Supporting Information

Valeriadimers A-C, three sesquiterpenoid dimers from *Valeriana officinalis* var. *latifolia*†

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† Electronic Supplementary Information (ESI) available: The extraction scheme, compound characterization, spectroscopic data, and CIF files of **1** and **3** are included herein. See DOI: 10.1039/b000000x/

‡ Zhu-zhen Han and Ji Ye contributed equally to this work.

Contents

Experimental section

Experimental section

General Experimental Procedures.

General. Optical rotations were obtained with a Perkin-Elmer 341 polarimer. IR spectra were recorded with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-500 spectrometer (**1, 2** in $CDCl_3$ and **3** in CD_3OD. HRESIMS were measured on an Agilent LC/MSD Trap XCT mass spectrometer. Materials for column chromatography were silica gel (200-300 mesh; Huiyou Silica Gel Development Co., Ltd.), Sephadex LH-20 (40-70 μm; Pharmacia Co., Ltd.), and YMC-Gel ODS-A (50 μm; YMC, Milford, MA). Preparative TLC (0.4-0.5 mm, 20×20 cm) was conducted with glass precoated silica gel GF_{254} (Huiyou Silica Gel Development Co., Ltd.). Spots were visualized under UV light (254 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating. The cell lines (HUVECs) used for the assay were purchased from the Shanghai institute of pharmaceutical industry. MTT was purchased from Sigma Chemical Co. and dimethyl sulfoxide (DMSO) from Merck, Sharp & Dohme, Ltd.

Plant Material. The roots of *Valeriana officinalis* var. *latifolia* were collected from Gaopo, Guizhou province in China in July 2007 and authenticated by Prof. Han-min Zhang, Second Military Medical University. A herbarium specimen (NO. 2007-08-18) was deposited in the School of Pharmacy, Second Military Medical University, Shanghai, China.

Extraction and Isolation: The dried root powder of *Valeriana officinalis* var. *latifolia* (9.8kg) were extracted with 50 L of 95% ethanol at room temperature (3×24 h). The EtOH solution was filtered and concentrated under reduced pressure to yield crude extract, which was suspended in H_2O (2.5 L) and partitioned successively with $CHCl_3$ (5×2.5 L) and EtOAc (2×2.5 L), respectively. The $CHCl_3$ extract (307 g) was subjected to silica gel column chromatography, eluted with a step gradient of petroleum ether-acetone (from 100: 0 to 0:100), to afford 15 fractions (1-15). Fr.5 (9.0 g) was submitted a ODS CC(CH_3OH-H_2O , 40-100%) , and purified by preparative TLC ($CHCl_3$- EtOAc, 10:1) to yield **1** (16.1 mg) and **2** (28.2 mg). Fr.9 (1.2 g) was purified by silica gel column chromatography ($CHCl_3$- Me_2CO , 4:1) to yield **3** (20.7 mg).

Valeriadimer A (**1**) Orthorhombic crystal; $[\alpha]_D^{25}$ +2.36 (c 0.12, CH_3OH); IR (KBr) ν_{max} 2942，

2920，1711，1456，1381cm^{-1}; ^{1}H NMR and ^{13}C NMR data see **Table S1**; ESI-MS *m/z*: 411 $[M+H]^+$, 433 $[M+Na]^+$, 445 $[M+Cl]^-$; HRESIMS *m/z*: 433.3113 $[M+Na]^+$ (calcd. for $C_{28}H_{42}O_2$, 433.3083).

Valeriadimer B (**2**) colorless oil; $[\alpha]_D^{25}$ +41.7 (c 0.20, CH_3OH); IR (KBr) ν_{max} 2924，2866，1705，1628,1452，1382, 1300, 1259, 1176cm^{-1}; ^{1}H NMR and ^{13}C NMR data see **Table S2**; ESI-MS *m/z*: 455 $[M+H]^+$, 477 $[M+Na]^+$, 489 $[M+Cl]^-$; HRESIMS *m/z*: 455.3154 $[M+H]^+$ (calcd. for $C_{29}H_{42}O_4$, 455.3156).

Valeriadimer C (**3**) Orthorhombic crystal; $[\alpha]_D^{25}$ -30.0 (c 0.35, CH_3OH); IR (KBr) ν_{max} 3573, 3512, 3354, 2978, 2927, 1460, 1379, 1147, 1084 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see **Table S3**; ESI-MS *m/z*: 505 $[M+H]^+$, 527 $[M+Na]^+$, 503 $[M-H]^-$; HRESIMS *m/z*: 527.3458 $[M+H]^+$ (calcd. for $C_{30}H_{48}O_6$, 527.3451).

Cell Viability Assays.

HUVECs ($4 \times 10^4 \sim 6 \times 10^4$ cells/well) were seeded into 96-well plates in RPMI 1640 containing 10% FBS. After attachment, the medium was replaced with RPMI 1640 containing 3% FBS. Cells were treated with **1**, **2**, and **3** for 48 h, respectively. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide, which is converted to formazan in surviving cells. The formazan was dissolved in 10% SDS–5% iso-butanol–0.01 M HCl. Optical density was measured (three times) at 570 nm with 630 nm as the reference and cell viability was normalized as the percentage of control.[1]

References and Notes

1 K. K. Shen, L. L. Ji, C. Y. Gong, Y. B. Ma, L. Yang, Y. Fan, M. Q. Hou, Z. T. Wang, *Biochem. Pharmacol*, 2012, **84**, 784 – 792.

Table S1. ^{1}H NMR and ^{13}C NMR Data of **1** (δ in ppm, J in Hz)

	1				
No	δ_H mult. (*J* in Hz)	δ_C	No	δ_H mult. (*J* in Hz)	δ_C
1	3.09 (dd, 11.0 ,2.0)	64.7 d	1′	2.64 (dd, 13.0, 4.0)	42.4 d
2a	2.13 (m)	28.7 t	2′a	2.00 (m)	29.1 t
2b	1.37 (overlap)		2′b	1.86 (m)	
3a	2.75 (m)	31.4 t	3′a	2.83 (dt, 7.5, 14.0)	39.0 t
3b	2.17 (m)		3′b	2.27(m)	
4		142.2 s	4′		215.7 s
5	5.03 (d, 8.3)	126.2 d	5′	1.78 (dd, 5.0, 1.5)	55.7 d
6	1.37 (overlap)	28.7 d	6′	0.52 (dd, 9.0, 5.0)	23.6 d
7	0.59,(dt, 1.5, 10.0)	35.3 d	7′	0.80 (t, 9.0)	18.6 d
8a	1.80 (m)	22.4 t	8′a	2.05 (m)	17.1 t
8b	0.95(overlap)		8′b	1.60 (dd, 16.0, 8.0)	
9a	2.12 (m)	41.1 t	9′a	1.34 (m)	33.5 t
9b	1.07 (overlap)		9′b	0.69 (m)	
10		61.9 s	10′		39.3 s
11		21.3 s	11′		19.5 s
12	1.08 (s)	30.0 q	12′	0.95 (s)	16.3 q
13	1.01 (s)	16.6 q	13′	1.03 (s)	29.9 q
14	1.12 (s)	19.4 q	14′	0.73 (s)	23.6 q

Data of **1** was recorded at 500 MHz and ^{13}C NMR spectroscopic at 125 MHz

Table S2. ^{1}H NMR and ^{13}C NMR Data of **2** (δ in ppm, J in Hz)

	2				
N	δ_H mult. (J in Hz)	δ_C	No	δ_H mult. (J in Hz)	δ_C
1	2.99 (dd, 11.5 ,3.5)	63.8 d	1′	5.48 (dd, 11.5, 5.0)	71.2 d
2a	2.27 (m)	28.7 t	2′a	2.16 (overlap)	26.9 t
2b	1.37 (m)		2′b	1.83 (overlap)	
3a	2.72 (dt, 14.0, 3.5)	24.1 t	3′a	2.92 (dt, 7.0, 14.5)	36.8 t
3b	2.17 (m)		3′b	2.33(m)	
4		133.1 s	4′		213.4 s
5	6.88 (d, 9.5)	144.4 d	5′	1.94 (dd, 4.5, 2.0)	54.3 d
6	1.48 (overlap)	29.5 d	6′	0.48 (dd, 8.5, 4.5)	22.9 d
7	0.94,(overlap)	38.2 d	7′	0.80 (overlap)	19.6 d
8a	1.88 (m)	23.0 t	8′a	1.81 (overlap)	16.2 t
8b	1.10 (overlap)		8′b	1.55 (dd, 15.0, 6.5)	
9a	2.17 (overlap)	41.1 t	9′a	1.46 (m)	31.9 t
9b	1.10 (overlap)		9′b	0.76 (m)	
10		61.4 s	10′		38.0 s
11		23.9 s	11′		19.7 s
12	1.14 (s)	29.3 q	12′	0.97 (s)	16.2 q
13	1.19 (s)	16.4 q	13′	1.00 (s)	30.2 q
14	0.99 (s)	18.2 q	14′	0.92 (s)	22.1 q
15		167.7 s			

Data of **2** were recorded in $CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR

Table S3. ^{1}H NMR and ^{13}C NMR Data of **3** (δ in ppm, J in Hz)

	3				
N	δ_H mult. (J in Hz)	δ_C	No	δ_H mult. (J in Hz)	δ_C
1a	1.94 (m)	25.2 t	1′a	1.80 (m)	26.2 t
1b	1.72 (overlap)		1′b	1.62 (overlap)	
2a	1.72 (overlap)	35.3 t	2′a	1.77 (m)	30.2 t
2b	1.62 (m)		2′b	1.65 (overlap)	
3		83.1 s	3′		94.0 s
4	1.27 (t, 10.5)	47.9 d	4′	1.52 (overlap)	46.0 d
5	0.70 (m)	29.5 d	5′	0.53 (m)	31.4 d
6	0.56 (m)	27.4 s	6′	0.67 (m)	27.5 s
7a	1.77 (overlap)	20.6 t	7′a	1.77 (overlap)	20.5 t
7b	0.94 (overlap)		7′b	0.94 (overlap)	
8a	1.72 (overlap)	45.1 t	8′a	1.72 (overlap)	45.0 t
8b	1.53 (overlap)		8′b	1.53 (overlap)	
9		75.1 s	9′		74.8 s
10	1.94 (m)	59.3 d	10′	1.81 (m)	59.1 d
11		20.5 s	11′		20.7 s
12	1.06 (s)	16.3 q	12′	1.05 (s)	16.4 q
13	1.03 (s)	28.7 q	13′	1.04 (s)	28.4 q
14	1.13 (s)	19.7 q	14′	1.14 (s)	20.2 q
15	5.12 (s)	103.0 d	15′	5.40 (s)	98.2 d

Data of **3** were recorded in CD_3OD at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR

Table S4. Cell viability of **1**, **2**, and **3** (Mean±SD, n=3)

	1	**2**	**3**	**VEGF**[a]	**DMSO**
concentration	20 μM	20 μM	20 μM	10 ng/mL	
viability	146.8±3.3%	140.7±4.7%	97.7±3.4%	149.5±1.4%	100%

[a] positive control

Figure S1 The chemical structures of **1-3**

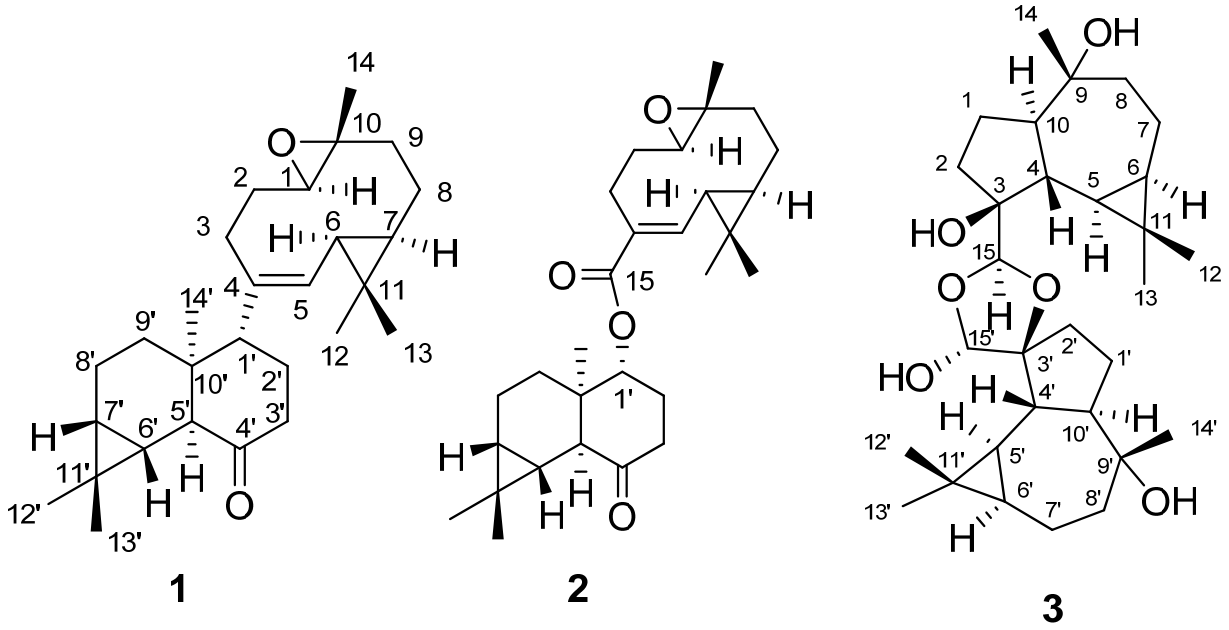
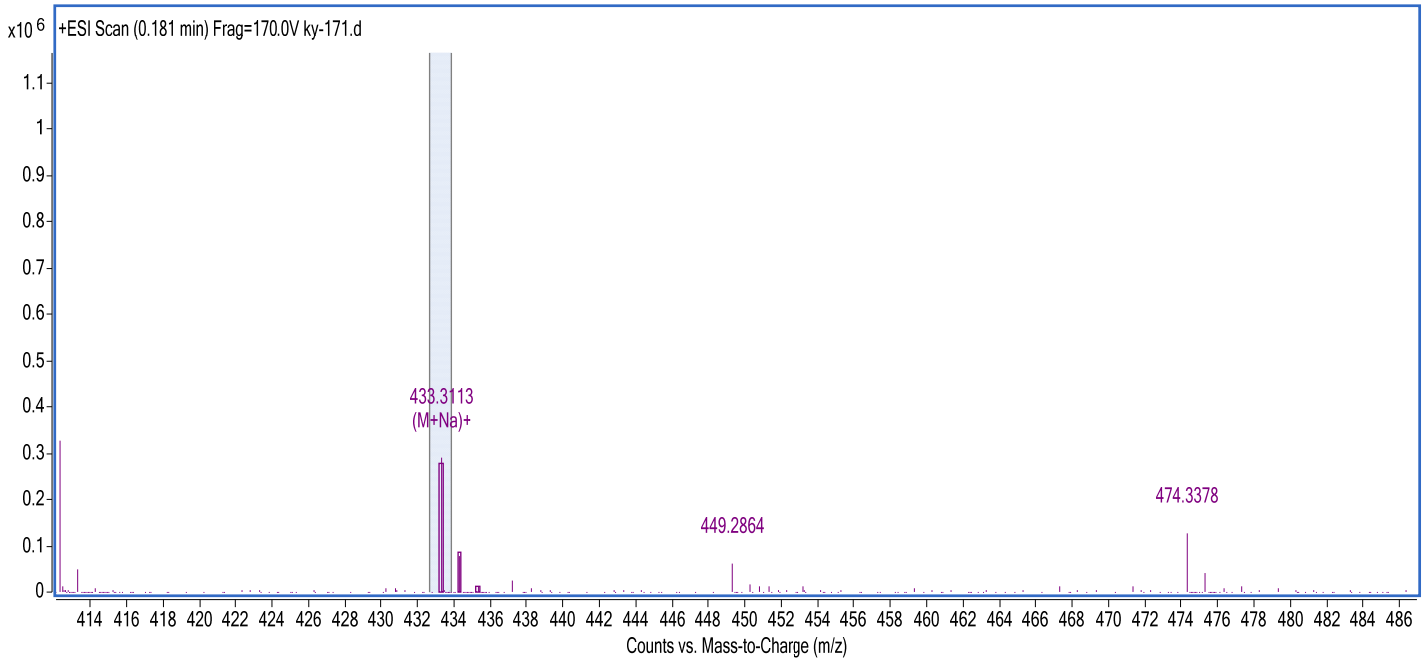


Figure S2 HRESIMS spectrum of **1**



m/z	Ion	Formula	Abundance
433.3113	(M+Na)+	C28 H42 Na O2	290709

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass
TRUE	C28 H42 O2	C28 H42 Na O2	433.3083	85.6		410.3221	410.3185

Figure S3 IR spectrum of **1**

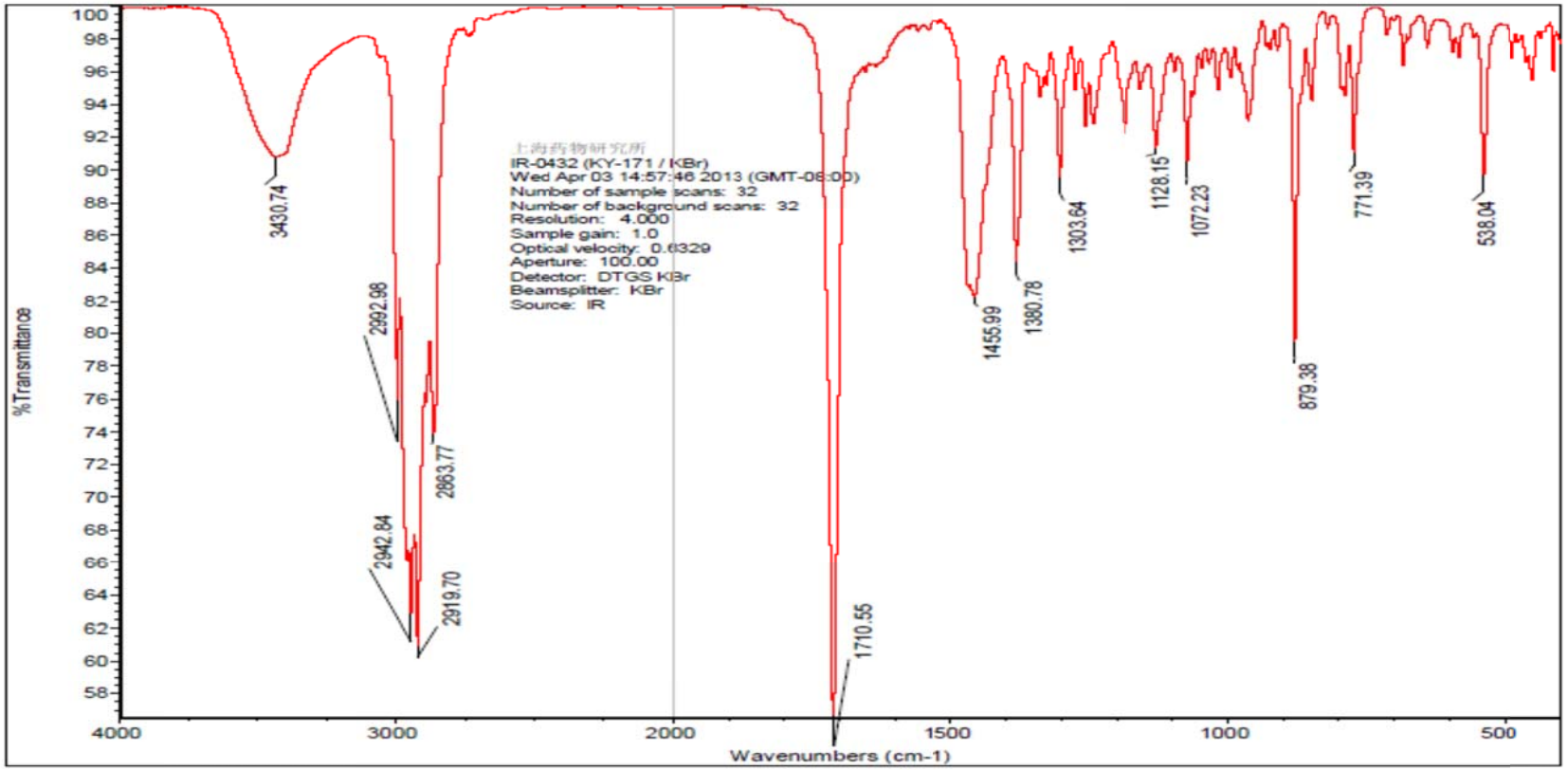


Figure S4 500 MHz ^{1}H NMR spectrum of **1** in $CDCl_3$

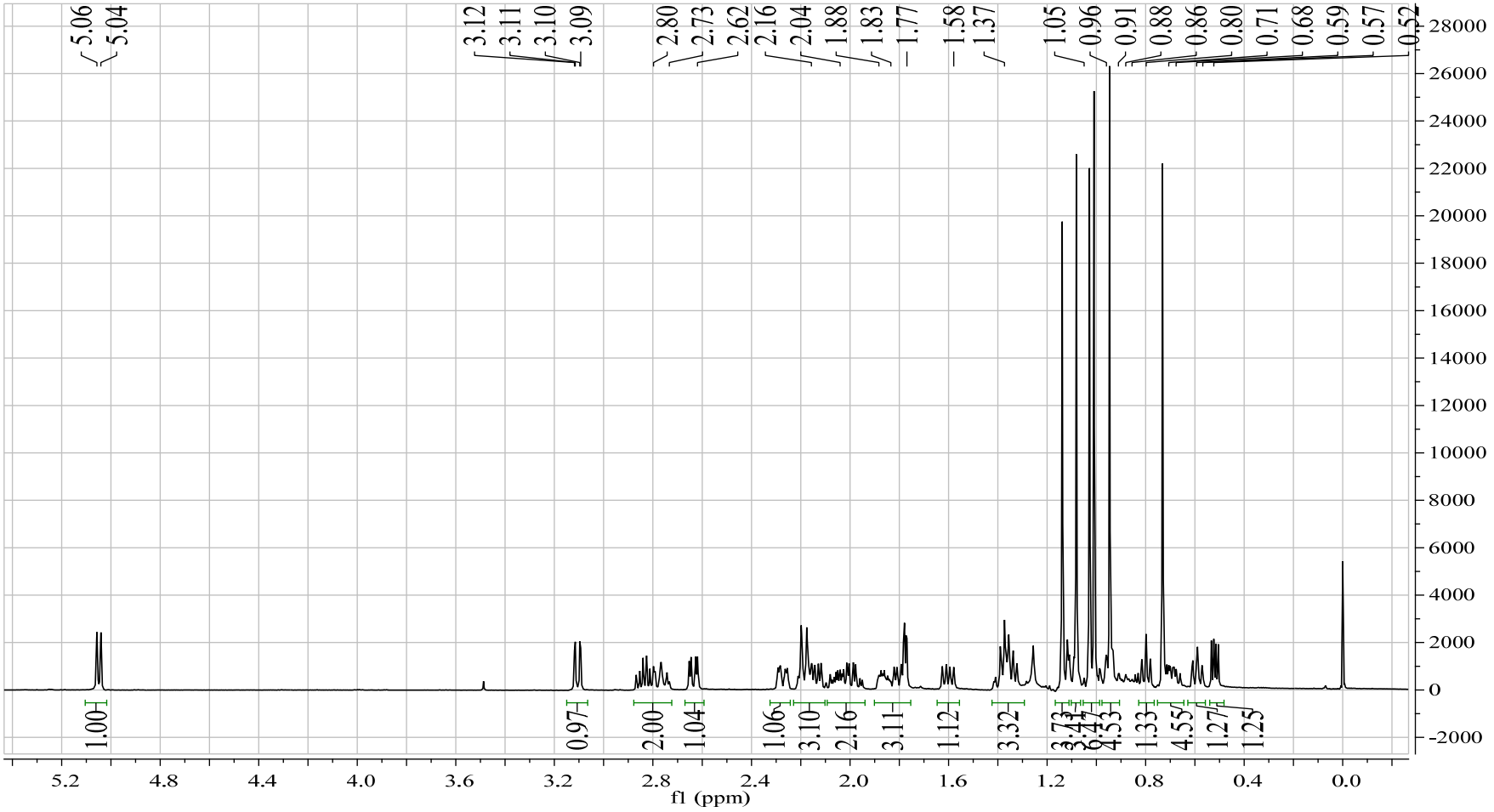


Figure S5 125 MHz ^{13}C NMR spectrum of **1** in $CDCl_3$

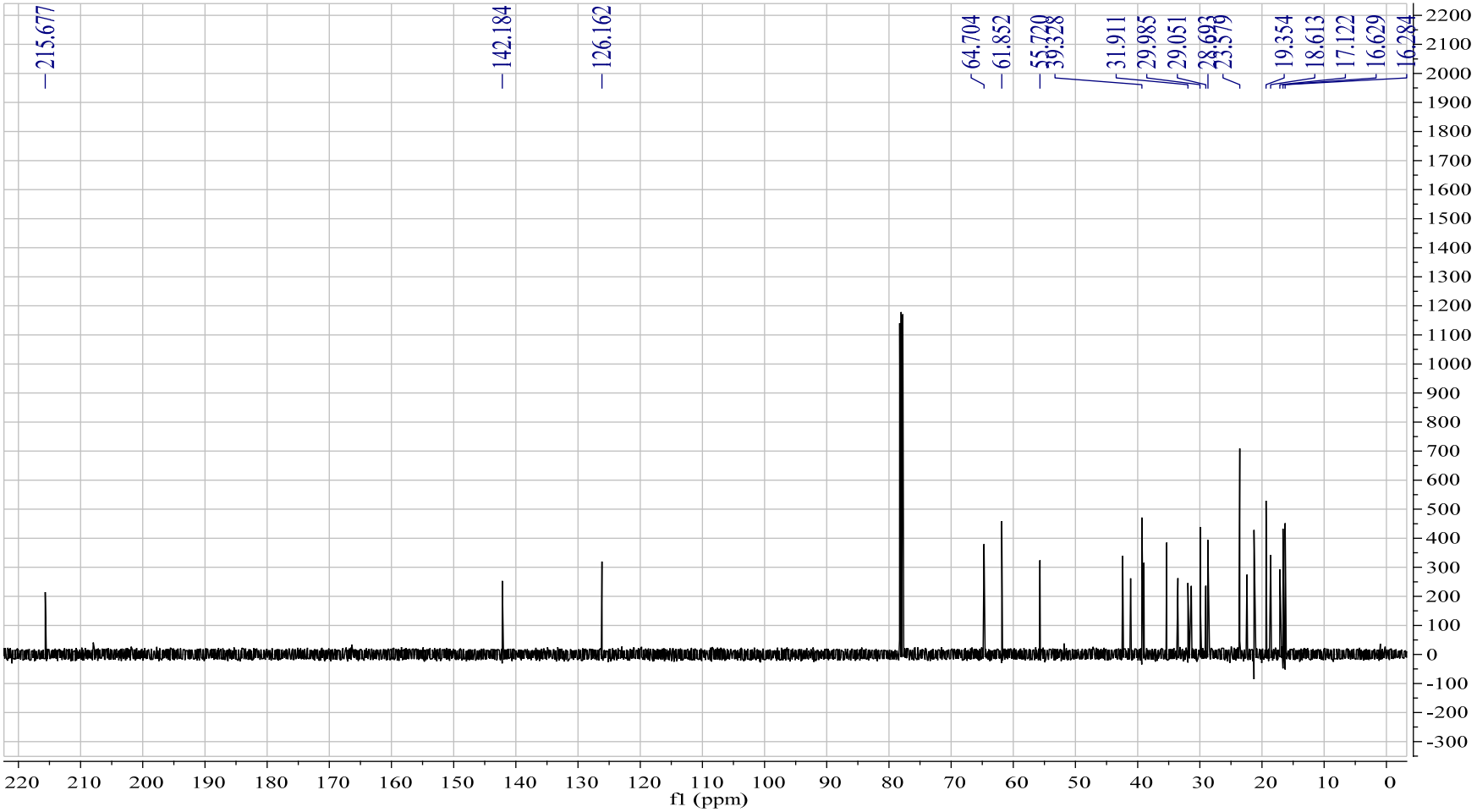


Figure S6 125 MHz DEPT NMR spectrum of **1** in $CDCl_3$

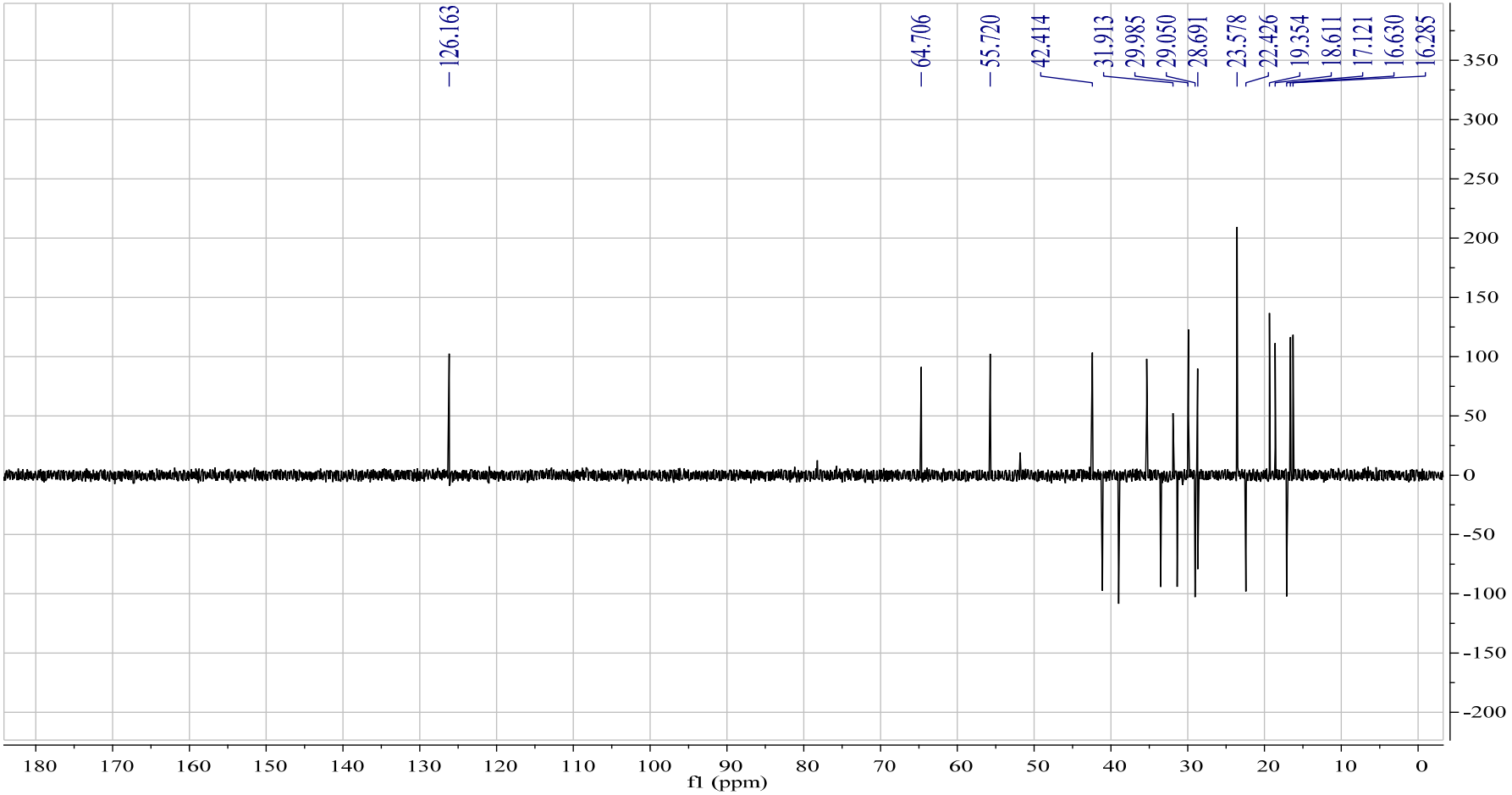


Figure S7 500 MHz ^{1}H-^{1}H COSY NMR spectrum of **1** in $CDCl_3$

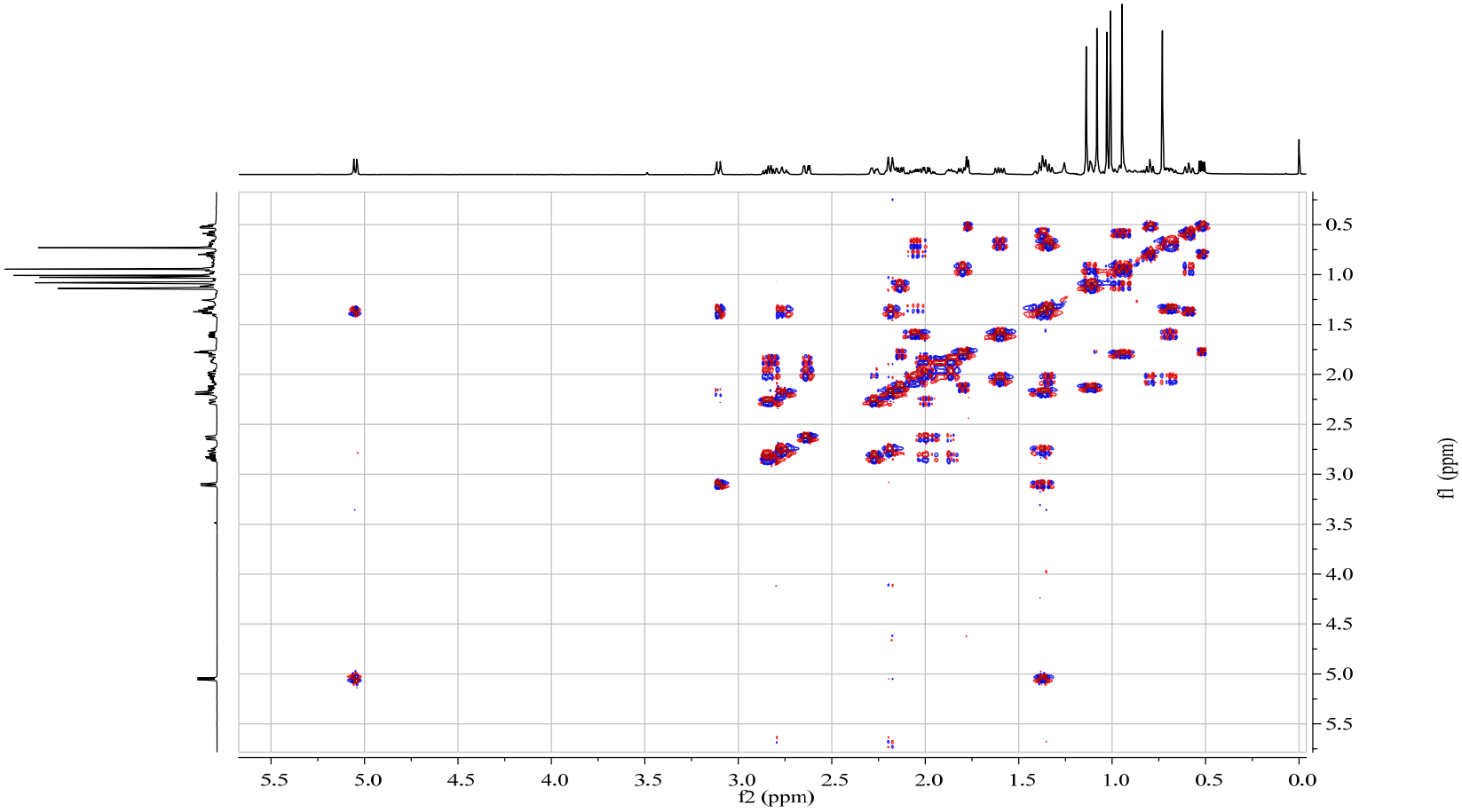


Figure S8 500 MHz HSQC NMR spectrum of **1** in $CDCl_3$

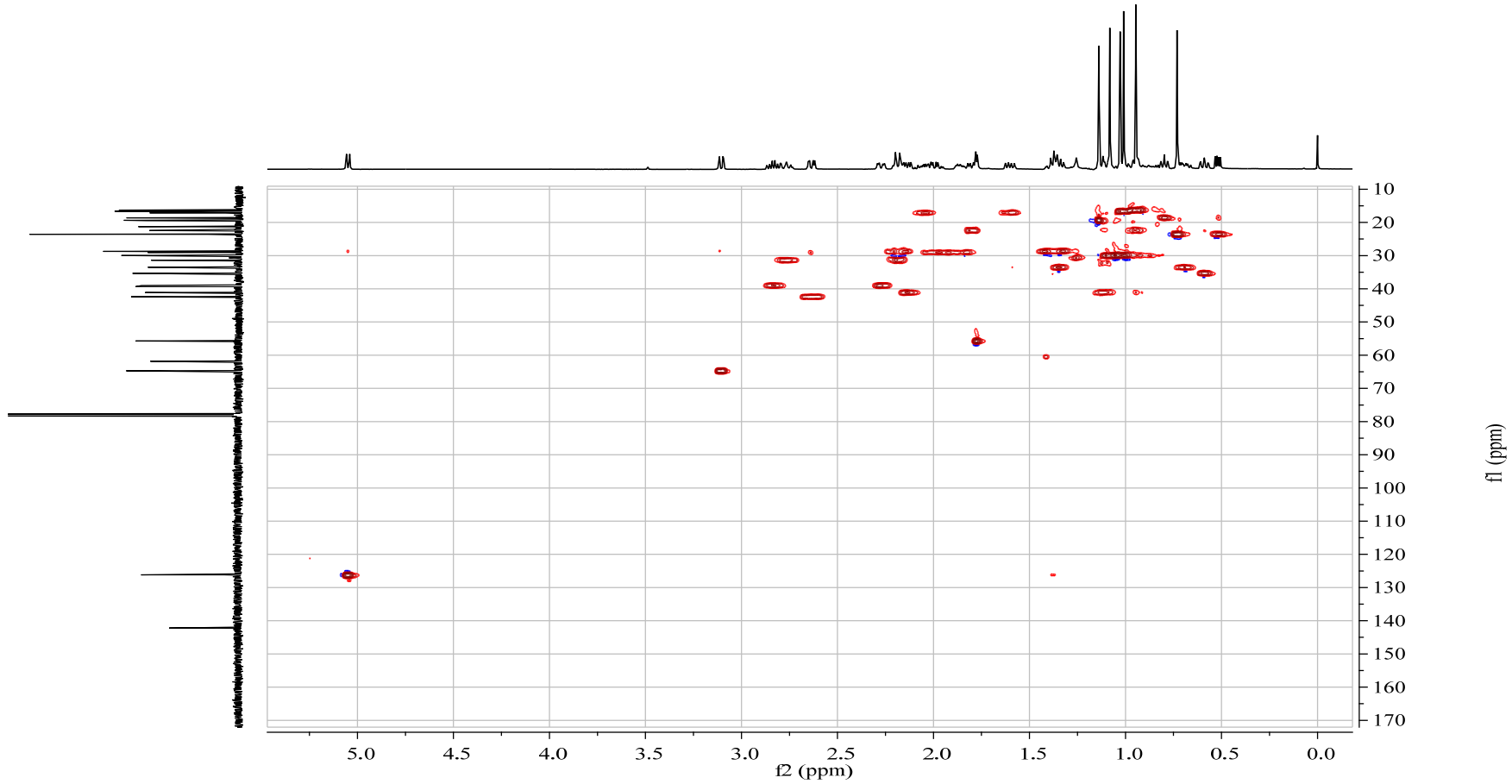


Figure S9 500 MHz HMBC NMR spectrum of **1** in $CDCl_3$

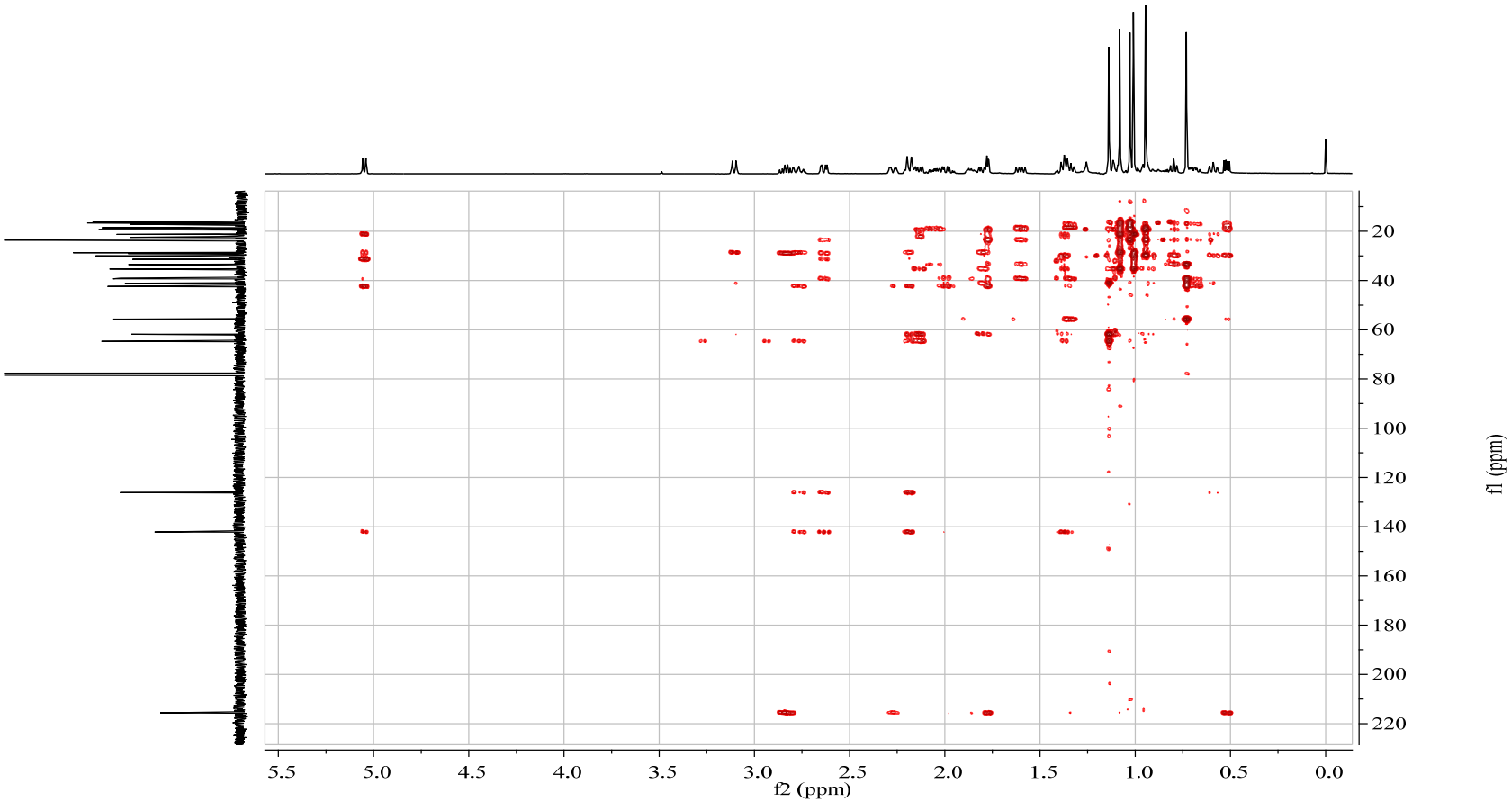


Figure S10 500 MHz NOESY NMR spectrum of **1** in $CDCl_3$

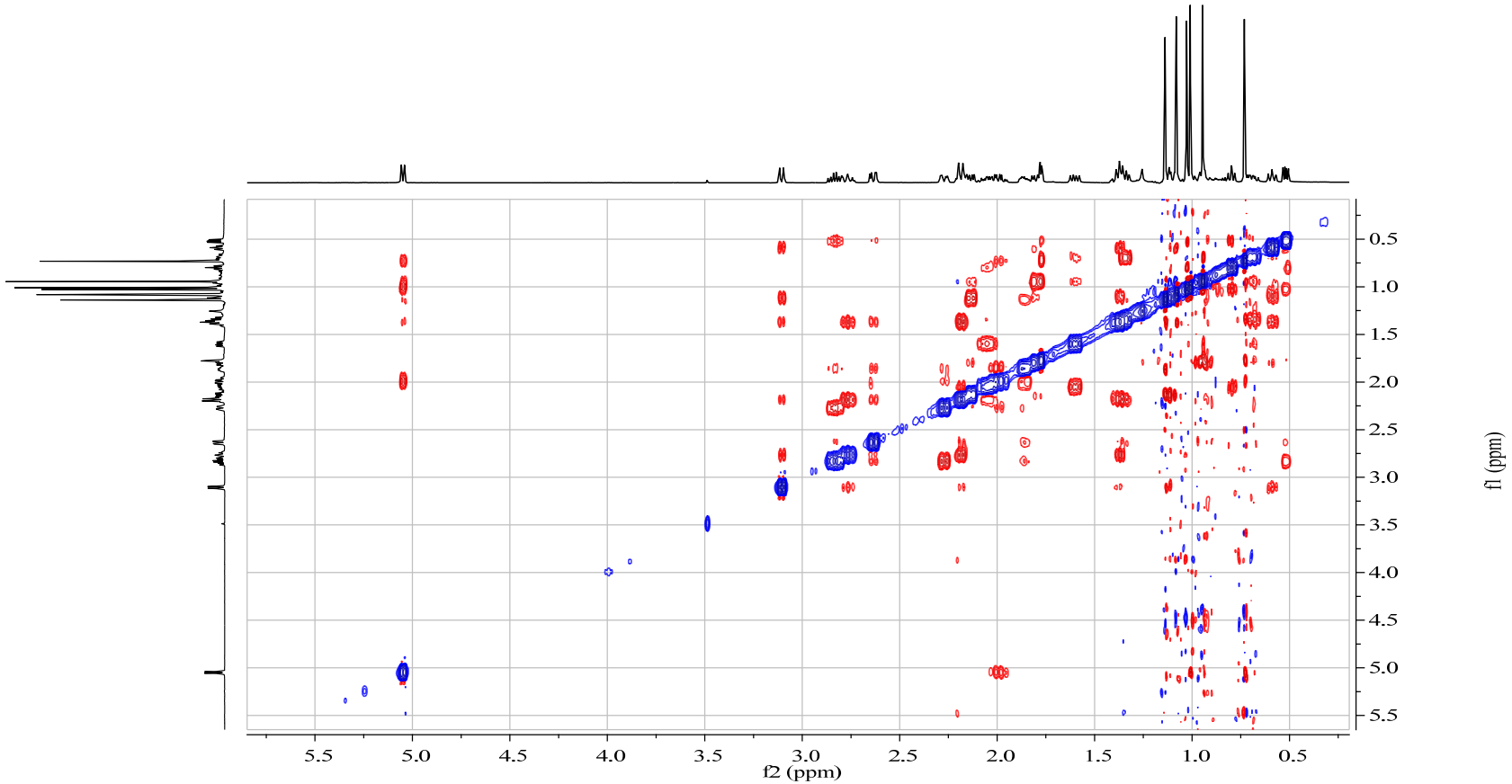


Figure S11 Single X-ray crystal structure and Packing diagram of **1**

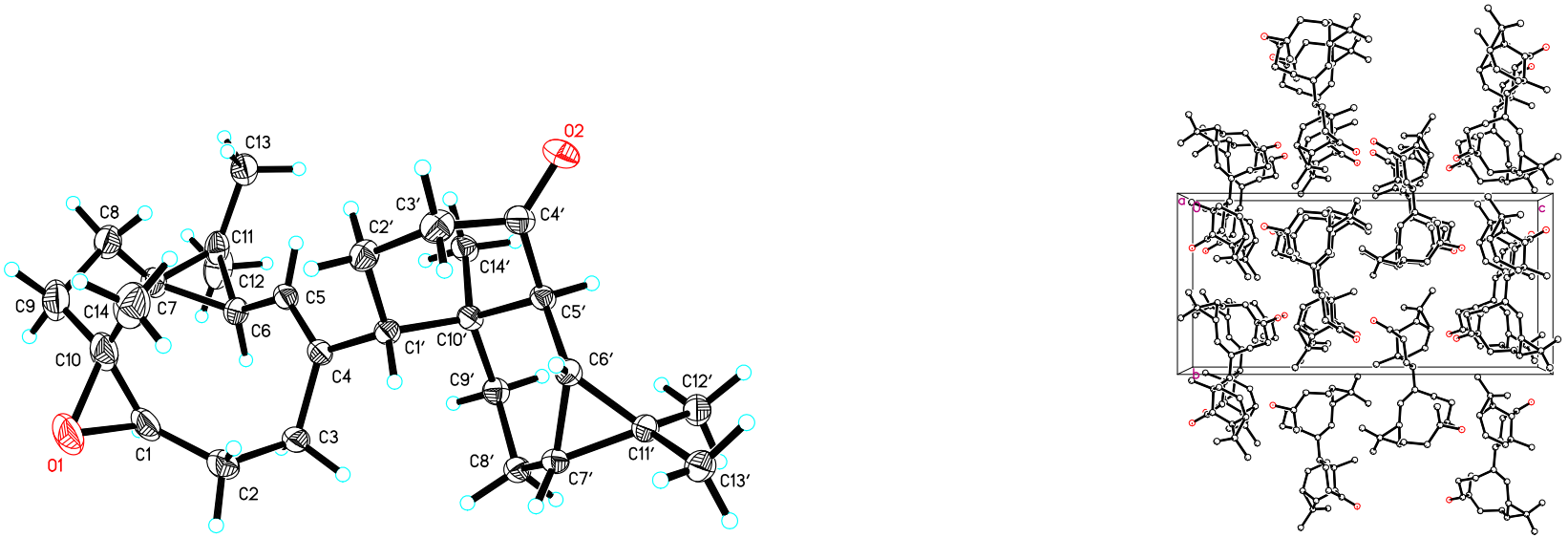
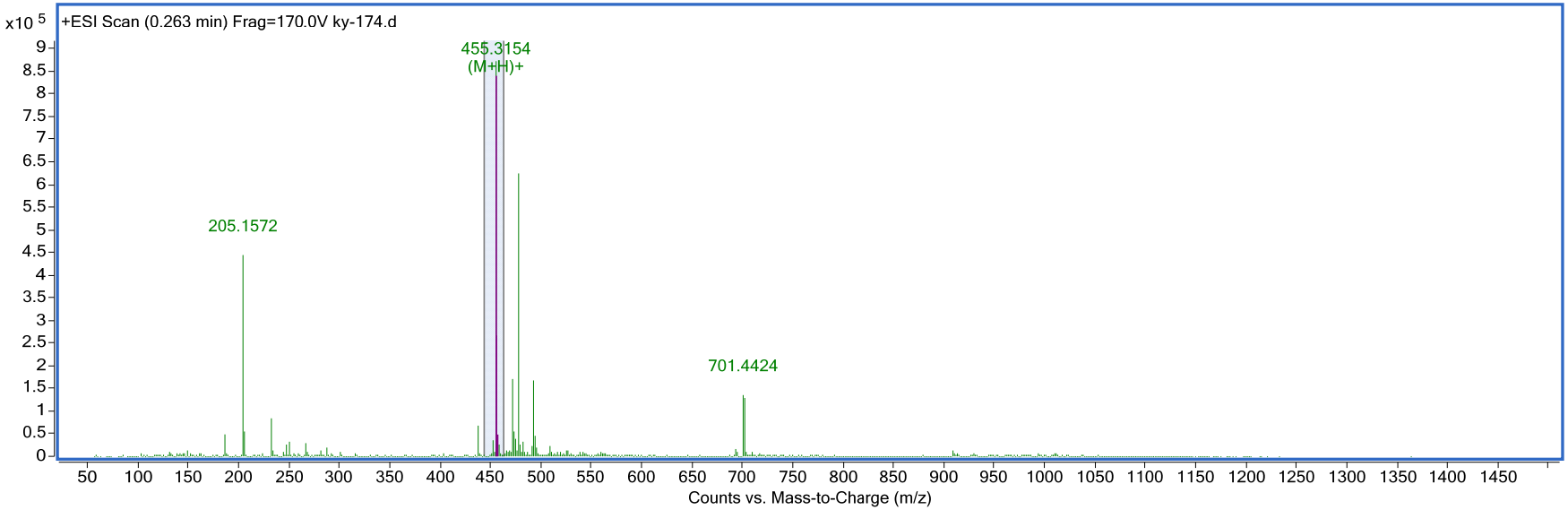


Figure S12 HRESIMS spectrum of **2**



m/z	Ion	Formula	Abundance
455.3154	(M+H)+	C29 H43 O4	873452.3

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Match	Mass Match	m/z
TRUE	C29 H42 O4	C29 H43 O4	455.3156	97.39		454.3081	454.3083	0.5	0.5	91.6	99.63	99.75	455.3154

Figure S13 IR spectrum of **2**

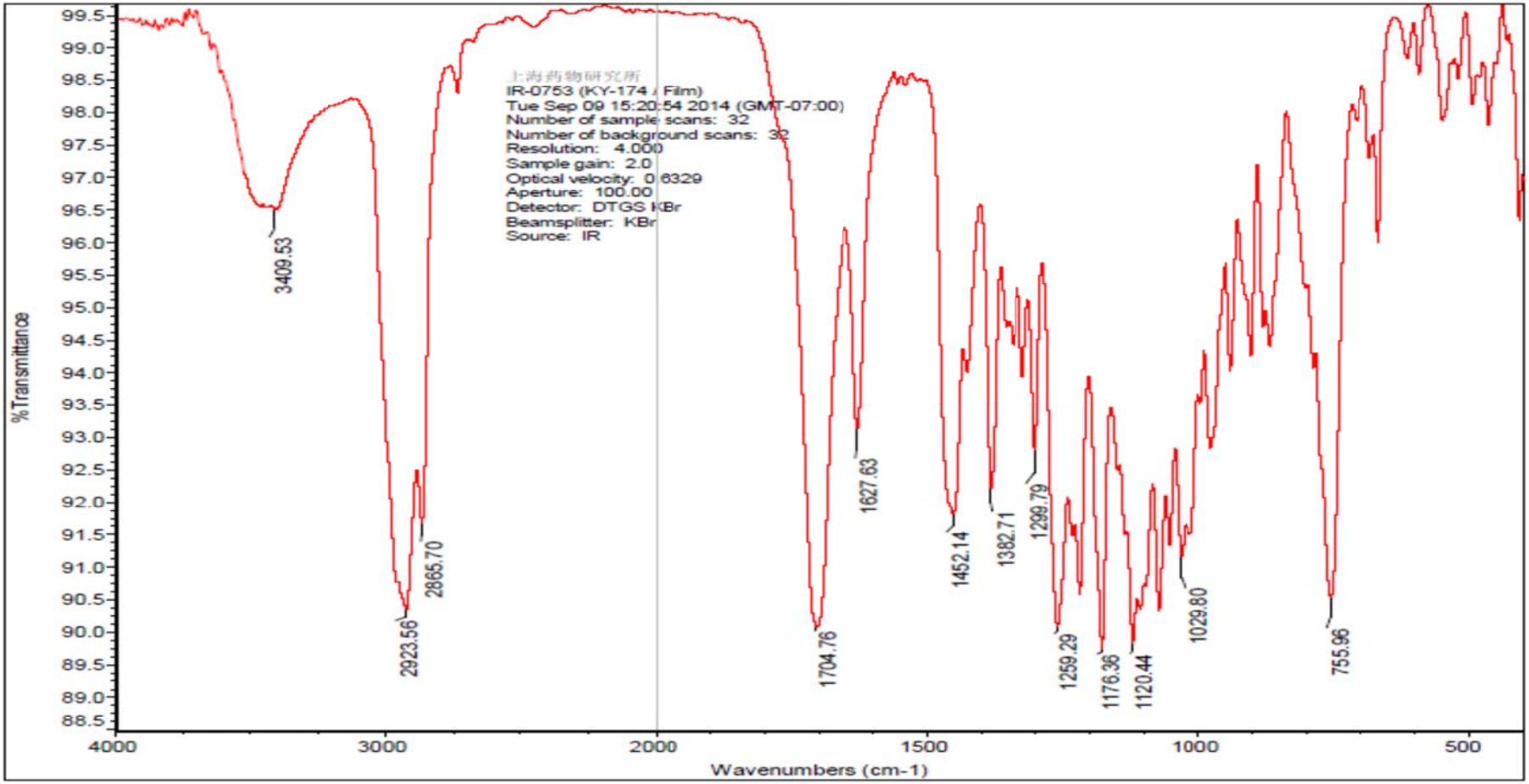


Figure S14 500 MHz ^{1}H NMR spectrum of **2** in $CDCl_3$

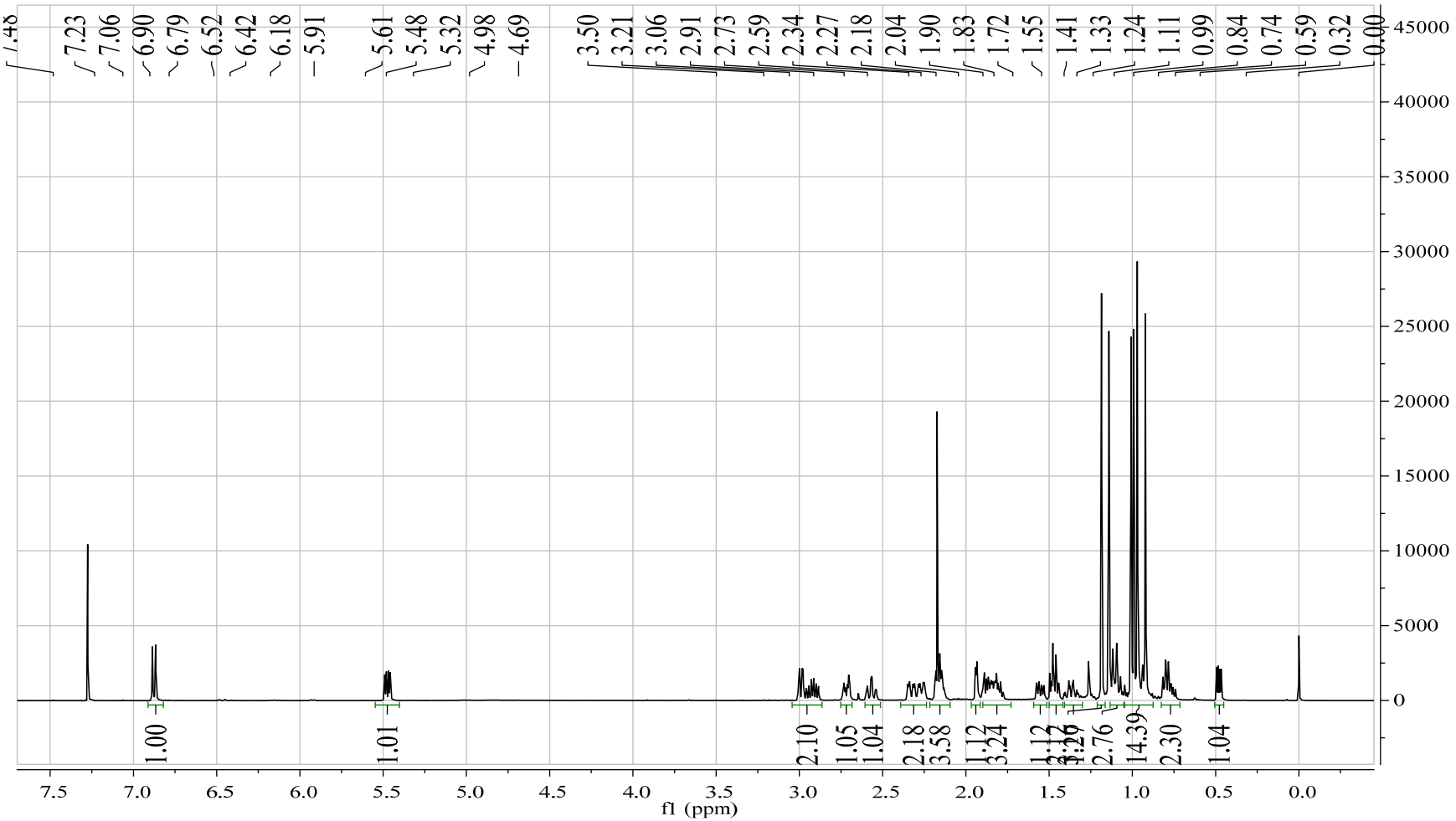


Figure S15 125 MHz ^{13}C NMR spectrum of **2** in $CDCl_3$

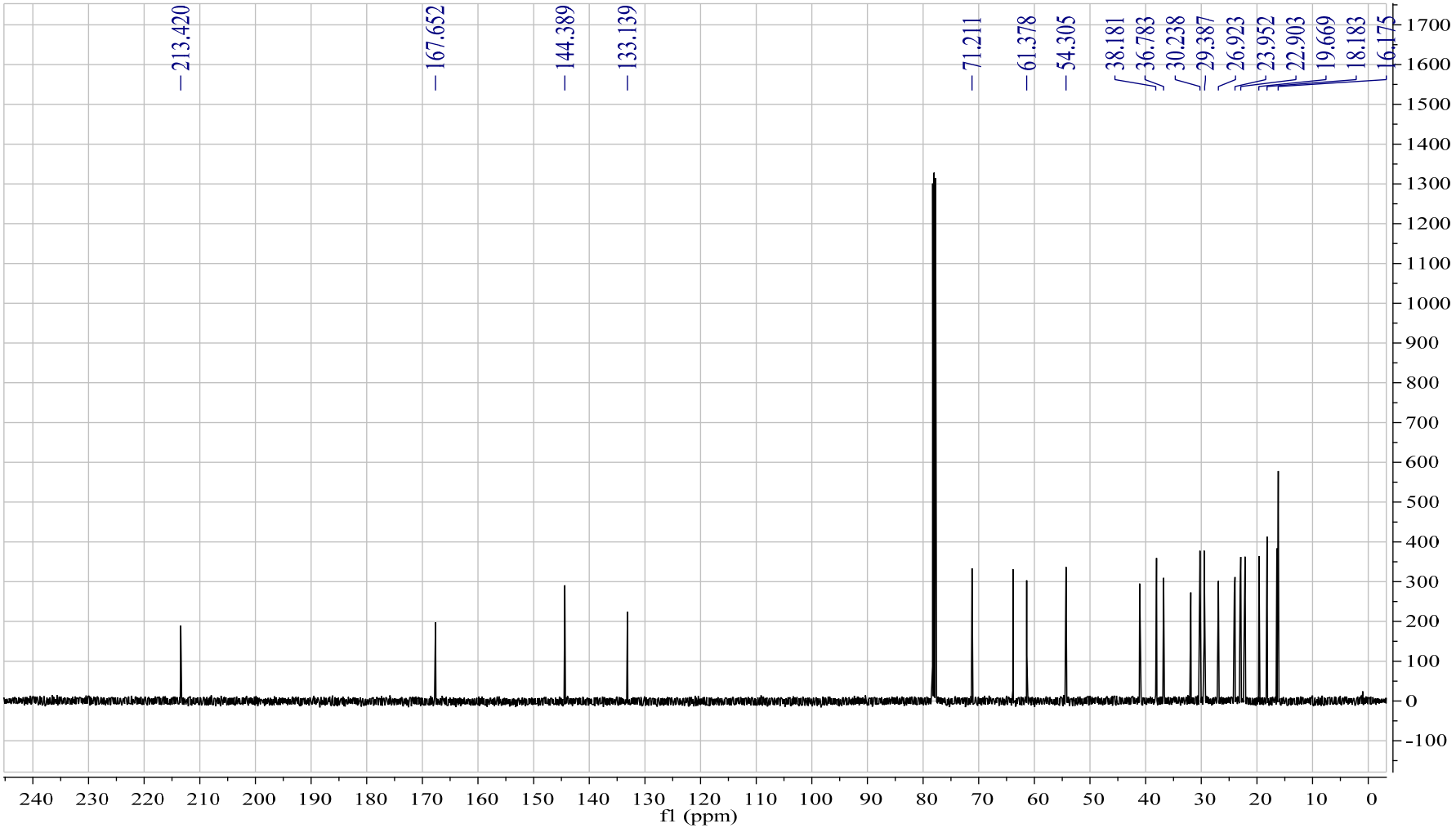


Figure S16 125 MHz DEPT NMR spectrum of **2** in $CDCl_3$

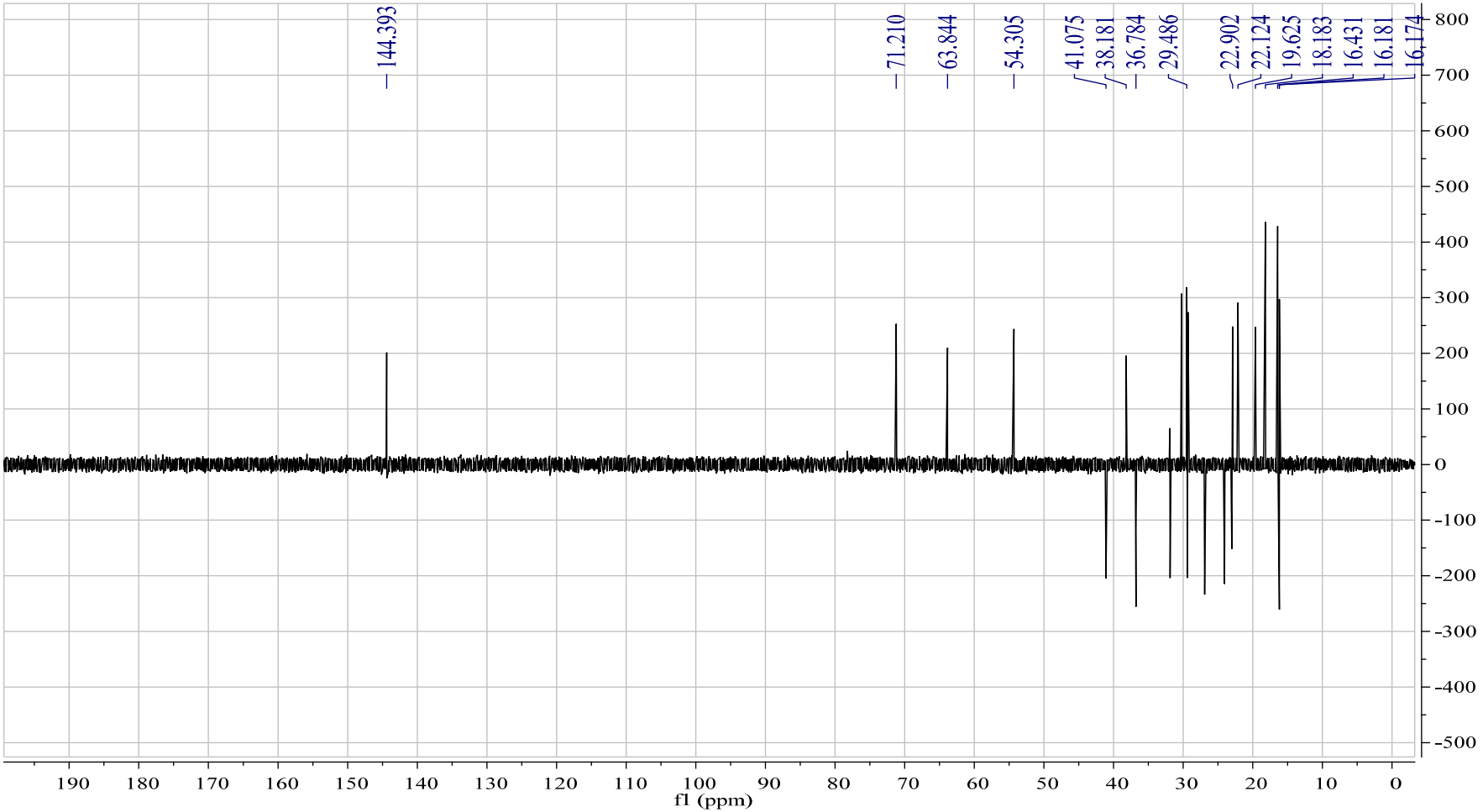


Figure S17 500 MHz ^{1}H-^{1}H COSY NMR spectrum of **2** in $CDCl_3$

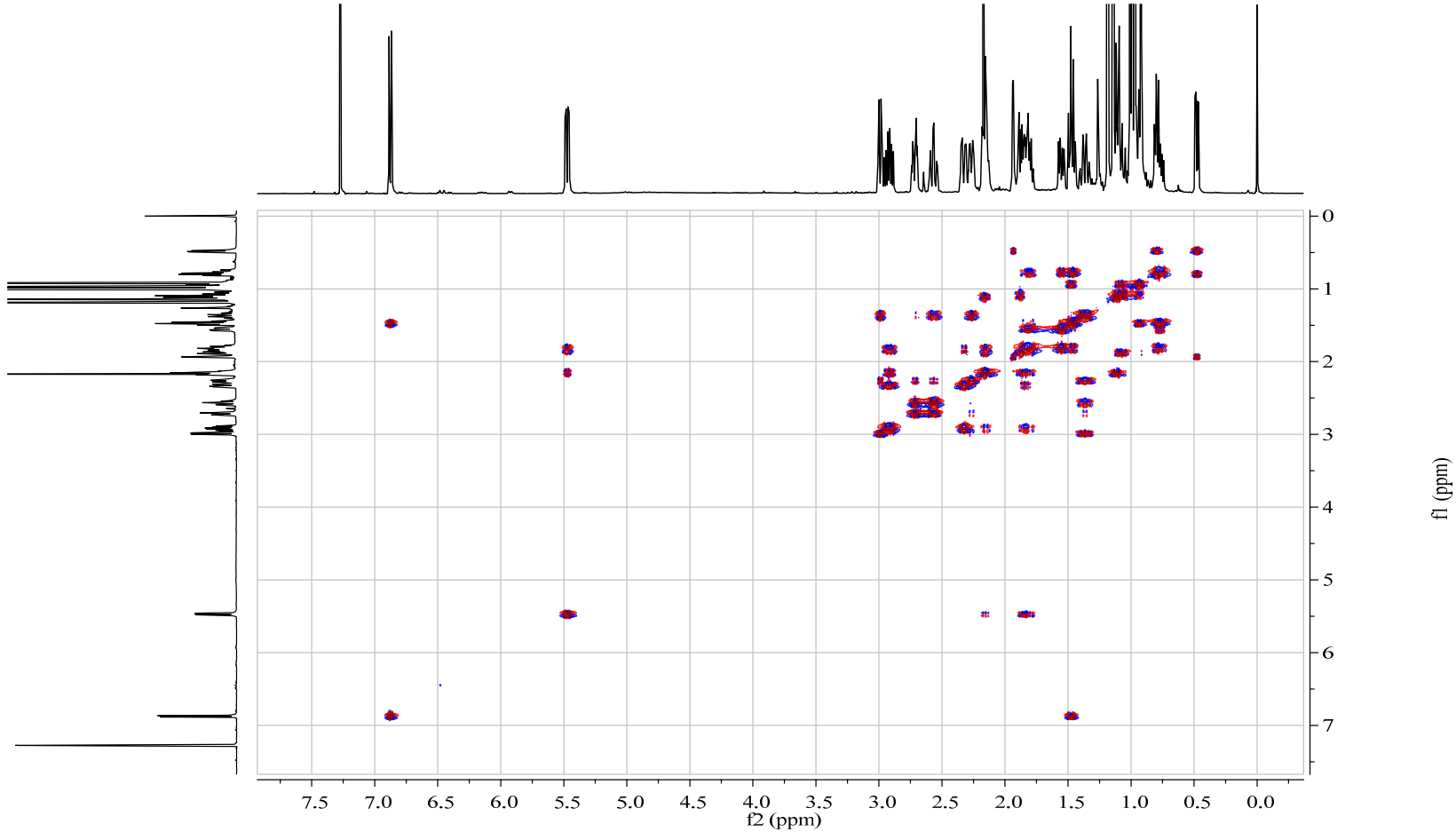


Figure S18 500 MHz HSQC NMR spectrum of **2** in $CDCl_3$

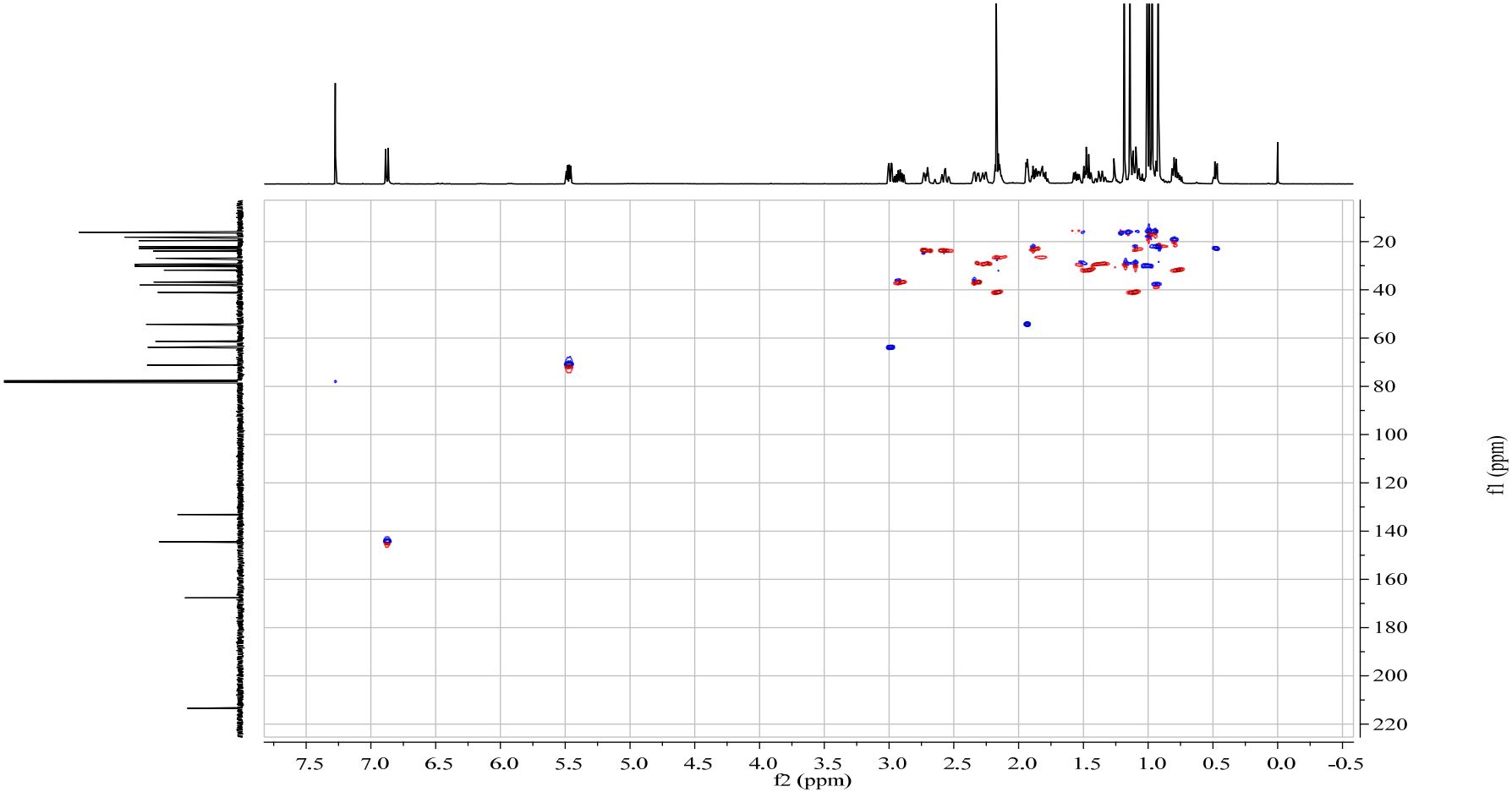


Figure S19 500 MHz HMBC NMR spectrum of **2** in $CDCl_3$

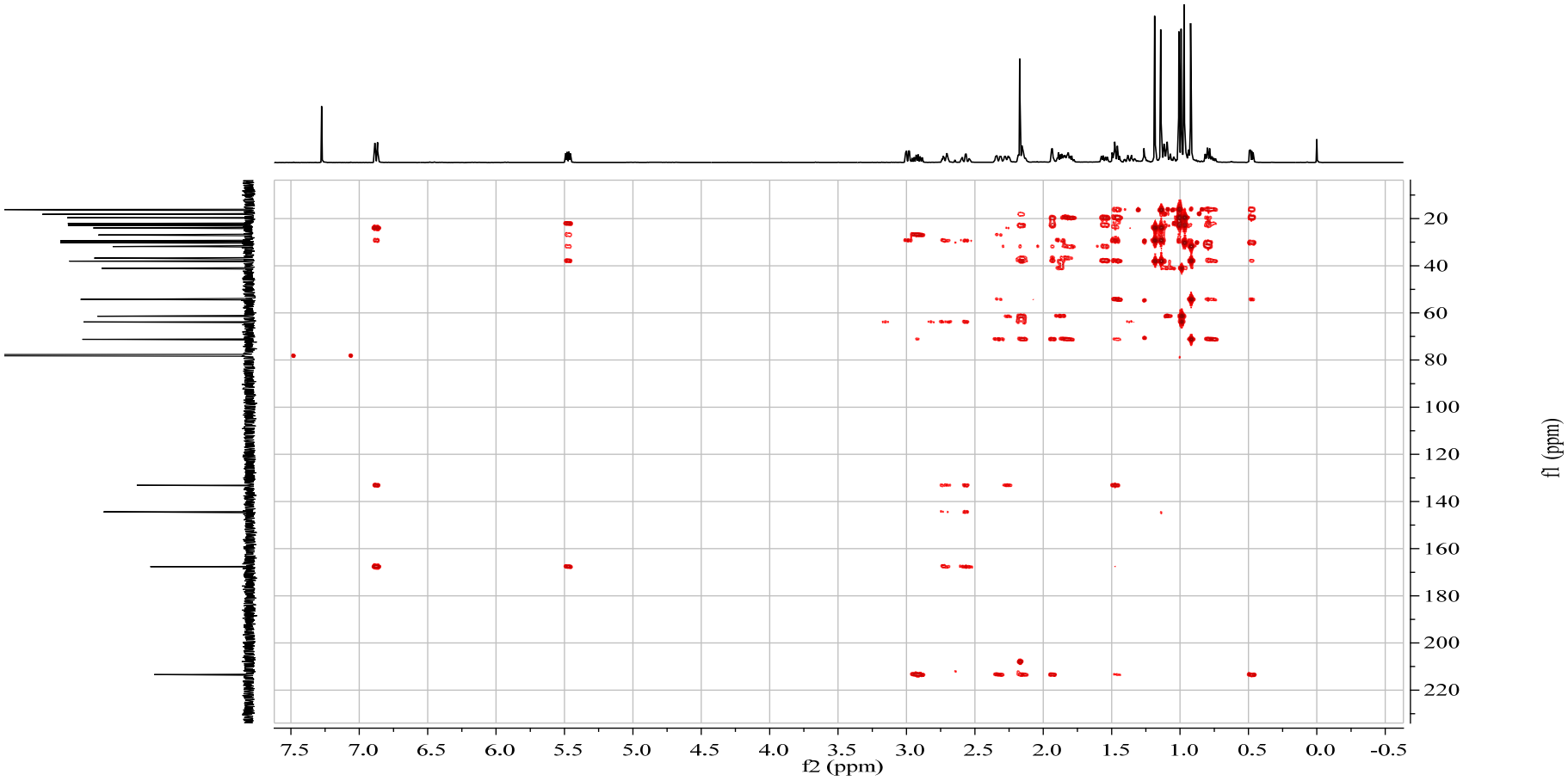


Figure S20 500 MHz NOESY spectrum of **2** in $CDCl_3$

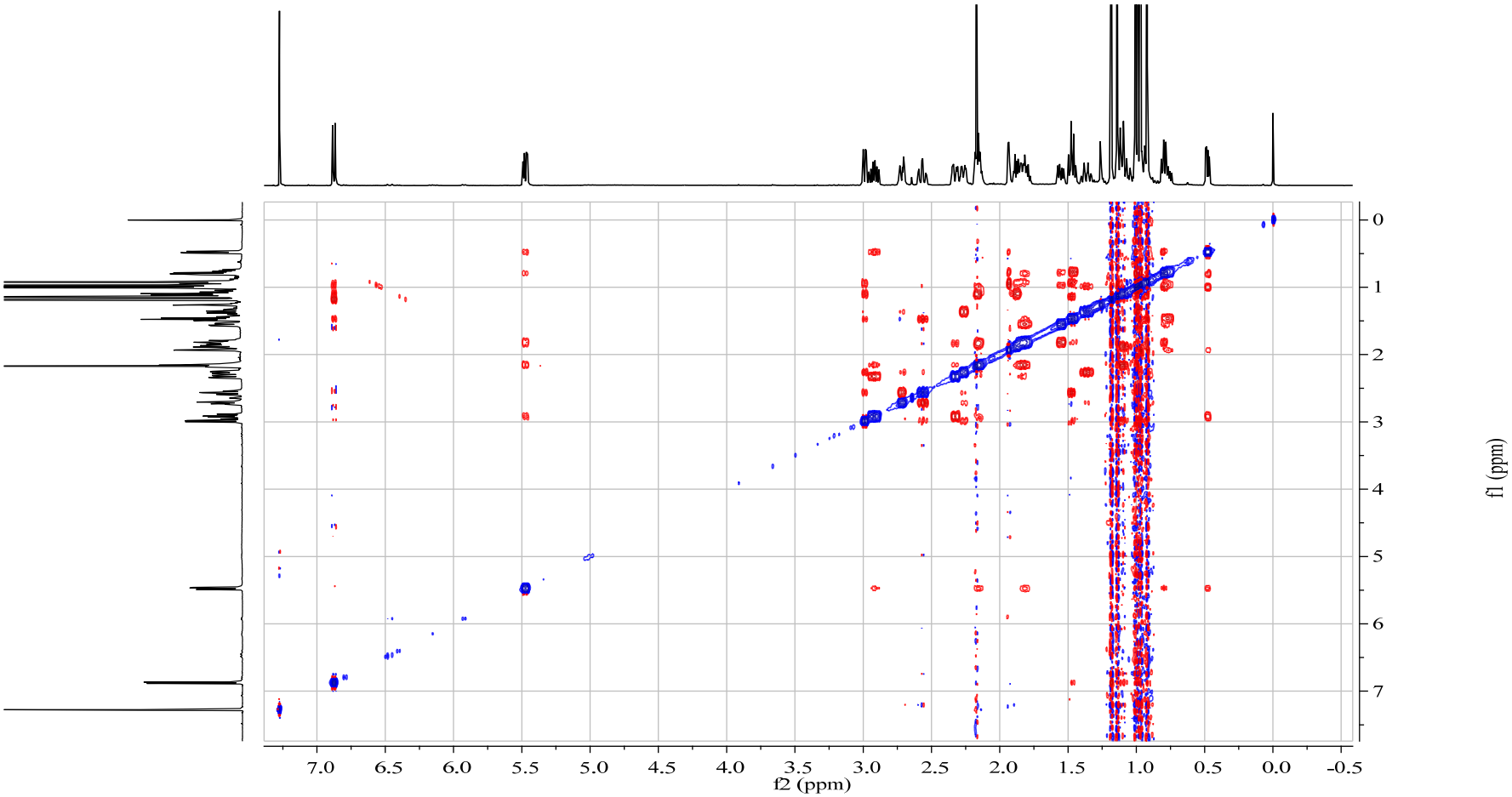
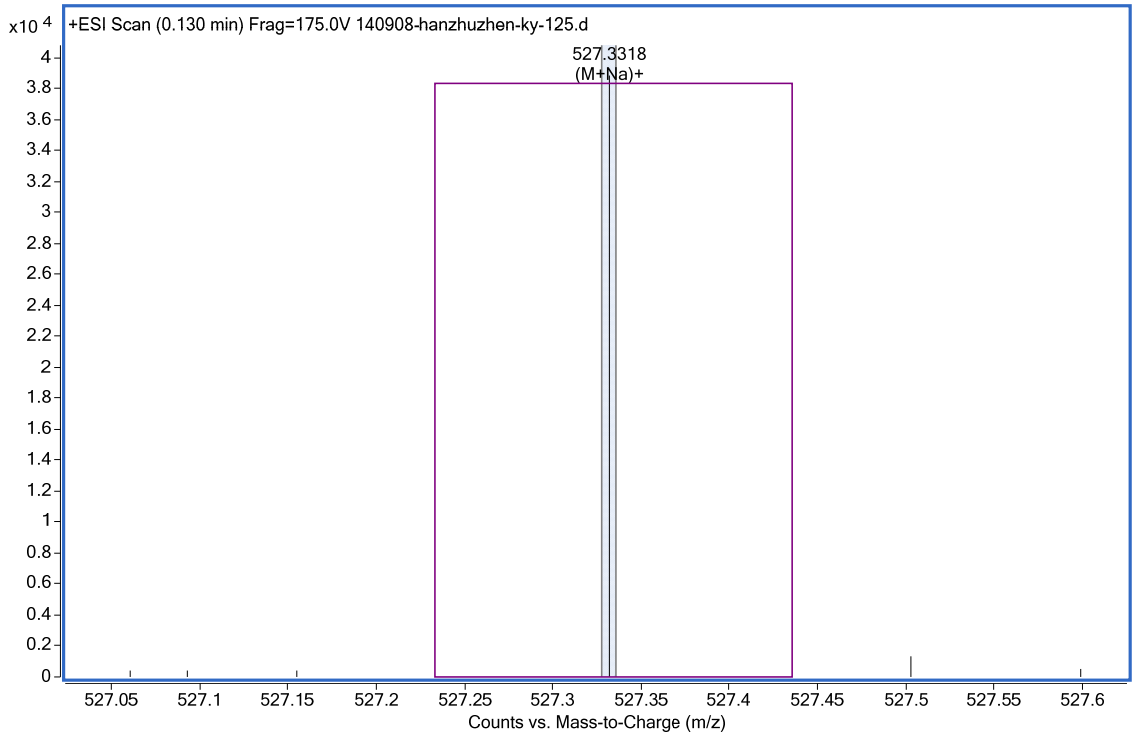


Figure S22 HRESIMS spectrum of **3**



527.3318		(M+Na)+		C30 H48 Na O6				
	Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass
	TRUE	C30 H48 O6	C30 H48 Na O6	527.3343	80.22		504.3426	504.3451

Figure S22 IR spectrum of **3**

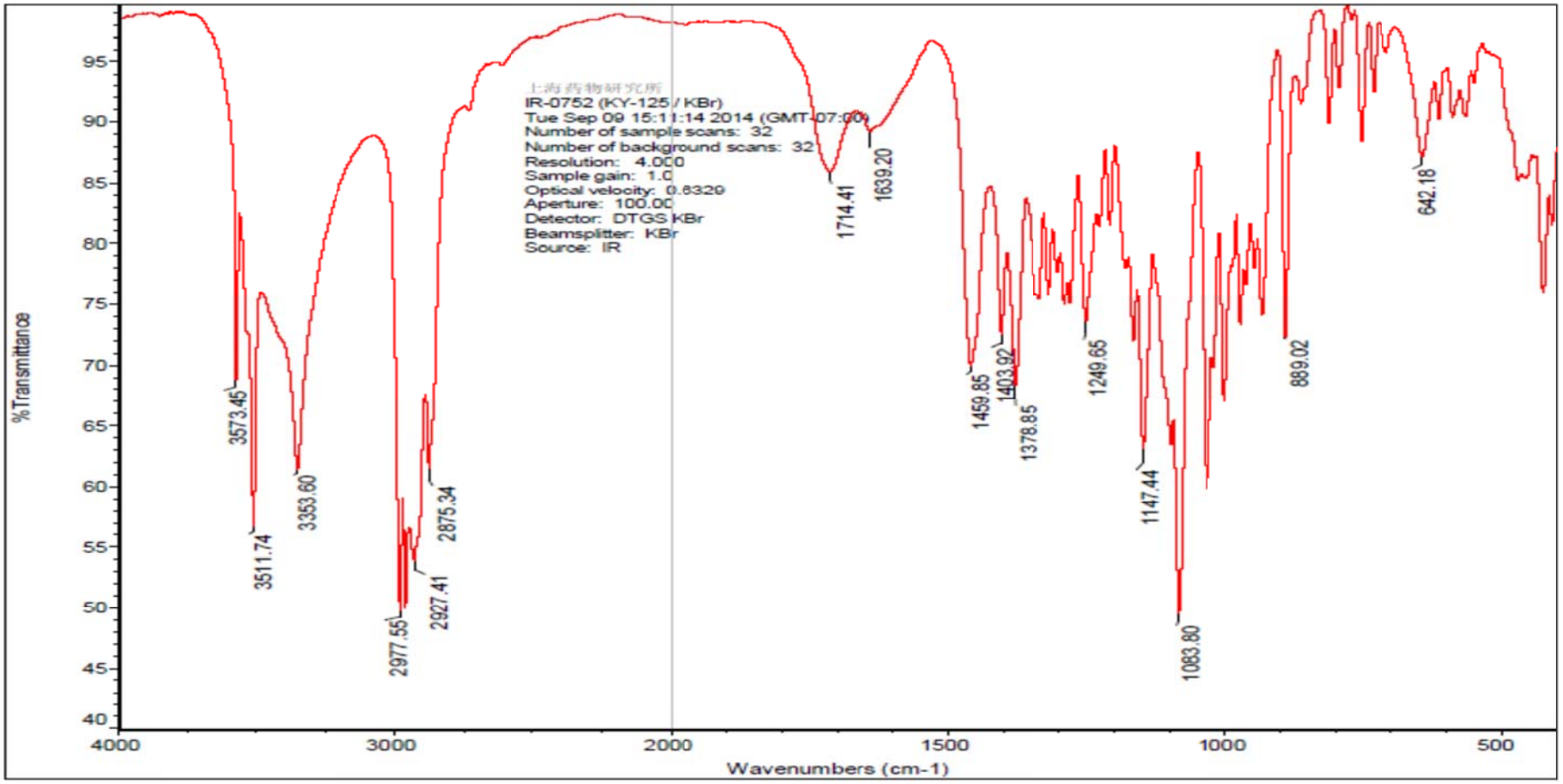


Figure S23 500 MHz ^{1}H NMR spectrum of **3** in CD_3OD

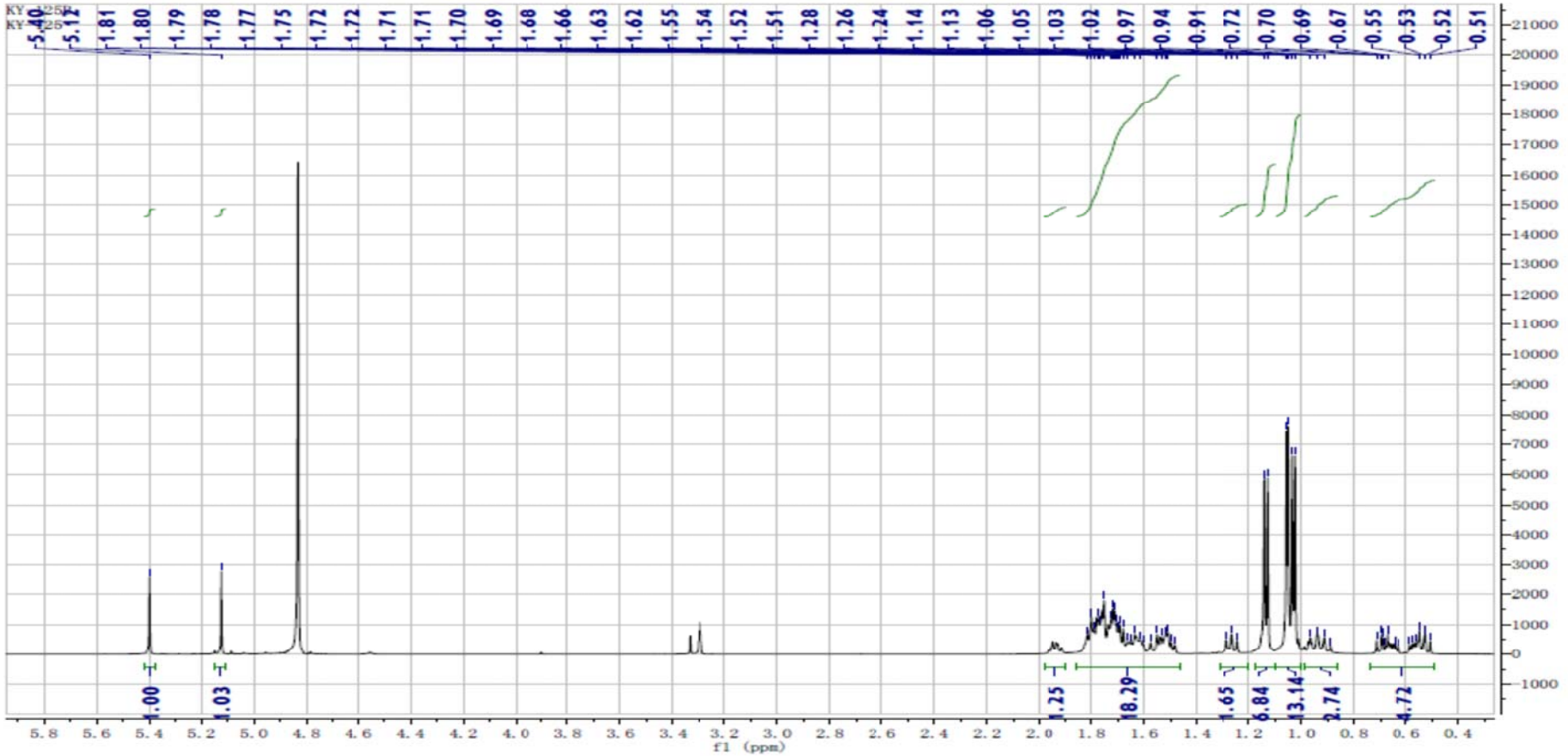


Figure S24 125 MHz ^{13}C NMR spectrum of **3** in CD_3OD

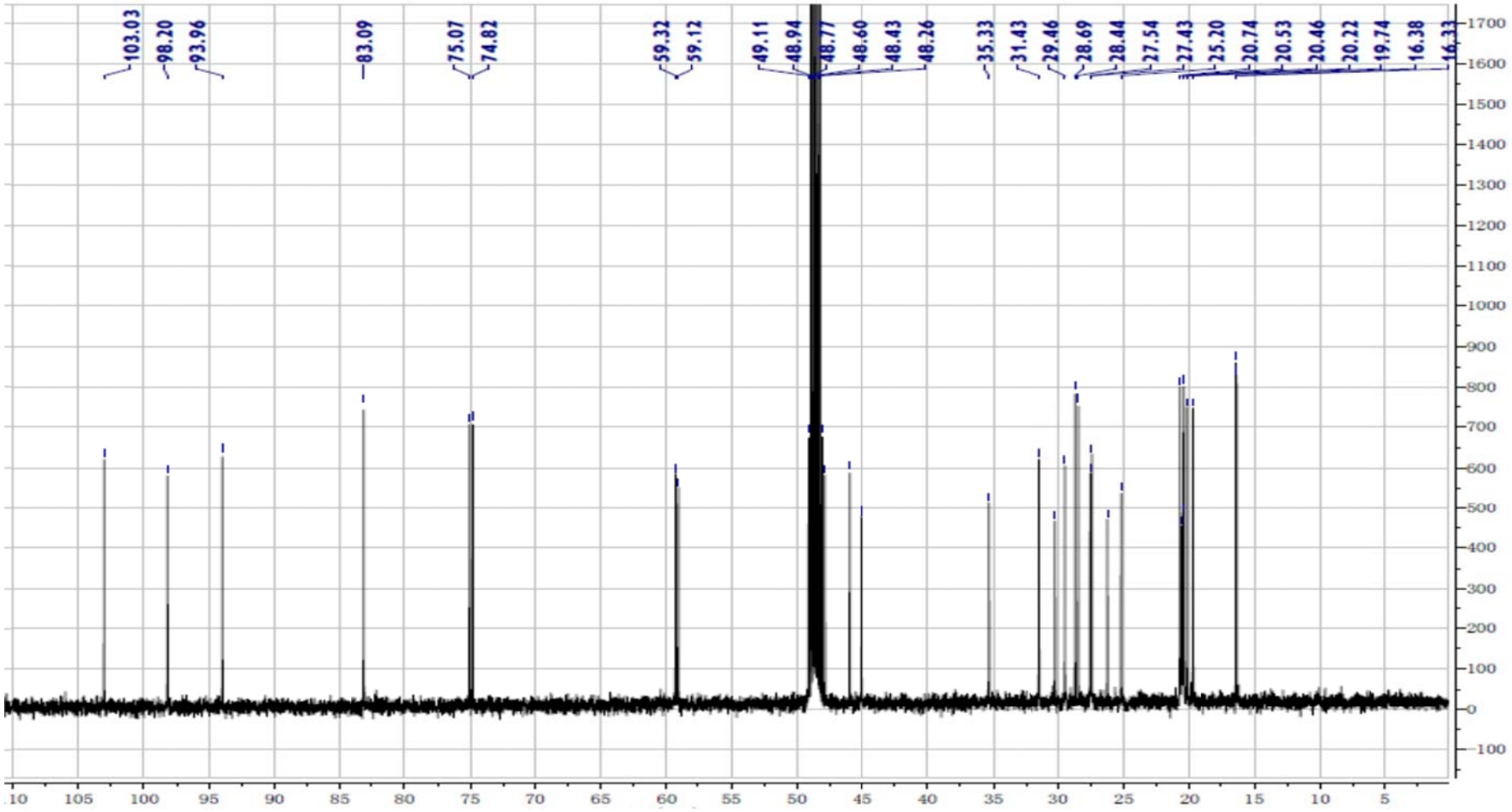


Figure S25 125 MHz DEPT spectrum of **3** in CD_3OD

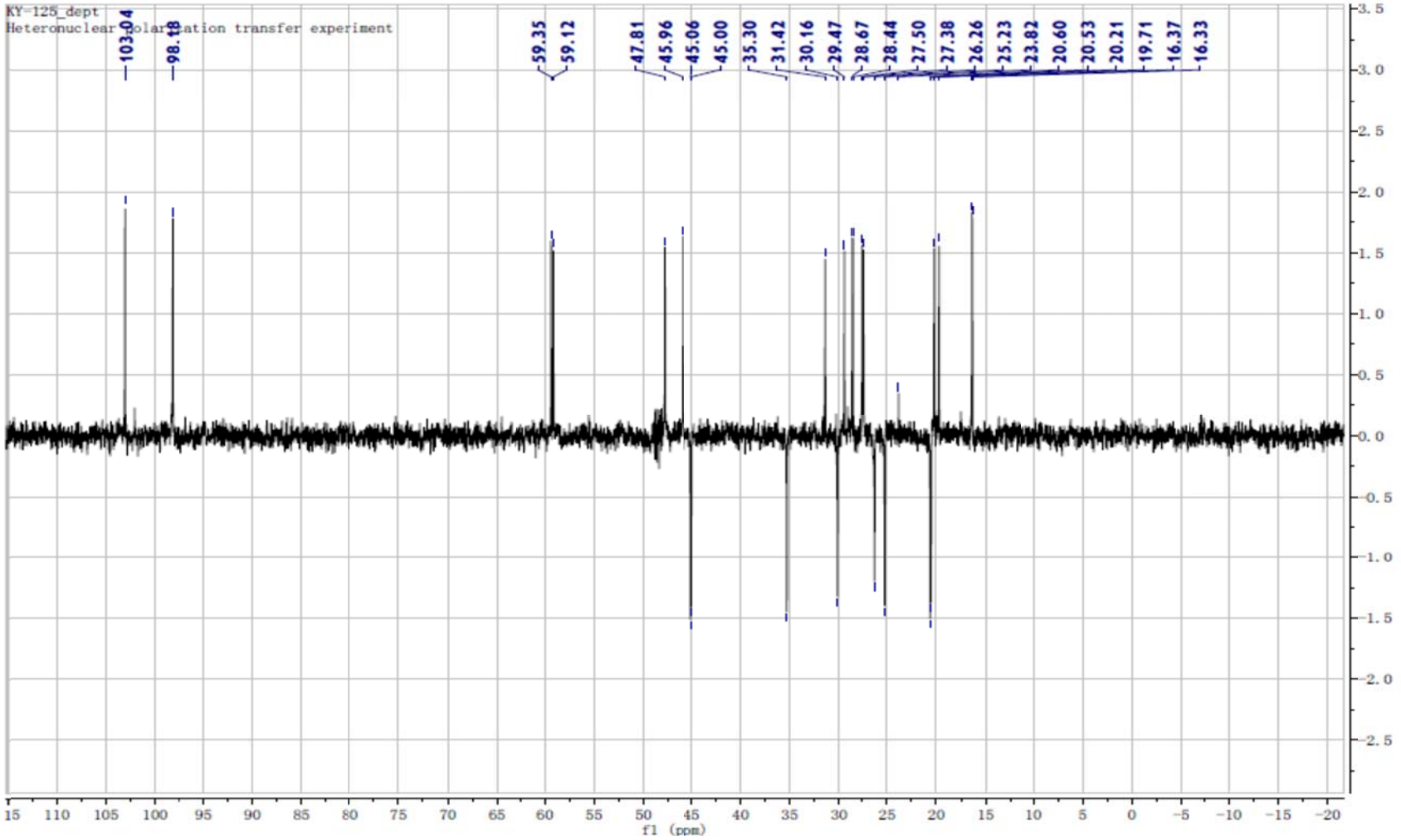


Figure S26 500 MHz ^{1}H-^{1}H COSY NMR spectrum of **3** in CD_3OD

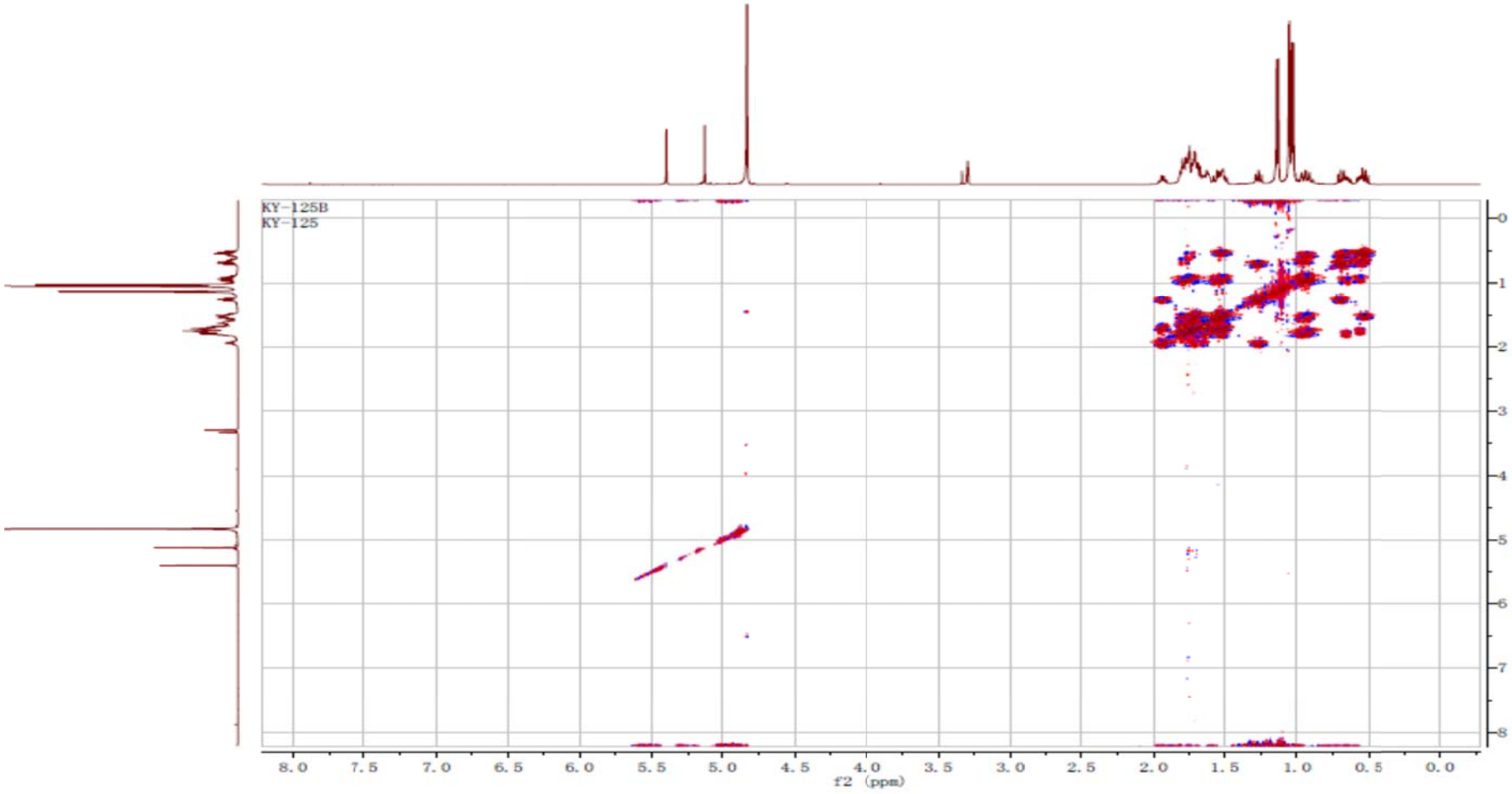


Figure S27 500 MHz HSQC NMR spectrum of **3** in CD_3OD

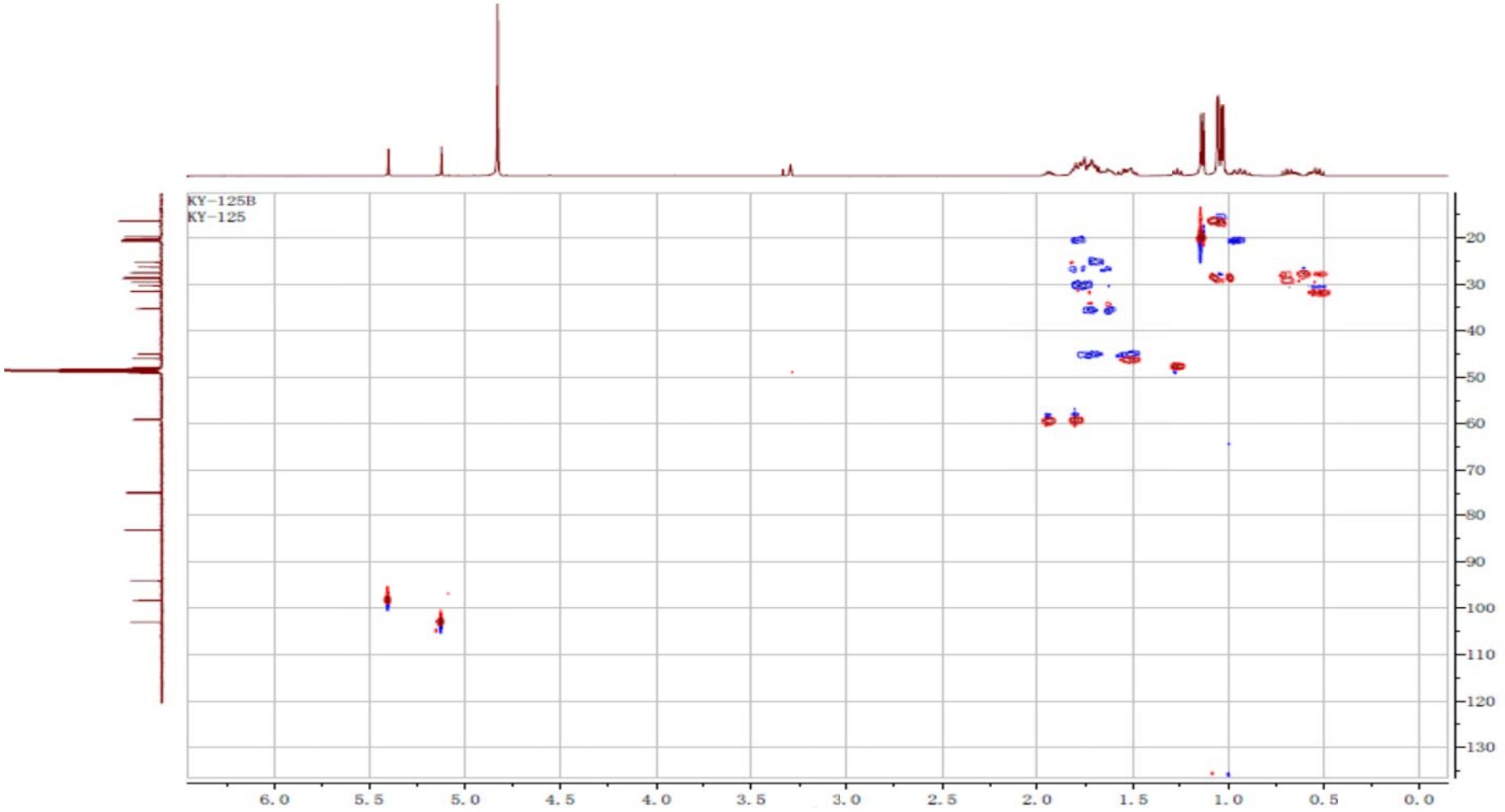


Figure S28 500 MHz HMBC NMR spectrum of **3** in CD_3OD

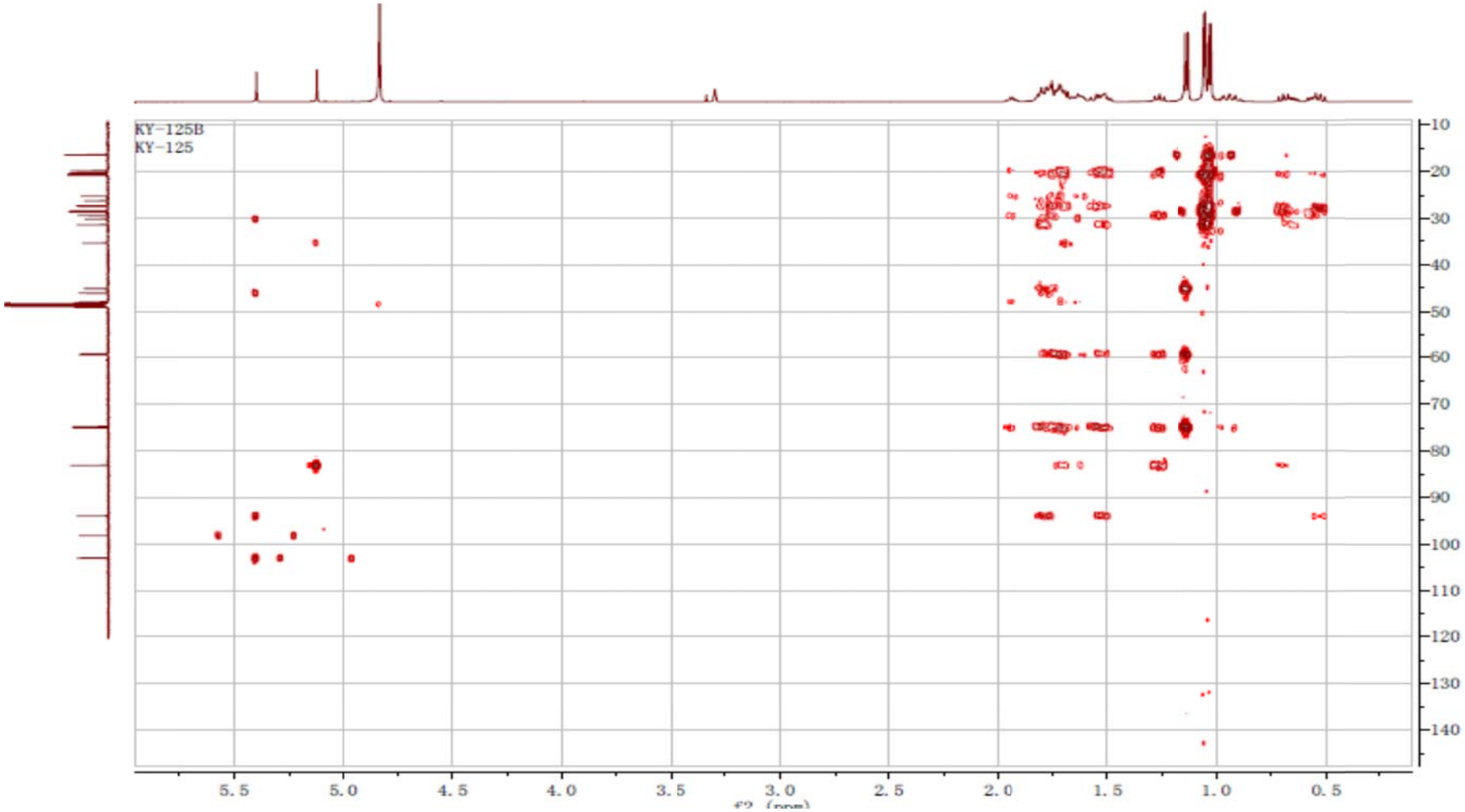


Figure S29 500 MHz NOESY NMR spectrum of **3** in CD_3OD

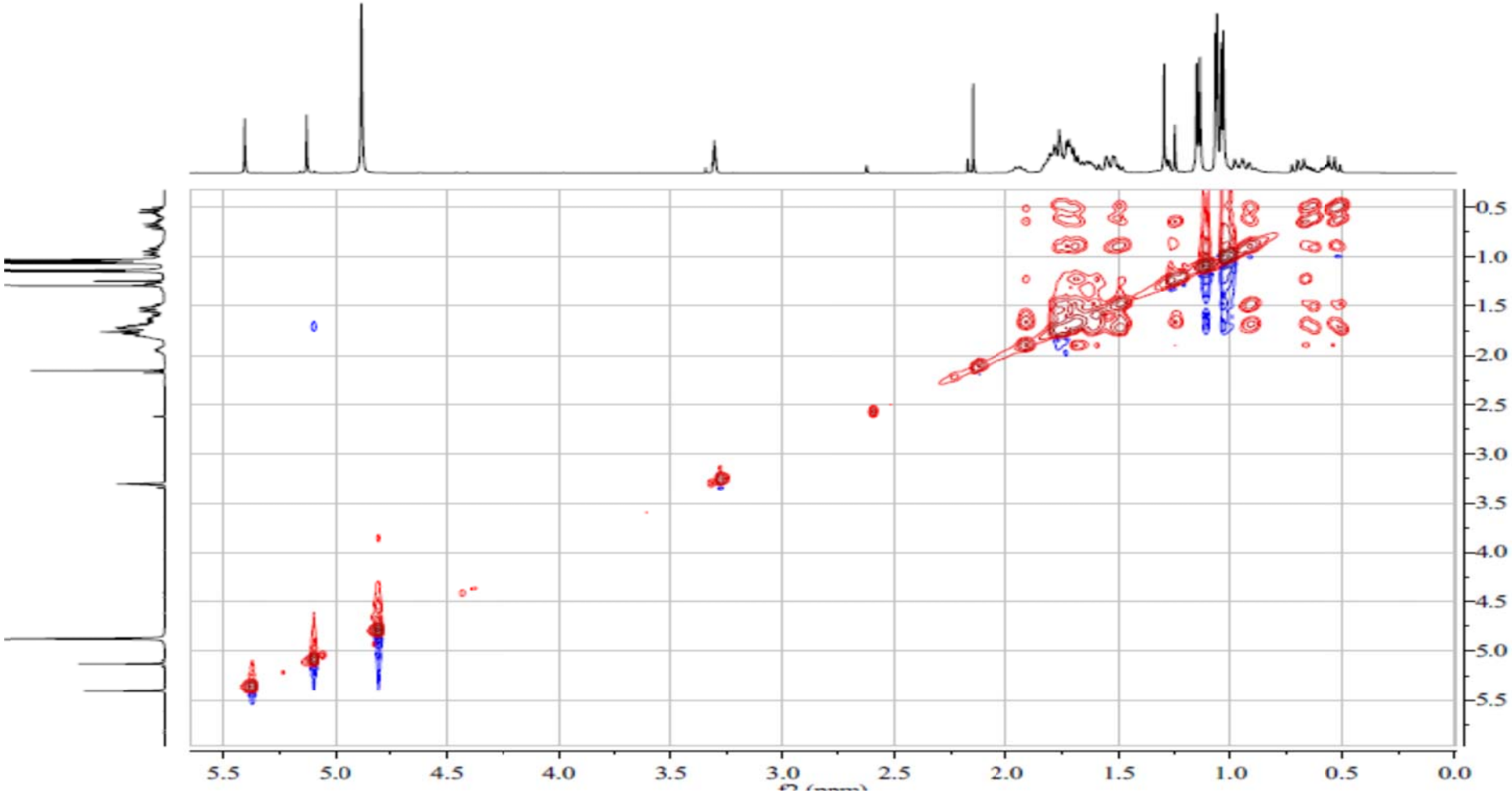
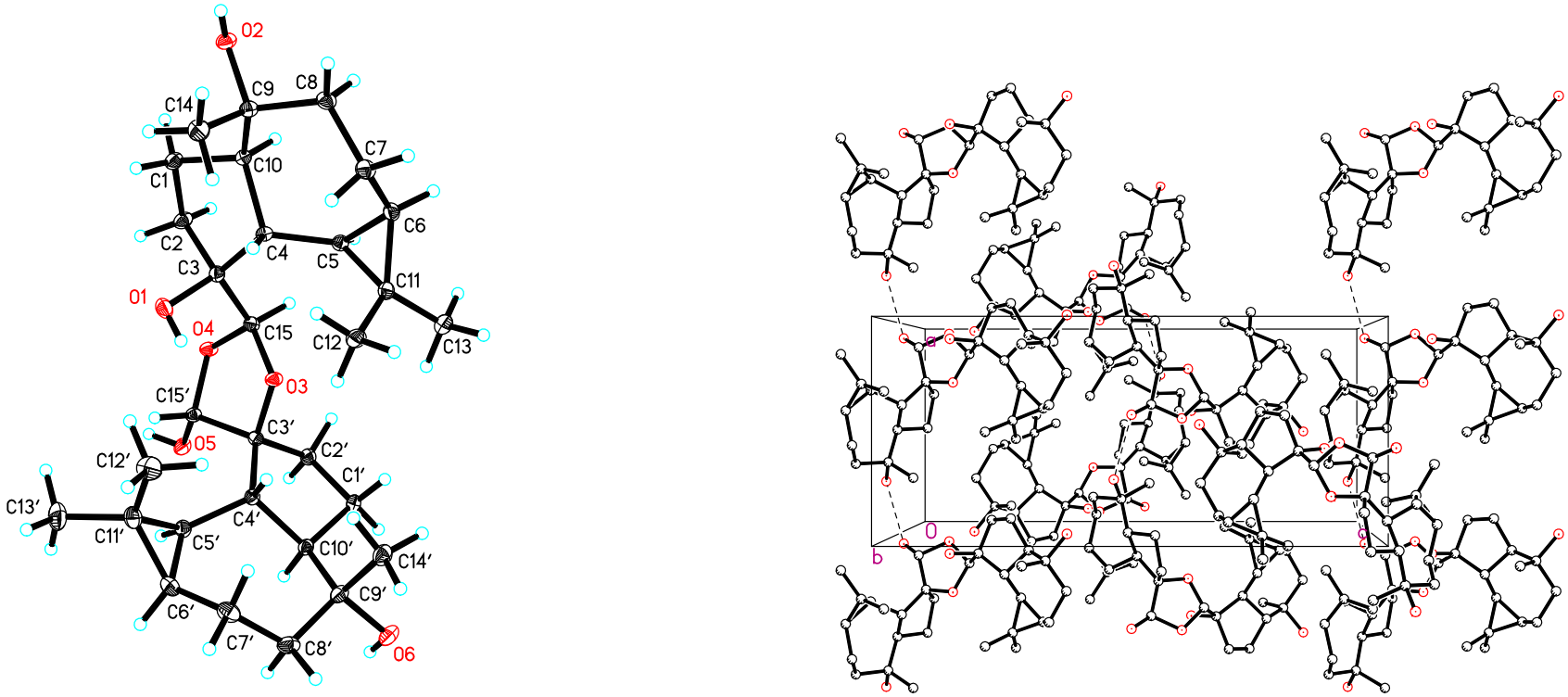


Figure S30 Single X-ray crystal structure and Packing diagram of **3**



Crystallographic data of 1

Indentification code	Cu_dm12499_0m
Empirical formula	$C_{28}H_{42}O_2$
Formula weight	410.62
Temperature	140 (2) K
Wavelength	1.54178Å
Crystal system	othorhombic
Space group	P 2(1) 2(1) 2(1)
Unit cell dimensions	a = 8.5312 (2) Å, α = 90 °; b = 11.5963 (2) Å, β = 90 °; c = 24.0459 (5) Å, γ = 90 °
Volume	2378.87(9) $Å^3$
Z	4
Calculated density	1.147 mg/m^3
Absorption coefficient	0.530 mm^{-1}
F(000)	904
Crystal size	0.28 × 0.25 × 0.13 mm^3
Theta range for data collection	4.23 to 69.65 º
Limiting indices	-9 <= h <= 10 -13 <= k <= 14 -29 <= l <= 29
Reflections collected / unique	18693/4410 [R(int) = 0.0379]
Completeness to theta = 64.99	99.3 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9343 and 0.8658
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4410/0/277
Goodness-of-fit on F^2	1.086
Final R indices [I>2σ(I)]	R_1 = 0.0350, wR_2 = 0.1078
R indices (all data)	R_1 = 0.0353, wR_2 = 0.1085
Absolute structure parameter (flack parameter)	-0.01 (18)
Largest diff. peak and hole	0.204 and -0.213 e. $Å^{-3}$

Single crystal for analysis was obtained from acetone solution. Data collection was performed

with a *Bruker APEX2 CCD* and graphite monochromated Cu*Ka* radiation (λ = 1.54178 Å) at 140 (2) K. *Bruker* SAINT. Program used to solve and refine structure: SHELXS-97, SHELXL-97, resp. Crystallographic data for have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 928251). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].

Crystallographic data of 3

Indentification code	Cu_dm116252_0m
Empirical formula	$C_{30}H_{48}O_6$
Formula weight	504.68
Temperature	173 (2) K
Wavelength	1.54178Å
Crystal system	othorhombic
Space group	P 2(1) 2(1) 2(1)
Unit cell dimensions	a = 9.0356 (2) Å, α = 90 °; b = 14.7212 (2) Å, β = 90 °; c = 20.2608 (3) Å, γ = 90 °
Volume	2694.99 (12) $Å^3$
Z	4
Calculated density	1.244 mg/m^3
Absorption coefficient	0.676 mm^{-1}
F(000)	1104
Crystal size	0.18 × 0.16 × 0.15 mm^3
Theta range for data collection	3.71 to 66.61 º
Limiting indices	-10 <= h <= 10 -17 <= k <= 17 -22 <= l <= 24
Reflections collected / unique	51177/4760 [R(int) = 0.0346]
Completeness to theta = 64.99	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9054 and 0.8880
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4760/0/335
Goodness-of-fit on F^2	1.027
Final R indices [I>2σ(I)]	R_1 = 0.0265, wR_2 = 0.0711
R indices (all data)	R_1 = 0.0270, wR_2 = 0.0715
Absolute structure parameter(flack parameter)	-0.08 (11)
Largest diff. peak and hole	0.173 and -0.157 e. $Å^{-3}$

Single crystal for analysis was obtained from 70% methanol-water solution. Data collection was performed with a *Bruker APEX2 CCD* and graphite monochromated Cu*Ka* radiation (λ = 1.54178

Å) at 140 (2) K. *Bruker* SAINT. Program used to solve and refine structure: SHELXS-97, SHELXL-97, resp. Crystallographic data for have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 931555). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].