

# New Cytotoxic Cerebrosides from the Red Sea Cucumber *Holothuria spinifera* Supported by *In-Silico* Studies

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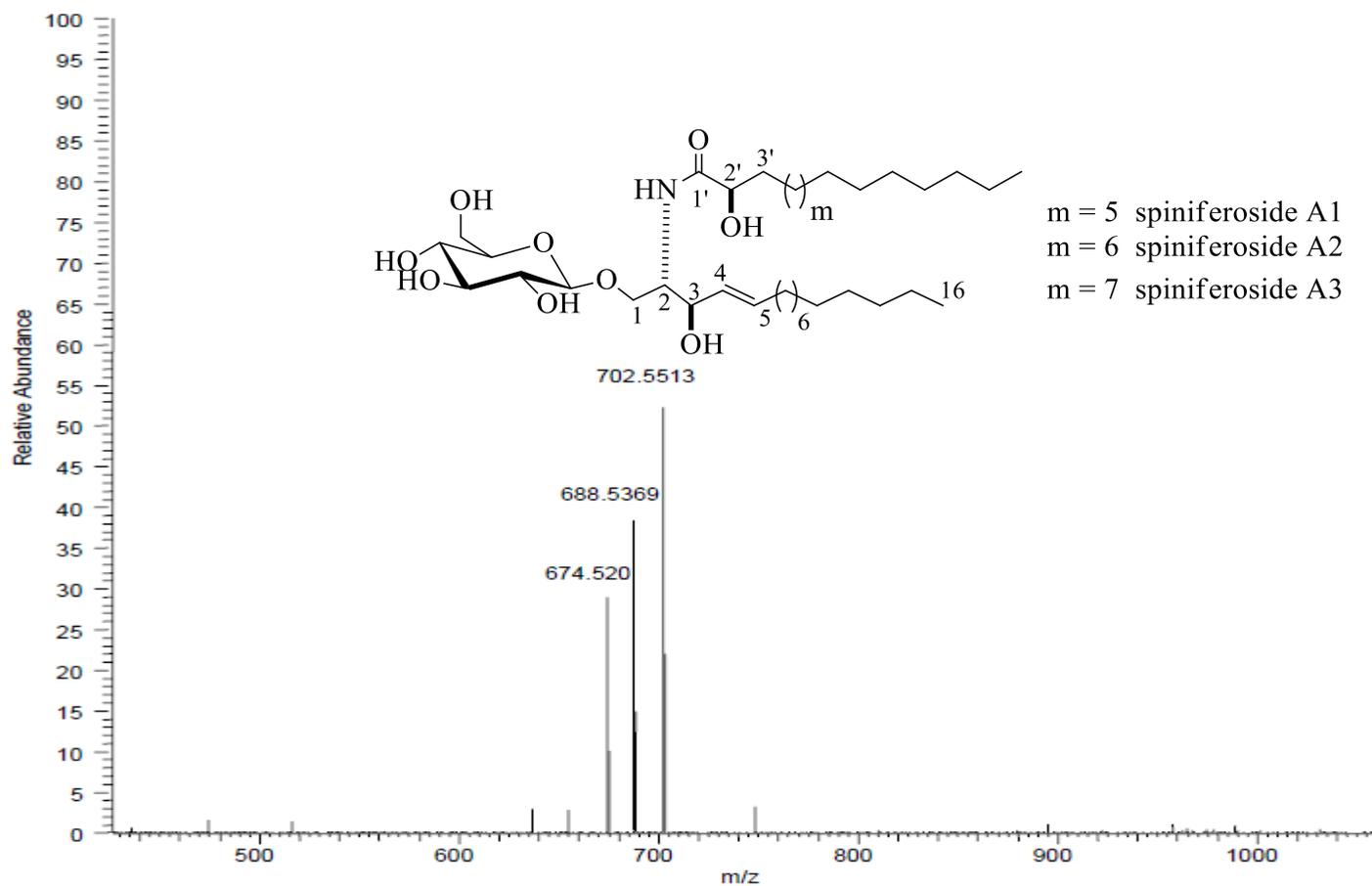


Figure S1. LC-HRESIMS of compound 1 (M+H)<sup>+</sup>.

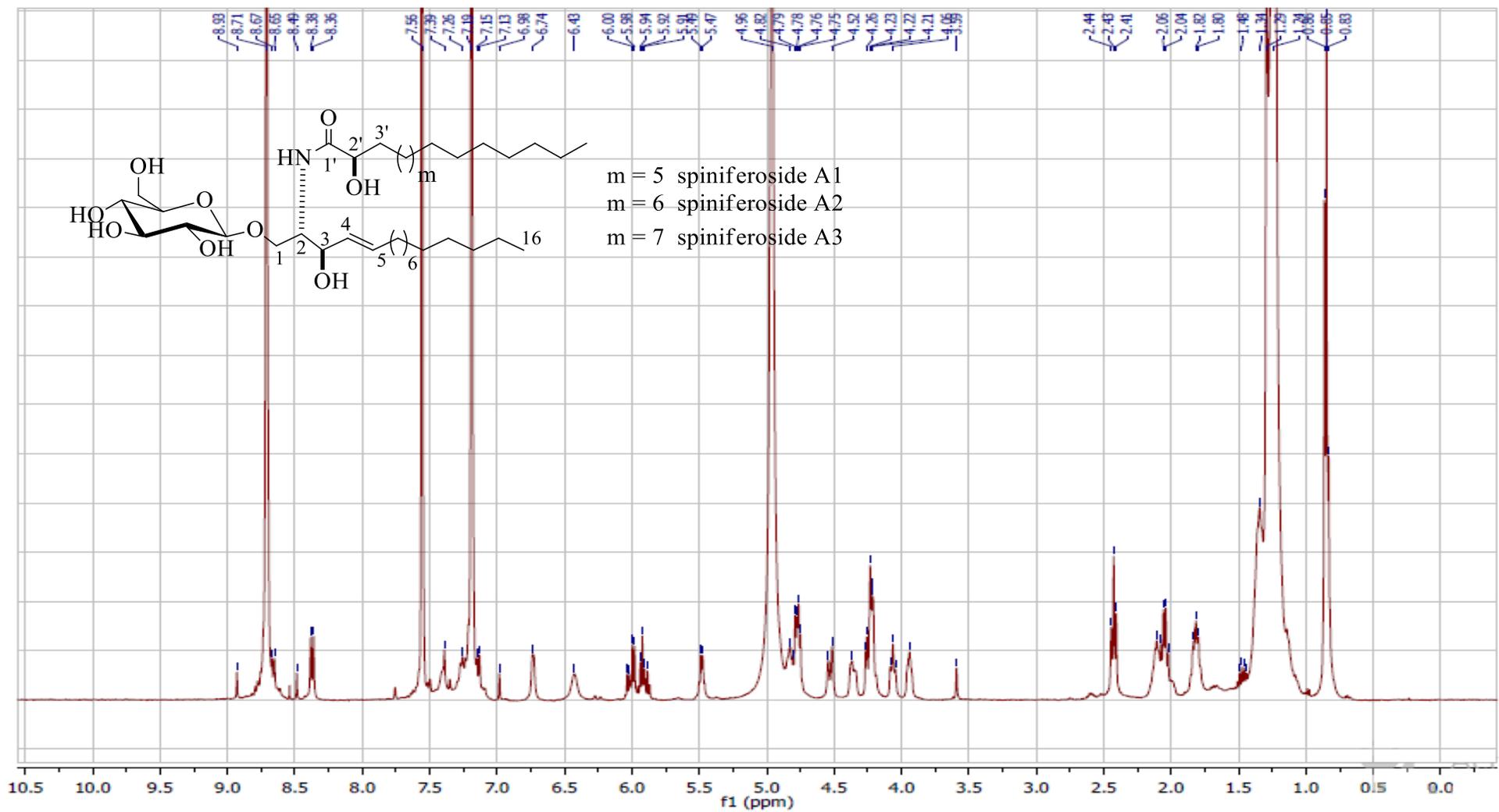


Figure S2.  $^1\text{H}$  NMR spectrum of compound 1 (in  $\text{C}_5\text{D}_5\text{N}$ , 400 MHz).

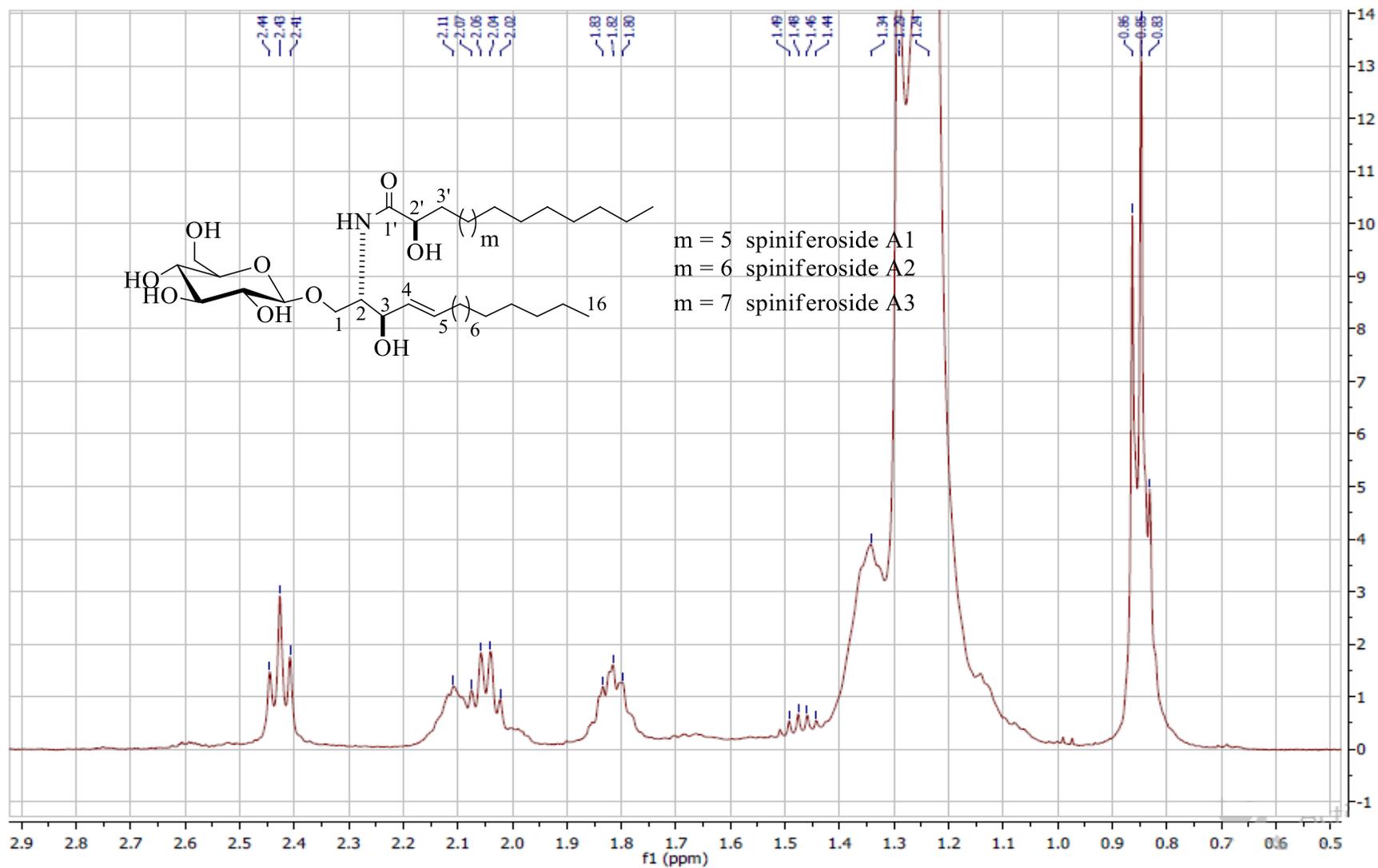
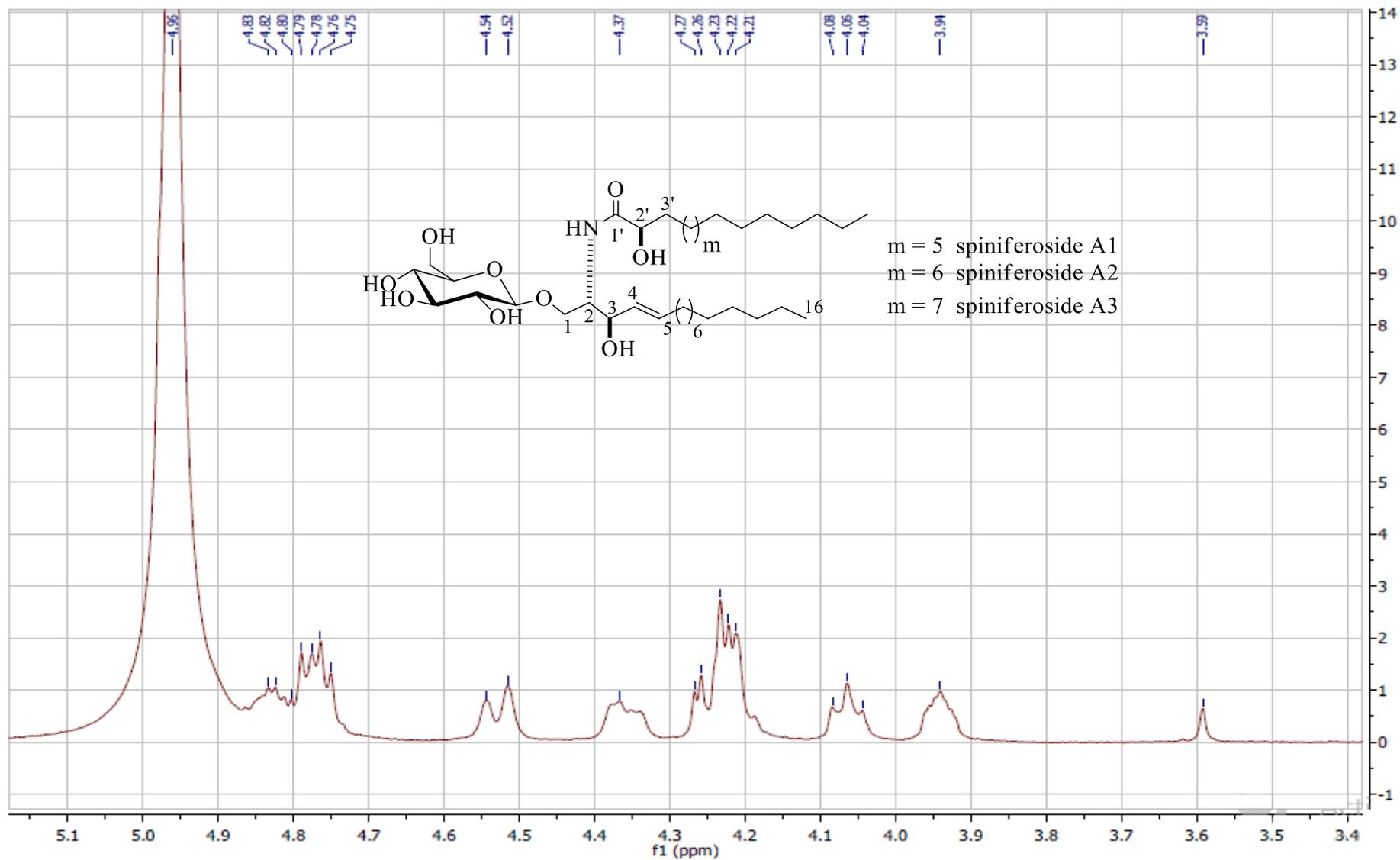


Figure S3. Partial expansion of the <sup>1</sup>H NMR spectrum of compound 1 (in C<sub>5</sub>D<sub>5</sub>N, 400 MHz).



**Figure S4.** Partial expansion of the  $^1\text{H}$  NMR spectrum of compound **1** (in  $\text{C}_5\text{D}_5\text{N}$ , 400 MHz).

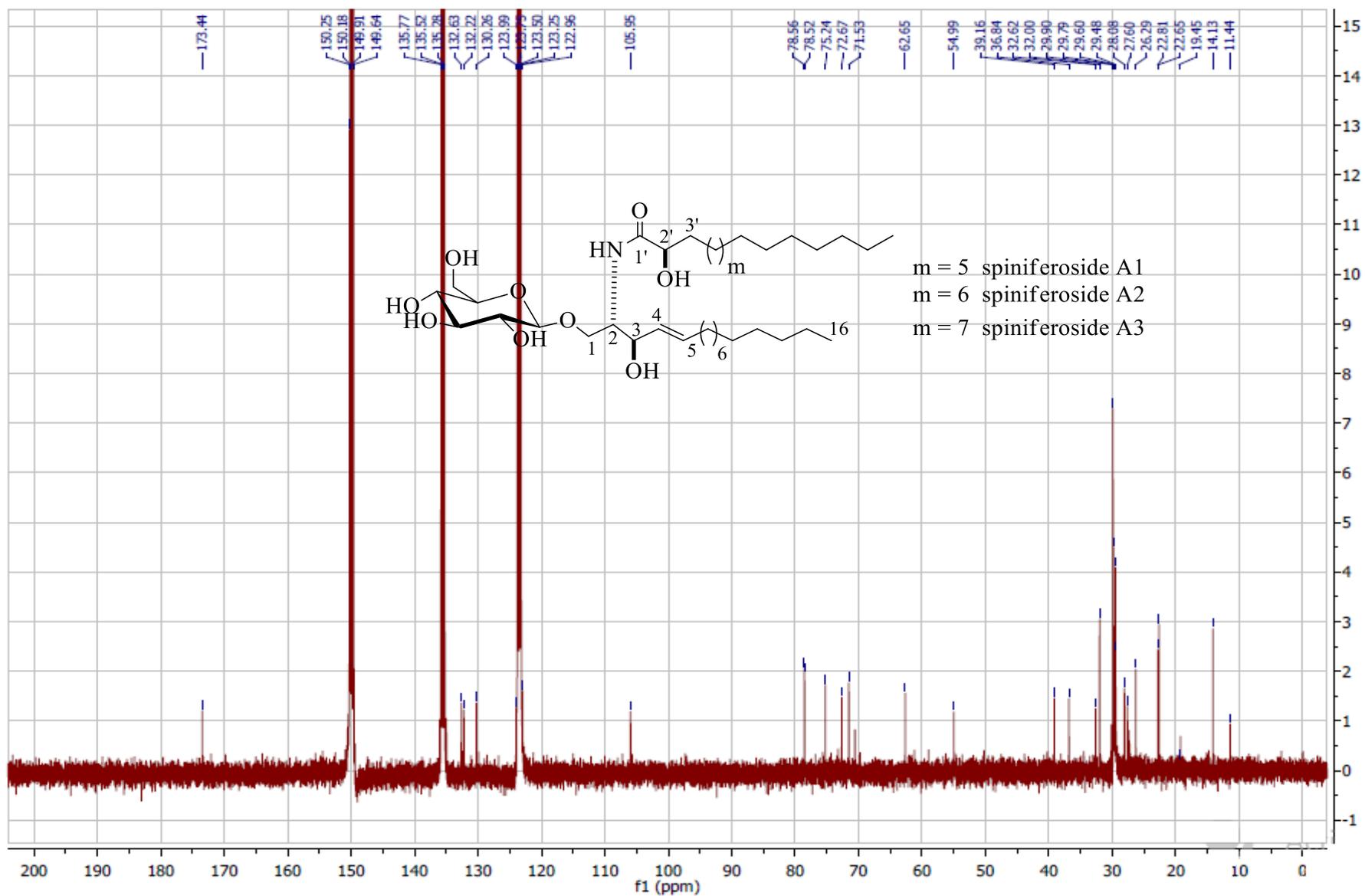
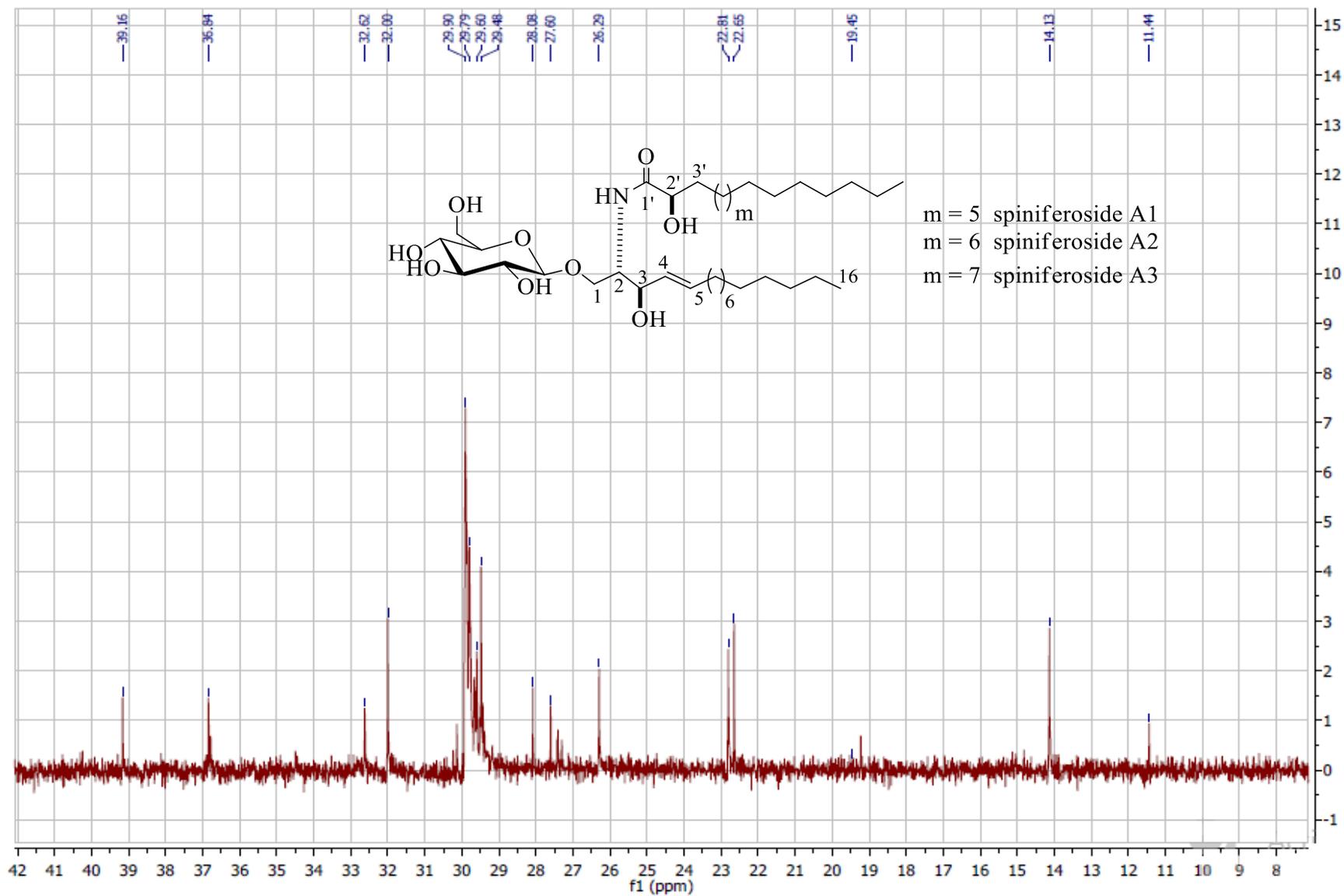
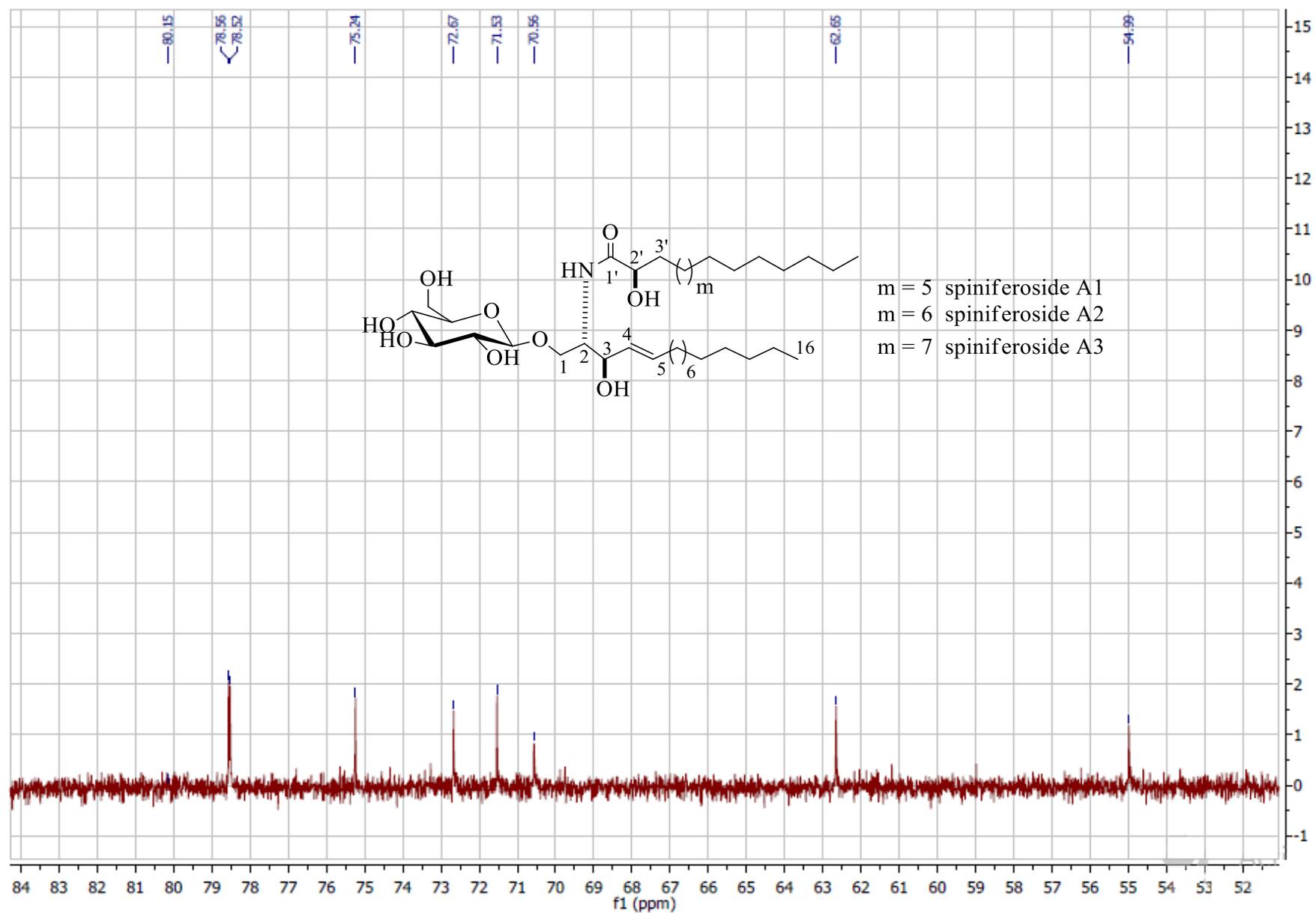


Figure S5.  $^{13}C$  NMR spectrum of compound 1 (in  $C_5D_5N$ , 100 MHz).



**Figure S6.** Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound 1 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).



**Figure S7.** Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound **1** (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

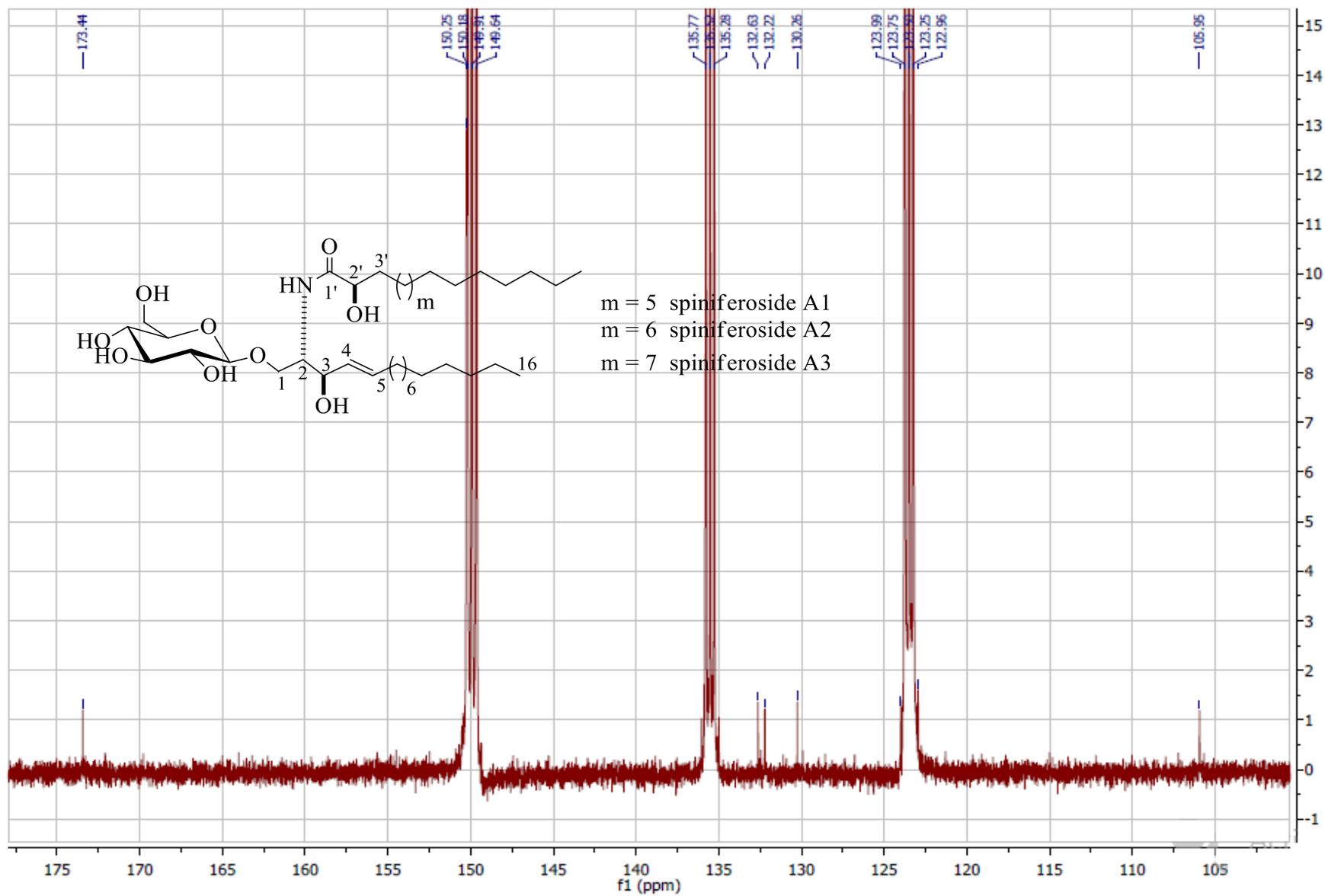
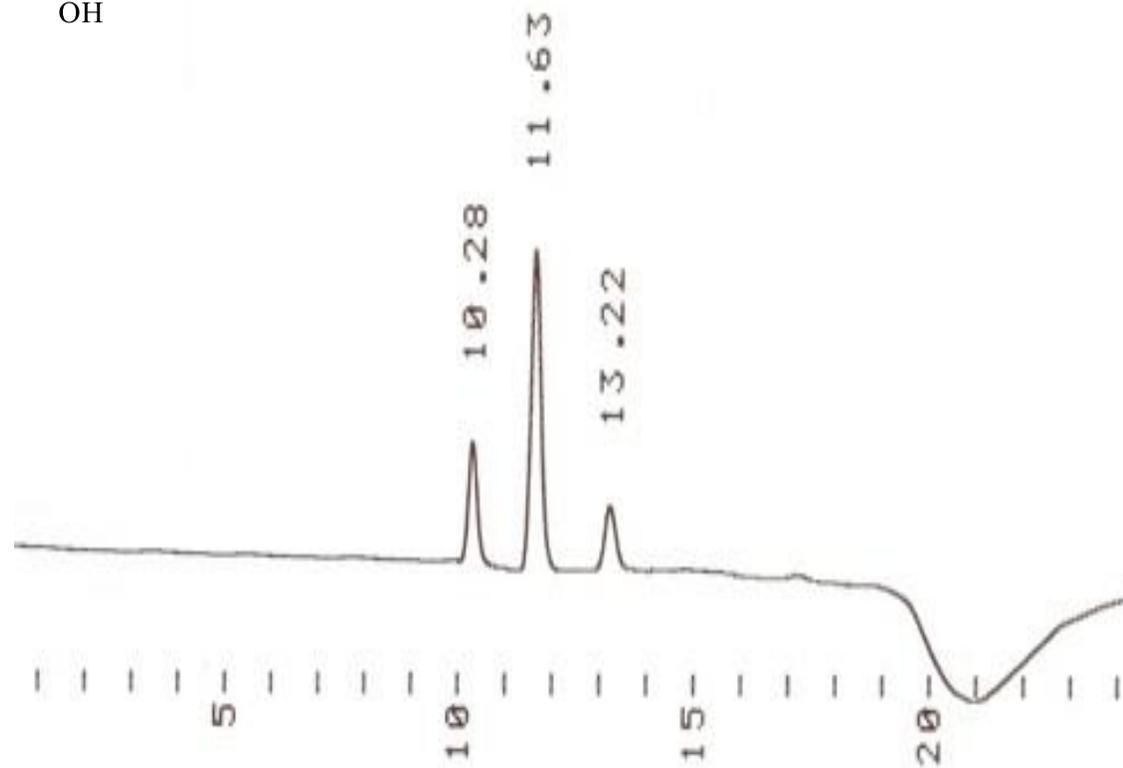
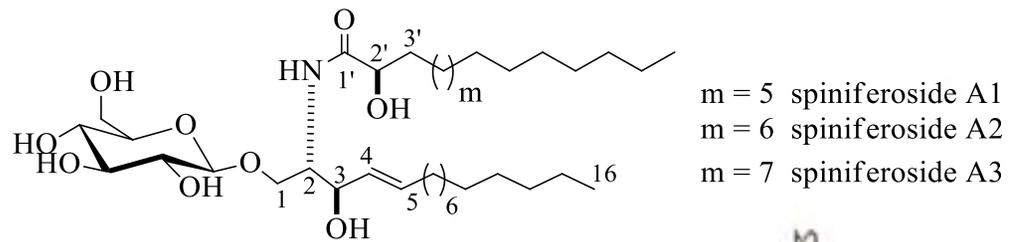


Figure S8. Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound **1** (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).



**Figure S9.** Chromatogram of semi-preparative HPLC purification of compound 1.

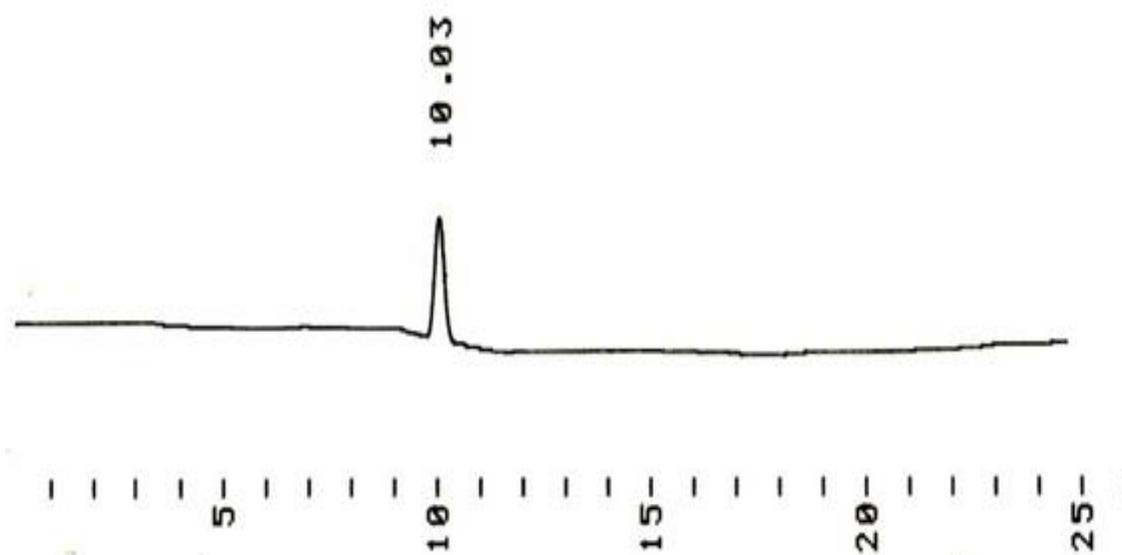
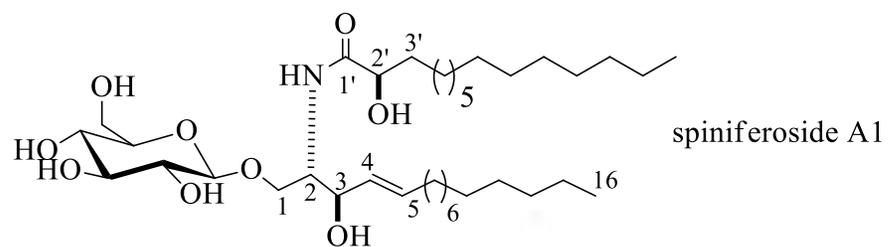


Figure S10. Chromatogram of semi-preparative HPLC purification of spiniferoside A1 (1a).

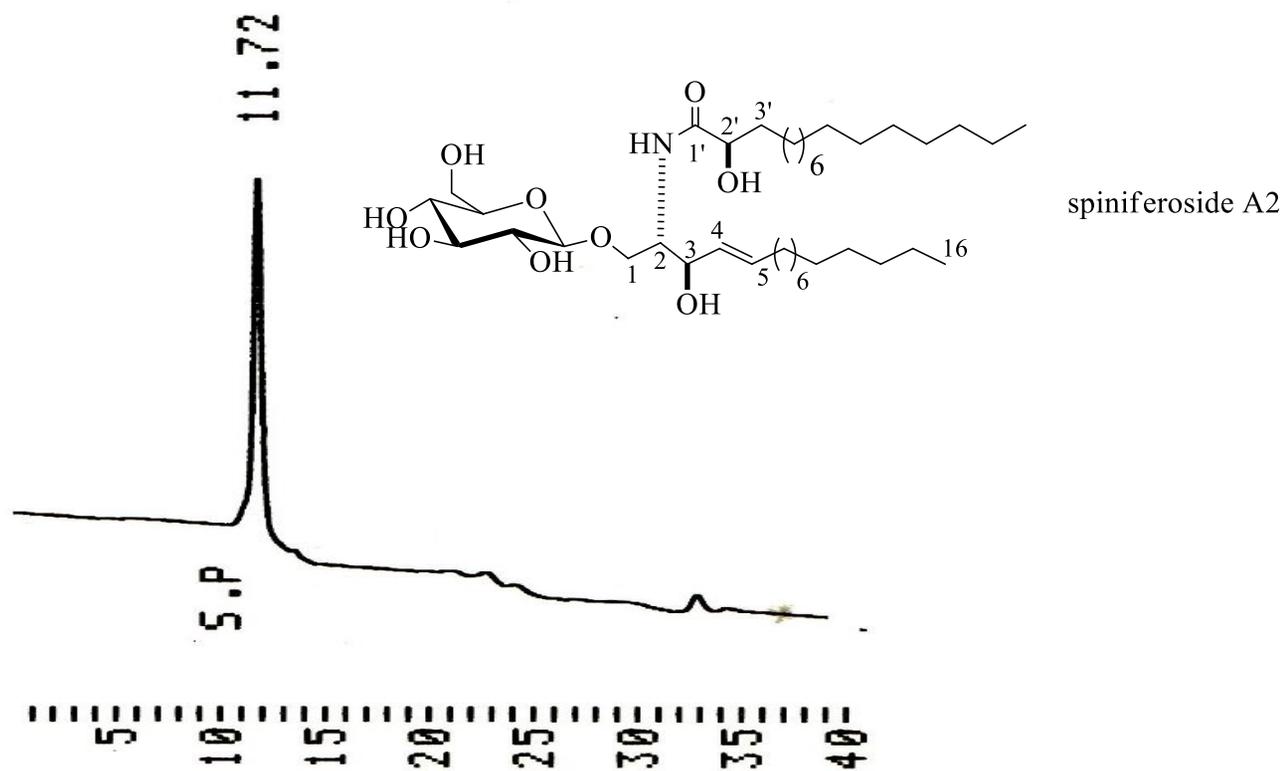
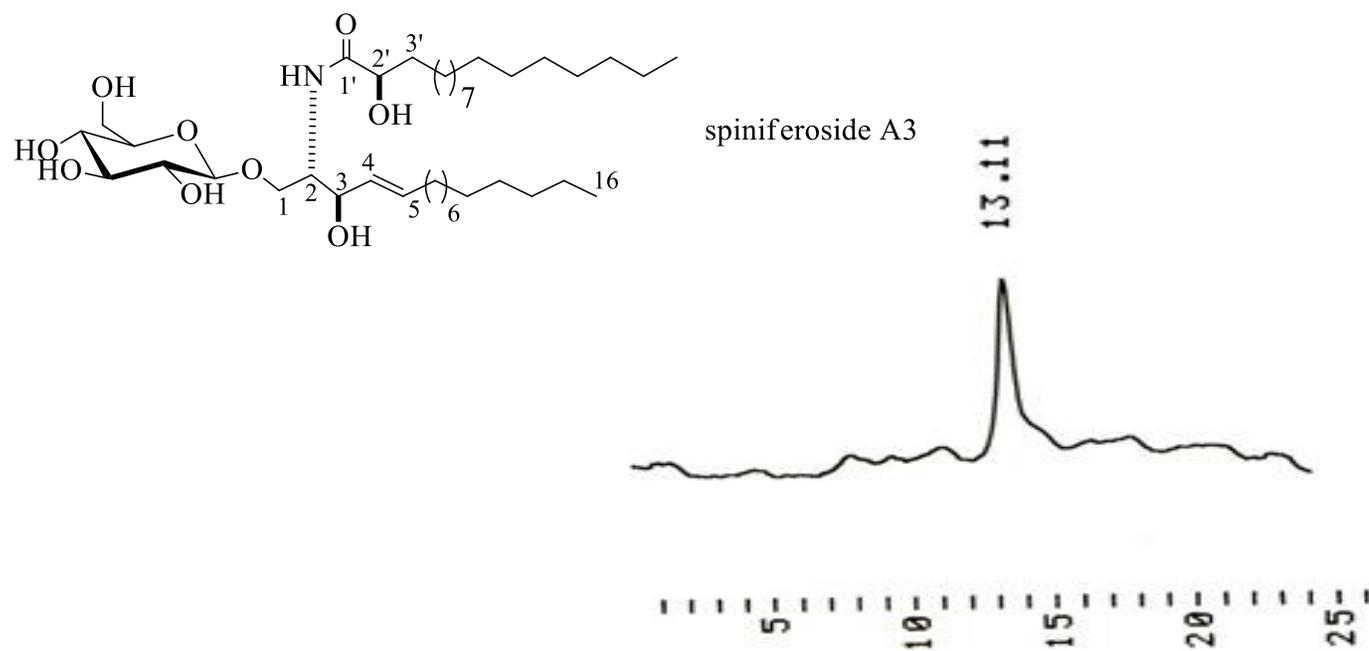


Figure S11. Chromatogram of semi-preparative HPLC purification of spiniferoside A2 (1b).



**Figure S12.** Chromatogram of semi-preparative HPLC purification of spiniferoside A3 (**1c**).

UR12a ESI POS

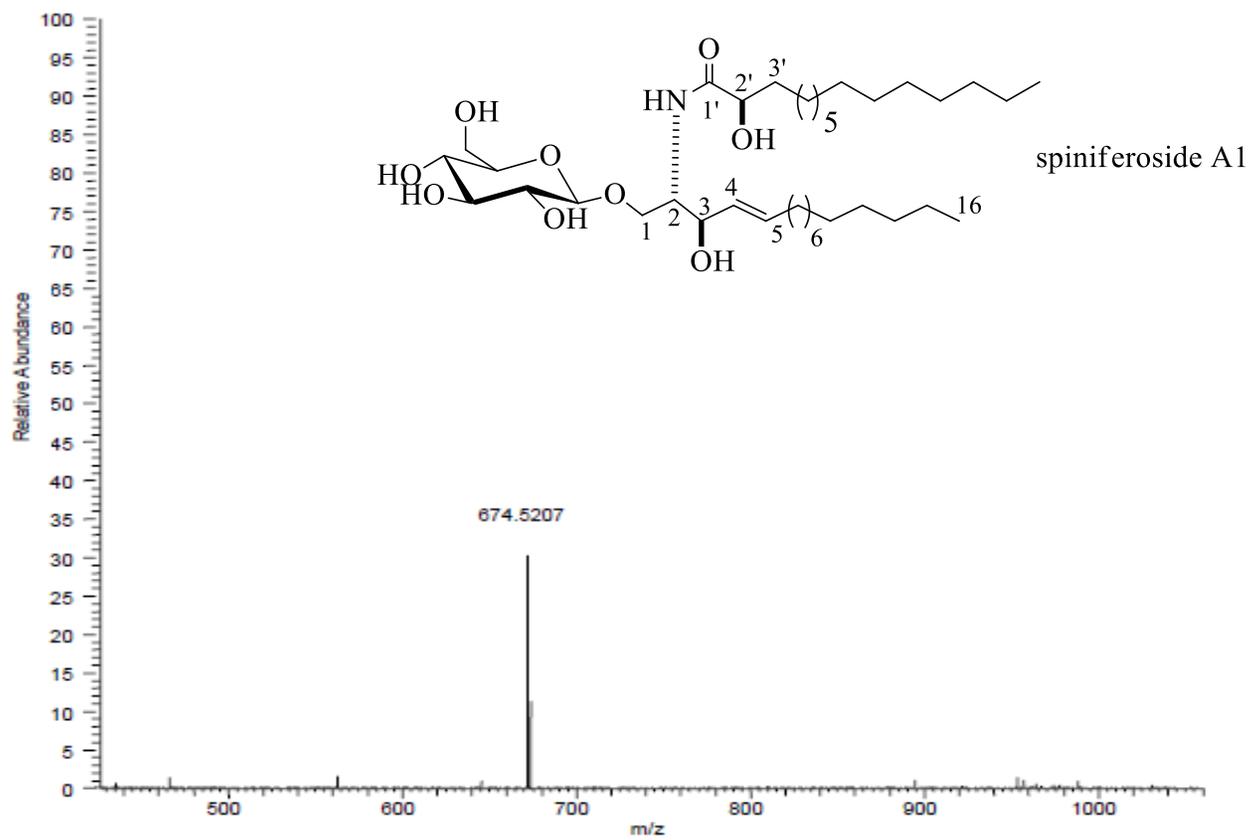
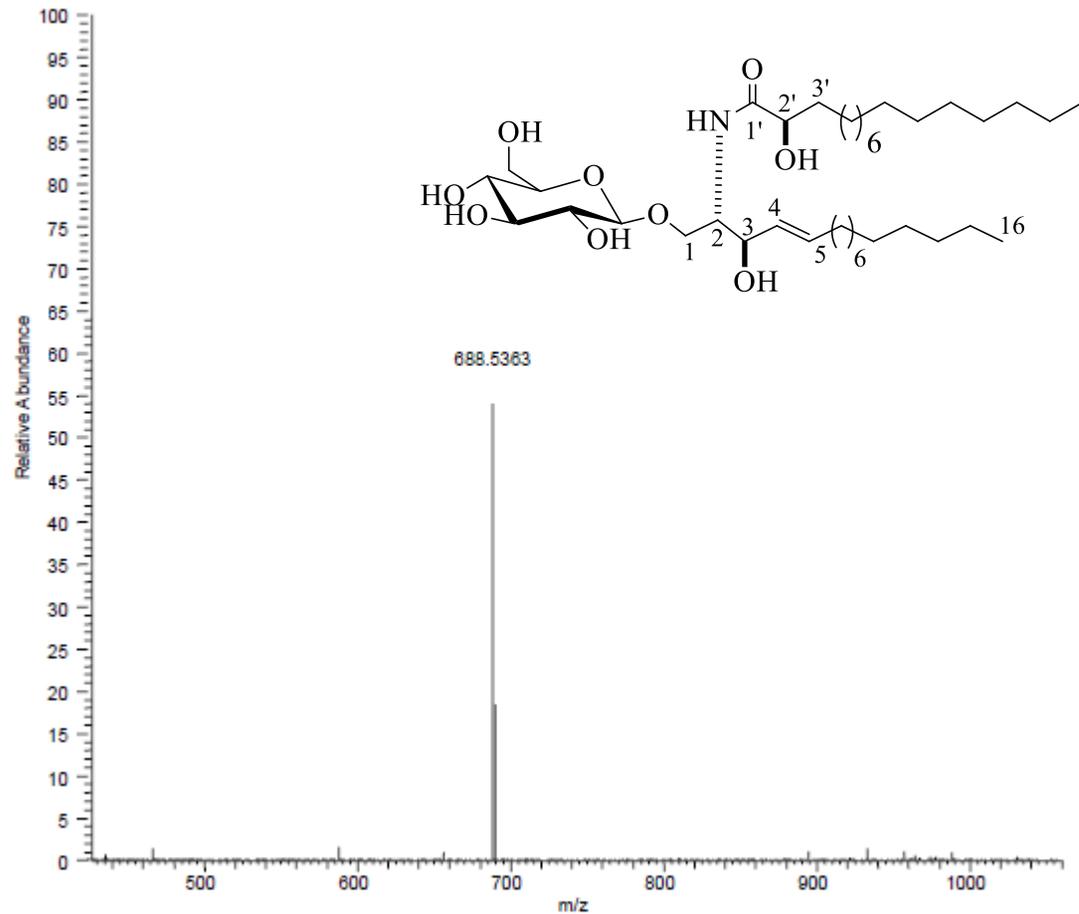


Figure S13. LC-HRESIMS for spiniferoside A1 (1a).

UR12b ESI POS



spiniferoside A2

Figure S14. LC-HRESIMS for spiniferoside A2 (1b).

UR12c ESI POS

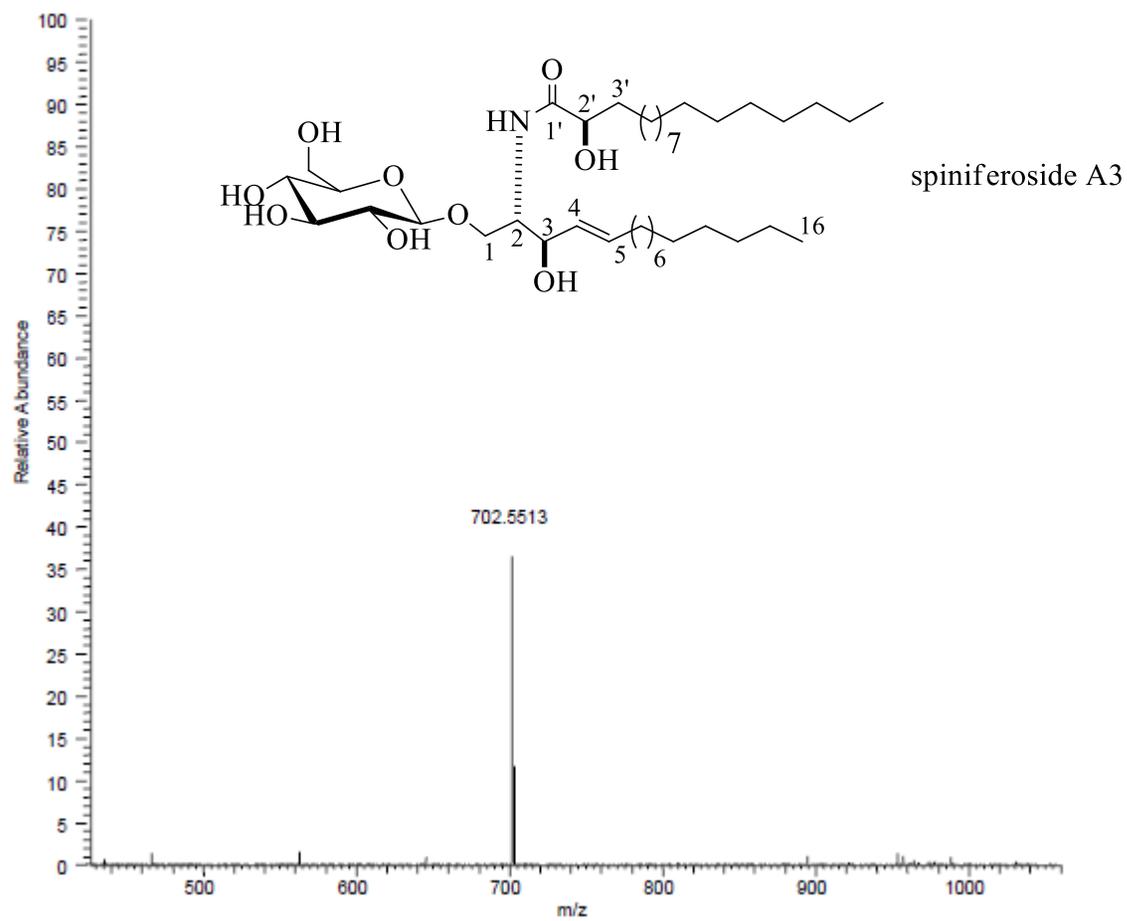


Figure S15: LC-HRESIMS for spiniferoside A3 (1c).

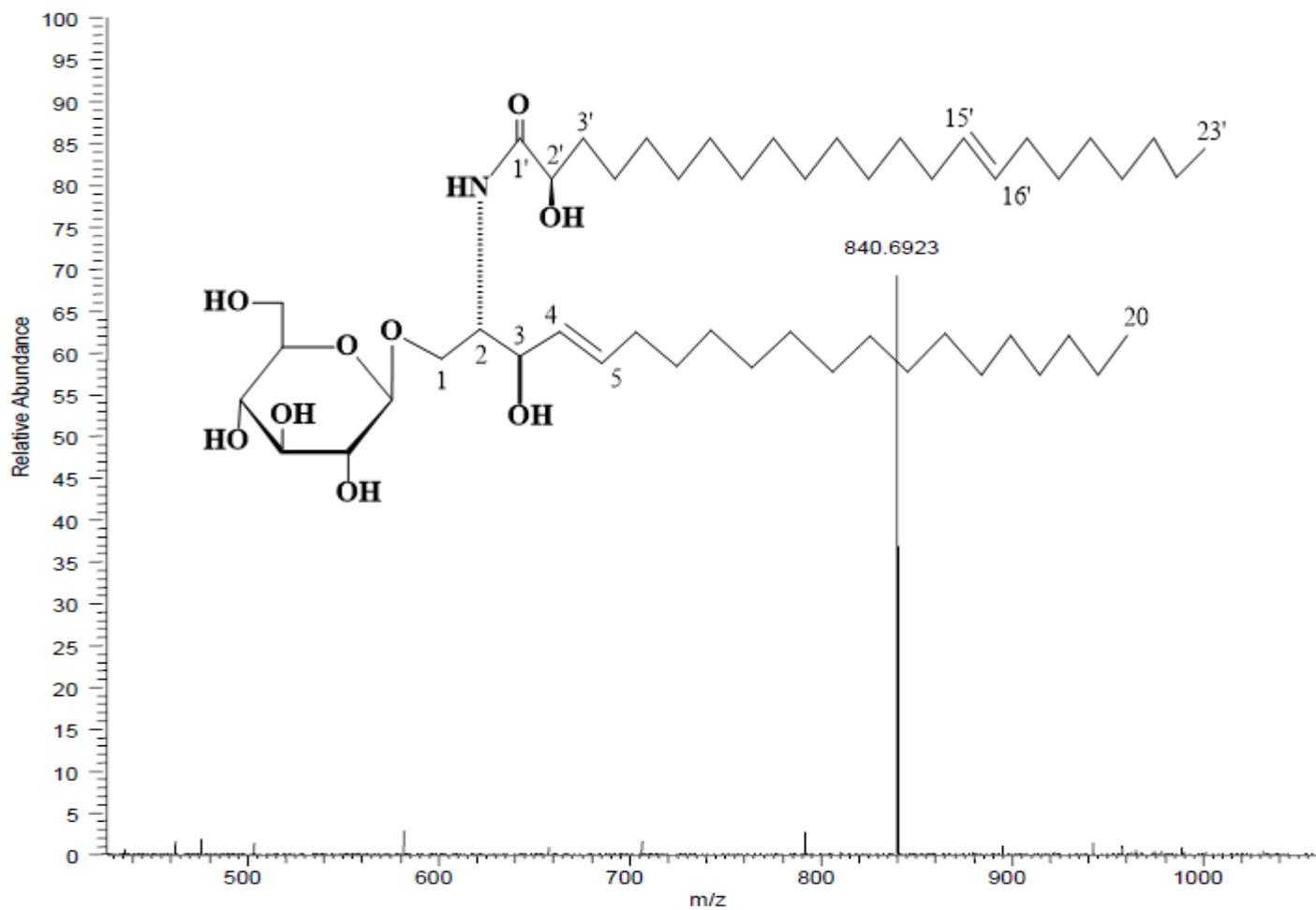


Figure S16. LC-HRESIMS of compound 2 (M+H)<sup>+</sup>

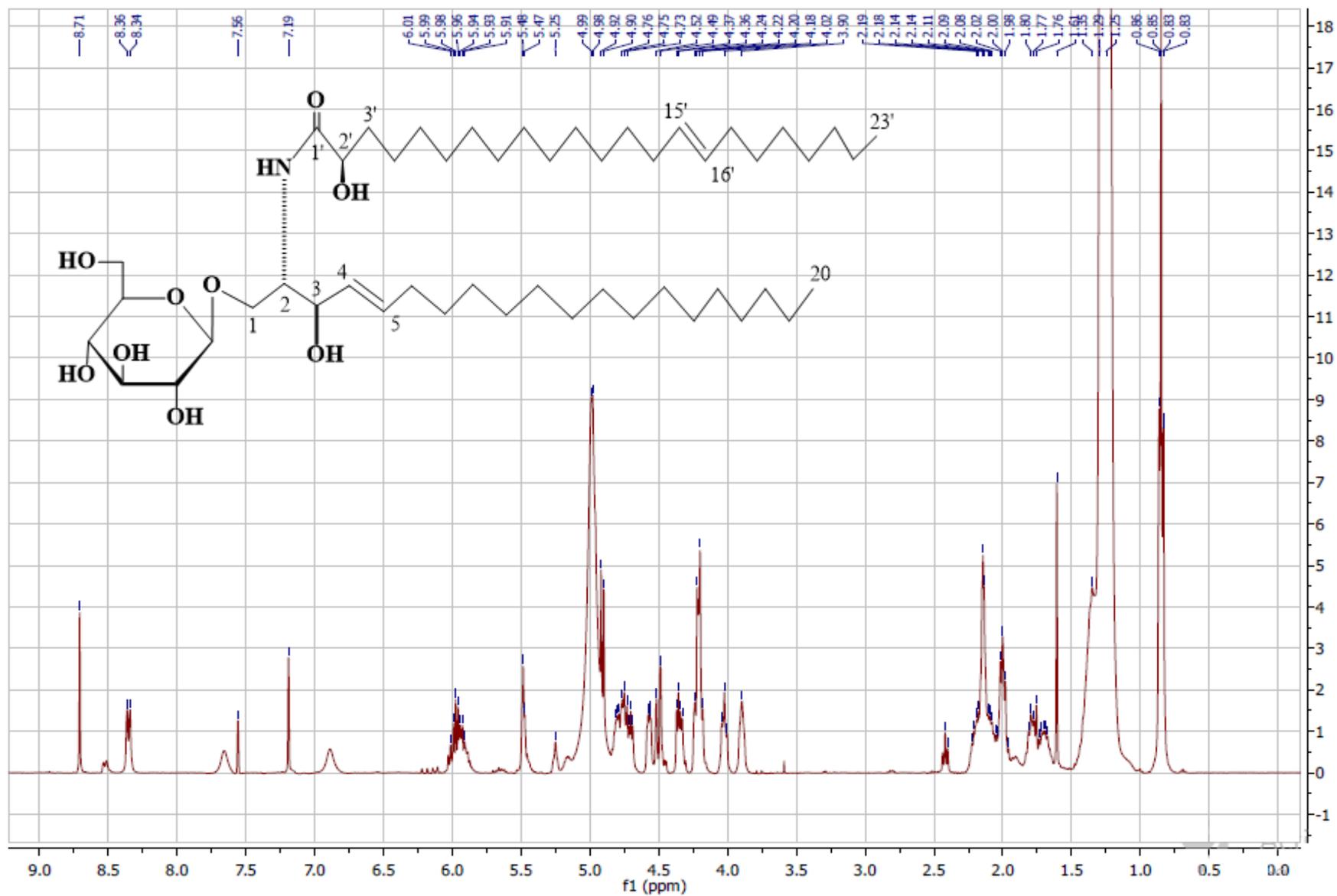


Figure S17.  $^1\text{H}$  NMR spectrum of compound 2 (in  $\text{C}_5\text{D}_5\text{N}$ , 400 MHz).

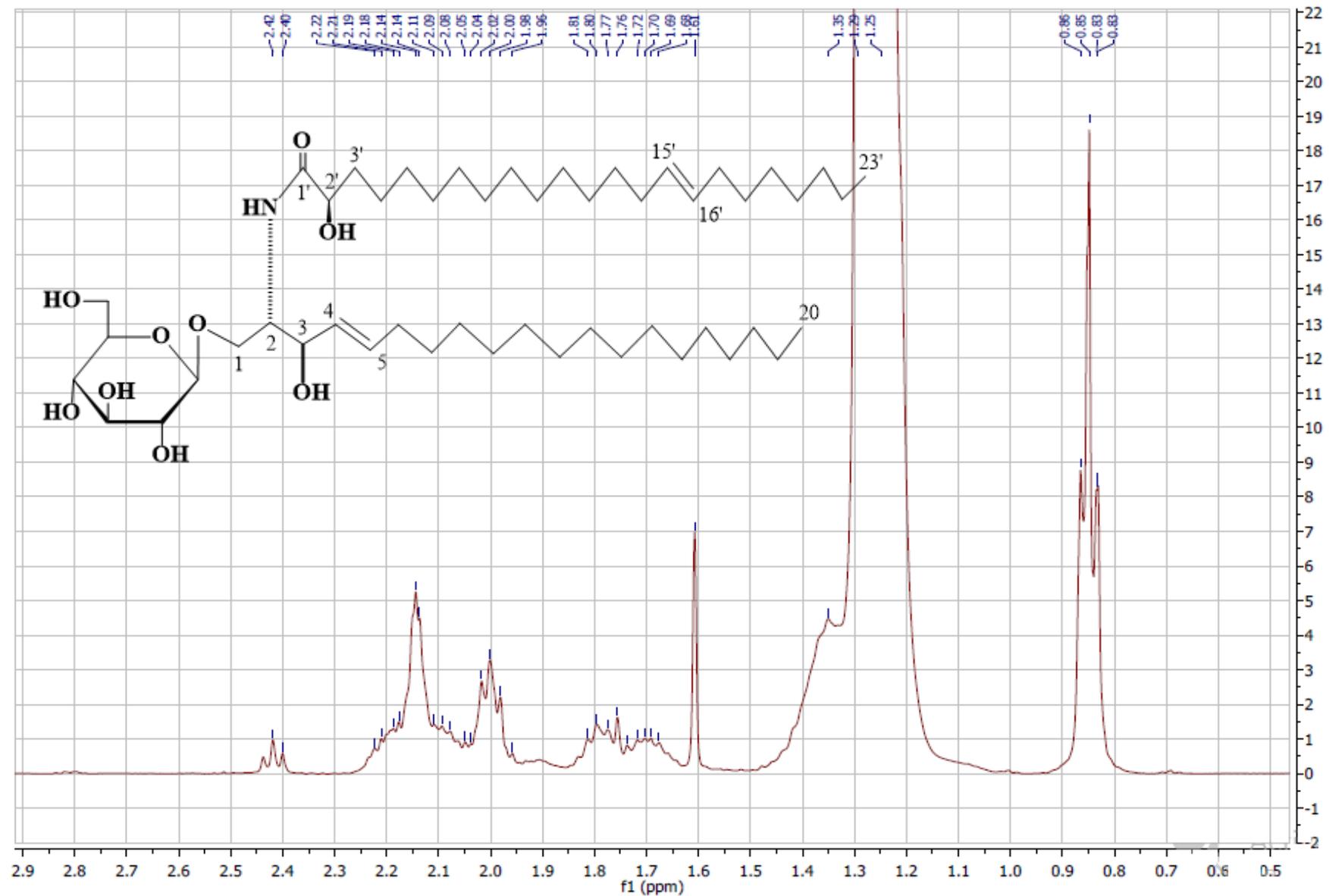


Figure S18. Partial expansions of the <sup>1</sup>H NMR spectrum of compound 2 (in C<sub>5</sub>D<sub>5</sub>N, 400 MHz).

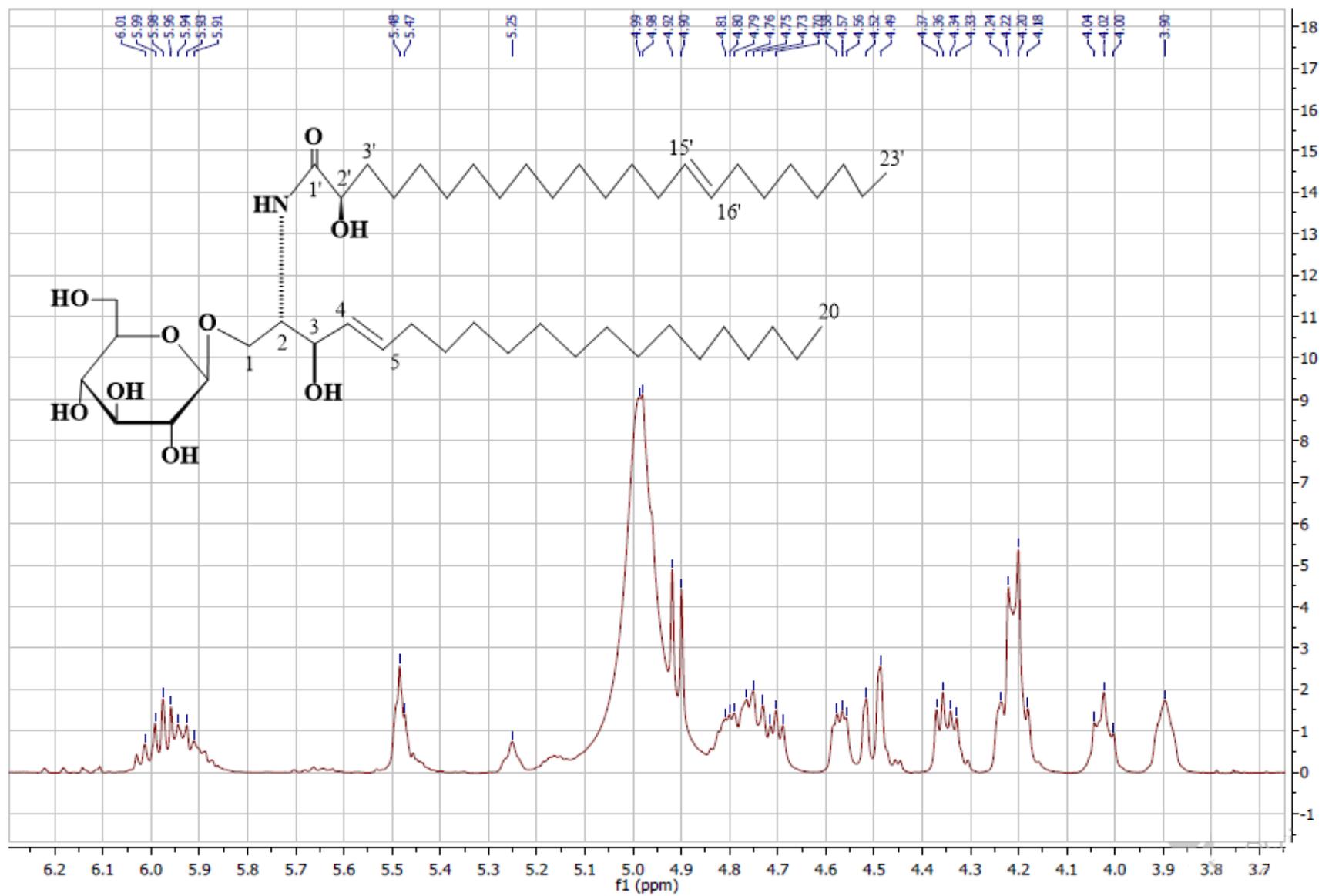


Figure S19. Partial expansions of the <sup>1</sup>H NMR spectrum of compound 2 (in C<sub>5</sub>D<sub>5</sub>N, 400 MHz).

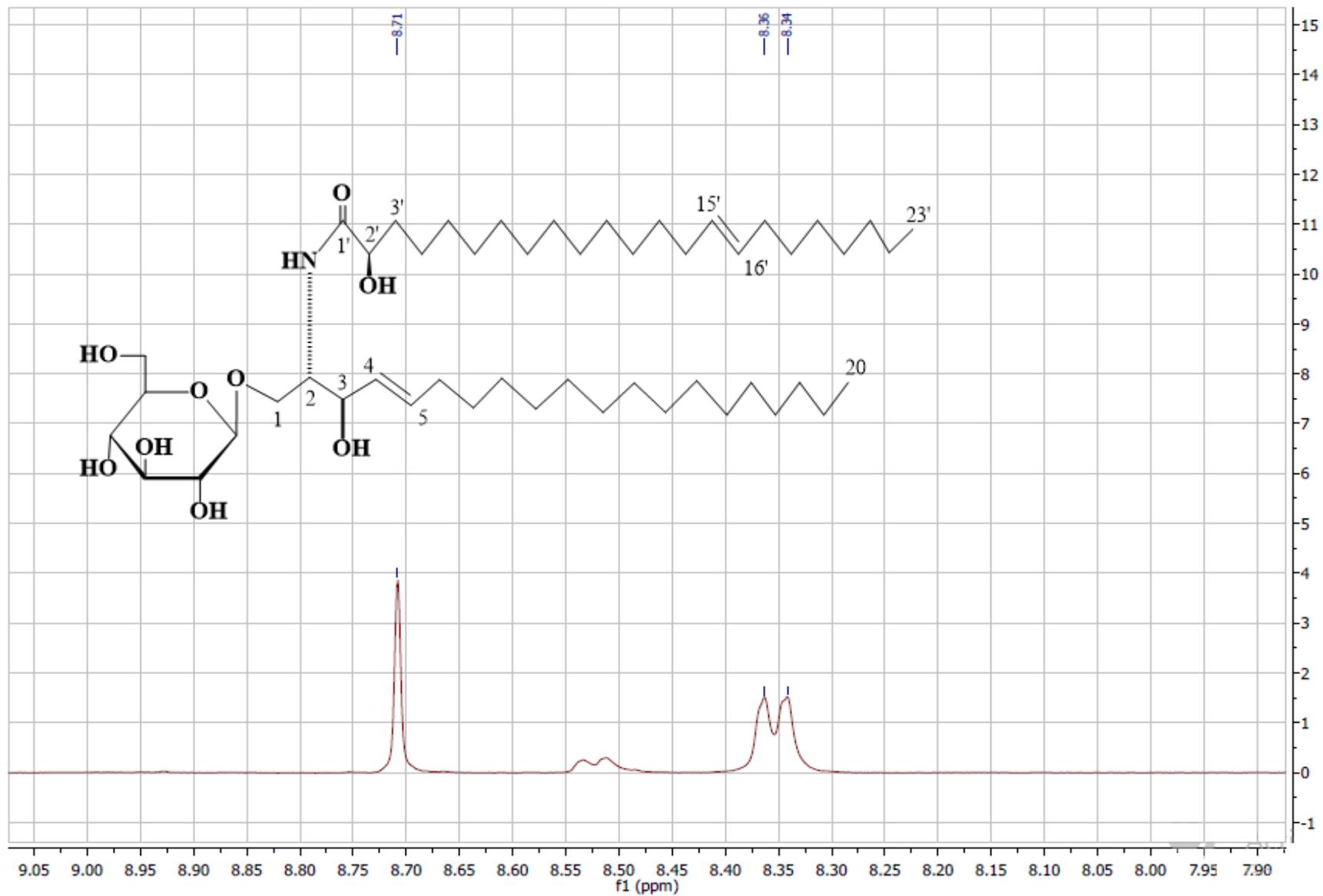


Figure S20. Partial expansions of the <sup>1</sup>H NMR spectrum of compound 2 (in C<sub>5</sub>D<sub>5</sub>N, 400 MHz).

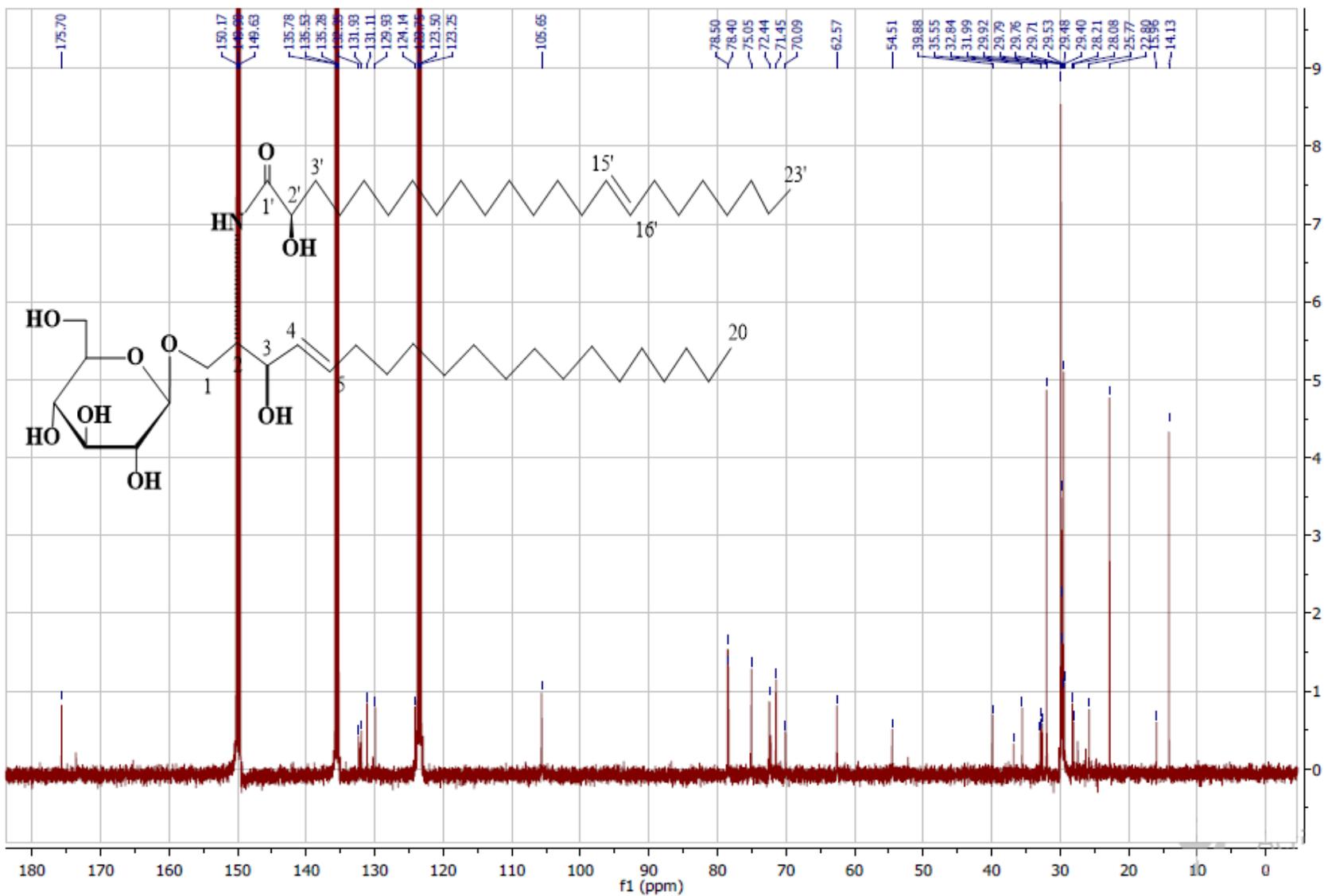


Figure S21.  $^{13}\text{C}$  NMR spectrum of compound 2 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

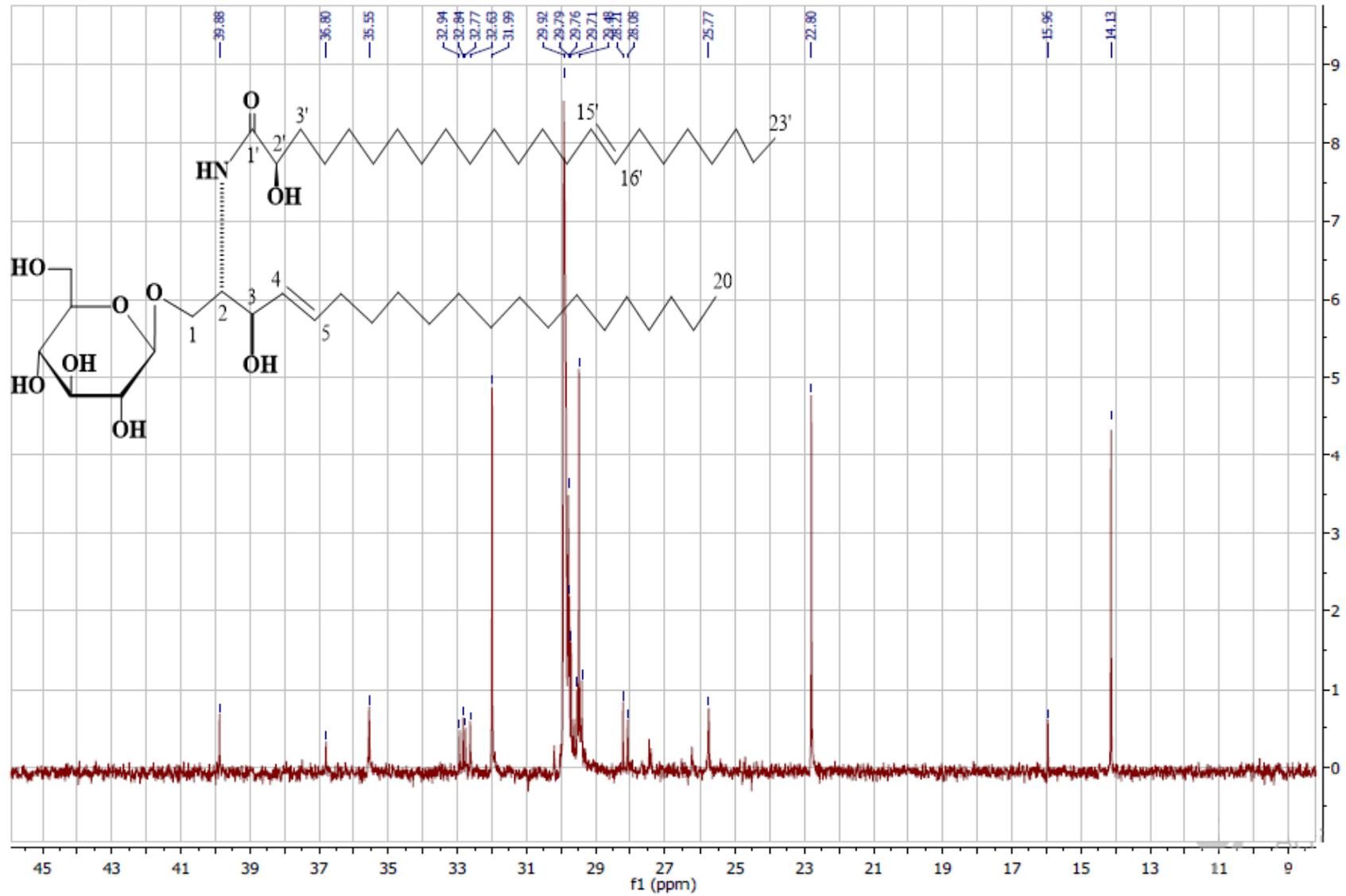


Figure S22. Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound 2 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

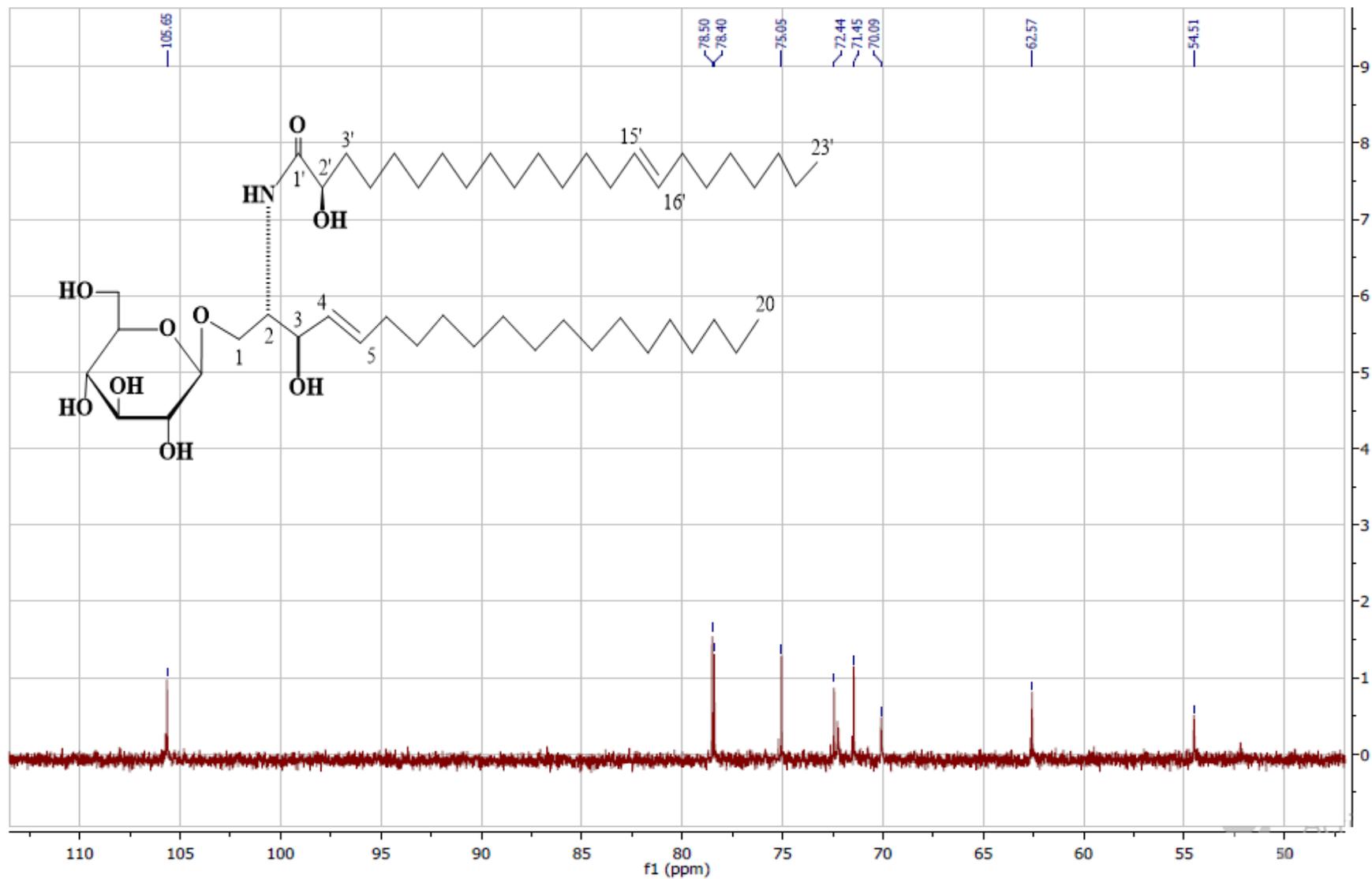


Figure S23. Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound 2 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

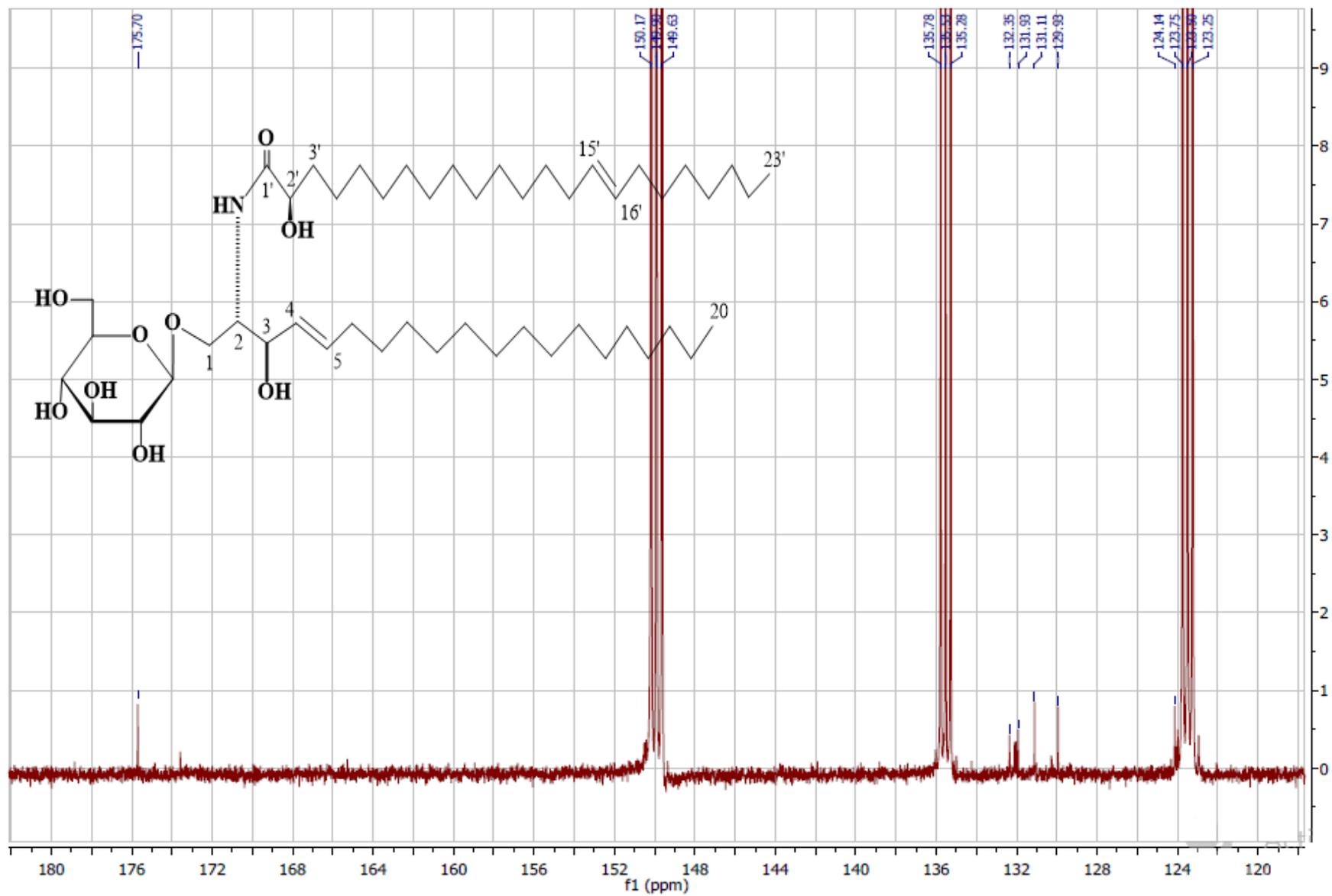
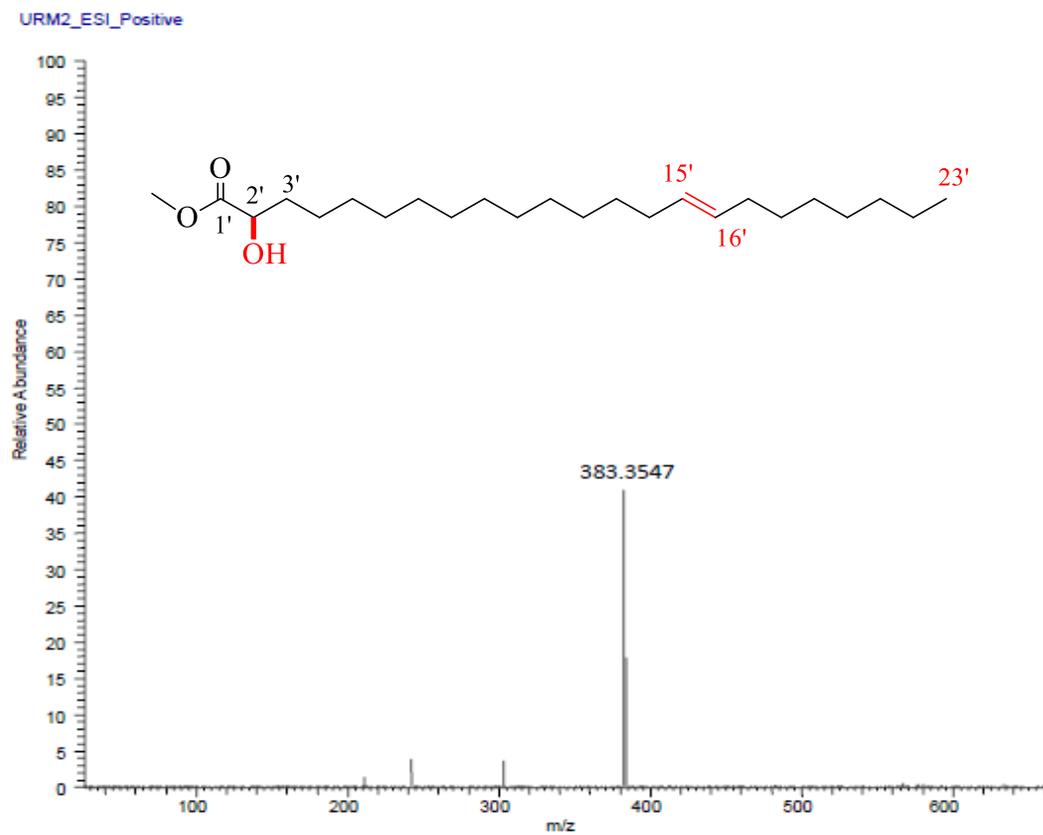
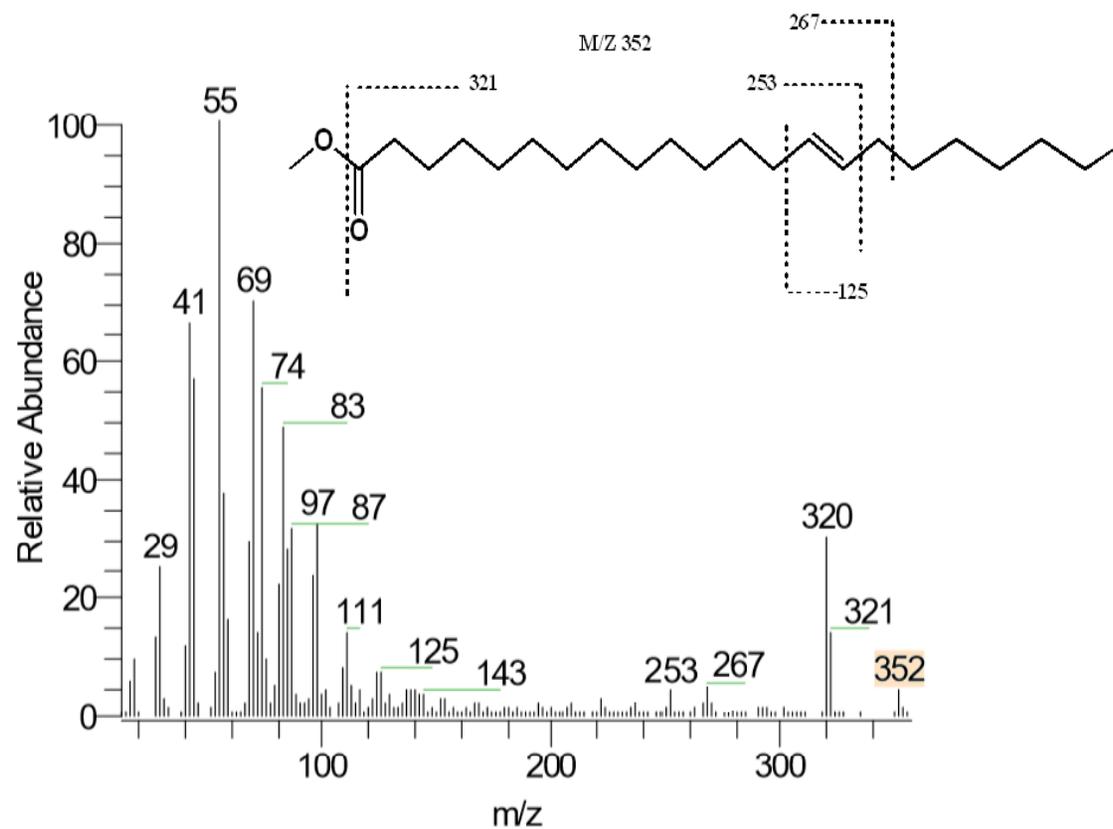


Figure S24. Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound 2 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).



**Figure S25.** LC-HRESIMS for  $\alpha$ -hydroxy fatty acid methyl ester after hydrolysis of compound **2**



**Figure S26.** GC-MS analysis of fatty acids methyl esters carried out after oxidation of  $\alpha$ -hydroxy fatty acid methyl ester (Compound 2)

UR6 ESI POS 2101

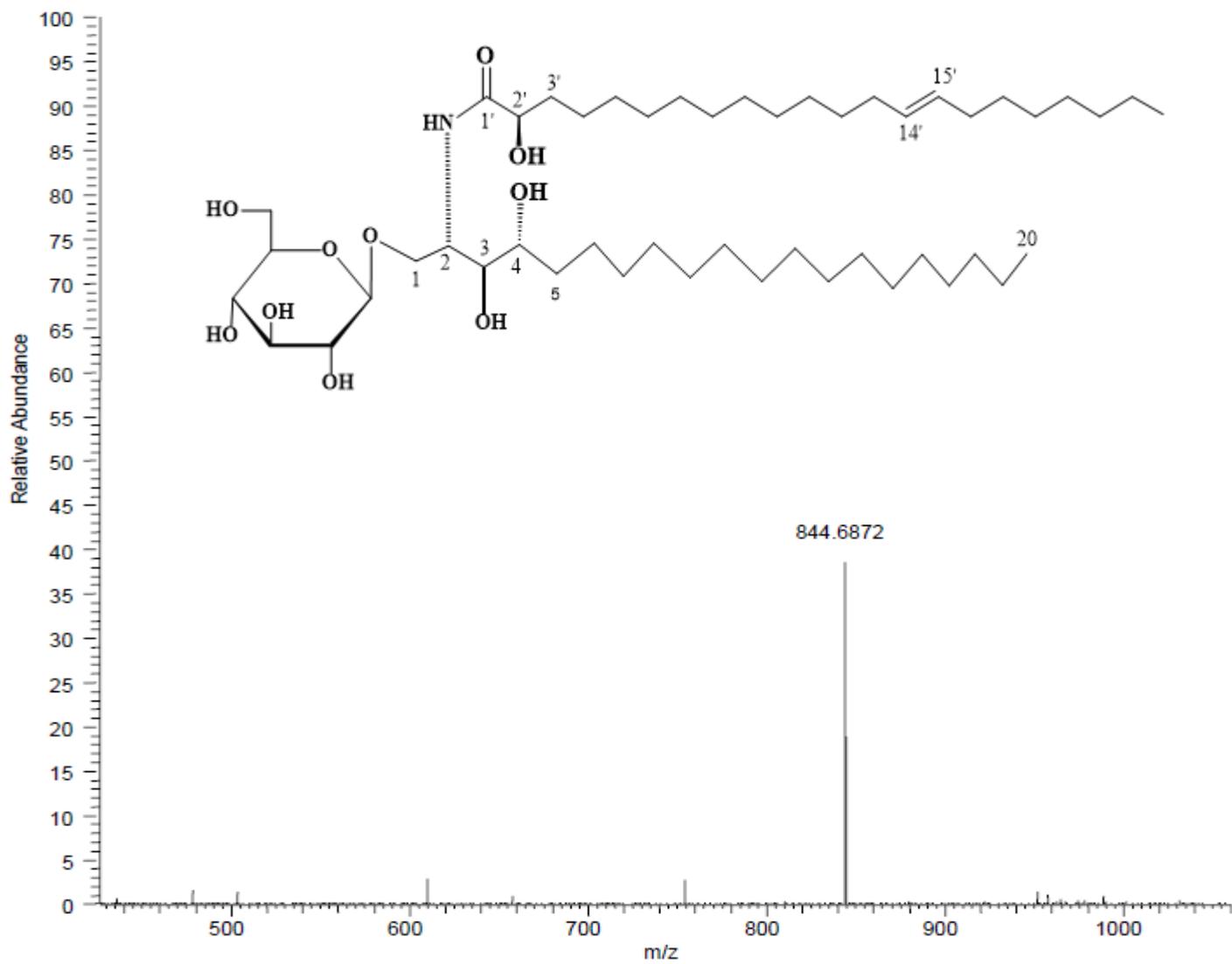


Figure S27. LC-HRESIMS of Compound 3 ( $M+H$ )<sup>+</sup>.

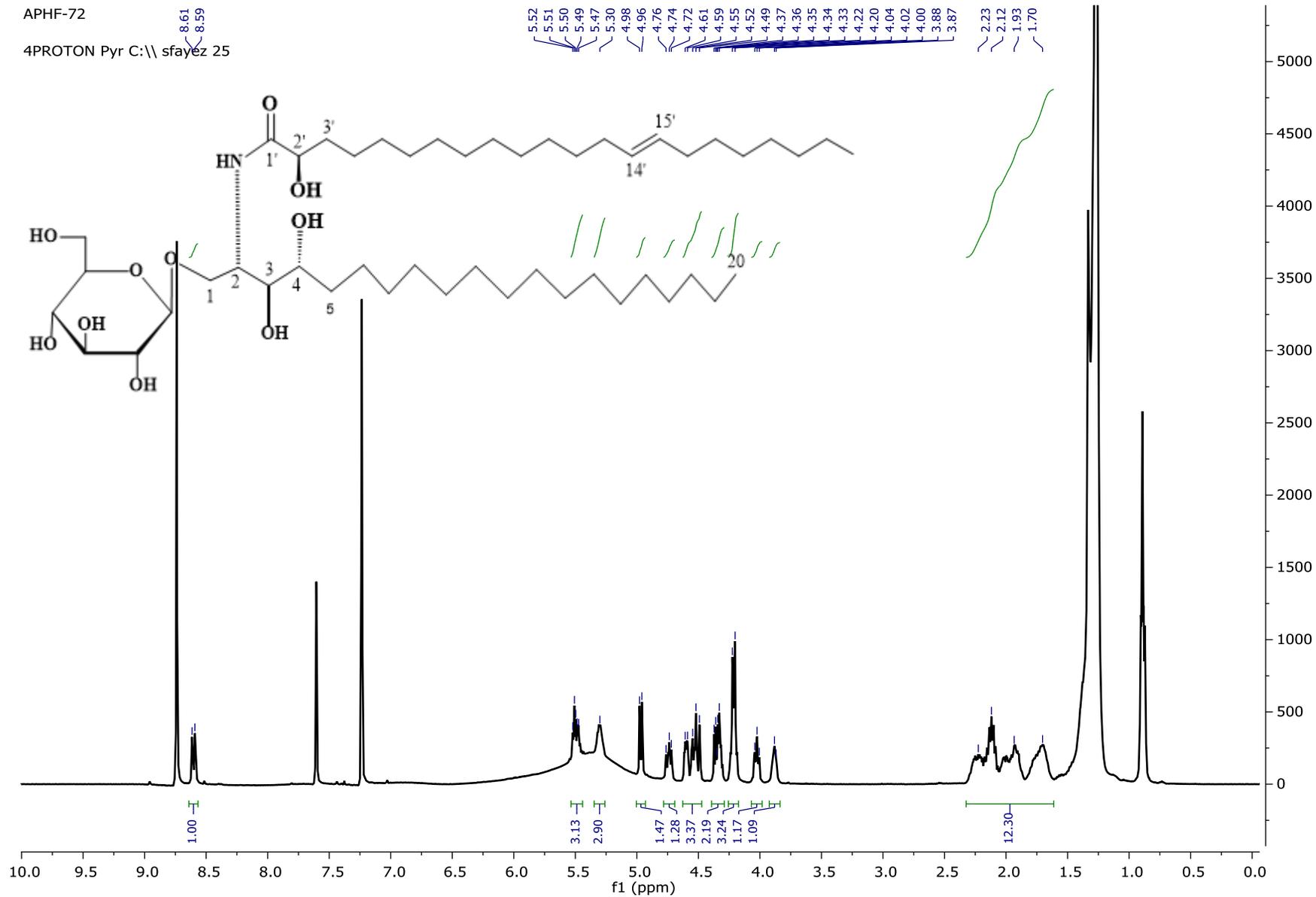


Figure S28.  $^1\text{H}$  NMR spectrum of compound 3 (in  $\text{C}_5\text{D}_5\text{N}$ , 400 MHz).

APHF-72

4PROTON Pyr C:\\ sfayez 25

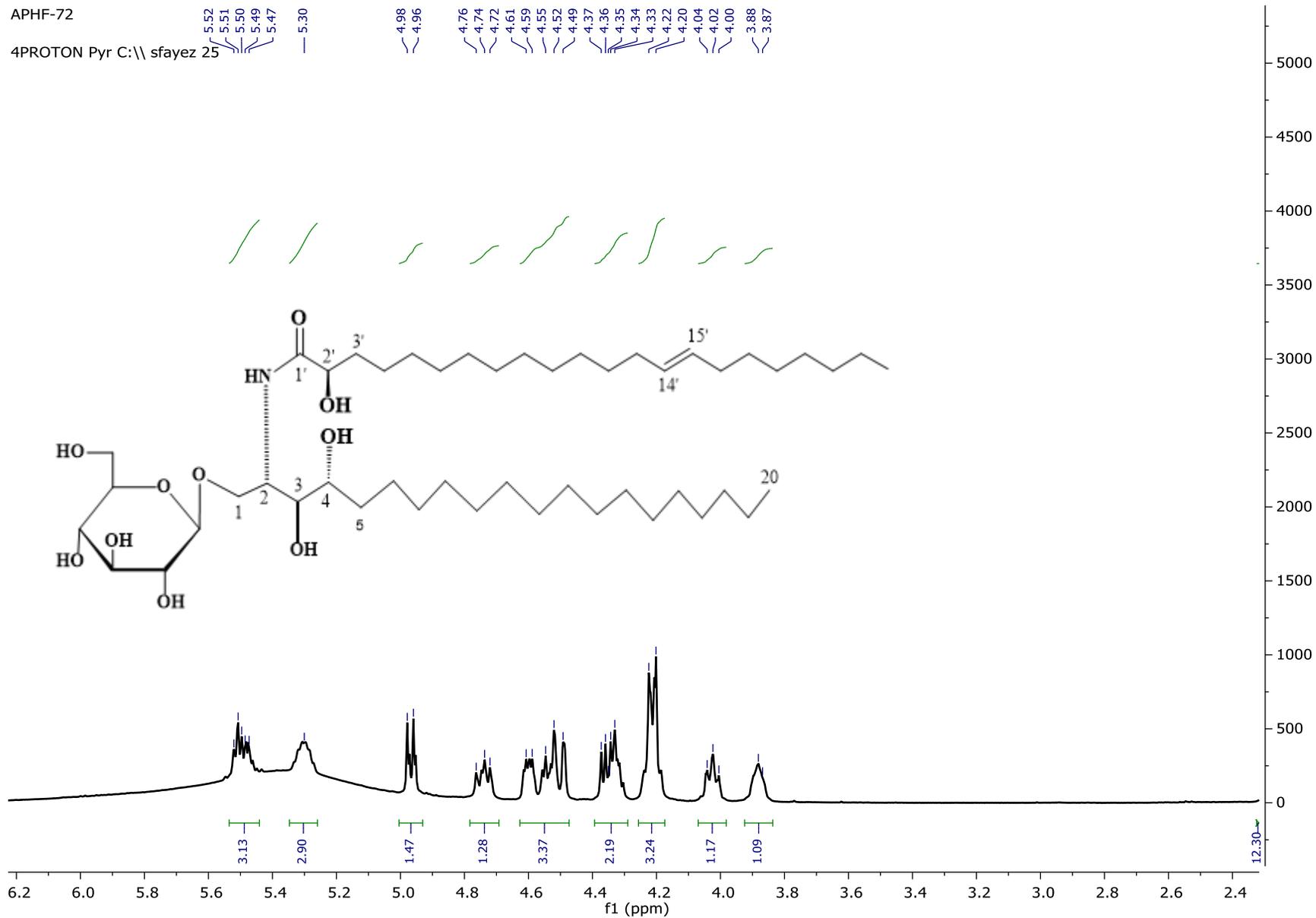


Figure S29. Partial expansion of the <sup>1</sup>H NMR spectrum of compound 3 (in C<sub>5</sub>D<sub>5</sub>N, 400 MHz).

APHF-72-  
4C13CPD Pyr C:\\ sfayez 12

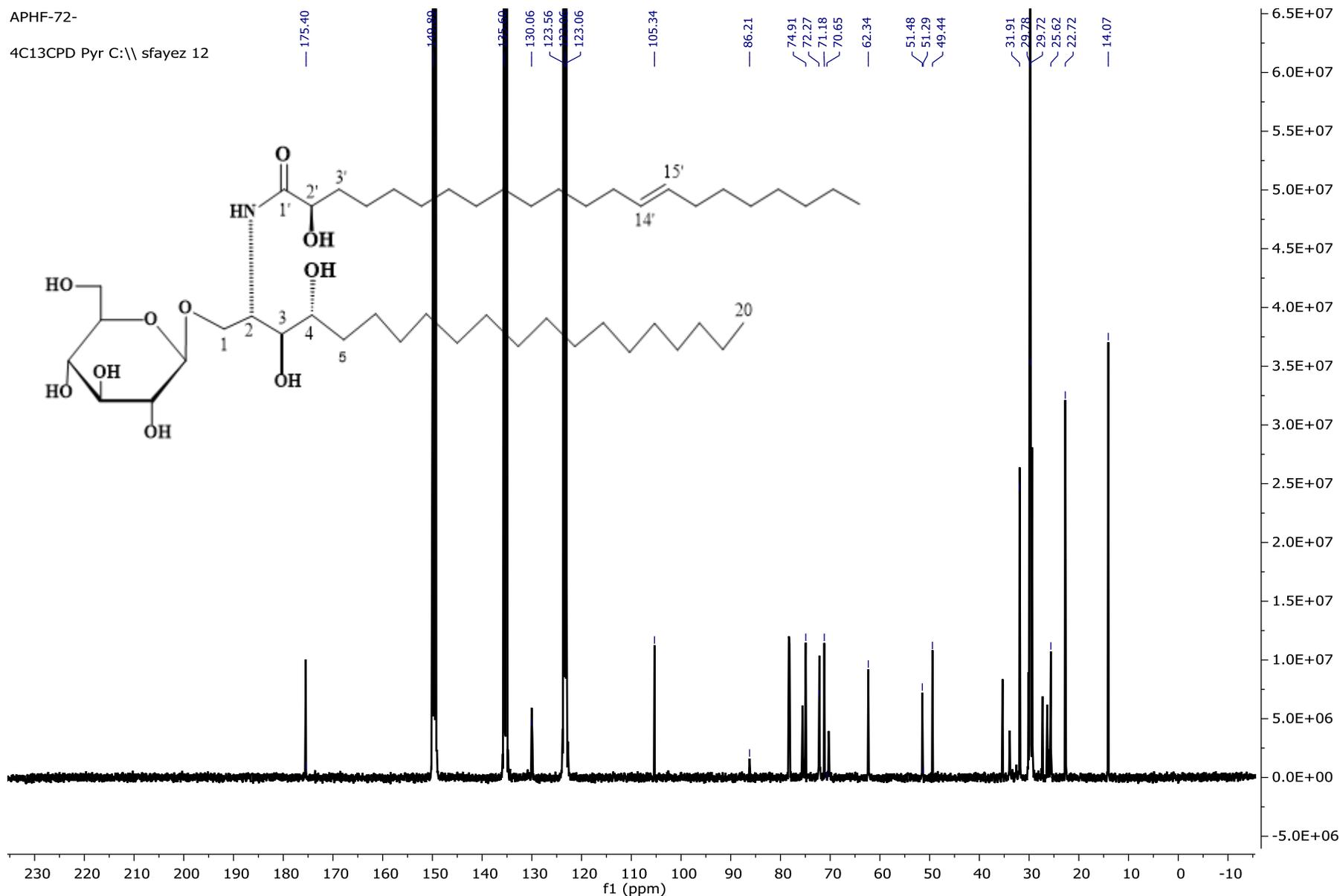


Figure S30.  $^{13}\text{C}$  NMR spectrum of compound 3 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

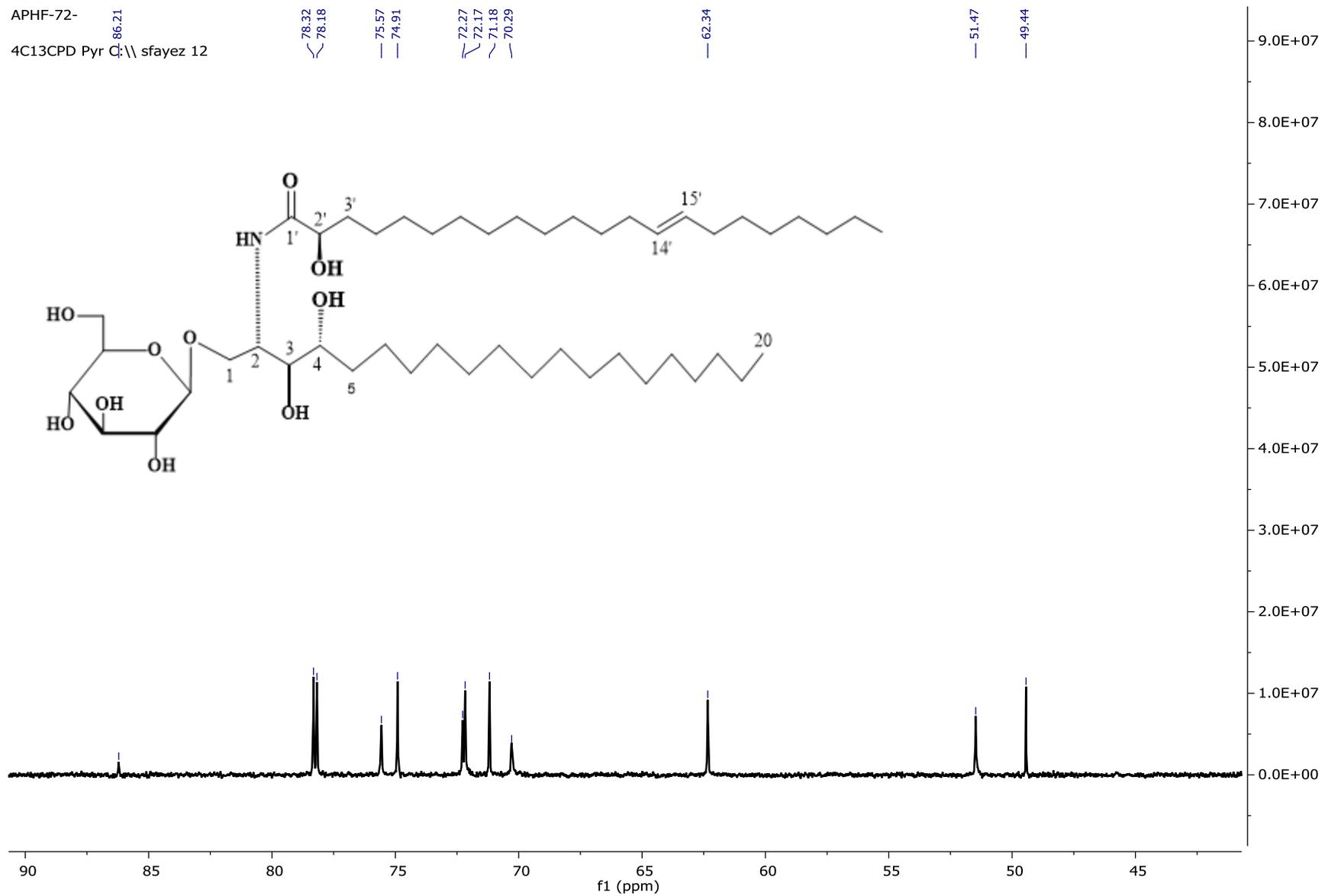


Figure S31. Partial expansion of the  $^{13}\text{C}$  NMR spectrum of compound 3 (in  $\text{C}_5\text{D}_5\text{N}$ , 100 MHz).

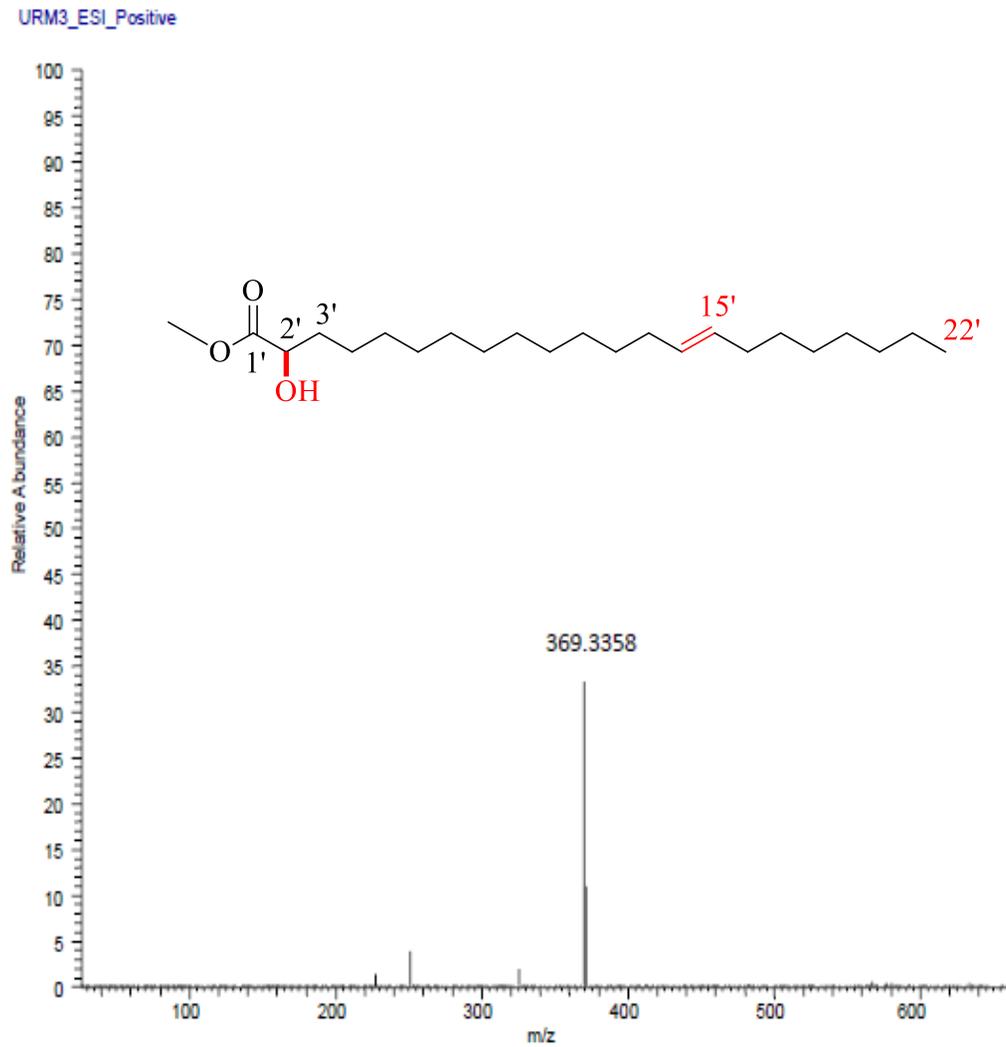
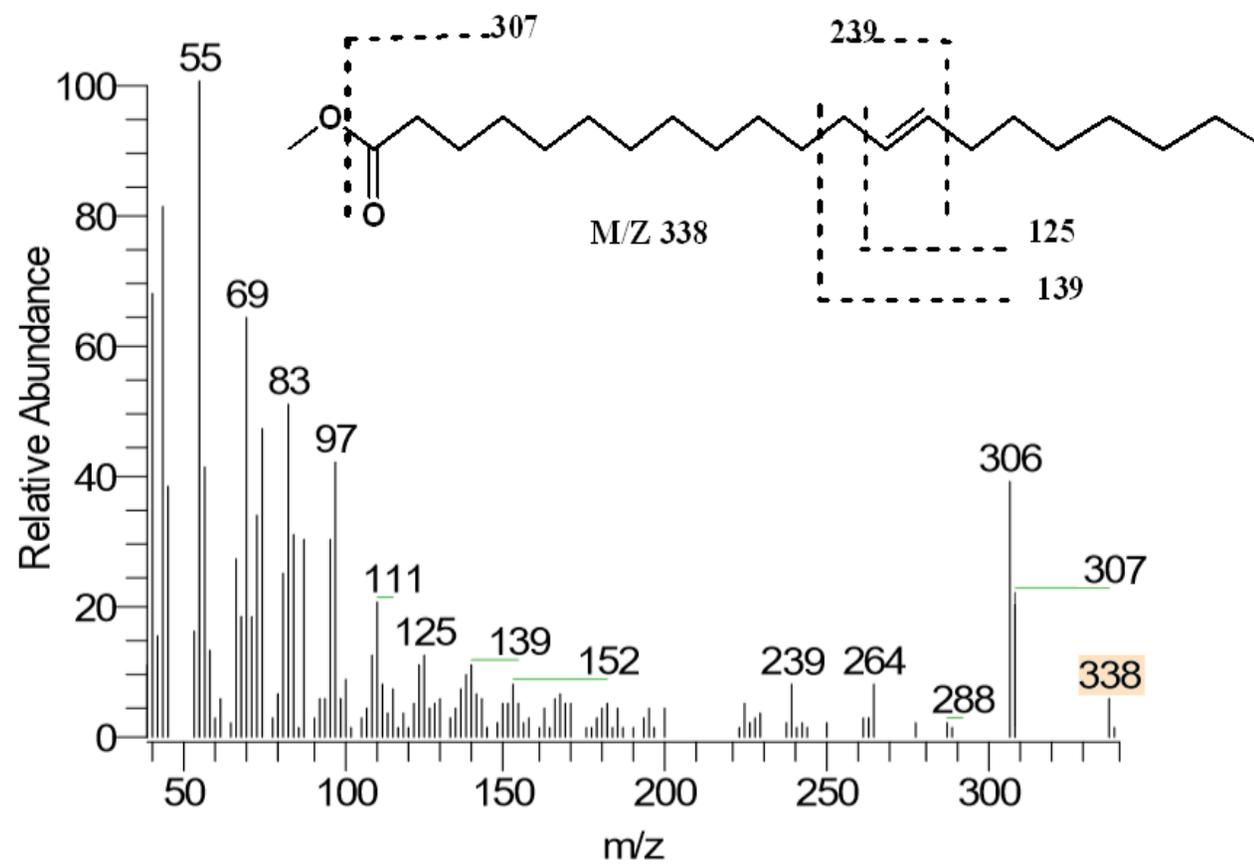


Figure S32. LC-HRESIMS for  $\alpha$ -hydroxy fatty acid methyl ester after hydrolysis of compound 3



**Figure S33.** GC-MS analysis of fatty acids methyl esters carried out after oxidation of  $\alpha$ -hydroxy fatty acid methyl ester (Compound 3)

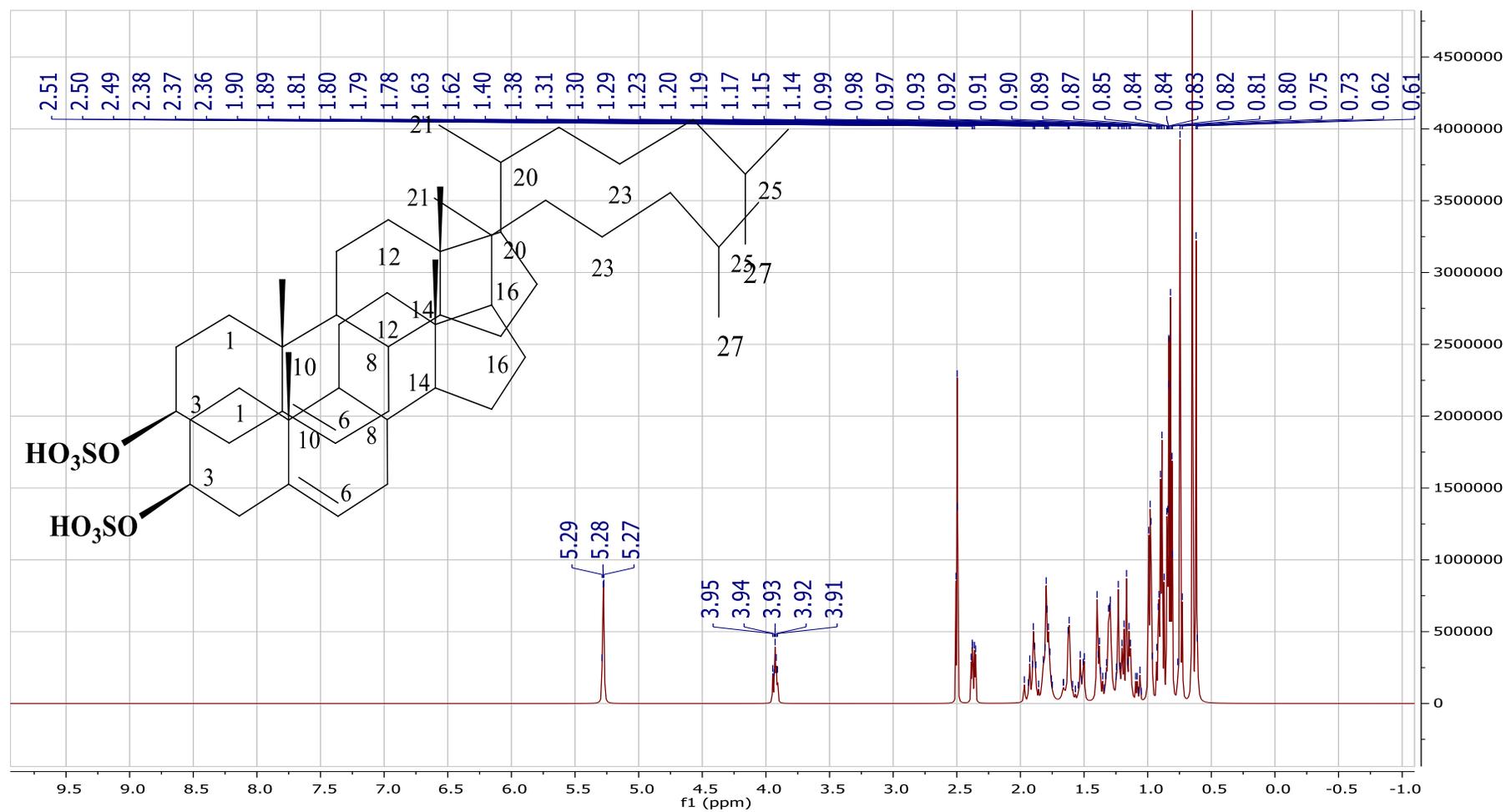
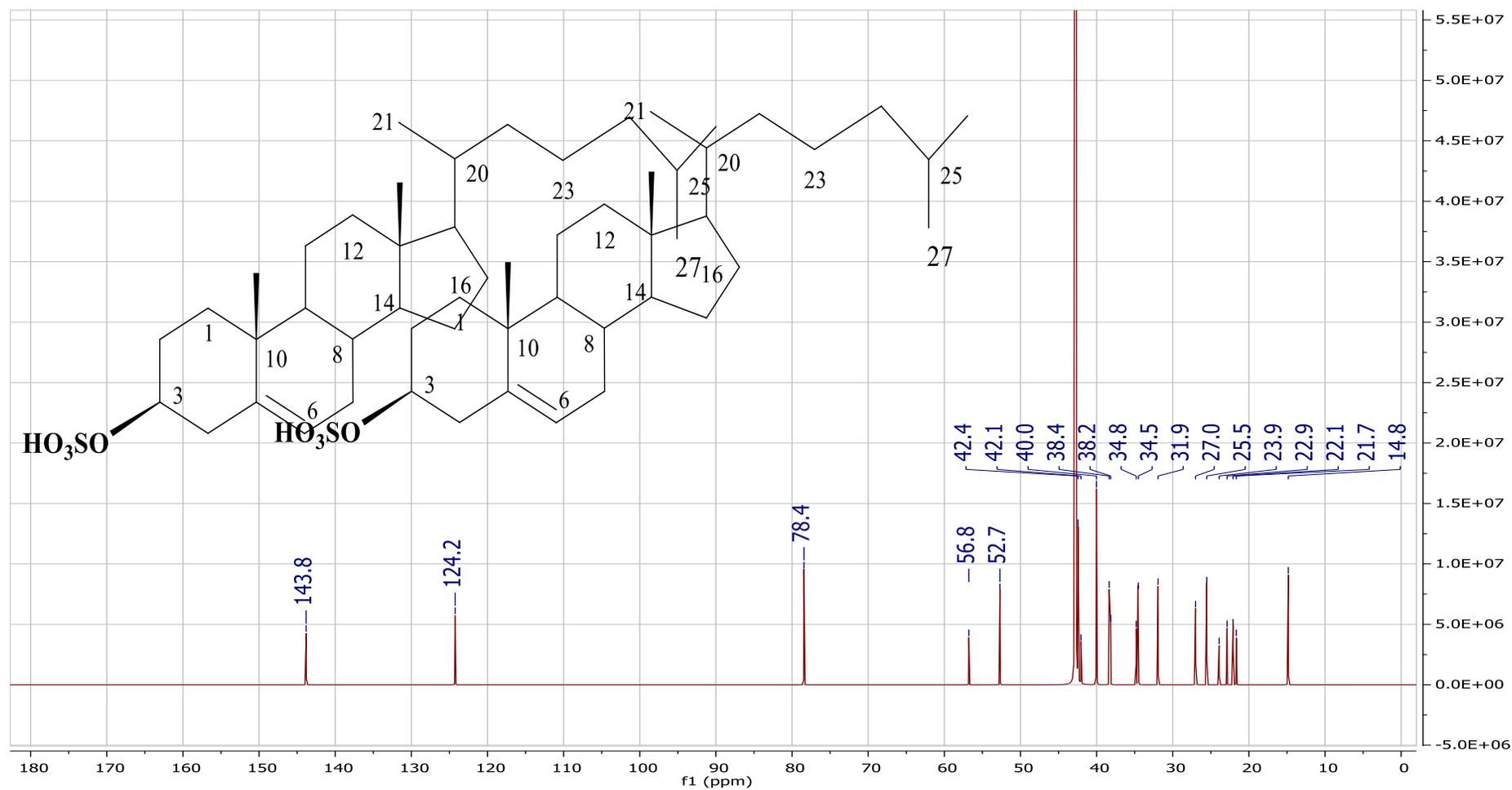
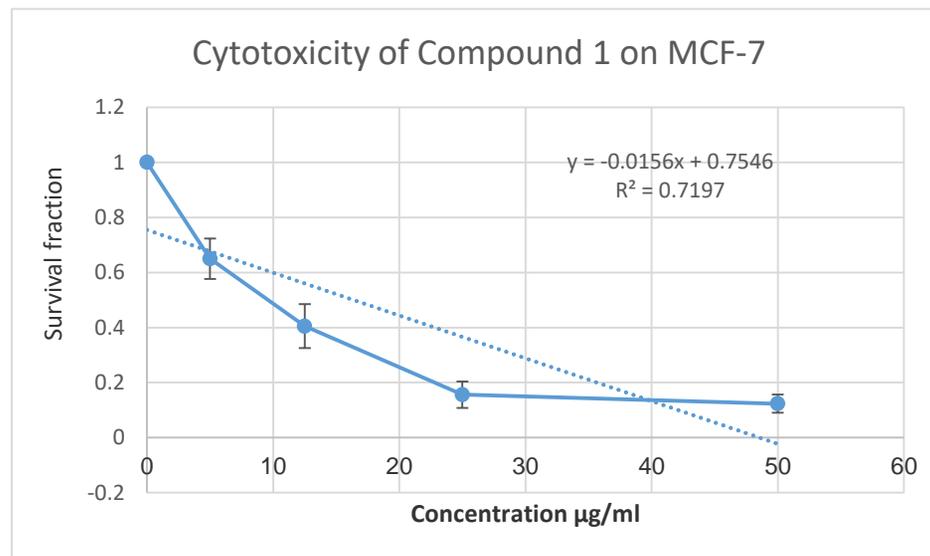


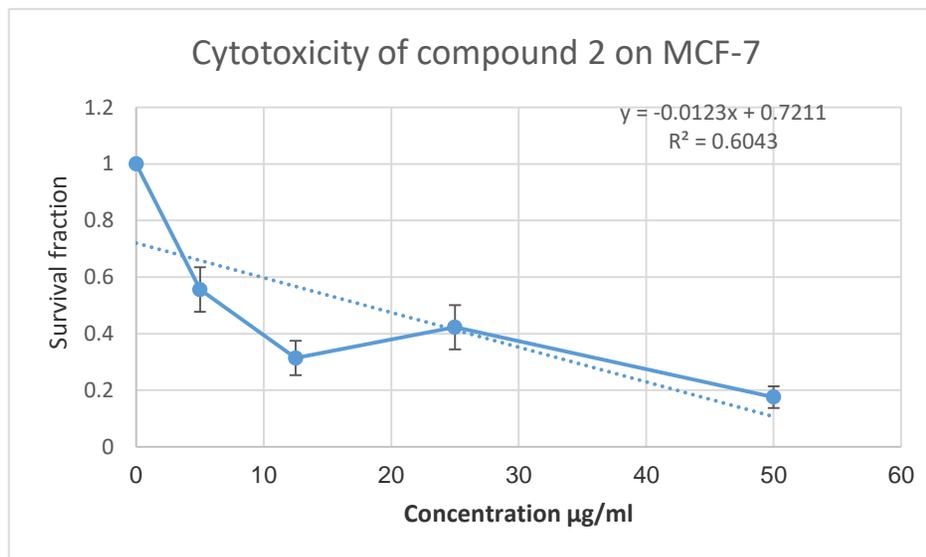
Figure S34.  $^1\text{H}$  NMR spectrum of compound 4 (in DMSO, 400 MHz)



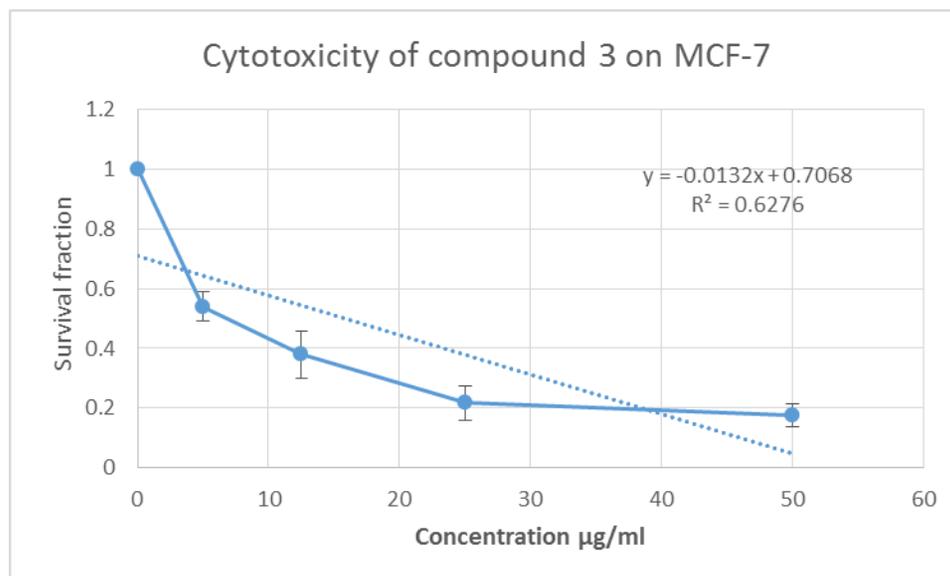
**Figure S35.**  $^{13}\text{C}$  NMR spectrum of compound 4 (in DMSO, 400 MHz)



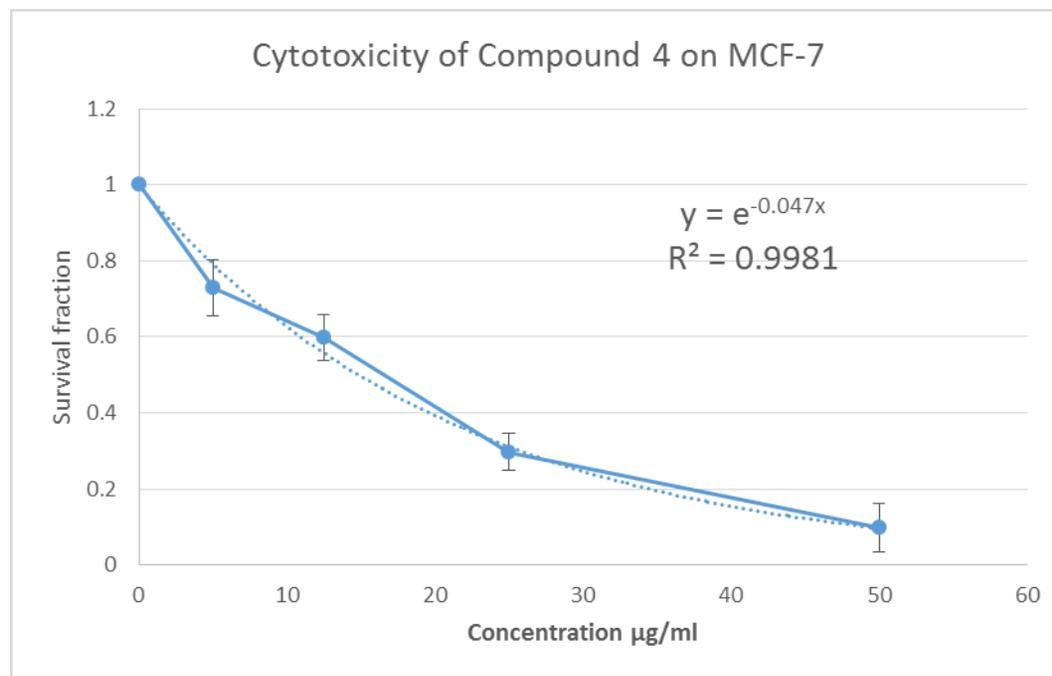
**Figure S36.** Cytotoxicity of compound 1 on MCF-7



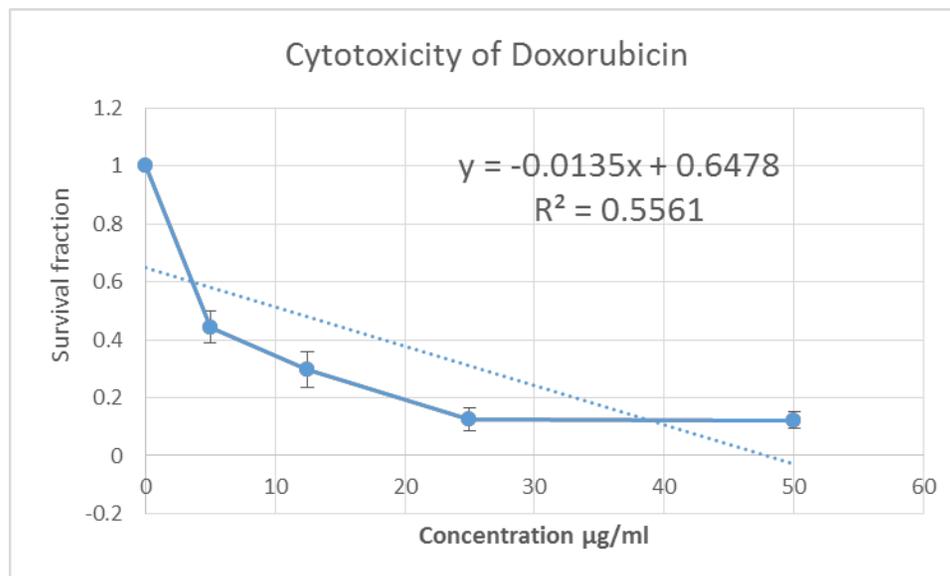
**Figure S37.** Cytotoxicity of compound 2 on MCF-7



**Figure S38.** Cytotoxicity of compound 3 on MCF-7



**Figure S39.** Cytotoxicity of compound 4 on MCF-7



**Figure S40.** Cytotoxicity of Doxorubicin on MCF-7