SUPPORTING INFORMATION

Cannabizetol, a Novel Cannabinoid: Chemical Synthesis, Anti-inflammatory Activity and Extraction from *Cannabis sativa* L.

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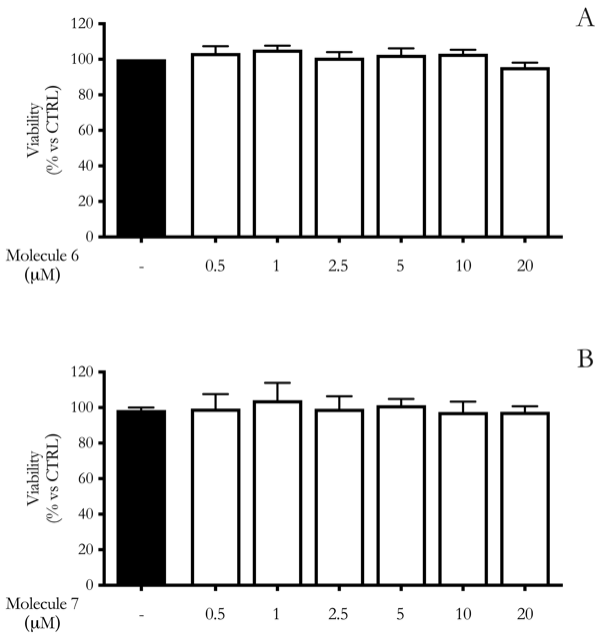
Contents | Pages

1. Continuous reaction conditions screening for cannabizetol

Supplementary Table S1: Reaction conditions screening for continuous synthesis of cannabizetol (**7**).

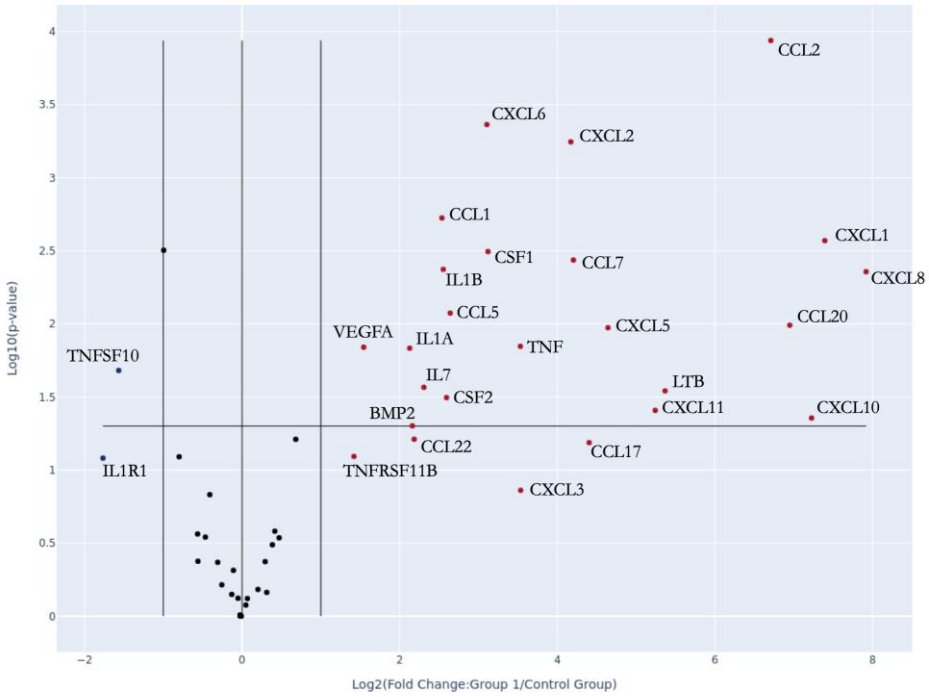
Entry	T (°C)	P (bar)	Res. time (min)	Molar ratio CBG:CH_2O	Catalyst Molar Ratio CBG:Cat.	Yield (%)
1	100	5	90	1:3	-	no product
2	120	5	90	1:3	-	traces
3	140	7	90	1:3	-	6
4	140	7	180	1:3	-	6
5	140	7	90	1:100	-	traces
6	140	7	90	1:0.5	-	12
7	140	7	90	1:0.5	*p*TSA (1:0.1)	degradation
8	140	7	90	1:0.5	TEA (1:5)	20
9	140	7	90	1:0.5	TEA (1:0.5)	12
10	140	7	90	1:0.5	TEA (1:1)	12
11	140	7	90	1:0.5	TEA (1:10)	12
12	140	7	60	1:0.5	TEA (1:5)	9
13	140	7	30	1:0.5	TEA (1:5)	3

2. Cytotoxicity tests

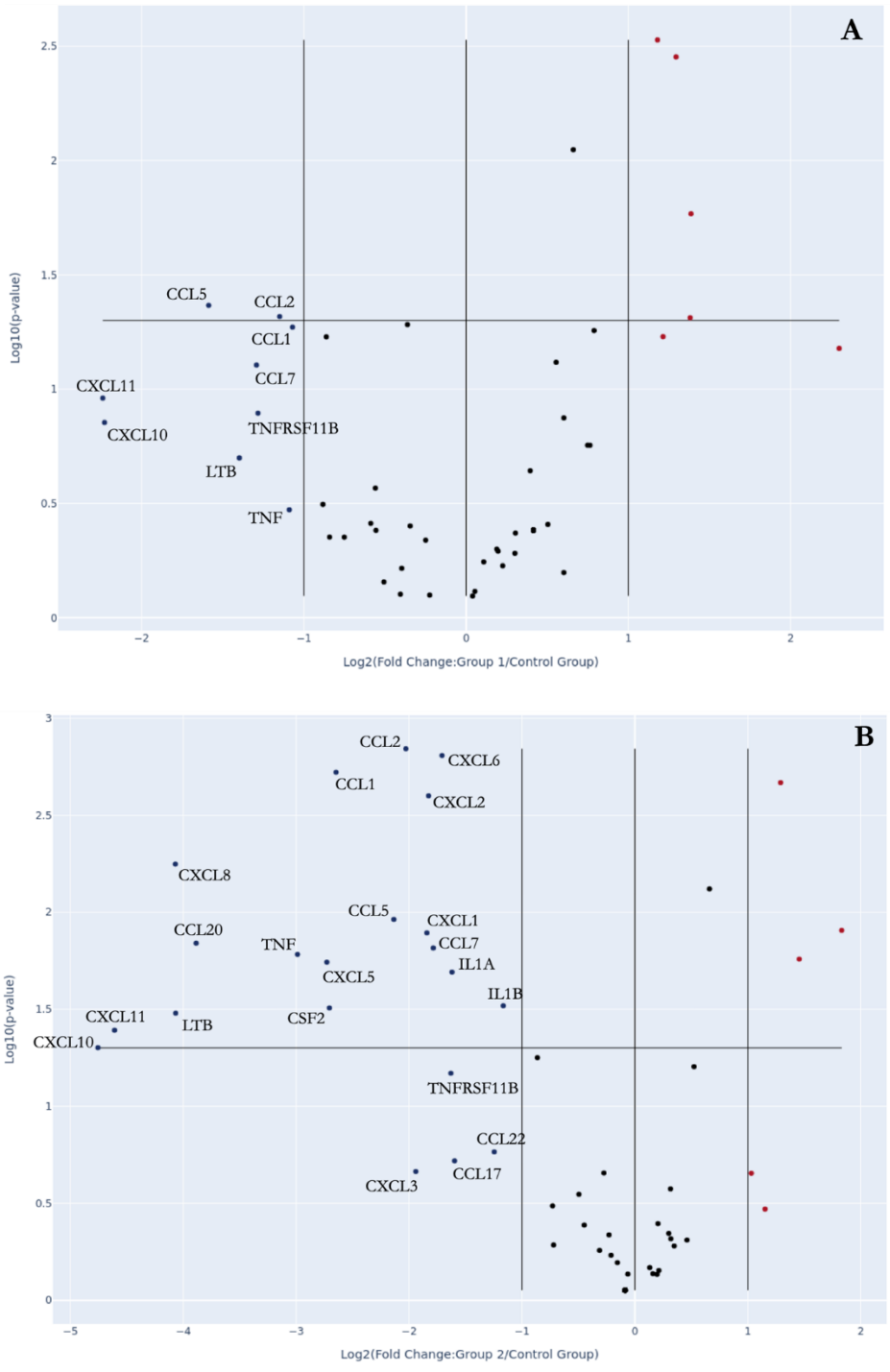


Supplementary Figure S1. Cytotoxicity assay (MTT test) of cannabinoids **6** (A) and **7** (B), in HaCaT cells treated with concentrations ranging from 0.5 to 20 μM. Data are reported as percentage in comparison to the control (mean ± SEM), which was arbitrarily assigned 100% value.

3. Gene expression tests

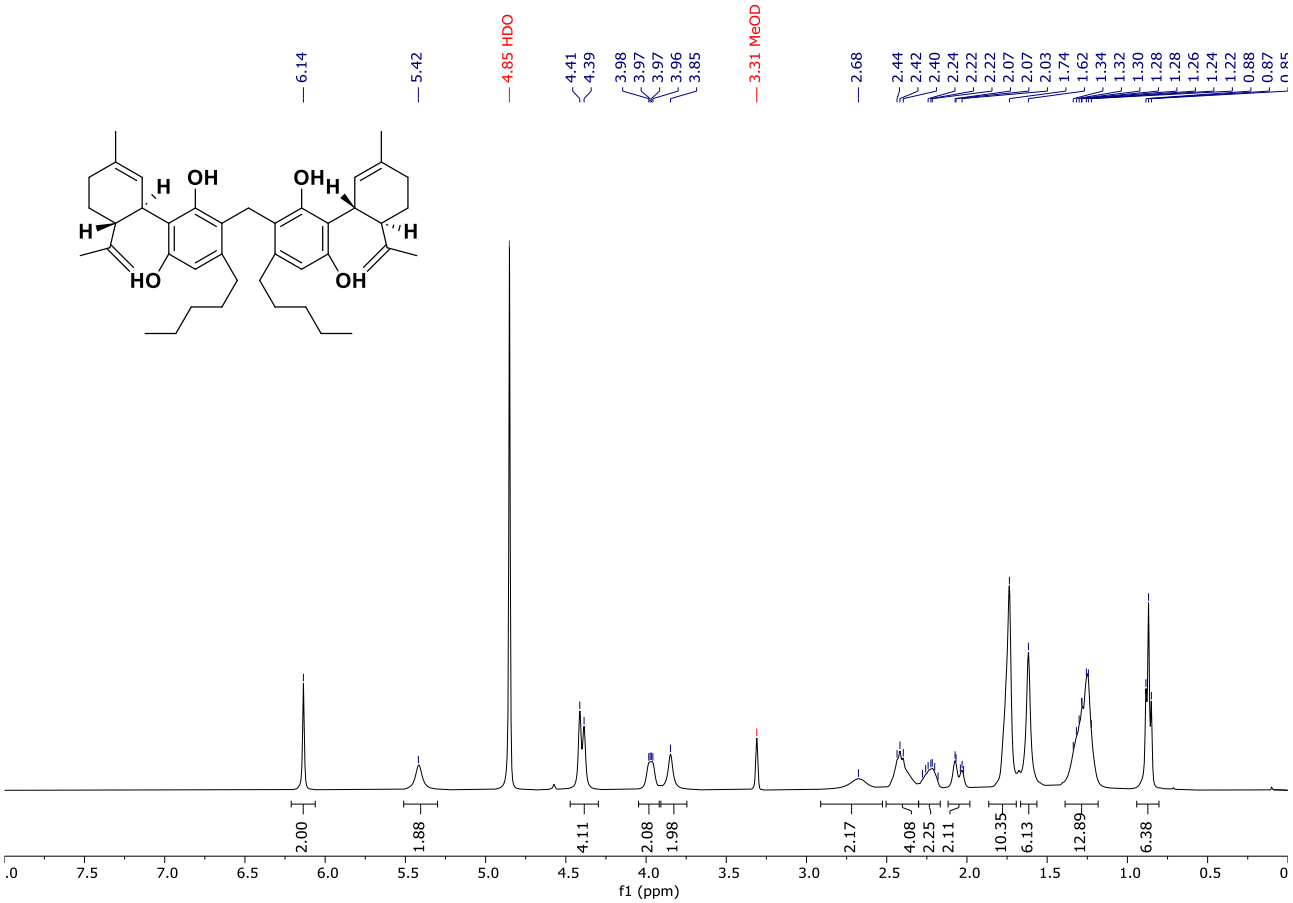


Supplementary Figure S2. Effect of TNFα (10 ng/mL) on gene expression of 84 genes related to the inflammatory process. The Volcano Plot identifies significant gene expression changes by plotting the $\log_2$ of the fold changes in gene expression on the x-axis versus their statistical significance on the y-axis (p-value threshold: 0.05). The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold (fold regulation threshold: 2).

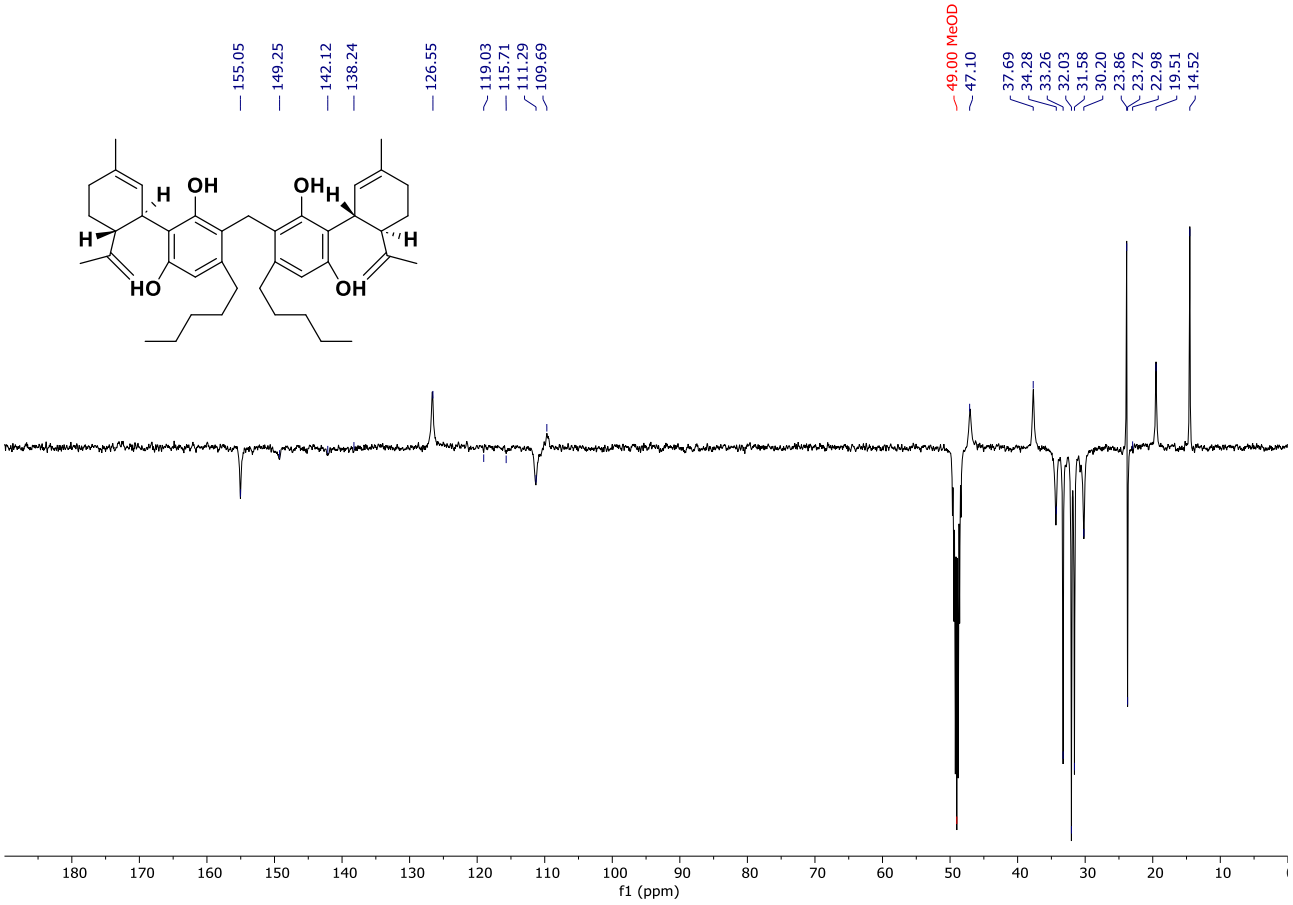


Supplementary Figure S3. Effect of cannabigerol (A) and compound **7** (B) at concentration of 5 μM on TNFα-induced gene expression of 84 genes related to the inflammatory process. The Volcano Plot identifies significant gene expression changes by plotting the $\log_2$ of the fold changes in gene expression on the x-axis versus their statistical significance on the y-axis (p-value threshold: 0.05). The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold (fold regulation threshold: 2).

4. [1]H and APT NMR spectra of cannabitwinol (6)

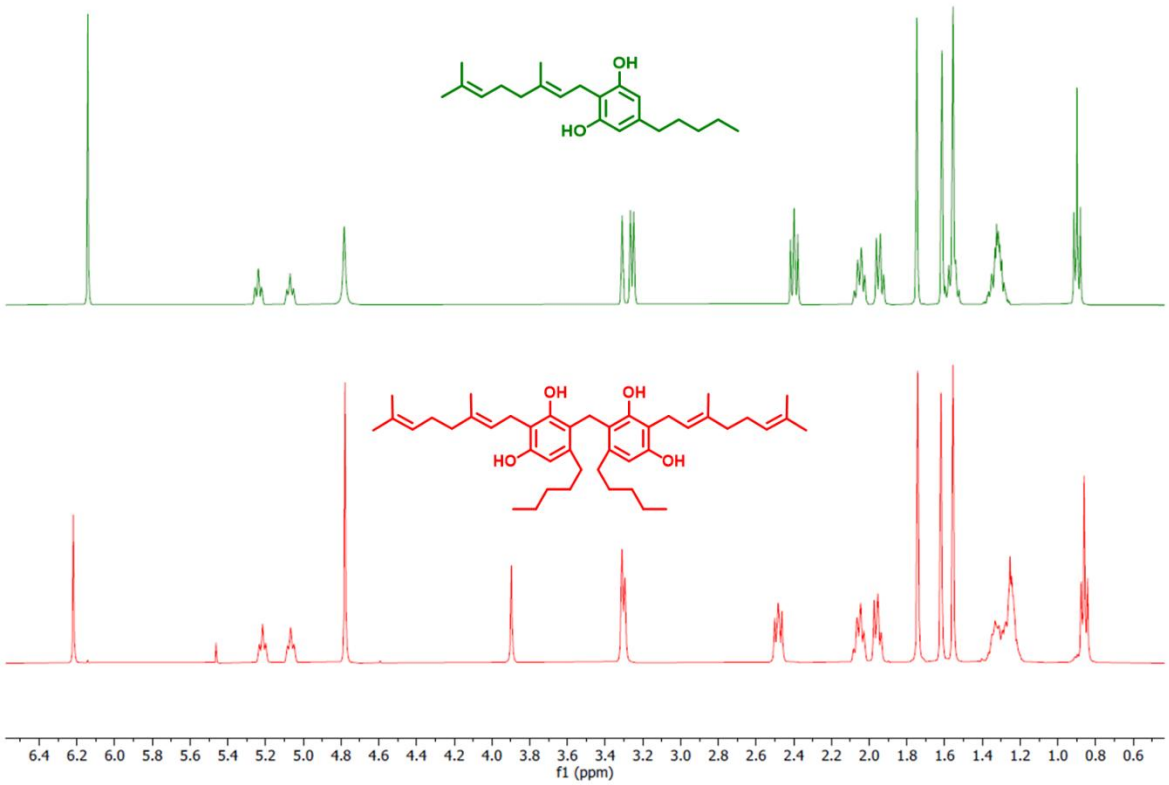


Supplementary Figure S4. [1]H-NMR spectra (400 MHz, CD_3OD) of **6.**

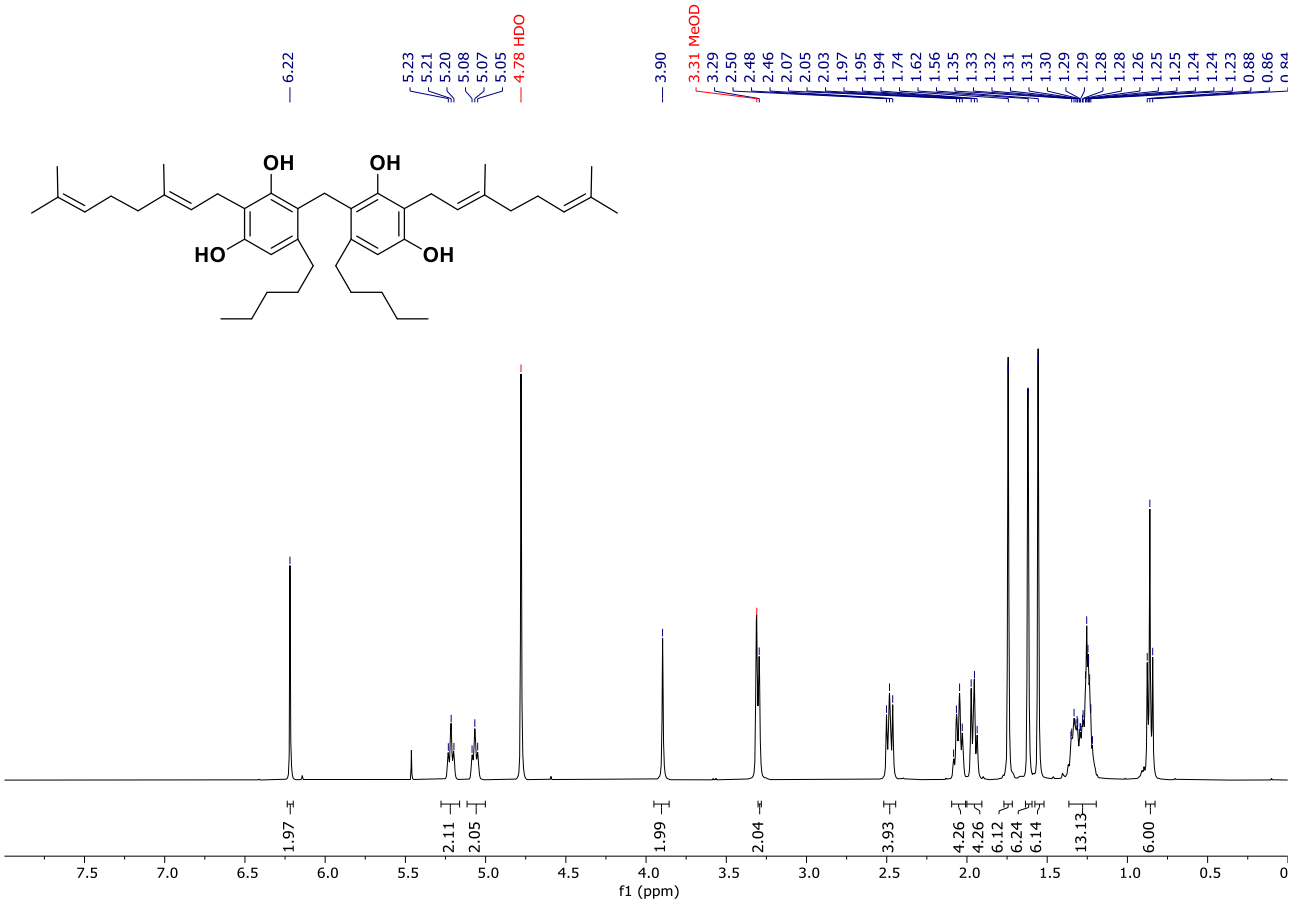


Supplementary Figure S5. APT NMR spectra (101 MHz, CD_3OD) of **6.**

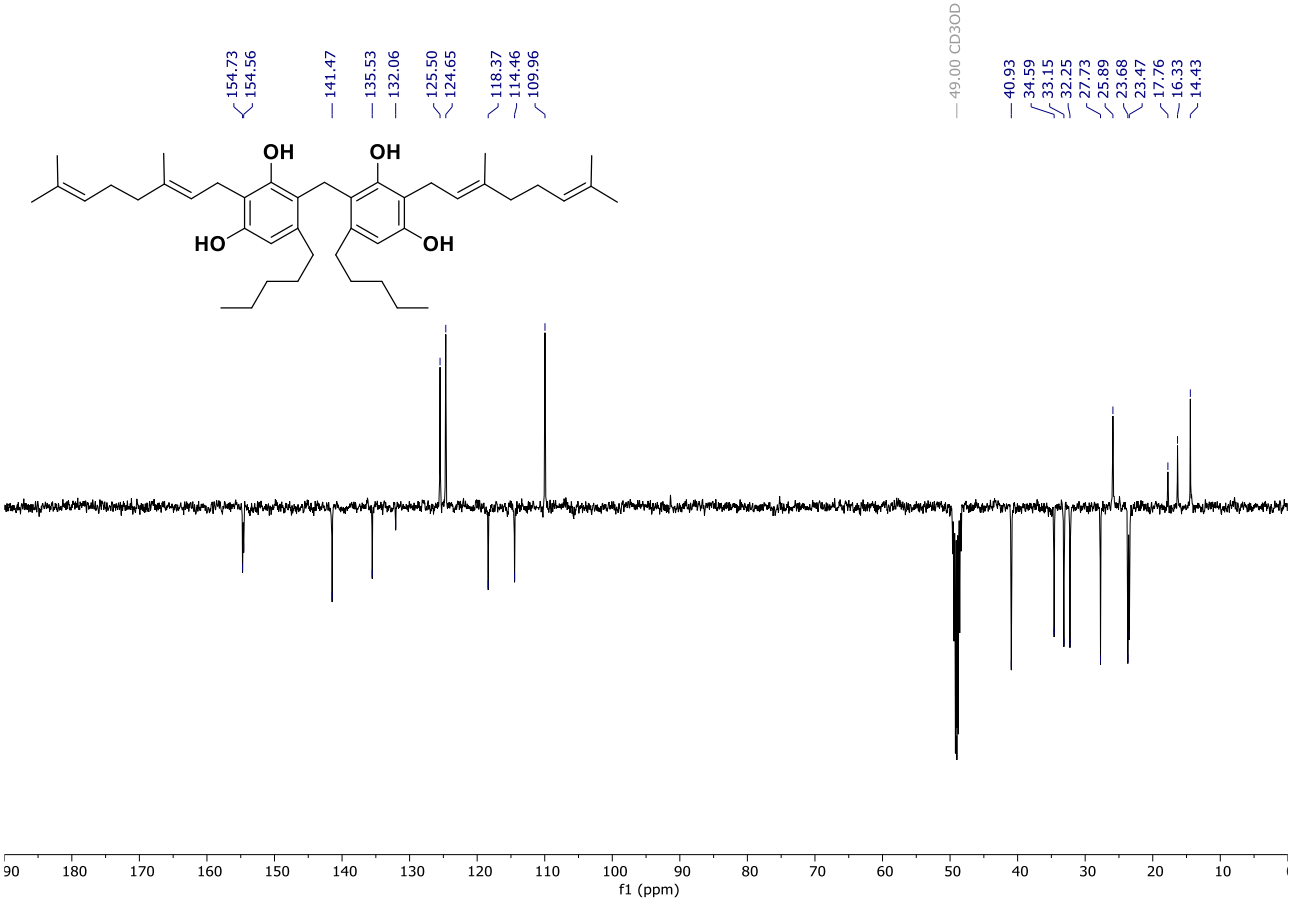
5. ^{1}H, APT and 2D NMR spectra of cannabizetol (7)



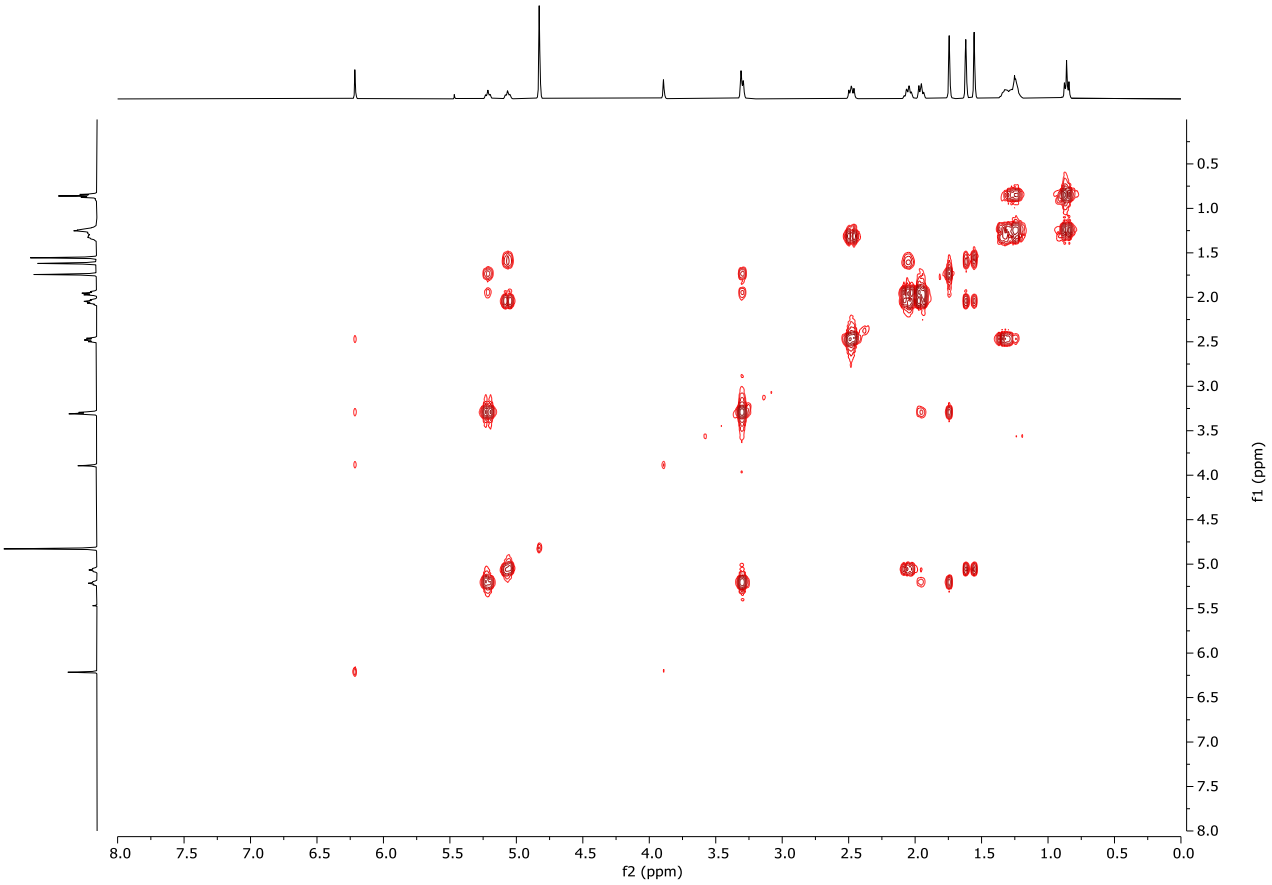
Supplementary Figure S6. Comparison of the ^{1}H NMR (400 MHz) spectra of CBG (on top, in green) and cannabizetol (on bottom, in red) in MeOH-d_4 at 25 °C.



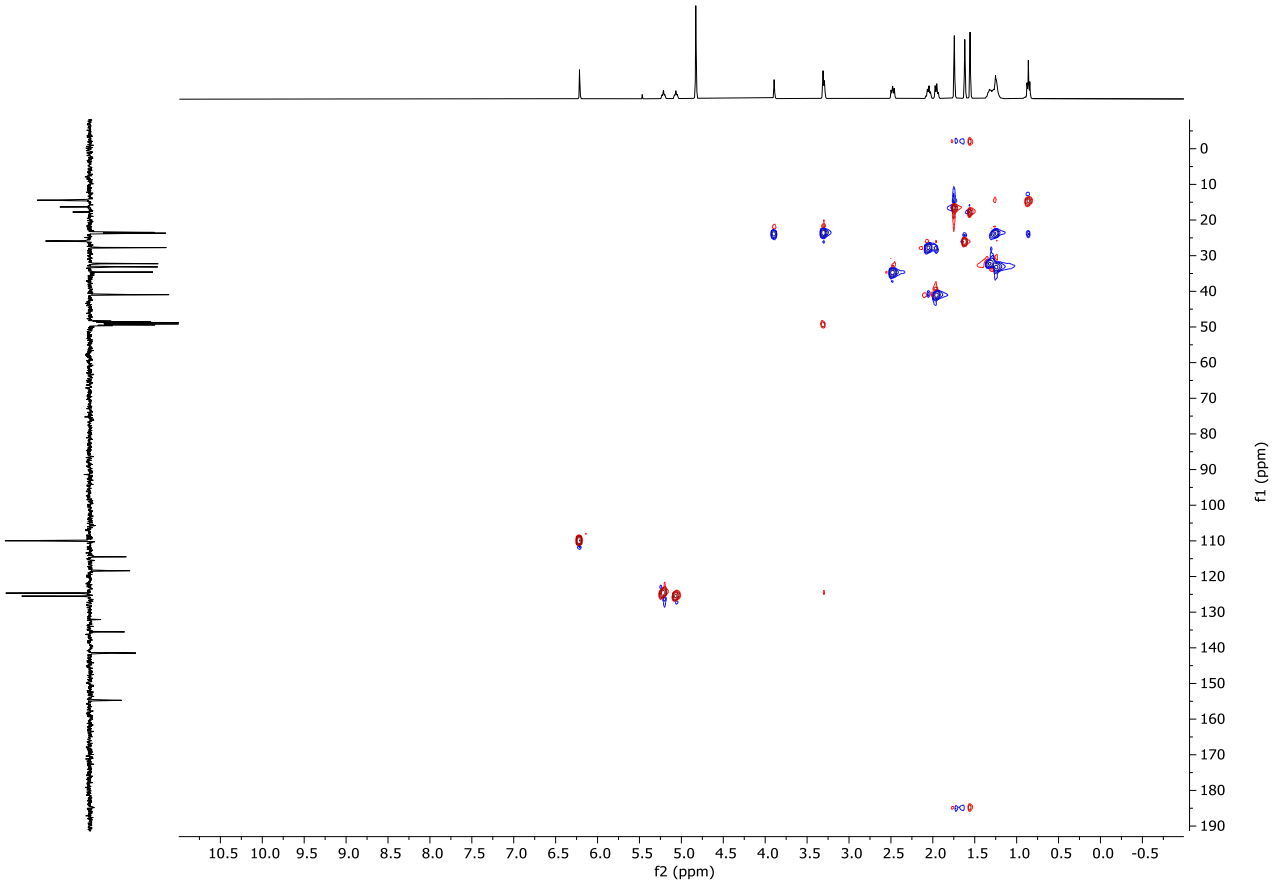
Supplementary Figure S7. ^{1}H-NMR spectra (400 MHz, MeOH-d_4) of **7.**



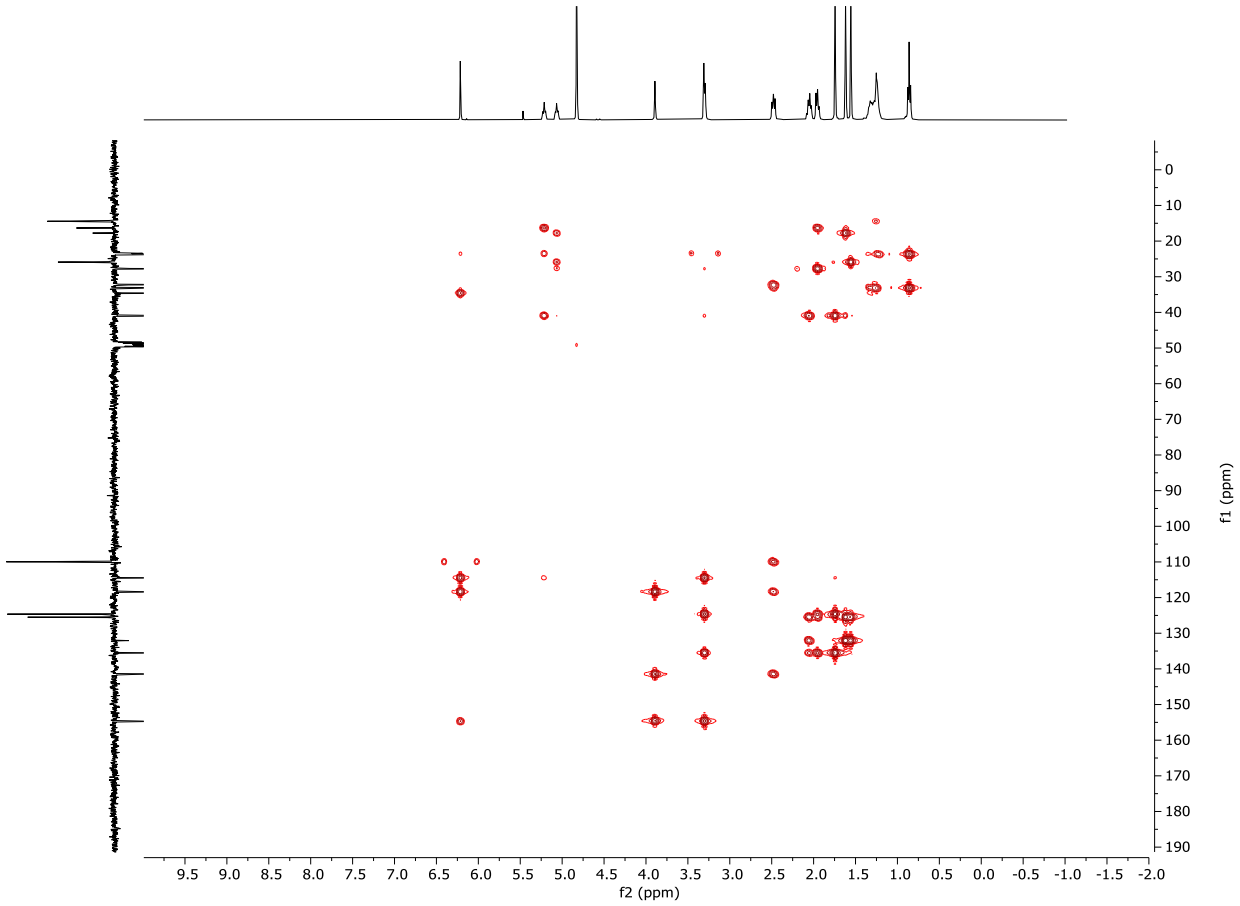
Supplementary Figure S8. APT NMR spectra (101 MHz, MeOH-d_4) of **7.**



Supplementary Figure S9. 2D NMR COSY spectra of compound **7** in MeOH-d_4.

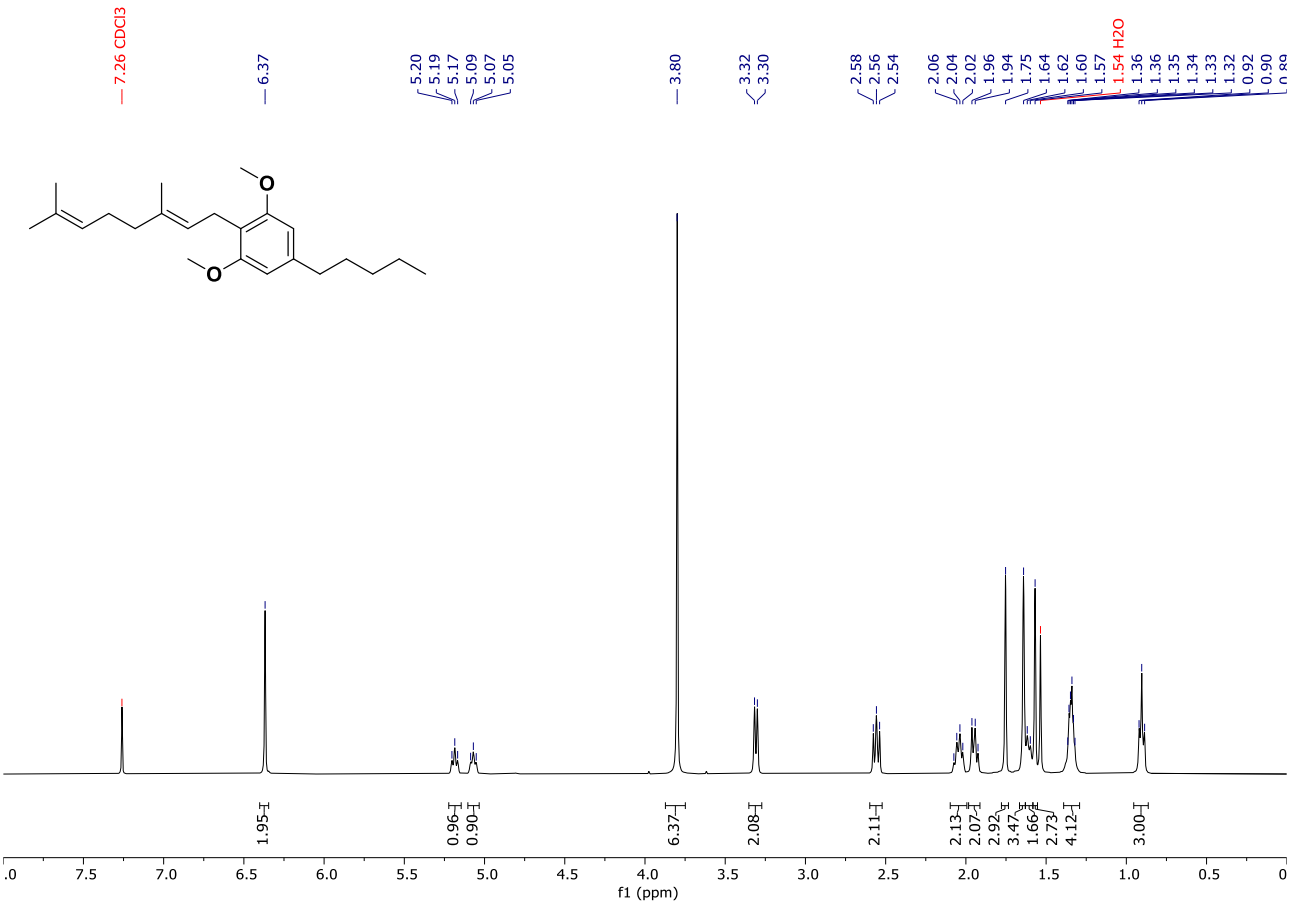


Supplementary Figure S10. 2D NMR HSQC spectra of compound **7** in MeOH-d_4.

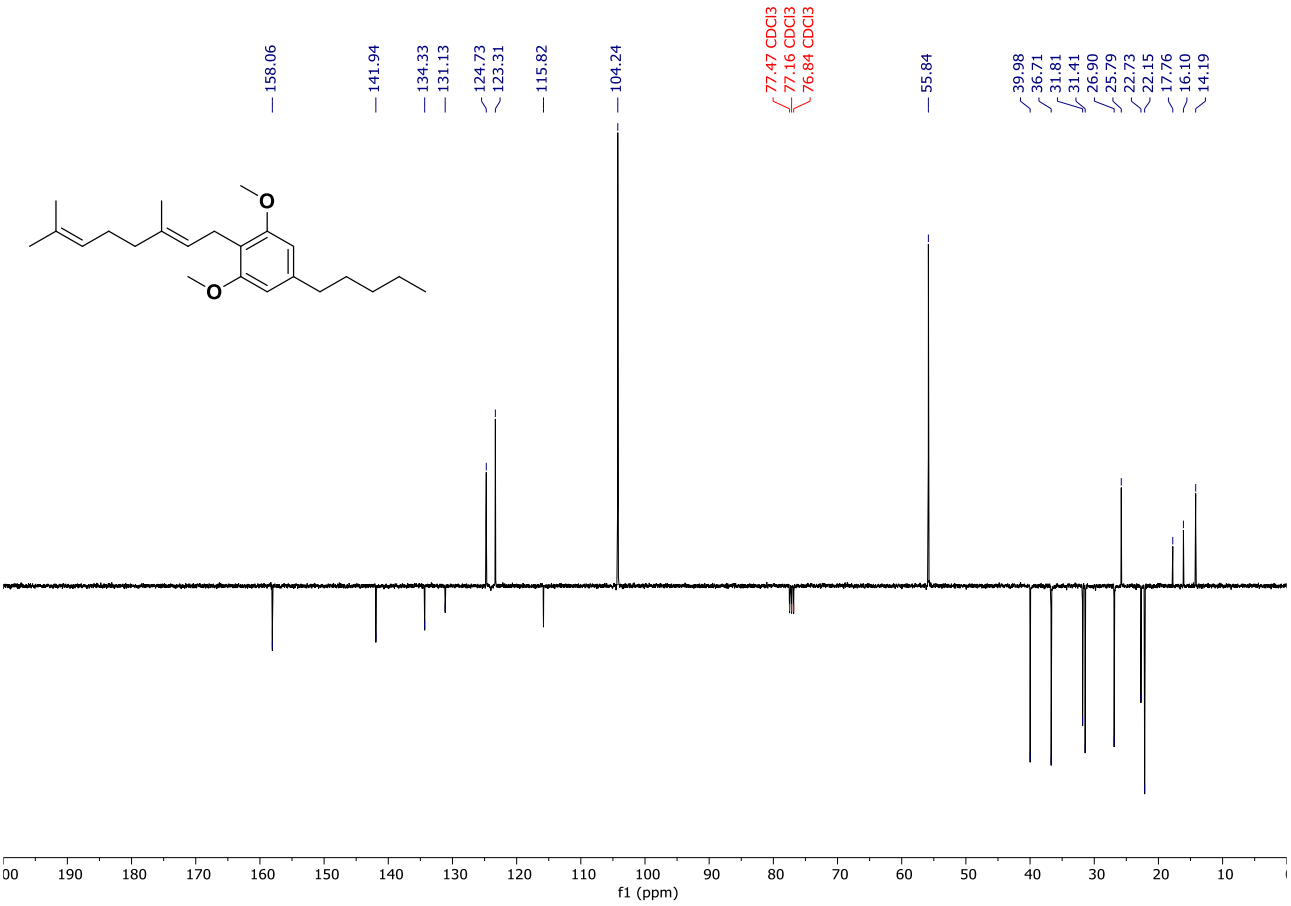


Supplementary Figure S11. 2D NMR HMBC spectra of compound **7** in MeOH-d_4.

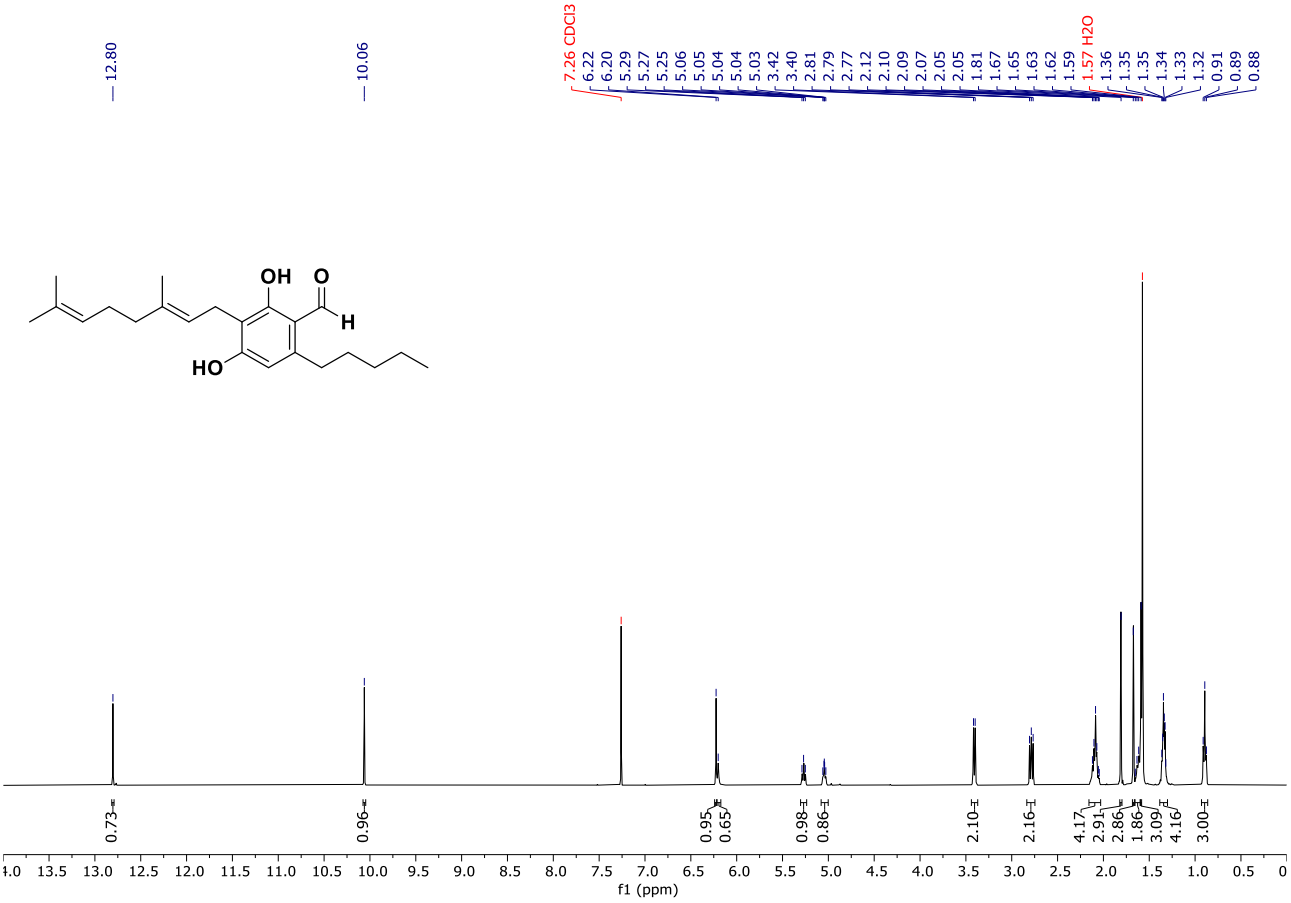
6. [1]H and APT NMR spectra of intermediates 8, 9, 10, 11, 12



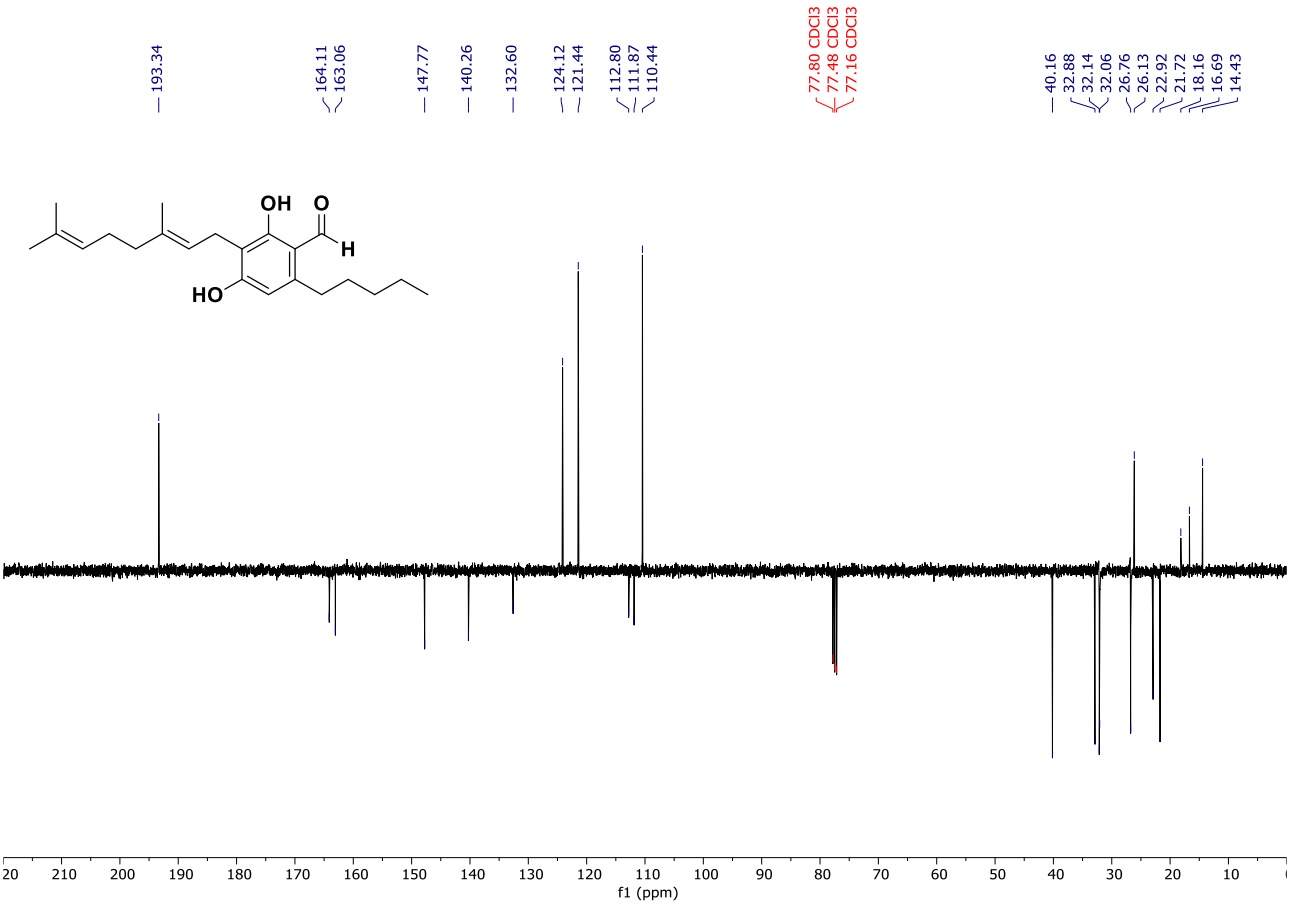
Supplementary Figure 12. [1]H-NMR spectra (400 MHz, $CDCl_3$) of **8.**



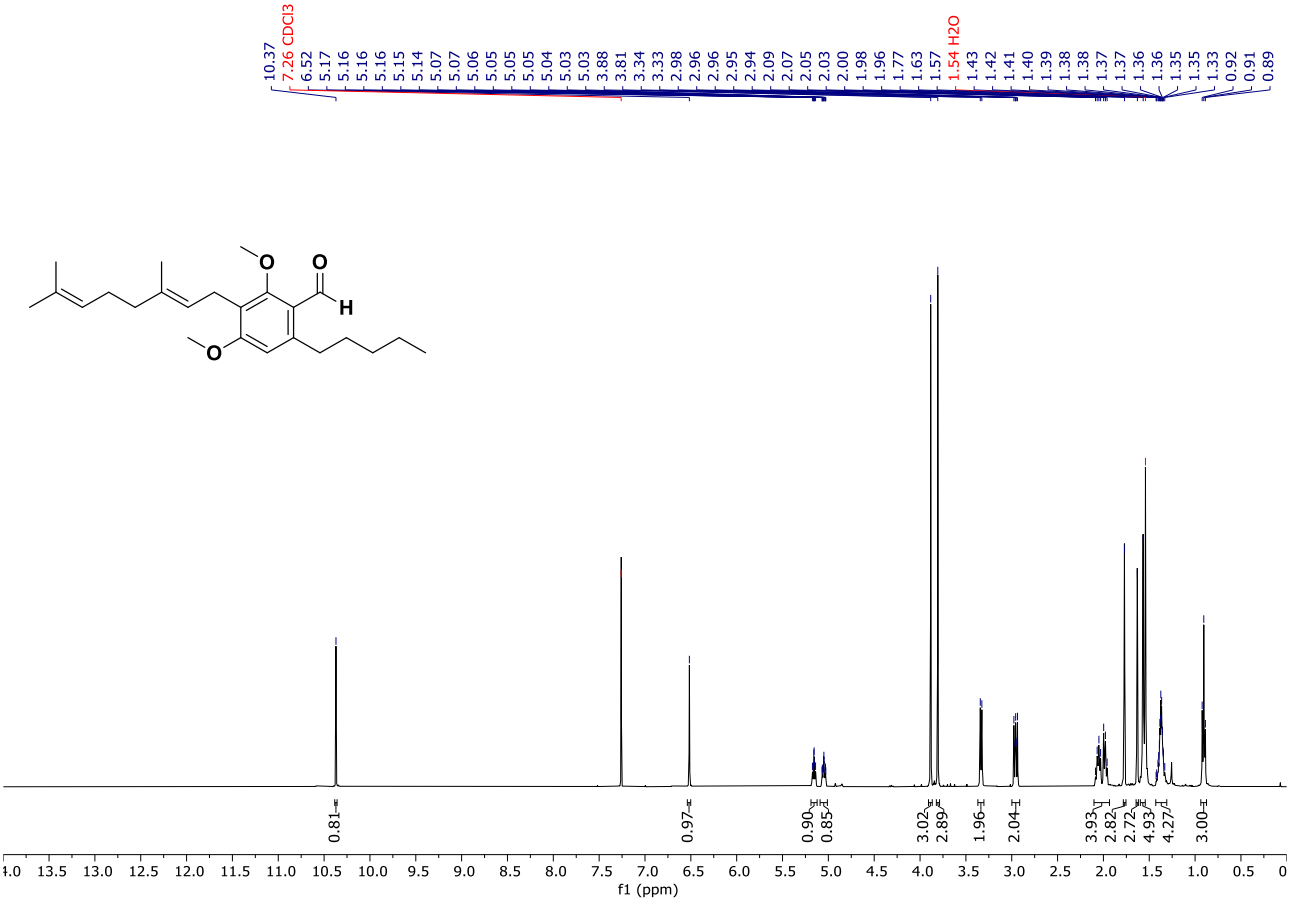
Supplementary Figure S13. APT NMR spectra (101 MHz, $CDCl_3$) of **8.**



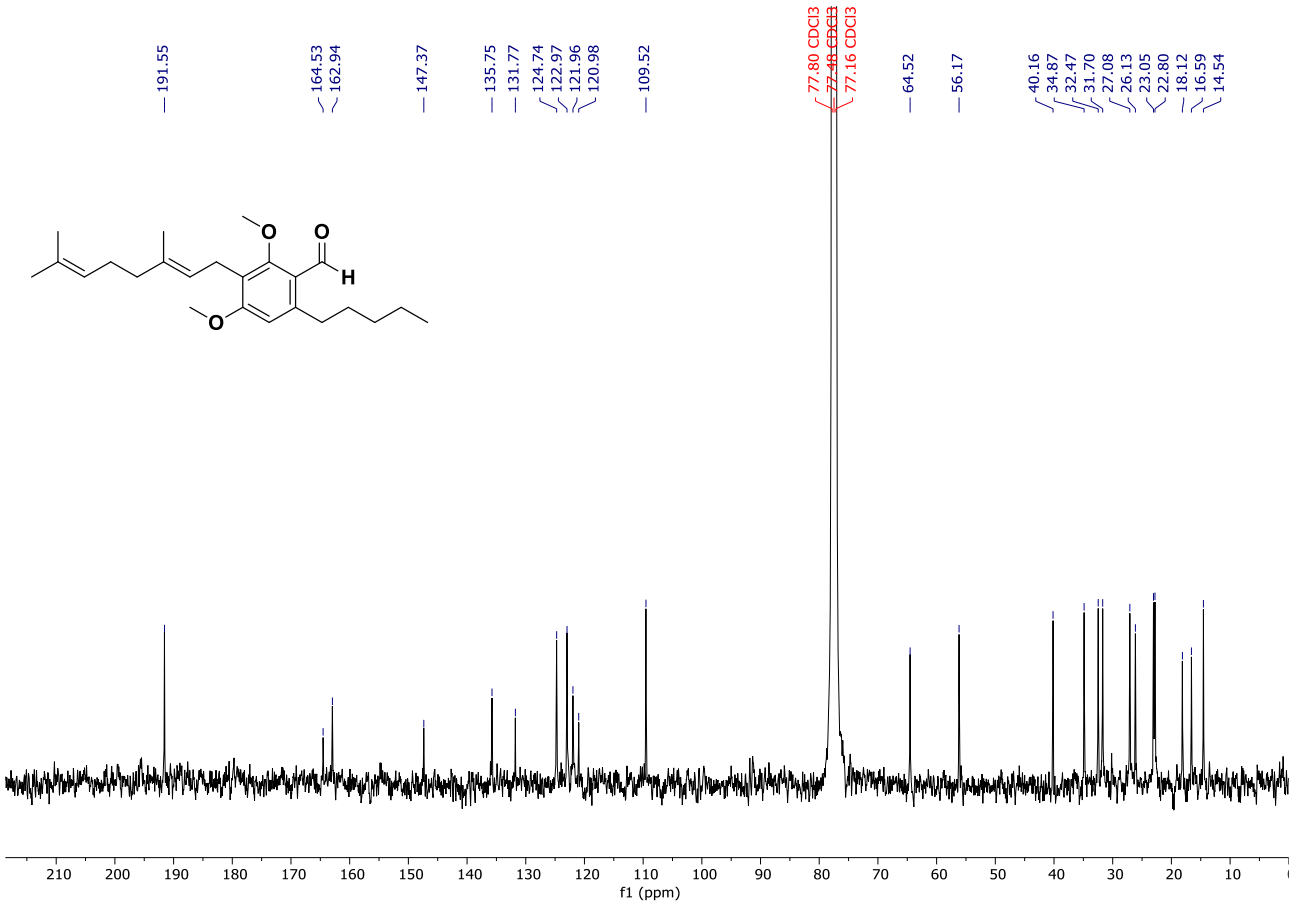
Supplementary Figure S14. ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of **9.**



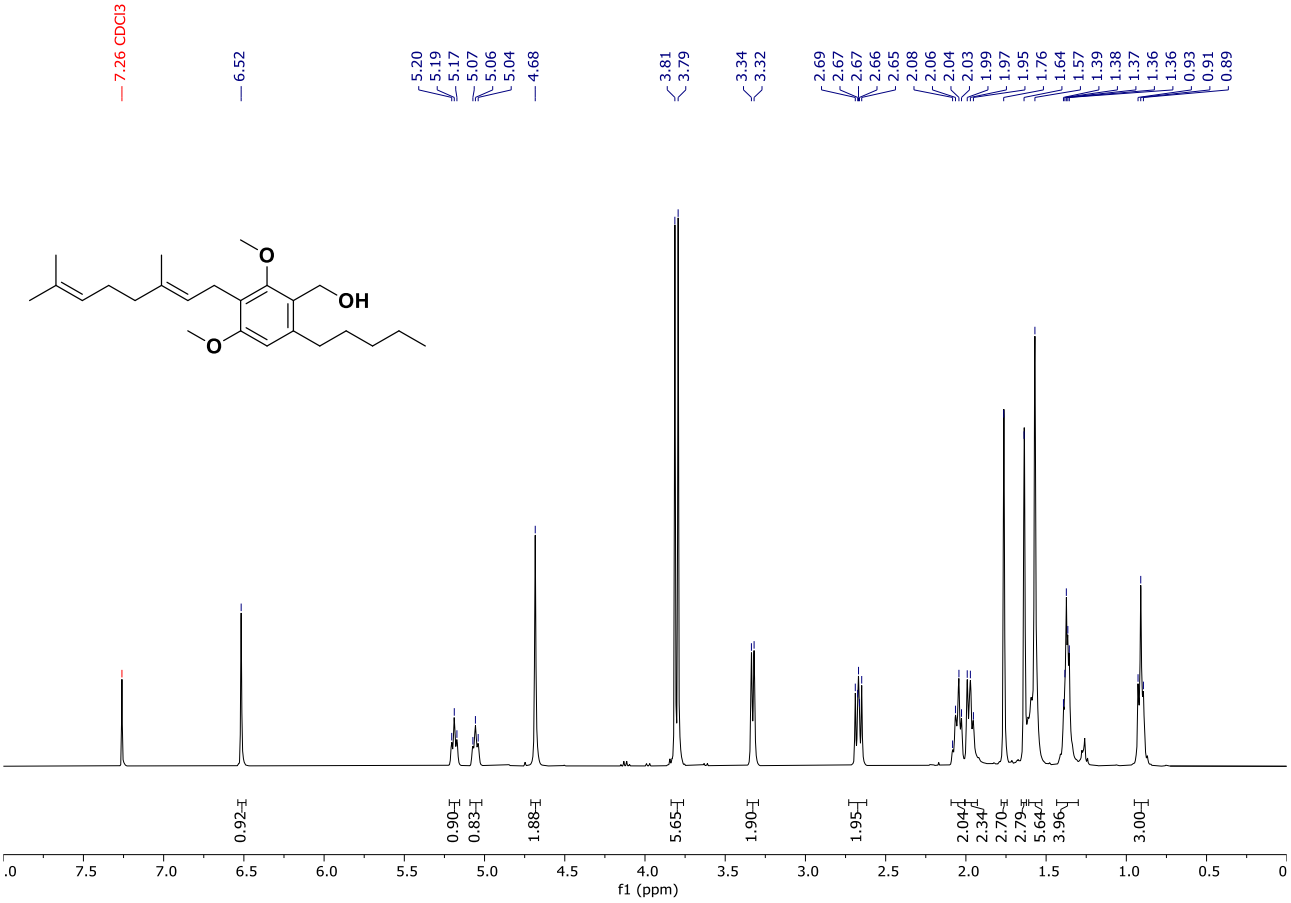
Supplementary Figure S15. APT NMR spectra (101 MHz, $CDCl_3$) of **9.**



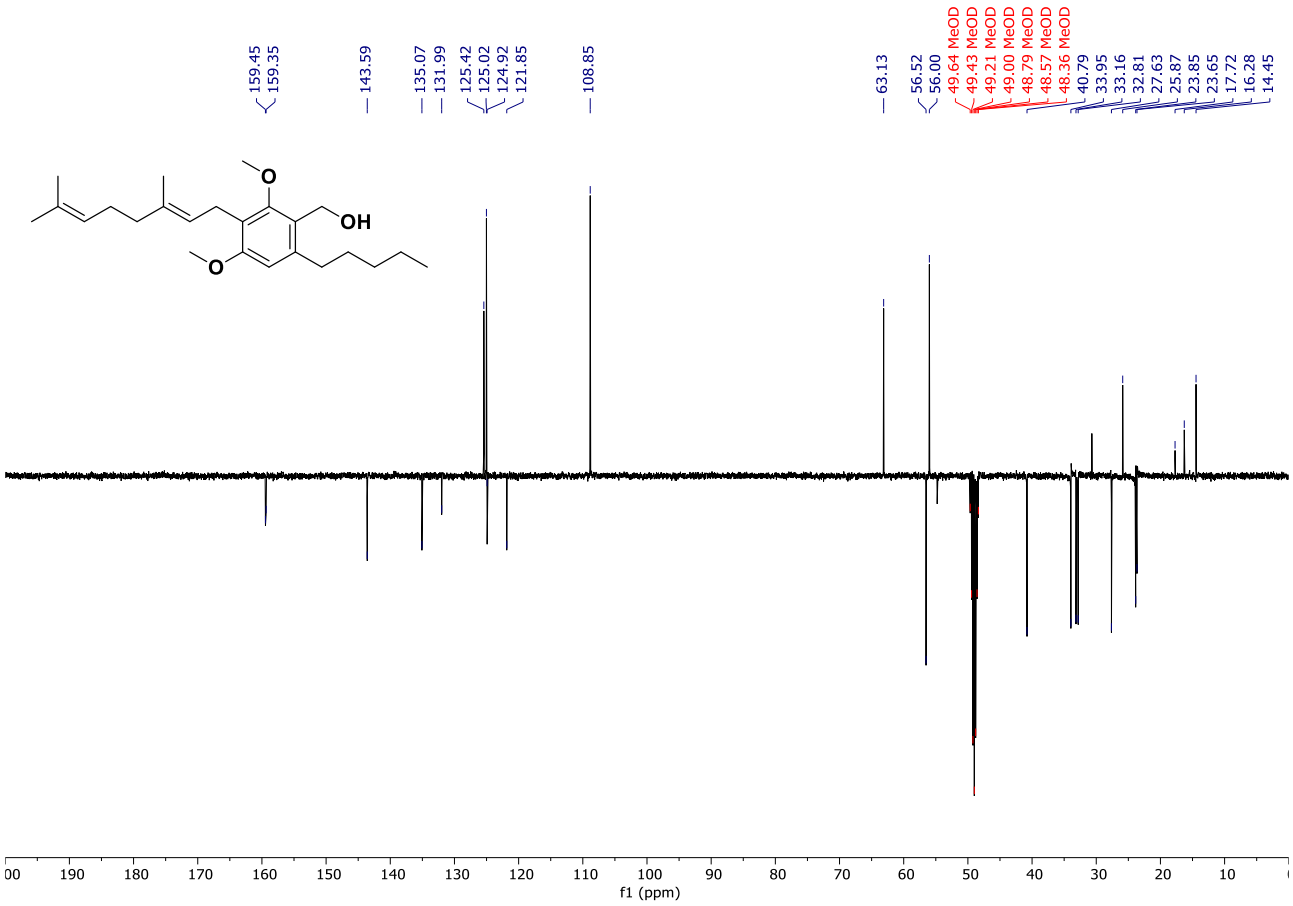
Supplementary Figure S16. ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of **10.**



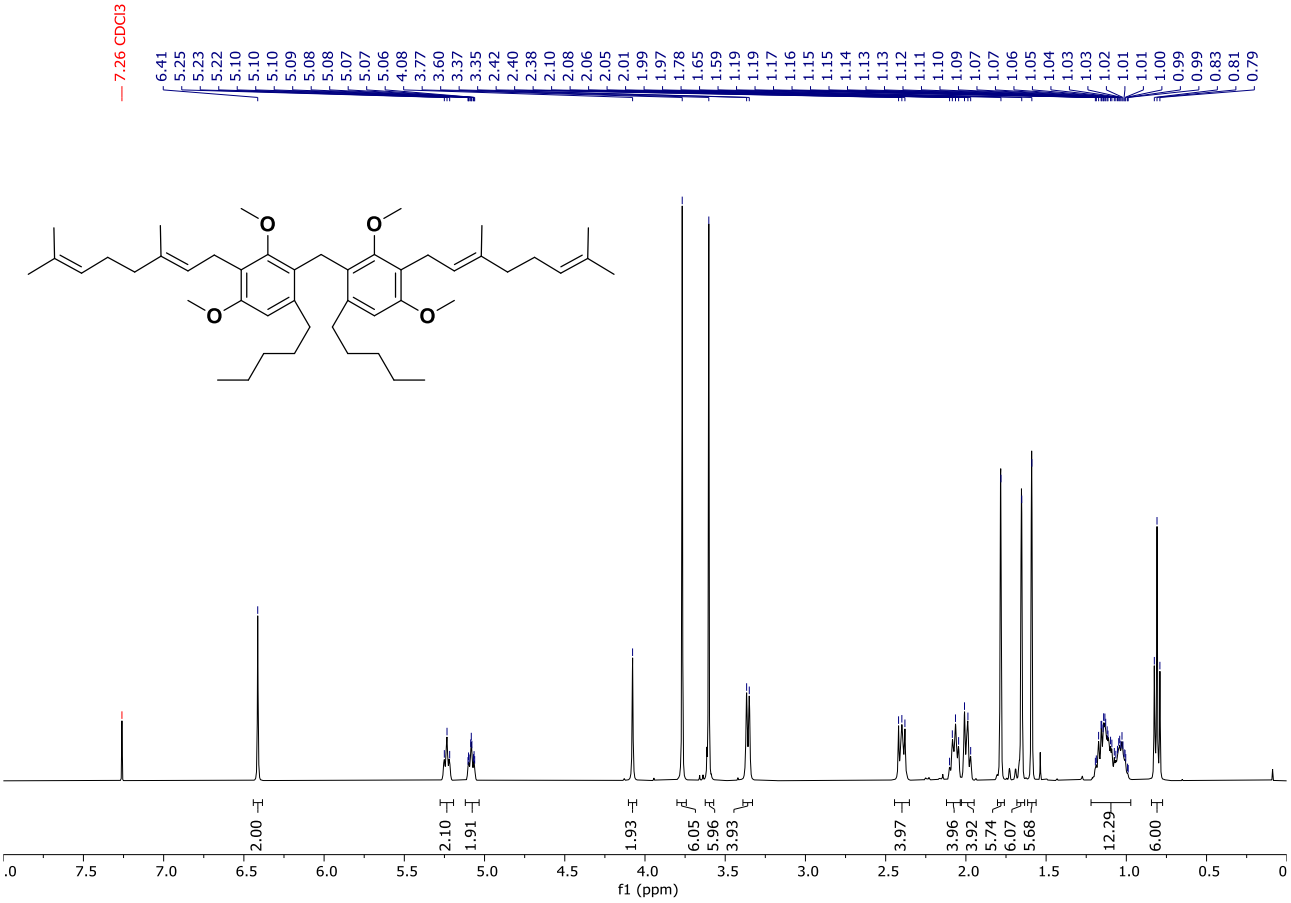
Supplementary Figure S17. ZGDC NMR spectra (101 MHz, $CDCl_3$) of **10.**



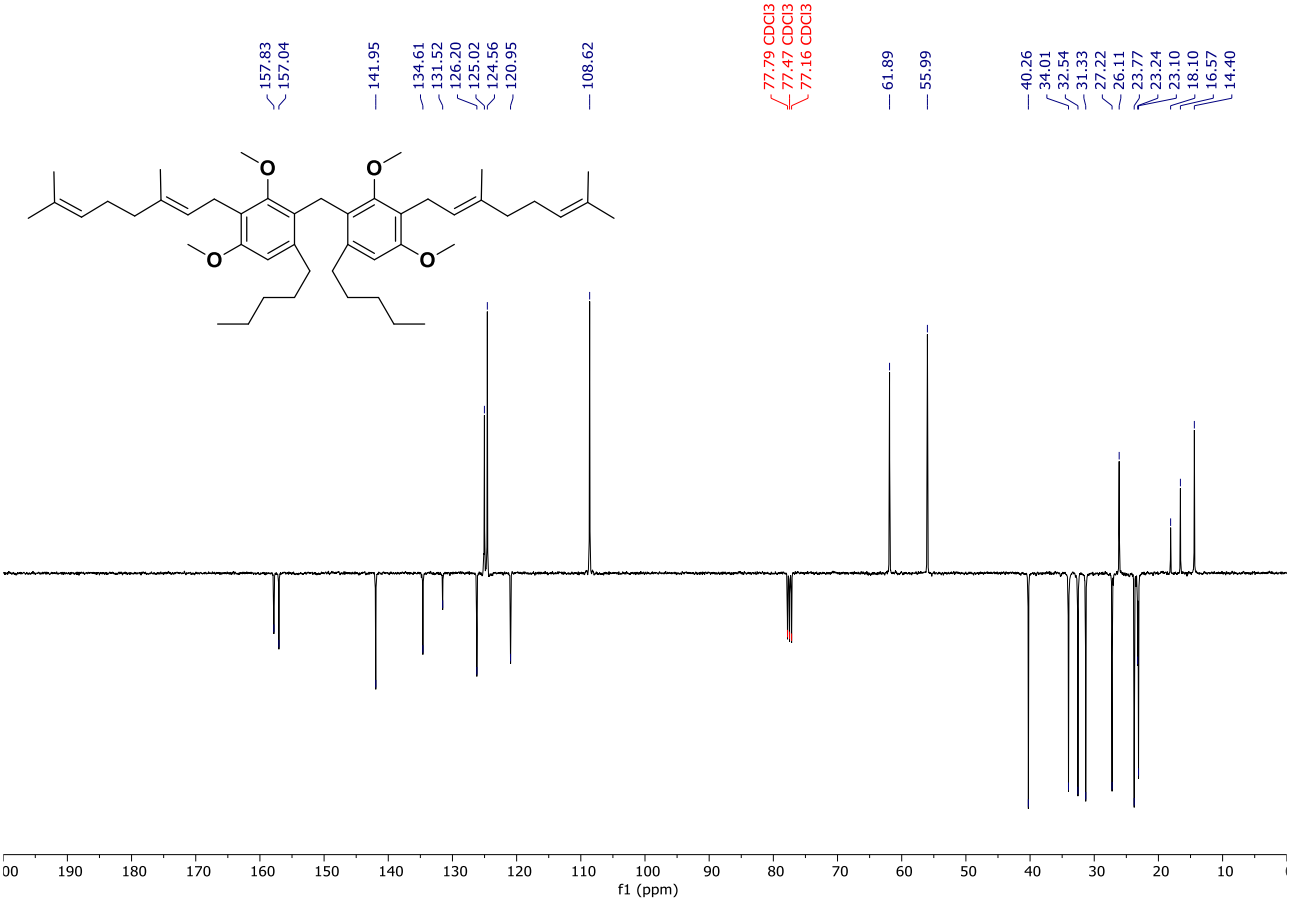
Supplementary Figure S18. ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of **11.**



Supplementary Figure S19. APT NMR spectra (101 MHz, MeOH-d_4) of **11.**

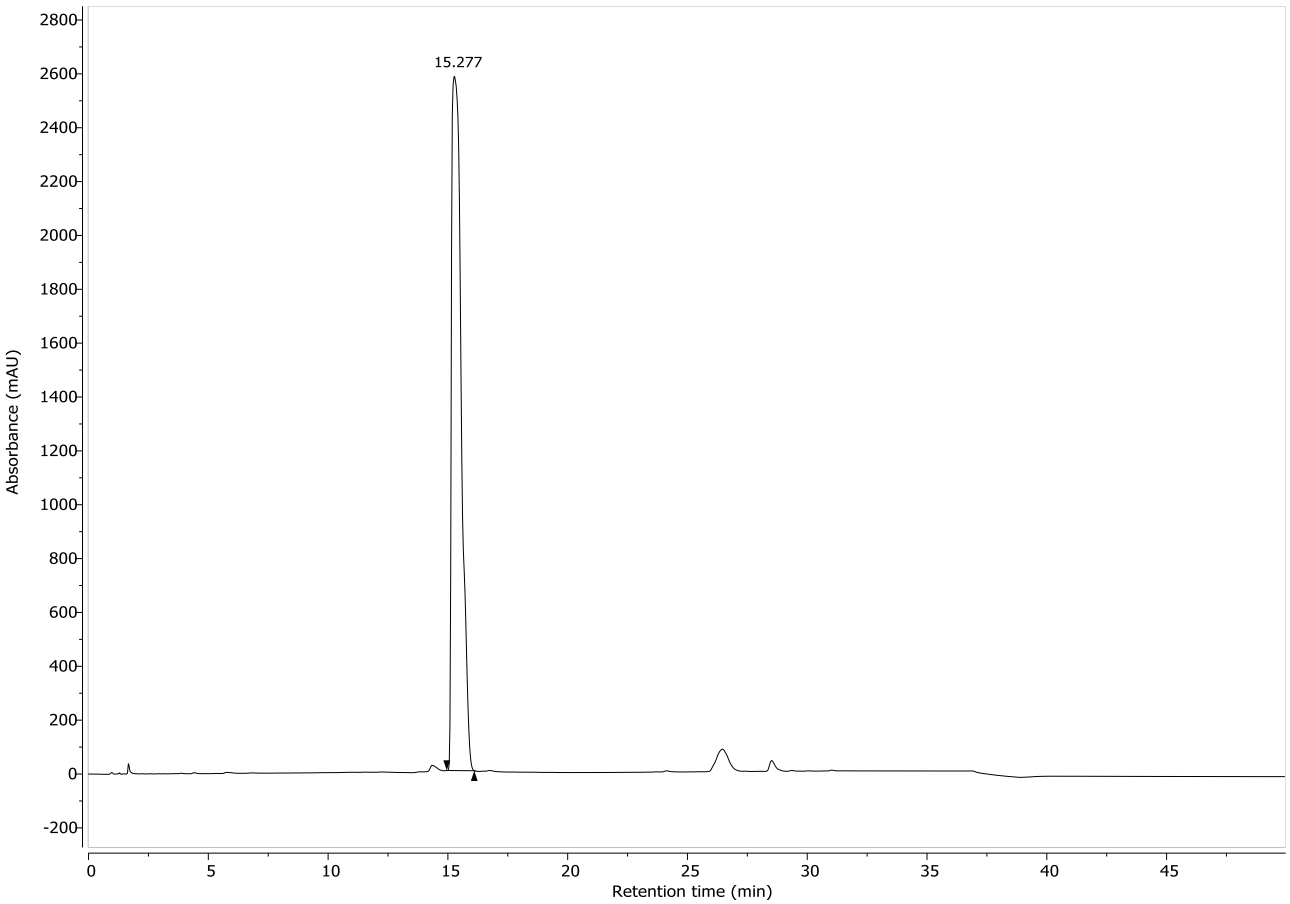


Supplementary Figure S20. ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of **12.**

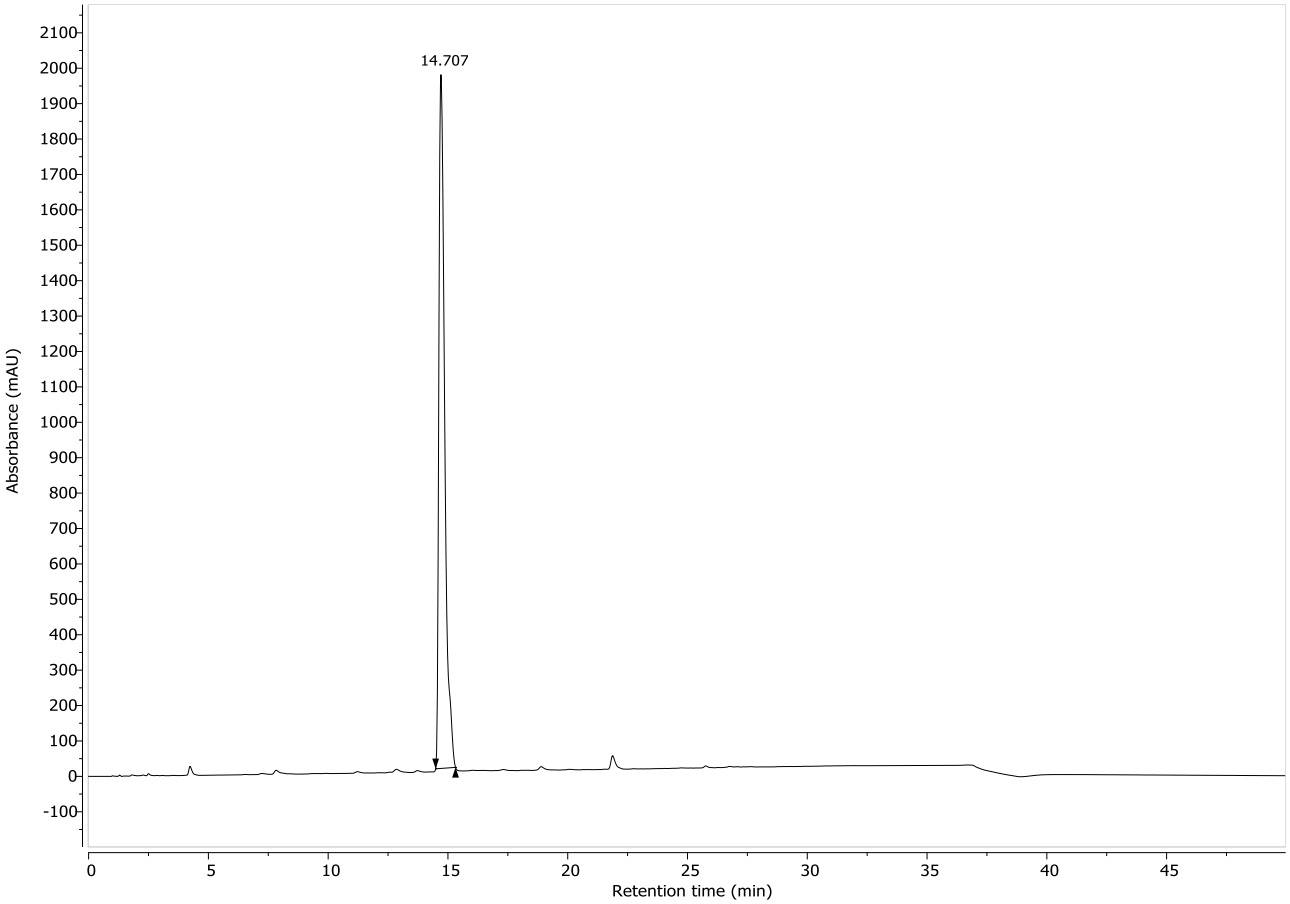


Supplementary Figure S21. APT NMR spectra (101 MHz, $CDCl_3$) of **12.**

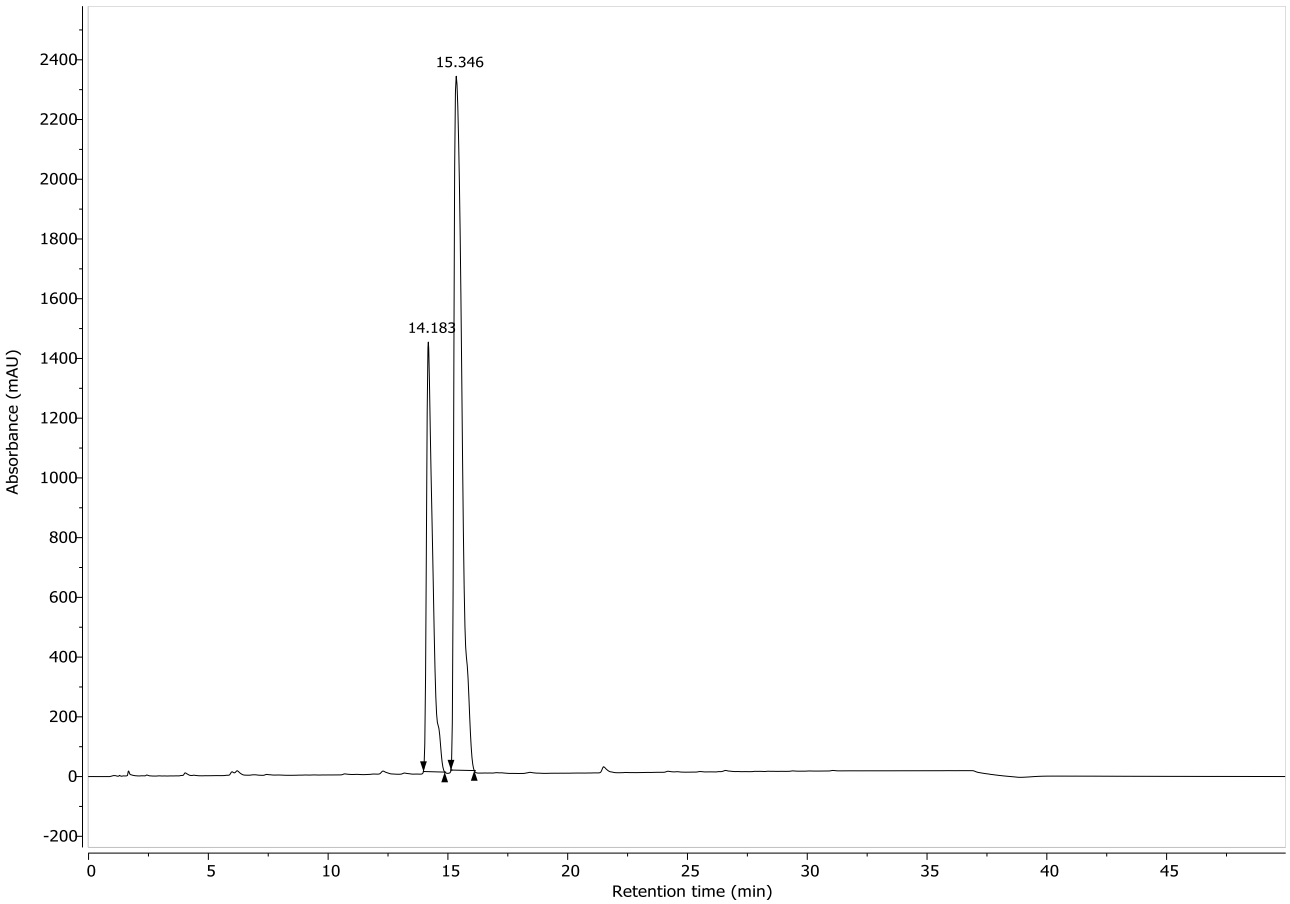
7. HPLC traces of cannabitwinol (6) and cannabizetol (7)



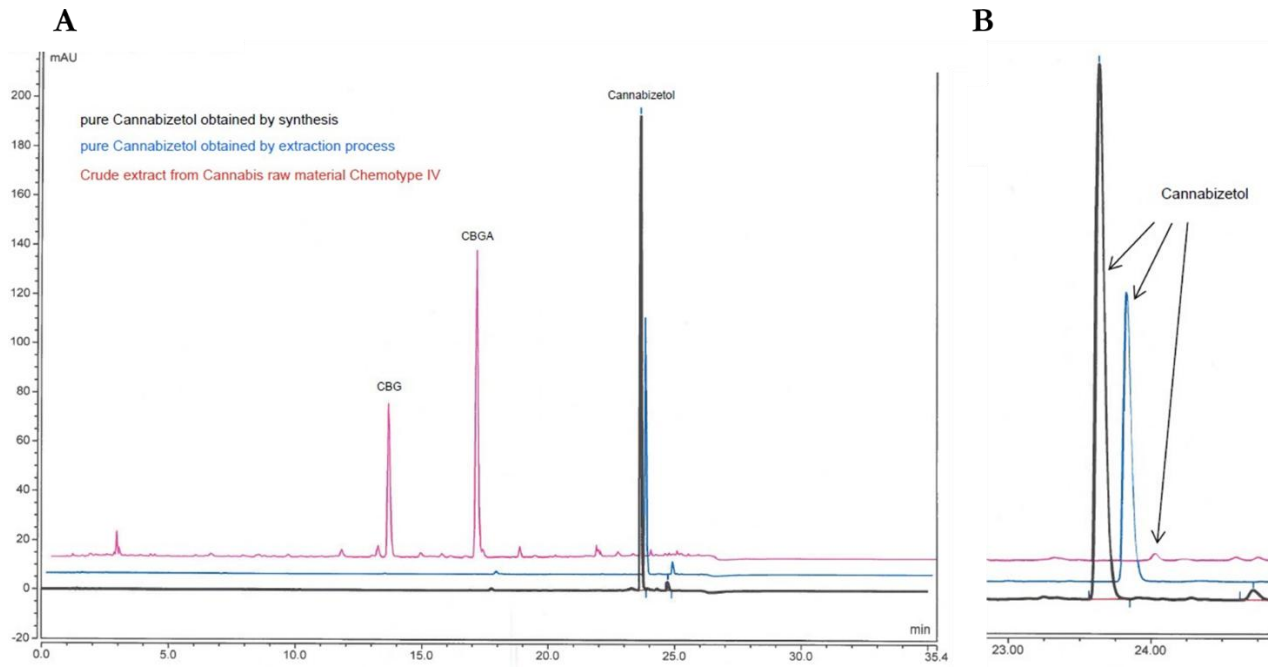
Supplementary Figure S22. HPLC chromatogram of compound **6**: Agilent 1100 Series System RP column ZORBAX SB C8 (3.5 µm x 4.6 x 150 mm), flow rate of 1.2 mL/min, UV spectra recorded at 254 nm and 220 nm with DAD detection, mobile phase H_2O/MeOH. Method: gradient for 30 min from 80% to 100% MeOH.



Supplementary Figure S23. HPLC chromatogram of compound **7**: Agilent 1100 Series System RP column ZORBAX SB C8 (3.5 µ m x 4.6 x 150 mm), flow rate of 1.2 mL/min, UV spectra recorded at 254 nm and 220 nm with DAD detection, mobile phase H_2O/MeOH. Method: gradient for 30 min from 80% to 100% MeOH.



Supplementary Figure S24. HPLC chromatogram of 1:1 mixture of compound **6** and **7**: Agilent 1100 Series System RP column ZORBAX SB C8 (3.5 µ m x 4.6 x 150 mm), flow rate of 1.2 mL/min, UV spectra recorded at 254 nm and 220 nm with DAD detection, mobile phase H_2O/MeOH. Method: gradient for 30 min from 80% to 100% MeOH.



Supplementary Figure S25. Superimposition (**A**) and close up view at 24 min (**B**) of HPLC chromatograms of crude extract from Cannabis sativa raw material (in red), pure cannabizetol (**7**) obtained from extraction process (in blue) and pure cannabizetol (**7**) obtained from synthesis (in black). HPLC condition according to Ph. Eur. monograph 07/2024:3151 (Cannabidiol).