Supporting Information

Detection of Carbohydrate Binding Proteins Using Magnetic Relaxation Switches.

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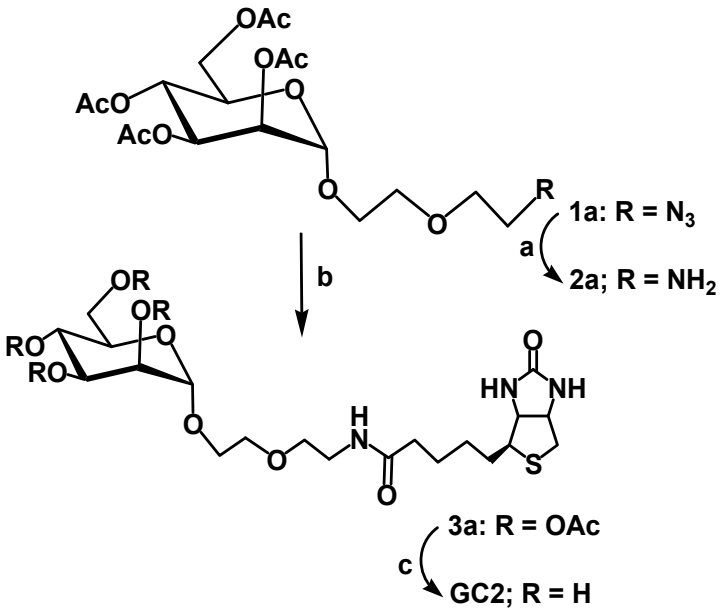
General Methods:

All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. Concanavalin A (ConA), *Galanthus Nivalis* lectin (GNL), *Ricinus communis* lectin (RCA120) and Ricin toxin were purchased from Vector Laboratories. ConA was dialysed in PBS buffer as reported by Cloninger et. al.[1] Ricin was also dialysed before use. Shiga toxins 1 and 2 were isolated as previously described.[2] 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. ^1H 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 ^1H 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.

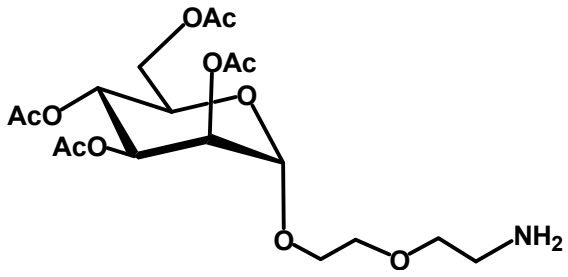
Abbreviations: 2-chloro-4,6-dimethoxy-1,3,5-triazine, CDMT; *N*–methyl morpholine, NMM; N,N Dimethyl formamide, DMF; Ethyl acetate, EtOAc; Triisopropylsilane, TIPS; Trifluroacetic acid,TFA; Acetonitrile, CH_3CN; Dichloromethane, DCM; Dichloroethane, DCE; Trifluoromethanesulfonic anhydride, Tf_2O; Trimethylsilyltrifluoromethanesulfonate, TMSOTf; Hydrazine acetate, $NH_2NH_2.HOAc$; Trichloroacetonitrile,Cl_3CCN; Triethylamine, NEt_3;Methanol, CH_3OH.

A. Synthesis of glycoconjugates



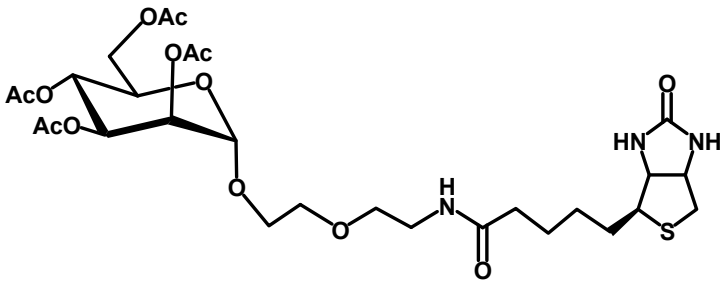
Scheme 1. *Reagents and conditions.* a. 10% Pd/C, EtOH, H_2, RT, 4h, 91%. (b) D-Biotin, NMM, CDMT, THF, 0 ^{0}C, 48h, 50%. (c) NaOMe, MeOH, RT, 4h, 82%.

Compound 2a: 2-(2-Amino-ethoxy)-ethyl 2,3,4,6 tetra-*O*-acetate α-D-mannopyranoside.



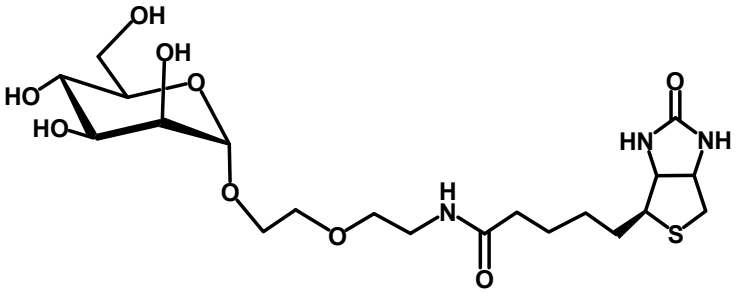
2-(2-Azido-ethoxy)-ethyl 2,3,4,6 tetra-*O*-acetate α-D-mannopyranoside, **1a** (0.90 g, 1.95 mmol) was dissolved in 4 mL of EtOAc and 4 mL of EtOH was added to it. Pd/C (450 mg) was added to it and mixture was stirred for 16 h under 1 atm hydrogen gas. Upon completion (TLC), the reaction mixture was filtered through a small pad of celite followed by washing with 3 X 10 mL EtOH. The solvent was evaporated in *vacuo*. The crude product was purified by flash column chromatography, eluting with 70:30 mixture of EtOAc and hexane, to give compound **2a** as a white solid (0.78 g, 91%). Spectral data matched reported values.[3]

Compound 3a: 2,3,4,6-Tetra-*O*-acetyl-(*S*-D-biotinoyl-5-amino-3-oxopentyl)-α-D-mannopyranoside.



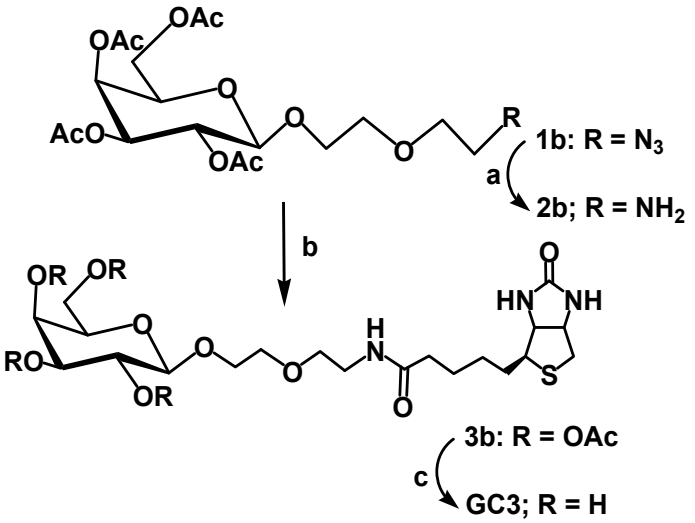
D-biotin (56.1 mg, 0.23 mmol) was dissolved in 1 mL of THF and cooled to 0 ^{0}C. CDMT (29.2 mg, 0.25 mmol) and NMM (55 µL, 0.50 mmol) was added and stirred overnight at 0 ^{0}C. In a separate flask, **2a** (50 mg, 0.12 mmol) was dissolved in 1 mL of THF and the contents were added to the previous solution. The mixture was stirred for 48 h, slowly warming to rt. Upon completion (TLC), the solvent was evaporated and the residue was dissolved in EtOAc and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated in *vacuo* and the product was purified by flash column chromatography, eluting with 90:10 mixture of CH_2Cl_2 and MeOH to give **3a** as a light yellow syrupy solid (48 mg, 63%).^{1}H NMR ($CDCl_3$): δ 6.67 (bs, 1H), 6.49 (bs, 1H), 5.73 (bs, 1H), 5.34-5.23 (m, 3H), 4.91 (s, 1H), 4.52-4.49 (m, 1H), 4.33-4.24 (m, 1H), 4.14-4.01 (m, 2H), 3.81-3.40 (m, 8H), 3.17-3.12 (m, 1H), 2.92 (dd, *J* = 4.8 Hz, 12.8 Hz), 2.74 (d, 12.8 Hz), 2.25 (t, 2H), 2.16 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.77-1.61 (m, 4H), 1.47-1.42 (m, 2H), 1.26-1.23 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 173.43, 170.71, 170.26, 170.03, 169.74, 164.01, 97.54, 70.17, 69.83, 69.63, 68.99, 68.48, 67.03, 66.18, 62.54, 61.82, 60.19, 55.63, 40.55, 39.15, 35.90, 28.26, 28.12, 25.64, 20.95, 20.79, 20.74. HRMS calculated for $[C_{28}H_{43}N_3O_{13}SNa]^+$: 684.2415. Found 684.2383.

Compound GC2: (*S*-D-biotinoyl-5-amino-3-oxopentyl)-α-D-mannopyranoside.



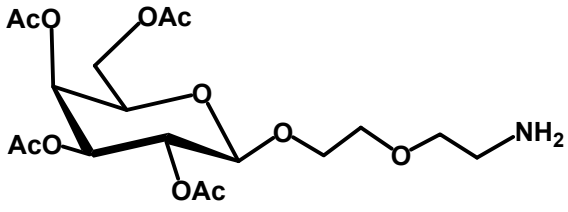
Compound **3a** (48 mg, 0.073 mmol) was dissolved in MeOH (2 mL) and a solution of NaOMe in MeOH (0.5M, 0.5 mL) was added. The reaction mixture was stirred at rt for 6h. The

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 water, to give **GC2** as a white solid. (29 mg, 82%) ^{1}H NMR (D_2O): ∂ 4.88 (d, *J* = 1.6 Hz, 1H), 4.62-4.59 (m, 1H), 4.44-4.41 (m, 1H), 3.96-3.95 (m, 1H), 3.89-3.34 (m, 10H), 3.41-3.31 (m, 3H), 3.02 (dd, *J* = 4.8, 12.8 Hz, 1H), 2.80 (d, *J* = 13.2 , 1H), 2.29 (t, 2H), 1.77-1.37 (m, 4H), 1.45-1.37 (m, 2H). ^{13}C NMR (D_2O): 176.94, 165.33, 99.89, 72.74, 70.51, 69.95, 69.32, 68.90, 66.72, 66.40, 62.08, 60.91, 60.24, 55.37, 39.68, 38.88, 35.46, 27.85, 27.67, 25.13. HRMS calculated for [$C_{20}H_{35}N_3O_9SNa]^+$: 516.1992. Found 516.1953.



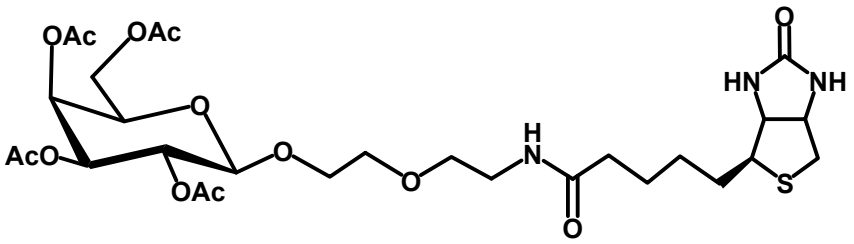
Scheme 2. *Reagents and conditions.* a. 10% Pd/C, EtOH, H_2, RT, 4h, 98%. (b) D-Biotin, NMM, CDMT, THF, 0 ^{0}C, 48h, 3b, 55%. (c) NaOMe, MeOH, RT, 4h, 85%.

Compound 2b: 2-(2-Amino-ethoxy)-ethyl 2,3,4,6 Tetra-*O*-acetate β-D-galactopyranoside.



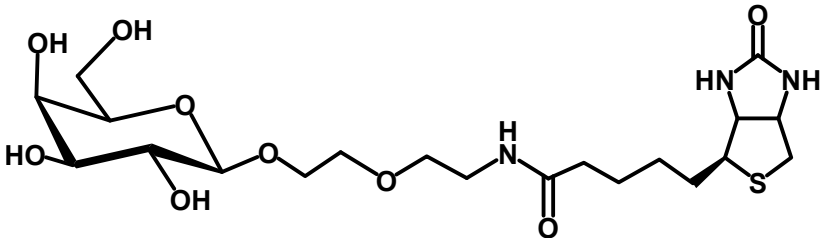
2b was synthesized in a similar manner from **1b** (50 mg, 0.11 mmol) as described for **2a** to yield 2b as a white solid (46 mg, 98%). Spectral data matched reported values.[4]

Compound 3b: 2,3,4,6-Tetra-*O*-acetyl-(*S*-D-biotinoyl-5-amino-3-oxopentyl)-β-D-galacto pyranoside.



3b was synthesized in a similar manner from **2b** (30 mg, 0.068 mmol) as described for **2a** to yield **3b** as a light yellow syrupy solid (25 mg, 55%). ^{1}H NMR ($CDCl_3$): ∂ 6.57 (bs, 1H), 6.24 (bs, 1H), 5.40 (d, *J* = 2.8 Hz, 1H), 5.21-5.17 (m, 1H), 5.06-5.03 (m, 1H), 4.55 (d, *J* = 8 Hz, 2H), 4.34 (bs, 1H), 4.21-4.09 (m, 3H), 4.06-3.92 (m, 3H), 3.72-3.38 (m, 6H), 3.17 (bs, 1H), 2.93-2.90 (m, 1H), 2.76 (d, *J* = 12.8 Hz , 1H), 2.34-2.20 (m, 2H), 2.15 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.83-1.60 (m, 4H), 1.49-1.46 (m, 2H), 1.27-1.24 (m, 3H). ^{13}C NMR ($CDCl_3$): ∂ 172.26, 170.47, 170.21, 170.17, 169.83, 101.49, 70.77, 70.73, 69.99, 69.75, 69.15, 68.92, 67.03, 61.84, 61.26, 60.23, 55.93, 55.46, 40.59, 39.10, 35.82, 29.71, 28.13, 25.53, 20.89, 20.73, 20.70, 20.63, 14.21. HRMS calculated for [$C_{28}H_{43}N_3O_{13}SNa]^+$: 684.2415. Found 684.2383.

Compound 4b: (*S*-D-biotinoyl-5-amino-3-oxopentyl)-α-D-galactopyranoside.



GC3 was synthesized in a similar manner from **3b** (20 mg, 0.032 mmol) as described for **3a** to yield **GC3** as a white solid (16 mg, 85%). ^{1}H NMR (D_2O): ∂ 4.63-4.59 (m, 1H), 4.44-4.41 (m, 2H), 4.09-4.00 (m, 2H), 3.92-3.91 (m, 1H), 3.85-3.63 (m, 12H), 3.55-3.51 (m, 2H), 3.41-3.34 (m, 6H), 3.02-2.97 (m, 2H), 2.80-2.76 (d, *J* = 12.8 Hz , 1H), 2.30-2.26 (m, 3H), 1.76-1.39 (m, 11H). ^{13}C NMR (D_2O): ∂ 102.90, 75.17, 72.75, 70.77, 69.55, 68.75, 68.64, 60.99, 60.26, 55.34, 38.14, 35.44, 27.67, 25.10. HRMS calculated for $[C_{20}H_{35}N_3O_9SNa]^+$: 516.1992. Found 516.1953.

B. Calculation of the amount of ligands on the bead.

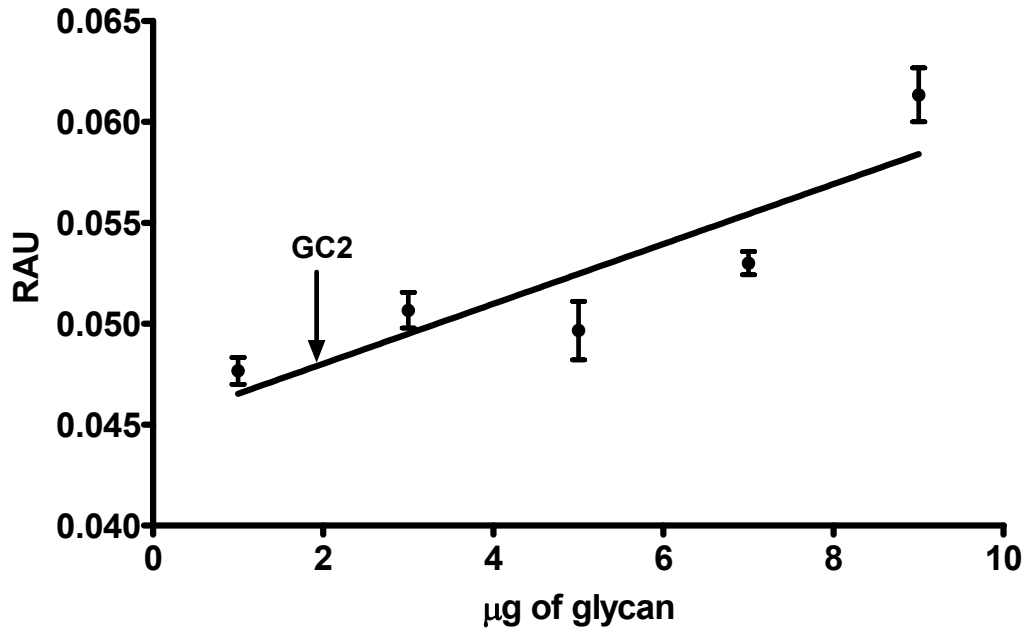


Figure 1. The plot of amount of glycan (μg) versus absorbance. The concentration of glycans on the bead for **GC2** is represented by an arrow.

C. Magnetic properties of the carbohydrate coated micrometer beads.

Property	Micrometer Beads	GC coated beads
Size (nm)	1000 (from manufacturer)	-
Settling	<5%[5]	-
Ligands/bead	-	**GC1**: (tetra antennary α-mannoside coated beads) 2 X 10^6 **GC2** (α-mannoside coated beads): 1 x 10^6 **GC3** (β-galactoside coated beads): 1 x 10^6
R_1 / S^{-1} per mM Fe	<1	<1
R_2 / S^{-1} per mM Fe	45	44
M / emu per g Fe	105 (from manufacturer)	-
Fe atoms per particle	2.8 x 10^9 (from manufacturer)	-

Table 1. Physical and chemical characterization of carbohydrate coated micrometer beads. The streptavidin coated beads were purchased from Invitrogen® (Catalog number: 650-01).

D. Comparison of Concanavalin A (ConA) and Galanthus Nivalis lectin (GNL).

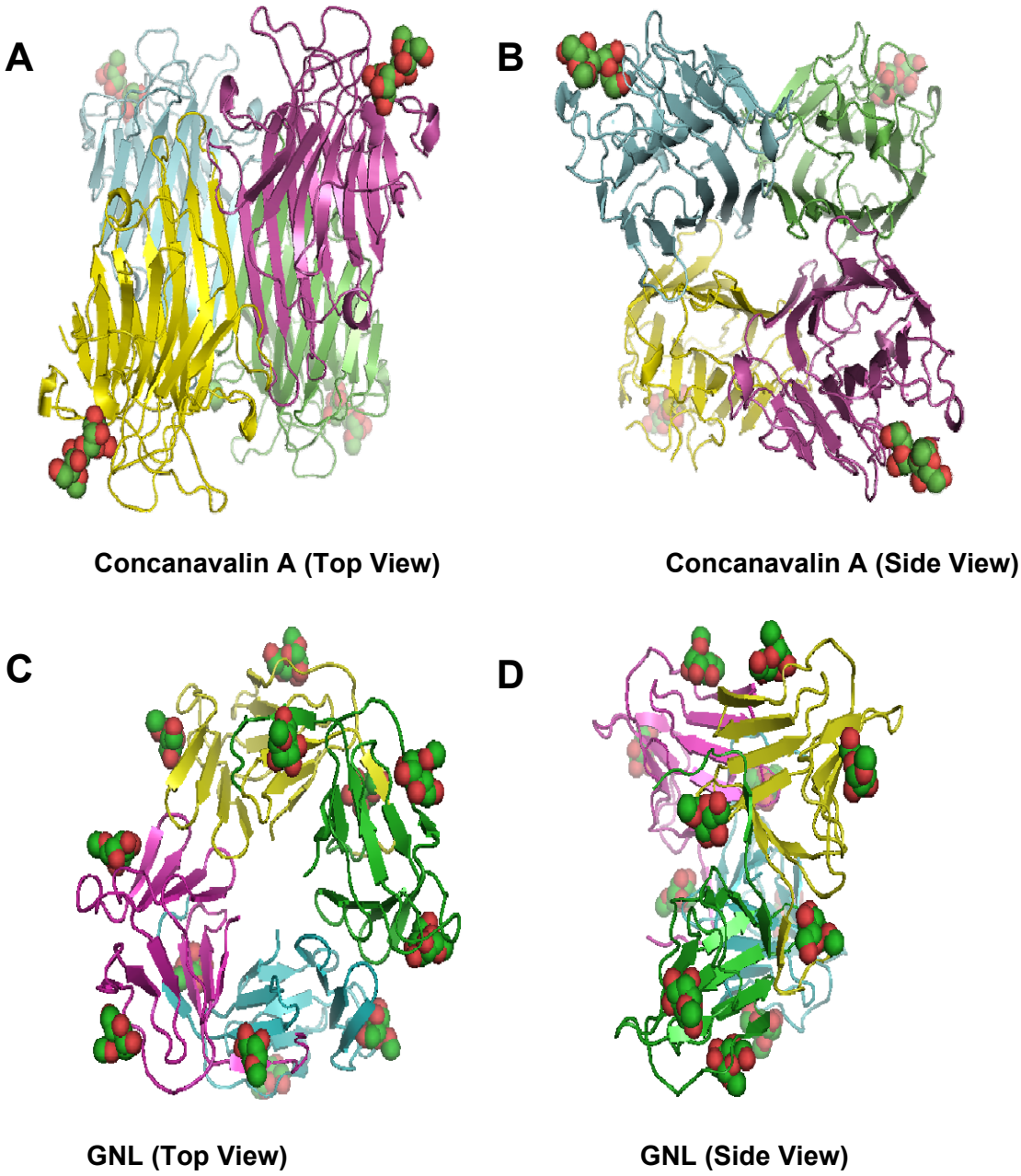


Figure 2. Top and Side view of **(A,B)** Concanavalin A[6] and **(C,D)** *Galanthus Nivalis* lectin (GNL)[7] depicting the different arrangement of the binding sites. The lectins and the glycans are represented in ribbon and ball-and-stick diagrams, respectively. The coordinates were downloaded from NCBI and the figure was generated using Pymol®.

E. Transverse relaxation measurements.

The parameters used for the instrument are given as below.

Status:		Settings:	
Serial No.	: ND2862	90 Pls.Len.[us]	: 2.28
MBox Temp. [C]	: 40.000	180Pls.Len.[us]	: 4.46
Temp. Err. [C]	: 0.000	DetAngle.B[deg]	: 316
Temp. Control	: ON	DetAngle.N[deg]	: 320
Parameters:		H Offs. [Steps]	: -345
Scans	: 8	NMR Freq. [MHz]	: 59.950000
Rd [s]	: 2.00	Pulse Atten[dB]	: 0
Gain [dB]	: 54	Instr.Gain [db]	: 56
Dig. Bw [Hz]	: 20000	DeadTime [ms]	: 0.0058
Ana. Bw	: narrow	Homog.Limit[ms]	: 0.20
Offset Comp.	: off	Instr. Rd [s]	: 2.00
Det. Mode	: magnitude	Stat Grad X [%]	: 0.00
Magnitude Mode	: PSD	Stat Grad Y [%]	: 0.00
Dig. Res.	: high	Stat Grad Z [%]	: 0.00
Dummy Shots	: 0		
Pulse Atten[dB]	: 0	SFC	: no
Gradient Unit	: none		

F. Analysis of the data to show ΔT_2 / min differences in the aggregation profiles.

Lectin	**Figures**	**ΔT_2 / min**
GNL (1.9 nM)	3A	1.25 ± 0.09
ConA (1.9 nM)		0.34 ± 0.12
Ricin (1.7 nM)	3B	1.01 ± 0.36
RCA120 (1.7 nM)		1.34 ± 0.08
GC1 (1.9 nM ConA)	3C	0.90 ± 0.61
GC2 (1.9 nM ConA)		0.34 ± 0.12
Antibody to RCA120 (0.85 nM)	3D	1.59 ± 0.05
RCA120 (0.85 nM)		0.63 ± 0.15

Table 2. Rate of increase in ΔT_2 per min for all curves from Figure 3 of the main manuscript.

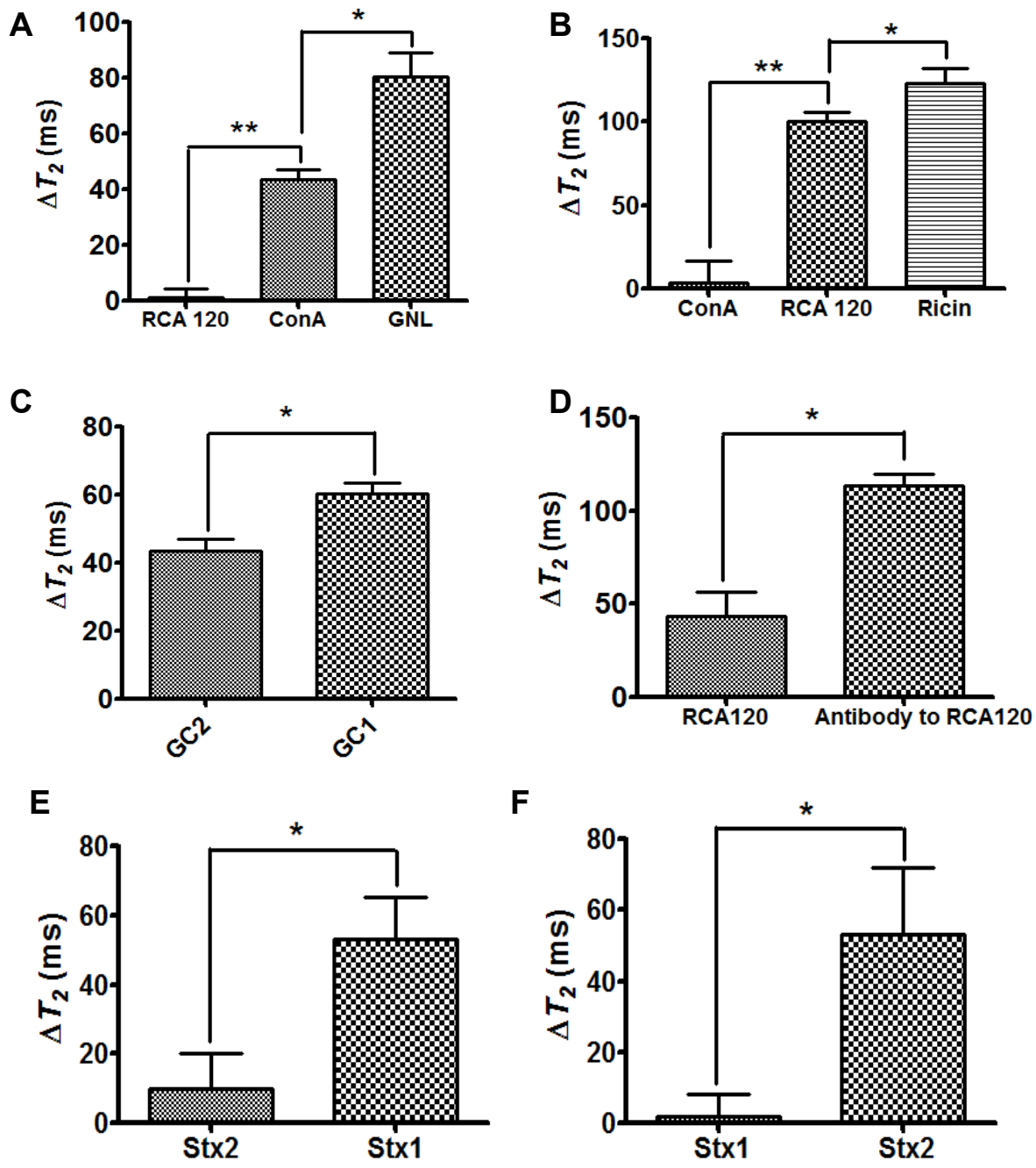
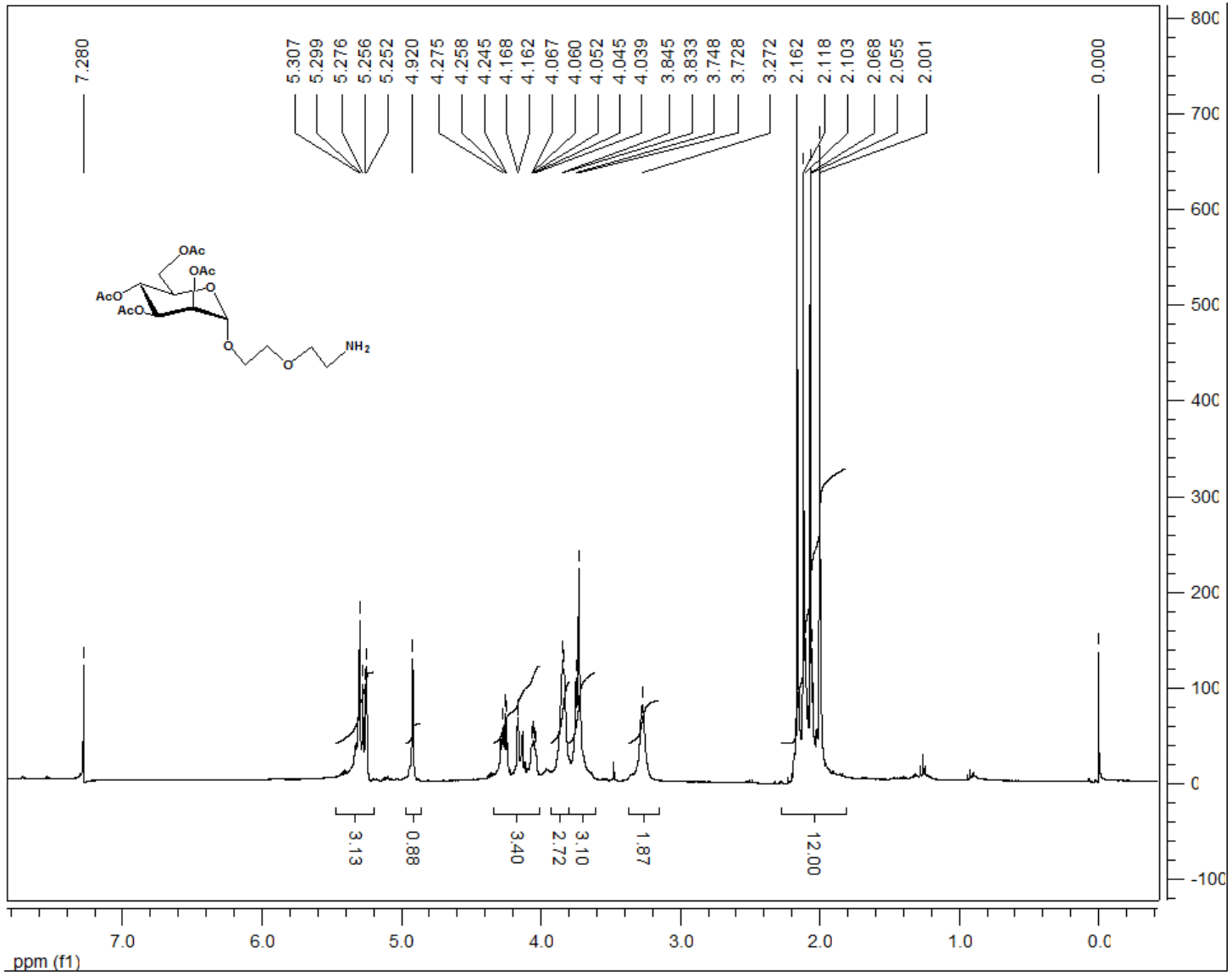
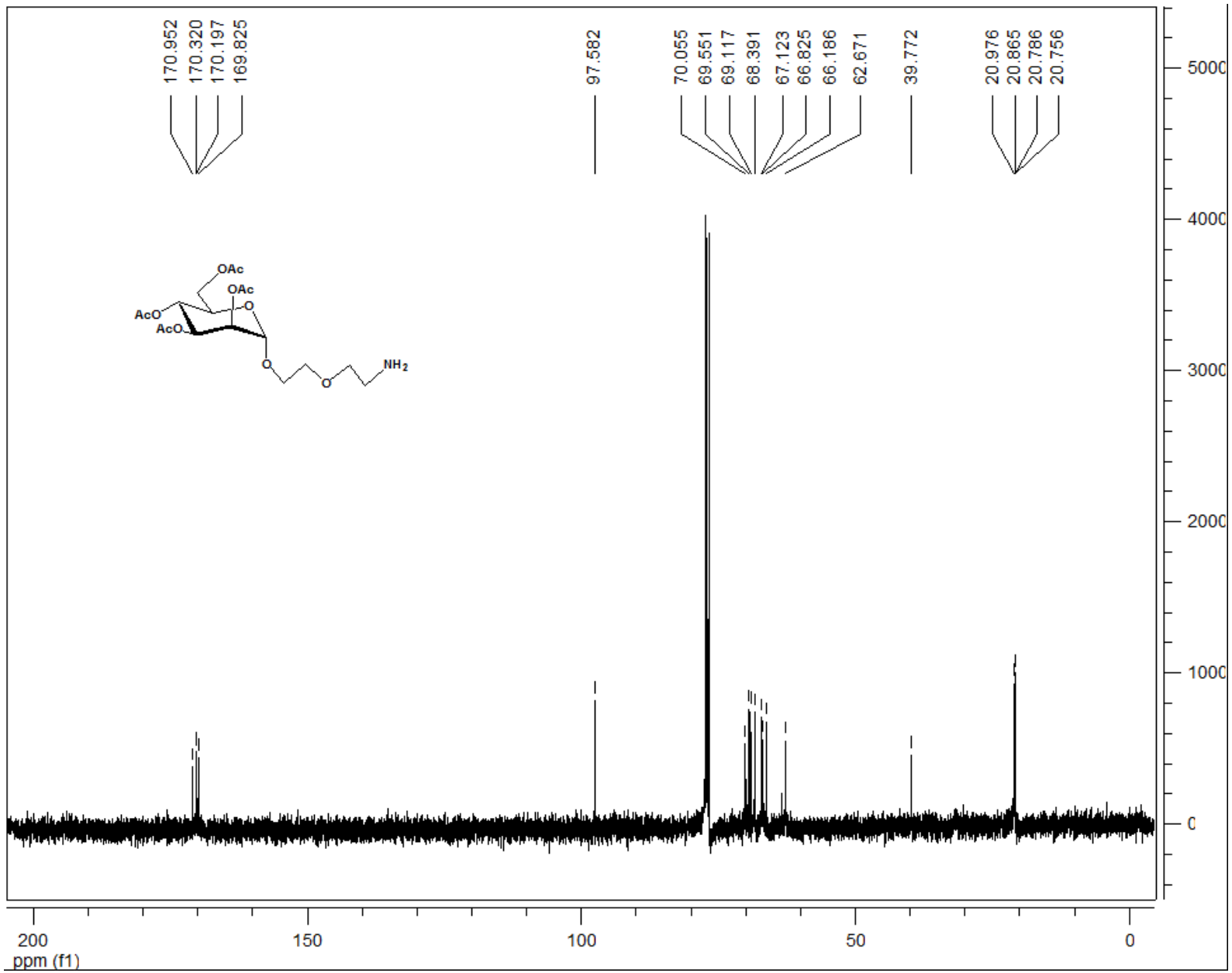


Figure 4: Statistical analysis of the binding of different proteins to carbohydrate coated beads. The data represents ΔT_2 at 85 min. (A) Detection of α-mannoside binding proteins using GC2 coated magnetic beads. (B) Detection of β-galactoside binding proteins using GC3 coated magnetic beads. (C) Comparison of binding of ConA to GC1 and GC2 coated magnetic beads. (D) Increase in sensitivity by using GC3 and antibody specific to RCA120. (E) Selective detection of Stx 1 using Pk coated magnetic beads. (E) Selective detection of Stx2 using NHAc-Pk coated magnetic beads. **: P<0.008, *: P<0.04.

G. ^{1}H and ^{13}C NMRs of all compounds used in this study.

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20.976
20.865
20.786
20.756
OAc
OAc
AcO
AcO
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O
O
NH2
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0
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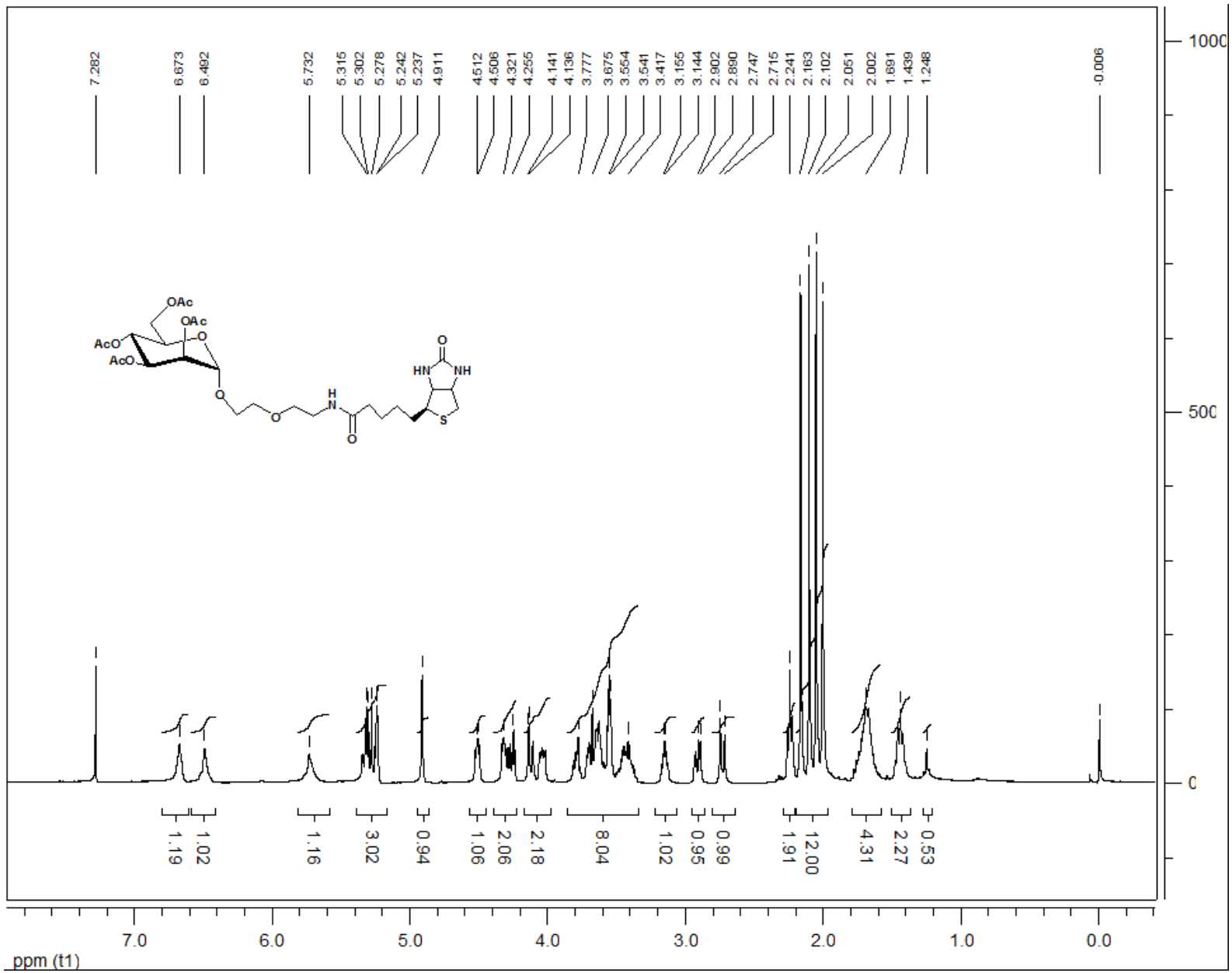
50
0
ppm (f1)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3.155
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2.715
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2.163
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2.051
2.002
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OAc
AcO
AcO
O
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N
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HN
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1.02
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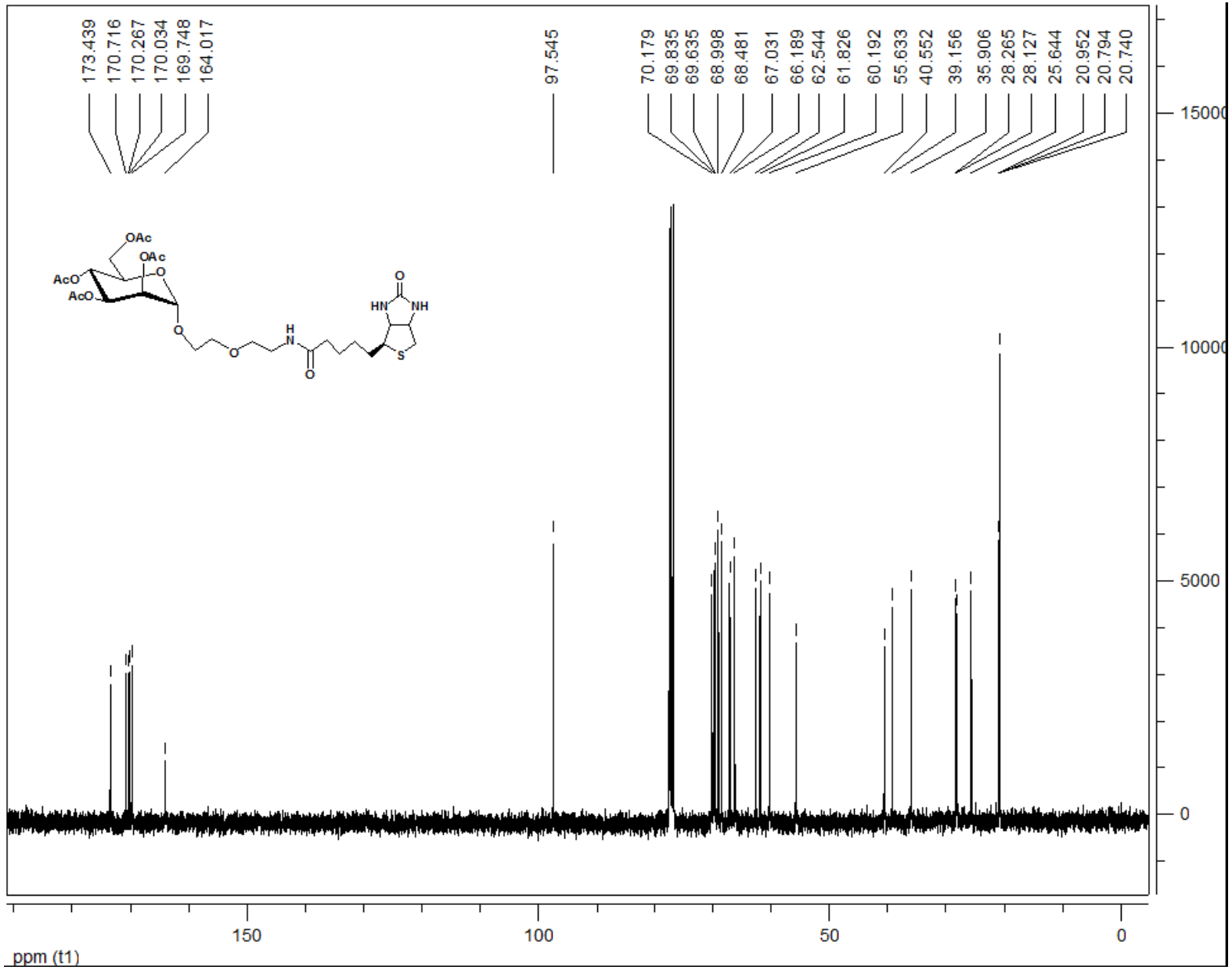
ppm (t1)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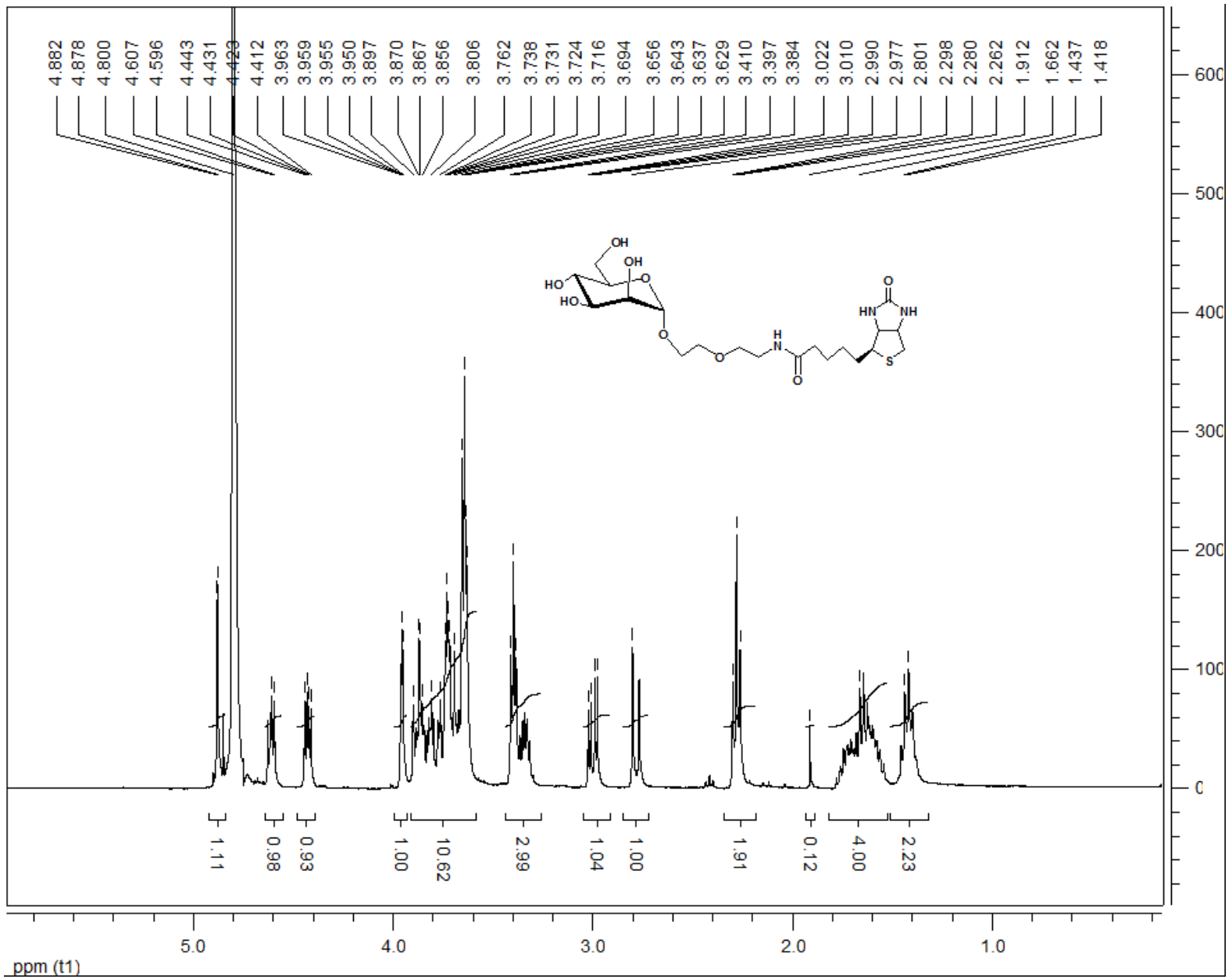
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O
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1.04
1.00
1.91
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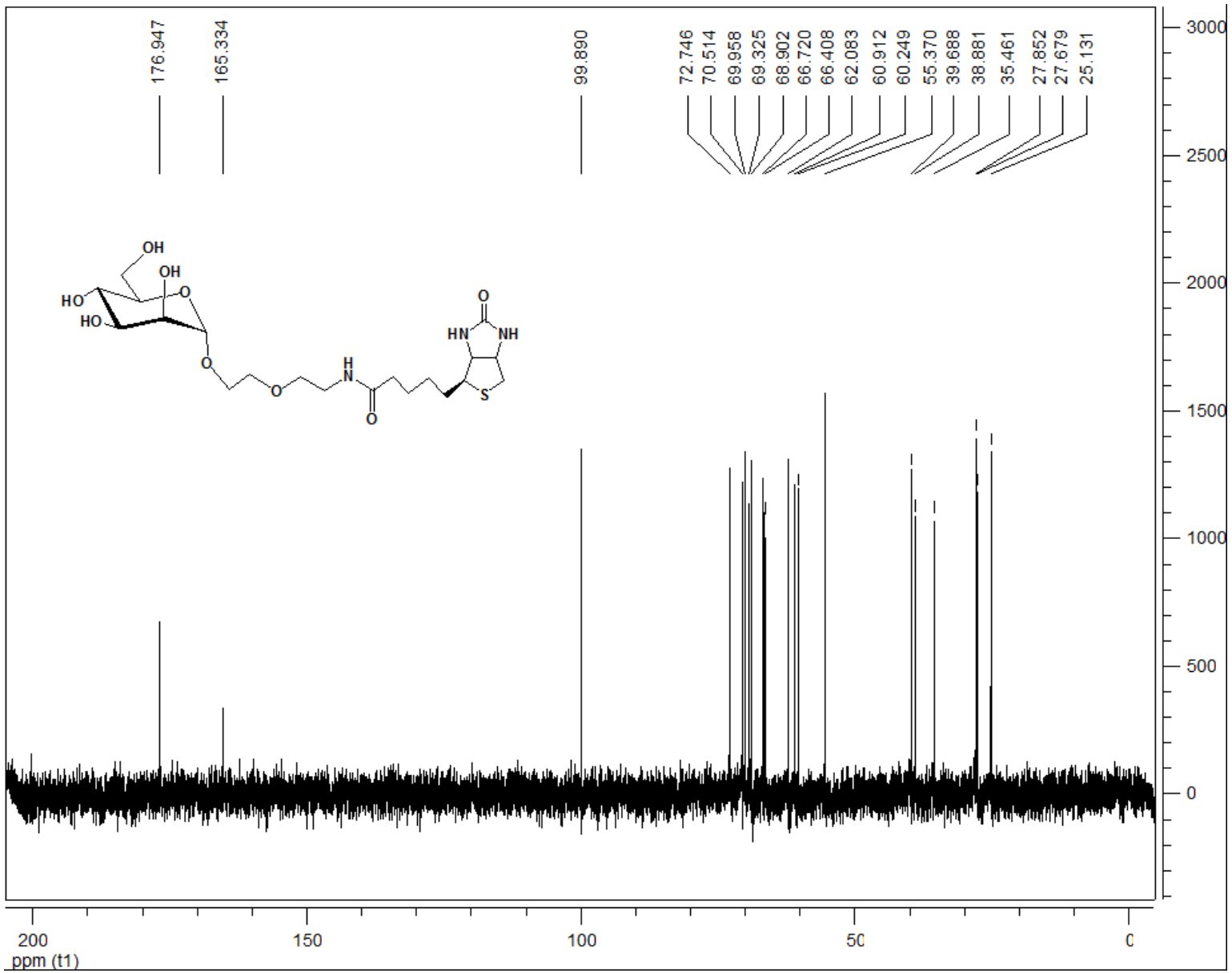
ppm (t1)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27.679
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O
O
H
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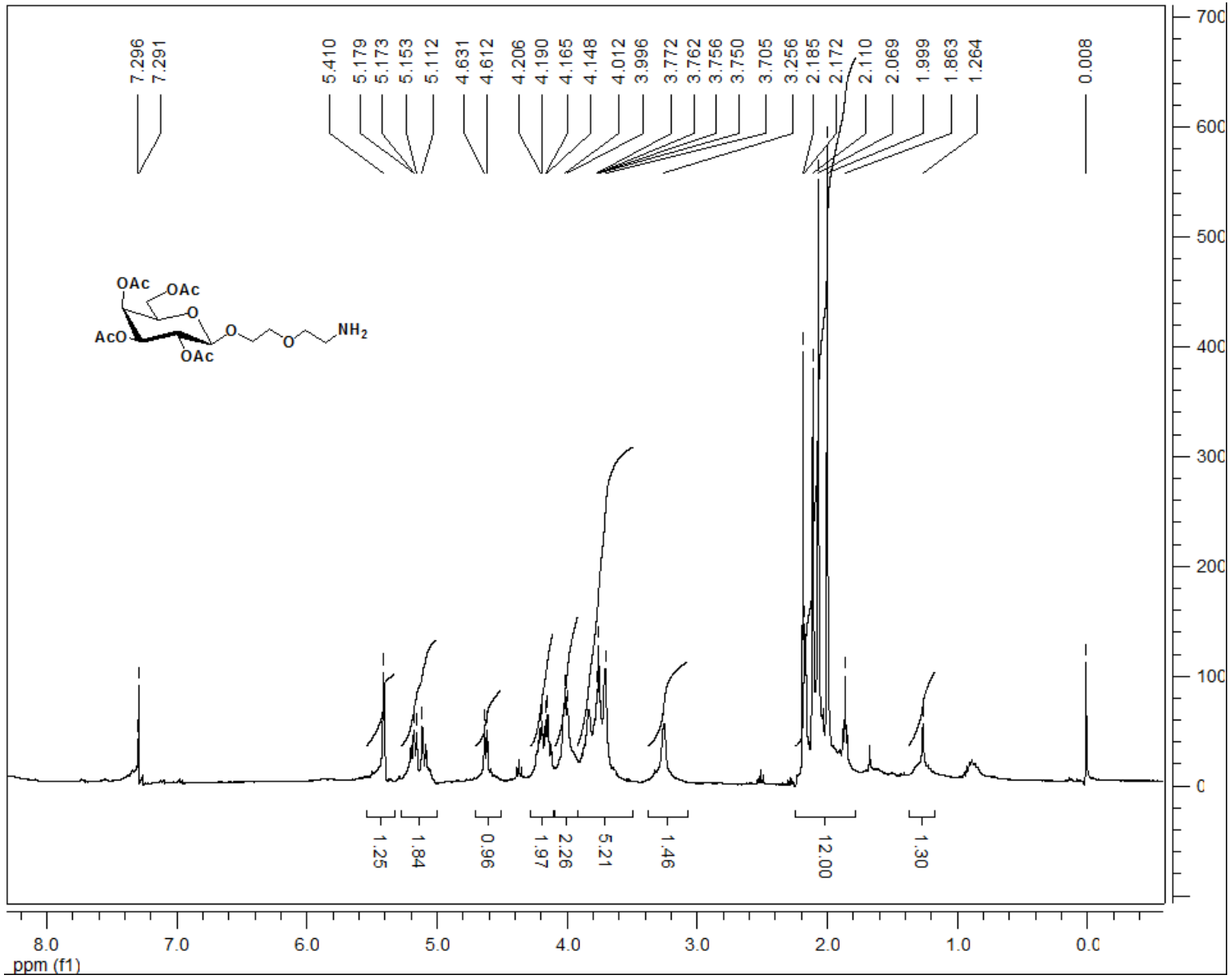
ppm (t1)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3.772
3.762
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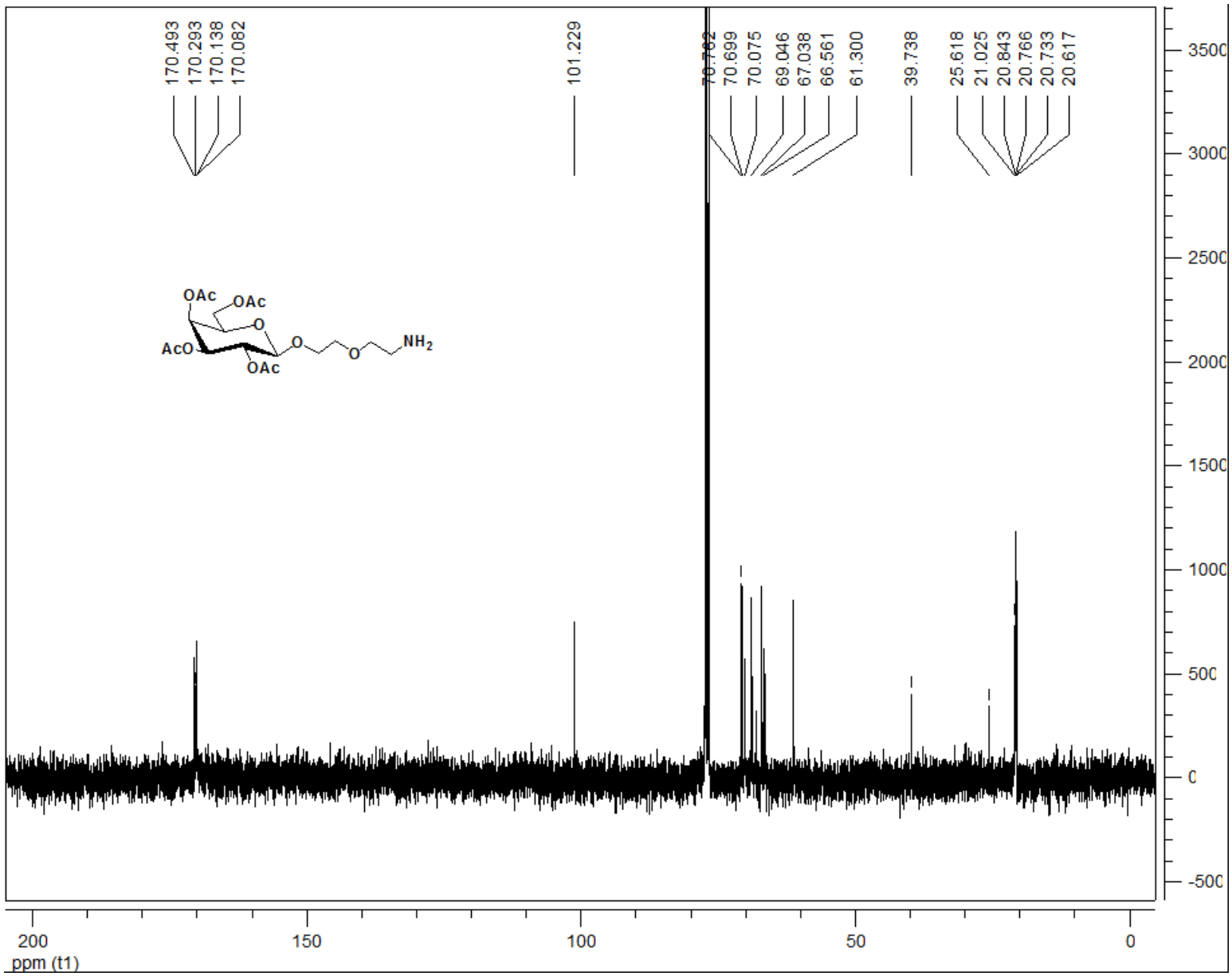
3.750
3.705
3.256
2.185
2.172
2.110
2.069
1.999
1.863
1.264
0.008
OAc
OAc
O
AcO
O
O
NH2
OAc
1.25
1.84
0.96
1.97
2.26
5.21
1.46
12.00
1.30
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)
700
600
500
400
300
200
100
0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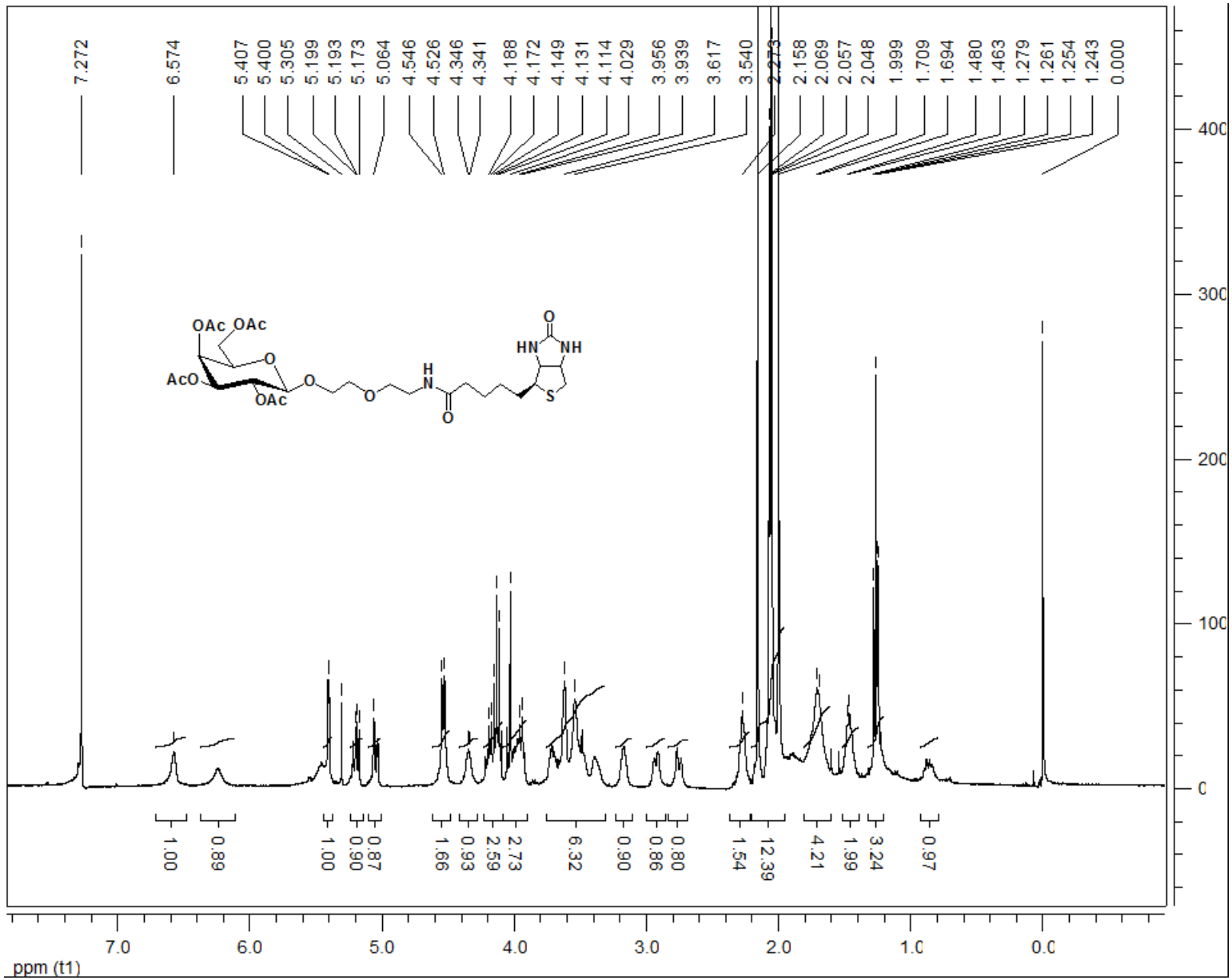
170.293
170.138
170.082
101.229
70.782
70.699
70.075
69.046
67.038
66.561
61.300
39.738
25.618
21.025
20.843
20.766
20.733
20.617
OAc
OAc
O
AcO
O
O
NH2
OAc
3500
3000
2500
2000
1500
1000
500
0
-500
200
150
100
50
0
ppm (t1)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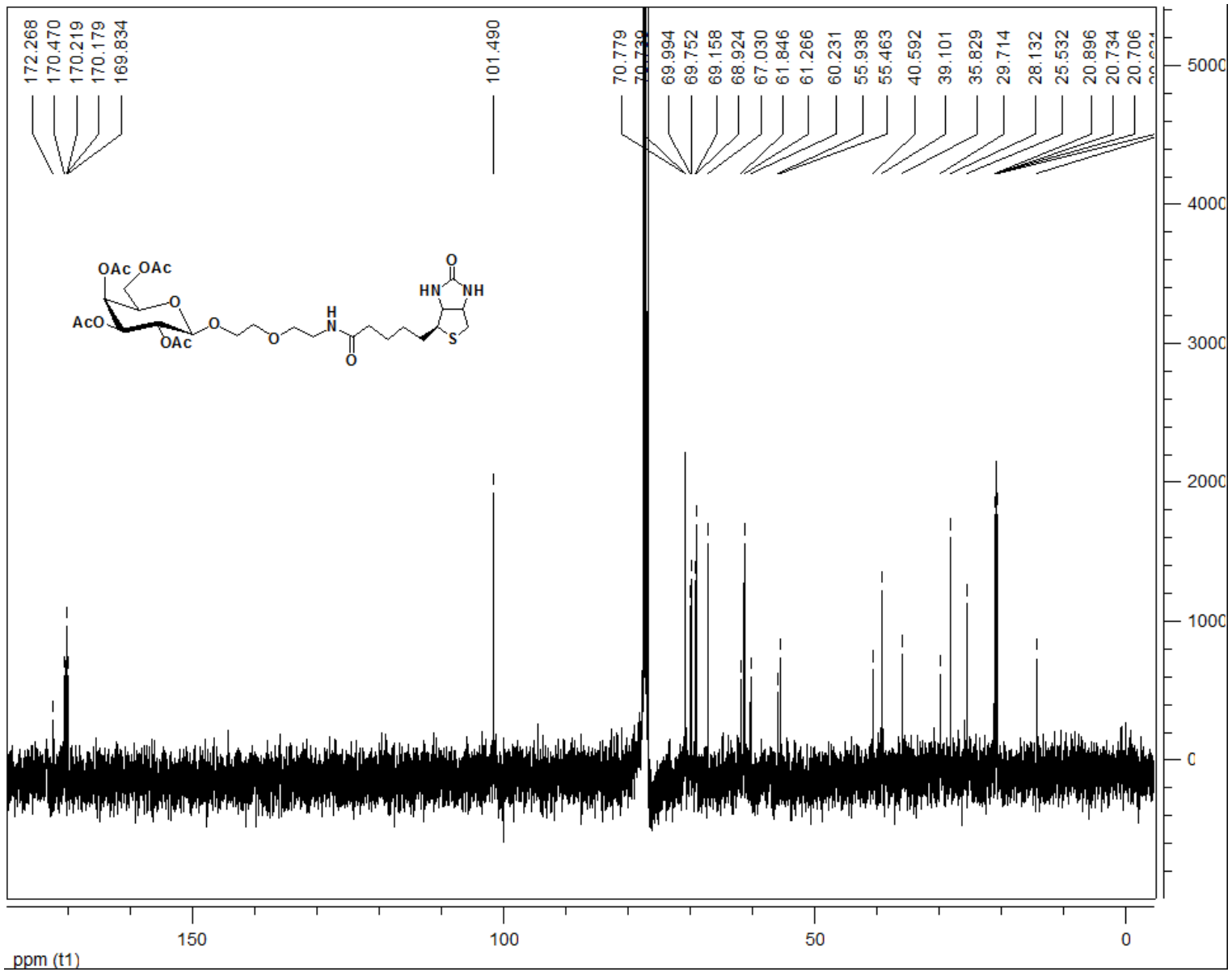
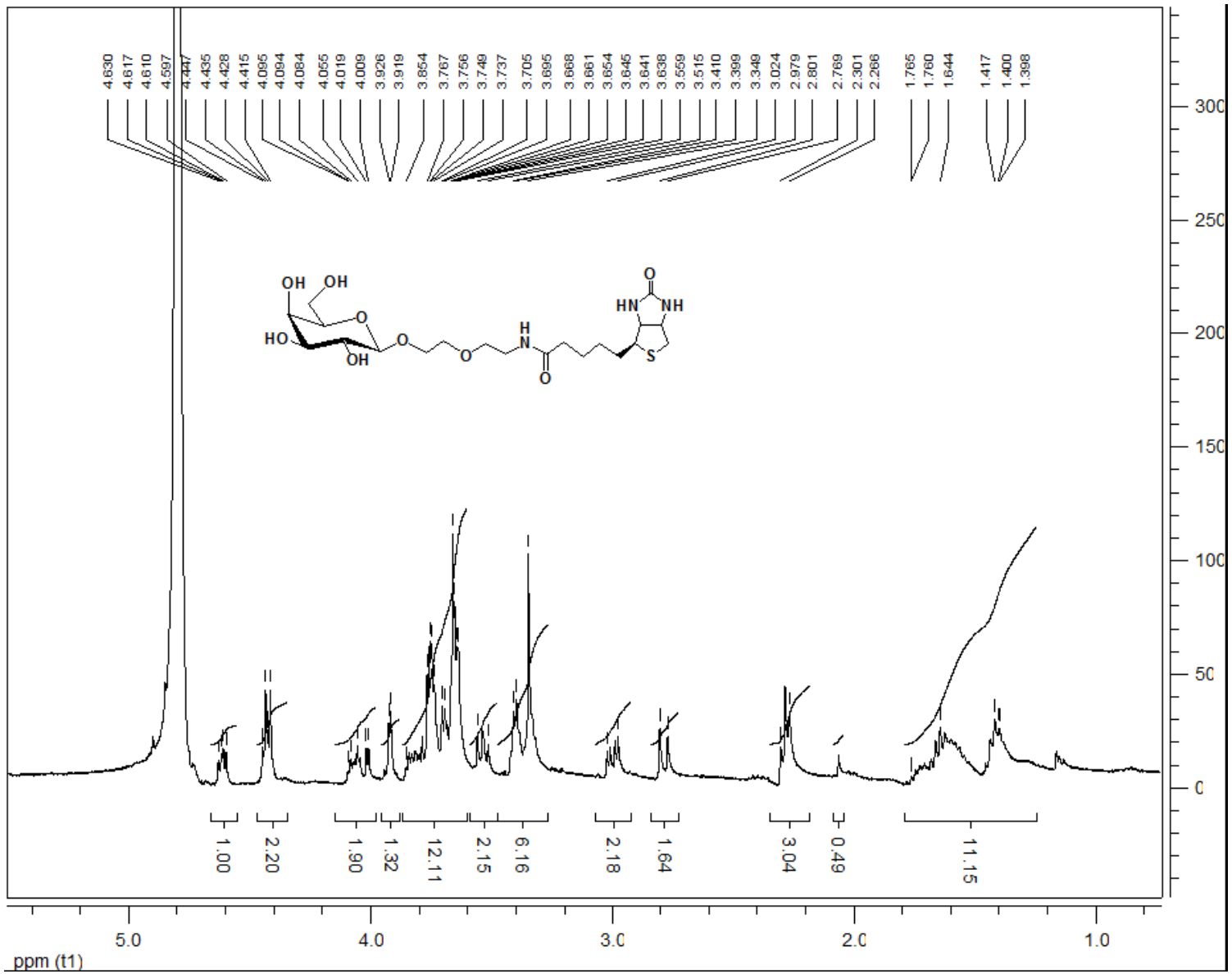
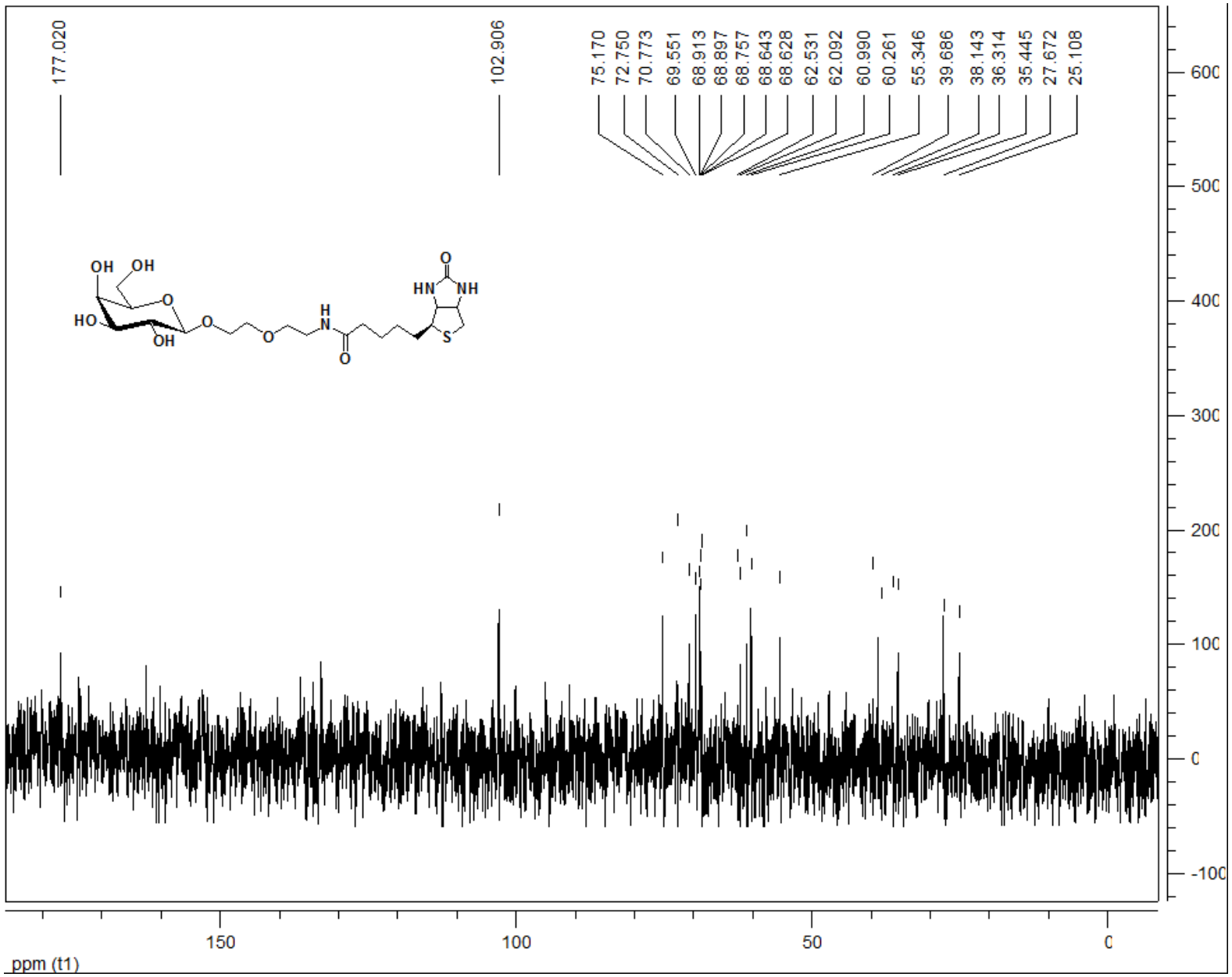
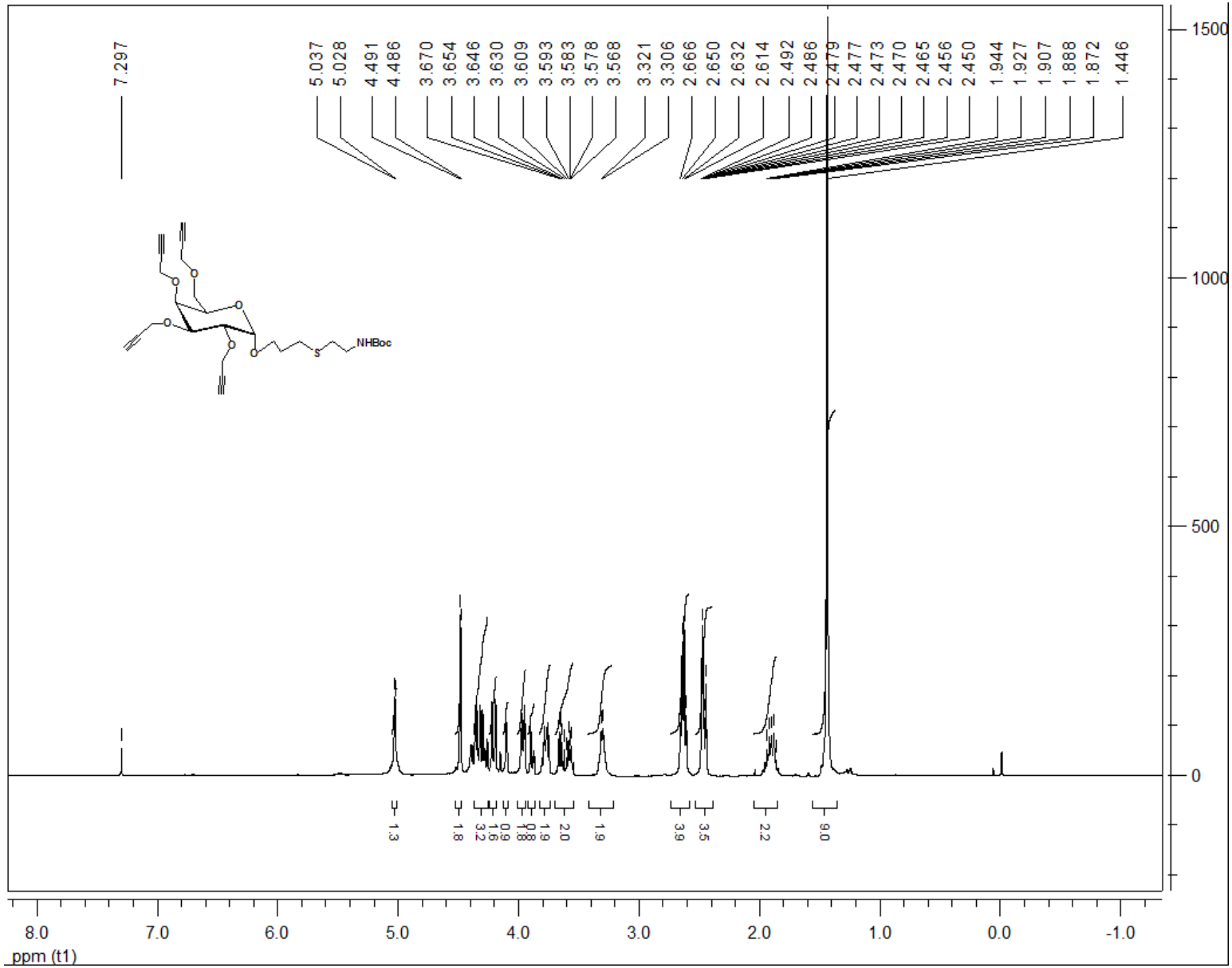
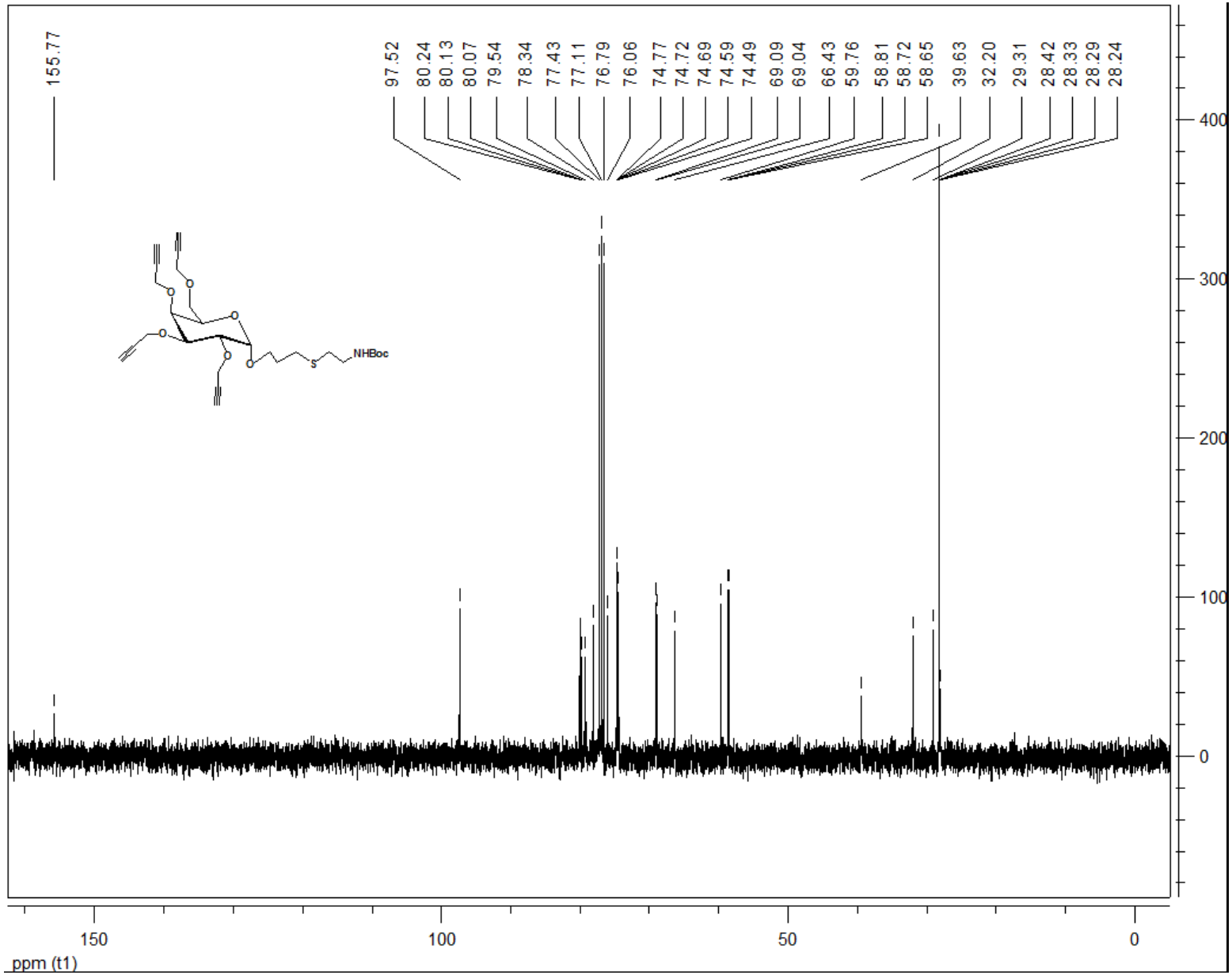
6.574
5.407
5.400
5.305
5.199
5.193
5.173
5.064
4.546
4.526
4.346
4.341
4.188
4.172
4.149
4.131
4.114
4.029
3.956
3.939
3.617
3.540
2.273
2.158
2.069
2.057
2.048
1.999
1.709
1.694
1.480
1.463
1.279
1.261
1.254
1.243
0.000
OAc OAc
AcO
OAc
O
HN
NH
S
1.00
0.89
1.00
0.90
0.87
1.66
0.93
2.59
2.73
6.32
0.90
0.86
0.80
1.54
12.39
4.21
1.99
3.24
0.97
400
300
200
100
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

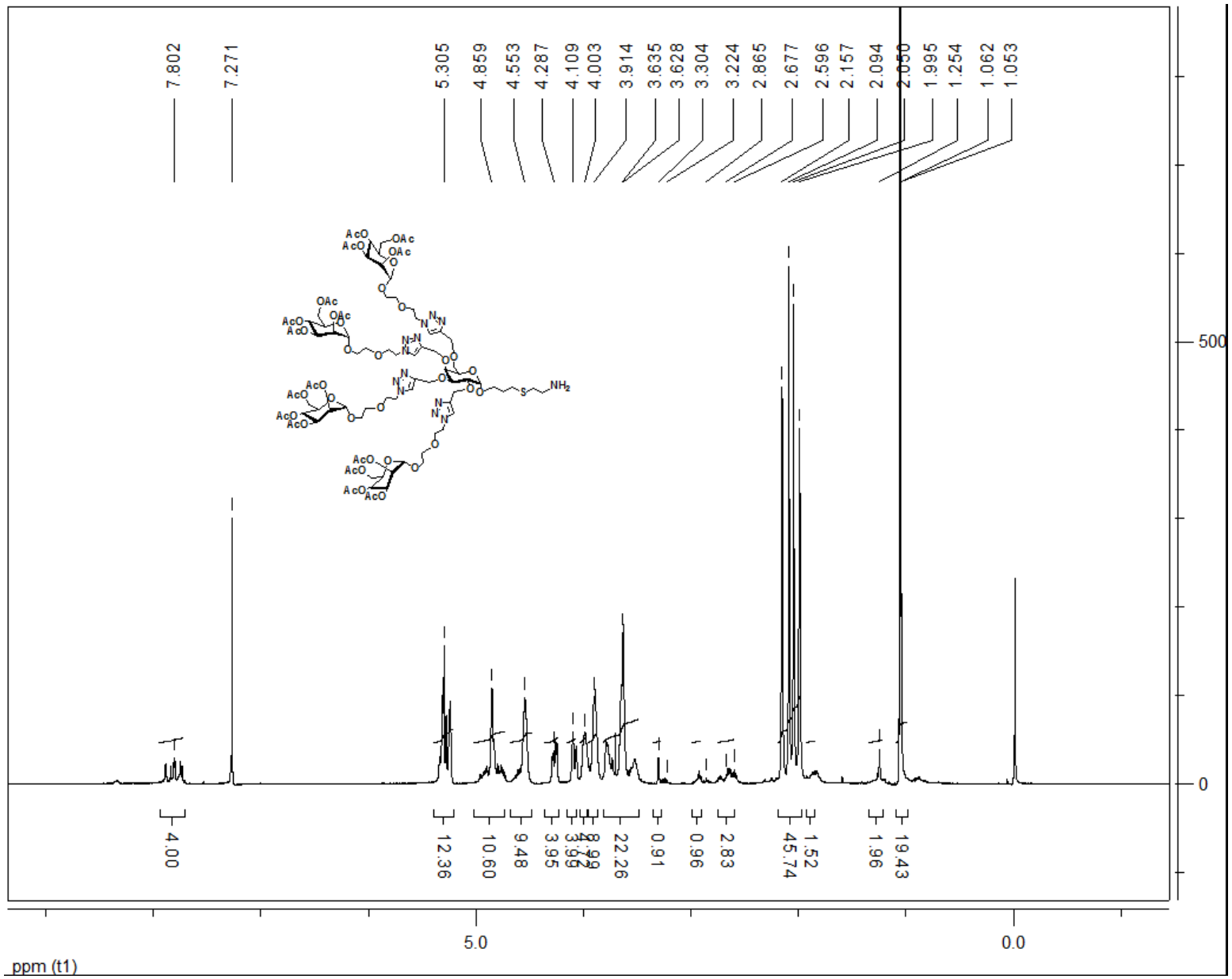

172.268
170.470
170.219
170.179
169.834
101.490
70.779
69.994
69.752
69.158
68.924
67.030
61.846
61.266
60.231
55.938
55.463
40.592
39.101
35.829
29.714
28.132
25.532
20.896
20.734
20.706
OAc OAc
AcO
OAc
O
HN
NH
S
5000
4000
3000
2000
1000
0
150
100
50
0
ppm (t1)

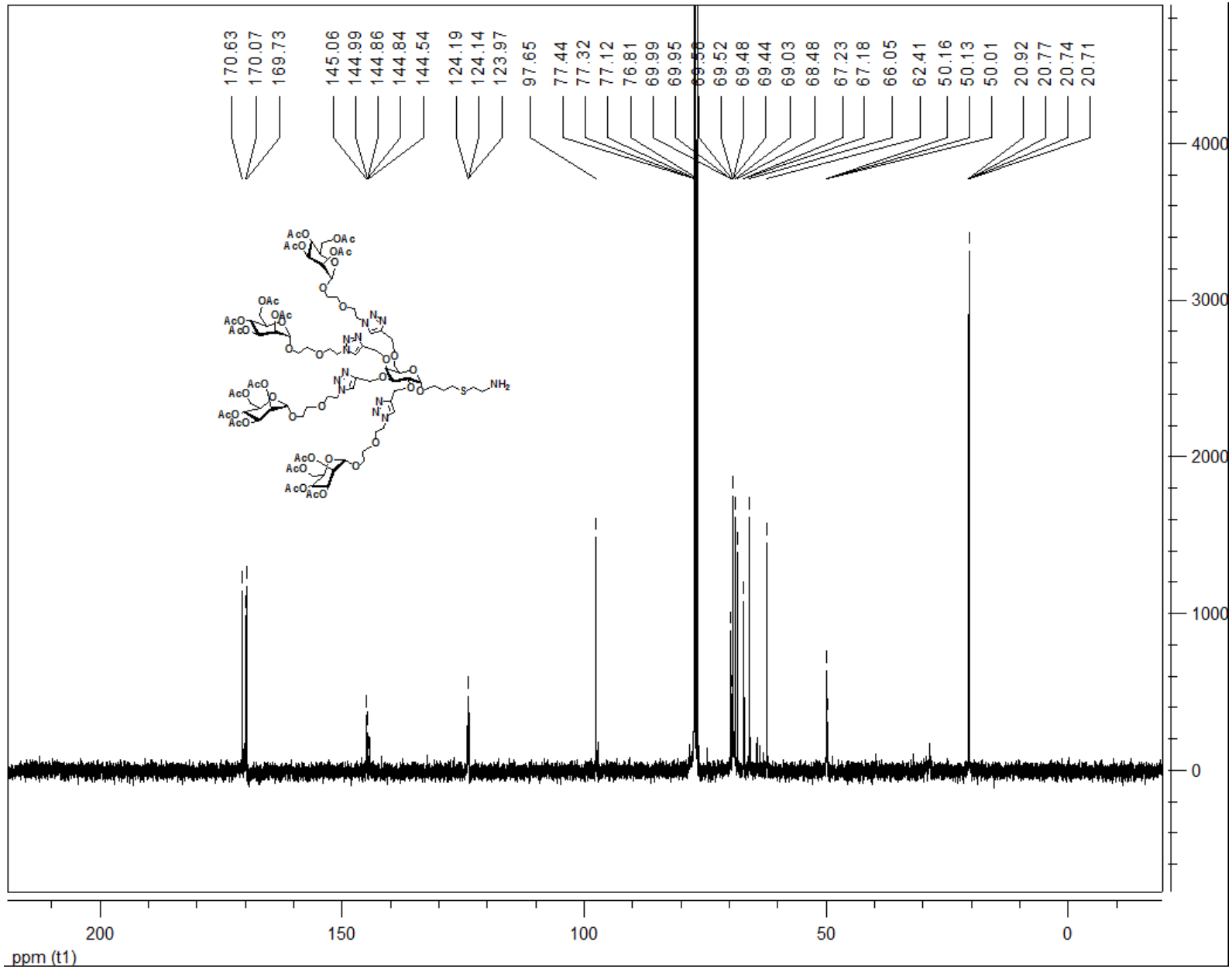

4.630
4.617
4.610
4.597
4.447
4.435
4.428
4.415
4.095
4.094
4.084
4.055
4.019
4.009
3.926
3.919
3.854
3.767
3.756
3.749
3.737
3.705
3.695
3.668
3.661
3.654
3.645
3.641
3.638
3.559
3.515
3.410
3.399
3.349
3.024
2.979
2.801
2.769
2.301
2.266
1.765
1.760
1.644
1.417
1.400
1.398
OH
OH
HO
OH
O
O
O
H
N
O
HN
NH
S
O
1.00
2.20
1.90
1.32
12.11
2.15
6.16
2.18
1.64
3.04
0.49
11.15
300
250
200
150
100
50
0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

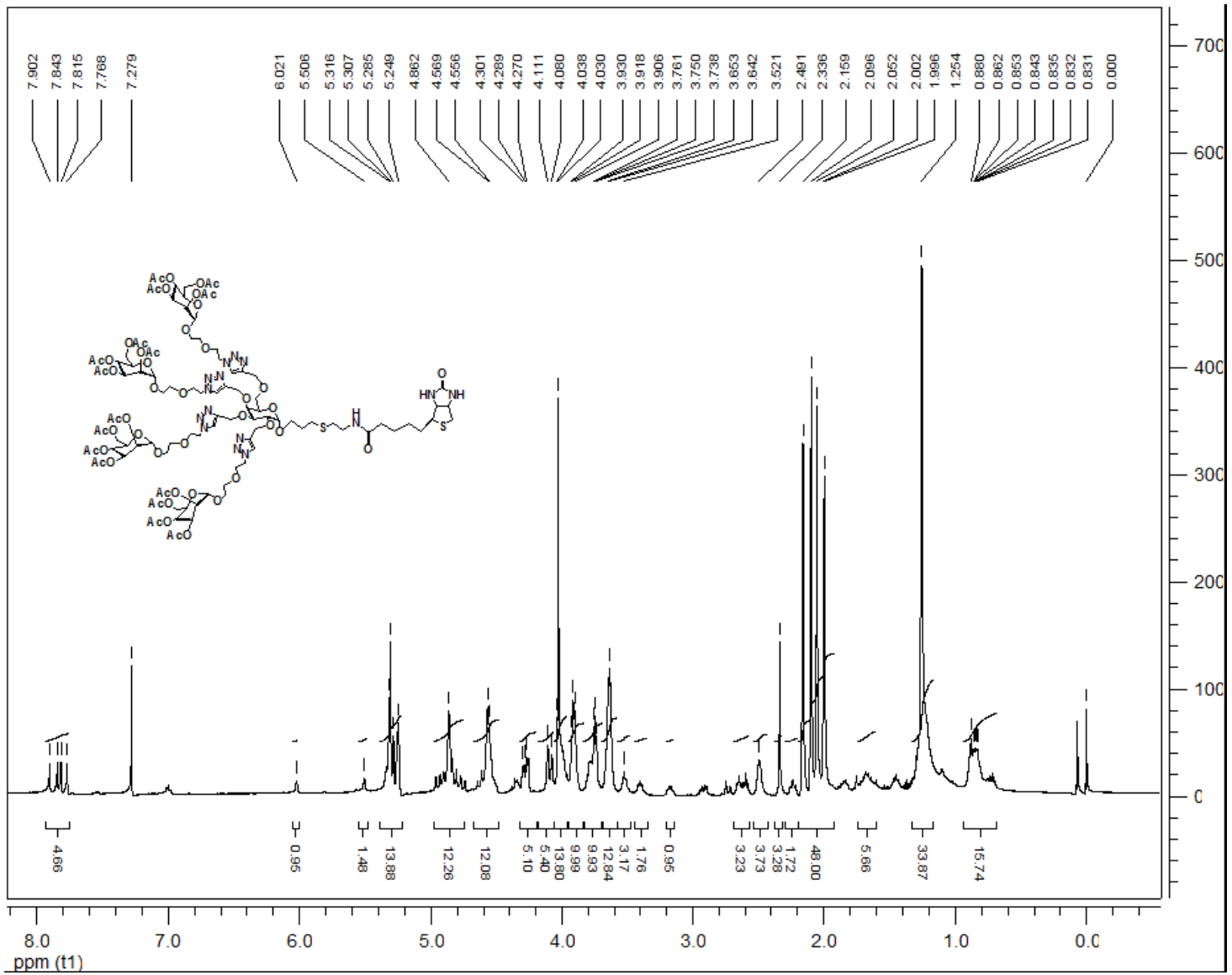

177.020
102.906
75.170
72.750
70.773
69.551
68.913
68.897
68.757
68.643
68.628
62.531
62.092
60.990
60.261
55.346
39.686
38.143
36.314
35.445
27.672
25.108
OH
OH
HO
OH
O
O
O
H
N
O
HN
NH
O
S
600
500
400
300
200
100
0
-100
150
100
50
0
ppm (t1)

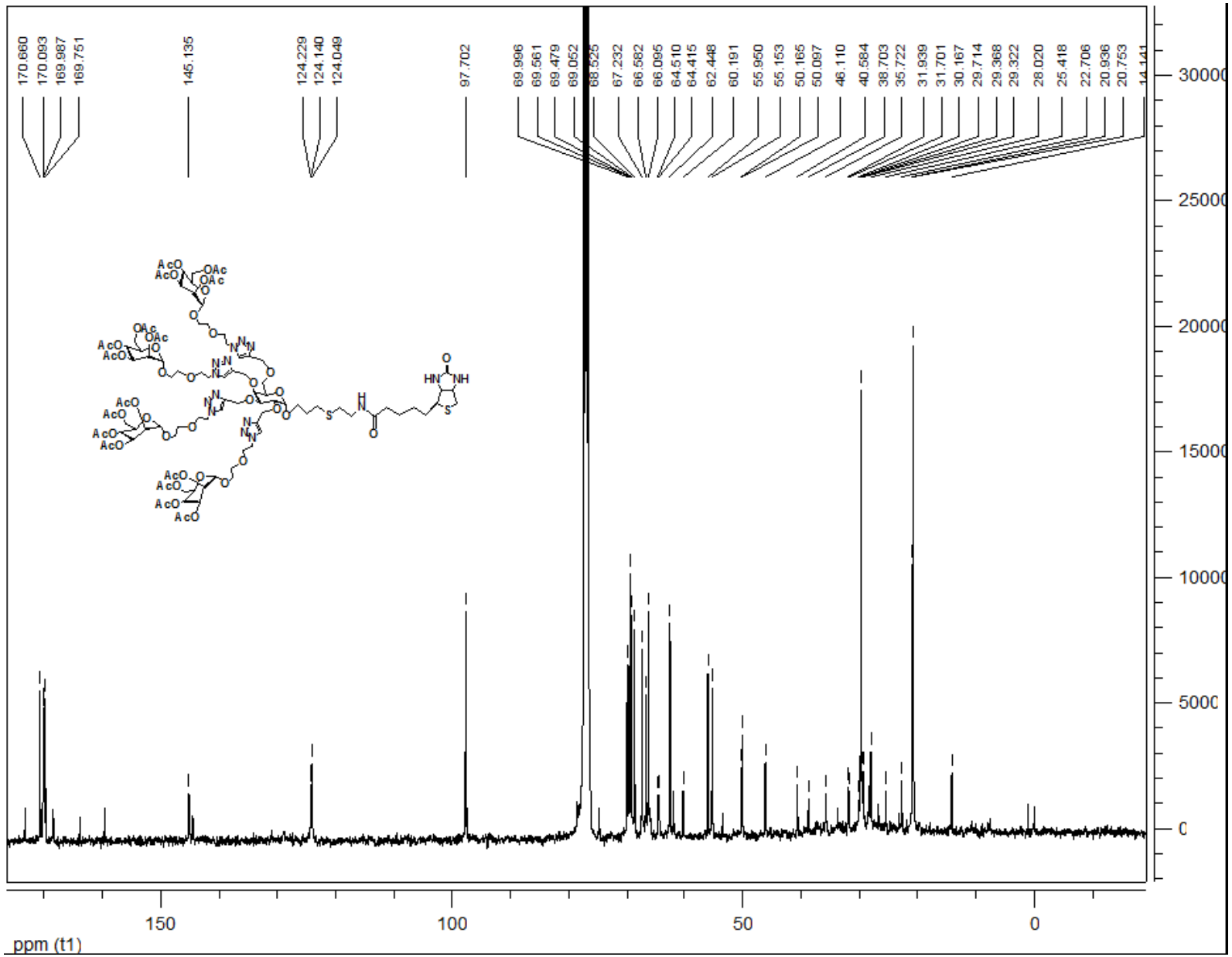

7.297
5.037
5.028
4.491
4.486
3.670
3.654
3.646
3.630
3.609
3.593
3.583
3.578
3.568
3.321
3.306
2.666
2.650
2.632
2.614
2.492
2.486
2.479
2.477
2.473
2.470
2.465
2.456
2.450
1.944
1.927
1.907
1.888
1.872
1.446
NHBoc
1.3
1.8
3.2
1.6
0.9
0.8
1.9
2.0
1.9
3.9
3.5
2.2
9.0
1500
1000
500
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
ppm (t1)

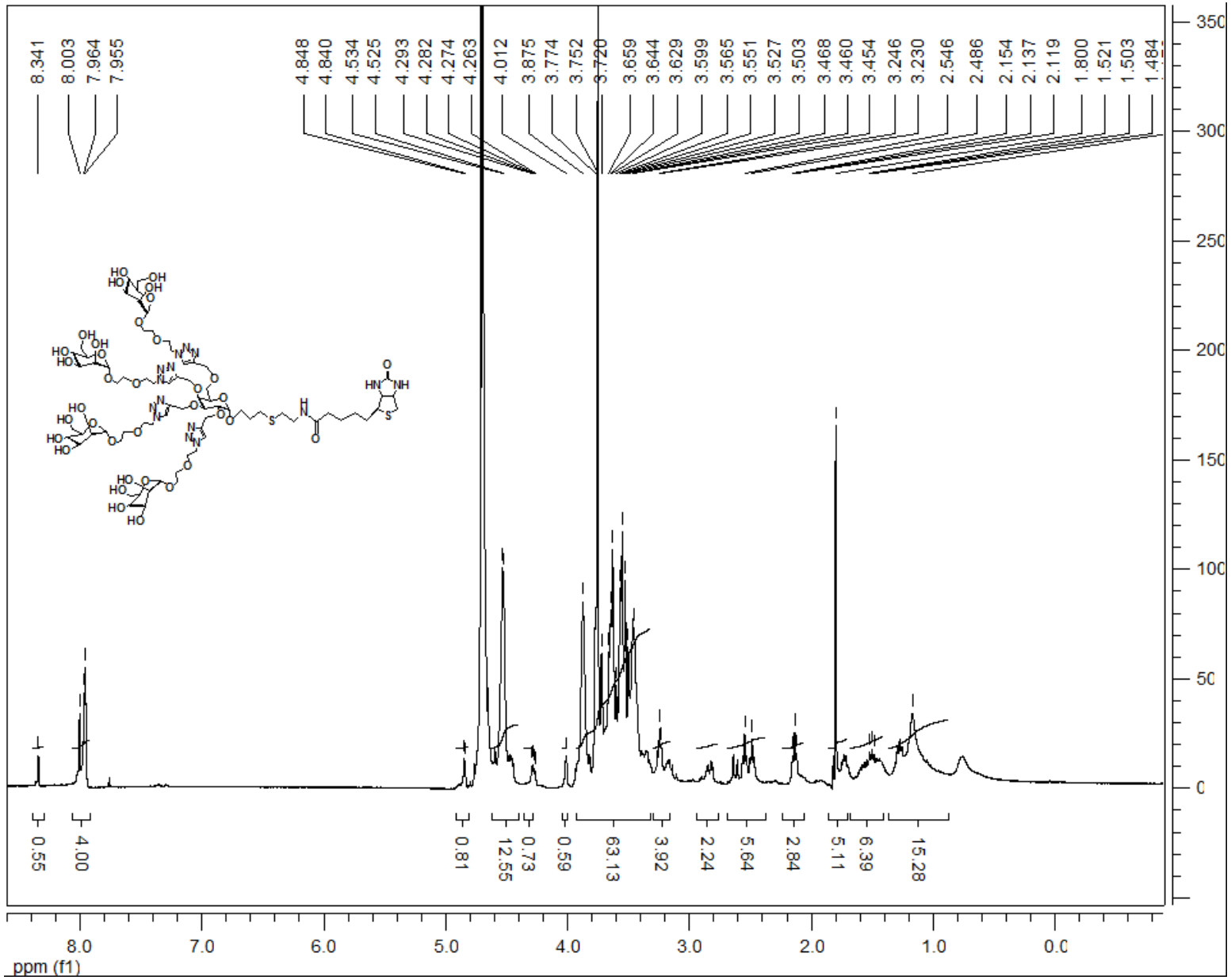

155.77
97.52
80.24
80.13
80.07
79.54
78.34
77.43
77.11
76.79
76.06
74.77
74.72
74.69
74.59
74.49
69.09
69.04
66.43
59.76
58.81
58.72
58.65
39.63
32.20
29.31
28.42
28.33
28.29
28.24
NHBoc
400
300
200
100
0
150
100
50
0
ppm (t1)

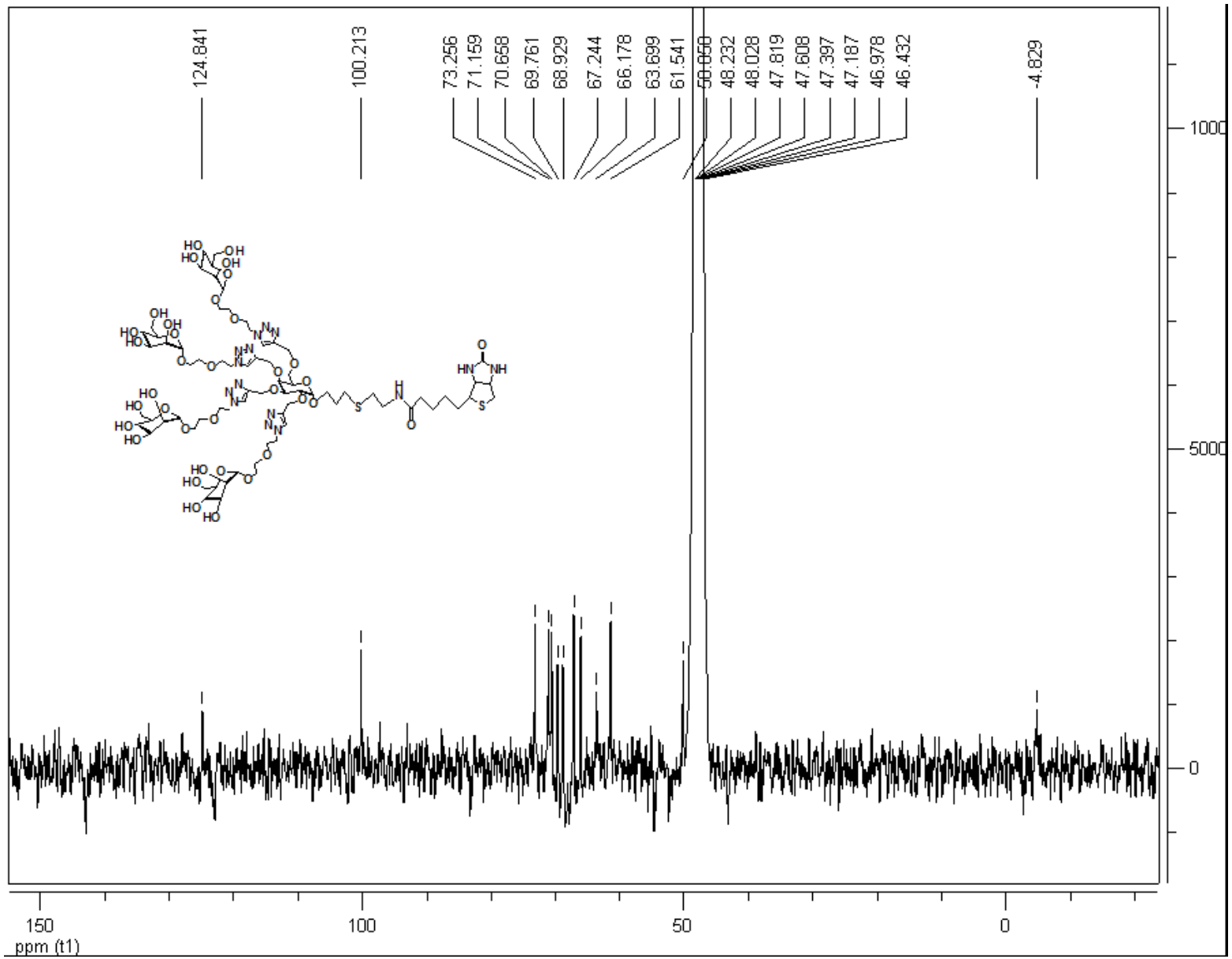

7.802
7.271
5.305
4.859
4.553
4.287
4.109
4.003
3.914
3.635
3.628
3.304
3.224
2.865
2.677
2.596
2.157
2.094
2.050
1.995
1.254
1.062
1.053
500
0
4.00
12.36
10.60
9.48
3.95
22.26
0.91
0.96
2.83
45.74
1.52
1.96
19.43
5.0
0.0
ppm (t1)


170.63
170.07
169.73
145.06
144.99
144.86
144.84
144.54
124.19
124.14
123.97
97.65
77.44
77.32
77.12
76.81
69.99
69.95
69.56
69.52
69.48
69.44
69.03
68.48
67.23
67.18
66.05
62.41
50.16
50.13
50.01
20.92
20.77
20.74
20.71
4000
3000
2000
1000
0
200
150
100
50
0
ppm (t1)


7.902
7.843
7.815
7.768
7.279
6.021
5.506
5.316
5.307
5.285
5.249
4.862
4.569
4.556
4.301
4.289
4.270
4.111
4.080
4.038
4.030
3.930
3.918
3.906
3.761
3.750
3.738
3.653
3.642
3.521
2.491
2.336
2.159
2.096
2.052
2.002
1.996
1.254
0.880
0.862
0.853
0.843
0.835
0.832
0.831
0.000
4.66
0.95
1.48
13.88
12.26
12.08
5.10
5.40
13.80
9.99
9.93
12.84
3.17
1.76
0.95
3.23
3.73
3.28
1.72
48.00
5.66
33.87
15.74
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


170.660
170.093
169.987
169.751
145.135
124.229
124.140
124.049
97.702
69.996
69.561
69.479
69.052
68.525
67.232
66.582
66.095
64.510
64.415
62.448
60.191
55.950
55.153
50.165
50.097
46.110
40.584
38.703
35.722
31.939
31.701
30.167
29.714
29.368
29.322
28.020
25.418
22.706
20.936
20.753
14.141
30000
25000
20000
15000
10000
5000
0
150
100
50
0
ppm (t1)


8.341
8.003
7.964
7.955
4.848
4.840
4.534
4.525
4.293
4.282
4.274
4.263
4.012
3.875
3.774
3.752
3.720
3.659
3.644
3.629
3.599
3.565
3.551
3.527
3.503
3.468
3.460
3.454
3.246
3.230
2.546
2.486
2.154
2.137
2.119
1.800
1.521
1.503
1.484
0.55
4.00
0.81
12.55
0.73
0.59
63.13
3.92
2.24
5.64
2.84
5.11
6.39
15.28
ppm (f1)


124.841
100.213
73.256
71.159
70.658
69.761
68.929
67.244
66.178
63.699
61.541
58.868
48.232
48.028
47.819
47.608
47.397
47.187
46.978
46.432
-4.829
1000
5000
0
150
100
50
0
ppm (t1)

H. References.

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