

Supplementary Materials

Acyclic Triterpenoids from *Alpinia katsumadai* Inhibit IL-6-Induced STAT3 Activation

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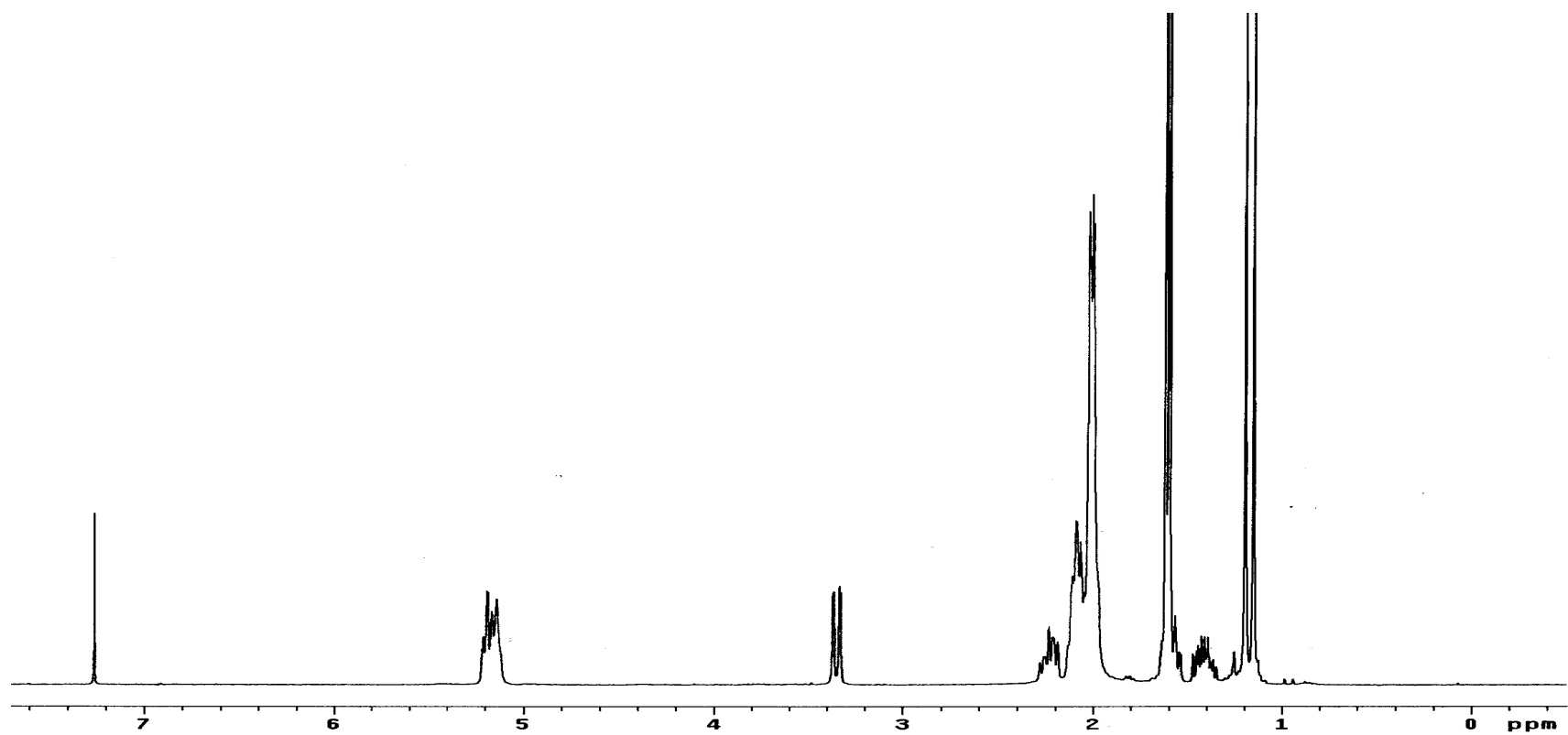


Figure S1. ^1H NMR spectrum of 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**1**) (300 MHz in CDCl_3).

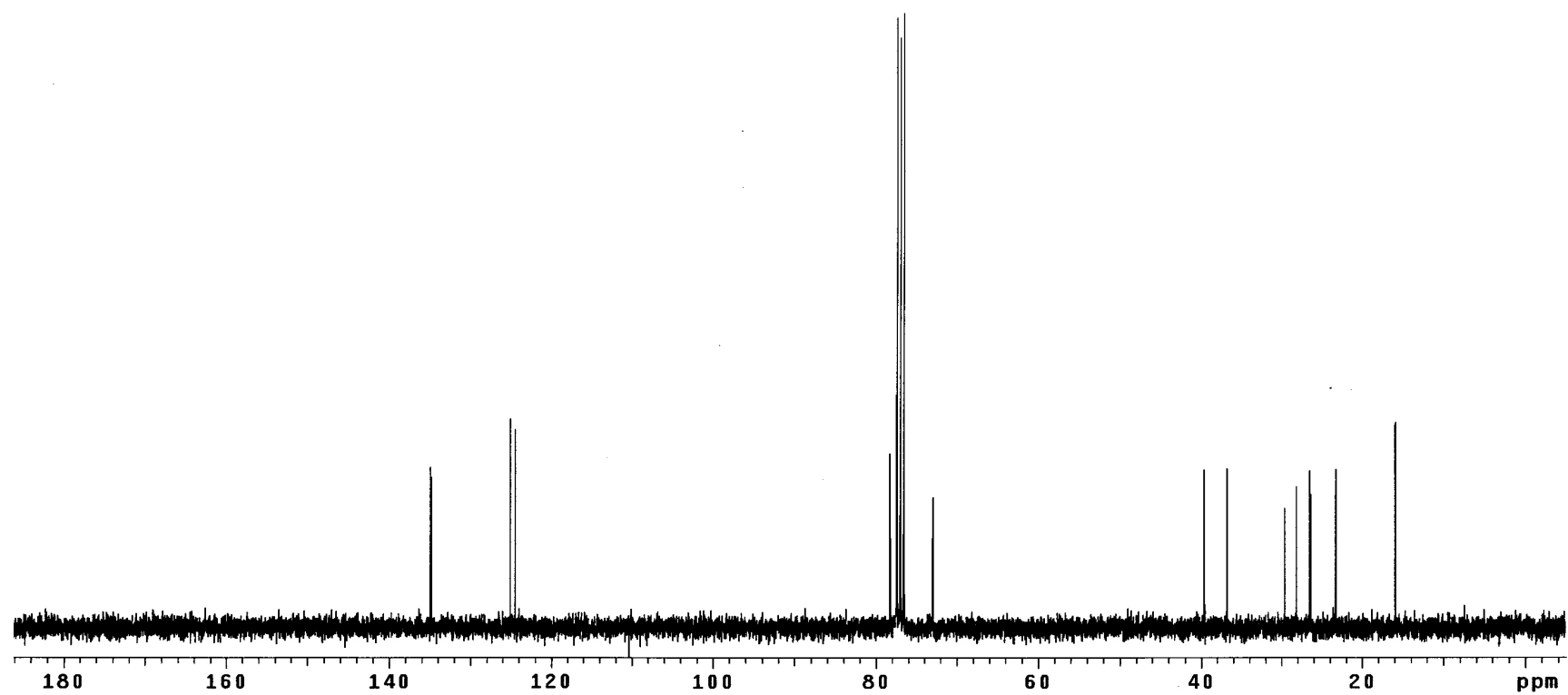


Figure S2. ^{13}C NMR spectrum of 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**1**) (75 MHz in CDCl_3).

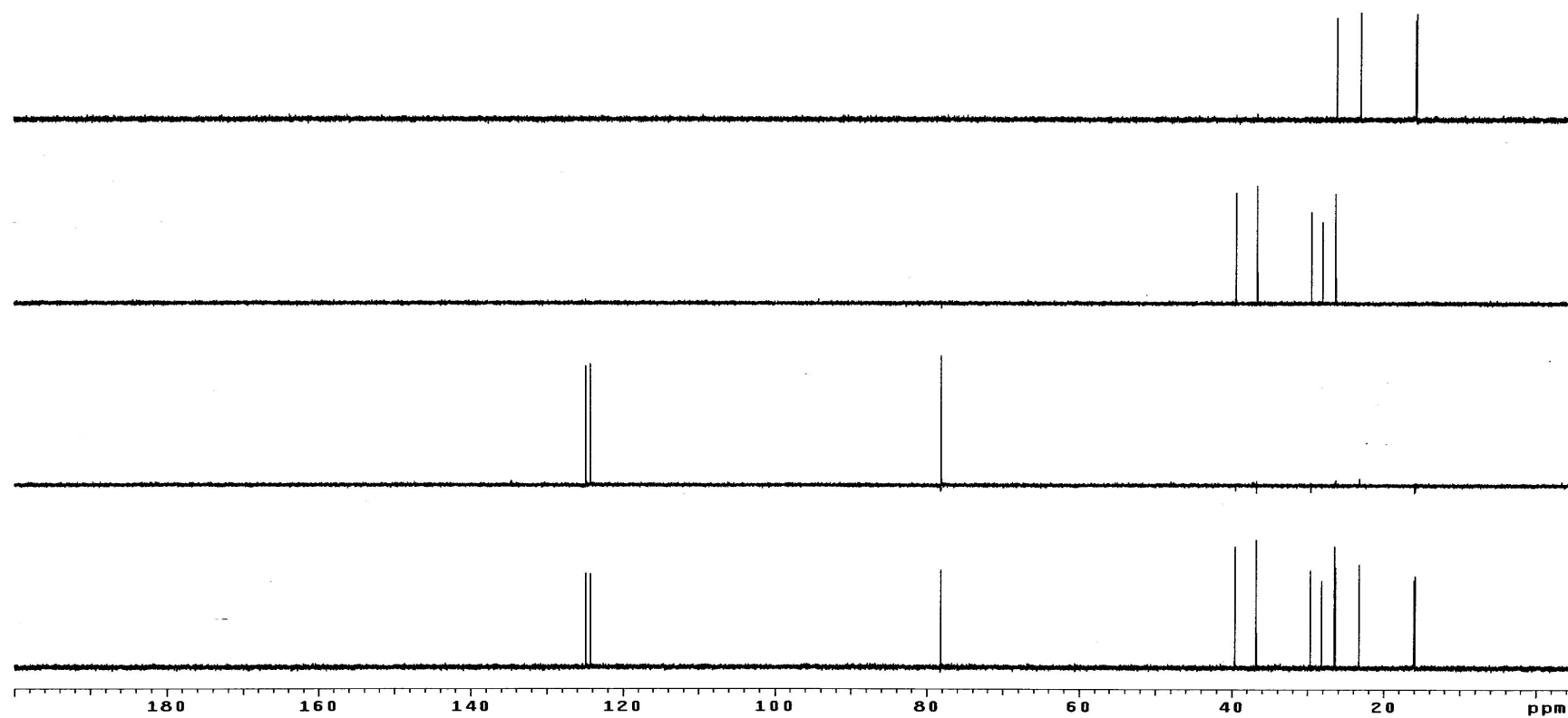


Figure S3. DEPT NMR spectrum of 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**1**) (75 MHz in CDCl₃).

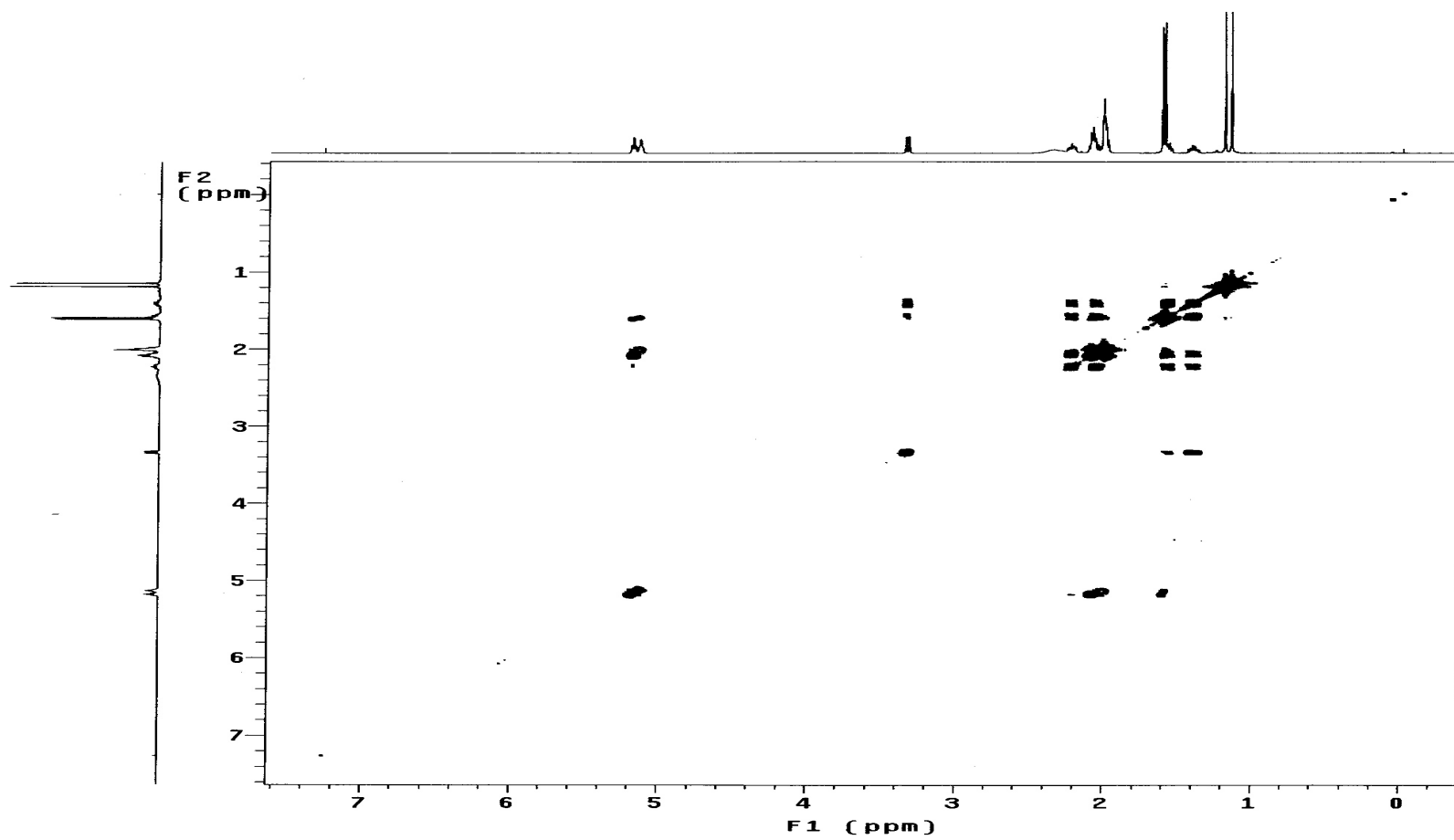


Figure S4. ^1H - ^1H COSY NMR spectrum of 2,3,22,23-tertrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (1).

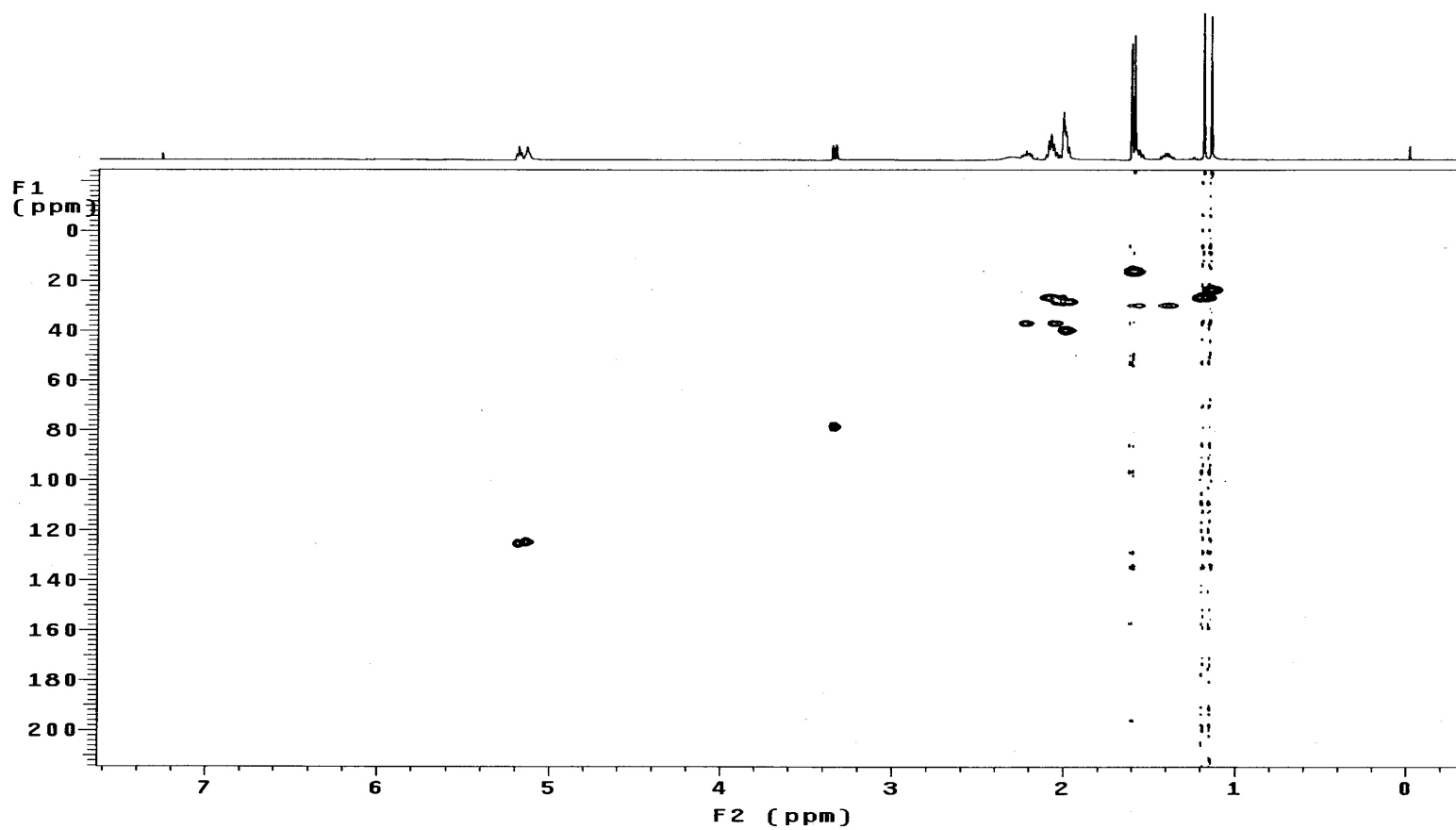


Figure S5. HMQC NMR spectrum of 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (1).

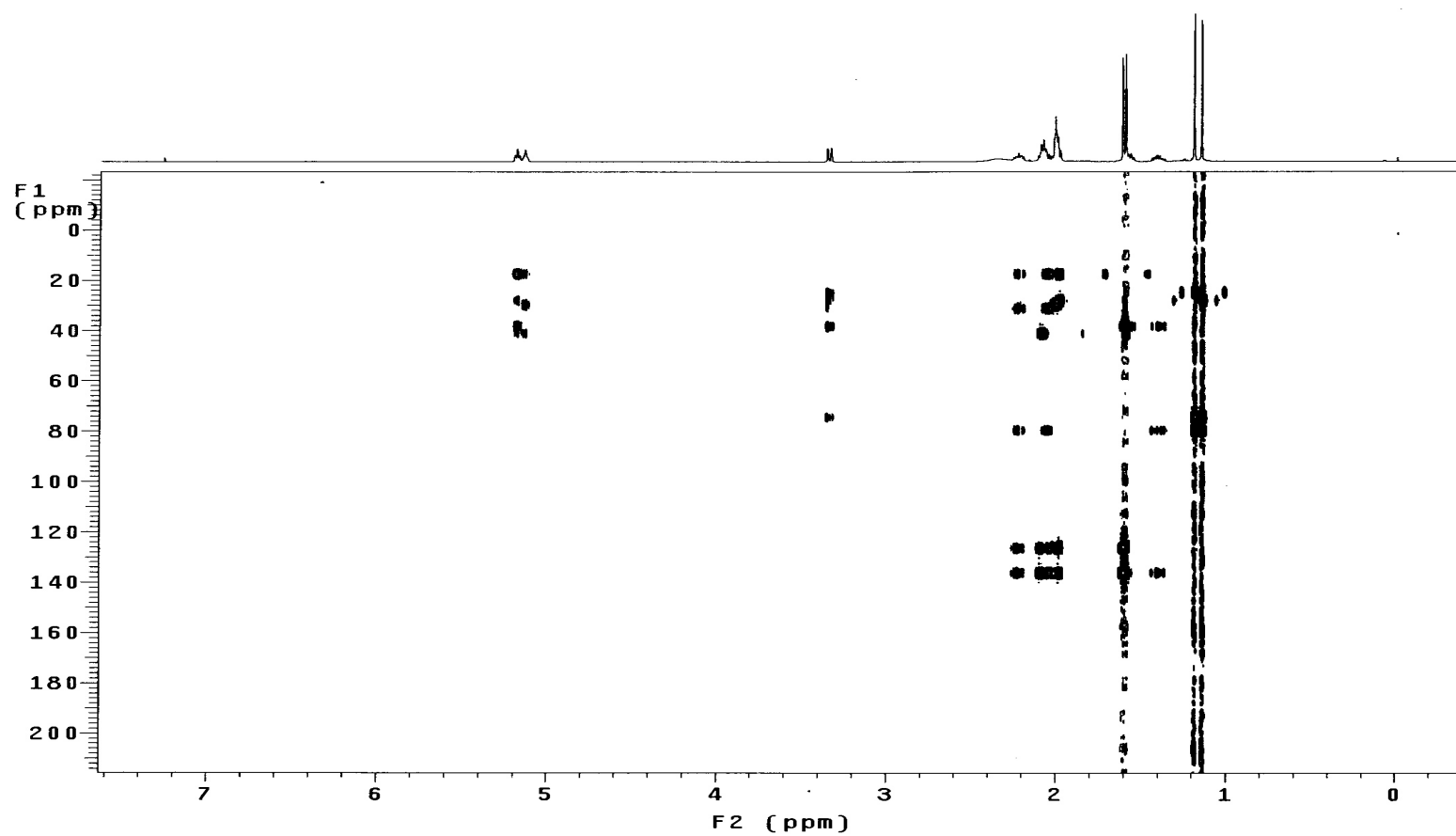


Figure S6. HMBC NMR spectrum of 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (1).

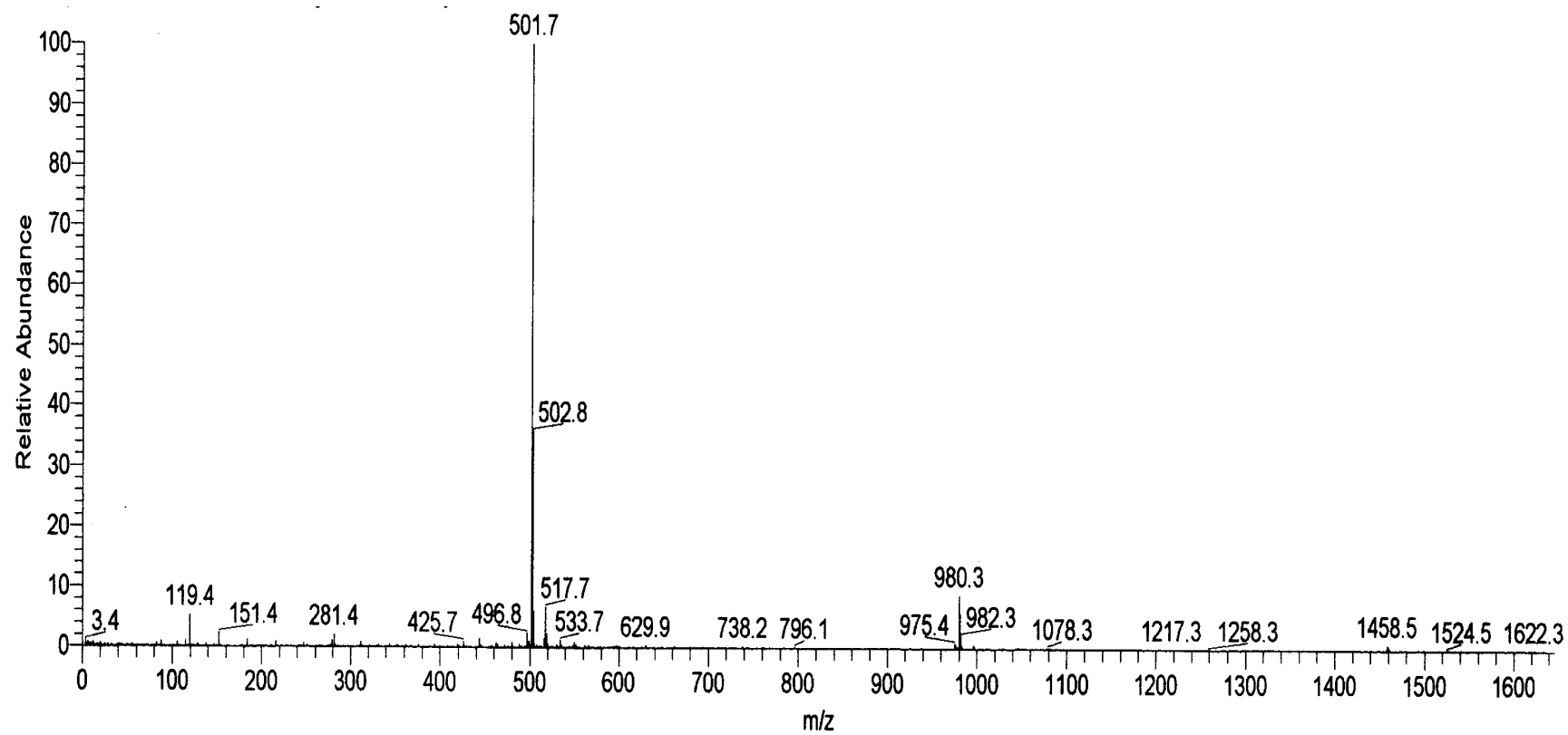


Figure S7. LRESIMS spectrum of 2,3,22,23-tertrahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (1).

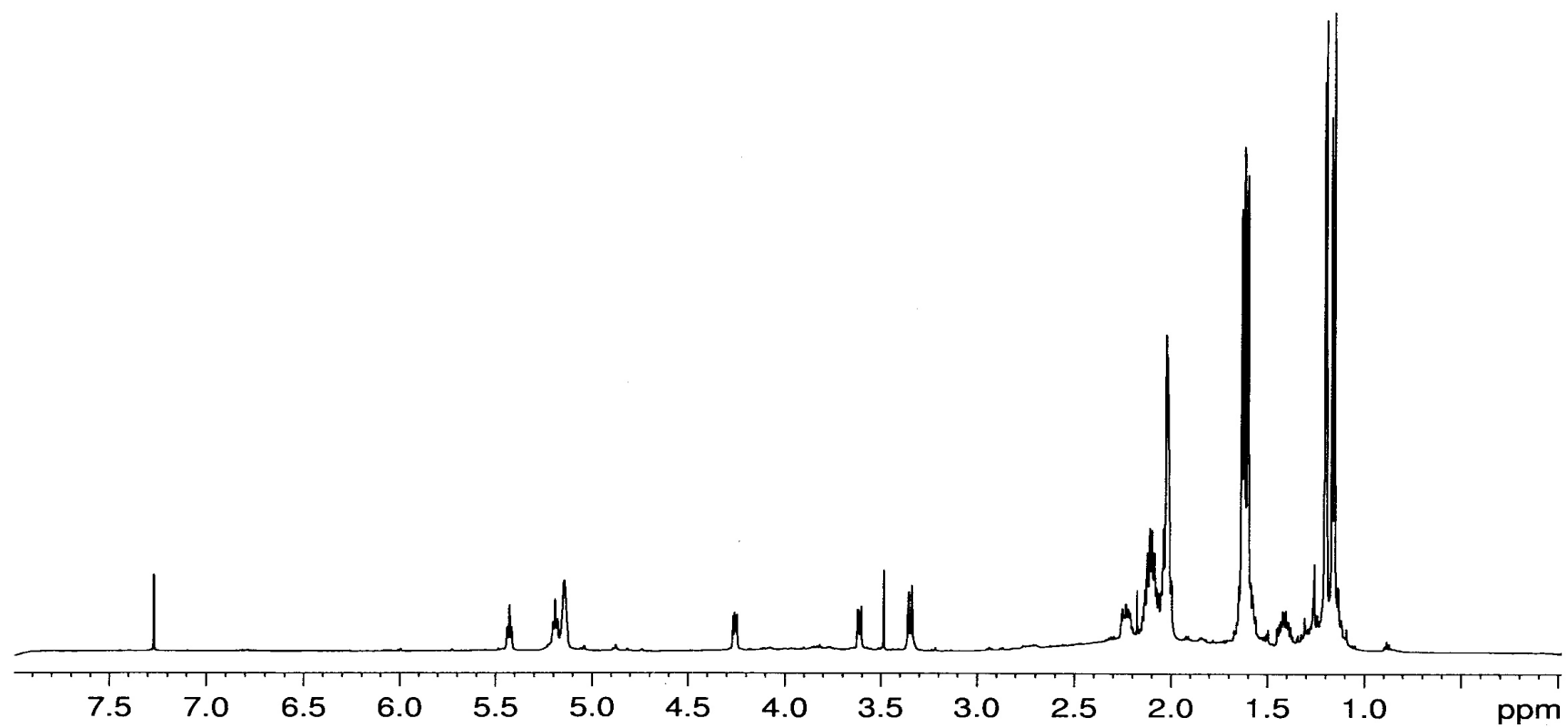


Figure S8. ^1H NMR spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**2**) (500 MHz in CDCl_3).

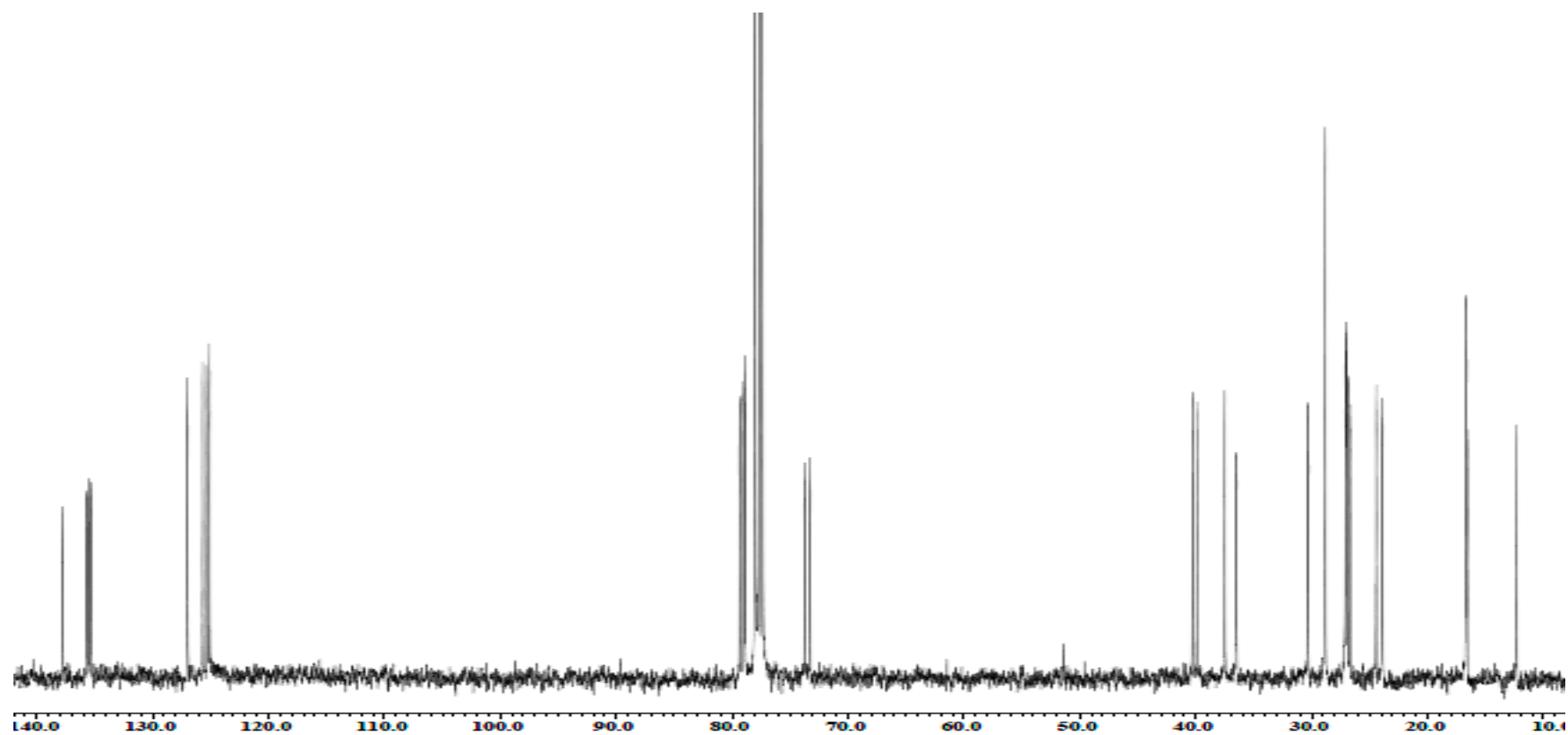


Figure S9. ^{13}C NMR spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**2**) (125 MHz in CDCl_3).

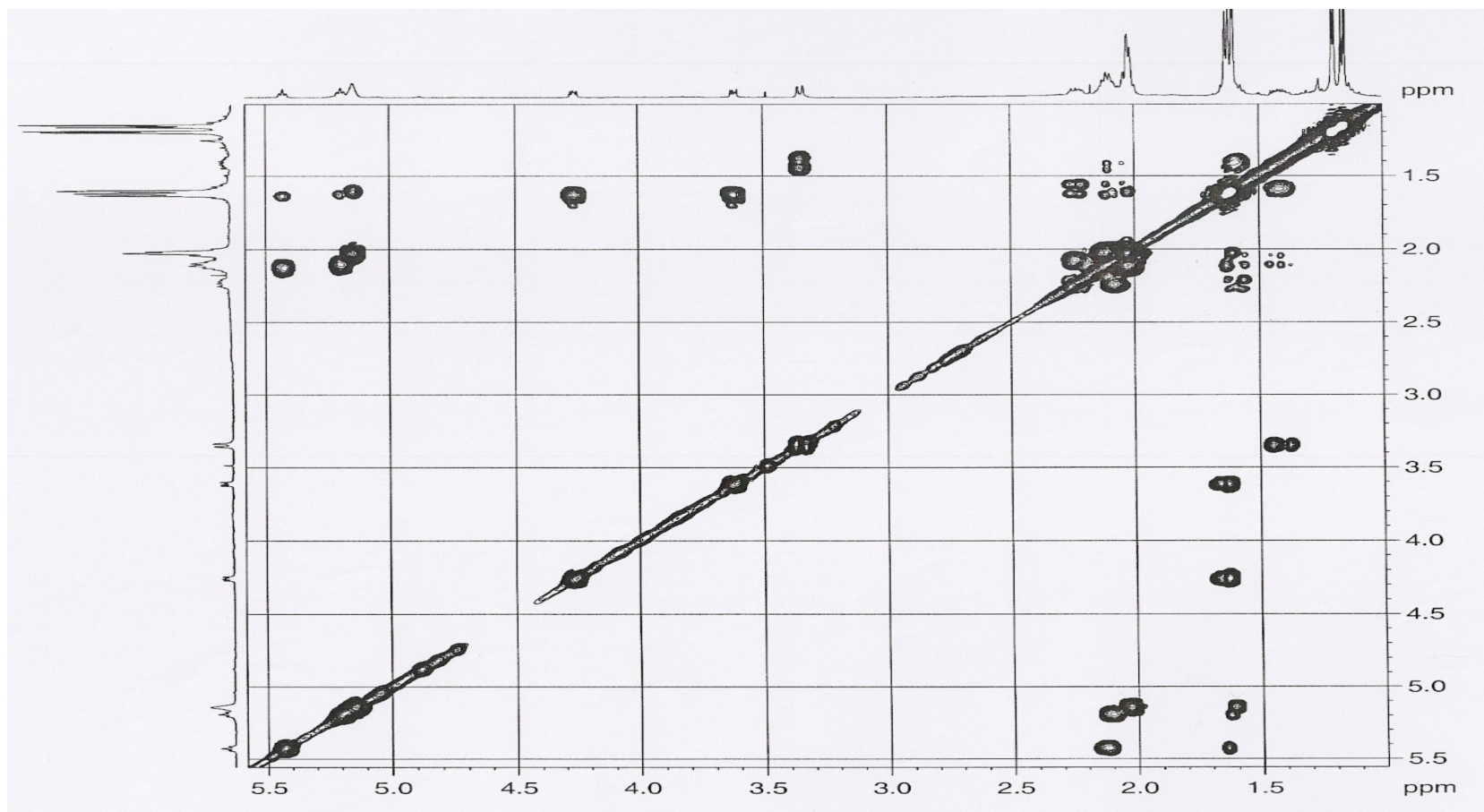


Figure S10. ^1H - ^1H COSY NMR spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (2).

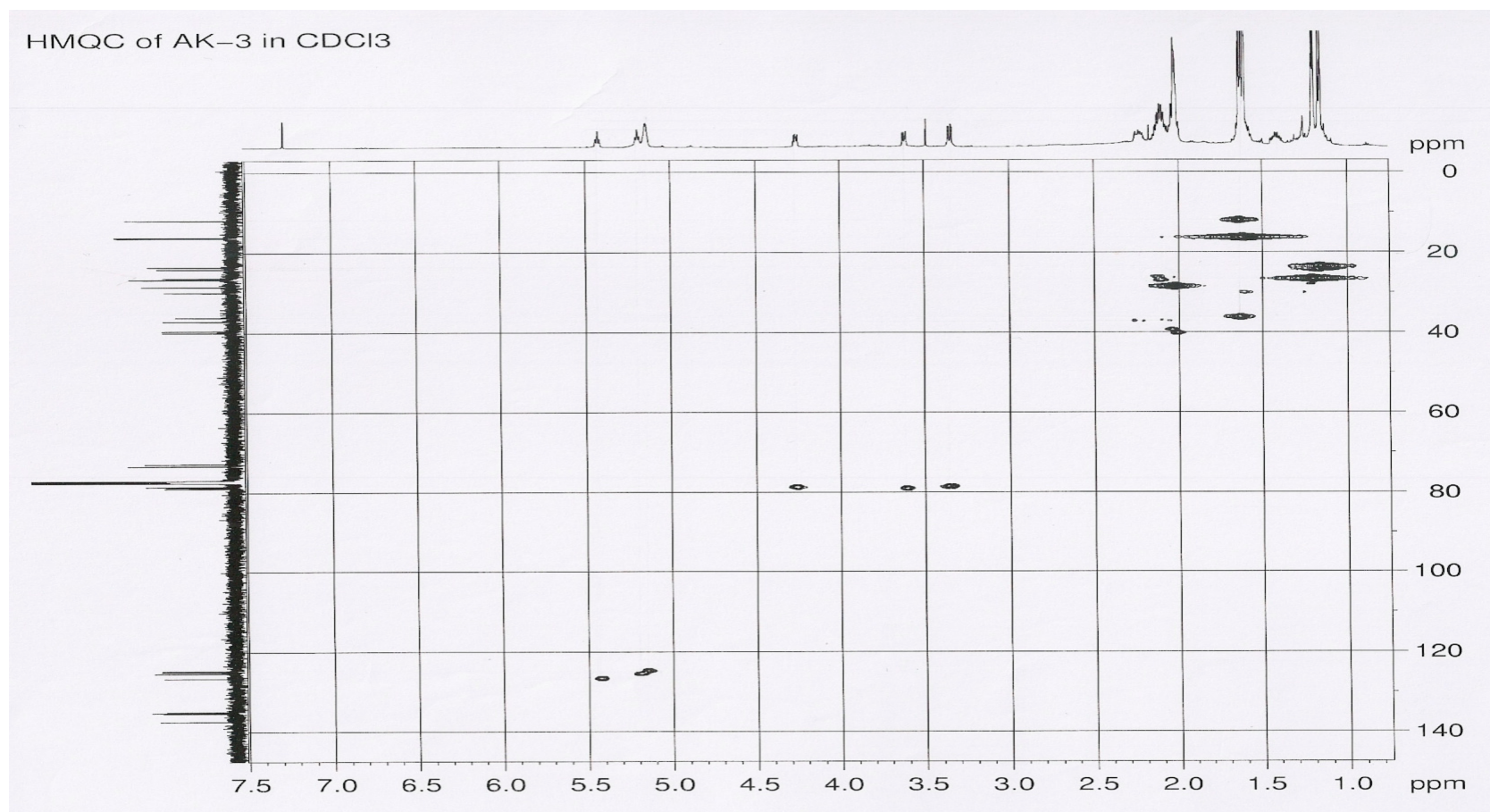


Figure S11. HMQC NMR spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (**2**).

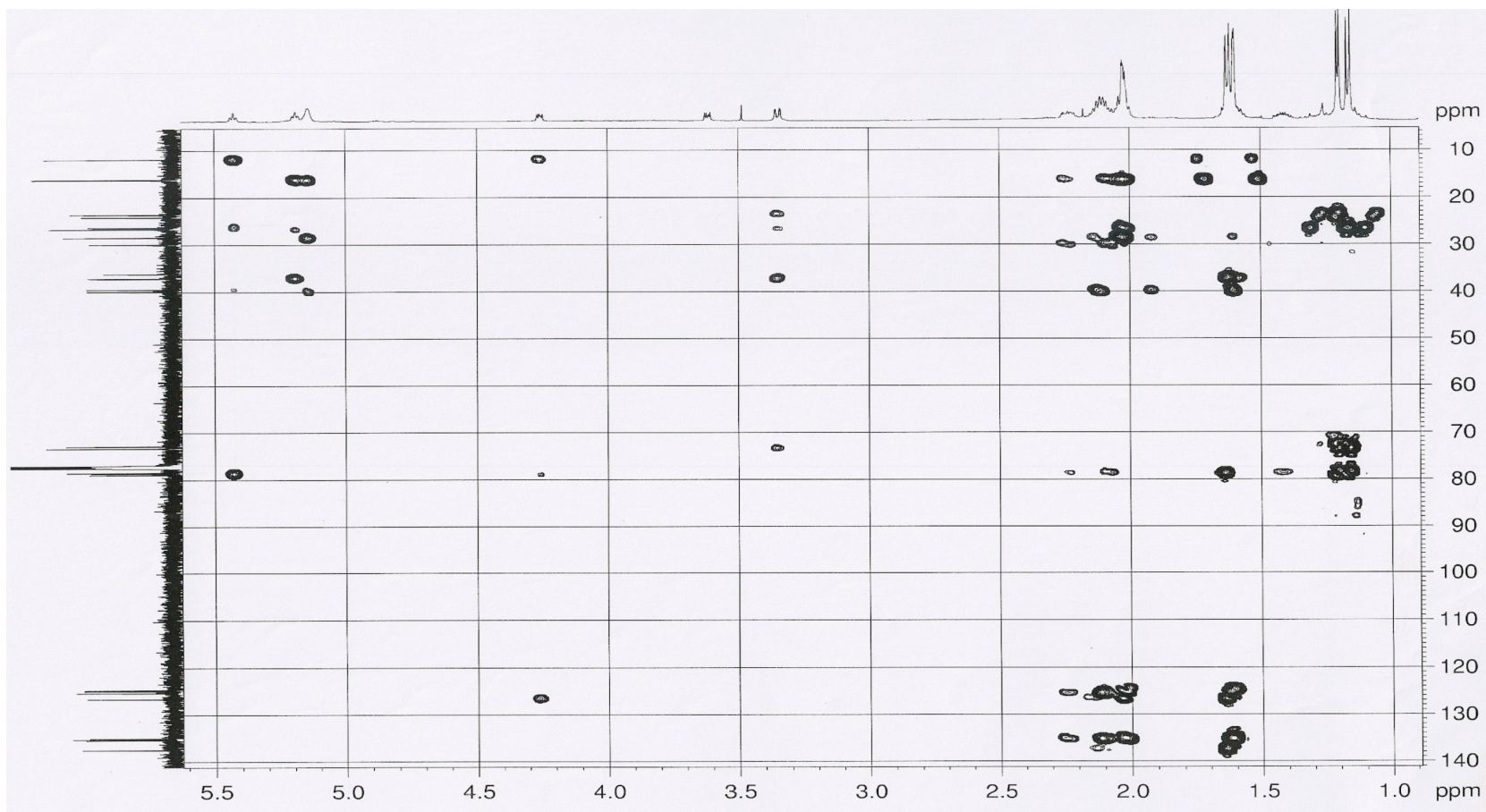


Figure S12. HMBC NMR spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (2).

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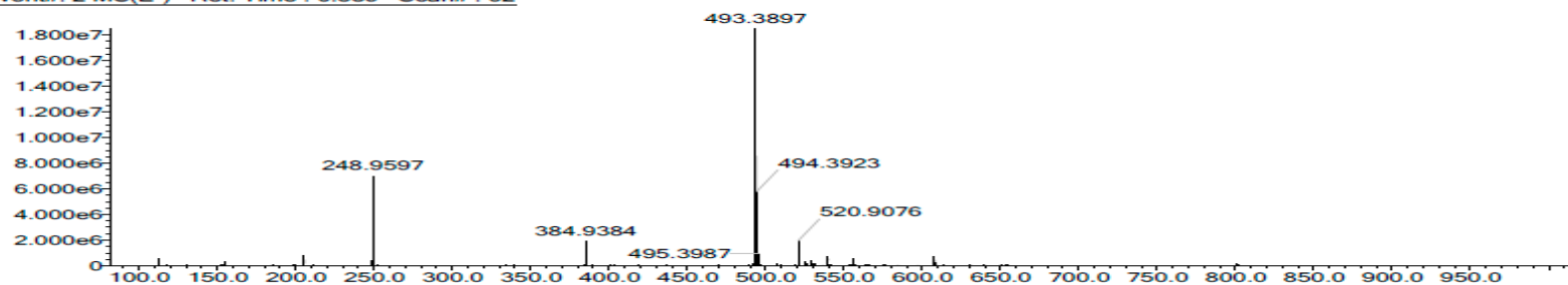
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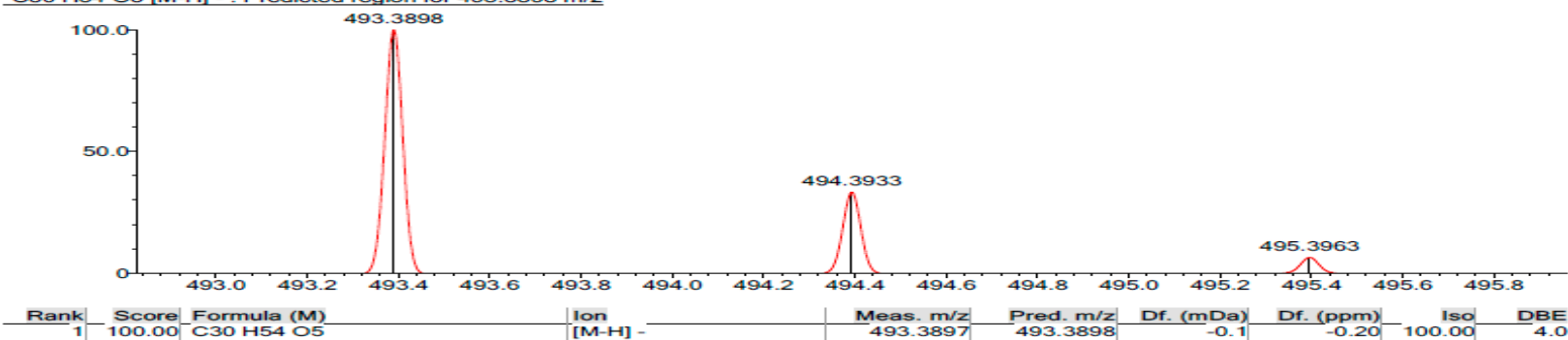


Figure S13. HRESIMS spectrum of 2,3,5,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-tetracos-6,10,14,18-tetraene (2).

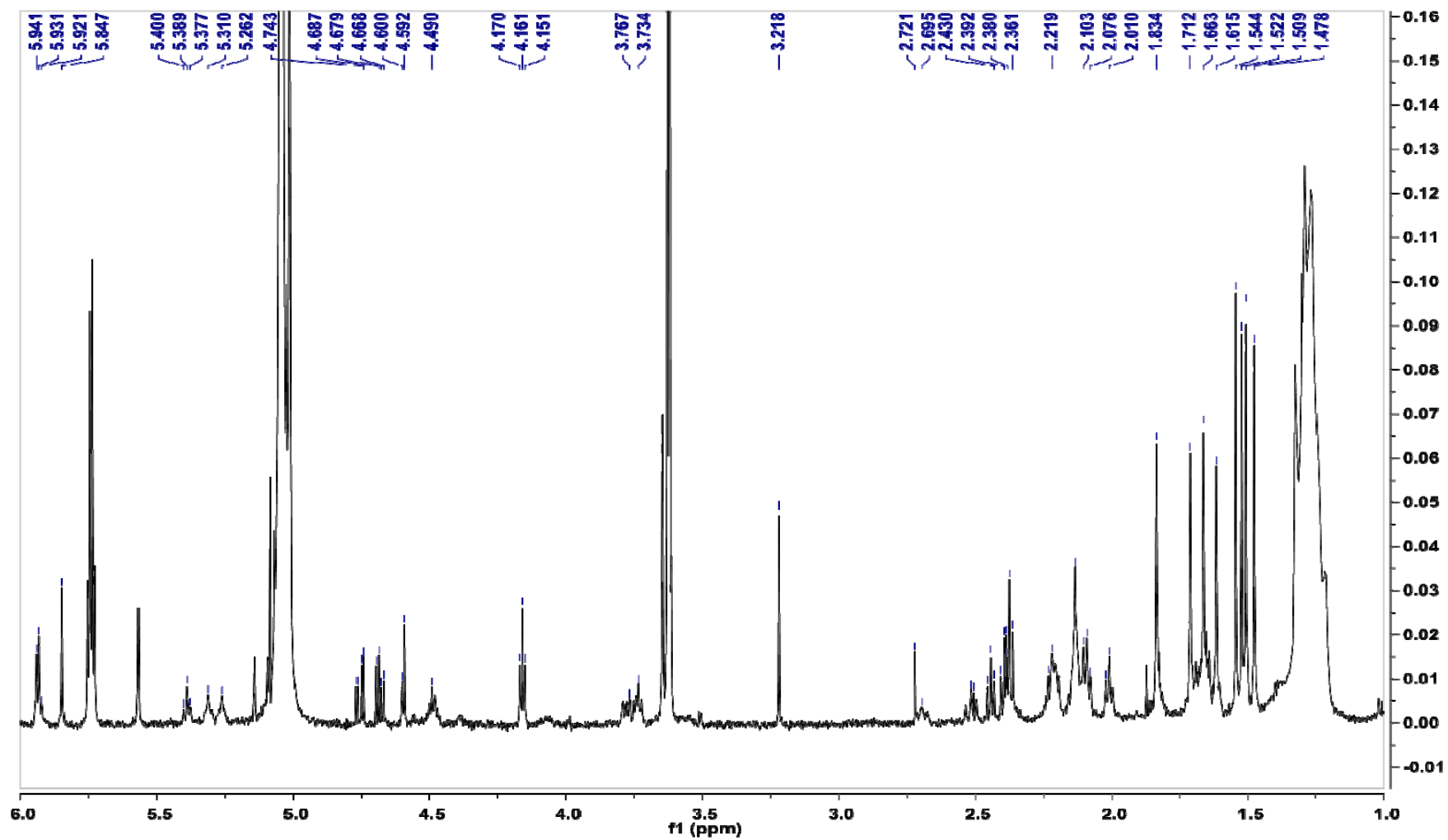


Figure S14. ^1H NMR spectrum of 5-mono-(*S*)-MTPA ester of 2 (2a) (600 MHz in $\text{pyridine-}d_5$).

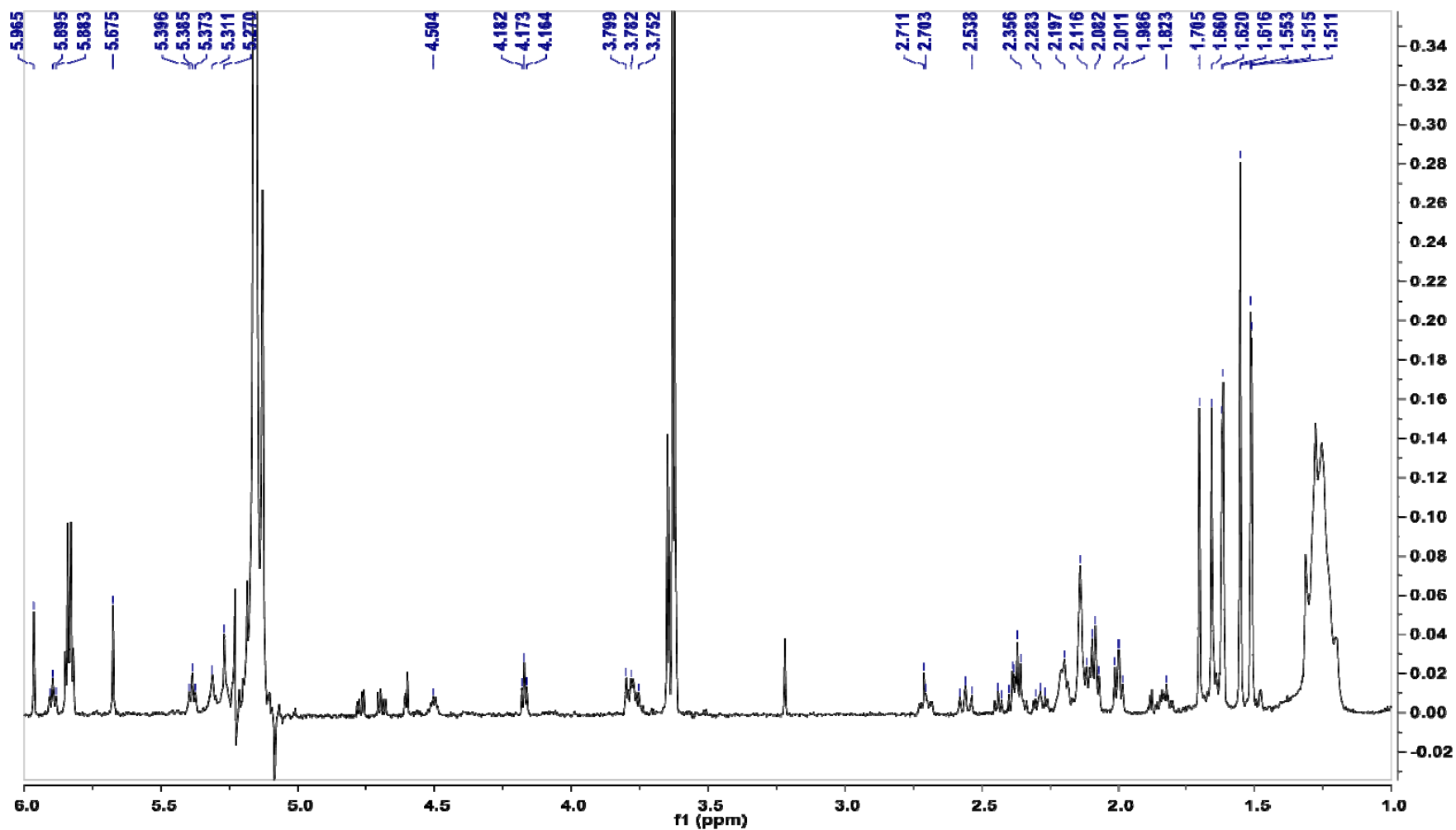


Figure S15. ^1H NMR spectrum of 5-mono-(*R*)-MTPA ester of **2** (**2c**) (600 MHz in pyridine- d_5).

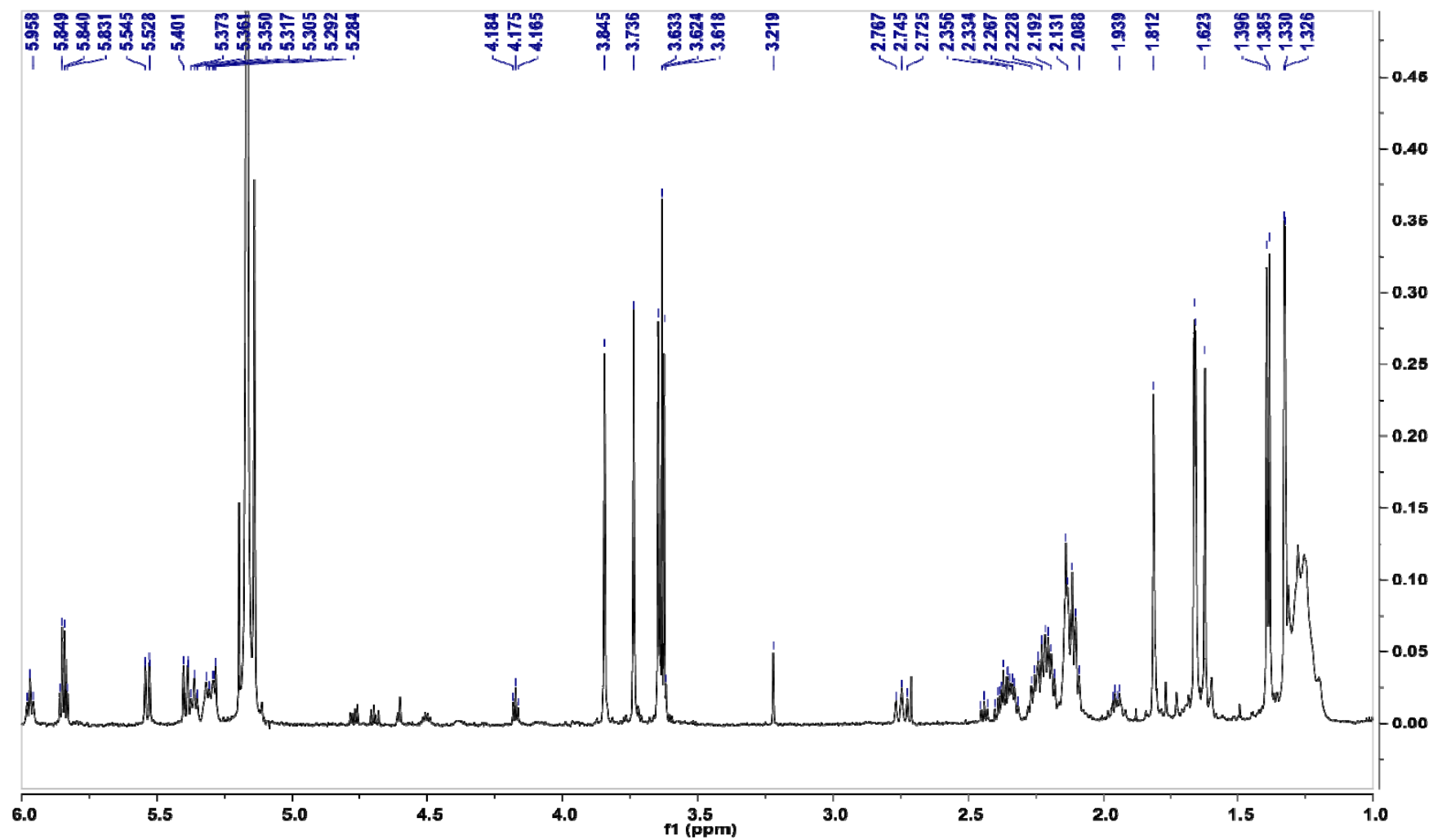


Figure S16. ^1H NMR spectrum of 3,5,22-tris-(*S*)-MTPA ester of 2 (**2b**) (600 MHz in $\text{pyridine-}d_5$).

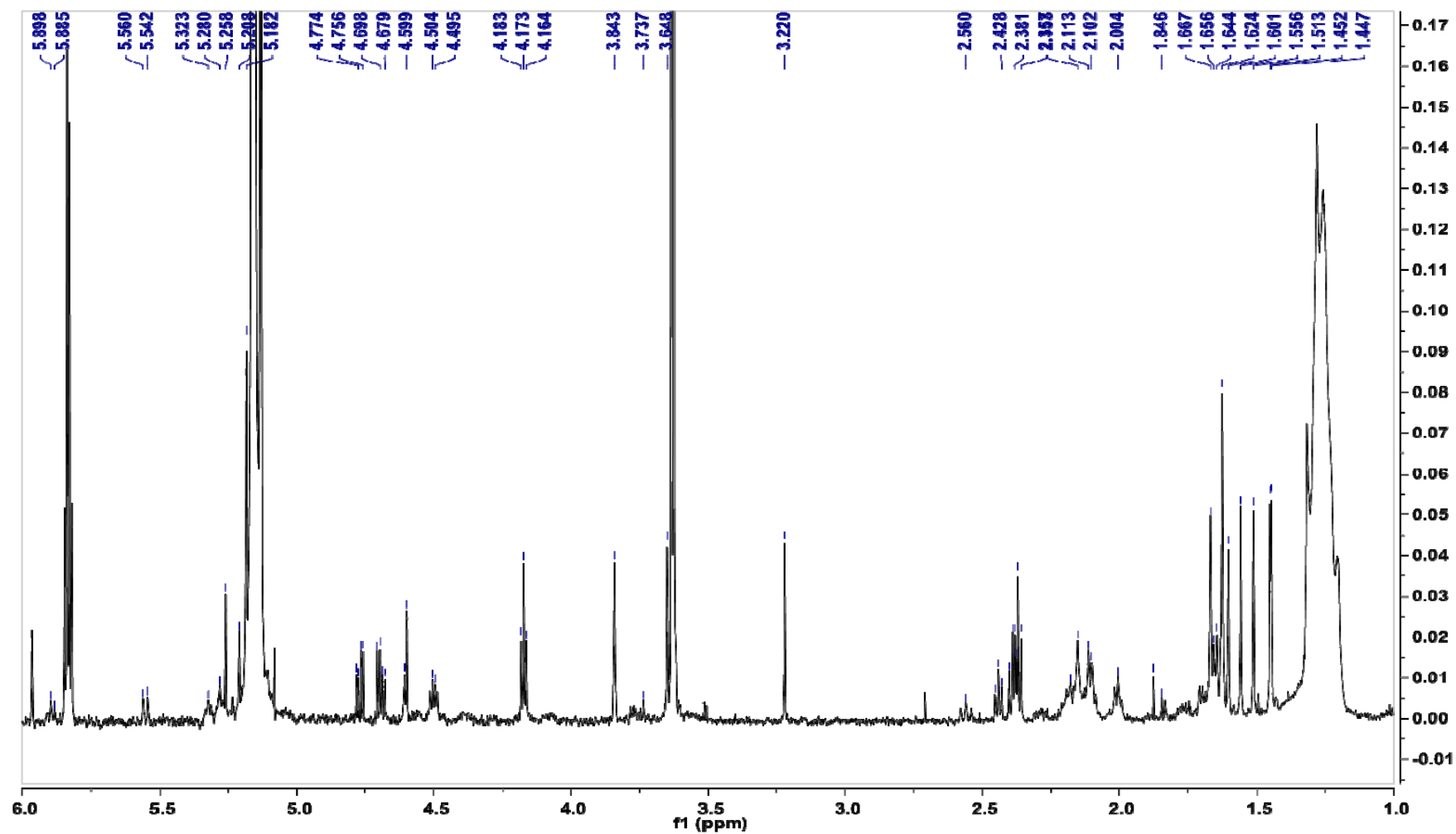


Figure S17. ^1H NMR spectrum of 3,5,22-tris-(*R*)-MTPA ester of 2 (2d) (600 MHz in $\text{pyridine-}d_5$).

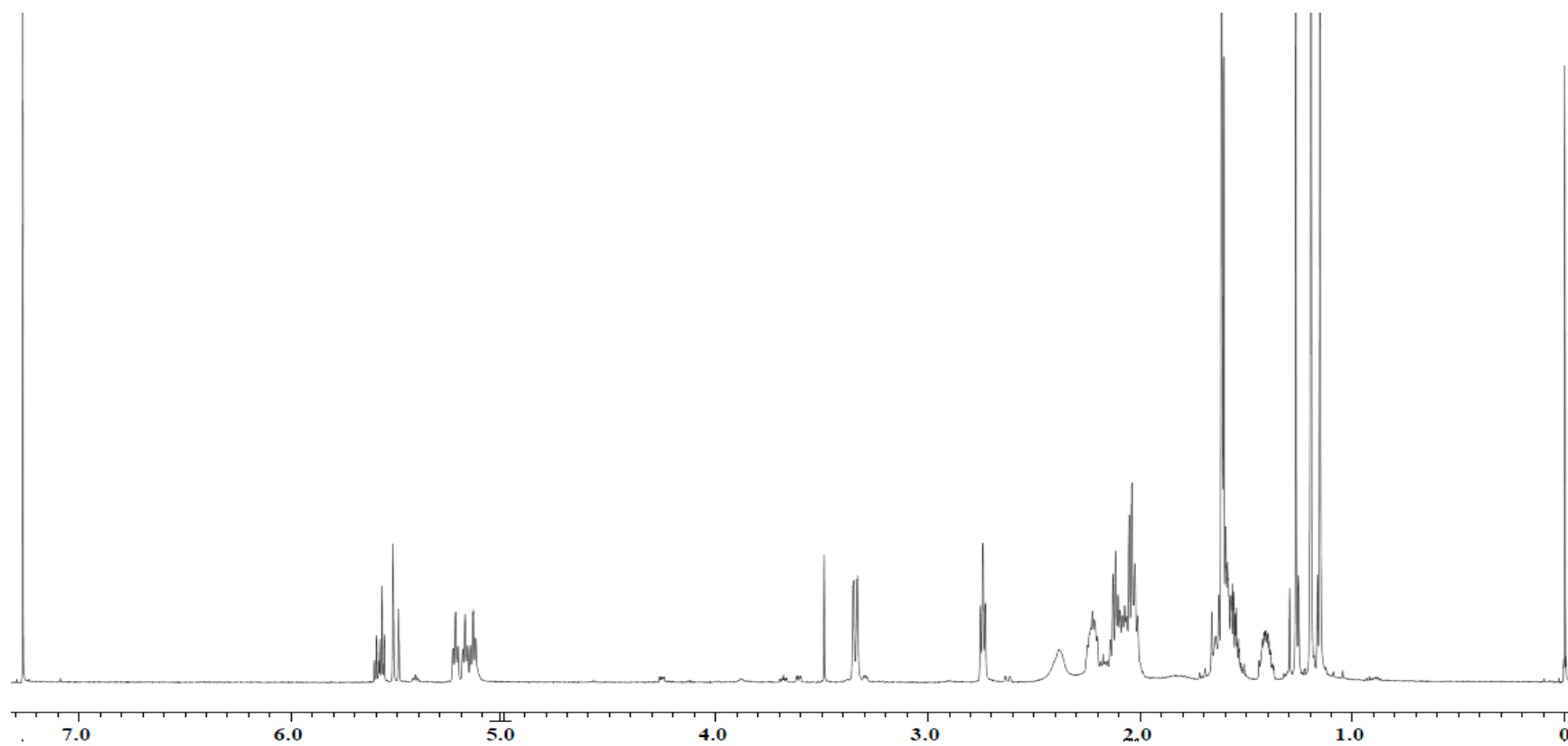


Figure S18. ^1H NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (3) (600 MHz in CDCl_3).

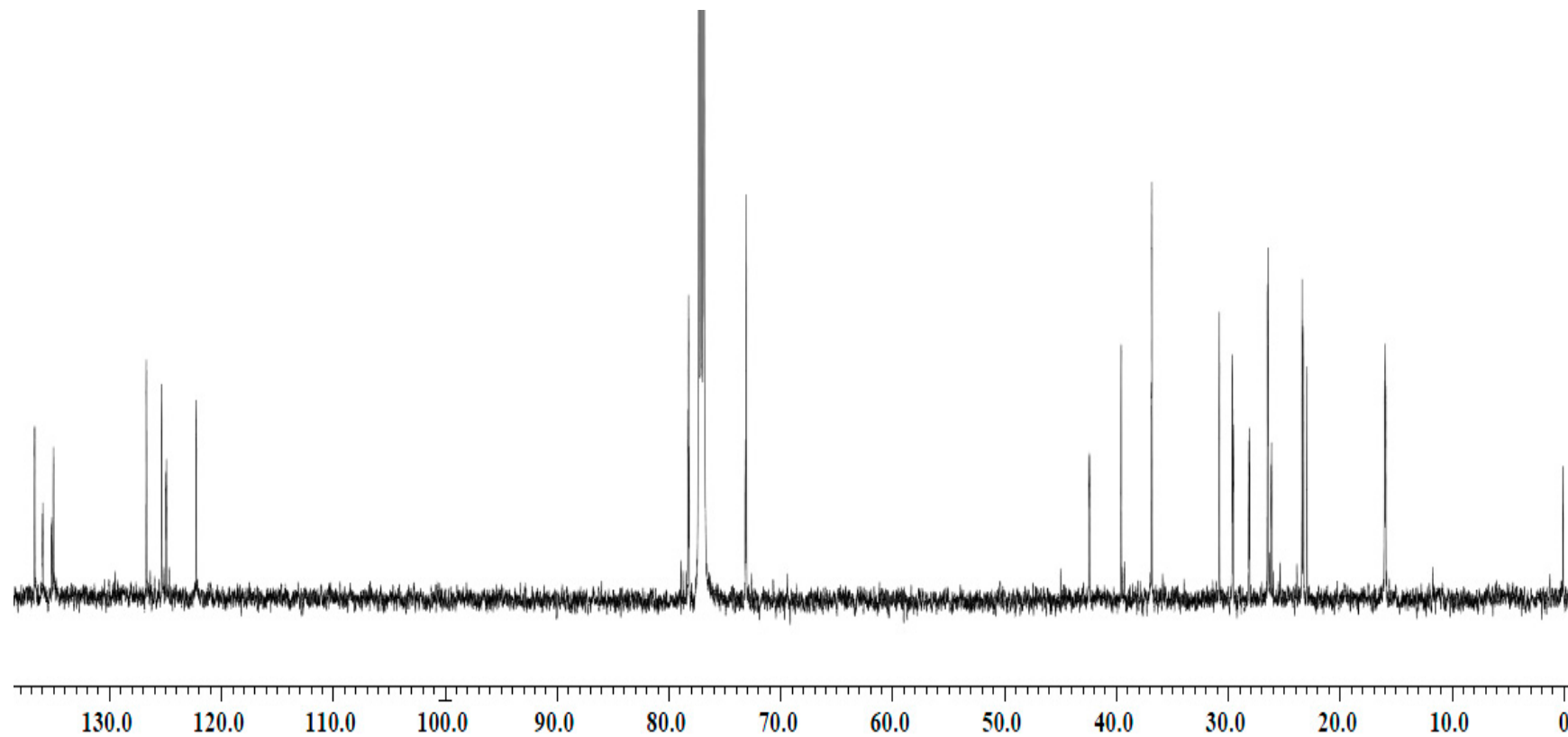


Figure S19. ^{13}C NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (**3**) (150 MHz in CDCl_3).

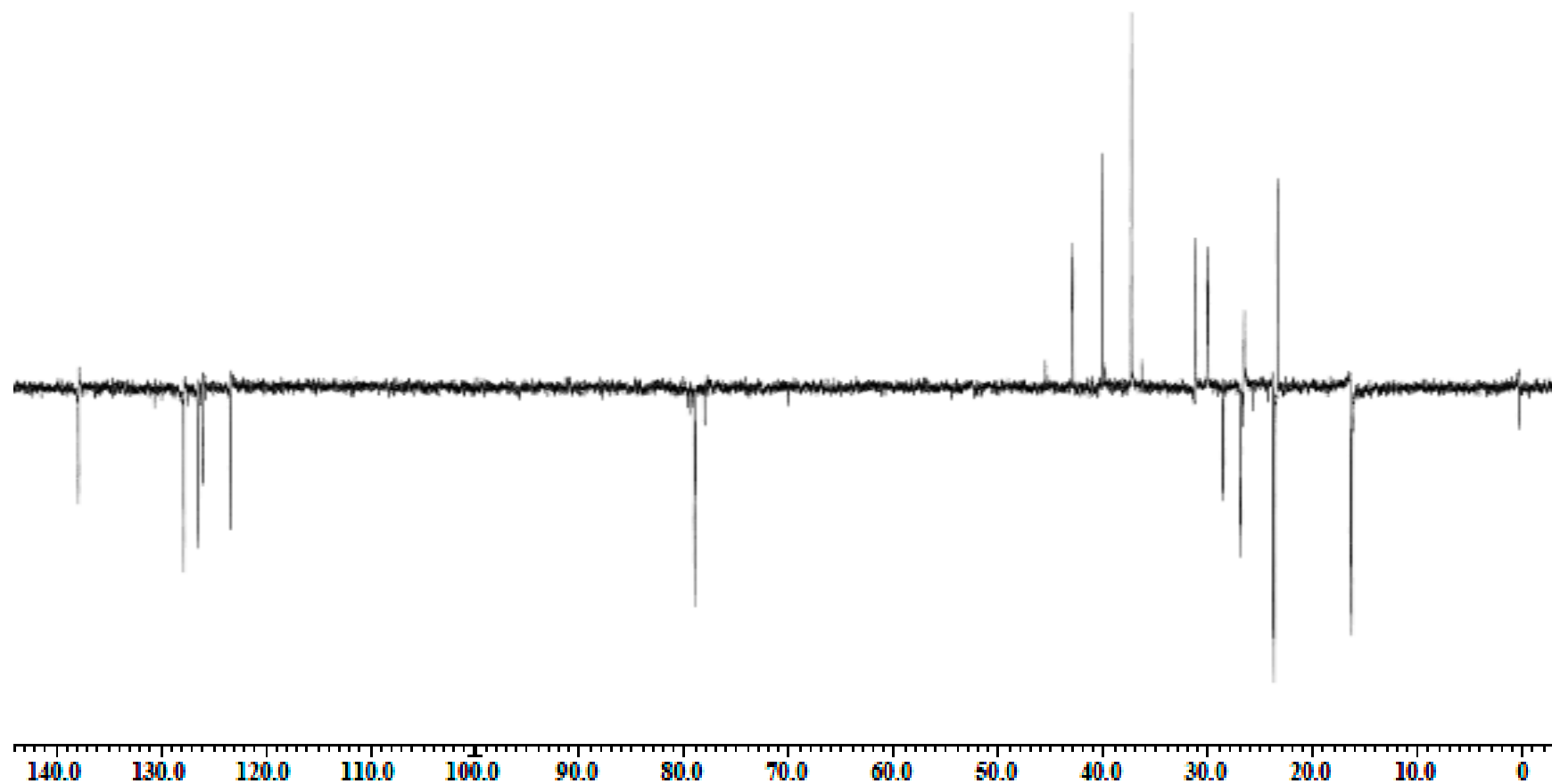


Figure S20. DEPT-135 NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (**3**) (150 MHz in CDCl₃).

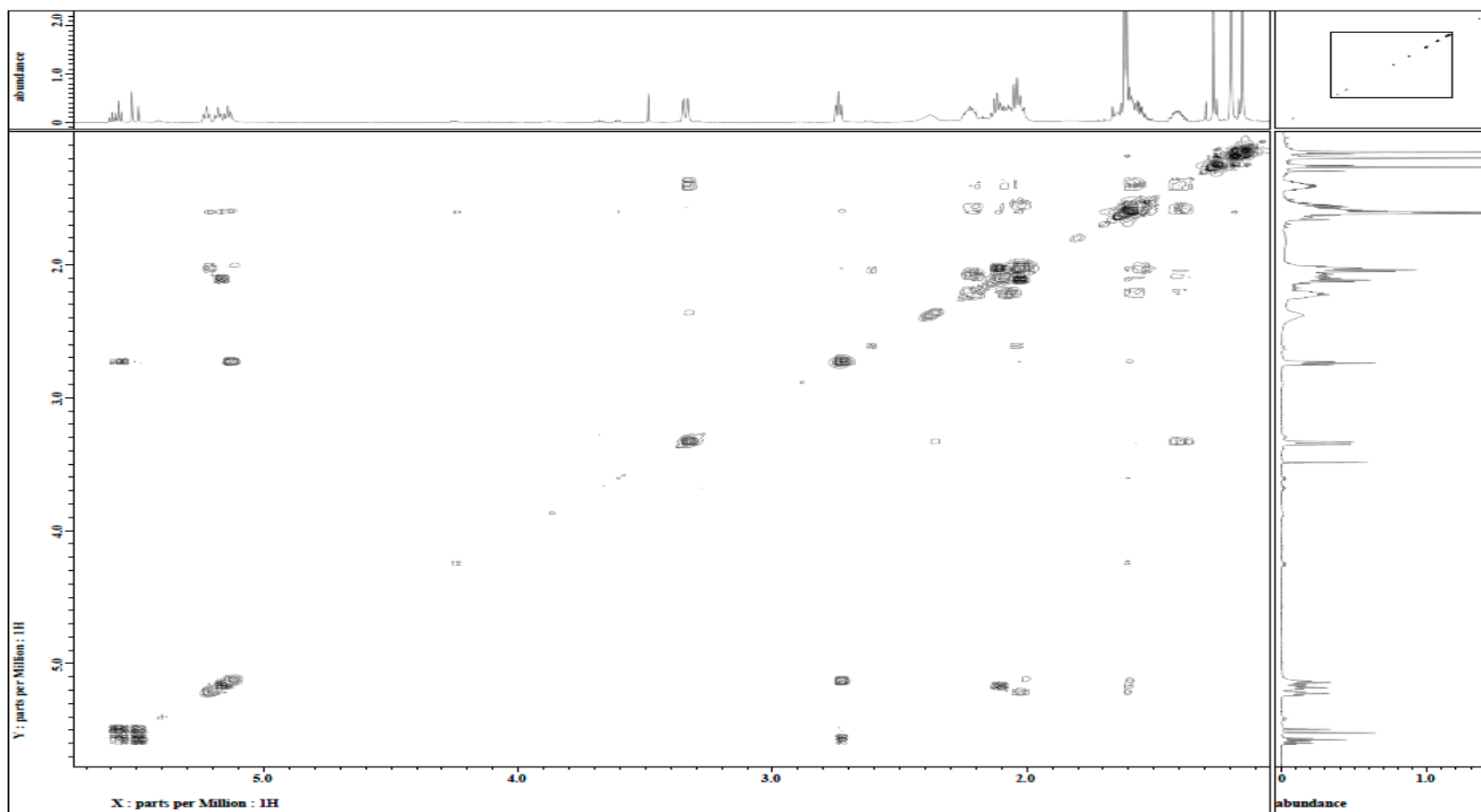


Figure S21. ^1H - ^1H COSY NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (3).

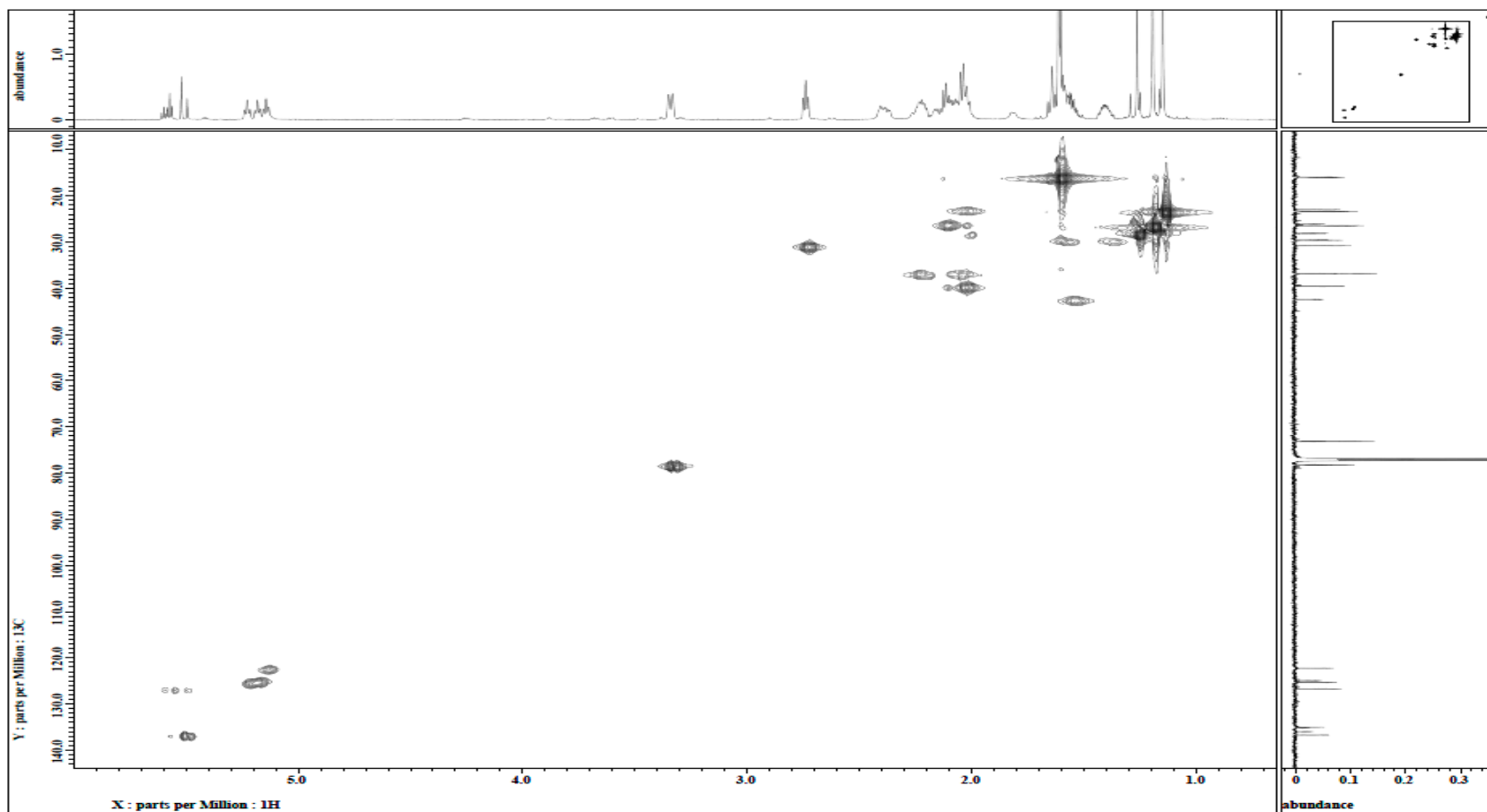


Figure S22. HSQC NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (3).

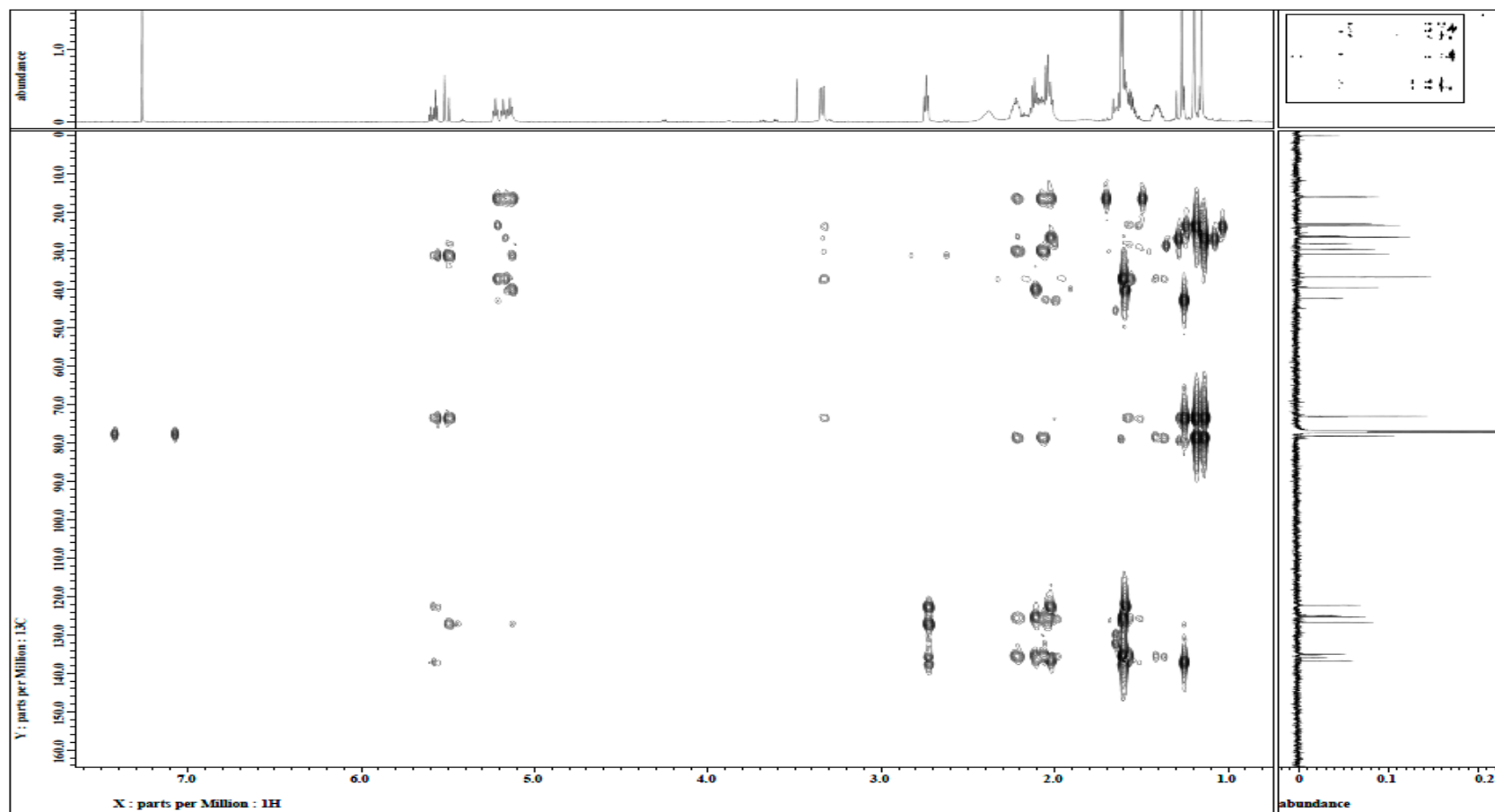


Figure S23. HMBC NMR spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (3).

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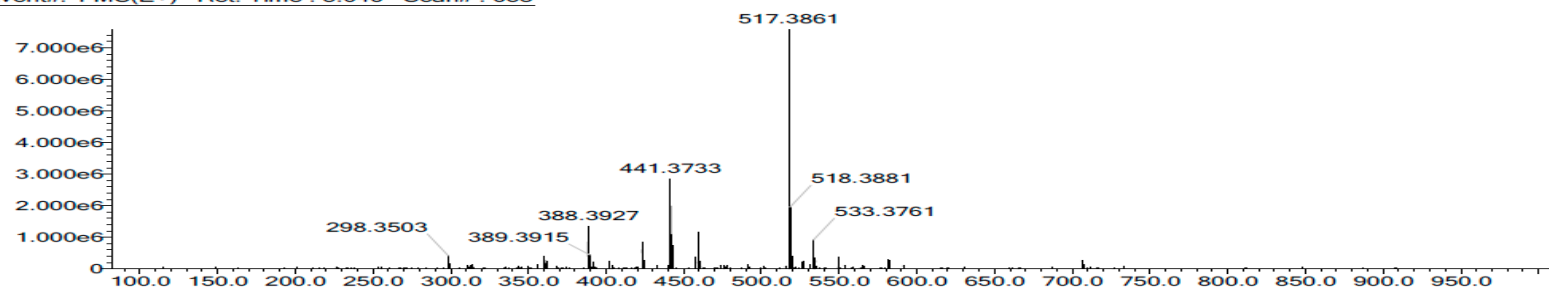
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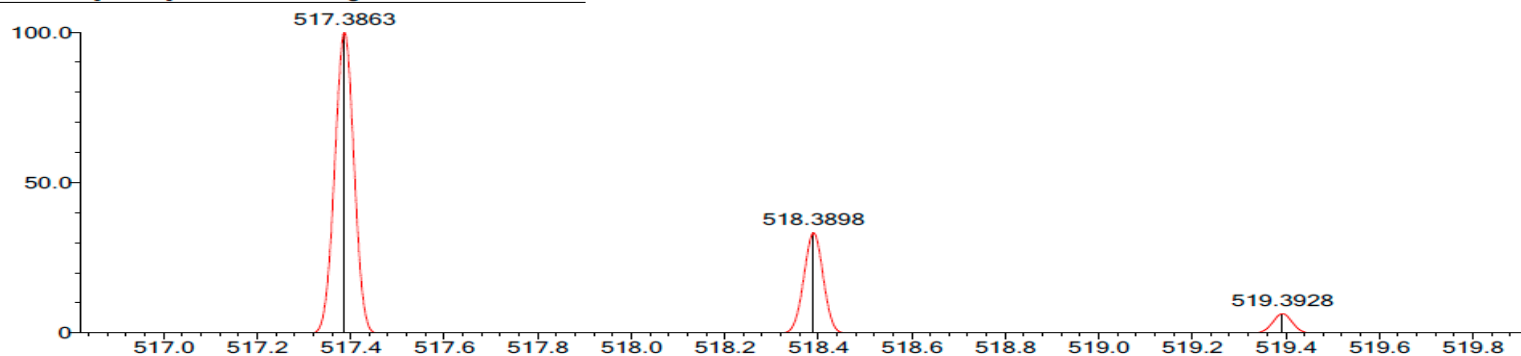
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C30 H54 O5 [M+Na] + : Predicted region for 517.3863 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
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Figure S24. HRESIMS spectrum of 2,3,6,22,23-Pentahydroxy-2,6,11,15,19,23-hexamethyl-tetracos-7,10,14,18-tetraene (3).

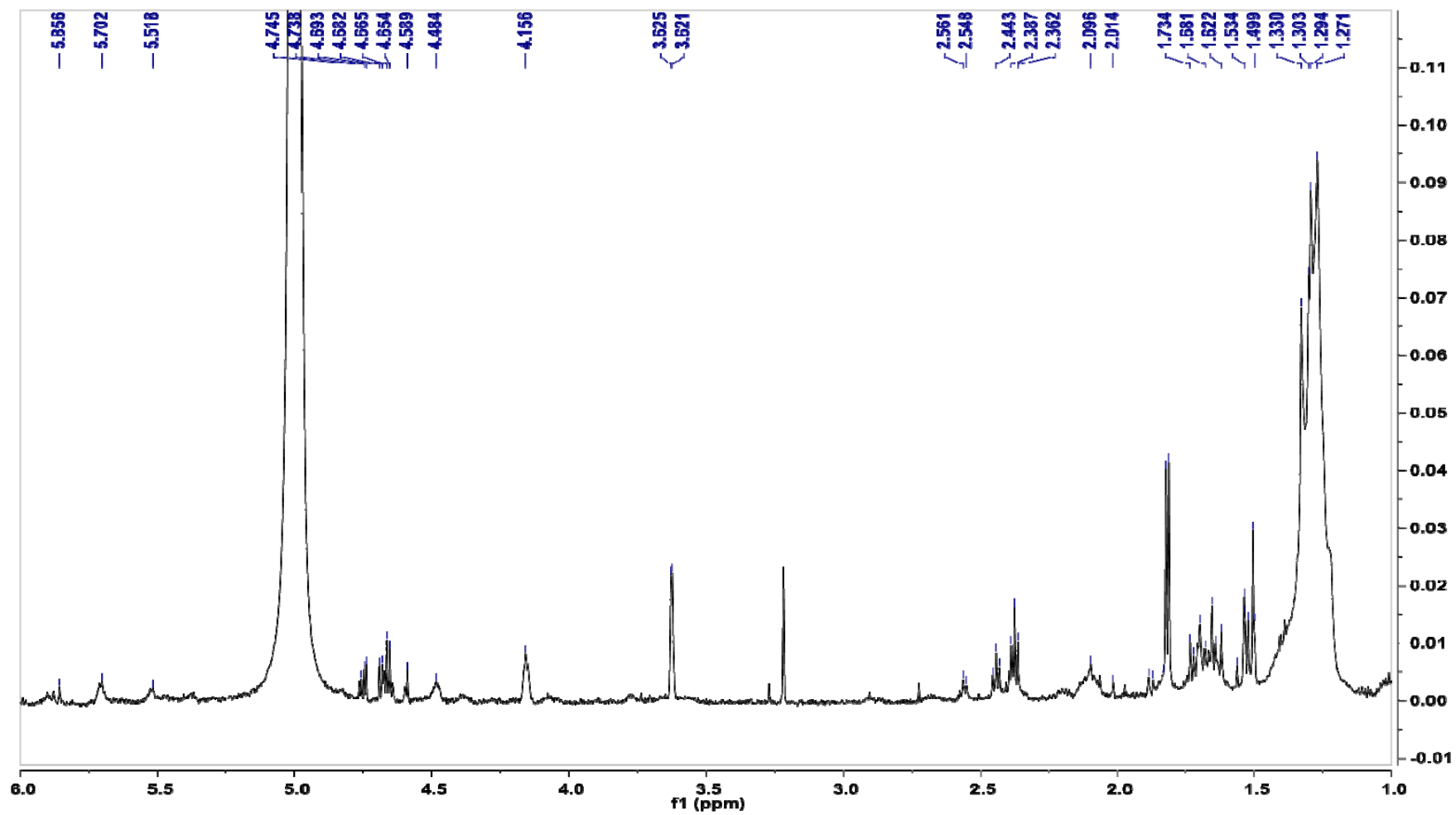


Figure S25. ^1H NMR spectrum of 3,22-bis-(*S*)-MTPA ester of **3** (**3a**) (600 MHz in pyridine- d_5).

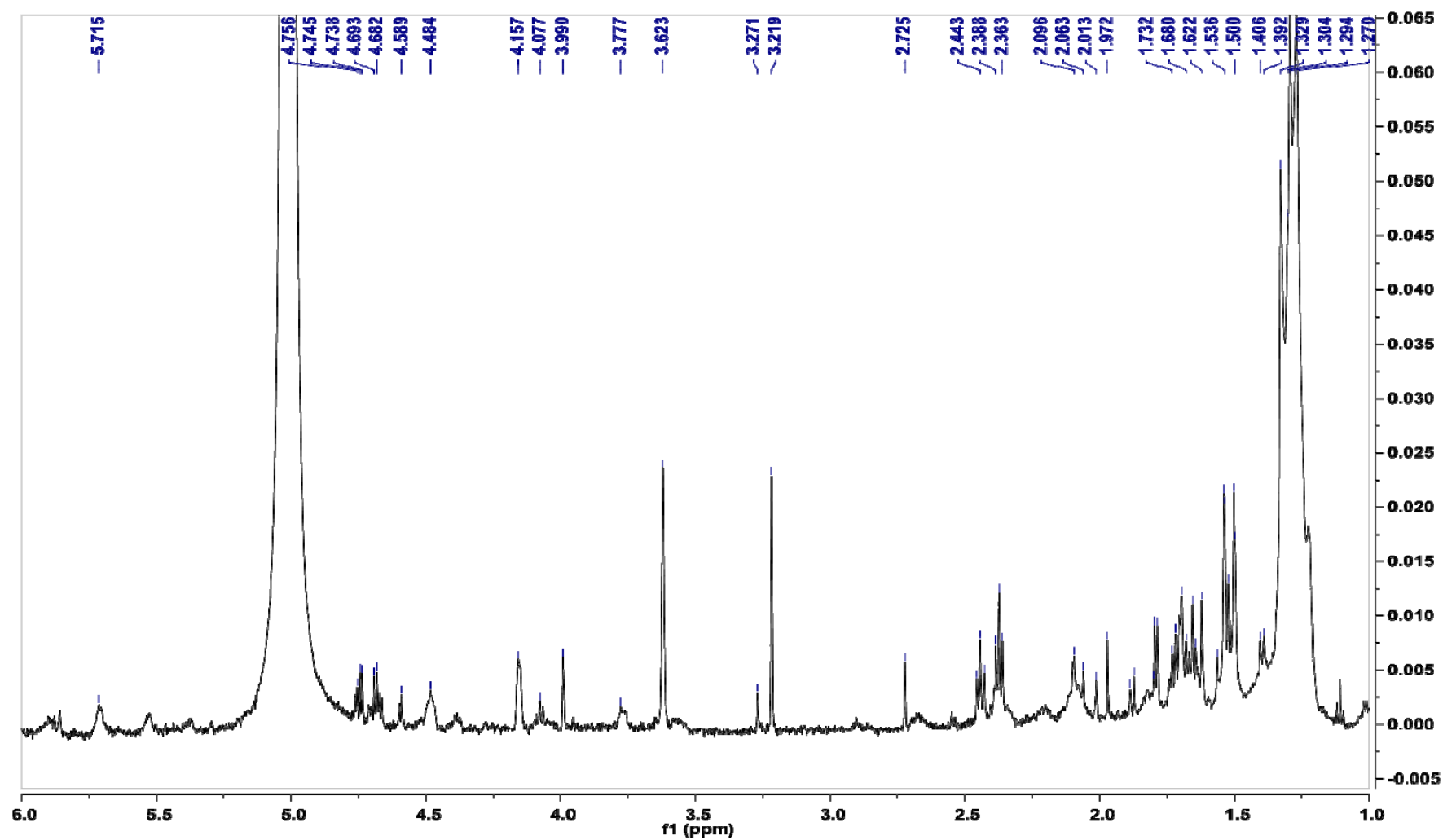


Figure S26. ^1H NMR spectrum of 3,22-bis-(*R*)-MTPA ester of **3** (**3b**) (600 MHz in pyridine- d_5).

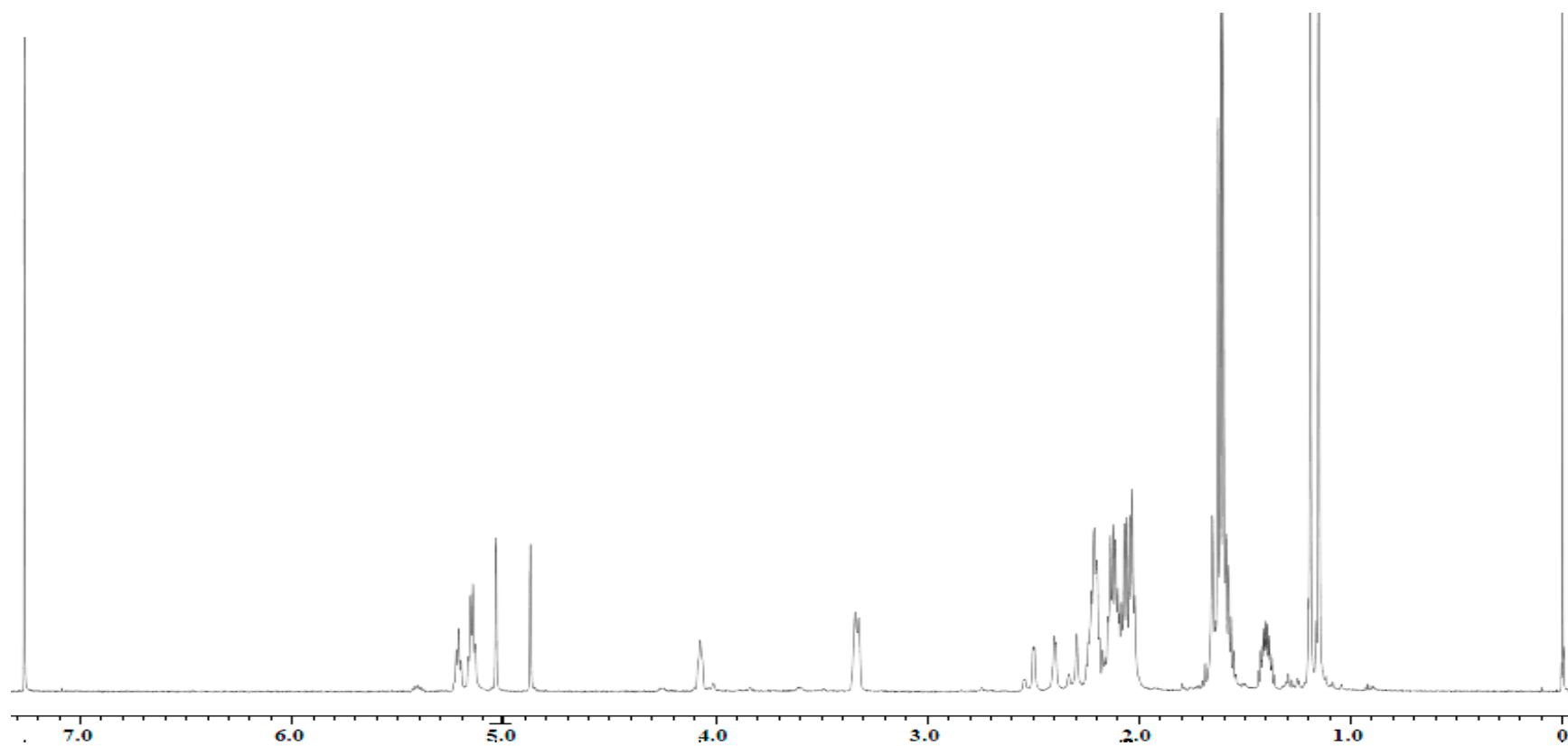


Figure S27. ^1H NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetrasa-10,14,18-triene (**4**) (600 MHz in CDCl_3).

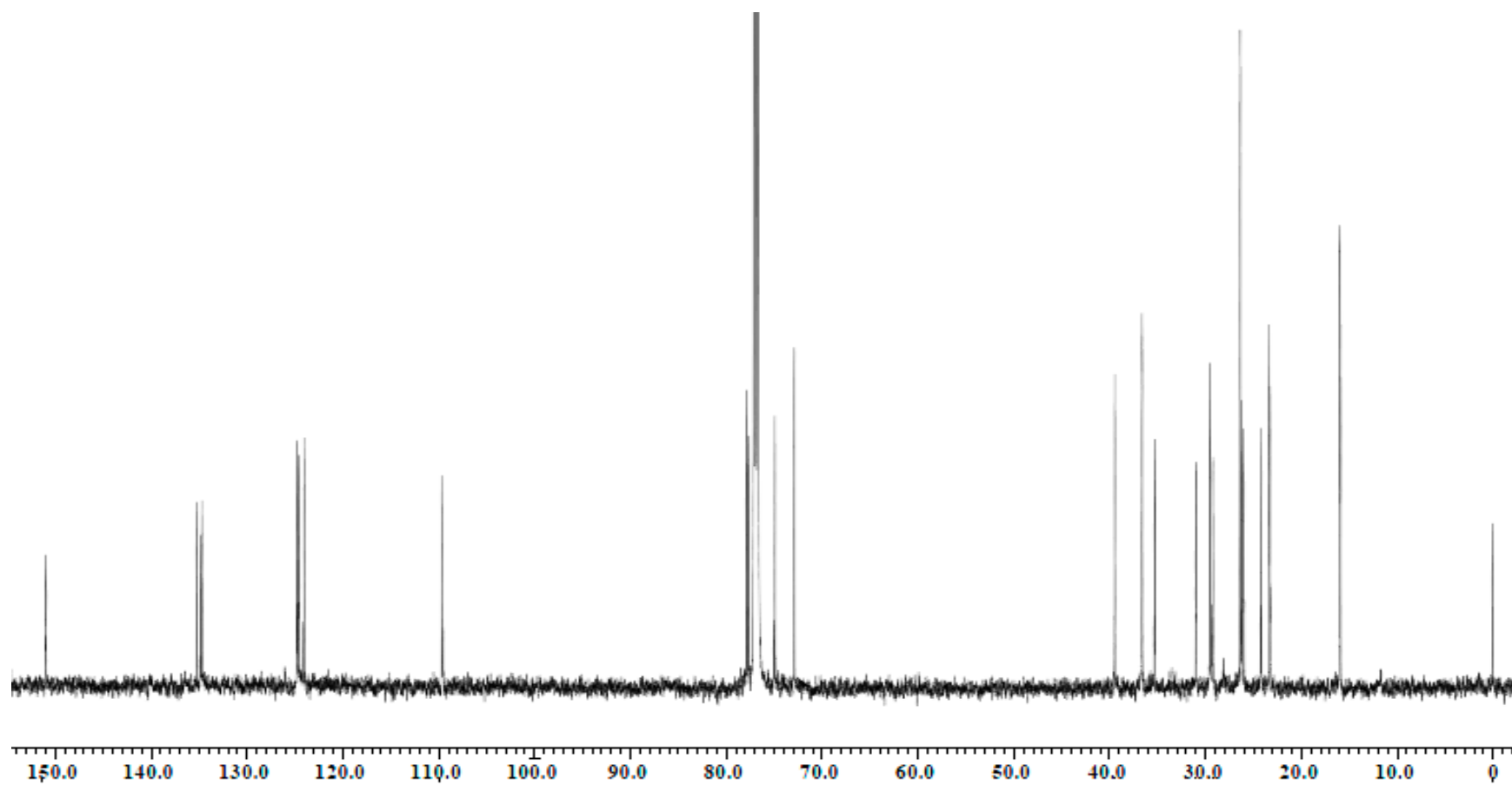


Figure S28. ^{13}C NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracosa-10,14,18-triene (**4**) (150 MHz in CDCl_3).

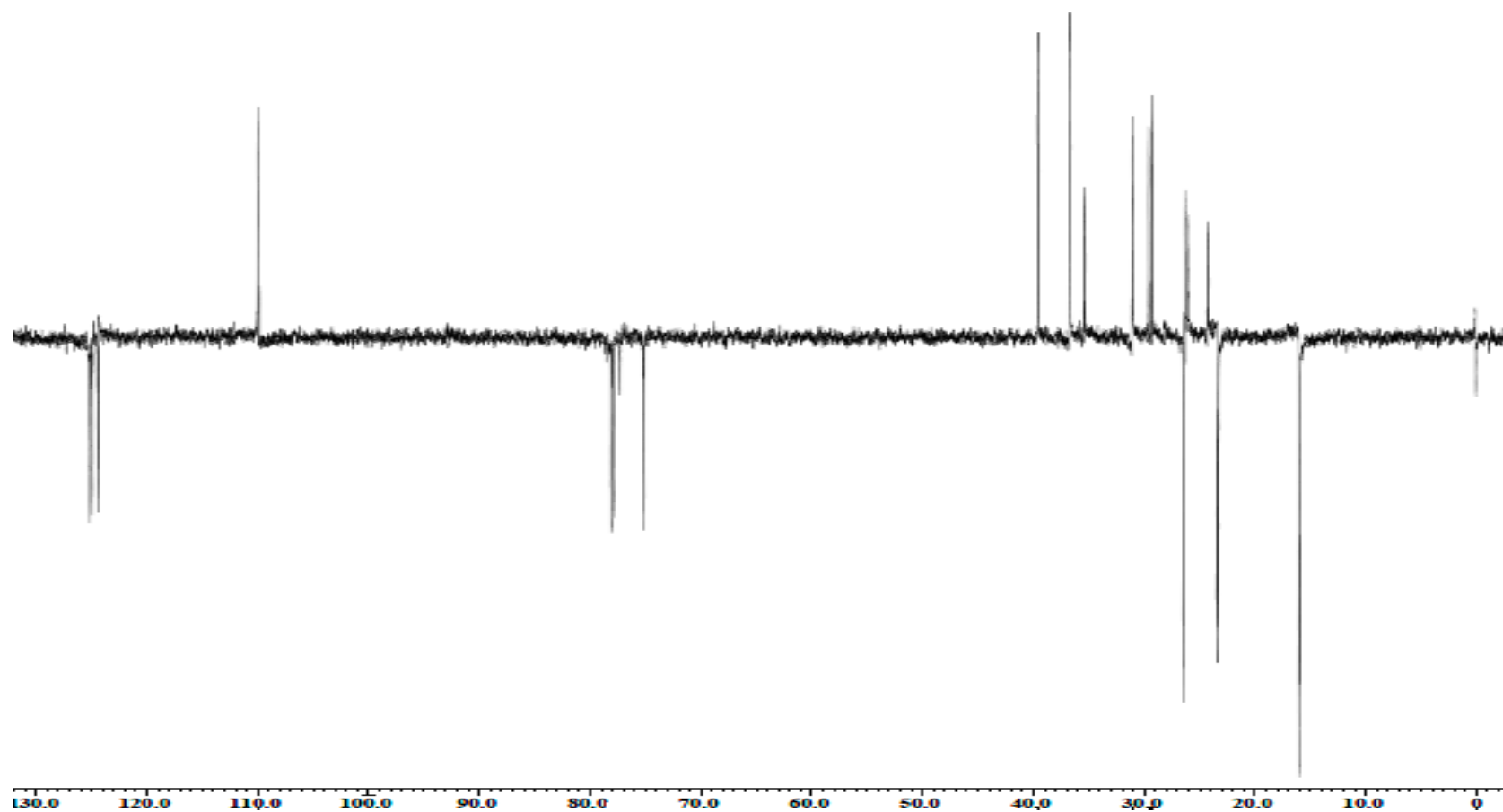


Figure S29. DEPT-135 NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracosa-10,14,18-triene (**4**) (150 MHz in CDCl₃).

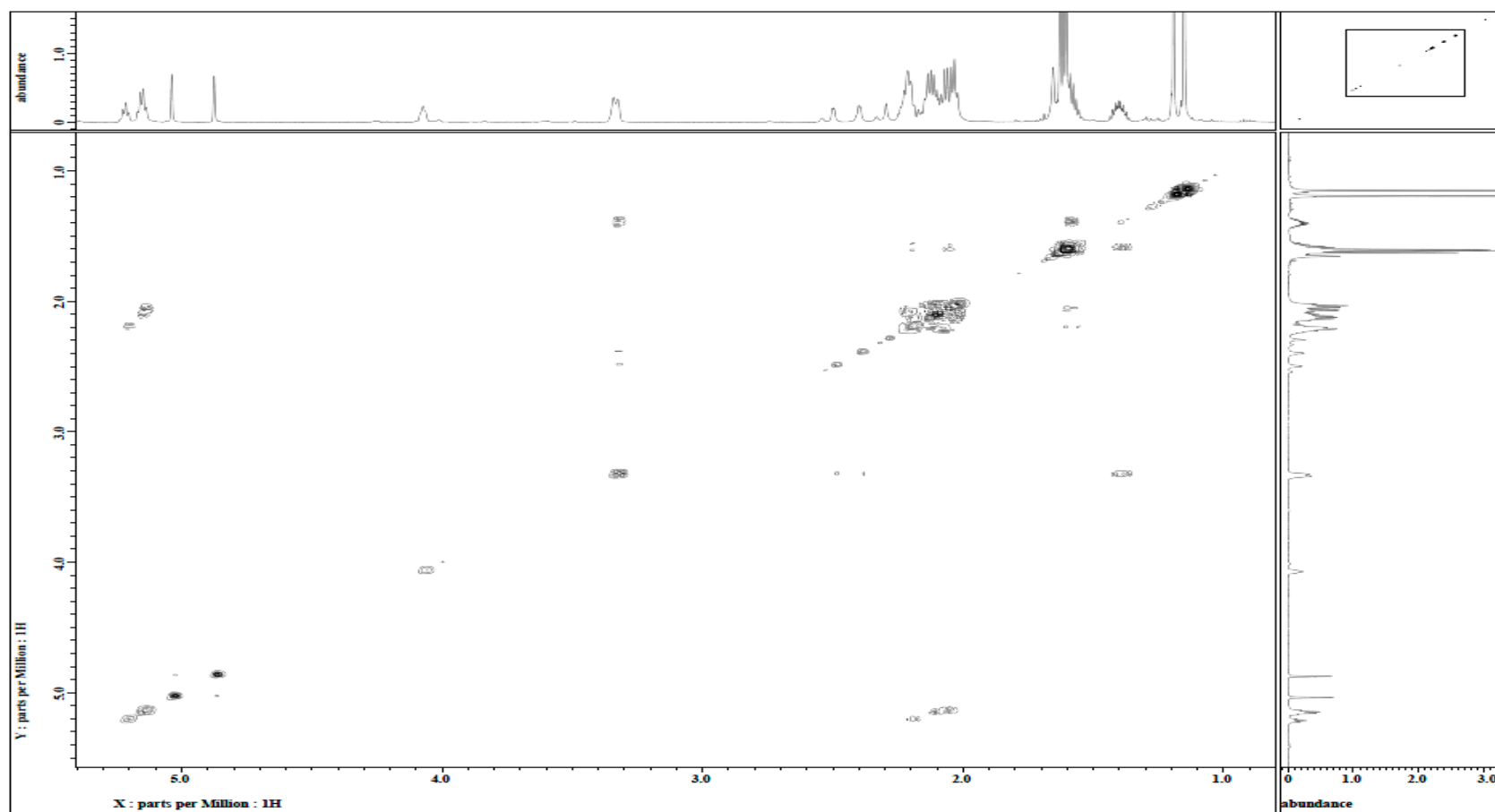


Figure S30. ^1H - ^1H COSY NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracos-10,14,18-triene (**4**).

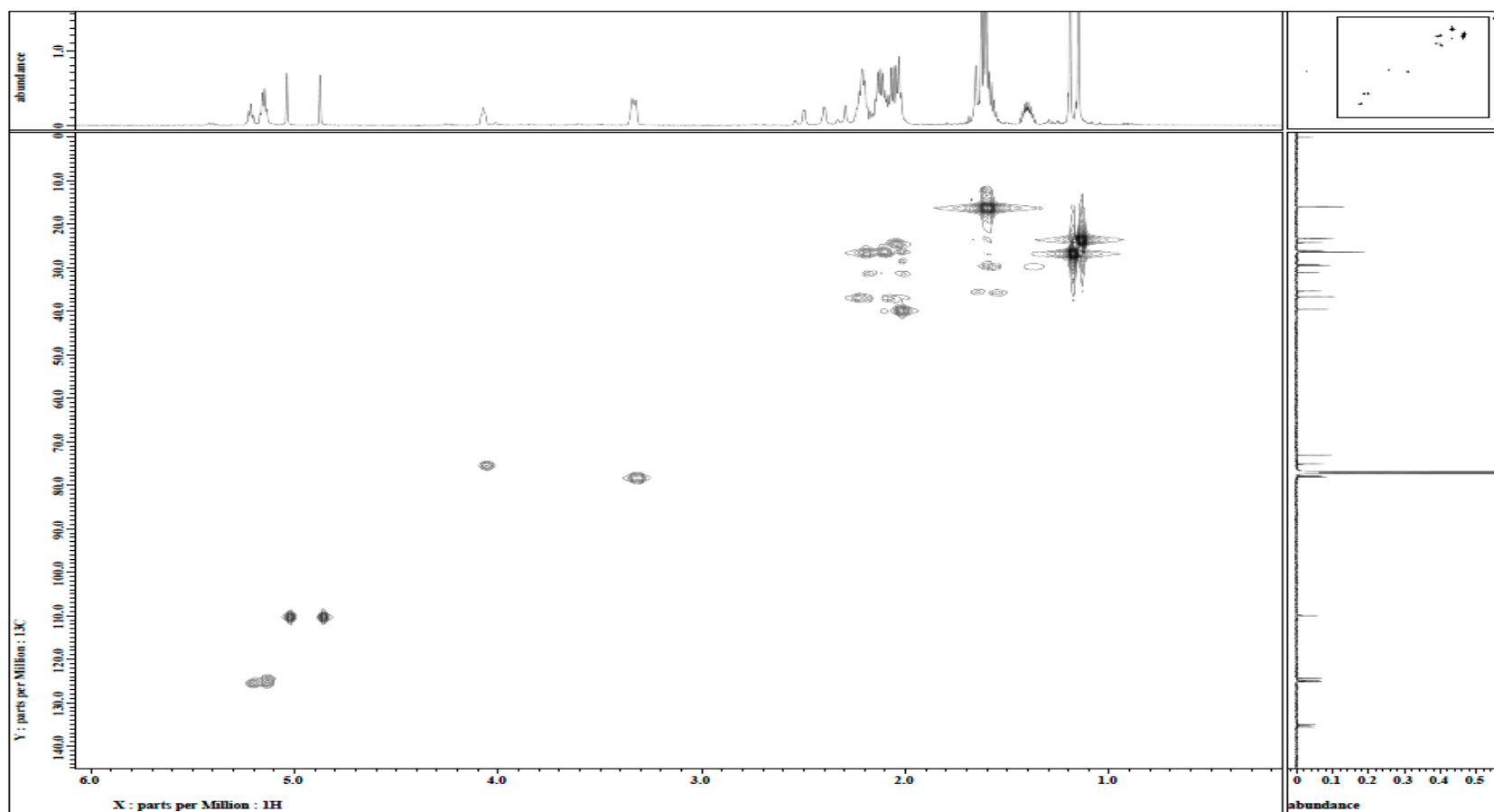


Figure S31. HMQC NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracosa-10,14,18-triene (4).

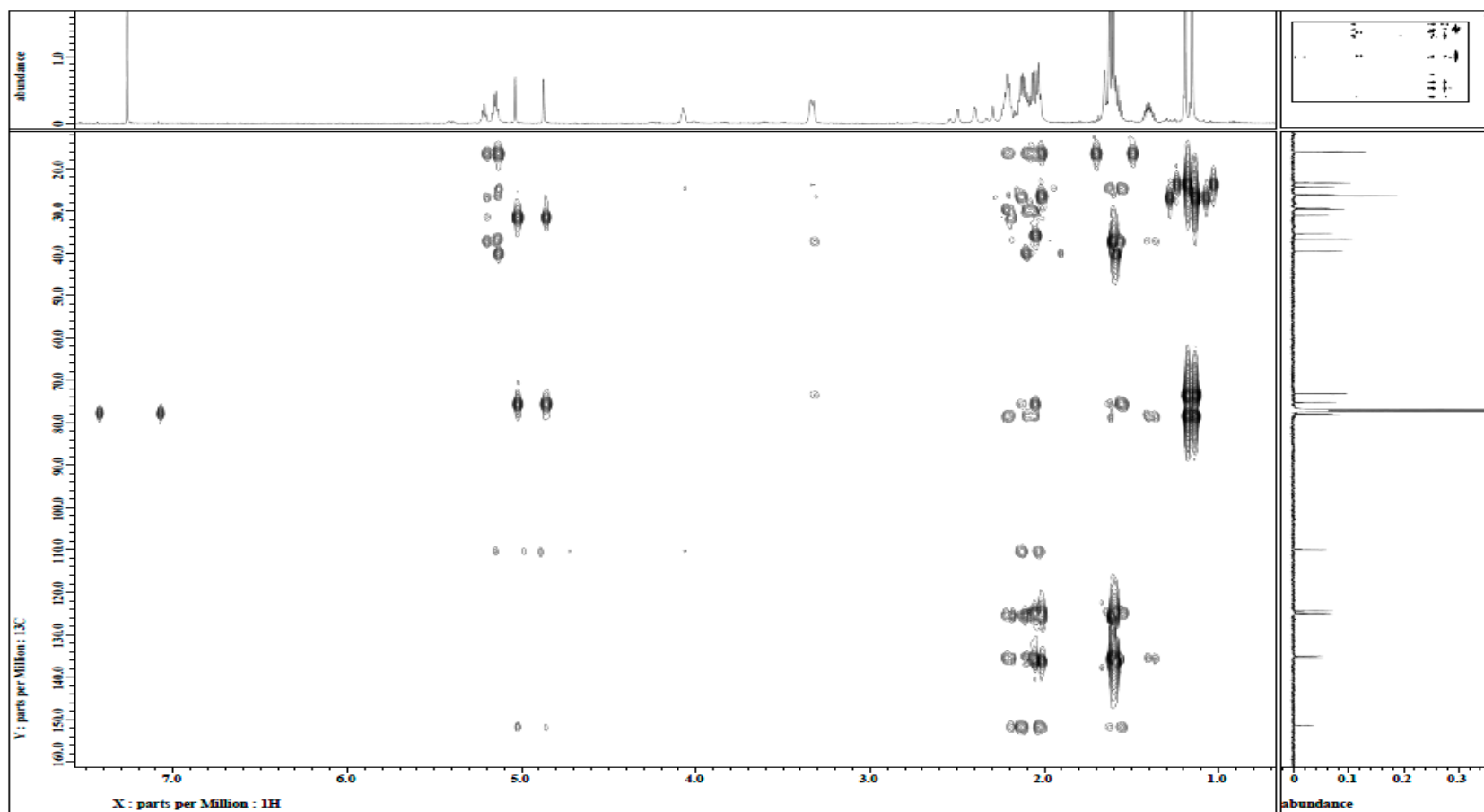


Figure S32. HMBC NMR spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracosa-10,14,18-triene (4).

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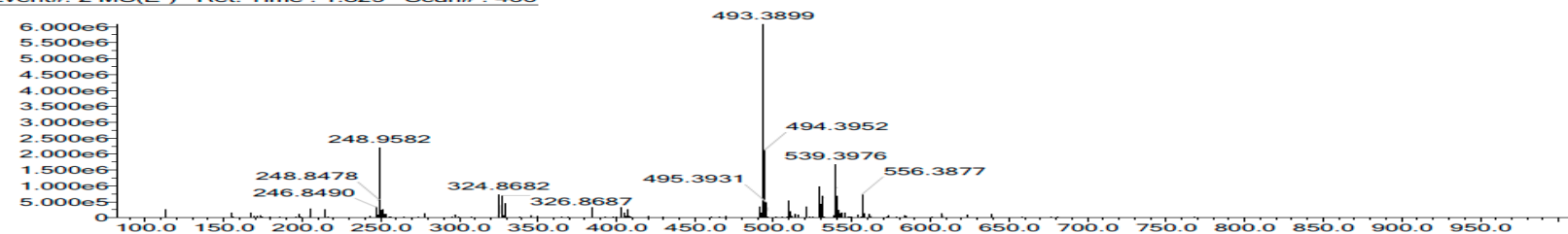
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N	3	0	0					

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 HC Ratio: unlimited
 Max Isotopes: all
 MSn Iso RI (%): 75.00

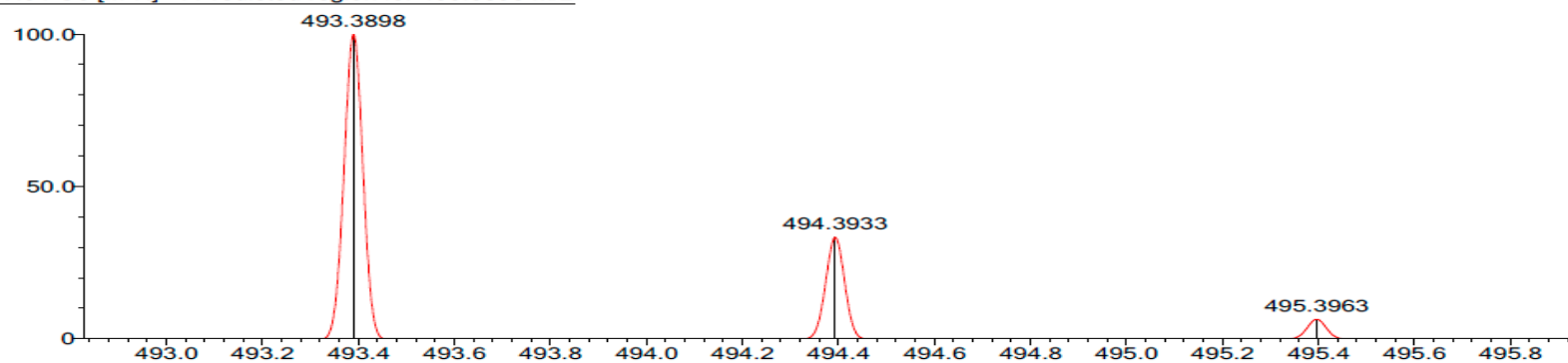
DBE Range: not fixed
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: no
 Isotope Res: 10000
 Max Results: 10

Event#: 2 MS(E-) Ret. Time : 1.829 Scan# : 466



C30 H54 O5 [M-H] - : Predicted region for 493.3898 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	88.71	C30 H54 O5	[M-H] -	493.3899	493.3898	0.1	0.20	88.71	4.0

Figure S33. HR-ESI MS spectrum of 2,3,6,22,23-pentahydroxy-2,10,15,19,23-hexamethyl-7-methylenetetracosa-10,14,18-triene (4).

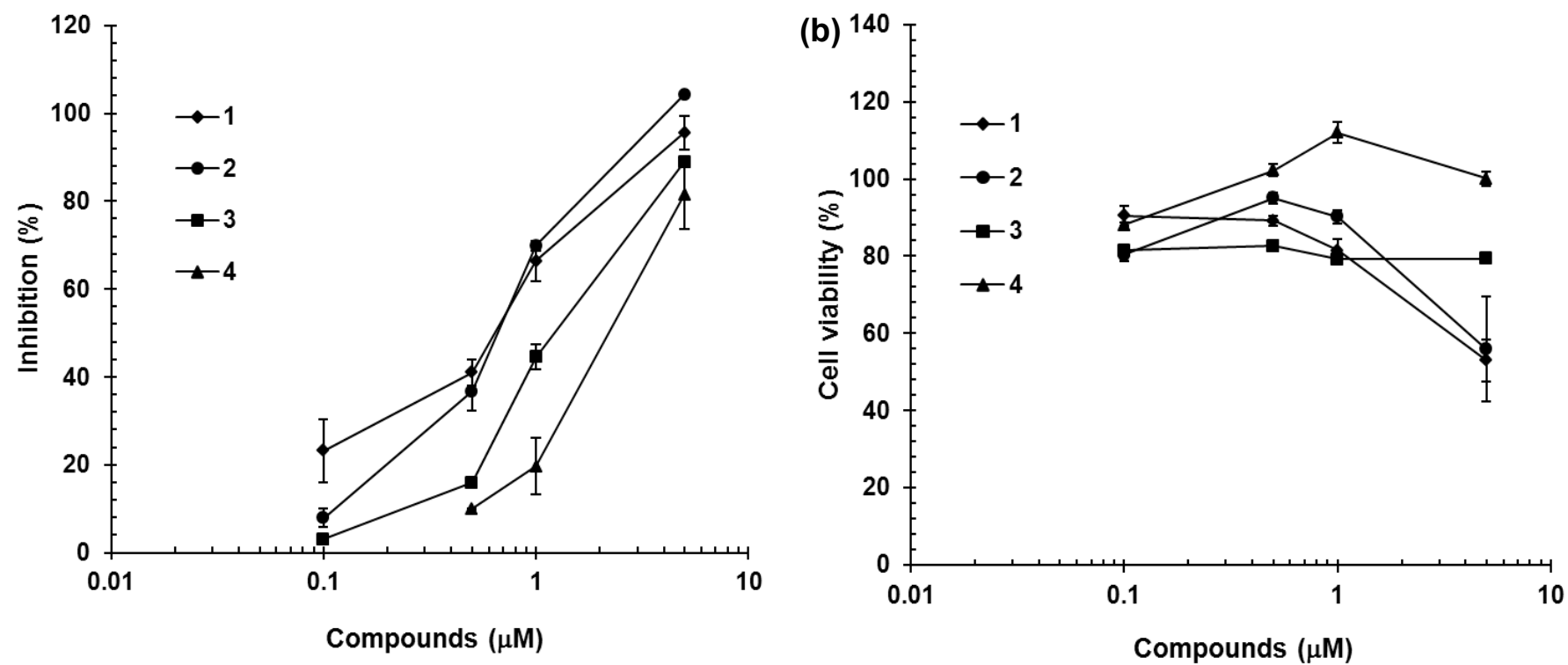


Figure S34. Inhibitory effects of compounds 2-4 on IL-6/STAT3 activation (a) and cell viability (b) in Hep3B cells.

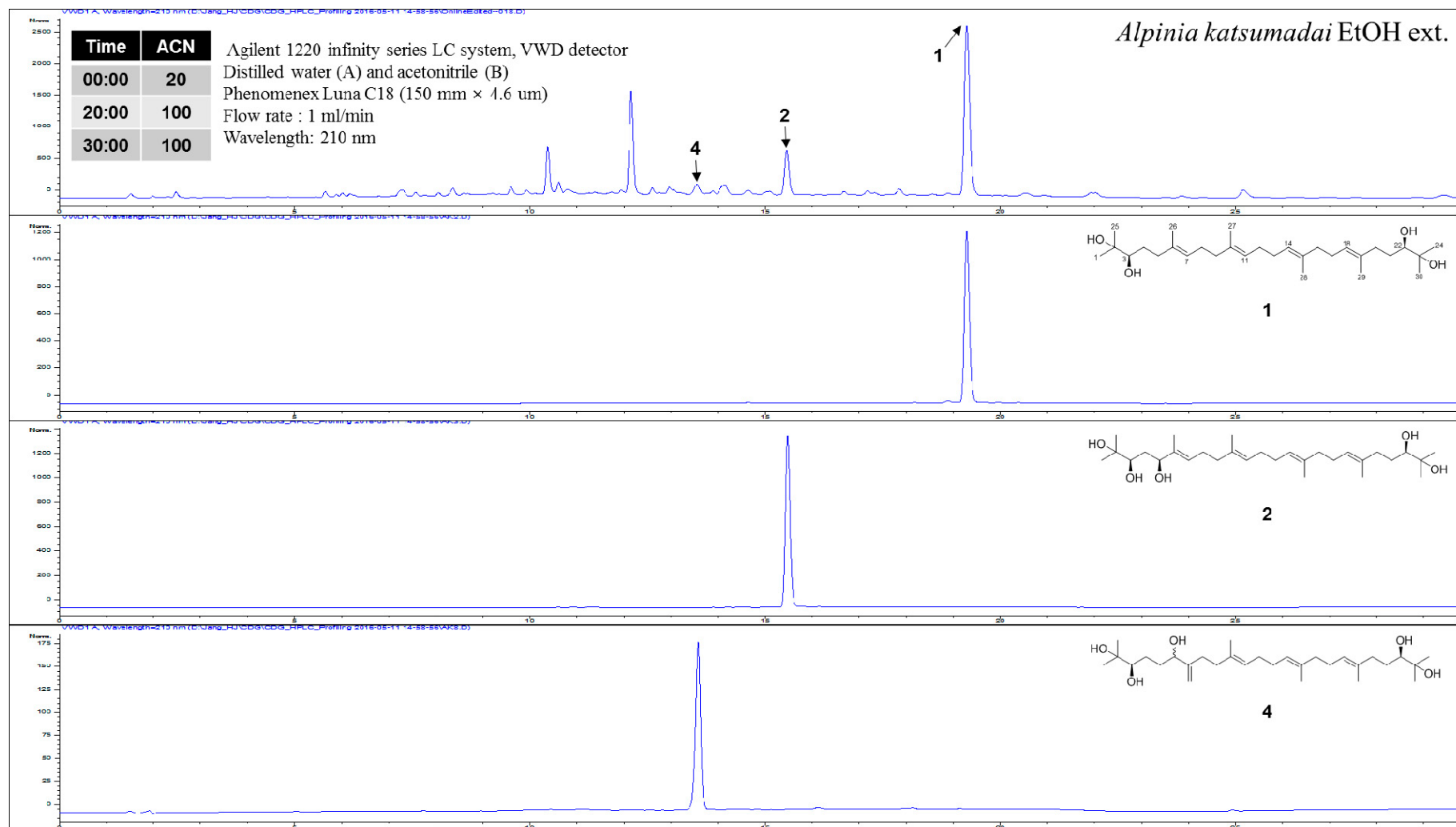


Figure S35. HPLC chromatogram of compounds **1**, **2**, and **4** of *A. katsumadai* ethanol-soluble extract (Agilent 1220 Infinity series; wavelength: 210 nm; column: Phenomenex Luna C₁₈; mobile phase: H₂O and acetonitrile; time of analysis: 30 min).

Table S1. Inhibitory effects of EtOH extract, CHCl₃ and H₂O layer of *A. katsumadai* on IL-6-induced STAT3 activation.

Samples (μg/mL)		Inhibition (%) ^a	Cytotoxicity (%) ^a
EtOH extract	0.5	45.3 ± 0.9	97.2 ± 0.8
	1	66.8 ± 3.6	101.4 ± 0.2
	5	102.7 ± 0.6	55.4 ± 1.6
CHCl ₃ layer	0.5	29.2 ± 1.9	90.6 ± 0.1
	1	42.7 ± 2.9	92.0 ± 0.4
	5	90.0 ± 0.1	95.6 ± 0.4
H ₂ O layer	0.5	17.4 ± 4.5	87.5 ± 0.4
	1	19.6 ± 1.4	86.1 ± 0.5
	5	50.3 ± 4.6	93.2 ± 0.7

^a The data are presented as the means from three independent experiments performed in duplicate.