Supporting Information

General.

Agents and chemicals were purchased from Sigma-Aldrich or TCI (shanghai). Dried dichloride methane (CH_2Cl_2) was obtained by distilling from calcium hydride and stored under argon atmosphere. Reagents were purchased commercially and used without further purification. All reactions were monitored by thin layer chromatography (TLC) with silica gel coated plates, UV light as visualizing agent or basic aqueous potassium permanganate as developing agent. NMR spectra were recorded on Agilent 500/54/ASP instrument and calibrated using residual protonated solvent (δ_H = 7.26 and δ_C = 77.16 for $CDCl_3$, δ_H = 3.31 and δ_C = 49.15 for CD_3OD, δ_H = 2.50 and δ_C = 39.51 for DMSO-d6) as an internal reference. Data for ^1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet or unresolved. Coupling constants (J) are reported in Hertz. Mass spectra were recorded by ESI-MS.

A TXN producer, *Streptomyces bottropensis* NRRL 12051 was purchased from American Agricultural Research Service (ARS). *Escherichia coli* DH5α competent cells were used for routine subcloning and plasmid preparations and it were grown in LB medium with appropriate antibiotics. pCC1FOS™ Vector (Epicentre) was used for fosmid library preparations. PCR amplification was carried out using either *Taq* DNA polymerase or PfuUltra^{TM} DNA polymerase with genomic DNA or fosmid pTG5001, pTG5002, or pTG5003 as a template, degenerate or specific primers were listed in Table S1/S2. Primer synthesis was performed at Invitrogen Shanghai Center. DNA sequencing was performed at the Shanghai Majorbio Pharm Technology Co., Ltd. The general genetic manipulations of *S. bottropensis*, production and analysis of TXN were performed as following described methods.

Sequence Analysis.

The open reading frames (ORFs) were deduced from the sequence by performing Frame Plot 4.0 beta program (http://nocardia.nih.go.jp/fp4) and BLAST methods. Amino acid sequence alignments were performed by the CLUSTALW method from BIOLOGYWORKBENCH 3.2 software (http://workbench.sdsc.edu).

Production and Analysis of Trioxacarcin (TXN).

S. bottropensis NRRL 12051 WT and recombinant strains were grown in a seed culture of TSB (tryptic soy broth) (3%) at 30 °C for 36 hr, then 5 mL of seeding culture suspension was transferred into a 500-mL flask containing 100 mL fermentation broth (soluble starch 6%，glucose 1%，yeast extract 1%, NaCl 0.3%, $MgSO_4.7H_2O$ 0.1%, KH_2PO_4 0.1%，$CuSO_4.5H_2O$ 7 mg, $FeSO_4.7H_2O$ 1 mg, $MnCl_2.4H_2O$ 0.8 mg, $ZnSO_4.7H_2O$ 0.2 mg, $CoCl_2.7H_2O$ 0.0006 mg, HP20, 5%) and incubated at 30 °C for 5d. The culture broth was centrifugalized (5000 rpm for 10 min), the culture filtrates were removed, and all the mycelia were collected including macro resin HP20 followed by acetone soaking and ultrasonic broking. The organic layer was evaporated under reduced pressure, and the water layer was extracted thrice with equal volume of ethyl acetate. Combined extract was finally concentrated to 2 mL. HPLC analysis was carried out on a Kromasil 100-C18 column (5μ, 4.6×250 mm). The column was equilibrated with 50% solvents A (H_2O and 0.1% HCOOH) and B (CH_3CN and 0.1% HCOOH) and developed with the following program: 0–5 min, 10% B; 5-25 min, a linear gradient from 10% B to 90% B; 25–27 min constant 90% B; 27-29 min with a linear gradient from 90% B to 10% B; 29-30min constant 10% B. This process was carried out at a flow rate of 1 mL/min and UV detection at 271 nm using an Agilent 1100 HPLC system. The identity of compound was confirmed by liquid chromatography/MS (LC-MS) analysis performed on a Thermo Scientific LCQ FLEET system under the same conditions. TXN-A showed $[M+Na]^+$ ion at m/z = 899.2, consistent with the molecular formula$C_{42}H_{52}O_{20}$, and HRMS (m/z): $[M+Na]^+$ calcd. 899.2944, found 899.2945.

Precursors Feeding and Isolation of TXN-A.

The fermentation was carried out as mentioned above, equal portions of ^{13}C-labeled precursor (a total of 0.7 g/L) was added to a fermentation culture by pulse feeding after 48, 56, 64, 72, 80, 88 hr of incubation. The crude extract was subjected to flash chromatography on silica gel with MeOH/CH_2Cl_2 gradient (0-10% MeOH in CH_2Cl_2). The eluent washed with MeOH/CH_2Cl_2 (2:100, and 3:100, v/v) was collected, and it was further analyzed by gel filtration chromatography (MeOH/$CHCl_3$, 1:1 v/v). The residues with green fluorescent fraction was collected and detected with TLC (MeOH/$CHCl_3$, 1:15, R_f = 0.6), collected and evaporated to give a yellow compound

TXN-A, this yellow compound in ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) was the same as that reported in ref.1. The cultures feeding with each isotope-labeled precursor were extracted and treated as described above, and the pure labeled TXN was isolated and spectral-analyzed by ^{13}C-NMR (125 MHz, $CDCl_3$) (Fig. S1, Table S3). $^{13}C_6$-L-Ile (a total of 0.35 g/L) was fed in to the fermentation cultures in the same way, and the labeled product was also analyzed by LC-MS and ^{13}C NMR (125 MHz, $CDCl_3$).

Construction of Gene Replacement and Complementation Mutants.

The gene replacement mutants of*orf-3, orf-1, orf+3, orf+11, txnA$_1$, txnA$_4$, txnRg$_1$, txnRg$_6$, txnC$_2$, txnC$_3$, txnC$_4$, txnO$_2$, txnO$_5$, txnO$_6$, txnO$_{12}$,* and *txnB$_4$,* were constructed using REDIRECT Technology similar to those previously published[2]. The internal region of each target gene was replaced by a apramycin resistance gene *aac(3)IV*, *oriT* (RK2) and was amplified with the following primers respectively: Orf-3-F/R, Orf-1-F/R, Orf+3-F/R, Orf+11-F/R,TxnA1-F/R, TxnA4-F/R, TxnRg$_1$-F/R, TxnRg$_6$-F/R, TxnC2-F/R, TxnC3-F/R, TxnC4-F/R, TxnO2-F/R, TxnO5-F/R, TxnO6-F/R, TxnO12-F/R, and TxnB4-F/R (Table S3), the PCR template were fosmids of pTG5001, pTG5002 and pTG5003. Mutant fosmids Δ*orf-3,* Δ*orf-1,* Δ*orf+3,* Δ*orf+11,* Δ*txnA$_1$,* Δ*txnA$_4$,* Δ*txnRg$_1$,* Δ*txnRg$_6$,* Δ*txnC$_2$,* Δ*txnC$_3$,* Δ*txnC$_4$,* Δ*txnO$_2$,* Δ*txnO$_5$,* Δ*txnO$_6$,* Δ*txnO$_{12}$,* and Δ*txnB$_4$* for gene replacement were constructed and introduced into *S.bottropensis* NRRL 12051 by conjugation from *E. coli* S17-1. The double crossover mutants (TG5002, TG5003, TG5006, TG5005, TG5001, TG5008, TG5004, TG5007, TG5009, TG5011, TG5010, TG5012, TG5013, TG5014, TG5015, and TG5016 (Table S2) were screened and selected with apramycin-resistant colonies whose genotype were tested by PCR (Yz--3-F/R, Yz--1-F/R, Yz-+3-F/R, Yz-+11-F/R, Yz-A1-F/R, Yz-A4-F/R, Yz-Rg$_1$-F/R, Yz-Rg$_6$-F/R, Yz-C2-F/R, Yz-C3-F/R, Yz-C4-F/R, Yz-O2-F/R, Yz-O5-F/R, Yz-O6-F/R, Yz-O12-F/R, and Yz-B4-F/R as primers, Table S3).

Anticancer Activity Assays of TXN-A and Analogues.

All cell-culture work was conducted in a class II biological safety cabinet. Jurkat cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). Jurkat cells

were grown to approximately 80% confluence, and then were collected, and pelleted by centrifugation (3 min at 1000 rpm). The supernatant was discarded and the cell pellet was resuspended in fresh medium, and the concentration of cells was determined using a hemacytometer. The cell suspension was diluted to a concentration of 10000 cells/50 μL. The wells of a pre-sterilized 96-well plate were charged with 50 μL per well of the diluted cellular suspension. Stock solutions of each compound in DMSO were diluted serially with RPMI-1640 medium (supplemented with FBS), and 50 μL resulting solutions were added to the wells containing cells to achieve final concentrations of 0.25 nM to 2 nM (TXN-A) or 0.25 nM to 2500 nM (all other compounds). After incubating at 37 ºC and 5% CO_2 for 48 h, 10 μL of CCK-8 (Vazyme^{TM}CCK-8 Cell Counting Kit) was added to each well. After incubating at 37 °C and 5% CO_2 for 4 h, The optical density was read out at λ = 450 nm with a microplate reader and the background was subtracted at λ = 630 nm. Cells incubated with 0.1% DMSO served as positive control. Three independent replicates were conducted.

1. R. P. Maskey, E. Helmke, O. Kayser, H. H. Fiebig, A. Maier, A. Busche and H. Laatsch, *J. Antibiot. (Tokyo)*. 2004, **57**, 771-779.

2. H. M. Ma, Q. Zhou, Y. M. Tang, Z. Zhang, Y. S. Chen, H. Y. He, H. X. Pan, M. C. Tang, J. F. Gao, S. Y. Zhao, Y. Igarashi and G.-L. Tang, *Chem. Biol*. 2013, **20**, 796-805.

Table S1. Summary of ^{13}C NMR data of TXN-A from incorporation of ^{13}C-labeled acetate

Carbon position	^{13}C-NMR	Enhanced signal [1-^{13}C]-acetate	Enhanced signal [2-^{13}C]-acetate	[1,2-$^{13}C_2$]-acetate feeding	
				*J*c-c/Hz	adjacent carbon
1	203.09	√		66.50	9a
2	67.95		√	46.50	3
3	36.68	√		46.50	2
4	67.55		√	58.63	4a
4a	126.66	√		58.63	4
5	116.98		√	76.00	6
6	142.89	√		76.00	5
7	114.90		√	85.63	8
8	151.77	√		86.00	7
8a	114.81		√	85.00	9
9	163.17	√		85.00	8a
9a	107.54		√	66.25	1
10	144.82		√	79.38	10a
10a	135.46	√		79.38	10
11	69.22	√		40.13	12
12	71.47		√	39.75	11
13	101.59		√	51.38	
14	68.34				
15	104.69			80.50	
16	99.76			79.88	
17	48.21			35.75	
18	20.39		√	53.63	
19	62.76			66.50	
16′	56.82				
16″	56.24				
1′	97.99				
2′	36.65				

3′	68.83					
4′	74.43					
5′	62.90					
6′	16.89					
7′	170.41	√			74.60	8′
8′	20.91		√		74.50	7′
9′	25.73					
1″	94.84					
2″	31.54					
3″	70.20					
4″	79.58					
5″	63.85					
6″	14.55					
7″	210.42		√			
8″	27.78		√			

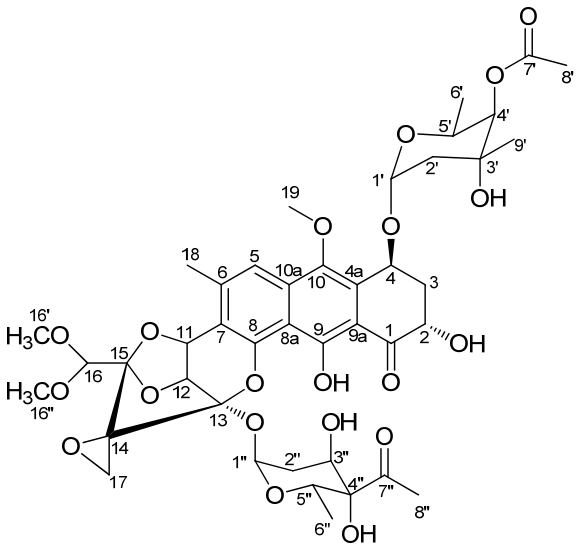


Table S2. Strains and plasmids used in this study

Strain/Plasmid	Characteristics	Reference
Strains		
E. coli DH5α	Host for general cloning	Invitrogen
E. coli S17-1	Donor strain for conjugation between *E. coli* and *S. bottropensis* NRRL *12051*	1
E. coli BW 25114	Host for PCR-targeting gene replacement on fosmids	2
S.bottropensis NRRL12051	Wild type strain, TXN producing	NRRL
*S.bottropensis*TG5001	Δ*txnA*$_1$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5002	Δ*orf-3* gene replacement mutant, TXN producing	This work
S.bottropensis TG5003	Δ*orf-1* gene replacement mutant, TXN producing	This work
S.bottropensis TG5004	Δ*txnRg*$_1$ gene replacement mutant, TXN low-producing	This work
S.bottropensis TG5005	Δ*orf+11* gene replacement mutant, TXN low-producing	This work
S.bottropensis TG5006	Δ*orf+3* gene replacement mutant, TXN producing	This work
S.bottropensis TG5007	Δ*txnRg*$_6$ gene replacement mutant, TXN low-producing	This work
S.bottropensis TG5008	Δ*txnA*$_4$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5009	Δ*txnC*$_2$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5010	Δ*txnC*$_4$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5011	Δ*txnO*$_2$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5012	Δ*txnO*$_5$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5013	Δ*txnC*$_3$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5014	Δ*txnO*$_6$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5015	Δ*txnO*$_{12}$ gene replacement mutant, TXN non-producing	This work
S.bottropensis TG5016	Δ*txnB*$_4$ gene replacement mutant, TXN non-producing	This work
Plasmids		
pSP72	*Ap*^{R}, *E. coli* subcloning vector	Promega
pCC1FOS-1™	fosmid vector for genomic library construction	Epicentre

pTG5001	pCC1FOS-1-based, *S.bottropensis* NRRL12051 fosmid containing TXN gene cluster (left part)	This work
pTG5002	pCC1FOS-1-based, *S.bottropensis* NRRL12051 fosmid containing TXN gene cluster (middle part)	This work
pTG5003	pCC1FOS-1-based, *S.bottropensis* NRRL12051 fosmid containing TXN gene cluster (right part)	This work
pTG5004	pTG5001 derivative for gene replacement of *orf-3*	This work
pTG5005	pTG5001 derivative for gene replacement of *orf-11*	This work
pTG5006	pTG5001 derivative for gene replacement of $txnA_1$	This work
pTG5007	pTG5001 derivative for gene replacement of $txnRg_1$	This work
pTG5008	pTG5001 derivative for gene replacement of $txnA_4$	This work
pTG5009	pTG5001 derivative for gene replacement of $txnB_4$	This work
pTG5010	pTG5002 derivative for gene replacement of $txnC_2$	This work
pTG5011	pTG5002 derivative for gene replacement of $txnC_4$	This work
pTG5012	pTG5002 derivative for gene replacement of $txnO_2$	This work
pTG5013	pTG5002 derivative for gene replacement of $txnO_5$	This work
pTG5014	pTG5002 derivative for gene replacement of $txnC_3$	This work
pTG5015	pTG5002 derivative for gene replacement of $txnO_6$	This work
pTG5016	pTG5003 derivative for gene replacement of $txnRg_6$	This work
pTG5017	pTG5003 derivative for gene replacement of $txnO_{12}$	This work
pTG5018	pTG5003 derivative for gene replacement of *orf+3*	This work
pTG5019	pTG5003 derivative for gene replacement of *orf+11*	This work

Ap^R, ampicillin resistance; Km^R, kanamycin resistance; Am^R, apramycin resistance.Tsr^R, thiostrepton resistance.

1 T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater and D. A. Hopwood, *Practical Streptomyces Genetics* 2000, (John Innes Foundation, Norwich, UK).

2 B. Gust, G. L. Challis, K. Fowler, T. Kieser and K. F. Chater, *Proc. Natl. Acad. Sci. USA* 2003, **100**, 1541–1546.

Table S3. Primers used in this study

Primers	Sequence (5'→ 3')	Restriction enzyme
KS-For	ATCACCGTGGCCTGYTTYGAYGCSATC	Degenerate
KS-Rev	CCGGTGTTGACSGSRTAGAACCANGC	Degenerate
4,6-DH-F	CSGGSGSSGCSGGSTTCATSGG	Degenerate
4,6-DH-R	GGGWRCTGGYRSGGSCCGTAGTTG	Degenerate
SK-PKS-II-F	GCGGTCTCGTACTCGGAG	Specific
SK-PKS-II-R	GCCGGCTCGGTATGTGATCG	Specific
SK-dUDP-F	GTAGTTGTTGGAGCAGCGG	Specific
SK-dUDP-R	CTCCACGTACGTCCGAGGC	Specific
Orf-3-F	GAACGAGCCGCGGCAATCGATTCCCAGGACCTGTTCCGCATTCCGGGGATCCGTCGACC	Specific
Orf-3-R	GCTCCGTGGCCTGGCGGCCTTGTACGTCGTCCTGTTCCATGTAGGCTGGAGCTGCTTC	Specific
Orf-1-F	GTCAGAGAGTCTTGAGGGACGAGGCGATGGCCTGGTCGGATTCCGGGGATCCGTCGACC	Specific
Orf-1-R	ATCCCGCGTTCGCCGCCATGGACTTCCACGCACCGTATCTGTAGGCTGGAGCTGCTTC	Specific
Orf+3-F	GGATGGTGGACACCTTCGTCGAAGAGGTCGGCAGGGTGGATTCCGGGGATCCGTCGACC	Specific
Orf+3-R	TGACGCCCAGCGCCCTGCGATACGGAGAGGCCATCAGGTTGTAGGCTGGAGCTGCTTC	Specific
Orf+11-F	CTTCGACGACGTCTGGAGGCTGCTGCTCGACGACCGGCTATTCCGGGGATCCGTCGACC	Specific
Orf+11-R	TCCAGCGCGCCGGTGACCAGTTGGGCGTAGATCGCGACCTGTAGGCTGGAGCTGCTTC	Specific
TxnA1-F	ACTTCTCACCGATGGCCGATCGGCCACACGCACCATCAGATTCCGGGGATCCGTCGACC	Specific
TxnA1-R	GTACTTCCCTCGCGGATCAGCTCCACCGCGTGTCCGATCTGTAGGCTGGAGCTGCTTC	Specific
TxnA4-F	GTCAGCGTCTGGCGTGGTCCGGCCTCGACCAGGAGCATGATTCCGGGGATCCGTCGACC	Specific
TxnA4-R	GGTCATCGGCATCGACGACGTCCGGCGTGCCCAGCCTCTTGTAGGCTGGAGCTGCTTC	Specific
TxnRg1-F	GAAGCGGAACTGCGGGTCATTGGCAGGCTCCTGGCAGCCATTCCGGGGATCCGTCGACC	Specific
TxnRg1-R	CCGGGTGCCGACCTTAGACGGTGCAGGCCATGGAACGGCTGTAGGCTGGAGCTGCTTC	Specific
TxnRg6-F	CCTCGACCCAACGCCCTTGGGCCGTCTTCGACATCGACGTGTAGGCTGGAGCTGCTTC	Specific
TxnRg6-R	GGATGGTGGACACCTTCGTCGAAGAGGTCGGCAGGGTGGATTCCGGGGATCCGTCGACC	Specific
TxnC2-F	CATGGCGGTCTTCGGGTGTGCTCGTGGCGCCGAGAACGTATTCCGGGGATCCGTCGACC	Specific
TxnC2-R	CAGACGGCGTTCACGGTGACGCCGGTCCGCGCCAGTTCCTGTAGGCTGGAGCTGCTTC	Specific
TxnC3-F	GGATGCCTCCGTACTGGTGGACACCGCCGCCACCGAAGCATTCCGGGGATCCGTCGACC	Specific
TxnC3-R	CACCACCGCAGGTAGTTCTCGGTCCGGTCCAGCAGTTCCTGTAGGCTGGAGCTGCTTC	Specific

TxnC4-F	GTTACGAAGCAGCCGGTGGGACCGTCCGCGTCGAGCGTGATTCCGGGGATCCGTCGACC	Specific
TxnC4-R	CCGCATCGACGAGCGGCACGGCCGGCTGGACATCCTCATTGTAGGCTGGAGCTGCTTC	Specific
TxnO2-F	AGATCCGGACACCGCTCCAGCAGCACGCGCAGGGCGATGATTCCGGGGATCCGTCGACC	Specific
TxnO2-R	GGACCGCGCCGACTTCCACCTGTGGTCGAGCGATCTGTCTGTAGGCTGGAGCTGCTTC	Specific
TxnO5-F	GGAAGAGCTTGCTCCACACGATCTCCAGCTCCAGGCGTGATTCCGGGGATCCGTCGACC	Specific
TxnO5-R	TCAACGCCTACATCAGCGACCTGATCCAGACCAAGCGCCTGTAGGCTGGAGCTGCTTC	Specific
TxnO6-F	GCGAACAGGTTGACATGACGCAGGAGTTCGTCCACCGCGATTCCGGGGATCCGTCGACC	Specific
TxnO6-R	GCAGTCGGTCTTCGACGAACTGCTCGACGACATCGAGGCTGTAGGCTGGAGCTGCTTC	Specific
TxnO12-F	GGAACGGACGGCGGTCTTCGCTTCCAGCCTGGCCAGCGGATTCCGGGGATCCGTCGACC	Specific
TxnO12-R	GGAGTTCCGTTCGGCGACCGTGACAAGTTCCACCGCTGGTGTAGGCTGGAGCTGCTTC	Specific
TxnB4-F	GAGGACCAGTACACGTGCGCACGCTCACCTATTGGCAGGATTCCGGGGATCCGTCGACC	Specific
TxnB4-R	CATGCACCATGAGCGAGGTCGCGACCACCGTGACGTCGTTGTAGGCTGGAGCTGCTTC	Specific
Yz--3-F	AGTGGTACGCCGTCACTC	Specific
Yz--3-R	CAGTGCTCTCGACCTGGC	Specific
Yz--1-F	AGCGACGCGTTCTCGGCTG	Specific
Yz--1-R	ACGCGCGGCGATGTGGACTG	Specific
Yz-+3-F	CGCATGCGTGACATCCTC	Specific
Yz-+3-R	CGATGTCCACCAGGTCGC	Specific
Yz-+11-F	CGCCTCGTGGTACATCACC	Specific
Yz-+11-R	AGGCTGCCTCCAGTGCCAC	Specific
Yz-A1-F	TGCGGAGAGTCGCCATCAC	Specific
Yz-A1-R	CGTGGATGGCGTCGAAGC	Specific
Yz-A4-F	GCGCGAACGATGTCAGCG	Specific
Yz-A4-R	GGACCTCATGGACGACGAG	Specific
Yz-Rg1-F	AGTCCACATCGCCGCGCGTG	Specific
Yz-Rg1-R	ACTGCGCGATCGGAGAGTCG	Specific
Yz-Rg6-F	GCACTCGGAACGTAGACG	Specific
Yz-Rg6-R	TTCGACACCCTCTCGGAGG	Specific
Yz-C2-F	CCATCGTTACCGGCGCGAC	Specific
Yz-C2-R	GCCGTAGTACTCGGCATGG	Specific
Yz-C3-F	CTGAACAACGCCGGGCTGG	Specific
Yz-C3-R	GCCAGCAGGCCTTCCTGTG	Specific
Yz-C4-F	GCCGTCCGACGGTCACCAG	Specific

Yz-C4-R	CGCGACAAGGCTCGCGGT	Specific
Yz-O2-F	TCTCGTACGGCACCGCCAG	Specific
Yz-O2-R	TCCGCTGTCGGTCATCTGC	Specific
Yz-O5-F	AAGGCCTGGCGAGCCTCAG	Specific
Yz-O5-R	CAGGACCGGCACGACTTCT	Specific
Yz-O6-F	AAGCCGGAGGTGTCGGTCG	Specific
Yz-O6-R	CATGGACTCGCCCGAGCAC	Specific
Yz-O12-F	CGTCGAGGGCCAGGTCCGG	Specific
Yz-O12-R	GTCCAGGACTTCGCGTTC	Specific
Yz-B4-F	GCTGACACCGGTGCACTCG	Specific
Yz-B4-R	GCACCATGAGCGAGGTCGCG	Specific

Legend: R, A or G; Y, C or T; M, A or C; K, G or T; S, C or G; W, A or T; H, A, C, or T; B, C, G, or T; V, A, C, or G; D, A, G, or T; N, any nucleotide.

Table S4. ^{1}H- and ^{13}C-NMR data of **4a** and **4b**

	4a		**4b**	
No.	δ_C	δ_H	δ_C	δ_H
1	174.41		174.93	
2	90.43		90.02	
3	168.74		168.88	
4	103.62	5.57（s,1H)	104.09	5.57 (s, 1H)
5	166.55		166.39	
6	119.85		119.28	
7	139.8		140.2	
8	103.8	6.30(d,2.4 Hz, 1H)	103.64	6.31 (d, 1.1 Hz, 1H)
9	164.2		164.41	
10	112.75	6.22(d,2.4Hz,1H)	113.02	6.23 （d， 1.3 Hz， 1H)
11	106.48		106.48	
12	38.88	3.57 (s, 2H)	38.82	3.60 (s, 2H)
13	203.12		203.24	
14	121.89		121.64	
15	159.18		158.59	
16	101.9	6.14(d, 2.2Hz, 1H)	101.87	6.14 (s, 1H)
17	162.56		162.35	
18	106.48	6.25(d,2.2 Hz, 1H)	106.08	6.31 (d, 1.1 Hz, 1H)
19	150.67		165.66	
20	39.02	2.45（Sept，6.8 Hz, 1H)	32.01	2.74 (sept, 6.2 Hz,1H)
21	32.43	1.37 (m, 1H), 1.46 (m, 1H)	22.18	1.06（d, 6.2 Hz,3H)
22	12.92	0.69 (t, 7.4 Hz, 3H)	22.18	1.06（d, 6.2 Hz,3H)
23	22.18	1.01 (d, 4.9 Hz, 3H)		

Table S5. ^{1}H- and ^{13}C-NMR data of **6a**

No.	δ_C	δ_H
1	165.26	
2	89.7	5.34 (s, 1H)
3	172.19	
4	101.61	5.79 (s, 1H)
5	167.7	
6	38.1	4.18 (q, 16.0 Hz, 2H)
7	136.23	
8	125.6	6.81 (d, 7.4 Hz, 1H)
9	135.4	7.32 (t, 7.9 Hz, 1H)
10	118.51	6.84 (d, 8.9 Hz, 1H)
11	159.47	
12	118.66	
13	192.44	
14	48.54	2.81(m, 1H),2.94(m, 1H)
15	100.82	
16	47.34	2.81(m, 1H),2.91(m, 1H)
17	194.14	
18	100.42	5.59 (s, 1H)
19	195.64	
20	42.95	2.23(m,1H)
21	27.18	1.44(m, 1H), 1.64(m, 1H)
22	11.71	0.88 (dd, 13.3, 7.2 Hz, 3H)
23	17.24	1.11 (dt, 8.0, 4.0 Hz, 3H)

Table S6. ^{1}H- and ^{13}C-NMR data of **9a**

No.	δ_C	δ_H
1	205.82	
2	34.13	
3	30.39	
4	63.18	5.34(4, 4.2 Hz, 1H)
4a	110.93	
5	120.69	7.85（s, 1H)
6	139.84	
6-Me	24.03	2.86 (s, 3H)
7	119.76	
8	151.59	
8a	117.8	
9	157.16	
9a	112.95	
10	157.99	
10a	133.33	
11	178.96	
12	109.8	6.46(s, 1H)
13	172.74	
14	73.12	
15	33.12	2.16 (m, 2H)
16	8.35	0.79 (t, 6.0 Hz, 3H)
17	26.94	1.55 (s, 3H)

Table S7. ^{1}H- and ^{13}C-NMR data of **12a** and **12b**

	12a		**12b**	
No.	δ_C	δ_H	δ_C	δ_H
1	203.11		202.9	
2	67.83	4.77 (dd, 12.2, 5.3 Hz, 1H)	67.94	4.78 (dd, 11.7, 4.1 Hz, 1H)
3	36.76	2.23 (m, 1H), 2.78 (m, 1H)	36.62	2.20 (m, 1H), 2.81 (m, 1H)
4	68.07	5.38 (d, 3.1 Hz, 1H)	67.46	5.39 (d, 2.8 Hz, 1H)
4a	126.58		126.48	
5	116.91	7.47 (s, 1H)	116.87	7.49 (s, 1H)
6	143.17		143.07	
6-Me	20.65	2.59 (s, 3H)	20.45	2.61 (s, 3H)
7	114.93		115	
8	151.78		151.83	
8a	115.5		114.78	
9	163.18		163.25	
9a	107.52		107.39	
10	144.99		144.83	
10-Me	62.84	3.83 (s, 3H)	62.75	3.84 (s, 3H)
10a	135.56		135.42	
11	69.32	4.86 (d, 3.7 Hz, 1H)	69.1	5.22(d, 4.1 Hz, 1H)
12	73.46	5.25(d, 3.7 Hz, 1H)	71.47	5.35(d, 4.1 Hz, 1H)
13	100.26		101.57	
14	68.98		68.31	
15	104.12		104.61	
16	99.02	4.75 (s, 1H)	99.6	4.75 (s, 1H)
16-OMe	56.82	3.47 (s, 3H)	56.81	3.45 (s, 3H)
16-OMe	57.27	3.61 (s, 3H)	56.84	3.61 (s, 3H)
17	50.9	3.02 (d, 5.3 Hz, 1H), 3.12 (d, 5.3 Hz, 1H)	47.97	2.86 (d, 5.7 Hz, 1H), 2.93 (d, 5.7 Hz, 1H)
1'	98.22	5.38 (d, 3.1 Hz, 1H)	97.96	5.37（t, 4.0 Hz, 1H)
2'	36.7	1.62 (d, 14.6 Hz, 1H), 1.96 (dd, 14.6, 4.0 Hz, 1H)	35.57	1.62 (d, 14.5 Hz, 1H), 1.96 (m, 1H)
3'	69.55		68.83	
3'- Me	25.83	1.07 (s, 3H)	25.71	1.07 (s, 3H)
4'	74.53	4.71 (s ,1H)	74.4	4.73 (s ,1H)
5'	63.03	4.54 q, 6.4 Hz, 1H)	62.91	4.54 (q, 6.3 Hz, 1H)
6'	17	1.24 (d, 6.4 Hz, 1H)	16.89	1.29 (d, 6.3 Hz, 1H)
4'-CO$\underline{C}$H$_3$	21.05	2.14 (s, 3H)	20.93	2.14 (s, 3H)
4'-$\underline{C}$OCH$_3$	170.57		170.41	
1"			94.83	5.79 （d, 3.0 Hz, 1H)
2"			29.71	2.07 (m, 1H), 2.20 (m, 1H)
3"			69.97	3.61 (m, 1H)
4"			74.57	
5"			64.37	4.76 (m, 1H)

6"		16.77	1.24 (overlap, 3H)
4"-Me		26.28	1.34 (s, 3H)

Table S8. ^{1}H- and ^{13}C-NMR data of **13a** and **13b**

13a			**13b**
No.	δ_C	δ_H	δ_H
1	202.37		
2	67.93	4.70 (m, 1H)	4.71 (m, 1H)
3	48.04	3.13 (m,2H)	3.13(m, 2H)
4	192.86		
4a	121.24		
5	116.41	7.75 (s, 1H)	7.78(s,1H)
6	143.25		
6-Me	18.68	2.61（s, 3H)	2.61(s, 3H）
7	117.84		
8	150.72		
8a	117.84		
9	161.58		
9a			
10	146.62		
10a	136.19		
11	69.64	5.29 (d, 3.2 Hz, 1H)	5.29 (d, 4.1 Hz, 1H)
12	71.51	5.49 (d, 3.2 Hz, 1H)	5.49 (d, 4.1 Hz, 1H)
13	101.86		
14	68.97		
15	104.5		
16	100.06	4.73 (d, 1.0 Hz, 1H)	4.69(s, 1H)
16-OMe	55.45	3.45 (d, 1.0 Hz, 3H)	3.42 （s, 3H)
16-OMe	55.52	3.60 (d, 1.0 Hz, 3H)	3.57 （s, 3H)
17	47.86	2.71 (d, 5.6 Hz, 1H) 2.86 (d, 5.6 Hz, 1H)	2.73 (d, 5.7 Hz, 1H) 2.87 (d, J5.7 Hz, 1H)
1"	94.23	5.84 (s, 1H)	5.80 (d, 3.2 Hz, 1H)
2"	31.1	2.38 (m, 1H), 2.05 (m, 1H)	
3"	70.12	3.66 (m, 1H)	
4"	79.1		
5"	64.38	4.94 (q, 6.3 Hz, 1H)	4.95 (q,6.3 Hz, 1H)
6"	13.61	1.14 (d, 6.3 Hz, 3H)	1.30 (d, 6.3 Hz, 3H)
4"-$\underline{C}OCH_3$	210.4		3.85 (q, 6.5Hz,1H)
4"-CO$\underline{C}H_3$	24.83	2.30 (s, 3H)	1.29 (d, 6.6 Hz, 3H)

Table S9. ^{1}H- and ^{13}C-NMR data of **14a**

No.	δ_C	δ_H
1	203.13	
2	68.07	4.76 (dd, 12.7, 5.3 Hz, 1H)
3	36.79	2.19(td, 13.3, 2.6 Hz,1H),2.78(m, 1H)
4	67.66	5.38（m, 1H)
4a	126.77	
5	117.08	7.50 (s, 1H)
6	142.93	
6-Me	20.44	2.61(s,3H)
7	115.24	
8	151.87	
8a	114.96	
9	163.3	
9a	107.62	
10	144.96	
10-Me	62.87	3.83 (s, 3H)
10a	135.57	
11	69.52	5.22 (d, 4.2 Hz, 1H)
12	71.85	5.35 (d, 4.2 Hz, 1H)
13	101.74	
14	68.55	
15	105.16	
16	67.66	3.72(d, 11.25 Hz, 1H), 3.79(d, 11.25 Hz, 1H)
16-OMe		
16-OMe	60.41	3.47 (s, 3H)
17	48.29	2.93 (d, 5.2 Hz, 1H), 2.87 (d, 5.2 Hz, 1H)
1'	98.14	5.37(m,1H)
2'	36.79	1.60 (d, 14.6 Hz, 1H), 1.94 (m, 1H)
3'	68.94	
3'- Me	25.83	1.05 (s, 3H)
4'	74.55	4.72(m,1H)
5'	63.03	4.51（m, 1H)
6'	16.99	1.22 (d, J = 6.8, 3H)
4'-CO$\underline{C}$H$_3$	21	2.12(s, 3H)
4'-$\underline{C}$OCH$_3$	170.47	
1"	94.95	5.83 (d, J = 2.3 Hz, 1H)
2"	31.67	2.11(m,1H),2.42(m, 1H)
3"	70.33	3.68(m, 1H)
4"	79.7	
5"	63.94	4.97（m, 1H)
6"	14.66	1.07(d, 6.4 Hz, 3H)
4"-CO$\underline{C}$H$_3$	27.95	2.47 (s, 3H)

4"-$\underline{C}OCH_3$	210.53

Table S10. ^{1}H- and ^{13}C-NMR data of **15a** and **15b**

	15a		**15b**	
No.	δ_C	δ_H	δ_C	δ_H
1	203.47		203.81	
2	32.6	2.67 (m, 1H), 3.01(m, 1H）	32.91	2.65 (m, 1H) 3.14 (m, 1H)
3	28.55	2.21(m,1H), 2.41(m, 1H)	27.74	2.26(m,1H), 2.43(m, 1H)
4	66.42	5.36 (m, 1H)	62.5	5.41 (m, 1H)
4a	126.98		130.41	
5	117.04	7.50 (d, 0.6 Hz, 1H)	116.18	7.49 (d, 0.6 Hz, 1H)
6	142.37		141.84	
6-Me	20.47	2.59 (s, 3H)	20.52	2.62(s, 3H)
7	114.97		114.38	
8	151.89		152.09	
8a	114.65		114.33	
9	163.43		162.97	
9a	109.44		109.31	
10	144.29		143.16	
10-Me	62.99	3.83 (s, 3H)	62.97	3.93(s,3H)
10a	135.26		135.5	
11	69.42	5.22 (d, 4.1 Hz, 1H)	70.42	5.25 (d, 4.1 Hz, 1H)
12	71.61	5.33 (d, 4.1 Hz, 1H)	71.45	5.36 (d, 4.1 Hz, 1H)
13	101.64		101.51	
14	69		69.5	
15	104.8		104.04	
16	99.84	4.75 (s, 1H)	99.89	4.78(s,1H)
16-OMe	56.93	3.47 (s, 3H)	56.92	3.50 (s, 3H)
16-OMe	56.33	3.61 (s, 3H)	56.15	3.64 (s, 3H)
17	48.37	2.97 (d, 5.7 Hz, 1H) 2.92 (d, 5.7 Hz, 1H)	48.33	2.98 (d, 5.7 Hz, 1H) 2.91 (d, 5.7 Hz, 1H)
1'	97.13	5.25 (d, 3.6 Hz, 1H)		
2'	36.65	1.92 (dd, 14.5, 4.0 Hz, 1H) 1.59 (d, 14.6 Hz, 1H)		
3'	68.5			
3'- Me	25.8	1.05 (s, 3H)		
4'	74.62	4.72 (s, 1H)		
5'	62.89	4.48 (d, 7.2 Hz, 1H)		
6'	17.04	1.21 (d, 6.8, 3H)		
4'-$CO\underline{C}H_3$	21.04	2.11 (s, 3H)		

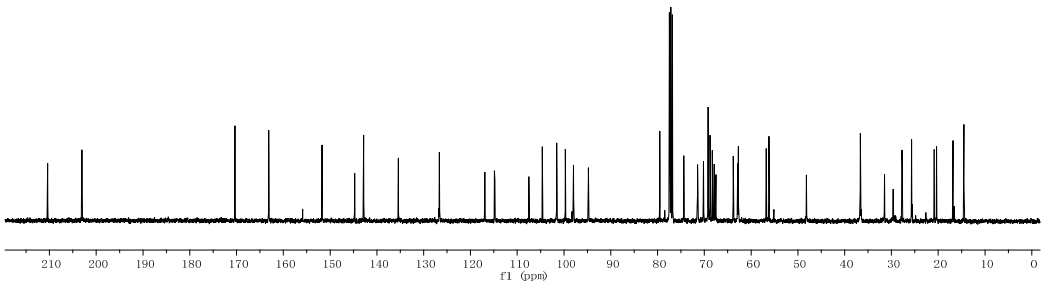
4'-$\underline{C}OCH_3$	170.54			
1"	94.93	5.83 (d, 2.6 Hz, 1H)	94.99	5.86 (d, 2.7 Hz, 1H)
2"	31.68	2.41(m,1H), 2.11(m, 1H)	31.77	2.45(m,1H), 2.13(m,1H)
3"	70.36	3.70(m,1H)	70.42	3.70(m,1H)
4"	79.74		79.79	
5"	63.93	5.00 (q, 6.4 Hz, 1H)	63.97	5.03 (q, 6.4 Hz, 1H)
6"	14.68	1.07(d, 6.4 Hz, 3H)	14.47	1.10(d,6.4 Hz, 3H）
4"-CO$\underline{C}H_3$	27.95	2.47 (s, 3H)	27.74	2.50 (s, 3H)
4"-$\underline{C}OCH_3$	210.58		211.4	

Table S11. HRESIMS data of compounds **4a~15b**

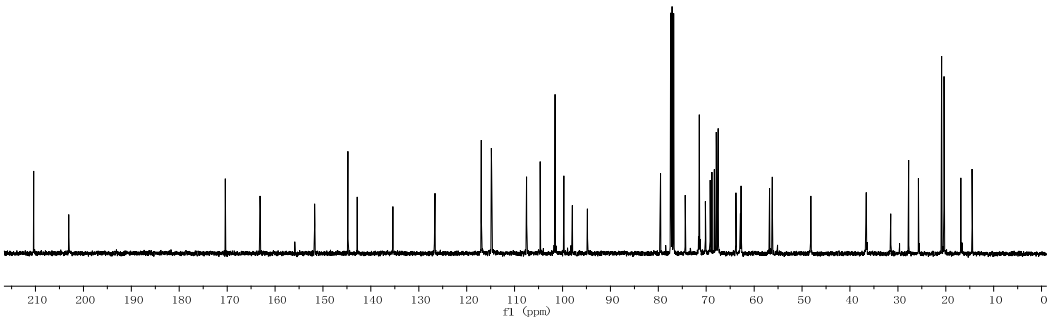
Comp. Name	Mass	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
4a	426.1309	$C_{23}H_{22}O_8$	426.1315	-1.37	$C_{23}H_{22}O_8$	$C_{23}H_{22}O_8$
4b	412.114	$C_{22}H_{20}O_8$	412.1158	-5.74	$C_{22}H_{20}O_8$	$C_{22}H_{20}O_8$
6a	428.149	$C_{23}H_{24}O_8$	428.1471	4.72	$C_{23}H_{24}O_8$	$C_{23}H_{24}O_8$
9a	398.135	$C_{22}H_{22}O_7$	398.1366	-3.41	$C_{22}H_{22}O_7$	$C_{22}H_{22}O_7$
12a	704.226	$C_{34}H_{40}O_{16}$	704.2316	-7.68	$C_{34}H_{40}O_{16}$	$C_{34}H_{40}O_{16}$
12b	848.304	$C_{41}H_{52}O_{19}$	848.3103	-7.39	$C_{41}H_{52}O_{19}$	$C_{41}H_{52}O_{19}$
13a	688.203	$C_{33}H_{36}O_{16}$	688.2003	3.7	$C_{33}H_{36}O_{16}$	$C_{33}H_{36}O_{16}$
13b	690.217	$C_{33}H_{38}O_{16}$	690.2160	1.14	$C_{33}H_{38}O_{16}$	$C_{33}H_{38}O_{16}$
14a	846.297	$C_{41}H_{50}O_{19}$	846.2946	2.35	$C_{41}H_{50}O_{19}$	$C_{41}H_{50}O_{19}$
15a	860.309	$C_{42}H_{52}O_{19}$	860.3103	-1.18	$C_{42}H_{52}O_{19}$	$C_{42}H_{52}O_{19}$
15b	674.22	$C_{33}H_{38}O_{15}$	674.2211	-2.14	$C_{33}H_{38}O_{15}$	$C_{33}H_{38}O_{15}$

Fig. S1 ^{13}C NMR data of TXN-A from the incorporation of ^{13}C-labeled acetate.

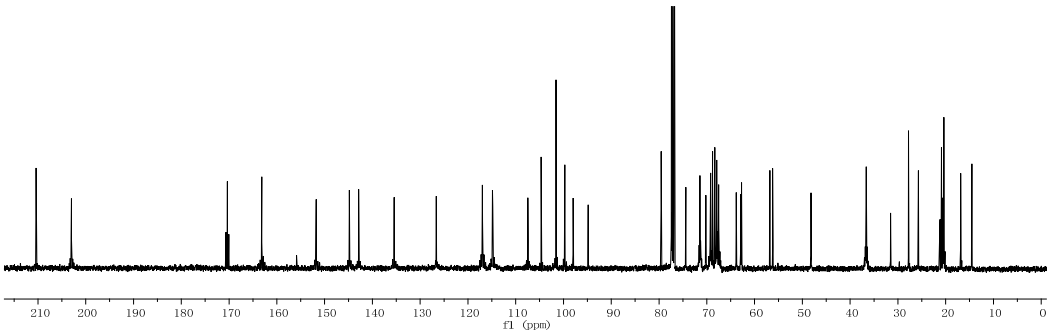
A



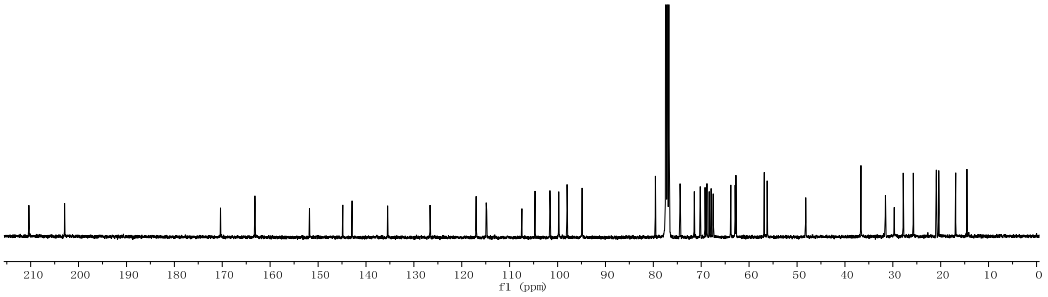
B



C

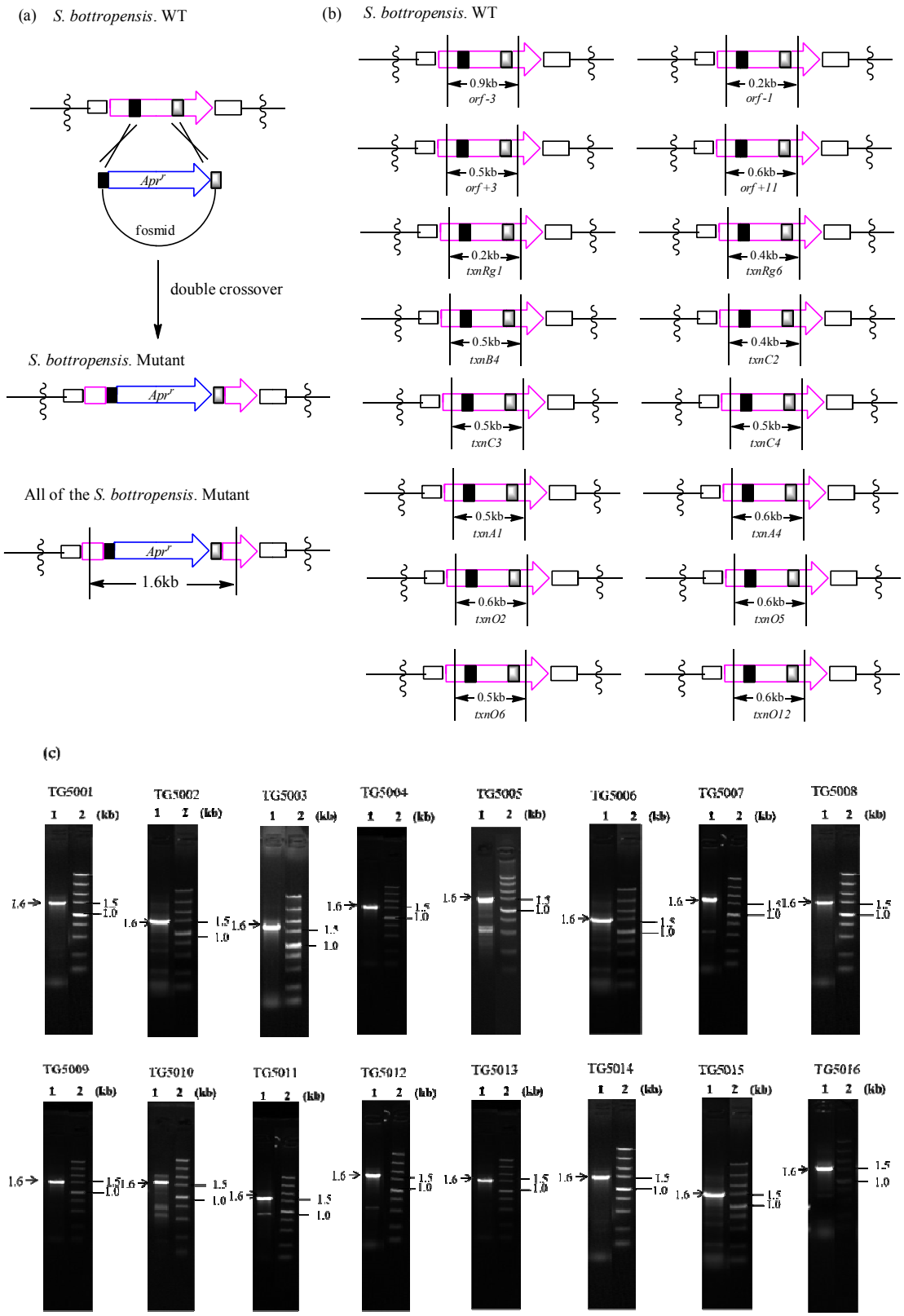


D



(A) [1-^{13}C]-acetate; (B) [2-^{13}C]-acetate; (C) [1,2-$^{13}C_2$]-acetate; (D) No feeding as control.

Fig. S2 Identification of the genotype of mutants via homologous recombination.



Genomic DNA from both the *S. bottropensis* wild type and mutant strains *S.* sp. TG5001 (Δ*txnA1*),

S. sp. TG5002 (Δ*orf-3*), *S*. sp. TG5003 (Δ*orf-1*), *S*. sp. TG5004 (Δ*txnRg1*), *S*. sp. TG5005 (Δ*orf+11*), *S*. sp. TG5006 (Δ*orf+3*), *S*. sp. TG5007 (Δ*txnRg6*), *S*. sp. TG5008 (Δ*txnA4*), *S*. sp. TG5009 (Δ*txnC2*), *S*. sp. TG5010 (Δ*txnC4*), *S*. sp. TG5011 (Δ*txnC3*), *S*. sp. TG5012 (Δ*txnO2*), *S*. sp. TG5013 (Δ*txnO5*), *S*. sp. TG5014 (Δ*txnO6*), *S*. sp. TG5015 (Δ*txnO12*), and *S*. sp. TG5016 (Δ*txnB4*) were extracted and tested by PCR analysis, respectively. A 1.6-kb (spectinomycin resistance gene fragment) band can be amplified from all of the mutants with specific primers. Wild type for *orf-3, orf-1, orf+3, orf+11, txnRg1, txnRg6, txnB4, txnC2, txnC3, txnC4, txnA1*, *txnA4, txnO2*, *txnO5*, *txnO6, txnO12* gene the corresponding PCR products is 0.9-kb, 0.2-kb, 0.5-kb, 0.6-kb, 0.2-kb, 0.4-kb, 0.5-kb, 0.4-kb, 0.5-kb, 0.5-kb, 0.5-kb, 0.6-kb, 0.6-kb, 0.6-kb, 0.5-kb and 0.6-kb, respectively). Lane 1, *Streptomyces* mutant strain; Lane 2 DNA marker.

Fig. S3 ^{1}H-NMR spectrum of compound **4a** in CD_3OD

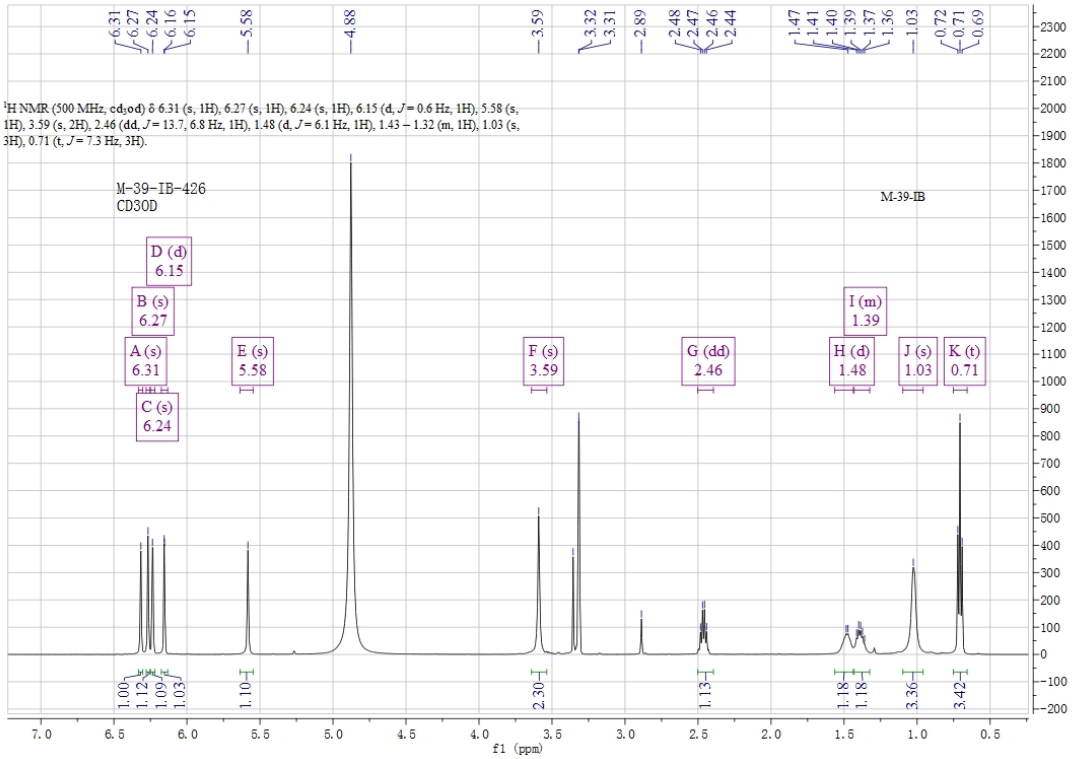


Fig. S4 ^{13}C-NMR spectrum of compound **4a** in CD_3OD

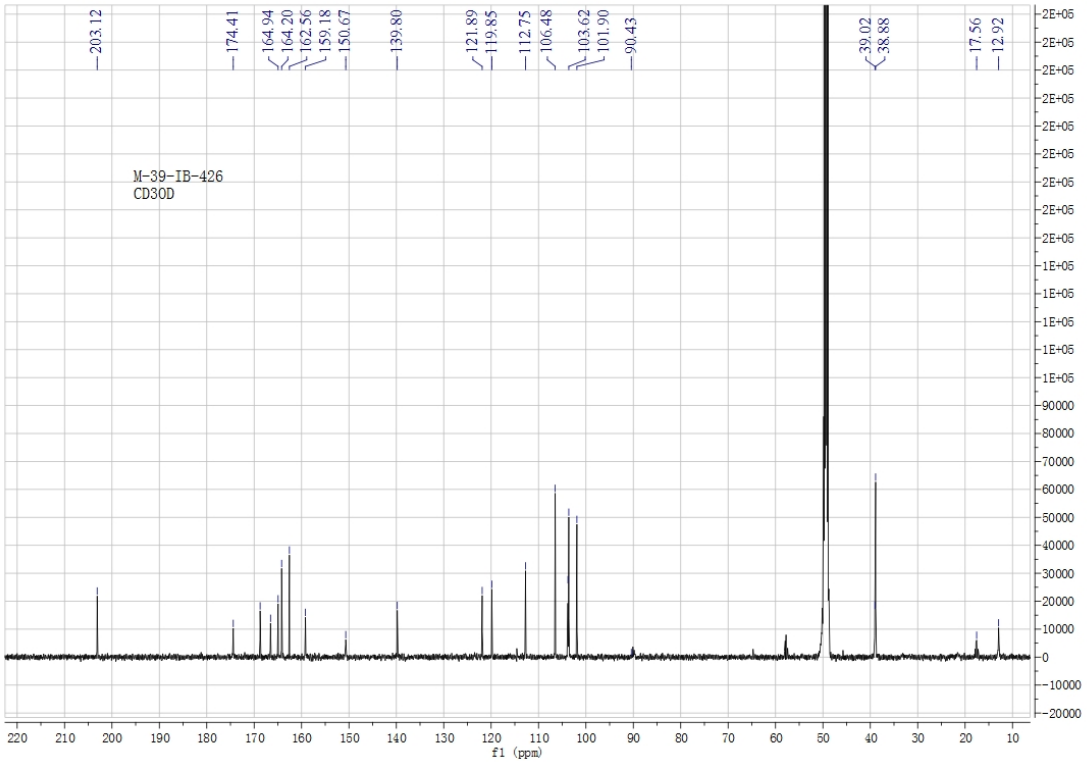


Fig. S5 ^{1}H-^{1}H COSY spectrum of compound **4a** in CD_3OD

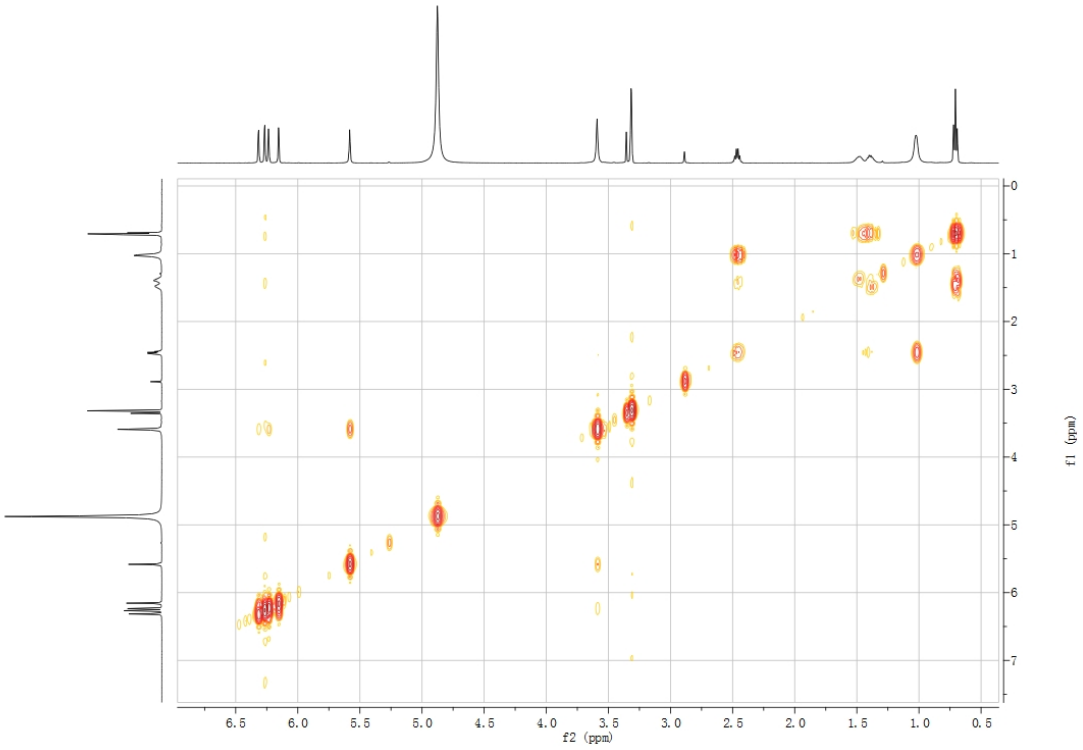


Fig. S6 HSQC NMR spectrum of compound **4a** in CD_3OD

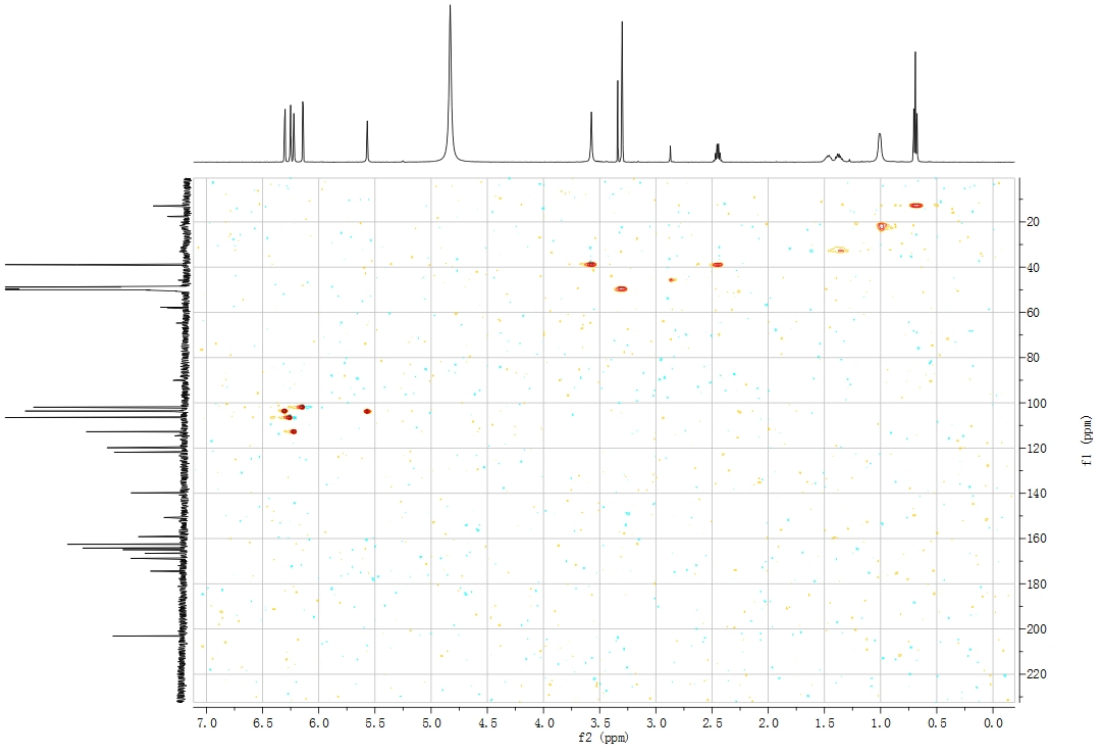


Fig. S7 HMBC NMR spectrum of compound **4a** in CD_3OD

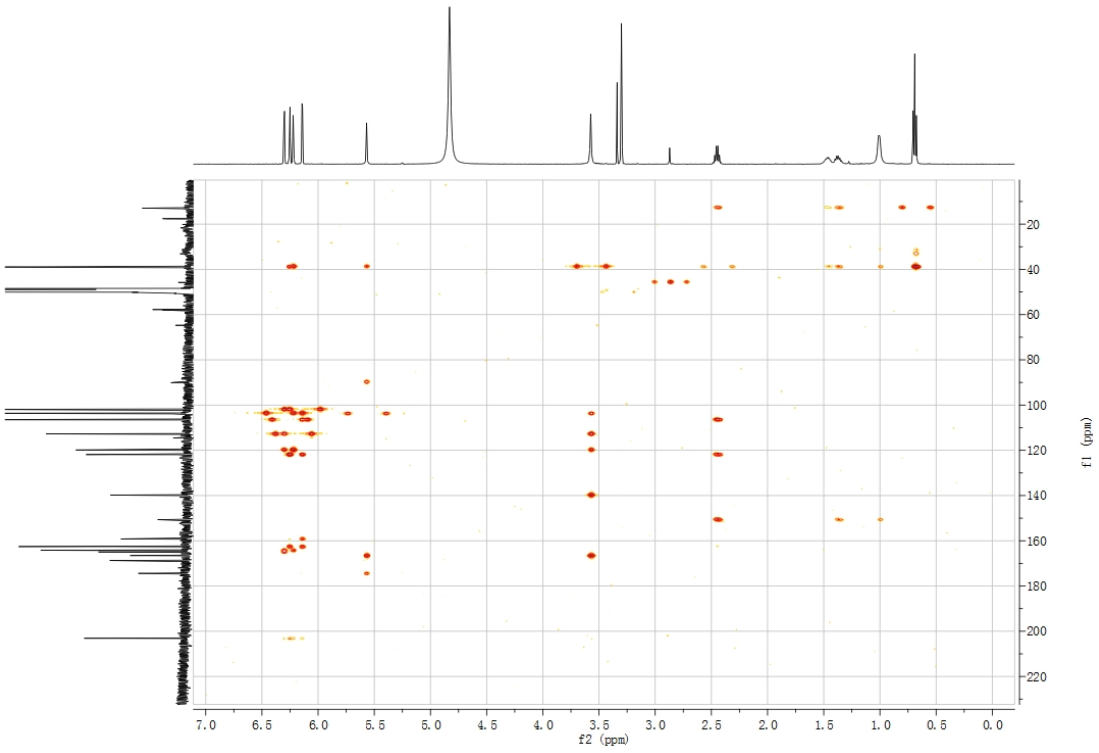


Fig. S8 ^{1}H-NMR spectrum of compound **4b** in CD_3OD

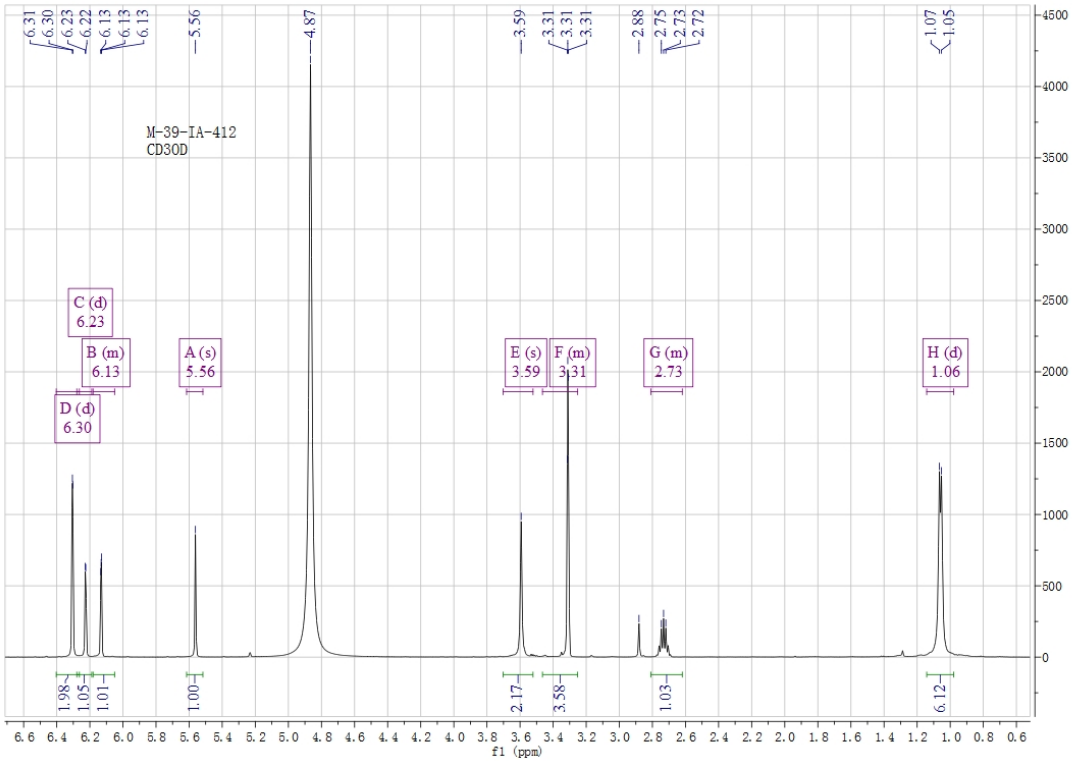


Fig. S9 ^{13}C-NMR spectrum of compound **4b** in CD_3OD

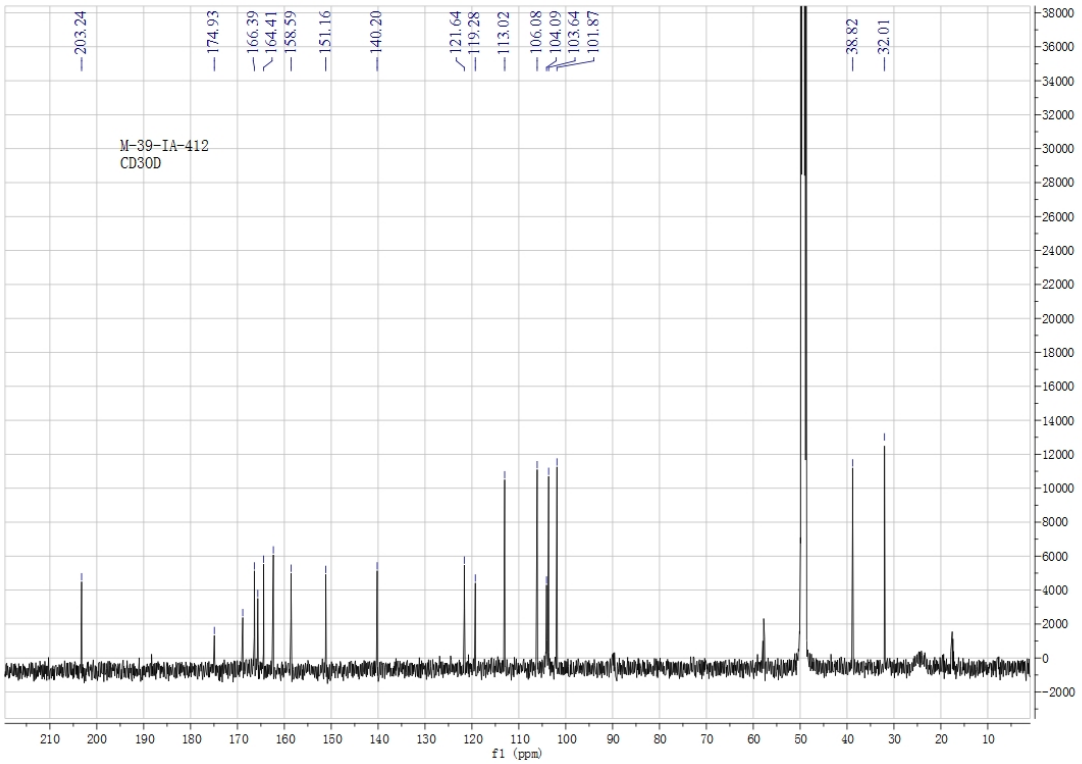


Fig. S10 ^{1}H-^{1}H COSY spectrum of compound **4b** in CD_3OD

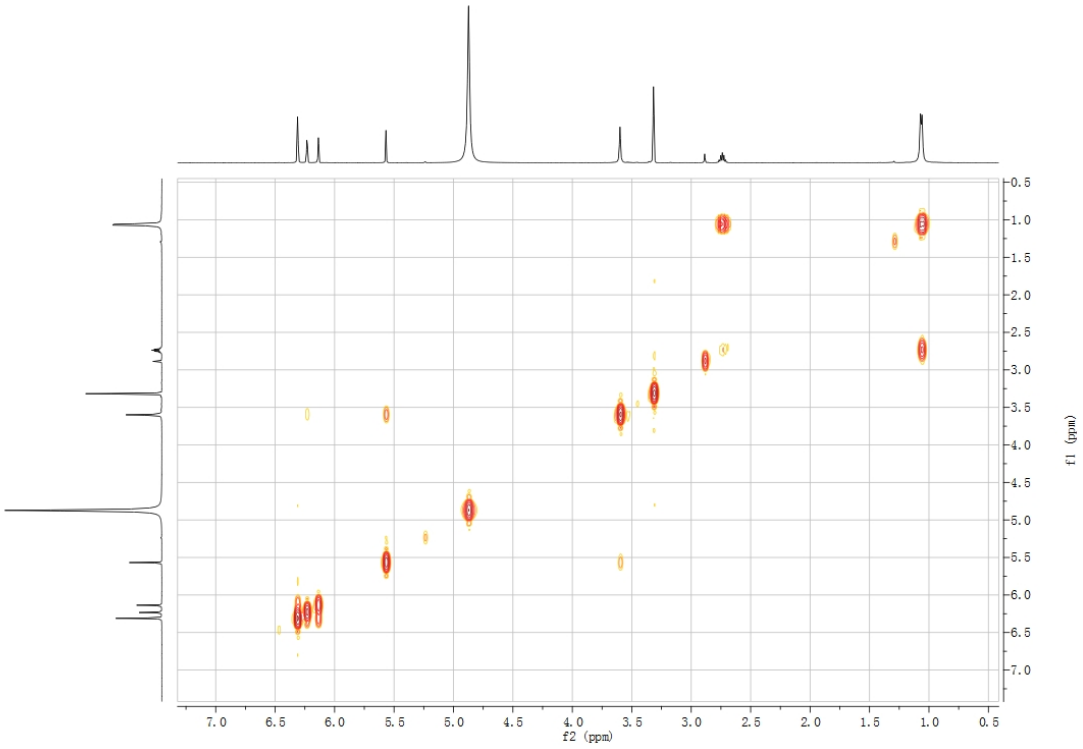


Fig. S11 HSQC NMR spectrum of compound **4b** in CD_3OD

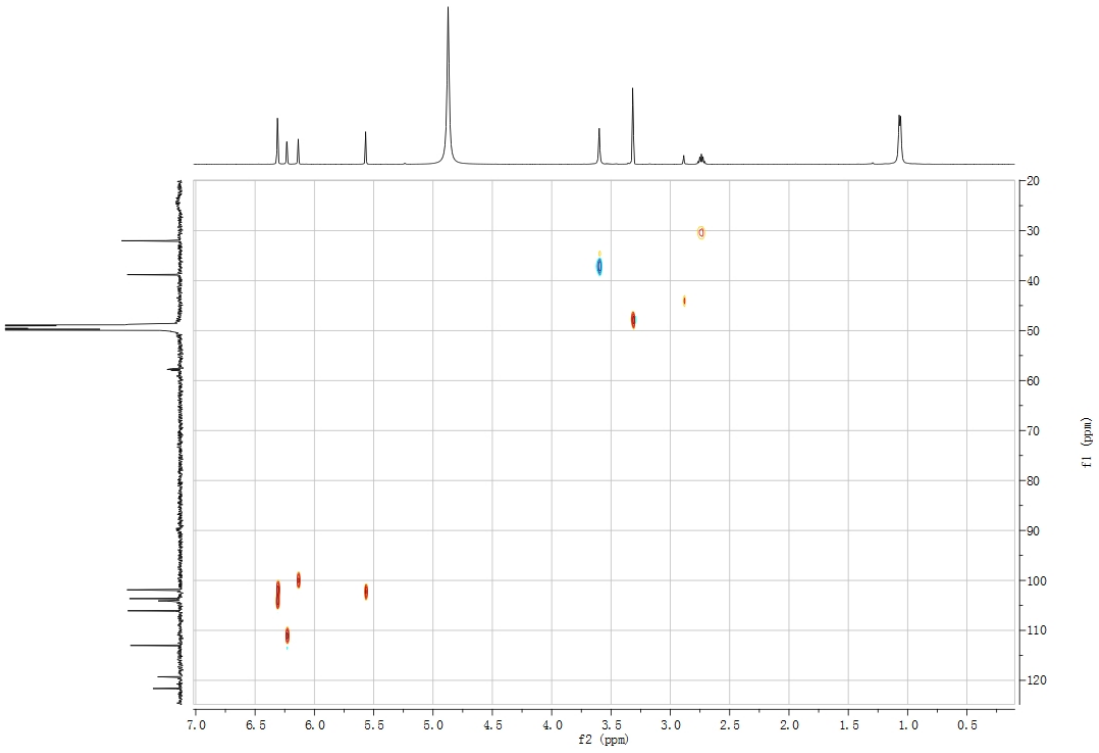


Fig. S12 HMBC NMR spectrum of compound **4b** in CD_3OD

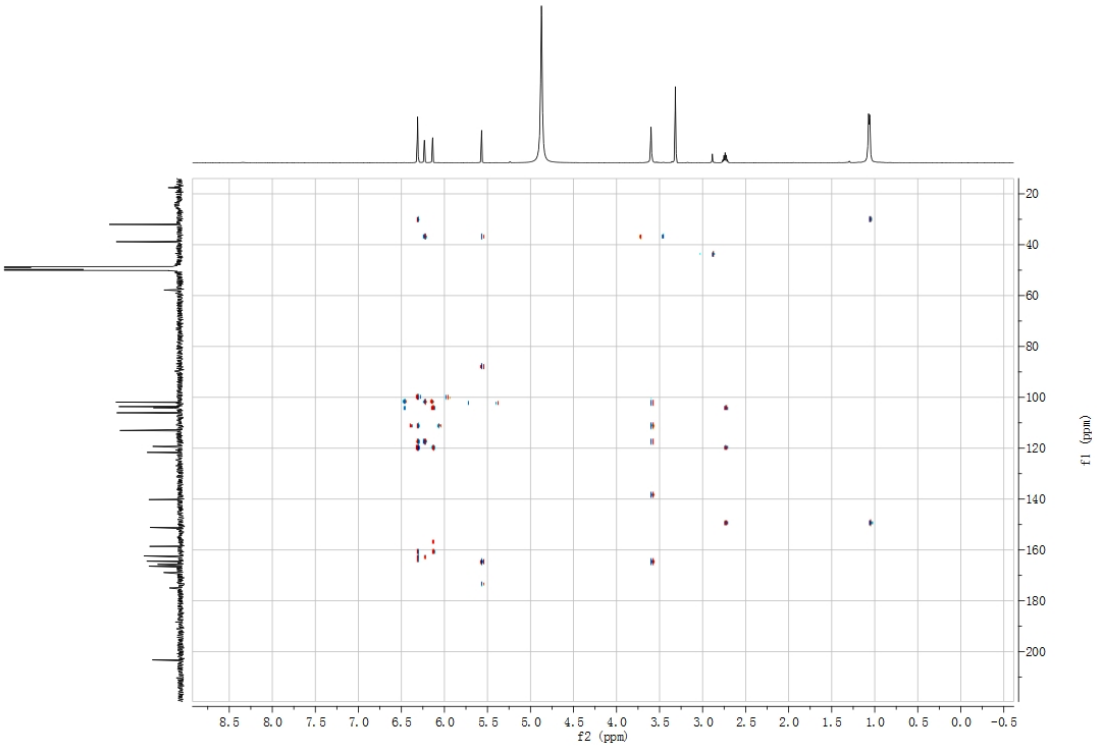


Fig. S13 ^{1}H-NMR spectrum of compound **6a** in $CDCl_3$

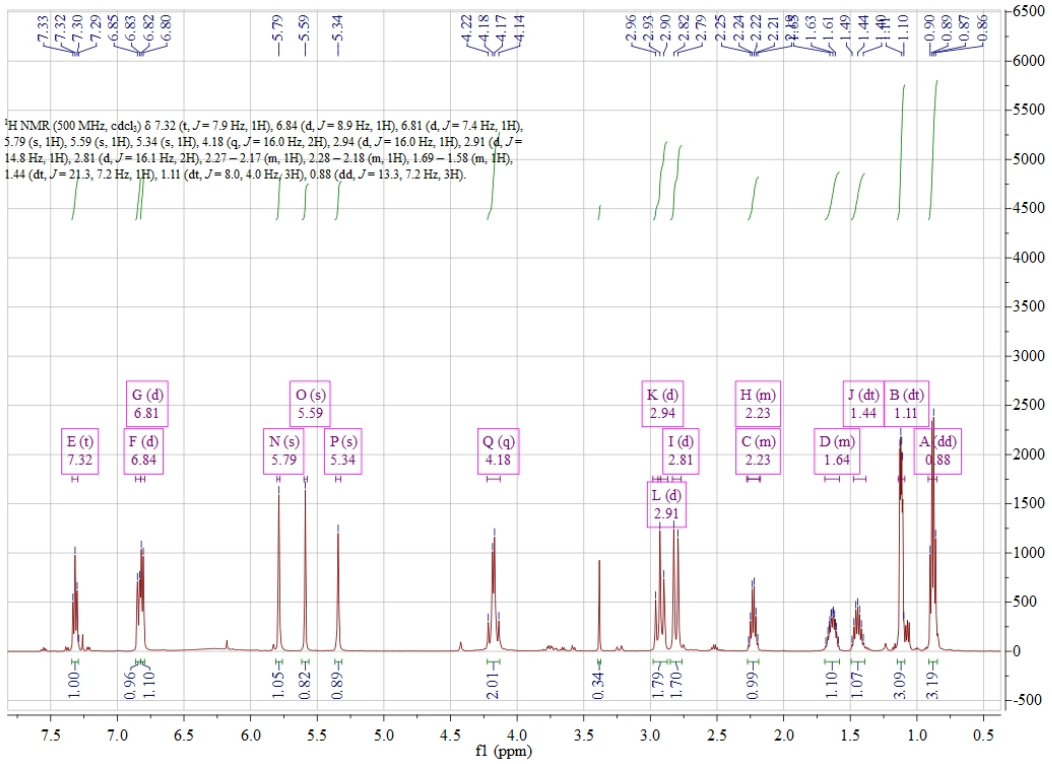


Fig. S14 ^{13}C-NMR spectrum of compound **6a** in $CDCl_3$

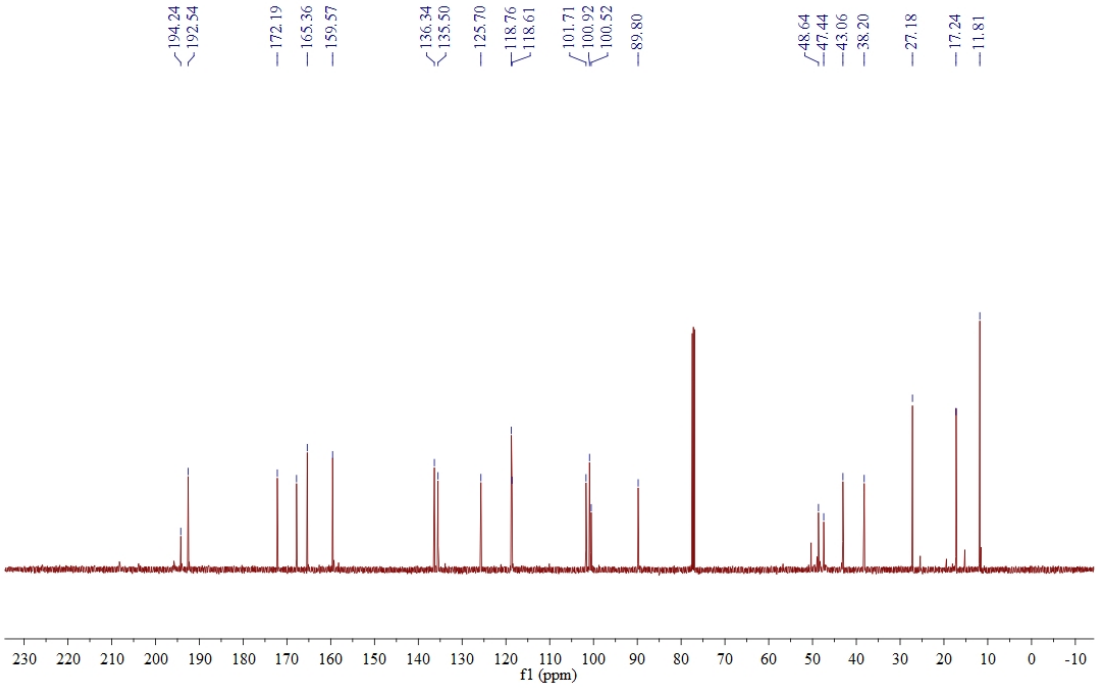


Fig. S15 ^{1}H-^{1}H COSY spectrum of compound **6a** in $CDCl_3$

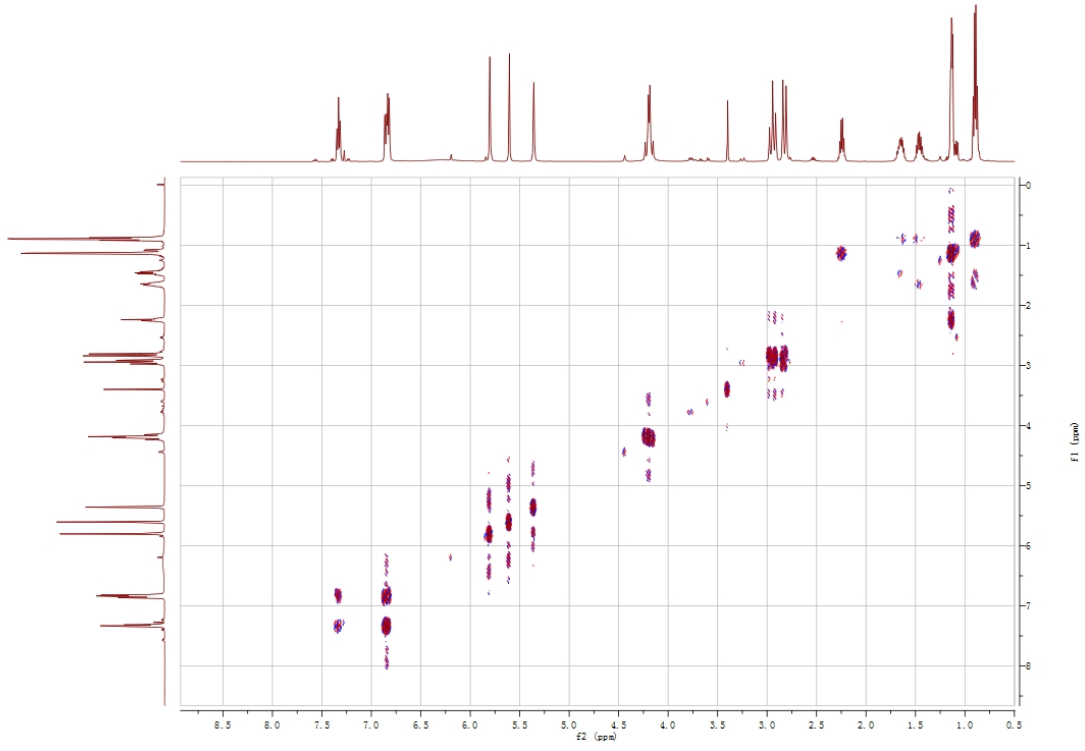


Fig. S16 HSQC NMR spectrum of compound **6a** in $CDCl_3$

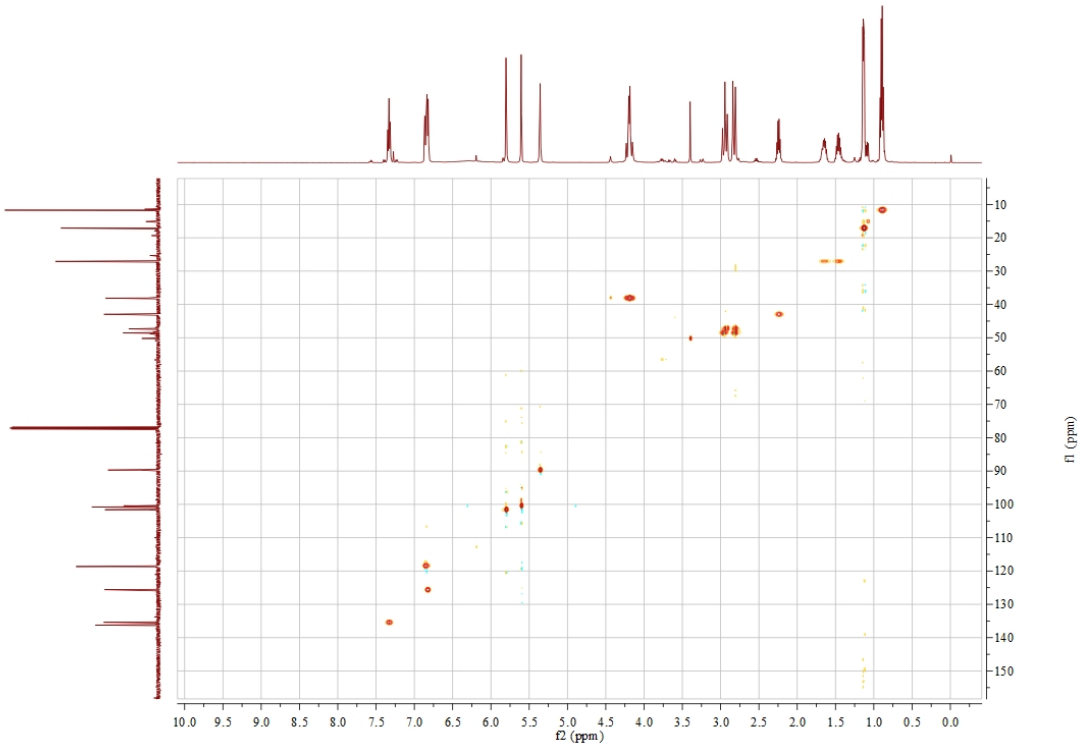


Fig. S17 HMBC NMR spectrum of compound **6a** in $CDCl_3$

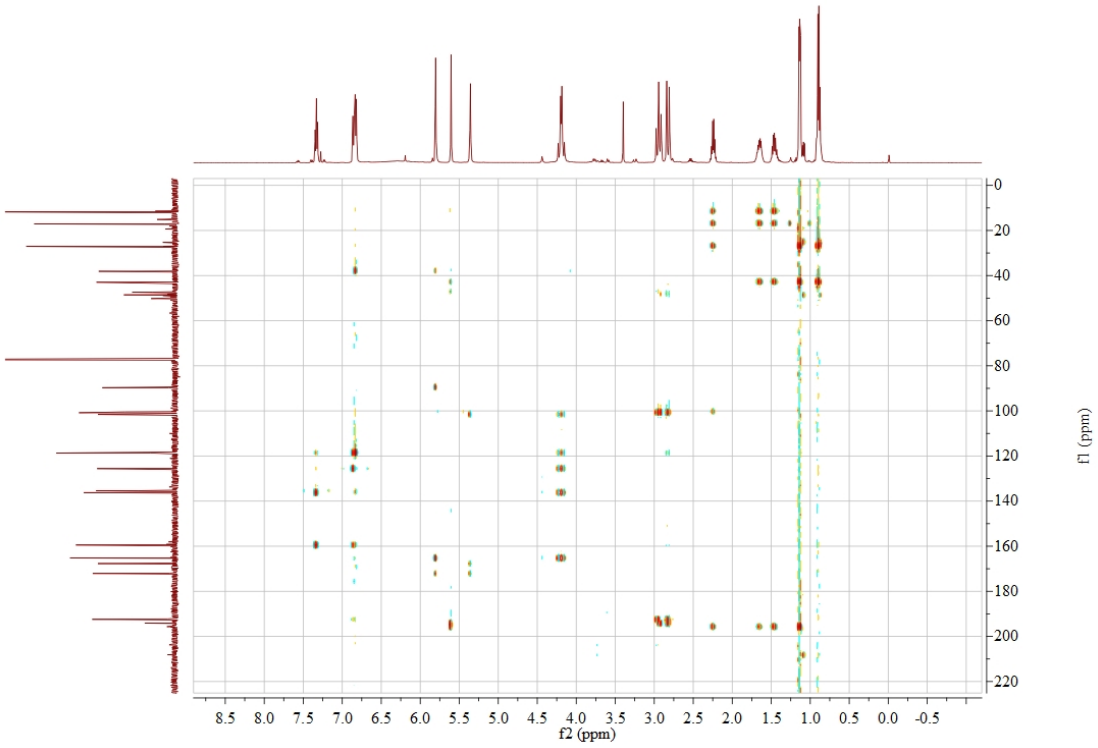


Fig. S18 ^{1}H-NMR spectrum of compound **9a** in DMSO-d6

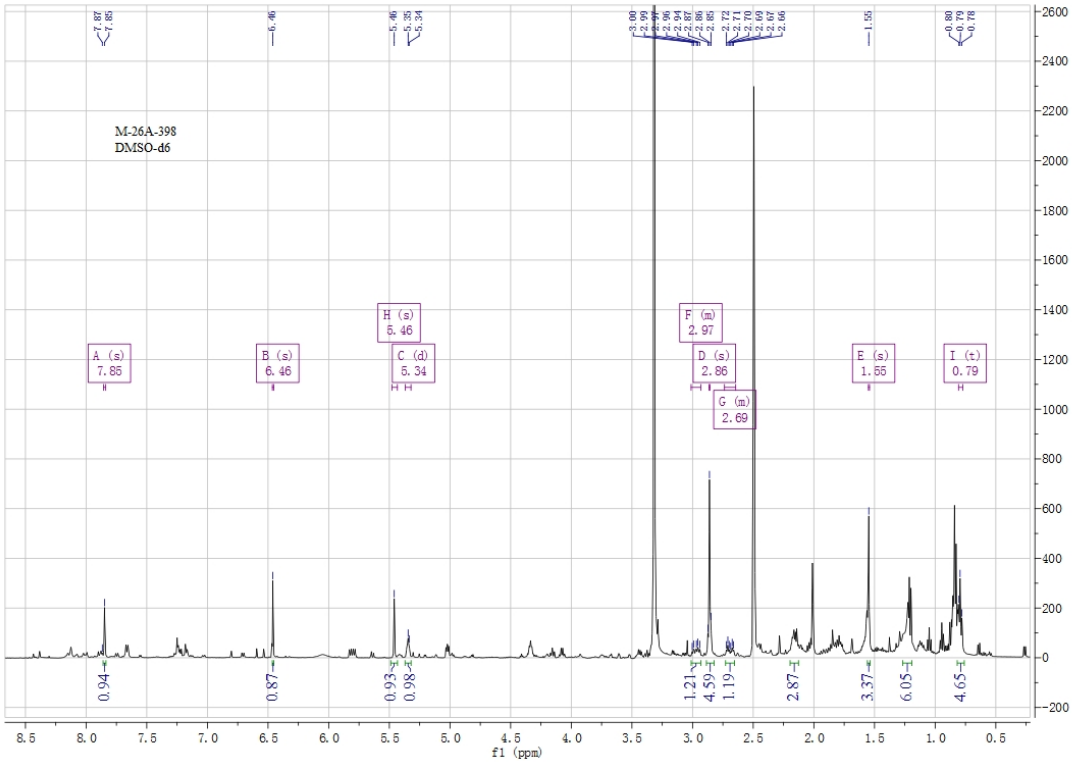


Fig. S19 ^{13}C-NMR spectrum of compound **9a** in DMSO-d6

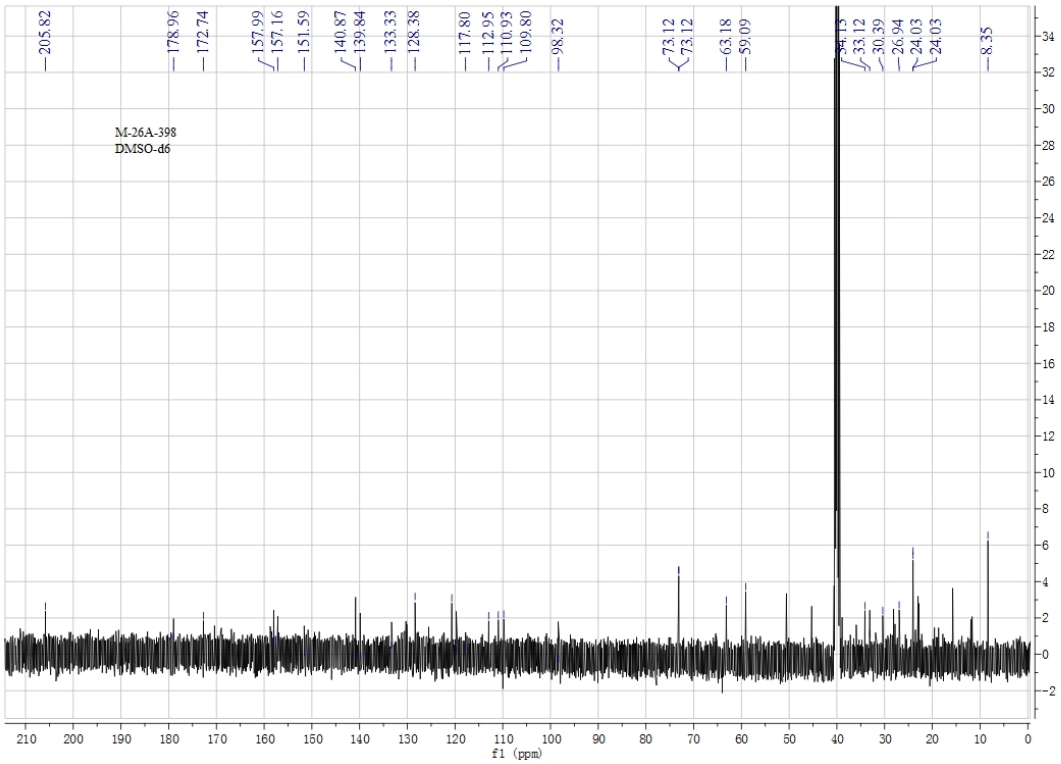


Fig. S20 HSQC NMR spectrum of compound **9a** in DMSO-d6

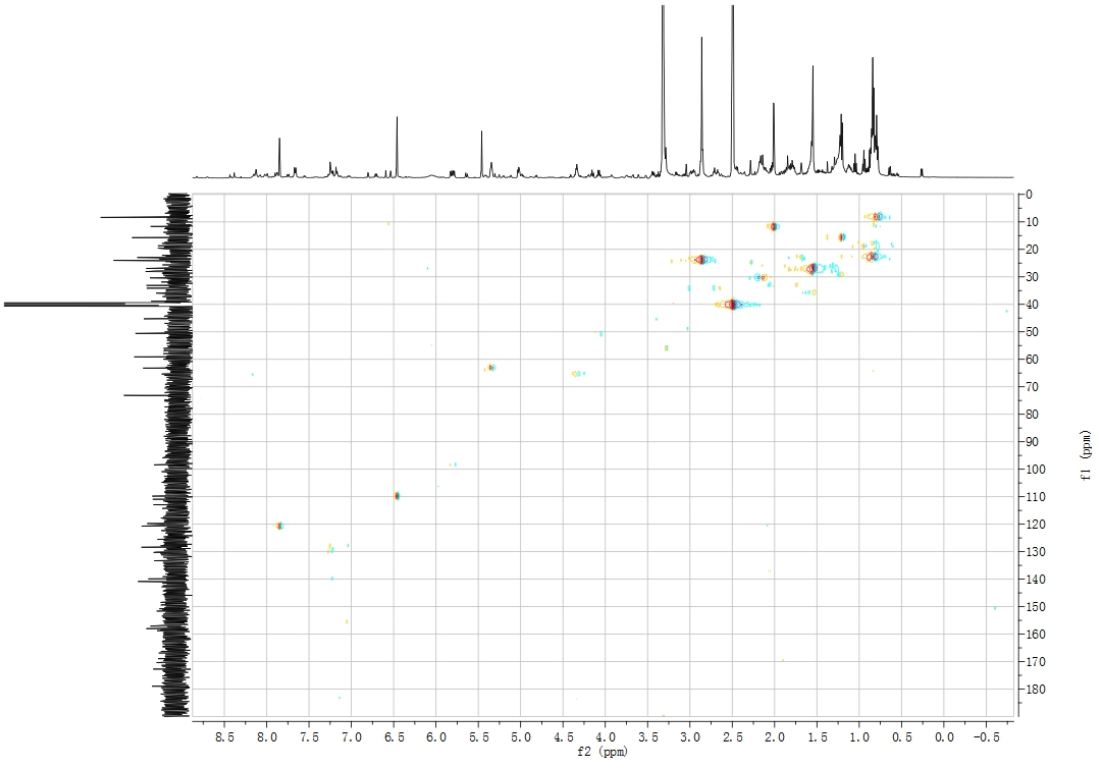


Fig. S21 HMBC NMR spectrum of compound **9a** in DMSO-d6

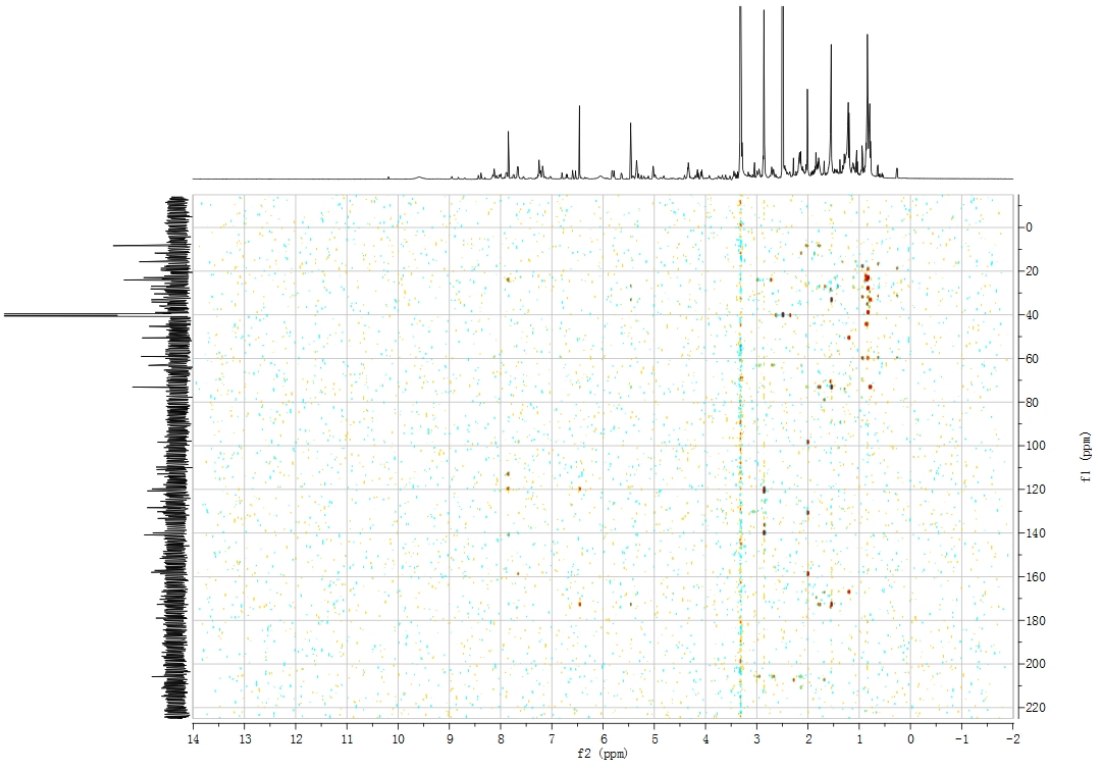


Fig. S22 ^{1}H-NMR spectrum of compound **12a** in $CDCl_3$

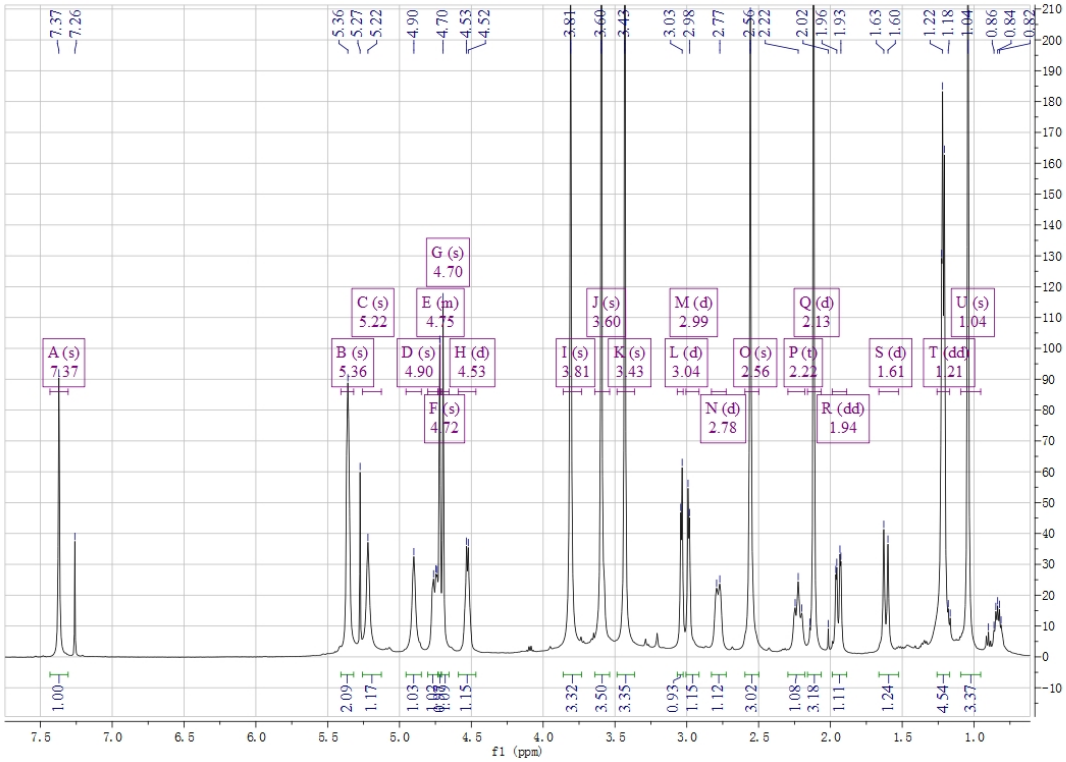


Fig. S23 ^{13}C-NMR spectrum of compound **12a** in $CDCl_3$

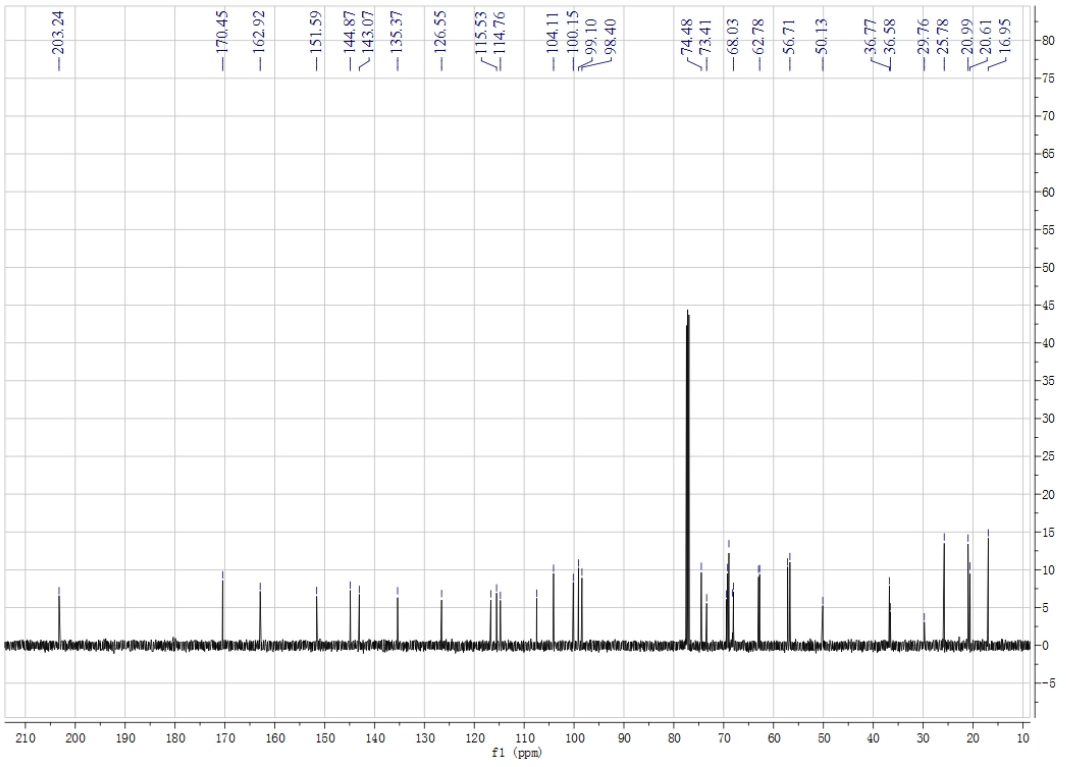


Fig. S24 HSQC NMR spectrum of compound **12a** in $CDCl_3$

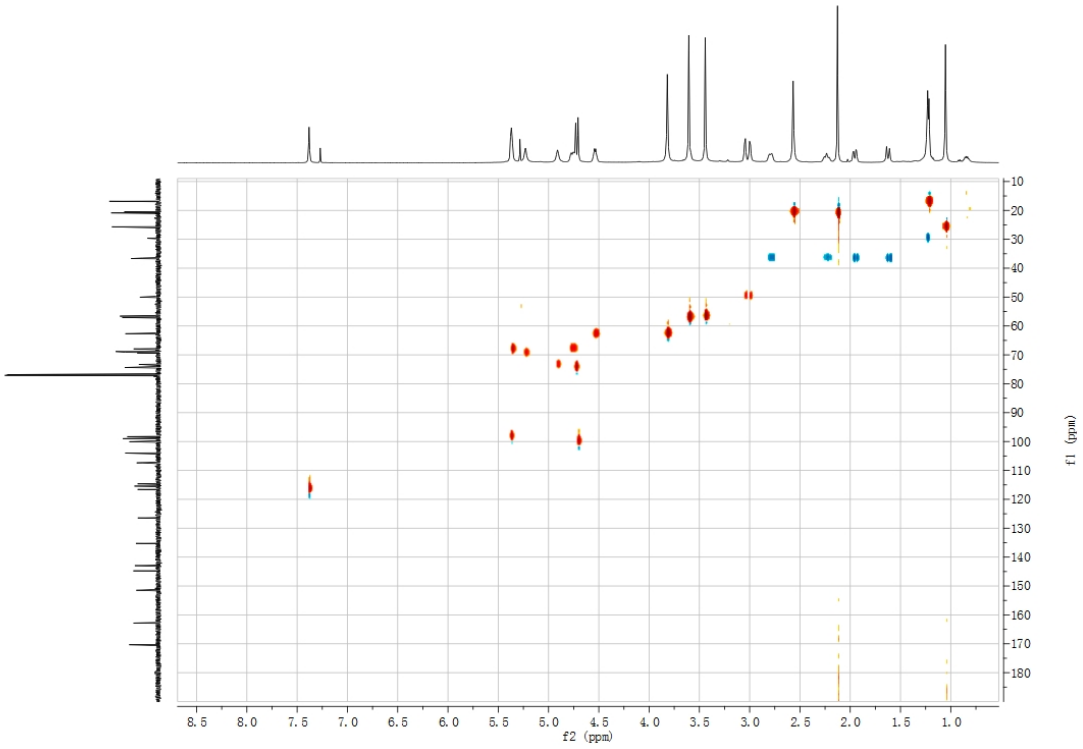


Fig. S25 HMBC NMR spectrum of compound **12a** in $CDCl_3$

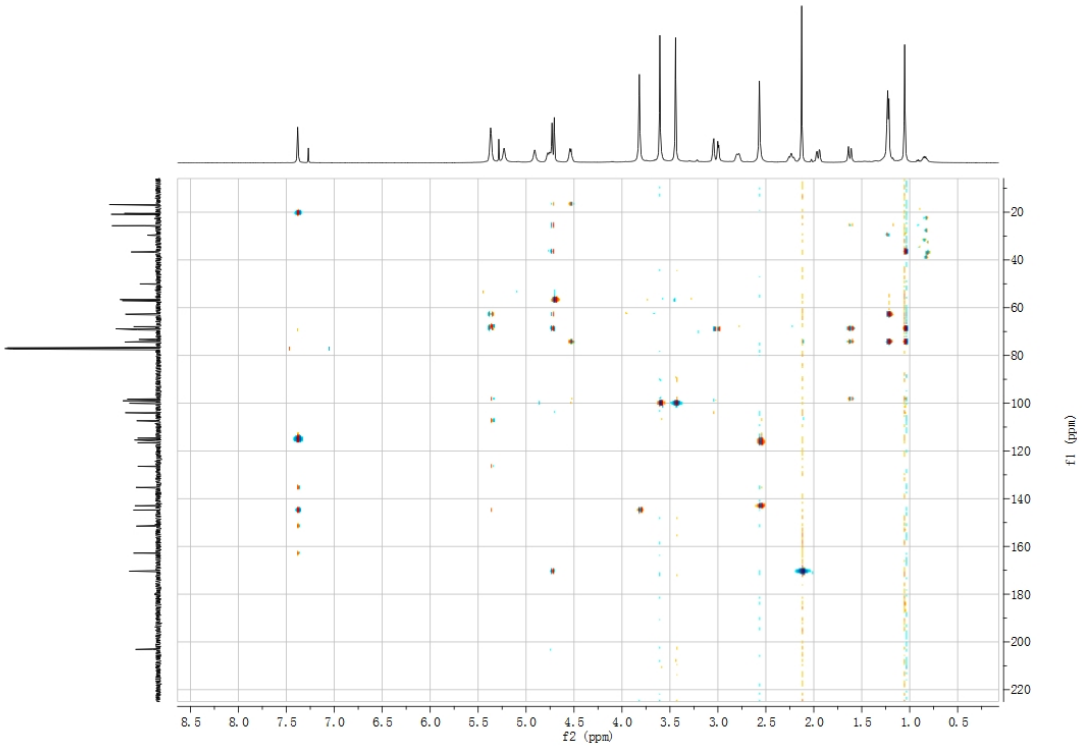


Fig. S26 ^{1}H-NMR spectrum of compound **12b** in $CDCl_3$

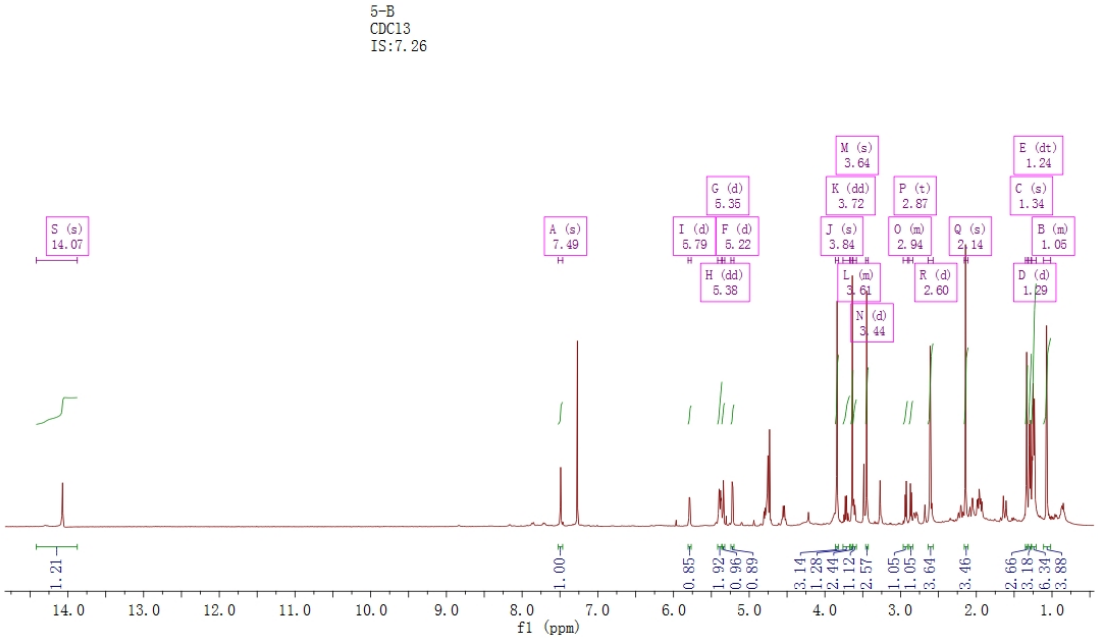


Fig. S27 ^{13}C-NMR spectrum of compound **12b** in $CDCl_3$

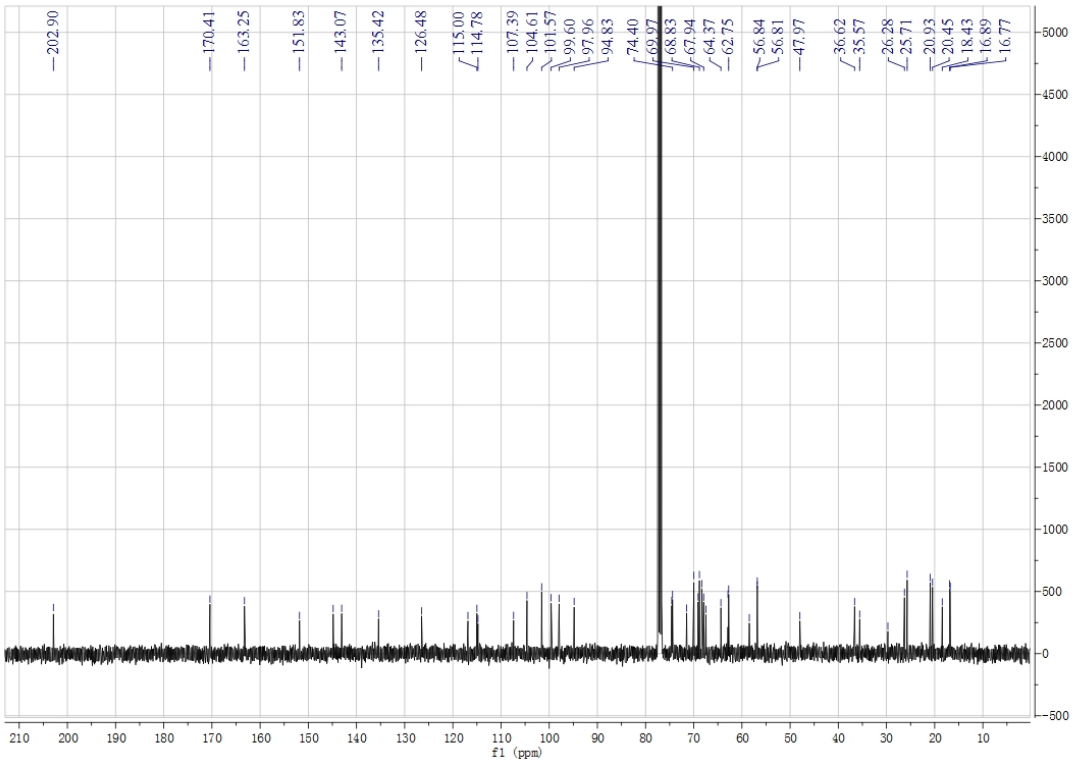


Fig. S28 ^{1}H-NMR spectrum of compound **13a** in CD_3OD

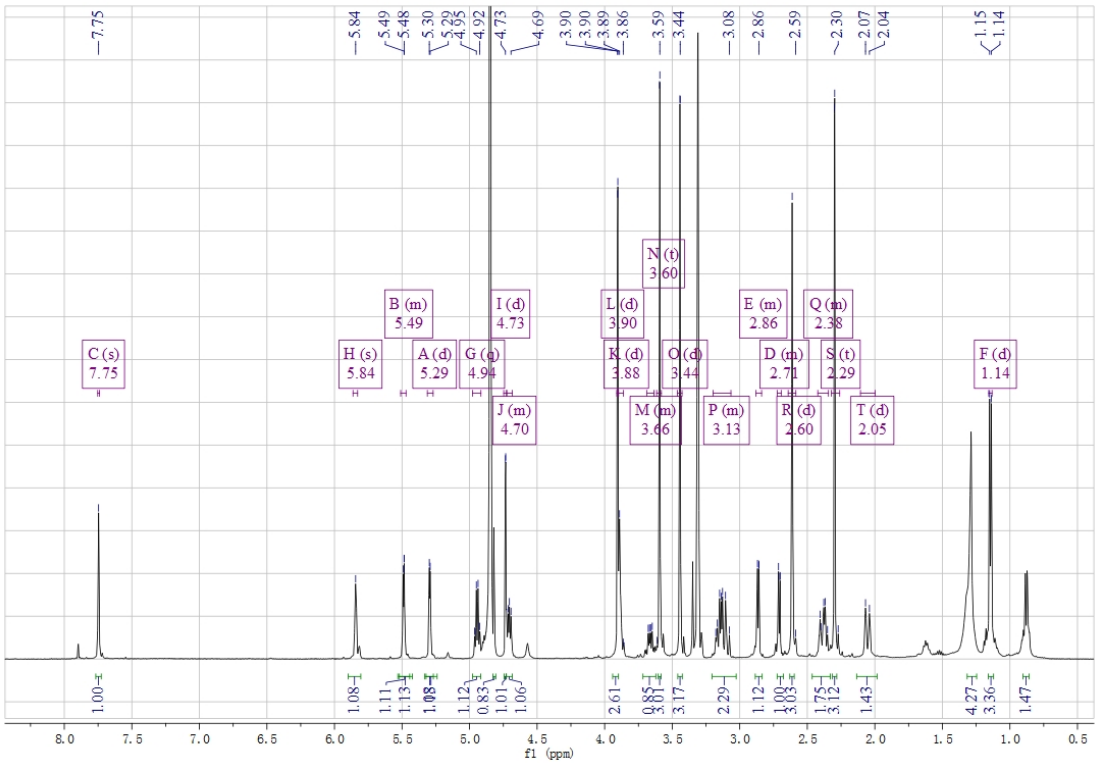


Fig. S29 ^{13}C-NMR spectrum of compound **13a** in CD_3OD

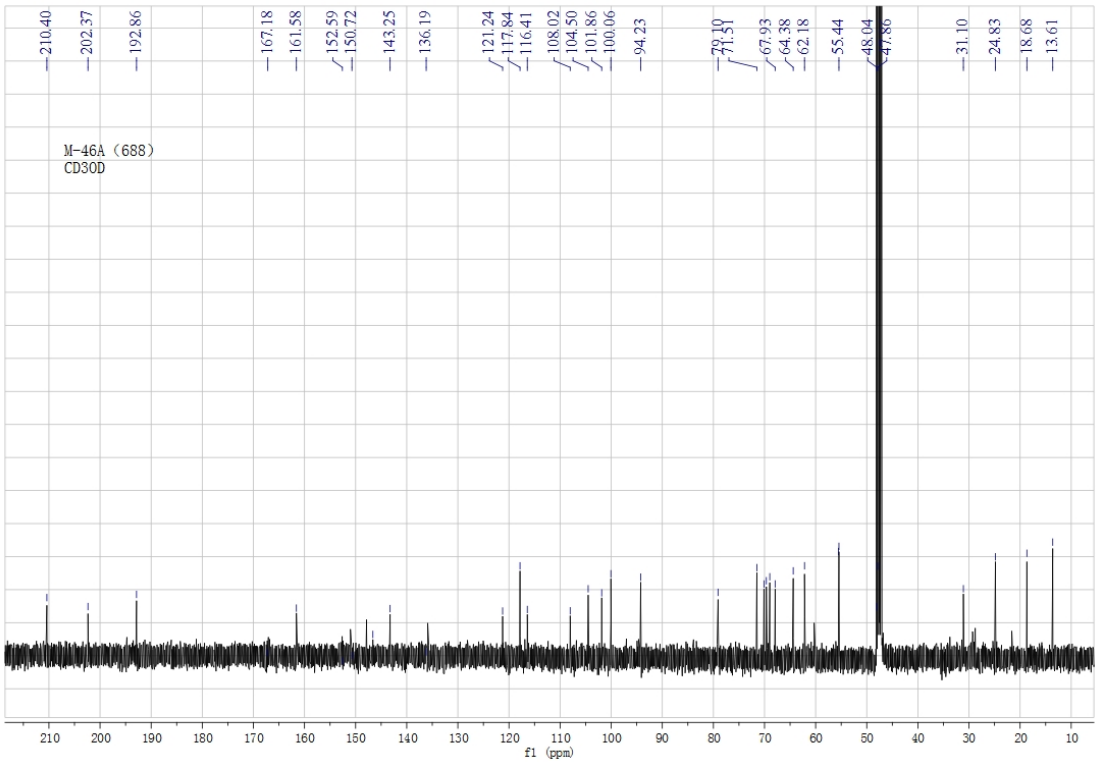


Fig. S30 HSQC NMR spectrum of compound **13a** in CD_3OD

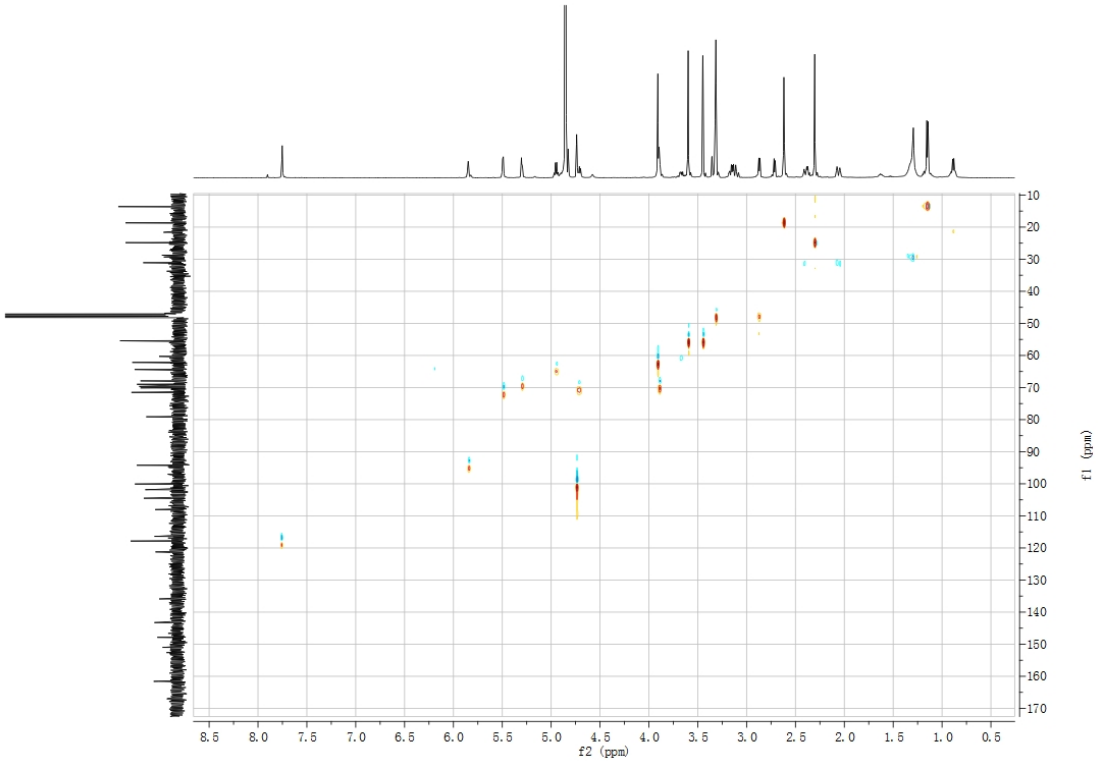


Fig. S31 HMBC NMR spectrum of compound **13a** in CD_3OD

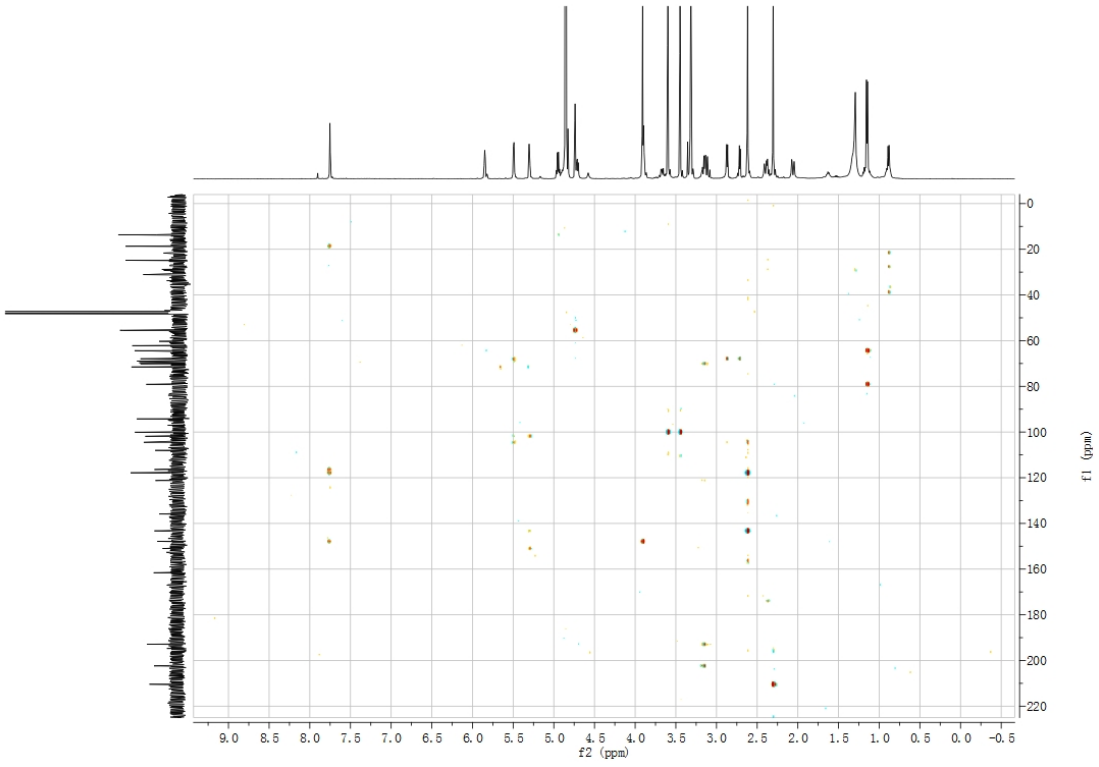


Fig. S32 ^{1}H-NMR spectrum of compound **13b** in CD_3OD

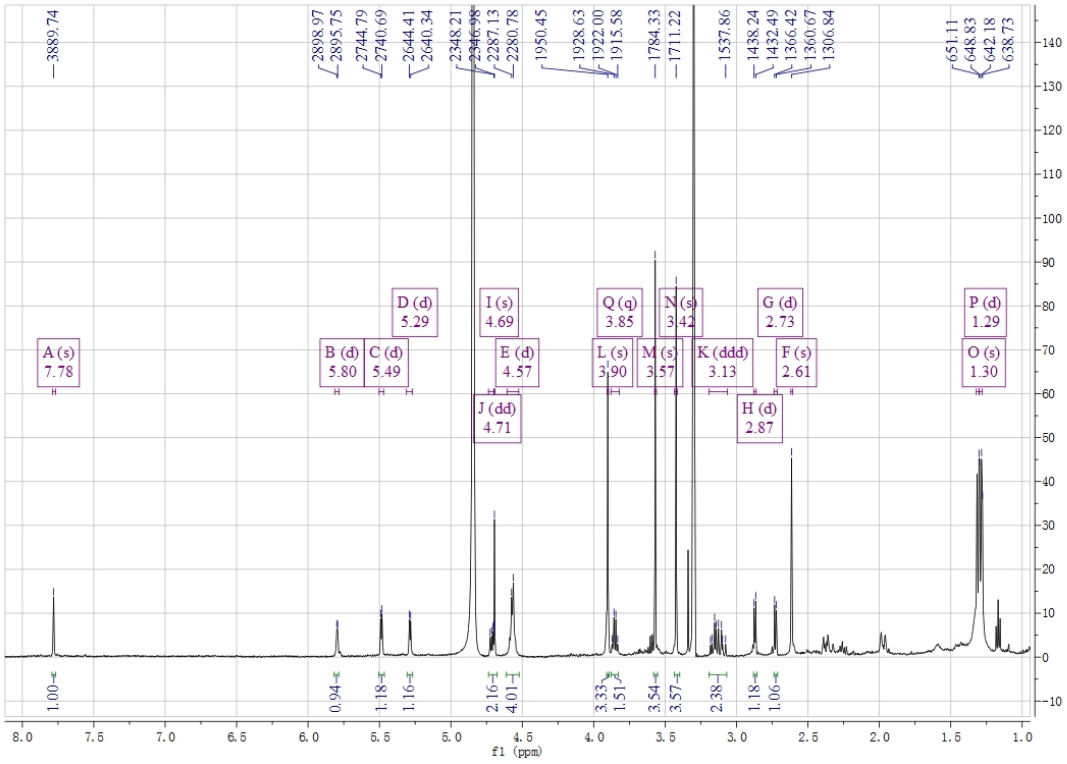


Fig. S33 ^{1}H-NMR spectrum of compound **14a** in $CDCl_3$

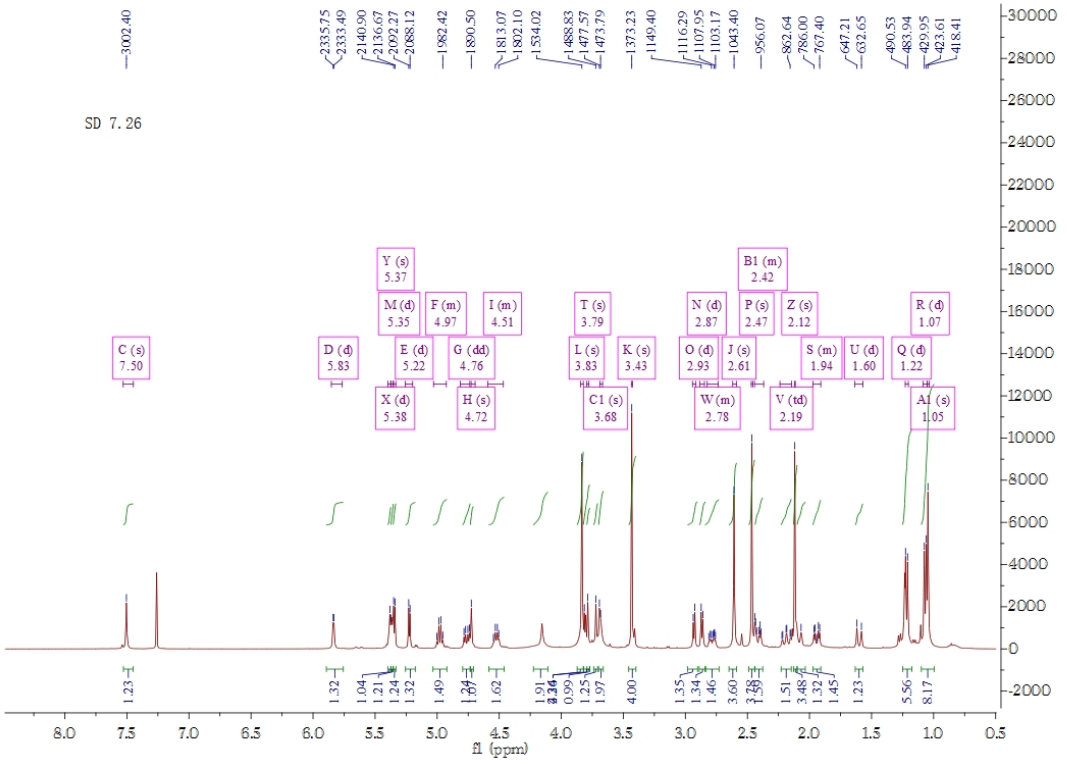


Fig. S34 ^{13}C-NMR spectrum of compound **14a** in $CDCl_3$

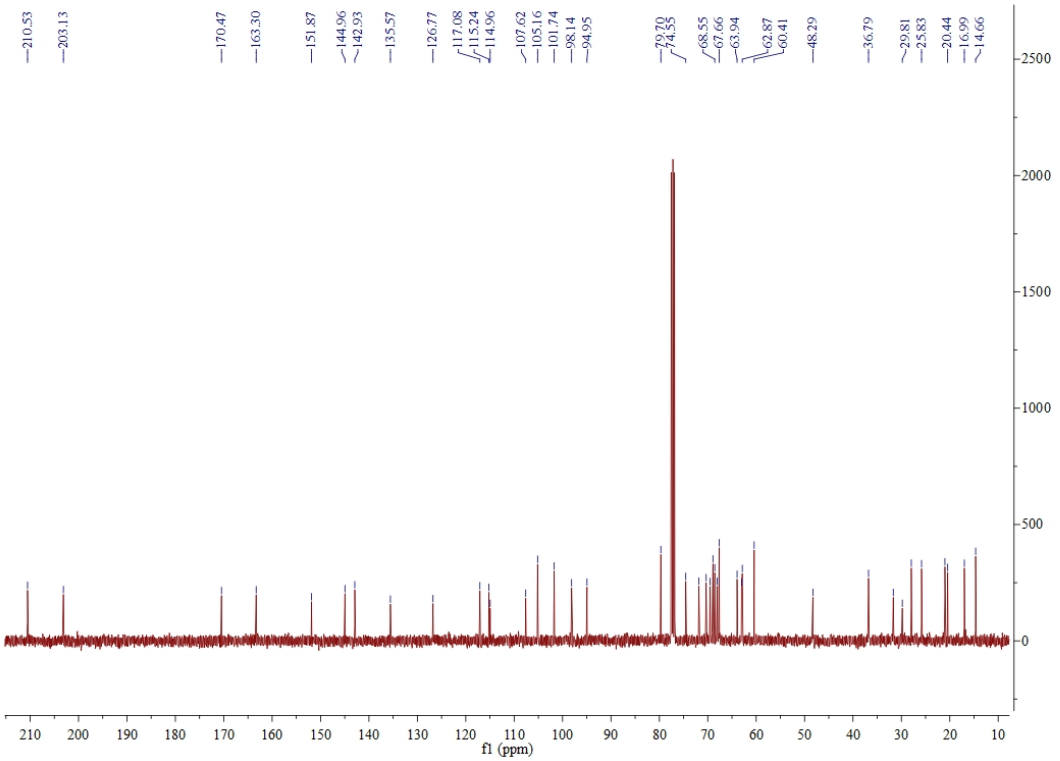


Fig. S35 HSQC NMR spectrum of compound **14a** in $CDCl_3$

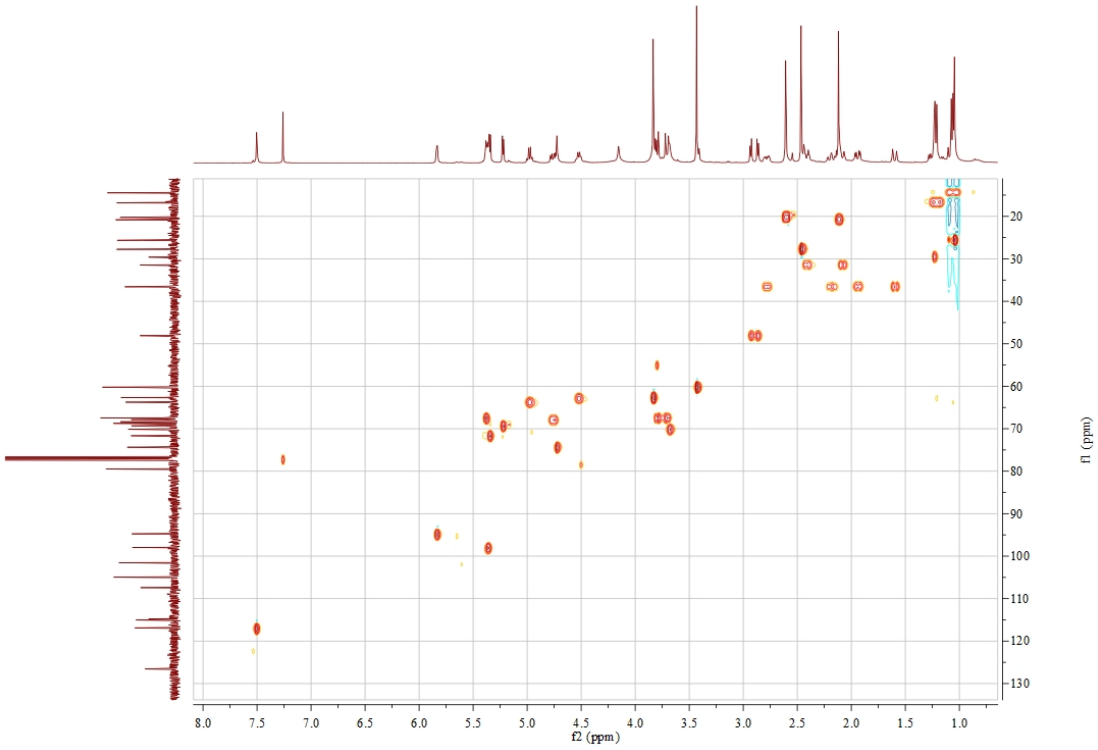


Fig. S36 ^{1}H-^{1}H COSY spectrum of compound **14a** in $CDCl_3$

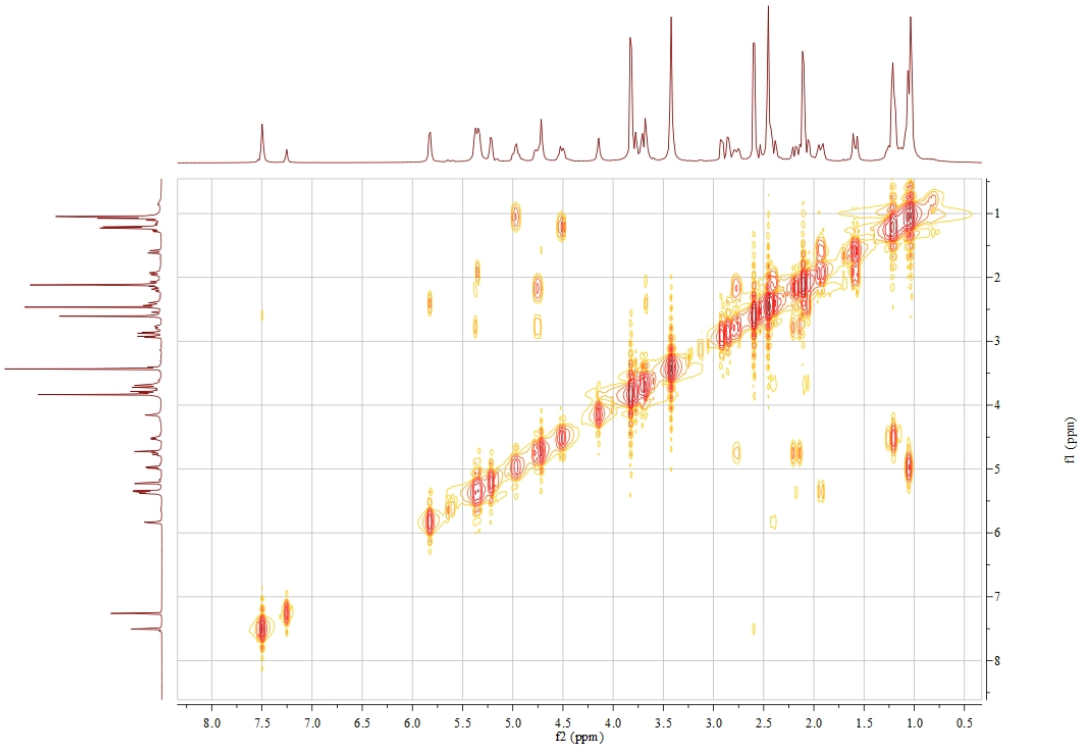


Fig. S37 HMBC NMR spectrum of compound **14a** in $CDCl_3$

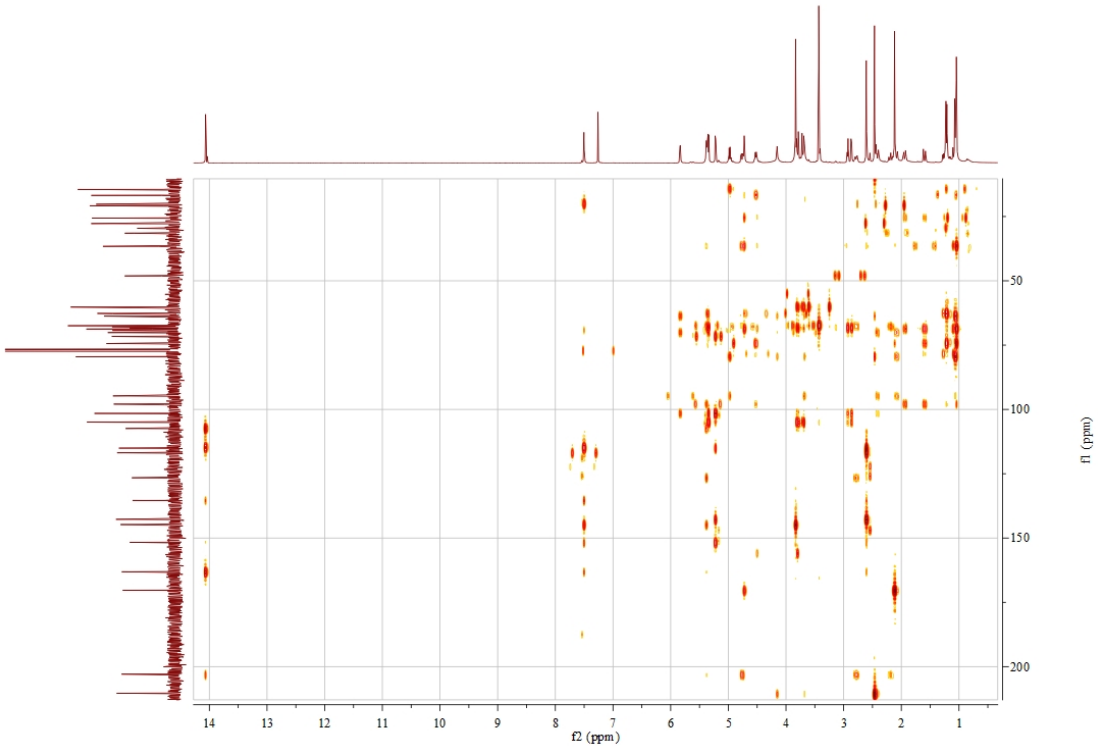


Fig. S38 ^{1}H-NMR spectrum of compound **15a** in $CDCl_3$

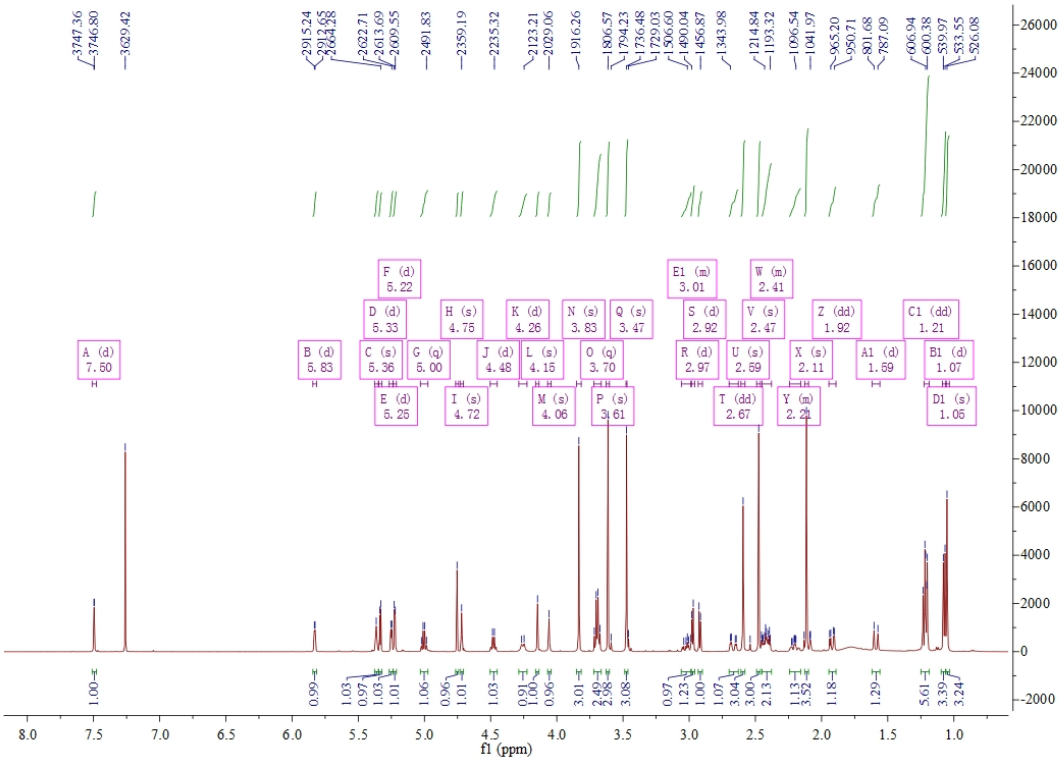


Fig. S39 ^{13}C-NMR spectrum of compound **15a** in $CDCl_3$

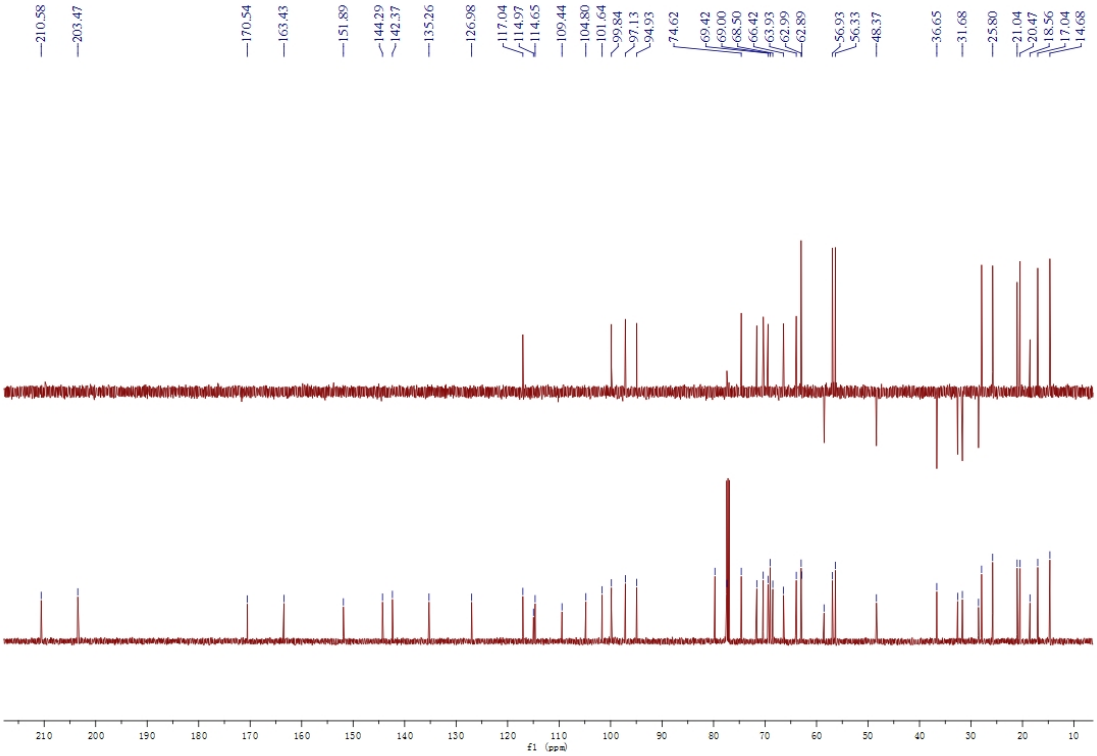


Fig. S40 ^{1}H-^{1}H COSY spectrum of compound **15a** in $CDCl_3$

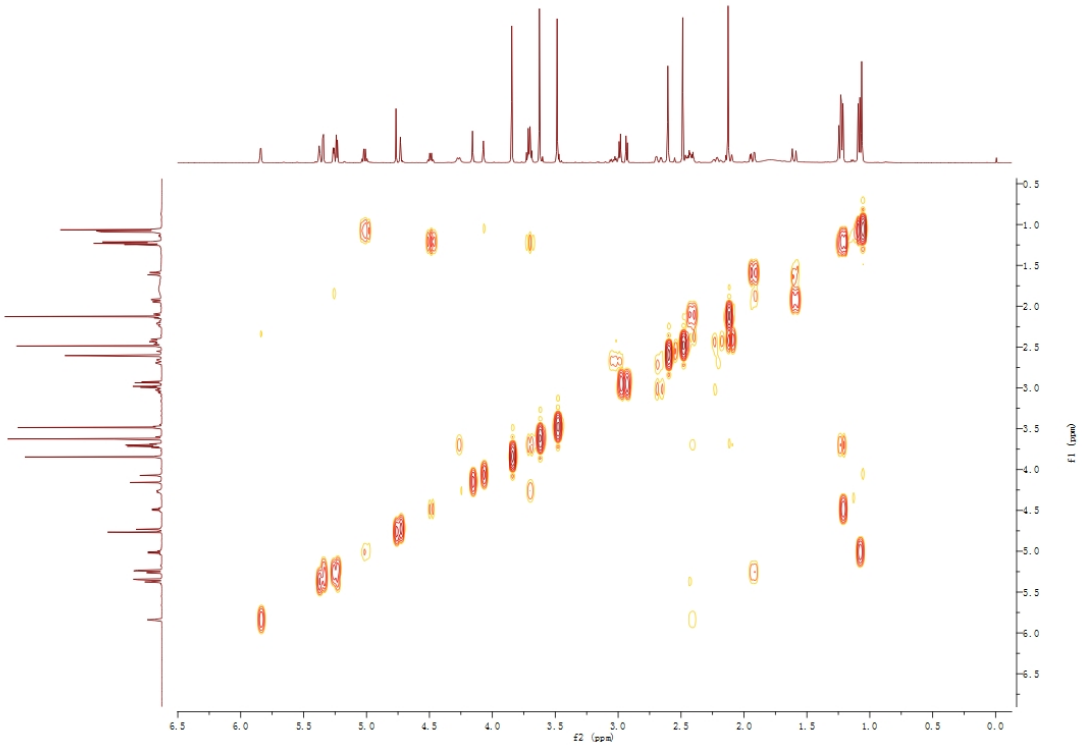


Fig. S41 HSQC NMR spectrum of compound **15a** in $CDCl_3$

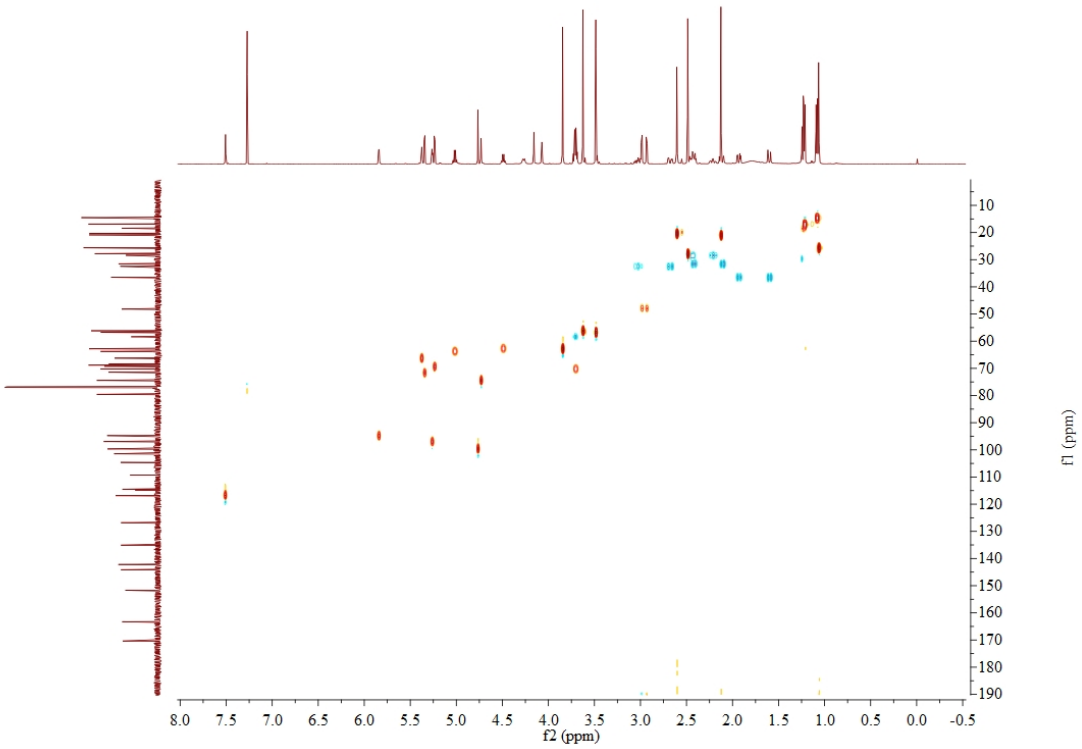


Fig. S42 HMBC NMR spectrum of compound **15a** in $CDCl_3$

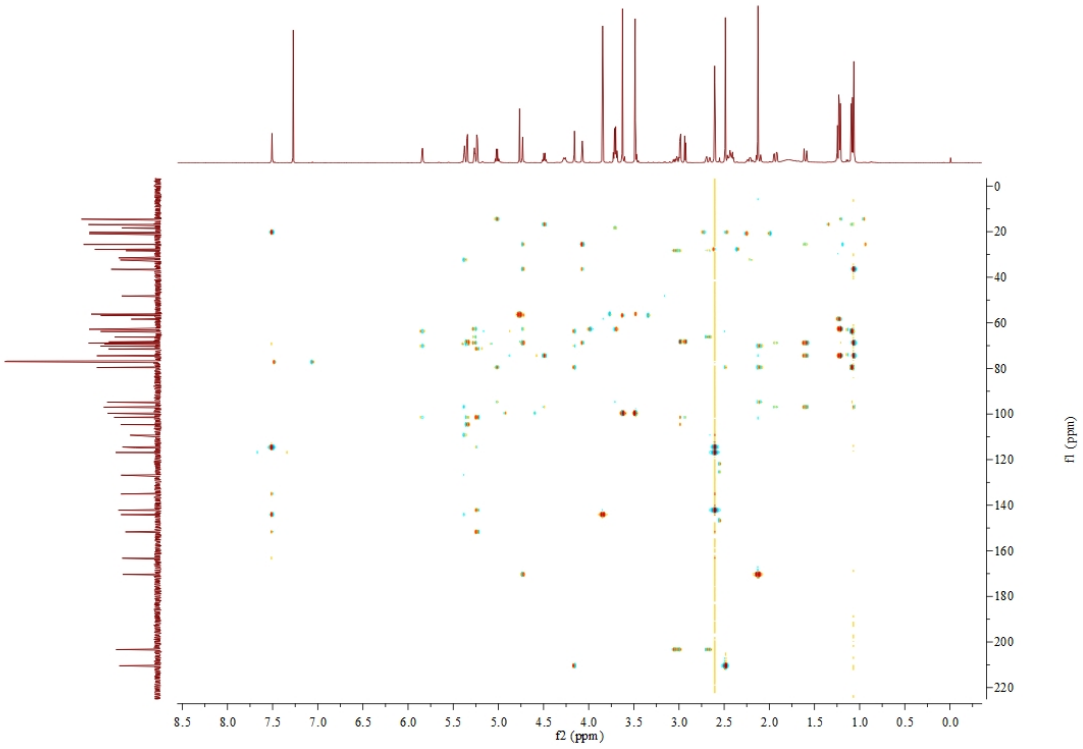


Fig. S43 ^{1}H-NMR spectrum of compound **15b** in $CDCl_3$

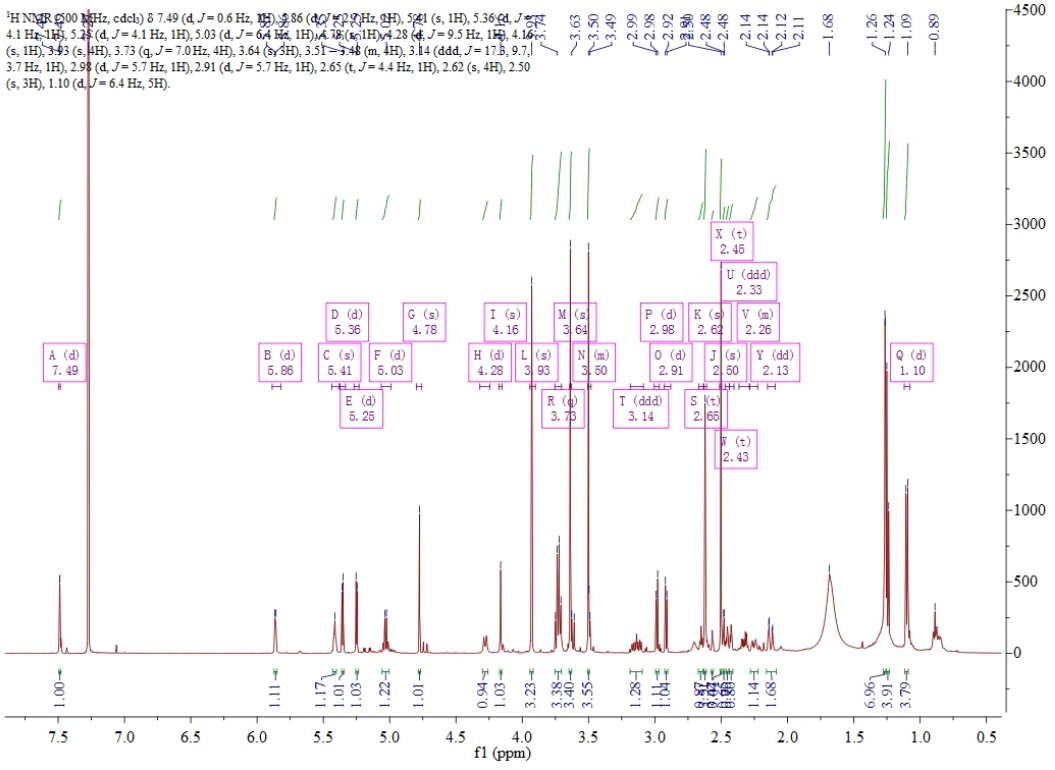


Fig. S44 ^{13}C-NMR spectrum of compound **15b** in $CDCl_3$

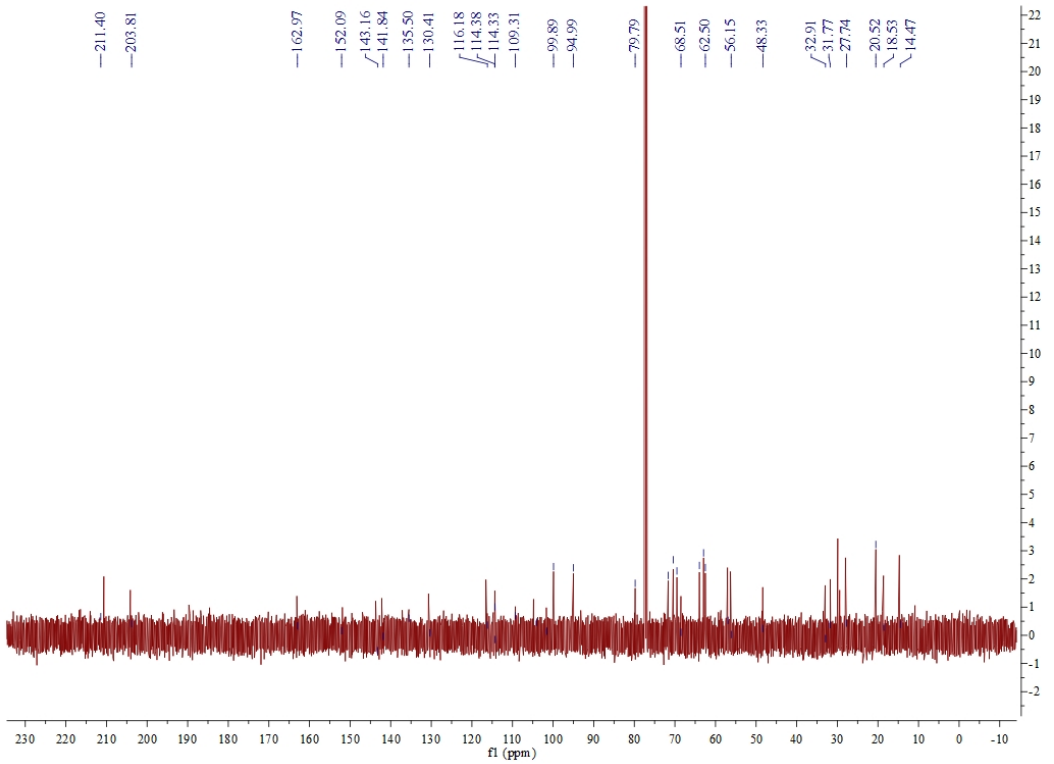


Fig. S45 Proposed pathway from intermediate **9** to **10**.