

# Large scale conversion of trilobolide into the payload of Mipsagargin: 8-*O*-(12-aminododecanoyl)-8-*O*-debutanoylthapsigargin

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# Product 1 (3-oxo-3-desangeloyl trilobolide)

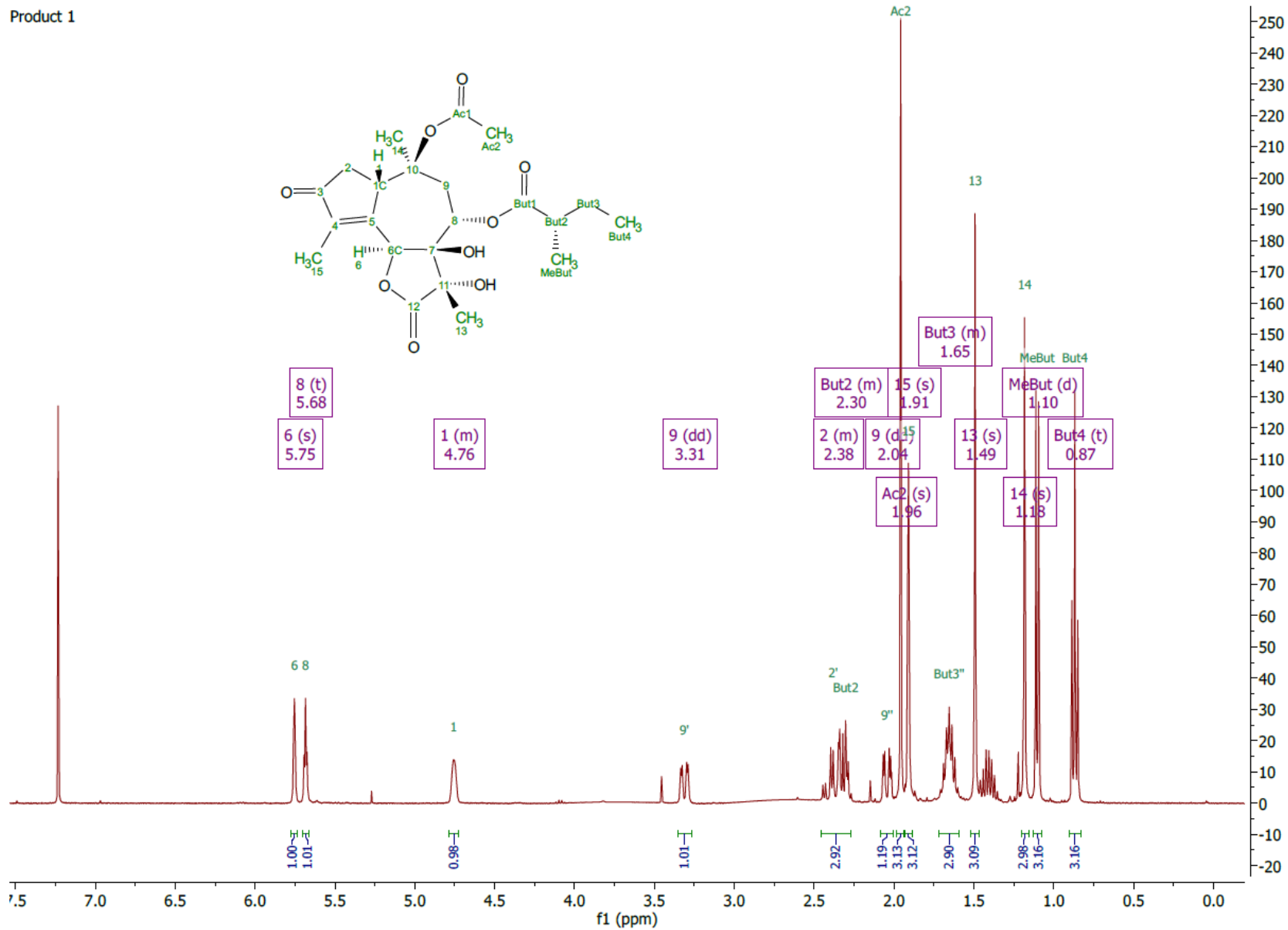


Figure S1A – <sup>1</sup>H NMR of product 1 (400 MHz, CDCl<sub>3</sub>)

Product 1

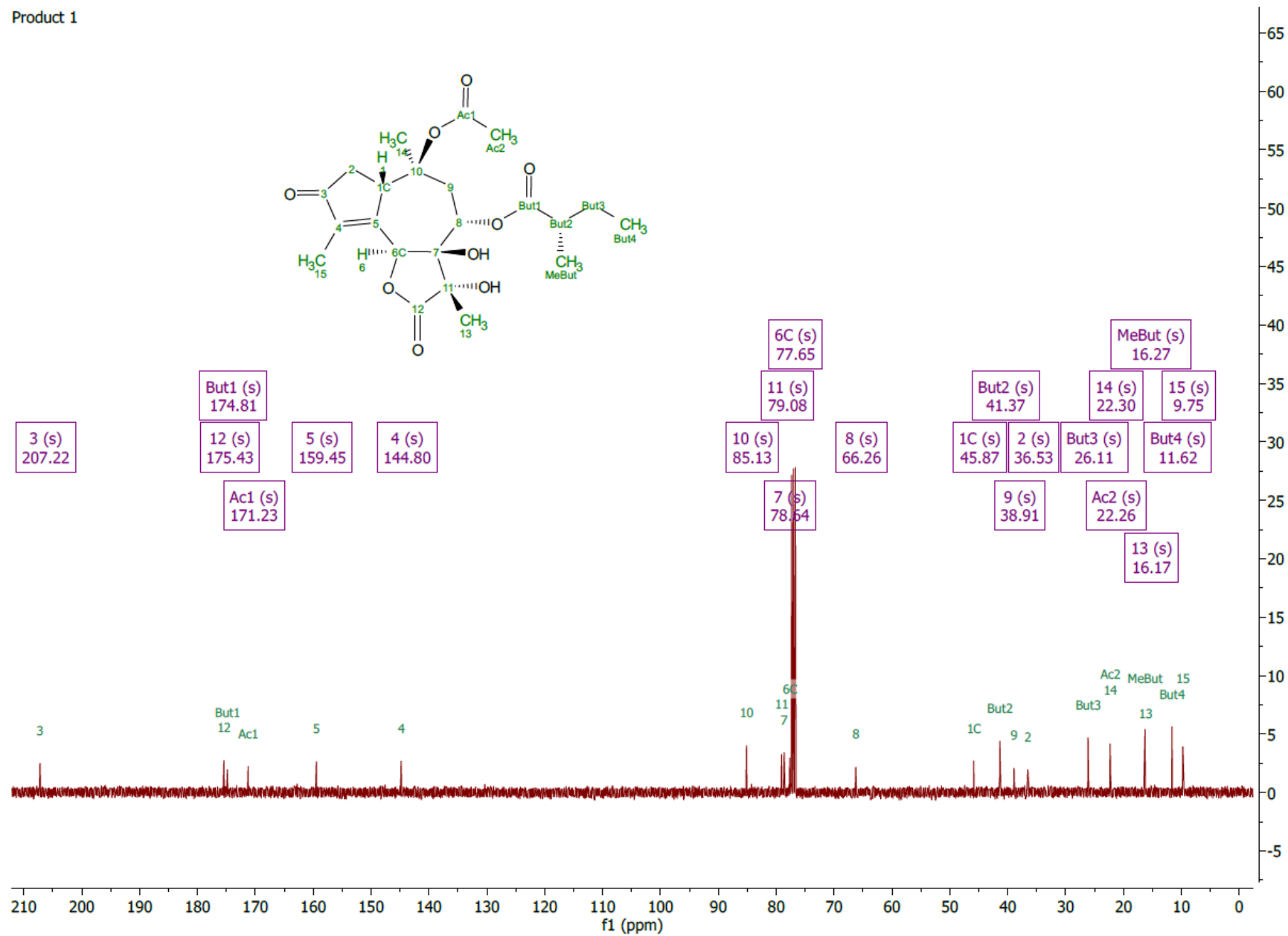


Figure S1B – <sup>13</sup>C NMR of product 1 (100 MHz, CDCl<sub>3</sub>)

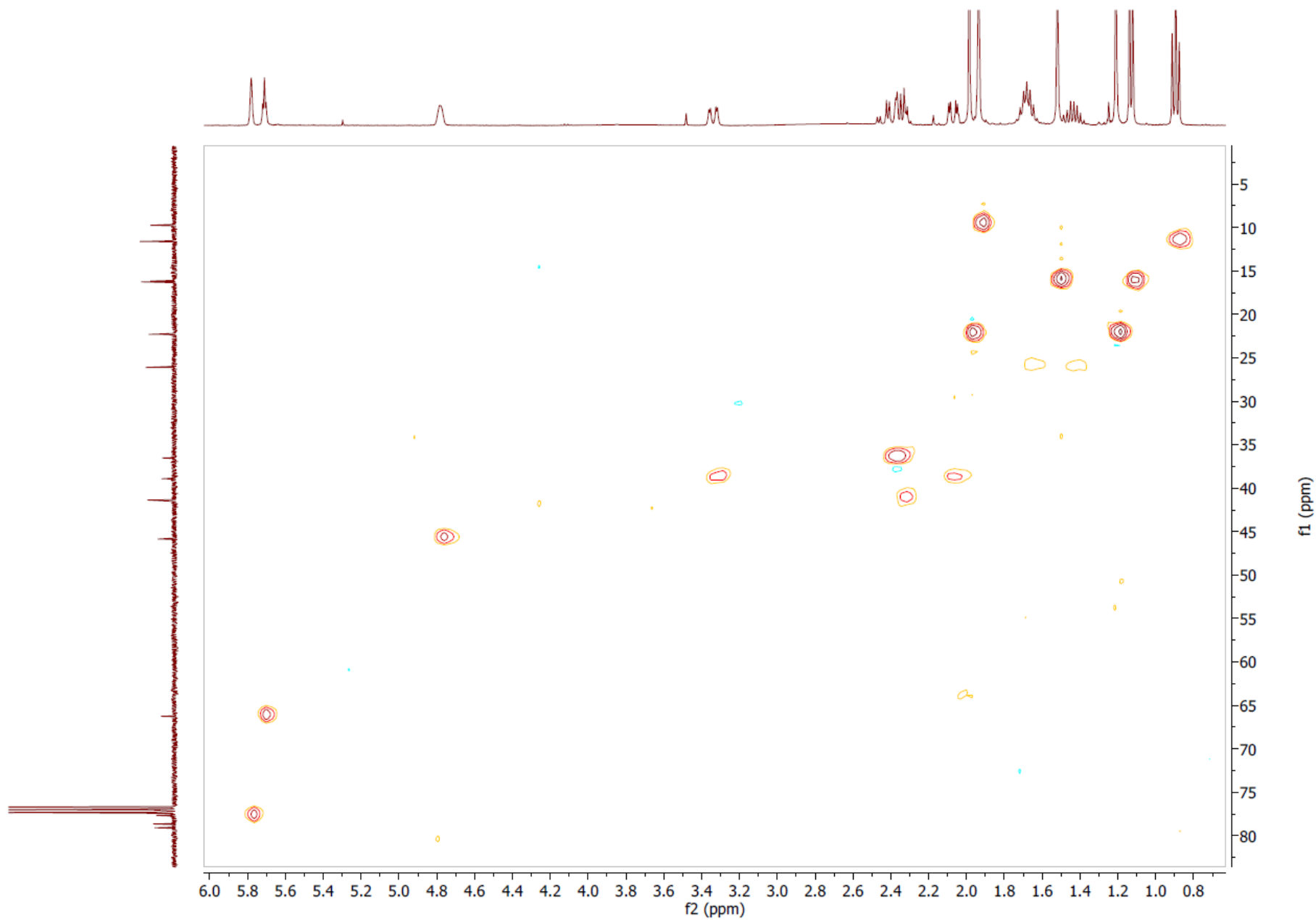
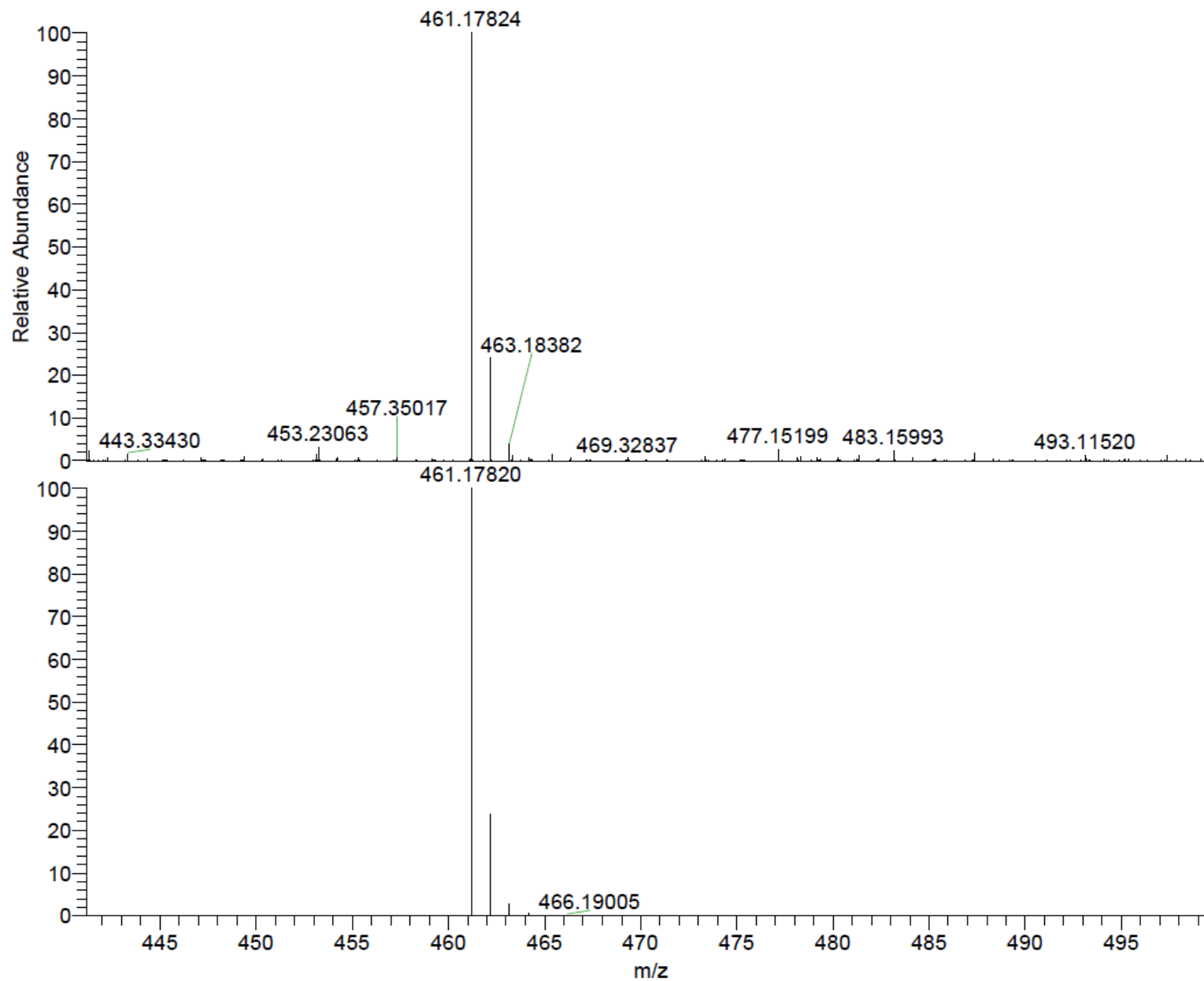


Figure S1C - HMQC of product 1



NL:  
 2.99E6  
 261\_Zimmermann\_ESIpos  
 \_TZ81\_1#69-80 RT:  
 1.00-1.16 AV: 12 T:  
 FTMS + c ESI Full ms  
 [200.00-2000.00]

NL:  
 7.70E5  
 C<sub>22</sub>H<sub>30</sub>O<sub>9</sub>+Na:  
 C<sub>22</sub>H<sub>30</sub>O<sub>9</sub>Na<sub>1</sub>  
 pa Chrg 1

Figure S1D - HRMS of product 1 - [C<sub>22</sub>H<sub>30</sub>O<sub>9</sub>+Na]<sup>+</sup> = 461.17824

# Product 2 (2-octanoyl-3-oxo-3-desangeloyl trilobolide)

Product 2

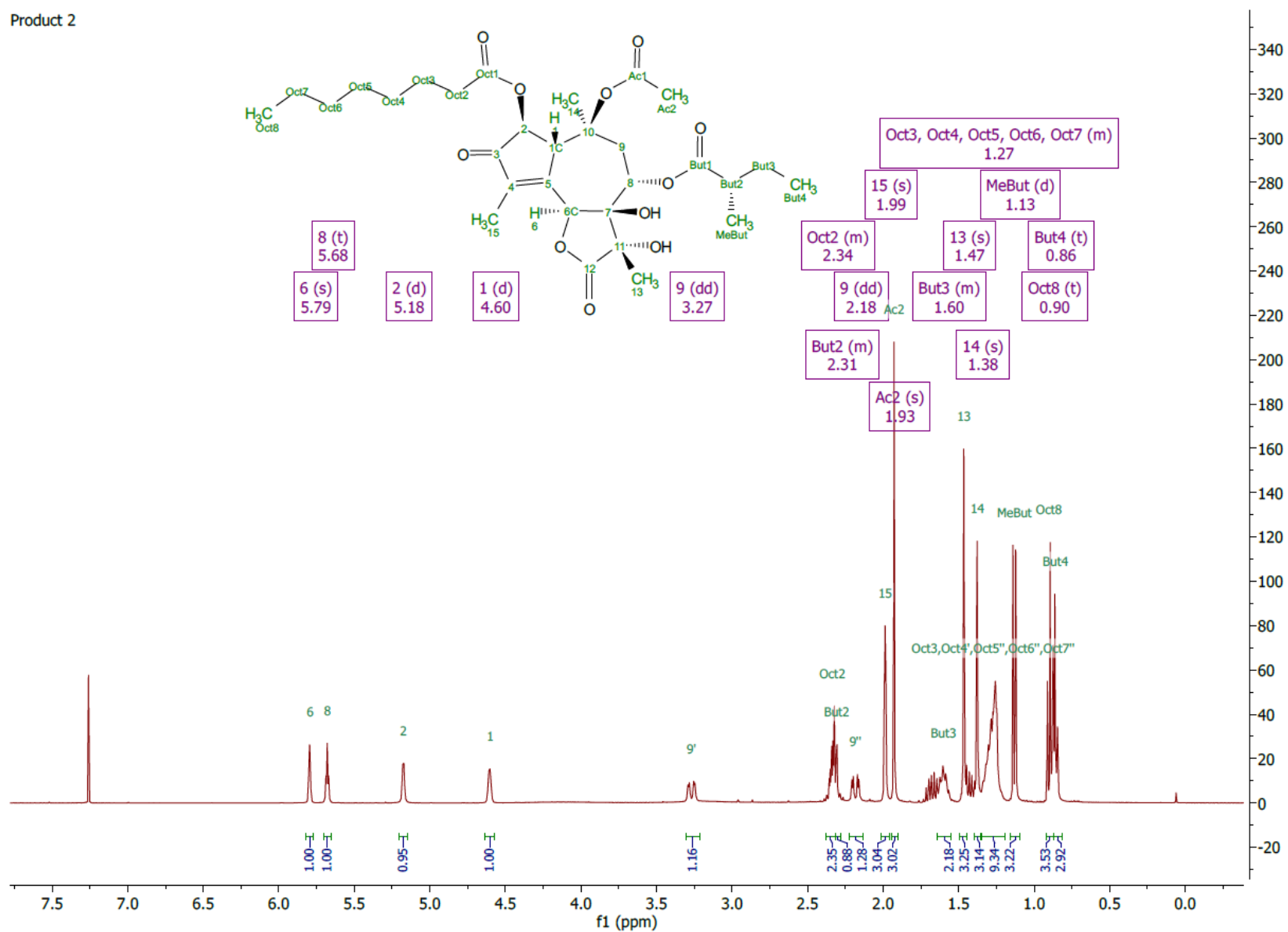


Figure S2A <sup>1</sup>H NMR of product 2 (400 MHz, CDCl<sub>3</sub>)

Product 2

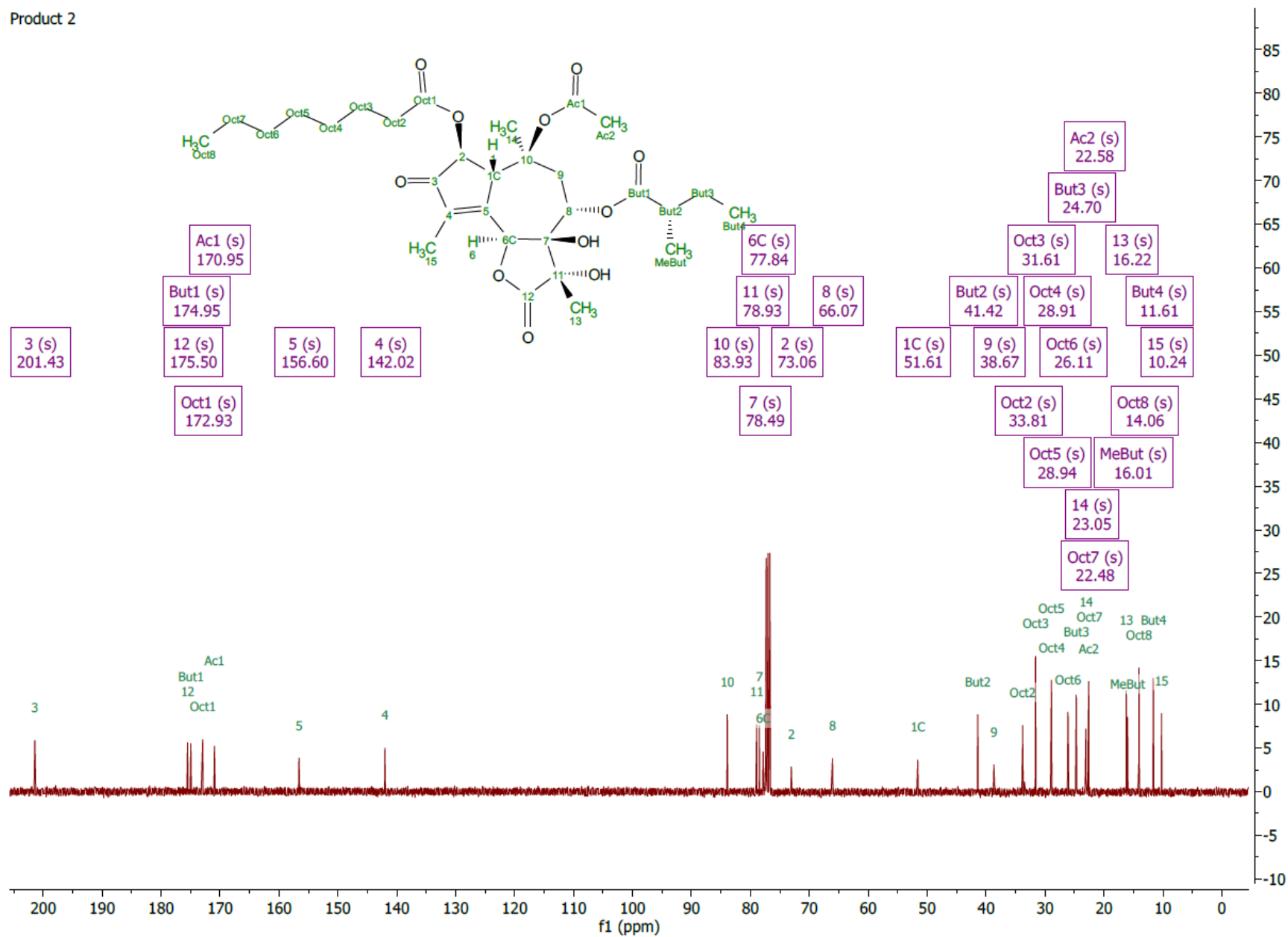
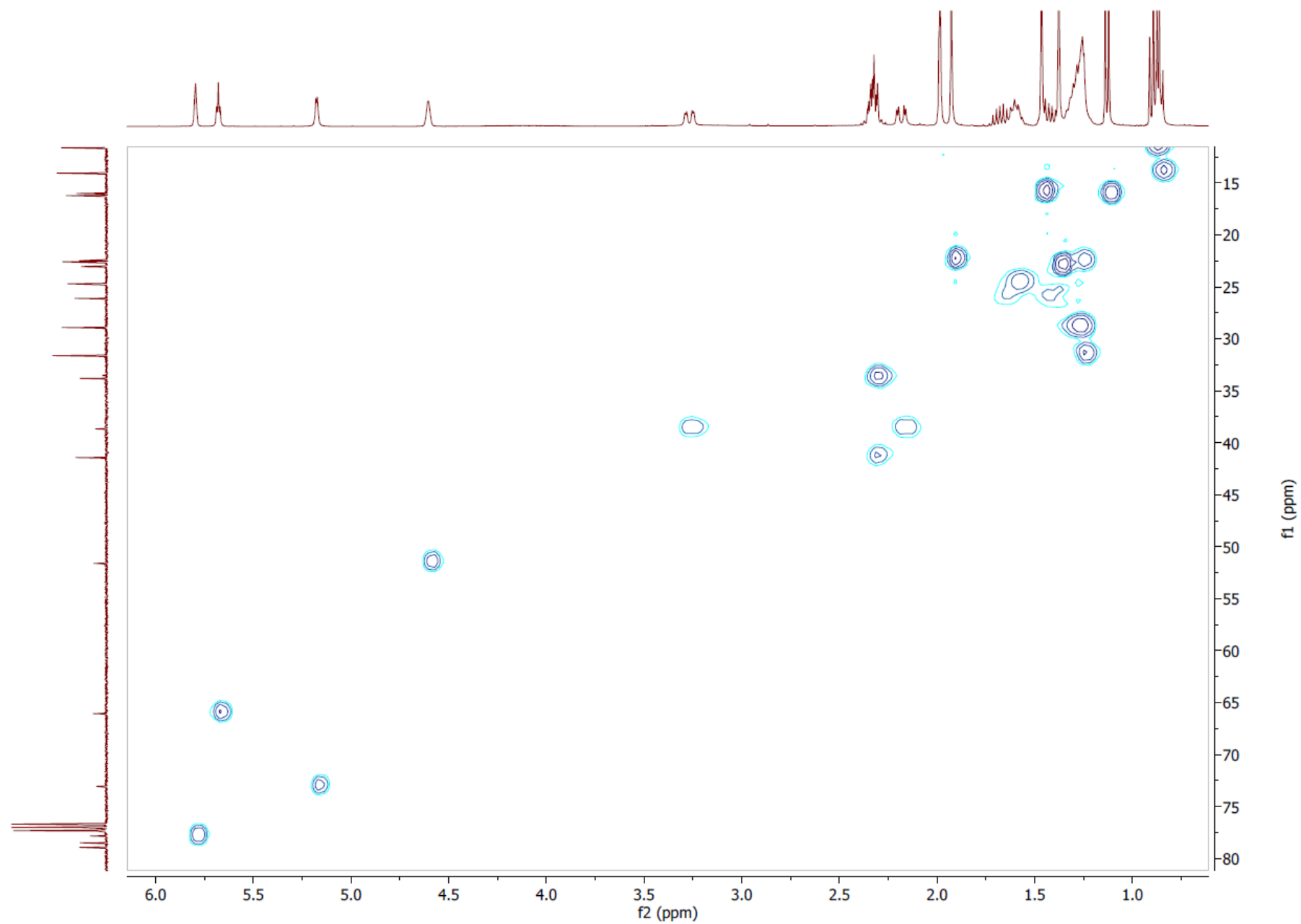
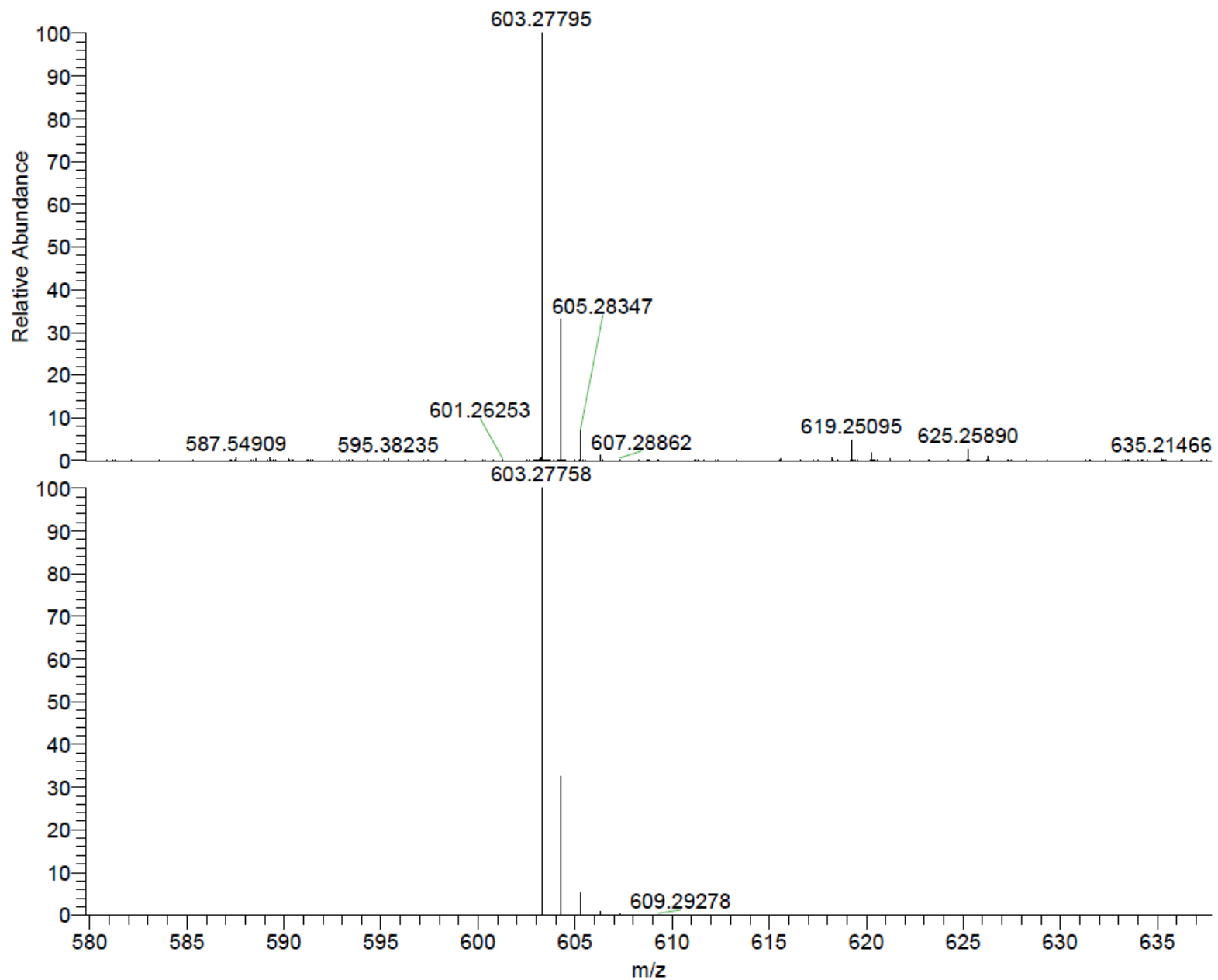


Figure S2B <sup>13</sup>C NMR of product 2 (100 MHz, CDCl<sub>3</sub>)



**Figure S2C** HMQC of product 2





NL:  
 1.26E7  
 261\_Zimmermann\_ESIpos  
 \_TZ82\_1#18-30 RT:  
 0.25-0.43 AV: 13 T:  
 FTMS + c ESI Full ms  
 [200.00-2000.00]

NL:  
 7.01E5  
 $C_{30}H_{44}O_{11} + Na$ :  
 $C_{30}H_{44}O_{11}Na_1$   
 pa Chrg 1

Figure S2D LCMS of product 2 -  $[C_{30}H_{44}O_{11} + Na]^+ = 603.27795$

# Product 3 ((3S)-hydroxy-2-octanoyl-3-desangeloyl trilobolide)

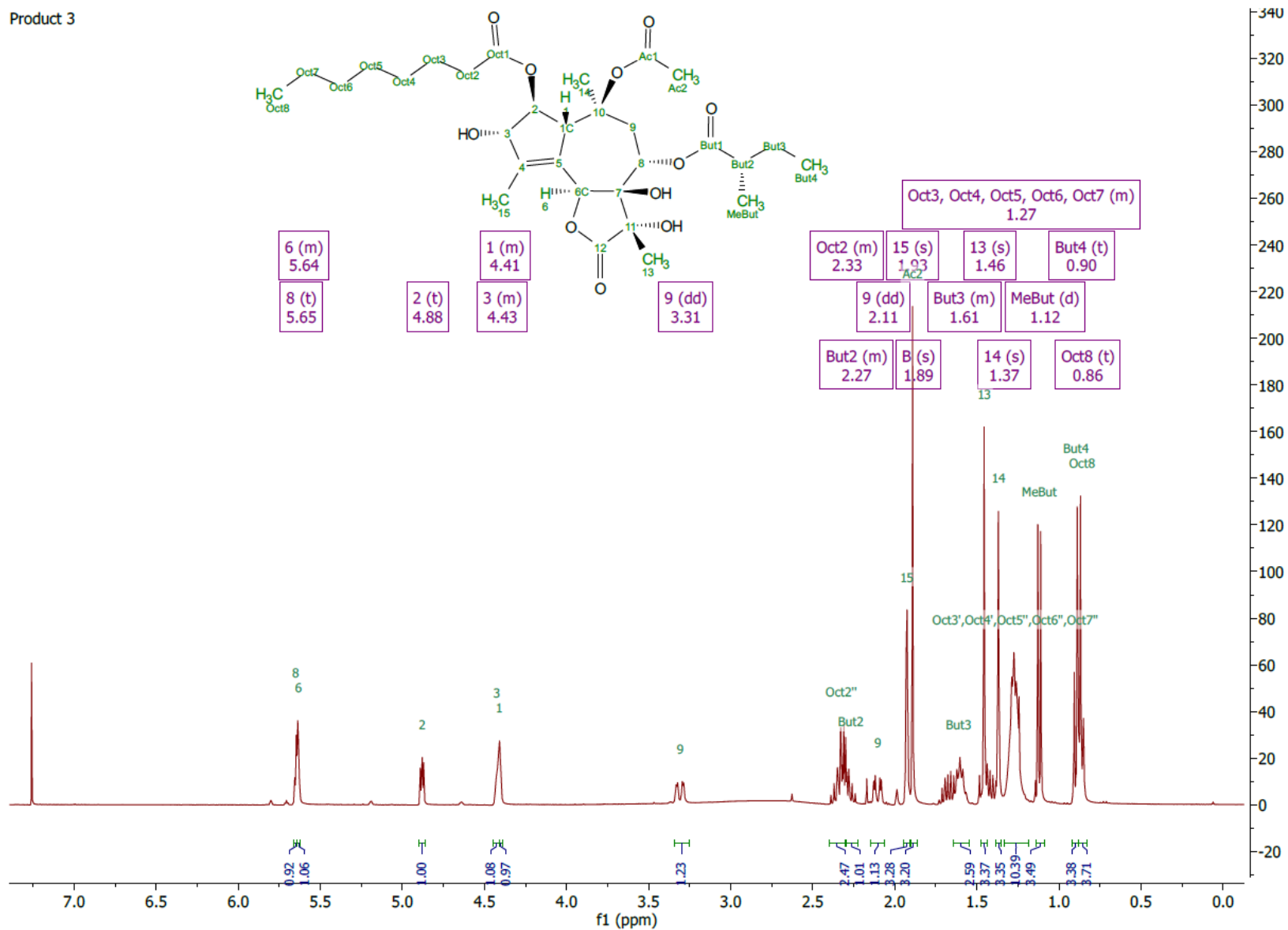


Figure S3A <sup>1</sup>H NMR of product 3 (400 MHz, CDCl<sub>3</sub>)

Product 3

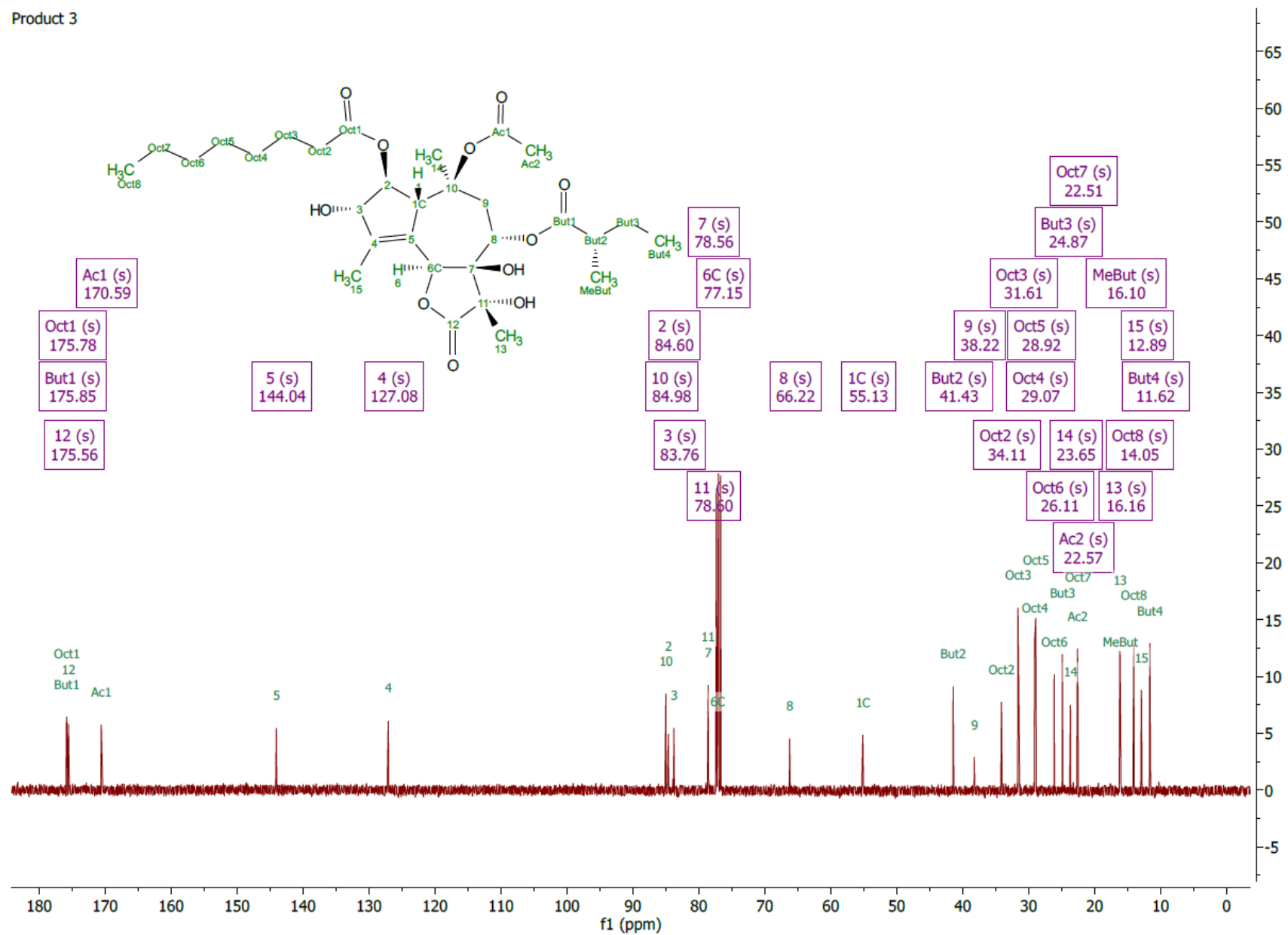


Figure S3B <sup>13</sup>C NMR of product 3 (100 MHz, CDCl<sub>3</sub>)

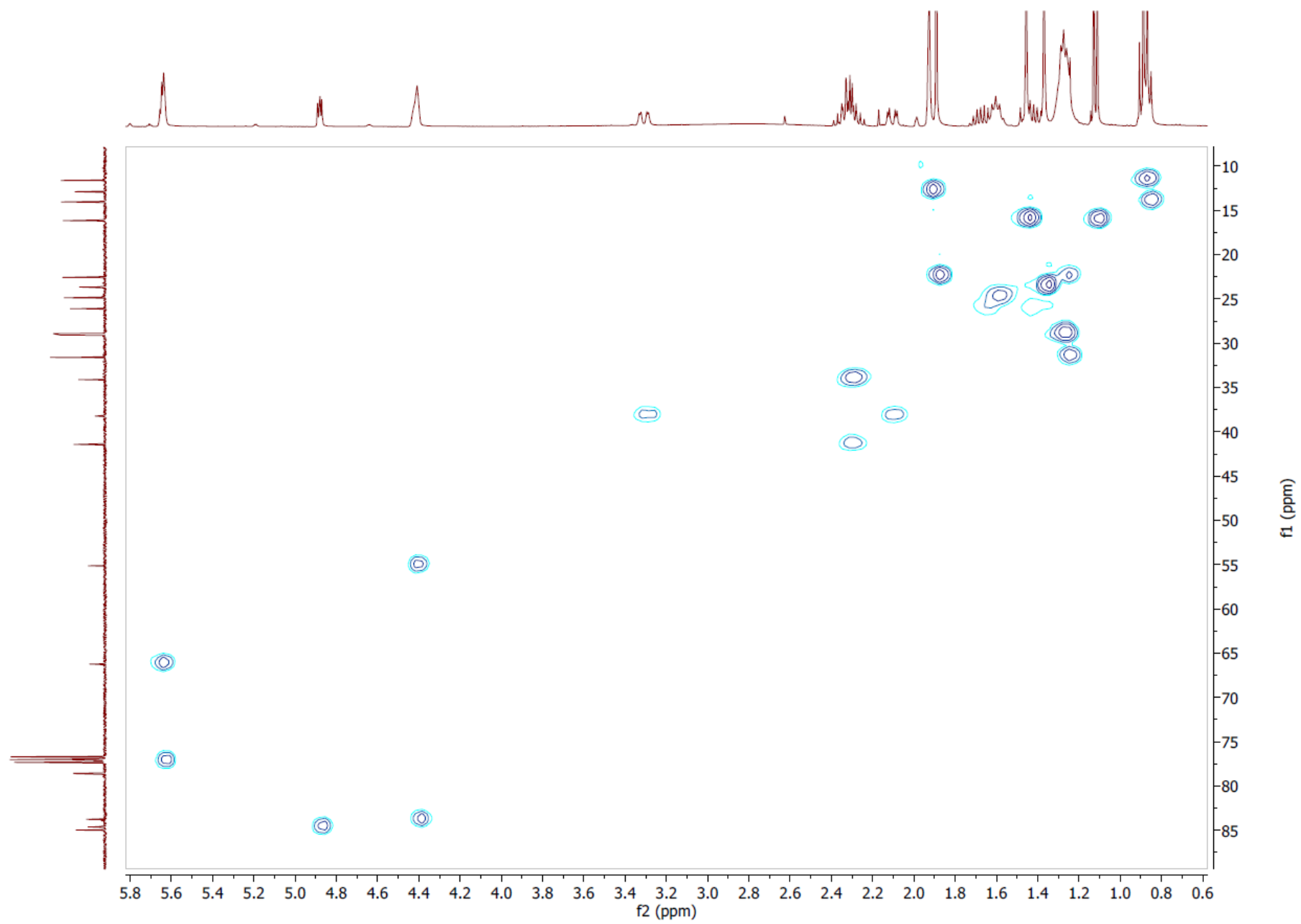
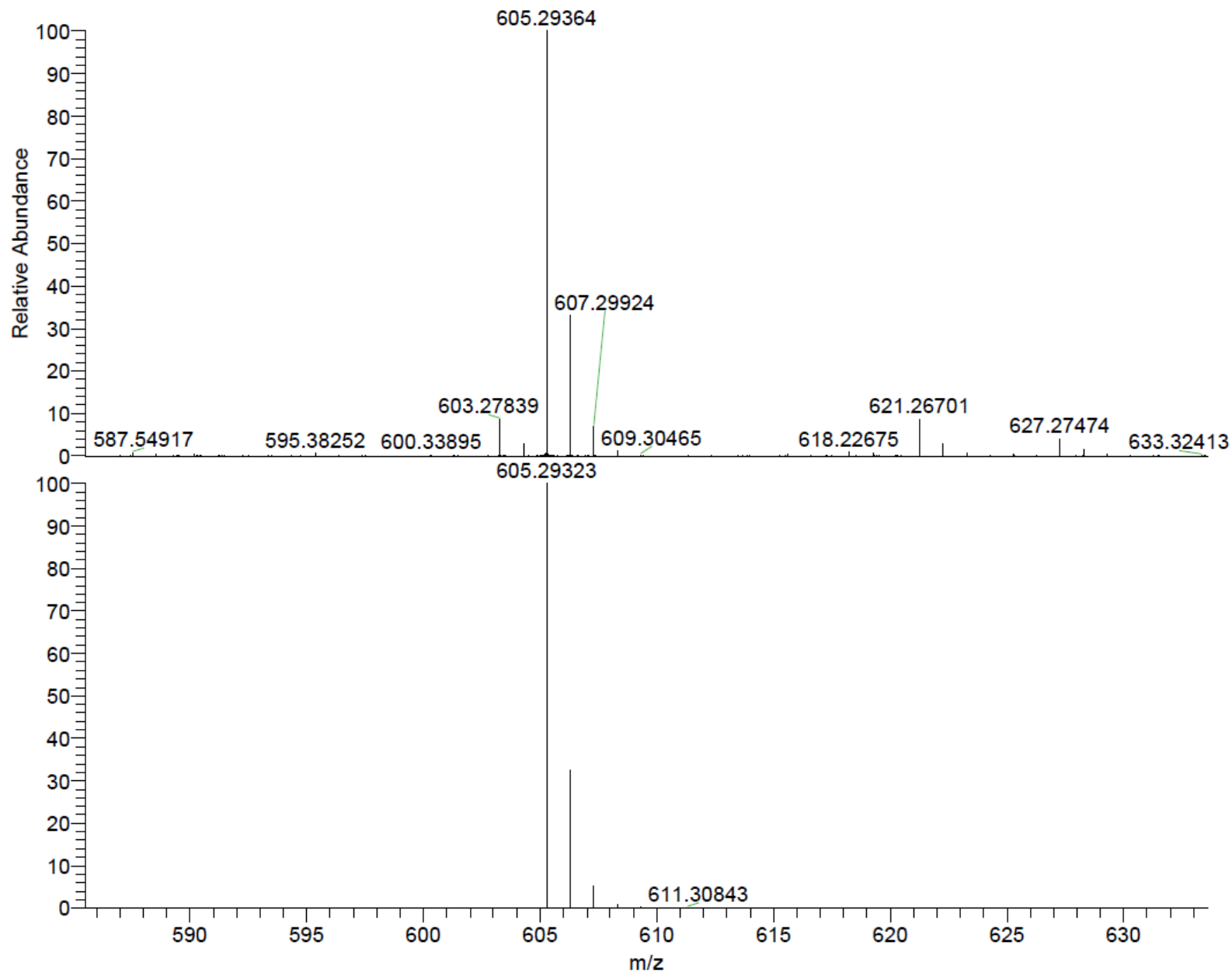


Figure S3C HMQC of product 3



NL:  
1.54E7  
261\_Zimmermann\_ESIpo  
s\_TZ84\_1#45-51 RT:  
0.65-0.74 AV: 7 T: FTMS  
+ c ESI Full ms  
[200.00-2000.00]

NL:  
7.01E5  
C<sub>30</sub>H<sub>46</sub>O<sub>11</sub>+Na:  
C<sub>30</sub>H<sub>46</sub>O<sub>11</sub>Na<sub>1</sub>  
pa Chrg 1

Figure S3D HRMS of product 3 - [C<sub>30</sub>H<sub>46</sub>O<sub>11</sub>+Na]<sup>+</sup> = 605.29364

# Product 4 (8-O-((2S)-Methylbutanoyl)-8-O-debutanoyl thapsigargin)

Product 4

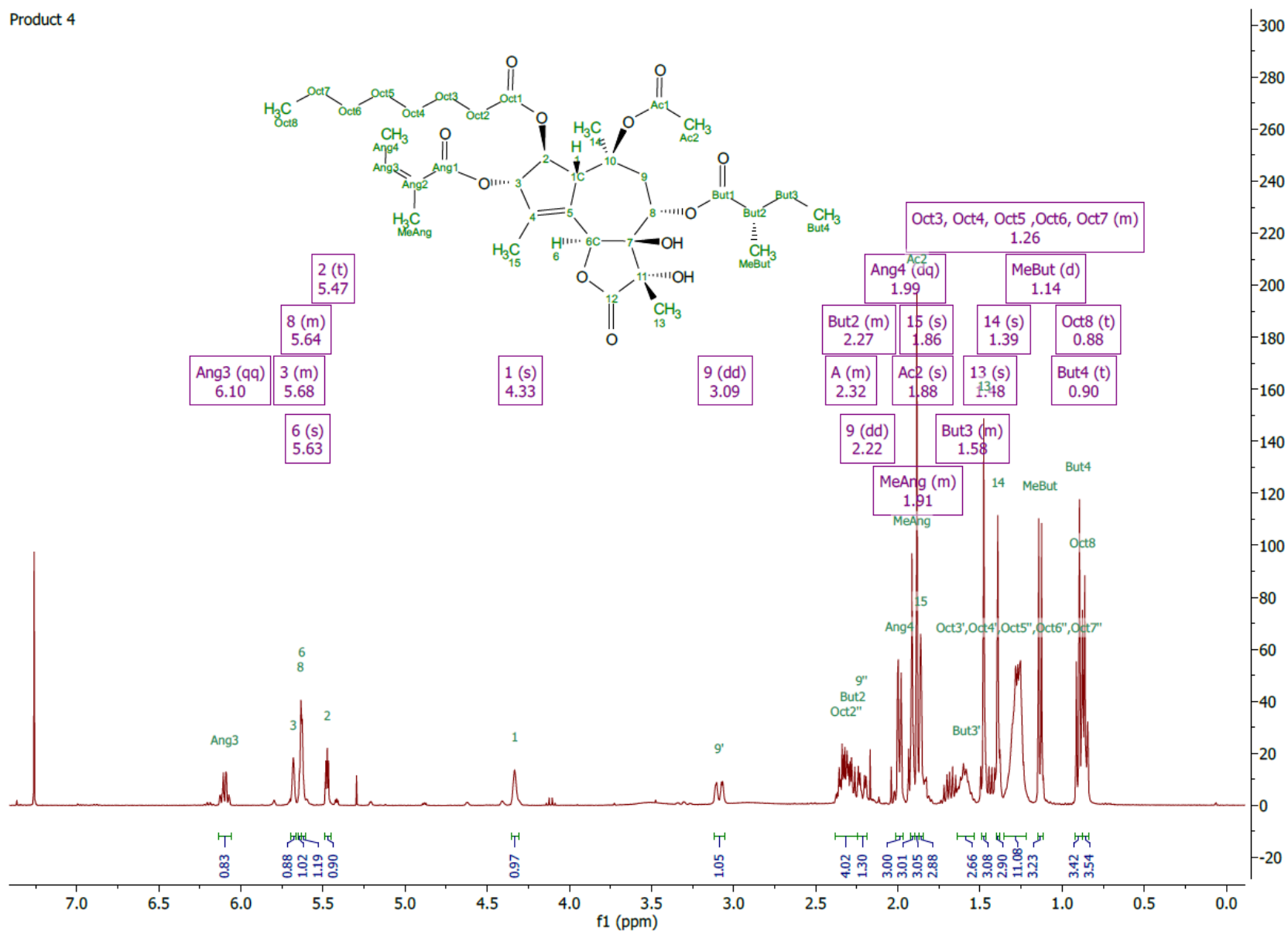


Figure S4A <sup>1</sup>H NMR of product 4 (400 MHz, CDCl<sub>3</sub>)

Product 4

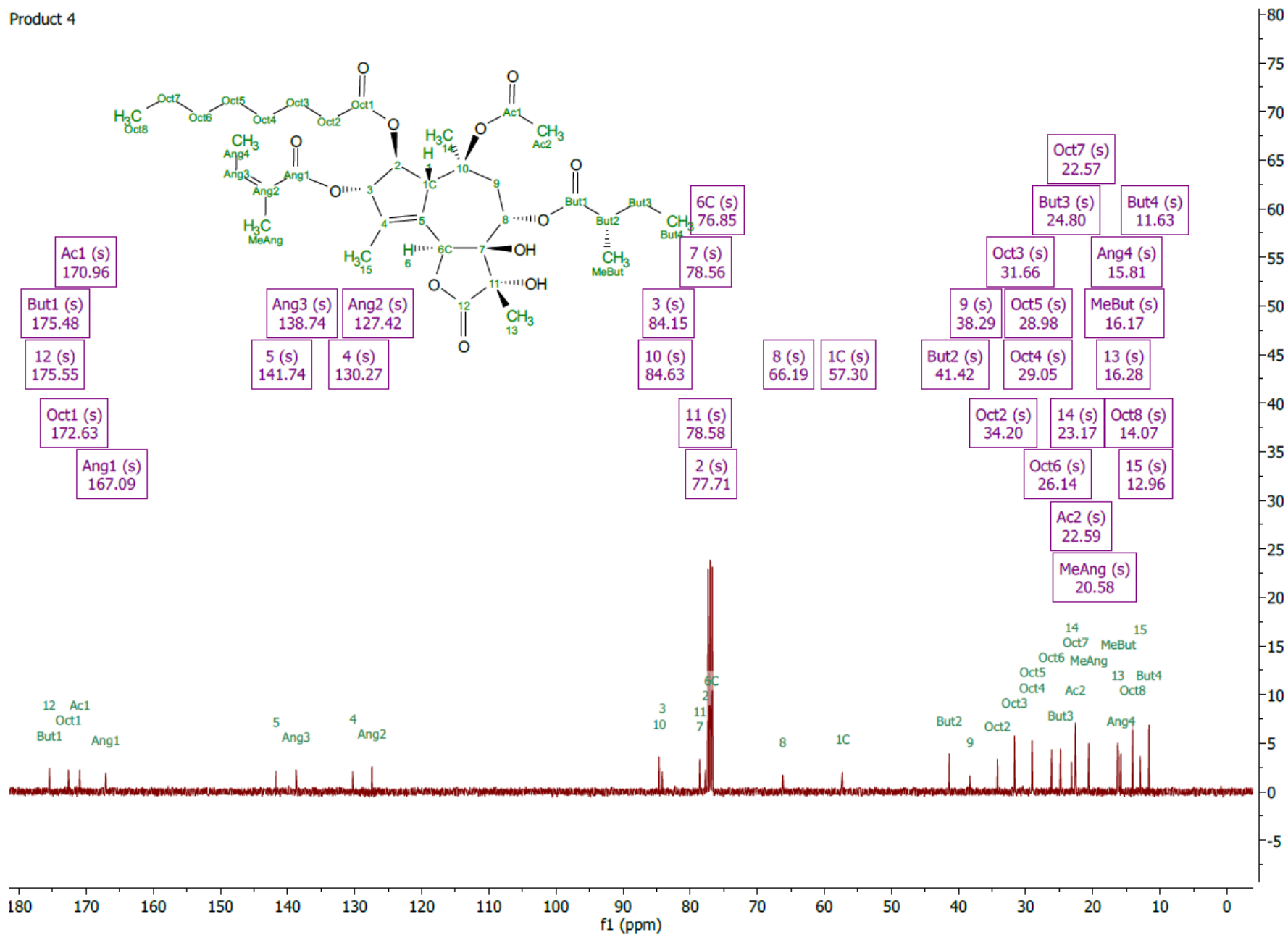


Figure S4B  $^{13}\text{C}$  NMR of product 4 (100 MHz,  $\text{CDCl}_3$ )

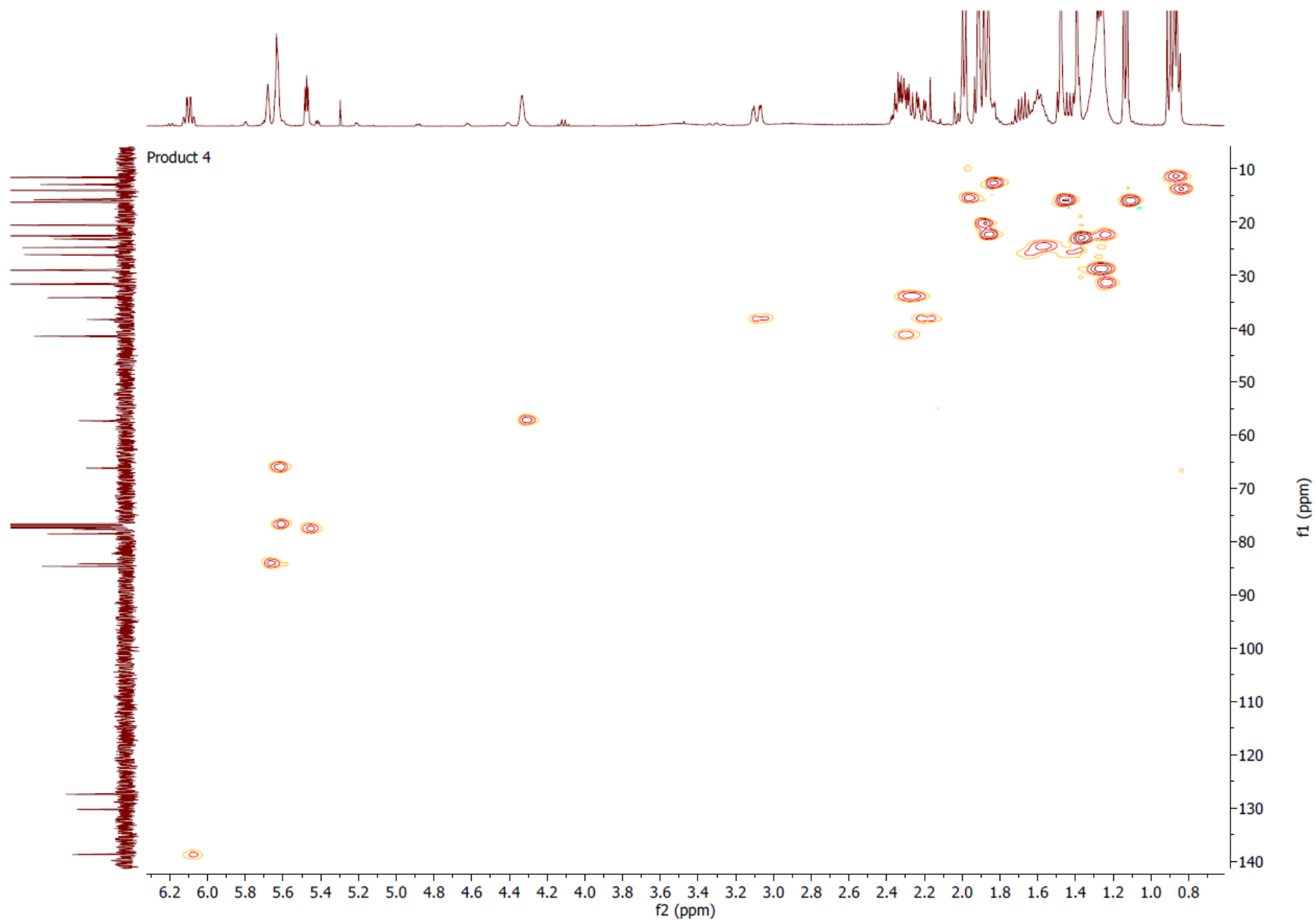
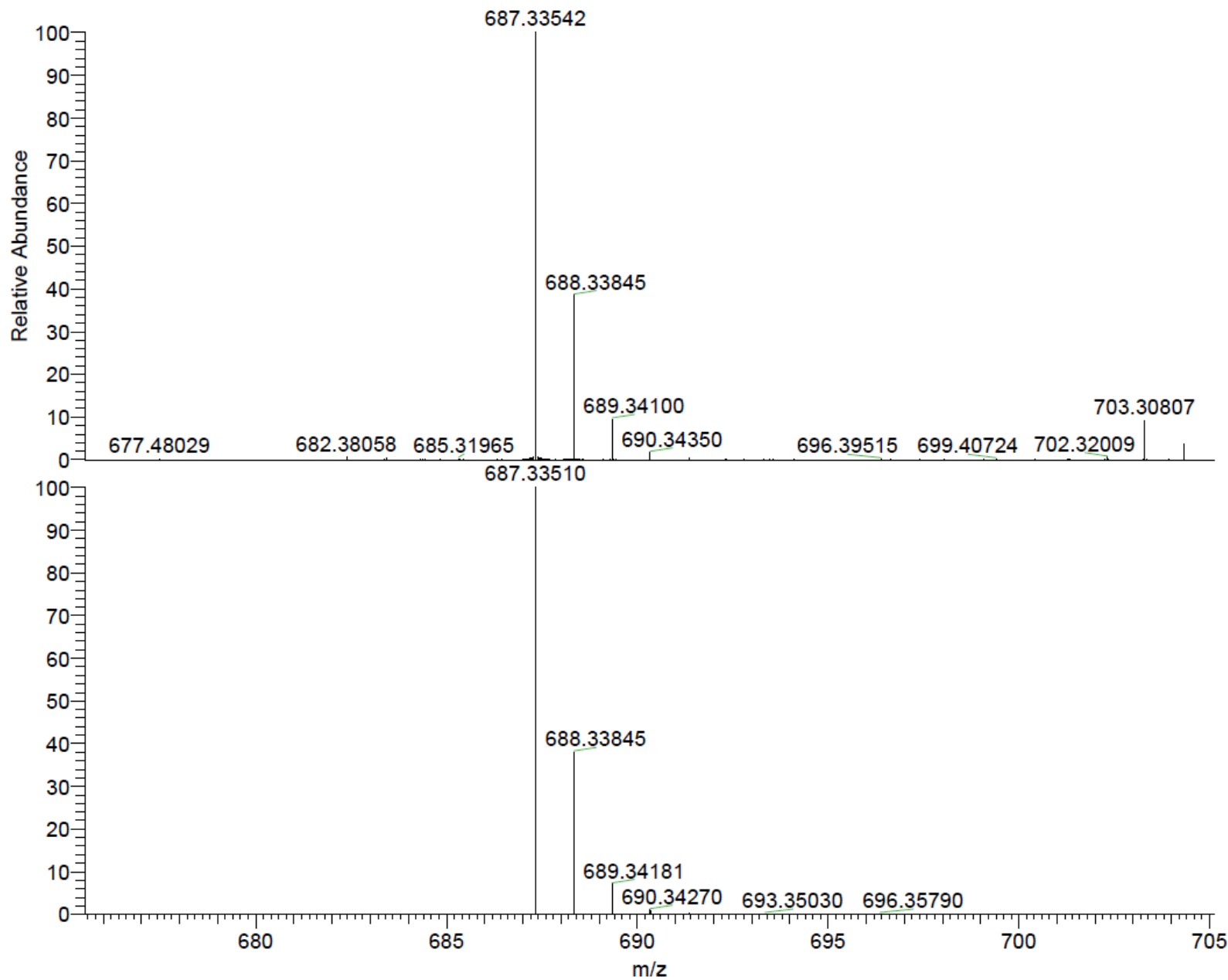


Figure S4C HMQC of product 4





NL:  
1.80E7  
261\_Zimmermann\_ESIpo  
s\_TZ87\_1#48-54 RT:  
0.69-0.78 AV: 7 T: FTMS  
+ c ESI Full ms  
[200.00-2000.00]

NL:  
6.63E5  
c<sub>35</sub> h<sub>52</sub> o<sub>12</sub> +Na:  
C<sub>35</sub> H<sub>52</sub> O<sub>12</sub> Na<sub>1</sub>  
pa Chrg 1

Figure S4D HRMS of product 4 -  $[C_{35}H_{52}O_{12}+Na]^+ = 687.33542$

# Product 5 (8-O-debutanoyl thapsigargin)

Product 5

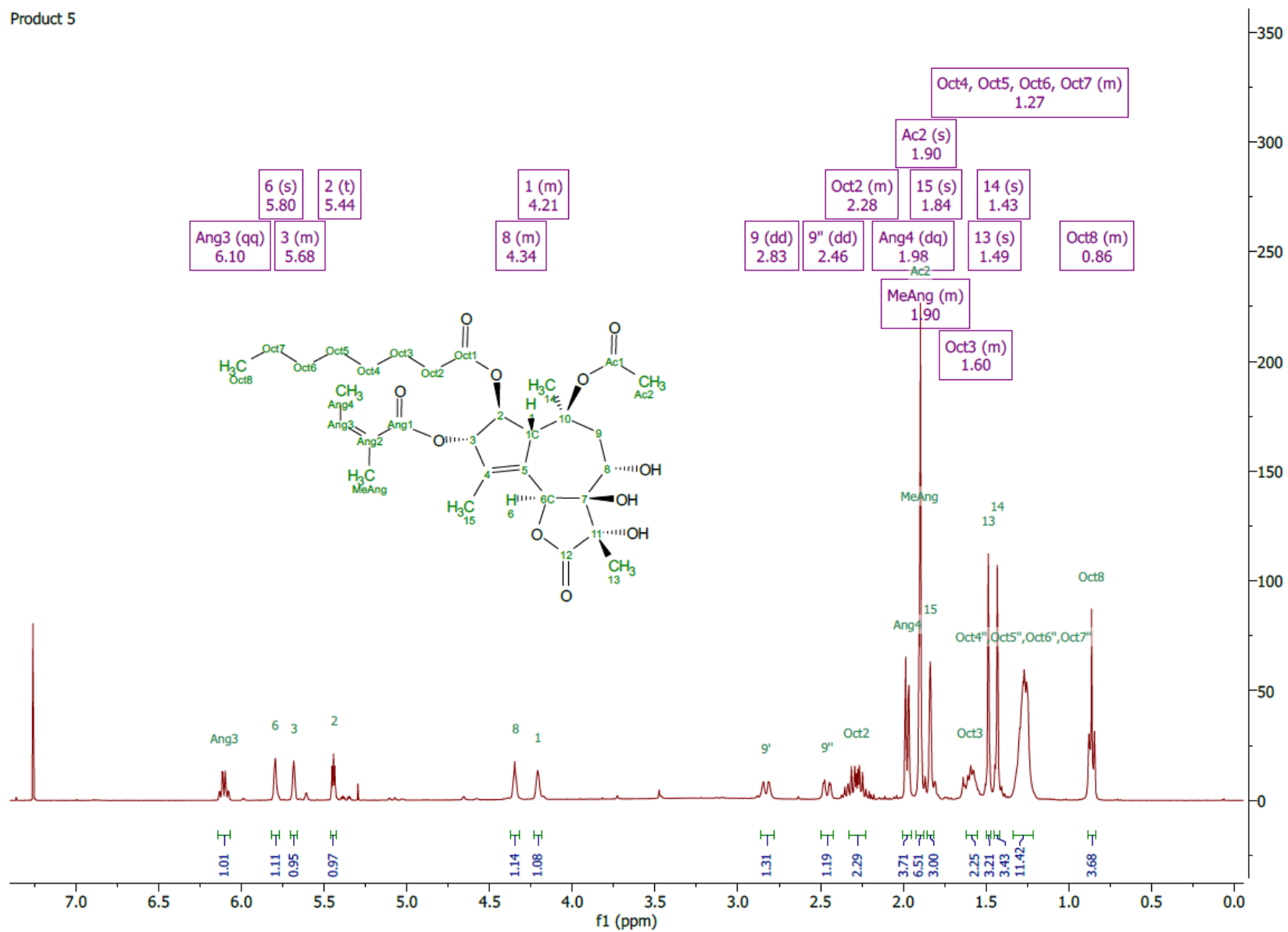


Figure S5A <sup>1</sup>H NMR of product 4 (400 MHz, CDCl<sub>3</sub>)

Product 5

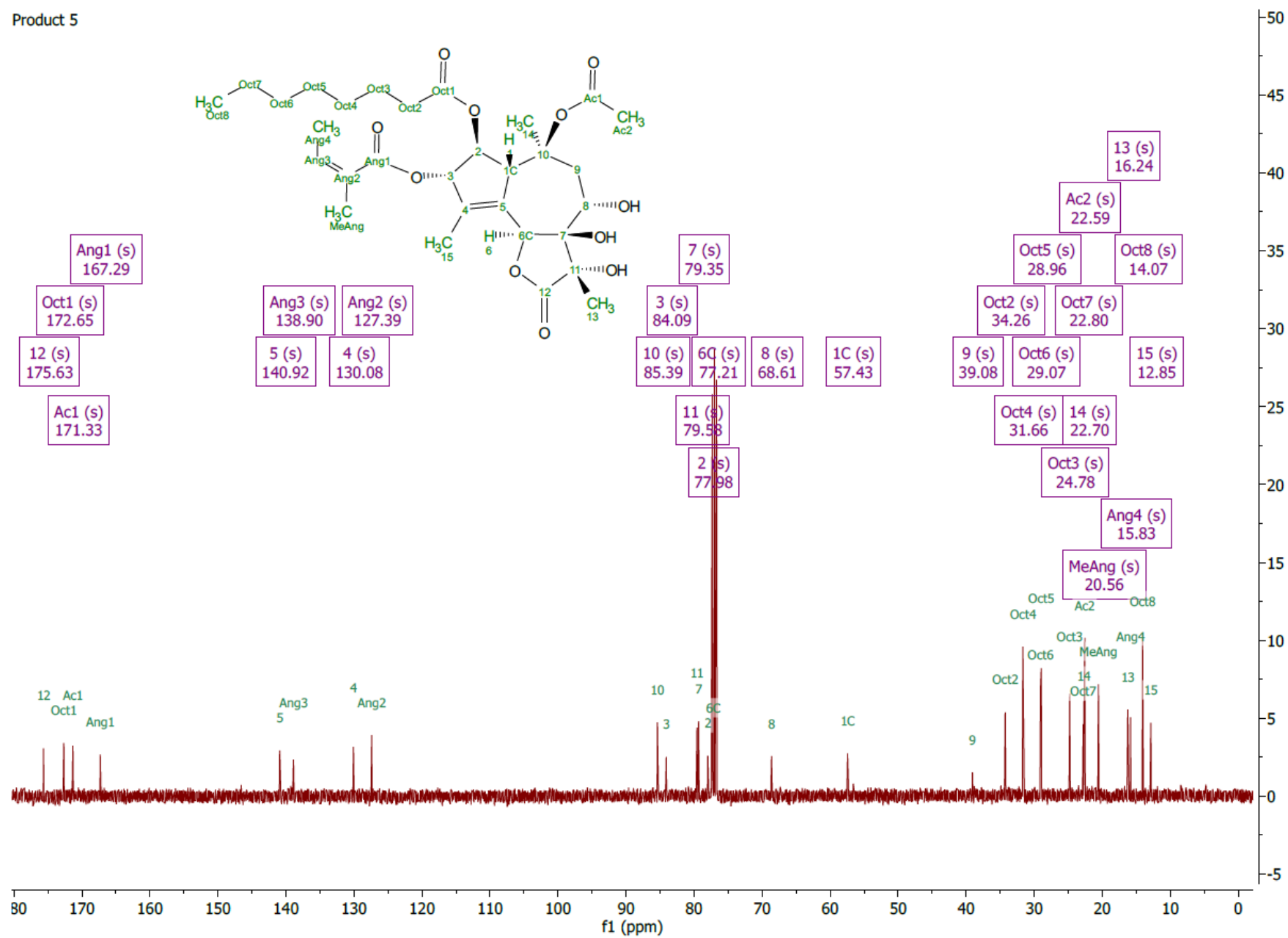


Figure S5B <sup>13</sup>C NMR of product 5 (100 MHz, CDCl<sub>3</sub>)

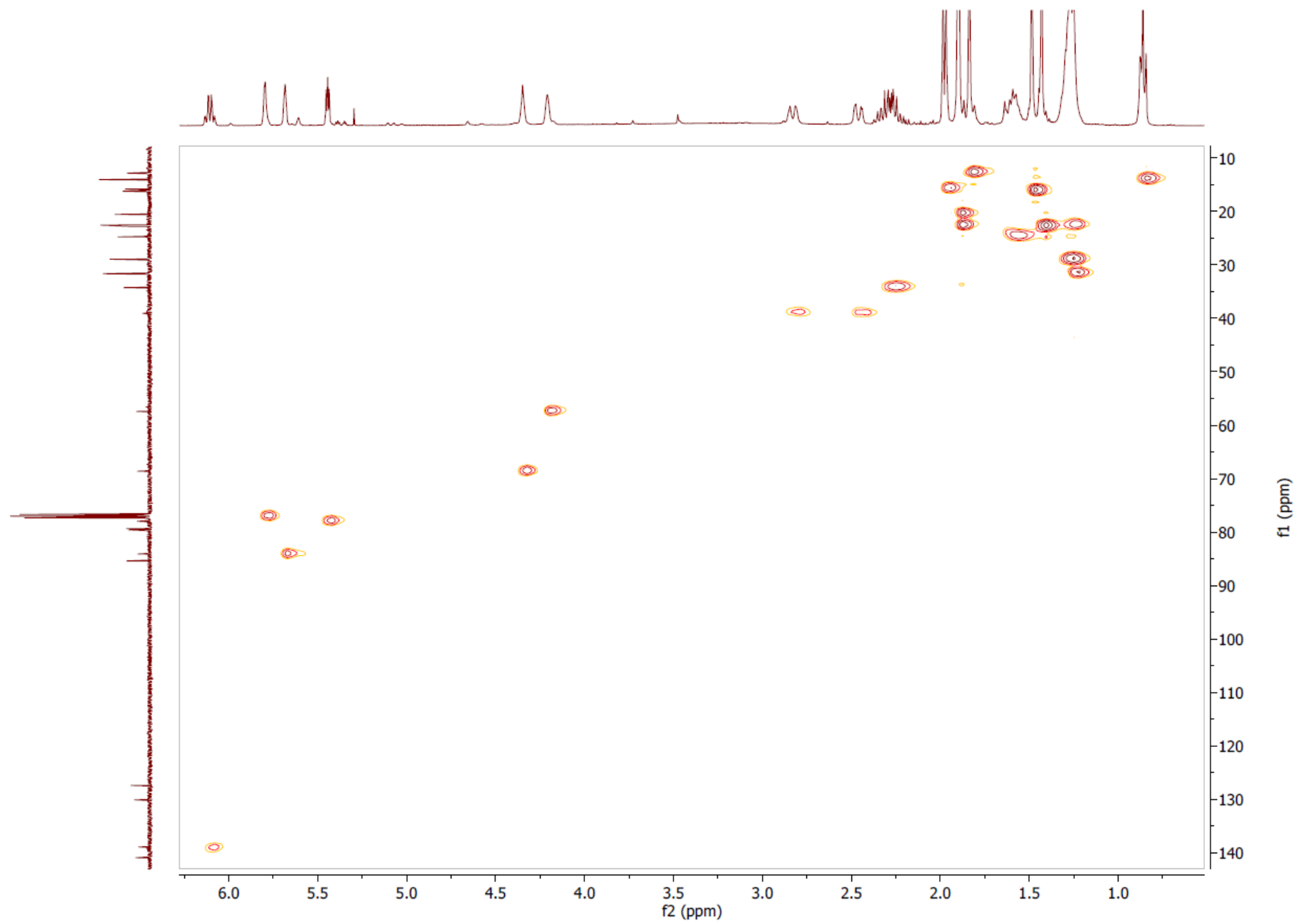


Figure S5C HMQC of product 5

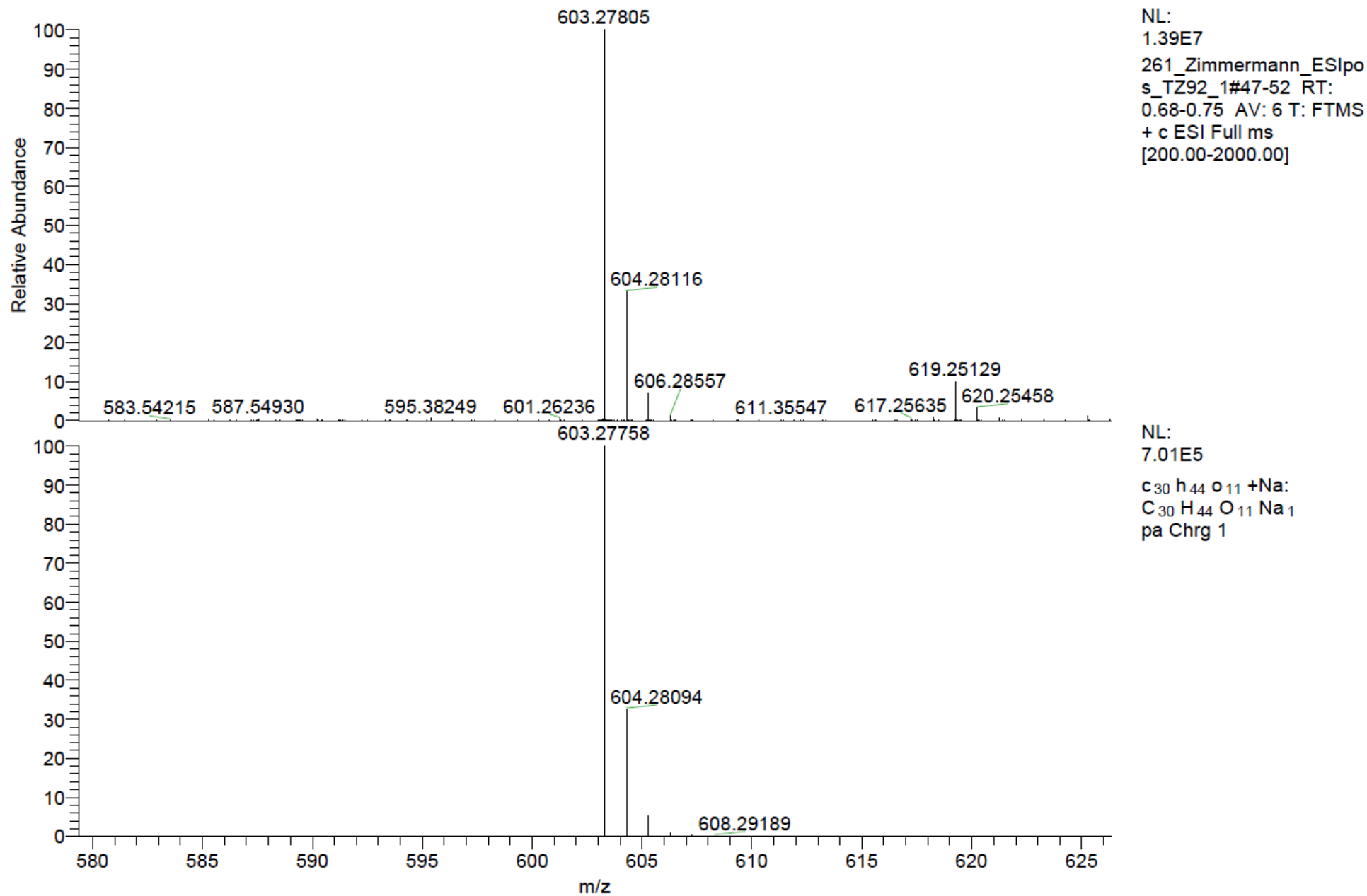


Figure S5D HRMS of product 5 -  $[C_{30}H_{44}O_{11}+Na]^+ = 603.27805$

# Product 6 (8-O-(Boc-12-aminododecanoyl)-8-O-debutanoyl thapsigargin)

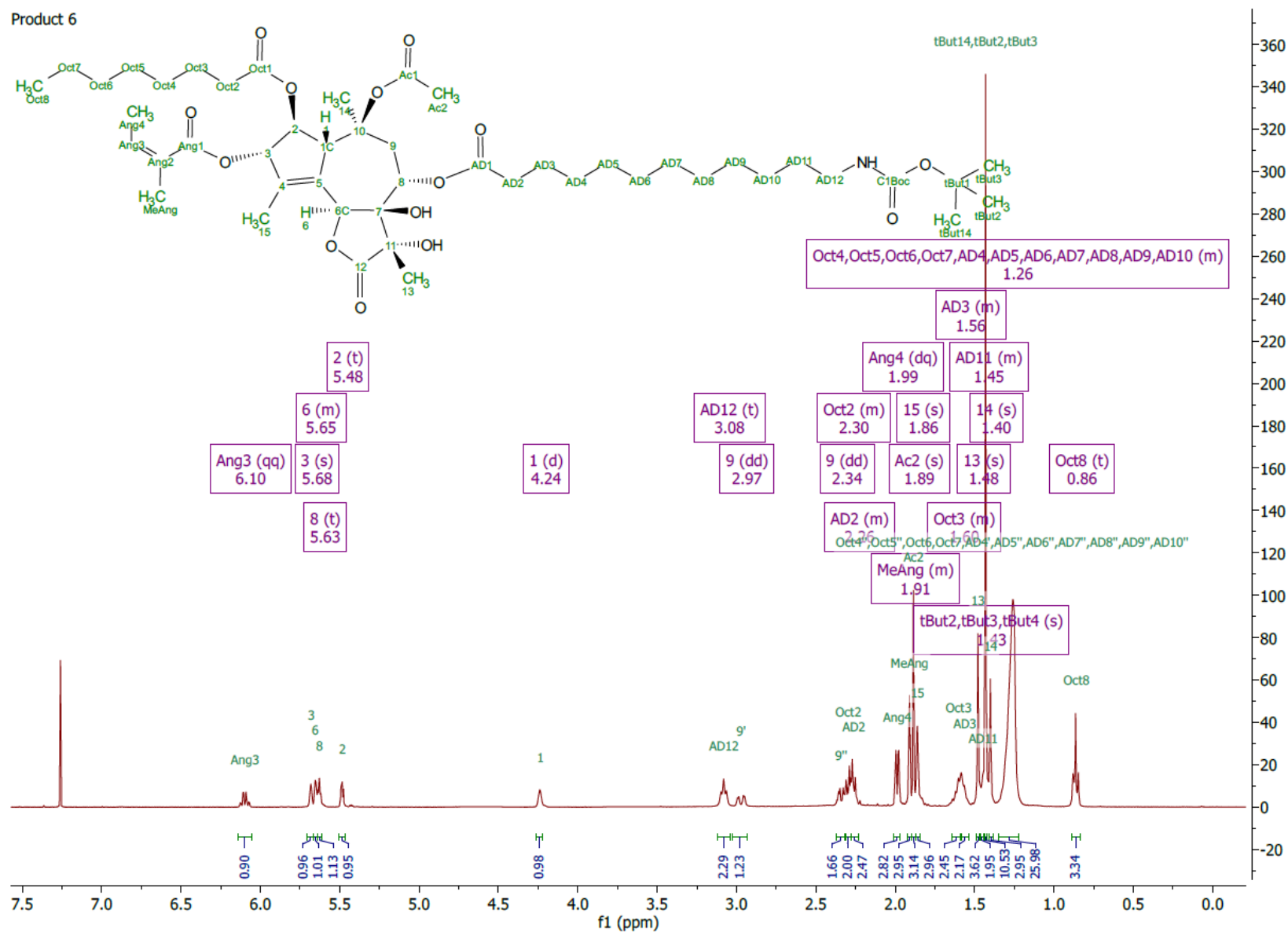


Figure S6A <sup>1</sup>H NMR of product 6 (400 MHz, CDCl<sub>3</sub>)

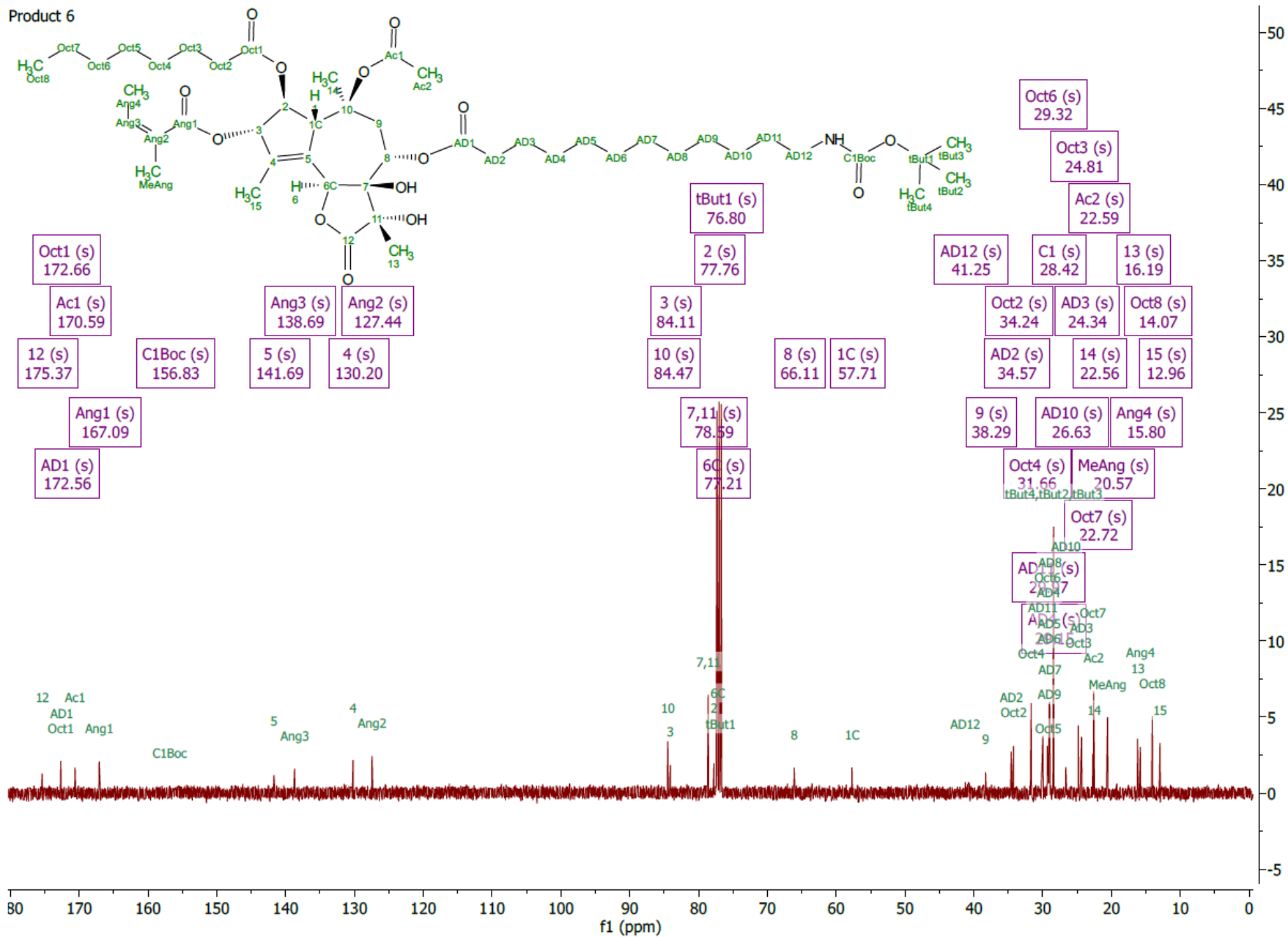


Figure S6B  $^{13}\text{C}$  NMR of product 6 (100 MHz,  $\text{CDCl}_3$ )

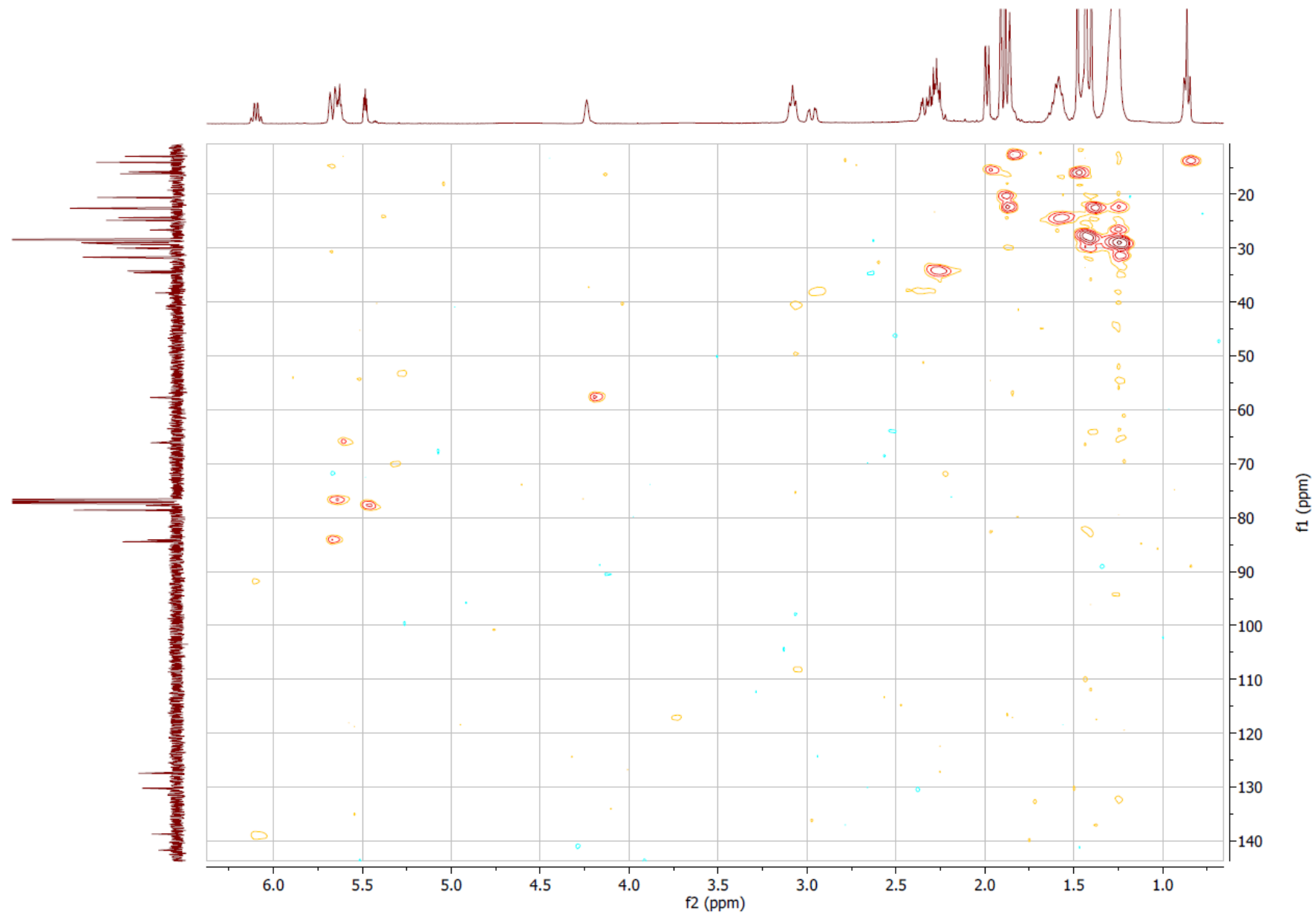


Figure S6C HMQC of product 6



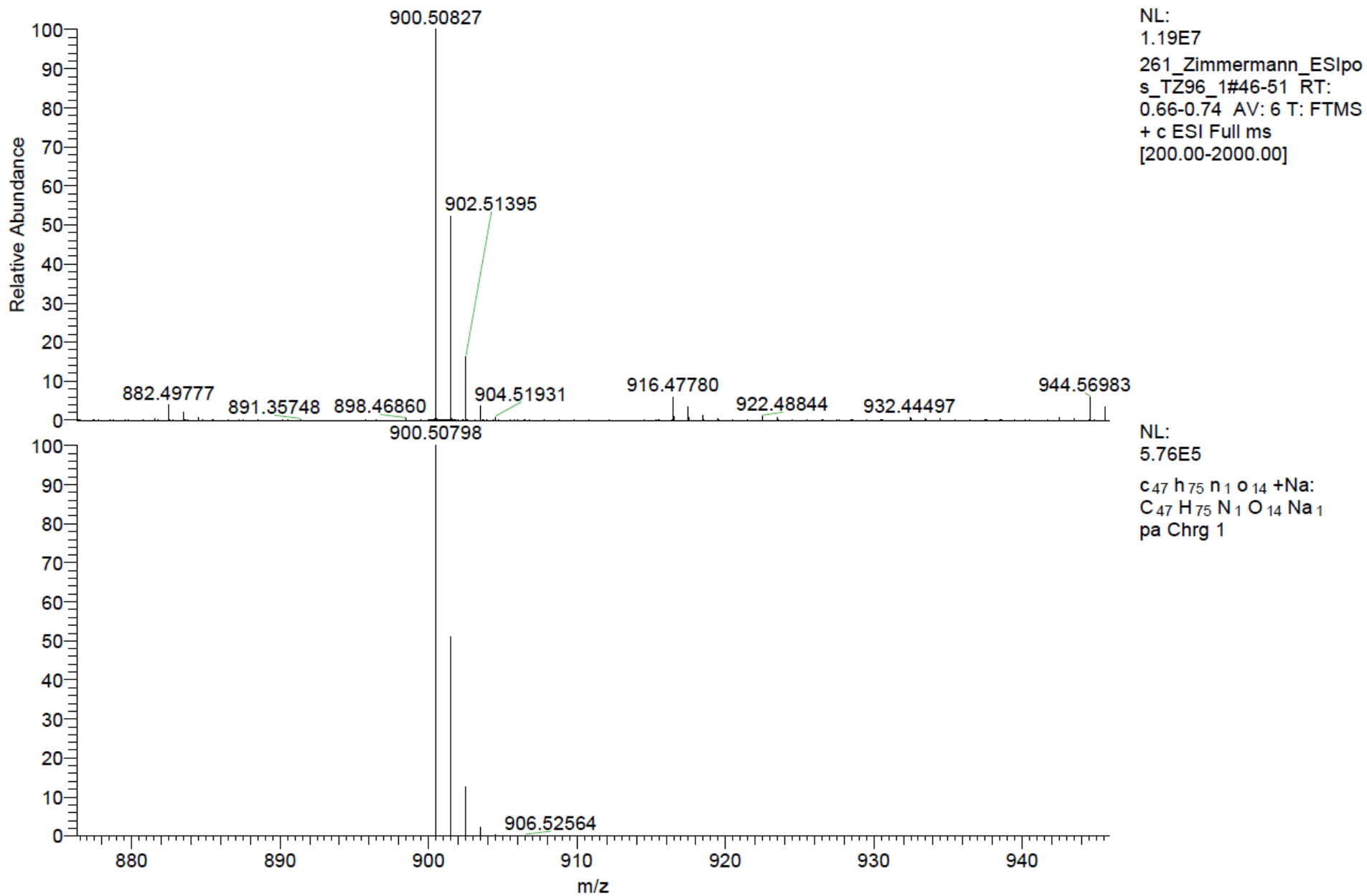


Figure S6D HRMS of product 6 -  $[C_{47}H_{75}NO_{14}+Na]^+ = 900.50827$

# Product 7 (8-O-(12-Aminododecanoyl)-8-O-debutanoyl thapsigargin)

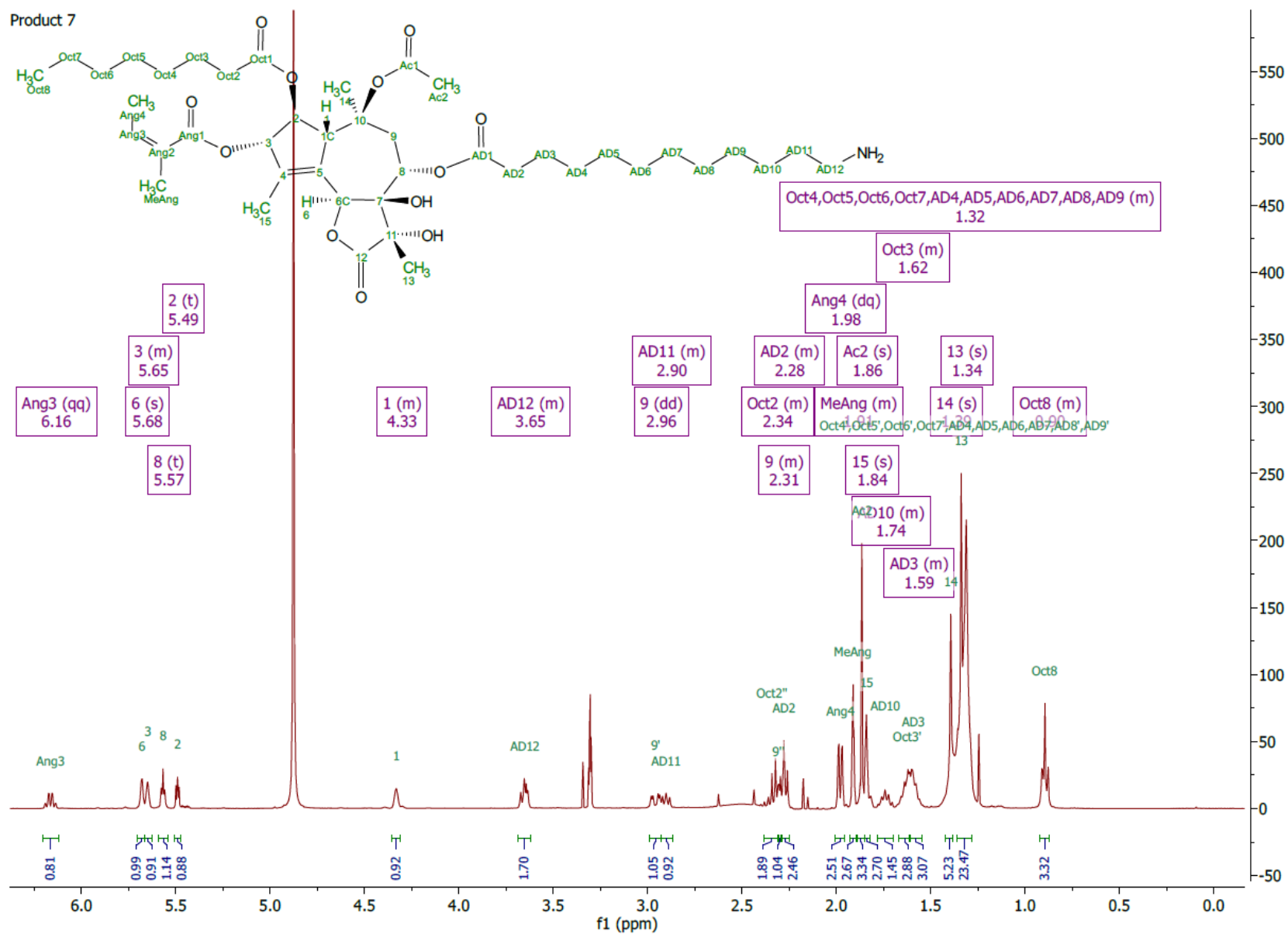


Figure S7A <sup>1</sup>H NMR of product 7 (400 MHz, CD<sub>3</sub>OD)

Product 7

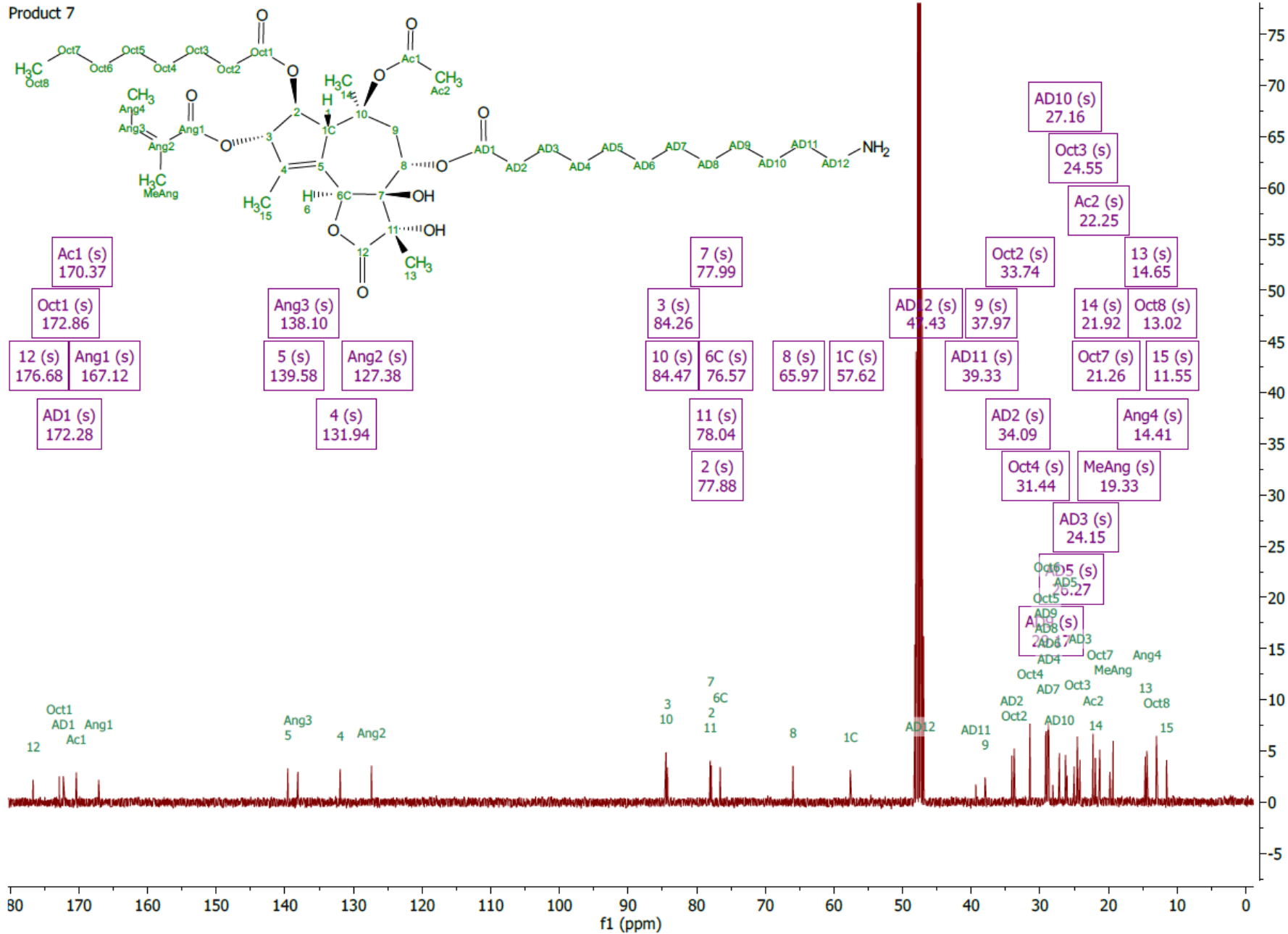


Figure S7B <sup>13</sup>C NMR of product 7 (100 MHz, CD<sub>3</sub>OD)

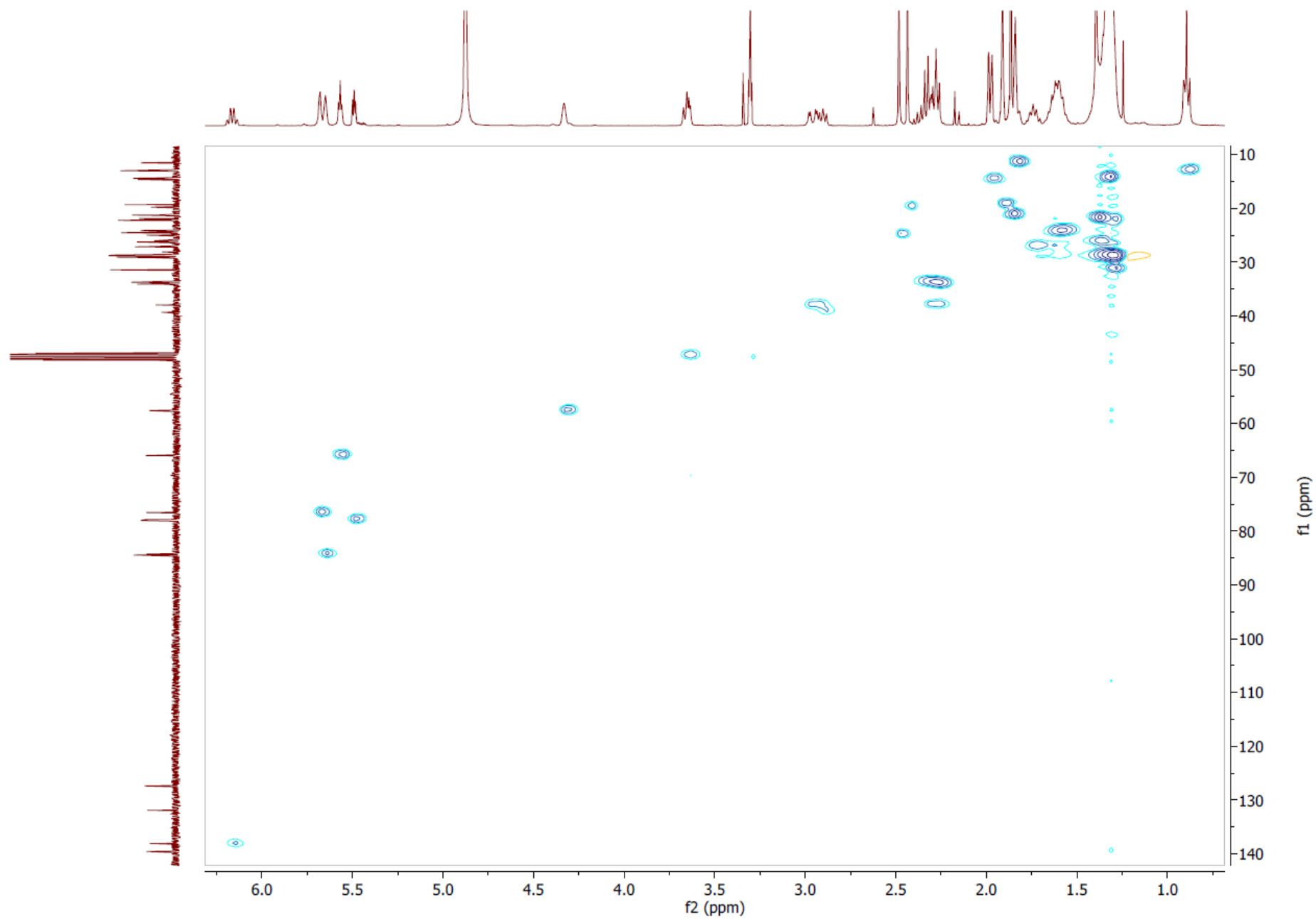


Figure S7C HMQC of product 7

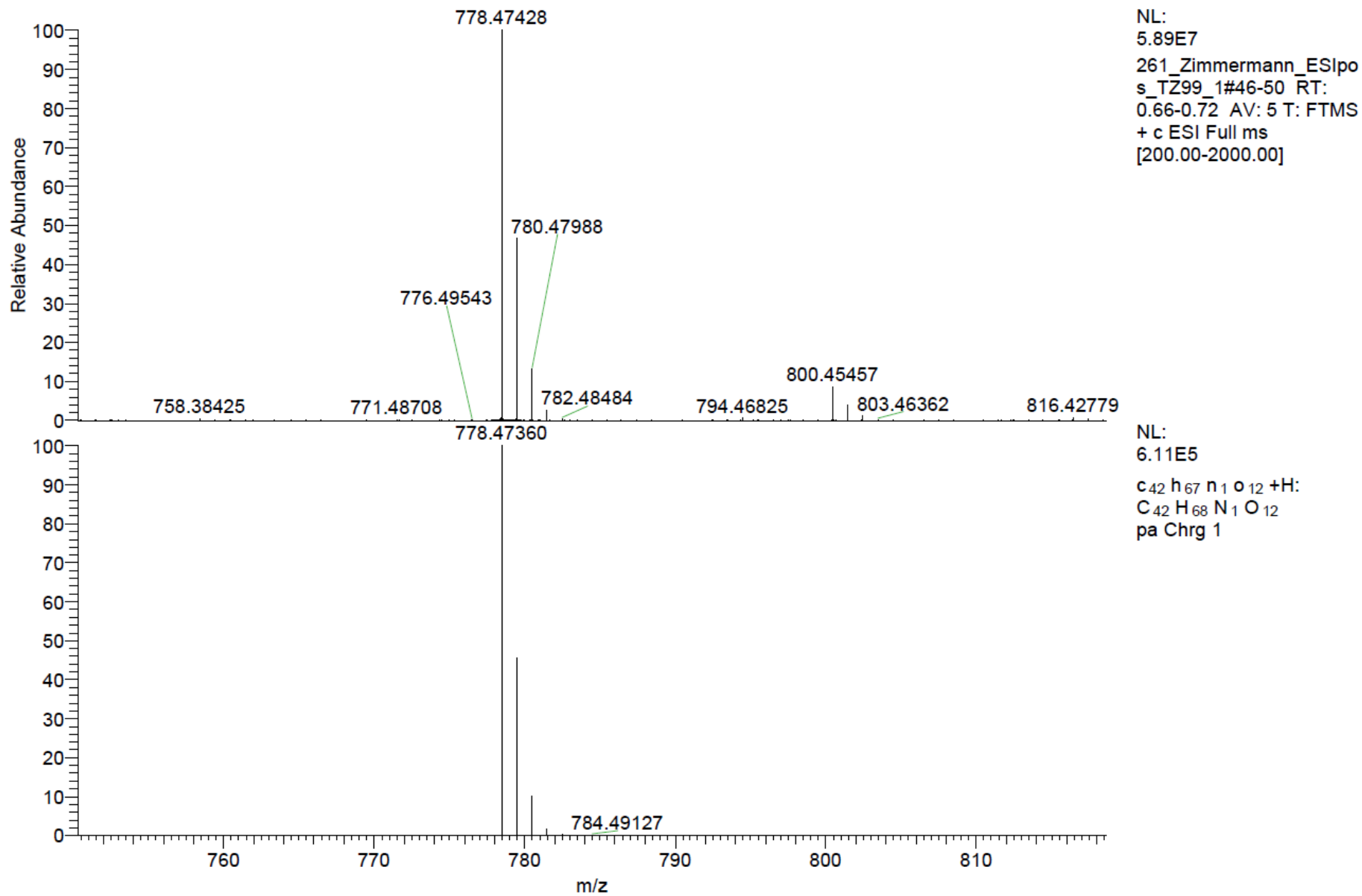


Figure S7D HRMS of product 7 -  $[C_{42}H_{67}NO_{12}+H]^+ = 778.47428$

# 2,4,6-trichlorobenzoic (Z)-2-methylbut-2-enoic anhydride (8)

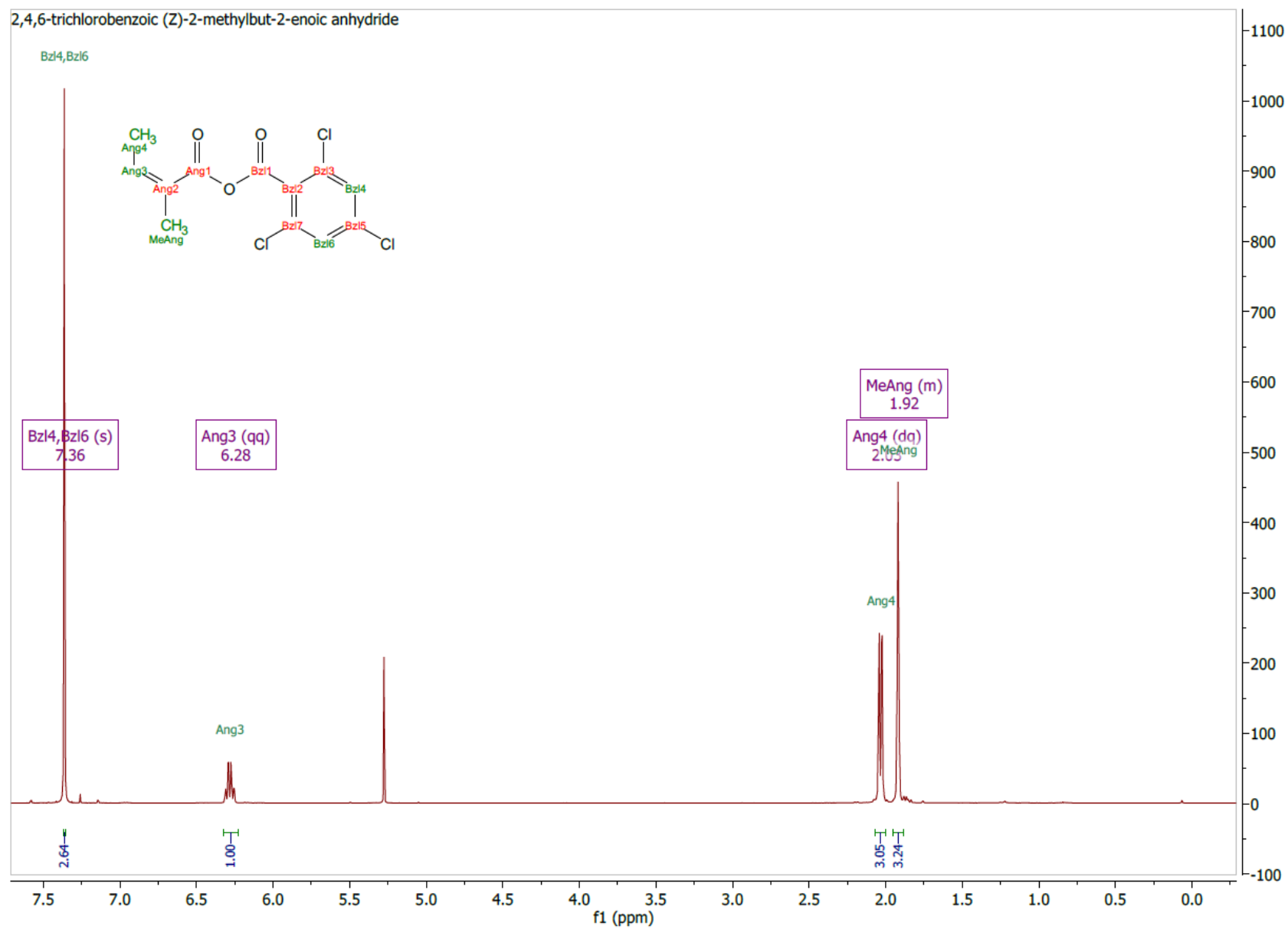


Figure S8A <sup>1</sup>H NMR of product 8 (400 MHz, CD<sub>3</sub>Cl)

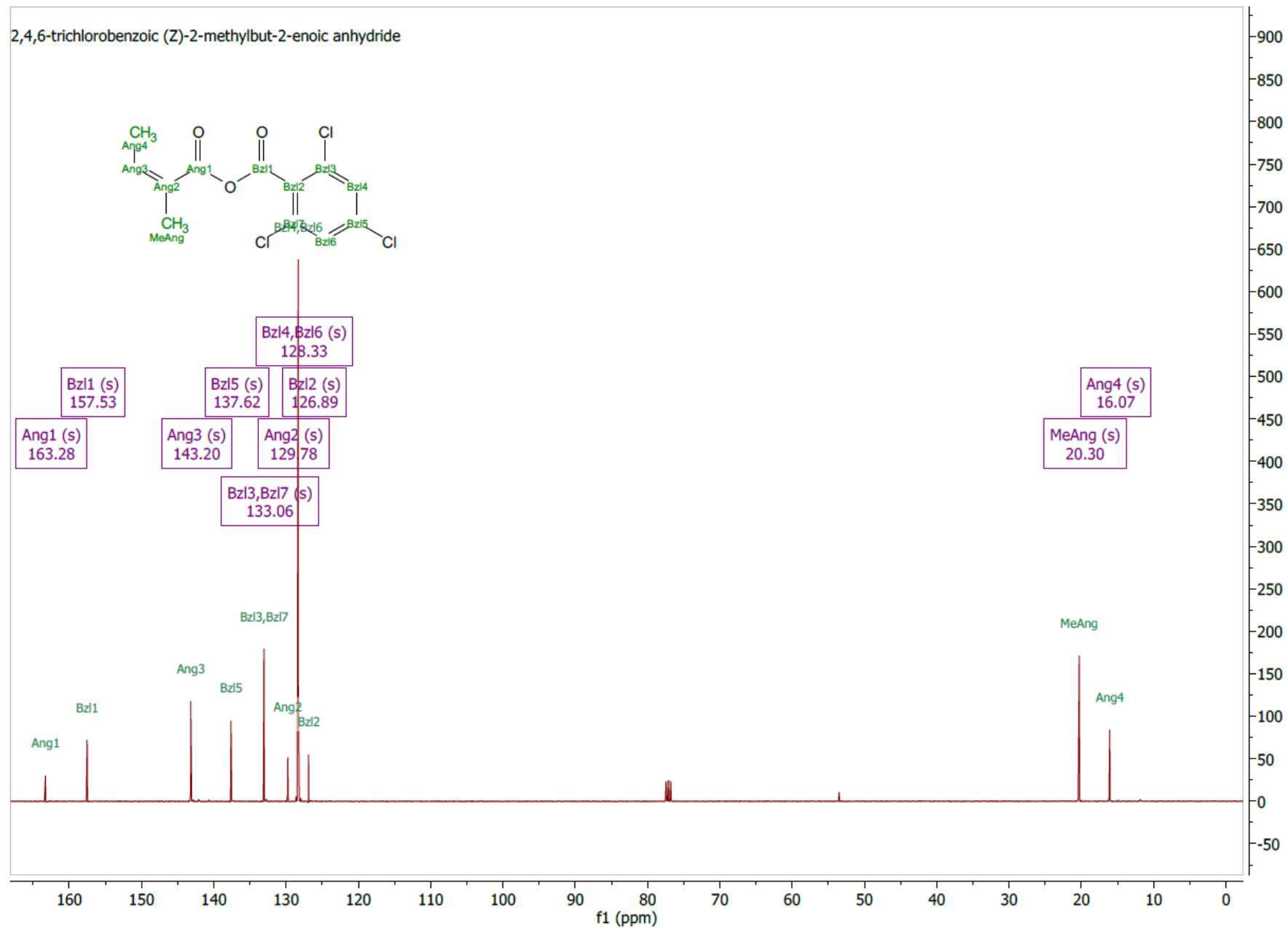


Figure S8B <sup>13</sup>C NMR of product 8 (100 MHz, CD<sub>3</sub>Cl)

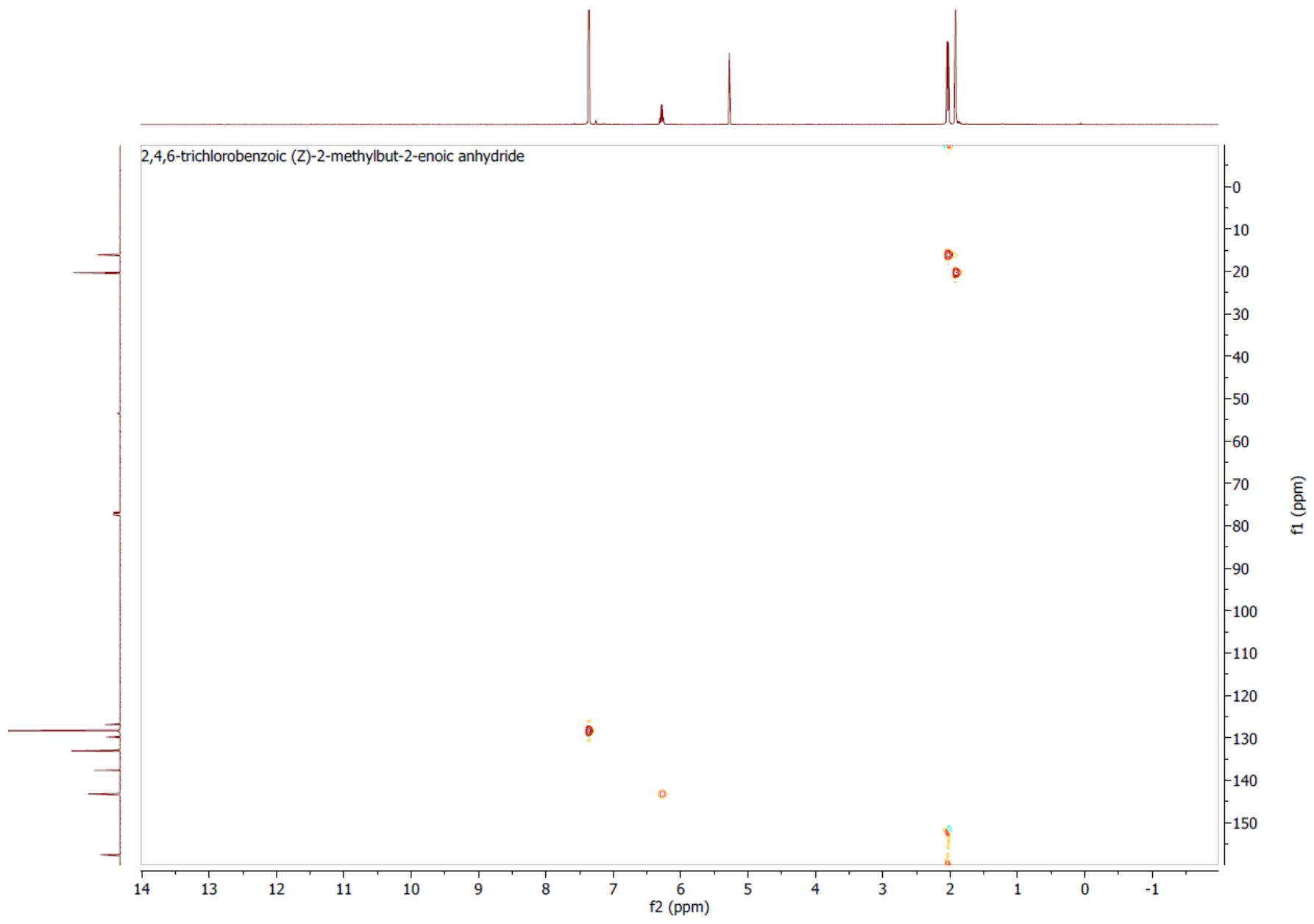


Figure S8C HMQC of product 8



# Boc-N-12-aminododecanoic acid (9)

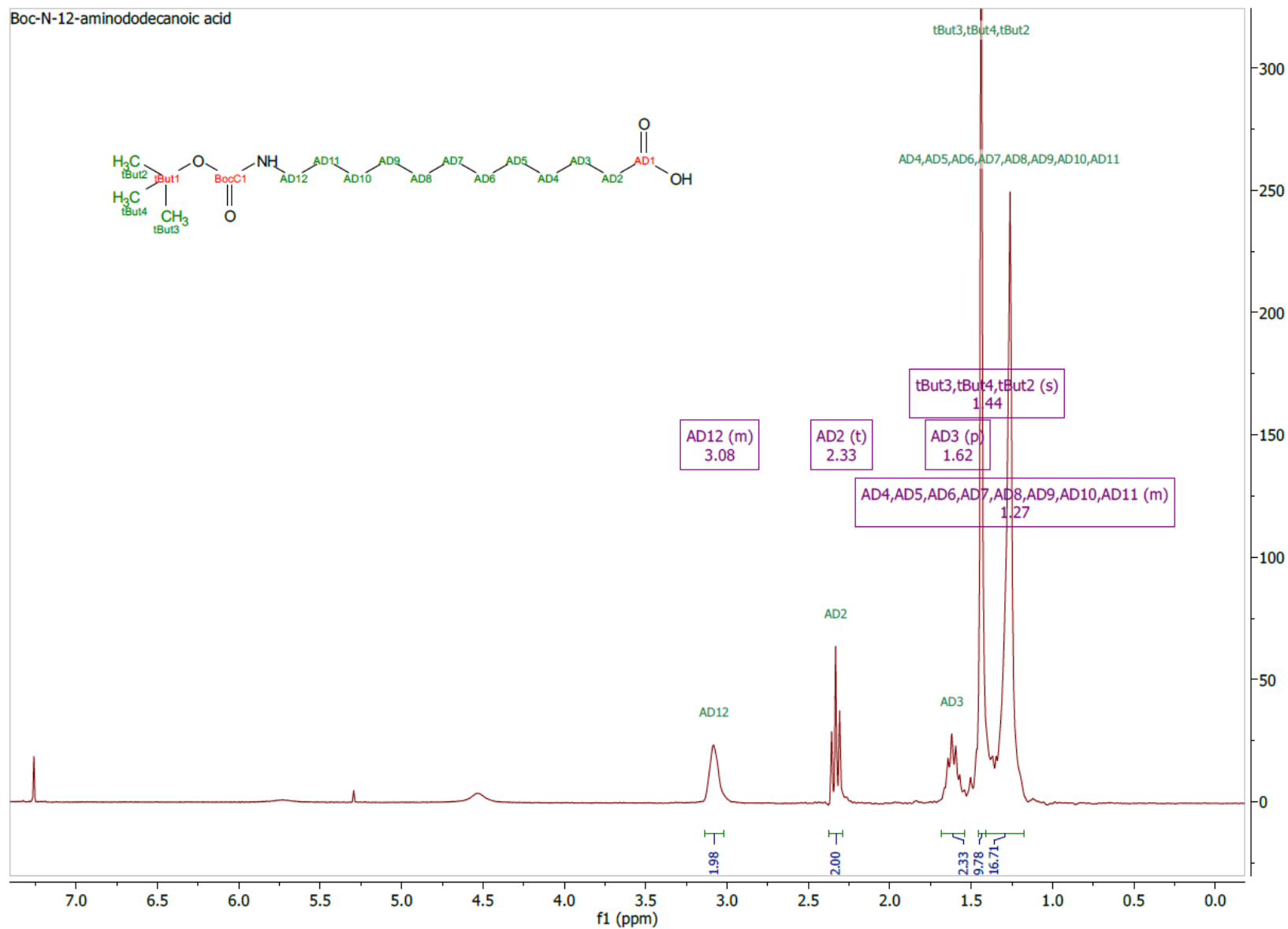
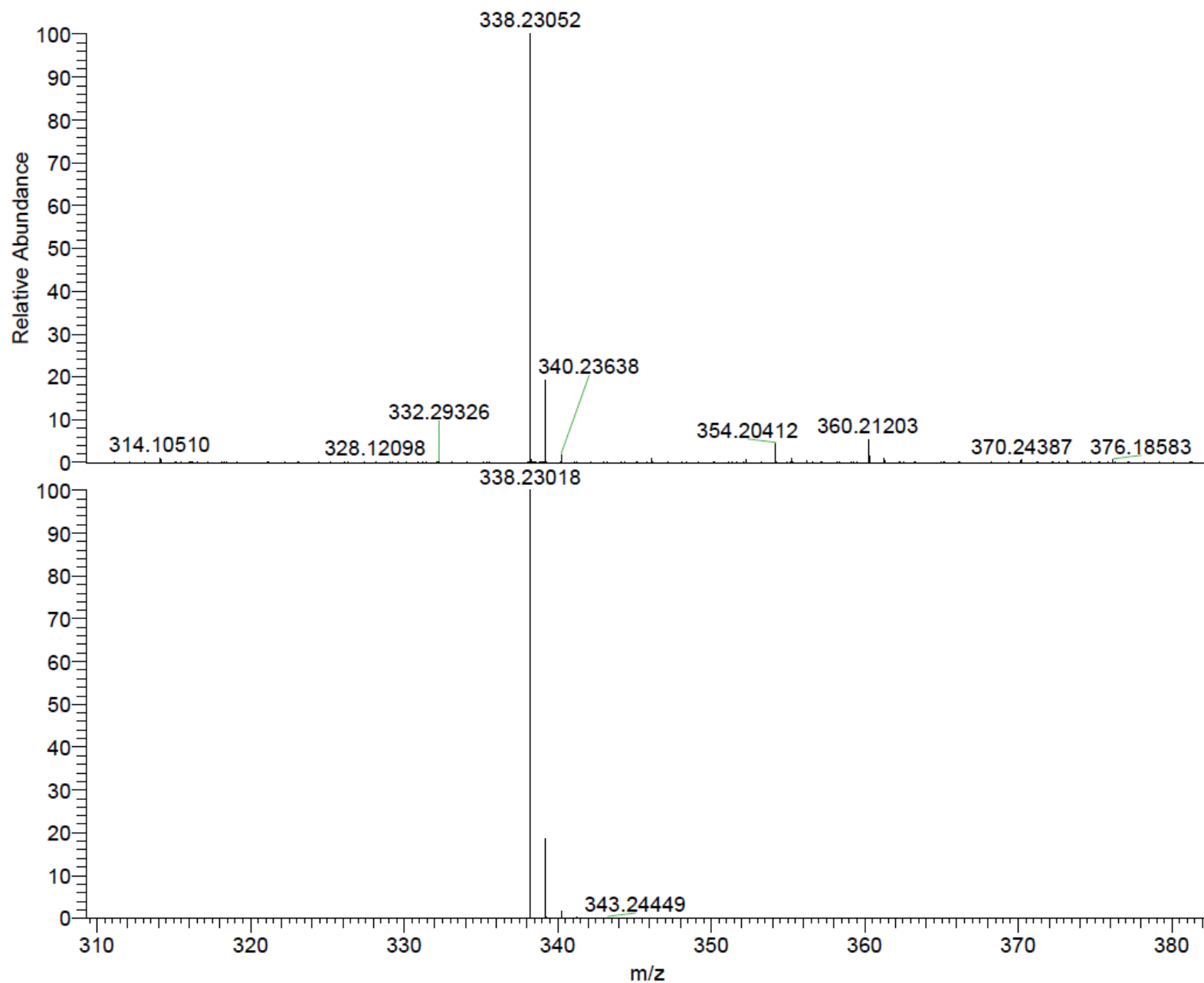


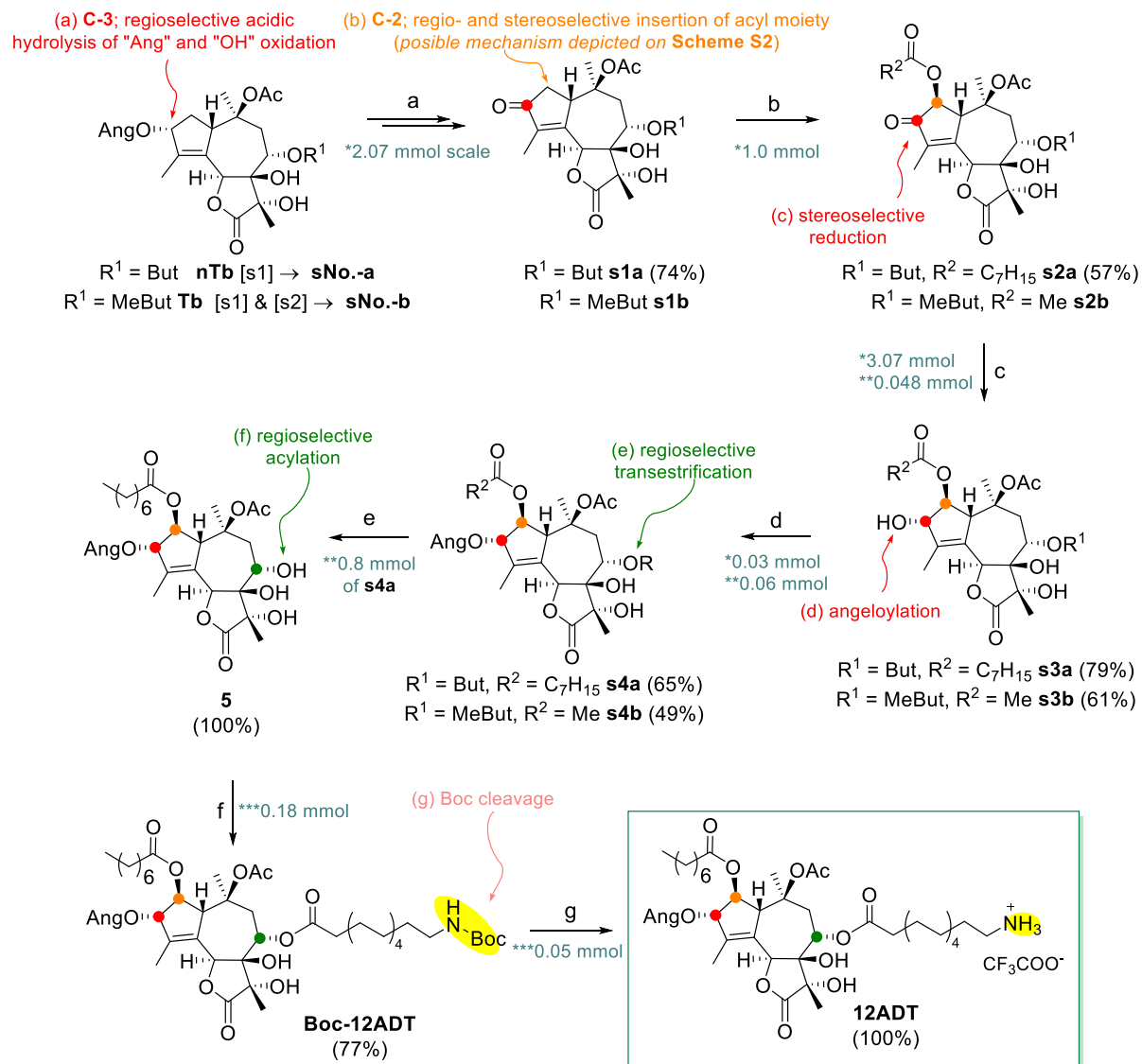
Figure S9A  $^1\text{H}$  NMR of product 9 (400 MHz,  $\text{CD}_3\text{Cl}$ )



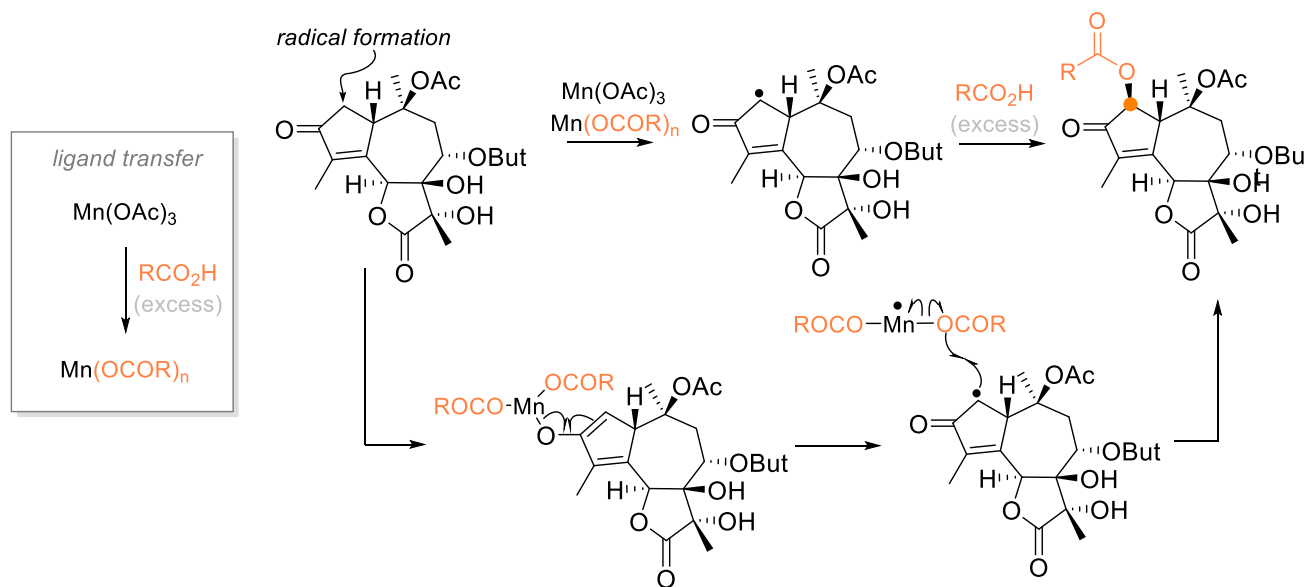
NL:  
2.32E7  
261\_Zimmermann\_ESIpos  
\_Boc-12AD\_1#50-56 RT:  
0.72-0.81 AV: 7 T: FTMS  
+ c ESI Full ms  
[200.00-2000.00]

NL:  
8.19E5  
c<sub>17</sub> h<sub>33</sub> n<sub>1</sub> o<sub>4</sub> +Na:  
C<sub>17</sub> H<sub>33</sub> N<sub>1</sub> O<sub>4</sub> Na<sub>1</sub>  
pa Chrg 1

Figure S9B HRMS of product 9 -  $[C_{17}H_{33}NO_4+Na]^+ = 338.23052$



**Scheme S1.** The reactions adopted from the literature published [s1\*, s2\*\*, s3\*\*\*]. *Reagents and conditions:* a)  $\text{CrO}_3$ , HF, AcCN, MW-95 °C, 2 h; b) octanoic acid,  $\text{Mn}(\text{OAc})_3 \cdot 2\text{H}_2\text{O}$ , 120 °C, 6 h; c)  $\text{Zn}(\text{BH}_4)_2$ ,  $\text{Et}_2\text{O}$ , -20 °C, 3.5 h, then  $\text{H}_2\text{O}$ ; d) *in situ* preparation of 2,4,6-trichlorobenzoic (Z)-2-methylbut-2-enoic anhydride = acyl chloride, AngOH, TEA, in toluene; 90 °C, 18 h; e) TEA, MeOH, 0 °C  $\rightarrow$  20 °C, 21 h; f) Boc-12-aminododecanoic acid, DCC, 4-DMAP, DCM, RT, 0 °C  $\rightarrow$  RT, 6 h; g) TFA, DCM,  $\text{H}_2\text{O}$ , RT, 45 min. Under the arrows, the scale of the starting material is specified in green.



**Scheme S2.** Proposed mechanism of  $\alpha$ -oxylation

## References

- [s1] Crestey, F.; Toma, M.; Christensen, S. B. Concise synthesis of thapsigargin from nortrilobolide. *Tetrahedron Lett.* **2015**, 56 (43), 5896-5898.
- [s2] Doan, N. T. Q.; Crestey, F.; Olsen, C. E.; Christensen, S. B. Chemo- and regioselective functionalization of nortrilobolide: Application for semisynthesis of the natural product 2-acetoxytrilobolide. *J. Nat. Prod.* **2015**, 78 (6), 1406-1414.
- [s3] Jakobsen, C. M.; Denmeade, S. R.; Isaacs, J. T.; Gady, A.; Olsen, C. E.; Christensen, S. B. Design, synthesis, and pharmacological evaluation of thapsigargin analogues for targeting apoptosis to prostatic cancer cells. *J. Med. Chem.* **2001**, 44, 4696-4703.