Supplementary Materials

Unusual Secondary Metabolites of the Aerial Parts of *Dionysia diapensifolia* Bioss. (Primulaceae) and Their Anti-Inflammatory Activity

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Table S1. Anti-proliferative activity of selected compounds isolated from diethyl ether subfraction of the methanolic extract of *D. diapensifolia* in MTT assay after 24 h shown as % cellular inhibition (MEAN±SEM) of n = 3. ***, **, * denote $p < 0.001$, $p < 0.01$, $p < 0.05$ vs. control. 74
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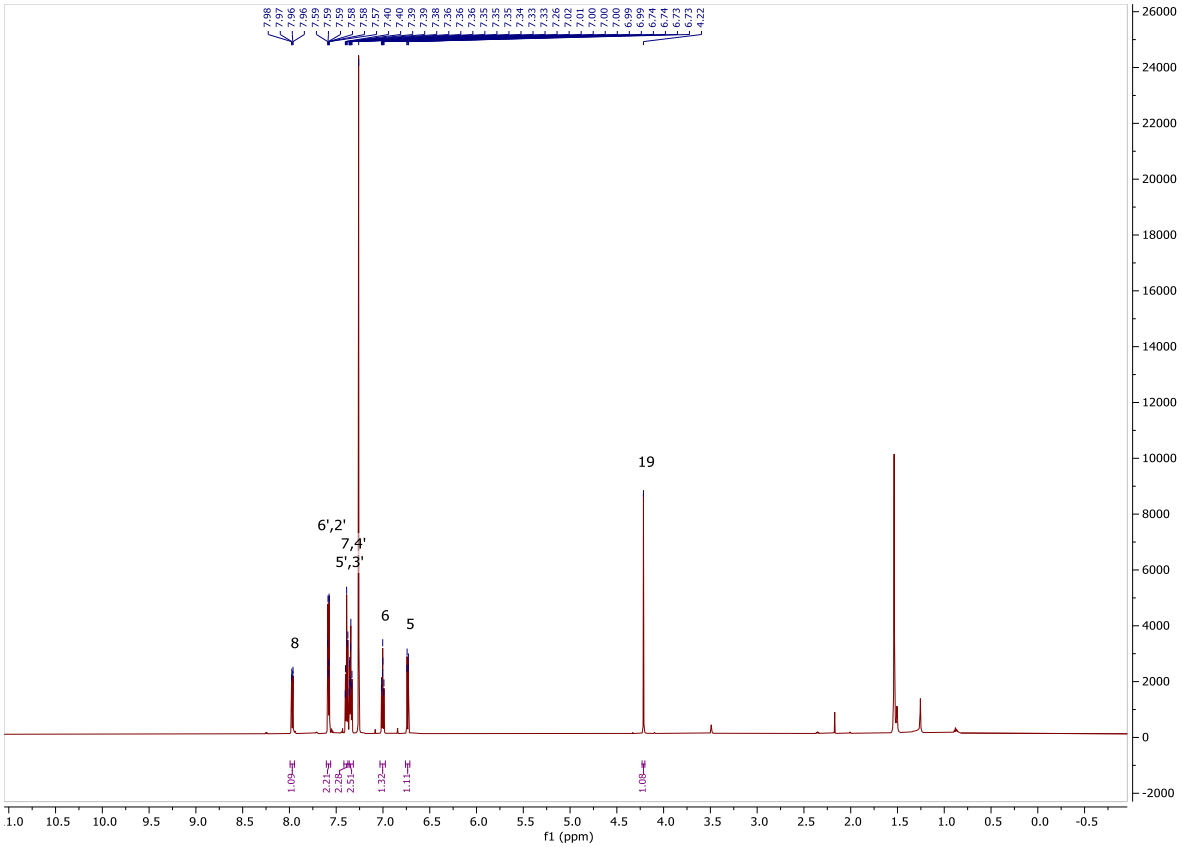

Figure S1. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 1.

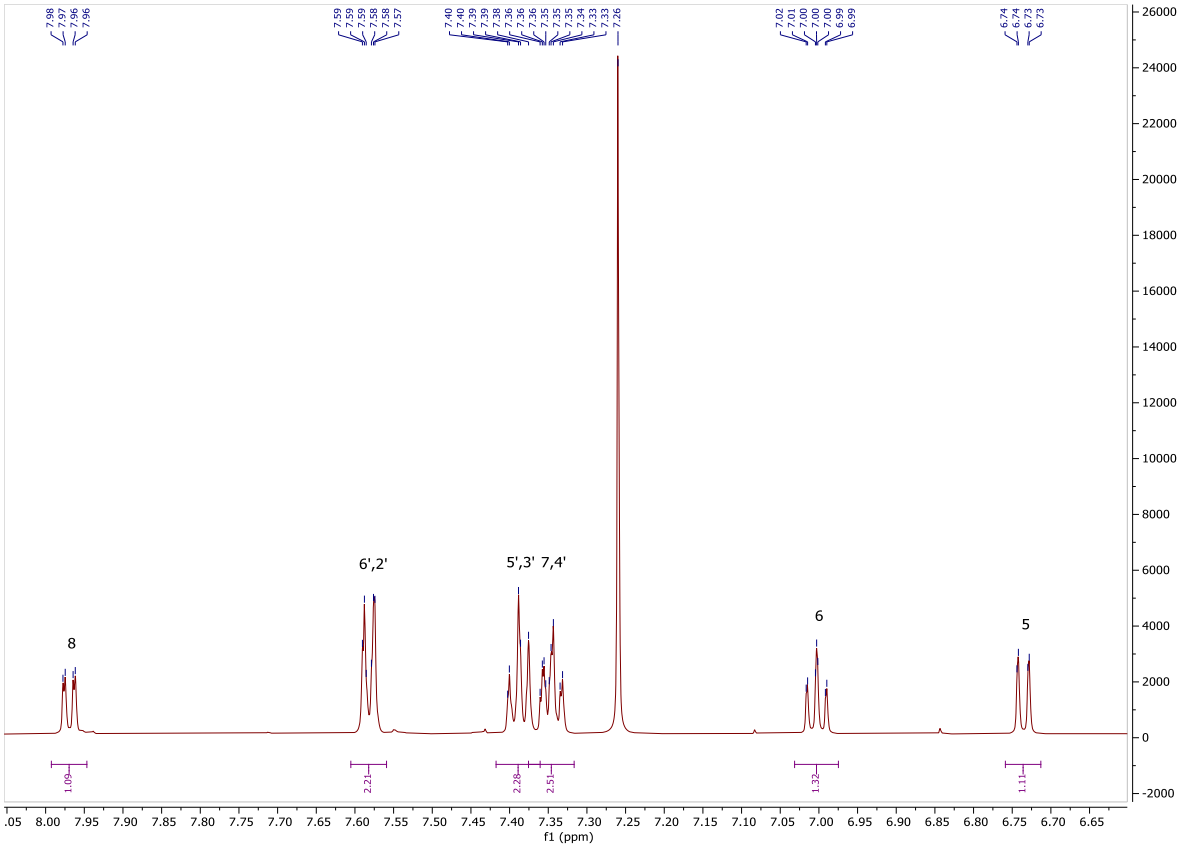

Figure S2. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 1 (insert δ 8.0 – 6.5 ppm).

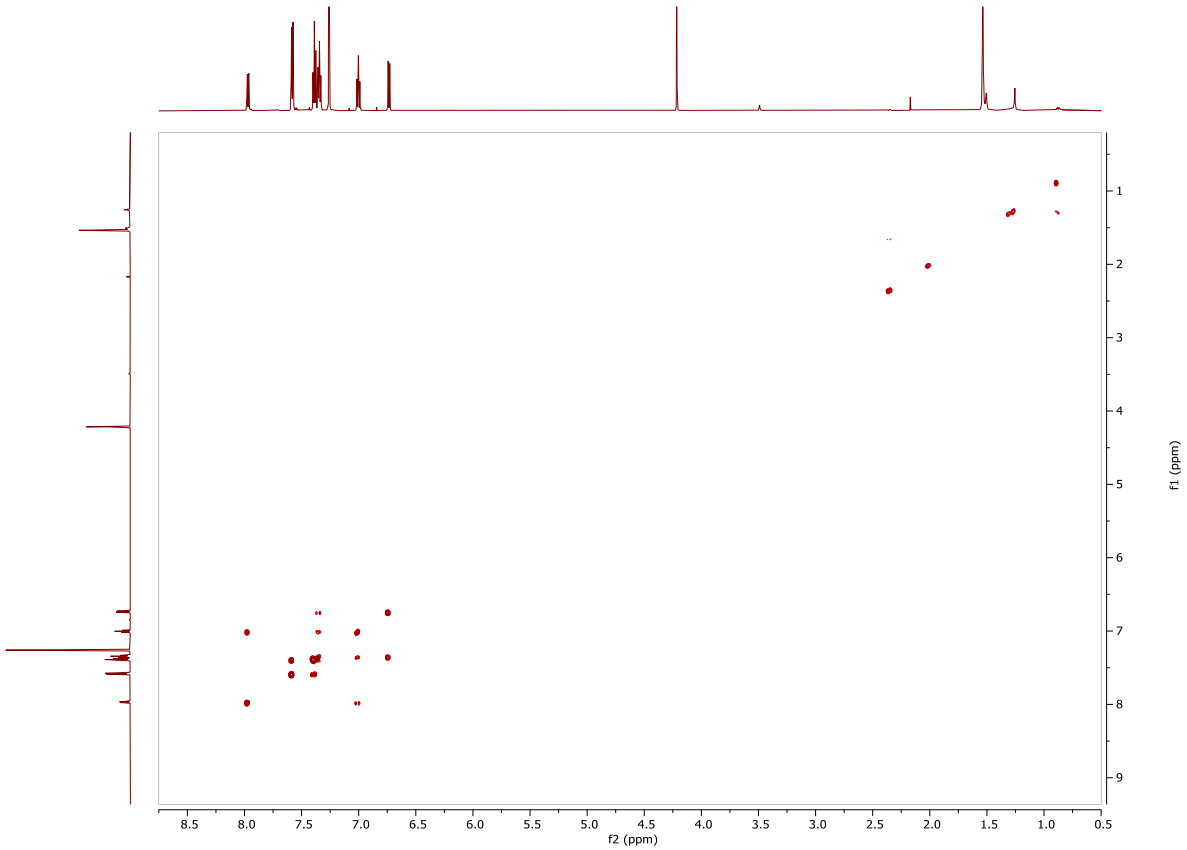

Figure S3. COSY spectrum (600.19 MHz, $CHCl_3$-*d*) of compound 1.

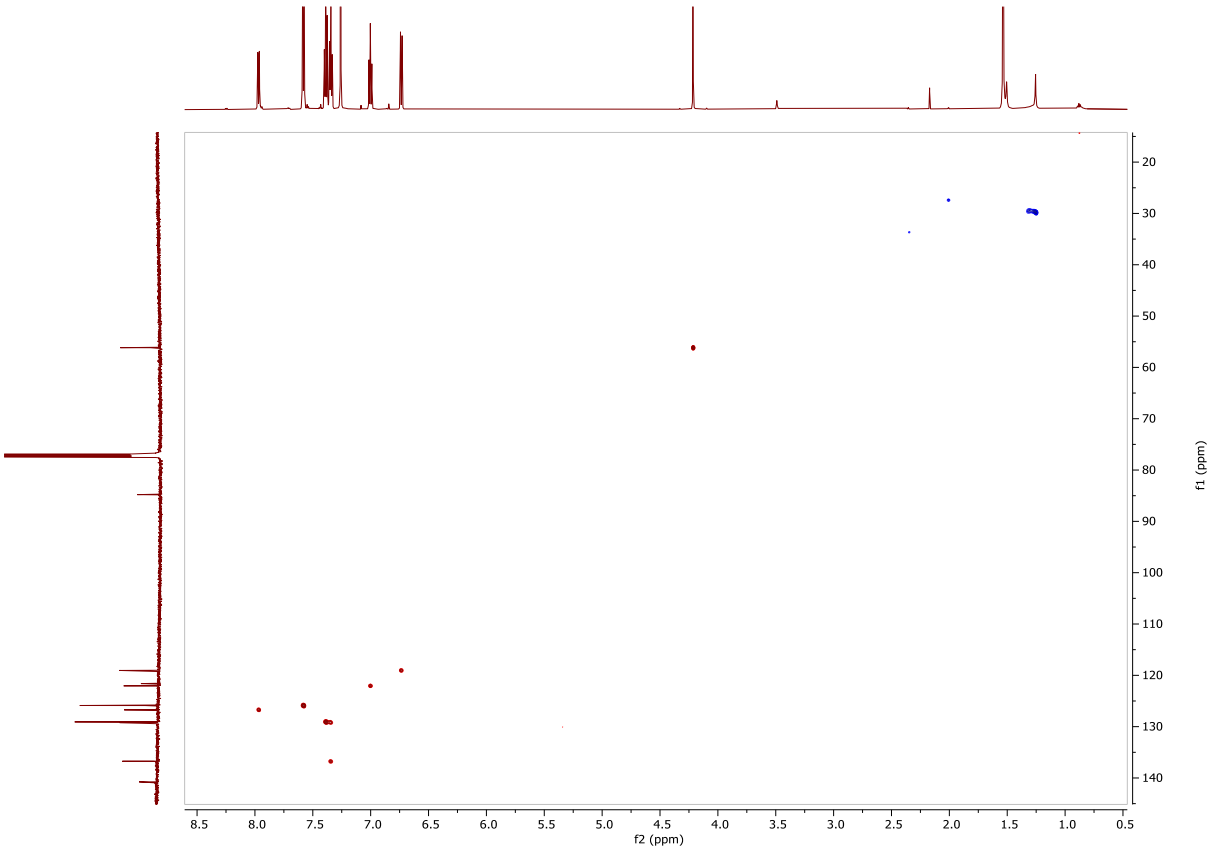

Figure S4. HSQC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 1.

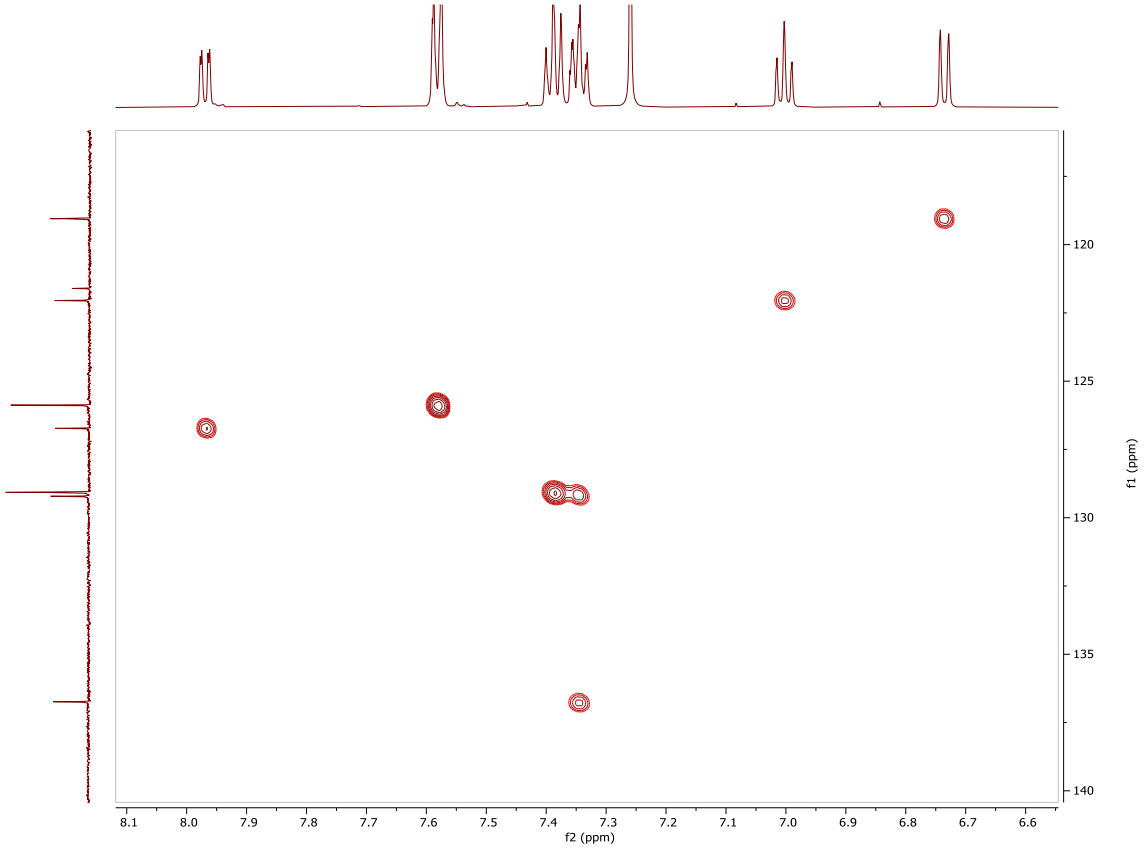

Figure S5. HSQC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 1 (insert δ 8.0 – 6.5 ppm).

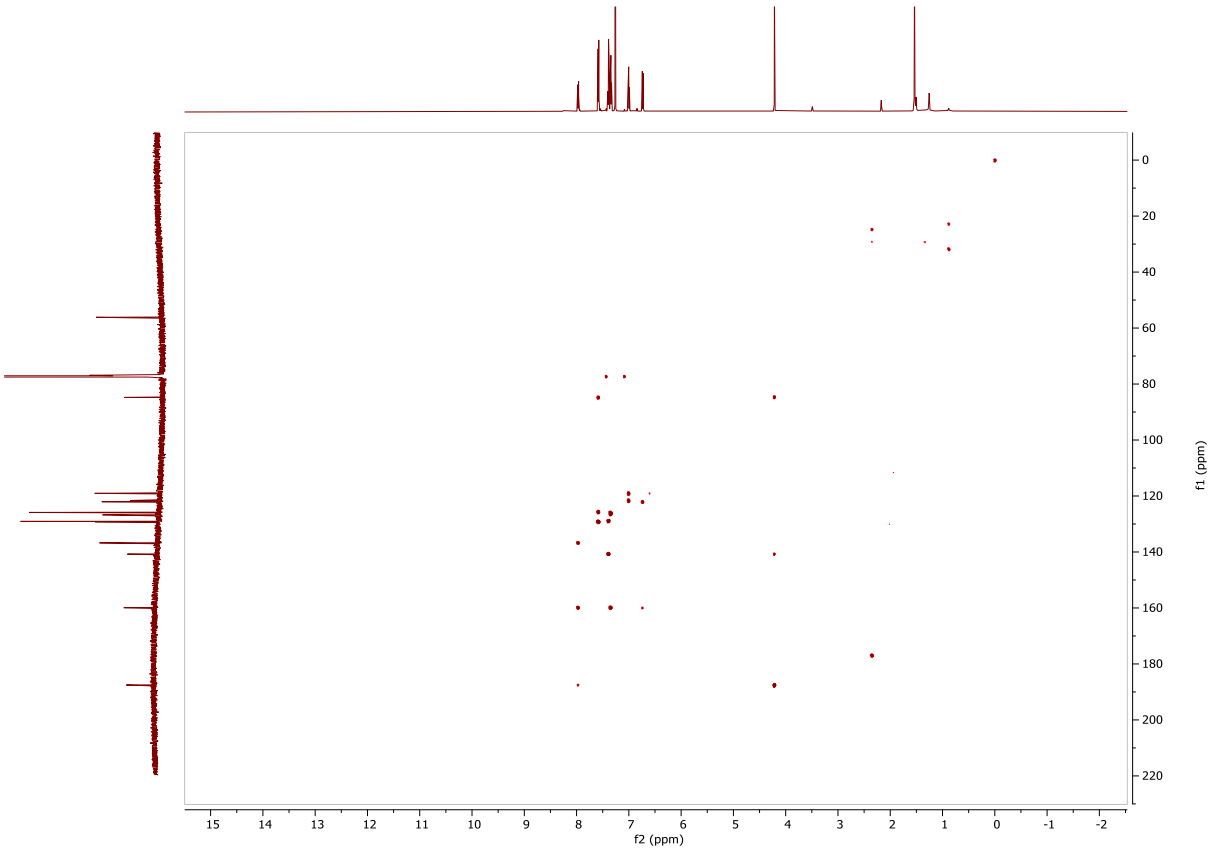

Figure S6. HMBC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 1.

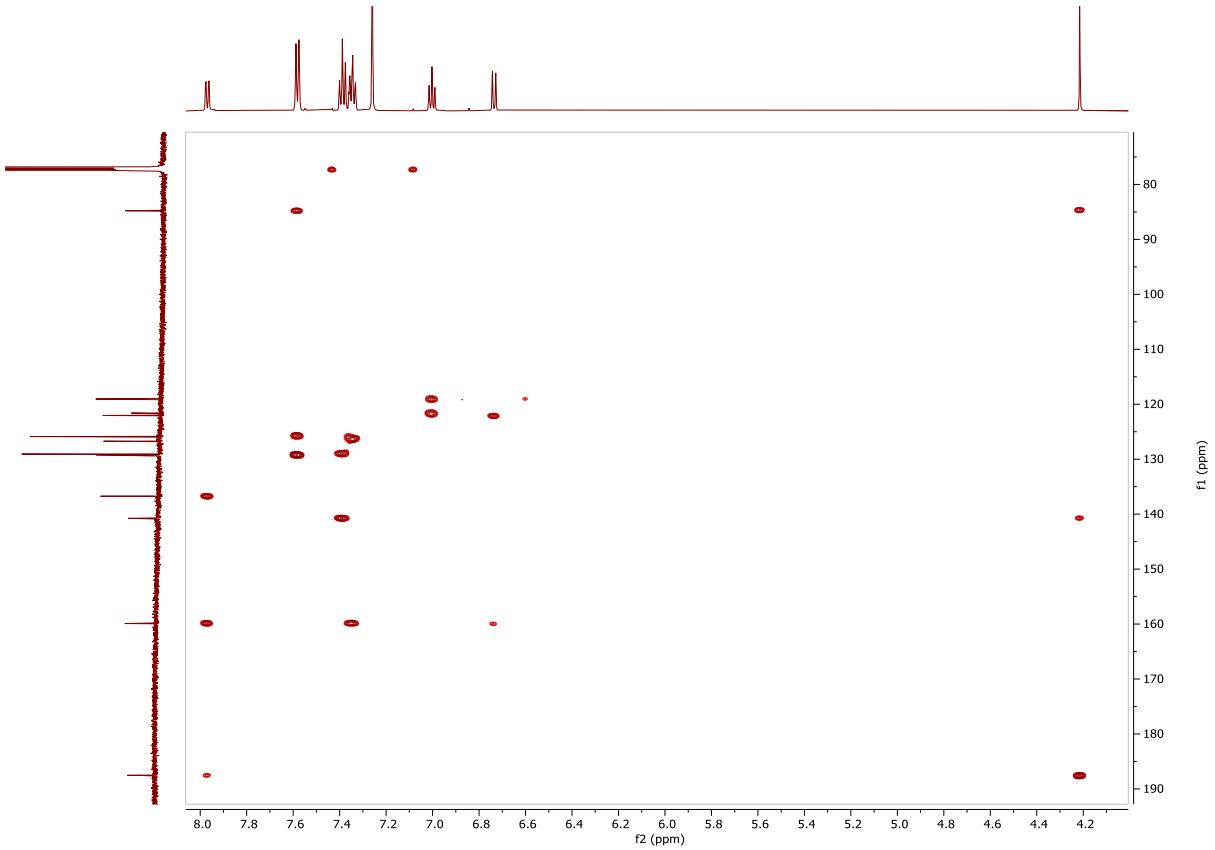

Figure S7. HSQC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 1 (insert δ 8.0 – 4.0 ppm).

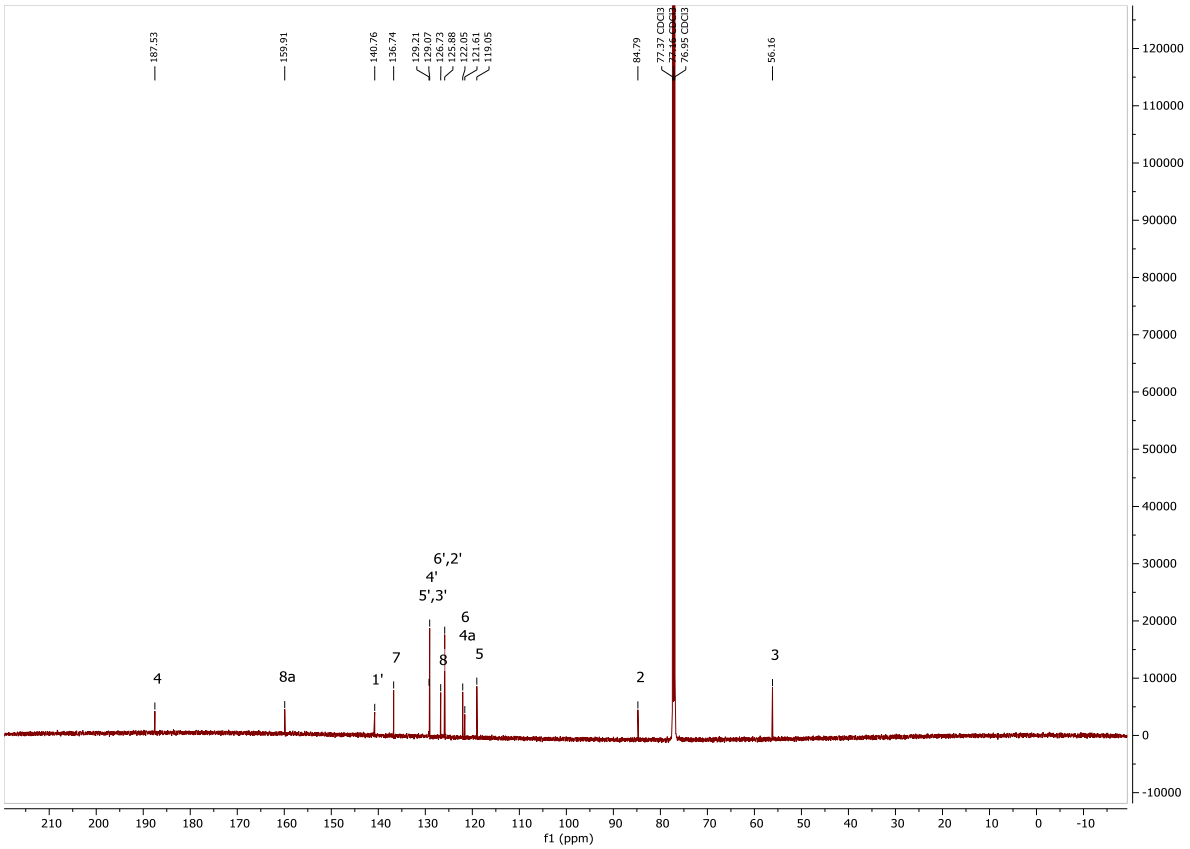

Figure S8. ^{13}C-NMR spectrum (150.91 MHz, $CHCl_3$-*d*) of compound **1**.

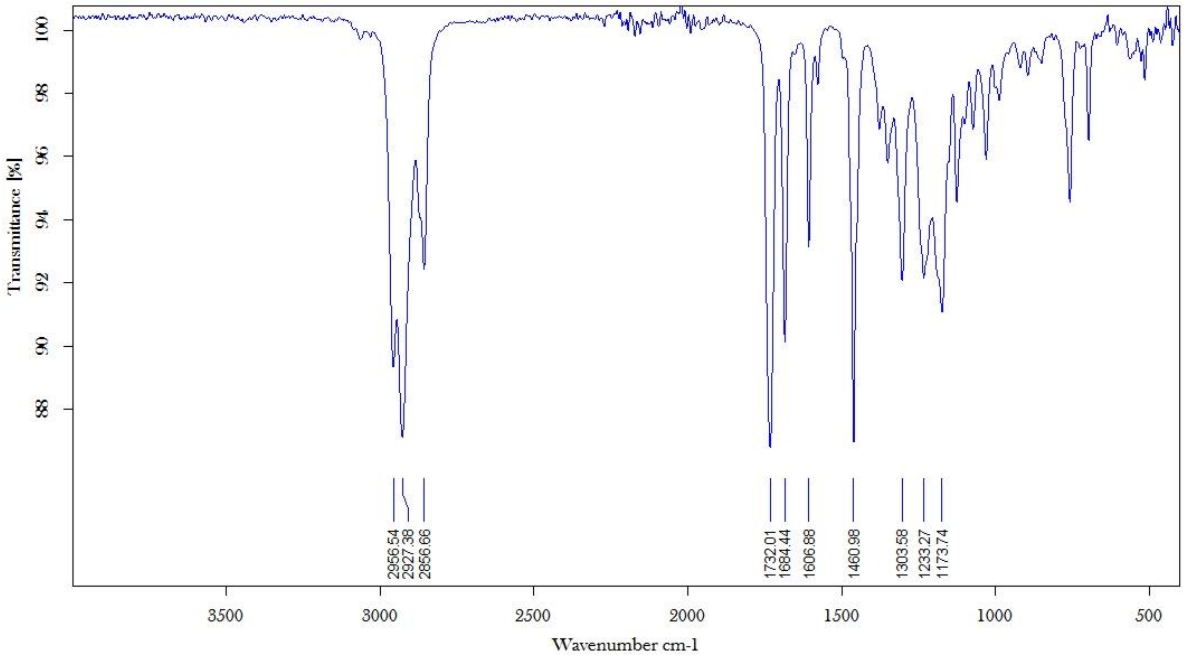

Figure S9. IR spectrum of compound 1.

Mass Spectrum SmartFormula Report

Analysis Info

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Comment	#7 - oeliger A. 1200784/0 Aceton		

Acquisition Parameter

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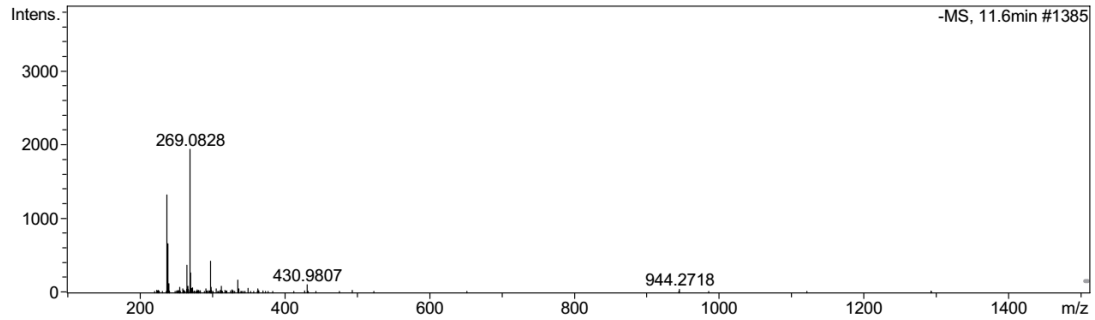
Scan End	1500 m/z	Set Collision Cell RF	452.4 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
237.0577	1	C 15 H 9 O 3	100.00	237.0557	-2.0	-8.5	277.2	11.5	even	ok

Figure S10. HRLCESIMS spectrum of compound 1.

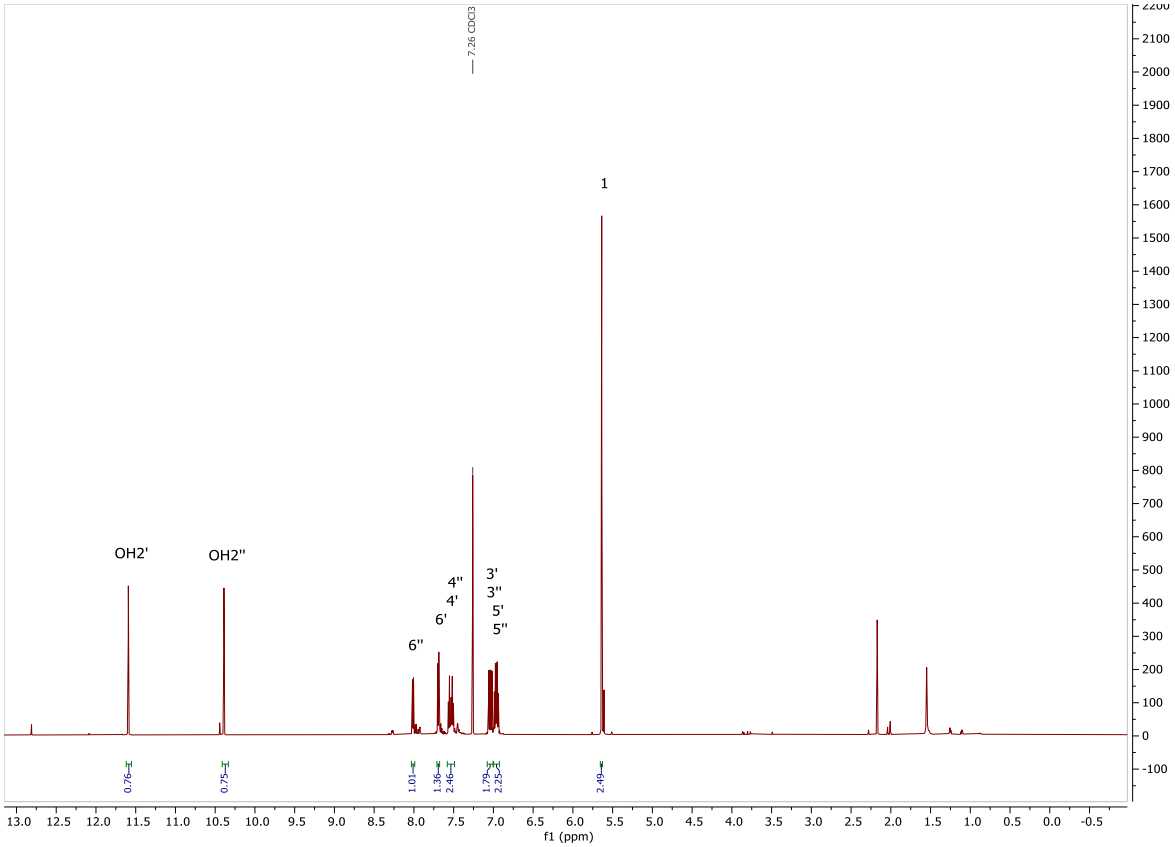


Figure S11. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 2.

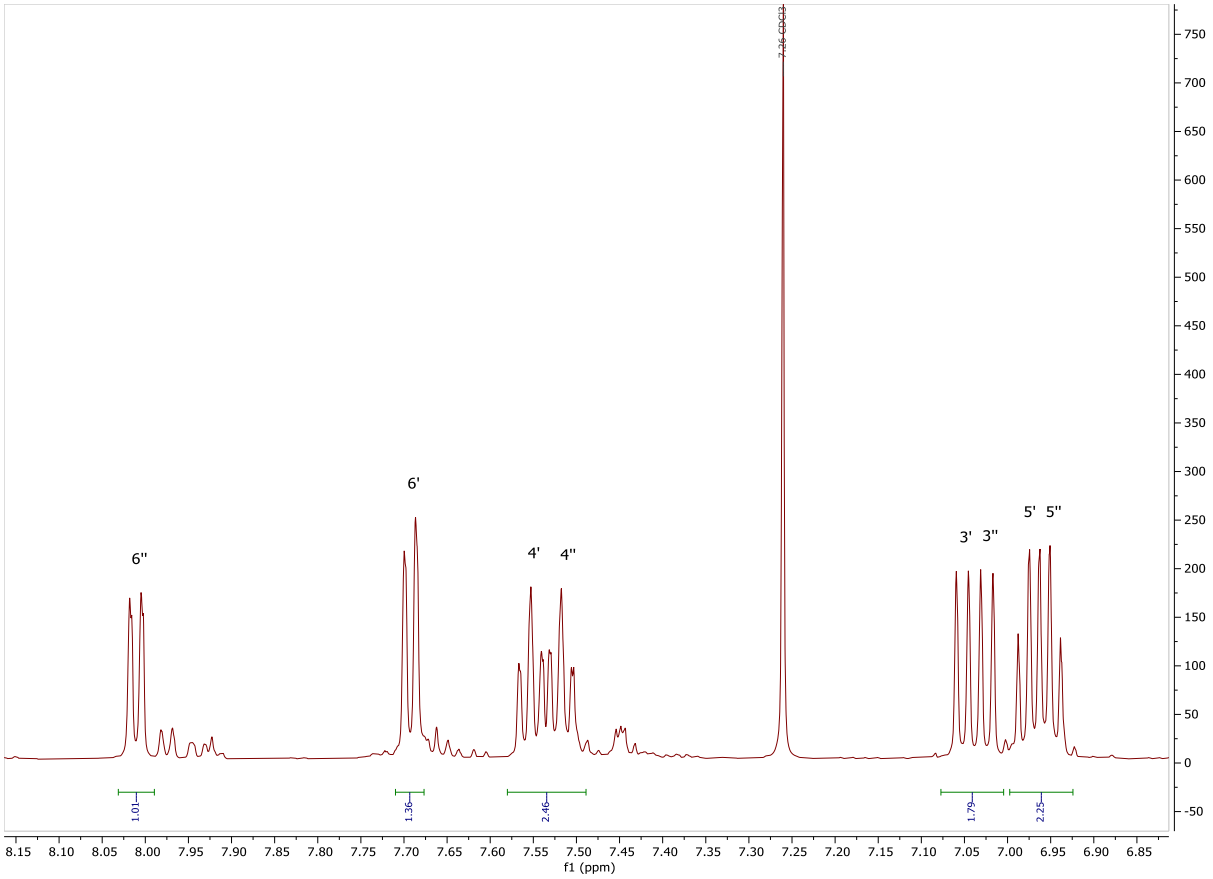


Figure S12. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 2 (insert δ 8.2 – 6.7 ppm).

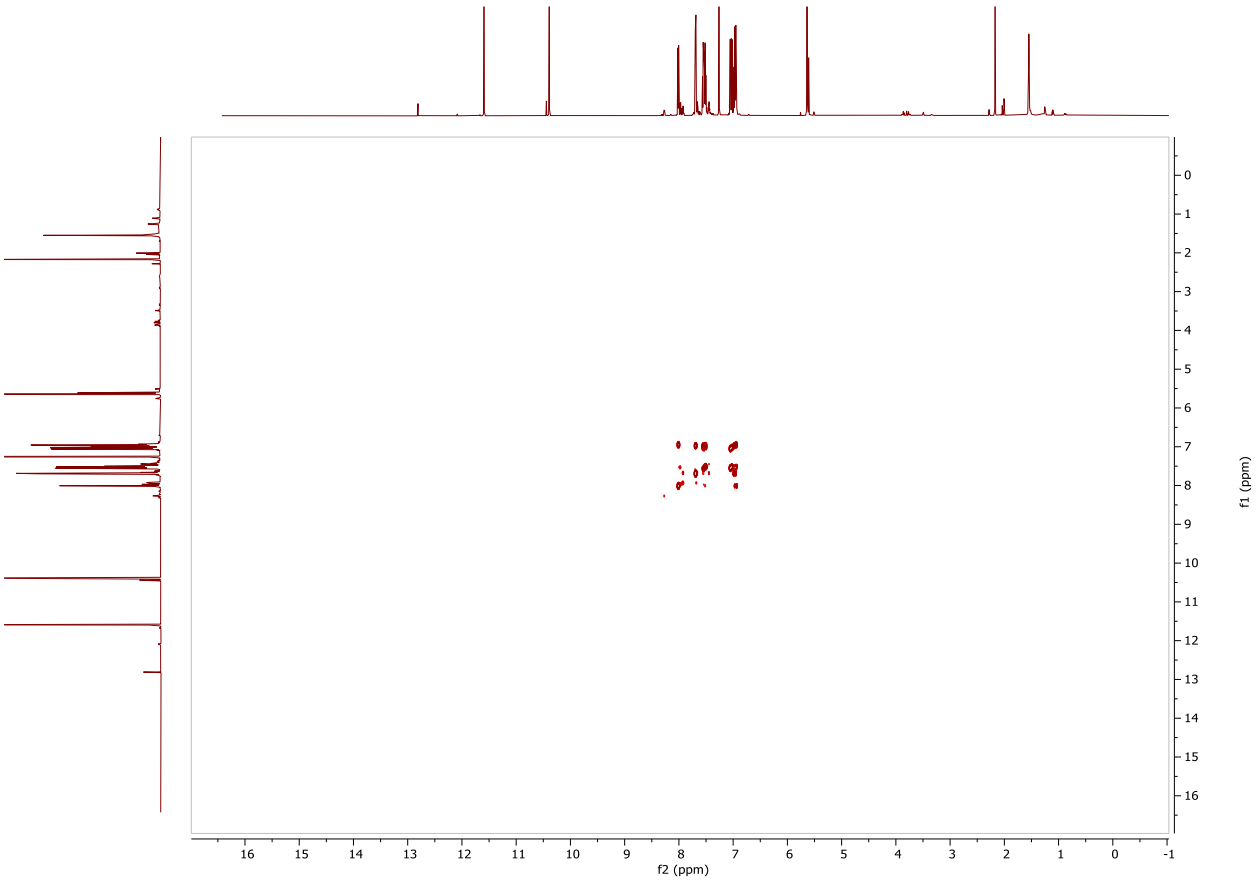


Figure S13. COSY spectrum (600.19 MHz, $CHCl_3$-*d*) of compound 2.

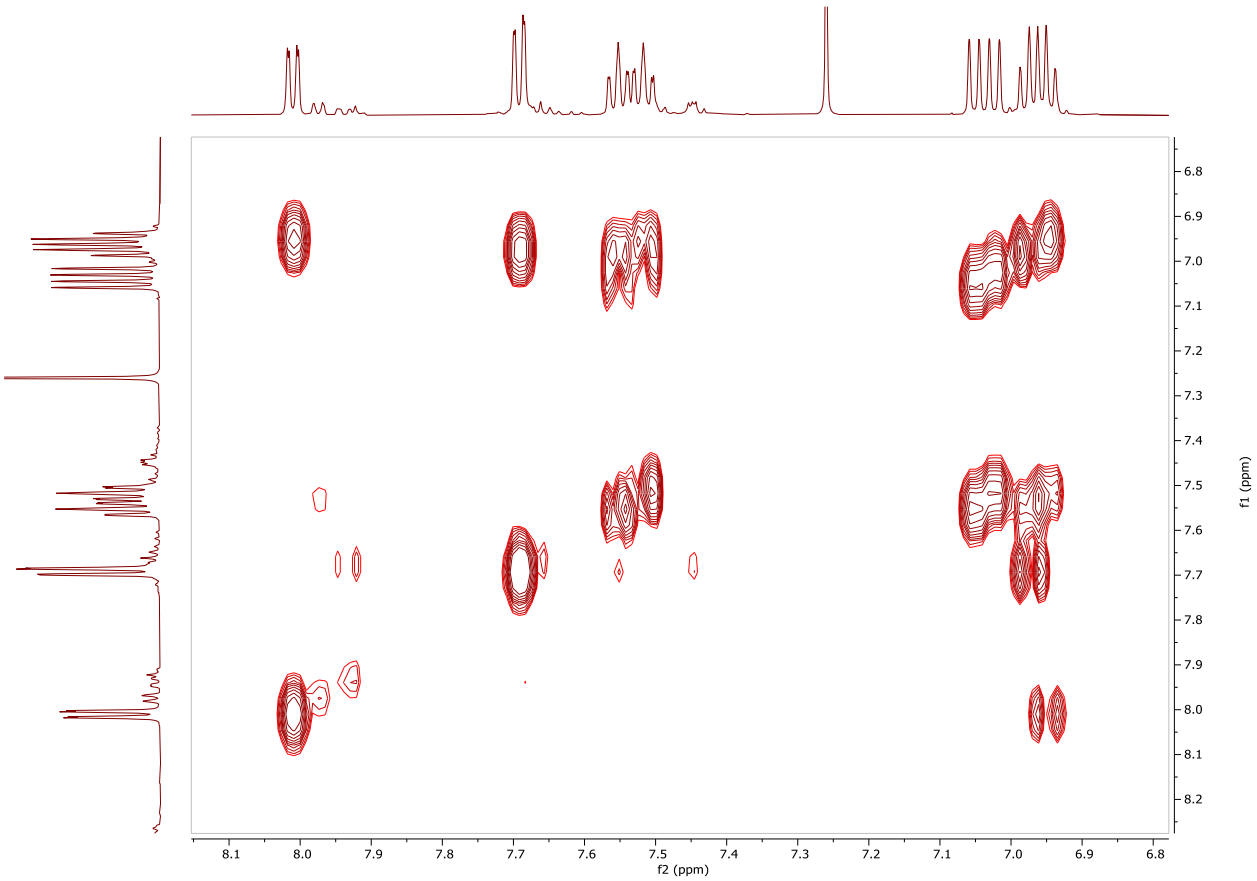


Figure S14. COSY spectrum (600.19 MHz, $CHCl_3$-*d*) of compound 2 (insert δ 8.2 – 6.7 ppm).

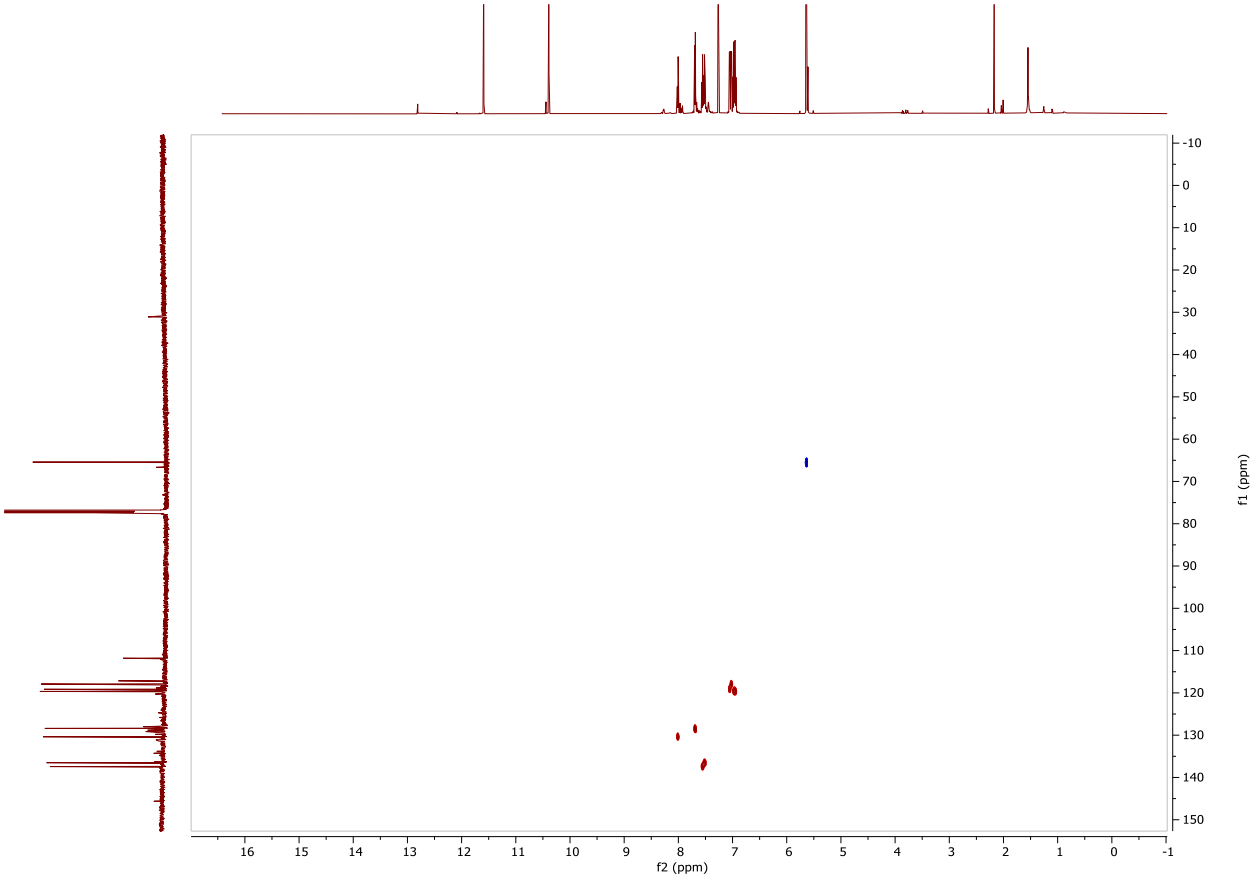


Figure S15. HSQC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 2.

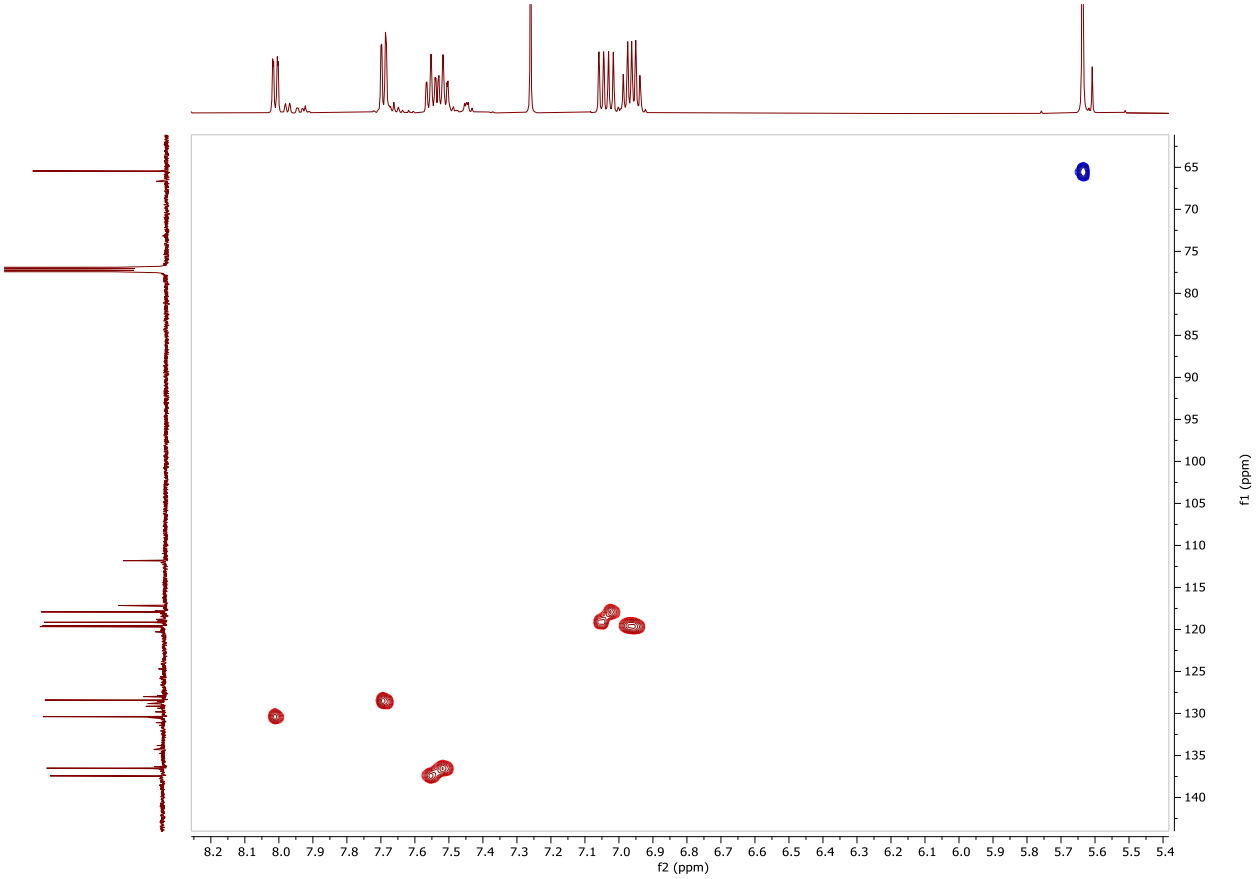


Figure S16. HSQC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 2 (insert δ 8.2 – 5.4 ppm).

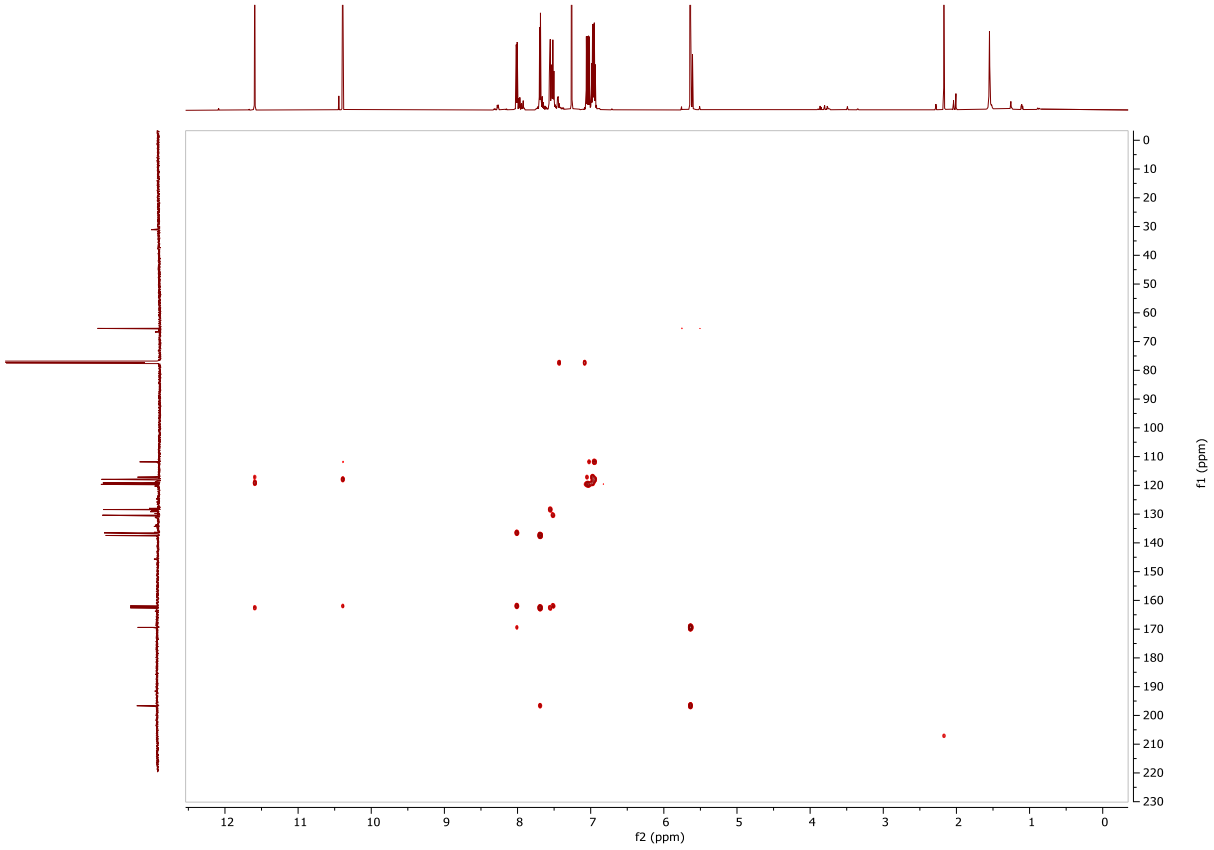


Figure S17. HMBC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 2.

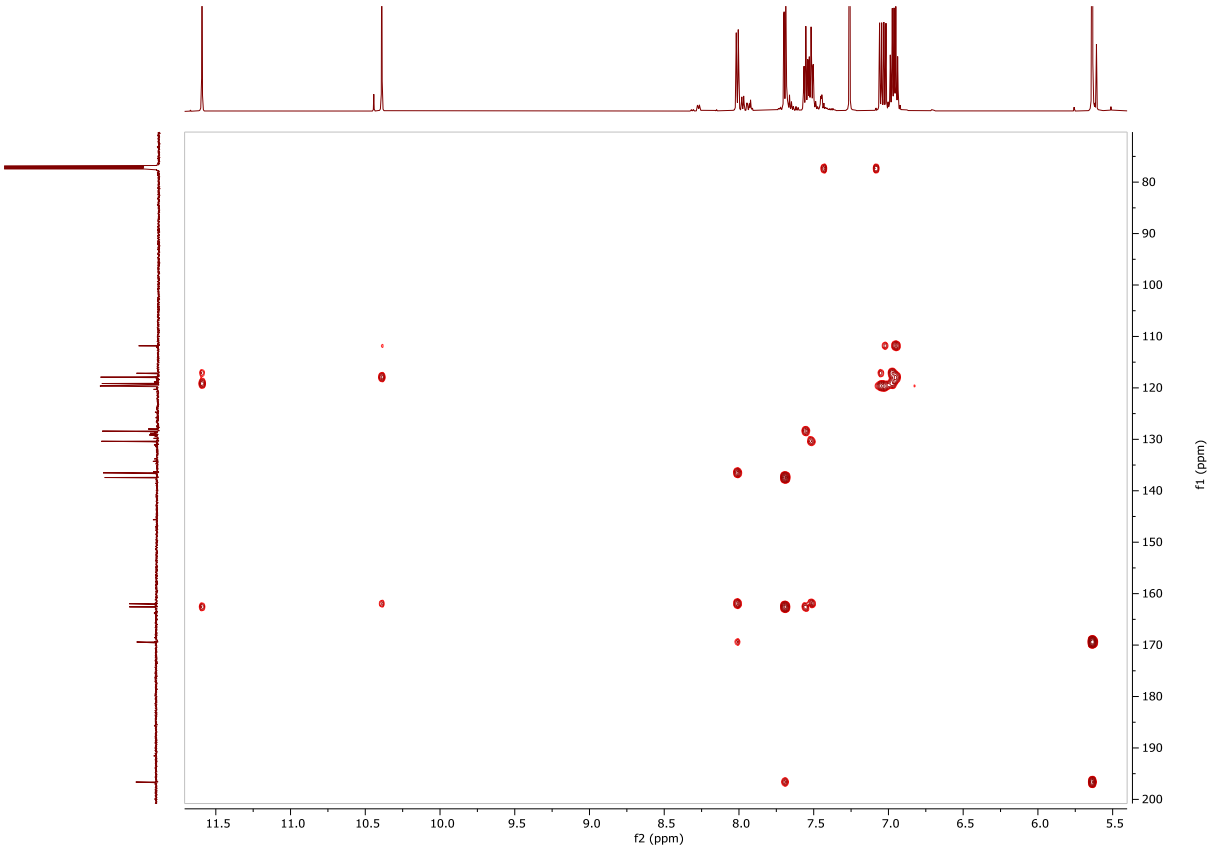


Figure S18. HMBC spectrum (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 22 (insert δ 11.7 – 5.5 ppm).

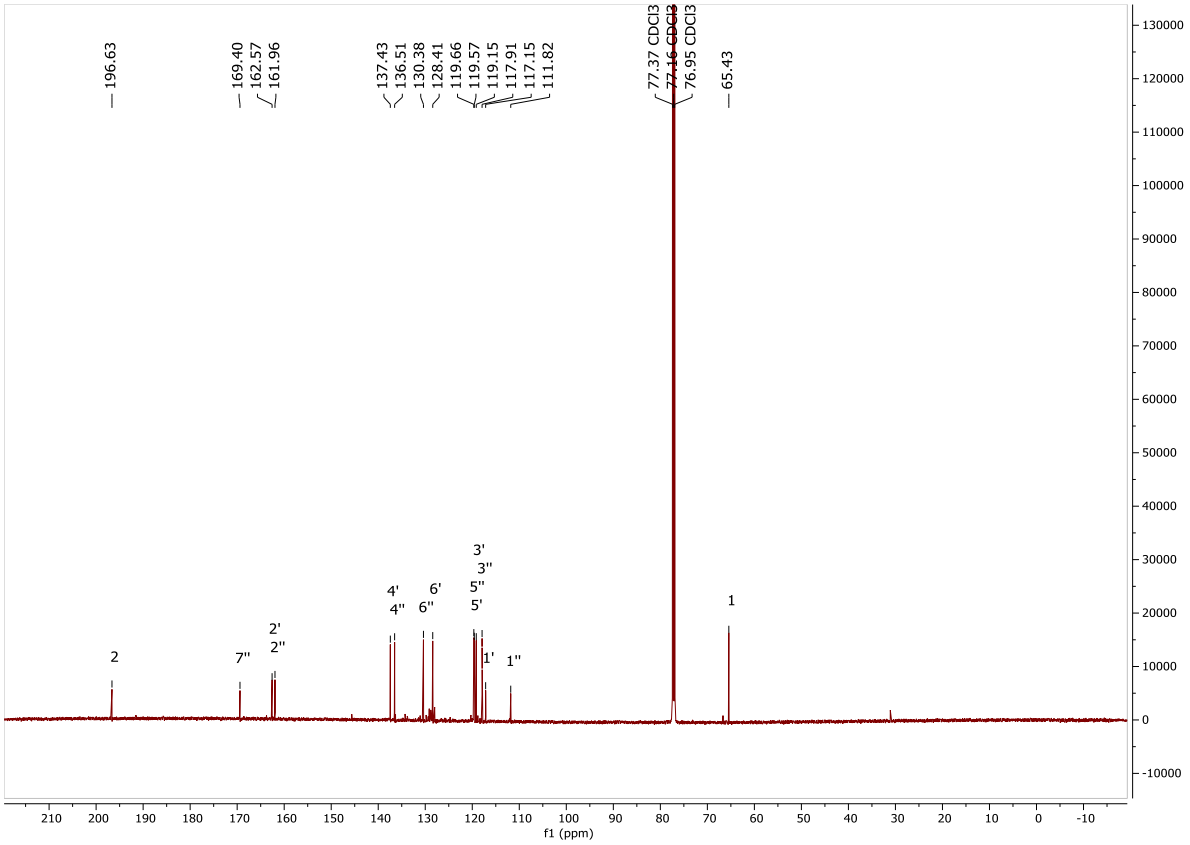


Figure S19. ^{13}C-NMR (600.19/150.91 MHz, $CHCl_3$-*d*) of compound 2.

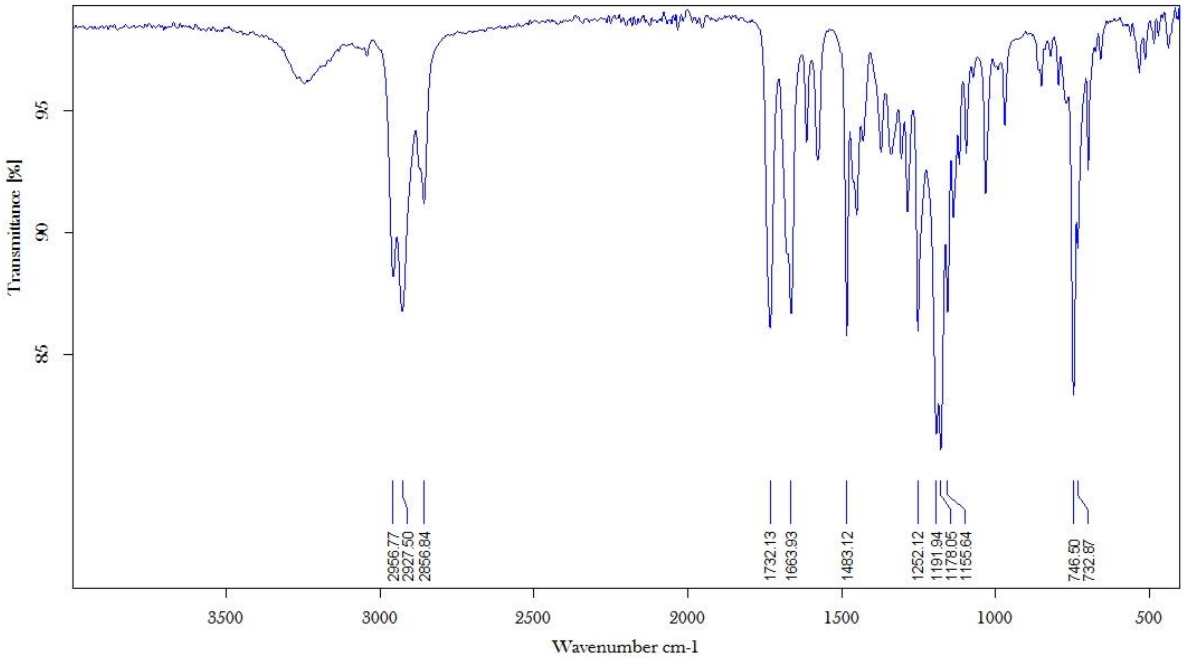


Figure S20. IR spectrum of compound 2.

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	F8PS9_THF_pos	Instrument / Ser#	micrOTOF-Q 10202
Comment	#7 - oeliger A. 1200784/0 Aceton		

Acquisition Parameter

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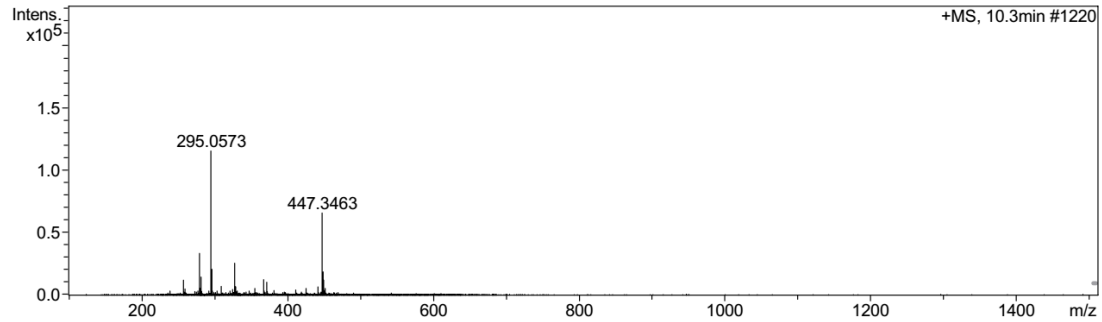
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	452.4 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
295.0573	1	C 15 H 12 Na O 5	100.00	295.0577	0.4	1.3	8.3	9.5	even	ok
	2	C 13 H 13 Na 2 O 5	28.64	295.0553	-2.0	-6.9	20.9	6.5	even	ok

Figure S21. HRLCESIMS spectrum of compound 2.

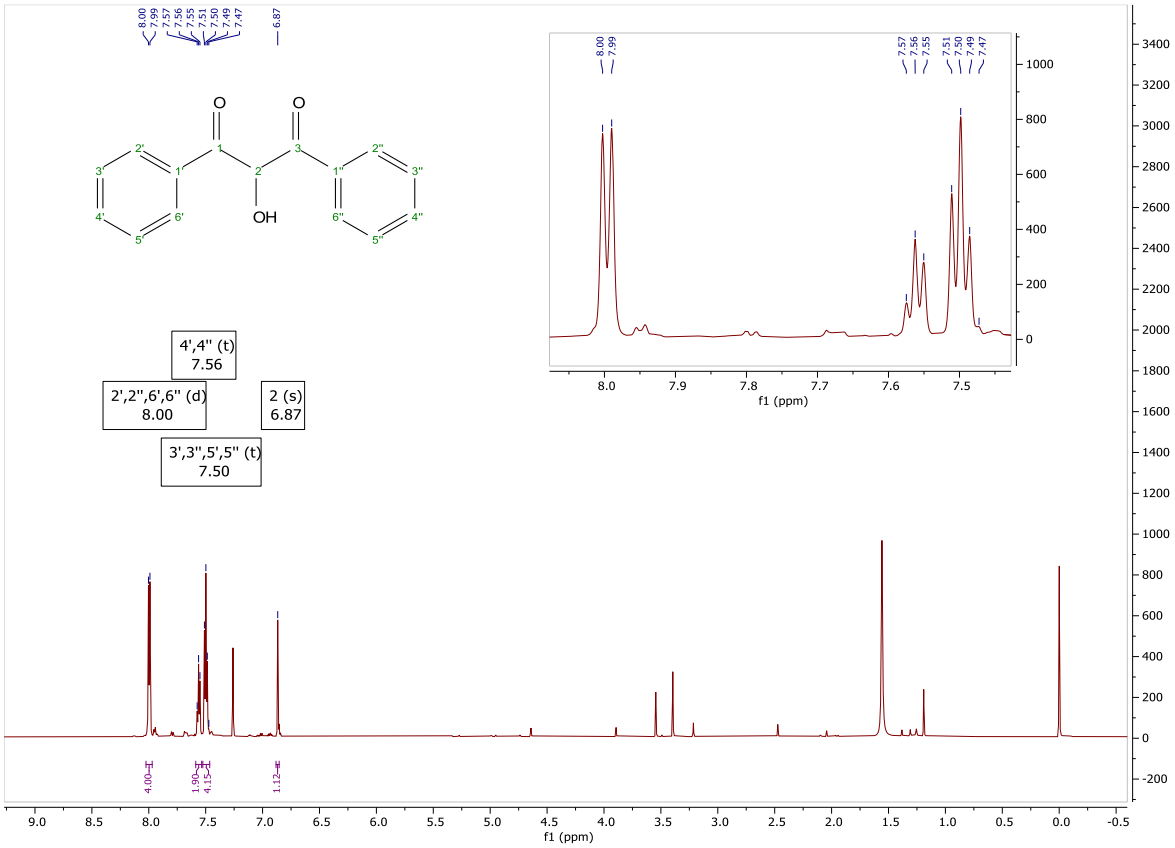


Figure S22. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 3.

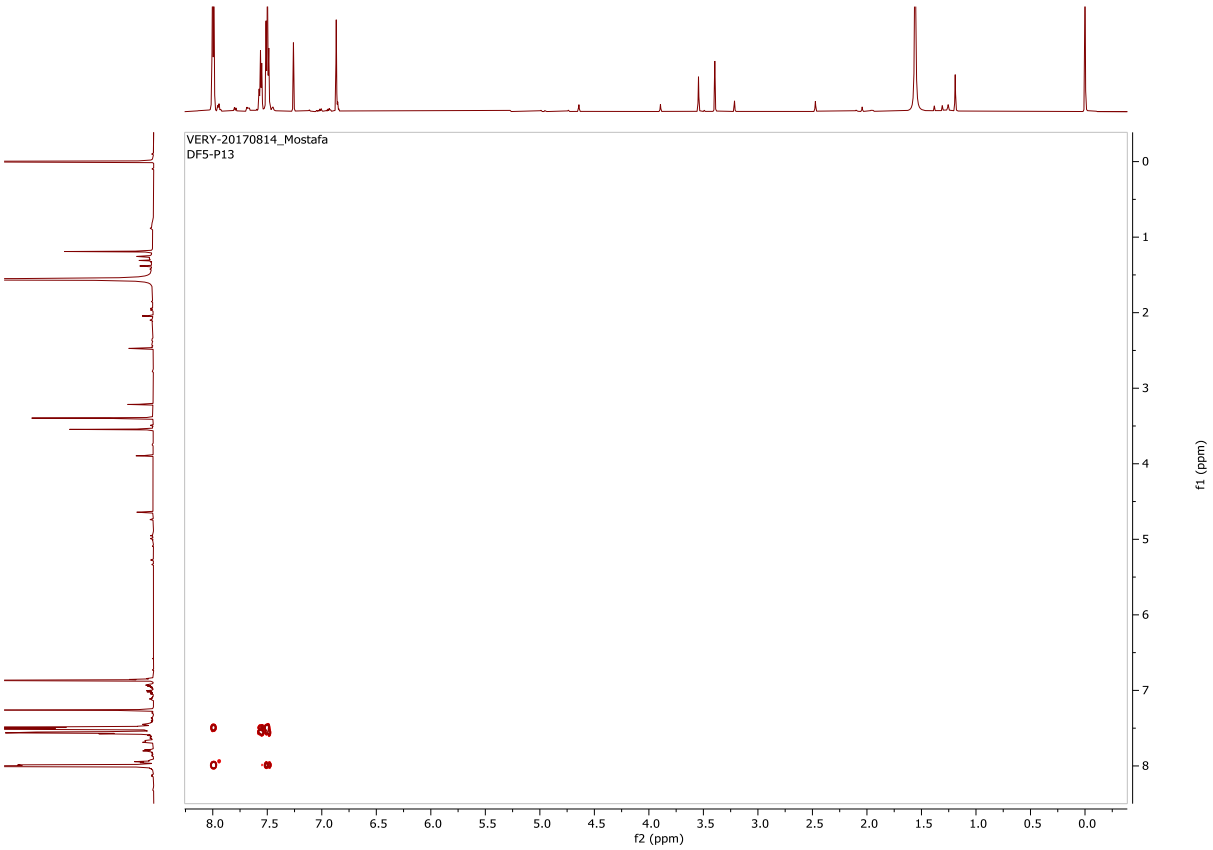


Figure S23. COSY spectrum (600.19 MHz, $CHCl_3$-*d*) of compound 3.

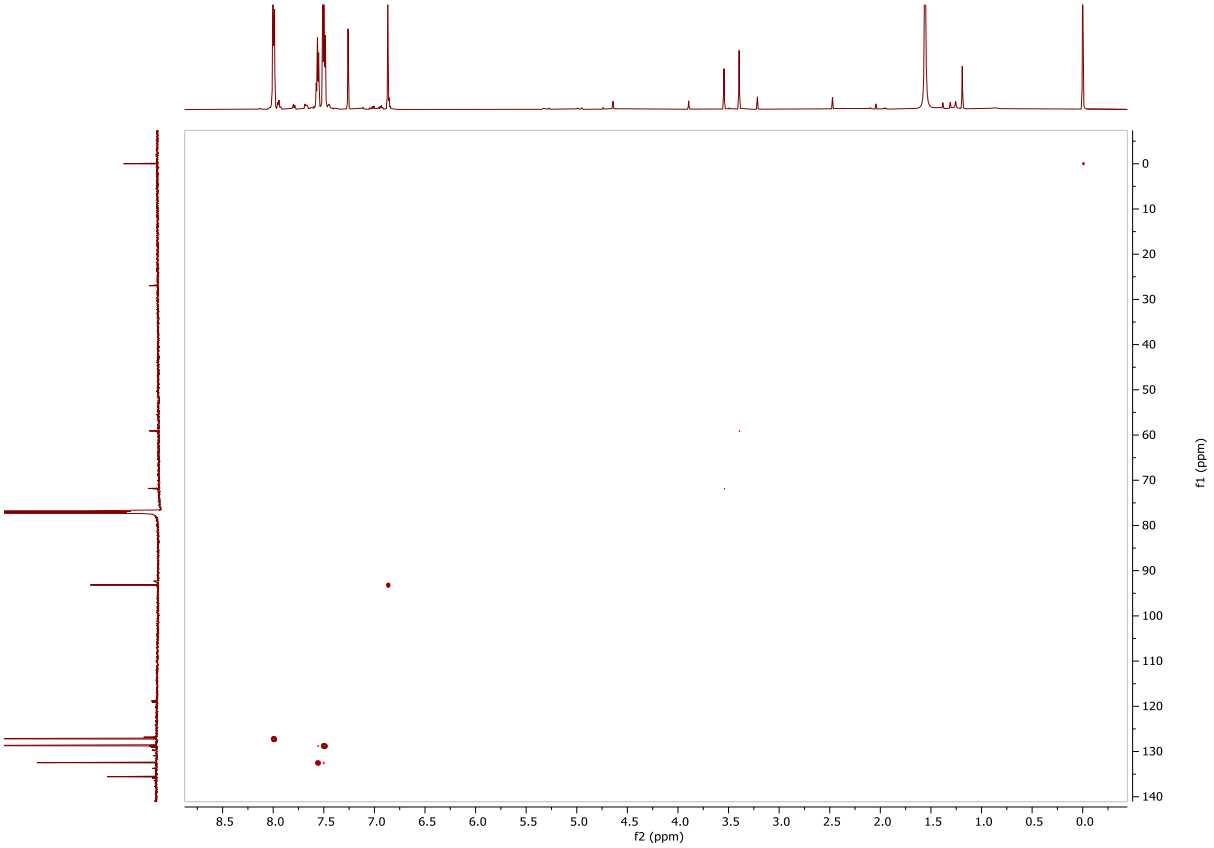


Figure S24. HSQC spectrum (600.19, 150.91 MHz, $CHCl_3$-*d*) of compound 3.

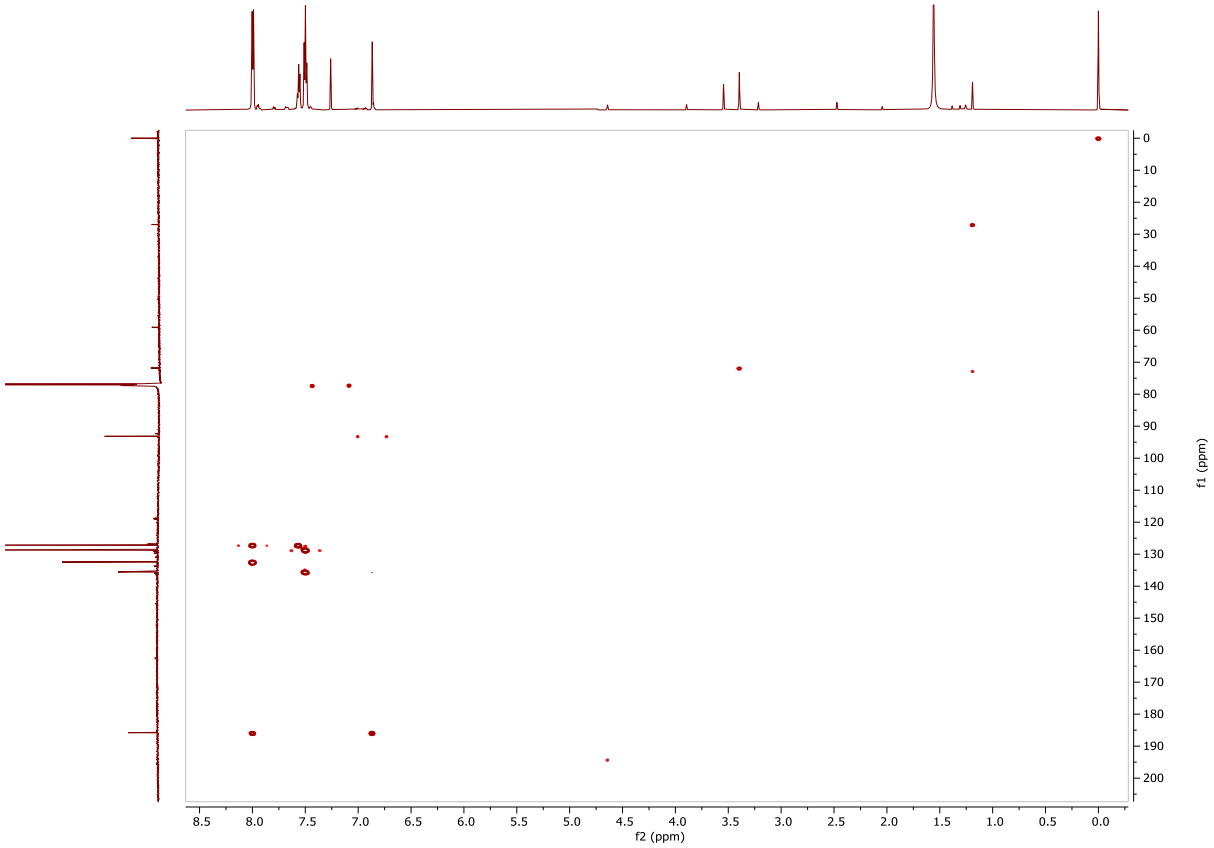


Figure S25. HMBC spectrum (600.19, 150.91 MHz, $CHCl_3$-*d*) of compound 3.

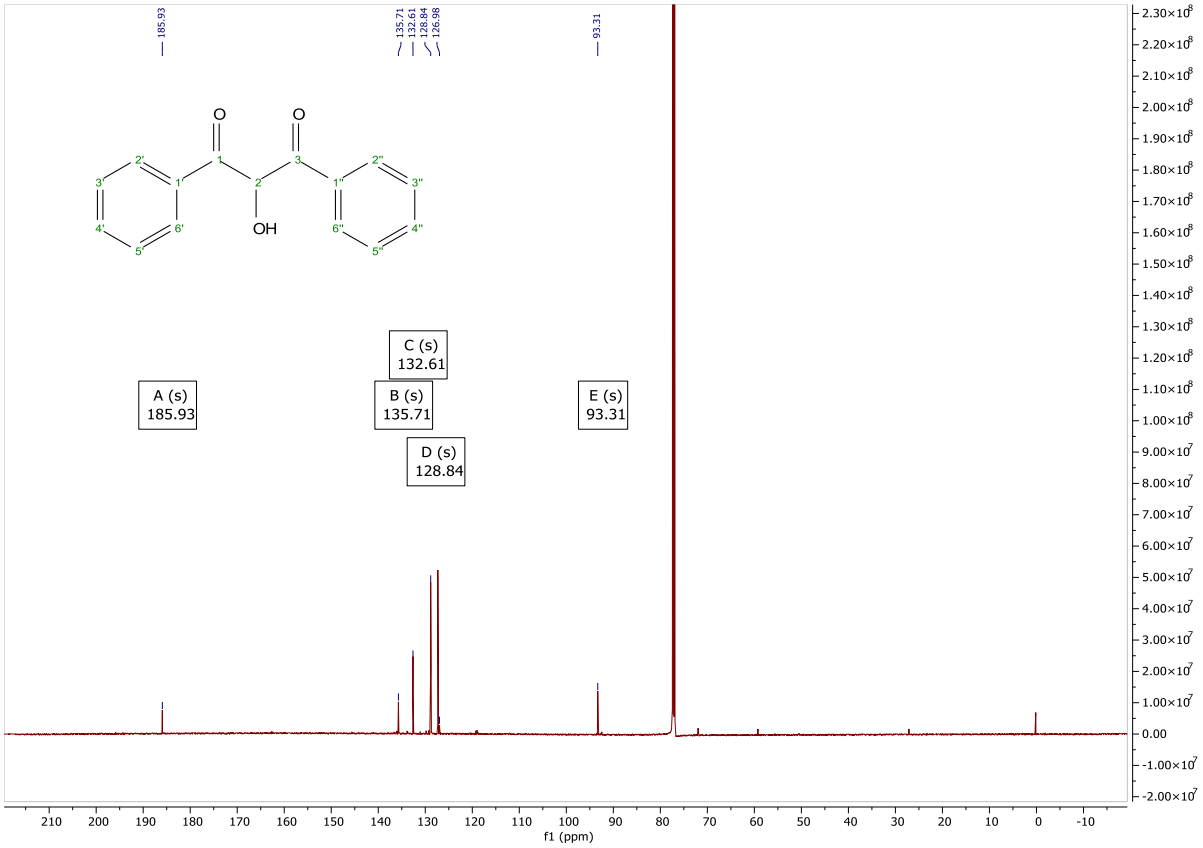


Figure S26. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 3.

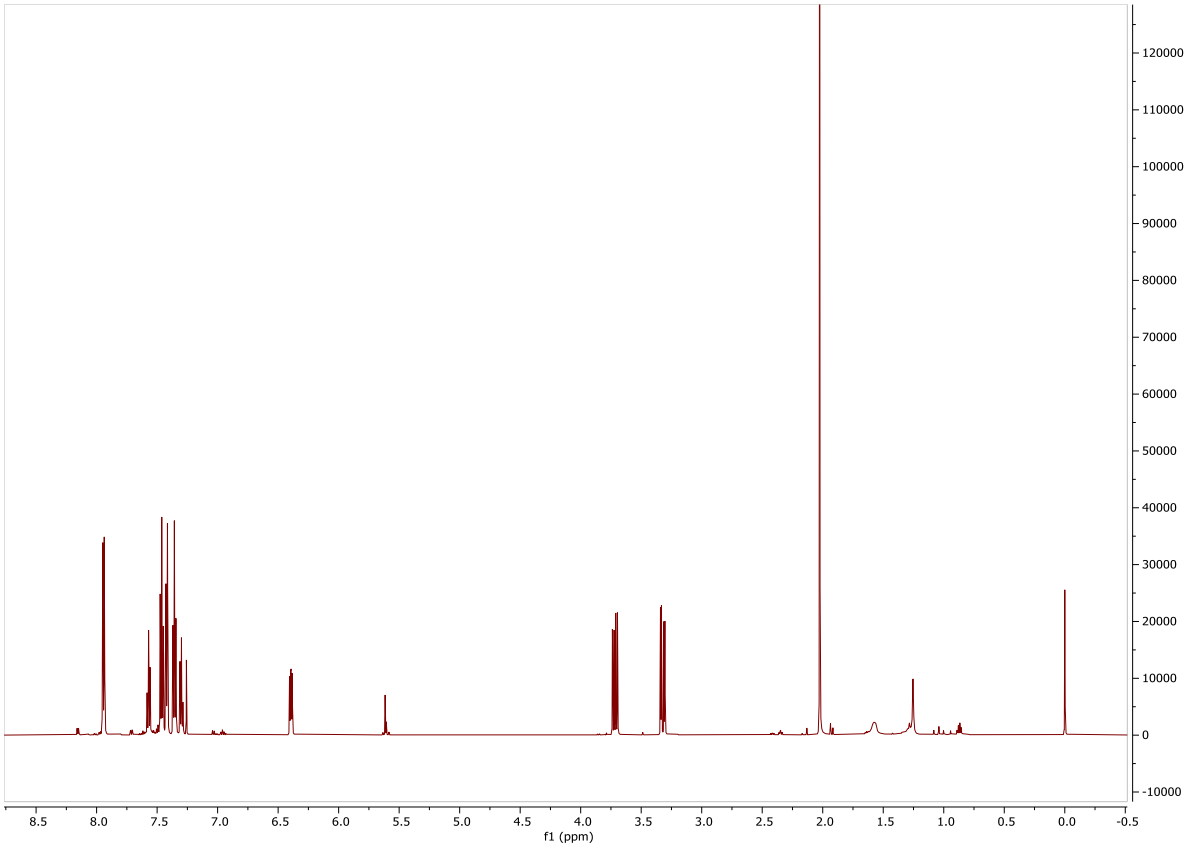


Figure S27. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 4.

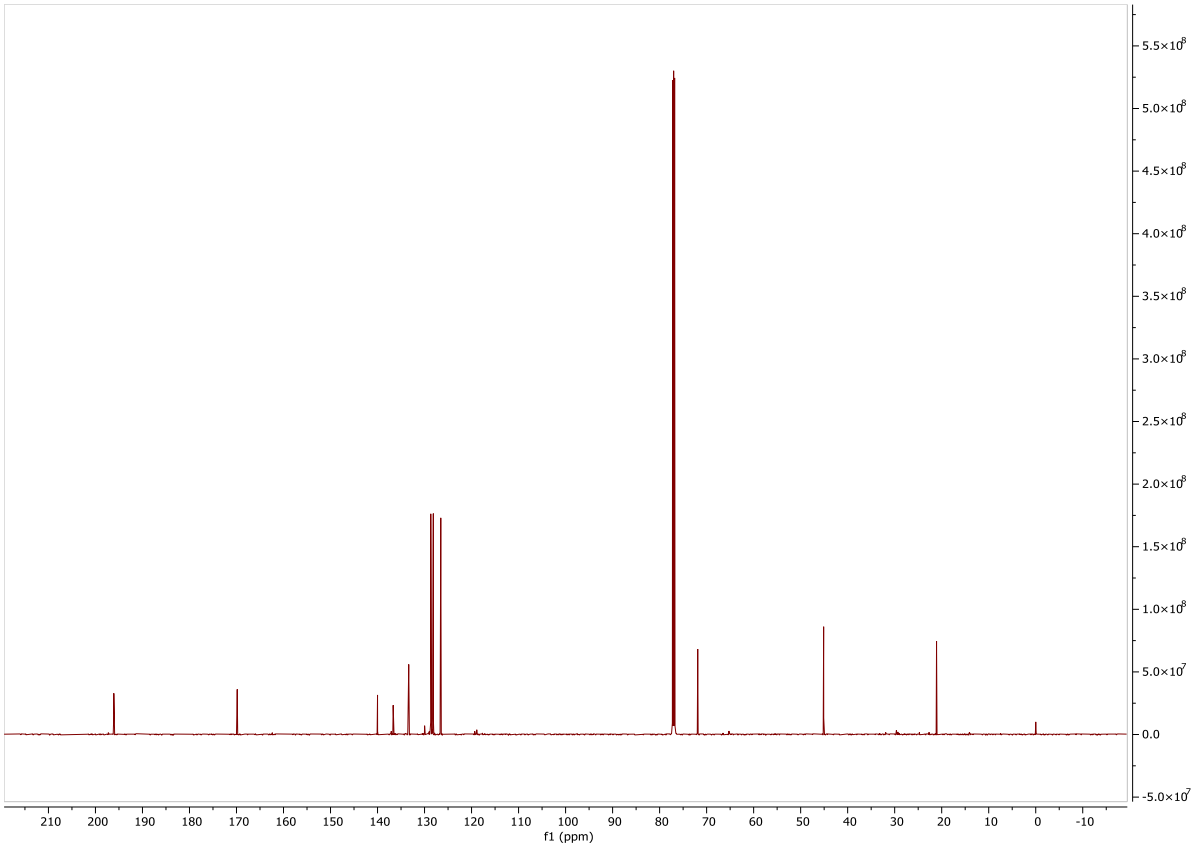


Figure S28. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 4.

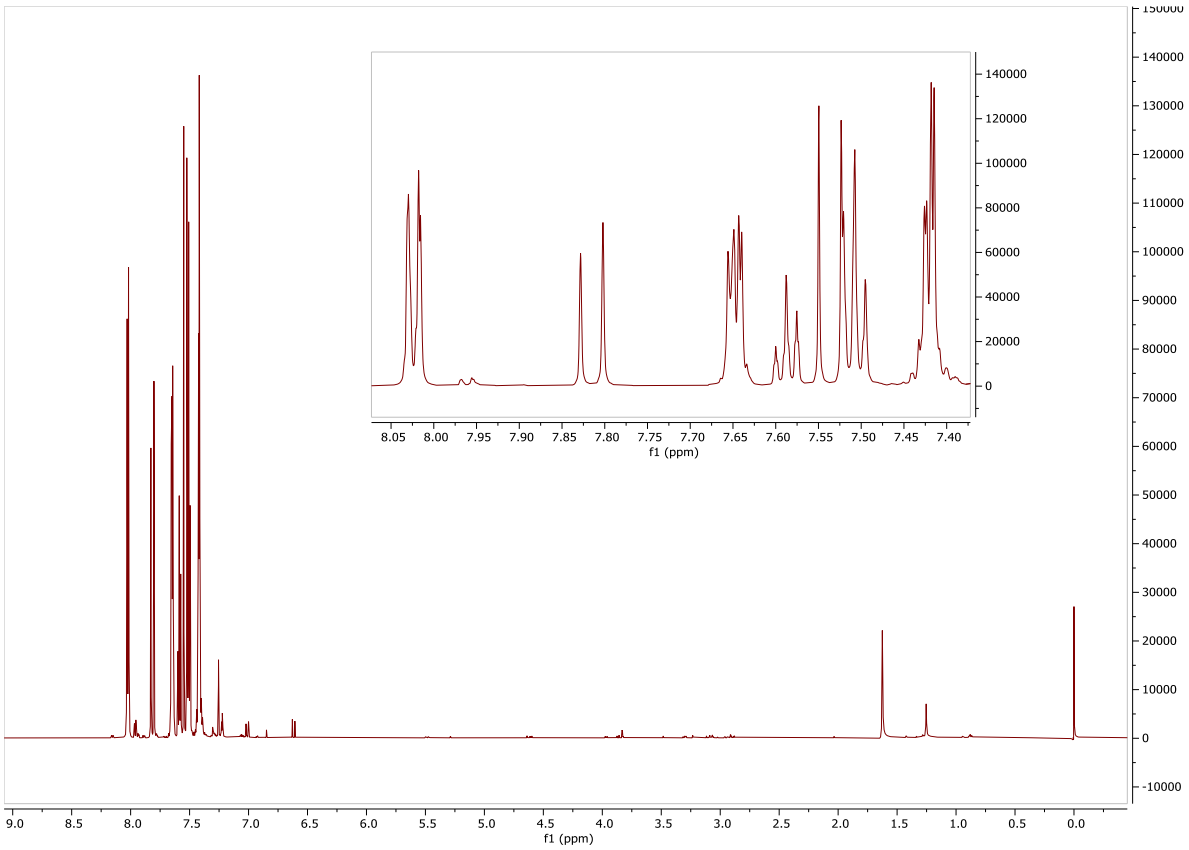


Figure S29. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 5.

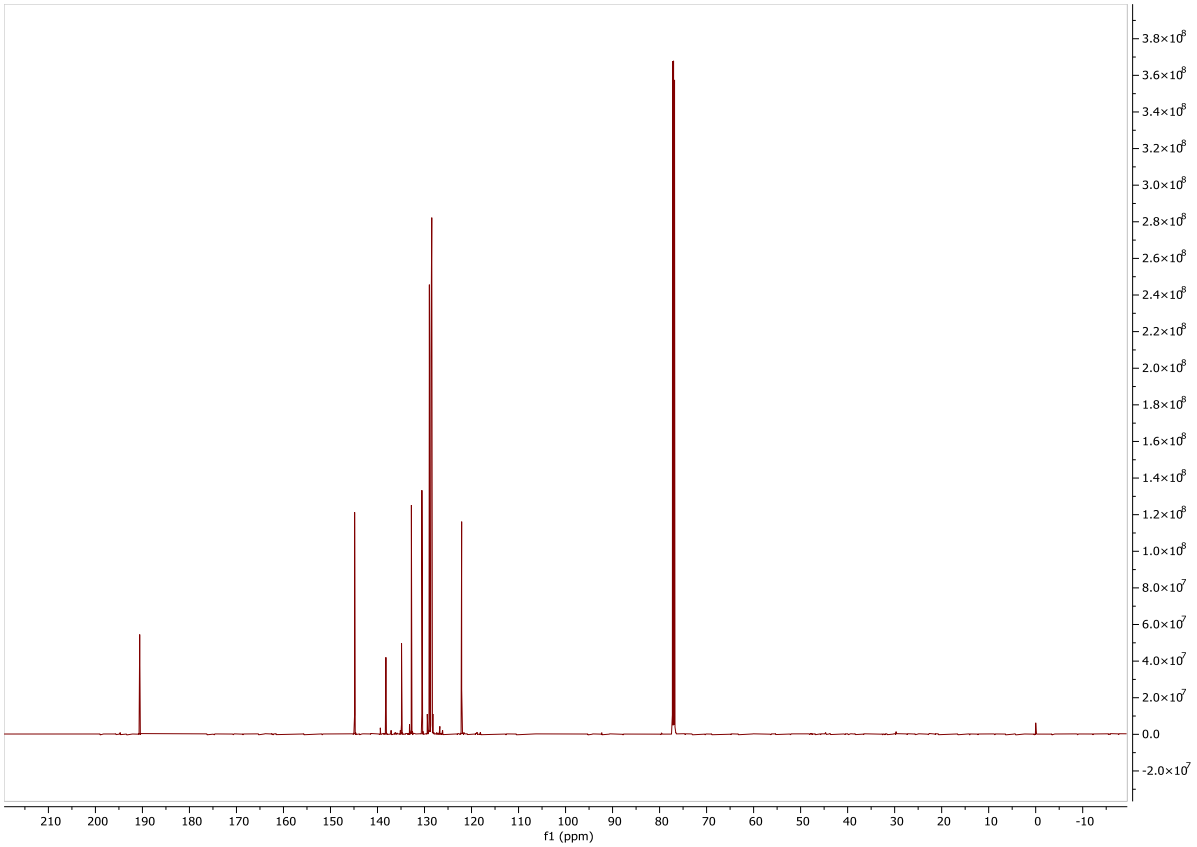


Figure S30. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 5.

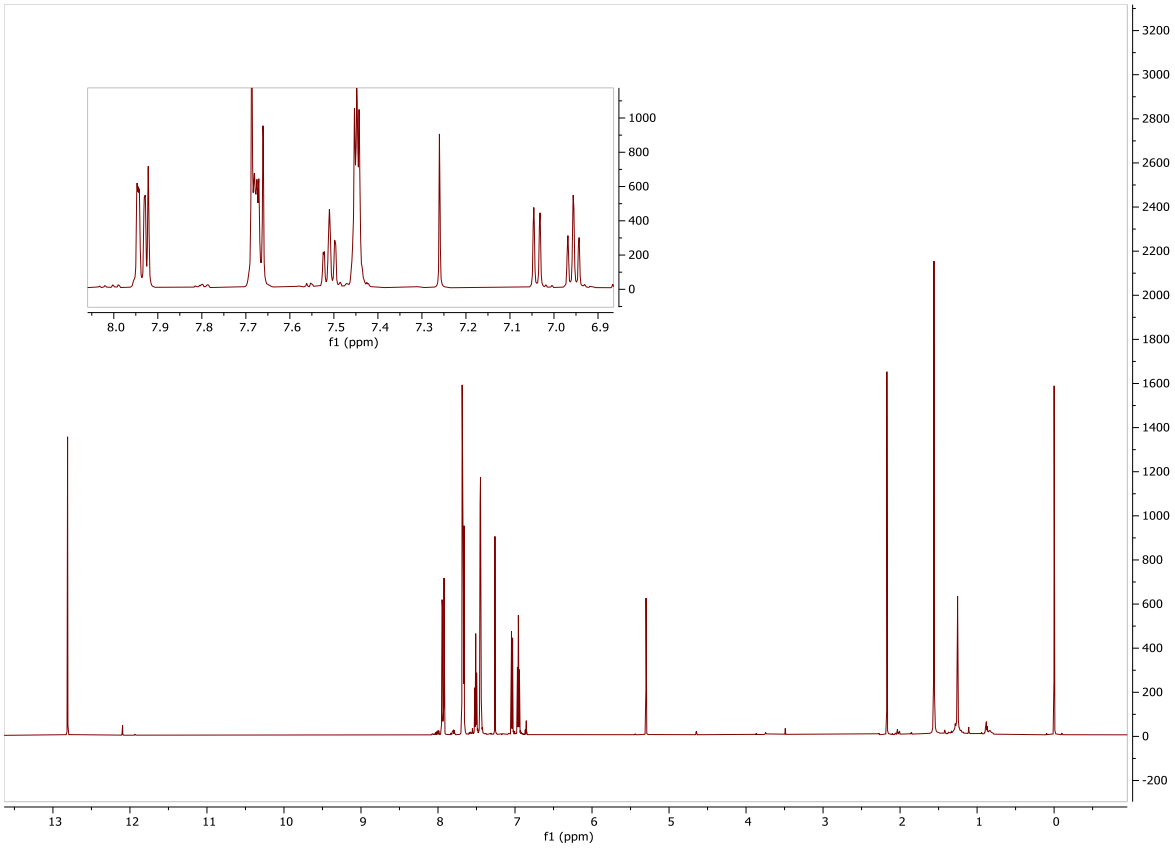


Figure S31. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 6.

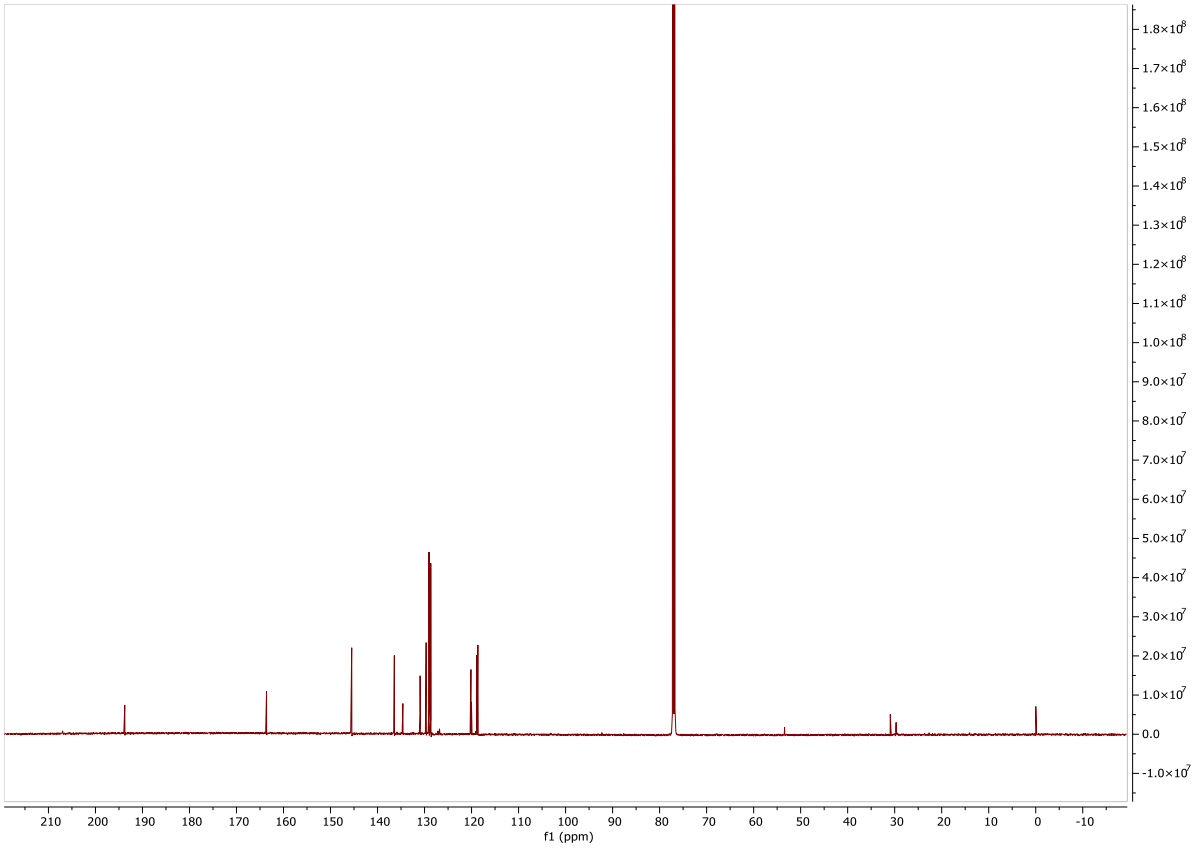


Figure S32. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 6.

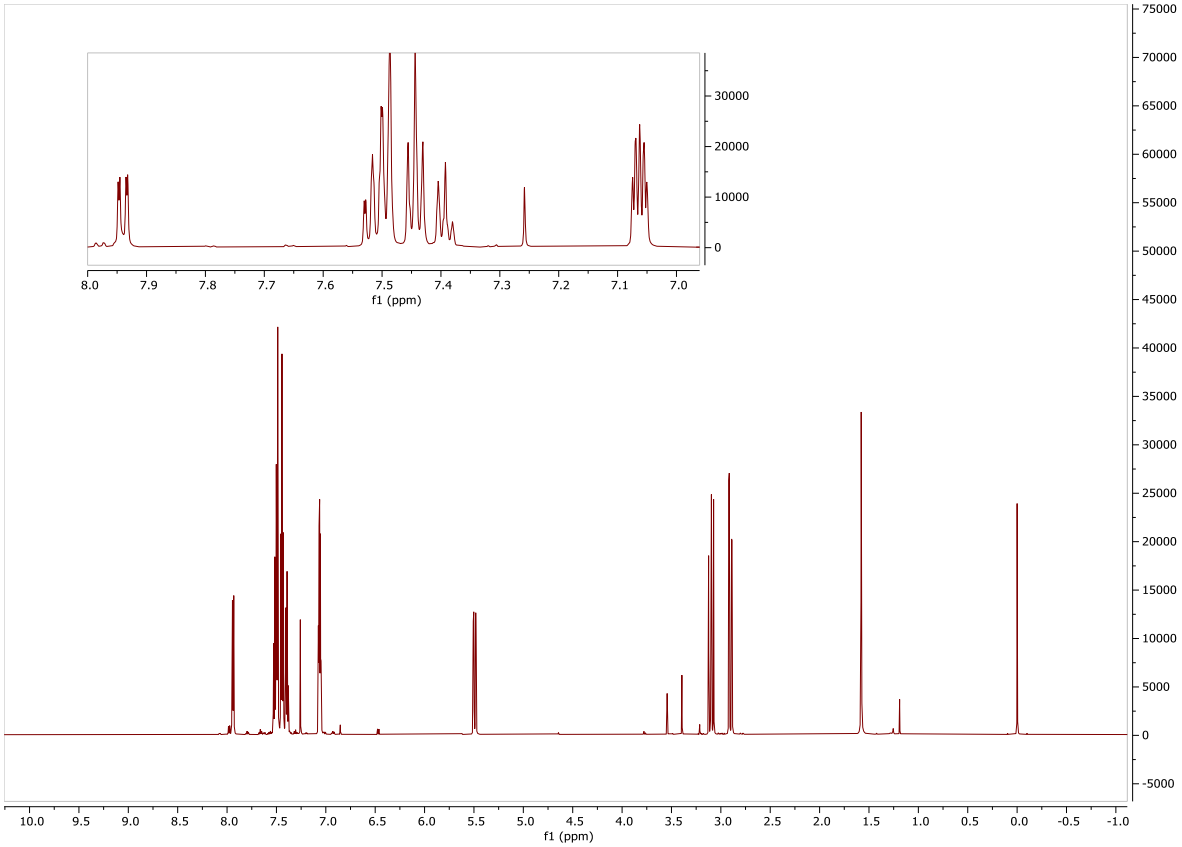


Figure S33. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 7.

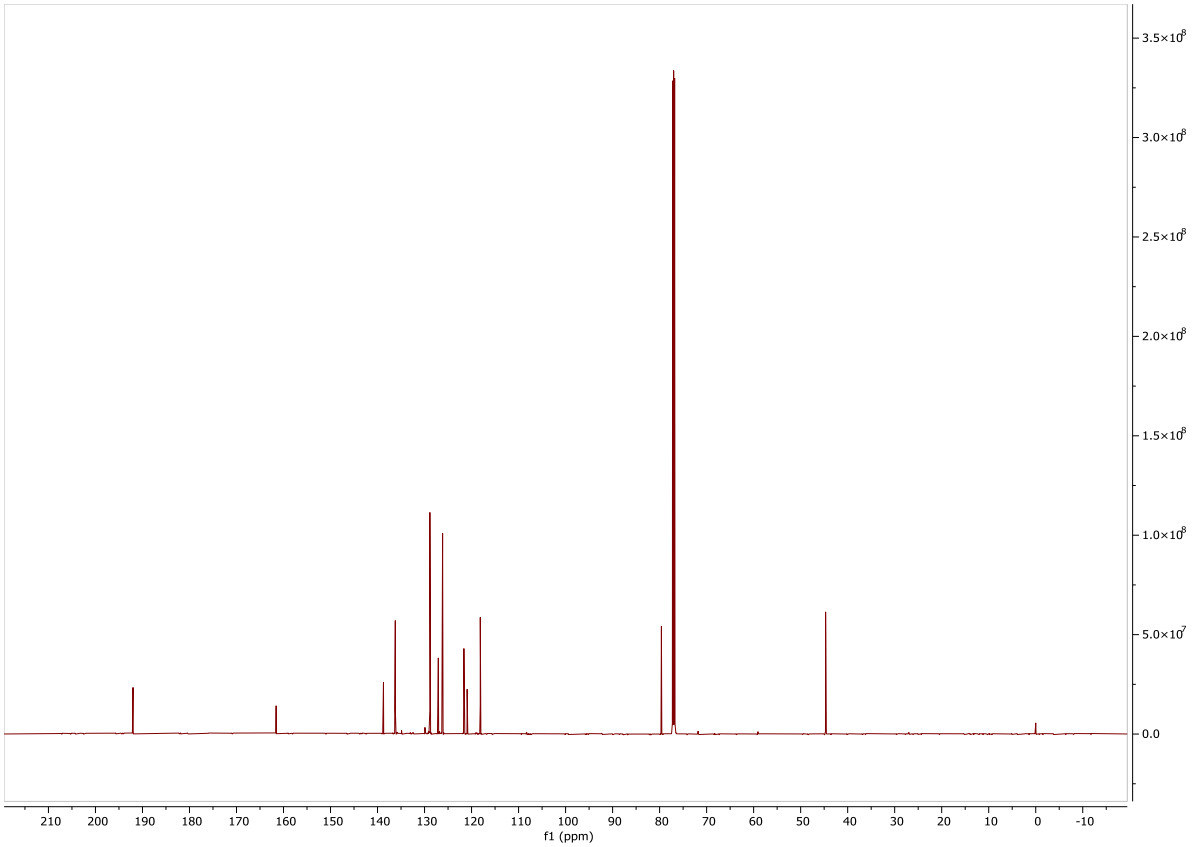


Figure S34. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 7.

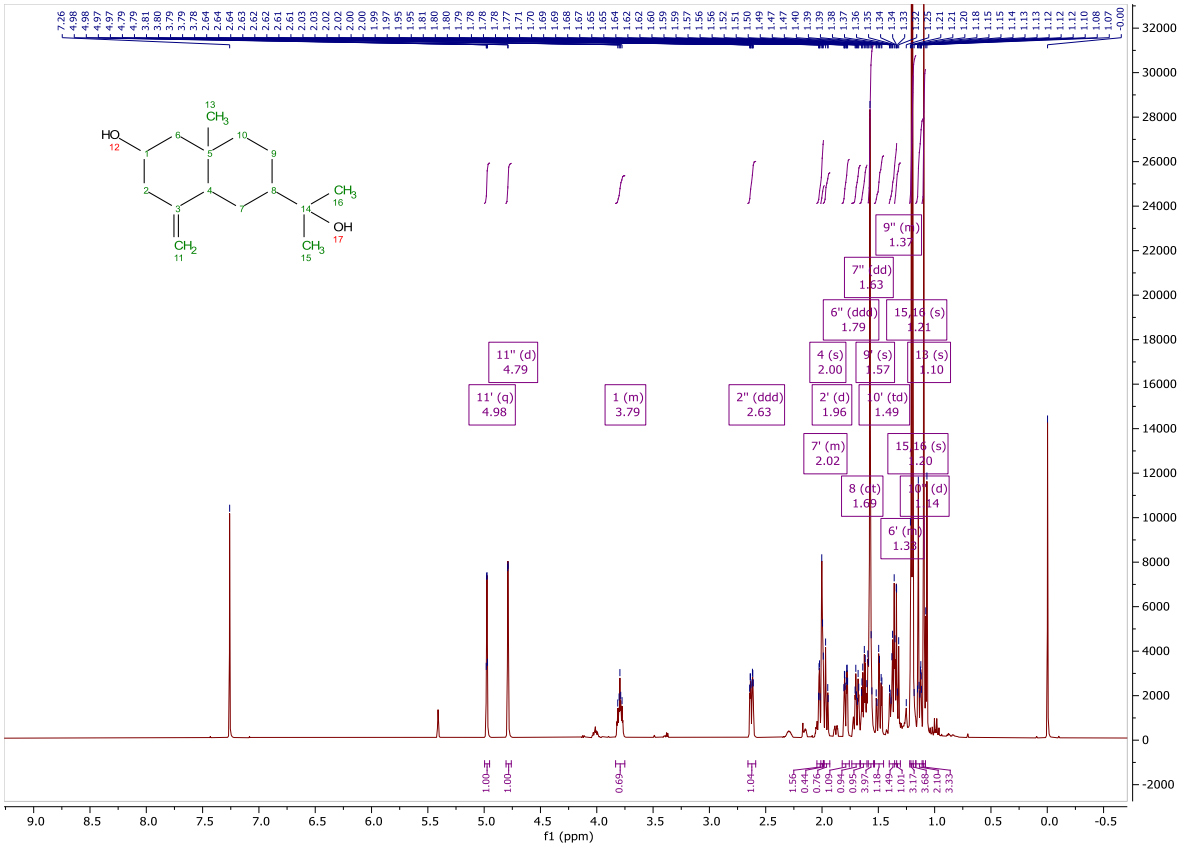


Figure S35. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 8.

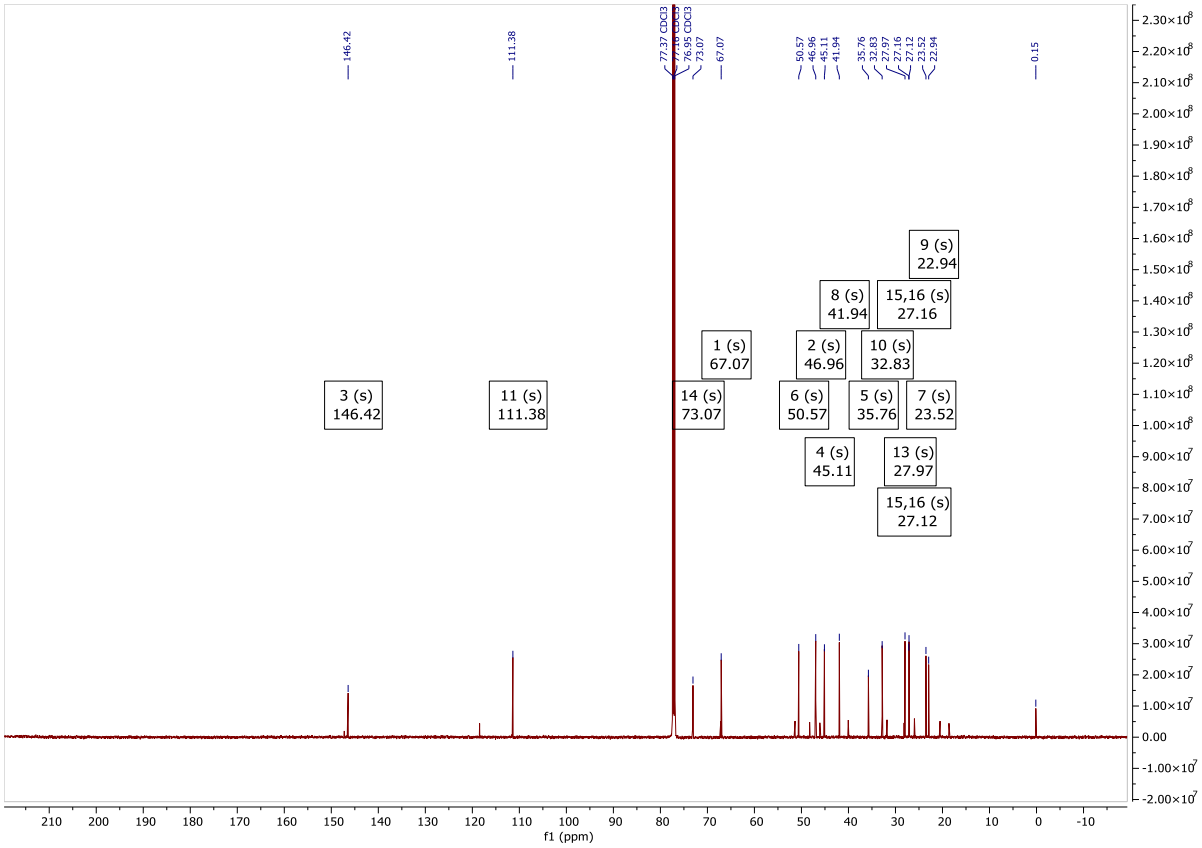


Figure S36. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 8.

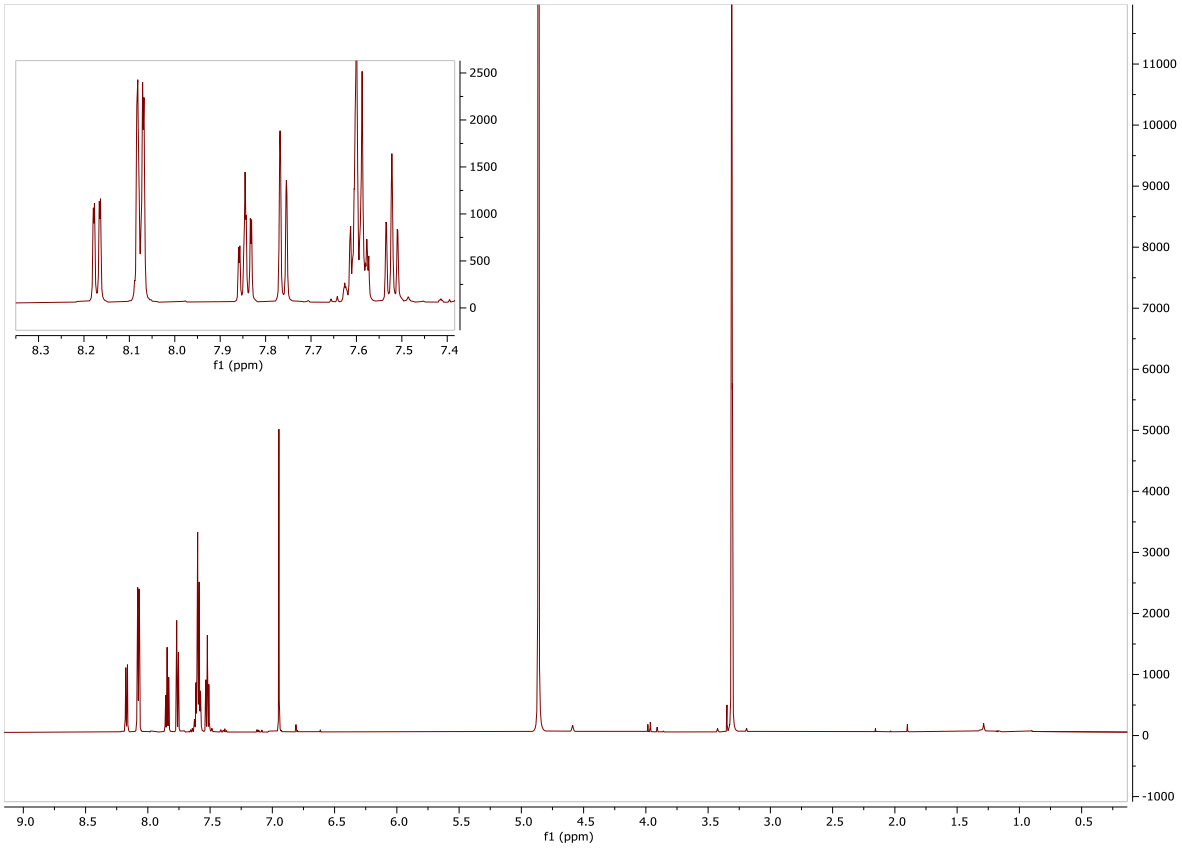


Figure S37. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 9.

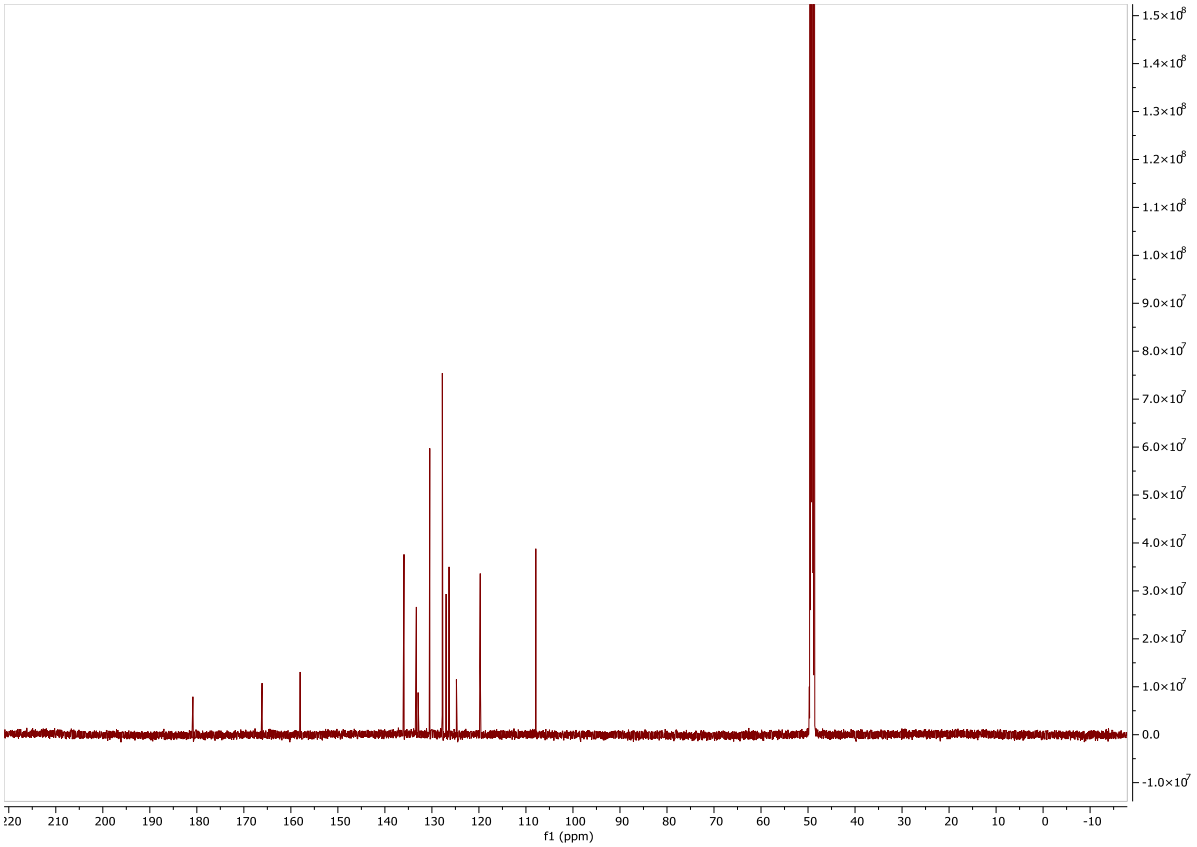


Figure S38. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 9.

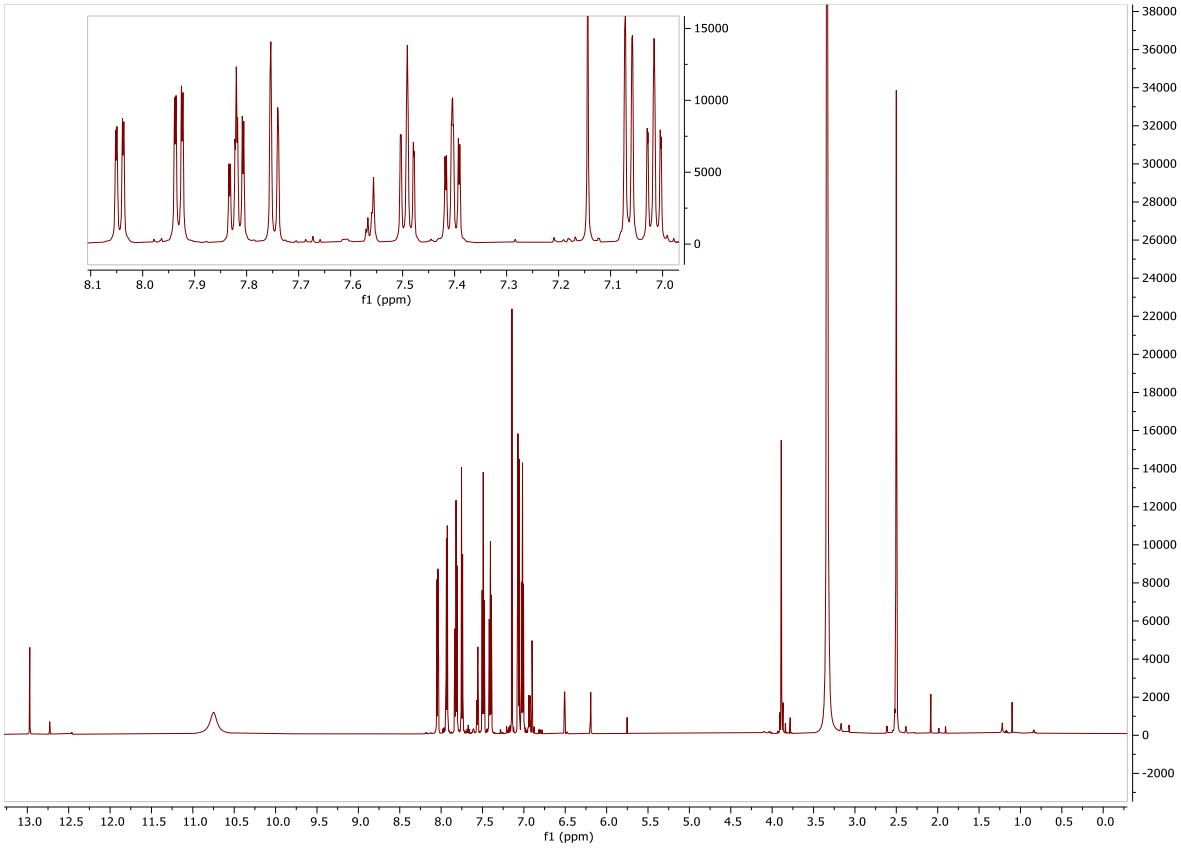


Figure S39. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 10.

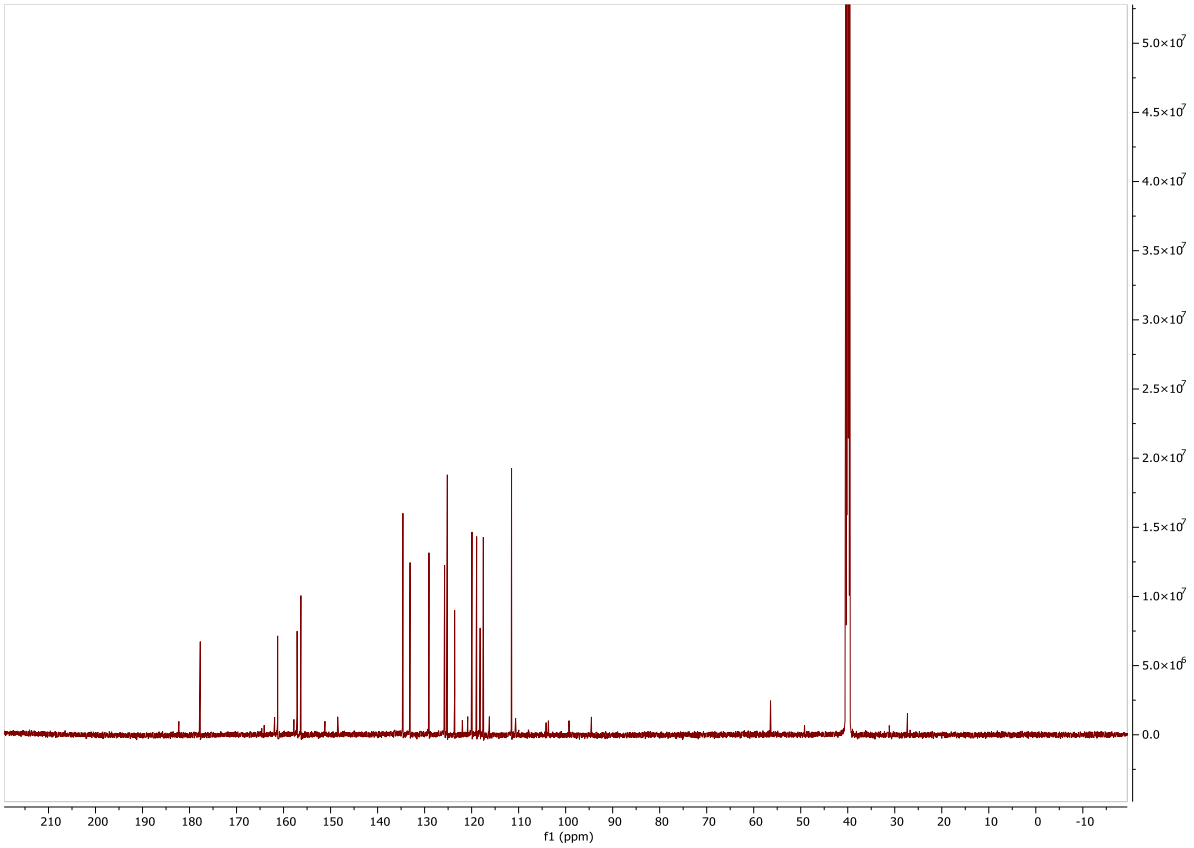


Figure S40. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 10.

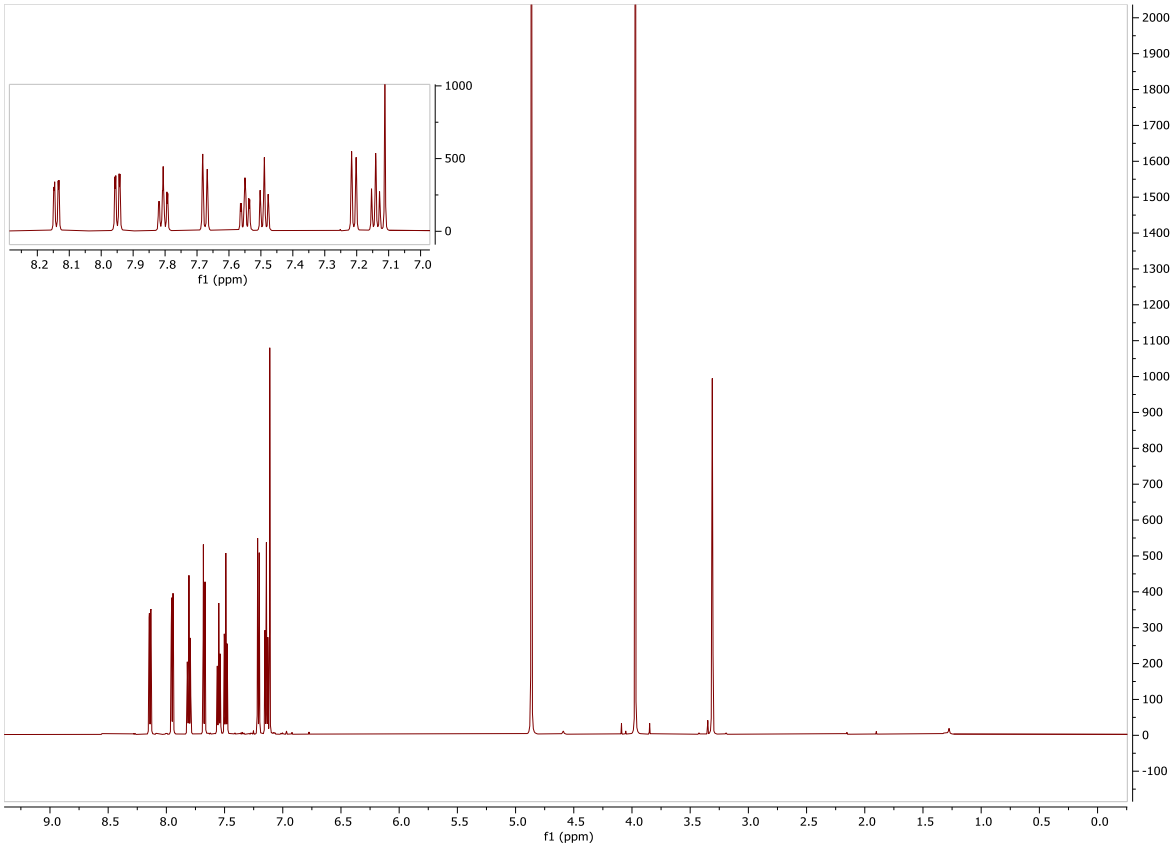


Figure S41. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 11.

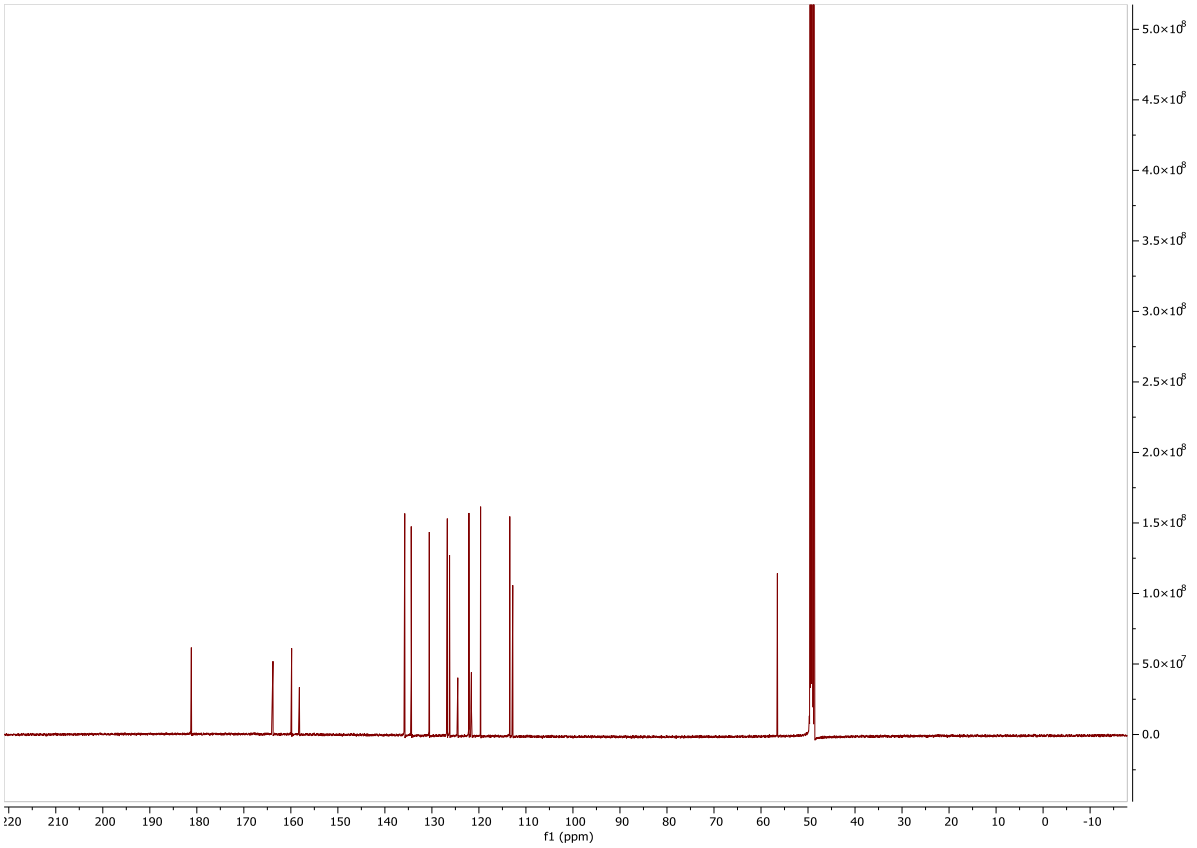


Figure S42. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 11.

Figure S43. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 12.

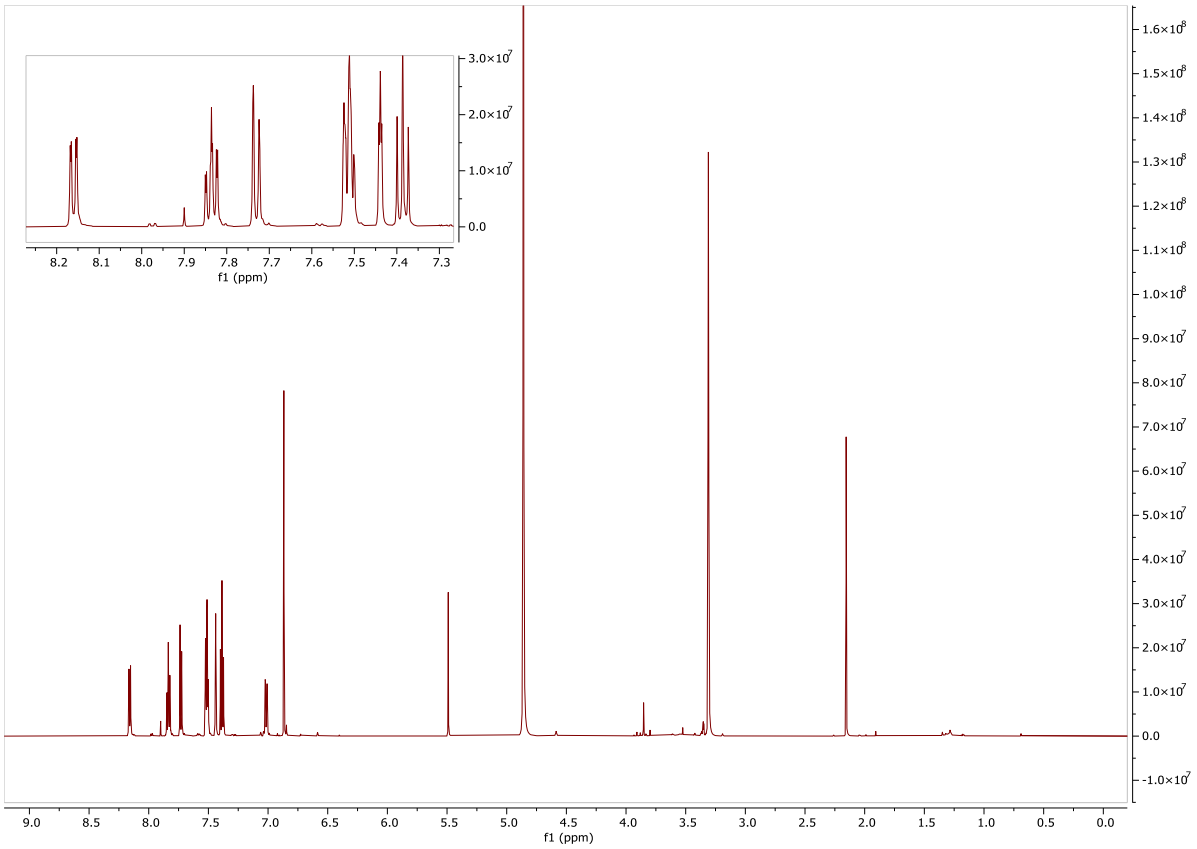


Figure S44. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 12.

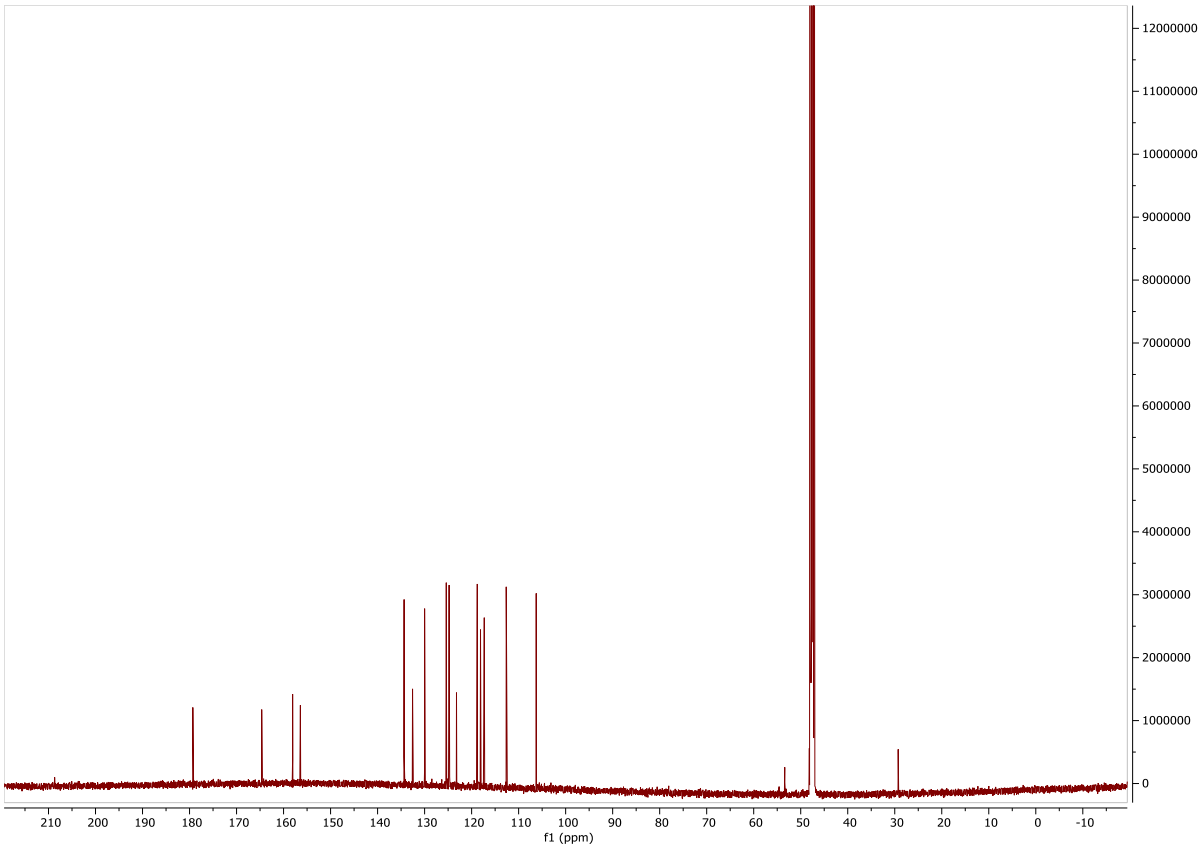


Figure S45. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 13.

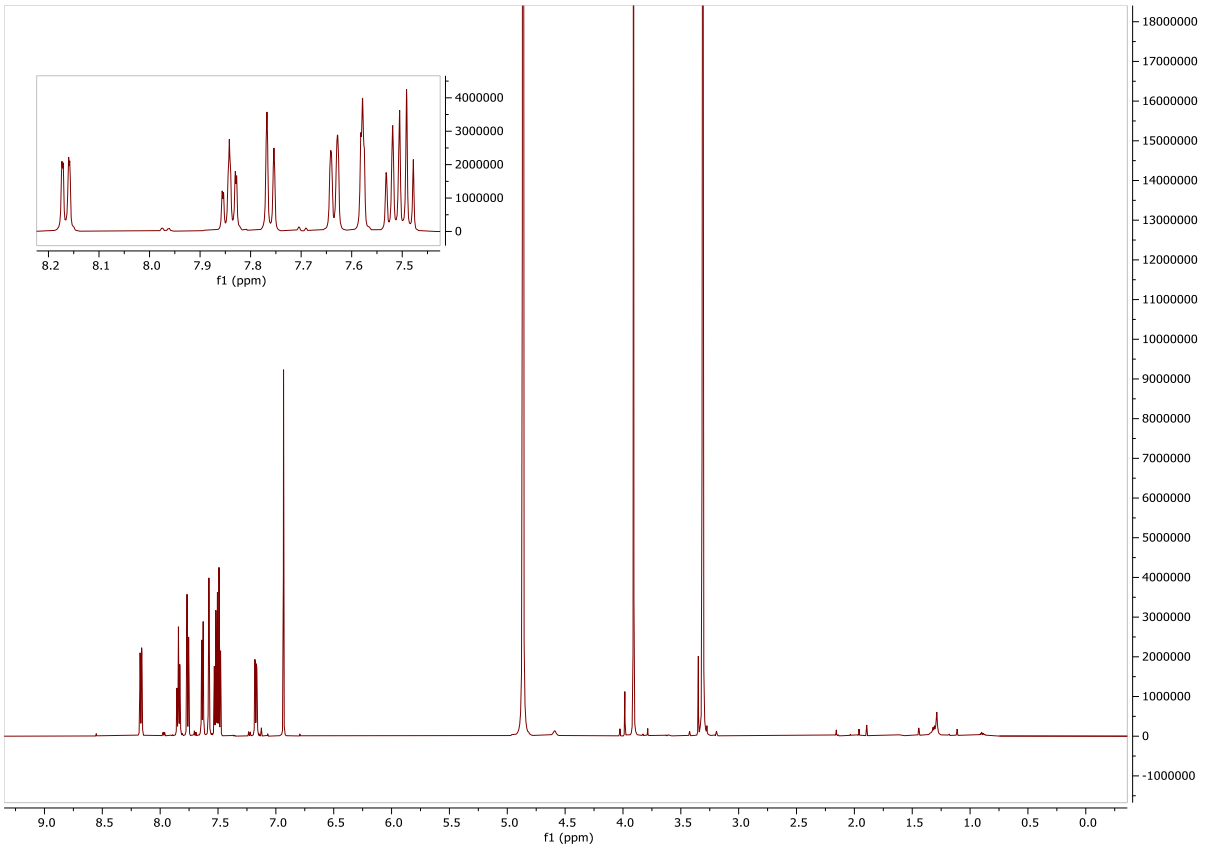


Figure S46. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 13.

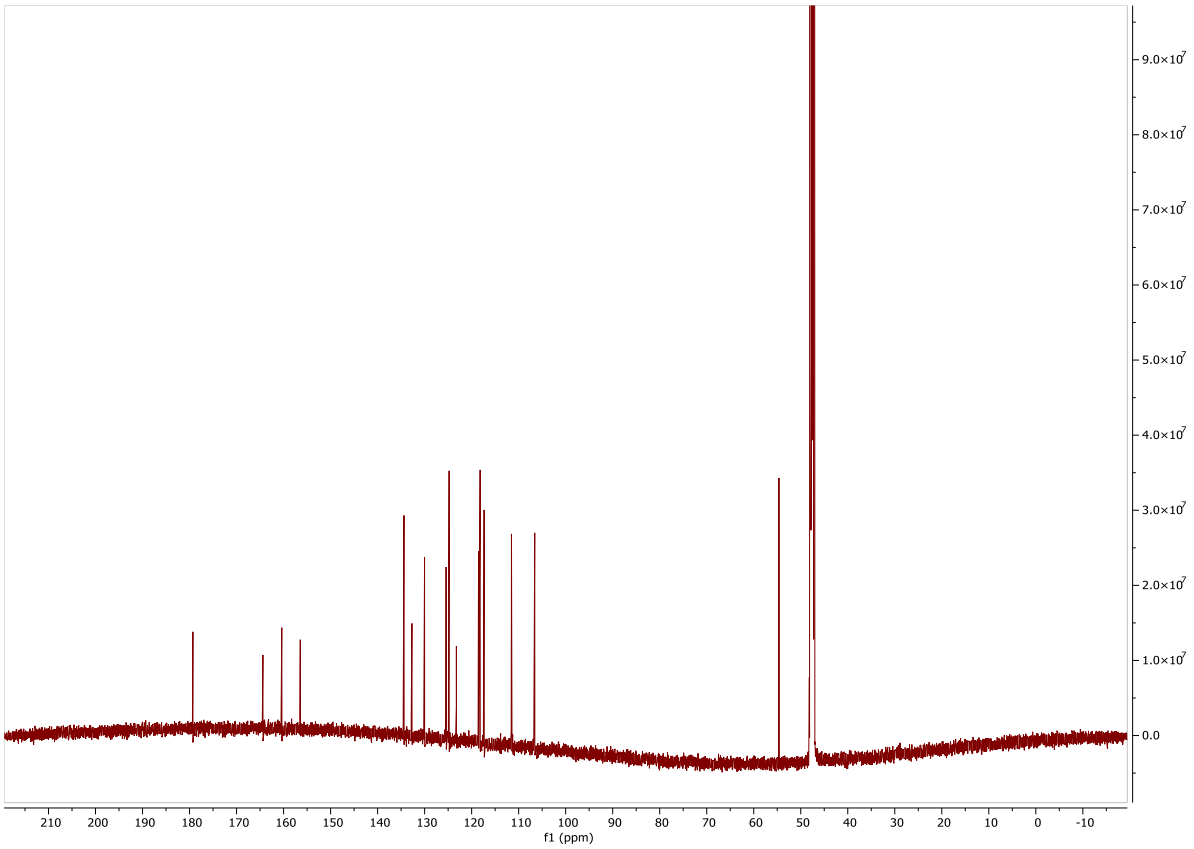


Figure S47. ^{1}H-NMR (600.19 MHz, Acetone-d_6) of compound 14.

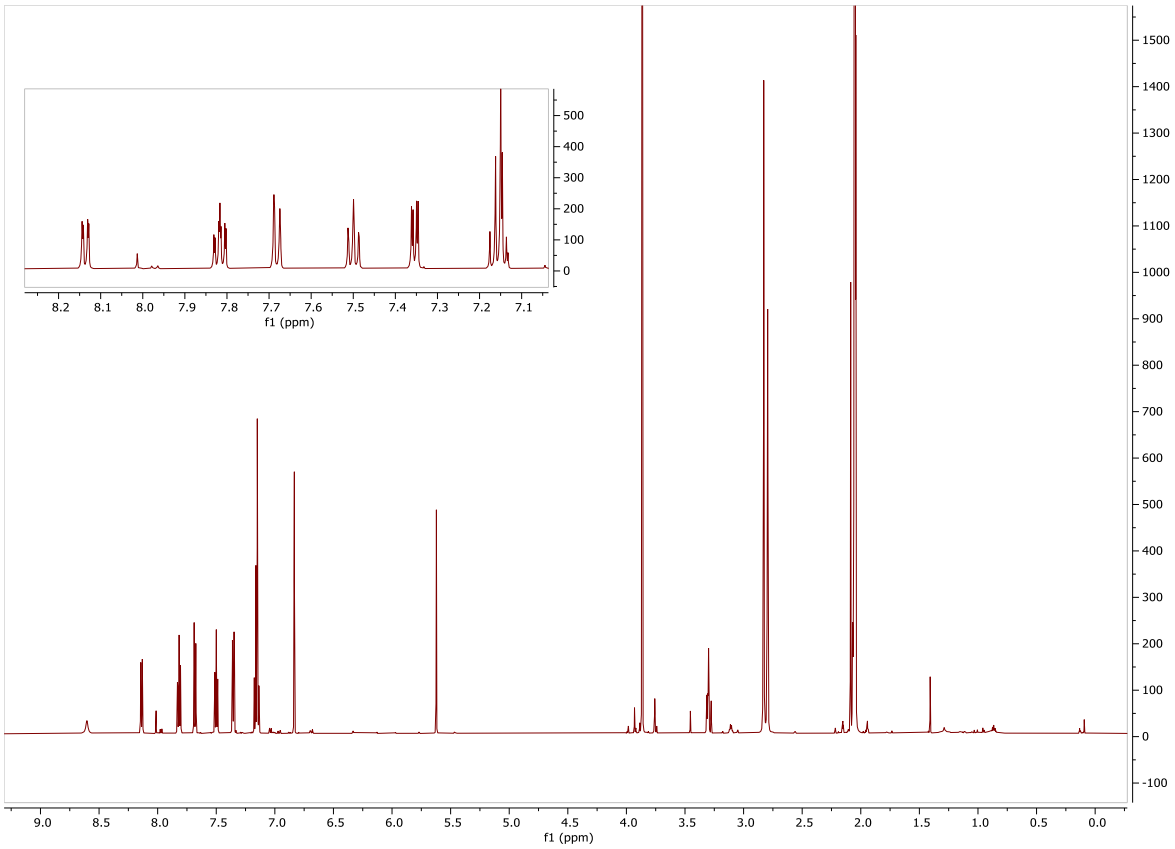


Figure S48. ^{13}C-NMR (150.91 MHz, Acetone-d_6) of compound 14.

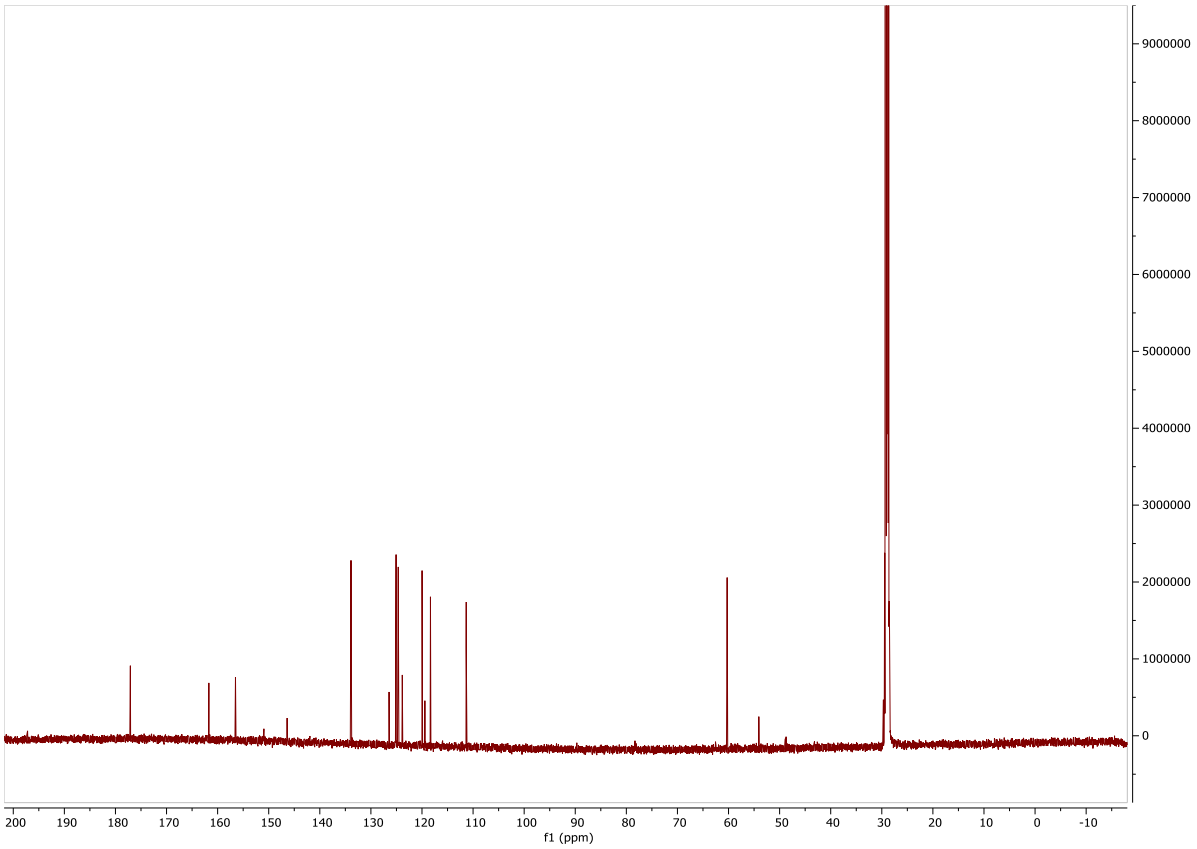


Figure S49. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 15.

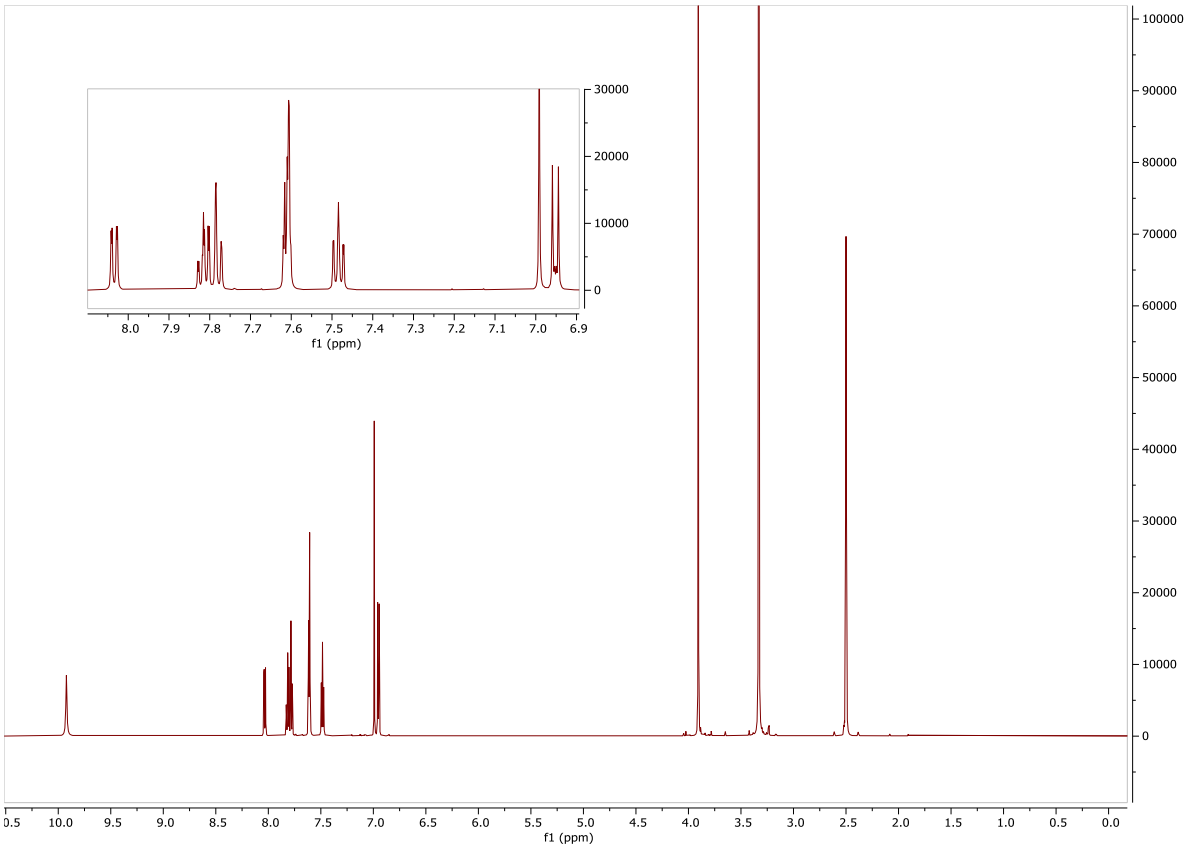


Figure S50. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 15.

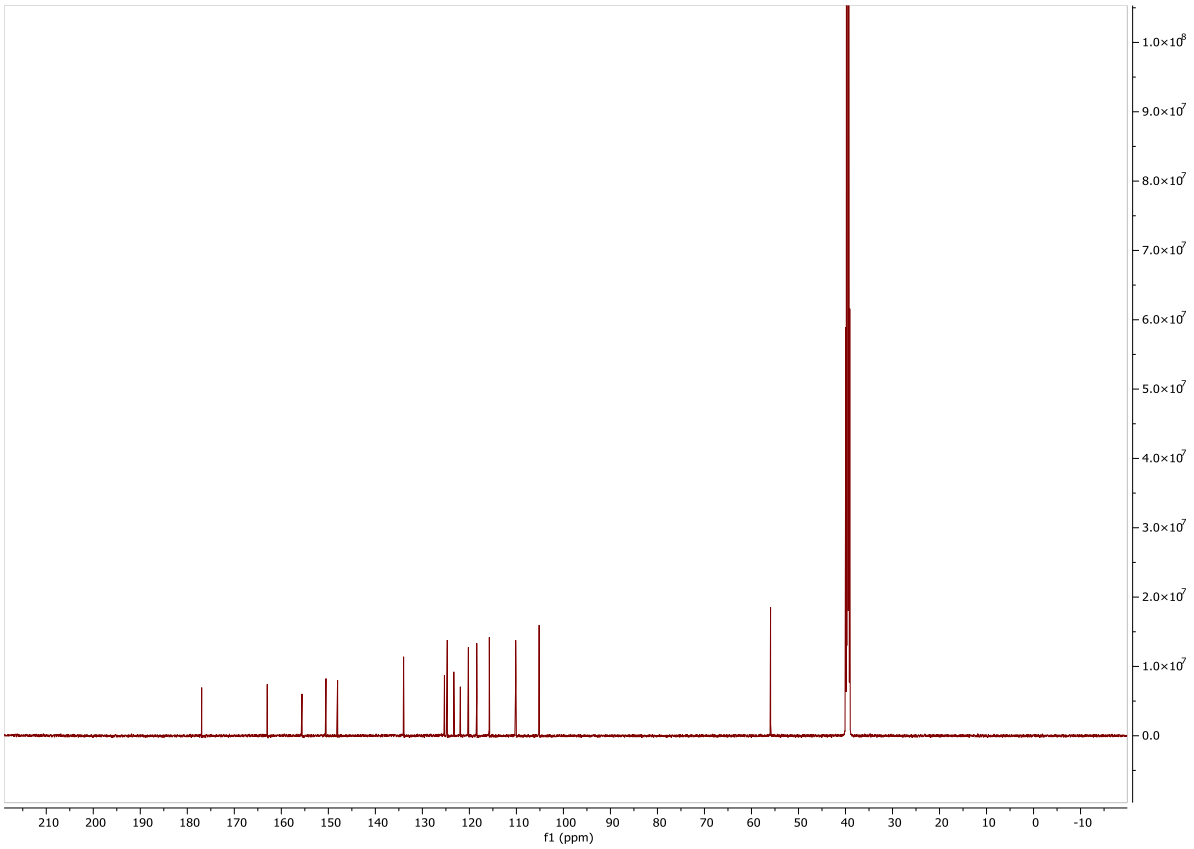


Figure S51. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 16.

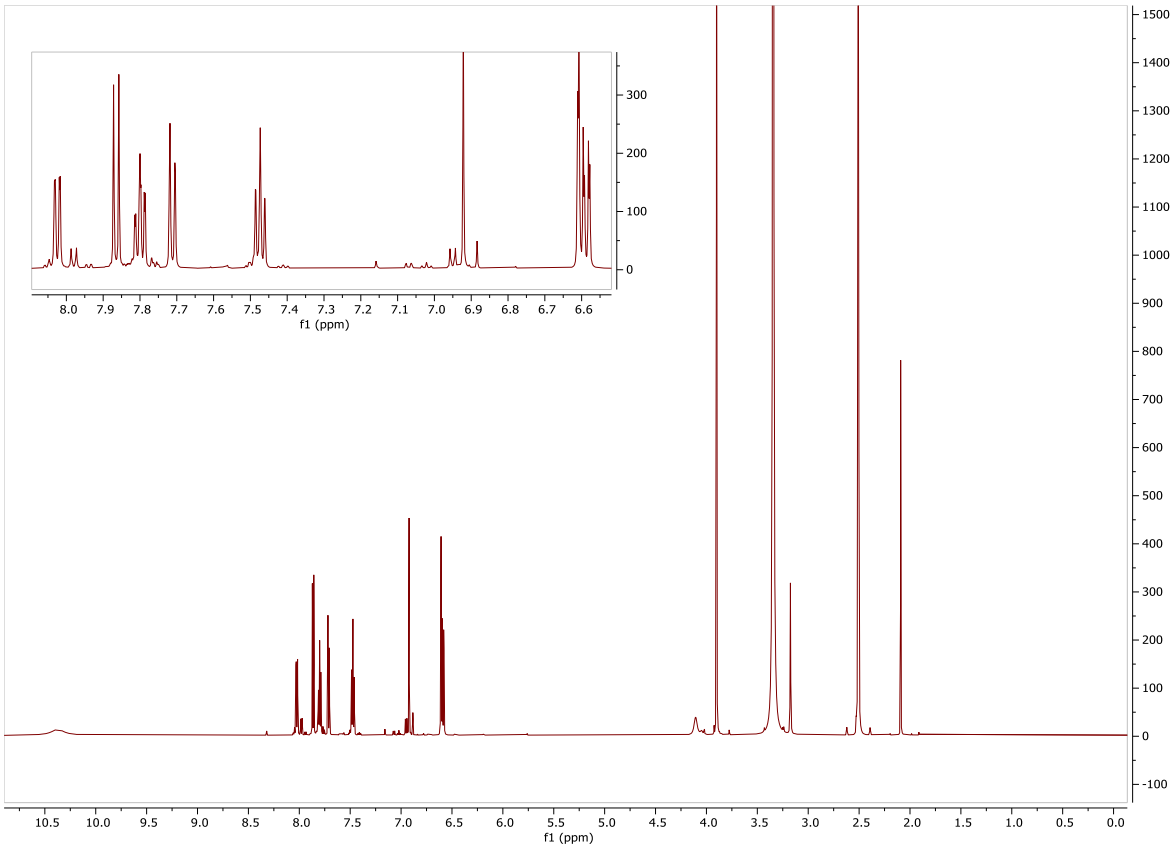


Figure S52. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 16.

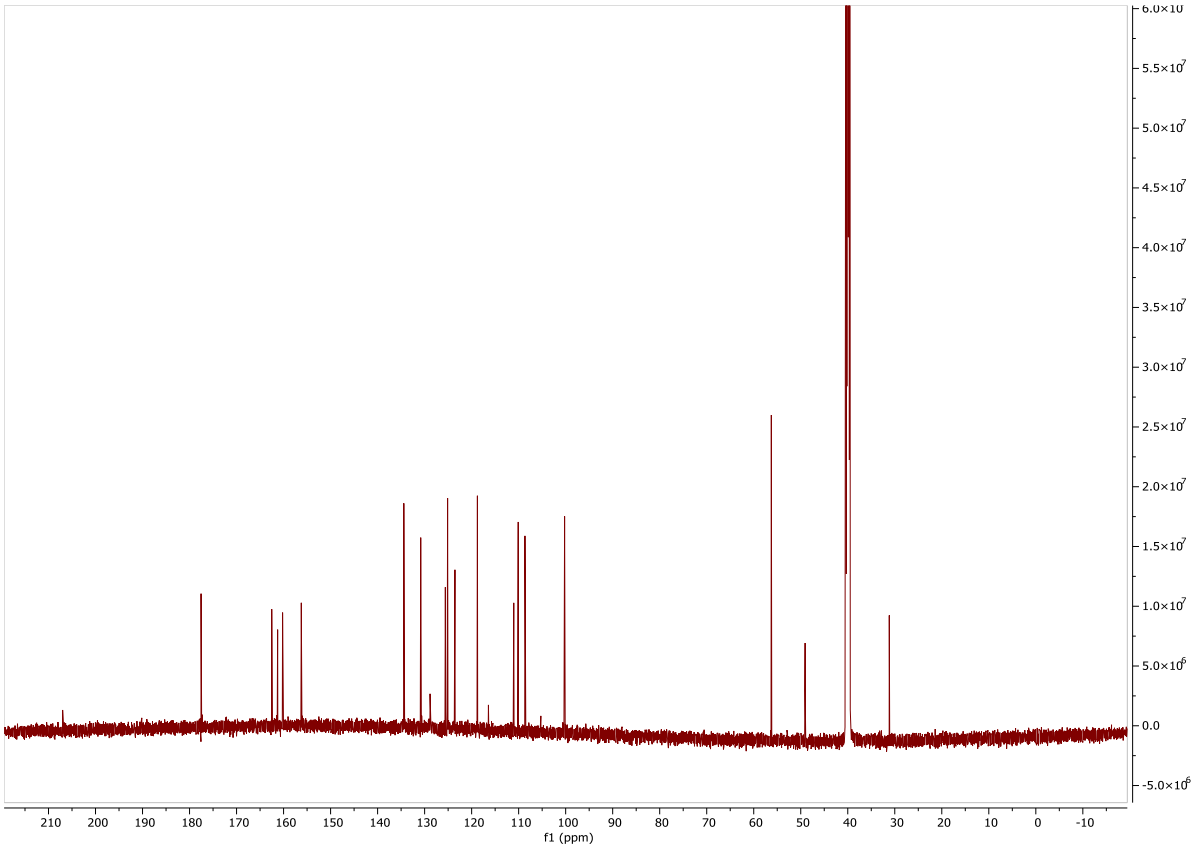


Figure S53. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 17.

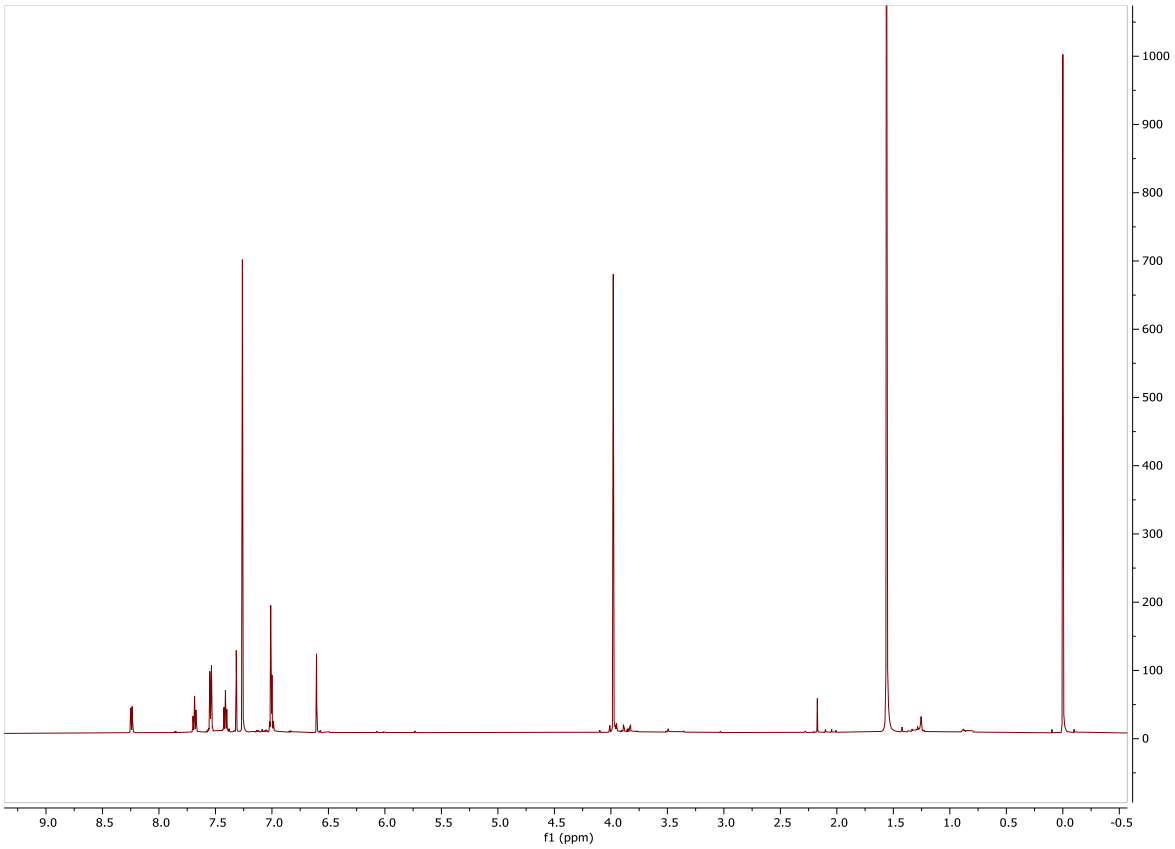


Figure S54. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 17.

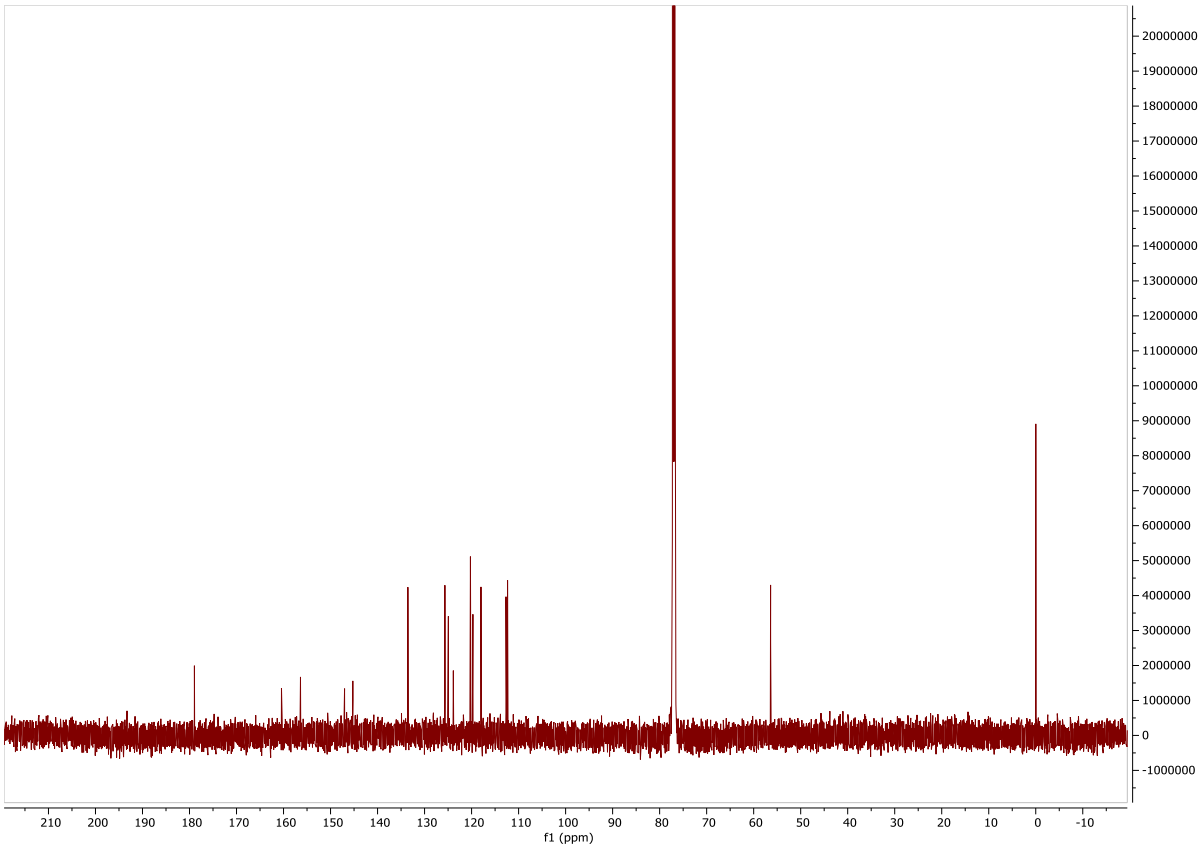


Figure S55. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 18.

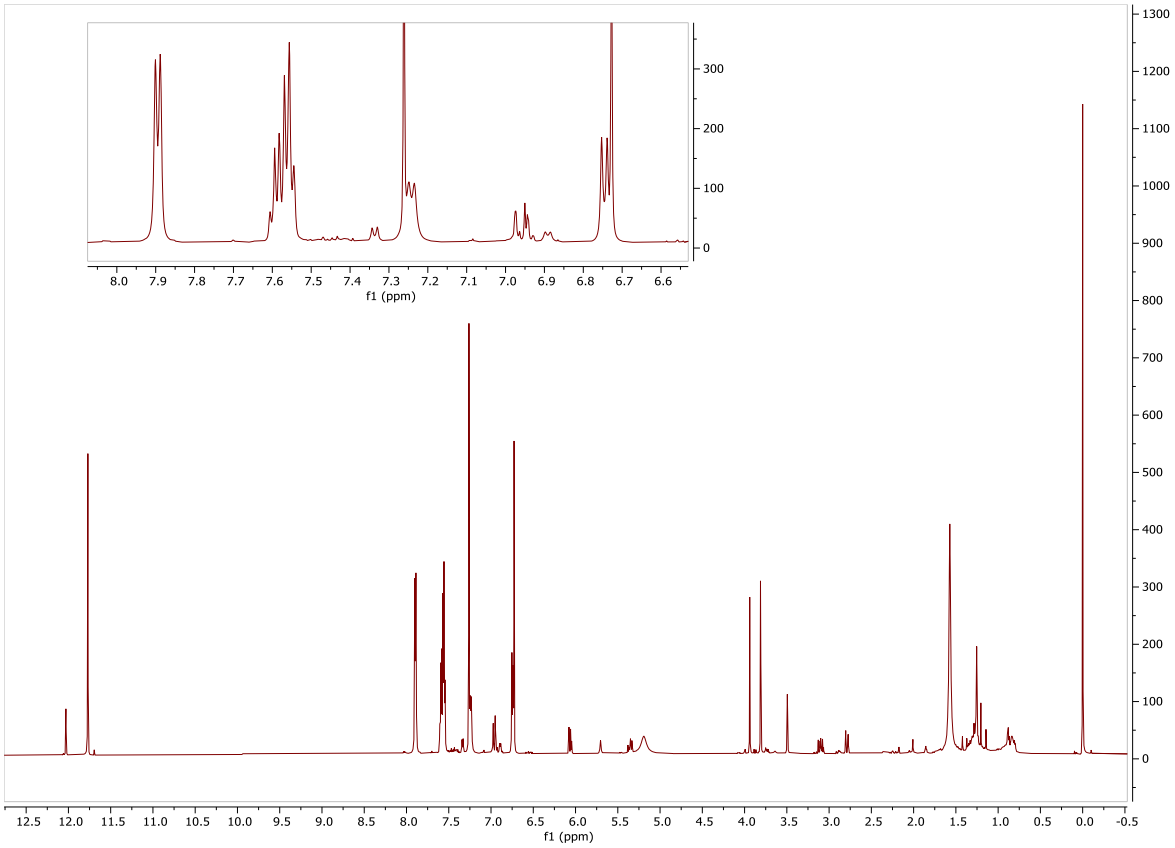


Figure S56. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 18.

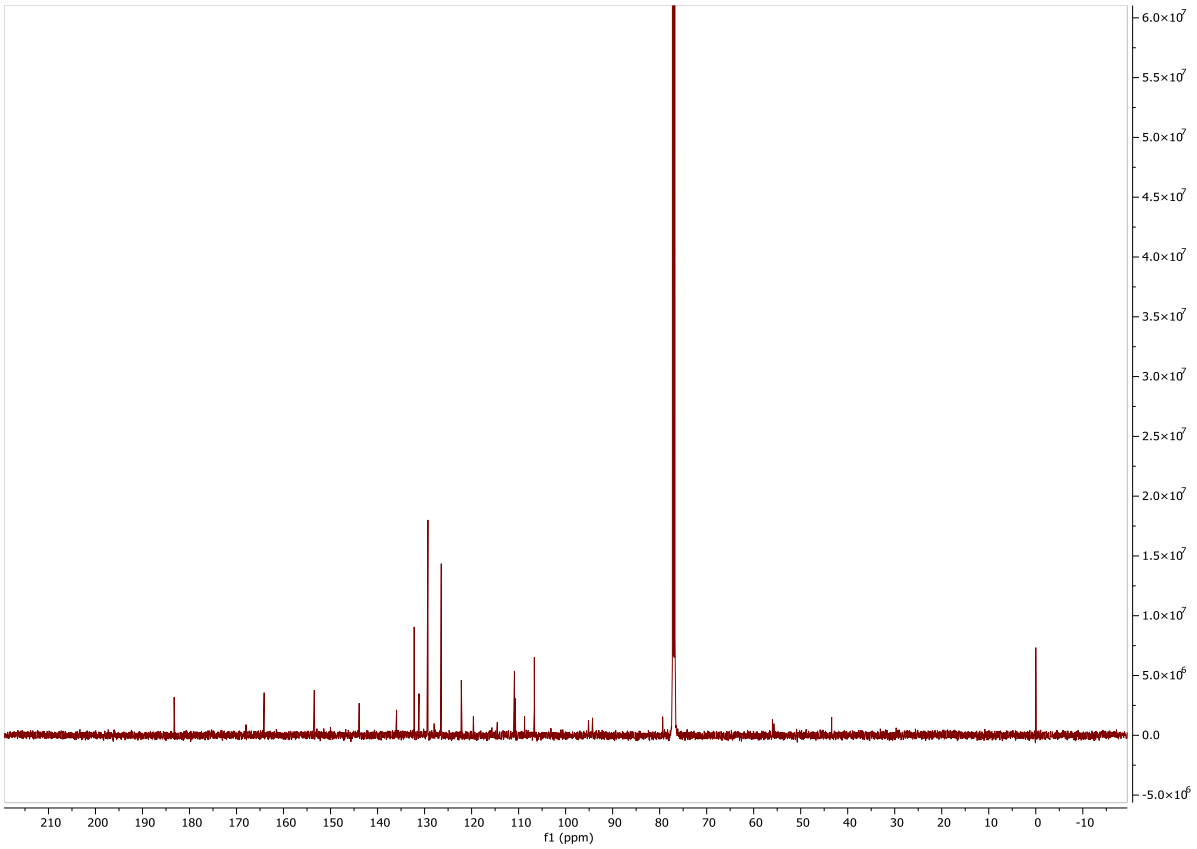


Figure S57. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 19.

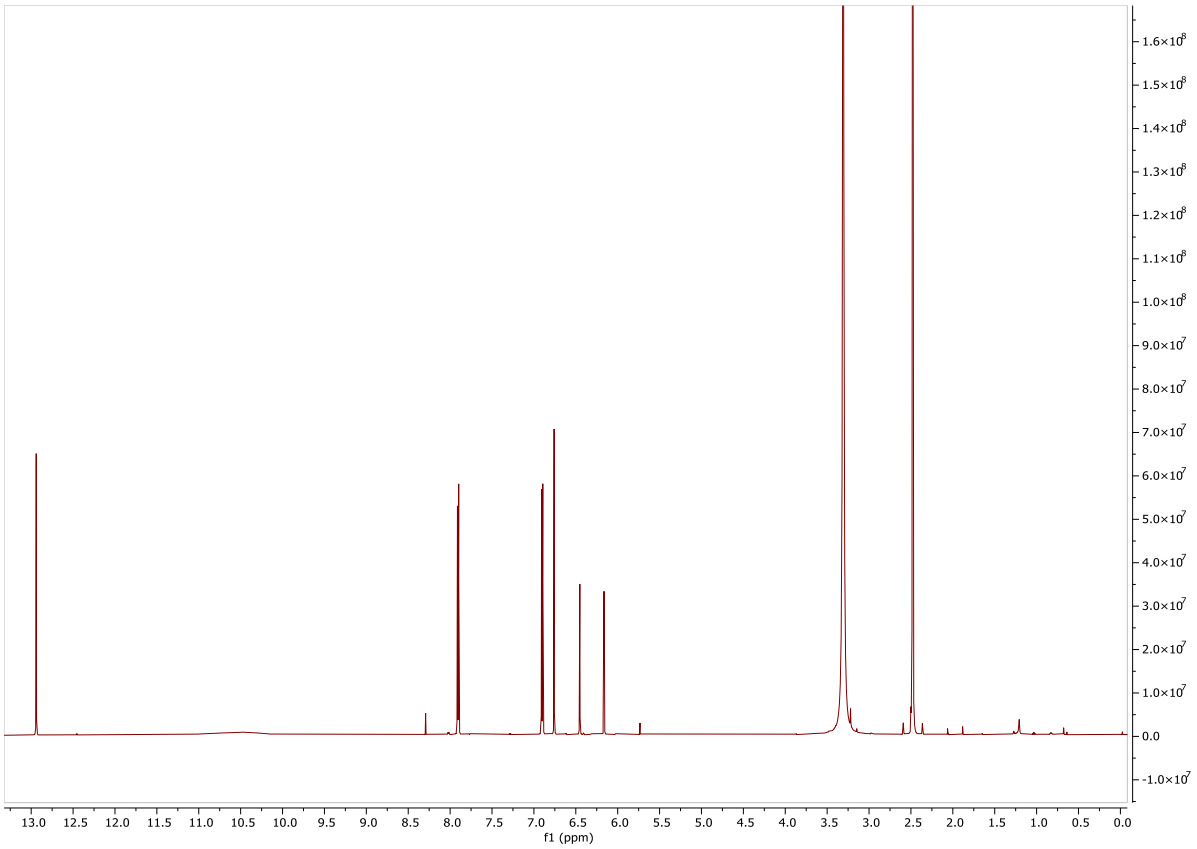


Figure S58. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 19.

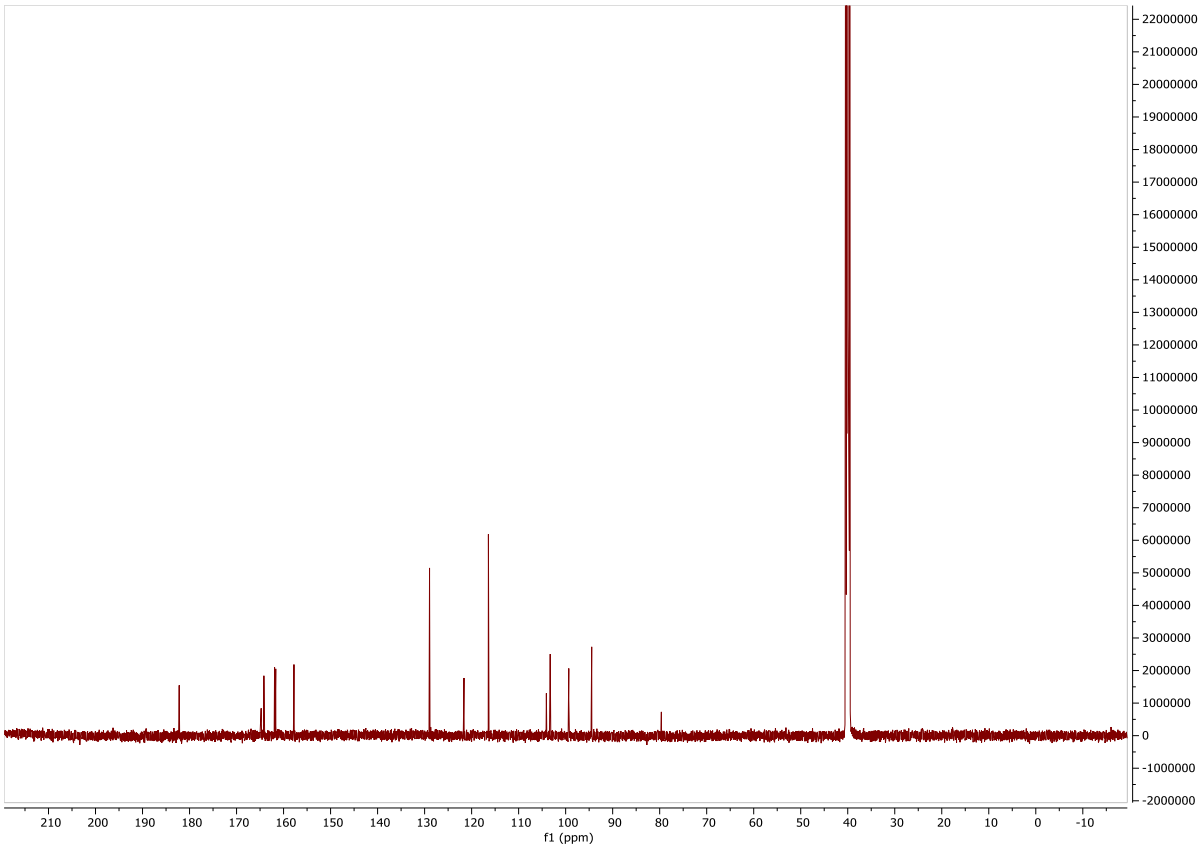


Figure S59. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 20.

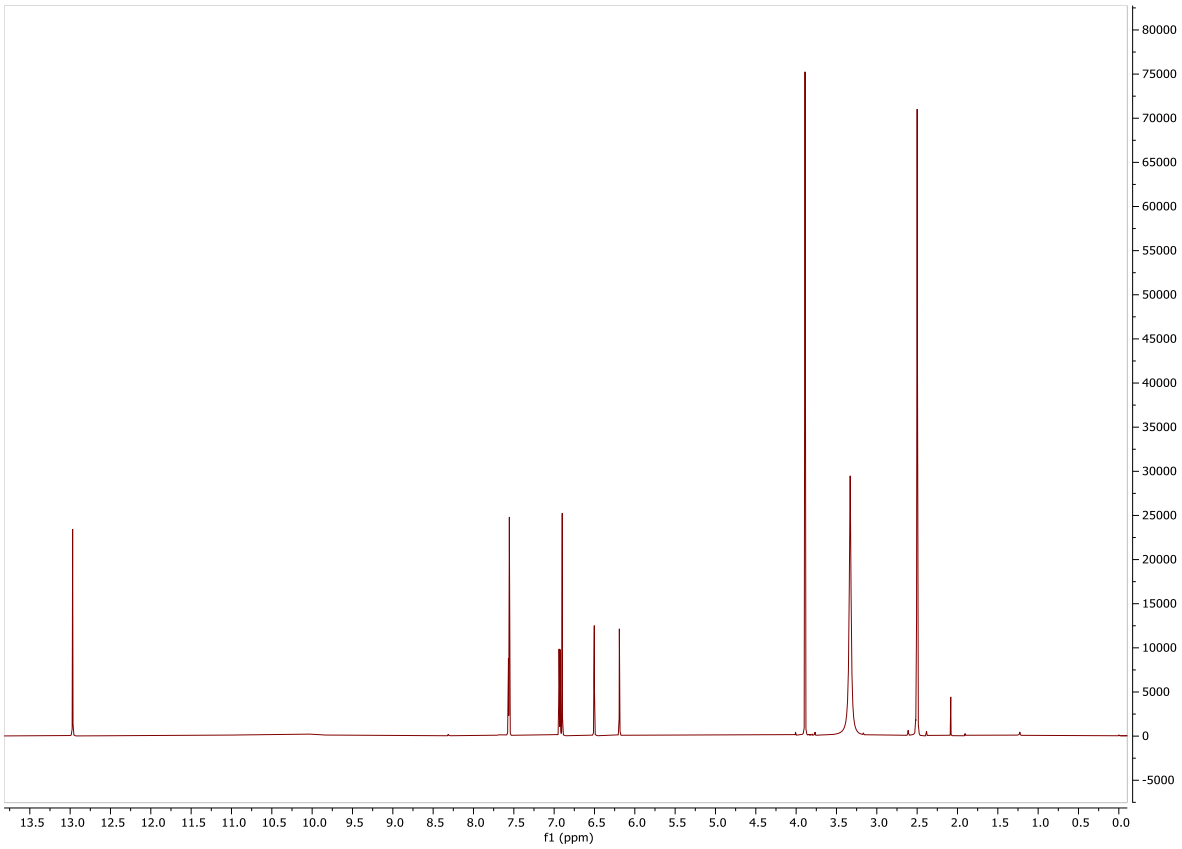


Figure S60. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 20.

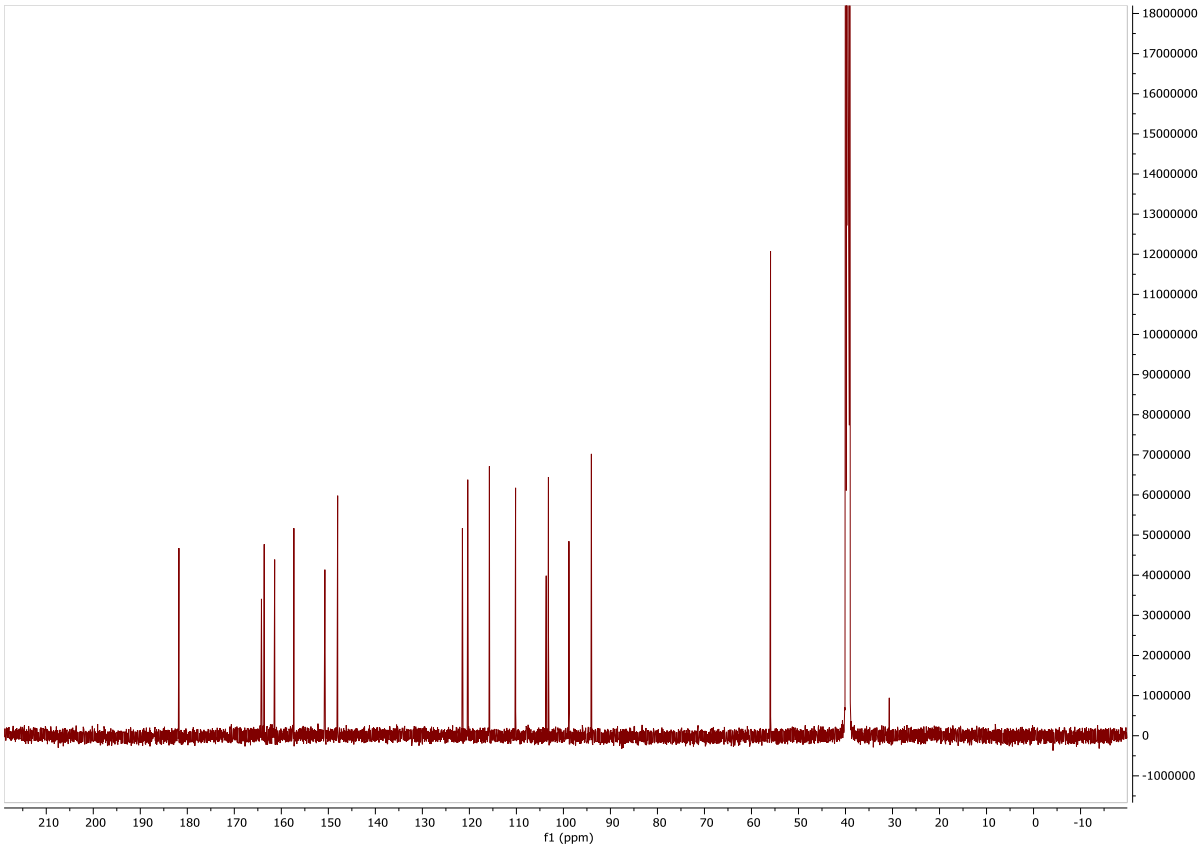


Figure S61. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 21.

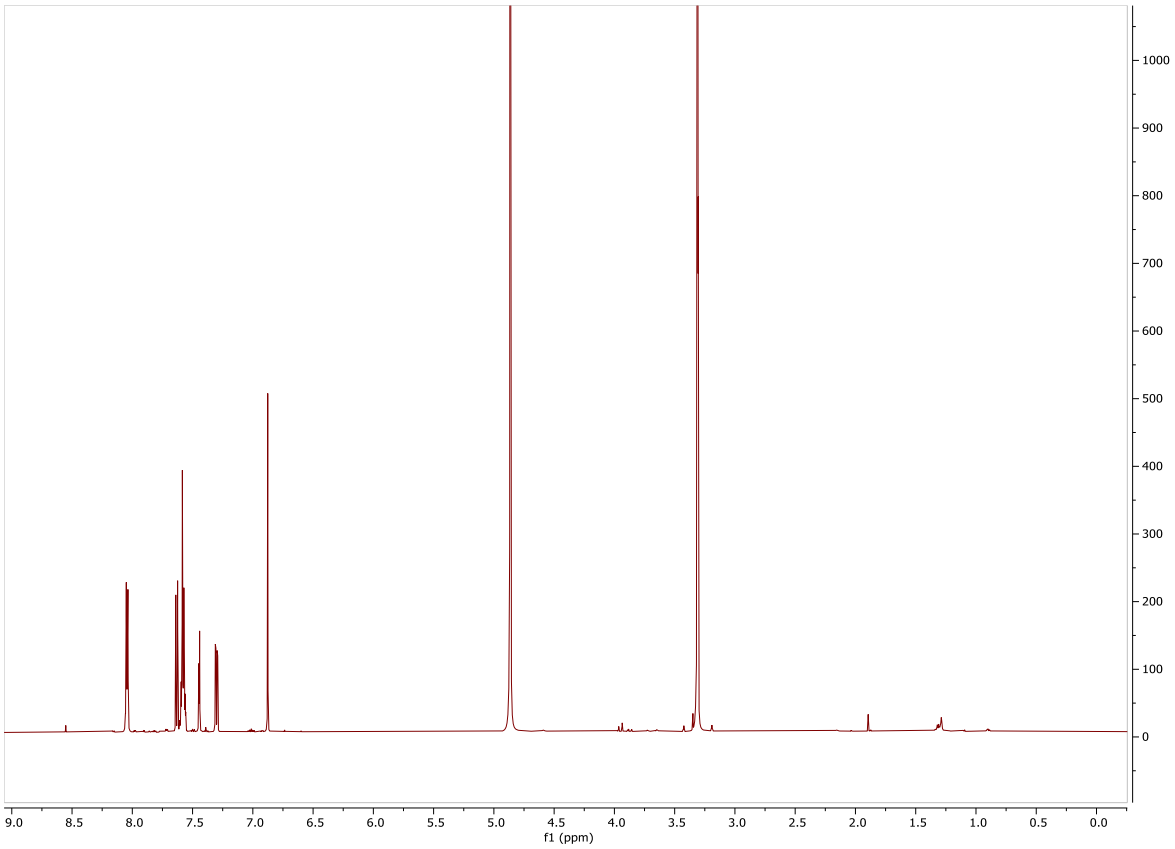


Figure S62. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 21.

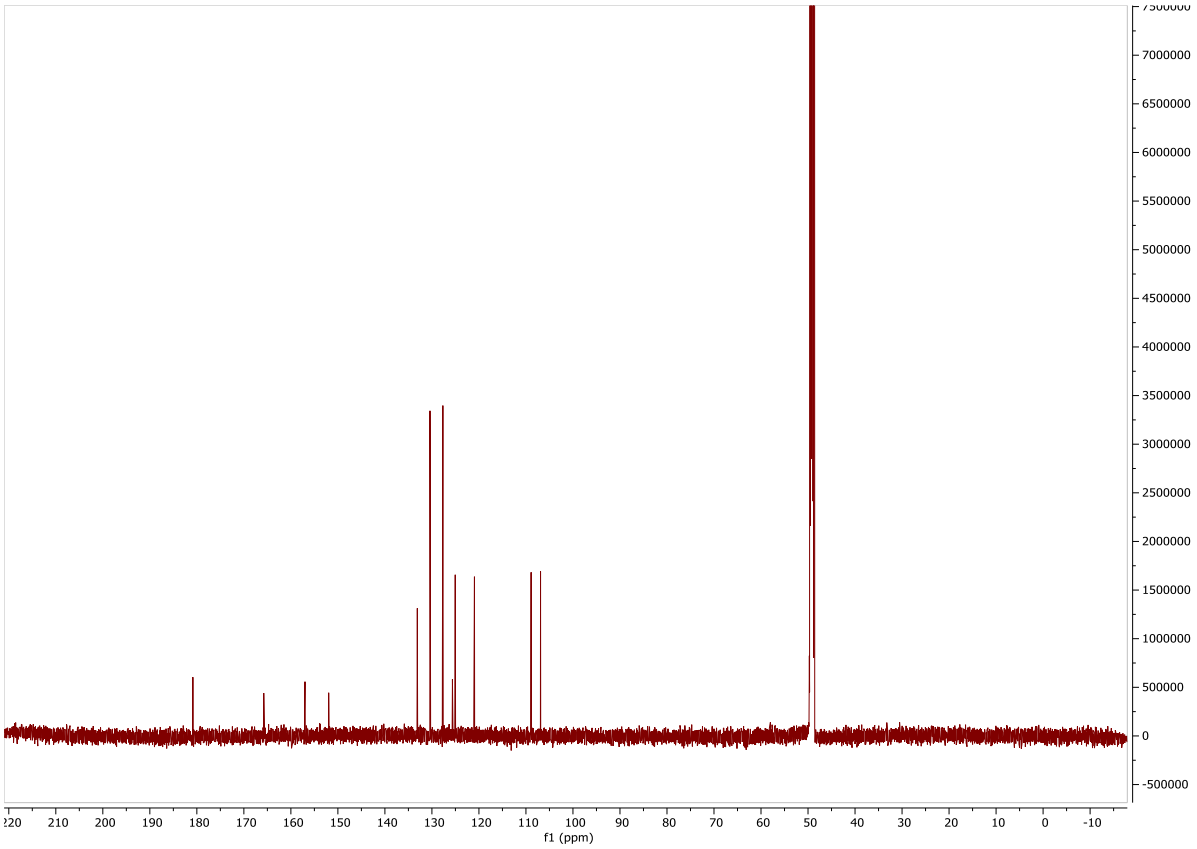


Figure S63. ^{1}H-NMR (600.19 MHz, $CHCl_3$-*d*) of compound 22.

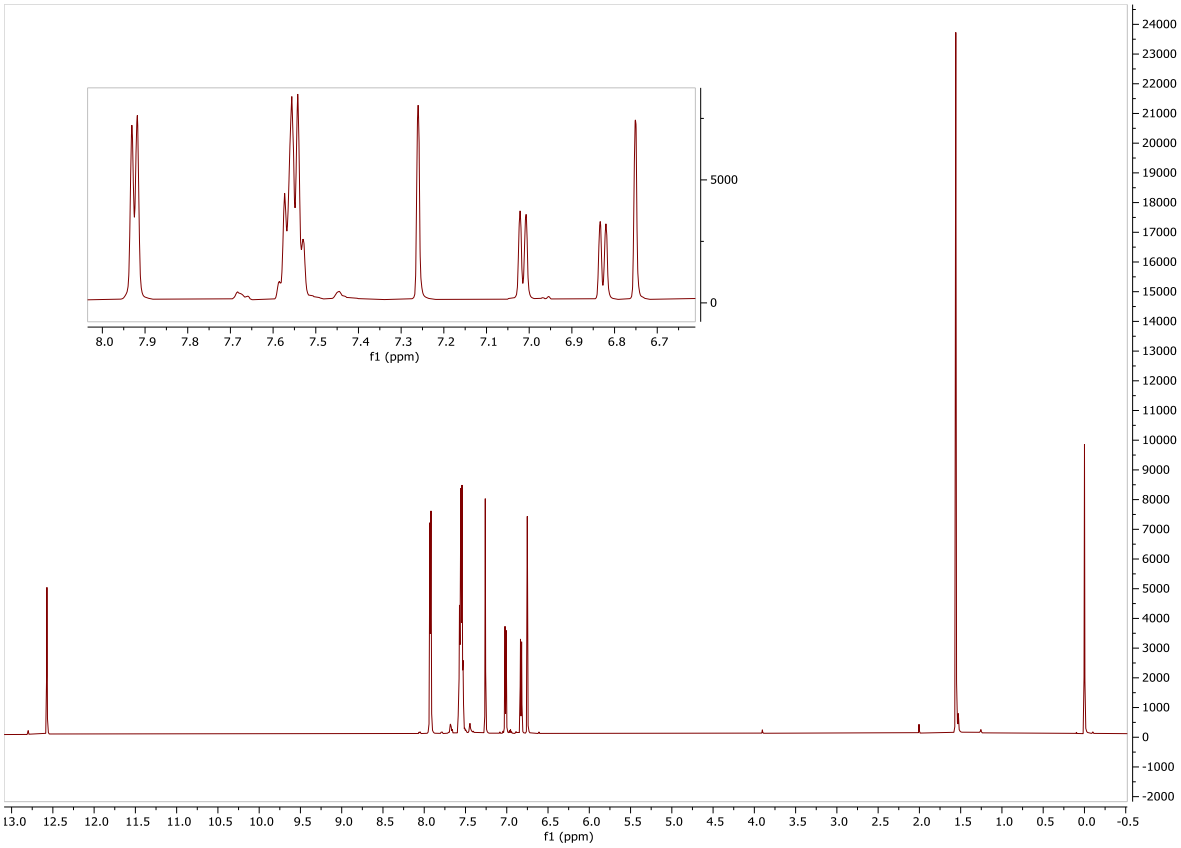


Figure S64. ^{13}C-NMR (150.91 MHz, $CHCl_3$-*d*) of compound 22.

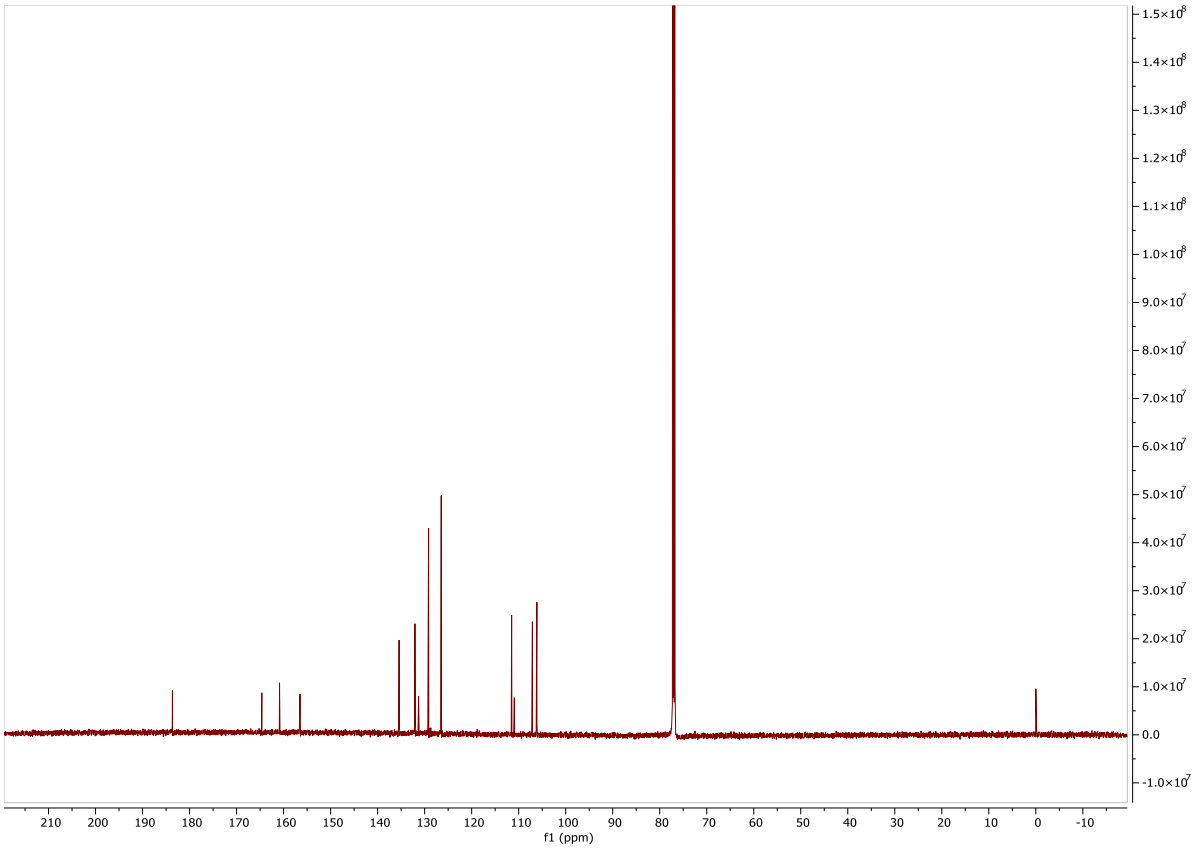


Figure S65. ^{1}H-NMR (600.19 MHz, Acetone-d_6) of compound 23.

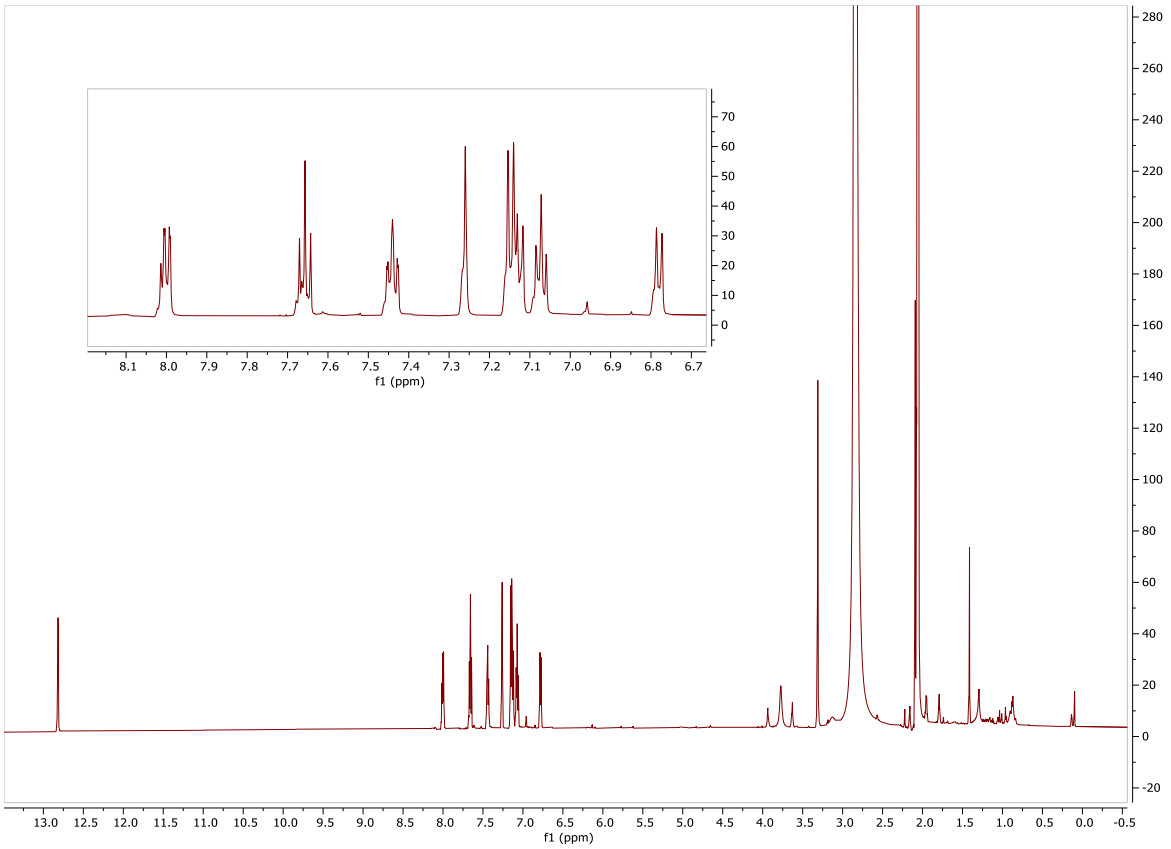


Figure S66. ^{13}C-NMR (150.91 MHz, Acetone-d_6) of compound 23.

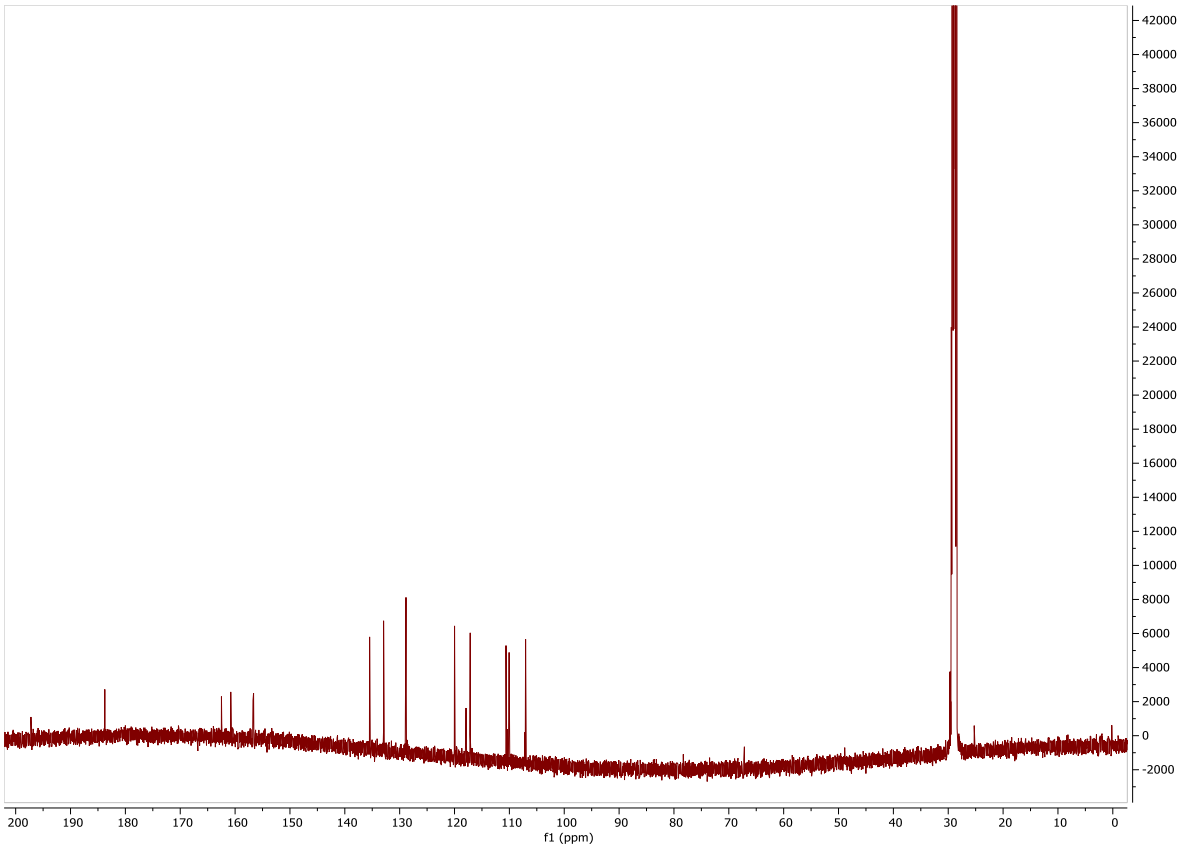


Figure S67. ^{1}H-NMR (600.19 MHz, MeOH-d_4) of compound 24.

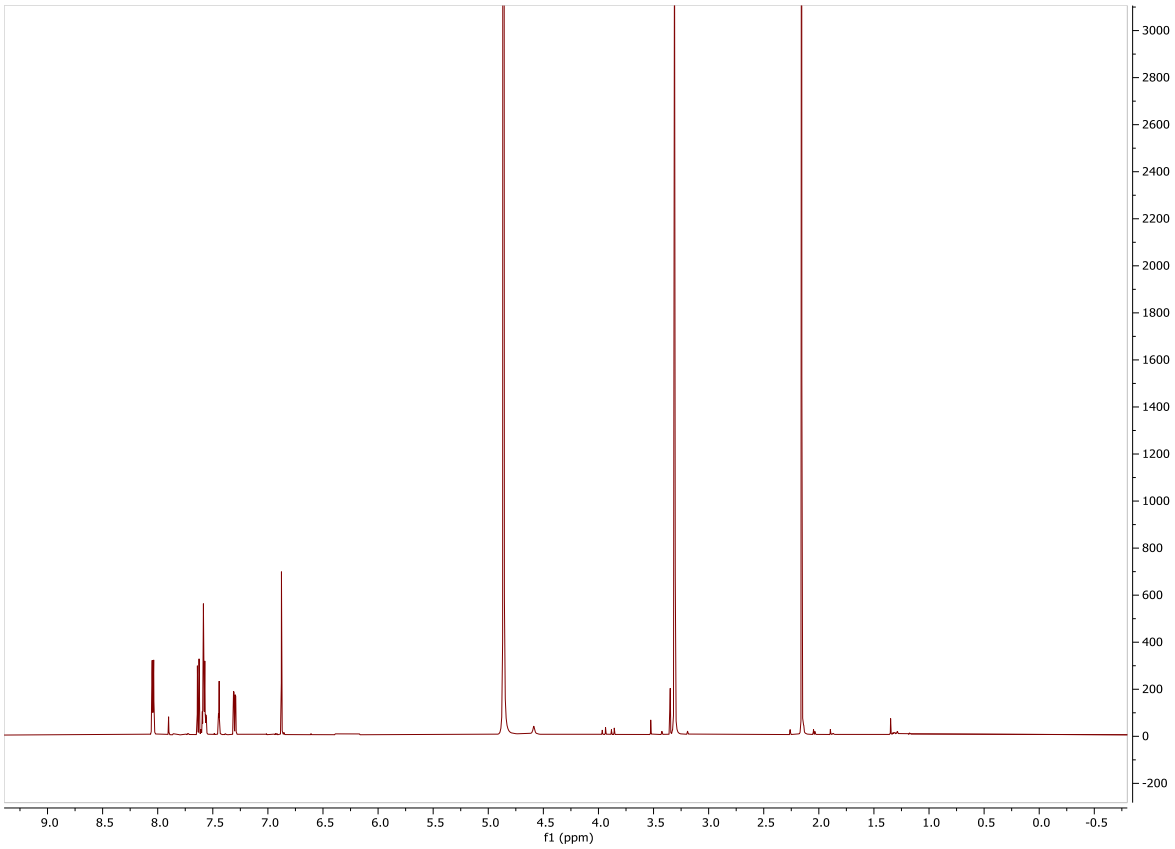


Figure S68. ^{13}C-NMR (150.91 MHz, MeOH-d_4) of compound 24.

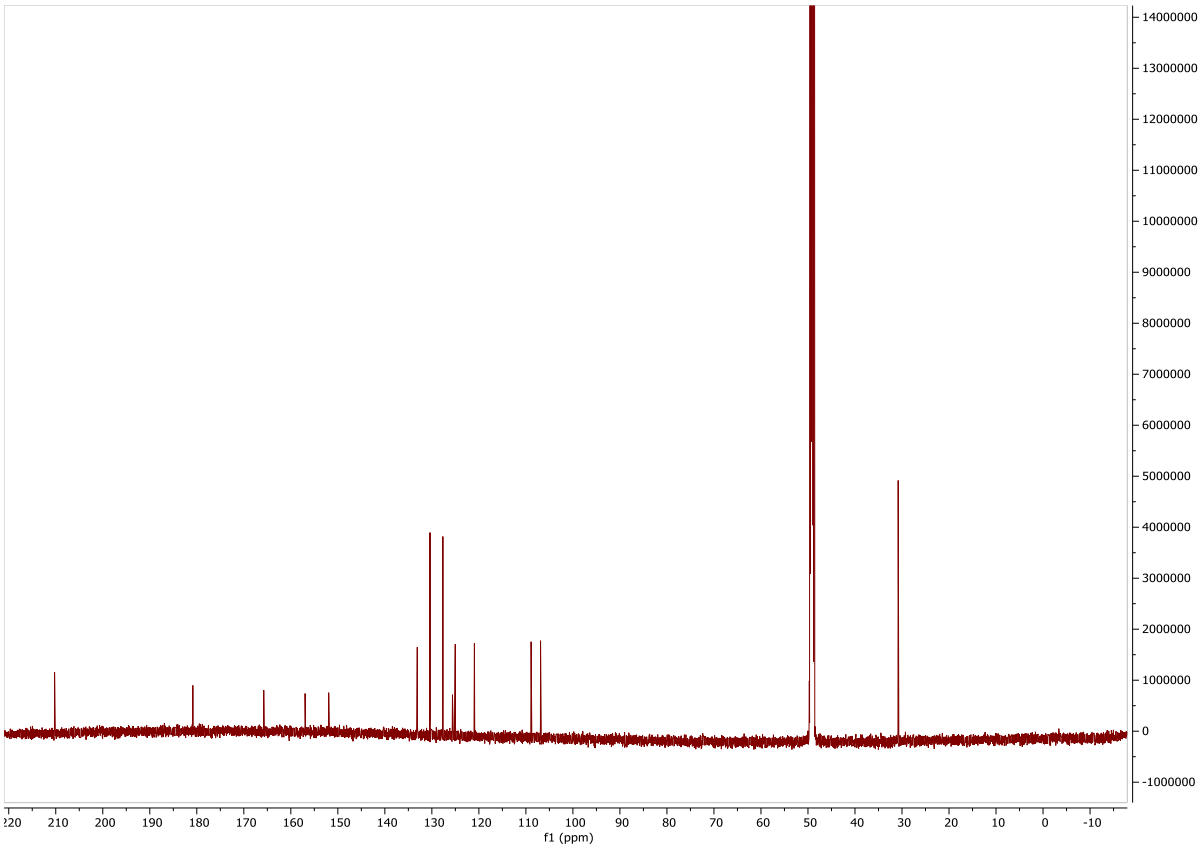


Figure S69. ^{1}H-NMR (600.19 MHz, DMSO-d_6) of compound 25.

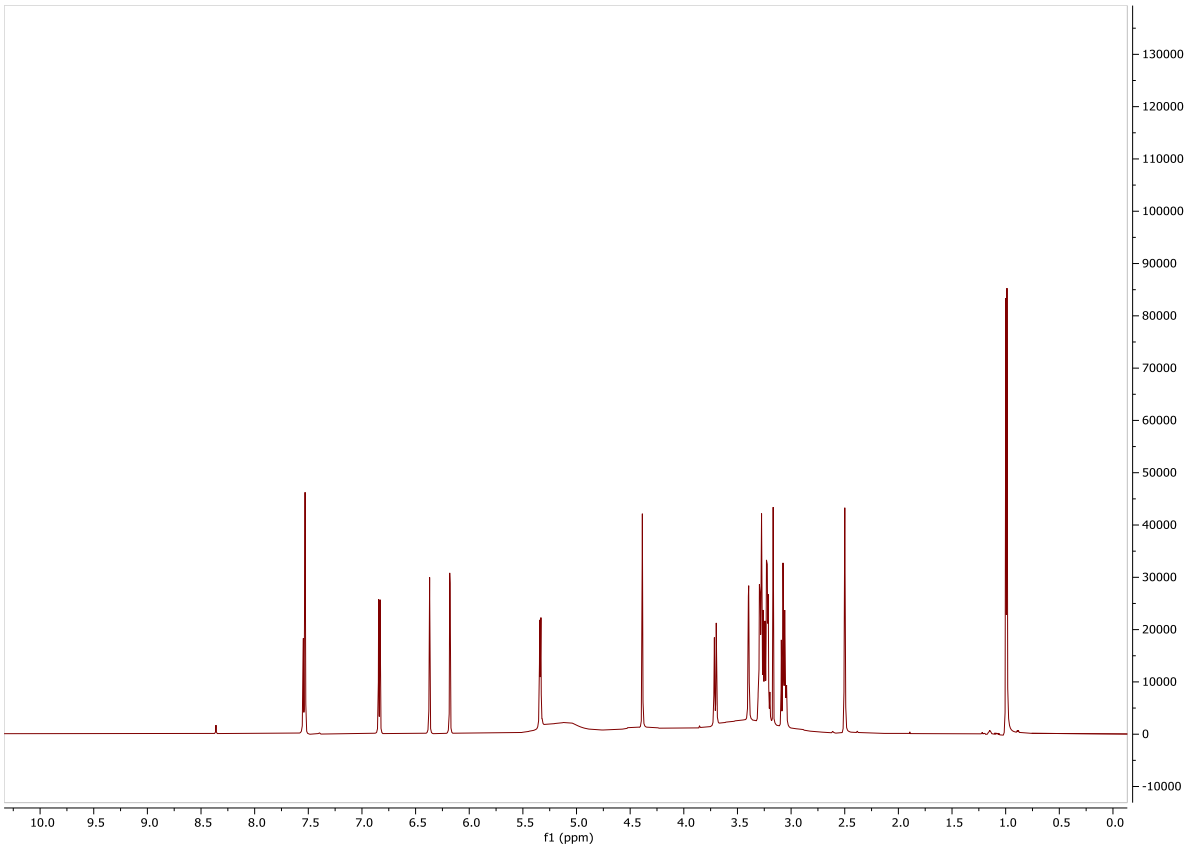


Figure S70. ^{13}C-NMR (150.91 MHz, DMSO-d_6) of compound 25.

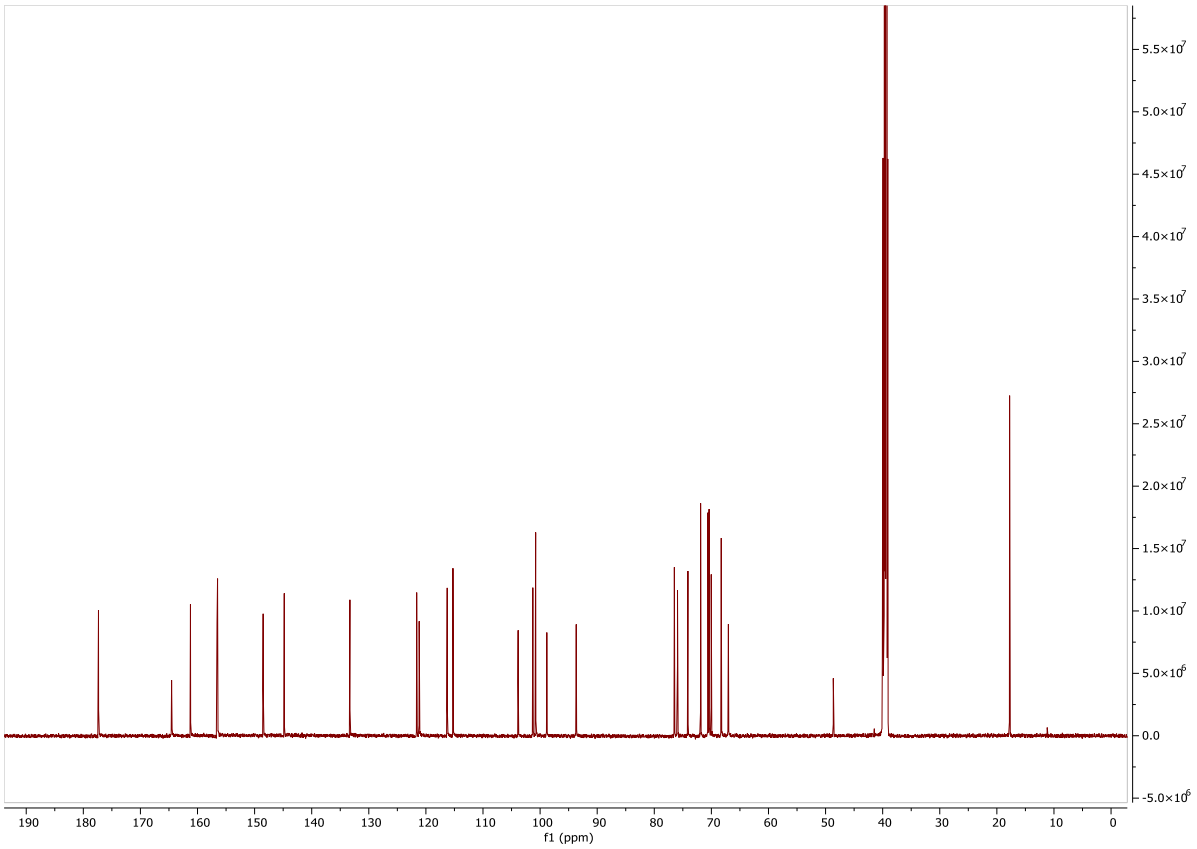
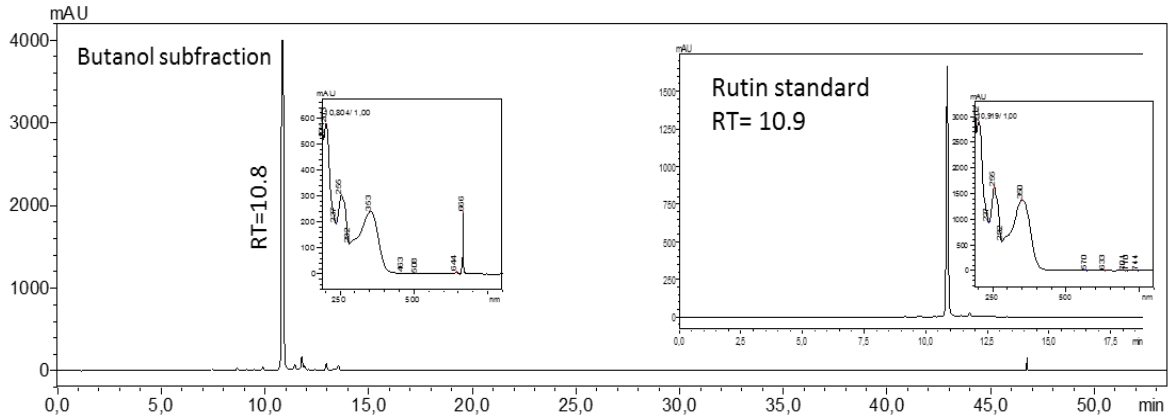


Table S1. Anti-proliferative activity of selected compounds isolated from diethyl ether subfraction of the methanolic extract of *D. diapensifolia* in MTT assay after 24 h shown as % cellular inhibition (MEAN±SEM) of n = 3. ***, **, * denote $p < 0.001$, $p < 0.01$, $p < 0.05$ vs. control.

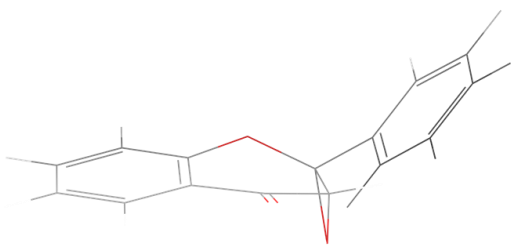
Compound	50 ~~µg/mL~~µM	10 ~~µg/mL~~µM	5 ~~µg/mL~~µM
1	0.00±0.00	0.67±0.67	3.33±2.40
2	0.13±0.13	0.00±0.00	4.72±2.48
3	2.40±2.40	2.27±2.27	0.50±0.50
4	1.93±1.93	0.03±0.03	0.70±0.70
6	69.43±0.18***	0.00±0.00	0.00±0.00
8	0.36±0.36	0.66±0.66	1.40±0.91
9	12.63±0.24*	6.23±3.24	3.80±3.10
10	40.33±0.88	1.00±0.58	0.00±0.00
11	3.90±1.17	6.73±1.34	8.03±1.14

Figure S71. HPLC-DAD chromatogram of *D. diapensifolia n*-butanol subfraction and rutin standard.

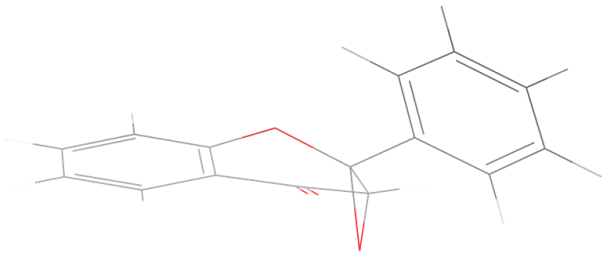


Analysis condition: stationary phase: Phenomenex Aqua C18 5µm, 150 × 4.6 mm; mobile Phase: A = H_2O + 0.02% TFA, B = acetonitrile; gradient: 0 min: B=2%; 20 min: B=50%; 40 min: B=98%, 50 min B=98%; temp.: 35°C; flow: 1 mL/min; butanol subfraction: 1 mg/mL, inj. vol. 10 µL; rutin standard: 2 mg/mL, inj. vol. 10 µL.

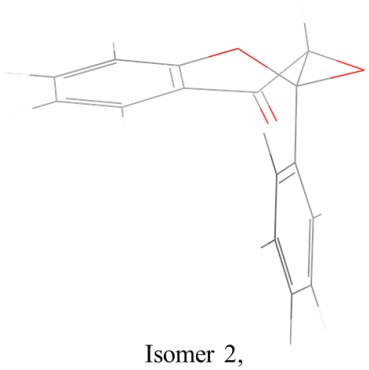
Figure S72. Low energy conformers of compound 1. Conformer generation was done on MacroModel 09 (Schrödinger Ltd.), using OPLS-3 as forcefield in gas phase. Conformers occurring in energy window of 5 kcal.mol^{-1} were further optimized in DFT/6-31G(d,p) level in the gas phase using Gaussian 16 v. A3 software [1].



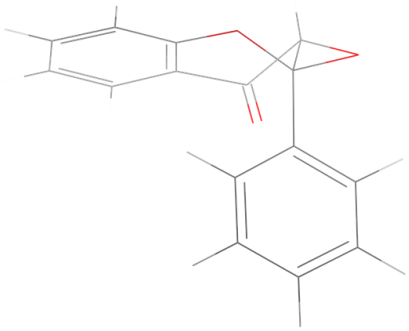
Isomer 1,
C1: 50%



Isomer 1,
C2: 50%



Isomer 2,
C1: 50%



Isomer 2,
C2: 50%

Table S2. Experimental chemical shifts and Boltzmann-averaged shielding tensors of two diastereomers of **1**, used for DP4+ chemical shift calculation.

Atom	δexp (ppm)	Isomer 1	Isomer 2
C	119	53.423	52.492
C	121.6	62.683	54.439
C	159	23.372	13.689
C	126.7	63.755	64.914
C	136.7	44.001	44.863
C	122	58.970	58.636
C	187.5	-9.164	0.477
C	56.2	117.895	114.574
C	84.8	97.934	89.100
C	140.8	47.319	51.255
C	125.9	55.482	52.460
C	129.1	52.892	53.108
C	129.2	51.508	50.935
C	129.1	52.892	53.111
C	125.9	55.481	52.463
H	4.22	28.148	23.798
H	6.74	23.319	24.089
H	8.00	24.147	23.760
H	7.35	23.703	24.298
H	7.00	24.193	27.547
H	7.58	23.723	23.978
H	7.39	23.883	24.077
H	7.35	23.875	24.012
H	7.39	23.883	24.077
H	7.58	23.723	23.978

Figure S73. The result sheet of DP4+ chemical shift probability calculation of two diastereomers of compound 1. Calculation of shift tensors were done using GIAO/mpw1pw91/6-311+(d,p)/CPCM in $CHCl_3$ in Gaussian 16 A.3 [Ref]. DP4+ probability calculation was done using the method originally published by Grimblat et. al [2].

1	Functional	Solvent?		Basis Set		Type of Data	
2	mPW1PW91	PCM		6-311+G(d,p)		Shielding Tensors	
3							
4		Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5	Isomer 6
5	sDP4+ (H data)	100.00%	0.00%	-	-	-	-
6	sDP4+ (C data)	99.99%	0.01%	-	-	-	-
7	sDP4+ (all data)	100.00%	0.00%	-	-	-	-
8	uDP4+ (H data)	79.58%	20.42%	-	-	-	-
9	uDP4+ (C data)	100.00%	0.00%	-	-	-	-
10	uDP4+ (all data)	100.00%	0.00%	-	-	-	-
11	DP4+ (H data)	100.00%	0.00%	-	-	-	-
12	DP4+ (C data)	100.00%	0.00%	-	-	-	-
13	DP4+ (all data)	100.00%	0.00%	-	-	-	-

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