

Supplementary Information

Gene Conversion-Associated Successive Engineering of Modular Polyketide Synthases

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Supplementary Tables

Supplementary Table 1. Bacterial strains used in this work

Strain	Characteristics	Source or Reference
<i>E. coli</i> strain		
<i>E. coli</i> DH5 α	Host for general cloning	Tiagen biotech
<i>E. coli</i> ET12567/pUZ8002	Donor strain used for <i>E. coli</i> - <i>Streptomyces</i> conjugation	Ref. 29
<i>Streptomyces</i> strain		
<i>S. cinnamoneus</i> ATCC 21532 (WT)	Wild-type strain for cinnamonycin production	Ref. 29
<i>S. coelicolor</i> A3(2)	CCR and HCD gene containing strain	GCA_008931305.1
Δ cmmB mutant	<i>cmmB</i> in-frame deletion mutant	Ref. 29
WT+mgmO	<i>mgmO</i> gene integrated strain of WT	This work
Δ cmmB+mgmO	<i>mgmO</i> gene integrated strain of Δ cmmB mutant	This work
S1	Cmm-AT1 replacement mutant with Cmm-AT4 in wild-type <i>streptomyces cinnamoneus</i>	This work
S2	Cmm-AT4 replacement mutant with Mgm-AT5 in wild-type <i>streptomyces cinnamoneus</i>	This work
S3	Cmm-AT5 replacement mutant with Mgm-AT5 in wild-type <i>streptomyces cinnamoneus</i>	This work
S3-CCR-HCD	Ethylmalonyl-CoA pathway key gene overexpression strain of mutant S3	This work
S4	Cmm-AT4 replacement mutant with Mgm-AT5 in S3	This work
S5	Cmm-AT5 replacement mutant with Mgm-AT5 in S4	This work
S5-mgmKS ₅	Cmm-KS5 replacement mutant with Mgm-KS5 in S5	This work
S5-mgmKS ₆	Cmm-KS6 replacement mutant with Mgm-KS6 in S5	This work
S5-mgmACP ₅	Cmm-ACP5 replacement mutant with Mgm-ACP in S5	This work
S6	<i>cmmB</i> in-frame deletion mutant in S5-mgmKS ₅	This work
S7	Mangromycin C produced strain	This work
S5-ASC	Mutants with “active site cap” region substitutions in Cmm-KS5	This work

Supplementary Table 2. Nucleotide sequences of gene conversion regions in *cmm* BGCAT₂, AT₆ region:

GGAACCAACGCCCACGTCATCCTGGAACAGGCACCACCAGAGACAGTGGAGGAGCAGGCGCC
 CGCCACTGGGCAGAGCGACGTGGTCGTGCCCTGGGTCTGCTCGGGCAAGACCGAAGCAGCCG
 TCGACGAACAGCTCGCACGGCTACGCCAATGGGCCAGCAACGGCCGGACGCCCGGCCAGTC
 GACGTGCCCCACGCCTTGGCCACGAGTCGCACGCACTTCTGAATACCGGGCCGCGGTCTAGG
 CCGAACCCACGAAGAACTTGTACCGCGCTGGCCTCGCCCGCATCGGTCTTACGAGGCCGGCG
 ACAAGGCCGCGAACTGGCGGTCTCTTCGCGGGACAGGGCTCACAACGCCCCGGCATGGGAC
 GCGAACTCCACGCCACCTACCCAGCCTTCGCCGACGCCTTCGACGCCATCCGCACCGAACTCG
 ACCAACACCTAGACCTGCCACTCACCCACATCATGTGGCACGACACCACCGGCCTACTCCACCA
 AACCGCCTATACCCAGGCCGCGCTCTTCGCCCTCGAAACAGCCCAATACAGGCTCATAGAAAGC
 TGGGGACTACGGCCAGAGGCACTGCTCGGGCACTCGATCGGGGAACTACCGCAGCCACGT
 CGCCGGCATCTGGTCTCTCGAAGACGCCTGCACCCTGGTCGCCGCACGCGGACGCCTCATGCA
 AACCTCCCCACCAGCGGCACCATGACCGCCCTCCAAGCCACAGAAAACGAAATCGCTCCTCTT
 CTCAACGAGCGAGTGAGCCTGGCAGCCATCAACGGCCCTCATCCGTCTCATCTCCGGCGAC
 AAAGACGCCGTCGACACCATCGCAACAACCGTCACCAACTGGGGCCGCAAAACCAAGAACTC
 CACGTACGCCACGCCTTCCACTCCCCCACATGGACCCATACTCGACGAATTCCAAACCATCG
 CCGAGTCCCTCACCTACCACCCACCCAGCTGACCATCATCTCCAACCTCACCGGCCAACCCAC
 CACCACCGACACCCTCACCCCCACCTACTGGACCCACCACATCCGCCAACCCGTCCGCTTCAA
 CGACGGCCTCACCCACCTCACCCACACACCTCCTCGAACTCGGGCCCCGACAGCACCTCAC
 CGCCCTCACCCAACAAACCCACCCCCACACCACCGCCACCCCCCTCCTCCGCAAAAACCAACC
 CGAACCCACACCAACCATCACCGCCCTCACCCACCTCCACACCACCGGCACCAACCCCAACTG
 GAACACCCTCCTCCCCACCACCCACCCACCCACGACCTCCCCACCTACCCCTTCCAACACCAC
 CACTACTGGCTG

AT₇ region:

GCACGAACGCGCACCTCATCCTTGAGGAATCACCCCAGGAGACGGCCGACACGGCGGATCCGA
 CCGCGCCCGTCGAGGCCAAGGACGTCGTCTGCGGTGGGTCTGCTCGGGCAAGTCACCGGAG
 GCGCTGGACGAGCAGCTGAGCCGGCTGCGGCGCTGGGCCGAGGACCGTCCCGACGTGCACC
 CCGCCGACACGGCGTACGCCCTCGCGACCACCCGCACCCACTTCGATCACCGGGCGGCCGTG
 GTGGGCAGCACCCGCGAAGAACTGATCGAGGCCCTGGGCTCGGTCTCGGTGATCCGCGGCAG
 GCGGCACGACGGCAGGCTGGCGATCCTCTTCGCGGGACAGGGCTCACAACGGCCCGGCATGG
 GGCGCGAACTCCACGCCACCTACCCAGCCTTCGCCGACGCCTTCGACGCCATCCGCACCGAAC
 TCGACCAACACCTAGACCAGCCACTCACCCACATCATGTGGCACGACACCACCGGCCTGCTCCA
 CCAAACCGCCTACACCCAGGCCGCGCTCTTCGCCCTCGAAACAGCCCAATACAGGCTCATAGAA
 AGCTGGGGACTACGGCCAGAGGCACTGCTCGGGCACTCGATCGGGGAACTACCGCAGCCCA
 CGTCGCCGGCATCTGGTCTCTCGAAGACGCCTGCACCCTGGTCGCCGCACGCGGACGCCTCAT
 GCAAACCCTCCCCACCAGCGGCACCATGACCGCCCTCCAAGCCACAGAAAACGAAATCGCTCC
 TCTTCTCAACGAGCGAGTGAGCCTGGCAGCCATCAACGGCCCTCATCCGTCTCATCTCCGG
 CGACAAAGACGCCGTGACACCATCGCAACAACCGTCACCAACTGGGGCCGCAAAACCAAGAA
 ACTCCACGTACGCCACGCCTTCCACTCCCCCACATGGACCCATACTCGACGAATTCCAAACC
 ATCGCCGAGTCCCTCACCTACCACCCACCCAGCTGACCATCATCTCCAACCTCACCGGCCAAC
 CCACCACCAACCGACACCCTCACCCACCTACTGGACCCACCACATCCGCCAACCCGTCCGCT
 TCAACGACGGCCTCACCCACCTCACCCACACACCTCCTCGAACTCGGGCCCCGACAGCACCC
 TCACCGCCCTCACCCAACAAACCCACCCCCACACCACCGCCACCCCCCTCCTCCGCAAAAACC
 ACCCCGAACCCACACCAACCATCACCGCCCTCACCCACCTCCACACCACCGGCACCAACCCCA
 ACTGGAACACCCTCCTCCCCACCACCCACCCACCCACGACCTCCCCACCTACCCCTTCCAACA
 CCACCCTACTGGCTCGAC

Supplementary Table 3. Gene annotations of *mgm* BGC.

Gene	Amino Acids	Proposed Function	Protein Homology	Identity (%)
<i>mgmO</i>	493	Tryptophan-halogenase	TiaM (GenBank: ADU85999.1)	63.95%
<i>mgmP</i>	600	3-hydroxybutyryl-coa dehydrogenase	WP_011030948.1	47.88%
<i>mgmF</i>	450	Crotonyl-coa carboxylase/reductase	WP_030863150.1	87.64%
<i>mgmL</i>	258	Type II thioesterase	CmmL	50.42%
<i>mgmN</i>	251	Transcriptional regulator	WP_104630222.1	93.15%
<i>mgmD1</i>	5523	Type I polyketide synthase	CmmD1	
<i>mgmD2</i>	3992	Type I polyketide synthase	CmmD2	
<i>mgmD3</i>	2397	Type I polyketide synthase	CmmD3	
<i>mgmE</i>	402	FAD-dependent monooxygenase	CmmE	55.50%
<i>mgmG</i>	282	ABC transporter	CmmG	62.01%
<i>mgmH</i>	315	ABC transporter	CmmH	62.03%
<i>mgmI</i>	330	Zinc-binding alcohol dehydrogenase	CmmI	57.23%
<i>mgmJ</i>	192	Transcriptional regulator	CmmJ	58.76%
<i>mgmC1</i>	356	Type III polyketide synthase	CmmC1	70.11%
<i>mgmC2</i>	238	enoyl-CoA hydratase/isomerase family protein	CmmC2	48.68%
<i>mgmC3</i>	485	Enoyl-CoA hydratase/isomerase family protein	CmmC3	58.55%
<i>mgmC4</i>	546	Thiamine pyrophosphate-binding protein	CmmC4	71.38%
<i>mgmC5</i>	486	Benzaldehyde dehydrogenase	CmmC5	69.20%
<i>mgmC6</i>	268	Enoyl-CoA-hydratase	VemD	79.85%
<i>mgmK</i>	1058	Transcriptional regulator	CmmK	36.20%

Supplementary Table 4. Plasmids and primers used to construct of exogenous gene integration mutants in this study.

Mutant Strain	Plasmid	Cloning Method	Fragment	Primer Name	Primer Sequence (5'→3')	Template
WT+mgmO ΔcmmB+mgmO	pSET152-mgmO	DNA ligation	mgmO-2	mgmO-P1	GGAATTC <u>CATA</u> TGACGCGGAA AGTCCTAG (<i>Nde</i> I)	Gene fragment synthesized by GenScript Biotechnology Co., Ltd
				mgmO-P2	CCG <u>GATATCTC</u> AGGCGACGAC GGCGTCGGC (<i>EcoRV</i>)	
			linearized pSET152- <i>ermE</i> *p digested with <i>Nde</i> I/ <i>EcoRV</i>			
S3-CCR-HCD	pSET152-CCR-HCD	Gibson assembly	CCR	CCR-HCD-P1	<u>ACTCCACAGGA</u> <u>GGACCCATATG</u> ACCGTGAAGG ACATCCTGG	genomic DNA of <i>S. coelicolor</i> A3(2)
				CCR-HCD-P2	<u>GGACTCCAGG</u> <u>AATGAGGTCAG</u> ATGTTCCGGAA GCGGTTGATG	
			HCD	CCR-HCD-P3	<u>CCTCATTCTG</u> <u>GAGTCCCGCG</u> ATGGCCACTCC CCTGTCCGAC	
				CCR-HCD-P4	<u>TTGGGCTGCA</u> <u>GGTCGACTCTA</u> GATTATCAGCG GCGGGCATGC TCG	
			linearized pSET152- <i>ermE</i> *p digested with <i>Nde</i> I/ <i>Xba</i> I			
S7	pSET152-CCR-HCD-mgmO	Gibson assembly	mgmO-1	CCR-HCD-mgmO-P1	<u>CATGCCCGCC</u> <u>GCTGATAATCG</u> AGTGTCCGTTT GAGTGGC	pSET152-mgmO
				CCR-HCD-mgmO-P2	<u>CTGCAGGTCG</u> <u>ACTCTAGGCGC</u> TGCAGGTCCG CGGATC	
			linearized pSET152-CCR-HCD digested with <i>Xba</i> I			

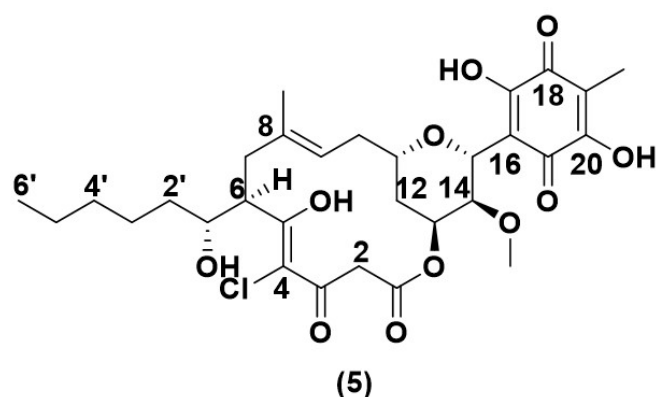
Supplementary Table 5. Plasmids and primers used to construct genetic manipulating mutants in this study.

Mutant Strain	Plasmid	Cloning Method	Fragment	Primer Name	Primer Sequence (5'→3')	Template		
S1	pKC1139-S1	Gibson assembly	S1-1	S1-P1	<u>AGCTATGACATGAT</u> <u>TACGAATTCGAGTT</u> CGGCTACCTGAGC ATCAC	genomic DNA of <i>S. cinnamoneus</i> ATCC 21532		
				S1-P2	<u>TCTTCGAGGATGA</u> <u>CGT</u> GGGCGTTGGT GCCGCTGAC			
			S1-2	S1-P3	<u>ACGTCATCCTCGA</u> AGAGGCAC		linearized pKC1139 digested with <i>EcoRI/HindIII</i>	
				S1-P4	<u>TGCTCGAACGGGT</u> <u>ACGTC</u> GGGCAGGTC CACCC			
			S1-3	S1-P5	<u>ACGTACCCGTTCG</u> <u>AGCAC</u> CGGCACTA C			
				S1-P6	<u>TAAAACGACGGCC</u> <u>AGTGCC</u> AAGCTTG ACGAGAACATCAA GAACGCGGTG			
S2 S4	pKC1139-S2	Gibson assembly	SAT4-1	S2-P1	<u>CAGCTATGACATG</u> <u>ATTACGAATTCATG</u> GCCACCAGTGAGG CGGTG	genomic DNA of <i>S. cinnamoneus</i> ATCC 21532		
				S2-P2	<u>TTCGAGGATGACG</u> <u>TGGG</u> CGTTG			
			SAT4-2	S2-P3	<u>CCACGTCATCCTC</u> <u>GAAC</u> CAGGCCCCCG C	Gene fragment synthesized by GenScript Biotechnology Co., Ltd		
				S2-P4	<u>CTCGAAGGCGTAC</u> <u>GTC</u> GGCAGCTCCA CCGTCCGACCCGA GC			
			SAT4-3	S2-P5	<u>ACGTACGCCTTCG</u> <u>AGC</u> GGC	genomic DNA of <i>S. cinnamoneus</i> ATCC 21532		
				S2-P6	<u>TAAAACGACGGCC</u> <u>AGTGCC</u> AAGCTTT CCTCCCTGCCGAG CGTCAG			
S3 S5	pKC1139-S3	Gibson assembly	SAT5-1	S3-P1	<u>CAGCTATGACATG</u> <u>ATTACGAATTC</u> TGC TCGACGACGGCGT CCTC	genomic DNA of <i>S. cinnamoneus</i> ATCC 21532		
				S3-P2	<u>CCTGTTCGAGGAT</u> <u>CACGT</u> GGGCGTTG GTGCCGCTG			
			SAT5-2	S3-P3	<u>GTGATCCTCGAAC</u> <u>AGG</u> CCCC	Gene fragment synthesized		
				S3-P4	<u>TACGTCGGCAGCT</u>			

					<u>CCACCGTCCGACC</u> CGAGC	by GenScript Biotechnology Co., Ltd
			SAT5-3	S3-P5	<u>TGGAGCTGCCGAC</u> <u>GTACGCGTTCGAG</u>	genomic DNA of S.
				S3-P6	<u>TAAAACGACGGCC</u> <u>AGTGCCAAGCTTA</u> GTTGAGGACCACG TCCACTC	<i>cinnamoneus</i> ATCC 21532
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>			
S5- mgmKS ₅	pKC1139- KS5	Gibson assembly	SKS5-1	KS5-P1	<u>CAGCTATGACATG</u> <u>ATTACGAATTTCGAC</u> GAGGAGGAGTGG CCTGTC	genomic DNA of S.
				KS5-P2	<u>ATGGACACGATGA</u> <u>CGACCGGGTCCCC</u> GTCCGCCGCGCCC GACC	<i>cinnamoneus</i> ATCC 21532
			SKS5-2	KS5-P3	<u>GACCCGGTCGTCA</u> <u>TCGTGTCC</u>	Gene fragment synthesized by GenScript Biotechnology Co., Ltd
				KS5-P4	<u>CTGTTTCGAGGATC</u> <u>ACGTGCGCGTTGG</u> TGCCGGAG	
			SKS5-3	KS5-P5	<u>CACGTGATCCTCG</u> <u>AACAGGC</u>	pKC1139-S3
				KS5-P6	<u>TAAAACGACGGCC</u> AGTGCCAAGCTTC AGGTGAGCCGGAA CAGCGAG	
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>			
S5- mgmKS ₆	pKC1139- KS6	Gibson assembly	SKS6-1	KS6-P1	<u>CAGCTATGACATG</u> <u>ATTACGAATTTCGTC</u> TTCCTGACCGCCTA CTACG	genomic DNA of S.
				KS6-P2	<u>ATCGCGATGGGCT</u> <u>CGTGACGCCGGCT</u> CTCGACCTCCTGC AGC	<i>cinnamoneus</i> ATCC 21532
			SKS6-2	KS6-P3	<u>CACGAGCCCATCG</u> <u>CGATCG</u>	Gene fragment synthesized by GenScript Biotechnology Co., Ltd
				KS6-P4	<u>AGGATGACGTGGG</u> <u>CGTTGGTGCCGGA</u> GATGCCGAAG	
			SKS6-3	KS6-P5	<u>AACGCCACGTCA</u> <u>TCCTGGAAC</u>	genomic DNA of S.
				KS6-P6	<u>TAAAACGACGGCC</u> <u>AGTGCCAAGCTTT</u> GCATGGCCACCAA GGAGGAC	<i>cinnamoneus</i> ATCC 21532
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>			
S5- mgmACP ₅	pKC1139- ACP5	Gibson assembly	ACP5-1	ACP5- P1	CAGCTATGACATGA TTACGAATTCTGGC ACTGGCCCAGGAG	genomic DNA of S. <i>cinnamoneus</i>

					TG	ATCC 21532	
				ACP5-P2	AGCCGTTCGGCCA GGGTGCCGCCGCC CTCGG		
				ACP5-2	ACP5-P3	ACCCTGGCCGAAC GGCTC	Gene fragment synthesized by GenScript Biotechnology Co., Ltd
					ACP5-P4	TCCTCCGGCACCA GCAGC	
				ACP5-3	ACP5-P5	AGCTGCTGGTGCC GGAGGAGCCGTC	genomic DNA of <i>S.</i> <i>cinnamoneus</i> ATCC 21532
					ACP5-P6	TAAAACGACGGCC AGTGCCAAGCTTG CTGATCCCGAACG ACGACAC	
				linearized pKC1139 digested with <i>EcoRI/HindIII</i>			
S6	pKC1139-cmmB (Ref. 1)	DNA ligation	cmmB-1	cmmB-P1	CG <u>GAATTC</u> CGATC ACCACCGGACTGA AG (<i>EcoRI</i>)	genomic DNA of <i>S.</i> <i>cinnamoneus</i> ATCC 21532	
				cmmB-P2	GCT <u>TCTAG</u> AGGATG GCTGCCGCTCATC CAG (<i>XbaI</i>)		
			cmmB-2	cmmB-P3	GCT <u>TCTAG</u> AGATCA CGGAGCCTCCAGC G (<i>XbaI</i>)		
				cmmB-P4	CCC <u>AAGCTT</u> CGGC TCGGCACCATGAA GACGTC (<i>HindIII</i>)		
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>				
S5-ASC	pKC1139-ASC	Gibson assembly	ASC-1	ASC-P1	<u>CAGCTATGACATG</u> <u>ATTAC</u> GAATTCGAC GAGGAGGAGTGGA CCTGTC	genomic DNA of <i>S.</i> <i>cinnamoneus</i> ATCC 21532	
				ASC-P2	<u>GTGGACATGACGG</u> <u>CCACCCCGC</u>		
			ASC-2	ASC-P3	<u>TGGCCGTCATGTC</u> <u>CAC</u> GGCCGATCTG TTCG	Gene fragment synthesized by GenScript Biotechnology Co., Ltd	
				ASC-P4	<u>GGCGAACGCGGT</u> <u>GCCGTC</u>		
			ASC-3	A230T-P5	<u>GACGGCACCGCGT</u> <u>TCGCC</u> GAGGGGGT GGGCATGGTGC	genomic DNA of <i>S.</i> <i>cinnamoneus</i> ATCC 21532	
				A230T-P6	<u>CTGTTGAGGATC</u> <u>AC</u> GTGGGCGTTGG TGCCGCTGAC		
			ASC-4	ASC-P7	CAC <u>GTGATCCTCG</u> <u>AACAGGC</u>	pKC1139-S3	
				ASC-P8	<u>TAAAACGACGGCC</u> <u>AGTGCC</u> AAGCTTC AGGTGAGCCGGAA CAGCGAG		
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>				

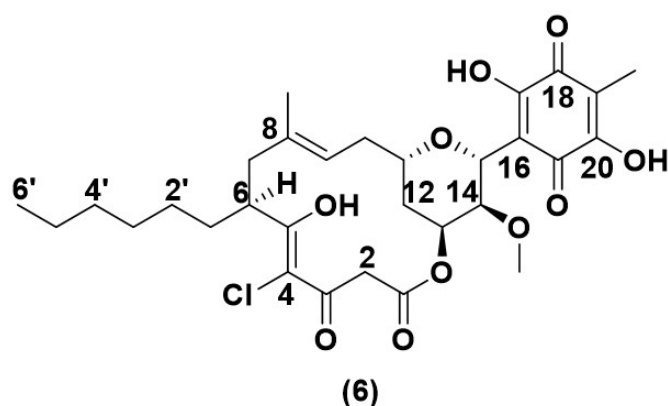
S5-A230T	pKC1139-A230T	Gibson assembly	A230T1	A230T-P1	<u>CAGCTATGACATG</u> <u>ATTAC</u> GAATTCGAC GAGGAGGAGTGGA CCTGTC	genomic DNA of <i>S. cinnamoneus</i> ATCC 21532
				A230T-P2	<u>TCCGCAAAGGCCT</u> <u>TGC</u> AGCG	
			A230T-2	A230T-P3	<u>TGCAAGGCCTTTG</u> <u>CGGACGGCGCGG</u> <u>A</u> CGGCACCGCCTT CGGTGAGGG	
				A230T-P4	<u>CTGTTGAGGATC</u> <u>AC</u> GTGGGCGTTGG TGCCGCTGAC	
			A230T-3	A230T-P5	CAC <u>GTGATCCTCG</u> <u>AAC</u> AGGC	pKC1139-S3
				A230T-P6	<u>TAAAACGACGGCC</u> <u>AGTGCC</u> AAGCTTC AGGTGAGCCGGA CAGCGAG	
			linearized pKC1139 digested with <i>EcoRI/HindIII</i>			

Supplementary Table 6. NMR data of compound **5**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		165.1	C
2a	3.55 (d, 1H, $J=15.7$ Hz)	46.5	CH ₂
2b	3.82 (d, 1H, $J=15.7$ Hz)		
3		188.3	C
4		110.4	C
5		188.7	C
6	3.41 (m, 1H)	47.6	CH
7a	2.11 (dd, 1H, $J=12.9, 3.2$ Hz)	39.7	CH ₂
7b	2.69 (t, 1H, $J=12.7$ Hz)		
8		134.6	C
9	5.19 (t, 1H, $J=8.4$ Hz)	122.6	CH
10a	2.16 (dd, 1H, $J=12.8$ Hz, 8.0Hz)	33.7	CH ₂
10b	2.23 (dt, 1H, $J=12.7$ Hz, 10.0Hz)		
11	3.26 (overlapped, 1H)	75.5	CH
12a	1.66 (m, 1H)	31.2	CH ₂
12b	1.85 (ddd, 1H, $J=16.8, 19.9, 4.2$ Hz)		
13	5.39 (q, 1H, $J=3.0$ Hz)	67.1	CH
14	3.25 (overlapped, 1H)	75.5	CH
15	4.96 (d, 1H, $J=1.8$ Hz)	75.1	CH
16		111.1	C
17		*	C
18		*	C
19		114.3	C
20		*	C
21		*	C
8-CH ₃	1.68 (s, 3H)	16.7	CH ₃
14-OCH ₃	3.40 (s, 3H)	59.3	CH ₃
19-CH ₃	1.94 (s, 3H)	7.8	CH ₃
1'	3.74 (d, 1H, $J=5.0$ Hz)	74.3	CH
2'a	1.40 (m, 1H)	35.8	CH ₂
2'b	1.58 (m, 1H)		
3'a	1.39 (m, 1H)	25.2	CH ₂
3'b	1.53 (m, 1H)		
4'	1.30 (m, 2H)	31.7	CH ₂
5'	1.32 (m, 2H)	22.6	CH ₂
6'	0.90 (t, 3H, $J=6.9$ Hz)	14.0	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in CDCl₃).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

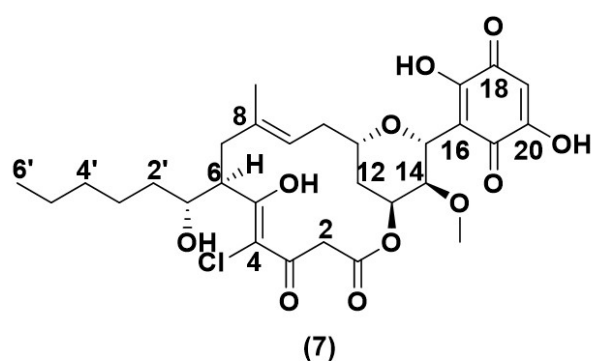
Supplementary Table 7. NMR data of compound **6**.

NO.	δ H (mult., J in Hz)	δ C	C-type
1		165.3	C
2a	3.54 (d, 1H, J=15.7Hz)	46.9	CH ₂
2b	3.82 (d, 1H, J=15.7Hz)		
3		189.7	C
4		109.1	C
5		188.5	C
6	3.29 (m, 1H)	42.0	CH
7a	2.07 (dd, 1H, J=13.0, 3.0Hz)	42.1	CH ₂
7b	2.47 (dd, 1H, J=13.5, 6.2Hz)		
8		135.4	C
9	5.16 (t, 1H, J=8.3Hz)	122.0	CH
10a	2.17 (m, 1H)	33.7	CH ₂
10b	2.21 (m, 1H)		
11	3.25 (overlapped, 1H)	75.5	CH
12a	1.67 (m, 1H)	31.1	CH ₂
12b	1.83 (ddd, 1H, J=14.0, 11.3, 2.5 Hz)		
13	5.39 (q, 1H, J=3.0Hz)	67.1	CH
14	3.25 (overlapped, 1H)	75.5	CH
15	4.95 (d, 1H, J =1.8Hz)	75.2	CH
16		111.2	C
17		*	C
18		*	C
19		114.1	C
20		*	C
21		*	C
8-CH ₃	1.66 (s, 3H)	16.8	CH ₃
14-OCH ₃	3.40 (s, 3H)	59.2	CH ₃
19-CH ₃	1.94 (s, 3H)	7.8	CH ₃
1'a	1.47 (m, 1H)	33.9	CH ₂
1'b	1.67 (m, 1H)		
2'a	1.27 (m, 1H)	27.2	CH ₂
2'b	1.34 (m, 1H)		
3'	1.27 (m, 2H)	29.3	CH ₂
4'	1.26 (m, 2H)	31.6	CH ₂
5'	1.28 (m, 2H)	22.6	CH ₂
6'	0.88 (t, 3H, J=7.0 Hz)	14.1	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-d₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

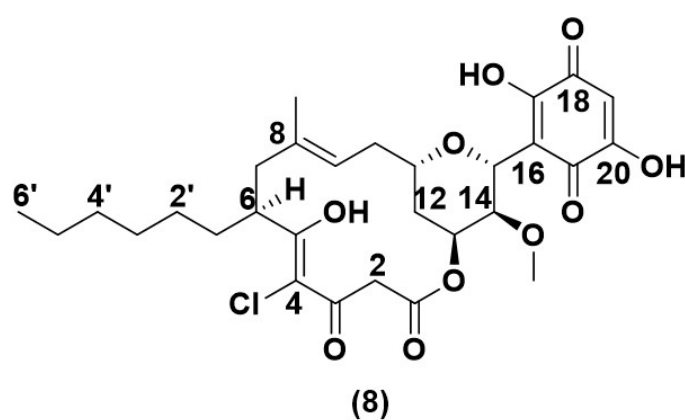
Supplementary Table 8. NMR data of compound 7.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		165.0	C
2a	3.55 (d, 1H, $J=15.7$ Hz)	46.7	CH ₂
2b	3.82 (d, 1H, $J=15.7$ Hz)		
3		188.2	C
4		110.4	C
5		188.8	C
6	3.41 (m, 1H)	47.7	CH
7a	2.11 (dd, 1H, $J=12.9, 3.2$ Hz)	39.6	CH ₂
7b	2.69 (t, 1H, $J=12.7$ Hz)		
8		134.7	C
9	5.19 (t, 1H, $J=8.4$ Hz)	122.6	CH
10a	2.18 (dd, 1H, $J=12.8, 6.9$ Hz)	33.7	CH ₂
10b	2.24 (dt, 1H, $J=12.7, 10.0$ Hz)		
11	3.26 (overlapped, 1H)	75.5	CH
12a	1.67 (m, 1H)	31.2	CH ₂
12b	1.86 (ddd, 1H, $J=14.0, 11.3, 2.5$ Hz)		
13	5.39 (q, 1H, $J=2.9$ Hz)	67.1	CH
14	3.26 (overlapped, 1H)	75.5	CH
15	4.98 (d, 1H, $J=1.8$ Hz)	75.1	CH
16		112.0	C
17		*	C
18		*	C
19	6.00 (s, 1H)	105.0	CH
20		*	C
21		*	C
8-CH ₃	1.68 (s, 3H)	16.8	CH ₃
14-OCH ₃	3.41 (s, 3H)	59.3	CH ₃
1'	1.47 (m, 1H)	33.9	CH
2'a	1.40 (m, 1H)	35.8	CH ₂
2'b	1.58 (m, 1H)		
3'a	1.39 (m, 1H)	25.2	CH ₂
3'b	1.53 (m, 1H)		
4'	1.31 (m, 2H)	31.9	CH ₂
5'	1.32 (m, 2H)	22.7	CH ₂
6'	0.90 (t, 3H, $J=6.9$ Hz)	13.9	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

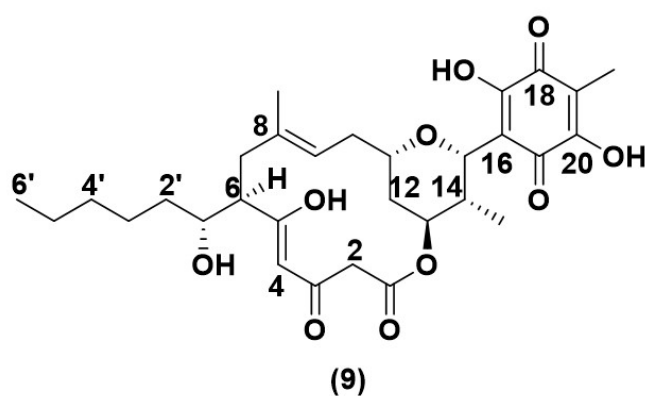
* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 9. NMR data of compound **8**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		165.2	C
2a	3.54 (d, 1H, $J=15.7$ Hz)	47.0	CH ₂
2b	3.83 (d, 1H, $J=15.7$ Hz)		
3		189.7	C
4		109.1	C
5		188.5	C
6	3.29 (m, 1H)	42.2	CH
7a	2.07 (dd, 1H, $J=12.9, 2.4$ Hz)	42.4	CH ₂
7b	2.48 (t, 1H, $J=12.6$)		
8		135.5	C
9	5.16 (t, 1H, $J=8.4$ Hz)	122.0	CH
10a	2.17 (m, 1H)	33.6	CH ₂
10b	2.21 (m, 1H)		
11	3.25 (overlapped, 1H)	75.5	CH
12a	1.68 (m, 1H)	31.1	CH ₂
12b	1.84 (ddd, 1H, $J=14.1, 11.2, 2.5$ Hz)		
13	5.39 (q, 1H, $J=3.0$ Hz)	67.1	CH
14	3.25 (overlapped, 1H)	75.5	CH
15	4.95 (d, 1H, $J=1.7$ Hz)	75.2	CH
16		111.2	C
17		*	C
18		*	C
19	5.99 (s, 1H)	105.0	CH
20		*	C
21		*	C
8-CH ₃	1.67 (s, 3H)	16.7	CH ₃
14-OCH ₃	3.39 (s, 3H)	59.2	CH ₃
1'a	1.47 (ddt, 1H, $J=14.3, 10.1, 5.2$ Hz)	33.9	CH ₂
1'b	1.67 (m, 1H)		
2'a	1.27 (m, 1H)	27.2	CH ₂
2'b	1.34 (m, 1H)		
3'	1.26 (m, 2H)	29.5	CH ₂
4'	1.26 (m, 2H)	31.6	CH ₂
5'	1.28 (m, 2H)	22.5	CH ₂
6'	0.88 (t, 3H, $J=6.9$ Hz)	14.0	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

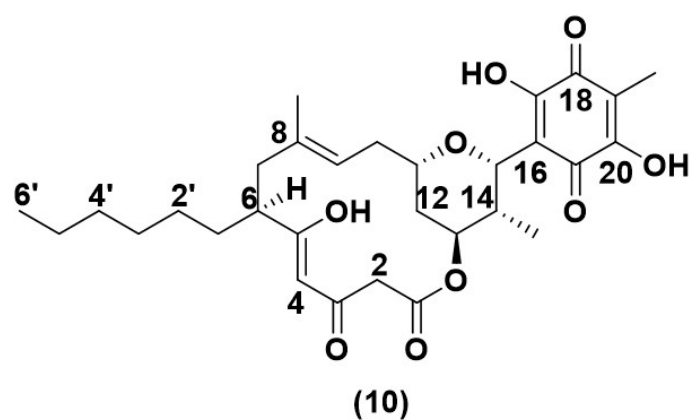
Supplementary Table 10. NMR data of compound **9**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.0	C
2	3.43 (td, 2H, $J=21.4, 7.4$ Hz)	47.5	CH ₂
3		190.0	C
4	5.48 (s, 1H)	101.2	CH
5		191.2	C
6	2.30 (ddd, $J=12.4, 5.7, 3.2$ Hz)	52.6	CH
7a	2.08 (dd, 1H, $J=13.1, 3.2$ Hz)	39.7	CH ₂
7b	2.59 (t, 1H, $J=12.7$ Hz)		
8		135.6	C
9	5.12 (overlapped, 1H)	121.6	CH
10a	2.16 (dt, 1H, $J=7.4, 2.7$ Hz)	34.5	CH ₂
10b	2.24 (m, 1H)		
11	3.36 (ddd, 1H, $J=12.9, 7.1, 4.3$ Hz)	76.0	CH
12	1.68 (m, 2H)	30.7	CH ₂
13	5.14 (overlapped, 1H)	72.1	CH
14	2.16 (dtd, 1H, $J=10.0, 7.2, 2.6$ Hz)	34.5	CH
15	5.21 (d, 1H, $J=2.7$ Hz)	76.0	CH
16		112.4	C
17		*	C
18		*	C
19		114.2	C
20		*	C
21		*	C
8-CH ₃	1.61 (s, 3H)	17.1	CH ₃
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.5	CH ₃
19-CH ₃	1.93 (s, 3H)	7.9	CH ₃
1'	3.69 (m, 1H)	73.7	CH
2'a	1.40 (m, 1H)	36.0	CH ₂
2'b	1.50 (m, 1H)		
3'a	1.35 (m, 1H)	25.4	CH ₂
3'b	1.49 (m, 1H)		
4'	1.31 (m, 2H)	31.7	CH ₂
5'	1.29 (m, 2H)	22.6	CH ₂
6'	0.89 (t, 3H, $J=6.9$ Hz)	14.1	

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 11. NMR data of compound **10**.

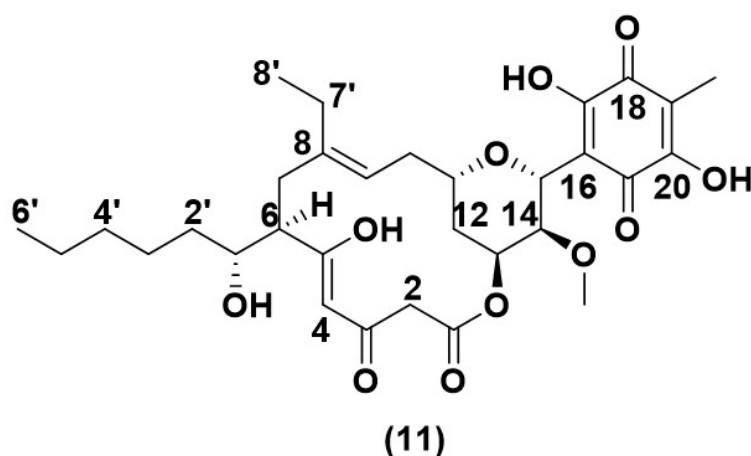


NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.2	C
2	3.42 (m, 2H)	48.5	CH ₂
3		191.4	C
4	5.44 (s, 1H)	99.8	CH
5		190.5	C
6	2.17 (overlapped, 1H)	47.2	CH
7a	2.02 (dd, 1H, $J=12.8, 3.0$ Hz)	42.5	CH ₂
7b	2.36 (t, 1H, $J=12.5$ Hz)		
8		136.2	C
9	5.09 (t, 1H, $J=8.0$ Hz)	121.3	CH
10	2.23 (m, 2H)	34.4	CH ₂
11	3.35 (m, 1H)	76.5	CH
12a	1.65 (ddd, 1H, $J=14.4, 11.1, 2.5$ Hz)	30.7	CH ₂
12b	1.69 (overlapped, 1H)		
13	5.14 (q, 1H, $J=2.8$ Hz)	72.1	CH
14	2.16 (overlapped, 1H)	35.0	CH
15	5.21 (d, 1H, $J=2.7$ Hz)	76.5	CH
16		112.4	C
17		*	C
18		*	C
19		114.1	C
20		*	C
21		*	C
8-CH ₃	1.59 (d, 3H, $J=1.4$ Hz)	17.0	CH ₃
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.6	CH ₃
19-CH ₃	1.93 (s, 3H)	7.8	CH ₃
1'a	1.38 (m, 1H)	33.2	CH ₂
1'b	1.68 (overlapped, 1H)		
2'	1.26 (overlapped, 2H)	29.3	CH ₂
3'	1.26 (overlapped, 2H)	27.7	CH ₂
4'	1.26 (overlapped, 2H)	31.6	CH ₂
5'	1.26 (overlapped, 2H)	22.6	CH ₂
6'	0.88 (t, 3H, $J=6.9$ Hz)	14.1	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

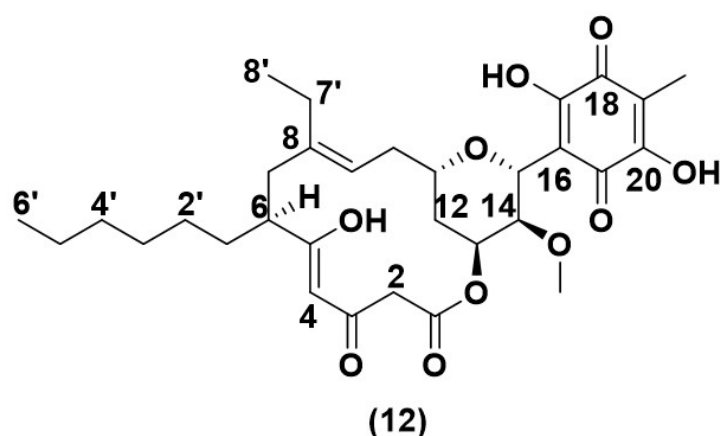
Supplementary Table 12. NMR data of compound 11.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		166.7	C
2a	3.43 (d, 1H, $J=14.1$ Hz)	47.3	CH ₂
2b	3.46 (d, 1H, $J=14.1$ Hz)		
3		189.3	C
4	5.46 (s, 1H)	101.3	CH
5		191.3	C
6	2.27 (ddd, 1H, $J=12.3, 5.7, 3.1$ Hz)	52.8	CH
7a	2.20 (overlapped, 1H)	35.5	CH ₂
7b	2.50 (t, 1H, $J=12.8$ Hz)		
8		141.1	C
9	5.04 (overlapped, 1H)	121.0	CH
10	2.22 (overlapped, 1H)	33.6	CH ₂
11	3.29 (t, 1H, $J=10.3$ Hz)	74.9	CH
12a	1.74 (d, 1H, $J=14.3$ Hz)	30.1	CH ₂
12b	1.80 (d, 1H, $J=14.3$ Hz)		
13	5.34 (q, 1H, $J=3.0$ Hz)	66.3	CH
14	3.28 (m, 1H)	75.8	CH
15	5.03 (overlapped, 1H)	74.6	CH
16		111.2	C
17		*	C
18		*	C
19		114.2	C
20		*	C
21		*	C
14-OCH ₃	3.37 (s, 3H)	59.2	CH ₃
19-CH ₃	1.94 (s, 3H)	7.7	CH ₃
1'	3.69 (ddd, 1H, $J=9.0, 5.8, 2.9$ Hz)	73.9	CH
2'a	1.41 (m, 1H)	35.9	CH ₂
2'b	1.50 (m, 1H)		
3'a	1.35 (m, 2H)	25.3	CH ₂
3'b	1.50 (m, 2H)		
4'	1.31 (m, 2H)	31.6	CH ₂
5'	1.31 (m, 2H)	23.2	CH ₂
6'	0.90 (t, 3H, $J=6.9$ Hz)	13.9	CH ₃
7'a	1.82 (m, 1H)	23.21	CH ₂
7'b	2.10 (dq, 1H, $J=14.8, 7.5$ Hz)		
8'	0.95 (t, 3H, $J=7.2$ Hz)	12.8	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

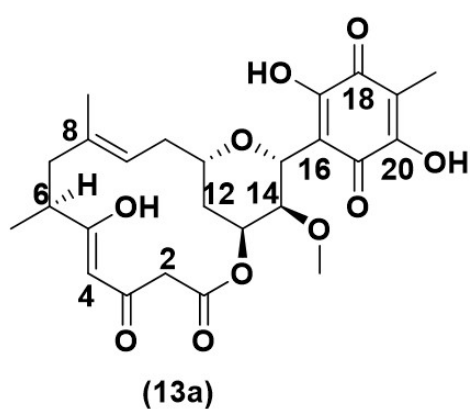
* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 13. NMR data of compound **12**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.0	C
2a	3.43 (d, 1H, $J=13.9$ Hz)	48.1	CH ₂
2b	3.48 (d, 1H, $J=13.9$ Hz)		
3		190.8	C
4	5.44 (s, 1H)	99.9	CH
5		190.8	C
6	2.17 (overlapped, 1H)	48.1	CH
7a	2.16 (overlapped, 1H)	38.3	CH ₂
7b	2.31 (m, 1H)		
8		141.6	C
9	5.04 (t, 1H, $J=8.3$ Hz)	120.6	CH
10	2.24 (overlapped, 2H)	33.6	CH ₂
11	3.30 (overlapped, 1H)	75.6	CH
12	1.79 (q, 2H, $J=2.5$ Hz)	30.2	CH ₂
13	5.37 (q, 1H, $J=2.9$ Hz)	66.4	CH
14	3.30 (overlapped, 1H)	75.6	CH
15	5.07 (d, 1H, $J=1.7$ Hz)	74.7	CH
16		111.2	C
17		*	C
18		*	C
19		114.2	C
20		*	C
21		*	C
14-OCH ₃	3.40 (s, 3H)	59.2	CH ₃
19-CH ₃	1.97 (s, 3H)	7.8	CH ₃
1'a	1.43 (ddd, 1H, $J=13.8, 8.7, 5.0$ Hz)	33.4	CH
1'b	1.71 (m, 1H)		
2'	1.29 (overlapped, 2H)	29.2	CH ₂
3'	1.29 (overlapped, 2H)	27.5	CH ₂
4'	1.29 (overlapped, 2H)	31.6	CH ₂
5'	1.30 (overlapped, 2H)	22.5	CH ₂
6'	0.91 (q, 3H, $J=6.2$ Hz)	14.1	CH ₃
7'a	1.86 (m, 1H)	23.4	CH ₂
7'b	2.11 (m, 1H)		
8'	0.98 (t, 3H, $J=7.5$ Hz)	13.0	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

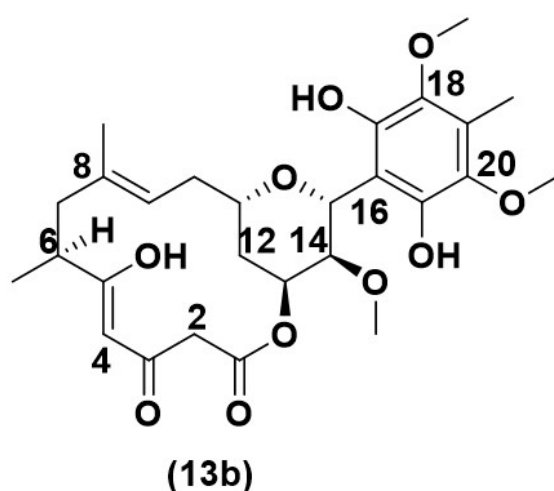
* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 14. NMR data of compound **13a**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2a	3.39 (d, 1H, $J=13.7$ Hz)	48.2	CH ₂
2b	3.45 (d, 1H, $J=13.7$ Hz)		
3		190.9	C
4	5.42 (s, 1H)	98.3	CH
5		190.9	C
6	2.33 (m, 1H)	41.1	CH
7a	1.98 (m, 1H)	43.3	CH ₂
7b	2.38 (overlapped, 1H)		
8		135.9	C
9	5.08 (t, 1H, $J=8.4$ Hz)	121.4	CH
10a	2.21 (m, 1H)	34.2	CH ₂
10b	2.35 (overlapped, 1H)		
11	3.29 (m, 1H)	74.7	CH
12a	1.60 (overlapped, 1H)	30.4	CH ₂
12b	1.78 overlapped, 1H)		
13	5.34 (q, 1H, $J=3.0$ Hz)	66.2	CH
14	3.27 (m, 1H)	75.7	CH
15	5.03 (d, 1H, $J=1$ Hz)	74.7	CH
16		111.1	C
17		*	C
18		*	C
19		114.0	C
20		*	C
21		*	C
6-CH ₃	1.18 (d, 3H, $J=6.3$ Hz)	18.4	CH ₃
8-CH ₃	1.58 (d, 3H, $J=1.6$ Hz)	18.2	CH ₃
14-OCH ₃	3.36 (s, 3H)	59.1	CH ₃
19-CH ₃	1.94 (s, 3H)	7.5	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in CDCl₃).

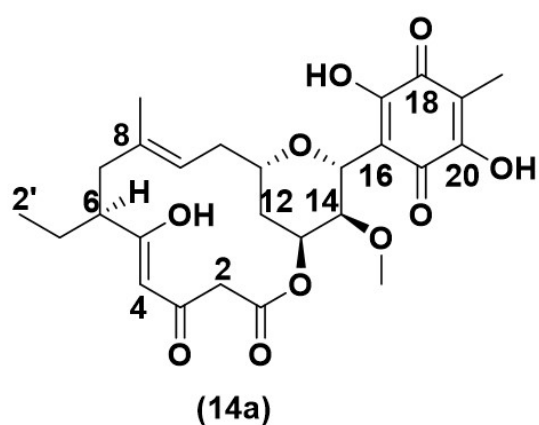
* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 15. NMR data of compound **13b**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2a	3.39 (d, 1H, $J=14.0$ Hz)	48.3	CH ₂
2b	3.44 (d, 1H, $J=14.0$ Hz)		
3		191.5	C
4	5.46 (s, 1H)	98.5	CH
5		191.2	C
6	2.35 (m, 1H)	41.4	CH
7a	1.99 (d, 1H, $J=10.66$ Hz)	44.0	CH ₂
7b	2.36 (m, 1H)		
8		135.5	C
9	5.11 (t, 1H, $J=8.4$ Hz)	121.8	CH
10a	2.21 (overlapped, 1H)	34.3	CH ₂
10b	2.29 (m, 1H)		
11	3.31 (m, 1H)	74.8	CH
12a	1.60 (overlapped, 1H)	30.7	CH ₂
12b	1.82 (ddd, 1H, $J=14.0, 11.3, 2.5$ Hz)		
13	5.32 (q, 1H, $J=2.9$ Hz)	67.9	CH
14	3.32 (m, 1H)	76.6	CH
15	5.26 (d, 1H, $J=1.6$ Hz)	75.4	CH
16		107.38	C
17		144.3	C
18		138.4	C
19		123.8	C
20		138.4	C
21		144.3	C
6-CH ₃	1.18 (d, 3H, $J=6.1$ Hz)	18.5	CH ₃
8-CH ₃	1.60 (s, 3H)	17.0	CH ₃
14-OCH ₃	3.21 (s, 3H)	59.6	CH ₃
18-OCH ₃	3.74 (s, 3H)	60.6	CH ₃
19-CH ₃	2.21 (s, 3H)	9.5	CH ₃
20-OCH ₃	3.74 (s, 3H)	60.6	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

Supplementary Table 16. NMR data of compound **14a**.

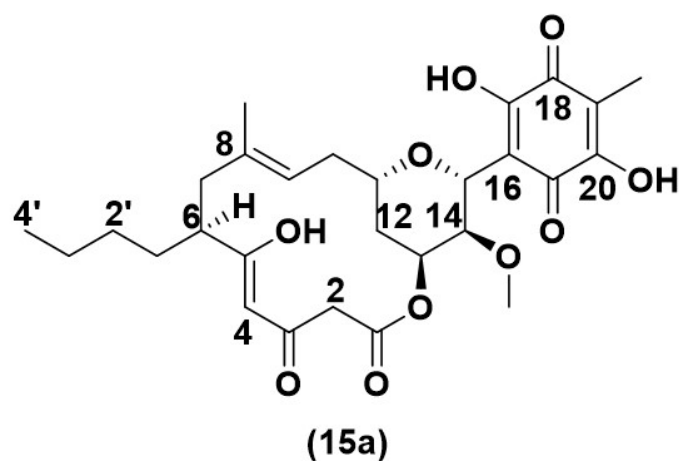


NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		166.9	C
2a	3.40 (d, 1H)	48.2	CH ₂
2b	3.46 (m, 1H)		
3		191.1	C
4	5.44 (s, 1H)	100.0	CH
5		190.1	C
6	2.09 (m, 1H)	48.9	CH
7a	2.04 (dd, 1H, $J=12.8, 3.2$ Hz)	42.1	CH ₂
7b	2.36 (t, 1H, $J=12.5$ Hz)		
8		135.9	C
9	5.09 (t, 1H, $J=8.4$ Hz)	121.5	CH
10	2.21 (m, 1H)	34.1	CH ₂
11	3.28 (overlapped, 1H)	75.3	CH
12	1.75 (ddd, 1H, $J = 14.0, 11.2, 2.6$ Hz)	30.4	CH ₂
13	5.35 (q, 1H, $J=3.0$ Hz)	66.3	CH
14	3.28 (overlapped, 1H)	75.3	CH
15	5.04 (d, 1H, $J=1.7$ Hz)	74.8	CH
16		111.2	C
17		*	C
18		*	C
19		114.2	C
20		*	C
21		*	C
8-CH ₃	1.59 (s, 3H)	17.0	CH ₃
14-OCH ₃	3.37 (s, 3H)	59.2	CH ₃
19-CH ₃	1.95 (s, 3H)	7.7	CH ₃
1'a	1.47 (m, 1H)	26.2	CH ₂
1'b	1.71 (m, 1H)		
2'	0.89 (t, 3H, $J=7.4$ Hz)	12.1	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

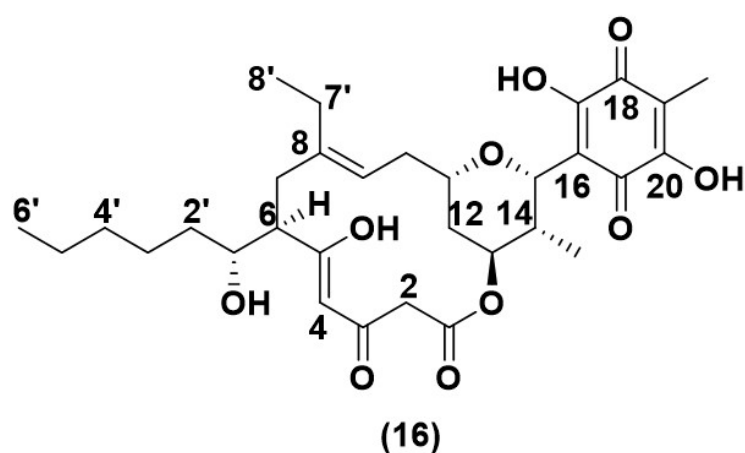
Supplementary Table 17. NMR data of compound **15a**.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2a	3.40 (d, 1H, $J=13.8$ Hz)	48.2	CH ₂
2b	3.46 (d, 1H, $J=13.8$ Hz)		
3		191.0	C
4	5.43 (s, 1H)	99.7	CH
5		190.5	C
6	2.16 (m, 1H)	46.9	CH
7a	2.02 (m, 1H)	42.5	CH ₂
7b	2.36 (t, 1H, $J=12.5$)		
8		136.0	C
9	5.08 (t, 1H, $J=8.4$ Hz)	121.4	CH
10	2.20 (t, 1H, $J=7.9$ Hz)	34.1	CH ₂
11	3.28 (overlapped, 1H)	74.7	CH
12a	1.70 (m, 1H)	30.4	CH ₂
12b	1.78 (ddd, 1H, $J=14.1, 11.3, 2.5$ Hz)		
13	5.35 (q, 1H, $J=2.9$ Hz)	66.3	CH
14	3.27 (overlapped, 1H)	75.7	CH
15	5.03 (d, 1H, $J=1.7$ Hz)	74.7	CH
16		111.2	C
17		*	C
18		*	C
19		114.1	CH
20		*	C
21		*	C
8-CH ₃	1.59 (s, 3H)	17.00	CH ₃
14-OCH ₃	3.37 (s, 3H)	59.3	CH ₃
1'a	1.40 (m, 1H)	33.0	CH ₂
1'b	1.69 (m, 1H)		
2'	1.23 (m, 1H)	30.0	CH ₂
3'	1.30 (m, 2H)	22.7	CH ₂
4'	0.88 (t, 3H, $J=7.2$ Hz)	13.9	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

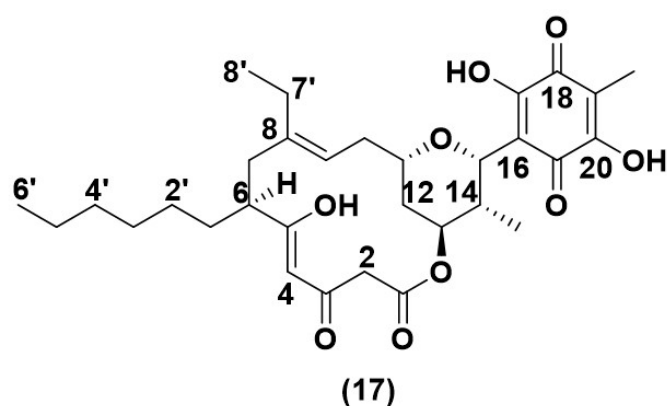
Supplementary Table 18. NMR data of compound **16**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		166.9	C
2a	3.42 (d, H, $J=14.1$ Hz)	47.7	CH ₂
2b	3.46 (d, 1H, $J=14.1$ Hz)		
3		189.4	C
4	5.47 (s, 1H)	101.4	CH
5		191.5	C
6	2.26 (overlapped, 1H)	52.5	CH
7a	2.17 (m, 1H)	35.4	CH ₂
7b	2.50 (t, 1H, $J=12.7$ Hz)		
8		141.0	C
9	5.04 (t, 1H, $J=8.3$ Hz)	120.9	CH
10	2.26 (overlapped, 2H)	52.5	CH ₂
11	3.36 (m, 1H)	76.5	CH
12a	1.66 (m, 1H)	30.2	CH ₂
12b	1.72 (m, 1H)		
13	5.14 (t, 1H, $J=2.9$ Hz)	72.1	CH
14	2.16 (m, 1H)	35.0	CH
15	5.21 (d, 1H, $J=2.7$ Hz)	76.5	CH
16		112.2	C
17		*	C
18		*	C
19		114.1	C
20		*	C
21		*	C
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.3	CH ₃
19-CH ₃	1.93 (s, 3H)	7.6	CH ₃
1'	3.68 (m, 1H)	72.1	CH
2'a	1.40 (m, 1H)	35.9	CH ₂
2'b	1.49 (m, 1H)		
3'a	1.35 (m, 1H)	25.1	CH ₂
3'b	1.48 (m, 1H)		
4'	1.29 (m, 2H)	31.6	CH ₂
5'	1.31 (m, 2H)	22.8	CH ₂
6'	0.89 (d, 3H, $J=7.1$ Hz)	13.9	CH ₃
7'a	1.83 (m, 1H)	23.2	CH ₂
7'b	2.10 (m, 1H)		
8'	0.96 (t, 3H, $J=7.5$ Hz)	12.8	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

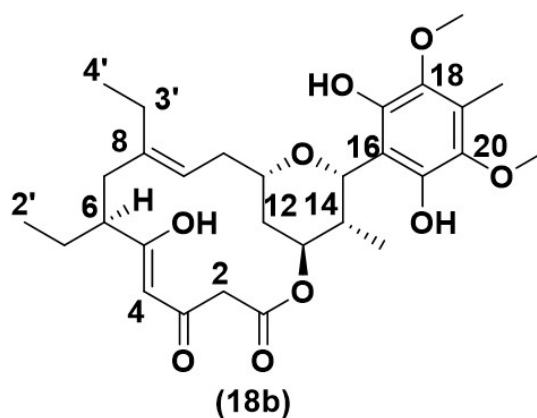
Supplementary Table 19. NMR data of compound 17.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2a	3.41 (d, 1H, $J=13.8$ Hz)	48.3	CH ₂
2b	3.44 (d, 1H, $J=13.8$ Hz)		
3		191.2	C
4	5.42 (s, 1H)	100.0	CH
5		190.5	C
6	2.13 (overlapped, 1H)	47.4	CH
7a	2.13 (overlapped, 1H)	38.2	CH ₂
7b	2.27 (t, 1H, $J=13.3$ Hz)		
8		141.6	C
9	5.01 (t, 1H, $J=8.3$ Hz)	120.1	CH
10	2.23 (m, 2H)	33.8	CH ₂
11	3.34 (m, 1H)	75.9	CH
12a	1.64 (overlapped, 1H)	30.4	CH ₂
12b	1.73 (overlapped, 1H)		
13	5.14 (q, 1H, $J=2.8$ Hz)	71.8	CH
14	2.16 (m, 1H)	35.0	CH
15	5.22 (d, 1H, $J=2.7$ Hz)	76.2	CH
16		112.2	C
17		*	C
18		*	C
19		114.1	C
20		*	C
21		*	C
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.5	CH ₃
19-CH ₃	1.93 (s, 3H)	7.6	CH ₃
1'a	1.40 (m, 1H)	33.8	CH ₂
1'b	1.67 (overlapped, 1H)		
2'	1.26 (overlapped, 2H)	29.3	CH ₂
3'	1.25 (overlapped, 2H)	27.1	CH ₂
4'	1.29 (overlapped, 2H)	31.6	CH ₂
5'	1.28 (overlapped, 2H)	22.4	CH ₂
6'	0.88 (t, 3H, $J=7.0$ Hz)	14.0	CH ₃
7'a	1.81 (m, 1H)	23.1	CH ₂
7'b	2.07 (m, 1H)		
8'	0.95 (t, 3H, $J=7.5$ Hz)	13.1	CH ₃

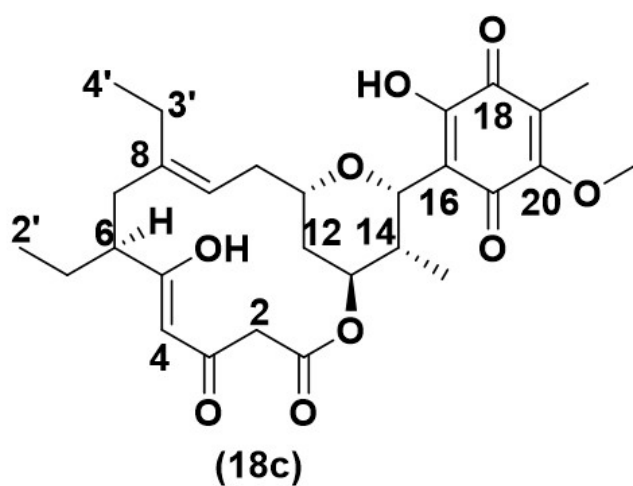
¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 20. NMR data of compound **18b**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.3	C
2	3.42(d, 2H, $J=13.8$ Hz)	48.4	CH ₂
3		191.3	C
4	5.46 (overlapped, 1H)	100.2	CH
5		190.5	C
6	2.07 (m, 1H)	49.2	CH
7a	2.14 (m, 1H)	38.1	CH ₂
7b	2.26 (d, 1H, $J=12.8$ Hz)		
8		141.3	C
9	5.04 (t, 1H, $J=8.1$ Hz)	121.1	CH
10a	2.22 (m, 1H)	34.1	CH ₂
10b	2.31 (m, 1H)		
11	3.38 (m, 1H)	75.8	CH
12a	1.68 (m, 1H)	30.5	CH ₂
12b	1.74 (m, 1H)		
13	5.17 (td, 1H, $J=22.2, 1.7$ Hz)	72.7	CH
14	2.18 (overlapped, 1H)	35.4	CH
15	5.46 (overlapped, 1H)	75.8	CH
16		108.5	C
17		141.7	C
18		138.7	C
19		123.4	C
20		138.7	C
21		141.7	C
14-CH ₃	0.98 (m, 3H)	11.6	CH ₃
18-OCH ₃	3.73 (s, 3H)	60.7	
19-CH ₃	2.20 (s, 3H)	9.4	CH ₃
20-OCH ₃	3.73 (s, 3H)	60.7	
1'a	1.46 (m, 1H)	26.4	CH
1'b	1.70 (m, 1H)		
2'	0.89 (t, 3H, $J=7.4$ Hz)	12.2	CH ₂
3'a	1.83 (dq, 1H, $J=14.8, 7.6$ Hz)	23.4	CH ₂
3'b	2.09 (overlapped, 1H)		
4'	0.95 (t, 3H, $J=7.6$ Hz)	12.4	CH ₂

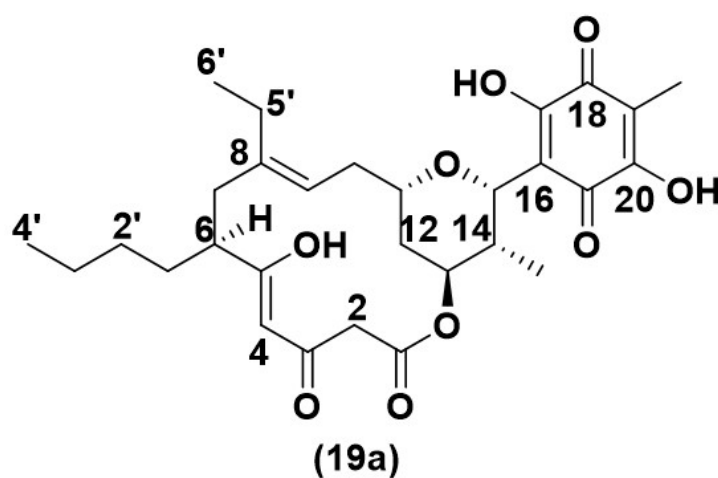
¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

Supplementary Table 21. NMR data of compound **18c**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2	3.43 (s, 1H)	48.4	CH ₂
3		191.5	C
4	5.44 (s, 1H)	100.0	CH
5		190.0	C
6	2.06 (m, 1H)	48.4	CH
7a	2.14 (m, 1H)	38.1	CH ₂
7b	2.27 (t, 1H, $J=12.5$ Hz)		
8		141.8	C
9	5.02 (t, $J=8.3$ Hz, 1H)	120.4	CH
10a	2.22 (m, 1H)	33.2	CH ₂
10b	2.36 (q, 1H, $J=7.7$ Hz)		
11	3.36 (ddt, 1H, $J=12.0, 9.7, 2.4$ Hz)	76.6	CH
12a	1.63 (m, 1H)	30.3	CH ₂
12b	1.73 (m, 1H)		
13	5.14 (q, 1H, $J=2.8$ Hz)	71.8	CH
14	2.18 (m, 1H)	35.1	CH
15	5.21 (d, 1H, $J=2.7$ Hz)	76.6	CH
16		114.6	C
17		155.3	C
18		183.2	C
19		125.8	C
20		155.5	C
21		182.00	C
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.4	CH ₃
19-CH ₃	1.94 (s, 3H)	8.0	CH ₃
20-OCH ₃	4.01 (s, 3H)	61.5	CH ₃
1'a	1.46 (m, 1H)	25.9	CH ₂
1'b	1.70 (m, 1H)		
2'	0.89 (t, 3H, $J=7.4$ Hz)	12.2	CH ₃
3'a	1.82 (m, 1H)	23.4	CH ₂
3'b	2.08 (m, 1H)		
4'	0.95 (t, 3H, $J=7.5$ Hz)	13.0	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

Supplementary Table 22. NMR data of compound **19a**.

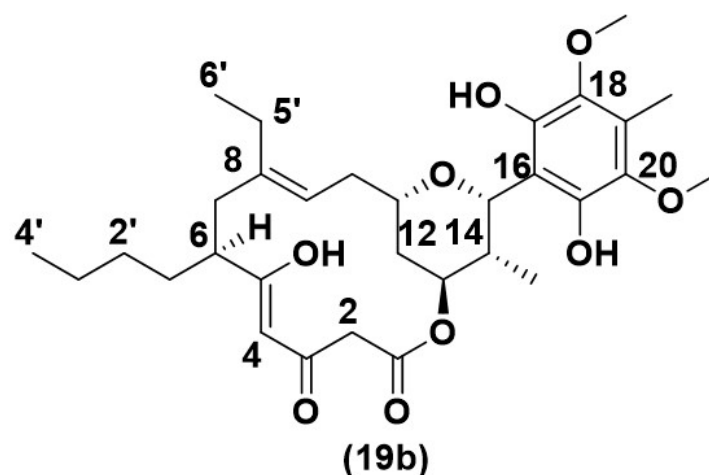


NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.1	C
2a	3.41(d, 1H, $J=13.8$ Hz)	48.4	CH ₂
2b	3.45(d, 1H, $J=13.8$ Hz)		
3		191.2	C
4	5.44 (s, 1H)	99.9	CH
5		190.5	C
6	2.13 (overlapped, 1H)	47.4	CH
7a	2.13 (overlapped, 1H)	38.6	CH ₂
7b	2.28 (t, 1H, $J=13.2$ Hz)		
8		141.7	C
9	5.01 (t, 1H, $J=8.3$ Hz)	121.4	CH
10	2.23 (m, 2H)	33.8	CH ₂
11	3.35 (m, 1H)	76.6	CH
12a	1.65 (m, 1H)	30.4	CH ₂
12b	1.72 (m, 1H)		
13	5.14 (q, 1H, $J=2.8$ Hz)	71.7	CH
14	2.16 (m, 1H)	34.7	CH
15	5.22 (d, 1H, $J=2.7$ Hz)	76.6	CH
16		112.3	C
17		*	C
18		*	C
19		114.0	C
20		*	C
21		*	C
14-CH ₃	1.01 (d, 3H, $J=7.4$ Hz)	11.9	CH ₃
19-CH ₃	1.93 (s, 3H)	7.5	CH ₃
1'a	1.40 (ddt, 1H, $J=13.4, 10.6, 5.8$ Hz)	33.3	CH
1'b	1.67 (ddd, 1H, $J=8.9, 4.4, 3.0$ Hz)		
2'	1.23 (m, 2H)	29.9	CH ₂
3'	1.29 (m, 2H)	22.6	CH ₂
4'	0.88 (t, 3H, $J=7.2$ Hz)	13.8	CH ₂
5'a	1.81 (dq, 1H, $J=14.7, 7.6$ Hz)	23.5	CH ₂
5'b	2.08 (tt, 1H, $J=14.2, 7.4$ Hz)		
6'	0.94 (t, 3H, $J=7.2$ Hz)	11.9	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

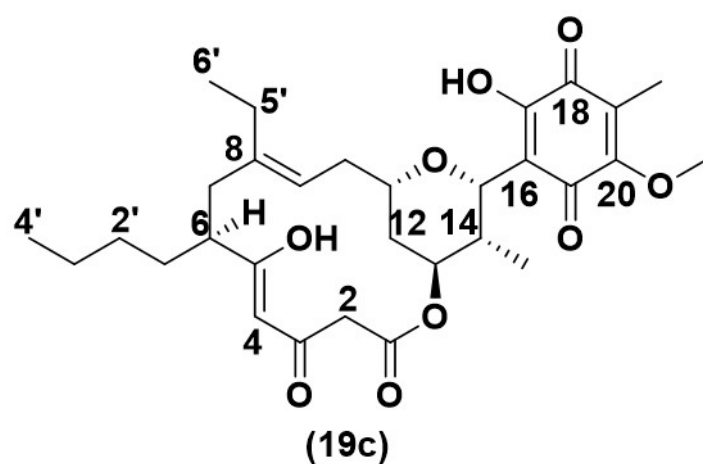
Supplementary Table 23. NMR data of compound **19b**.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		167.3	C
2a	3.40 (d, 1H, $J=14.0$ Hz)	38.4	CH ₂
2b	3.44 (d, 1H, $J=14.0$ Hz)		
3		191.4	C
4	5.45 (s, 1H)	100.1	CH
5		190.6	C
6	2.15 (overlapped, 1H)	47.4	CH
7a	2.14 (overlapped, 1H)	38.4	CH ₂
7b	2.27 (m, 1H)		
8		141.6	C
9	5.04 (t, 1H, $J=8.3$ Hz)	121.2	CH
10a	2.22 (m, 1H)	34.2	CH ₂
10b	2.31 (m, 1H)		
11	3.38 (m, 1H)	75.9	CH
12a	1.68 (m, 1H)	30.5	CH ₂
12b	1.74 (m, 1H)		
13	5.17 (q, 1H, $J=2.7$ Hz)	72.7	CH
14	2.19 (m, 1H)	35.4	CH
15	5.46 (d, 1H, $J=2.8$ Hz)	77.1	CH
16		108.5	C
17		*	C
18		138.6	C
19		123.6	C
20		138.6	C
21		*	C
14-CH ₃	0.97 (d, 3H, $J=7.4$ Hz)	11.9	CH ₃
18-OCH ₃	3.73 (s, 3H)	60.6	CH ₃
19-CH ₃	2.20 (s, 3H)	9.4	CH ₃
20-OCH ₃	3.73 (s, 3H)	60.6	CH ₃
1'a	1.41 (m, 1H)	33.04	CH ₂
1'b	1.69 (m, 1H)		
2'	1.25 (overlapped, 2H)	27.5	CH ₂
3'	1.30 (overlapped, 2H)	22.8	CH ₂
4'	0.88 (t, 3H, $J=6.8$ Hz)	13.8	CH ₃
5'a	1.83 (m, 1H)	23.5	CH ₂
5'b	2.09 (m, 1H)		
6'	0.94 (t, 3H, $J=7.6$ Hz)	11.9	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

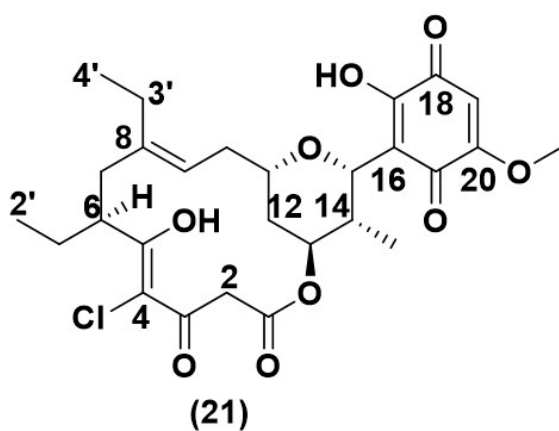
* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Table 24. NMR data of compound **19c**.

NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		166.5	C
2	3.45 (s, 2H)	48.4	CH ₂
3		191.00	C
4	5.45 (s, 1H)	99.9	CH
5		190.5	C
6	2.15 (overlapped, 1H)	47.4	CH
7a	2.16 (overlapped, 1H)	38.4	CH ₂
7b	2.29 (t, 1H, $J=13.2$ Hz)		
8		141.8	C
9	5.03 (t, 1H, $J=8.3$ Hz)	120.6	CH
10	2.25 (m, 2H)	33.9	CH ₂
11	3.37 (m, 1H)	76.1	CH
12a	1.64 (m, 1H)	30.4	CH ₂
12b	1.76 (m, 1H)		
13	5.16 (q, 1H, $J=2.7$ Hz)	72.0	CH
14	2.20 (m, 1H)	35.3	CH
15	5.23 (d, 1H, $J=2.7$ Hz)	76.9	CH
16		114.3	C
17		155.3	C
18		183.2	C
19		125.5	C
20		155.4	C
21		181.7	C
14-CH ₃	1.03 (d, 3H, $J=7.2$ Hz)	11.4	CH ₃
19-CH ₃	1.96 (s, 3H)	8.3	CH ₃
20-OCH ₃	4.03 (s, 3H)	61.2	CH ₃
1'a	1.43 (m, 1H)	33.1	CH ₂
1'b	1.70 (m, 1H)		
2'	1.27 (m, 2H)	29.8	CH ₂
3'	1.32 (m, 2H)	22.6	CH ₂
4'	0.90 (t, 3H, $J=7.2$ Hz)	14.0	CH ₃
5'a	1.84 (m, 1H)	23.5	CH ₂
5'b	2.10 (m, 1H)		
6'	0.96 (t, 3H, $J=7.5$ Hz)	13.0	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

Supplementary Table 25. NMR data of compound **21**.



NO.	δ_H (mult., J in Hz)	δ_C	C-type
1		165.1	C
2a	3.53(d, 1H, $J=15.6$ Hz)	47.0	CH ₂
2b	3.53(d, 1H, $J=15.6$ Hz)		
3		189.6	C
4		109.2	C
5		188.8	C
6	3.19 (m, 1H)	43.8	CH
7a	2.23 (overlapped, 1H)	37.8	CH ₂
7b	2.36 (t, 1H, $J=12.7$ Hz)		
8		141.3	C
9	5.10 (t, 1H, $J=8.3$ Hz)	120.9	CH
10	2.20 (overlapped, 1H)	33.4	CH ₂
11	3.35 (m, 1H)	76.6	CH
12	1.67 (m, 1H)	30.8	CH ₂
13	5.17 (brs, 1H)	72.5	CH
14	2.17 (m, 1H)	34.9	CH
15	5.16 (brs, 1H)	77.4	CH
16		114.9	C
17		156.0	C
18		*	C
19	5.80 (s, 1H)	104.5	CH
20		158.7	C
21		180.0	C
14-CH ₃	0.99 (overlapped, 3H)	11.4	CH ₃
20-OCH ₃	3.80 (s, 3H)	56.3	
1'a	1.53 (m, 1H)	27.0	CH ₂
1'b	1.69 (m, 1H)		
2'	0.94 (t, 3H, $J=7.4$ Hz)	11.6	CH ₃
3'	1.88 (m, 1H)	23.0	CH ₂
4'	0.98 (overlapped, 3H)	12.8	CH ₃

¹H NMR: 600 MHz; ¹³C NMR: 150 MHz (in DMSO-*d*₆).

* The interconversion of the benzoquinone moiety led to the missing of these carbon signals.

Supplementary Figure

Compare these results against the new Clustered nr database ? BLAST

DescriptionsGraphic SummaryAlignmentsTaxonomy

Sequences producing significant alignmentsDownloadSelect columnsShow100?

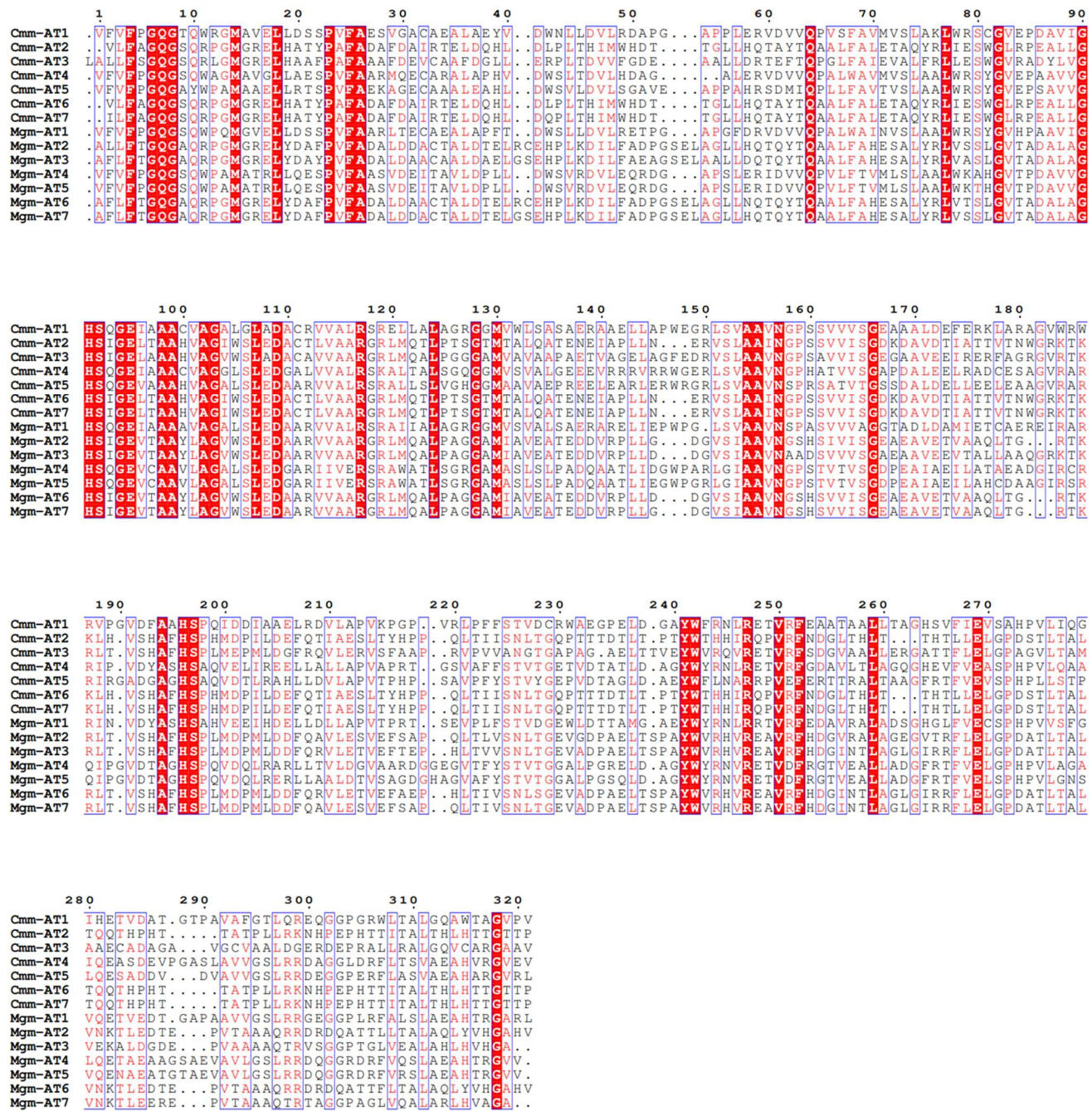
☒ select all 100 sequences selectedGenPeptGraphicsDistance tree of resultsMultiple alignmentMSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	type I polyketide synthase [Streptomyces cinnamoneus]	Streptomyces ci...	848	1411	100%	0.0	100.00%	3925	WP_099197735.1
☒	type I polyketide synthase [Streptomyces mangrovisoli]	Streptomyces m...	680	1241	100%	0.0	78.64%	3992	WP_046583961.1
☒	type I polyketide synthase [Streptomyces cinnamoneus]	Streptomyces ci...	672	1893	100%	0.0	80.14%	5475	WP_240003137.1
☒	type I polyketide synthase [Streptomyces cinnamoneus]	Streptomyces ci...	671	1892	100%	0.0	80.14%	5475	WP_243469264.1
☒	SDR family NAD(P)-dependent oxidoreductase [Streptomyces sp. NBC_00659]	Streptomyces s...	657	1200	100%	0.0	76.06%	3919	WP_329297130.1
☒	SDR family NAD(P)-dependent oxidoreductase [Streptomyces sp. NBC_01393]	Streptomyces s...	653	1200	100%	0.0	76.48%	3918	WTZ06591.1
☒	type I polyketide synthase [Streptomyces hygrosopicus]	Streptomyces h...	649	3043	100%	0.0	74.30%	8595	WP_236256936.1
☒	type I polyketide synthase [Streptomyces hygrosopicus]	Streptomyces h...	649	3051	100%	0.0	74.30%	8606	MEU5273606.1
☒	type I polyketide synthase [Streptomyces hygrosopicus]	Streptomyces h...	649	3037	100%	0.0	74.30%	8605	MEU1908929.1
☒	type I polyketide synthase [Saccharothrix saharensis]	Saccharothrix s...	648	1271	100%	0.0	75.23%	4484	WP_141974657.1
☒	type I polyketide synthase [Streptomyces sp. 5-10]	Streptomyces s...	647	647	100%	0.0	74.07%	1620	WP_191070427.1
☒	type I polyketide synthase [Streptomyces hygrosopicus]	Streptomyces h...	647	3038	100%	0.0	74.07%	8582	MEU7368327.1

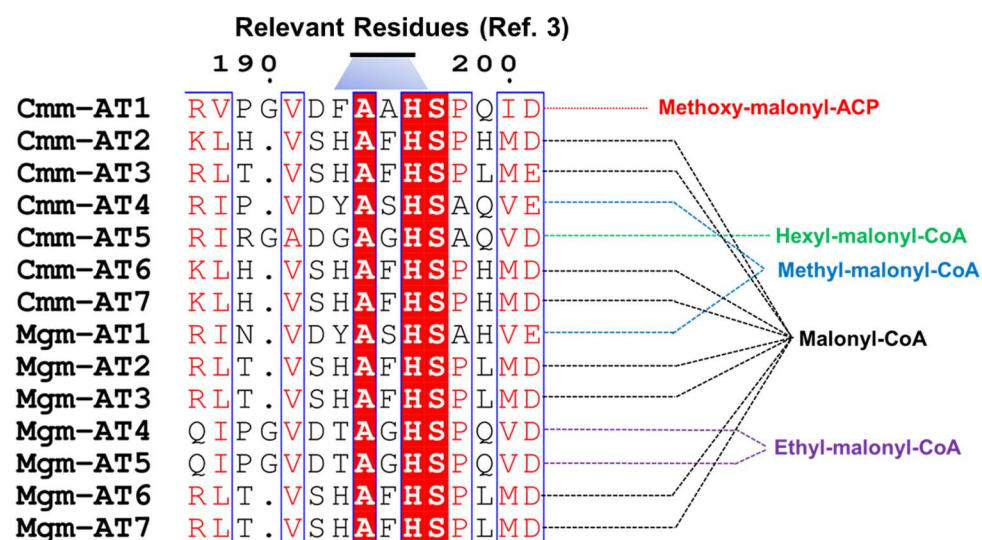
Supplementary Fig. 1. BLAST search results using CmmD2-KS₅ as the probe.

MgmD2-KS₅ are circled in red square.

a

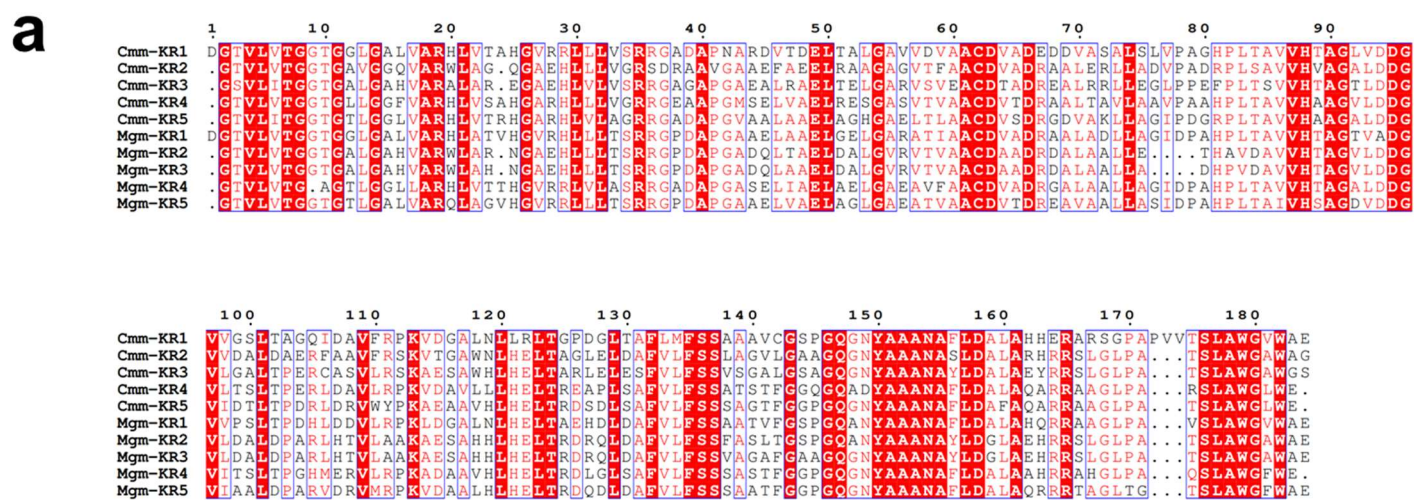


b

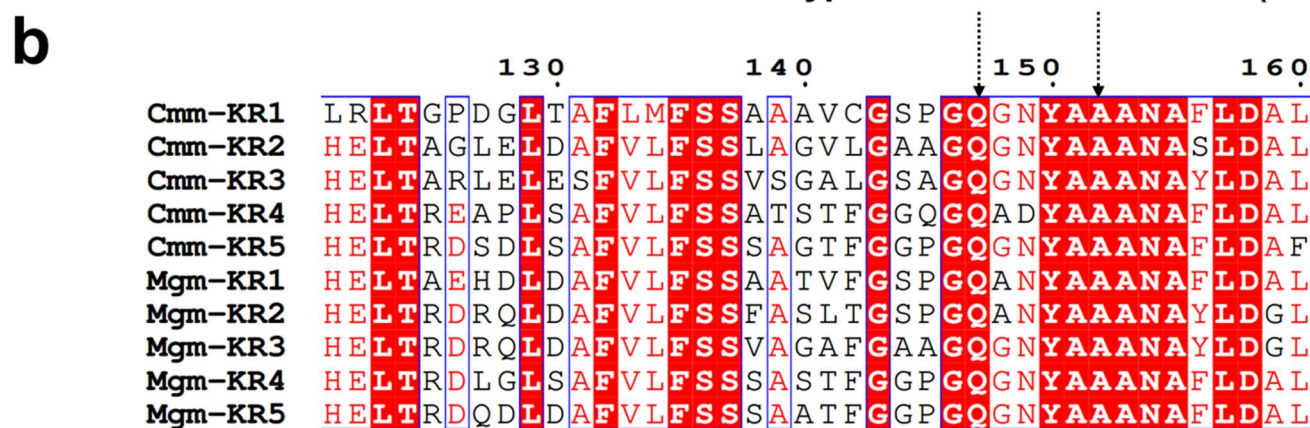


Supplementary Fig. 2. Amino acid sequence alignments of AT domains between *cmn* and *mgm* BGCs.

(a) AT domains. (b) The substrate-specific motif¹, covering residues 193-196, in AT domains. The corresponding extender unit of each AT is labeled.



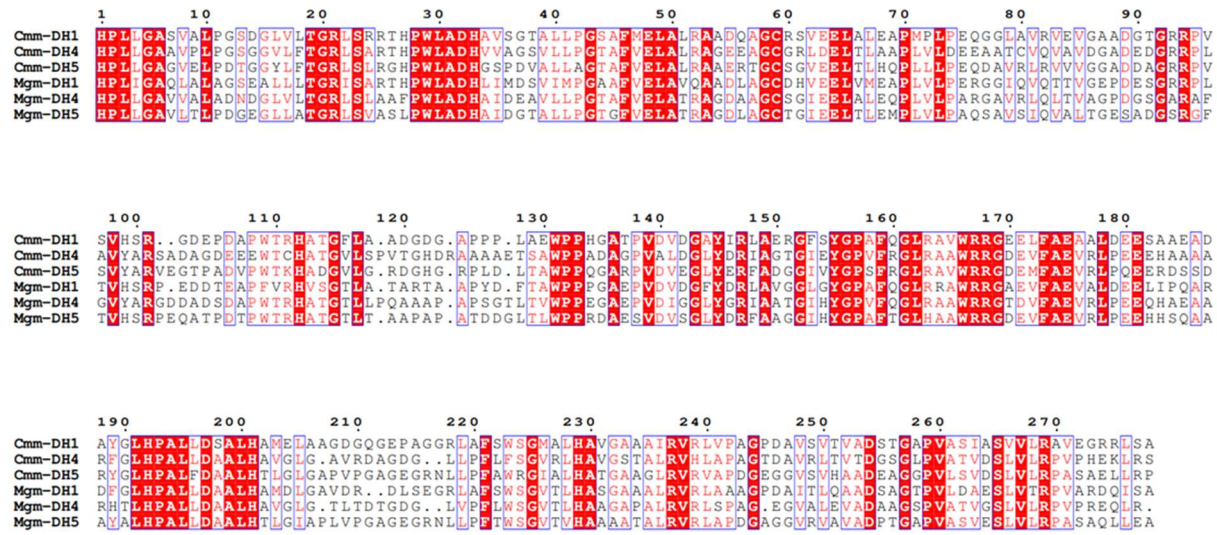
B1-type KR relevant residues (Ref. 4)



Supplementary Fig. 3. Amino acid sequence alignments of KR domains between *cmk* and *mgm* BGCs.

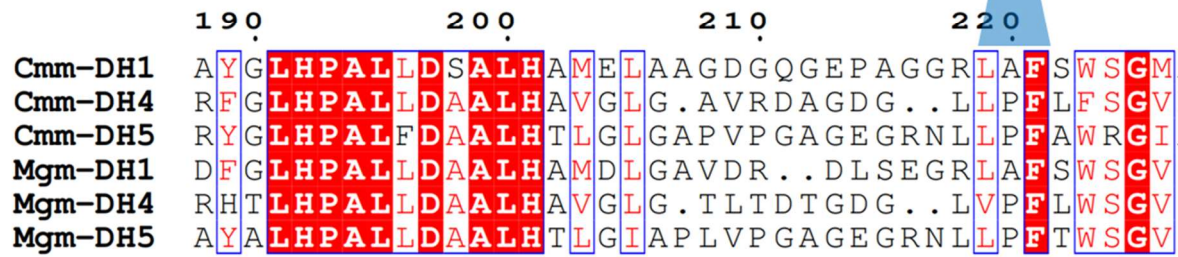
(a) KR domains. (b) Comparison of the signature amino acids of B1-type KR².

a

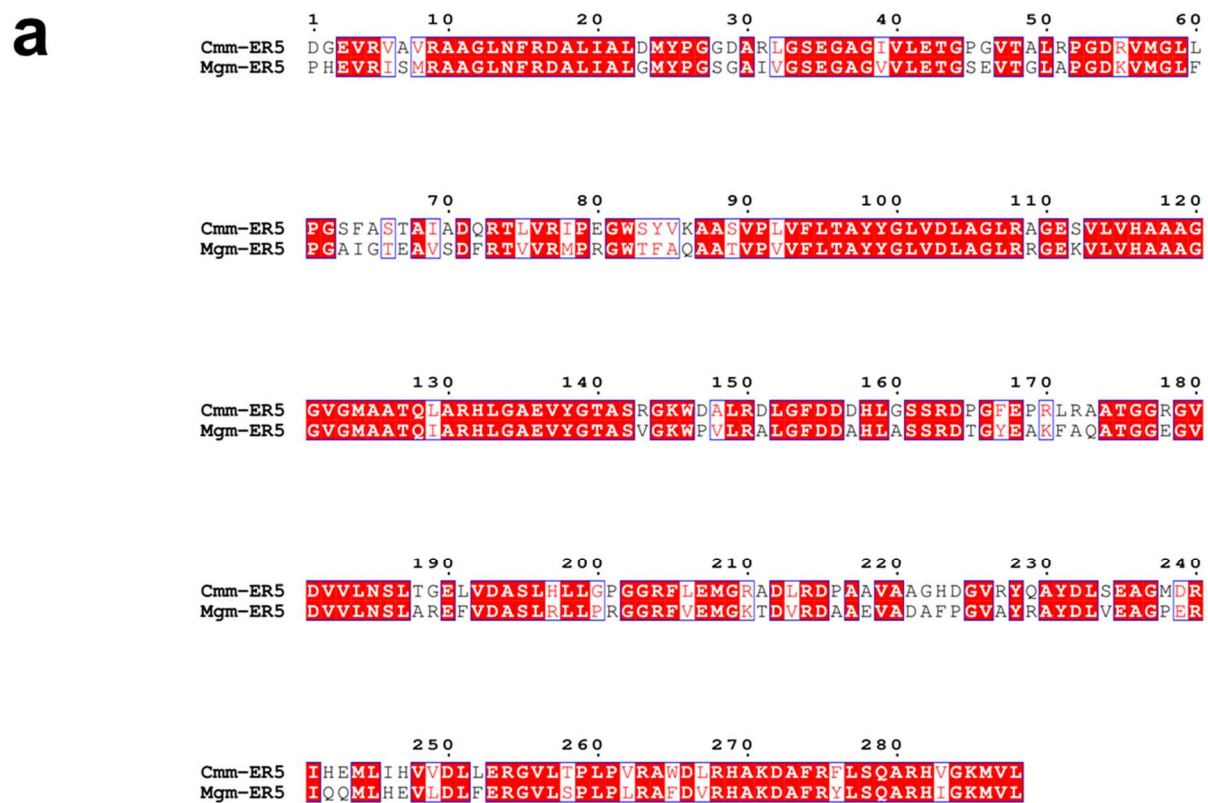


“LAF” motif (Ref. 2)

b

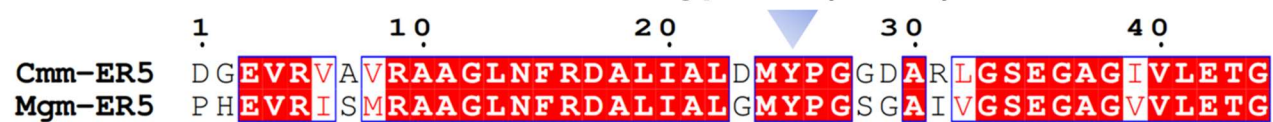


Supplementary Fig. 4. Amino acid sequence alignments of DH domains between *cmc* and *mgm* BGCs. (a) DH domains. (b) Comparison of the catalytically inactive signature motif “LAF” of Cmm-DH1 with Mgm-DH1.



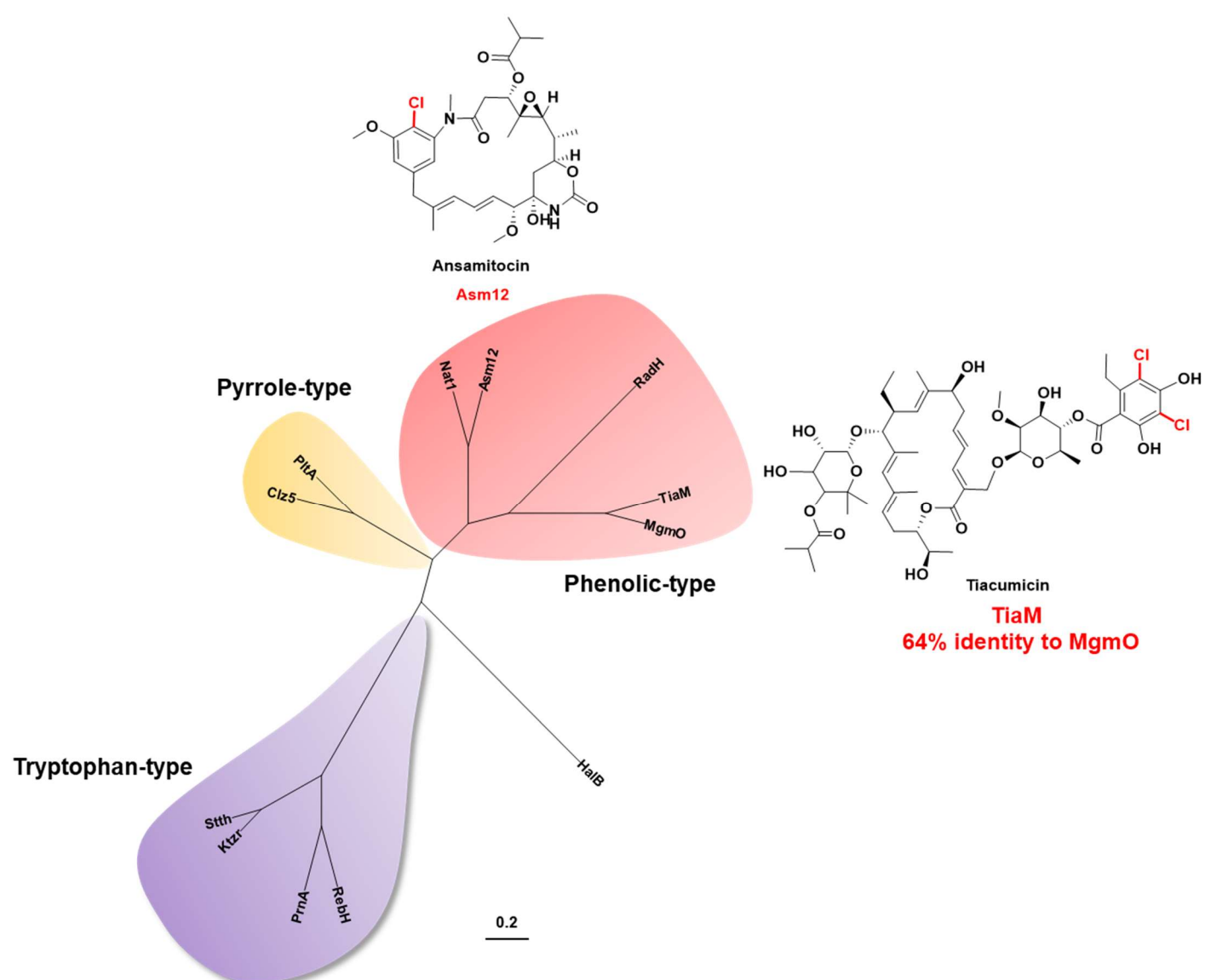
b

Signature residue “Y” of
L-type ER (Ref. 4)



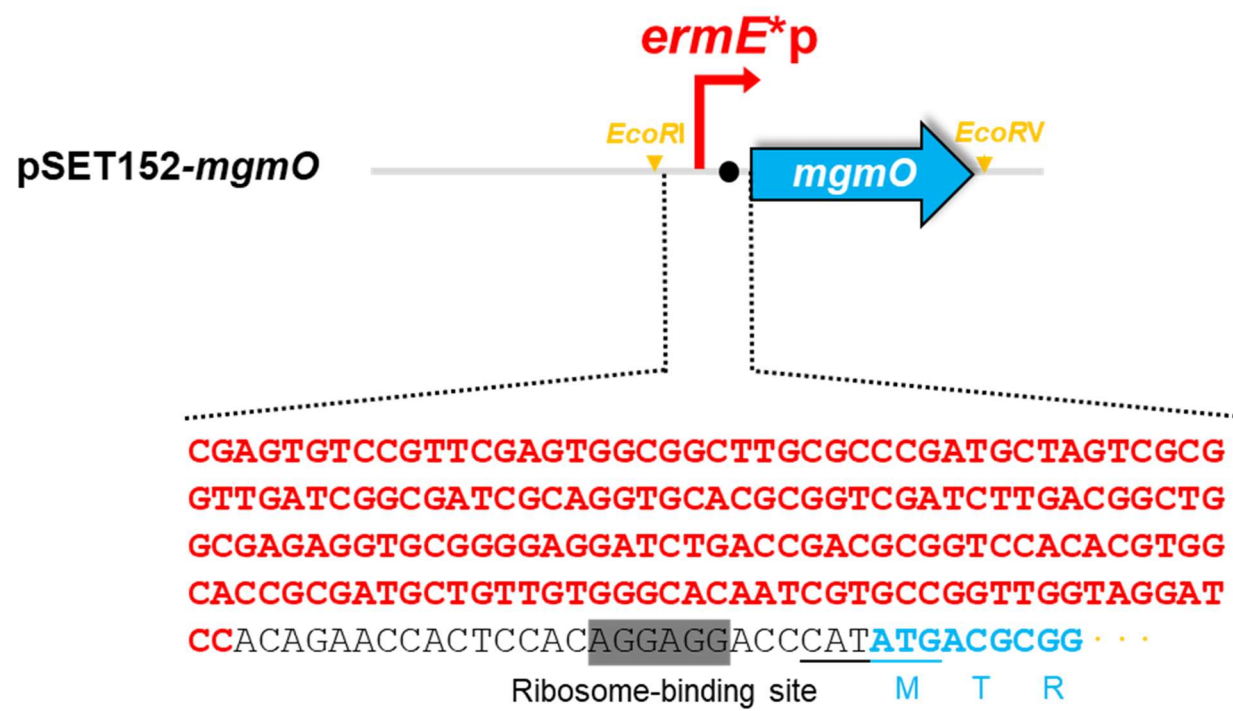
Supplementary Fig. 5. Amino acid sequence alignments of ER domains between *cmm* and *mgm* BGCs.

(a) ER domains. (b) Comparison of the signature amino acid “Y” of L-type ER².



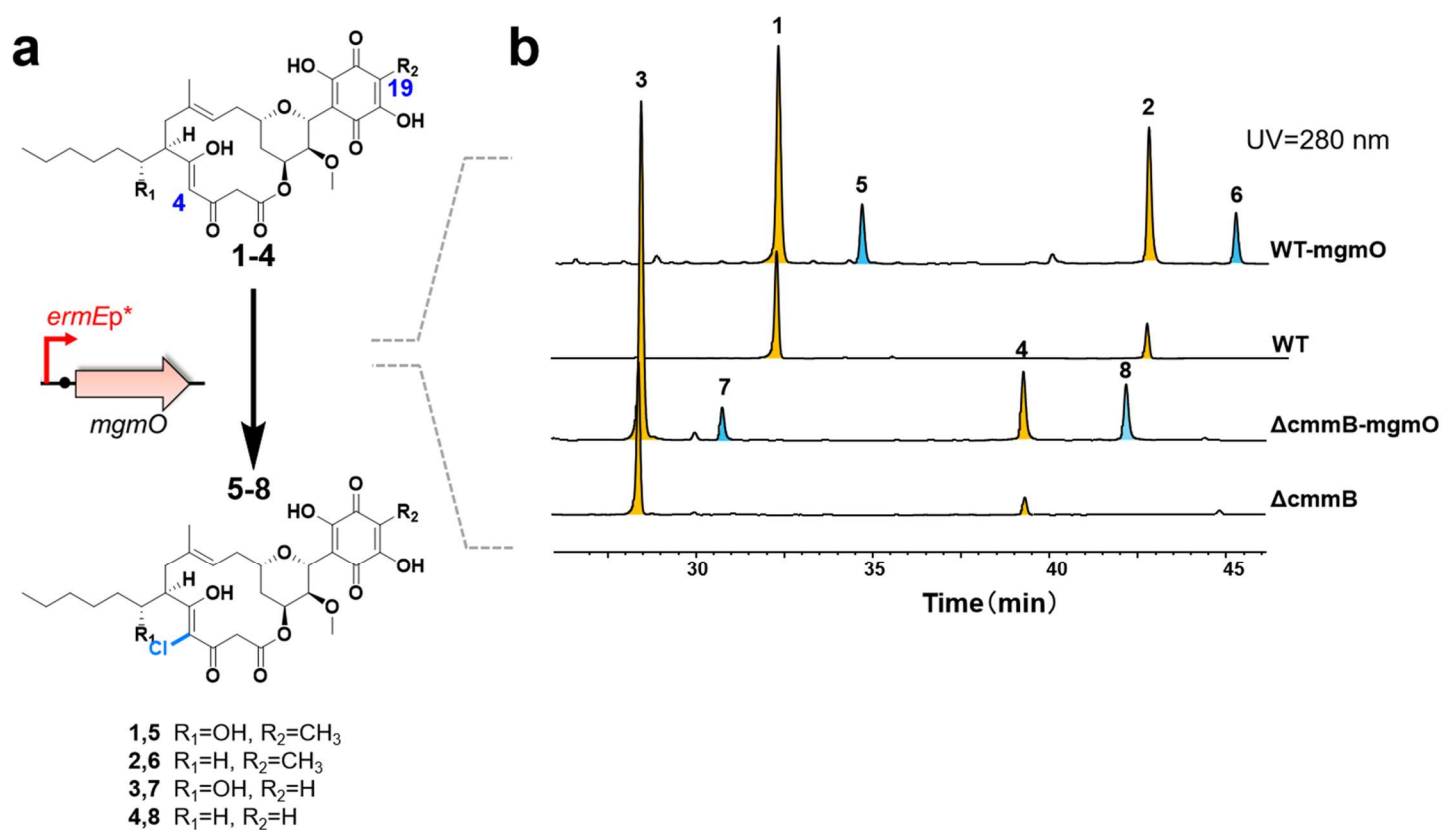
Supplementary Fig. 6. Phylogenetic tree analysis of FAD-dependent halogenases³.

MgmO (WP_046591495.1); TiaM (ADU85999.1); RadH (C5H881.1); Asm12 (AAM54090.1); Nat1 (ADM46362.1); PltA (Q4KCZ0.1); Clz5(A0A345BJN5.1); SttH (E9P162.1); Ktzr (A8CF74.2); PrnA (P95480.1); RebH (Q8KHZ8.1); HalB (AAQ04685.1).



Supplementary Fig. 7. Nucleotide sequence of plasmid pSET1252-mgmO.

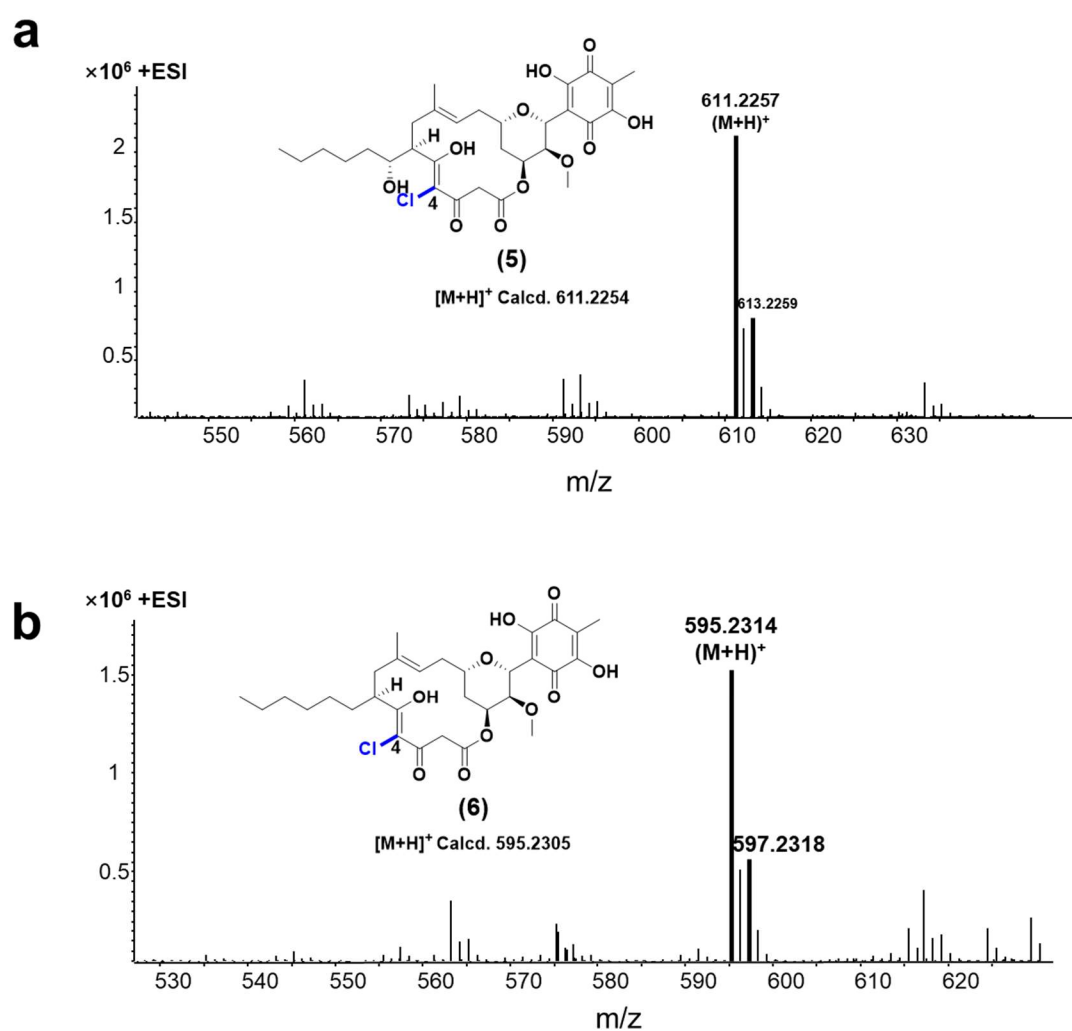
The "*ermE**_p" nucleotide sequence is displayed in red bold font.



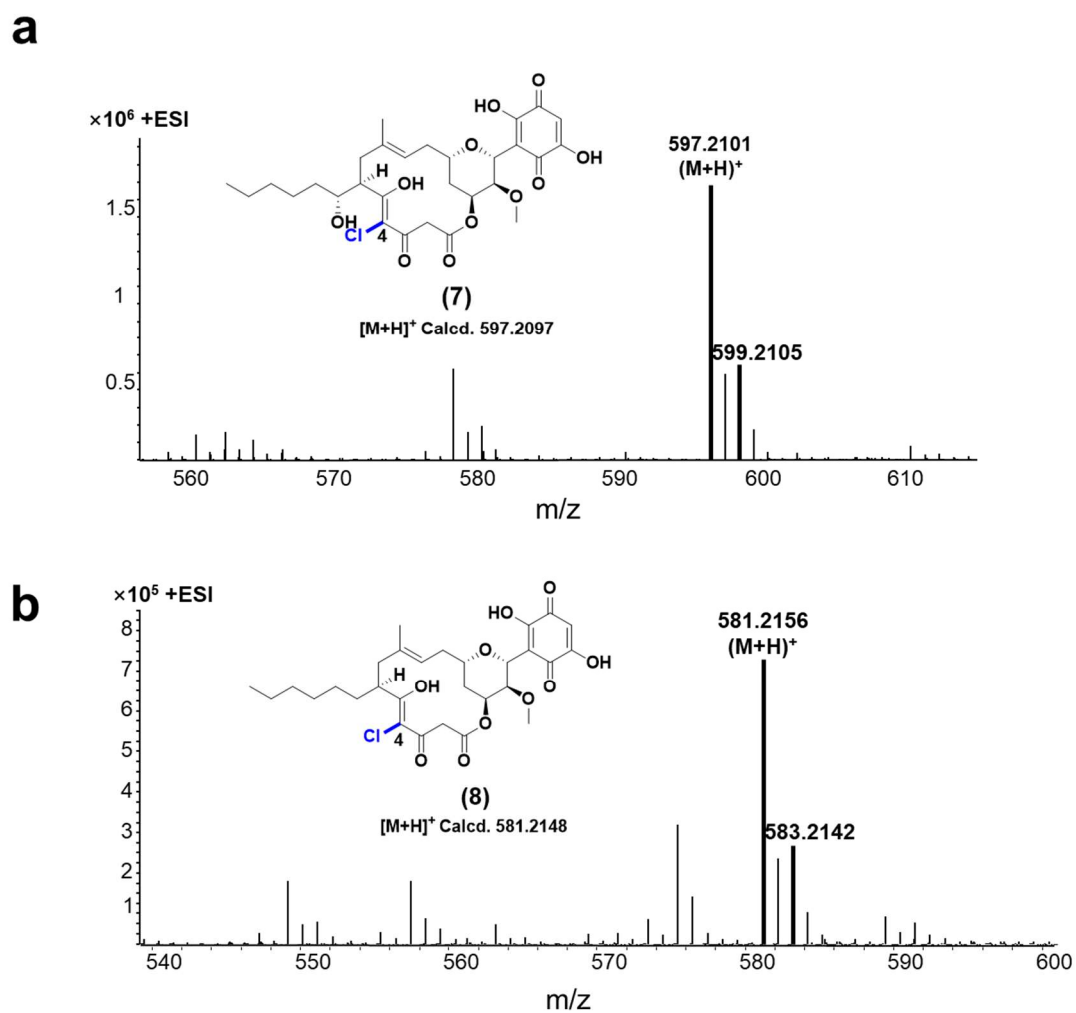
Supplementary Fig. 8. Olefin chlorination catalyzed by MgmO.

a. Cinnamomycin **1-4** converted to chlorinated-cinnamomycin **5-8** by MgmO. *ermE**p: the promoter used.

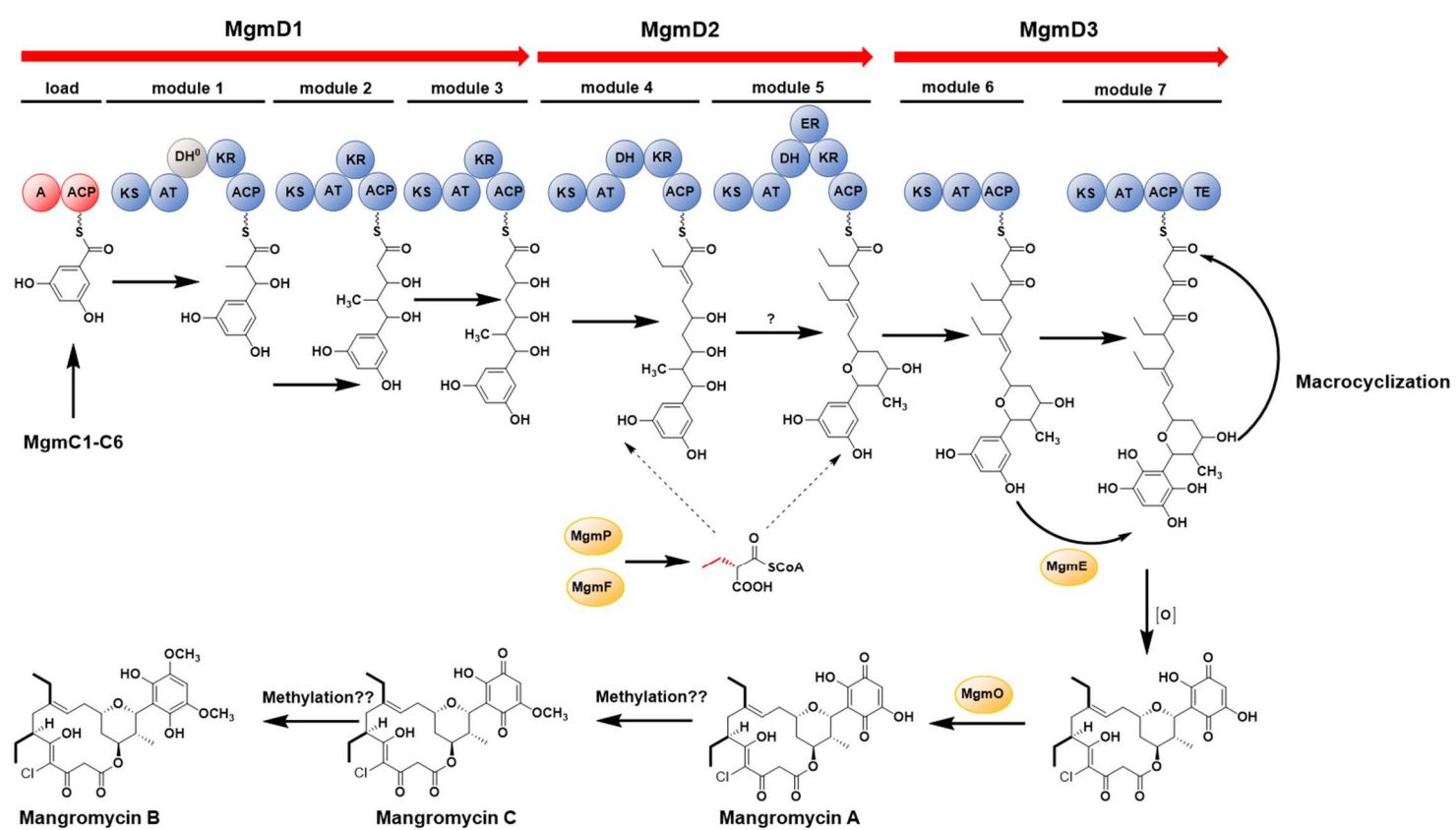
b. HPLC analyses of mutant strains after fermentation. Cinnamomycin **1-4** are colored as yellow. Chlorinated cinnamomycin **5-8** are colored as blue.



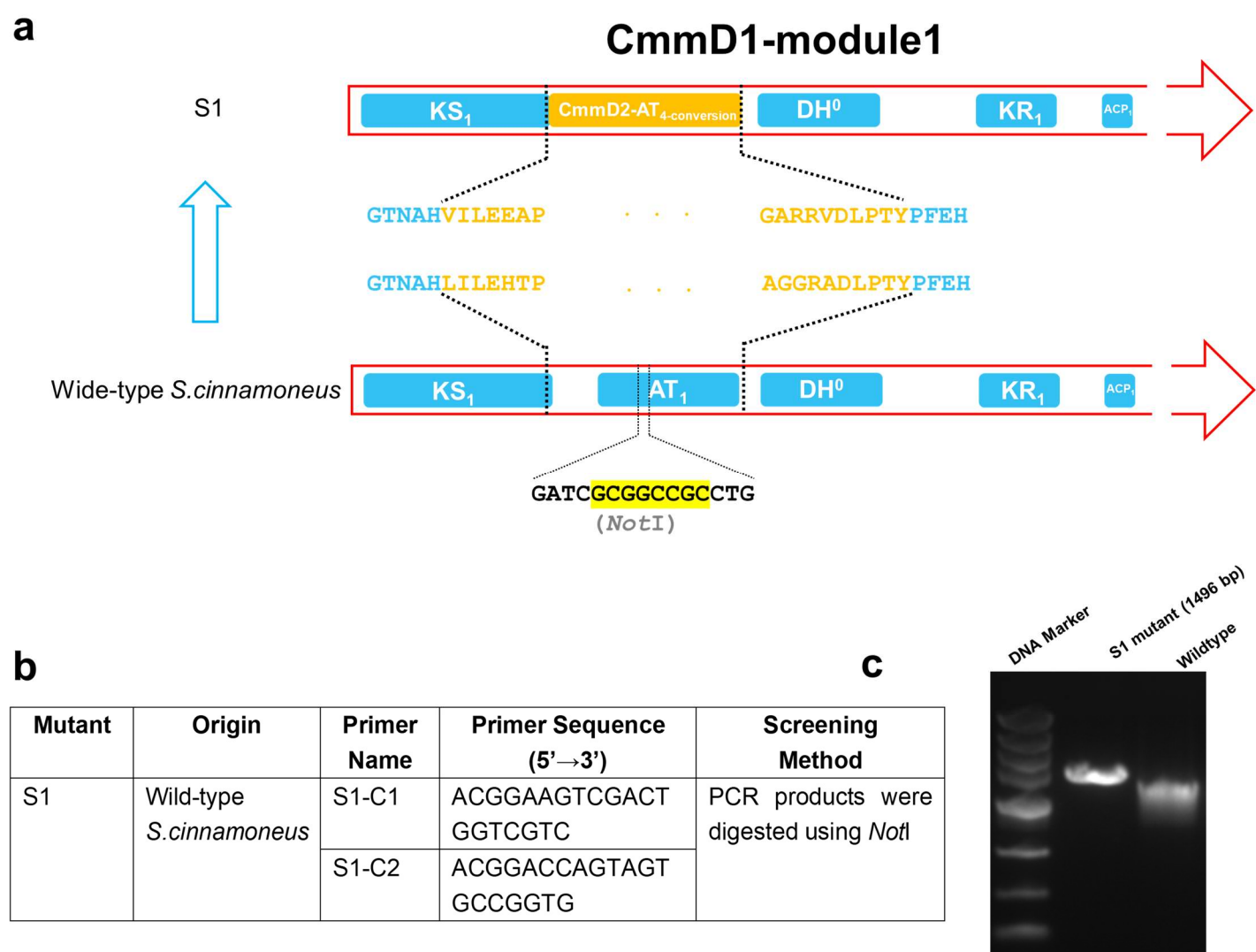
Supplementary Fig. 9. HR-ESI-MS spectra of chlorinated derivatives **5** (a) and **6** (b).



Supplementary Fig. 10. HR-ESI-MS spectra of chlorinated derivatives **7** (a) and **8** (b).

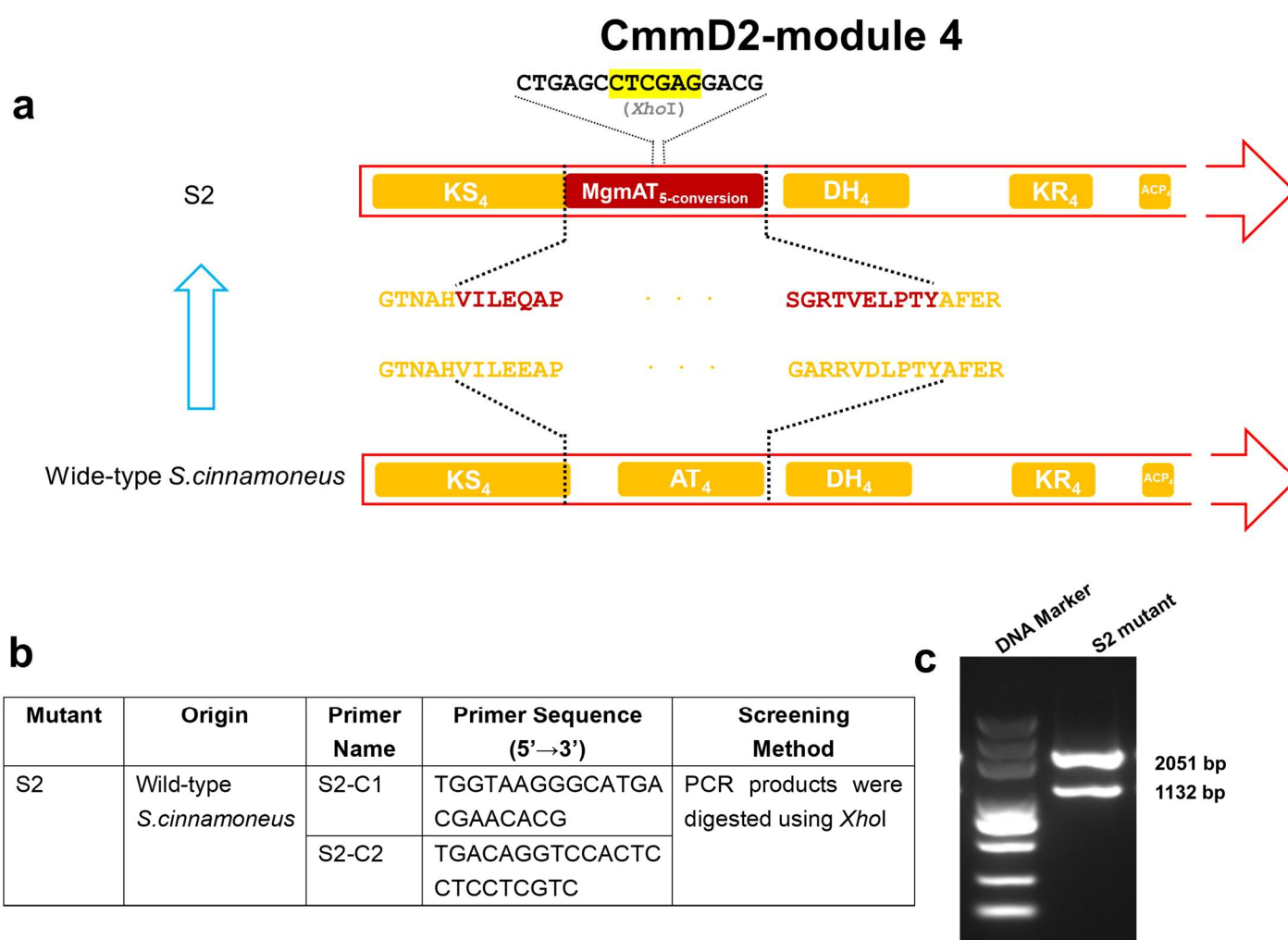


Supplementary Fig. 11. Proposed biosynthetic pathway of mangromycin **A-C**.



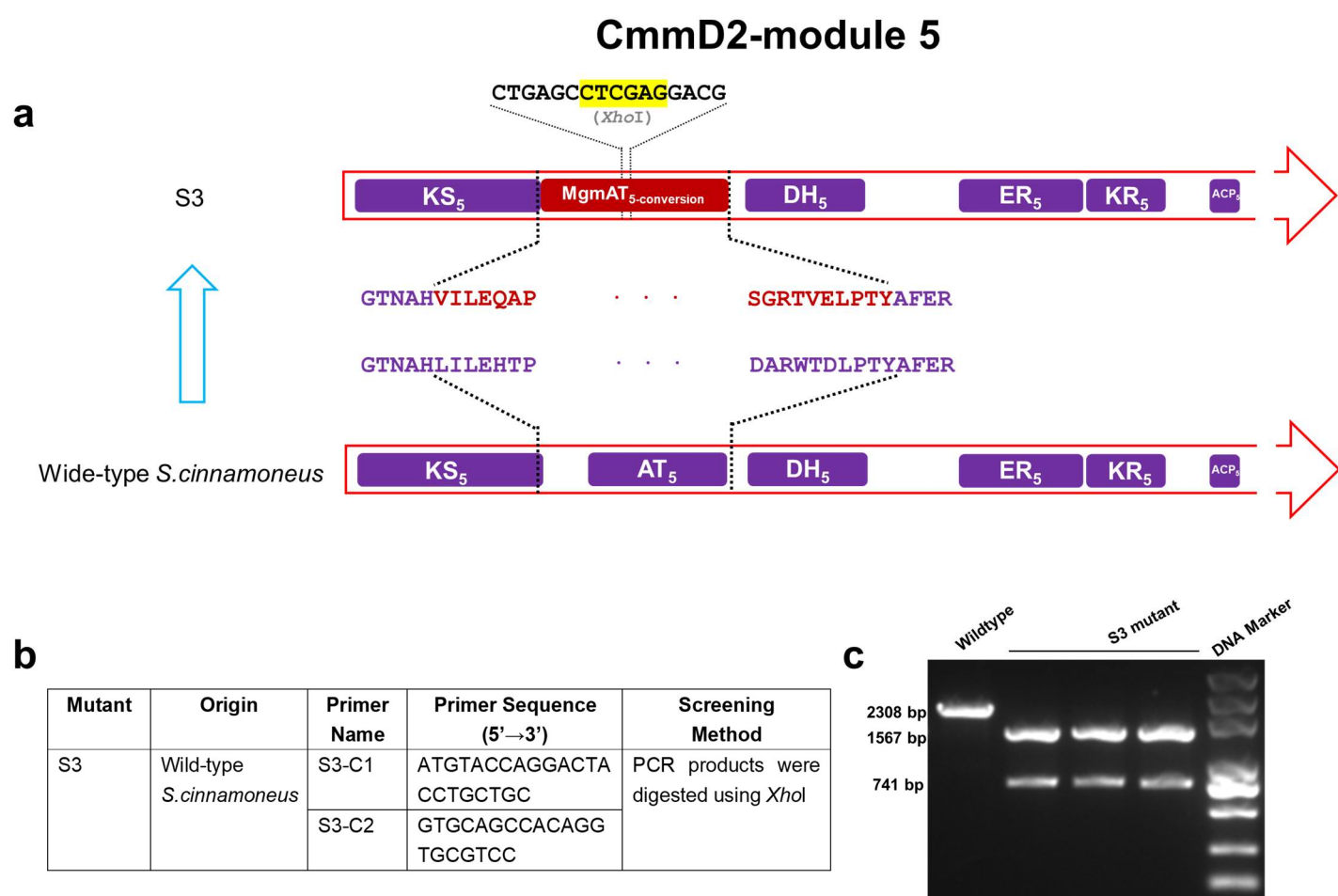
Supplementary Fig. 12. Construction and confirmation of mutant S1 strain.

(a) Schematic diagram of construction of mutant S1, *NotI* restriction enzyme cutting site used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S1 by PCR and restriction enzyme digesting. The blue squares represent domains from CmmD1-module 1. *NotI* enzyme digestion always caused fragment blurring on the gel. Thus, S1 strain was confirmed by DNA sequencing.



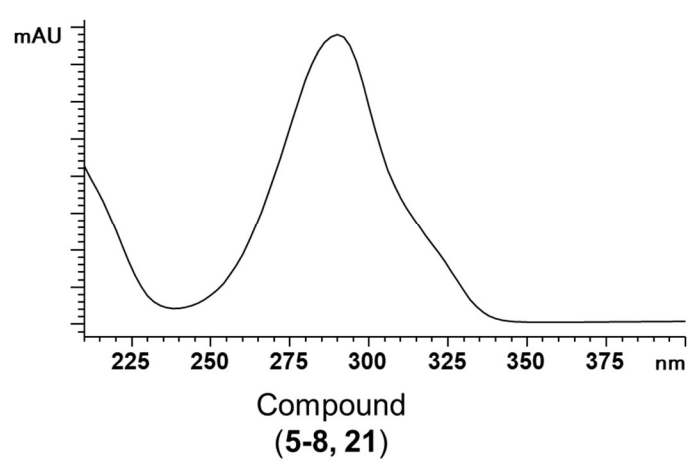
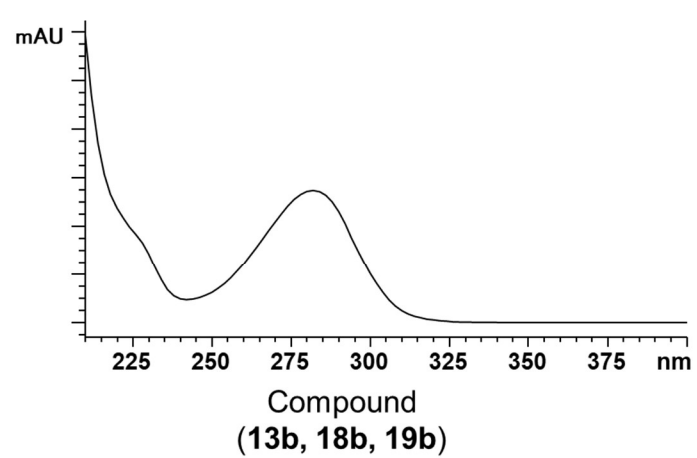
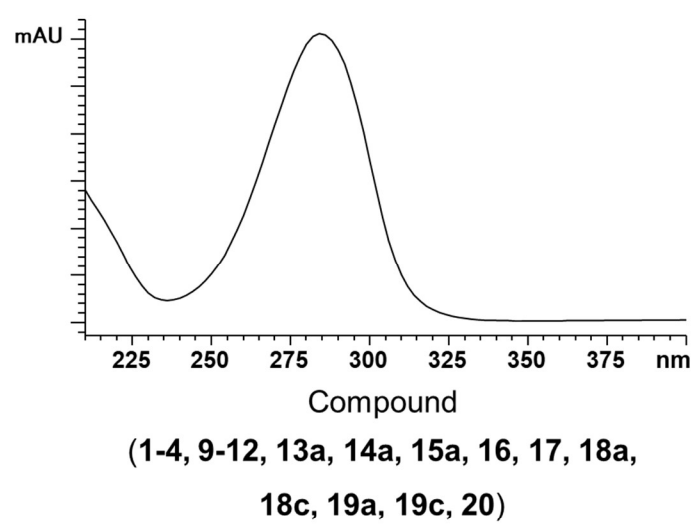
Supplementary Fig. 13. Construction and confirmation of mutant S2 strain.

(a): Schematic diagram of construction of mutant S2, *XhoI* restriction enzyme cutting site used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S2 by PCR and restriction enzyme digesting. The yellow squares represent domains from CmmD2-module 4. Due to the introduction of *XhoI* digestion site, the PCR product in the mutant S3 could be cleaved into two bands of 2051bp and 1132bp.

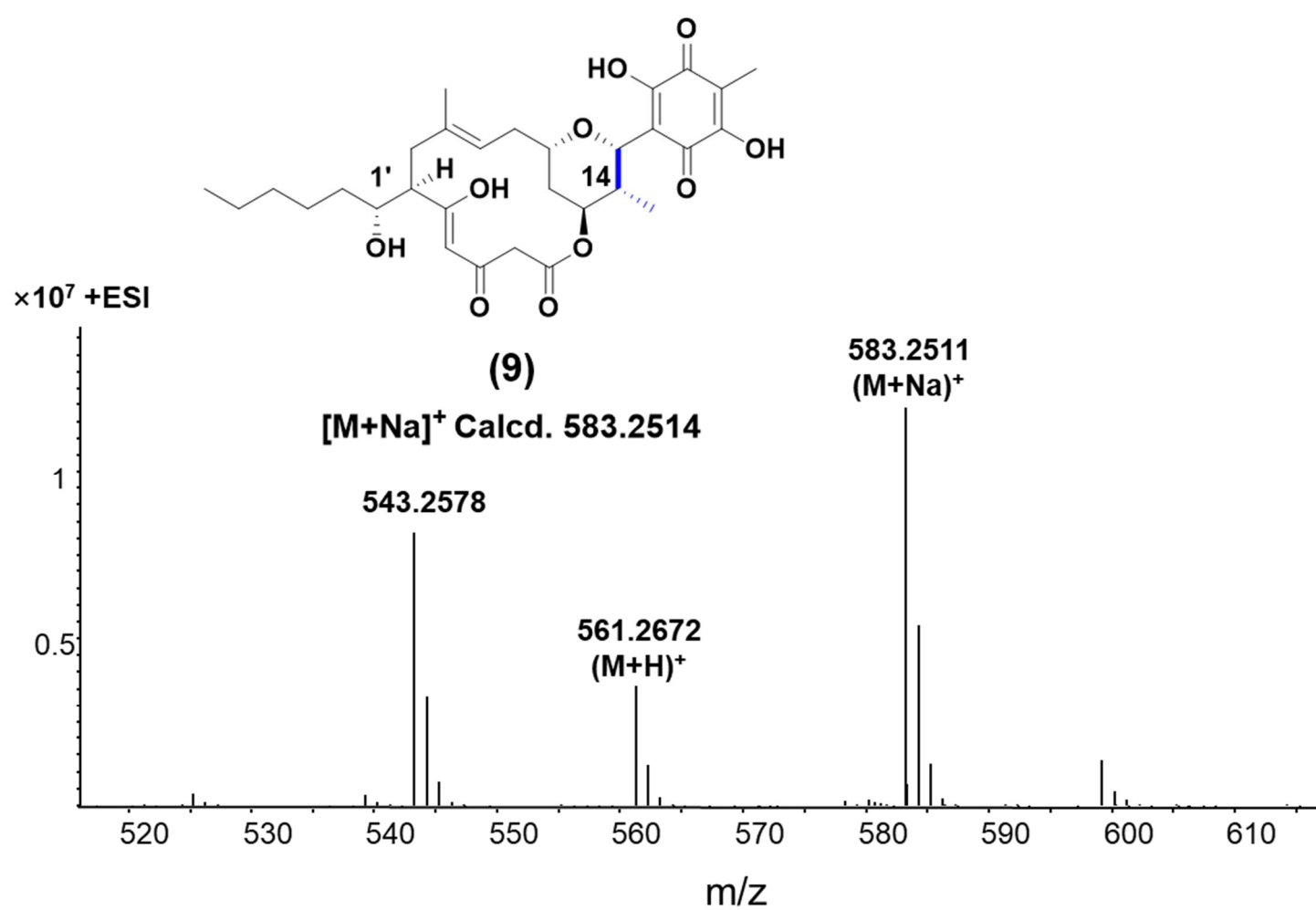


Supplementary Fig. 14. Construction and confirmation of mutant S3 strain.

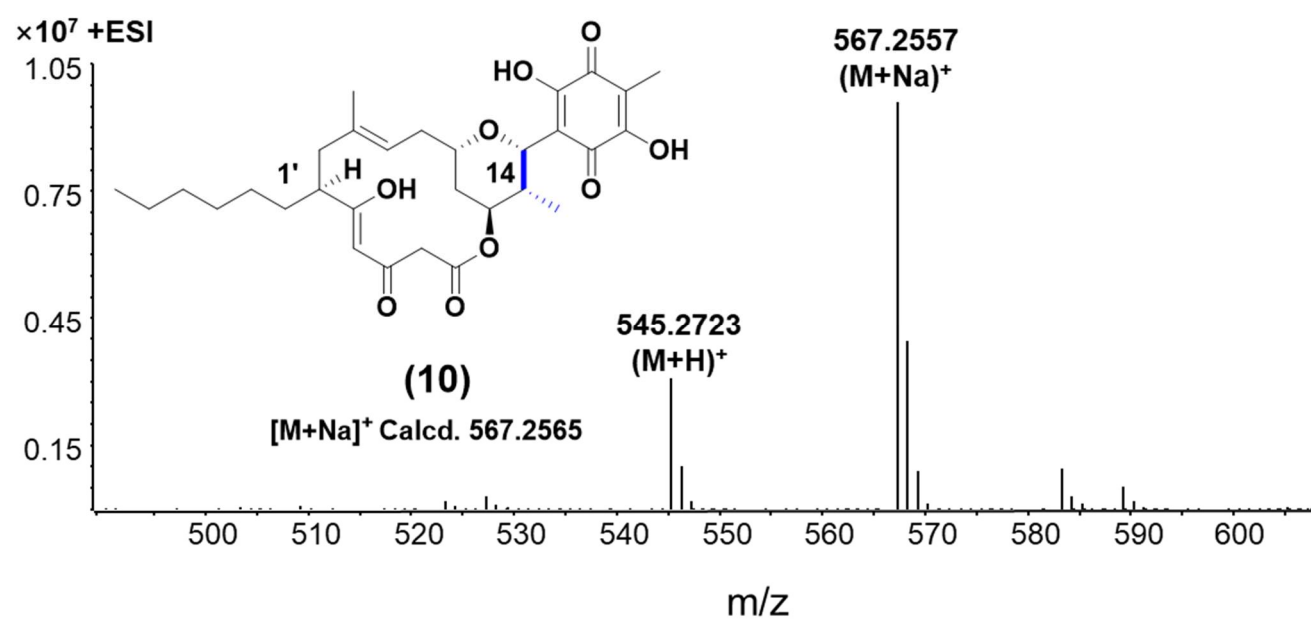
(a) Schematic diagram of construction of mutant S3, *XhoI* restriction enzyme cutting site used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S3 by PCR and restriction enzyme digesting. The purple squares represent domains from CmmD2-module 5. Due to the introduction of *XhoI* digestion site, the PCR products in mutant S3 could be cleaved into two bands of 1567 bp and 741bp, whereas the one in wild-type remained to be 2308bp.



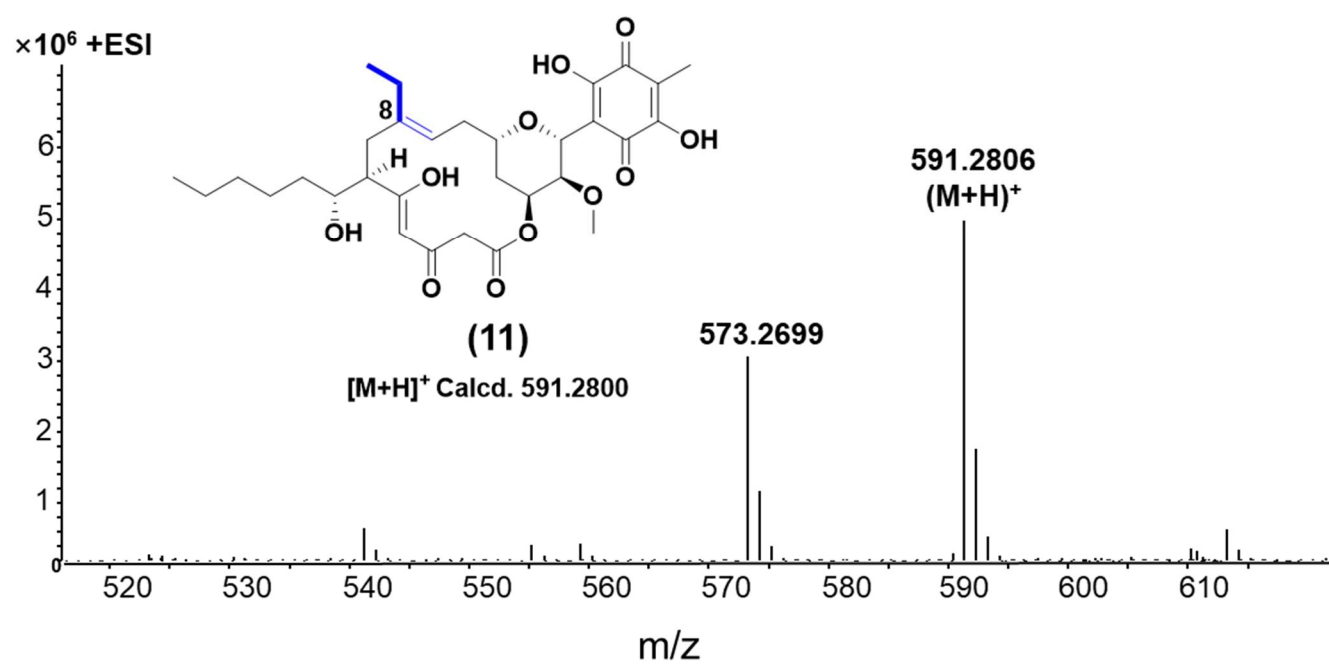
Supplementary Fig. 15. UV-Vis spectra of compounds **1-21**.



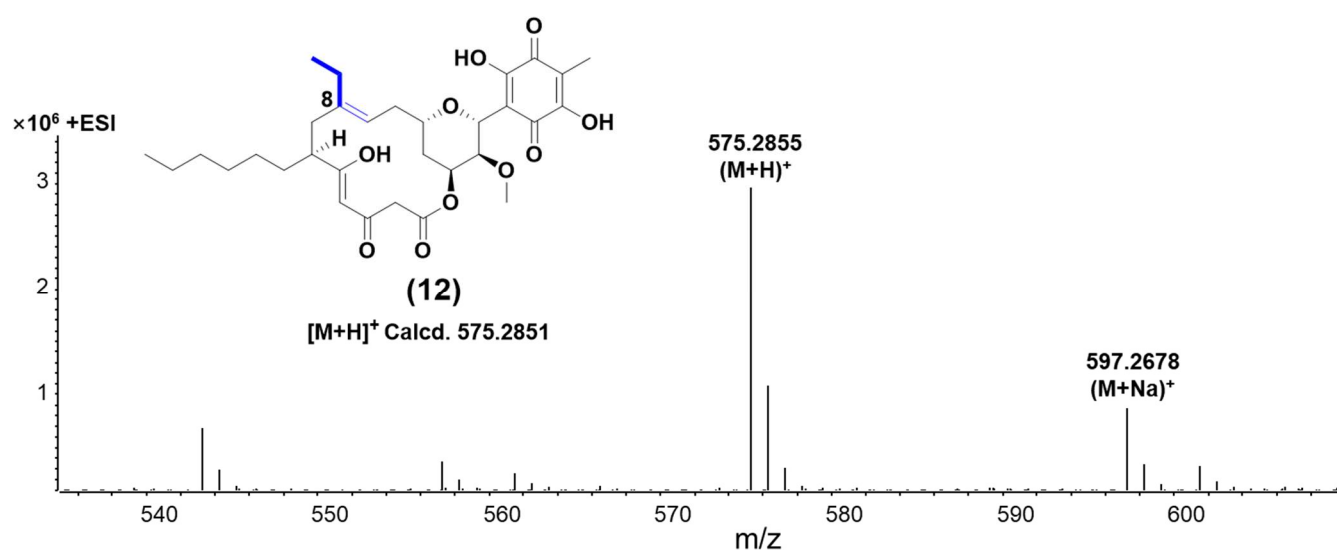
Supplementary Fig. 16. HR-ESI-MS of derivatives **9**.



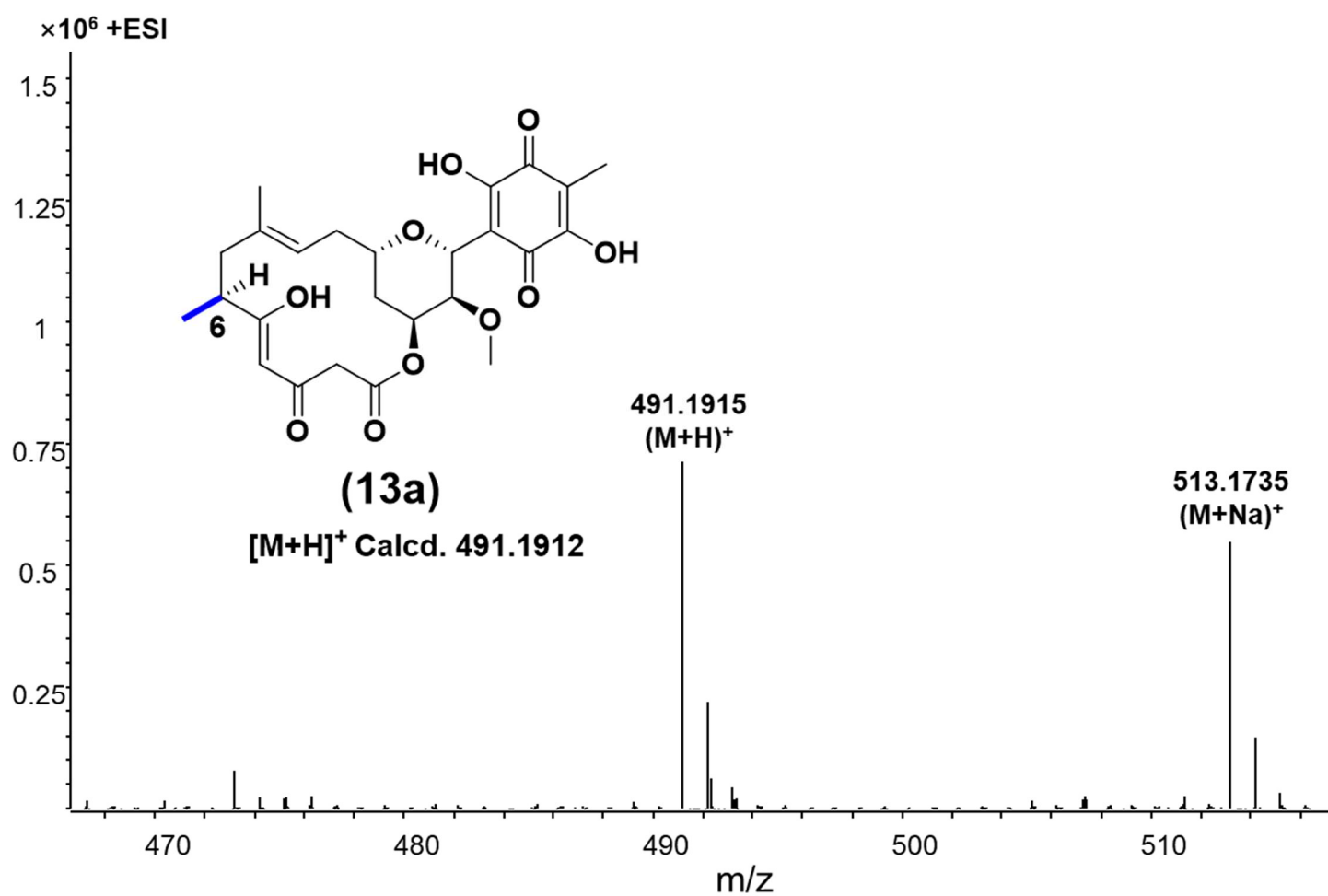
Supplementary Fig. 17. HR-ESI-MS spectrum of compound **10**.



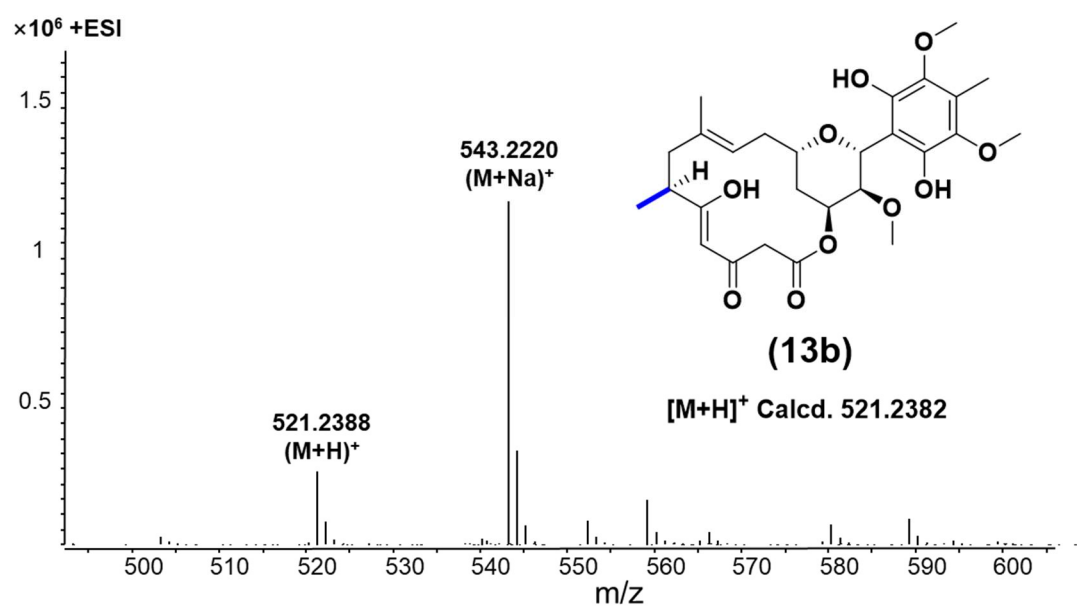
Supplementary Fig. 18. HR-ESI-MS spectrum of compound **11**.



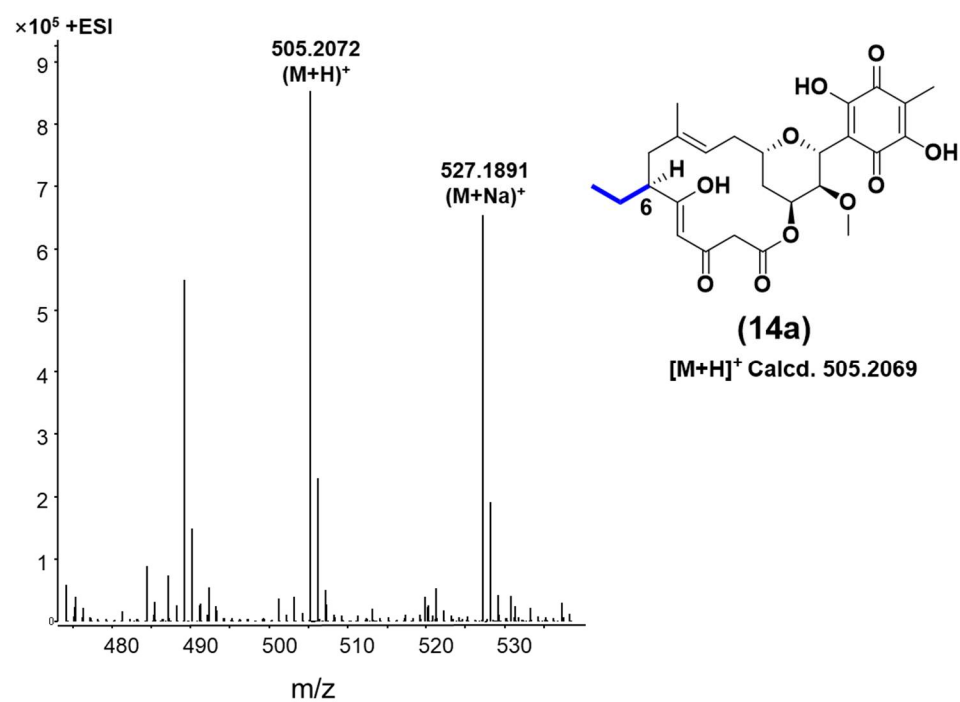
Supplementary Fig. 19. HR-ESI-MS spectrum of compound **12**.



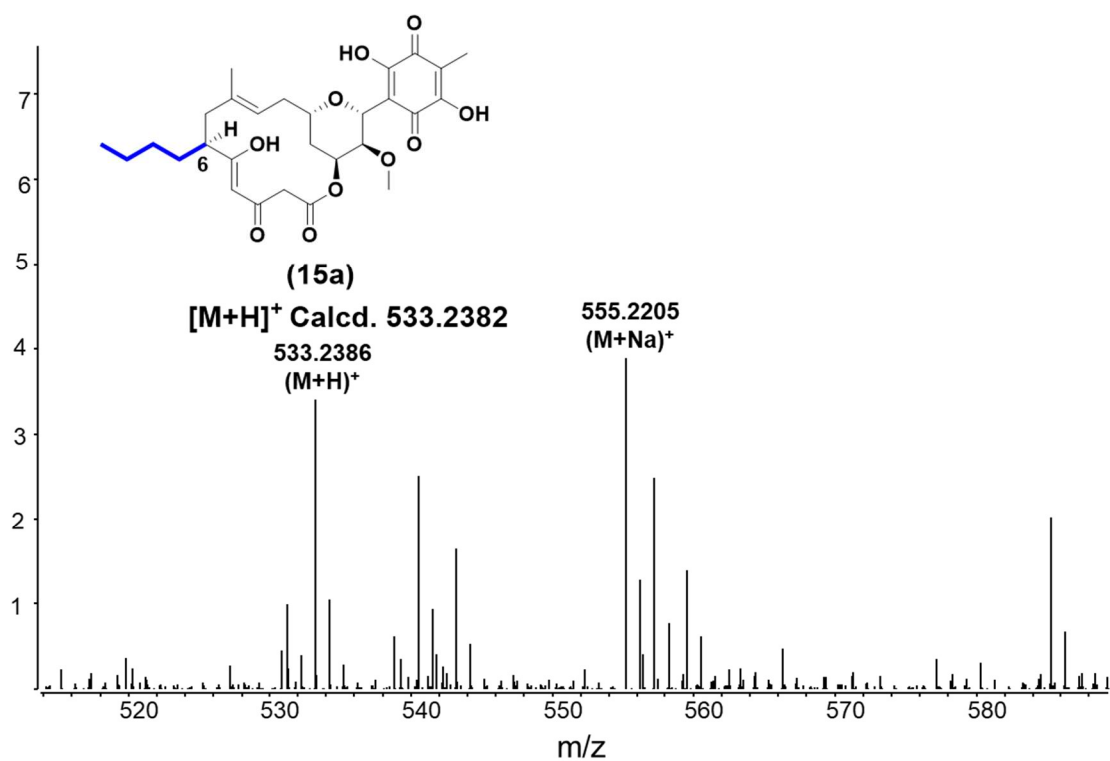
Supplementary Fig. 20. HR-ESI-MS spectrum of compound **13a**.



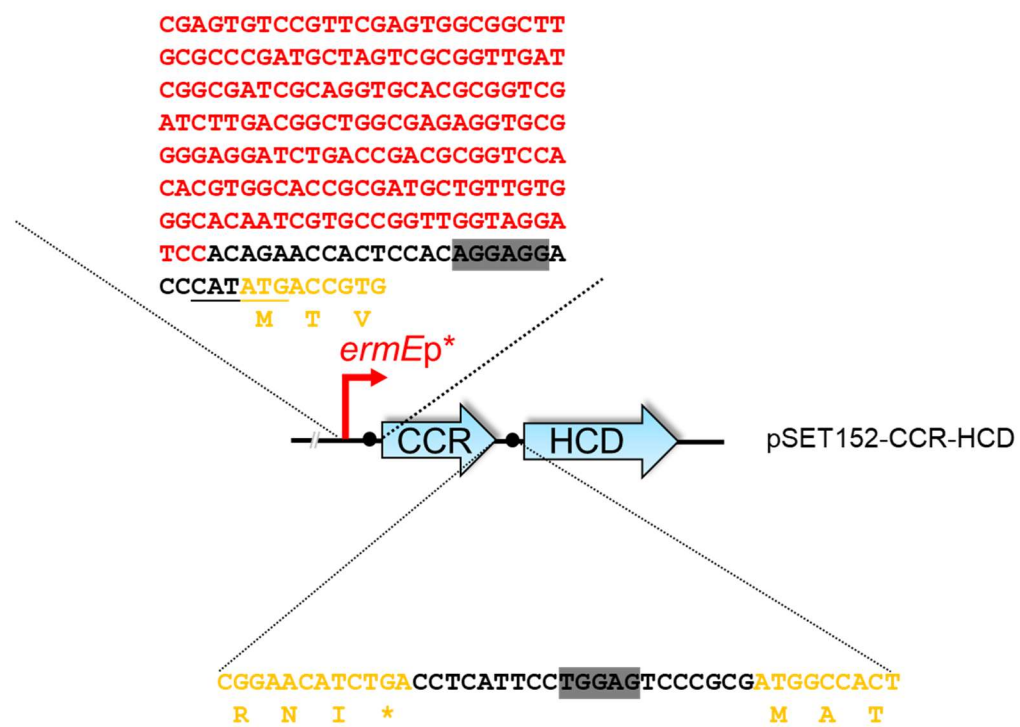
Supplementary Fig. 21. HR-ESI-MS spectrum of compound **13b**.



Supplementary Fig. 22. HR-ESI-MS spectrum of compound **14a**.

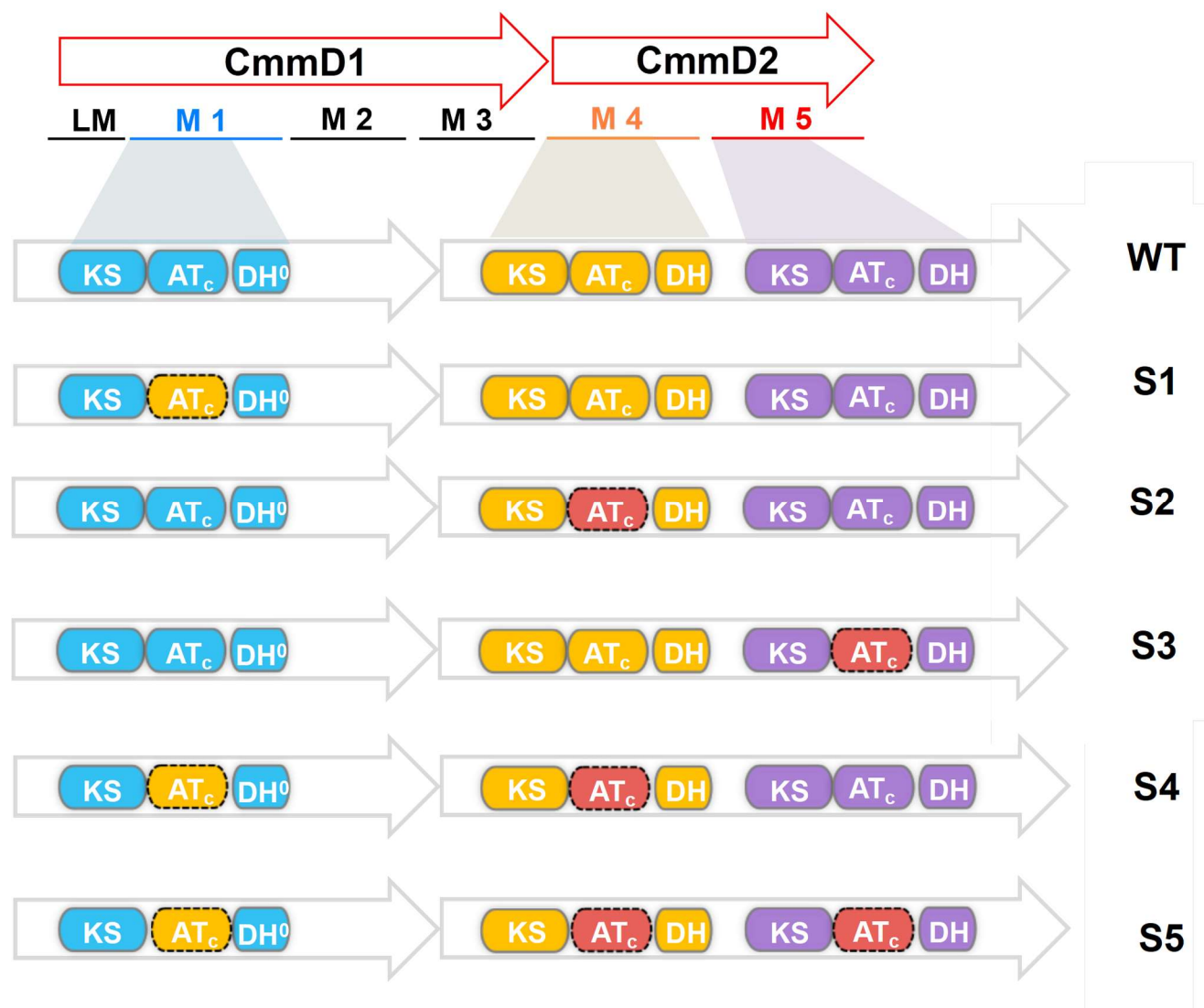


Supplementary Fig. 23. HR-ESI-MS spectrum of compound **15a**.

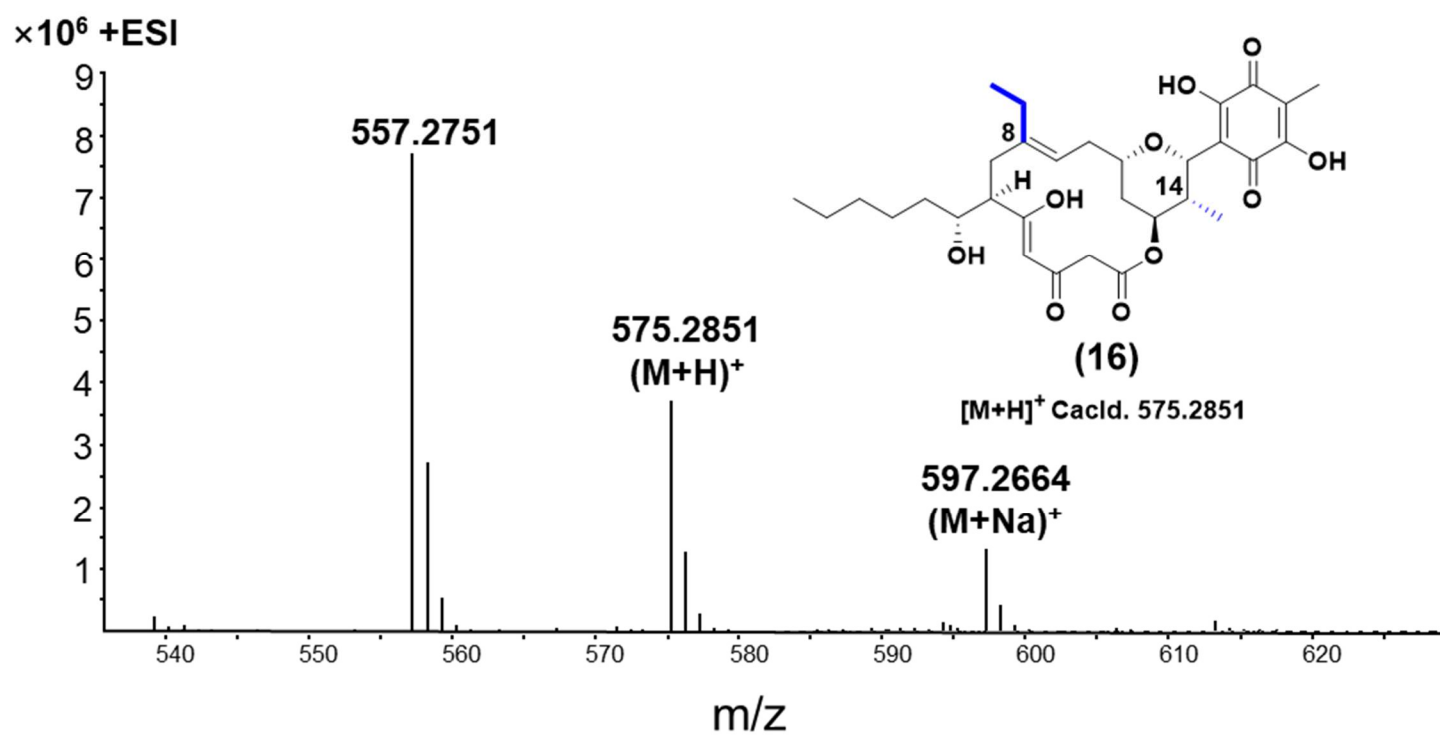


Supplementary Fig. 24. Nucleotide sequence of plasmid pSET1252-CCR-HCD.

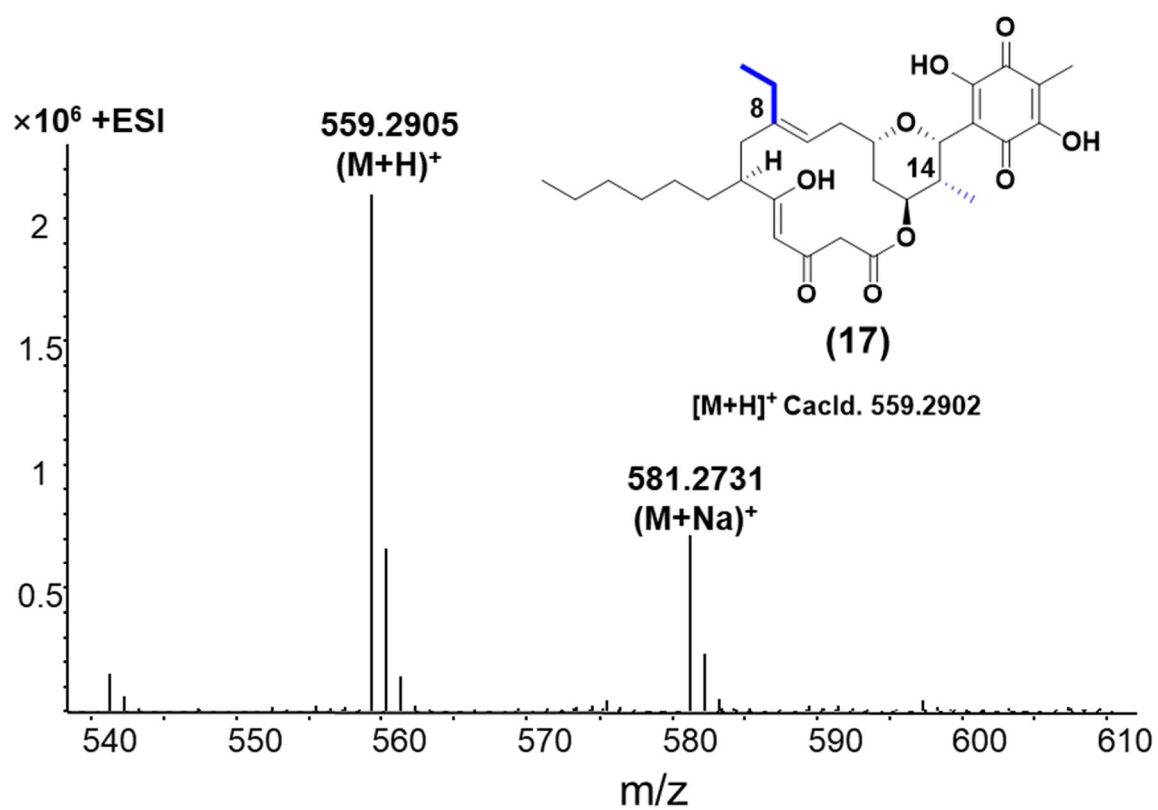
The "*ermE**p" nucleotide sequence is displayed in red bold font. Black dots represent ribosome- binding sites. The nucleotide sequences of CCR (WP_019328626.1) and HCD (WP_011030948.1) are displayed in yellow bold font.



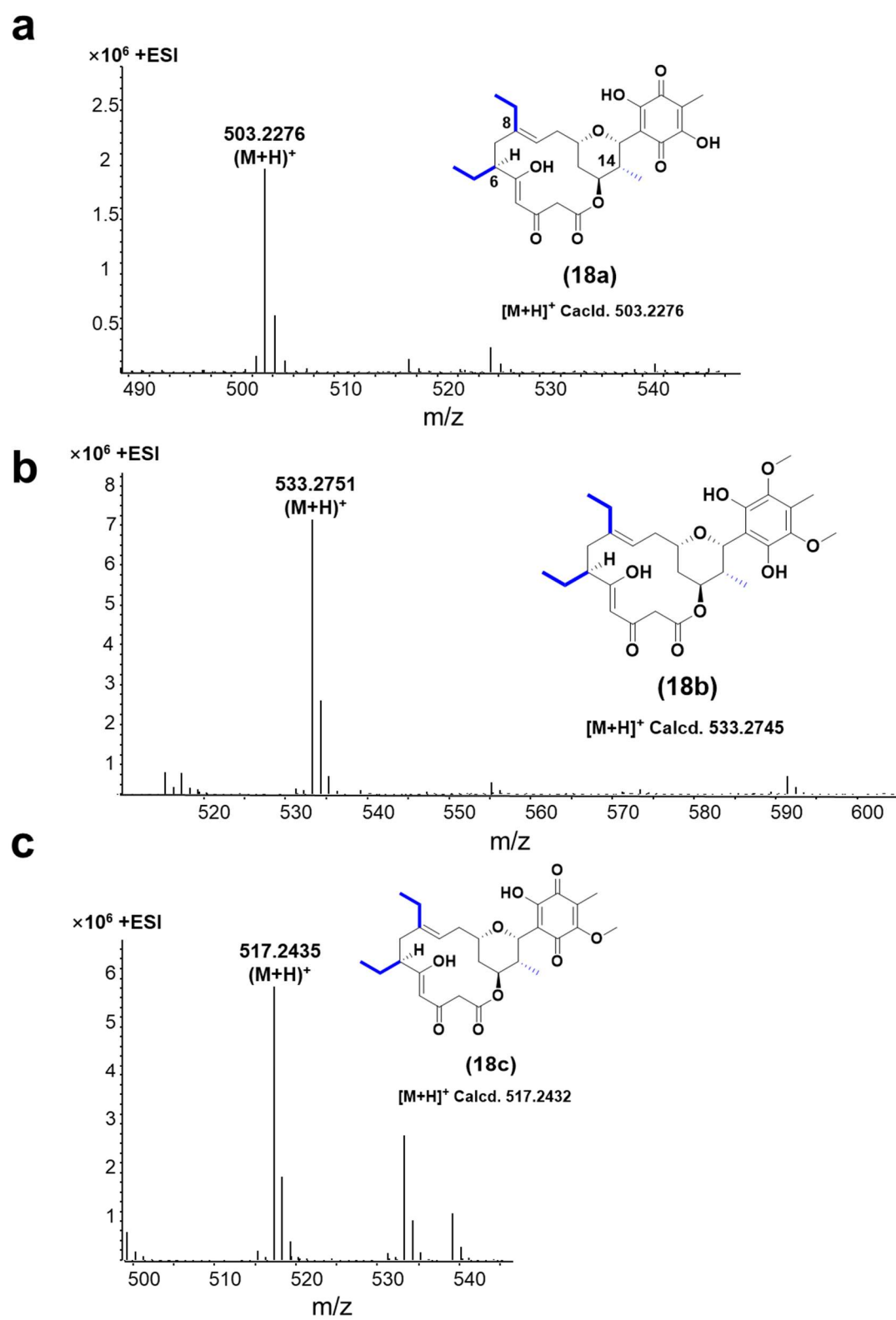
Supplementary Fig. 25. The generation of mutant S4 and S5 by successive domain replacements. AT_c colored in yellow represent CmmD2-AT₄; AT_c colored in red represent MgmD2-AT₅.



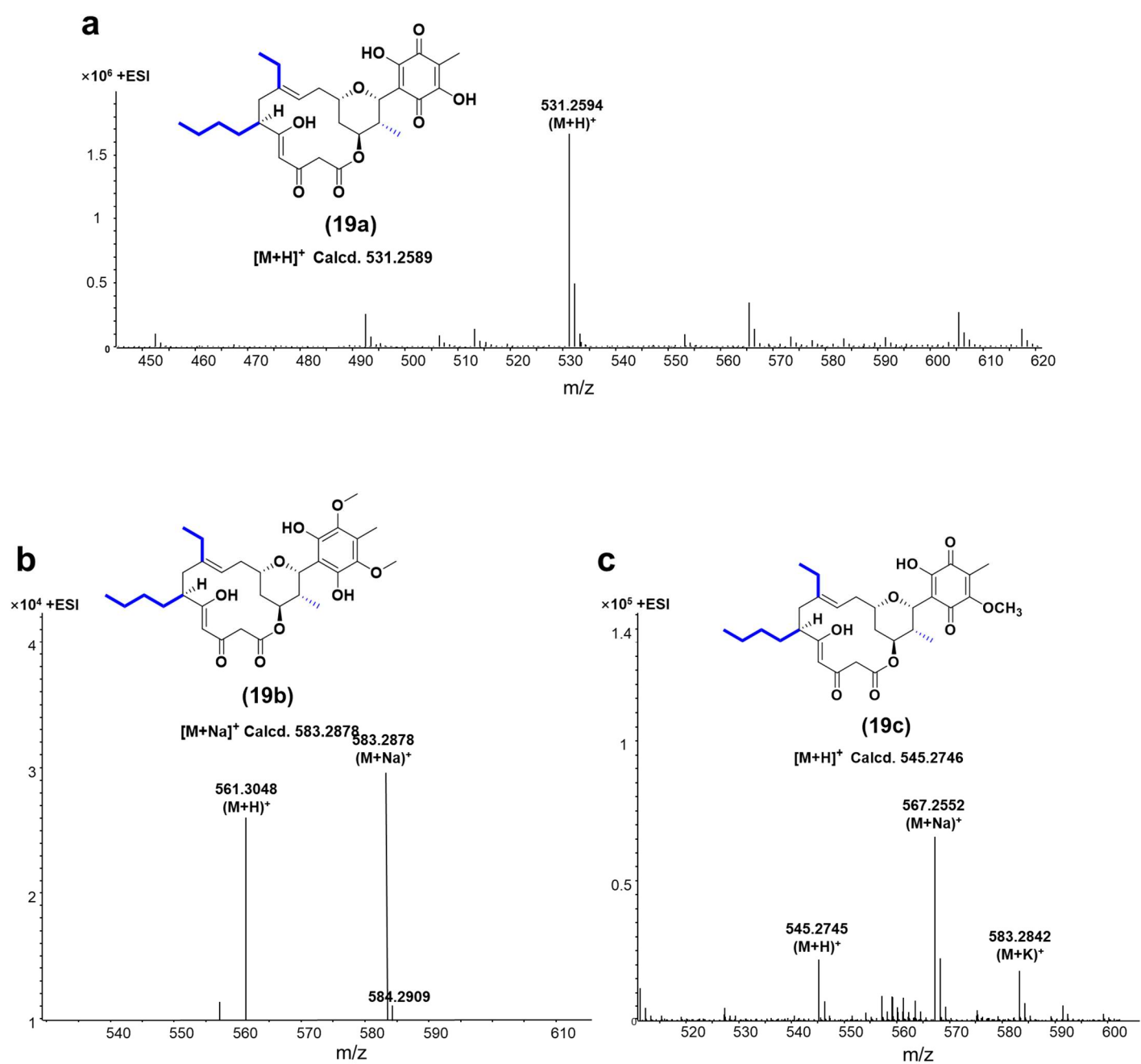
Supplementary Fig. 26. HR-ESI-MS spectrum of compound **16**.



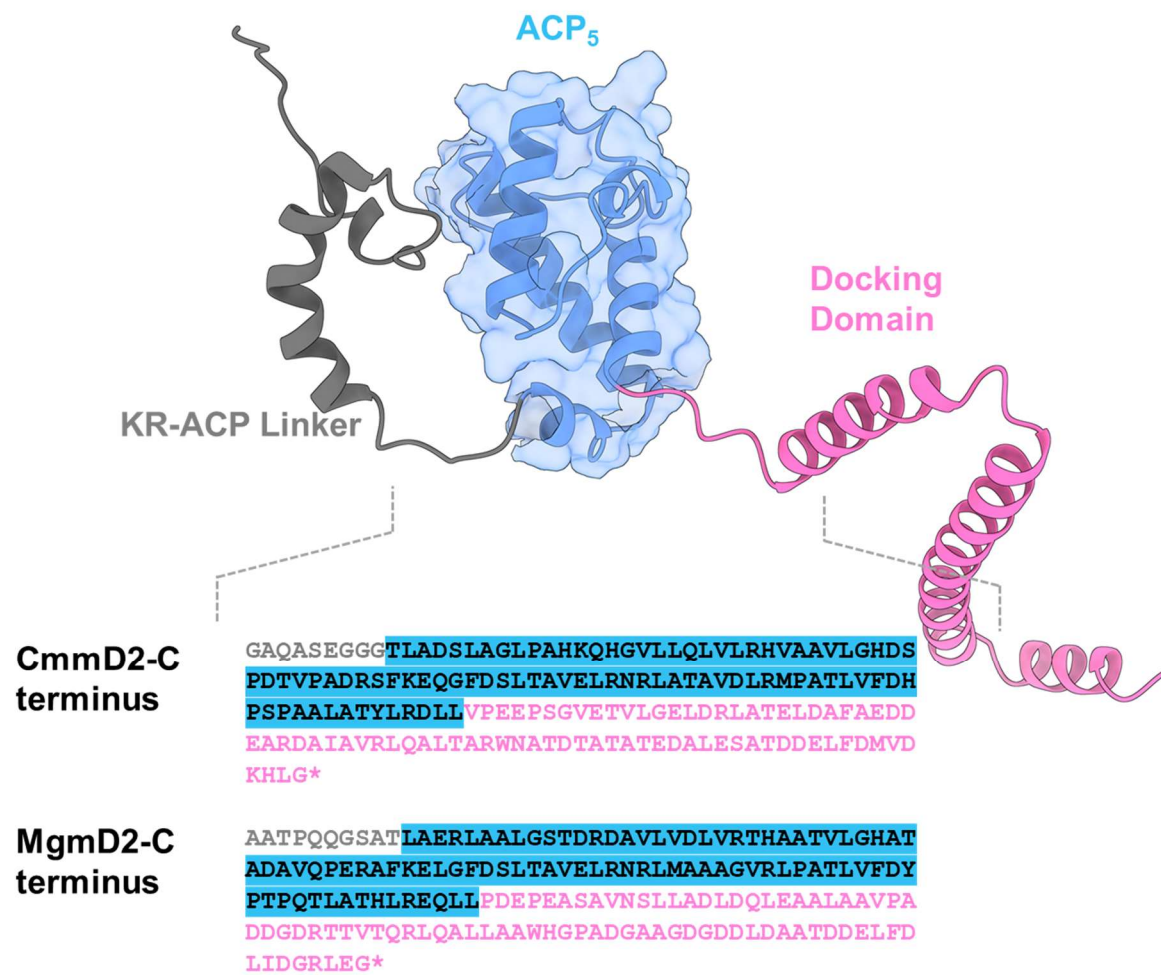
Supplementary Fig. 27. HR-ESI-MS spectrum of compound **17**.



Supplementary Fig. 28. HR-ESI-MS spectrums of compounds **18a-18c**.

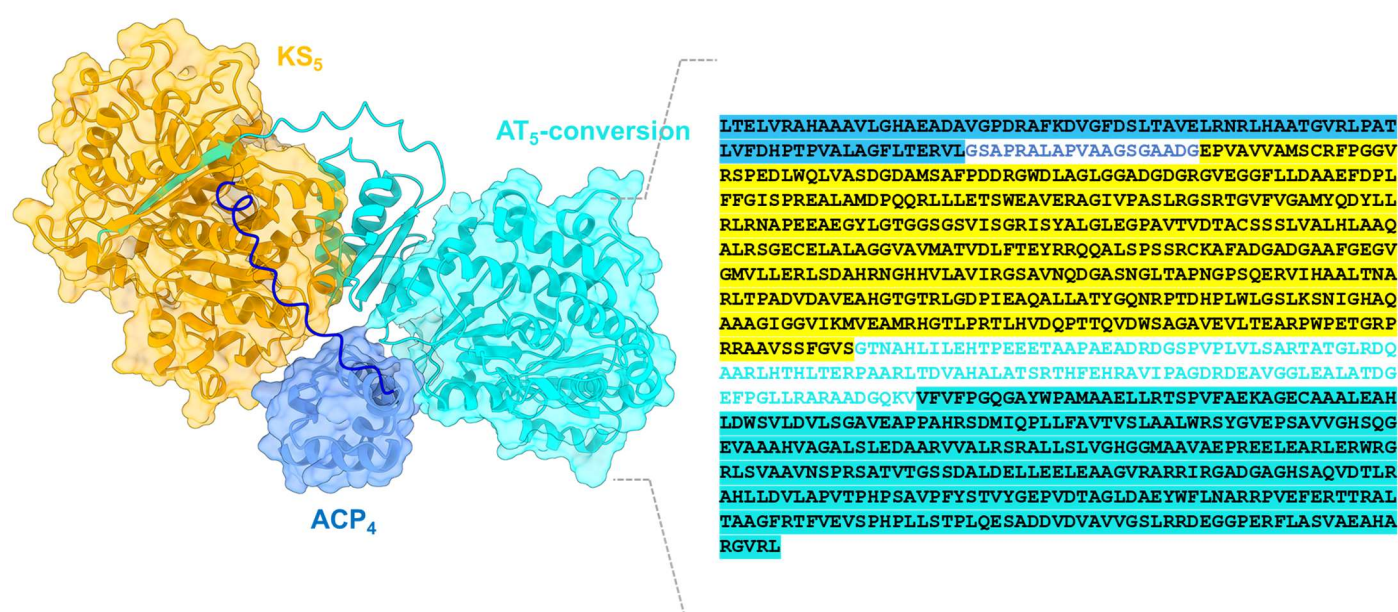


Supplementary Fig. 29. HR-ESI-MS spectrums of compounds **19a-19c**.



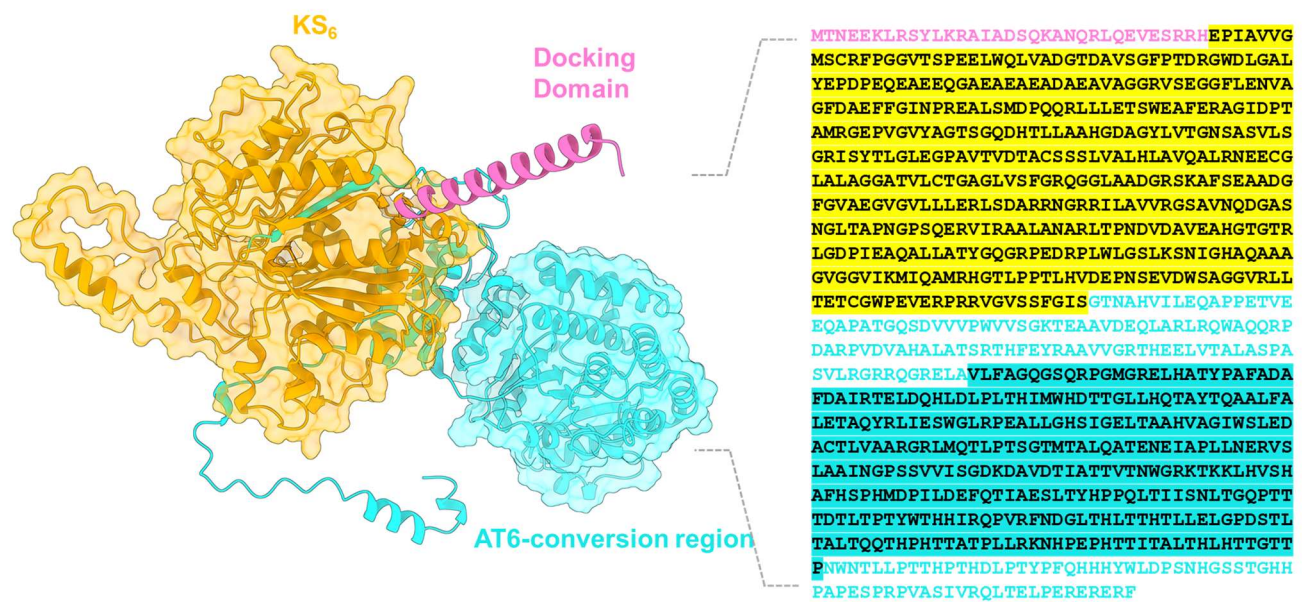
Supplementary Fig. 30. Structural modeling of CmmD2-ACP₅ region.

Model was created using AlphaFold 2.0 and visualized using Chimera X software. CmmD2-ACP₅ domain and MgmD2-ACP₅ domain are highlighted in blue. C-terminal docking domains of CmmD2 and MgmD2 are highlighted in pink, and the upstream linkers of ACP domains are colored in grey.



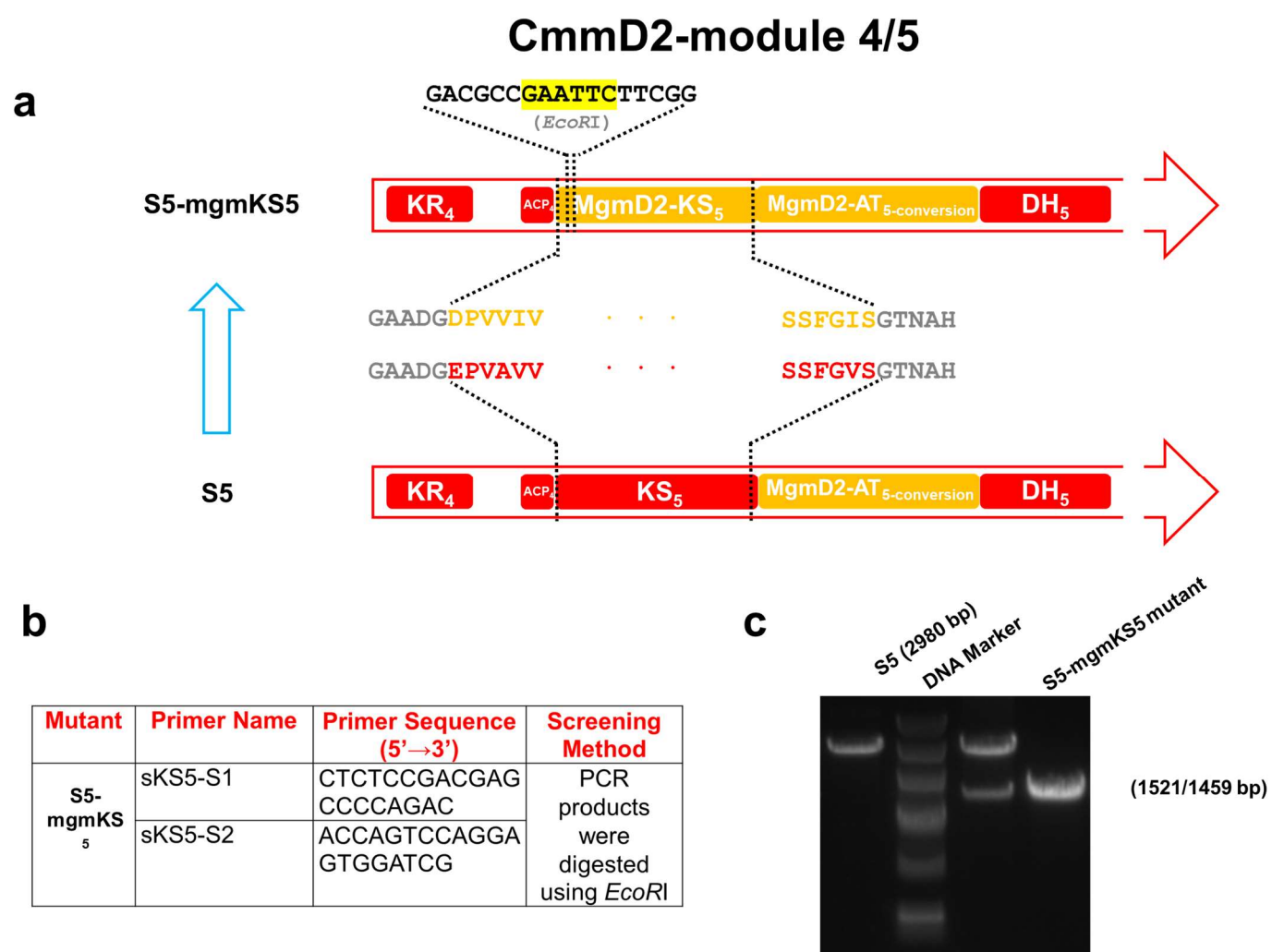
Supplementary Fig. 31. Structural modeling of CmmD2-(ACP₄-KS₅-AT₅) subunit.

Model was created using AlphaFold 2.0 and visualized using Chimera X software. The CmmD2-KS₅ domain is highlighted in yellow. CmmD2-ACP₄ domain and ACP₄-KS₅ linker are highlighted in blue, and the downstream CmmD2-AT₅ domain is highlighted in cyan.



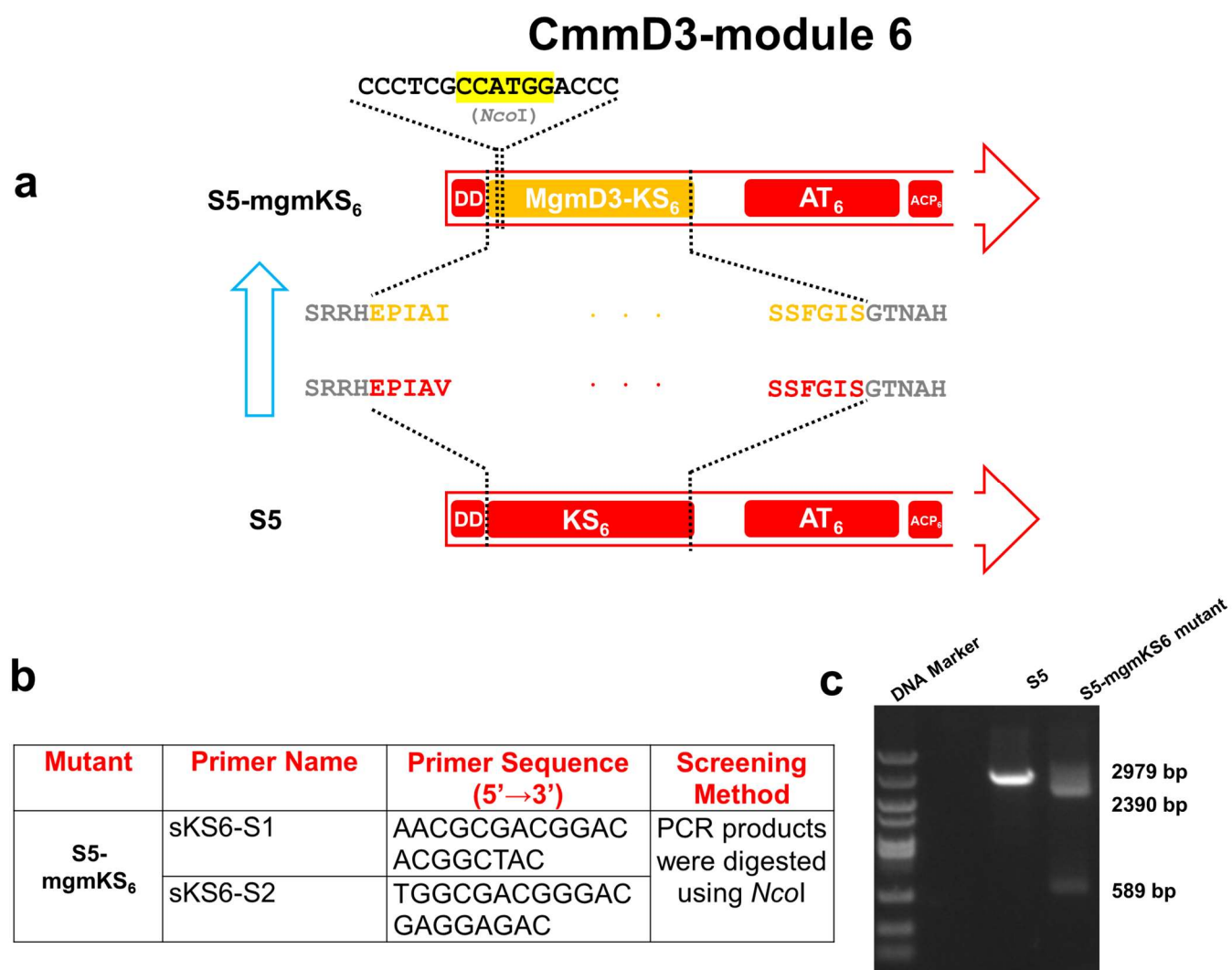
Supplementary Fig. 32. Structural modeling of CmmD3-(Docking domain-KS₆-AT₆) subunit.

Model was created using alphafold 2.0 and visualized using Chimera X software. The CmmD3-KS₆ domain is highlighted in yellow, indicating the replacement boundaries of the KS domain for mutant S5-mgmKS₆. The N-terminal docking domain of CmmD3 is highlighted in pink, and the downstream AT₆ domain is highlighted in cyan.



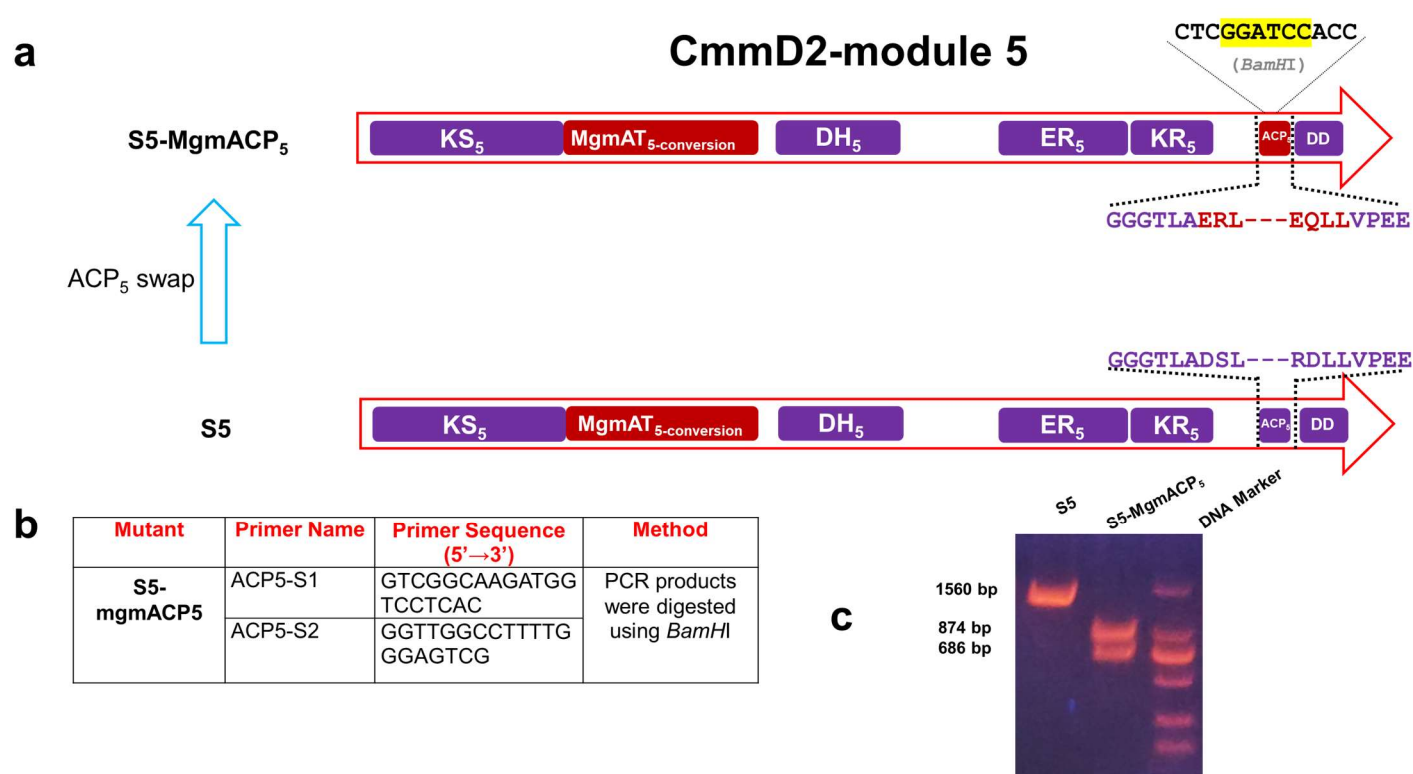
Supplementary Fig. 33. Construction and confirmation of mutant S5-mgmKS₅ strain.

(a) Schematic diagram of construction of mutant S5-mgmKS₅. *EcoRI* restriction sites used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S5-mgmKS₅ by PCR and restriction enzyme digesting. The red squares represent domains from CmmD2. Due to the introduction of *EcoRI* digestion site, the PCR product in mutant S5-mgmKS₅ could be cleaved into two bands of 1521 bp and 1459bp, whereas the one in mutant S5 remained to be 2980 bp.



Supplementary Fig. 34. Construction and confirmation of mutant S5-mgmKS₆ strain.

(a) Schematic diagram of construction of mutant S5-mgmKS₆, *Nco*I restriction sites used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S5-mgmKS₆ by PCR and restriction enzyme digesting. The red squares represent domains from CmmD3, and DD is the abbreviation of docking domain. Due to the introduction of *Nco*I digestion site, the PCR product in mutant S5-mgmKS₆ could be cleaved into two bands of 2390 bp and 589 bp, whereas the one in mutant S5 remained to be 2979 bp. The red squares represent domains from CmmD3, and DD is the abbreviation of docking domain.

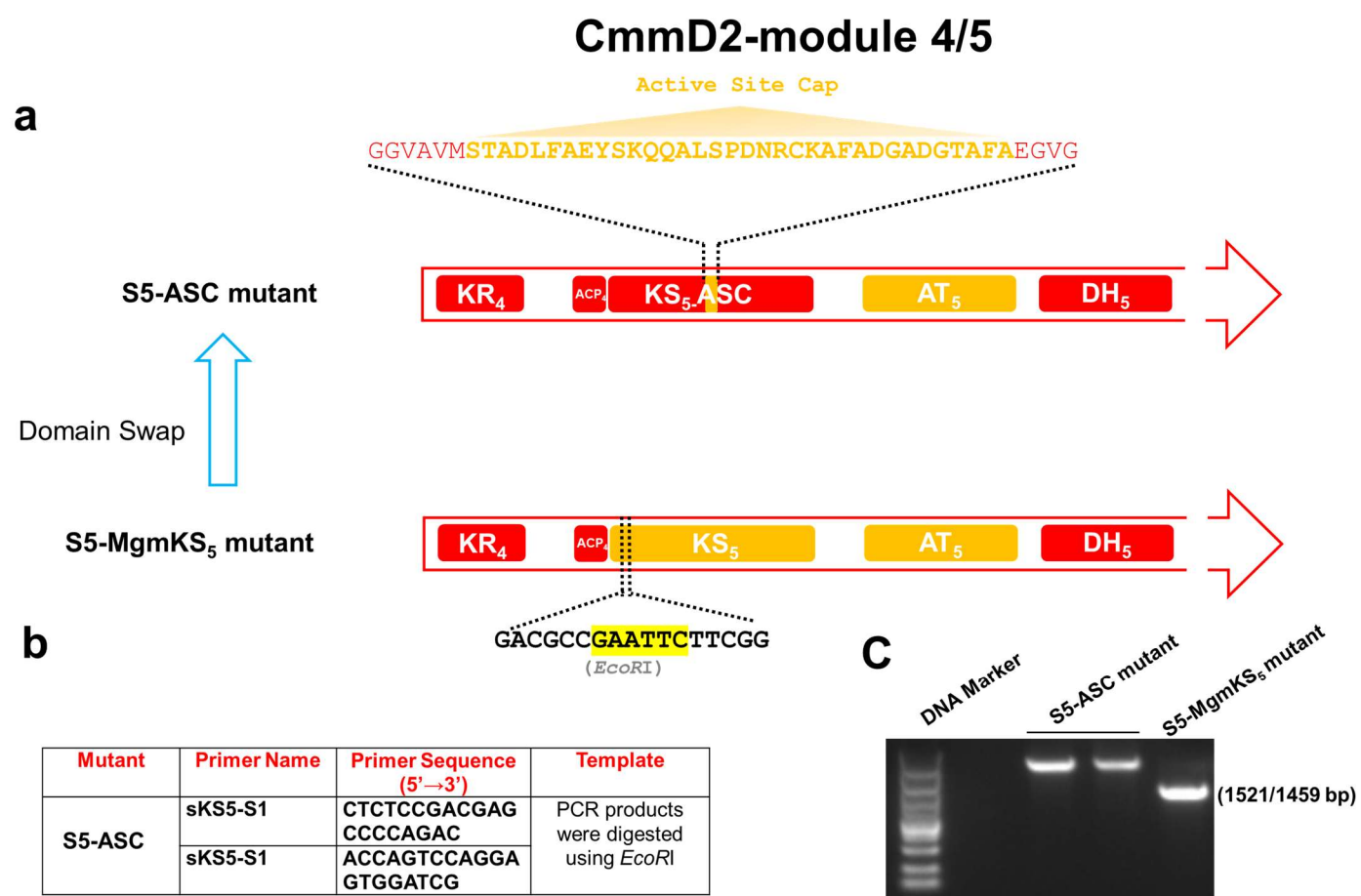


Supplementary Fig. 35. Construction and confirmation of mutant S5-mgmACP₅ strain.

(a) Schematic diagram of construction of mutant S5-mgmACP₅, *Bam*HI restriction sites used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S5-mgmACP₅ by PCR and restriction enzyme digesting. The purple squares represent domains from CmmD2, and DD is the abbreviation of docking domain. Due to the introduction of *Bam*HI digestion site, the PCR product in mutant S5-mgmACP₅ could be cleaved into two bands of 874 bp and 686 bp, whereas the one in wild-type remained to be 1560 bp. The purple squares represent domains from CmmD2, and DD is the abbreviation of docking domain.

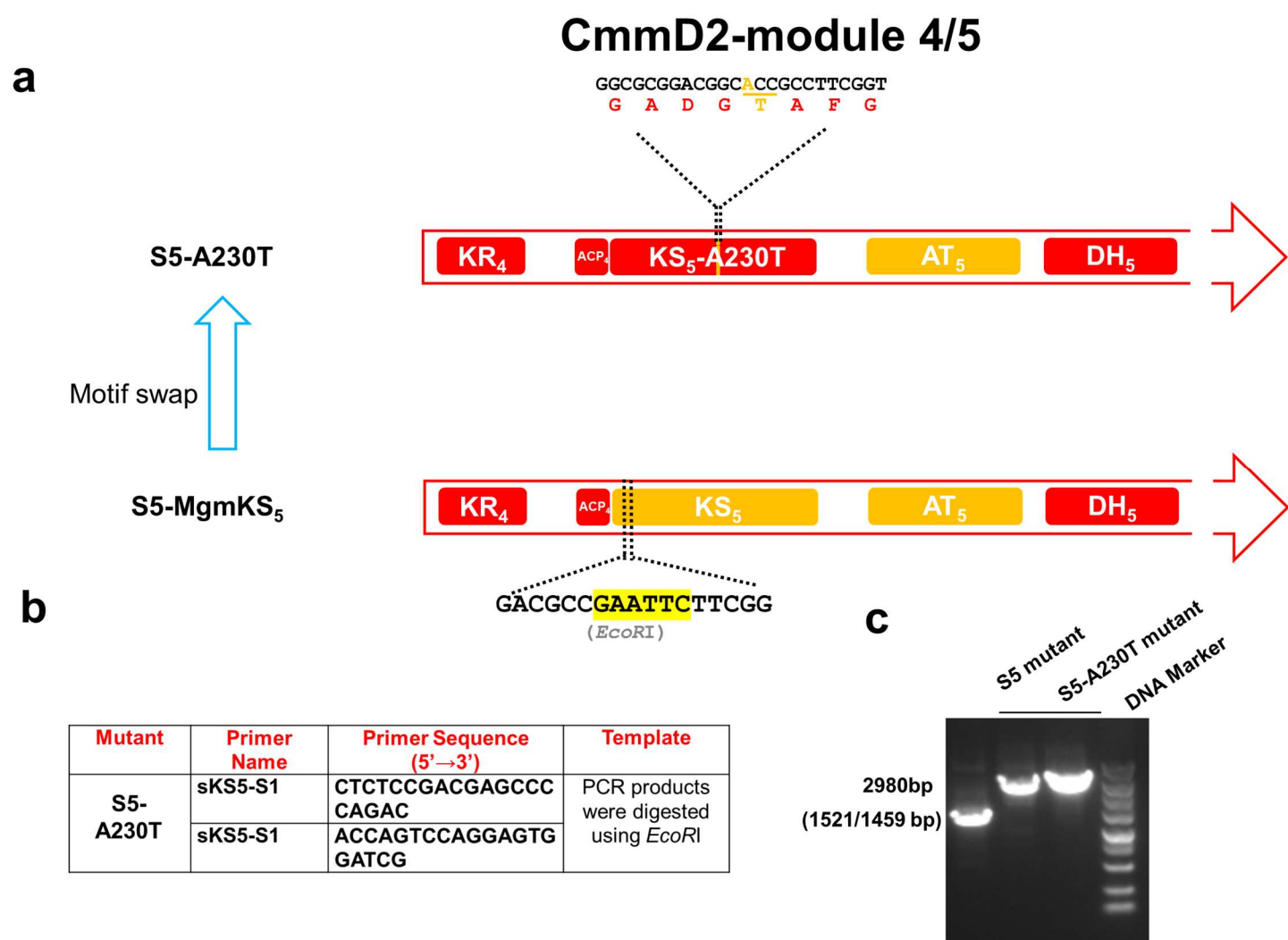


Supplementary Fig. 36. Amino acid sequence alignments of Mgm-KS5, Cmm-KS5 and EryKS3⁴.



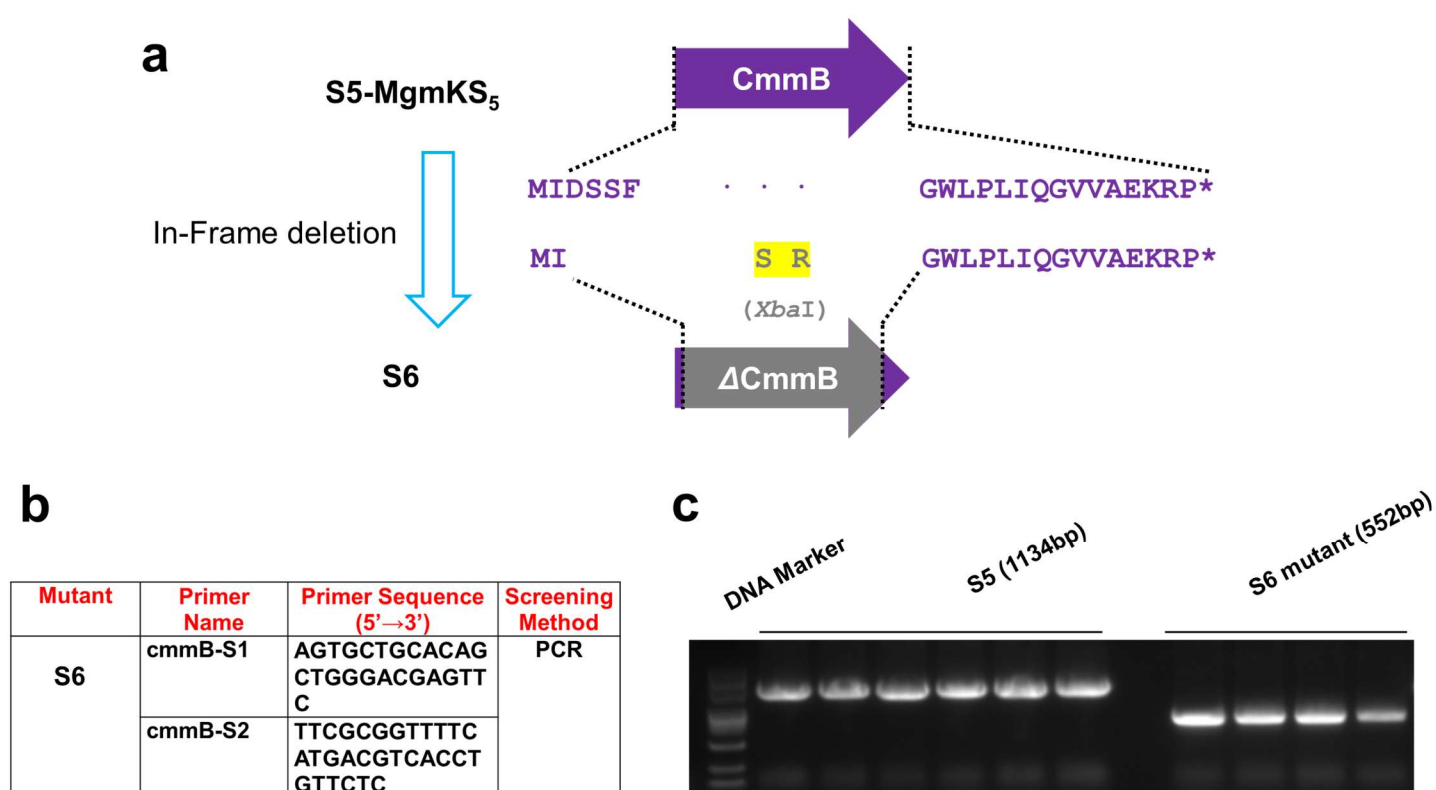
Supplementary Fig. 37. Construction and confirmation of mutant S5-ASC.

(a) Schematic diagram of construction of mutant S5-mgmKS₅. *EcoRI* restriction sites used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S5-ASC by PCR and restriction enzyme digesting. The red squares represent domains from CmmD2, and yellow squares represent domains from MgmD2. Due to the absence of *EcoRI* digestion site, the PCR products in mutant S5-ASC could be cleaved into two bands of 1521 bp and 1459bp, whereas the one in S5-mgmKS₅ remained to be 2980 bp.



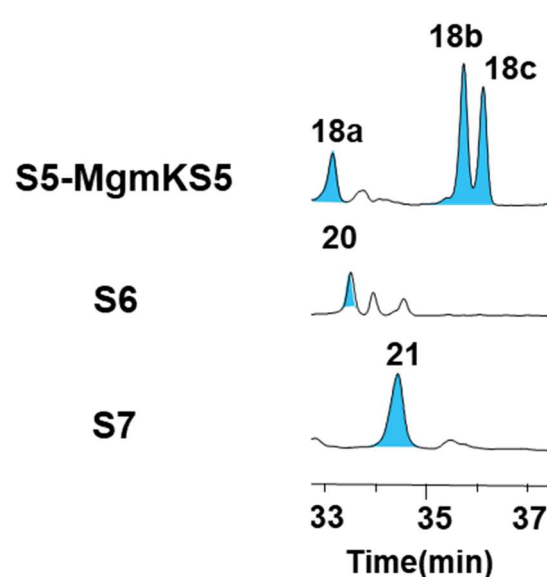
Supplementary Fig. 38. Construction and confirmation of S5-A230T mutant strain.

(a) Schematic diagram of construction of mutant S5-A230T. *EcoRI* restriction sites used for screening is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S5-A230T by PCR and restriction enzyme digesting. The red squares represent domains from CmmD2, and yellow squares represent domains from MgmD2. Due to the absence of *EcoRI* digestion site, the PCR product in mutant S5-A230T could be cleaved into two bands of 1521 bp and 1459bp, whereas the one in mutant S5-mgmKS₅ remained to be 2980 bp.

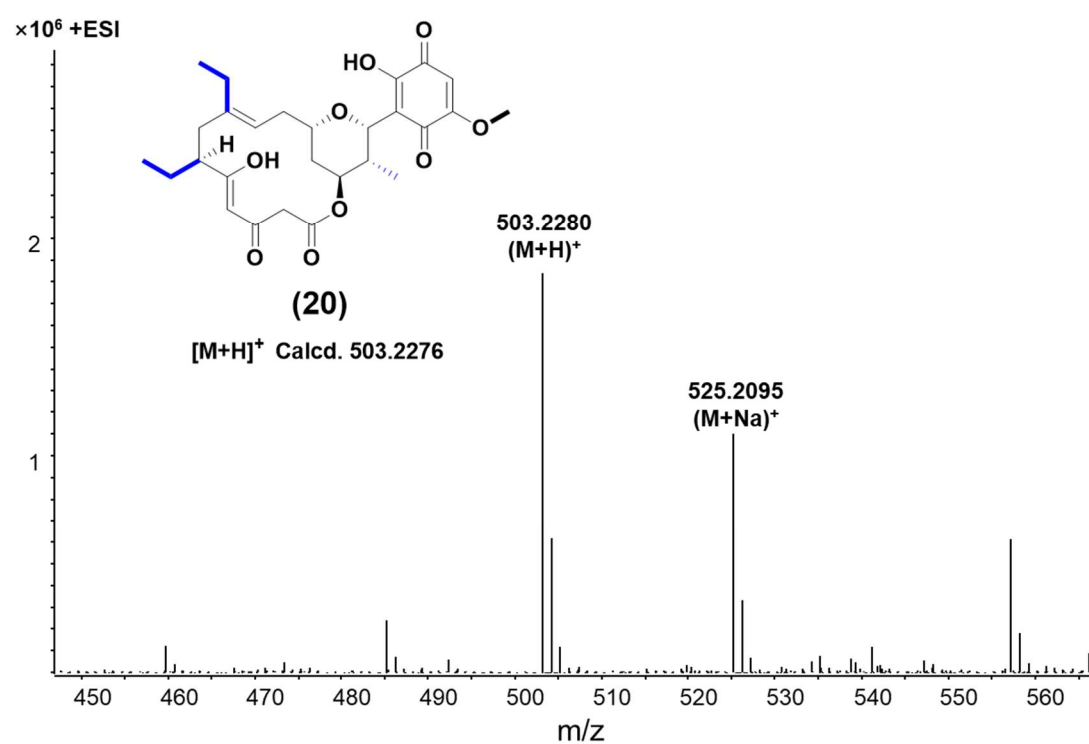
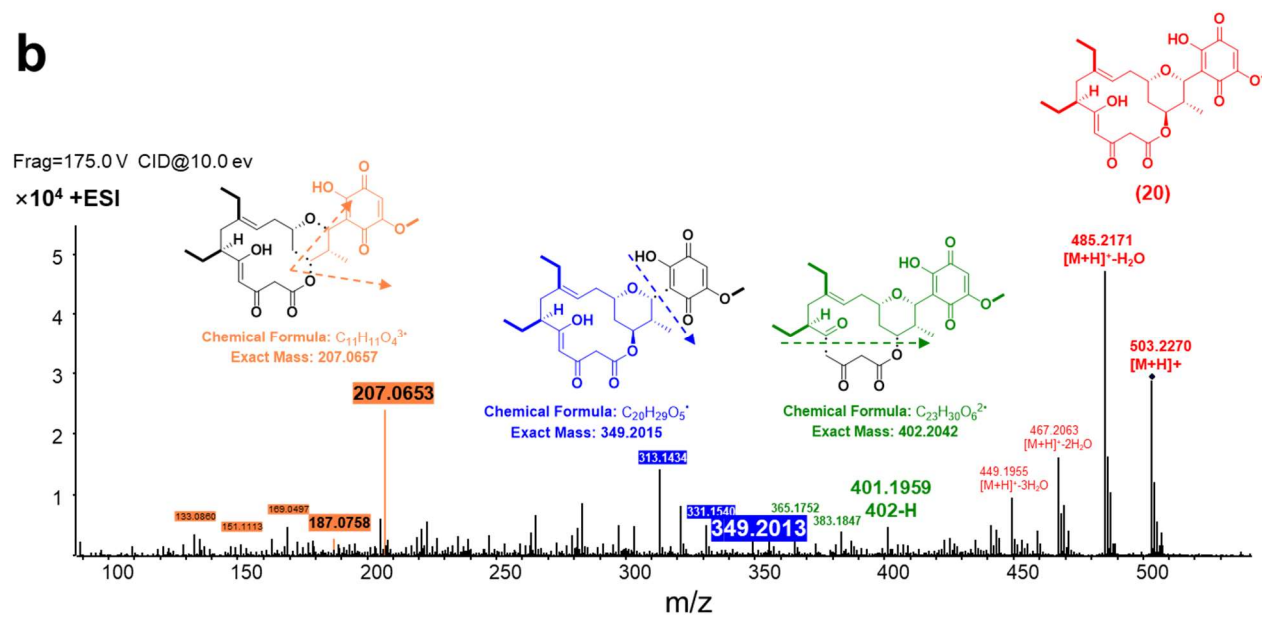


Supplementary Fig. 39. Construction and confirmation of mutant S6 strain.

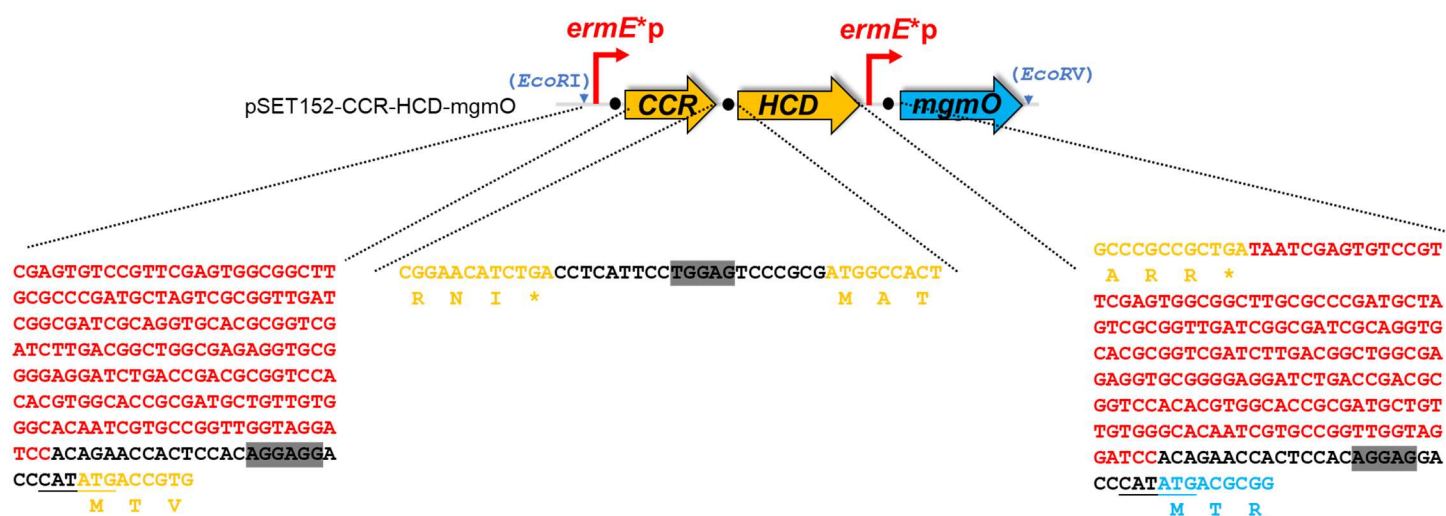
(a) Schematic diagram of construction of mutant S6, *XbaI* restriction sites is highlighted in yellow; (b) Primers and methods for mutant strain screening; (c) Agarose gel screening of mutant S6. Due to the absence of the gene fragment, the PCR products in mutant S6 were expected to be 552bp, whereas the one in mutant S5-mgmKS₅ remained to be 1134 bp.



Supplementary Fig. 40. HPLC chromatograms of the fermentation broth from engineered strains of S6 and S7 at $\lambda = 280$ nm. The desired products are shown in blue peaks. Three independent experiments were repeated.

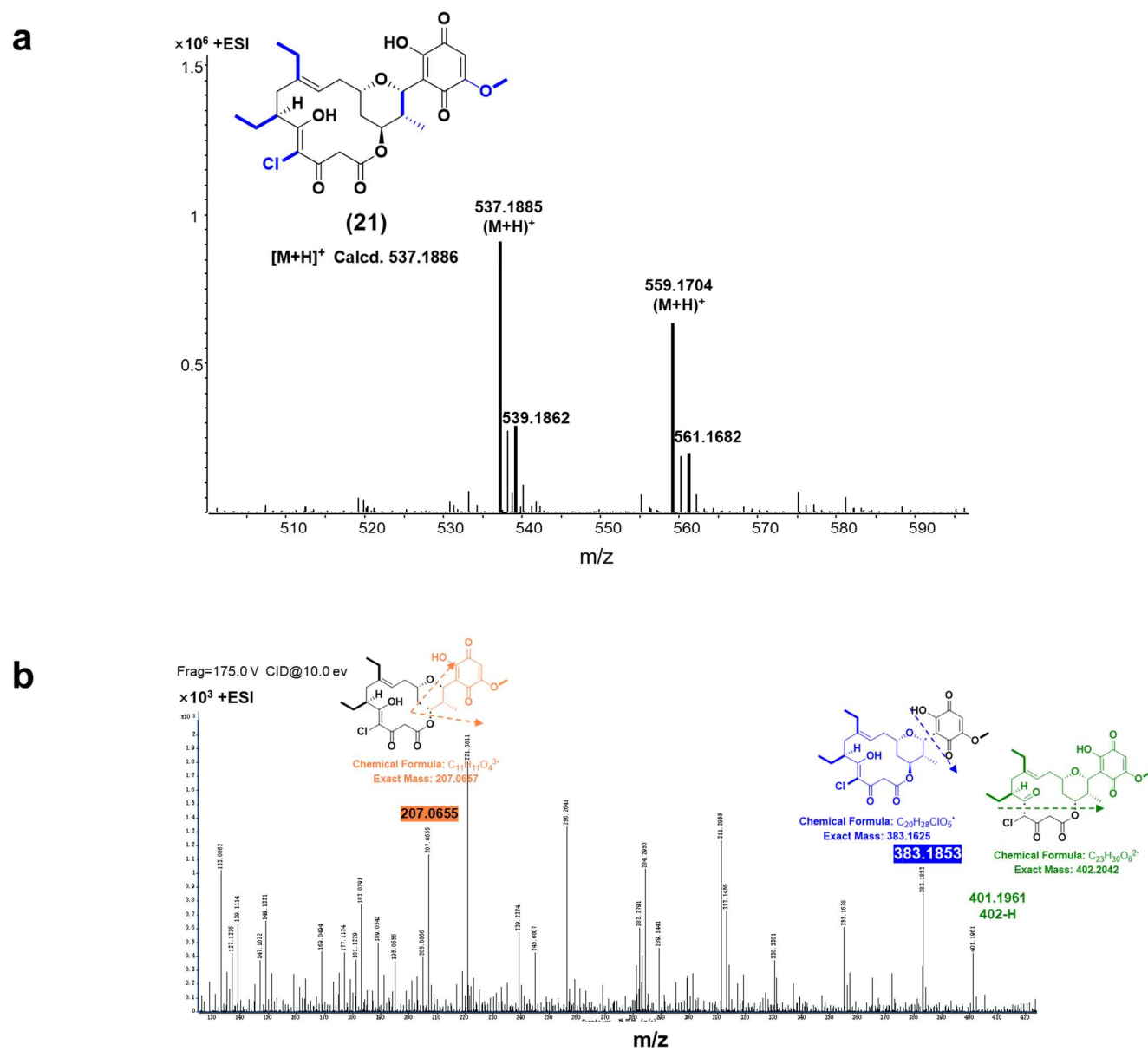
a**b**

Supplementary Fig. 41. LC-ESI-HRMS (a) and MS/MS (b) analysis of compound **20**.



Supplementary Fig. 42. Nucleotide sequence of plasmid pSET1252-CCR-HCD-mgmO.

The "*ermE**" nucleotide sequence is displayed in red bold font. The nucleotide sequences of CCR and HCD are displayed in yellow bold font. The nucleotide sequences of *mgmO* are displayed in blue bold font.



Supplementary Fig. 43. LC-ESI-HRMS spectrum (**a**) and MS/MS analysis (**b**) of compound **21**.

Supplementary References

1. Dunn, B.J. & Khosla, C. (2013). Engineering the acyltransferase substrate specificity of assembly line polyketide synthases. *J. R. Soc. Interface* *10*, 20130297. <https://doi.org/10.1098/rsif.2013.0297>.
2. Keatinge-Clay, A. T. (2016) Stereocontrol within polyketide assembly lines. *Nat. Prod. Rep.* *33*, 141-149. <https://doi.org/10.1039/c5np00092k>.
3. Latham, J., et al. (2018). Development of halogenase enzymes for use in synthesis. *Chem. Rev.* *118*, 232-269. <https://doi.org/10.1021/acs.chemrev.7b00032>.
4. Murphy, A. C., et al. (2016). Broadening substrate specificity of a chain-extending ketosynthase through a single active-site mutation. *Chem. Commun.* *52*, 8373-8376. <https://doi.org/10.1039/c6cc03501a>.