Supporting Information

Production of 5α-androstene-3, 17-dione from phytosterols by co-expression of 5α-reductase and glucose-6-phosphate dehydrogenase in engineered *Mycobacterium neoaurum*

Yunqiu Zhao[1], Yanbing Shen[1]*, Sai Ma[1], Jianmei Luo[1], Ouyang Wei[1], Haijie Zhou[1], Rui Tang[2], Min Wang*

[1] Key Laboratory of Industrial Fermentation Microbiology (Tianjin University of Science and Technology), Ministry of Education, Tianjin Key Laboratory of Industrial Microbiology, Tianjin Engineering Research Center of Microbial Metabolism and Fermentation Process Control, College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, P.R. China.

* Corresponding Author

Tel.: +86 22 60601256

Fax: +86 22 60602298

E-mail: minw@tust.edu.cn; shenyb@tust.edu.cn

Supplement materials

Supplementary Table 1A. Strains used in this study.

Supplementary Table 1B. Plasmids and primers used in this study.

Supplementary Table 2. ^{1}H and ^{13}C data of the purified product.

Supplementary Fig. 1A. Gene cloning and construction of the recombinant plasmid pMV261-$5\alpha_{T.\ denticola}$ for 5α-reductase production in *E. coli* DH 5α, identification of recombinant plasmids. The length of 5α-reductase from *T. denticola* is 774 bp. The length of pMV261 is 4488 bp. M, DL5000 marker; lane 1-2, the amplified 5α-reductase gene by PCR; Lane 3-4, the recombinant plasmid pMV261-*5α* digested by *Bam*H I/*Hin*d III.

Supplementary Fig. 1B. Gene cloning and construction of the recombinant plasmid pMV261-*5α-G62*$_{M.\ neoaurum}$ for G6PDH2 production in *E. coli* DH 5α, identification of recombinant plasmids. The length of G6PDH2 is 1521 bp. The length of recombinant plasmid pMV261-*5α* is 5262 bp. M, DL5000 marker; lane 5-6, the amplified G6PDH2 gene by PCR; lane 7-8, the recombinant plasmid pMV261-*5α-G6PDH2*$_{M.\ neoaurum}$ digested by *Hin*d III.

Supplementary Fig. 2. Lineweaver-Burk plot of 5α-reductase.

Supplementary Fig. 3. TLC results of biotransformation of PS and AD by *MNR* M3Δ*ksdd*/261 and *MNR* M3Δ*ksdd*/261-$5\alpha_{T.\ denticola}$.

Lane 1, standards of PS (purple), 5α-AD (yellow), AD (green). Lane 2-3, biotransformation of PS and AD respectively by *MNR* M3Δ*ksdd*/261; lane 4-5,

biotransformation of PS and AD respectively by *MNR* M3Δ*ksdd*/261-*5α*.

Supplementary Fig. 4. GC-MS analysis of standard 5α-AD (up) and purified product (down).

Supplementary Fig. 5A. ^{1}H-NMR spectrum of the purified product.

Supplementary Fig. 5B. ^{13}C-NMR spectrum of the purified product.

Supplementary Fig. 6A. HMBC spectrum of the purified product.

Supplementary Fig. 6B. HSQC spectrum of the purified product.

Supplementary Fig. 6C. ^{1}H-^{1}H COSY spectrum of the purified product.

Supplementary Fig. 6D. ^{1}H-^{1}H NOESY spectrum of the purified product.

Supplementary Table 1A. Strains used in this study.

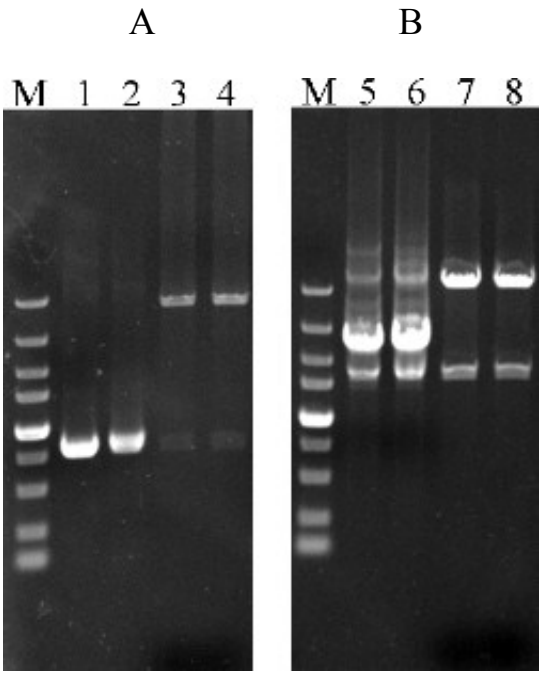
Name	Description	Sources
Strains		
Escherichia coli DH 5α	*E. coli* cloning host	Transgen Biotech
Mycobacterium neoaurum	Source of G6PDH gene	This lab
Saccharomyces cerevisiae	Source of G6PDH gene	This lab
MNR M3Δ*ksdd*	ksdd-deletion mutant of *MNR* M3	This lab
MNR M3Δ*ksdd*/261	*MNR* M3Δ*ksdd* electro-transformed with pMV261 as control	This study
MNR M3Δ*ksdd*/261-$5\alpha_{Rat}$	*MNR* M3Δ*ksdd* expressing 5α-reductase gene from *Rat*	This study
MNR M3Δ*ksdd*/261-$5\alpha_{T.\ denticola}$	*MNR* M3Δ*ksdd* expressing 5α-reductase gene from *T. denticola*	This study
MNR M3Δ*ksdd*/261-5α-$G62_{S.\ cerevisiae}$	*MNR* M3Δ*ksdd* expressing 5α-reductase from *T. denticola* and G6PDH from *S. cerevisiae*	This study
MNR M3Δ*ksdd*/261-5α-$G62_{M.\ neoaurum}$	*MNR* M3Δ*ksdd* expressing 5α-reductase from *T. denticola* and G6PDH from *M. neoaurum*	This study

Supplementary Table 1B. Plasmids and primers used in this study.

Name	Description	Sources
Plasmids		
pUC57-*5α*	The codon-optimized 5α-reductase gene delivered by pUC57, Amp^R	GENEWIZ Biotechnology Co., Ltd
pMV261	Shuttle vector of *Mycobacterium* and *E. coli*, Phsp60, Kan^R	Dr. W. R. Jacobs Jr. for providing pMV261
pMV261-$5\alpha_{Rat}$	pMV261 containing 5α-reductase gene from Rat, Kan^R	This study
pMV261-$5\alpha_{T.\ denticola}$	pMV261 containing 5α-reductase gene from *T. denticola*, Kan^R	This study
pMV261-*G6PDH*	pMV261 containing G6PDH gene, Kan^R	This study
pMV261-*5α*-$G62_{S.\ cerevisiae}$	pMV261 containing 5α-reductase from *T. denticola* and G6PDH from *S. cerevisiae*, Kan^R	This study
pMV261-*5α*-$G62_{M.\ neoaurum}$	pMV261 containing 5α-reductase from *T. denticola* and G6PDH from *M. neoaurum*, Kan^R	This study
Primers 5'-3'		
P1	CGCGGATCCATGGAGCGGCTCATCTTCATCTTC (*Bam*H I)	
P2	CCCAAGCTTTCAGAAAATGAACGGGAAGACGC (*Hin*d III)	
P3	CCGGAATTCATGAGCACAGCCGAGGCAT (*Eco*R I)	
P4	AACAAGCTTTCACGGCCGCCGCCACTC (*Hin*d III)	
P5	CCCAAGCTTTAAGTAGCGGGGTTGCCGTCACC (*Hin*d III)	
P6	AACAAGCTTTCACGGCCGCCGCCACTC (*Hin*d III)	

Notes: Amp^R ampicillin-resistant, Kan^R kanamycin-resistant, the restriction enzyme sites were underlined.

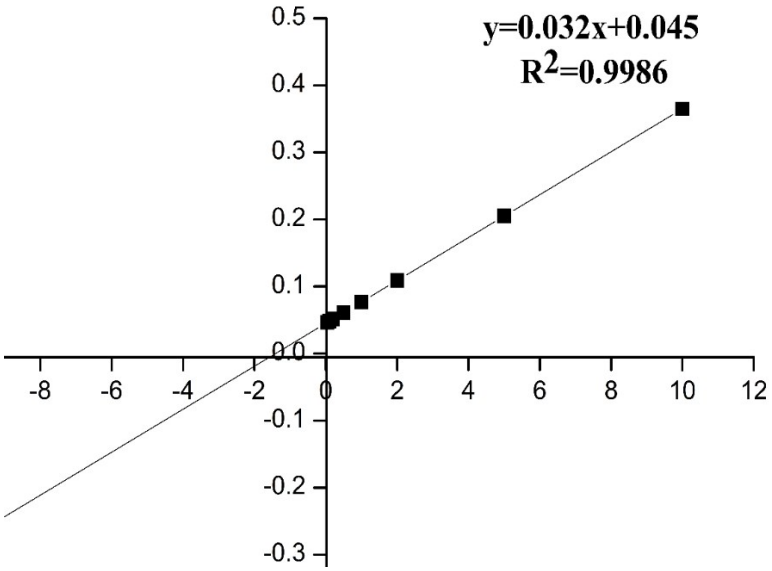
Supplementary Fig. 1



Supplementary Fig. 1A. Gene cloning and construction of the recombinant plasmid pMV261-*5α*$_{T.\ denticola}$ for 5α-reductase production in *E. coli* DH 5α, identification of recombinant plasmids. The length of 5α-reductase from *T. denticola* is 774 bp. The length of pMV261 is 4488 bp. M, DL5000 marker; lane 1-2, the amplified 5α-reductase gene by PCR; Lane 3-4, the recombinant plasmid pMV261-*5α* digested by *Bam*H I/*Hin*d III.

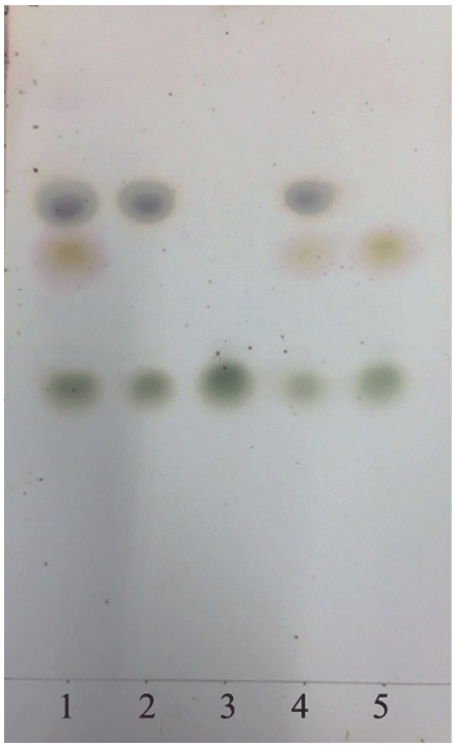
Supplementary Fig. 1B. Gene cloning and construction of the recombinant plasmid pMV261-*5α*-*G62*$_{M.\ neoaurum}$ for G6PDH2 production in *E. coli* DH 5α, identification of recombinant plasmids. The length of G6PDH2 is 1521 bp. The length of recombinant plasmid pMV261-*5α* is 5262 bp. M, DL5000 marker; lane 5-6, the amplified G6PDH2 gene by PCR; lane 7-8, the recombinant plasmid pMV261-*5α*-*G6PDH2*$_{M.\ neoaurum}$ digested by *Hin*d III.

Supplementary Fig. 2



Supplementary Fig. 2. Lineweaver-Burk plot of 5α-reductase.

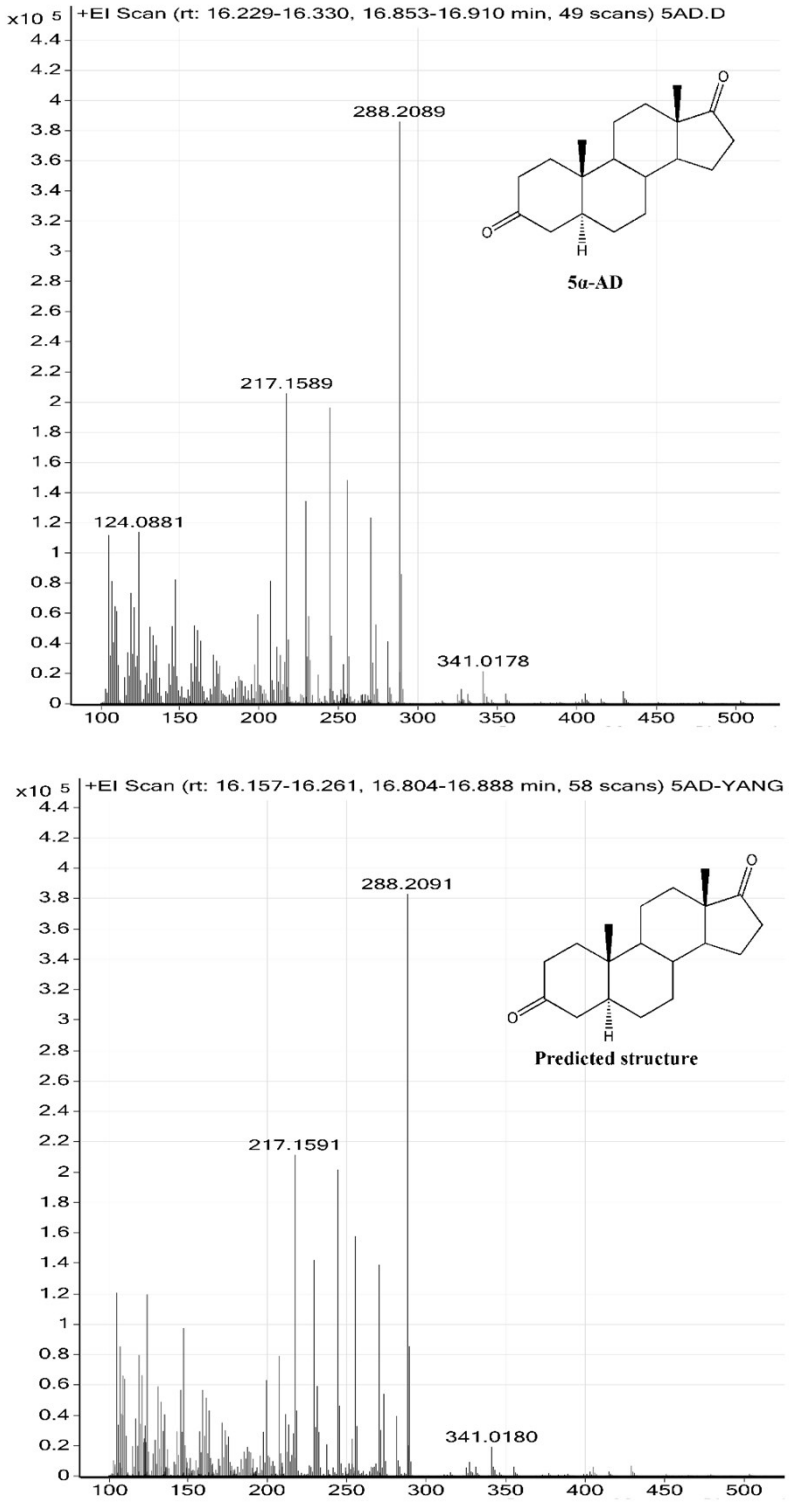
Supplementary Fig. 3



Supplementary Fig. 3. TLC results of biotransformation of PS and AD by *MNR* M3Δ*ksdd*/261 and *MNR* M3Δ*ksdd*/261-$5\alpha_{T.\ denticola}$.

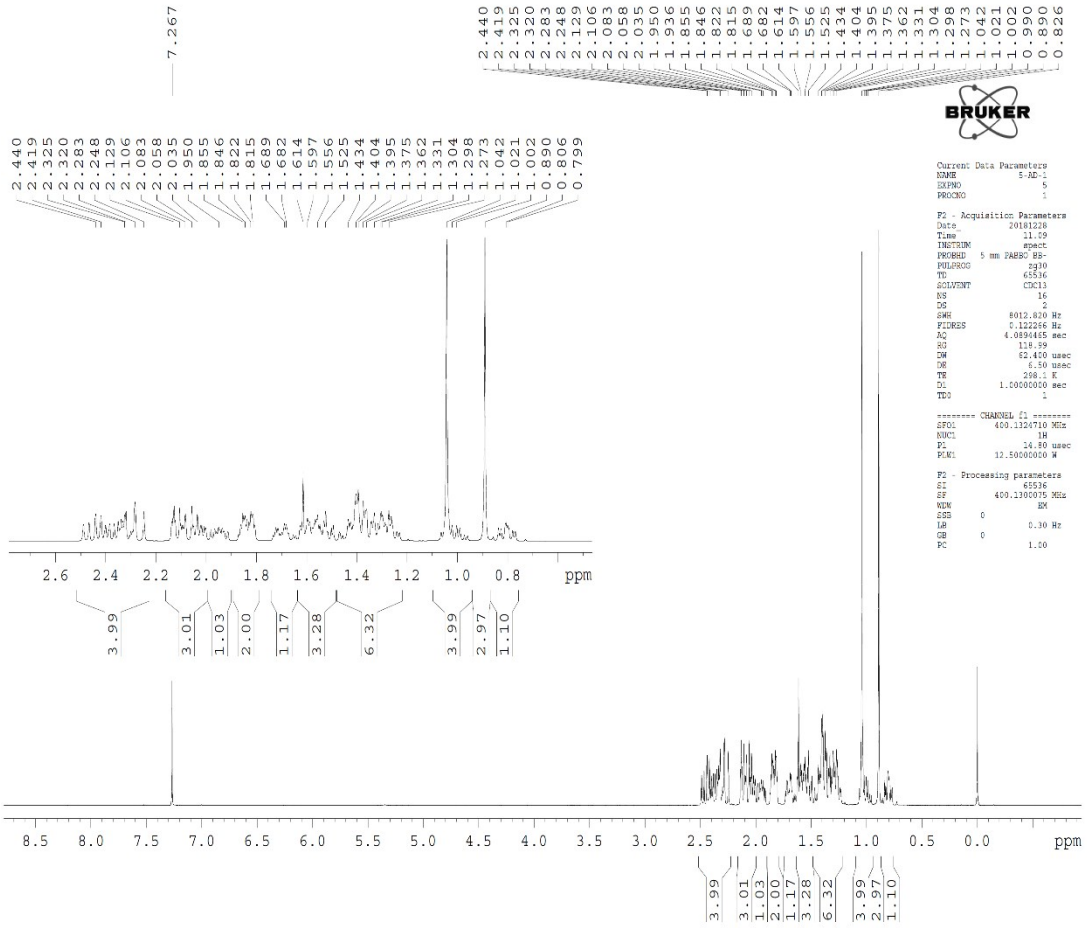
Lane 1, standards of PS (purple), 5α-AD (yellow), AD (green). Lane 2-3, biotransformation of PS and AD respectively by *MNR* M3Δ*ksdd*/261; lane 4-5, biotransformation of PS and AD respectively by *MNR* M3Δ*ksdd*/261-*5α*.

Supplementary Fig. 4



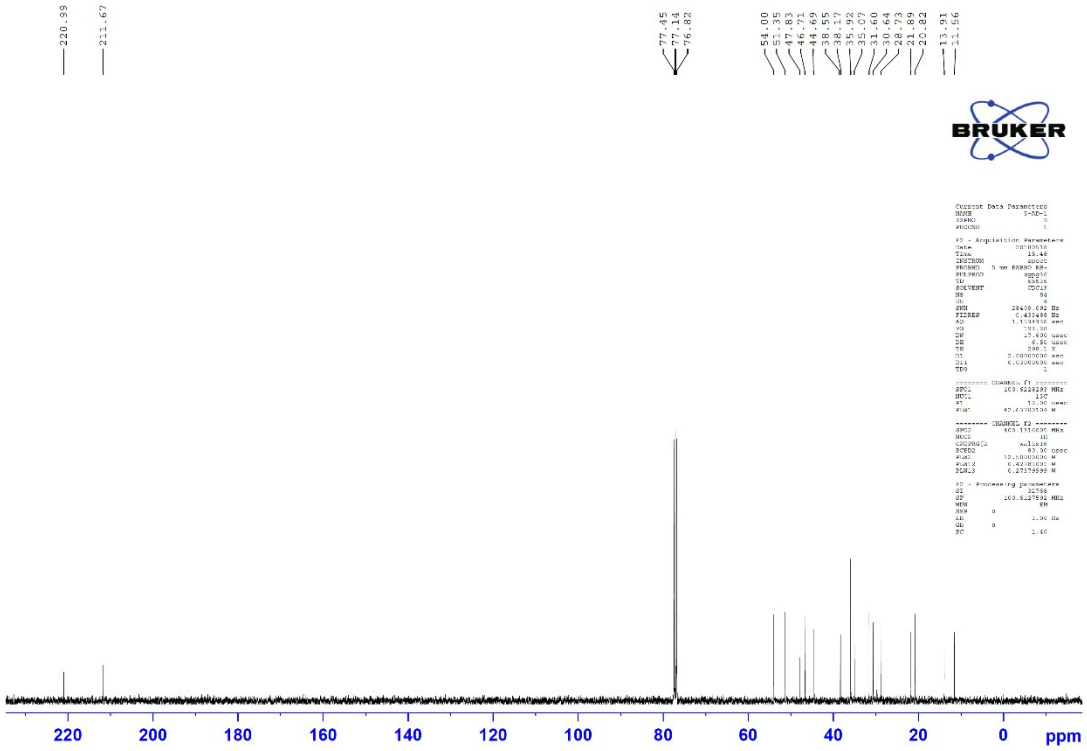
Supplementary Fig. 4. GC-MS analysis of standard 5α-AD (up) and purified product (down).

Supplementary Fig. 5A



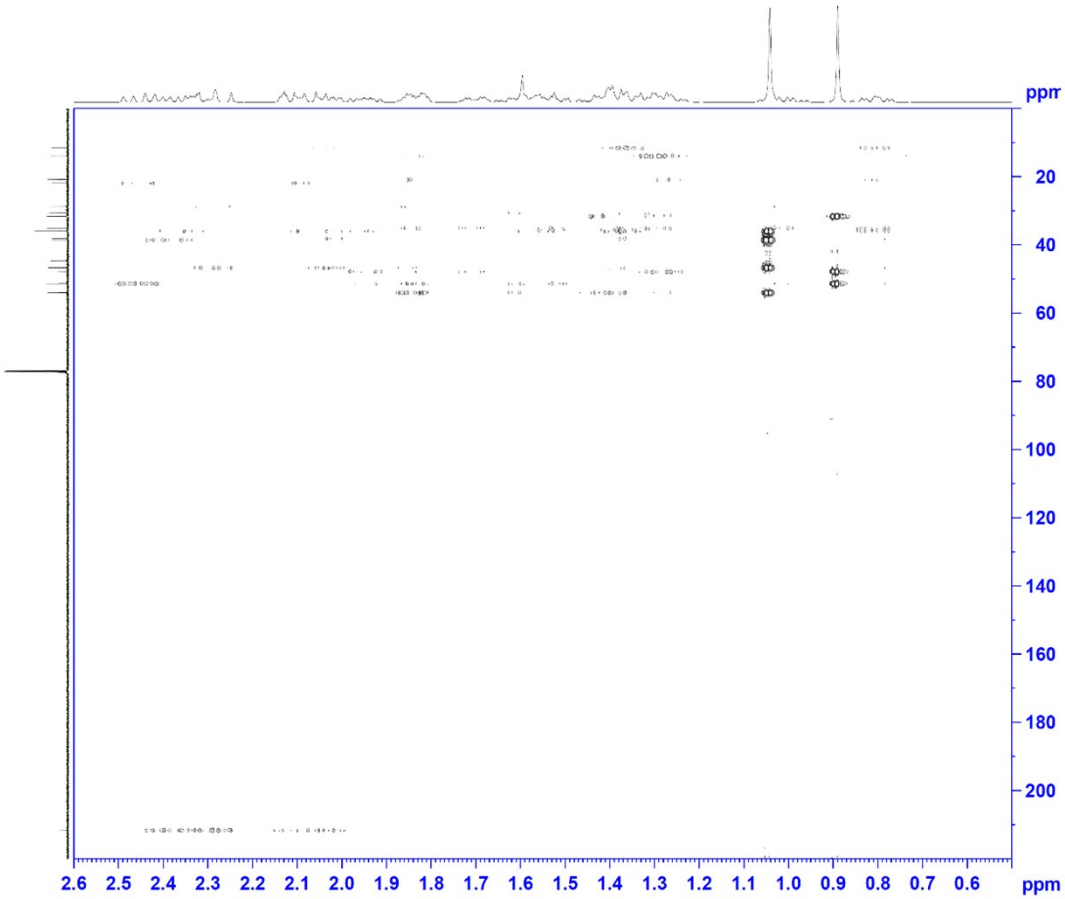
Supplementary Fig. 5A. ^{1}H-NMR spectrum of the purified product.

Supplementary Fig. 5B



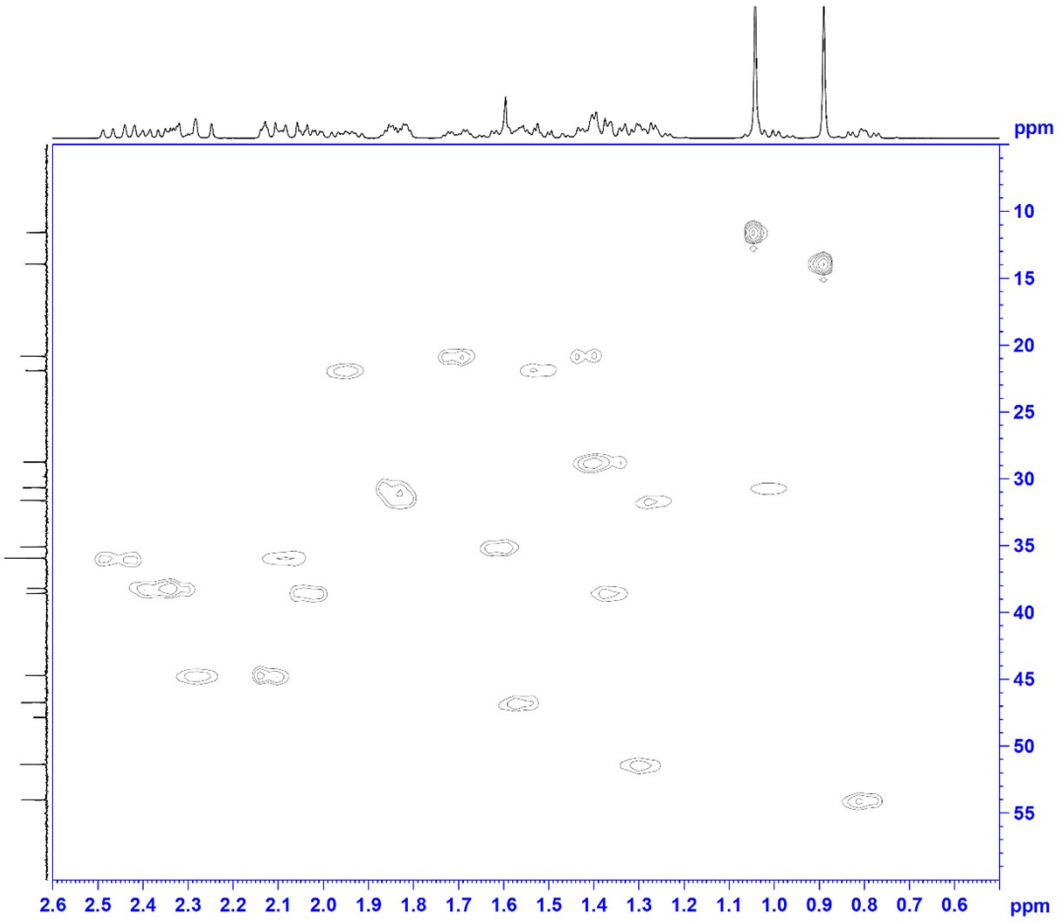
Supplementary Fig. 5B. ^{13}C-NMR spectrum of the purified product.

Supplementary Fig. 6A



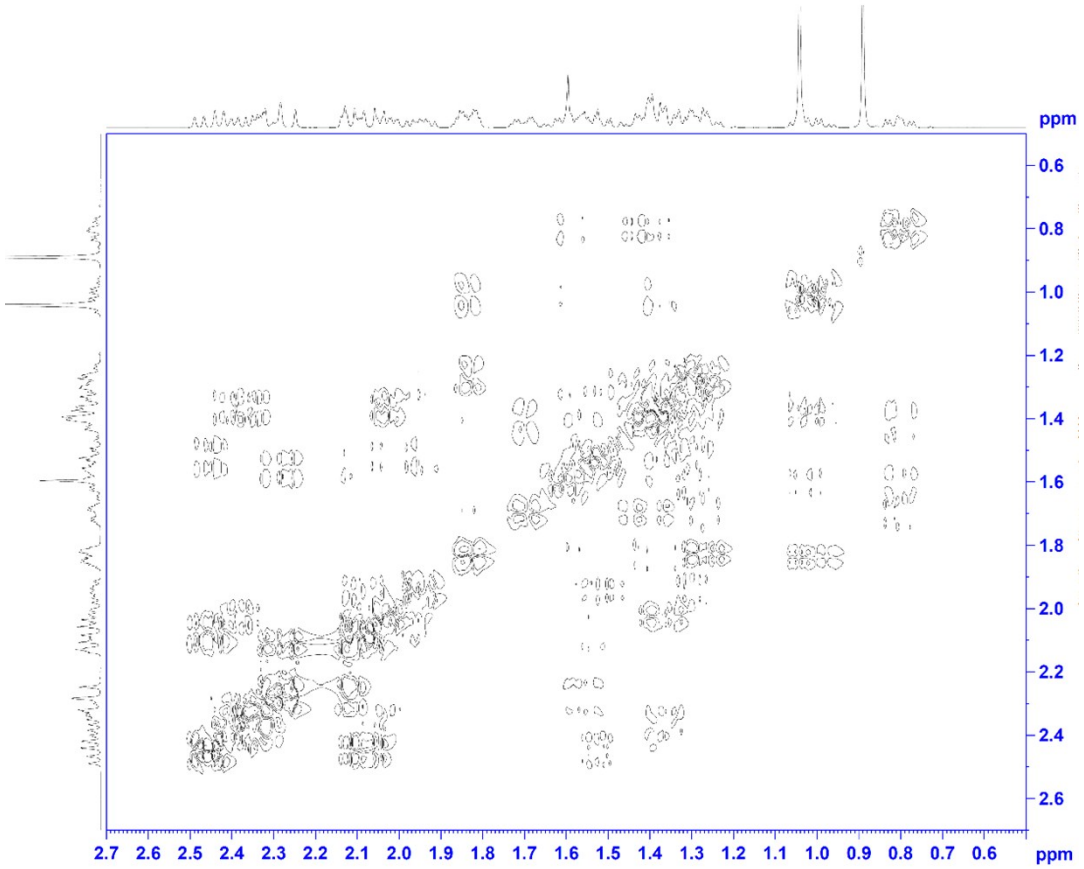
Supplementary Fig. 6A. HMBC spectrum of the purified product.

Supplementary Fig. 6B



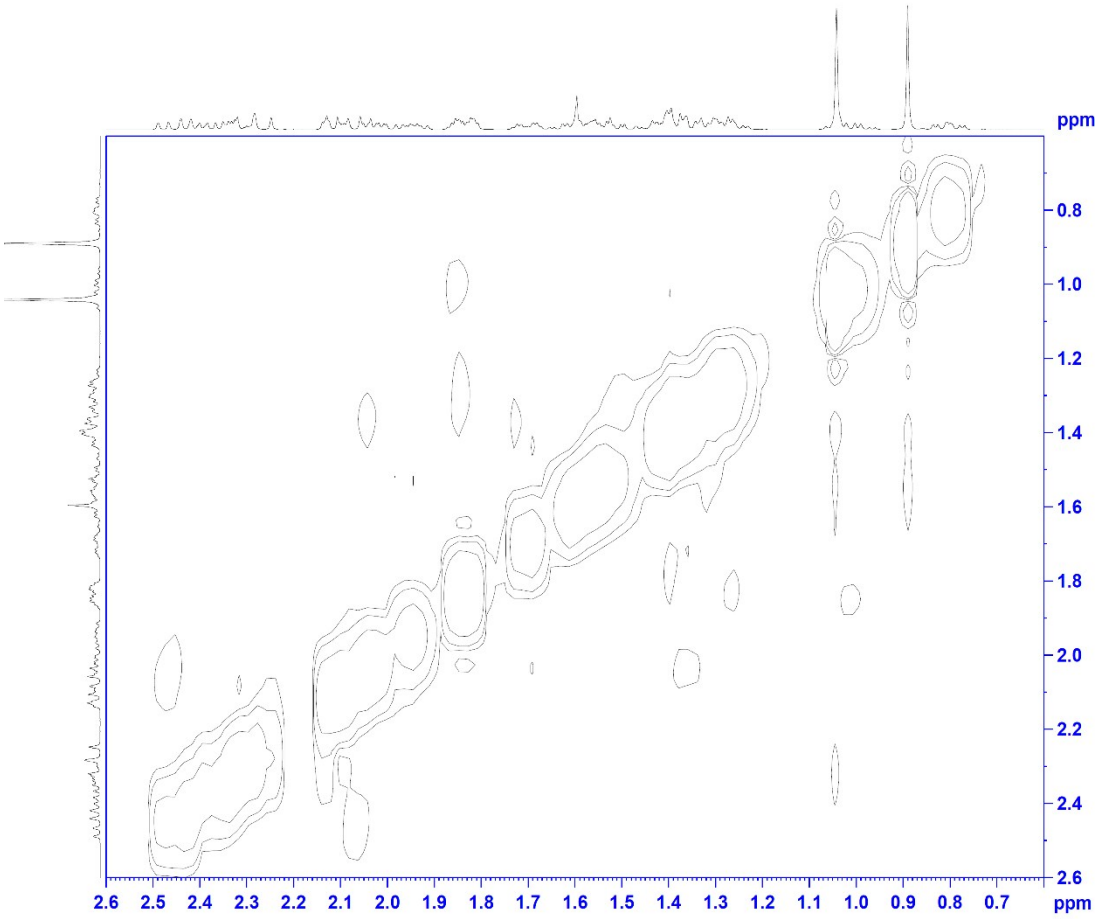
Supplementary Fig. 6B. HSQC spectrum of the purified product.

Supplementary Fig. 6C



Supplementary Fig. 6C. ^{1}H-^{1}H COSY spectrum of the purified product.

Supplementary Fig. 6D



Supplementary Fig. 6D. ^{1}H-^{1}H NOESY spectrum of the purified product.

Supplementary Table 2. ^{1}H and ^{13}C data of the purified product.

Carbon No.	^{13}C (δ, ppm)	^{1}H (δ, ppm)
1	38.55	1.95, 1.82
2	38.17	2.43, 2.28
3	211.67	–
4	44.69	2.35, 2.06
5	46.71	1.43
6	28.73	1.72, 1.41
7	31.60	1.62, 1.40
8	35.07	1.33
9	51.35	0.80
10	35.92	–
11	20.82	1.55, 1.26
12	30.64	1.52, 1.28
13	47.83	–
14	54.00	1.02
15	21.89	2.06, 1.85
16	35.92	2.10, 2.42
17	220.99	–
18	13.91	0.89
19	11.56	1.04