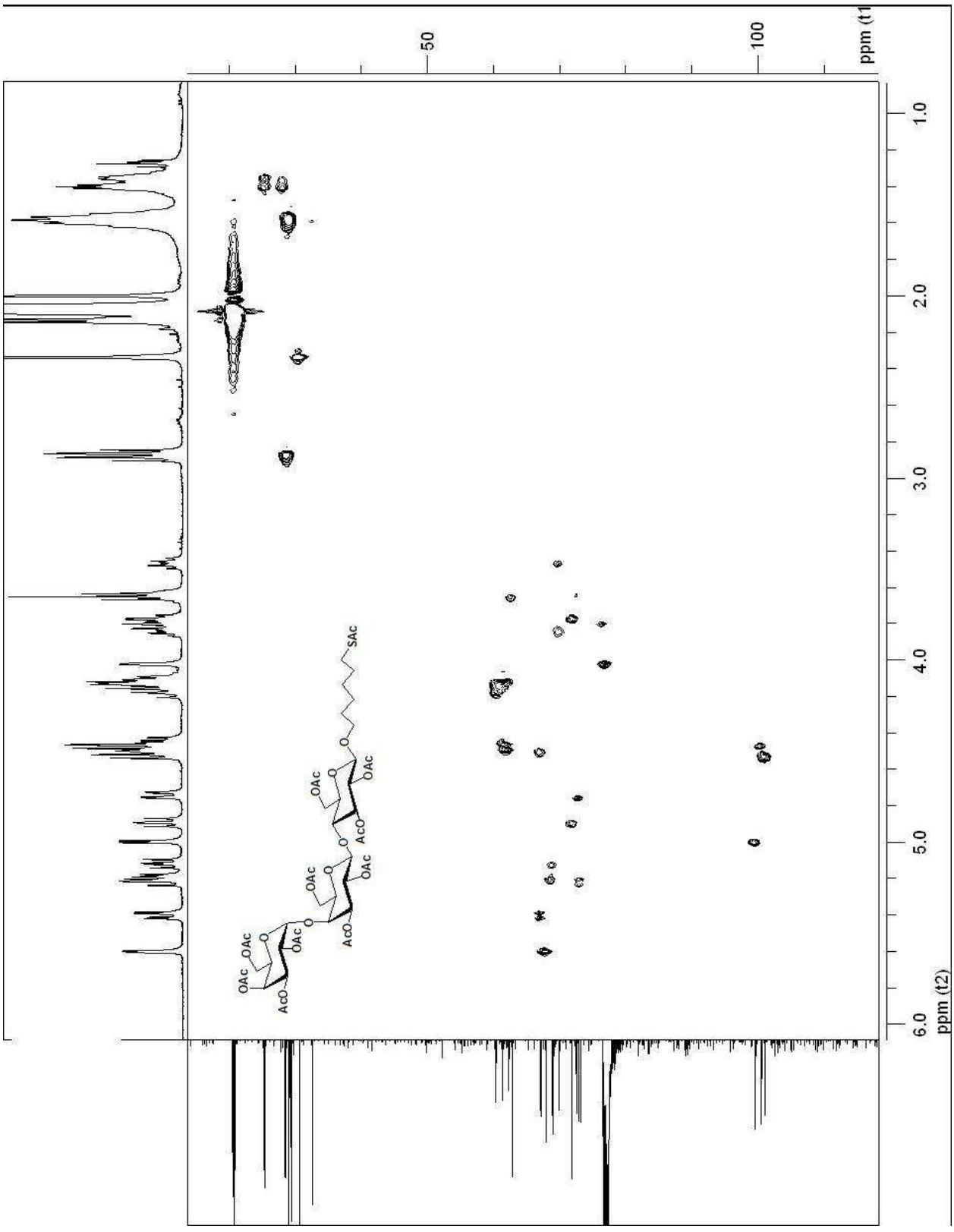
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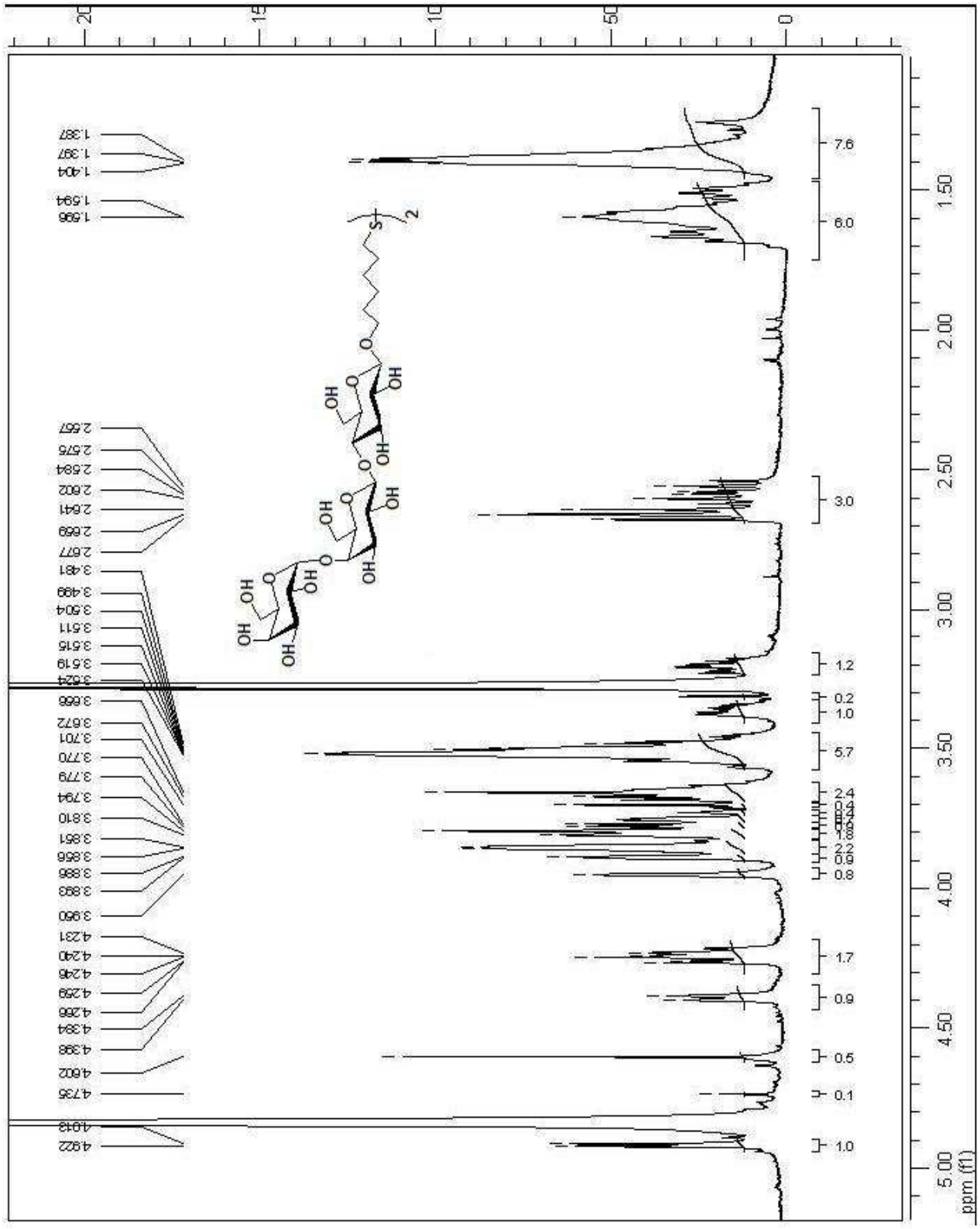

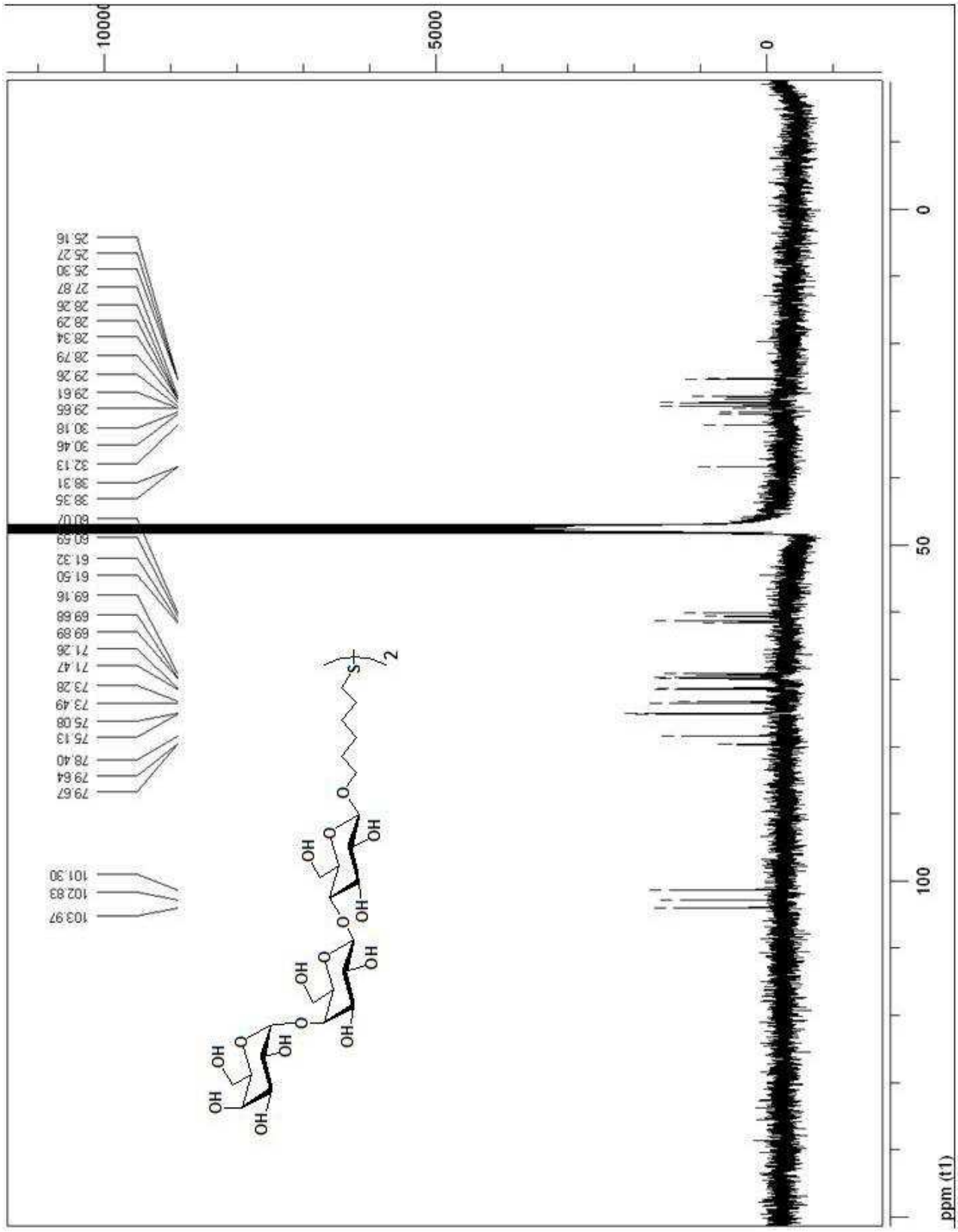
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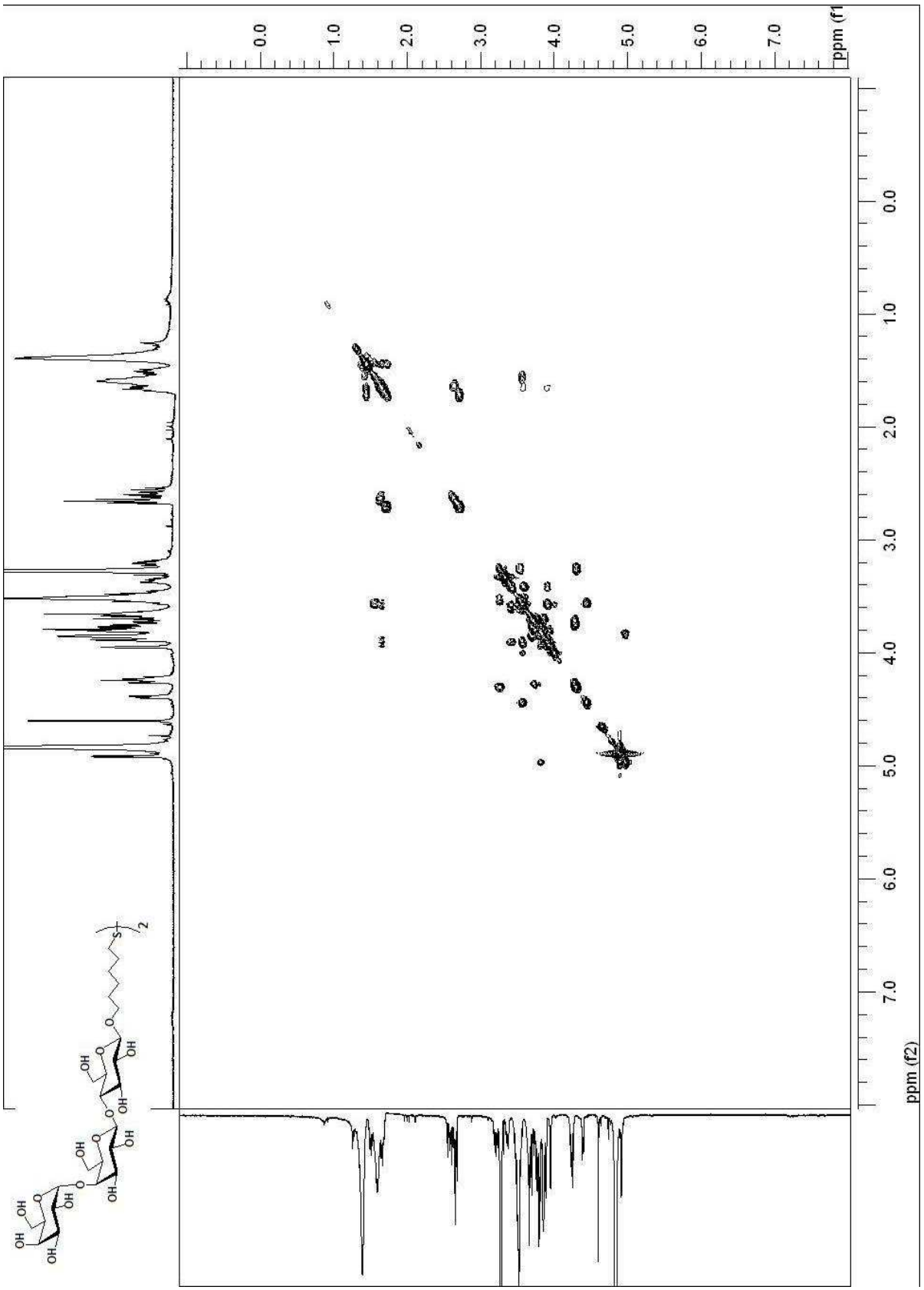
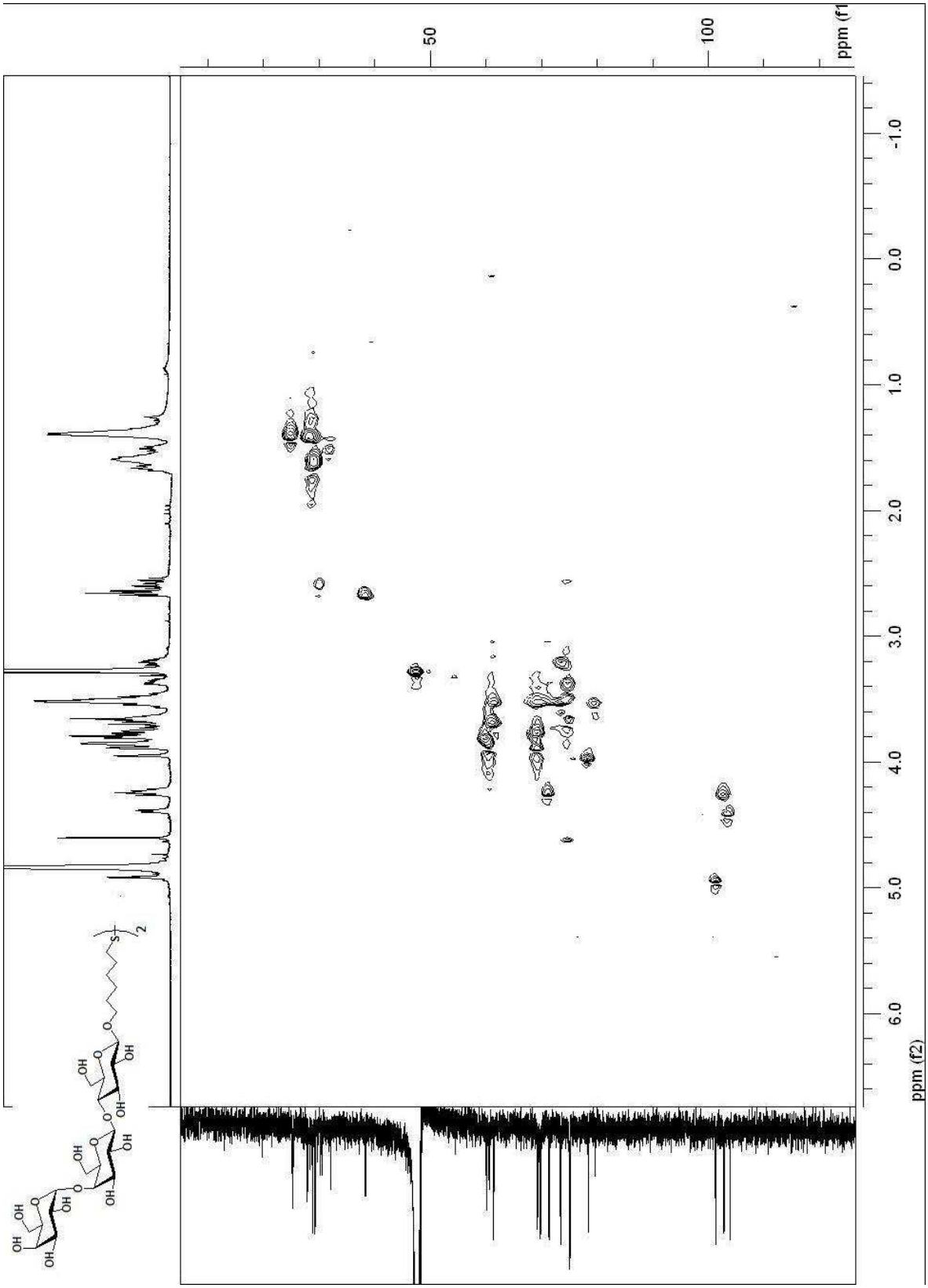
100
ppm (t1)


0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
ppm (f1)
ppm (f2)
OH
O
S
2


50
100
ppm (f1
-1.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
ppm (f2)
OH
O
S
2

Characterization of gold NPs

The gold NPs were characterized using NMR, UV-VIS and TEM analysis. Typical spectra of gold NPs using different techniques are shown in figures 1-3.

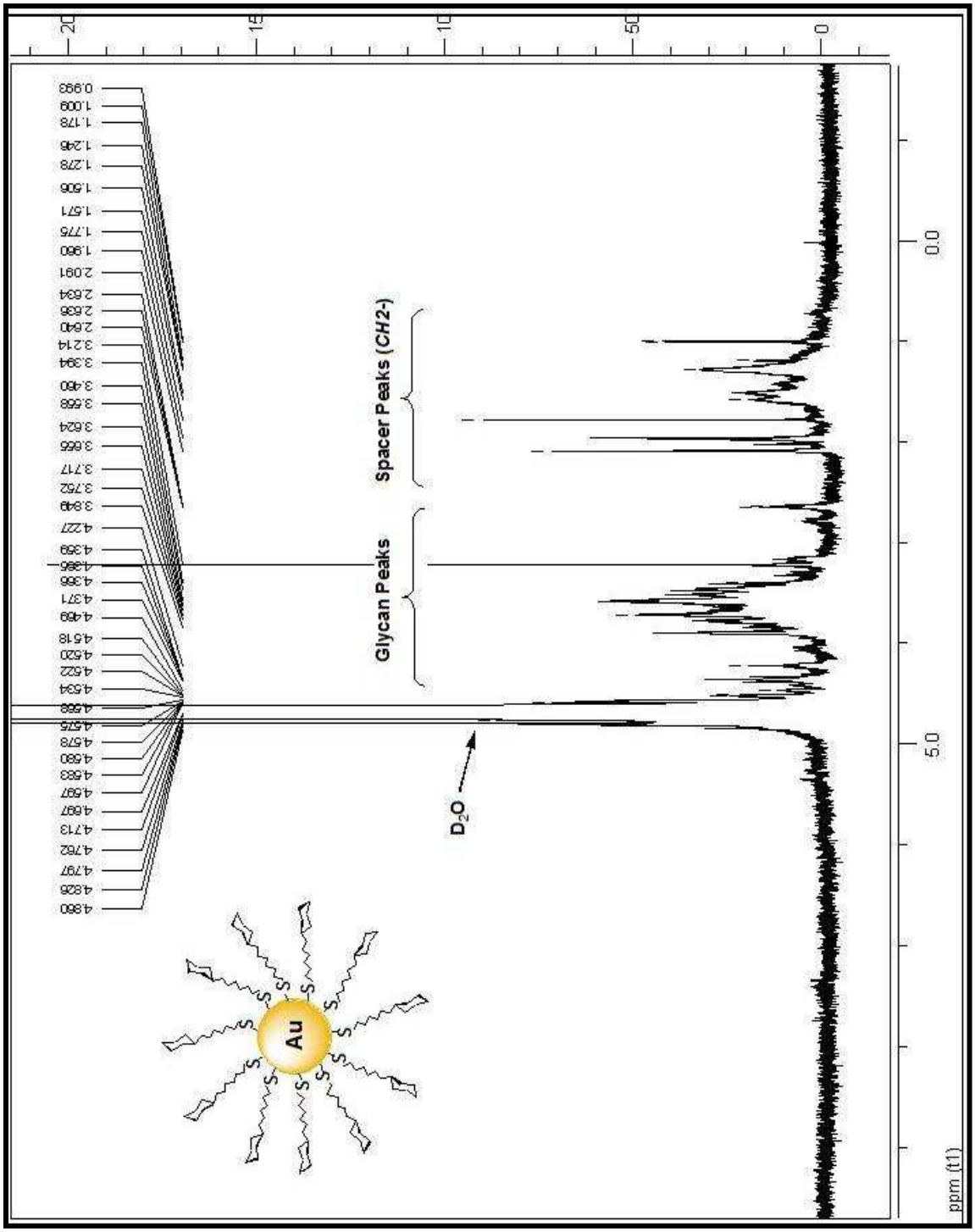


Figure 1. ^{1}H NMR spectra of GNP1

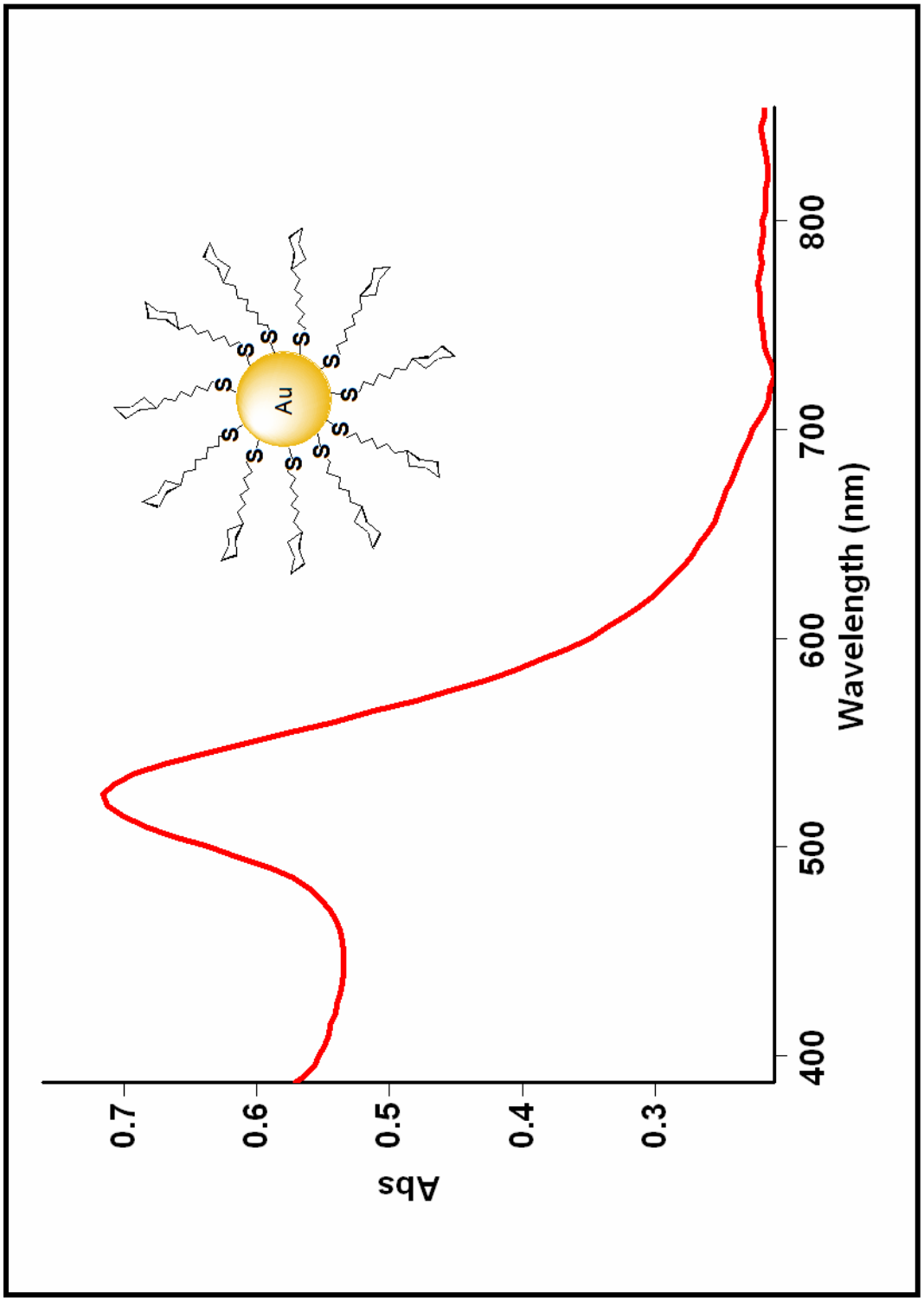
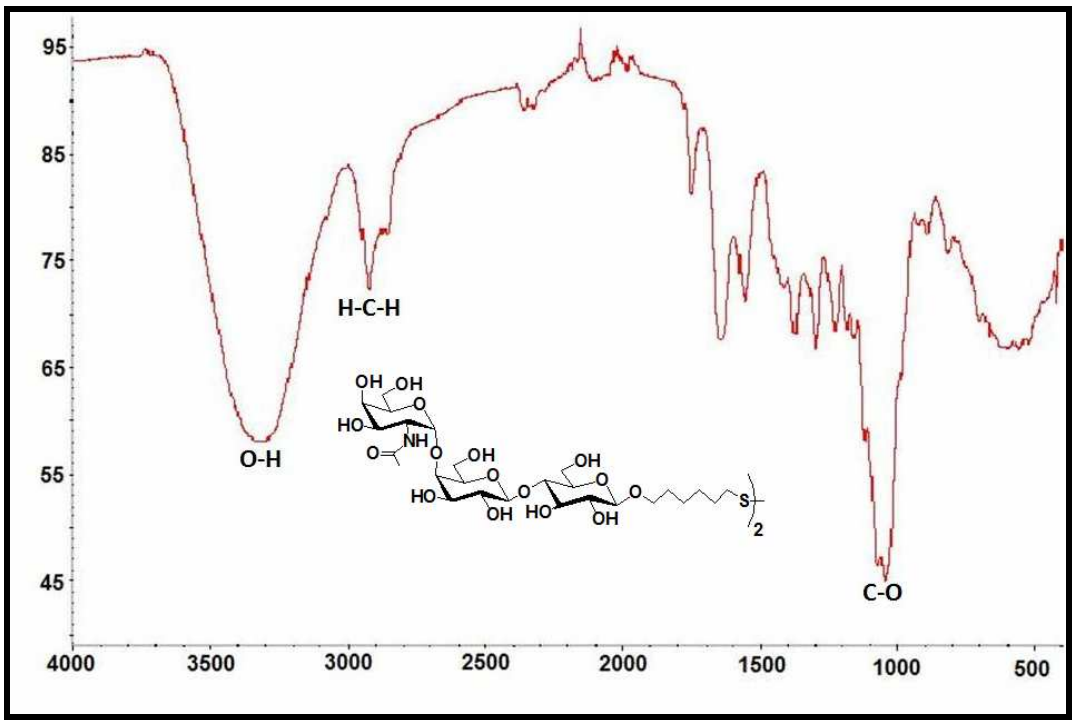


Figure 2. UV-VIS spectra of GNP1.The surface plasmon resonance is clearly visible at 515 nm.

A



B

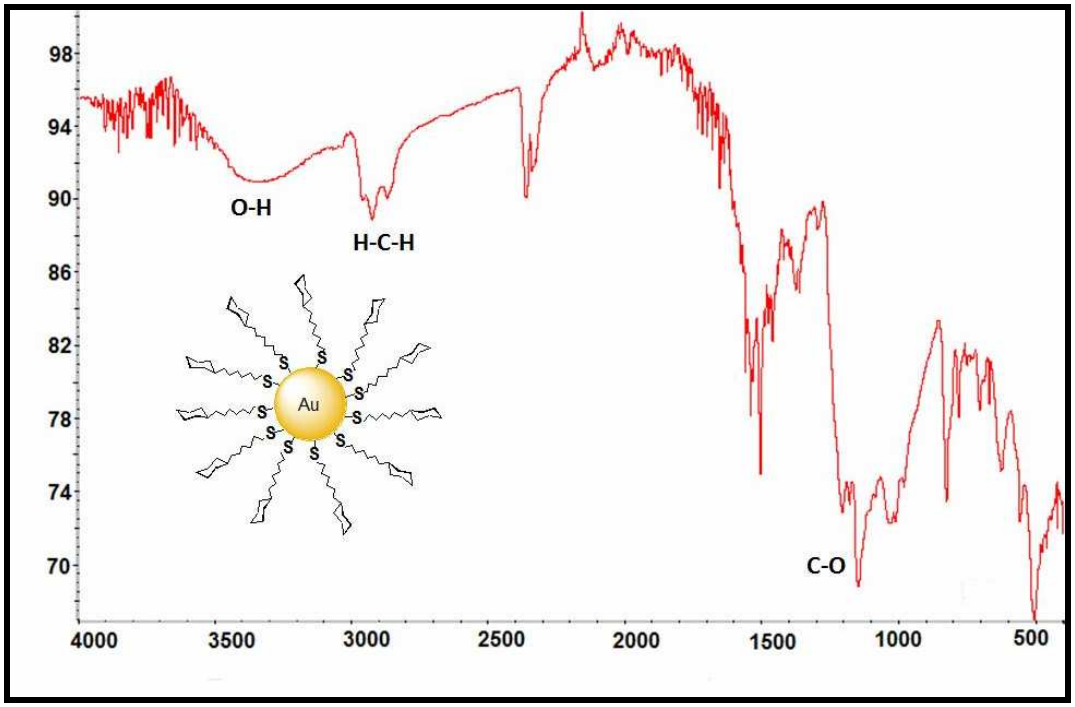
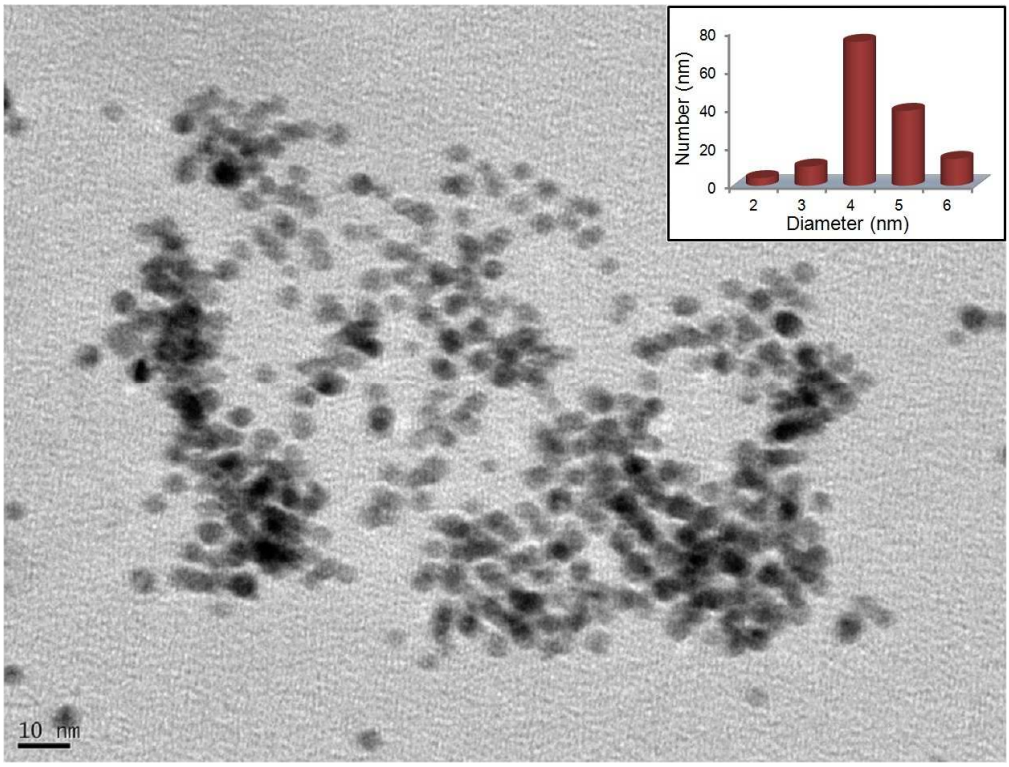
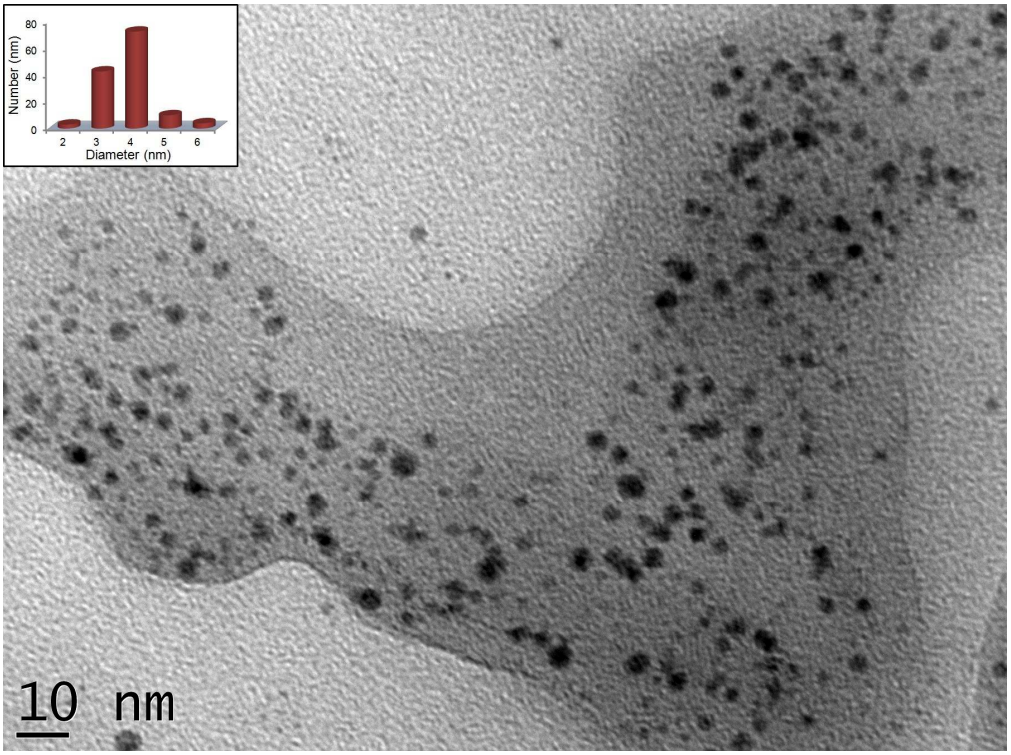


Figure 3. **(A)** IR spectra of LG2, the NHAcGal-Gal-Glu ligand. **(B)** IR spectra of the GNP2.

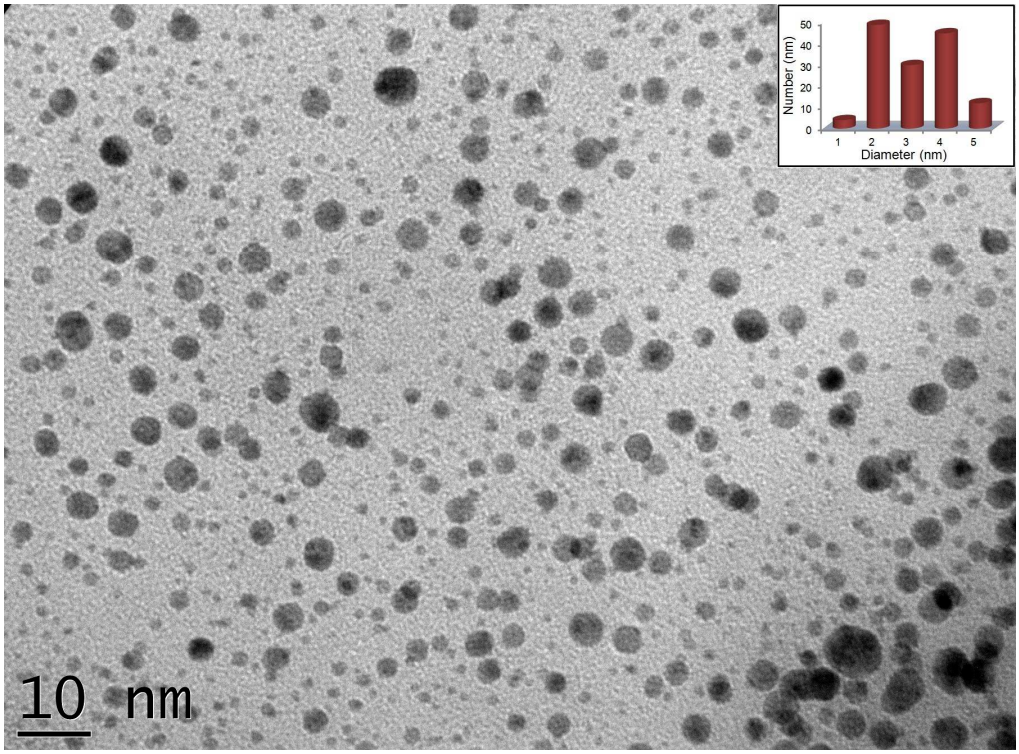
A)


Number (nm)
80
60
40
20
0
2
3
4
5
6
Diameter (nm)
10 nm

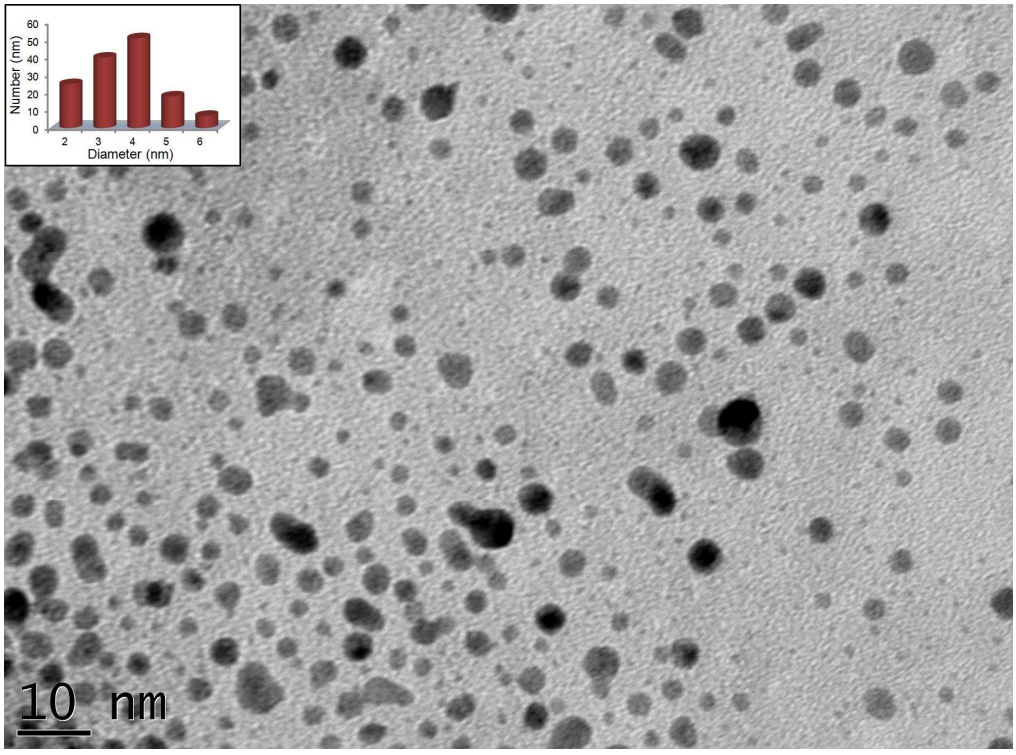
B)


Number (nm)
80
60
40
20
0
2
3
4
5
6
Diameter (nm)
10 nm

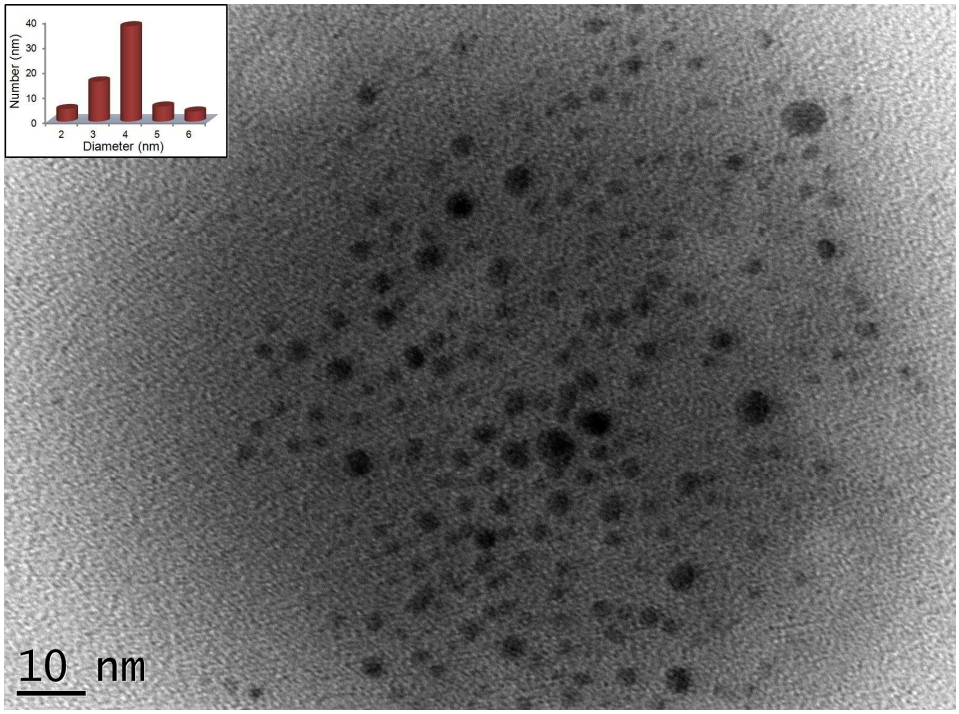
C)


Number (nm)
50
40
30
20
10
0
1
2
3
4
5
Diameter (nm)
10 nm

D)


Number (nm)
60
50
40
30
20
10
0
2
3
4
5
6
Diameter (nm)
10 nm

E)



F)

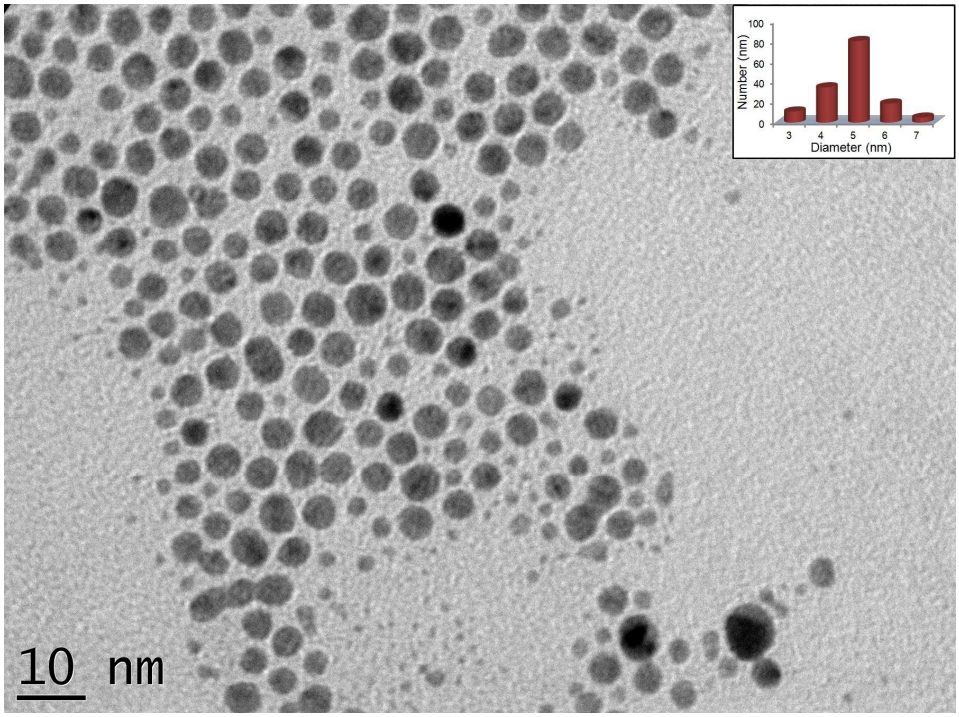


Figure 4. TEM image of GNP1 **(A)**, GNP2 **(B)**, GNP3 **(C)**, gold NP with LG2:OEG – 75:25 **(D)**, 50:50 **(E)**, 25:75 **(F)**. Inset: The size distribution of the NPs.

Determination of number of sugars per nanoparticle.

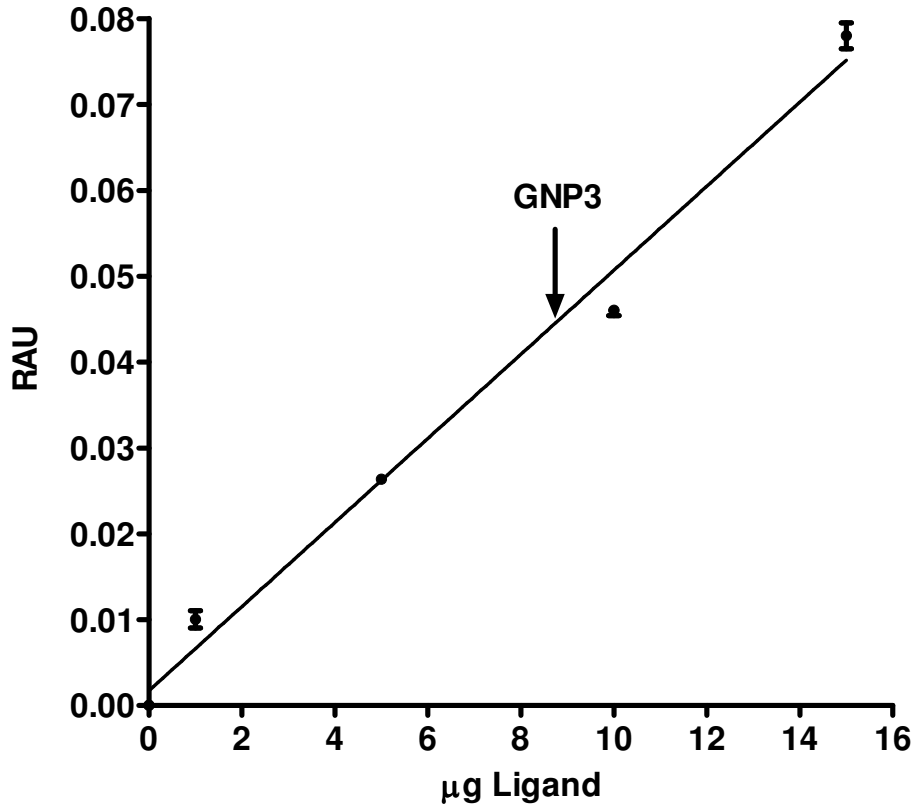
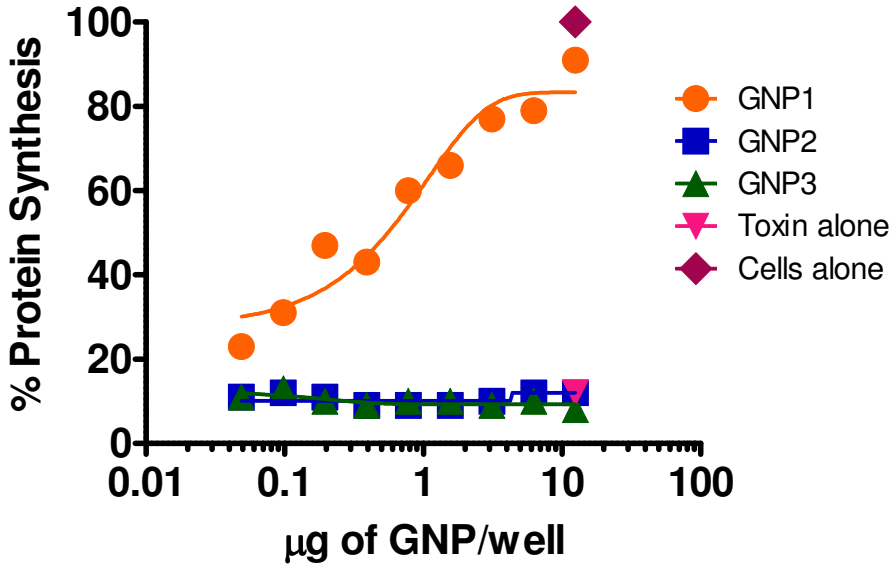


Figure 5. The plot of concentration of free ligand versus absorbance.

GNPs	Amount of Sugar on 1 mg of NPs (μg/mg)	No. of sugar per NPs
GNP1	105	70
GNP2	95	65
GNP3	85	125

Table 1. Number of sugars per nanoparticle.

A)



B)

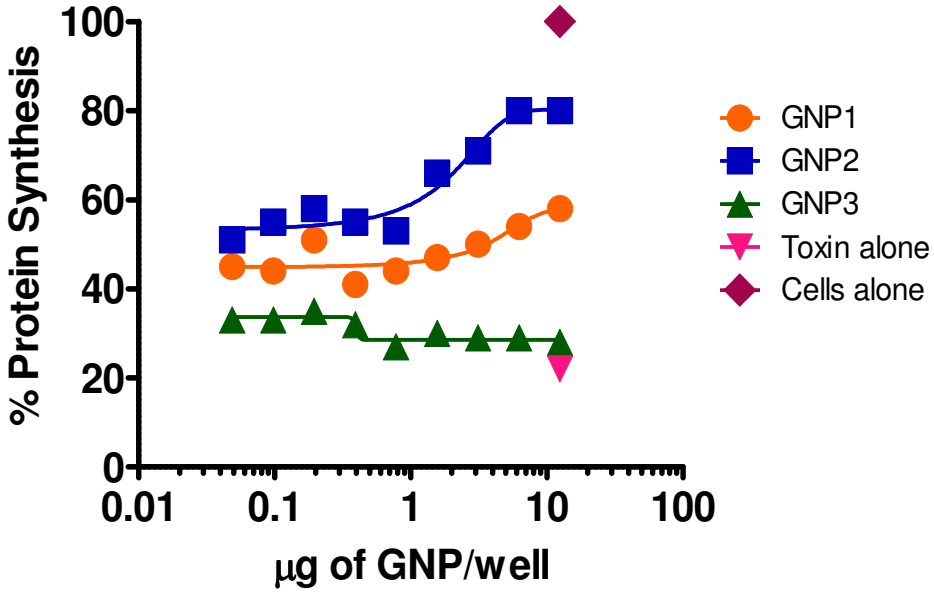


Figure 6. **(A)** Inhibition of 6.4 ng of Stx1 with varying concentrations of GNPs. **(B)** Inhibition of 6.4 ng of Stx 2 with varying concentrations of GNPs

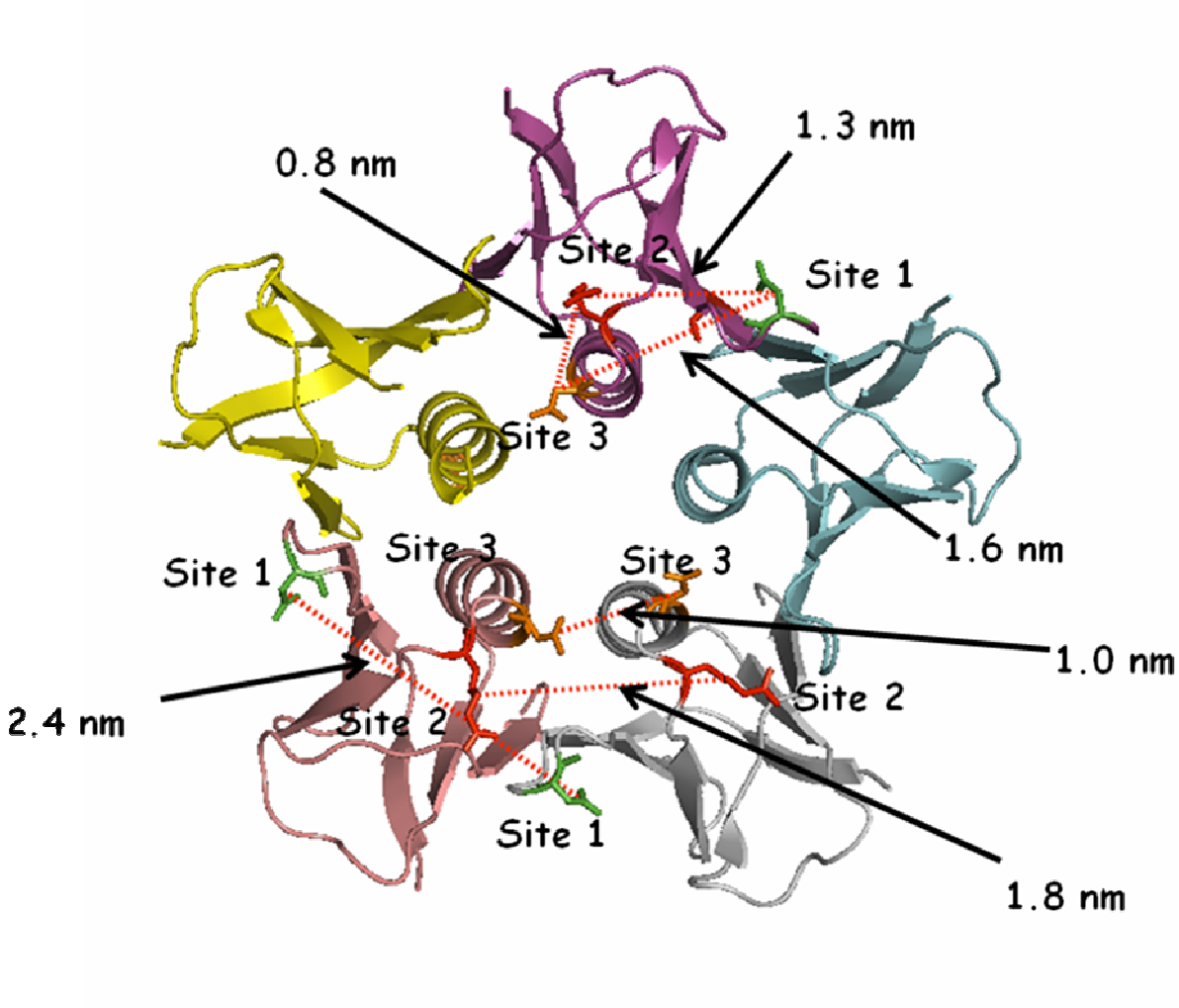


Figure 7. Representation of Shiga 2a depicting the distances between the three theoretical binding sites based on studies with Stx1.(*4*) The coordinates were downloaded from NCBI(*5*) and the figure was generated using Pymol®. The distance between site 1 and 2 was calculated to be ~ 1.3 nm, distance between site 2 and 3 was calculated to be 0.8 nm and the distance between site 1 and 3 was calculated to be ~ 1.6 nm. Also the distance between binding sites 2 of adjacent B subunits was calculated to be 1.8 nm.

References:

(1) Kale, R. R., McGannon, C. M., Fuller-Schaefer, C., Hatch, D. M., Flagler, M. J., Gamage, S. D., Weiss, A. A., and Iyer, S. S. (2008) Differentiation between Structurally Homologous Shiga 1 and Shiga 2 Toxins by Using Synthetic Glycoconjugates. *Angew Chem Int Ed Engl 47*, 1265-1268.

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