Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis, Relaxation Properties and *In vivo* Assessment of Carborane-GdDOTA-Monoamide Conjugate as MRI Blood Pool Contrast Agent

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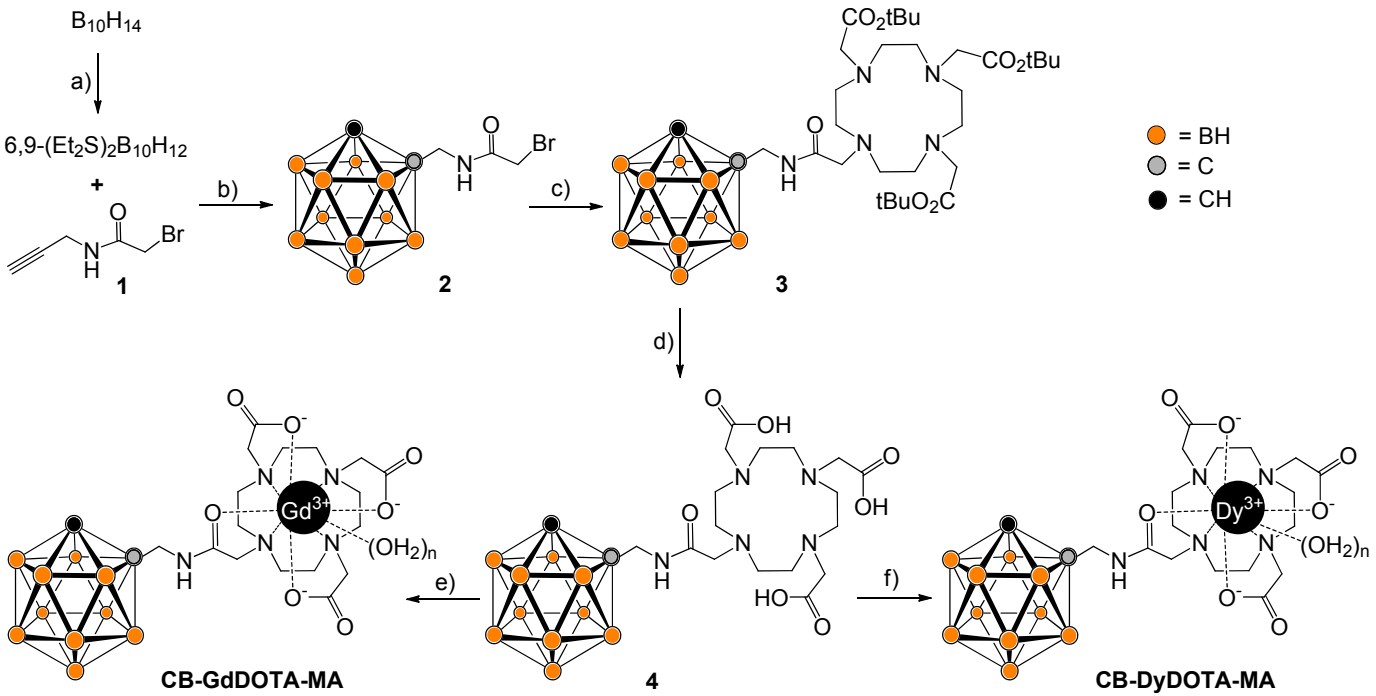
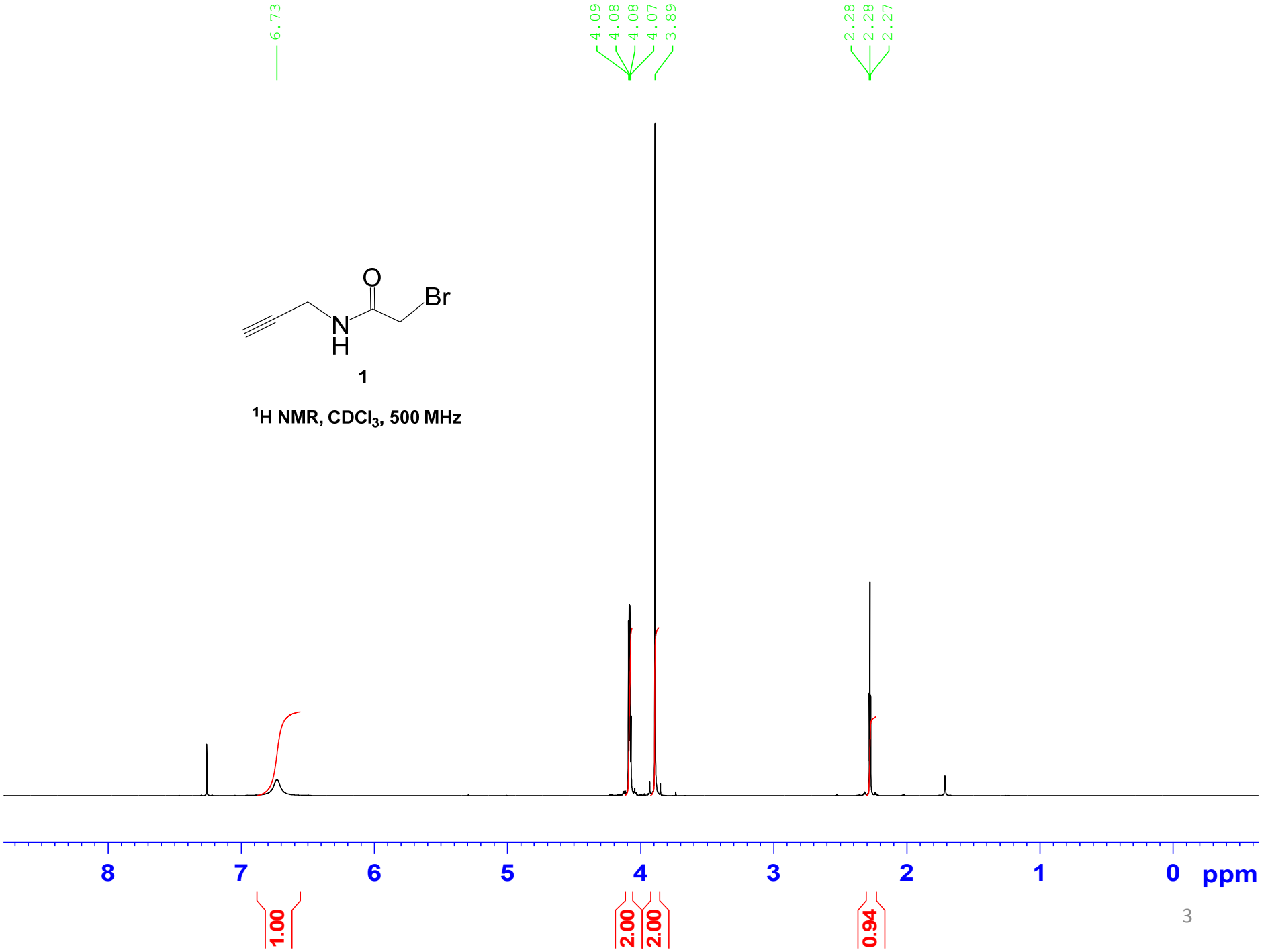
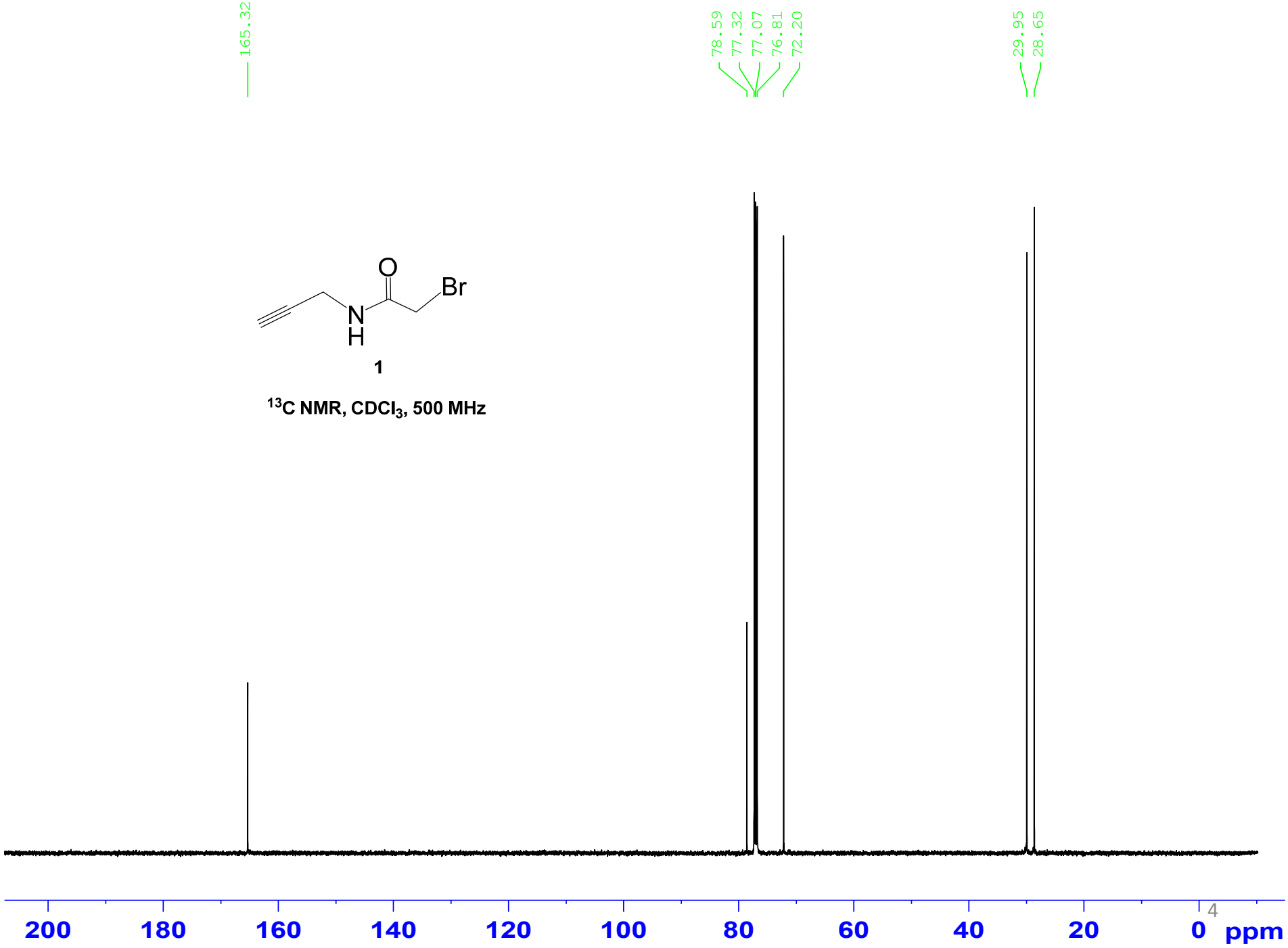
Contents:



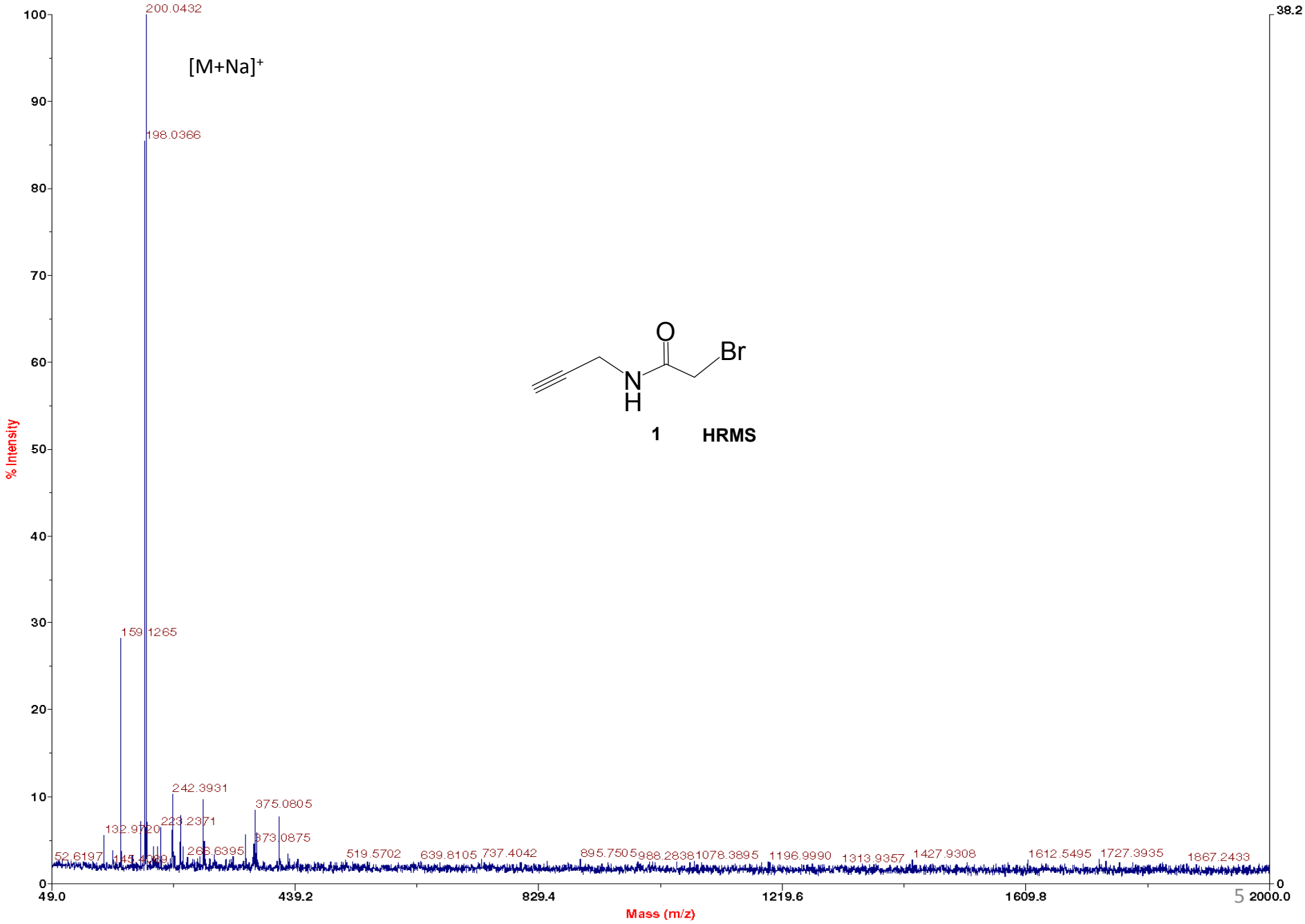
Scheme 1 Synthesis of **CB-GdDOTA-MA**. Reagents and conditions: a) Et_2S, Toluene, 85 °C, 2 h. b) Acetonitrile, 65 °C, 12 h, 41%. c) DO3A-*t*-Bu-ester, *N, N*-diisopropylethylamine, 85 °C for 12 h, 60%. d) Formic acid, 60 °C, 12 h, 82%. e) $GdCl_3.6H_2O$, H_2O-MeOH (1:1), pH 6.5, RT, 12 h, 73%. f) $DyCl_3.6H_2O$, H_2O-MeOH (1:1), pH 6.5, RT, 12 h, 68%.


6.73
4.09
4.08
4.08
4.07
3.89
2.28
2.28
2.27
O
Br
N
H
1
^{1}H NMR, CDCl$_3$, 500 MHz
8
7
6
5
4
3
2
1
0
ppm
1.00
2.00
2.00
0.94

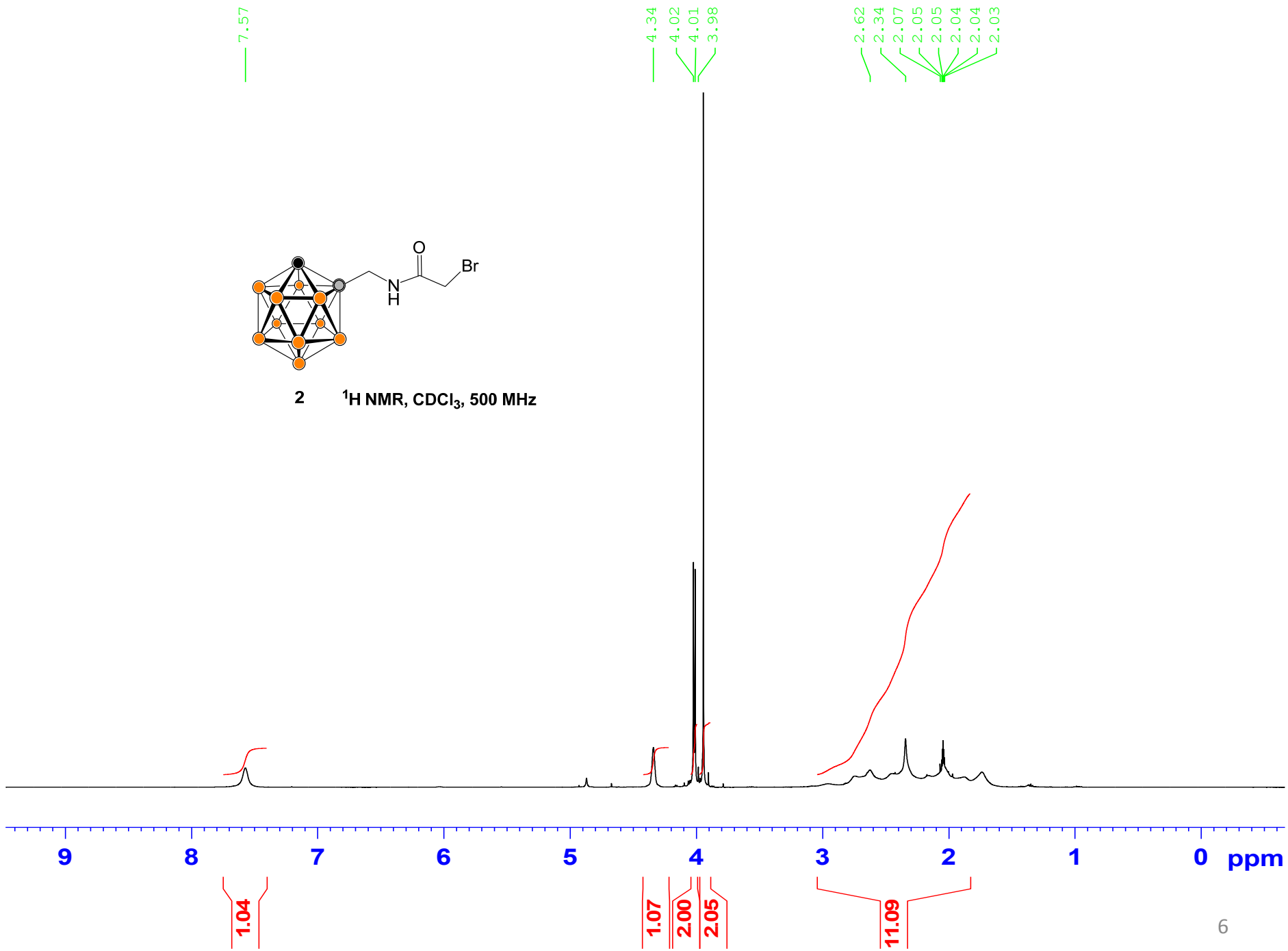


1

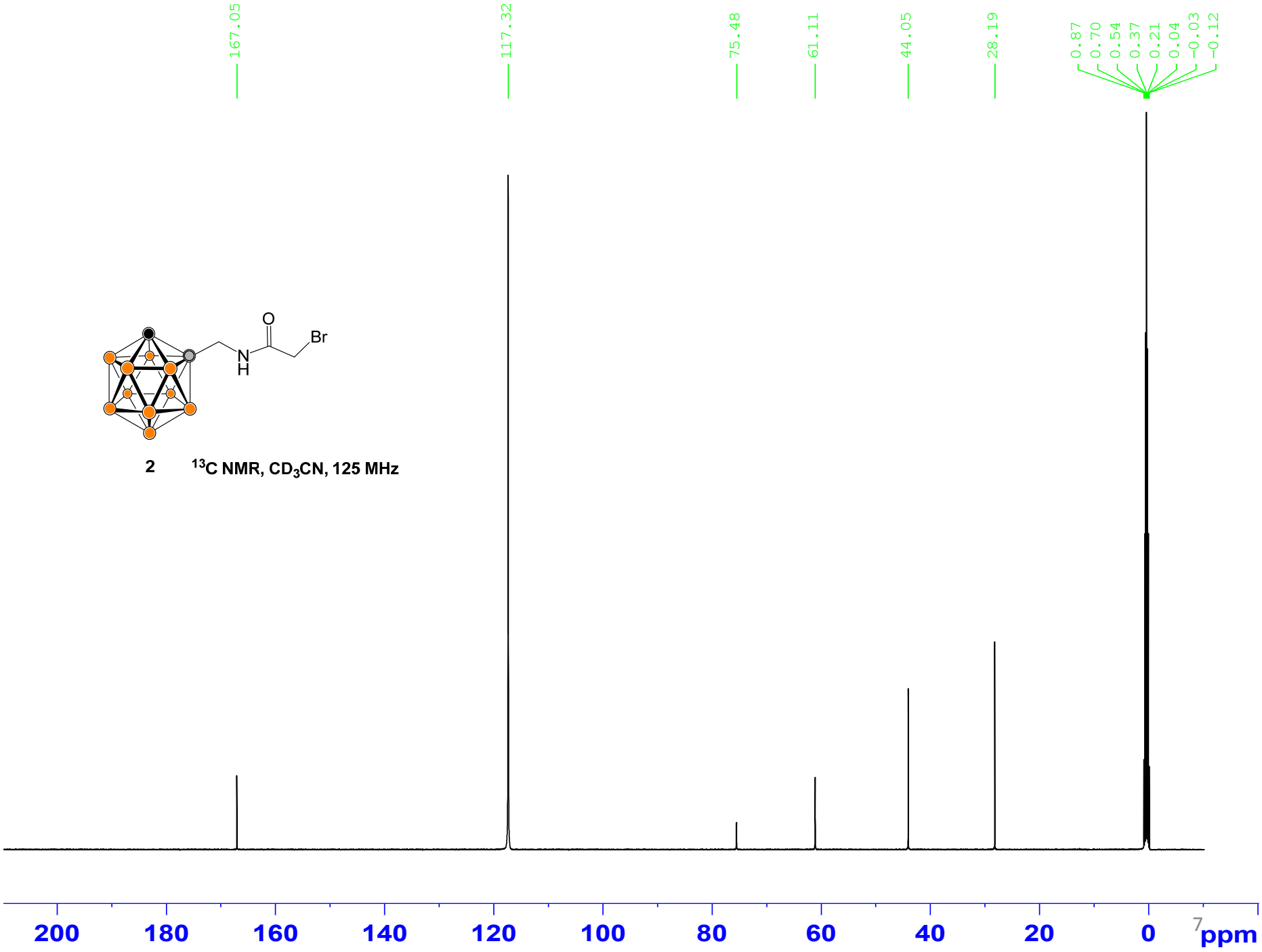
^{13}C NMR, $CDCl_3$, 500 MHz


200.0432
[M+Na]+
198.0366
159.1265
242.3931
375.0805
223.2371
373.0875
52.6197
519.5702
639.8105
737.4042
895.7505
988.2838
1078.3895
1196.9990
1313.9357
1427.9308
1612.5495
1727.3935
1867.2433
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
38.2
49.0
439.2
829.4
1219.6
1609.8
2000.0
O
Br
N
H

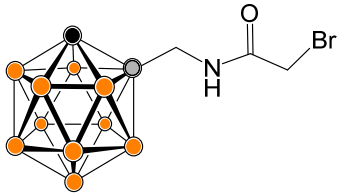
1 **HRMS**



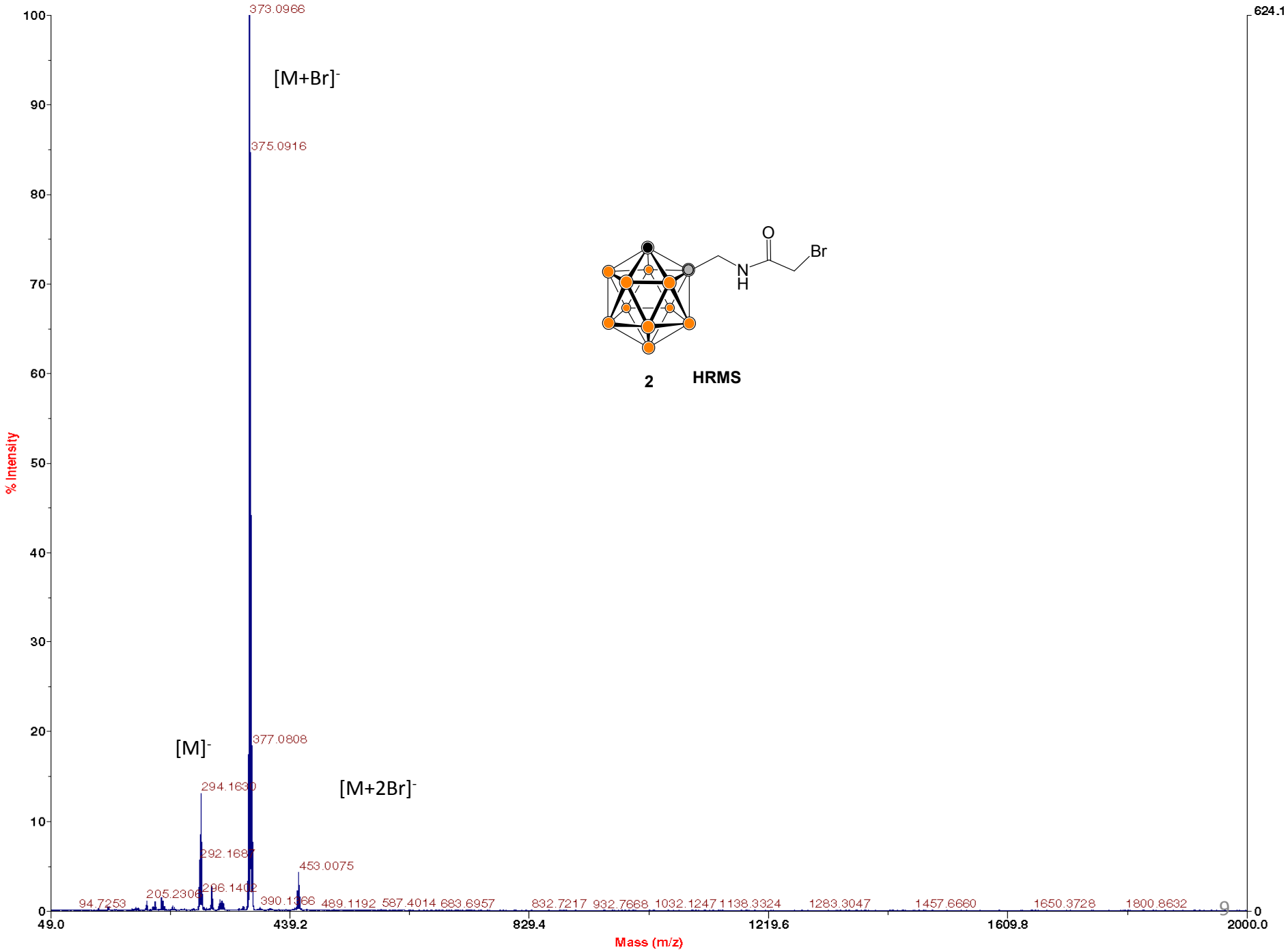
2 **^{1}H NMR, $CDCl_3$, 500 MHz**



2 ^{13}C NMR, CD_3CN, 125 MHz

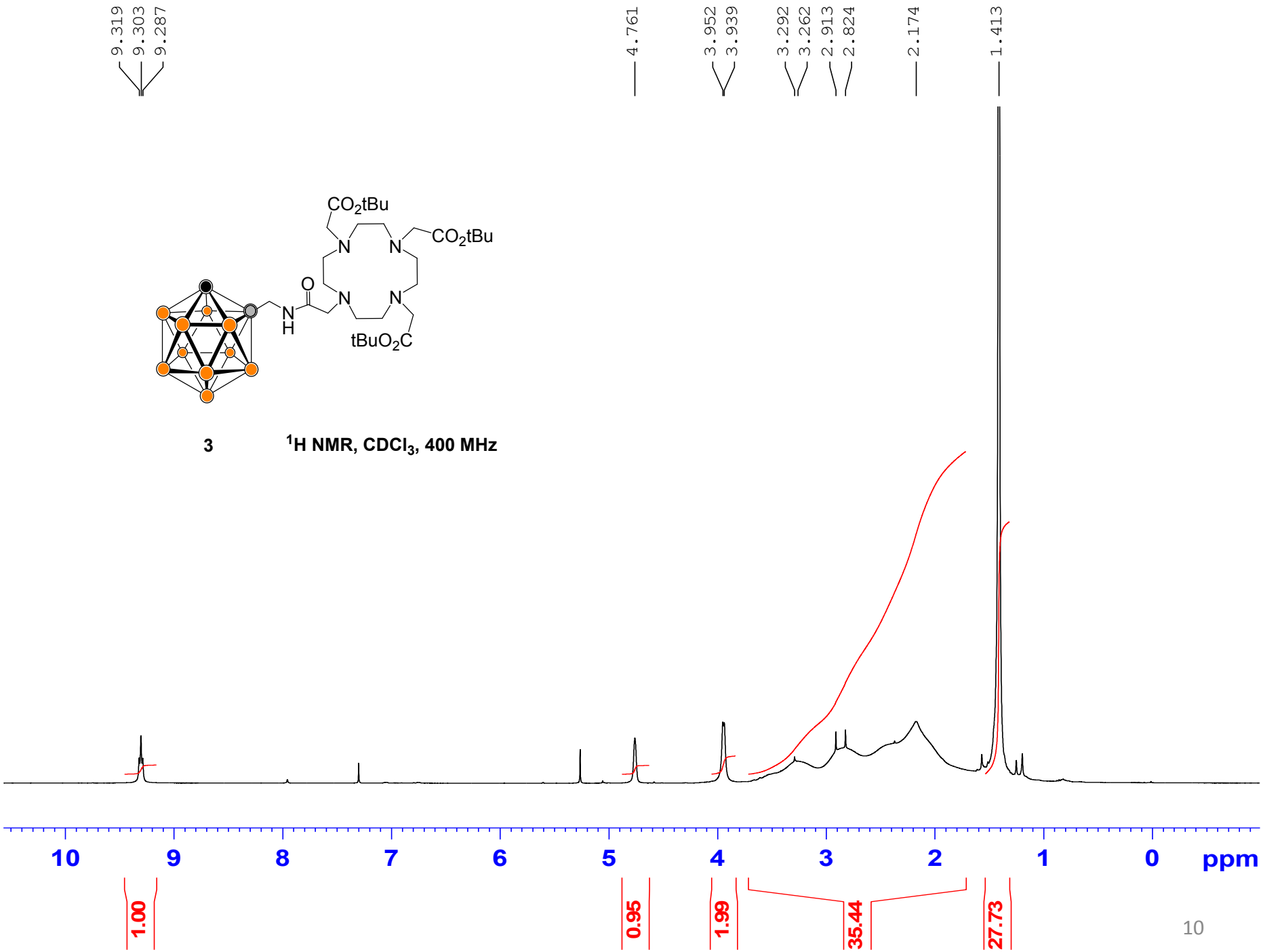


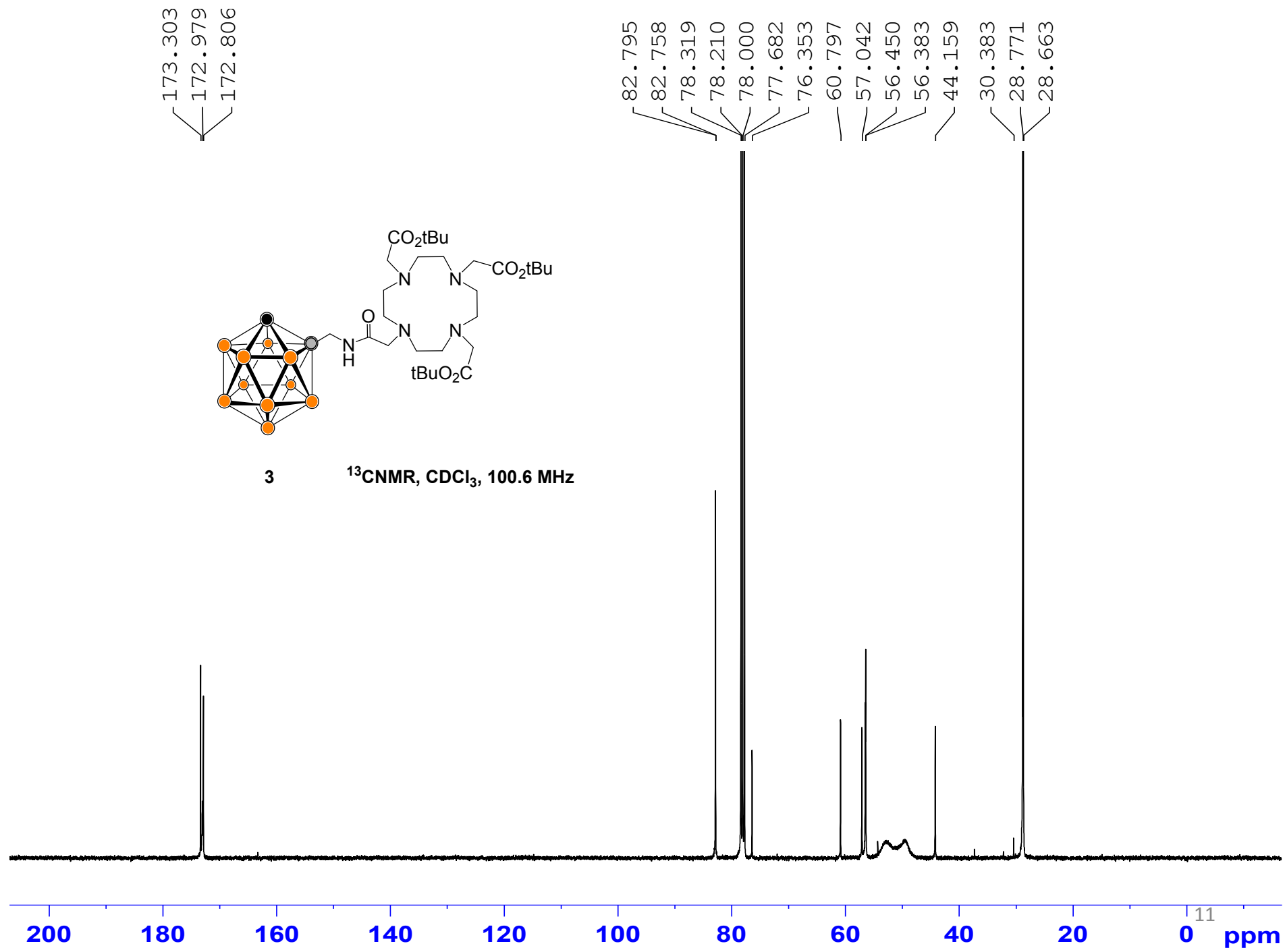
2 **^{11}B NMR, CD_3CN, 160 MHz**


373.0966
[M+Br]-
375.0916
[M]-
377.0808
[M+2Br]-
294.1630
292.1687
453.0075
296.1402
205.2306
94.7253
390.1366
489.1192
587.4014
683.6957
832.7217
932.7668
1032.1247
1138.3324
1283.3047
1457.6660
1650.3728
1800.8632
624.1
100
90
80
70
60
50
40
30
20
10
0
% Intensity
49.0
439.2
829.4
1219.6
1609.8
2000.0
Mass (m/z)

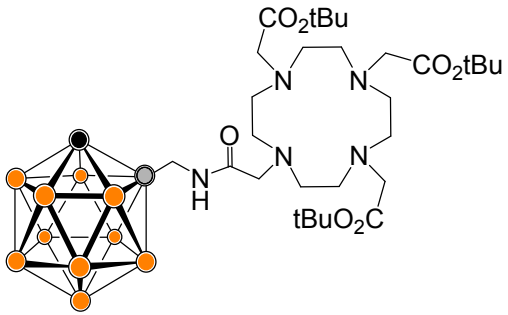
O
Br
N
H

2 **HRMS**

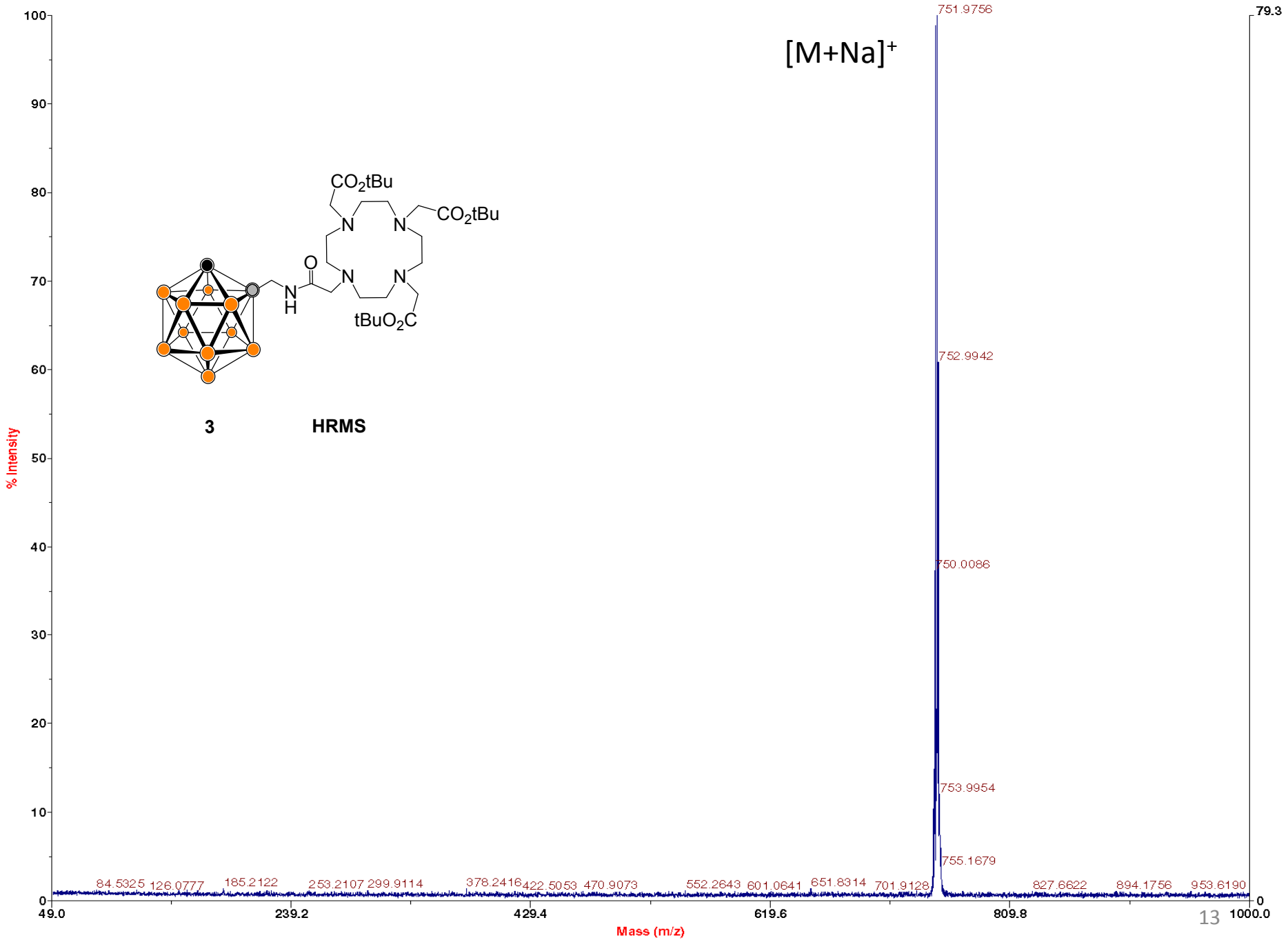

9.319
9.303
9.287
4.761
3.952
3.939
3.292
3.262
2.913
2.824
2.174
1.413
CO2tBu
CO2tBu
N
N
O
N
N
N
H
tBuO2C
3
1H NMR, CDCl3, 400 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.95
1.99
35.44
27.73

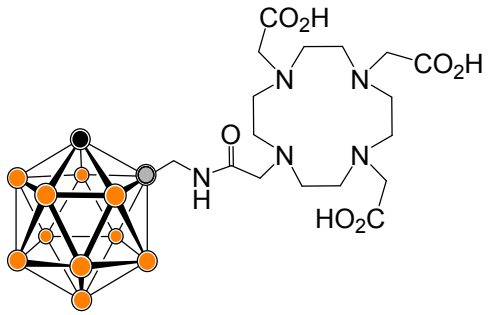


3 **^{13}CNMR, $CDCl_3$, 100.6 MHz**

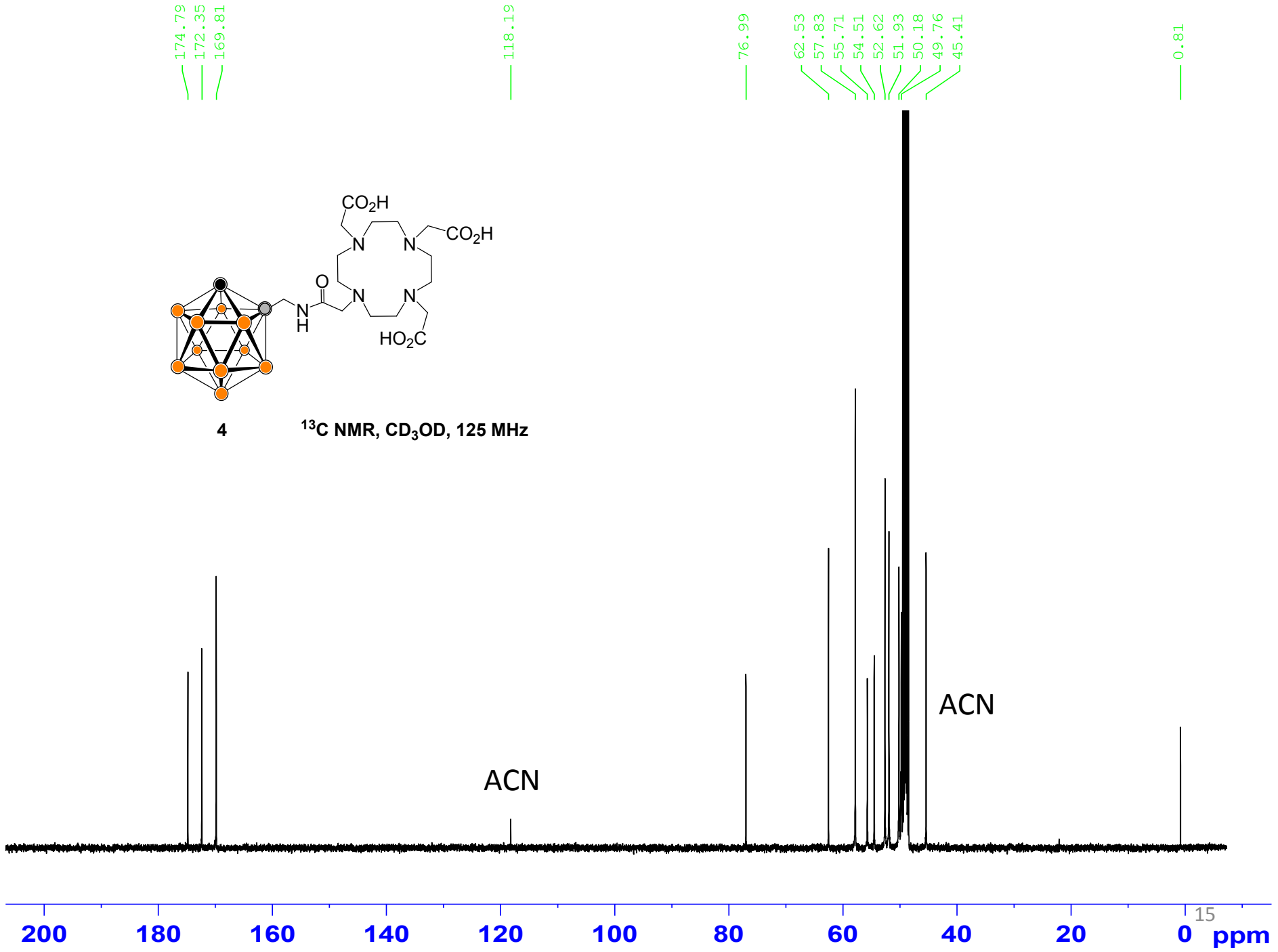


3 **^{11}B NMR (dc), $CDCl_3$, 128 MHz**

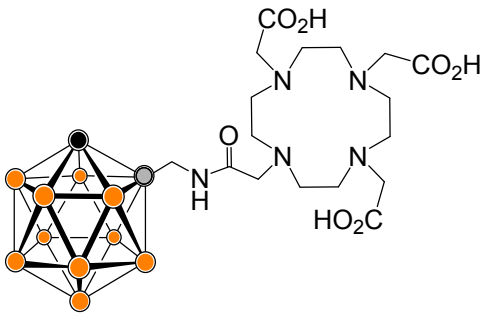

[M+Na]+
CO2tBu
CO2tBu
tBuO2C
O
N
H
3
HRMS
% Intensity
Mass (m/z)
751.9756
752.9942
750.0086
753.9954
755.1679
84.5325
126.0777
185.2122
253.2107
299.9114
378.2416
422.5053
470.9073
552.2643
601.0641
651.8314
701.9128
827.6622
894.1756
953.6190
79.3
49.0
239.2
429.4
619.6
809.8
1000.0



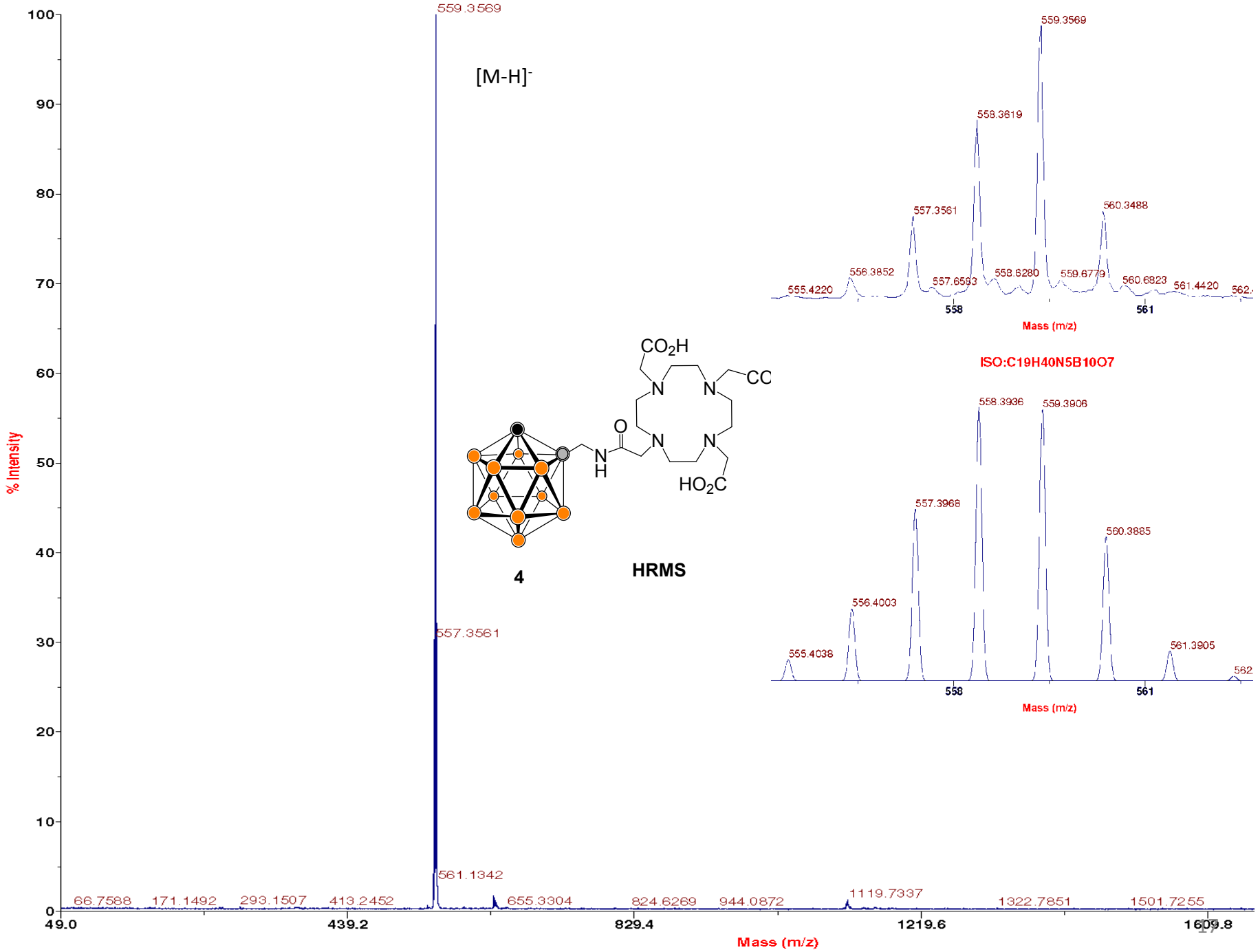
4 **^{1}H NMR, CD_3OD, 500 MHz**

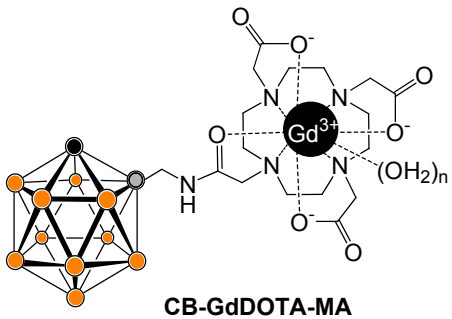


4 **^{13}C NMR, CD_3OD, 125 MHz**



4 **^{11}B NMR, CD_3OD, 160 MHz**

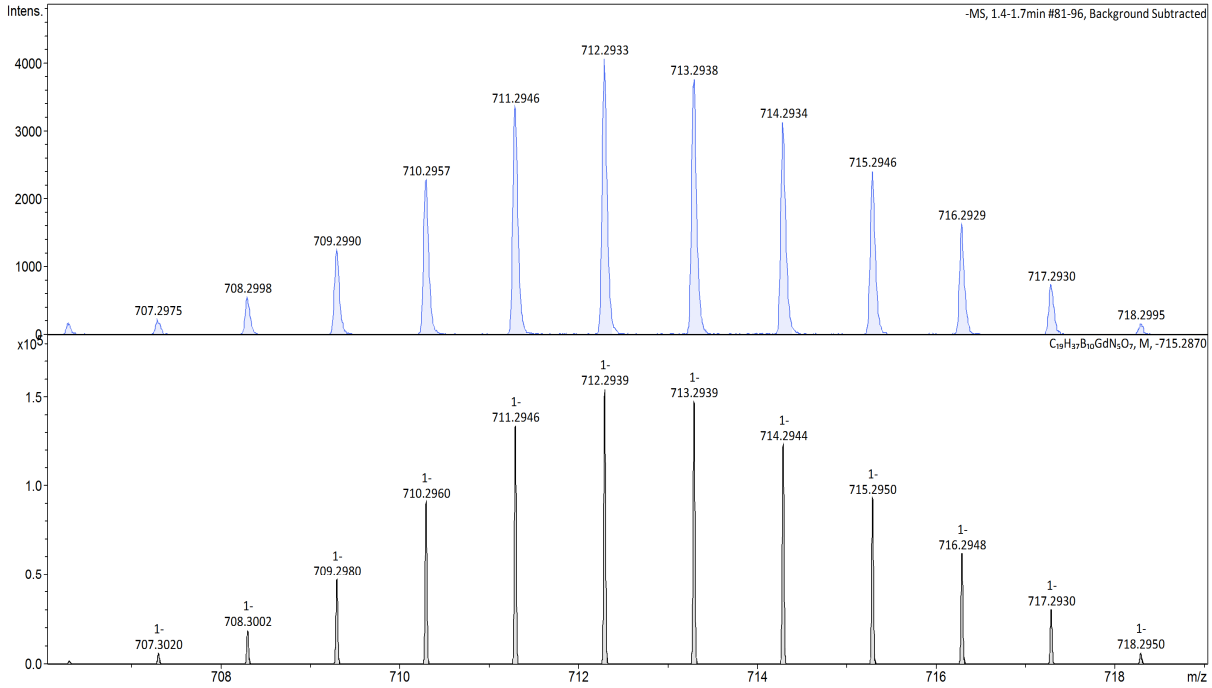

559.3569
[M-H]-
100
90
80
70
60
50
40
30
20
10
0
% Intensity
557.3561
561.1342
66.7588
171.1492
293.1507
413.2452
655.3304
824.6269
944.0872
1119.7337
1322.7851
1501.7255
49.0
439.2
829.4
1219.6
1609.8
Mass (m/z)
CO2H
CC
N
N
O
N
N
N
H
HO2C
4
HRMS
559.3569
558.3619
557.3561
560.3488
556.3852
555.4220
557.6583
558.6280
559.6779
560.6823
561.4420
562.
558
561
Mass (m/z)
ISO:C19H40N5B10O7
558.3936
559.3906
557.3968
560.3885
556.4003
555.4038
561.3905
562
558
561
Mass (m/z)

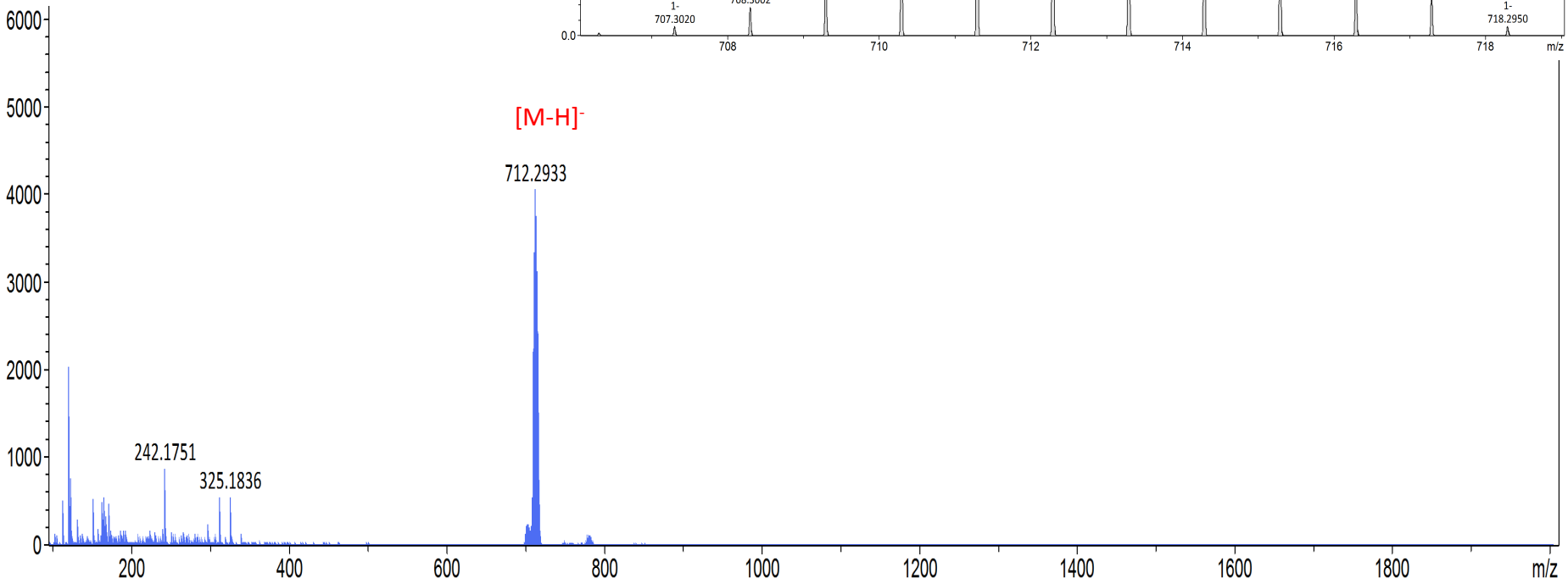


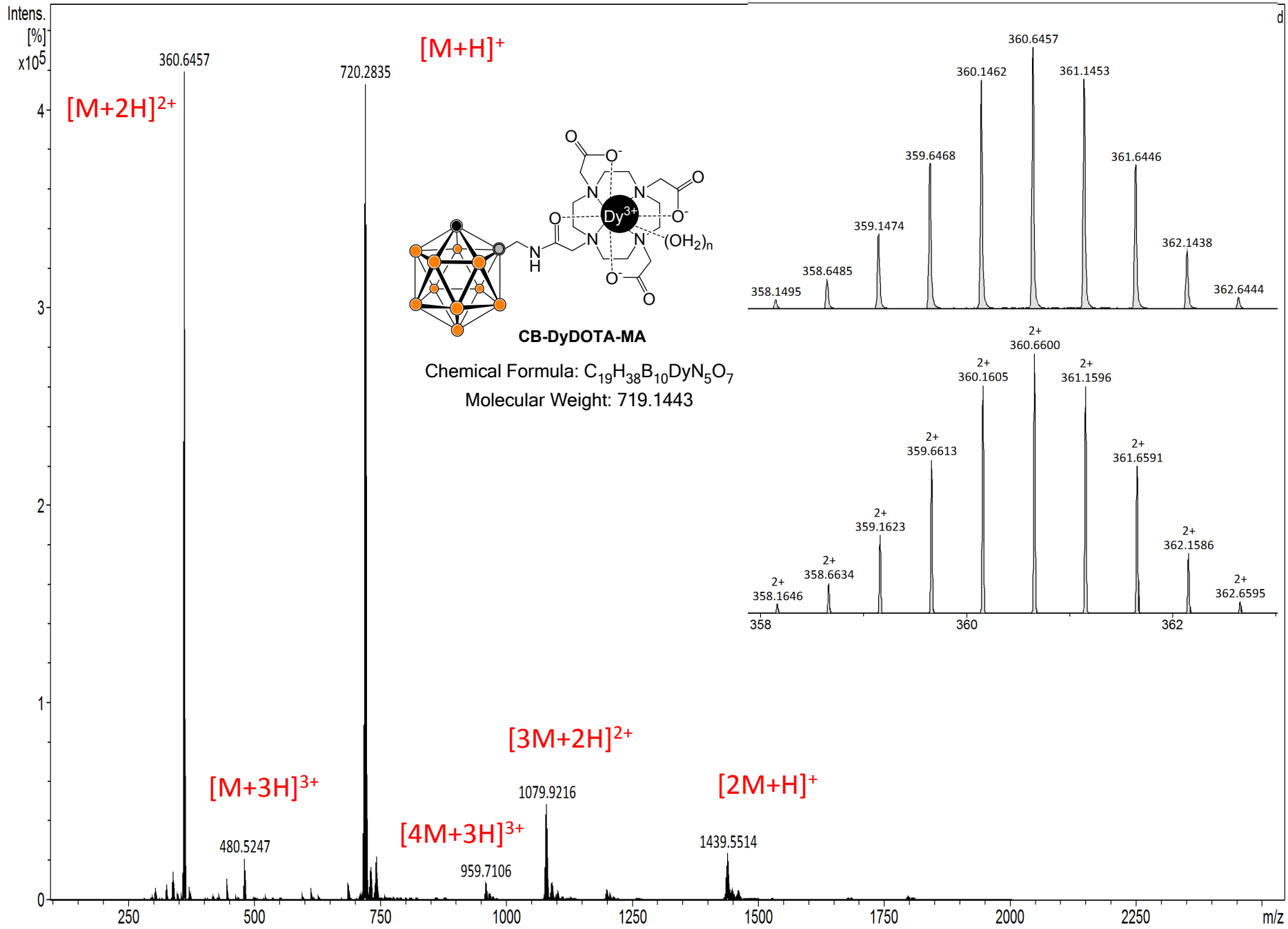
CB-GdDOTA-MA

Chemical Formula: $C_{19}H_{38}B_{10}GdN_5O_7$

Molecular Weight: 713.8943






Intens.
[%]
x10^5
4
3
2
1
0
360.6457
720.2835
[M+2H]2+
[M+H]+
[M+3H]3+
480.5247
[3M+2H]2+
1079.9216
[4M+3H]3+
959.7106
[2M+H]+
1439.5514
250
500
750
1000
1250
1500
1750
2000
2250
m/z
CB-DyDOTA-MA
Chemical Formula: C19H38B10DyN5O7
Molecular Weight: 719.1443
d
360.6457
360.1462
361.1453
359.6468
361.6446
359.1474
362.1438
358.6485
358.1495
362.6444
2+
360.6600
2+
360.1605
2+
361.1596
2+
359.6613
2+
361.6591
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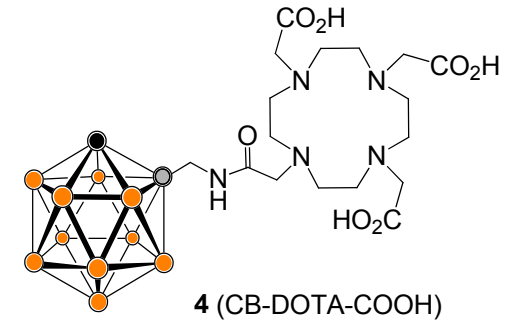
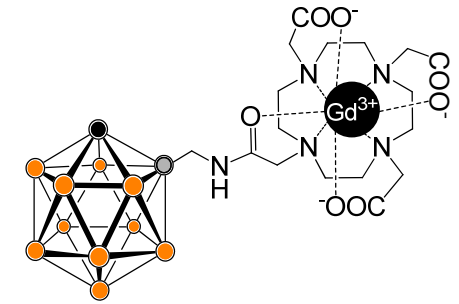
2+
358.1646
2+
362.6595
358
360
362



4 (CB-DOTA-COOH)



CB-GdDOTA-MA

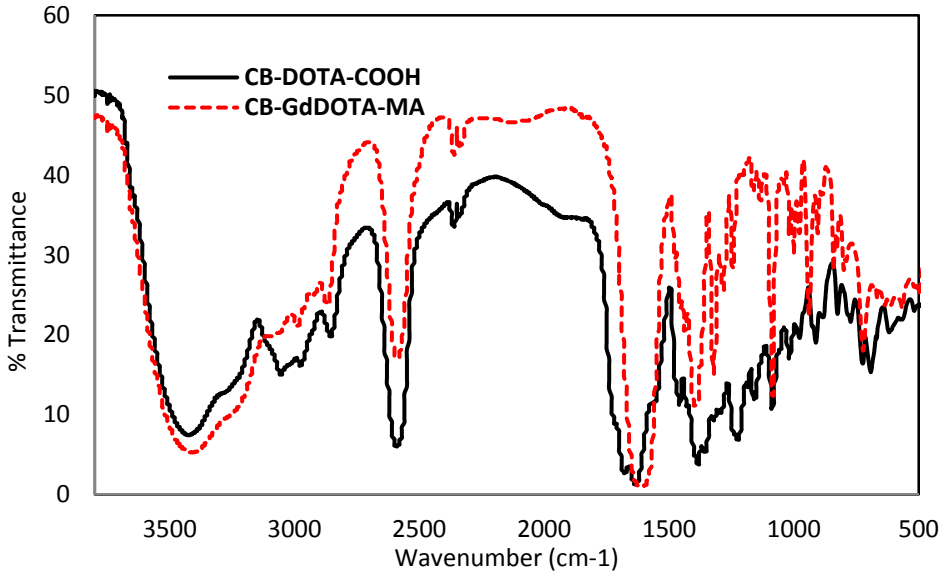


Fig. S1 IR spectrums of compound **4** and **CB-GdDOTA-MA**.

Analytical HPLC analysis of **CB-GdDOTA-MA**
Instrument: Alliance Waters 2695.
Column: Agilent Zorbax SB-C18, 5 µm (4.6 x 250 mm).
Eluent: 1 mL/min; gradient: B 0% to 100% over 45 min,
B 100% 45-60 min (A: water, B: acetonitrile).
Detector: Evaporative Scattering Detector (SEDEX 75,
operated at 45 °C and 3.5 bars using N_2).

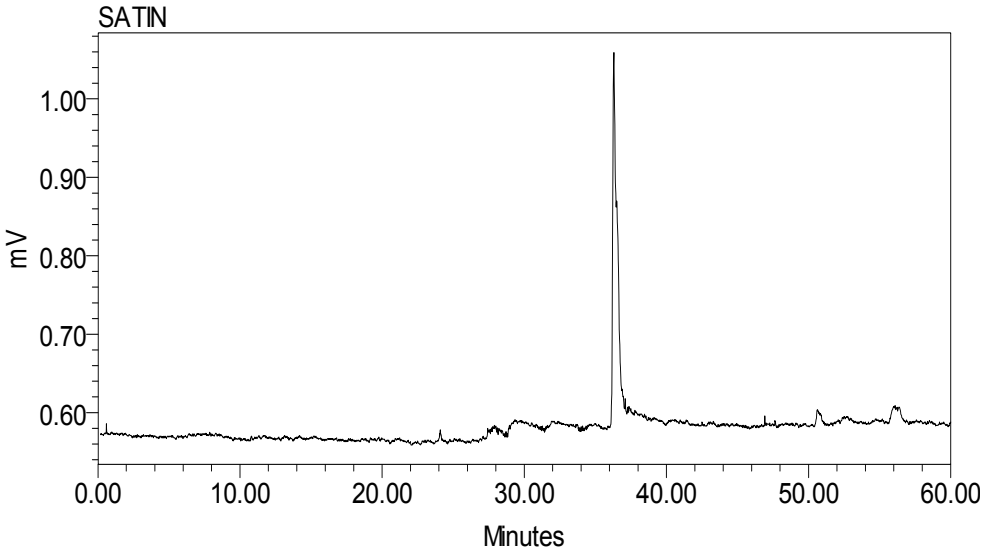


Fig. S2 HPLC trace of **CB-GdDOTA-MA**.

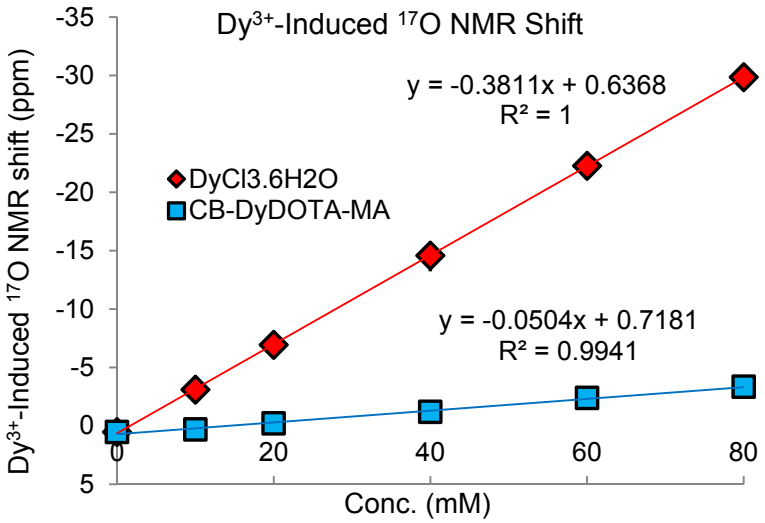


Fig. S3 Determination of Hydration number (q) for **CB-DyDOTA-MA**.

Determination of hydration number (q) for CB-DyDOTA-MA: Varying concentrations of **CB-DyDOTA-MA** 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 using a Bruker Ascend 400 MHz NMR instrument at RT with the deuterium signal locked. A graph was plotted between the Δδ and the concentration for both **CB-DyDOTA-MA** 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;

$$(\Delta\delta) = q\Delta[Dy(ligand)_n(H_2O)_q]/[H_2O] \quad \text{(I)}$$

The slope of a plot of the Δδ 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 **CB-DyDOTA-MA**.

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 = -381 ppm dm^3 mol^{-1} or,

qΔ/[H2O] = -381 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] = -381 ppm dm^3 mol^{-1} /9 or

Δ/[H2O] = - 42.3 ppm dm^3 mol^{-1}

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

For complex **CB-DyDOTA-MA**, Slope = qΔ/[H2O], or

q = slope/(Δ/[H2O]) or q = -50.4/-42.3, hence

q = 1.2

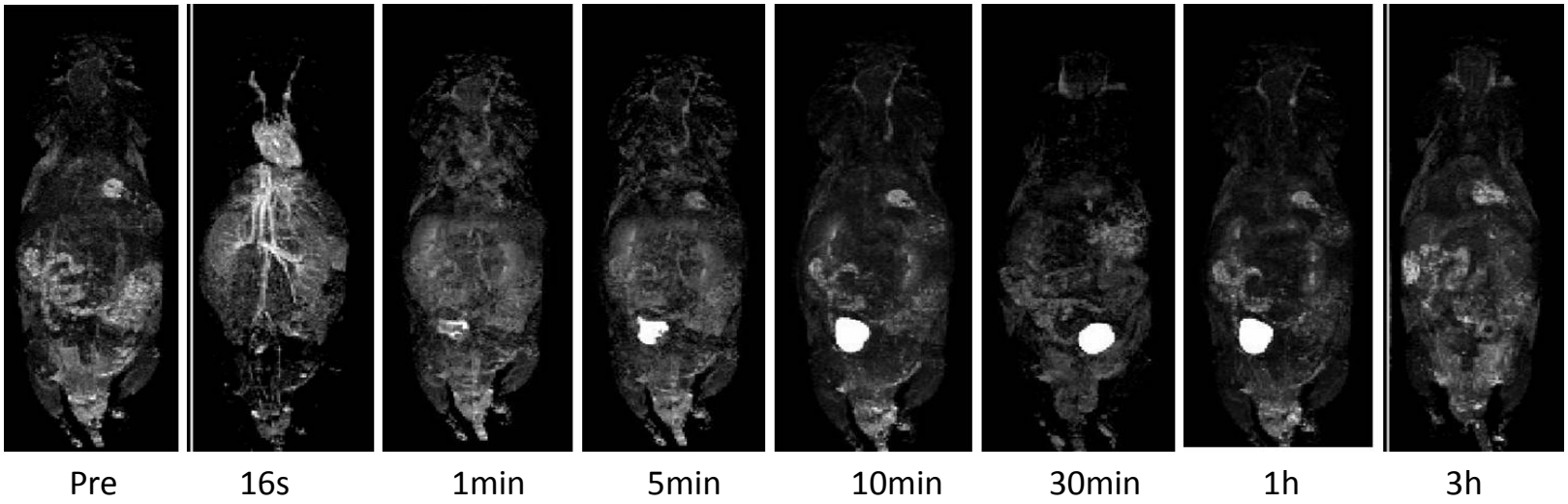


Fig. S4 Representative *in vivo* T1-weighted MRA scans of a mouse at various time points p.i. of Omniscan at a Gd dose of 1.2 mmol/kg.