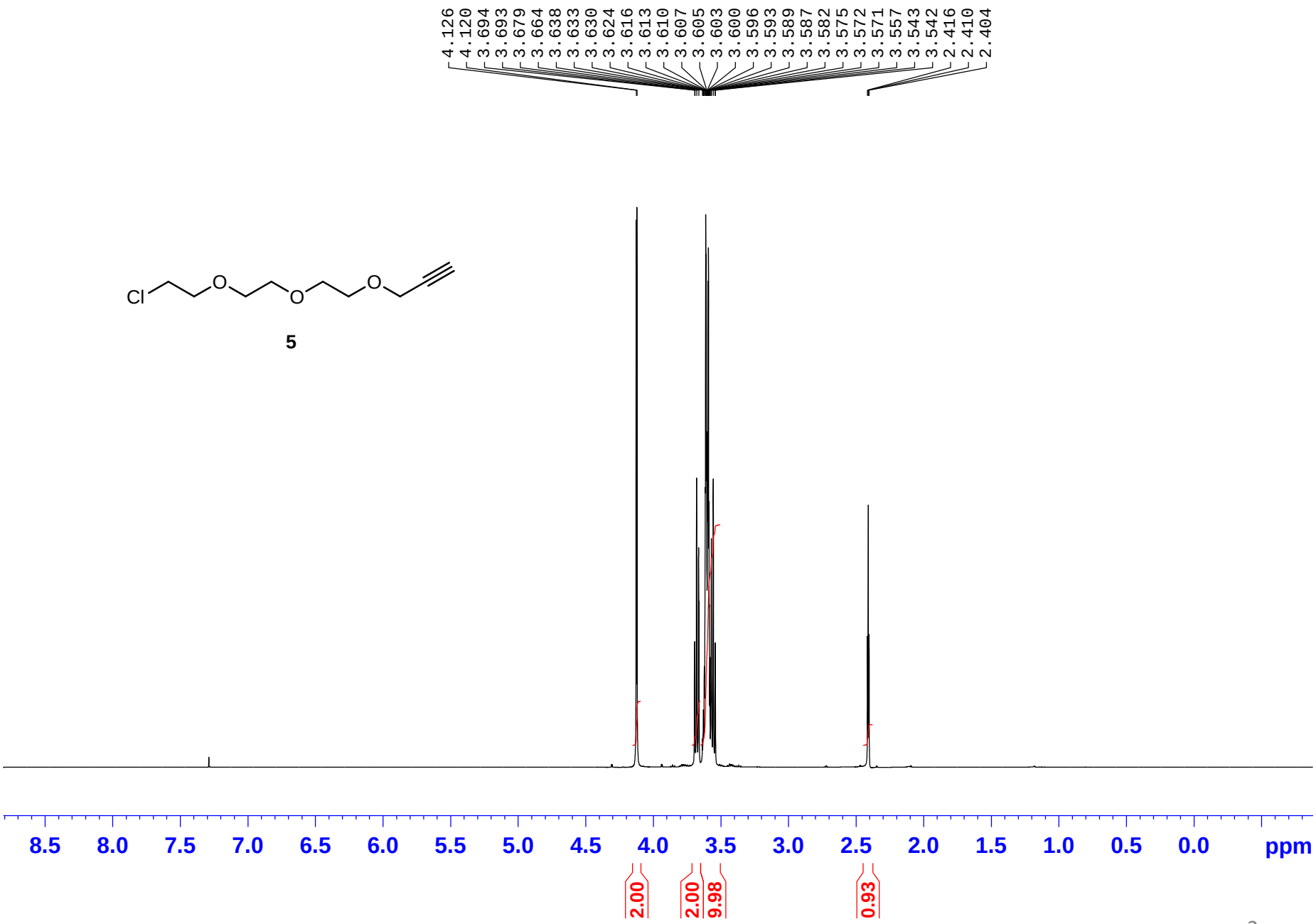
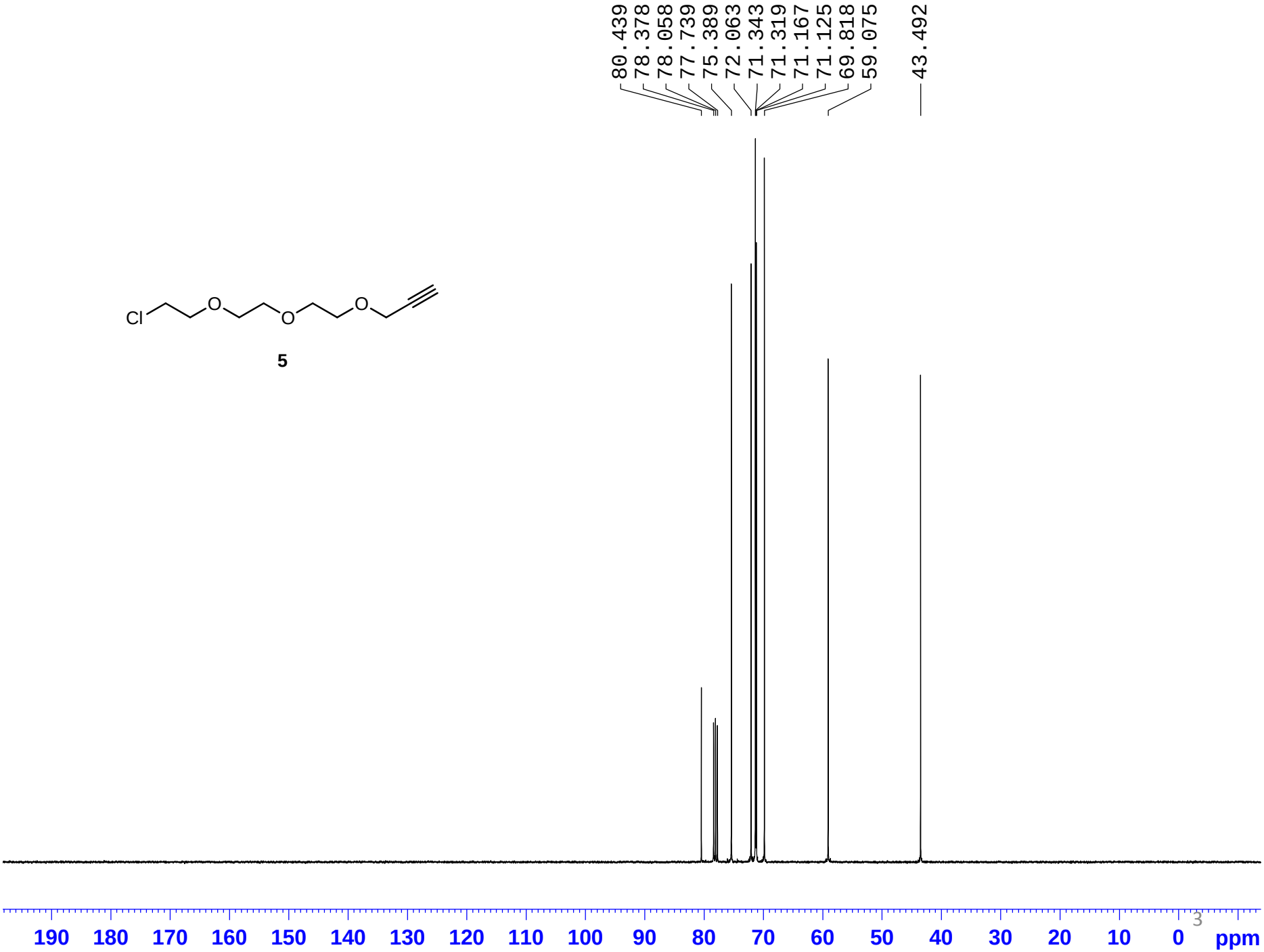
Synthesis and Relaxivity Studies of a DOTA-based Nanomolecular Chelator Assembly Supported by an Icosahedral *Closo*-B_{12}^{2-} -Core for MRI: A Click Chemistry Approach

Lalit N. Goswami, Lixin Ma, Peter J. Kueffer, Satish S. Jalisatgi and M. Frederick Hawthorne*
International Institute of Nano and Molecular Medicine, School of Medicine, University of Missouri, Columbia, Missouri 65211-3450
*Author to whom correspondence should be address; Email: hawthornem@missouri.edu;
Tel.: 001-573-882-7016; Fax: 001-573-884-6900.

Supporting Information (SI)

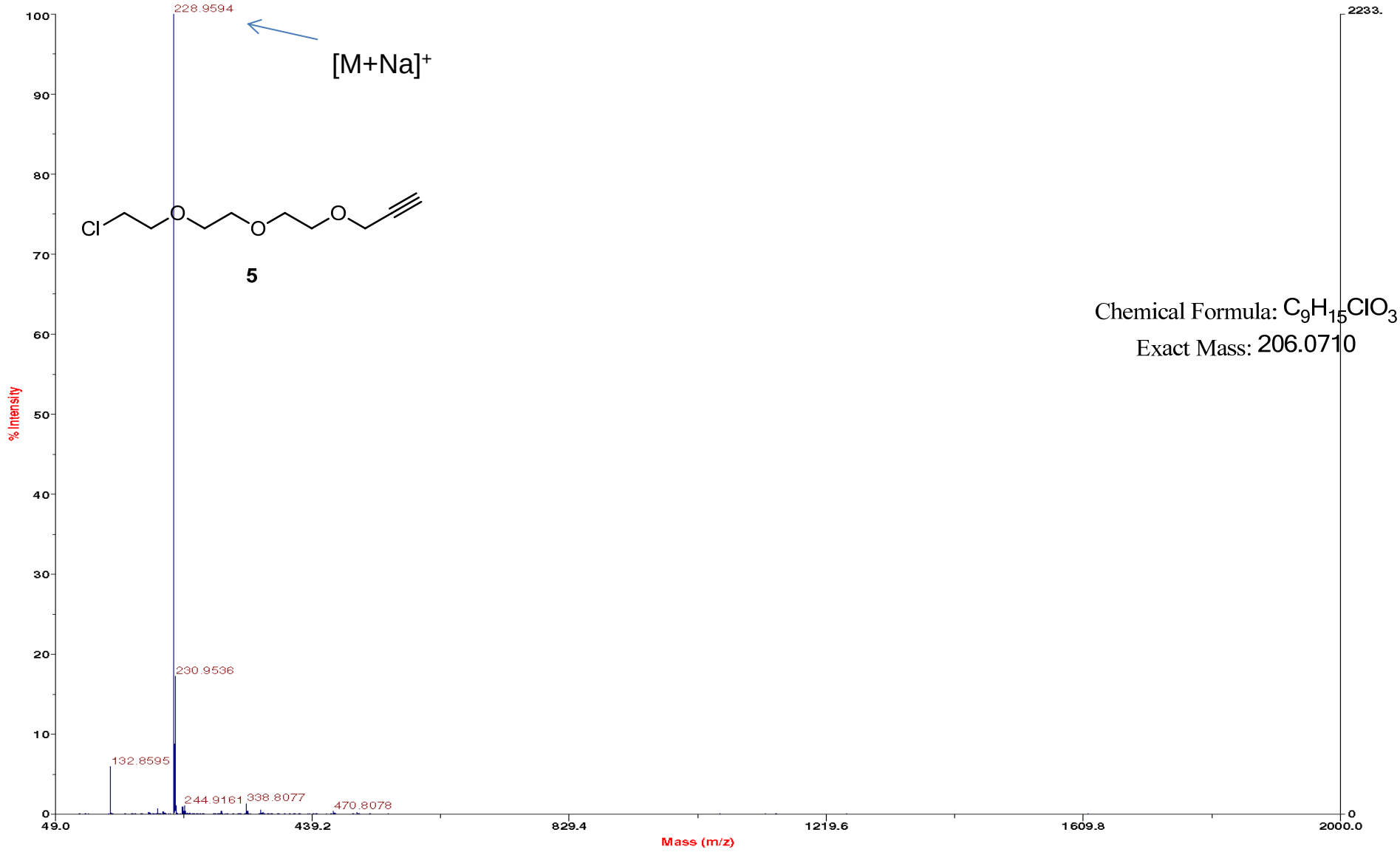

4.126
4.120
3.694
3.693
3.679
3.664
3.638
3.633
3.630
3.624
3.616
3.613
3.610
3.607
3.605
3.603
3.600
3.596
3.593
3.589
3.587
3.582
3.575
3.572
3.571
3.557
3.543
3.542
2.416
2.410
2.404
Cl
O
O
O
5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
2.00
2.00
9.98
0.93


80.439
78.378
78.058
77.739
75.389
72.063
71.343
71.319
71.167
71.125
69.818
59.075
43.492
Cl
O
O
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

5

Applied Biosystems Mariner System 5268

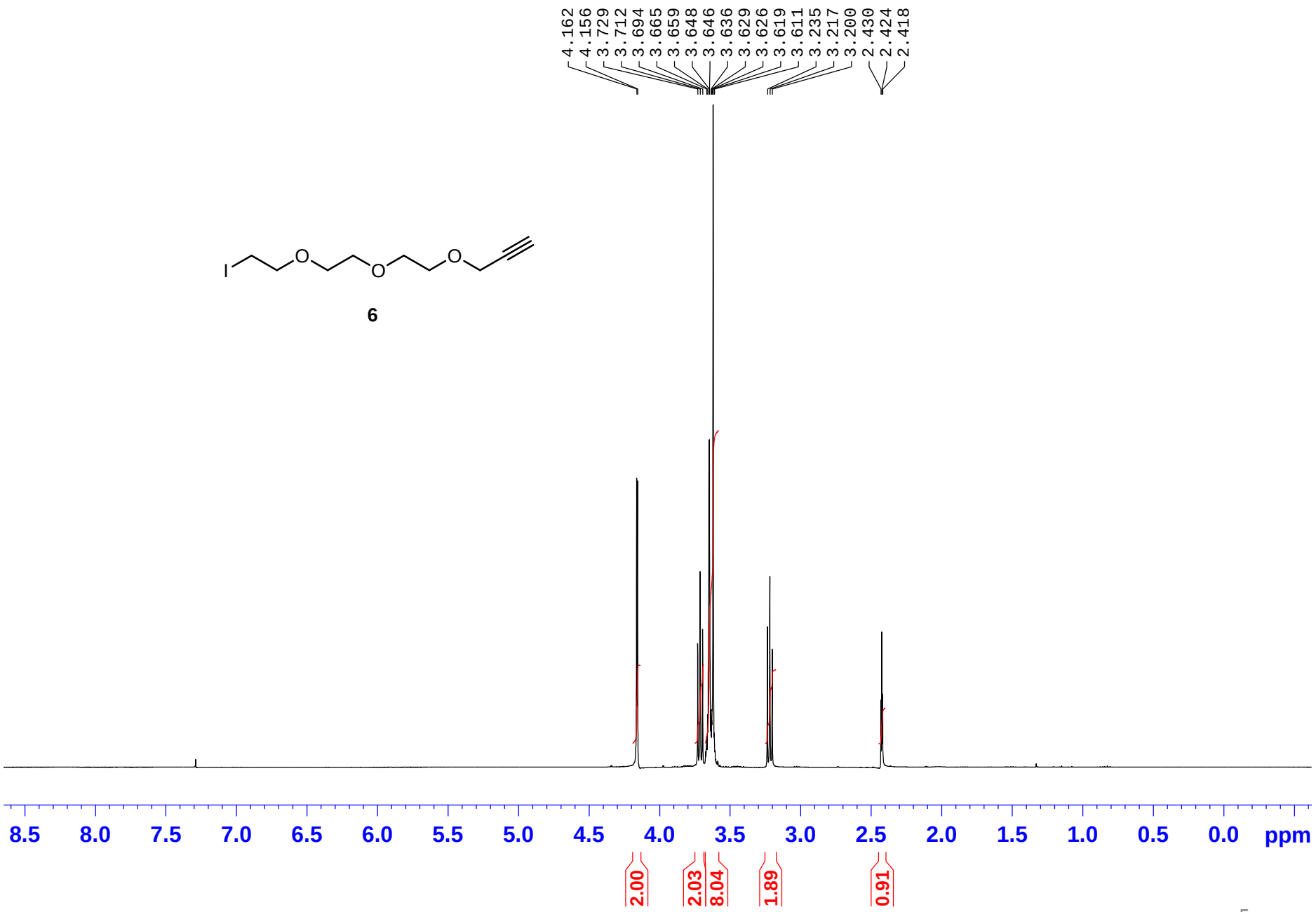
Mariner Spec /1:32 ASC[BP = 229.0, 2233]

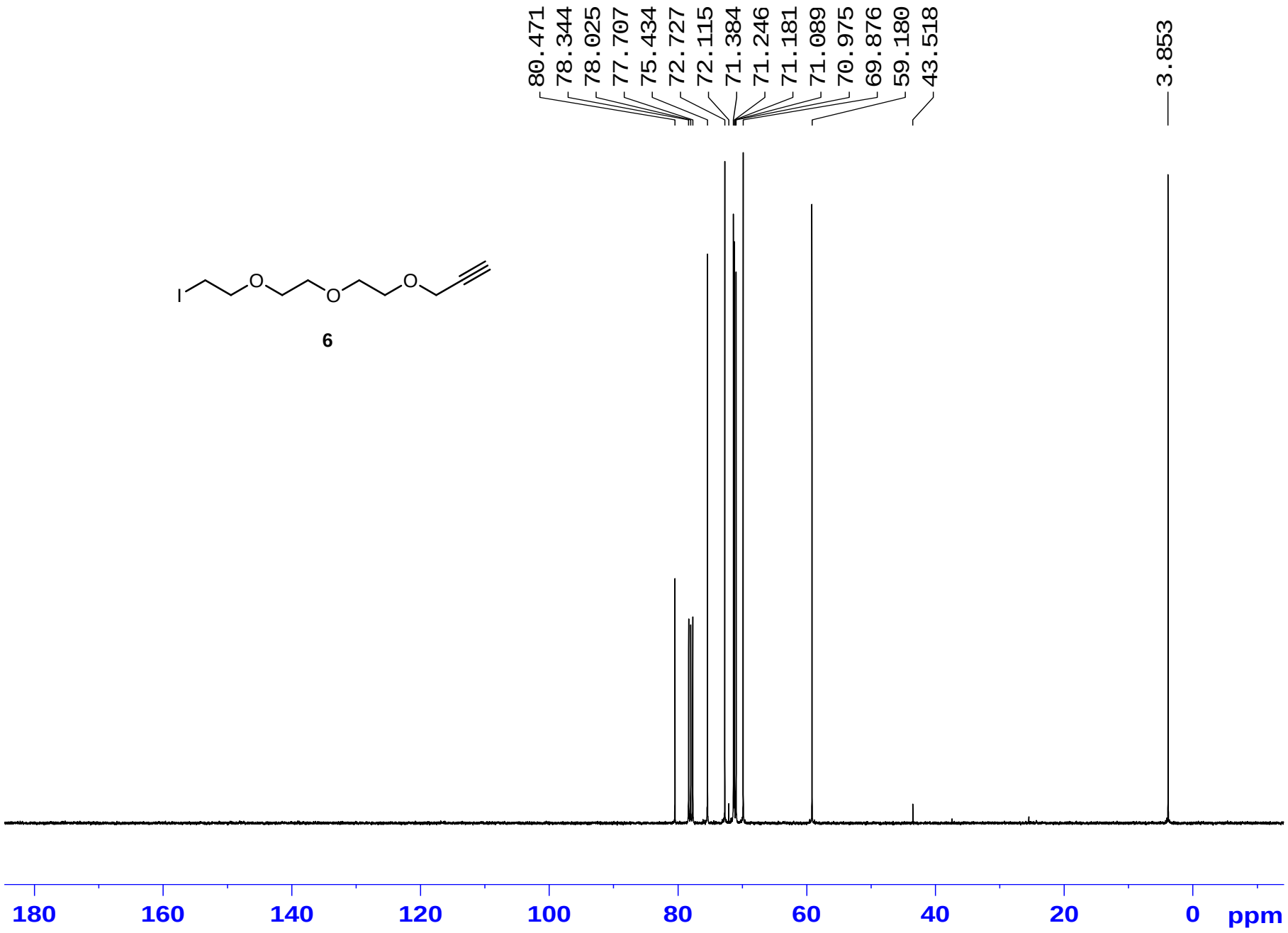


Chemical Formula: $C_9H_{15}ClO_3$

Exact Mass: 206.0710

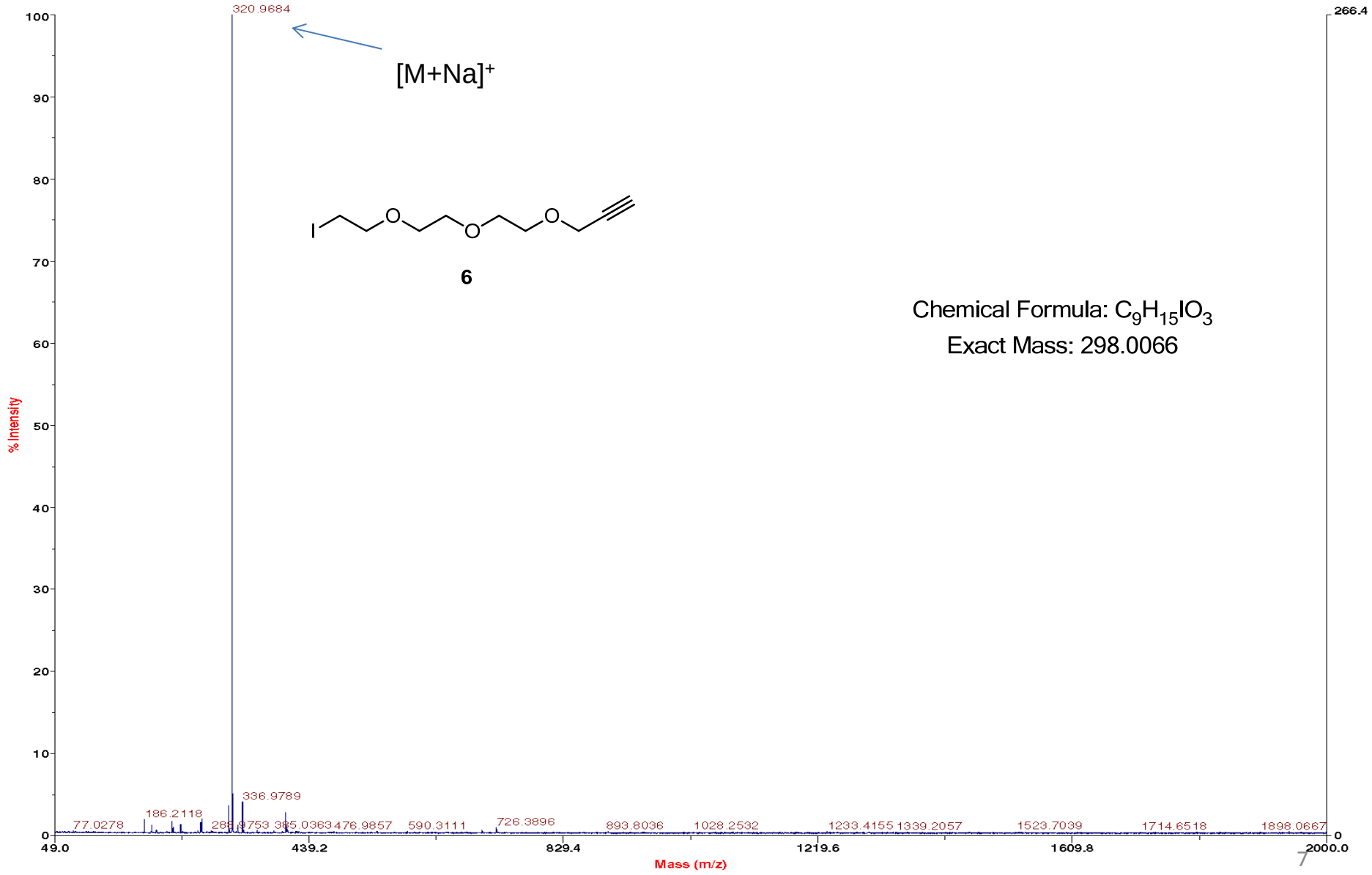
Acquired: Apr 01 15:06:00 2010
Mariner Mass Spectrum
C:\Mariner\Data\2010\Apr\01 Thur\LNG-508M003.dat


4.162
4.156
3.729
3.712
3.694
3.665
3.659
3.648
3.646
3.636
3.629
3.626
3.619
3.611
3.235
3.217
3.200
2.430
2.424
2.418
I
O
O
O
6
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
ppm
2.00
2.03
8.04
1.89
0.91


80.471
78.344
78.025
77.707
75.434
72.727
72.115
71.384
71.246
71.181
71.089
70.975
69.876
59.180
43.518
3.853
I
O
O
O
6
180
160
140
120
100
80
60
40
20
0
ppm

Applied Biosystems Mariner System 5268

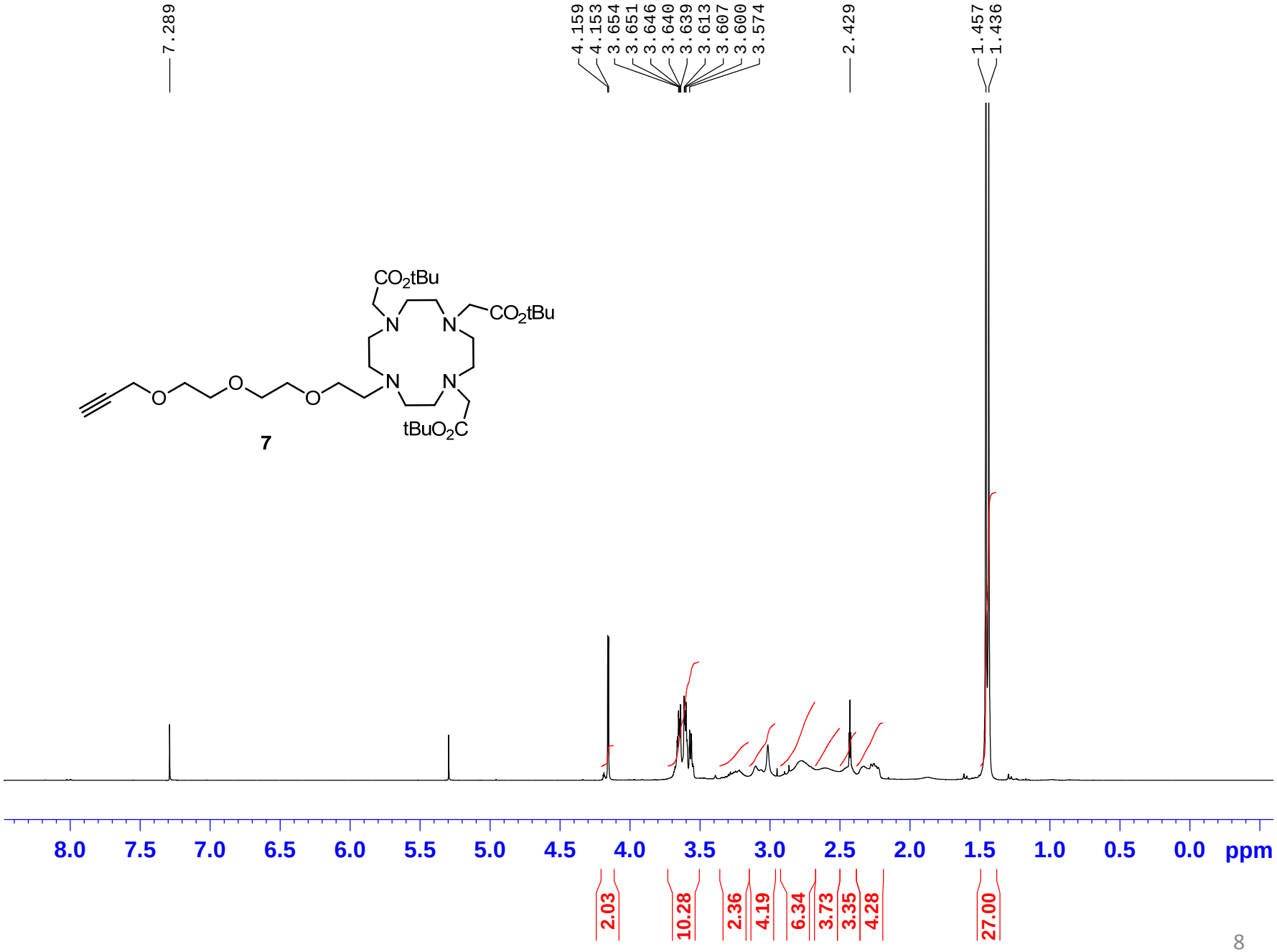
Mariner Spec /1:32 ASC[BP = 321.0, 266]

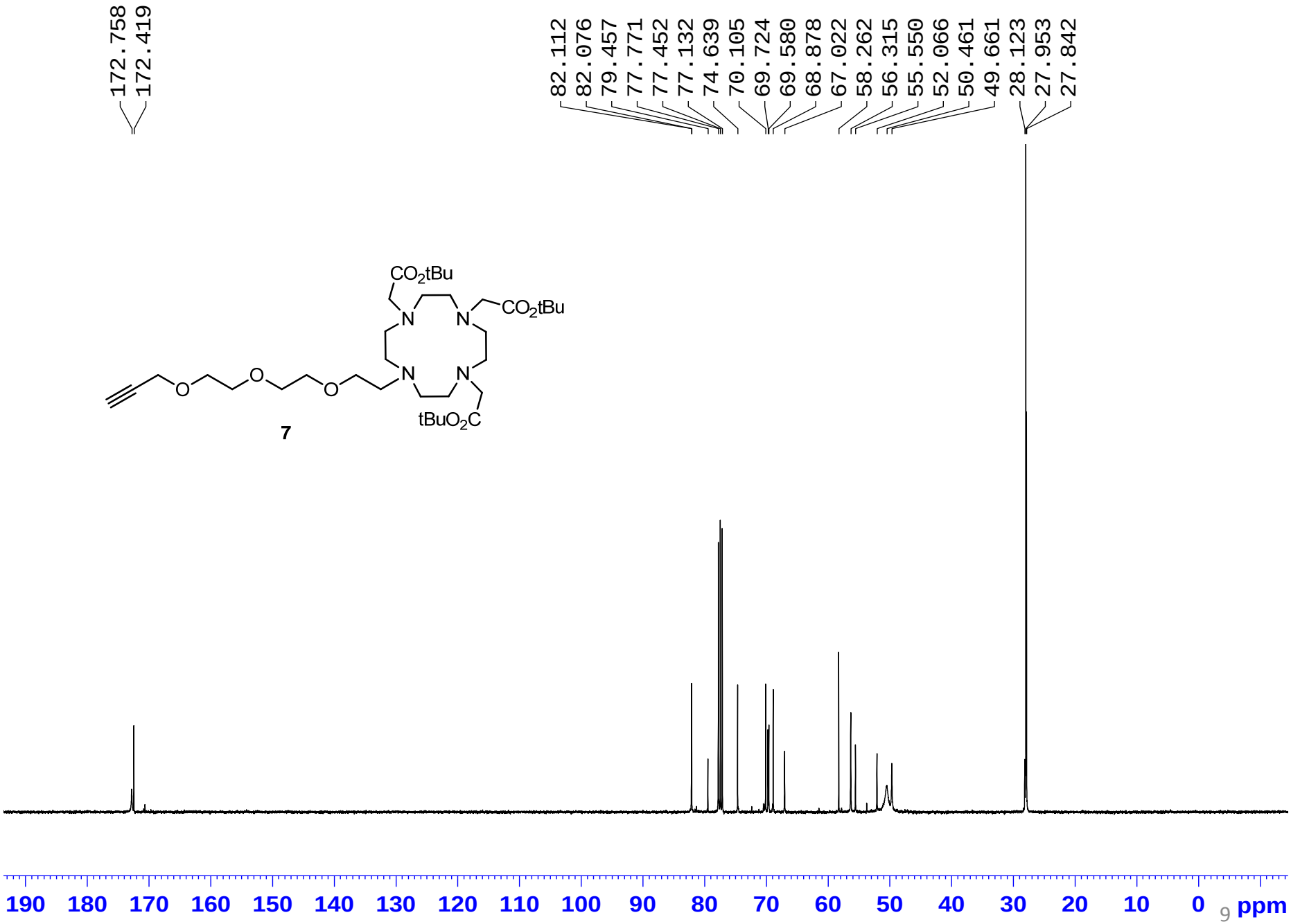


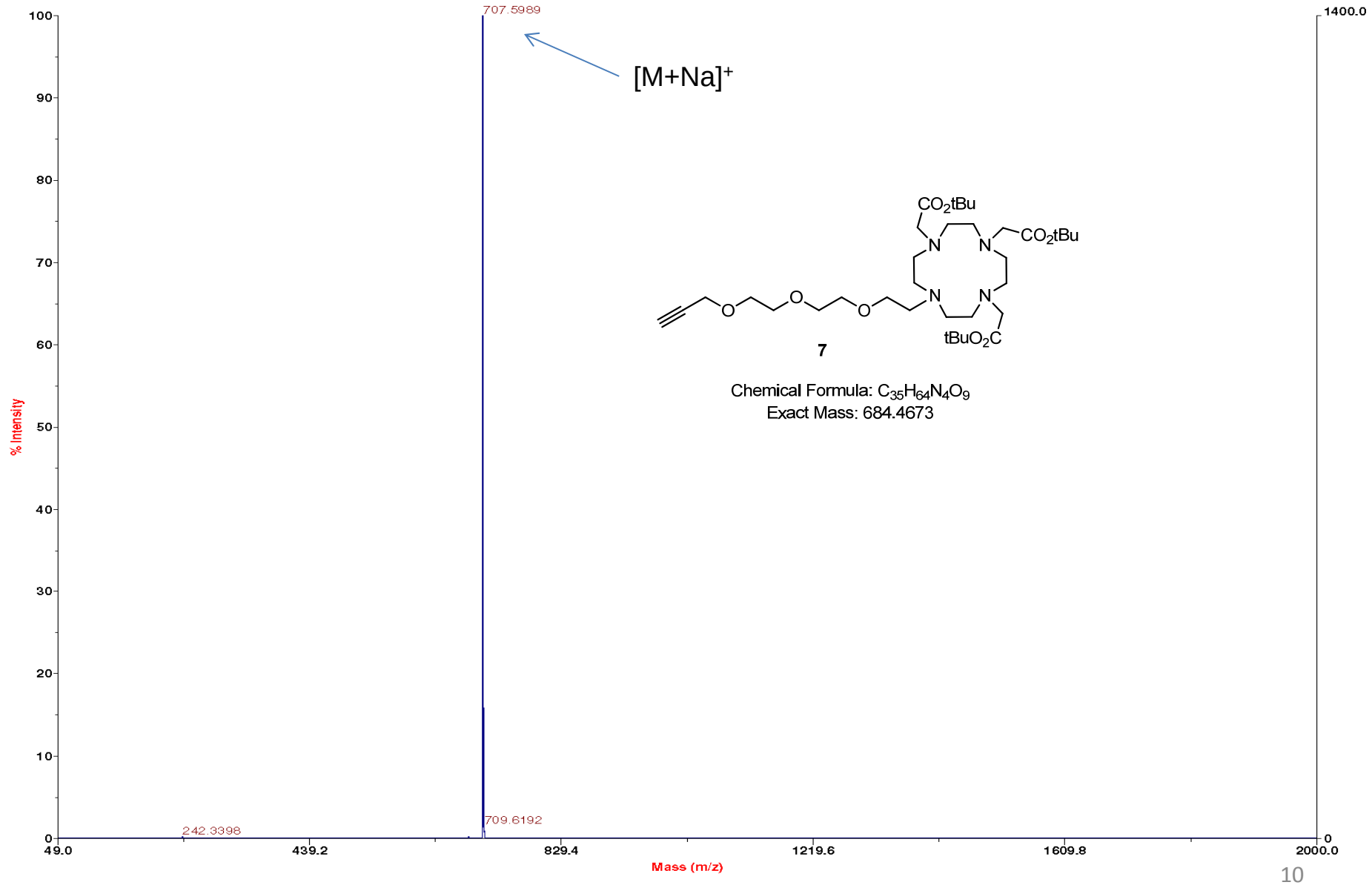
6

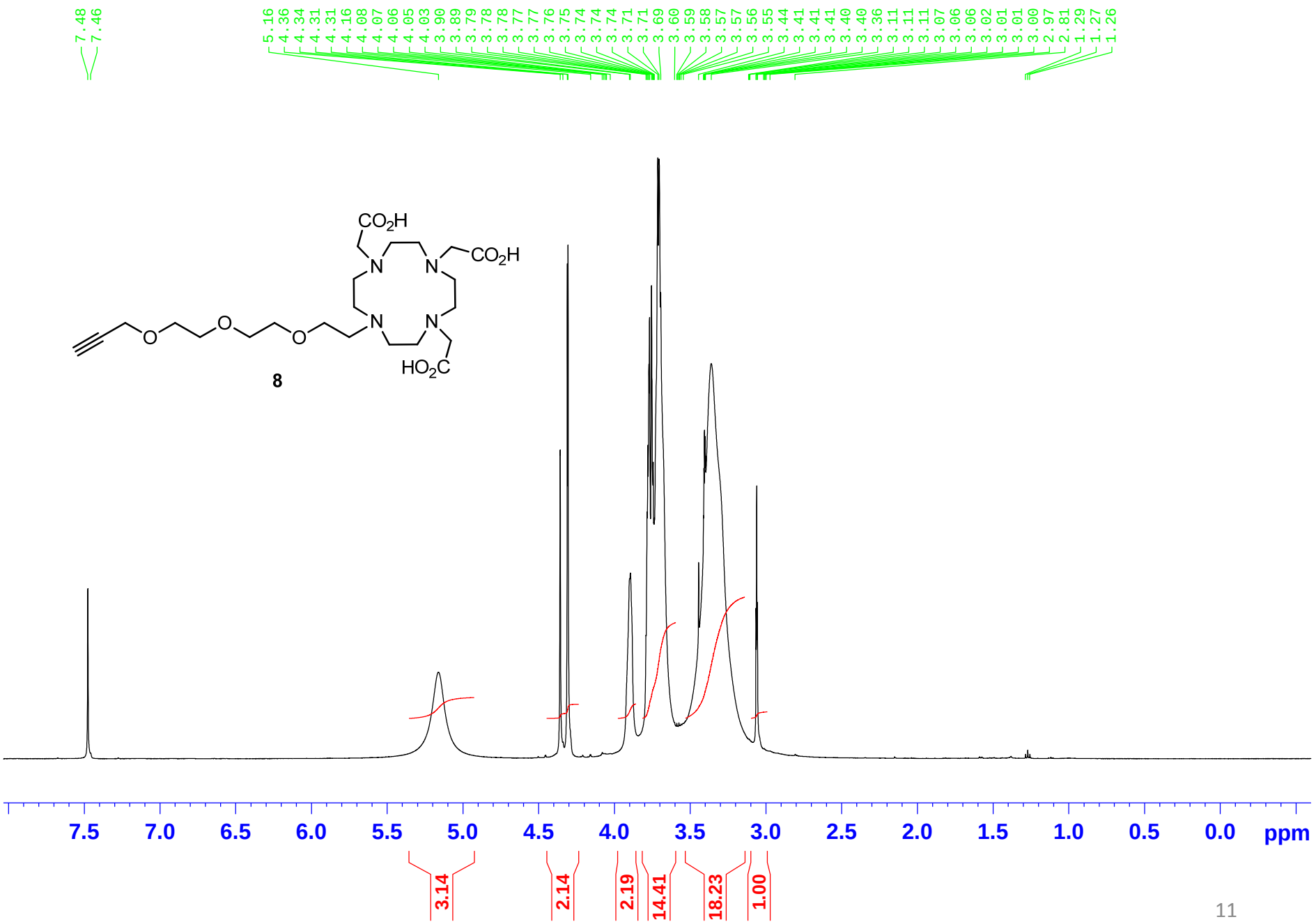
Chemical Formula: $C_9H_{15}IO_3$

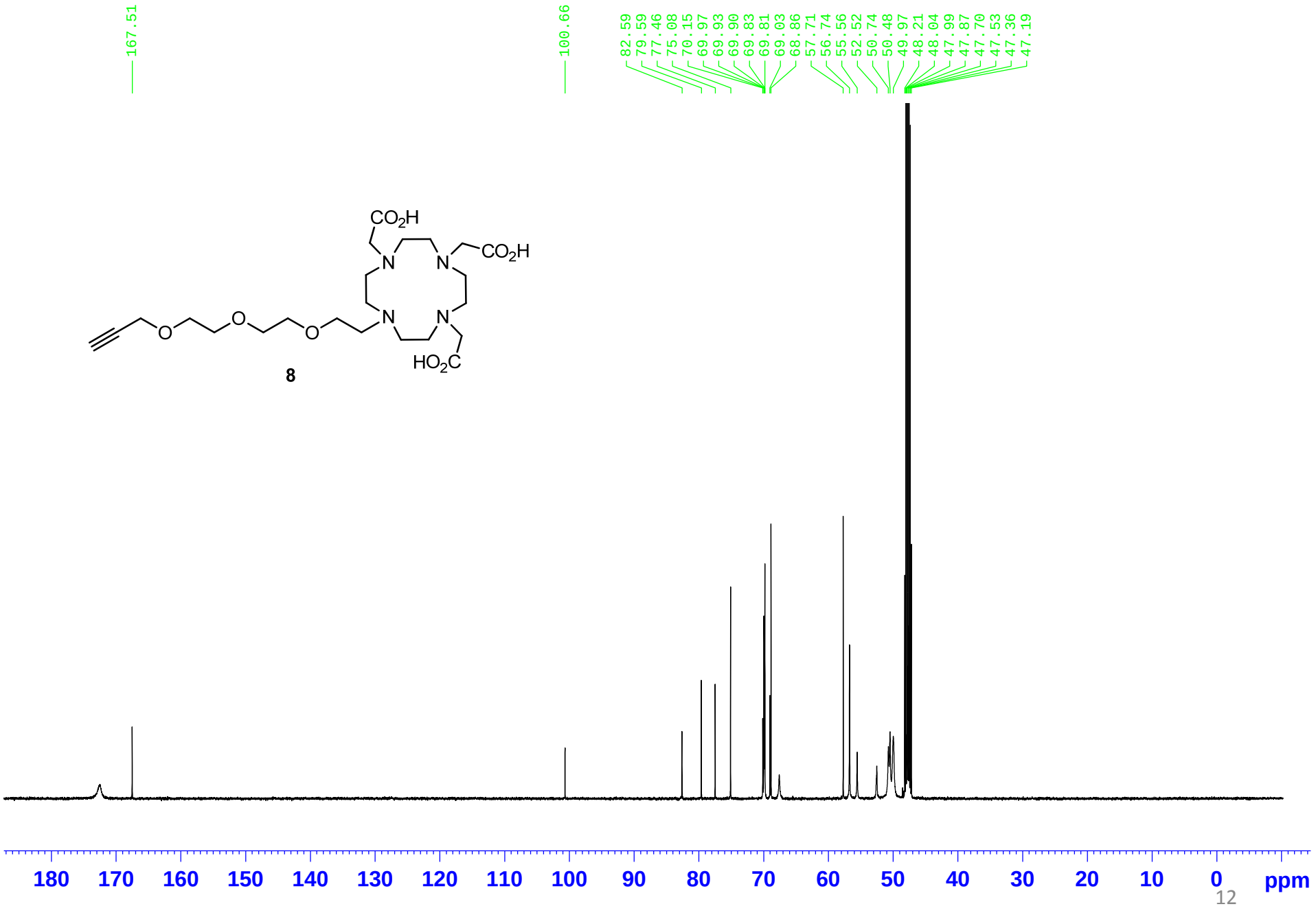
Exact Mass: 298.0066


7.289
4.159
4.153
3.654
3.651
3.646
3.640
3.639
3.613
3.607
3.600
3.574
2.429
1.457
1.436
CO2tBu
CO2tBu
tBuO2C
7
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
ppm
2.03
10.28
2.36
4.19
6.34
3.73
3.35
4.28
27.00

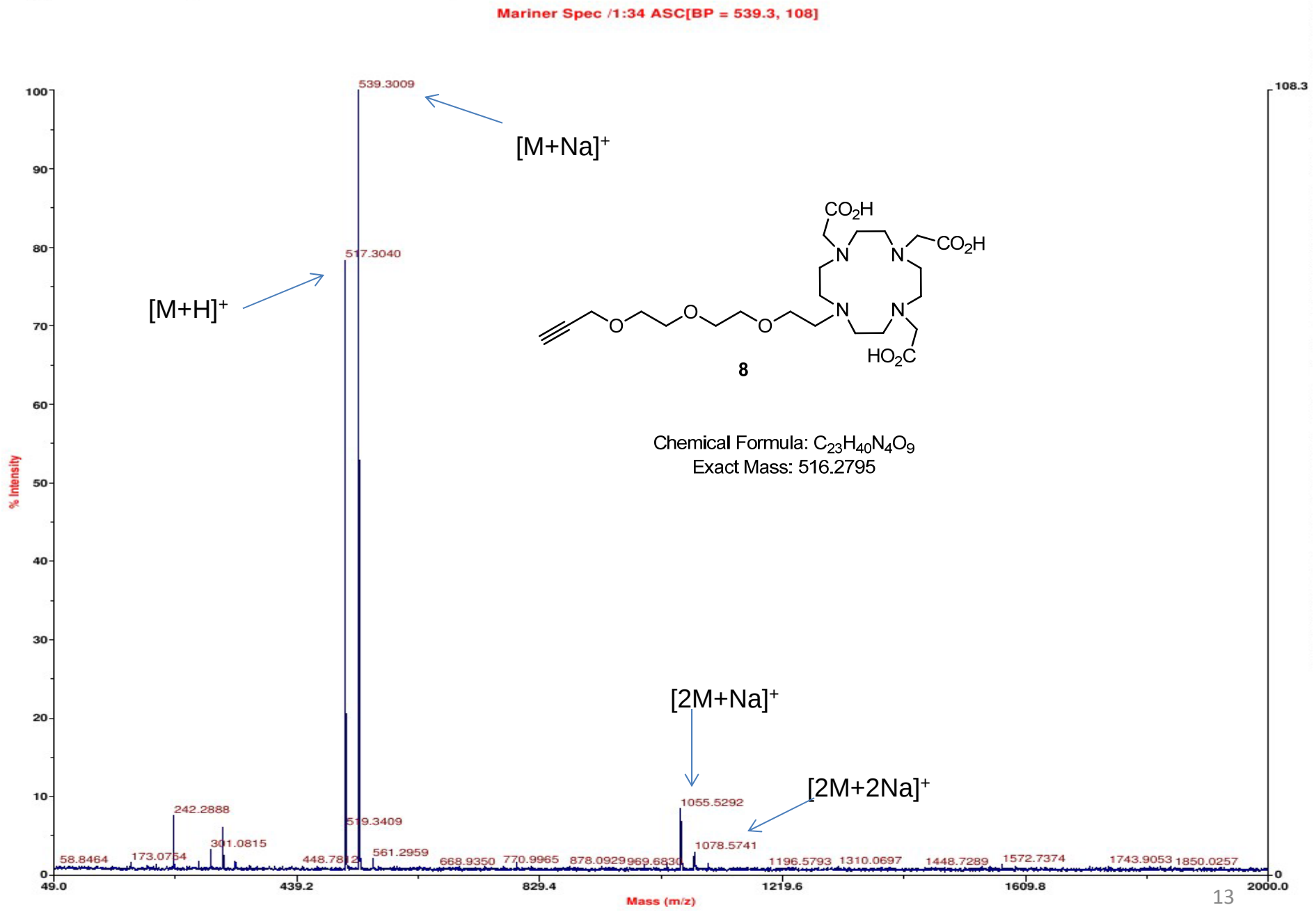

172.758
172.419
82.112
82.076
79.457
77.771
77.452
77.132
74.639
70.105
69.724
69.580
68.878
67.022
58.262
56.315
55.550
52.066
50.461
49.661
28.123
27.953
27.842
CO2tBu
CO2tBu
tBuO2C
7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


Applied Biosystems Mariner System 5268
Mariner Spec /1:40 (T /0.00:0.69) ASC[BP = 707.6, 1400]
707.5989
[M+Na]+
CO2tBu
CO2tBu
tBuO2C
7
Chemical Formula: C35H64N4O9
Exact Mass: 684.4673
% Intensity
Mass (m/z)
242.3398
709.6192
100
90
80
70
60
50
40
30
20
10
0
49.0
439.2
829.4
1219.6
1609.8
2000.0
1400.0


7.48
7.46
5.16
4.36
4.34
4.31
4.31
4.16
4.08
4.07
4.06
4.05
4.03
3.90
3.89
3.79
3.78
3.78
3.77
3.77
3.76
3.75
3.74
3.74
3.74
3.71
3.71
3.69
3.60
3.59
3.58
3.57
3.57
3.56
3.55
3.44
3.41
3.41
3.41
3.40
3.40
3.36
3.11
3.11
3.11
3.07
3.06
3.02
3.01
3.01
3.00
2.97
2.81
1.29
1.27
1.26
CO2H
CO2H
N
N
N
N
O
O
O
HO2C
8
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
ppm
3.14
2.14
2.19
14.41
18.23
1.00

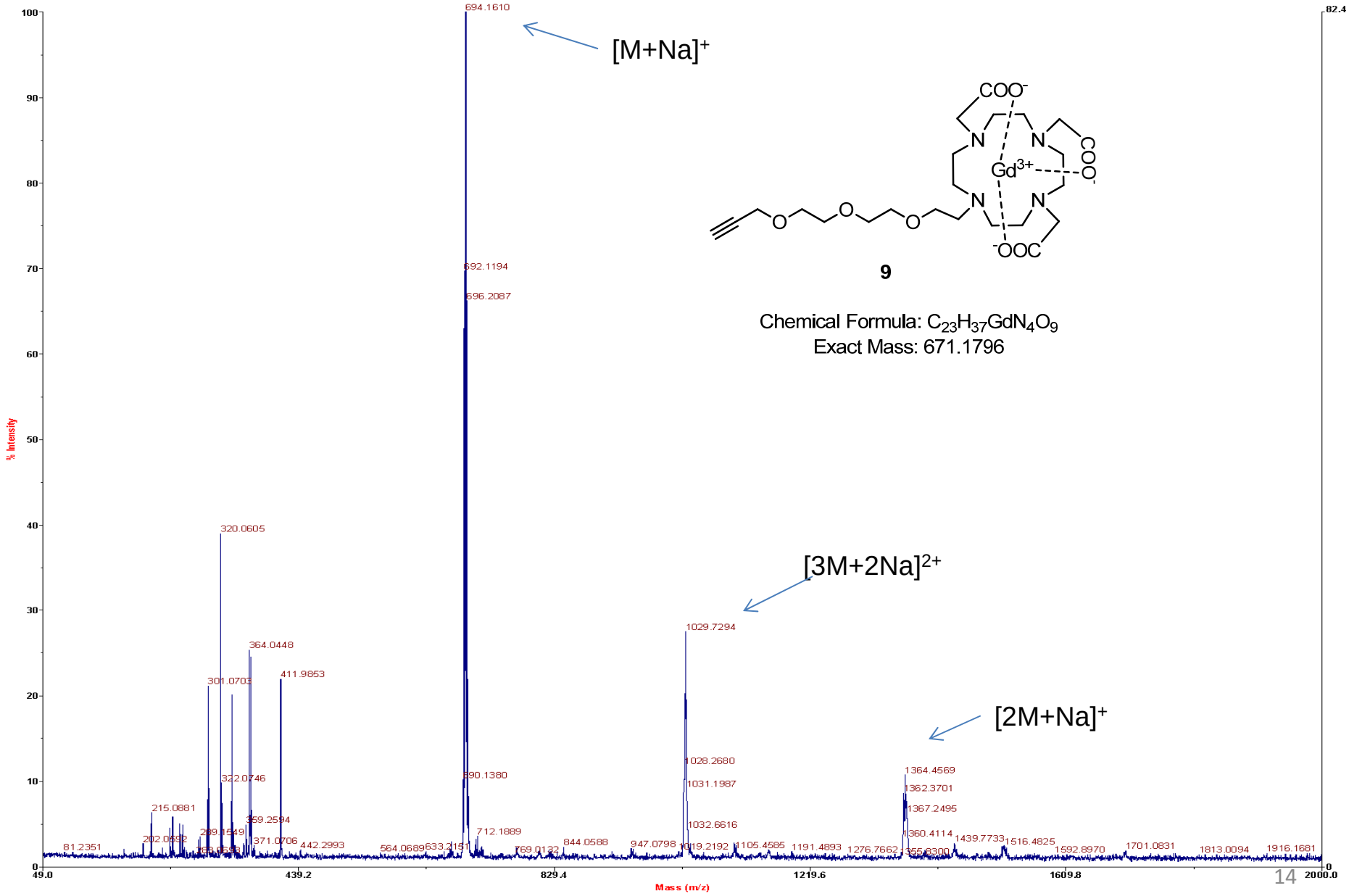

167.51
100.66
82.59
79.59
77.46
75.08
70.15
69.97
69.93
69.90
69.83
69.81
69.03
68.86
57.71
56.74
55.56
52.52
50.74
50.48
49.97
48.21
48.04
47.99
47.87
47.70
47.53
47.36
47.19
CO2H
CO2H
HO2C
N
N
N
N
O
O
O
8
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

Applied Biosystems Mariner System 5268



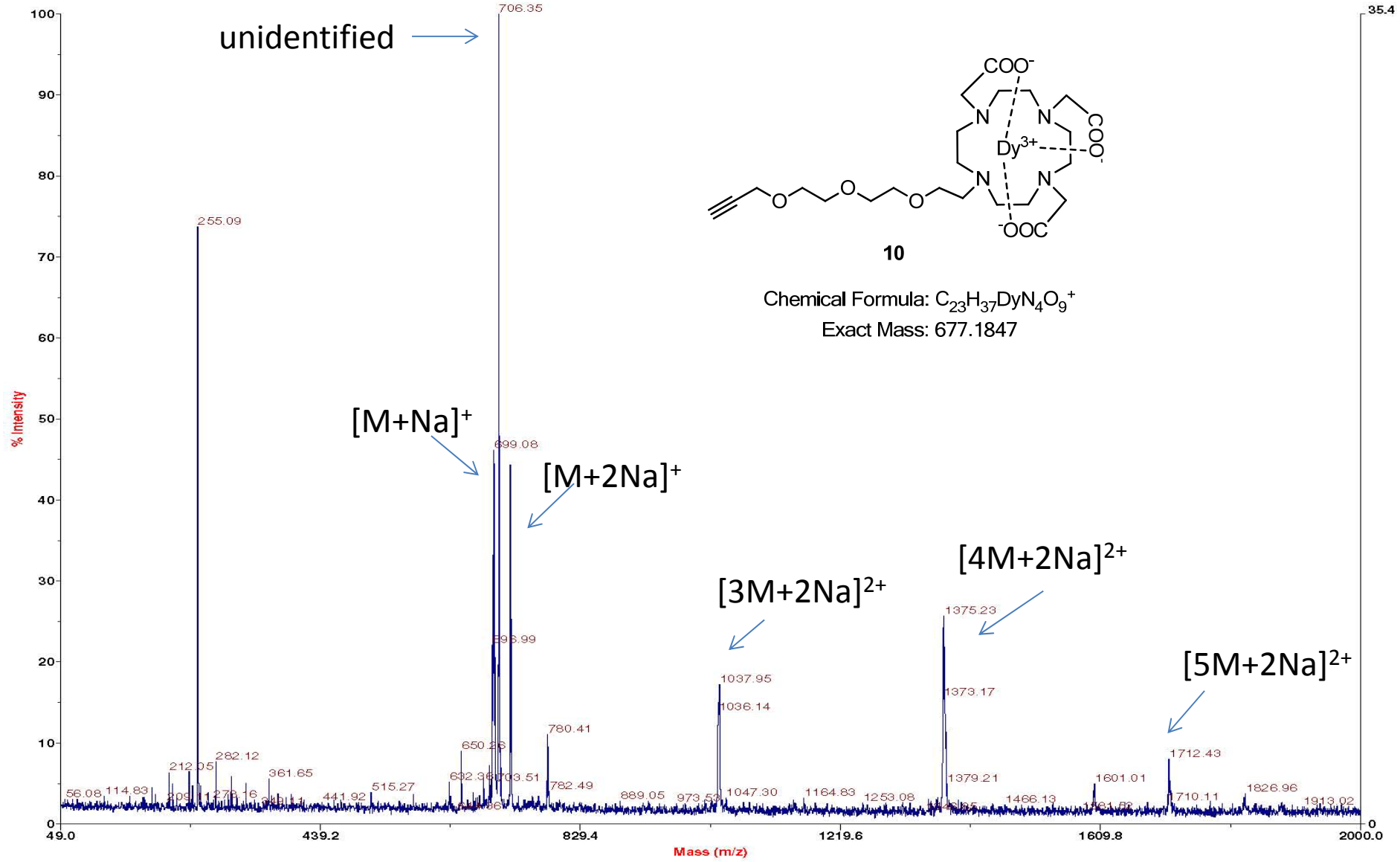
Applied Biosystems Mariner System 5268

Mariner Spec #1:27 ASC[BP = 694.2, 82]

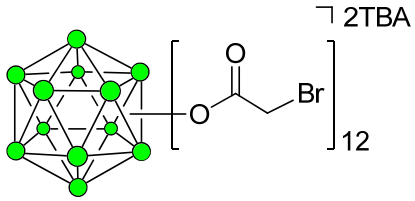


9

Chemical Formula: $C_{23}H_{37}GdN_4O_9$
Exact Mass: 671.1796

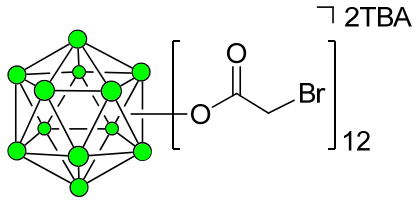

Applied Biosystems Mariner System 5219
Mariner Spec /1:24 ASC[BP = 706.4, 35]
unidentified
706.35
255.09
699.08
696.99
[M+Na]+
[M+2Na]+
[3M+2Na]2+
[4M+2Na]2+
[5M+2Na]2+
1037.95
1036.14
1375.23
1373.17
780.41
650.26
212.05
282.12
361.65
515.27
703.51
782.49
1047.30
1164.83
1253.08
1379.21
1466.13
1601.01
1712.43
1710.11
1826.96
1913.02
% Intensity
Mass (m/z)
49.0
439.2
829.4
1219.6
1609.8
2000.0
35.4
COO-
Dy3+
-OOC
10
Chemical Formula: C23H37DyN4O9+
Exact Mass: 677.1847
Acquired: Sep 04 16:50:00 2012
Mariner Mass Spectrum
C:\Mariner\Data\2012\Sep\04 Tue\LNG-1018C002.dat

B12-bromoacetic ester
B11 CDCl3 {C:\Bruker\TOPSPIN} goswami 10



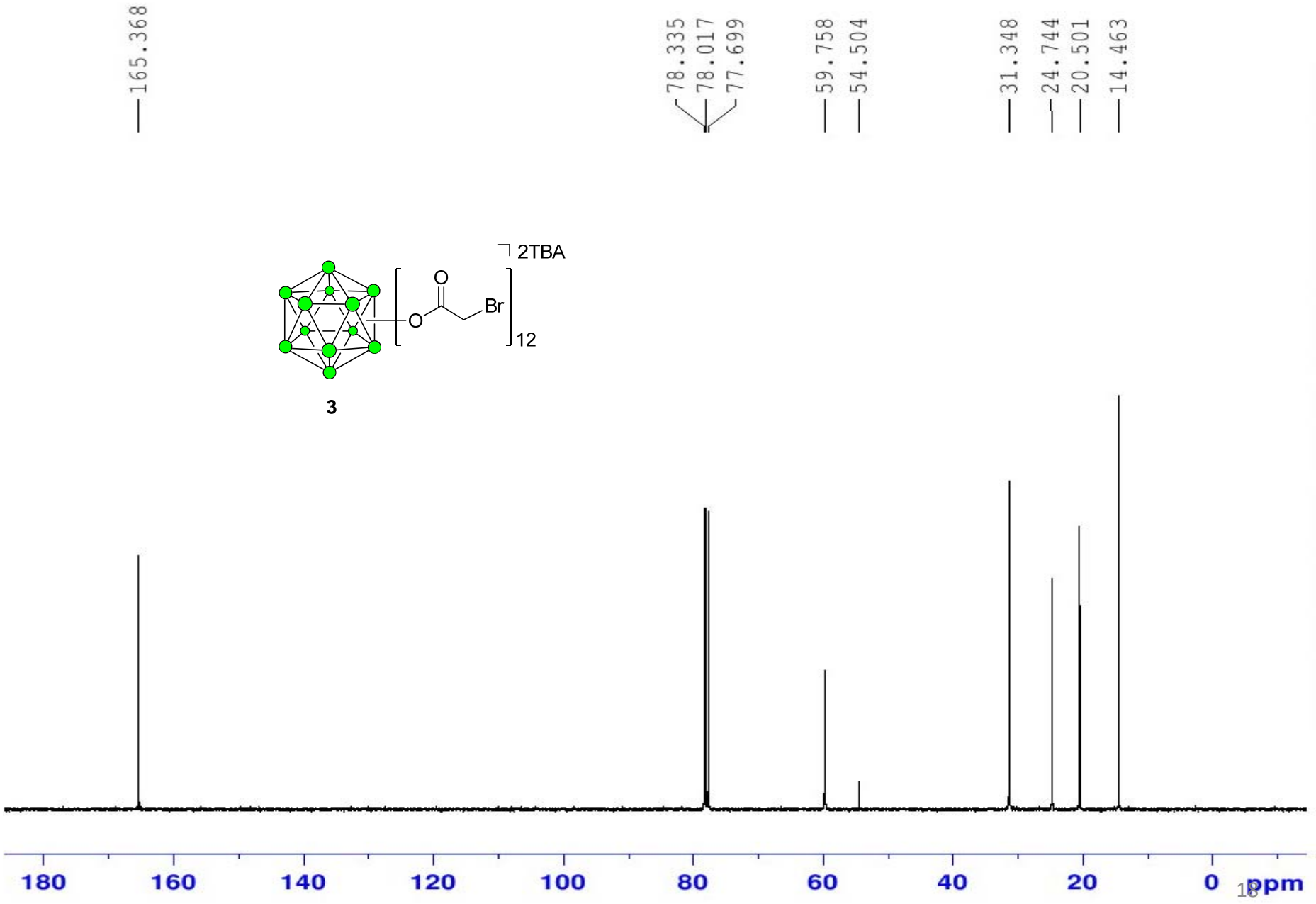
3

B12-bromoacetic ester
proton CDCl3 {C:\Bruker\TOPSPIN} goswami 9



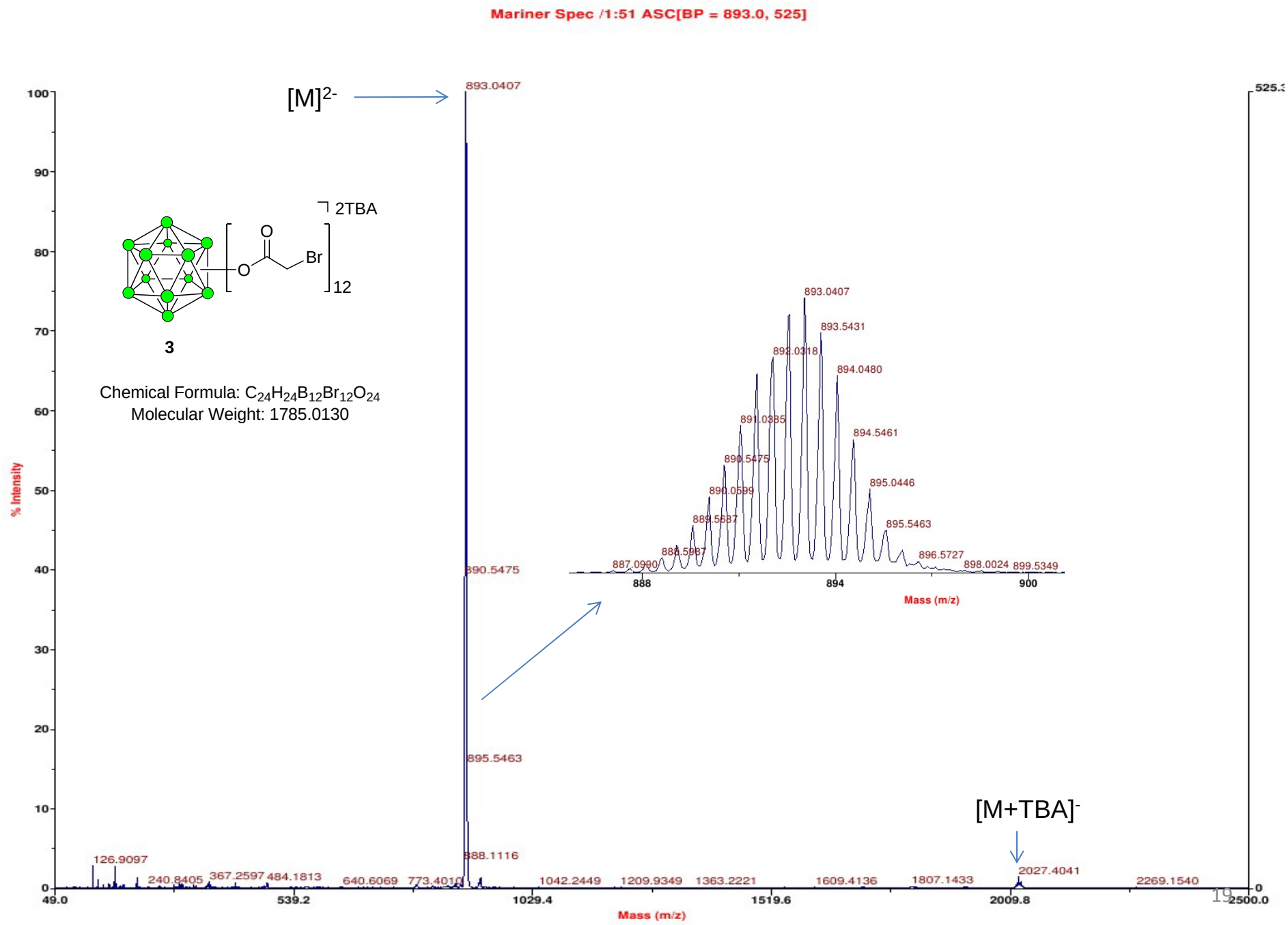
3

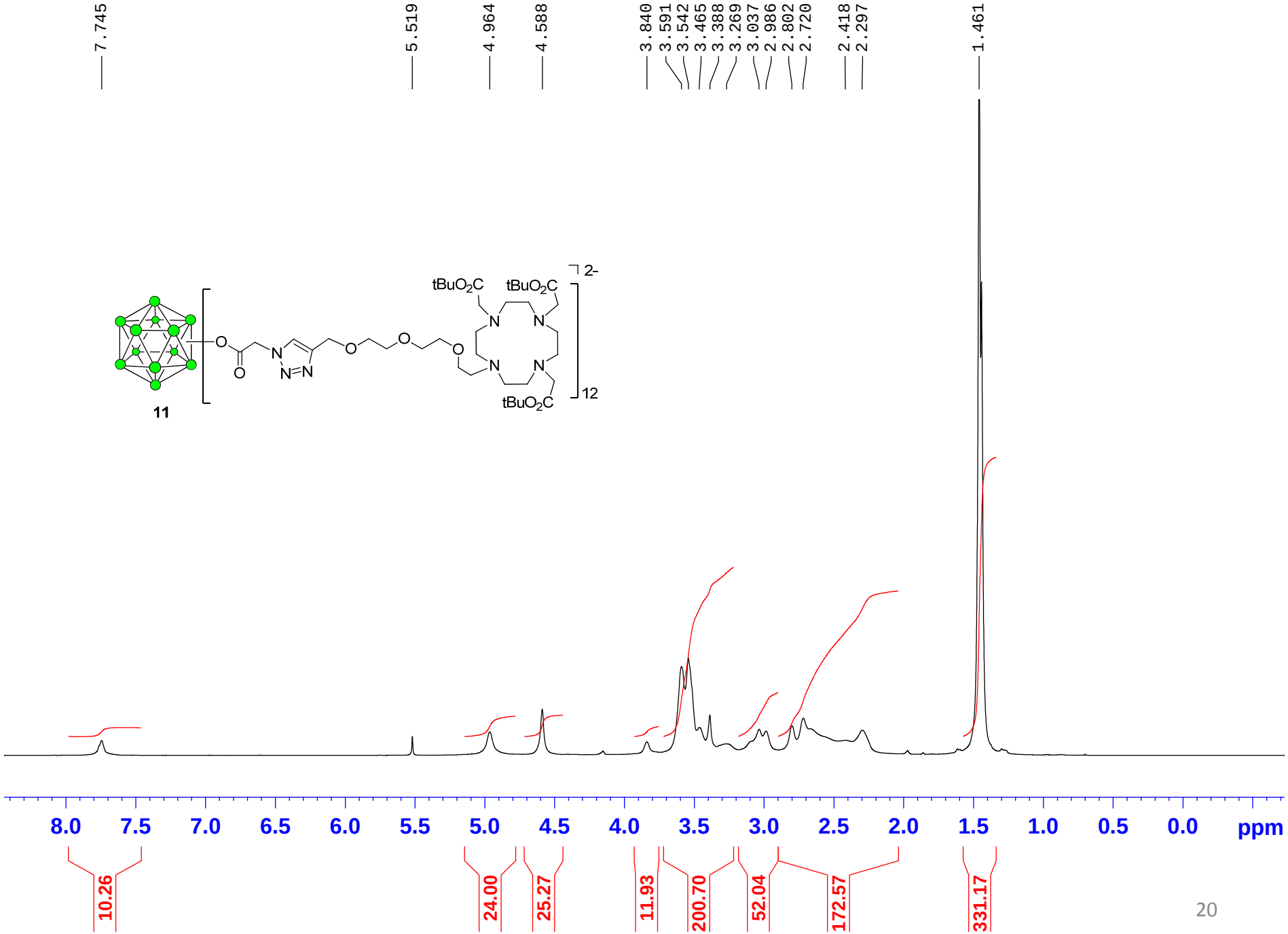
B12-bromoacetic ester
C13 CDCl3 {C:\Bruker\TOPSPIN} goswami 10

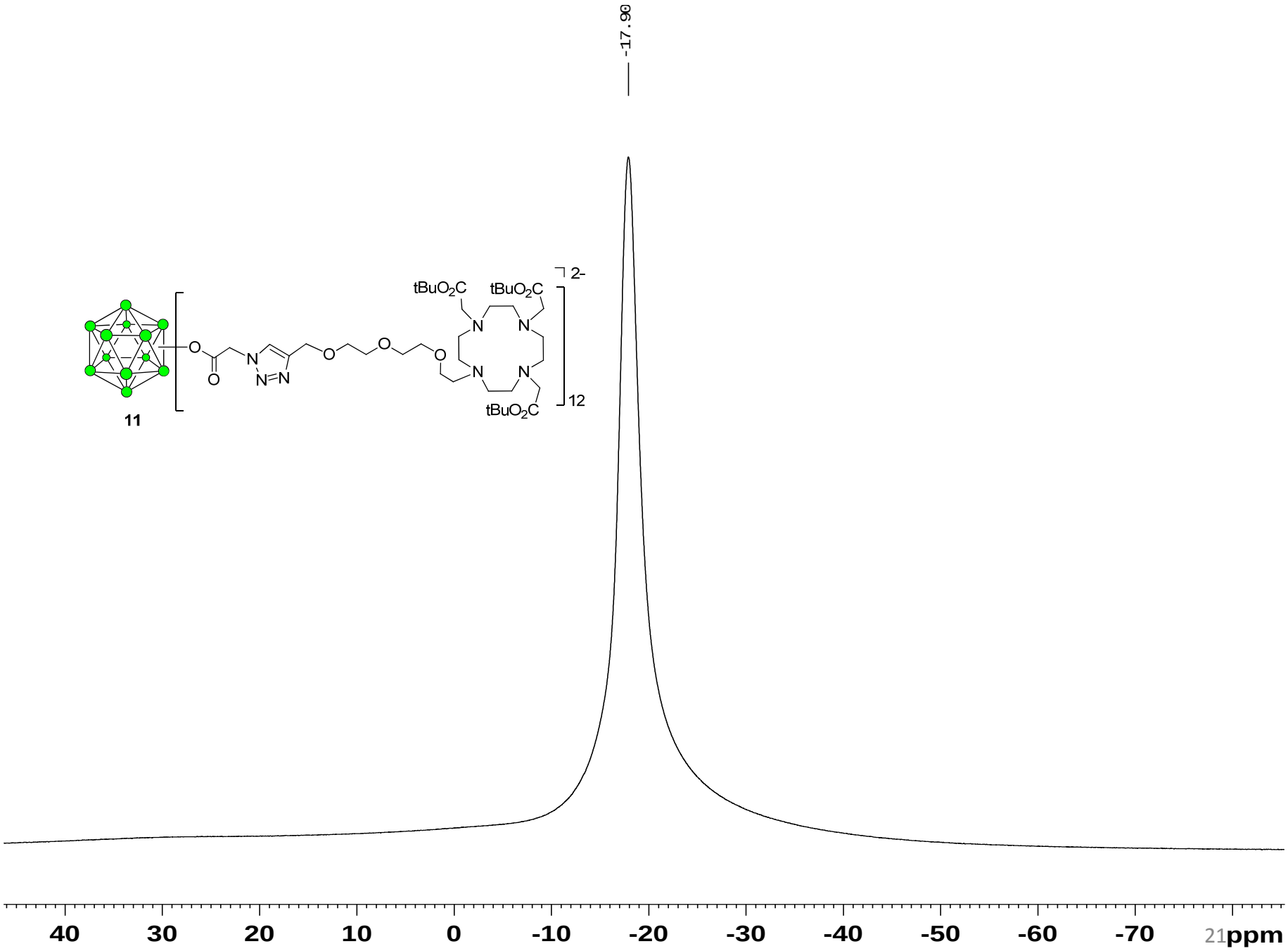


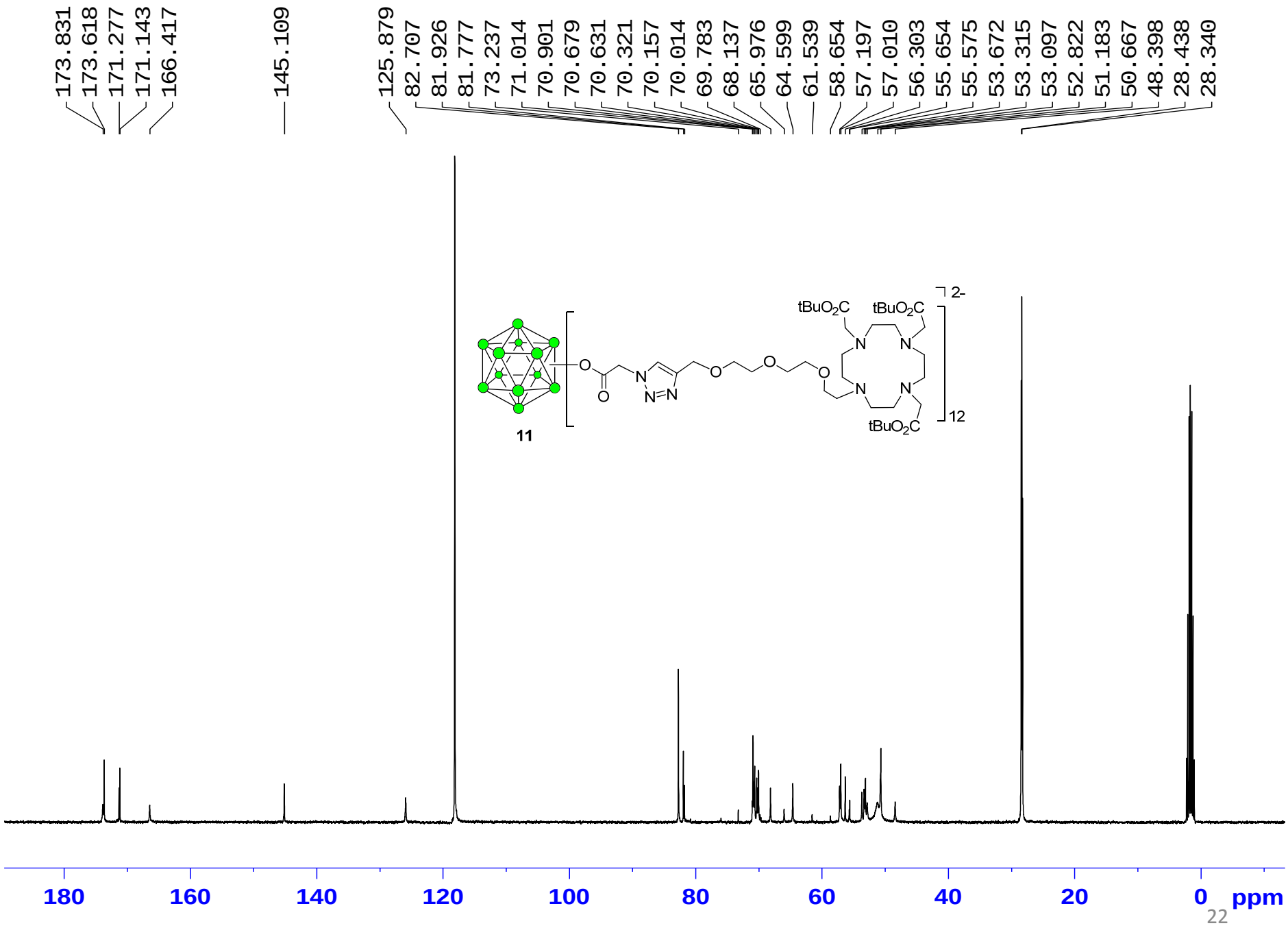
3

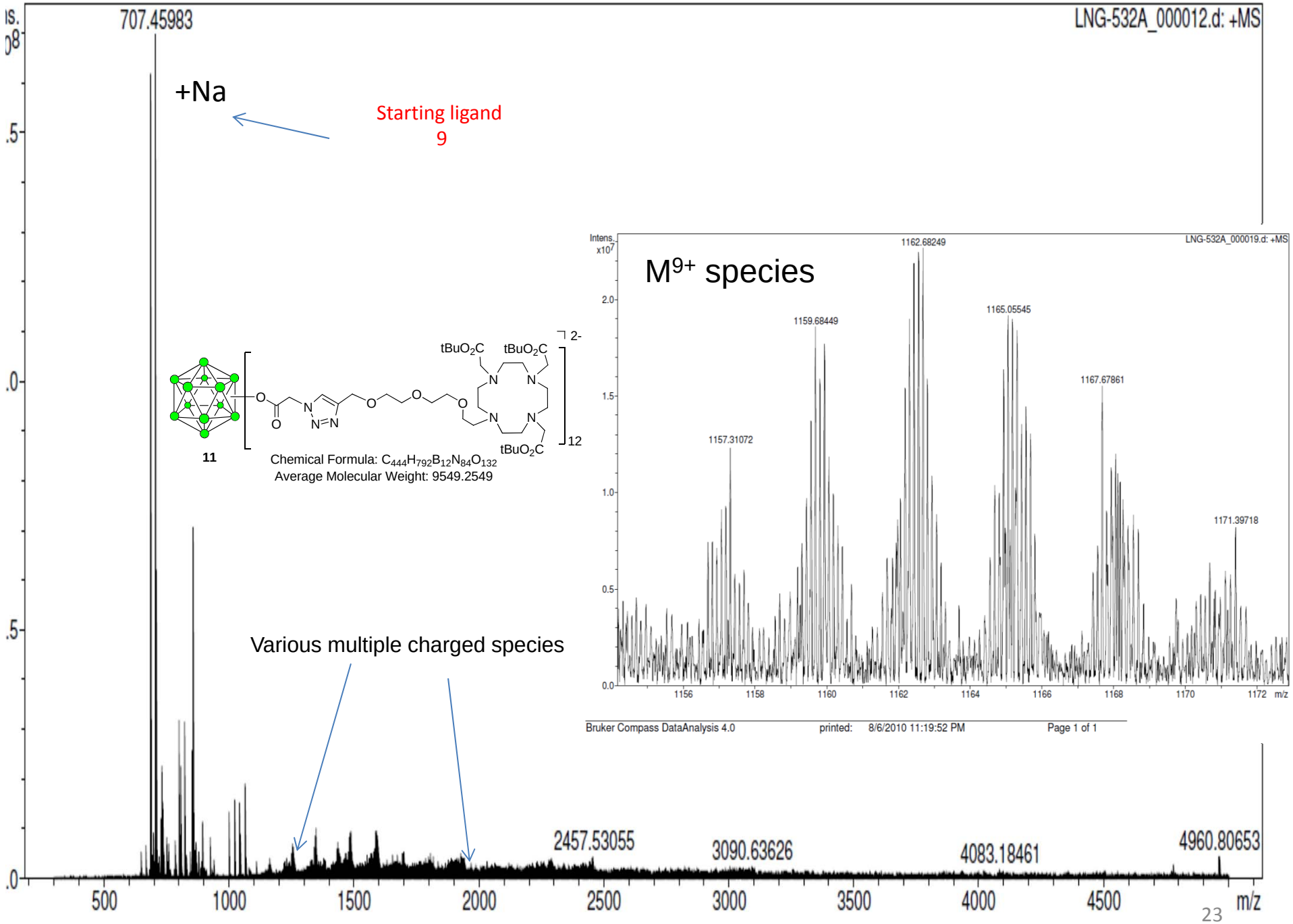
Applied Biosystems Mariner System 5268

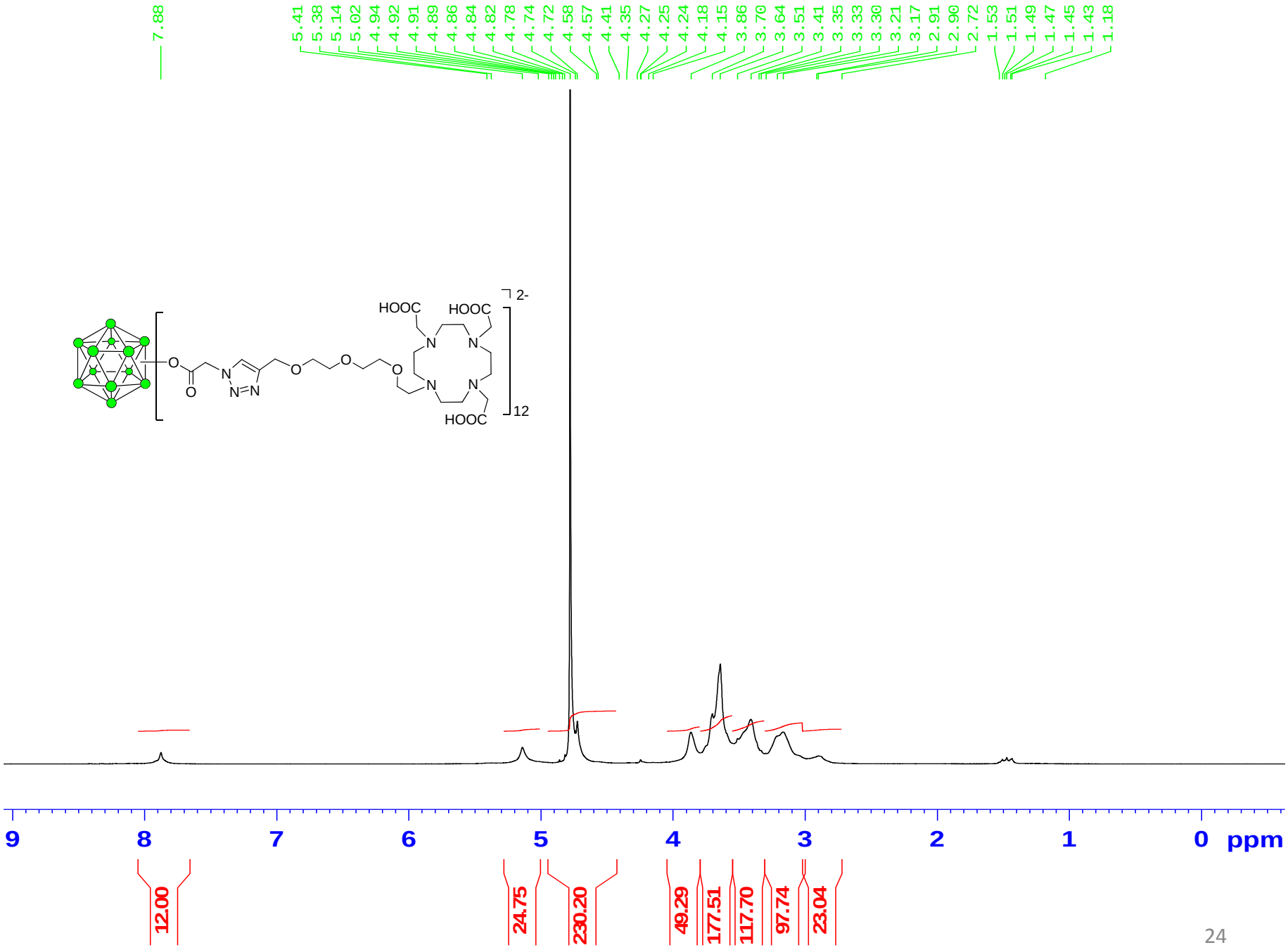


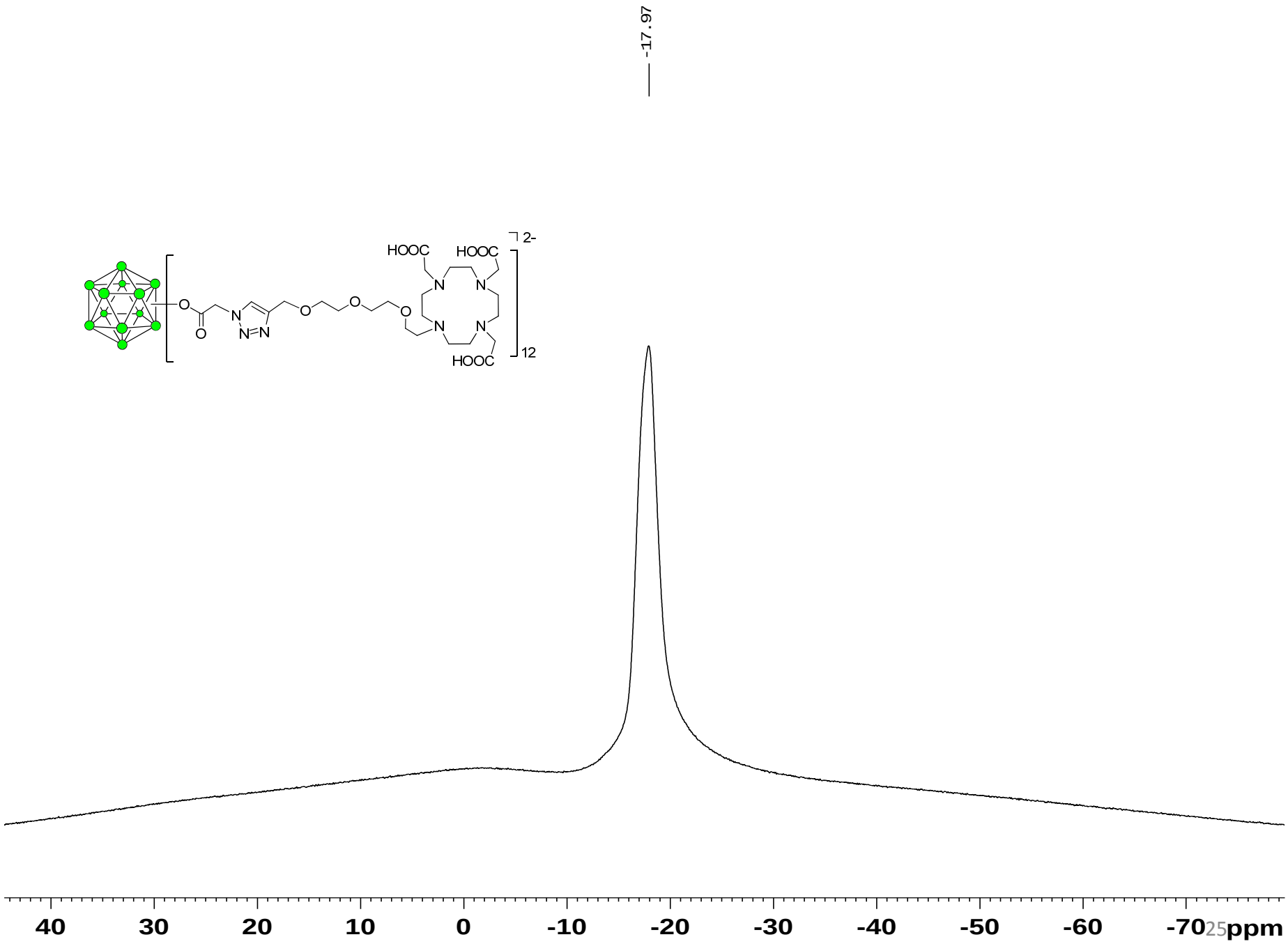

7.745
5.519
4.964
4.588
3.840
3.591
3.542
3.465
3.388
3.269
3.037
2.986
2.802
2.720
2.418
2.297
1.461
tBuO2C
tBuO2C
tBuO2C
2-
12
11
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
10.26
24.00
25.27
11.93
200.70
52.04
172.57
331.17

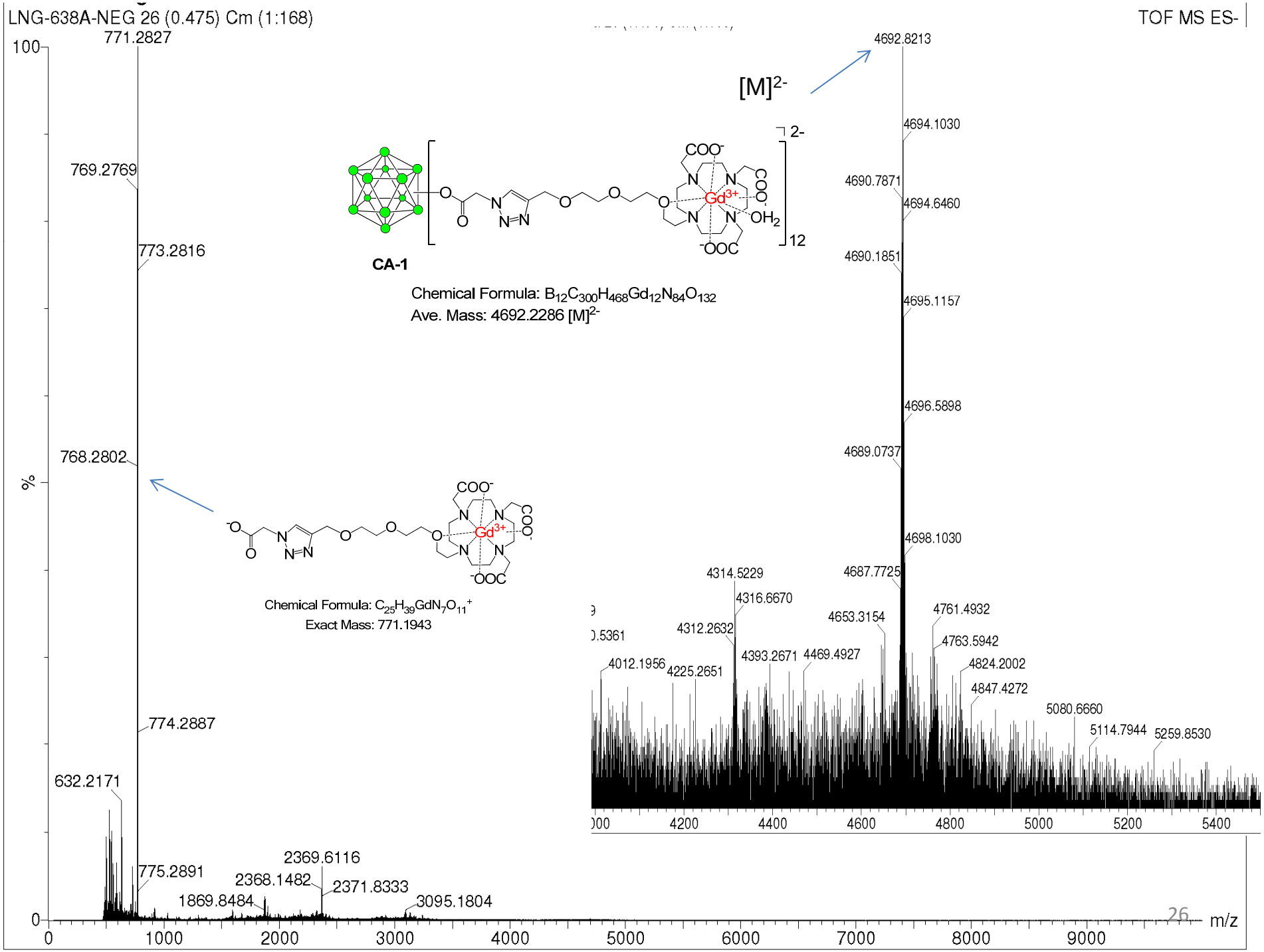

-17.90
2-
12
11
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
ppm

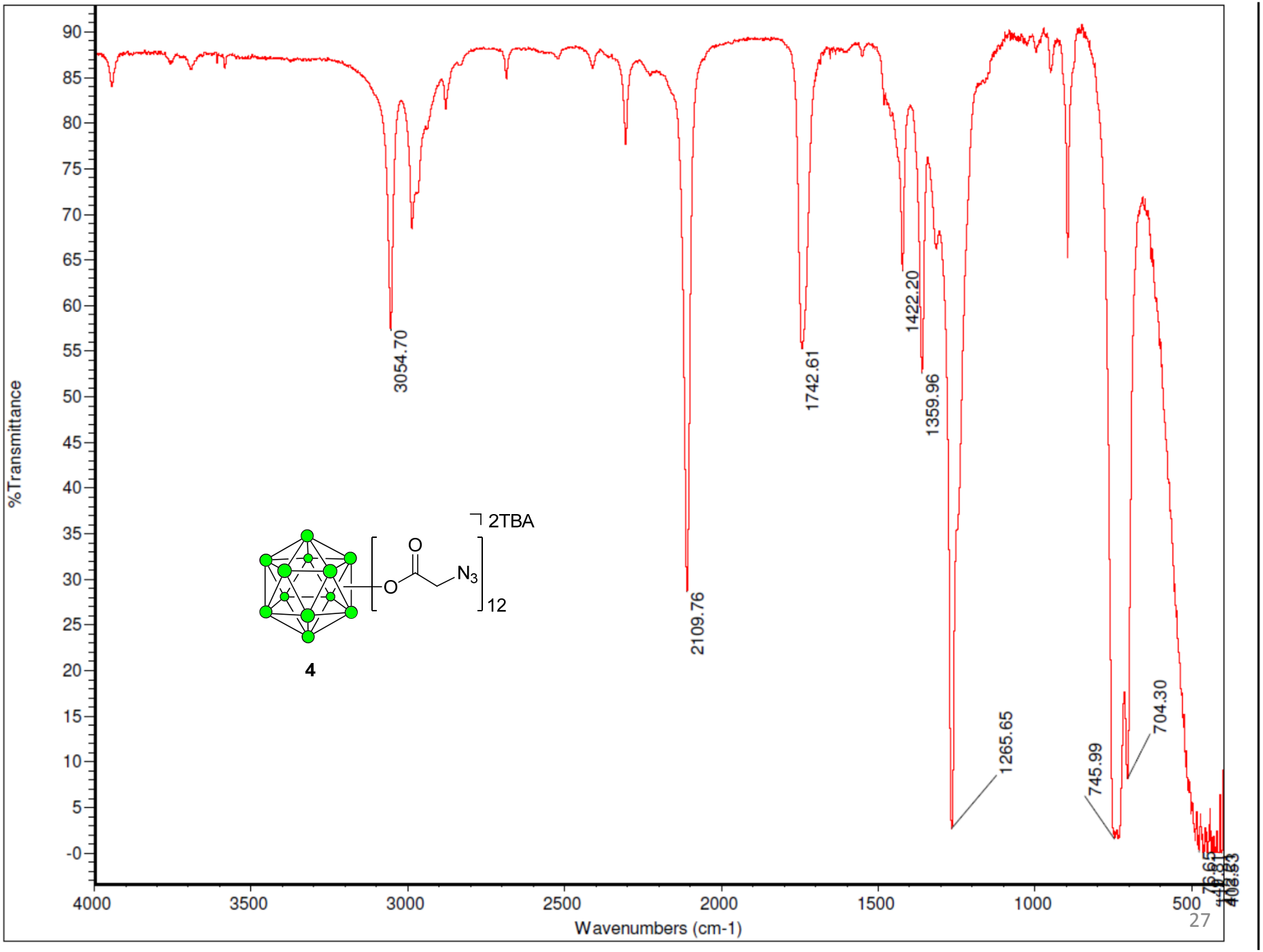

173.831
173.618
171.277
171.143
166.417
145.109
125.879
82.707
81.926
81.777
73.237
71.014
70.901
70.679
70.631
70.321
70.157
70.014
69.783
68.137
65.976
64.599
61.539
58.654
57.197
57.010
56.303
55.654
55.575
53.672
53.315
53.097
52.822
51.183
50.667
48.398
28.438
28.340
tBuO2C
tBuO2C
tBuO2C
tBuO2C
2-
12
11
180
160
140
120
100
80
60
40
20
0
ppm

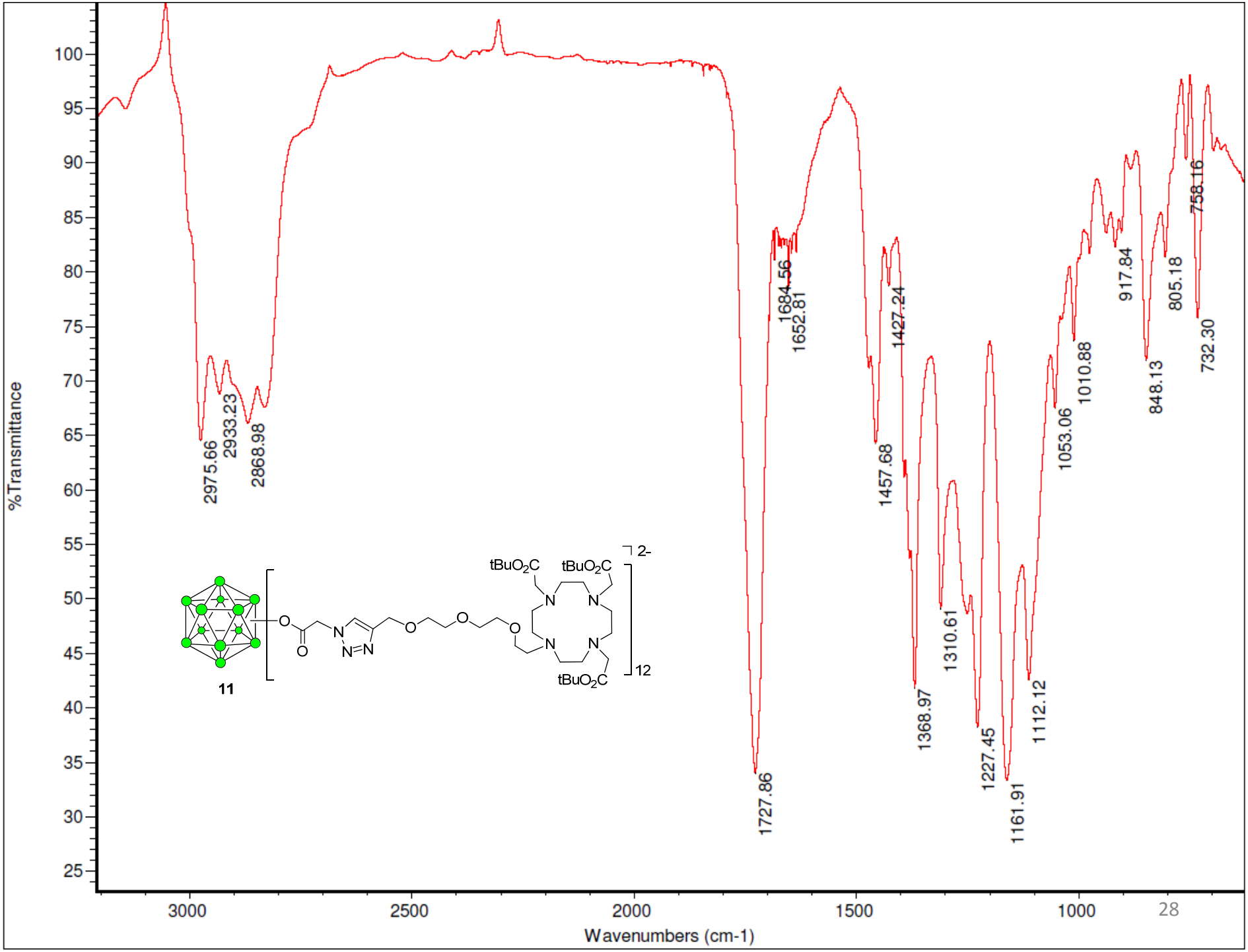

707.45983
LNG-532A_000012.d: +MS
+Na
Starting ligand
9
tBuO2C
tBuO2C
2-
12
tBuO2C
11
Chemical Formula: C444H792B12N84O132
Average Molecular Weight: 9549.2549
M9+ species
Intens.
x10^7
LNG-532A_000019.d: +MS
1162.68249
1159.68449
1165.05545
1167.67861
1157.31072
1171.39718
2.0
1.5
1.0
0.5
0.0
1156
1158
1160
1162
1164
1166
1168
1170
1172
m/z
Bruker Compass DataAnalysis 4.0
printed: 8/6/2010 11:19:52 PM
Page 1 of 1
Various multiple charged species
2457.53055
3090.63626
4083.18461
4960.80653
500
1000
1500
2000
2500
3000
3500
4000
4500
m/z

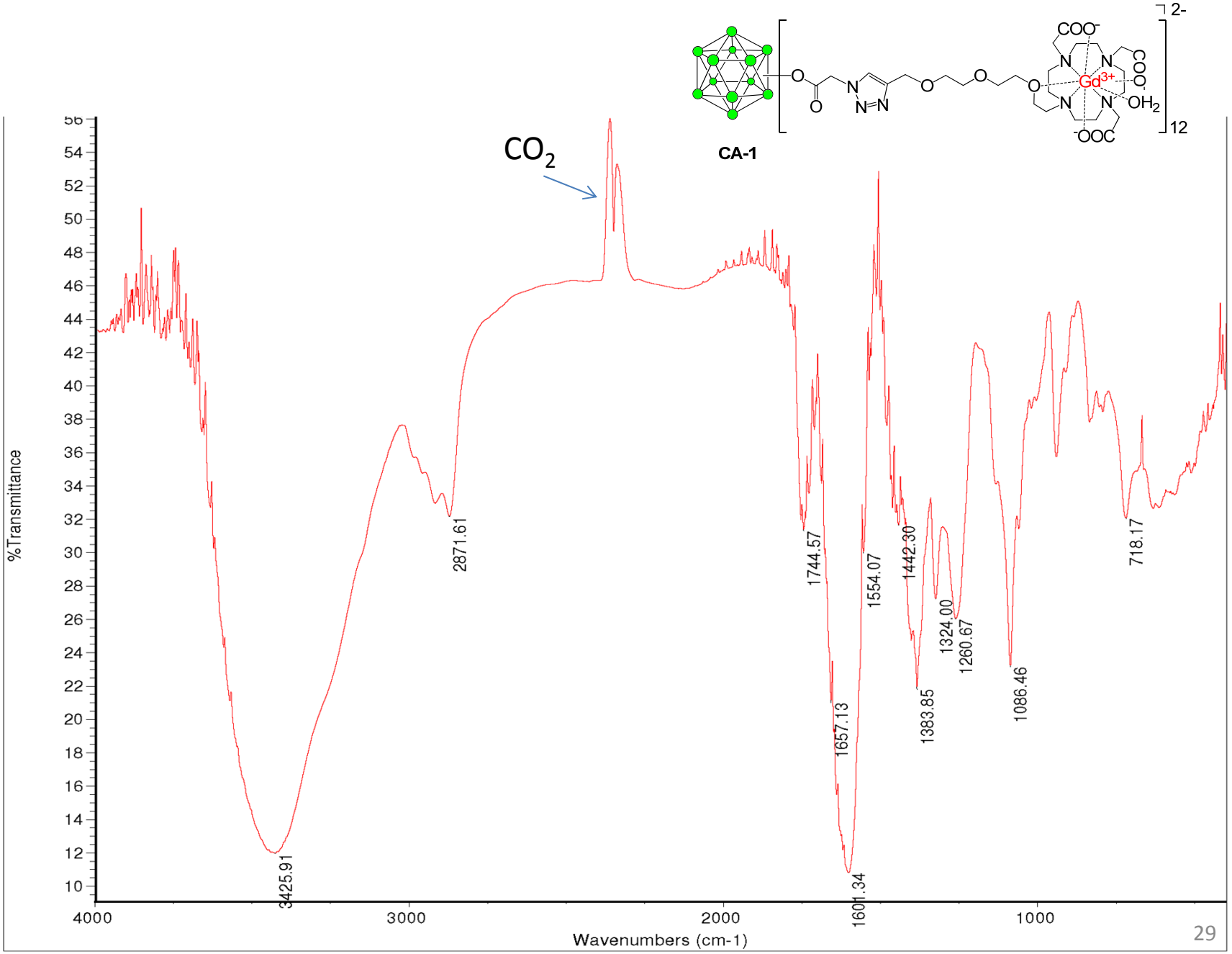

7.88
5.41
5.38
5.14
5.02
4.94
4.92
4.91
4.89
4.86
4.84
4.82
4.78
4.74
4.72
4.58
4.57
4.41
4.35
4.27
4.25
4.24
4.18
4.15
3.86
3.70
3.64
3.51
3.41
3.35
3.33
3.30
3.21
3.17
2.91
2.90
2.72
1.53
1.51
1.49
1.47
1.45
1.43
1.18
HOOC
HOOC
HOOC
2-
12
9
8
7
6
5
4
3
2
1
0
ppm
12.00
24.75
230.20
49.29
177.51
117.70
97.74
23.04


-17.97
HOOC
HOOC
N
N
N
N
HOOC
2-
12
O
O
N
N=N
O
O
O
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
25
ppm

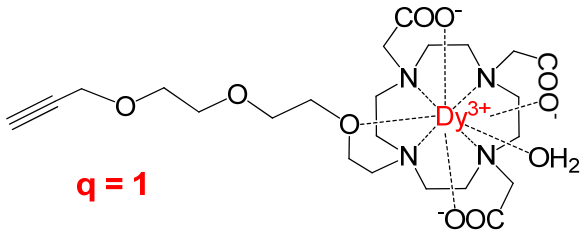

LNG-638A-NEG 26 (0.475) Cm (1:168)
TOF MS ES-
100
%
0
771.2827
769.2769
773.2816
768.2802
774.2887
632.2171
775.2891
2369.6116
2368.1482
2371.8333
1869.8484
3095.1804
4692.8213
[M]2-
4694.1030
4690.7871
4694.6460
4690.1851
4695.1157
4696.5898
4689.0737
4698.1030
4687.7725
4314.5229
4316.6670
4312.2632
4653.3154
4761.4932
4763.5942
4393.2671
4469.4927
4824.2002
4847.4272
4012.1956
4225.2651
5080.6660
5114.7944
5259.8530
CA-1
2-
12
Chemical Formula: B12C300H468Gd12N84O132
Ave. Mass: 4692.2286 [M]2-
Chemical Formula: C25H39GdN7O11+
Exact Mass: 771.1943
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
4200
4400
4600
4800
5000
5200
5400
m/z
26


%Transmittance
Wavenumbers (cm-1)
3054.70
2109.76
1742.61
1422.20
1359.96
1265.65
745.99
704.30
2TBA
O
N3
O
12
4

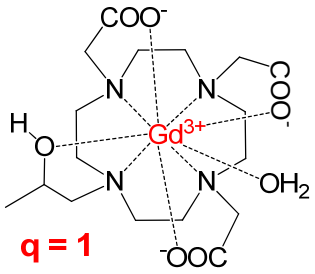

%Transmittance
Wavenumbers (cm-1)
2975.66
2933.23
2868.98
1727.86
1684.56
1652.81
1457.68
1427.24
1368.97
1310.61
1227.45
1161.91
1112.12
1053.06
1010.88
917.84
848.13
805.18
758.16
732.30
11
tBuO2C
2-
12


CA-1
CO_2
%Transmittance
Wavenumbers (cm-1)
3425.91
2871.61
1744.57
1657.13
1601.34
1554.07
1442.30
1383.85
1324.00
1260.67
1086.46
718.17

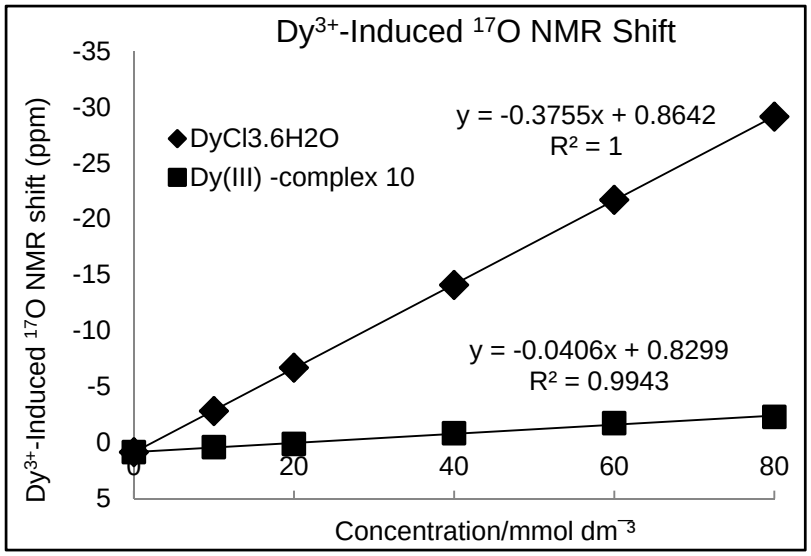
Determination of Hydration number (q) for DO3A Dy^{3+} complex 10



DOTA-Dy^{3+} complex **10**



ProHance
(BARACCO)



Varying concentrations of **10** and $DyCl_3.6H_2O$ over the range 10-80 mmol dm^{-3} were prepared in 80% D_2O-H_2O and the pH of the solutions was adjusted to pH 7.0. The ^{17}O NMR experiments were performed at 400 MHz using a Bruker Avance instrument at RT with the deuterium signal locked. A graph was plotted between the d.i.s. (Δδ) and the concentration for both **10** and the $DyCl_3.6H_2O$ solutions and slope was obtained. The Δδ value for a complex with the general formula, $Dy(ligand)_n(H_2O)_q$, is given by the following relation;

$$(\Delta\delta) = q\Delta[Dy(ligand)_n(H_2O)_q]/[H_2O] \qquad (I)$$

The slope of a plot of the d.i.s. versus the Dy^{3+} concentration is proportional to the **q** value of the complex (Figure). The **q** value was obtained by linearly fitting the Δδ value and was found to be 2 for complex **10**.

From the graph, which fit well to a straight line, the slope was calculated. From relation I, the slope of the graph can be equated with the following;

Slope = qΔ/[H2O],

From the graph, the slope was calculated as,

Slope = -375 ppm dm^3 mol^{-1} or,

qΔ/[H2O] = -375 ppm dm^3 mol^{-1}

For $DyCl_3$, the value of **q** was assumed to be 9, because the coordination number of Dy(III) in such complexes known to be 9. Hence, the value of Δ/[H2O] is calculated as follows:

Δ/[H2O] = -375 ppm dm^3 mol^{-1} /9 or

Δ/[H2O] = - 41.7 ppm dm^3 mol^{-1}

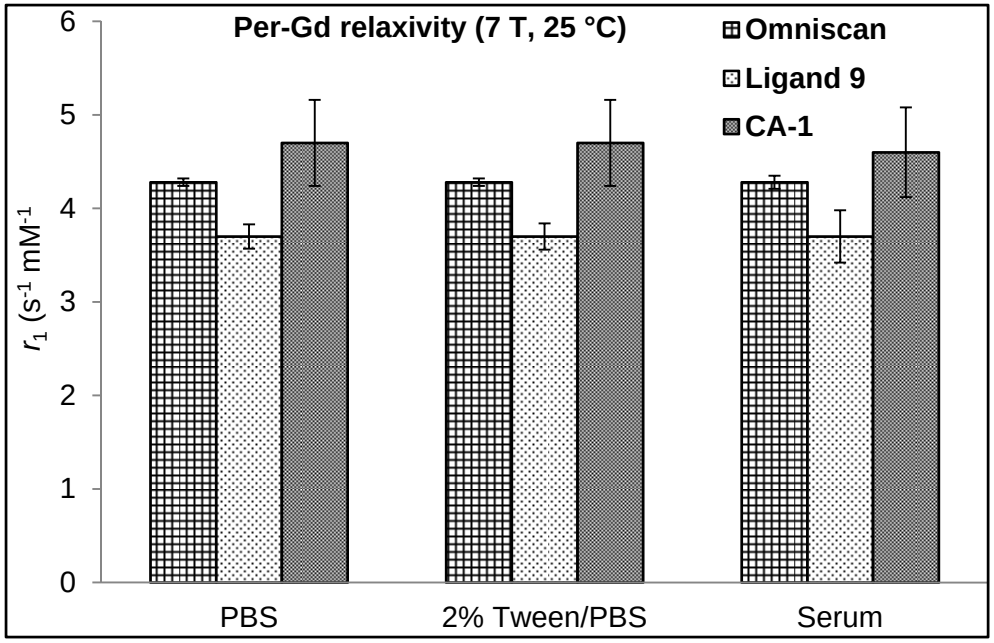
From this calculation, a value of q for the DOTA-Dy^{3+} complex **10** is calculated as follows:

For complex **10**, Slope = qΔ/[H2O], or

q = slope/(Δ/[H2O]) or q = -40.6/-41.7, hence

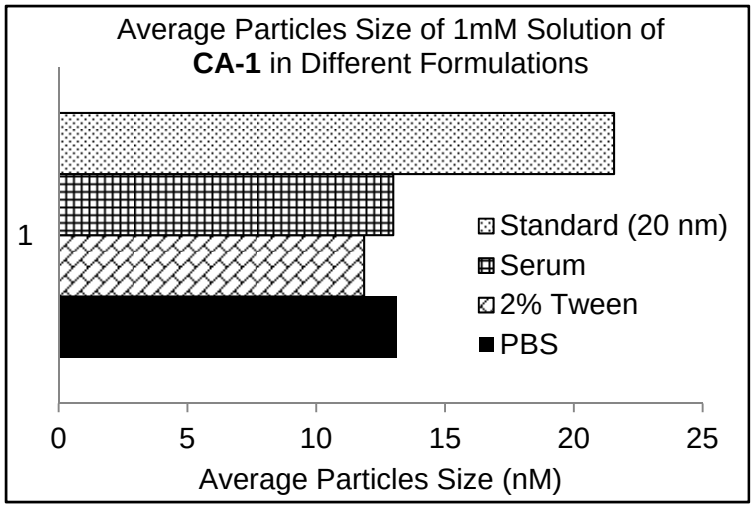
q = 1

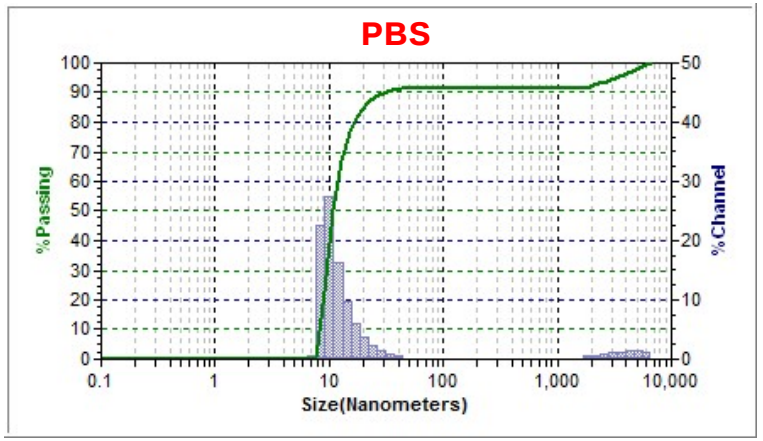
Figure. Comparison of the per-Gd r_1 values of **CA-1**, ligand **9** and Omniscan.

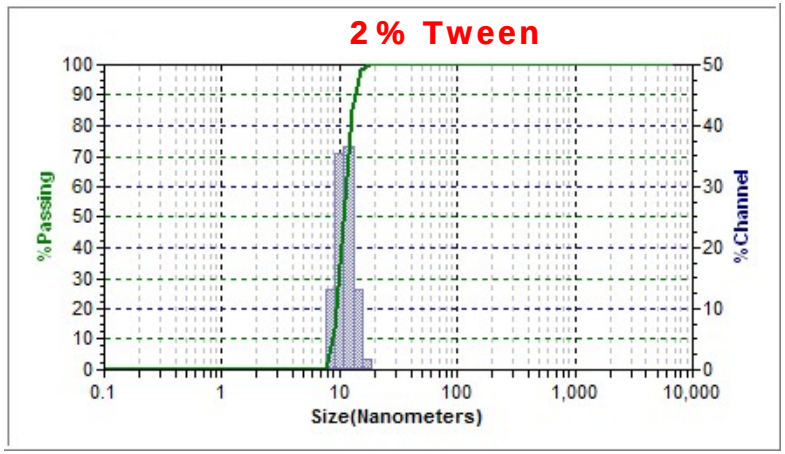


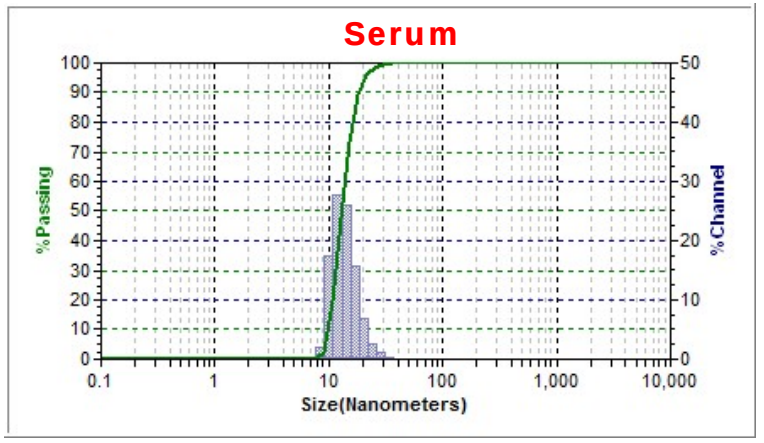
Dynamic Light Scattering Analysis of **CA-1**

1mM solutions;
Standard: Gold nanoparticles (20 nm)

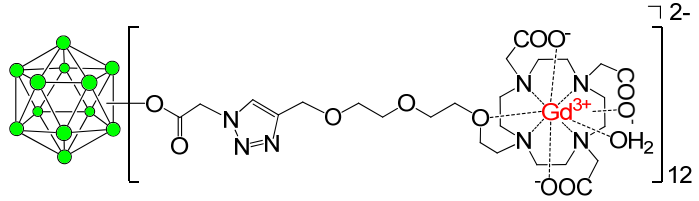








SE-HPLC analysis of CA-1



CA-1

- **Column:**
  - **BioSep-SEC-S2000 (Phenomenex)**
  - **250 X 2.0mm**
- **Mobile phase:**
  - **PBS, pH 6.6**
  - **Flow rate: 0.1 ml/min**
- **Detector:**
  - **UV-256 nm**
- **Injection:**
  - **5.0 mg/ml PBS stock solution**
  - **5ul injection**