

Synthesis and Bioactivity of Ancorinoside B, a Marine Diglycosyl Tetramic Acid

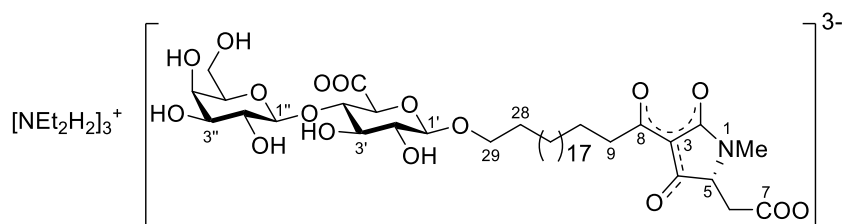
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NMR-Comparison of isolated and synthetic Ancorinoside B tris(diethylammonium) salt

Table S1. Comparison of ^{13}C and ^1H NMR shifts of isolated and synthetic Ancorinoside B tris(diethylammonium) salt

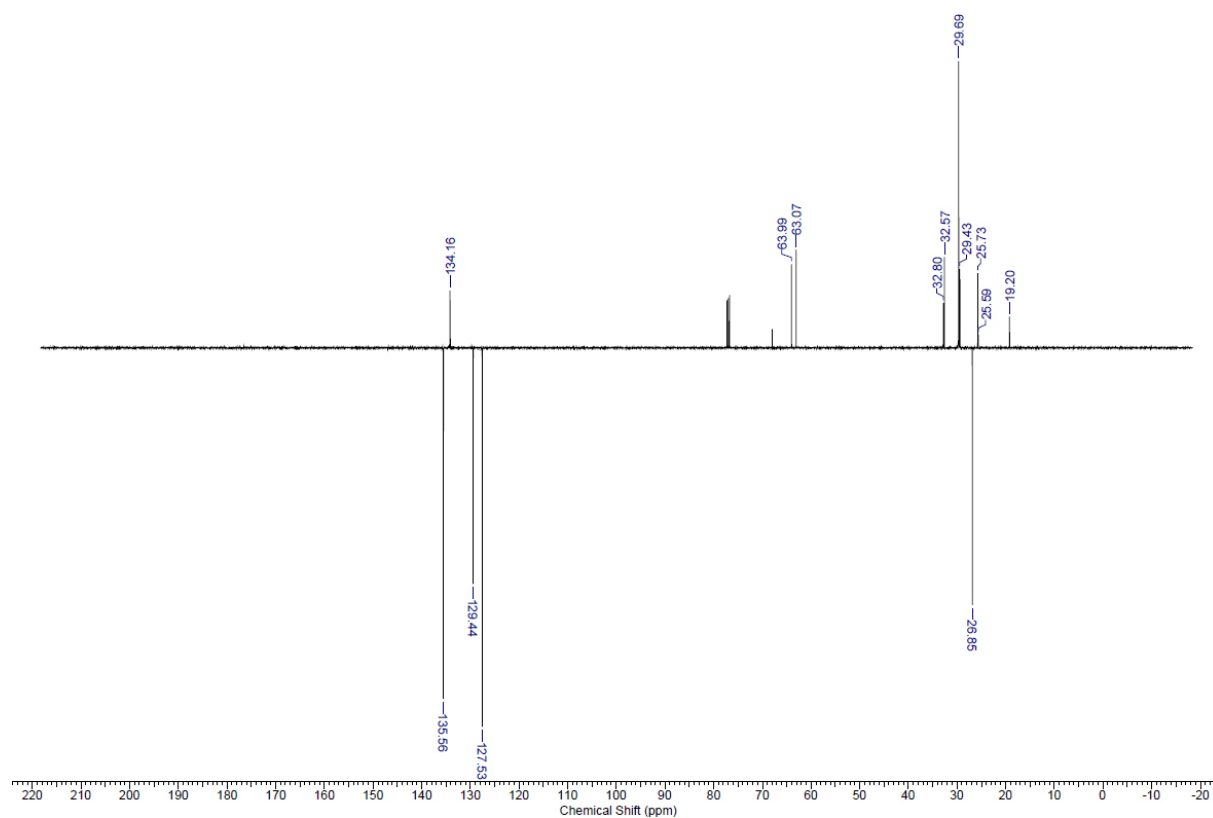
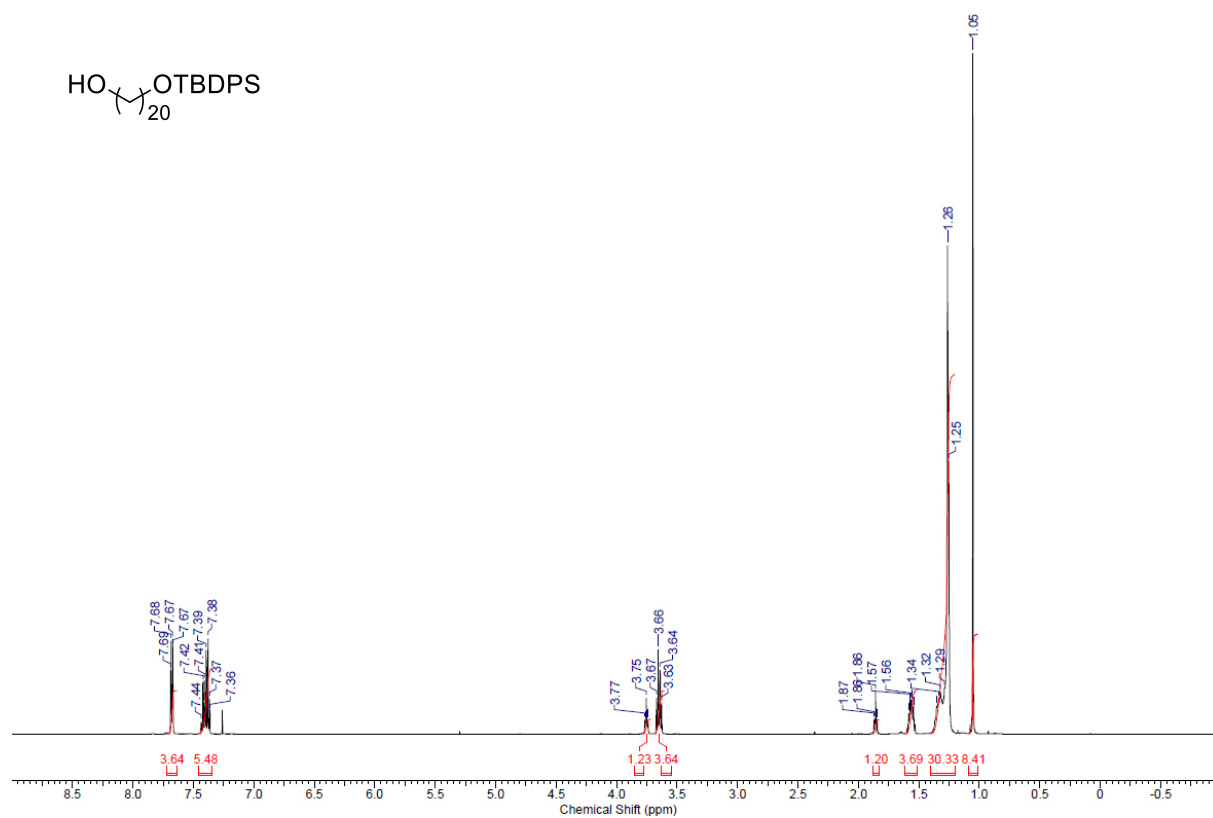
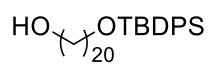


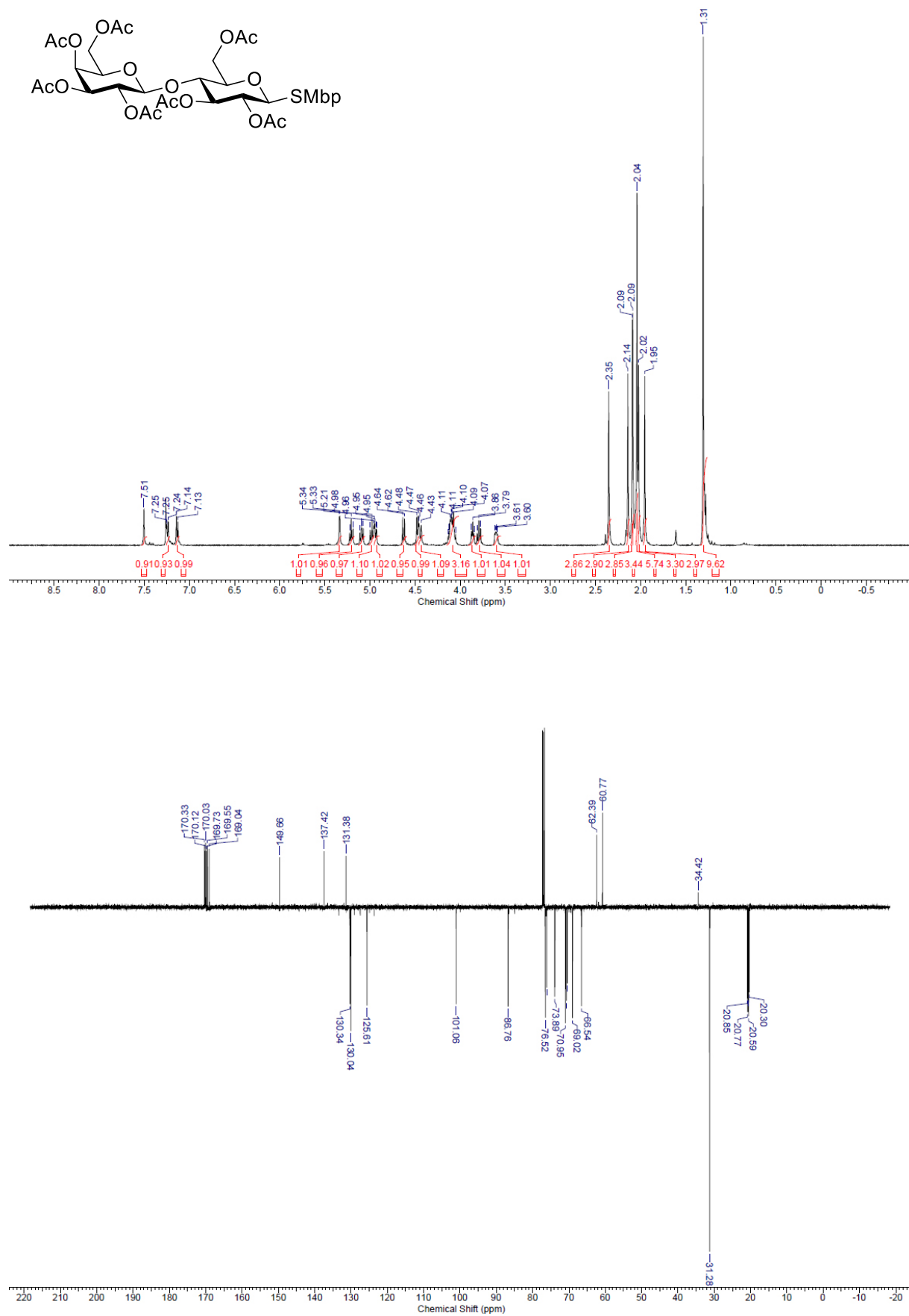
Pos.	natural $[\text{NEt}_2\text{H}_2]^+_3[\mathbf{2-3H}]^{3-}$ in CD_3OD^7		synthetic $[\text{NEt}_2\text{H}_2]^+_3[\mathbf{2-3H}]^{3-}$ in CD_3OD	
	δ_{C}	δ_{H} (mult., J [Hz])	δ_{C}	δ_{H} (mult., J [Hz])
2	175.7		176.1	
3	102.3		102.4	
4	195.7		196.7	
5	63.6	3.88 (dd, 8.1, 4.2)	64.7	3.90-3.95 (m)
6	39.1	2.78 (dd, 16.2, 4.2), 2.37 (dd, 16.2, 8.1)	41.7	2.76-2.80 (m), 2.13 (dd, 15.3, 9.2)
7	177.1		179.9	
8	197.0		197.8	
9	40.5	2.78 (m), 2.73 (m)	41.1	2.80-2.84 (m), 2.70-2.76 (m)
10	27.3	1.55 (quin, 7.3)	27.6	1.51-1.58 (m)
11	30.8	1.32 (m)	31.0	1.24-1.43 (m)
12-26	30.8	1.27 (m)	31.0	1.24-1.43 (m)
27	27.2	1.36 (m)	27.3	1.24-1.43 (m)
28	30.8	1.60 (quin, 7.3)	30.9	1.58-1.66 (m)
29	71.0	3.88 (m), 3.51 (m)	71.2	3.82-3.89 (m), 3.52-3.54 (m)
NMe	27.4	2.88 (s)	27.8	2.91 (s)
1'	104.2	4.29 (d, 7.7)	104.3	4.30 (d, 7.9)
2'	74.6	3.28 (t, 8.1)	74.7	3.27-3.29 (m)
3'	76.7	3.50 (t, 8.9)	76.8	3.49-3.52 (m)
4'	83.5	3.59 (t, 9.2)	83.6	3.59-3.62 (m)
5'	77.2	3.72 (d, 9.6)	77.3	3.69-3.73 (m)
6'	176.2		176.5	
1''	105.5	4.35 (d, 7.7)	105.6	4.35 (d, 7.3)
2''	72.7	3.53 (dd, 10.0, 7.7)	72.8	3.54-3.56 (m)
3''	75.0	3.47 (dd, 10.0, 3.8)	75.1	3.48 (dd, 9.9, 3.5)
4''	70.3	3.78 (d, 3.8)	70.4	3.77-3.79 (m)
5''	77.2	3.56 (m)	77.4	3.56-3.59 (m)
6''	62.5	3.76 (dd, 11.5, 7.3), 3.68 (d, 11.5, 4.6)	62.6	3.73-3.77 (m) 3.66-3.69 (m)
$3\text{HN}(\text{CH}_2\text{CH}_3)_2$	- ^a	- ^a	43.6	3.02 (q, 7.0)
$3\text{HN}(\text{CH}_2\text{CH}_3)_2$	- ^a	- ^a	11.9	1.24-1.43 (m)

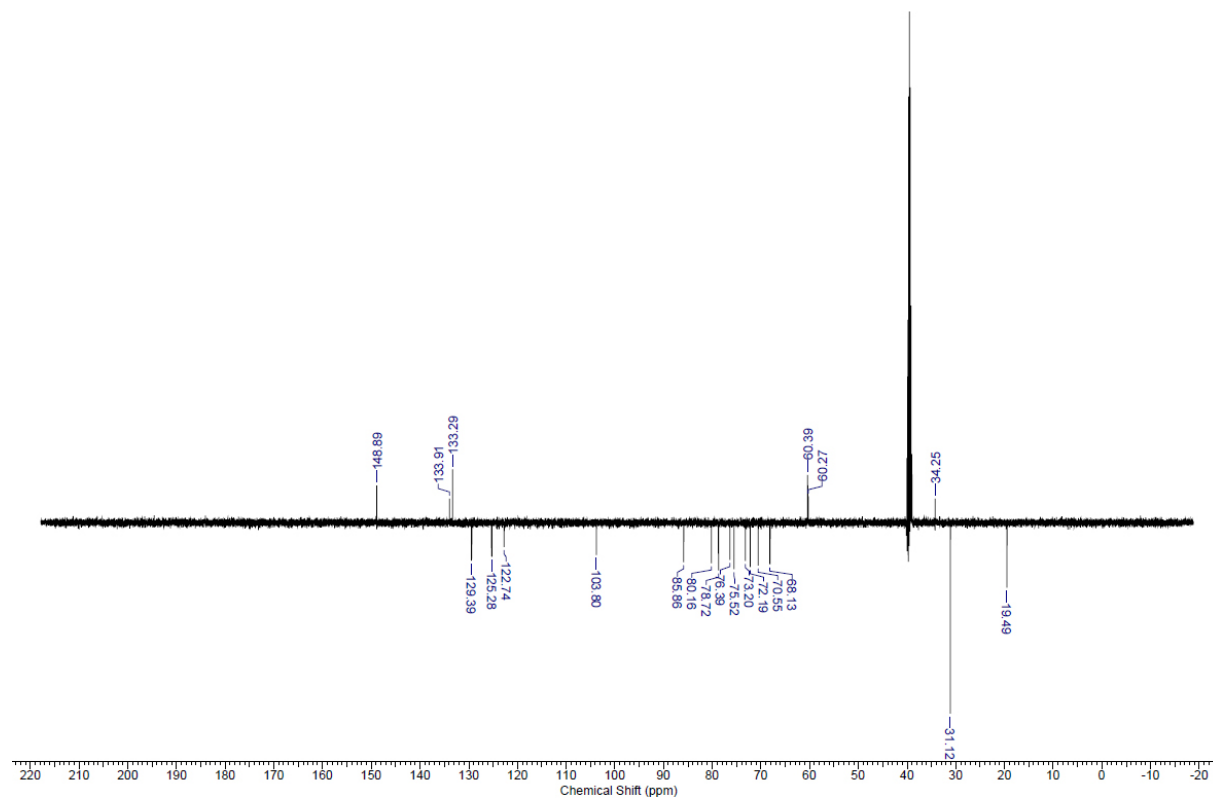
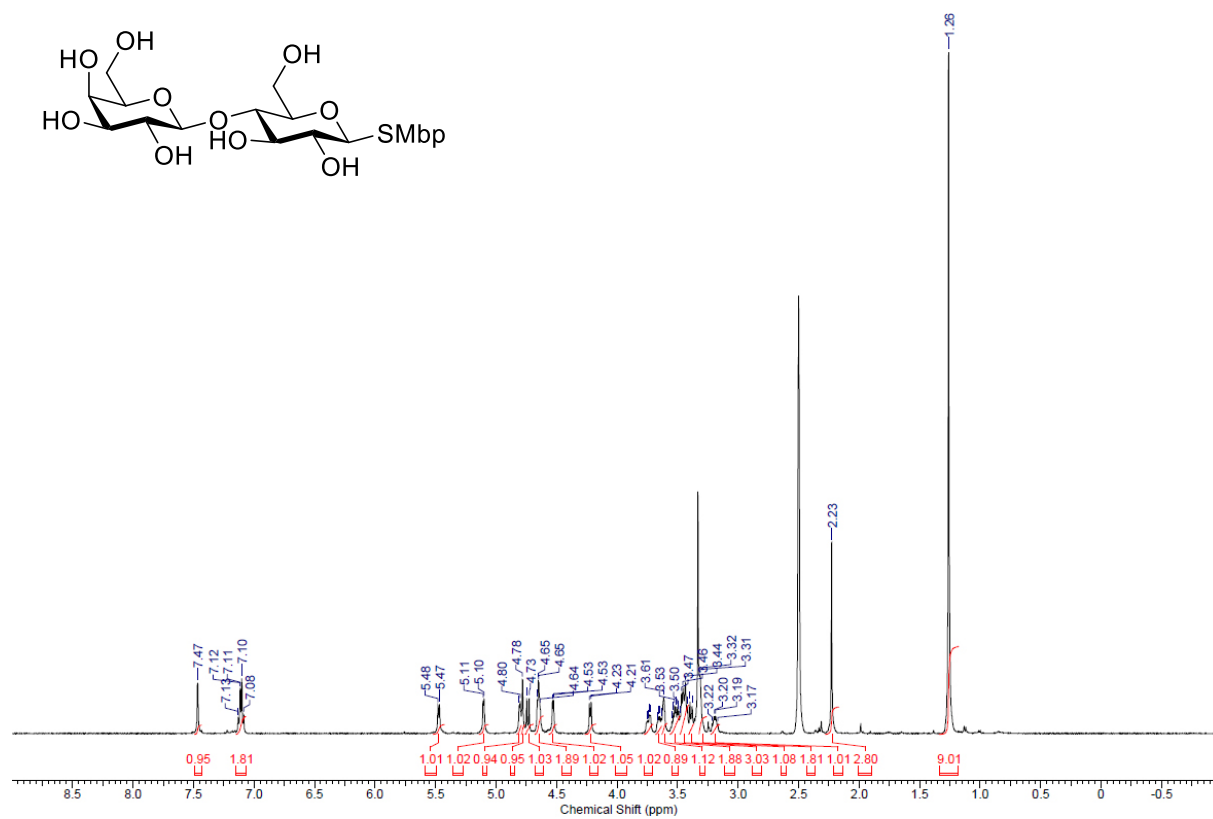
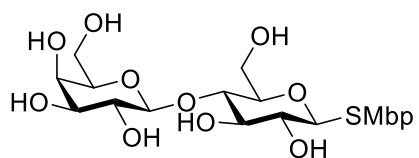
^a The literature does not state the shifts of the diethylammonium groups

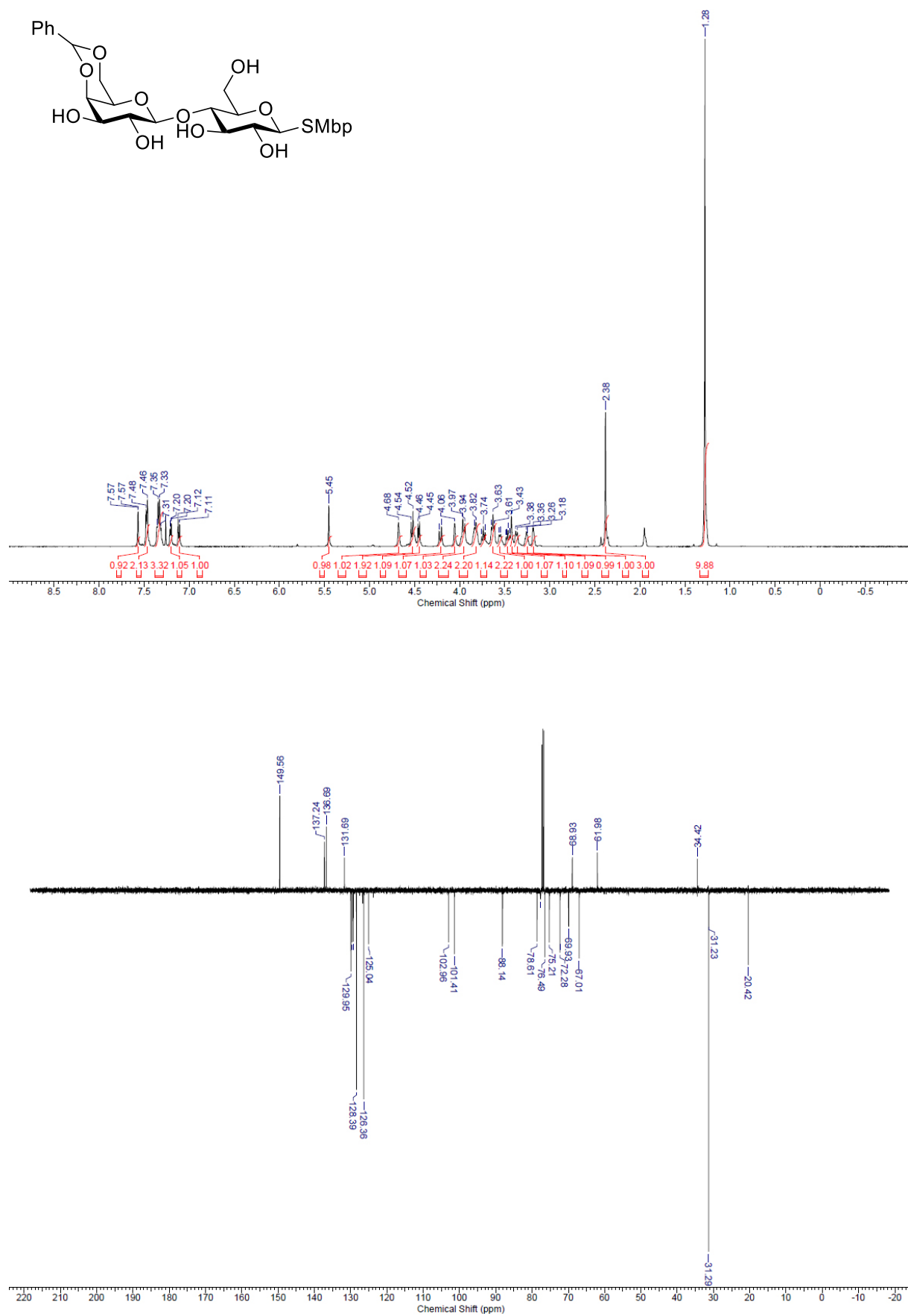
[7] Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; van Soest, R. W. M.; Fusetani, N. Ancorinosides B-D, inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP), from the marine sponge *Penares sollasi* Thiele. *Tetrahedron* **2001**, *57*, 1229-1234.

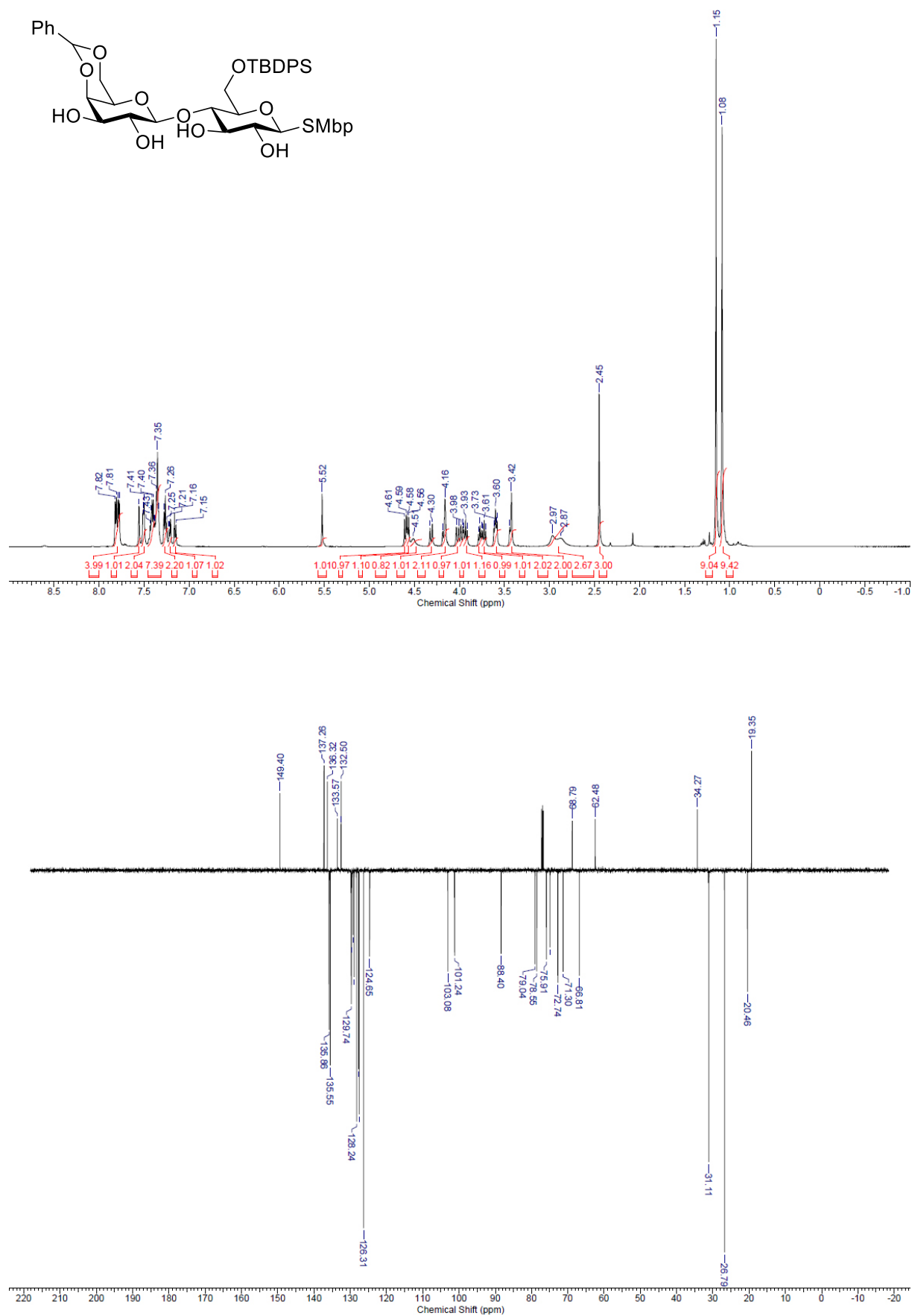
NMR spectra

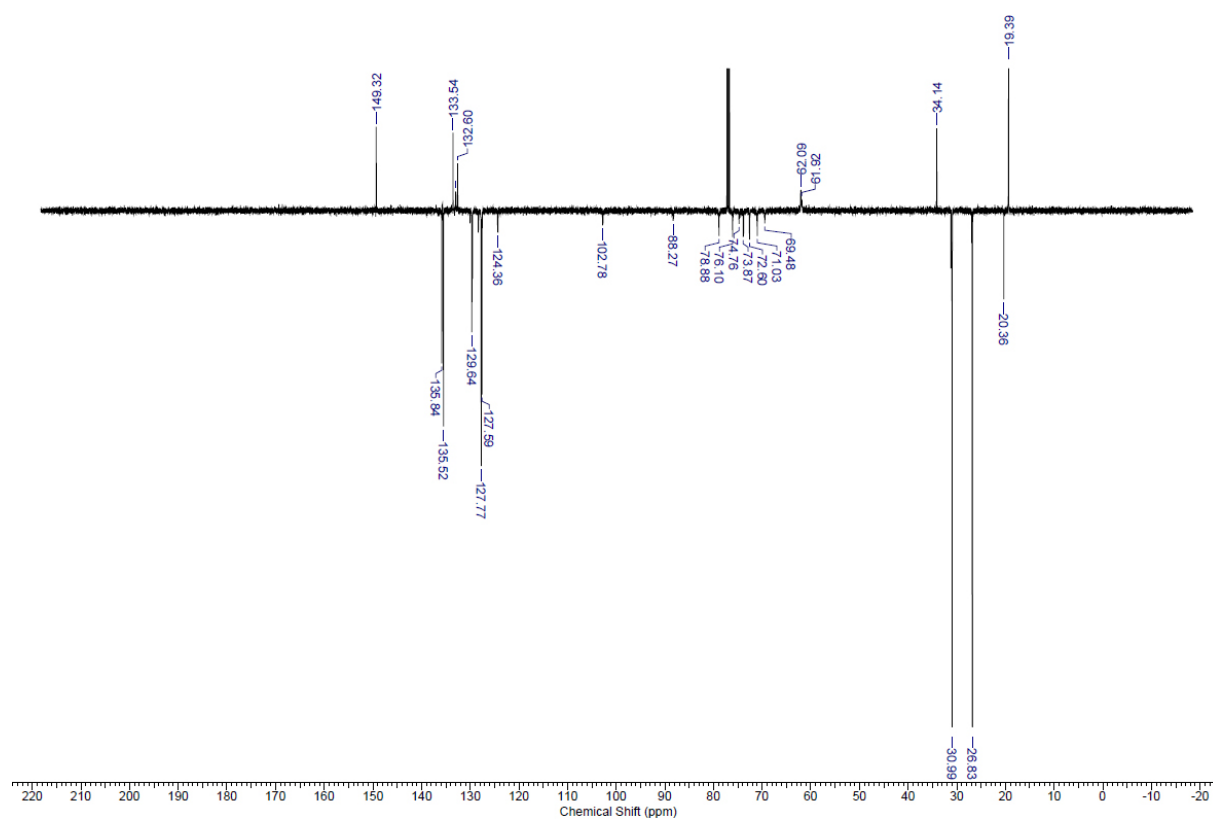
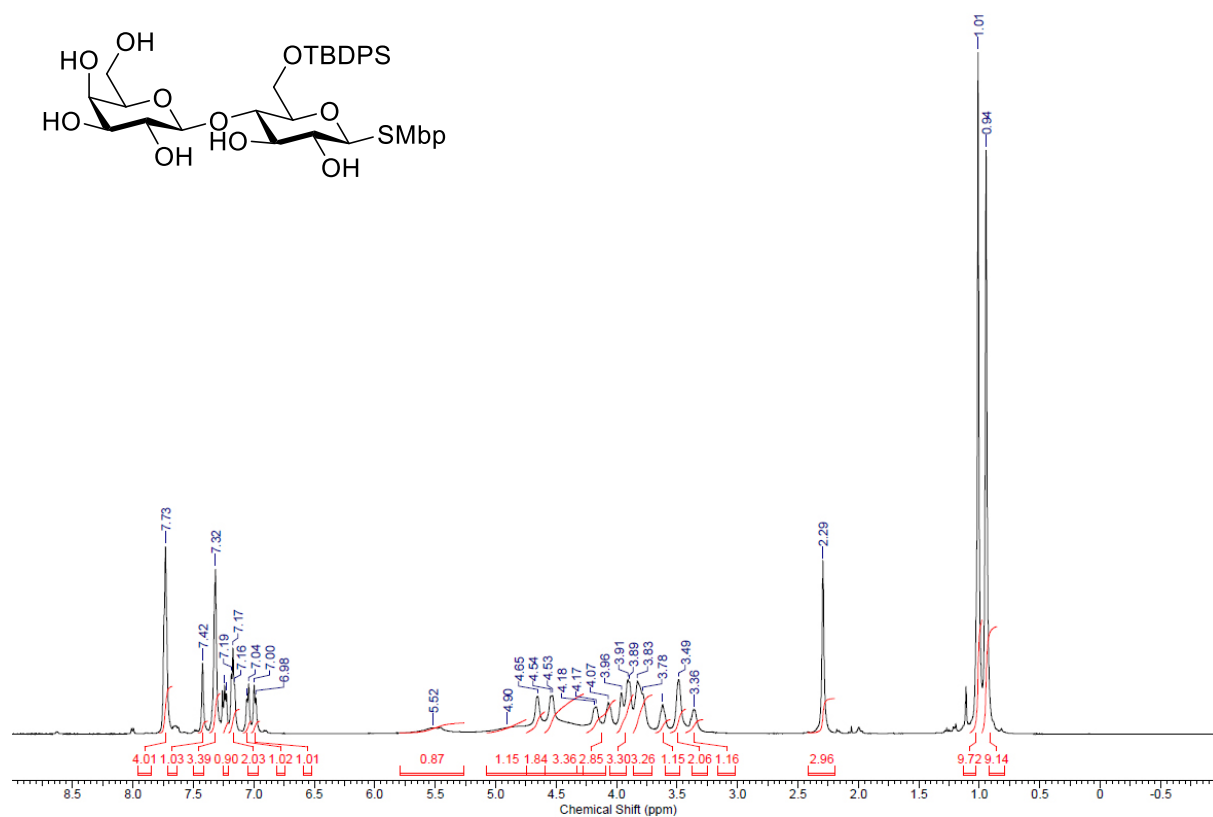
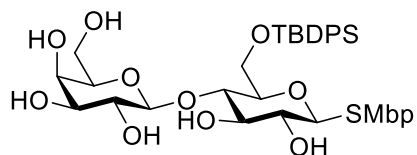


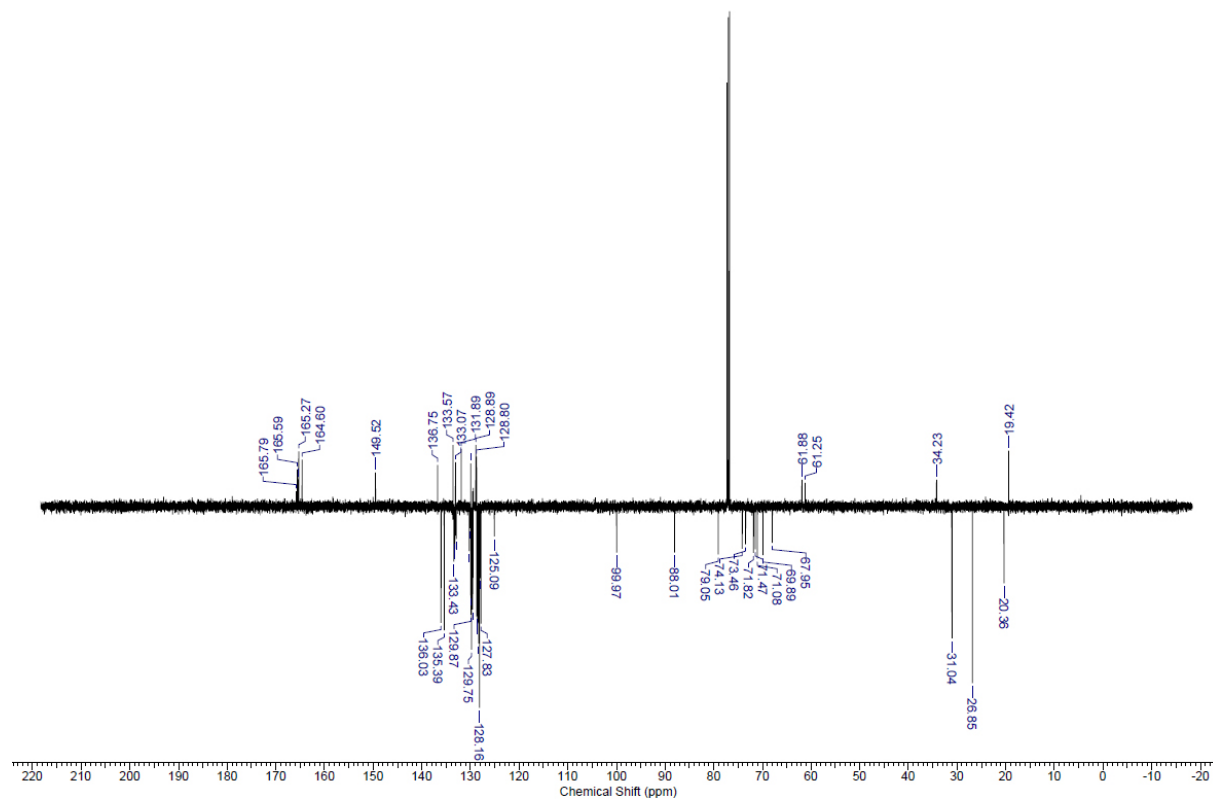
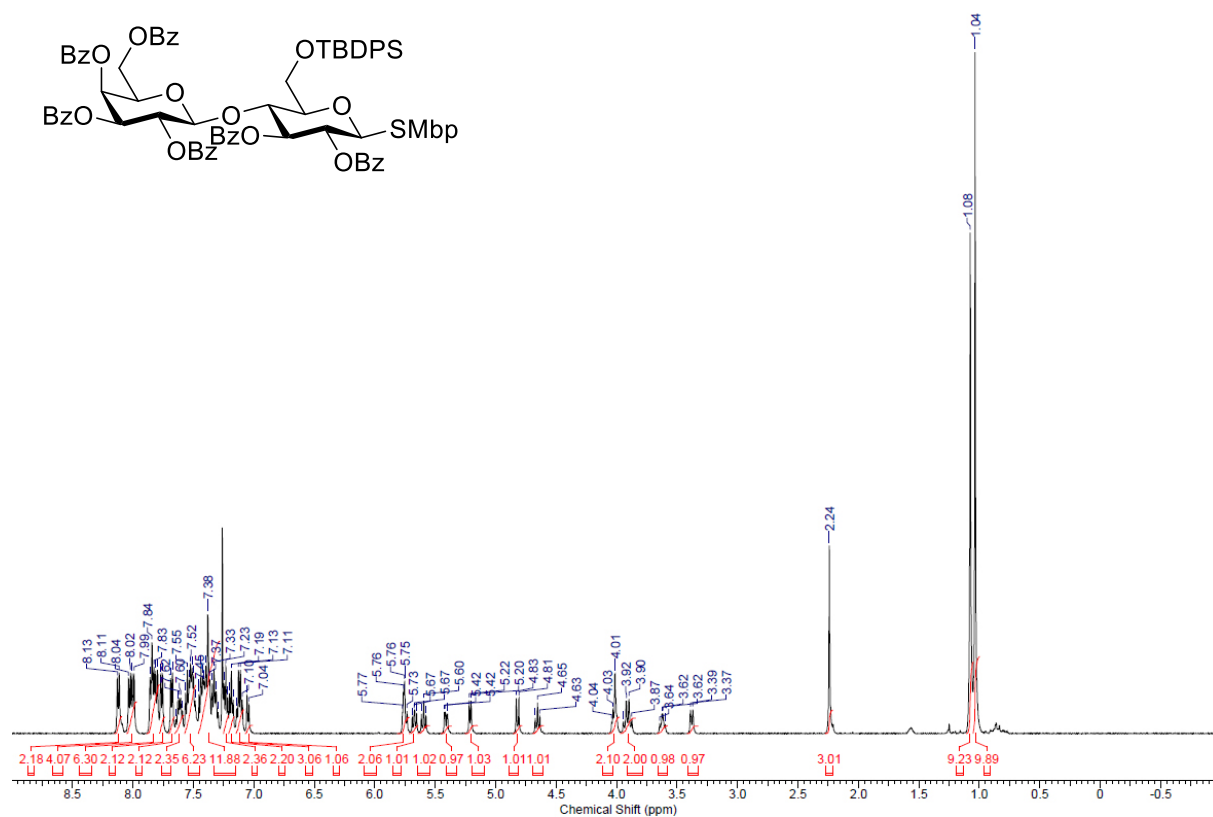
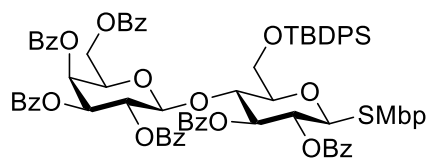


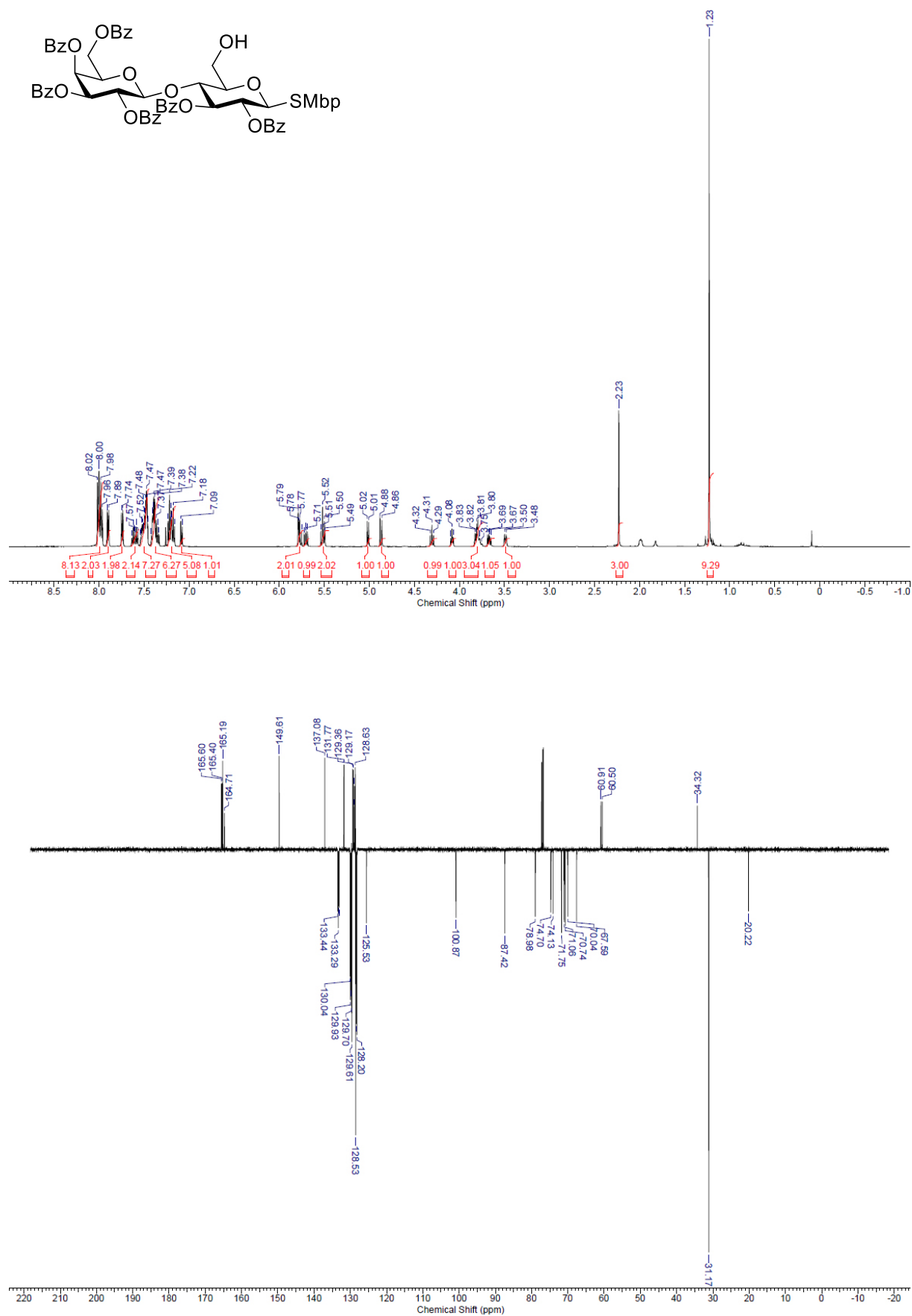


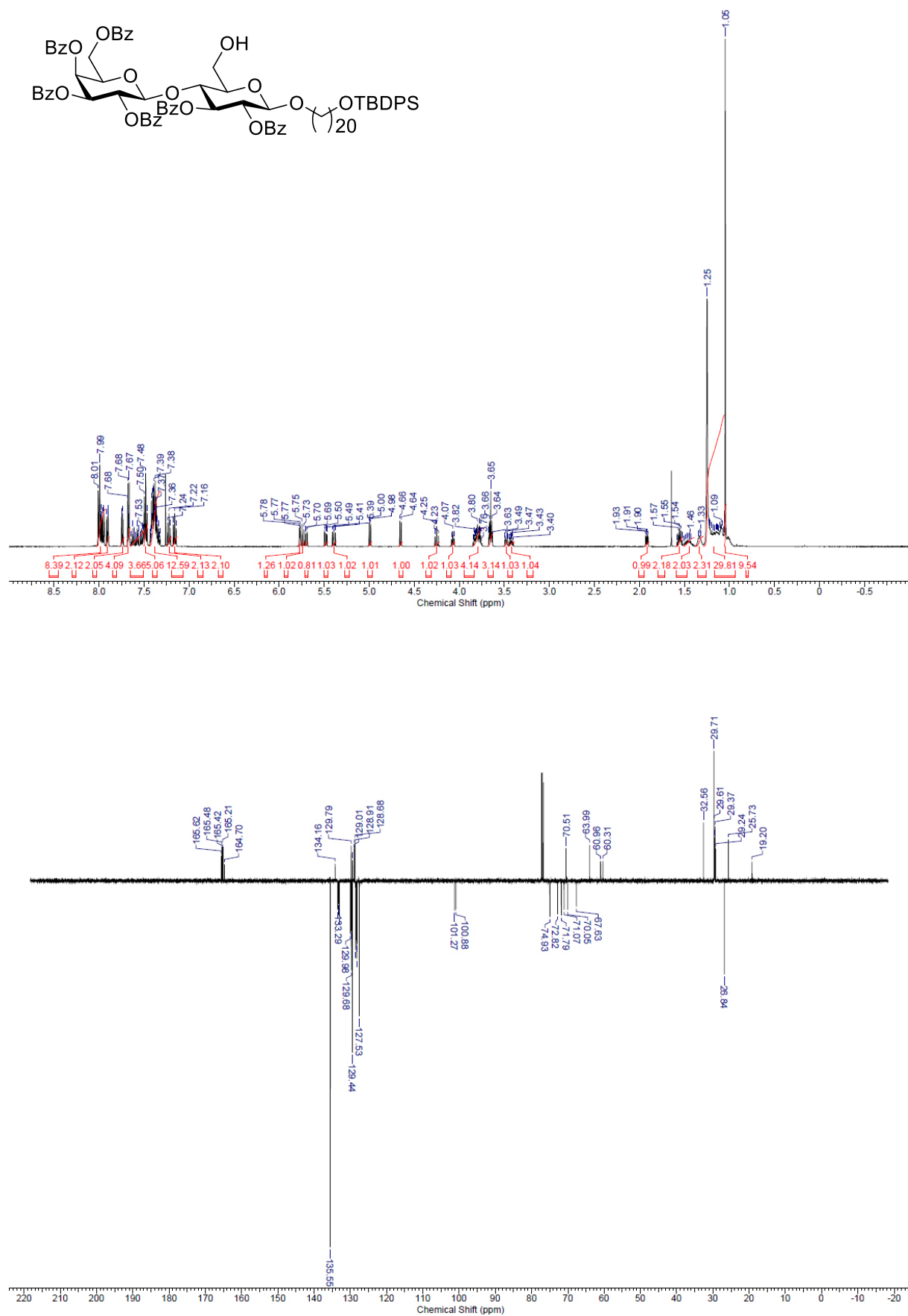


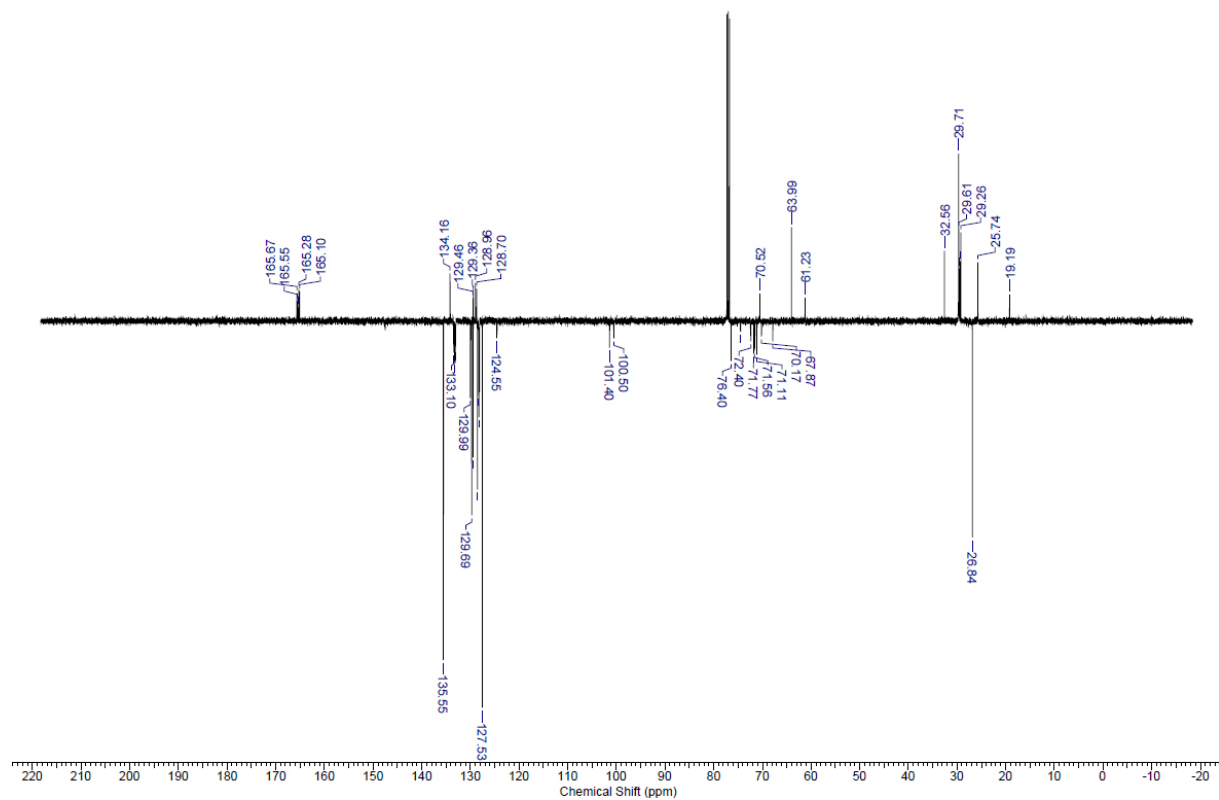
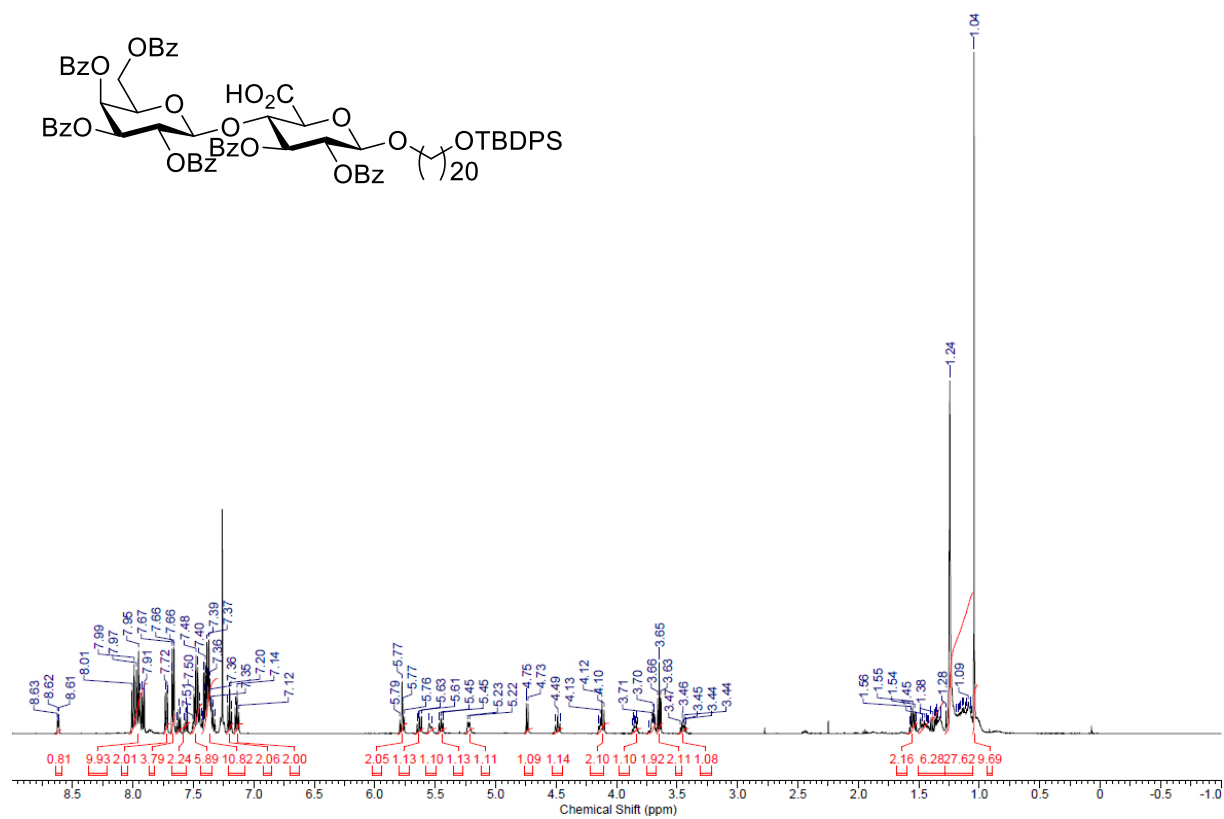


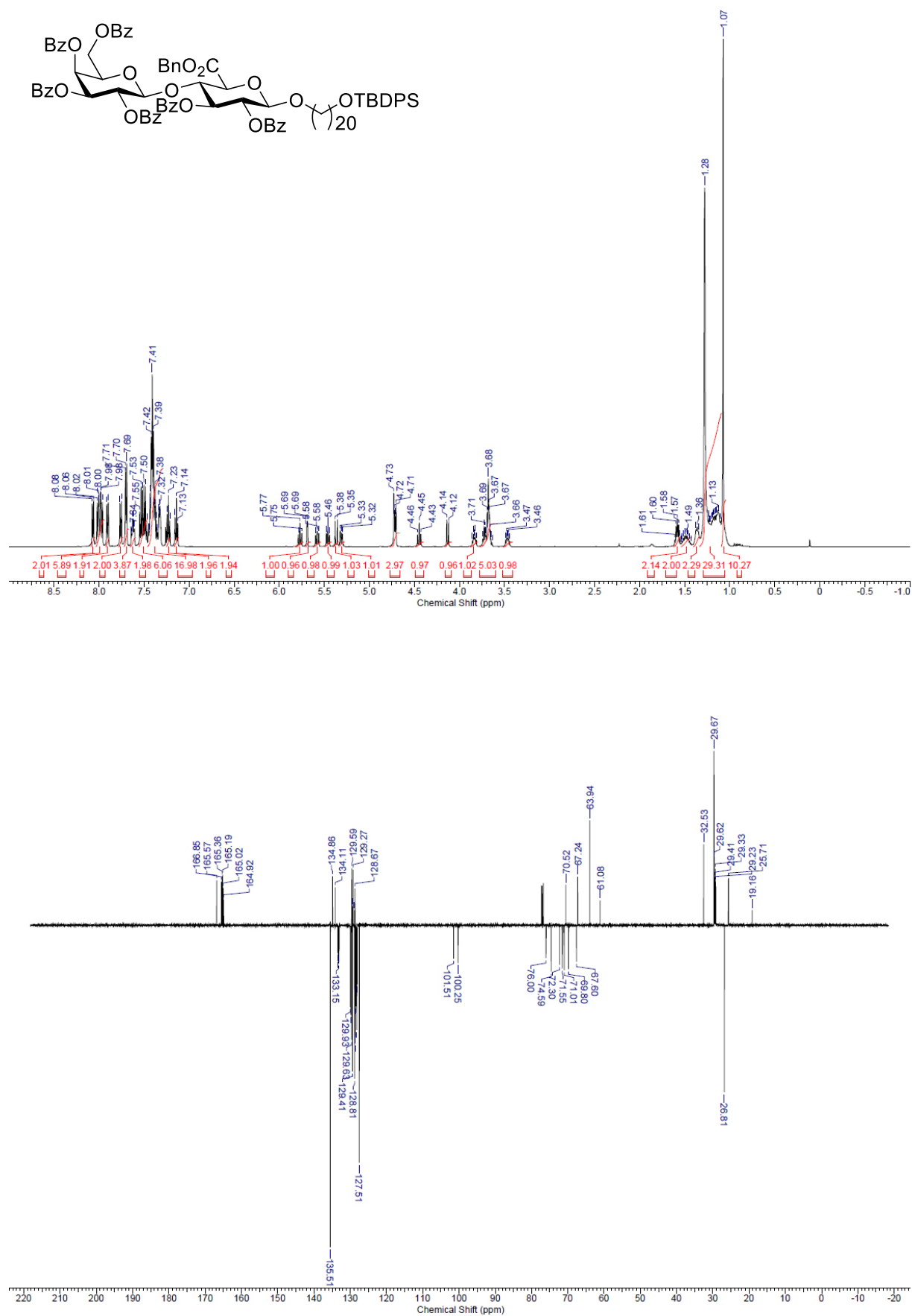


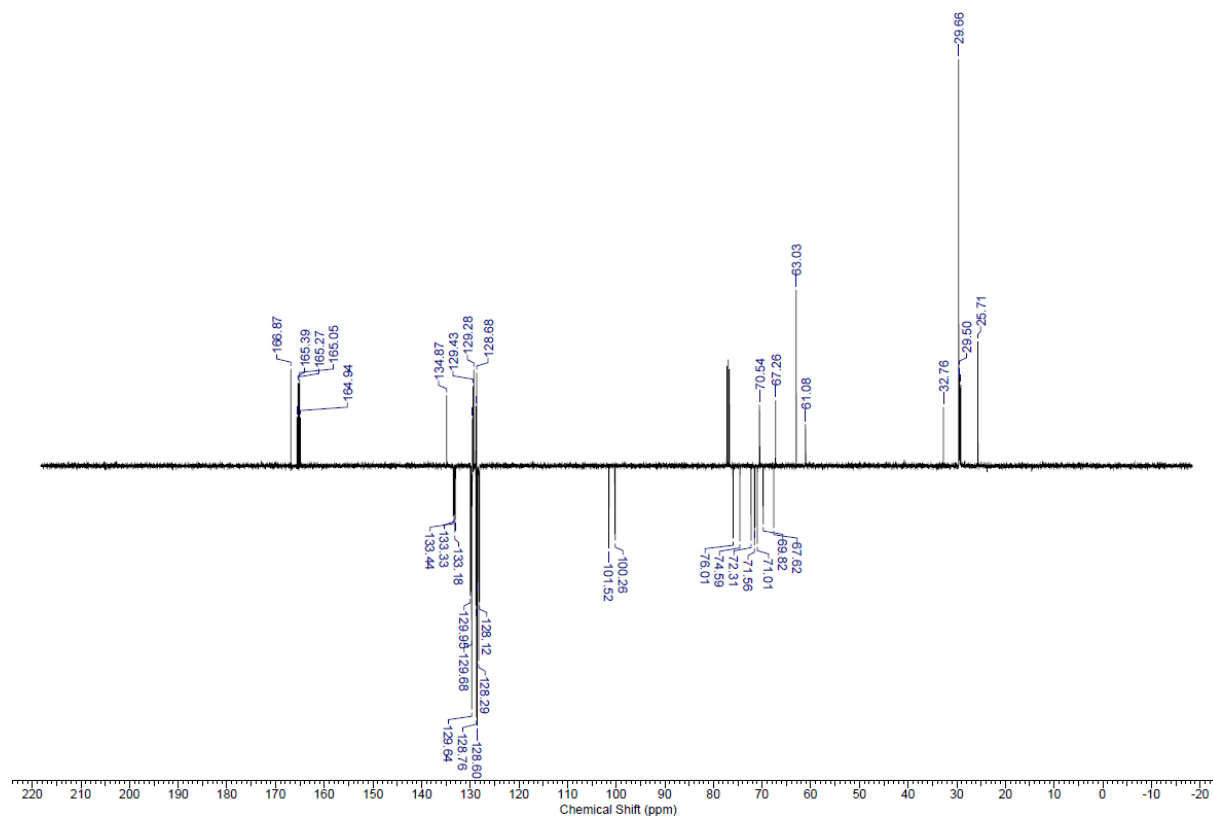
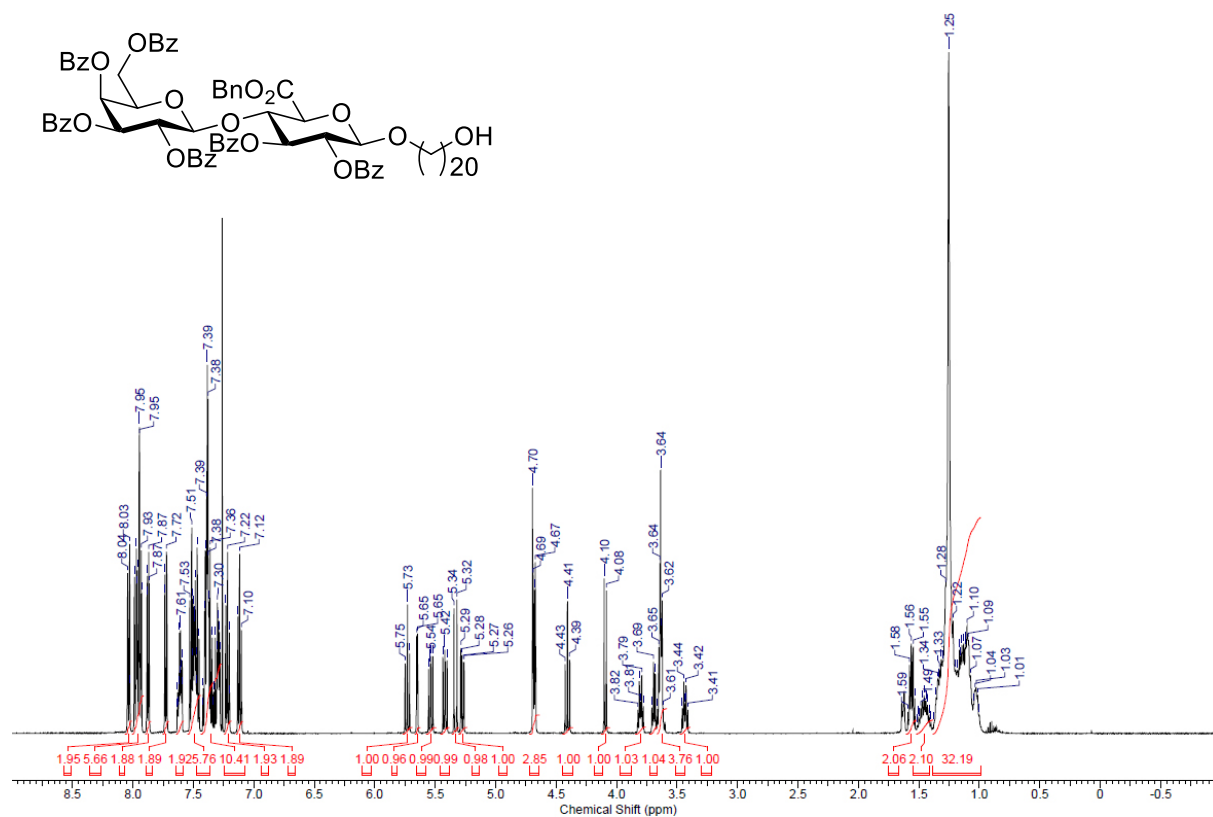


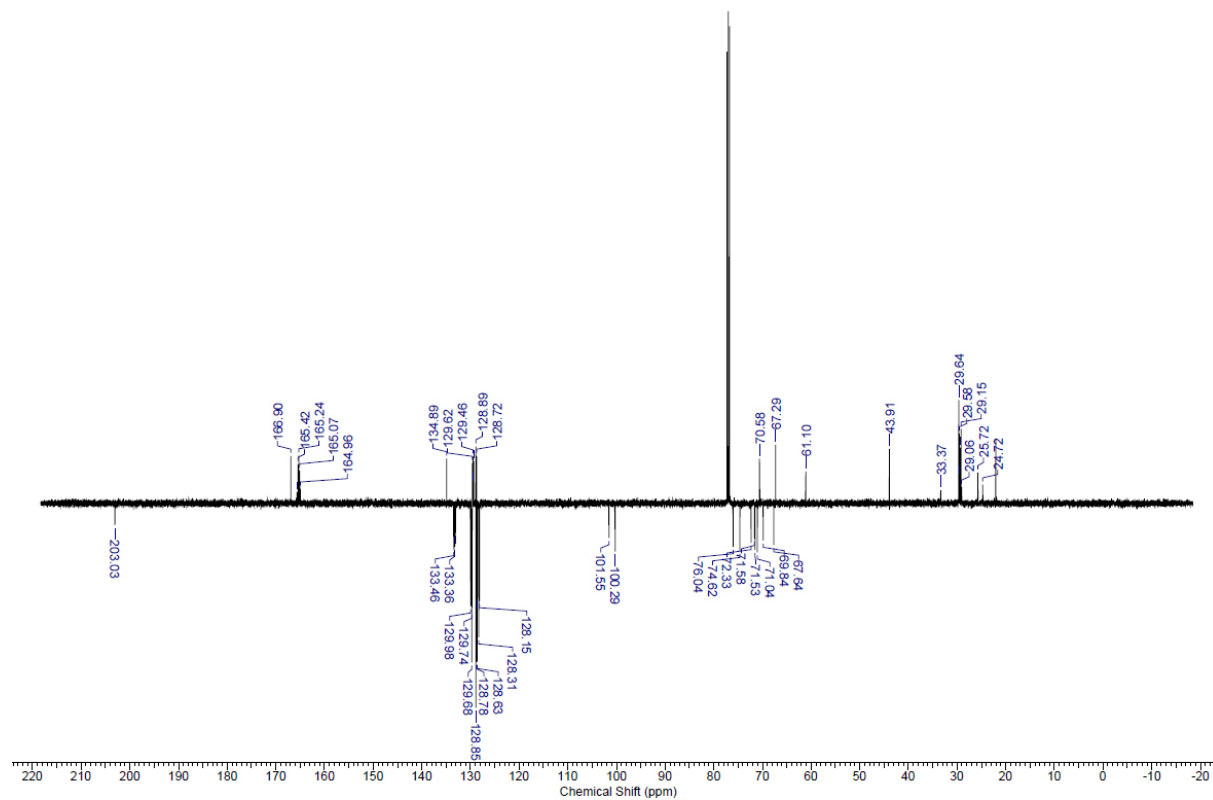
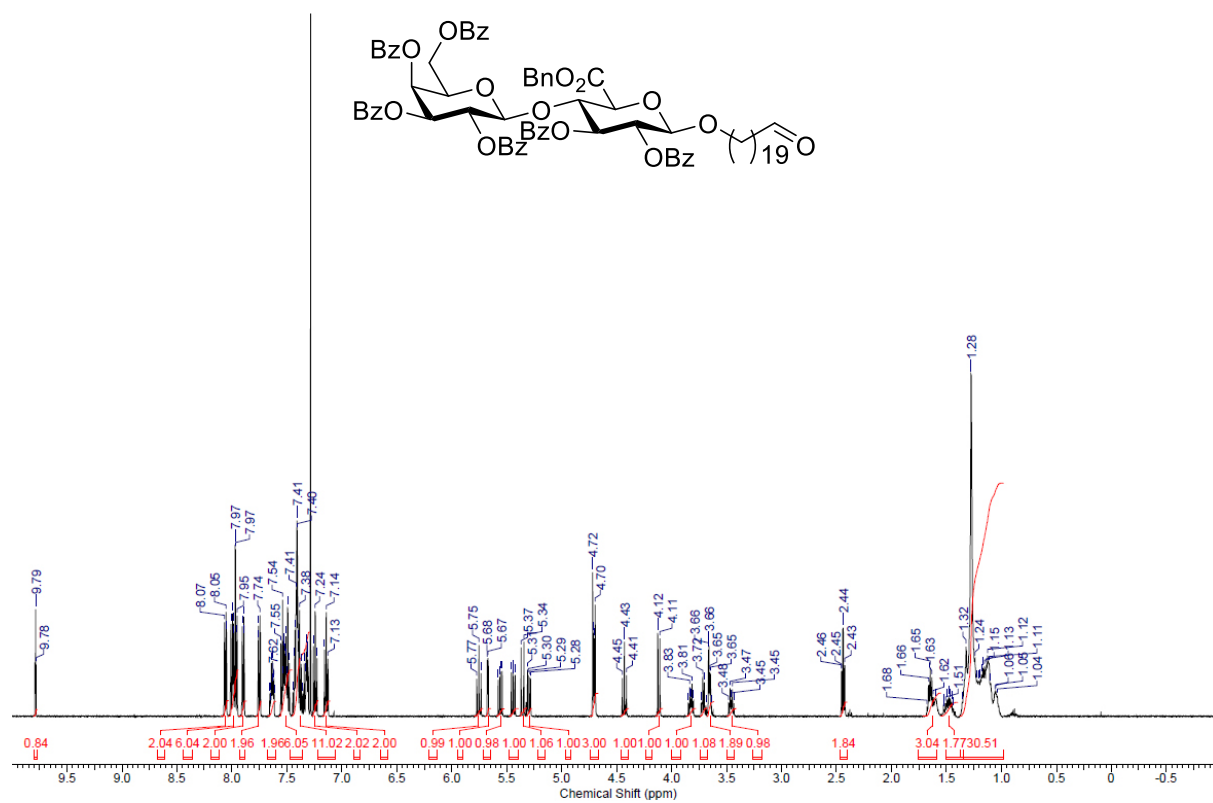


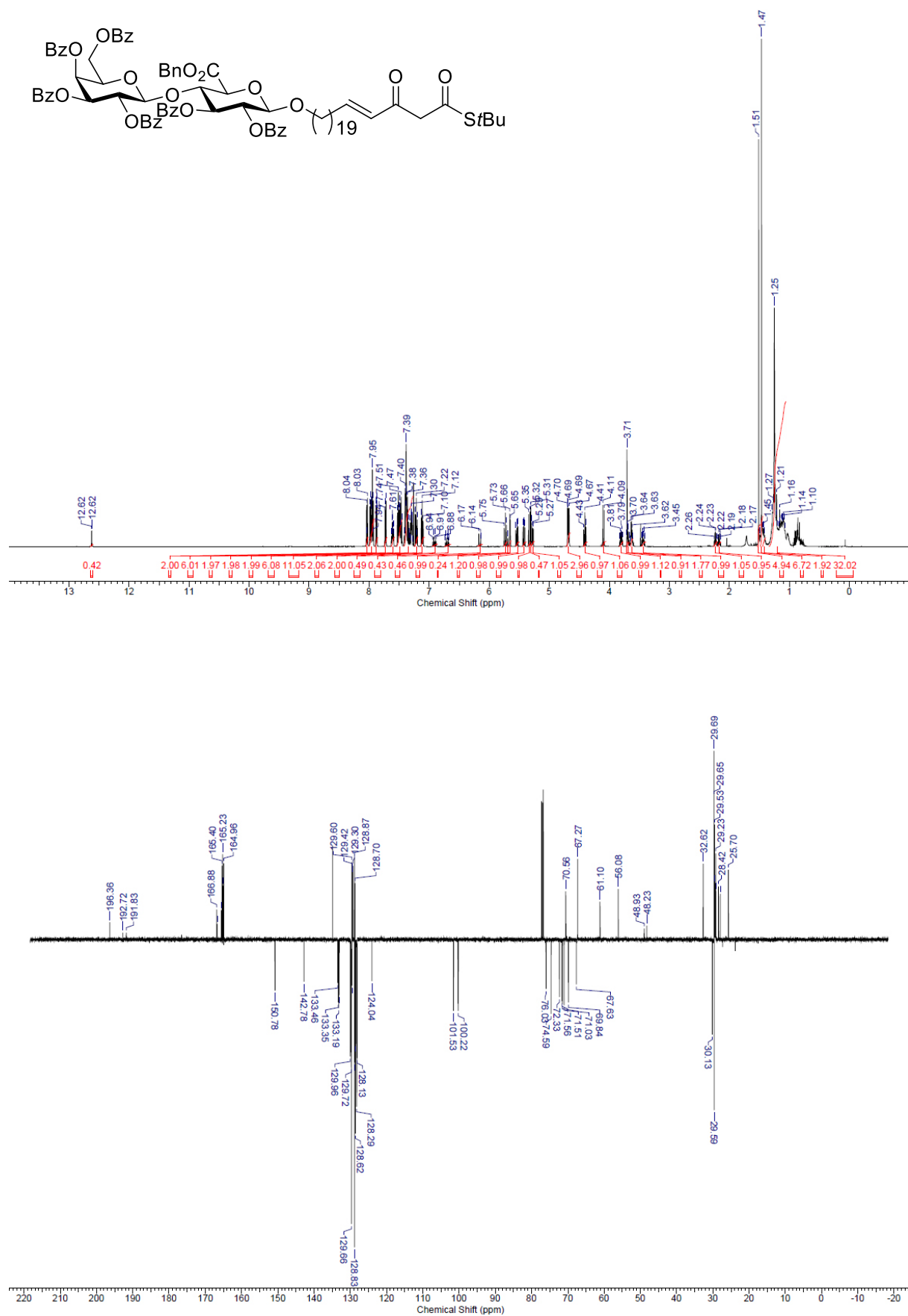


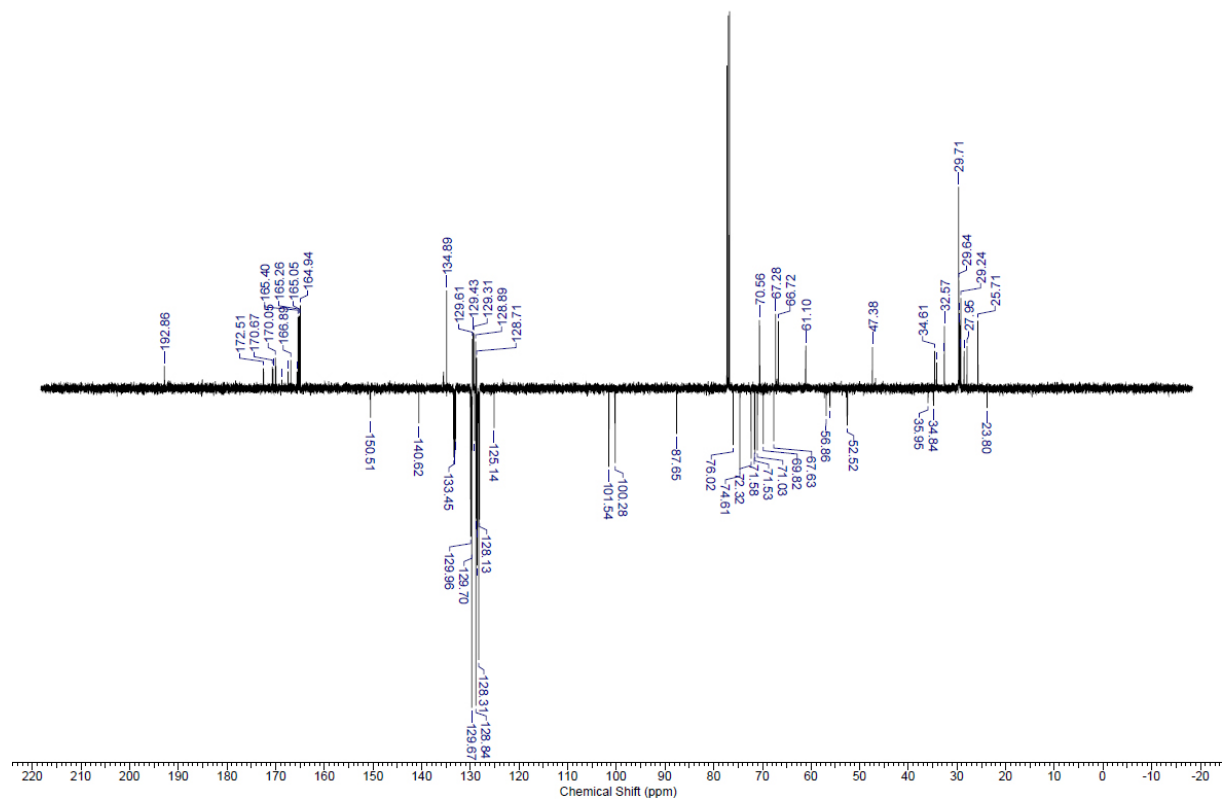
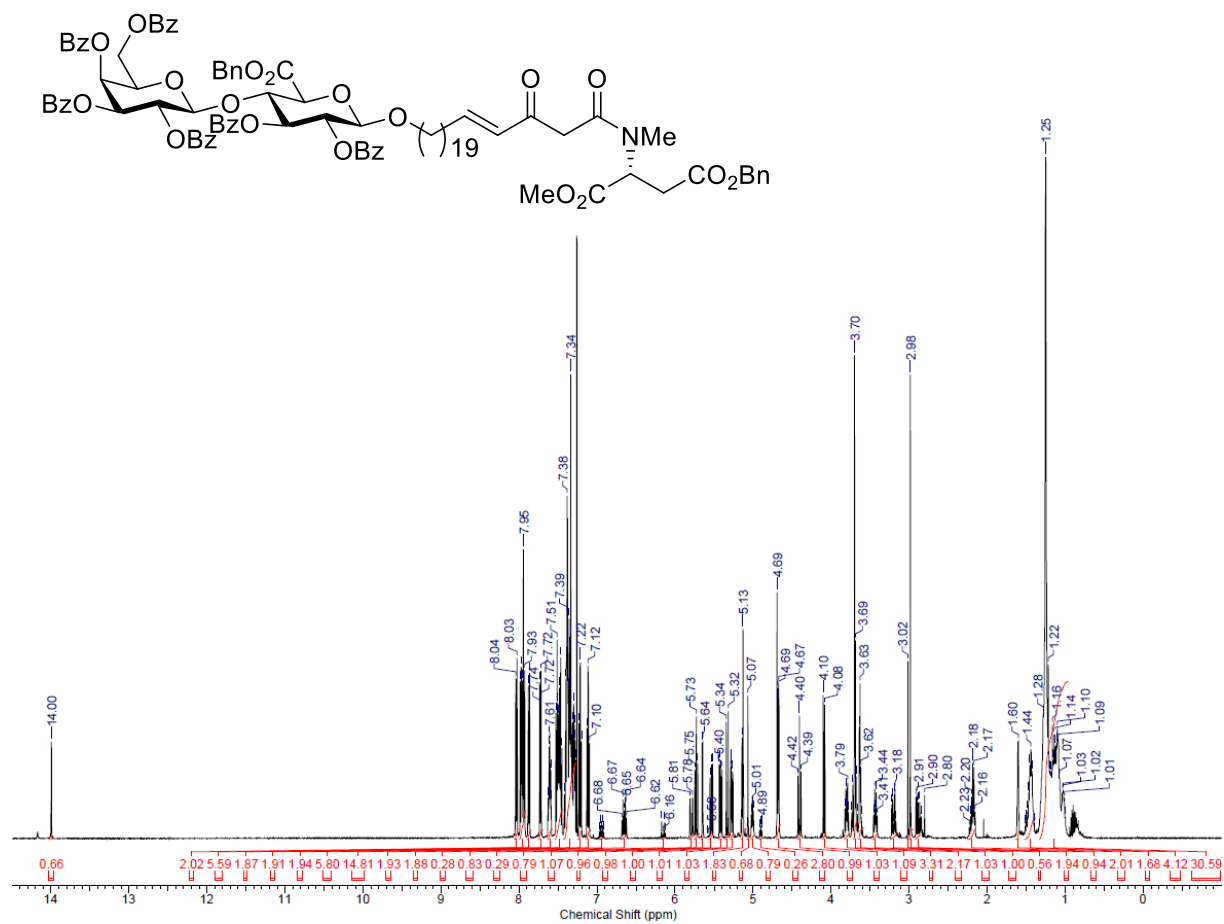


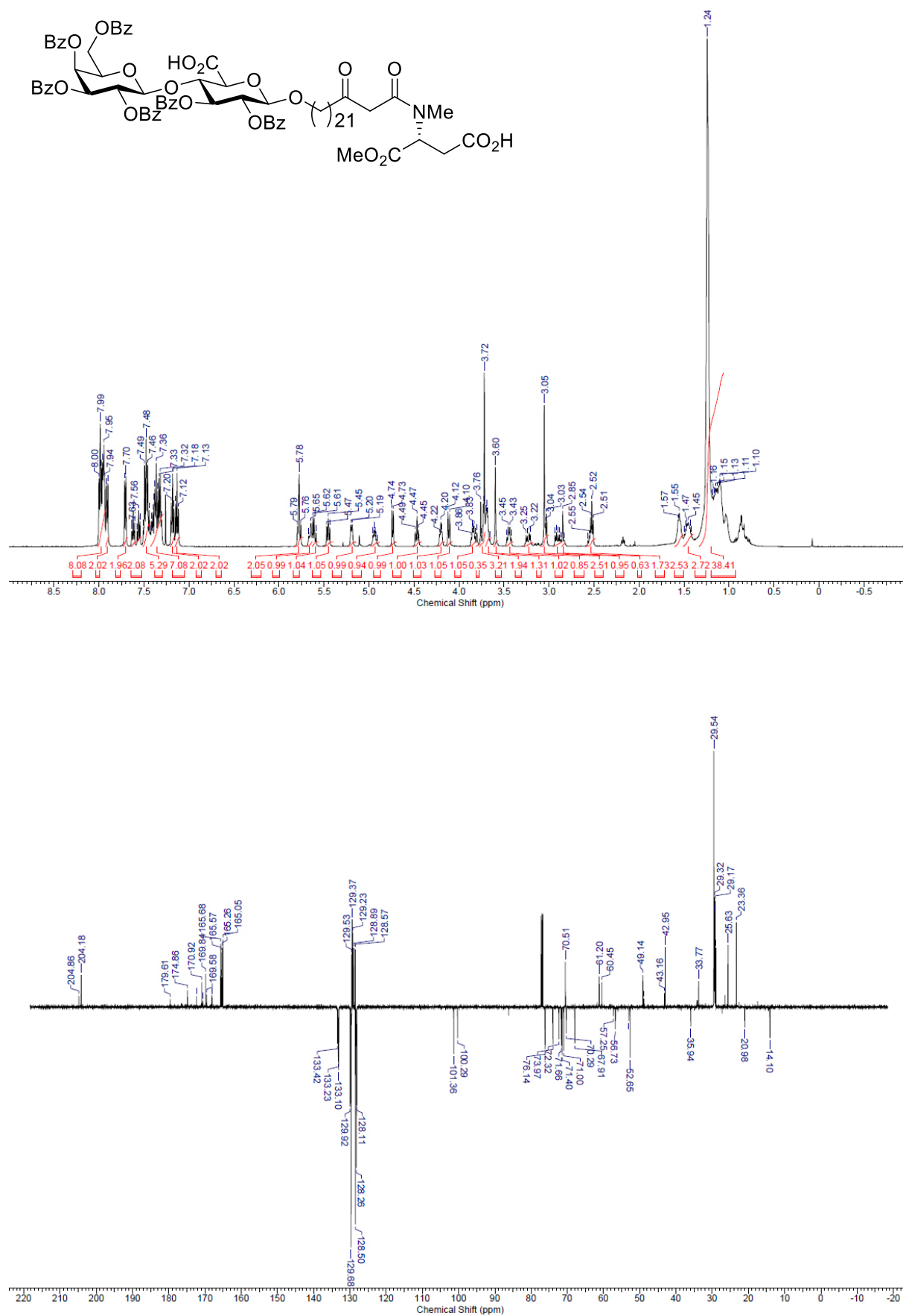


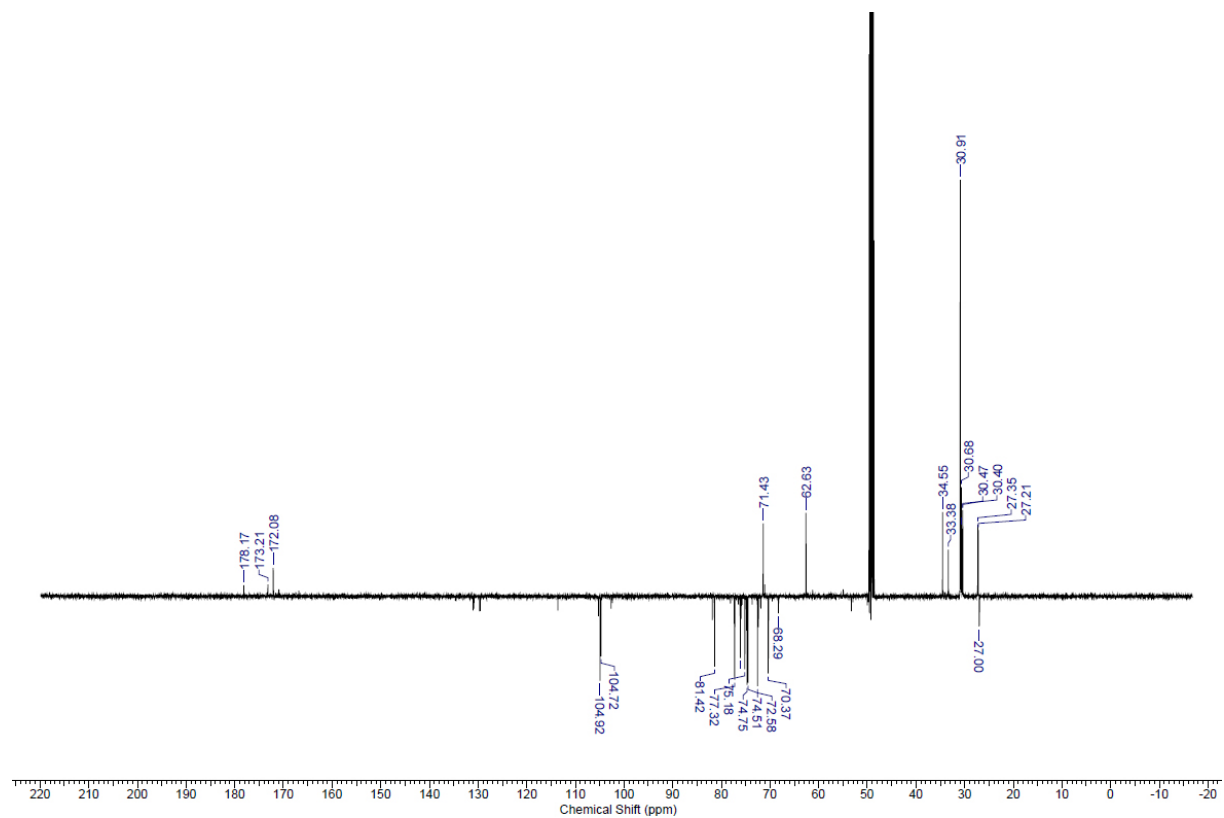
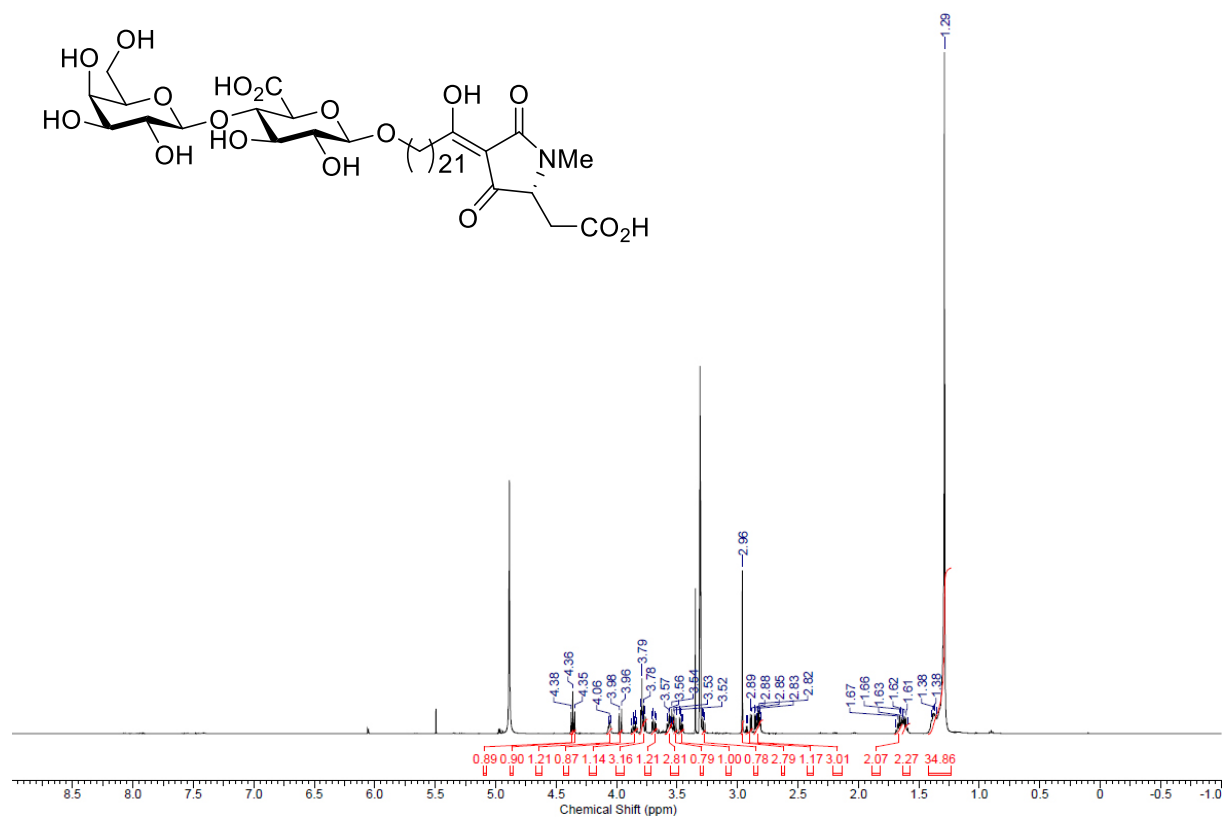




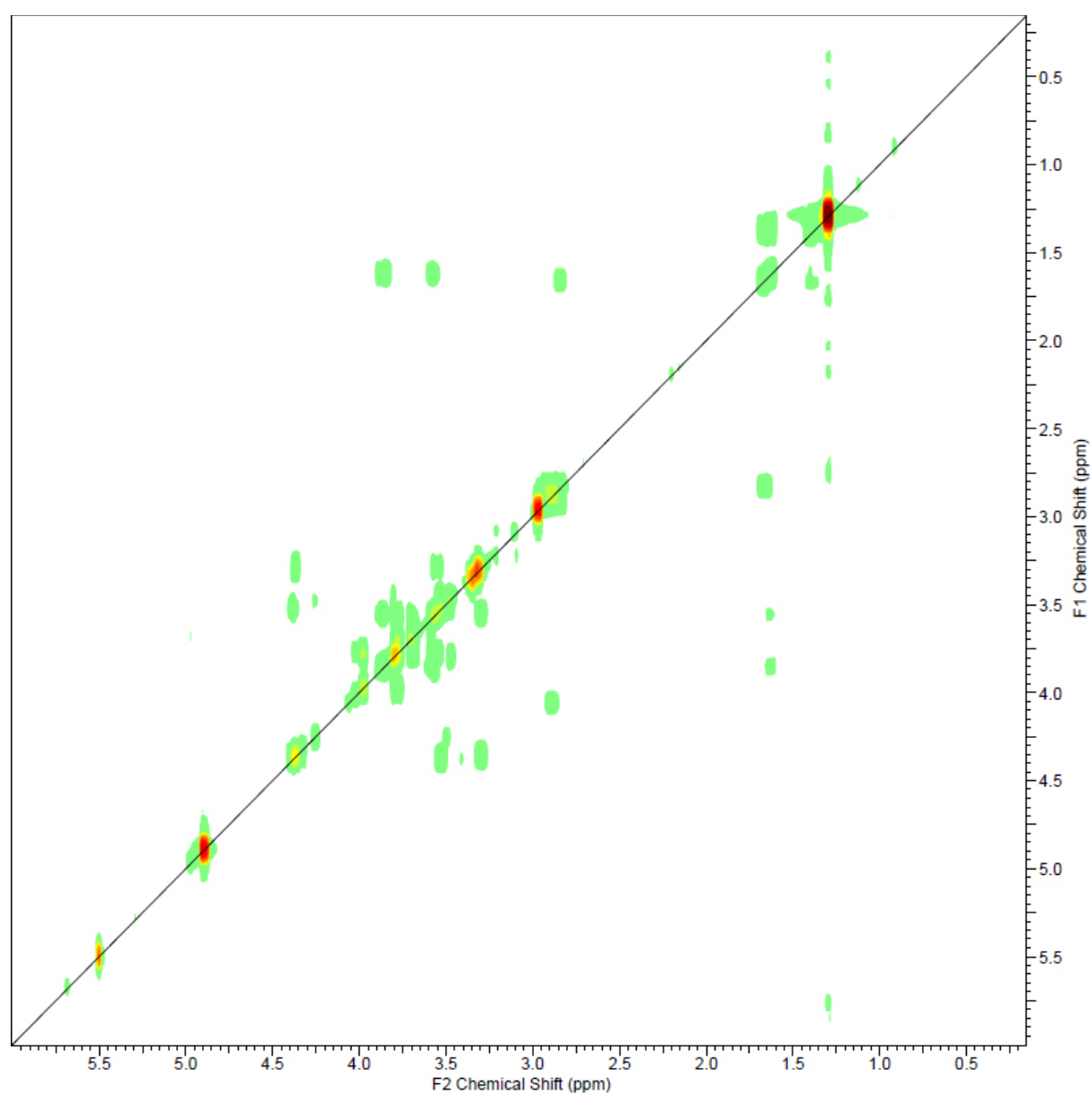




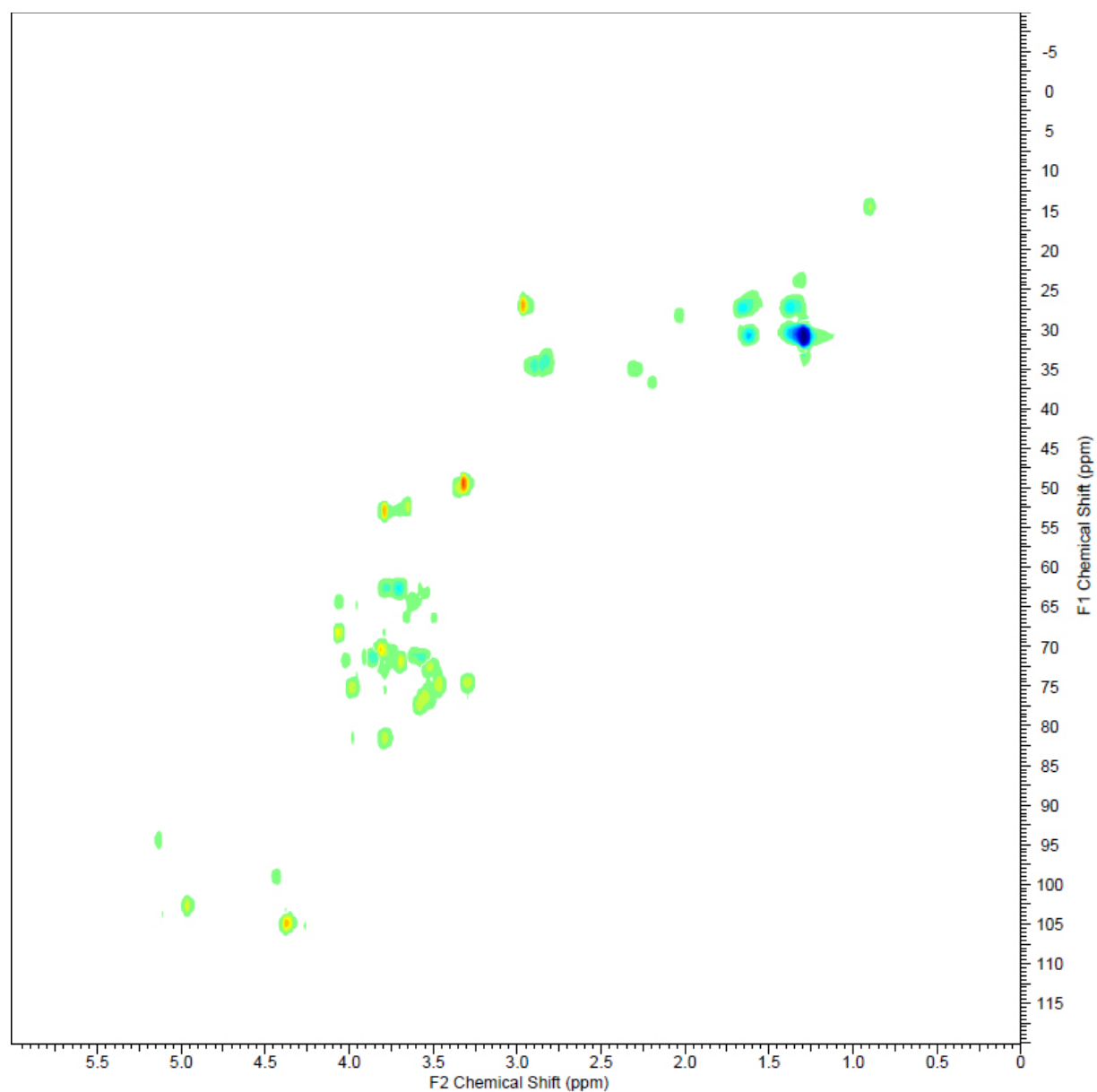




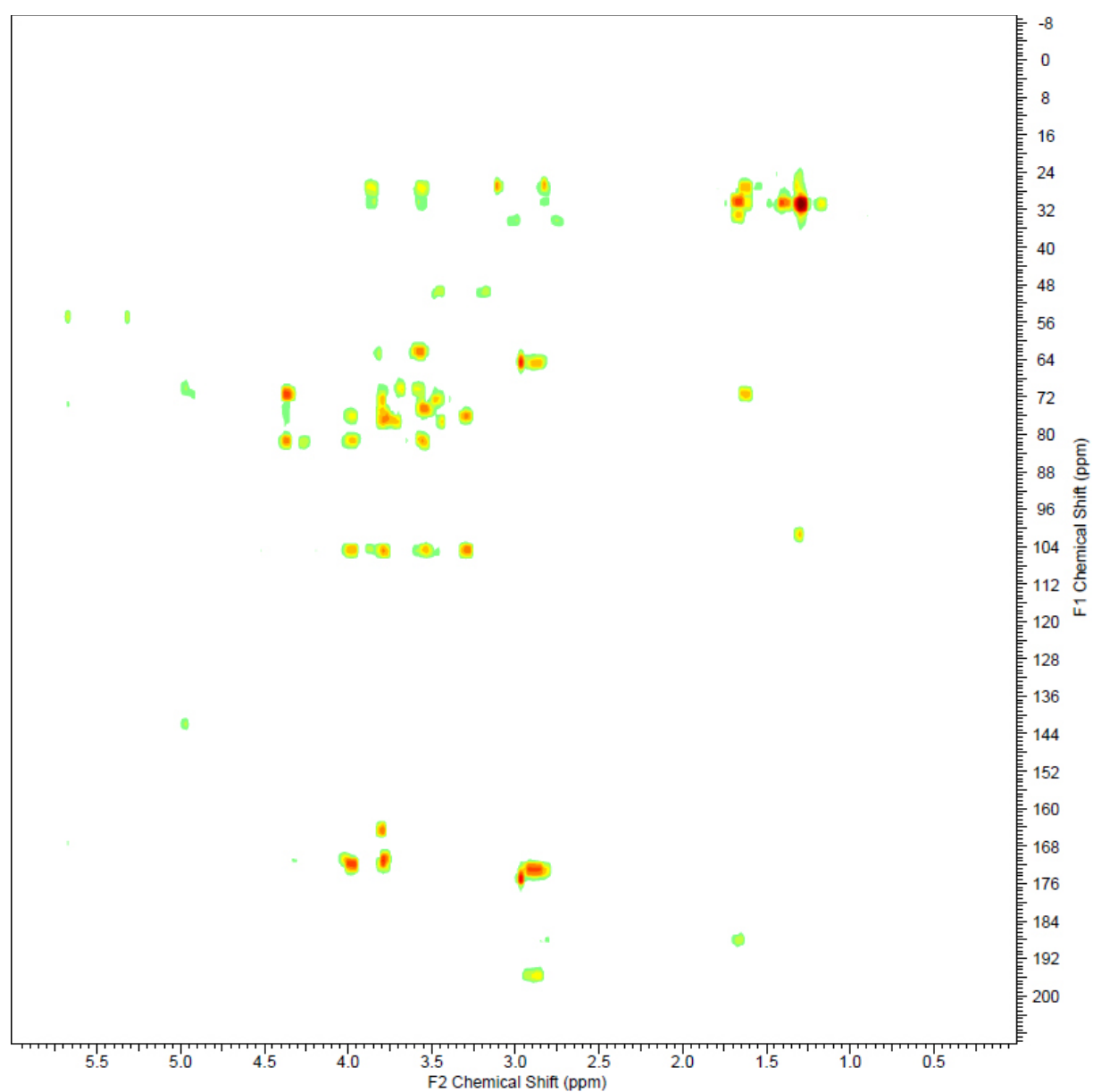
COSY of **2**



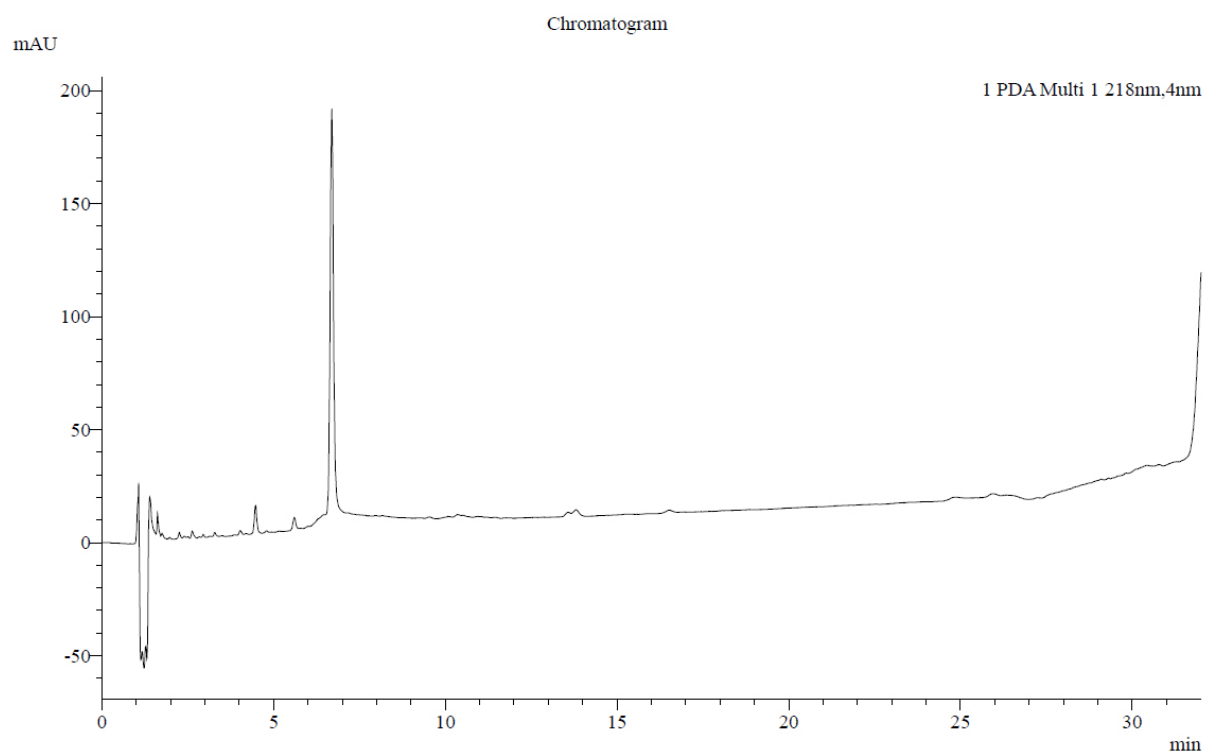
HSQC of **2**



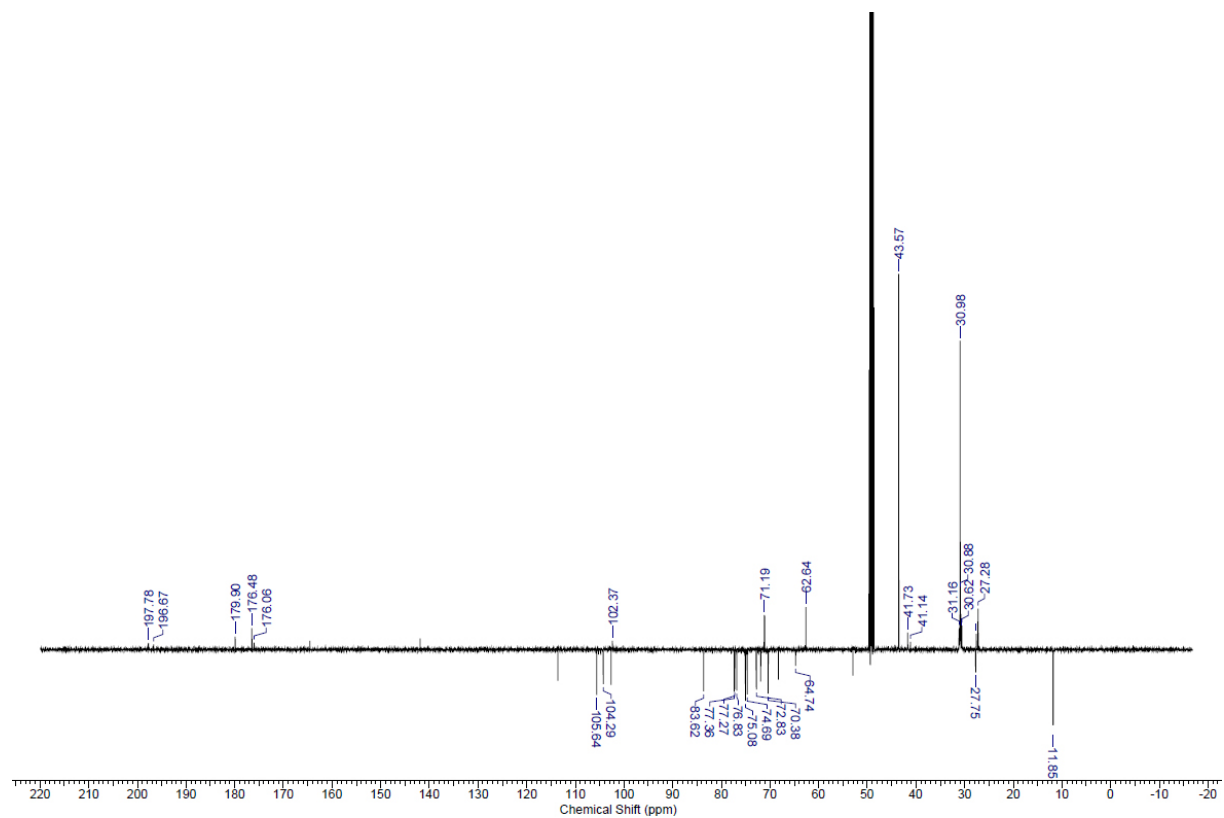
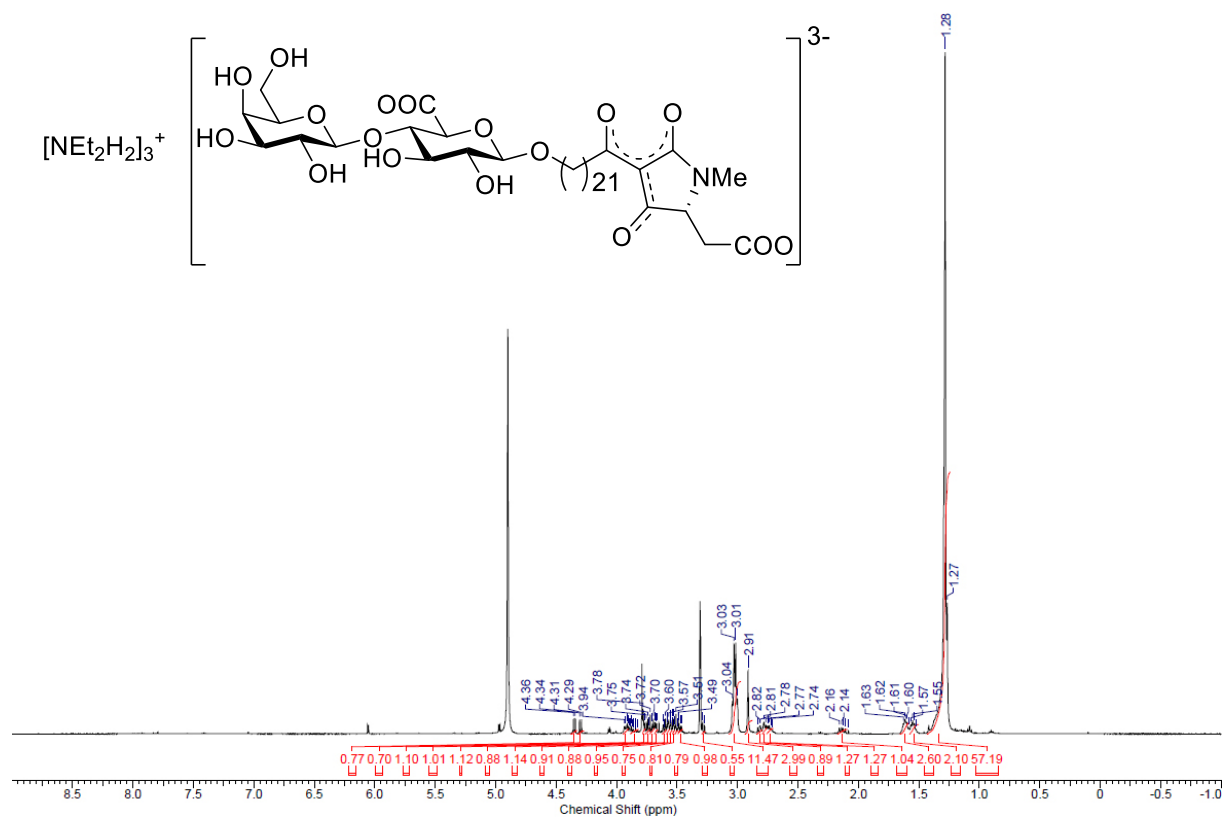
HMBC of 2



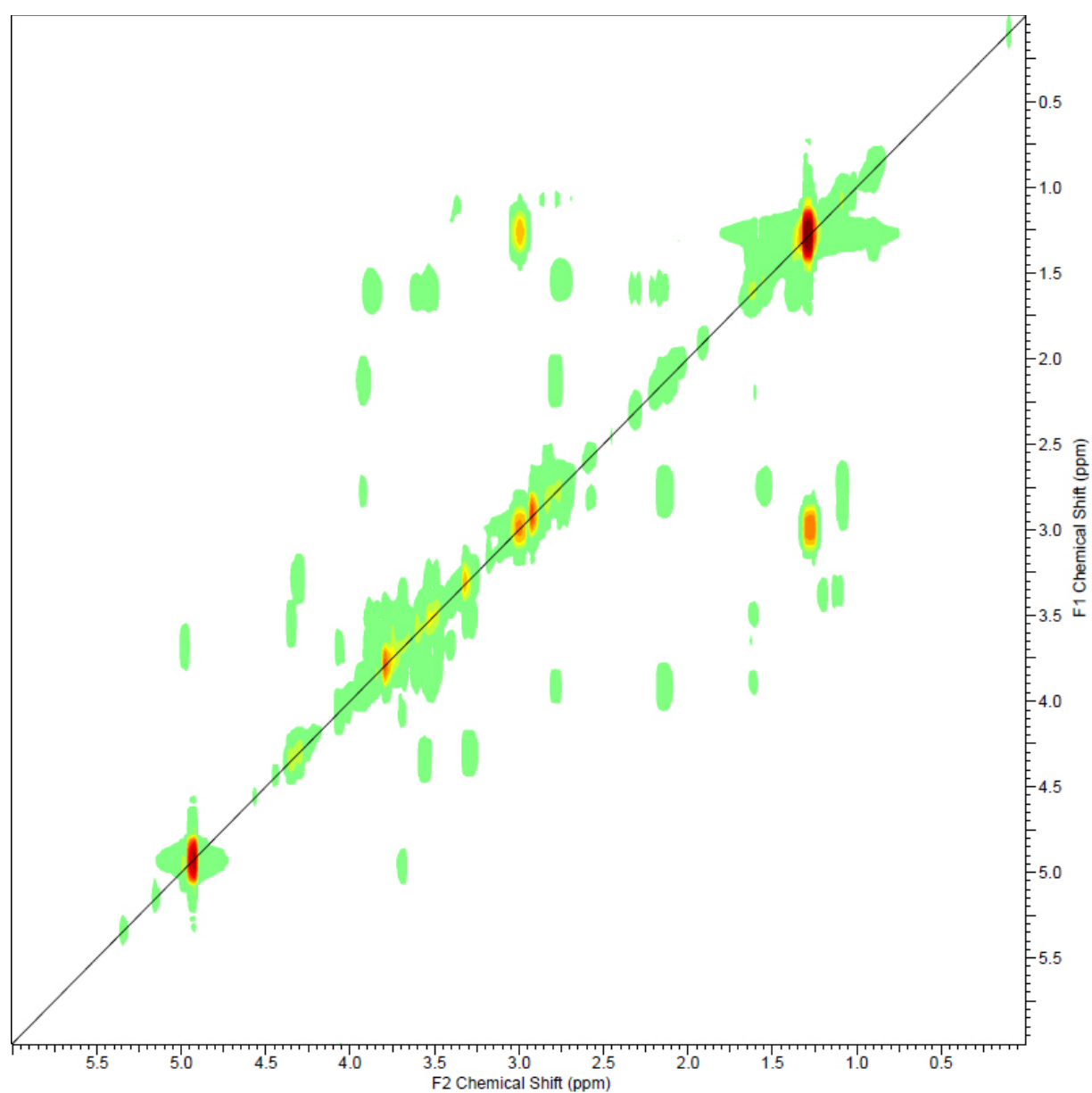
HPLC chromatogram of **2**



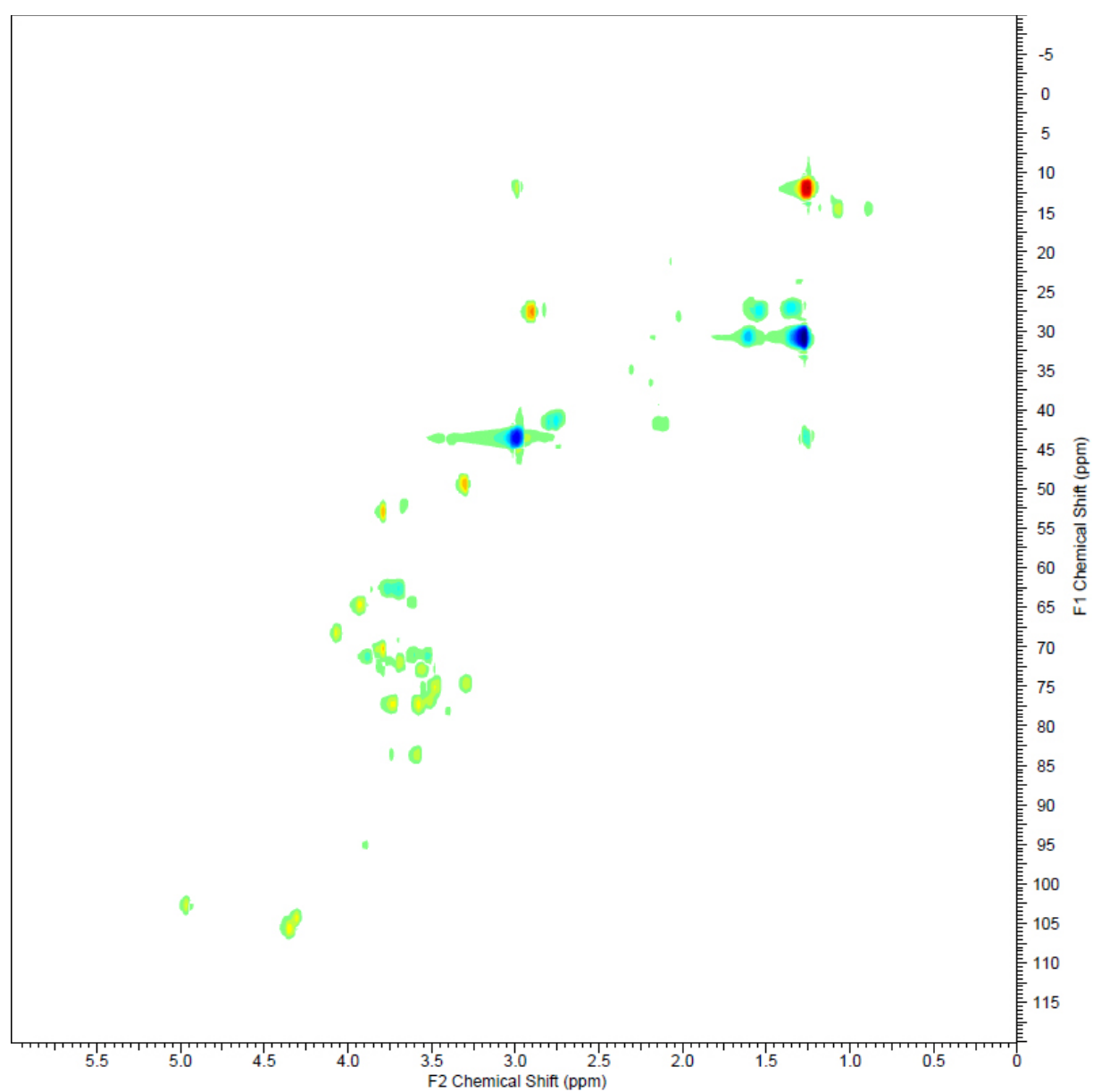
HPLC program: start at 70% MeCN in H₂O with 0.1% HCOOH to 85% MeCN in 25 min for 5 min;
Flow rate 1.0 mL/min, column: Knauer Eurospher II 100-3 C18, 150 x 4 mm



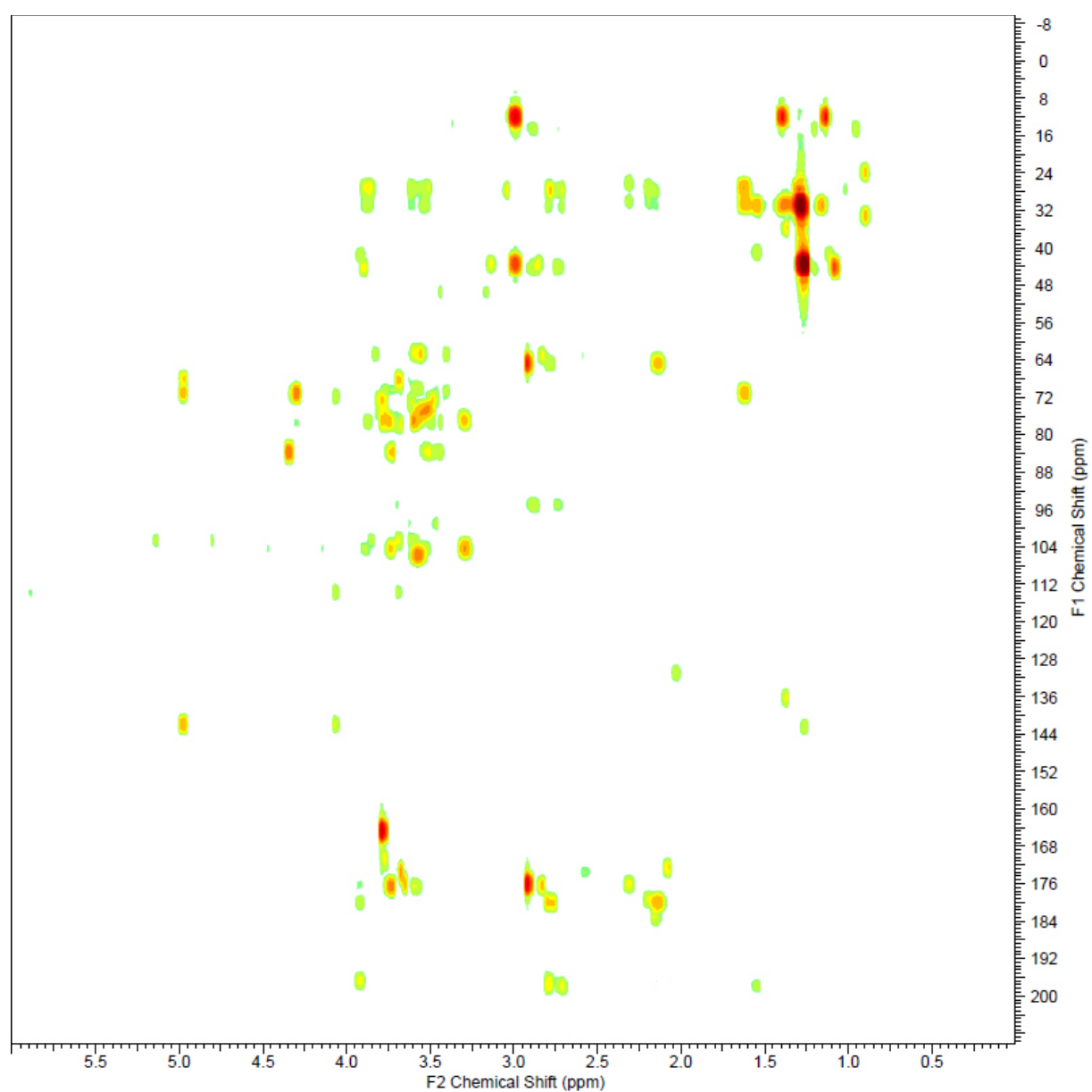
COSY of $[\text{NEt}_2\text{H}_2]_3^+ [\mathbf{2-3H}]^{3-}$



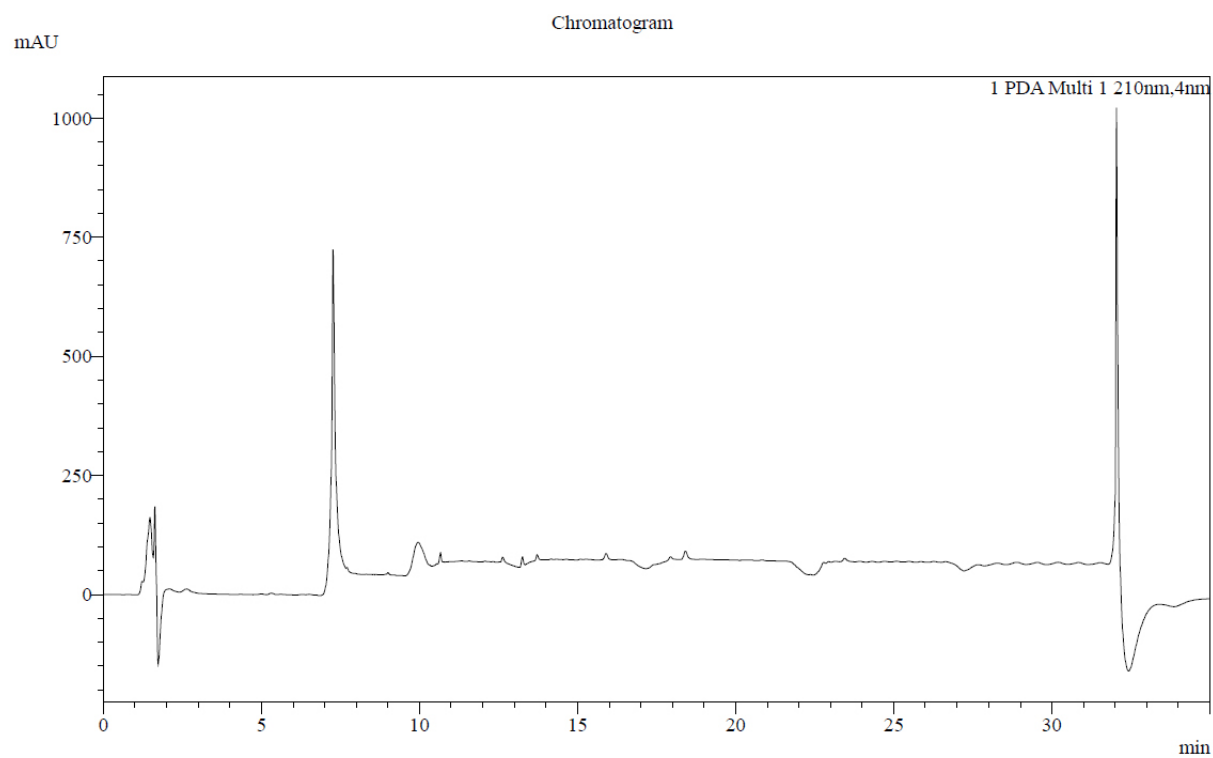
HSQC of $[\text{NEt}_2\text{H}_2]_3^+ [\mathbf{2}\text{-}^3\text{H}]^{3-}$



HMBC of $[\text{NEt}_2\text{H}_2]_3^+ [\text{2-3H}]^{3-}$



HPLC chromatogram of $[\text{NEt}_2\text{H}_2]_3^+ [\mathbf{2-3H}]^{3-}$



HPLC program: start at 40% MeCN in H₂O to 95% MeCN in 25 min for 5 min;
Flow rate 1.0 mL/min, column: Knauer Eurospher II 100-3 C18, 150 x 4 mm

HRMS of ancorinoside B (2)

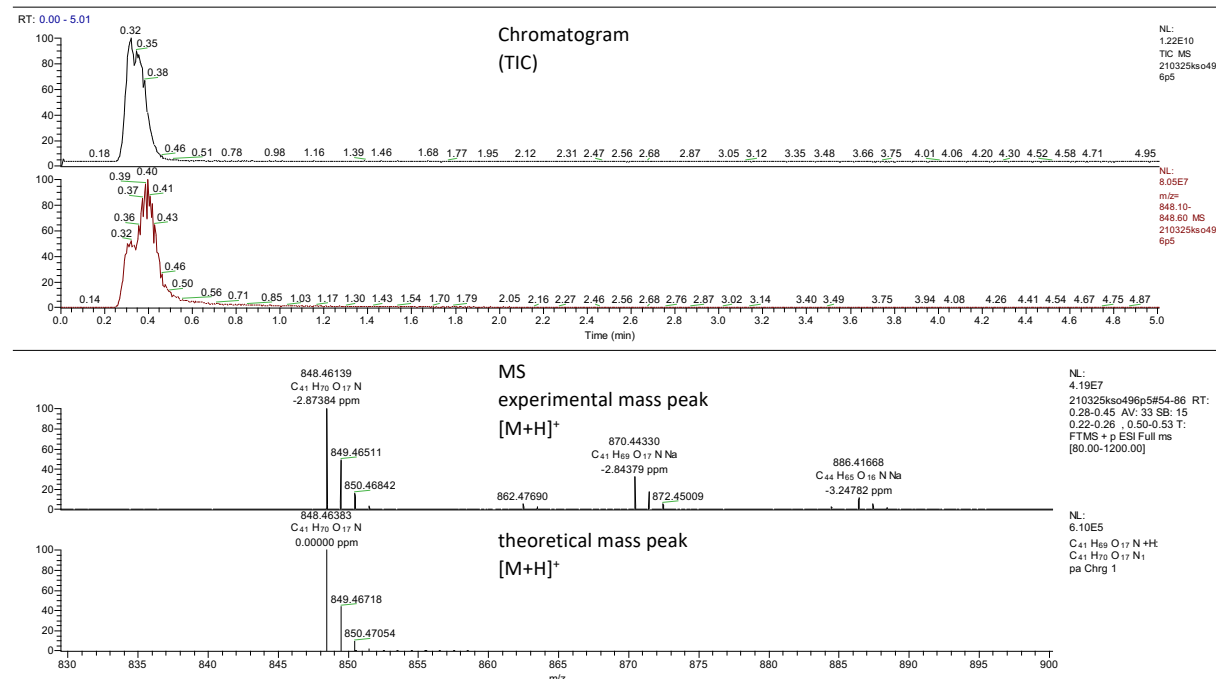
Analysis without HPLC-column, full scan mode 80-1200 amu, iso80: isocratic with 80% MeCN

ESI positive:

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03/26/21 12:51:28

KS496_5 C41H69NO17 ESI+

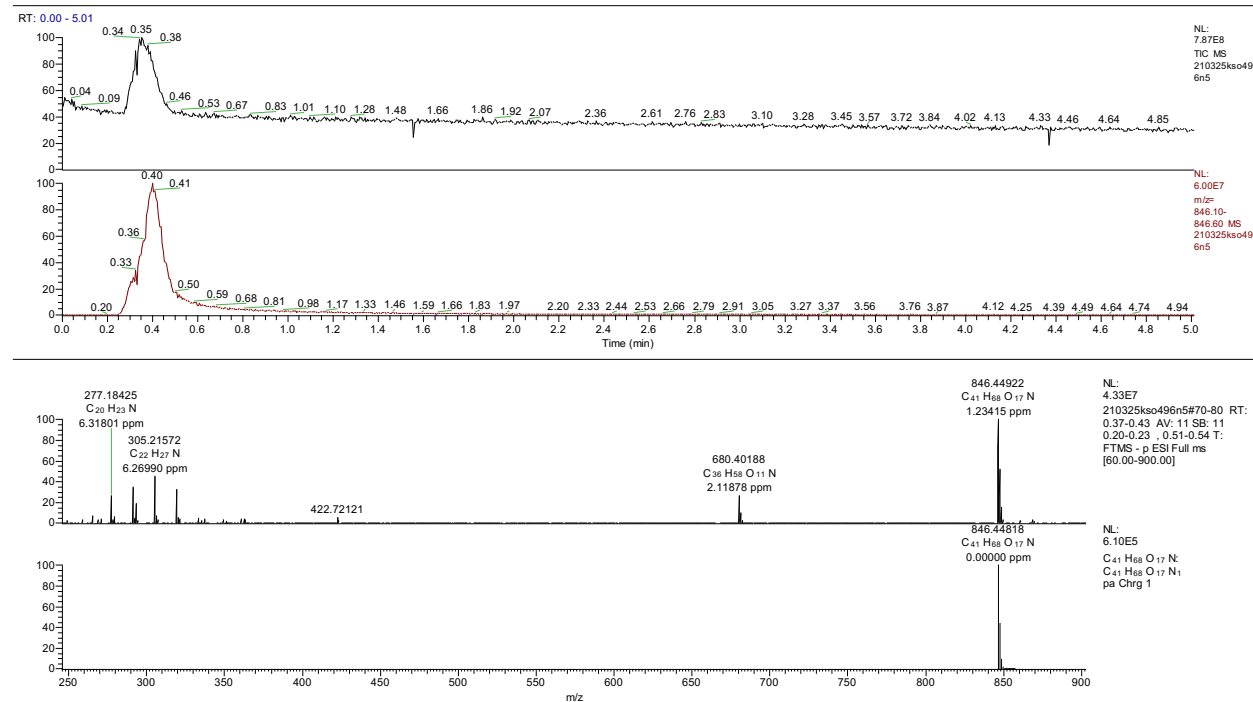


ESI negative:

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03/25/21 15:45:47

KS496_5 C41H69NO17 ESI-



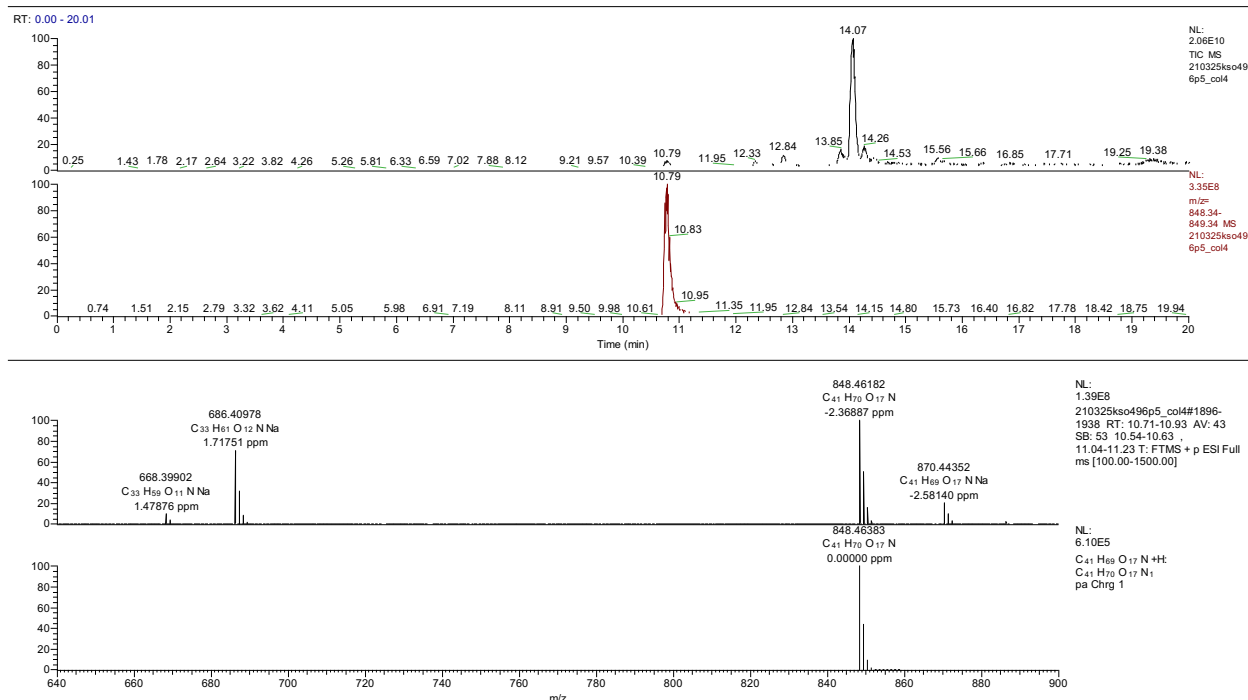
Analysis with HPLC-column (Accupore RP-MS 150 x 2.1 mm, 2.6 μ m), full scan mode 100-1500 amu, gradient from 40% to 90% MeCN

ESI positive

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03/26/21 13:41:38

KS496_5 C41H69NO17 col4 ESI+

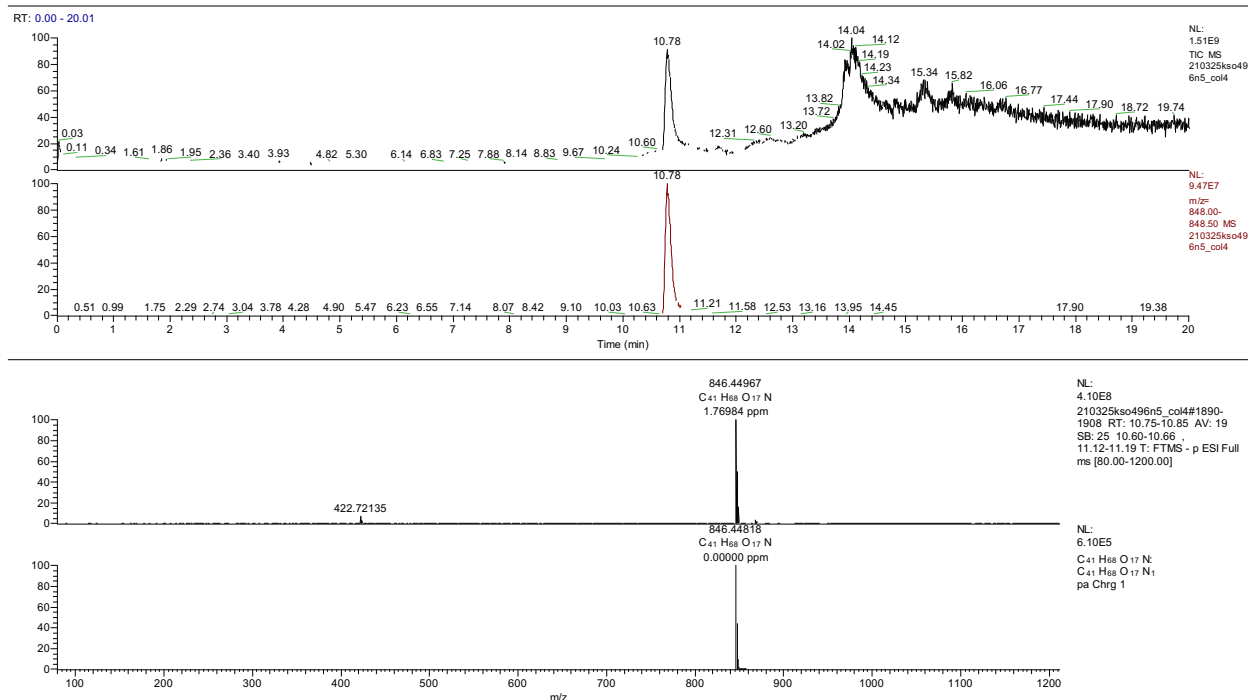


ESI negative

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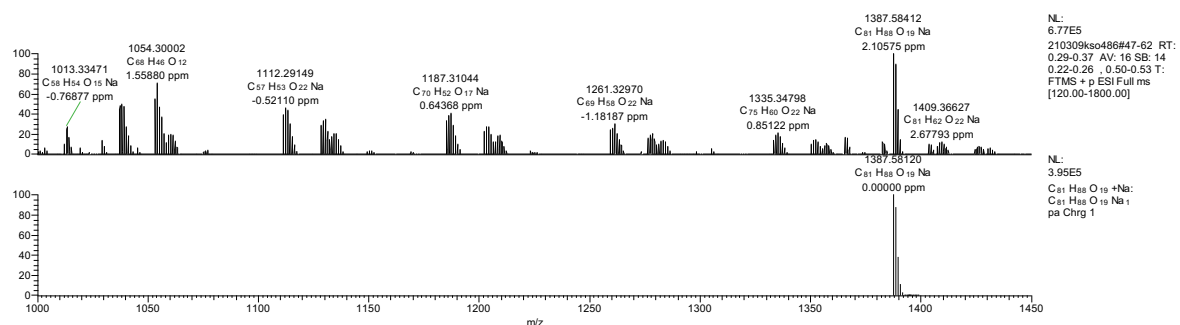
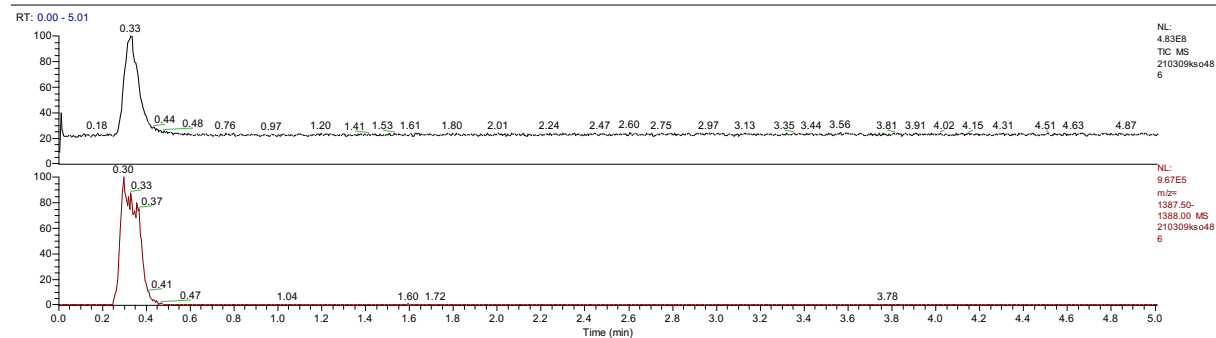


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KS 486

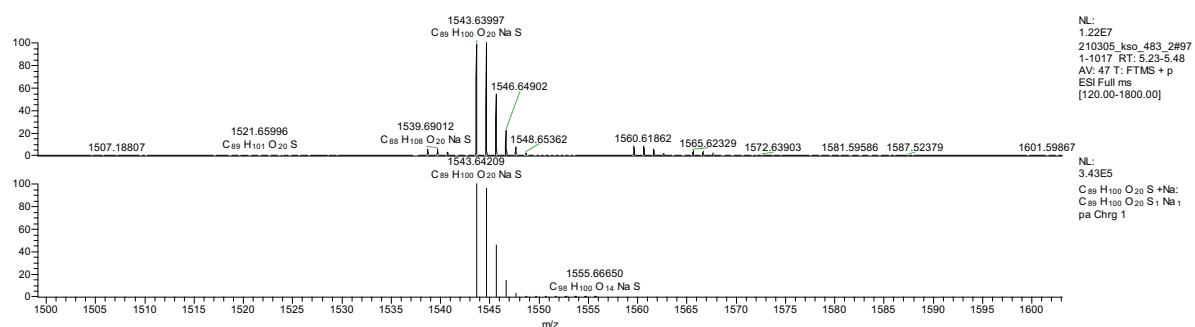
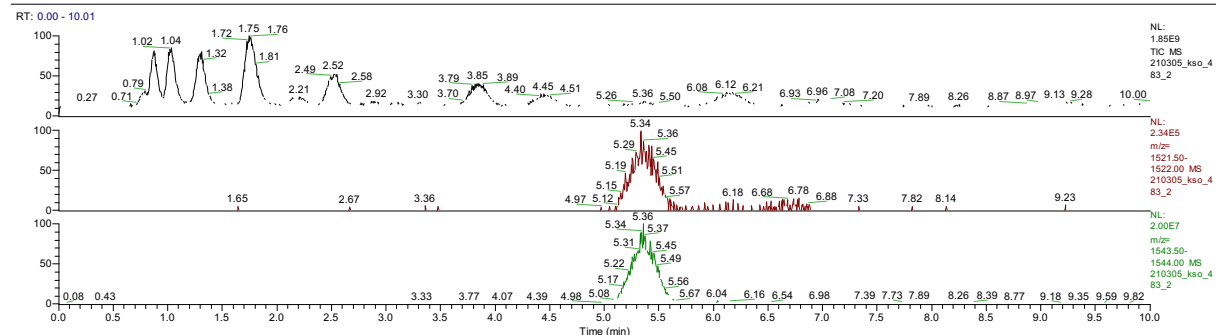


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KS 483 (col2iso95)

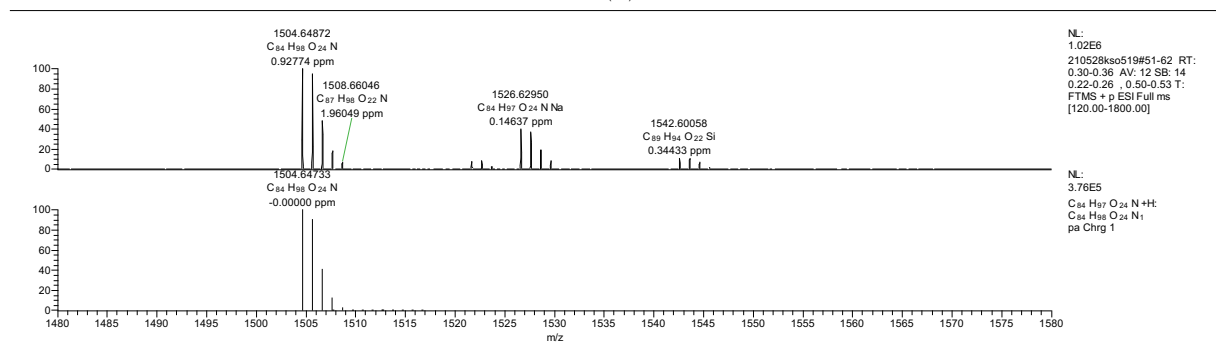
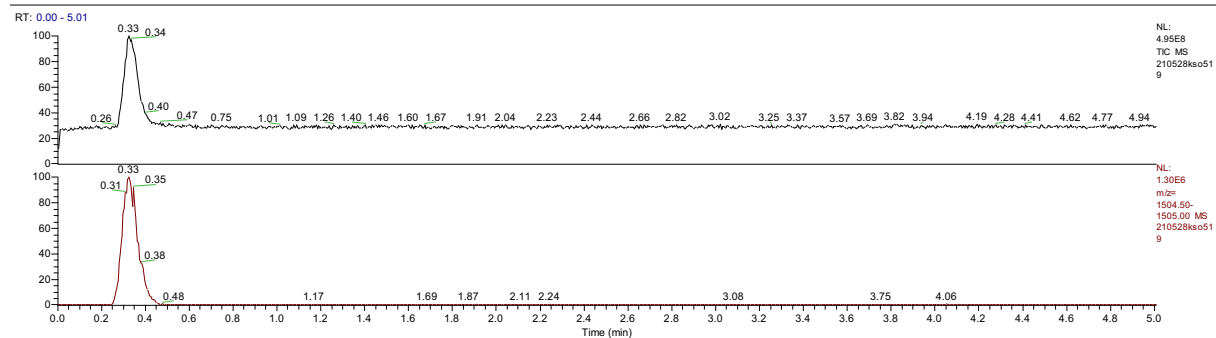


HRMS of 5

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05/28/21 10:43:25

KS519



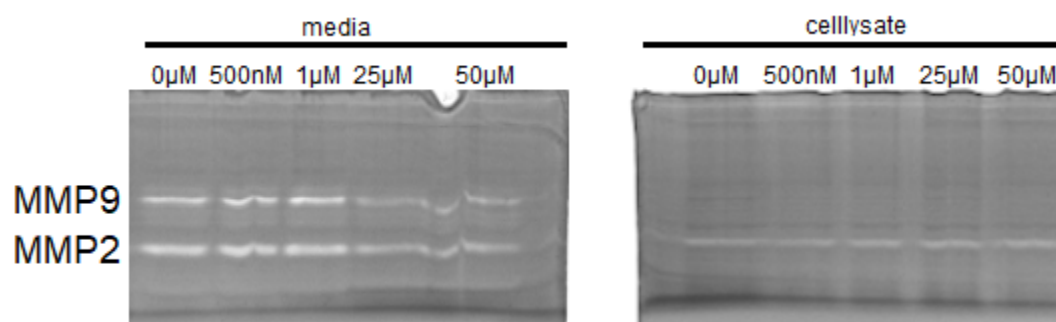


Figure S1. Effect of ancorinoside B (**2**) (500 nM, 1 μM, 25 μM, and 50 μM) on the cell-associated and secreted activities of MMP-2 and MMP-9 in 518A2 melanoma cells after 24 h exposure. Cell lysate and media samples were subjected to gelatin zymography. Control (DMSO)-treated probes are listed as 0 μM. Images are representative of two independent experiments.