SUPPORTING INFORMATION

Synthesis of *N*-acetylglucosamine and *N*-acetylallosamine resorcinarene-based multivalent β-thio-glycoclusters: unexpected affinity of *N*-acetylallosamine ligands towards Wheat Germ Agglutinin

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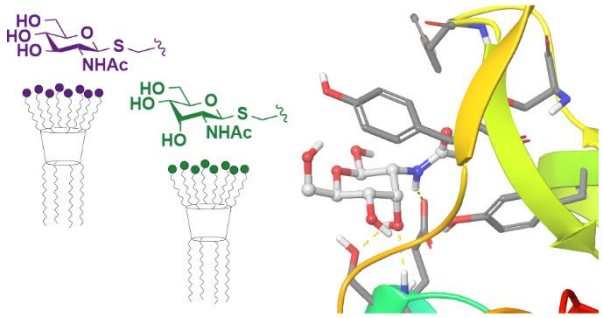

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Keywords: *N*-acetylglucosamine, *N*-acetylallosamine, thioglycoside, resorcinarene, glycoresorcinarene, glycocalixarene, glycocluster, multivalent ligand, lectin, WGA

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General methods

Solvents were distilled before use. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck). The compounds were detected with 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was performed on silica gel 60 from Merck, by elution with the solvents indicated in each case. 2-Propynyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**1**) was prepared by our reported method.[1] Calix[4]resorcinarenes **8**[2] and **9**[3] were synthesized following previously reported methods and their structures confirmed by NMR and HRMS. Reactions under microwave irradiation were carried out in an Anton-Paar Monowave 300 instrument with a System Internal IR probe type (T = 110 °C, t = 50 min). ^{1}H and $^{13}C\{^{1}H\}$ Nuclear Magnetic Resonance (NMR) spectra were recorded at 25 °C at 500 and 125.7 MHz, respectively, in a Bruker Avance Neo 500 spectrometer. ^{1}H and ^{13}C chemical shifts are reported in parts per million relative to tetramethylsilane or the residual solvent peak ($CHCl_3$: ^{1}H: δ 7.26 ppm, ^{13}C: δ 77.2 ppm). *J* values are given in Hz. Assignments of ^{1}H, ^{13}C were determined by analysis of coupling constants and assisted by 2D ^{1}H COSY and ^{1}H-^{13}C HSQC experiments. High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF in a Bruker micrOTOF-Q II spectrometer. Optical rotations were determined in a Perkin-Elmer 343 polarimeter, at 20 °C in a 1 dm cell. Turbidimetric assay was performed in an HP8452-A diode array spectrophotometer. Fluorescence spectra were recorded with a Cary Eclipse spectrophotometer equipped with two Czerny-Turner monochromators and a 15 W Xe pulse lamp (pulse width: 2–3 μs, power: 60–75 kW). Isothermal Titration Calorimetry experiments were carried out in a NanoITC calorimeter (TA Instruments) equipped with 200 μL cells and a 50 μL syringe, and data fitting was performed with Nano Analyze software.

Compound 8

Compound **8** was synthesized from resorcinol and dodecanal as previously described.[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83–9.15 (8 H, m, 8 × O*H*), 7.21 (4 H, s, 4 × b-H), 6.11 (4 H, s, 4 × a-H), 4.30 (4 H, t, $J_{CHAr2,CH2}$ = 7.4, C*H*Ar_2), 2.21 (8 H, m, C$H_2(CH_2)_9CH_3$), 1.44–1.18 (72 H, m, CH_2), 0.88 (12 H, t, $J_{CH3,CH2}$ = 6.9, CH_3). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$) δ 150.7 (C-c), 125.0 (C-d), 124.0 (C-b), 102.9 (C-a), 33.4 (*C*HAr_2), 33.3 (*C*$H_2(CH_2)_9CH_3$), 32.1, 30.0, 29.9 (× 3), 29.8, 29.6, 28.2, 22.8 (*C*H_2) 14.3 (*C*H_3). ESI-HRMS: *m/z* $[M+H]^+$ calcd for $C_{72}H_{113}O_8$: 1105.8430, found: 1105.8430.

Compound 9

Compound **9** was synthesized from resorcinol and acetaldehyde as previously described.[3] ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (8 H, s, 8 × O*H*), 6.77 (4 H, s, 4 × b-H), 6.14 (4 H, s, 4 × a-H), 4.45 (4 H, q, $J_{CHAr2,CH3}$ = 7.2, C*H*Ar_2), 1.30 (12 H, d, $J_{CHAr2,CH3}$ = 7.2, CH_3); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$) δ 151.9 (C-c), 125.3 (C-b), 123.2 (C-d), 102.2 (C-a), 28.6 (*C*HAr_2), 21.7 (*C*H_3). ESI-HRMS: *m/z* $[M+H]^+$ calcd for $C_{32}H_{33}O_8$: 545.2170, found: 545.2136.

2-(2-azidoethoxy)ethanol (11)

To a solution of commercial 2-(2-cloroethoxy)ethanol (2.0 mL, 18.6 mmol) in anh. DMF (25 mL), NaN_3 (3.60 g, 55.6 mmol) was added. The resulting suspension was heated to 90 °C for 18 h. Then, the solvent was evaporated under vacuum and the residue was dissolved in EtOAc (30 mL). The solution was extracted with LiCl 5% (3 × 10 mL) and water (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure, giving 2.27 g of product **11** (93%) as a colorless liquid. Spectral data was coincident with that of the bibliography.[4]

2-(2-azidoethoxy)-1-iodoethane (12)

To a solution of compound **11** (2.27 g, 17.3 mmol) in DCM (130 mL) at 0 °C, imidazole (1.53 g, 22.5 mmol), Ph_3P (5.88 g, 22.5 mmol) and I_2 (5.70 g, 22.5 mmol) were sequentially added. The reaction mixture was stirred at 0 °C for 30 min. Then, it was allowed to reach room temperature and stirred for an additional 1 h. A solution of $NaHSO_3$ 10% (150 mL) was added and the mixture was vigorously stirred for 5 min.

Layers were separated and the aqueous phase was extracted with DCM (3 × 40 mL). The organic extracts were combined and concentrated under vacuum. The residue was purified through column chromatography (hexane/EtOAc 1:0 → 8:2), obtaining 3.48 g of **12** as a pale-yellow liquid (84%). Spectral data was coincident with that of the bibliography.[5]

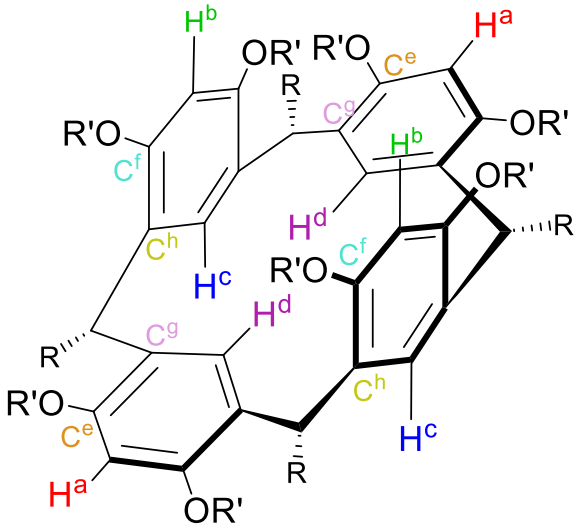

Figure S1. Schematic representation of the resorcinarene aromatic core in its flattened boat conformation with C_{2v} symmetry.

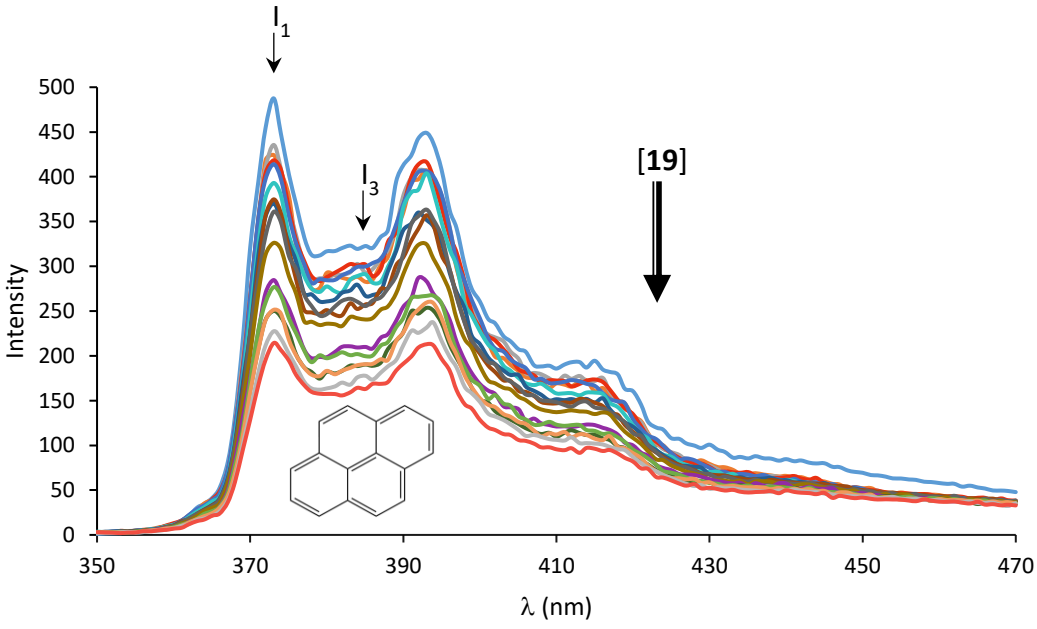

Figure S2. Fluorescence emission spectra of pyrene solutions with increasing amounts of glycoresorcinarene **19**. I_1 and I_3 corresponds to the intensity values at λ = 373 nm and λ = 383 nm respectively.

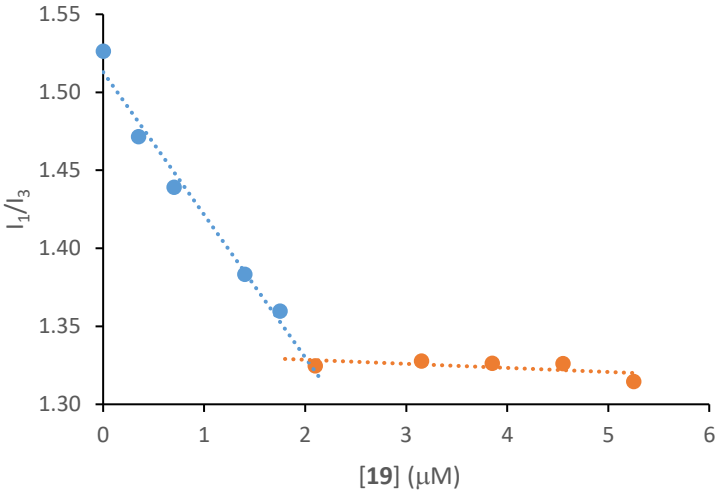

Figure S3. I_1/I_3 plotted against concentration for each addition of glycoresorcinarene **19**. The CMC value was obtained from the intersection of the represented curves.

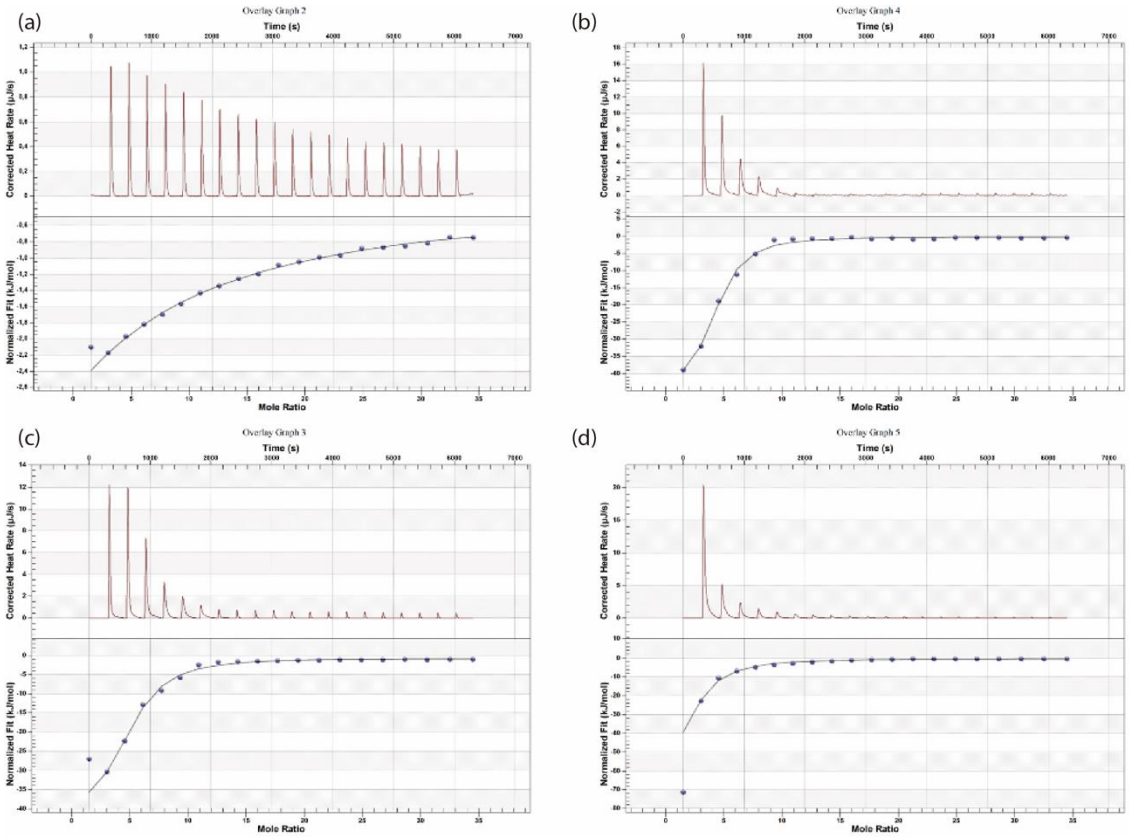

Figure S4. Interaction analysis of synthetic glycoresorcinarenes with WGA by ITC. Integrated heats of interaction between WGA and (*a*) GlcNAc, (*b*) **18**, (*c*) **19** and (*d*) **20** at 298 K. The independent model was implemented using NanoAnalyze software to obtain the fitting curve for the experimental data.

References

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2 L. Abis, E. Dalcanale, A. Du vosel and S. Sperala, *J. Org. Chem.*, 1988, **53**, 5475–5475.

3 A. G. S. Högberg, *J. Org. Chem.*, 1980, **45**, 4498–4500.

4 W. Gan, X. Cao, Y. Shi and H. Gao, *J. Polym. Sci.*, 2020, **58**, 84–90.

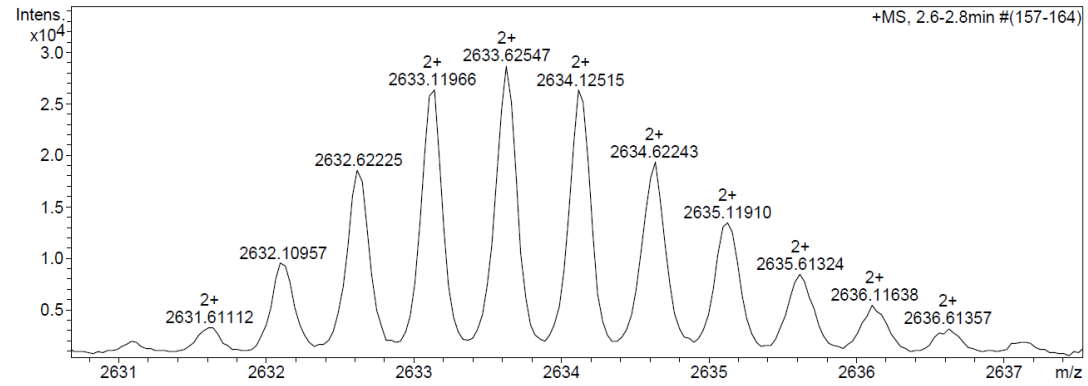
5 Y. S. Wang, S. Bai, Y. Y. Wang and Y. F. Han, *Chem. Commun.*, 2019, **55**, 13689–13692.

HRMS Spectra for compounds 15–20

Compound 15

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	600 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2800 m/z	Set Collision Cell RF	700.0 Vpp	Set Divert Valve	Source

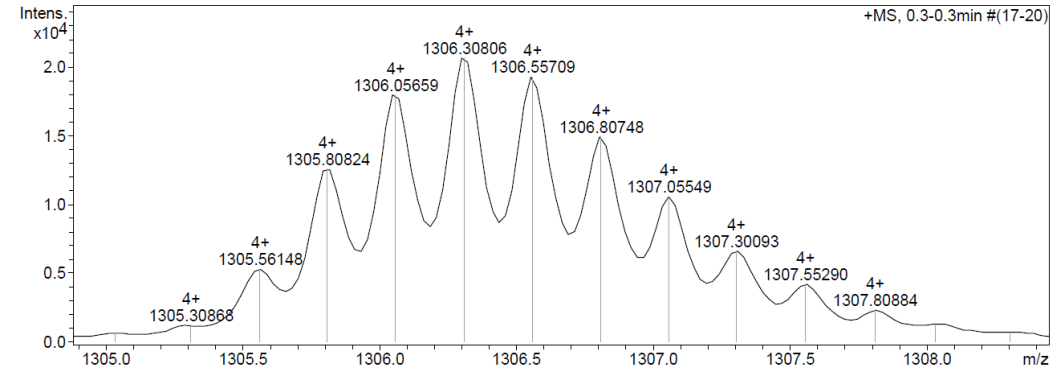


Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	e¯ Conf	N-Rule	mSigma
2632.10957	1	C 240 H 352 N 32 Na 2 O 80 S 8	2632.10048	-3.45	-6.18	even	ok	62.8

Compound 16

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	102 m/z	Set End Plate Offset	-450 V	Set Dry Gas	4.0 l/min
Scan End	6500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	e¯ Conf	N-Rule	mSigma
1305.56148	1	C 240 H 356 N 32 O 80 S 8	1305.56291	1.09	6.46	even	ok	35.7

Compound 17

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
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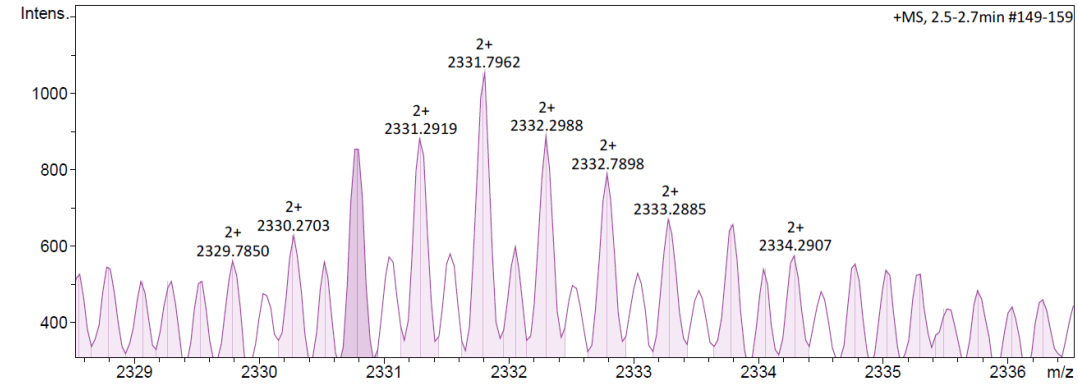
Scan End	5000 m/z	Set Collision Cell RF	800.0 Vpp	Set Divert Valve	Source

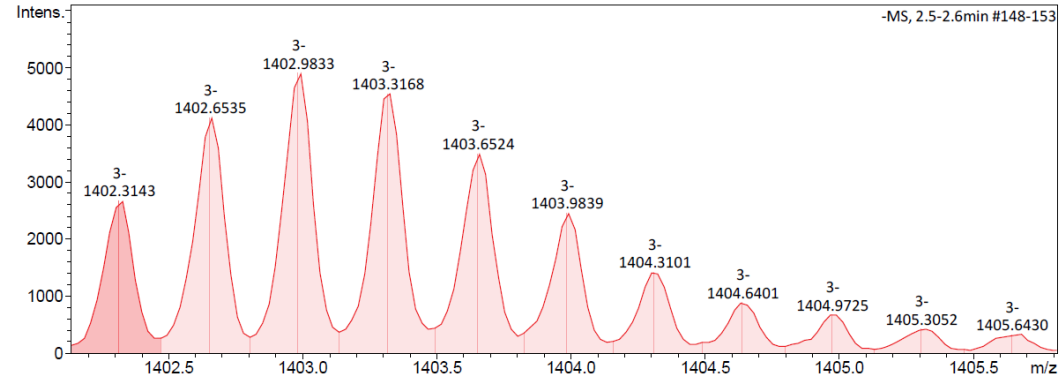


Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
2330.7836	1	C200H276N32O80S8	2330.8134	12.8	312.7	2	100.00	79.0	even	ok

Compound 18

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	800 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	1000.0 Vpp	Set Divert Valve	Source

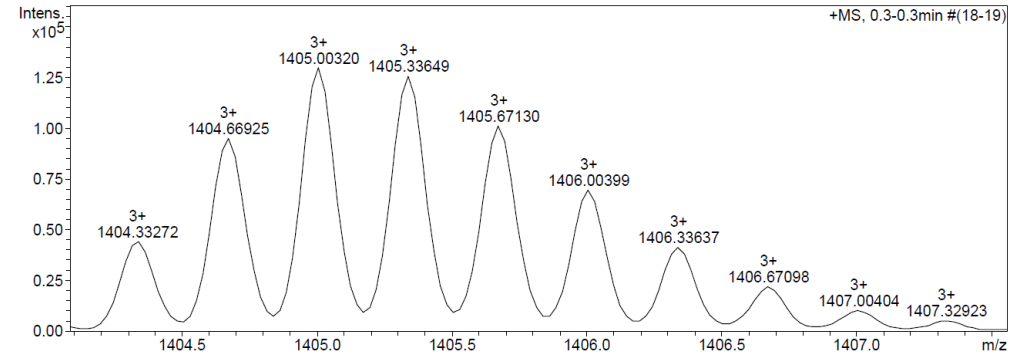


Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
1402.3143	1	C192H301N32O56S8	1402.3157	1.0	91.1	1	100.00	58.5	even	ok

Compound 19

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	650 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	700.0 Vpp	Set Divert Valve	Source

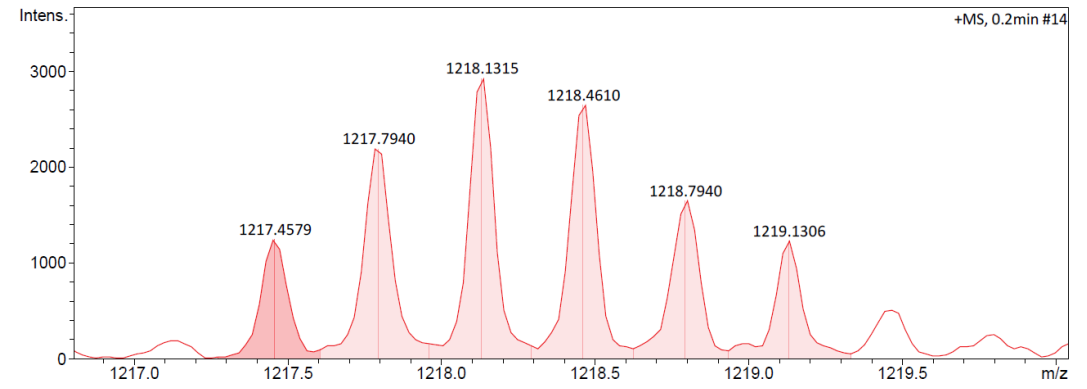


Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma
1404.33272	1	C 192 H 307 N 32 O 56 S 8	1404.33026	-1.75	-3.08	55.5	ok	even	15.1

Compound 20

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.5 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	250 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	7.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Source



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HO
HO
HO
O
S
NHAc

2

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1.02
1.00
1.01
2.00
2.02
0.92
2.97
4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0
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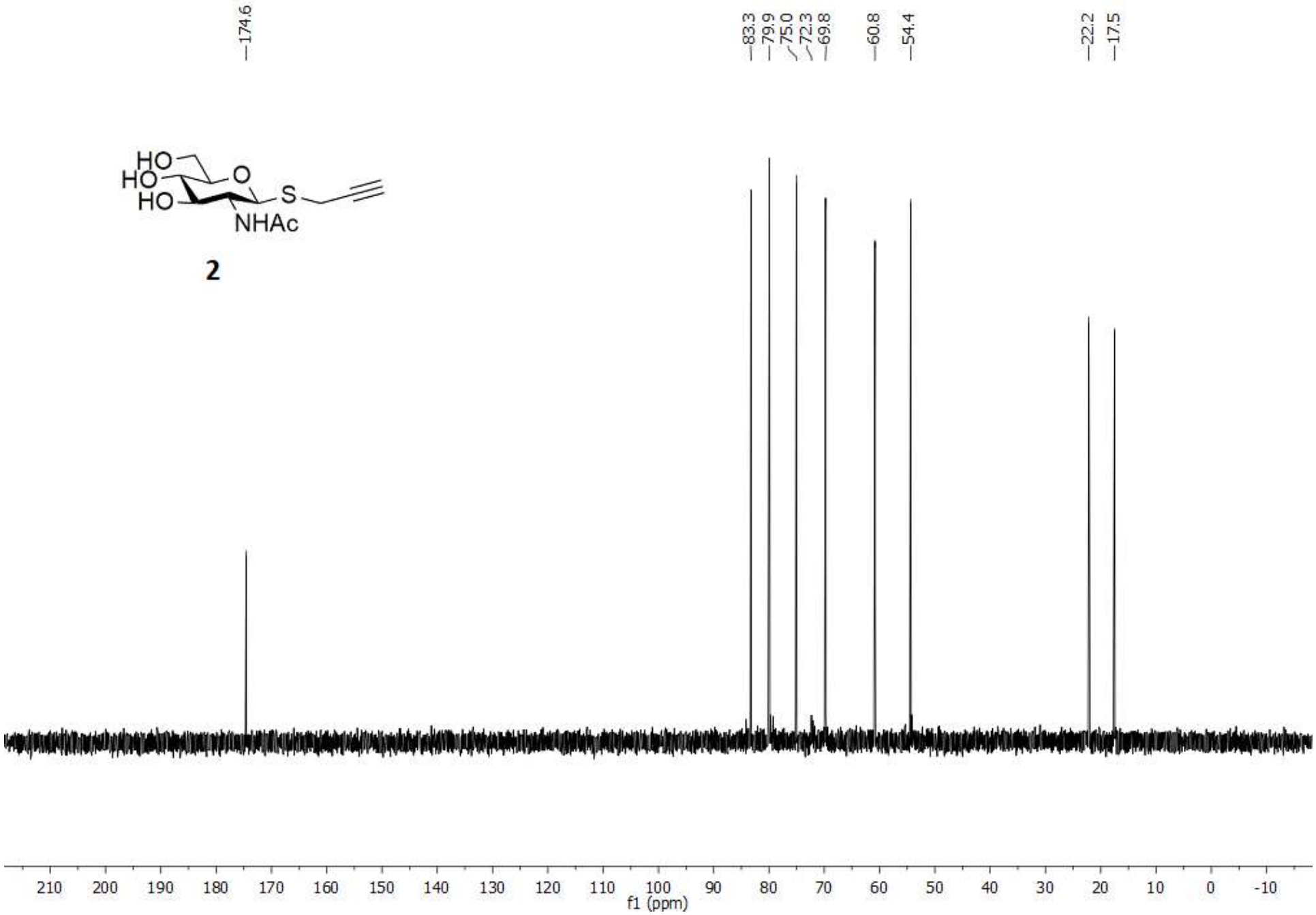
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HO
O
S
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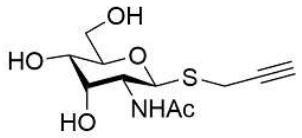
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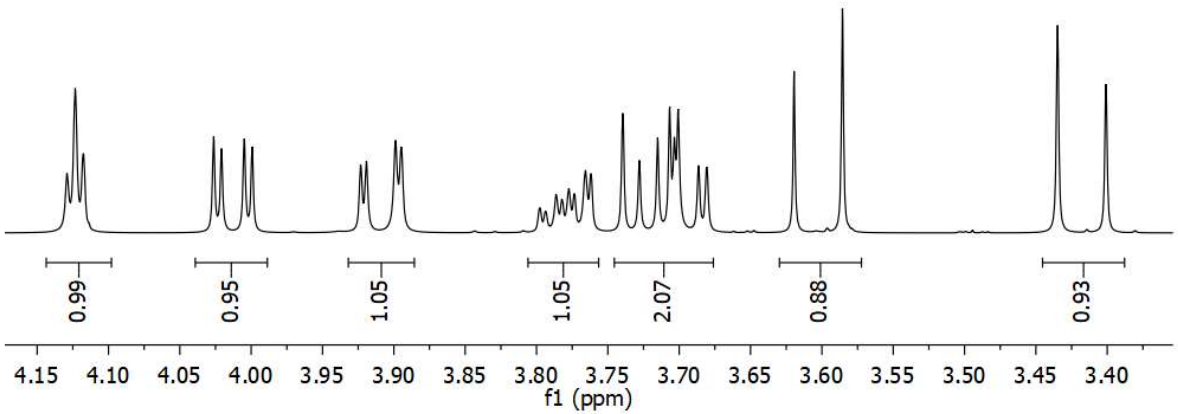
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f1 (ppm)

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1.05
2.07
0.88
0.93
3.28
5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0
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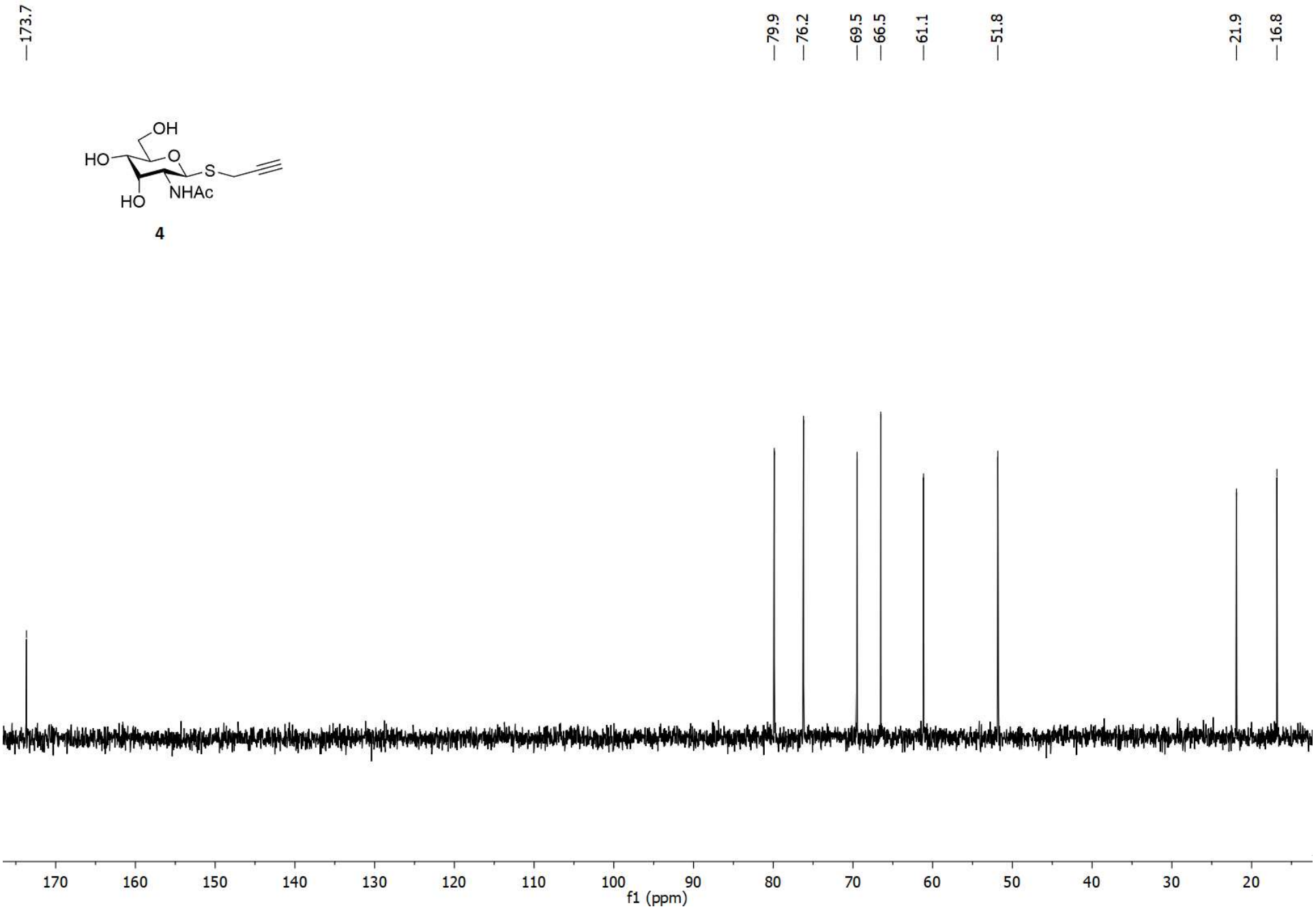
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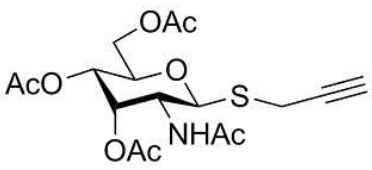
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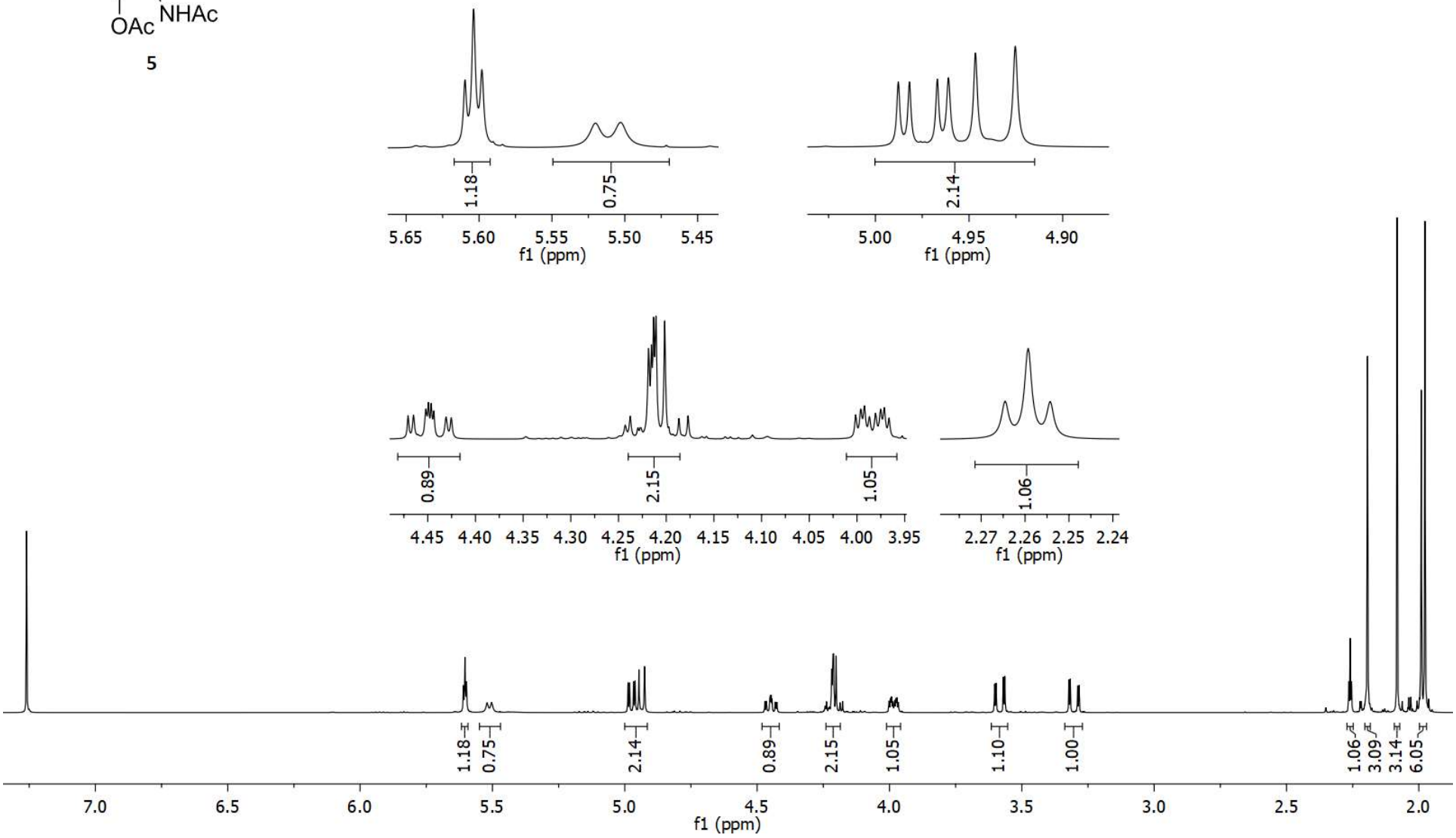
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f1 (ppm)
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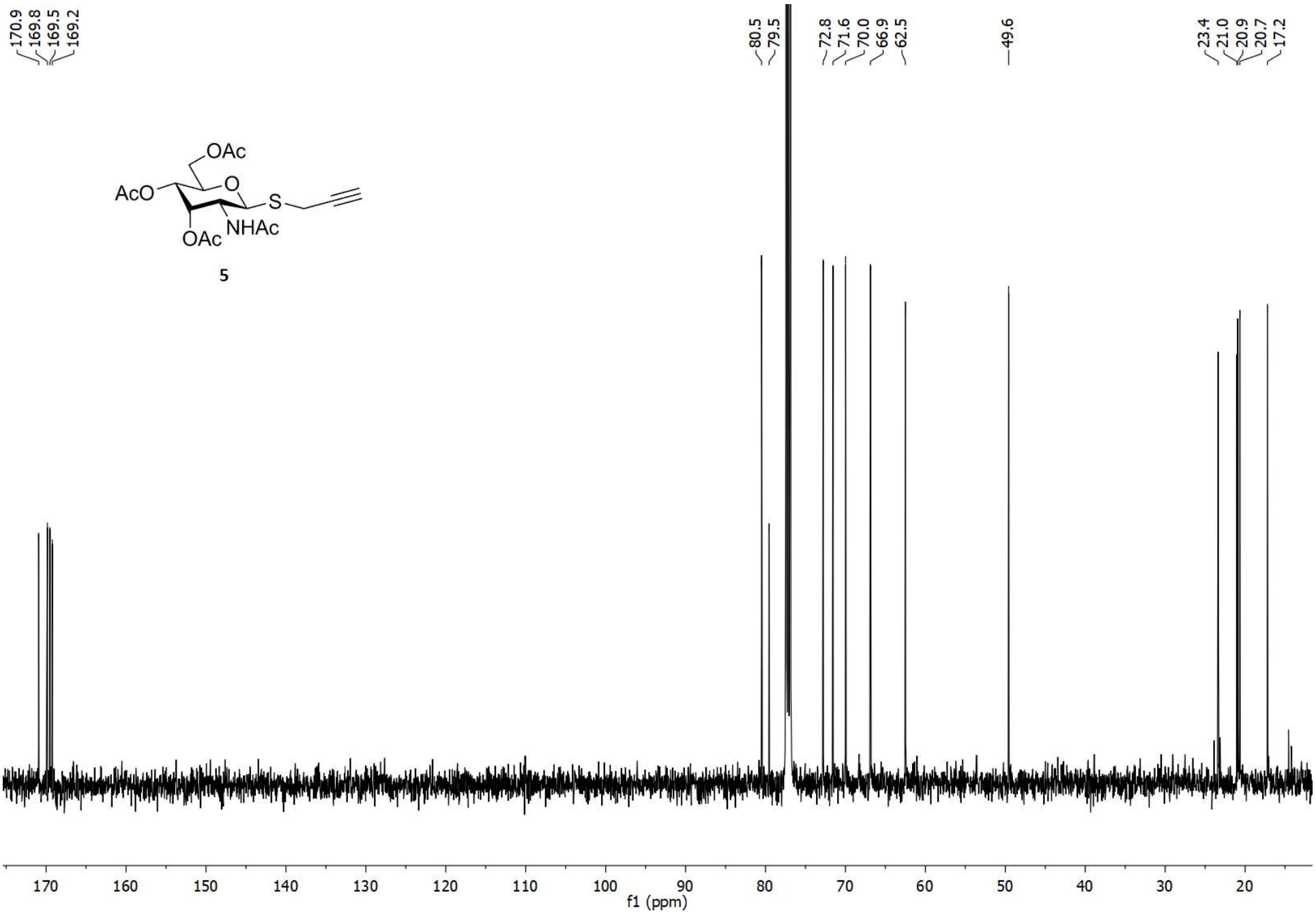
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AcO
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OAc
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150
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130
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f1 (ppm)


Ph
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O
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HO
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NHAc

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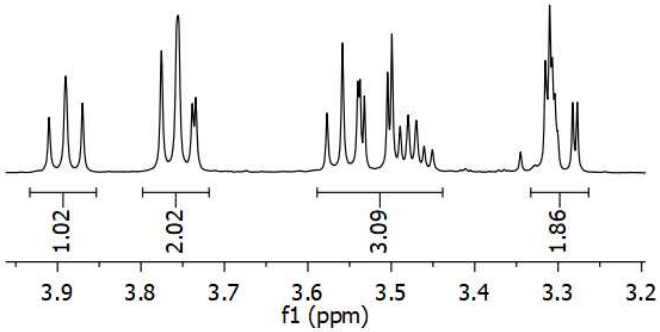
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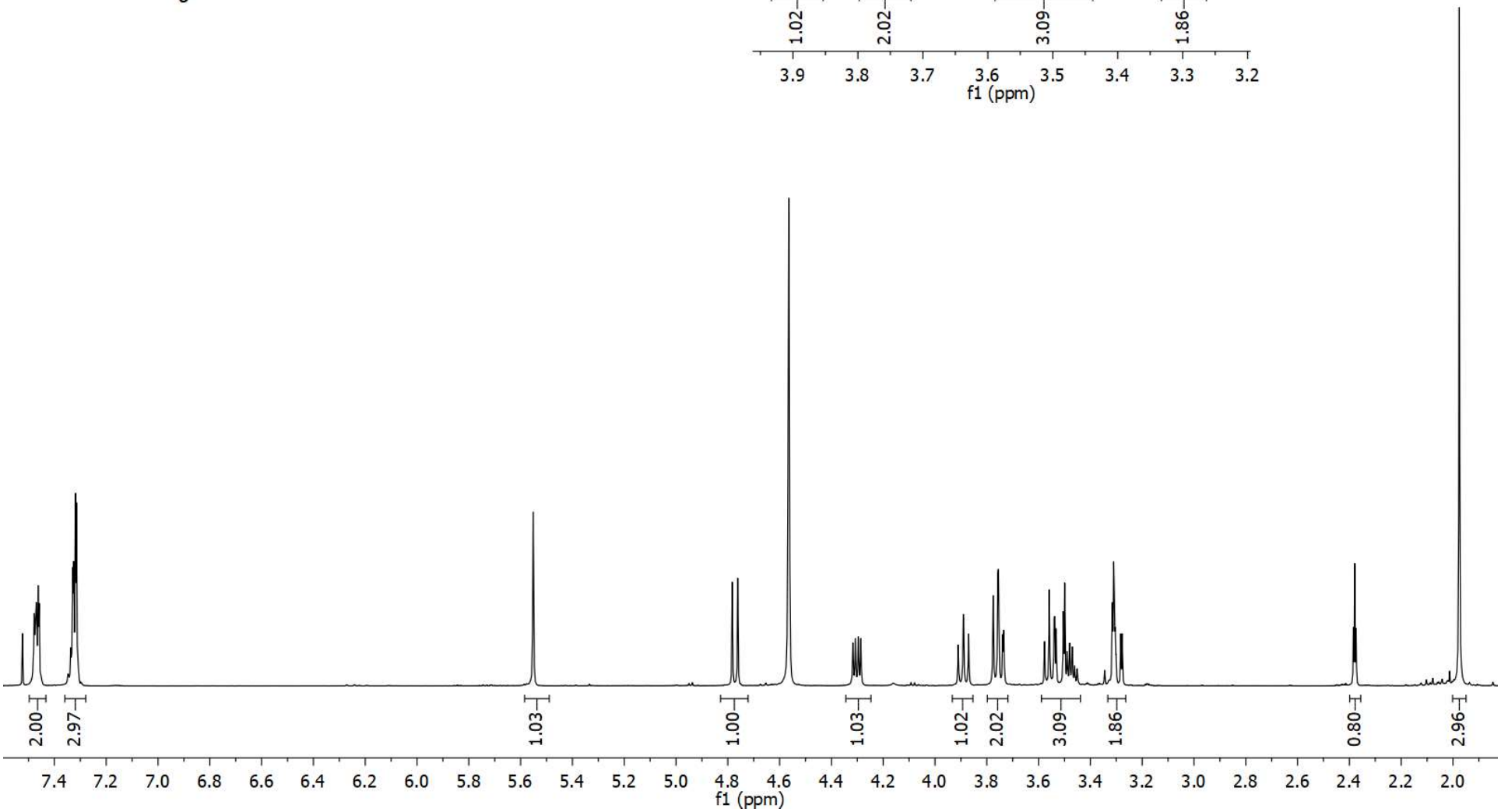
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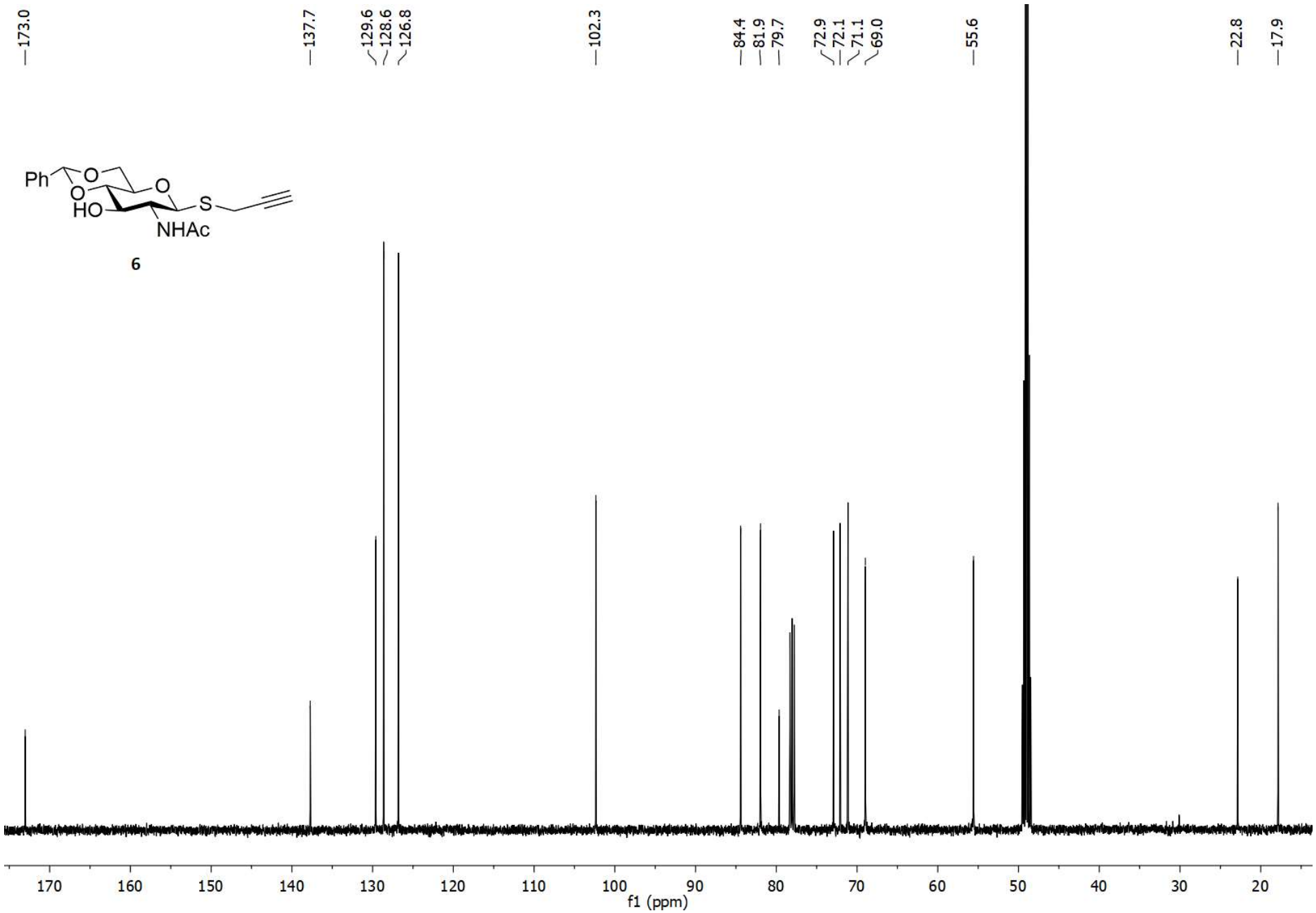
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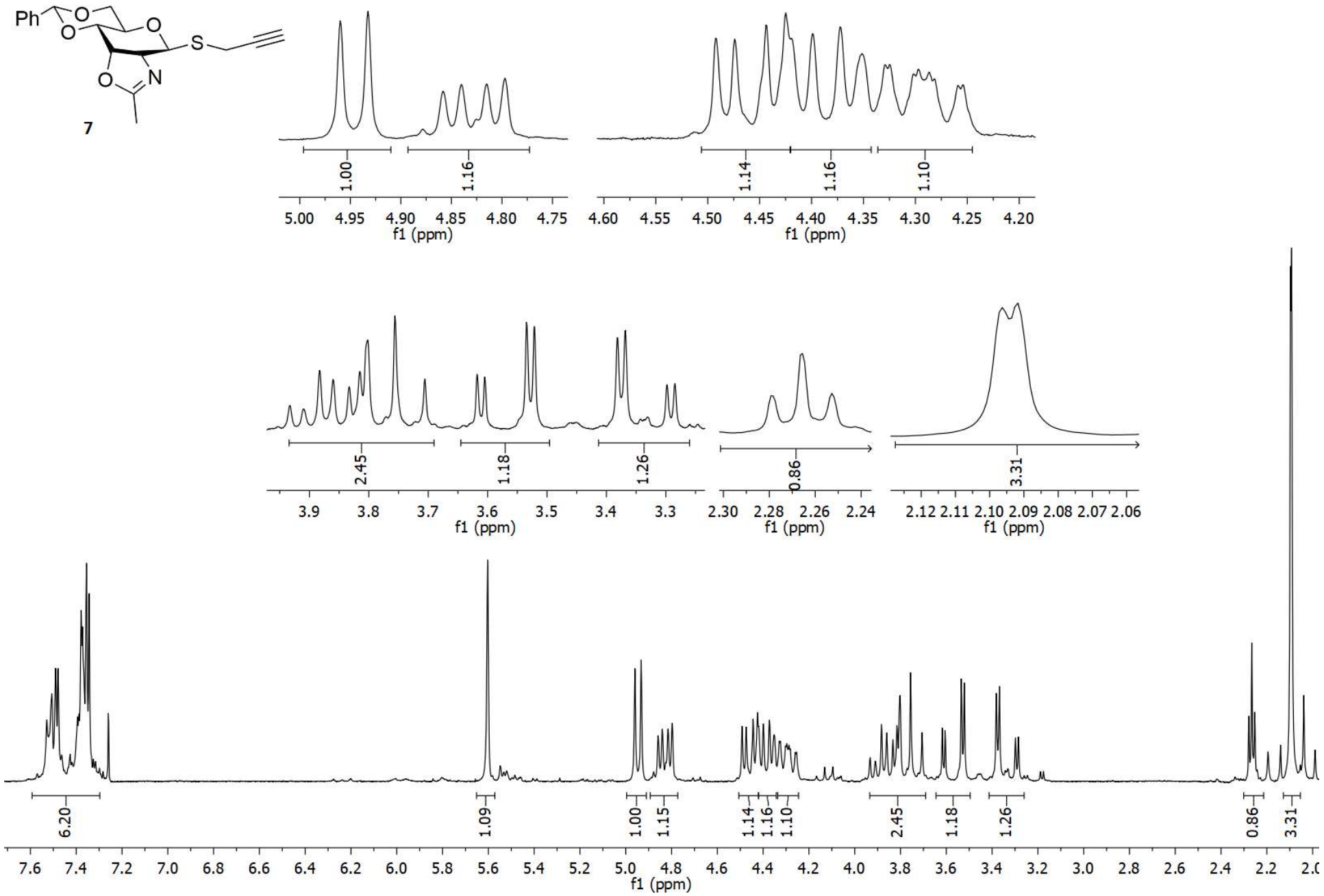
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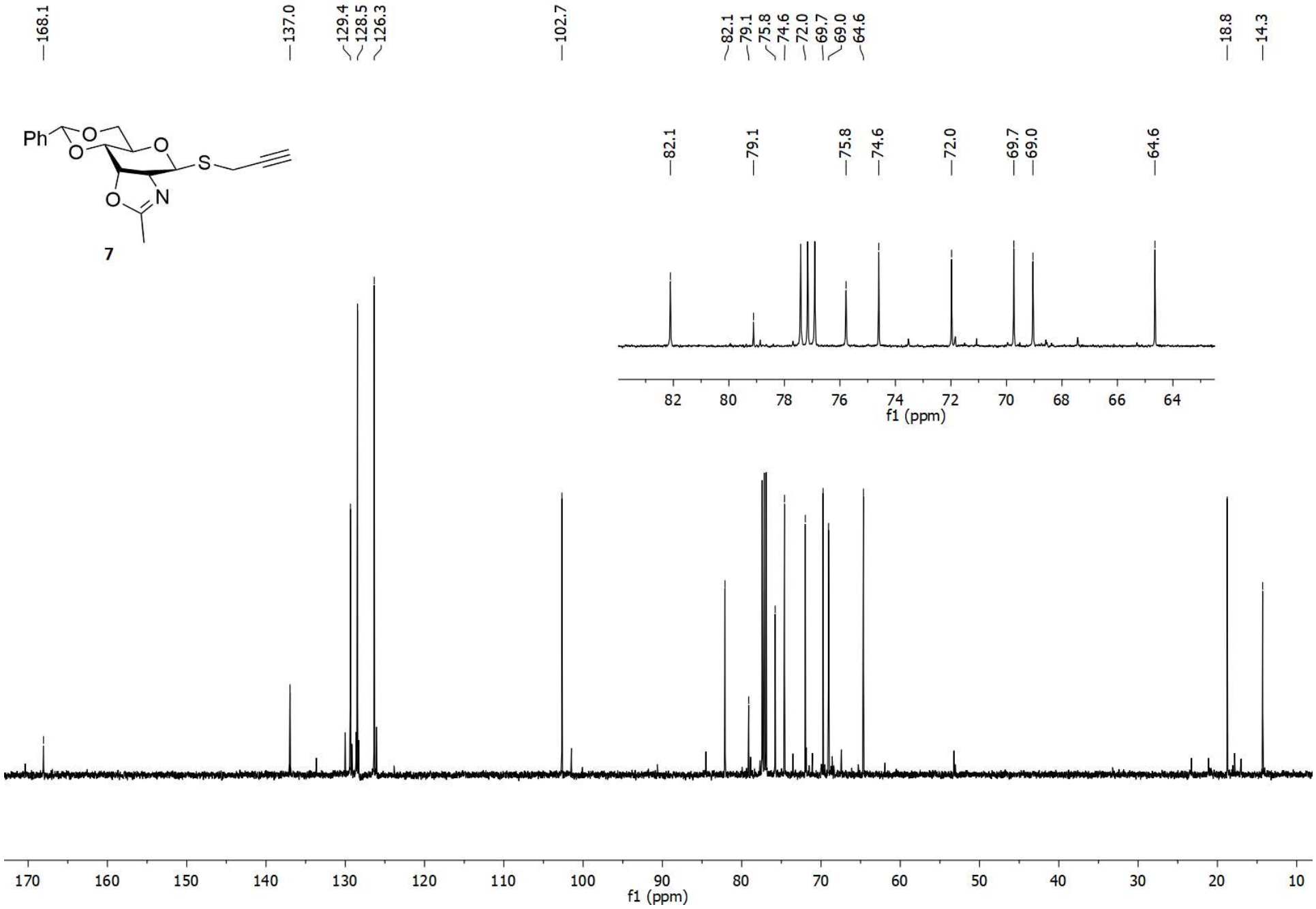
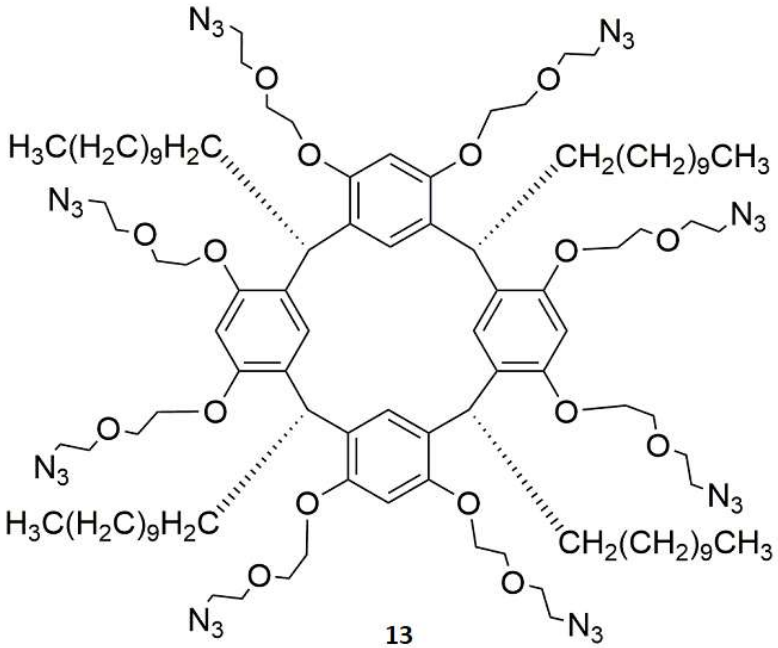
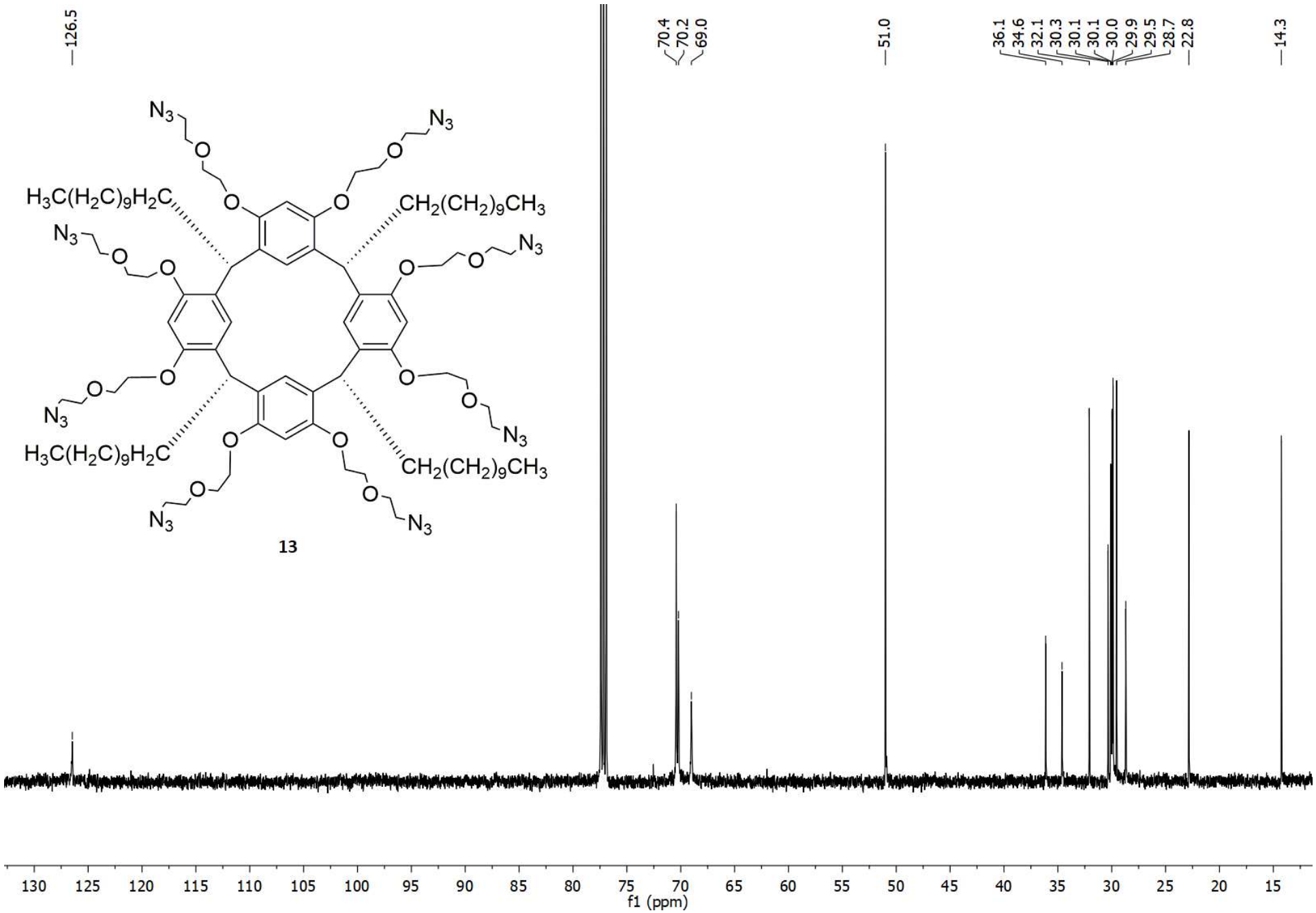
7
1.00
1.16
5.00 4.95 4.90 4.85 4.80 4.75
f1 (ppm)
1.14
1.16
1.10
4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20
f1 (ppm)
2.45
1.18
1.26
3.9 3.8 3.7 3.6 3.5 3.4 3.3
f1 (ppm)
0.86
2.30 2.28 2.26 2.24
f1 (ppm)
3.31
2.12 2.11 2.10 2.09 2.08 2.07 2.06
f1 (ppm)
6.20
1.09
1.00
1.15
1.14
1.16
1.10
2.45
1.18
1.26
0.86
3.31
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2
f1 (ppm)

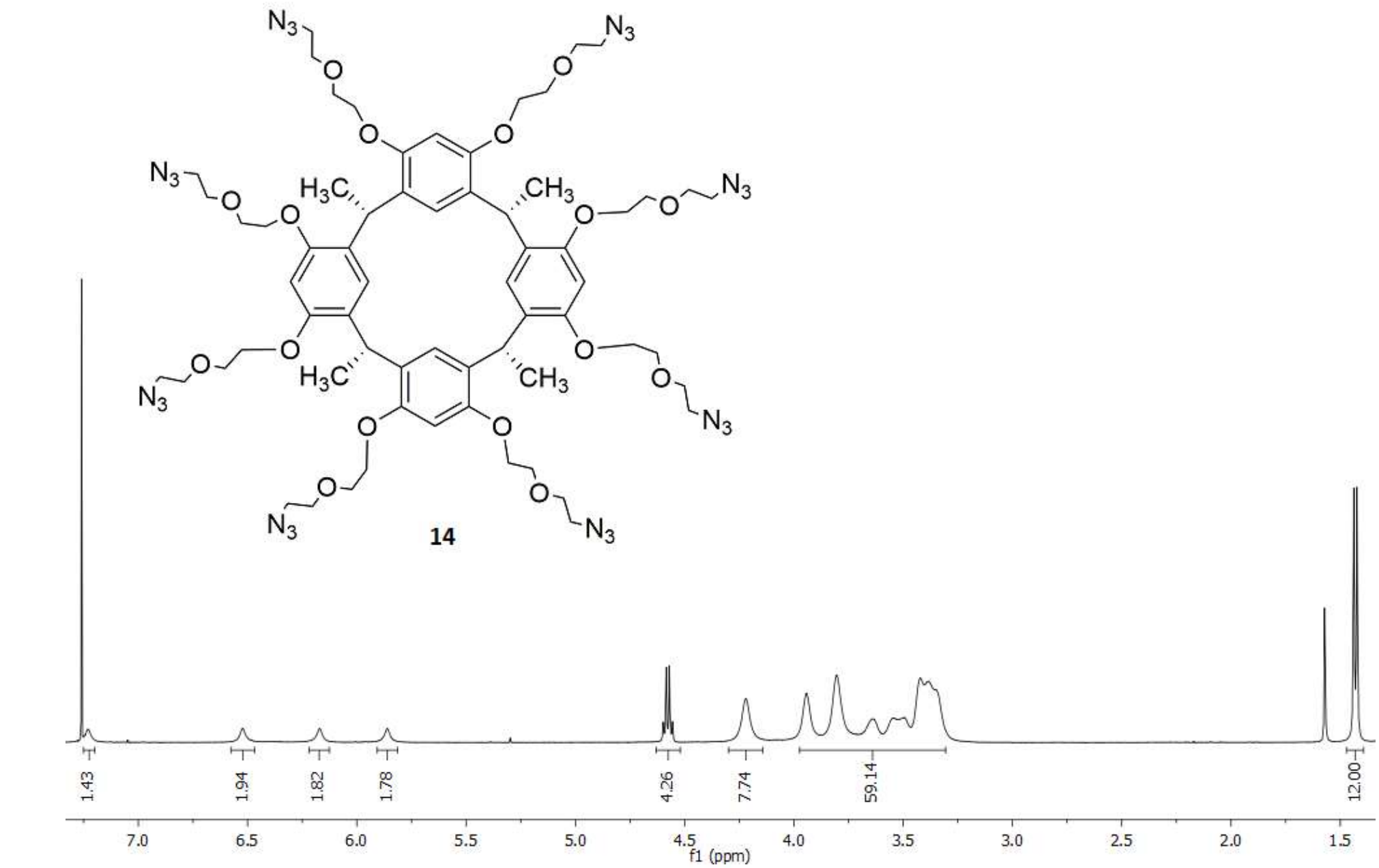

168.1
137.0
129.4
128.5
126.3
102.7
82.1
79.1
75.8
74.6
72.0
69.7
69.0
64.6
18.8
14.3
Ph
O
O
O
S
O
N
7
82.1
79.1
75.8
74.6
72.0
69.7
69.0
64.6
82
80
78
76
74
72
70
68
66
64
f1 (ppm)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

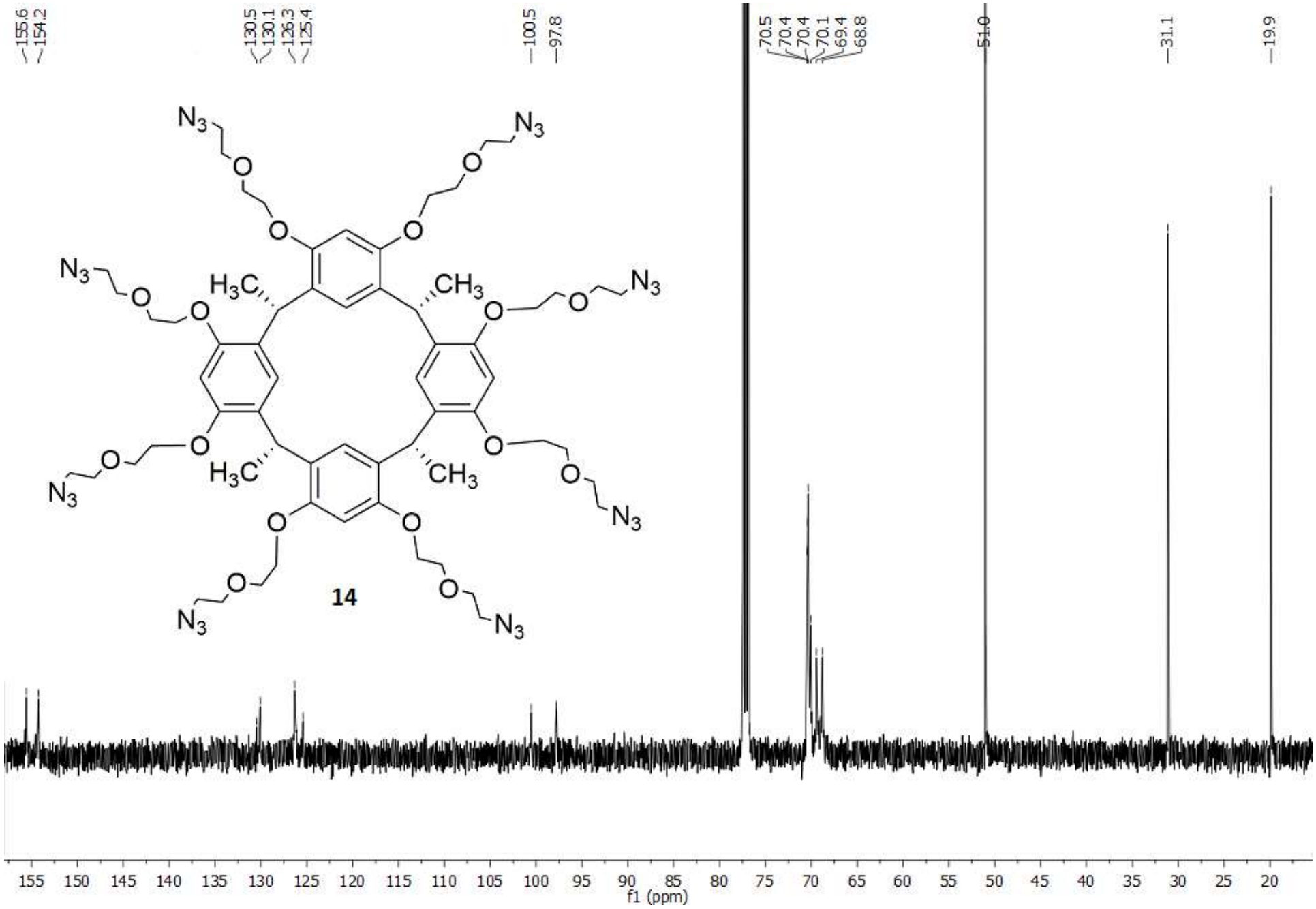

N3
O
H3C(H2C)9H2C
CH2(CH2)9CH3

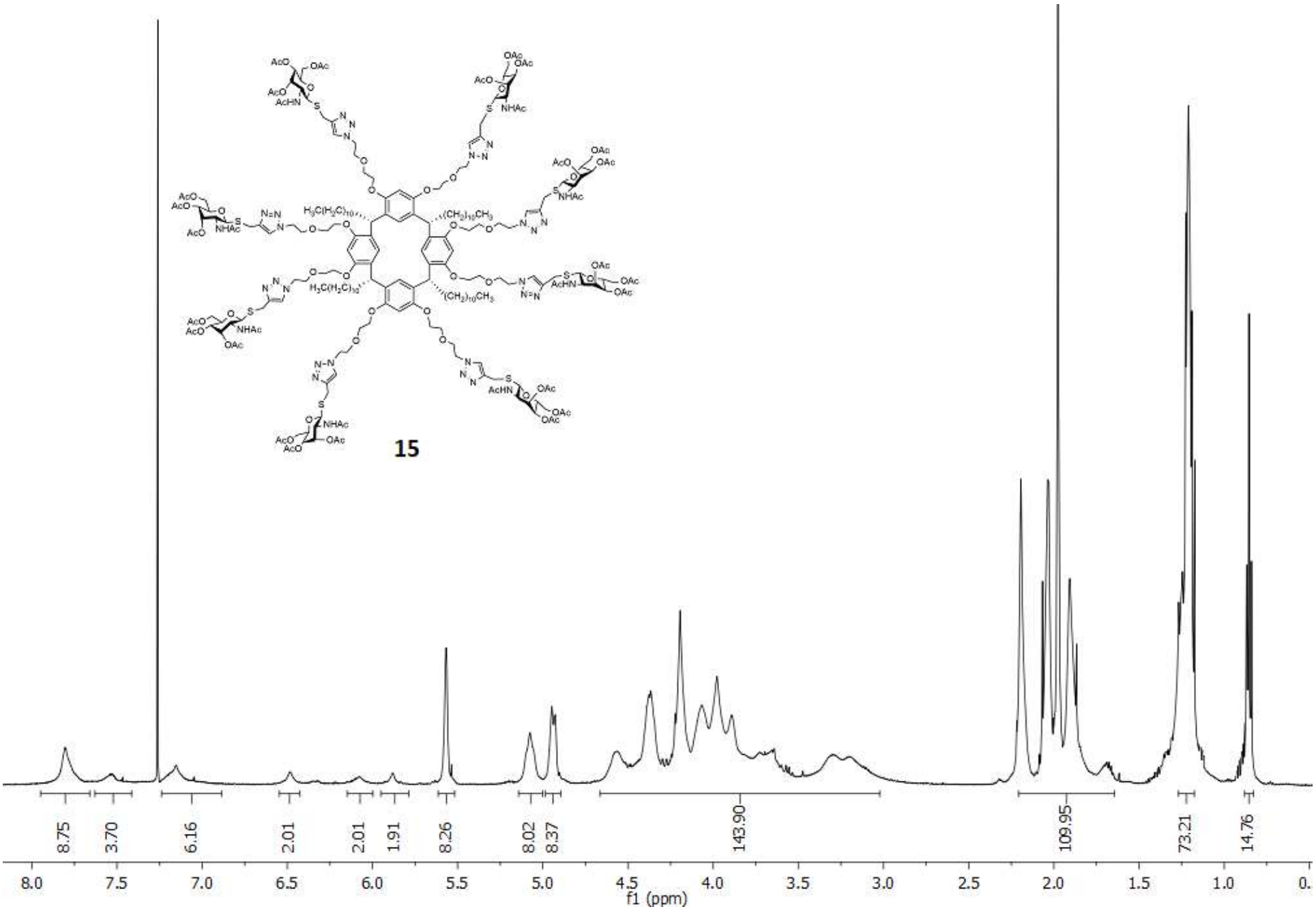
13

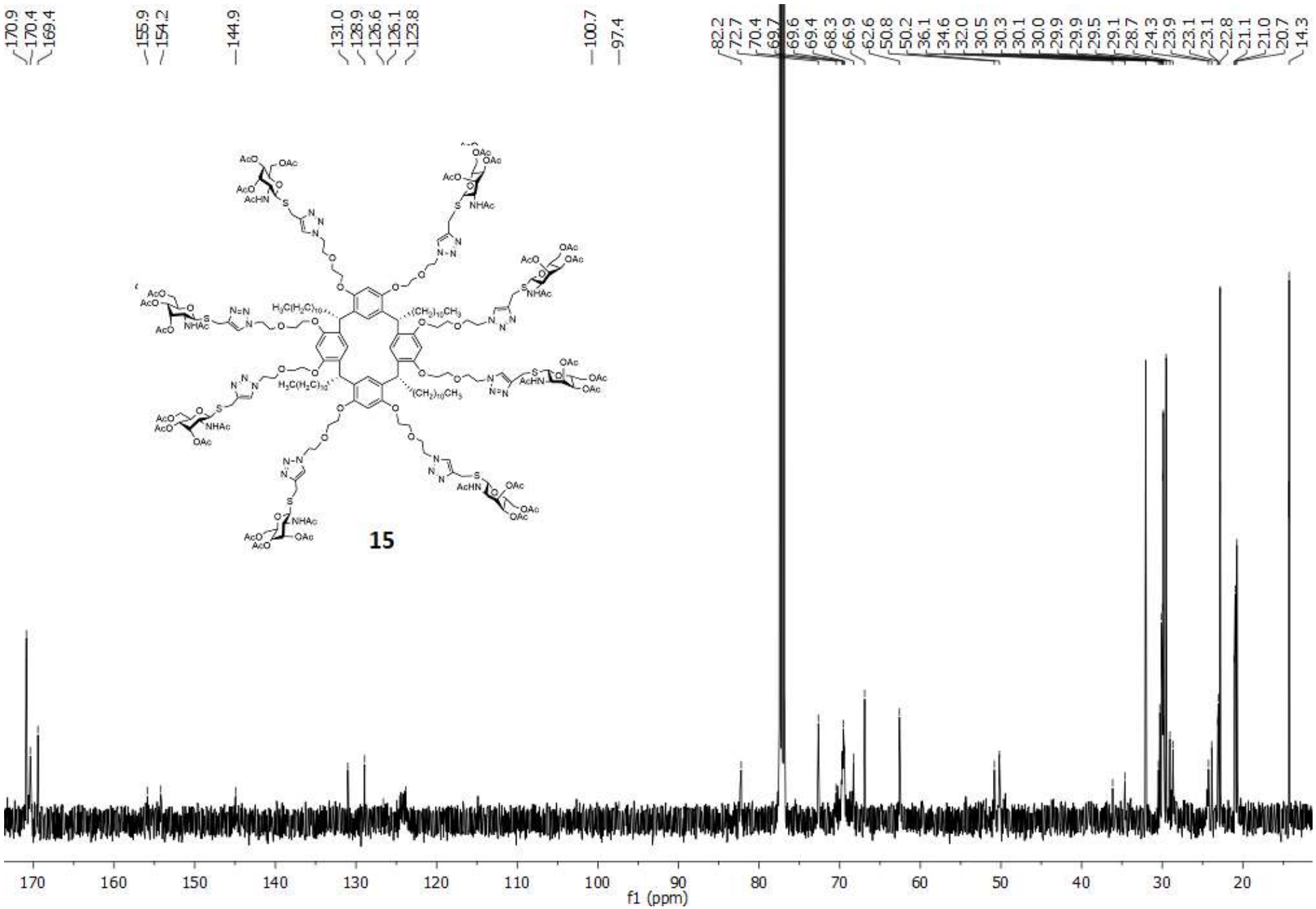
4.00
63.28
7.48
72.71
12.61
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

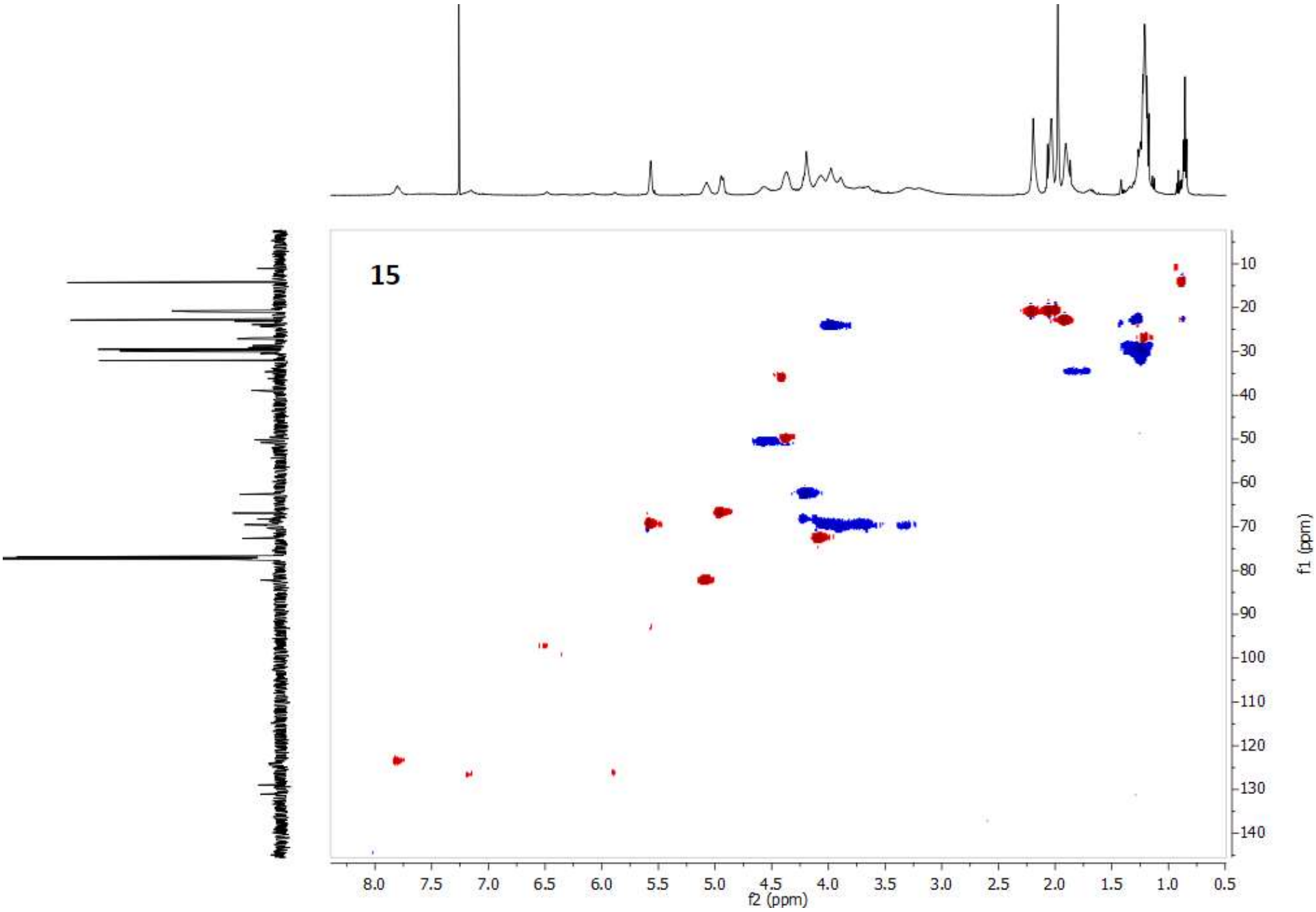

126.5
70.4
70.2
69.0
51.0
36.1
34.6
32.1
30.3
30.1
30.1
30.0
29.9
29.5
28.7
22.8
14.3
N3
H3C(H2C)9H2C
CH2(CH2)9CH3
13
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
f1 (ppm)

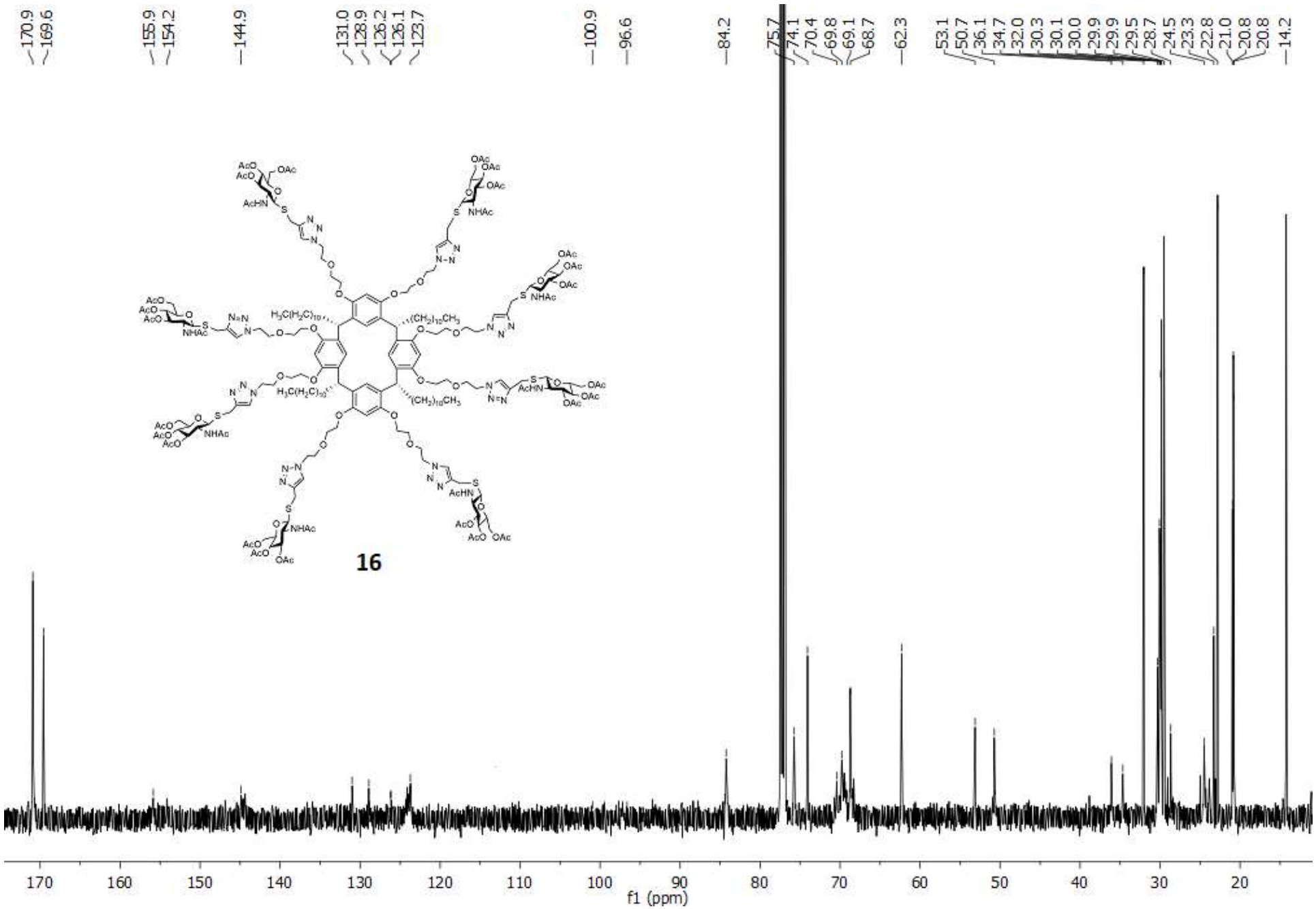

14
1.43
1.94
1.82
1.78
4.26
7.74
59.14
12.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

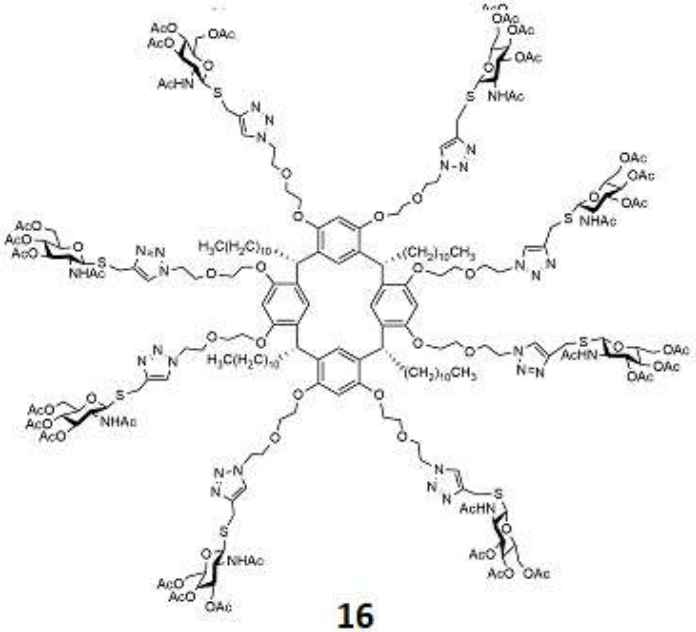

155.6
154.2
130.5
130.1
126.3
125.4
100.5
97.8
70.5
70.4
70.4
70.1
69.4
68.8
51.0
31.1
19.9
14
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)


15
8.75
3.70
6.16
2.01
2.01
1.91
8.26
8.02
8.37
143.90
109.95
73.21
14.76
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.
f1 (ppm)


170.9
170.4
169.4
155.9
154.2
144.9
131.0
128.9
126.6
126.1
123.8
100.7
97.4
82.2
72.7
70.4
69.7
69.6
69.4
68.3
66.9
62.6
50.8
50.2
36.1
34.6
32.0
30.5
30.3
30.1
30.0
29.9
29.9
29.5
29.1
28.7
24.3
23.9
23.1
23.1
22.8
21.1
21.0
20.7
14.3
15
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

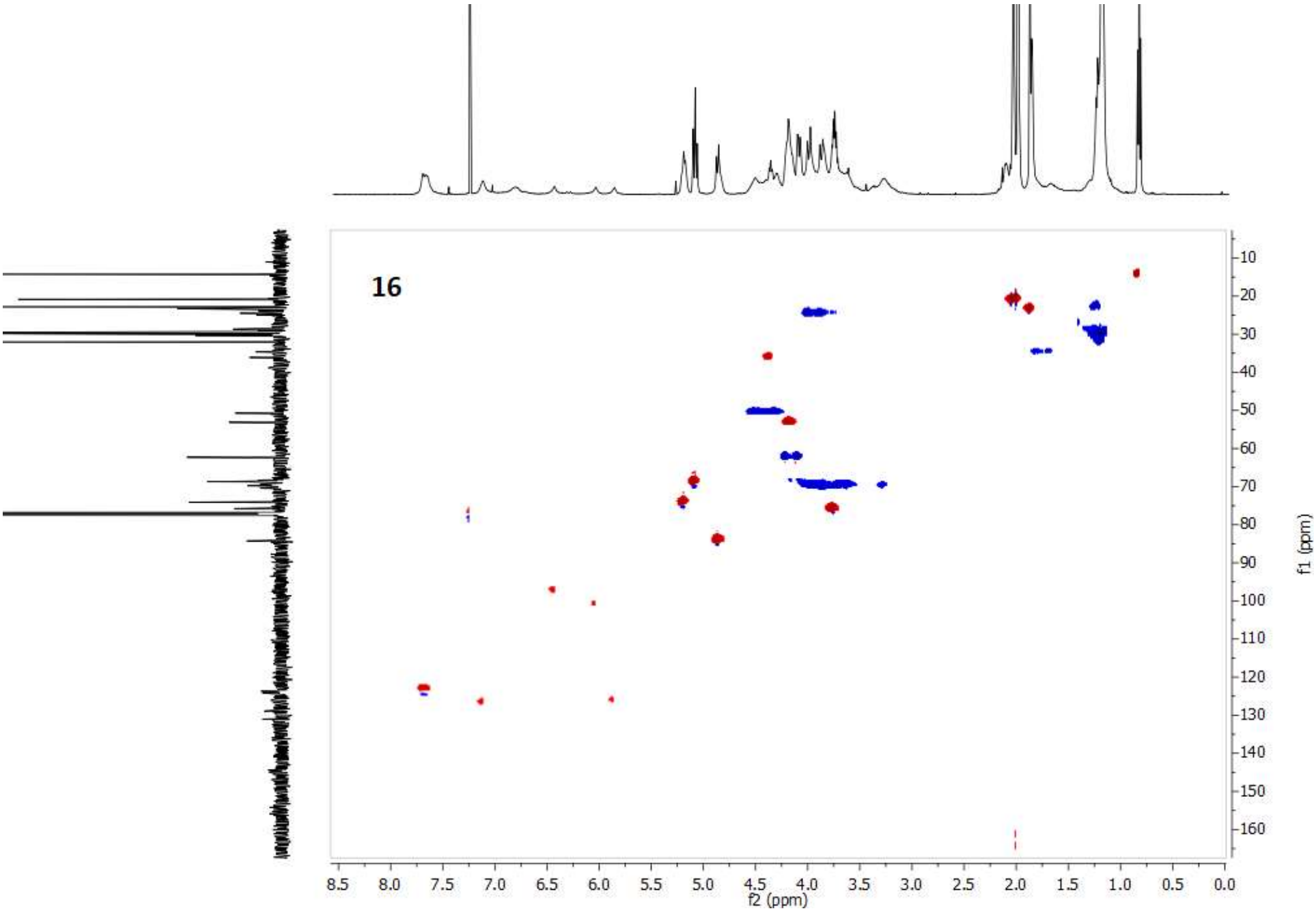

15
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)

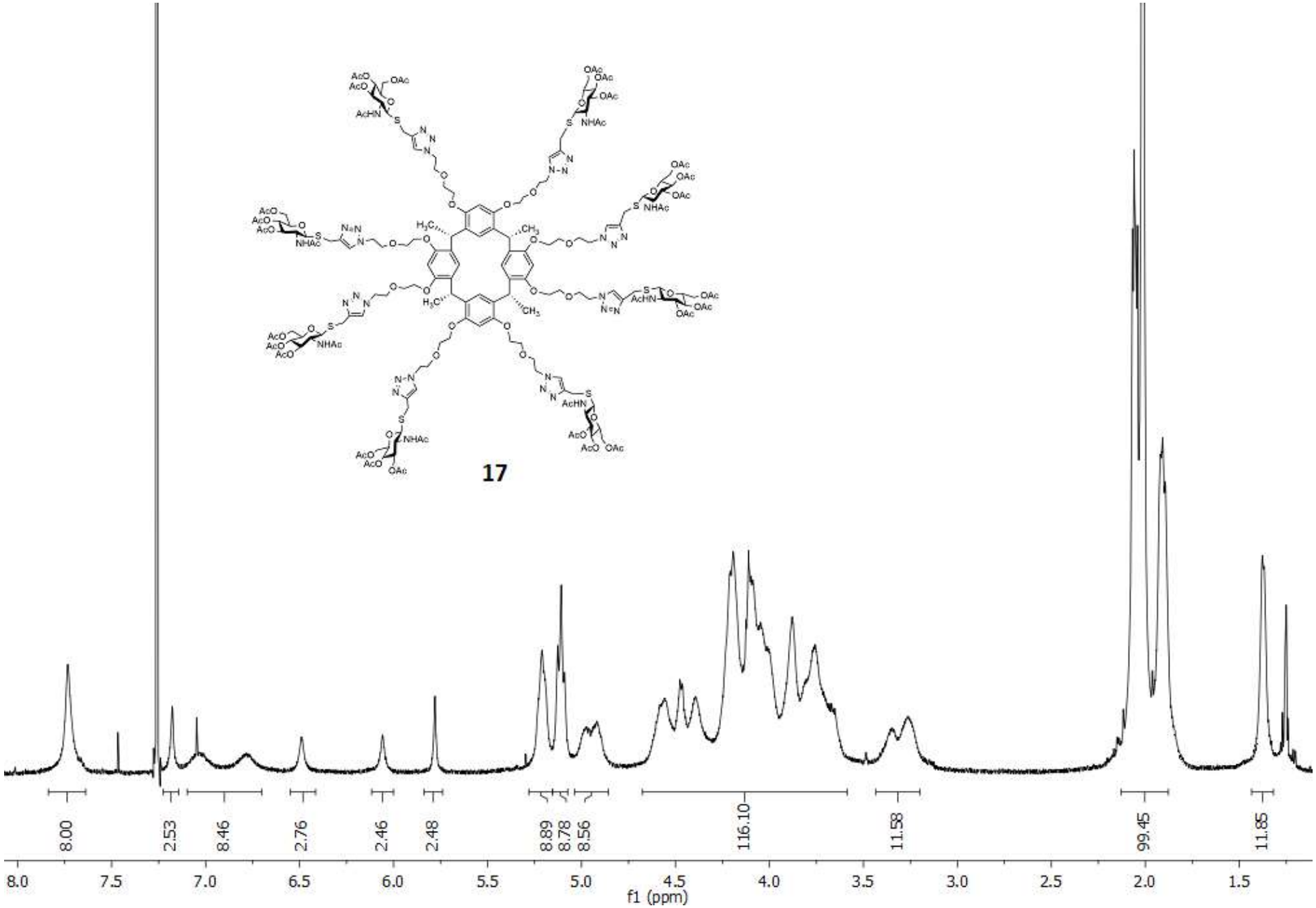

170.9
169.6
155.9
154.2
144.9
131.0
128.9
126.2
126.1
123.7
100.9
96.6
84.2
75.7
74.1
70.4
69.8
69.1
68.7
62.3
53.1
50.7
36.1
34.7
32.0
30.3
30.1
30.0
29.9
29.9
29.5
28.7
24.5
23.3
22.8
21.0
20.8
20.8
14.2
16
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

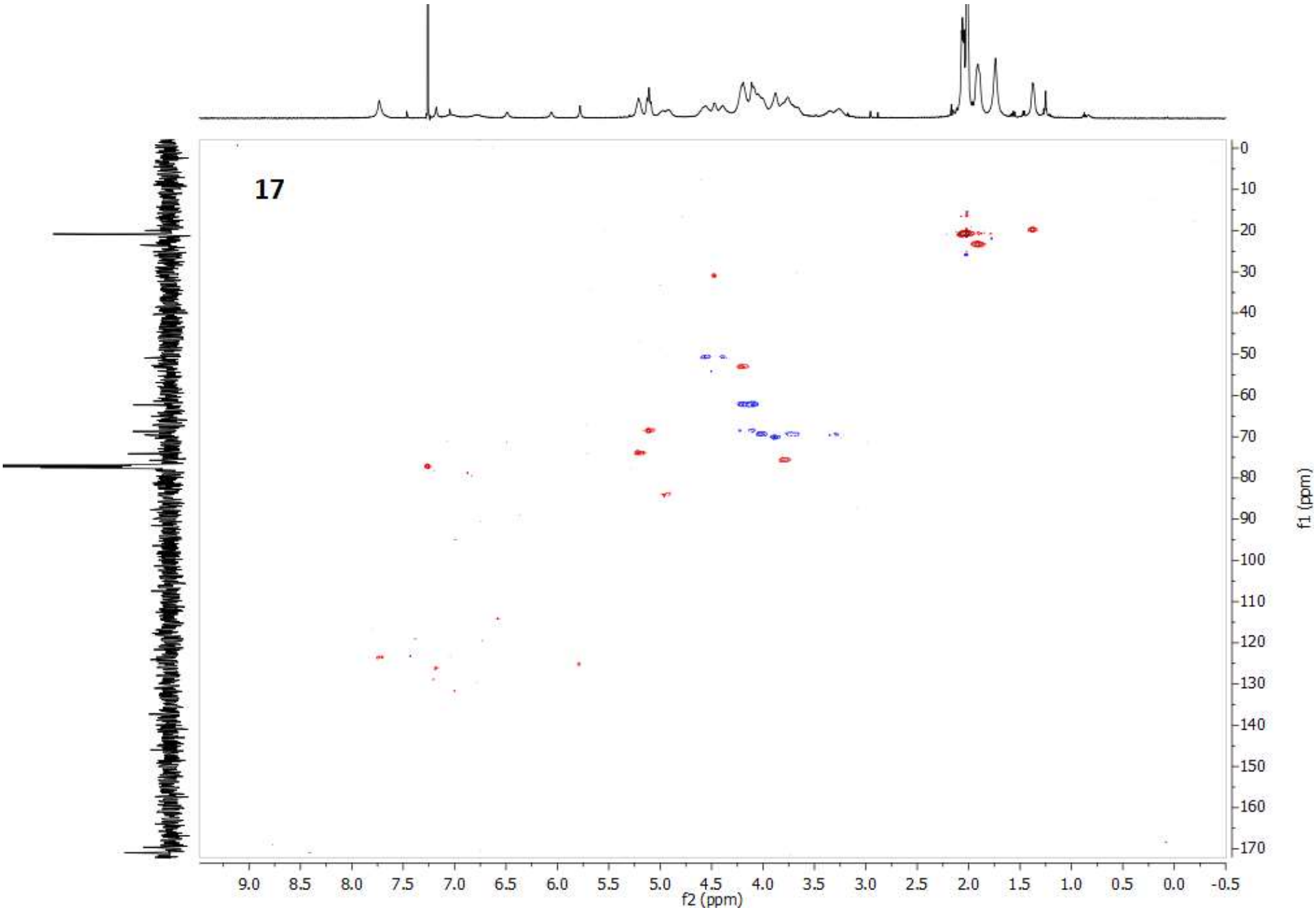


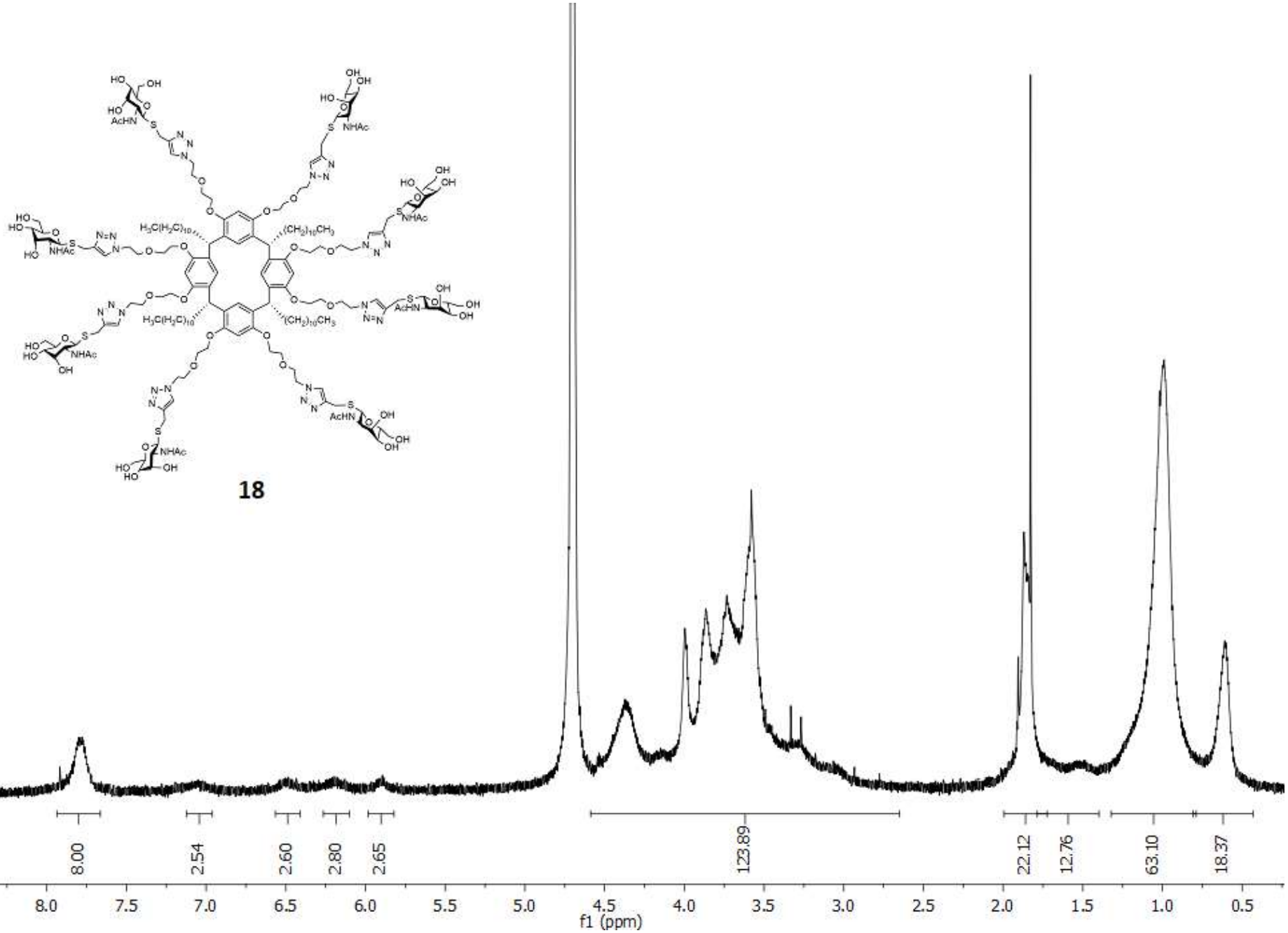
16

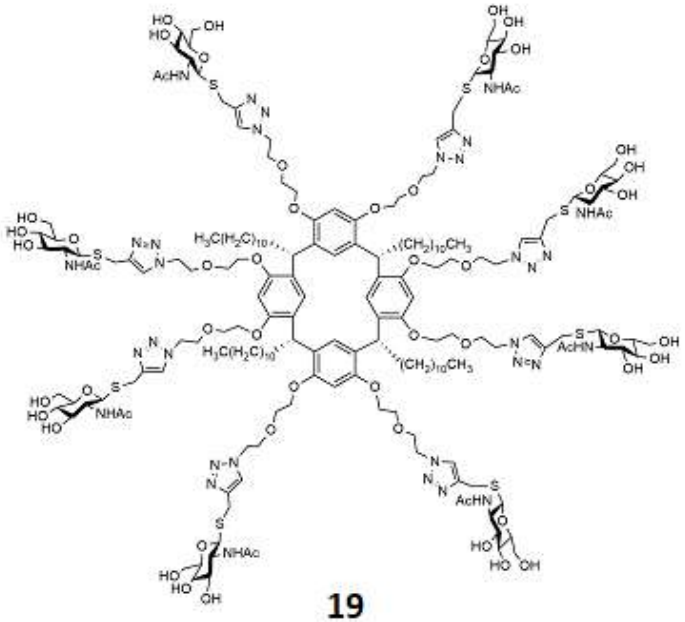
8.49
9.54
2.00
1.64
1.56
8.30
9.04
8.00
124.68
9.31
22.53
50.00
33.24
72.32
14.33
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


16
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
f1 (ppm)


17
8.00
2.53
8.46
2.76
2.46
2.48
8.89
8.78
8.56
116.10
11.58
99.45
11.85
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

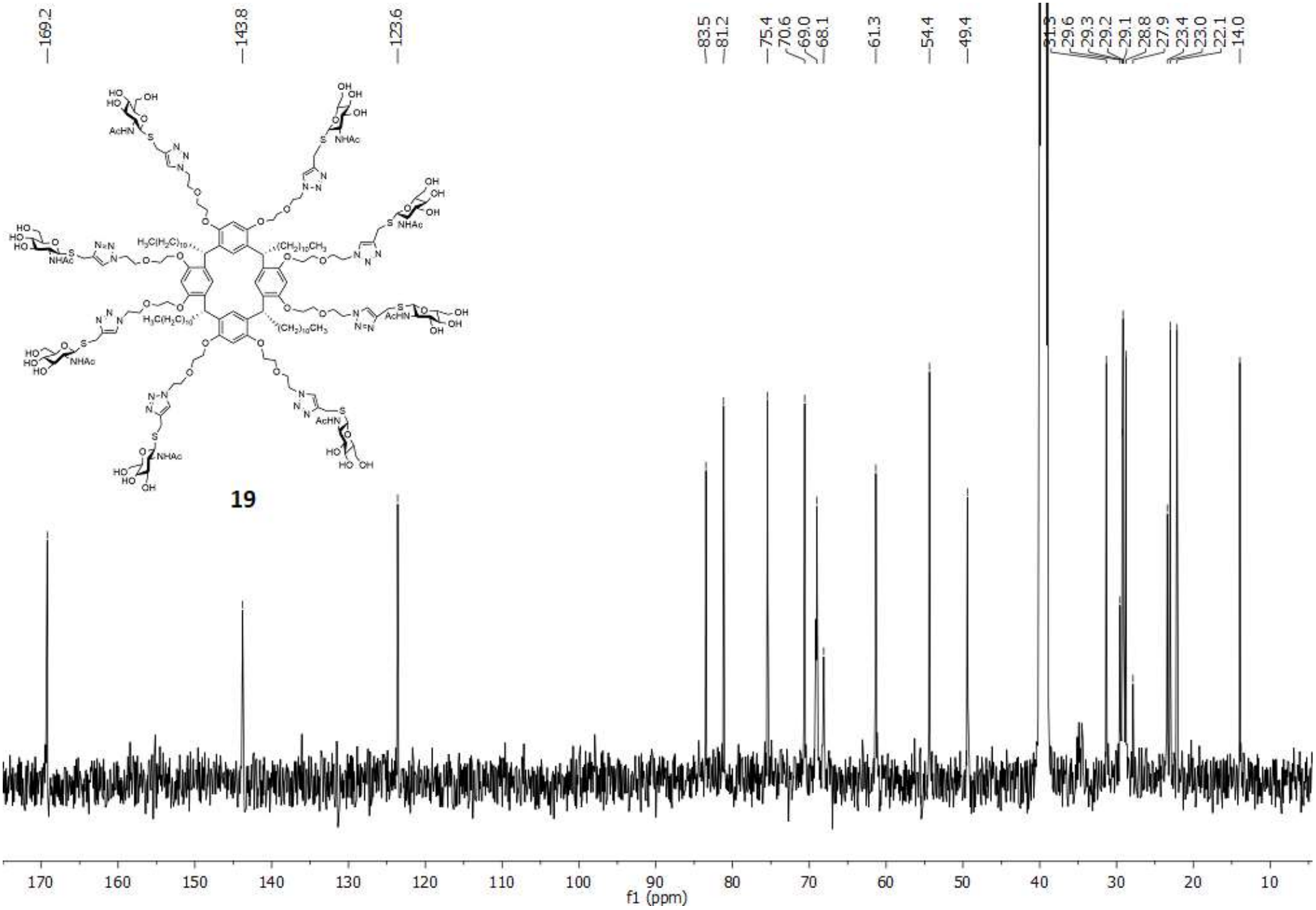

17
f2 (ppm)
f1 (ppm)

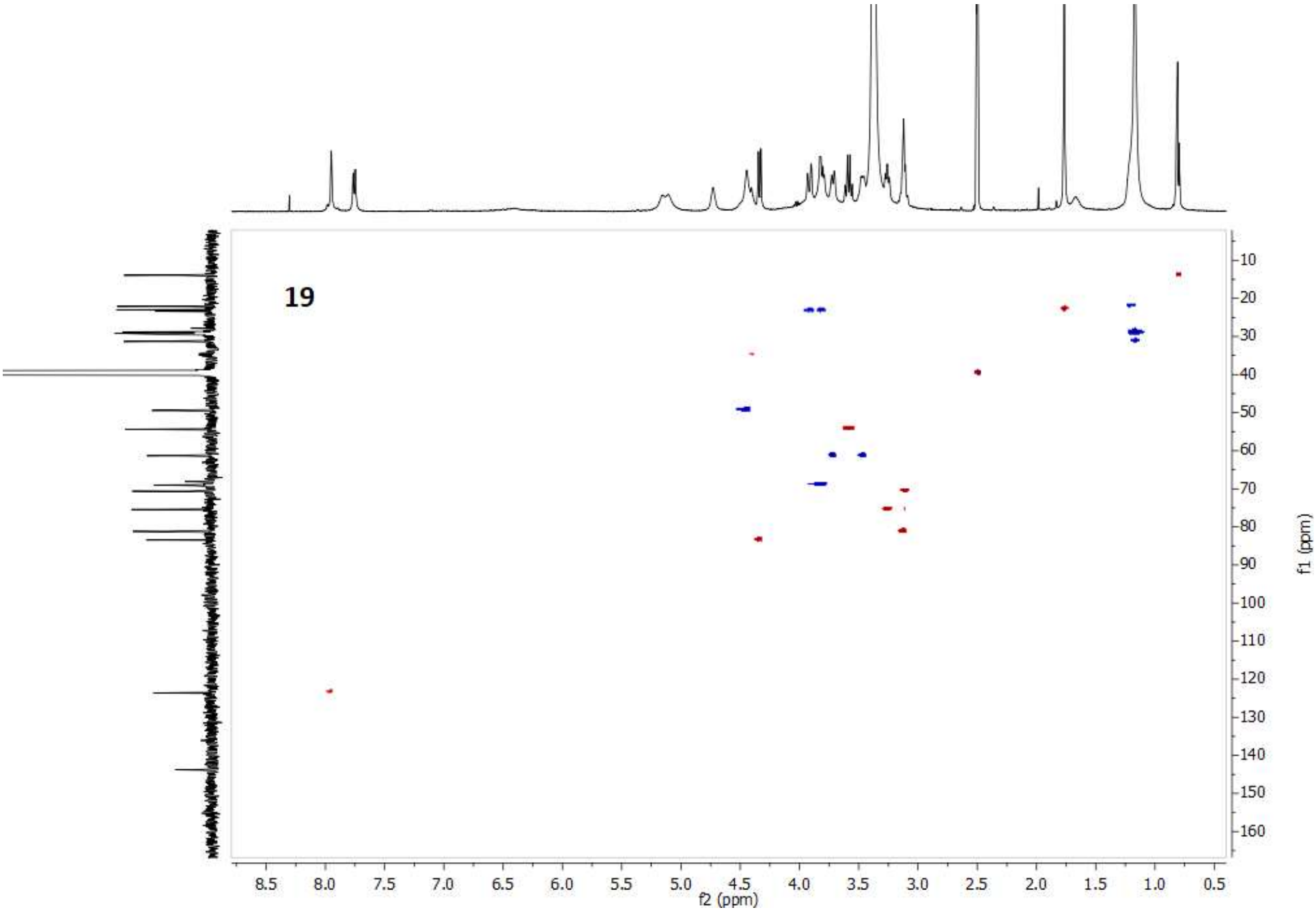

18
8.00
2.54
2.60
2.80
2.65
123.89
22.12
12.76
63.10
18.37
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

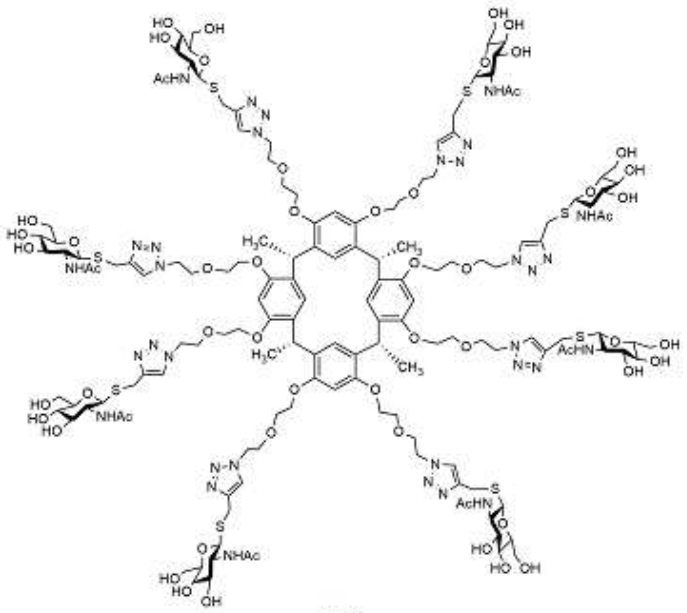


19

7.94
8.00
16.06
8.30
19.47
9.40
12.72
20.62
14.04
13.95
13.49
13.66
20.49
34.53
82.62
14.61
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

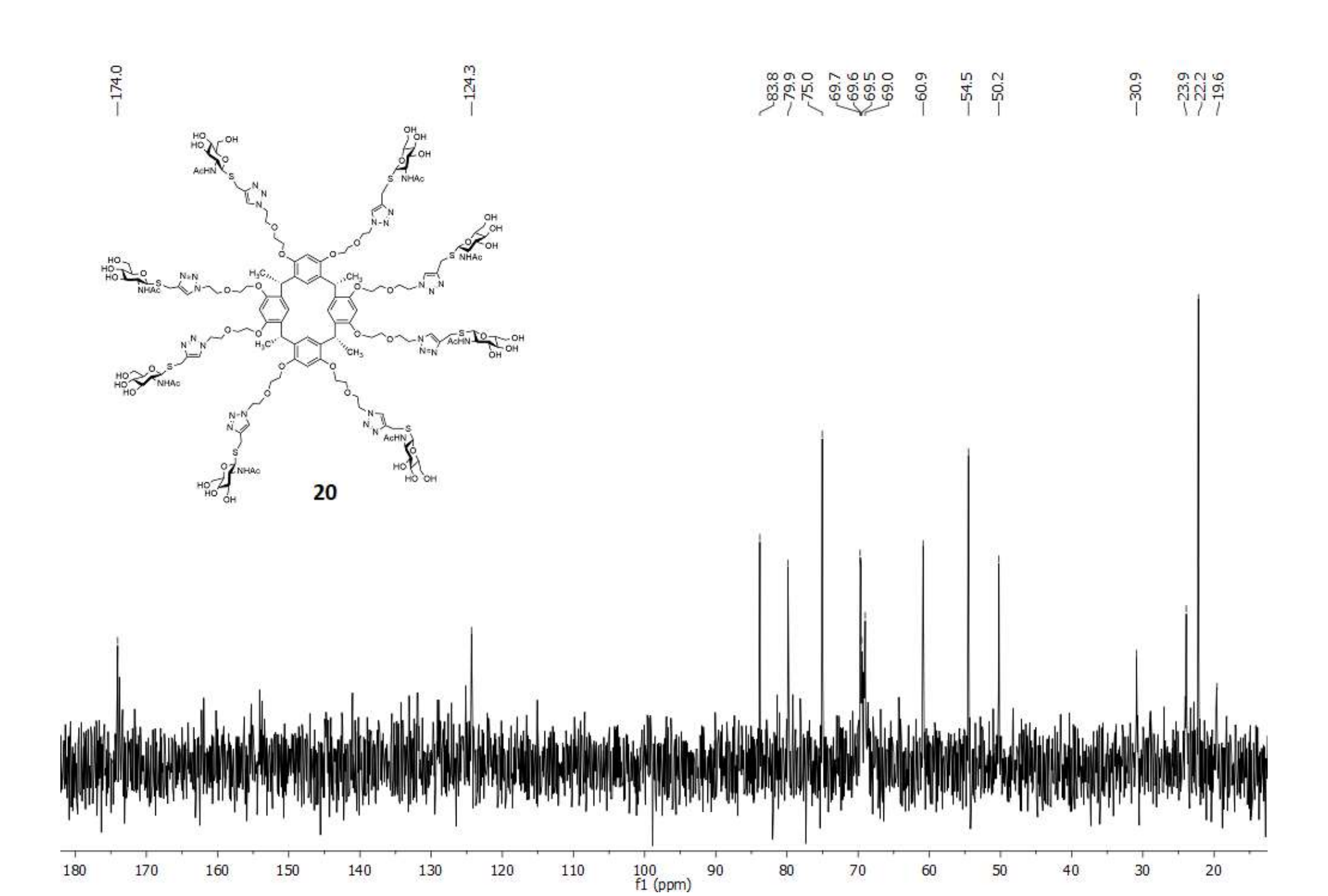

169.2
143.8
123.6
83.5
81.2
75.4
70.6
69.0
68.1
61.3
54.4
49.4
31.3
29.6
29.3
29.2
29.1
28.8
27.9
23.4
23.0
22.1
14.0
19
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

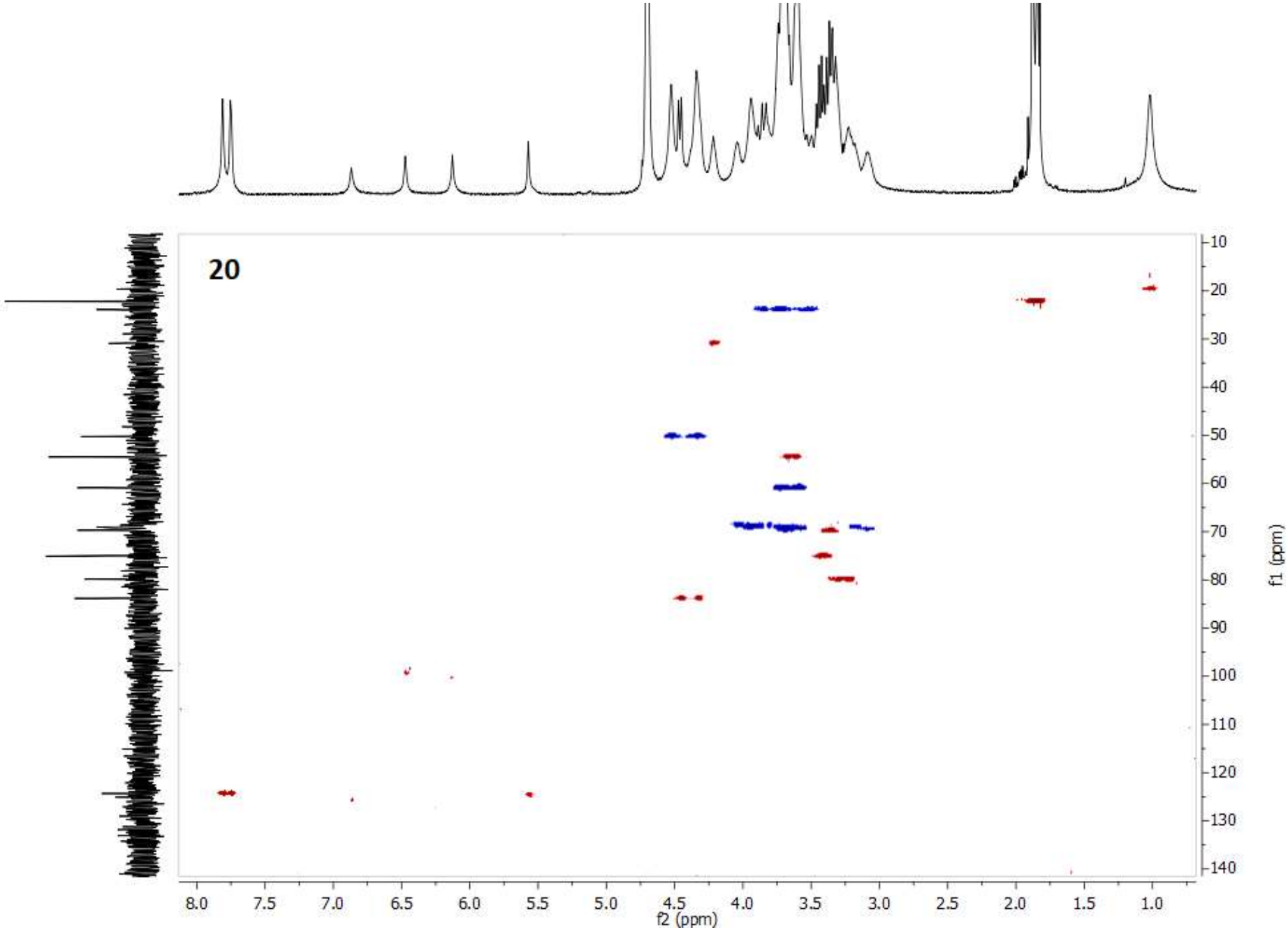

19
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
f1 (ppm)



20

7.35
1.91
2.12
1.99
2.00
134.98
23.21
11.66
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


174.0
124.3
83.8
79.9
75.0
69.7
69.6
69.5
69.0
60.9
54.5
50.2
30.9
23.9
22.2
19.6
20
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


20
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)