Supporting information for

Fluorescent Glutamine and Asparagine as Promising Probes for Chemical Biology

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1. UV and Fluorescent Instrumentation and characterization.

UV-Vis absorption spectra were collected on a Jasco V-750 UV-Visible spectrophotometer using a 1.0 cm quartz cuvette at room temperature over a wavelength range of 200-600 nm. Fluorescence spectra were recorded on a FlouroMax-4 steady state spectrofluorometer using a 1.0 cm quartz cuvette at 293 K. The fluorescence spectra of **7a** and **7b** were collected in various solvents at excitation wavelength (λ_{ex}) of 323 nm with an excitation slit 5 nm and emission slit 5 nm. The data were analyzed using related software.

1.1. Fluorescence quantum yield measurement. Quinine sulfate standard solution (10^{-3} M) was prepared by dissolving (0.0037 g) in 0.1 N H_2SO_4 (0.005 L). 10^{-5} M Quinine sulfate was prepared by diluting 10^{-3} M concentration in 0.1N H_2SO_4. Stock solutions of **7a** and **7b** (10^{-3} M) were prepared by dissolving (0.00150 g) of **7a** and (0.00160 g) of **7b** in different solvents (0.005 L). Further dilutions (10^{-5} M concentration) were made by diluting aliquots of 10^{-3} M concentrations in relevant solvents. Each solution was mixed using vortex. Fluorescence spectra of quinine sulfate was recorded at an excitation wavelength of 345 nm and emission wavelength set at 360-800 nm and using an excitation and emission slit width of 5 nm. The emission and excitation spectra of **7a** and **7b** were set at 323 nm and 338-700 nm respectively. The data were compared with standard quinine sulfate (Φ_r= 0.54 in 0.1N H_2SO_4) using equation (Eq.1). The quantum yields of **7a** and **7b** (Φ_S) are calculated and the results were incorporated in table 1.

$$\phi_s = \phi_r \frac{F_s A_r \eta_s^{\,2}}{F_r A_s \eta_r^{\,2}} \quad \ldots \text{(Eq. 1)}$$

Where, A_r and A_s *are* the integrated absorbance spectrum area (area under the absorbance curve, peak area); F_r and F_s are the relative integrated fluorescent intensities (area under the fluorescence curve, peak area) of the reference and samples respectively. η_r and η_s are respectively the refractive indices of the solvents in which the reference standard and samples are prepared.

1.2 Photostability study. A series of fluorescence spectra (λ_{ex}=323 nm, excitation slit of 5 nm and emission slit of 5 nm) of **7a** and **7b** samples (10^{-5} M concentration in water) were recorded at 293 K over a period of 2 h. 61 scans were collected over a period of 2 h and each spectrum was recorded 120 seconds after the previous spectrum until 61 spectra was recorded. An insignificant

decrement in the fluorescence intensity (λ_{em}= 464 nm) of **7a** (5.8 %) and **7b** (7.8 %) was observed, which demonstrates that the compounds **7a** and **7b** were resistant to photobleaching.

1.3. Solvachromatic study. Solvent study was carried out by dissolving requisite amount of compounds **7a** and **7b** in Water, Methanol, DMF, DMSO and PBS buffer respectively. Due to the low solubility of **7a** and **7b** in Acetonitrile (CAN), stock solution was prepared in 1:1 solvent mixture of ACN:Water. The solutions were mixed using a vortex and fluorescence emission spectra (λ_{ex}= 323 nm, excitation slit of 5 nm and emission slit of 5 nm) were recorded at 295 K.

1.4. Optical Rotation measurement

Optical rotation was measured by optical spectrophotometry using a Rudolph Research Analytical Autopol III Automatic Polarimeter. The specific rotation ([α]) of the FAA **7a** and **7b** (1mg/mL in MeOH) was measured by optical spectrophotometry using a Rudolph Research Analytical Autopol III Automatic Polarimeter at 589 nm wavelength, 303 K and with an optical path length 100 mm, and calculated according to given formula:

Specific Rotation $[\alpha]^{T}_{\lambda} = \alpha/l\,x\,c$

Where, α = degree of rotation, l = optical path length (in dm), c = concentration in g/mL

2. Cell Culture and Confocal imaging

HT-29 cell line obtained from NCCS, Pune, India was seeded on glass coverslip placed in Tarson six-well cell culture plate at 5000 cells per well. Cells were grown overnight in DMEM (Dulbecco's Modified Eagle Medium) media with 10 % FBS (Fetal bovine serum) at 37 °C in a 5% CO_2 incubator (Thermo Scientific, India). Before treatment with fluorescent derivative compounds, cells were washed with PBS to removed unbound cells. Finally, to investigate the biocompatibility of chemically synthesized fluorescent amino acids (FAAs) in live cells, 5 μM of the final concentration of both **7a** and **7b** were added separately in the well and allowed to incubate for 1 hr. Cells were washed 4 times to remove the surface-bound and free FAAs before microscopy. Confocal microscopy images were obtained using a confocal laser scanning microscope-880 (Zeiss, Germany).

3. **Protein extraction and fluorescence spectrum analysis**

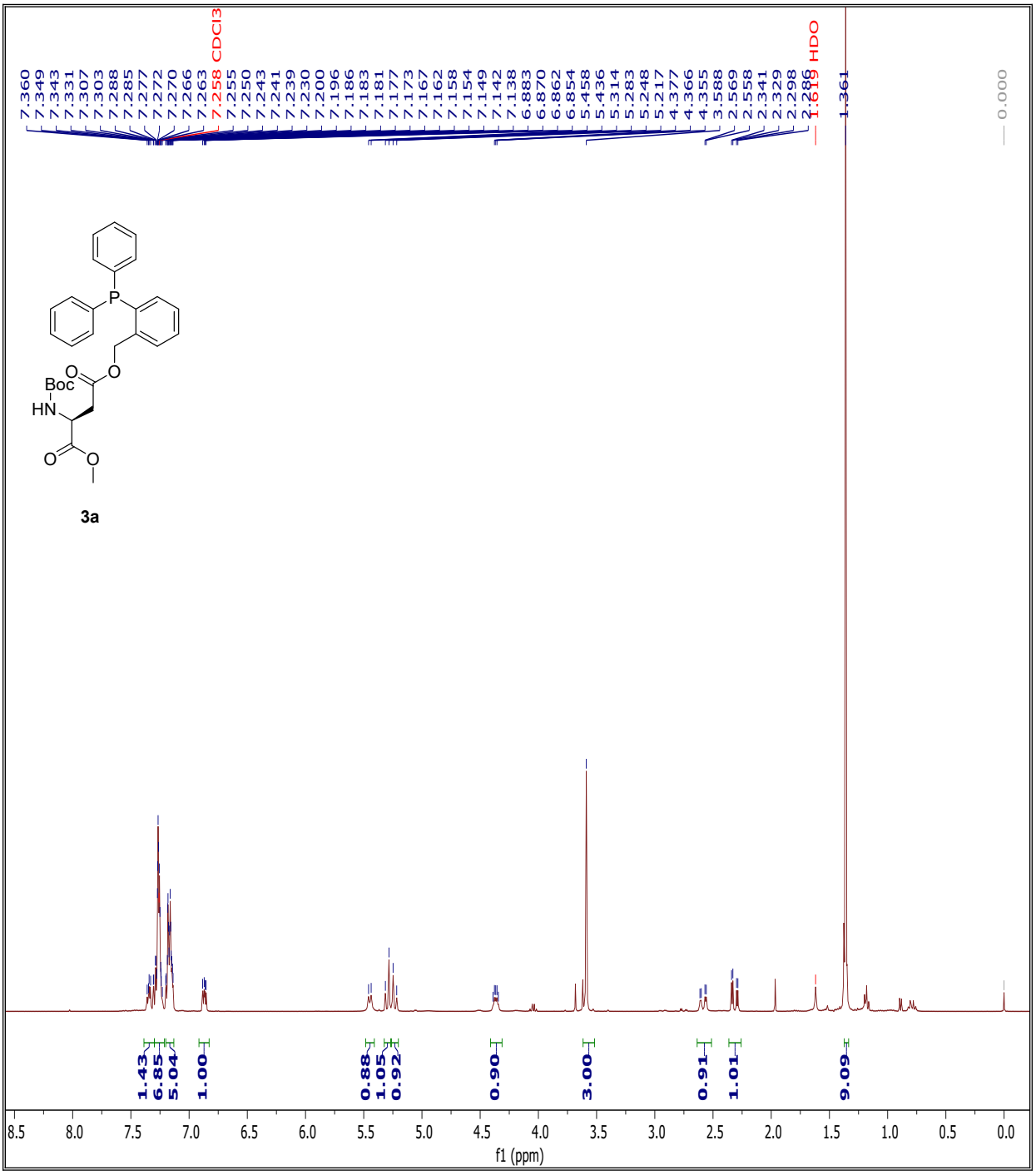
To further explore the genetically incorporation of an unnatural fluorescent amino acid into a peptide, around 50,000 cells were treated with both the compound with a final concentration of 5 μM and incubated overnight. Cells were washed with PBS and total protein was extracted using RIPA buffer.[1] The fluorescence spectrum of total protein of cells treated with **7a** and **7b** and non-treated cells were captured using Fluorolog Horiba, USA.

4. **References**

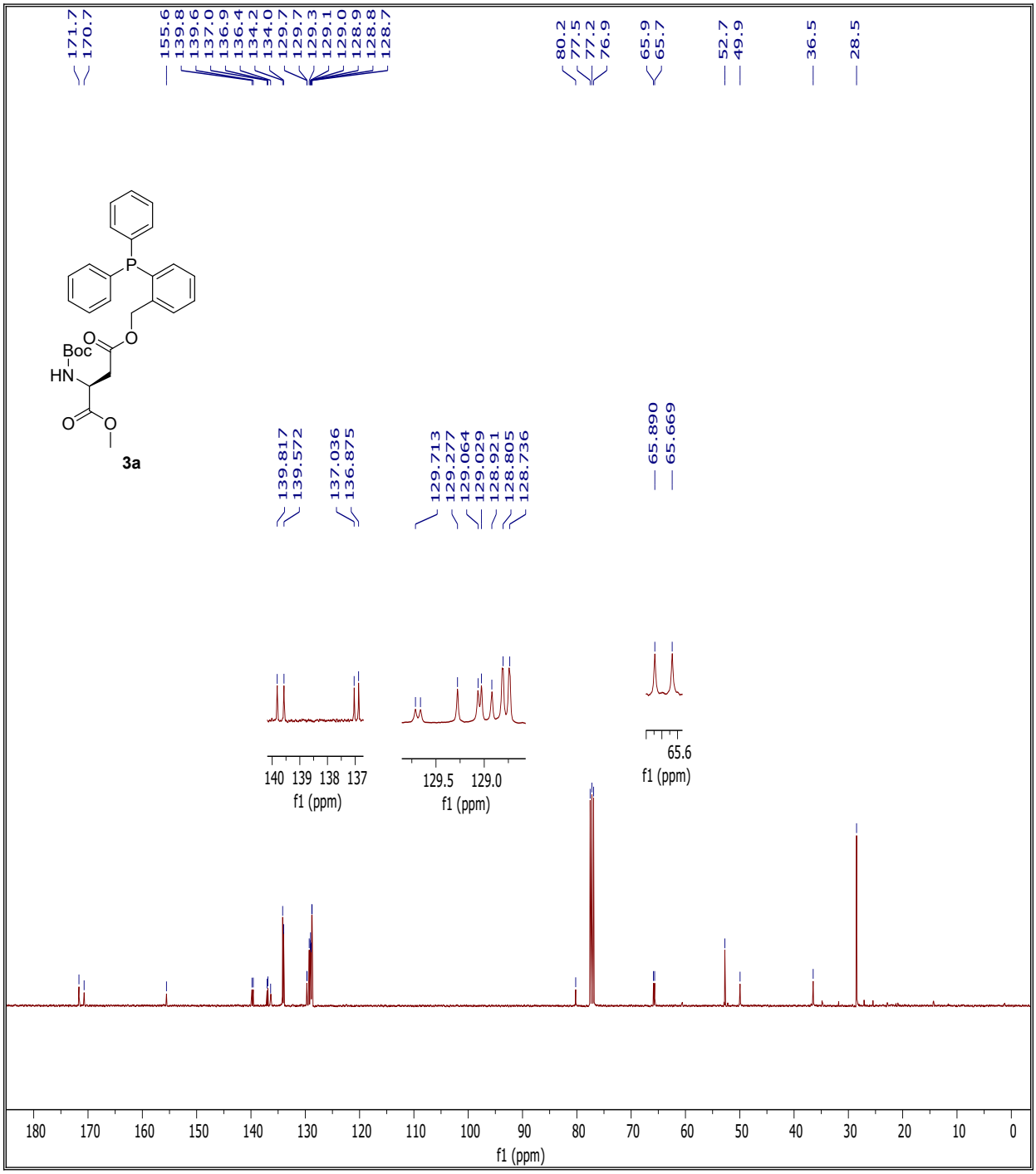
1. Y. Liu, W. F. Bodme, *PNAS*. 2006, **103,** 976.

5. NMR Spectra of Reported Compounds

^{1}H NMR of 3a



^{13}C NMR of 3a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129.0
128.9
128.8
128.7
80.2
77.5
77.2
76.9
65.9
65.7
52.7
49.9
36.5
28.5
Boc
HN
3a
139.817
139.572
137.036
136.875
129.713
129.277
129.064
129.029
128.921
128.805
128.736
65.890
65.669
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f1 (ppm)
129.5 129.0
f1 (ppm)
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f1 (ppm)
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f1 (ppm)

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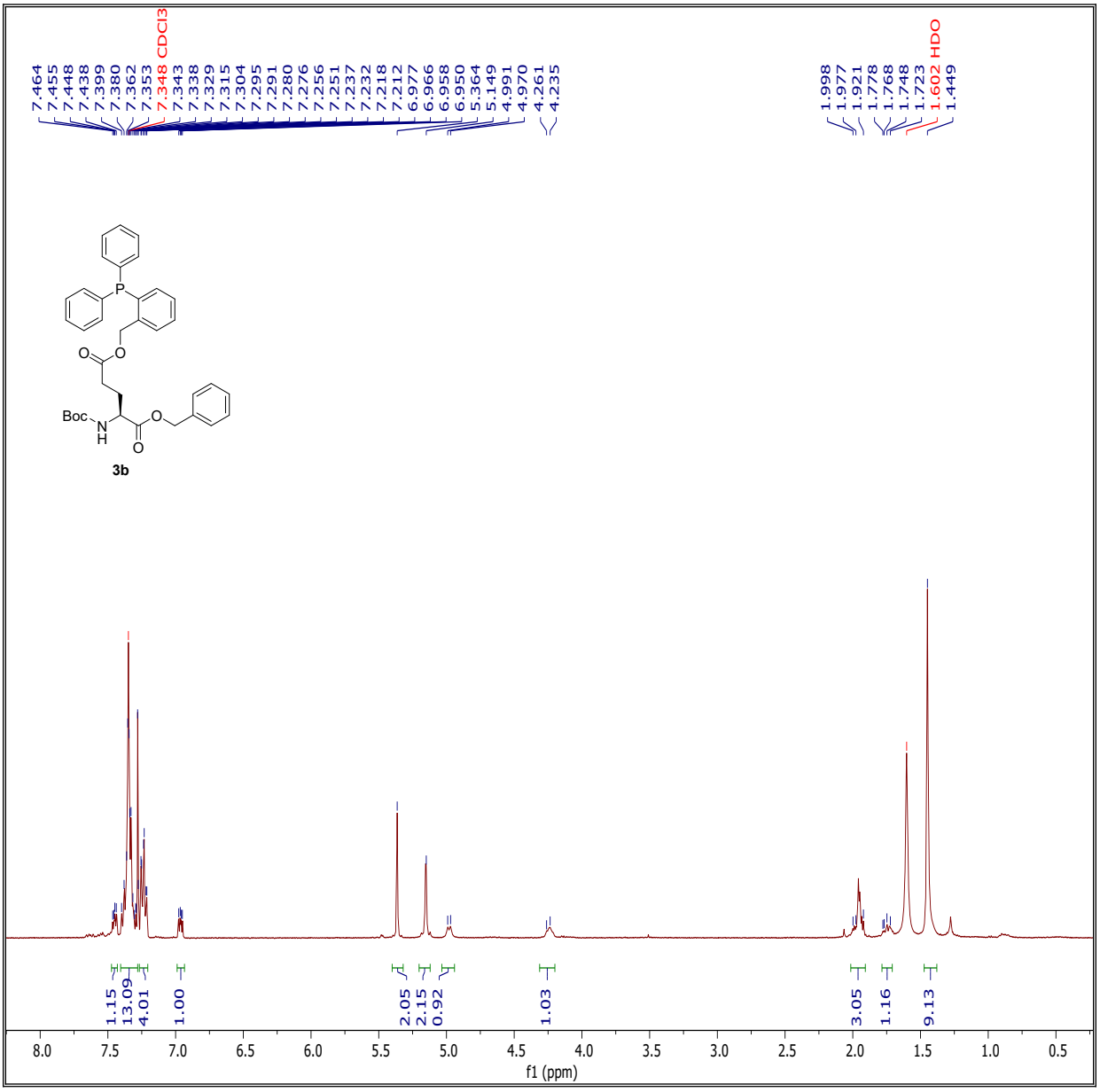
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7.295
7.291
7.280
7.276
7.256
7.251
7.237
7.232
7.218
7.212
6.977
6.966
6.958
6.950
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4.970
4.261
4.235
1.998
1.977
1.921
1.778
1.768
1.748
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Boc
N
H
O
O
O
O
P
3b
1.15
13.09
4.01
1.00
2.05
2.15
0.92
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3.05
1.16
9.13
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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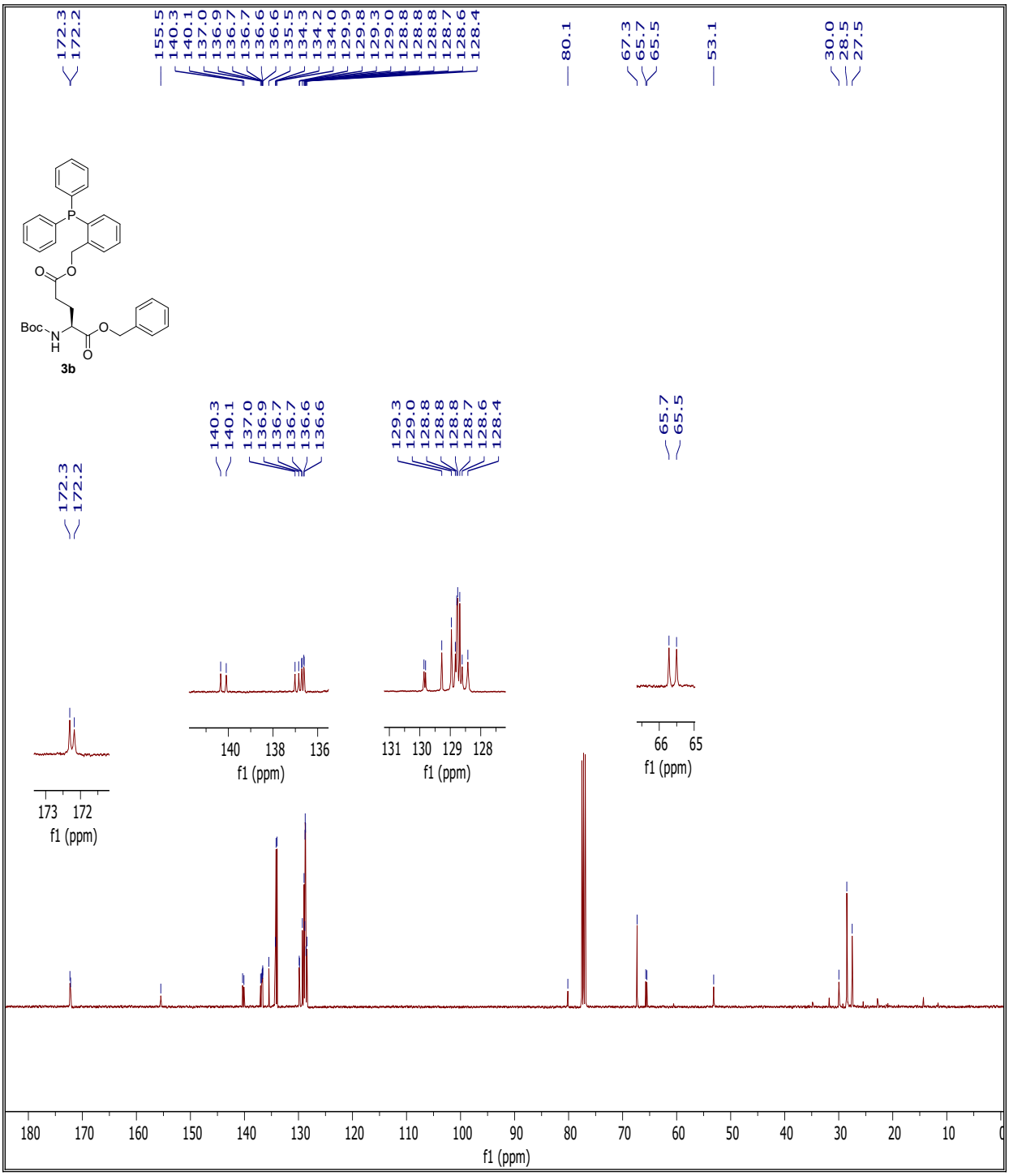
^{13}C NMR of 3b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129.9
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129.0
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128.8
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128.7
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80.1
67.3
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65.5
53.1
30.0
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Boc
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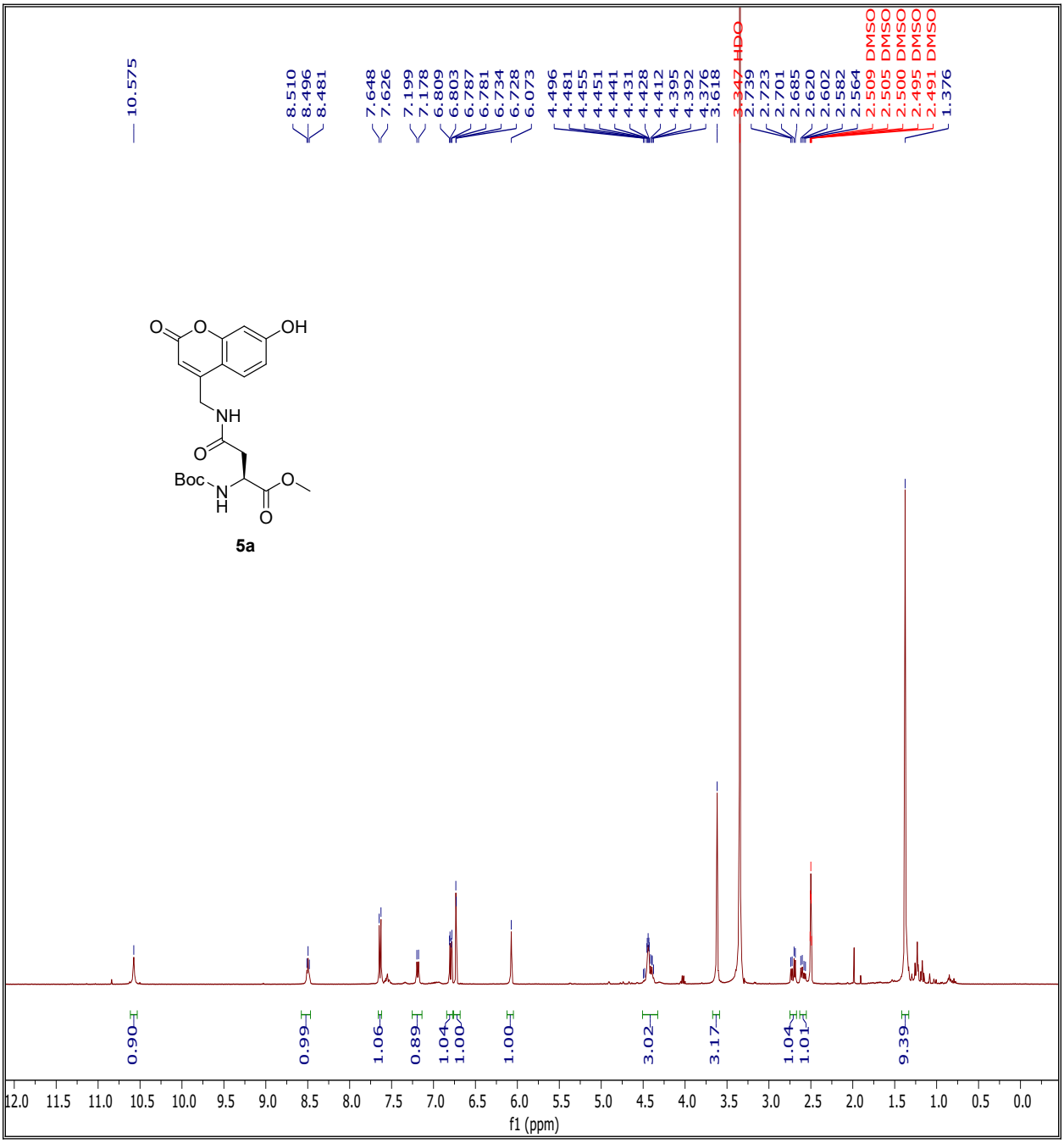
P
3b
f1 (ppm)

^{1}H NMR of 5a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4.481
4.455
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4.441
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4.412
4.395
4.392
4.376
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O
Boc
N
H
O
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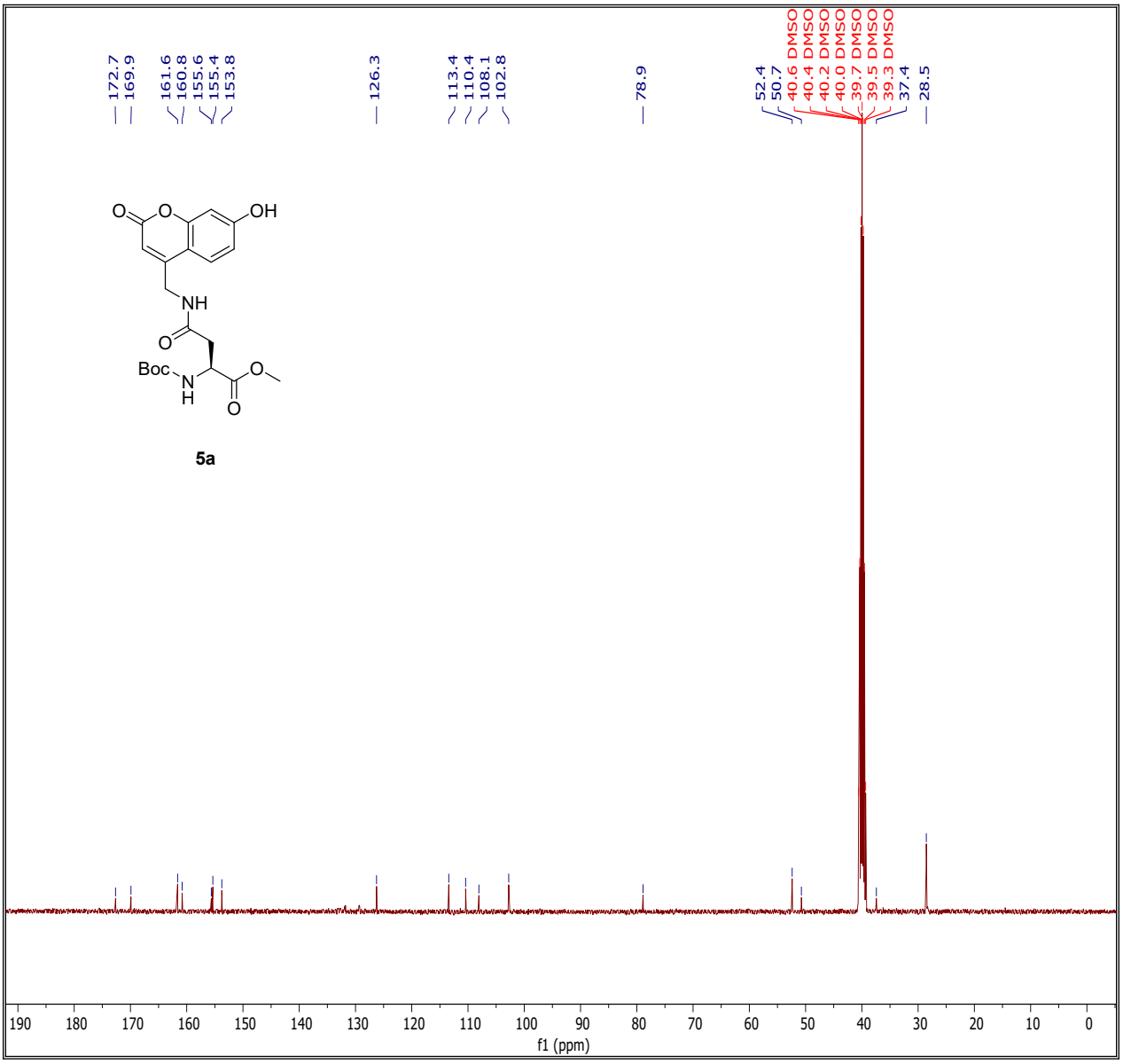
5a
0.90
0.99
1.06
0.89
1.04
1.00
1.00
3.02
3.17
1.04
1.01
9.39
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

^{13}C NMR of 5a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O
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Boc
N
H
O
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5a
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180
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160
150
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f1 (ppm)

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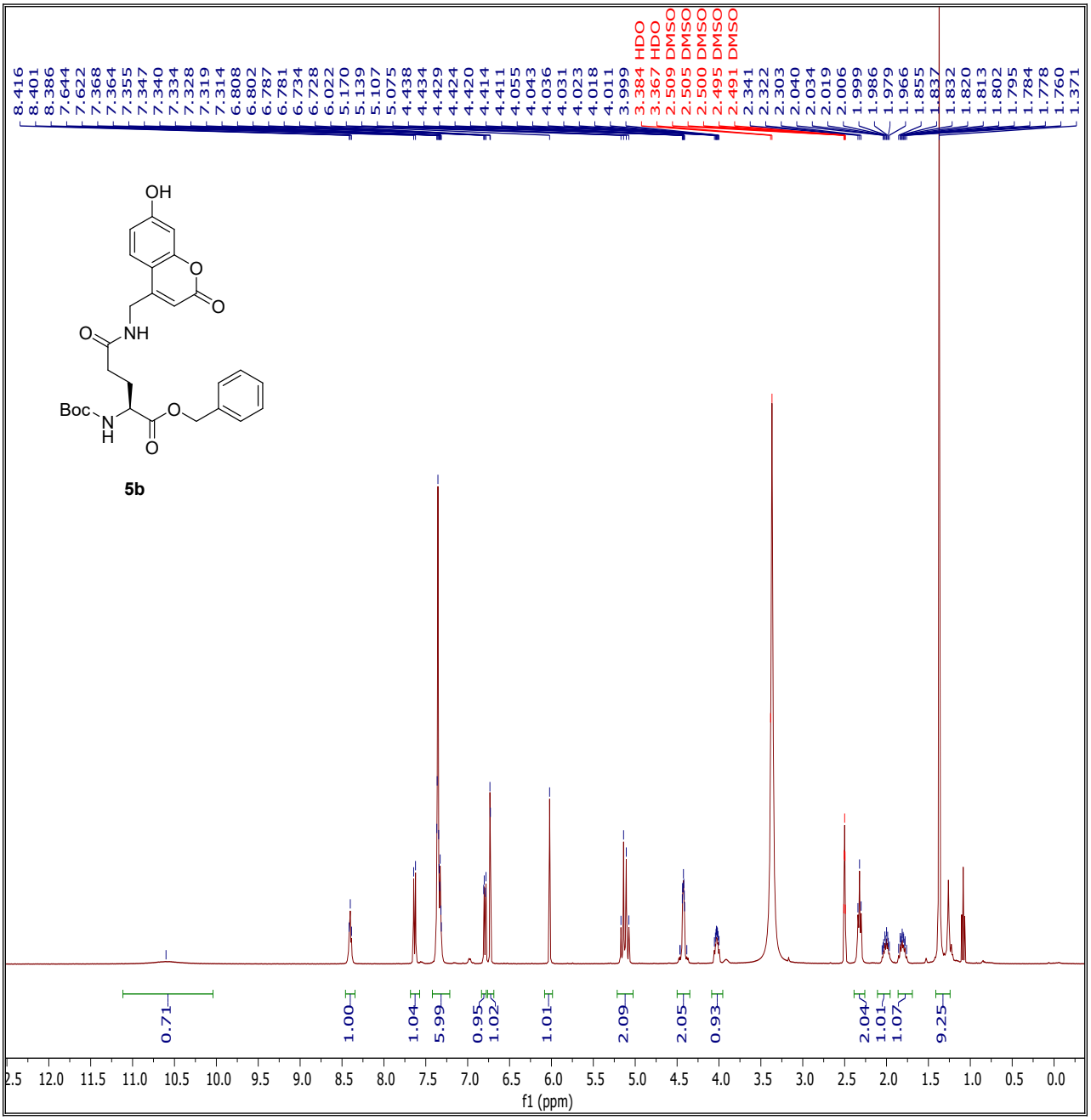
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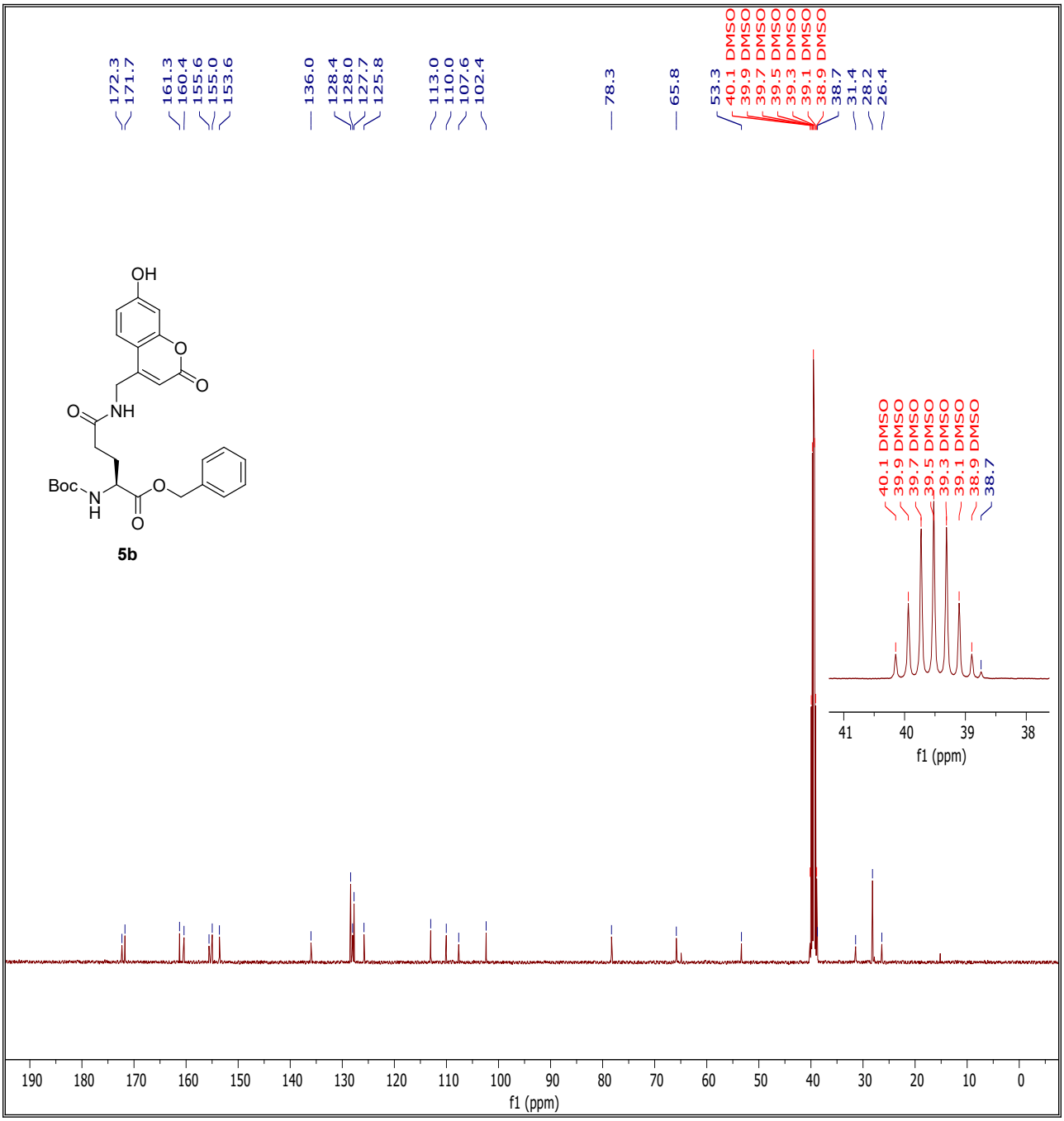
^{13}C NMR of 5b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53.3
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28.2
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Boc
N
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5b
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39.7 DMSO
39.5 DMSO
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39.1 DMSO
38.9 DMSO
38.7
41
40
39
38
f1 (ppm)
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180
170
160
150
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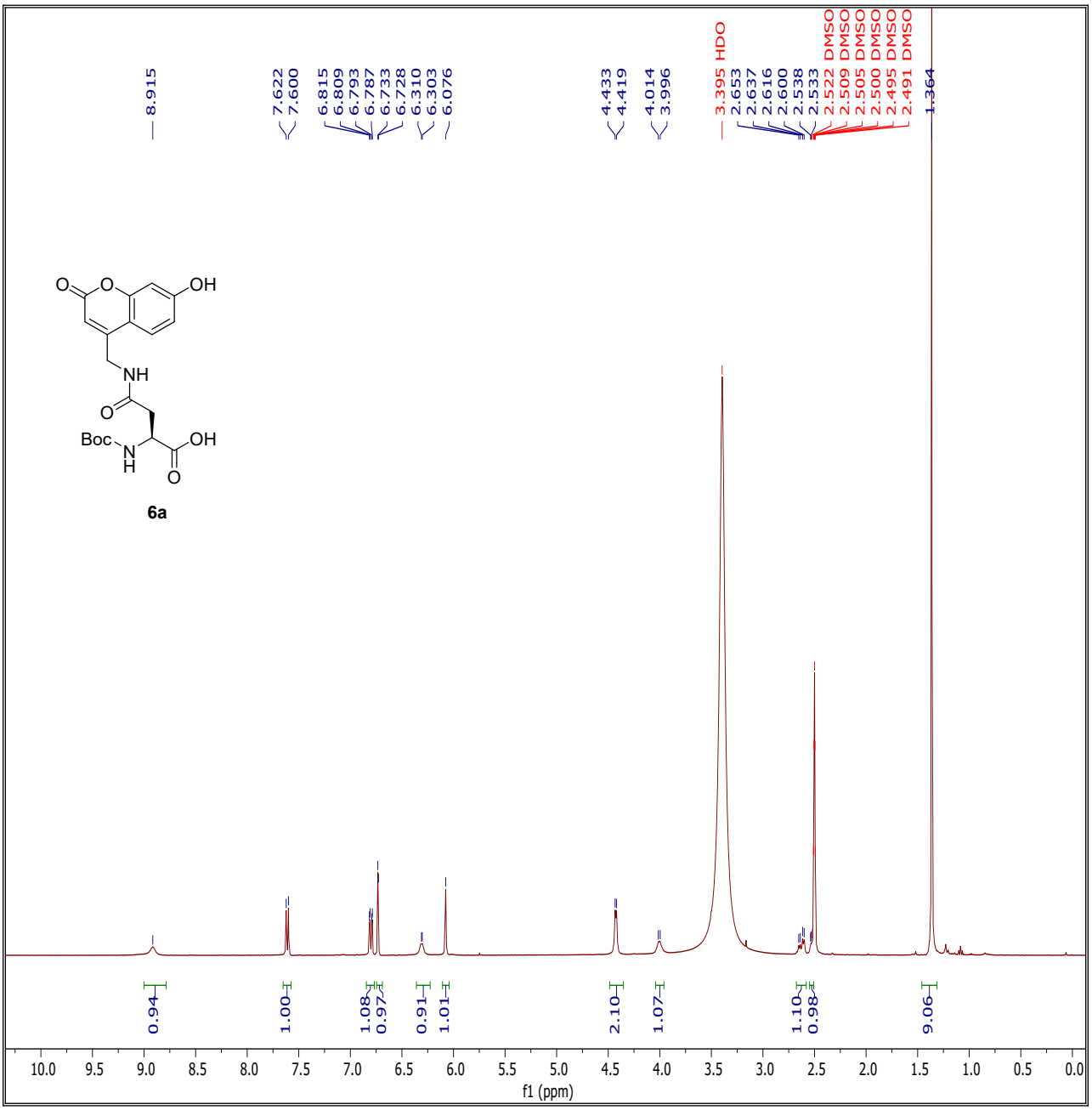
f1 (ppm)

^{1}H NMR of 6a



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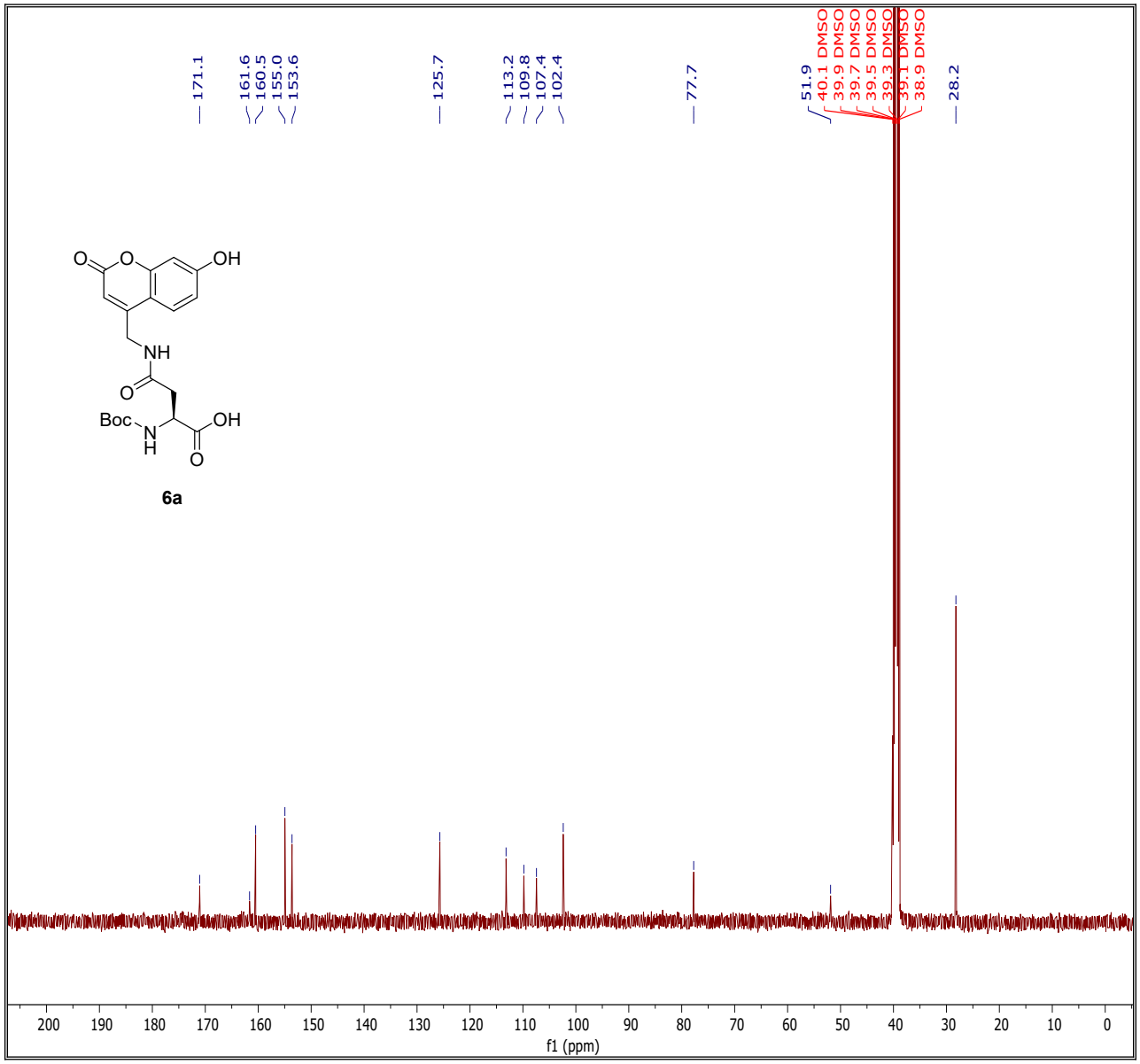
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28.2
O
O
OH
NH
O
Boc
N
H
OH
O
6a
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190
180
170
160
150
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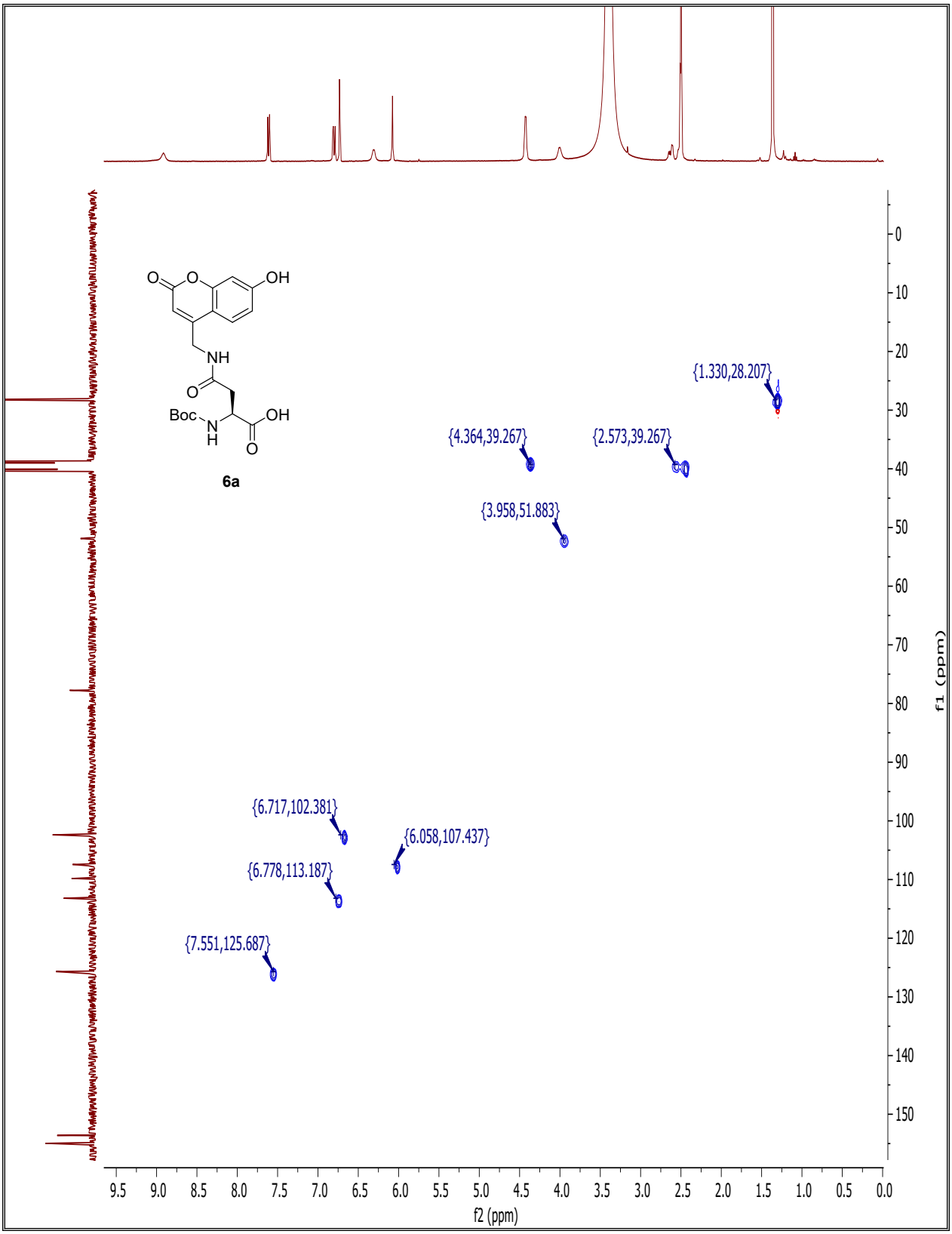
HSQC of 6a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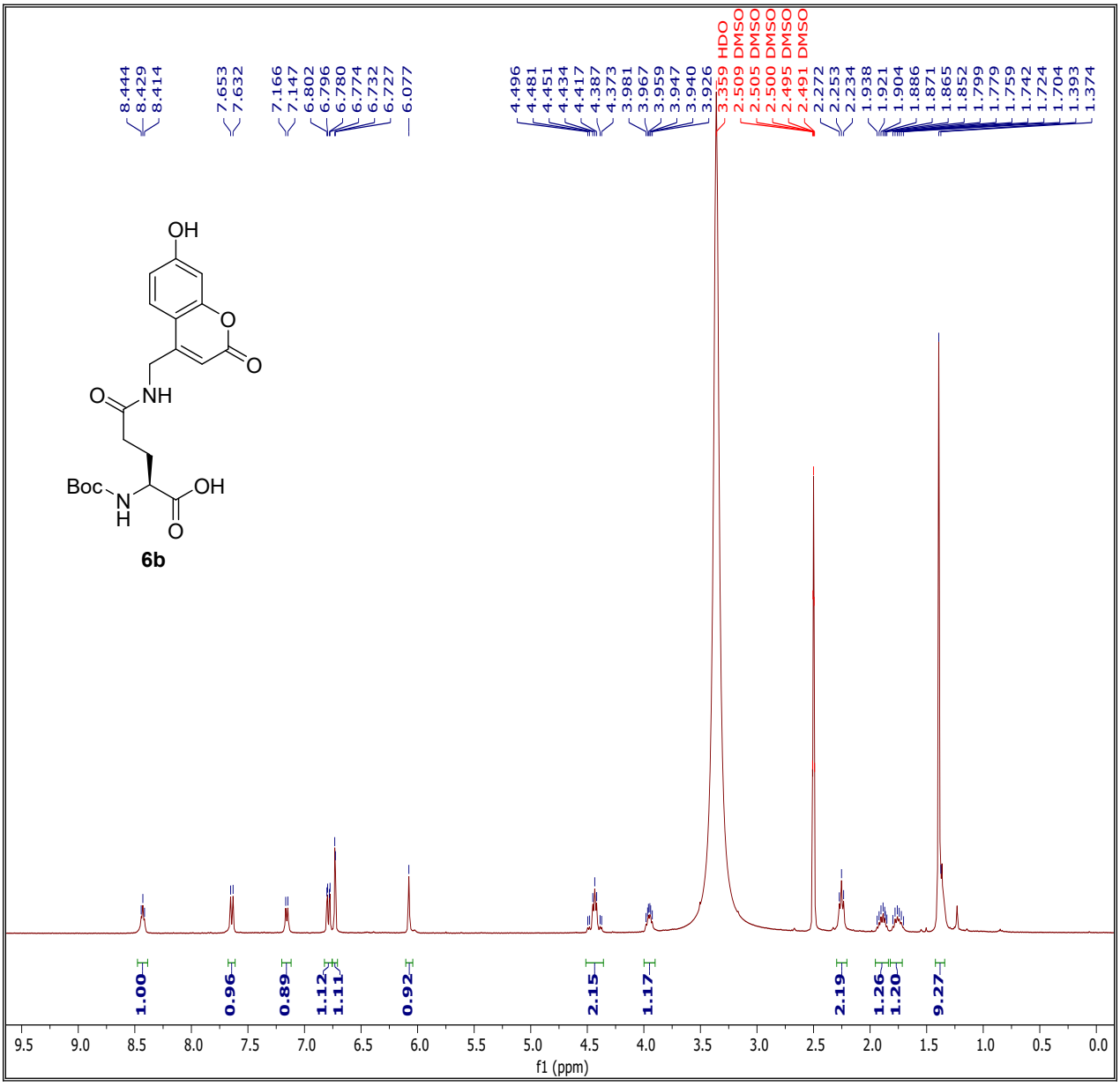
f2 (ppm)
f1 (ppm)

^{1}H NMR of 6b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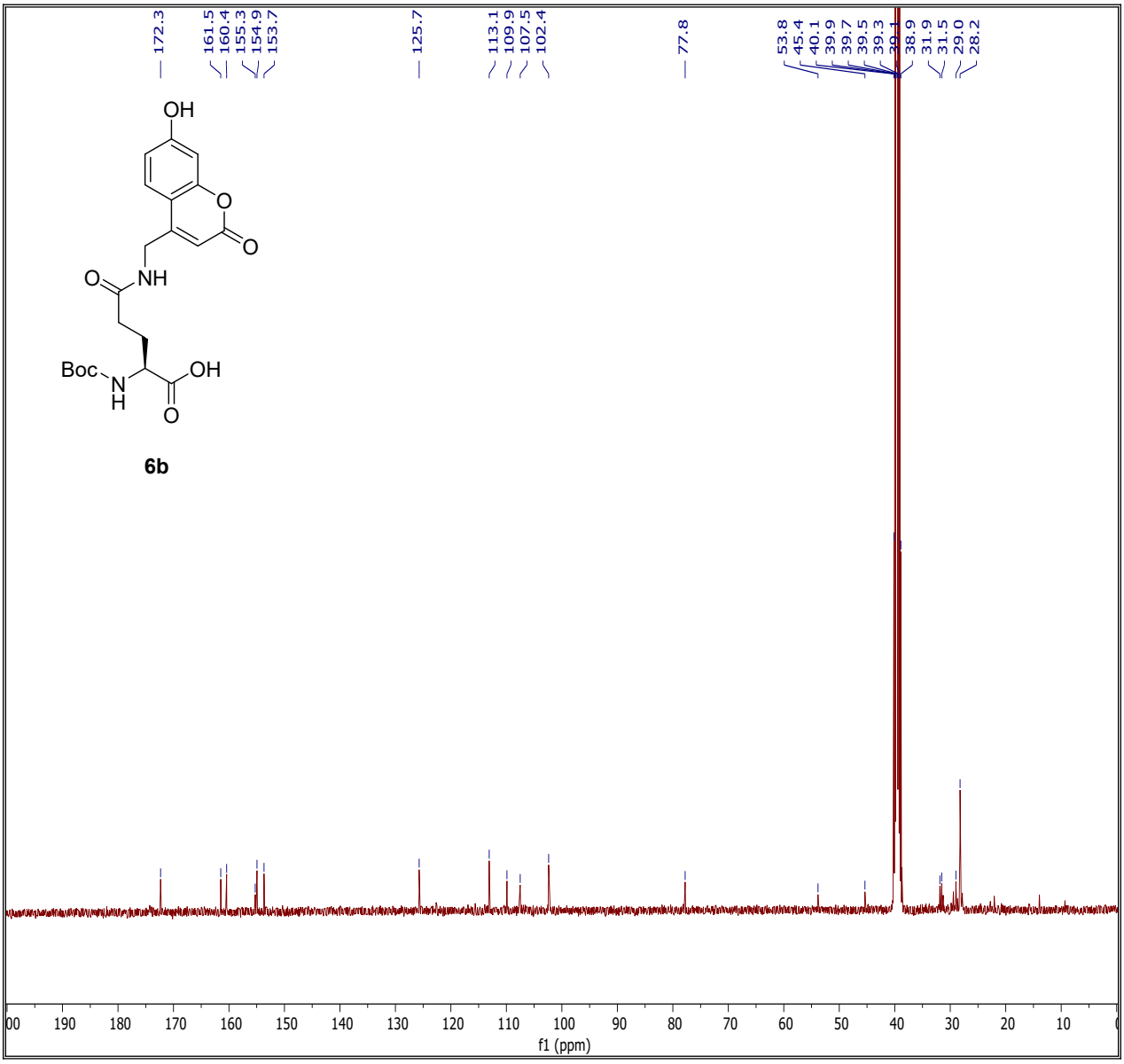
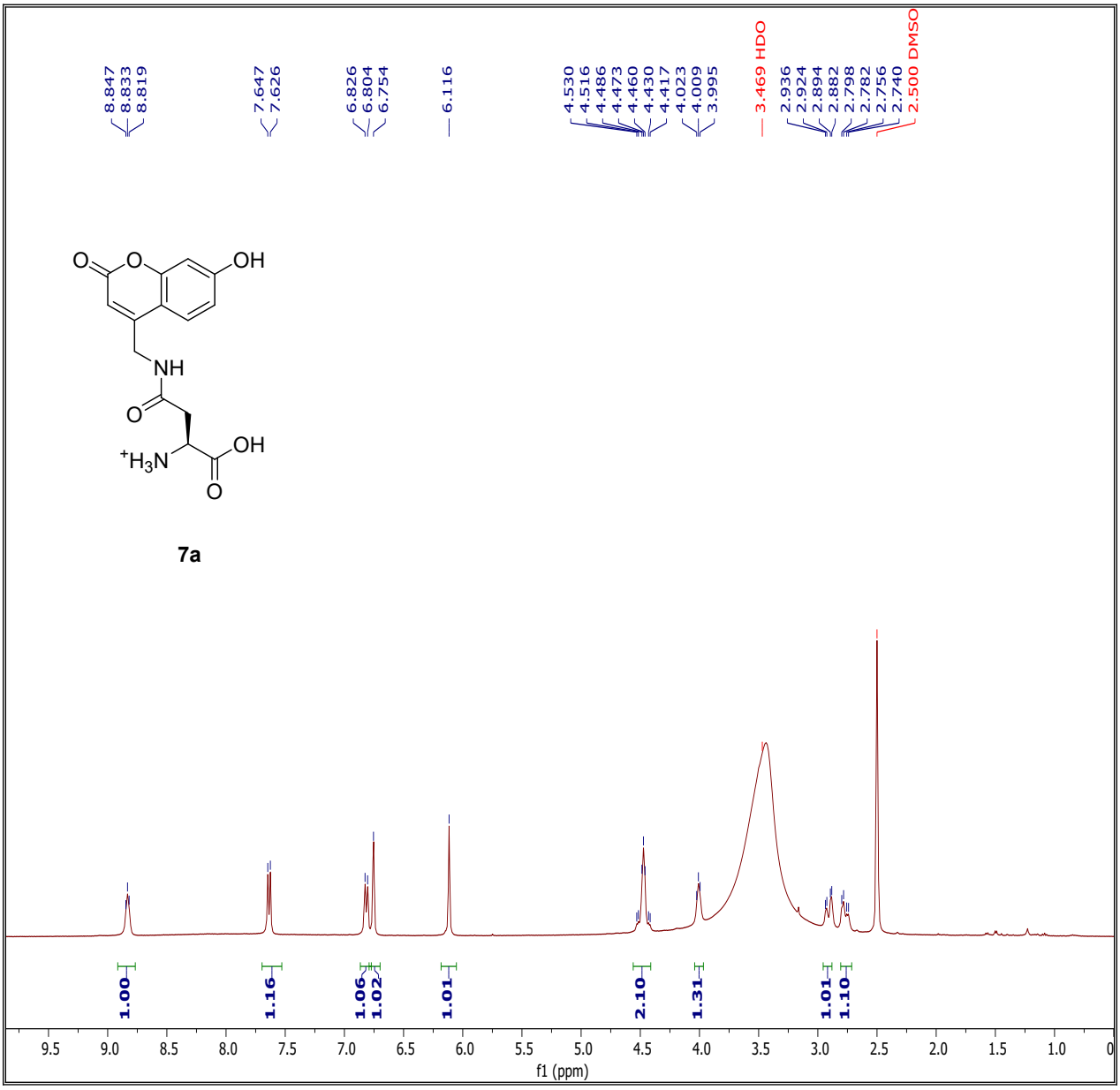
^{13}C NMR of 6b



6b

^{1}H NMR of 7a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7a
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1.06
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1.01
2.10
1.31
1.01
1.10
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8.0
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6.0
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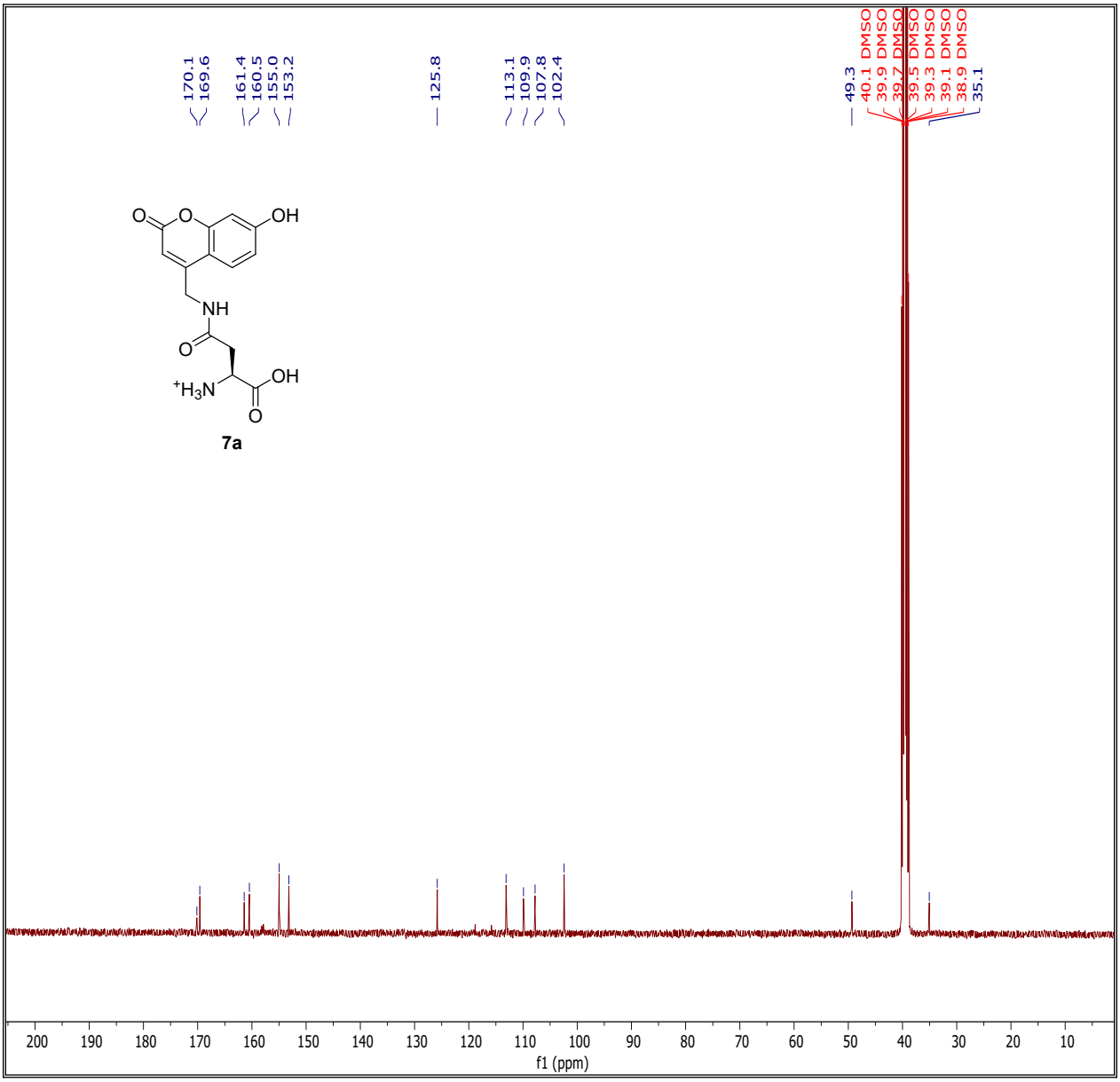
^{13}C NMR of 7a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O
O
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7a
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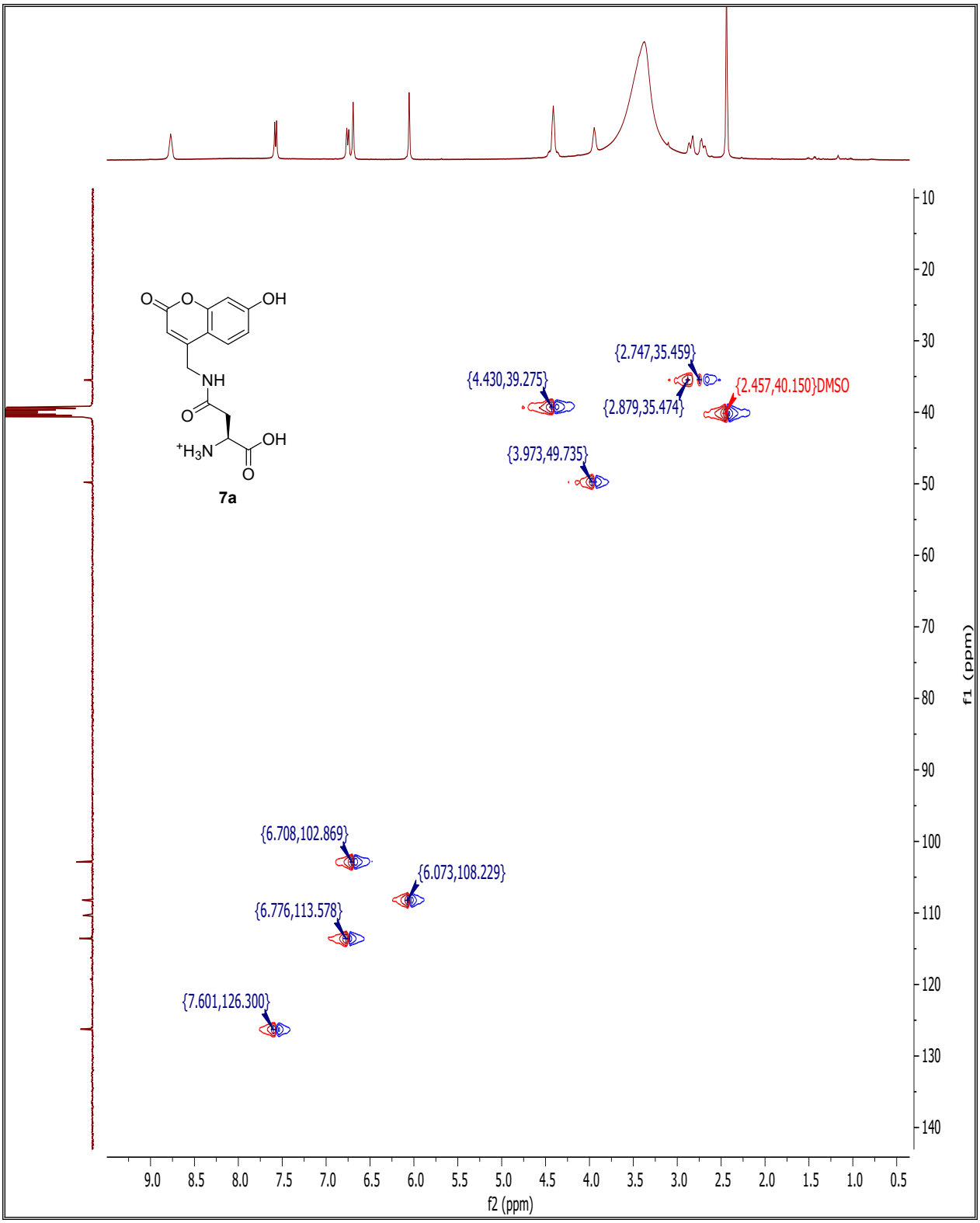
f1 (ppm)

HSQC of 7a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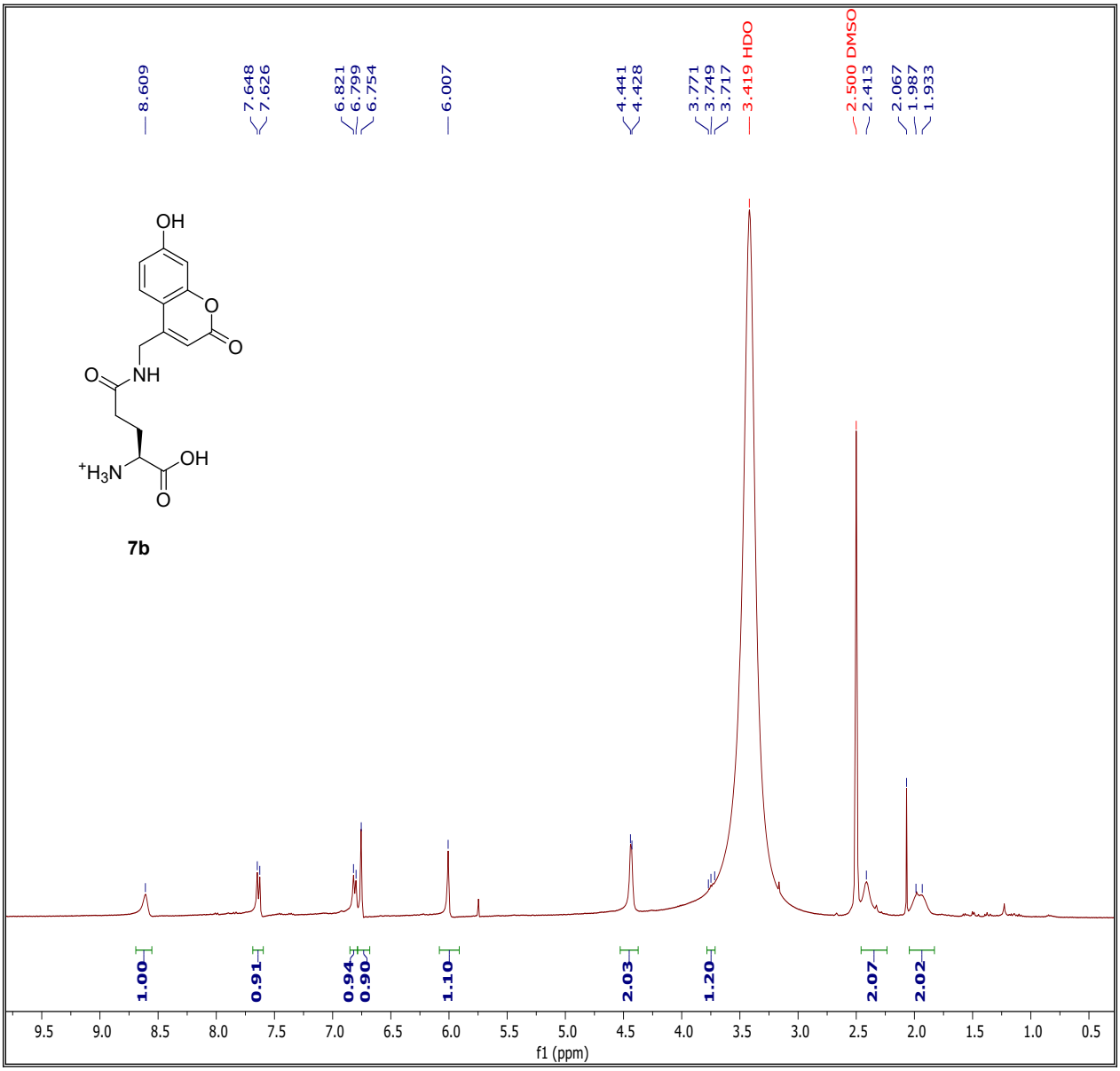
7a
f1 (ppm)
f2 (ppm)

^{1}H NMR of 7b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2.067
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1.933
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O
NH
O
+H3N
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7b
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0.90
1.10
2.03
1.20
2.07
2.02
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
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5.0
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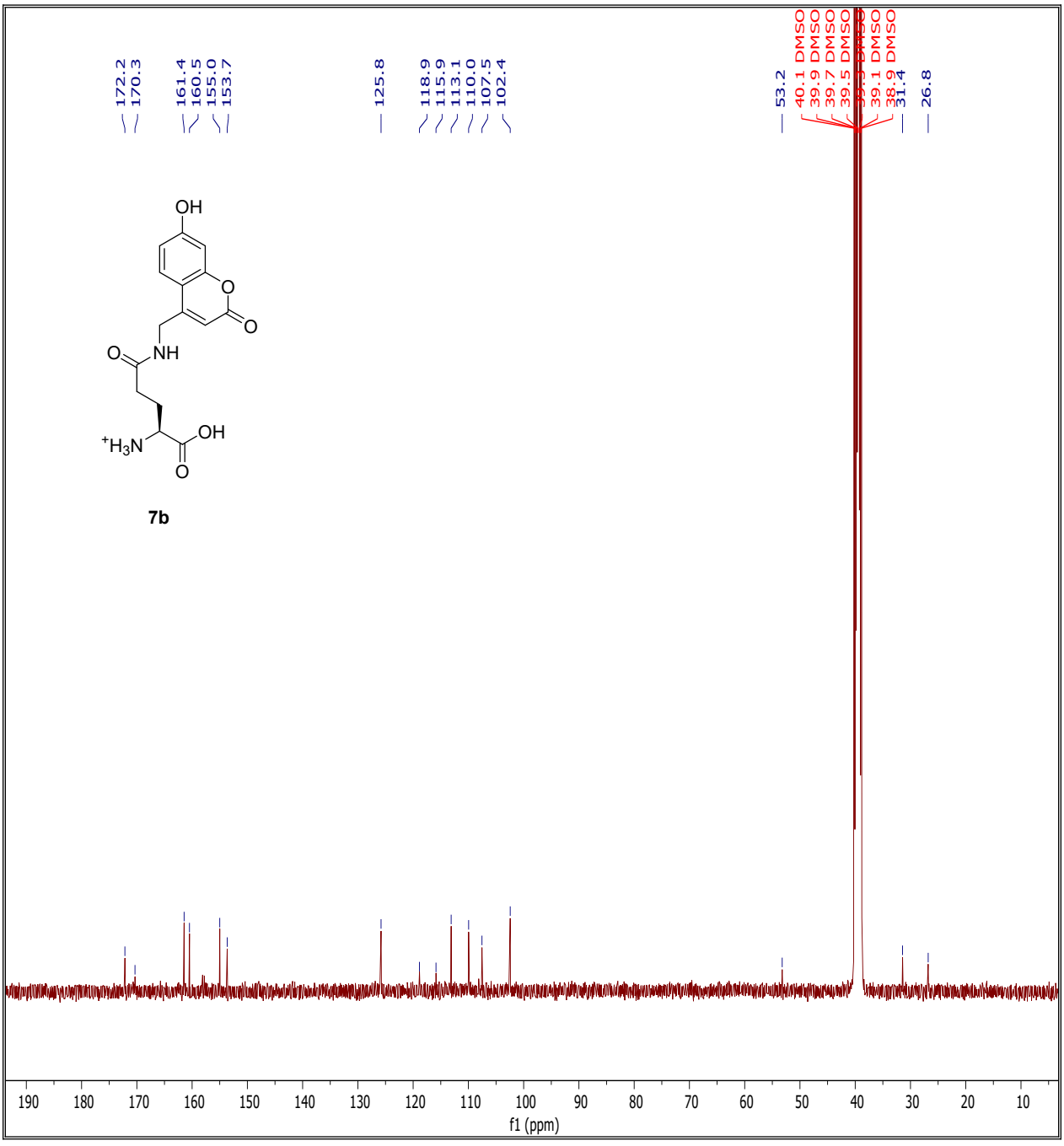
1.5
1.0
0.5
f1 (ppm)

^{13}C NMR of 7b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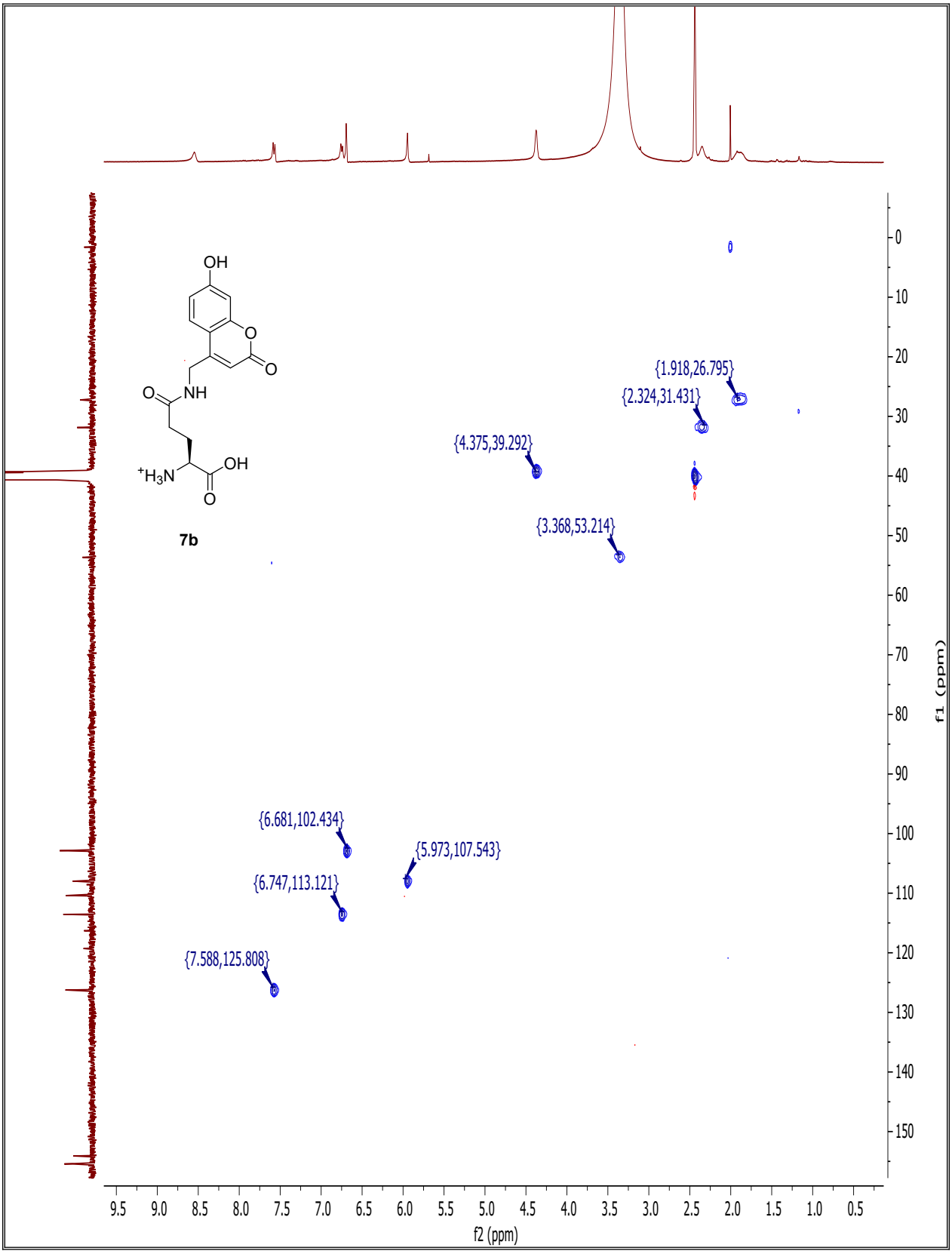
115.9
113.1
110.0
107.5
102.4
53.2
40.1 DMSO
39.9 DMSO
39.7 DMSO
39.5 DMSO
39.3 DMSO
39.1 DMSO
38.9 DMSO
31.4
26.8
OH
O
O
O
NH
+H3N
OH
O
7b
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

HSQC of 7b


OH
O
O
NH
O
+H3N
OH
O
7b
{1.918,26.795}
{2.324,31.431}
{4.375,39.292}
{3.368,53.214}
{6.681,102.434}
{5.973,107.543}
{6.747,113.121}
{7.588,125.808}
f2 (ppm)
f1 (ppm)