Enzyme-Catalysed Conversion of Atranol and Derivatives into Dimeric Hydrosoluble Materials: Application to the Preparation of a Low-Atranol Oakmoss Absolute

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Supporting information: Table of Contents

1. Equipment

^{1}H NMR and ^{13}C NMR spectra were recorded on BRUCKER AC spectrometers (200 and 400 MHz). ^{1}H NMR spectra are reported as follows: chemical shifts in ppm (δ) relative to the chemical shift of TMS at 0 ppm, integration, multiplicities (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), and coupling constants (Hz). ^{13}C NMR spectra chemical shifts are reported in ppm (δ) relative to $CDCl_3$ at 77.16 ppm. Identity was assessed by comparison with data from authentic samples or literature data for **1** and its synthetic intermediates.

Column chromatography was carried out on silica gel (spherical 15–30 µm, neutral 63–200 µm, Geduran Si 60, Merck KGaA).

GC/MS analyses were performed using a Shimadzu QP2010 gas chromatograph (conditions: carrier gas, He; injector and detector temperatures, 250 °C; injected volume, 0.5 µL; split ratio, 1/100; pressure, 180 kPa; SLB-5ms capillary column (thickness: 0.25 mm, length: 30 m, inside diameter: 0.25 mm); temperature program, 60–315 °C at 10 °C min−1, and 10 min at 315 °C) coupled to a mass selective detector. Mass spectra were obtained by electron ionization at 70 eV, m/z 35–400, source temperature 250 °C; only the most abundant ions are given.

HPLC analyses were carried out using a Agilent 1100 chromatograph equipped with a UV photodiode array detector and a mass analyzer Thermo LCQ advantage, ionization by electrospray, and a quadrupole mass detector. Chromatographic conditions: water/ACN 85/15 for 5 min then to 0:100 in 45 min and 5 min at 0/100 at a 0.3 mL/min flow rate, column Marcherey–Nagel Nucleodur C18, 150 x 2.0 mm, 3 µm particle size, injection volume: 10 µL.

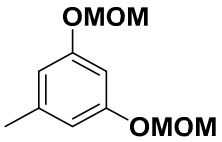
High resolution mass spectrometry (HRMS) was performed at ERINI platform (Grasse, France) using a Waters UPLC coupled with a Waters Xevo G2 QTOF spectrometer.

Enantioselective SFC was performed on a Jasco Extrema apparatus equipped with Daicel ChiralPak IA column coupled with a dual wavelength 190 to 600 nm UV-4070/75 detector. Pressure: 150 bars; flow: 4 mL/min; MeOH: 15%; wavelength: 245nm.

Polarimetry was performed on an Anton Paar MCP150 polarimeter at 20 °C in MeOH/H_2O 10:6 v.v. Wavelength: 589 nm.

Materials: Dimethylformamide (DMF), tetrahydrofuran (THF), methanol (MeOH), ethanol (EtOH), and cyclohexane (CHX) were purchased from Sigma-Aldrich and dried and/or distilled according to conventional procedures. Orcinol, $POCl_3$, MOMCl, NaH, n-BuLi, AcCl, Na_2CO_3, $NaHCO_3$, H_2O_2 (30% w/w in water), and HRP were purchased from Sigma-Aldrich and used as received.

2. Synthesis of atranol 2 from orcinol



1,3-di(methoxymethoxy)-5-methylbenzene: Orcinol (0.8 g, 6.5 mmol) was dissolved in freshly dried and distilled DMF (40 mL). To this solution was added NaH (0.58 g as a 60% dispersion in mineral oil, 14.5 mmol). The mixture was stirred under a nitrogen atmosphere at 0 °C in a round-bottomed flask equipped with a refrigerant and a bubbler allowing to monitor H_2 evolution. After 15 min, MOM-Cl (1.10 mL, 14.5 mmol) was added and the mixture stirred during pendant 18 h. Water was then carefully added (30 mL) and the resulting mixture extracted with Et_2O (5 x 30 mL). Organic layers were then pooled and washed with a 2 M aqueous NaOH solution (3 x 20 mL) and brine (20 mL). After drying over $MgSO_4$, filtration and solvent removal, a yellow oil was obtained, which was submitted to column chromatography over silica gel (petroleum ether/EtOAc 9:1) to yield the MOM-protected orcinol as a colorless liquid (1.13 g, 95%). Rf 0.7 (petroleum ether/EtOAc 8:2). **^{1}H NMR** ($CDCl_3$, 200 MHz): δ ppm 6.6-6.5 (m, 3H, ArH), 5.15 (s, 4H), 3.48 (s, 6H) 2.32 (s, 3H). **^{13}C NMR** ($CDCl_3$, 50 MHz): δ ppm 158.2 (C), 140.3 (C), 110.4 (CH), 102.1 (CH), 94.4 (CH), 55.9 (OCH_3), 21.7 (CH_3). **MS (EI)** ***m/z:*** 212 (8), 182 (1), 152 (3), 136 (2), 123 (1), 108 (2), 91 (1), 77 (2), 45 (100).

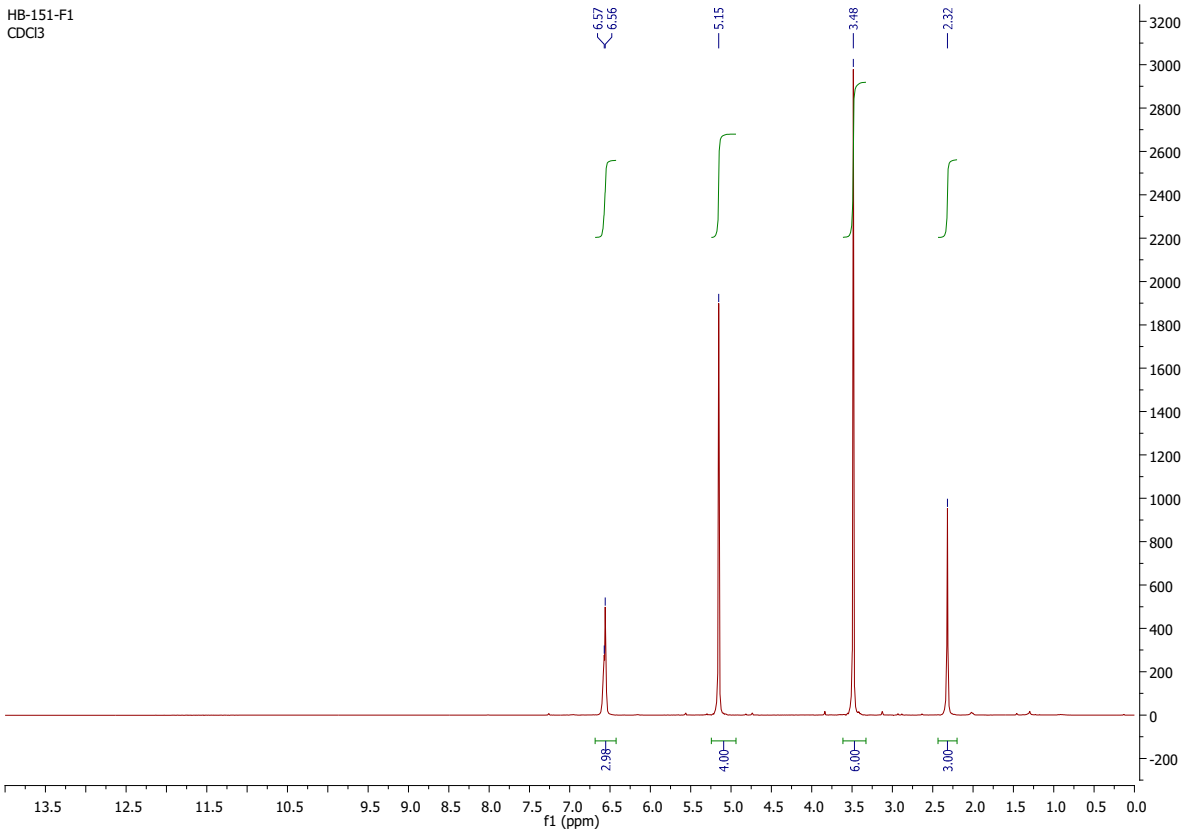


Figure S 1. ^{1}H-NMR spectrum of 1,3-di(methoxymethoxy)-5-methylbenzene.

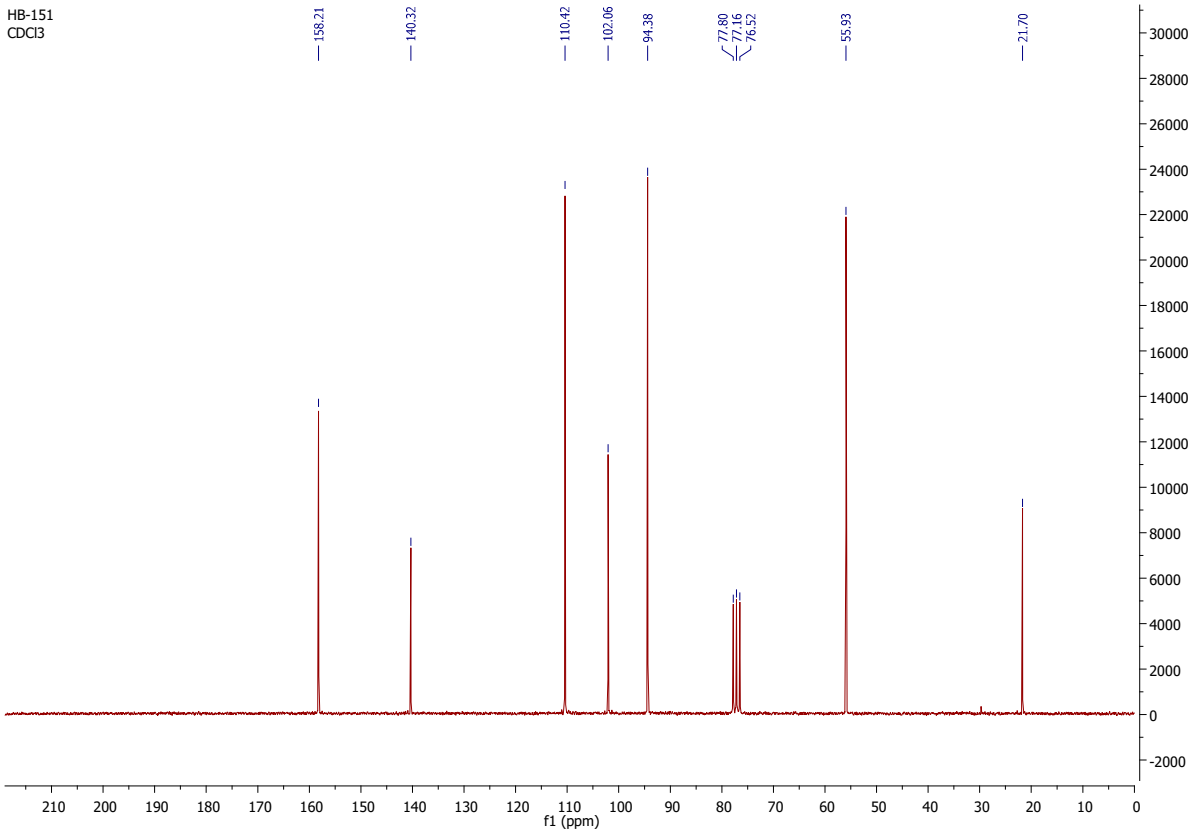
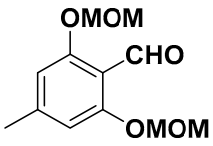


Figure S 2. ^{13}C-NMR spectrum of 1,3-di(methoxymethoxy)-5-methylbenzene.



1,3-di(methoxymethoxy)-4-methylbenzaldehyde: 1,3-di(methoxymethoxy)-5-methylbenzene (0.95 g, 4.5 mmol) was dissolved in freshly distilled THF (50 mL), and stirred under a nitrogen atmosphere at 0 °C while *n*-buthyllithium was added dropwise (3.4 mL as a 1.6 M solution in hexane, 5.4 mmol). The mixture was stirred over 1.5 h while allowed to warm to room temperature, and the reaction was quenched with DMF (0.7 mL, 9 mmol). The resulting mixture was washed with water (50 mL) and extracted with Et_2O (4 x 30 mL). Organic layers were pooled and washed with water (40 mL) and brine (40 mL), dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. The resulting yellow oil was submitted to column chromatography over silica gel (petroleum ether/EtOAc 95:5) to yield a yellow solid (0.81 g, 75%). **^{1}H NMR** ($CDCl_3$, 200 MHz): δ ppm 10.40 (s, 1H, CHO), 6.58 (s, 2H, ArH), 5.17 (s, 4H), 3.42 (s, 6H) 2.26 (s, 3H). **^{13}C NMR** ($CDCl_3$, 50 MHz): δ ppm 188.7 (CHO), 159.4 (C), 147.3 (C), 113.7 (CH), 109.3 (CH), 94.6 (CH), 56.3 (OCH_3), 22.5 (CH_3). **MS *m/z*** *:* 240 (2), 209 (2), 195 (3), 179 (4), 178 (10), 165 (3), 164 (4), 136 (2), 77 (2), 46 (3), 45 (100).

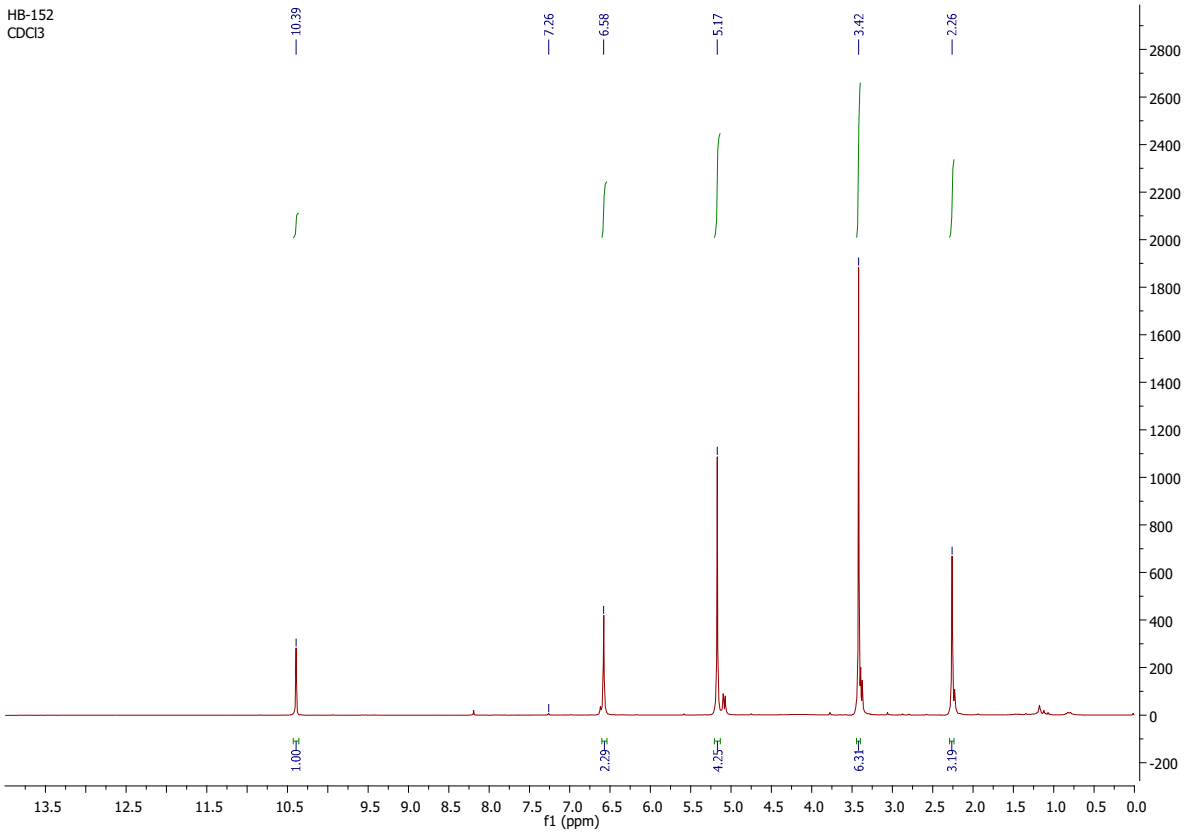


Figure S 3. ^{1}H-NMR spectrum of 1,3-di(methoxymethoxy)-4-methylbenzaldehyde.

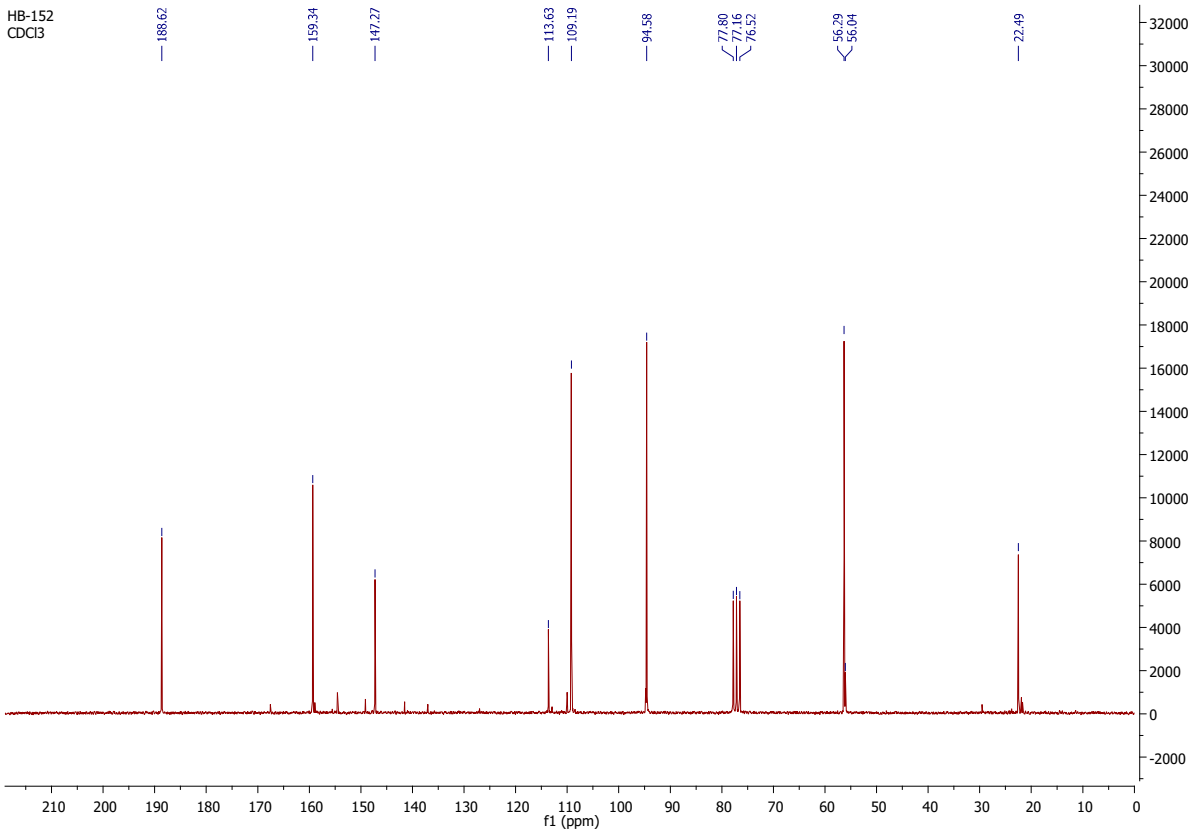
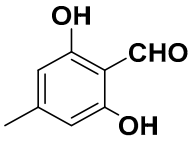


Figure S 4. ^{13}C-NMR spectrum of 1,3-di(methoxymethoxy)-4-methylbenzaldehyde.



Atranol 2: 1,3-di(methoxymethoxy)-4-methylbenzaldehyde (0.315 g, 1.3 mmol) was dissolved in 30 mL of MeOH at room temperature under a nitrogen atmosphere. Acetyl chloride was then added dropwise (60 µl, 0.9 mmol). The mixture was stirred for 20 h and concentrated *in vacuo*. An aqueous HCl solution was then added (30 mL, 0.1 M) and the resulting mixture extracted with EtOAc (3 x 150 mL). Organic layers were pooled and dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. The resulting light yellow oil was submitted to column chromatography over silica gel (petroleum ether/EtOAc 8:2), and atranol **1** was obtained as a light yellow oil (0.164 g, 90%). **^{1}H NMR** (acetone-d_6, 200 MHz): δ ppm 10.71 (s, 2H, OH), 10.26 (s, 1H, CHO), 6.25 (s, 2H, ArH), 2.23 (s, 3H). **^{13}C NMR** (acetone-d_6, 50 MHz): δ ppm 194.2 (CHO), 163.1 (C), 151.6 (C), 109.3 (C), 108.4 (CH), 22.4 (CH_3). **MS *m/z*** *:* 152 (84), 151 (100), 134 (6), 123 (4), 106 (16), 95 (9), 77 (14), 69 (6), 67 (11), 55 (12).

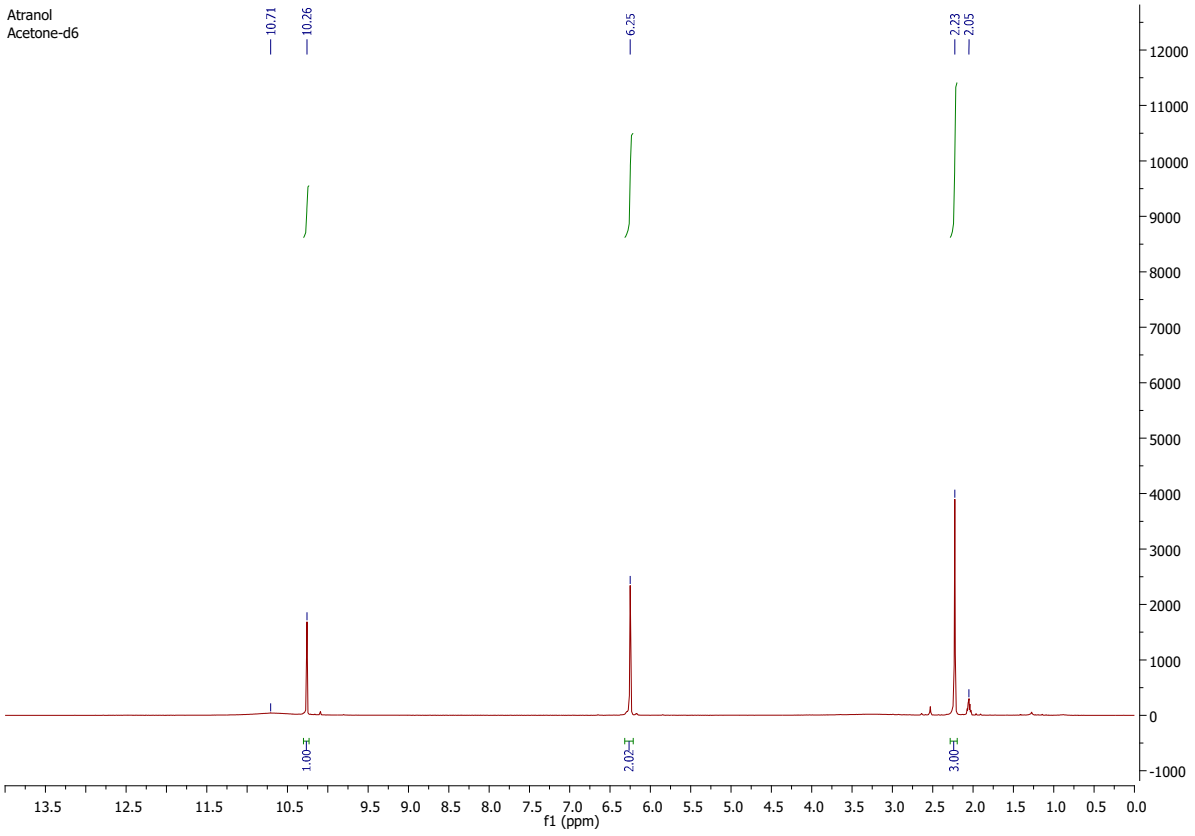


*Figure S 5. ^{1}H-NMR spectrum of atranol **2**.*

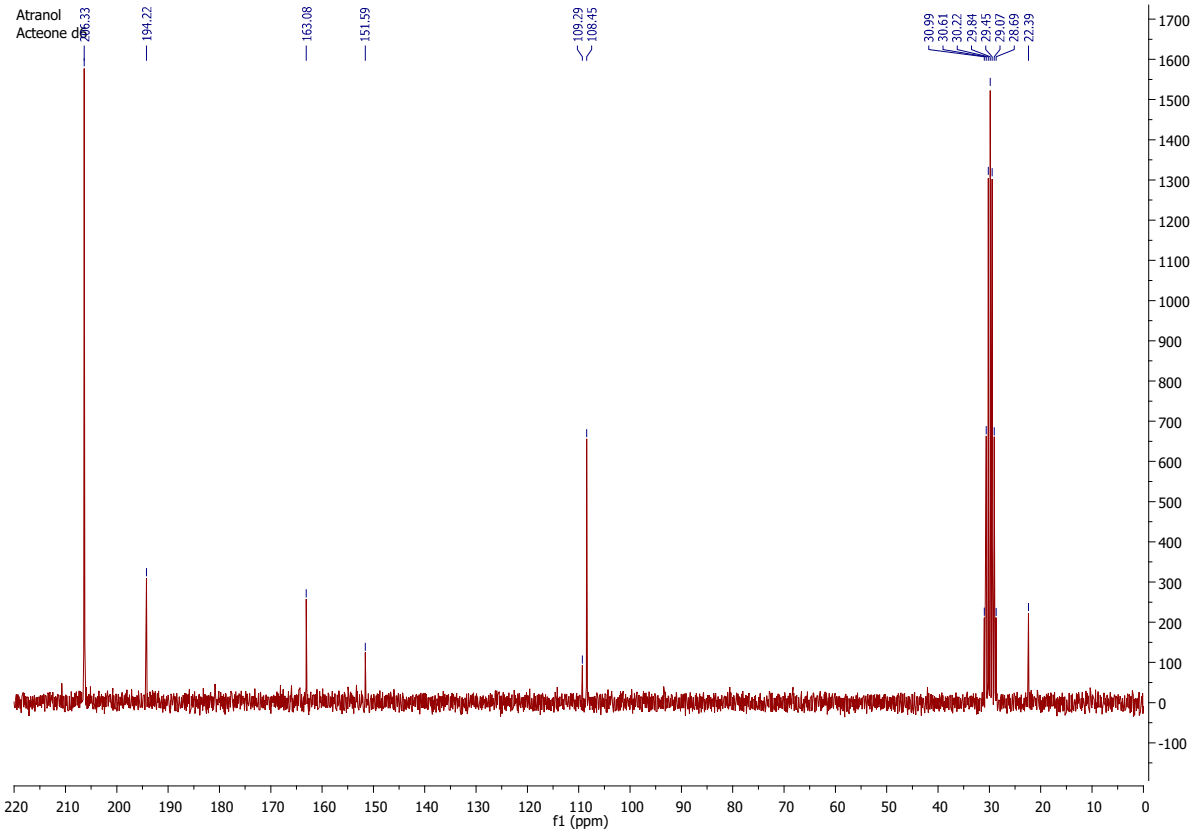


*Figure S 6. ^{13}C-NMR spectrum of atranol **2**.*

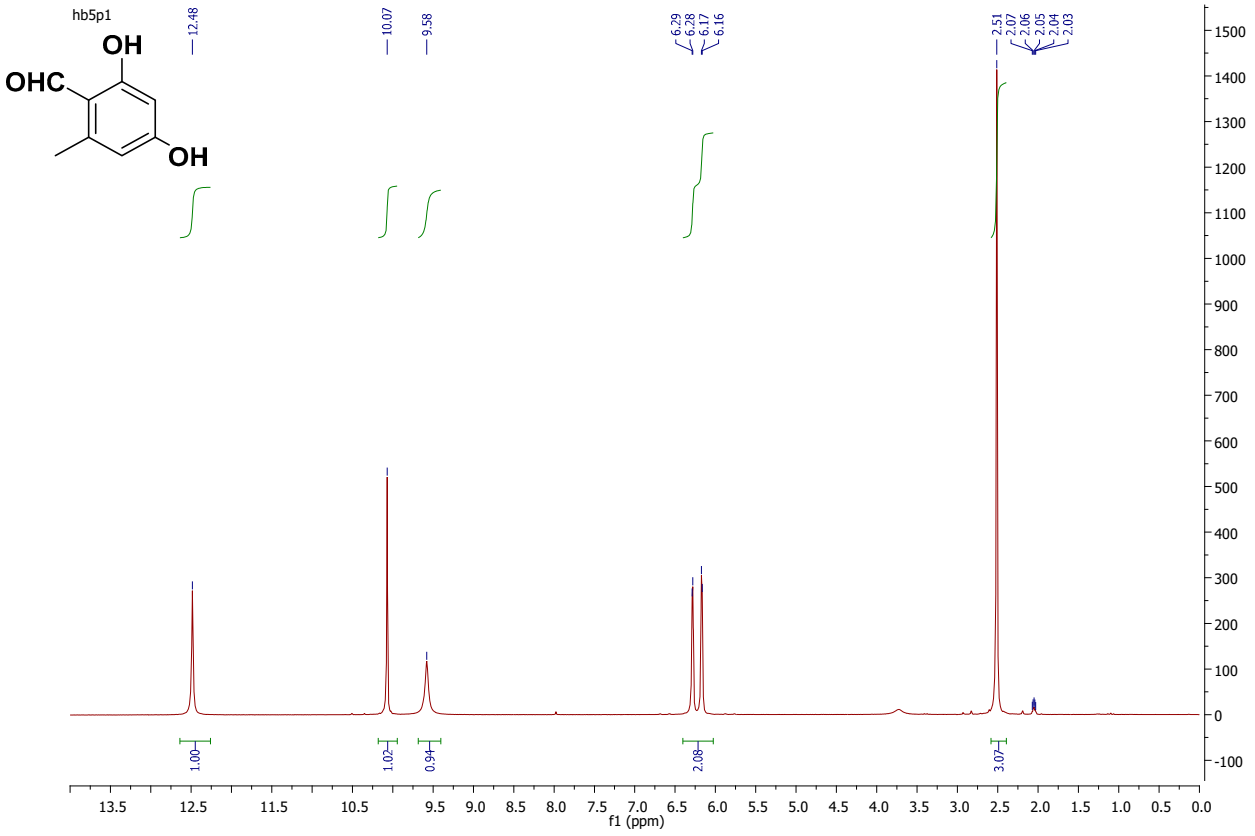


Figure S 7. ^{1}H-NMR spectrum of isoatranol.

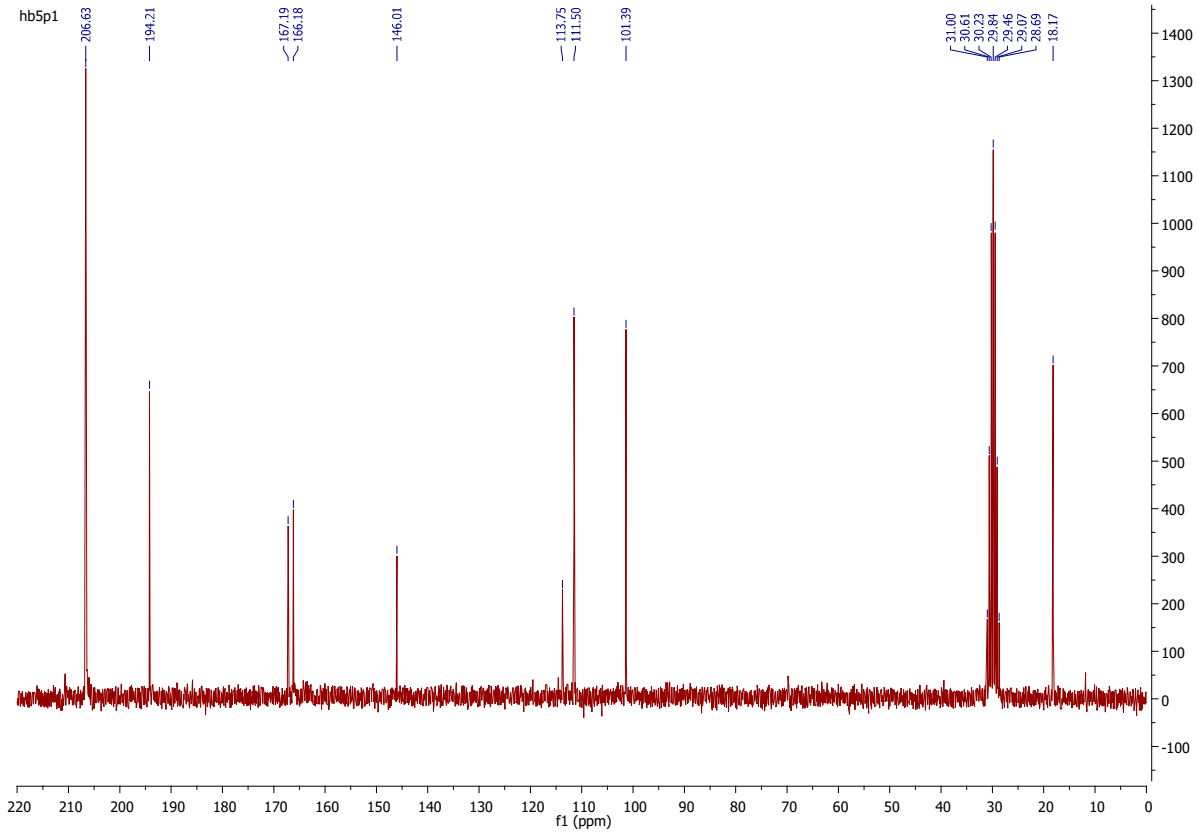
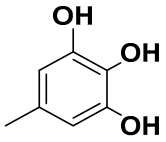


Figure S 8. ^{13}C-NMR spectrum of isoatranol.



5-Methylpyrogallol 5: Atranol (0.152 g, 1 mmol) and sodium percarbonate (0.236 g, 1.5 mmol) were dissolved in THF/water 3:7 mixture (5 mL). The reaction mixture is then stirred at room temperature for 2 hours. After completion of the reaction, aqueous 0.1 M HCl solution was added (5 mL) and the mixture extracted with EtOAc (2 x 10 mL). Organic layers were pooled and dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. An orange solid was obtained (0.119 g, 85%). **^{1}H NMR** (acetone-d_6, 200 MHz): δ ppm 7.64 (s, 2H, OH), 7.02 (s, 1H, OH), 6.20 (s, 2H, ArH), 2.10 (s, 3H). **^{13}C NMR** (acetone-d_6, 50 MHz): δ ppm 146.3 (C), 131.0 (C), 129.2 (C), 108.5 (CH), 20.9 (CH_3). **MS *m/z :*** 140 (100), 139 (34), 134 (6), 123 (11), 122 (19), 121 (9), 94 (35), 77 (4), 66 (37), 65 (21), 55 (6), 53 (17).

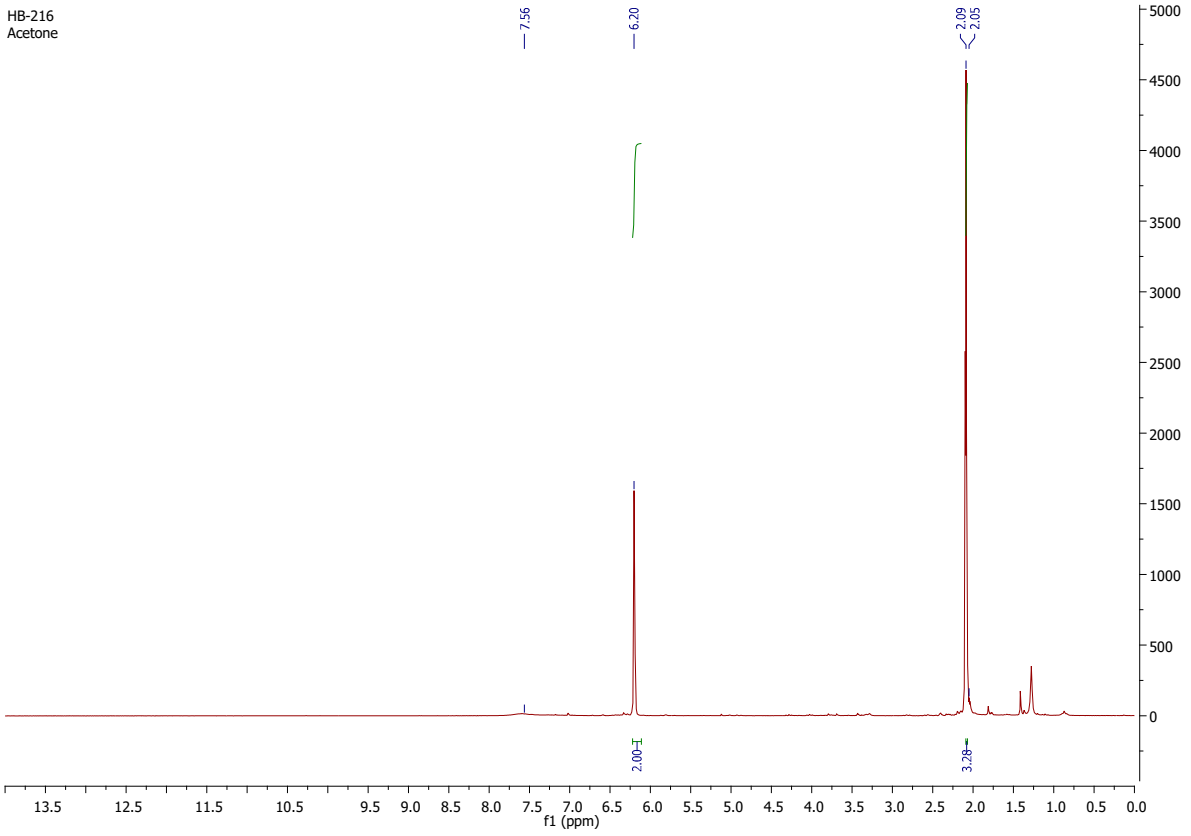


*Figure S 9. ^{1}H-NMR of 5-methylpyrogallol **5**.*

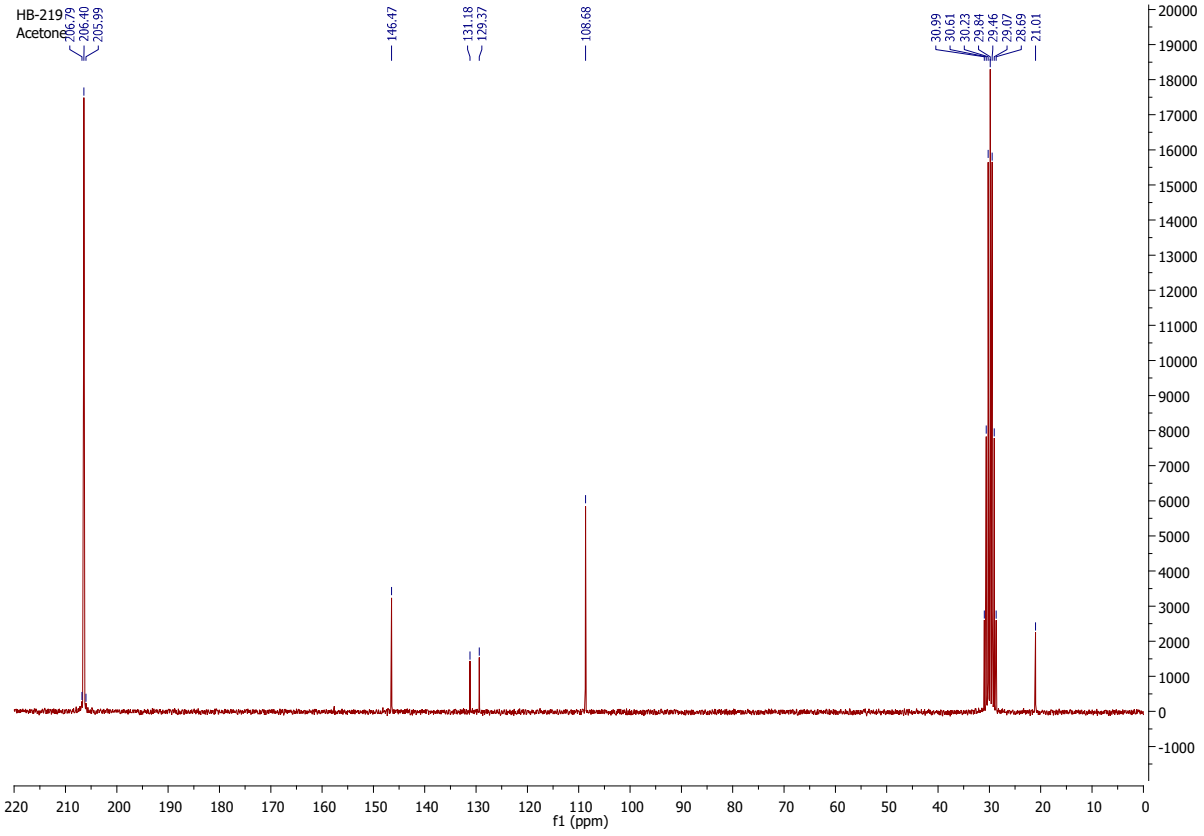
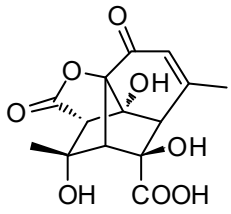


*Figure S 10. ^{13}C-NMR of 5-methylpyrogallol **5**.*

3. Dimerization of atranol 2 into 6



6

Dimerization of atranol **2** into **6**: HRP (124 U/mg, 4 mg) was dissolved in 65 mL of pH 9 carbonate buffer (20 mM) containing atranol **2** (200 mg, 1.32 mmol). The reaction flask was covered with aluminum foil to avoid peroxide decomposition. Reaction was initiated by the slow addition of a 30% aqueous H_2O_2 solution at 0.1 mL/h to ensure the final addition of 2 equivalents of hydrogen peroxide (0.264 mL) with respect to atranol. After 6 hours at room temperature, an aqueous HCl solution (0.1 M) was added until pH 4 was reached. This aqueous layer was extracted with ethyl acetate (3 x 70 mL). The aqueous phases were concentrated by rotary evaporation to give **6** as a white powder (305 mg, 75%).^{1}H NMR (D_2O, 400 MHz): δ ppm 6.37 (t, 1H), 3.97 (d, 1H), 3.37 (s, H), 2.68 (s, 2H), 2.62 (s, H), 2.14 (s, 3H), 1.79 (s, 3H). ^{13}C RMN: (D_2O, 100 MHz) δ ppm 197.2 (C), 178.0 (C), 171.5 (C), 164.1 (C), 127.1 (CH), 88.7 (C), 87.1 (C), 85.7 (C), 77.7 (C), 60.1 (CH), 54.4 (CH), 52.9 (CH), 25.7 (CH_3), 23.6 (CH_3). MS (ESI): $[M - H]^-$ ion was observed at *m/z* 309, $[2M - H]^+$ at *m/z* 619, $[2M - 2H + Na]^+$ at *m/z* 641. α(20 °C, 589 nm)= −0.002° ± 0.000 (triplicates).

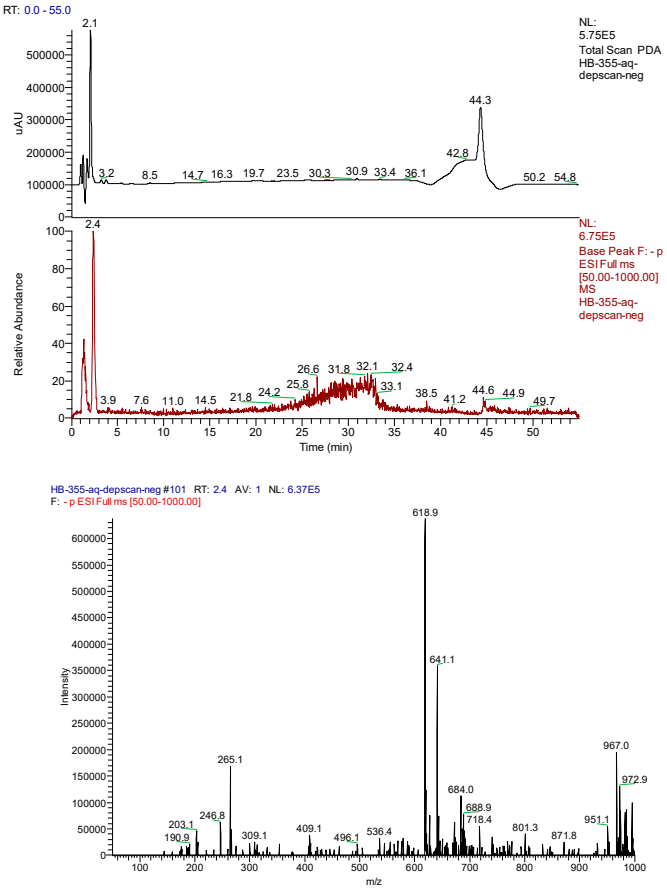
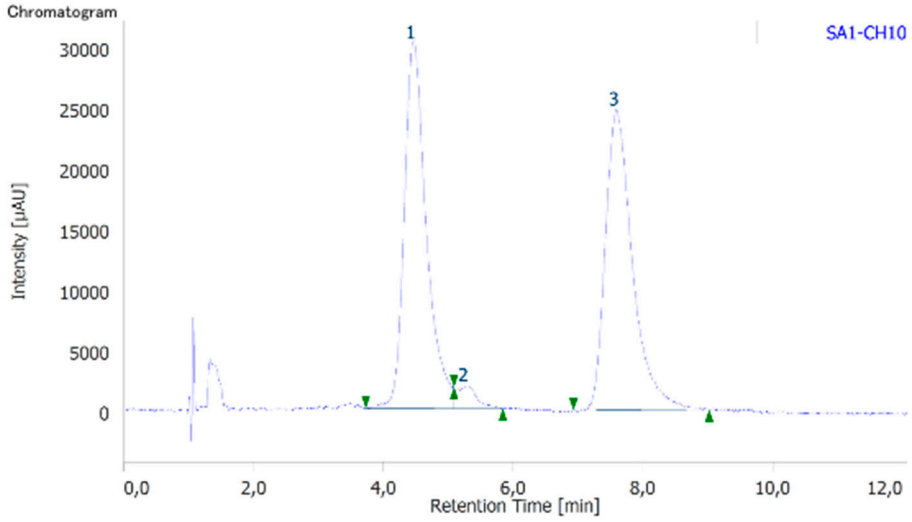


*Figure S 11. HPLC-MS of **6**.*

SA1-IA-150-4-15MeOH(245nm) SA1 27/07/2018 10:14:41

Chromatogram Information	
User Name	aurelien
Date Modified	27/07/2018 09:51:21
HPLC System Name	SFC-4000
Injection Date	27/07/2018 09:39:15
Volume	10,00 [µL]
Acquisition Time	25,0 [min]
Acquisition Sequence	SA1-IA-150-4-15MeOH(245nm)
Control Method	SFC-150-4-15MeOH (245nm)



Peak Information

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
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2	Unknown	10	5,268	40138	1891	2,717	3,315	N/A	N/A	N/A	N/A	
3	Unknown	10	7,590	720932	24812	48,798	43,513	N/A	1736	N/A	1,518	

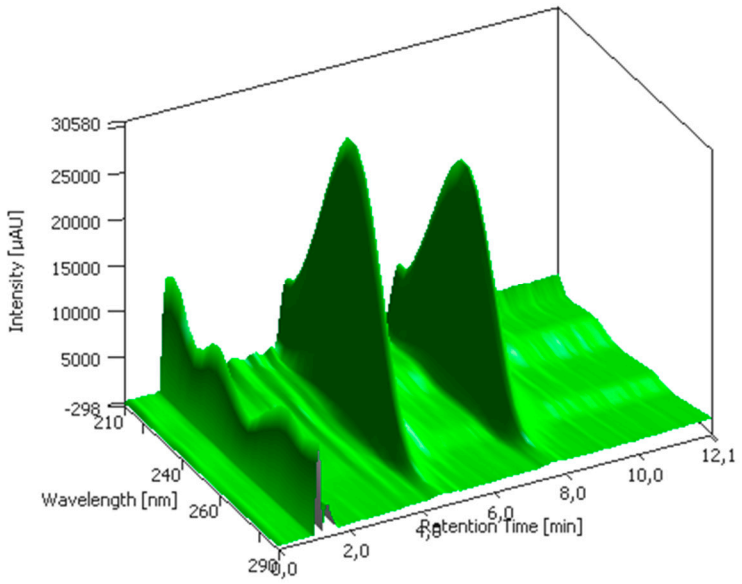


*Figure S 12. Enantioselective-SFC analysis of dimer **6**.*

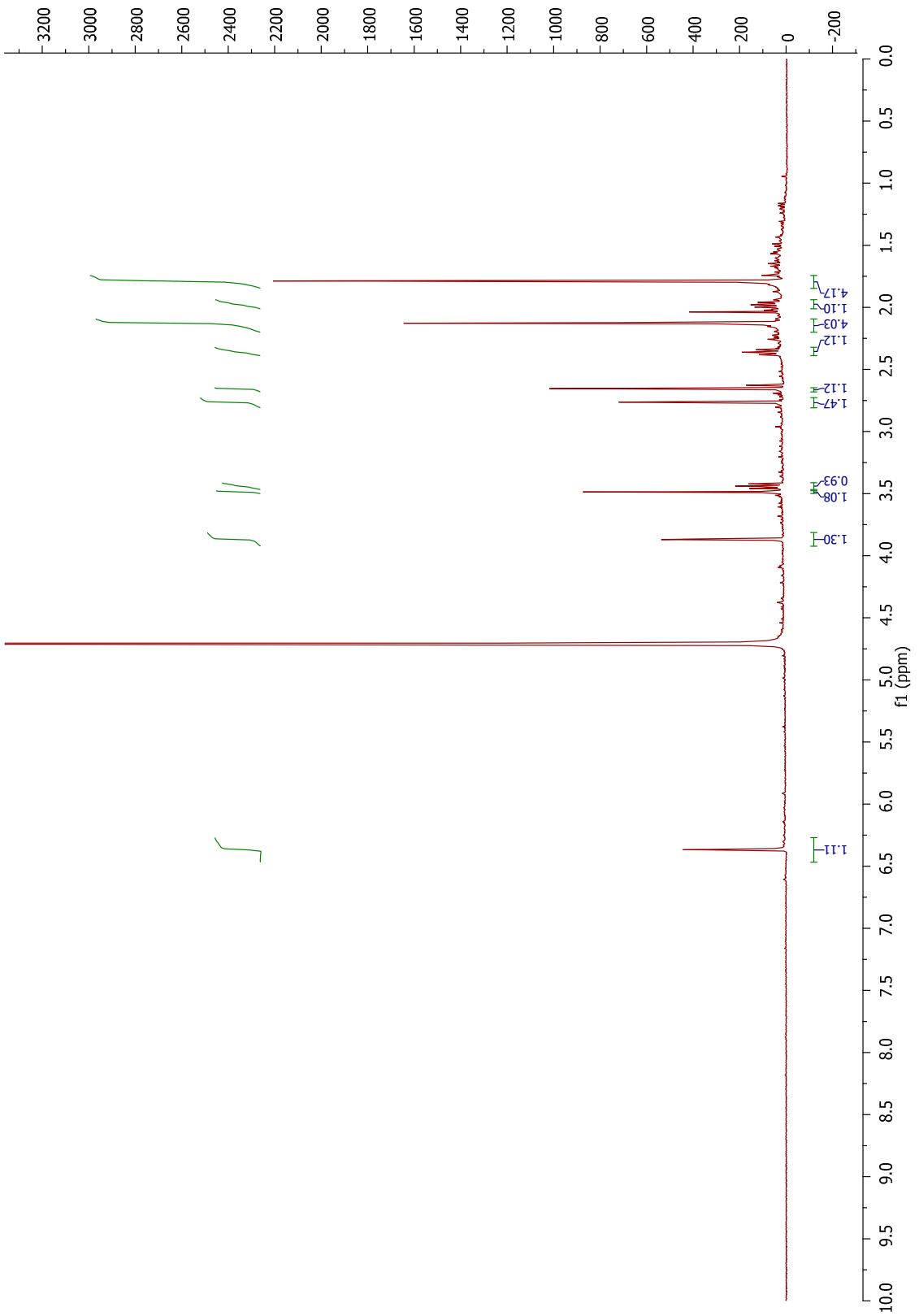


*Figure S 13. ^{1}H-NMR spectrum of **6**.*

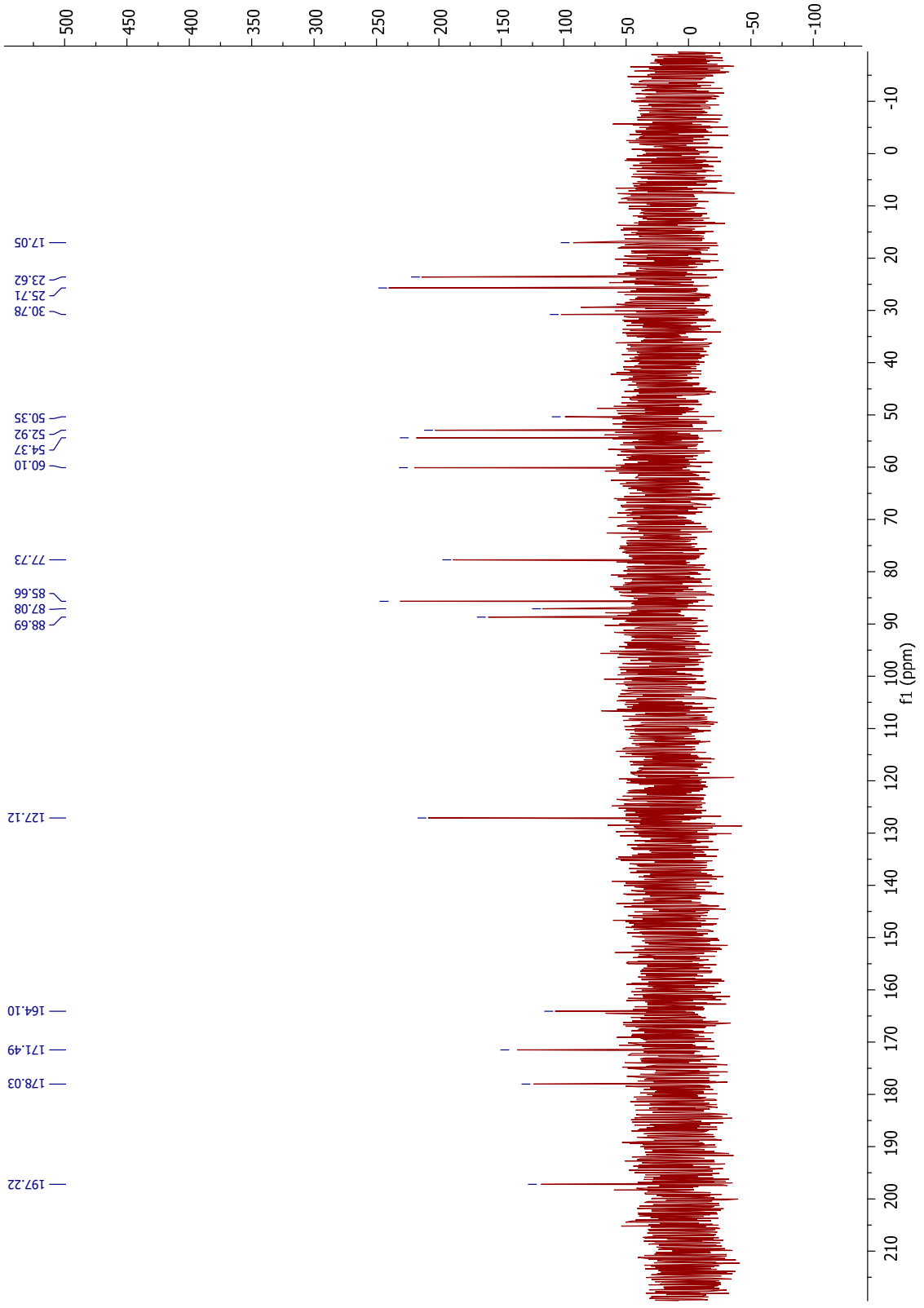


*Figure S 14. ^{13}C-NMR spectrum of **6**.*

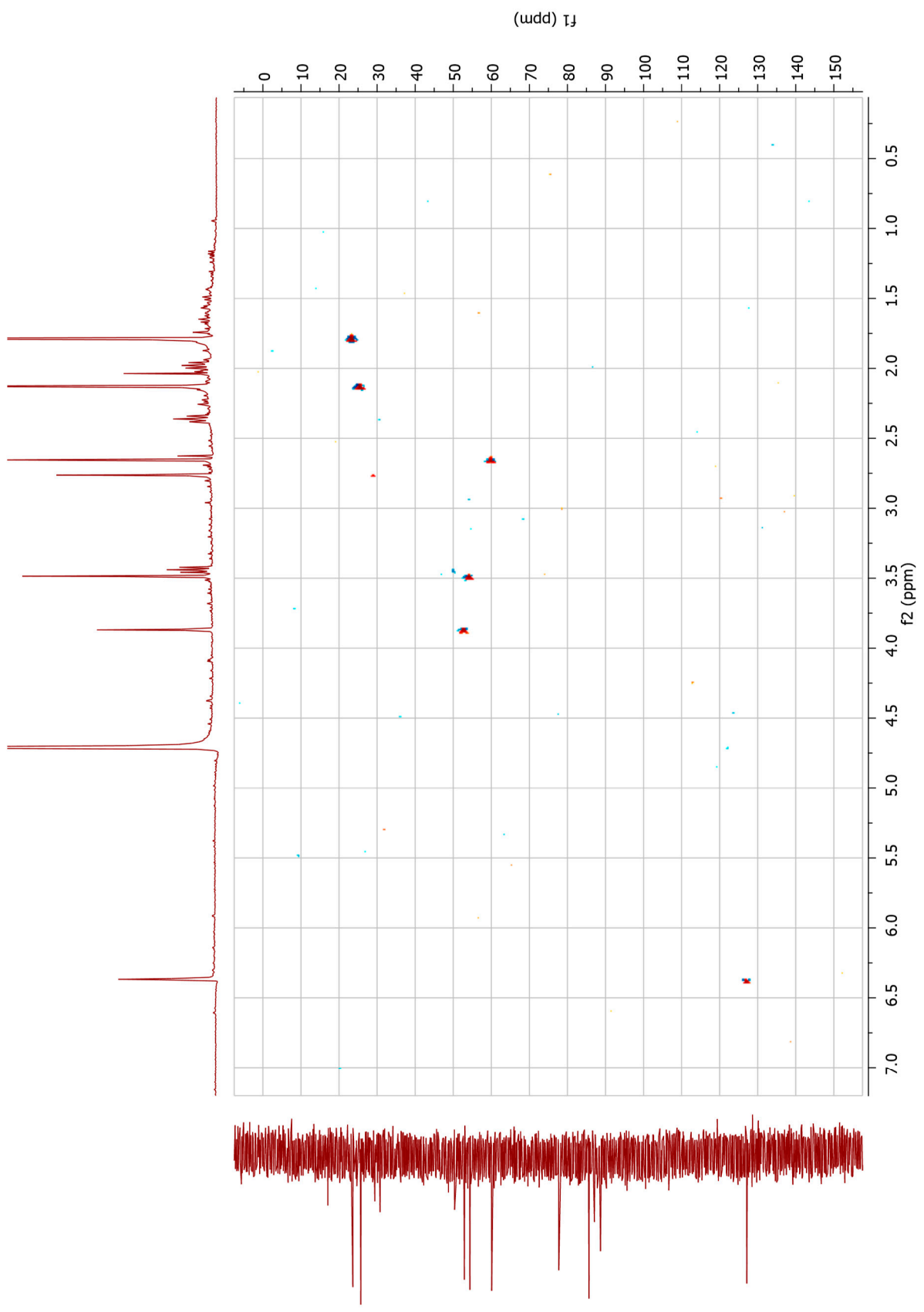

f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f2 (ppm)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

Figure S *15*. HMQC spectrum of **6**.

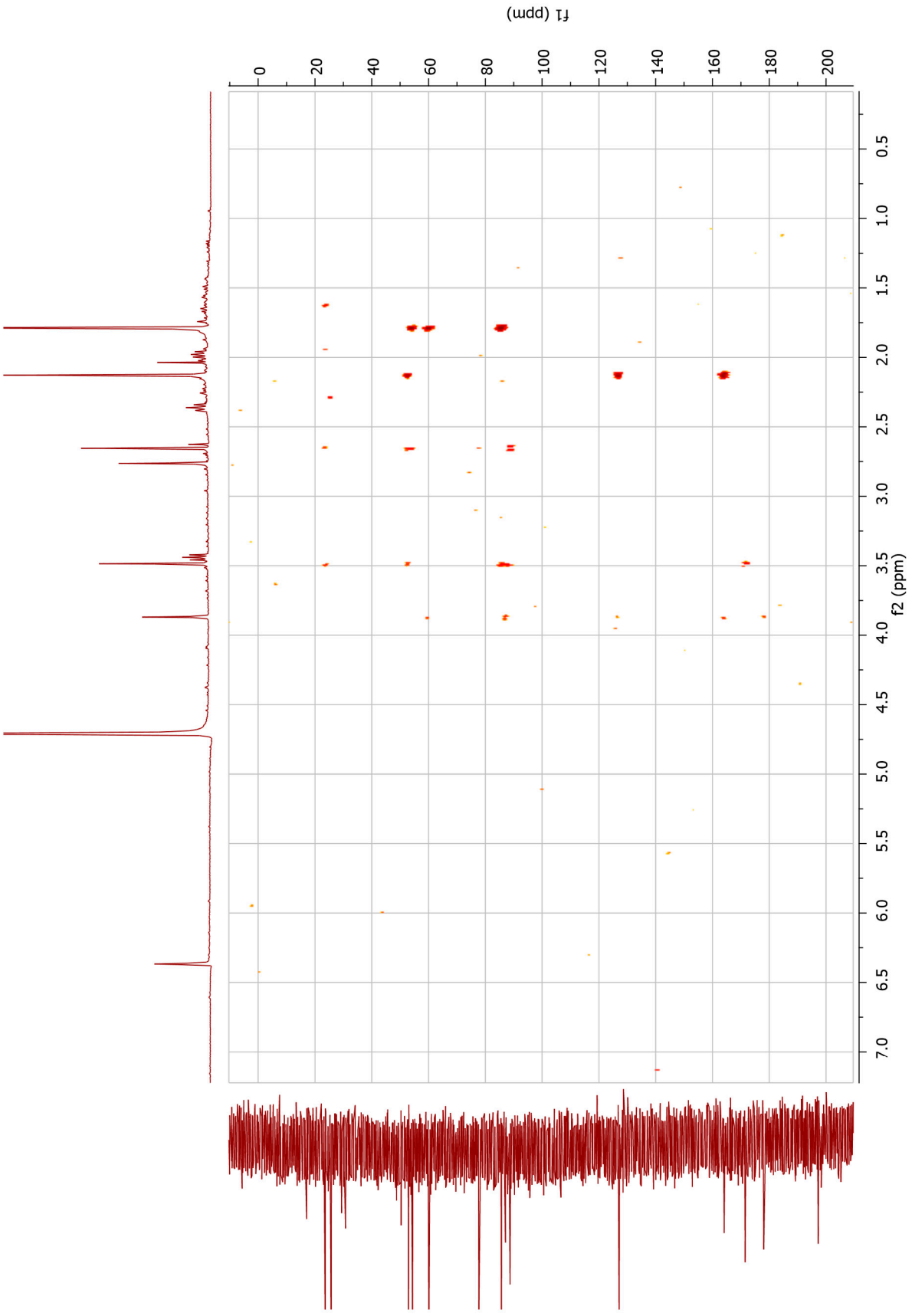
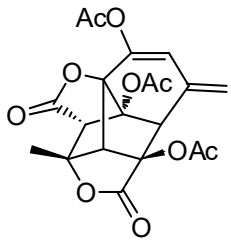


*Figure S 16. HMBC spectrum of **6**.*



7

Acetylated dimer 7: To a solution of dimer **6** (0.100 g, 0.32 mmol) in freshly distilled dichloromethane (2 mL) were added Et_3N (0.28 mL, 2.1 mmol) and Ac_2O (0.2 mL, 2.1 mmol). The mixture was stirred overnight at room temperature under a nitrogen atmosphere. After concentration *in vacuo*, water was added (10 mL) and the resulting solution extracted with EtOAc (2 x 10 mL). The organic layers were pooled, dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. A colorless oil was obtained (0.12 g, 90%). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ ppm 6.12 (s, 1H), 5.26 (s, 1H), 5.16 (s, 1H), 3.88 (s, 1H), 3.86 (s, 1H), 3.62 (s, 1H), 2.23 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.67 (s, 3H). **^{13}C NMR**: ($CDCl_3$, 100 MHz) δ ppm 170.9 (CO), 169.8 (CO), 169.7 (CO), 169.5 (CO), 169.1 (CO), 140.6 (C), 134.3 (C), 120.9 (C), 88.6 (C), 87.3 (C), 80.9 (C), 77.7 (C), 58.6 (CH), 55.5 (CH), 50.6 (CH), 23.2 (CH_3), 20.9 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20,5 (CH_3). **MS *m/z*** *:* 418 (1), 376 (20), 334 (7), 290 (2), 273 (3), 266 (1), 246 (2), 231 (3), 214 (6), 182 (23), 175 (3), 162 (6), 141 (28), 140 (100), 91 (1), 77 (2), 69 (2), 43 (100).

HRMS: 419.0950, calculated for $[M.H]^+$ $C_{20}H_{19}O_{10}$ 419.0978. Δ = −6.7 ppm.
377.0859, calculated for $[M(-CH_3CO+H).H]^+$ $C_{18}H_{17}O_9$ 377.0873. Δ = −3.7 ppm.
335,0754, calculated for $[M(-2CH_3CO+2H).H]^+$ $C_{16}H_{15}O_8$ 335,0767. Δ = −3,9 ppm.

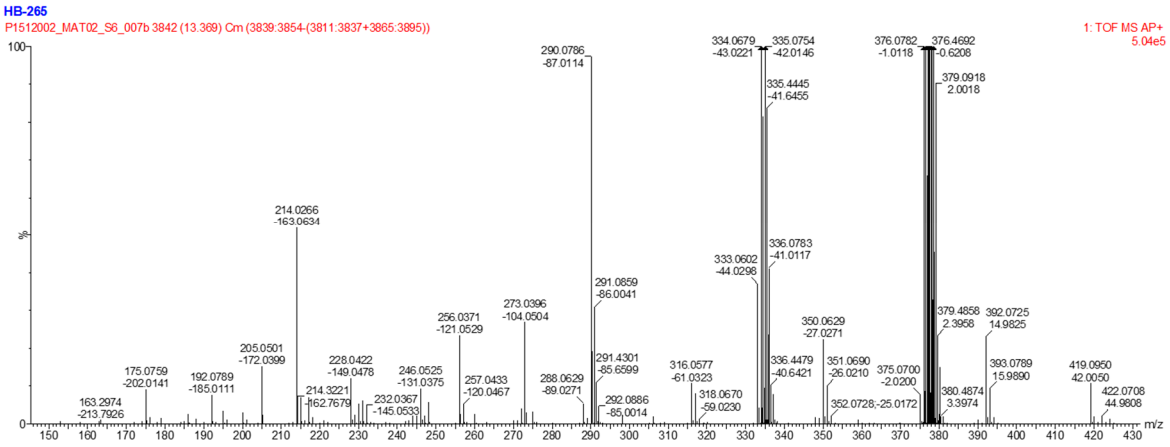


*Figure S 17. HMRS spectrum of **7**.*

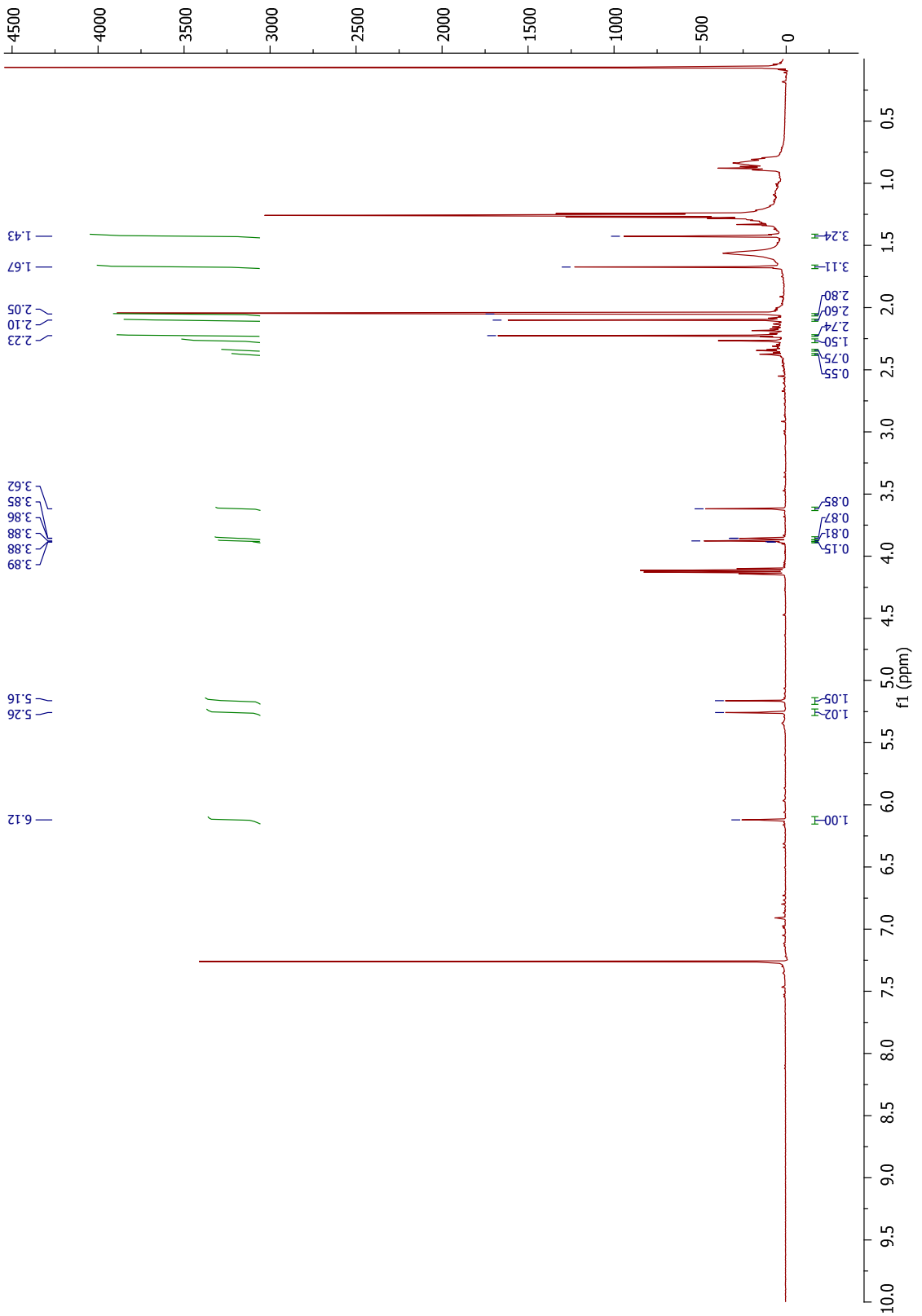


*Figure S 18. ^{1}H-NMR spectrum of **7**.*

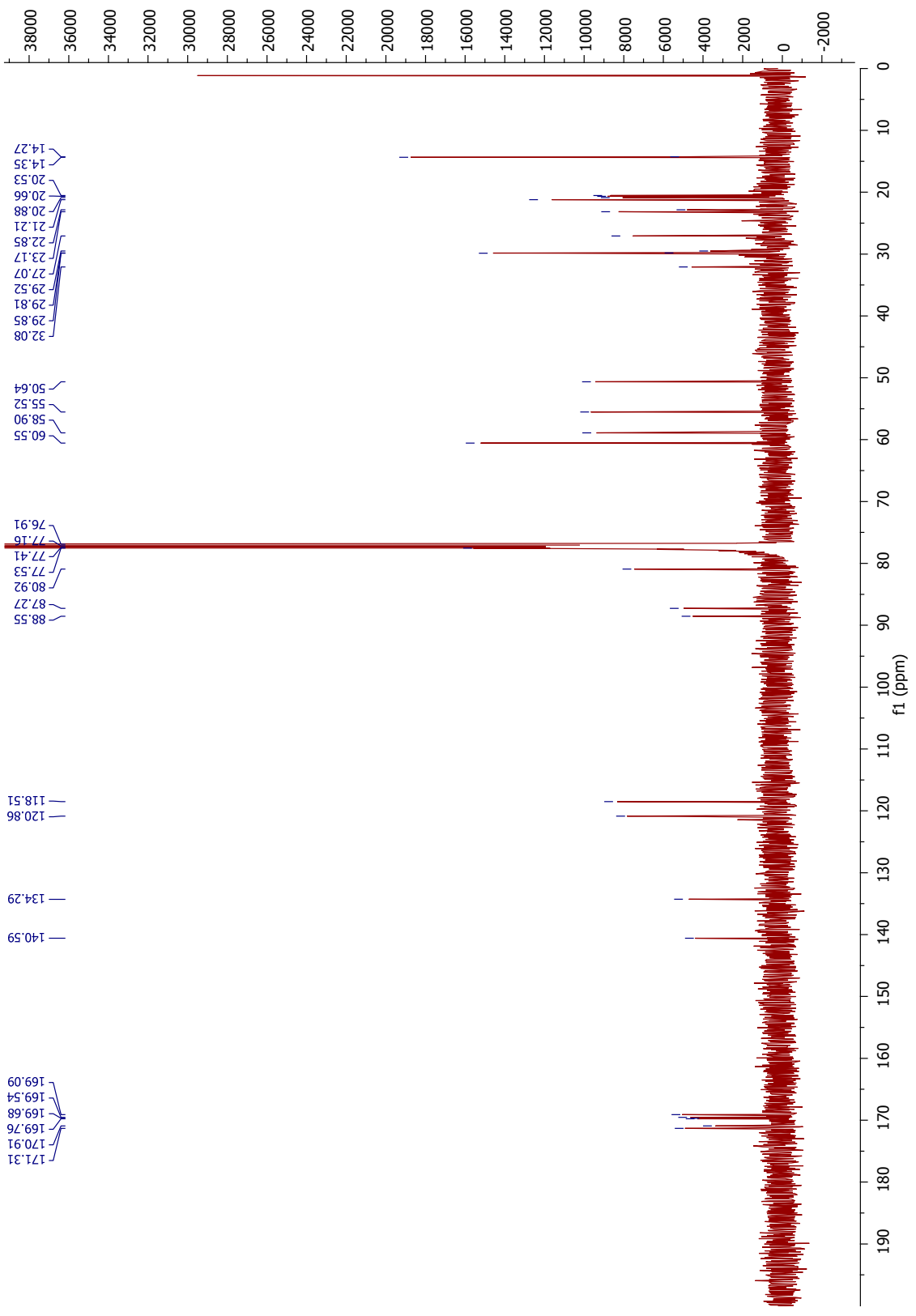


*Figure S 19. ^{13}C-NMR spectrum of **7**.*

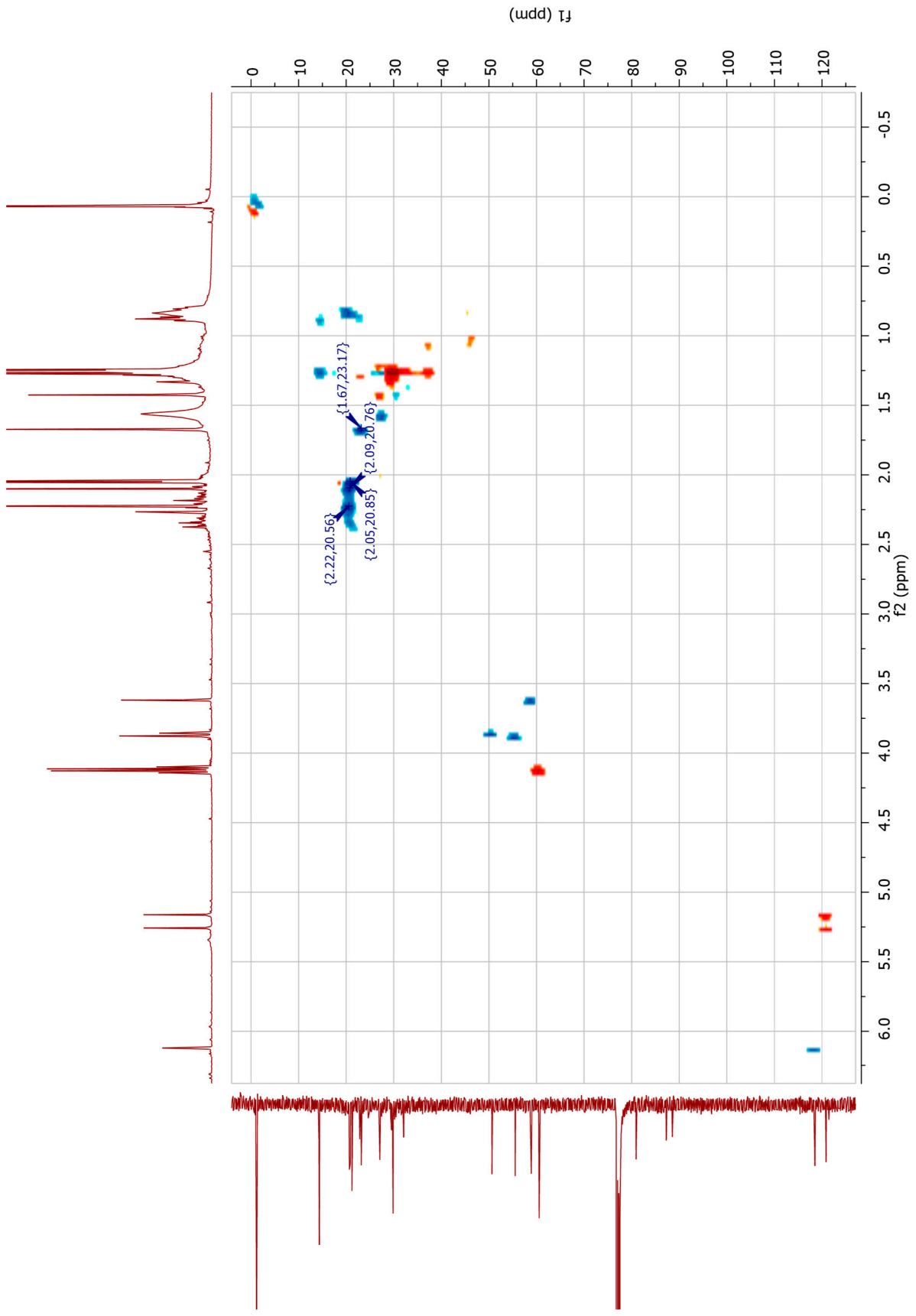


*Figure S 20. HMQC spectrum of **7**.*

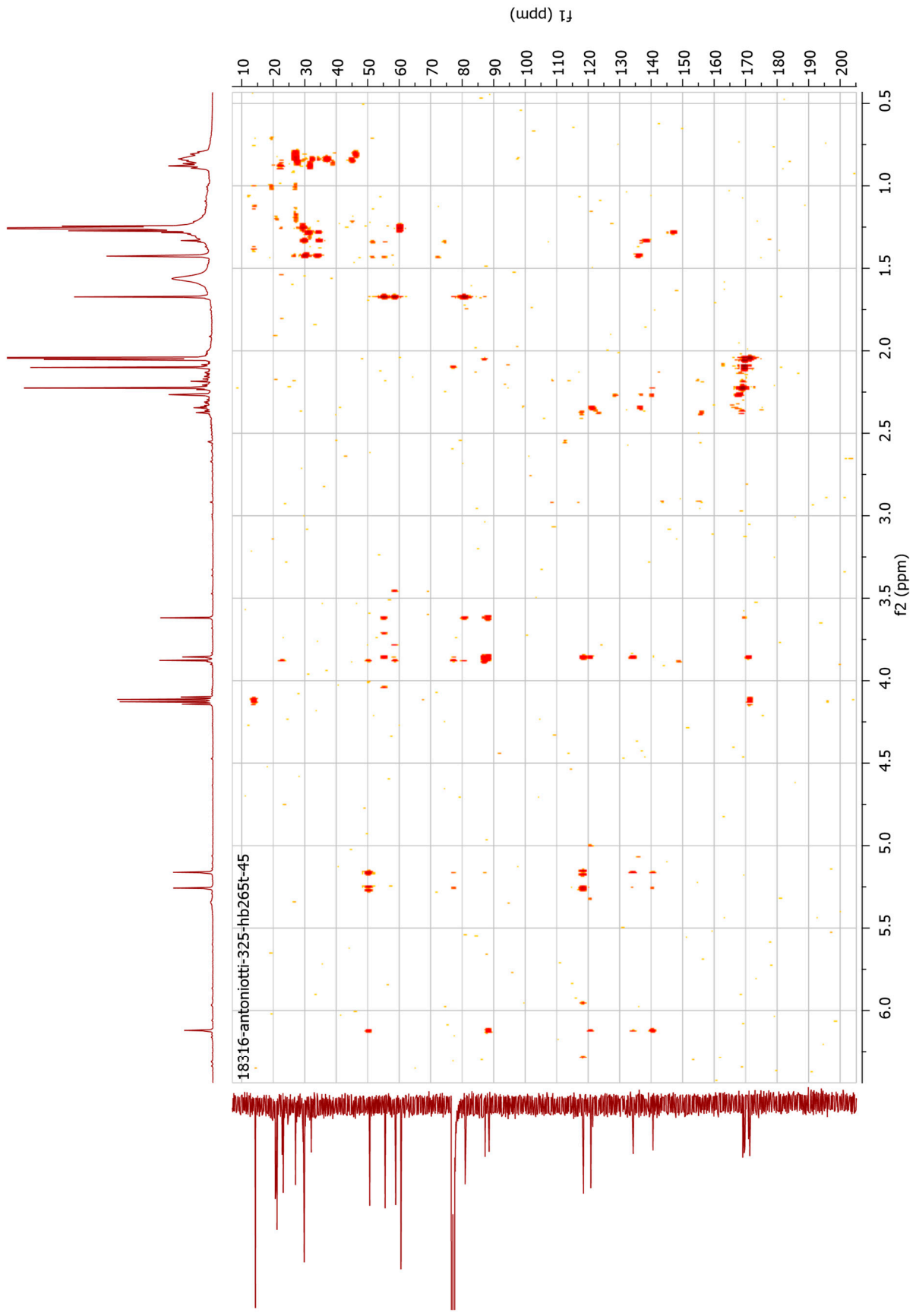


*Figure S 21. HMBC spectrum of **7**.*

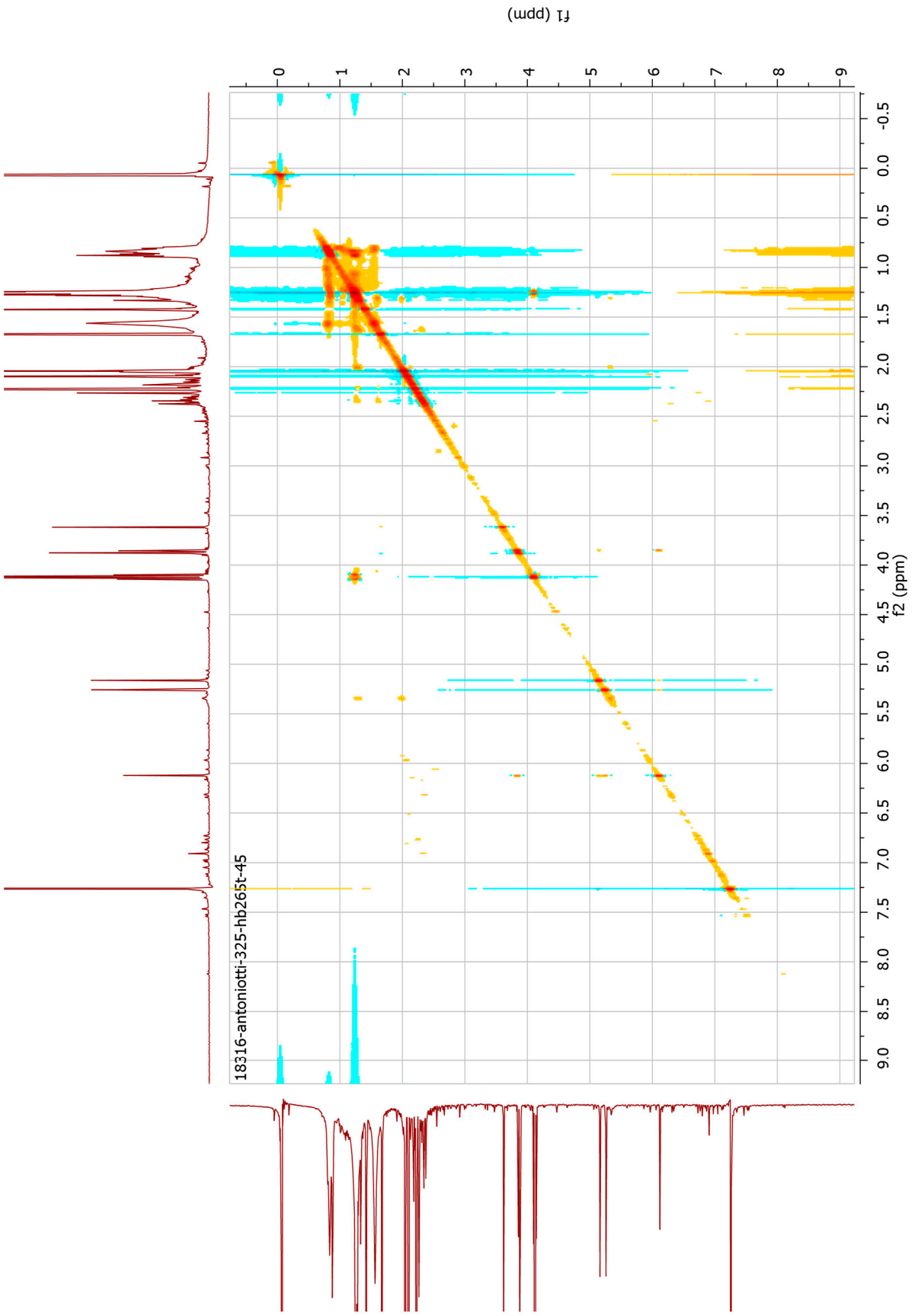


*Figure S 22. COSY of **7**.*

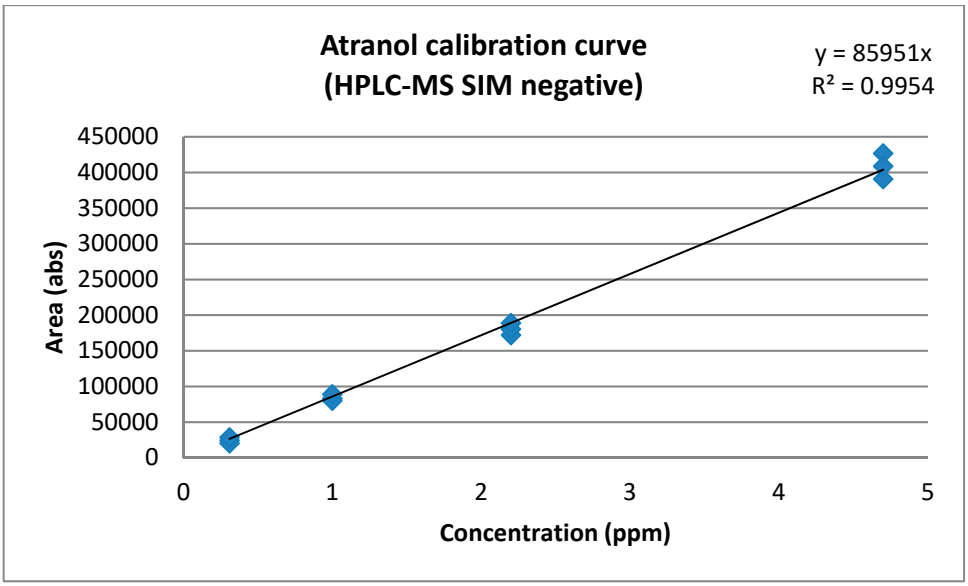


*Figure S 23. Calibration curve of **2**.*