Spiroamentotaxols A−D: Unprecedented 6/6/6/5/6/6/6/6 spiro-octacyclic bis-diterpene heterodimers from the endangered conifer *Amentotaxus yunnanensis* and their bioactivities

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1. Experimental details

1.1 General experimental procedures

NMR spectra were recorded on a Bruker Avance III 400 MHz or 600 MHz spectrometer. Chemical shifts are expressed in δ (ppm) and referenced to the residual solvent signals. Semi-preparative HPLC was conducted on a Shimadzu LH-20AT system with a SPD-M20A prominence diode array (PDA) detector and four ODS columns (Waters X-Bridge: 250×10 mm, 5 μm; YMC-Pack ODS-A: 250 × 10 mm, 5 μm; Cosmosil 5C_{18}-MS-II: 250 × 10 mm, 5 μm; Thermo Gold PFP: 250 × 10 mm, 5 μm). Optical rotations were measured on an Anton Paar MCP 4100 automatic polarimeter. IR spectrum was measured on a Nicolet 5700 FTIR spectrometer. ECD spectra were recorded on a JASCO-1500 spectropolarimeter. HRESIMS were acquired on AB SCIEX Triple TOF 5600 spectrometer. X-ray data were collected on a Bruker D8 Venture diffractometer. Melting points were obtained on a Melting Point Apparatus WRX-4 (Shanghai Yice Apparatus & Equipments CO. Ltd., PR China). Column chromatography (CC) was carried out using silica gel (100−200 or 200−300 mesh, Qingdao Marine Chemical Co. Ltd., PR China), MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

1.2 LC-ESI-MS analysis of *A. yunnanensis* extract

Fresh twigs and needles (80 g) of *A. yunnanensis* were extracted using 90% MeOH at room temperature three times (twelve hours soaking for each time), and the combined extracts were concentrated in vacuo to give a residue (6.7 g). The residue was analyzed using a Shimadzu LC-20A system coupled to a Shimadzu LCMS-2020 mass spectrometer. LC-MS was performed using a Shim-pack GISI-HP column ($C_{18,}$ 3 μm, 2.1×150 mm) with a flow rate of 0.2 mL/min, and the column temperature was maintained at 40°C. The mobile phase consisted of H_2O (A) and MeOH (B) with a linear gradient: 40−40% B (0.0−5.0 min); 40−100% B (5.1−25.0 min); 100−100% B (25.1−45.0 min). The analysis of the LC-MS data is shown in **Figure S9**.

Another 80 g sample of twigs and needles from *A. yunnanensis* was extracted with ethyl acetate (EtOAc) using ultrasonic treatment at room temperature, repeated three times with each extraction lasting twelve hours. The combined extracts were then concentrated in vacuo to give a residue (3.1 g). The residue was analyzed using a

Shimadzu LC-20A system coupled with a Shimadzu LCMS-2020 mass spectrometer. LC-MS was performed using the same Shim-pack GISI-HP column (C_{18}, 3 μm, 2.1×150 mm) at a flow rate of 0.2 mL/min, with the column temperature maintained at 40°C. The mobile phase consisted of H_2O (A) and MeOH (B) with a linear gradient: 40−100% B (0.0−30.0 min); 100−100% B (30.1−50.0 min). The analysis of the LC-MS data is shown in **Figure S10**. The spiroamentotaxols were detected in both the MeOH and EtOAc extracts. These findings support the conclusion that these heterodimers are genuine natural products, rather than artifacts resulting from the isolation procedures.

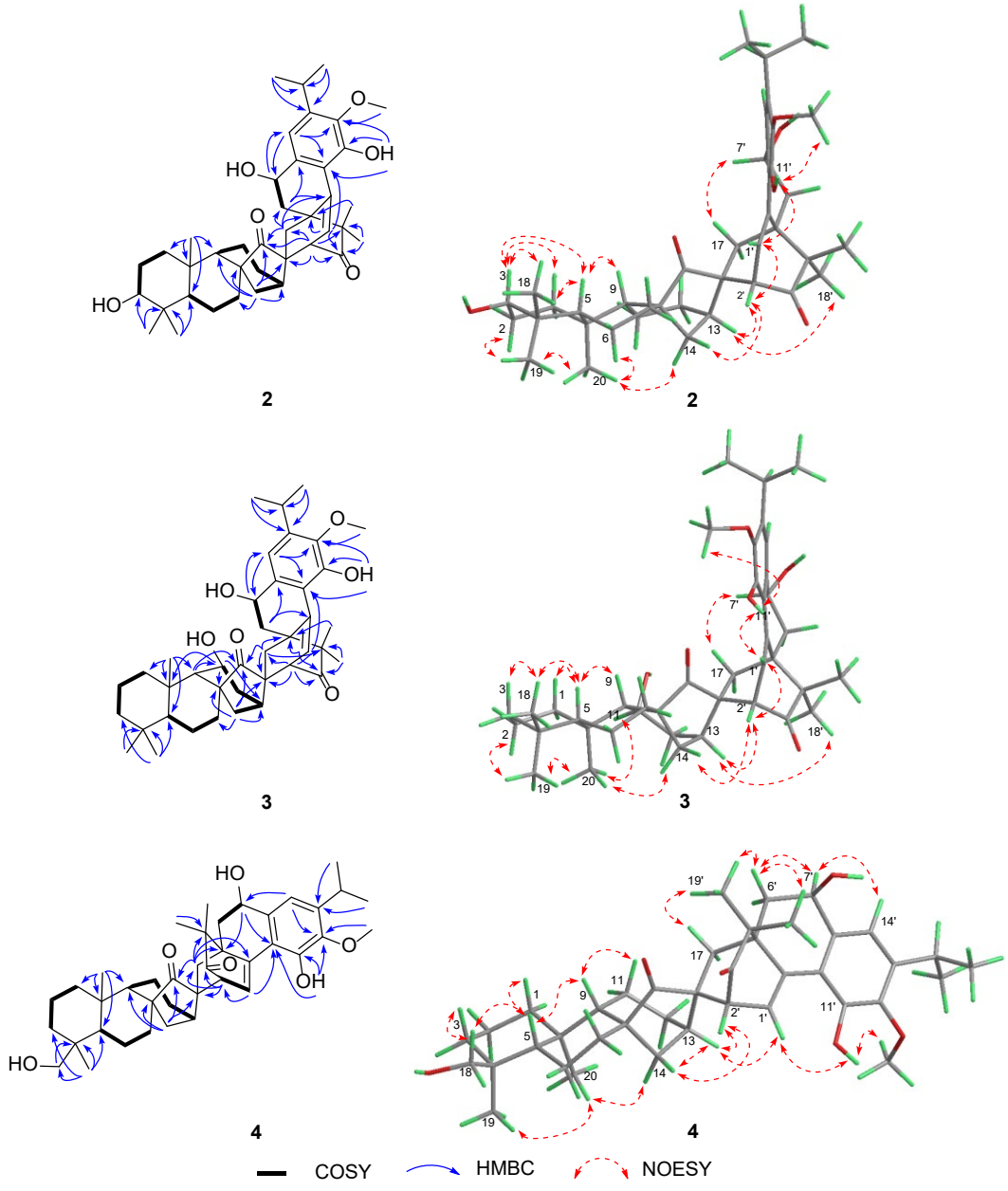


Figure S1. 2D NMR correlations of compounds **2**−**4**.

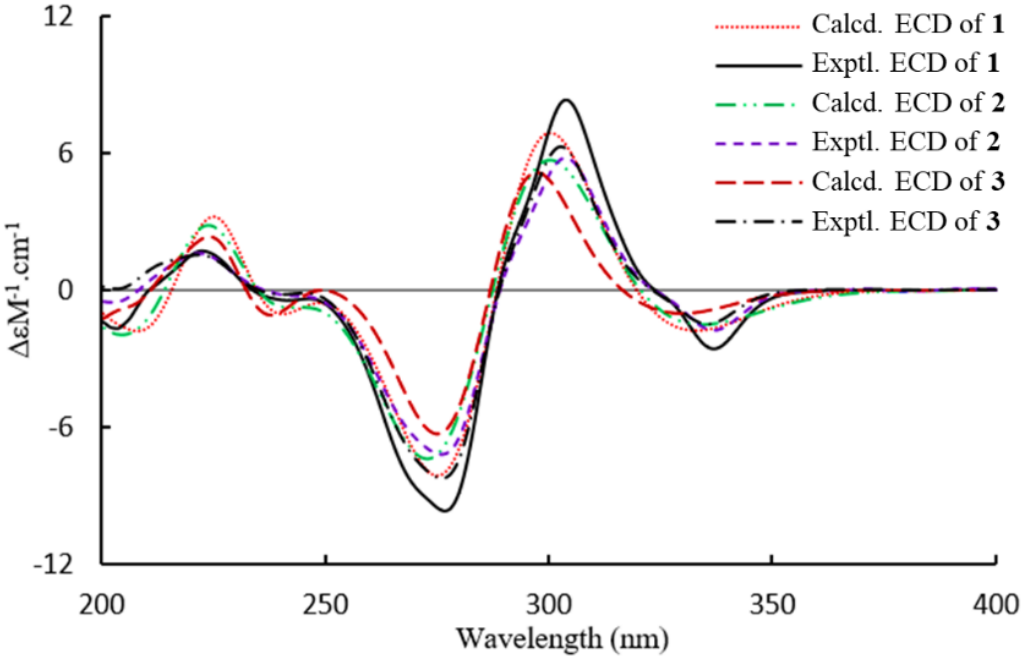


Figure S2. Calculated and experimental ECD spectra of compounds **1**−**3** (in MeOH).

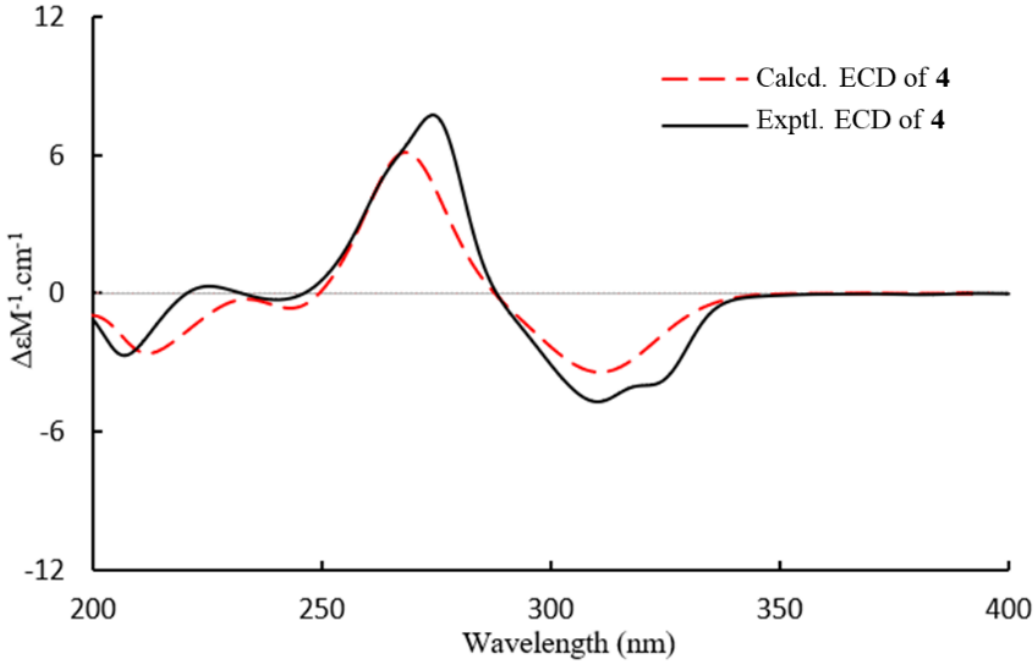


Figure S3. Calculated and experimental ECD spectra of compound **4 (**in MeOH).

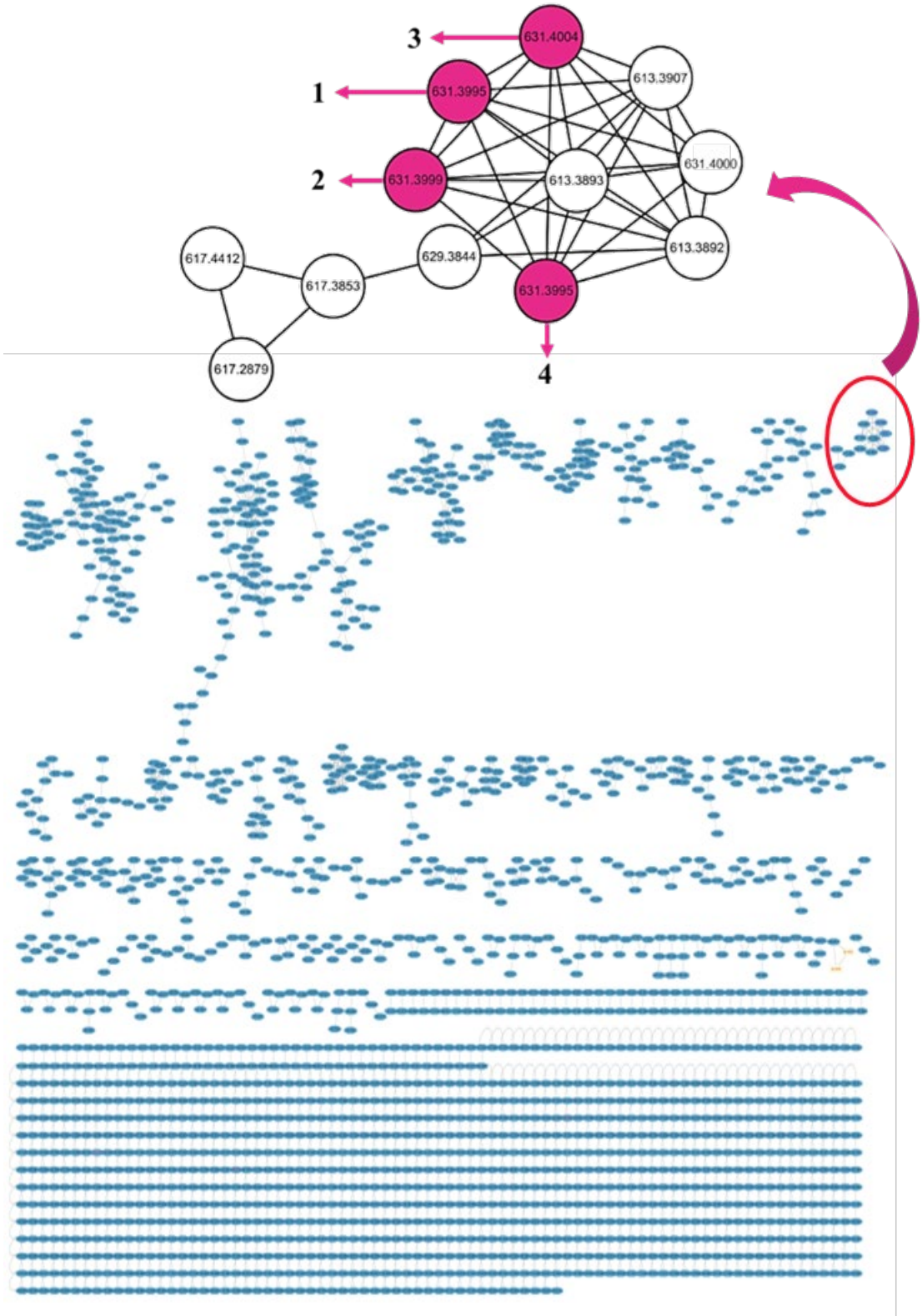


Figure S4. MoIN of *A. yunnanensis* MeOH extract. (underneath): Organized landscape of the complete network. (above): Molecular cluster of the predicted heterodimers.

Table S1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (δ in ppm, J in Hz, in $CDCl_3$) of compounds **1a** and **1b**. [a]

No.	**1a**		**1b**	
	δ_H	δ_C	δ_H	δ_C
1	α: 1.72, m; β: 0.73, m	38.9	α: 1.73, m; β: 0.73, m	38.8
2	α: 1.19, m; β: 1.33, m	18.6	α: 1.17, m; β: 1.30, m	18.7
3	α: 1.28, m; β: 1.36, m	35.1	α: 1.26, m; β: 1.38, m	35.0
4		37.5		37.4
5	1.19, br d (12.1)	48.6	1.19, br d (12.5)	48.5
6α	α: 1.47, m	17.6	α: 1.49, m	17.6
6β	β: 1.62, m		β: 1.63, m	
7	α: 1.81, m; β: 1.81, m	27.6	α: 1.80, m; β: 1.80, m	27.6
8		52.1		52.1
9	1.08, m	52.9	1.10, m	53.0
10		39.6		39.6
11	α: 1.64, m; β: 1.31, m	18.4	α: 1.64, m; β: 1.31, m	18.4
12	α: 1.88, m; β: 1.32, m	34.4	α: 1.86, m; β: 1.31, m	34.4
13	2.15, br s	38.4	2.18, br d (2.6)	38.6
14α	2.43, br d (12.1)	35.2	2.40, br d (12.5)	35.0
14β	1.83, br d (12.1)		1.82, br d (12.5)	
15		221.1		221.7
16		48.3		51.8
17	2.02, d (13.1); 1.79, d (13.1)	36.3	2.18, d (13.0); 1.67, d (13.0)	33.8
18	0.77, s	17.3	0.75, s	17.3
19	3.40, d (11.0); 3.12, d (11.0)	72.1	3.38, d (11.2); 3.07, d (11.2)	72.0
20	1.12, s	17.9	1.11, s	17.9
1'	7.13, d (7.0)	120.1	6.62, d (6.7)	119.0
2'	3.32, d (7.0)	53.6	3.23, d (6.7)	52.9
3'		213.2		214.4
4'		46.7		47.3
5'		50.0		47.4
6'	5.80, d (9.8)	128.1	α: 1.70, br dd (15.0, 14.3) β: 1.88, br dd (15.0, 6.9)	27.9
7'	6.53, d (9.8)	128.8	α: 2.66, ddd (15.2, 6.9, 3.7) β: 2.81, ddd (15.2, 14.3, 4.4)	27.6
8'		128.9		139.7
9'		114.4		118.9
10'		143.3		143.8
11'		147.6		146.7
12'		144.2		142.7
13'		140.3		139.7
14'	6.56, s	117.2	6.54, s	116.2
15'	3.23, m	26.6	3.23, m	26.5
16'	1.25, d (6.5)	23.7	1.24, d (6.5)	23.9
17'	1.25, d (6.5)	23.4	1.24, d (6.5)	23.5
18'	1.09, s	19.6	1.05, s	20.3
19'	0.93, s	25.4	0.98, s	24.9
OCH_3	3.81, s	62.0	3.78, s	61.8

[a]Assignments were made by a combination of 1D and 2D NMR experiments.

Table S2. X-ray crystallographic data for **1**.

Identification code	231116zpj_yns_27	
Empirical formula	1/4(2.$C_{40}H_{54}O_6$.3H_2O)	
Formula weight	328.93	
Temperature	173.00 K	
Wavelength	1.34139 Å	
Crystal system	Triclinic	
Space group	P1	
Unit cell dimensions	a = 7.3136(4) Å	α = 79.809(3)°.
	b = 11.2985(5) Å	β = 85.535(3)°.
	c = 21.3261(11) Å	γ = 88.994(3)°.
Volume	1729.15(15) $Å^3$	
Z	4	
Density (calculated)	1.263 Mg/m^3	
Absorption coefficient	0.436 mm^{-1}	
F(000)	714	
Crystal size	0.17 × 0.17 × 0.05 mm^3	
Theta range for data collection	3.619 to 55.155°.	
Index ranges	-8<=h<=8, -13<=k<=13, -25<=l<=25	
Reflections collected	47022	
Independent reflections	12854 [R(int) = 0.1093]	
Completeness to theta = 53.594°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7508 and 0.5717	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	12854 / 7 / 890	
Goodness-of-fit on F^2	1.040	
Final R indices [I>2σ(I)]	R_1 = 0.0626, wR_2 = 0.1498	
R indices (all data)	R_1 = 0.0903, wR_2 = 0.1647	
Absolute structure parameter	0.13(19)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.250 and -0.332 e.$Å^{-3}$	

Table S3. X-ray crystallographic data for **2**.

Identification code	240717zzy	
Empirical formula	C40 H58.50 O8.25	
Formula weight	671.36	
Temperature	170.00 K	
Wavelength	1.34139 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 11.9633(5) Å	α = 90°.
	b = 14.1368(6) Å	β = 90°.
	c = 43.3418(19) Å	γ = 90°.
Volume	7330.1(5) $Å^3$	
Z	8	
Density (calculated)	1.217 Mg/m^3	
Absorption coefficient	0.427 mm^{-1}	
F(000)	2916	
Crystal size	0.17 × 0.17 × 0.05 mm^3	
Theta range for data collection	2.860 to 54.960°.	
Index ranges	-14<=h<=14, -17<=k<=16, -52<=l<=51	
Reflections collected	84677	
Independent reflections	13910 [R(int) = 0.1283]	
Completeness to theta = 53.594°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7508 and 0.5142	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	13910 / 1 / 905	
Goodness-of-fit on F^2	1.033	
Final R indices [I>2σ(I)]	R_1 = 0.0733, wR_2 = 0.1914	
R indices (all data)	R_1 = 0.1118, wR_2 = 0.2173	
Absolute structure parameter	0.17(18)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.461 and -0.285 e. $Å^{-3}$	

Table S4. X-ray crystallographic data for **1a**.

Identification code	240522zpj_27r1	
Empirical formula	C40 H52 O5	
Formula weight	612.81	
Temperature	170.00 K	
Wavelength	1.34139 Å	
Crystal system	Monoclinic	
Space group	P 1 2_1 1	
Unit cell dimensions	a = 7.4099(5)Å	α = 90°.
	b = 11.3371(8) Å	β = 94.441(4)°.
	c = 19.5146(14) Å	γ = 90°.
Volume	1634.4(2) $Å^3$	
Z	2	
Density (calculated)	1.245 Mg/m^3	
Absorption coefficient	0.402 mm^{-1}	
F(000)	7664	
Crystal size	0.17 × 0.17 × 0.05 mm^3	
Theta range for data collection	3.926 to 54.936°.	
Index ranges	-9<=h<=9, -13<=k<=13, -23<=l<=23	
Reflections collected	30184	
Independent reflections	6174 [R(int) = 0.0761]	
Completeness to theta = 53.594°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7508 and 0.5220	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6174 / 2 / 426	
Goodness-of-fit on F^2	1.039	
Final R indices [I>2σ(I)]	R_1 = 0.0485, wR_2 = 0.1157	
R indices (all data)	R_1 = 0.0665, wR_2 = 0.1272	
Absolute structure parameter	0.05(19)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.165 and -0.207 $e.Å^{-3}$	

- **ECD calculations for 1−4.**

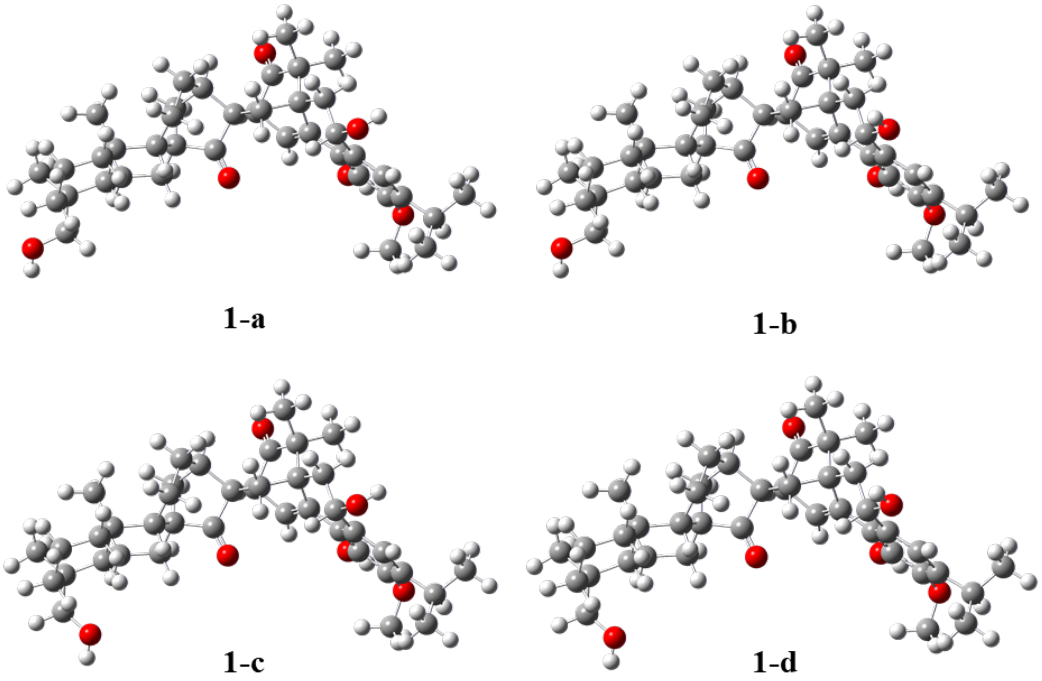


Figure S5. The optimized low-energy reoptimized MMFF conformers of **1** at B3LYP/6-31g level in gas.

Table S5. Boltzmann populations and relative binding free-energies (ΔG) of conformations of **1**.

Species	Boltzmann Population (%)	ΔG† (kJ/mol)
conformer **1-a**	0.0044	3.14
conformer **1-b**	0.0005	4.37
conformer **1-c**	0.8834	0
conformer **1-d**	0.1117	1.23

† Relative binding free energy

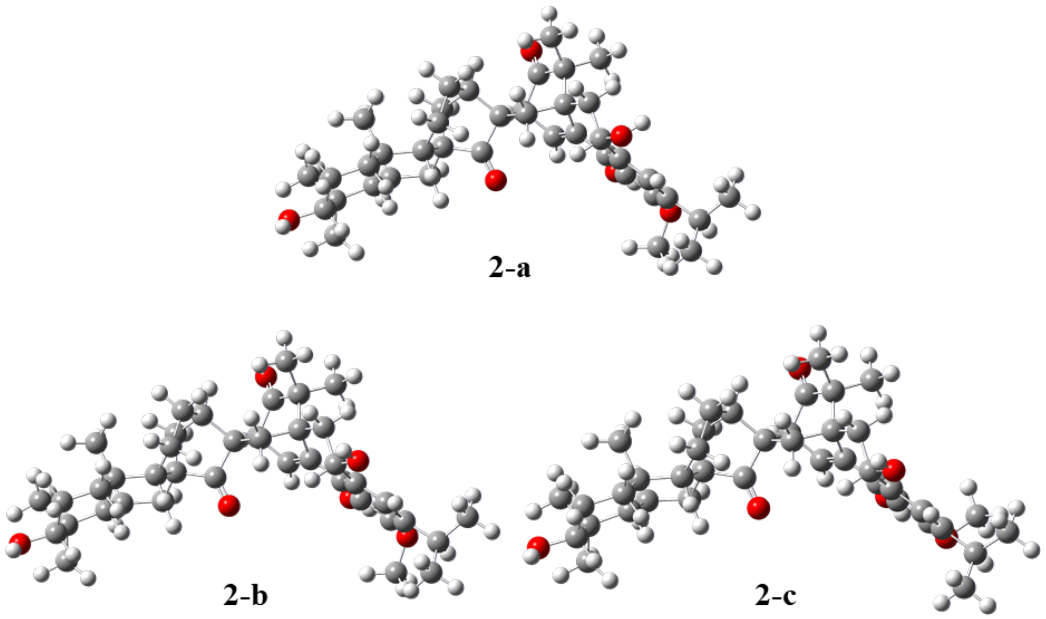


Figure S6. The optimized low-energy reoptimized MMFF conformers of **2** at B3LYP/6-31g level in gas.

Table S6. Boltzmann populations and relative binding free-energies (ΔG) of conformations of **2**.

Species	Boltzmann Population (%)	ΔG† (kJ/mol)
conformer **2-a**	0.8667	0
conformer **2-b**	0.0935	1.32
conformer **2-c**	0.0398	1.82

† Relative binding free energy

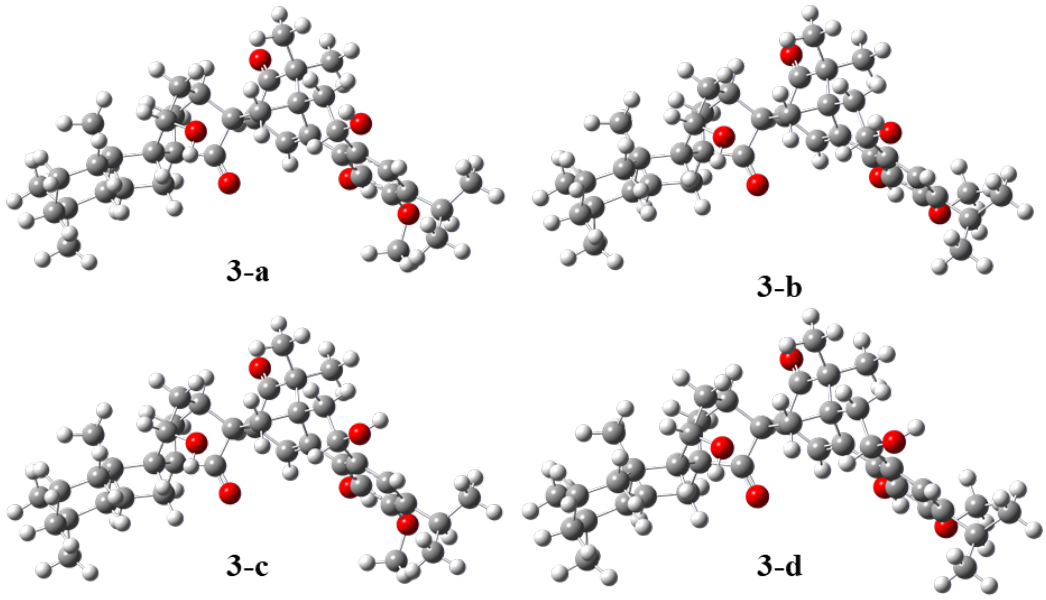


Figure S7. The optimized low-energy reoptimized MMFF conformers of **3** at B3LYP/6-31g level in gas.

Table S7. Boltzmann populations and relative binding free-energies (ΔG) of conformations of **3**.

Species	Boltzmann Population (%)	ΔG† (kJ/mol)
conformer **3-a**	0.0828	1.19
conformer **3-b**	0.0305	1.79
conformer **3-c**	0.6212	0
conformer **3-d**	0.2655	0.50

† Relative binding free energy

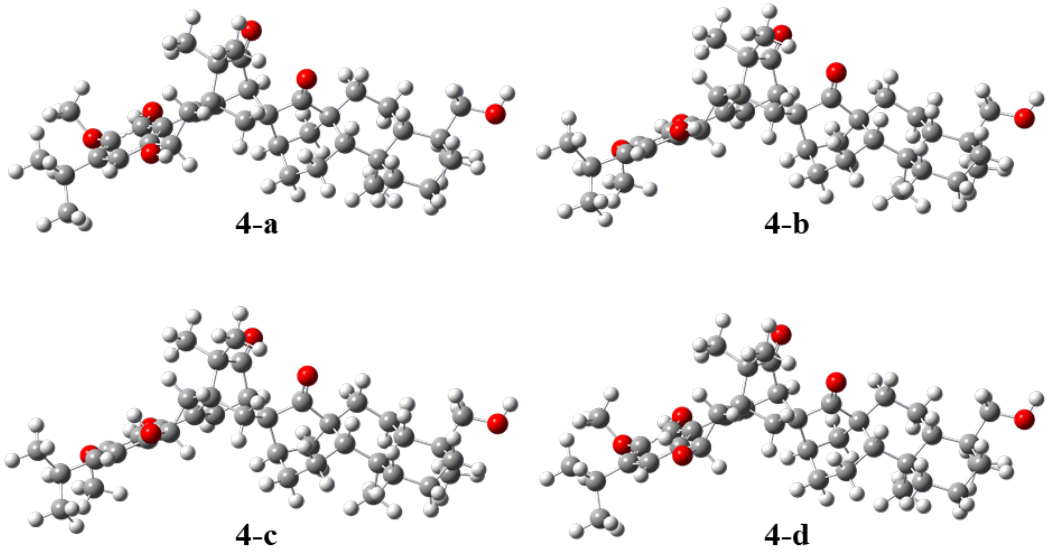


Figure S8. The optimized low-energy reoptimized MMFF conformers of **4** at B3LYP/6-31g level in gas

Table S8. Boltzmann populations and relative binding free-energies (ΔG) of conformations of **4**.

Species	Boltzmann Population (%)	ΔG† (kJ/mol)
conformer **4-a**	0.0293	1.74
conformer **4-b**	0.0618	1.30
conformer **4-c**	0.5527	0
conformer **4-d**	0.3562	0.26

† Relative binding free energy

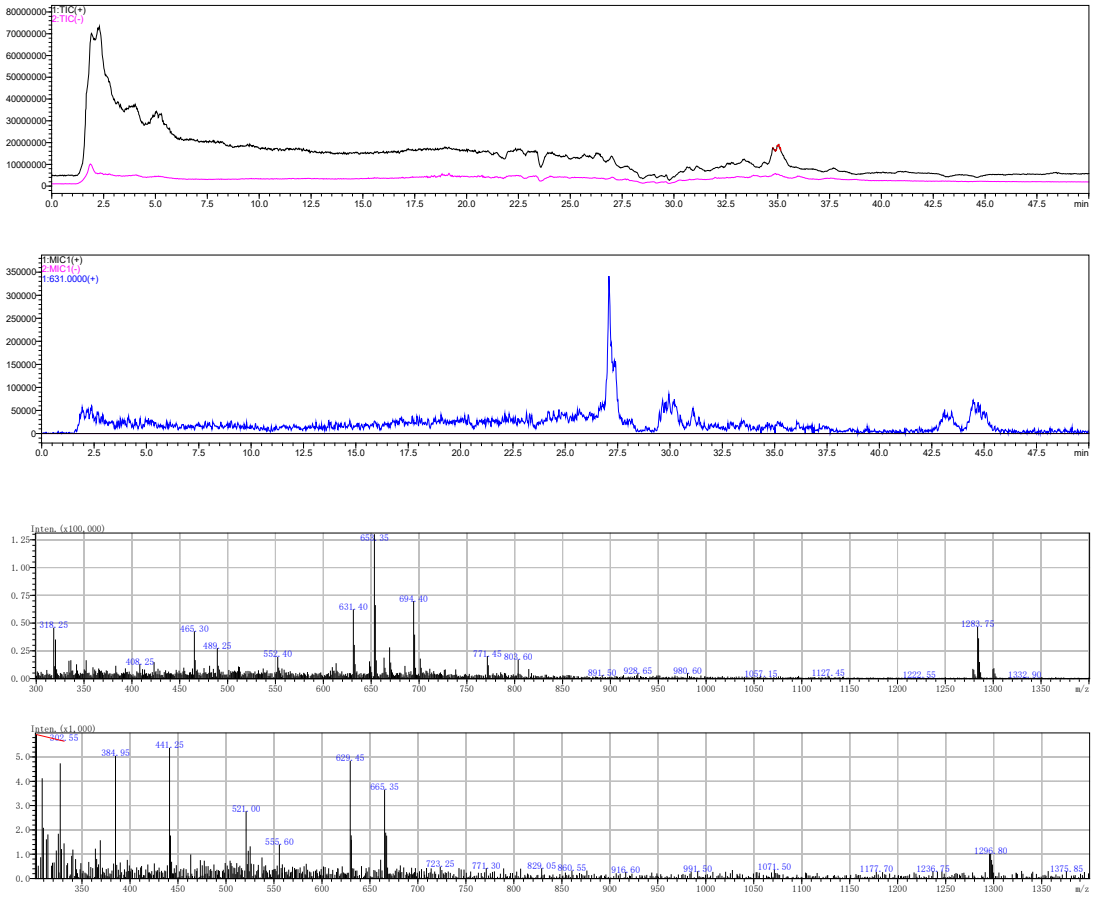


Figure S9. TIC and MIC (*m/z* $[M+H]^+$ 631) of the 90% MeOH/H_2O extract (top) and key MS spectra (t_R = 27.1 min) of *m/z* 631 ($[M+H]^+$) and *m/z* 629 ($[M-H]^-$) (bottom).

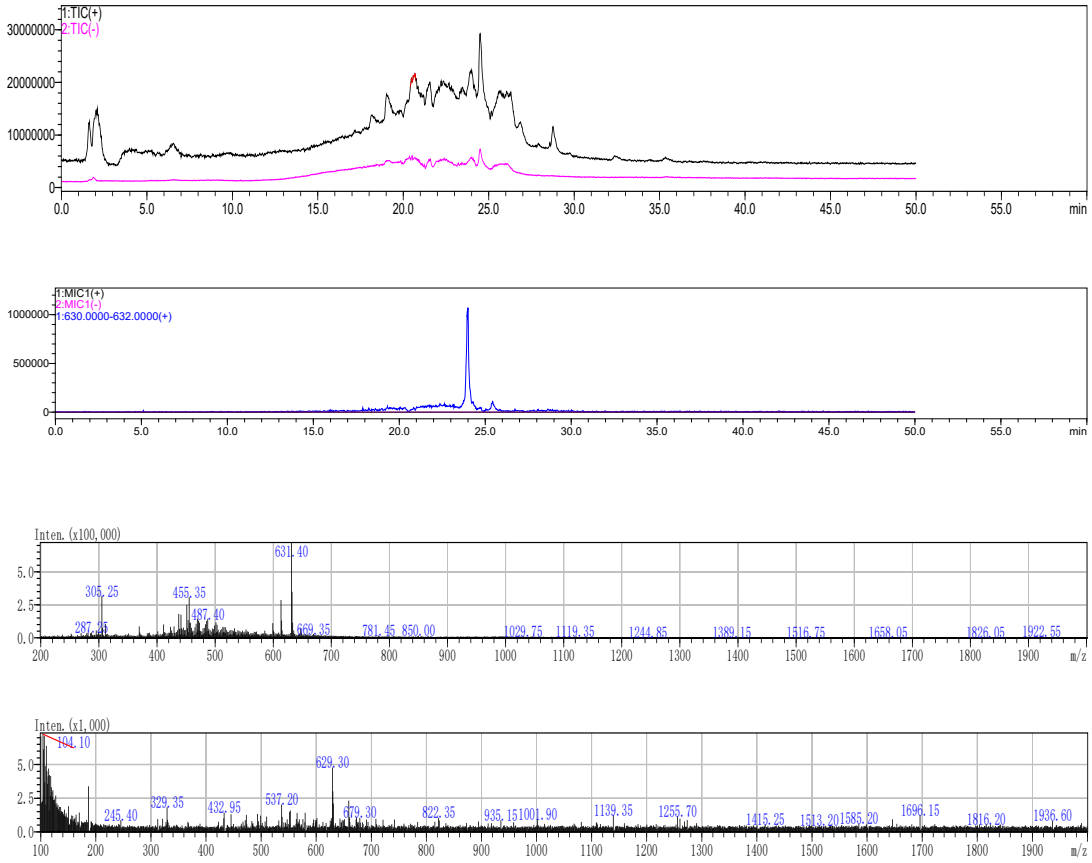


Figure S10. TIC and MIC (*m/z* $[M+H]^+$ 631) of the EtOAc extract (top) and key MS spectra (t_R = 24.1 min) of *m/z* 631 ($[M+H]^+$) and *m/z* 629 ($[M-H]^-$) (bottom).

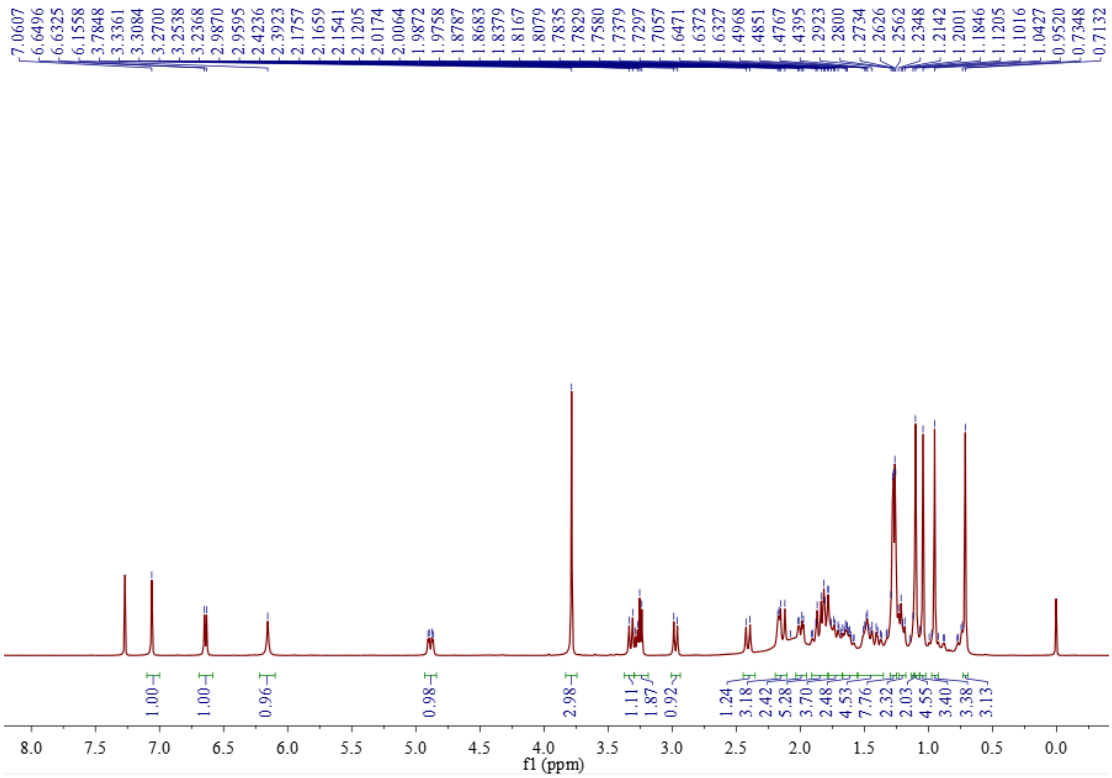


Figure S11. ^{1}H NMR spectrum of **1** in $CDCl_3$ (400 MHz)

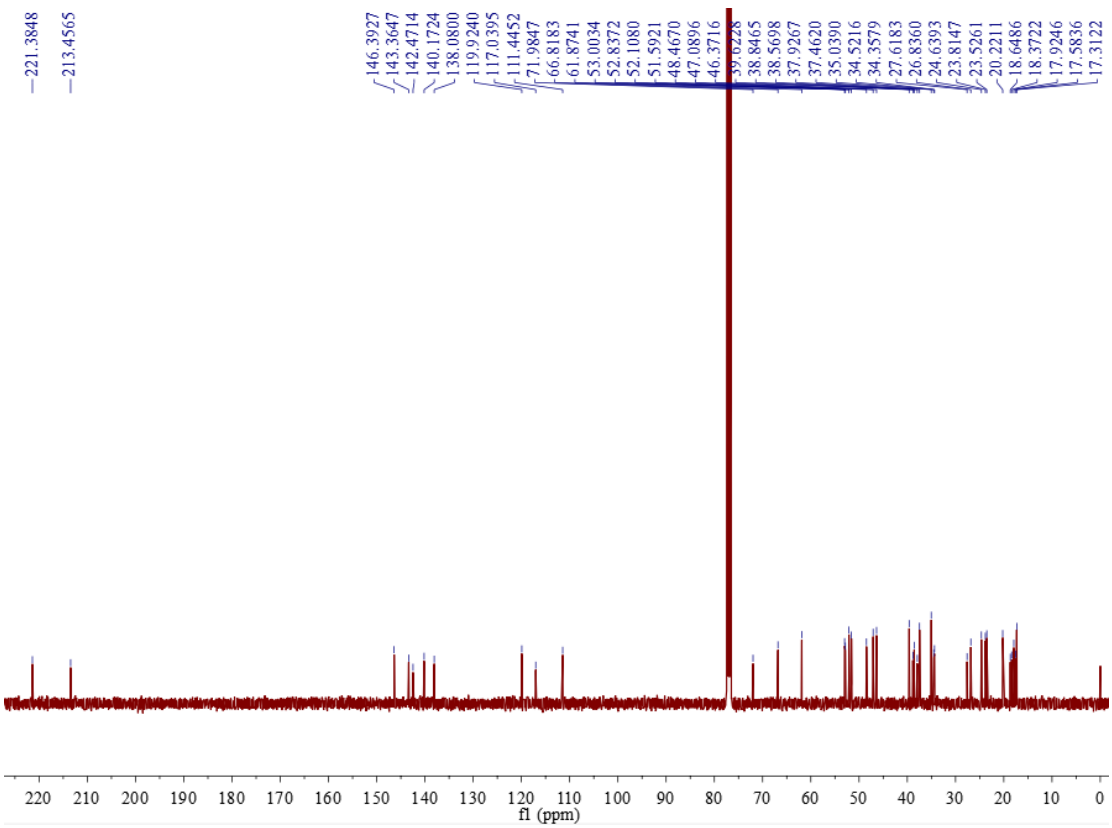


Figure S12. ^{13}C NMR spectrum of **1** in $CDCl_3$ (100 MHz)

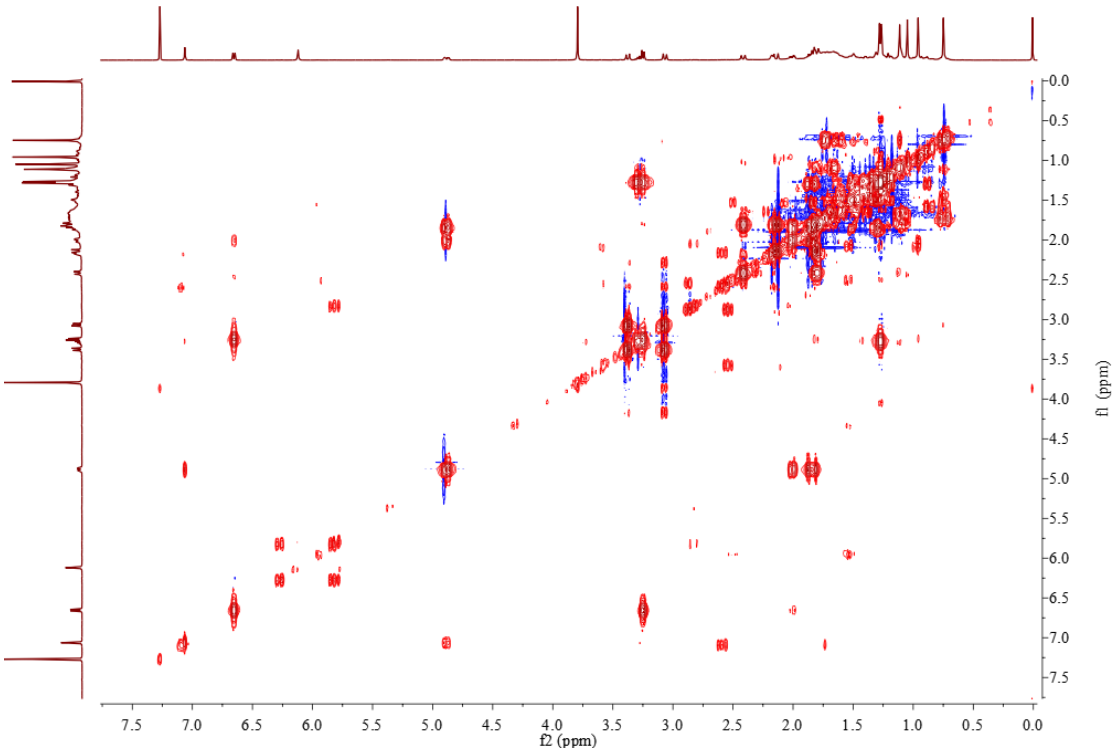


Figure S13. ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$ (400 MHz)

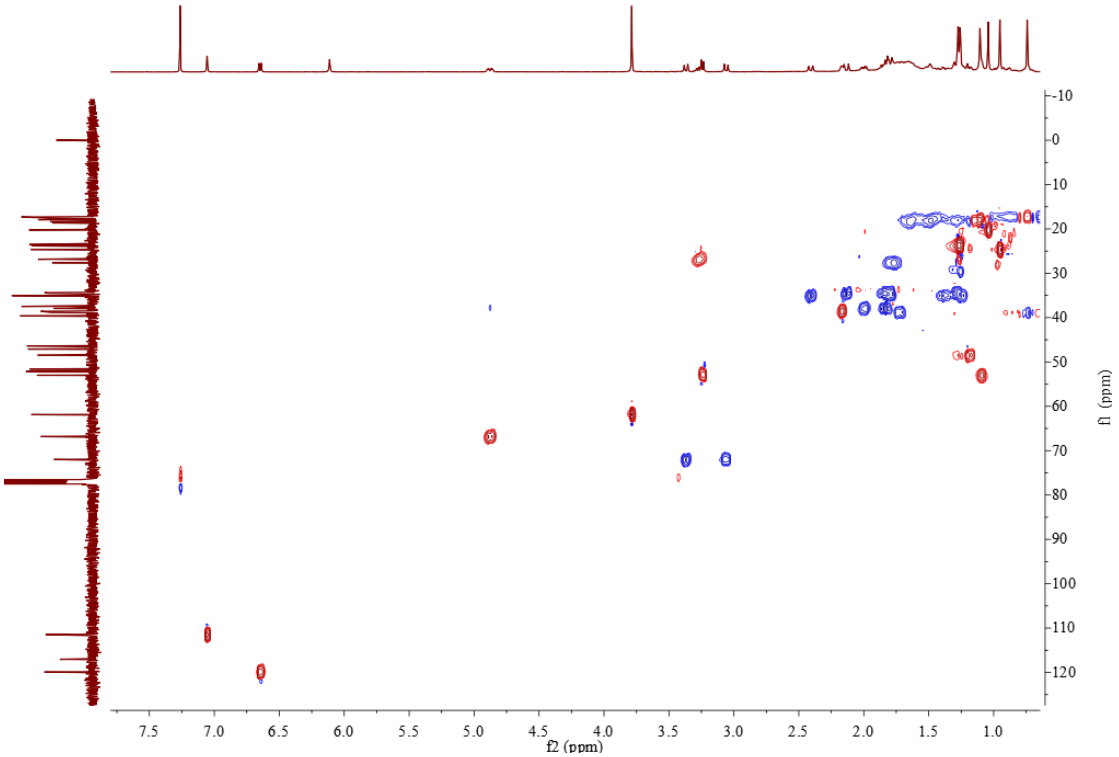


Figure S14. HSQC spectrum of **1** in $CDCl_3$ (400 MHz)

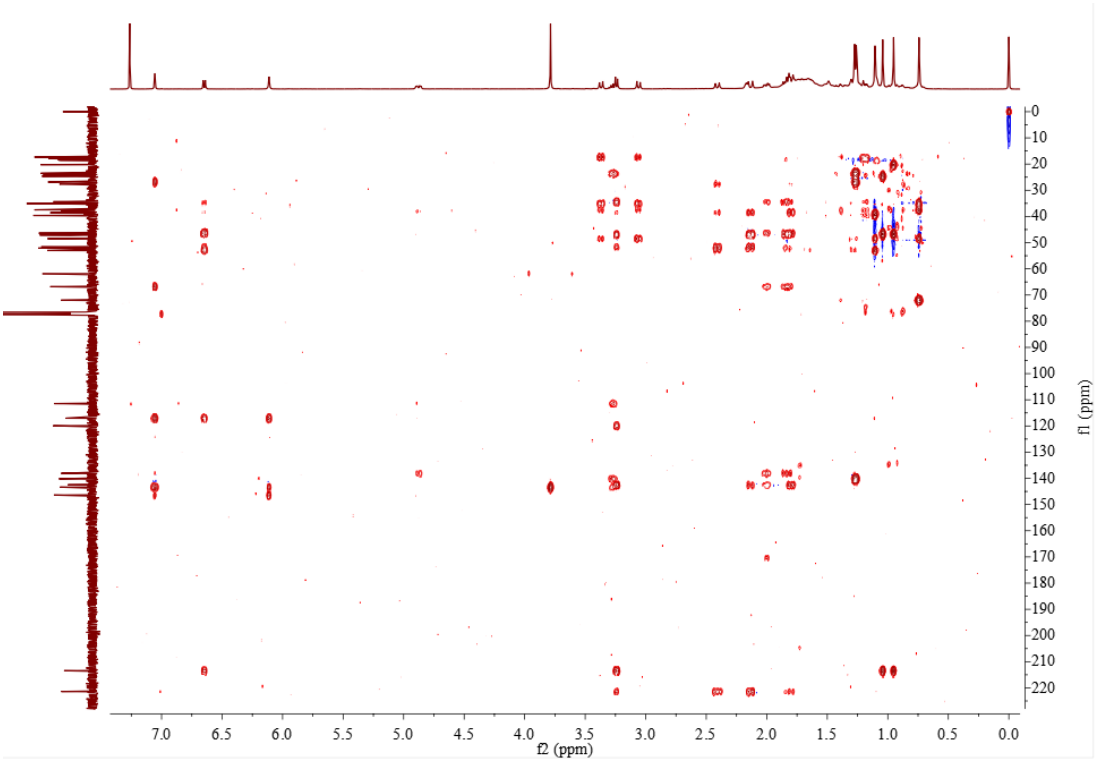


Figure S15. HMBC spectrum of **1** in $CDCl_3$ (400 MHz)

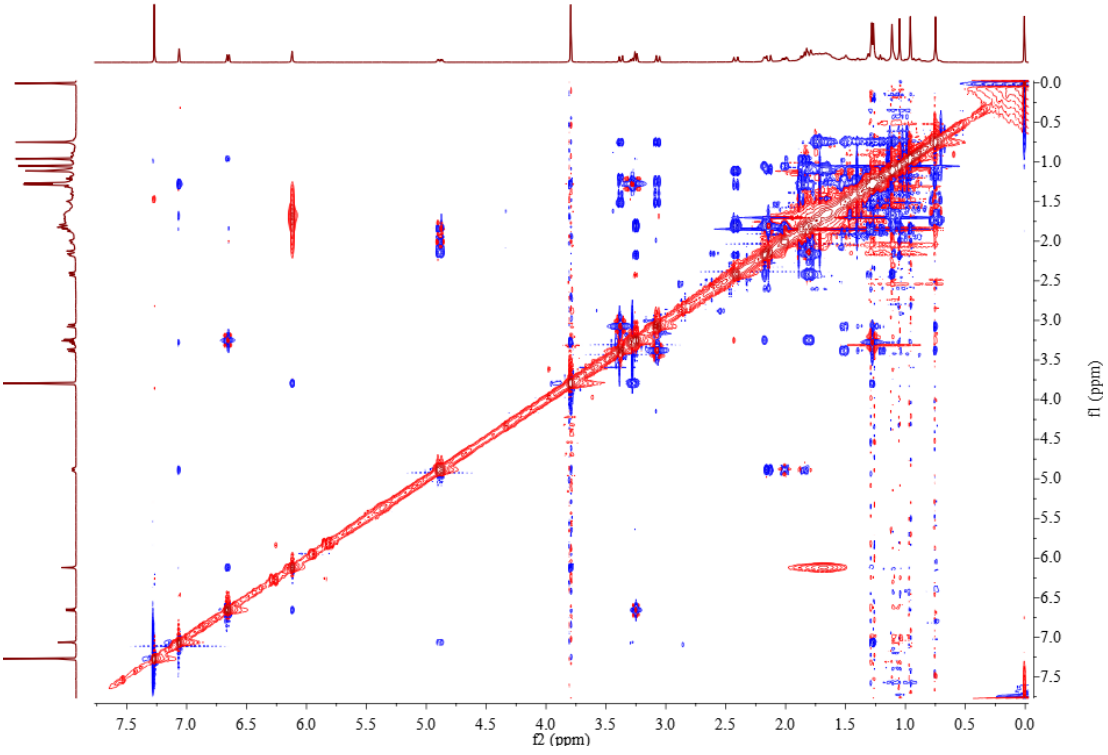
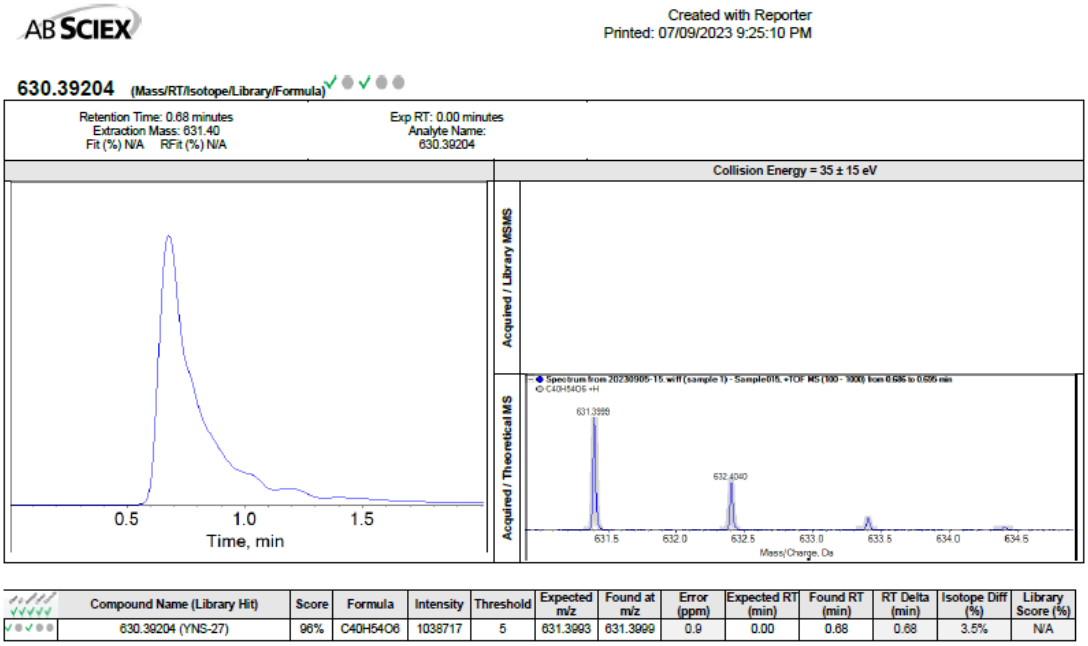


Figure S16. NOESY spectrum of **1** in $CDCl_3$ (400 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
630.39204 (YNS-27)	96%	C40H54O6	1038717	5	631.3993	631.3999	0.9	0.00	0.68	0.68	3.5%	N/A

Figure S17. HRESIMS report of **1**

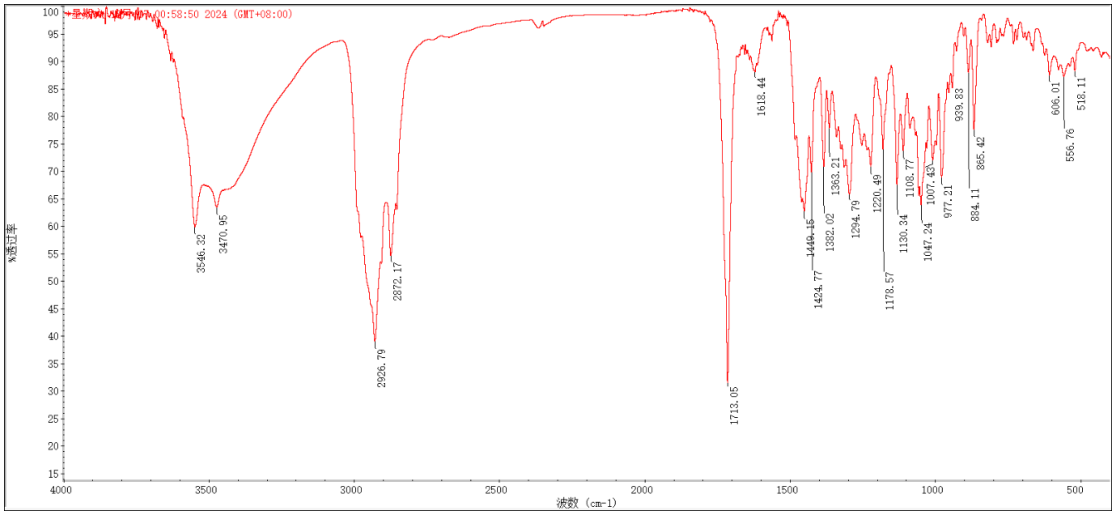


Figure S18. IR spectrum of **1**

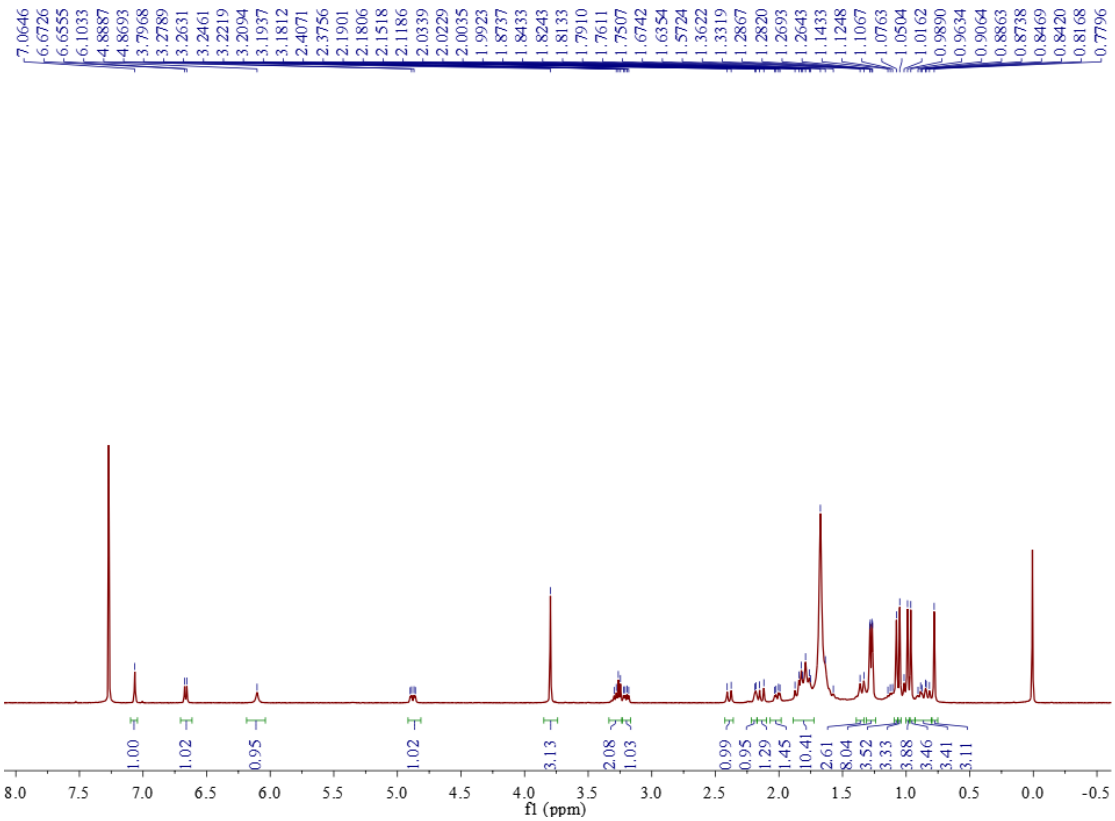


Figure S19. ^{1}H NMR spectrum of **2** in $CDCl_3$ (400 MHz)

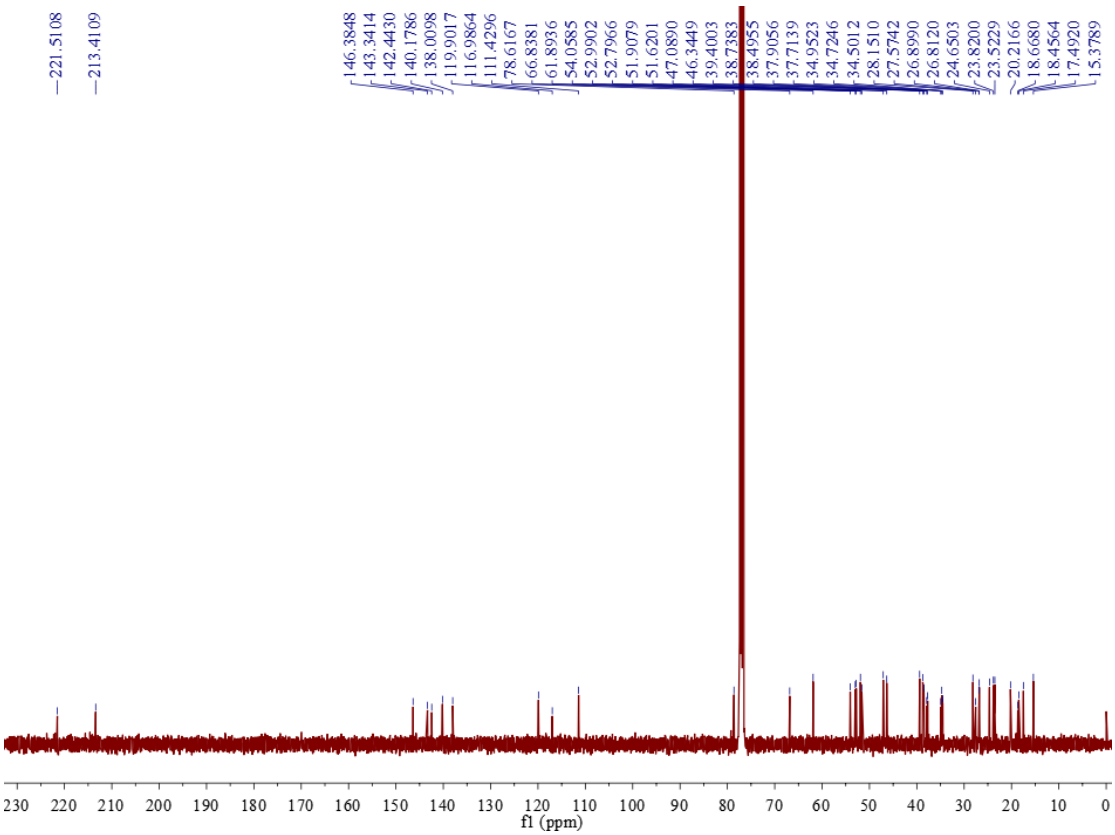


Figure S20. ^{13}C NMR spectrum of **2** in $CDCl_3$ (100 MHz)

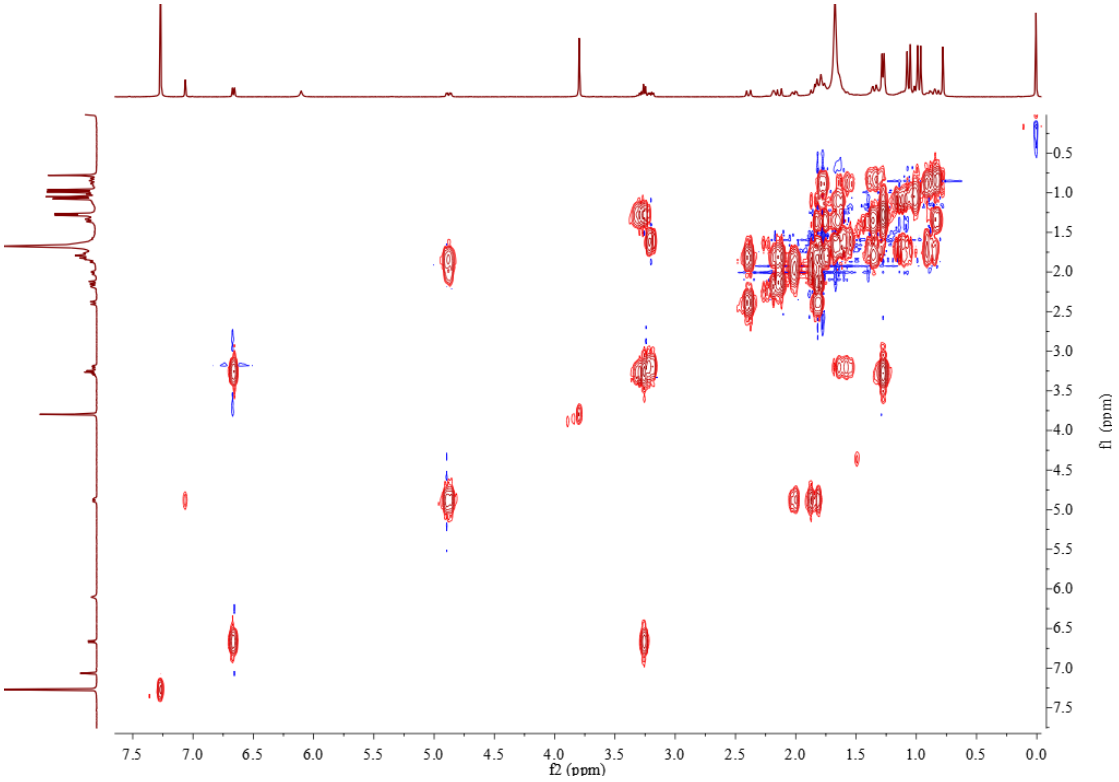


Figure S21. ^{1}H-^{1}H COSY spectrum of **2** in $CDCl_3$ (400 MHz)

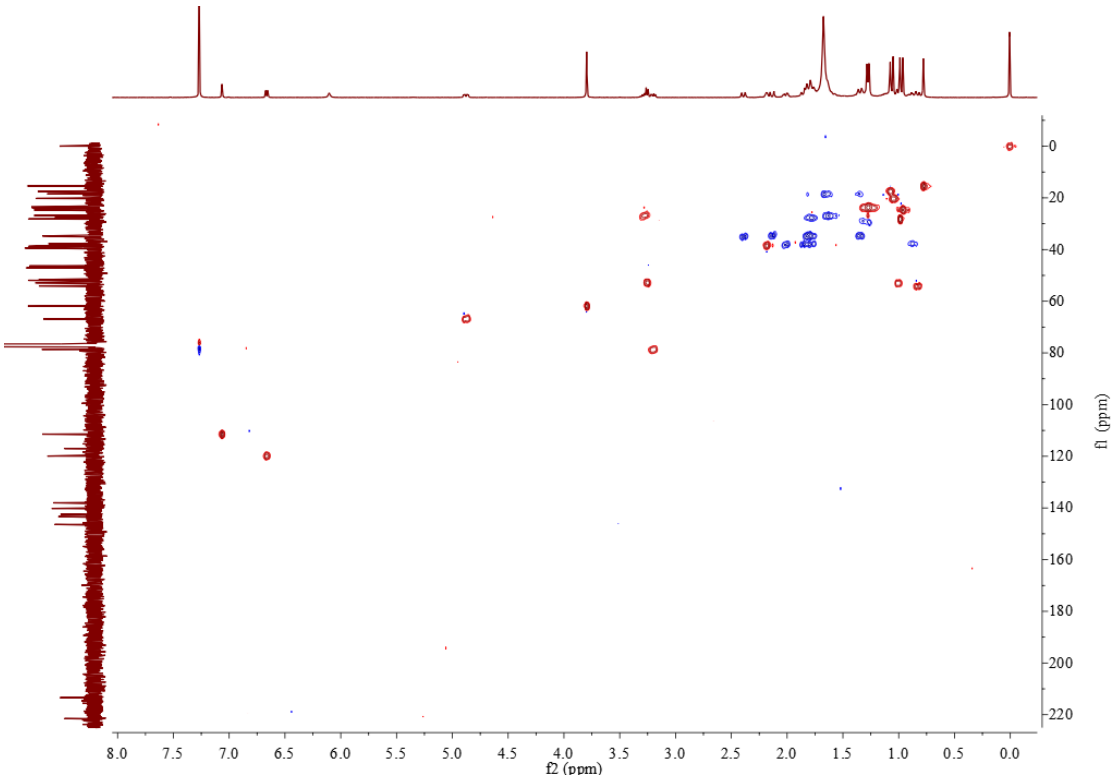


Figure S22. HSQC spectrum of **2** in $CDCl_3$ (400 MHz)

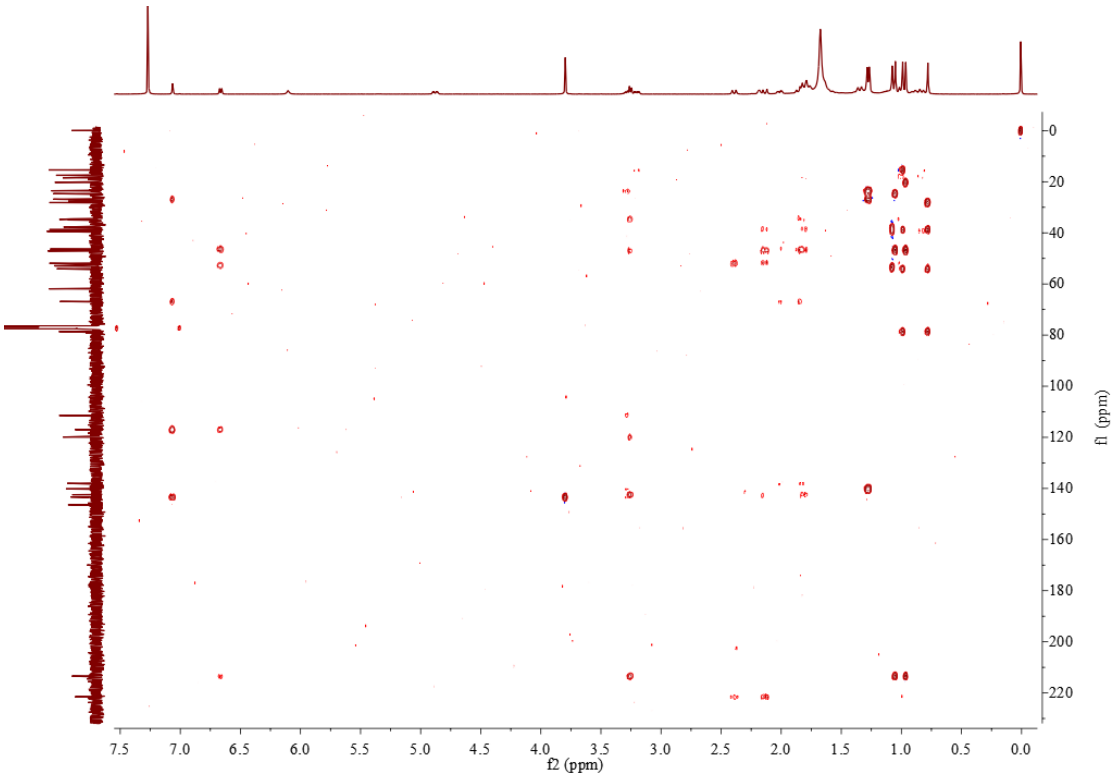


Figure S23. HMBC spectrum of **2** in $CDCl_3$ (400 MHz)

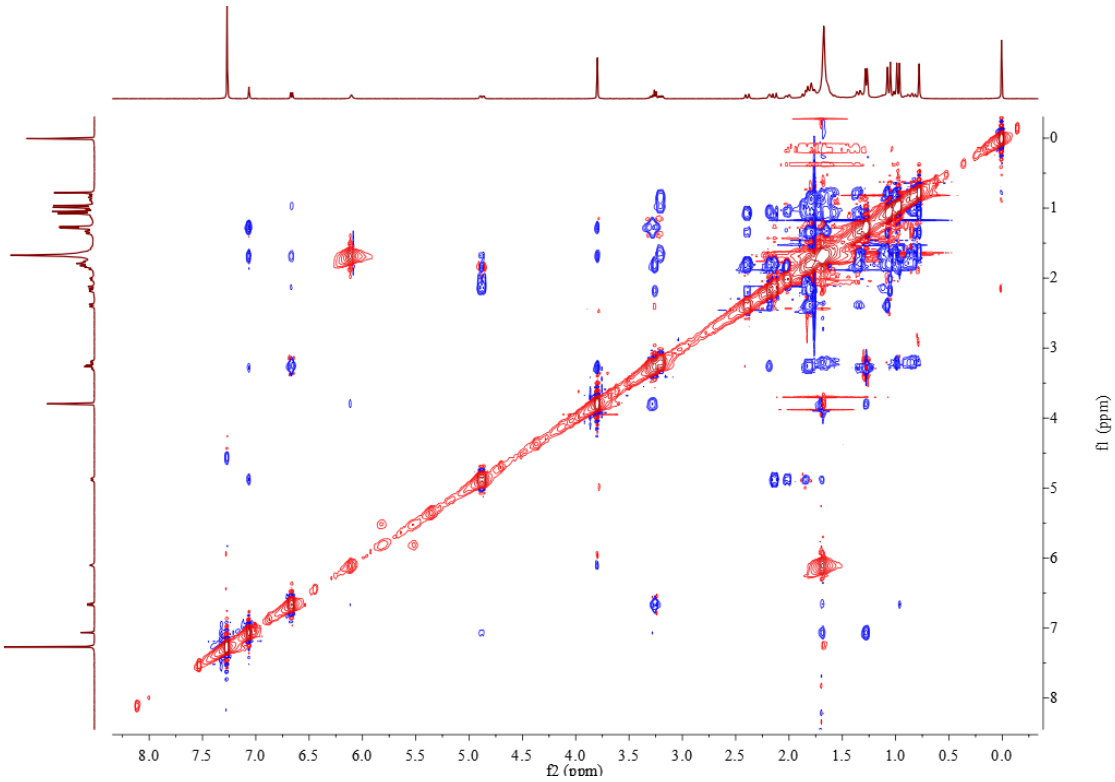
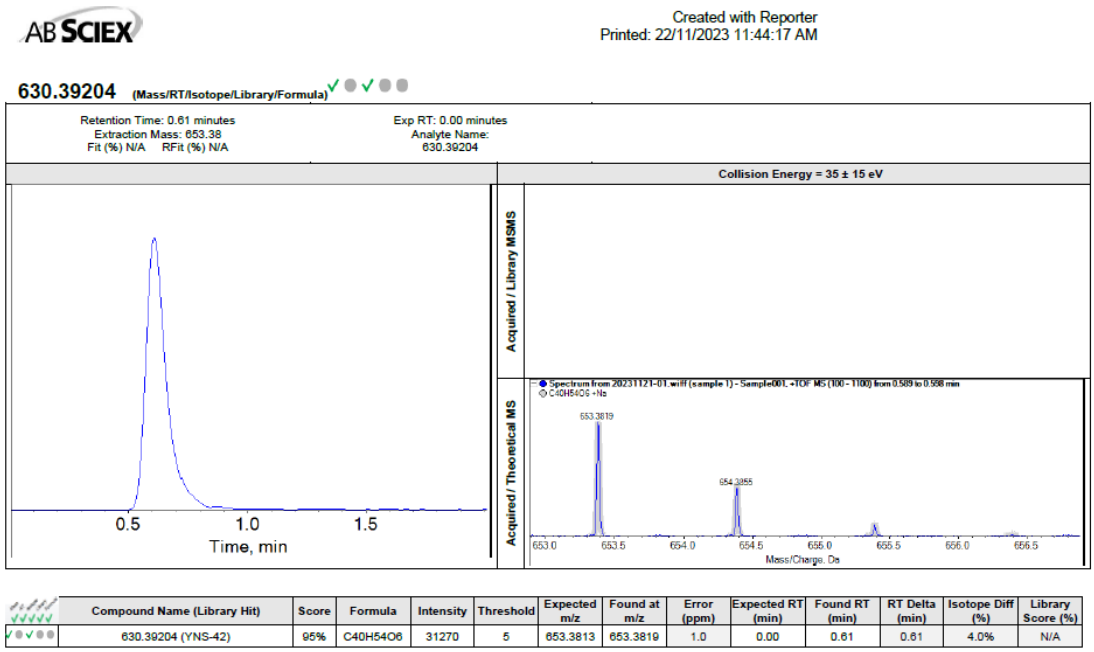


Figure S24. NOESY spectrum of **2** in $CDCl_3$ (400 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
630.39204 (YNS-42)	95%	C40H54O6	31270	5	653.3813	653.3819	1.0	0.00	0.61	0.61	4.0%	N/A

Figure S25. HRESIMS report of **2**

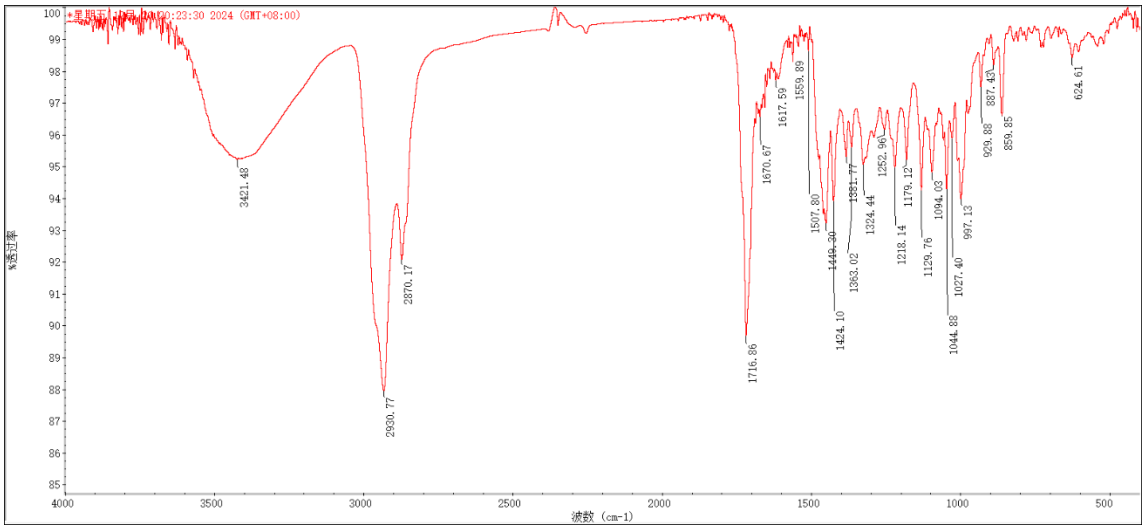


Figure S26. IR spectrum of **2**

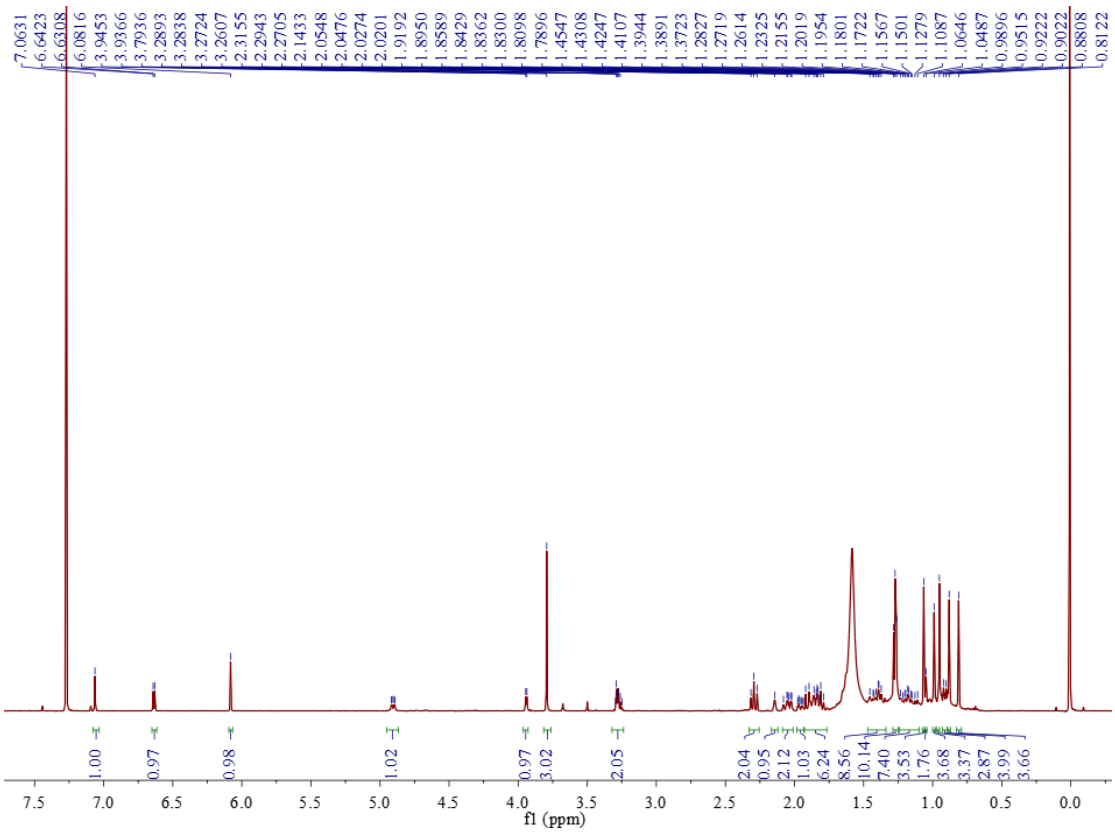


Figure S27. ^{1}H NMR spectrum of **3** in $CDCl_3$ (600 MHz)

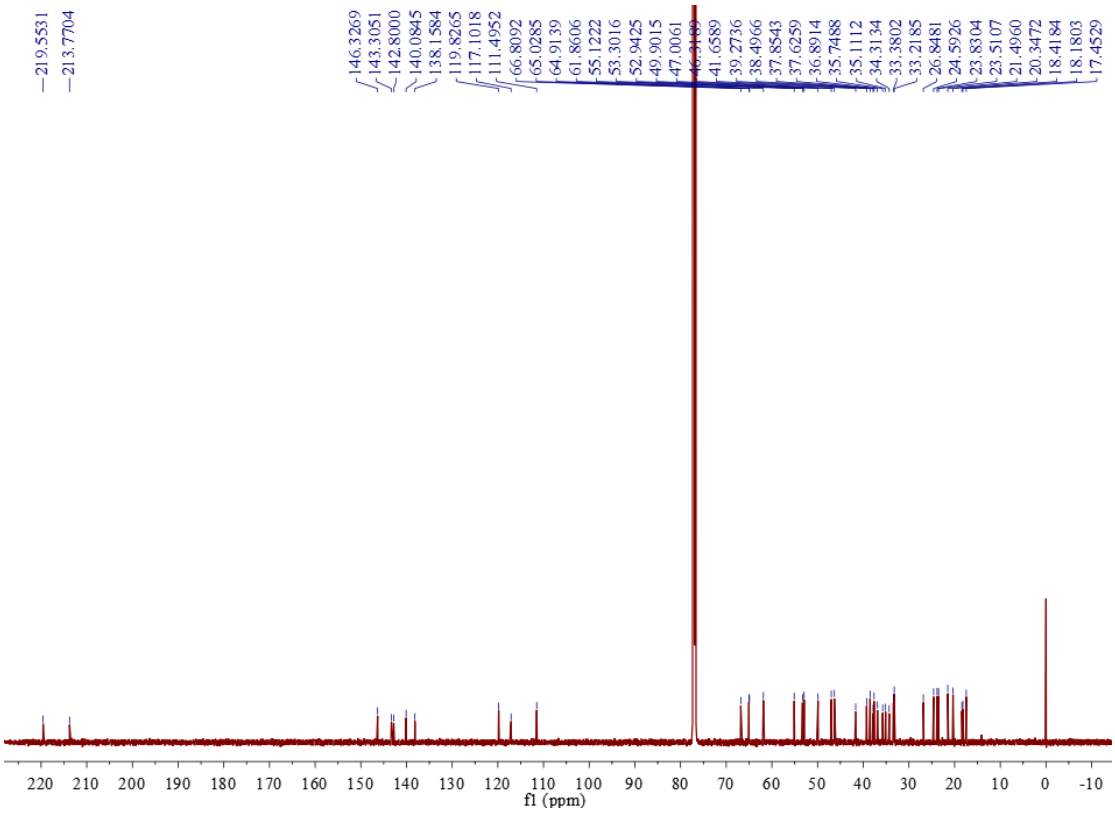


Figure S28. ^{13}C NMR spectrum of **3** in $CDCl_3$ (150 MHz)

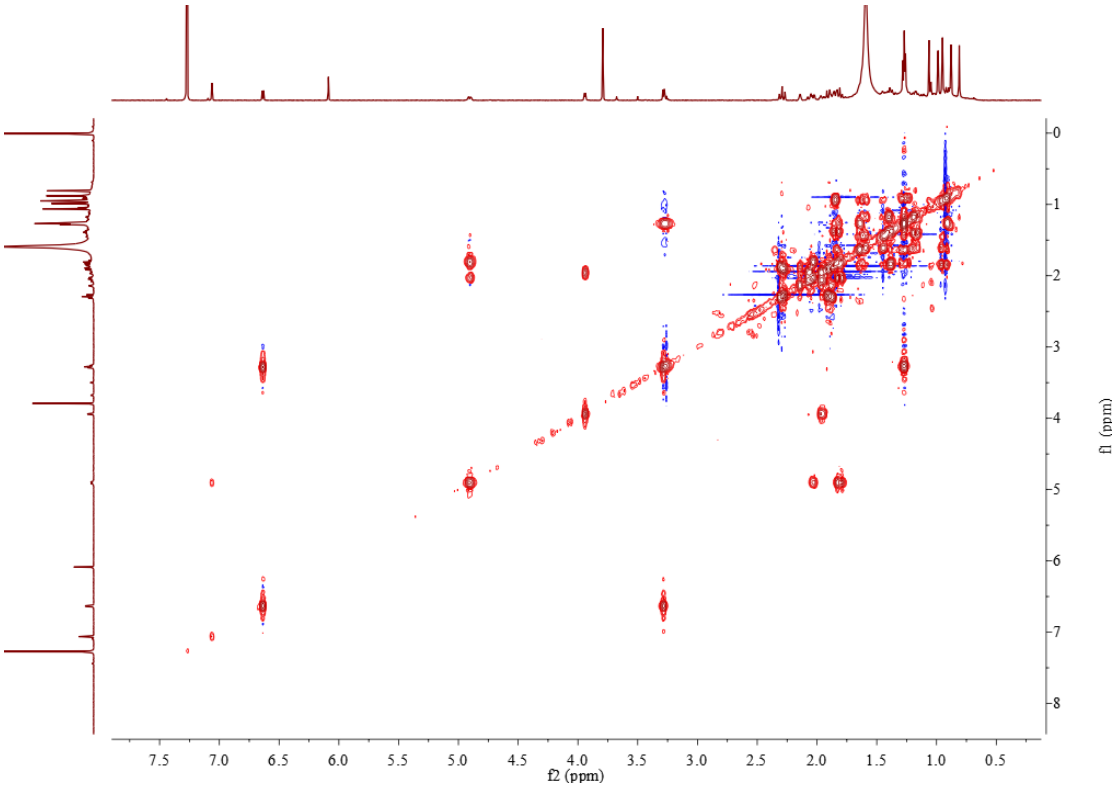


Figure S29. ^{1}H-^{1}H COSY spectrum of **3** in $CDCl_3$ (600 MHz)

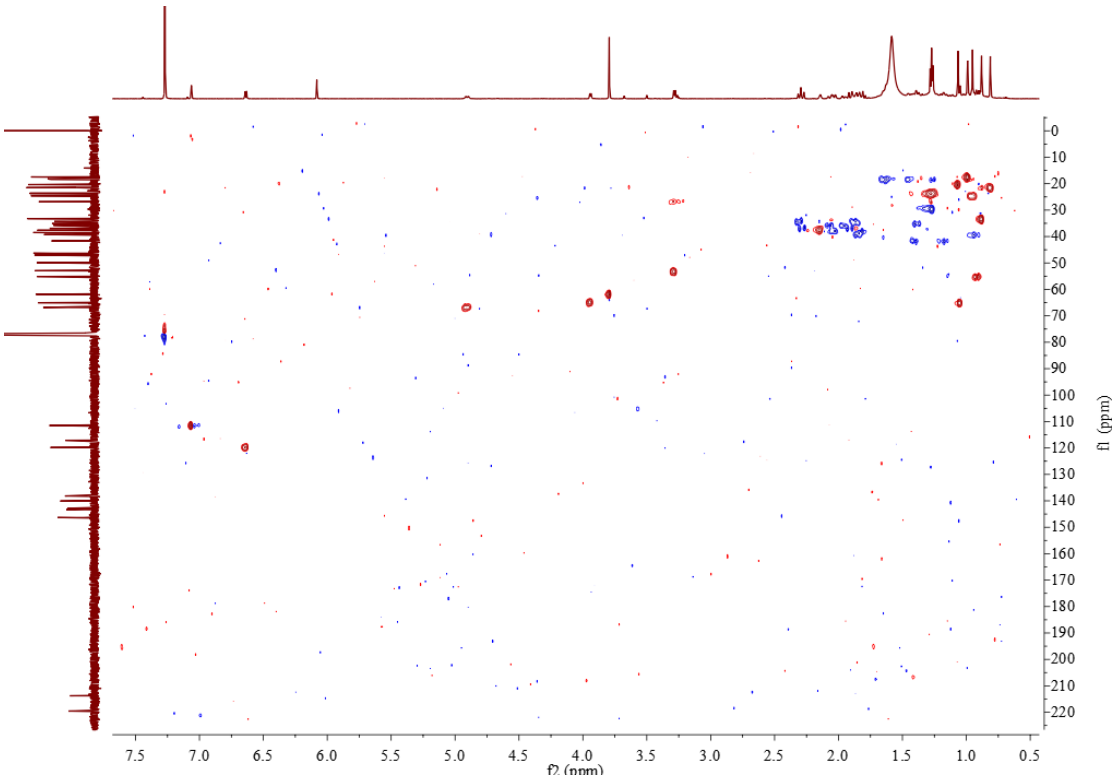


Figure S30. HSQC spectrum of **3** in $CDCl_3$ (400 MHz)

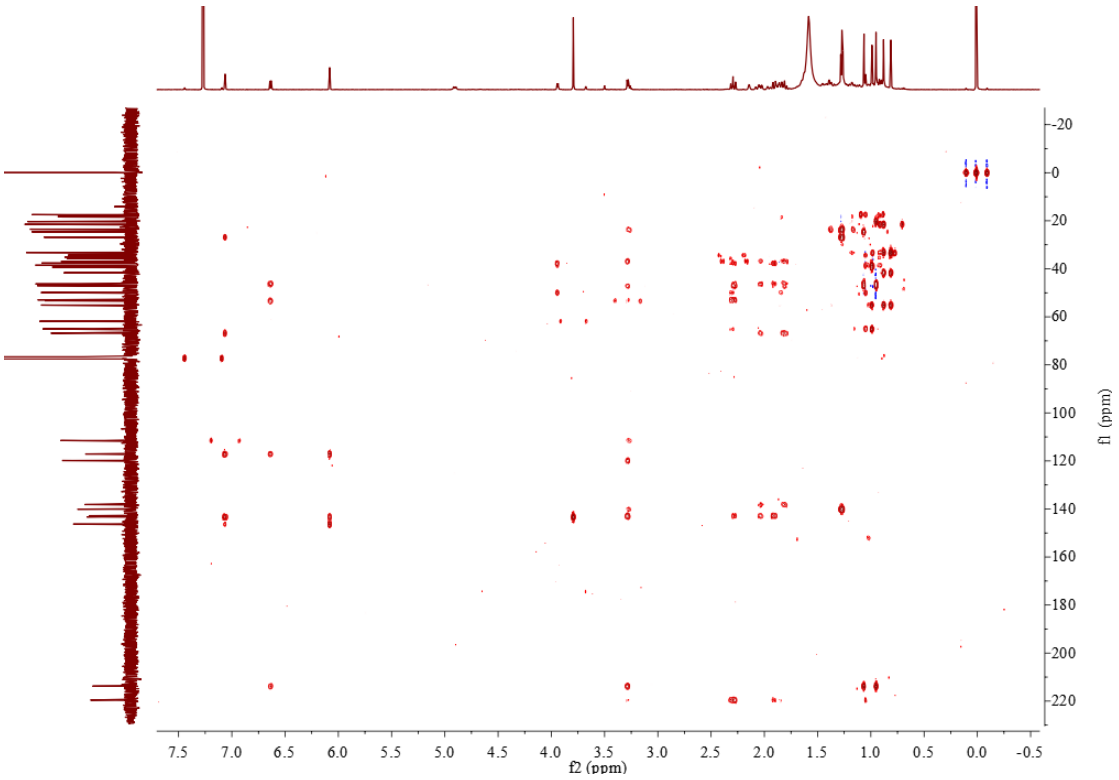


Figure S31. HMBC spectrum of **3** in $CDCl_3$ (600 MHz)

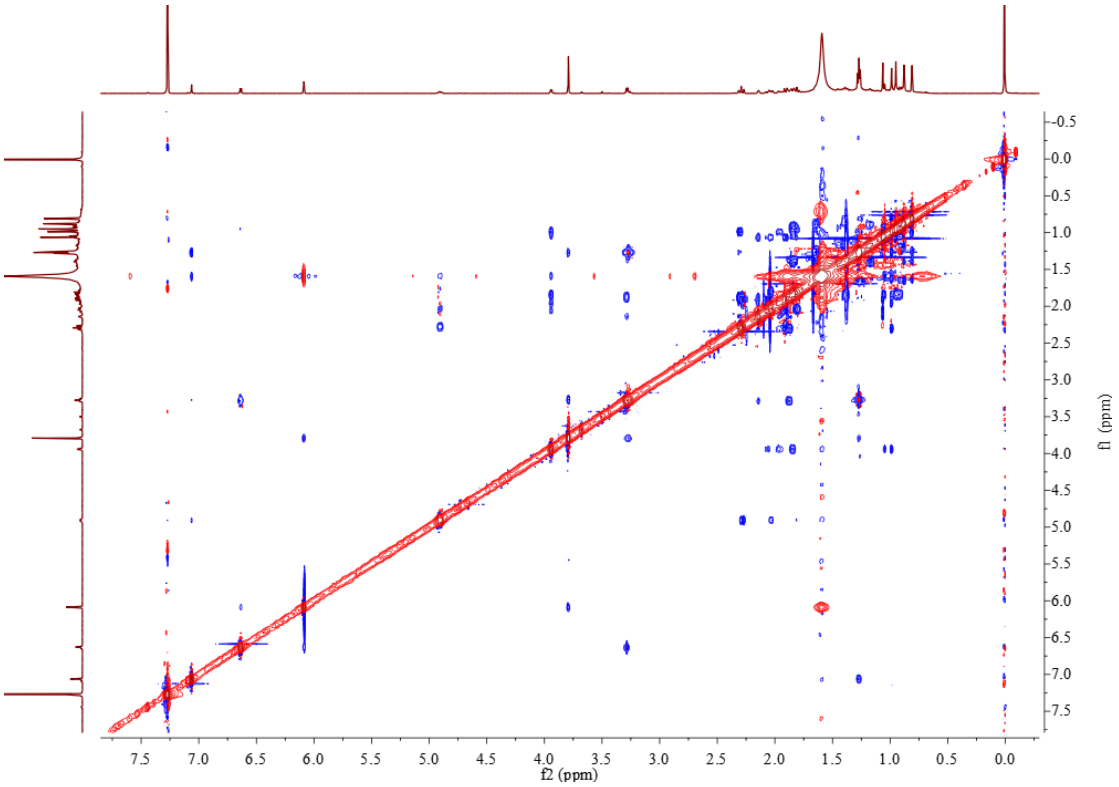
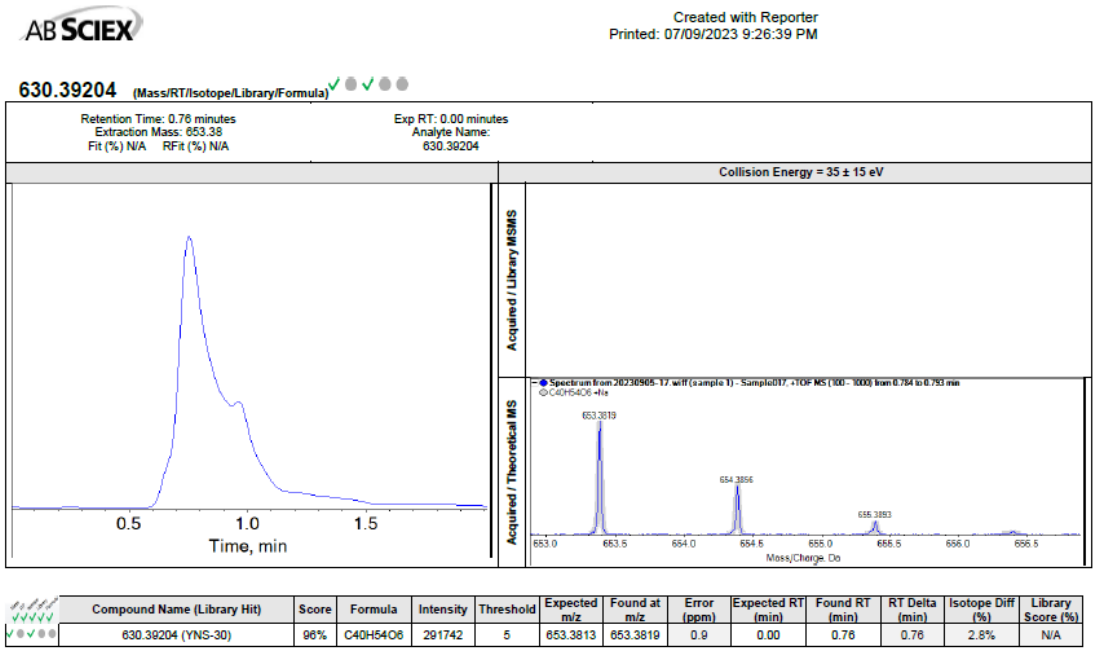


Figure S32. NOESY spectrum of **3** in $CDCl_3$ (600 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
630.39204 (YNS-30)	96%	C40H54O6	291742	5	653.3813	653.3819	0.9	0.00	0.76	0.76	2.8%	N/A

Figure S33. HRESIMS report of **3**.

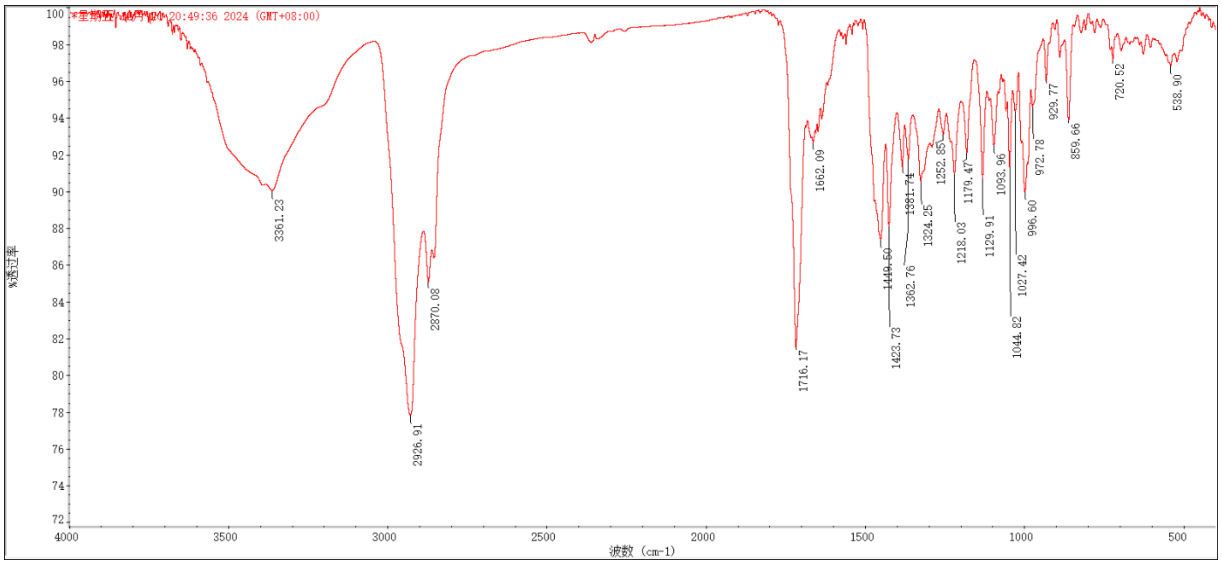


Figure S34. IR spectrum of **3**.

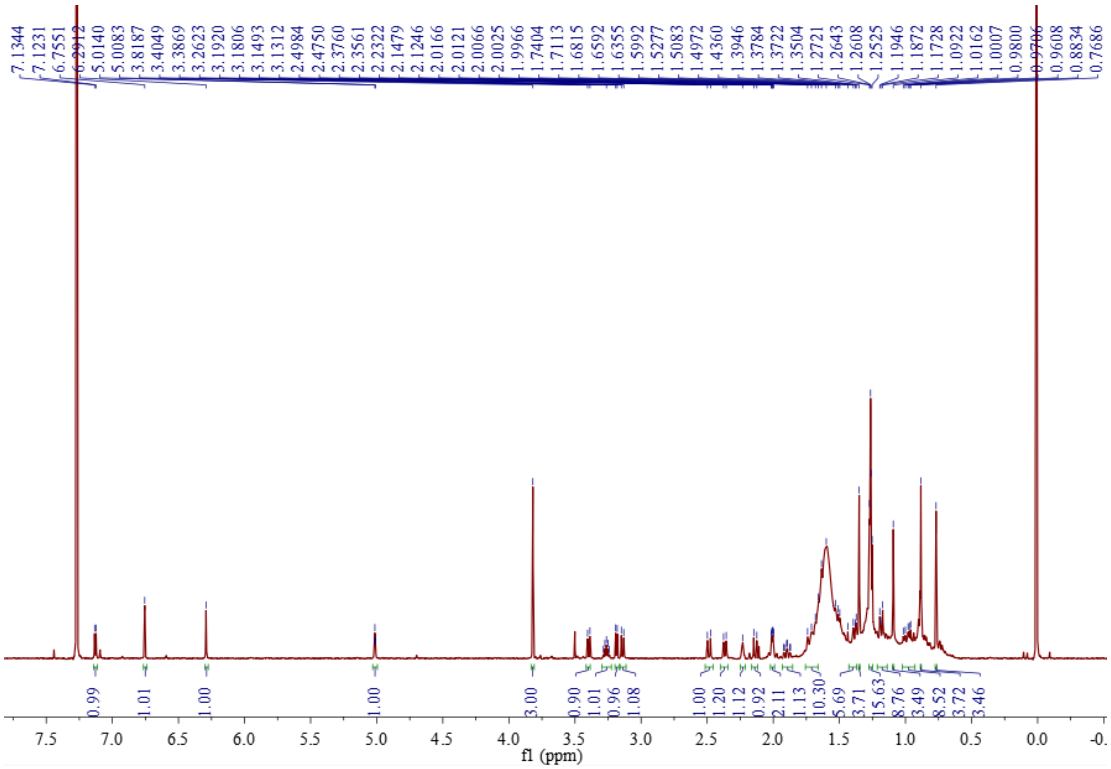


Figure S35. ^{1}H NMR spectrum of **4** in $CDCl_3$ (600 MHz)

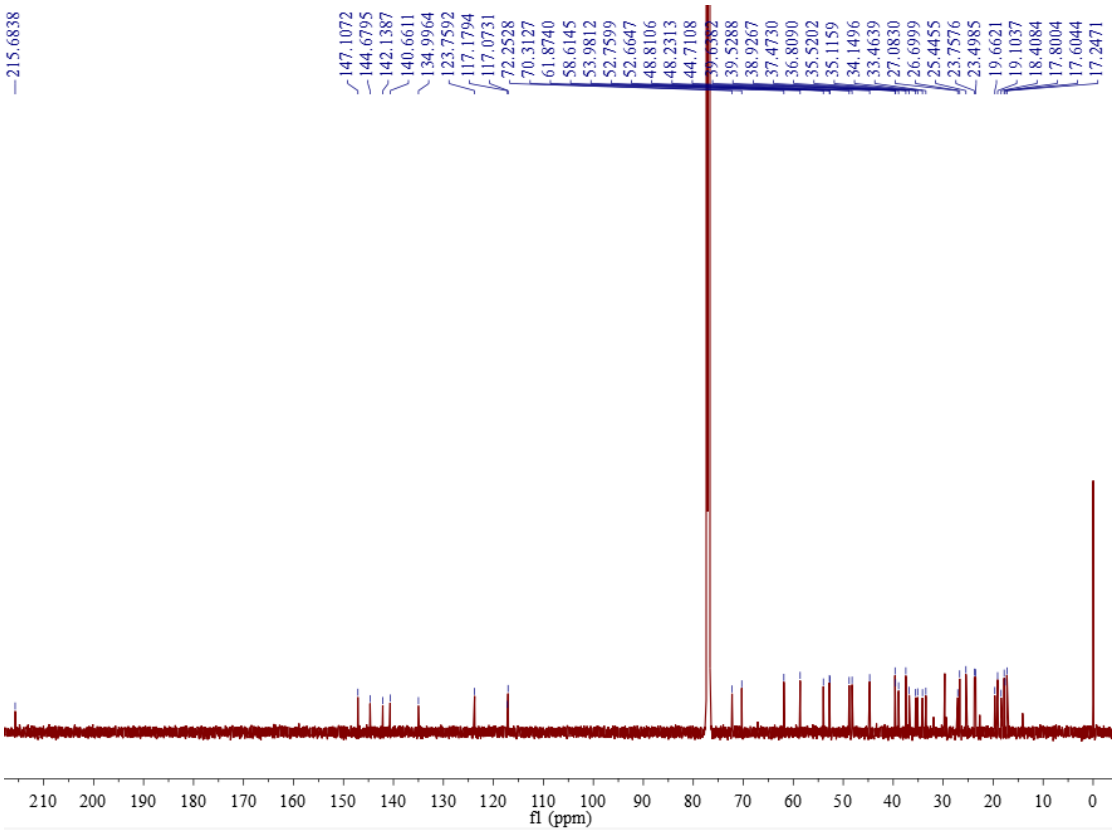


Figure S36. ^{13}C NMR spectrum of **4** in $CDCl_3$ (150 MHz)

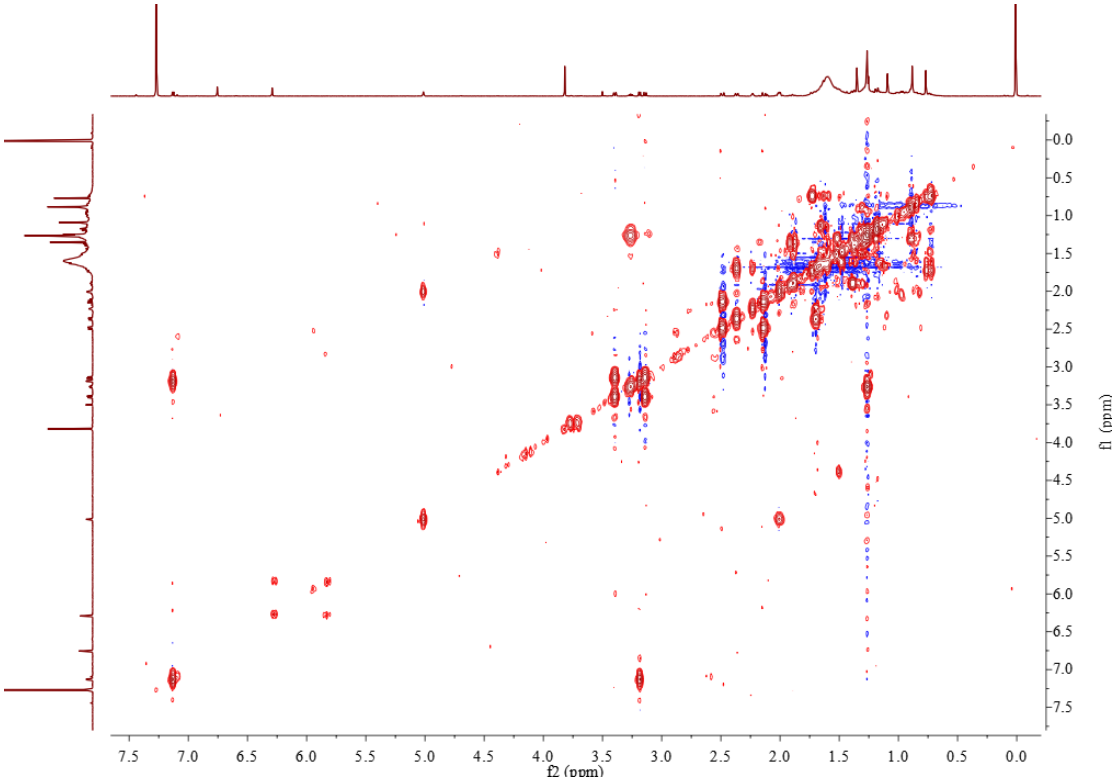


Figure S37. ^{1}H-^{1}H COSY spectrum of **4** in $CDCl_3$ (600 MHz)

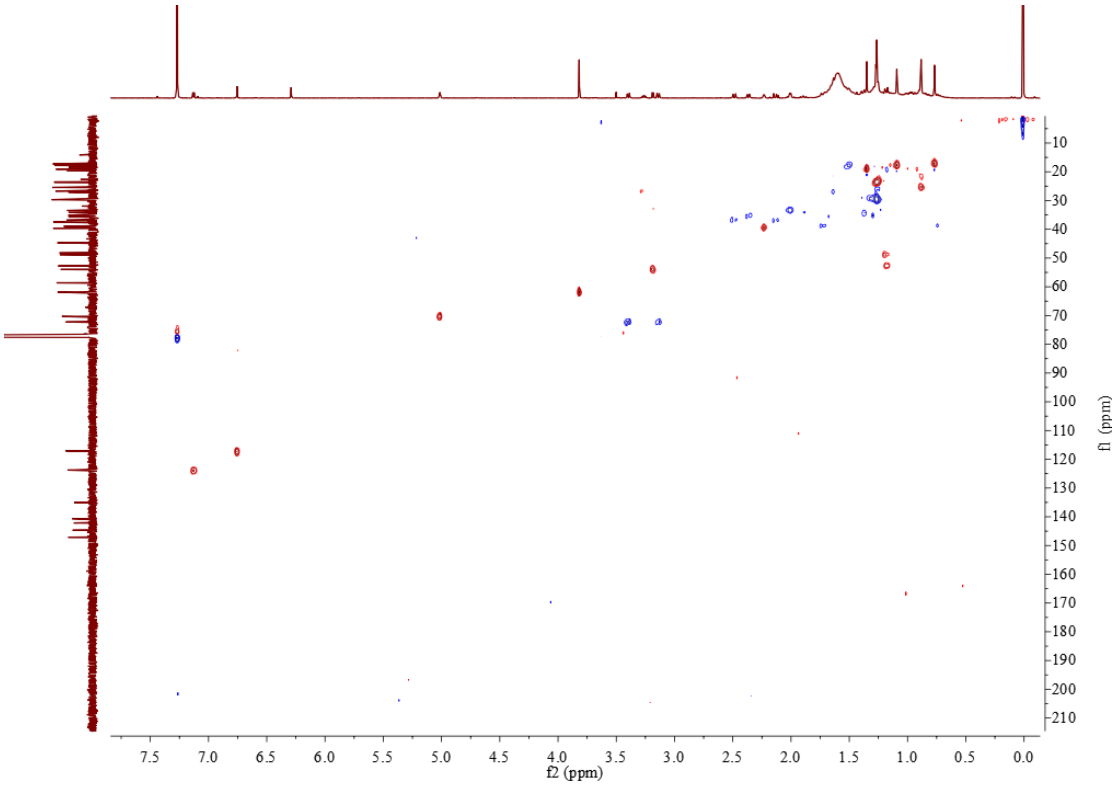


Figure S38. HSQC spectrum of **4** in $CDCl_3$ (600 MHz)

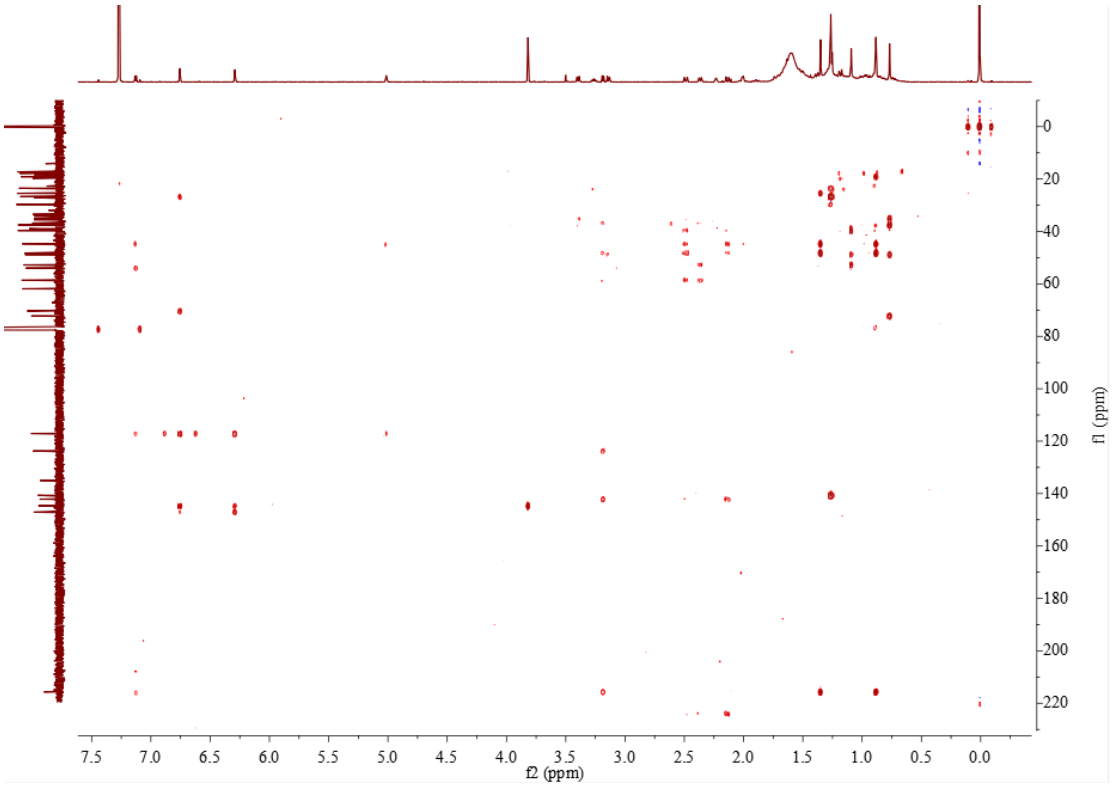


Figure S39. HMBC spectrum of **4** in $CDCl_3$ (600 MHz)

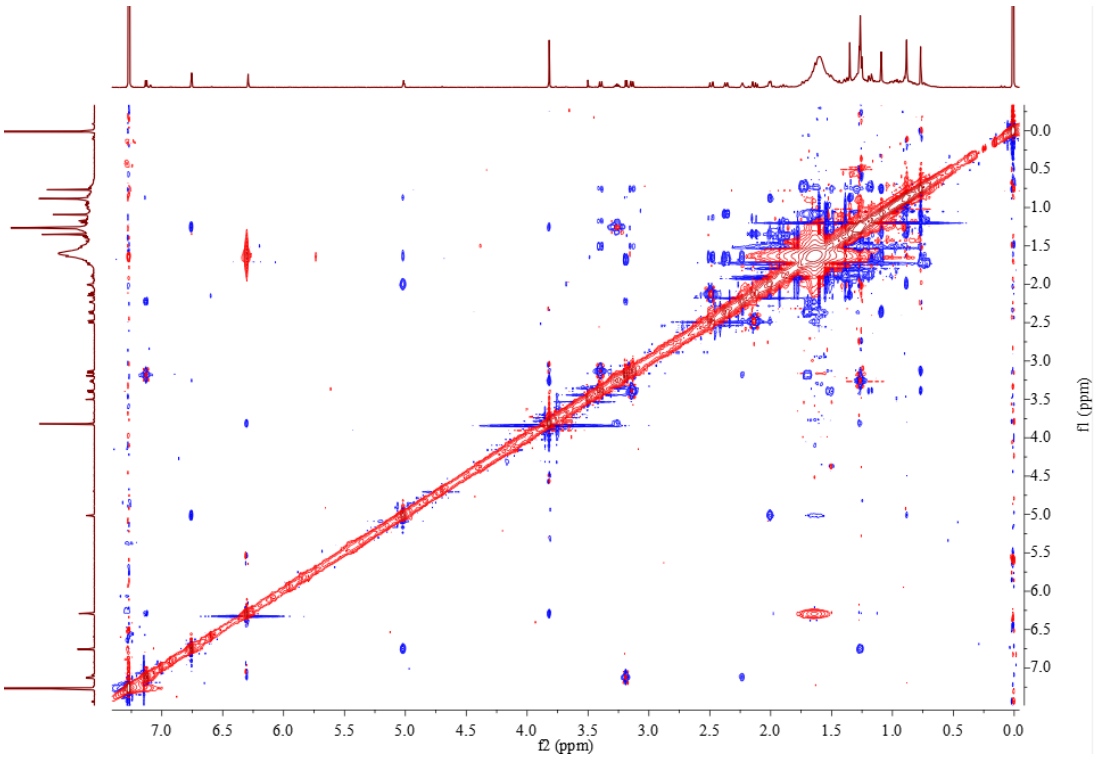
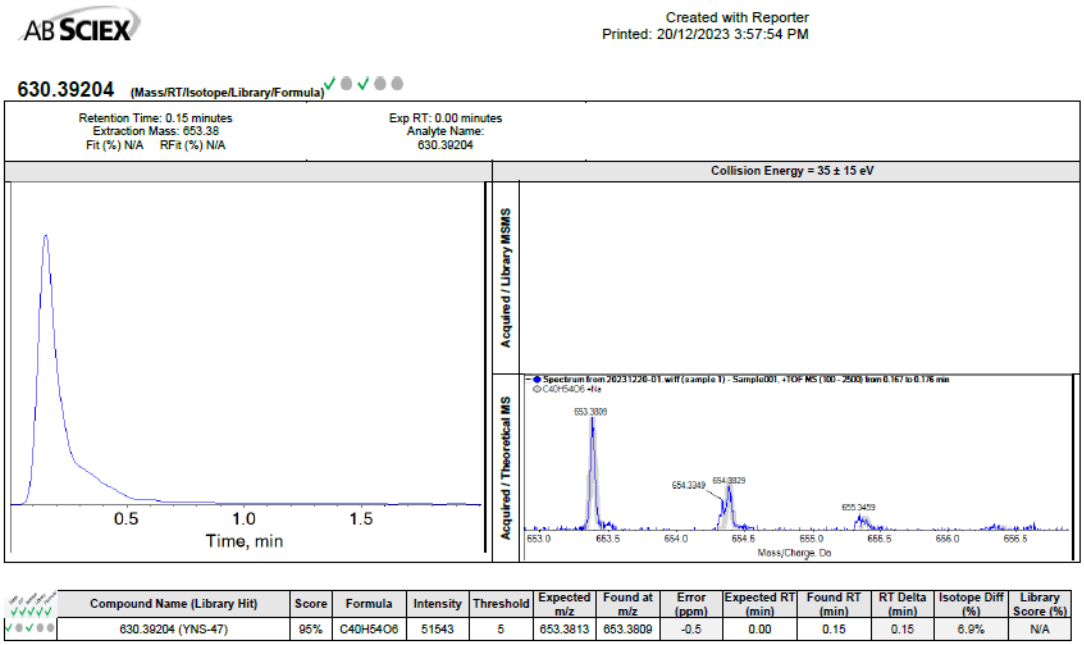


Figure S40. NOESY spectrum of **4** in $CDCl_3$ (400 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
630.39204 (YNS-47)	95%	C40H54O6	51543	5	653.3813	653.3809	-0.5	0.00	0.15	0.15	6.9%	N/A

Figure S41. HRESIMS report of **4**

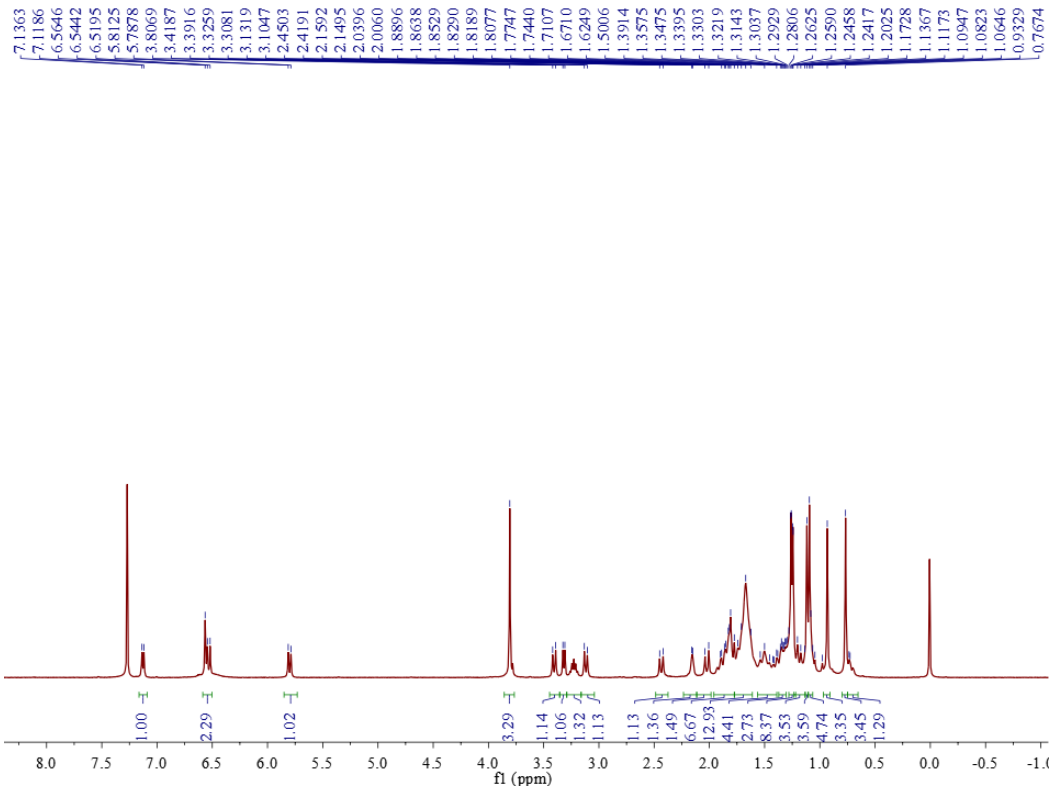


Figure S42. ^{1}H NMR spectrum of **1a** in $CDCl_3$ (400 MHz)

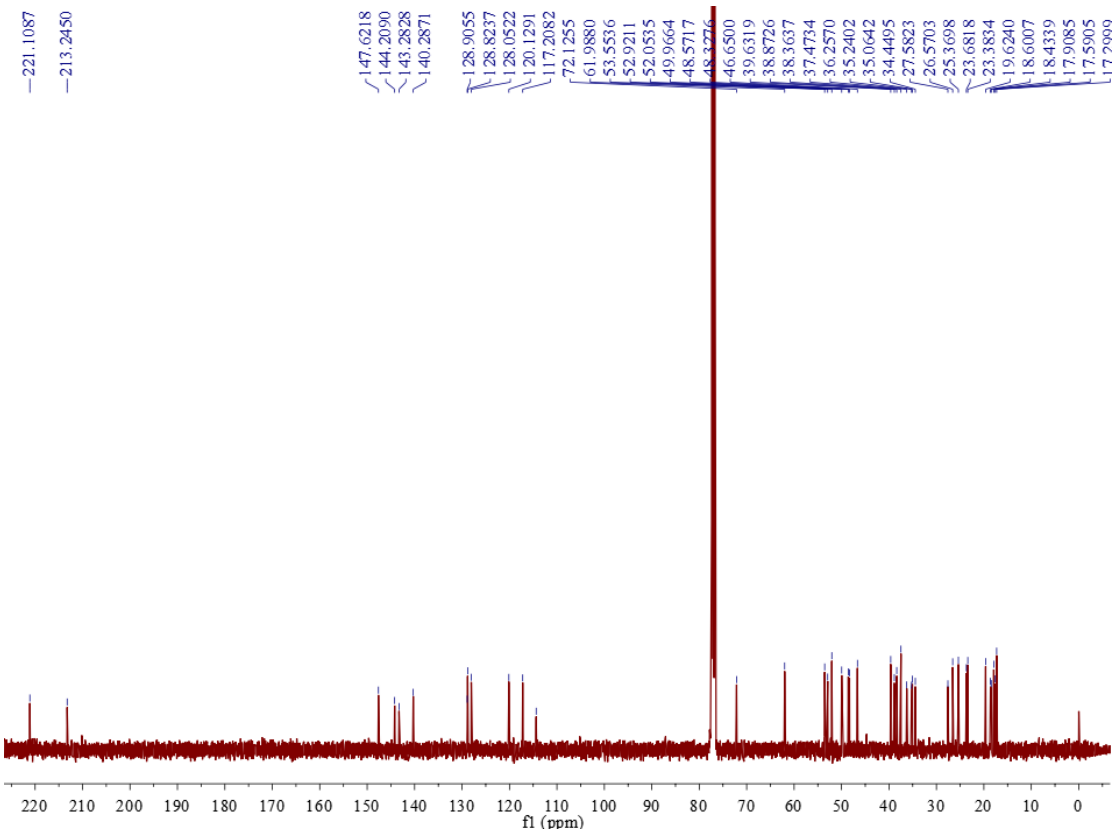
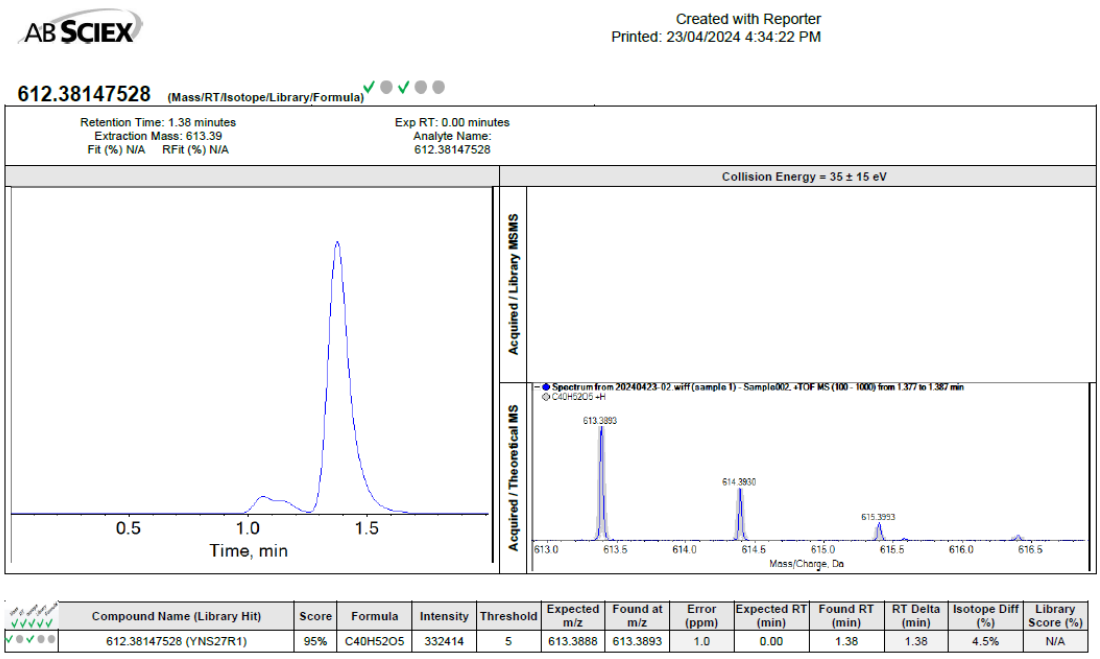


Figure S43. ^{13}C NMR spectrum of **1a** in $CDCl_3$ (100 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
612.38147528 (YNS27R1)	95%	C40H52O5	332414	5	613.3888	613.3893	1.0	0.00	1.38	1.38	4.5%	N/A

Figure S44. HRESIMS report of **1a**

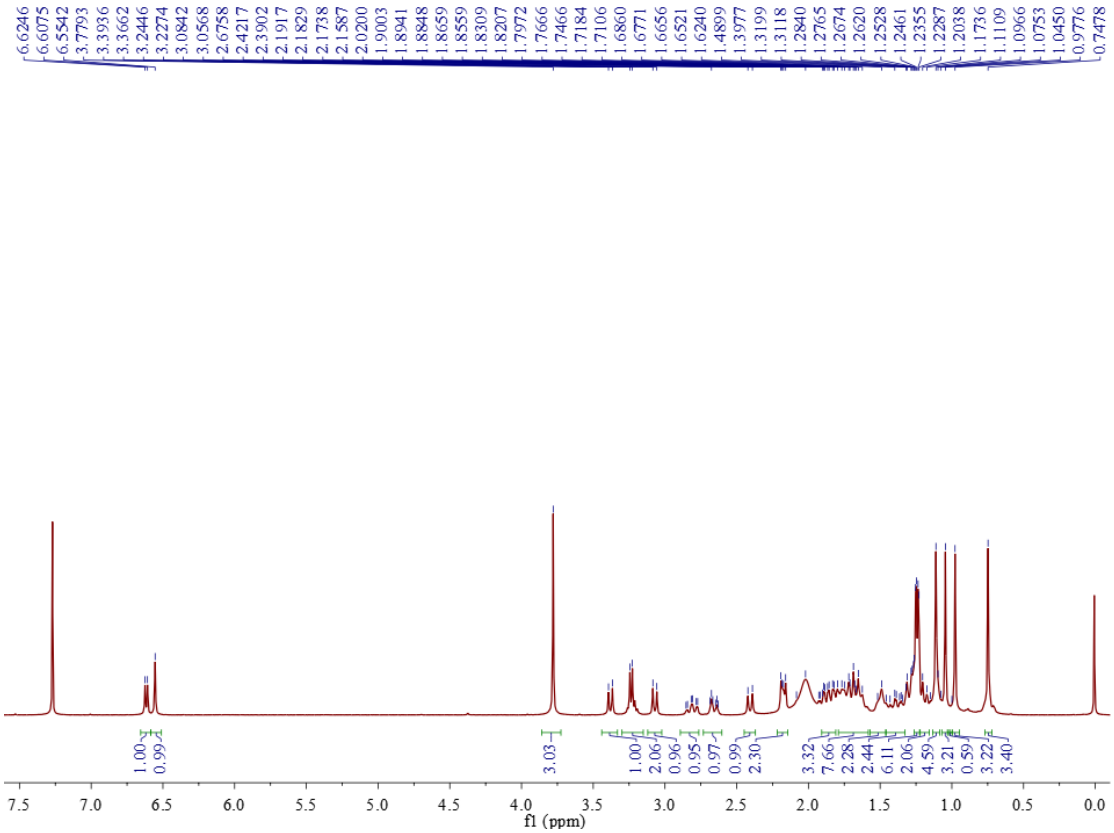


Figure S45. ^{1}H NMR spectrum of **1b** in $CDCl_3$ (400 MHz)

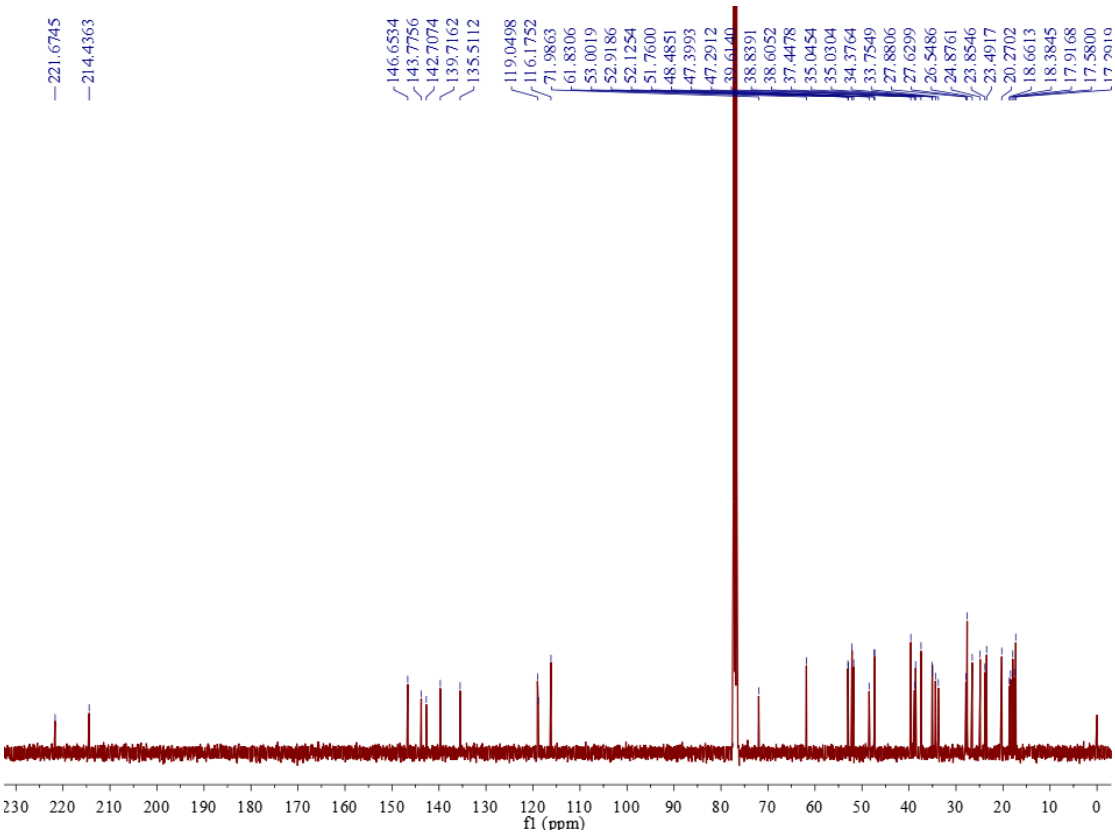
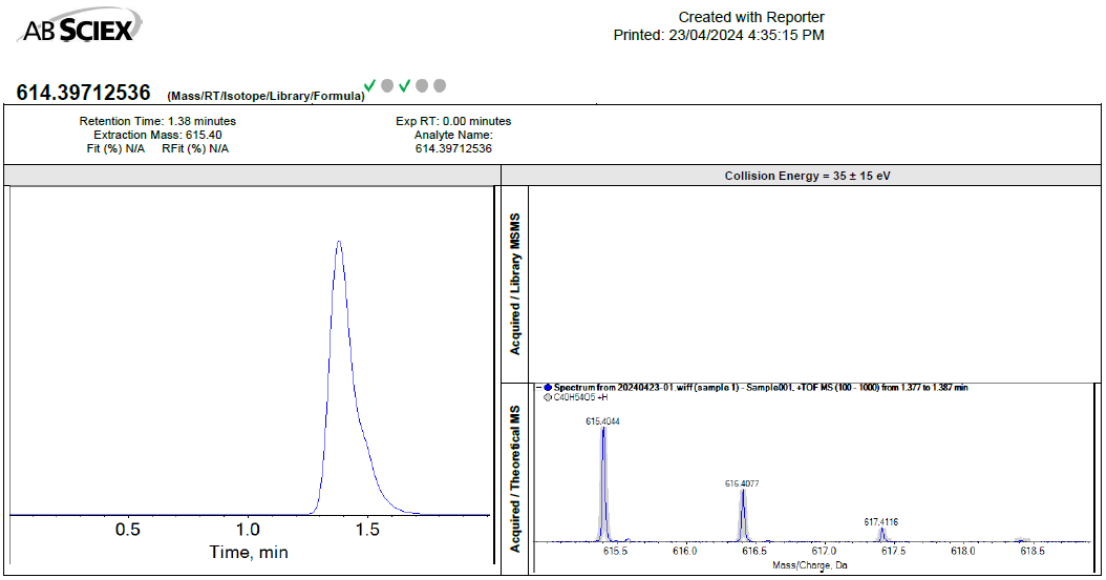


Figure S46. ^{13}C NMR spectrum of **1b** in $CDCl_3$ (100 MHz)



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score (%)
614.39712536 (YNS27R2)	99%	C40H54O5	237216	5	615.4044	615.4044	-0.1	0.00	1.38	1.38	0.9%	N/A

Figure S47. HRESIMS report of **1b**