Electronic Supplementary Information

Guanidyl modification of the 1-azabicyclo[3.1.0]hexane ring in ficellomycin essential for its biological activity

Sumire Kurosawa[a], Kenichi Matsuda[a], Fumihito Hasebe[a], Taro Shiraishi[a], Kazuo Shin-ya[c], Tomohisa Kuzuyama[a,b] and Makoto Nishiyama*[a,b]

a. Biotechnology Research Center, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8567, Japan.
b. Collaborative Research Institute for Innovative Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8567, Japan
c. National Institute of Advanced Industrial Science and Technology, 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan.

Address correspondence to: Makoto Nishiyama. Fax, +81-3-5841-8030; E-mail, umanis@mail.ecc.u-tokyo.ac.jp

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Table S1. Oligonucleotides used in this study.

Oligonucleotide	Sequence (Restriction enzyme sites are underlined)	Description (Restriction enzyme)
Δ*fic15* UpFw	5'-TGCTCTAGAAAGCTTCACCTCGTCGGCCGTCAC -3'	gene deletion (*Xba*I and *Hin*dIII)
Δ*fic15* UpRv	5'-CCGGAATTCGCTGGCCGAGGCGATCCA-3'	gene deletion (*Eco*RI)
Δ*fic15* DnFw	5'-CCGGAATTCGACTCGTGACCGGTGGGT-3'	gene deletion (*Eco*RI)
Δ*fic15* DnRv	5'-CCCAAGCTTGGCTCGCCGACGACATCG-3'	gene deletion (*Hin*dIII)
Δ*fic15* compFw	5'- CCCAAGCTTCGCGGCCGGCCCGTGAGA-3'	gene complementation (*Hin*dIII)
Δ*fic15* compRv	5'-TGCTCTAGACGCTCAACGGACGGGCTC-3'	gene complementation (*Xba*I)
Δ*fic15* checkFw	5'-GGCCGGACACGCCGAGTA-3'	colony PCR
Δ*fic15* checkRv	5'-TACCGGGCTGCCGGAATG-3'	colony PCR
Δ*fic16* UpFw	5'-TGCTCTAGAAAGCTTCGCGAGCCGTTCCTGGTT-3'	gene deletion (*Xba*I and *Hin*dIII)
Δ*fic16* UpRv	5'-CCGGAATTCGGCTGACCGGAAACCGAC-3'	gene deletion (*Eco*RI)
Δ*fic16* DnFw	5'-CCGGAATTCCATGGCGTGGTTCACCCT-3'	gene deletion (*Eco*RI)
Δ*fic16* DnRv	5'-CCCAAGCTTCTGCAGACCCTGCCCCTG-3'	gene deletion (*Hin*dIII)
Δ*fic16* compFw	5'-CCCAAGCTTCCCCGTGAACCACATGCG-3'	gene complementation (*Hin*dIII)
Δ*fic16* compRv	5'-TGCTCTAGACGGTCAGCCCAGCACGTC-3'	gene complementation (*Xba*I)
Δ*fic16* checkFw	5'-CGGGGACTCGTGACCGGT-3'	colony PCR
Δ*fic16* checkRv	5'-ATCCTCACCGCCGAGCCG-3'	colony PCR
Δ*fic22*UpFw	5'-CCCAAGCTTGTACGGGCCGGTGGTCGC-3'	gene deletion (*Hin*dIII)
Δ*fic22* UpRv	5'-CGGACTAGTATCGTGGCGCTGAGCCCG-3'	gene deletion (*Spe*I)
Δ*fic22* DnFw	5'-CGGACTAGTCATGTGAGGTGTCCTTTC-3'	gene deletion (*Spe*I)
Δ*fic22* DnRv	5'-CCCAAGCTTGGCTACCCCTGGACCGTG-3'	gene deletion (*Hin*dIII)
Δ*fic22* compFw	5'-CCCAAGCTTAGGAACACCTCGTCGACC-3'	gene complementation (*Hin*dIII)
Δ*fic22* compRv	5'-CCCAAGCTTCTACTCCCCCCAGTCCTC-3'	gene complementation (*Xba*I)
Δ*fic22* checkFw	5'-GACCGCGTCGCAGTCCTC-3'	colony PCR
Δ*fic22* checkRv	5'-GTGACCGTCGAGGAGCAC-3'	colony PCR

Fic13-pHis8Fw	5'-CGCGGATCCATGAGTGATTTCGCAGGA-3’	overexpression (*Bam*HI)
Fic13-pHis8Rv	5'-CCCAAGCTTCTACGGCTGGGCTGGCGG-3'	overexpression (*Hin*dIII)
Fic15-pHis8Fw	5'-CGCGGATCCATGTCCATCGACGCCGTA-3’	overexpression (*Bam*HI)
Fic15-pHis8Rv	5'-CCCAAGCTTTCAACGGACGGGCTCGGT-3'	overexpression (*Hin*dIII)
Fic16-pHis8Fw	5'-CGCGGATCCATGAACCCGACCCCGGAC-3’	overexpression (*Bam*HI)
Fic16-pHis8Rv	5'-CCCAAGCTTTCAGCCCAGCACGTCGGC-3'	overexpression (*Hin*dIII)
Fic36-pHis8Fw	5'-CGCGGATCCATGAGCCTGGTCAATGTC-3’	overexpression (*Bam*HI)
Fic36-pHis8Rv	5'-CCCAAGCTTCTACCGGTAGGTGGCGAG-3'	overexpression (*Hin*dIII)

Table S2. Characterization of **2**-DNP

NMR chemical shift

(Solvent: *d6*-DMSO, ^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz)

	δ_C	δ_H	(m, J_H in Hz)
1	168.9		
2	59.0	4.23	(dd, 3.2, 6.6)
3	60.4	4.15	(ddd, 3.3, 8.1, 11.5)
4a	31.1	2.75	(dd, 11.9, 18.0)
4b		1.82	(dd, 7.8, 18.1)
5	211.0		
6	41.7	2.33	(dd, 2.3, 6.3)
7a	28.1	2.20	(d, 2.2)
7b		1.71	(d, 6.4)
N8		9.77	(d, 6.7)
9	148.0		
10	117.3	7.29	(d, 9.8)
11	130.4	8.18	(dd, 2.7, 9.7)
12	134.8		
13	124.3	8.86	(d, 2.8)
14	130.1		

Optical rotation

$[\alpha]_D^{28} = -6.9$ (c 0.0034, water)

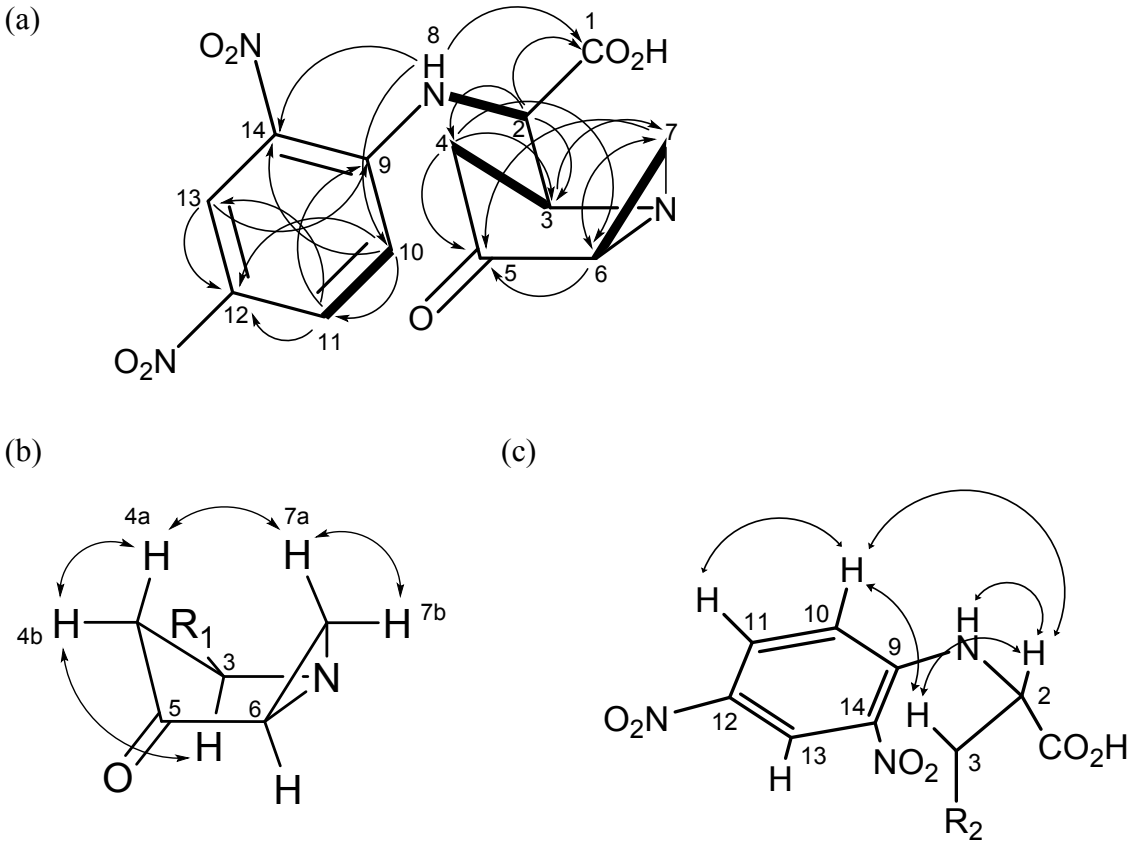

Fig. S1 Structure determination of **2**-DNP. (a) The key correlations of HMBC and double quantum filtered-COSY (DQF-COSY) are shown. (b)(c) The relative stereochemistry of **2**-DNP. The key correlations of NOESY are shown in the part of structure containing (b) the azabicyclo hexane ring or (c) DNP unit, respectively.

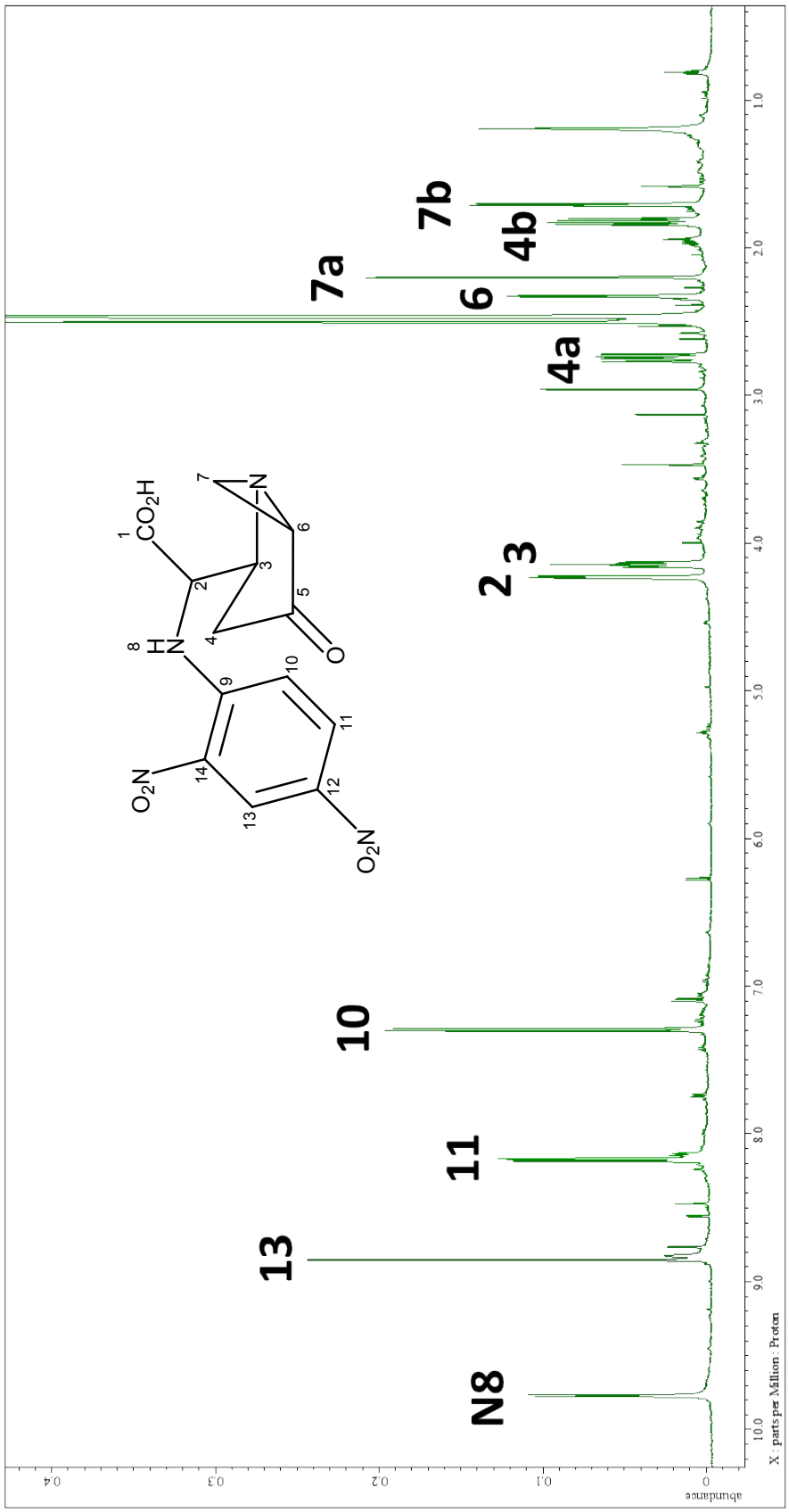

Fig. S2 ^{1}H NMR spectrum for **2**-DNP

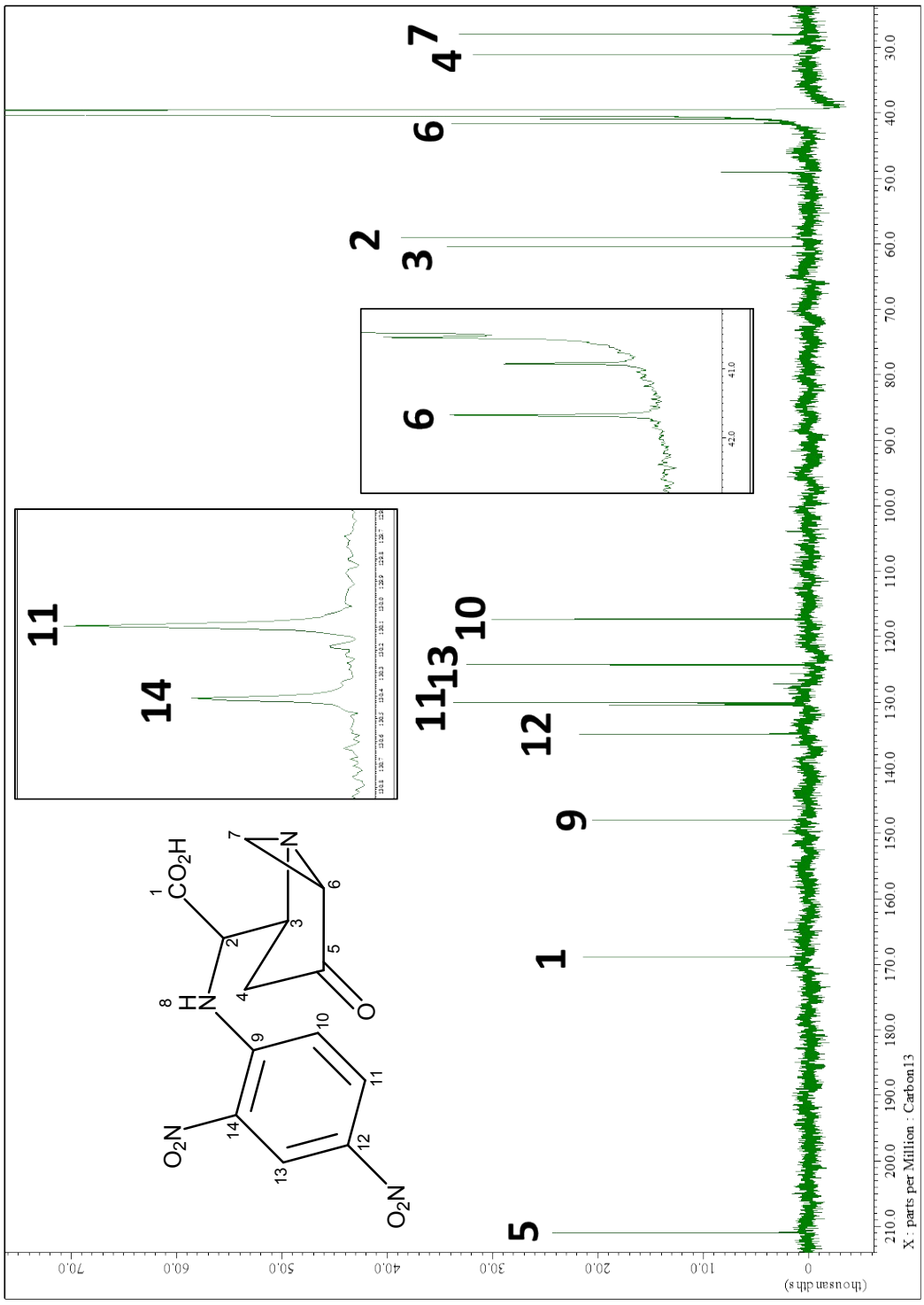

Fig. S3 ^{13}C NMR spectrum for **2**-DNP

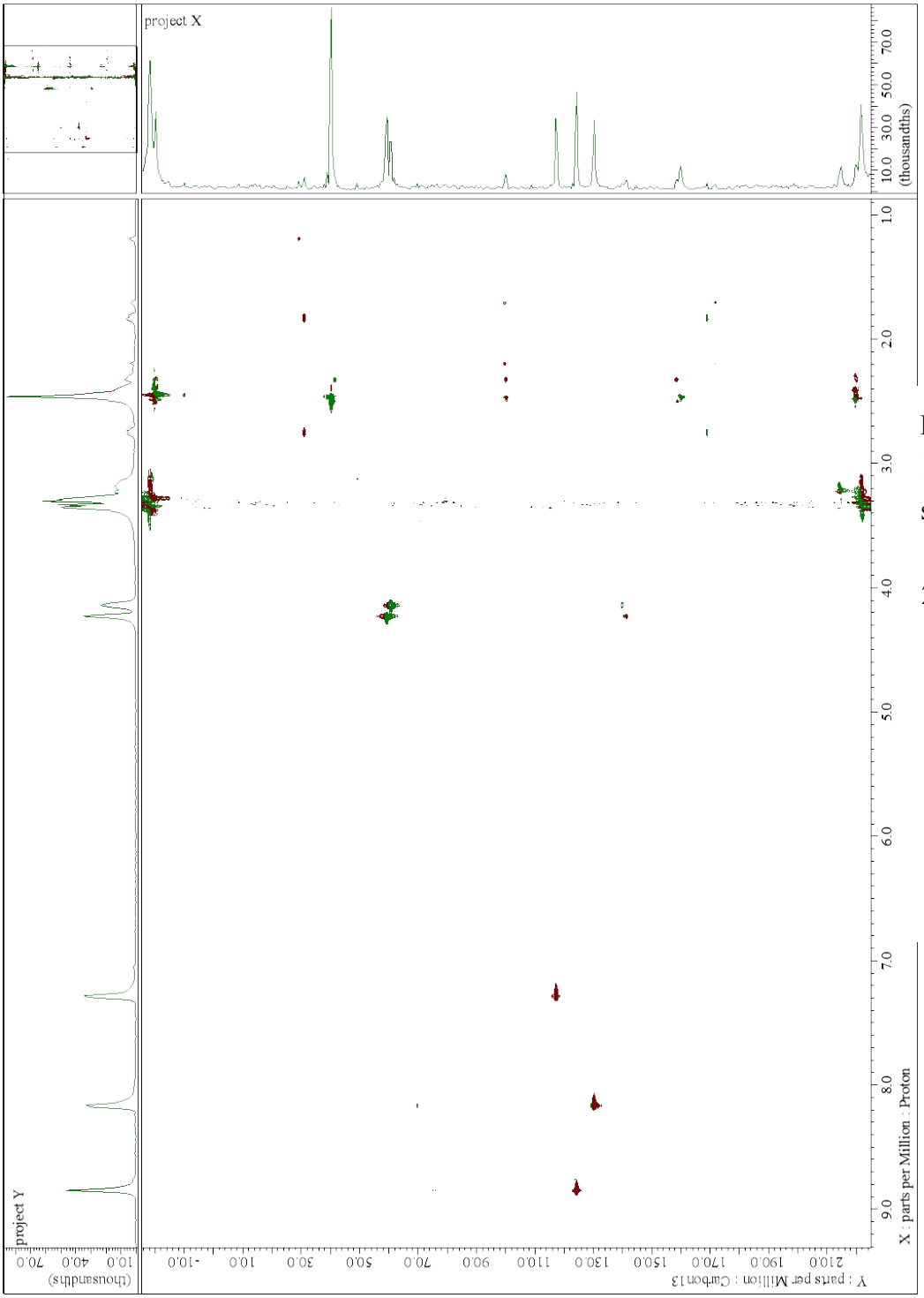

Fig. S4 HSQC spectrum for **2**-DNP

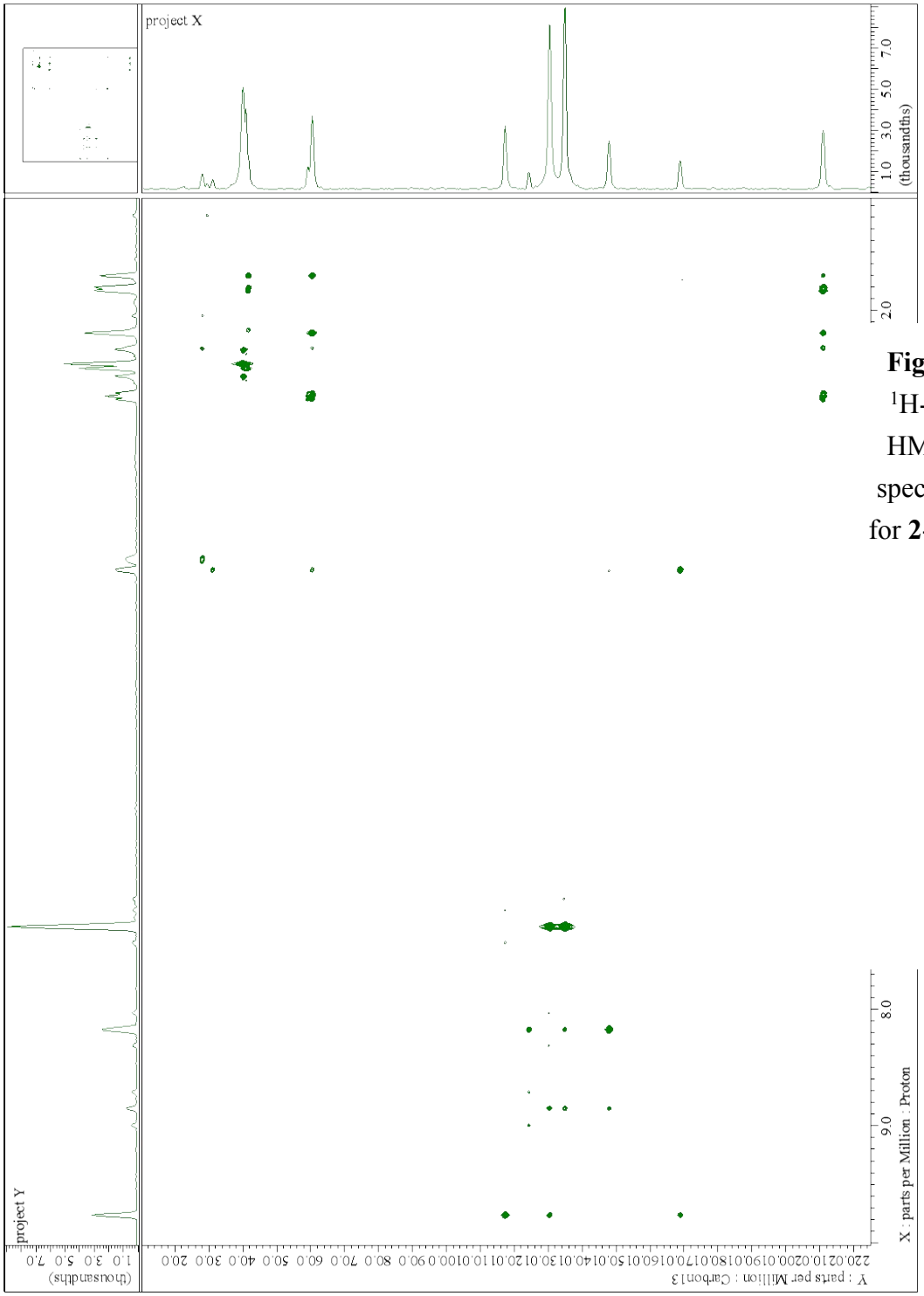

Fig. S5 ^{1}H-^{13}C HMBC spectrum for **2**-DNP

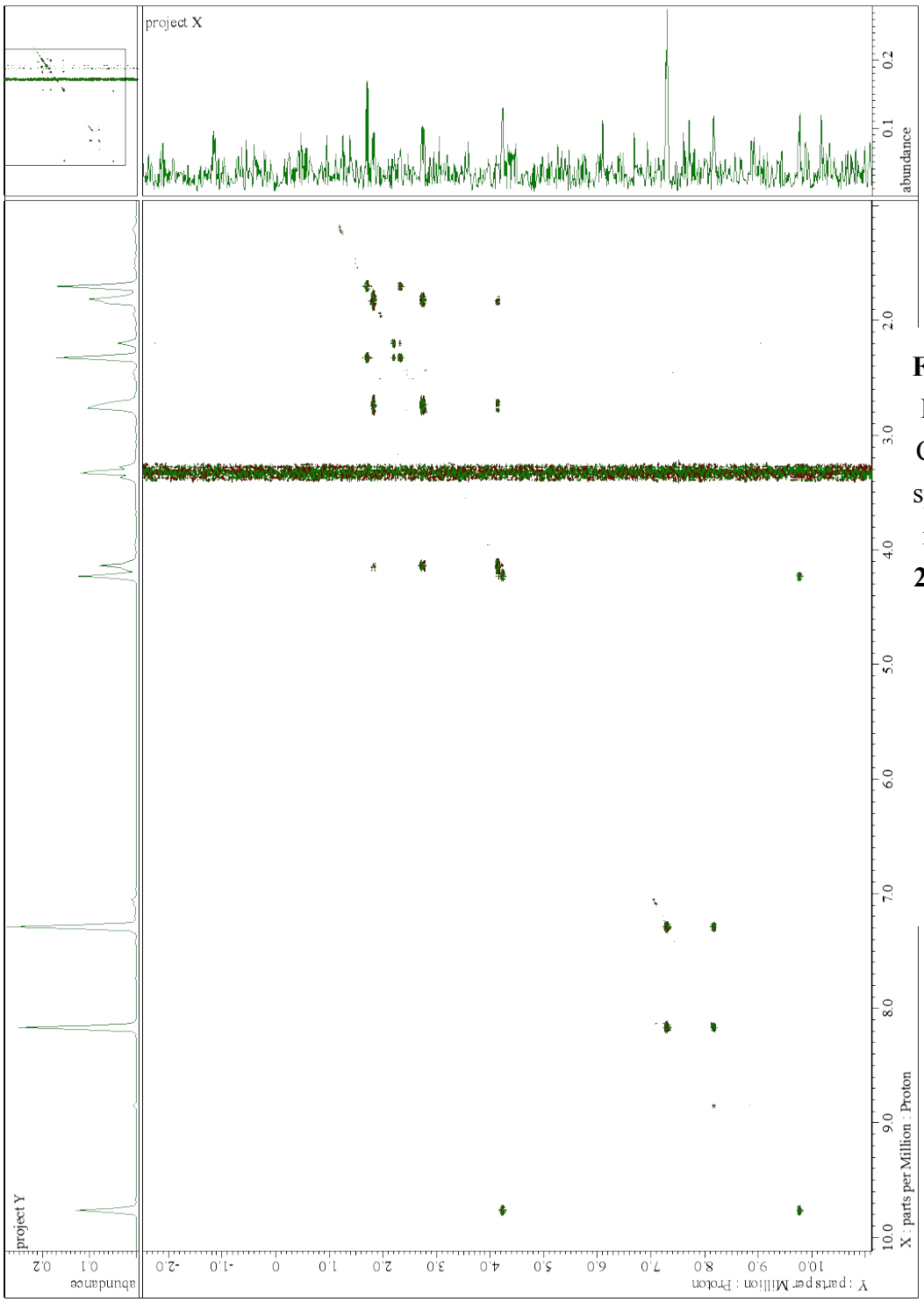

Fig. S6 DQF-COSY spectrum for **2**-DNP

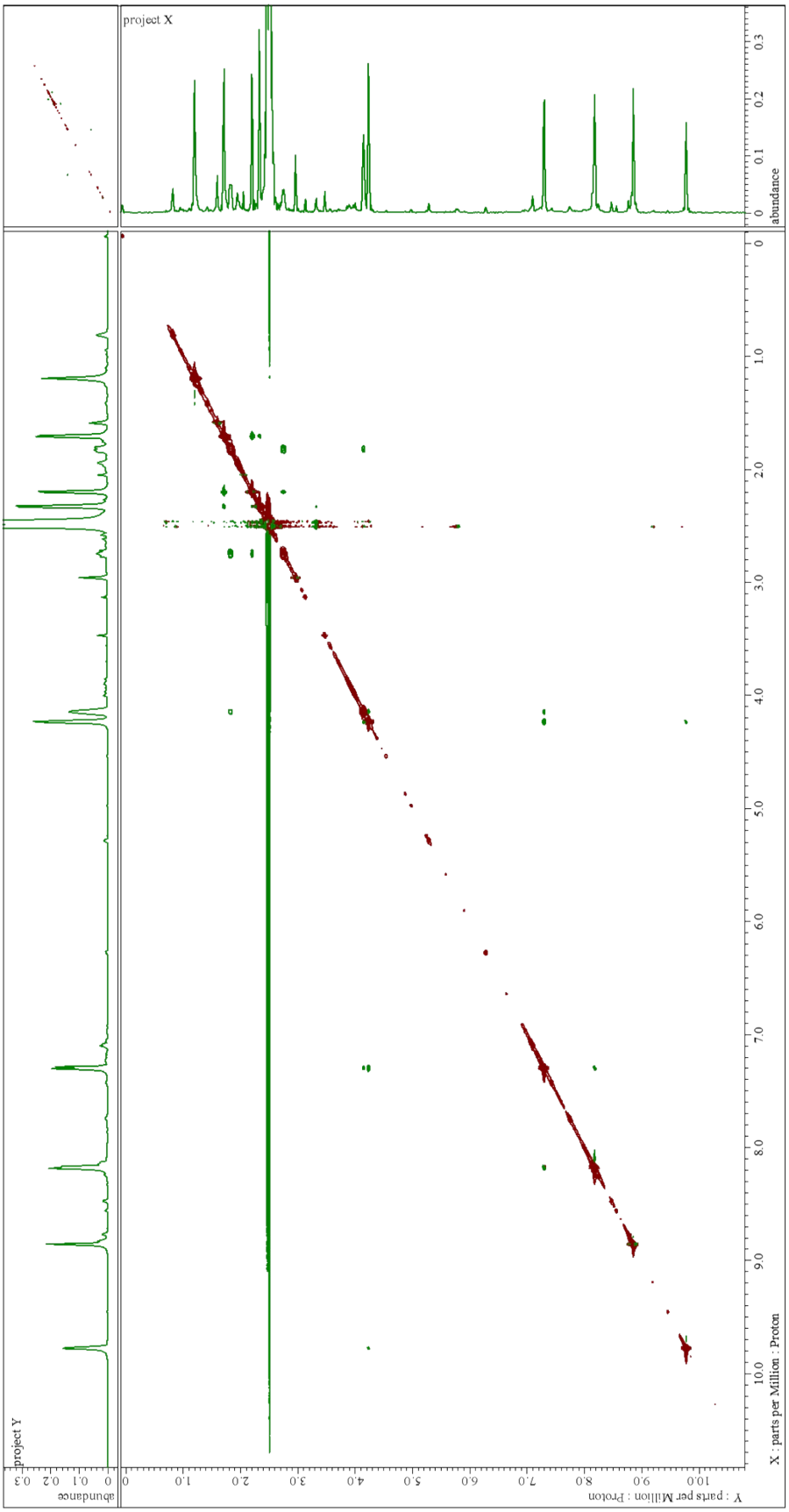

Fig. S7 NOESY spectrum for **2**-DNP

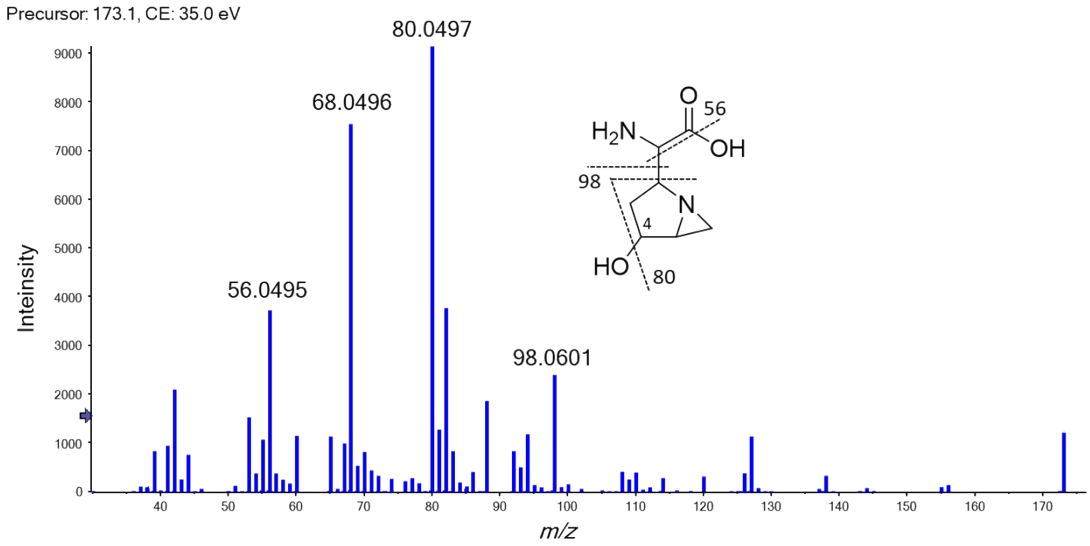

Fig. S8 MS/MS analysis of **1**. The *m/z* values with 98 and 80 of the azabicyclo hexane ring-containing fragments indicated the presence and absence of the hydroxy group at the C4 position of the ring.

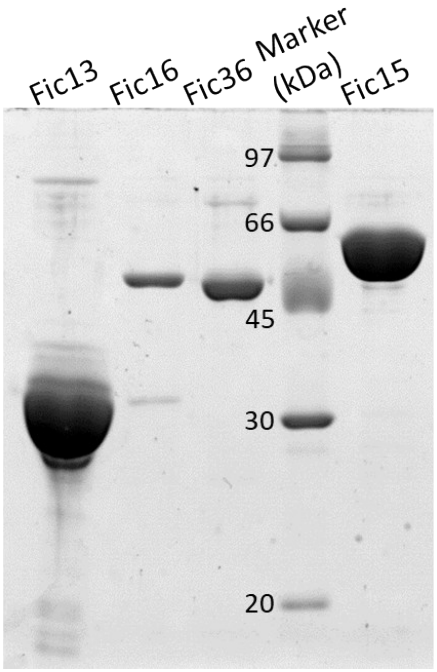

Fig. S9 SDS-PAGE of Fic13, Fic16, Fic36, and Fic15. All enzymes were purified with proposed mass of 28, 45, 41, and 49 kDa, respectively.

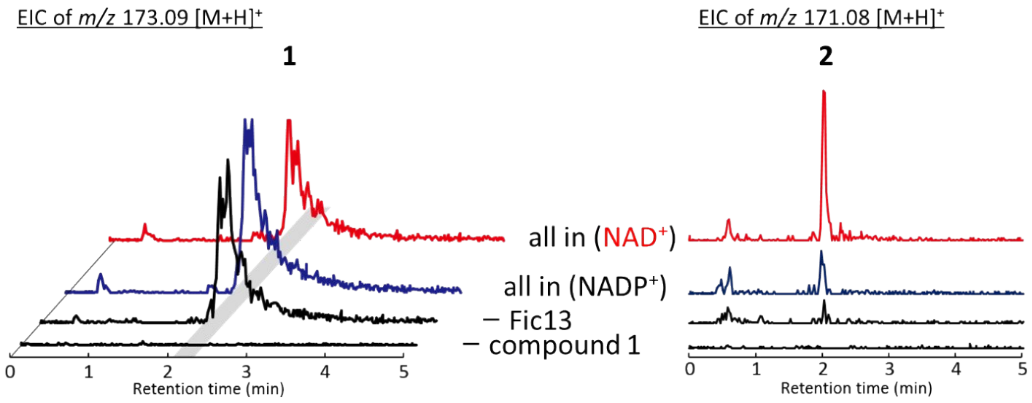

Fig. S10 LC-MS analysis of the *in vitro* reaction mixture for Fic13. EIC chromatograms for **1** and **2** (*m/z* 173.09 $[M + H]^+$ and *m/z* 171.08 $[M + H]^+$) are shown.

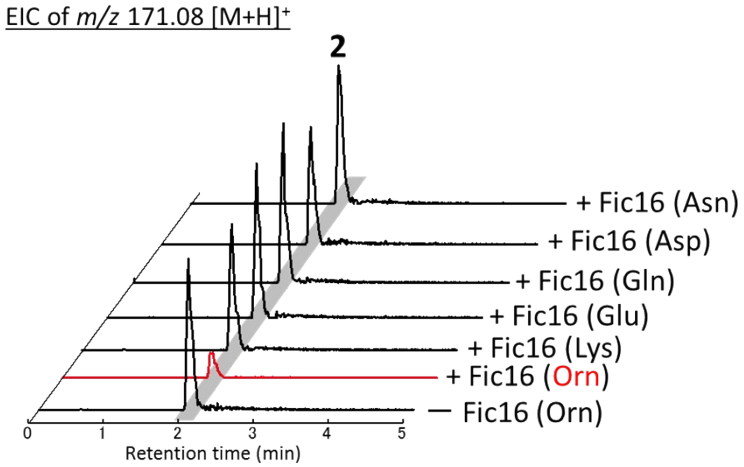

Fig. S11 LC-MS analysis of the *in vitro* reaction mixture for Fic16 using various amino acids as substrates. EIC chromatograms for **2** (*m/z* 171.08 $[M + H]^+$) are shown.

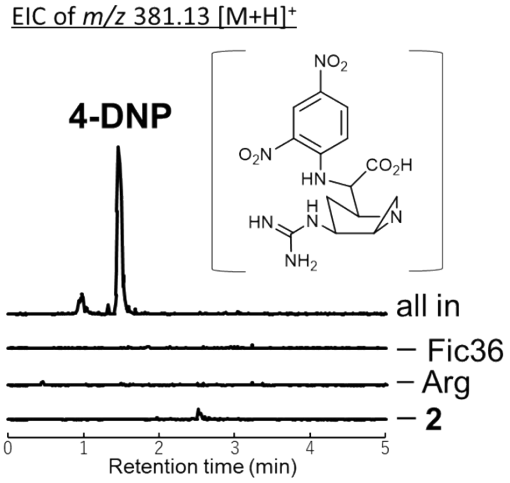

Fig. S12 LC-MS analysis of the *in vitro* coupling reaction mixture of Fic16 and Fic36. EIC chromatograms for **4**-DNP (*m/z* 380.13 $[M+H]^+$) are shown.

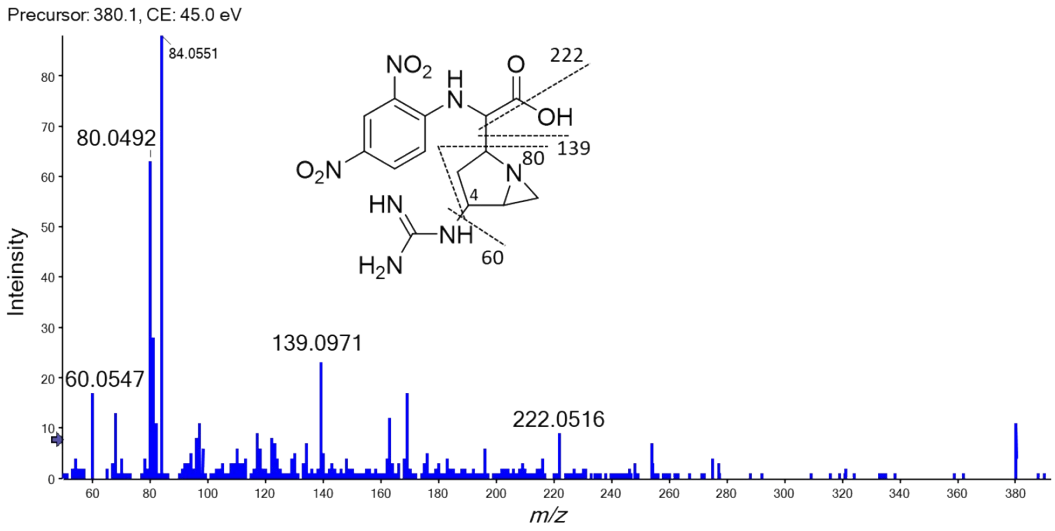

Fig. S13 MS/MS analysis of **4**-DNP. The *m/z* value of 60 was annotated as the guanidyl unit and those of 139 and 80 of the azabicyclo hexane ring-containing fragments indicated the presence and absence of the guanidyl unit at the C4 position of the ring. The *m/z* volume of 222 was comparable to the fragment with the DNP unit at the α-amino group.

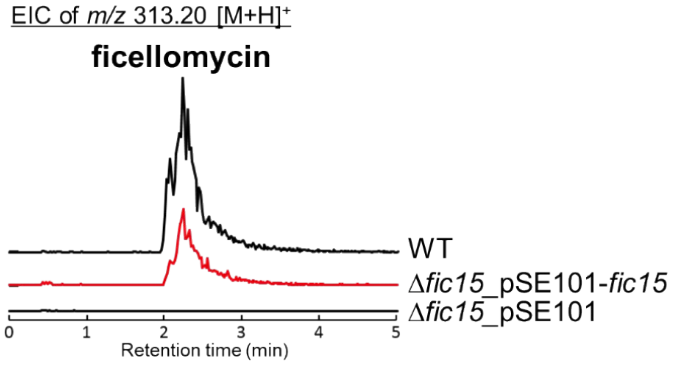

Fig. S14 Metabolite analysis of *S. ficellus* Δ*fic15*. Extracted ion count (EIC) chromatograms for ficellomycin (*m/z* 313.20 $[M + H]^+$) is shown.