



UNIVERSITAT DE
BARCELONA

Design and synthesis of sphingosine-1-phosphate lyase inhibitors and fluorogenic probes for the development of HTS assays

Pol Sanllehí Figuerola

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Supporting information for the doctoral thesis entitled:

Design and synthesis of sphingosine-1-phosphate lyase inhibitors and fluorogenic probes for the development of HTS assays

Author: Pol Sanllehí Figuerola

Supervisors: Dr. Antonio Delgado Cirilo and Dr. Jordi Bujons Vilas

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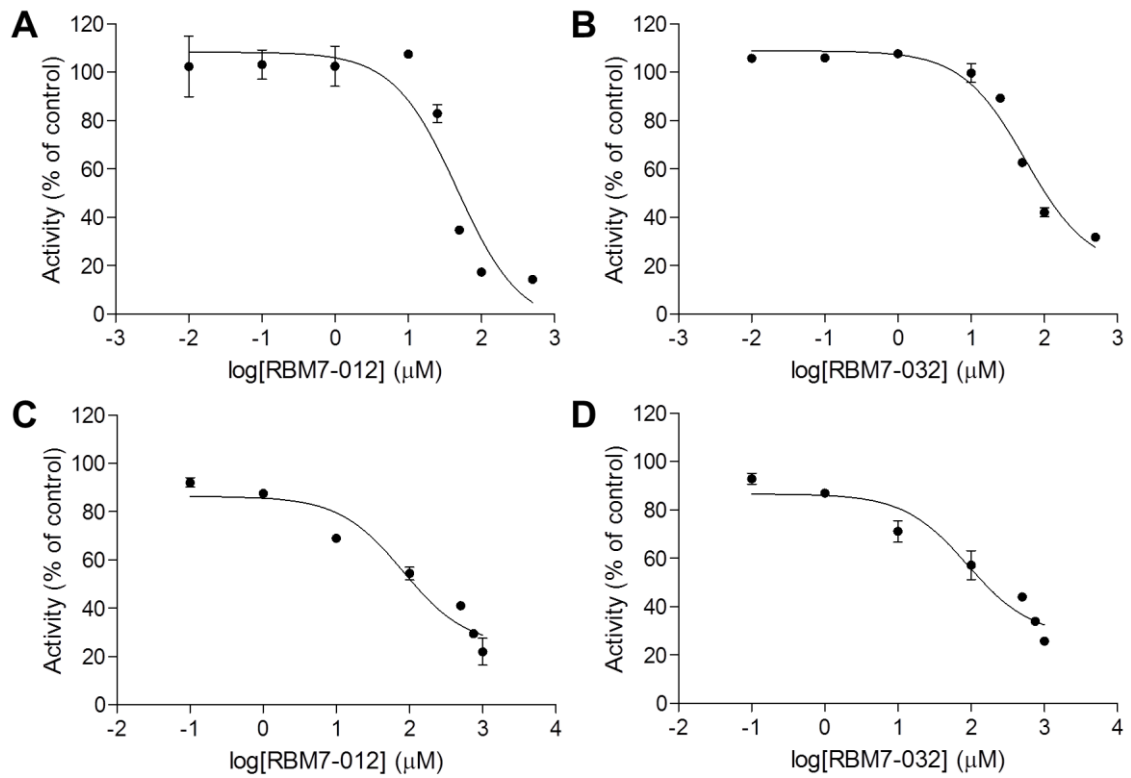


Figure S1. Activity (% of control) of StS1PL (A,B; final concentration: 25 $\mu\text{g}/\text{mL}$) and hS1PL (C,D; final concentration: 3 $\mu\text{g}/\text{mL}$) at graded concentrations of **RBM7-012** and **RBM7-032** using **RBM13** as substrate (final concentration: 125 μM). Data correspond to one representative experiment of two independent assays, each performed in triplicates, with similar results.

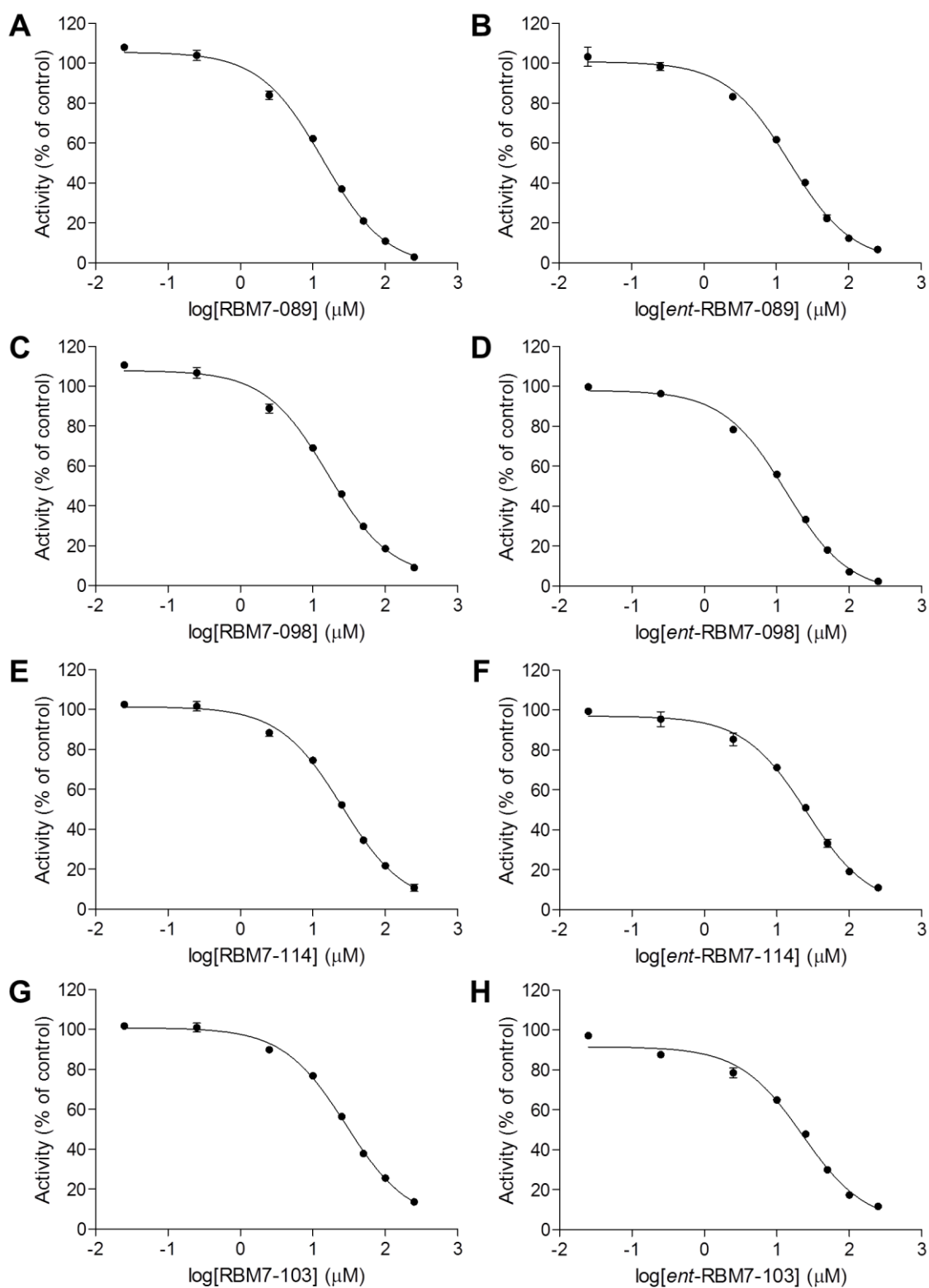


Figure S2. Activity (% of control) of StS1PL (final concentration: 25 μg/mL) at graded concentrations of *anti*- (A,B) or *syn*- (C,D) 2-azido-S1P stereoisomers and *anti*- (E,F) or *syn*- (G,H) 2-azido-dhS1P stereoisomers. **RBM13** was used as substrate at a final concentration of 125 μM. Data correspond to one representative experiment of 2–3 independent assays, each performed in triplicates, with similar results.

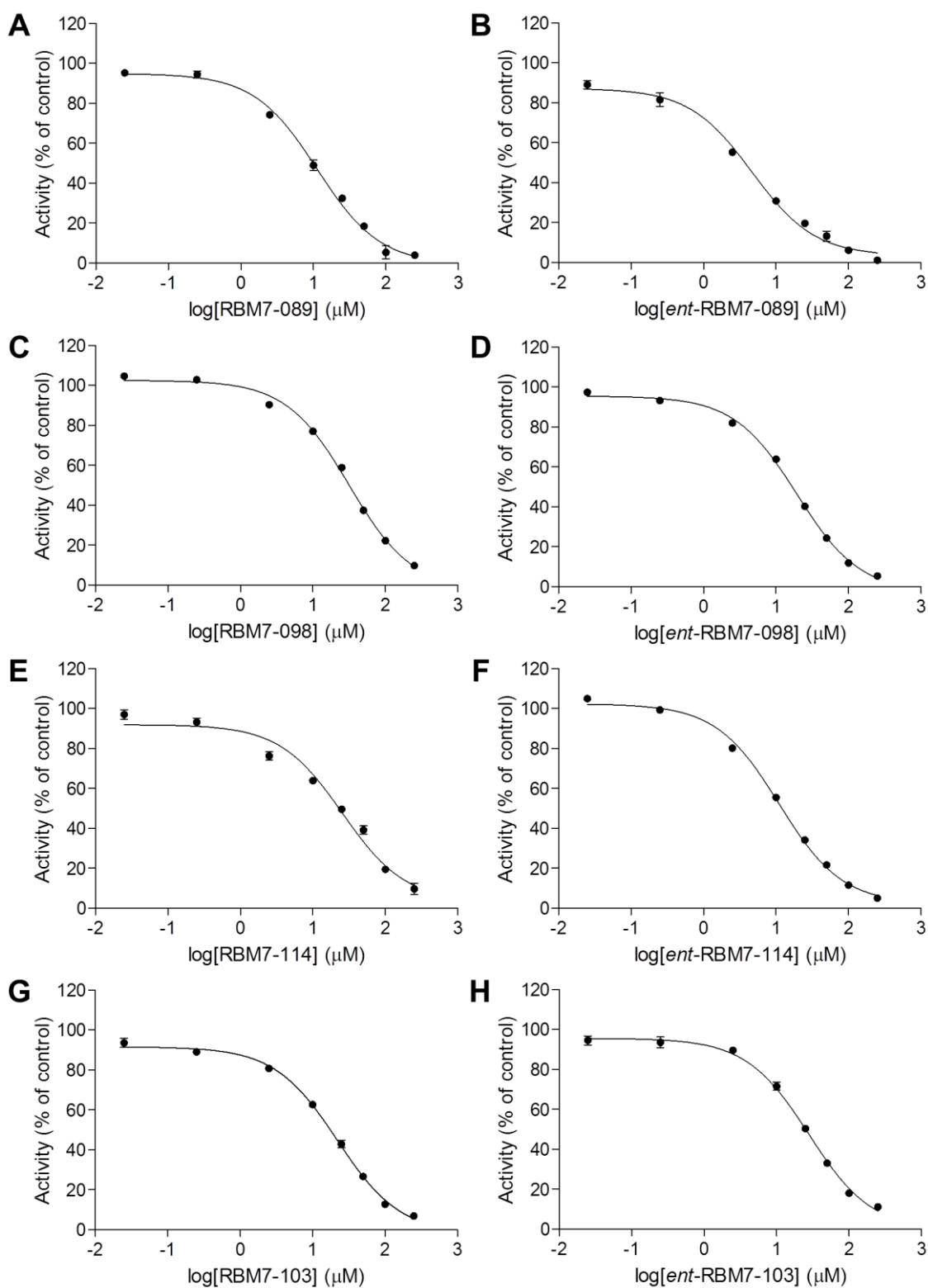


Figure S3. Activity (% of control) of hS1PL (final concentration: 3 $\mu\text{g}/\text{mL}$) at graded concentrations of *anti*- (A,B) or *syn*- (C,D) 2-azido-S1P stereoisomers and *anti*- (E,F) or *syn*- (G,H) 2-azido-dhS1P stereoisomers. **RBM13** was used as substrate at a final concentration of 125 μM . Data correspond to one representative experiment of 2–3 independent assays, each performed in triplicates, with similar results.

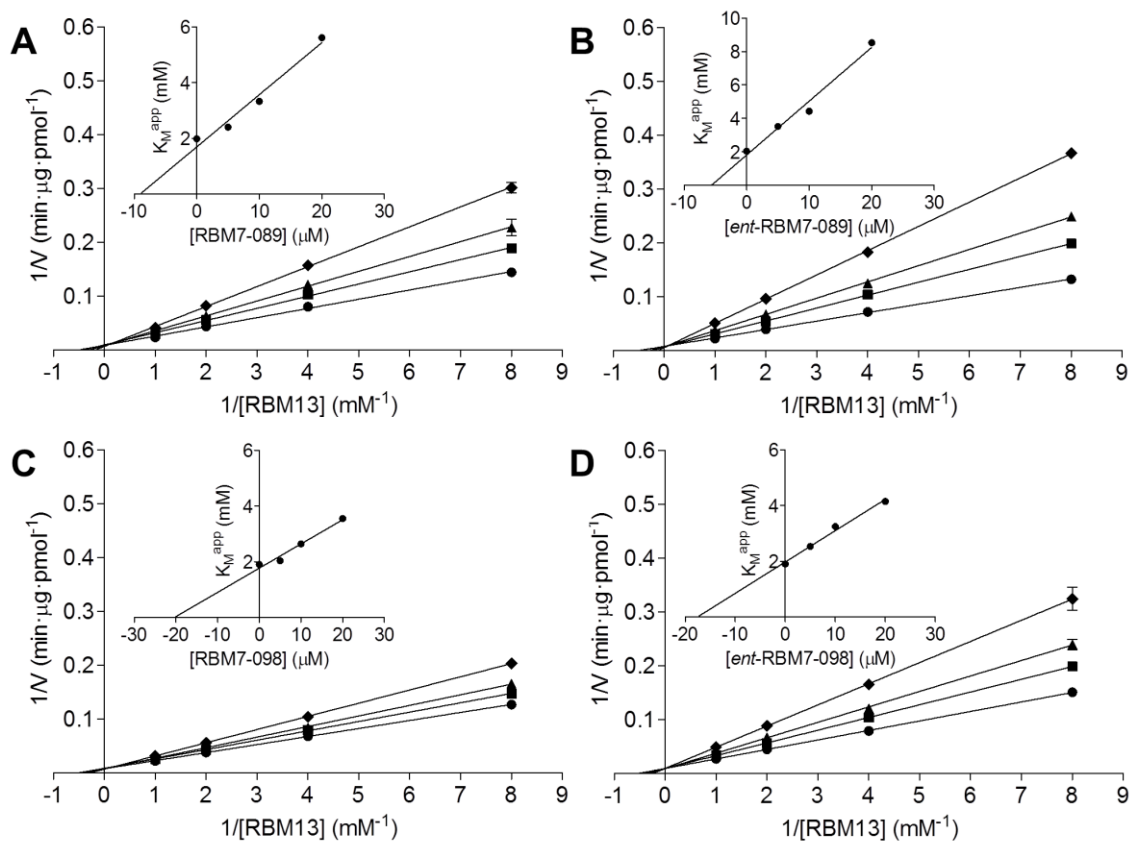


Figure S4. Lineweaver–Burk plots for inhibition of hS1PL (final concentration: 3 $\mu\text{g/mL}$) by *anti*- (A,B) and *syn*- (C,D) 2-azido-S1P stereoisomers at different concentrations of substrate (RBM13) and inhibitor (circle, 0 μM ; square, 5 μM ; triangle, 10 μM ; rhombus, 20 μM). Regression lines arise from data obtained in two different experiments with triplicates.

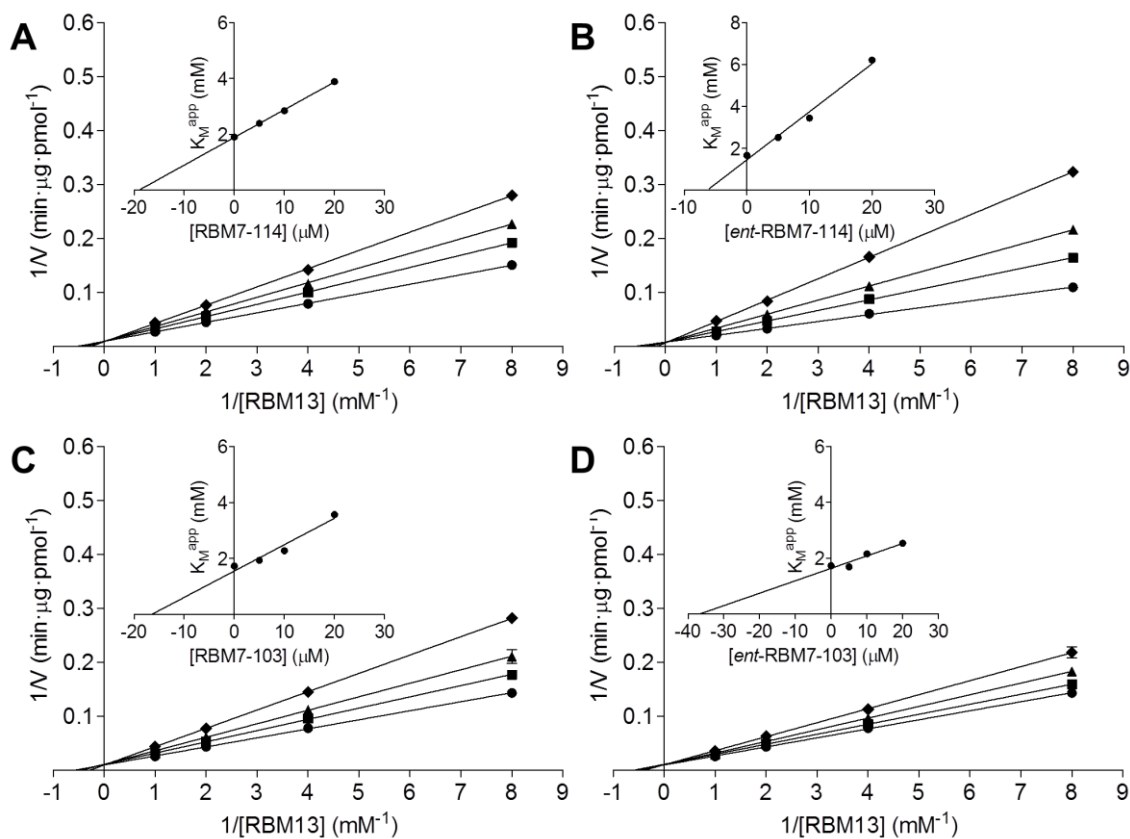


Figure S5. Lineweaver–Burk plots for inhibition of hS1PL (final concentration: 3 $\mu\text{g}/\text{mL}$) by *anti*- (A,B) and *syn*- (C,D) 2-azido-dhS1P stereoisomers at different concentrations of substrate (**RBM13**) and inhibitor (circle, 0 μM ; square, 5 μM ; triangle, 10 μM ; rhombus, 20 μM). Regression lines arise from data obtained in two different experiments with triplicates.

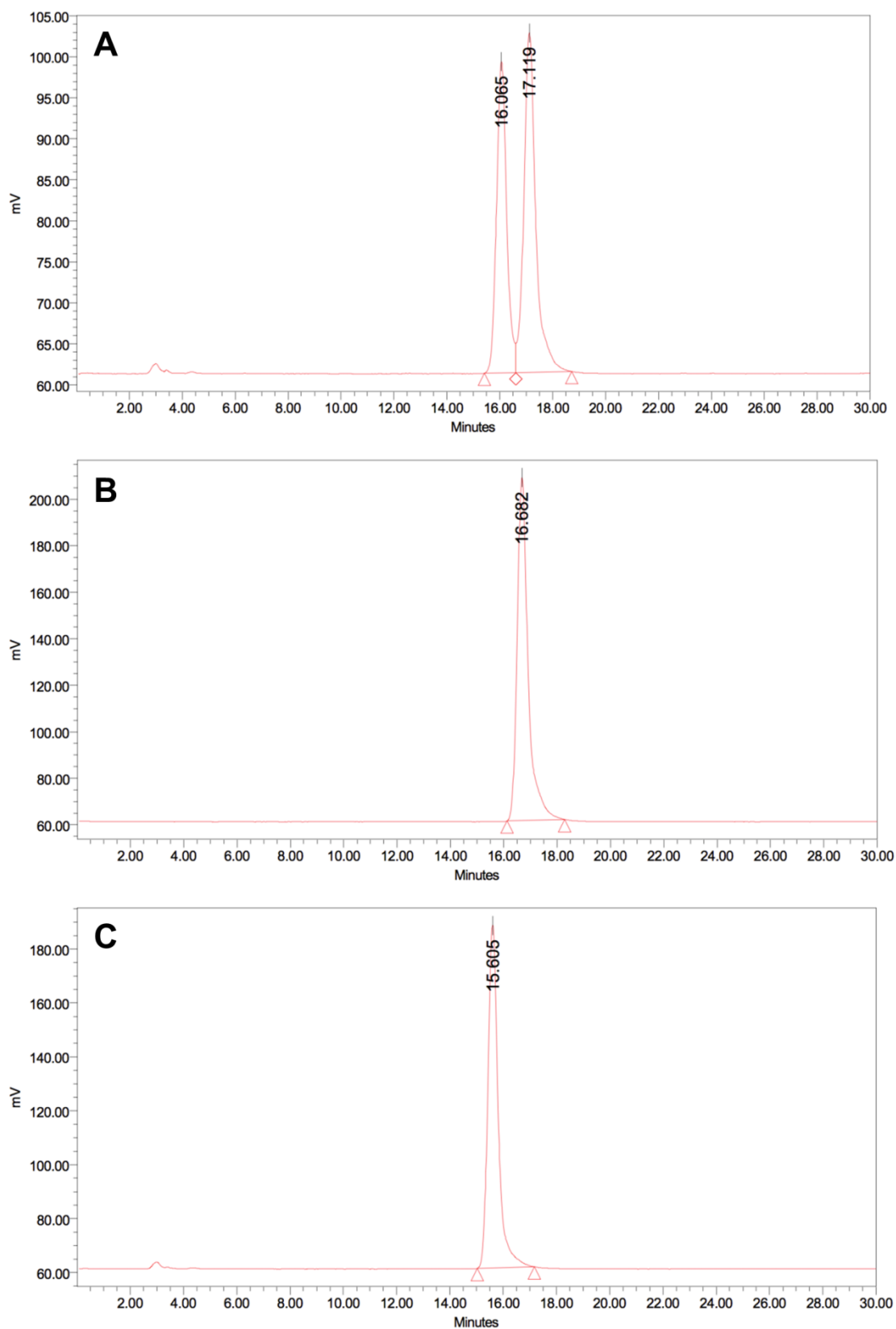


Figure S6. HPLC chromatograms (ELS detector) of a 1:1 mixture (A) of enantioenriched **RBM7-087** (B) and *ent*-**RBM7-087** (C).

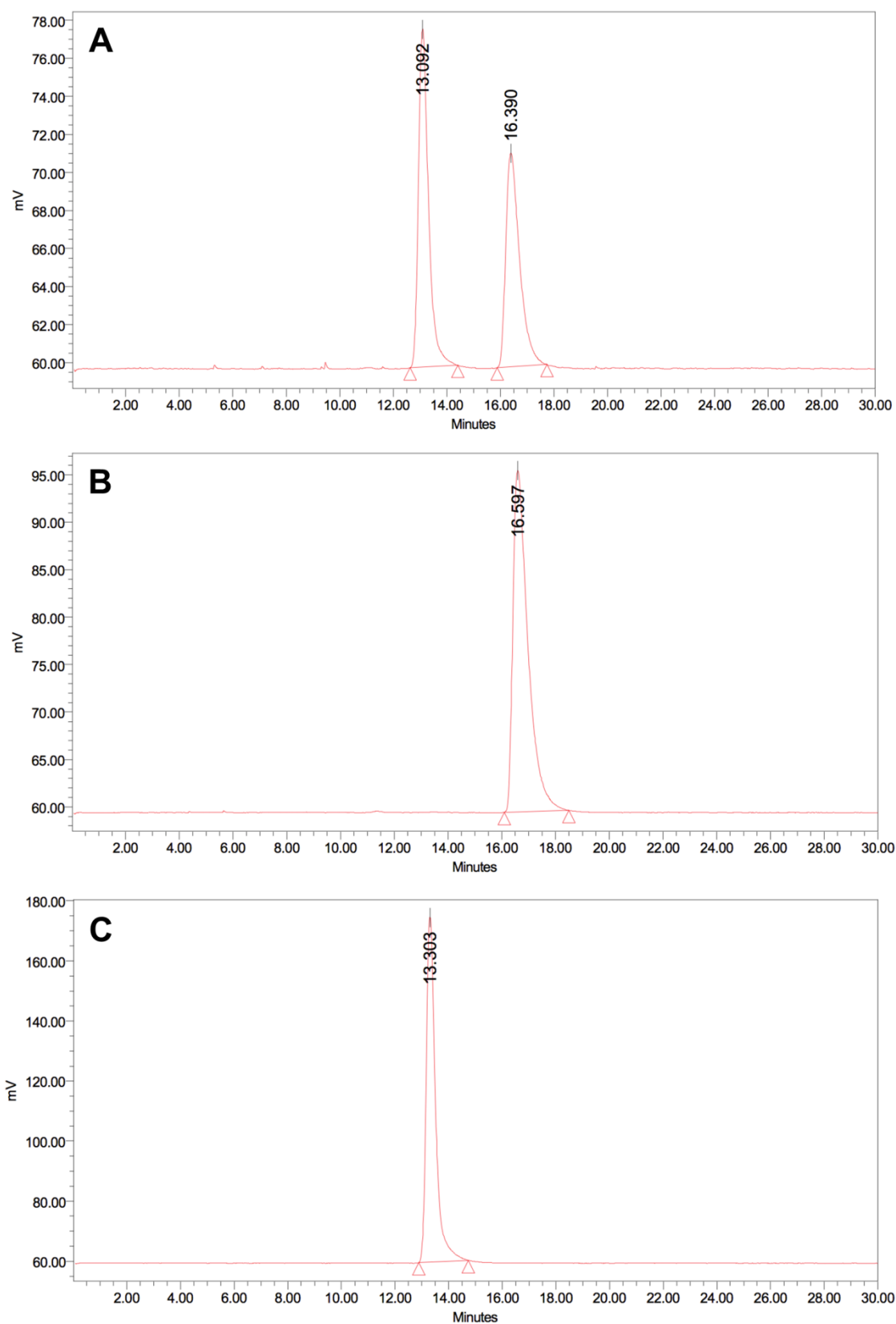


Figure S7. HPLC chromatograms (ELS detector) of a 1:1 mixture (A) of enantioenriched RBM7-096 (B) and *ent*-RBM7-096 (C).

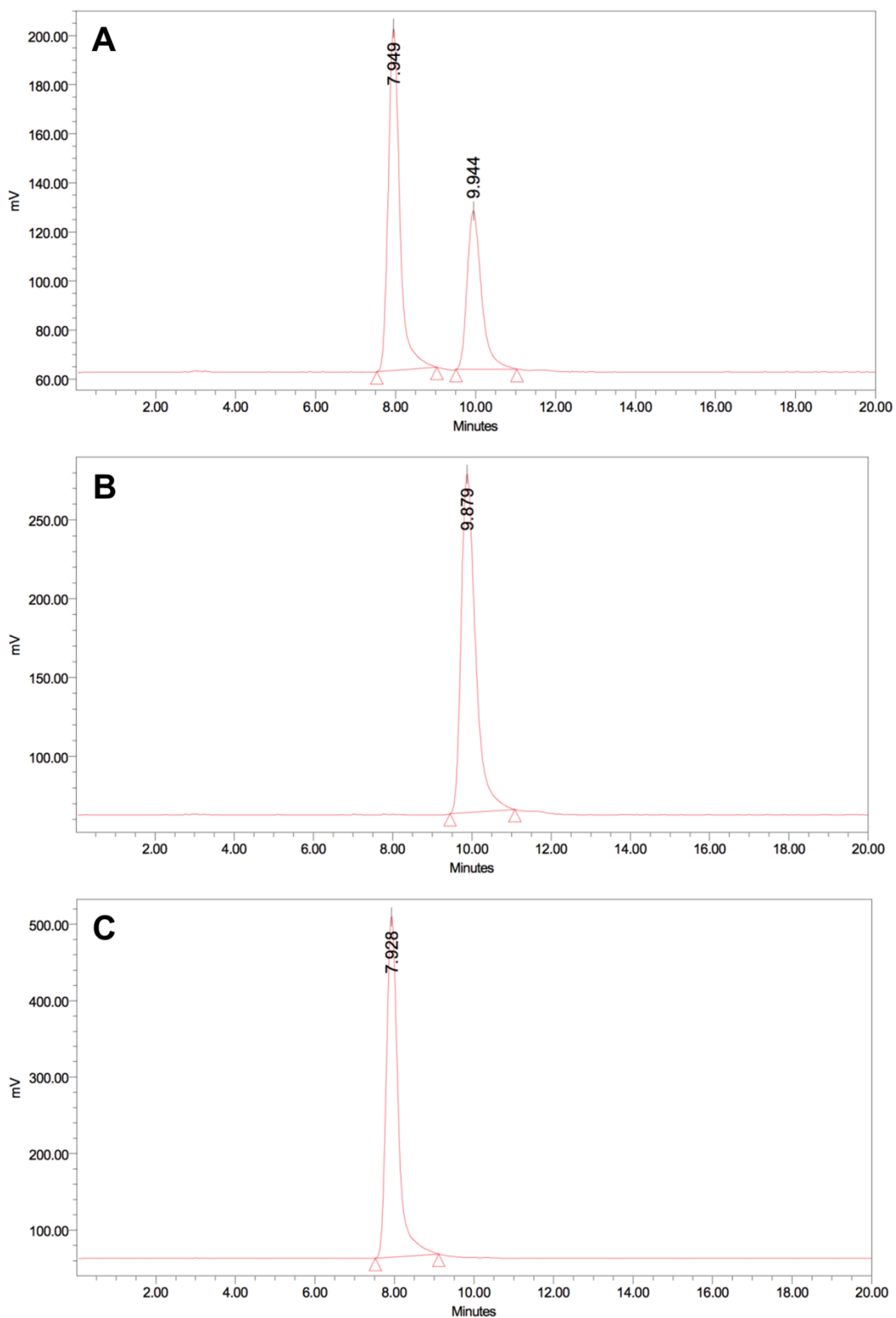


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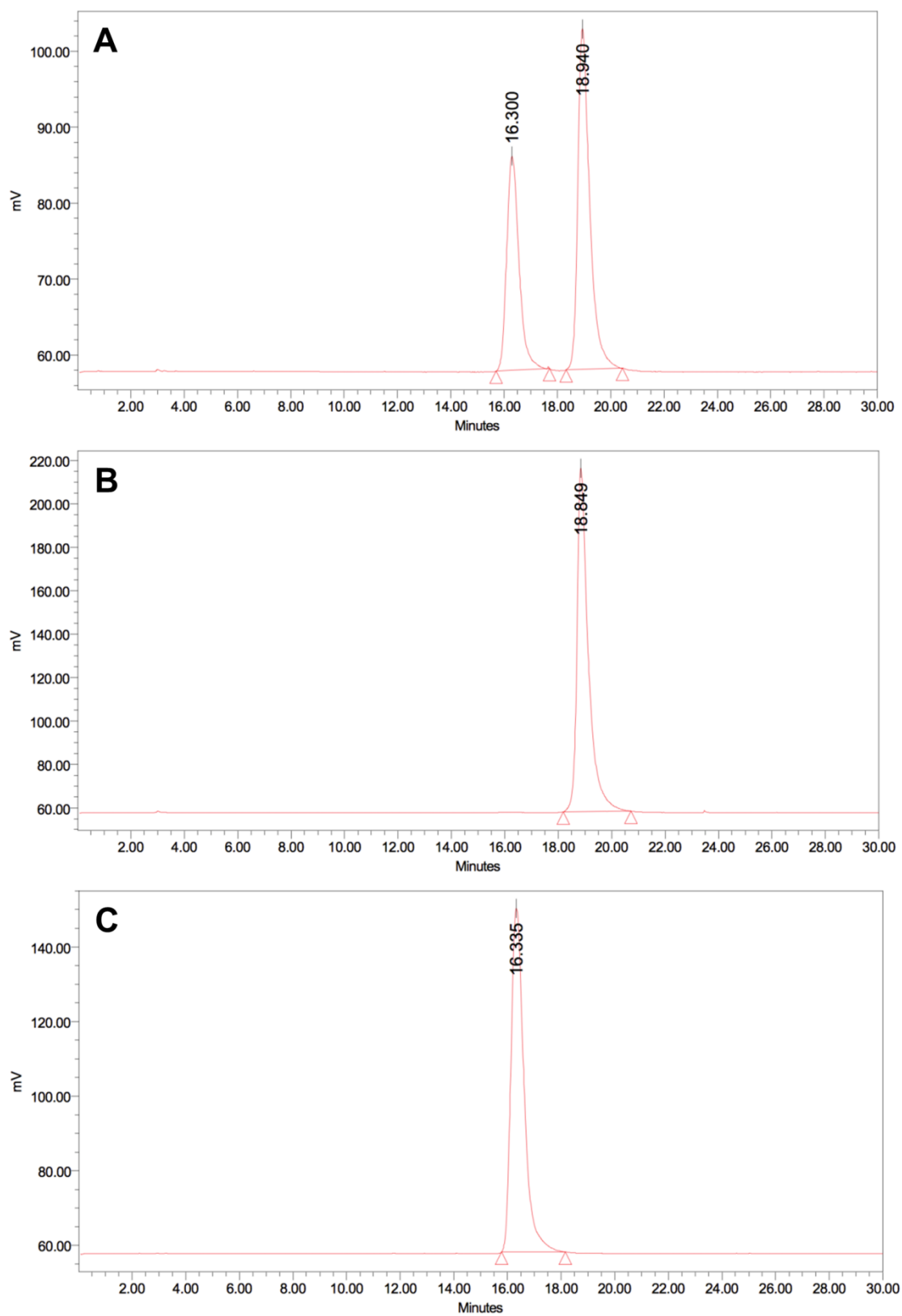


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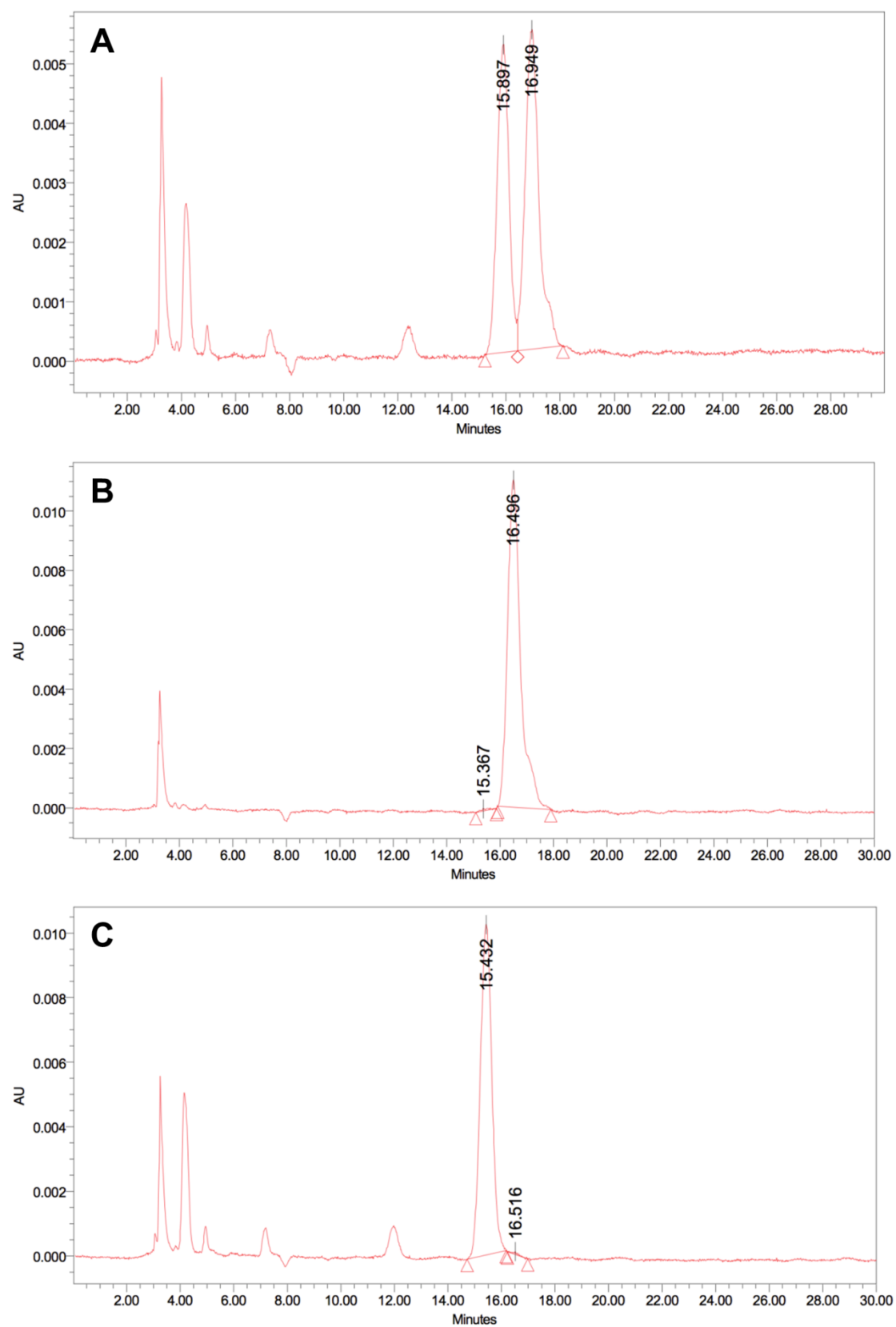


Figure S10. HPLC chromatograms (PDA detector) of a 1:1 mixture (A) of enantioenriched RBM7-087 (B) and *ent*-RBM7-087 (C).

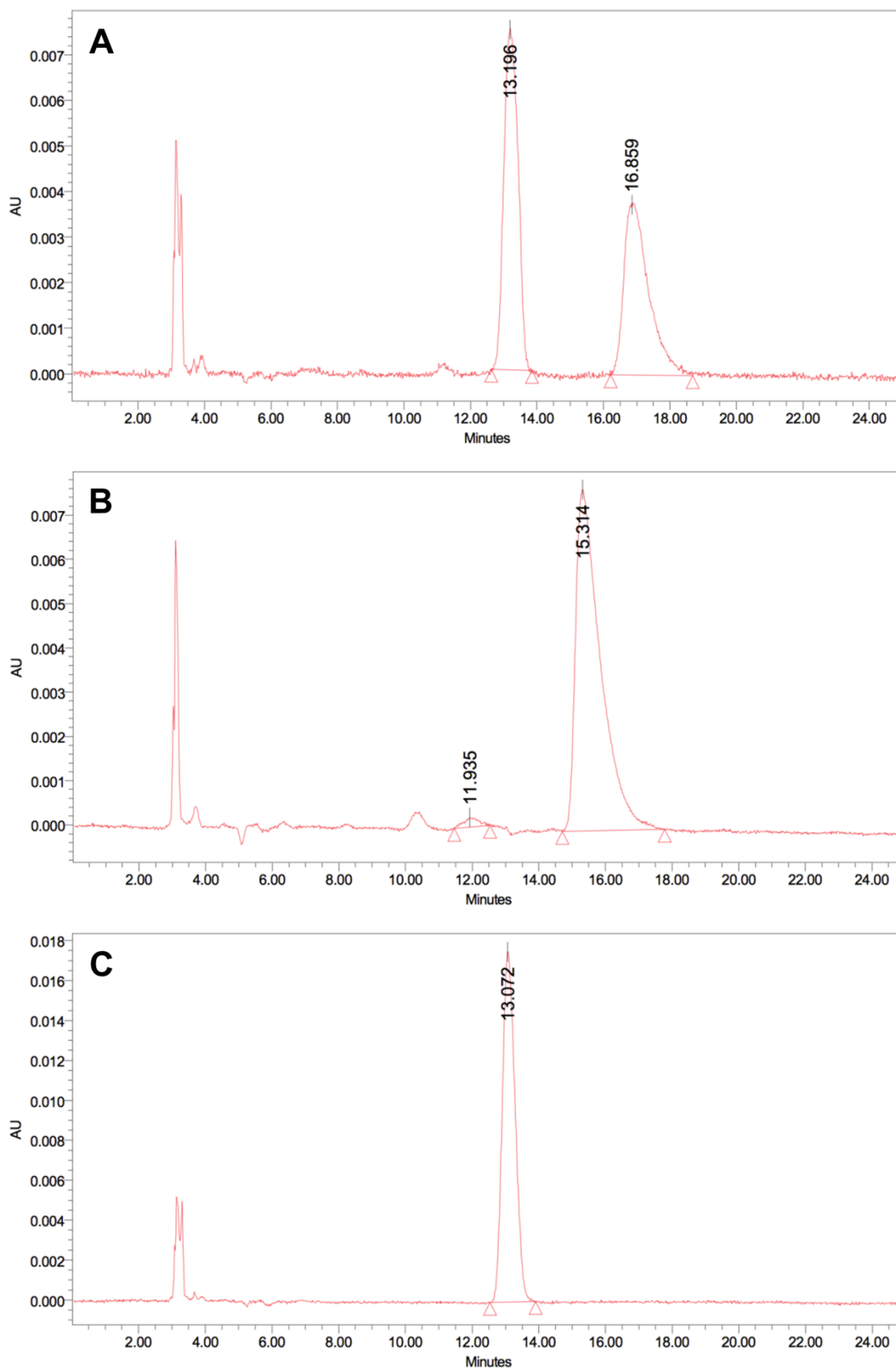


Figure S11. HPLC chromatograms (PDA detector) of a 1:1 mixture (A) of enantioenriched **RBM7-096** (B) and *ent*-**RBM7-096** (C).

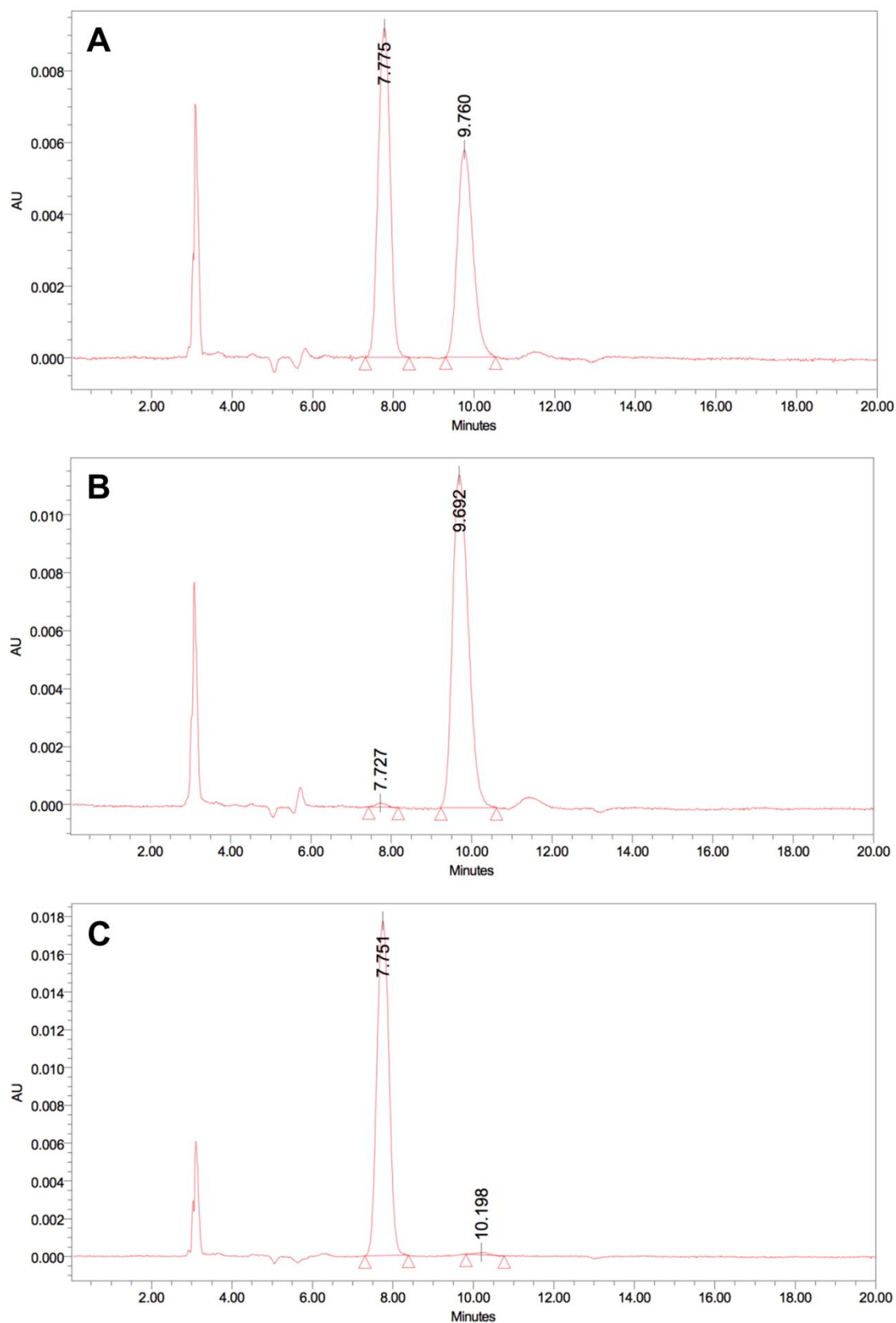


Figure S12. HPLC chromatograms (PDA detector) of a 1:1 mixture (A) of enantioenriched RBM7-112 (B) and *ent*-RBM7-112 (C).

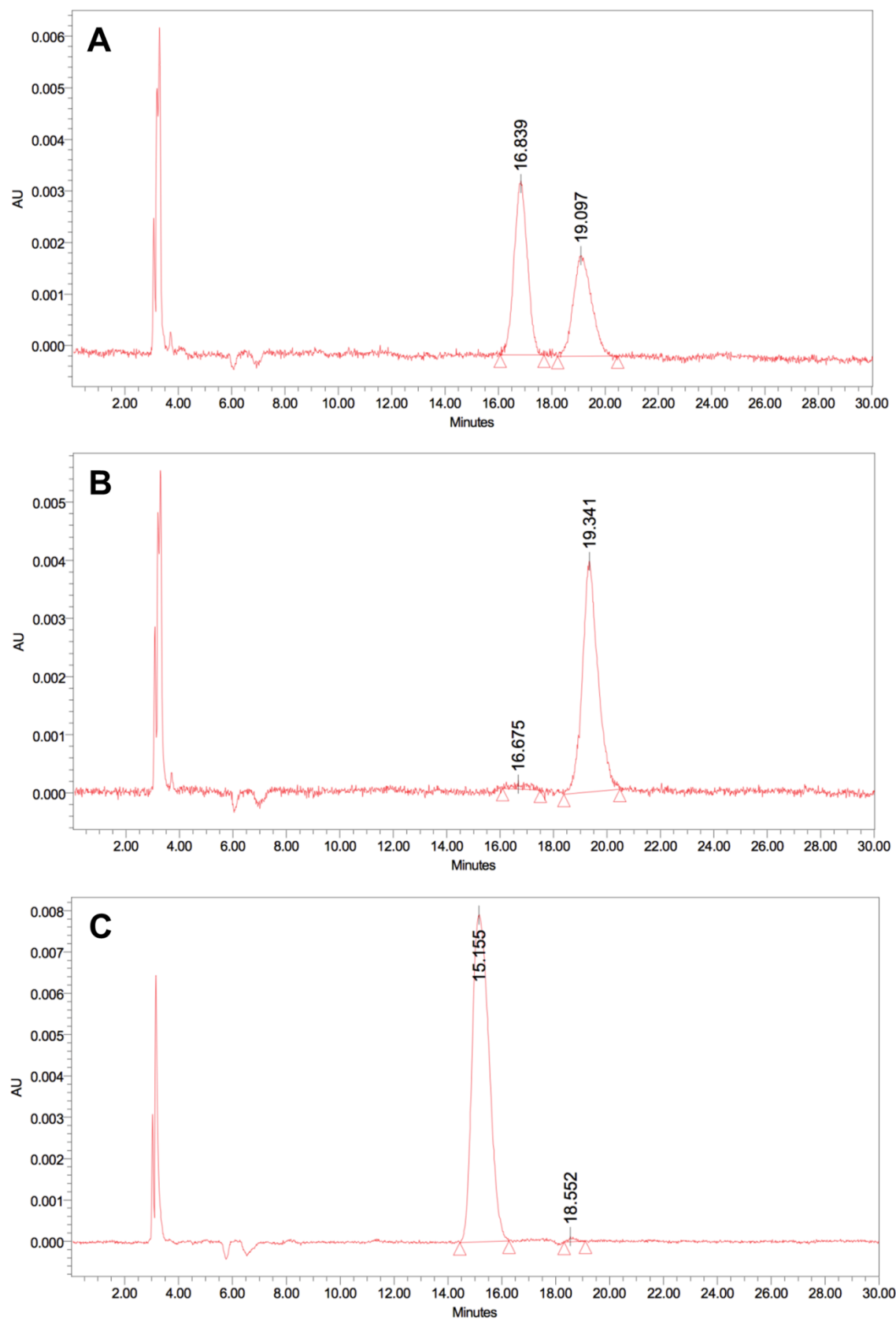
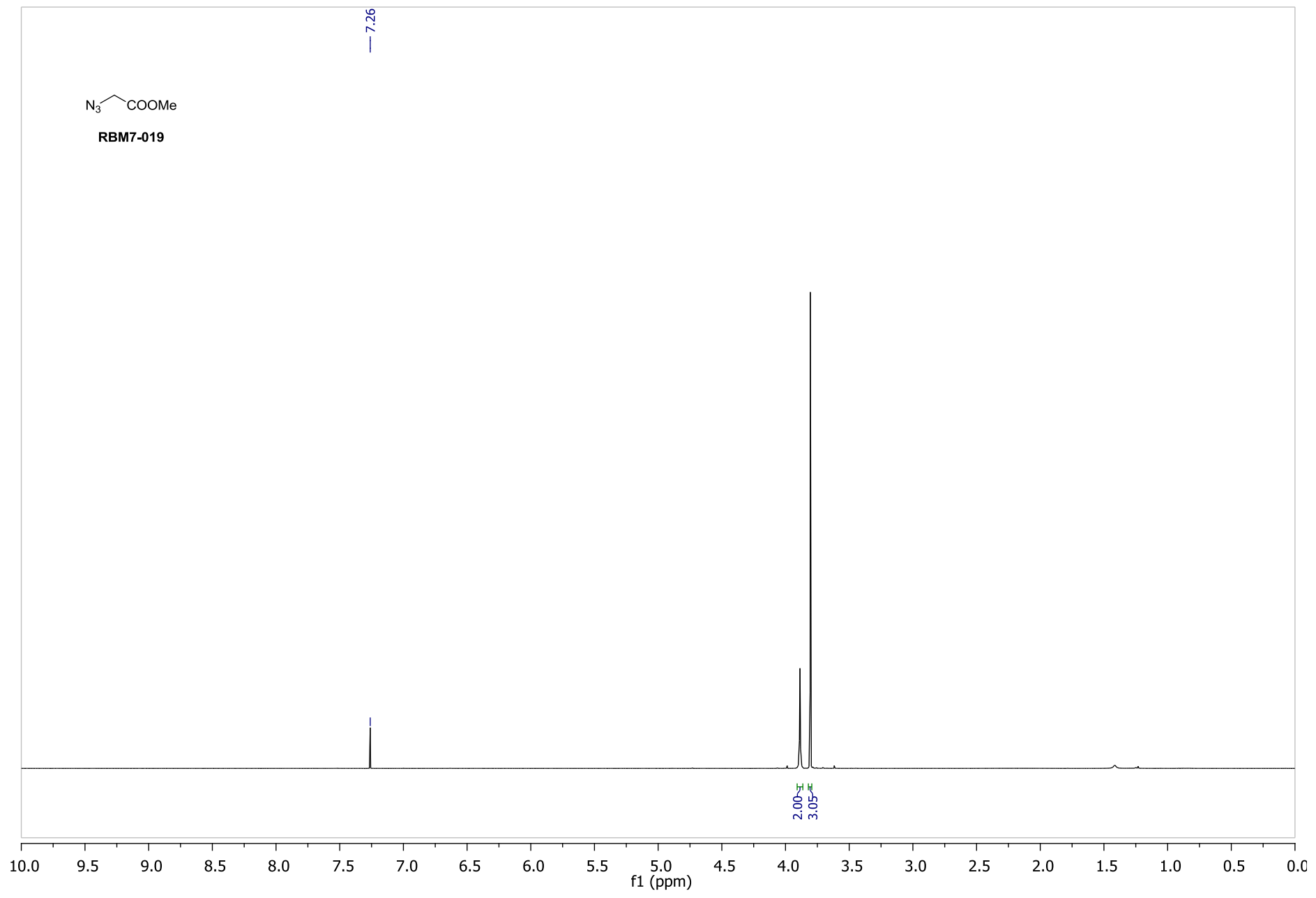
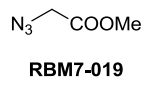
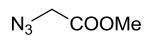


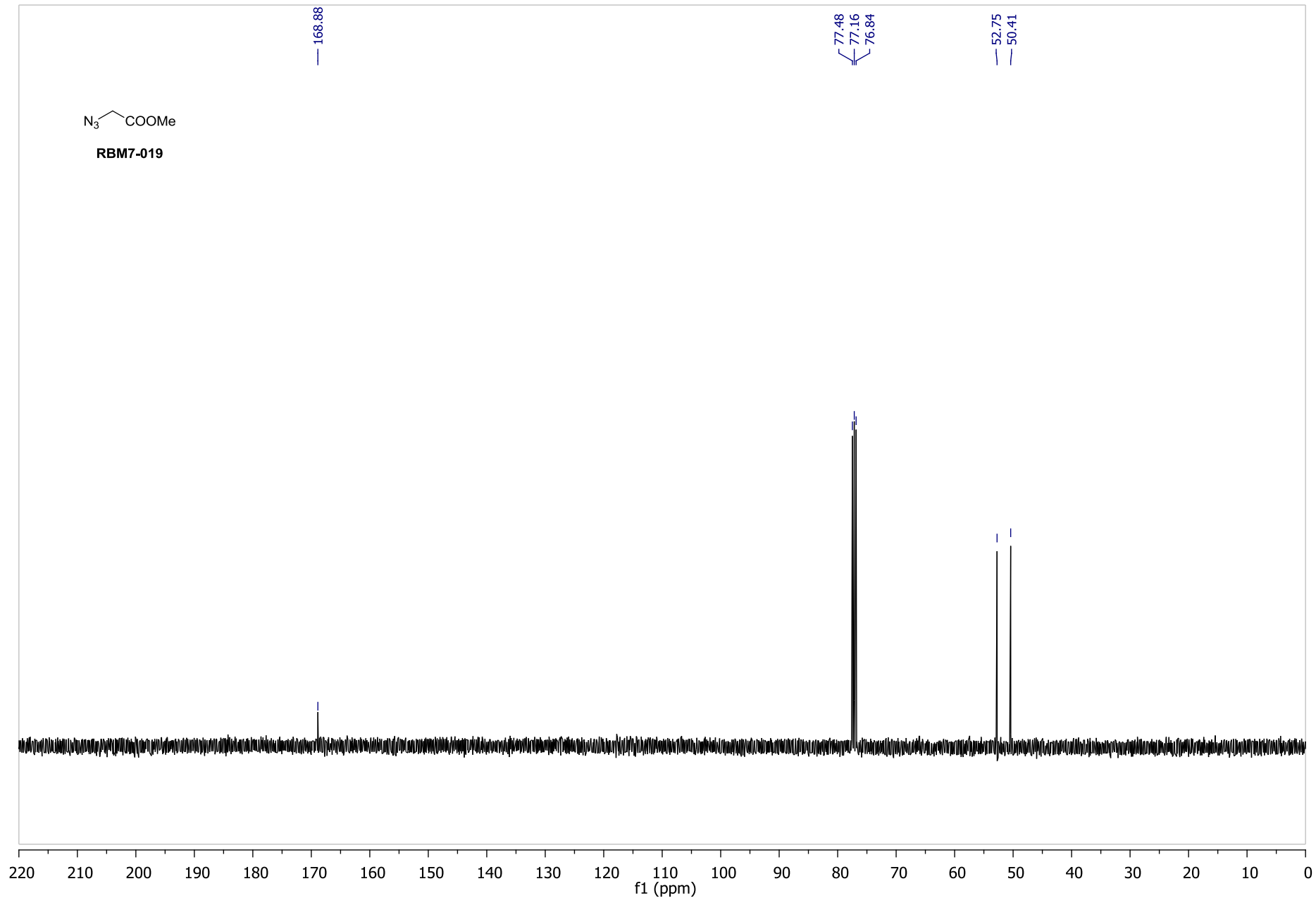
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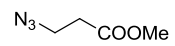
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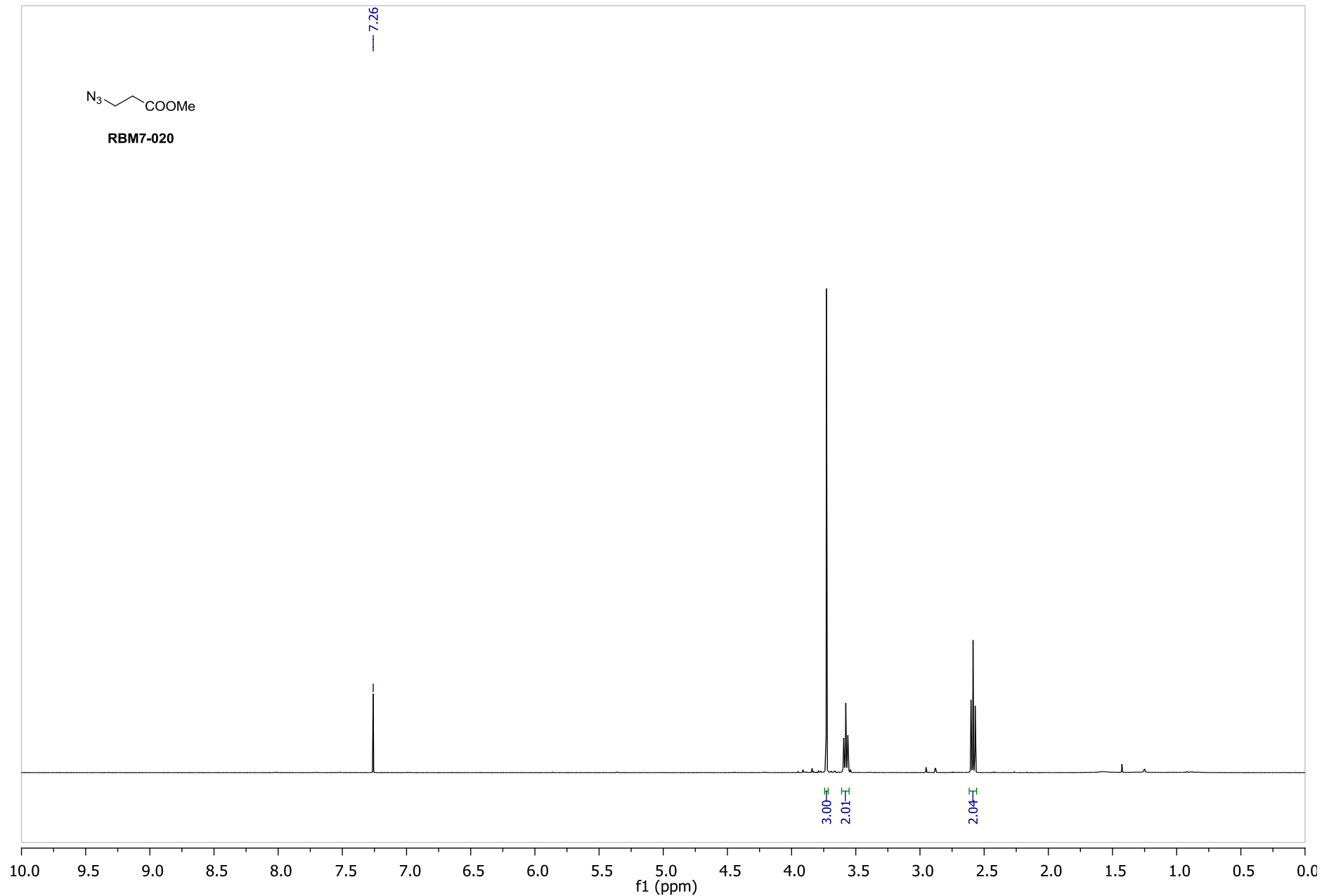


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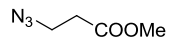


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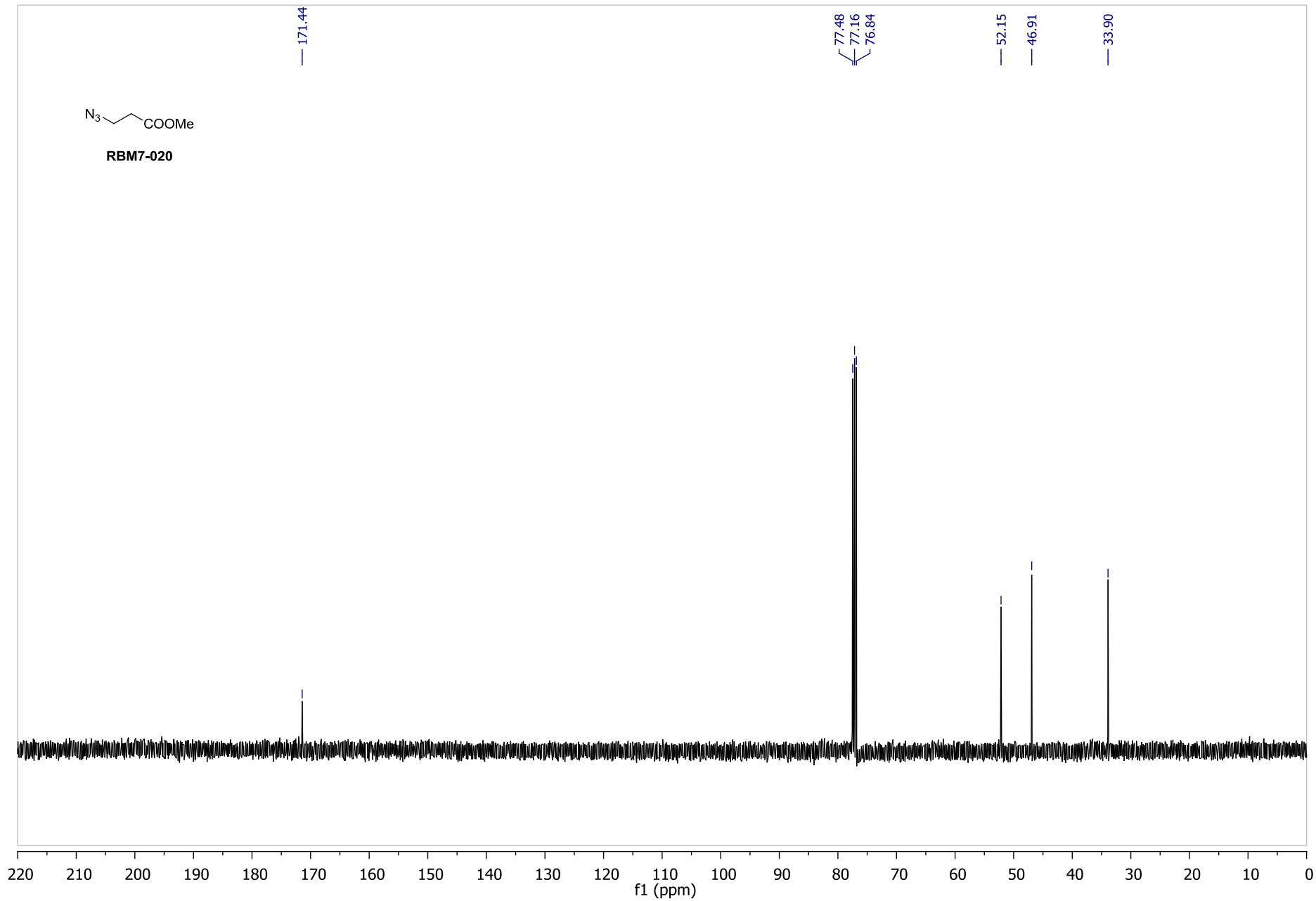
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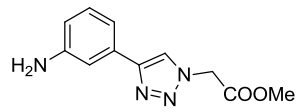
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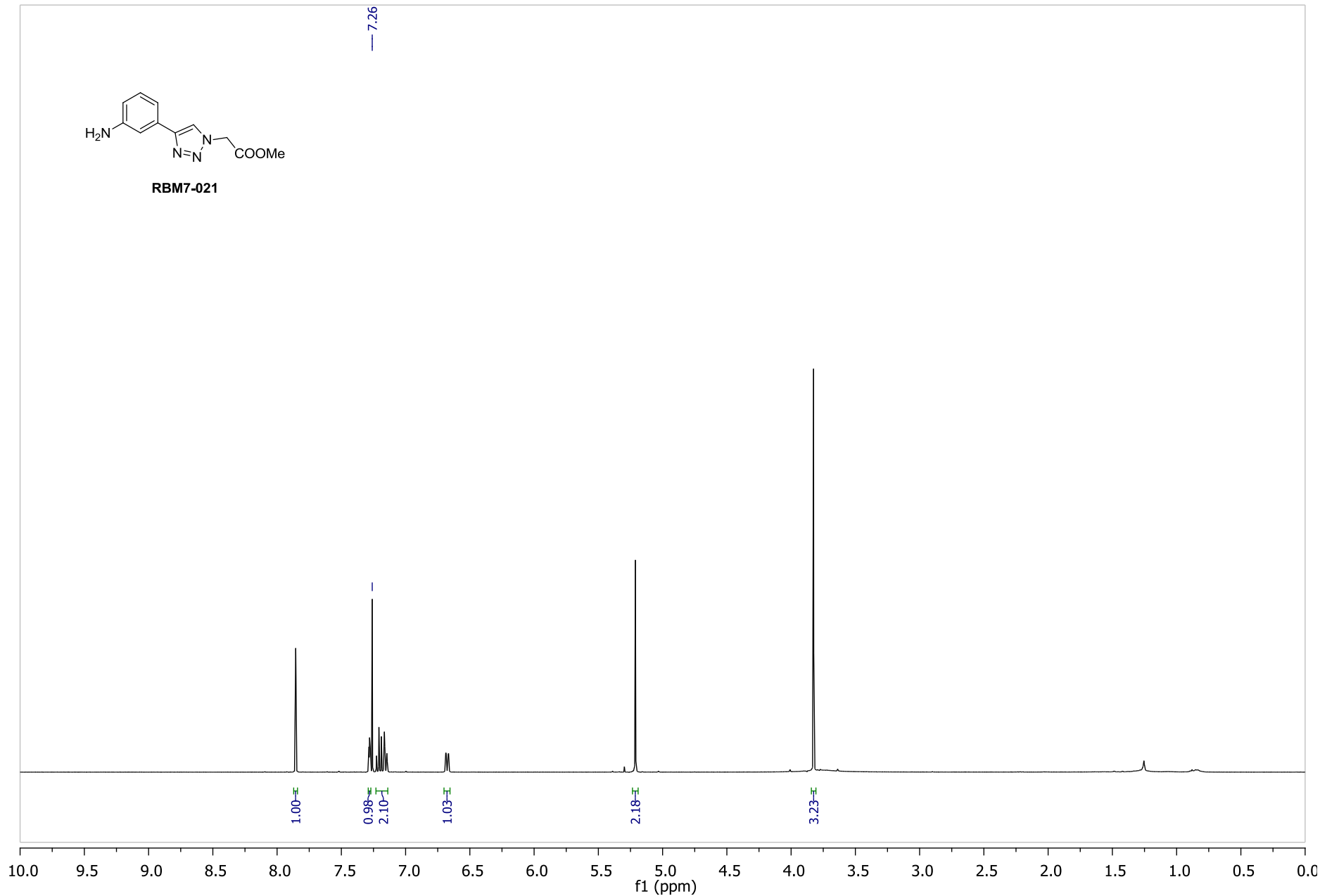
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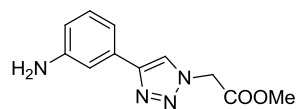


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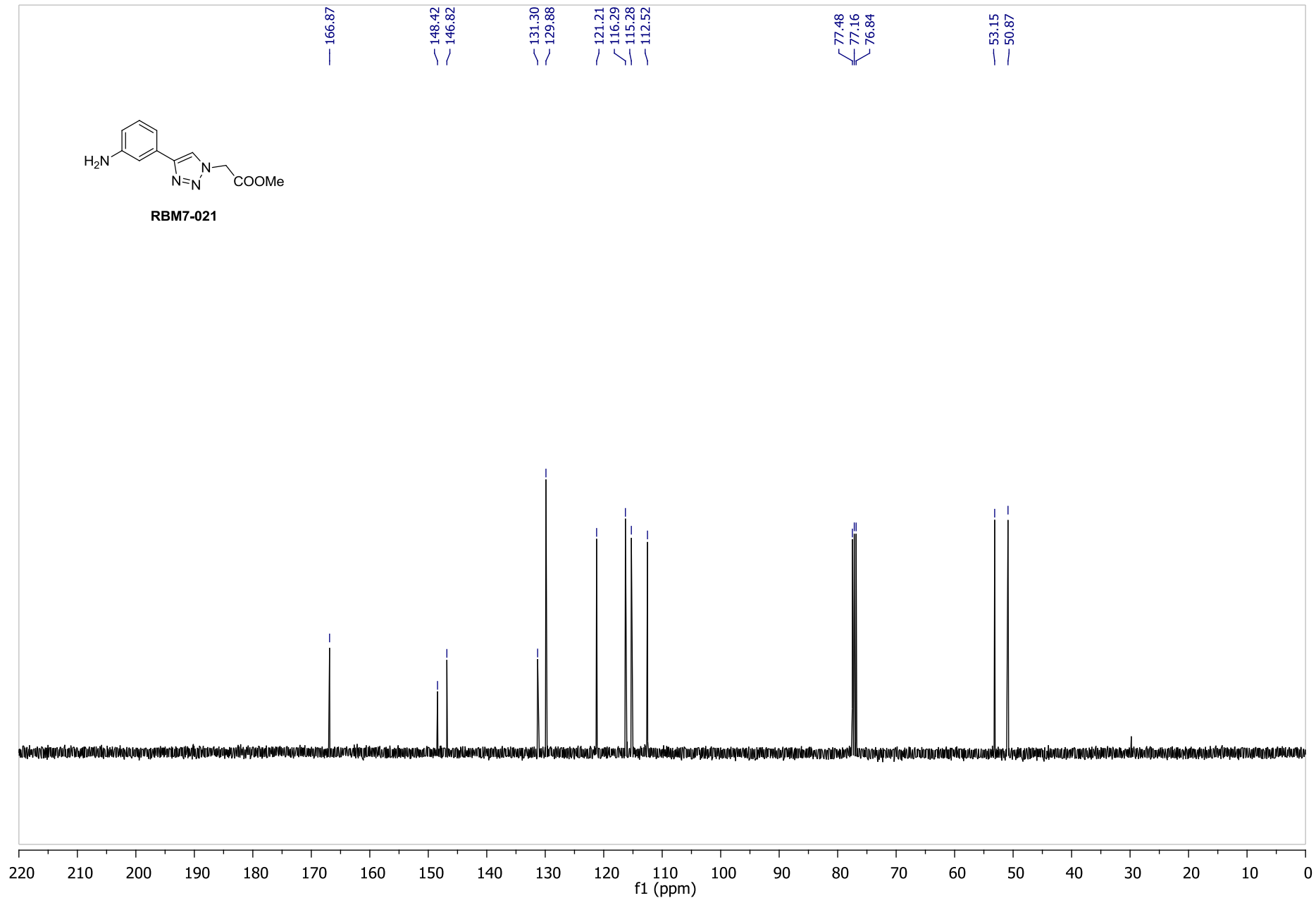


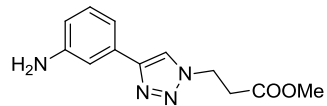
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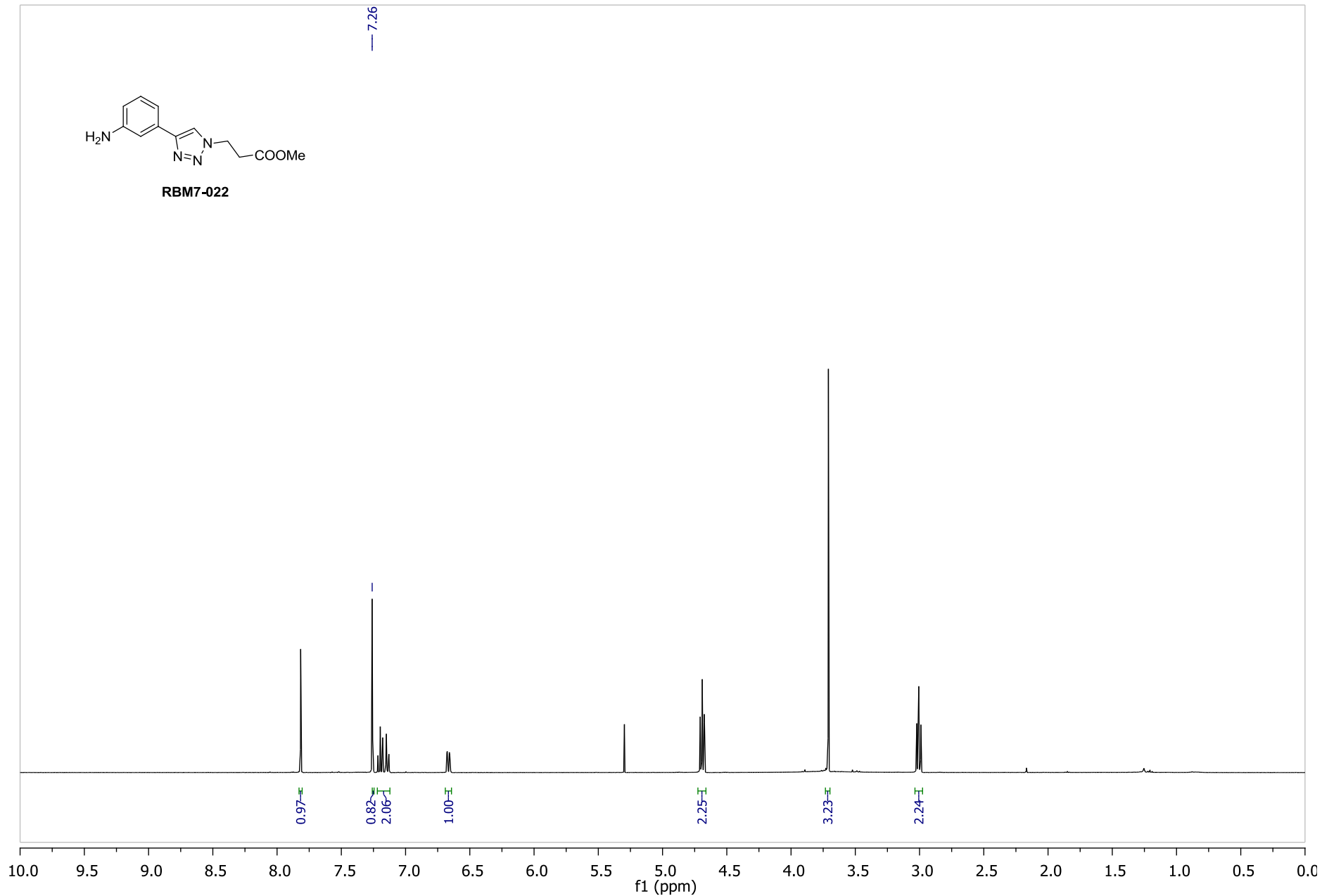


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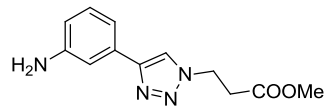




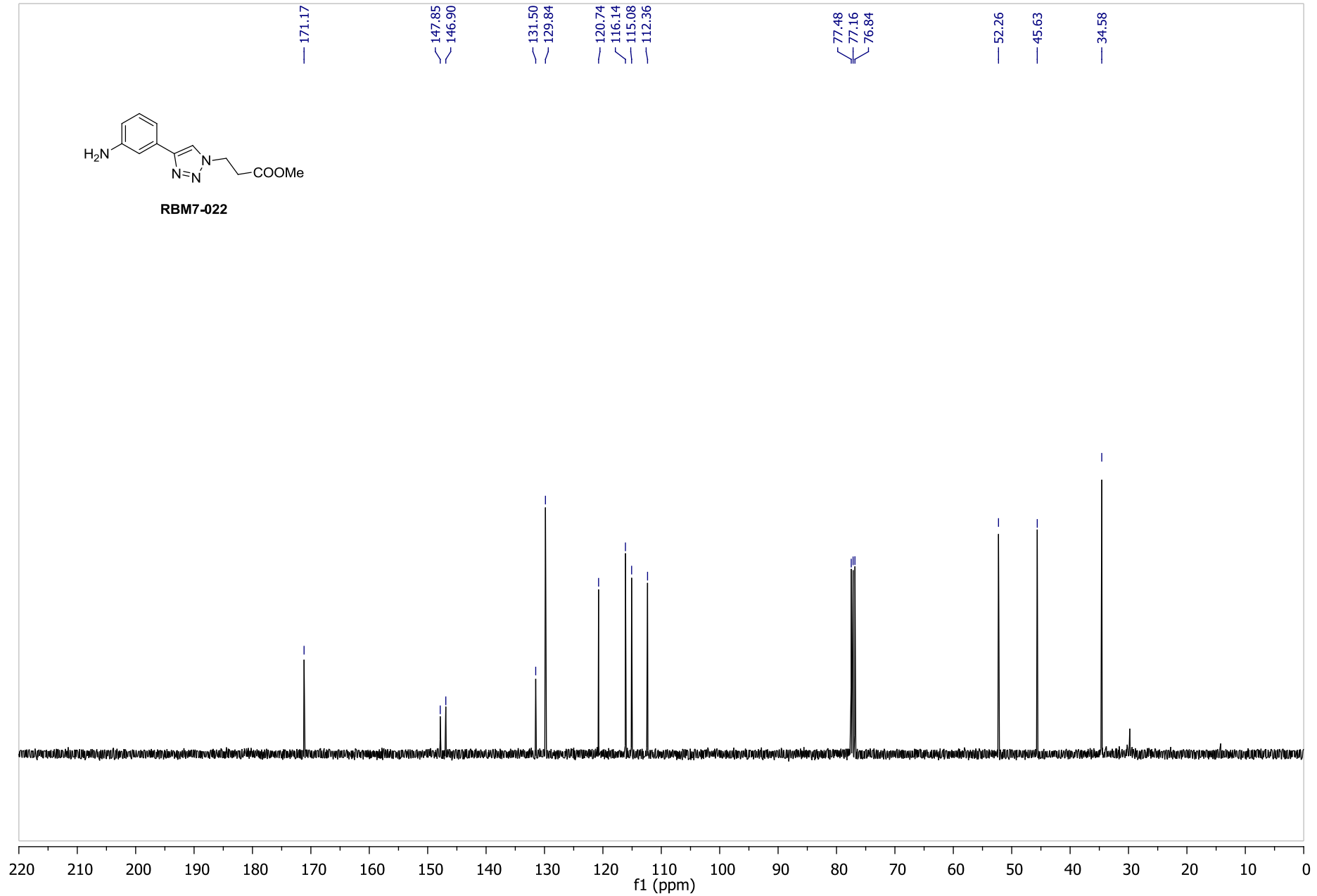
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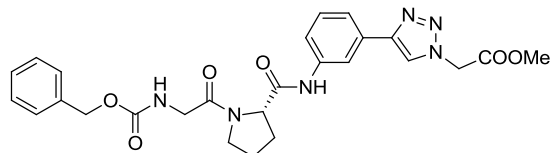
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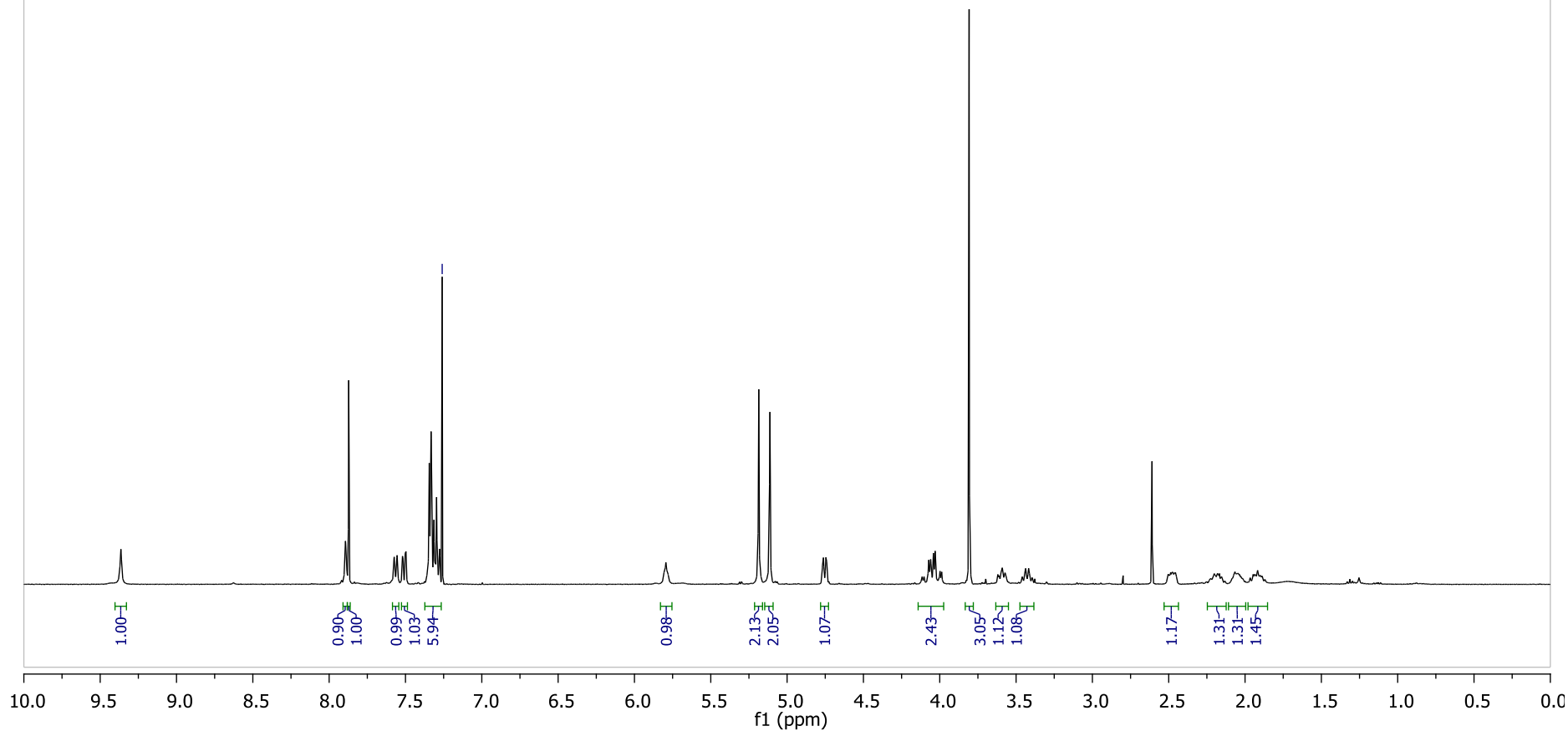


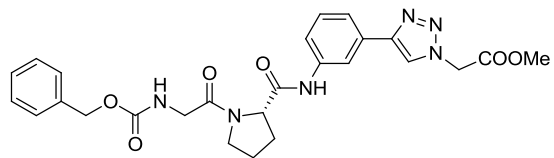
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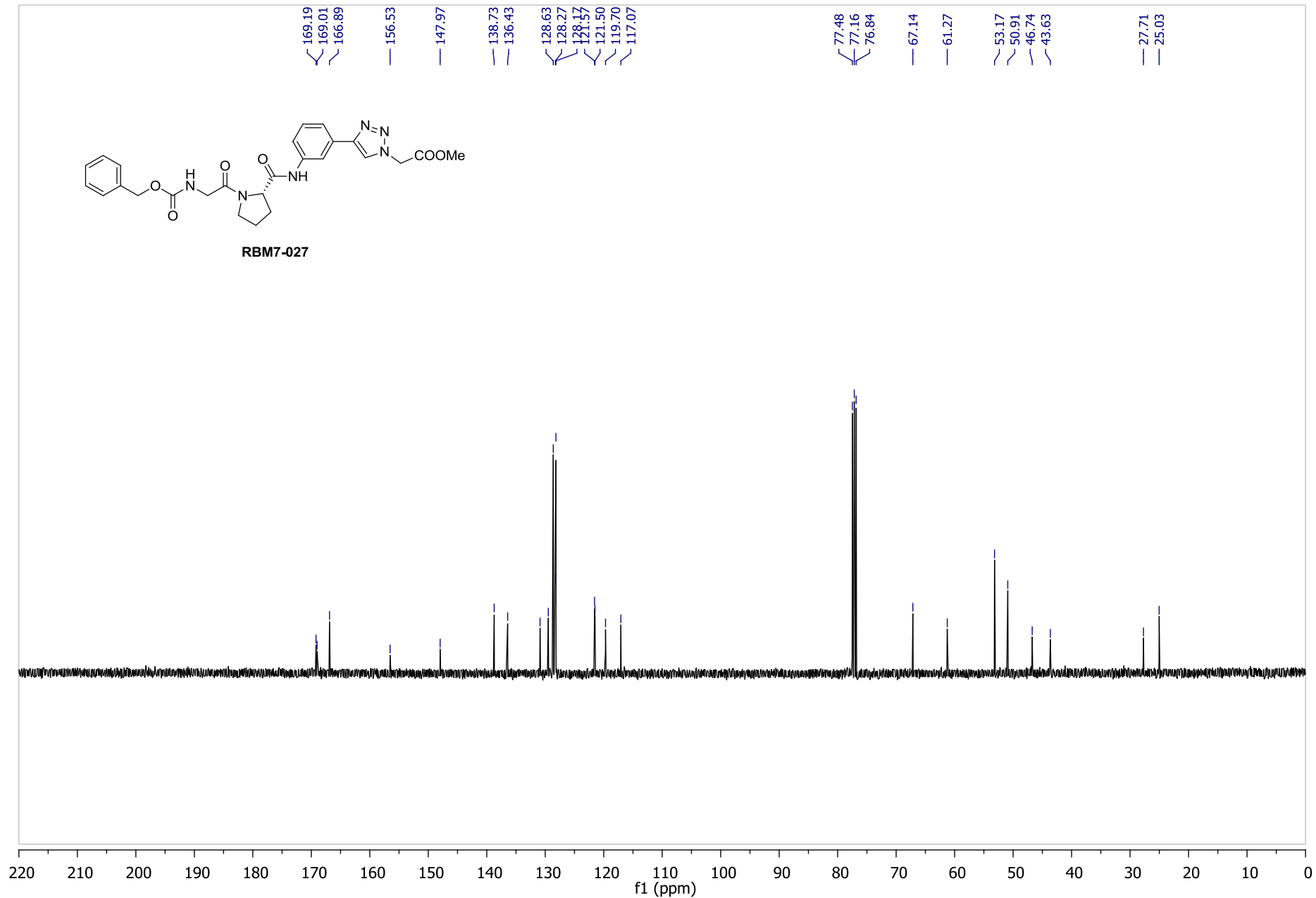
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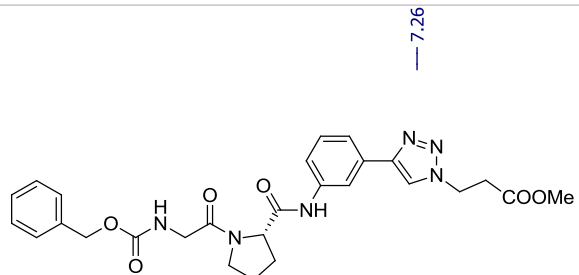
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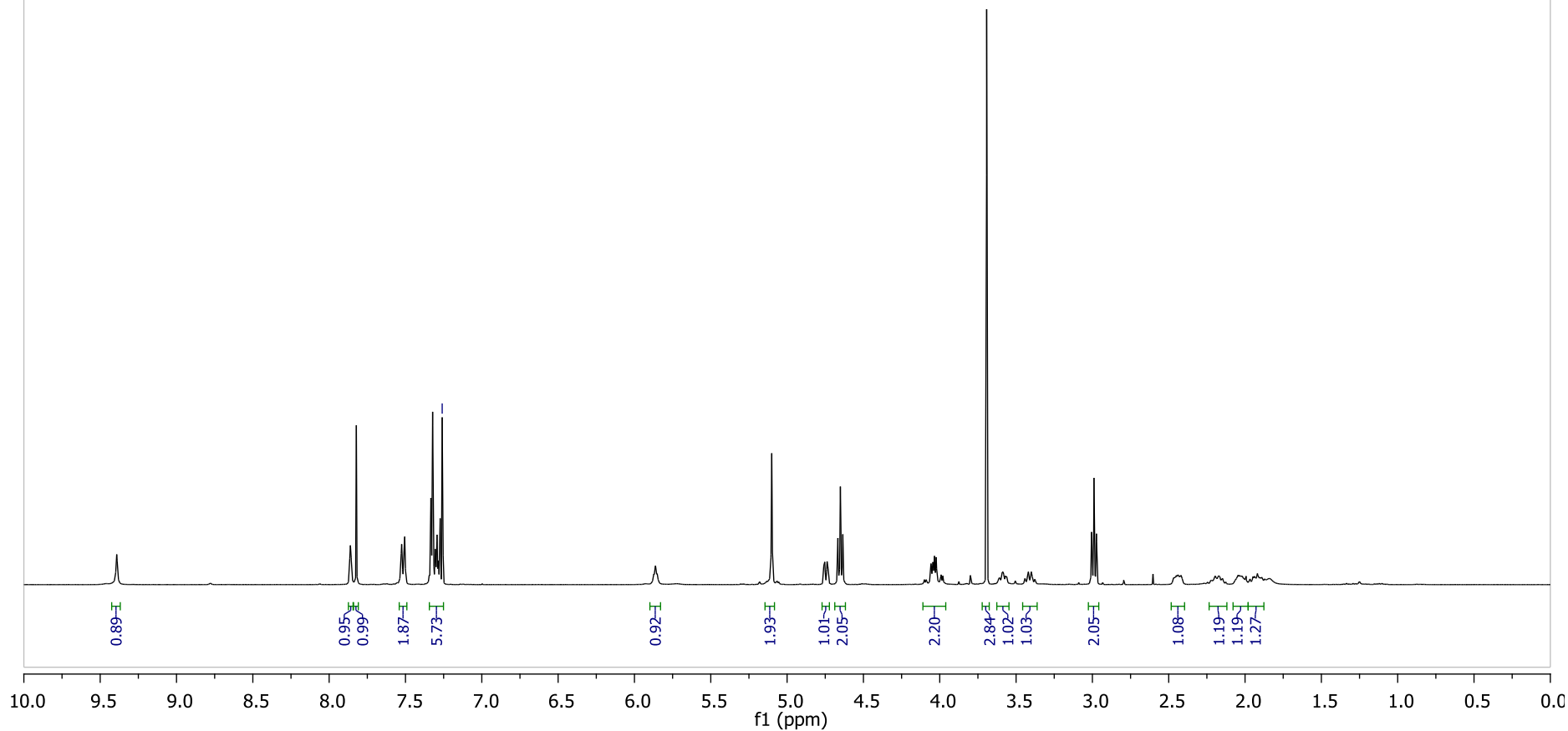
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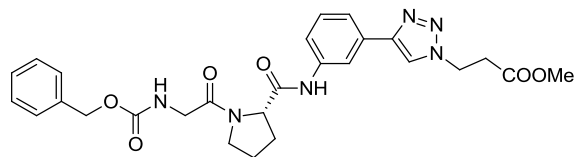




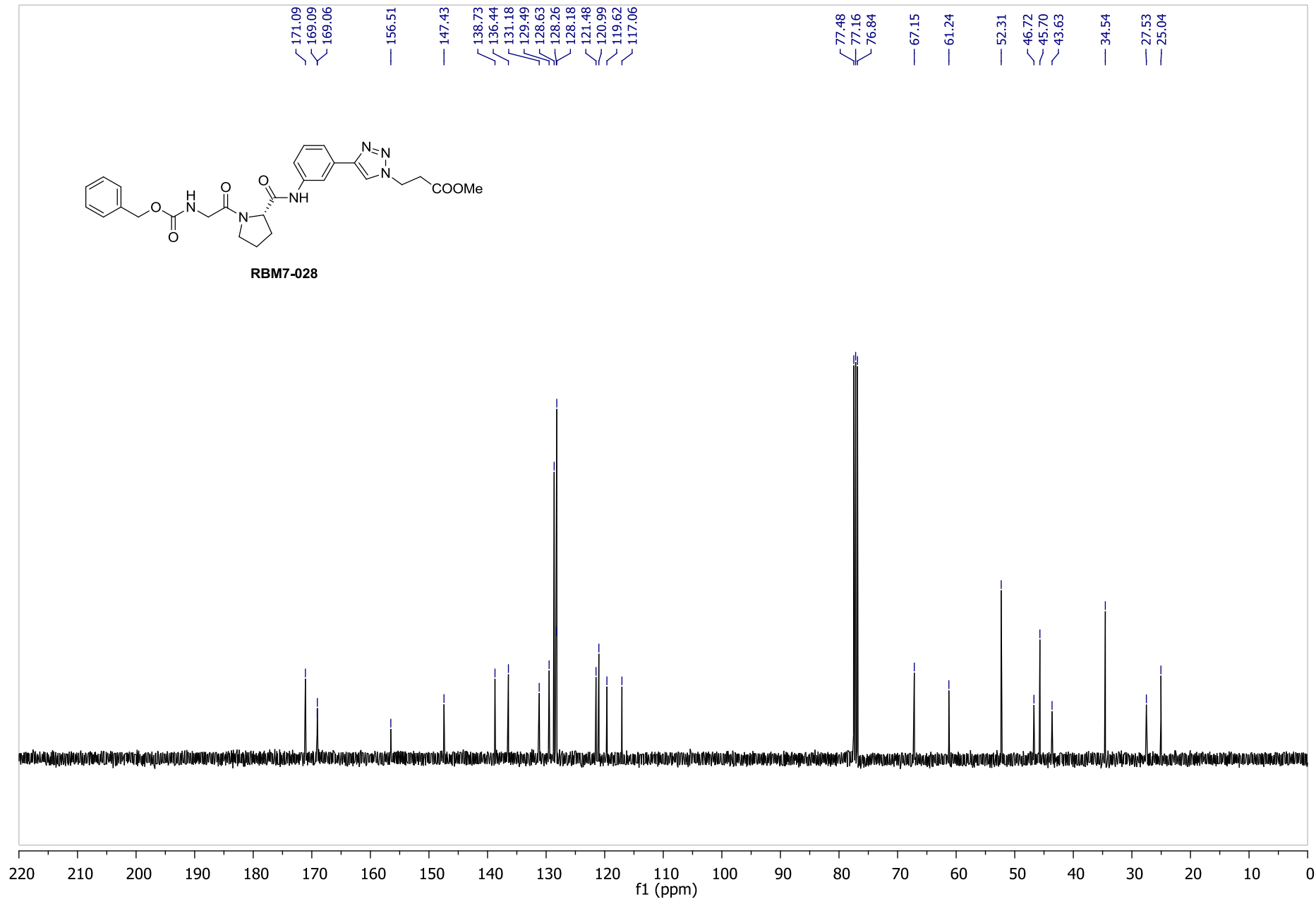
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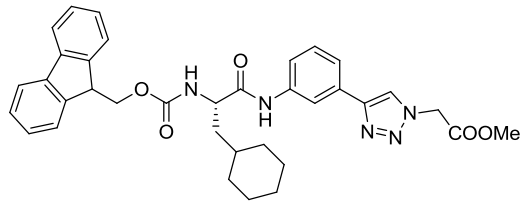
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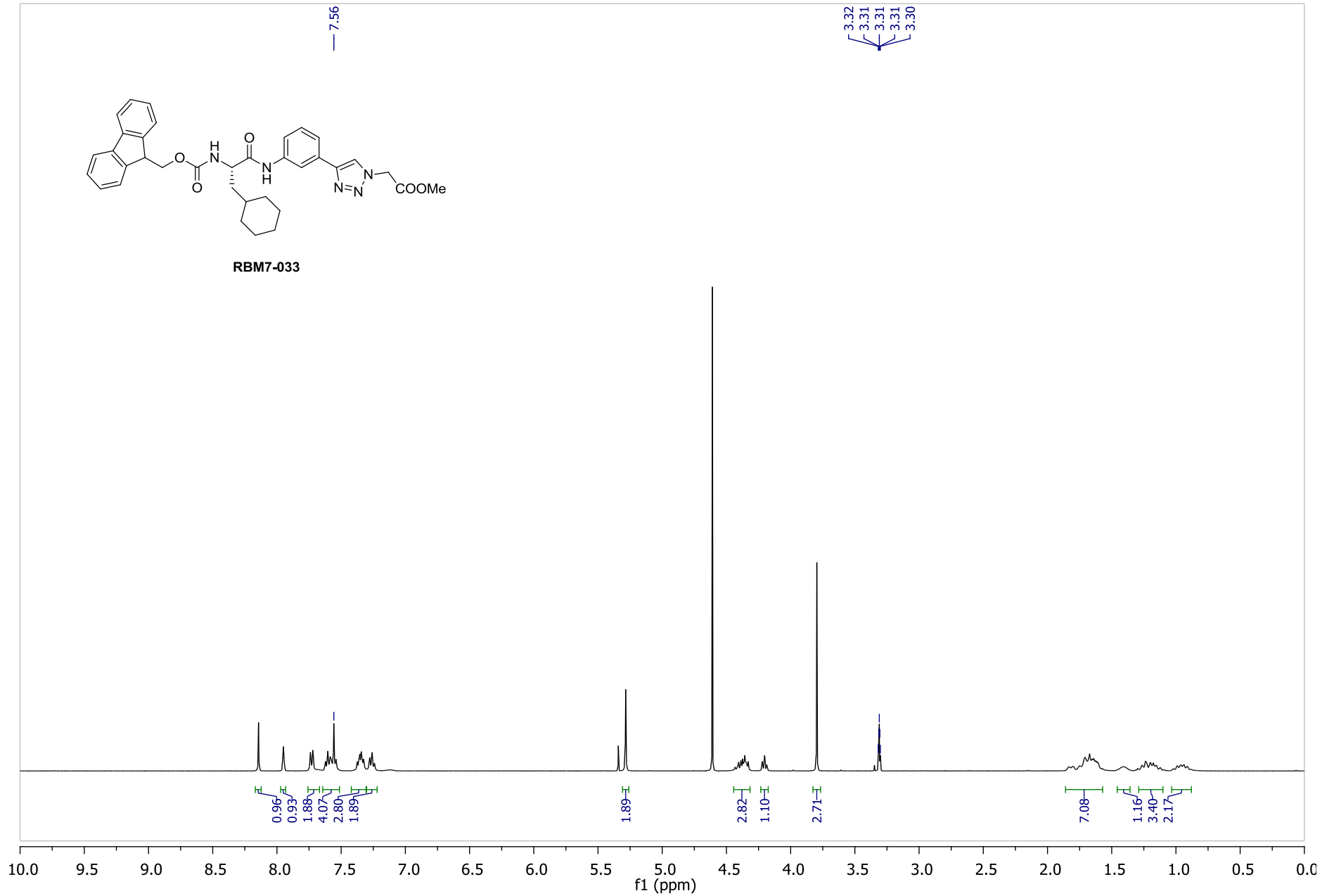


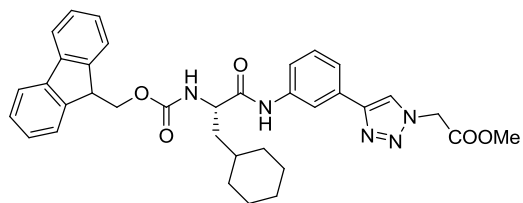
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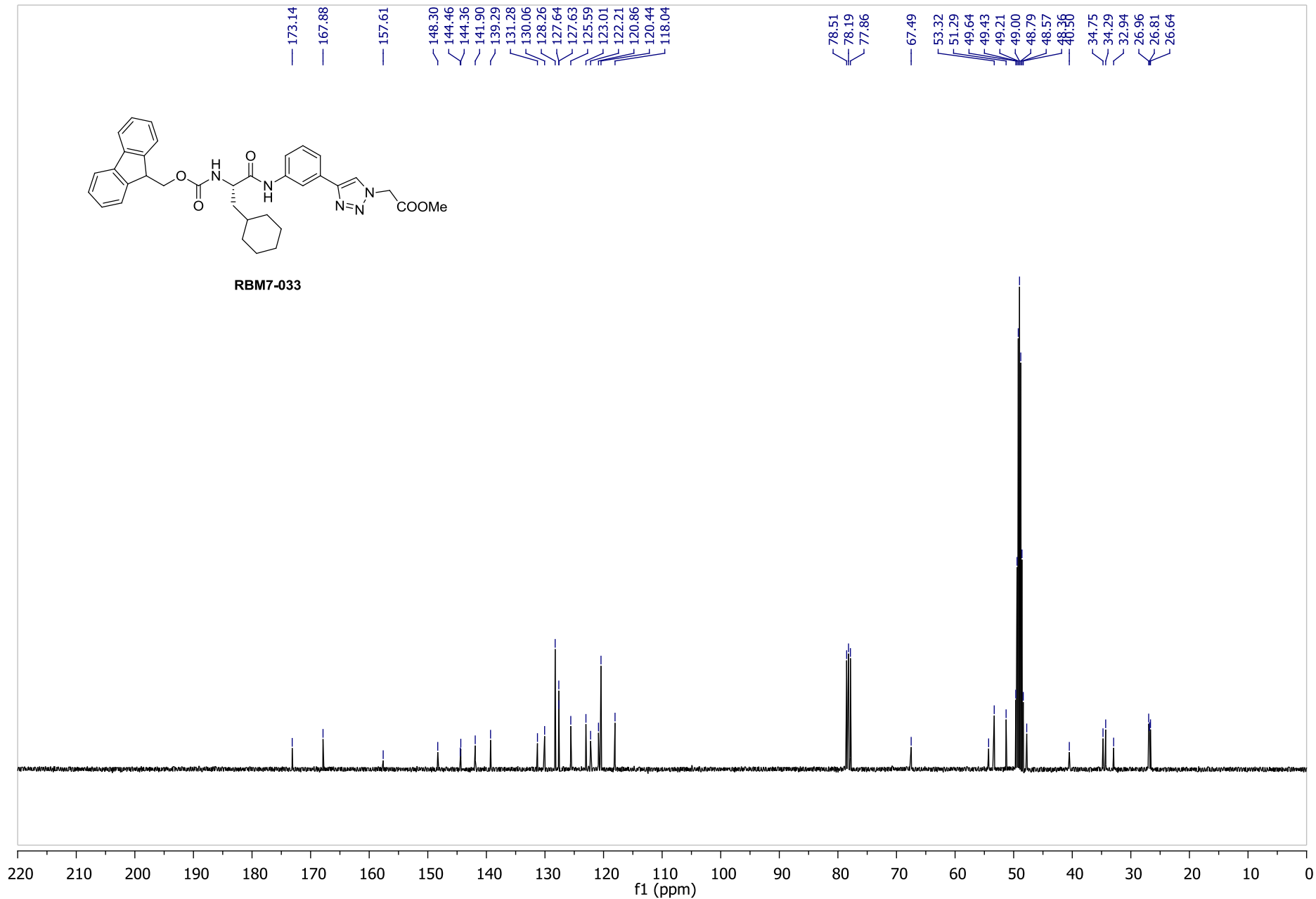


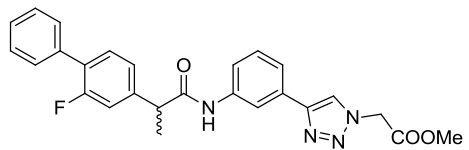
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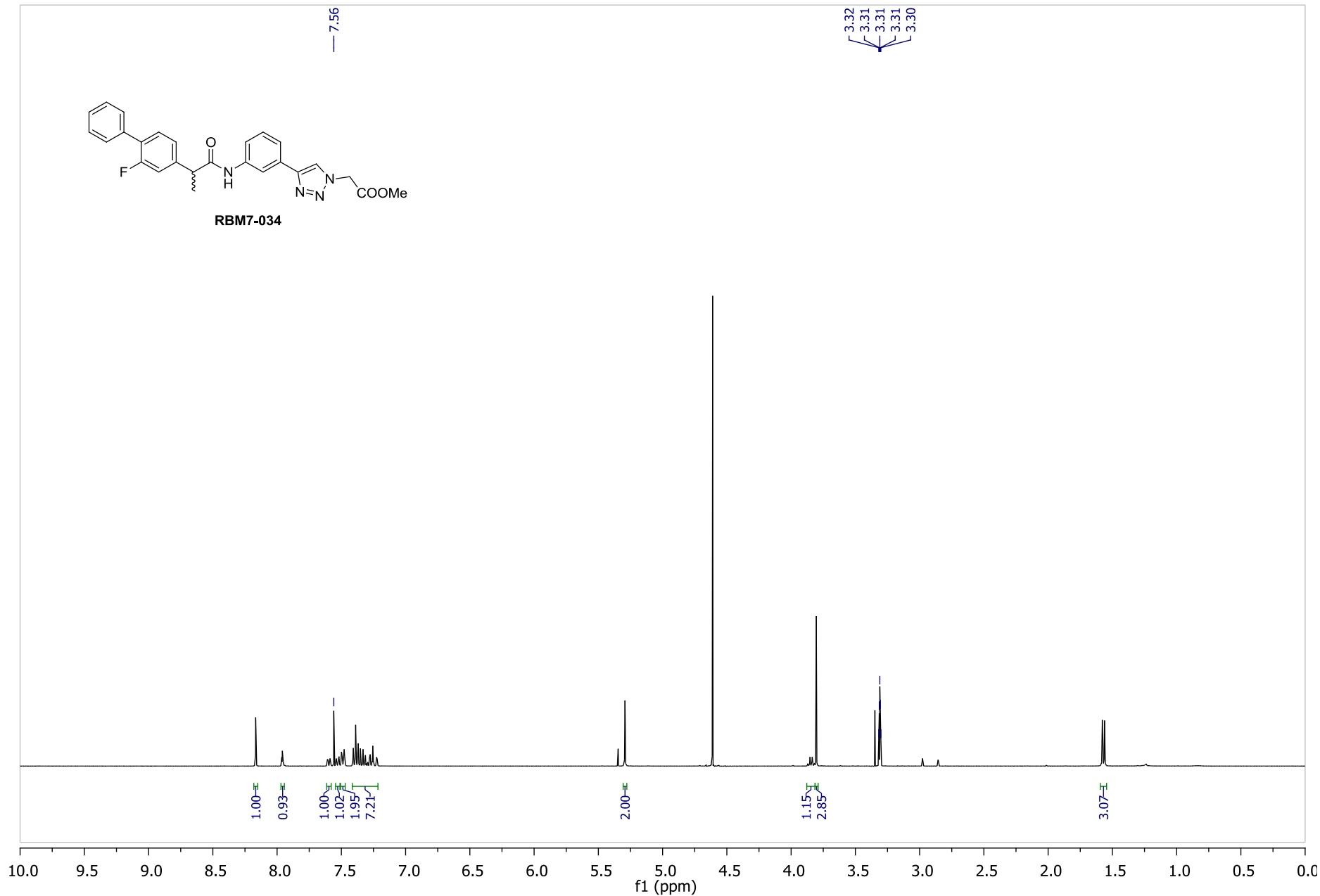


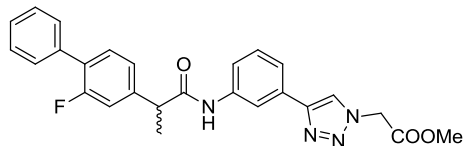
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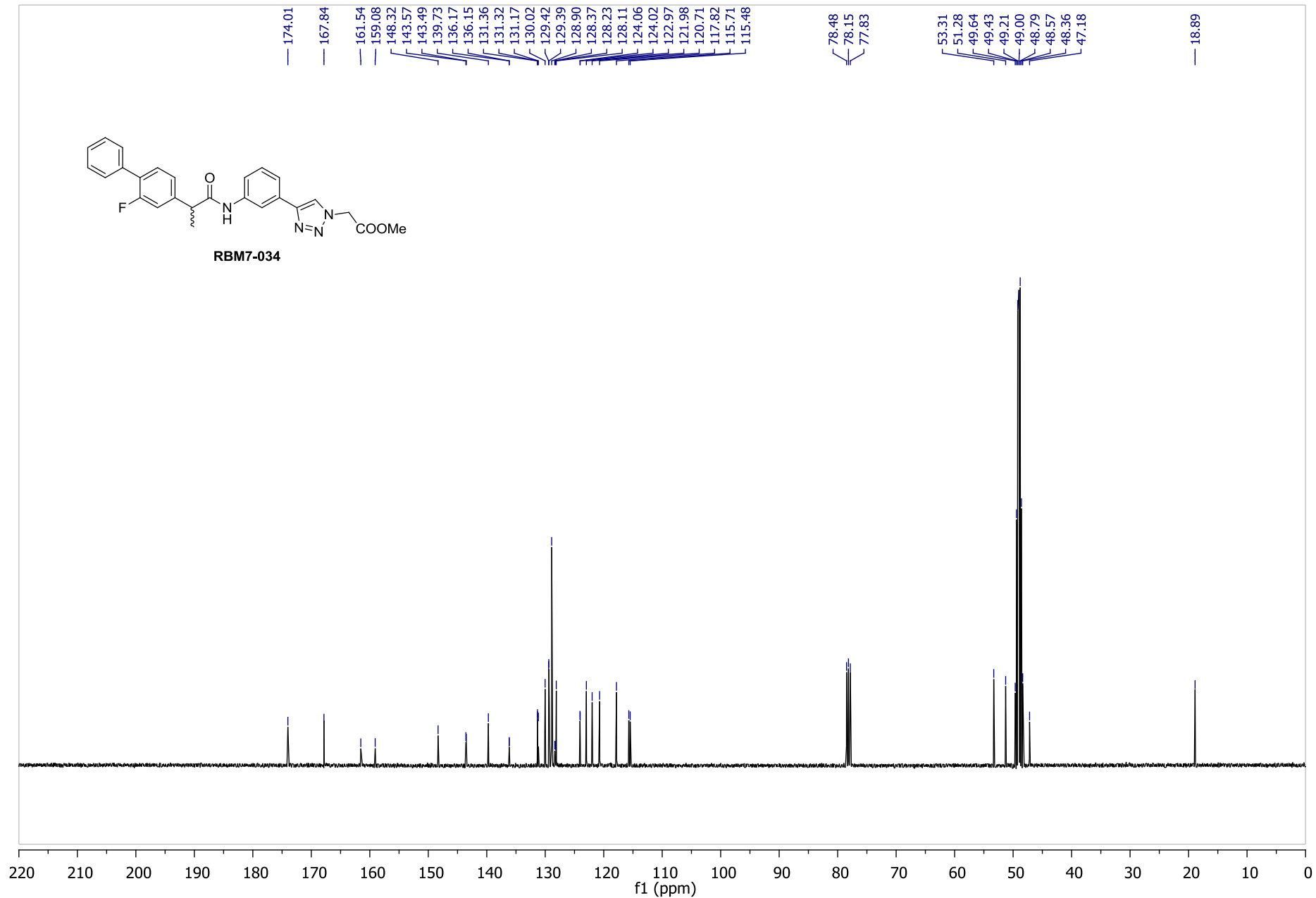


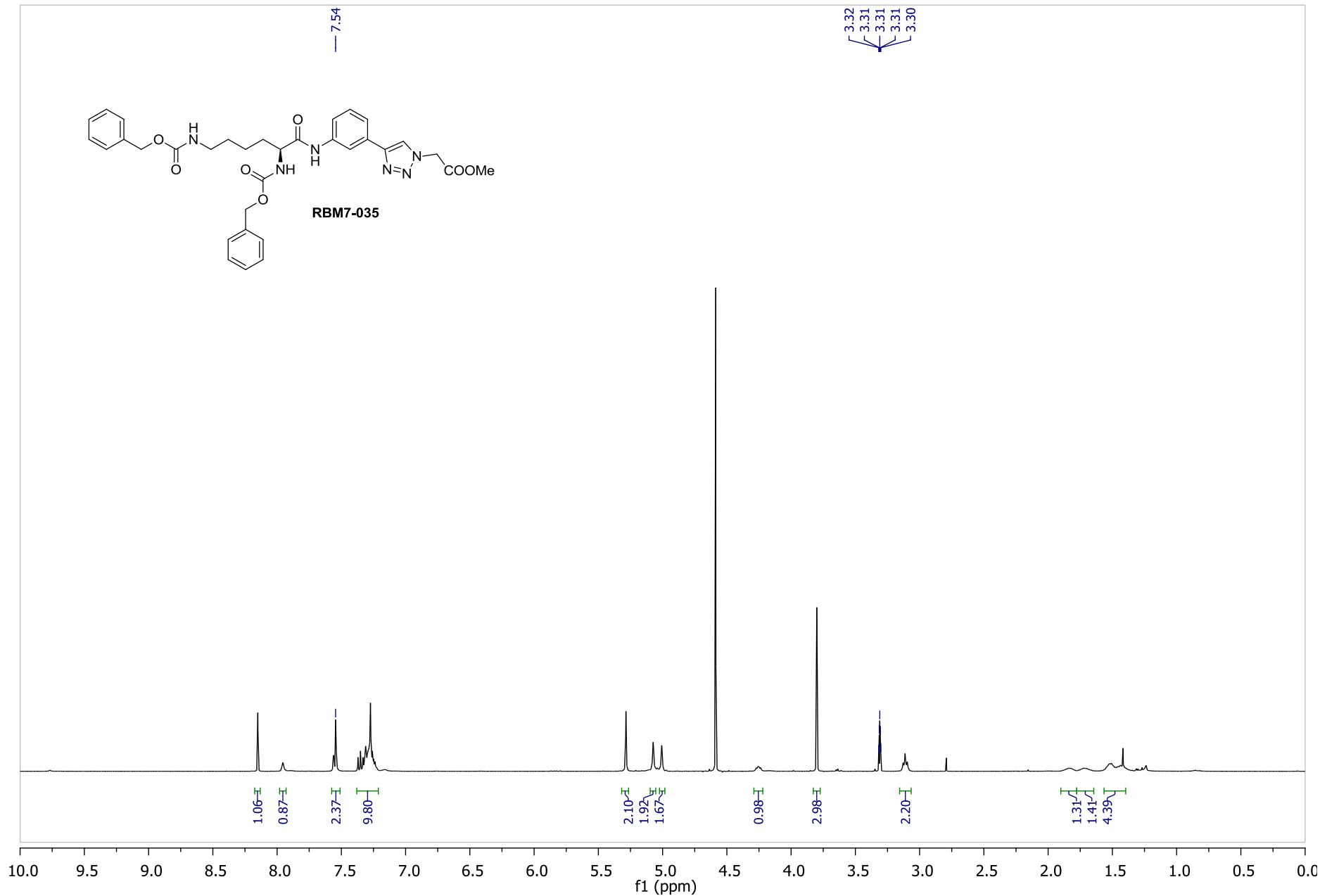
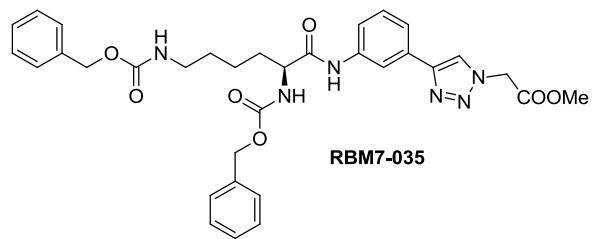
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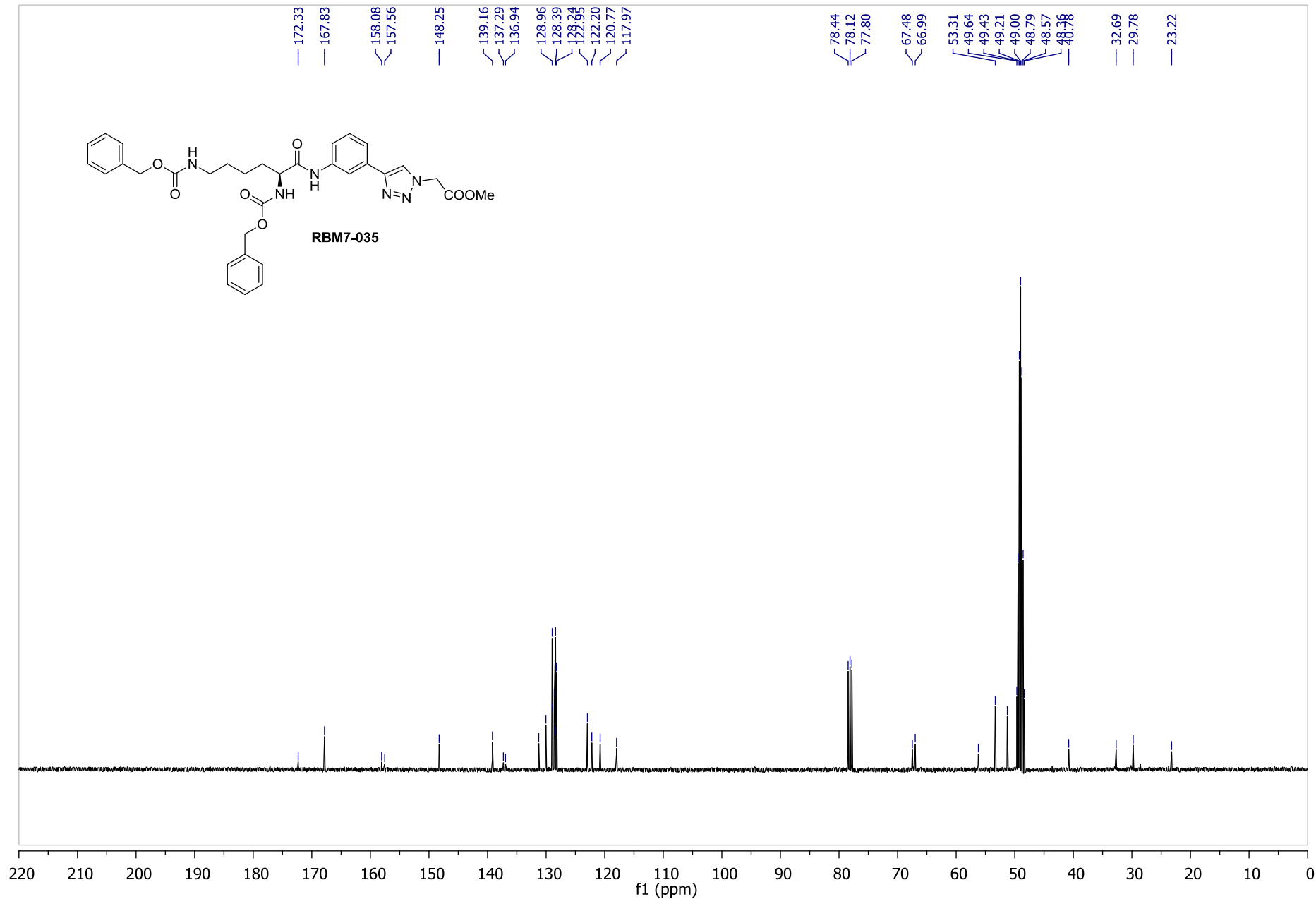
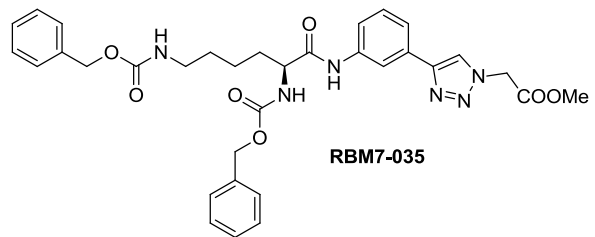


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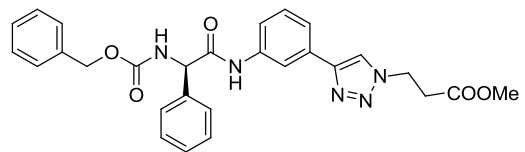




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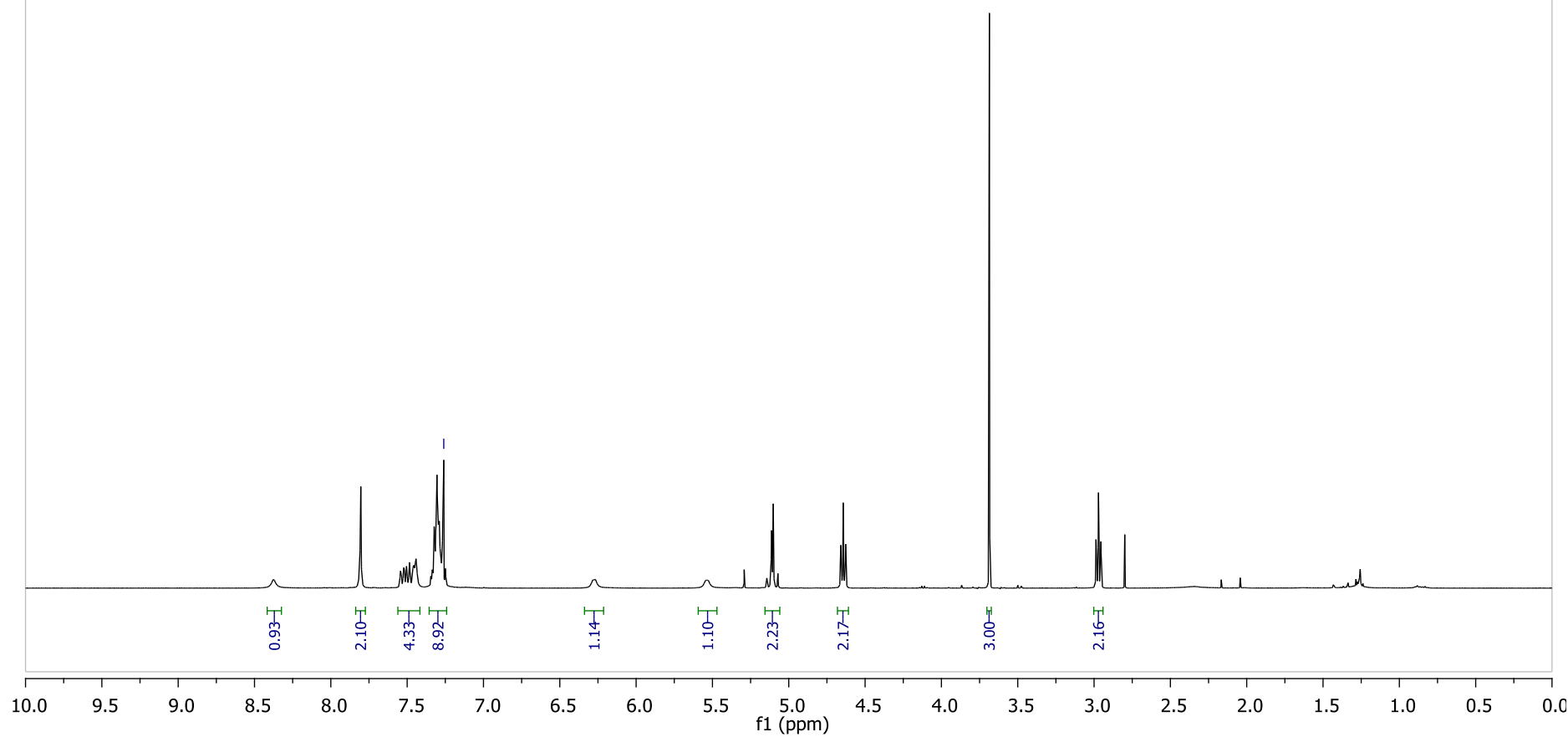


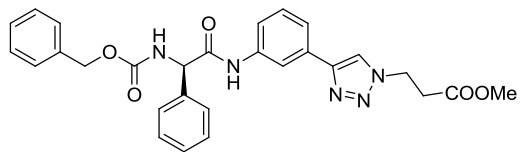
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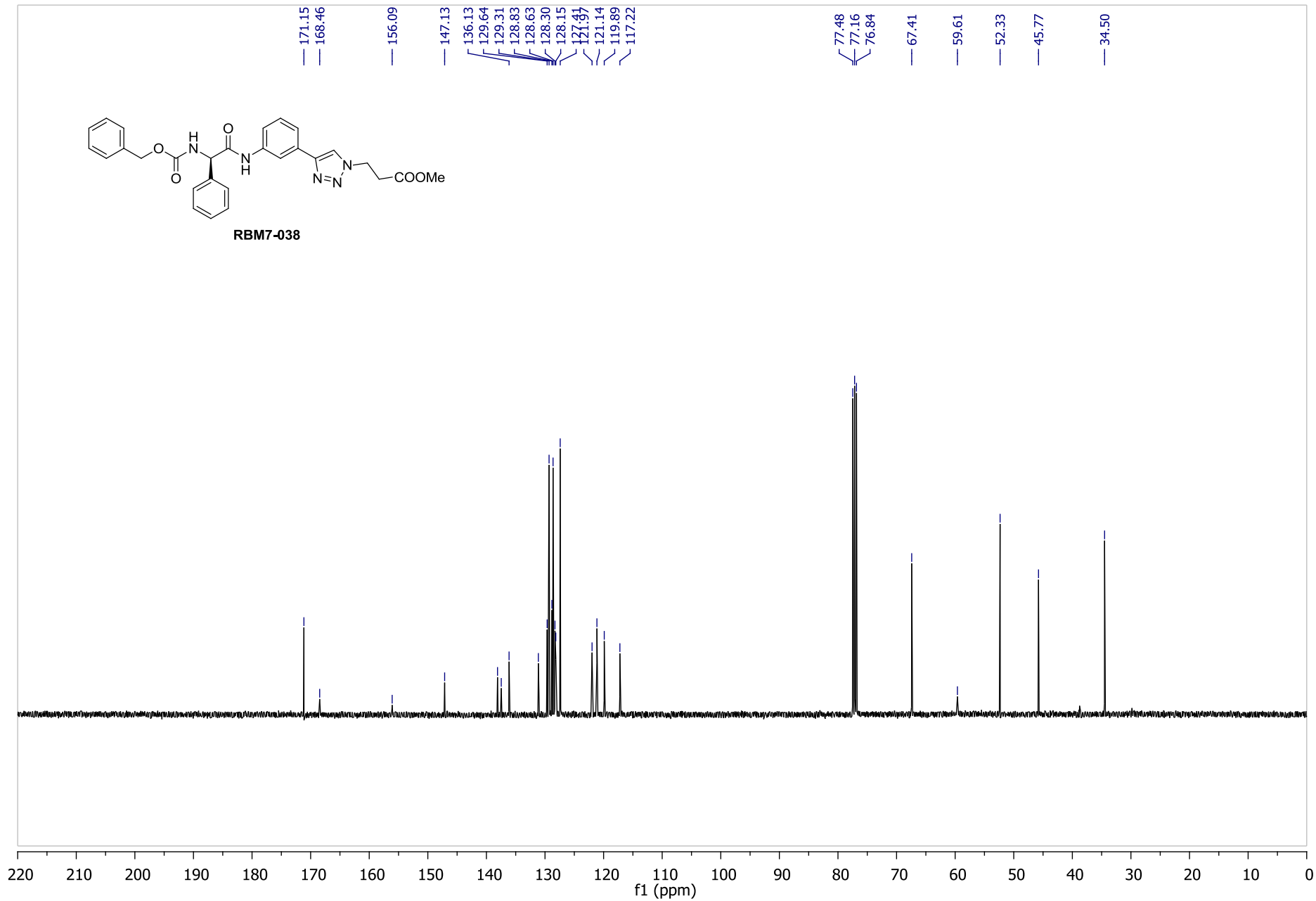
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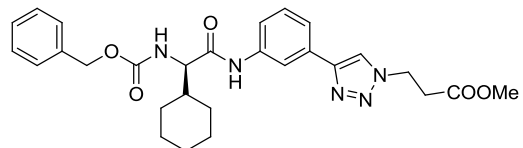
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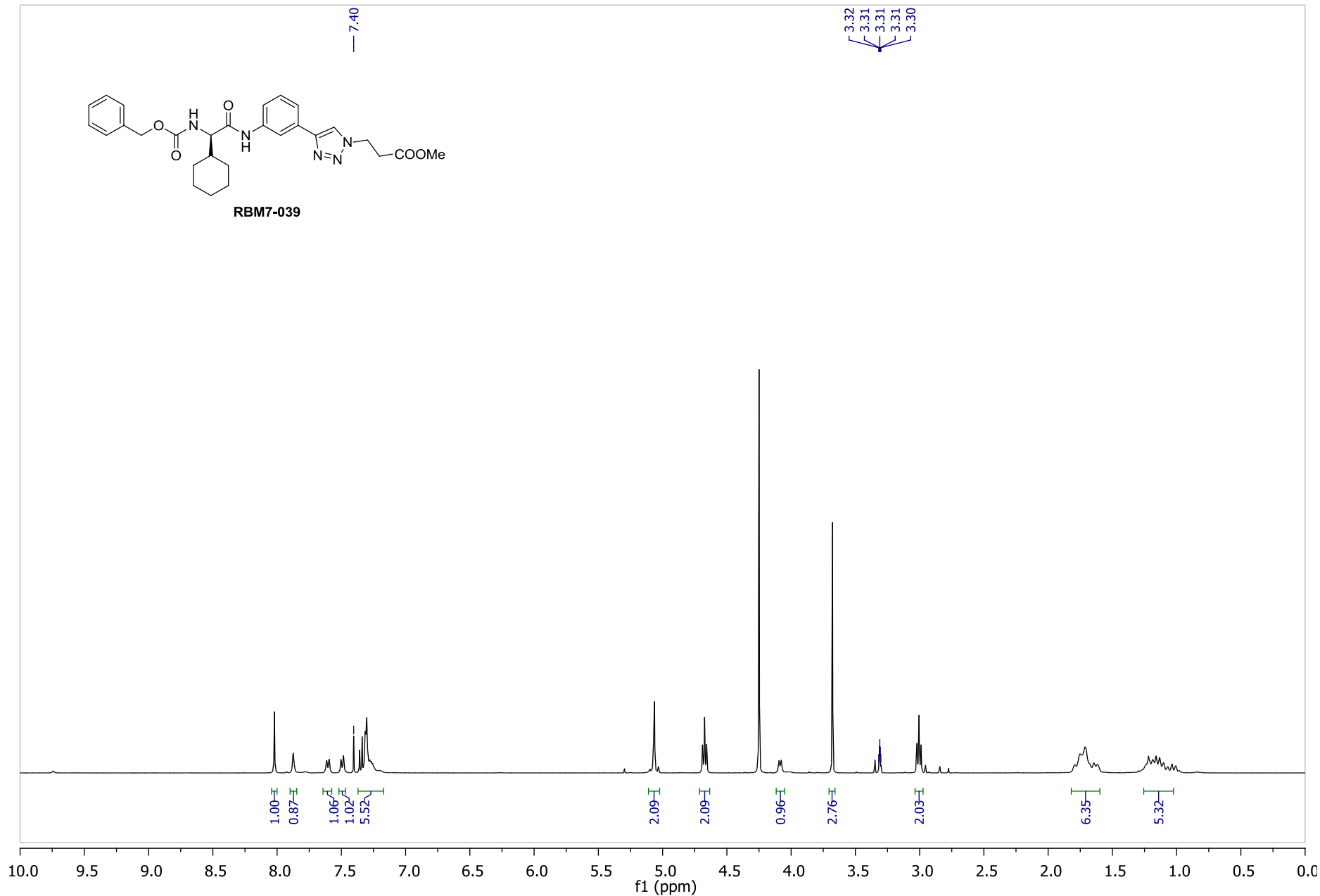


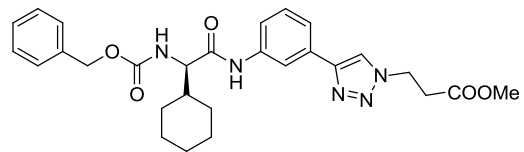
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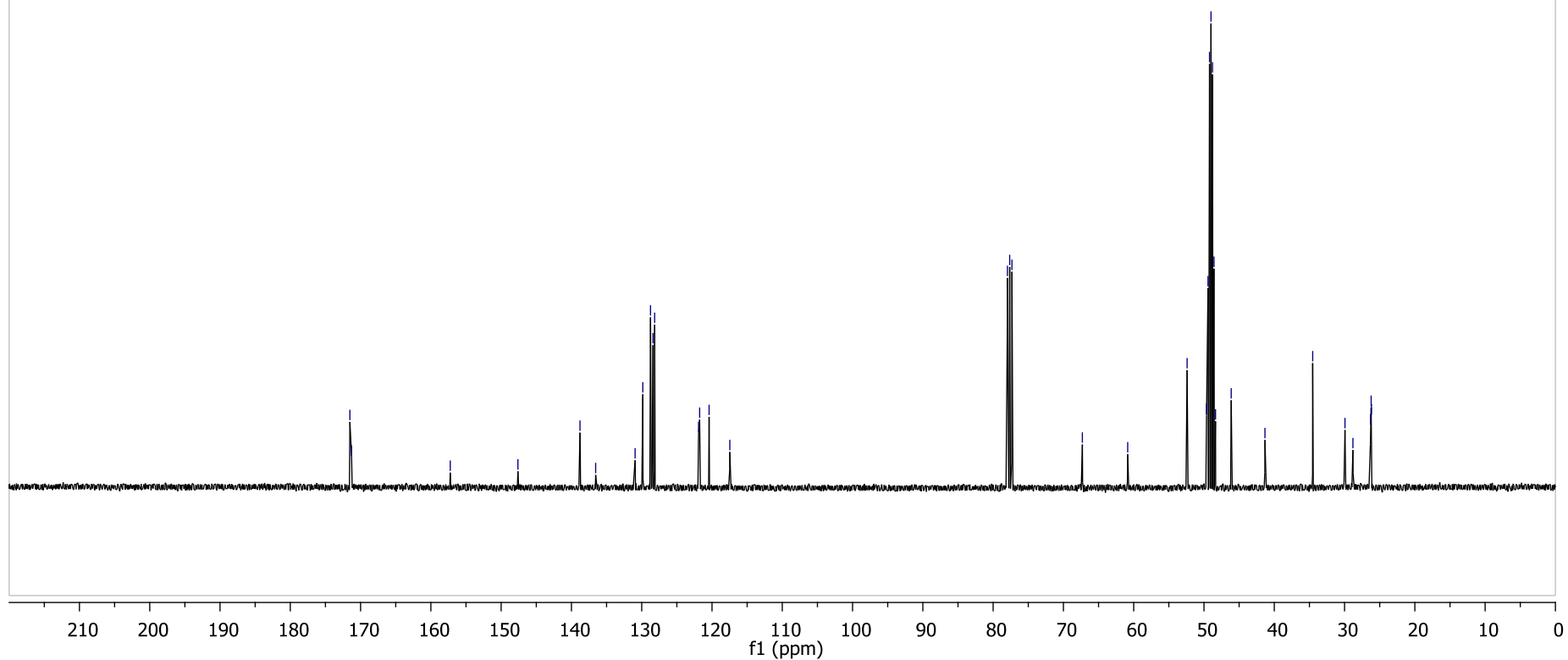
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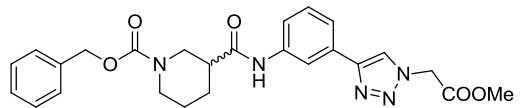




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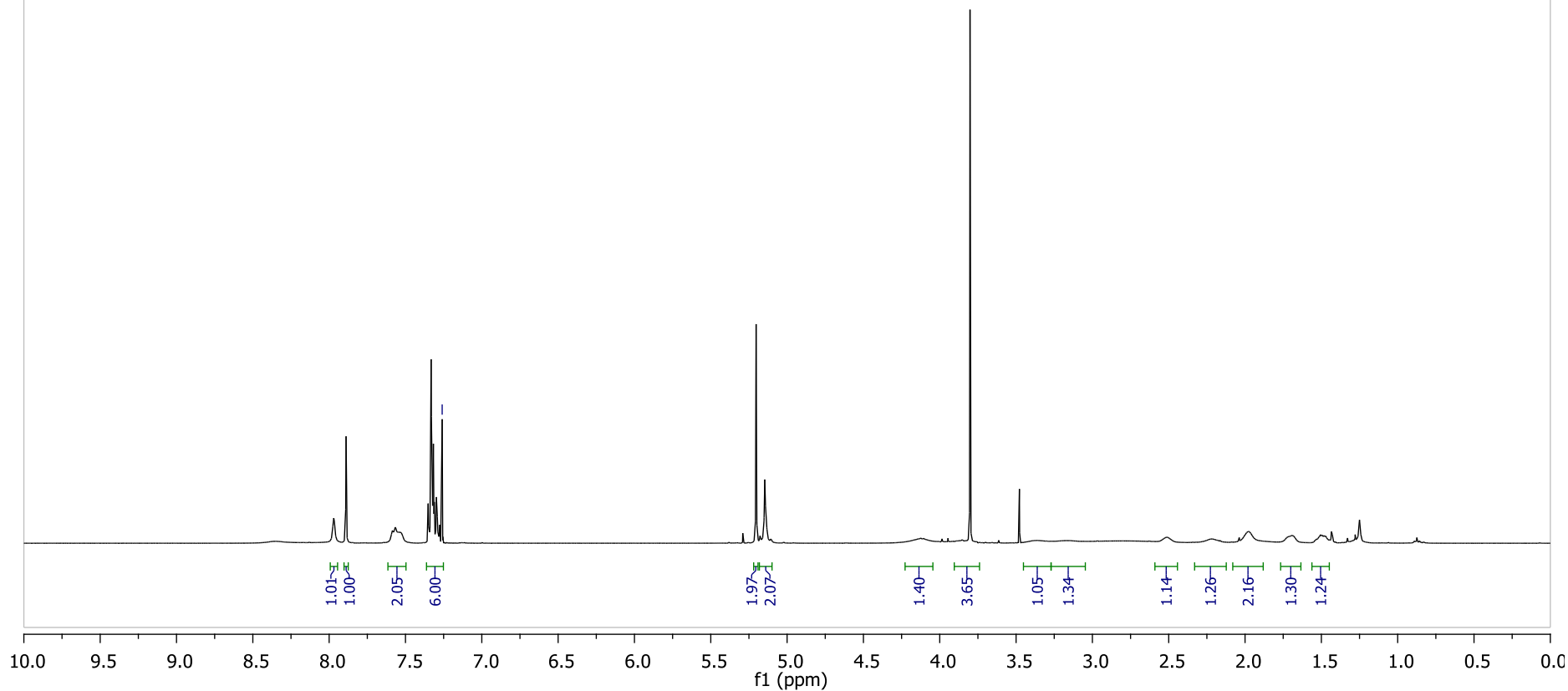
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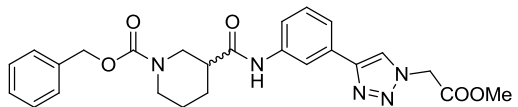


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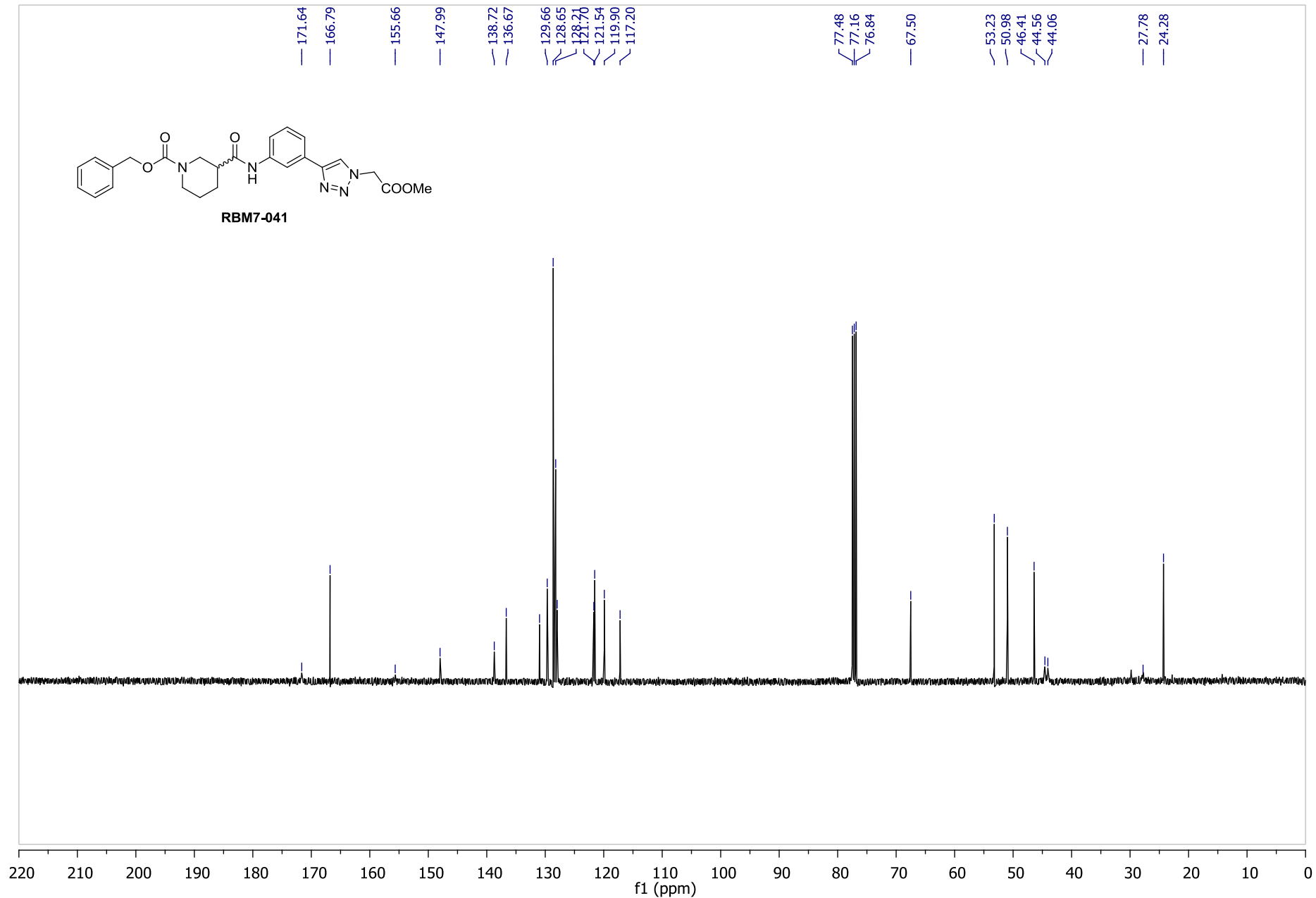
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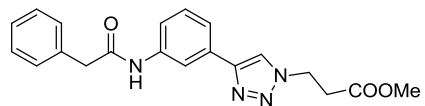


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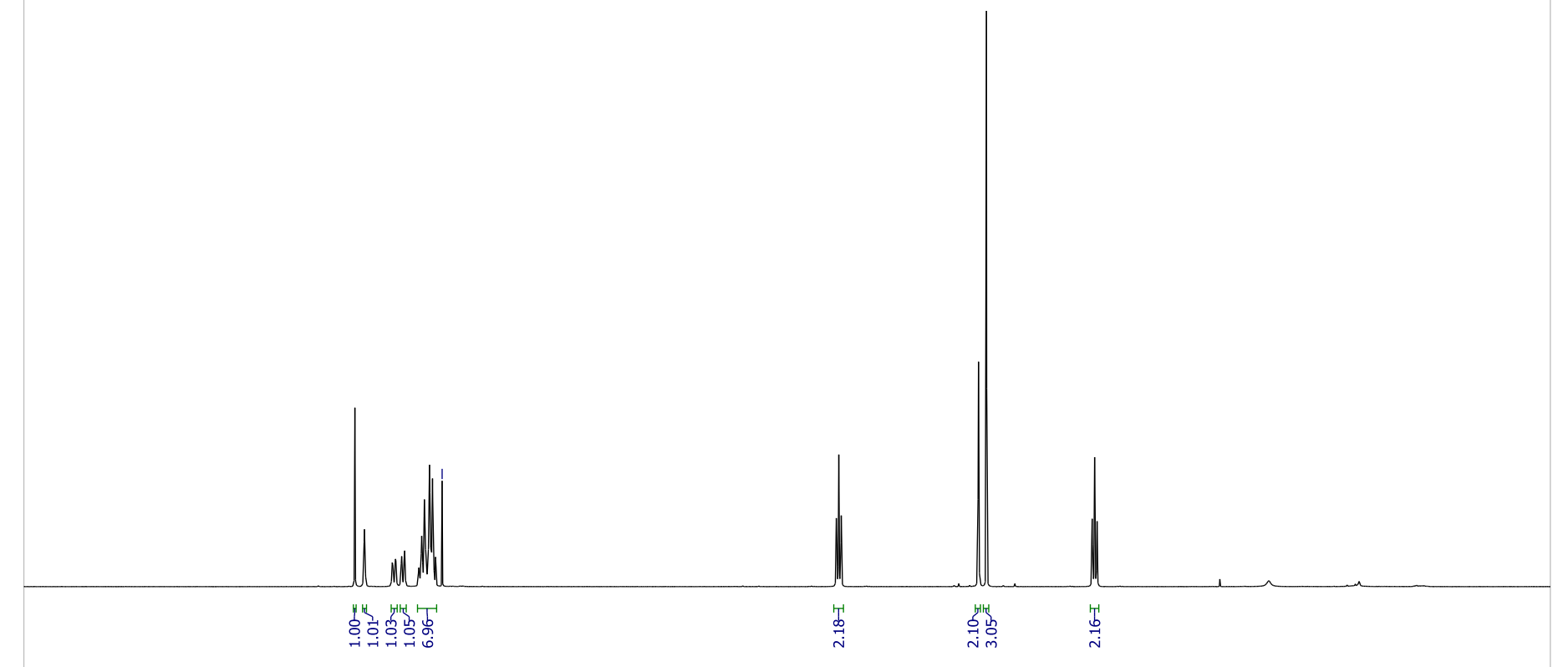
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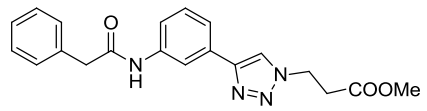
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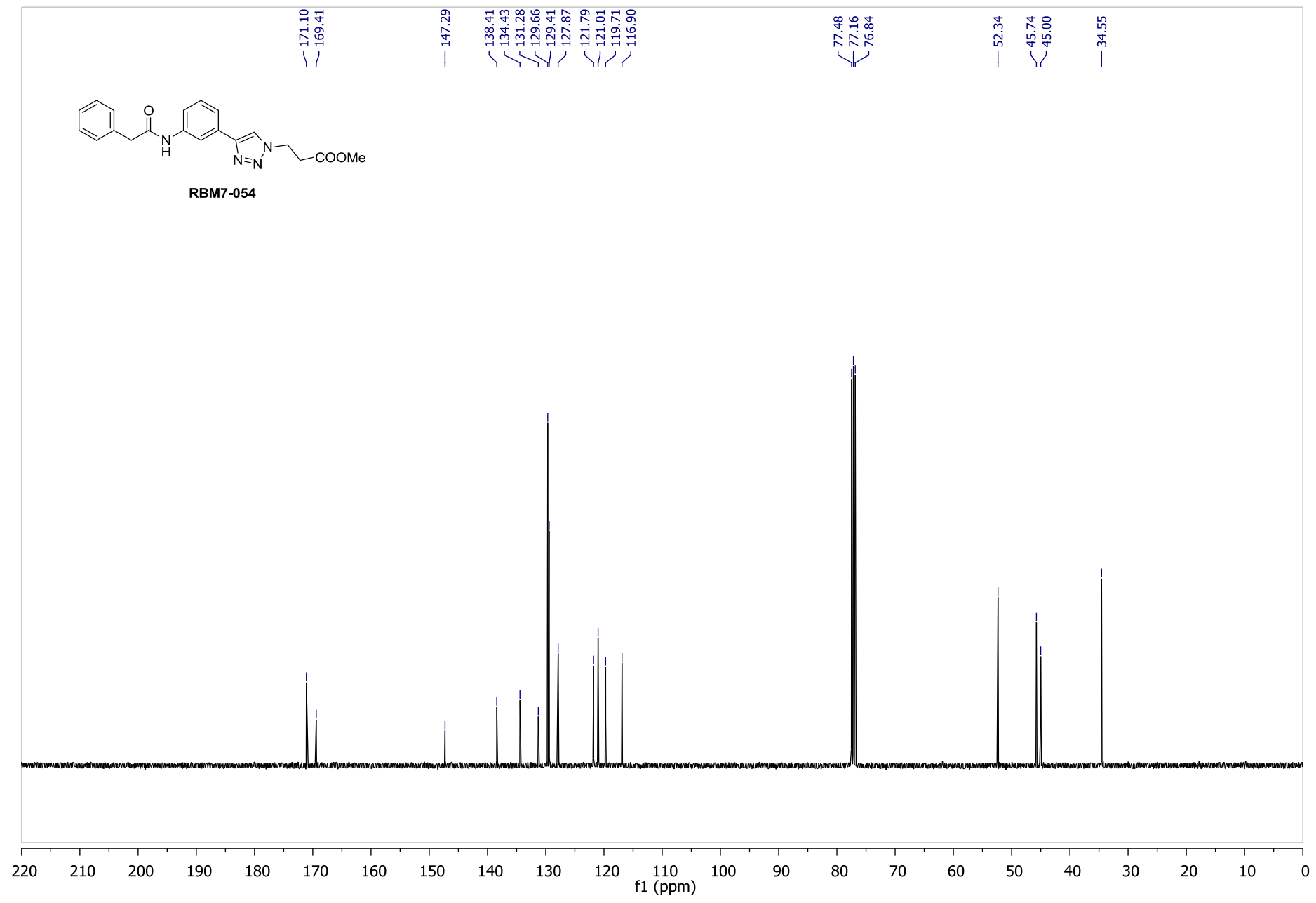


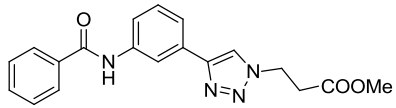
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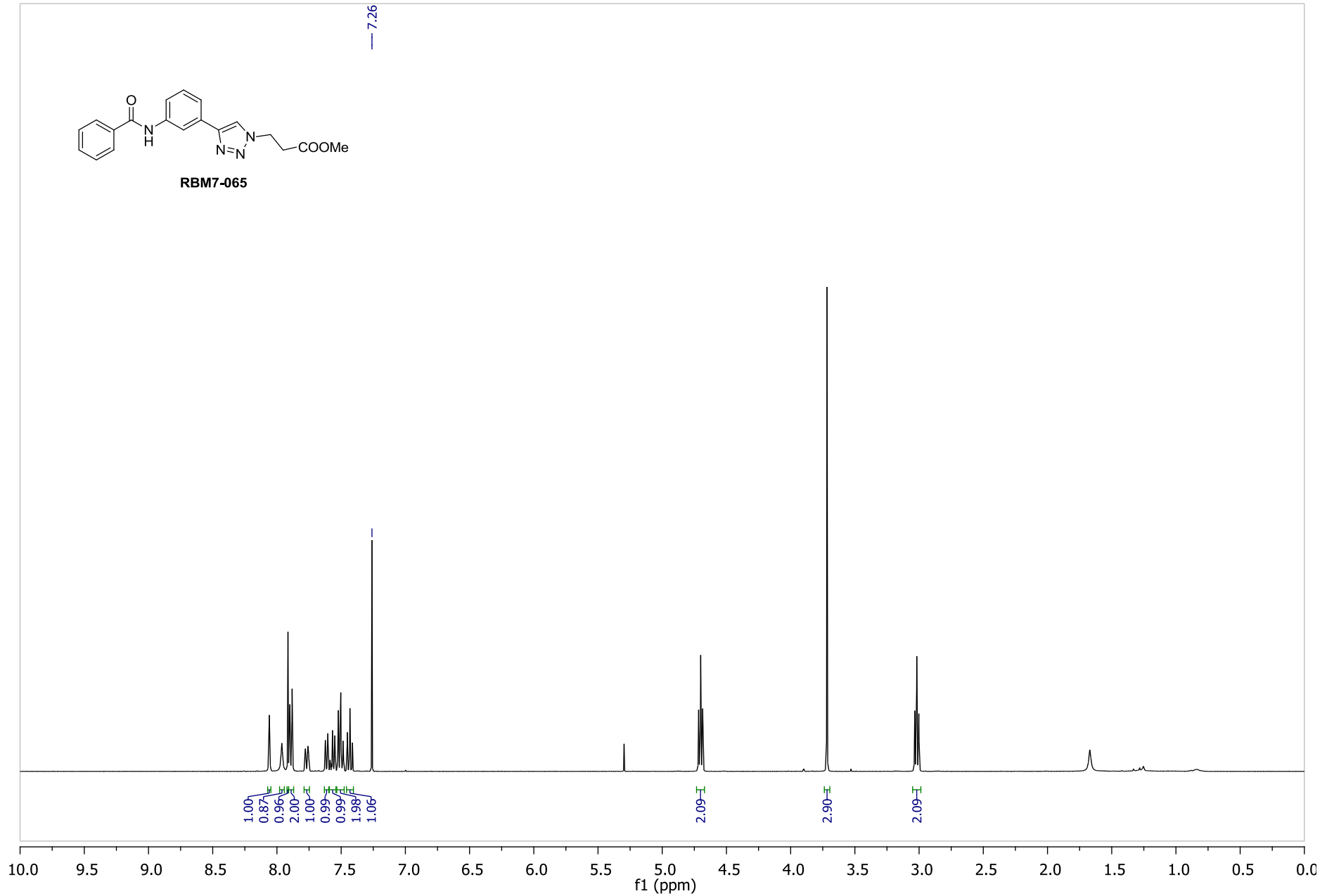
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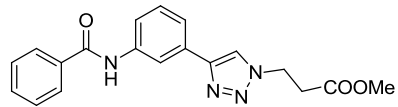


RBM7-065

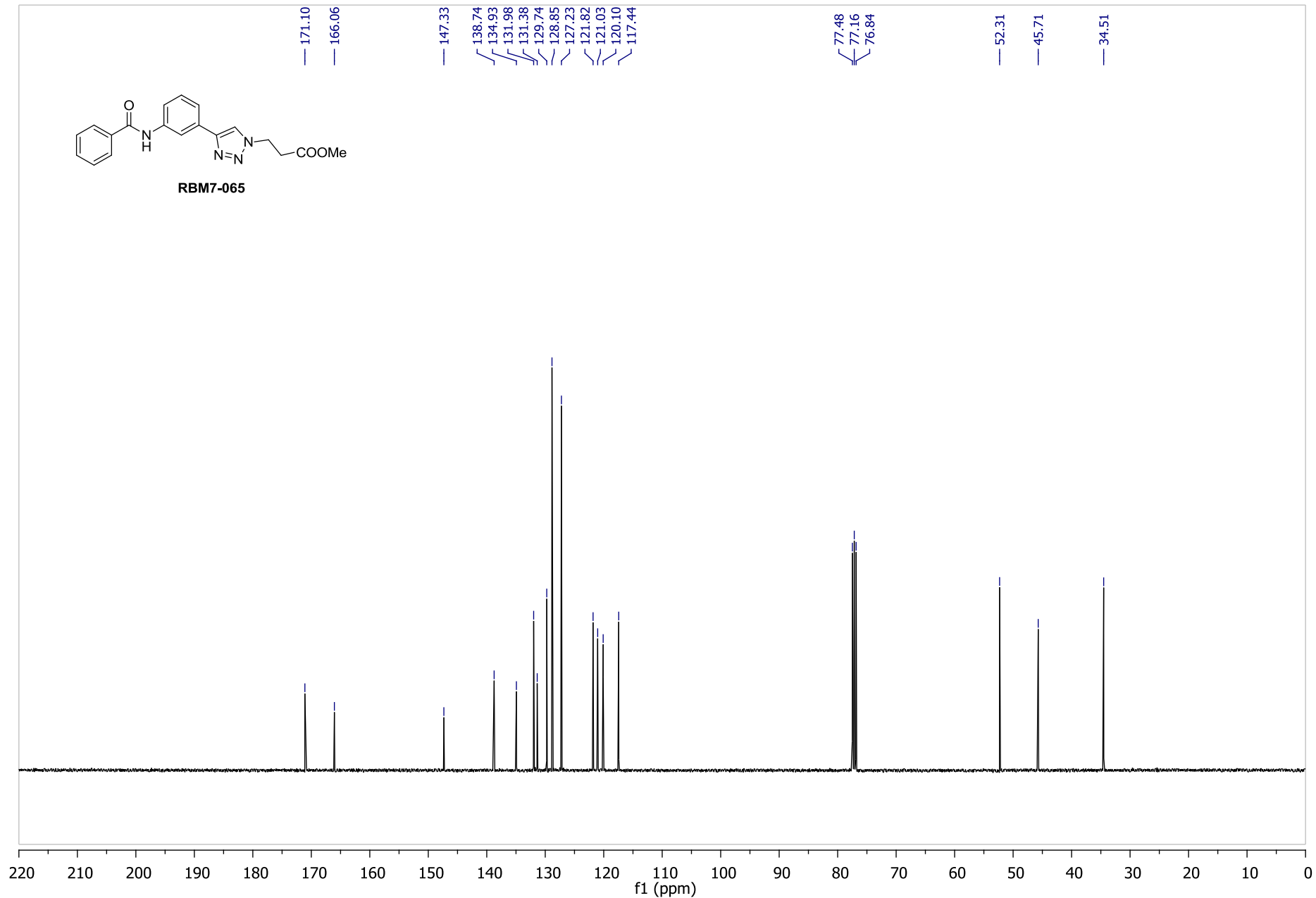
7.26

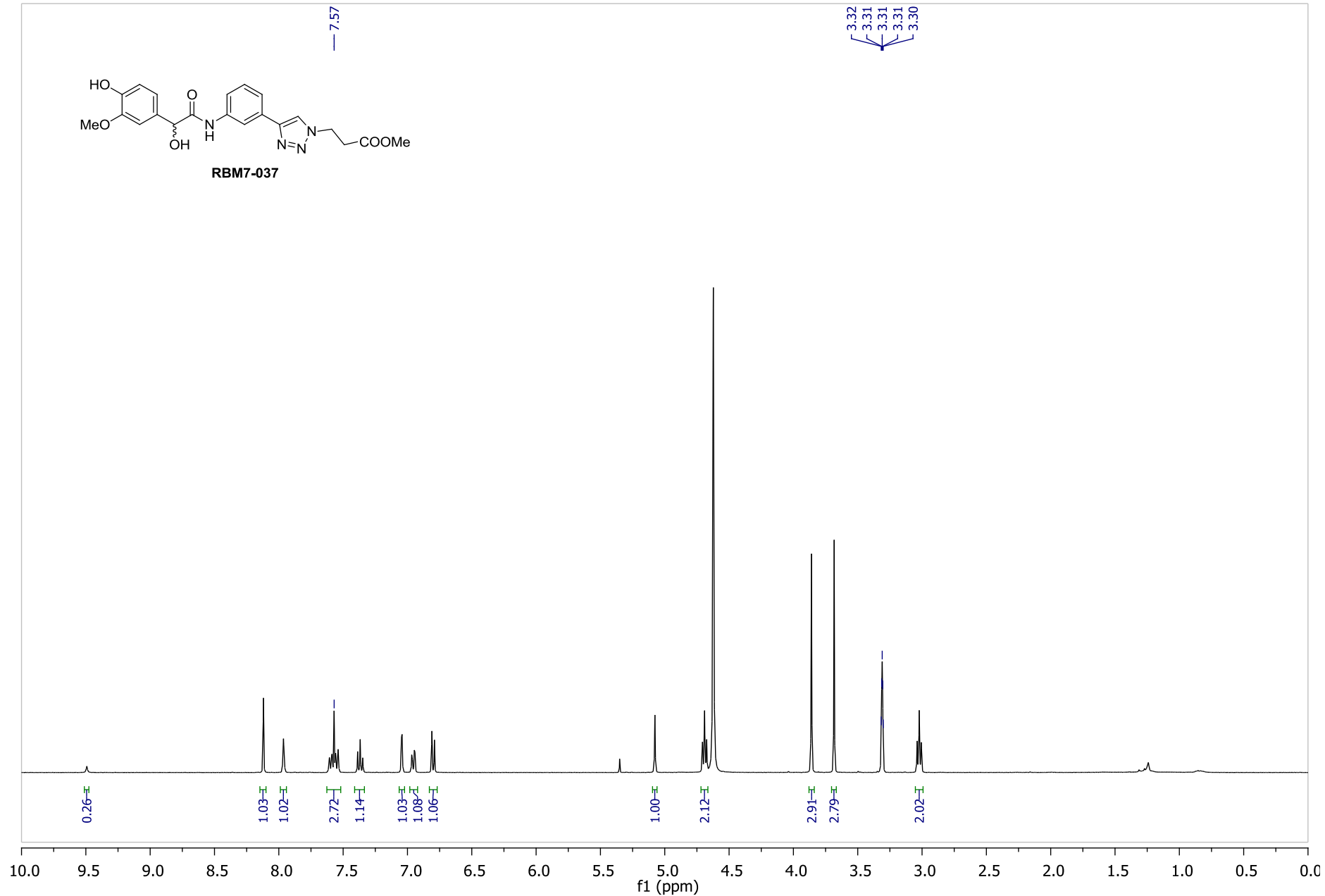
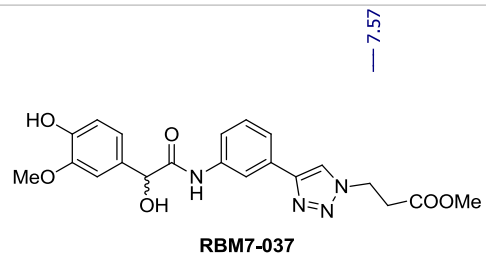


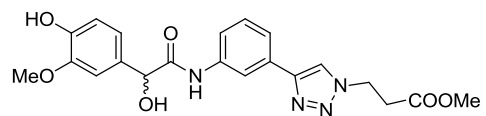
S40



RBM7-065







RBM7-037

172.61
171.63

147.90
147.67
146.59

138.46

131.73
131.23

129.96

122.13

121.91

120.37

120.06

117.58

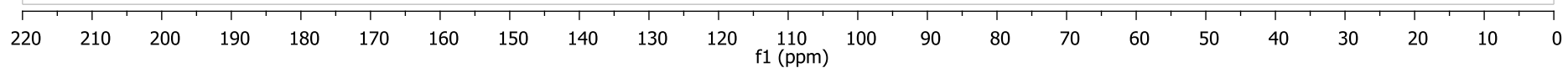
115.31

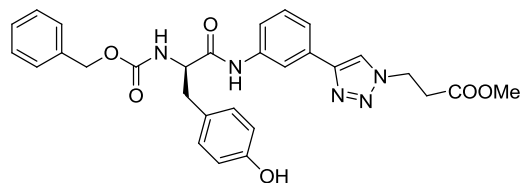
110.38

78.13
77.81
77.49
74.69

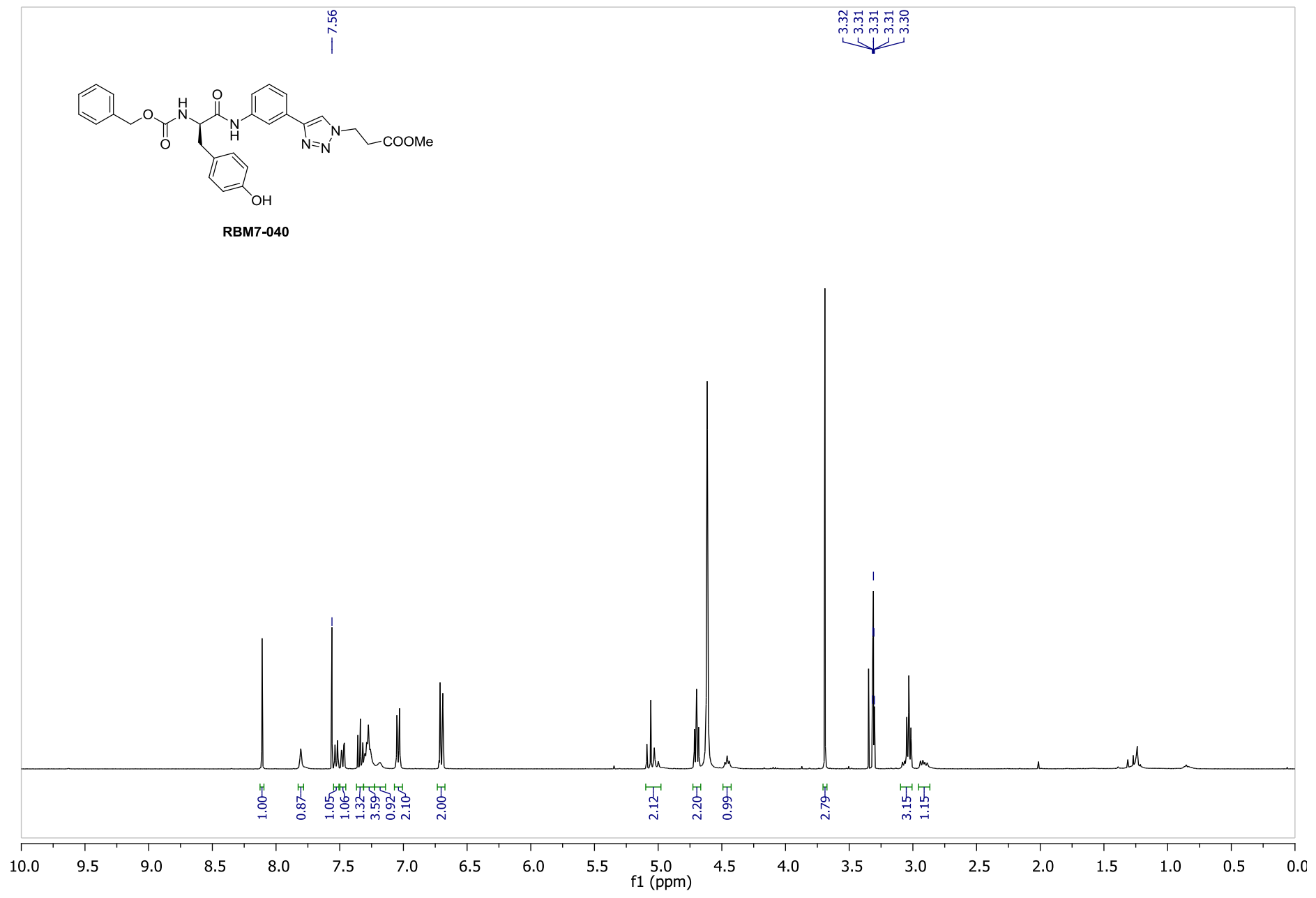
56.06
52.43
49.64
49.43
49.21
49.00
48.79
48.57
48.36
46.20

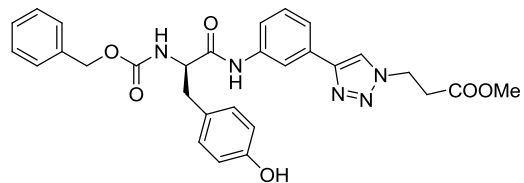
34.59





RBM7-040

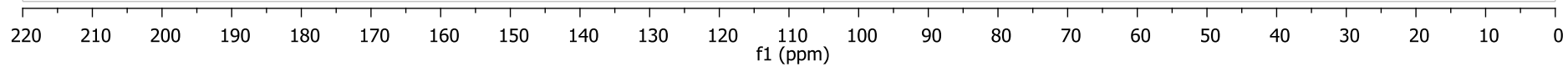




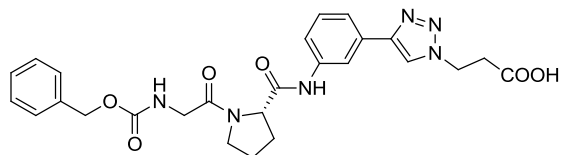
RBM7-040

171.94
171.76
157.38
156.47
147.87
139.02
137.06
130.95
128.98
128.56
128.28
122.23
120.94
118.16
115.91

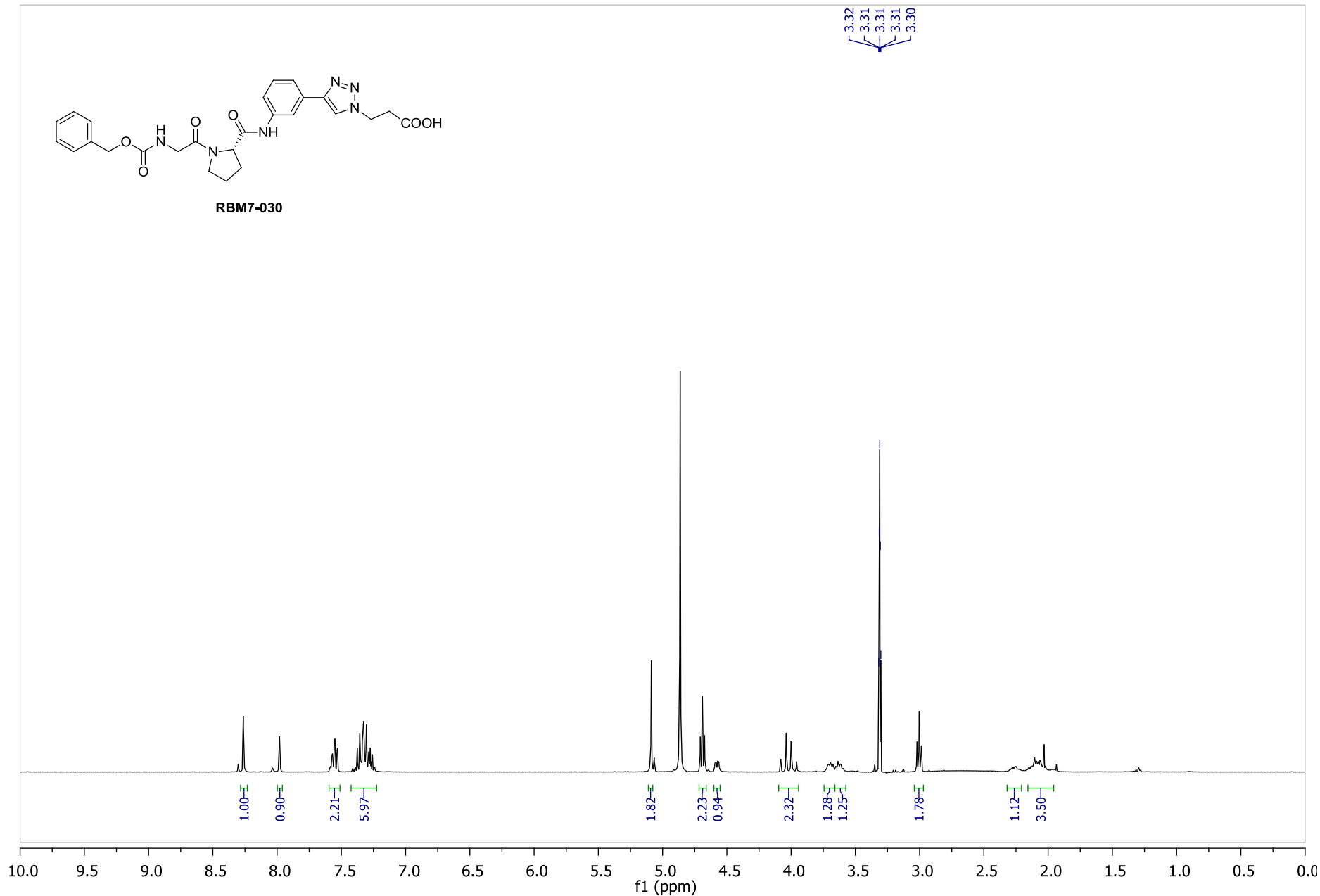
78.59
78.27
77.94
67.39
57.91
52.47
49.43
49.21
49.00
48.79
38.57
34.71

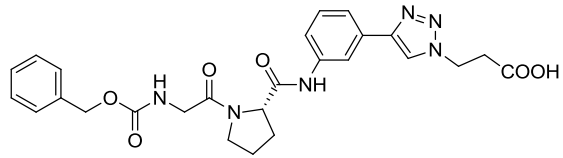


S45

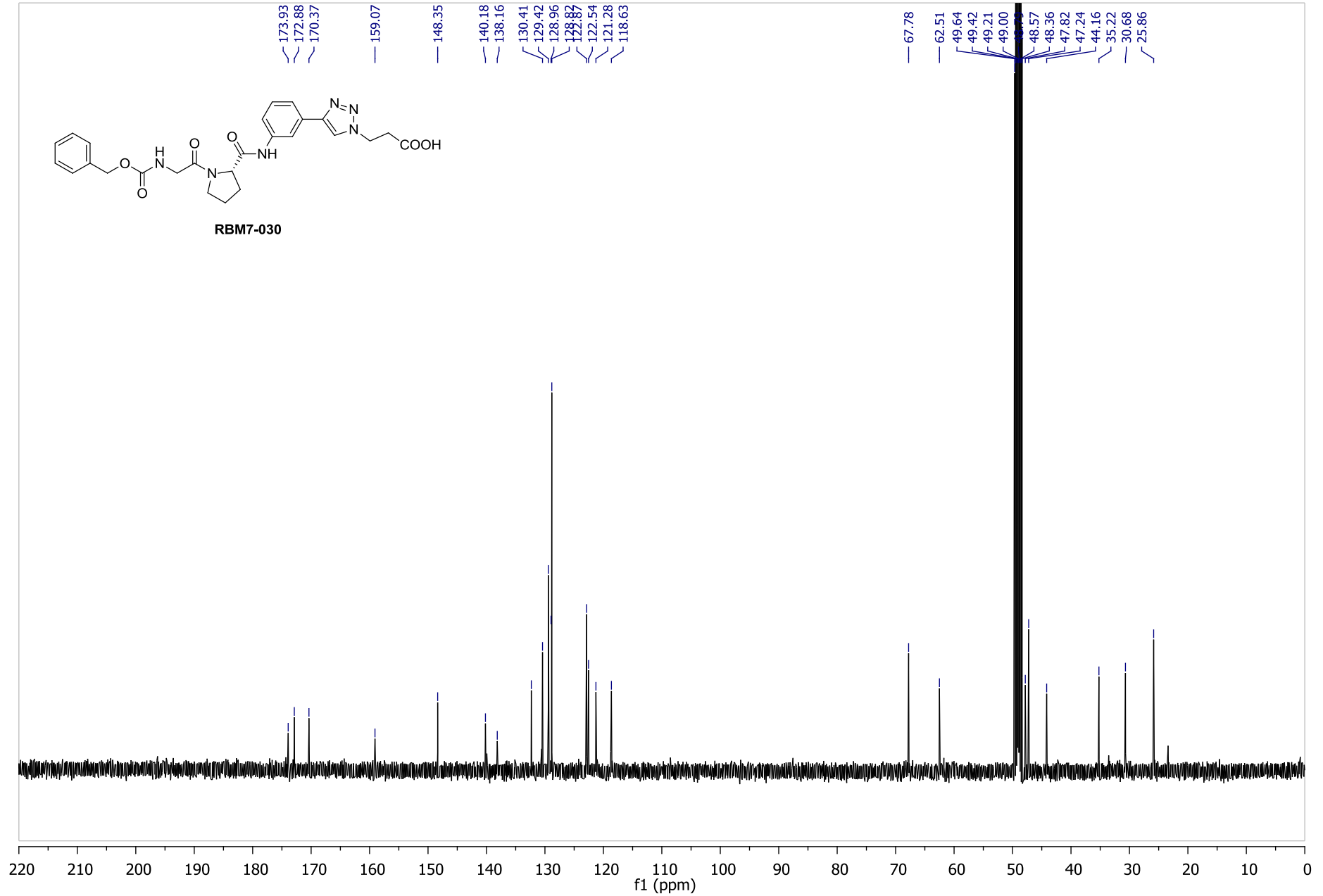


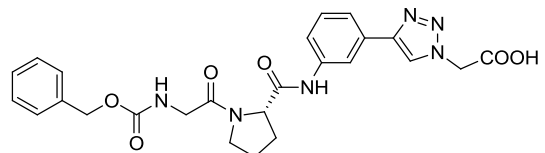
RBM7-030



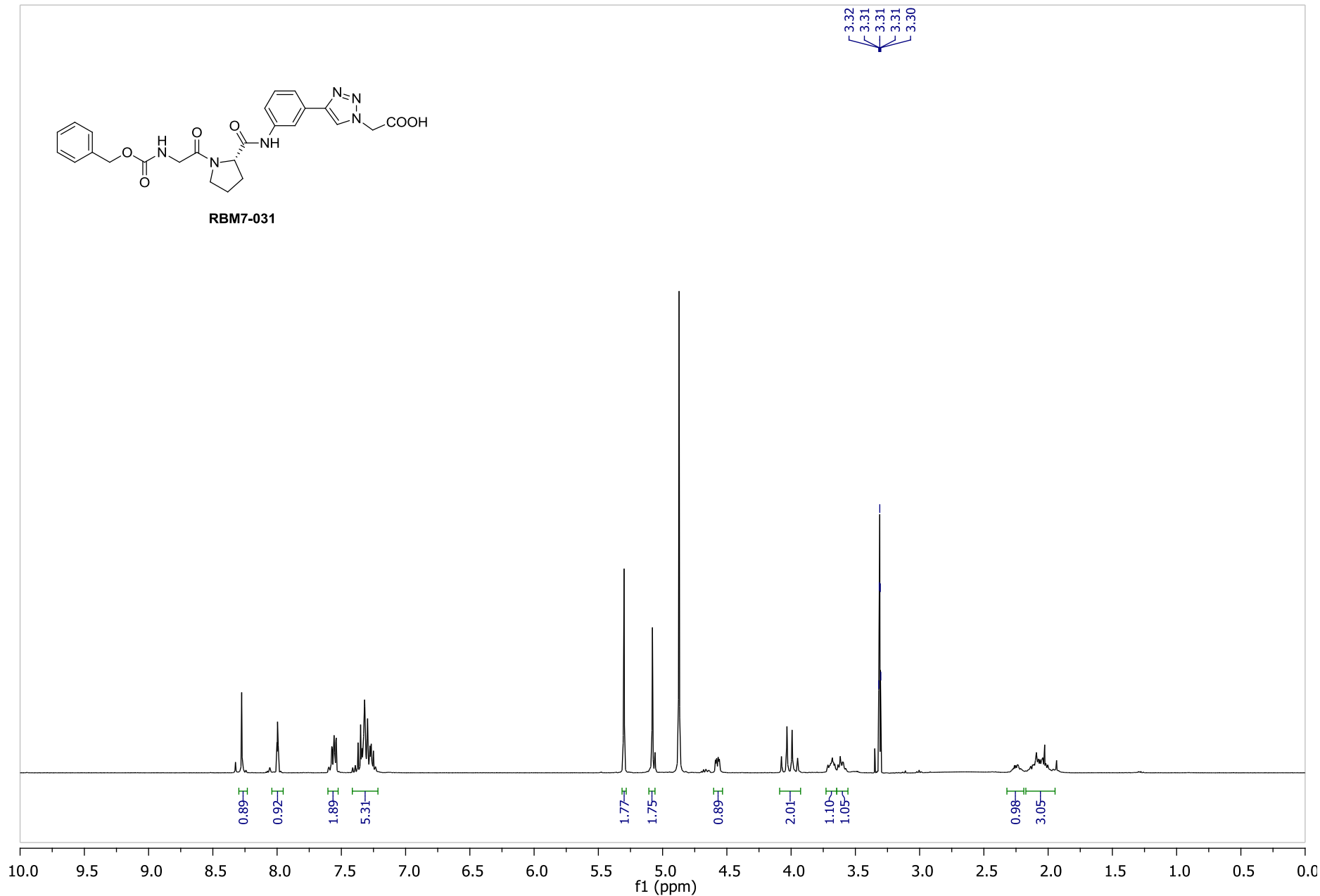


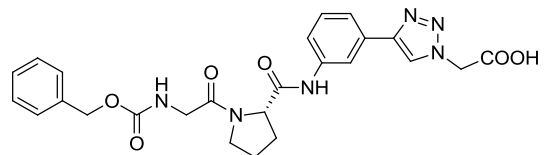
RBM7-030



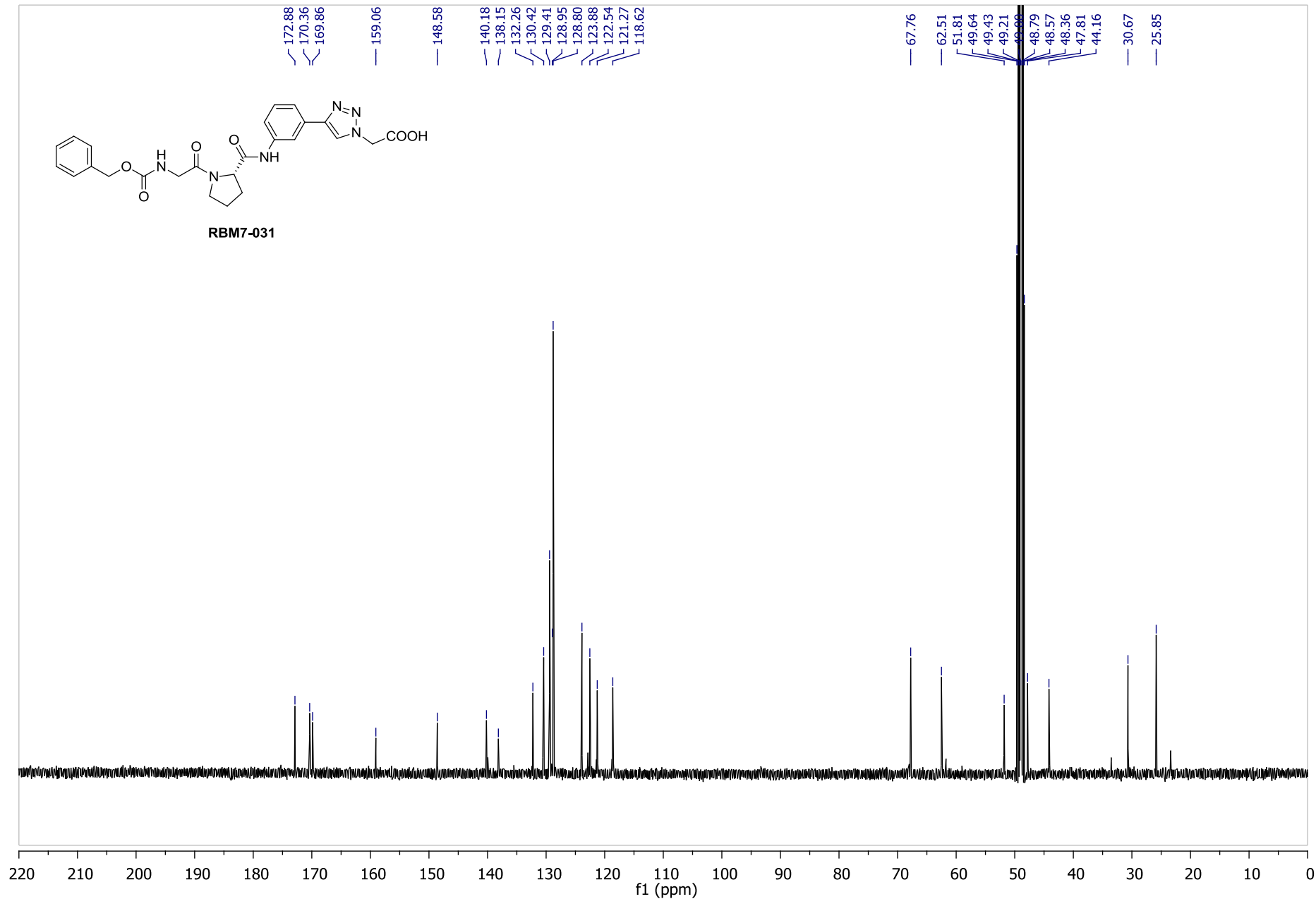


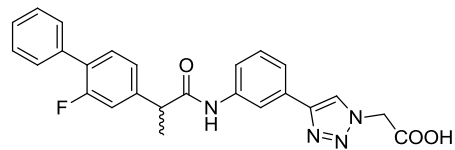
RBM7-031



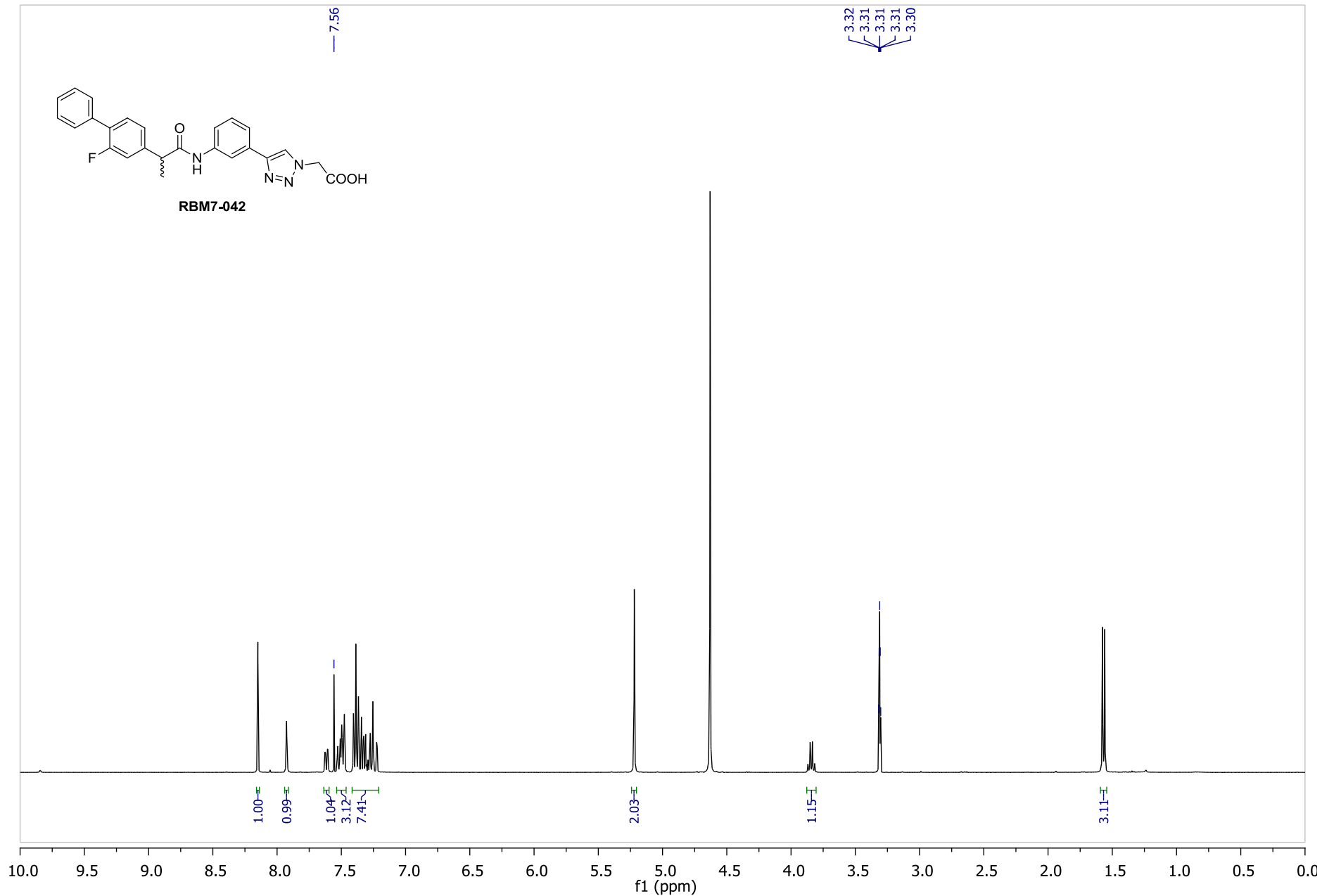


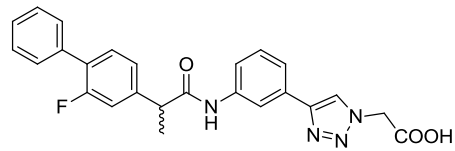
RBM7-031



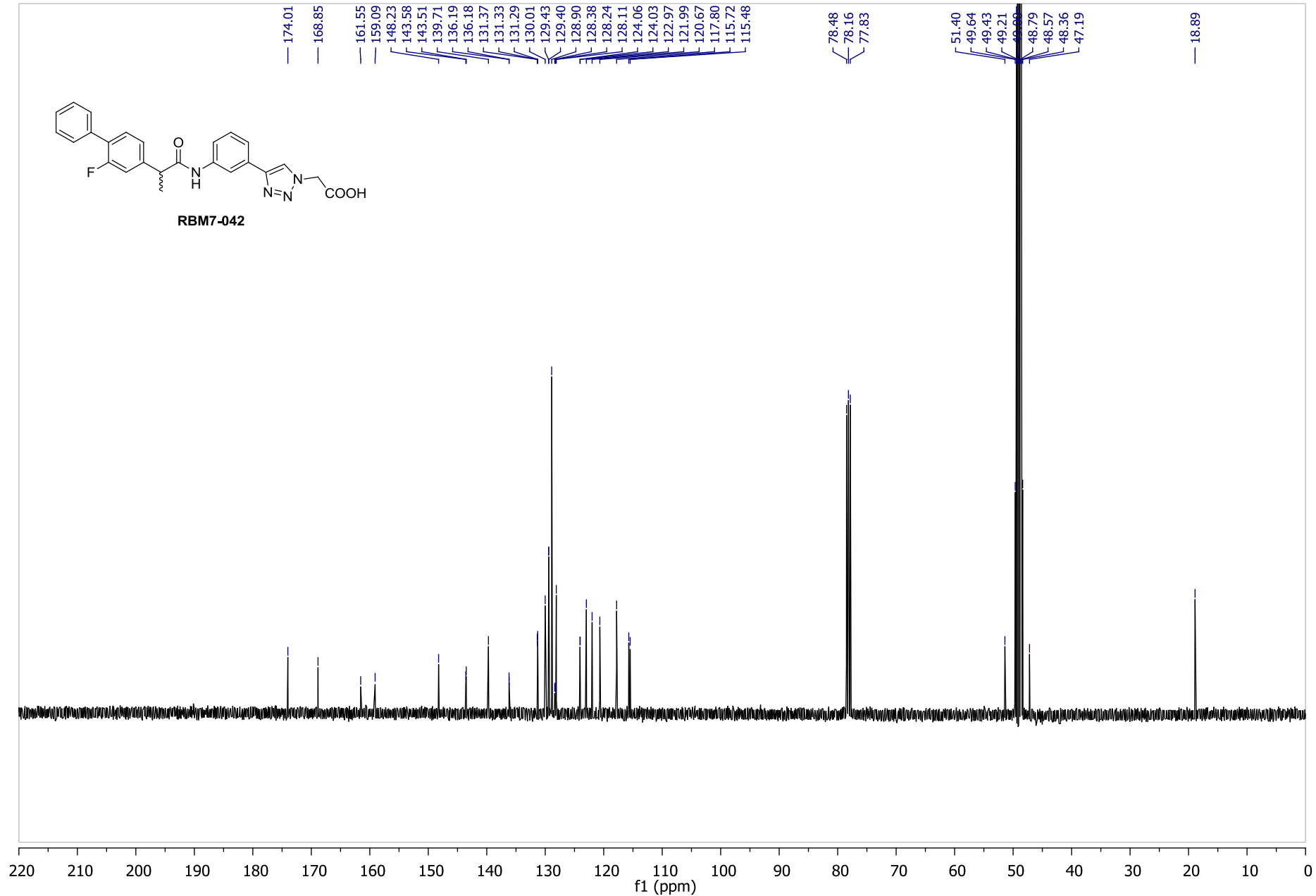


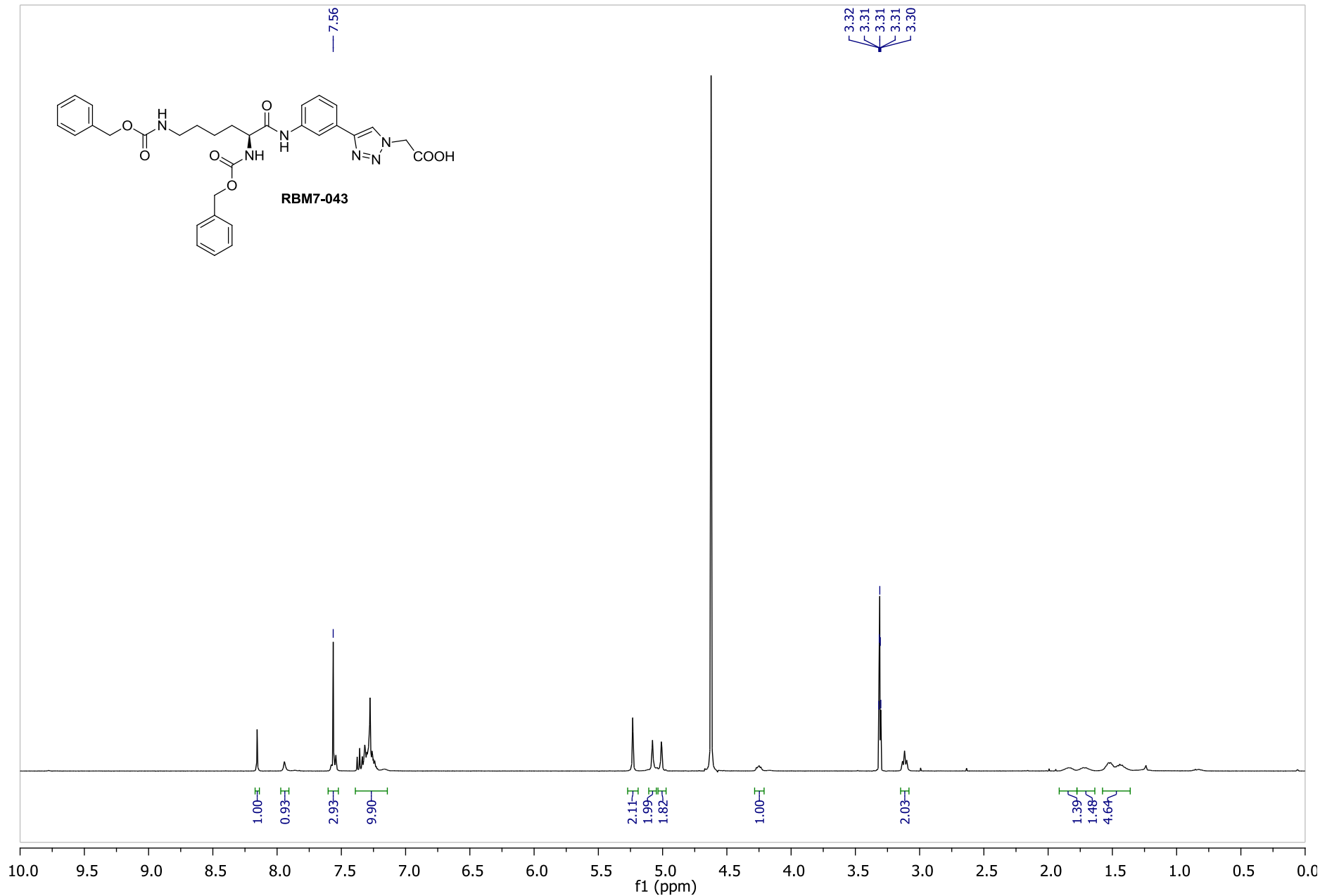
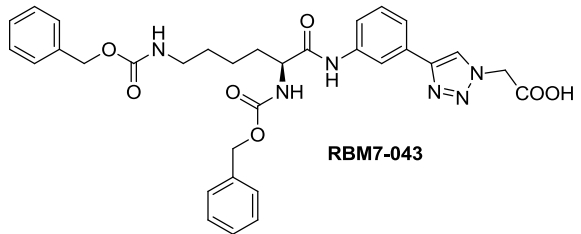
RBM7-042



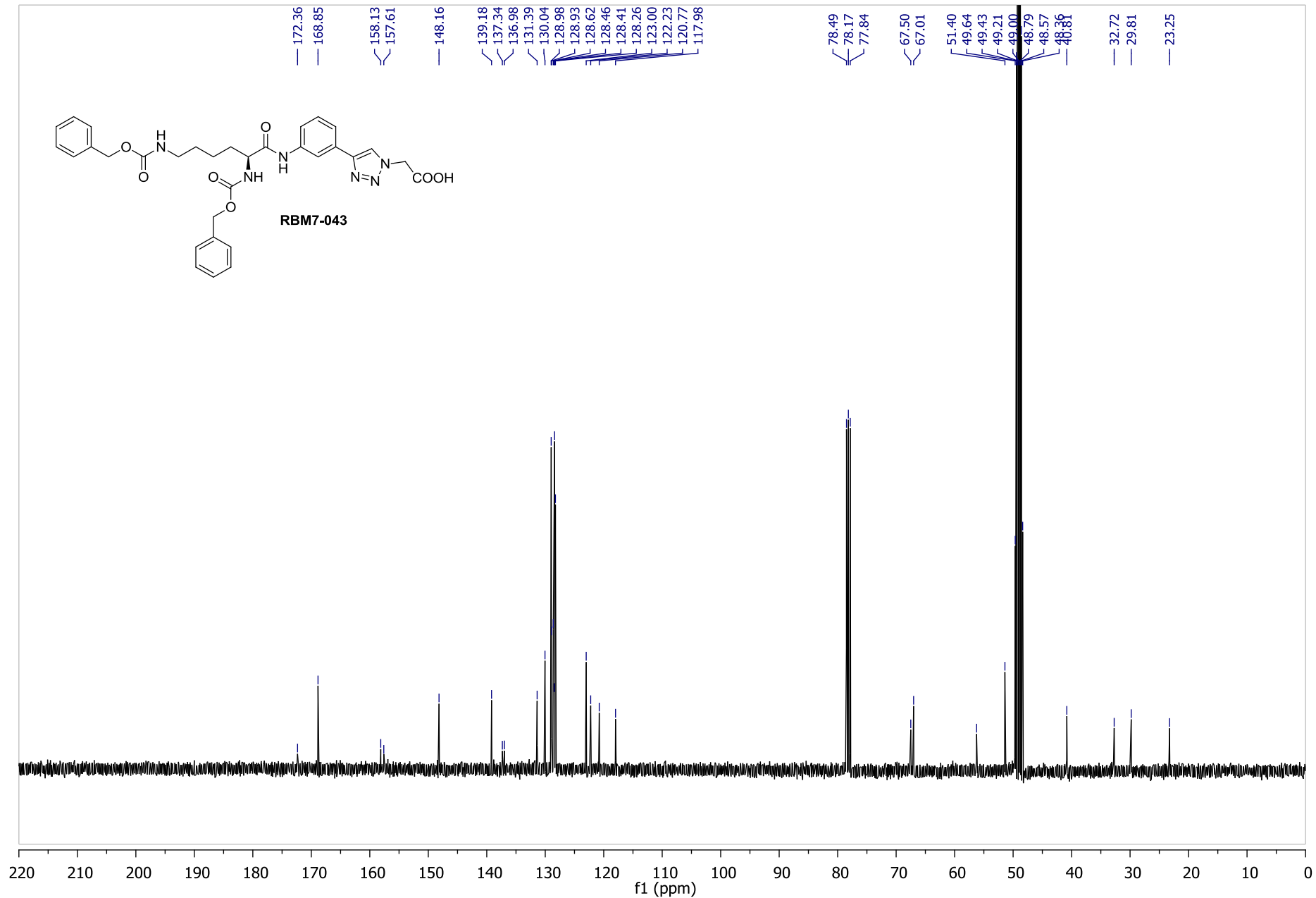
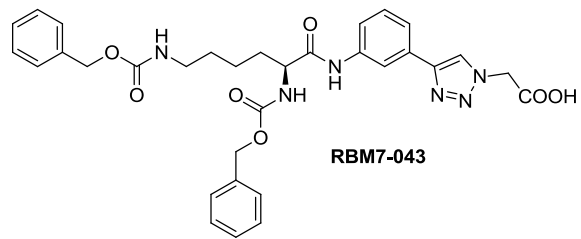


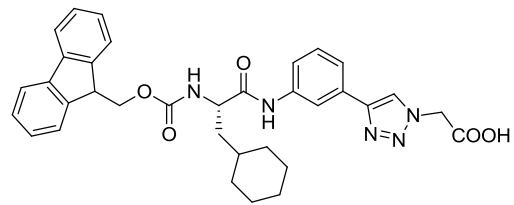
RBM7-042



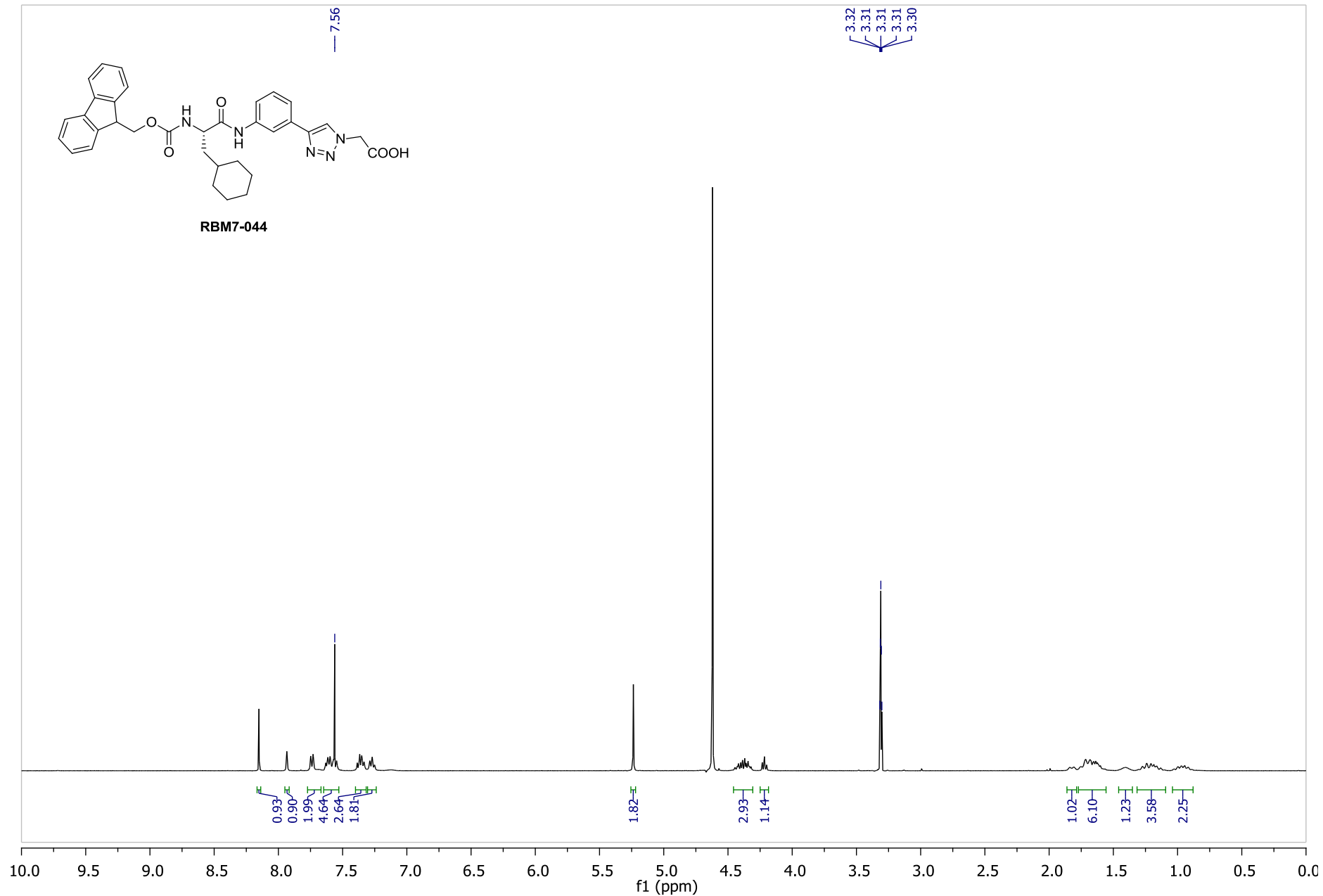


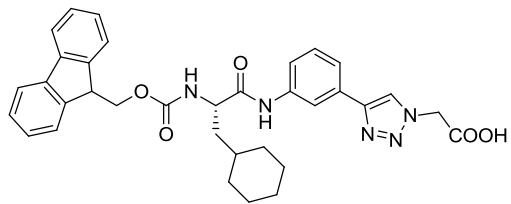
S52



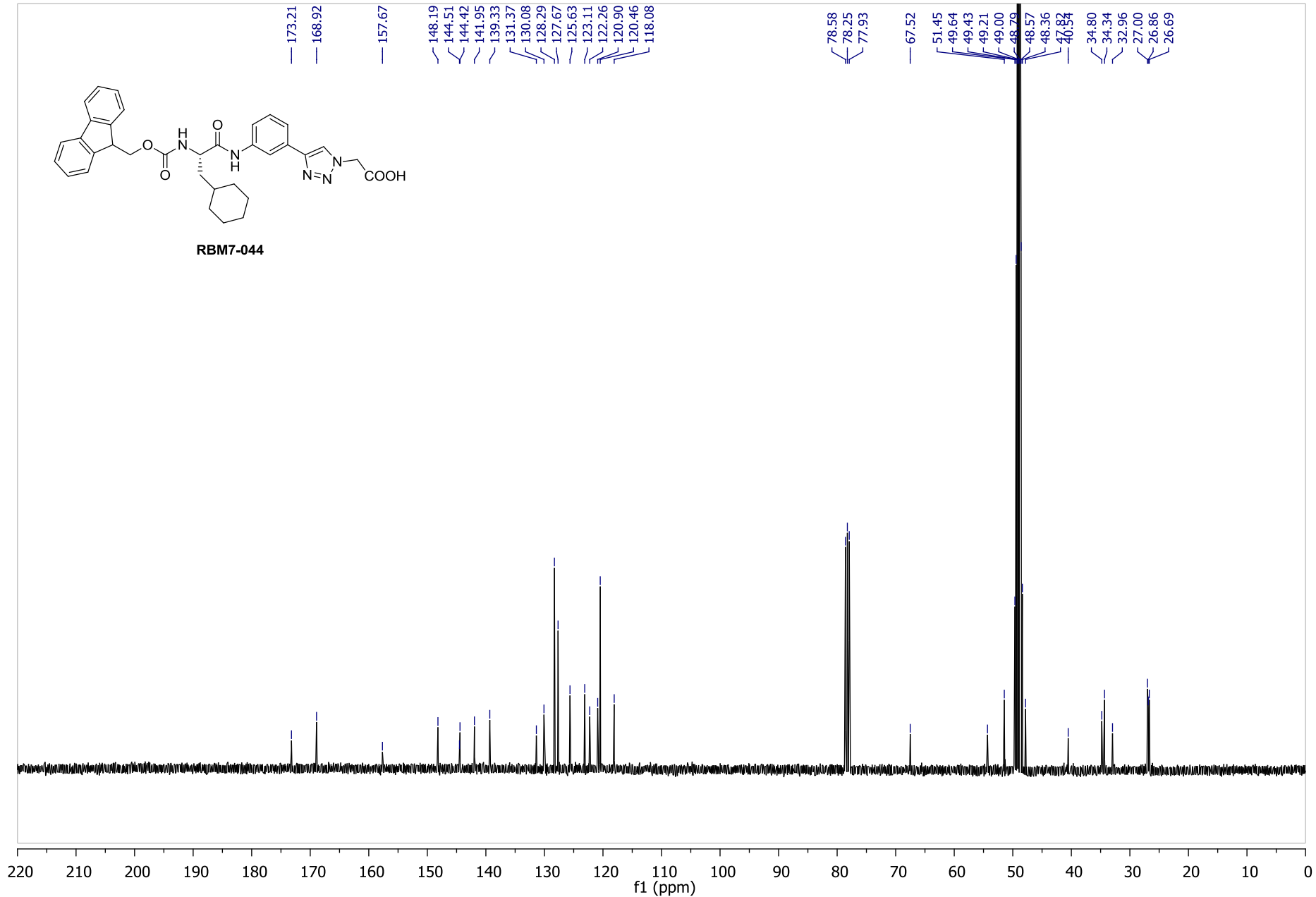


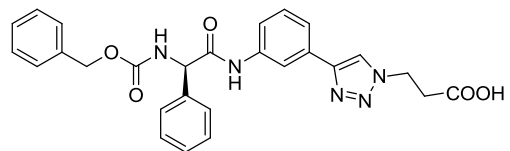
RBM7-044



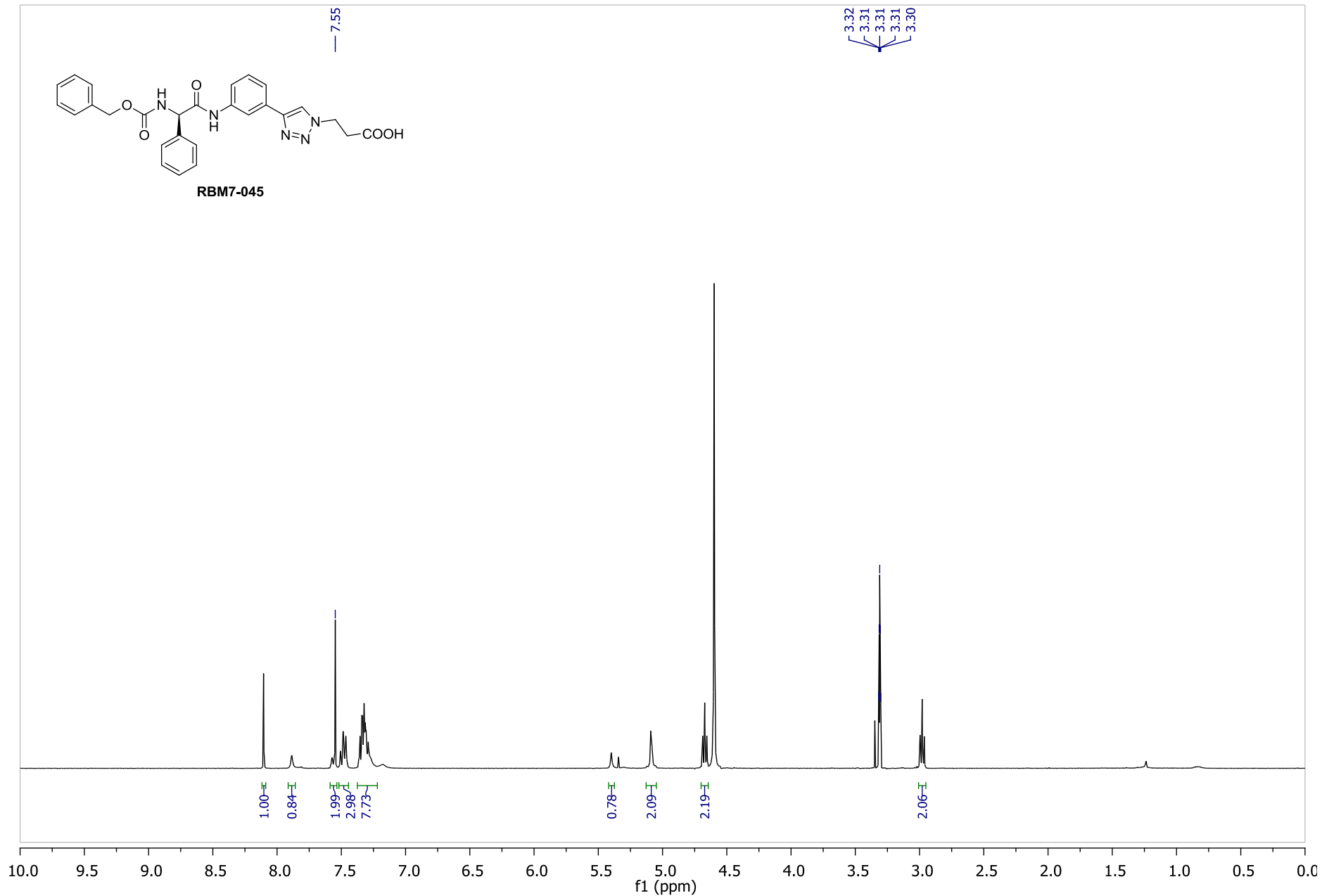


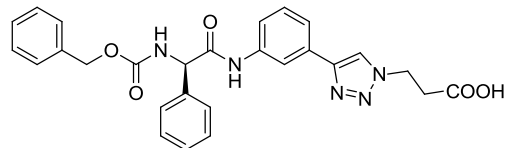
RBM7-044



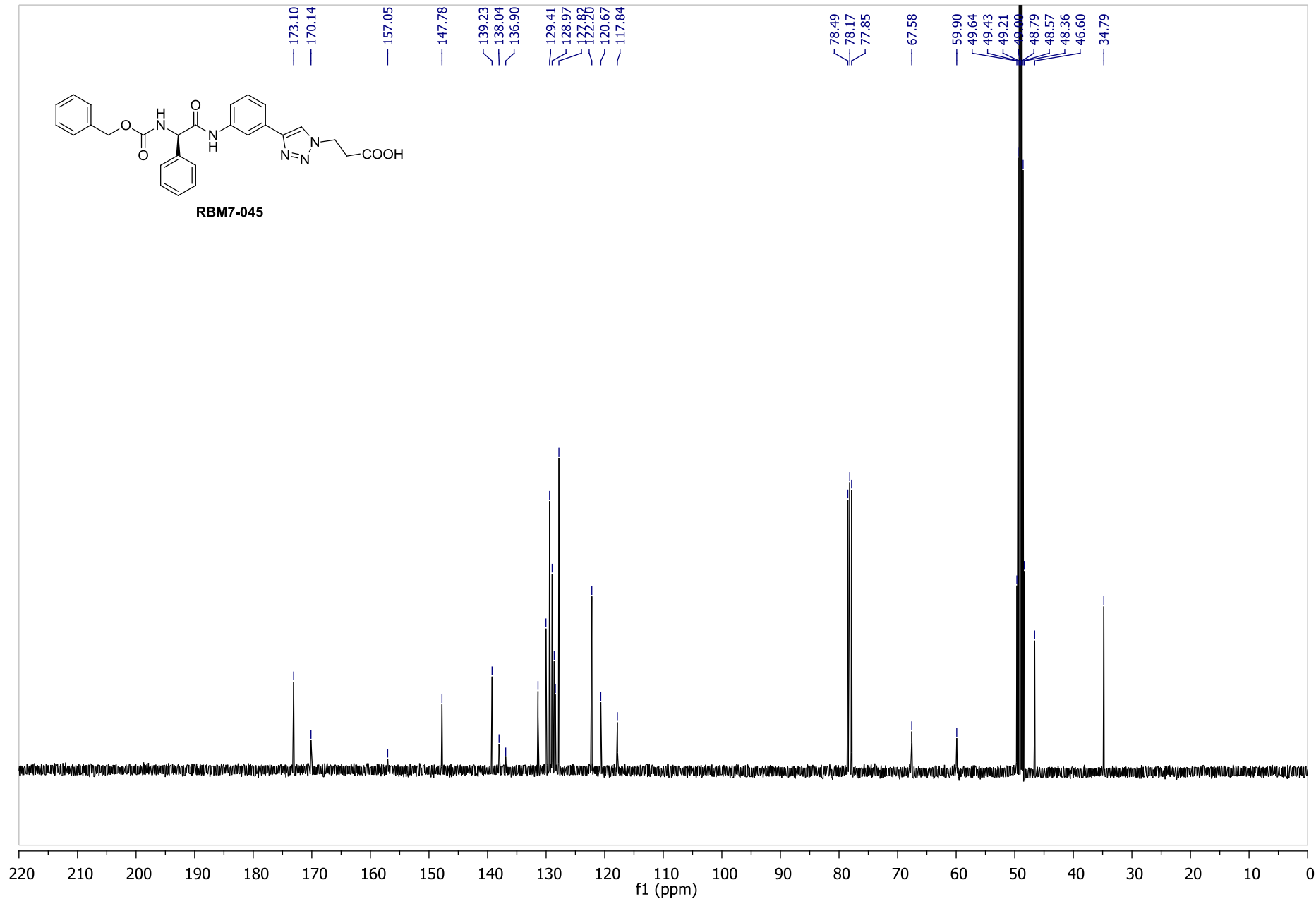


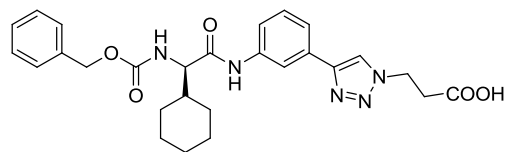
RBM7-045



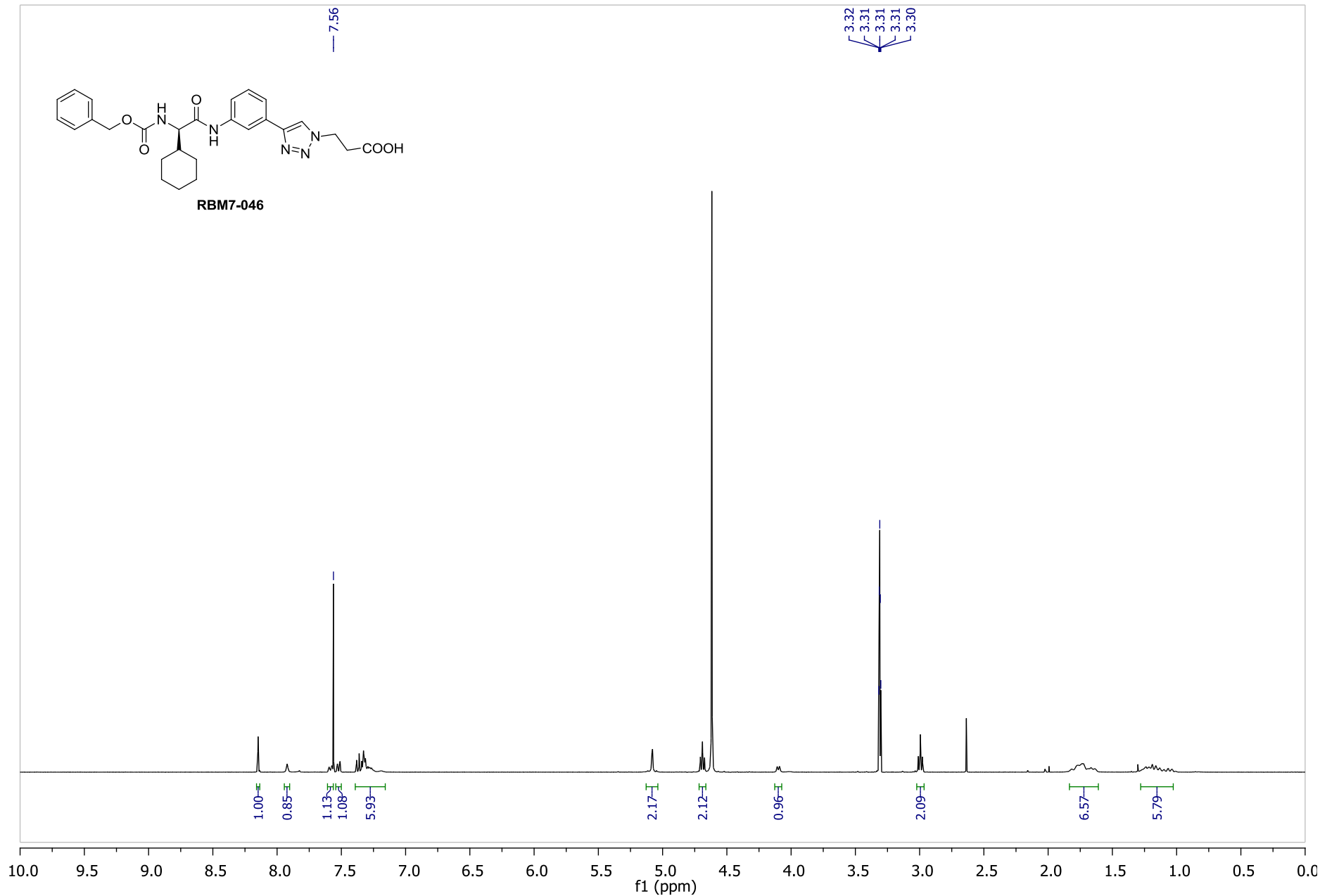


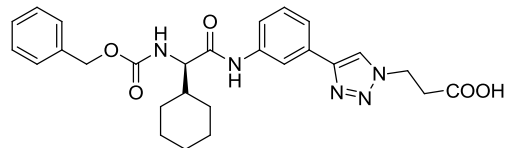
RBM7-045



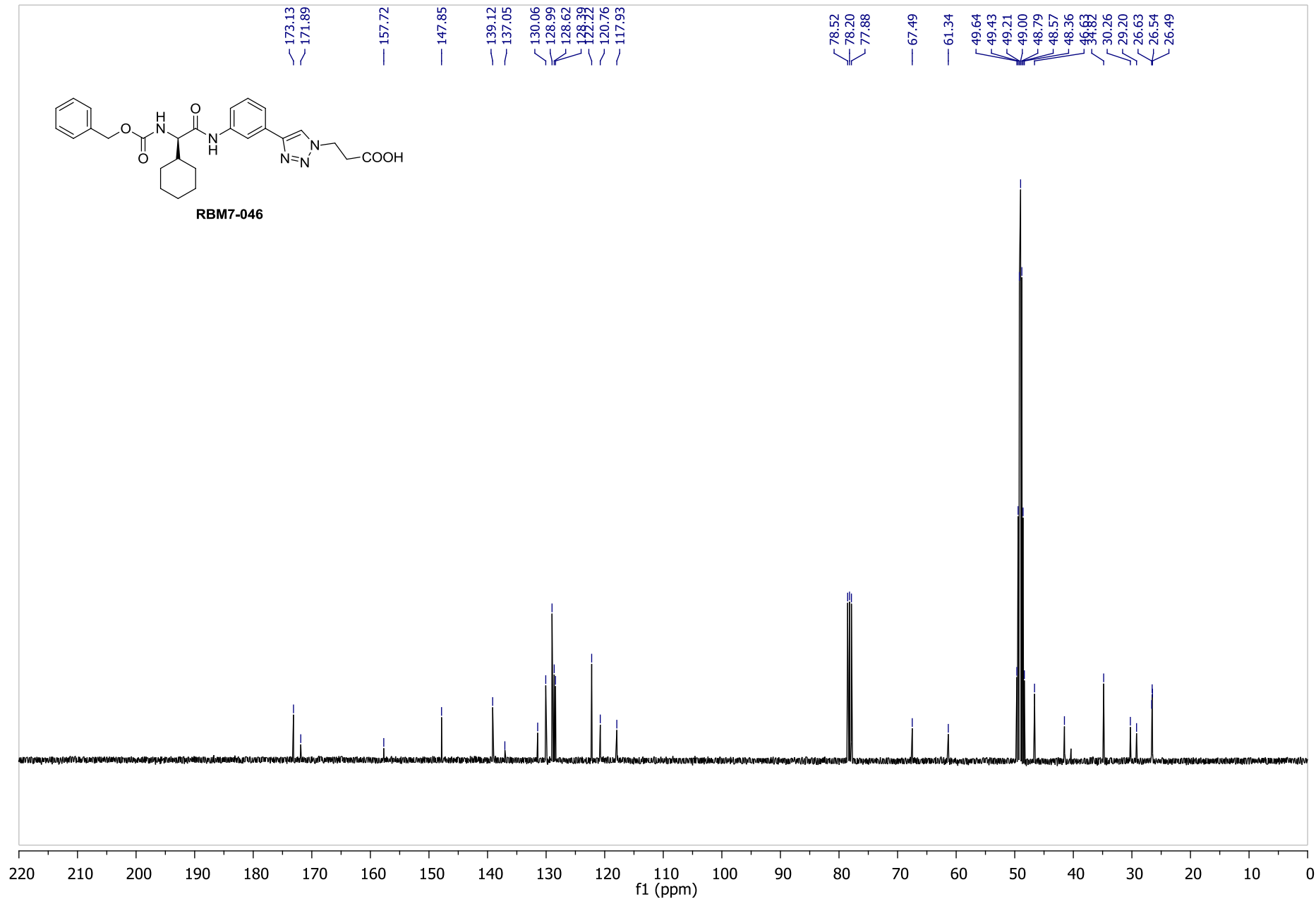


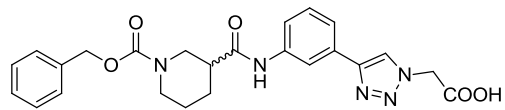
RBM7-046



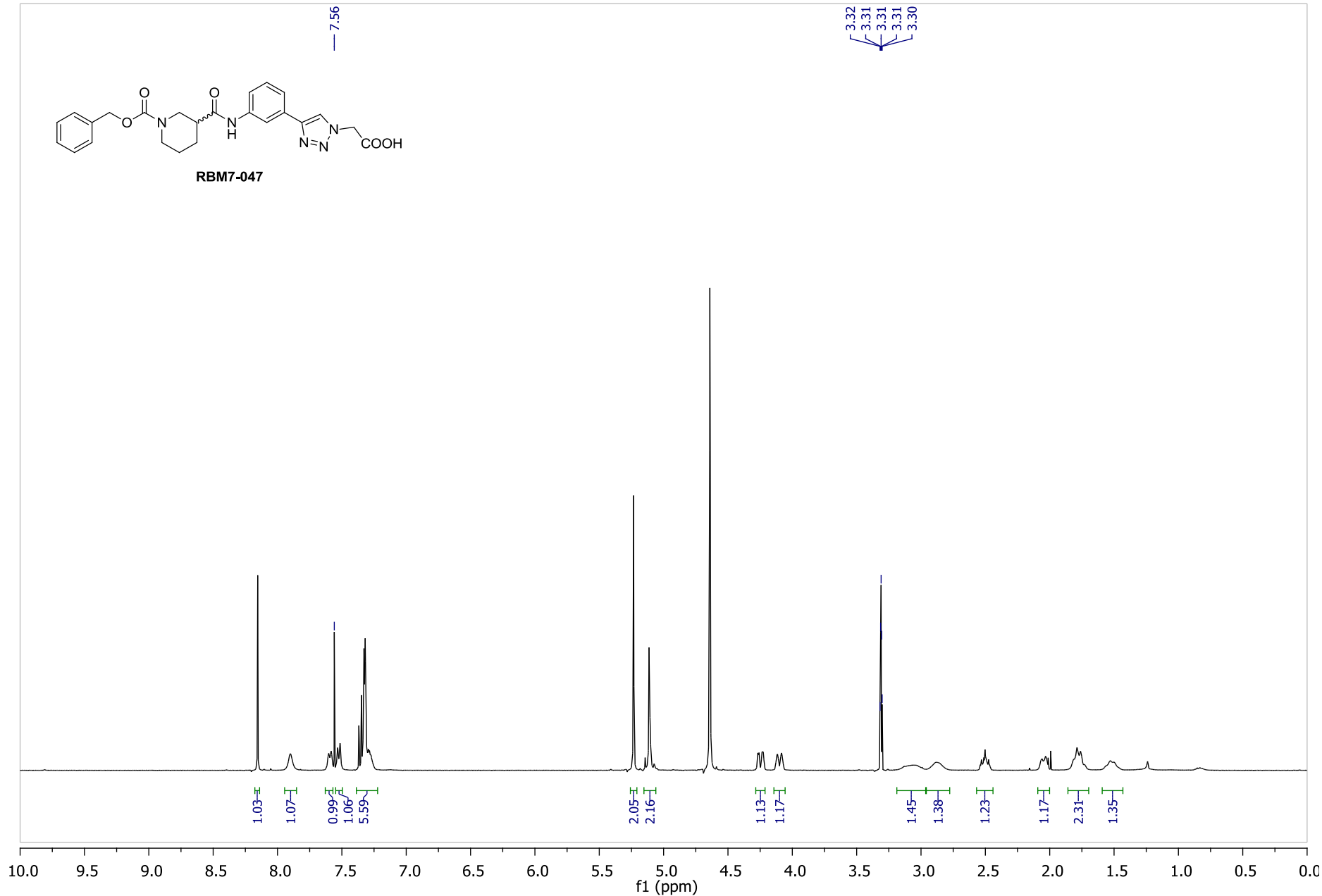


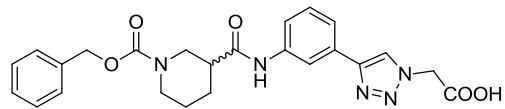
RBM7-046



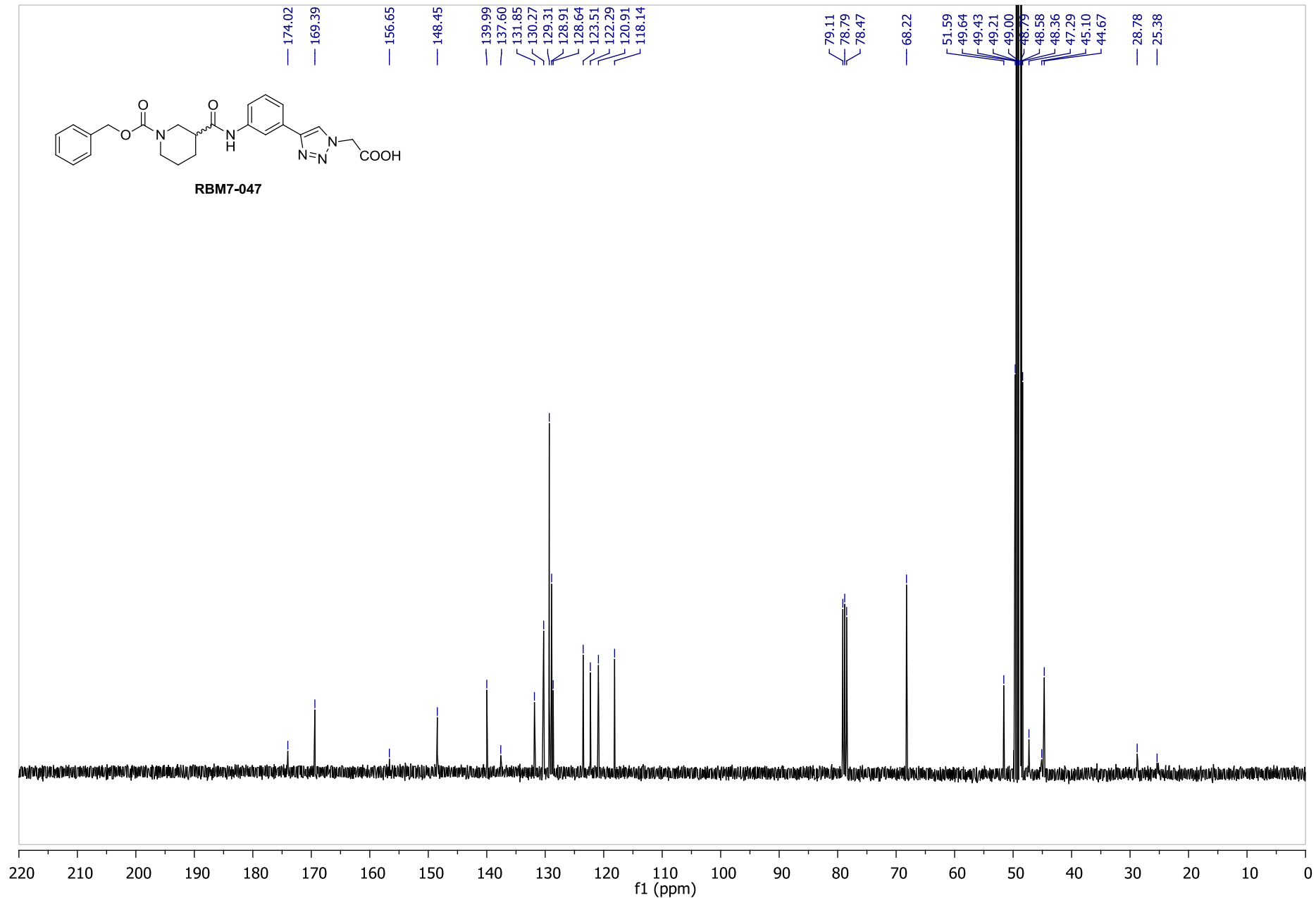


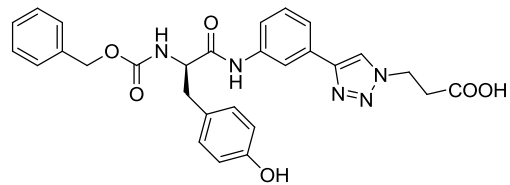
RBM7-047



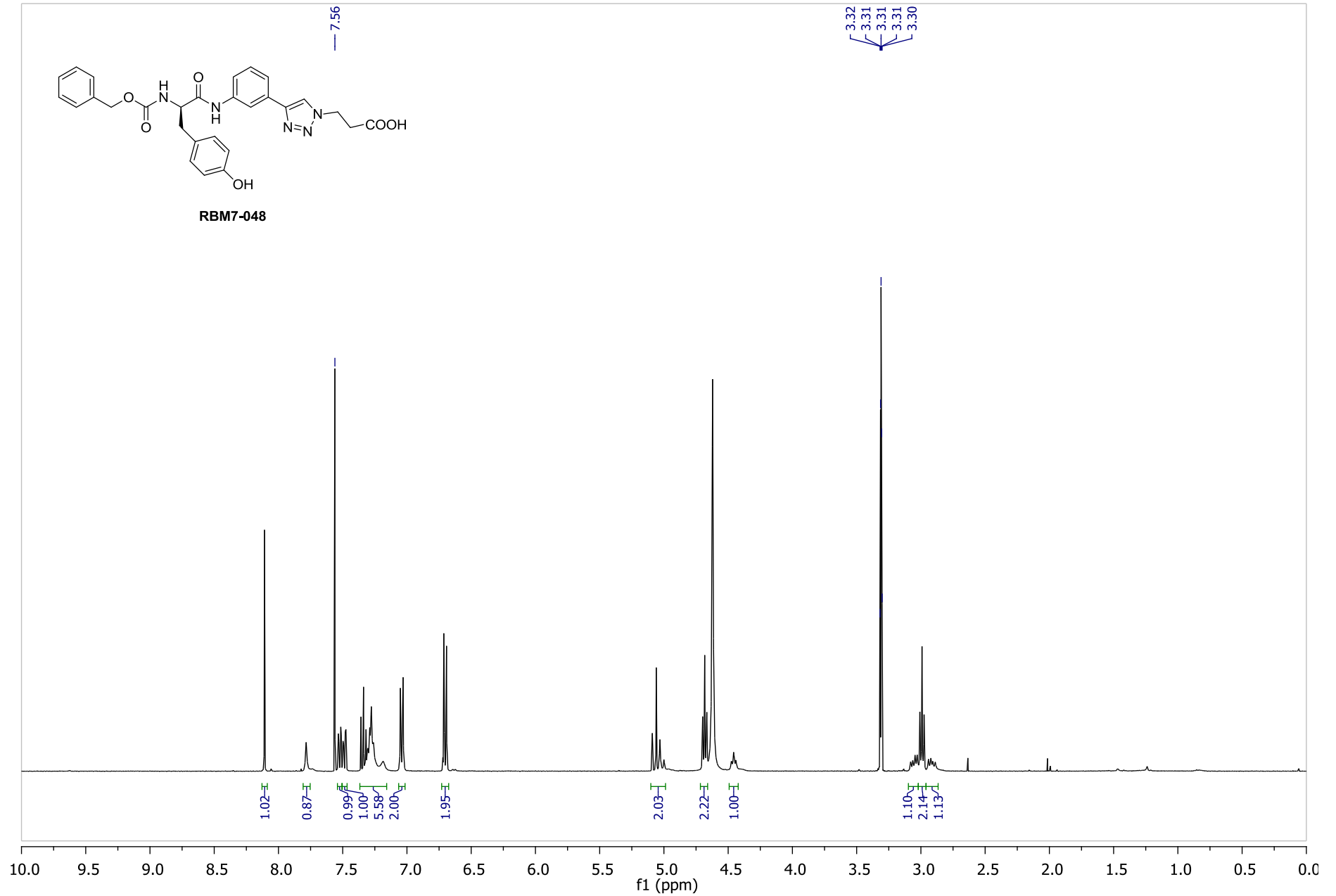


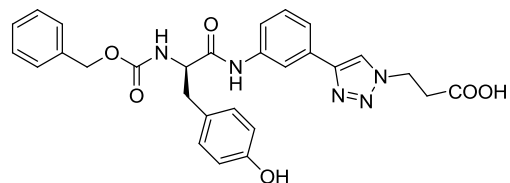
RBM7-047



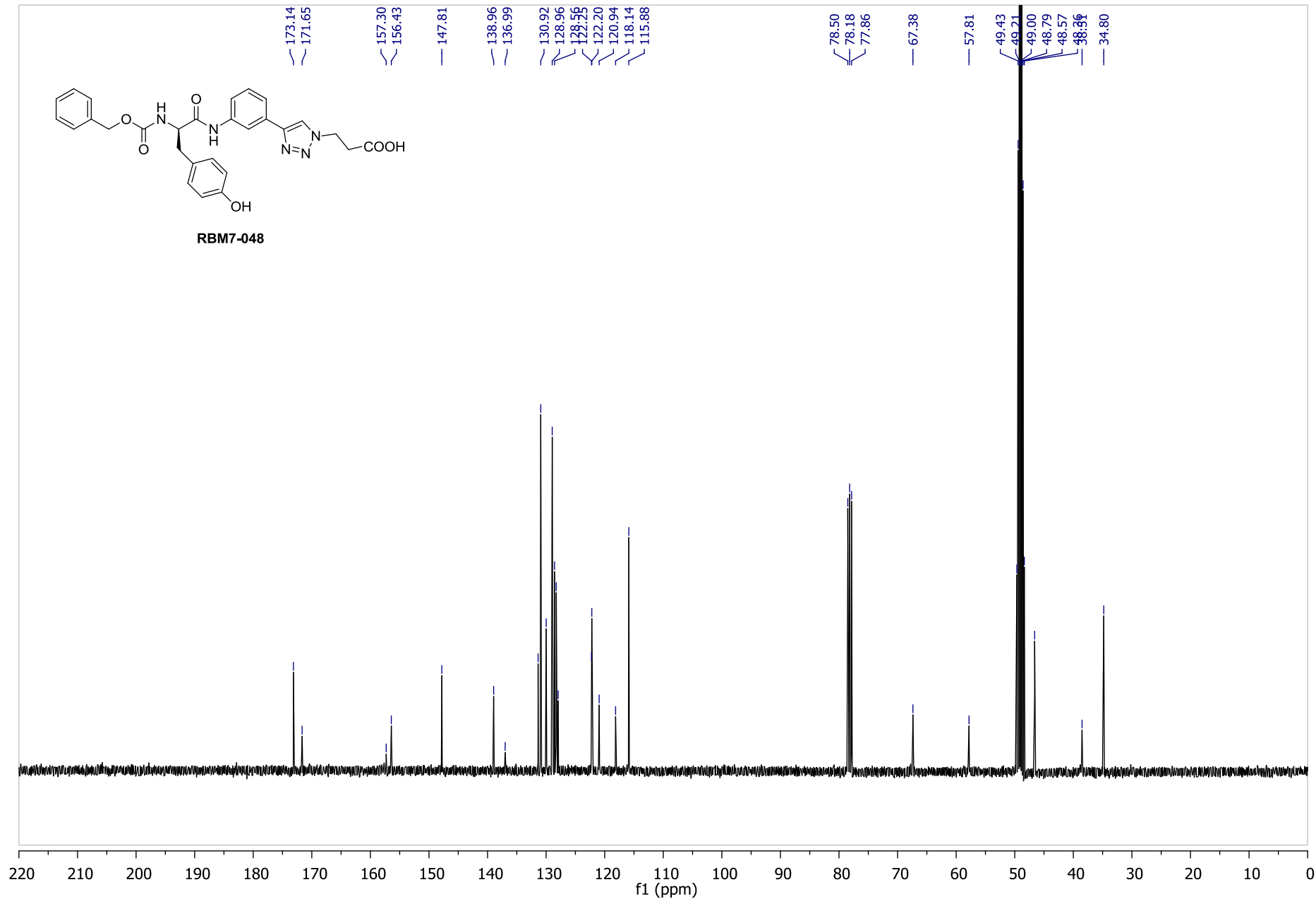


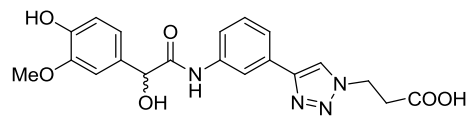
RBM7-048





RBM7-048

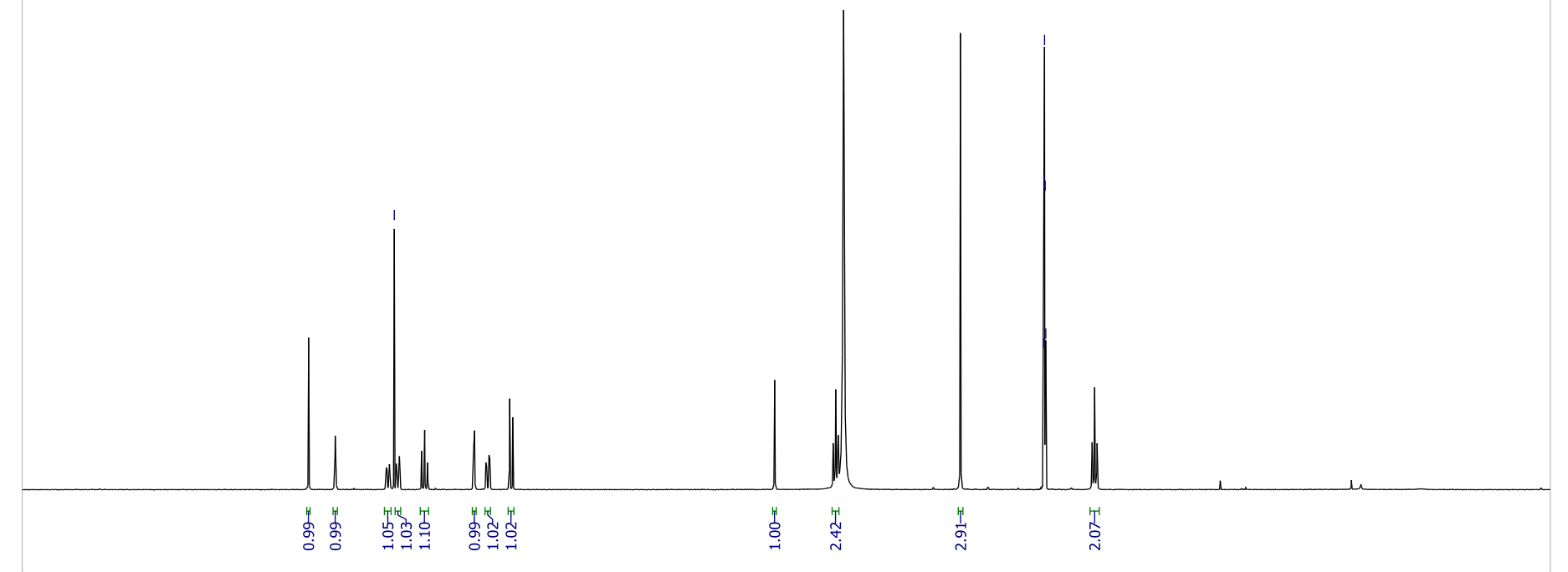


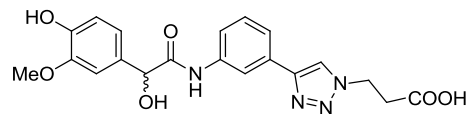


RBM7-049

7.57

3.32
3.31
3.31
3.31
3.30





RBM7-049

173.24
172.96

148.15
147.77
146.83

138.66

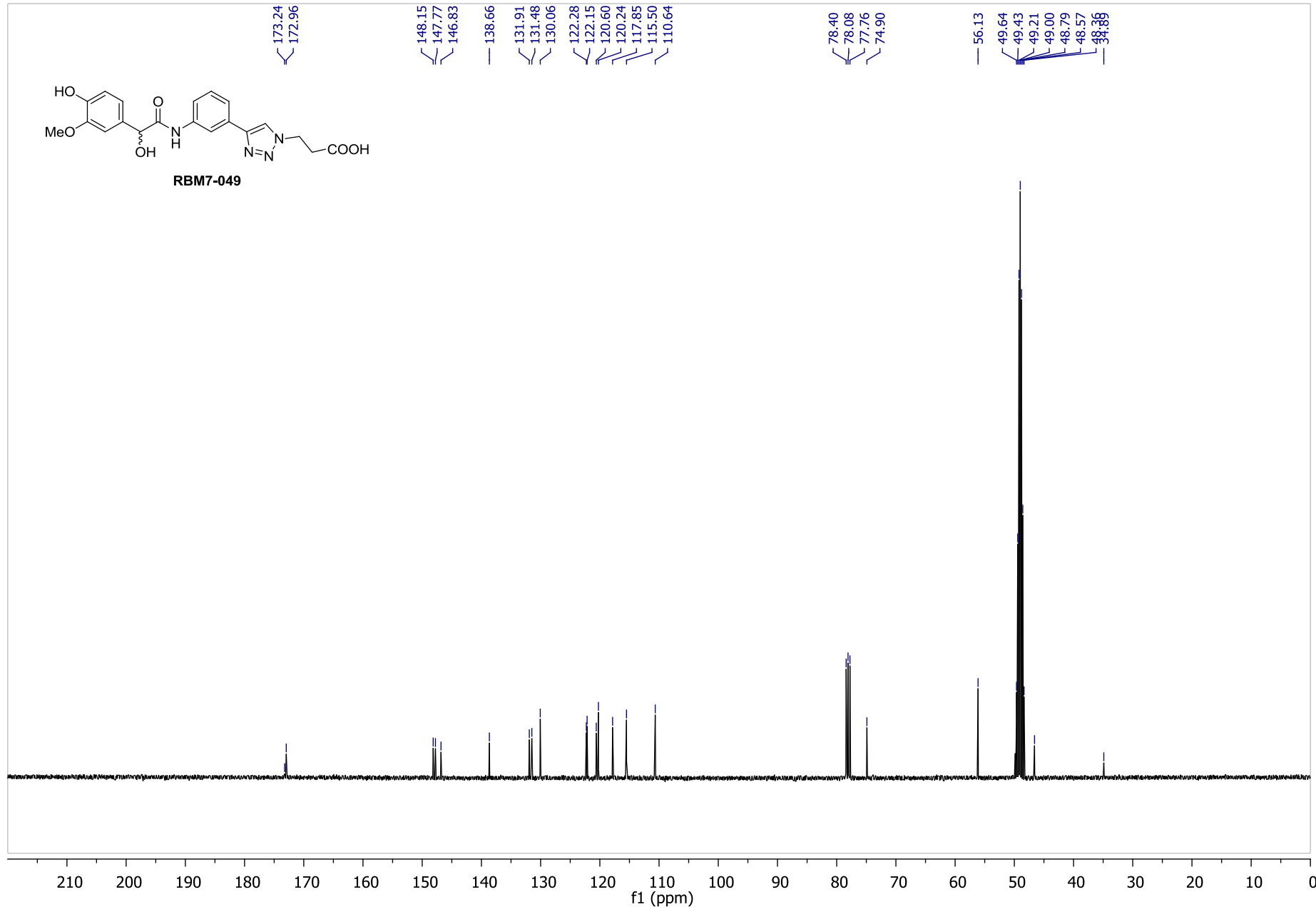
131.91
131.48
130.06

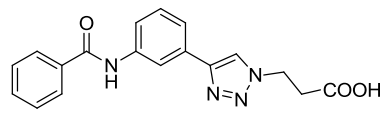
122.28
122.15
120.60

120.24
117.85
115.50
110.64

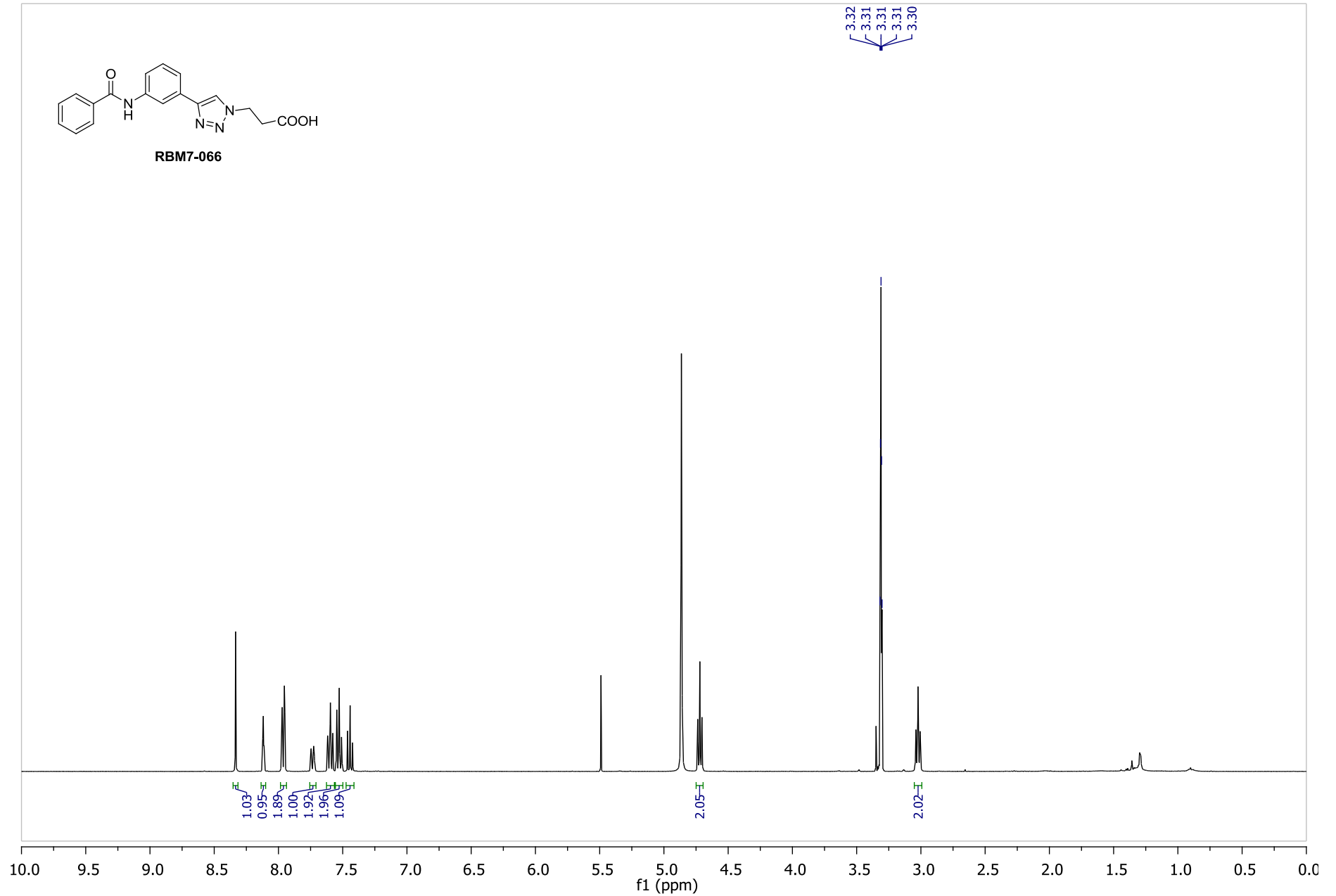
78.40
78.08
77.76
74.90

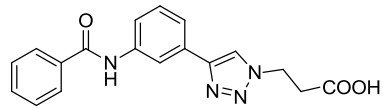
56.13
49.64
49.43
49.21
49.00
48.79
48.57
38.86



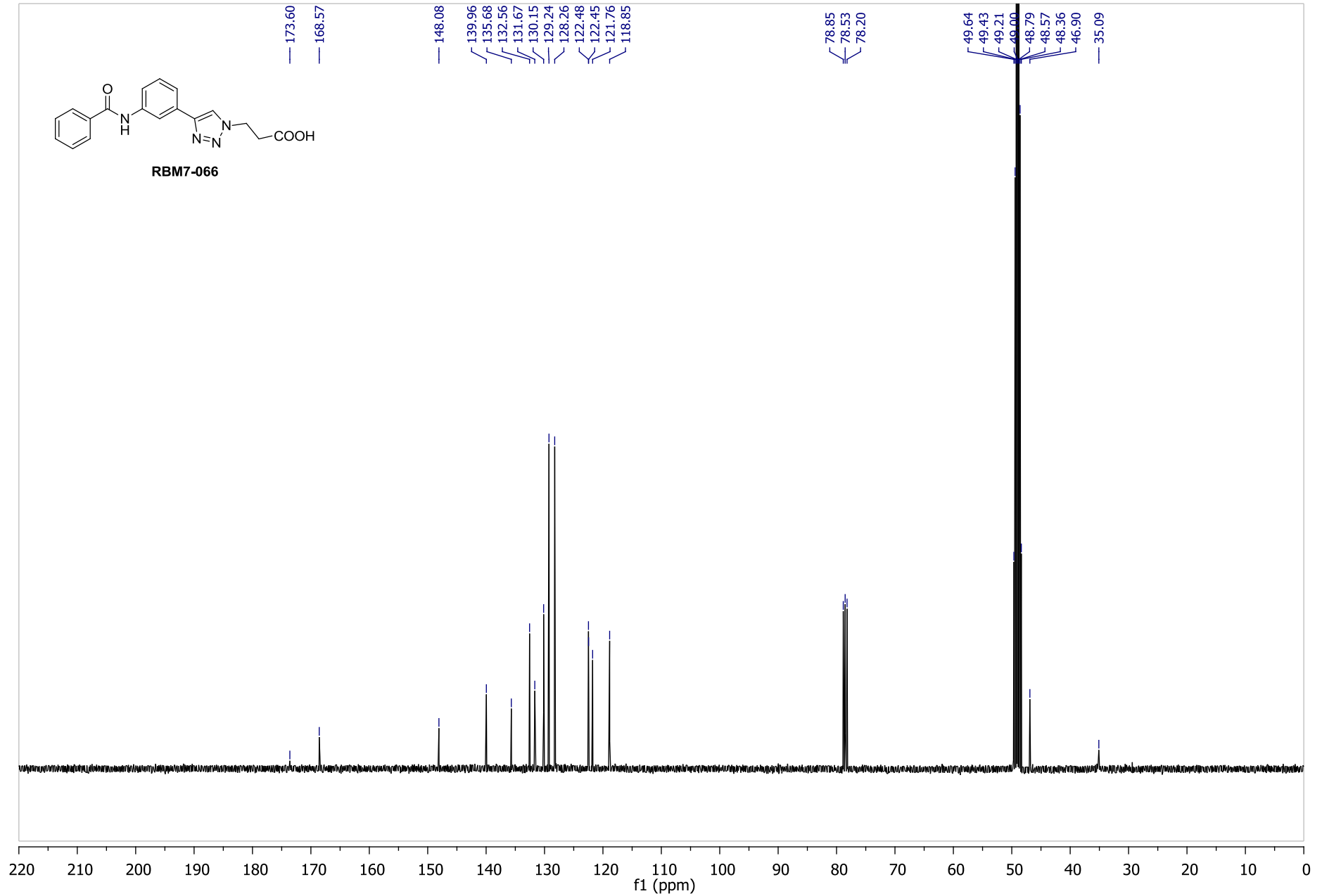


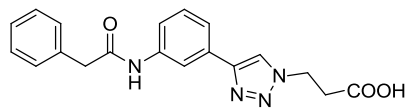
RBM7-066



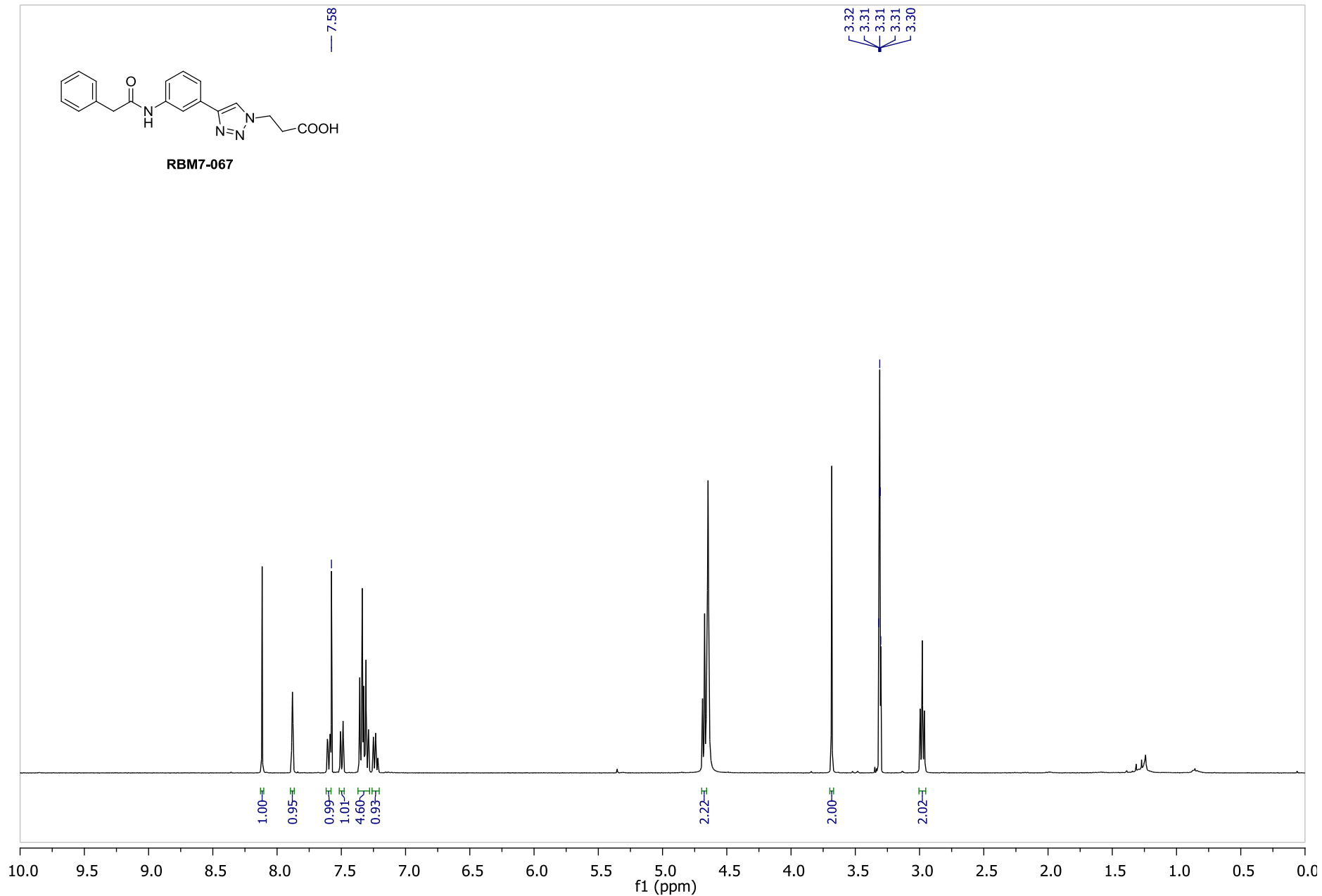


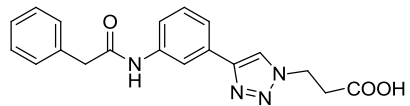
RBM7-066



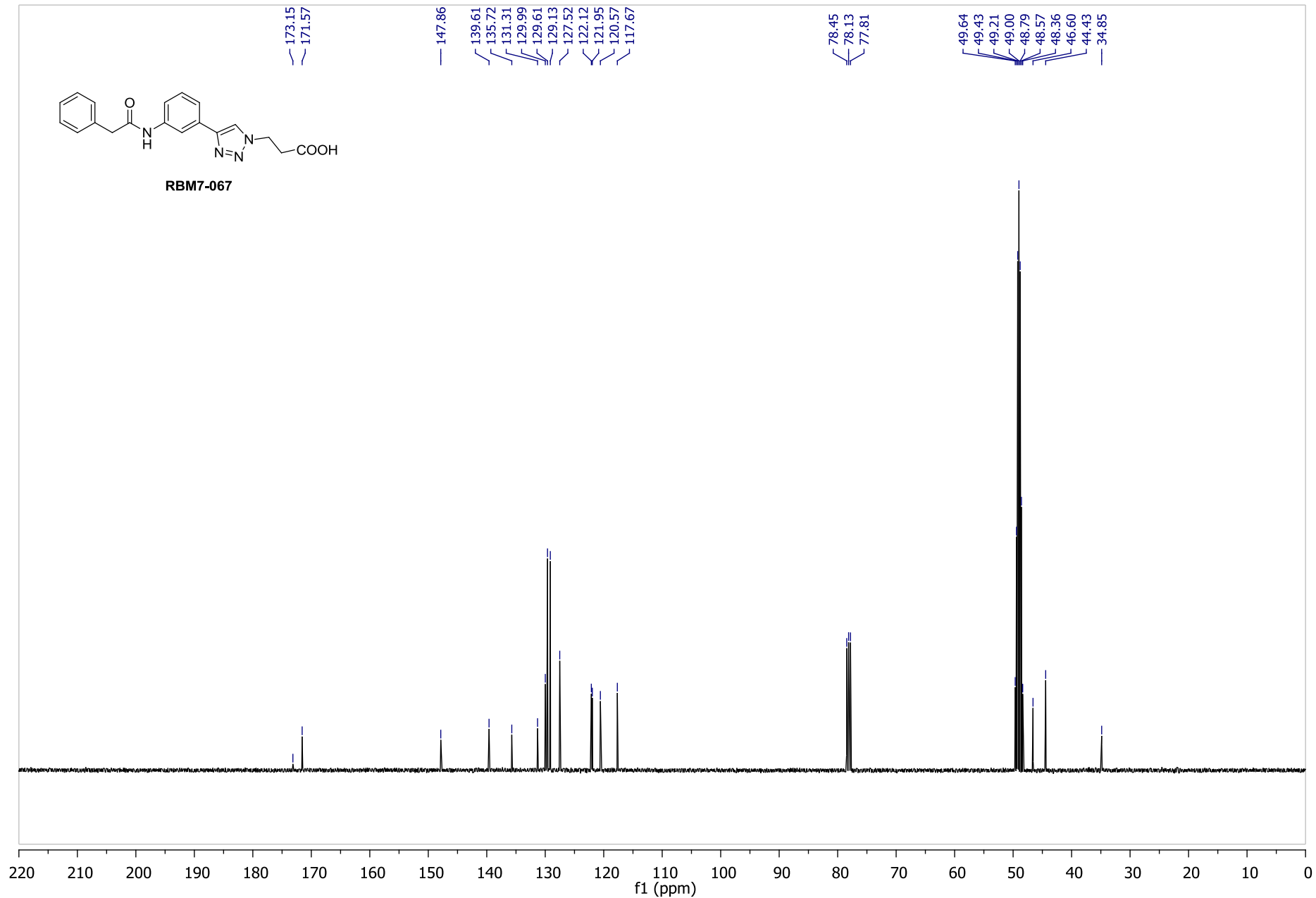


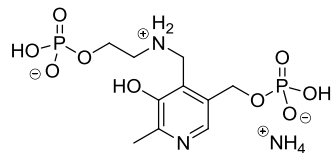
RBM7-067



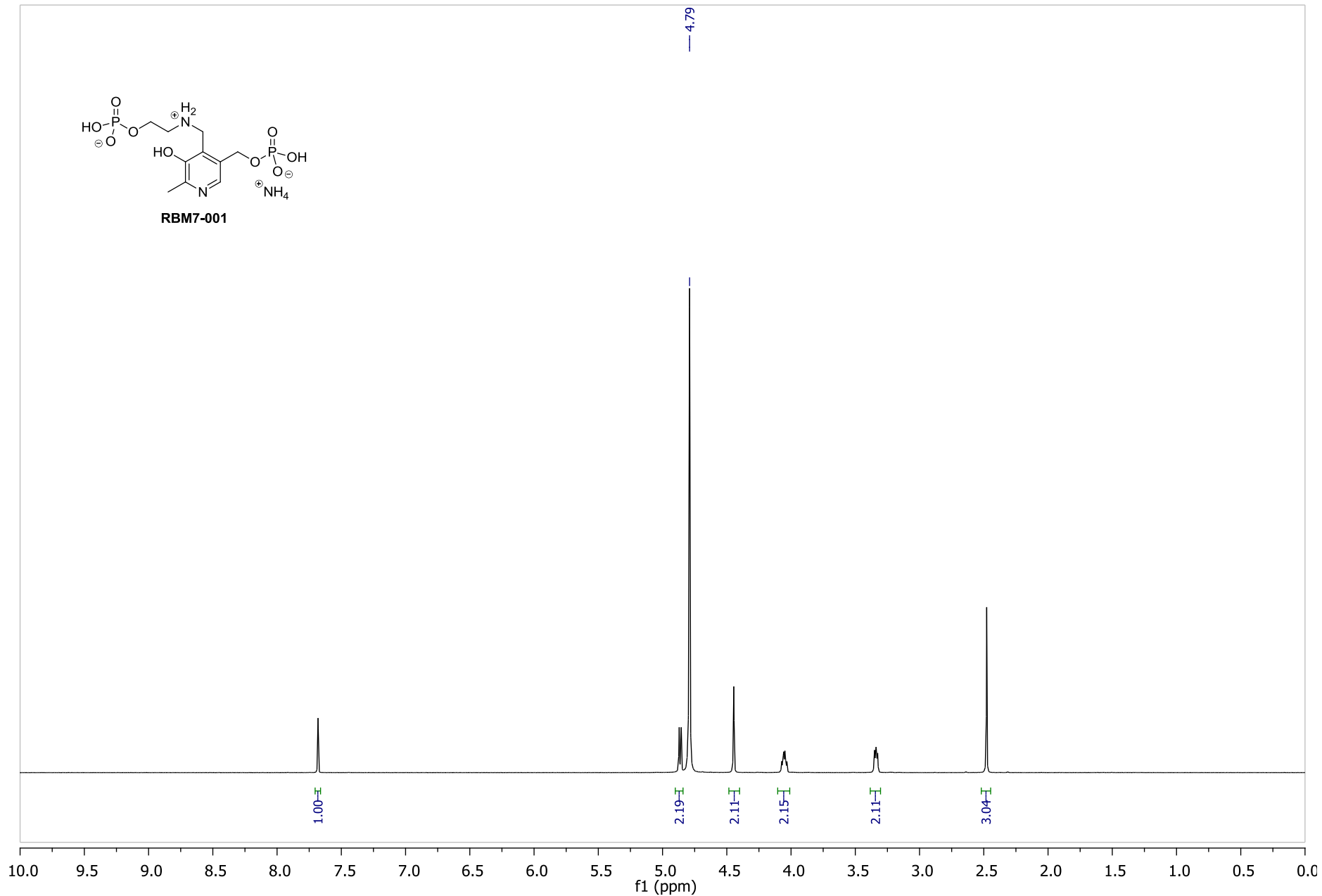


RBM7-067

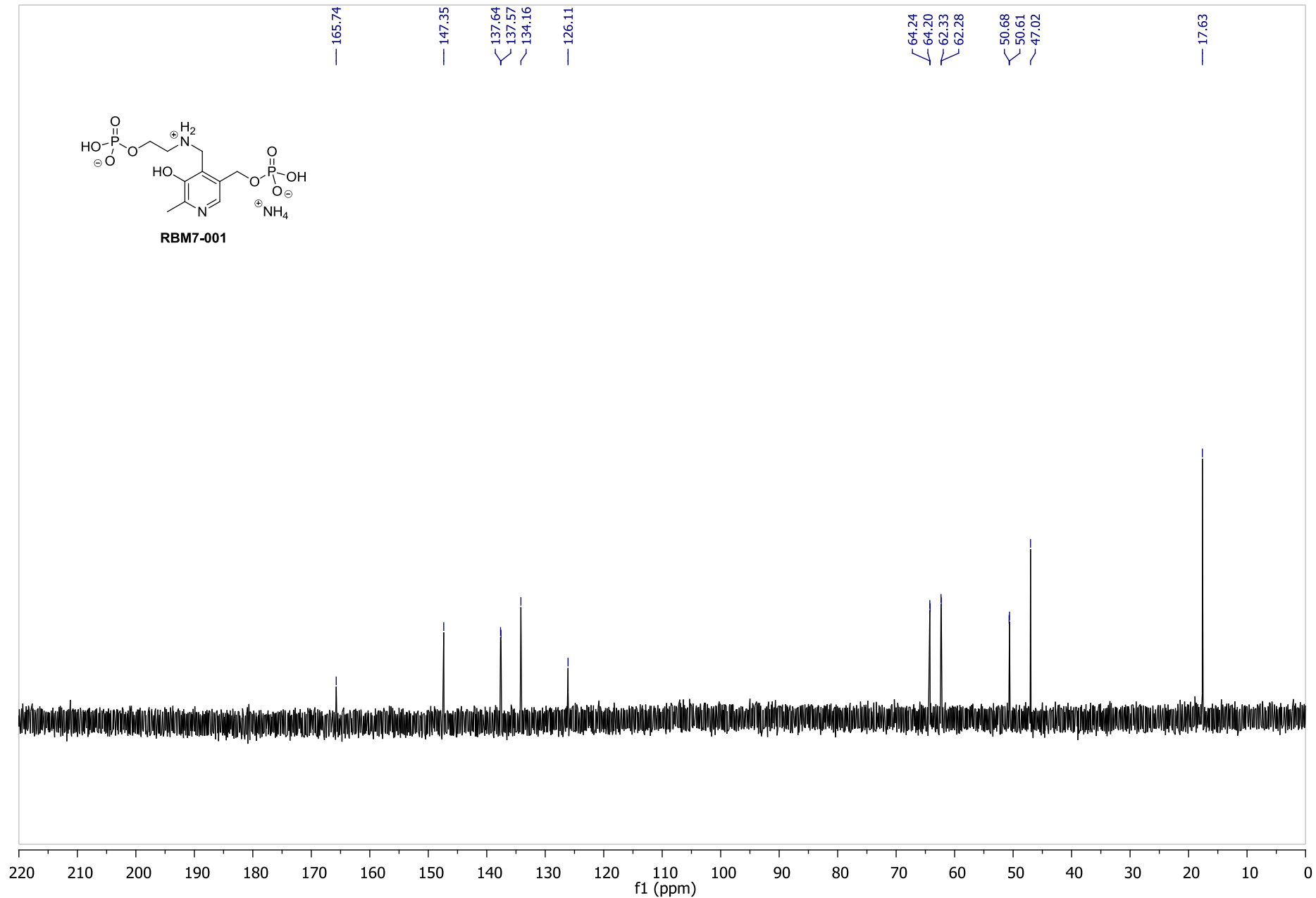
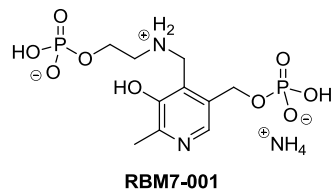


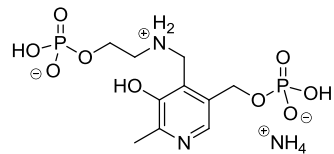


RBM7-001

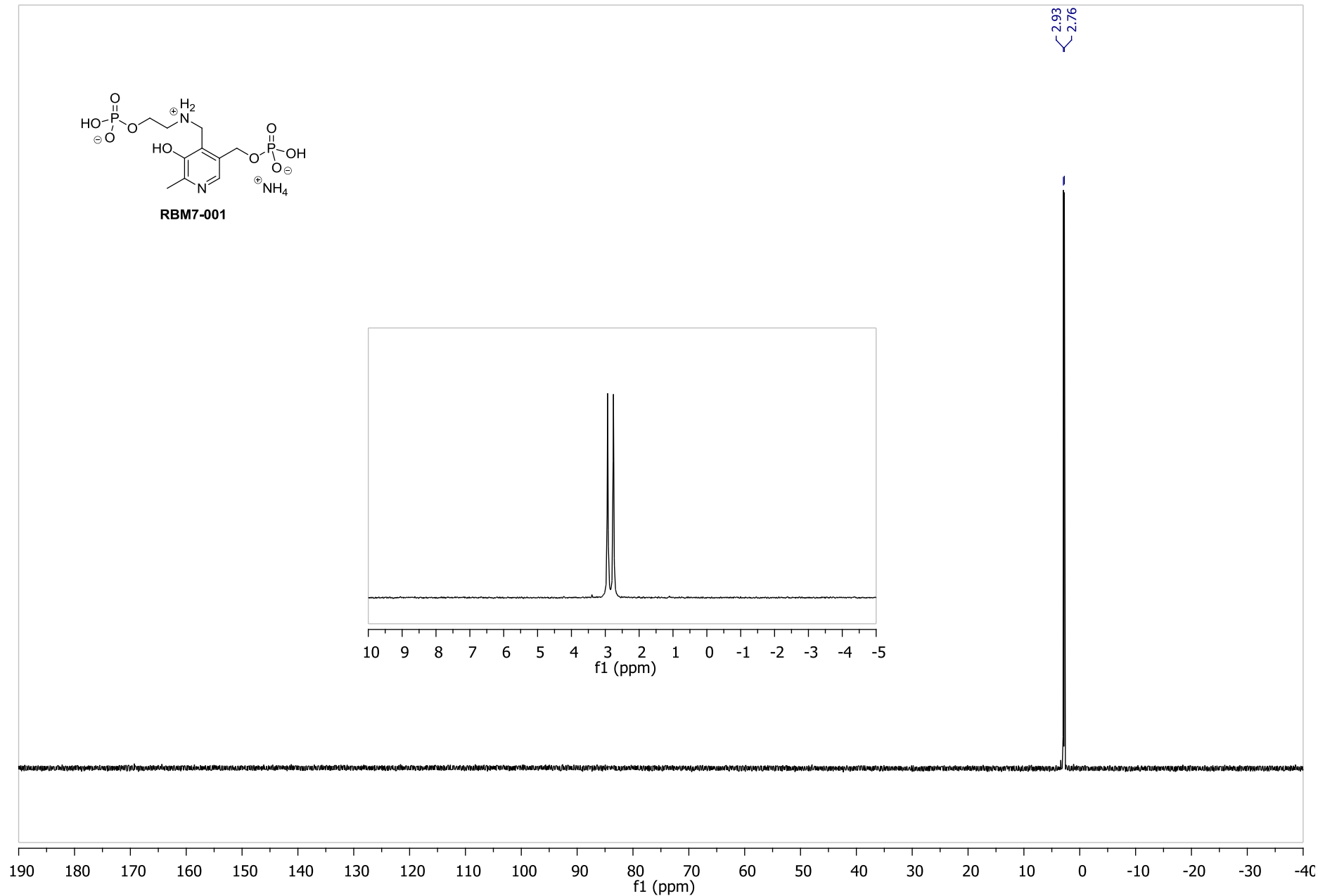


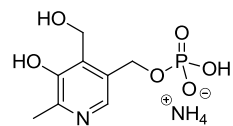
S70



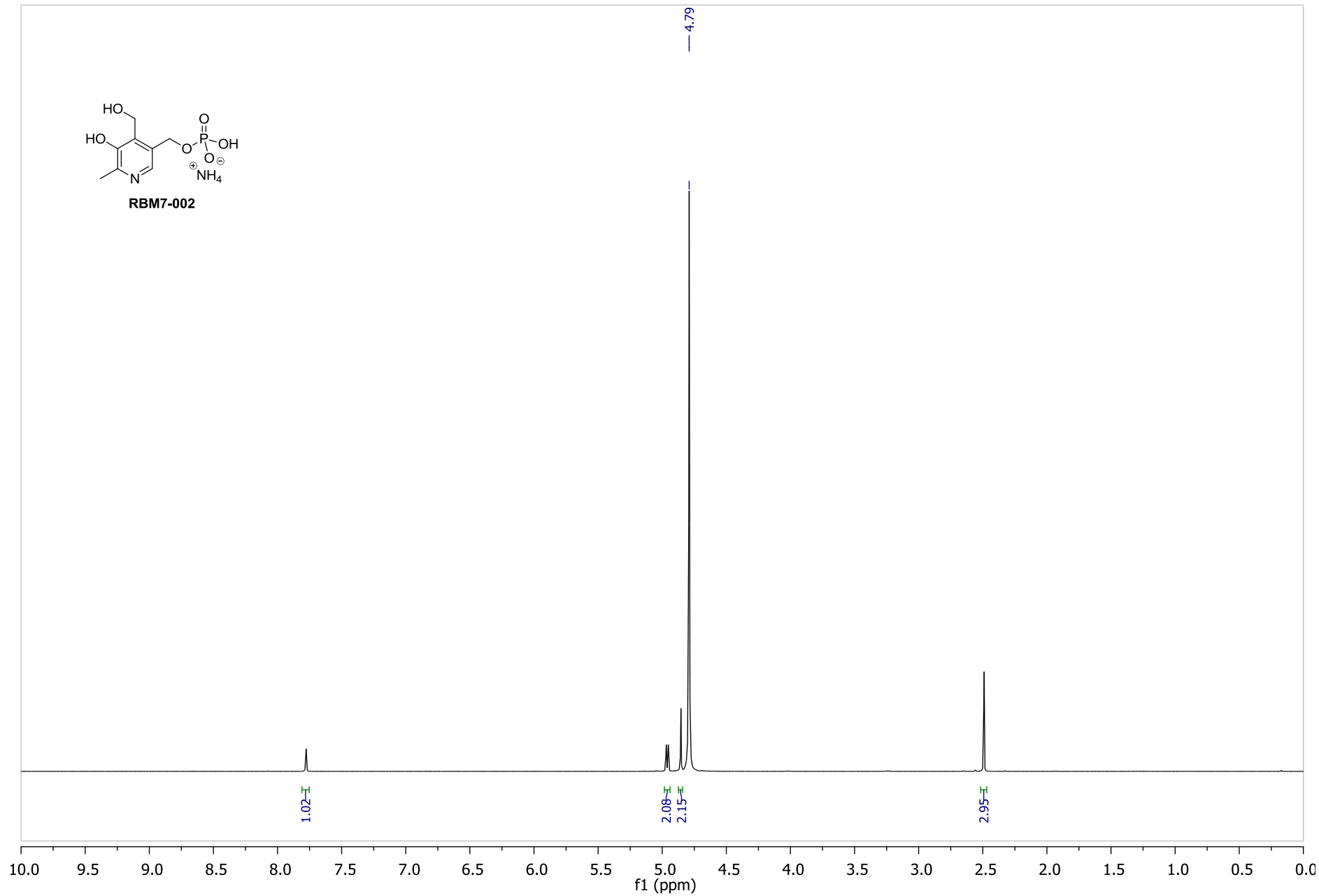


RBM7-001

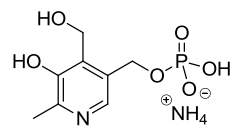




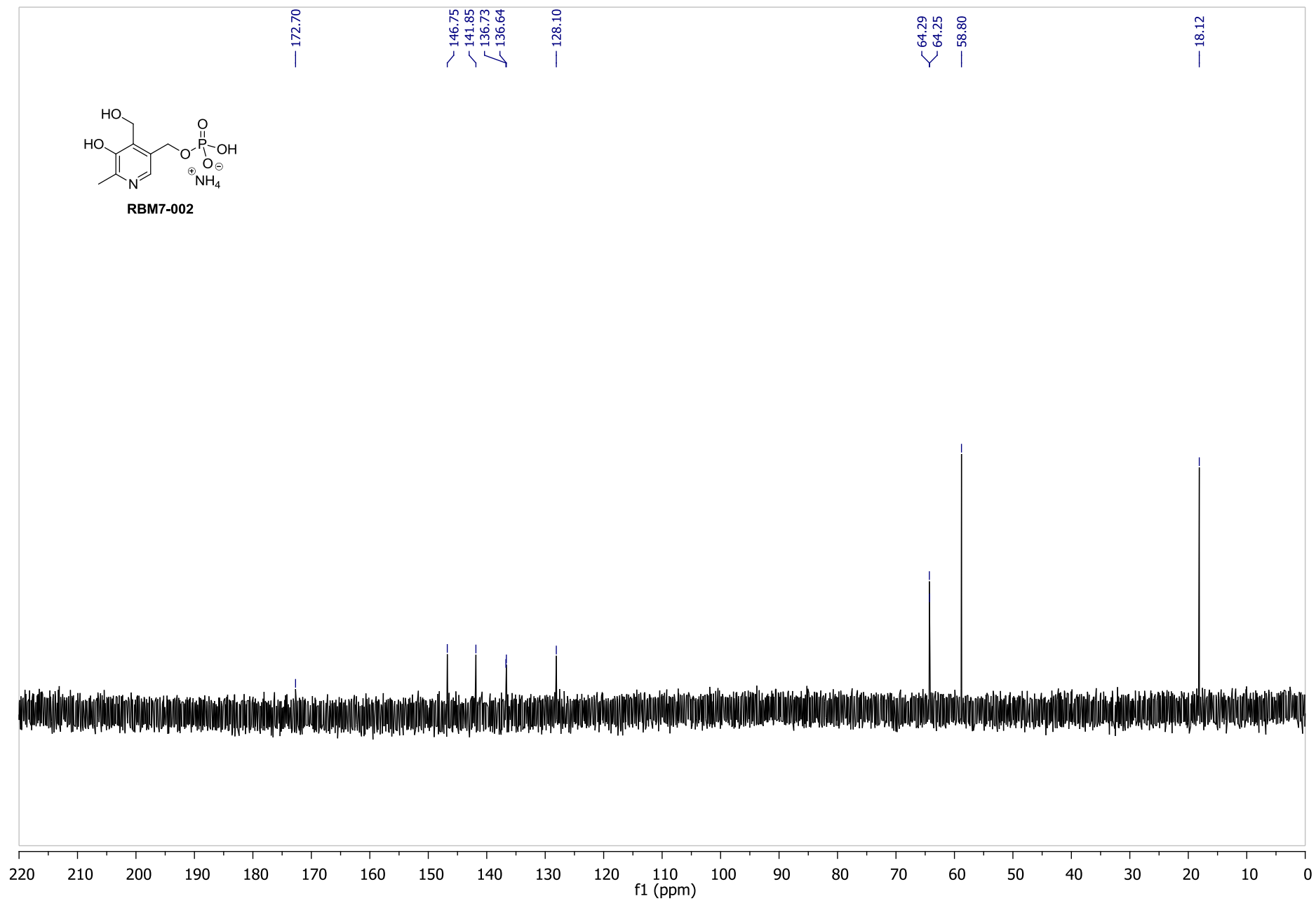
RBM7-002

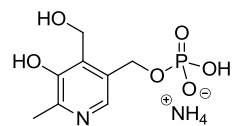


S73



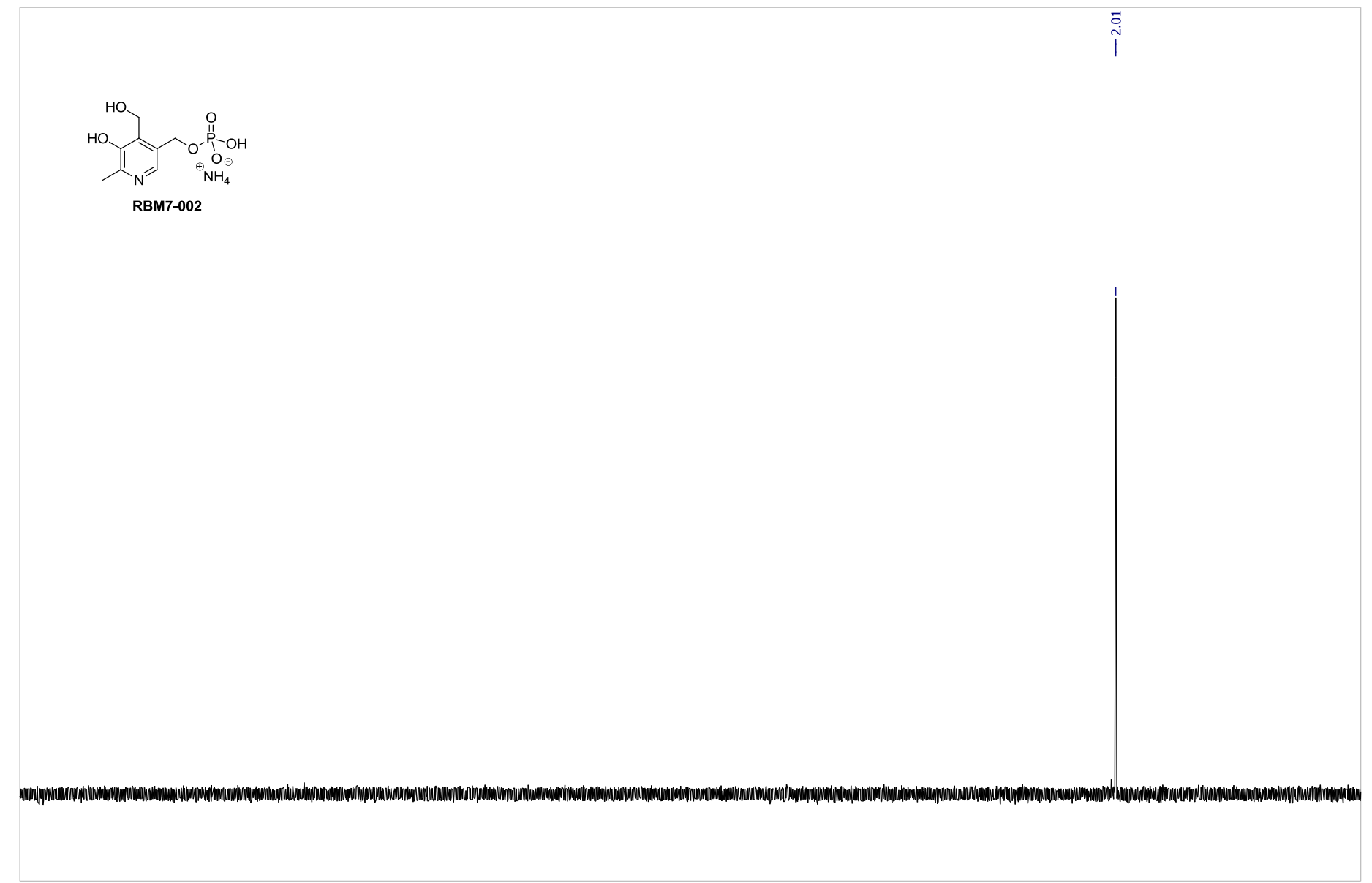
RBM7-002



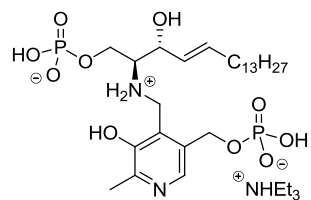


RBM7-002

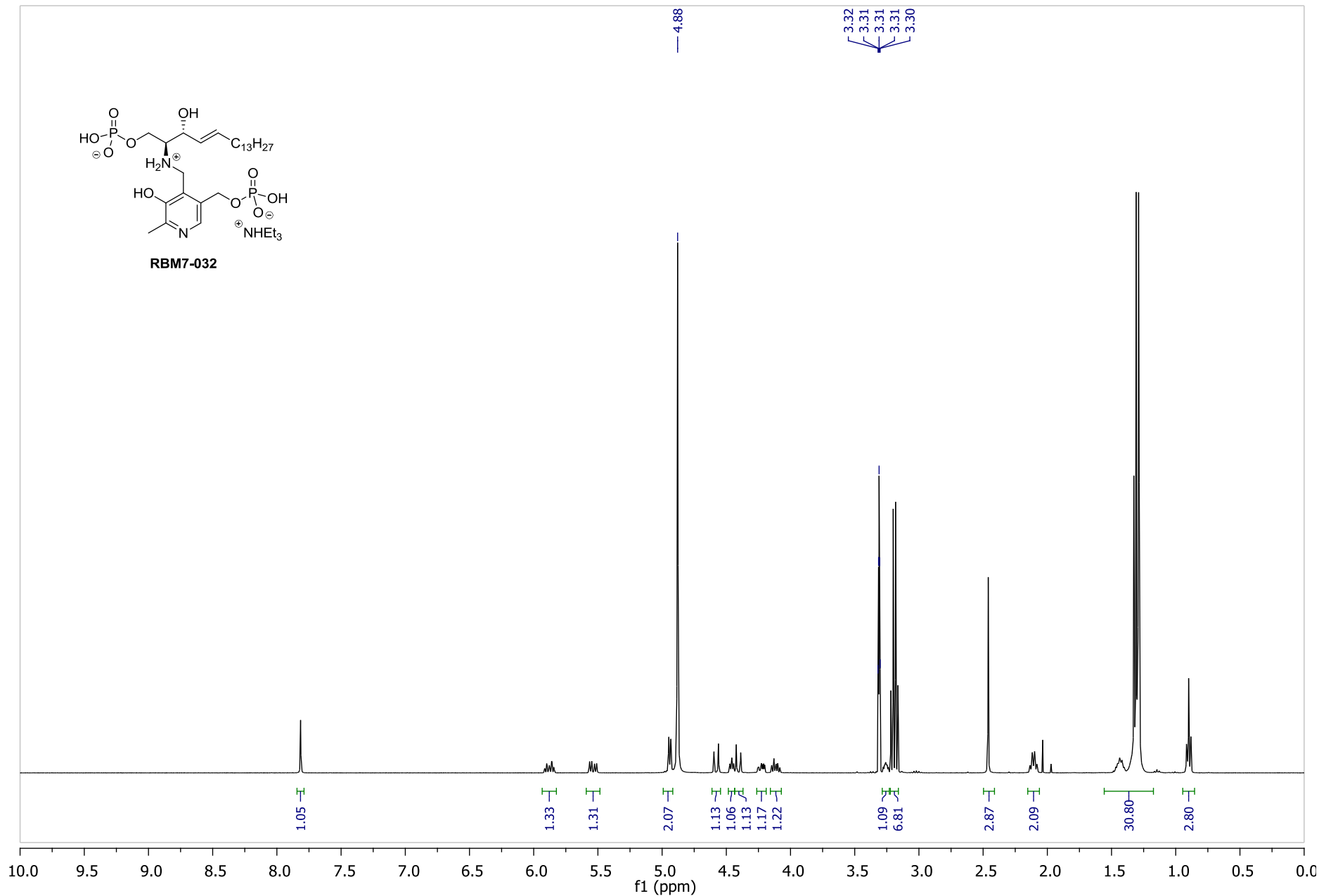
— 2.01

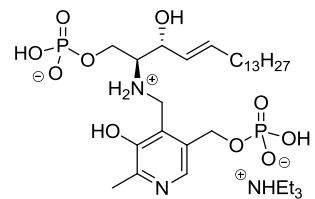


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)



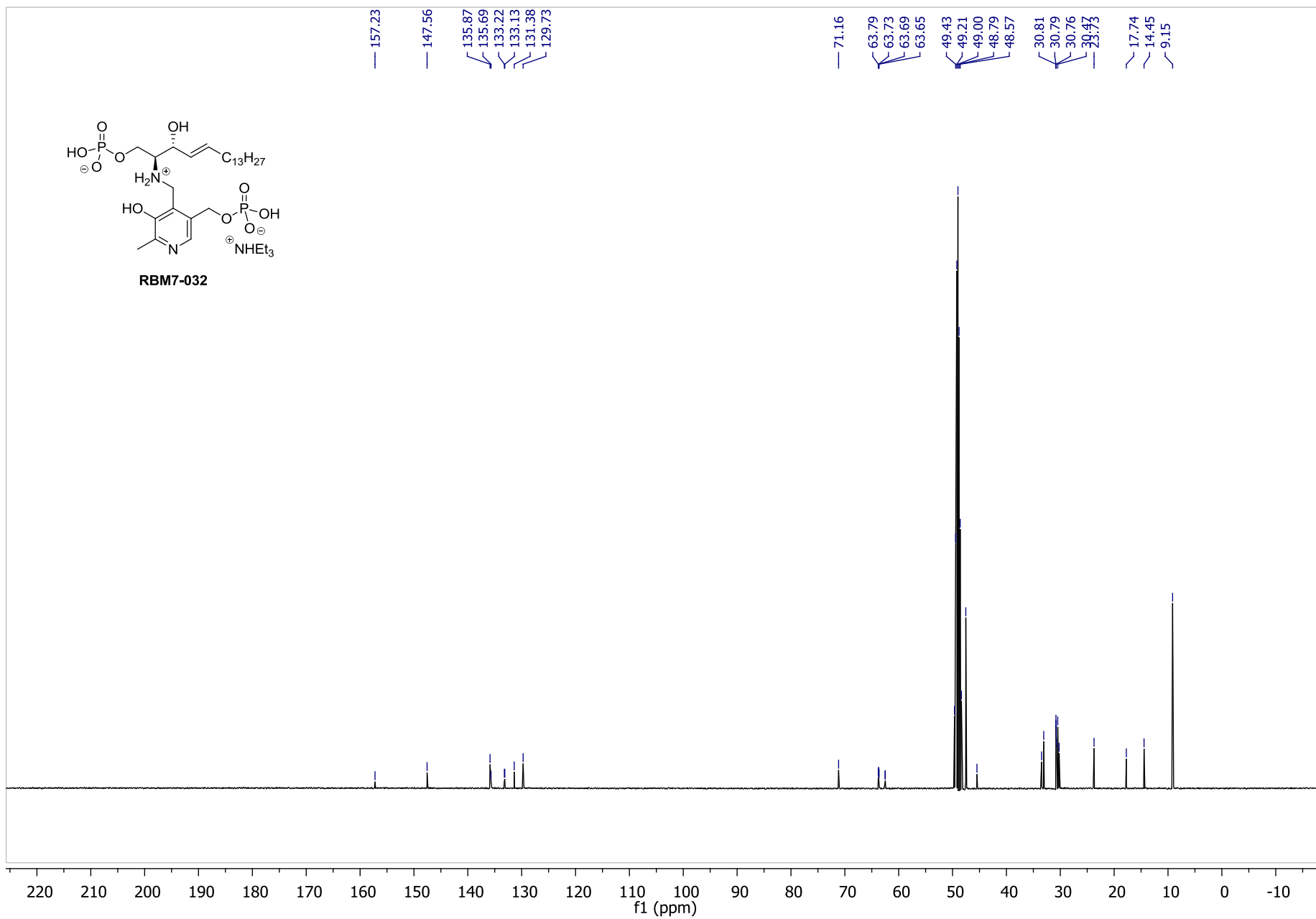
RBM7-032

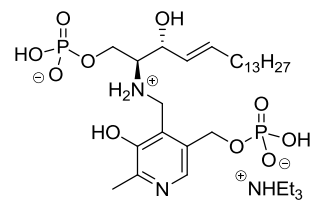




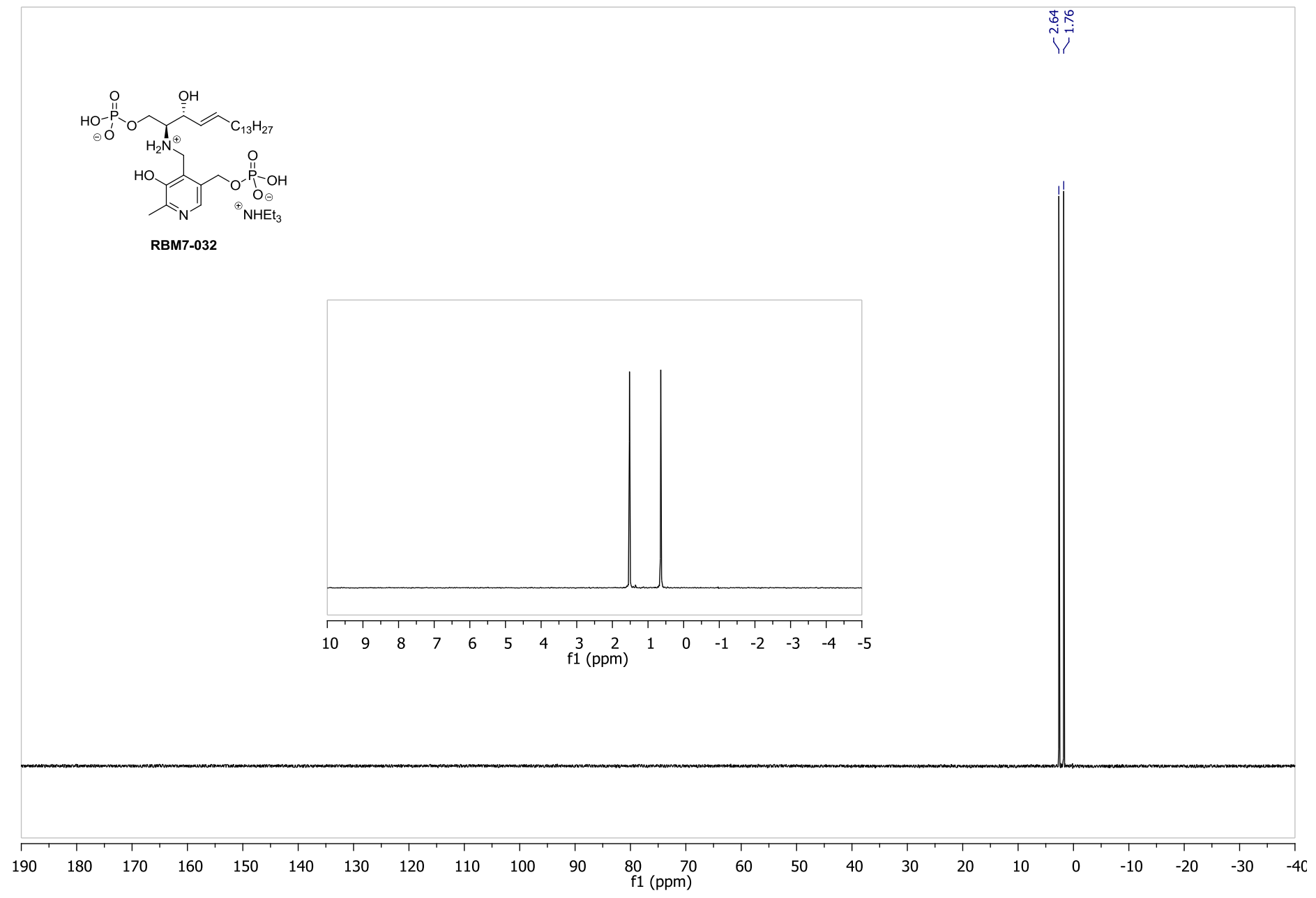
RBM7-032

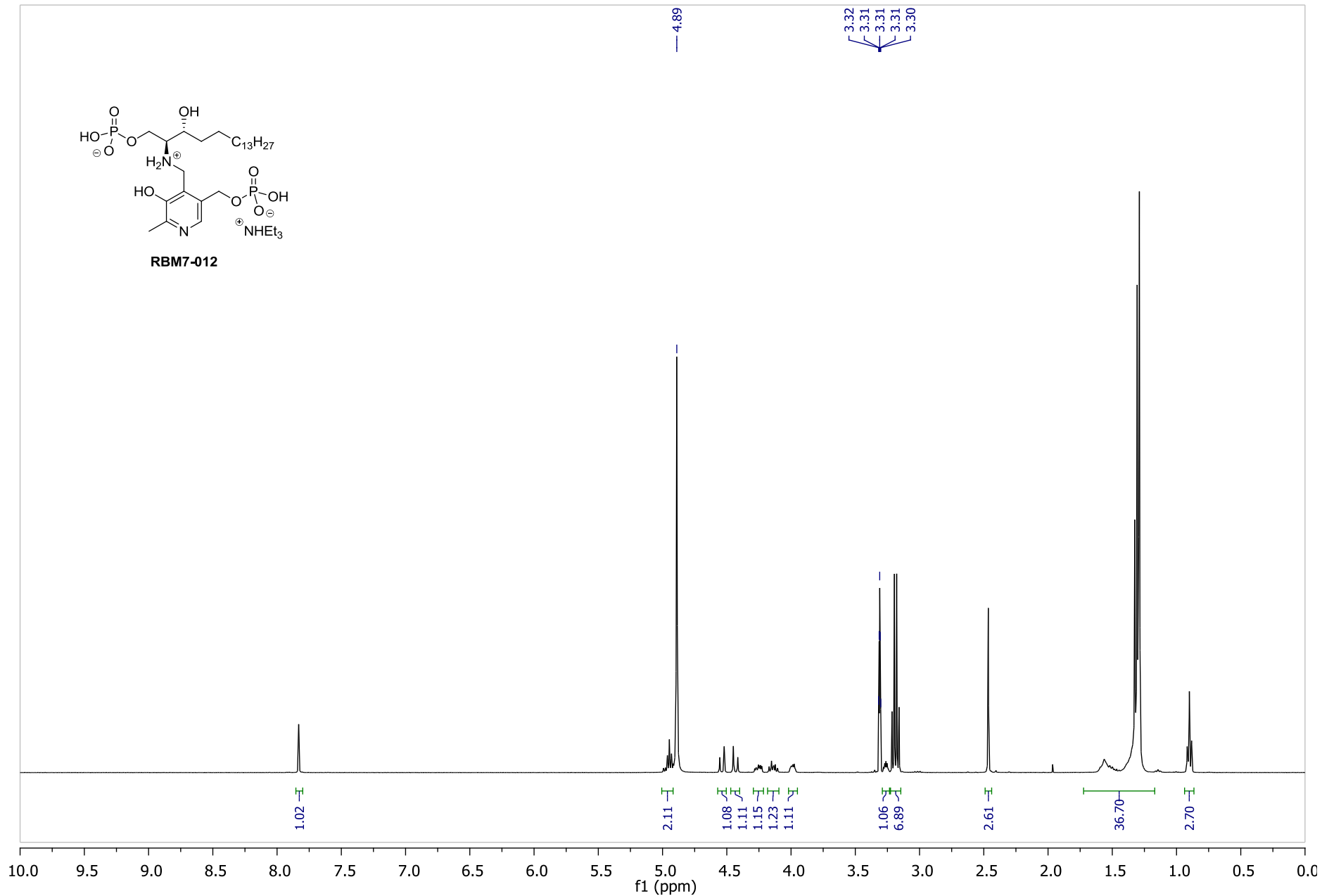
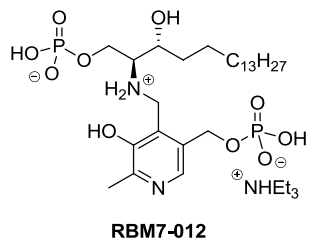
- 157.23
- 147.56
- ↙ 135.87
- ↘ 135.69
- ↙ 133.22
- ↘ 133.13
- ↙ 131.38
- ↘ 129.73
- 71.16
- ↙ 63.79
- ↘ 63.73
- ↙ 63.69
- ↘ 63.65
- ↙ 49.43
- ↘ 49.21
- ↙ 49.00
- ↘ 48.79
- ↙ 48.57
- ↙ 30.81
- ↘ 30.79
- ↙ 30.76
- ↘ 30.73
- ↙ 17.74
- ↘ 14.45
- ↙ 9.15

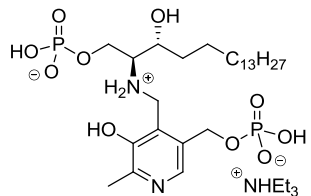




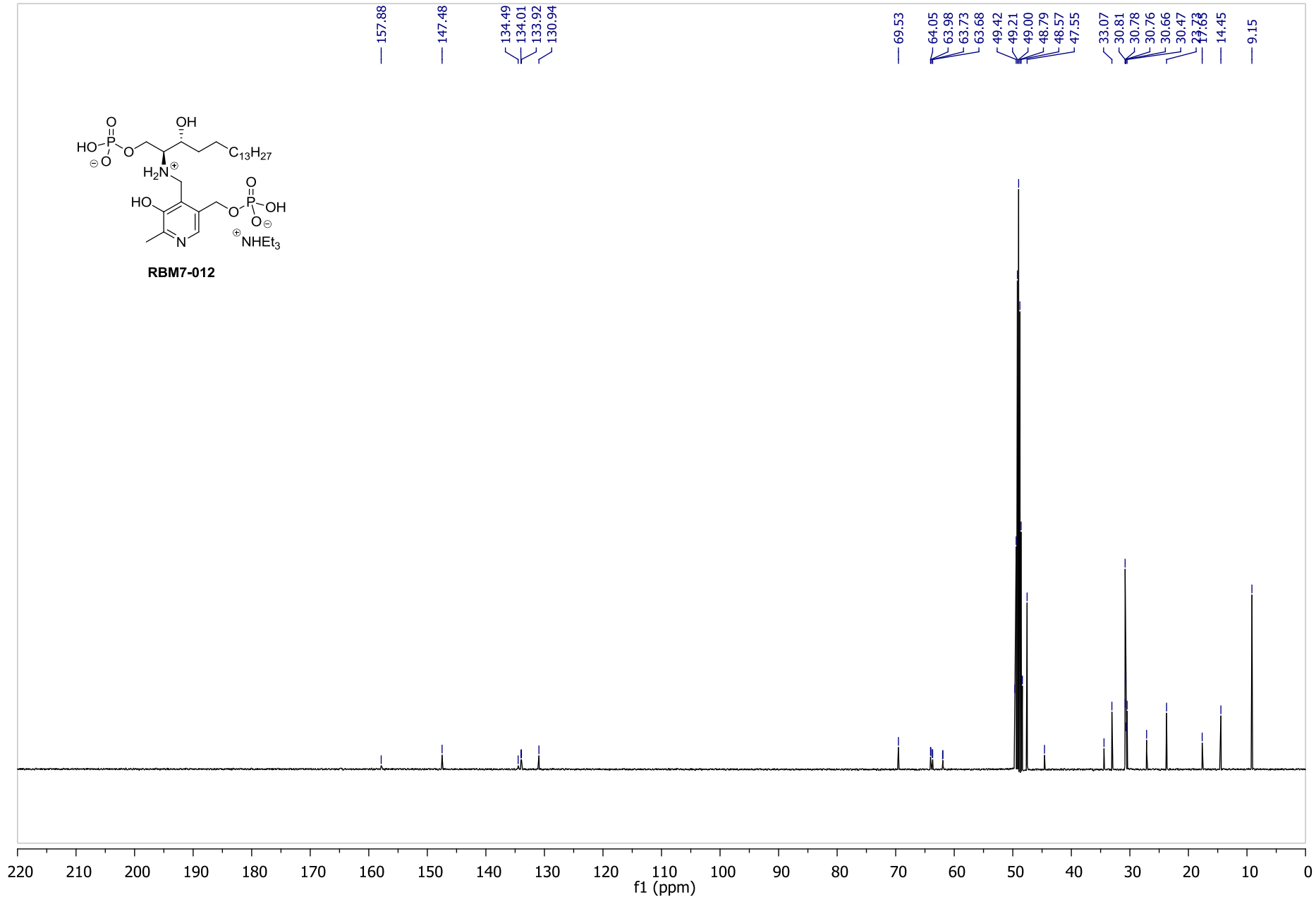
RBM7-032

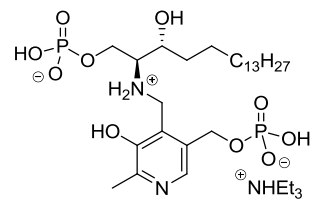






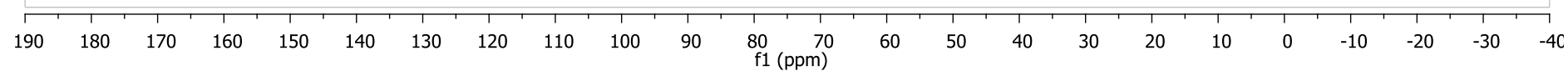
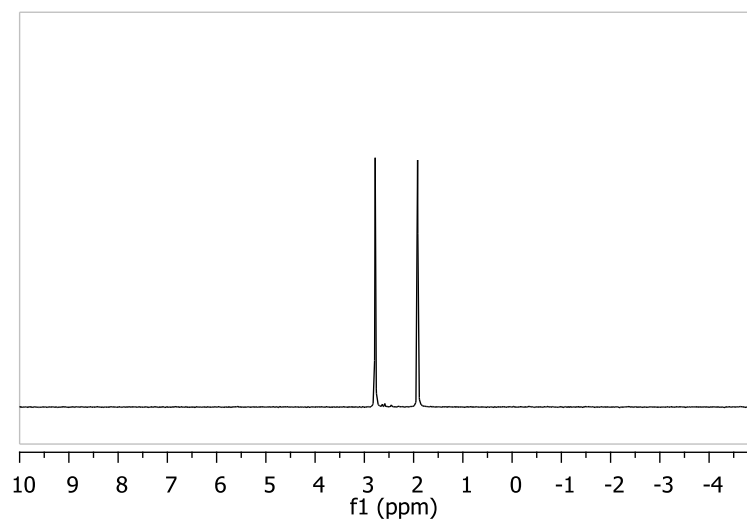
RBM7-012



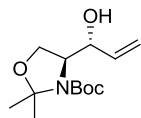


RBM7-012

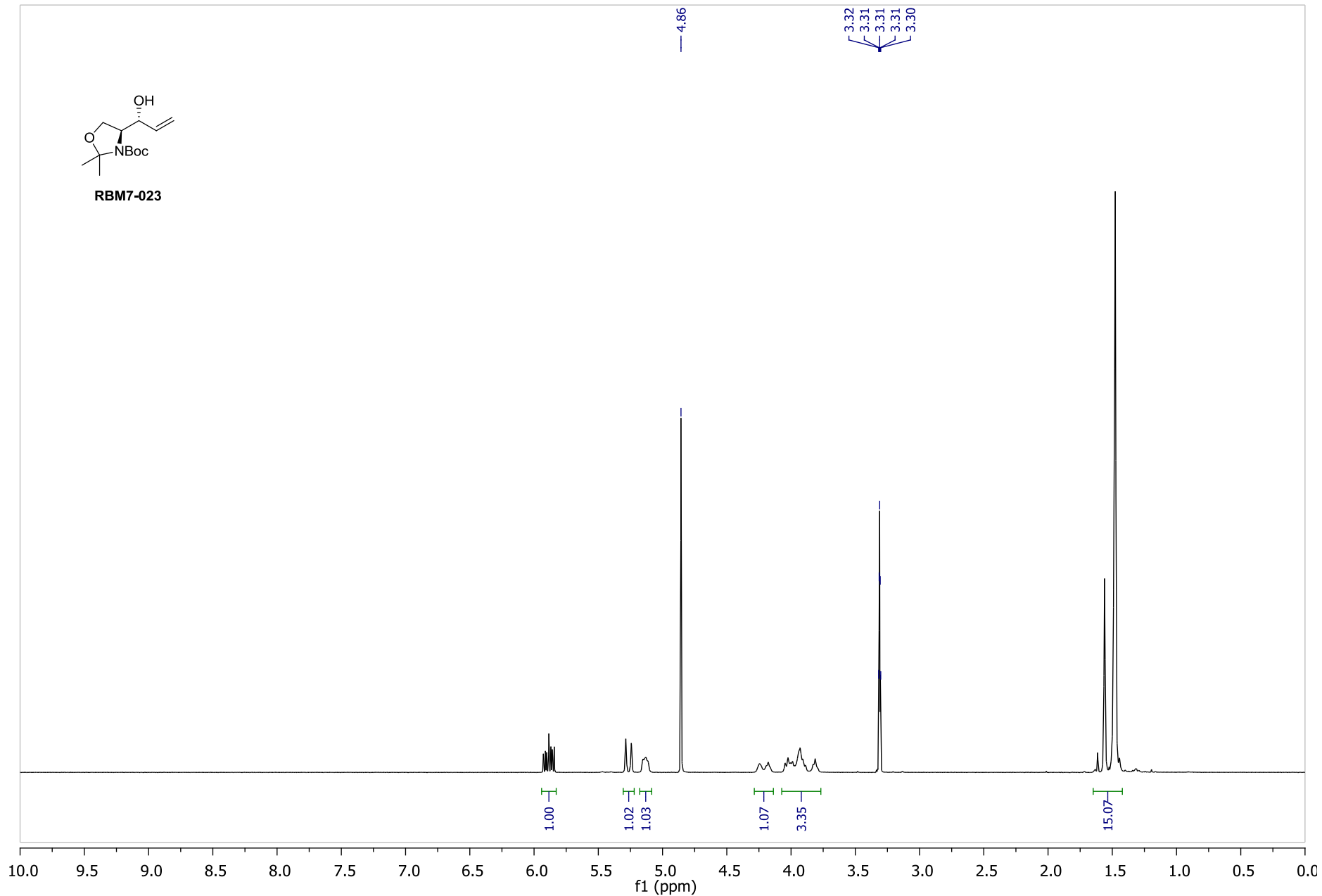
2.78
1.92

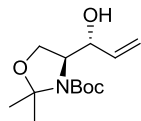


S81

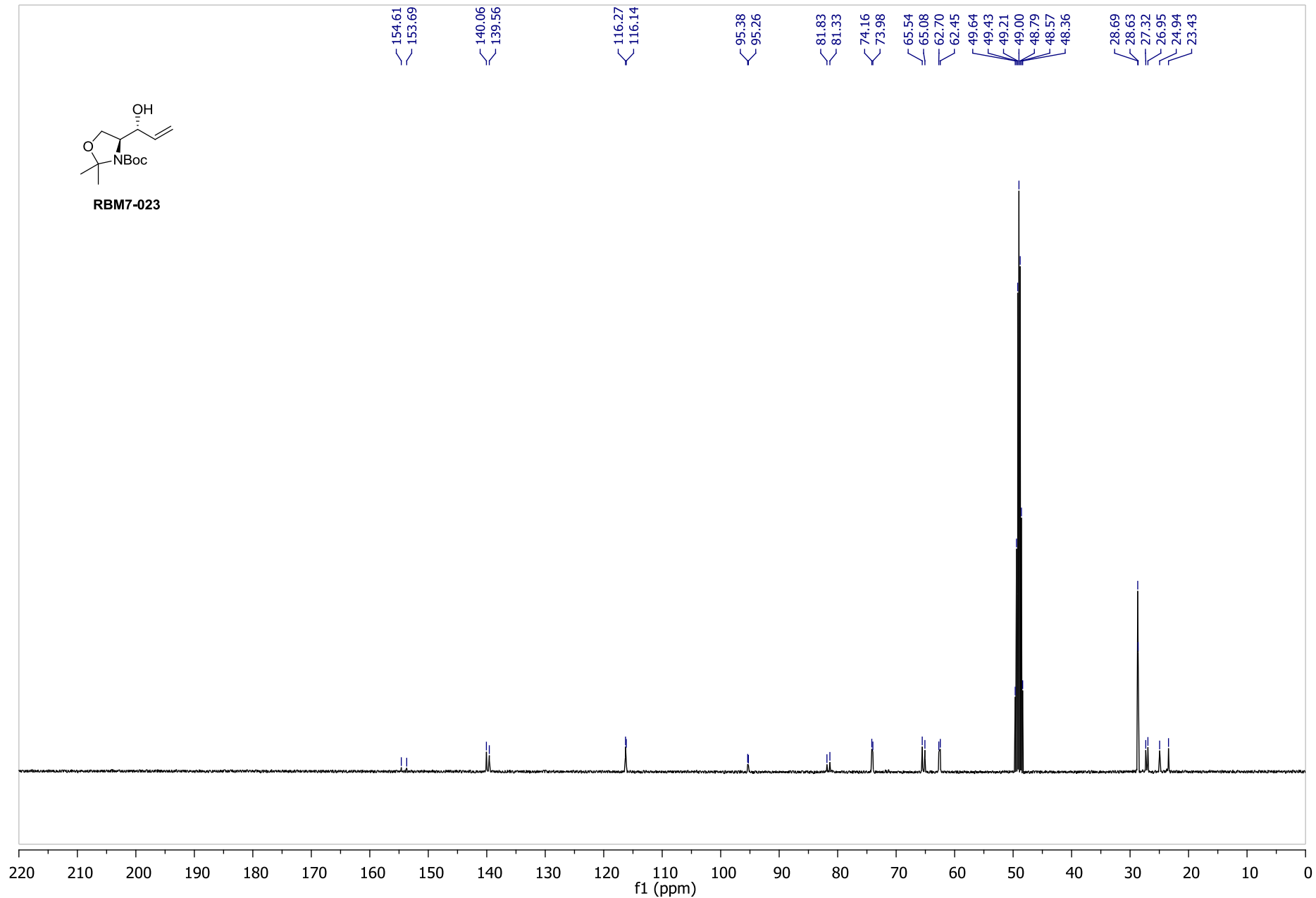


RBM7-023

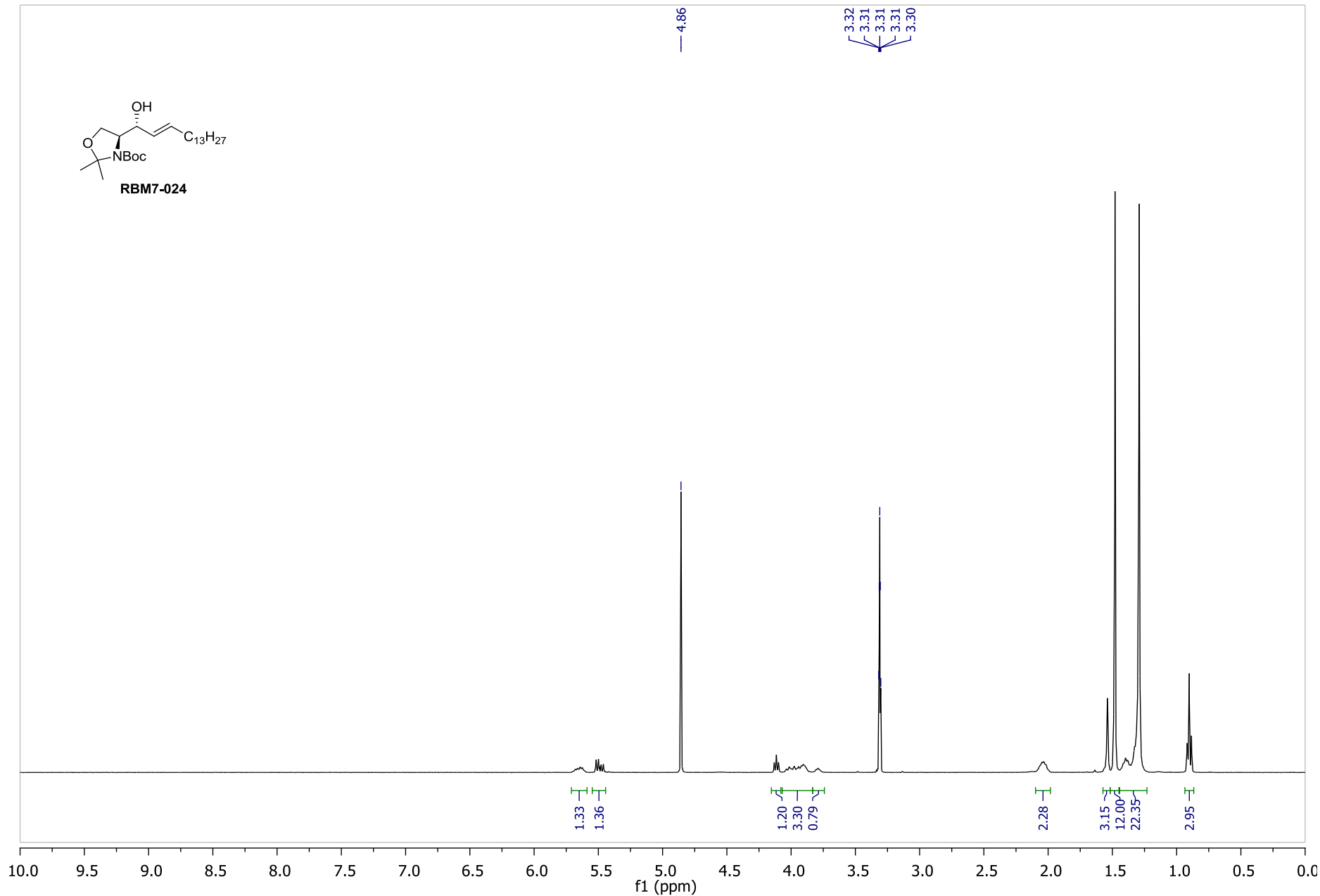
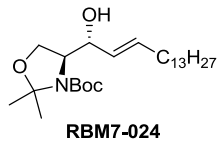




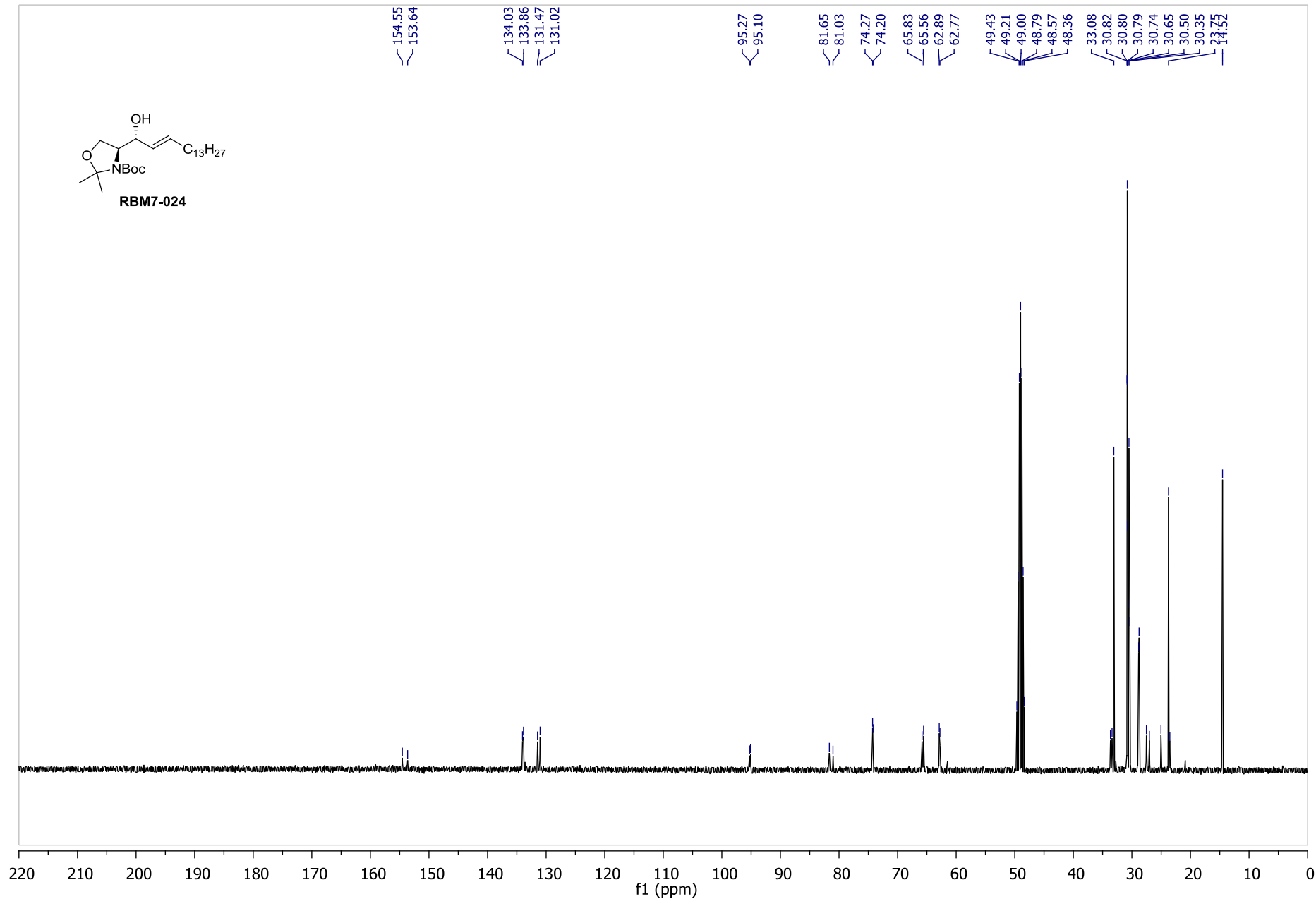
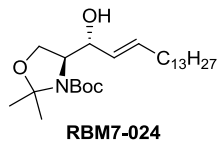
RBM7-023



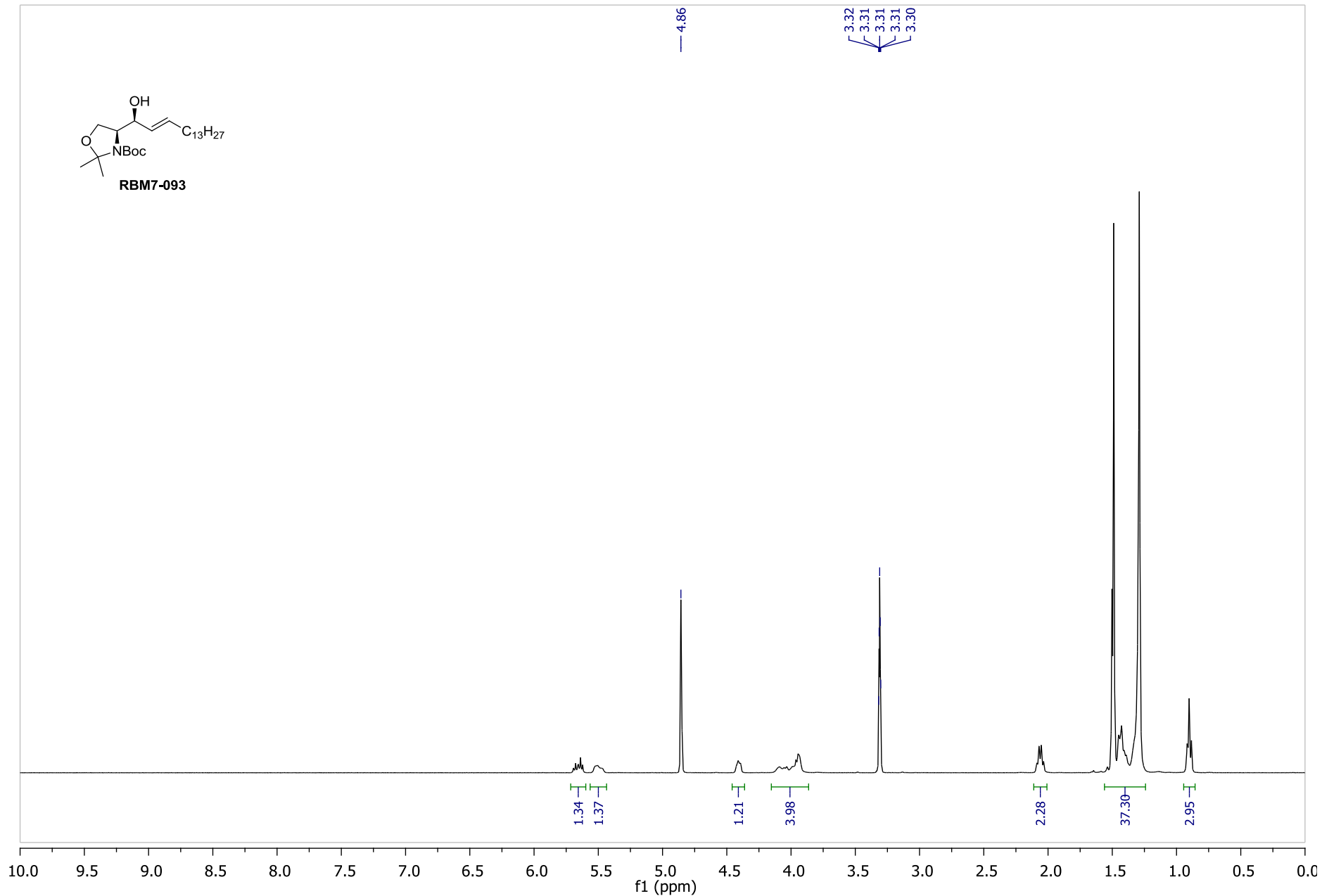
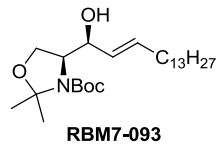
S83



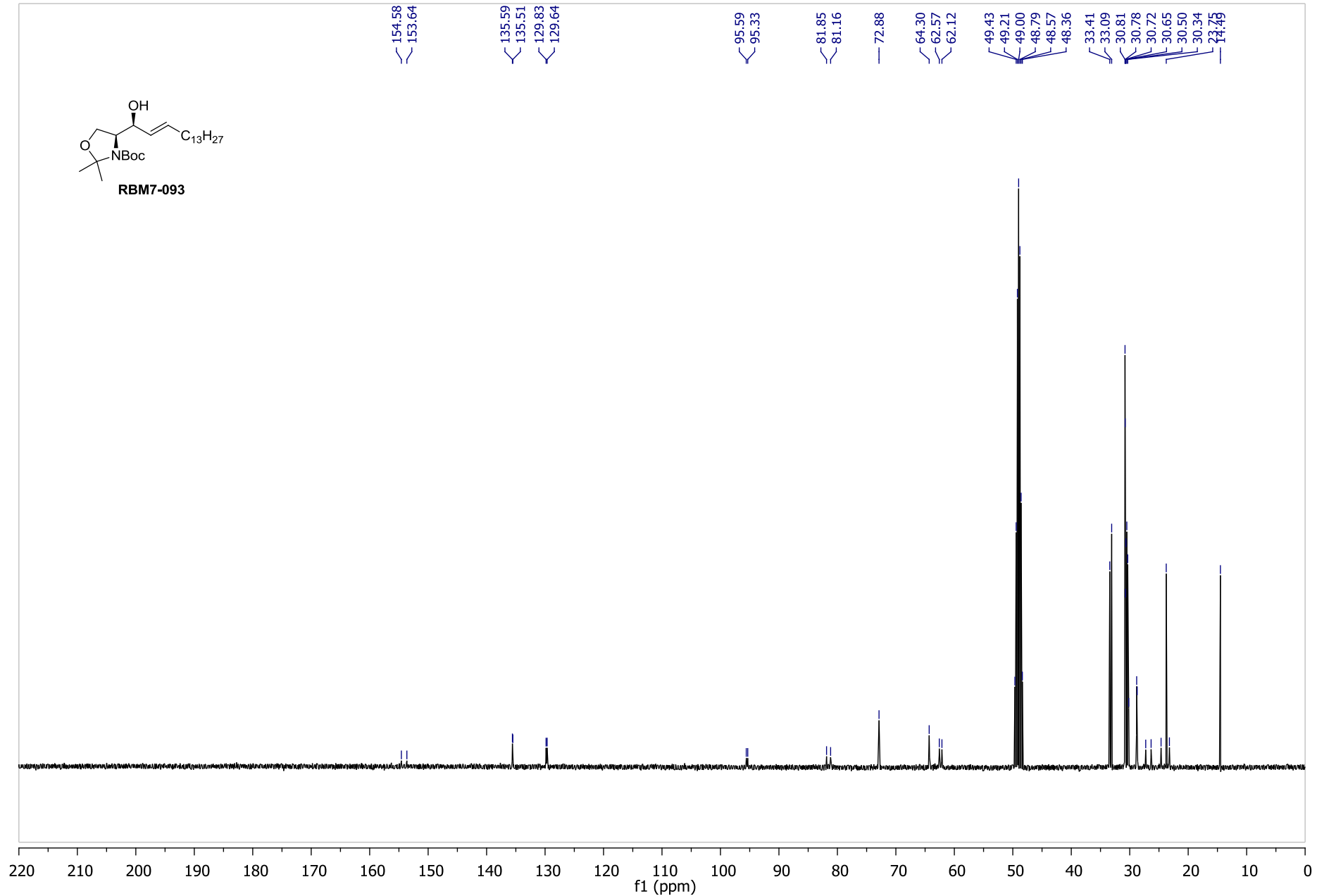
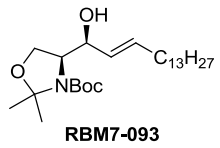
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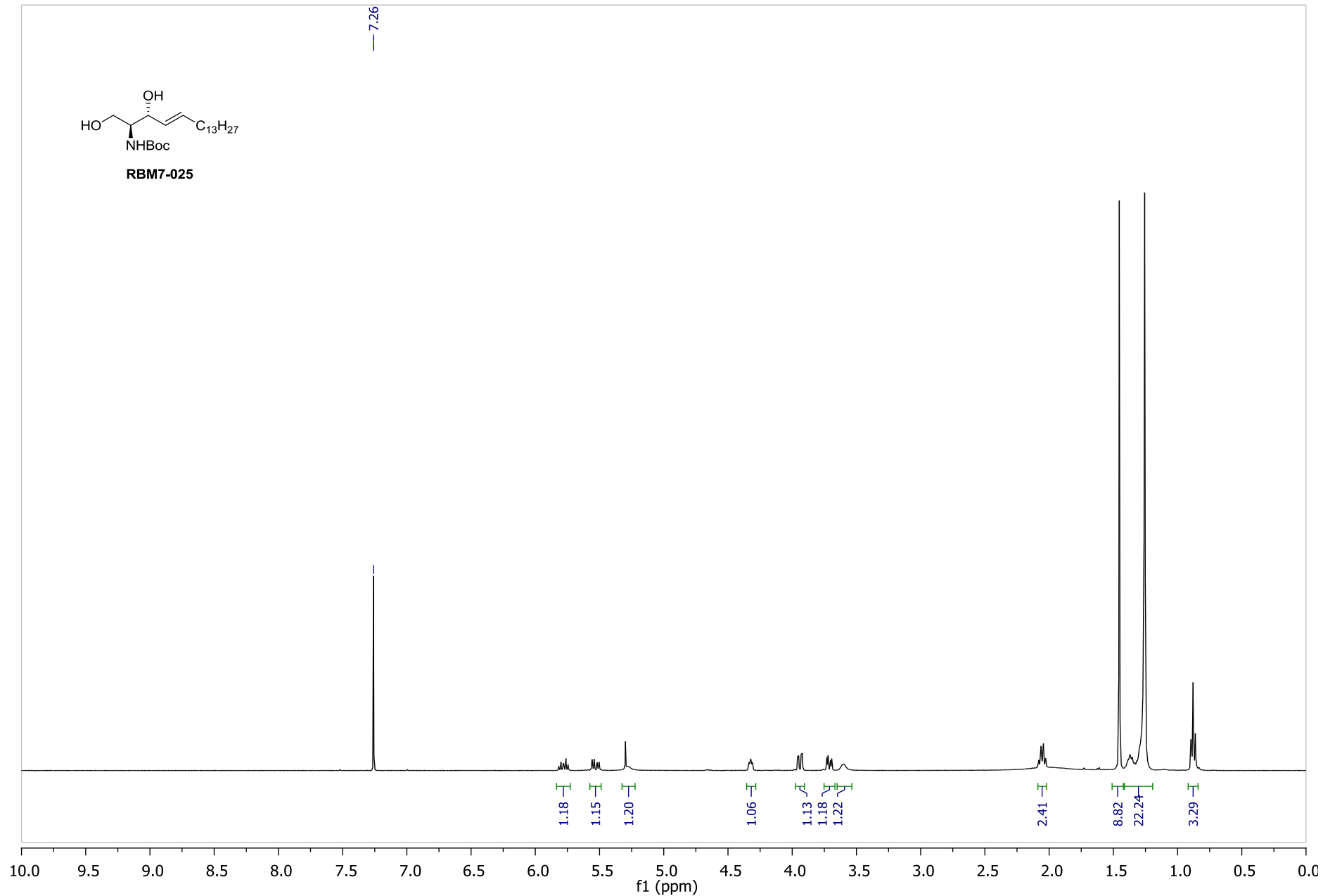
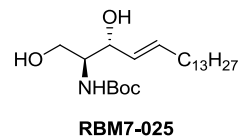
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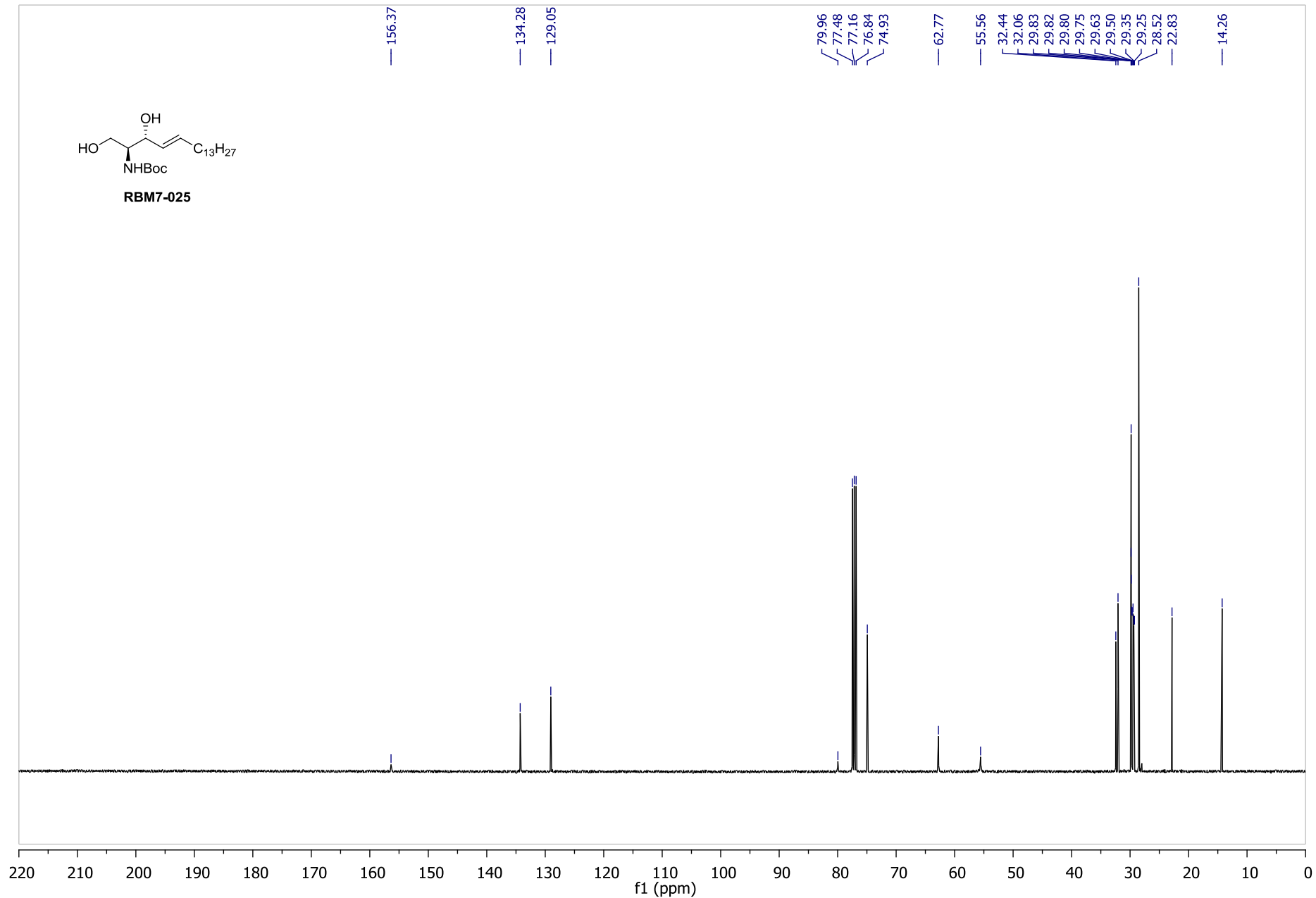
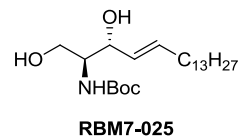
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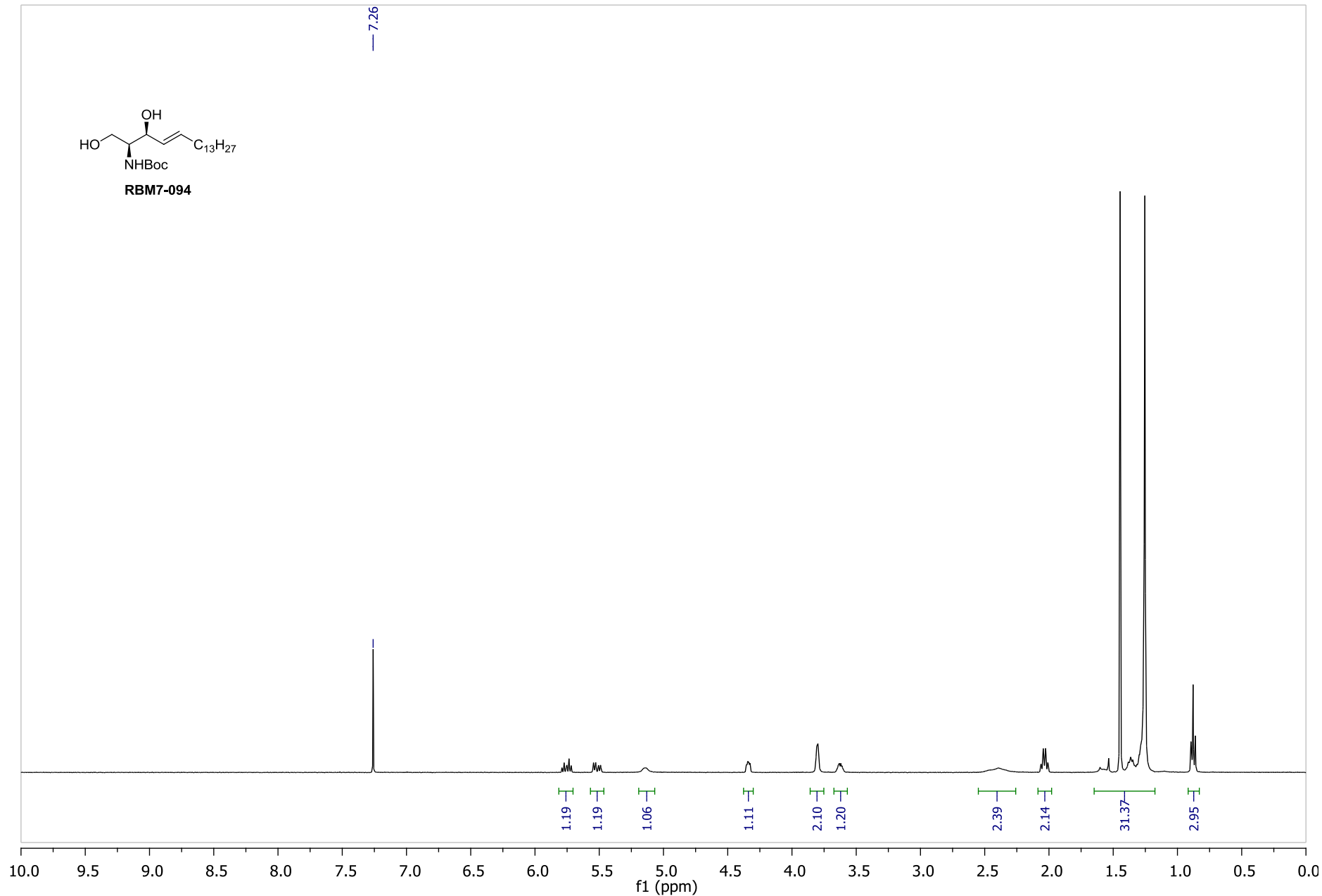
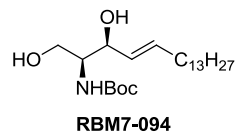
S87



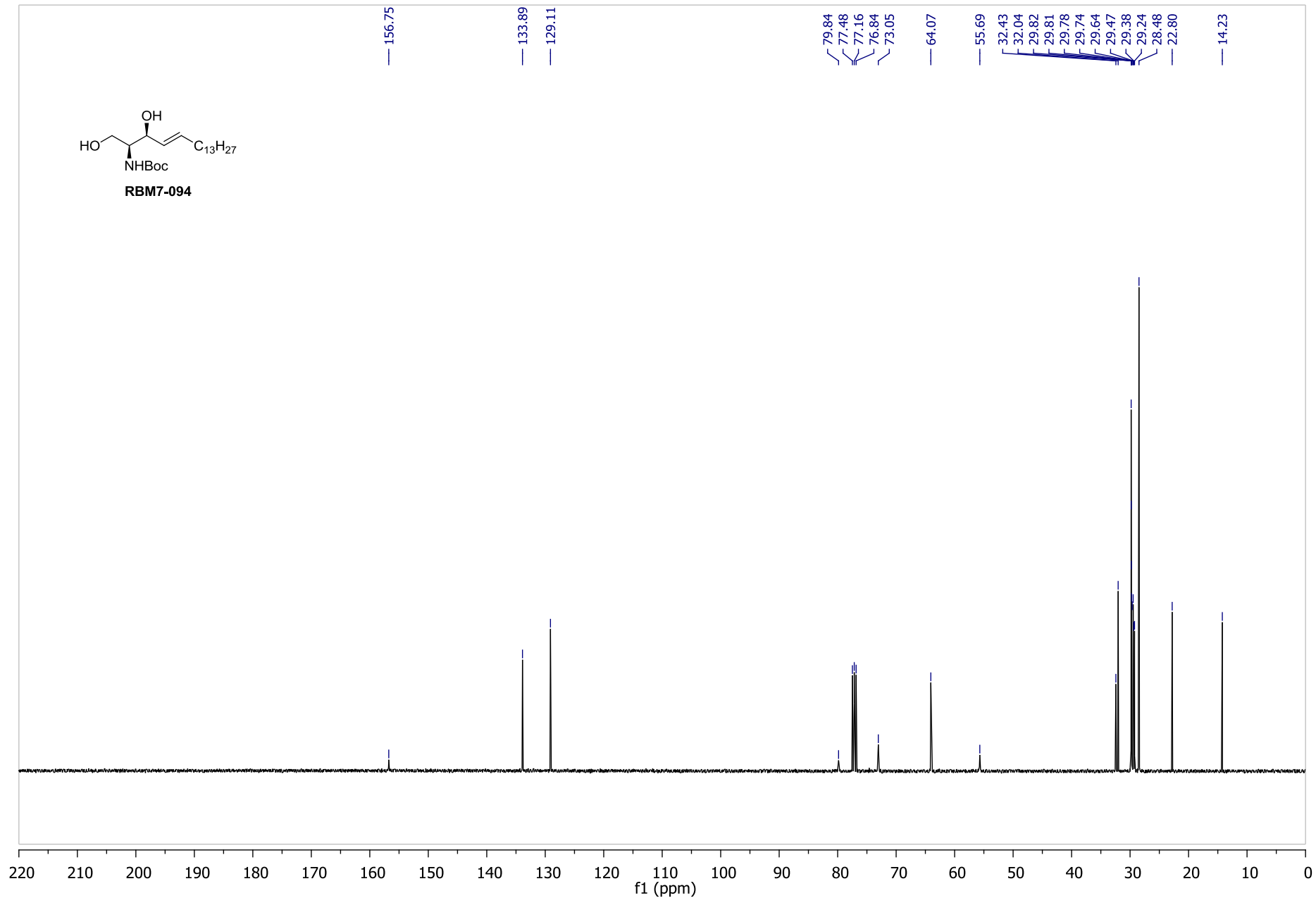
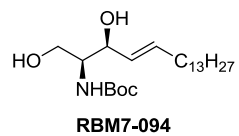
S88



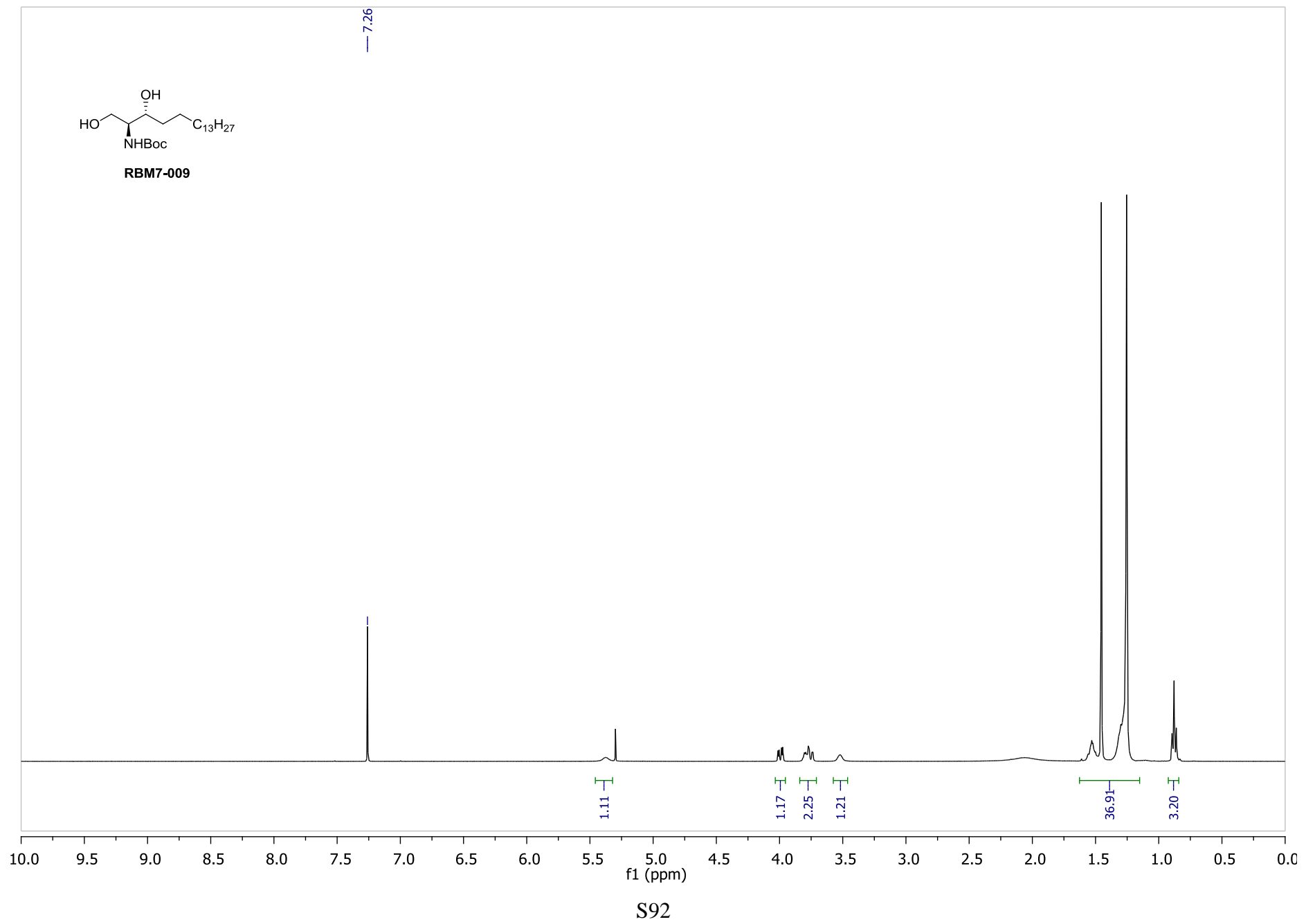
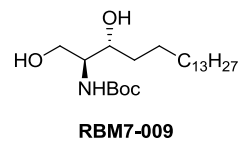
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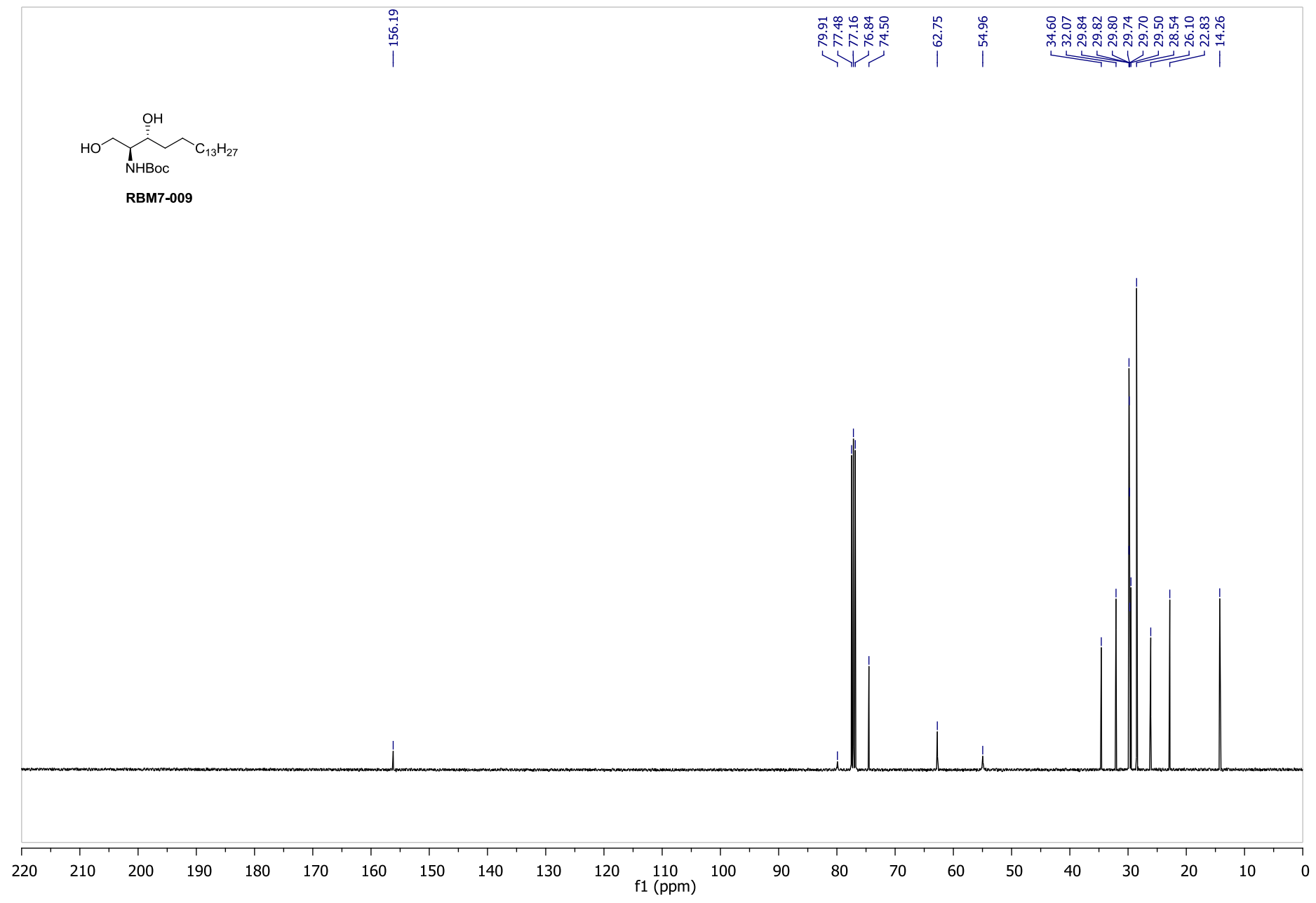
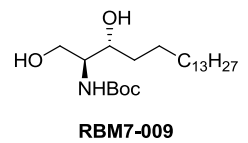


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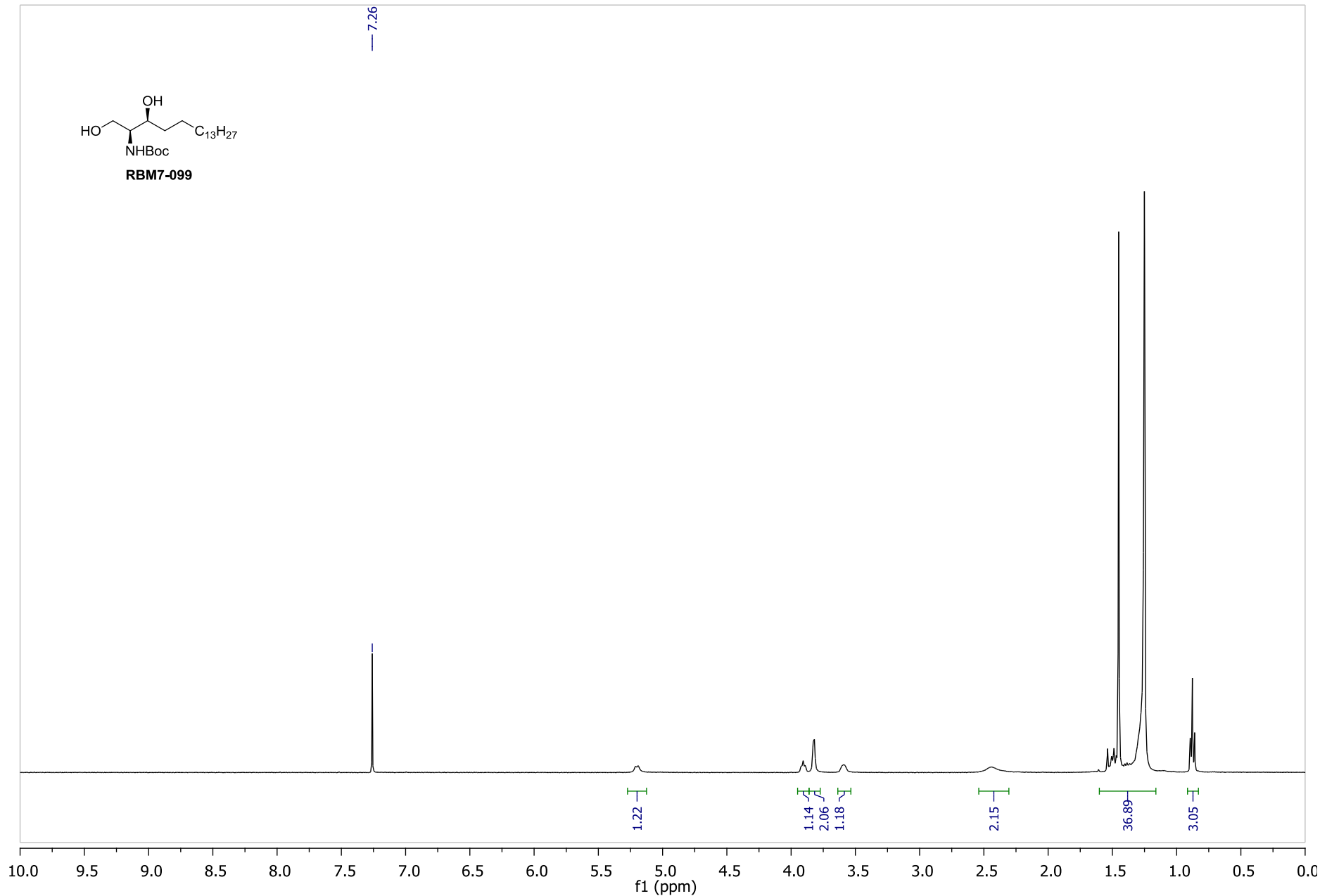
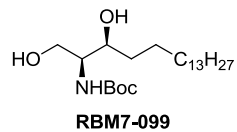


S91

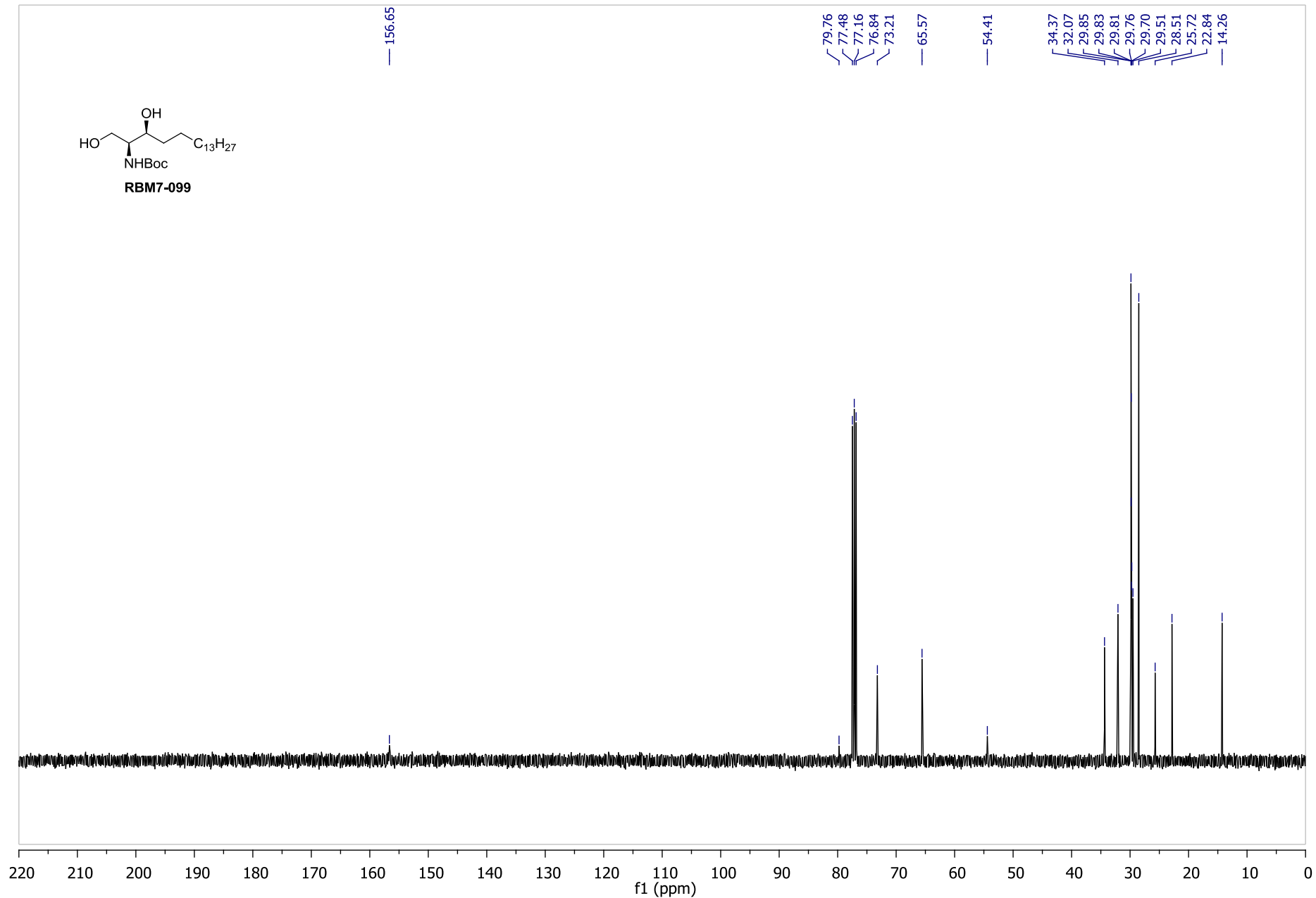
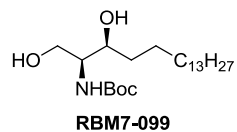




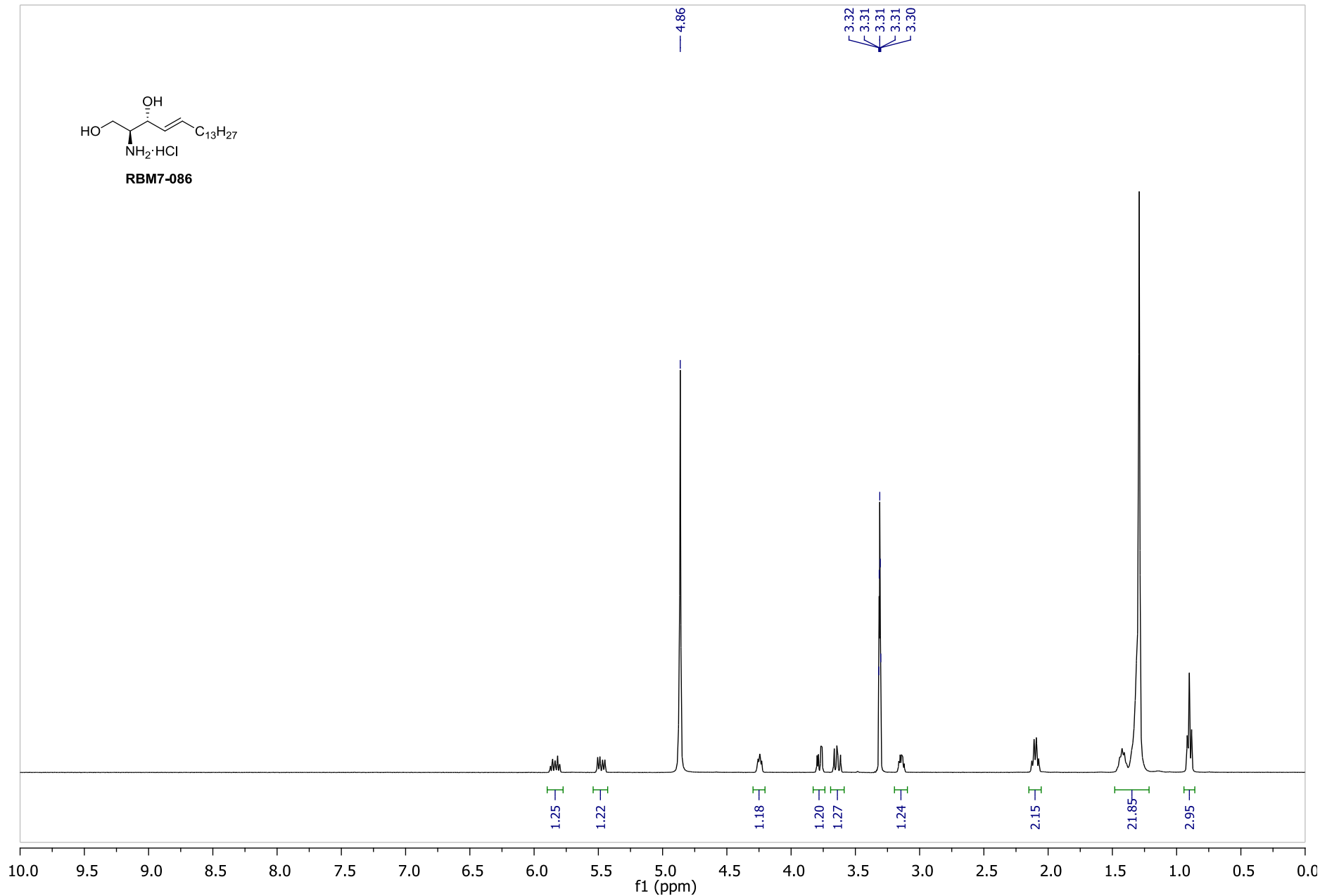
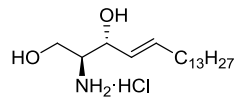
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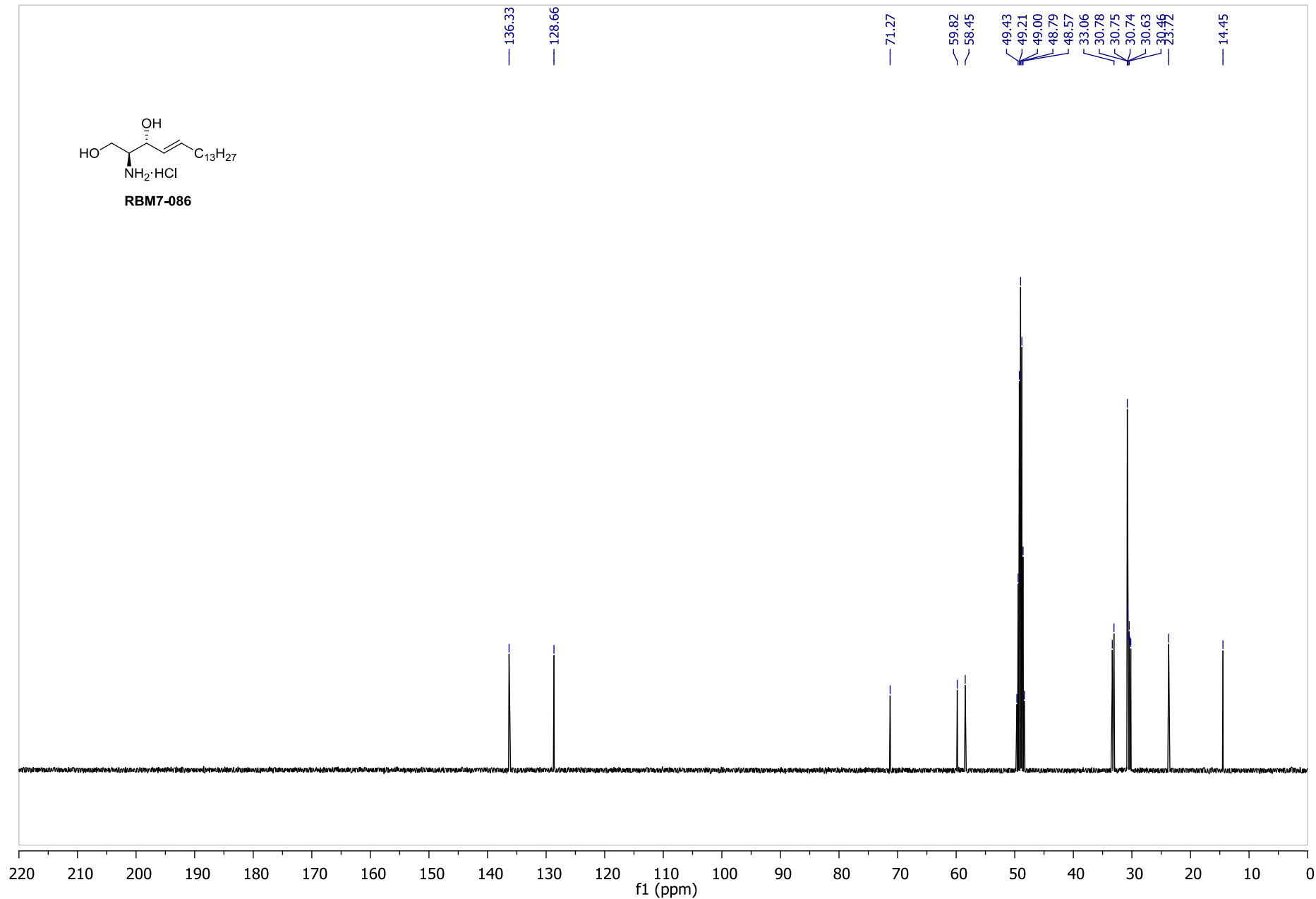
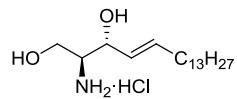
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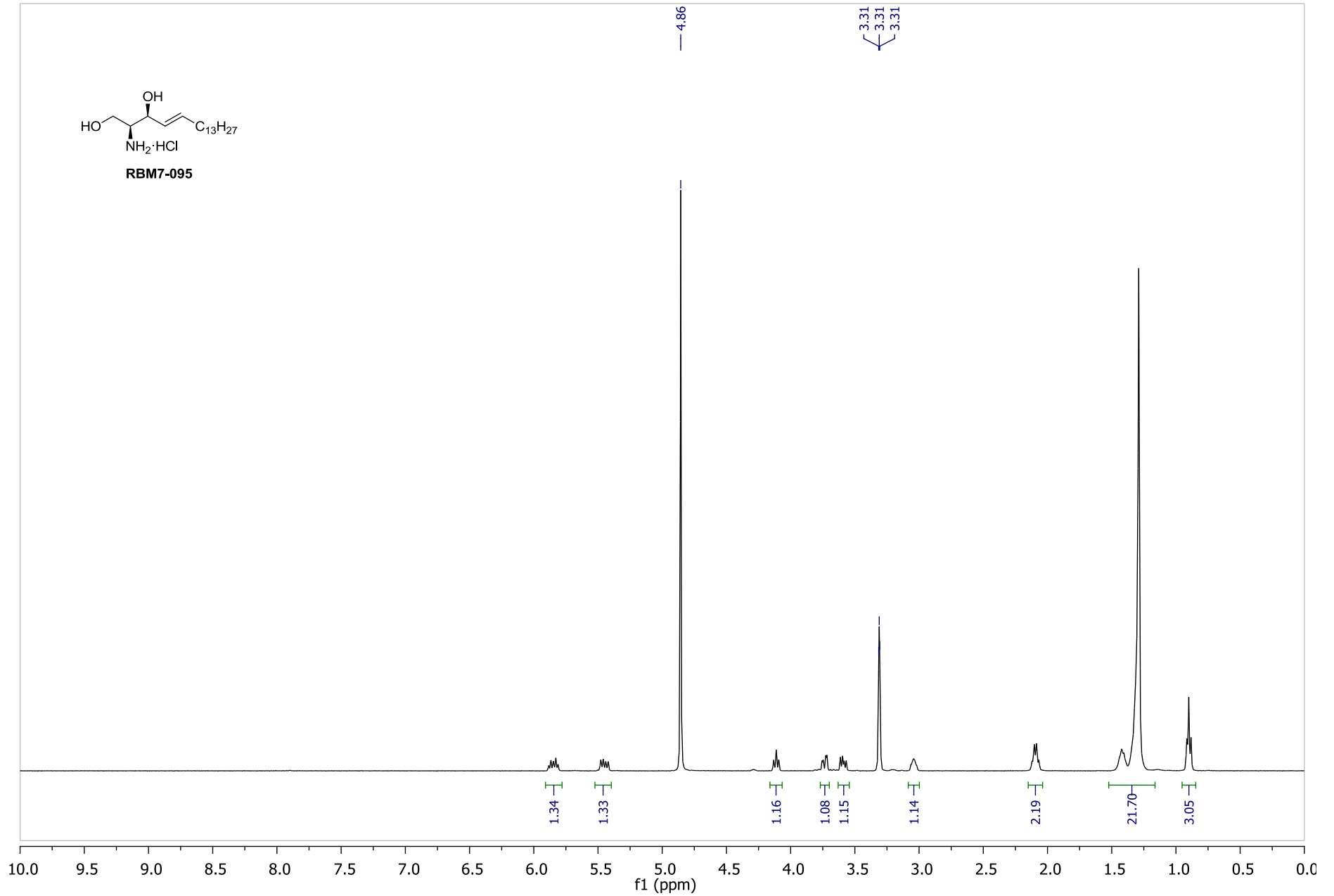
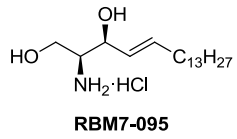
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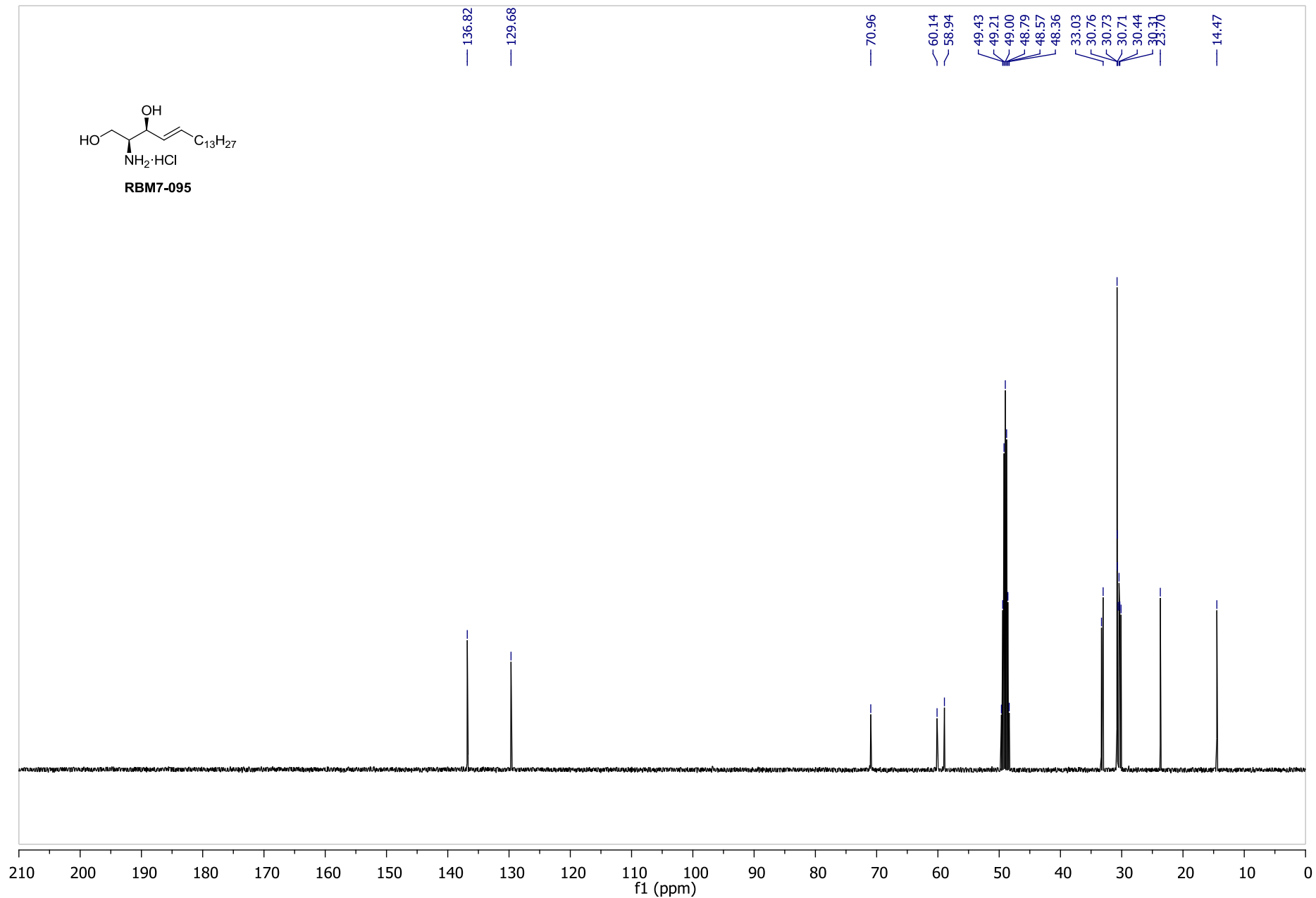
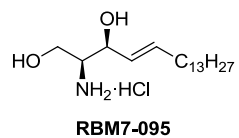
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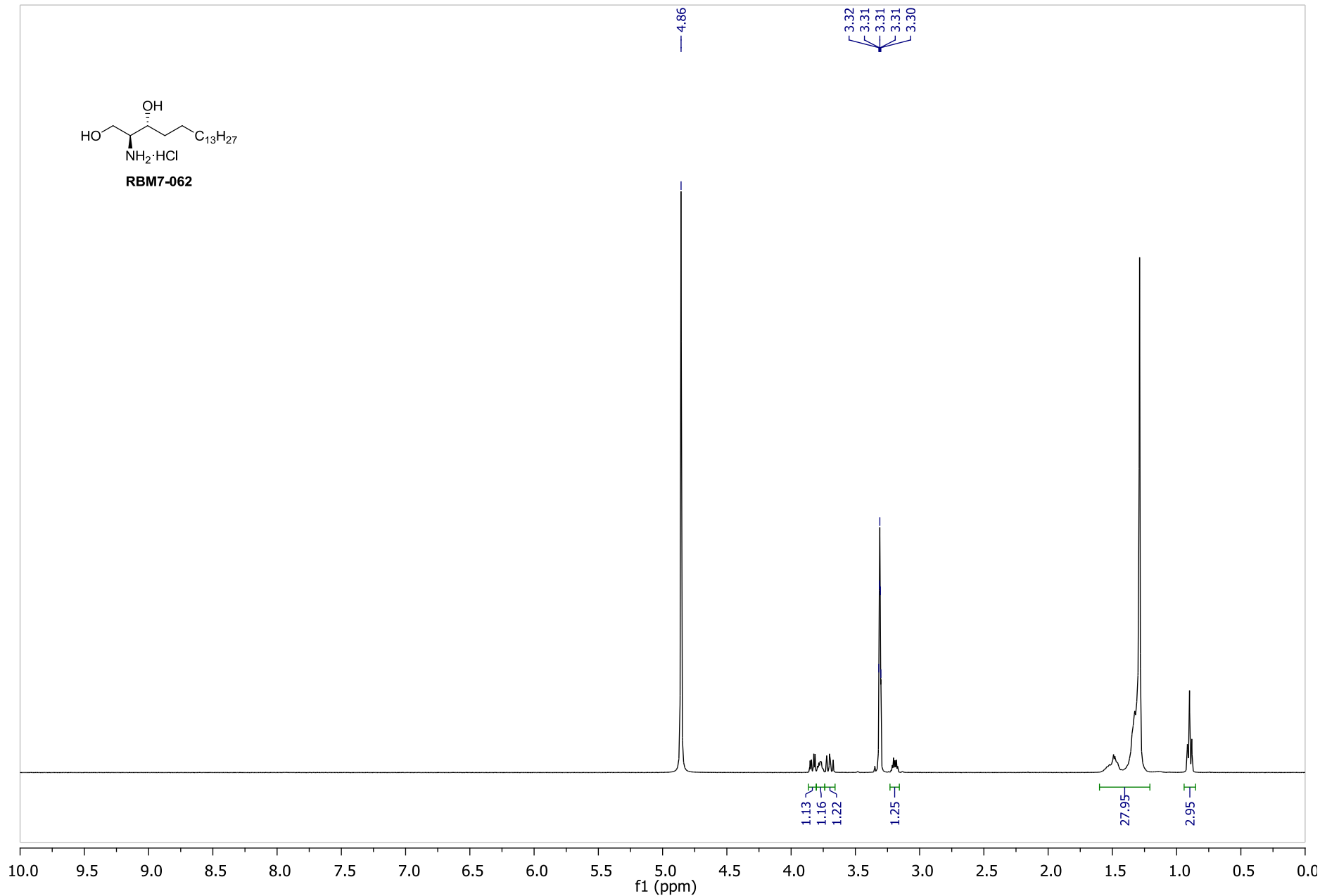
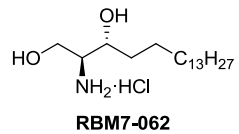
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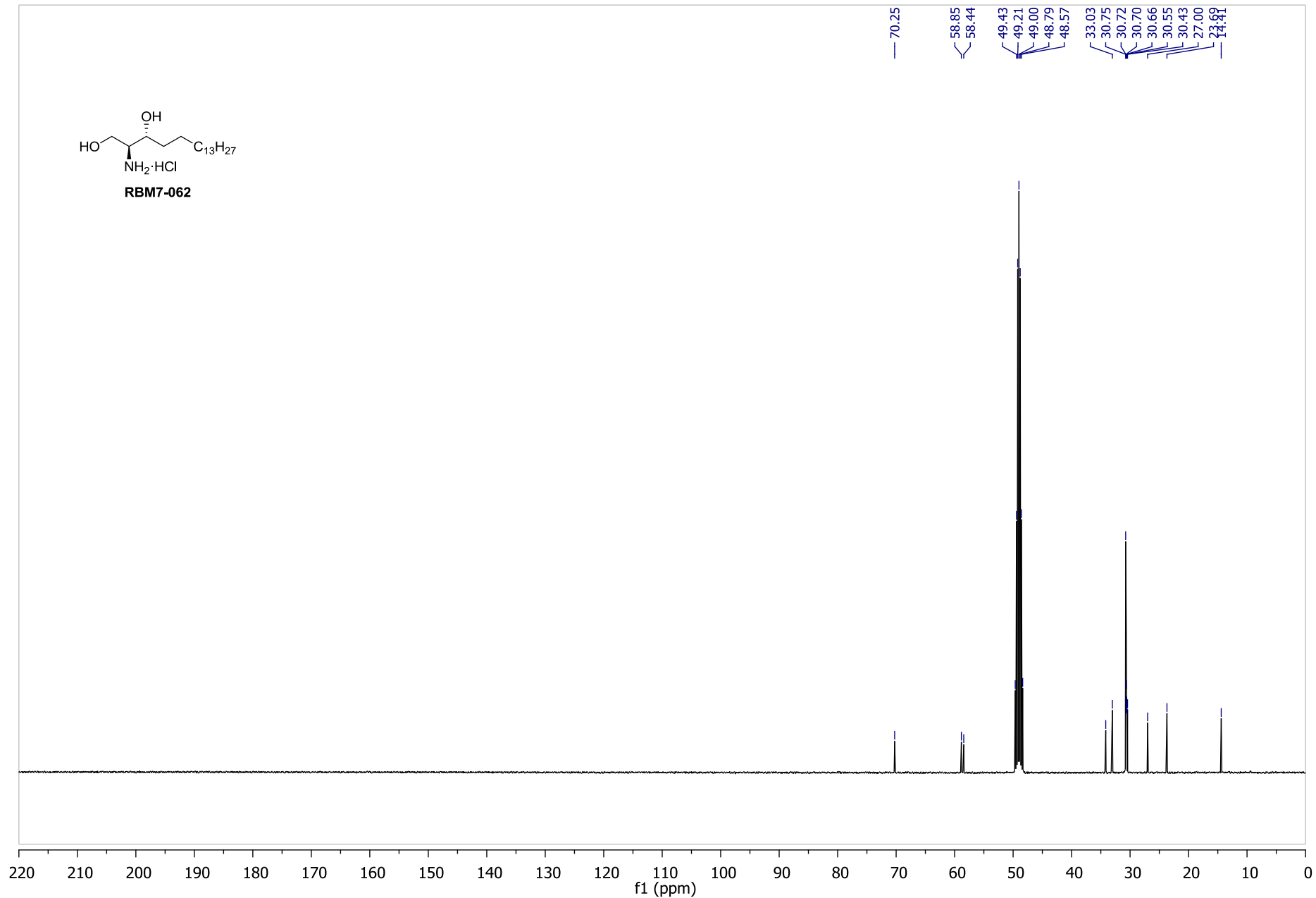
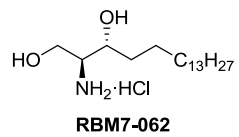
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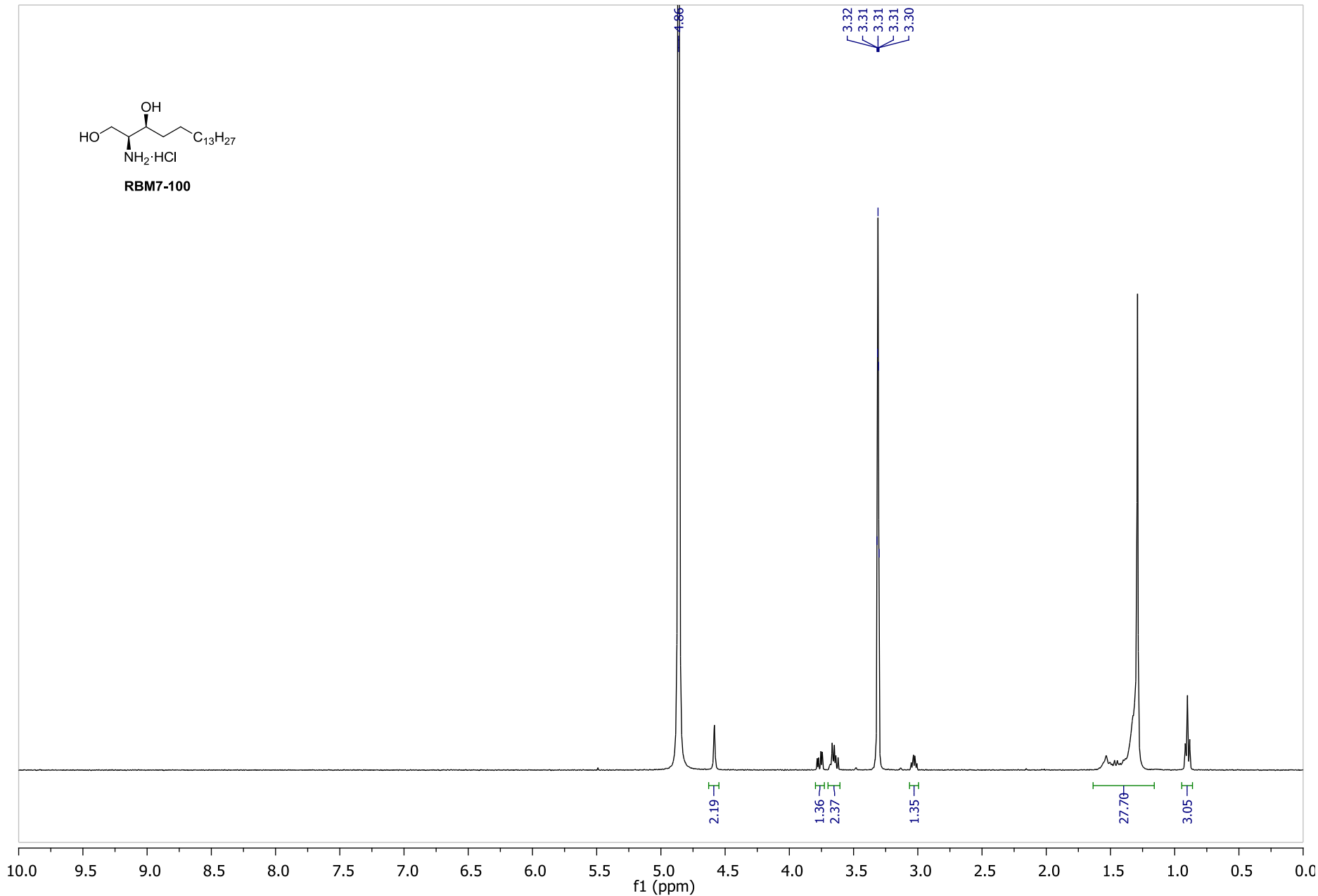
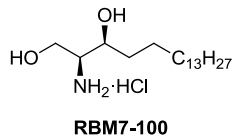
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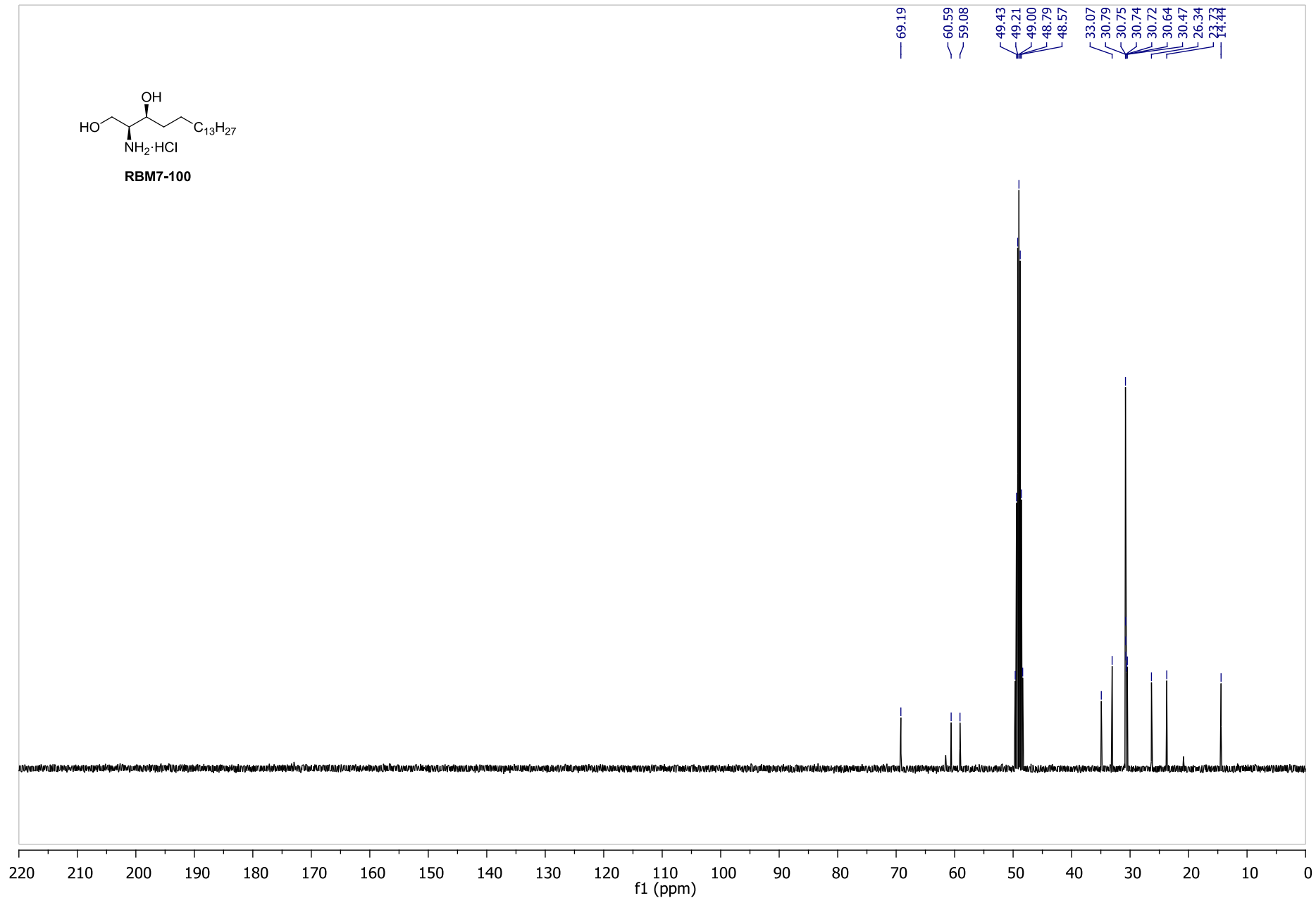
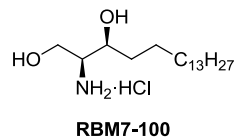
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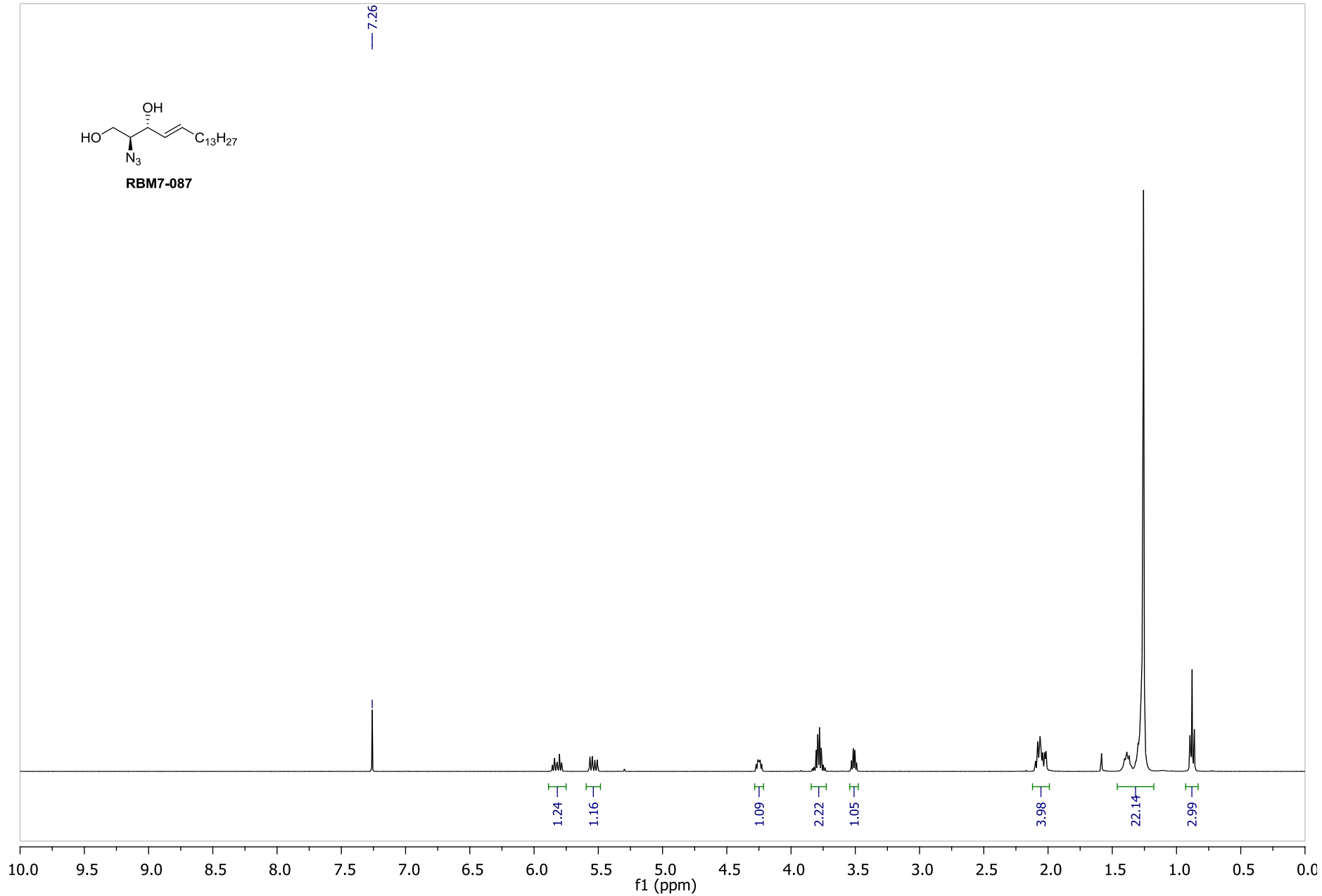
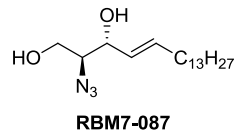
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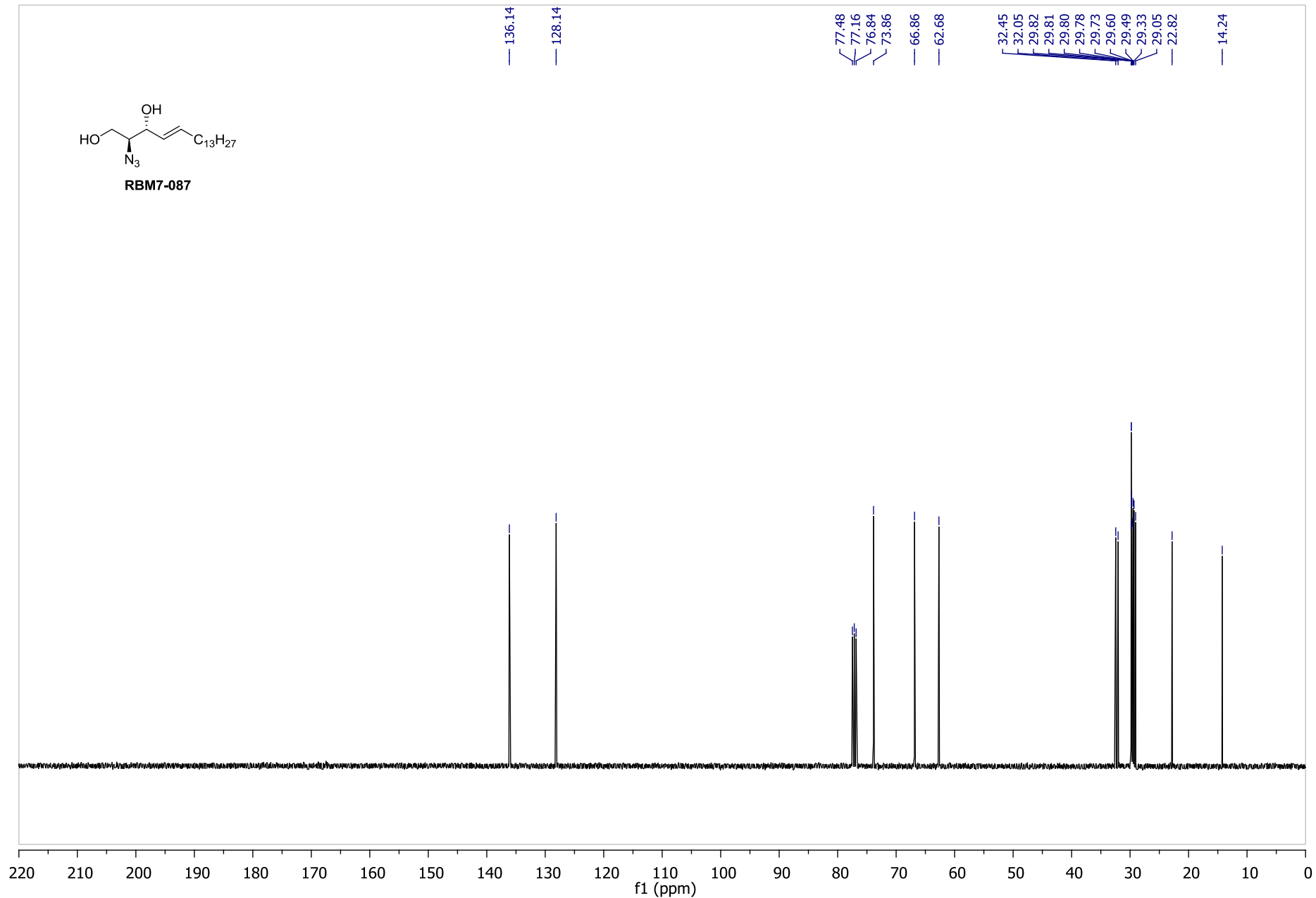
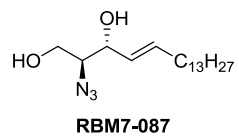
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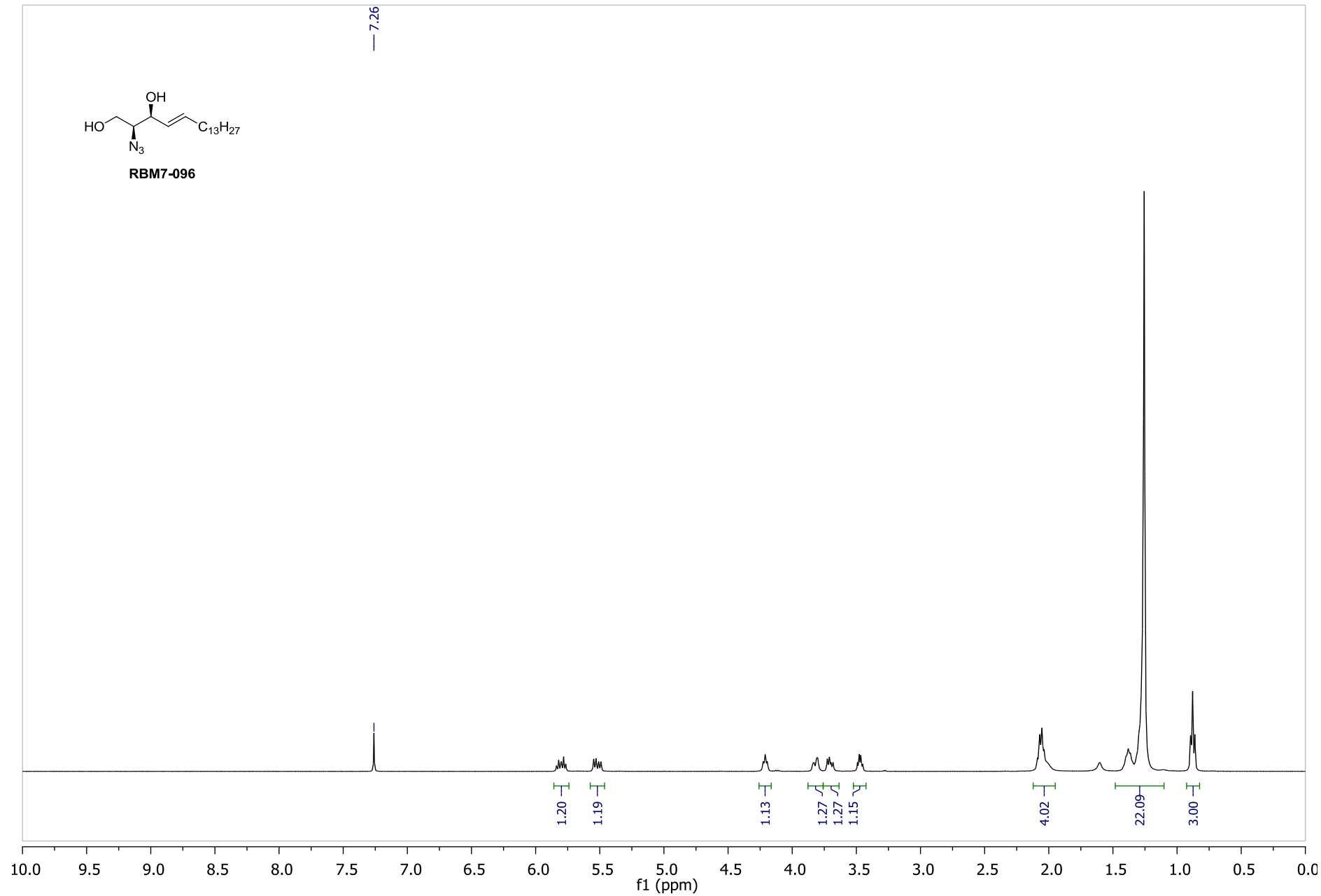
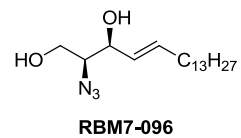
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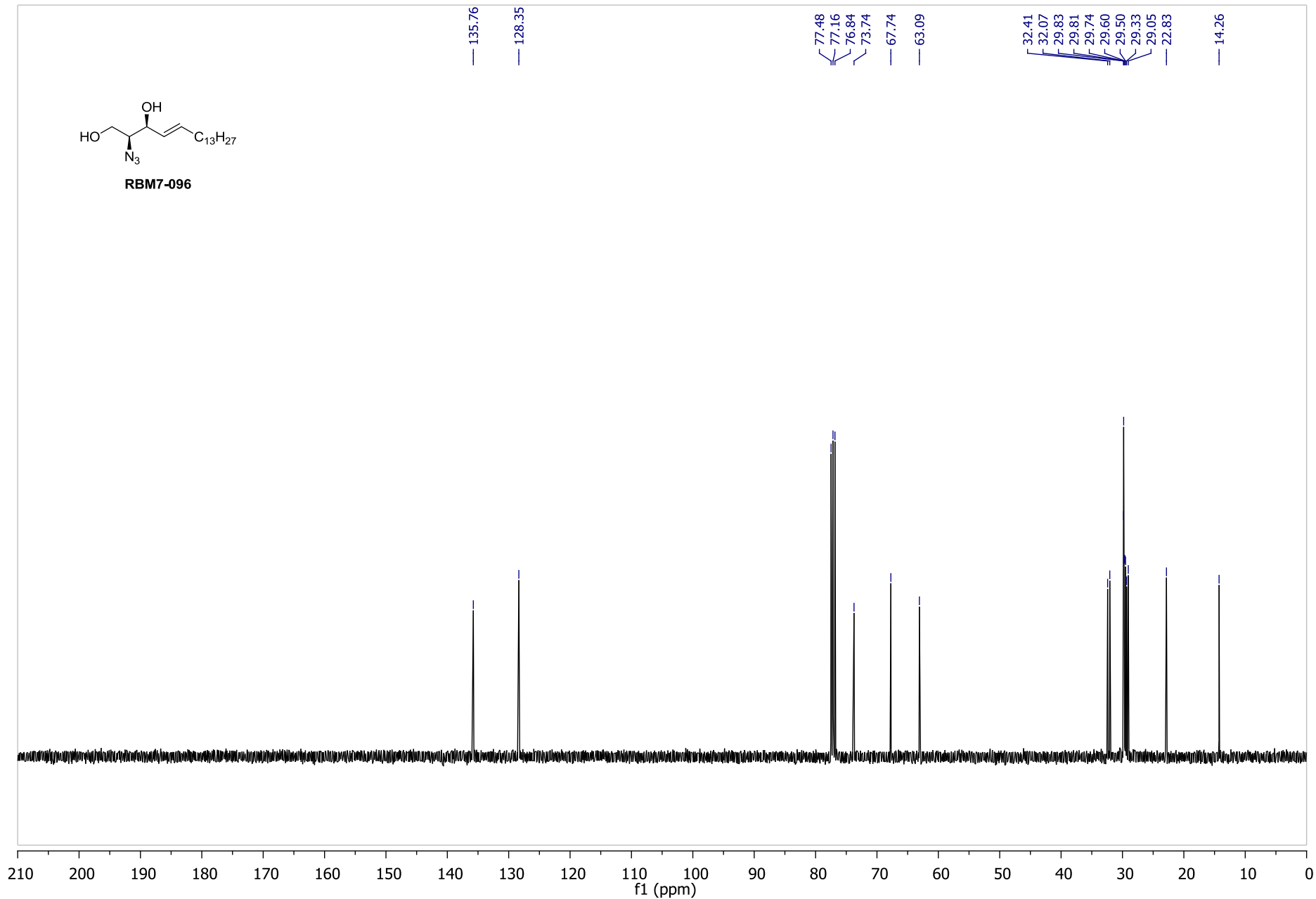
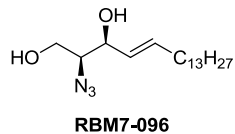
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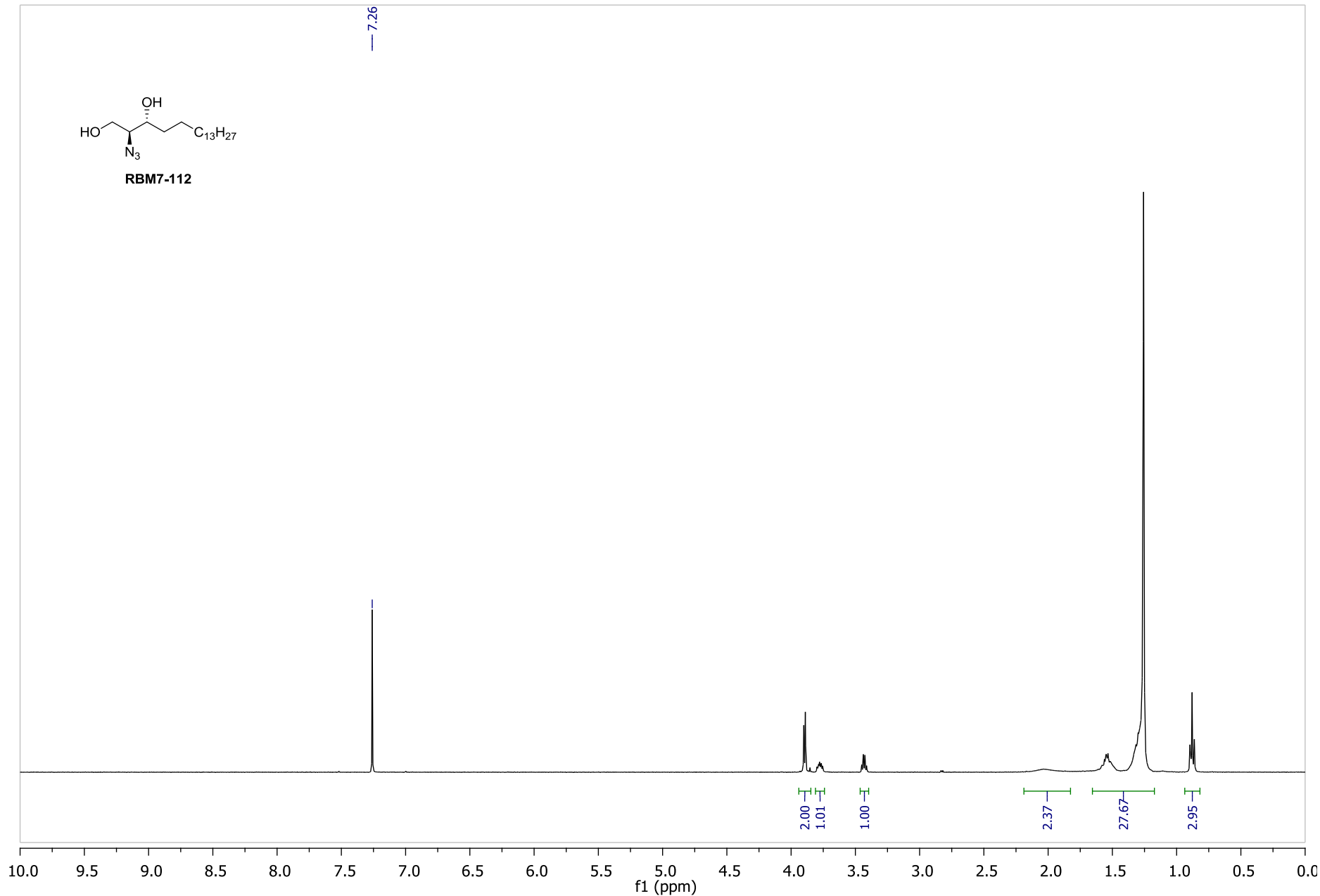
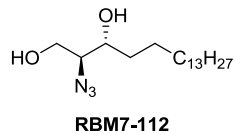
S105



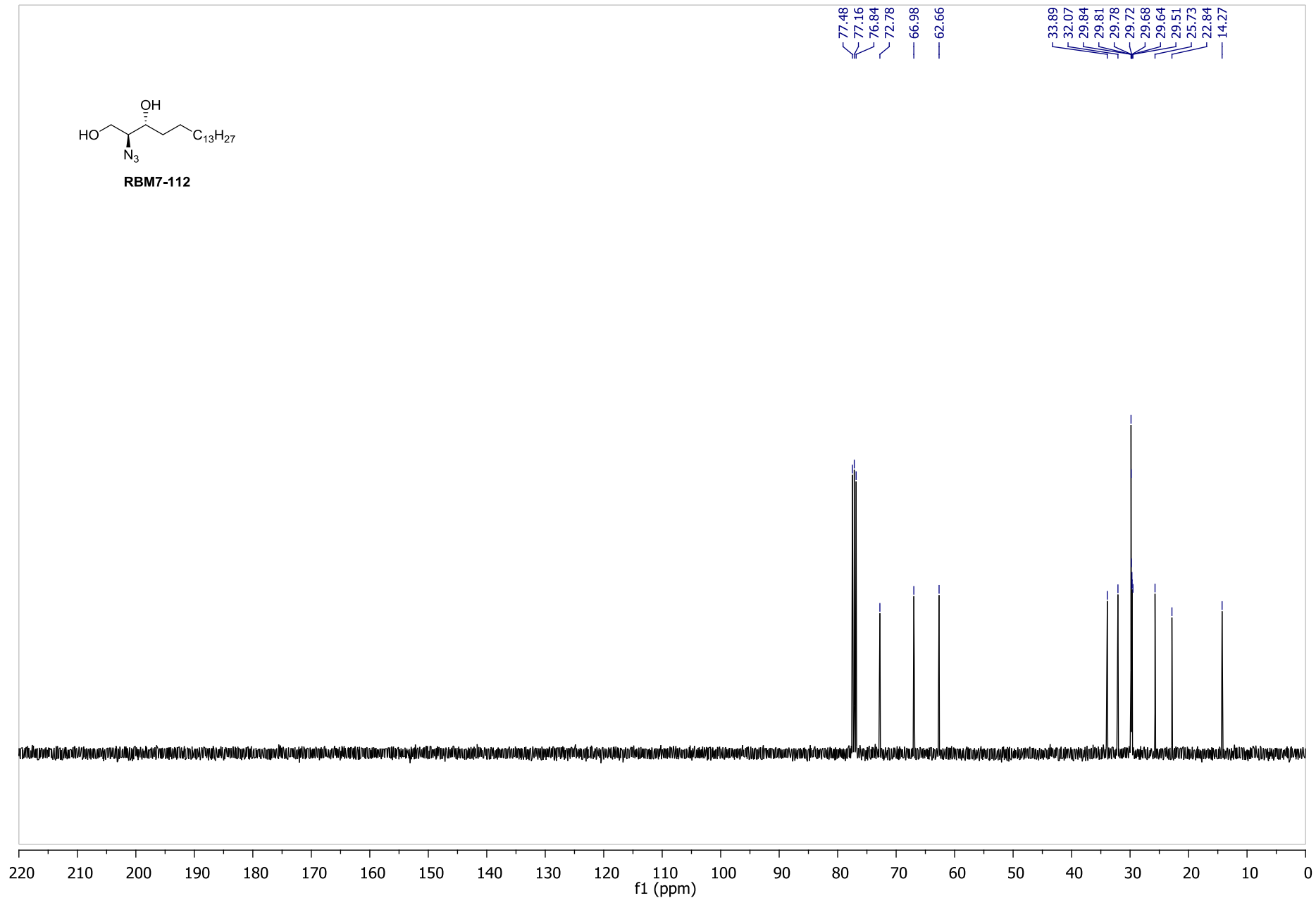
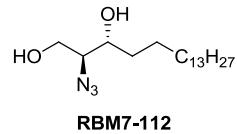
S106



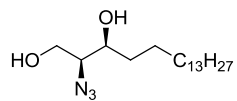
S107



S108

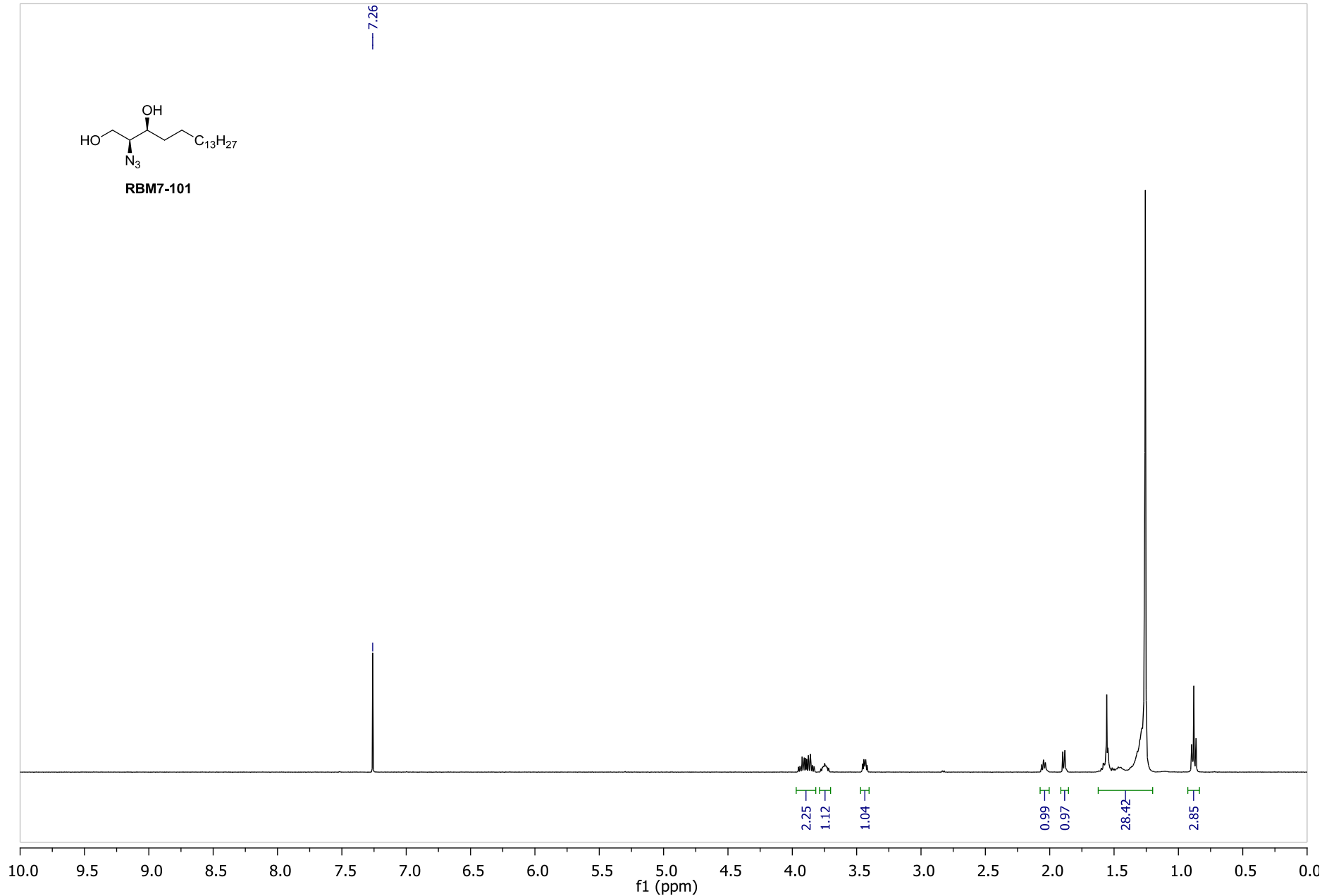


S109

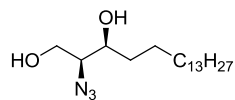


RBM7-101

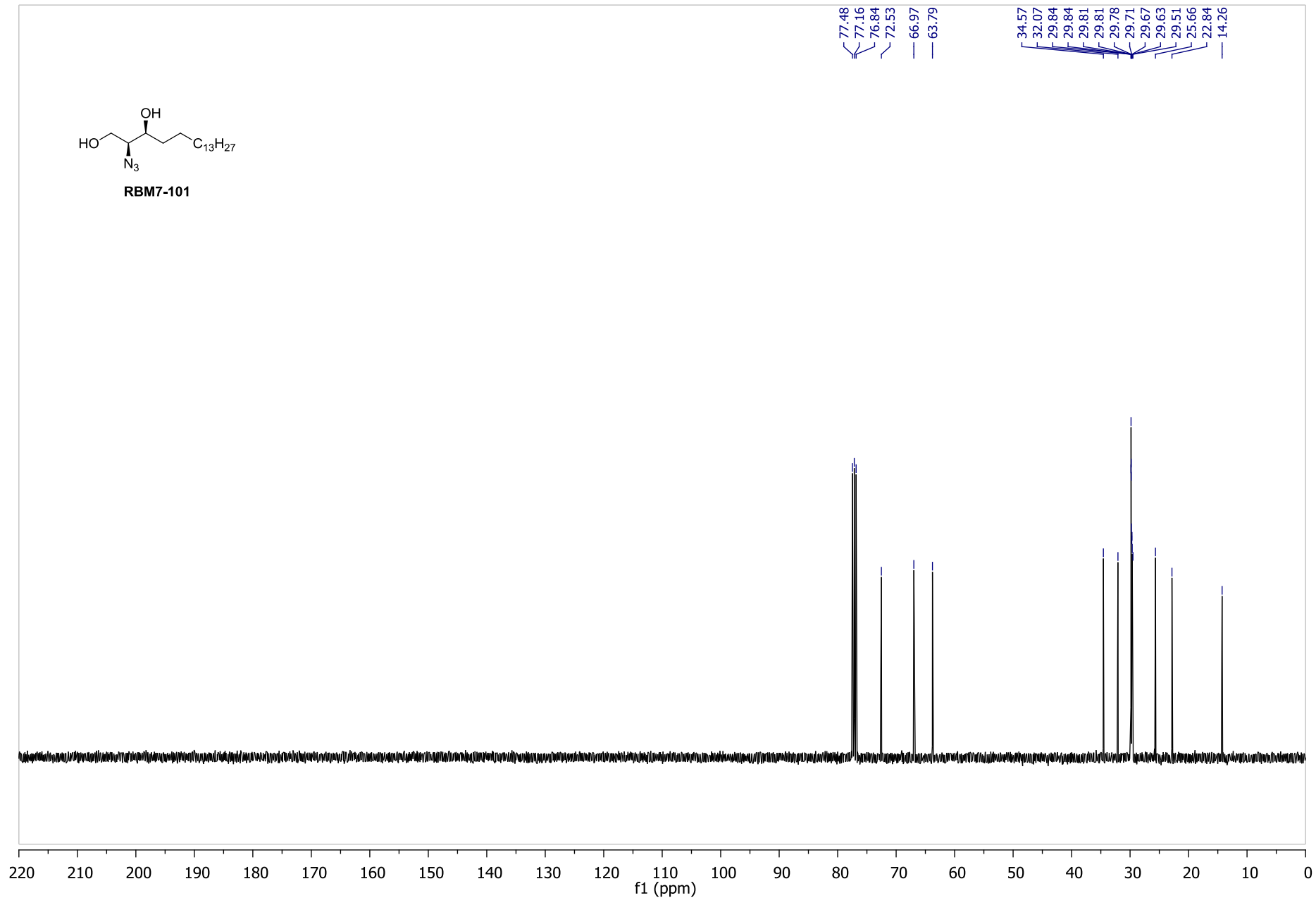
7.26

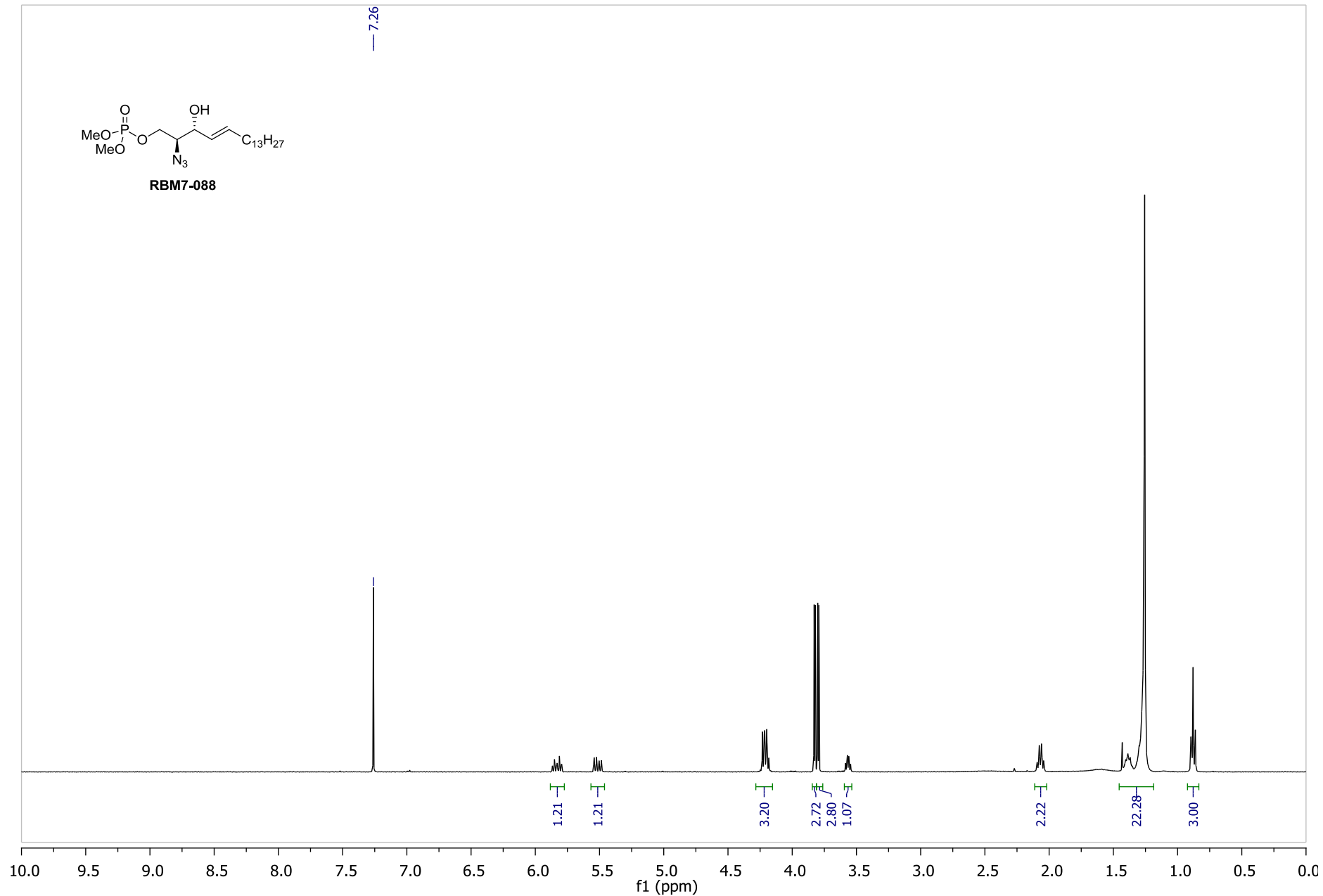
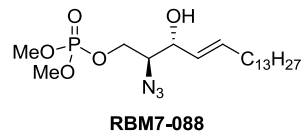


S110

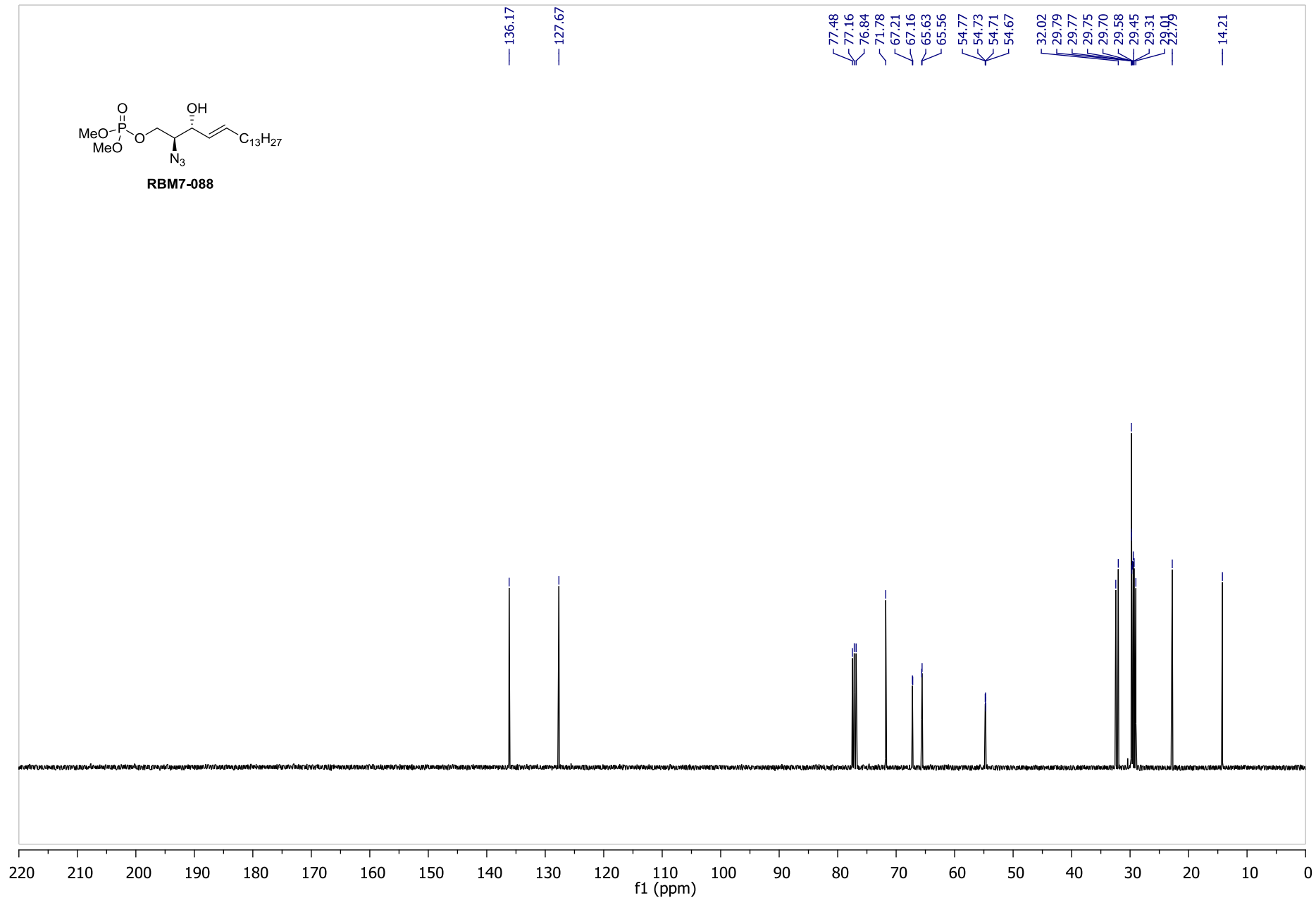
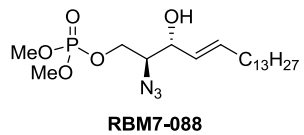


RBM7-101

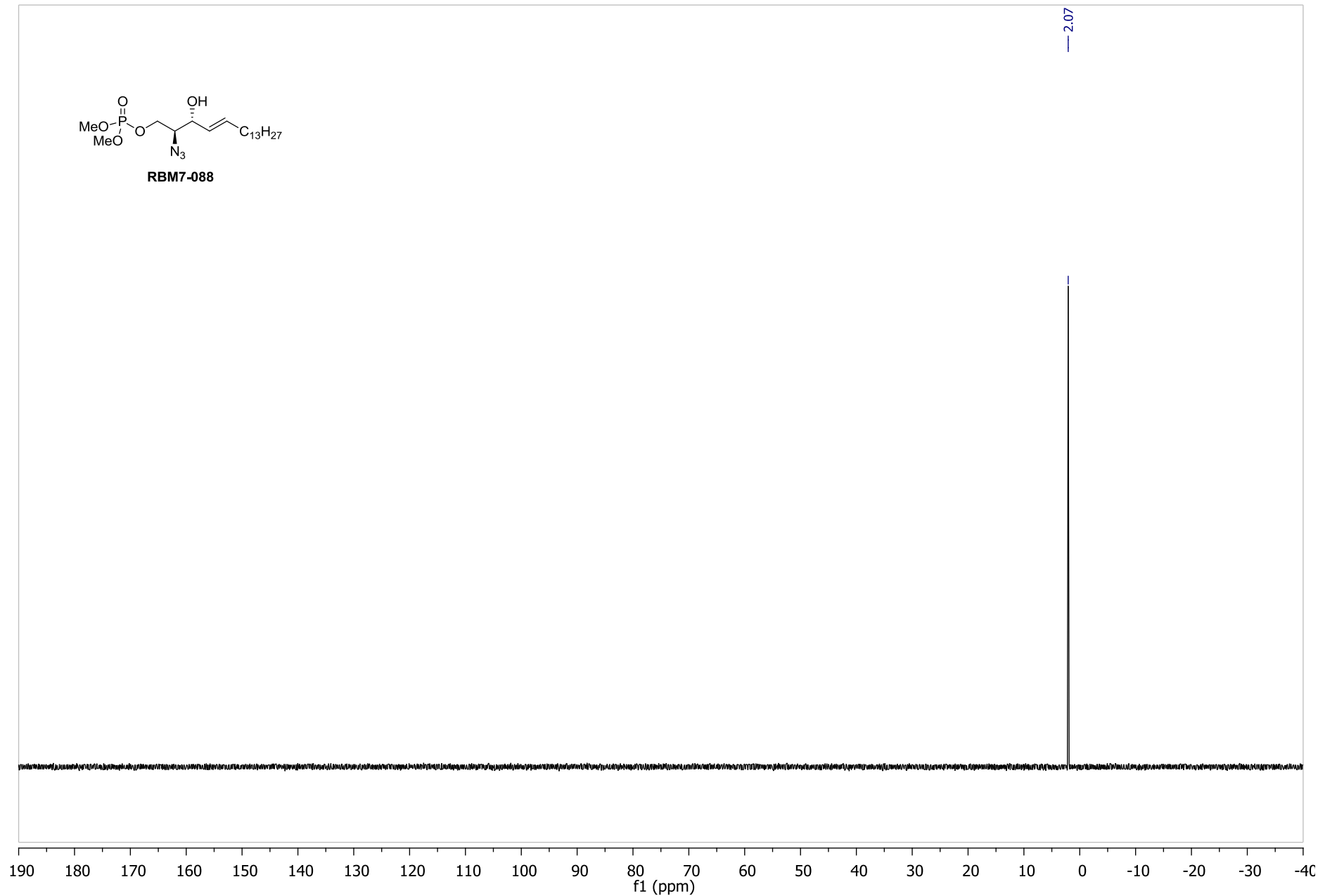
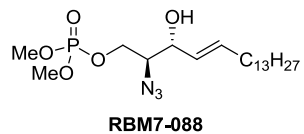




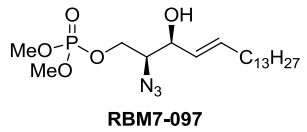
S112



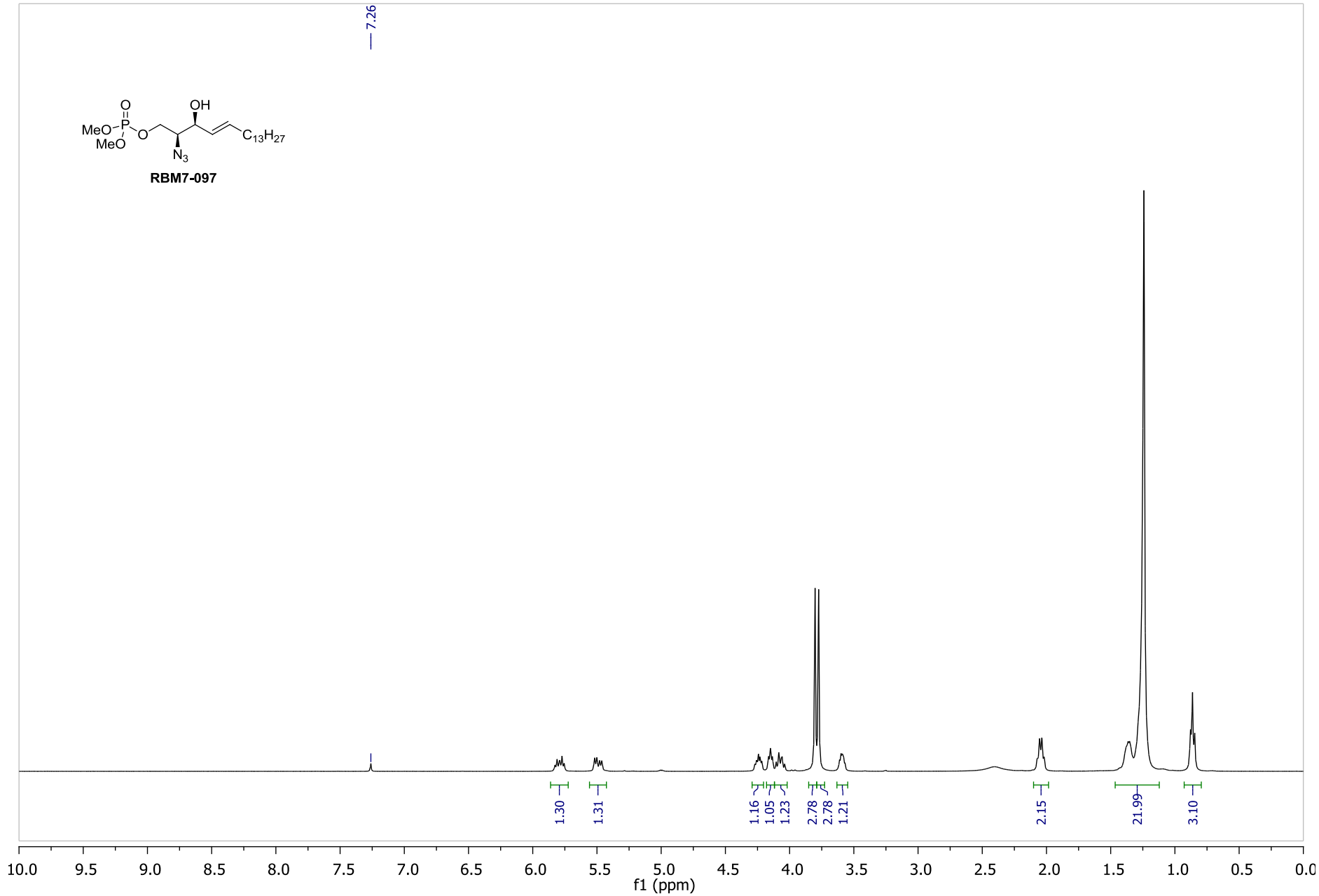
S113



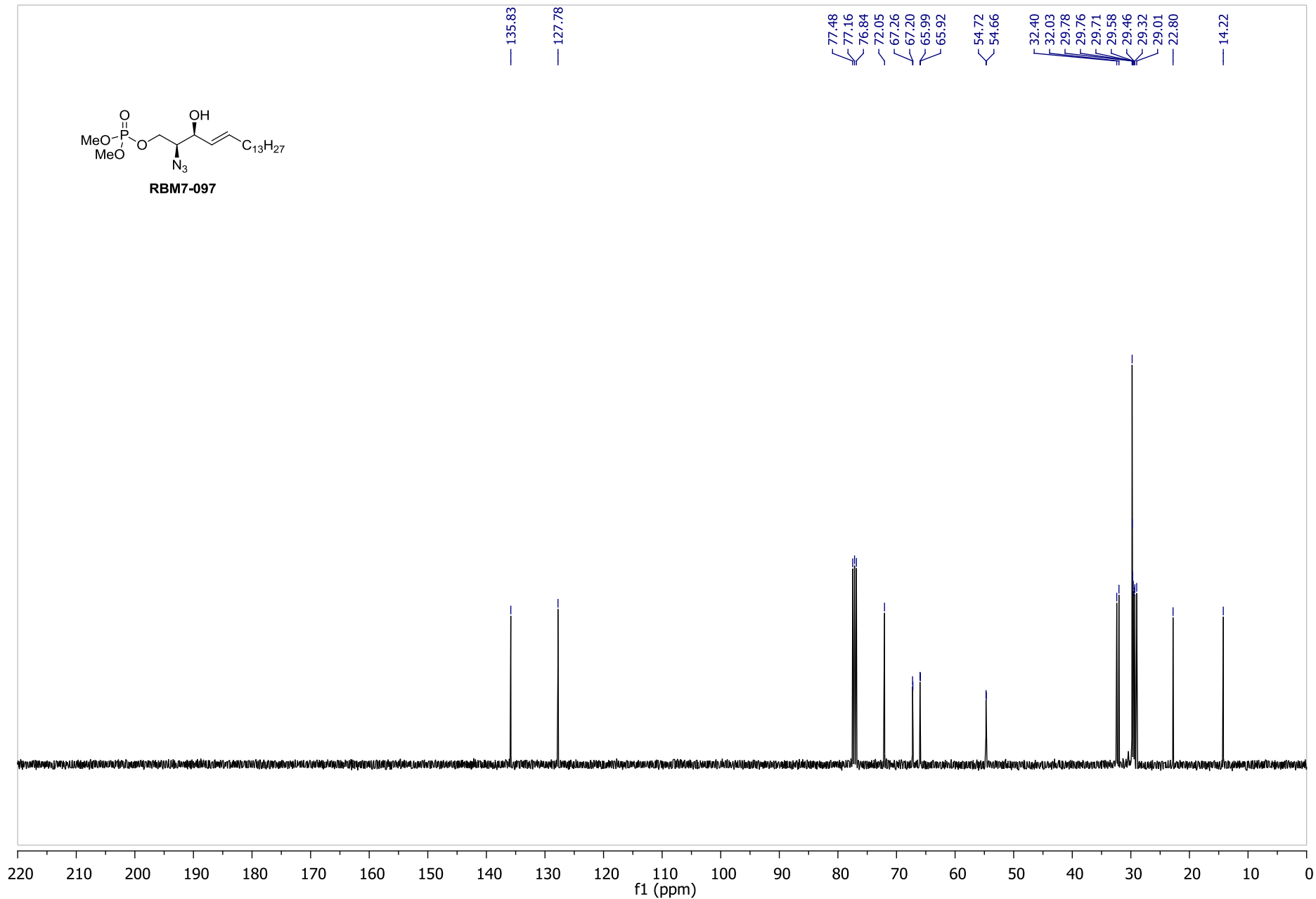
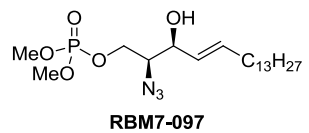
S114



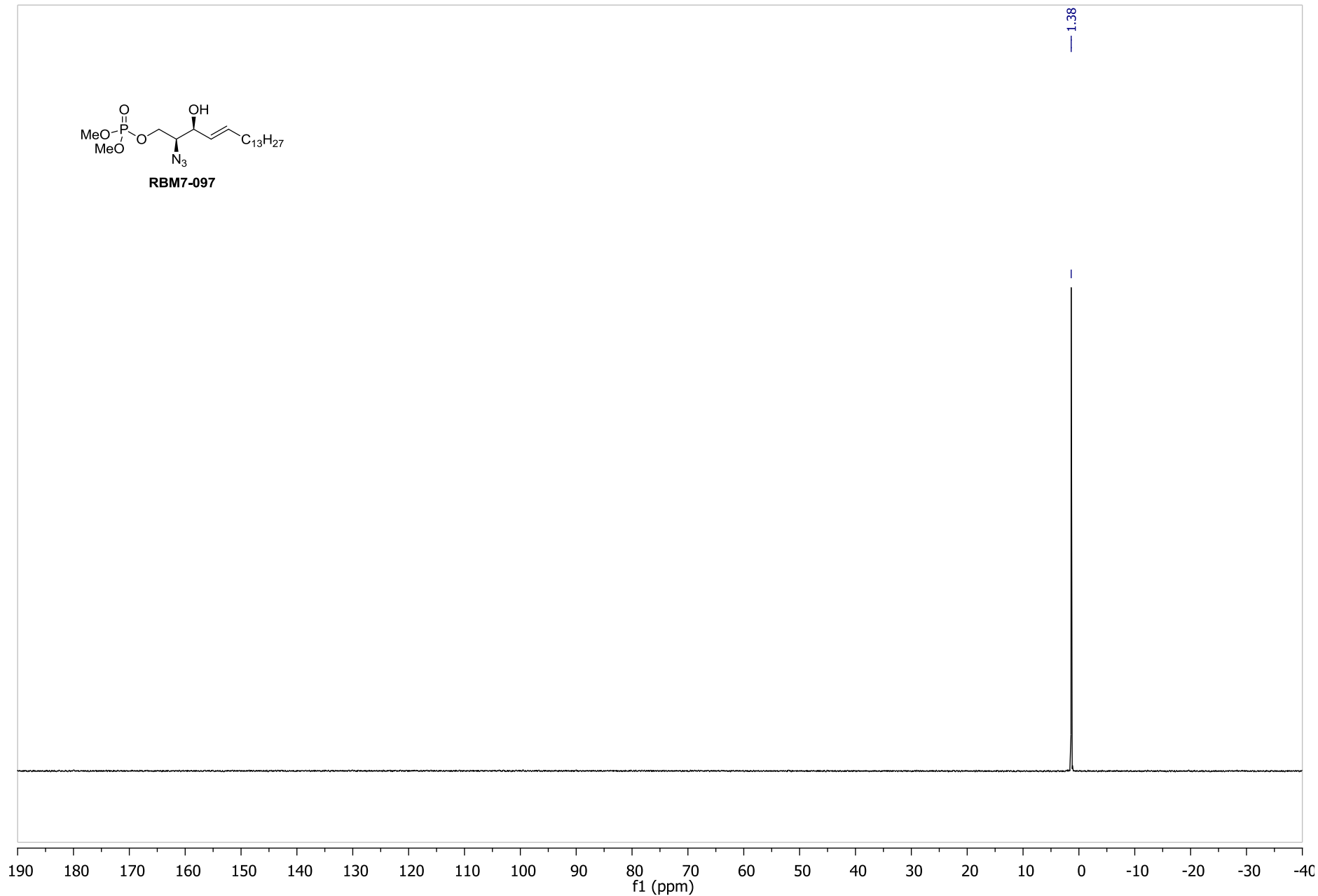
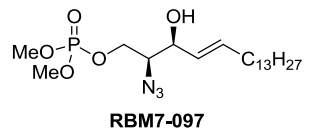
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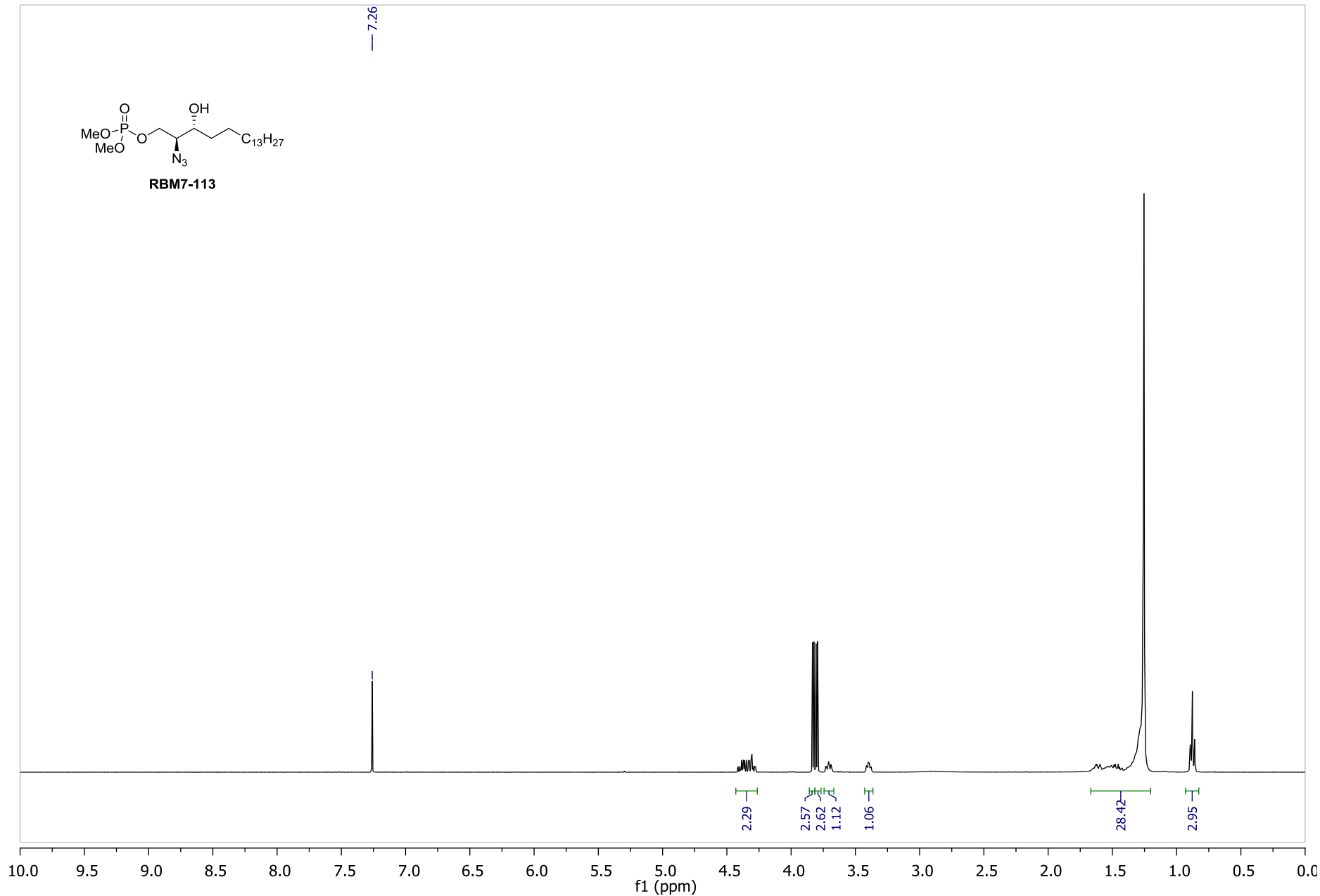
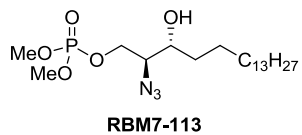
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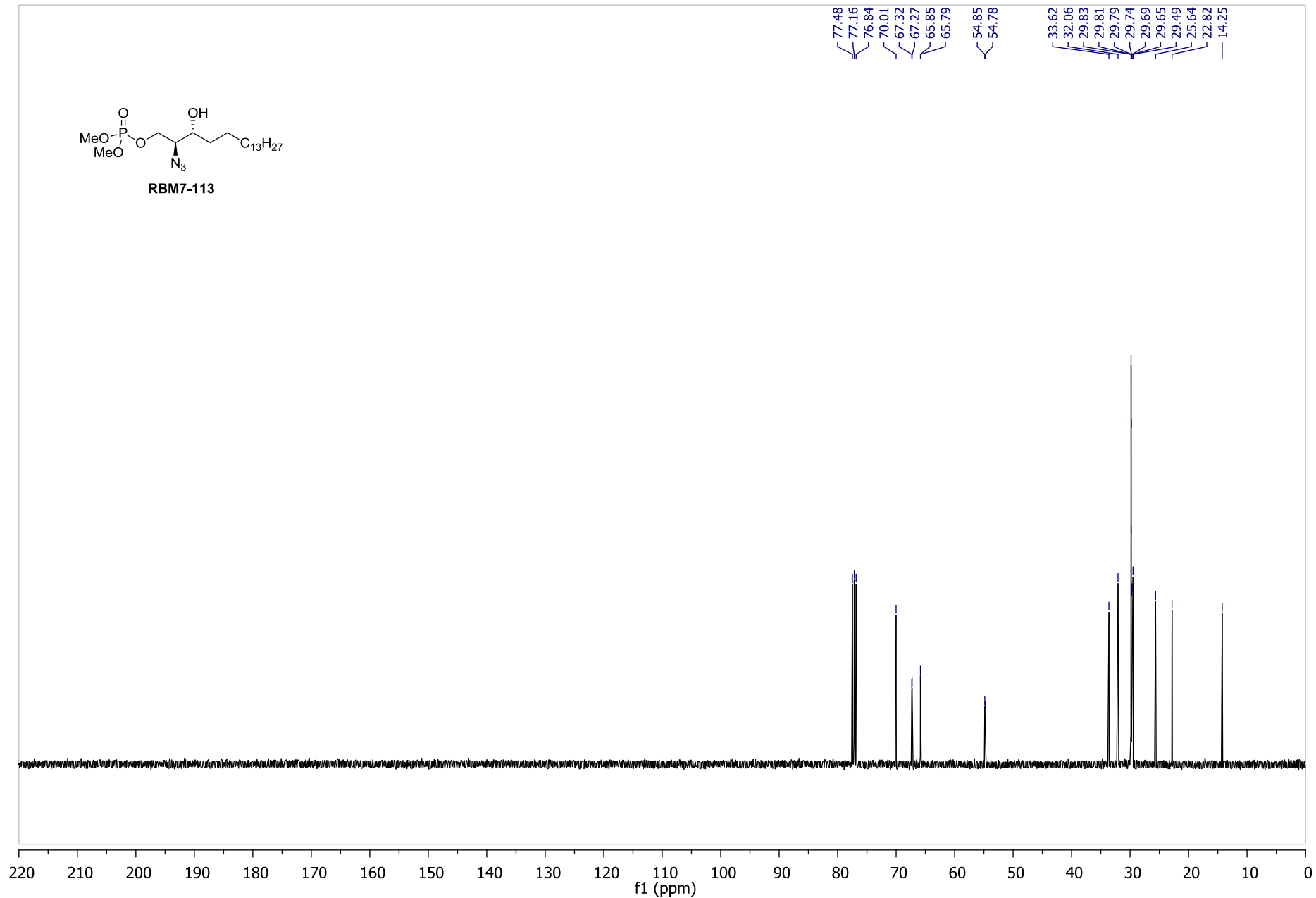
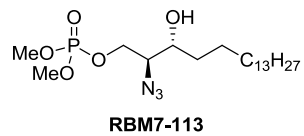
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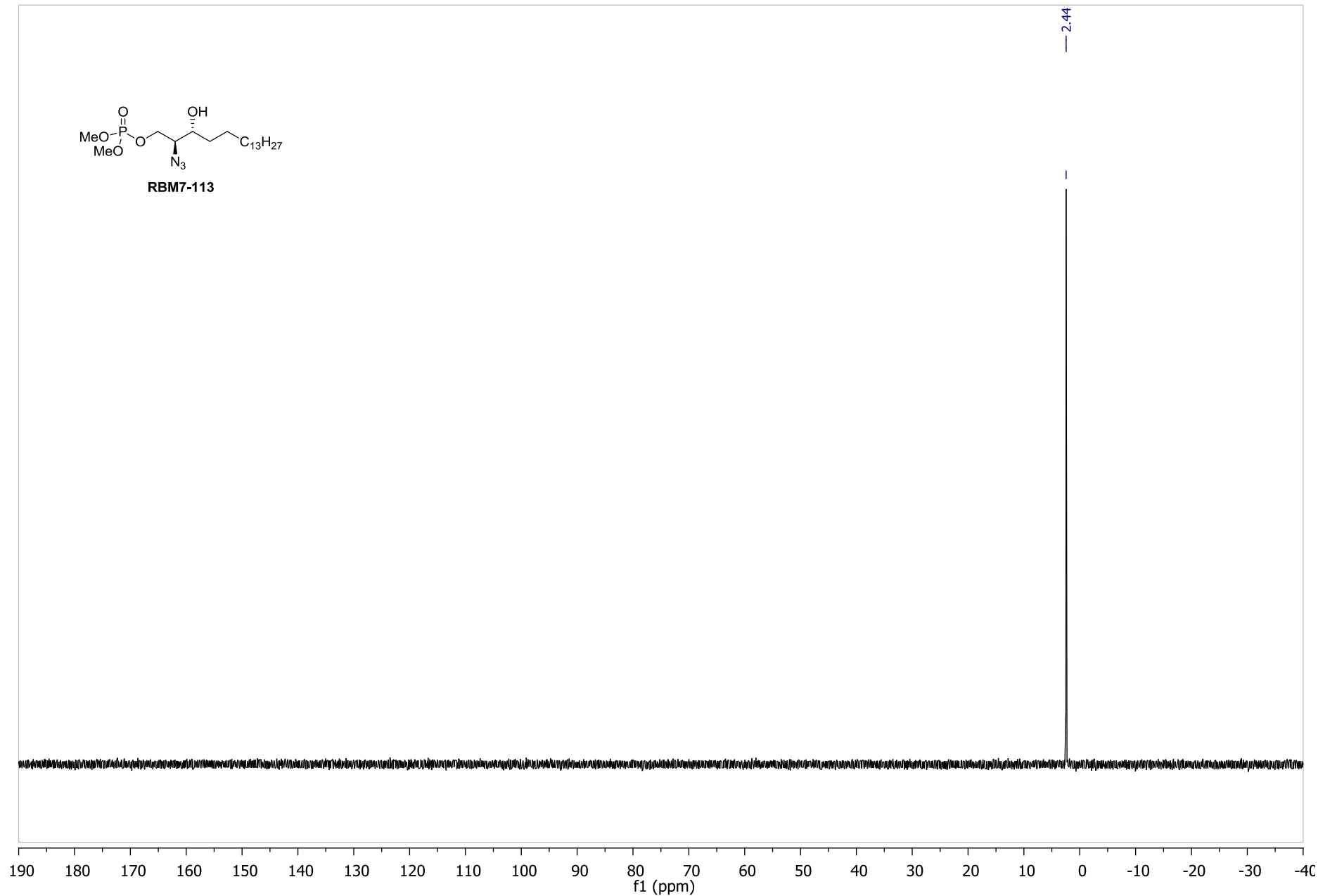
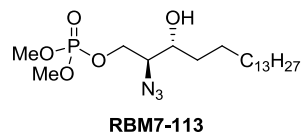
S117



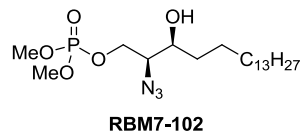
S118



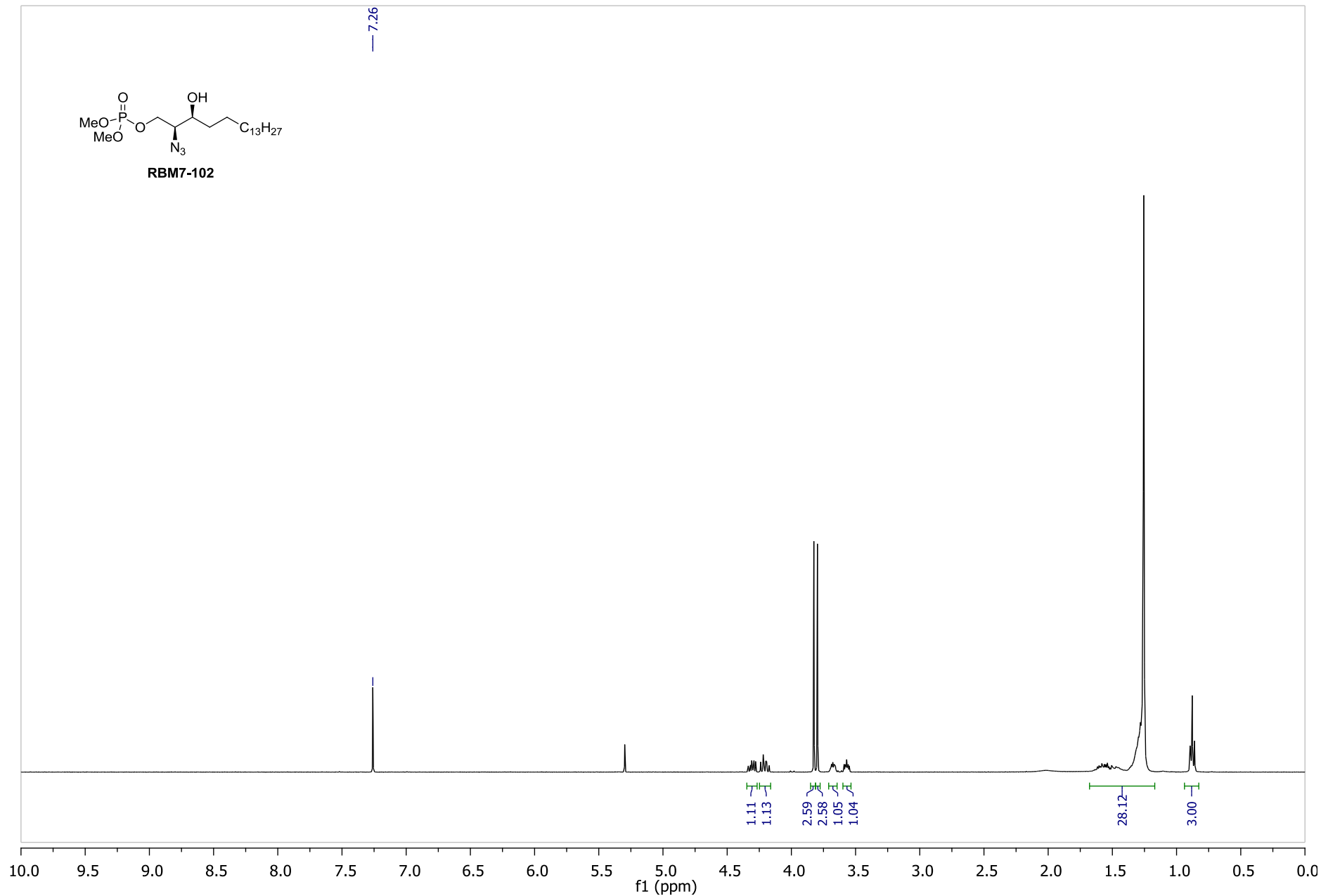
S119



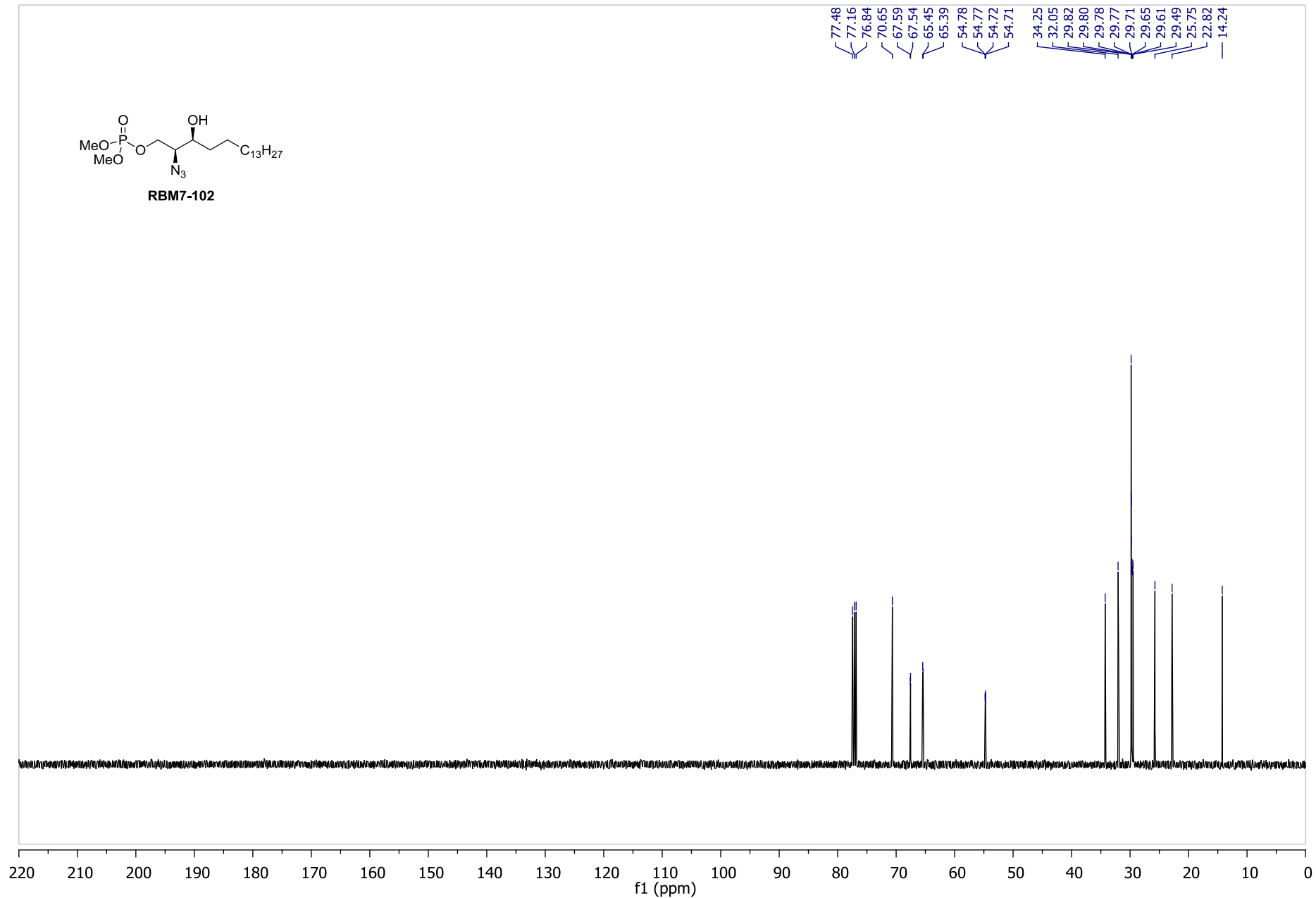
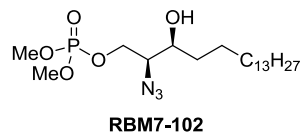
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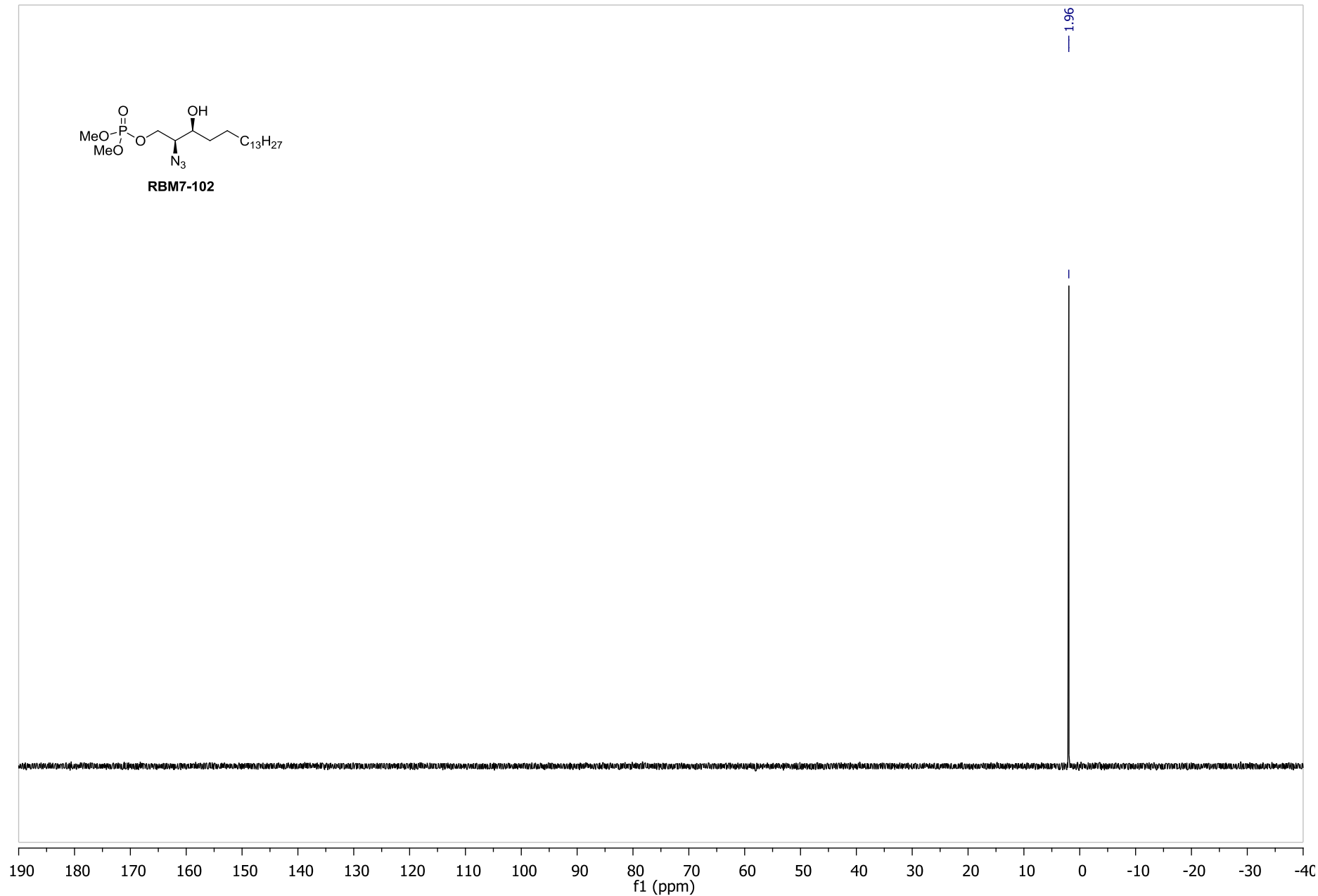
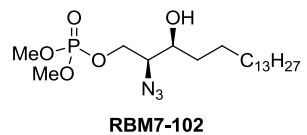
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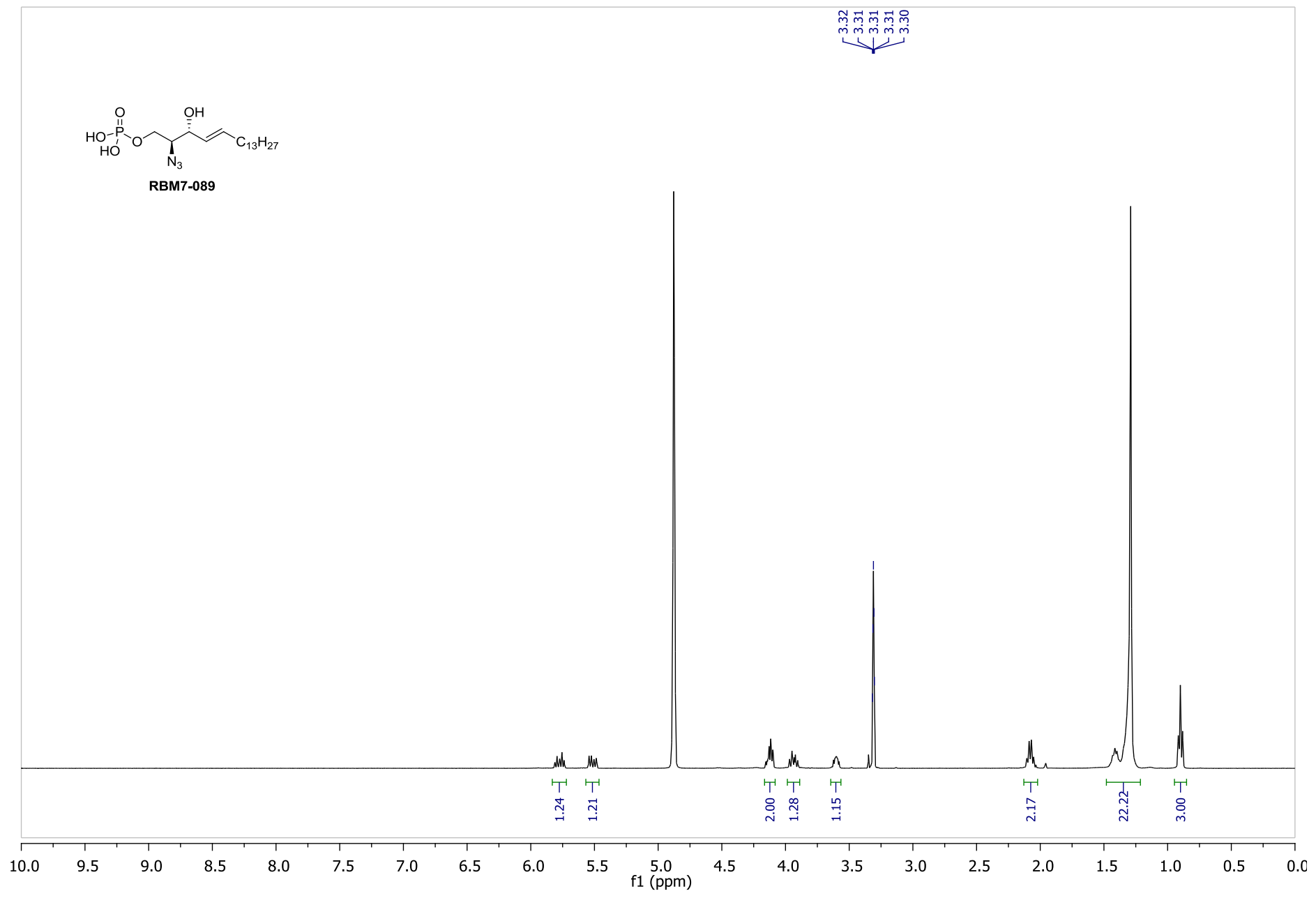
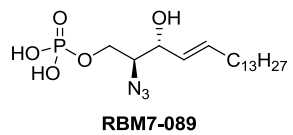
S121



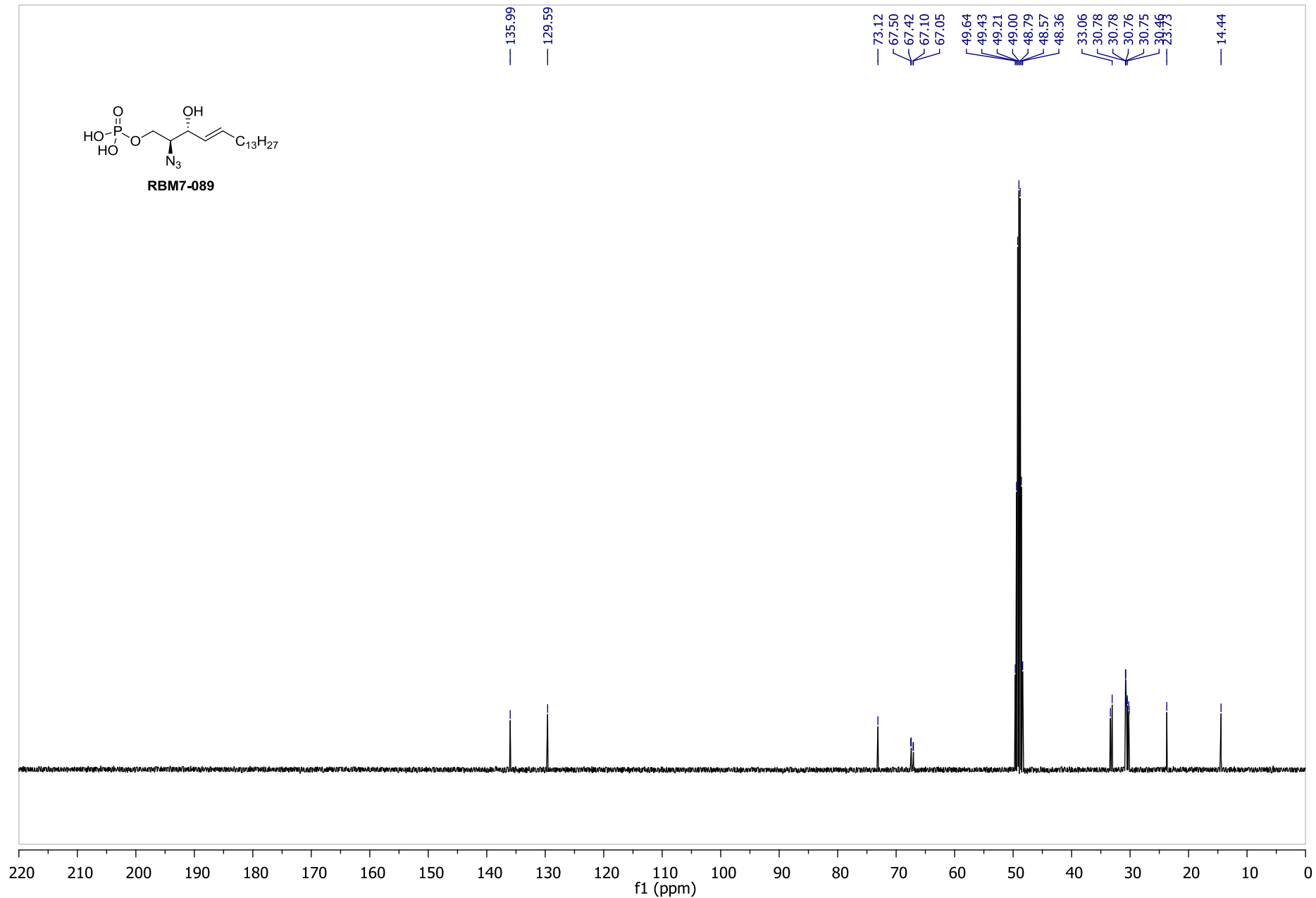
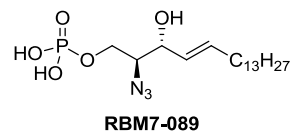
S122



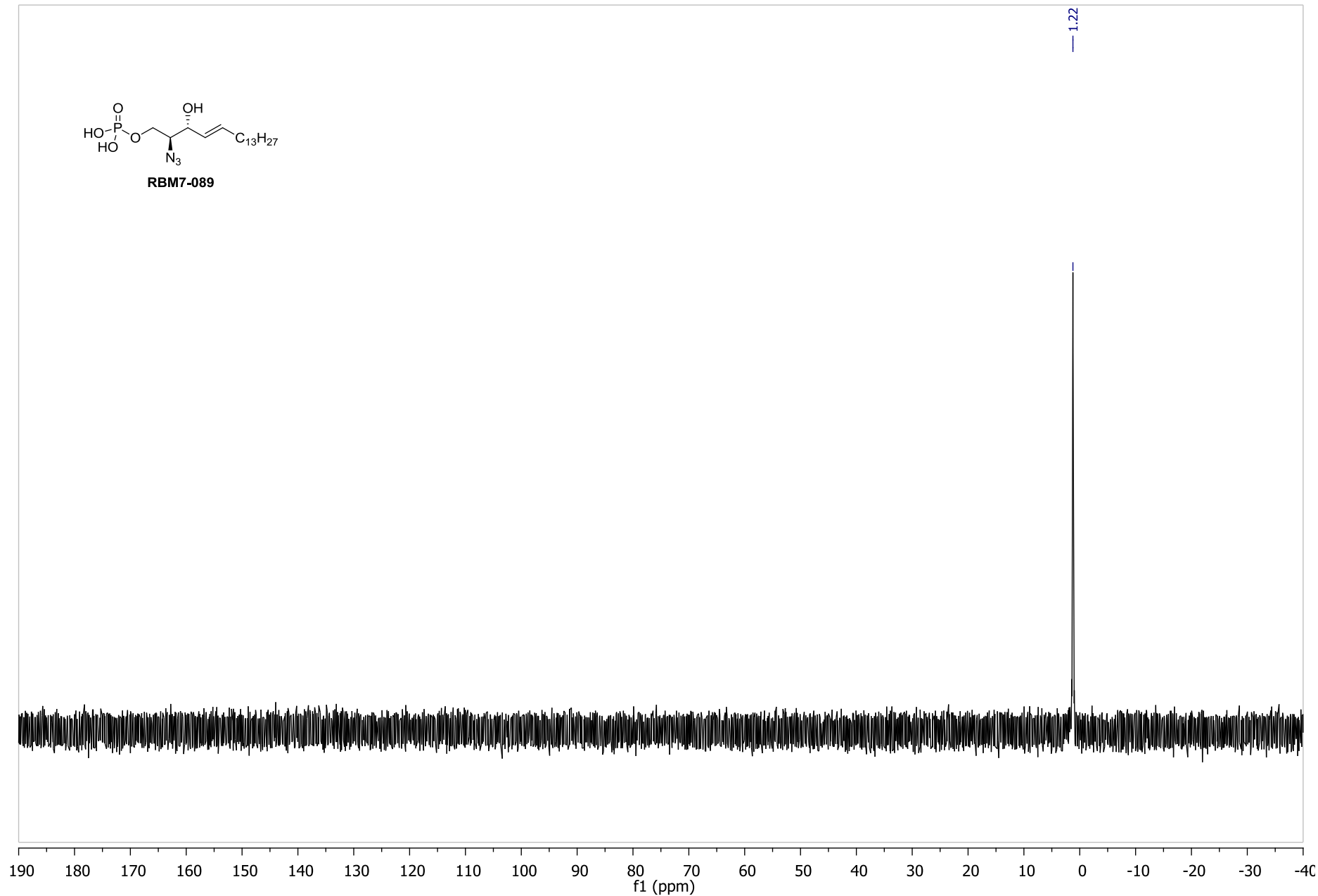
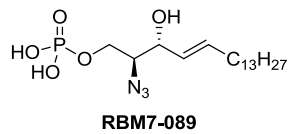
S123



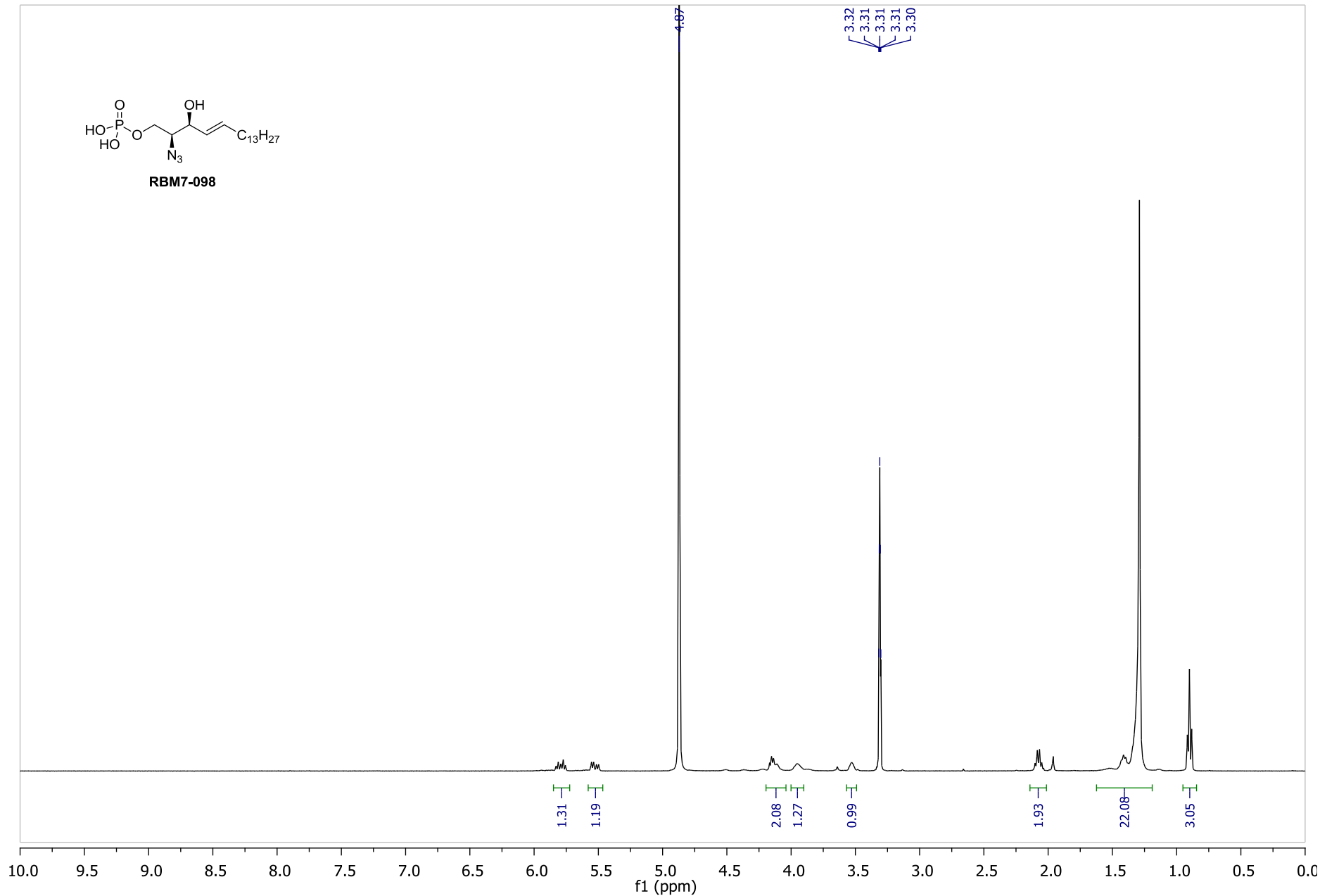
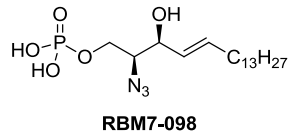
S124



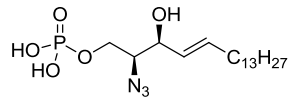
S125



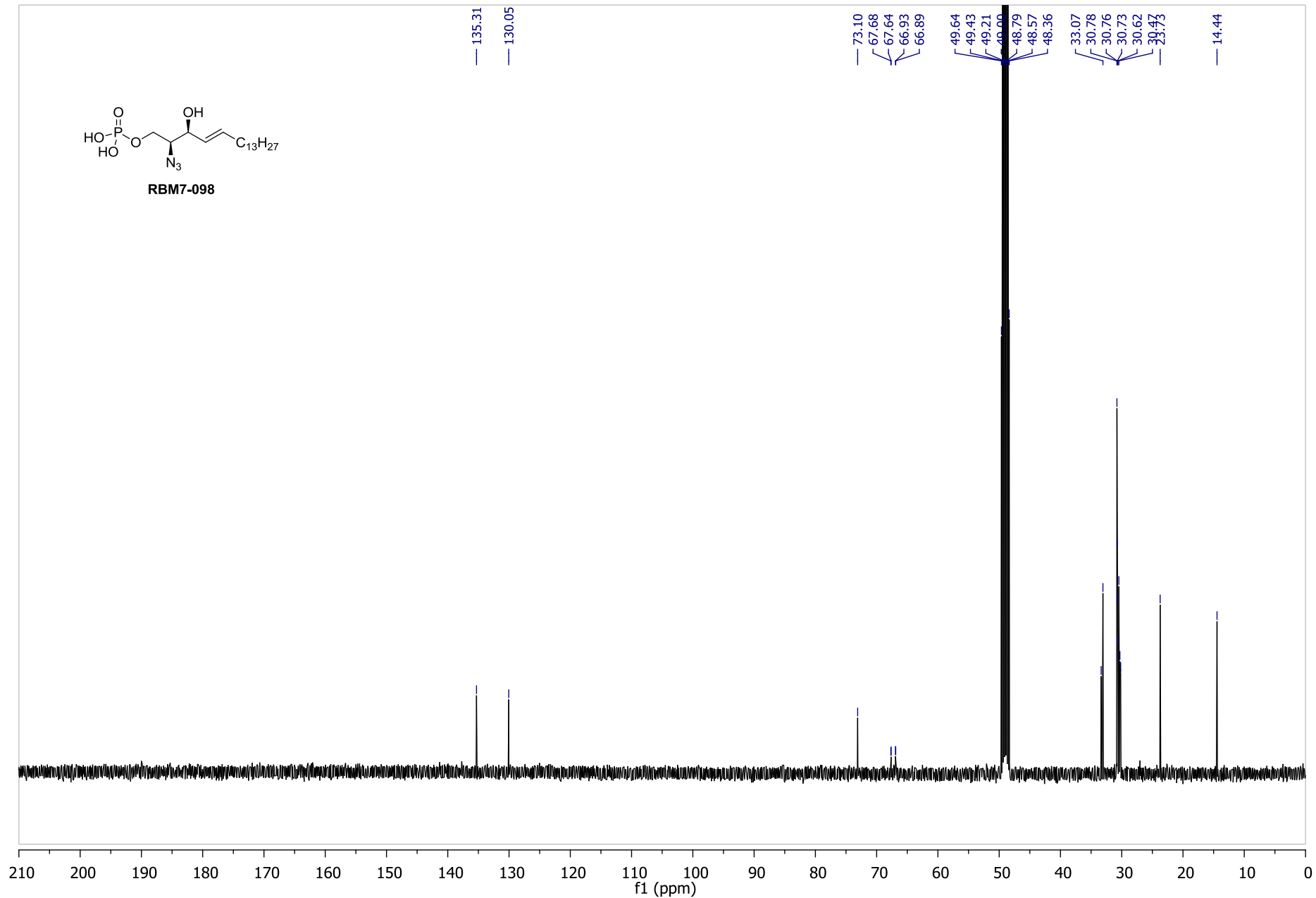
S126

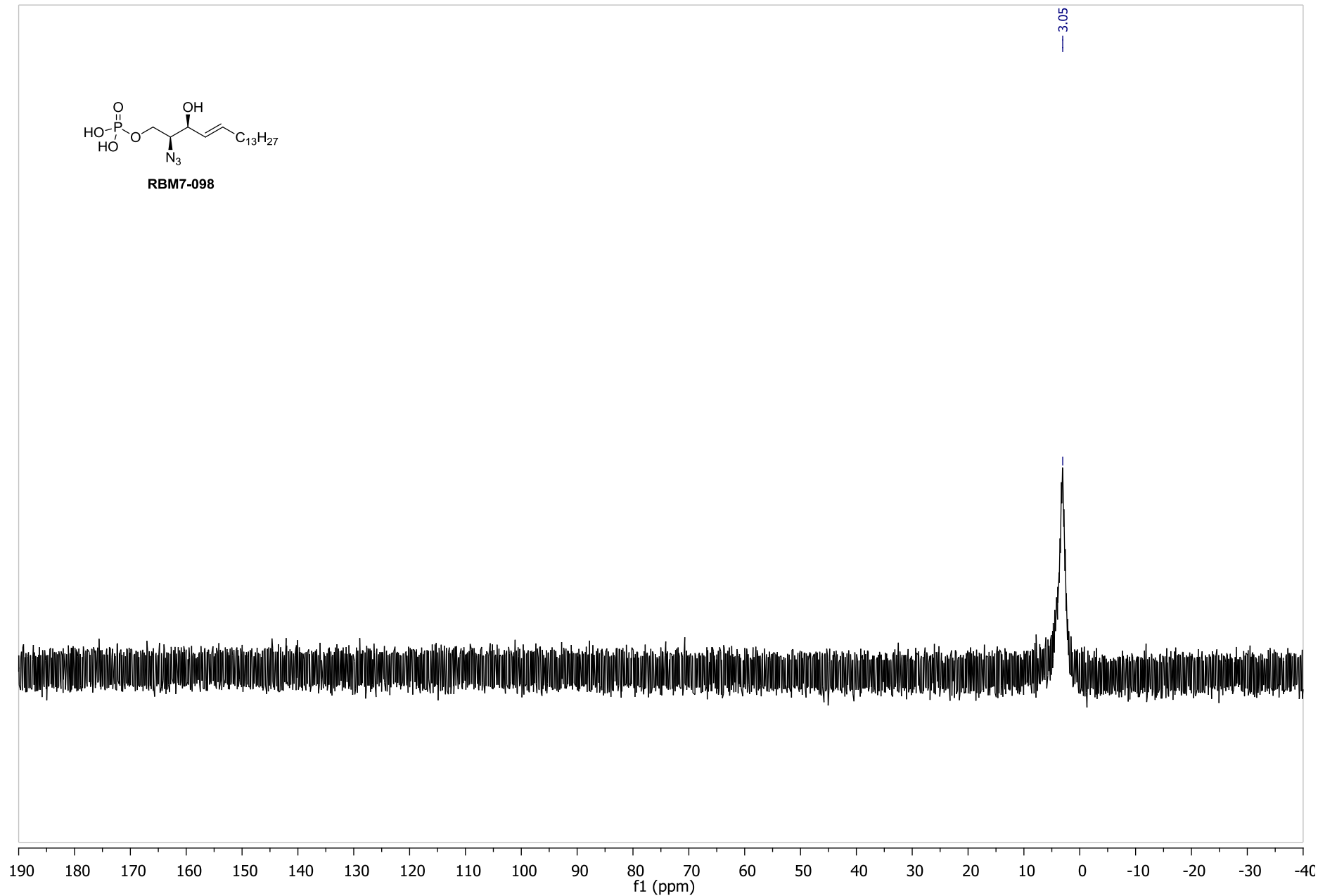
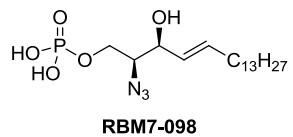


S127

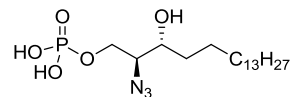


RBM7-098

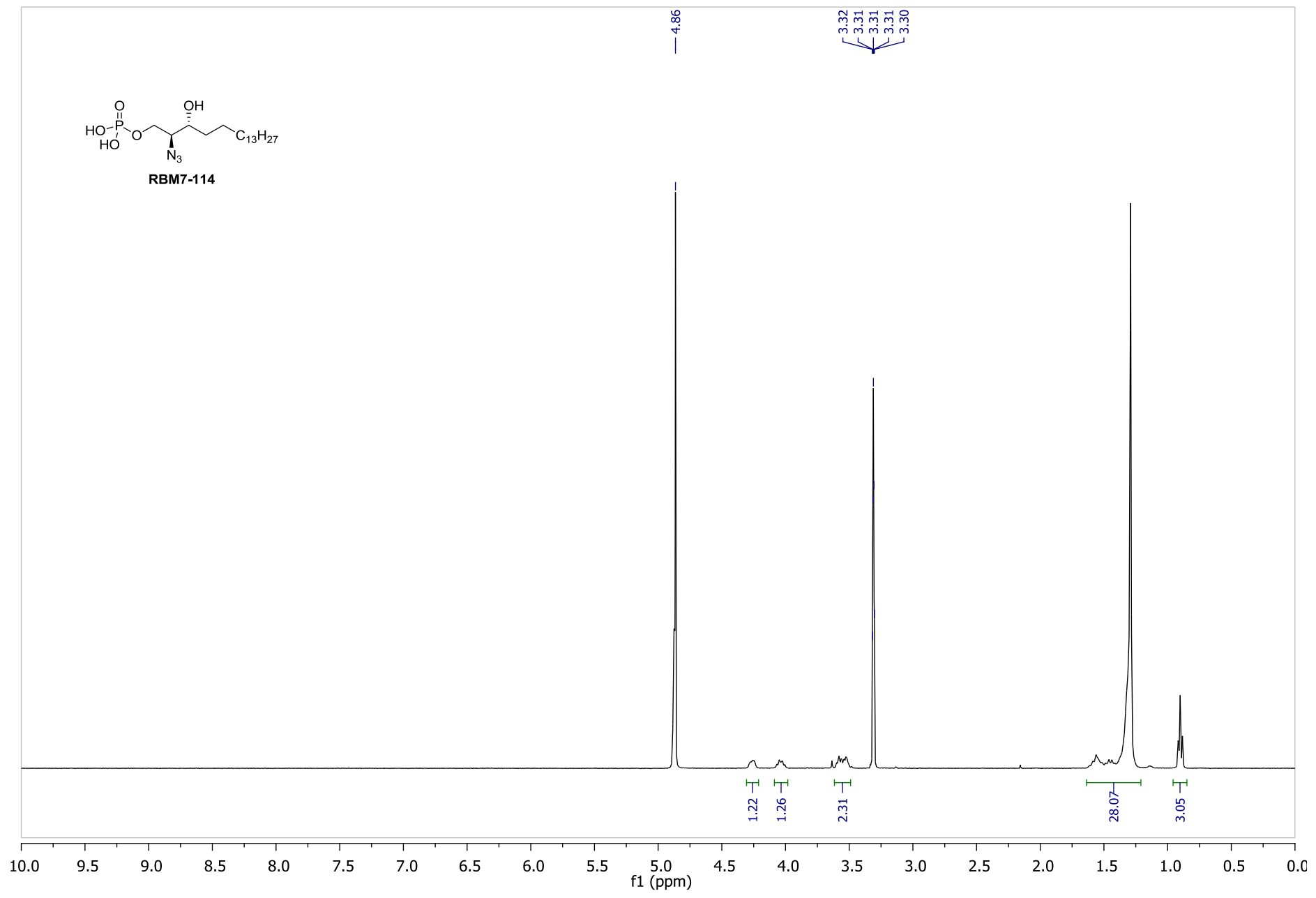


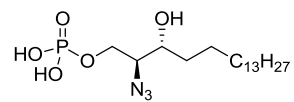


S129

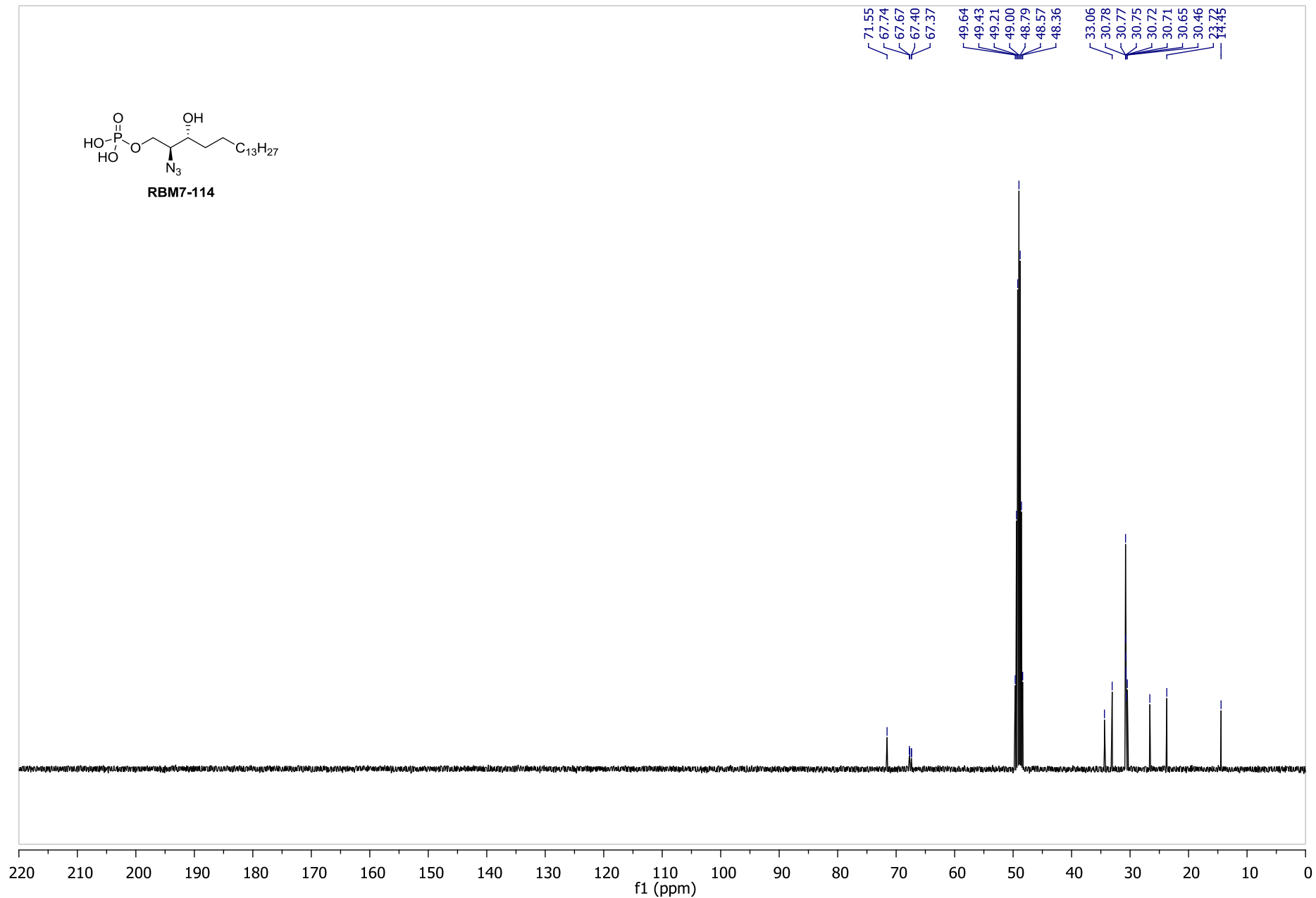


RBM7-114

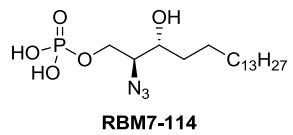




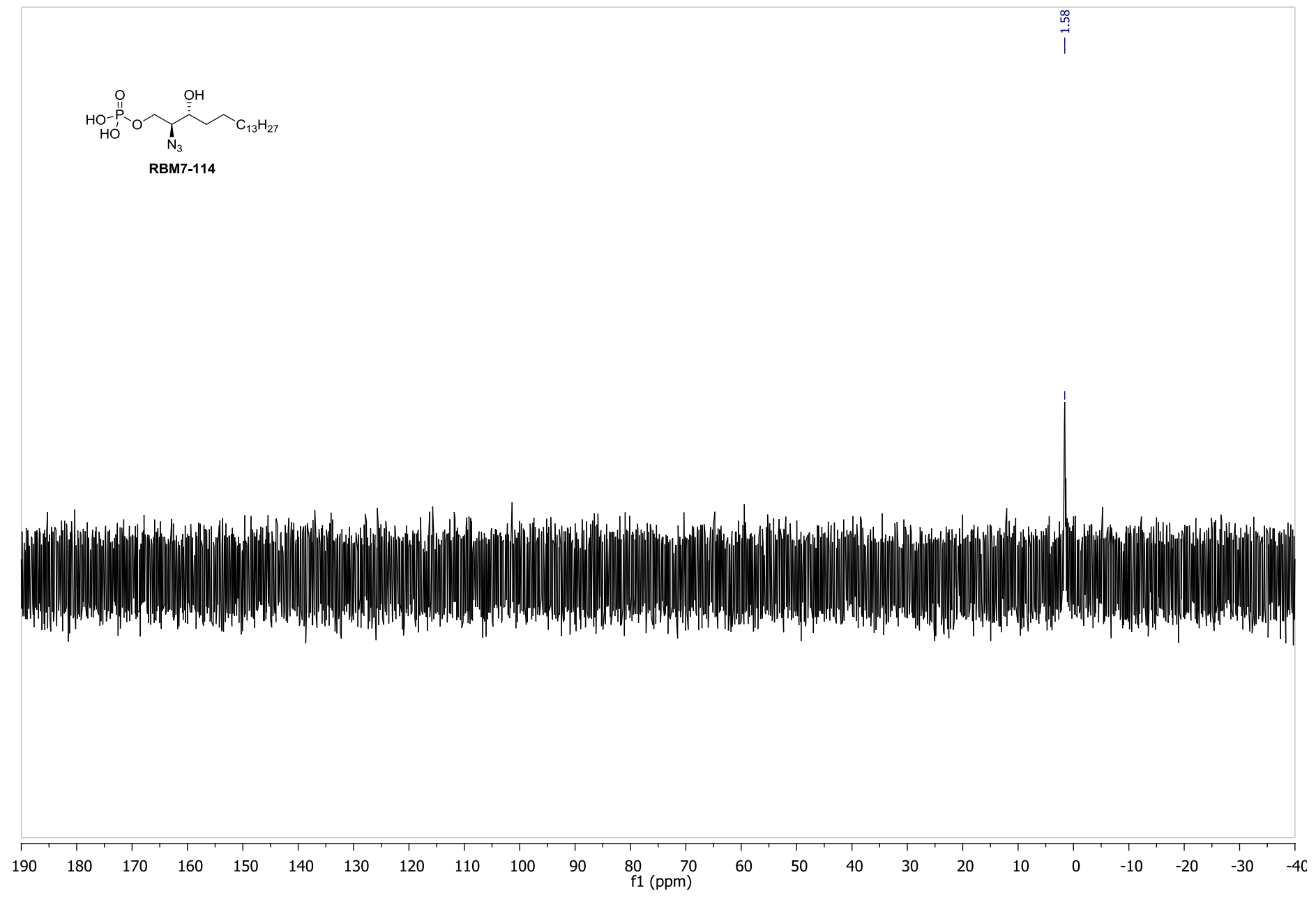
RBM7-114



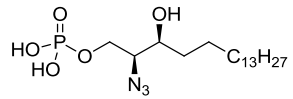
S131



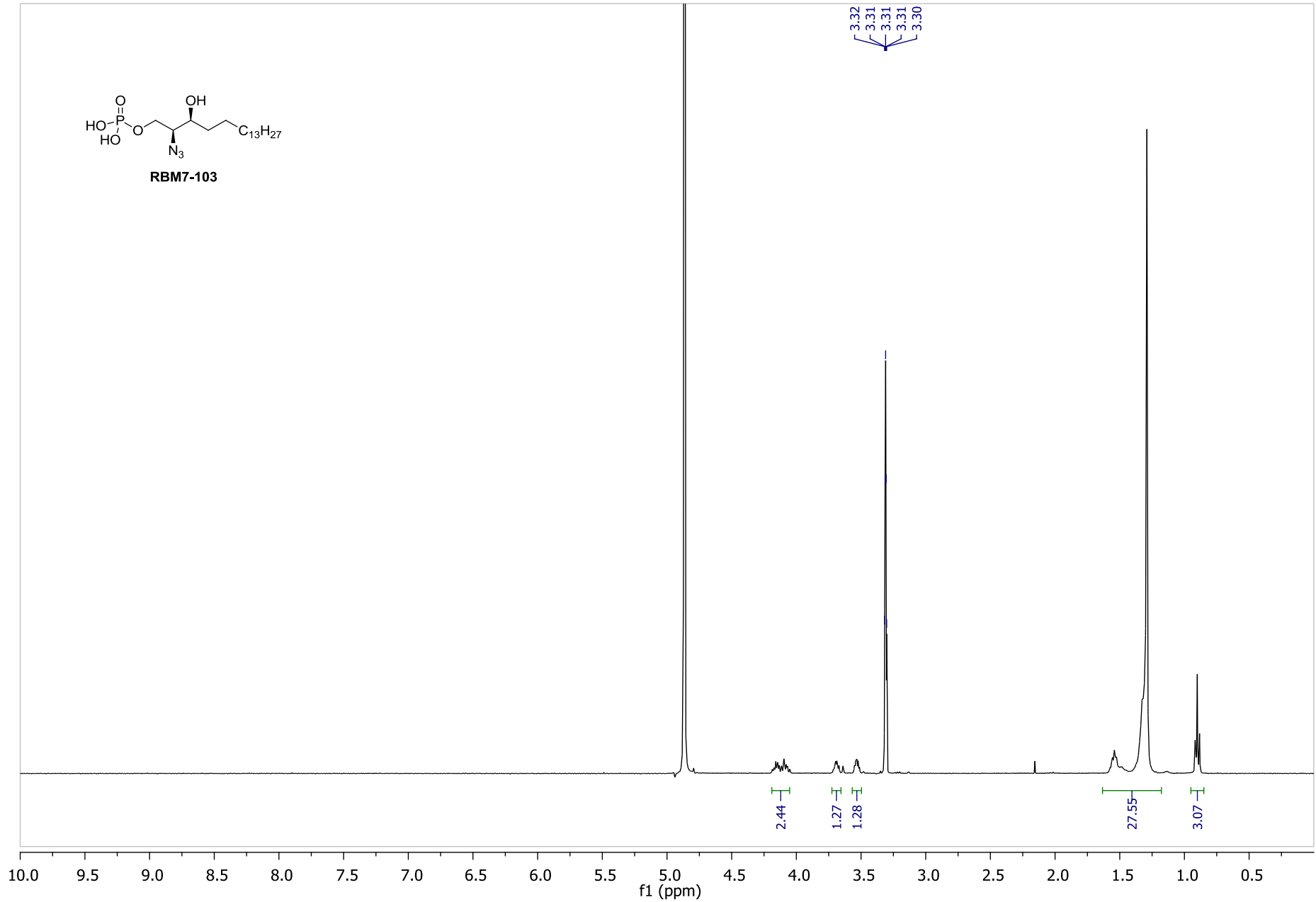
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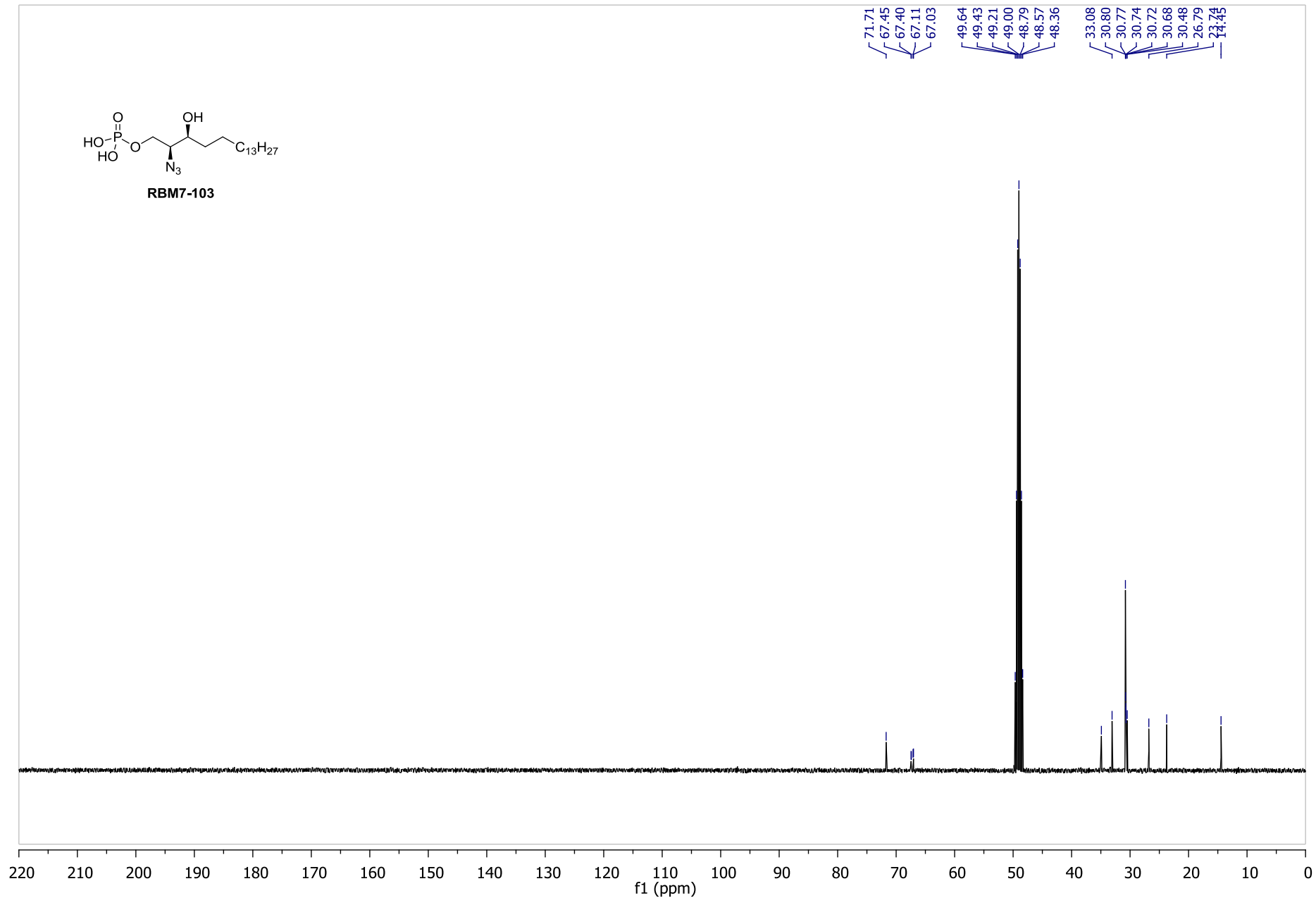
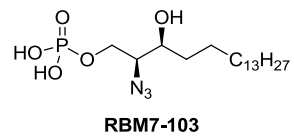


S132

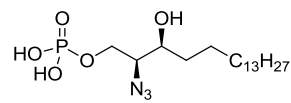


RBM7-103

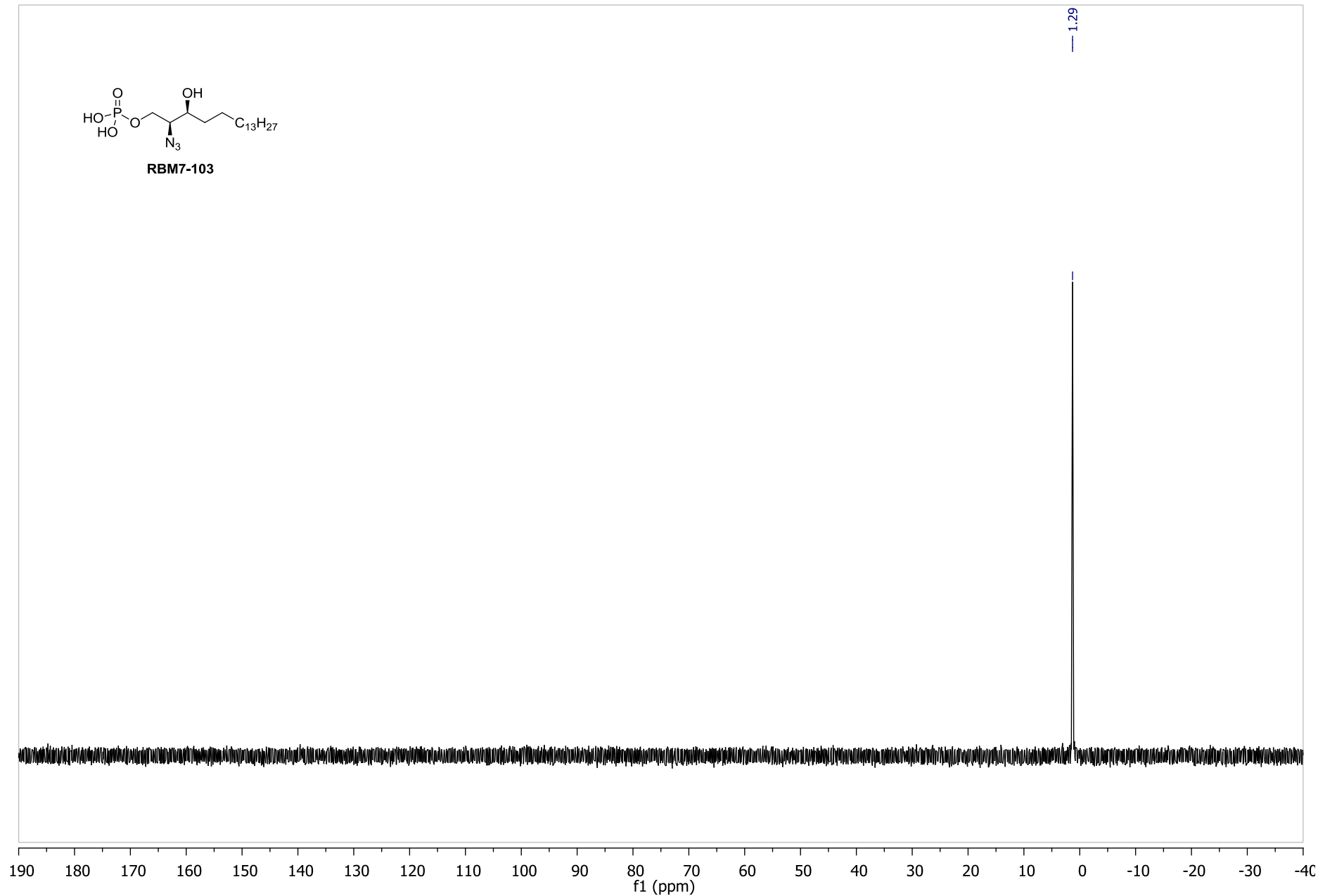




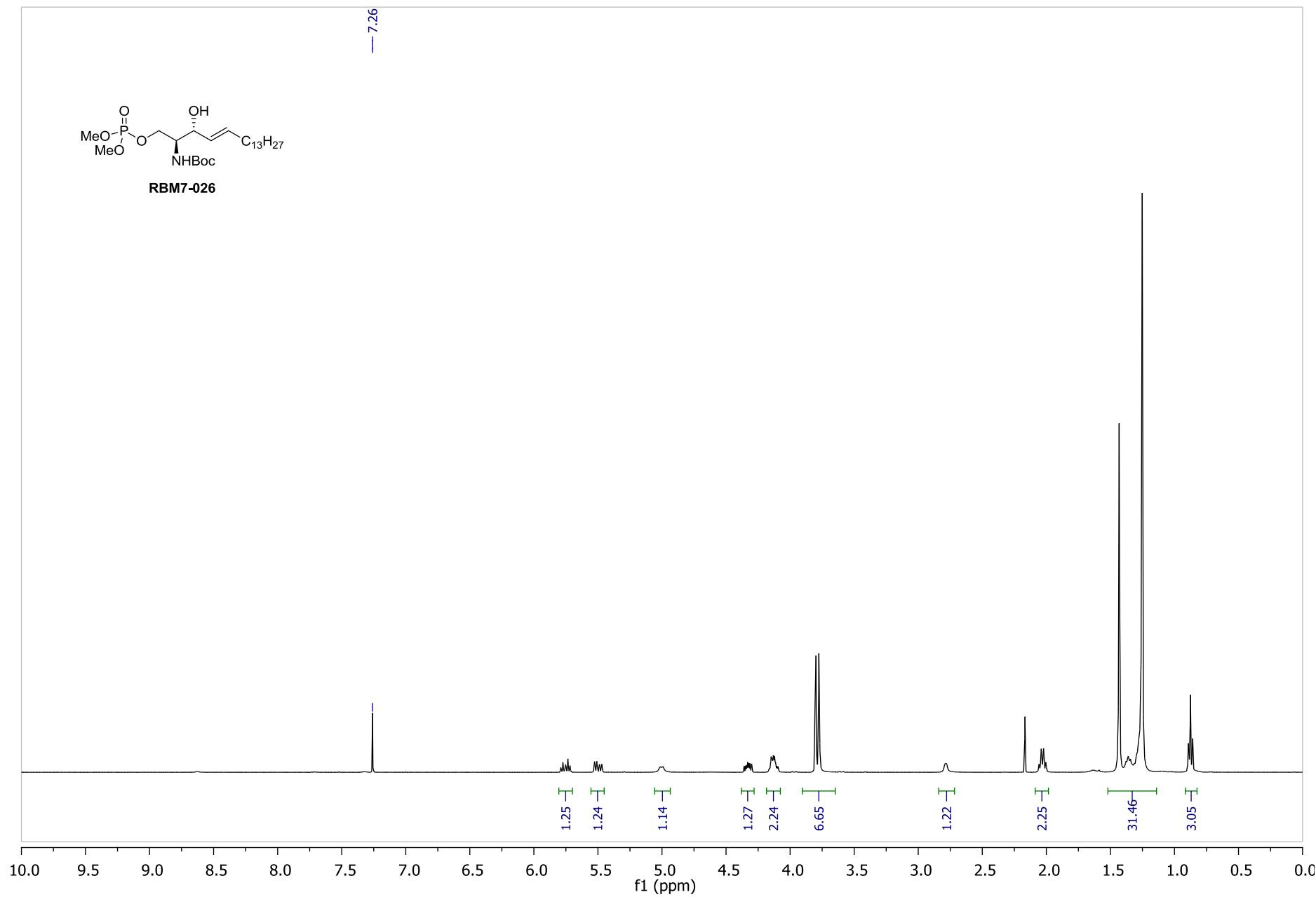
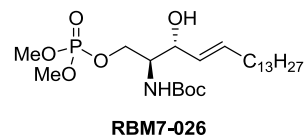
S134



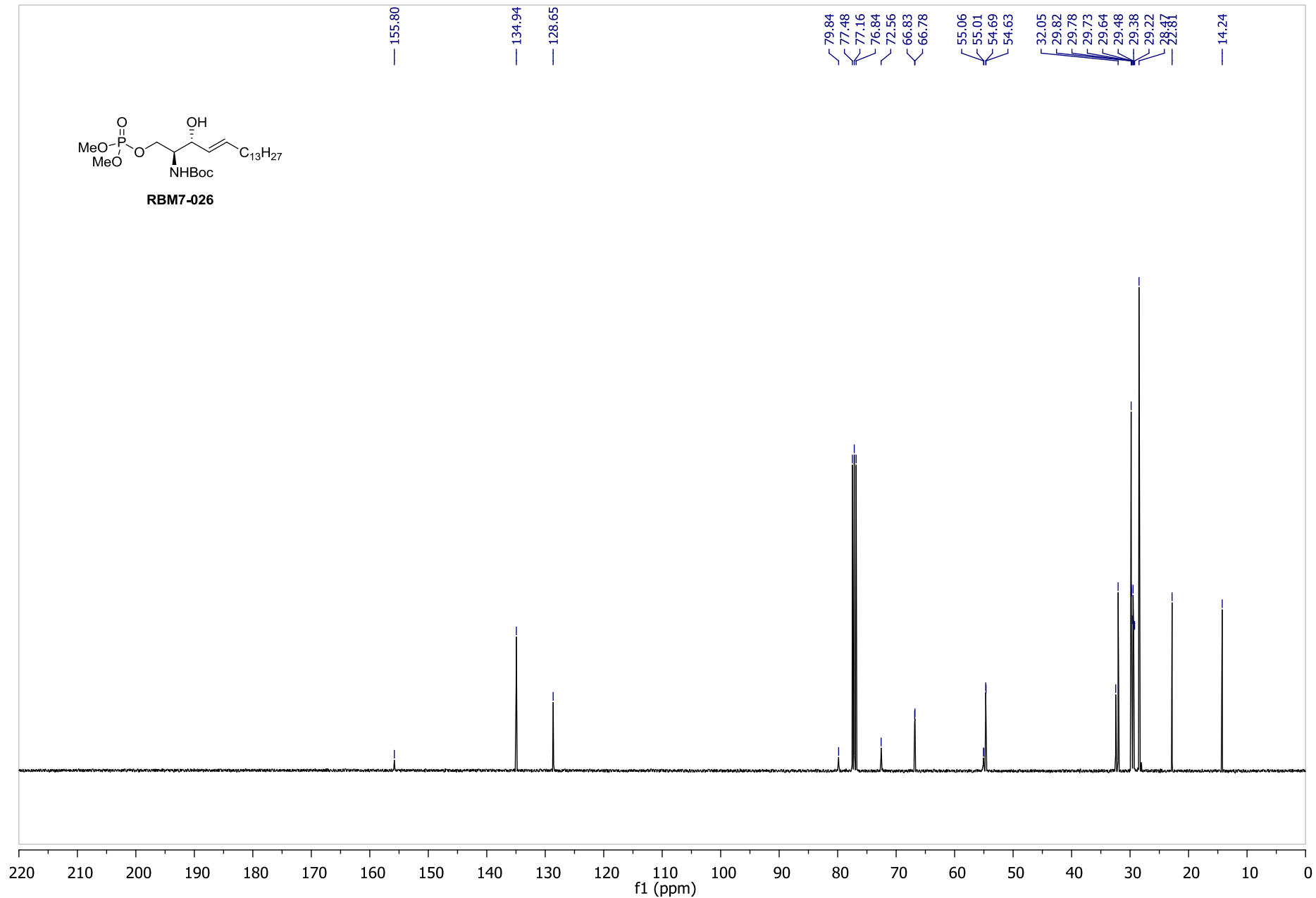
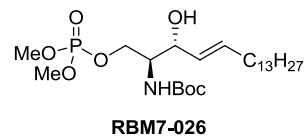
RBM7-103



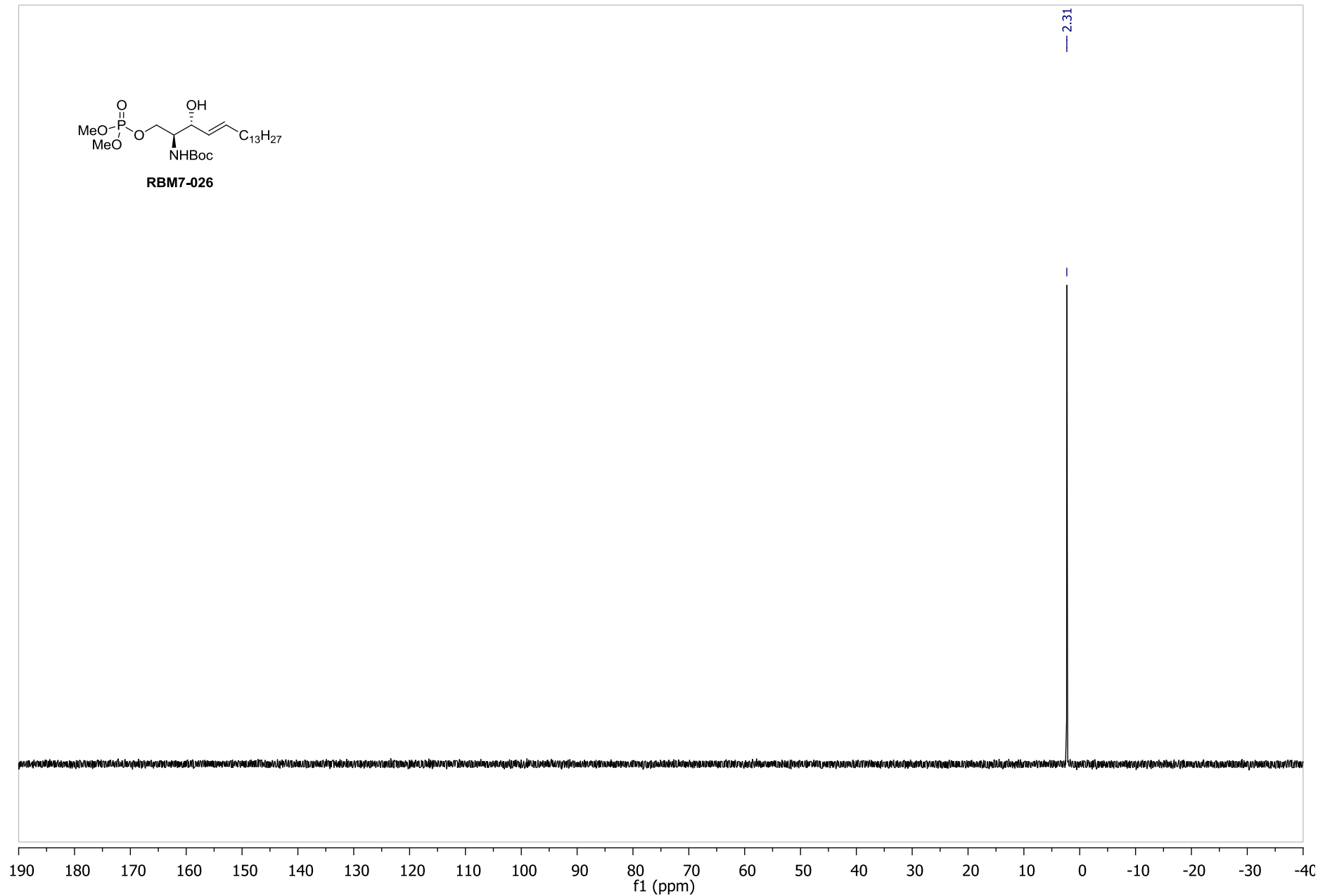
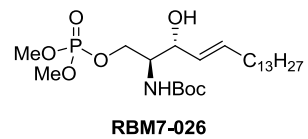
S135



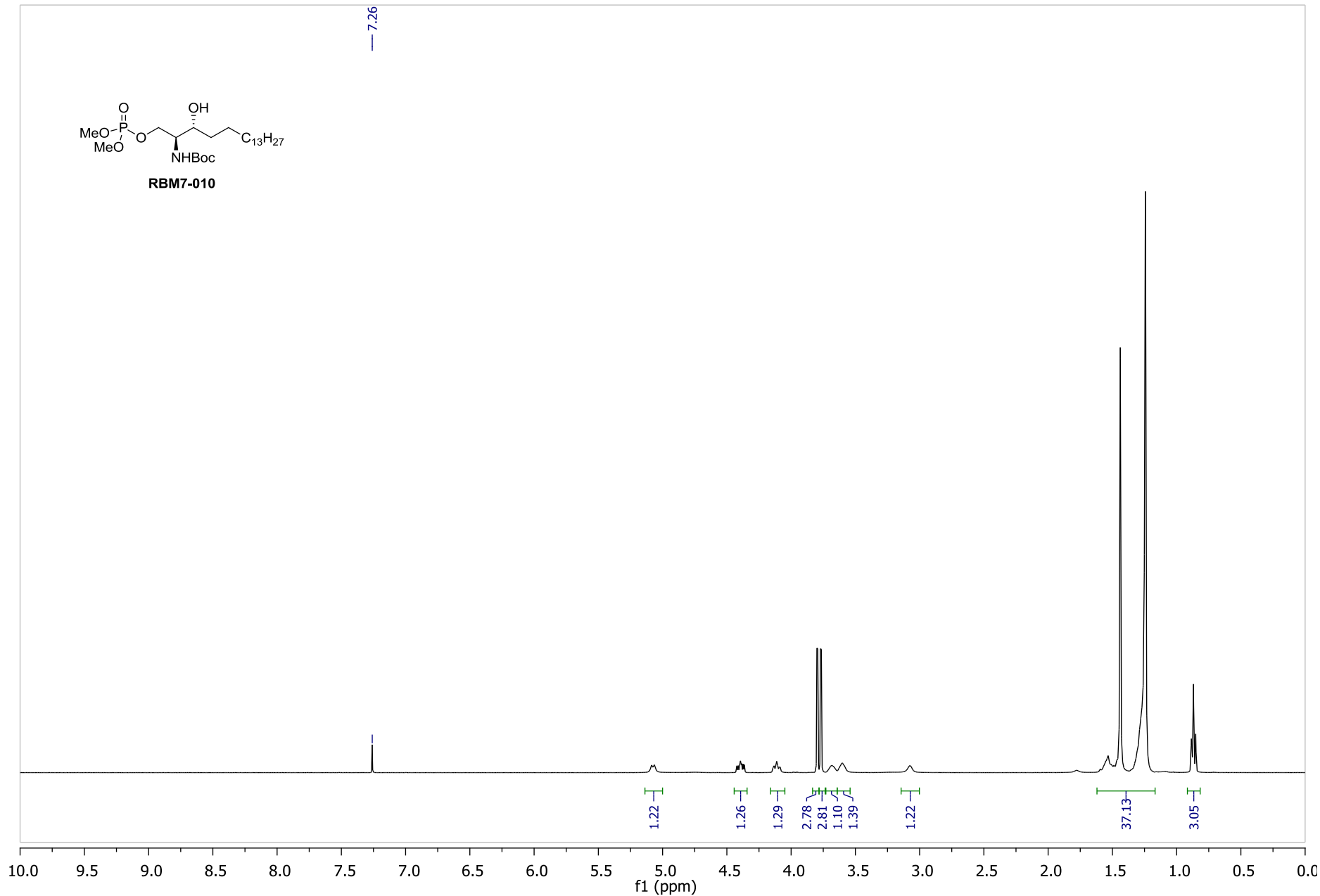
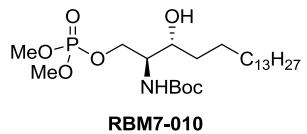
S136



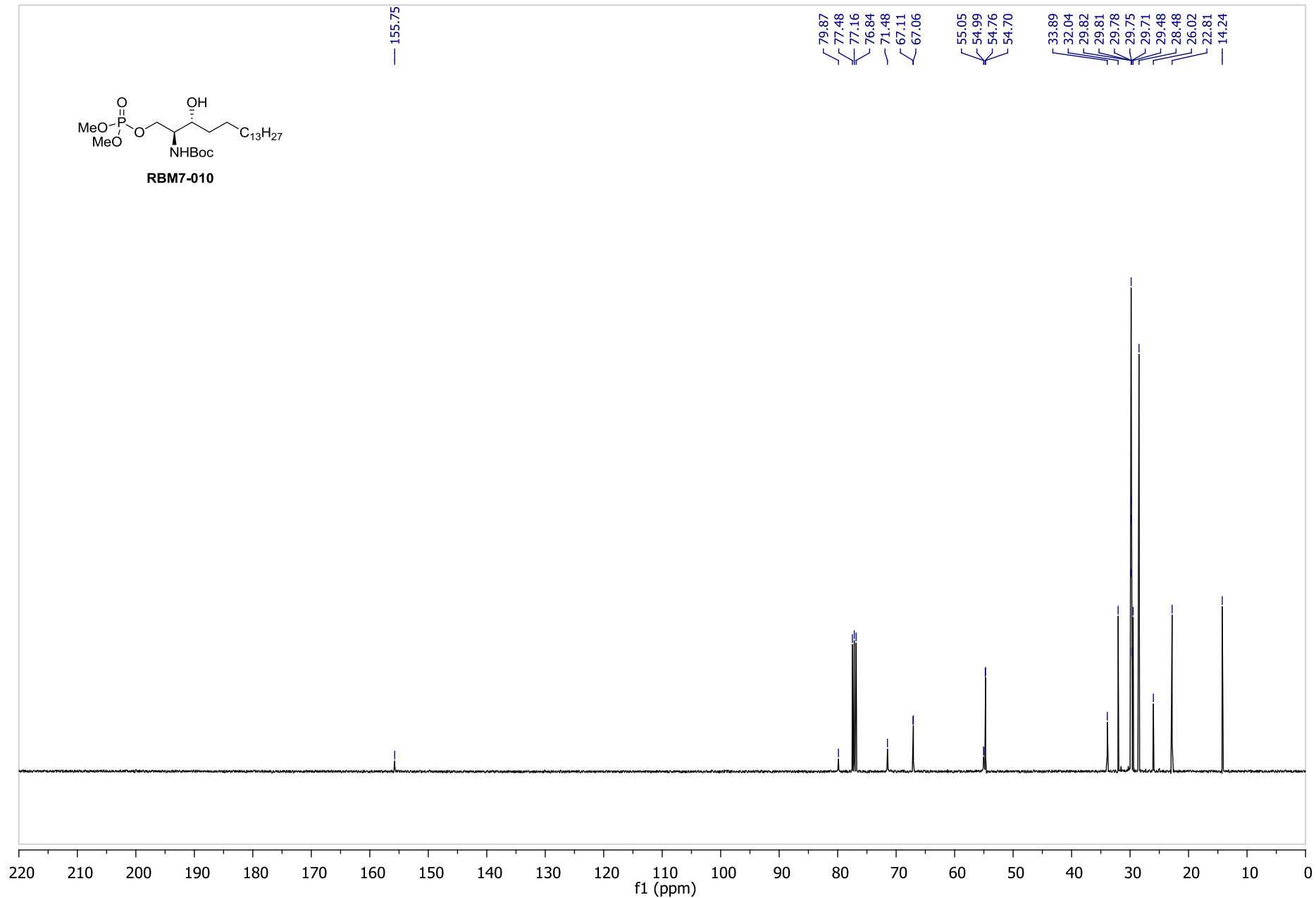
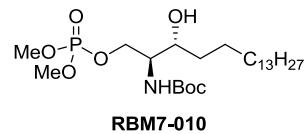
S137



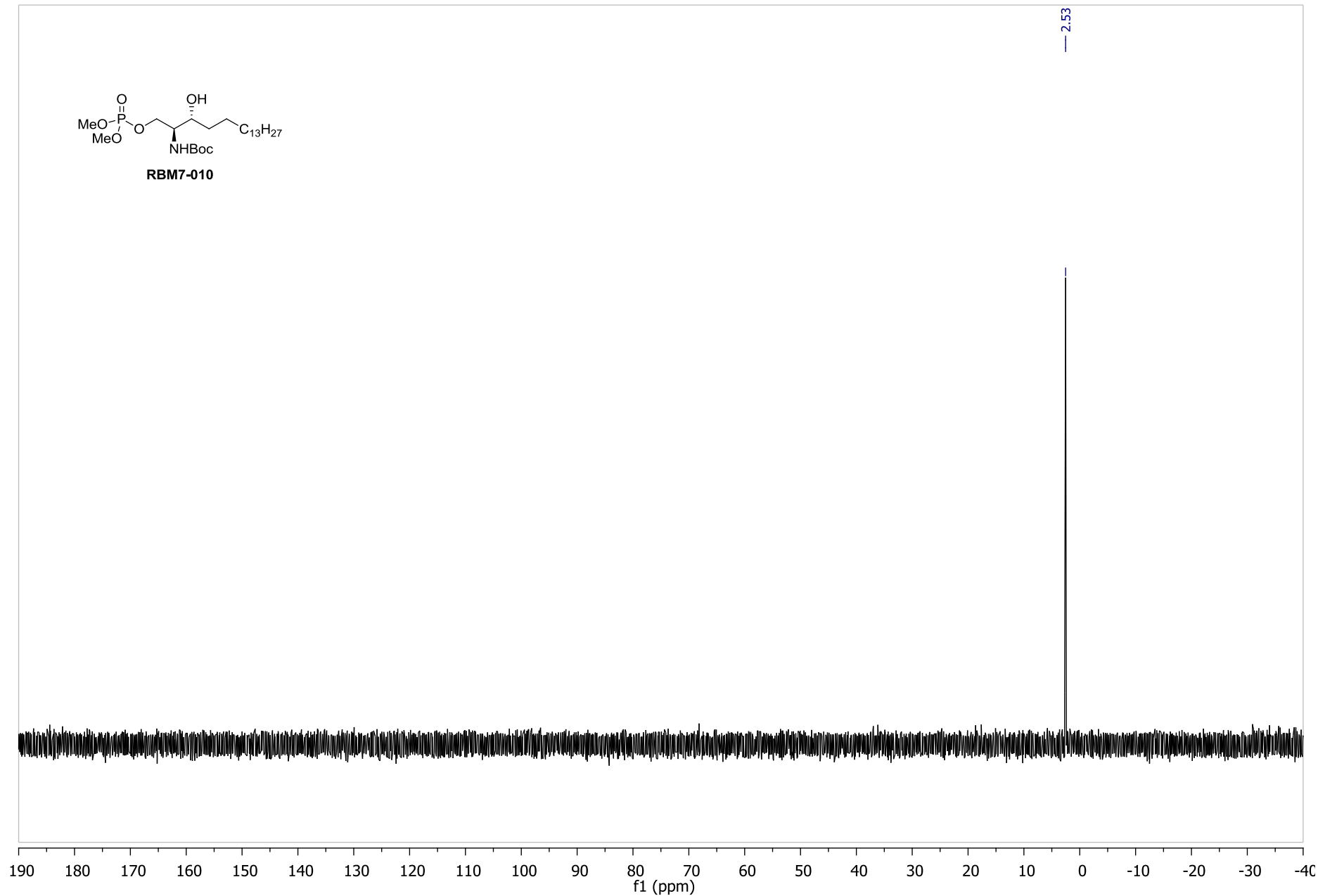
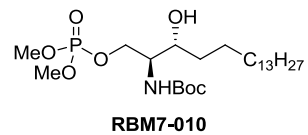
S138



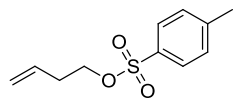
S139



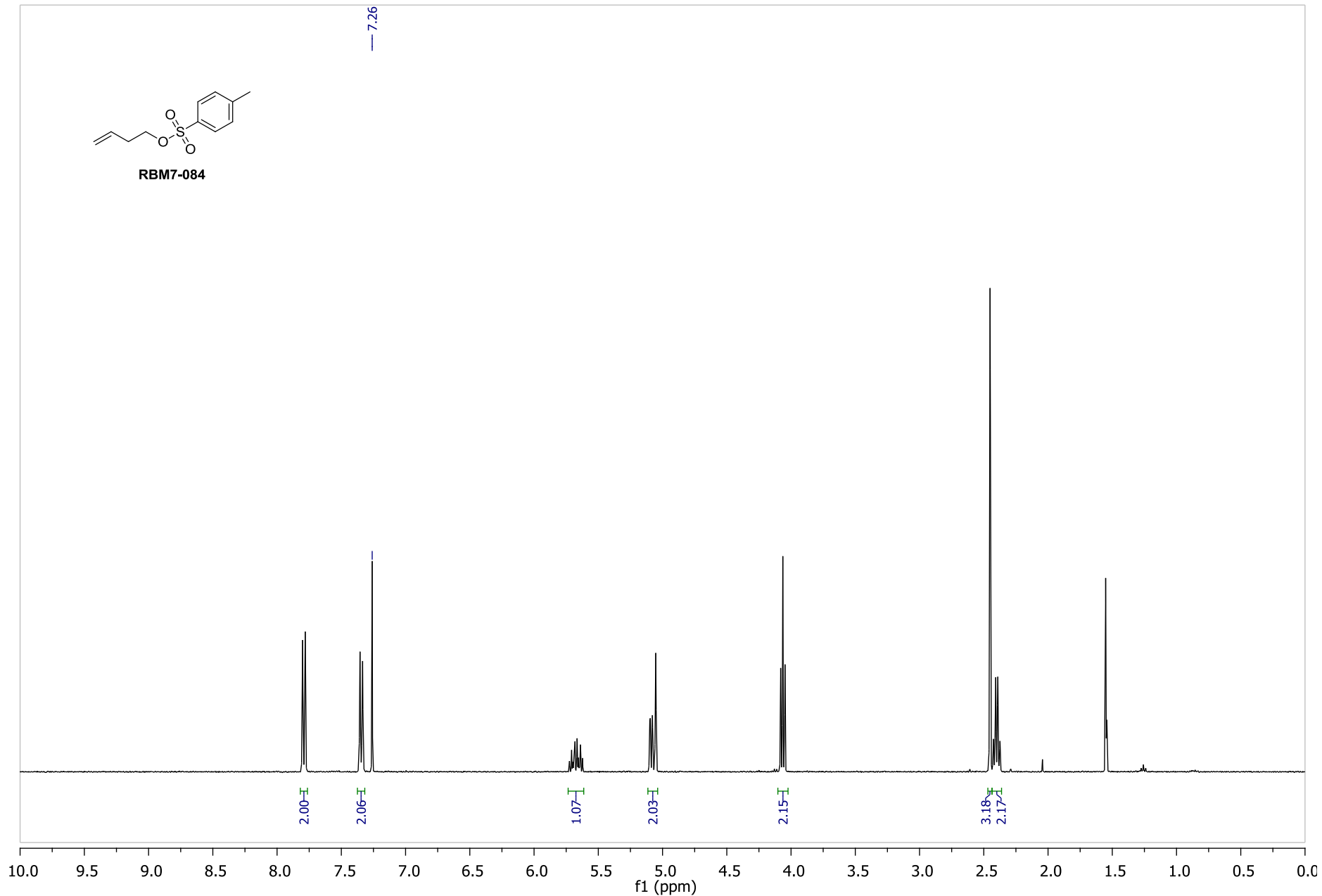
S140



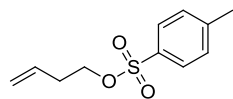
S141



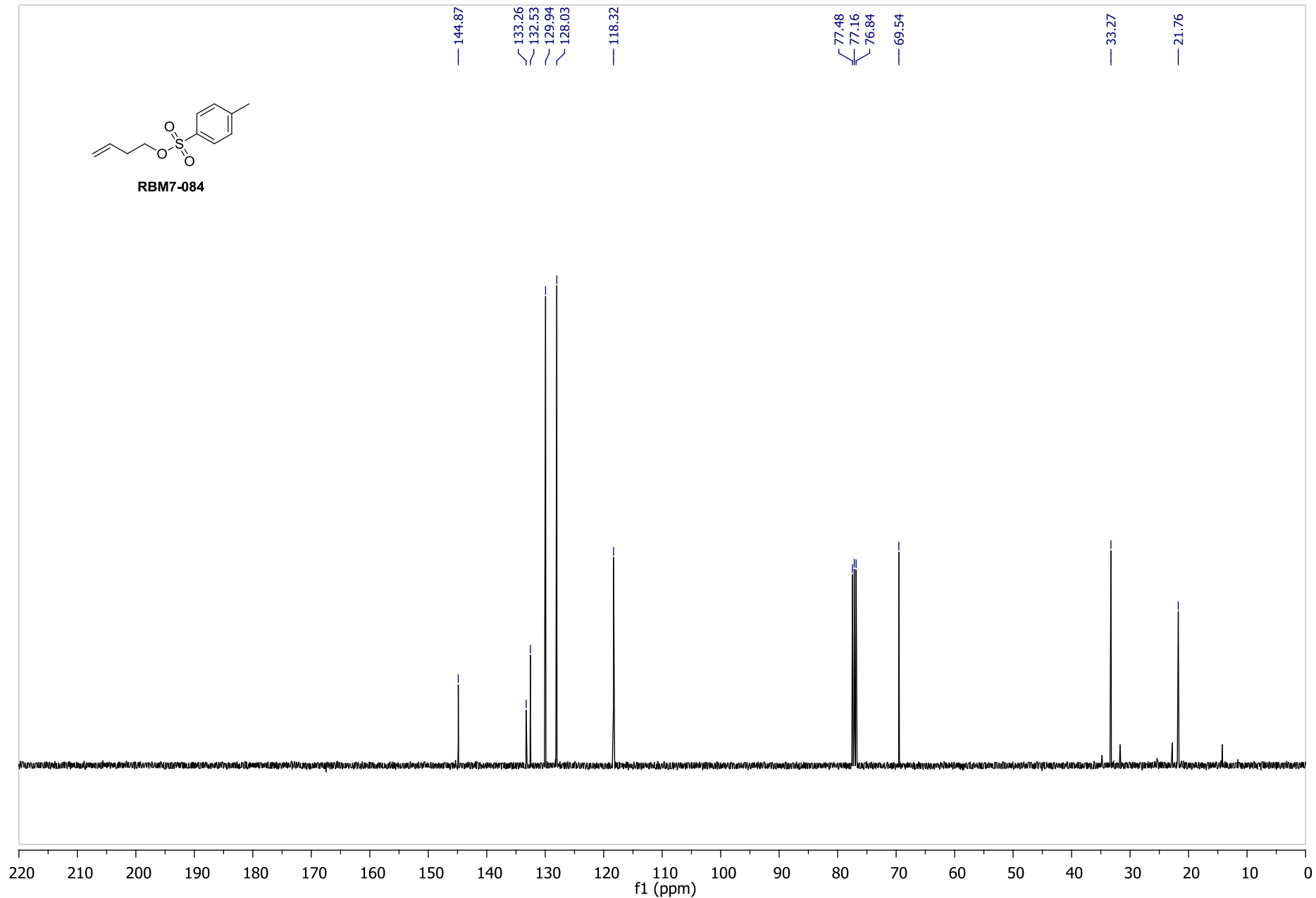
RBM7-084

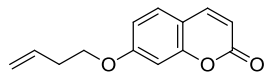


S142



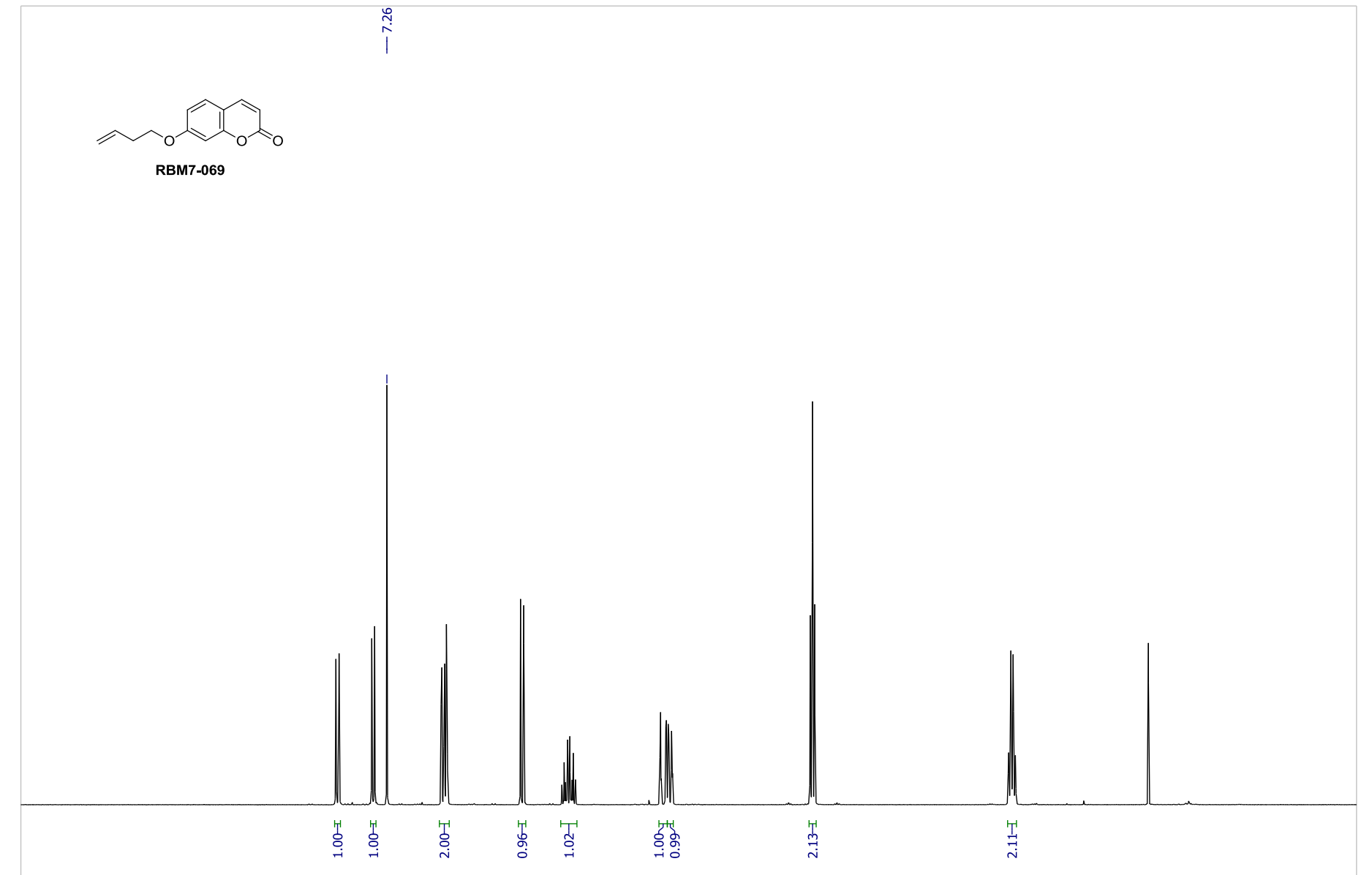
RBM7-084



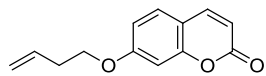


RBM7-069

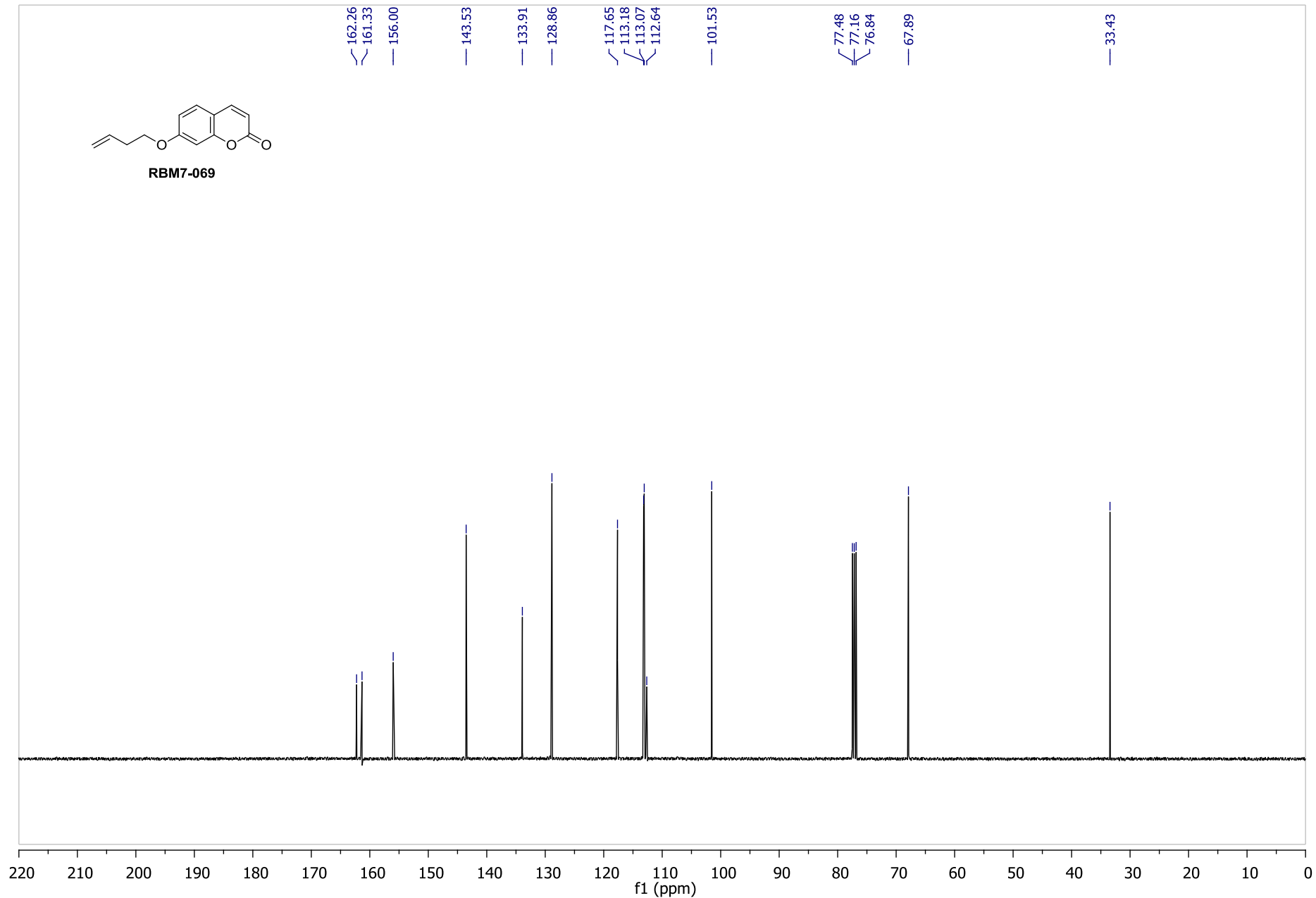
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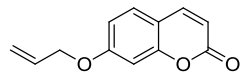
S144



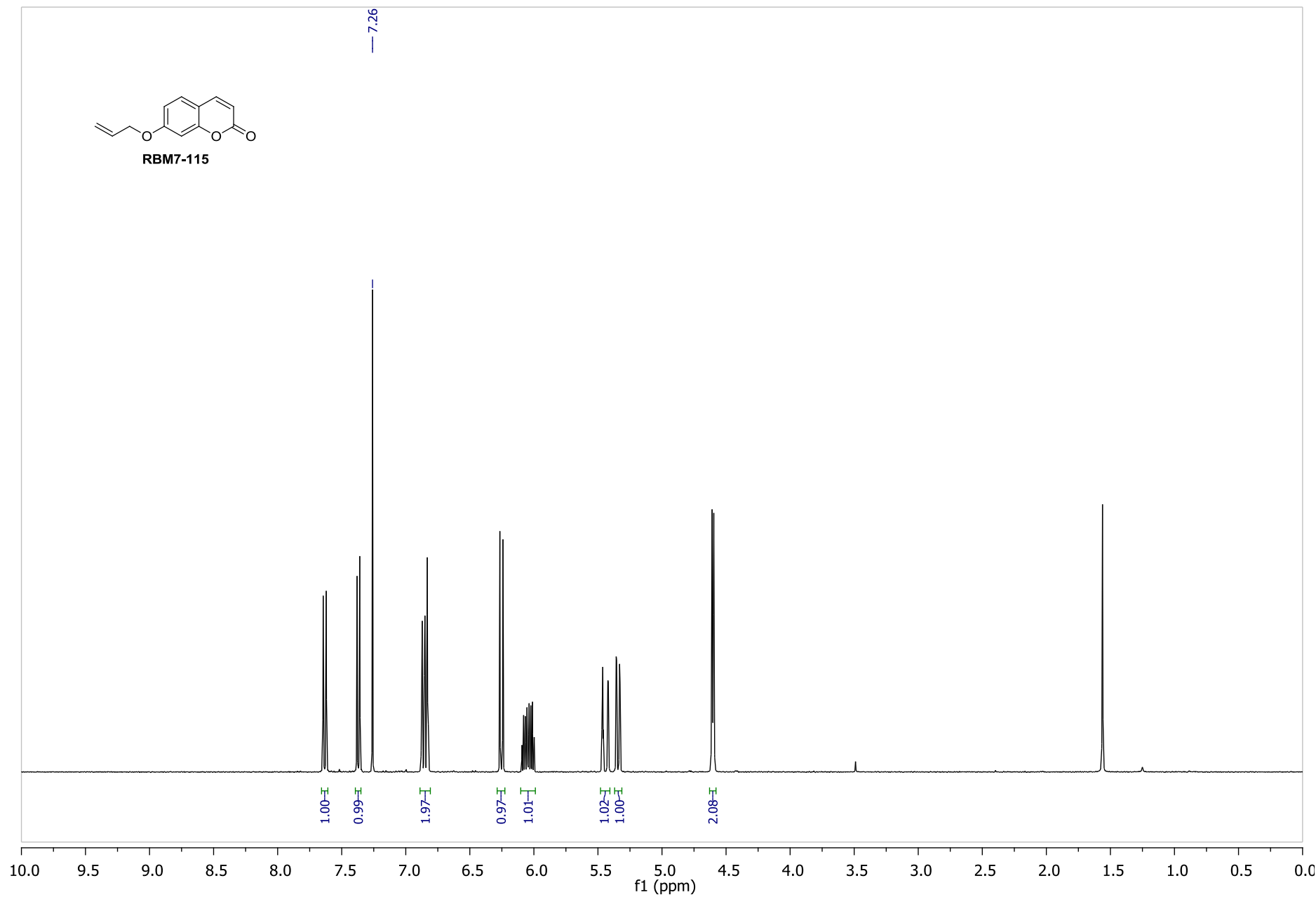
RBM7-069

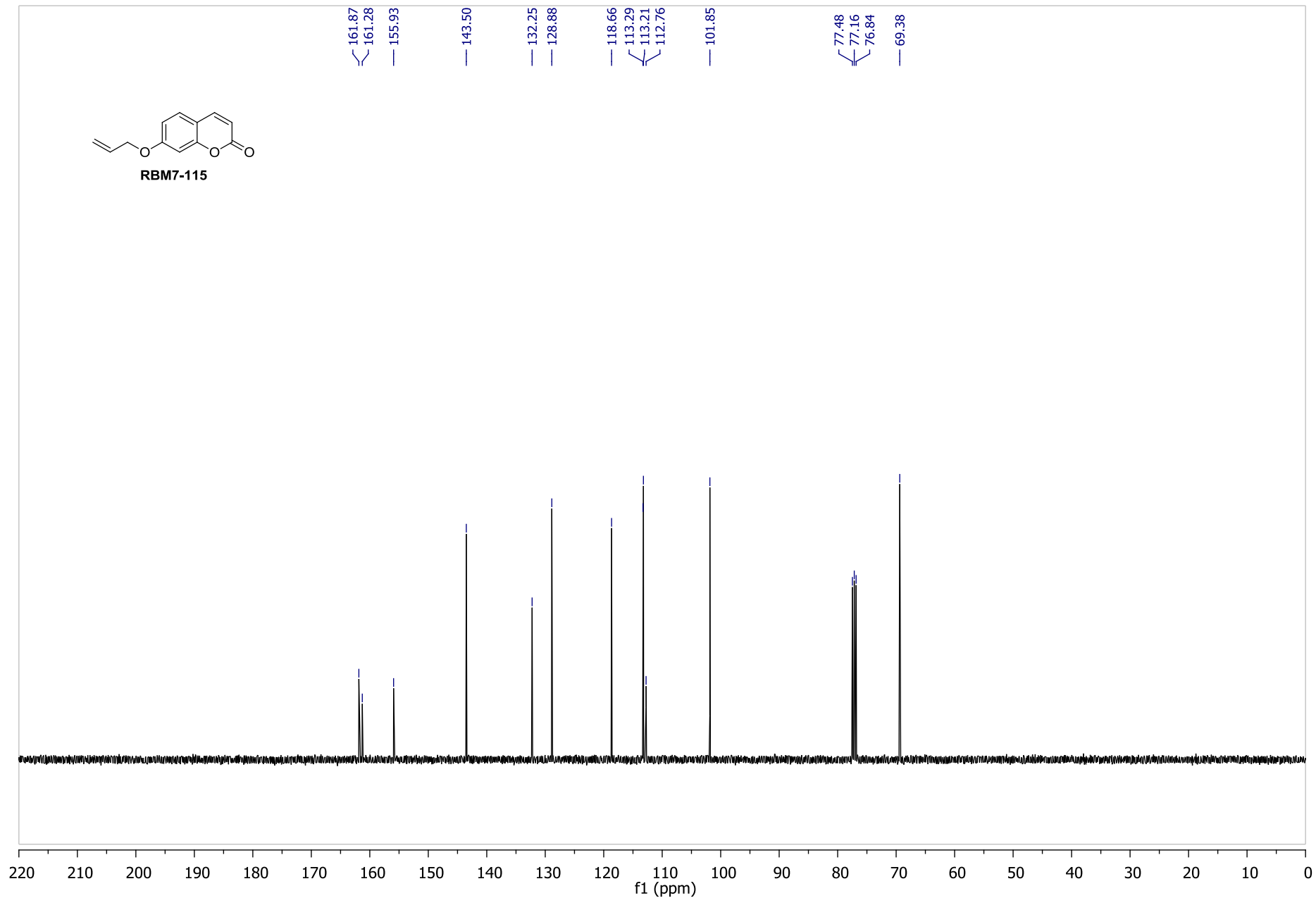
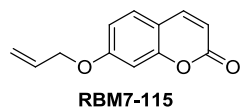


S145

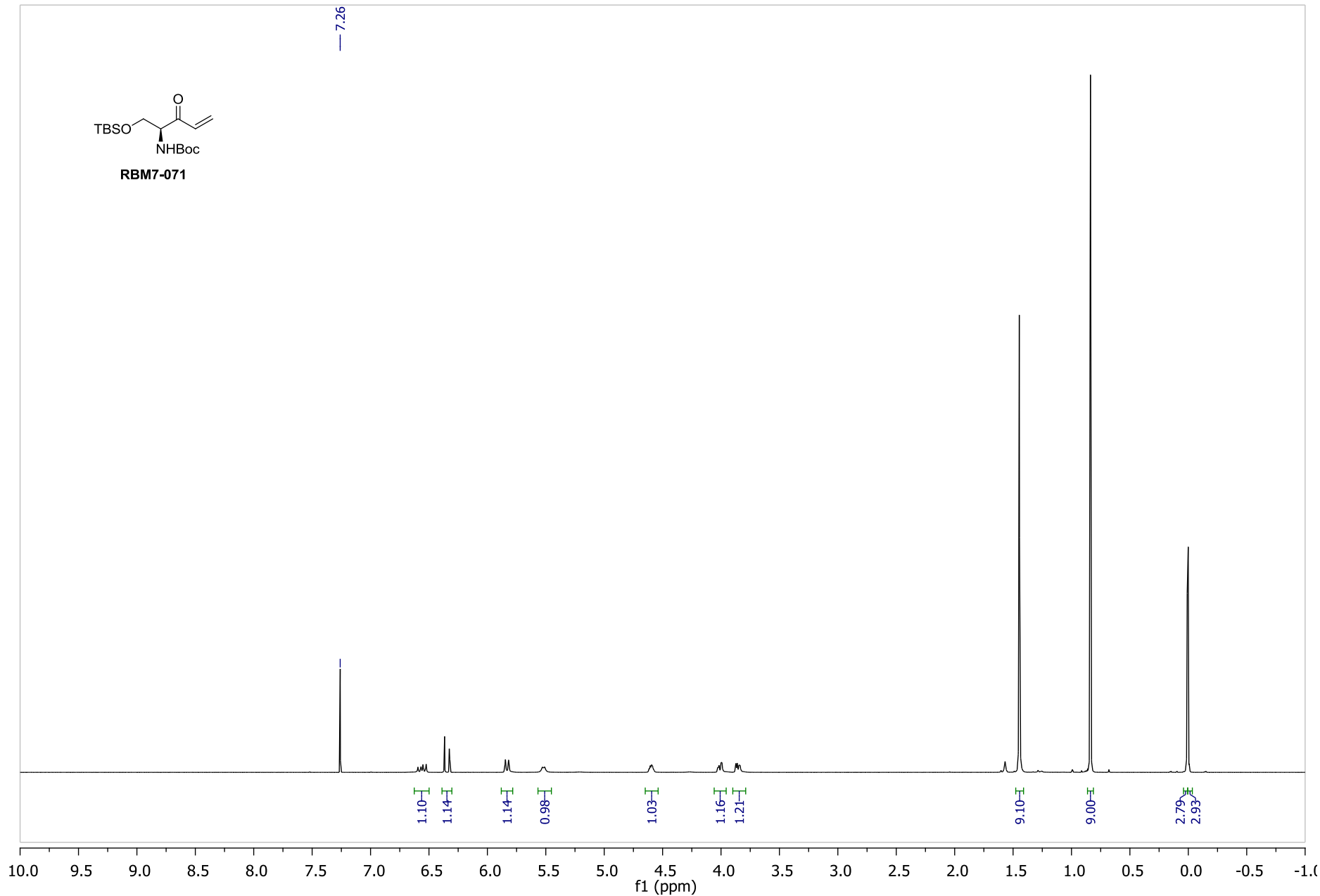
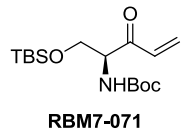


RBM7-115

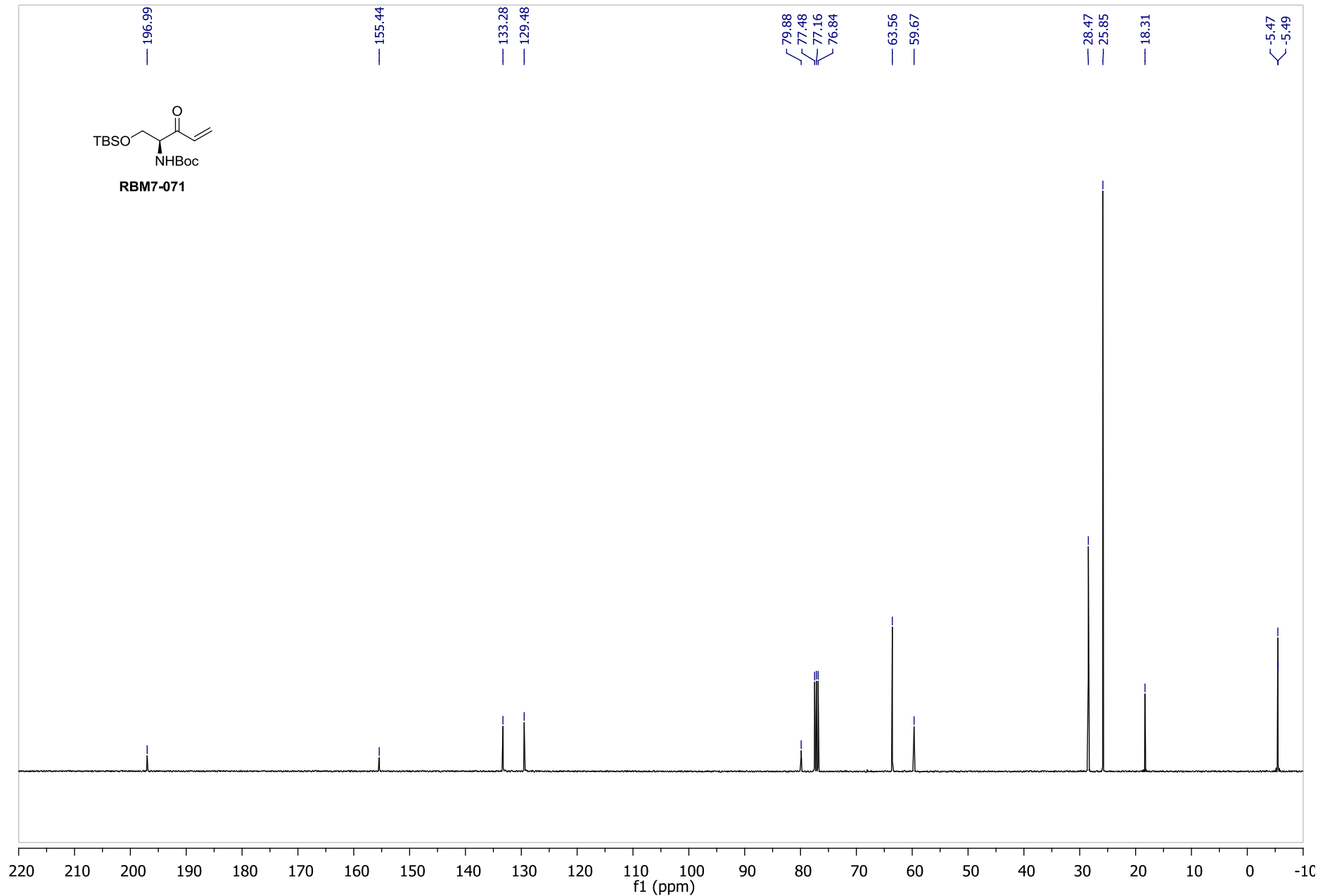
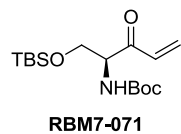


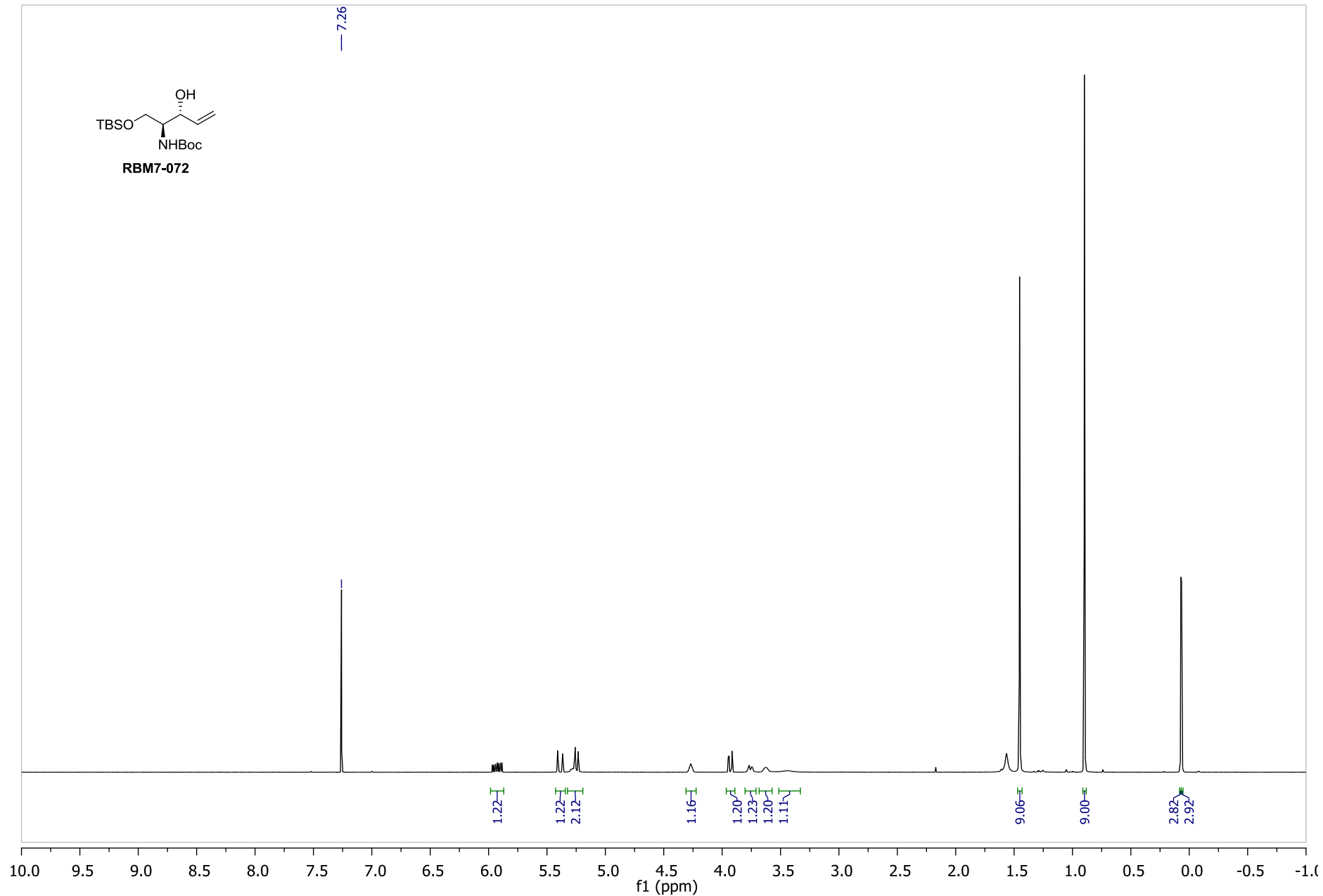
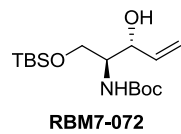


S147

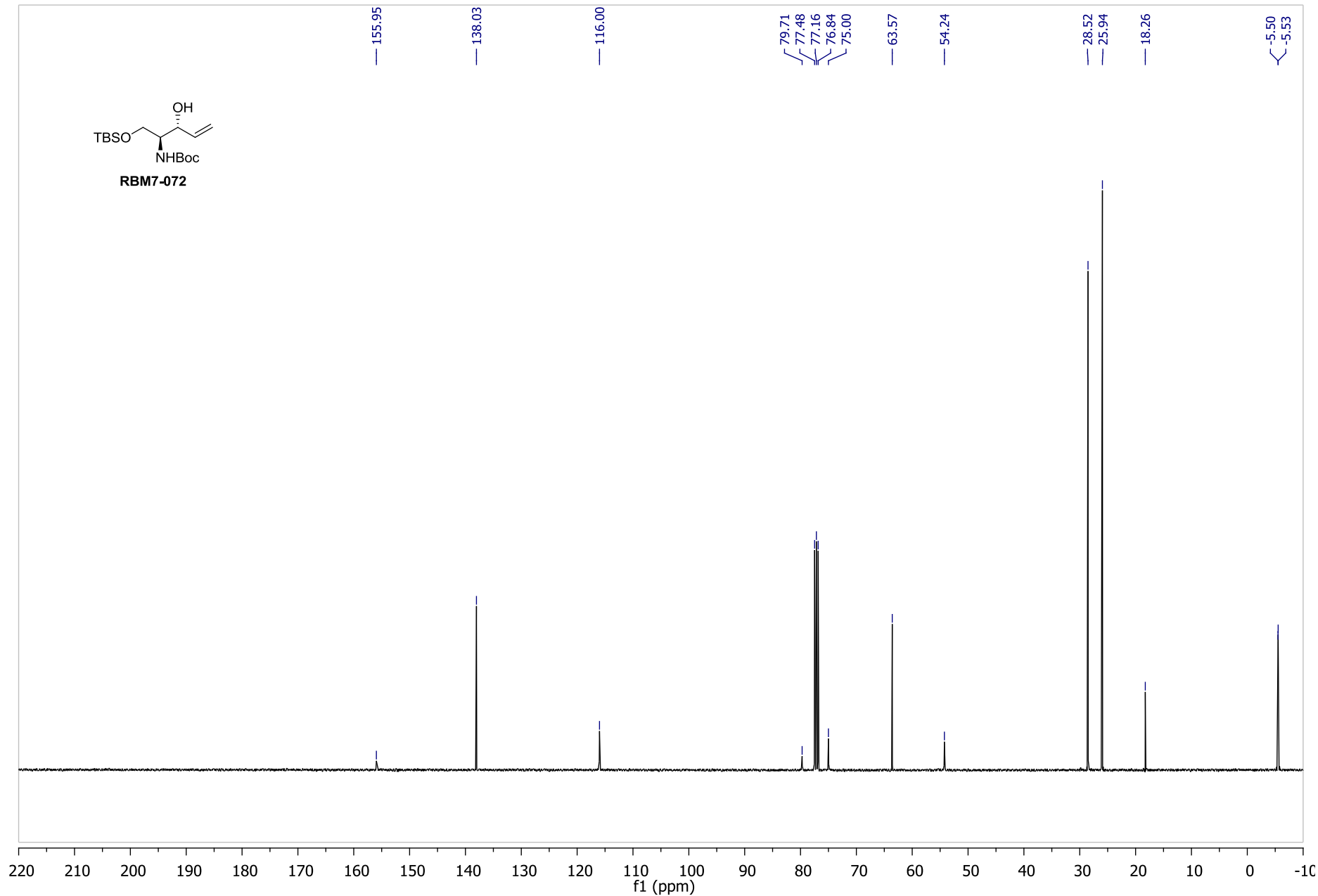
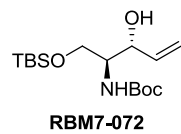


S148

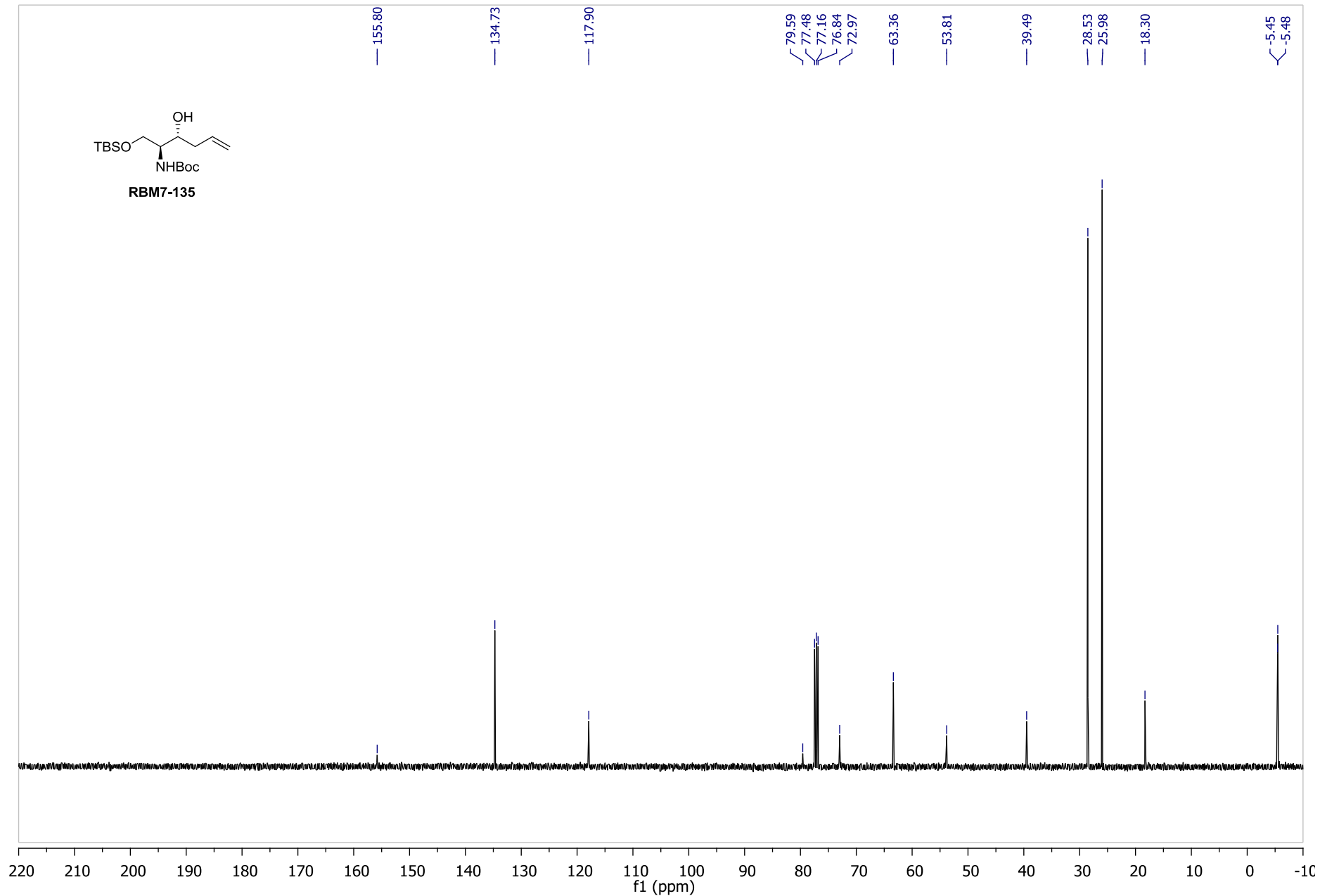
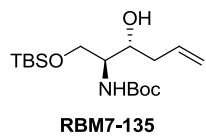




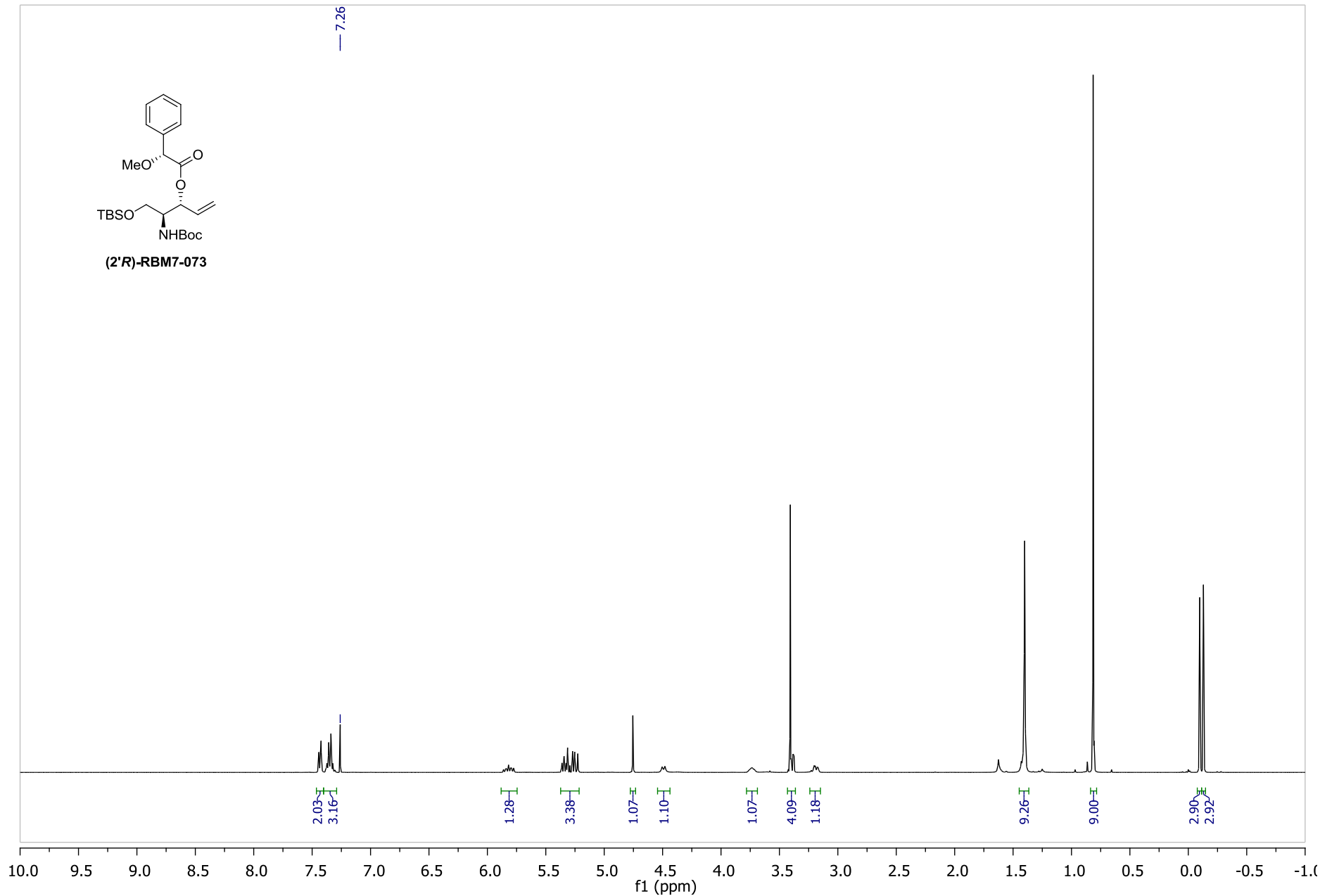
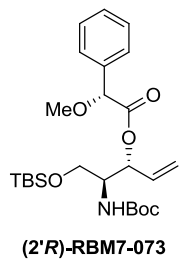
S150



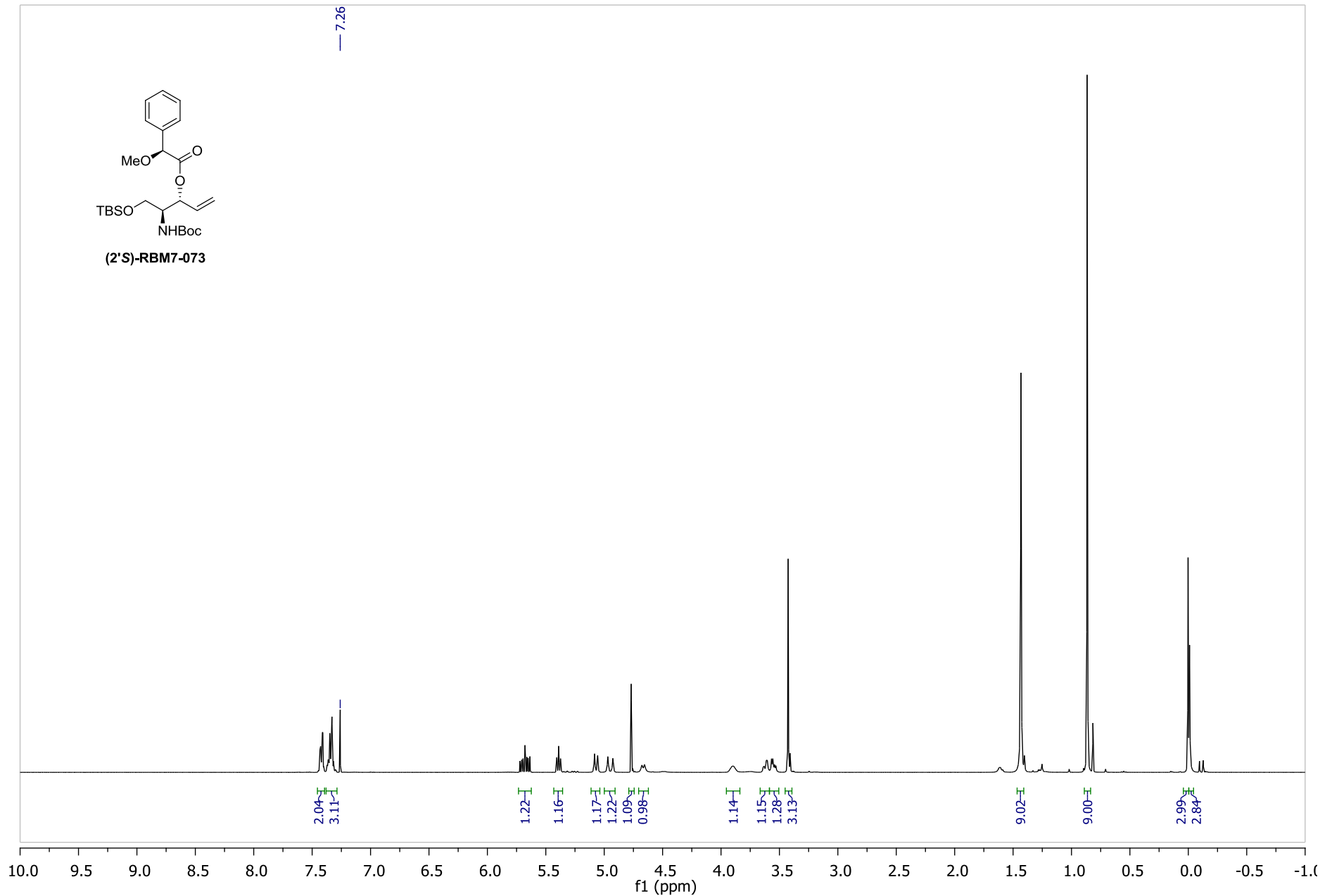
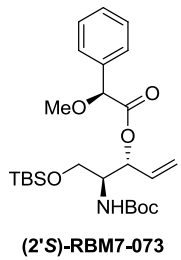
S151



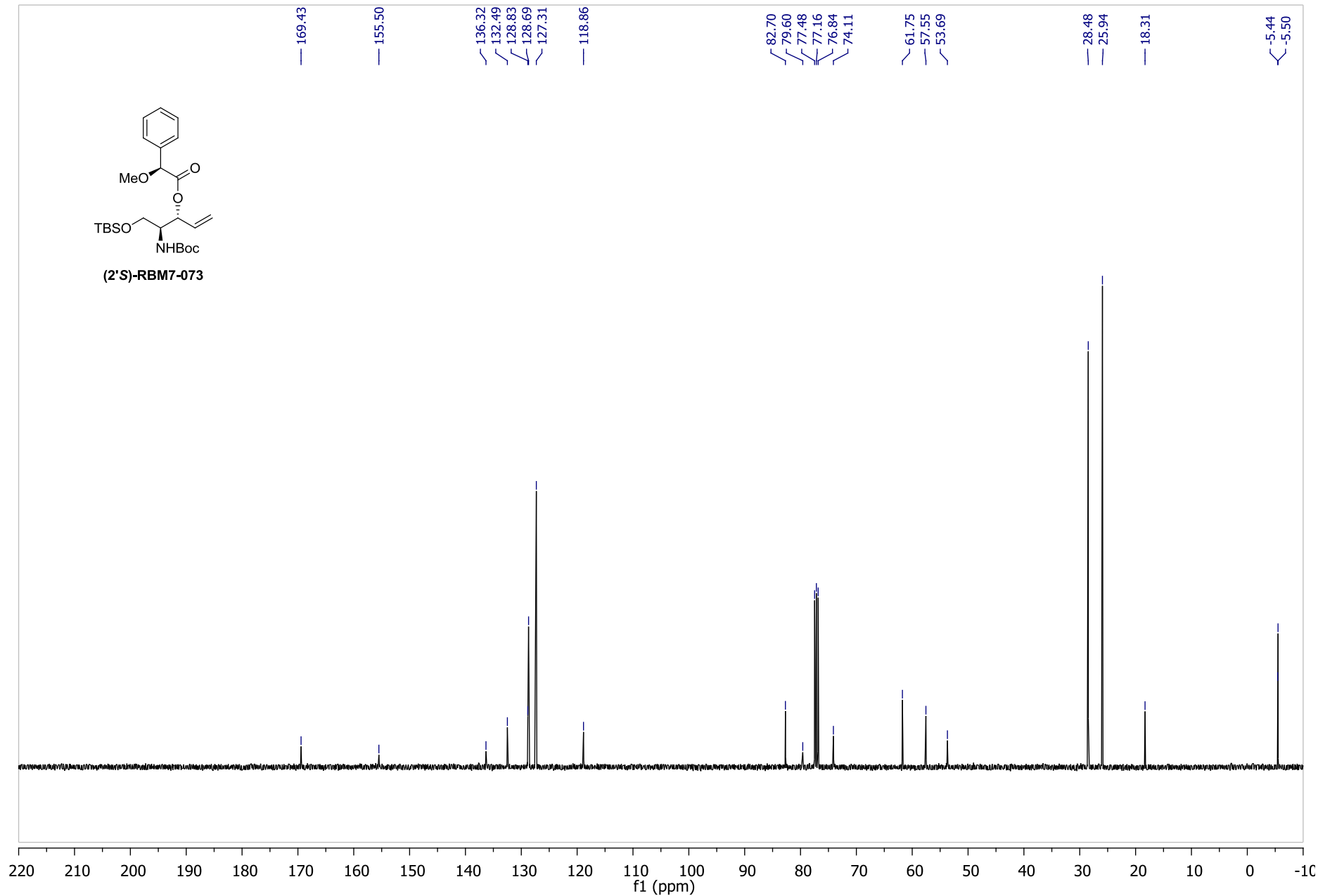
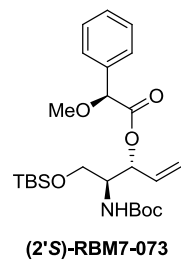
S153



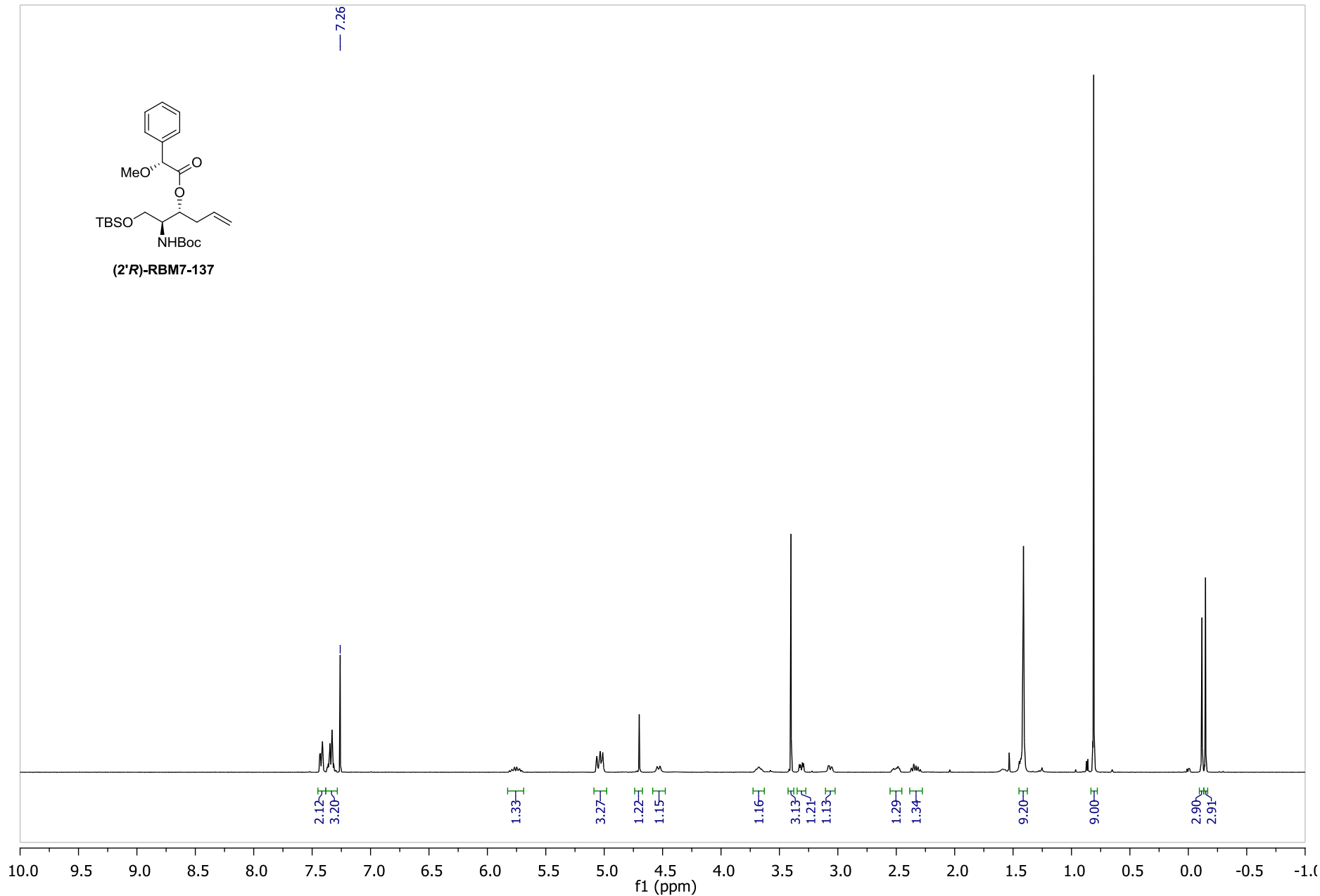
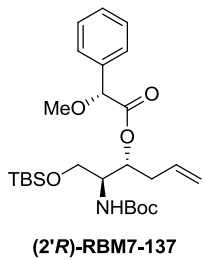
S154



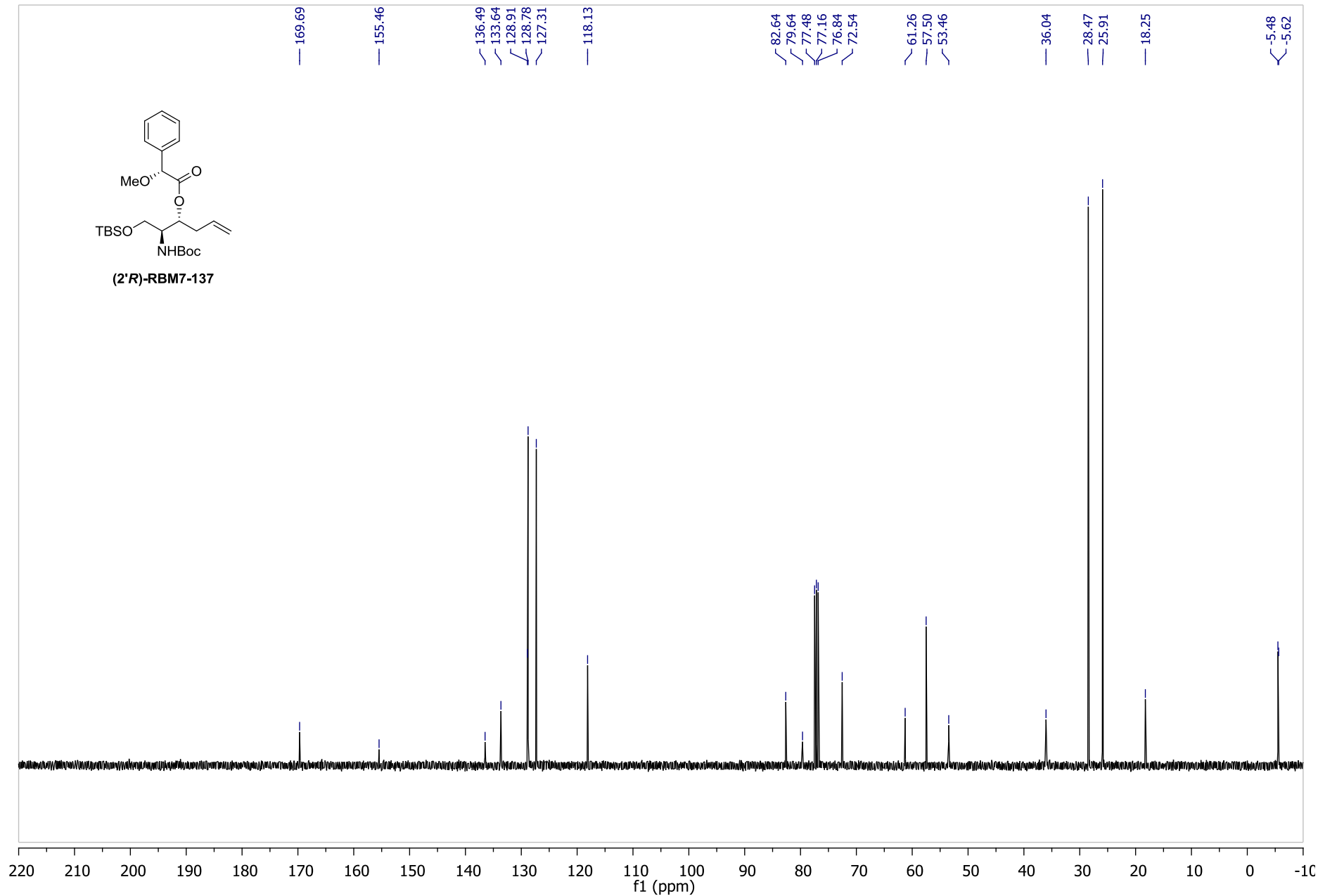
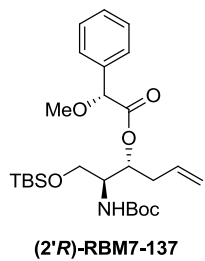
S156



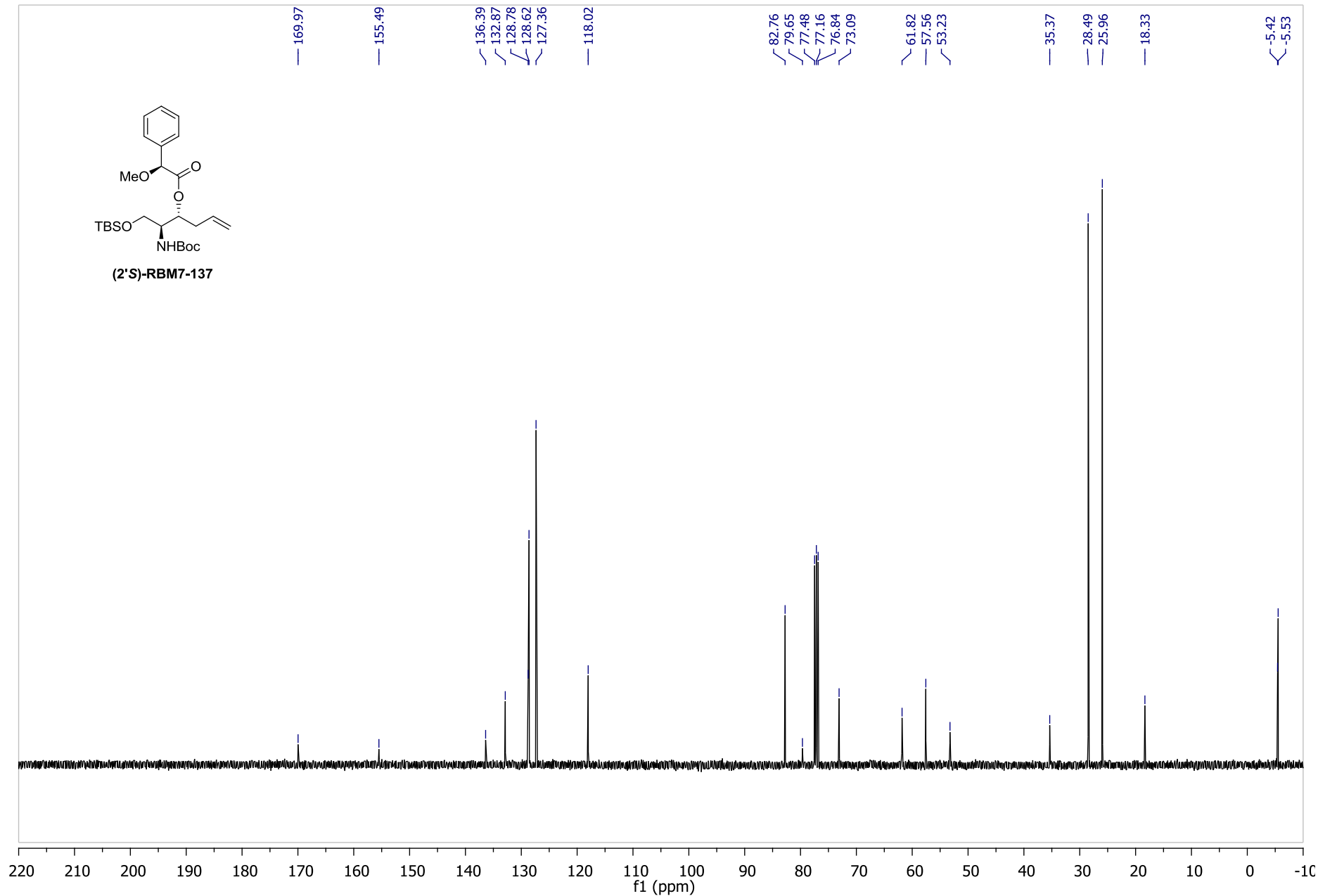
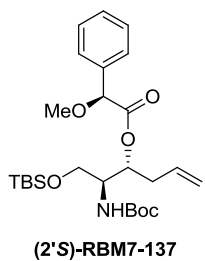
S157



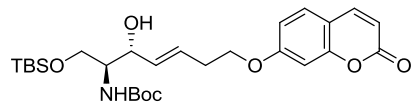
S158



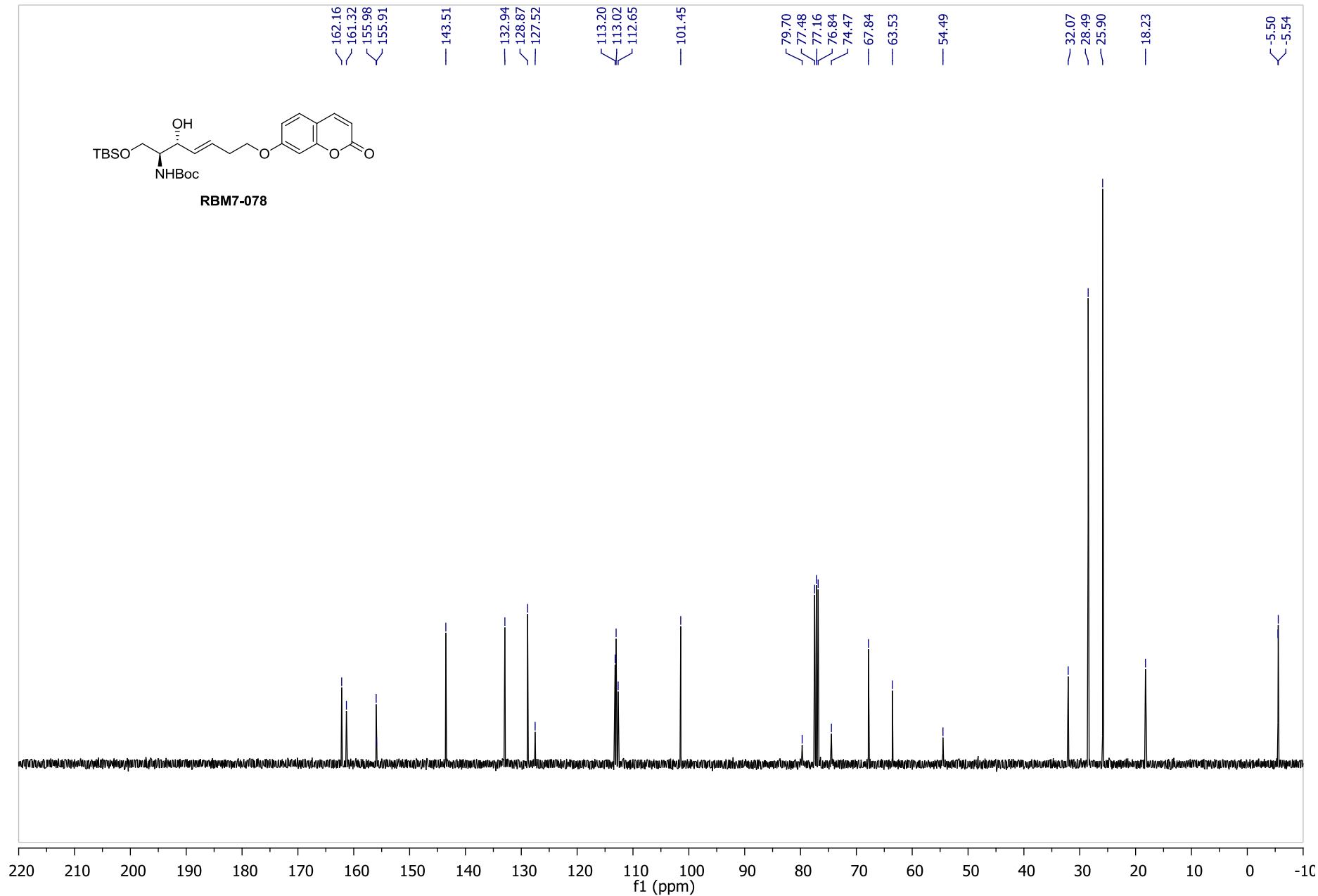
S159

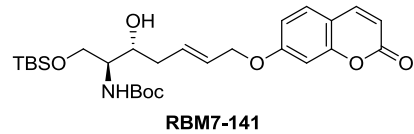


S161

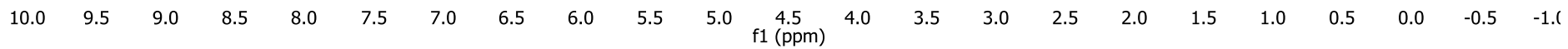


RBM7-078

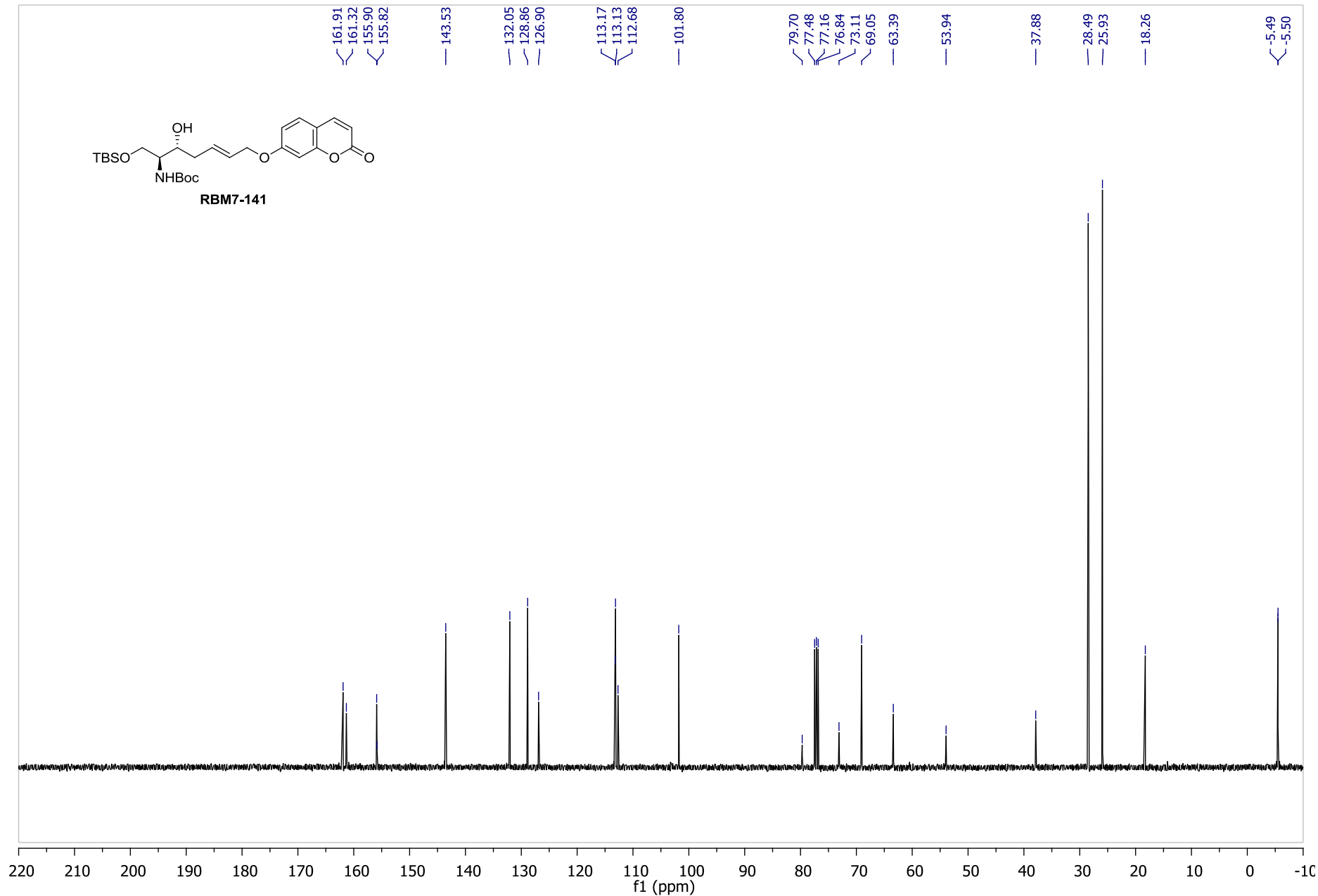
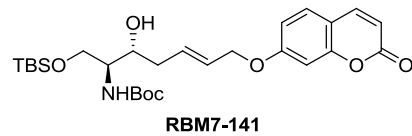




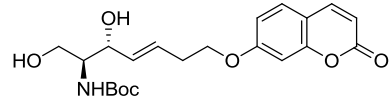
7.26



S164

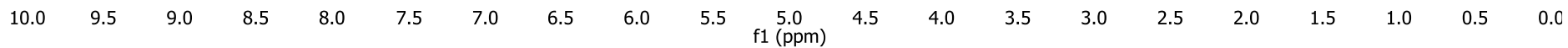


S165

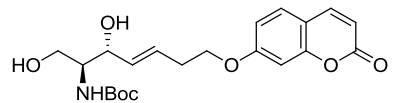


RBM7-079

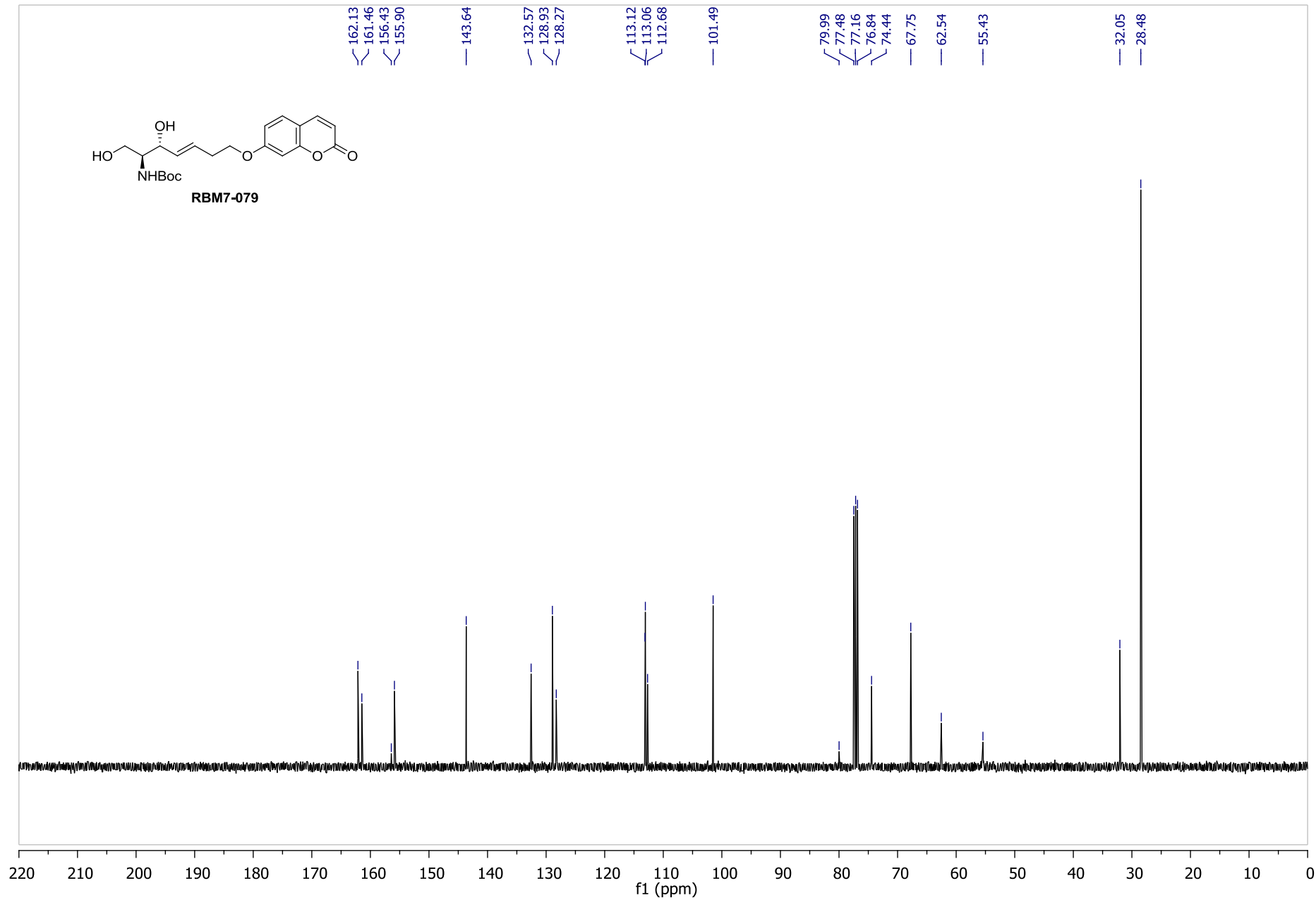
7.26

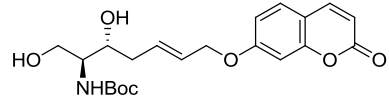


S166



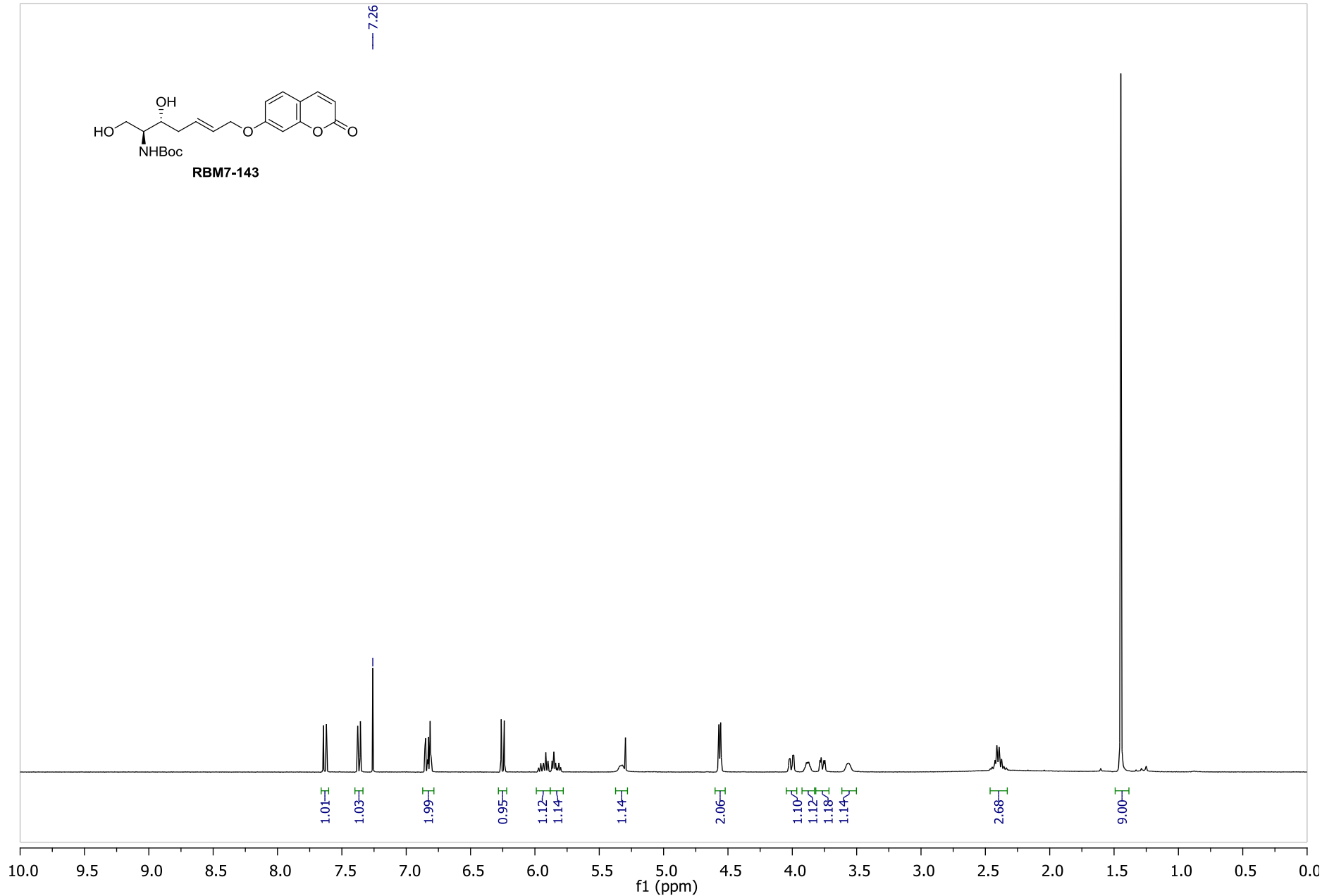
RBM7-079



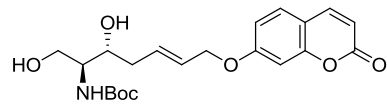


RBM7-143

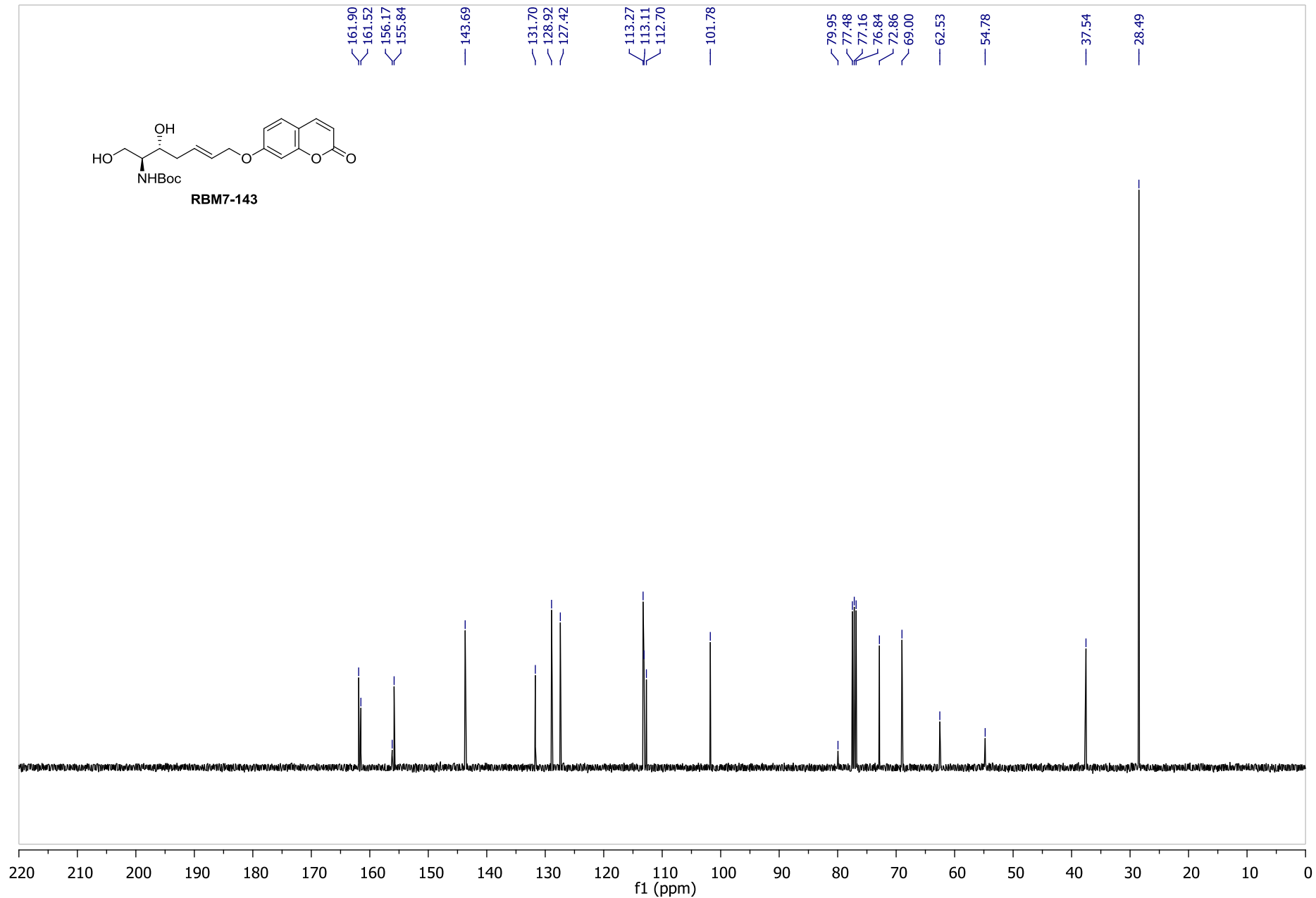
7.26

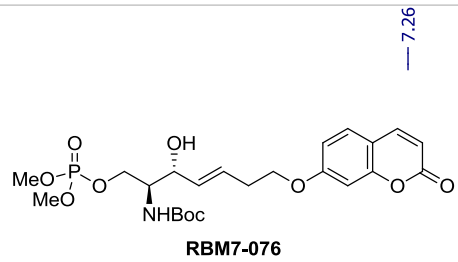


S168

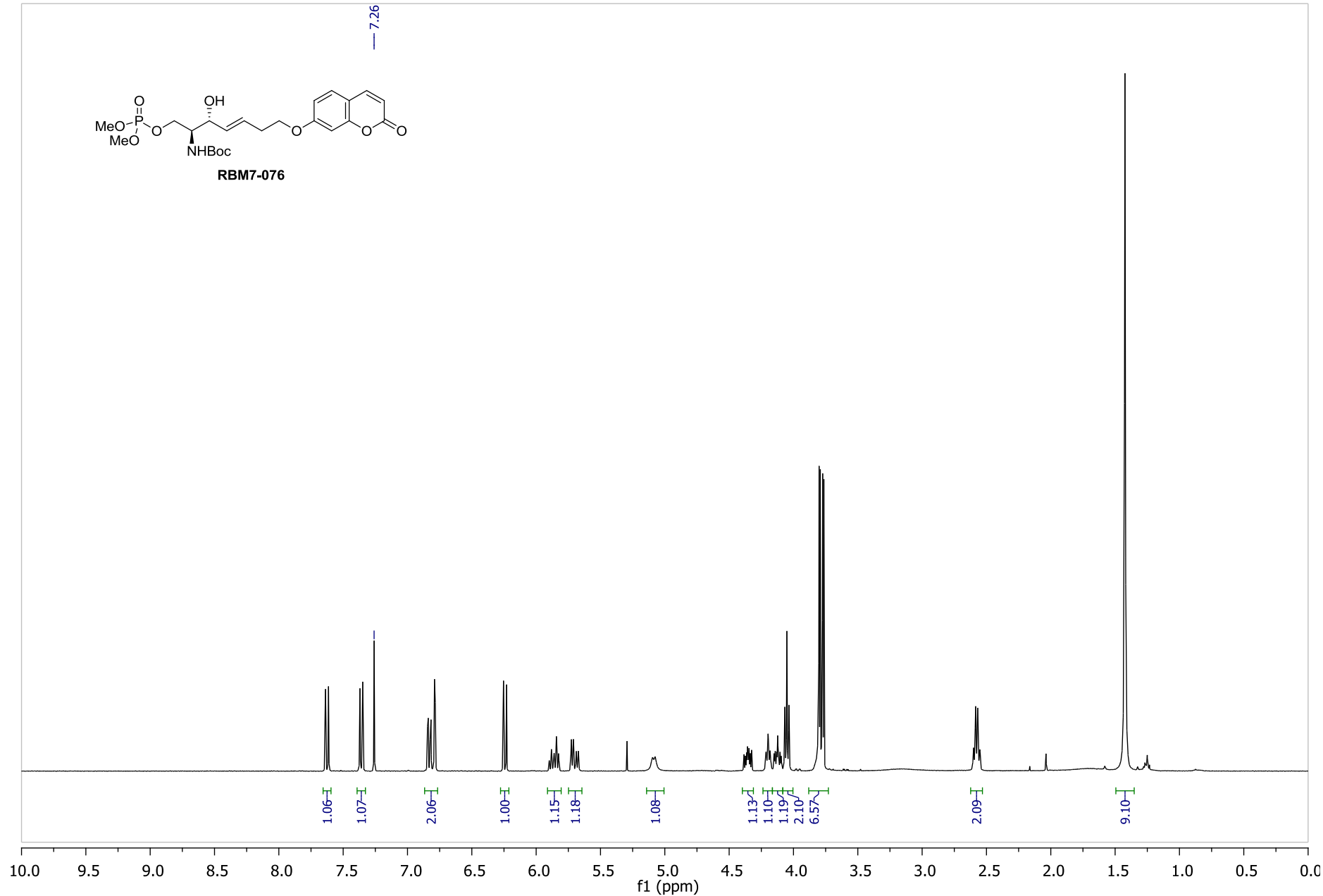


RBM7-143

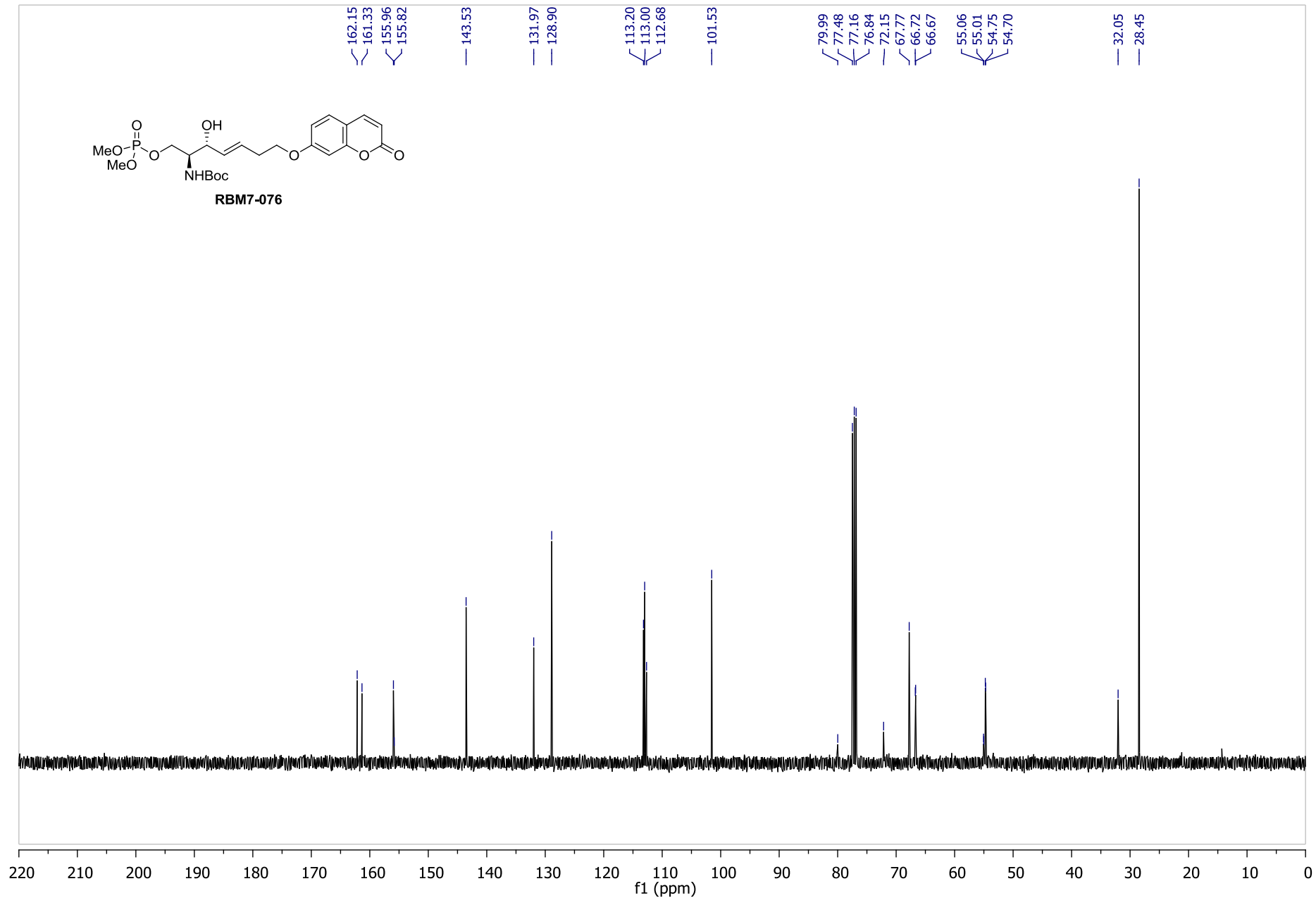
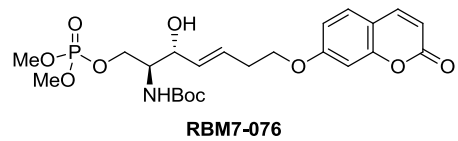




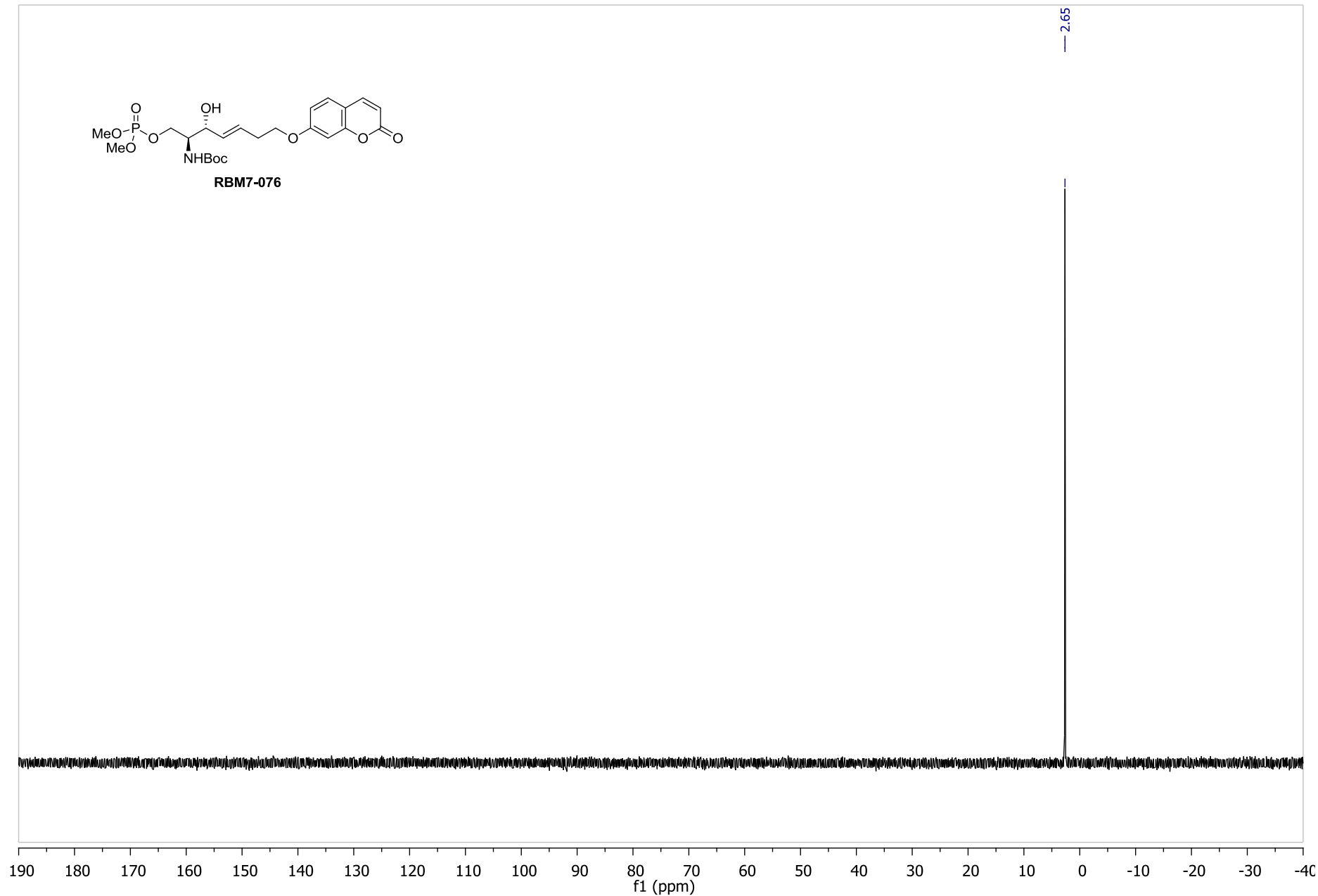
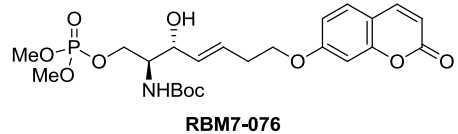
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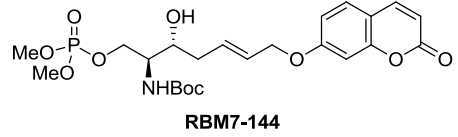
S170



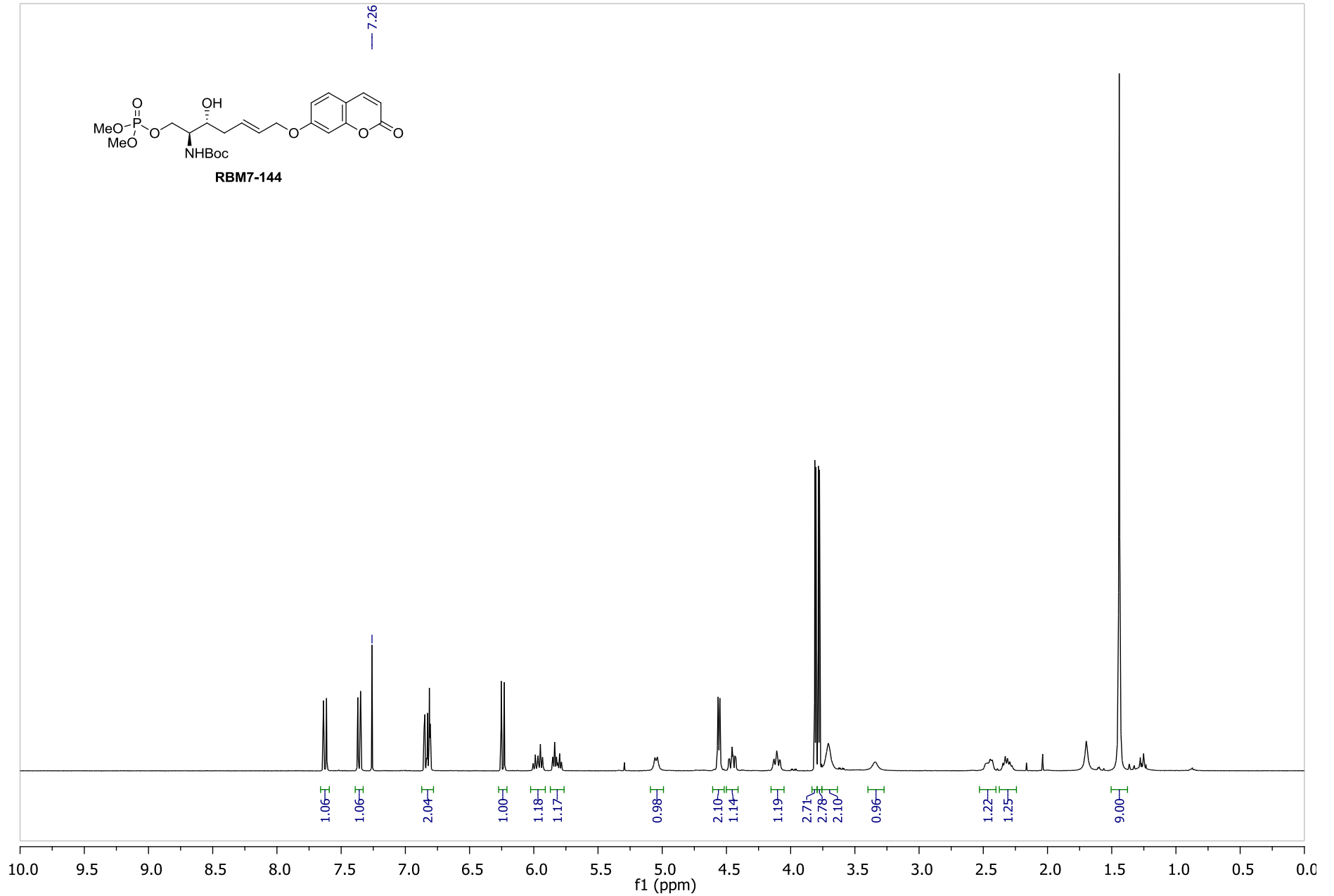
S171



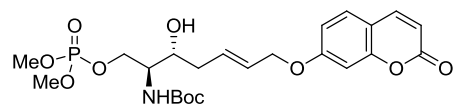
S172



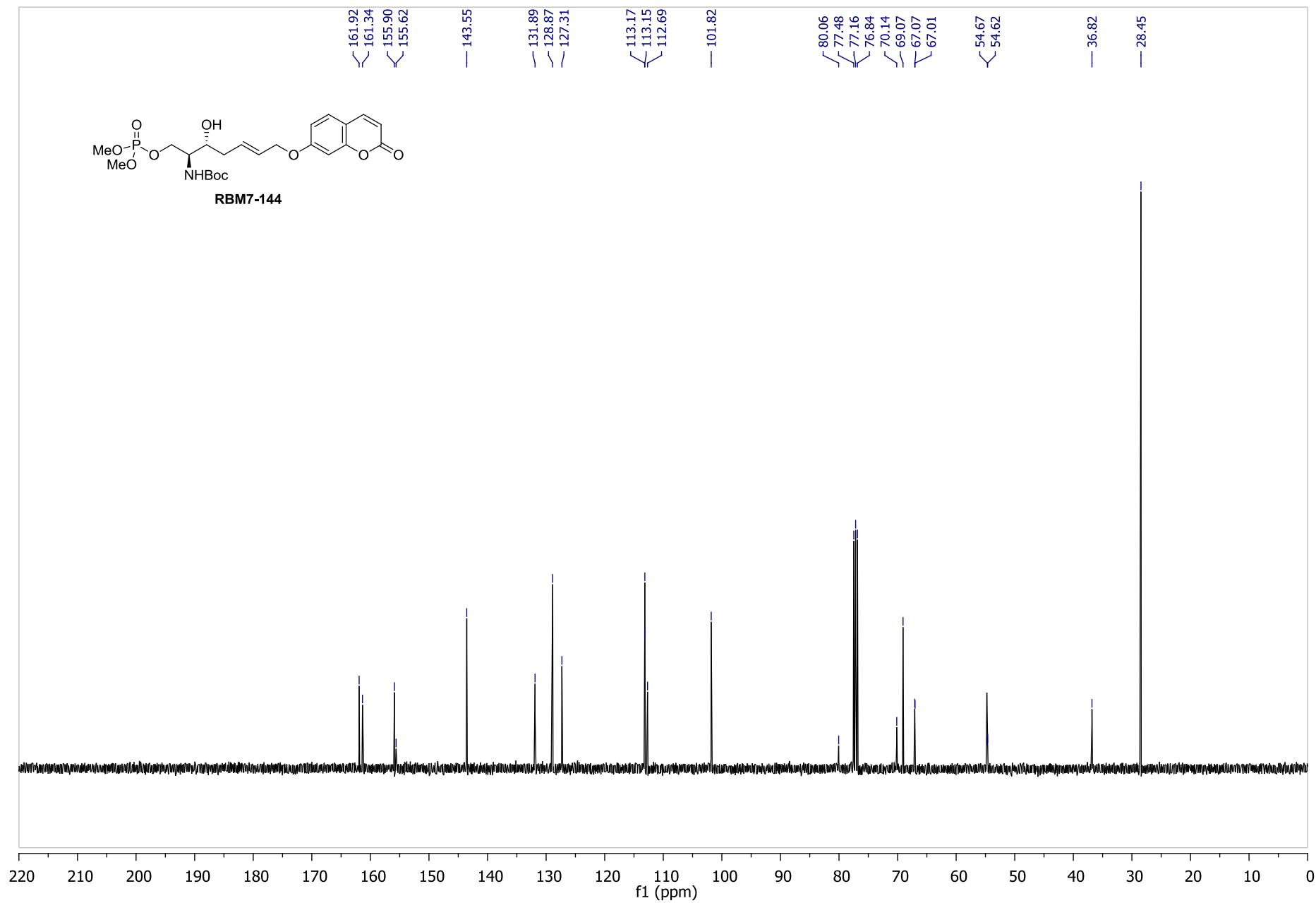
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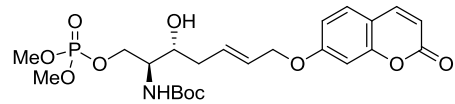


S173

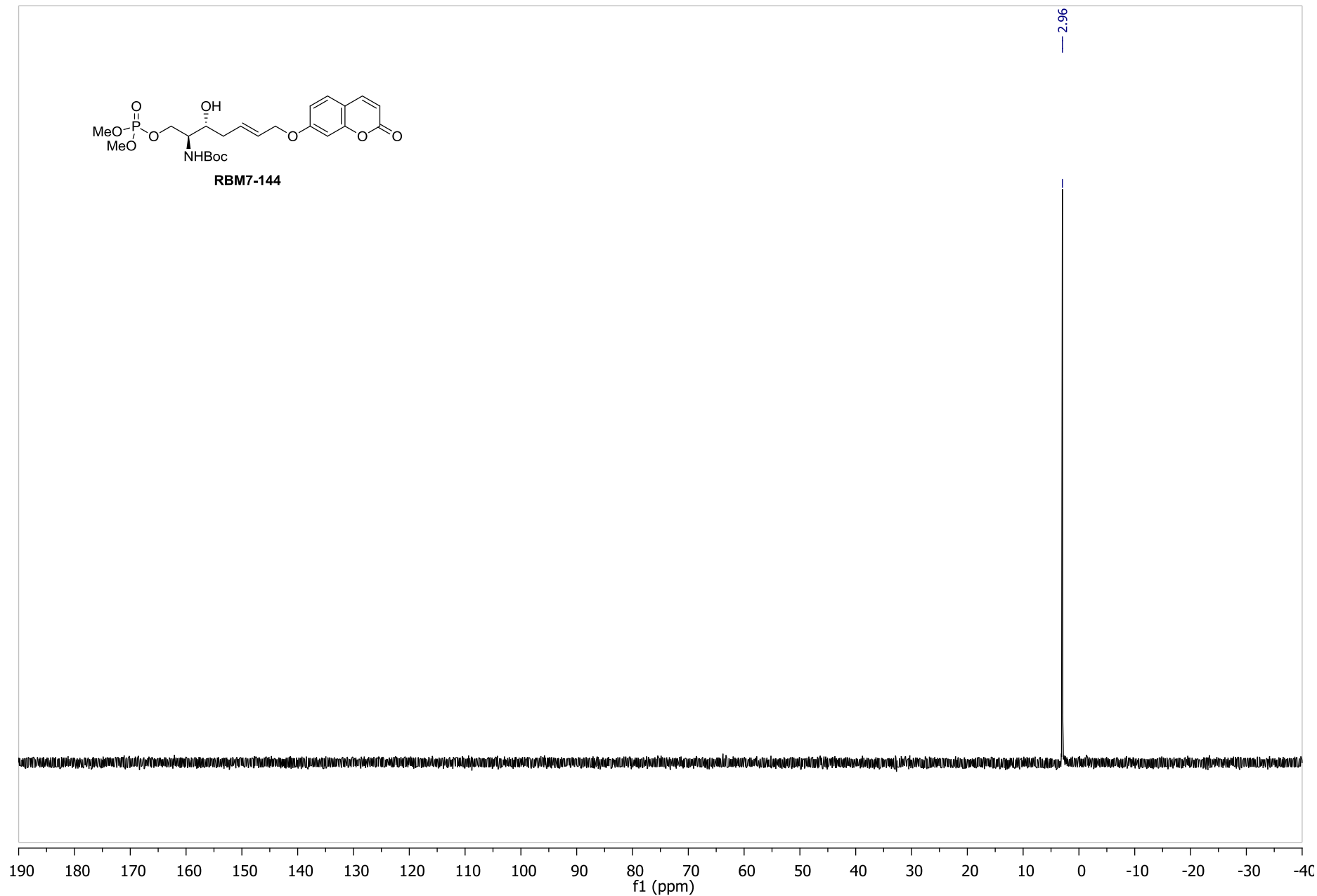


RBM7-144

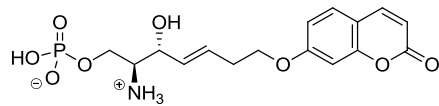




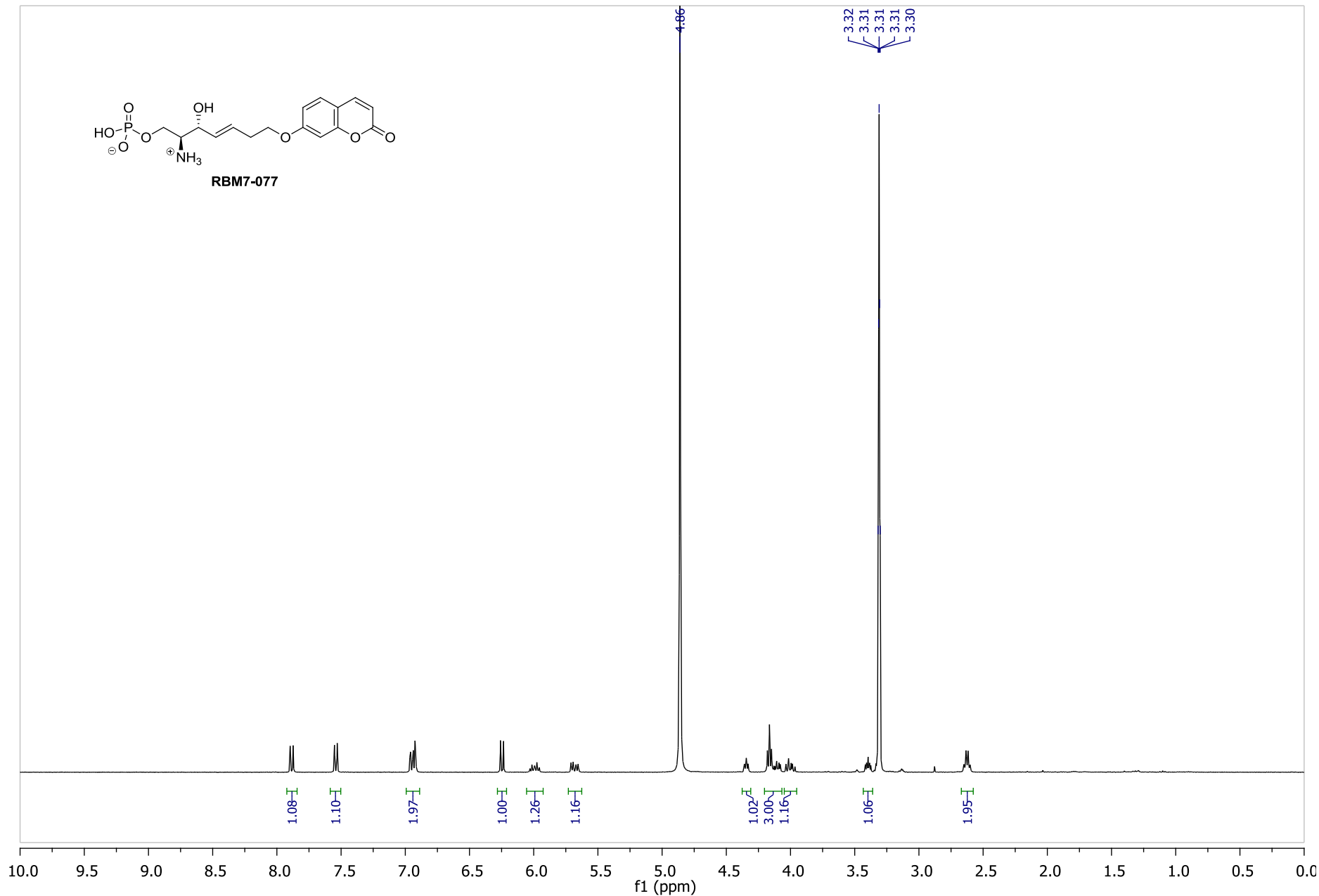
RBM7-144

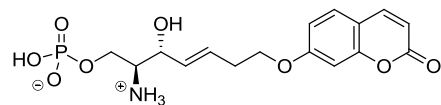


S175

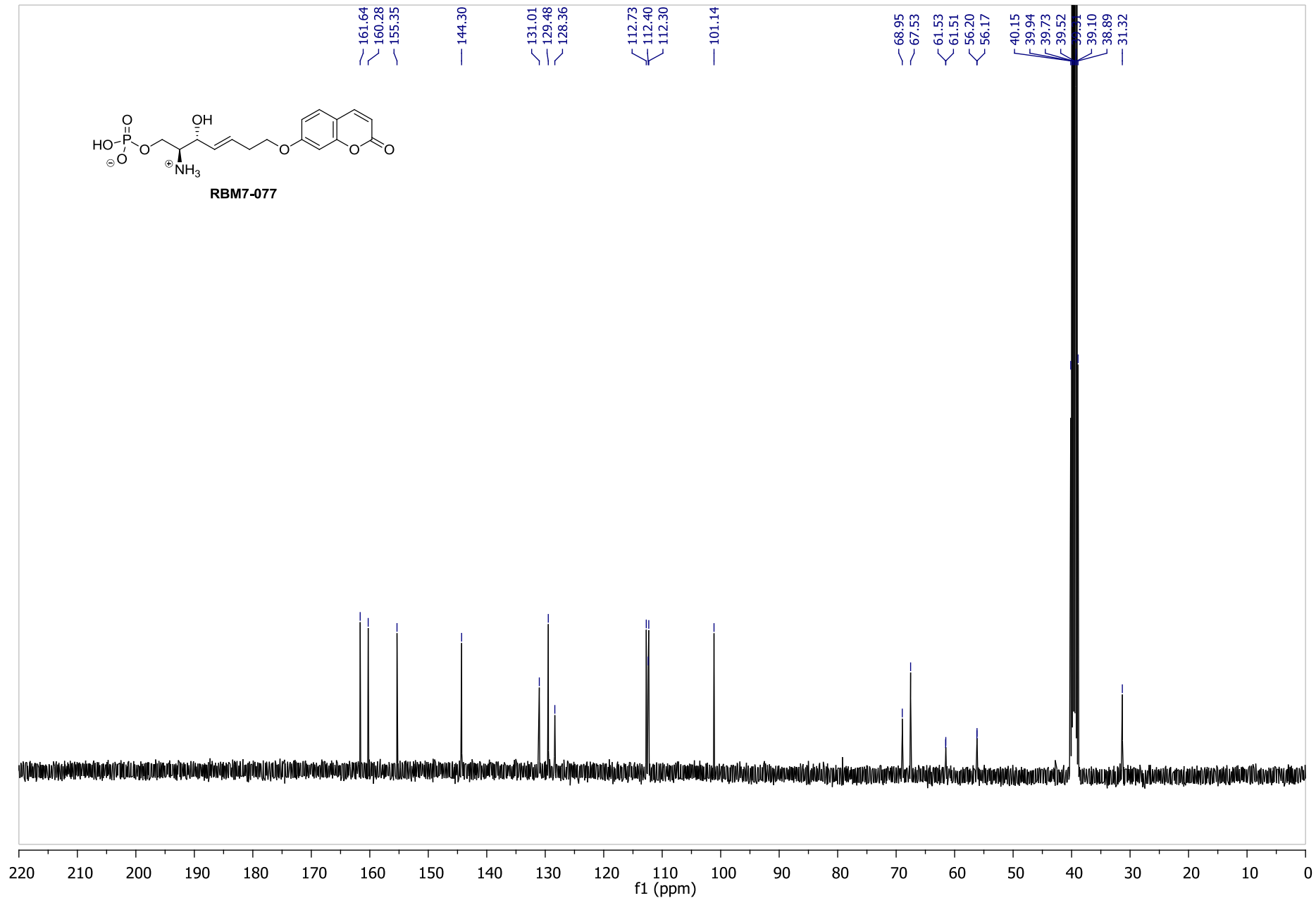


RBM7-077

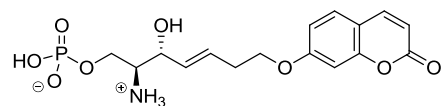




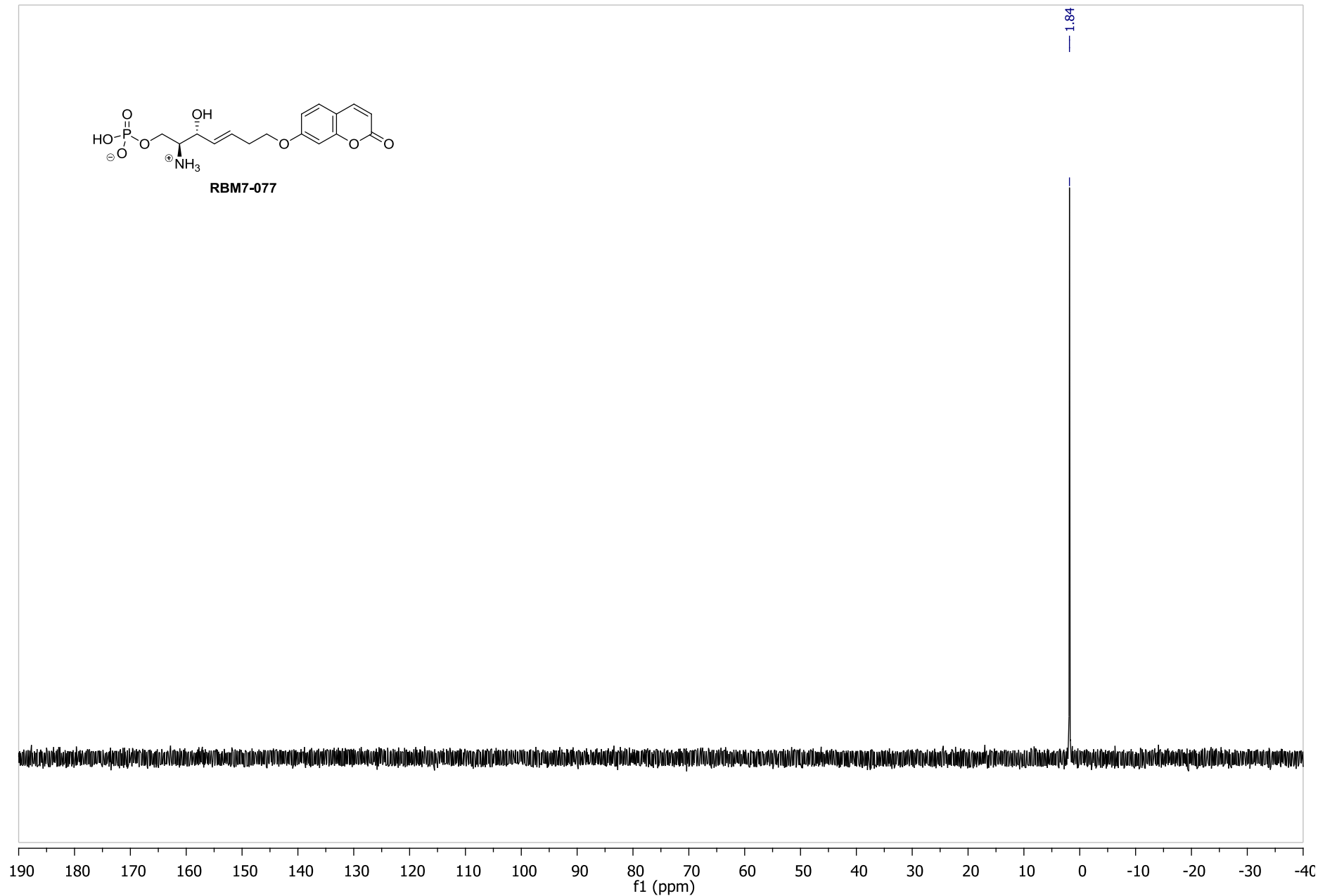
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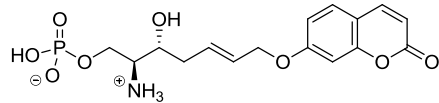


S177

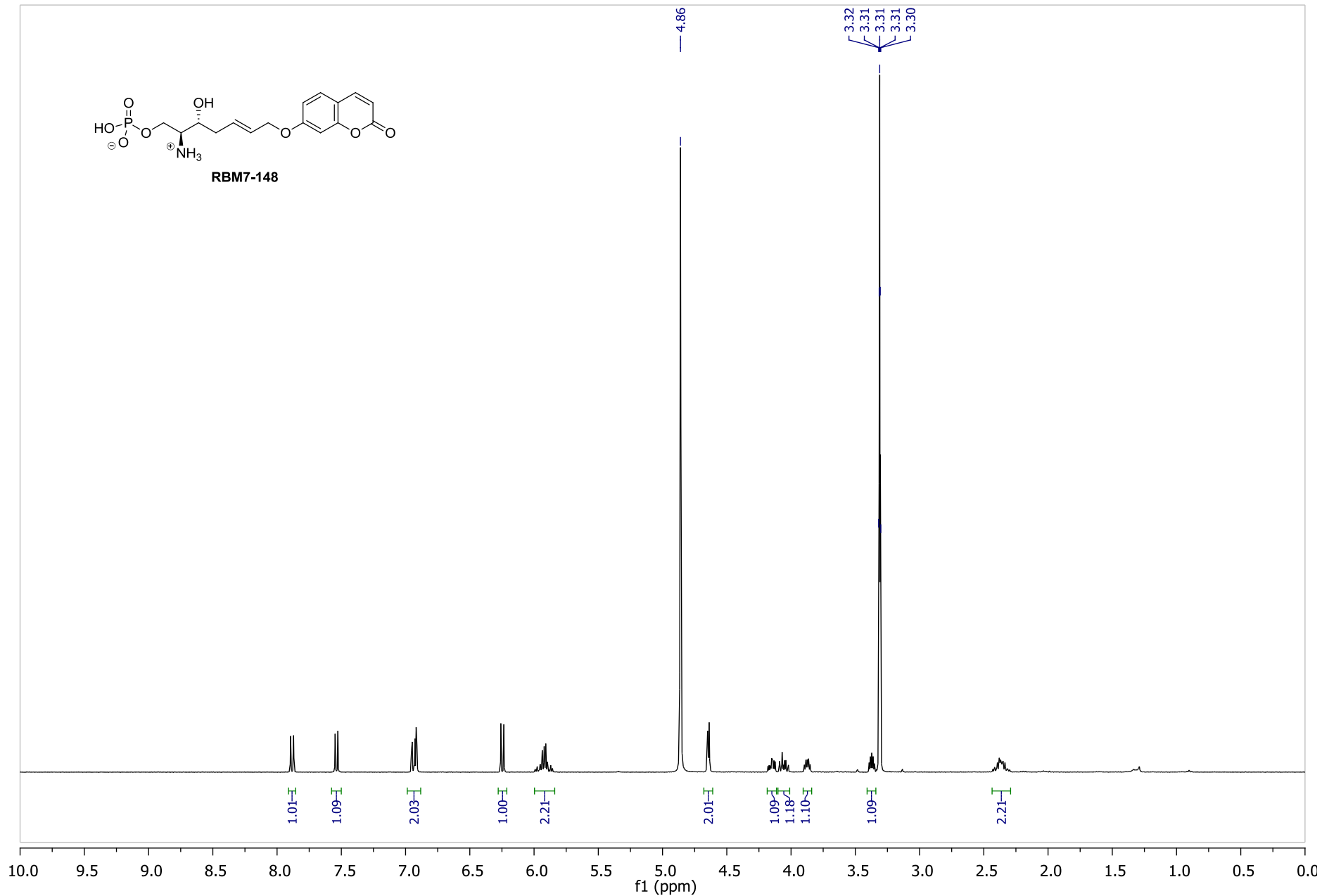


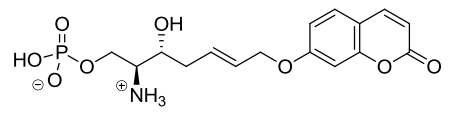
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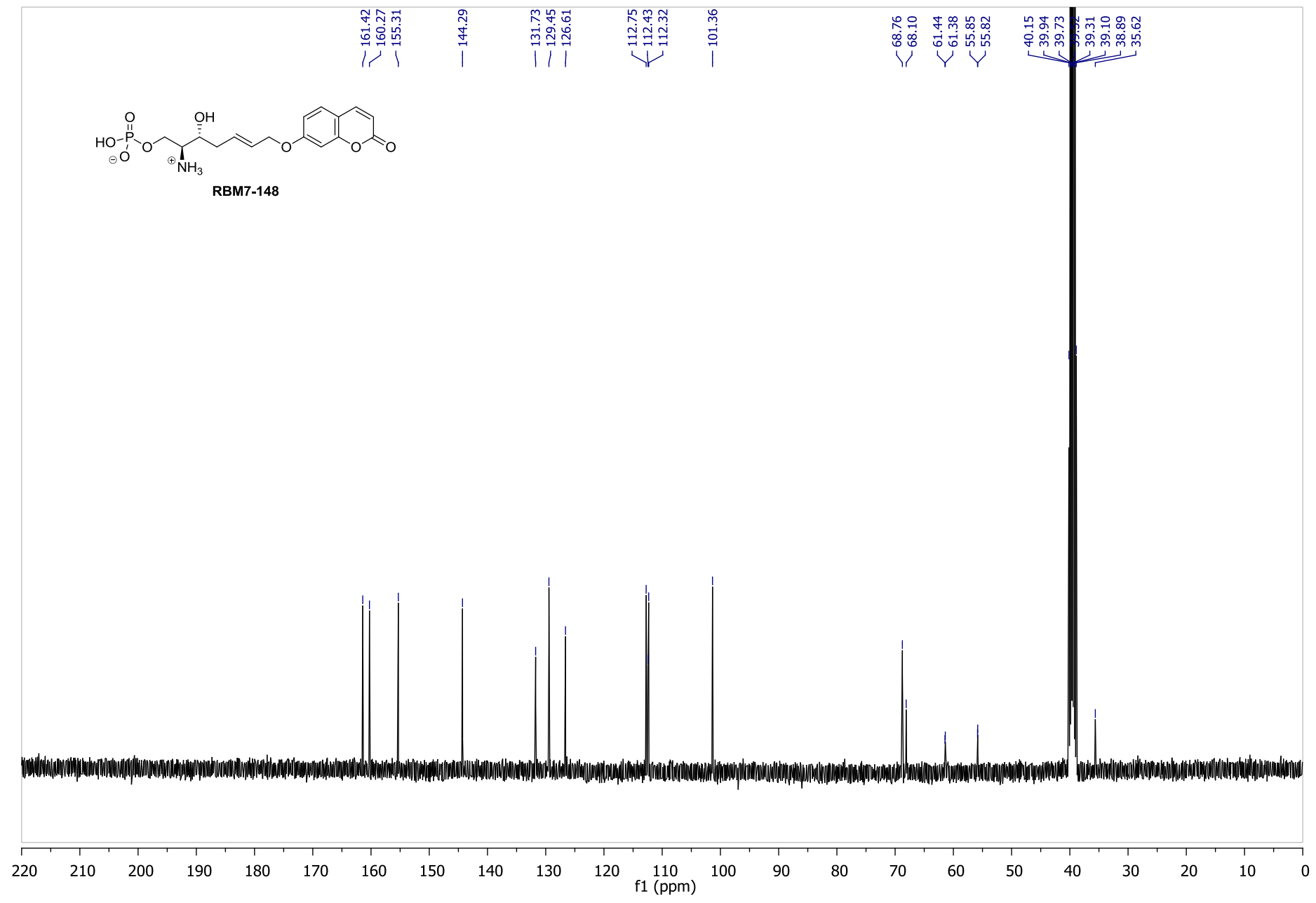


RBM7-148

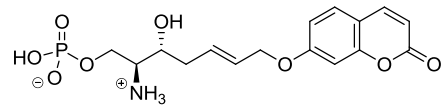




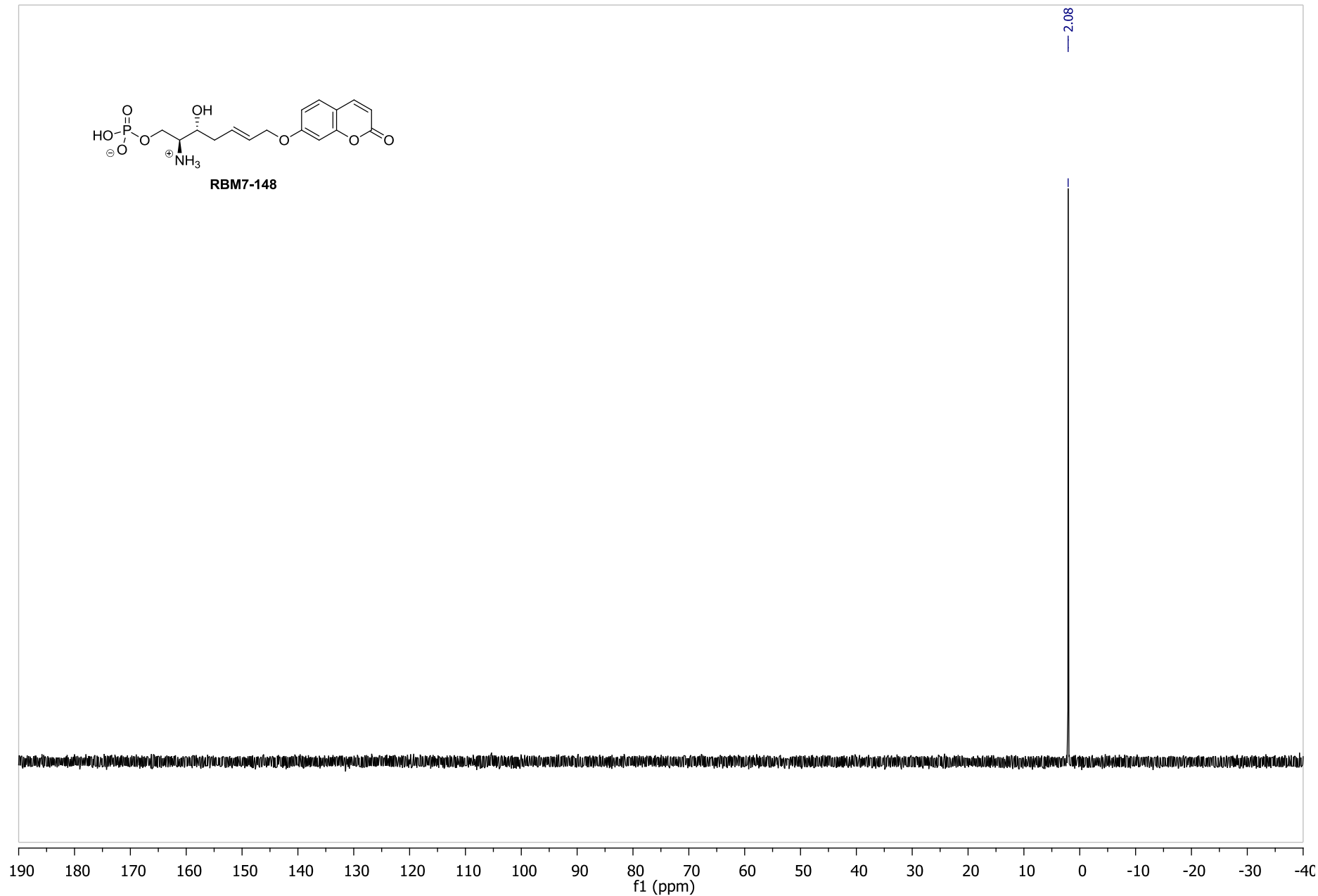
RBM7-148



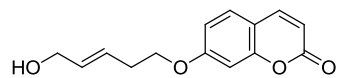
S180



RBM7-148

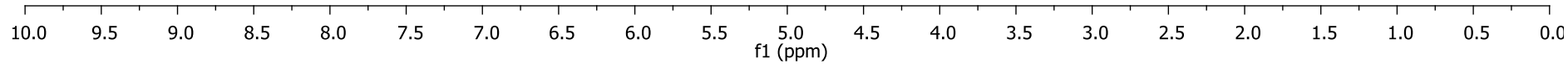


S181

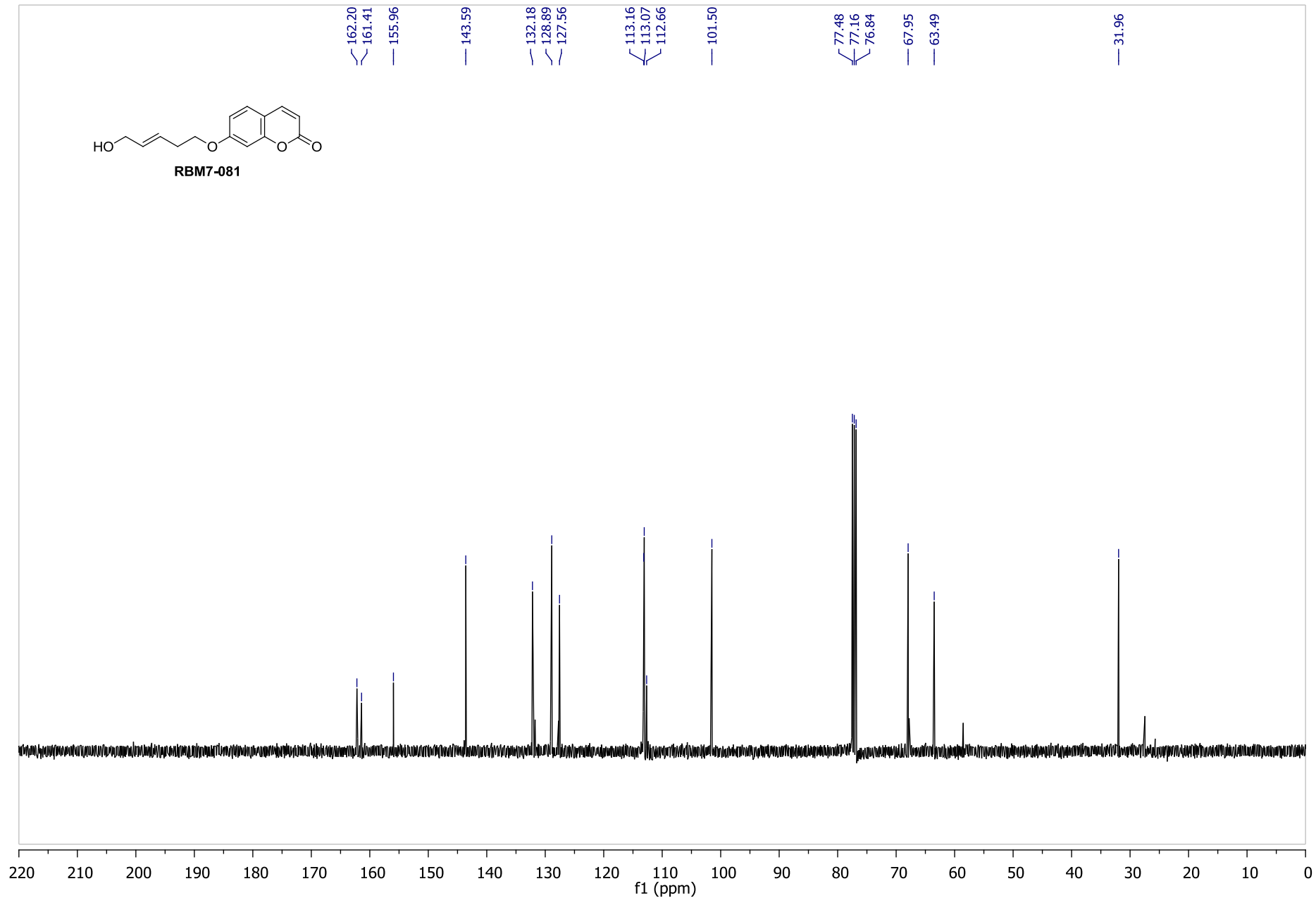


RBM7-081

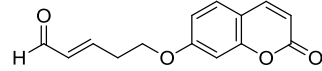
7.26



S182

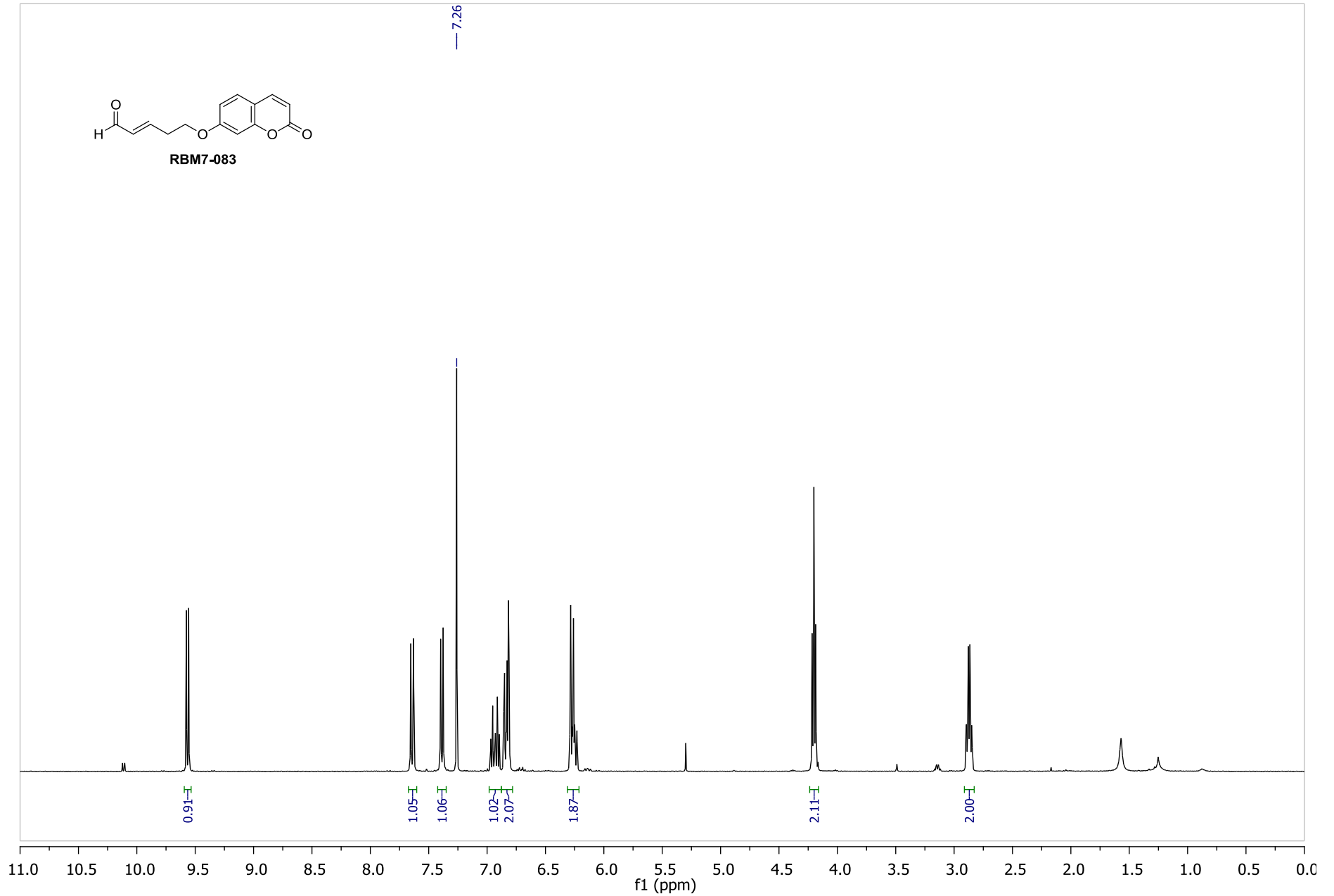


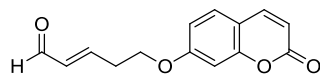
S183



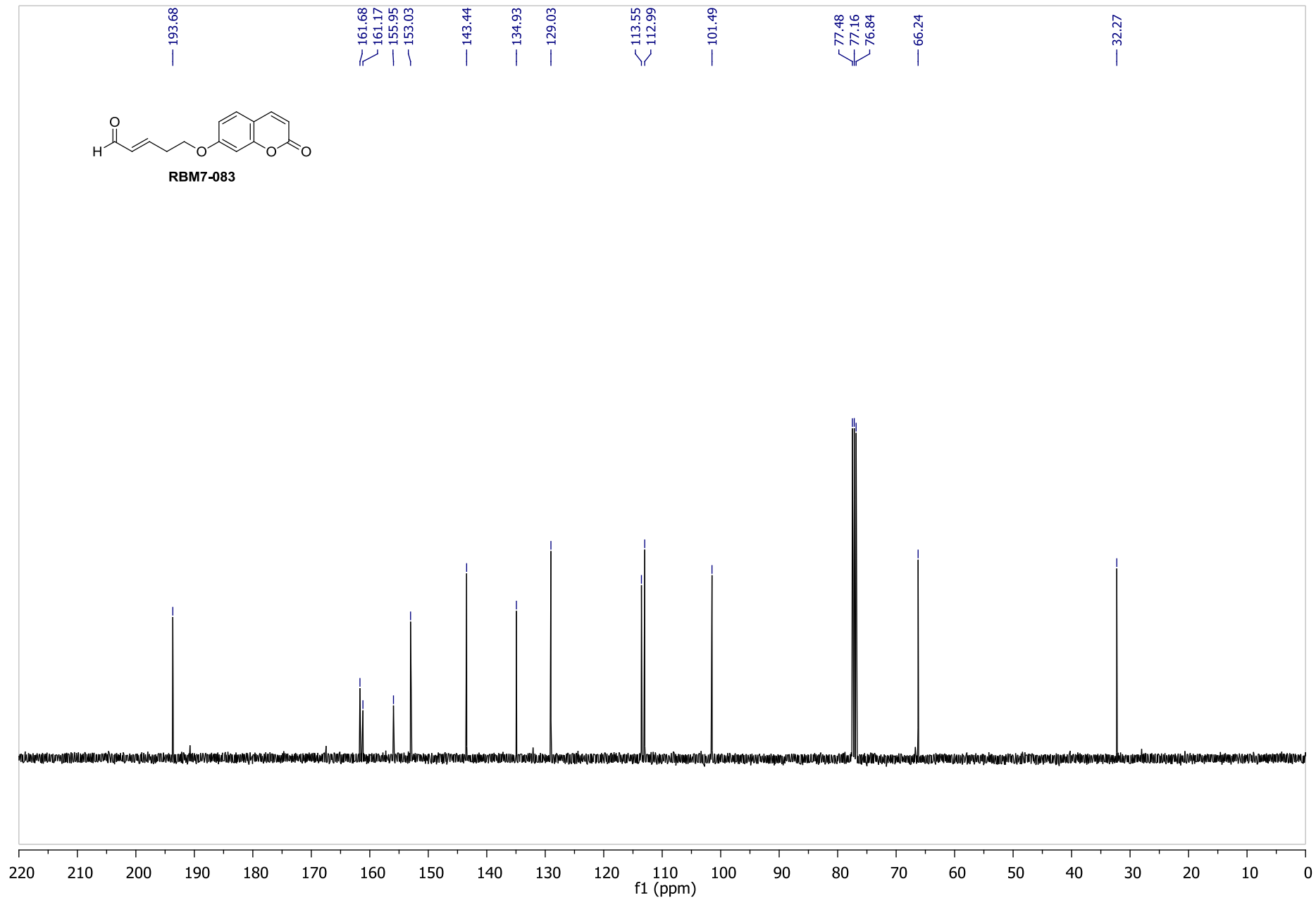
RBM7-083

7.26

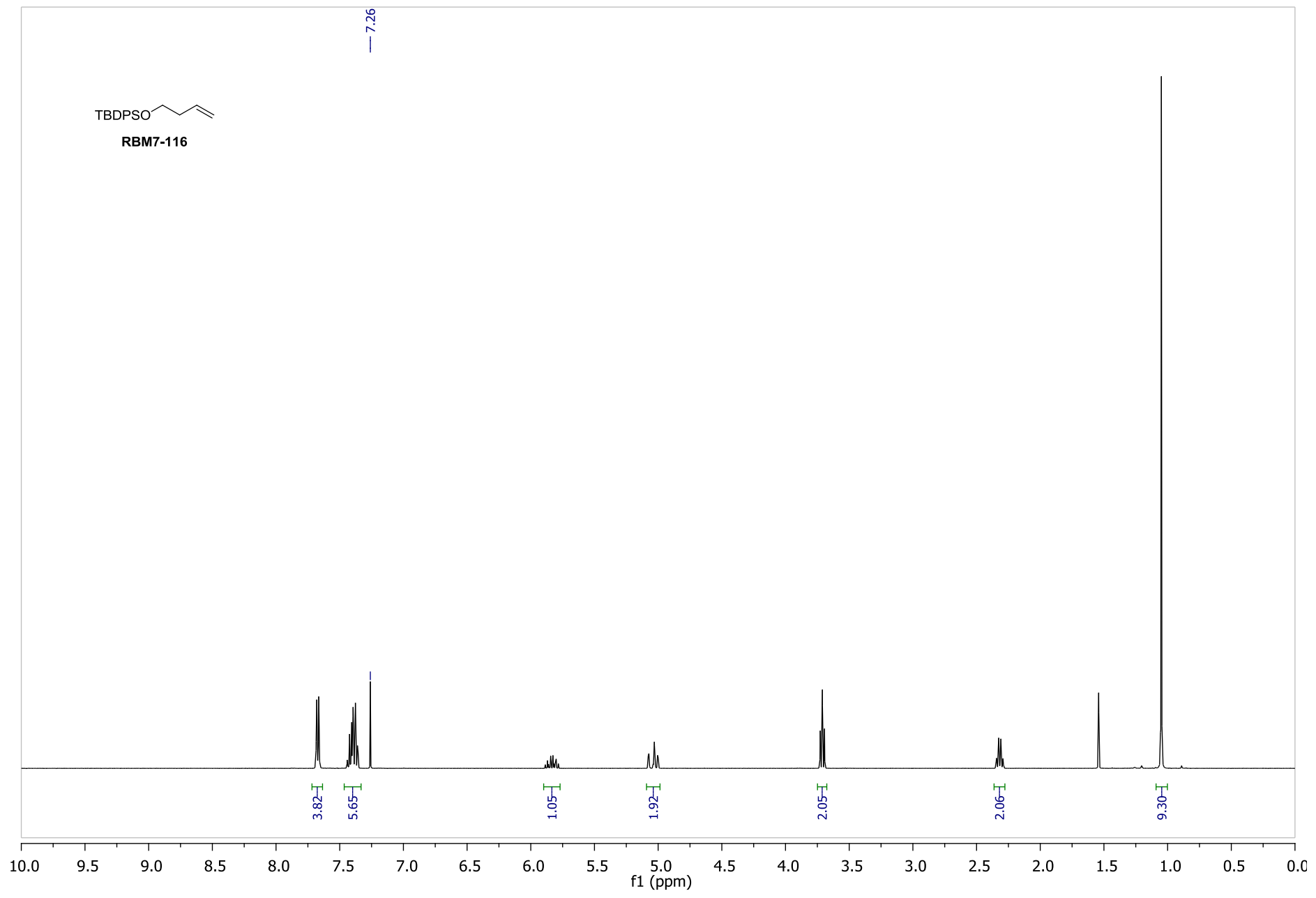
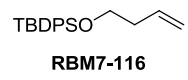




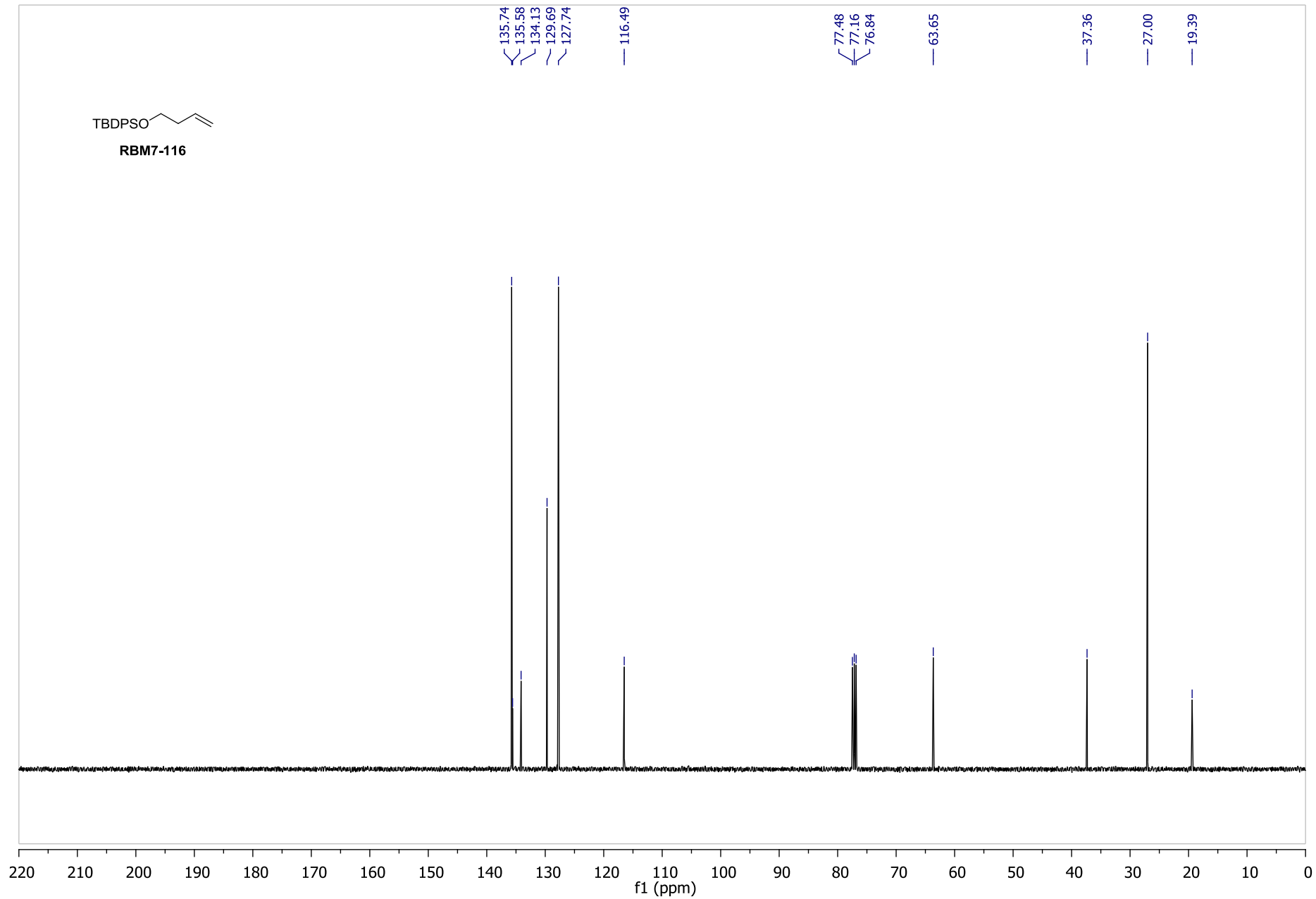
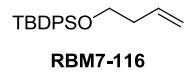
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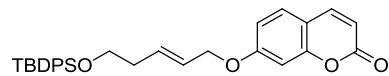
S185



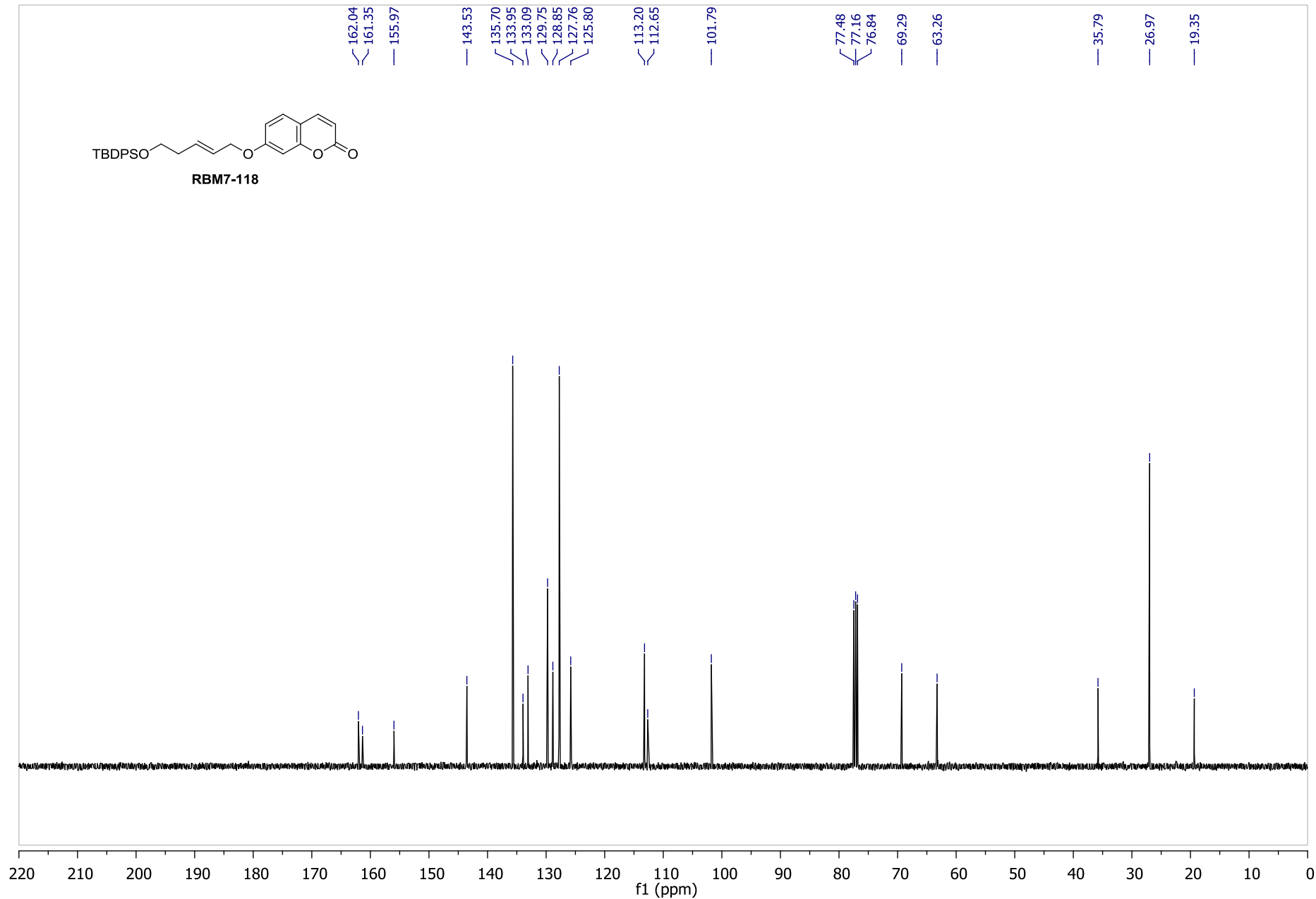
S186

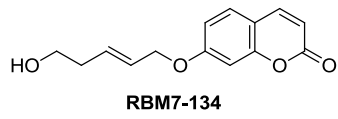


S187

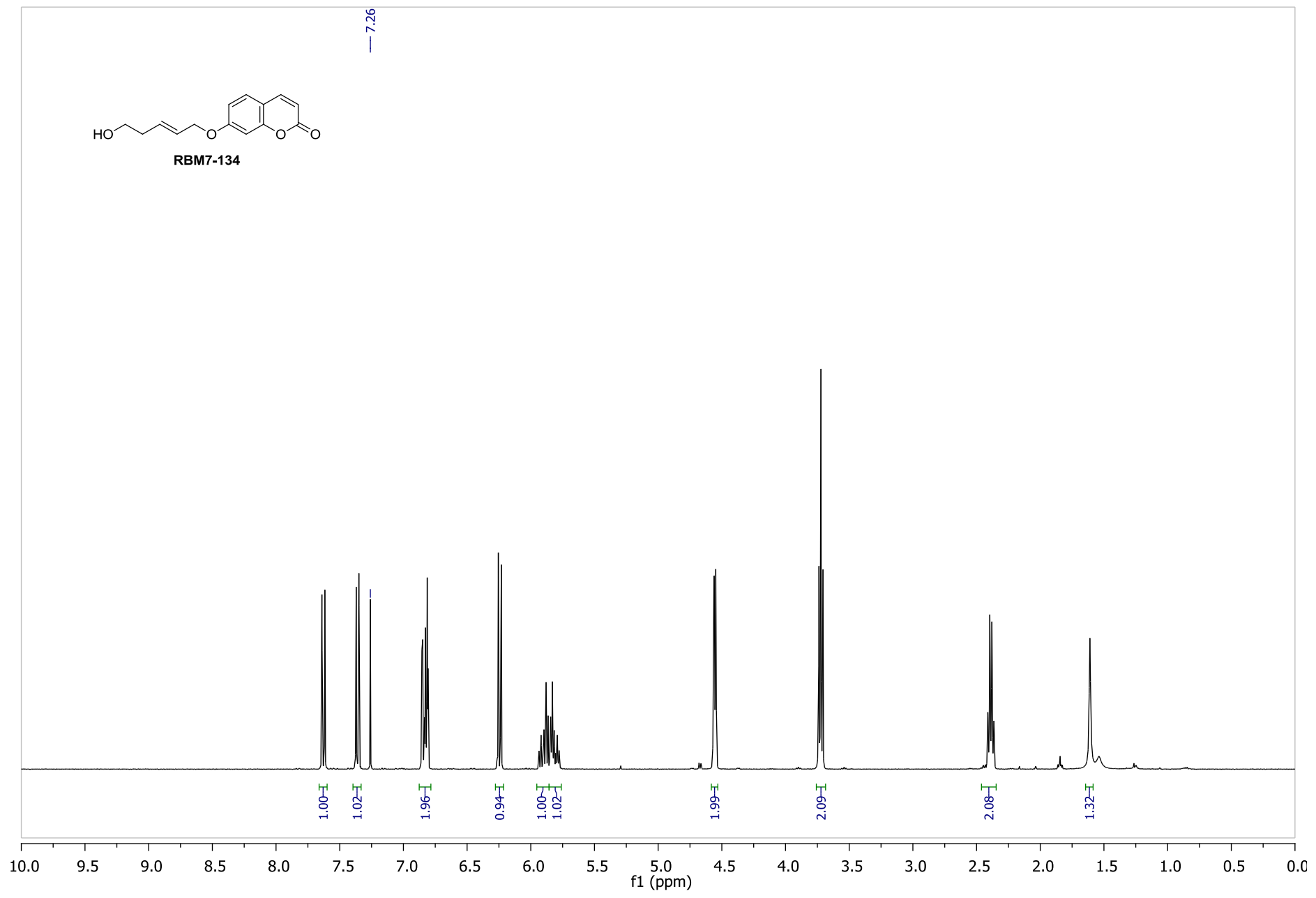


RBM7-118

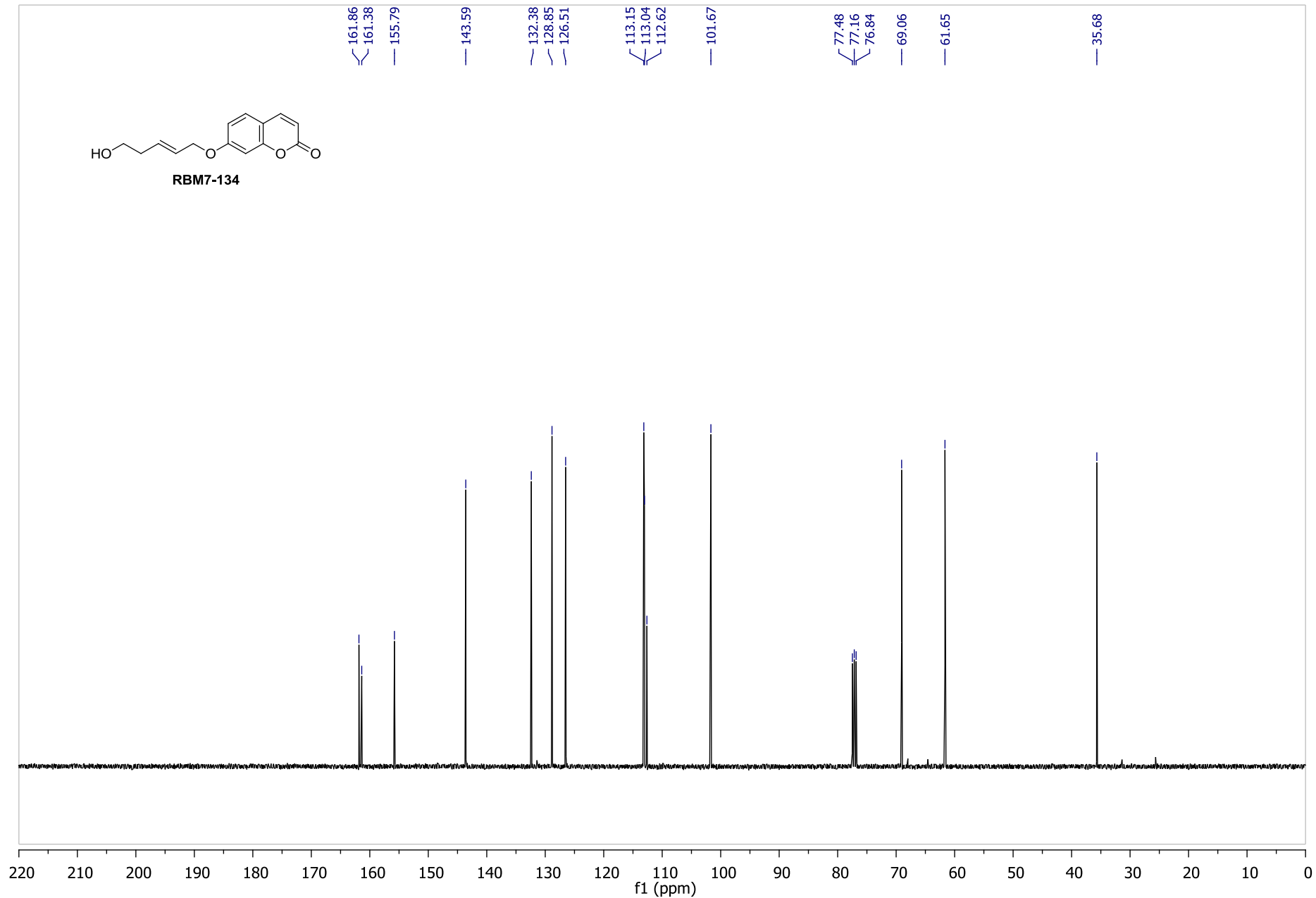
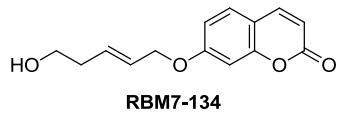




7.26



S190



S191