Supporting information:

Characterization of Yatakemycin Gene Cluster Revealing A Radical S-adenosylmethionine Dependent Methyltransferase and Highlighting Spirocyclopropane Biosynthesis

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Table S1. PCR primers used in this study

Primers	Sequence	Enzyme
YTM-G-For	5'-CGCAGCCCCTGTCTGGCGGATCTGC-3'	
YTM-G-Rev	5'-GCGCACAGCCGTTCACCCATCAGCG-3'	
YTM-L-For	5'-AACTCCATCAGCTCCGAGCCCTTCG-3'	
YTM-L-Rev	5'-TCGGCGAGTTGGTCCACGAGATGGG-3'	
YTM-N-For	5'-ACGCCGAATCCCGCGACACACGTGG-3'	
YTM-N-Rev	5'-AGGCCGGTACCCGGCACGCTCAGGG-3'	
YTM-GD1-For	5'-TAAAAGCTTCTGGATGAGGACGAAGAACAGCTGG-3'	*Hin*dIII
YTM-GD1-Rev	5'-TAATCTAGACATGATGACCTCGTACCTCAGCCC-3'	*Xba*I
YTM-GD2-For	5'-TAATCTAGAGCGTACCTCGGCAAGGACAAGCACG-3'	*Xba*I
YTM-GD2-Rev	5'-TAAGAATTCCACGTCGCTGTCCGTGATGAGCAGC-3'	*Eco*RI
ytm-ed-For	5'-TCCGCACCGCATGGCCGAACTGTCGGCCGGTTCAGC ATGATTCCGGGGATCCGTCGACC-3'	
ytm-ed-Rev	5'-GCGCAGCAGGGCCAGCCGGCGGGTCGTCAGTTCGTC TCATGTAGGCTGGAGCTGCTTC-3'	
ytm-jd-For	5'-GAAGGGGCCGCGGACGTCCTGTCCACCGCGGTCCAC ATGATTCCGGGGATCCGTCGACC-3'	
ytm-jd-Rev	5'-CTGGACCGGGAAGGCCTCCAGGTATCCCTCGATGTCT CATGTAGGCTGGAGCTGCTTC-3'	
orf(+1)d-For	5'-CCGTGCAGCCGTGAGCGAGGAACCCGACGTCACCGA ATGATTCCGGGGATCCGTCGACC-3'	
orf(+1)d-Rev	5'-CCTGAACTGCACCGCCGCCCGCGTCACTTCCGCCCCT CATGTAGGCTGGAGCTGCTTC-3'	
orf(+2)d-For	5'-GACGACCTTGTTGCAACGGTCCTCCCGGTTCTTCGAA	

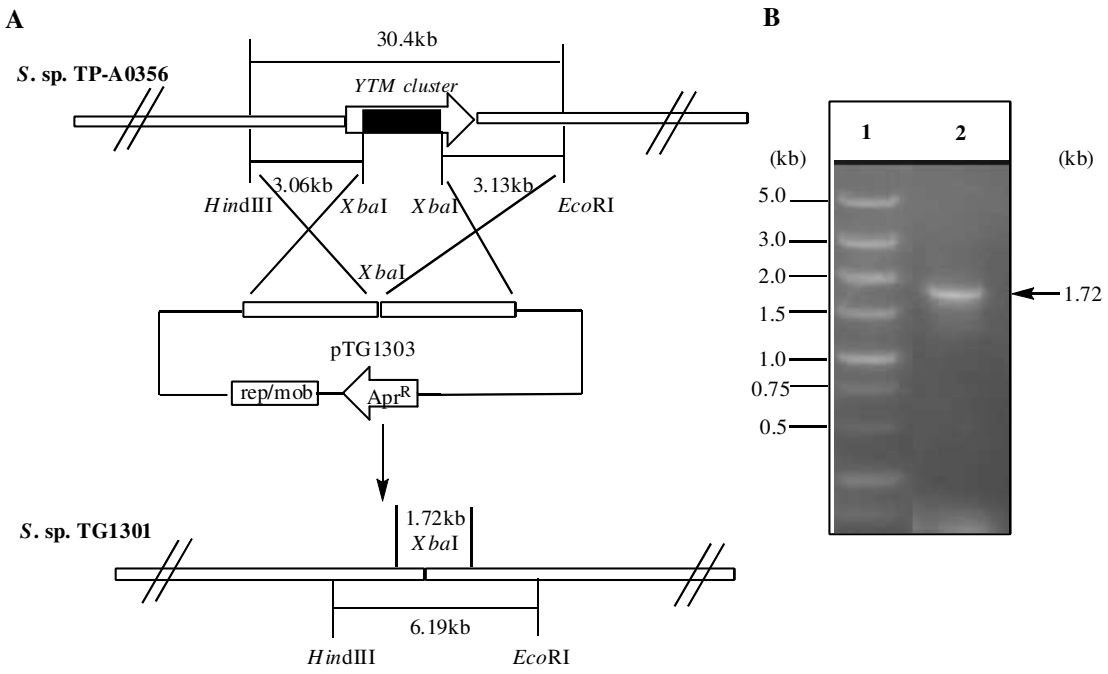
	TGATTCCGGGGATCCGTCGACC-3’	
orf(+2)d-Rev	5’-CGGCGGCGGGAACGCCGCGTCCAGGACGGTGAGTAC TCATGTAGGCTGGAGCTGCTTC-3’	
ytm-td-For	5’-GCCAAGGAGACCATCACCCGCAACTTCCTGGACACG ATGATTCCGGGGATCCGTCGACC-3’	
ytm-td-Rev	5’-CTCGGGCCTGACCTGCCAGTTGAGGATCTCGGTGCTT CATGTAGGCTGGAGCTGCTTC	
ytm-E-For	5’-TCCGCACCGCATGGCCGAACTG-3’	
ytm-E-Rev	5’-GCGCAGCAGGGCCAGCCGGCG-3’	
ytm-T-For	5’-GCCAAGGAGACCATCACCCGC-3’	
ytm-T-Rev	5’-CTCGGGCCTGACCTGCCAGTTG-3’	
ytm-J-For	5’-GAAGGGGCCGCGGACGTCCTG-3’	
ytm-J-Rev	5’-GACATCGAGGGATACCTGGAG-3’	
orf(+1)-For	5’-GTGAGCGAGGAACCCGACG-3’	
orf(+1)-Rev	5’-GCACCGCCGCCCGCGTCAC-3’	
orf(+2)-For	5’-GACGACCTTGTTGCAACGGTC-3’	
orf(+2)-Rev	5’-GAACGCCGCGTCCAGGACGG-3’	
YTM-tC-For	5’-TAAGAATTCCTGCTGCGGACCGAACGCCTGC-3’	*Eco*RI
YTM-tC-Rev	5’-TAACATATGCCGGTGACGCGTTCGGCGAGG-3’	*Nde*I
YtkT-For	5’- AAAAGGATCCACATATGACCGAAGCGCCCAAC	*Bam*HI
YtkT-Rev	5’- AAAGAATTCTCAAAGCTTGACAATGTTCAGCAACCG	*Eco*RI

Table S2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for YTM-T in pyridine-d_5[a]

Position	^{13}C	^{1}H
1-NH	-	13.7, s
2	133.8, s	-
3	108.3, d	7.56, d (2.0)
3a	119.0, s	-
3b	122.4, s	-
4	28.7, t	3.28, t (8.0)
5	54.4, t	4.70, t (8.0)
6a	129.6, s	-
7	141.1, s	-
8	135.0, s	-
8a	136.3, s	-
2-*CO*SCH$_3$	183.9, s	-
2-COS*CH$_3$*	11.6, q	2.53, s
7-OH	-	12.0, s
8OCH$_3$	60.9, q	4.07, s
1'-NH	-	13.4, s
2'	130.6, s	-
3'	107.8, d	7.72, d (2.0)
3'a	122.4, s	-
3'b	117.4, s	-
4'a	107.7, d	7.34, d (3.5)
5'	125.6, d	8.14, d (3.5)
6'a	132.8, s	-
7'	100.0, d	8.74, s
8'	144.5, s	-
8'a	128.0, s	-
2'-CO	162.4, s	-
1''-NH	-	13.1, s
2''	129.6, s	-
3''	111.2, d	7.36, d (2.0)
3''a	122.8, s	-
4''	95.0, d	7.28, s
5''	145.5, s	-
6''	151.2, s	-
7''	107.1, s	7.67, s
7''a	134.3, s	-
2''-CO	162.4, s	-
6''-OCH$_3$	56.4, q	3.88, s

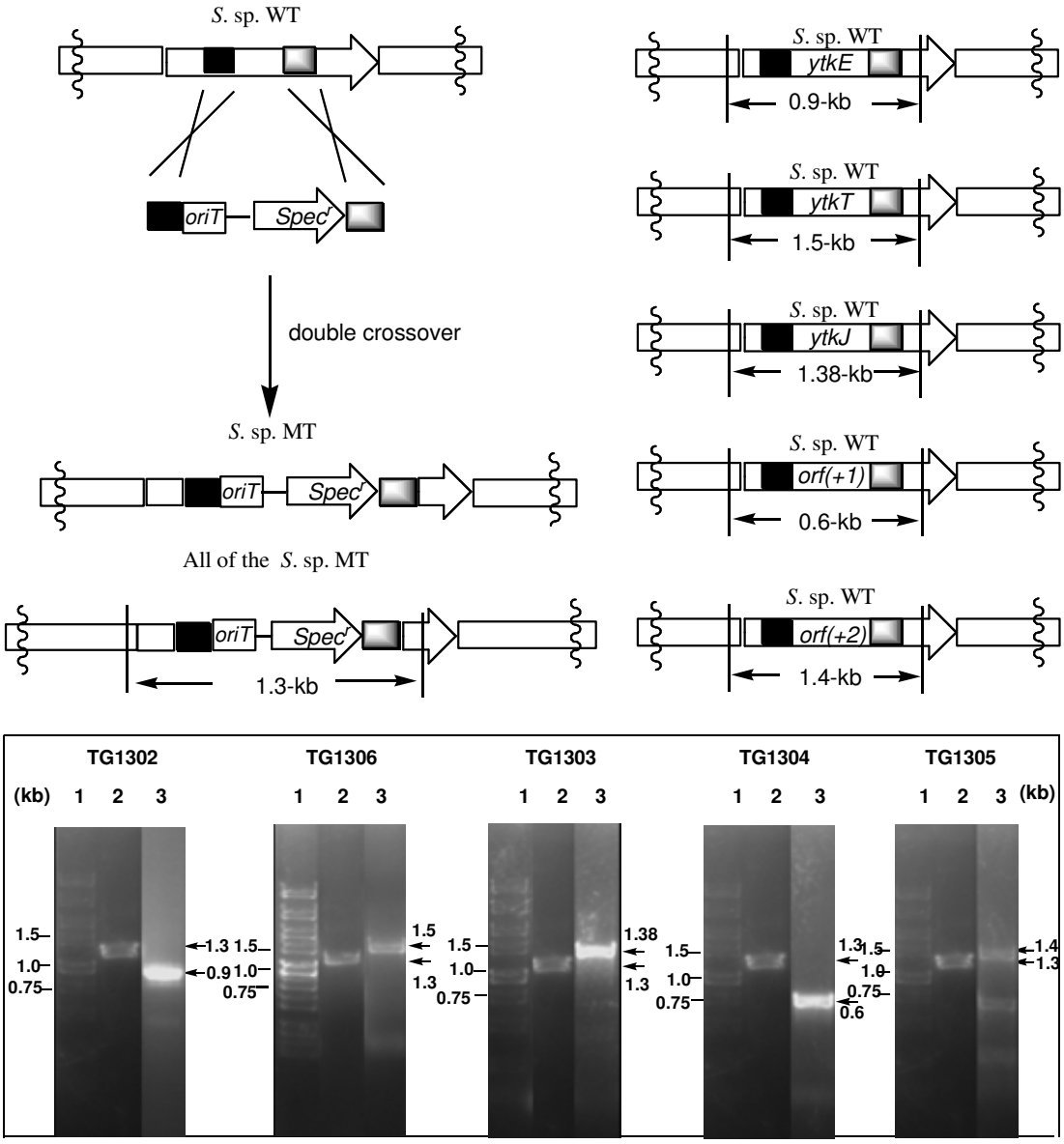
[a]Signals were assigned with the aid of ^{1}H-^{1}H COSY, HMQC and HMBC experiments.

Figure S1. Construction of YTM gene cluster deletion mutant strain *Streptomyces* sp TG1301 *via* homologous recombination.



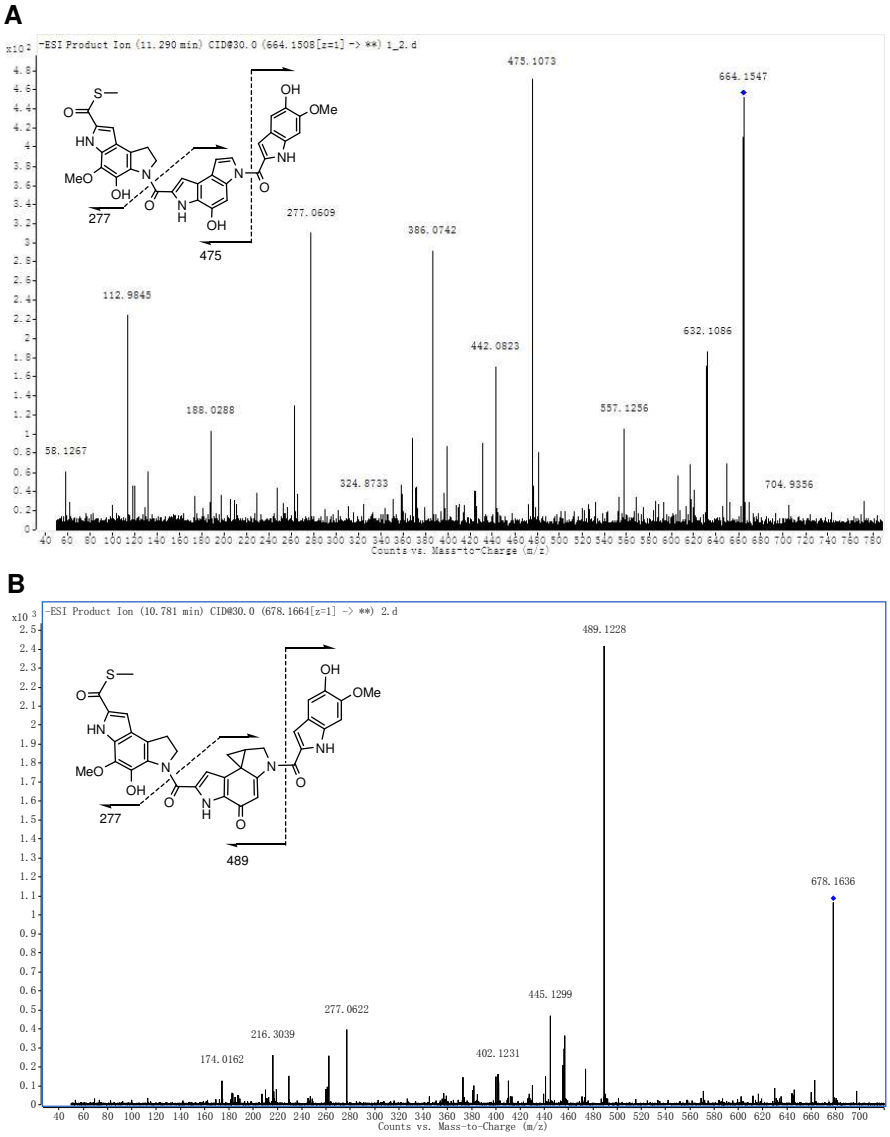
(A) A 30.4-kb gene fragment in wild type strain was shifted to 6.19-kb in TG1301 mutant strain, as would be predicted for the deletion of a 24.2-kb fragment of YTM gene cluster. (B) Genomic DNA from TG1301 mutant strain was used as template. PCR analysis of the genotype using ACTGACTTCATGGTGCAGGC and CTCGTACGGCCCCACGTACAG as primers. Lane 1, DNA marker; lane 2, *Streptomyces* mutant strain TG1301.

Figure S2. Inactivation of *ytkE*, *ytkT*, *ytkJ*, *orf(+1)*, *orf(+2)* in strain *Streptomyces* sp TP-A0356 *via* homologous recombination.



Genomic DNA from both the *Streptomyces* sp TP-A0356 wild type and mutant strains *S.* sp. TG1302 (Δ*ytkE*), *S.* sp. TG1306 (Δ*ytkT*), *S.* sp. TG1303 (Δ*ytkJ*), *S.* sp. TG1304 (Δ*orf+1*) and *S.* sp. TG1305 (Δ*orf+2*) were extracted, tested by PCR analysis, respectively. A 1.3-kb (spectinomycin resistance gene fragment) band can be amplified from all of the mutants with specific primers (Table S1) and wild type as control (for *ytkE*, *ytkT*, *ytkJ*, *orf+1*, *orf+2* gene, the corresponding PCR products are 0.9-kb, 1.5-kb, 1.38-kb, 0.6-kb, and 1.4-kb, respectively). Lane 1 DNA marker; lane 2, *Streptomyces* mutant strain; lane 3, *Streptomyces* wild type strain.

Figure S3. Structure elucidation of YTM-T from recombinant *Streptomyces* sp. TG1306 by MS/MS analysis.



(A) YTM-T. (B) YTM as control.

Figure S4. ^{1}H NMR (500 MHz) spectrum of the new compound **8**.

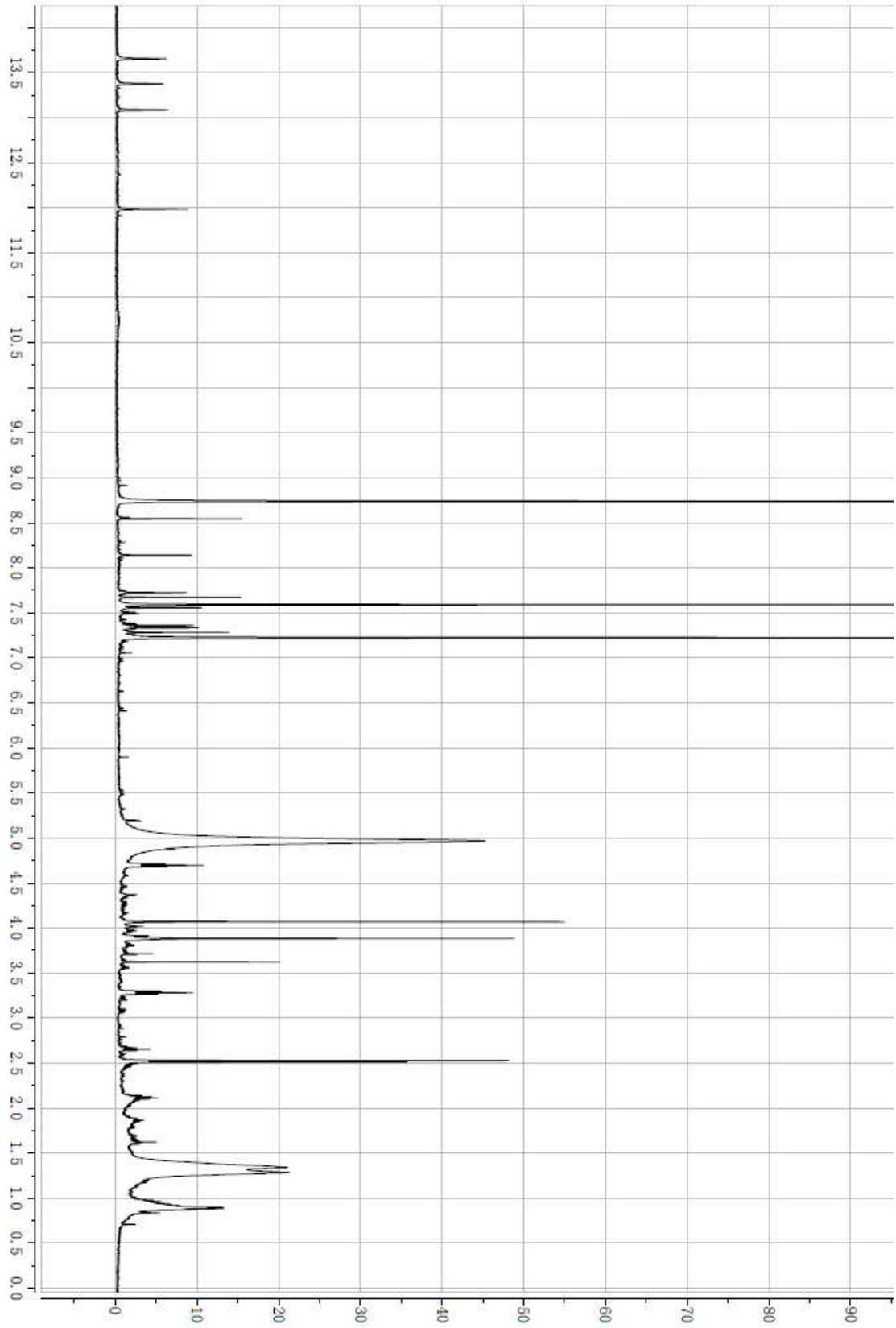


Figure S5. ^{13}C NMR (125 MHz) spectrum of the new compound **8**.

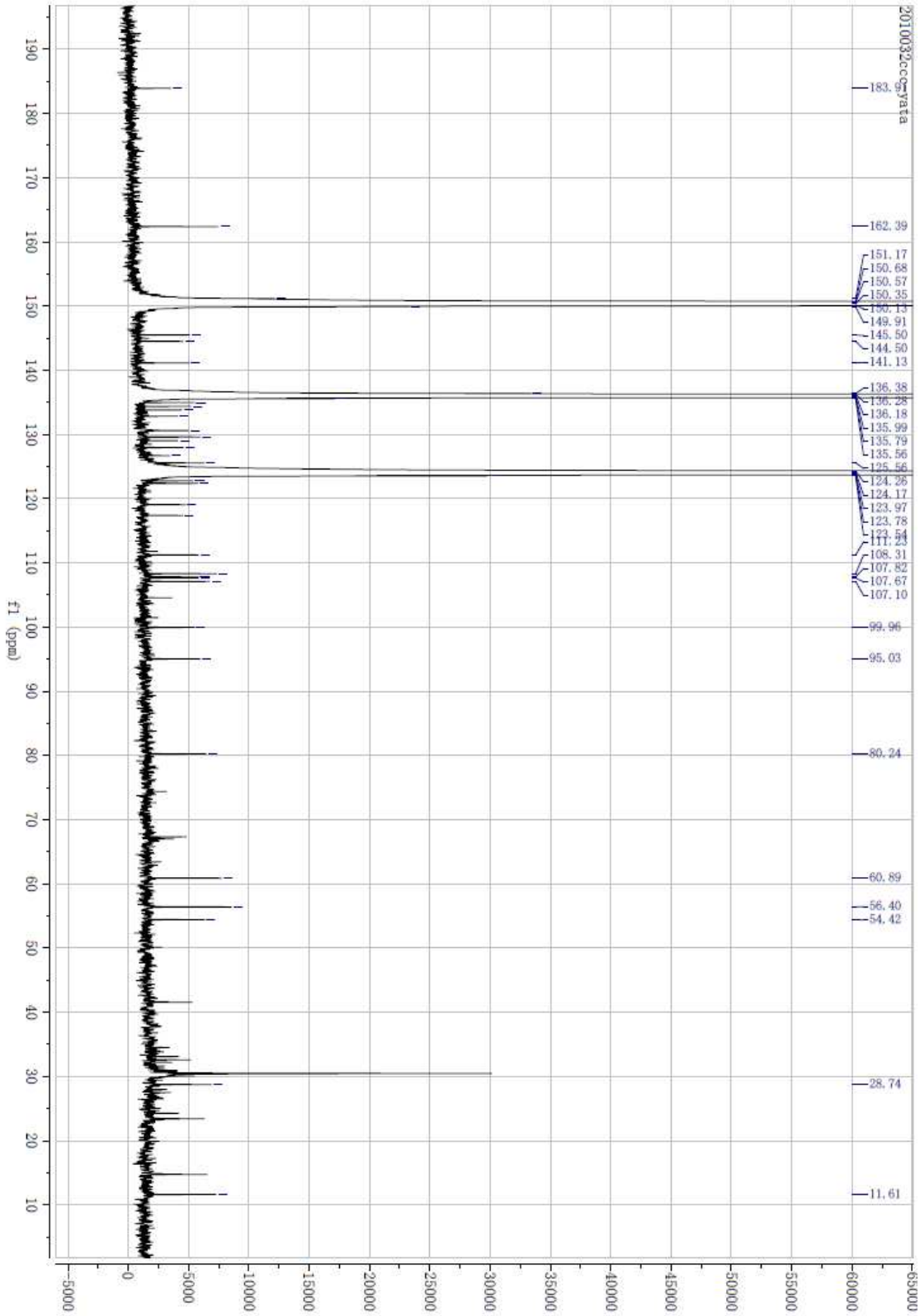


Figure S6. DEPT 135 (400 MHz) spectrum of the new compound **8**.

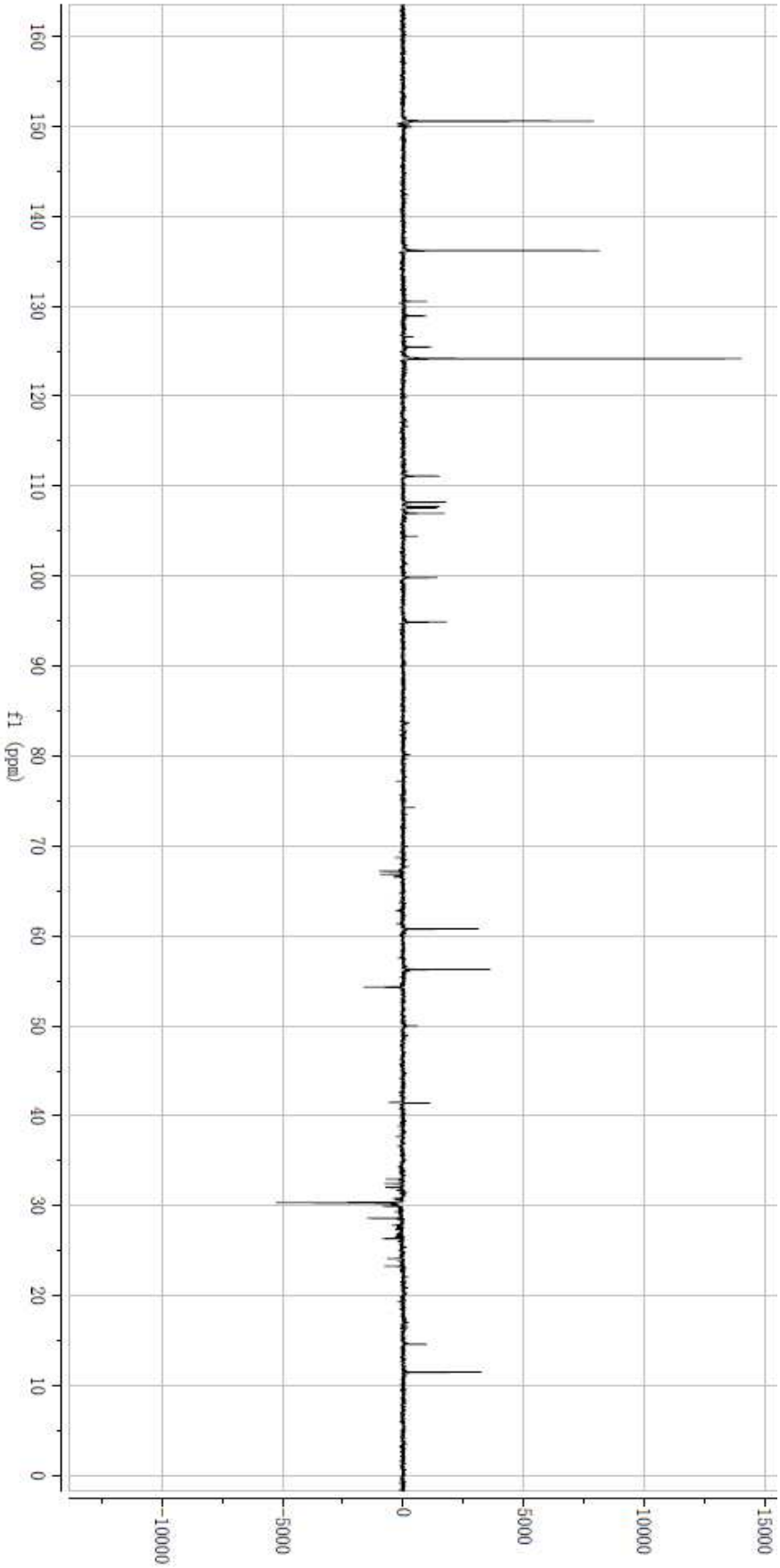


Figure S7. HMQC (500 MHz) spectrum of the new compound **8**.

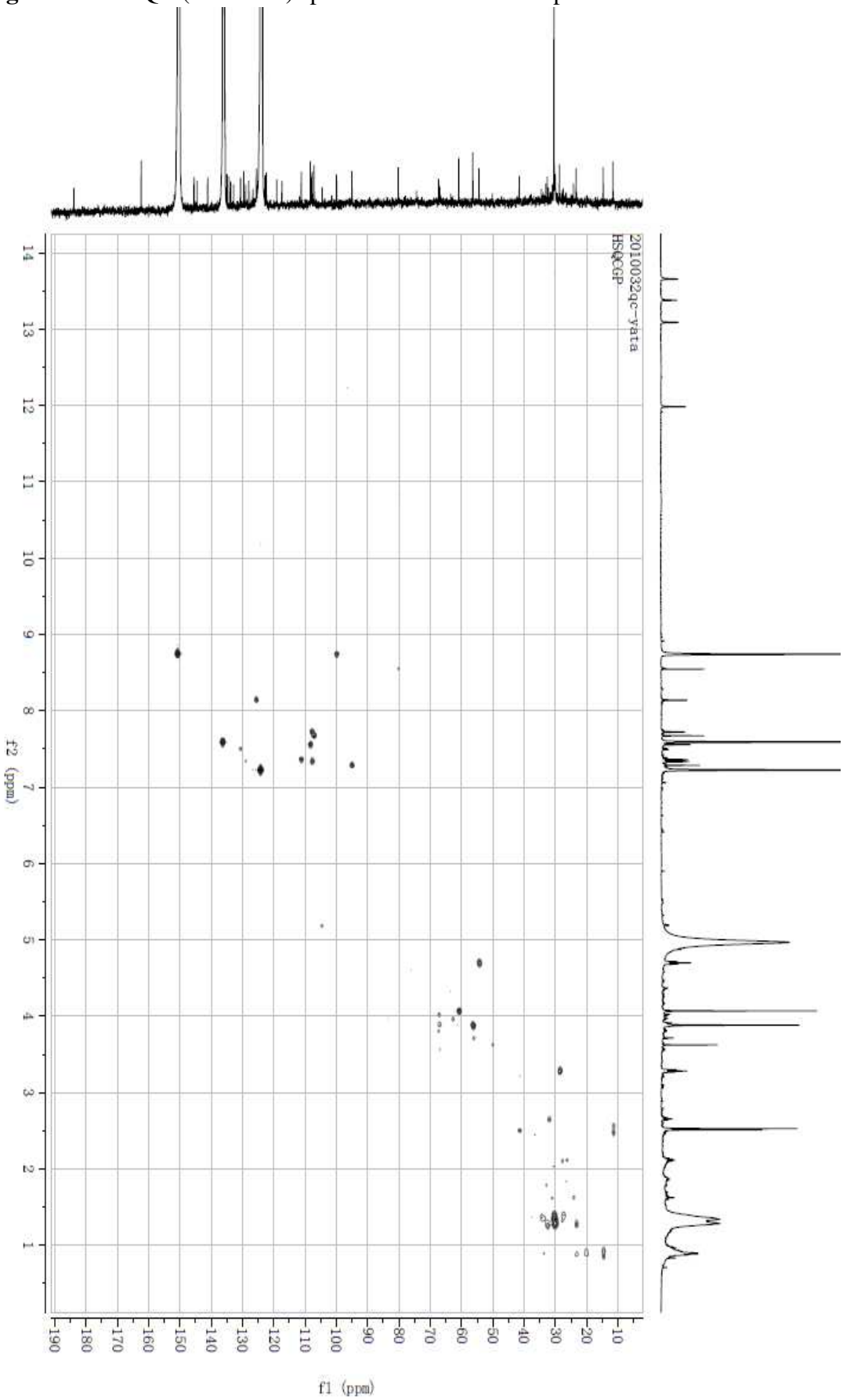


Figure S8. ^{1}H-^{1}H COSY (500 MHz) spectrum of the new compound **8**.

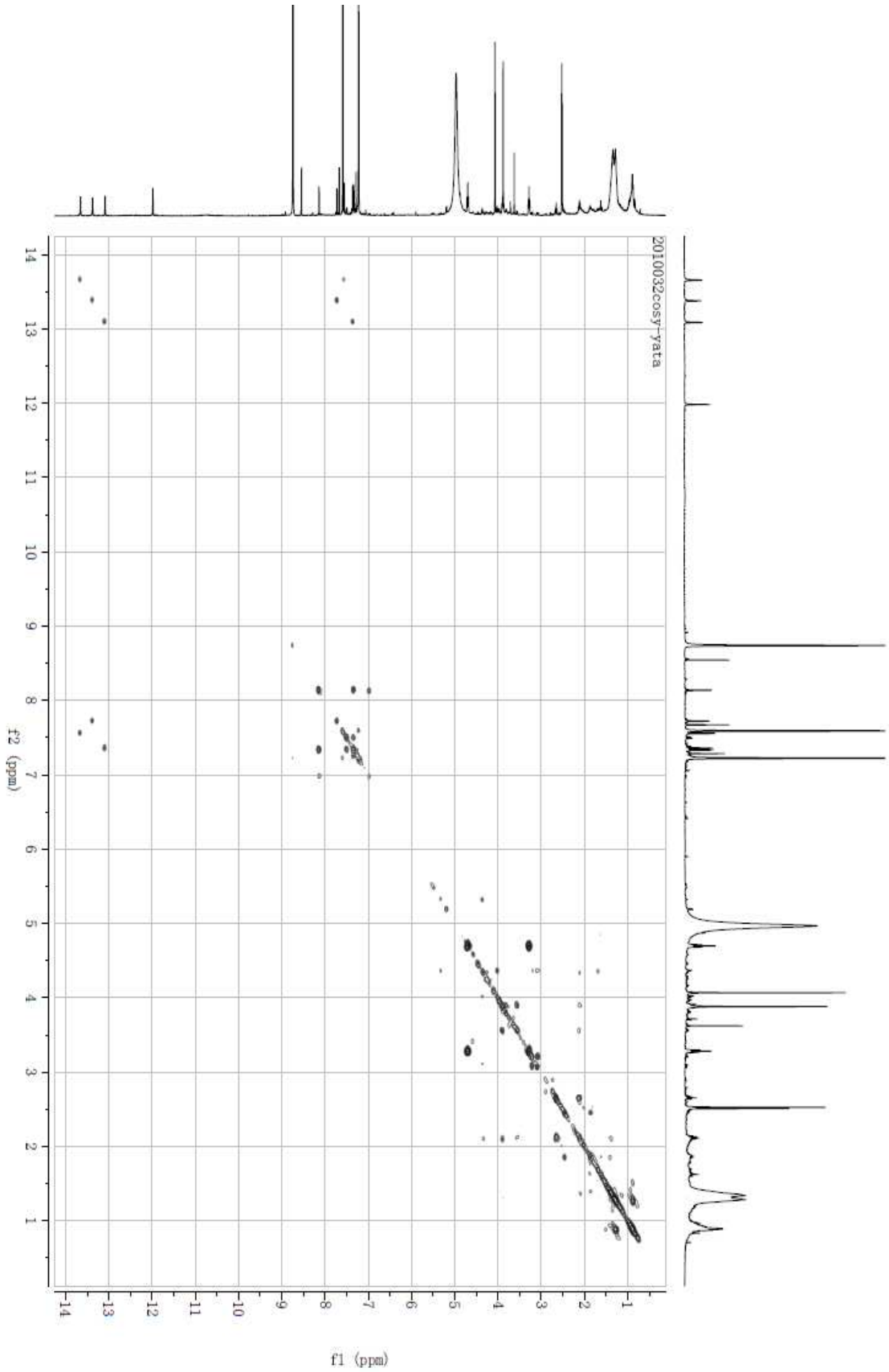


Figure S9. HMBC (500 MHz) spectrum of the new compound **8**.

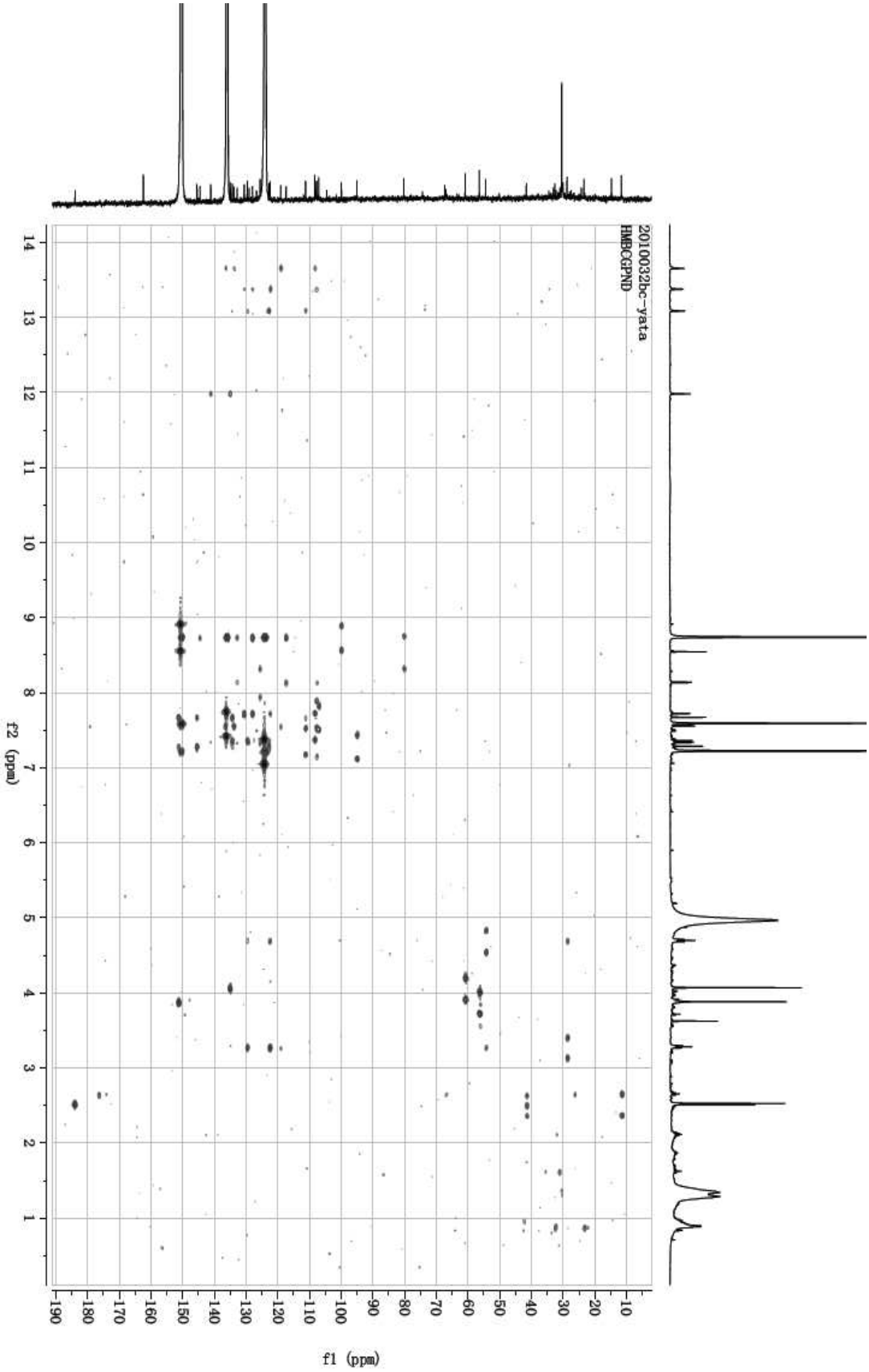
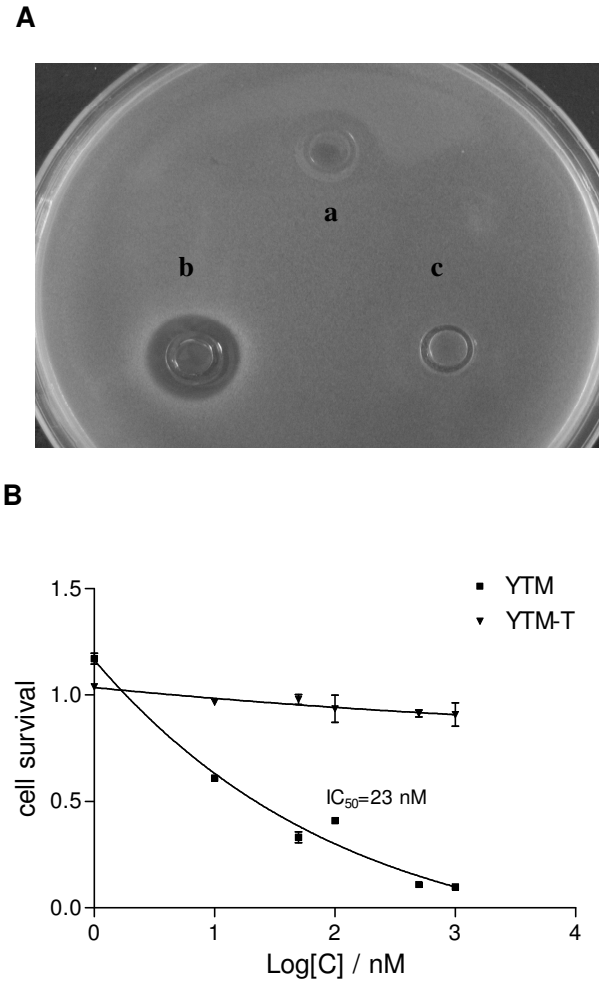


Figure S10. Bioassay of YTM and YTM-T (**8**).



(A) Determination of antifungi activity against *Saccharomyces cerevisiae* Y190. (a) 50 μL methanol as negative control, (b) 0.34 ng YTM in 50 μL methanol, and (c) 0.34 ng YTM-T in 50 μL methanol. (B) Assessing the cytotoxicity against cancer hela cell line. (■) YTM, and (▼) YTM-T.

Figure S11. HR-MALDI-MS spectrum of the enzymatic production catalyzed by YtkT, new compound **9**.