Supplementary Information

Characterization of the Flavoenzyme XiaK as an *N*-Hydroxylase and Implications in Indolosesquiterpene Diversification†

Qingbo Zhang,‡[a] Huixian Li,‡[ab] Lu Yu,[c] Yu Sun,[d] Yiguang Zhu,[a] Hanning Zhu,[a] Liping Zhang,[a] Shu-Ming Li,[e] Yuemao Shen,[f] Changlin Tian,[c] Ang Li,[d] Hung-wen Liu,[g*] and Changsheng Zhang[a*]

[a]CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, China;

[b]Institute of Marine Natural Products, School of Marine Sciences, Sun Yat-sen University, Guangzhou, 135 West Xingang Road, Guangzhou 510006, China;

[c]Hefei National Laboratory of Microscale Physical Sciences, School of Life Science, University of Science and Technology of China, Hefei, 230027, China, and High Magnetic Field Laboratory, Chinese Academy of Sciences, Hefei, 230031, P. R. China;

[d]State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China;

[e]Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Deutschhausstrasse 17a, 35037 Marburg, Germany;

[f]State Key Laboratory of Microbial Technology, School of Life Science, Shandong University, Jinan 250100, China;

[g]Division of Chemical Biology and Medicinal Chemistry, College of Pharmacy and Department of Chemistry, University of Texas at Austin, Austin, TX, 78712, USA.

‡ Q. Zhang and H. Li contributed equally to this work.

Correspondence and requests for materials should be addressed to C.Z. (email: czhang2006@gmail.com) or H.w.L. (h.w.liu@mail.utexas.edu)

Contents

Author Contributions
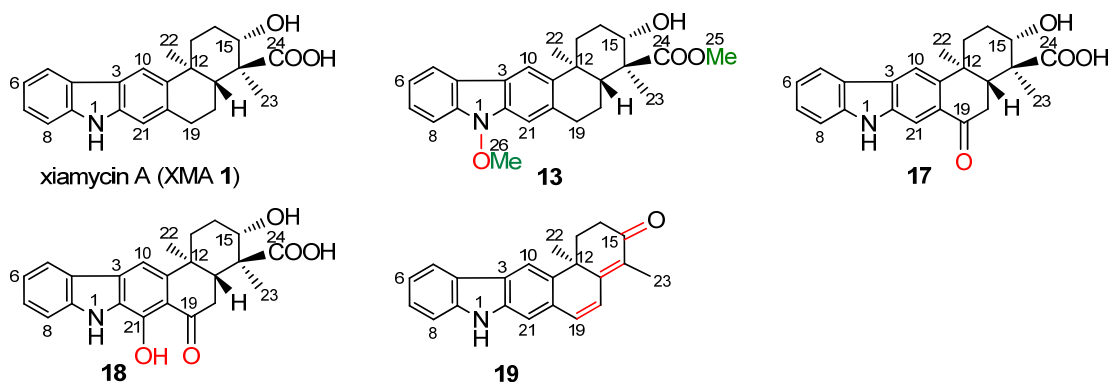
Q.Z., H.L. and C.Z. conceived and designed the study. Q.Z., Y.S and A.L. performed chemical studies. H.L., Y.Z. and H.Z. performed molecular and biochemical experiments. L.Y. and C.T. performed EPR experiments, L.Z. performed bioinformatics analysis. S.-M.L, Y.-M.S., A.L., H.w.L. and C.Z. analyzed and discussed the results. Q.Z., H.L., L.Y., H.w.L. and C.Z. prepared the manuscript.

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

Strains/Plasmids	Characteristic(s)	Sources
E.coli		
DH5α	Host strain for cloning	Invitrogen
ET12567	Donor strain for conjugation	[1]
BL21(DE3)	Host strain for protein expression	Novagen
Streptoymes sp.		
S. coelicolor YF11	Host strain for gene cluster and protein expression	[2]
S. pactum SCSIO 02999	Wild type, xiamycin/oxiamycin producer	[3]
XM47i	*XiaP* gene in-frame deletion mutant of SCSIO 02999	This work
Plasmids		
BT340	Cm^r, express FLP-recombinase to form in-frame deletion genes	[4]
pUZ8002	Km^r, including *tra* for conjugation	[5]
pSET152	Ap^r, integrative vector for *Streptoymes* heterologous expression	[6]
pPWW50A	Ap^r, *amp* in pPWW50 was replaced by *aac(3)IV* and *oriT*	[7]
pET28a	Km^r	Novagen
pCSG2407	cosmid carrying the intact *xia* gene cluster	[3]
pCSG2517	A pCSG2407 derivative where *xiaP* was disrupted by *aac(3)IV*	[3]
pCSG2547	A pCSG2517 derivative where *xiaP* was in-frame deleted	This work
pCSG2562	A pCSG2557 derivative where *neo* was disrupted by *aac(3)IV*	This work
pCSG2671	about 30 kb *Eco*R Ifragment containing the *xia* gene cluster from pCSG2407 inserted into pSET152 digested with *Eco*RI	This work
pCSG2607	1.8 kb *xiaK* PCR fragment (*Nde*I/*Bgl*II) from SCSIO 02999 inserted into pET28a for *E.coli* expression inserted into pET28a (*Nde*I/*Bam*HI)	This work
pCSG2701	1.8 kb *xiaK* PCR fragment (*Nde*I/*Bgl*II) from SCSIO 02999 inserted into pPWW50A(*Nde*I/*Bam*HI)	This work

Primers	Target gene	Sequence
For diagnostic PCR		
XiaP-1F	*xiaP*	5'- GCGAACTGGCAGCGATTGTG -3'
XiaP-1R		5'- GCGGATCTGGTAGGCGATGC -3'
orf1-1F	*orf1*	5'- GTACTGCGTGAAGCCCGACC -3'
orf1-1R		5'- CCCAGAACTCGGCGGTGTAC -3'
For gene cloning		
XiaK-3F	*xiaK*	5'-GGAAGA<u>CATATG</u>TTGGACGTAGAAGTGCC-3', *Nde*I
XiaK-3R		5'- CAACAT<u>AGATCT</u>CAGGAAGGTGTGG -3', *Bgl* II

Table S2 The ^{1}H NMR data of compounds **13**, **17–19**.

xiamycin A (XMA **1**)   **13**   **17**

18   **19**

No.	1[a] δH (mult, *J* in Hz)	13[b] δH (mult, *J* in Hz)	17[c] δH (mult, *J* in Hz)	18[c] δH (mult, *J* in Hz)	19[c] δH (mult, *J* in Hz)
NH	10.9 (s)[c]				
5	7.98 (d, 8.0)	8.09 (d, 8.0)	8.16 (d, 8.0)	8.07 (d, 7.8)	8.07 (d, 7.8)
6	7.10 (ddd, 8.0, 7.0, 1.0)	7.20 (ddd, 8.0, 7.0, 1.0)	7.20 (m)	7.18 (dd, 7.8, 7.2)	7.15 (ddd, 8.4, 7.2, 1.2)
7	7.30 (ddd, 8.0, 7.0, 1.0)	7.40 (ddd, 8.0, 7.0, 1.0)	7.47 (m)	7.44 (dd, 7.8, 7.2)	7.36 (ddd, 8.4, 7.2, 1.2)
8	7.36 (d, 8.0)	7.49 (d, 8.0)	7.47 (m)	7.50 (d, 8.4)	7.43 (d, 7.8)
10	7.93 (s)	8.05 (s)	8.12 (s)	7.55 (s)	8.16 (s)
13	2.60 (td, 13.0, 3.0);	2.54 (m);	2.73 (m);	2.62 (m);	2.98 (m);
	1.79 (m)	1.59 (m)	1.94 (m)	1.93 (m)	2.35 (m)
14	1.90 (m)	1.75 (m)	1.96 (m)	1.93 (m)	2.85 (m); 2.67 (m)
15	4.11 (dd, 9.0, 7.5)	3.88 (m)	4.13 (m)	4.10 (m)	
15-OH	4.71 (m)	4.80 (d, 5.0)			
17	2.21 (dd, 12.5, 2.0)	1.99 (m)	2.67 (m)	2.58 (m)	
18	2.00 (m);	1.93 (m);	2.96 (m);	3.01 (dd, 12, 14.5);	6.76 (d, 9.6)
	1.56 (m)	1.26 (m)	2.43 (d, 17.5)	2.39 (dd,12,14.5)	
19	3.09 (m)	3.09 (m); 2.96 (m)			7.05 (d, 9.6)
21	7.07 (s)	7.17 (s)	8.06 (s)		7.35 (s)
22	1.30 (s)	1.21 (s)	1.39 (s)	1.32 (s)	1.49 (s)
23	1.26 (s)	1.14 (s)	1.33 (s)	1.30 (s)	1.92 (s)
24-OH	12.1 (s)[c]				
25		3.62 (s)			
26		4.04 (s)			

[a] 500 MHz for ^{1}H NMR CD₃OD, tetramethylsilane (TMS) as an internal standard;

[b] 500MHz for ^{1}H NMR and DMSO-d_6, TMS as an internal standard;

[c] 600 MHz for ^{1}H NMR CD₃OD, TMS as an internal standard.

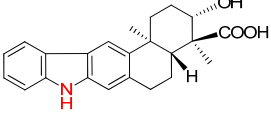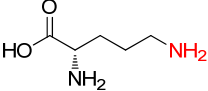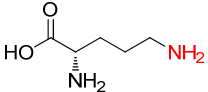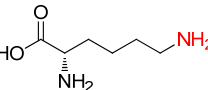**Table S3** The ^{13}C NMR data of compounds **13**, **17–19**.

xiamycin A (XMA **1**)　　　**13**　　　**17**

18　　　**19**
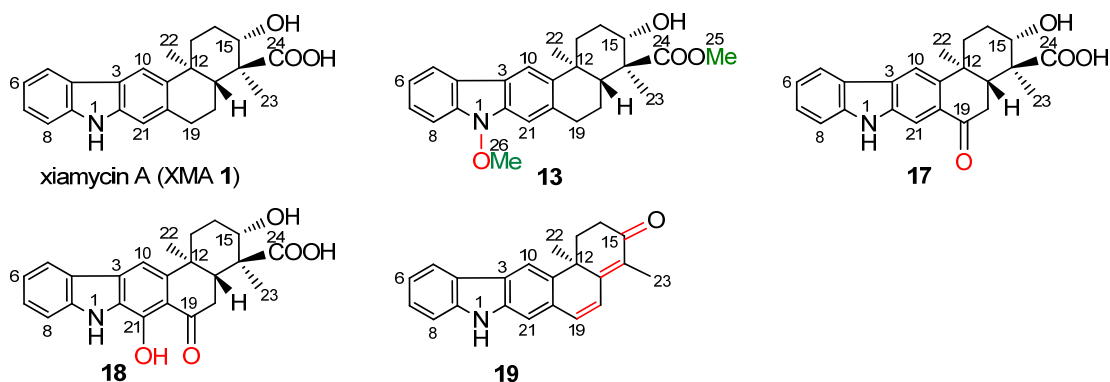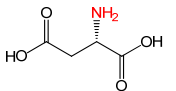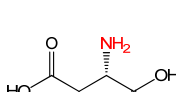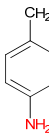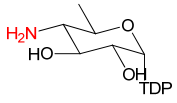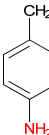
No.	**1**[a]	**13**[b]	**17**[c]	**18**[c]	**19**[c]
2	138.1 C	136.2 C	139.6 C	128.1 C	139.5 C
3	121.1 C	118.2 C	129.5 C	130.8 C	125.8 C
4	122.6 C	119.9 C	123.8 C	124.2 C	124.4 C
5	118.7 CH	120.4 CH	122.2 CH	122.2 CH	121.2 CH
6	117.5 CH	120.0 CH	120.3 CH	120.5 CH	120.2 CH
7	124.2 CH	125.9 CH	128.6 CH	128.6 CH	127.2 CH
8	109.7 CH	108.5 CH	112.2 CH	112.8 CH	112.0 CH
9	140.0 C	138.0 C	143.9 C	143.3 C	142.6 C
10	114.5 CH	116.4 CH	116.1 CH	106.7 CH	116.7 CH
11	139.9 C	142.2 C	147.5 C	147.4 C	138.0 C
12	36.4 C	36.8 C	38.2 C	38.7 C	40.7 C
13	37.0 CH$_2$	37.2 CH$_2$	38.2 CH$_2$	38.2 CH$_2$	35.0 CH$_2$
14	26.6 CH$_2$	27.4 CH$_2$	28.3 CH$_2$	28.3 CH$_2$	35.2 CH$_2$
15	74.4 CH	73.9 CH	76.8 CH	76.2 CH	200.7 C
16	53.0 C	53.5 C	54.3 C	54.3 C	129.4 C
17	45.7 CH	46.0 CH	46.8 CH	46.6 CH	160.2 C
18	20.7 CH$_2$	20.8 CH	38.7 CH$_2$	38.6 CH$_2$	123.6 CH
19	30.1 CH$_2$	30.2 CH$_2$	201.3 C	206.9 C	136.7 CH
20	132.3 C	133.7 C	130.2 C	112.4 C	130.3 C
21	109.1 CH	107.7 CH	110.7 CH	152.2 C	112.0 CH
22	24.7 CH$_3$	25.4 CH$_3$	24.8 CH$_3$	24.9 CH$_3$	31.4 CH$_3$
23	9.7 CH$_3$	10.9 CH$_3$	11.21 CH$_3$	11.5 CH$_3$	10.7 CH$_3$
24	180 C	177.3 C	180 C	181.3 C	
25		51.8 CH$_3$			
26		63.4 CH$_3$			

[a] 125 MHz for ^{13}C NMR, CD$_3$OD, tetramethylsilane (TMS) as an internal standard;

[b] 150 MHz for ^{13}C NMR, DMSO-d_6 and TMS as an internal standard;

[c] 150 MHz ^{13}C NMR, CD$_3$OD and TMS as an internal standard.

Table S4 Comparison of the kinetic parameters of XiaK and other *N*-oxidases.

Enzyme	Substrate	K_m [μM]	k_{cat} [min^{-1}]	k_{cat}/K_m [μM^{-1} min^{-1}]
XiaK		16.2 ± 3.3	260.4 ± 14.1	16.1
PvdA[8]		600 ± 70	24 ± 3	0.04
SidA[9]		490 ± 70	102 ± 6	0.21
NbtG[10]		350 ± 50	18.6 ± 1.2	0.053
CreE[11]		124.8 ± 5.0	68.4 ± 0.5	0.55
FzmM[12]		790 ± 70	148.8 ± 0.4	0.19
PrnD[13]		379 ± 34	6.5 ± 0.62	0.017
CalE10[14]		7.6 ± 1.2	0.04 ± 0.01	0.0053
AurF[15]		5.24 ± 0.64	6.21 ± 0.52	1.21

Note: PvdA, SidA, NbtG, CreE and FzmM are flavoenyzmes, while PrnD, CalE10 and AurF are P450 enzymes. Shown in the table (except XiaK) are the kinetic parameters for an N-hydroxylation reaction to convert a primary amine to a hydroxylamine.

Fig. S1 SDS-PAGE analysis of purified recombinant XiaK and the determination of FAD as the non-covalent binding cofactor in XiaK.
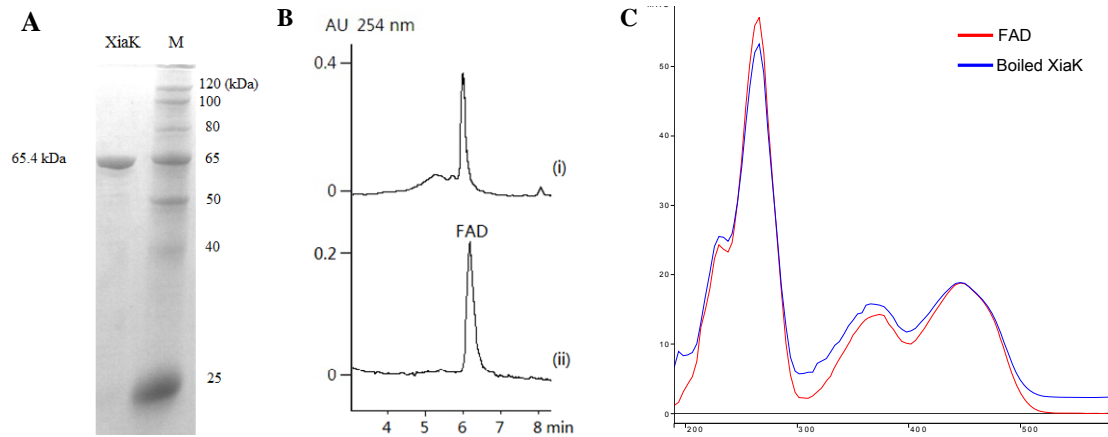
(A) SDS-PAGE analysis of XiaK. The expected size of N-His$_6$-tagged XiaK (65.4 kDa) was indicated. Lane M, protein molecular weight standards (ProteinRulerTM Ⅲ, TransGen Biotech). (B) HPLC analysis of boiled XiaK. (i) boiled XiaK; (ii) FAD standard. (C) Comparison of UV-Vis spectra of the supernatants of boiled XiaK and the FAD standard.

Fig. S2 UV-Vis spectral and LC-MS analysis of XiaK reaction products with XMA (**1**).
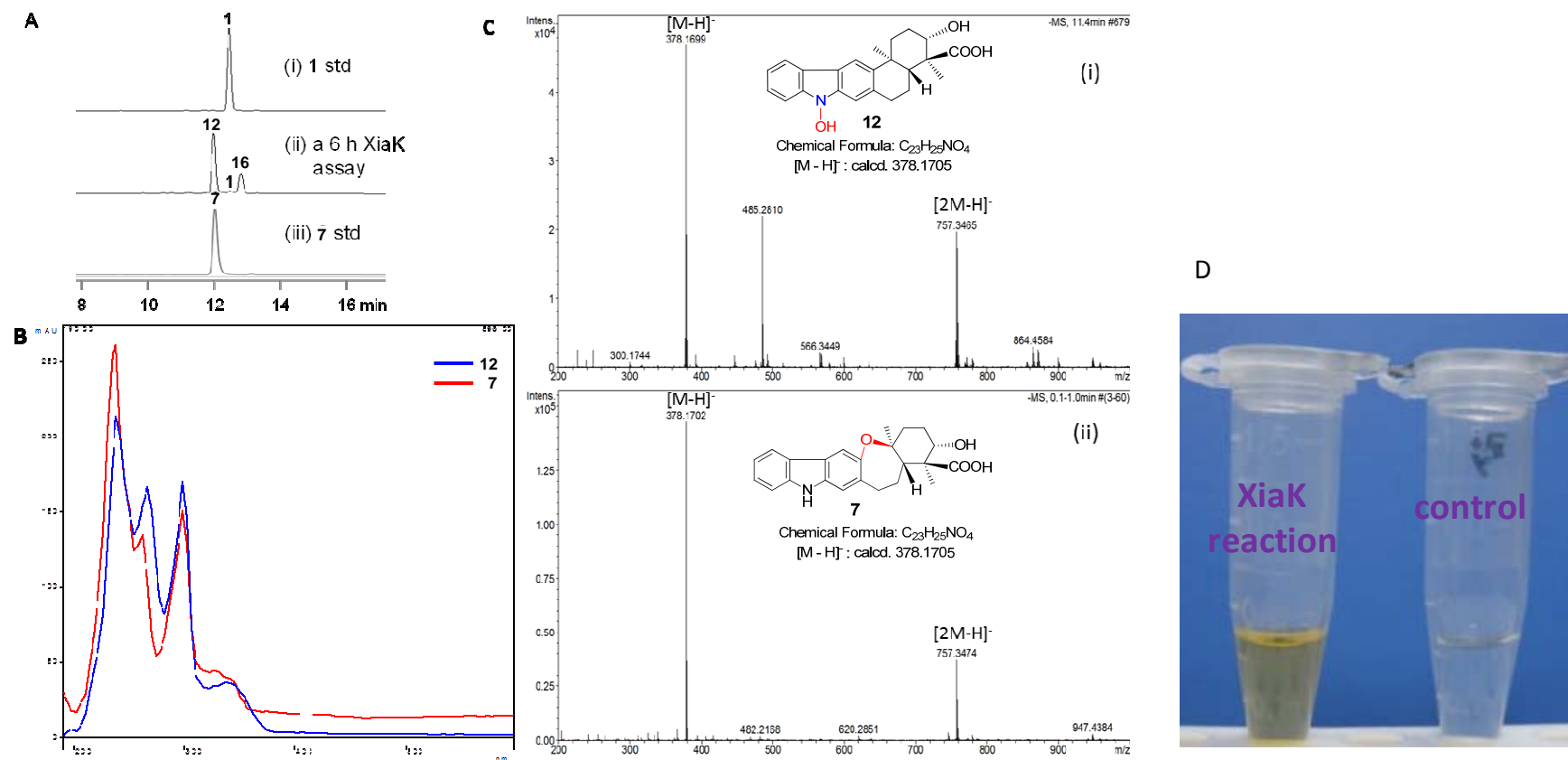
(A) HPLC analysis of XiaK-catalyzed reaction. (i) XMA (**1**) standard; (ii) a XiaK assay comprising of 300 μM XMA (**1**), 1 mM NADPH, and 5 μM XiaK in 50 mM Na_2HPO_4-NaH_2PO_4 buffer (pH 8.0) for 6 h at 30 °C; (iii) OXM (**7**) standard. (B) The UV-vis spectral comparison of NOXM (**12**) and OXM (**7**). (C): LC-HRESIMS spectra of **12** (i) and **7** (ii); (D) The yellow color of a typical XiaK reaction with **1** for 2 h versus a control lacking XiaK.

Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)
(A) The HRESIMS spectrum of **13**
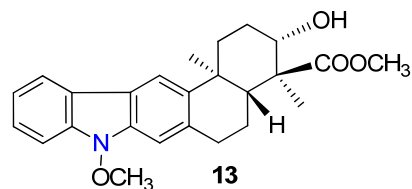
Chemical Formula: $C_{25}H_{29}NO_4$
$[M + Na]^+$: Calcd. 430.1989
$[M + K]^+$: Calcd. 446.1733

Meas. m/z	#	Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
430.1986	1	C 25 H 29 N Na O 4	100.00	430.1989	0.6	0.3	10.6	11.5	even	ok
446.1728	1	C 25 H 29 K N O 4	100.00	446.1728	0.0	0.0	9.7	11.5	even	ok
837.4082	1	C 50 H 58 N 2 Na O 8	100.00	837.4085	0.3	0.3	20.8	22.5	even	ok
853.3823	1	C 50 H 58 K N 2 O 8	100.00	853.3825	0.2	0.2	12.5	22.5	even	ok

Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)

(**B**) The ^{1}H-NMR spectrum of **13**

Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)

(**C**) The ^{13}C-NMR spectrum of **13**

Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)

(**D**) The DEPT135 spectrum of **13**
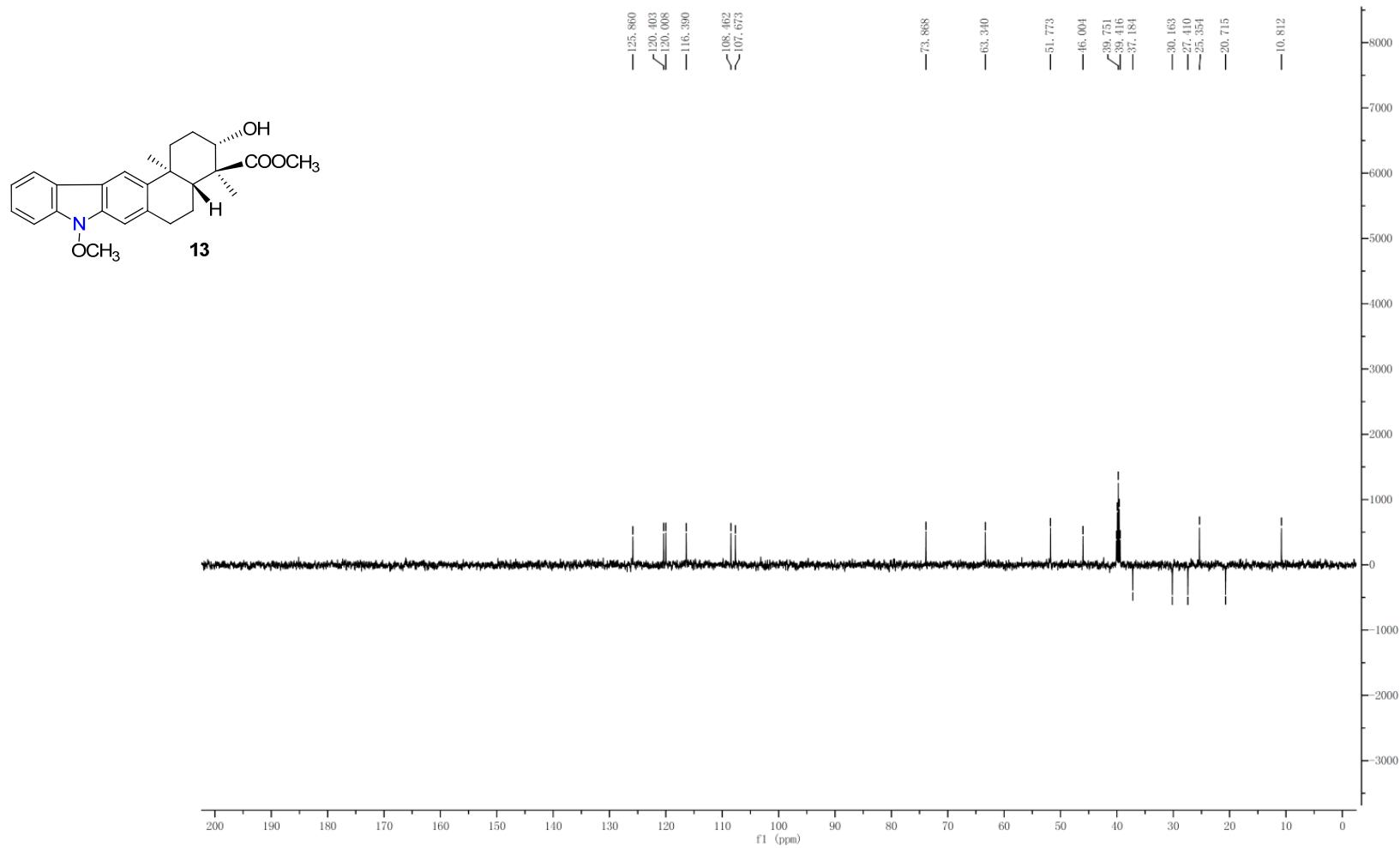
Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)

(**E**) The HSQC spectrum of **13**
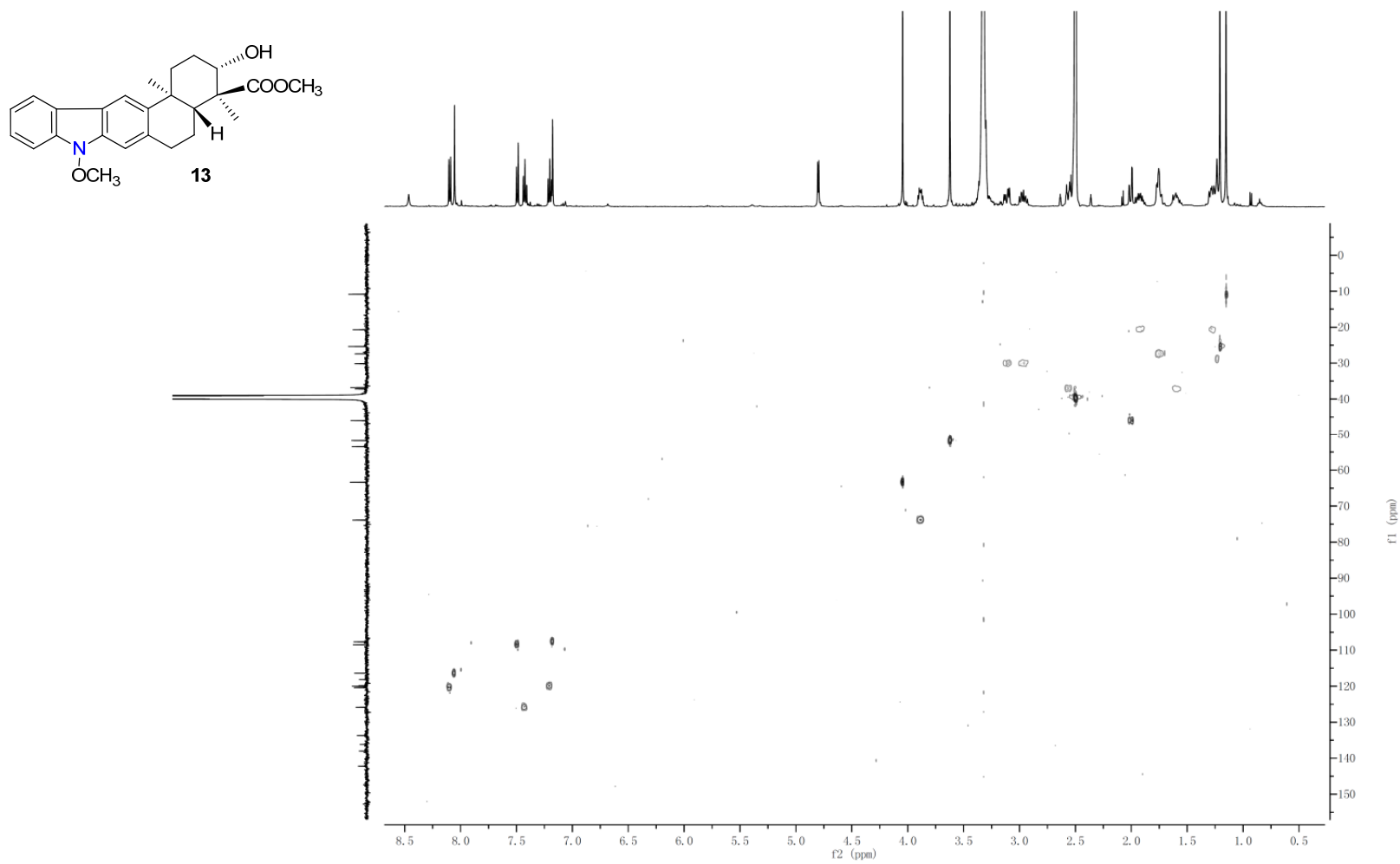
Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)
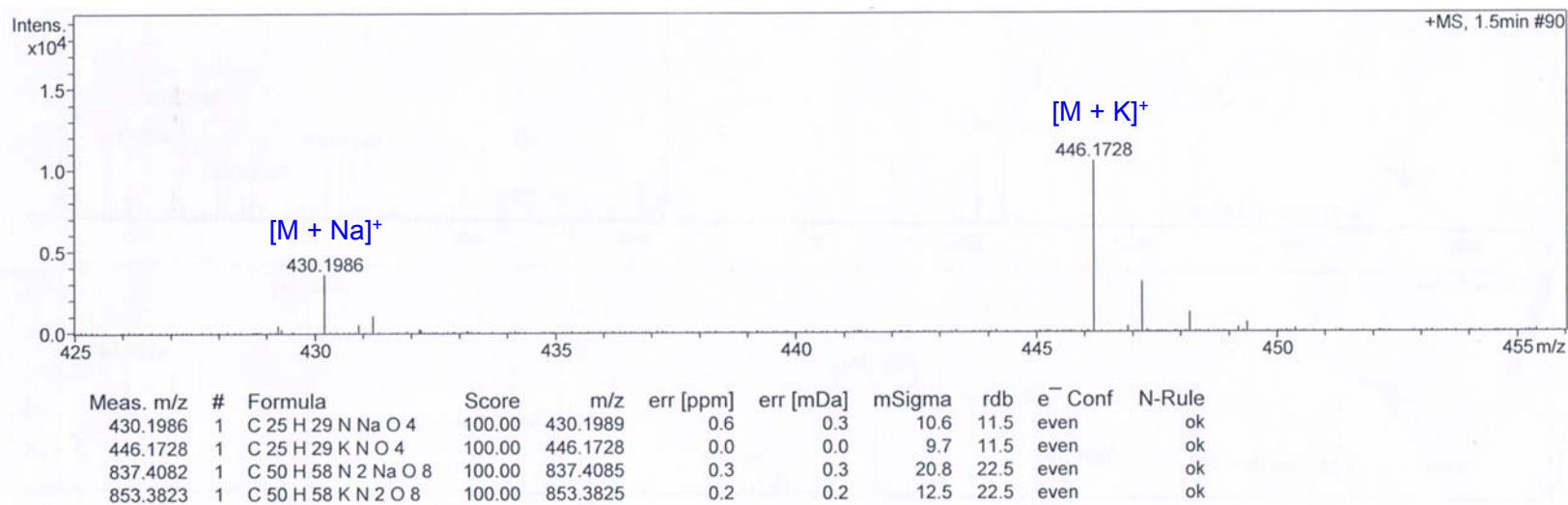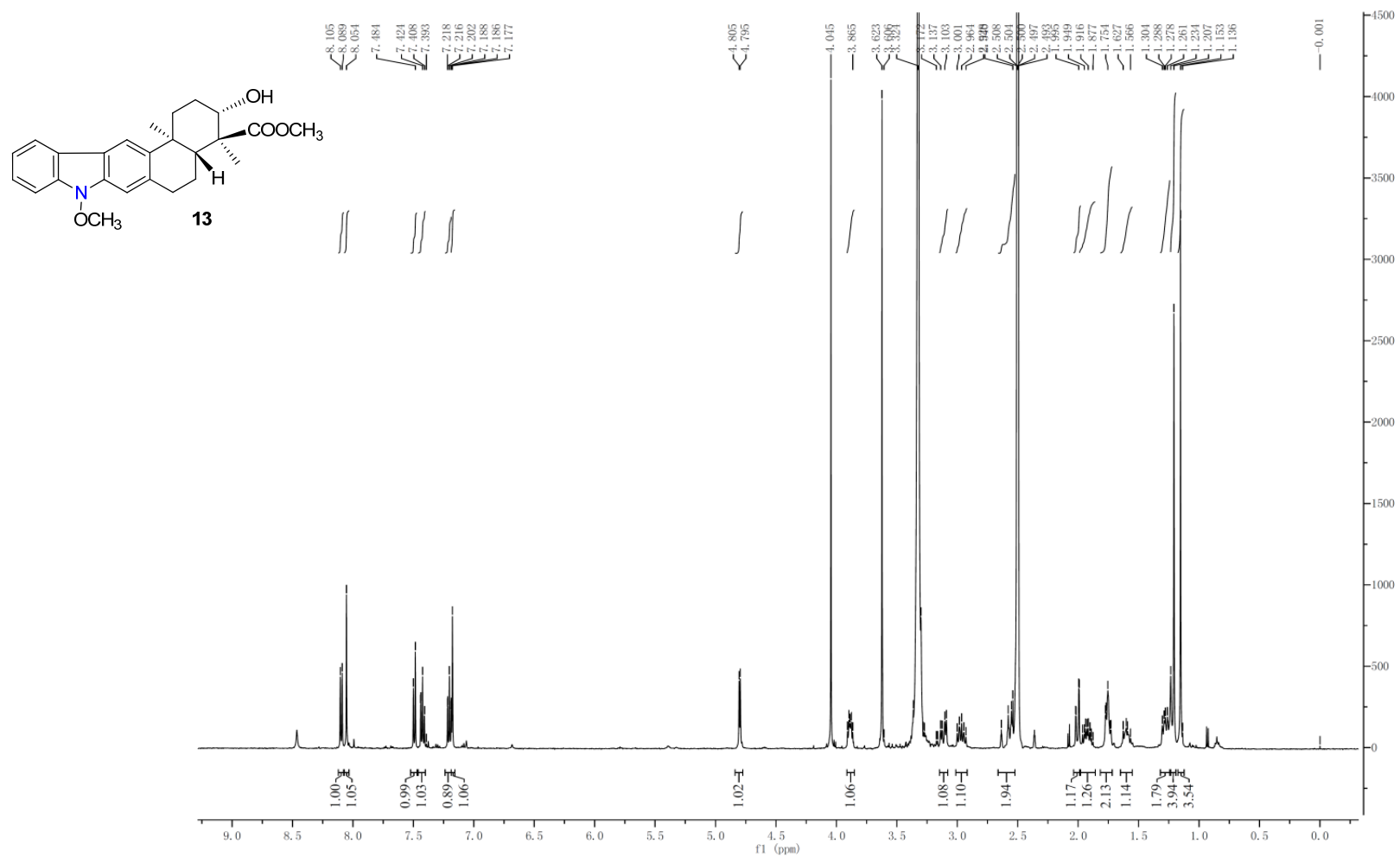(**F**) The ^{1}H-^{1}H COSY spectrum of **13**
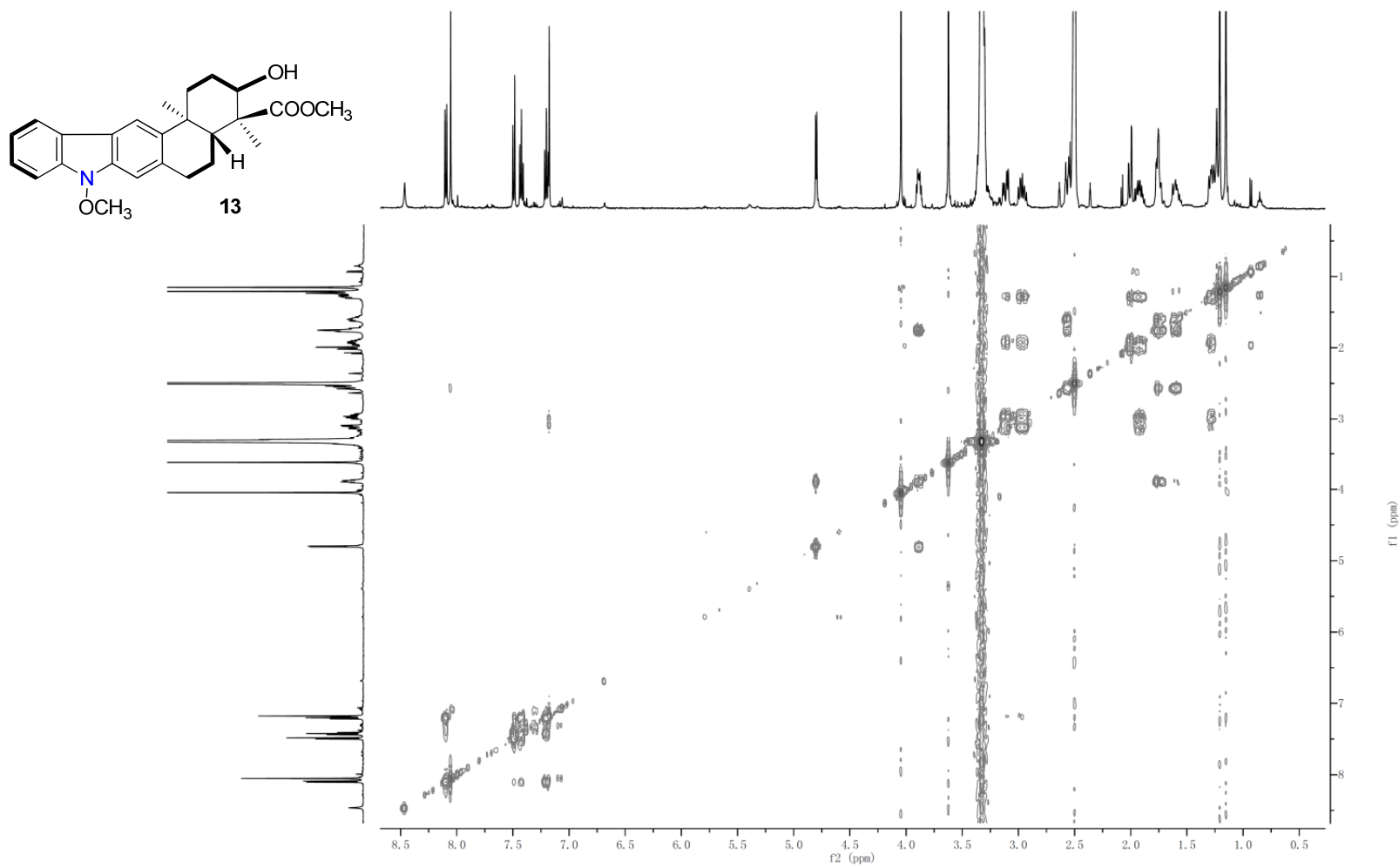
Fig. S3 The spectral data of **13**. (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, DMSO-d_6)
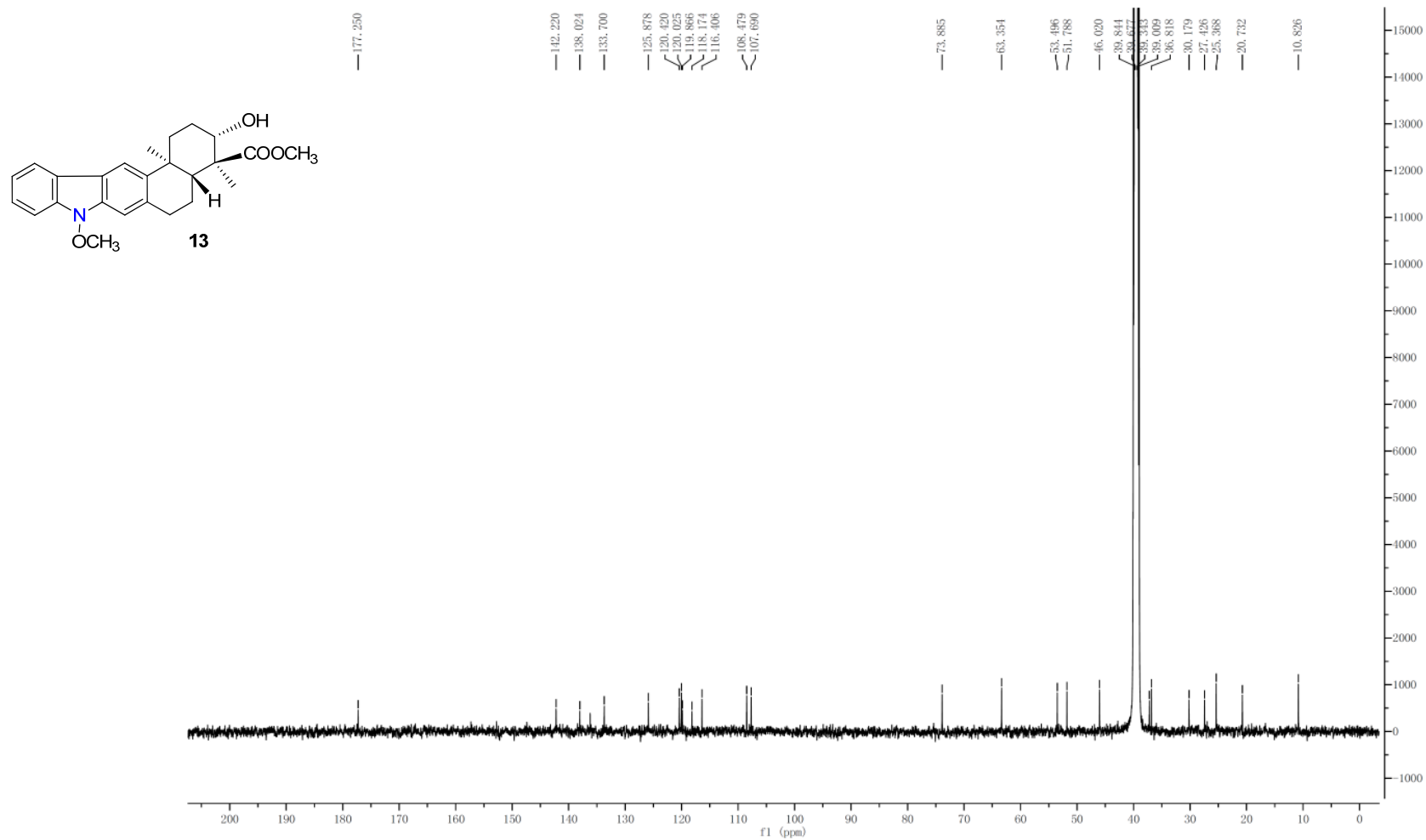
(**G**) The HMBC spectrum of **13**
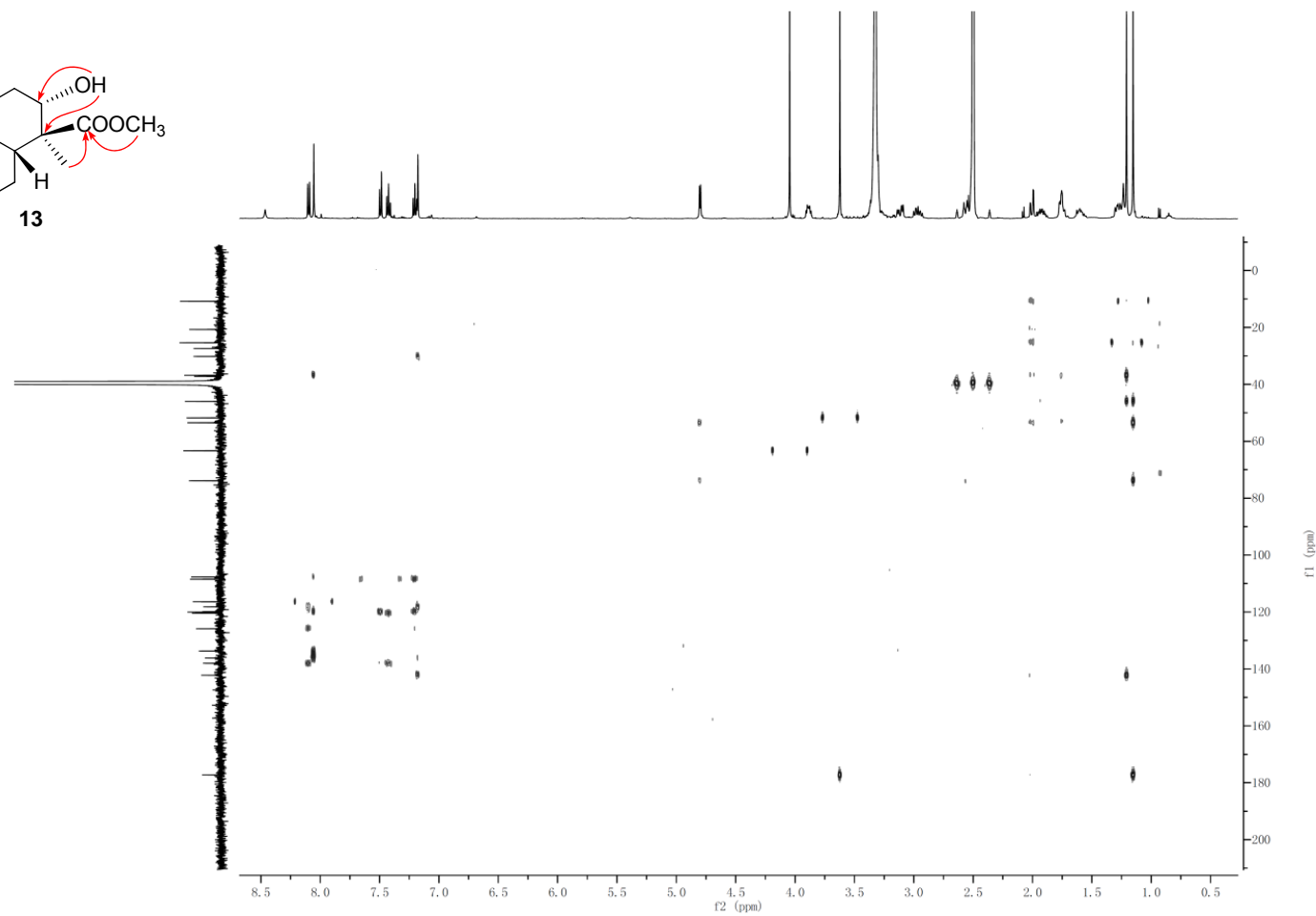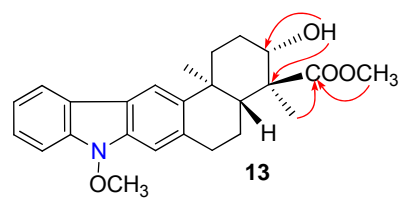
Fig. S4 The EPR spectra of XMA (**1**) and OXM (**7**)
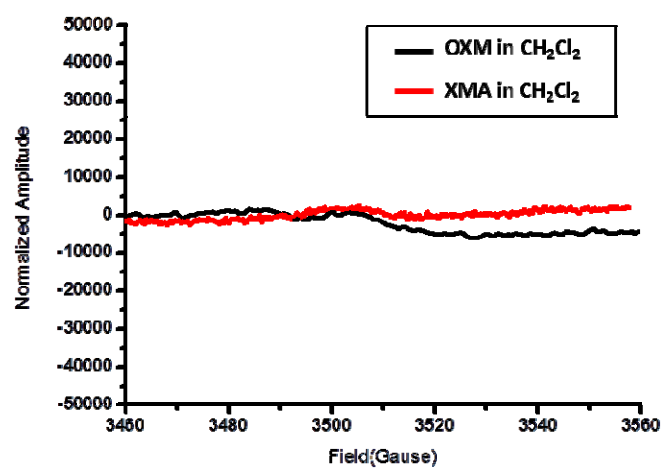
The EPR data acquisition parameters for free radical analysis were as follows: Frequency, 9.390 GHz; Microwave power, 1 mW; Modulation Frequency, 100 kHz; Modulation Amplitude, 0.5 Gauss; Sweep Time, 40 s/scan; Different number of scans were accumulated for each sample until a reasonable S/N ratio was achieved.

Fig. S5 ^{15}N-labelling related studies.

(A) HRESIMS spectrum of chemically synthesized ^{15}N-labeled **2**.
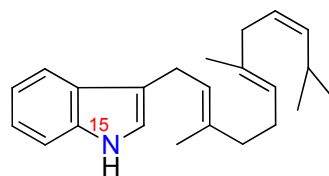
[15]N-**2**

Chemical Formula: $C_{23}H_{31}{}^{15}N$

$[M + H]^+$: Calcd. 323.2505

Meas. m/z	#	Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
323.2496	1	C 23 H 32 ^15N	100.00	323.2500	1.2	0.4	15.4	8.5	even	-
345.2297	1	C 23 H 31 Na ^15N	39.18	345.2319	6.5	2.2	43.0	8.5	even	-
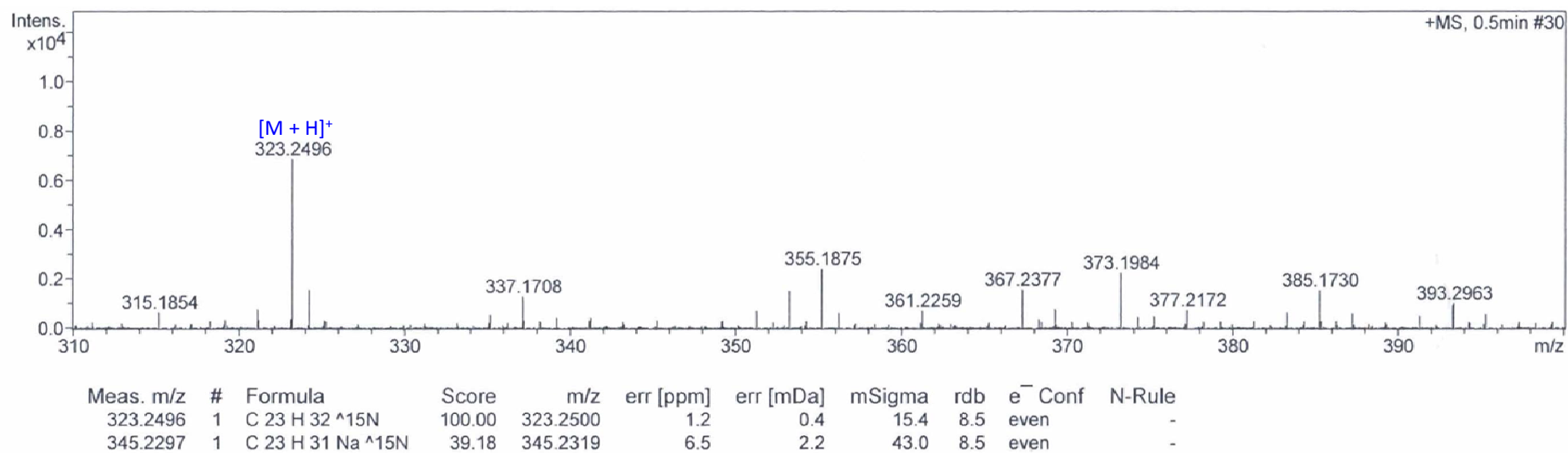
Fig. S5 ^{15}N-labelling related studies.

(**B**) ^{1}H NMR spectrum of chemically synthesized ^{15}N-labeled **2**. (500 MHz for ^{1}H NMR in CDCl$_3$)
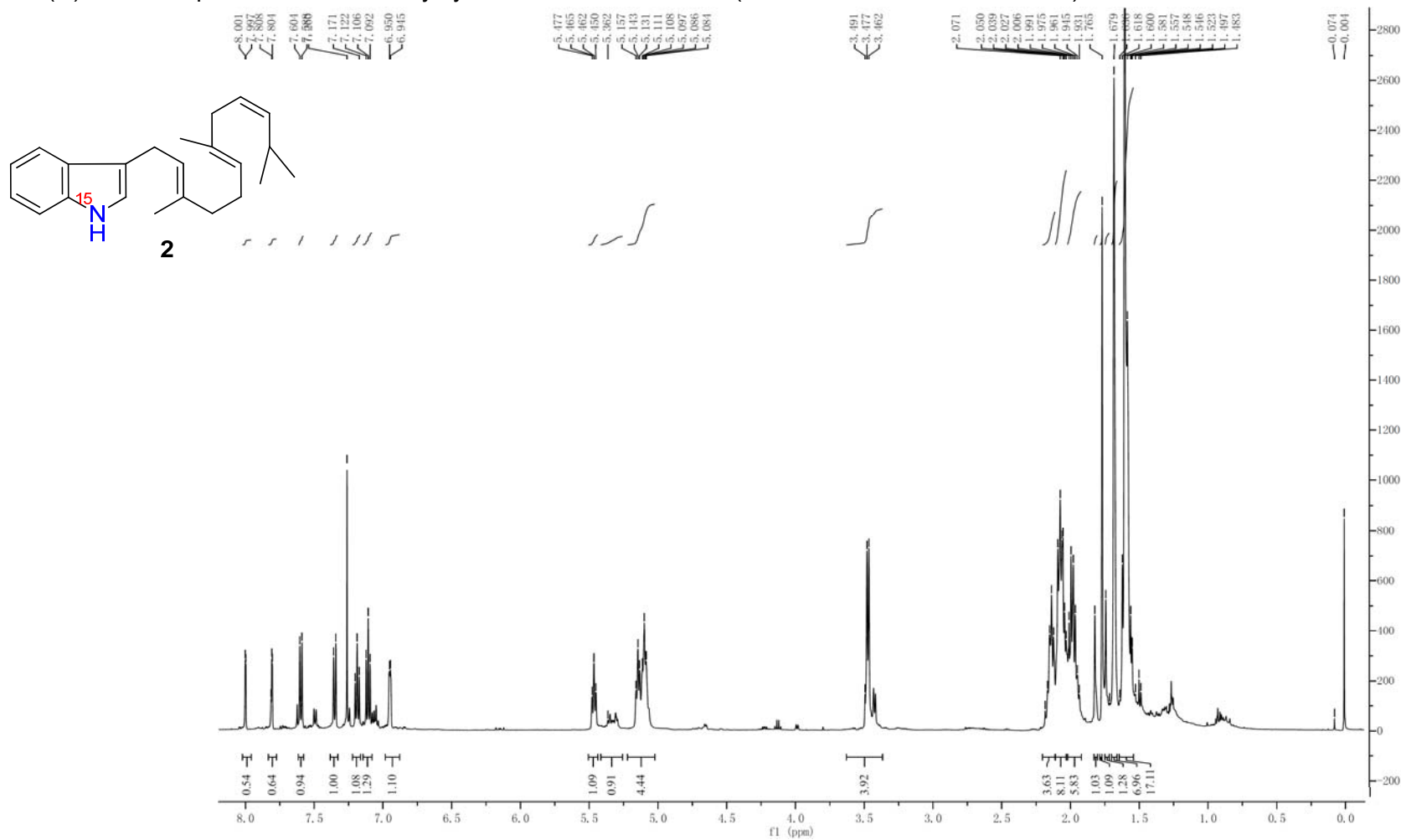
Fig. S5 ^{15}N-labelling related studies.

(**C**) HPLC analysis of feeding experiments of ^{15}N-labeled **2** in *S. pactum* XM47i. (i) feeding of ^{15}N-labeled **2** (147 mg) in *S. pactum* XM47i cultured in AM-4 meida (2 L); (ii) **2** standard; (iii) XMA (**1**) standard.
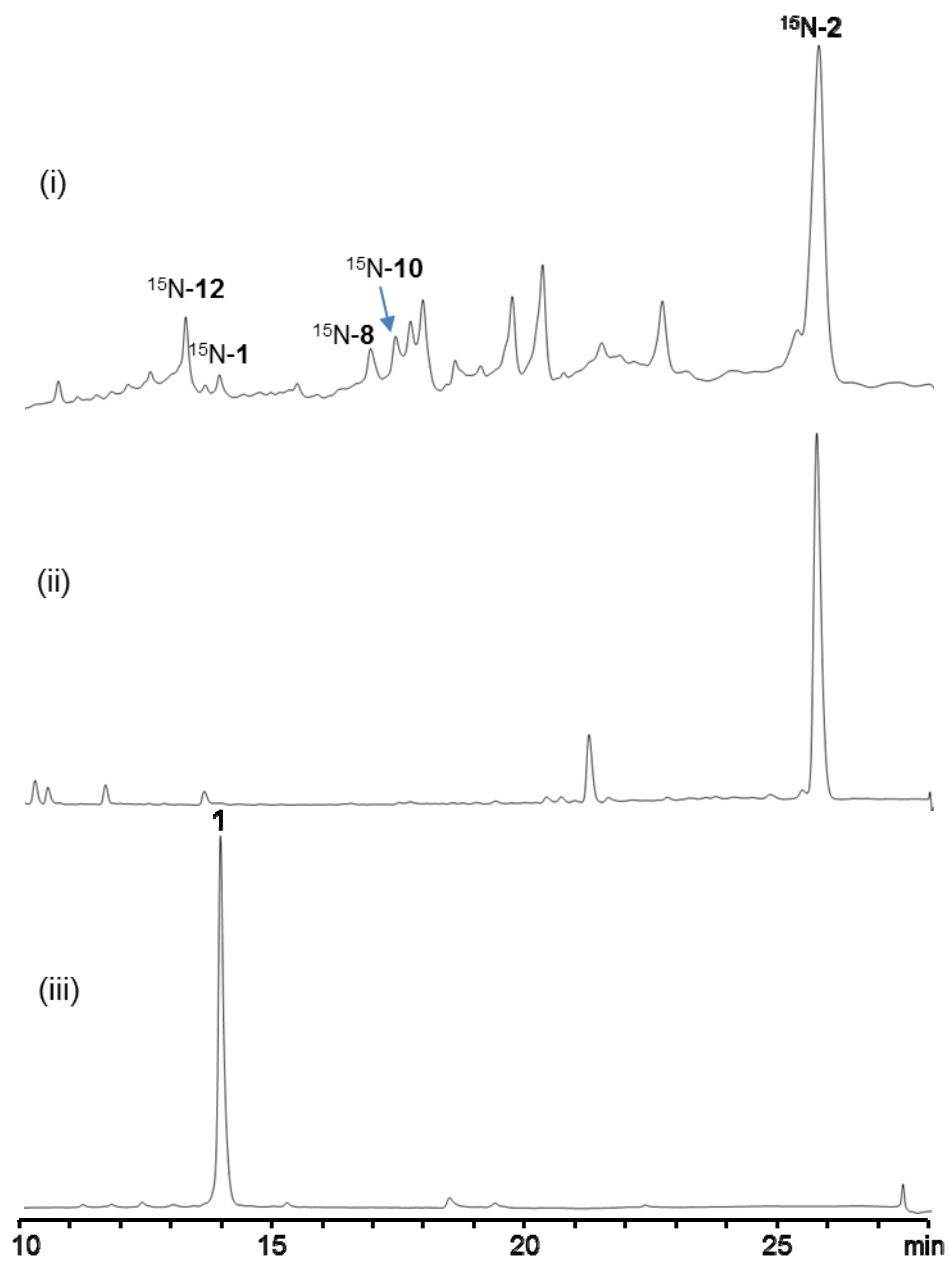
Fig. S5 ^{15}N-labelling related studies.

(**D**) HRESIMS of ^{15}N-labeled **1** isolated from *S. pactum* XM47i and ^{15}N-labeled **12** prepared from a XiaK reaction with ^{15}N-labeled **1**.

S21
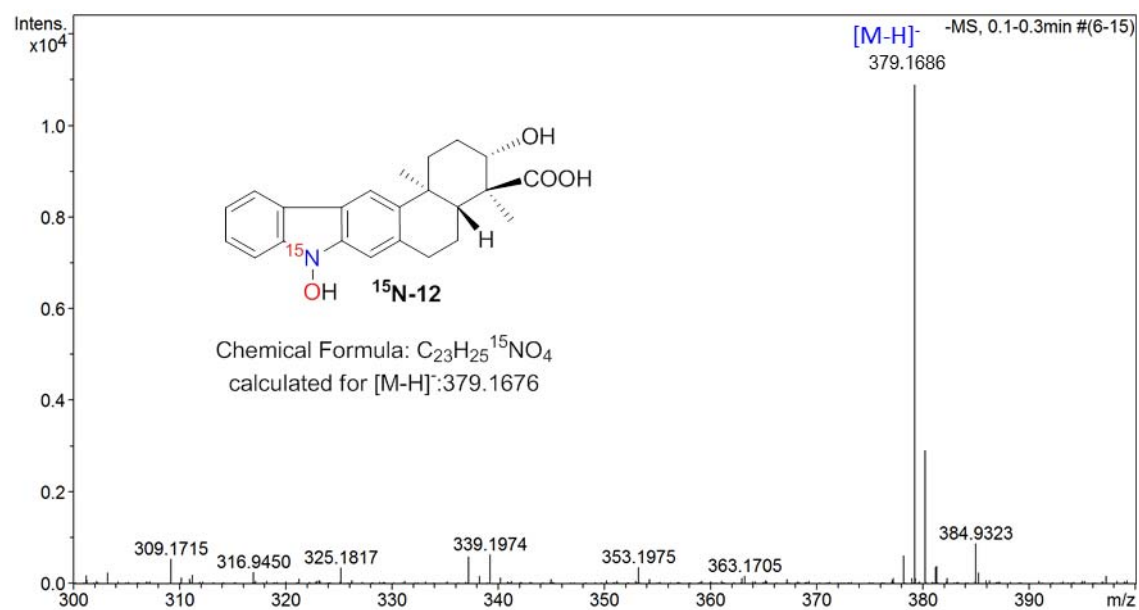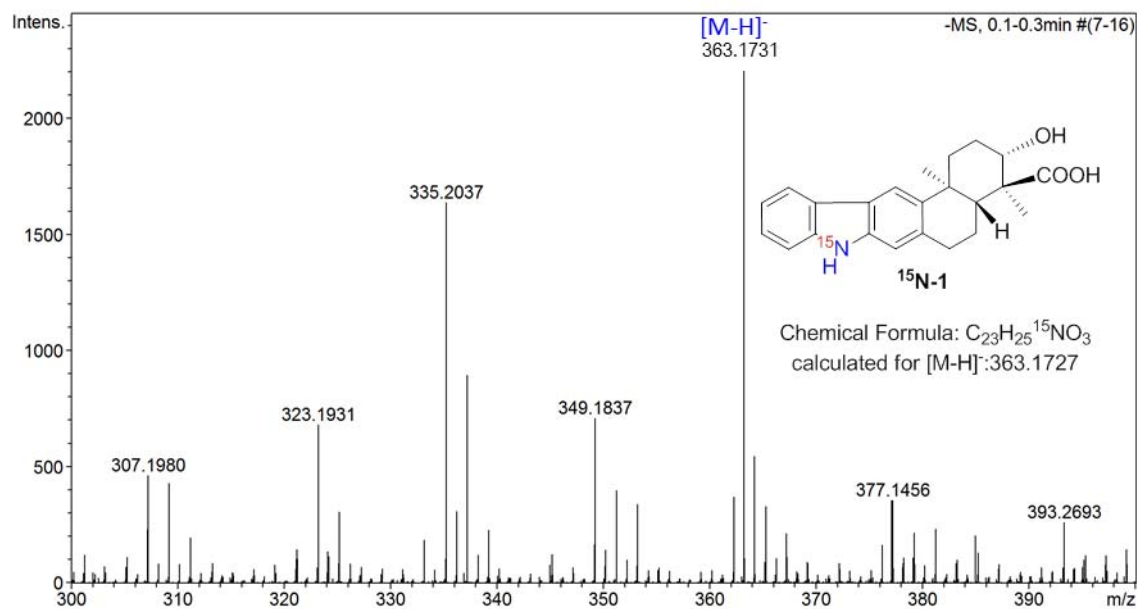
Fig. S6 Construction of the in-frame deletion mutant XM47i (Δ*xiaPi*).
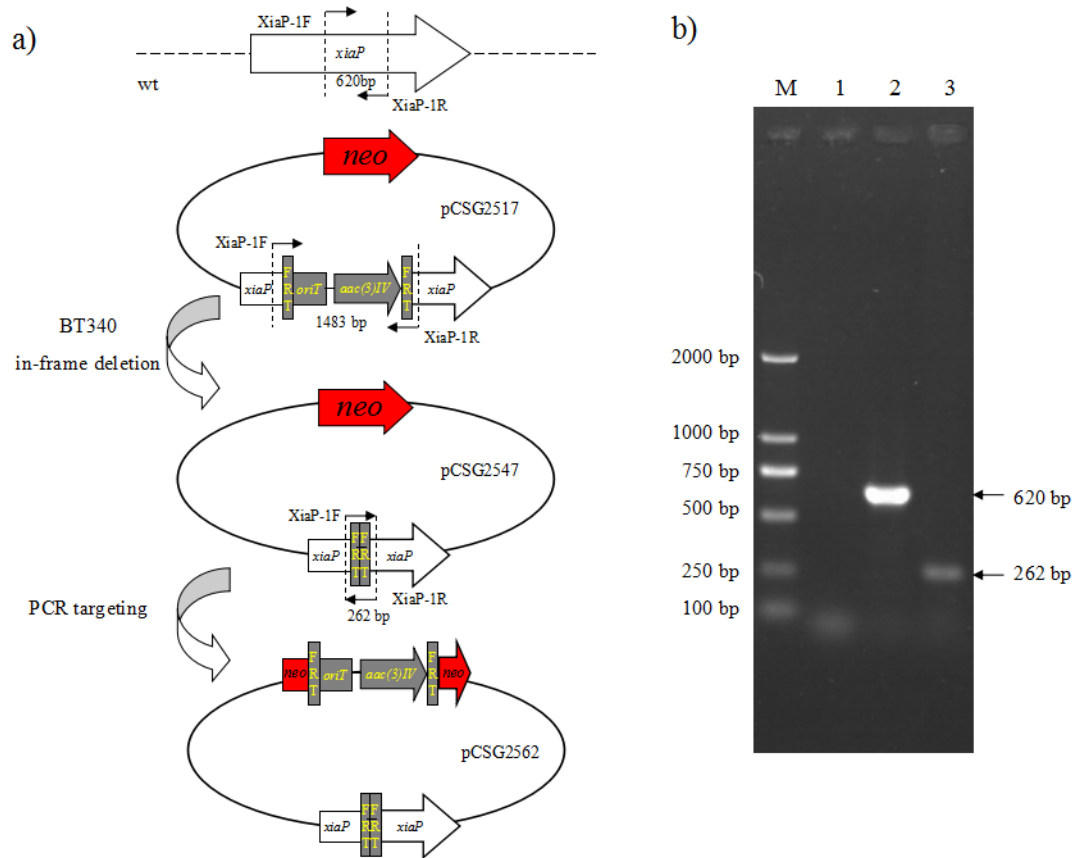
The *xiaP* gene in-frame deletion mutations were individually confirmed by diagnostic PCR. (a) Construction of the *xiaP* in-frame deletion vector pCSG2562. Location of the diagnostic PCR primers XiaP-1F and XiaP-1R (Table S1) was indicated. Sizes of PCR products, 620 bp for the wild type strain *S. pactum* SCSIO 02999 and 262 bp for the mutant XM47i, were also indicated. (b) Gel electrophoresis of PCR products. DNA templates were from: DNA marker DL 2000 (Takara, lane M); ddH$_2$O (negative control, lane 1); wild type strain *S. pactum* SCSIO 02999 (lane 2) and mutant XM47i (lane 3).

Fig. S7 Comparison of theoretical simulation and experimental EPR spectra of [14]N-**12** and [15]N-**7**.

EPR of [14]N-**12**

EPR of [15]N-**12**

g value=2.0051
A value=7.36 Gauss

g value=2.005
A value=10.6 Gauss
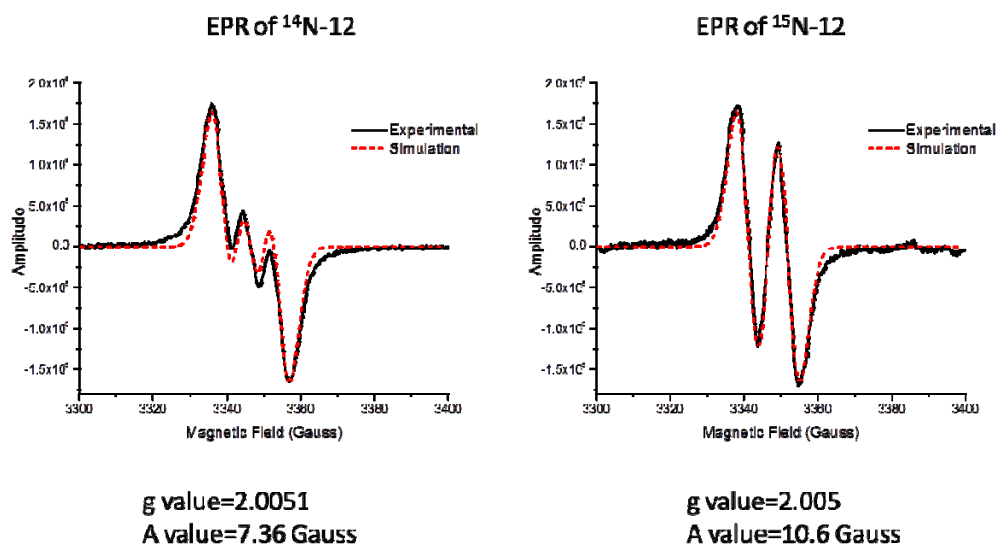
Fig. S8 Estimation of the spin concentration of **12**.

$y = 0.0971x + 0.4766$

71.1 µM
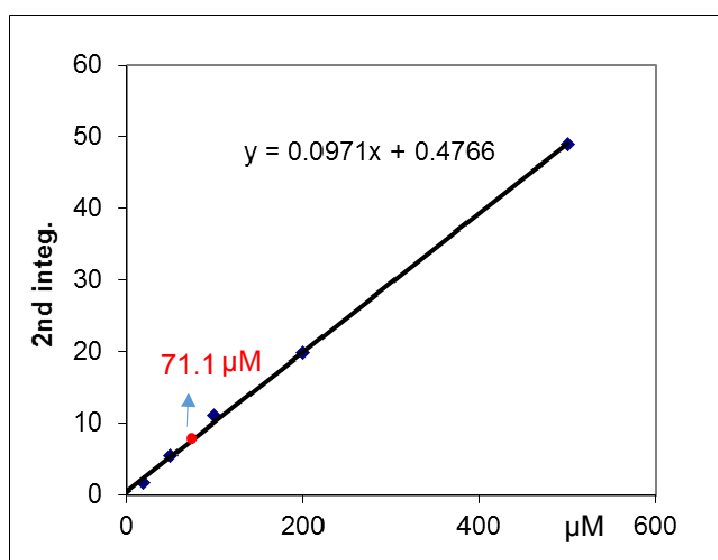
MTSL (1-oxyl-2,2,5,5- tetramethyl-Δ3-pyrroline-3-methyl methanethiosulfonate) was used as a standard. A set of dilution with different concentration (20, 50, 100, 200, and 500 µM) was used as spin concentration estimation; the spin concentration (red spot •) of **12** at a concentration of 2 mM in MeOH was estimated to be 71.1 µM, indicating a ratio of around 3.6%.

Fig. S9 Phylogenetic analysis of XiaK with other flavoenzymes.
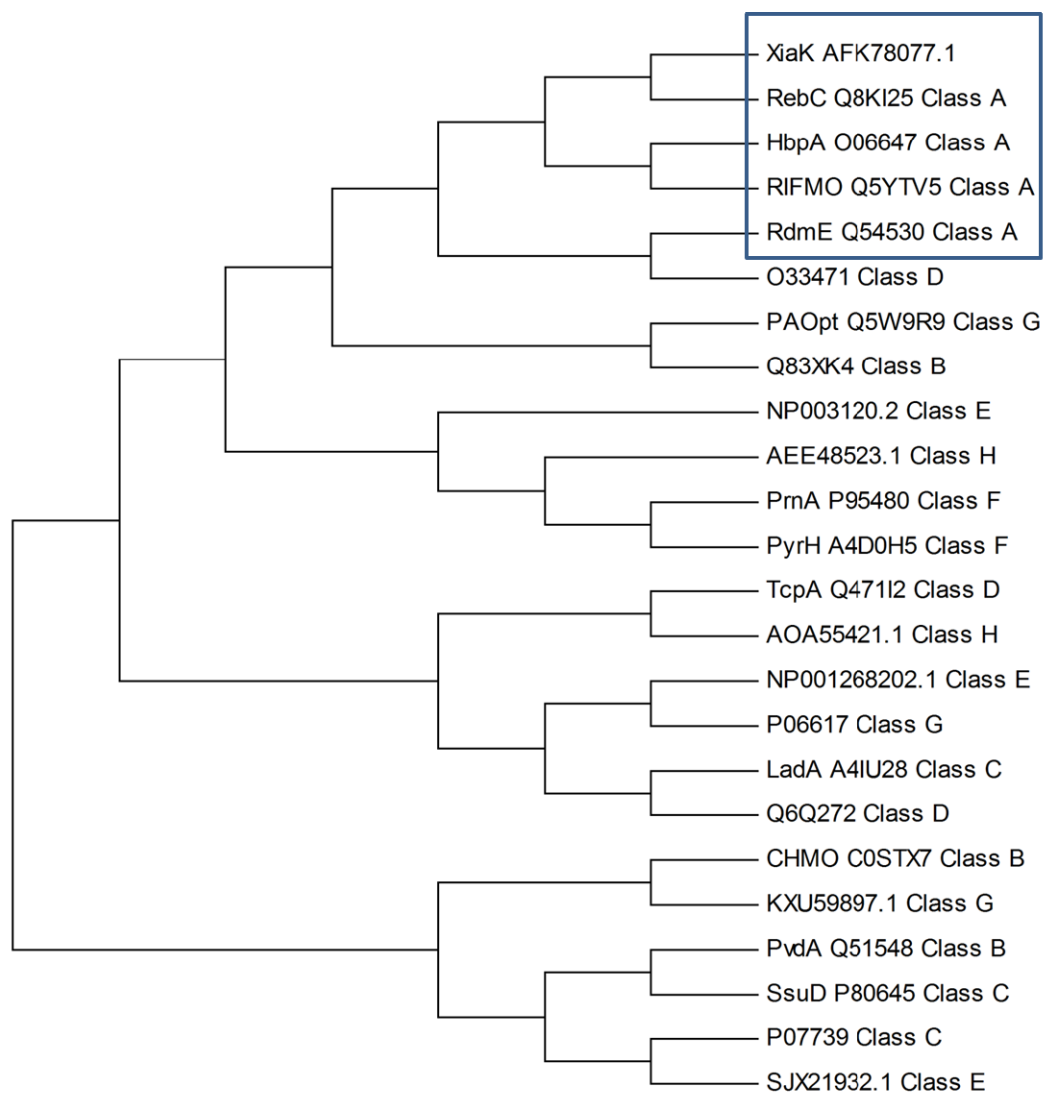
Fig. S10 Comparison of well aligned XiaK structure model and the crystal structures of Class A flavoprotein monooxygenases

The 3D-model of XiaK (cyan carton) was build by I-TASSER online server and structures alignment was processed with Pymol. A survey of the Protein Data Bank shows that the closes structural homologs of XiaK to be RdmE (yellow, PDB code 3IHG[16]), HbpA (salmon, PDB code 5BRT[17]), RifMO (magenta, PDB code 5KOW[18]) and RebC (grey, PDB code 2R0C[19]), which are all class A flavoprotein monooxygenases. According to Phylogenetic analysis, sequence and structural comparisions, XiaK is considered to be a member of class A flavoprotein monooxygenases.[20-22]

Fig. S11 Sequence Comparison of XiaK and four class A flavoprotein monooxygenases.

```
                          β1              α1                  β2                              α2
RdmE_Q54530           →→→→→→→      000000000000        →→→→→→                        00000000
                   1        10          20          30          40          50
                   .        .           .           .           .           .
RdmE_Q54530        .MNDHEVDVLVVGAGLGGLSTAMFLARQGVRVLVVERRPGLSPYPRAAGQNPRTMELLRI
XiaK_AFK78077.1    .MLDVEVPVLVVGGGGCGLAASVFLSDVGVRHLVVERRESTSVLPRAHYLNQRTMEVFRQ
HbpA_O06647        MSNSAETDVLIVGAGPACAMSATLLASLGIRSLMINRWRSTSPGPRSHIINQRTMEILRD
RebC_Q8KI25        MNAPIETDVLILGGCPVGMALALDLAHRQVGHLVVEQTDGTITHPRVGTIGPRSMELFRR
RIFMO_Q5YTV5       ..GSHMIDVIIAGGGPTGLMLAGELRLHGVRTVVLEKEPTPNQHSRSRGLHARSIEVMDQ

                        α3                      TT       β3              β4         α4        η1
RdmE_Q54530         000000                              →→→→→         →→→→→      000000    0000
                  60          70          80          90          100         110
                   .           .           .           .           .           .
RdmE_Q54530        GGVADEVRADDIRGTQGDFVIRLAESVRG.....EILRTVSESFDDMVAATEPCTPAGW
XiaK_AFK78077.1    HGVADDVYGISCPLPNMSEIAWRTSLGGDGPADGRELHRMEAFGGGGTAGPYAAHSTGPS
HbpA_O06647        IGLEESAKSLAVPKEYMGEHVYATSLAGEEFG....RIPAWASHPQAHAEHELASPSRY
RebC_Q8KI25        WGVAKQIRTAGWPGDHPLDAAWVTRVGGHEVYR.......IPLGTADTRATPEHTPEPD
RIFMO_Q5YTV5       RGLLERFLAHGEQFRVGGFFAG...................LAAEWPADLDTAHSYV

                             α5              β5          β6       η2        β7          TT       β8
RdmE_Q54530        0000000000000            →→→→        →→→→     000      →→→→→            →→→→→
                 120         130         140         150         160         170
                   .           .           .           .           .           .
RdmE_Q54530        AMLSQDKLEPILLAQARKHG.GAIRFGTRLLSFRQHDDDAGAGVTARLAGPDGEYDLRAG
XiaK_AFK78077.1    TNLPQHRLEPLLRRHAERRAPGSLLFHHQMEEFTQDEHGVLARVTDRSTGEVG..TVRAR
HbpA_O06647        CDLPQLYFEPMVVSEAAALRG.ADVRFLTEYLGHVEDQDGVTARLLDHVSGAEY..EVRAK
RebC_Q8KI25        AICPQHWLAPLLAEAVGERL....RTRSRLDSFEQRDDHVRATITDLRTGATR..AVHAR
RIFMO_Q5YTV5       LAIPQVVTERLLTEHATELG.AEIRRGCEVAGLDQDADGVTAELADGTR......LRAR

                →→→→→          α6                          ..    β9       η3      TT     β10
RdmE_Q54530                 00000                             →→→→→→→     000          →→→→→→→
                 180         190         200         210         220
                   .           .           .           .           .
RdmE_Q54530        YLVGADGNRSLVRESLGIGRYGHG..TLTHMVGVIFDADLSGIMEPGTTGWYYLHHP...
XiaK_AFK78077.1    YLLGADGGR.GVGDALGIRMDGAG..GGFTIISVHFSADLSRWWSDPVLMTHIFGLDGEV
HbpA_O06647        YIIGADGAHSLVAQNAGLPFFEGGQMGIGDSGSINIEFSADLSSLCEHRKGDMYWMFRAGSG
RebC_Q8KI25        YLVACDGASSPTRKALGIDAPPRHRTQVFRNILFRAPELRSLLGERAALFFFLMLSS...
RIFMO_Q5YTV5       YLVGCDGGRSTVRRLLGVDFPGEPTRVETLLADVRIDVPVETLTAVVAEVRKTQLRFG..

                      β11          TT    β12          η4        α7
RdmE_Q54530        →→→→→                →→→→→       000    00000000000             ─────
                 230         240         250         260         270         280
                   .           .           .           .           .           .
RdmE_Q54530        ....EFKGTFGPTDRPDRHTLFVEYDPDEGERPEDFTPQRCVELIGLALDAPEVKPELVD
XiaK_AFK78077.1    SVLVASGPDWGAASQEWALHFRVPPGTAAGGLDADTITGRVRELLKLPADLGVRIHHVSE
HbpA_O06647        ...INGVGVAALRMIRPWNKWICVWGYEKSKGTPEITKEEAKKIIHEIIGTDEIPVEVGP
RebC_Q8KI25        ..........SLRFPLRALDGRGLYRLTVGVDDASKSTMDSFELVRRAVAFDTEIEVLSD
RIFMO_Q5YTV5       ...........AVPAGDGFFRLIVPAQGLSADRAAPTLDELKRCLHATAGTDFGVHSPRW

                  β13             β14 β15          β16          TT     α8
RdmE_Q54530        →→→→→            →→TT→→→         →→→→                00000000000000000000
                   290          300         310         320         330         340
                   .           .           .           .           .           .
RdmE_Q54530        IQGWEMAARIAERWREGRVFLAGDAAKVTPPTGGMSGNAAVADGFDLAWKLAAVLQGGQAG
XiaK_AFK78077.1    YR...PEAVVARSFRSGRVFLAGDAAHRQPPAAGGGLNTAIQDVHNLAWKLGAVLEGRAG
HbpA_O06647        ISTWTINQQYAVRNTSGRVFCMGDAVHRHTPMGGLGLNTSVQDAYNLAWKLALVLKGQAA
RebC_Q8KI25        SE.WHLTHRVADSFSAGRVFLTGDAAHTLSPSGGFGMNTGIGSAADLGWKLAATLRGWAG
RIFMO_Q5YTV5       LSRFGDATRLAERYRTGRVLLAGDAAHIHPPTGGQLNLGIQDAFNLGWKLAAAIGGWAP

                                α9
RdmE_Q54530        00000000000000000000000000000000
                 350         360         370
                   .           .           .
RdmE_Q54530        AGLLDTYEDERKVAAELVVAEALAIYAQR.................................
XiaK_AFK78077.1    DALLDSYEAERLPVARRNVGAMGCLLNYGSLFAALGM..AHGKPEATEASIRELLADTP
HbpA_O06647        PTLLDSYDAERSPVAKQIVERAFKSLSTFPPVFEALSPPAPTESEMAEALVRLKDASEE
RebC_Q8KI25        PGLLATYEEERRPVAITSLEEANVNLRRTMDRELPPGLH....................D
RIFMO_Q5YTV5       PDLLDSYHDERHPVAAEVLDNTRAQMTLLS............................

                    η5                     α10
RdmE_Q54530        000          00......000          TT            T...T       T
                 380         390         400         410         420
                   .           .           .           .           .
RdmE_Q54530        MAPHMAEVWDKSVGYP.......ETLLGFRYRSSAVLATDDD.PARVENP...LTPSGRP
XiaK_AFK78077.1    MGETRRSVVRREVFGTQRIEYQAHDIELGHHYASGAVVADGTP.PPPRDPWGAVHHPTTRP
HbpA_O06647        GAKRRAALRKAMDATIIGLGGGHGVELNQRYVSRAVFPDGTPDPGFVRDQEFFYQASTRP
RebC_Q8KI25        DGPRGERIRAAVAEKLERSGARREFDAPGIHFGHTYRSSIVCGEPETEVATGGWRPSARP
RIFMO_Q5YTV5       ..............LDPGPRAVRRLMAELVEFPDVNR

                 T      β17     TT   β18   η6                β19          α11              β20
RdmE_Q54530             →→→→→        →→→→  0000            →→→→→        0000000000000000    →→→→
                 430         440         450         460         470         480
                   .           .           .           .           .           .
RdmE_Q54530        GFRGPHVLVSRHGERLSTVDLFG..DGWTLLAGELGADWVAAAEAVSAELGVPVRAYRVG
XiaK_AFK78077.1    GHRLPHVWLRAGDARRSTHDLVPGDGGFLLLTGPLGEGWAAAAKKAAADHGVAITVVPVG
HbpA_O06647        GAHLPHVWLTENQRRISTLDLCG.KGRFTLLTGLSGAAWKHEAEQVSQSLGIELKVCVIG
RebC_Q8KI25        GARAPHAWLTP...TTSTLDLFG..RGFVLLSFGTTDGVEAVTRAFADRHVPLETVTCHA
RIFMO_Q5YTV5       HLIEKITAIAVRYDLGDGHDIVGRRLRDIPLTEGRLYERMRGGRGLLLDRTGRLSVSGWS
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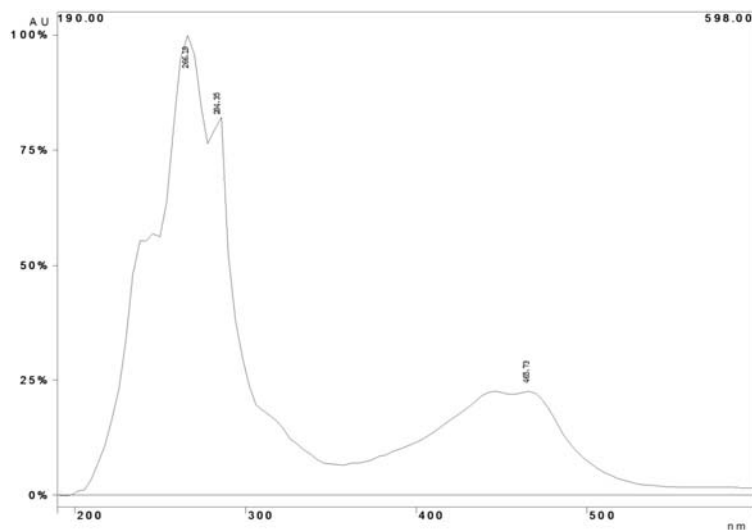
XiaK consists of a conserved motif of glycine residues GXGXXG and secondary structure of βαβ–Rossmann fold, which are characteristics of FAD binding domain.

Fig. S12 UV-Vis and HRESIMS spectra of **16**.

(A) UV-vis spectrum of **16**.
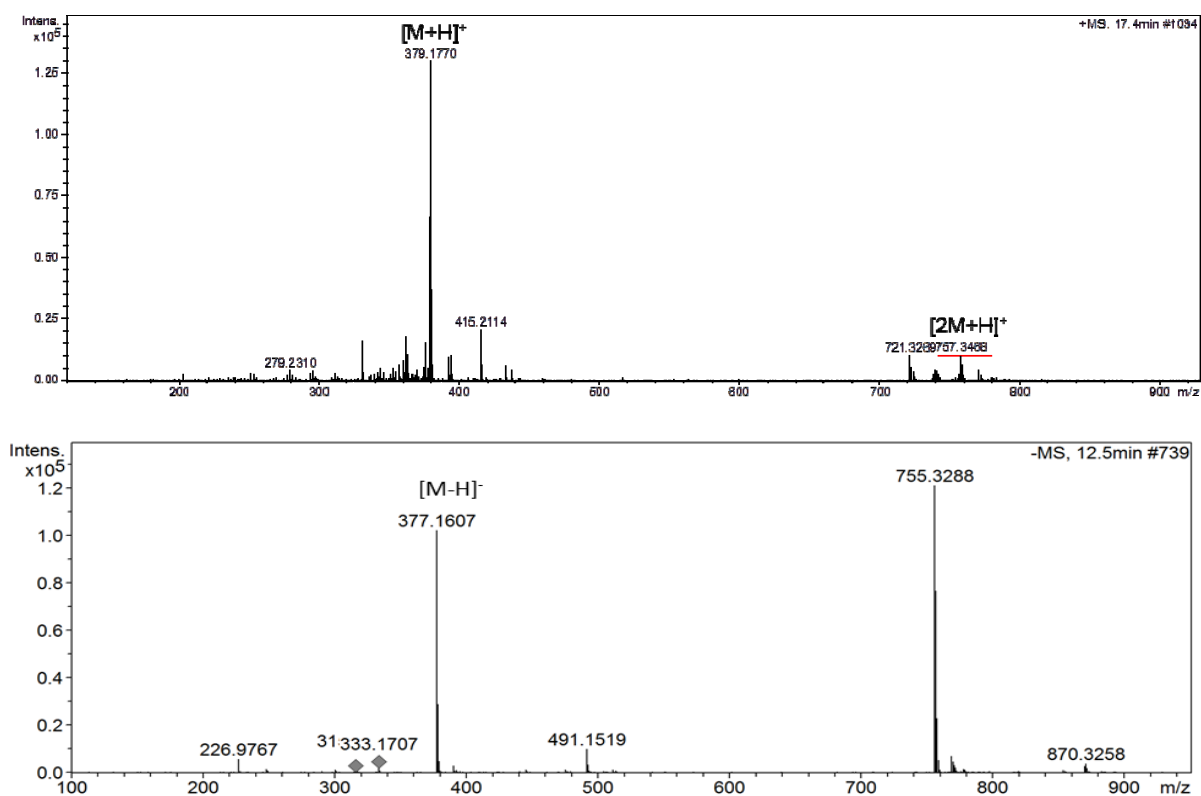

(B) HRESIMS spectrum of **16**.

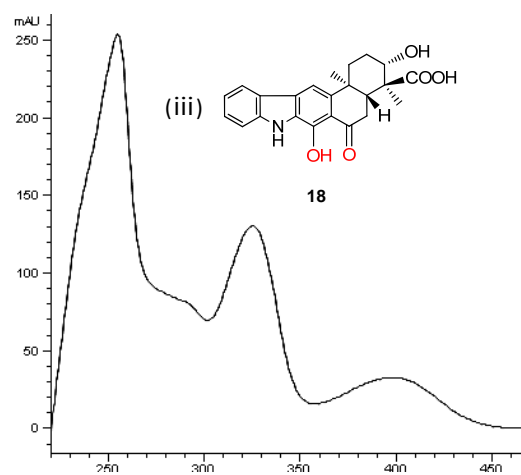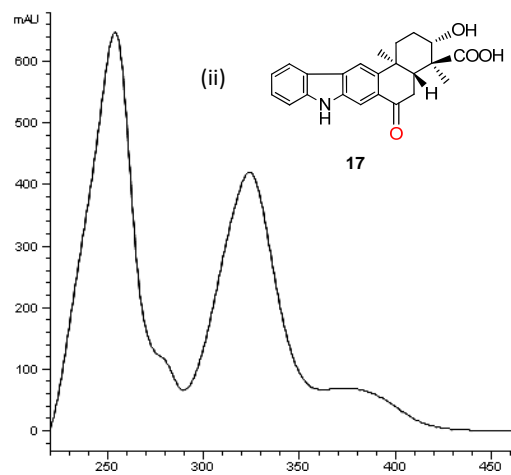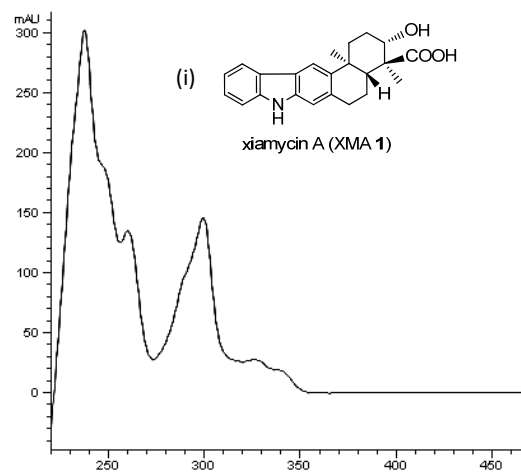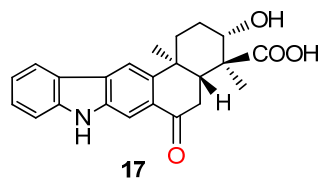**Fig. S13** Comparison of UV-vis spectra of **1**, **17** and **18**.

Fig. S14 The spectral data of **17** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
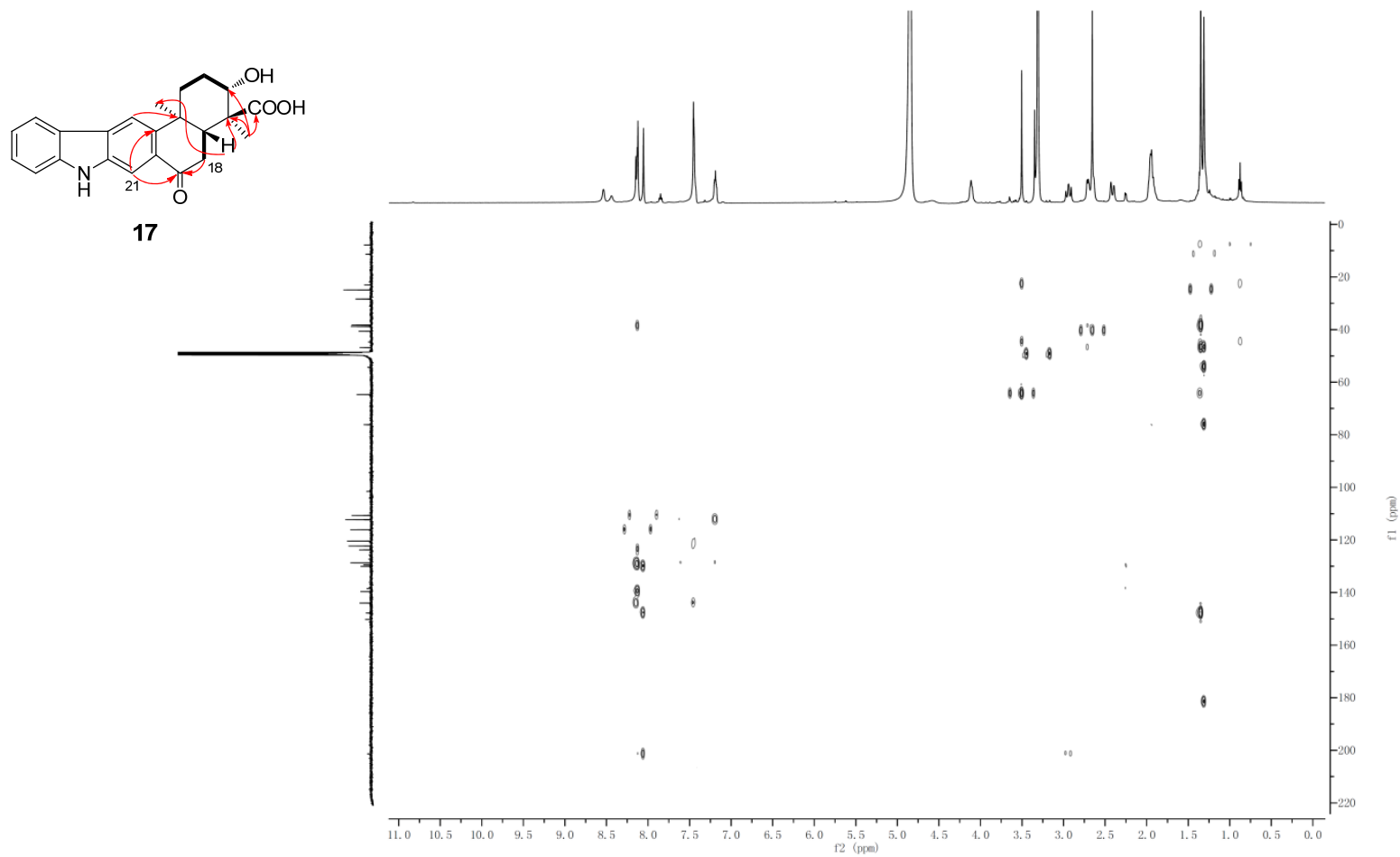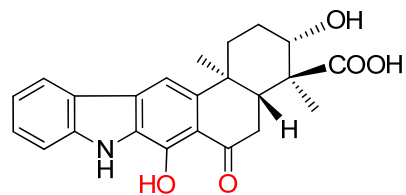
(**A**) The HRESIMS spectrum of **17**.

17

Chemical Formula: C$_{23}$H$_{23}$NO$_4$

[M - H]$^-$: calcd. 376.1554
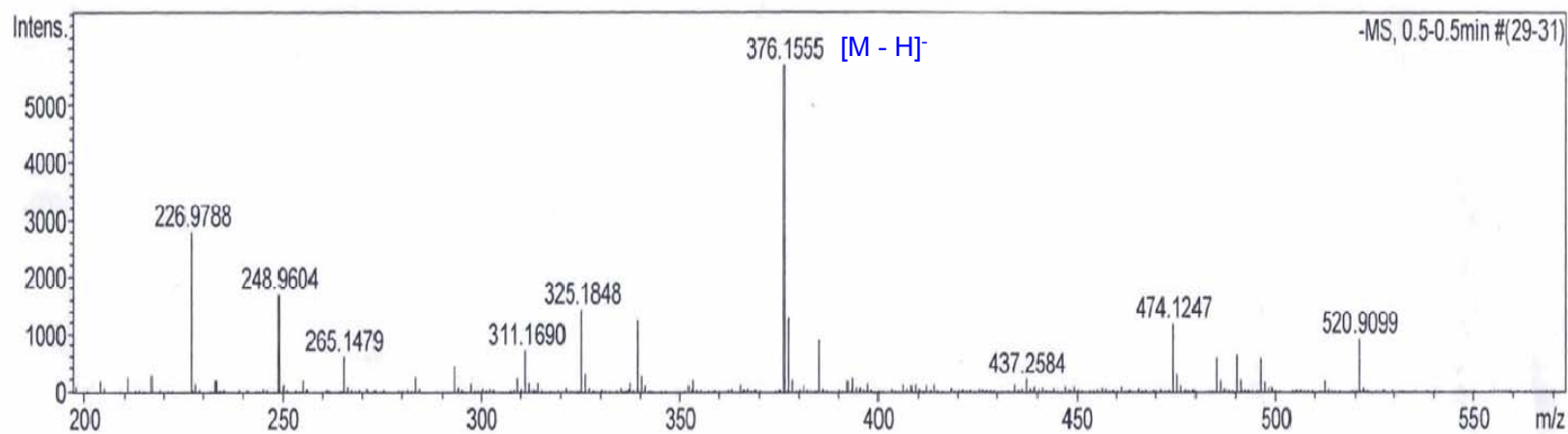
Fig. S14 The spectral data of **17**. (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)

(**B**) The ¹H-NMR spectrum of **17**.
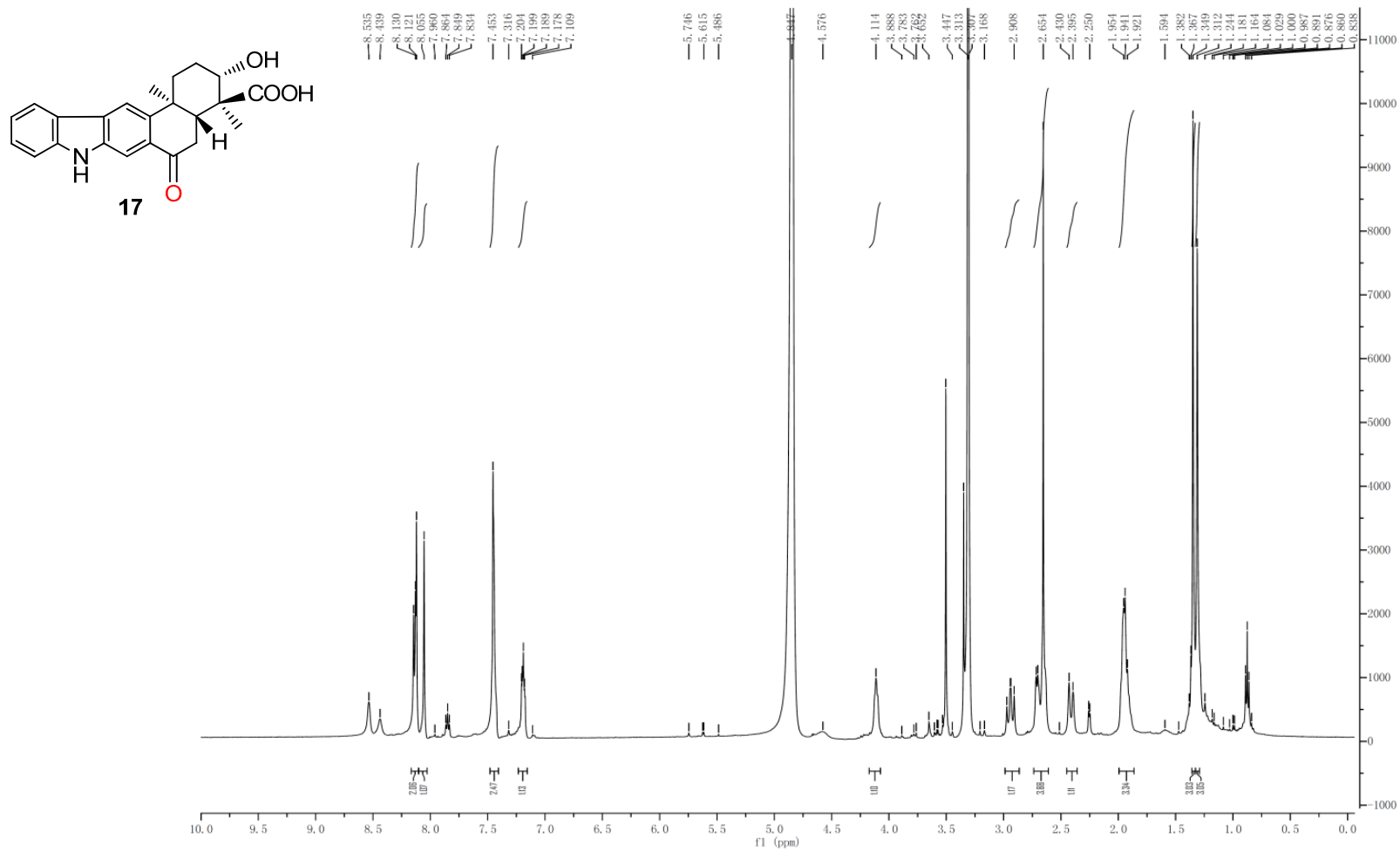
Fig. S14 The spectral data of **17** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)

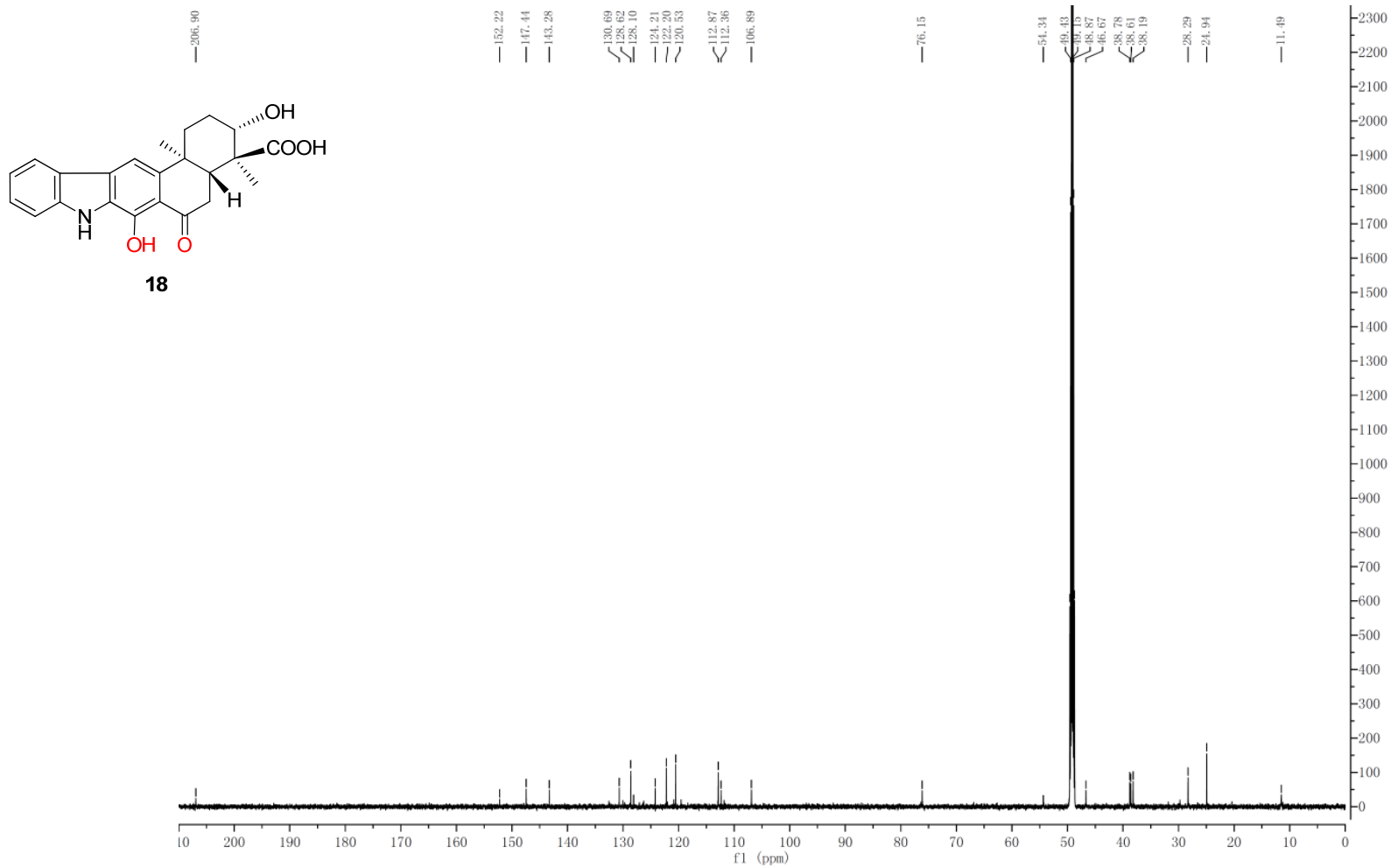(**C**) The ^{13}C-NMR spectrum of **17**.
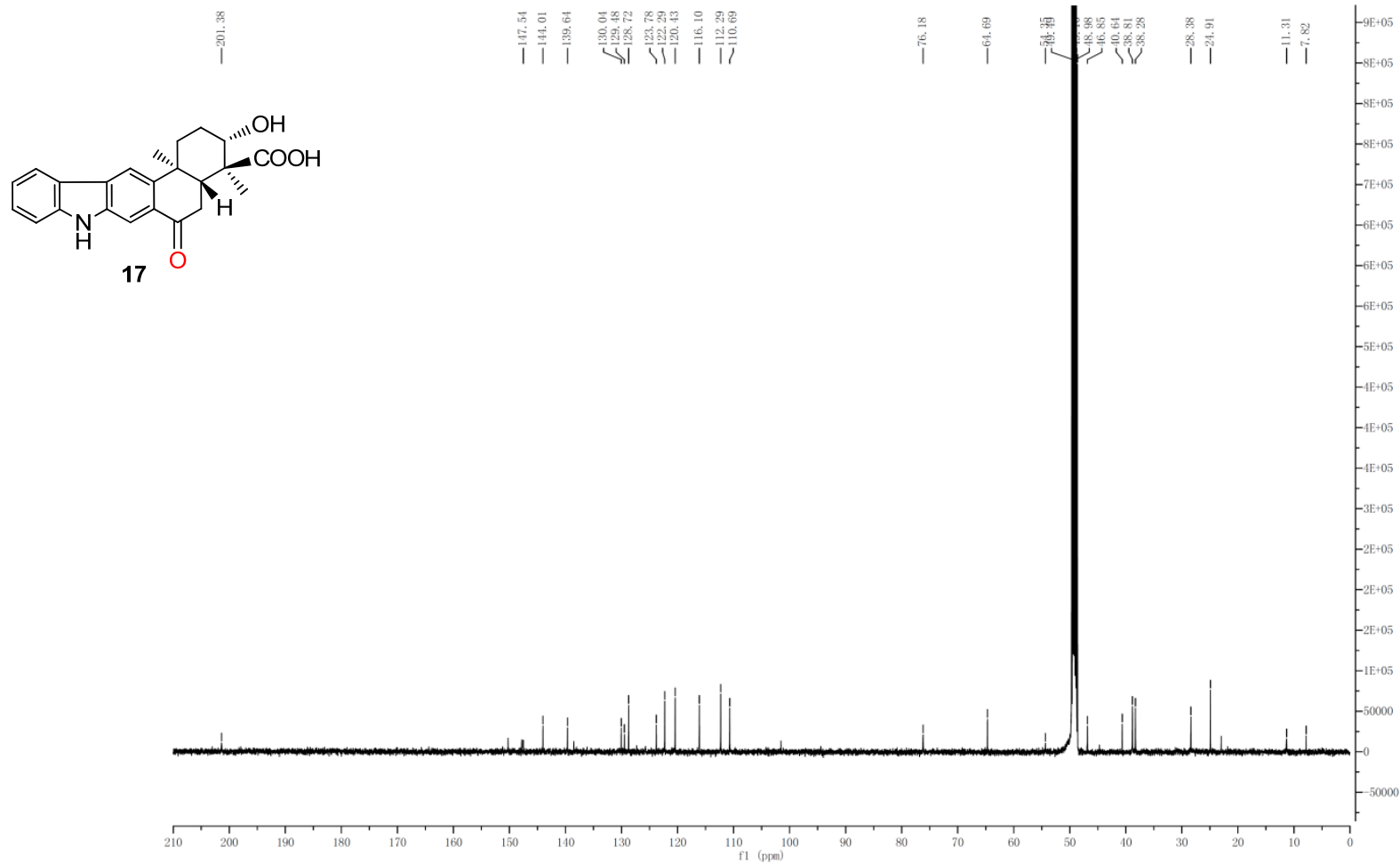
Fig. S14 The spectral data of **17** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
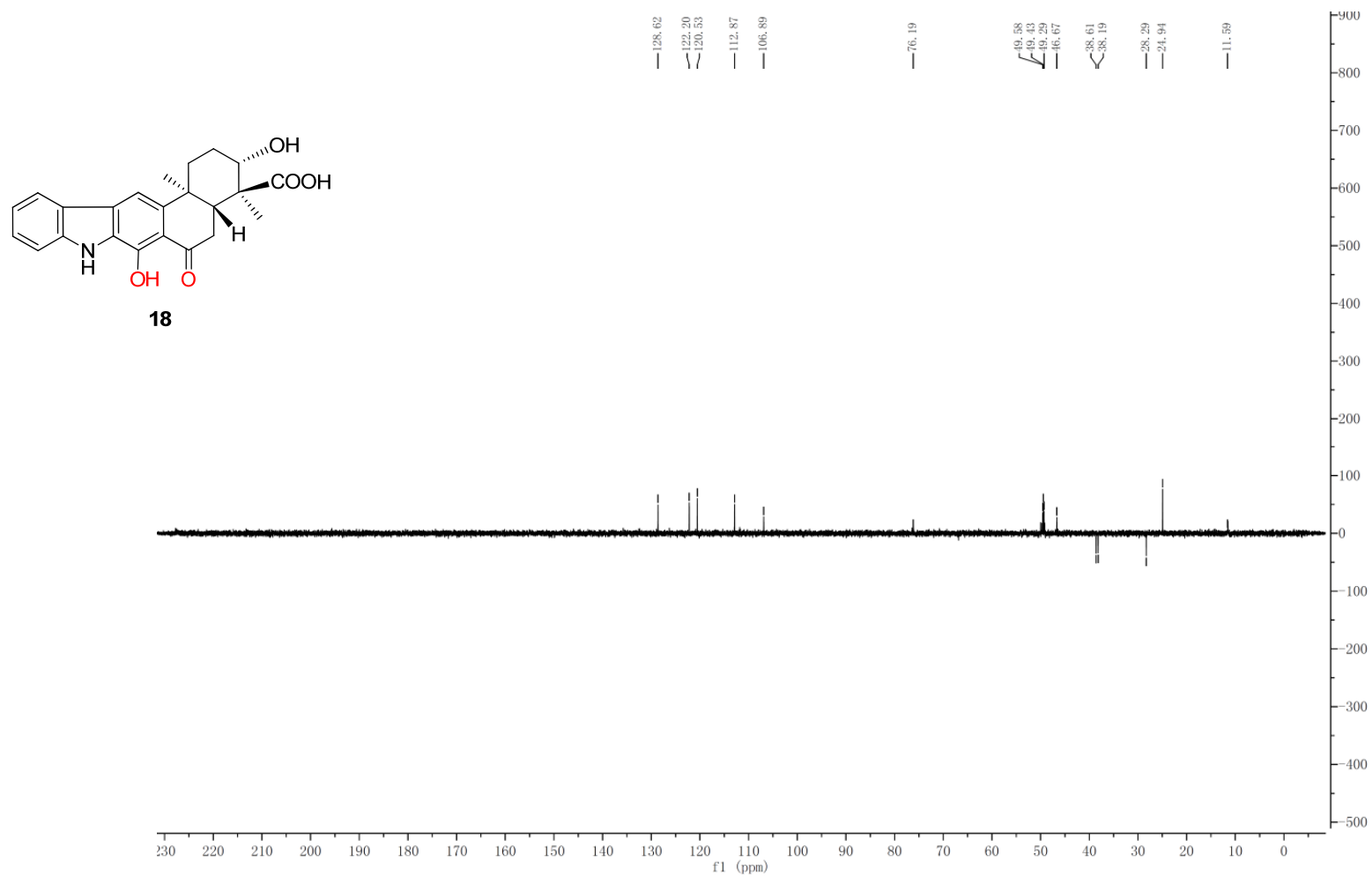(**D**) The DEPT135 spectrum of **17**.

Fig. S14 The spectral data of **17** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
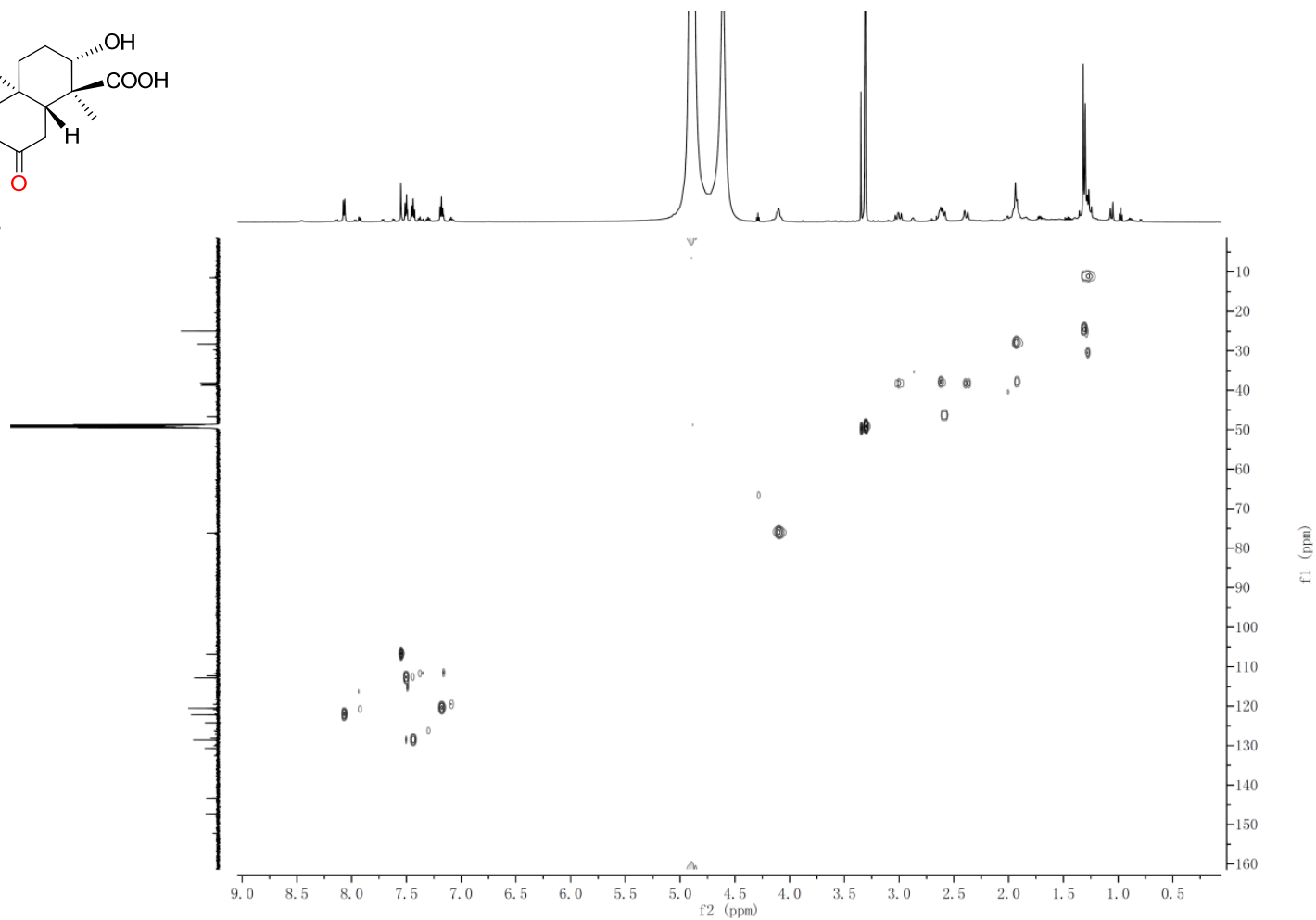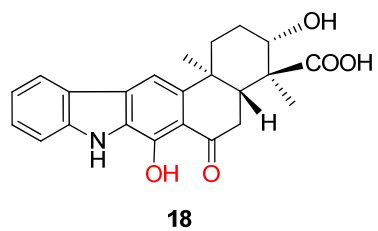
(**E**) The HSQC spectrum of **17**
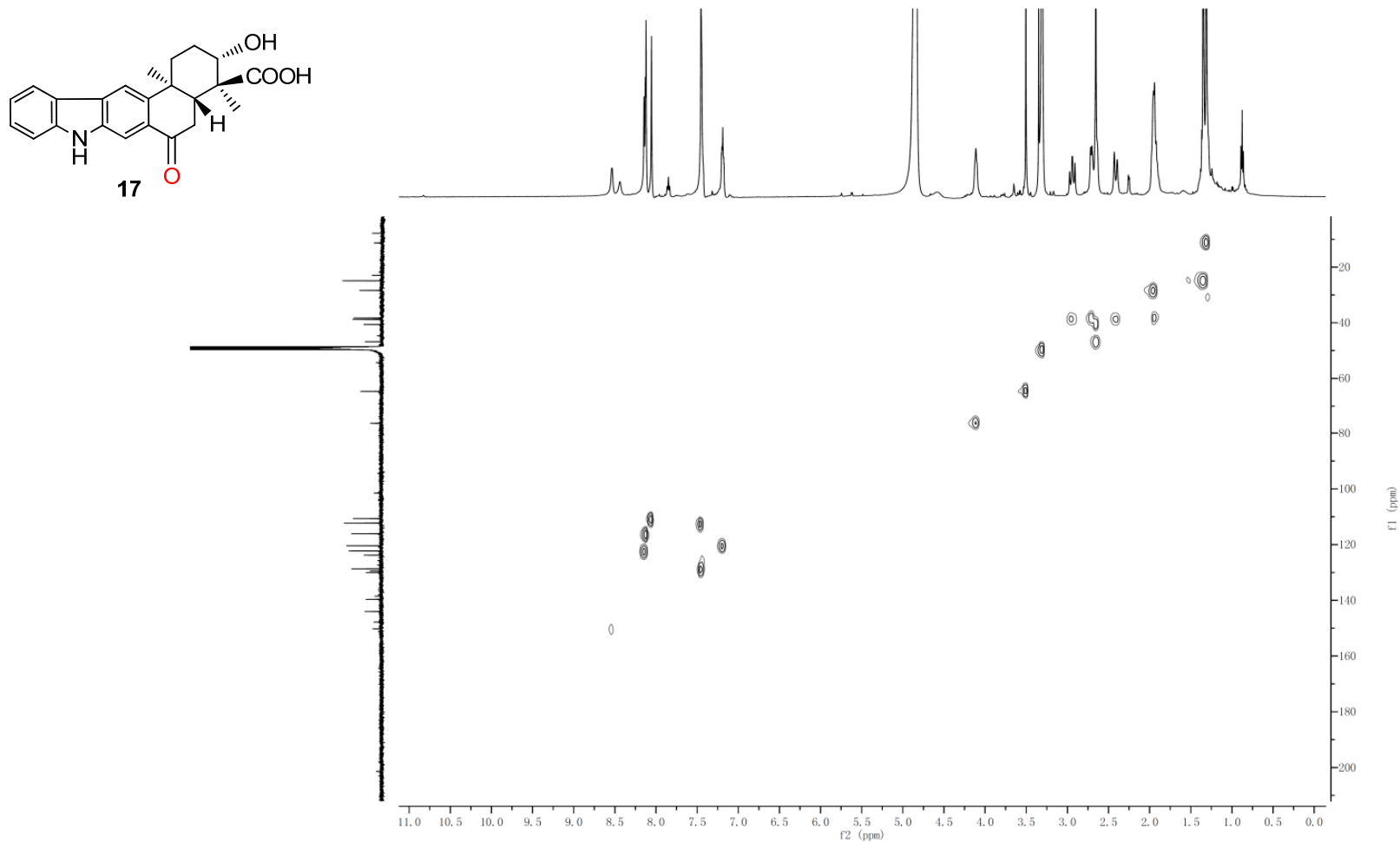
Fig. S14 The spectral data of **17** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**F**) The ^{1}H-^{1}H COSY spectrum of **17**
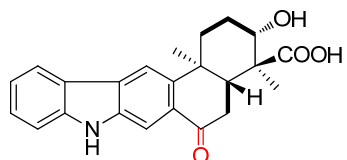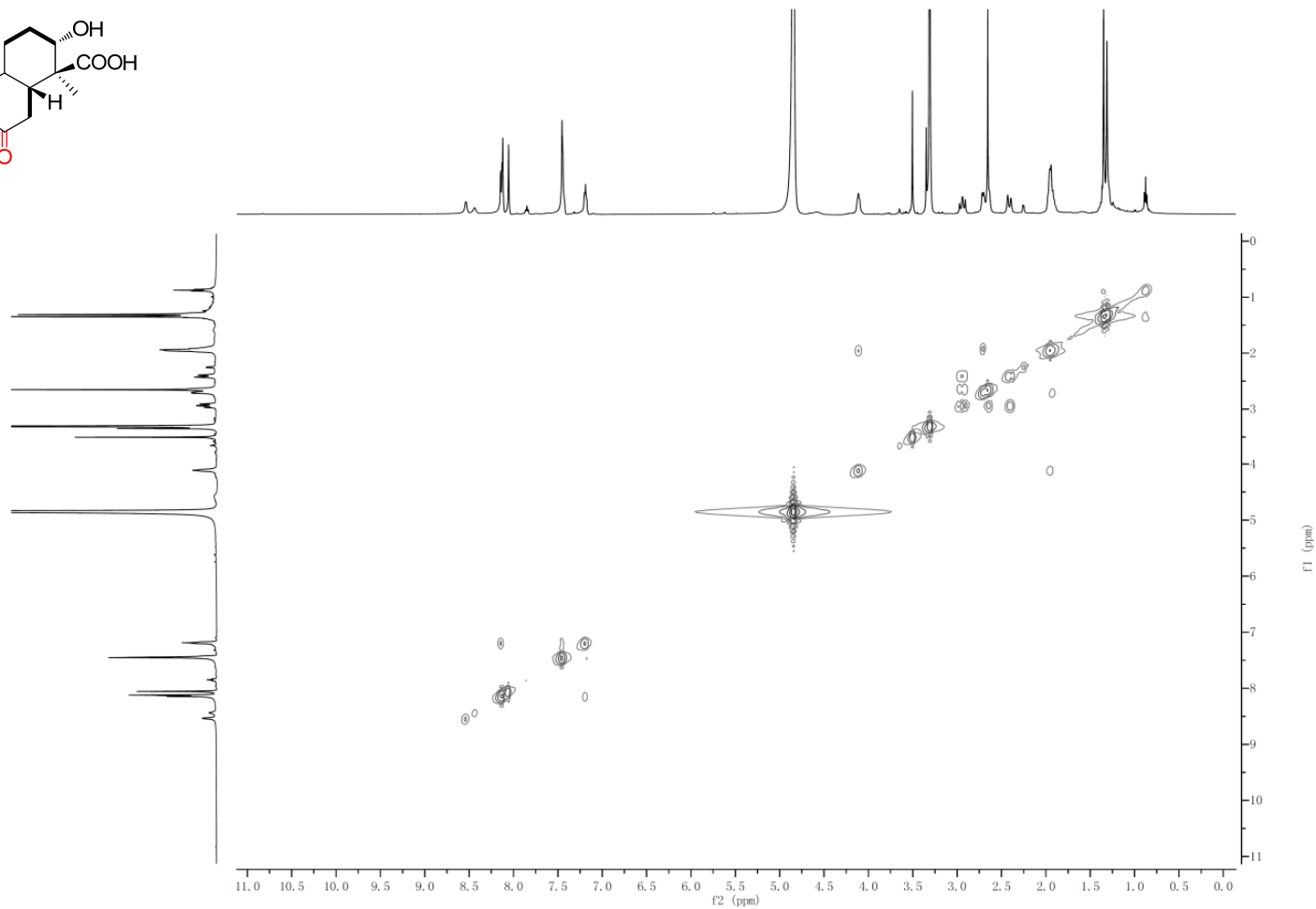
Fig. S14 The spectral data of **17** (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)
(**G**) The HMBC spectrum of **13**

17

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
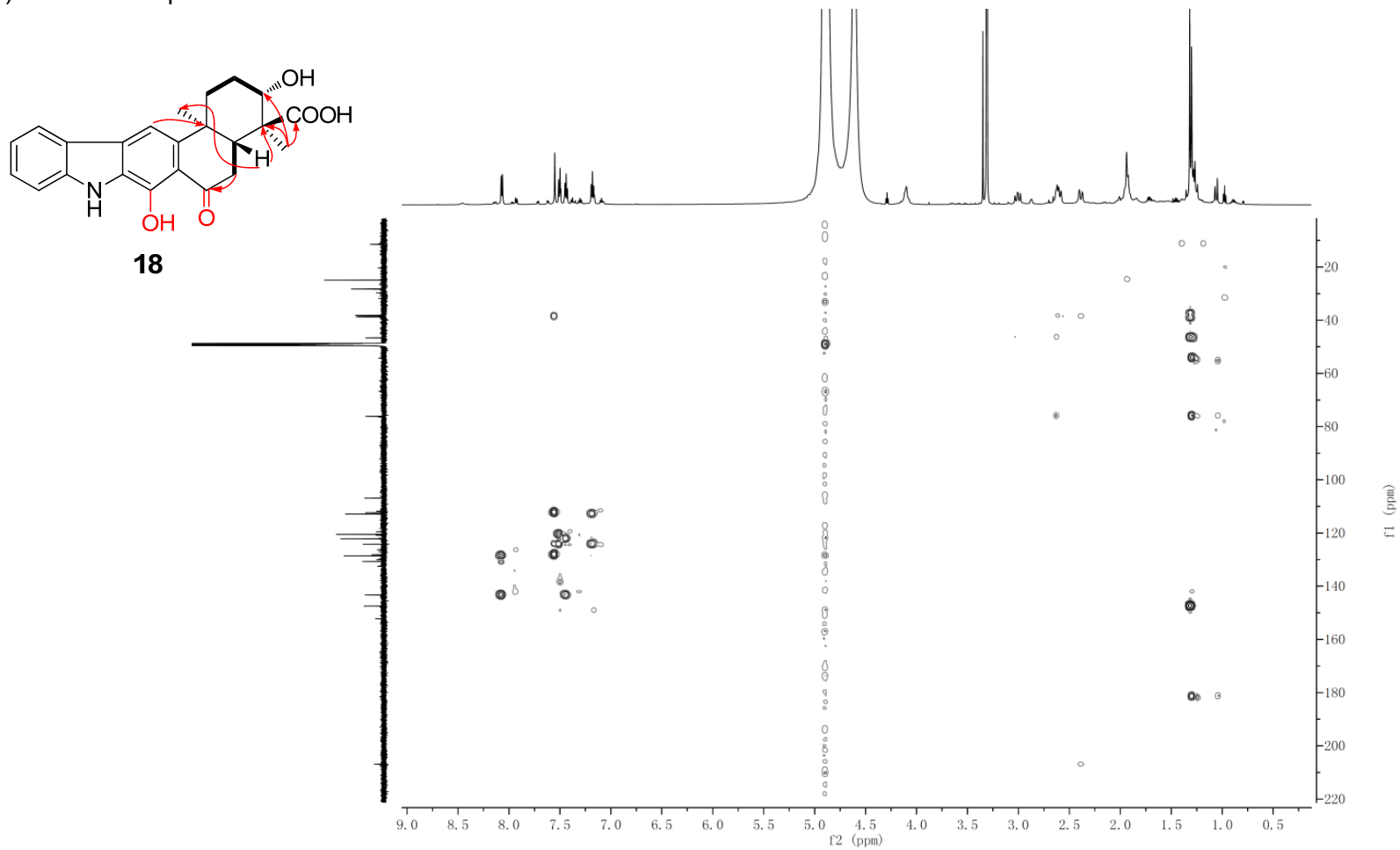(**A**) The HRESIMS spectrum of **18**.

18
Chemical Formula: C$_{23}$H$_{23}$NO$_5$
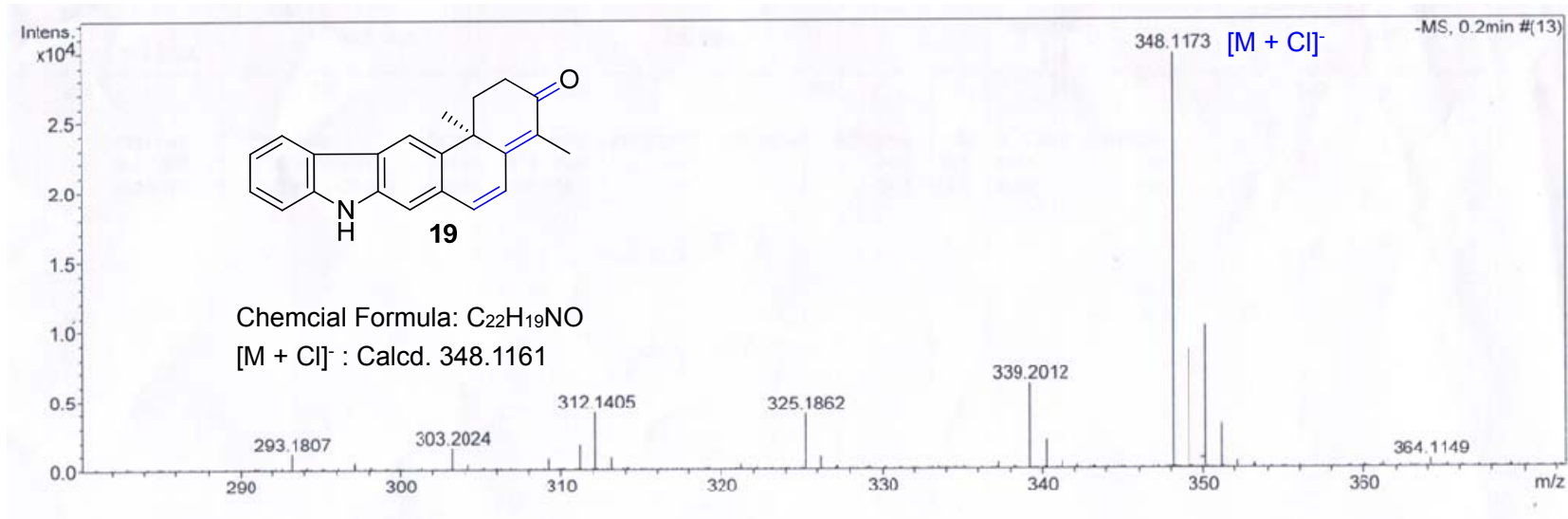[M - H]$^-$: calcd. 392.1503
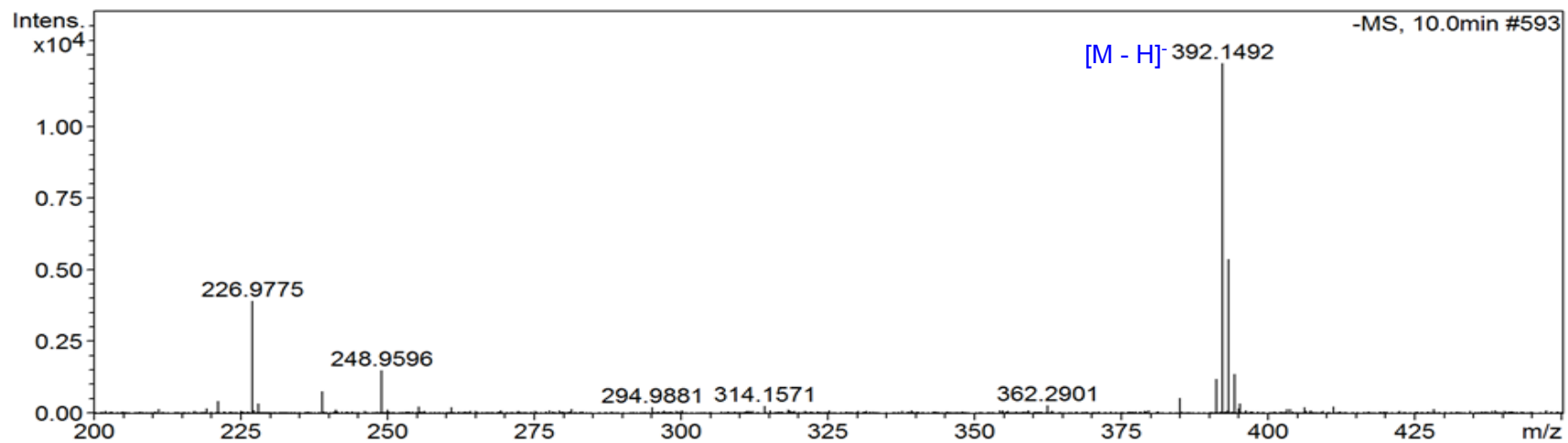
Fig. S15 The spectral data of **18** (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)

(**B**) The ¹H-NMR spectrum of **18**.

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)

(**C**) The ^{13}C-NMR spectrum of **18**.

18

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**D**) The DEPT135 spectrum of **18**.

18

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**E**) The HSQC spectrum of **18**.

18

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)

(**F**) The ^{1}H-^{1}H COSY spectrum of **18**.

Fig. S15 The spectral data of **18** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**G**) The HMBC spectrum of **18**.

Fig. S16 The spectral data of **19** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**A**) The HRESIMS spectrum of **19**

Chemcial Formula: C$_{22}$H$_{19}$NO
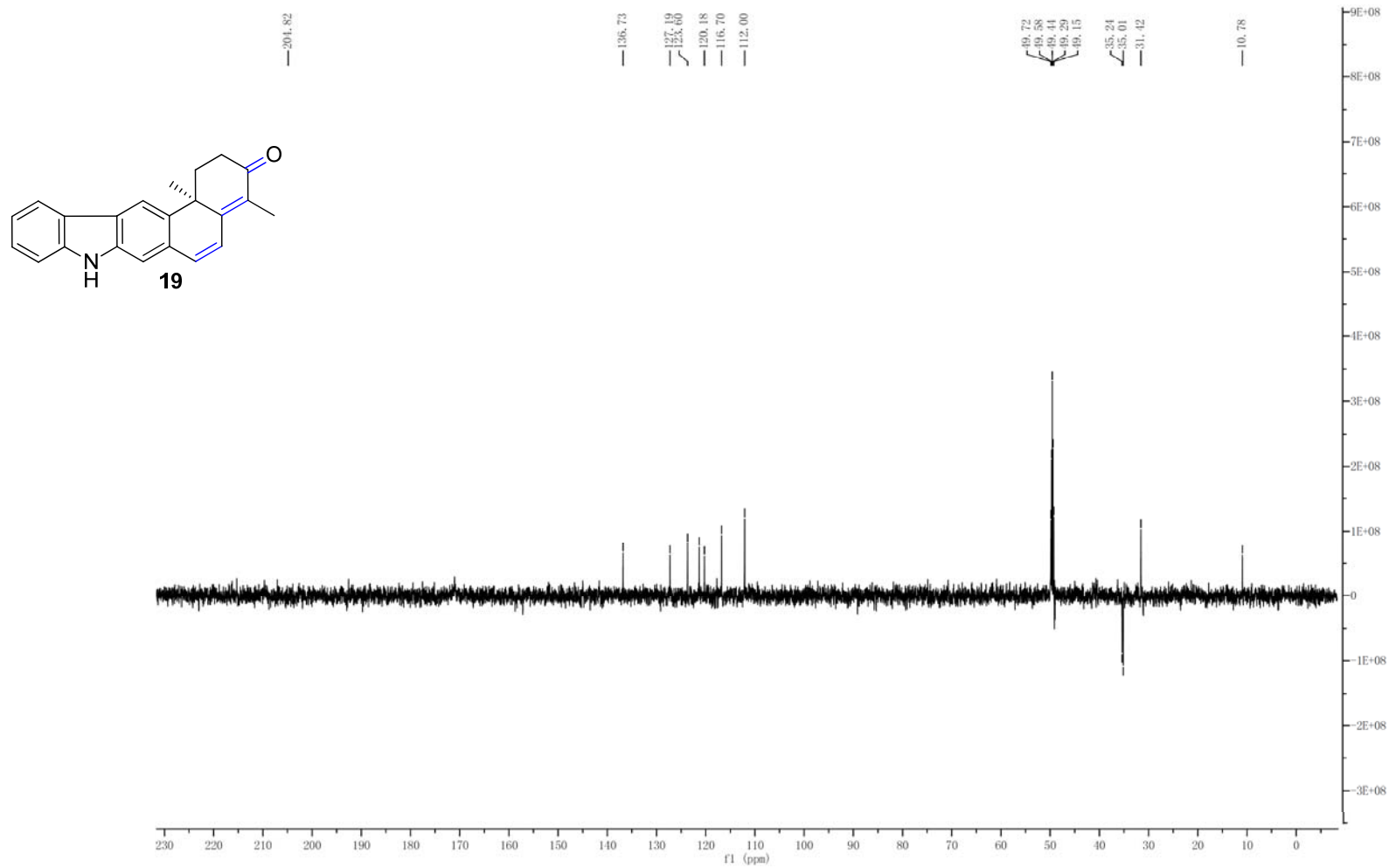[M + Cl]$^-$: Calcd. 348.1161

Fig. S16 The spectral data of **19** (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)

(**B**) The ¹H-NMR spectrum of **19**
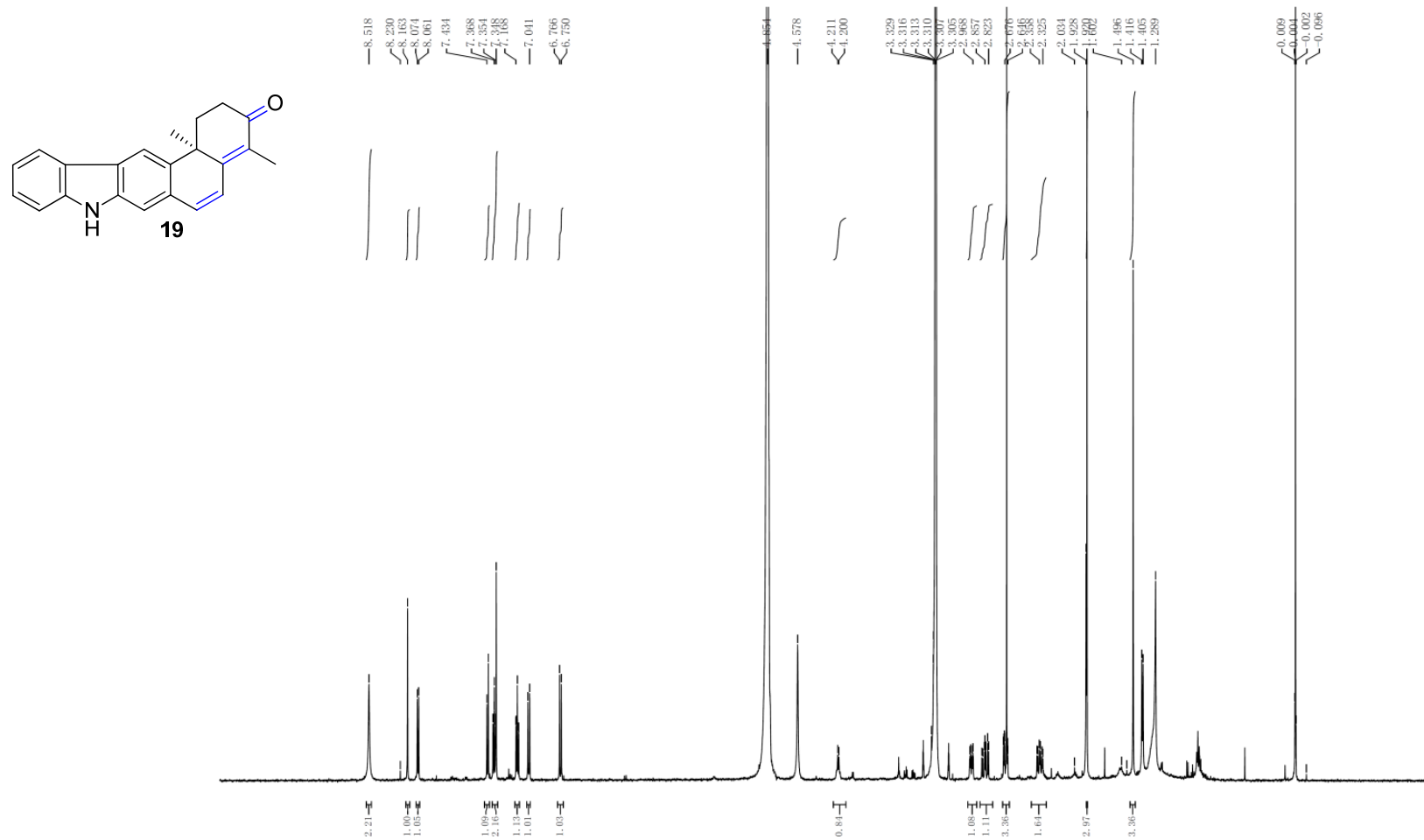
Fig. S16 The spectral data of **19** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)

(**C**) The ^{13}C-NMR spectrum of **19**
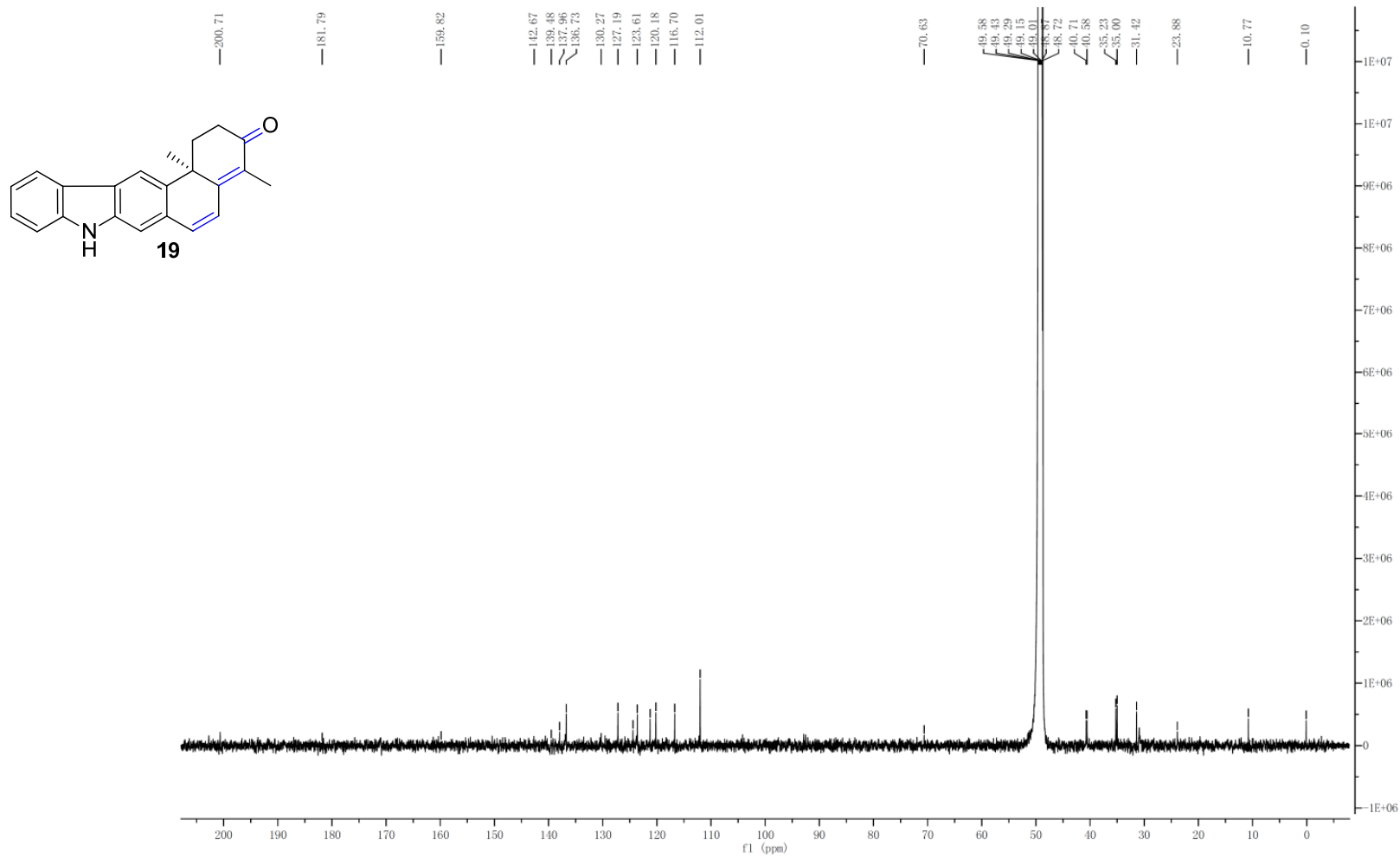
Fig. S16 The spectral data of **19** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
(**D**) The DEPT135 spectrum of **19**

Fig. S16 The spectral data of **19** (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)
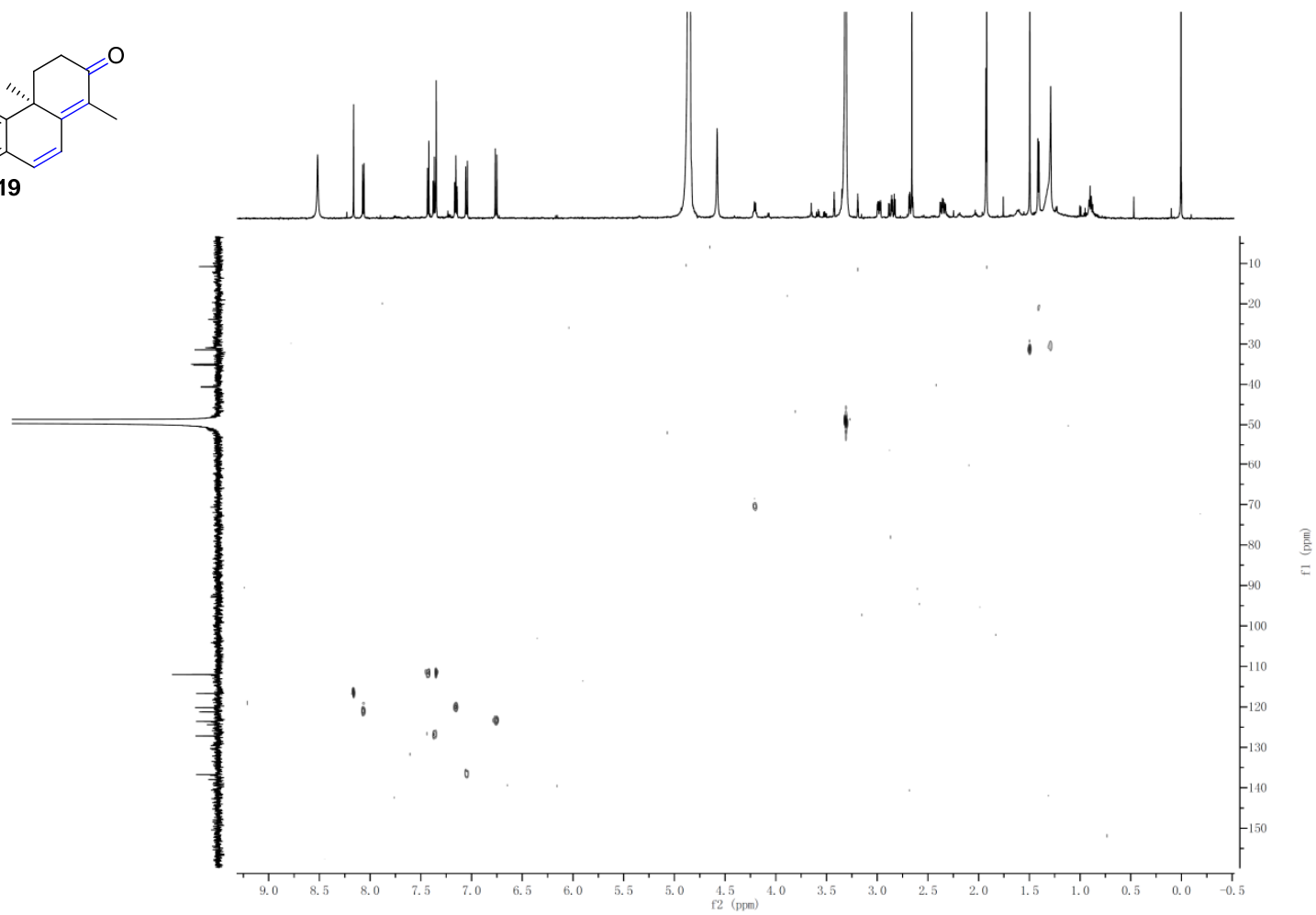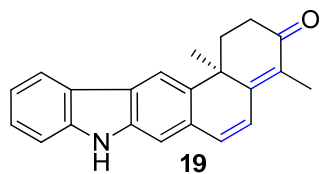(**E**) The HSQC spectrum of **19**

Fig. S16 The spectral data of **19** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
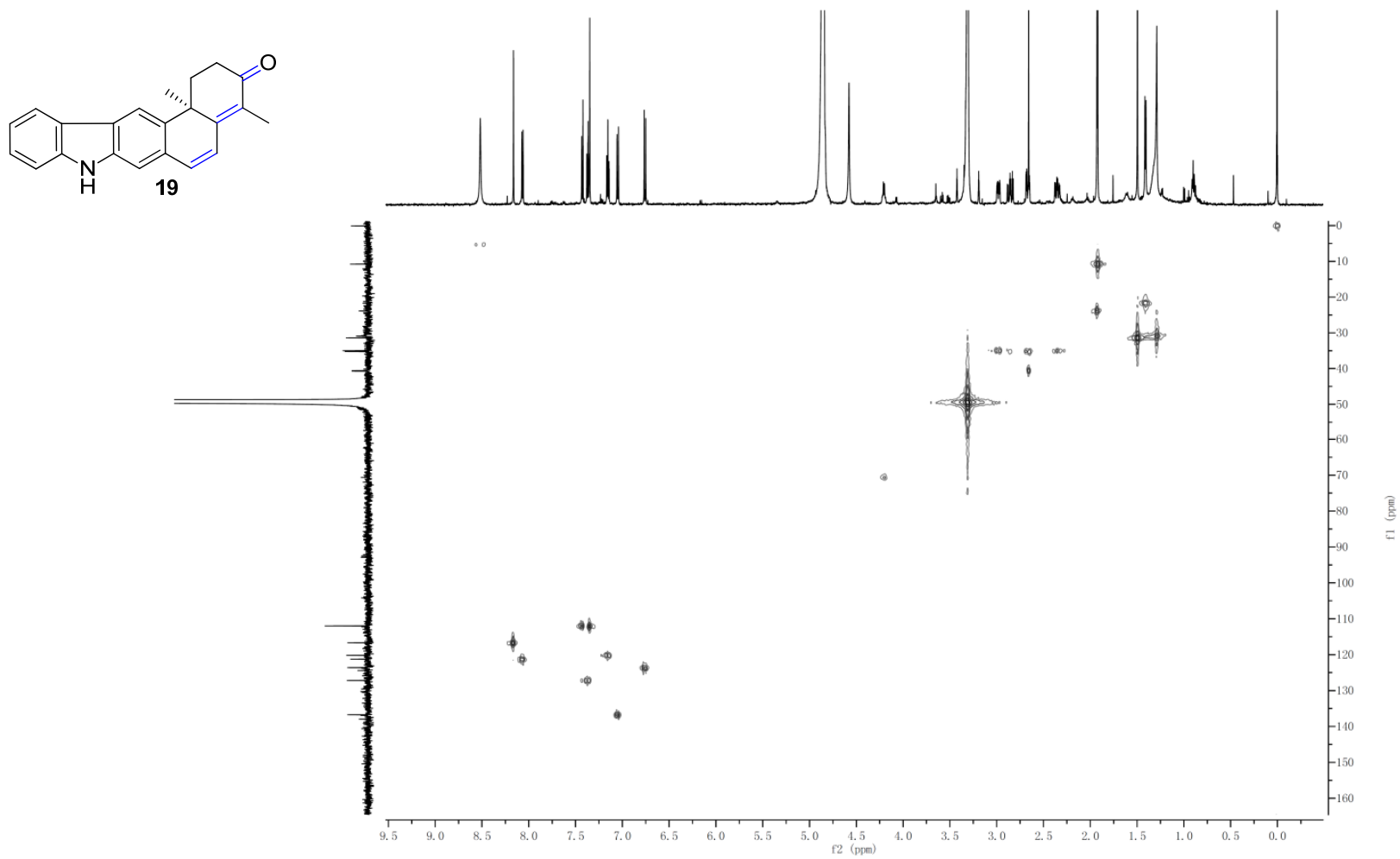
(**F**) The HMQC spectrum of **19**

Fig. S16 The spectral data of **19** (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, CD$_3$OD)
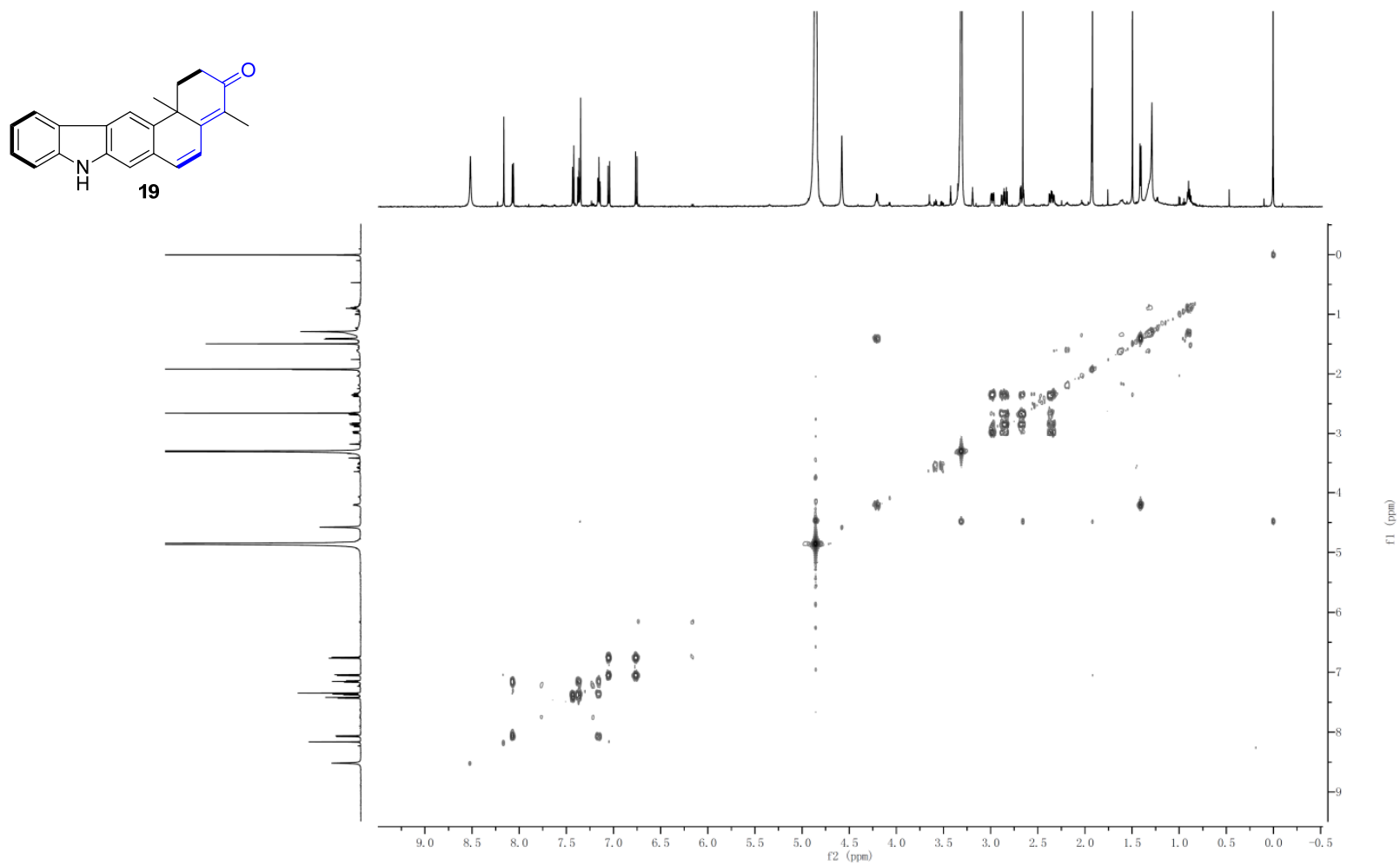(**G**) The ^{1}H-^{1}H COSY spectrum of **19**

Fig. S16 The spectral data of **19** (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR, CD₃OD)
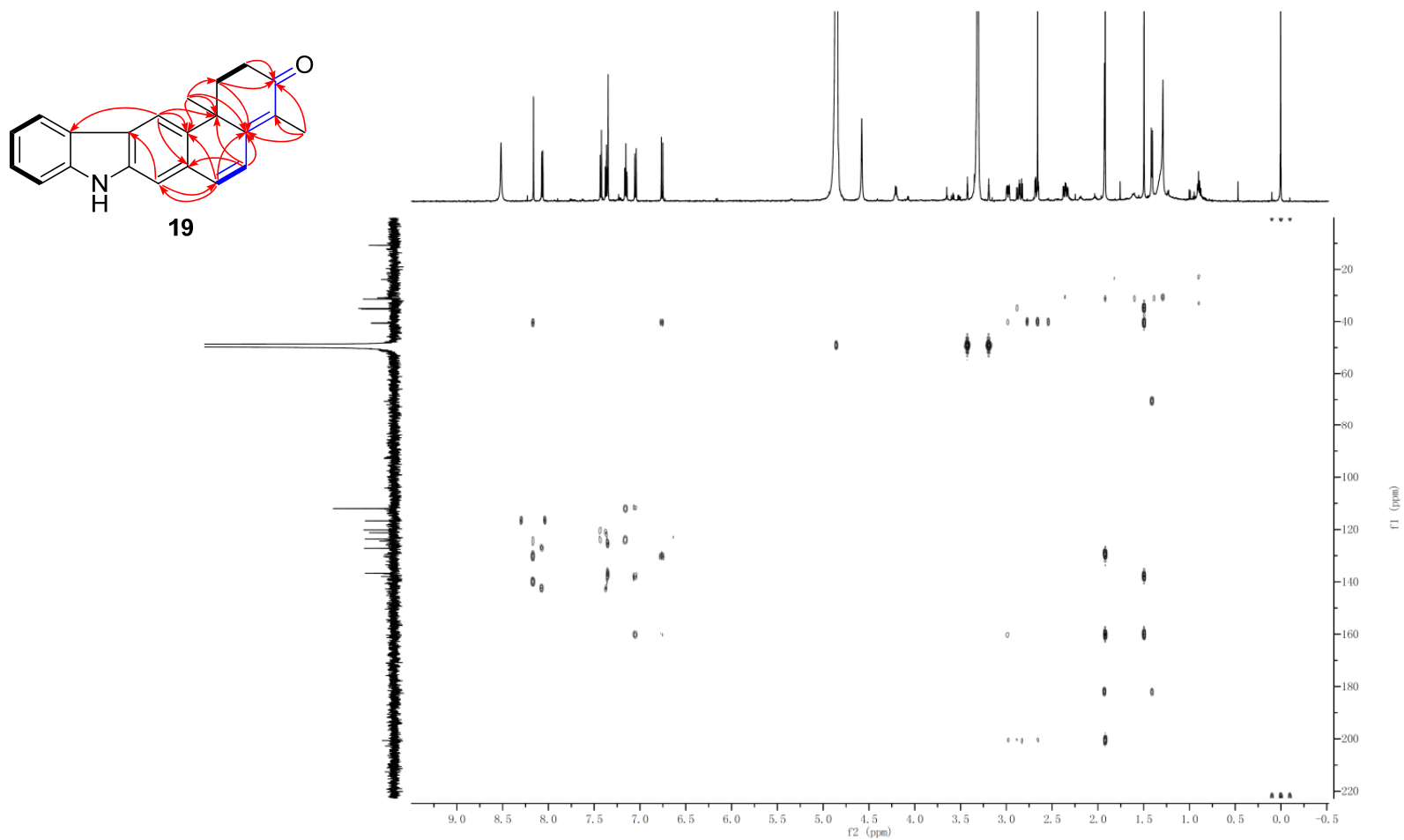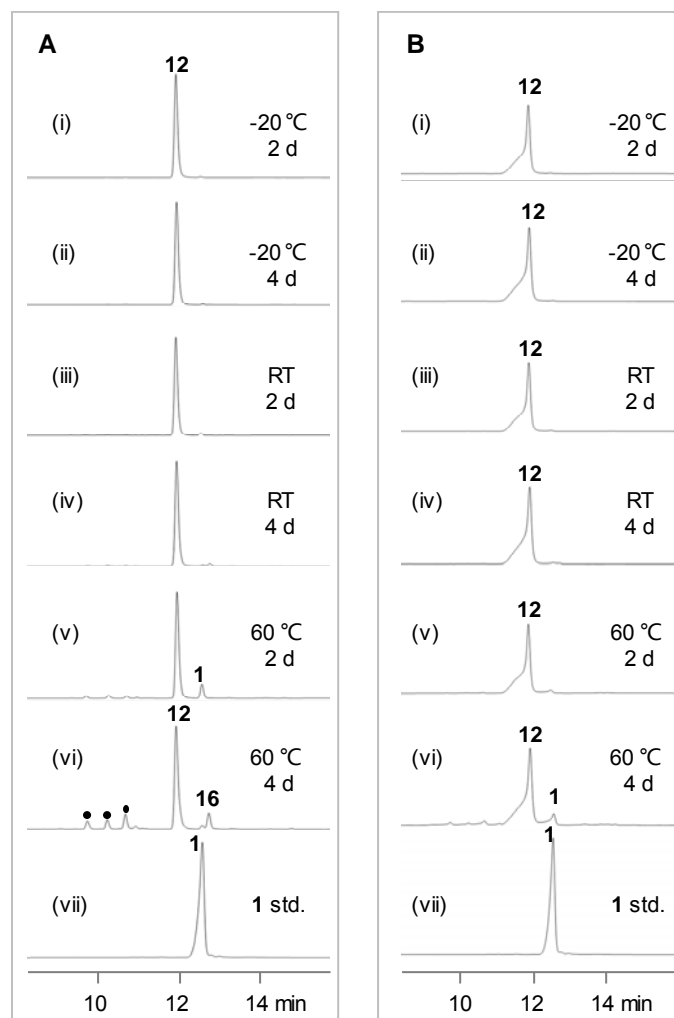(**H**) The HMBC spectrum of **19**

Fig. S17 Stability of compounds **12** stored in organic solvents.

(A): **NOXM** (**12**) dissolved in H$_2$O/AcN (1:1, v/v); (B): **12** dissolved in MeOH. (i) stored at -20 °C for 2 days; (ii) stored at -20°C for 4 days; (iii) stored at room temperature for 2 days; (iv) stored at room temperature for 4 days; (v) incubated at 60 °C for 2 days; (vi) incubated at 60 °C for 4 days; (vii) XMA (**1**) standard; the filled black circles (●) denote multiple XMA-related products which have not been isolated for structure elucidation.

Fig. S18 The hypothesized mechanism for the formation of DXMs A-C (**8**-**10**).
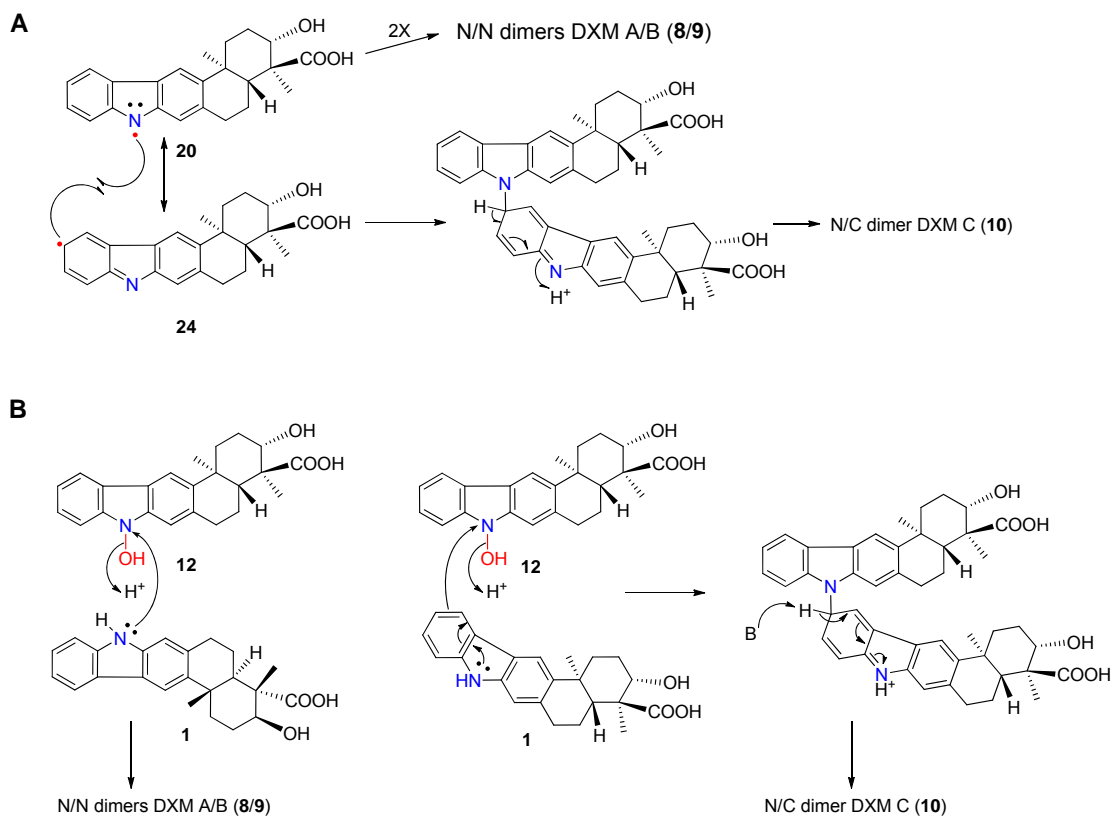
Supplementary Reference

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