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Supporting Information

for

Isolation and biosynthesis of daturamycins from *Streptomyces* sp. KIB-H1544

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Spectroscopic data for compounds 1–3, HRMS–ESI data for compound 8, annotation of genes in the *dat* biosynthetic gene cluster, list of biological material, vectors, and primers used in this study

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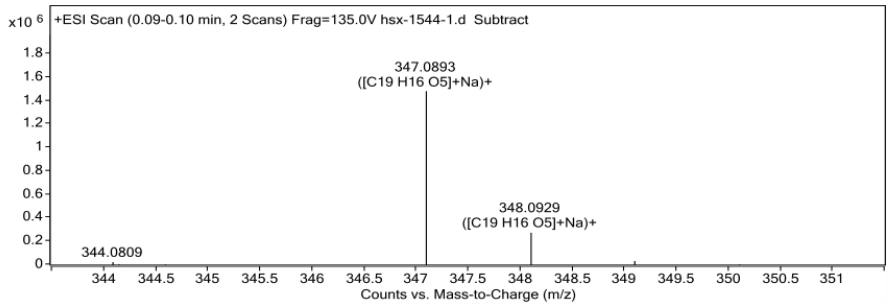
Table of contents

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
247.0752	1	155253.95		
279.1016	1	152539.56		
307.0968	1	1684749.63		
308.1003	1	311080.25		
342.1341	1	351820.19		
347.0893	1	1478410.75	C19 H16 O5	(M+Na)+
348.0929	1	277042.75	C19 H16 O5	(M+Na)+
363.0633	1	213484.89		
671.1899	1	786305.25		
672.1934	1	309542.5		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C19 H16 O5	324.0998	347.0890	347.0893	-0.30	-0.86	12.0000

Figure S1: HRMS–ESI spectrum of compound **1**.

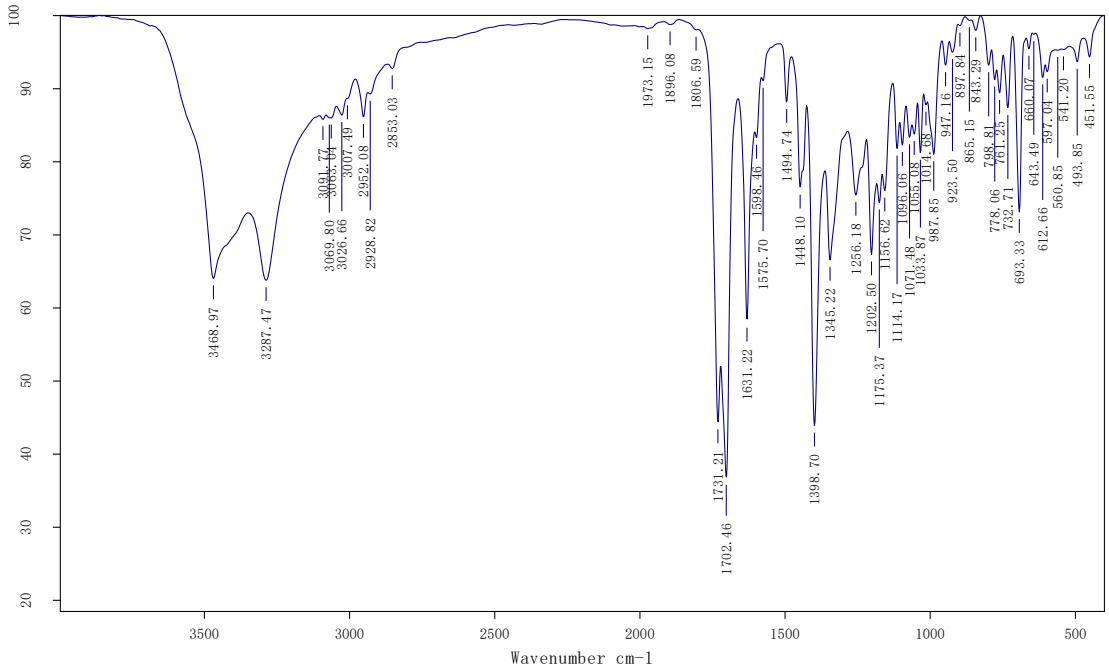


Figure S2: IR spectrum of compound **1** in MeOH.

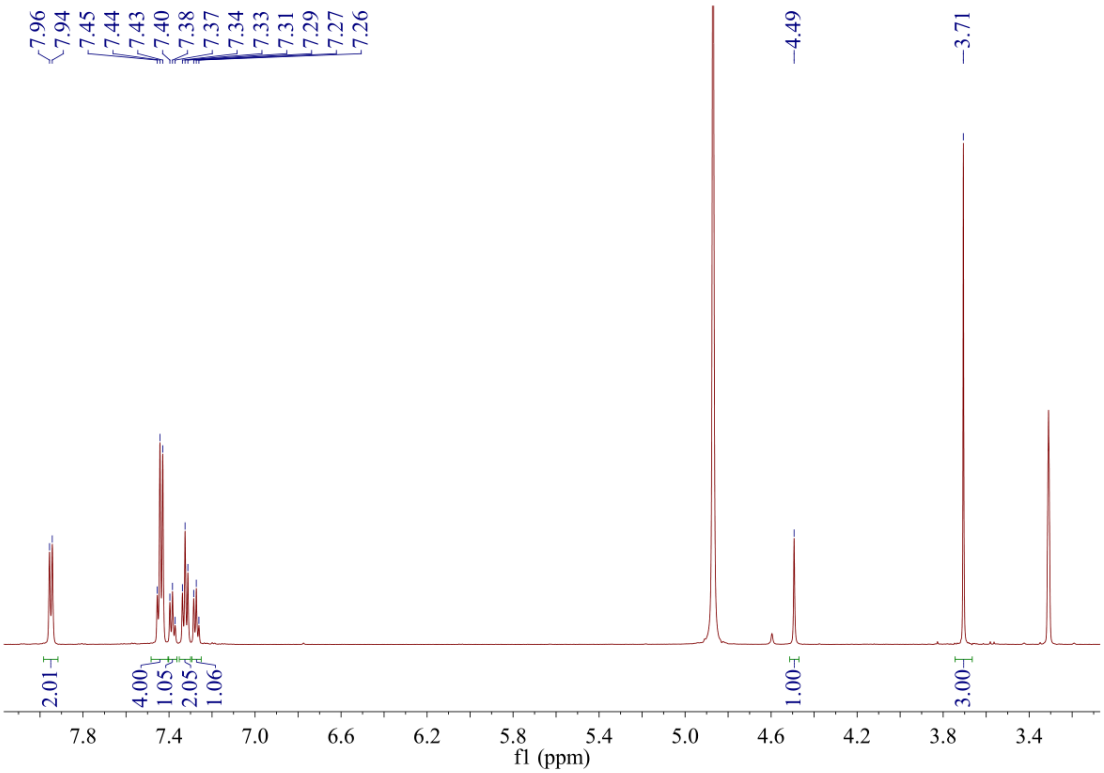


Figure S3: ^{1}H NMR spectrum of compound **1** (600 MHz, CD_3OD).

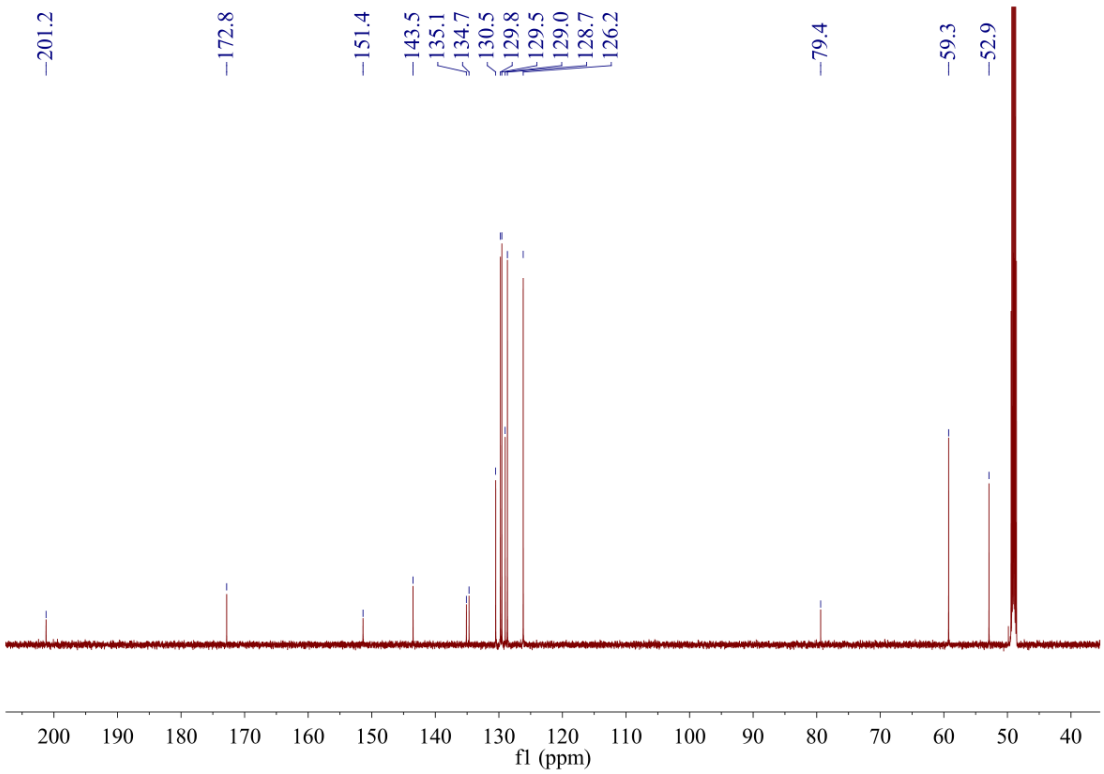


Figure S4: ^{13}C NMR spectrum of compound **1** (150 MHz, CD_3OD).

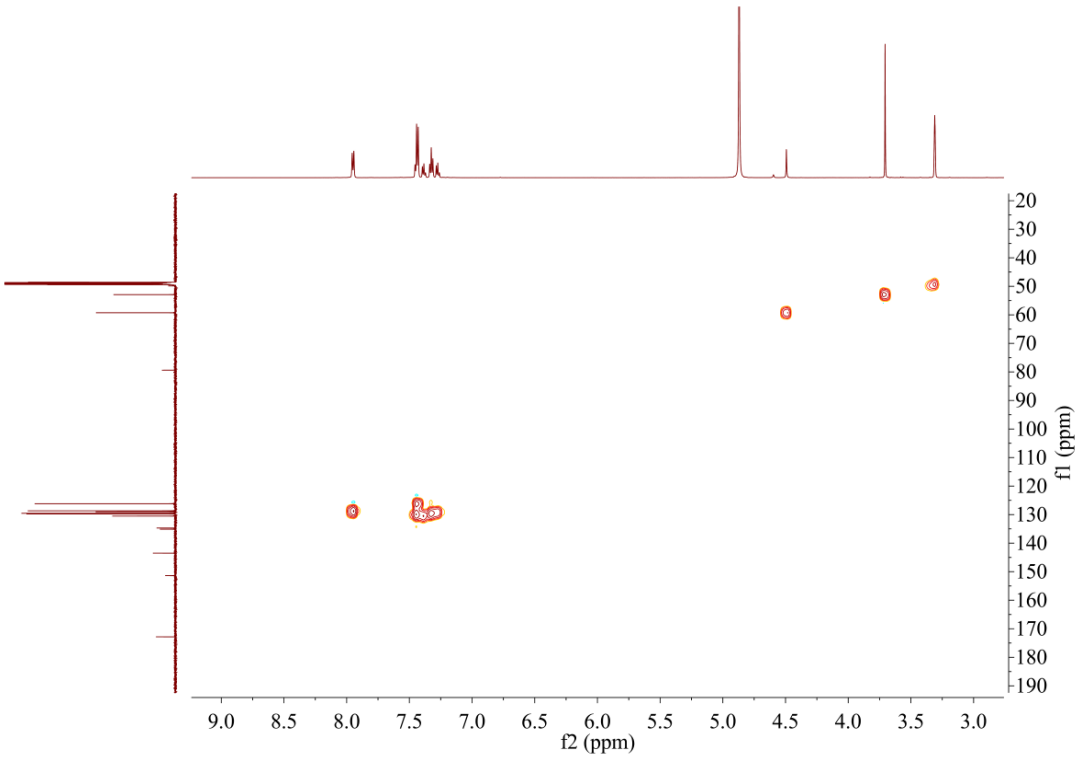


Figure S5: HSQC spectrum of compound **1** (600 MHz, CD_3OD).

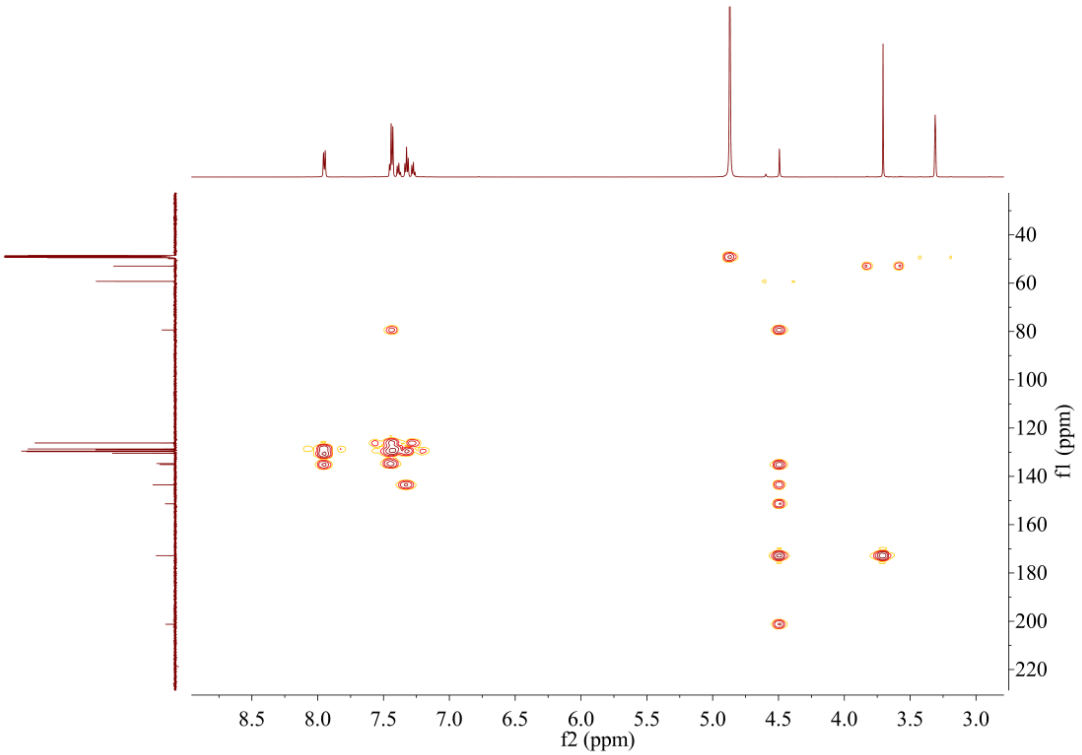
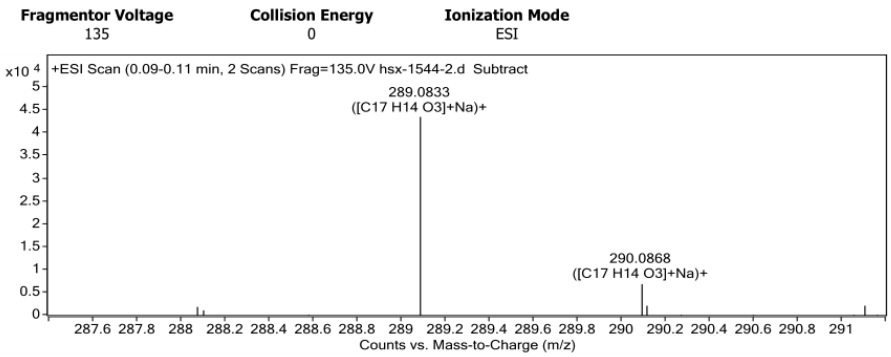


Figure S6: HMBC spectrum of compound **1** (600 MHz, CD_3OD).



Peak List

m/z	z	Abund	Formula	Ion
105.0341	1	30080.09		
221.0956	1	29522.43		
242.2837	1	19240.53		
249.0907	1	122767.83		
250.0938	1	22958.38		
267.1009	1	52670.34		
289.0833	1	43571.73	C17 H14 O3	(M+Na)+
294.1174	1	41010.38		
319.0938	1	20896.53		
437.1934	1	43131.52		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C17 H14 O3	266.0943	289.0835	289.0833	0.20	0.69	11.0000

Figure S7: HRMS–ESI spectrum of compound **2**.

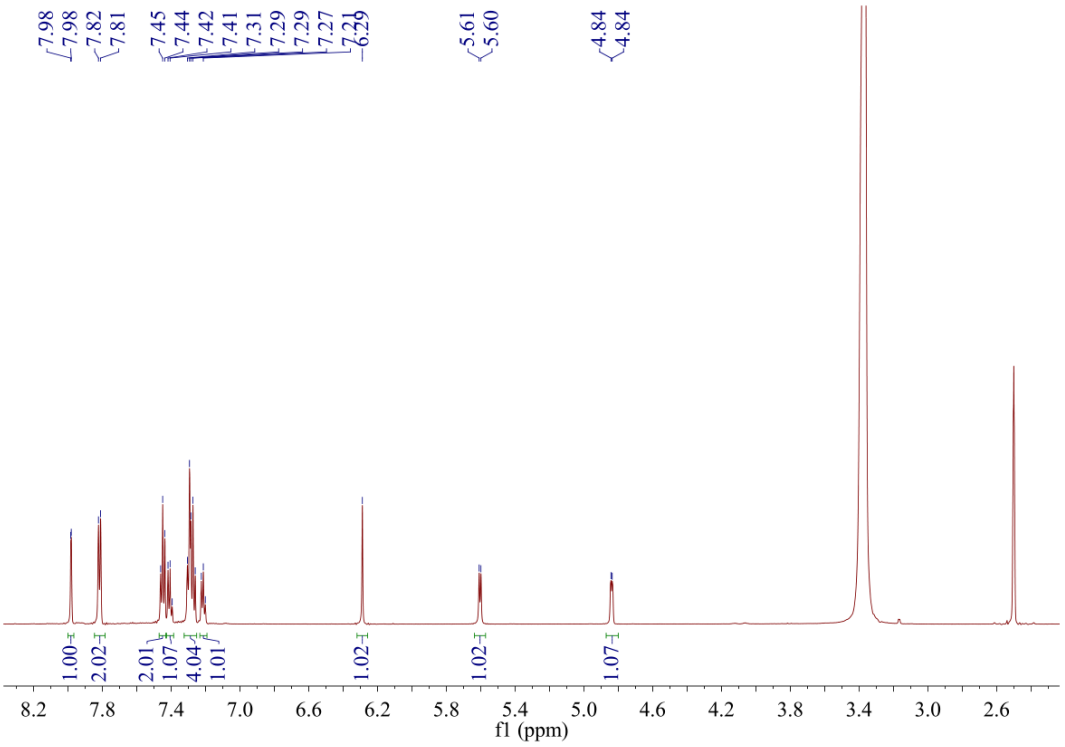


Figure S8: ^{1}H NMR spectrum of compound **2** (600 MHz, DMSO-d_6).

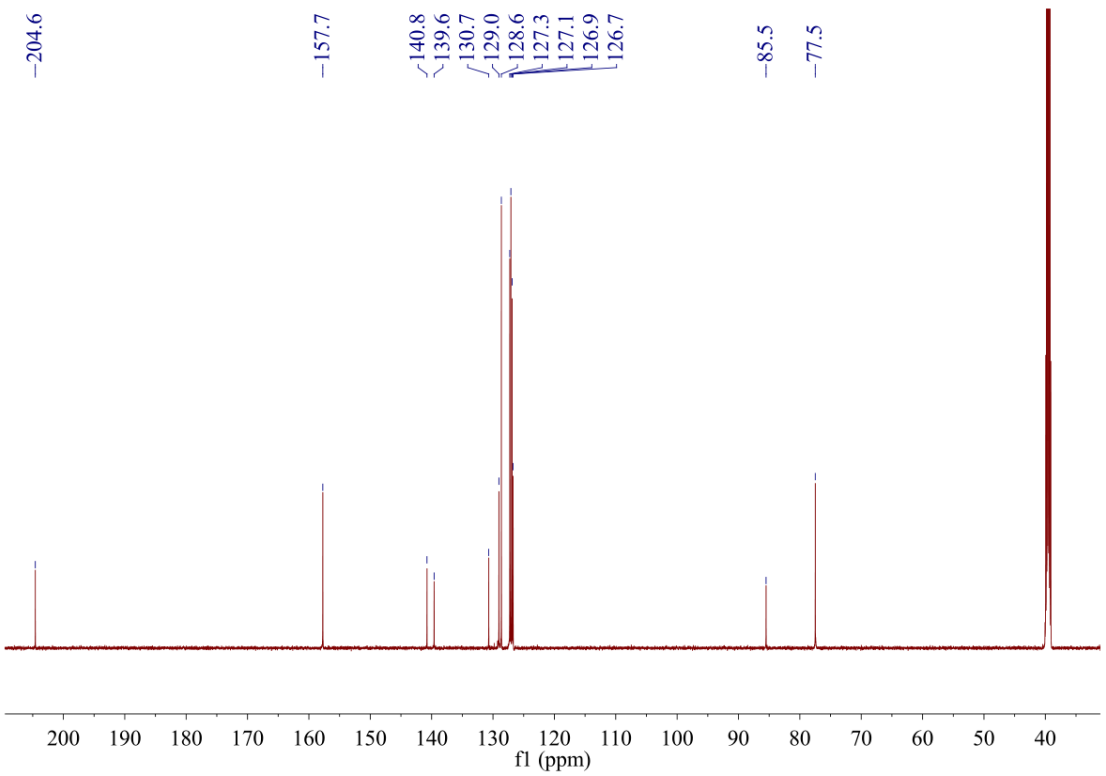


Figure S9: ^{13}C NMR spectrum of compound **2** (150 MHz, DMSO-d_6).

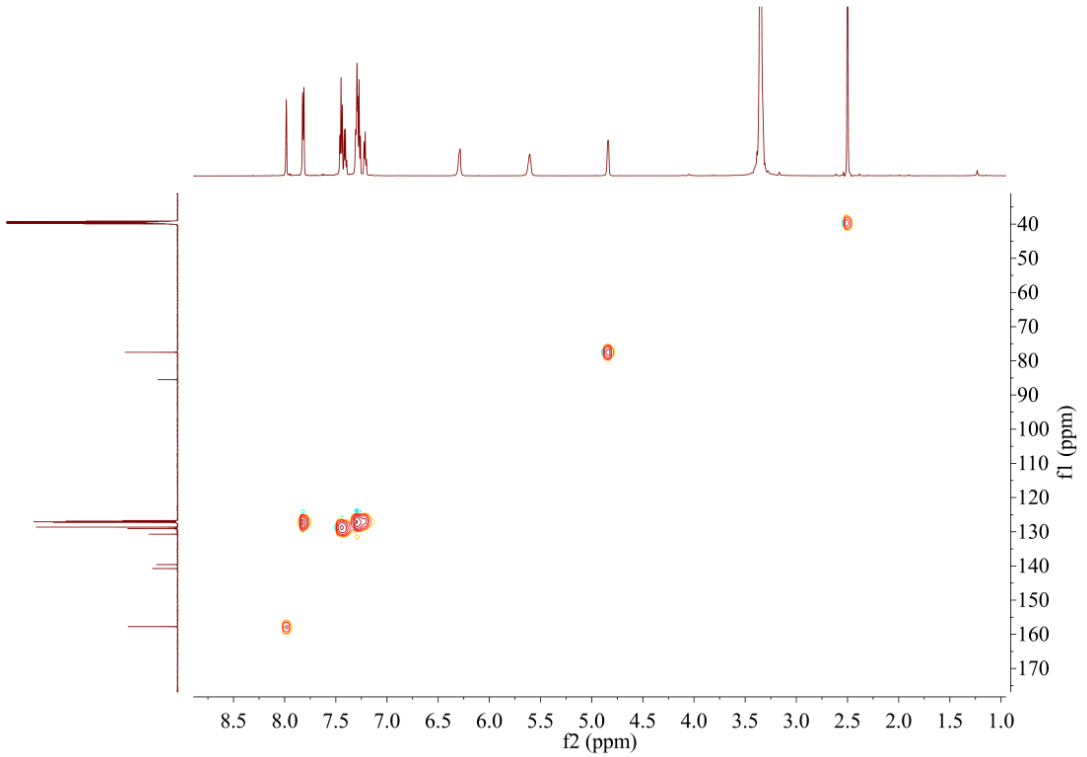


Figure S10: HSQC spectrum of compound **2** (600 MHz, DMSO-d_6).

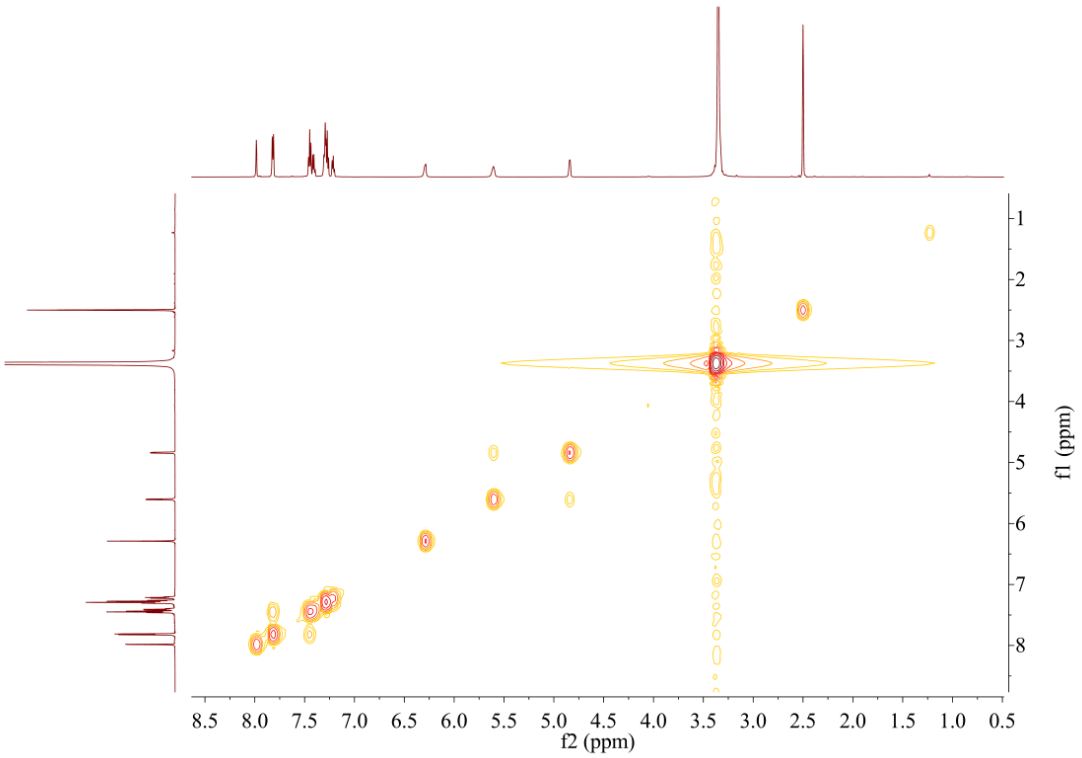


Figure S11: ^{1}H-^{1}H COSY spectrum of compound **2** (600 MHz, DMSO-*d*$_6$).

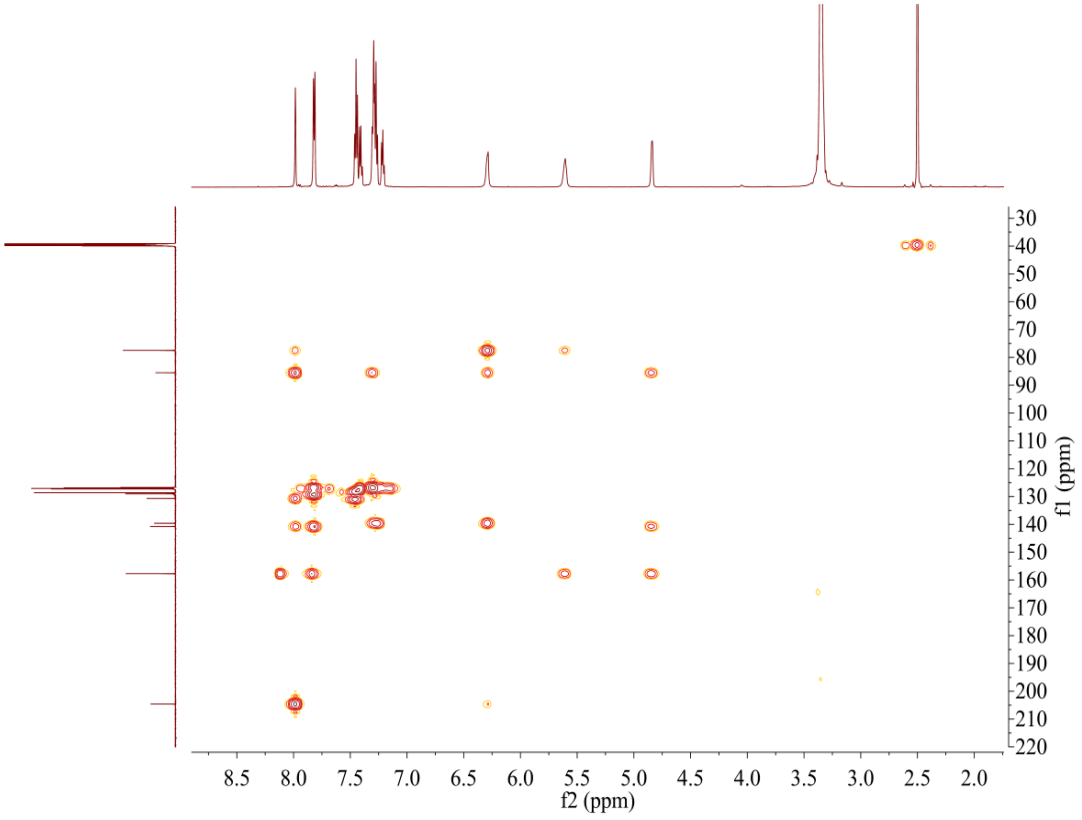


Figure S12: HMBC spectrum of compound **2** (600 MHz, DMSO-*d*$_6$).

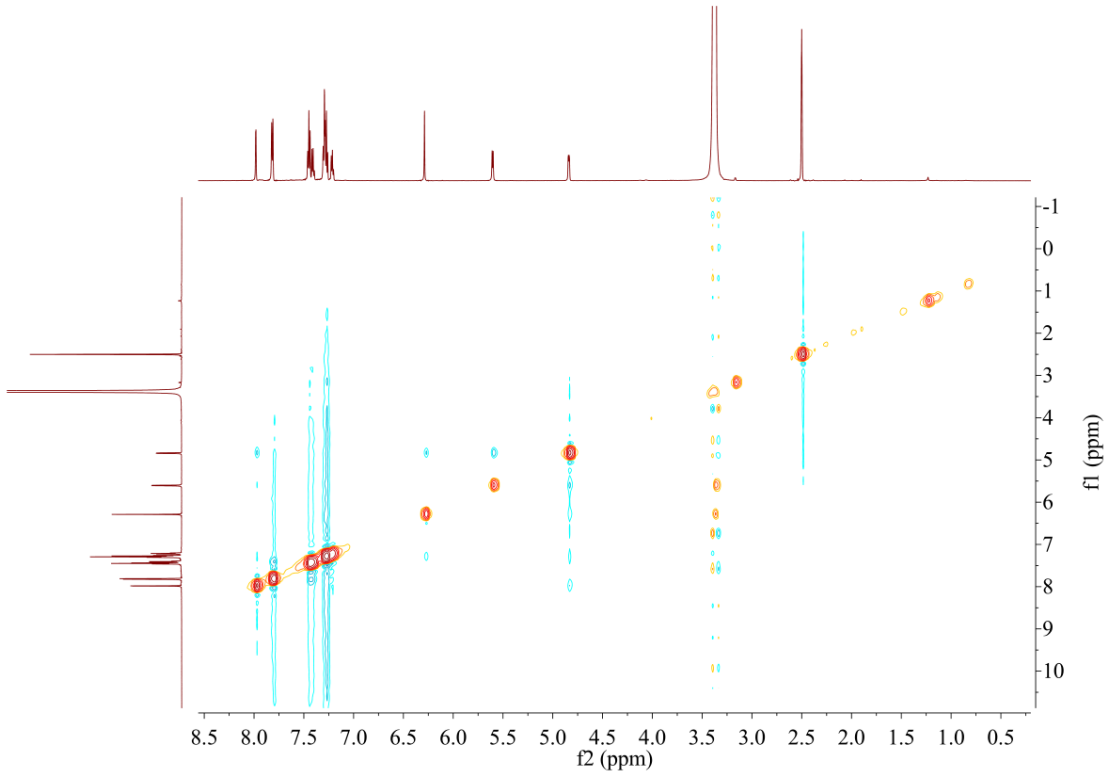


Figure S13: NOESY spectrum of compound **2** (600 MHz, DMSO-d_6).

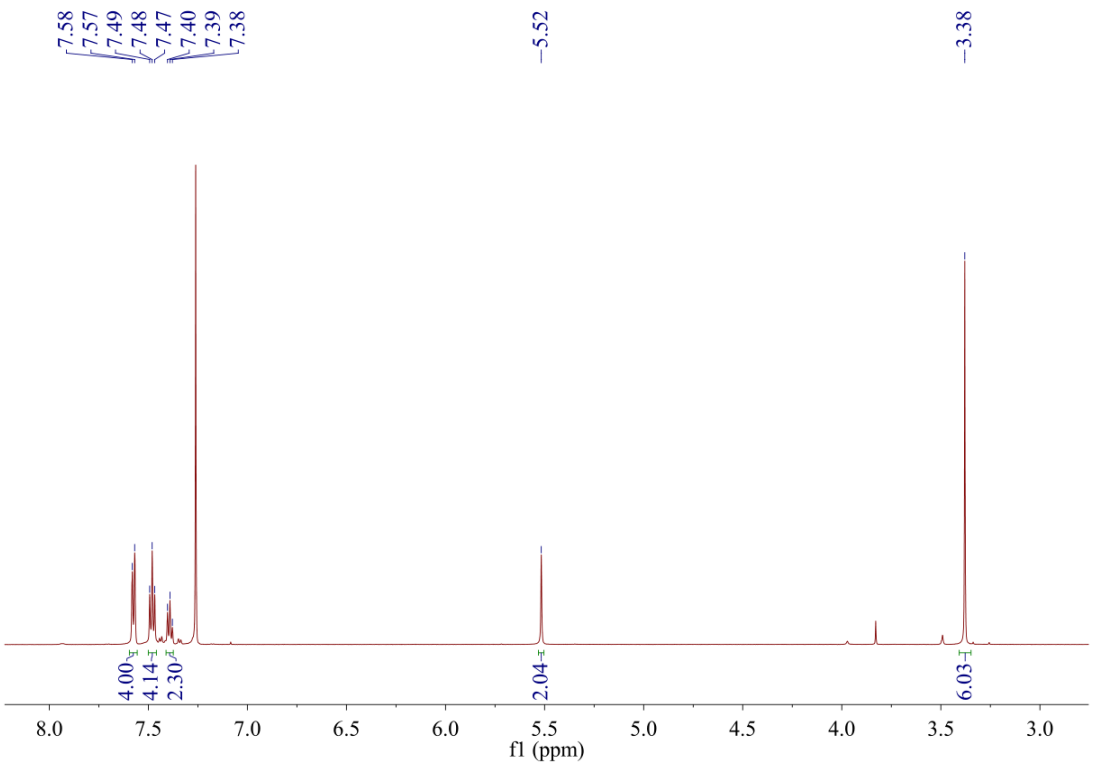


Figure S14: ^{1}H NMR spectrum of compound **3** (600 MHz, $CDCl_3$).

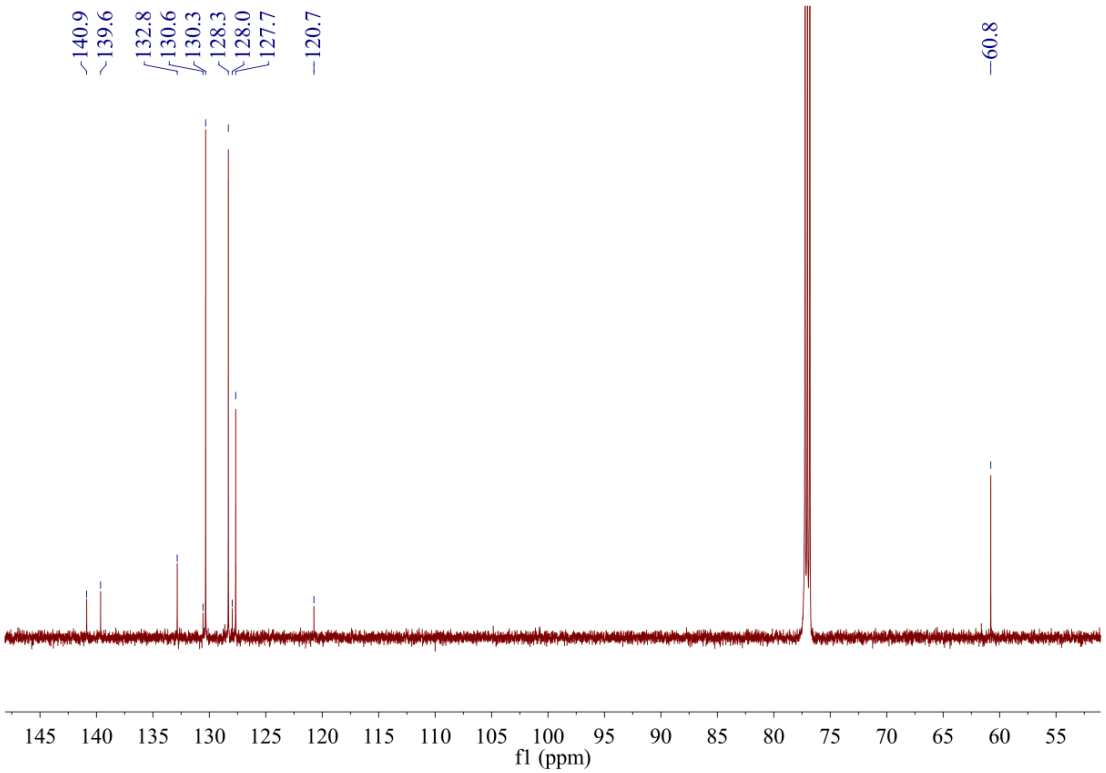


Figure S15: ^{13}C NMR spectrum of compound **3** (150 MHz, $CDCl_3$).

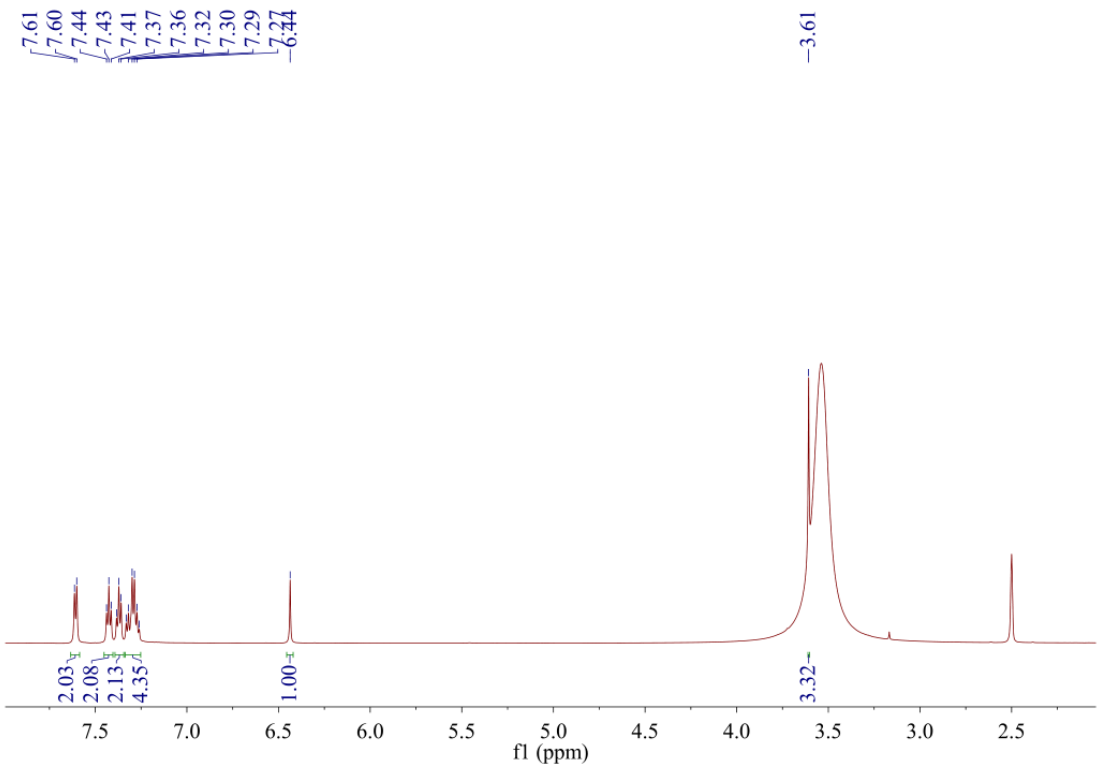


Figure S16: ^{1}H NMR spectrum of compound **4** (600 MHz, DMSO-d_6).

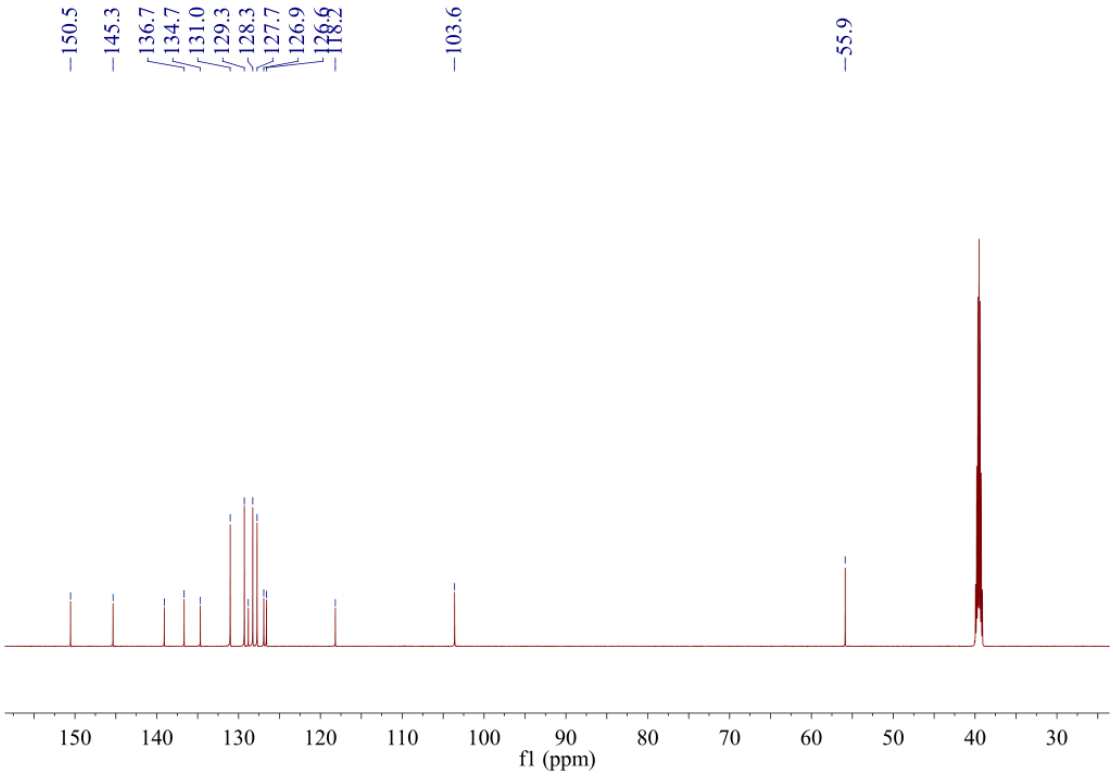


Figure S17: ^{13}C NMR spectrum of compound **4** (150 MHz, DMSO-d_6).

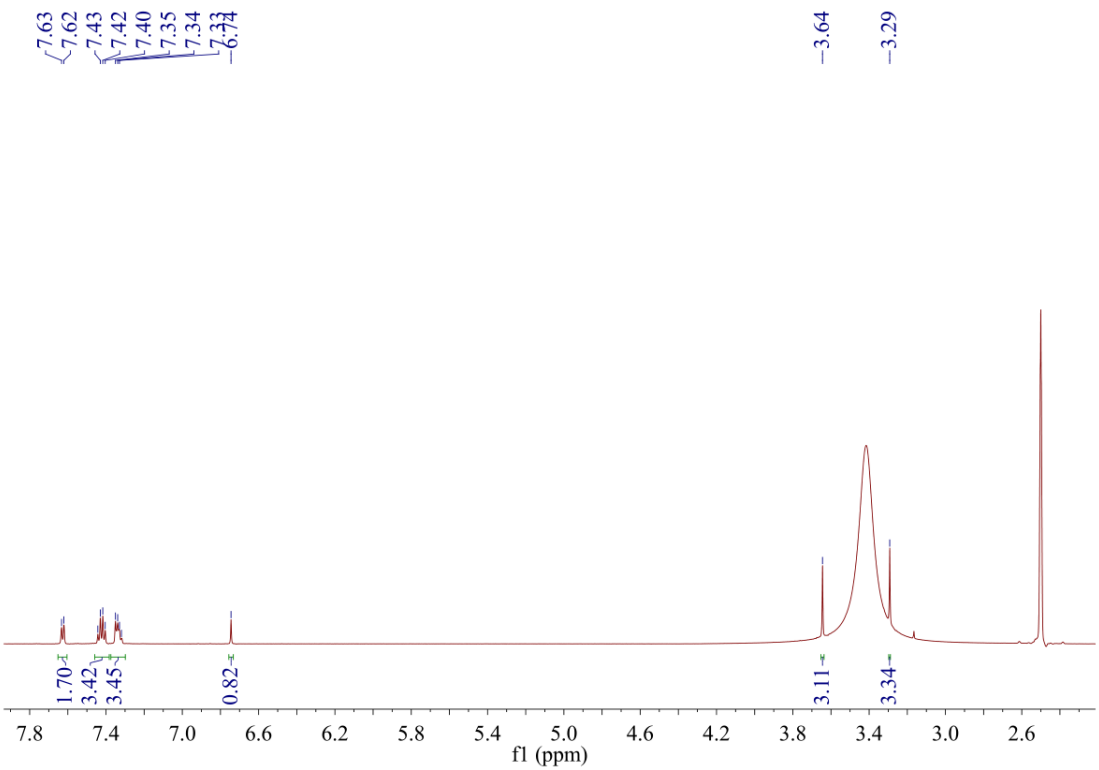


Figure S18: ^{1}H NMR spectrum of compound **5** (600 MHz, DMSO-d_6).

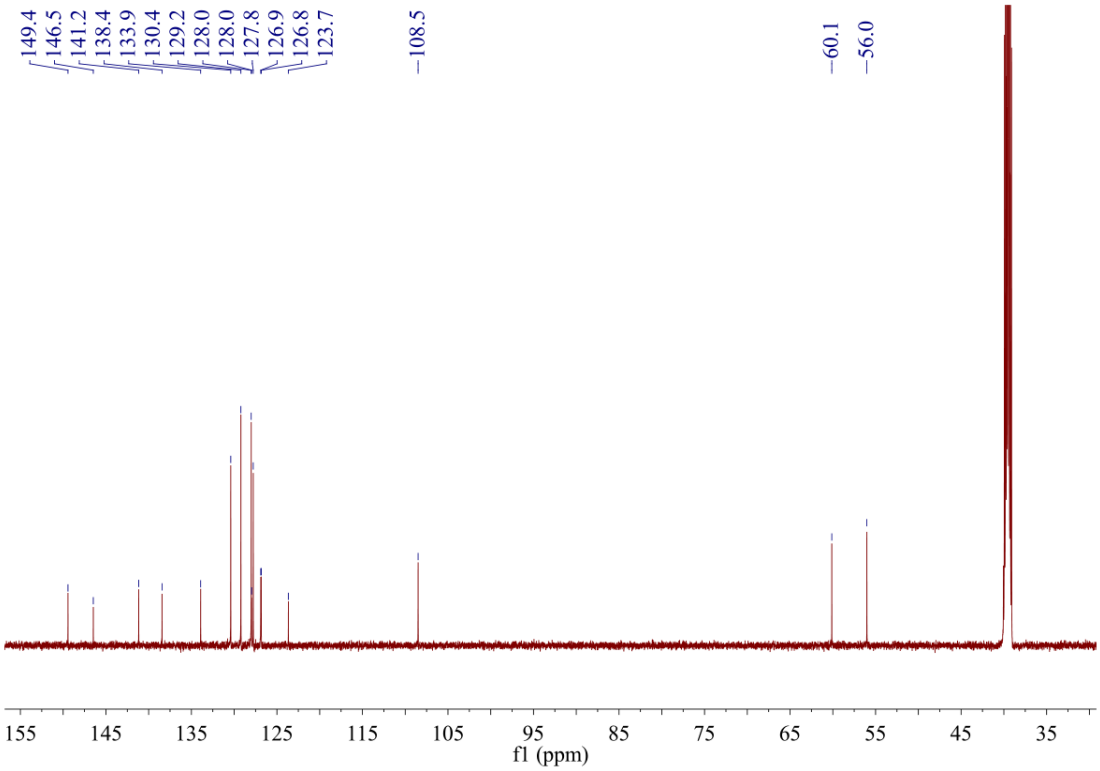


Figure S19: ^{13}C NMR spectrum of compound **5** (150 MHz, DMSO-d_6).

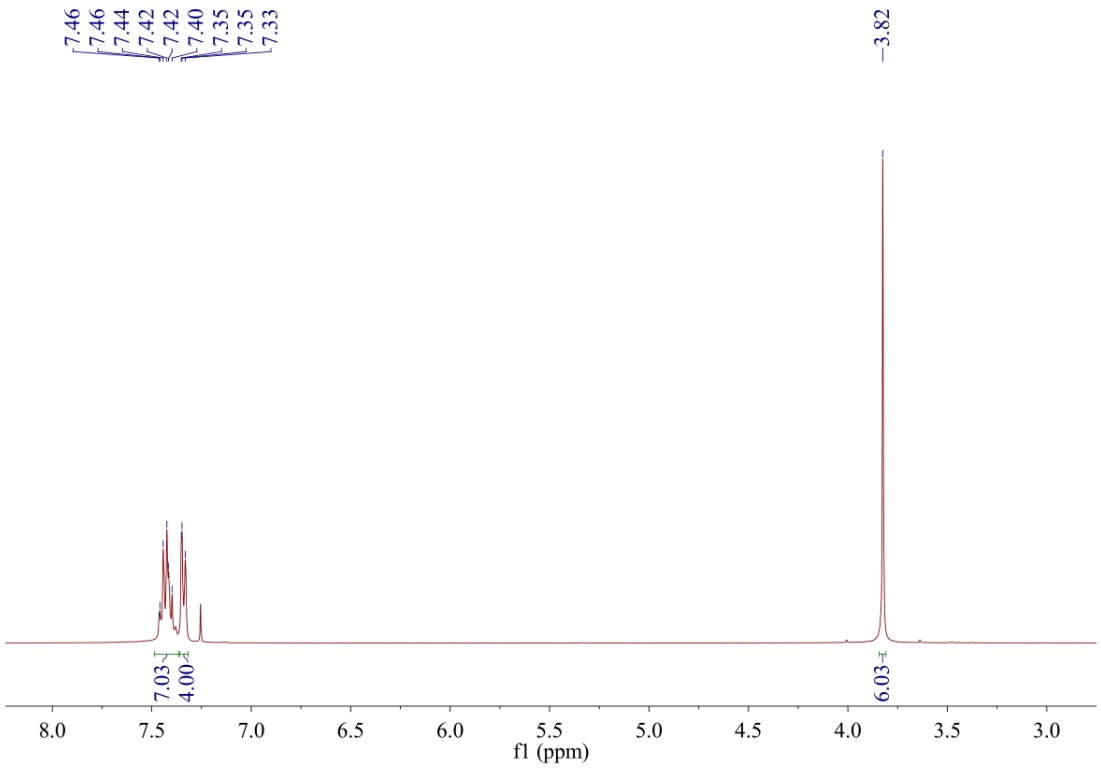


Figure S20: ^{1}H NMR spectrum of compound **6** (600 MHz, $CDCl_3$).

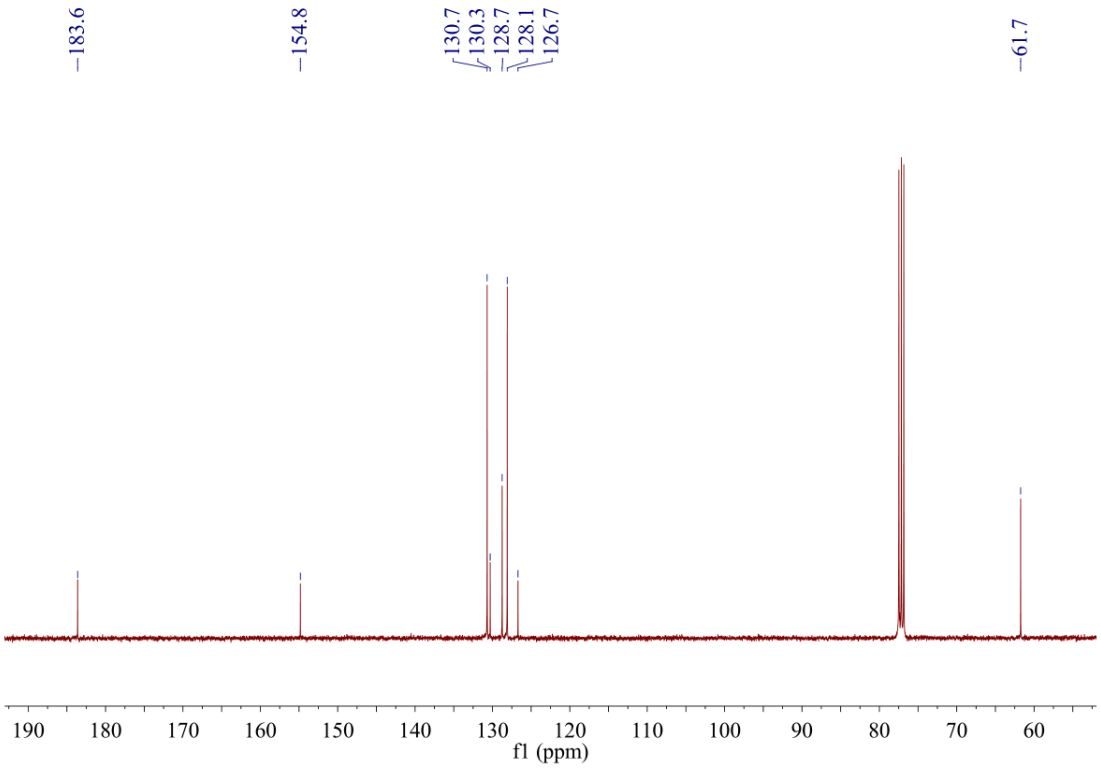
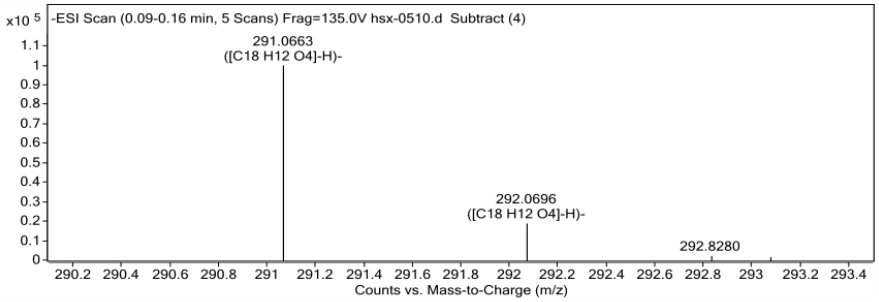


Figure S21: ^{13}C NMR spectrum of compound **6** (150 MHz, $CDCl_3$).

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
91.0555	1	81520.14		
92.928	1	111959.84		
94.9251	1	75297.28		
163.0398	1	93943.83		
268.8008	1	22055.07		
291.0663	1	100265.45	C18 H12 O4	(M-H)-
955.9718	1	569816.38		
956.9743	1	106831.3		
957.9695	1	185085.13		
958.9699	1	32834.93		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C18 H12 O4	292.0736	291.0663	291.0663	0.00	0.00	13.0000

Figure S22: HRMS–ESI spectrum of compound **8**.

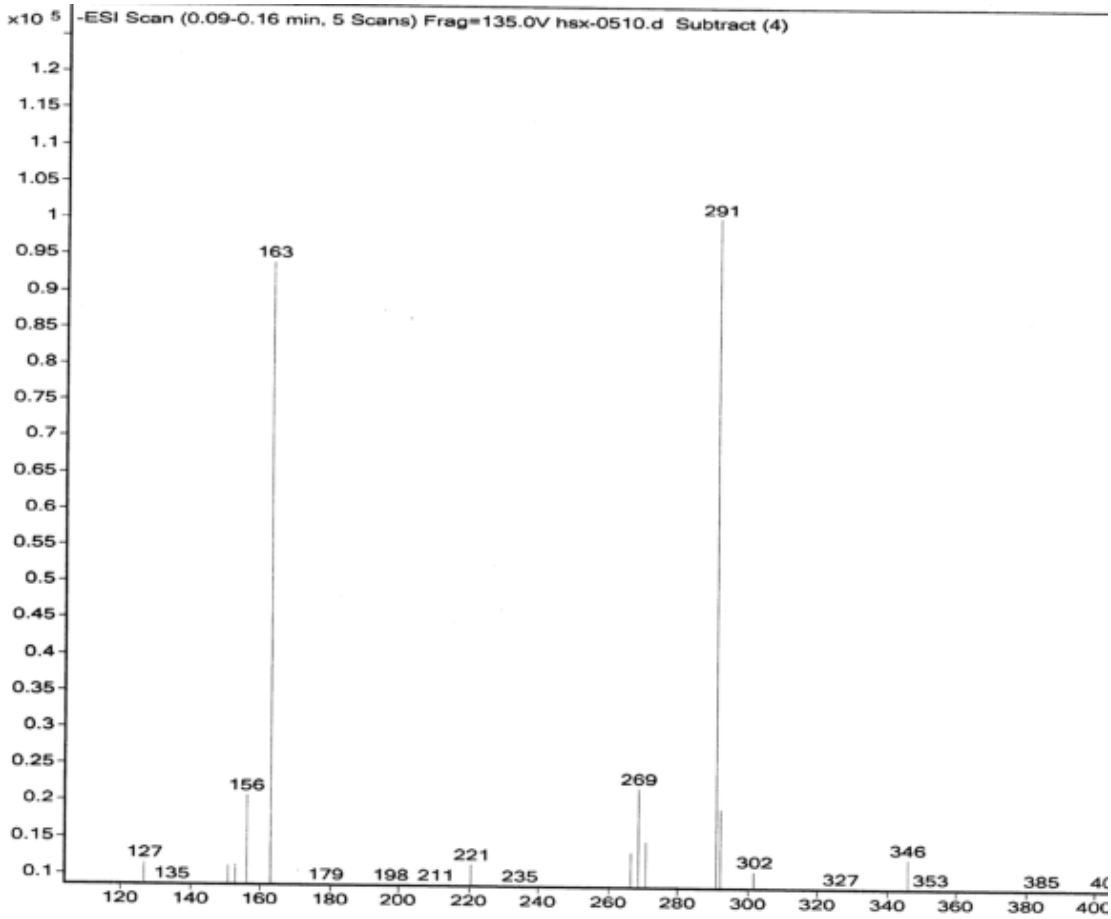


Figure S23: MS analysis of DatA reaction mixture.

Table S1: Annotations of ORFs in the *dat* biosynthetic gene cluster.

ORF	bp/AA	Proposed function	Identity (%)	Accession
datA	2763/920	peptide synthetase	95	KKZ69024.1
datB	927/308	dehydratase	96	KKZ69023.1
datC	405/134	limonene-1,2-epoxide hydrolase	98	KKZ69022.1
Orf1	897/298	hypothetical protein VO63_36430	91	KKZ69021.1
Orf2	681/226	ACP phosphodiesterase	95	KKZ72150.1
Orf3	1368/455	phospho-2-dehydro-3-deoxyheptonate aldolase	96	KKZ72151.1
Orf4	1194/397	chorismate synthase	91	WP_028797313.1
Orf5	315/104	chorismate mutase	84	KKZ72152.1
datD	1443/480	bilirubin oxidase	81	KKZ72238.1
Orf6	366/121	HxlR family transcriptional regulator	99	KKZ72153.1
Orf7	468/155	hypothetical protein VO63_19350	93	KKZ72154.1
datE	930/309	NADPH-dependent oxidoreductase	95	KKZ72155.1

Table S2: Strains and plasmids used and generated in this study.

Stains/Plasmids	Characteristics	Source
Streptomyces		
S. sp. KIB-H1544	Native producer	Isolated
KIB-H1544-Δ*datA*	*datA* inactivation mutant of *S*. sp. KIB-H1544	This study
Escherichia coli		
DH5*α*	Host strain for cloning	Invitrogen
BL21(DE3)	Host strain for expression of protein	Laboratory stock
BW25113/pIJ790	Host strain for PCR targeting	Laboratory stock
ET12567/pUZ8002	Donor strain for conjugation	Laboratory stock
XL1-blue MR	Host strain for genomic library	Agilent Technologies
Plasmids		
pIJ773	*Apr*^{r}, Plasmid containing the apramycin resistance gene	Laboratory stock
pSuperCos I-21A4	*Kan*^{r}, Cosmid containing *dat* gene cluster used for knockout	This study
p21A4-*ΔdatA*	*Apr*^{r}, gene inactivation clone used for mutant *ΔdatA*	This study
pETDuet-sfp	*Amp*^{r}, plasmid for the expression of protein	Laboratory stock
pETDuet-sfp-DatA	*Amp*^{r}, plasmid for the expression of *datA*	This study
pETDuet-sfp-EchA	*Amp*^{r}, plasmid for the expression of *echA*	This study

Abbreviations: *Kan*^{r}, kanamycin resistance; *Apr*^{r}, apramycin resistance; *Amp*^{r}, ampicillin resistance.

Table S3: Primers used in this study.

Target gene	Primer names	Sequences
Primers for screening libraries		
1544SL-1	1544SL-1F	TTCCTGCGACTGGTCTCCCG
	1544SL-1R	CGCCGTTGACGATGATGCTG
1544SL-2	1544SL-2F	CGAAGAACAACGAGGTGTG
	1544SL-2R	CGCTTGAAGTAGAGCATCG
Primer pairs used for mutant producer strain construction		
ΔdatA	datA-F	ATGACCGTGGCCATCCCATCGACCCGGACCGTCGCGGACATTCCGGGGATCCGTCGACC
	datA-R	CCGACATGCCGCGGACGTGGCCGGACGGGGAGTACGTGCTGTAGGCTGGAGCTGCTTC
Primer pairs used for PCR verification of the double cross-over mutant		
ΔdatA	datA-F-YZ	TACTACGCCGAGAGCGACG
	datA-R-YZ	CACCTCGTTGTTCTTCGCG
Primer pairs used for protein expression		
DatA	Duet-DatA-F	cgcggatccgATGACCGTGGCCATCCCAT
	Duet-DatA-R	cgagctcTACTTGCCGTCGCCGAGCG
EchA	Duet-EchA-F	cgcggatccgATGACGTCACAACCCTGGA
	Duet-EchA-R	cgagctcCTATGCCCGGCCCGTGGCC