Supplementary Information

Functional characterization of the halogenase SpmH and discovery of new deschloro tryptophan dimers

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Contents

Experiment methods

General information

Melting point was measured using an SGWX-4 digital display micromelting point instrument. Optical rotations were measured on a 341 Polarmeter (PerkinElmer, Inc.). UV spectra were measured on a U-2900 spectrophotometer (Hitachi). ECD spectra were recorded on a Chirascan circular dichroism spectrometer (Applied Photophysics Co.). IR spectra were obtained on an Affinity-1 FT-IR spectrometer (Shimadzu). 1D and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer (BrukerBiospin) with tetramethylsilane (TMS) as the internal standard. Mass spectrometric data were obtained on a quadrupole-time-of-flight mass spectrometry (Bruker Maxis 4G) for HRESIMS. Materials for column chromatography (CC) were silica gel (200–300 mesh; Jiangyou Silica gel development, Inc.) and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB). HPLC was carried out using a reversed-phase column (Phenomenex Gemini C18, 250 mm × 4.6 mm, 5 μm; Phenomenex, USA) with UV detection at 254 nm. Semi-preparative HPLC was performed on a Hitachi HPLC station (Hitachi-L2130) with Diode Array Detector (Hitachi L-2455) using a Gemini C18 column (250 mm × 10.0 mm, 5 μm; Phenomenex, USA).

Bacterial strains, plasmids, and reagents

Bacterial strains and plasmids are listed in Table S1. The strain *Streptomyces* sp. SCSIO 03032 was previously described[1] and was deposited at the China Center for Type Culture Collection (CCTCC) under the accession no. CCTCC M2011258. The strain SCSIO 03032 was grown at 28°C, either in ISP4-Agar media containing 3 % sea salt for growth and sporulation or in production media modified-A1BFe+C (starch 1 %, yeast extract 0.4 %, peptone 0.2 %, sea salt 3 %, pH 7.2−7.4) for fermentation. Chemicals, enzymes, and other molecular biological reagents were purchased from standard commercial sources and used according to the manufacturer's recommendations.

DNA isolation, manipulation and sequencing

DNA isolation and manipulations in *Escherichia coli* and *Streptomyces* were carried out according to standard procedures.[2, 3] Primers (Table S2) were synthesized at the Shanghai Invitrogen Biotech Co., Ltd. PCR amplifications were carried out on an Authorized Thermal Cycler (Eppendorf AG). DNA sequencing was performed at the Invitrogen Biotech Co., Ltd. (Guangzhou).

Gene inactivation experiments

The lambda-RED-mediated gene replacements were performed as standard procedures using mycelium as the recipients. The gene disruption experiments in *Streptomyces* sp. SCSIO 03032 were carried out using the previously reported genetic manipulation system.[4] Details for *spmH*-gene disruptions were described in Fig. S2. Each mutant was diagnosed by PCR analysis and confirmed by sequencing the PCR product. Three independent single clones of double-crossover mutant were inoculated into 50 mL of modified-A1BFe+C media and cultured at 28 °C for 6−8 days. The production of SPM/IDM/LNMs was monitored via HPLC analysis on an Agilent 1260 Infinity series. HPLC was carried out using a reversed-phase column (ZORBAX SB-C18, 150 × 4.6 mm, 5 μm, Agilent, USA) with UV detection at 254 nm under the following program: solvent system (solvent A, 10 % acetonitrile in water supplementing with 0.1 % formic acid; solvent B, 90 % acetonitrile in water); 5 % B to 50 % B (linear gradient, 0−7 min), 50 % B to 100 % B (linear gradient, 7−21 min), 100 % B (21−25 min), 100 % B to 5 % B (25−26 min), 5 % B (26−30 min); flow rate at 1 mL min^{-1}.

Overexpression and purification of SpmH

The *spmH* gene was PCR amplified from the genomic DNA of *Streptomyces* sp. SCSIO 03032 using primers 91EF and 91ER (Table S2). PCR products were digested with *Nde*I/*Bam*HI and were subsequently inserted into pET28a linearized with *Nde*I/*Bam*HI, to yield the *spmH* expression plasmid pCSG361 after sequence

confirmation. The plasmid pCSG361 was then introduced into *E. coli* BL21(DE3) for overexpression of *spmH*. When the cultures in LB media containing kanamycin (50 μg mL^{-1}) were grown to an OD_{600} of 0.5 at 37 °C, the production of SpmH was induced by the addition of IPTG to a final concentration of 0.1 mM. The cultures were grown at 16 °C for an additional 20 h. The cells were then collected by centrifugation, and washed with 50 mM Tris-HCl (pH 8.0) twice. The cell pellets were resuspended in the binding buffer (300 mM KCl, 50 mM potassium phosphate, 10 mM imidazole, pH 7.4) and sonicated. Purification of His_6-tagged recombinant SpmH was conducted using Ni-NTA affinity chromatography according to the manufacturer's manual (Novagen, USA). After desalting with PD-10 column (GE Healthcare, USA), the purified SpmH was stored in the storage buffer (10 % glycerol, 1 mM DTT, 100 mM potassium phosphate, pH 7.4) at −80°C for further experiments.

Enzyme assay

A typical *in vitro* enzyme reaction of SpmH was conducted in 100 μL potassium phosphate buffer (50 mM, pH 7.4) comprising of 150 μM L-Trp (or compounds **1**−**7**), 5 μM spmH, 10 μM FAD, 20 mM DTT and 50 mM NaCl. The reactions were incubated at 30 °C for 12 h and then were quenched by mixing with 100 μL of ice-cold MeOH. In a time course assays of the SpmH-catalyzed reaction of L-Trp, samples were taken at 0 min, 30 min, 60 min, 2 h, 4 h, 6 h, 8 h and 10 h.(Fig. S13). HPLC analysis of the enzyme reactions was carried out on the Agilent 1260 Infinity series instrument (Agilent Technologies Inc., USA) using a reversed-phase column (ZORBAX SB-C18, 150 × 4.6 mm, 5 μm, Agilent, USA) with UV detection at 220 nm under the following program: solvent system (solvent A, 0.1 % TFA/H_2O; solvent B, 0.1 % TFA/MeCN); 0 % B to 15 % B (linear gradient, 0−10 min), 15 % B to 20 % B (linear gradient, 10−20 min), 20 % B to 25 % B (linear gradient, 20−25 min), 25 % B to 0 % B (linear gradient, 25−25.1 min), 0 % B (25.1−30 min); flow rate at 1 mL min^{-1}. The product 5-Cl-L-Trp were identified by comparison of UV/ESIMS spectra and the retention time with the standard (Fig. S12−S13). For compounds **1**−**7**, the

reactions were incubated at 30 °C for 20 h and then were quenched by mixing with 100 μL of ice-cold MeOH. HPLC analysis of the enzyme reactions were detection at 254 nm under the following program: solvent system (solvent A, 10 % acetonitrile in water supplementing with 0.1 % formic acid; solvent B, 90 % acetonitrile in water); 5 % B to 50 % B (linear gradient, 0−7 min), 50 % B to 100 % B (linear gradient, 7−21 min), 100 % B (21−25 min), 100 % B to 5 % B (25−26 min), 5 % B (26−30 min); flow rate at 1 mL min^{-1}.

Fermentation and isolation

The Δ*spmH* mutant of *Streptomyces* sp. SCSIO 03032 was grown and maintained on ISP4-Agar containing 3 % natural sea salt. A few loops of spores of Δ*spmH* mutant were inoculated into 50 mL of seed medium (modified A1BFe+C: starch 1.0%, yeast extract 0.4 %, peptone 0.2 %, $CaCO_3$ 0.2 %, natural sea salt 3 %, pH 7.2−7.4). Then, a total of 20 L fermentation was performed by inoculating 10 mL of the seed culture into a 1000 mL Erlenmeyer flask containing 200 mL of the production medium, and culturing on a rotary shaker (200 rpm) at 28 ºC for 7 days. On the fifth day, a 20 mL (5 vol %) portion of the sterilized polystyrene resin (Amberlite XAD-16) was added into the production medium.

The mycelia and polystyrene resin were separated by filtration through a metal sieve (40 mesh). The mycelia parts were extracted three times, each with 6 L of acetone, and the acetone was removed under vacuum. The resins parts were washed twice with H_2O and transferred to a glass column. The glass column was eluted with 4 L of acetone. The acetone fractions were concentrated under vacuum to afford an aqueous residue, which was extracted four times with 1 L of EtOAc. The EtOAc extracts were combined with mycelia parts extract, and concentrated under vacuum to yield a total extract (5.0 g). The total extract was subjected to silica gel column chromatography (CC) and eluted with gradient cyclohexane-acetone (10:0 to 0:10, v/v) to obtain twelve fractions (Frs. 1−12). Fr. 5 was successively separated on Sephadex LH-20 CC using $CHCl_3$-MeOH (1:1, v/v) as the eluent to afford compound **1** (20.0

mg). Fr. 6 was chromatographed on Sephadex LH-20 CC eluting with $CHCl_3$-MeOH (1:1, v/v), followed by semi-preparative HPLC using MeCN-H_2O (60:40, v/v) as the mobile phase to afford compound **2** (5.5 mg, t_R = 27.0 min). Fr. 7 was further purified by repetitive Sephadex LH-20 CC ($CHCl_3$-MeOH, 1:1, v/v) to get compounds **3** (10.2 mg), **5** (8.6 mg) and **6** (22.5 mg). Compound **4** (5.0 mg, t_R = 13.5 min) was obtained from Fr. 8 by semi-preparative HPLC eluting with gradient MeCN-H_2O (40:60 to 65:35, v/v). Compound **7** (10.0 mg) was purified by Sephadex LH-20 CC eluting with $CHCl_3$-MeOH (1:1, v/v) from Fr. 10.

Cytotoxicity assay

The human nervous glioma and astrocytoma cell line SF-268, human breast adenocarcinoma cell line MCF-7, human hepatocellular carcinoma cell line HepG2, and human lung cancer cell line A549 were obtained from the American Type Culture Collection (ATCC). All of the cell lines were cultured in RPMI 1640 medium, supplemented with 10 % FBS (v/v) at 37 °C in a humidified atmosphere of 5 % CO_2 (v/v). Cells were cultured in 96-well plates for 24 h. Then the cells were treated with compounds **1**−**7** at various concentrations for 72 h. After incubated for another 4 h with 30 μL aliquot of MTT solution (5 mg mL^{-1} in PBS), the medium was discarded, and 100 μL of DMSO was added to dissolve the produced formazan. The absorbance was measured at 570 nm using a microplate Reader (Thermo scientific multiskan MK3, USA). Each well was performed in triplicate in 3 independent experiments. The concentration giving 50 % inhibition (IC_{50}) was determined from the dose-response curves using Prism software and presented as the mean ± SD.

Calculation details for compounds **1**, **2** and **4**

The Conformational analysis of **1**, **2** and **4** were performed in Sybyl 8.1 software using MMFF94s force field, which afforded 1, 3 and 1 selected conformers for **1**, **2** and **4** respectively, with an energy cutoff of 10 kcal mol^{-1} to the global minima. All of the obtained conformers were optimized using the B3LYP/6-31+G(d) level in gas phase by using Gaussian09 software.[5] TDDFT ECD calculations for the optimized conformers were performed at the B3LYP/6-31+G(d) level. The overall calculated ECD curves of all the compounds were weighted by Boltzmann distribution after a UV correction of 3, 3 and −18 nm, respectively. The ECD curves were produced by SpecDis 1.64 software.[6]

Physicochemical and spectroscopic data

Spiroindimicin G (1)

White amorphous powder. $[\alpha]_D^{20}$ +155.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 236 (3.62), 268 (3.38), 310 (2.99), 369 (2.83) nm; IR (KBr) ν_{max}: 3028, 2949, 2831, 1699, 1686, 1603, 1487, 1435, 1265, 1132, 1122, 1020 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+6.9), 228 (−38.1), 255 (+20.9), 310 (+12.2) nm; HRESIMS *m/z*: 428.1610 $[M + H]^+$ (calcd for $C_{25}H_{22}N_3O_4$, 428.1605); ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table S4.

Spiroindimicin H (2)

White amorphous powder. $[\alpha]_D^{20}$ +174.3 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.44), 230 (3.44), 279 (3.43), 307 (3.18), 355 (2.76) nm; IR (KBr) ν_{max}: 3394, 2949, 2831, 1697, 1686, 1603, 1489, 1433, 1332, 1261, 1248, 1119, 1093, 1018 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 206 (−36.6), 223 (+7.1), 235 (−7.3), 255 (+41.9), 275 (−5.3), 288 (+4.2), 319 (+8.8) nm; HRESIMS *m/z*: 428.1599 $[M + H]^+$ (calcd for $C_{25}H_{22}N_3O_4$, 428.1605); ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table S5.

Indimicin F (3)

Colorless blocks ($CHCl_3$/MeOH), m.p. 287−288 °C. $[\alpha]_D^{20}$ +119.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 238 (3.58), 288 (2.95) nm; IR (KBr) ν_{max}: 3362, 3294, 2912, 2833, 1688, 1672, 1605, 1435, 1393, 1335, 1015 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 223 (+20.2), 251 (−5.0), 277 (+3.5), 307 (−13.0), 352 (+3.2) nm; HRESIMS *m/z*: 394.1530 $[M + Na]^+$ (calcd for $C_{23}H_{21}N_3O_2Na$, 394.1526). ^1H (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table S6.

Indimicin H (4)

Yellow amorphous powder. $[\alpha]_D^{20}$ +327.2 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 236 (3.48), 290 (2.97), 355 (2.76) nm; IR (KBr) ν_{max}: 3213, 2982, 2949, 2827, 1661, 1542, 1423, 1387, 1250, 1103, 1018 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 230 (+20.1), 252 (−18.0), 307 (−13.7), 357 (+11.0) nm; HRESIMS *m/z*: 384.1718 $[M - H]^-$ (calcd for $C_{24}H_{22}N_3O_2$, 384.1722); ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table S7.

Table S1 Strains and plasmids used and generated in this study.

Strains/Plasmids	Characteristic(s)	Sources
Strains		
E. coli		
DH5	Host strain for cloning	Invitrogen
BW25113	Host strain for PCR targeting	[7]
ET12567	Donor strain for conjugation	[8]
BL21(DE3)	Host strain for protein expression	Novagen
Streptomyces		
SCSIO 03032	Wild type, spiroindimicins/indimicins/lynamicins producer	[1]
SPM261	*spmH* gene disrupted mutant of SCSIO 03032	This study
Plasmids		
SuperCos 1	Amp^r and Km^r, cosmid vector	Stratagene
pIJ773	Apr^r, source of *aac(3)IV*	[9]
pIJ790	Cm^r , including λ-RED (*gam*, *bet*, *exo*) for PCR-targeting	[9]
pUZ8002	Km^r, including *tra* for conjugation	[10]
pET28a	Km^r, expression vector	Novagen
pCSG205	Strain SCSIO 03032 genomic library cosmid	[4]
pCSG261	pCSG205 derivative where *spmH* was disrupted by *aac(3)IV*	This study
pCSG361	1.56 kb *Nde*I/*Bam*HI *spmH* PCR fragment from genomic DNA of strain SCSIO 03232 into pET28a	This study

Table S2 Primers used in this study.

Primers	Gene target	Sequences (5'–3', restriction sites are underlined)	
For gene replacement			
91tarF	*spmH*	GACCACTCCCAGCCGTTCGACCGCCAGTGCTTCGTGACCattccggggatccgtcgacc	
91tarR		CTCCTCGTCGATGAGGTGCGGCAGTGCCTGCTCCATCCGtgtaggctggagctgcttc	
For confirmation			
91testF	*spmH*	GGCTACAAGCTCGCCATCCG	
91testR		CGCAGCAGGTCGAACTCCGC	
For protein expression			
91EF	*spmH*	GGAGTACCATATGATCAGGAAAGTGGCC	(*Nde*I)
91ER		GCGGCGGATCCCTACGGGGTGTGGATGC	(*Bam*HI)

Table S3 Crystal data and structure refinement for **3**

Empirical formula	0.5 (C_{23} H_{21} N_3 O_2)
Formula weight	185.71
Temperature/K	99.97(19)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	10.59660(10)
b/Å	7.86090(10)
c/Å	11.87830(10)
α/°	90
β/°	114.1430(10)
γ/°	90
Volume/Å^3	902.898(17)
Z	4
ρ_{calc}g/cm^3	1.366
μ/mm^{-1}	0.711
F(000)	392.0
Crystal size/mm^3	0.3 × 0.2 × 0.2
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/	8.156 to 147.466
Index ranges	$-12 \le h \le 11$, $-9 \le k \le 9$, $-14 \le l \le 14$
Reflections collected	8825
Independent reflections	3527 [R_{int} = 0.0163, R_{sigma} = 0.0166]
Data/restraints/parameters	3527/1/257
Goodness-of-fit on F^2	1.098
Final R indexes [I >= 2σ (I)]	R_1 = 0.0291, wR_2 = 0.0736
Final R indexes [all data]	R_1 = 0.0295, wR_2 = 0.0739
Largest diff. peak/hole/e Å^{-3}	0.16/−0.27
Flack parameter	0.05(7)

The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using SHELXL-2014 package software[11]. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as deposition number CCDC 1873447 for **3**.

Table S4 1D and 2D NMR data of **1** (δ in ppm, J in Hz, $CDCl_3$)[a]

No		δ_H mult (J)	δ_C	^1H ^1H COSY	HMBC
1 (NH)		9.21 s			C-2, 3, 4, 5
2			113.9		
3			133.6		
4			140.8		
5			119.8		
6			161.2		
7		4.08 s	52.0		C-6
8			160.2		
9		3.60 s	51.6		C-8
2’	a	4.01 d (8.7)	64.1		C-4, 3’, 10’, 2’’
	b	3.59 d (8.7)			C-3’, 4’, 9’, 2’’
3’			51.9		
4’			131.6		
5’		6.57	122.9	H-6’	C-3’, 7’, 9’
6’		6.58	119.0	H-5’, 7’	C-4’, 8’
7’		7.14 dd (7.9, 7.9)	128.7	H-6’, 8’	C-5’, 9’
8’		6.70 d (7.9)	108.4	H-7’	C-4’, 6’
9’			153.6		
10’		2.90 s	36.7		C-2’, 9’
1’’ (NH)		8.58 s			C-2’’, 3’’, 4’’, 9’’
2’’			156.8		
3’’			111.6		
4’’			121.9		
5’’		8.18 d (7.8)	120.9	H-6’’	C-3’’, 7’’, 9’’
6’’		7.20 dd (7.8, 7.8)	121.0	H-5’’, 7’’	C-4’’, 8’’
7’’		7.16 dd (7.8, 7.8)	122.0	H-6’’, 8’’	C-5’’, 9’’
8’’		7.24 d (7.8)	112.1	H-7’’	C-4’’, 6’’
9’’			140.0		

[a]Spectra recorded at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR; Overlapped signals are reported without designating multiplicity.

Table S5 1D and 2D NMR data of **2** (δ in ppm, J in Hz, $CDCl_3$)[a]

No		δ_H mult (J)	δ_C	^{1}H-^{1}H COSY	HMBC
1 (NH)		9.66 s			
2			116.7		
3			123.9		
4			132.0		
5			123.0		
6			161.0		
7		3.49 s	51.9		C-6
8			160.4		
9		4.02 s	51.6		C-8
2’	a	3.93 d (8.6)	74.6		C-4, 3’, 10’, 5’’
	b	3.83 d (8.6)			C-3’, 4’, 9’, 5’’
3’			49.2		
4’			141.1		
5’		6.52 d (7.3)	123.4	H-6’	C-3’, 7’, 9’
6’		6.47 dd (7.3, 7.3)	117.7	H-5’, 7’	C-4’, 8’
7’		7.09 dd (7.3, 7.3)	127.5	H-6’, 8’	C-5’, 9’
8’		6.64 d (7.3)	106.3	H-7’	C-4’, 6’
9’			152.7		
10’		2.95 s	35.6		C-2’, 9’
1’’ (NH)		8.15 br s		H-2’’	C-2’’, 3’’, 9’’
2’’		7.97 d (2.2)	121.3	H-1’’	C-3’’, 4’’, 9’’
3’’			106.6		
4’’			123.3		
5’’			137.3		
6’’		7.05 d (7.6)	116.8	H-7’’	C-4’’, 8’’
7’’		7.14 dd (7.6, 7.6)	125.1	H-6’’, 8’’	C-5’’, 9’’
8’’		7.17 d (7.6)	108.4	H-7’’	C-4’’, 6’’
9’’			134.1		

[a]Spectra recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR; Overlapped signals are reported without designating multiplicity.

Table S6 1D and 2D NMR data of **3** (δ in ppm, J in Hz, DMSO-d_6.)[a]

No	δ_H mult (J)	δ_C	^{1}H-^{1}H COSY	HMBC	NOESY
1 (NH)					
2		167.0			
3		127.2			
4		145.7			
5	4.83 d (8.8)	83.0	OH	C-2, 3	
10	2.80 s	25.5		C-2, 5	
2’	4.12 s	71.2		C-3’, 4’, 10’, 11’, 3’’	H-11’
3’		46.5			
4’		132.4			
5’	7.40 d (7.5)	123.9	H-6’	C-3’, 7’, 9’	
6’	6.81 dd (7.5, 7.5)	118.8	H-5’, 7’	C-4’, 8’	
7’	7.11	127.7	H-6’, 8’	C-5’, 9’	
8’	6.58 d (7.5)	107.5	H-7’	C-4’, 6’	
9’		152.5			
10’	2.72 s	22.9		C-2’, 9’	
11’	1.54 s	34.5		C-4, 2’, 4’	H-2’
1’’ (NH)	11.9 s			C-2’’, 3’’, 4’’, 9’’	
2’’		133.1			
3’’		103.9			
4’’		123.3			
5’’	8.45 d (8.0)	122.5	H-6’’	C-3’’, 7’’, 9’’	
6’’	7.07 dd (8.0, 8.0)	119.8	H-5’’, 7’’	C-4’’, 8’’	
7’’	7.13	121.5	H-6’’, 8’’	C-5’’, 9’’	
8’’	7.44 d (8.0)	111.7	H-7’’	C-4’’, 6’’	
9’’		136.4			
OH	6.49 d (8.8)		H-5	C-4, 5	

[a]Spectra recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR; Overlapped signals are reported without designating multiplicity.

Table S7 1D and 2D NMR data of **4** (δ in ppm, J in Hz, $CDCl_3$)[a]

No	δ_H mult (J)	δ_C	^{1}H-^{1}H COSY	HMBC	NOESY
1 (NH)					
2		87.7			
3		149.2			
4		125.4			
5		168.1			
10	2.88 s	22.2		C-2, 5	
11	1.72 s	22.9		C-3	H-5''
2'	4.07 s	72.4		C-3', 4', 10', 11', 3''	H-11'
3'		46.5			
4'		131.7			
5'	7.95 dd (7.5, 1.0)	127.6	H-6'	C-3', 7', 9'	
6'	6.85 ddd (7.5, 7.5, 1.0)	119.4	H-5', 7'	C-4', 8'	
7'	7.12 ddd (7.5, 7.5, 1.0)	127.8	H-6', 8'	C-5', 9'	
8'	6.50 dd (7.5, 1.0)	107.5	H-7'	C-4', 6'	
9'		152.2			
10'	2.78 s	35.5		C-2', 9'	
11'	1.59 s	24.1		C-4, 2', 4'	H-2'
1'' (NH)	8.89 s			C-2'', 3'', 4'', 9''	
2''		135.8			
3''		106.1			
4''		123.2			
5''	8.24 d (7.7)	122.2	H-6''	C-3'', 7'', 9''	H-11
6''	7.24 dd (7.7, 7.7)	121.7	H-5'', 7''	C-4'', 8''	
7''	7.29 dd (7.7, 7.7)	123.1	H-6'', 8''	C-5'', 9''	
8''	7.46 d (7.7)	111.8	H-7''	C-4'', 6''	
9''		136.8			

[a]Spectra recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR; Overlapped signals are reported without designating multiplicity.

Fig. S1 Chemical structures of compounds **8**−**19**

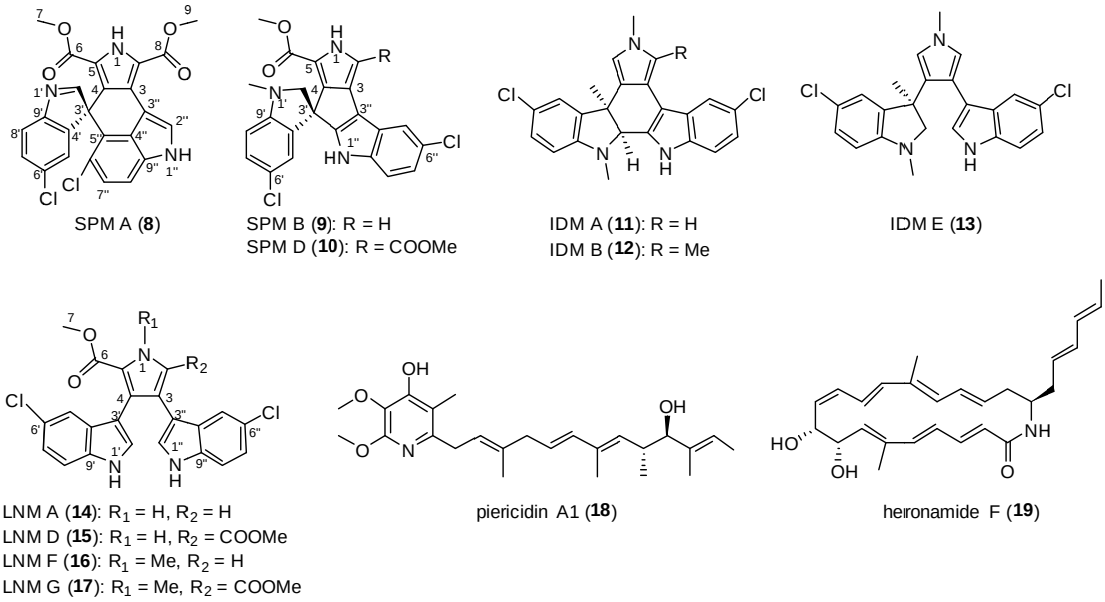
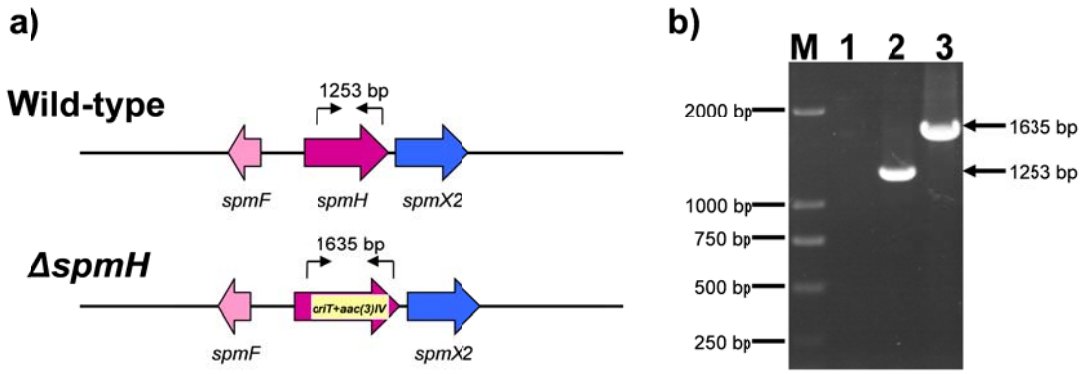


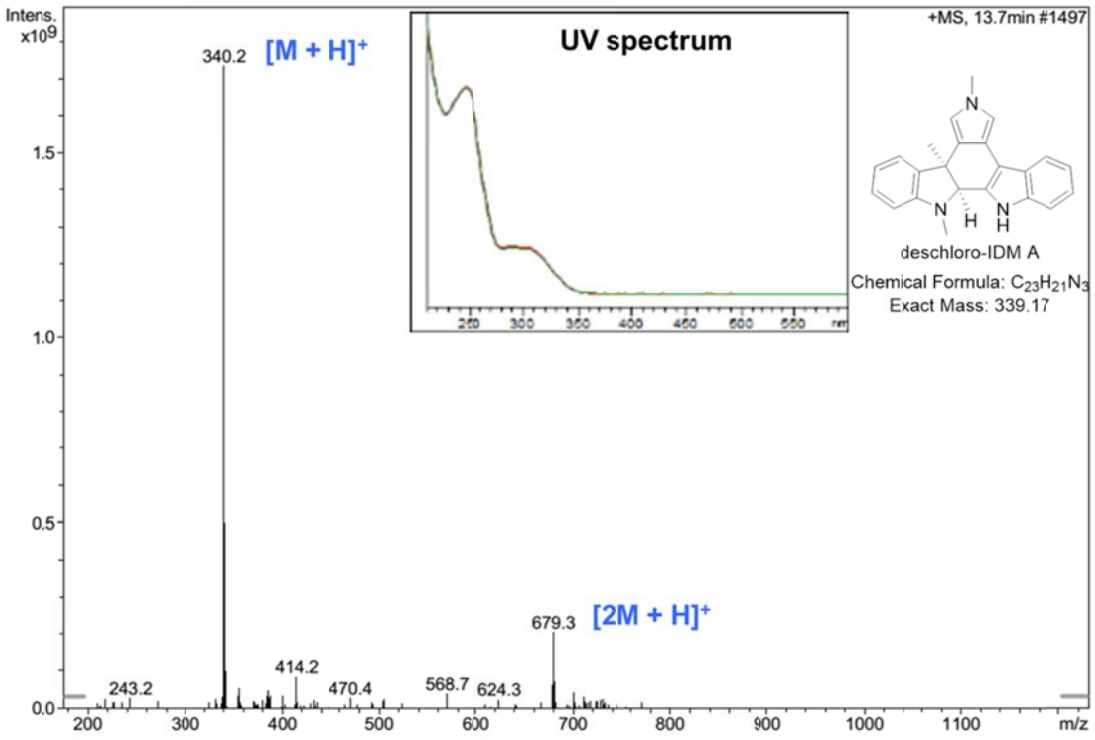
Fig. S2 Construction of *spmH*-inactivation mutant SPM261



Construction of *spmH*-inactivation mutant SPM261. **(a)** Depiction of *spmH*-inactivation. SPM261 was constructed by replacing a 987 bp *spmH* fragment with a 1369 bp DNA fragment containing *oriT* and *acc3(IV)* in pCSG205, resulting from a double cross-over recombination event. Location of the diagnostic PCR primers 91testF and 91testR (Table S2) was indicated. Sizes of PCR products, 1253 bp for the wild type strain *Streptomyces* sp. SCSIO 03032 and 1635 bp for the mutant SPM261, were also indicated. **(b)** Gel electrophoresis of PCR products. DNA templates were from: ddH_2O (negative control, lane 1), wild type strain *Streptomyces* sp. SCSIO 03032 (lane 2), mutant 261 (lane 3) and DNA marker DL 2000 (Takara, lane M).

Fig. S3 UV and MS spectra of deschloro-IDMs A and B

(A) The ESIMS (positive mode) and UV spectra of deschloro-IDM A



(B) The ESIMS (positive mode) and UV spectra of deschloro-IDM B

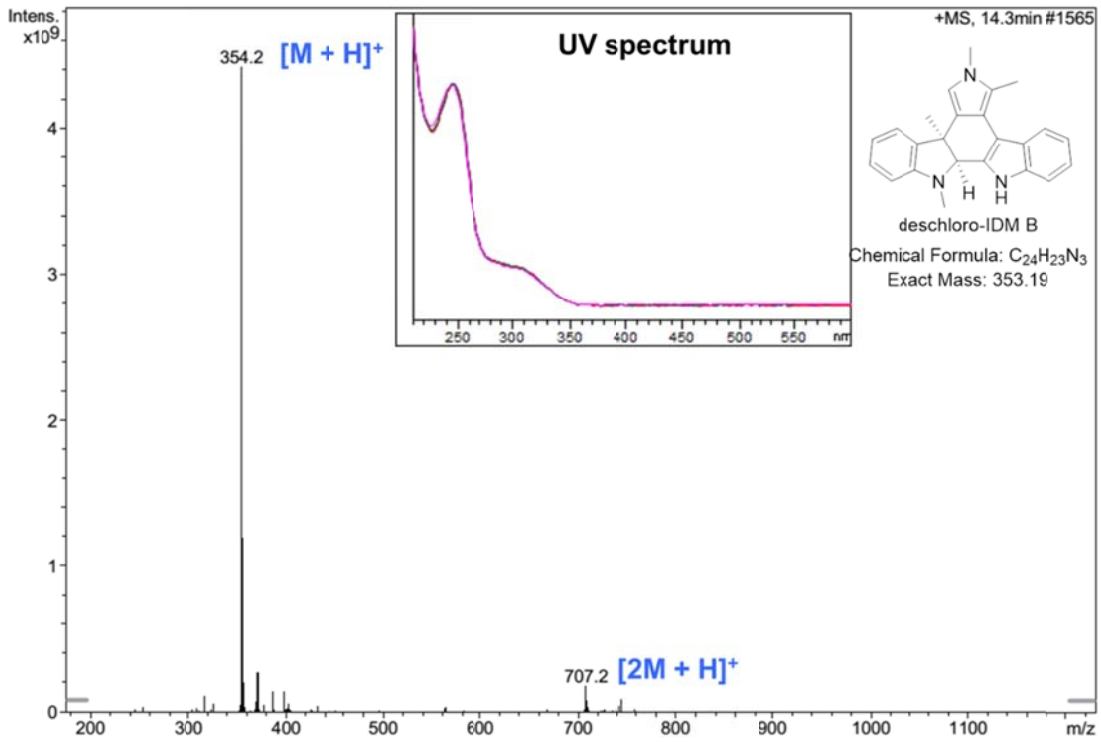


Fig. S4 The spectral data of **1**

(A) HR-ESI-MS spectrum of **1**

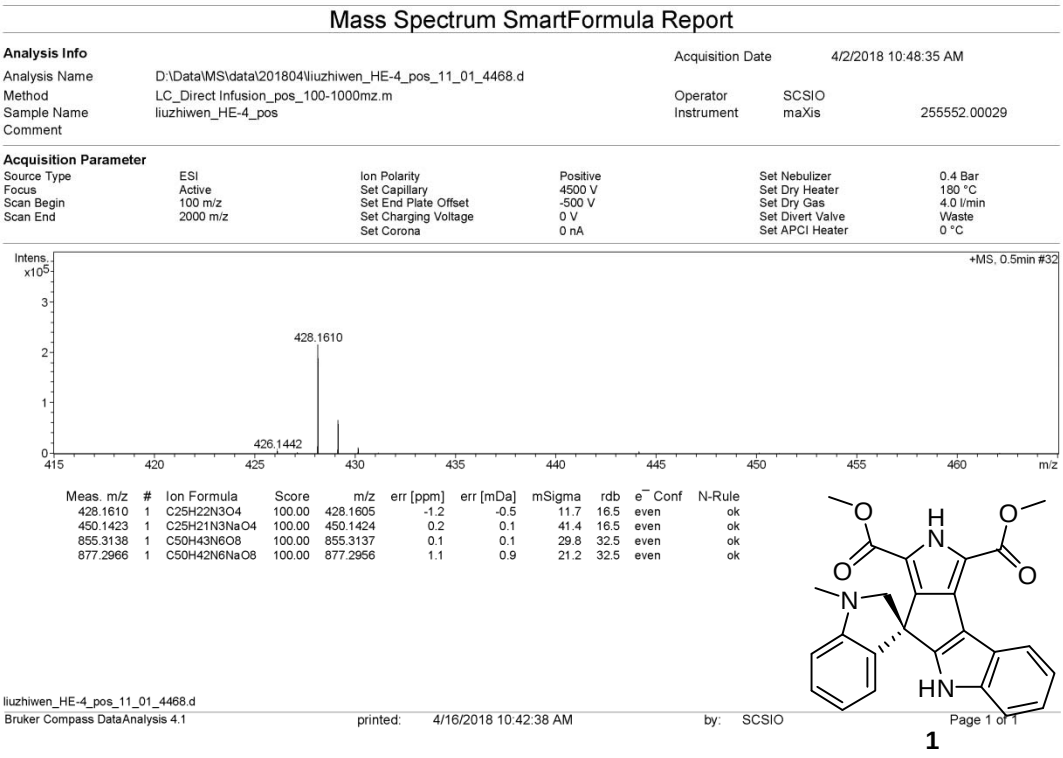


Fig. S4 The spectral data of **1**

(B) IR spectrum of **1**

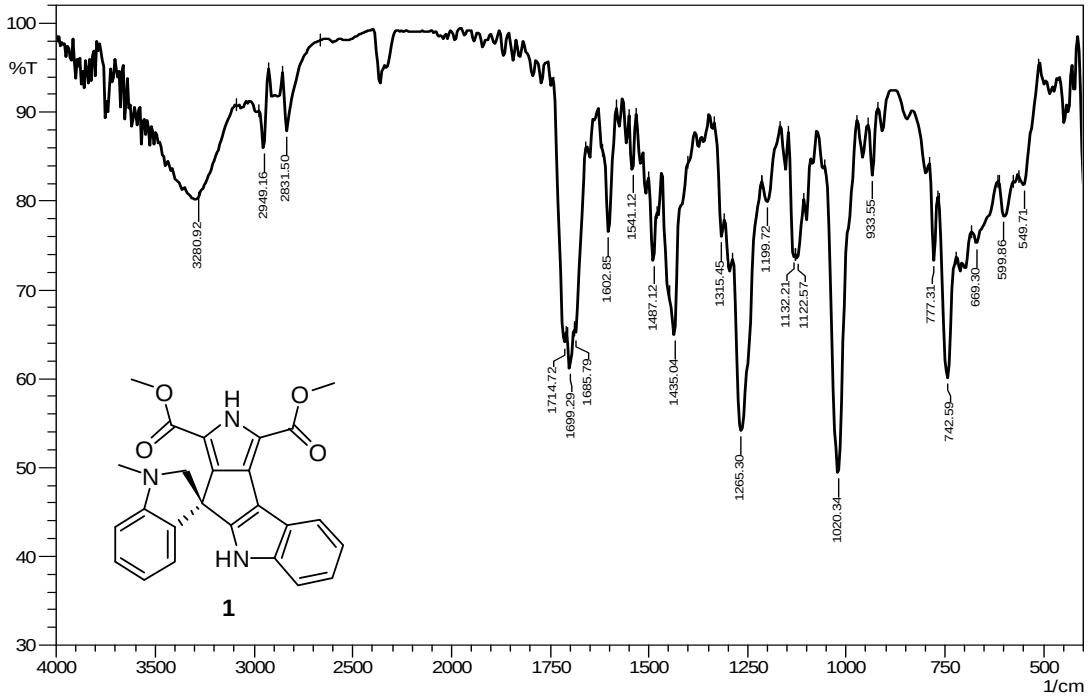
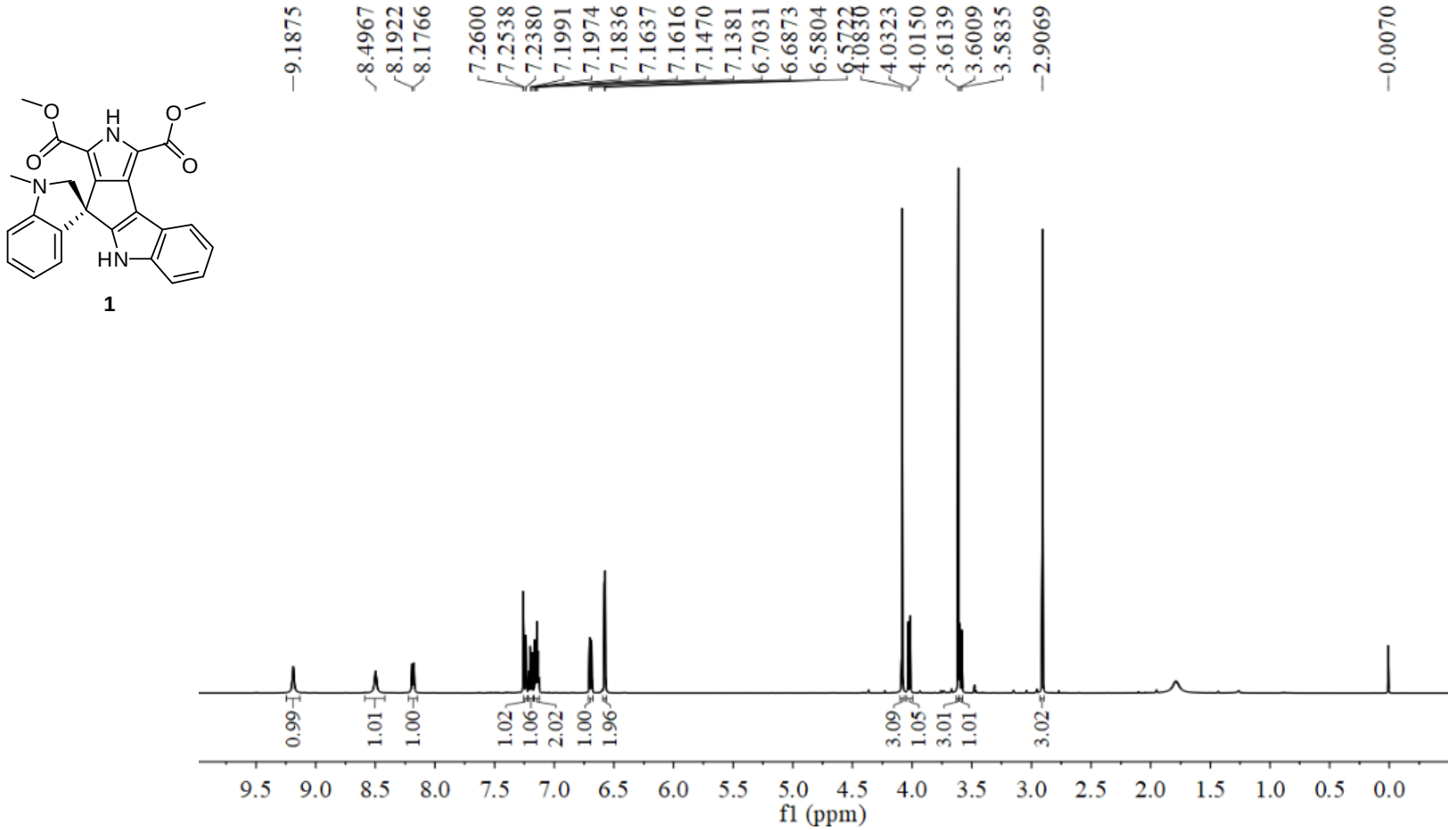


Fig. S4 The spectral data of **1**

(C) ^{1}H NMR spectrum of **1** ($CDCl_3$)



1

Fig. S4 The spectral data of **1**

(D) ^{13}C NMR spectrum of **1** ($CDCl_3$)

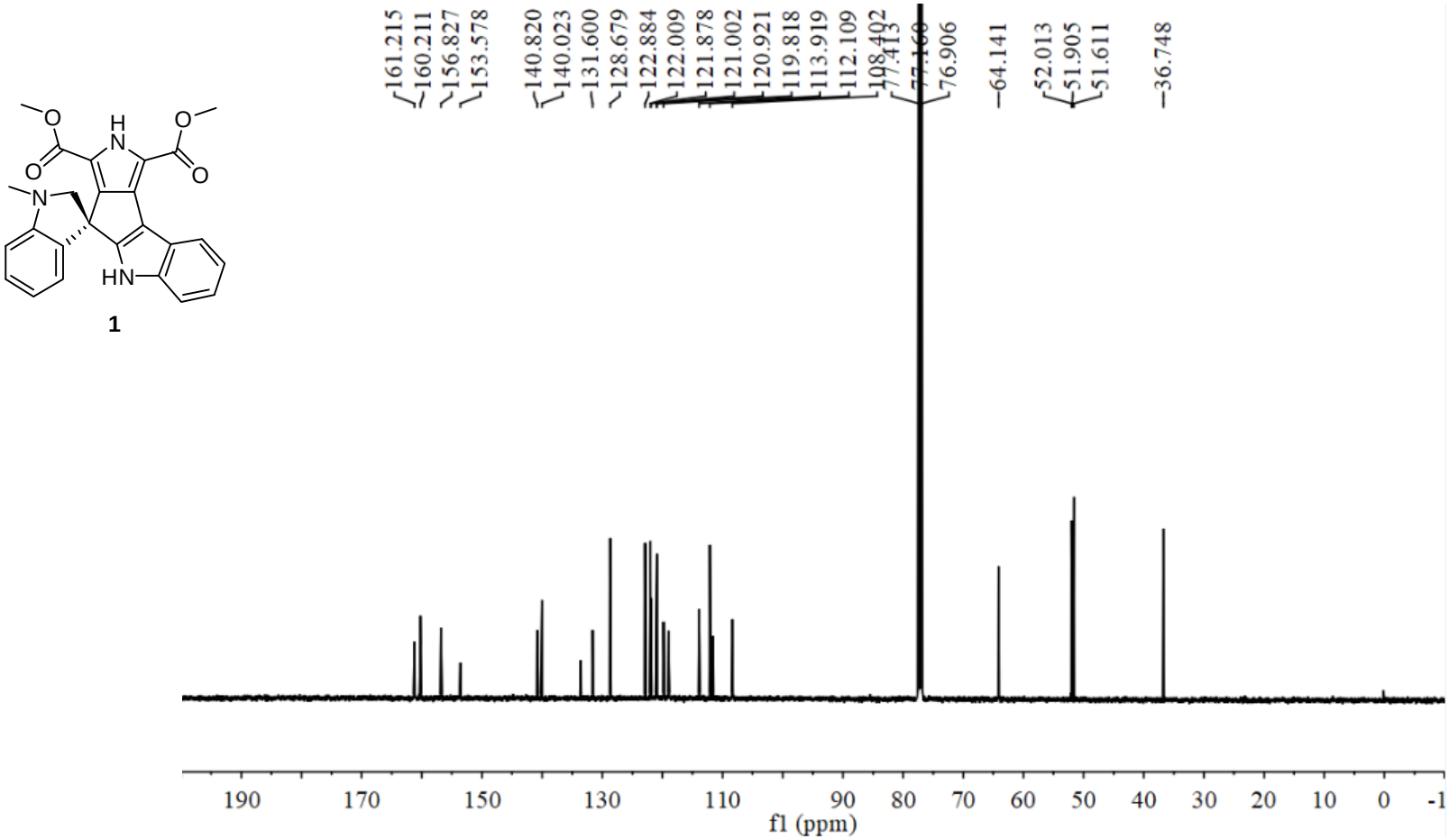


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Fig. S4 The spectral data of **1**

(E) DEPT135 and ^{13}C NMR spectra of **1** ($CDCl_3$)

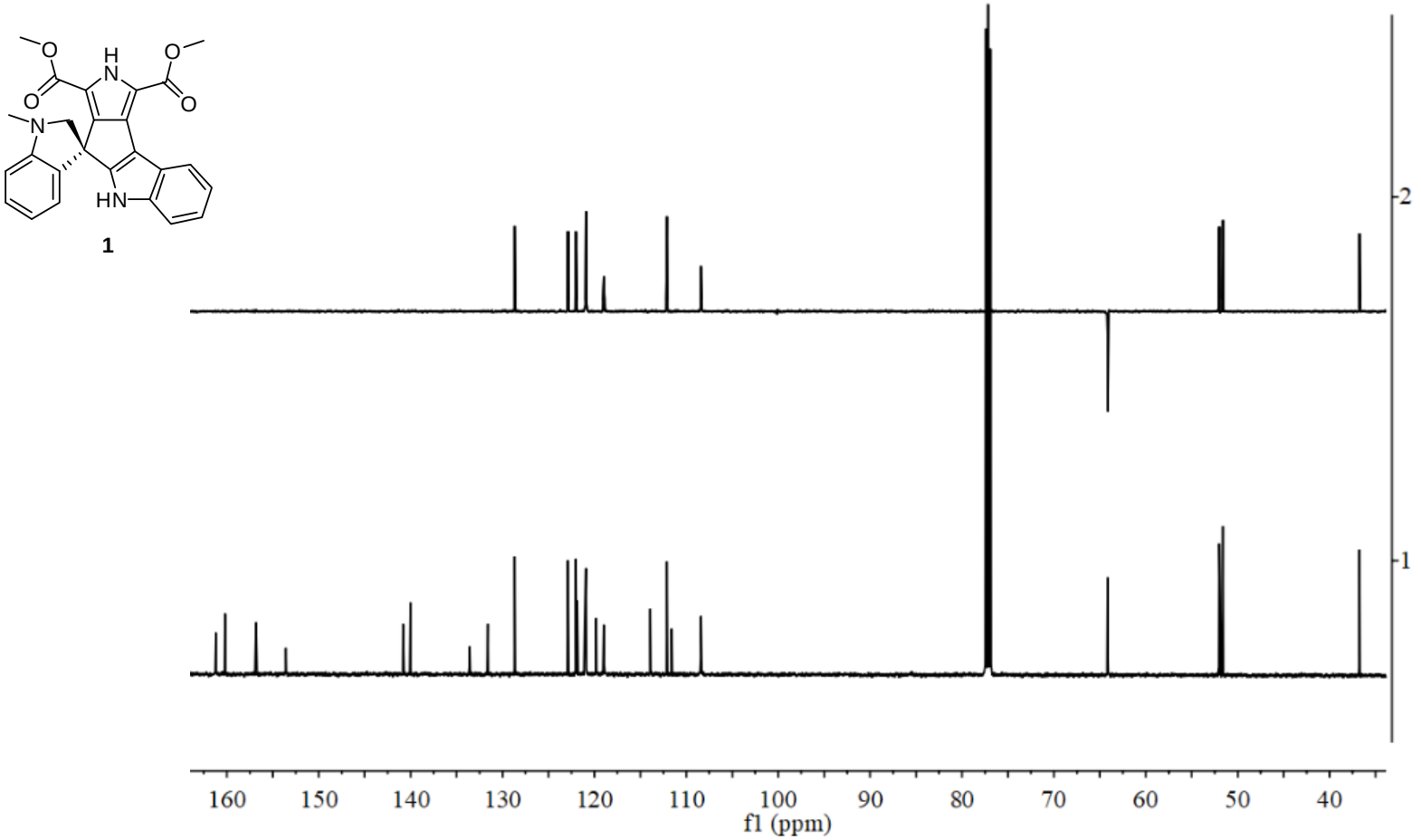


Fig. S4 The spectral data of **1**

(F) ^{1}H-^{1}H COSY spectrum of **1** ($CDCl_3$)

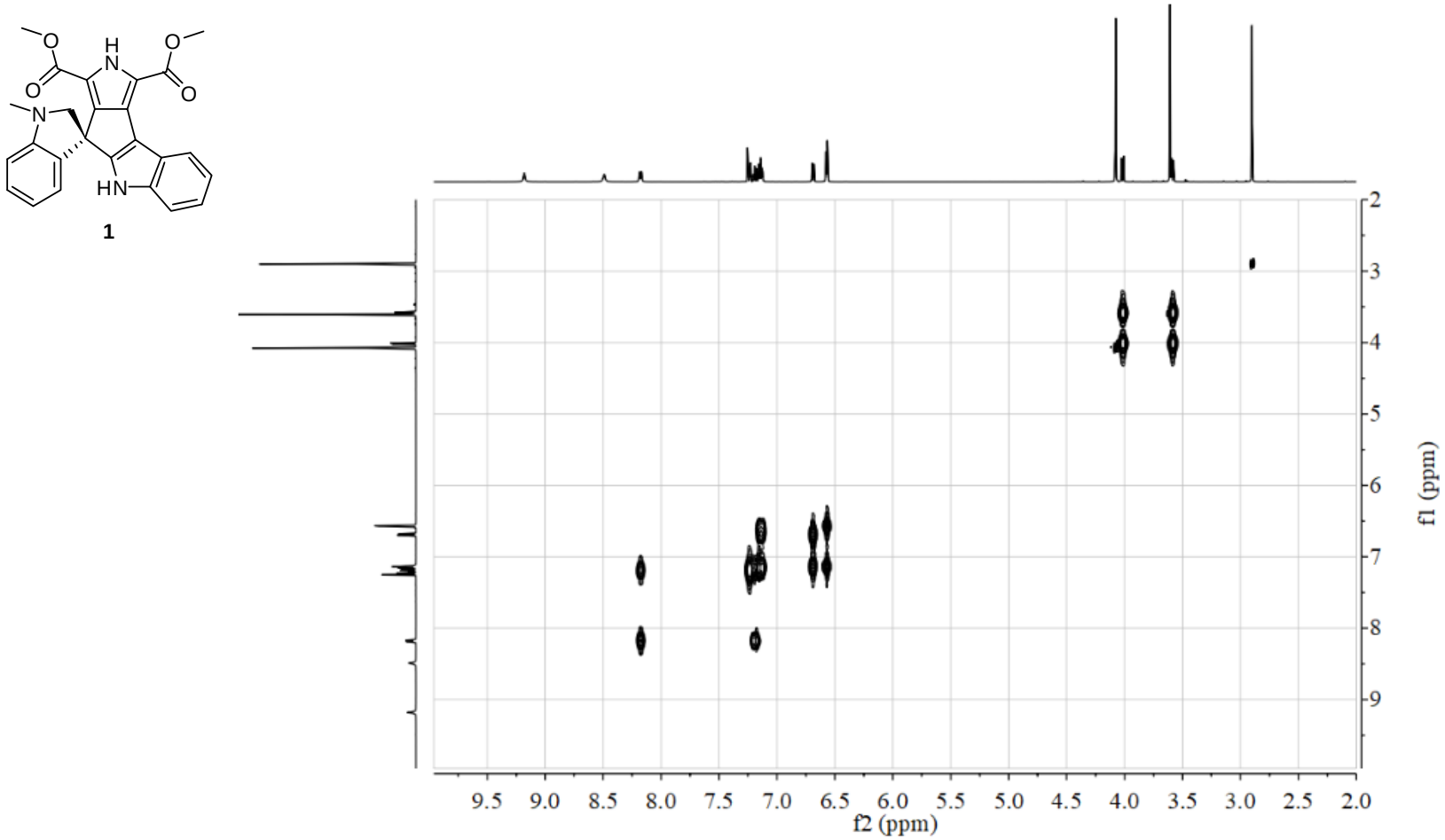
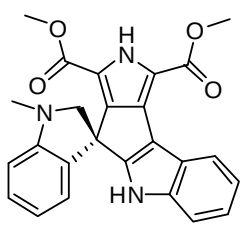


Fig. S4 The spectral data of **1**

(G) HSQC spectrum of **1** ($CDCl_3$)



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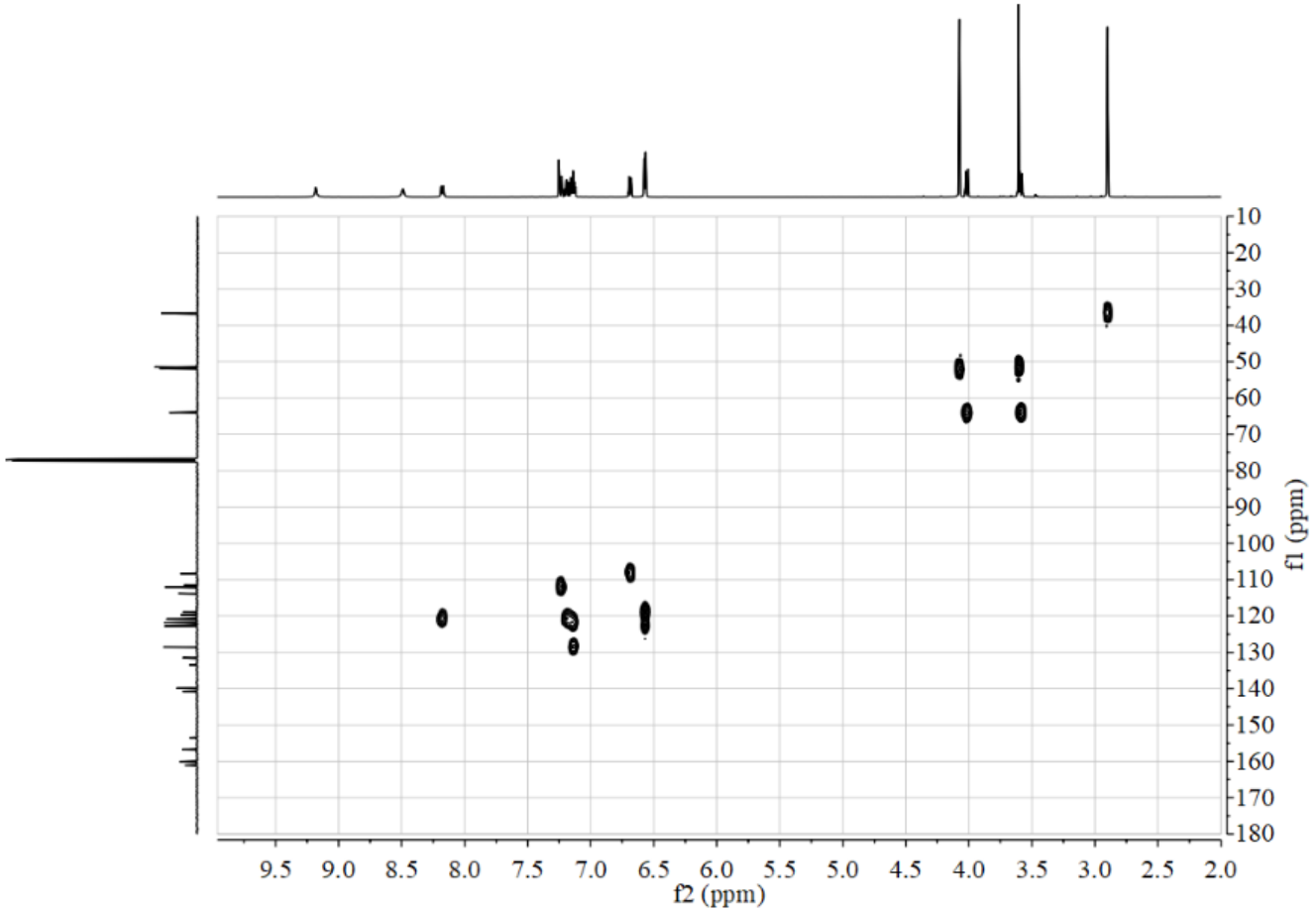
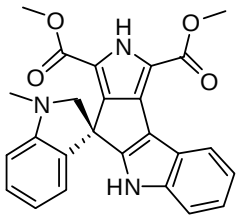


Fig. S4 The spectral data of **1**

(H) HMBC spectrum of **1** ($CDCl_3$)



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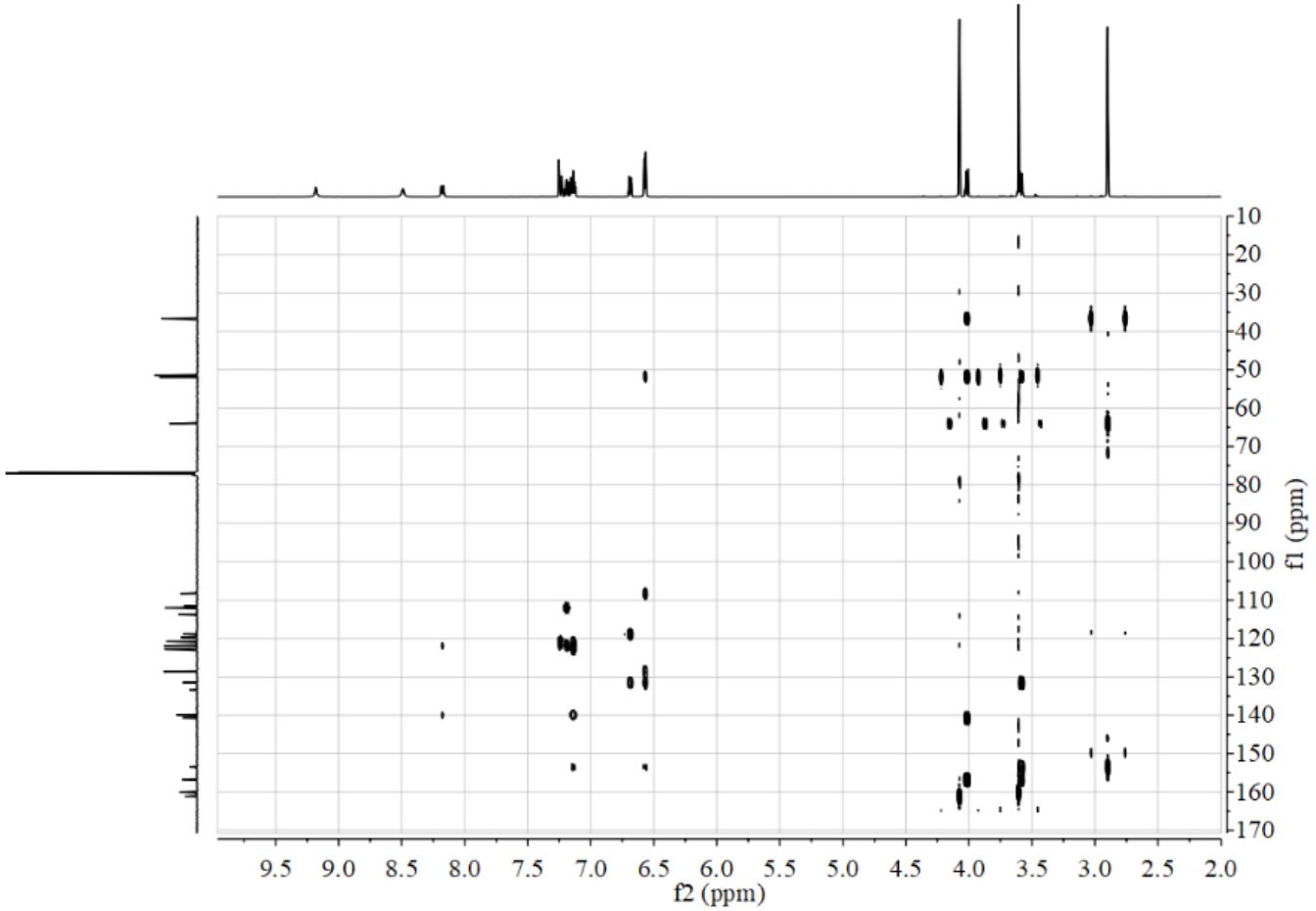
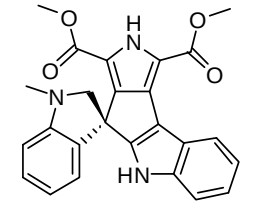


Fig. S4 The spectral data of **1**

(I) ECD spectra of compounds **1** and **SPM D**



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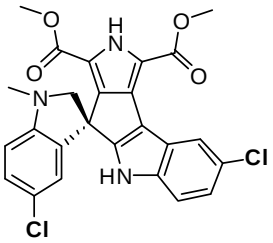


SPM D

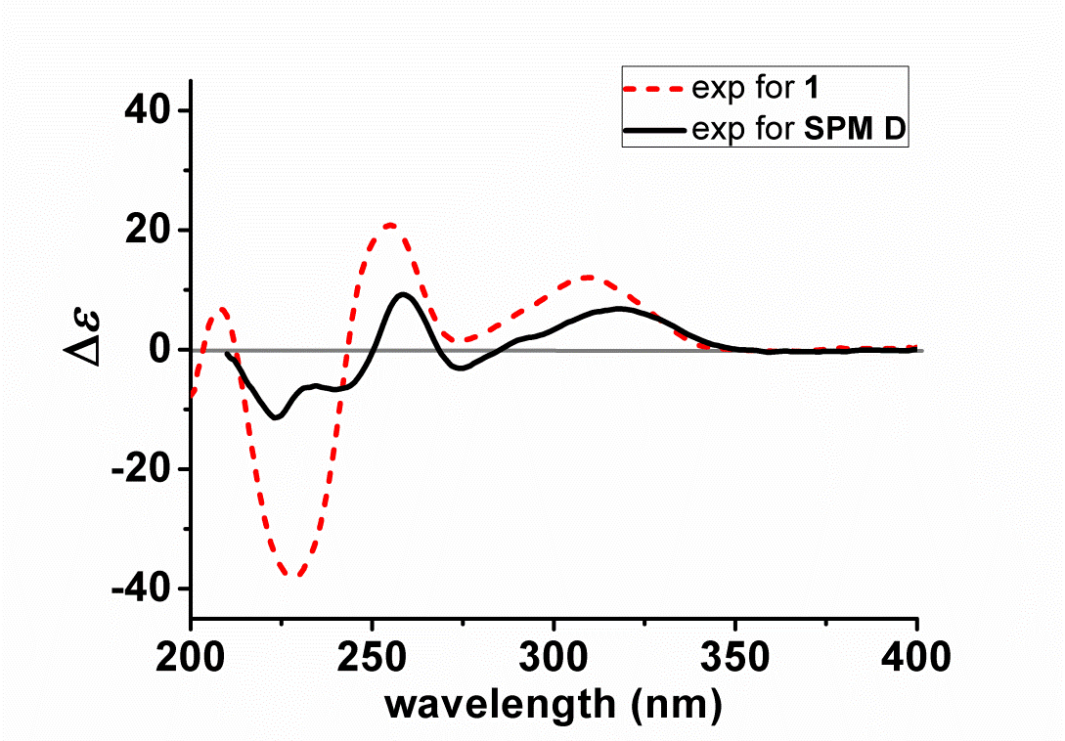


Fig. S5 The spectral data of **2**

(A) HR-ESI-MS spectrum of **2**

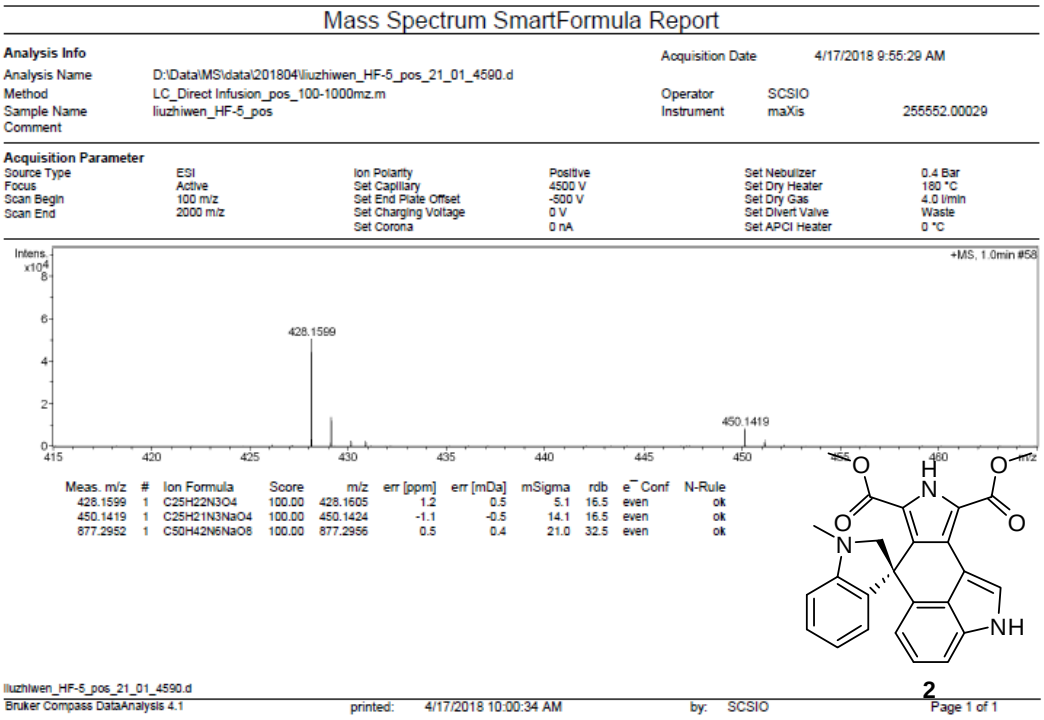


Fig. S5 The spectral data of **2**

(B) IR spectrum of **2**

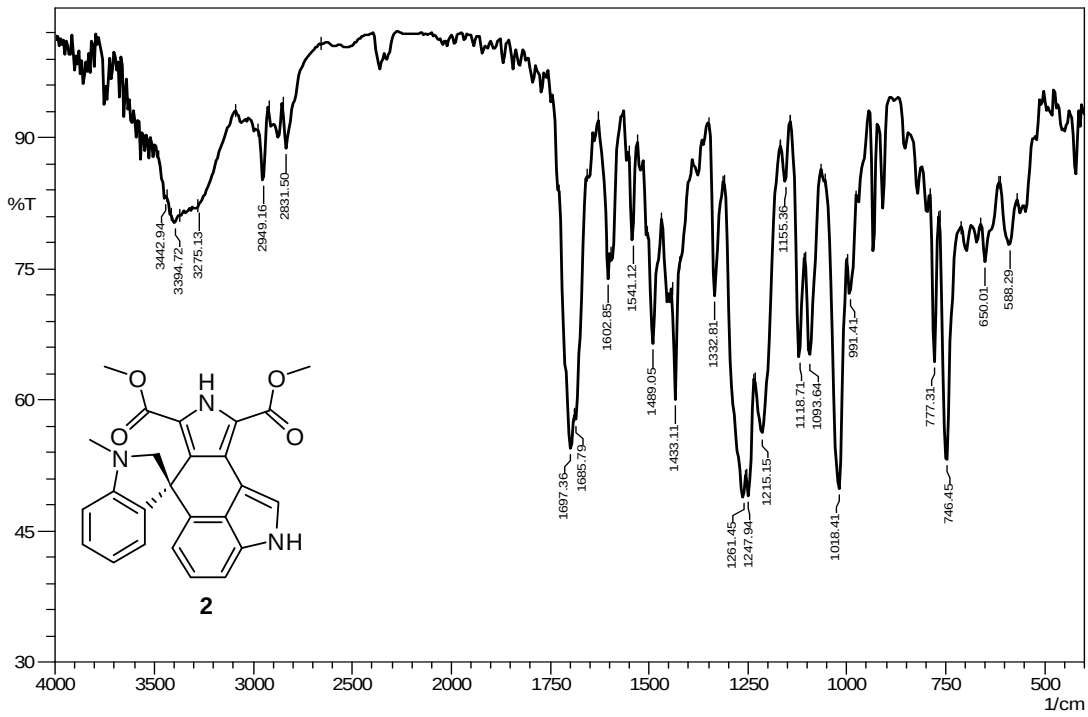


Fig. S5 The spectral data of **2**

(C) ^{1}H NMR spectrum of **2** ($CDCl_3$)

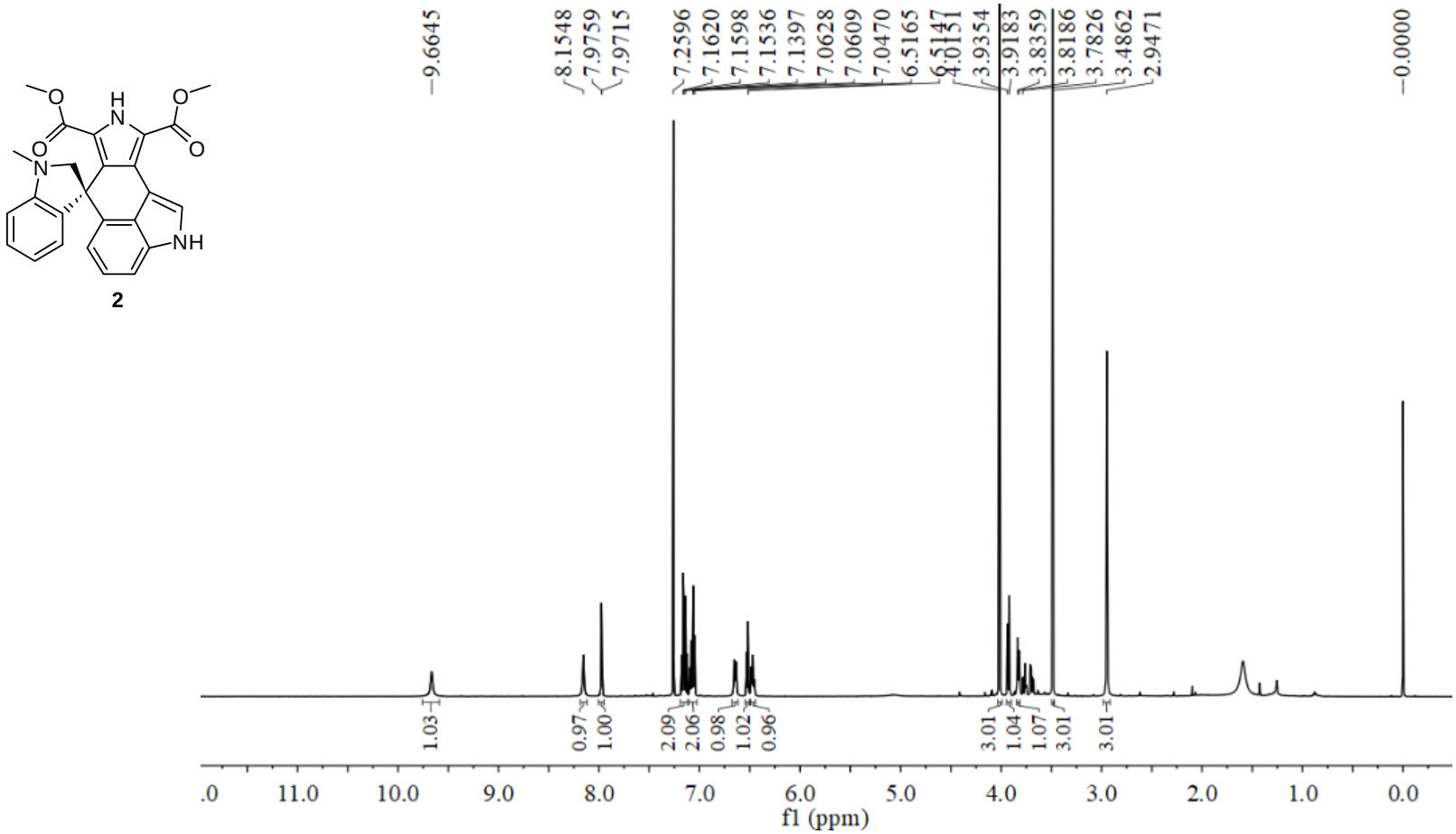
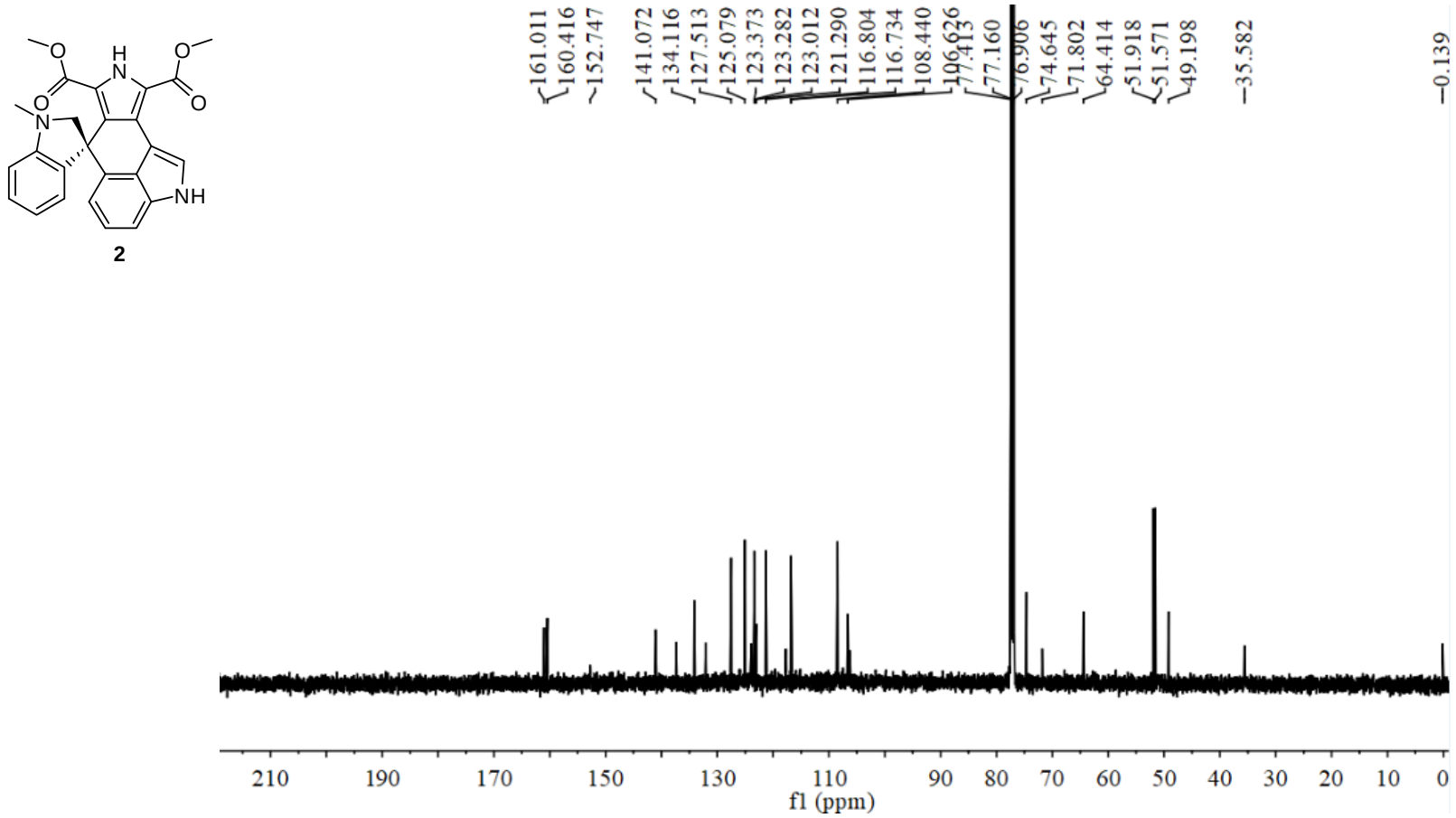


Fig. S5 The spectral data of **2**

(D) ^{13}C NMR spectrum of **2** ($CDCl_3$)



2

Fig. S5 The spectral data of **2**

(E) DEPT135 and ^{13}C NMR spectra of **2** ($CDCl_3$)

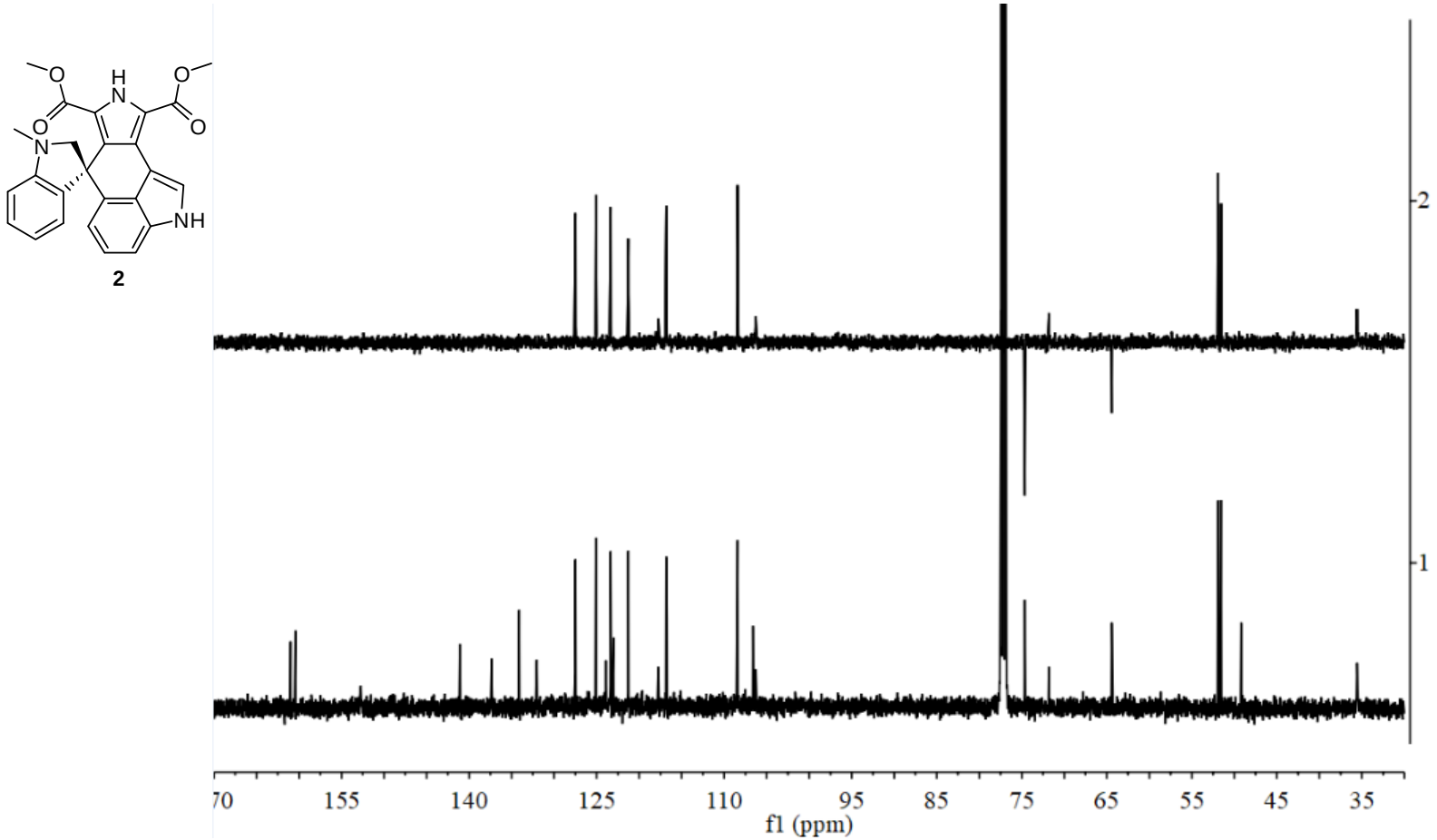


Fig. S5 The spectral data of **2**

(F) ^{1}H-^{1}H COSY spectrum of **2** ($CDCl_3$)

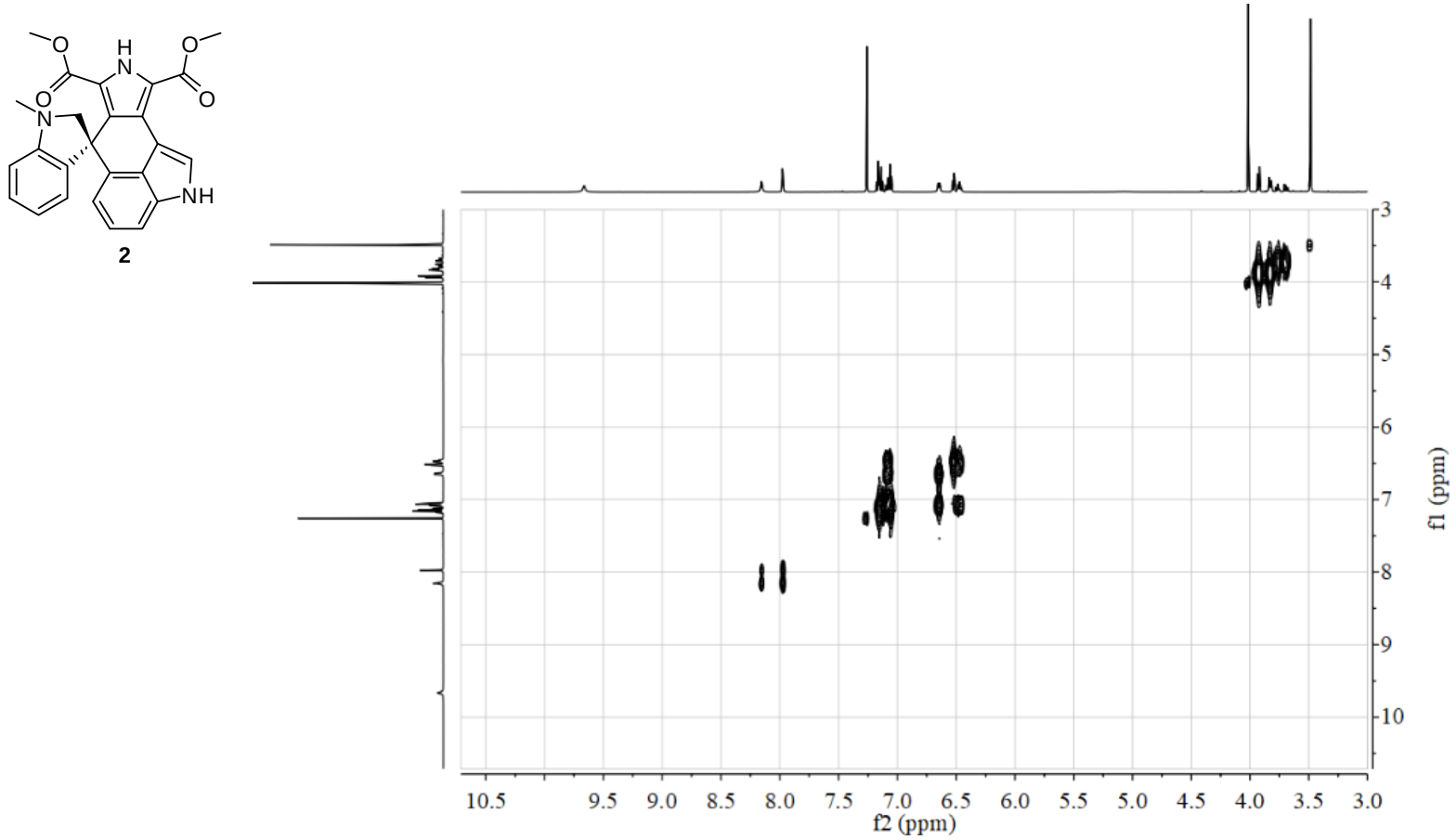


Fig. S5 The spectral data of **2**

(G) HSQC spectrum of **2** ($CDCl_3$)

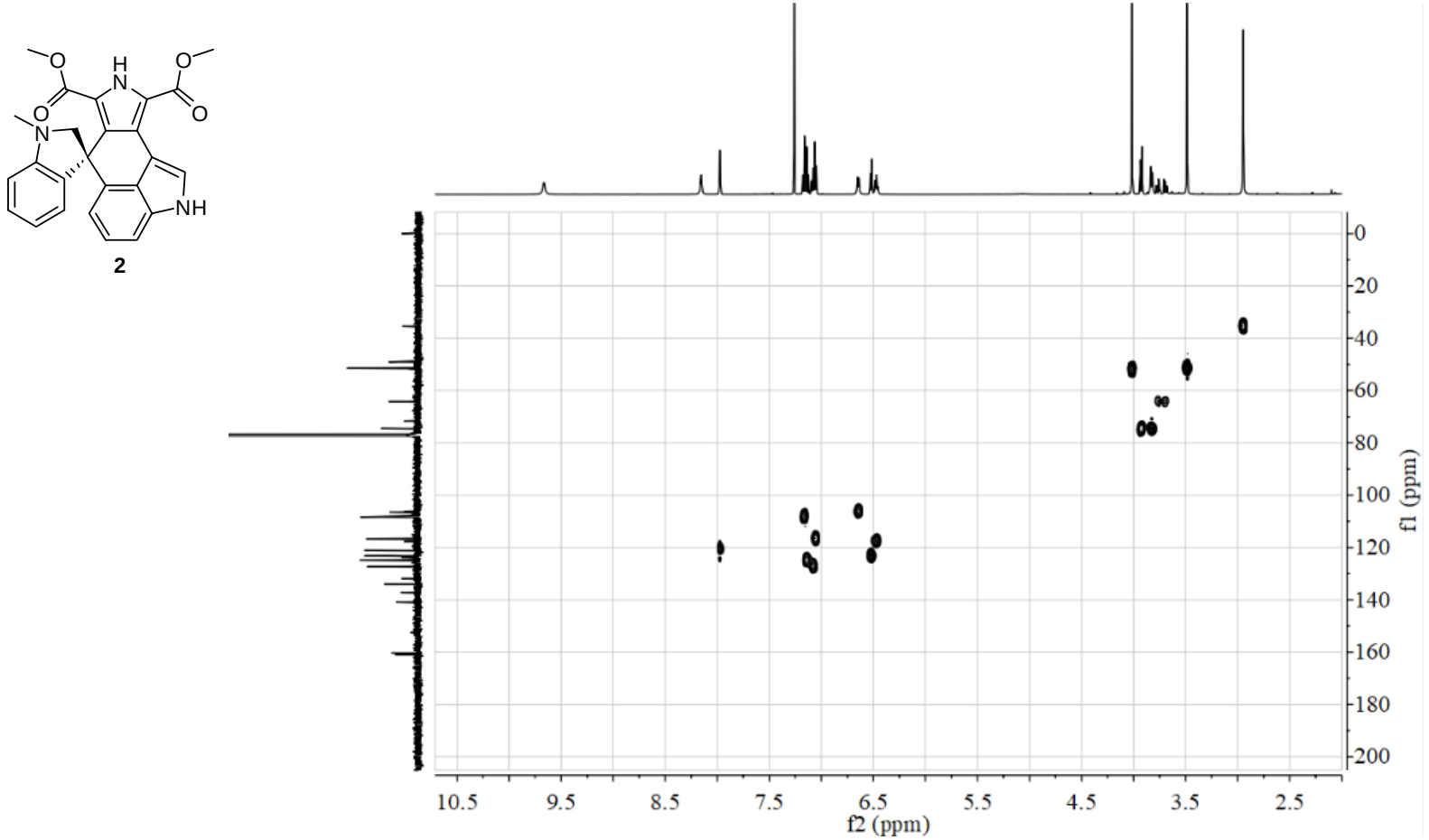
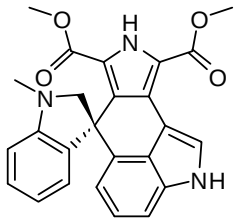


Fig. S5 The spectral data of **2**

(H) HMBC spectrum of **2** ($CDCl_3$)



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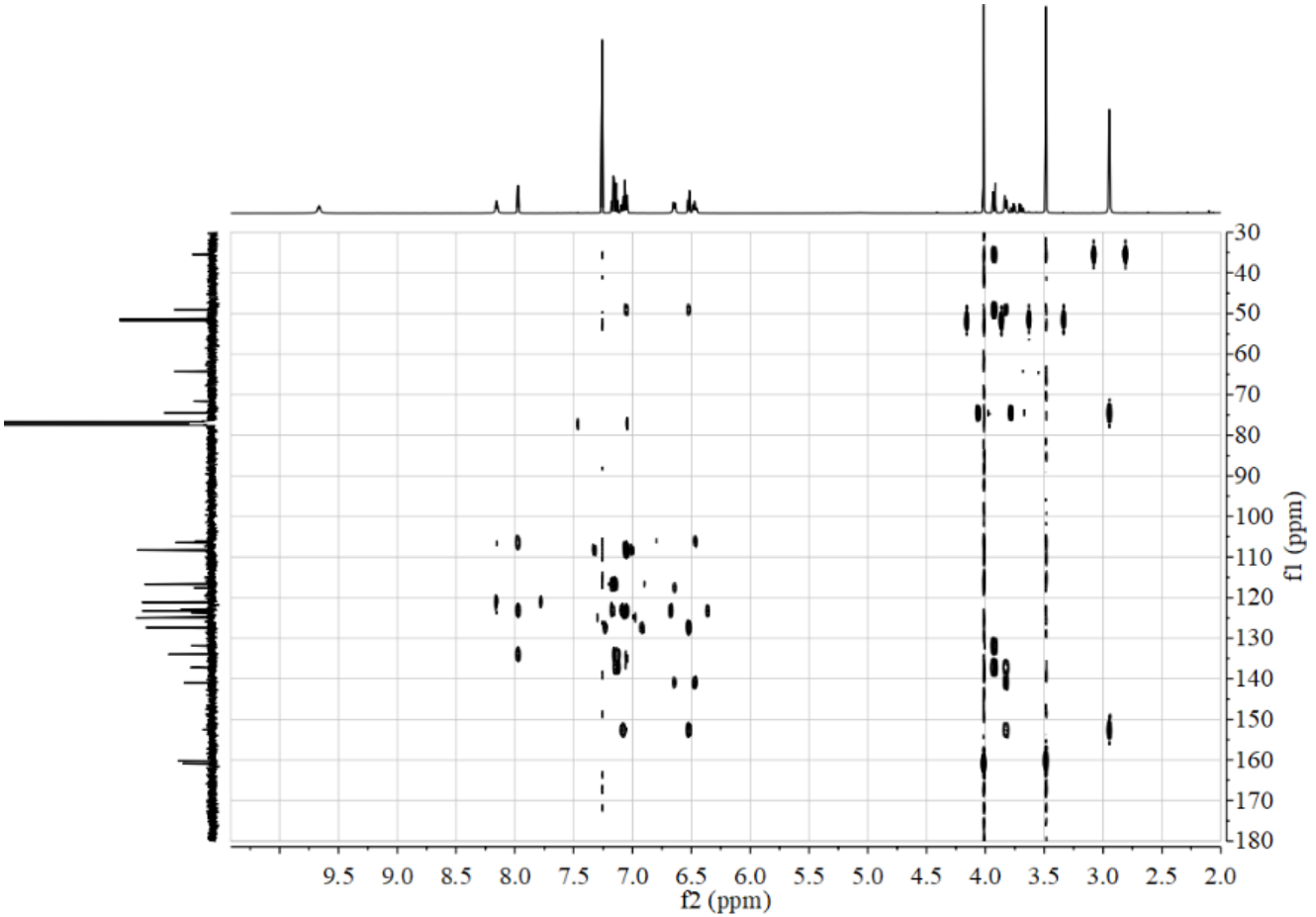


Fig. S6 The spectral data of **3**

(A) HR-ESI-MS spectrum of **3**

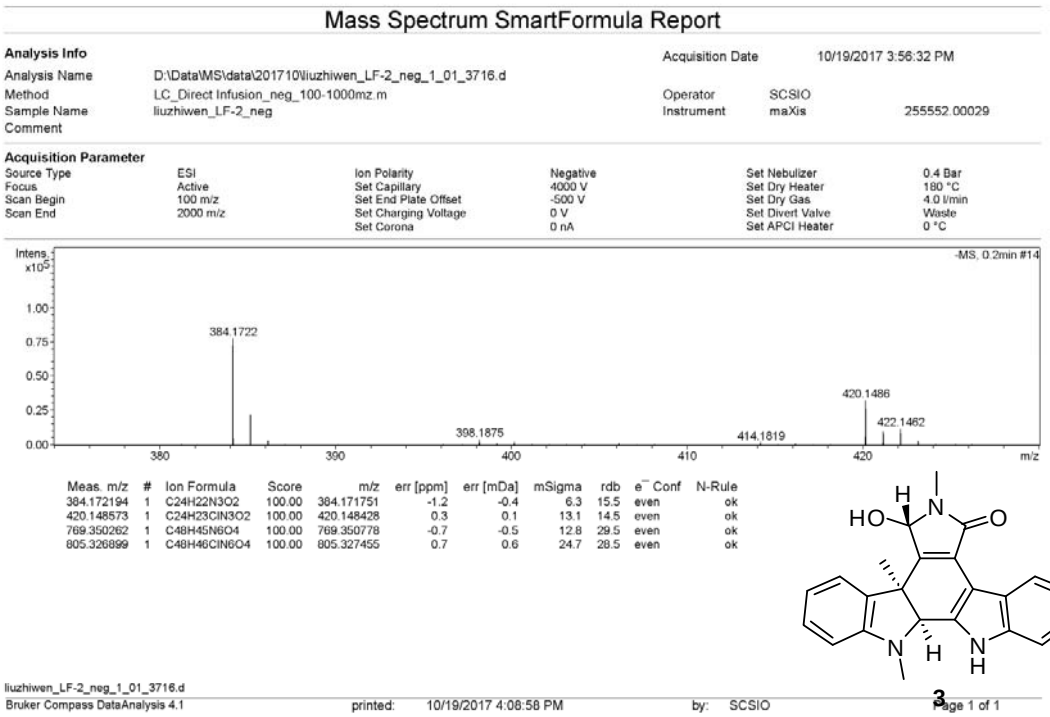


Fig. S6 The spectral data of **3**

(B) IR spectrum of **3**

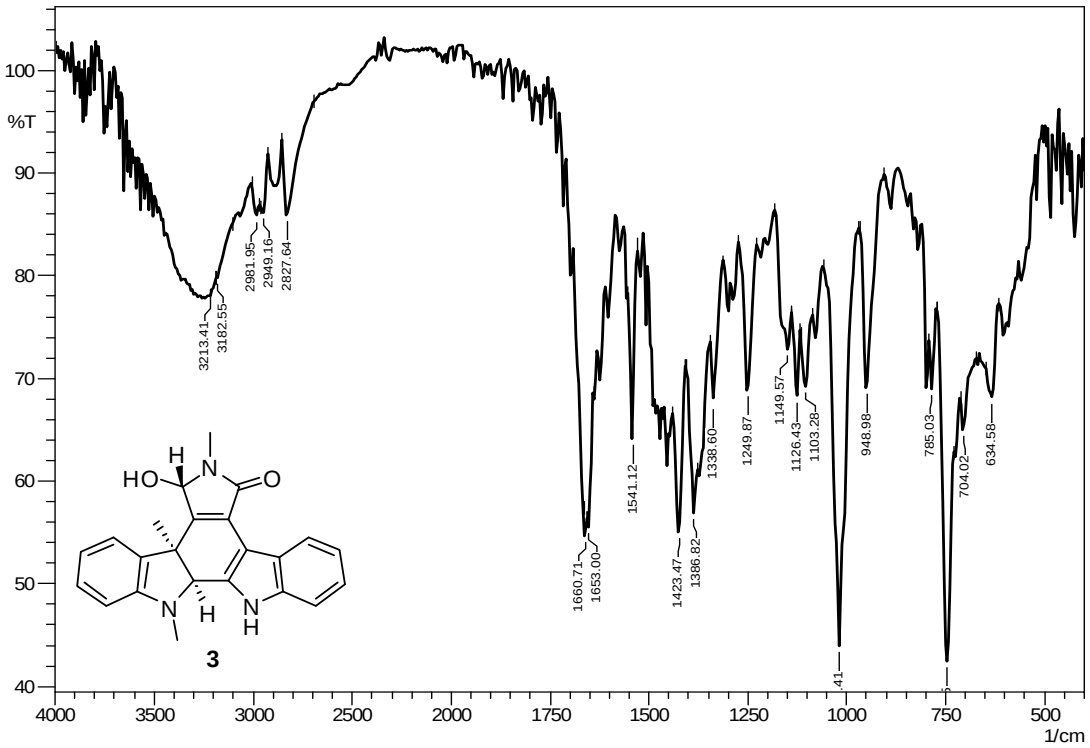


Fig. S6 The spectral data of **3**

(C) ^{1}H NMR spectrum of **3** (DMSO-d_6)

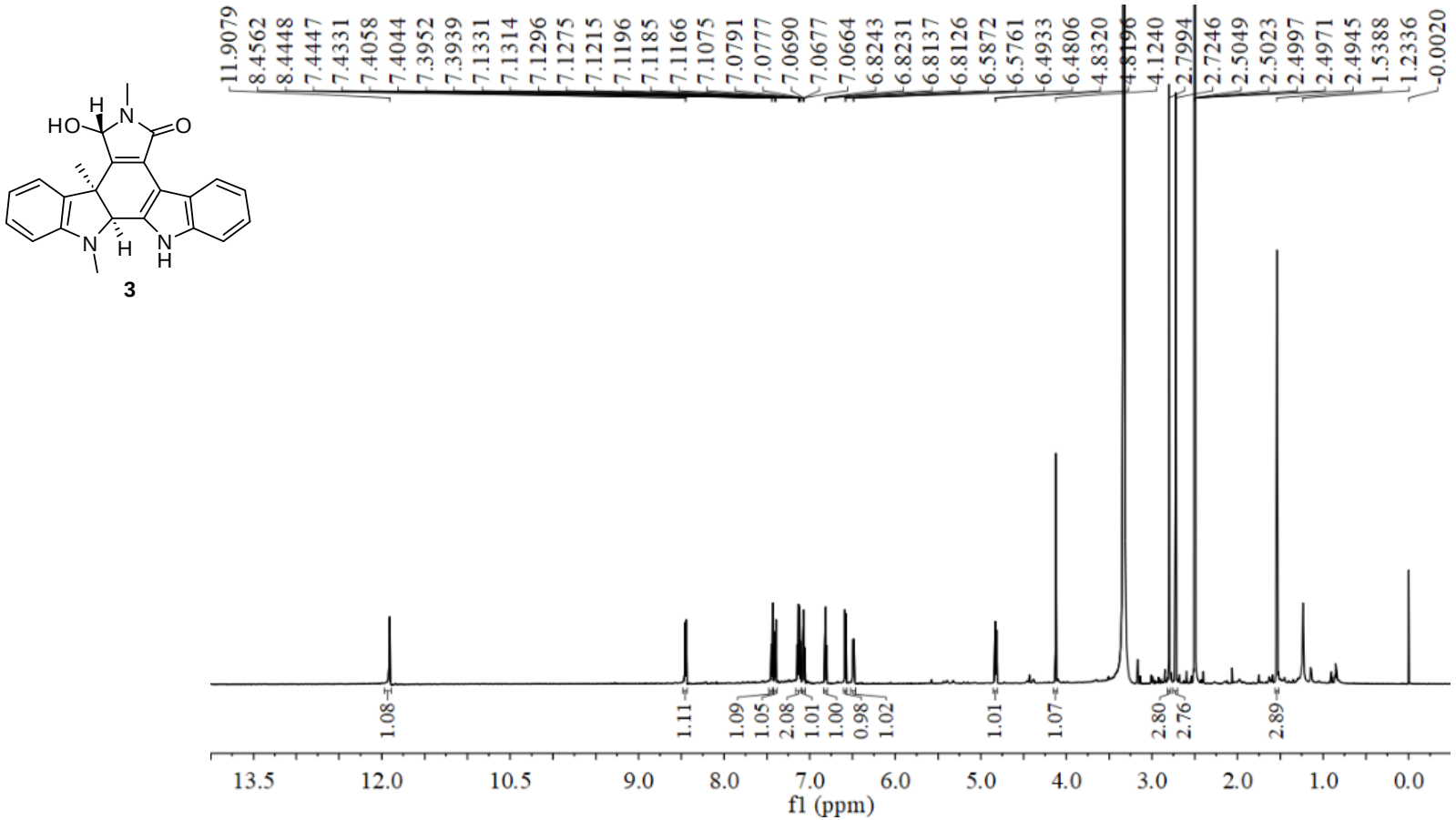


Fig. S6 The spectral data of **3**

(D) ^{13}C NMR spectrum of **3** (DMSO-d_6)

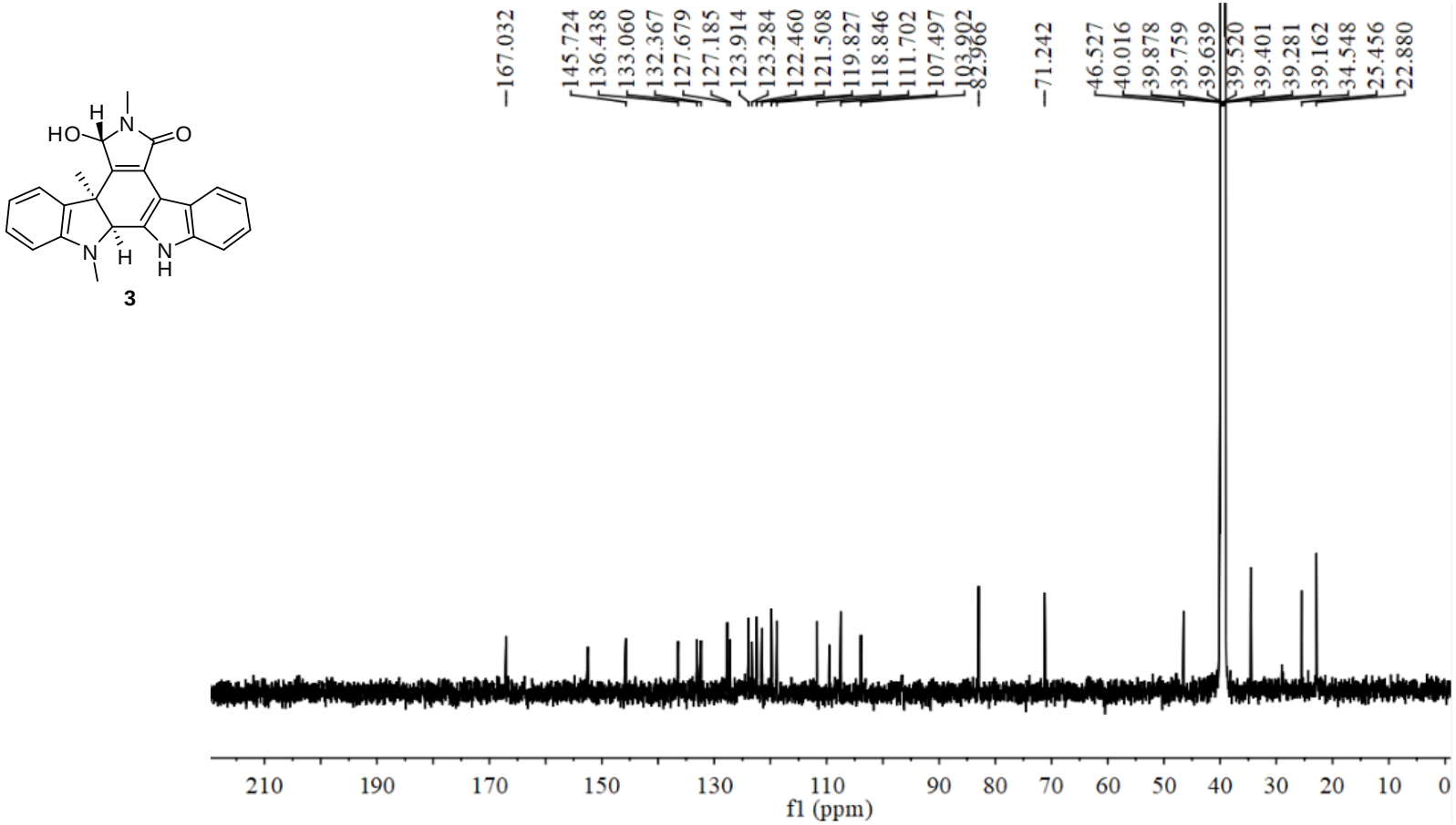


Fig. S6 The spectral data of **3**

(E) DEPT135 and ^{13}C NMR spectra of **3** (DMSO-d_6)

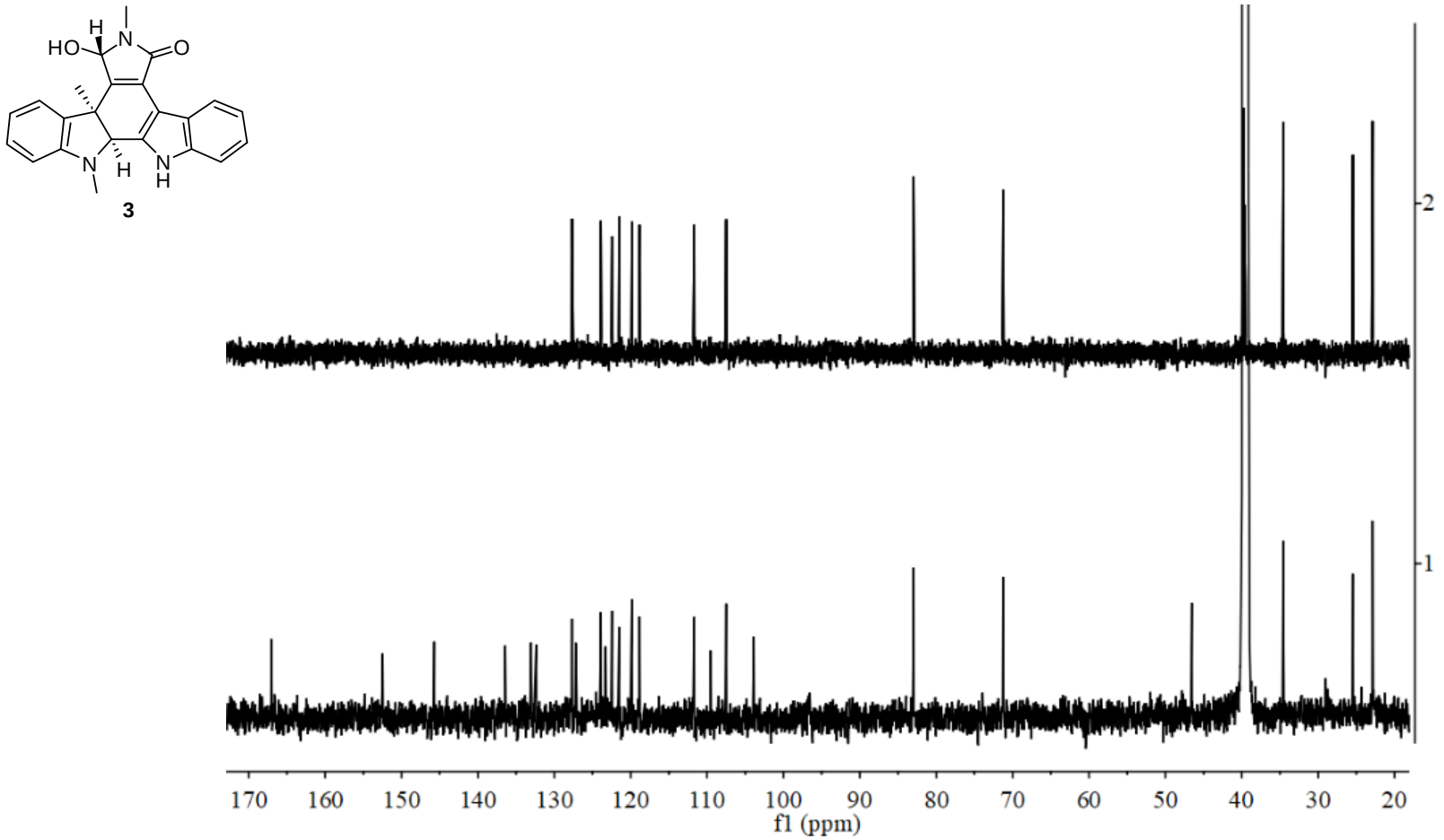
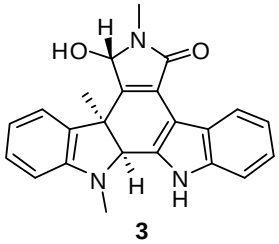


Fig. S6 The spectral data of **3**

(F) ^{1}H-^{1}H COSY spectrum of **3** (DMSO-d_6)



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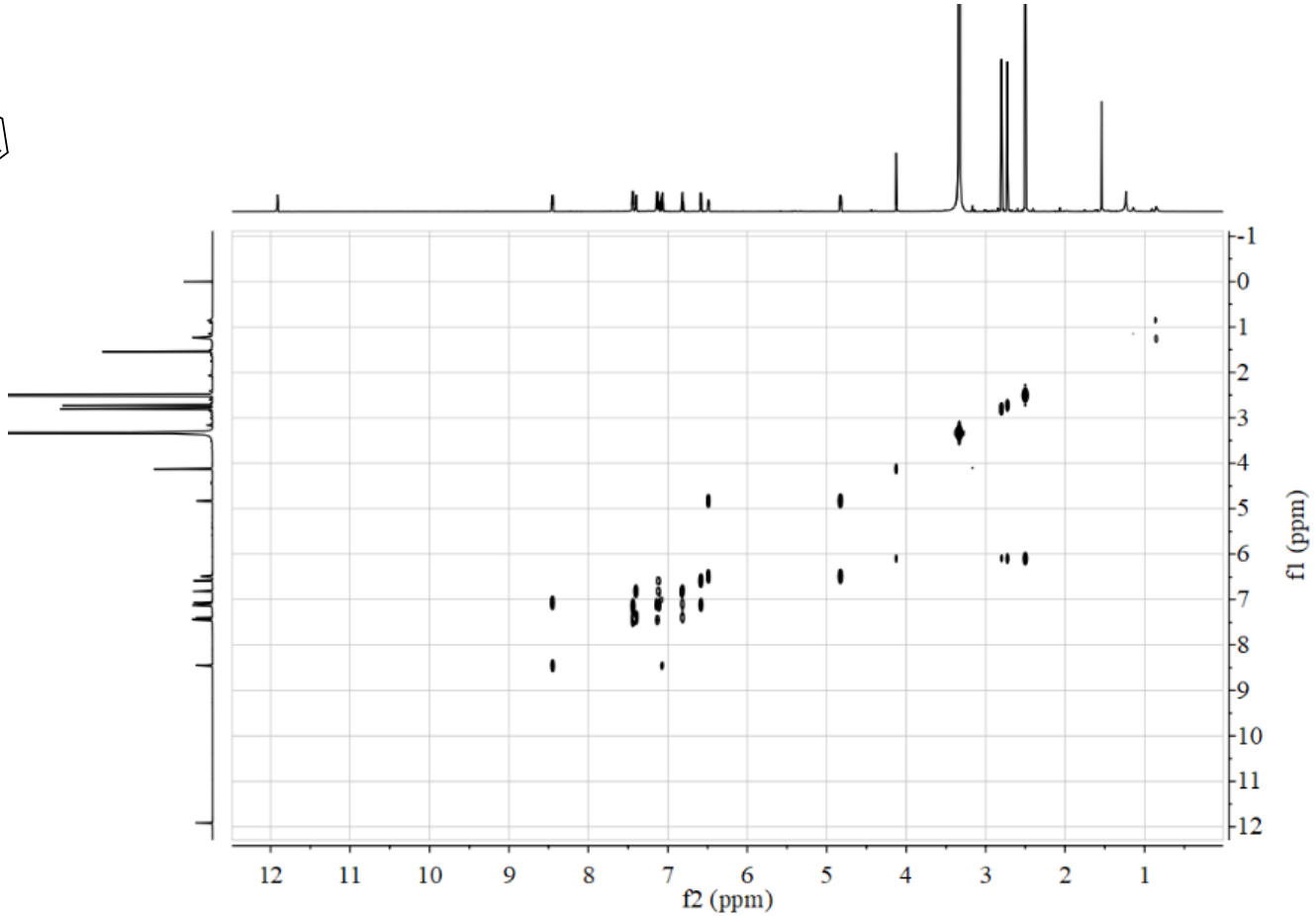


Fig. S6 The spectral data of **3**

(G) HSQC spectrum of **3** (DMSO-d_6)

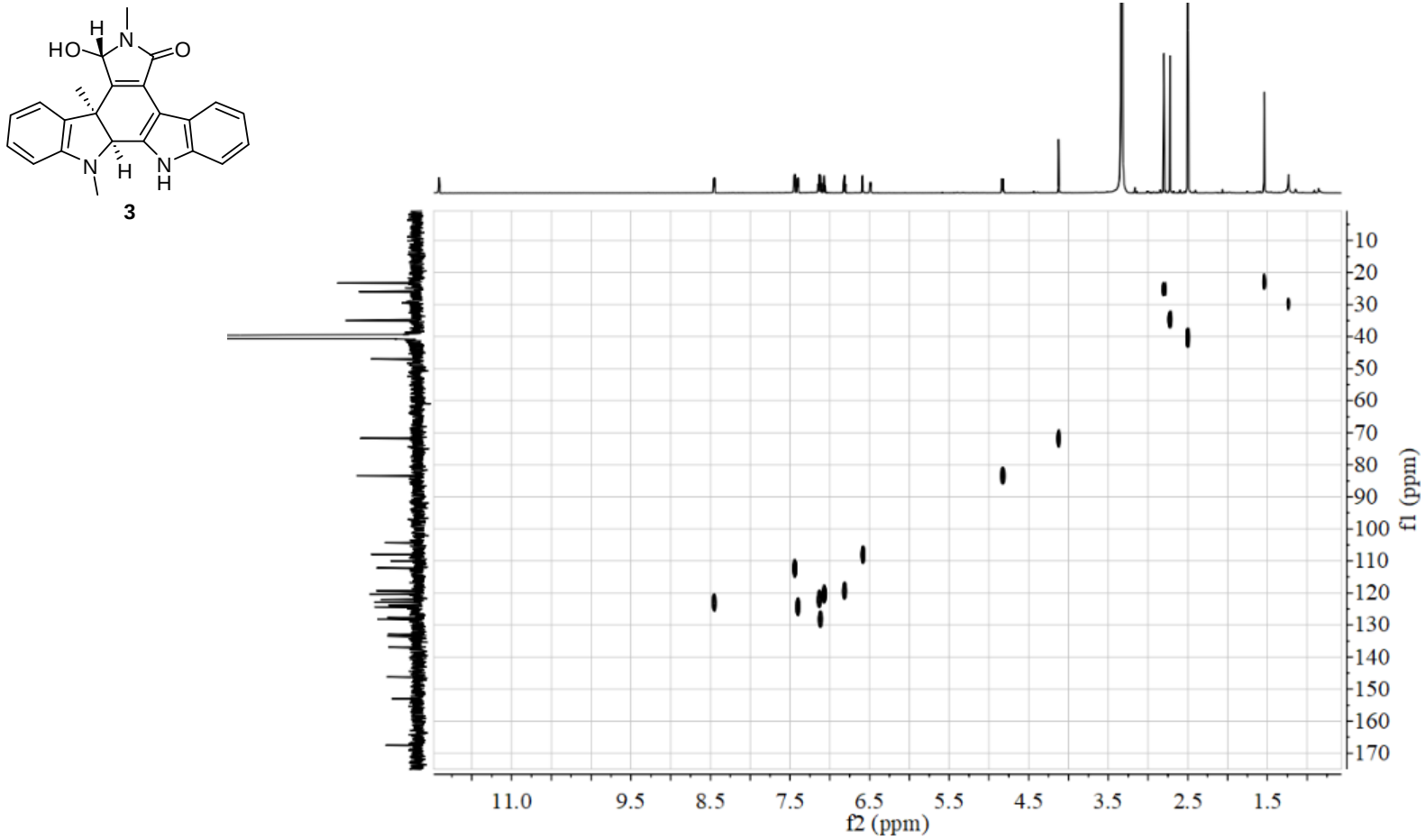


Fig. S6 The spectral data of **3**

(H) HMBC spectrum of **3** (DMSO-d_6)

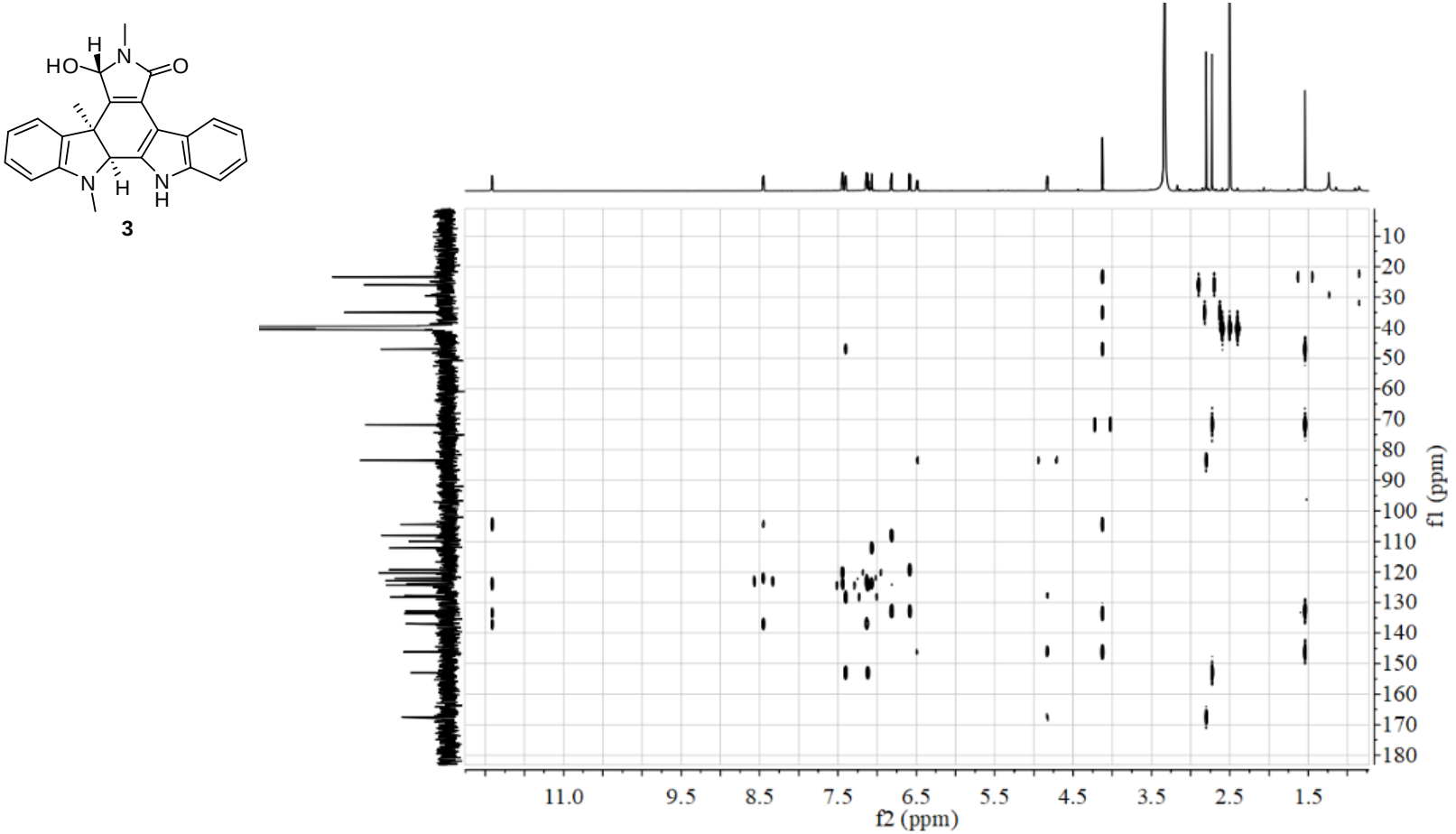


Fig. S6 The spectral data of **3**

(B) NOESY spectrum of **3** (DMSO-d_6)

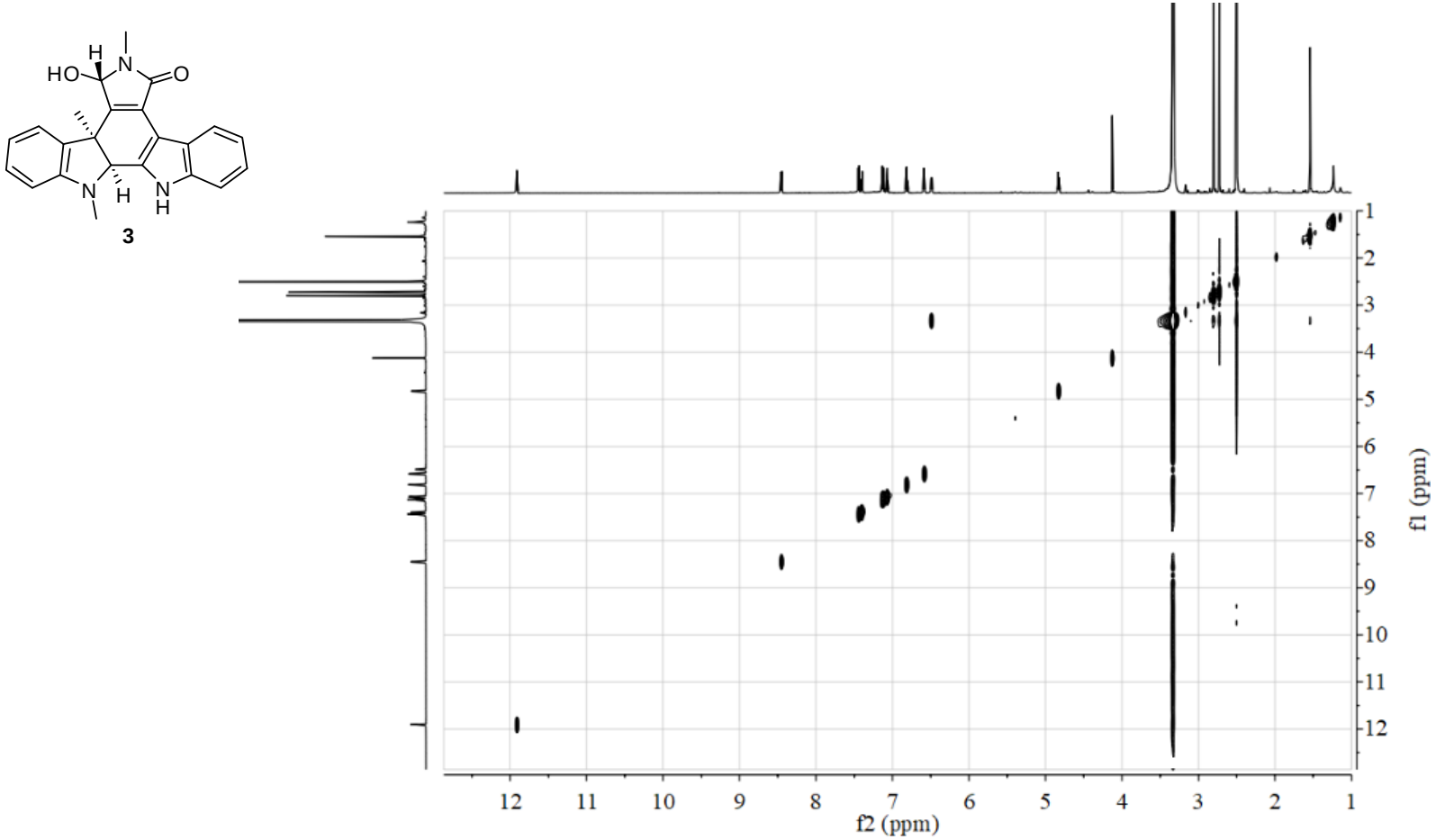


Fig. S7 The spectral data of **4**

(A) HR-ESI-MS spectrum of **4**

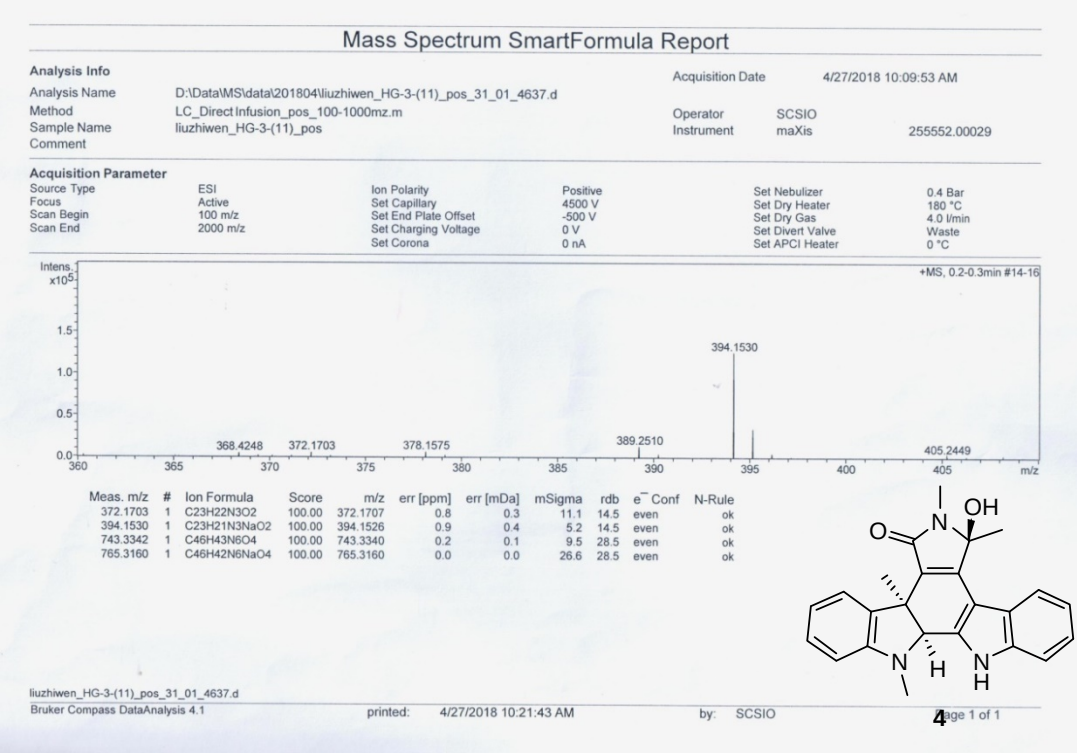


Fig. S7 The spectral data of **4**

(B) IR spectrum of **4**

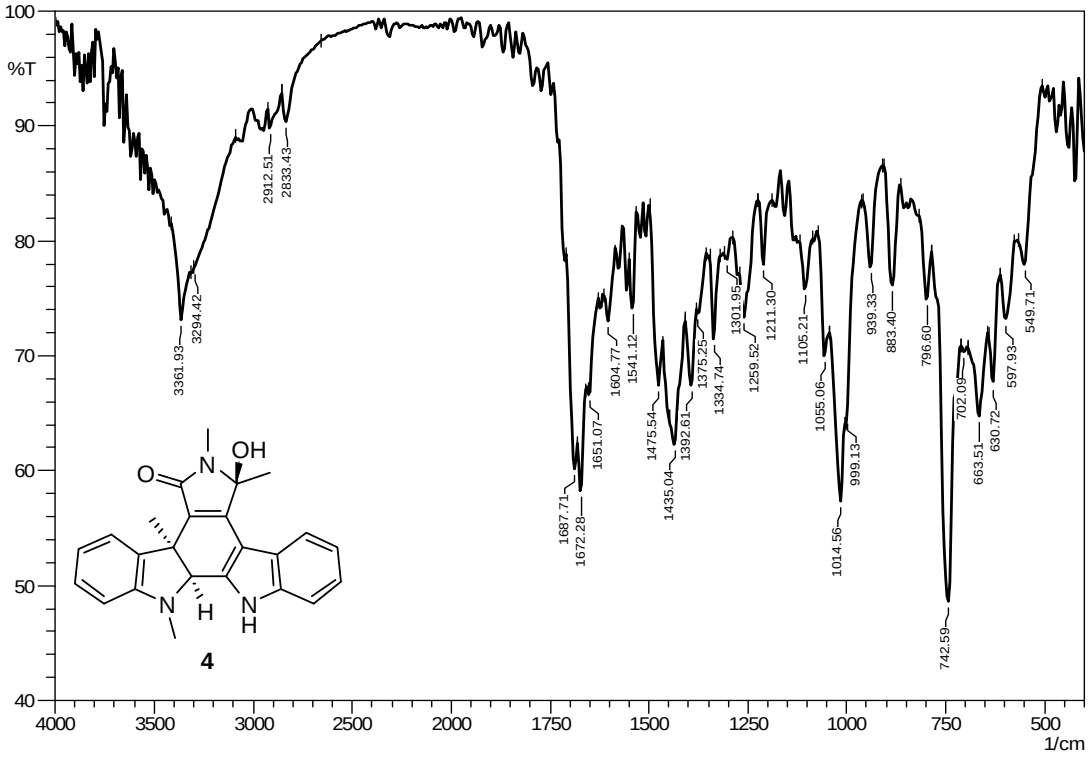


Fig. S7 The spectral data of **4**

(C) ^{1}H NMR spectrum of **4** ($CDCl_3$)

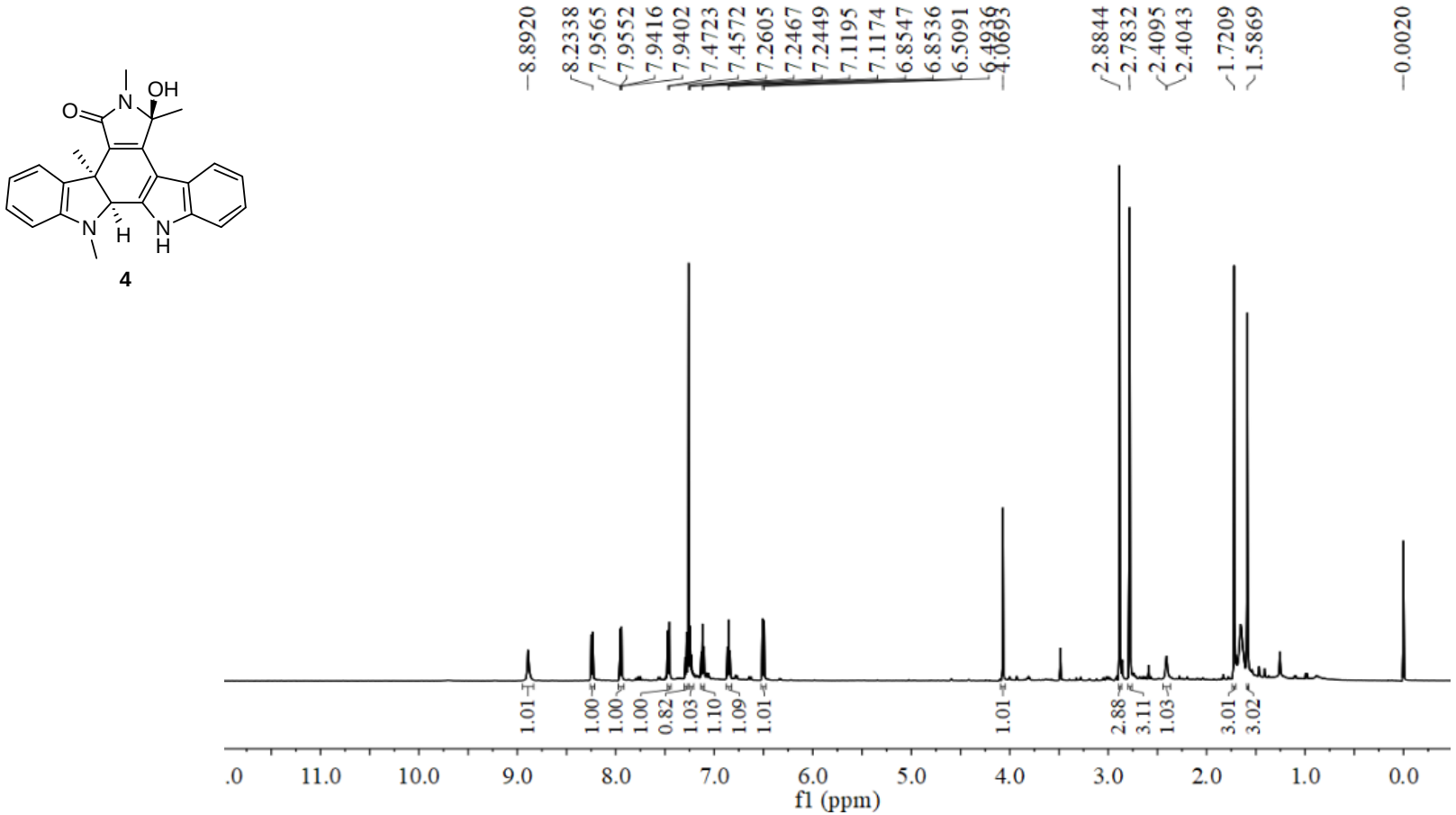
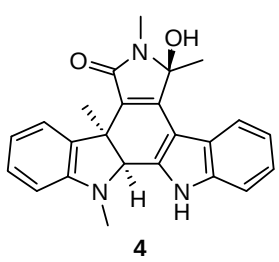


Fig. S7 The spectral data of **4**

(D) ^{13}C NMR spectrum of **4** ($CDCl_3$)



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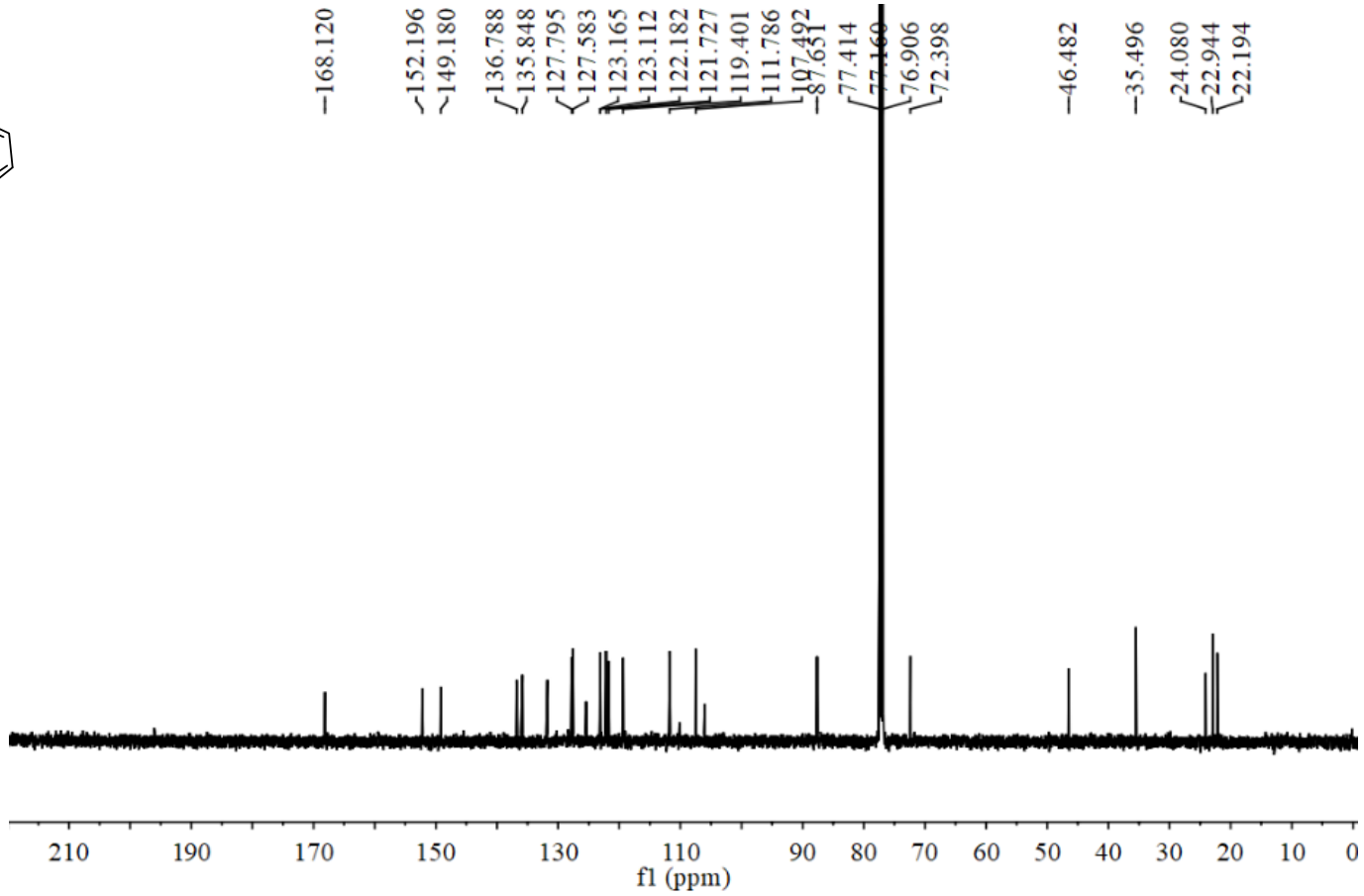
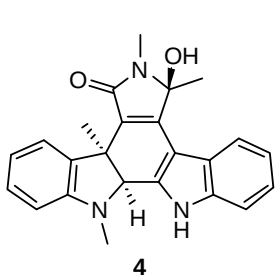


Fig. S7 The spectral data of **4**

(E) DEPT135 and ^{13}C NMR spectra of **4** ($CDCl_3$)



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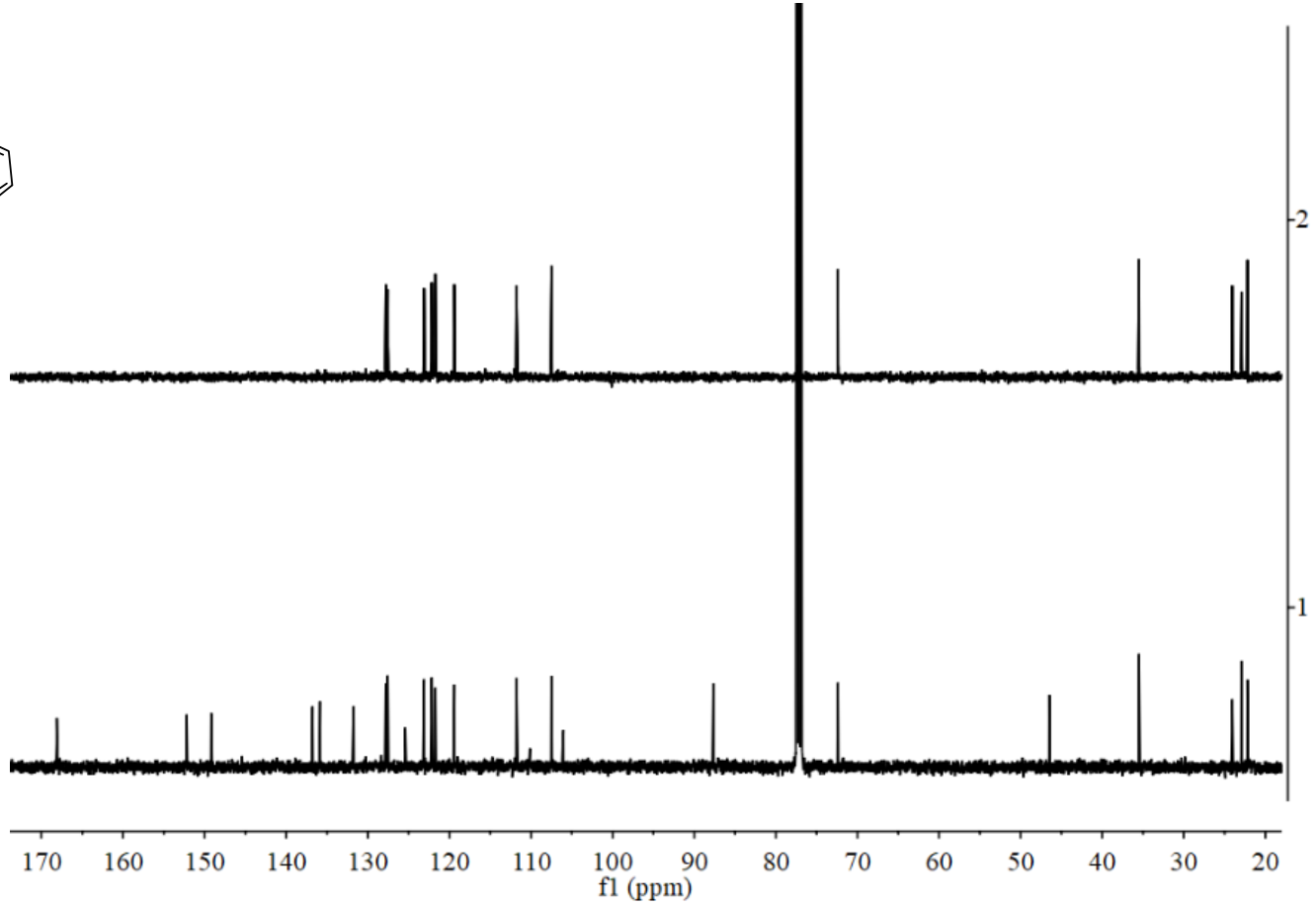


Fig. S7 The spectral data of **4**

(F) ^{1}H-^{1}H COSY spectrum of **4** ($CDCl_3$)

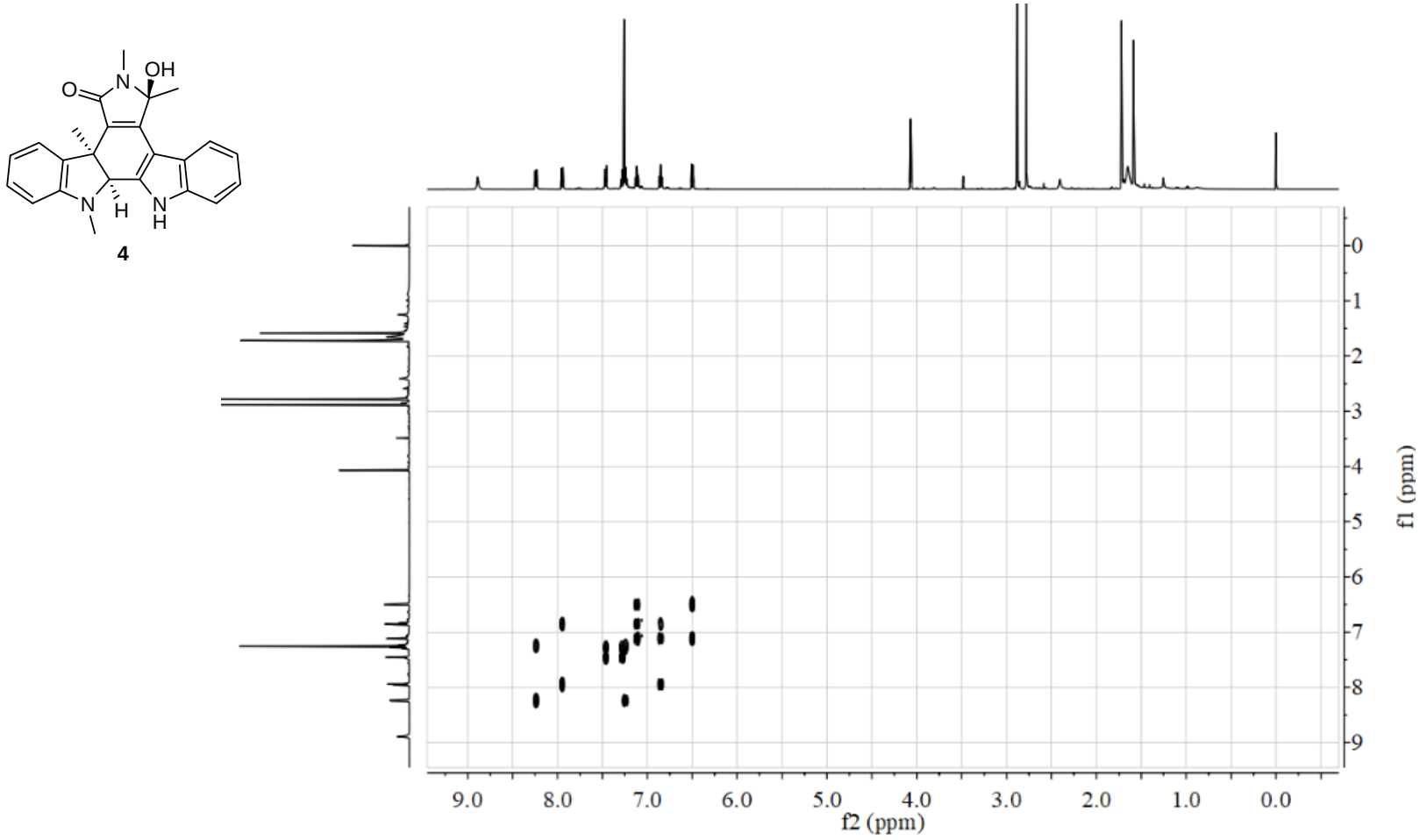


Fig. S7 The spectral data of **4**

(G) HSQC spectrum of **4** ($CDCl_3$)

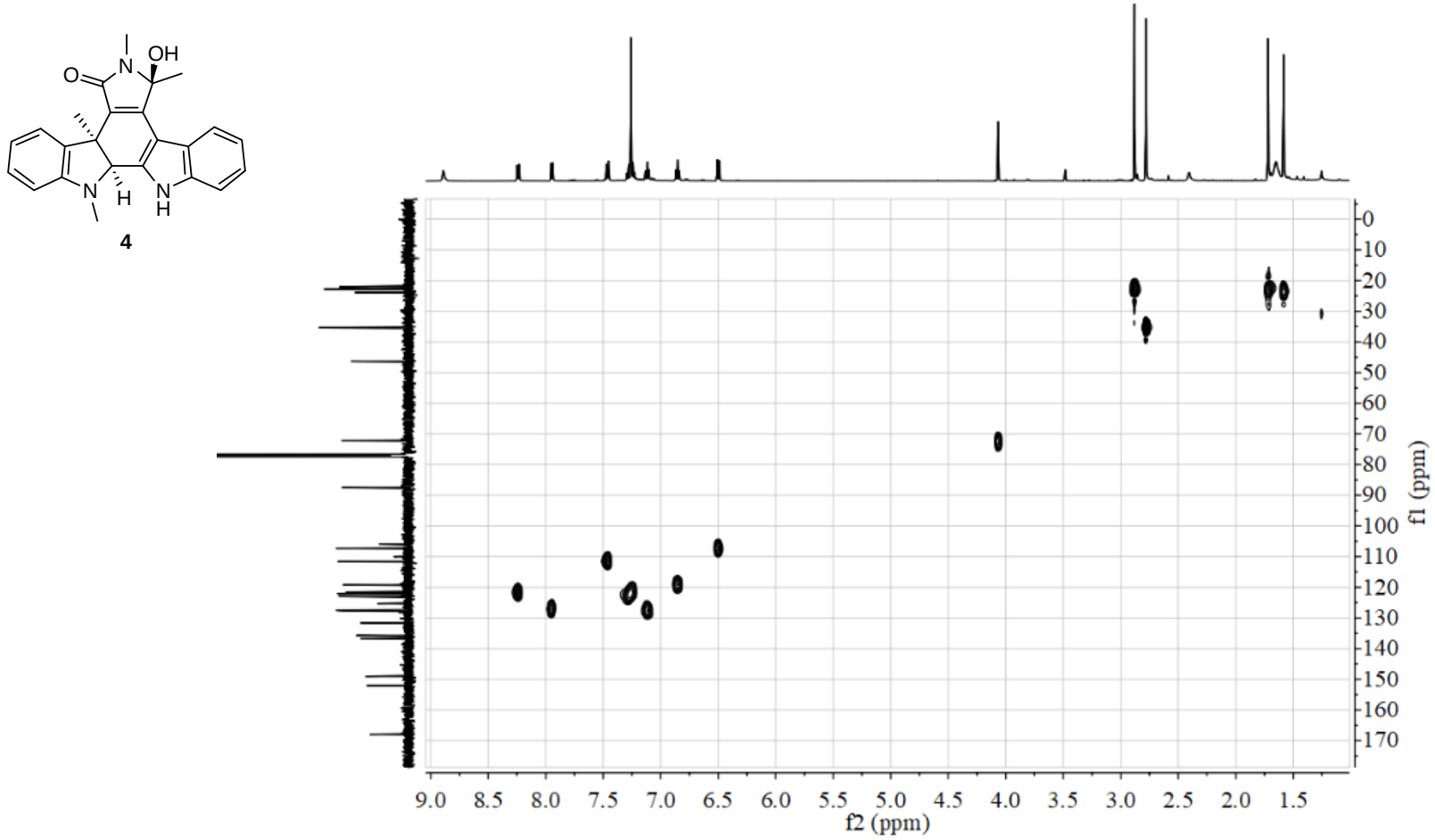


Fig. S7 The spectral data of **4**

(H) HMBC spectrum of **4** ($CDCl_3$)

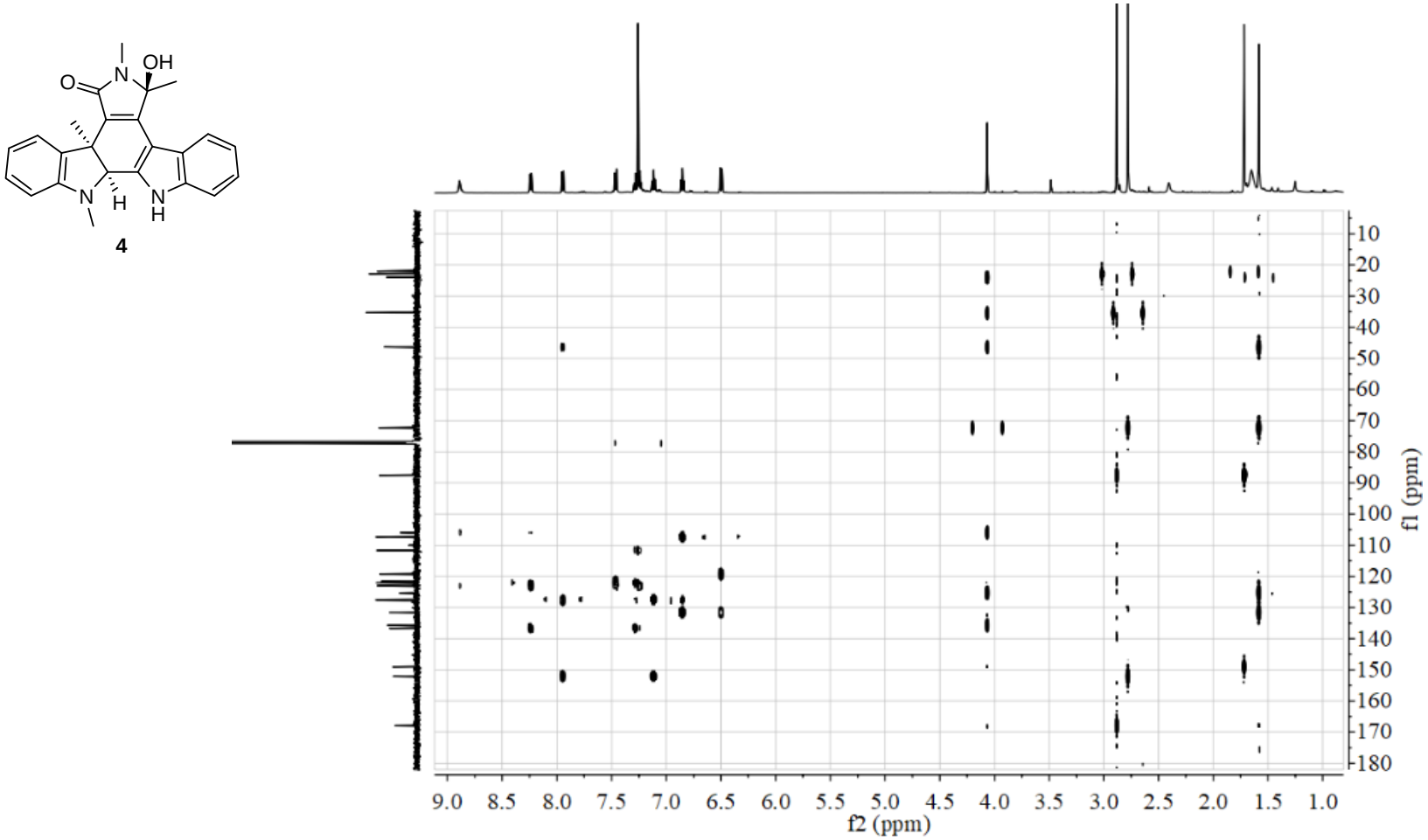
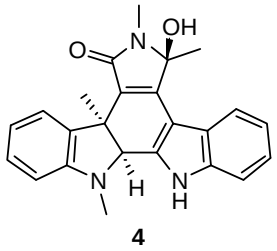


Fig. S7 The spectral data of **4**

(I) NOESY spectrum of **4** ($CDCl_3$)



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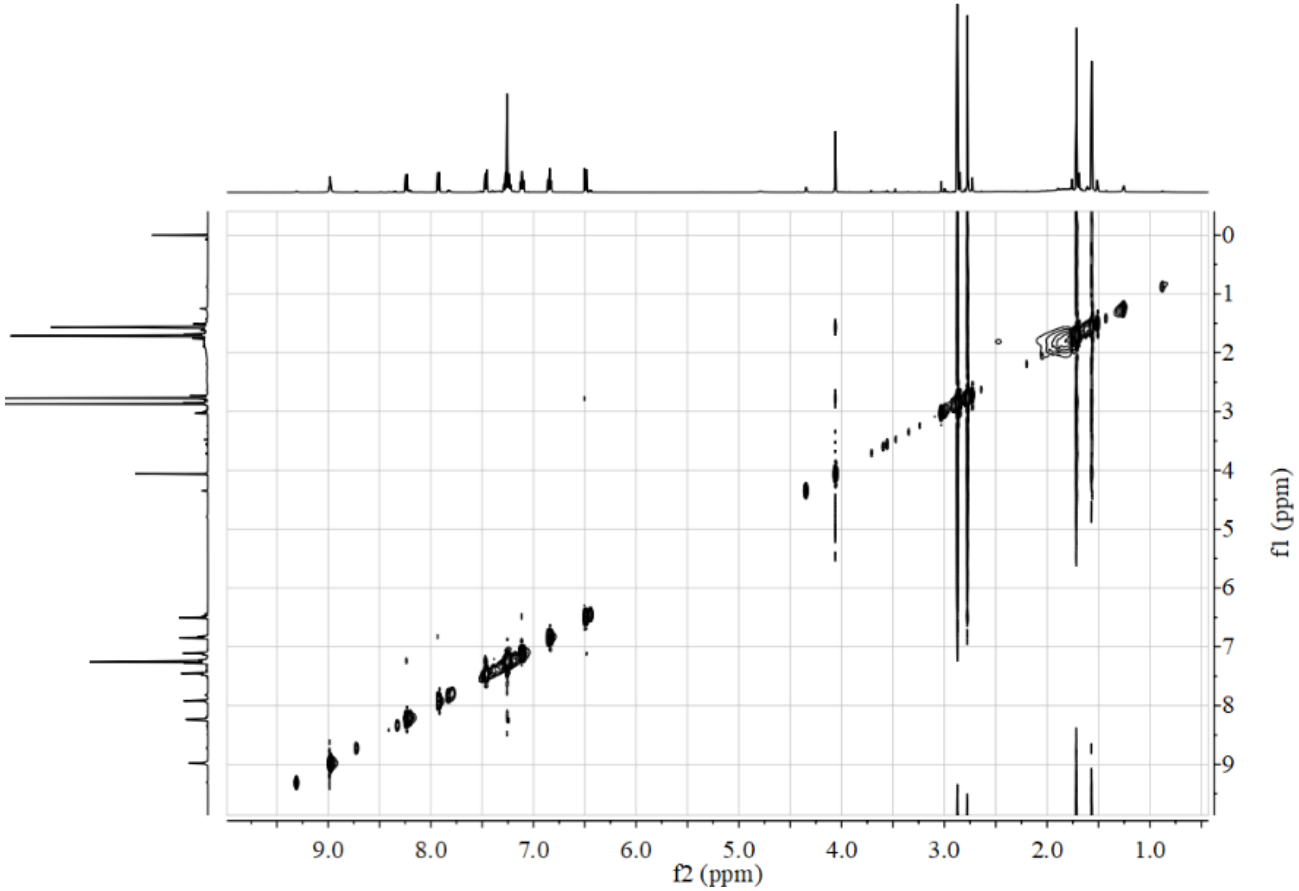
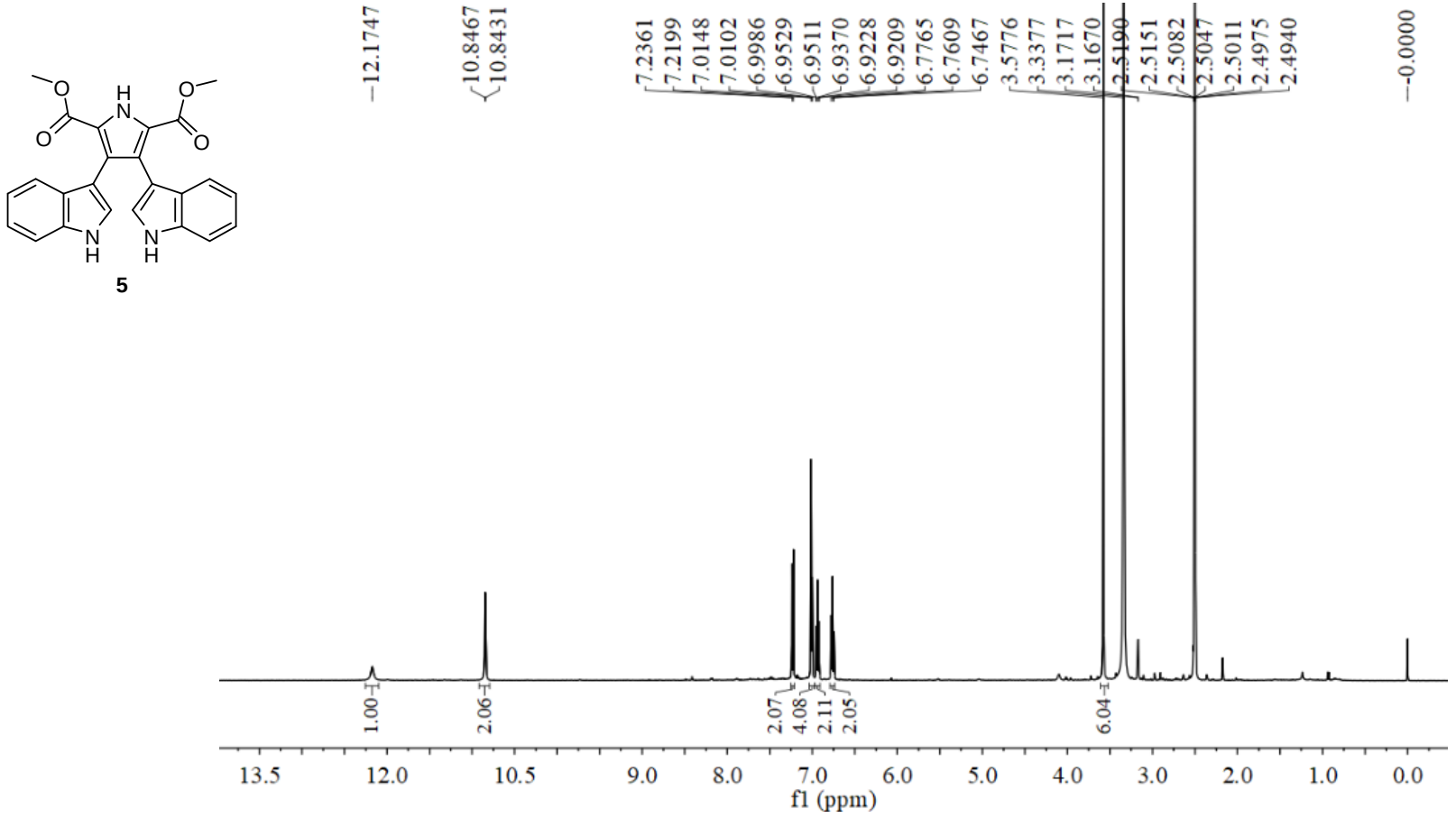


Fig. S8 The spectral data of **5**

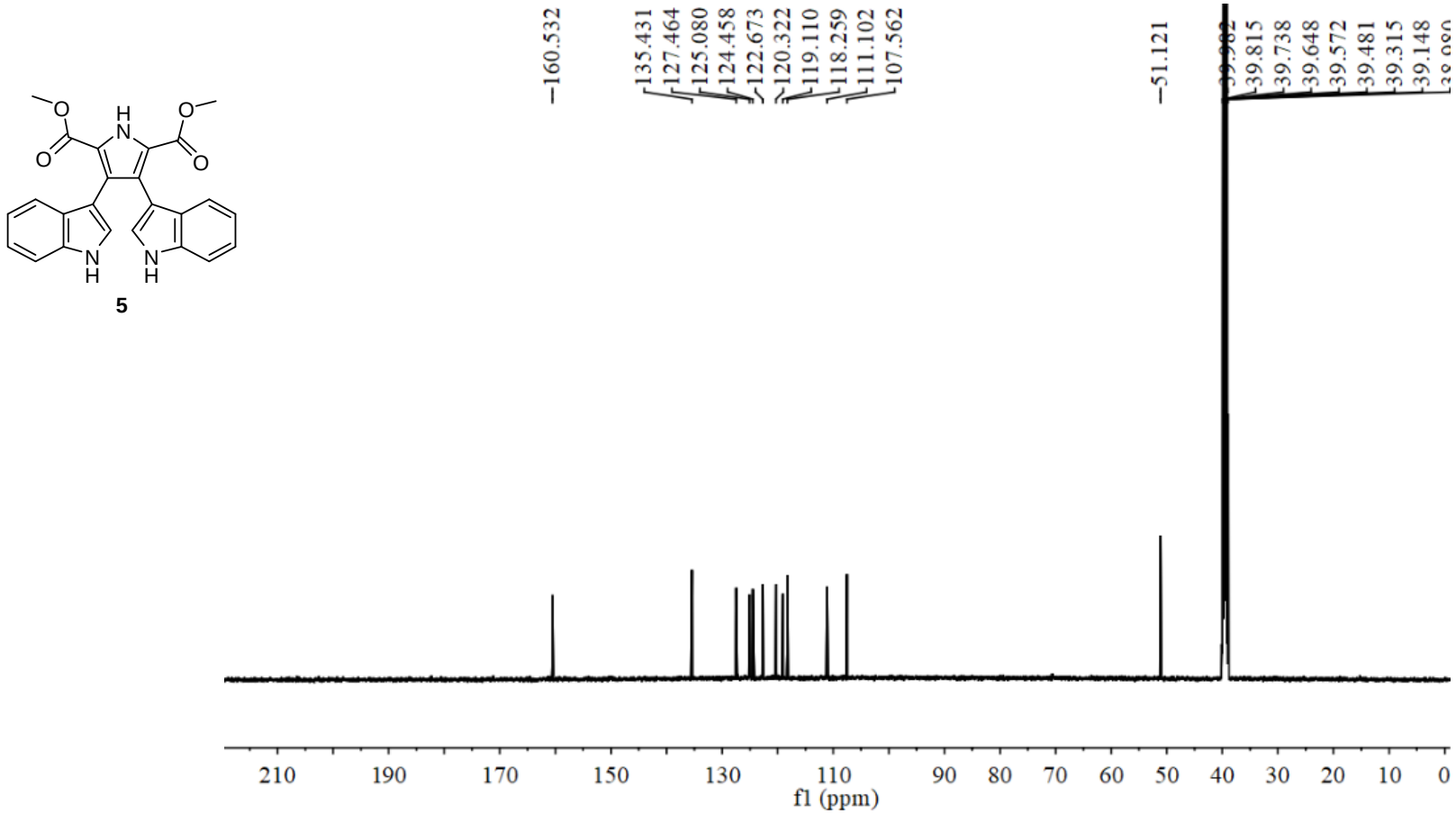
(A) ^{1}H NMR spectrum of **5** (DMSO-d_6)



5

Fig. S8 The spectral data of **5**

(B) ^{13}C NMR spectrum of **5** (DMSO-d_6)



5

Fig. S9 The spectral data of **6**

(A)^{1}H NMR spectrum of **6** (DMSO-d_6)

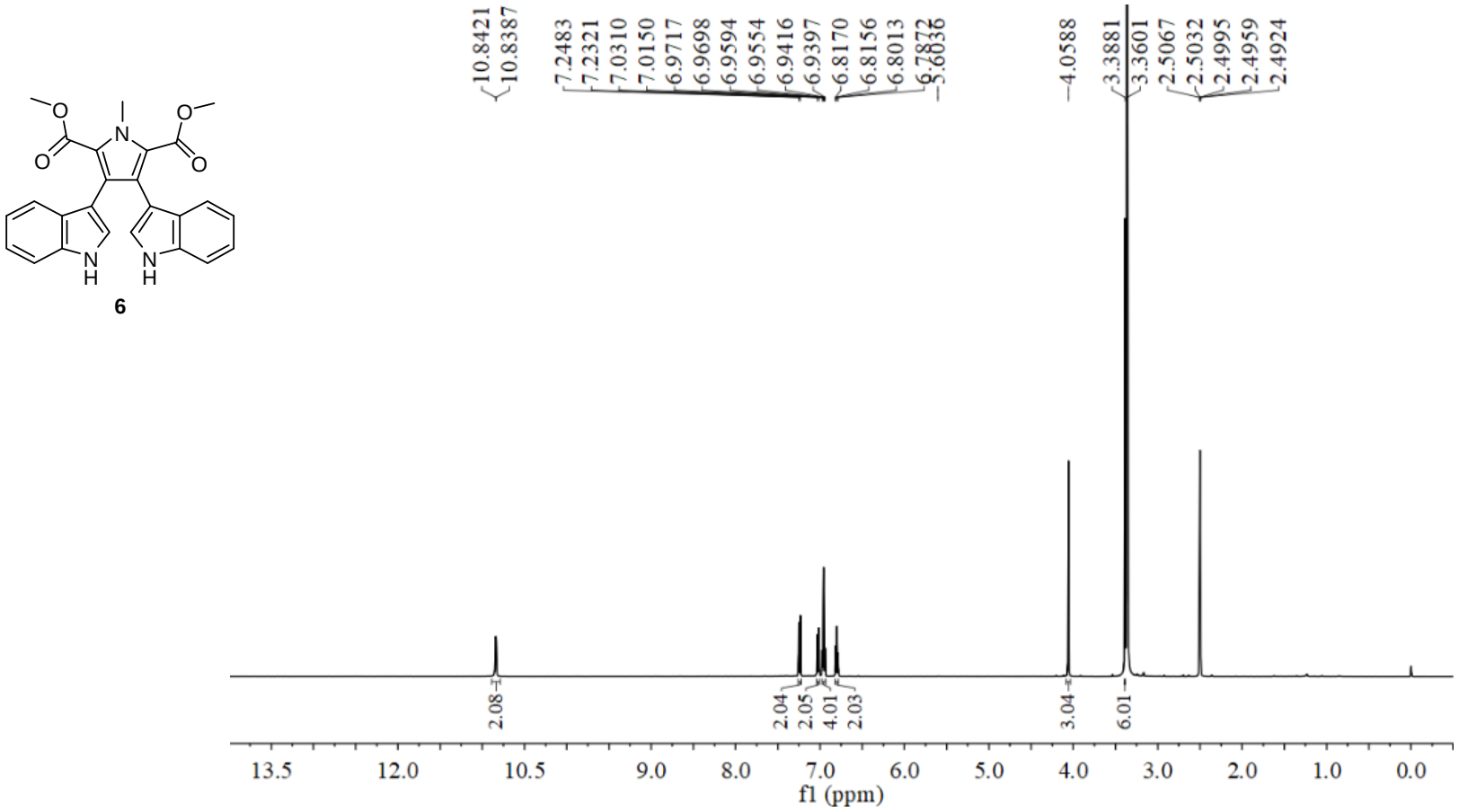
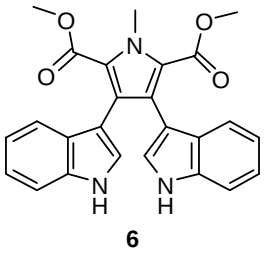


Fig. S9 The spectral data of **6**

(A)^{13}C NMR spectrum of **6** (DMSO-d_6)



6

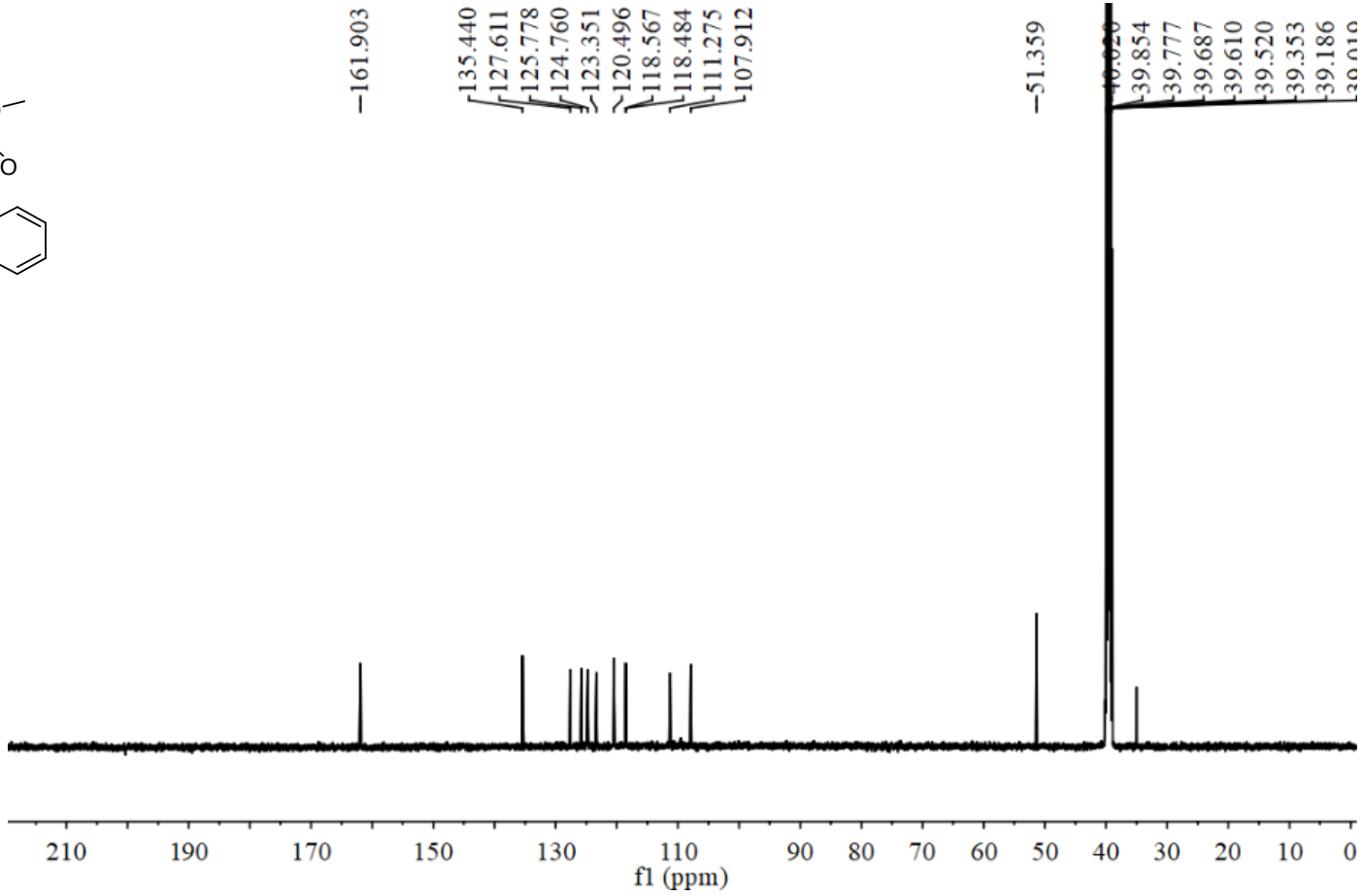


Fig. S10 The spectral data of **7**

(A) ^{1}H NMR spectrum of **7** ($CDCl_3$)

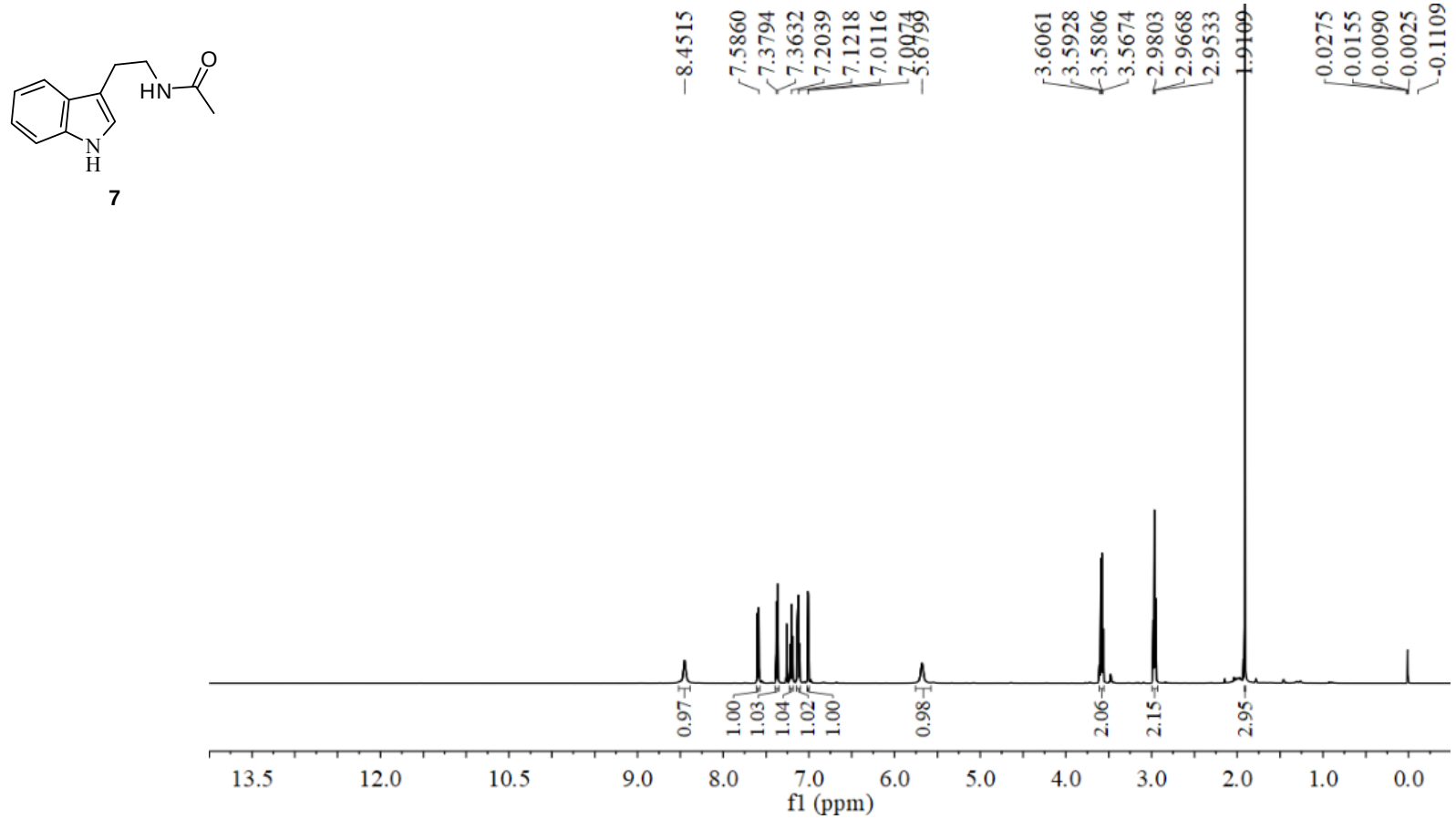


Fig. S10 The spectral data of **7**

(B) ^{13}C NMR spectrum of **7** ($CDCl_3$)

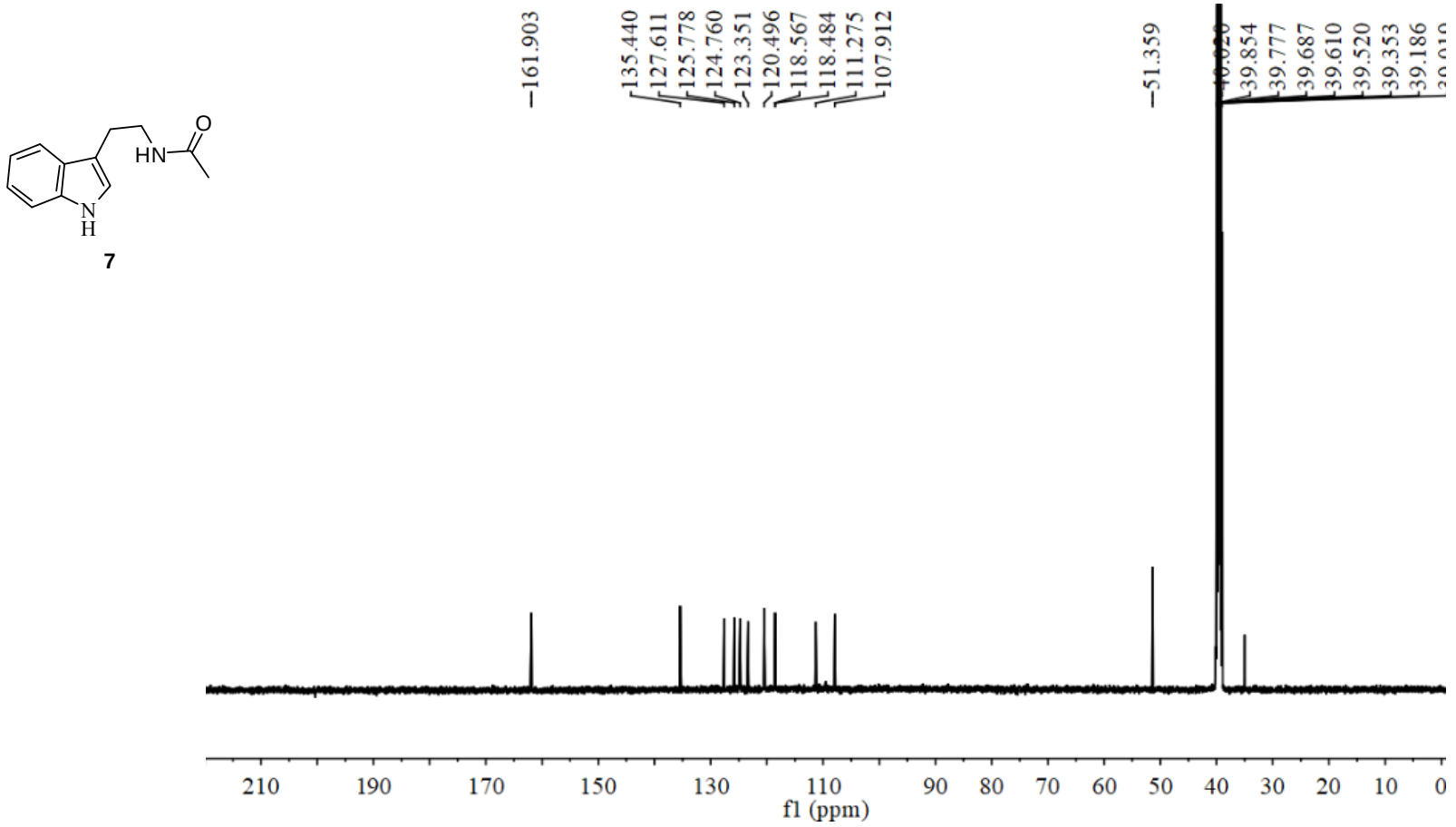
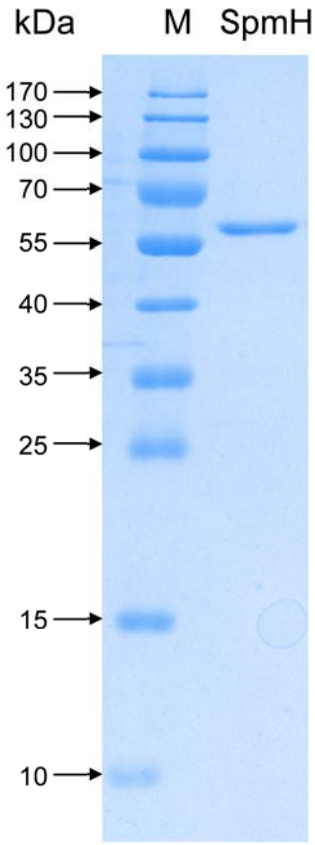


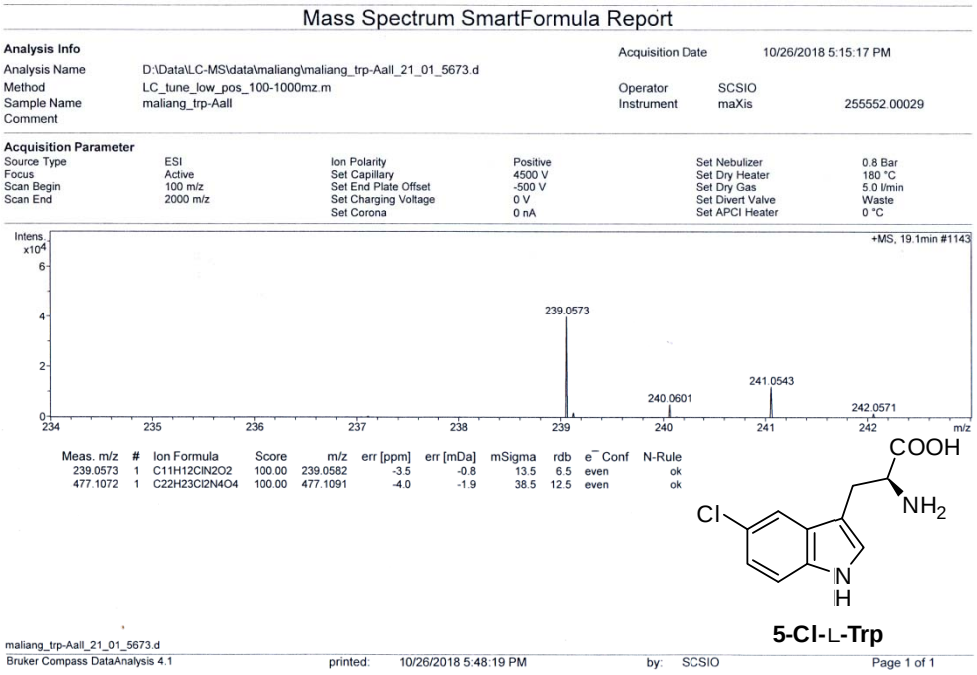
Fig. S11 SDS-PAGE analysis of purified recombinant SpmH



SDS-PAGE analysis of purified N-His_6-tagged SpmH. Lane M, protein molecular weight marker. The acrylamide percentage of SDS-PAGE gels is 12%.

Fig. S12 HRESIMS and UV spectra of 5-Cl-L-Trp

(A) HRESIMS spectrum of 5-Cl-L-Trp



(B) UV spectrum of 5- Cl-L-Trp

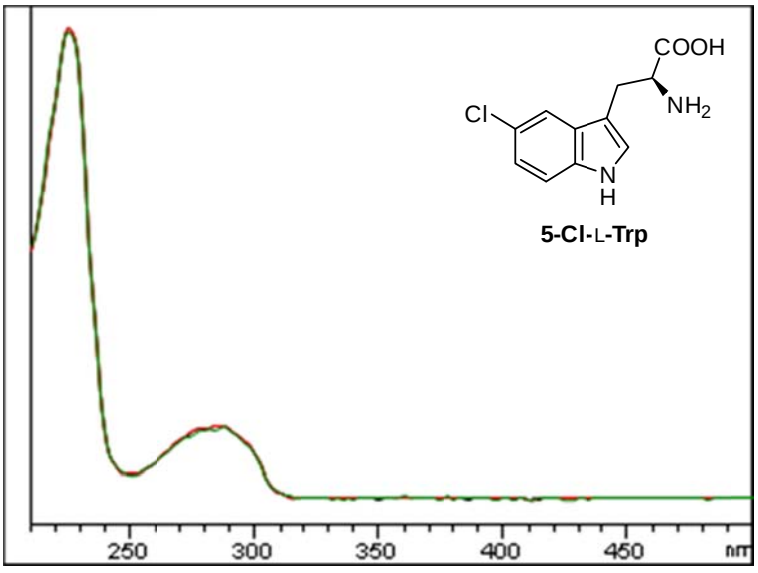
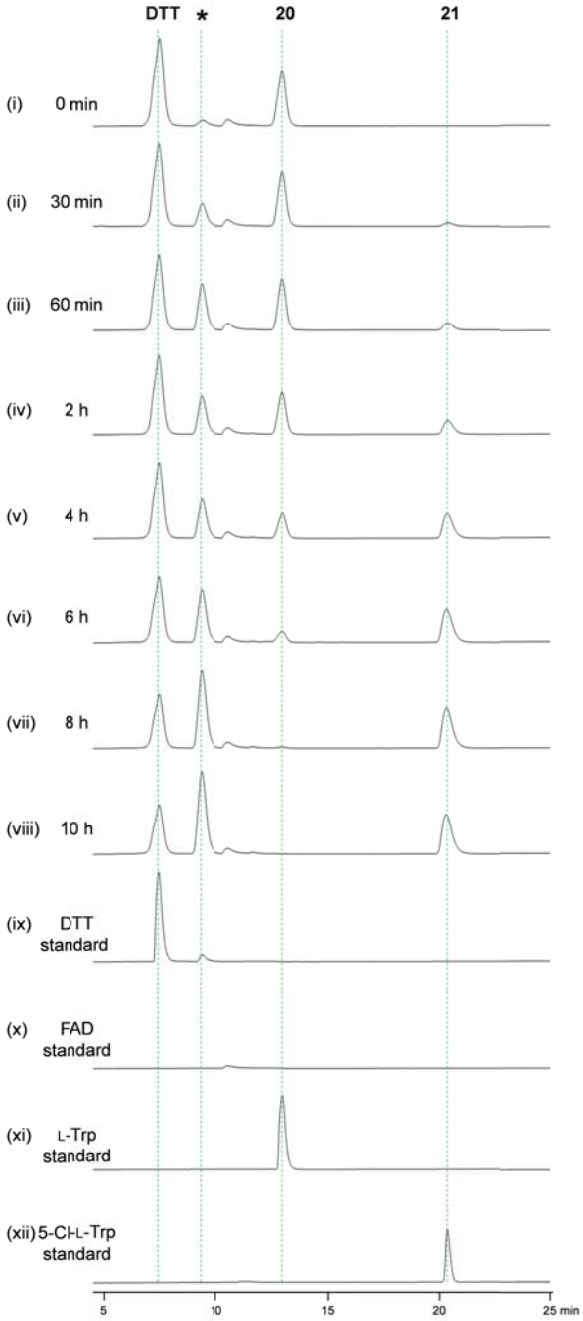
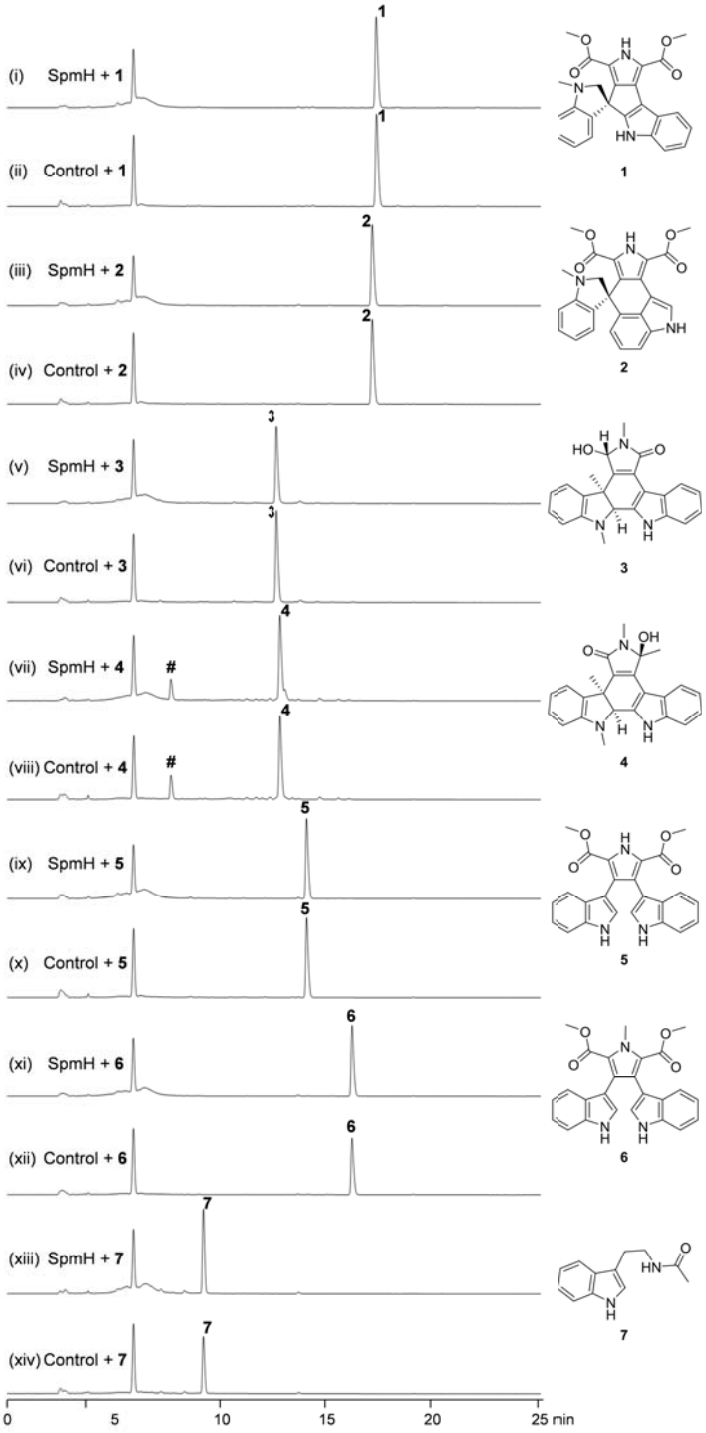


Fig. S13 HPLC analysis of a time course of SpmH enzyme assays



A SpmH assay containing 150 μM L-Trp, 5 μM SpmH, 10 μM FAD, 20 mM DTT and 50 mM NaCl for (i) 0 min, (ii) 30 min, (iii) 60 min, (iv) 2 h, (v) 4 h, (vi) 6 h, (vii) 8 h, (viii) 10 h, (ix) standard DTT, (x) standard FAD, (xi) standard L-Trp, (xii) standard 5-Cl-L-Trp. A typical SpmH assay was performed in potassium phosphate buffer (50 mM, pH 7.4) at 30 °C. The peak with an asterisk symbol denotes an unknown product derived from oxidation of DTT.

Fig. S14 HPLC analysis of SpmH enzyme assays with different substrates



HPLC analysis of SpmH enzyme assays with UV detection at 254 nm. A typical *in vitro* SpmH assay was conducted in 100 μL of reaction mixture in potassium phosphate buffer (50 mM, pH 7.4), comprising of 150 μM substrate, 5 μM SpmH, 10 μM FAD, 20 mM DTT and 50 mM NaCl at 30 °C for 20 h. The positive control of enzyme assay with L-Trp was set to confirm that the SpmH has the activity. The negative control of enzyme assays were minus the SpmH. The peaks marked with symbol # in trace (vii, viii) were an uncharacterized compound derived from compound **4**.

Supplementary References

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