Supporting information

Glycosylated asterisks are among the most potent low valency inducers of Concanavalin A aggregation

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Concanavalin A preparation

In a 10 mL centrifuge tube, approximately 5.0 mg of Concanavalin A (Con A was purchased from Sigma catalogue number: L7647) was dissolved without stirring in 10 mL of HEPES buffer with 100mM $CaCl_2$ (pH = 7.5). The tube was stored at 4 ºC for 12 hours to allow the Con A to dissolve. Afterwards, the solution was placed in a dialysis tube and dialyzed against a 1-L of tris buffered saline solution (TBS) for 4 hours. This was repeated with fresh TBS solutions, followed by dialysis against a 1L of phosphate-buffered saline solution (PBS) for

16 hours. The dialysis was done to remove any excess Ca^{2+} from the lectin solution. The Con A solution was removed from the tube and stored at 4 ºC until needed. ($C_{Con\ A}$ = 0.19 mg / mL).

Blood preparation

Fresh whole rabbit blood on heparin was obtained in a 4 mL vial. Alsever's solution was added to the blood to make up a 60:40 v/v solution. The blood was separated into 2 mL aliquots in 15 mL centrifuge tubes. These were then diluted to 12 mL with Alsever's solution. The cells were pelleted by centrifugation (1500 rpm x 15 min), and the layer of white blood cells and plasma proteins was removed with a pipette. This process was repeated 2 more times using PBS instead of Alsever's solution. The blood was then made up in the assay buffer solution, PBS w/ 0.5%BSA. Hemagglutination assays were all performed on blood obtained from the same rabbit during the same day.

Concanavalin A titration

Decreasing amounts of Con A were incubated with red blood cells to determine the lectin concentration needed to agglutinate the cells. Serial two-fold dilutions of Con A were made in a 96 well V-bottomed microtiter plate. To each well 50 µL of the blood solution was added and incubated for 1 hour at 27 ºC. After this time the wells were examined and the amount of Con A required to agglutinate the cell suspension was determined by mixing the solution in the well one time. The agglutinated wells showed a colourless solution with a red precipitate. This was then considered to be 1 unit (C = 0.023 mg/mL). For the inhibition assay a 2 unit Con A solution was made up. The inhibited wells showed a red colour solution.

Inhibiting dose determination

Following standard protocols,[1,2] and starting with a concentration of 25 mg/mL, serial two-fold dilutions of the inhibitors were made on two parallel plates. The inhibitor solutions were incubated with two times the agglutinating dose of Con A solution for an hour and a half at 27°C. After this time the blood solution was added and the wells were mixed and incubated for 2 hours at 27 ºC. The minimum concentration causing inhibition (MIC) was determined visually and this was the inhibiting dose. Each value was obtained by averaging the results of three independent experiments. The error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions of the assay. The parameter β represents the relative activity of inhibition/ligand in comparison to the monovalent analogue. β/N represents the relative activity/sugar concentration.

Blank and control experiments

Blank controls were performed in parallel to all assays with 50µL erythrocytes solution (6% in PBS) + 50µl of PBS on one column to ensure non agglutination and with 50µlL erythrocytes solution (6% in PBS) + 25 µL of PBS + 25µL of Con A (0,023mg/mL) on another column to ensure agglutination. The β-glucose asterisk **9** was used as a negative control to assess non-specific activity of the scaffold, as it is the most closely related compound available to compare. Similarly, the aniline amides of **2** and **4**, *N*-Phenyl 2-

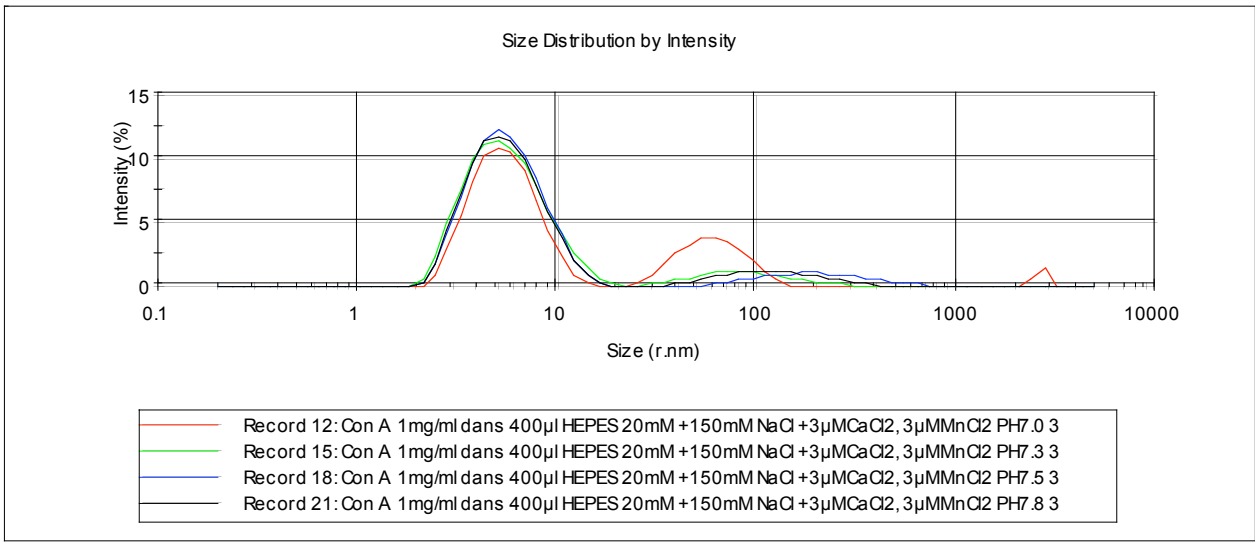
(1) Osawa, T.; Matsumoto, I. *Methods Enzymol*. **1972**, *28*, 323-327.
(2) Wolfenden, M. L.; Cloninger, M. J. *Bioconjugate Chem*. **2006**, *17*, 958-966.

(glucopyranosyloxy)acetamide and *N*-Phenyl 2-(mannopyranosyloxy)acetamide, respectively, were used to probe for sub-site assisted aglycone binding effects.
The hexa-acetamido asterisk (precursor to **1**) was ruled out as a control due to lack of solubility; the polyamino asterisk **1** was similarly regarded as a poor control, as polyamines are known to have a large range of activities and thus will be a very poor model for the glycosylated asterisk.

Dynamic light scattering experiments (DLS)

Preliminary DLS experiments were performed on a Zêtasizer Nano ZS from Malvern Instruments. Ligands and protein solutions were each centrifuged (13000 rpm / 10 minutes) and then filtered on polyethersulfone membrane (Minisart®, 0.2 μm, Sartorius). Both lectin and asterisk **10** were checked in different conditions of pH and time to ensure the absence of aggregates.

In a series of experiments aiming to study the effect of the pH and time on Con A, we prepared a series of solutions containing Con A (1mg/ml) in HEPES buffer (20mM) with different pH (7.0; 7.3;7.5; 7.8). Since Con A is a non spherical particle, the distribution of the hydrodynamic radius were checked by both intensity and volume, which showed primarily particles with radius below 10nm. The same solutions were checked after 4 and 24h to ensure the absence of large aggregates (Figure 1).



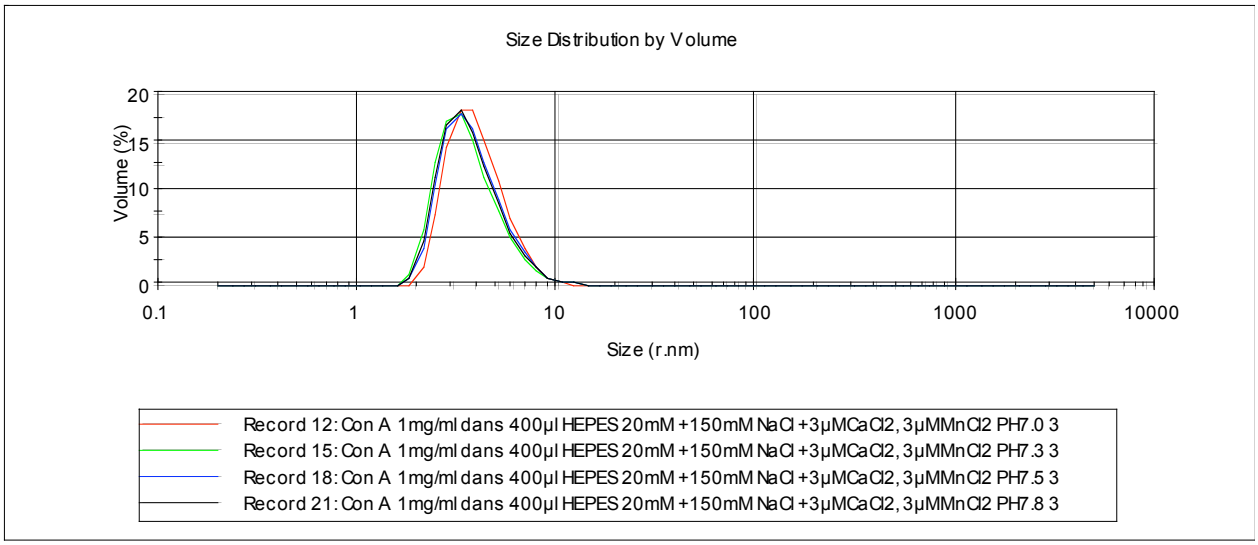
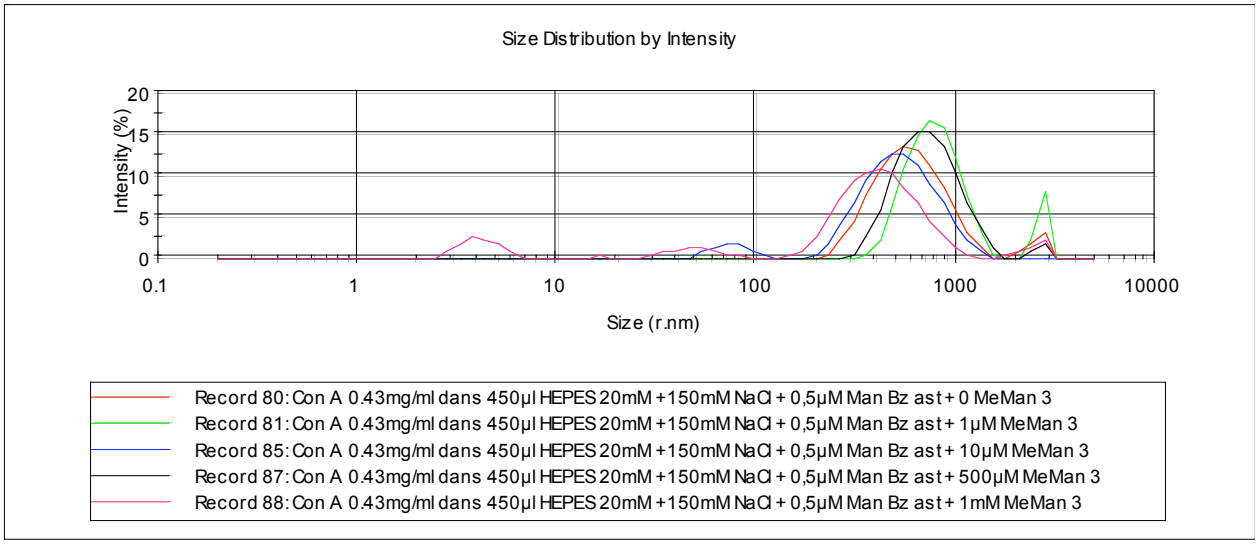


Figure 1 Con A vs pH

In a series of solutions containing con A (0.43mg/ml), asterisk **10** (0.5µM) and different concentrations of Me-α-Man (0, 1, 10, 500, 1000µM), it took almost 2000 times the concentration of **10** (1 mM) of the Me-α-Man to offset the effect of the asterisk (pink curve, Figure 2).



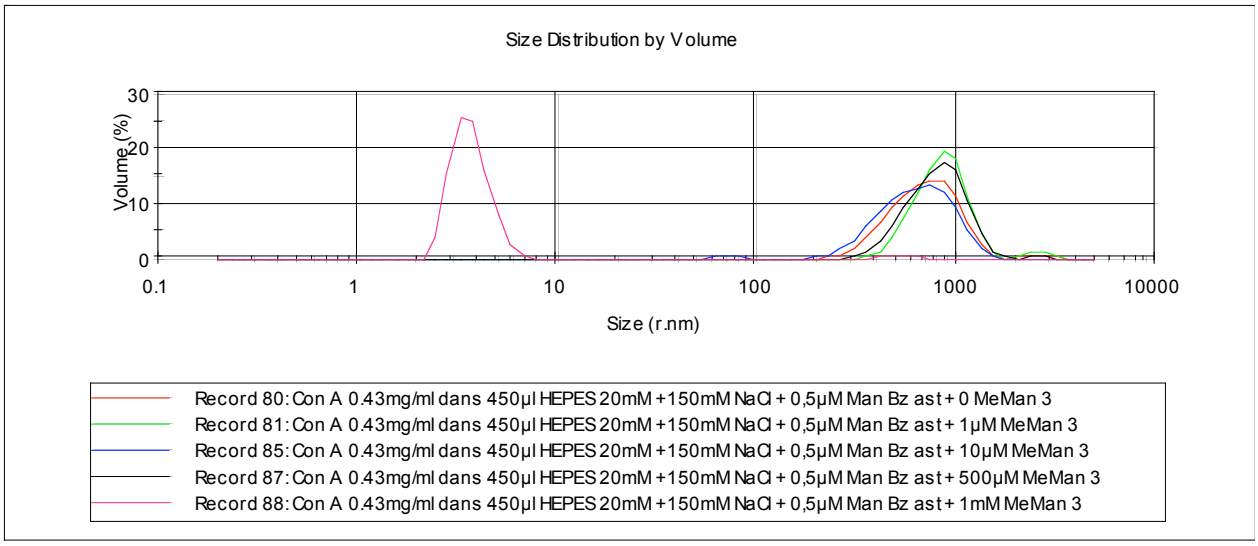
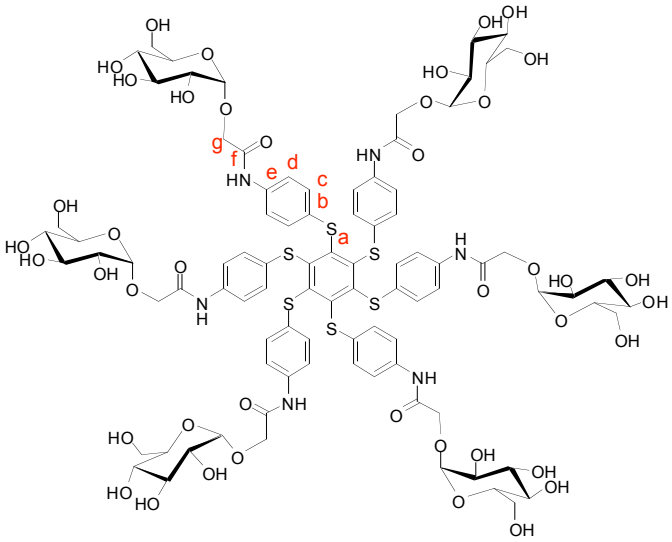


Figure 2 Con A+ **10** + Me-α-Man

General experimental procedures

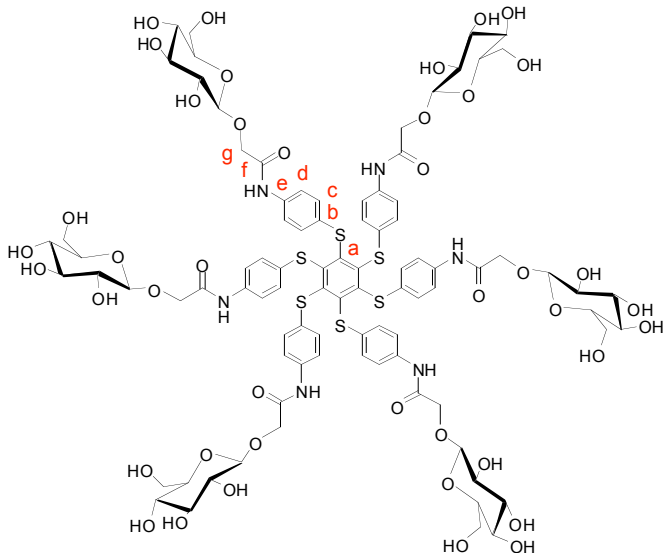
Flash chromatography was performed using Merck Gerudan Si 60 silica gel (40-65µM). Most chemicals were purchased from Aldrich, Acros and Fluka. TLC was performed on Kieselgel 60 F254 plates from Merck. Detection was carried out under UV light or by spraying with 20% ethanolic sulfuric acid followed by burning. DMF extra dry, purchased from Acros, was used for the reactions. Tetrahydrofuran was distilled over sodium and benzophenone under Argon prior to use. NMR spectra were recorded on a Bruker DRX 200, 300 and 500 MHZ. Chemical shifts are given in ppm, relative to internal TMS (δ=0.00 for ^{1}H and ^{13}C NMR) or solvent peaks and coupling constants are reported in Hz. ^{1}H and ^{13}C attributions were done systematically using ^{1}H, ^{13}C, DEPT experiments. Optical rotations were measured on a Perkin–Elmer 241 polarimeter (Na-D line) at ambient temperature. Melting points were measured on a Buchi oil apparatus. Infrared absorption spectra were recorded on a Perkin Elmer instrument with a Universal ATR accessory (contact crystal: Diamond/ZnSe).

Hexakis-(4-(α-D-glucopyranosyl)-N-acetamido-thiophenyl)-benzene (8):



M.p. : 190-200°C; TLC (SiO_2; PrOH /NH_4OH/H_2O : 6/4/1) R_f = 0.1; $[\alpha]_D$= +58 (C= 0.6; H_2O) IR (ν, cm^{-1}; KBr) 3368 (OH, NH) 1669 (C=O amides) ^{1}H NMR (300.13 MHz, $DMSO_{d6}$) δ= 9.94 (s; 6H; NH) 7.52 (d; 12H; J=7.7; Hd) 6.95 (d; 12H; J=7.5; Hc) 5.72 (s; 6H; OH) 4.97-4.95(m; 12H;OH) 4.82 (d; 6H; J=3.3; H1) 4.51 (t; 6H; J= 5.3; OH6) 4.15 (dd; 12H; J_1=16.2; J_2 =44.6; H6) 3.66-3.11 (m; 48H; H2, H3, H4, H5, Hg) ppm. ^{13}C NMR (75.46 MHz, $DMSOd_6$) δ= 169.0 (Cf) 147.2(Ca) 137.4(Ce) 132.6 (Cb) 129.1 (Cc) 121.3 (Cd) 100.8 (C1) 74.3 (C2) 74.1 (C3) 72.5 (C4) 70.8 (C5) 68.0 (C6) 61.6 (Cg) ppm. Elemental analyses: calcd for ($C_{90}H_{108}N_6O_{42}S_6$ + 15H_2O) C 44.88, H 5.78, N 3.49; Found C 44.74, H 5.45, N 3.39.

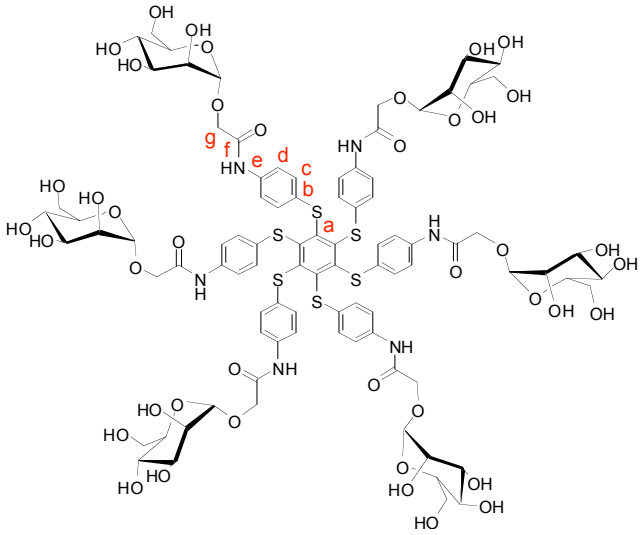
Hexakis-(4-(β-D-glucopyranoside)-N-acetamido-thiophenyl)-benzene (9):



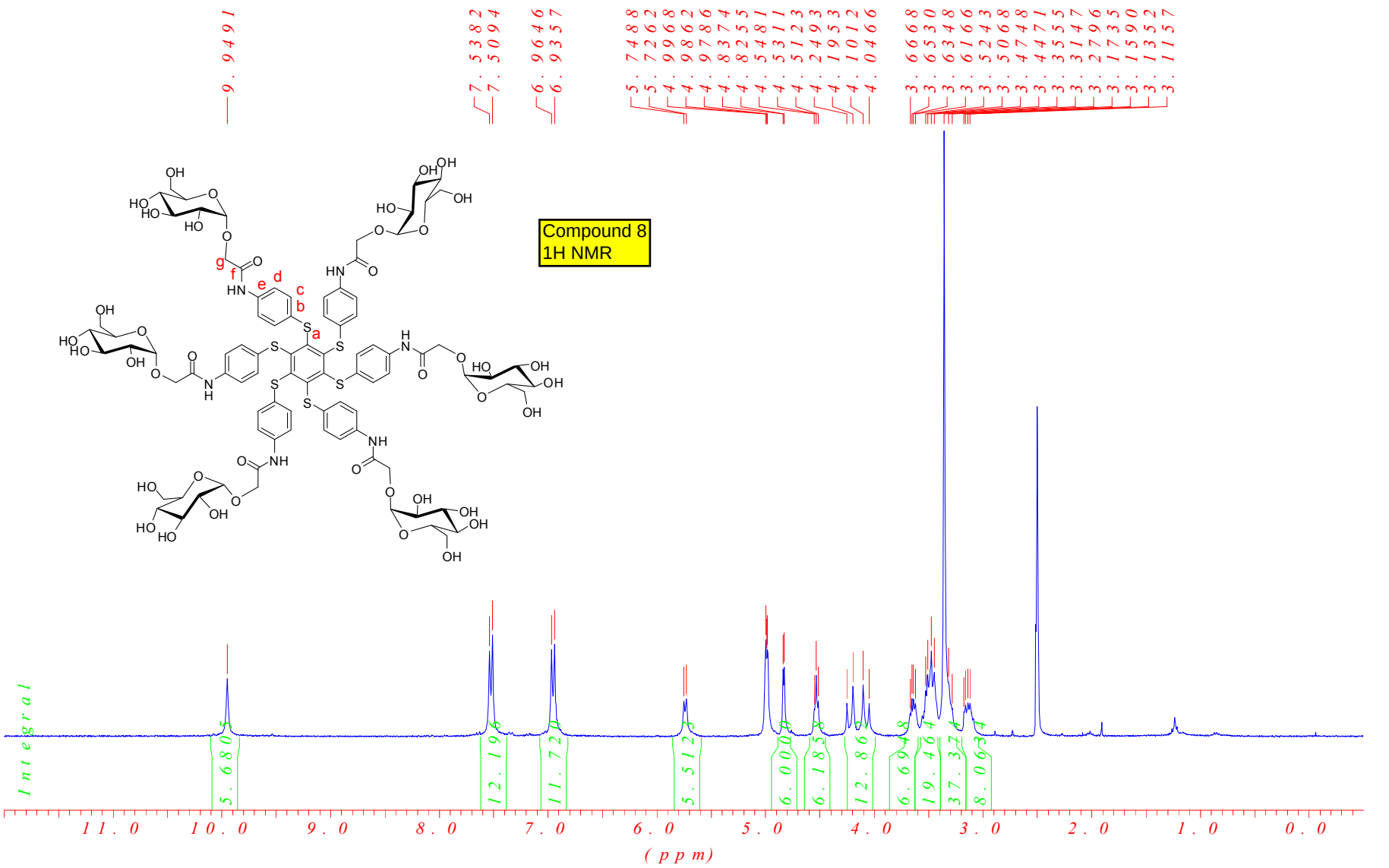
M.p. : 210-215°C; TLC (SiO_2; PrOH/NH_4OH/H_2O: 6/2/1) R_f = 0.1; $[\alpha]_D$=+98 (C=0.2; H_2O) IR (ν, cm^{-1}; KBr) 3391.9 (OH, NH) 1663.8 (C=O) ^{1}HNMR (300.13 MHz, $DMSO_{d6}$) δ=9.81 (s; 6H; NH) 7.53 (d; 12H; J=8.4Hz; Hd) 6.97 (d; 12H; J=8.4; Hc) 6.00 (d; 6H; J=3.4; OH) 5.14 (d; 6H; J=4.3; OH) 5.01 (d; 6H; J=5.2; OH2) 4.60 (t; 6H; J=5.8; OH6) 4.35-4.30(m; 12H; H1, Hg) 4.15 (d; 6H; J=16.2; Hg) 3.70-3.64 (m; 6H; H6) 3.48-3.44 (m; 6H; H6) 3.17-

3.15(m; 24H; H2, H3, H4, H5) ppm. ^{13}C NMR (75.46 MHz, $DMSO_{d6}$) δ =168.1 (Cf) 136.5(Ce) 131.8 (Cb) 128.8 (Cc) 120.3 (Cd) 103.3 (C1) 77.1 (C2) 76.2 (C3) 73.4 (C4) 69.8 (C5) 68.3 (C6) 60.8 (Cg) ppm. Elemental analyses calcd for ($C_{90}H_{108}N_6O_{42}S_6$ + $12H_2O$): C 45.91, H 5.65, N 3.62; Found C 45.54, H 5.10, N 4.07.

Hexakis-(4-(α-D-mannopyranosyl)-N-acetamido-thiophenyl)-benzene (10):



M.p.: 145-150°C. TLC (SiO_2; PrOH:NH_4OH:H_2O/ 60:25:15) R_f = 0.1. $[\alpha]_D$=+9.5 (C=0.2; H_2O) IR (KBr) ν=3381cm^{-1} (OH, NH); 1673 (C=O amides). ^{1}H NMR (300.13MHz, $DMSO_{d6}$) δ=9.75 (s; 6H; NH) 7.52 (d; 12H; J=8.4Hz; Hd) 6.93 (d; 12H; J=8.4; Hc) 4.82 (d; 6H; J=4.2; OH) 4.76 (d; 6H; J=5.2; OH) 4.74 (s; 6H; H1) 4.60 (d; 6H; J=6.1; OH) 4.52 (t; 6H; J=5.8; OH6) 4.13 (s, 12H; Hg) 3.82 (m; 6H; H5) 3.67-3.53 (m; 12H; H6) 3.44-3.31(m; H2, H3, H4) ppm. ^{13}C NMR (75.4MHz, $DMSO_{d6}$) δ=167.8 (Cf) 146.5 (Ca) 136.7 (Ce) 131.8 (Cb) 128.2 (Cc) 120.8 (Cd) 100.0 (C1) 74.5 (C2) 70.7 (C3) 69.8 (C4) 66.9 (C5) 65.5 (C6) 61.1 (Cg) ppm. Elemental analyses calcd for ($C_{90}H_{108}N_6O_{42}S_6$ + $14H_2O$): C 45.22, H 5.73, N 3.52. Found C 45.01, H 5.30, N 3.41.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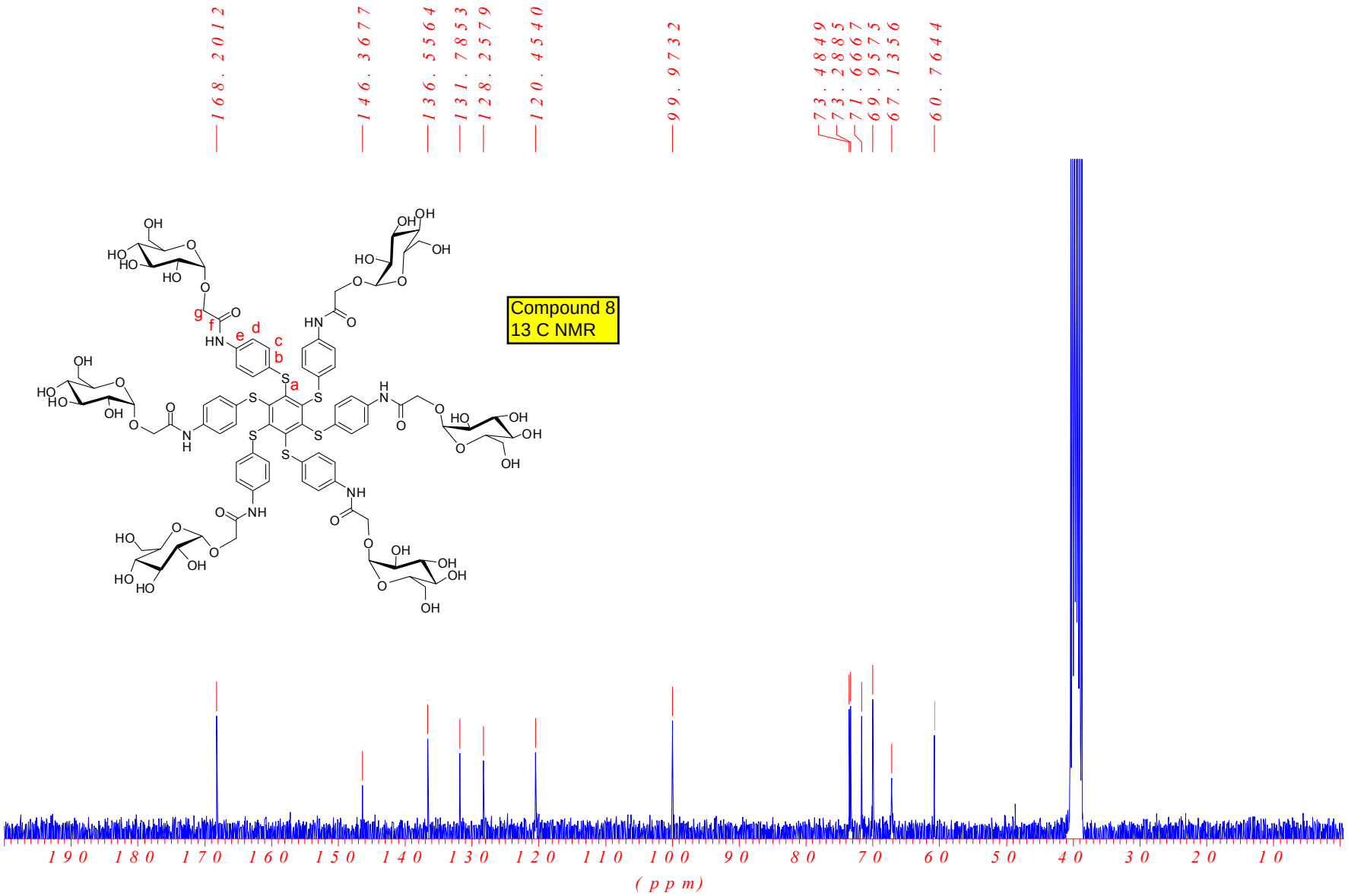
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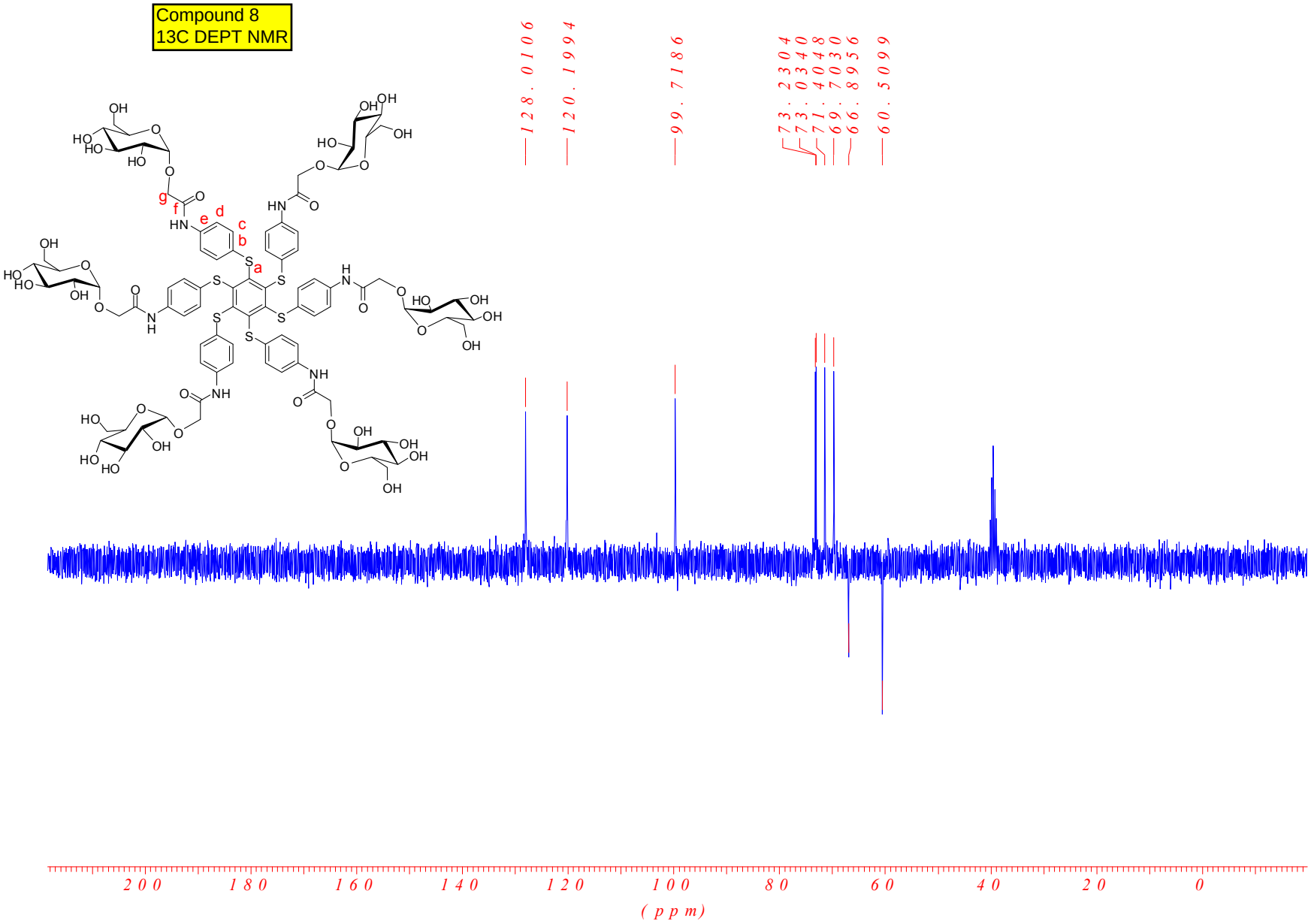
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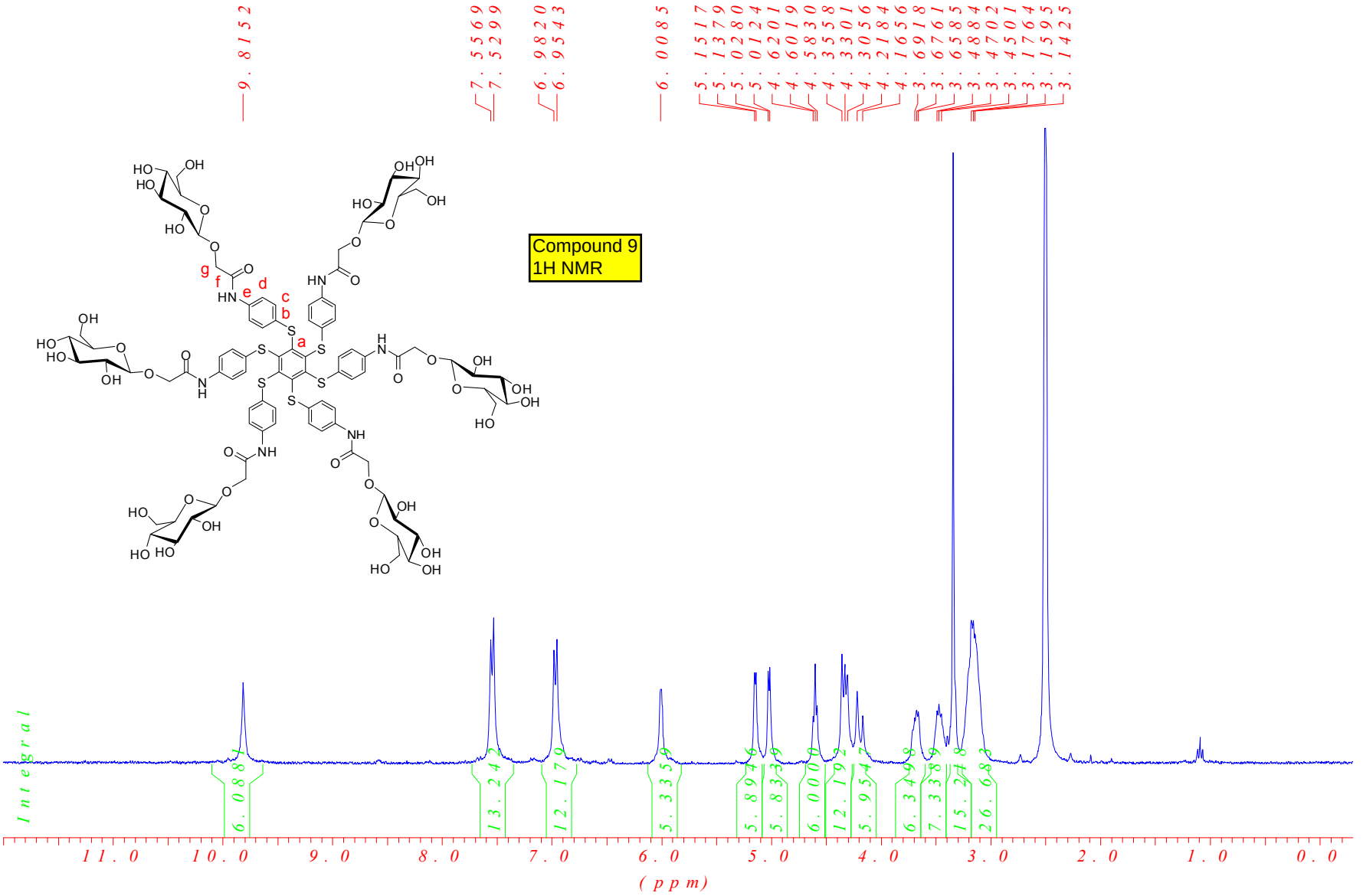
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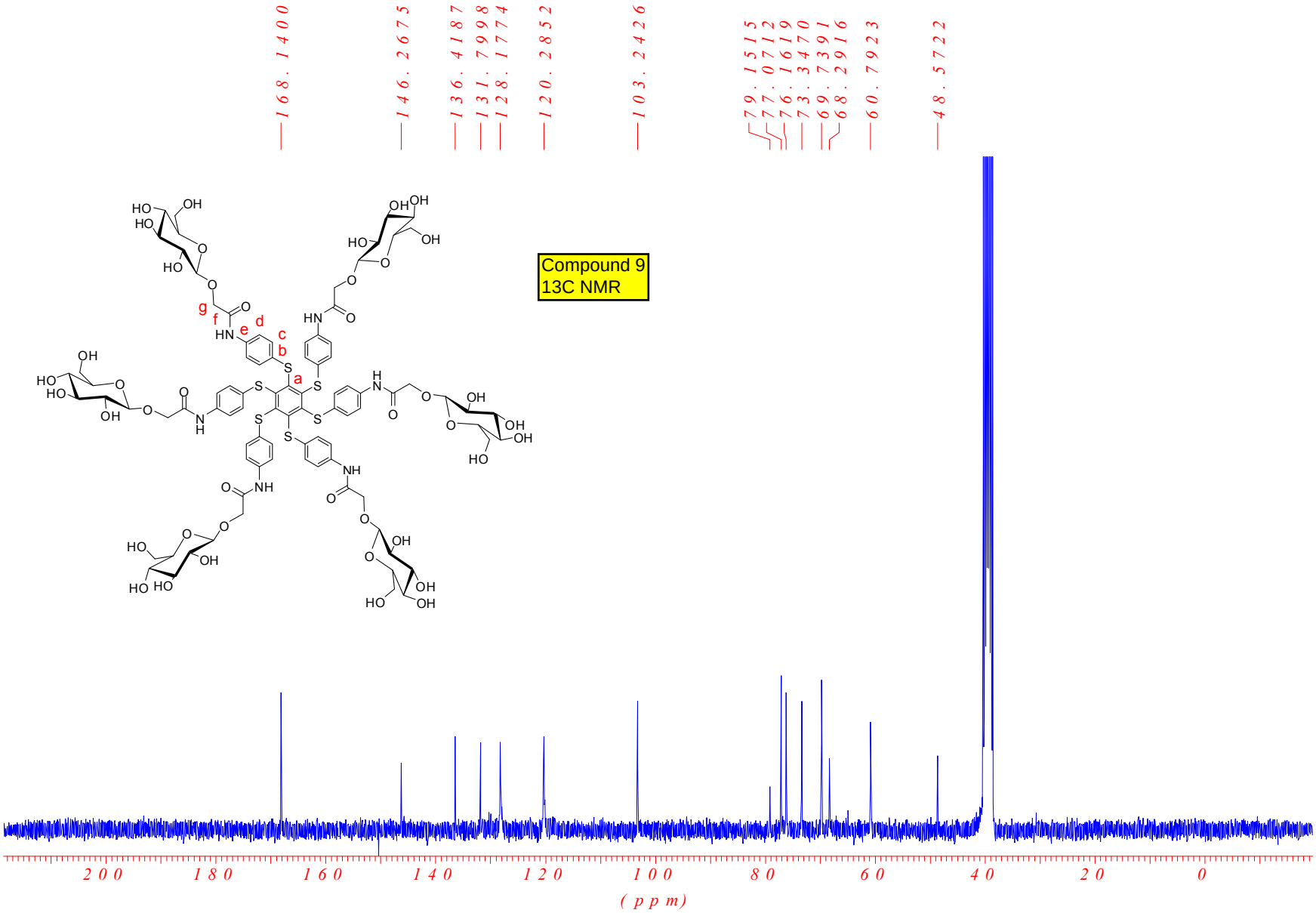
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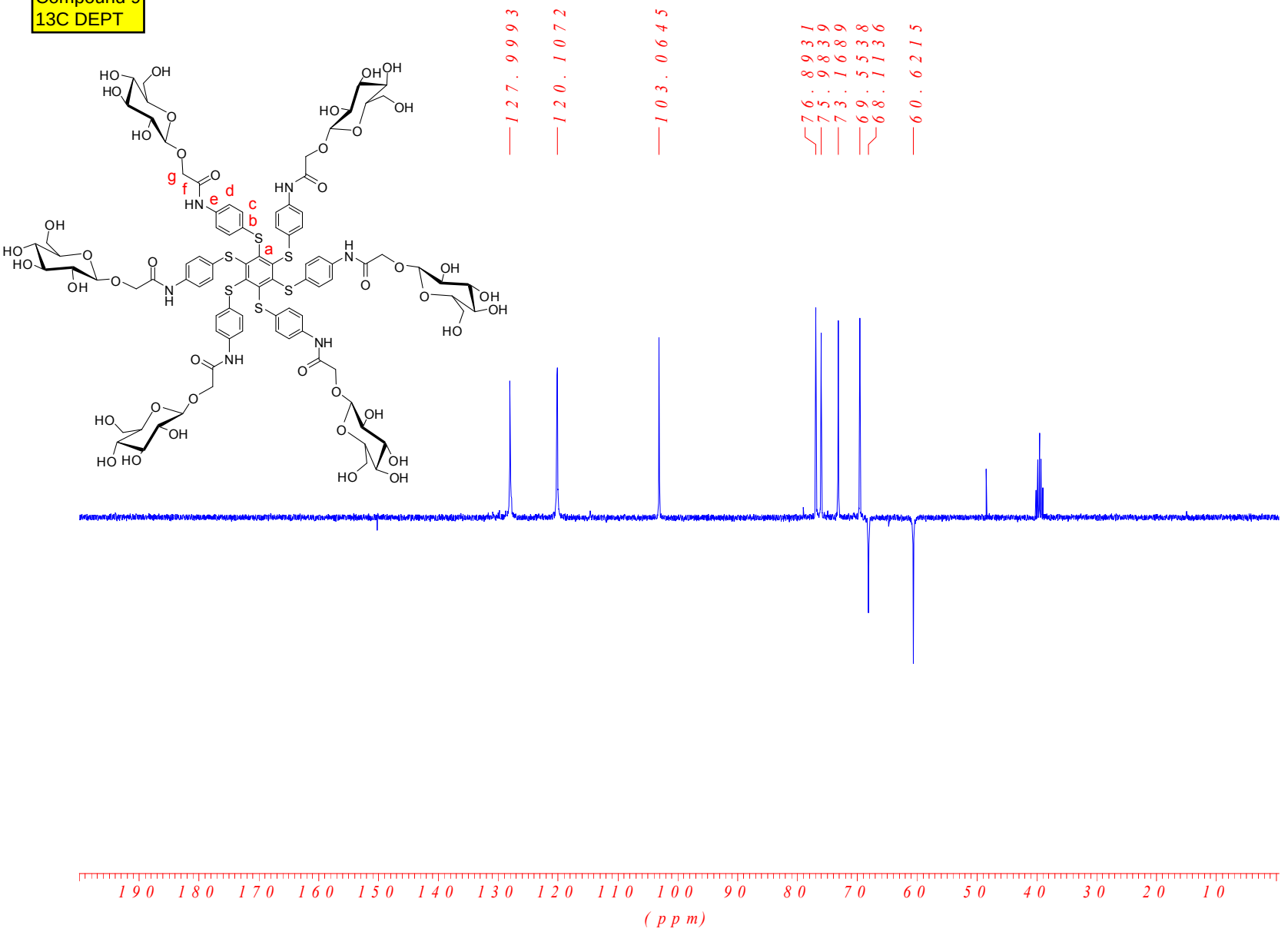

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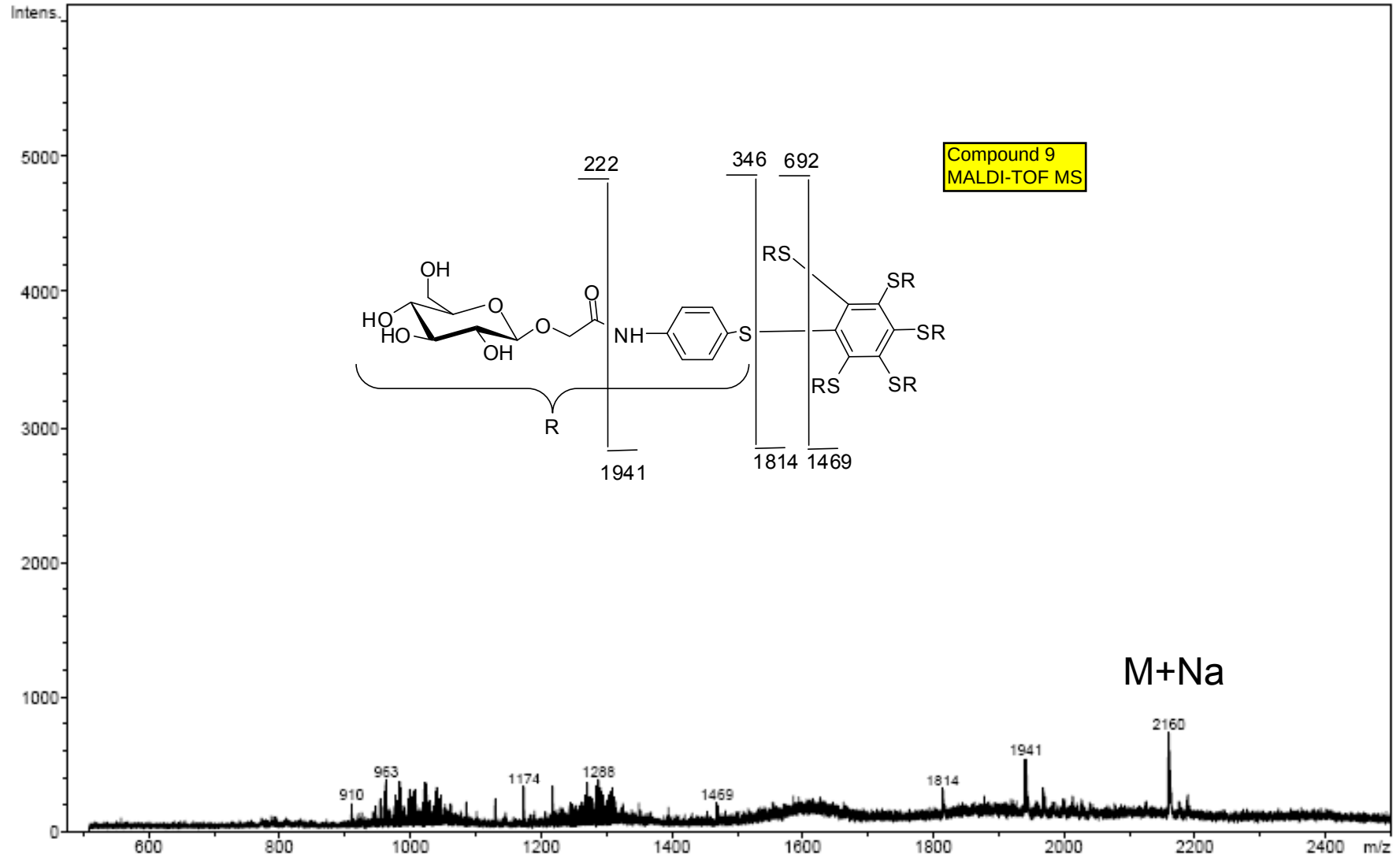

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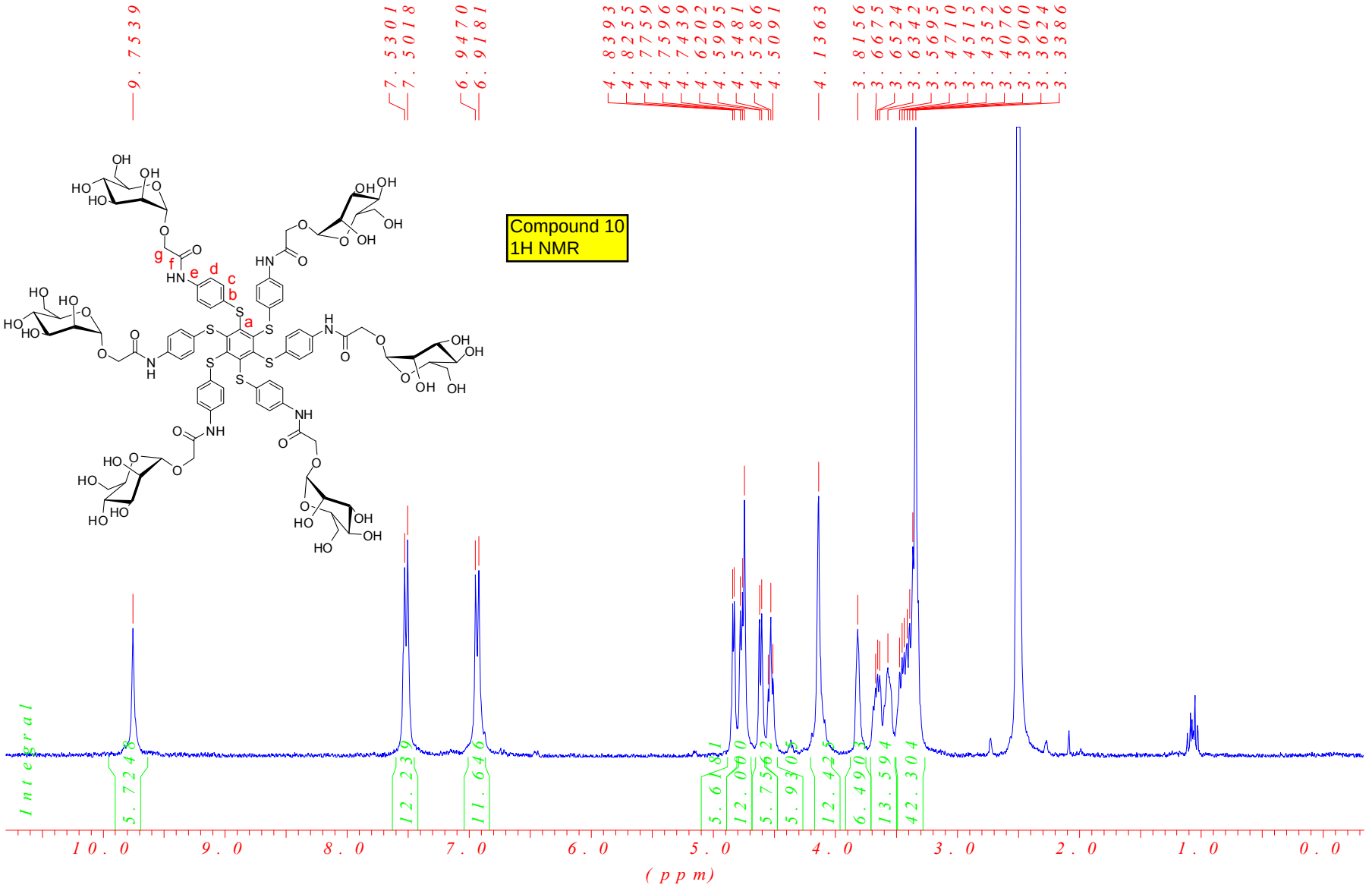
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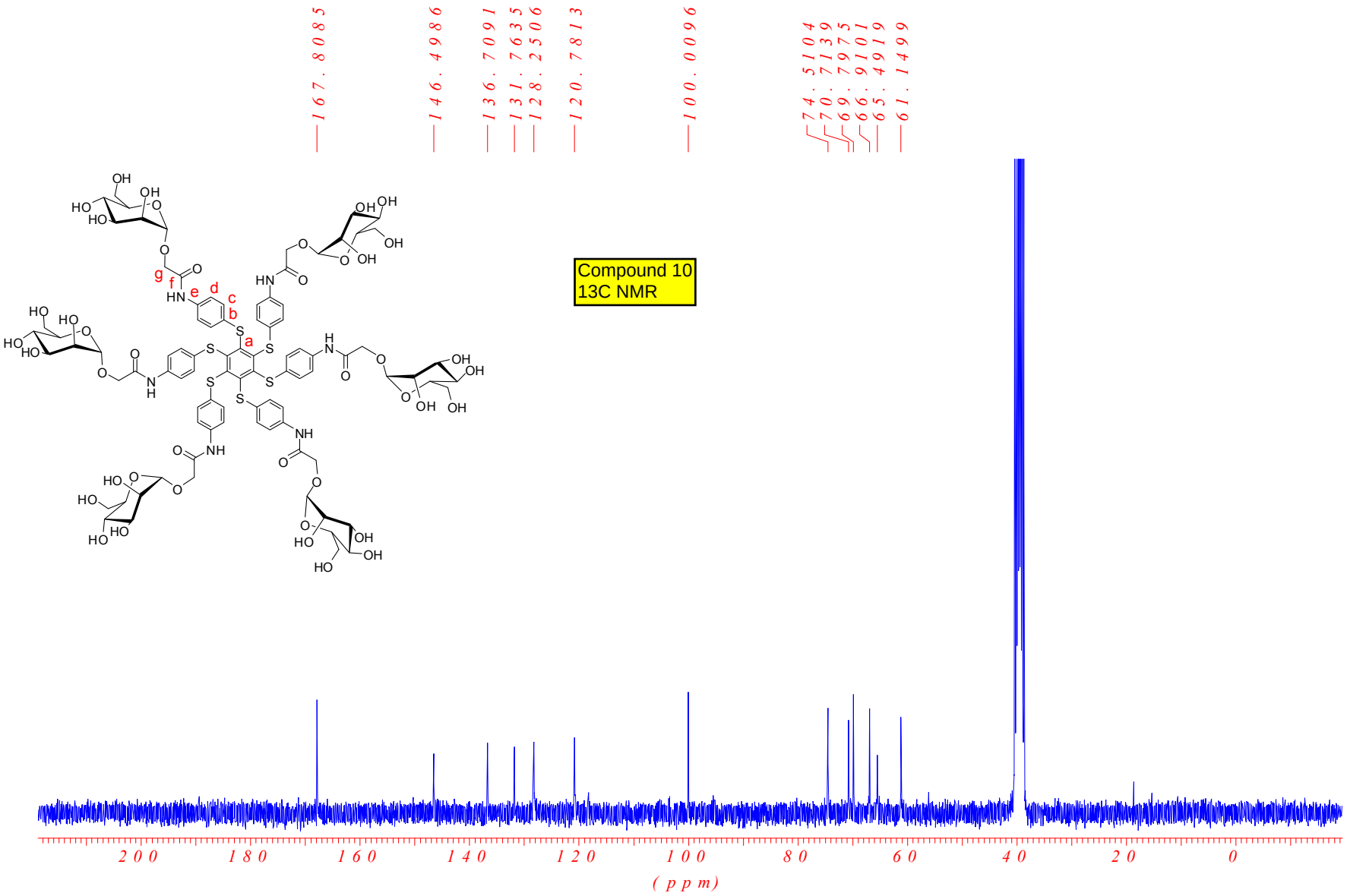
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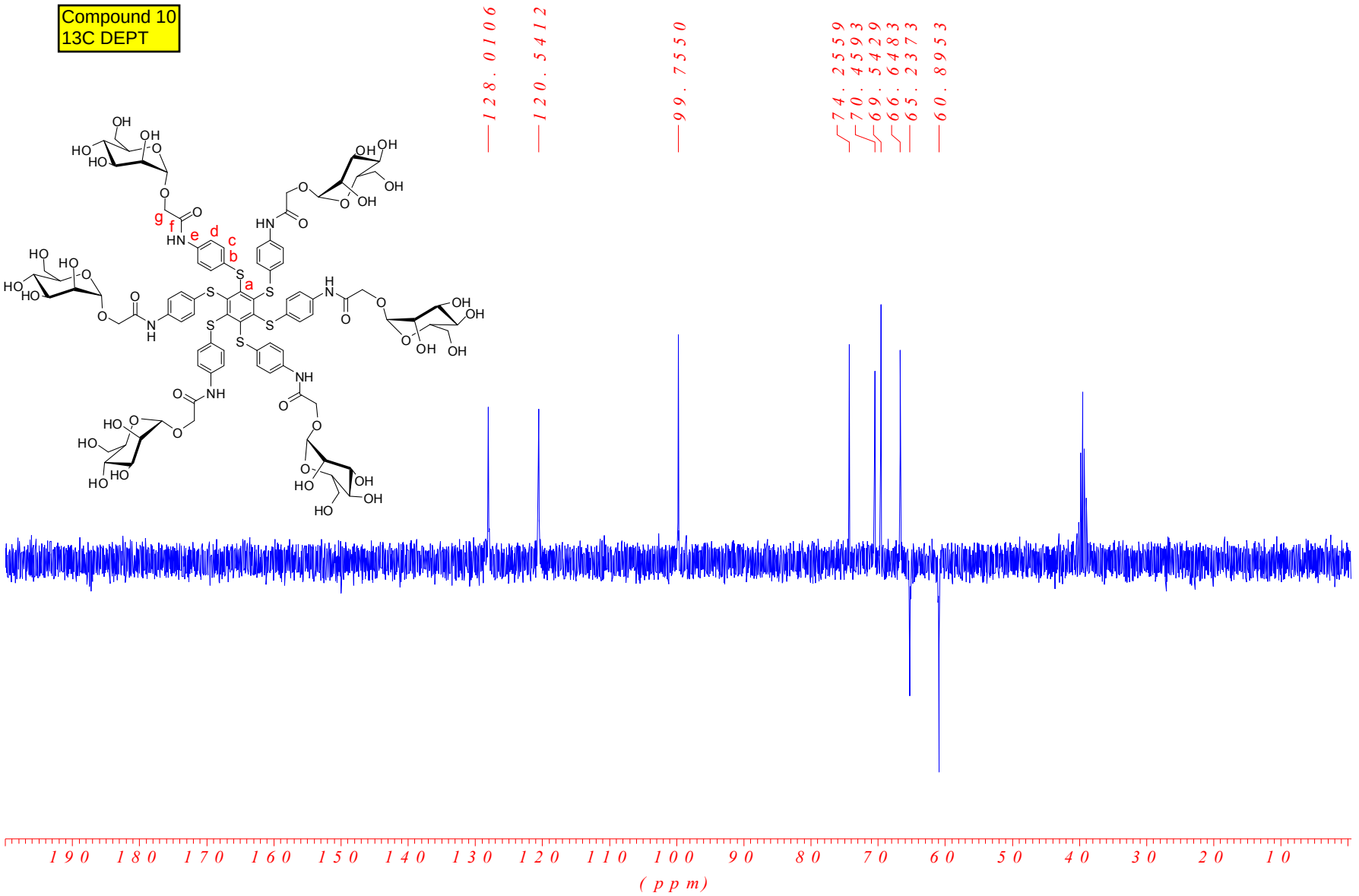
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