Supplementary Information for

A fungal ketoreductase domain that displays substrate-dependent stereospecificity

Hui Zhou[1§], Zhizeng Gao[3§], Kangjian Qiao[1], Jingjing Wang[1], John C. Vederas[3]*, Yi Tang[1,2]*

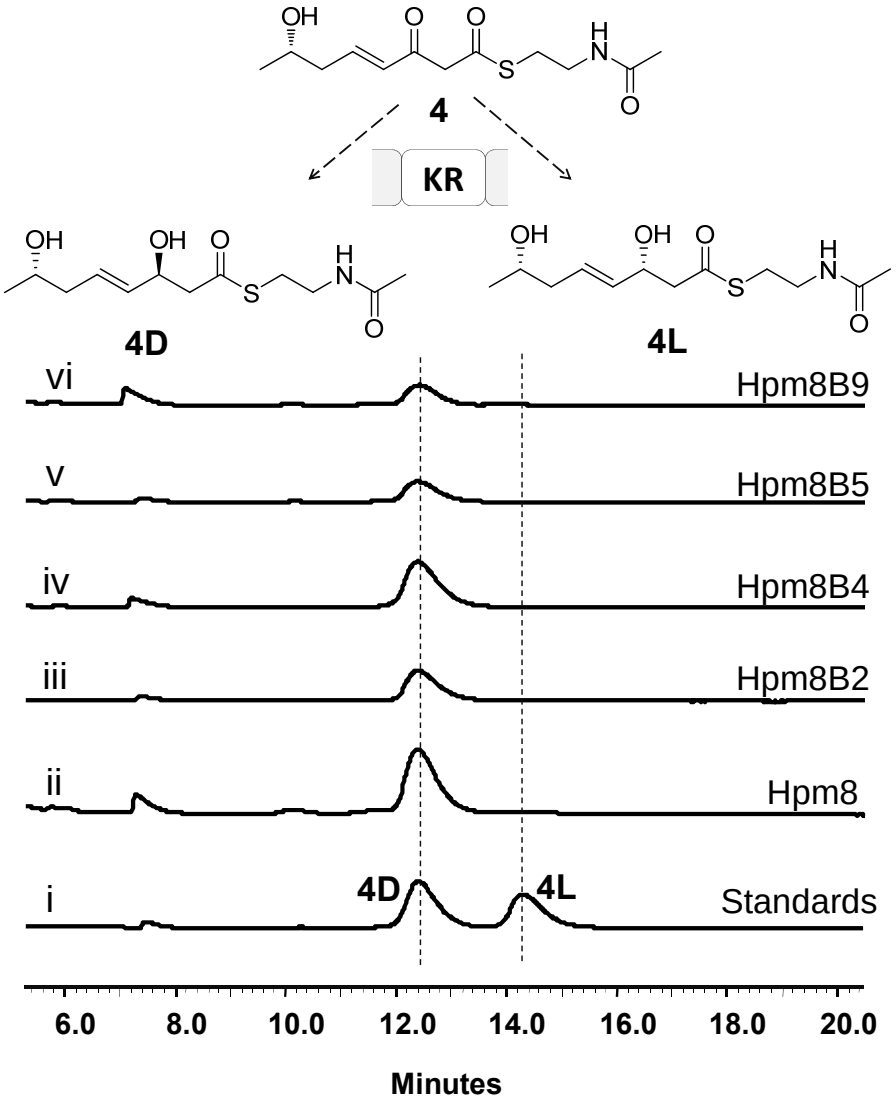
[1]Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, USA, [2]Department of Chemistry and Biochemistry, University of California, Los Angeles, USA and [3]Department of Chemistry, University of Alberta, Canada

[§]These authors contributed equally to this work.

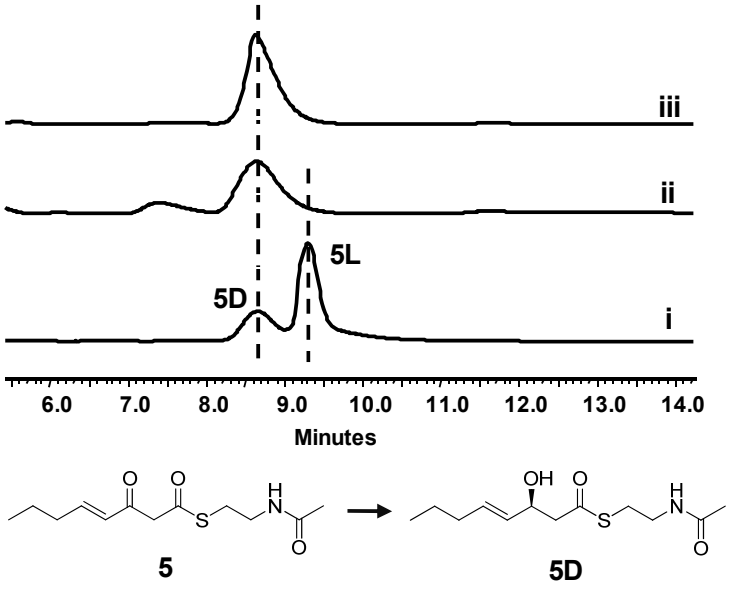
*Correspondence e-mail: yitang@ucla.edu, john.vederas@ualberta.ca

Table of Contents

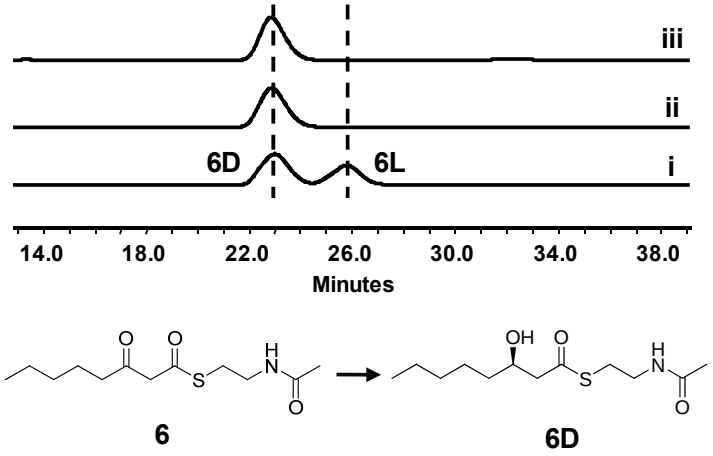
1. Supplementary Results



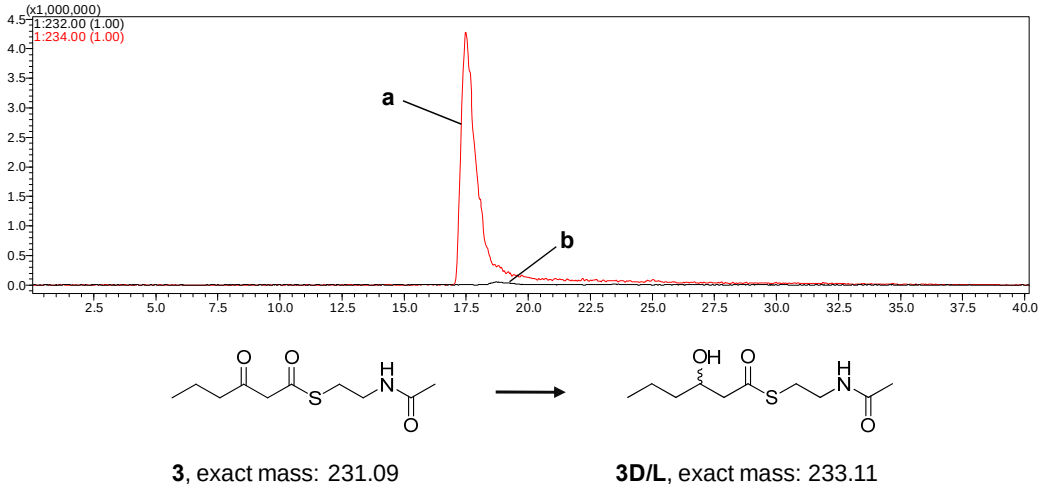
Supplementary Figure 1. The chiral HPLC traces (240 nm) of (i) the mixture of the chemical standards **4L** and **4D**, and the reductive products generated by (ii) Hpm8, (iii) Hpm8B2, (iv) Hpm8B4, (v) Hpm8B5 or (vi) Hpm8B9 starting from (*S,E*)-7-hydroxy-3-oxooct-4-enoyl SNAC **4**.



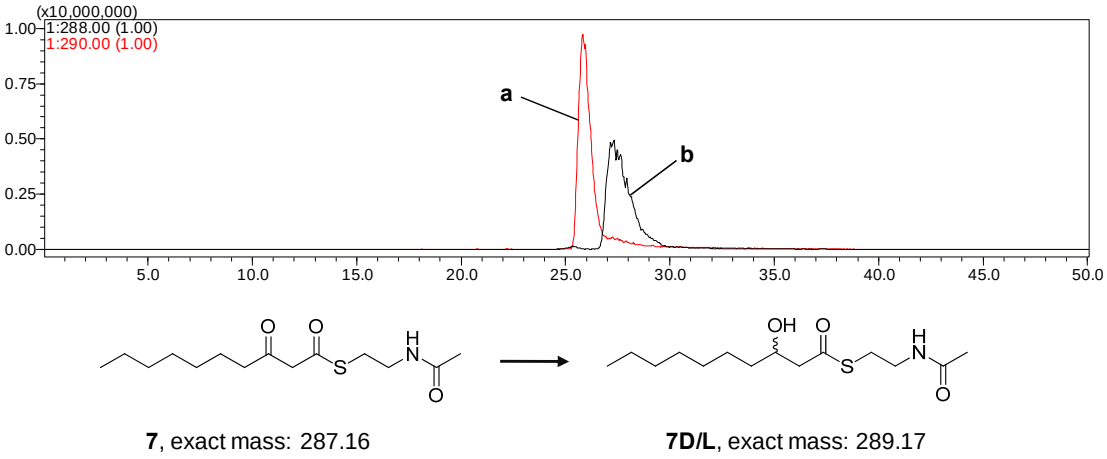
Supplementary Figure 2. (i) The chiral HPLC traces of the mixture of the chemical standards **5D** and **5L**, and the chiral HPLC traces of the reductive products by (ii) Hpm8 or (iii) Hpm8B4 with the substrate **5**. The chromatograms above were obtained by monitoring at 240 nm.



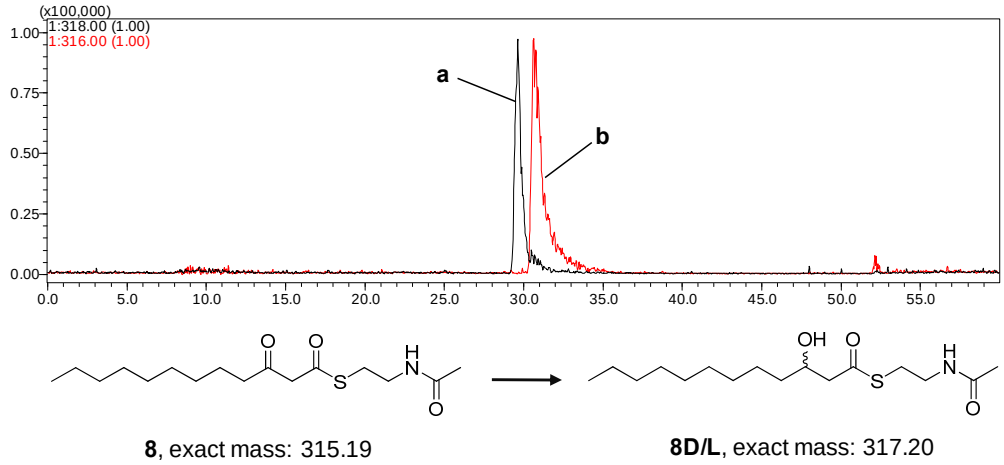
Supplementary Figure 3. (i) The chiral HPLC traces of the mixture of the chemical standards **6D** and **6L**, and the chiral HPLC traces of the reductive products by (ii) Hpm8 or (iii) Hpm8B4 with the substrate **6**. The chromatograms above were obtained by monitoring at 240 nm.



Supplementary Figure 4. The LCMS analysis of the in vitro Hpm8 KR assay on substrate **3**. The traces shown are the selected ion monitoring of desired ions in the positive ionization mode. Trace **a** is $[M+H]^+$ at 234 for **3D/L** and trace **b** is $[M+H]^+$ at 232 for **3**.

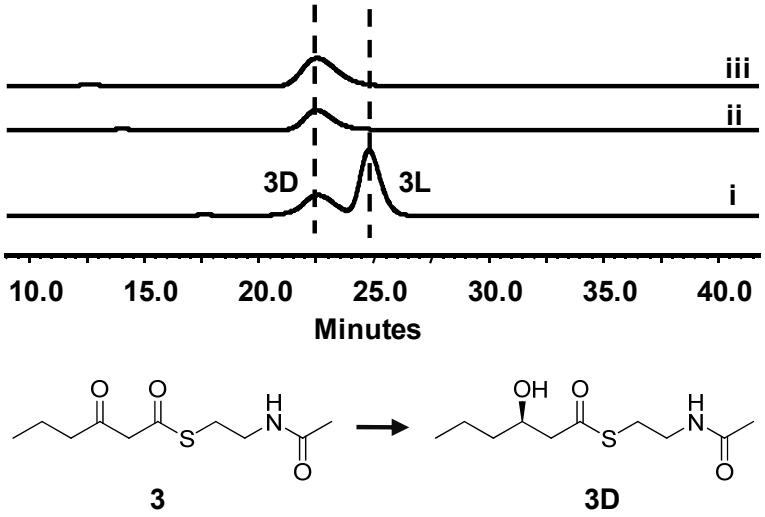


Supplementary Figure 5. The LCMS analysis of the in vitro Hpm8 KR assay on substrate **7**. The traces shown are the selected ion monitoring of desired ions in the positive ionization mode. Trace **a** is $[M+H]^+$ at 290 for **7D/L** and trace **b** is $[M+H]^+$ at 288 for **7**.

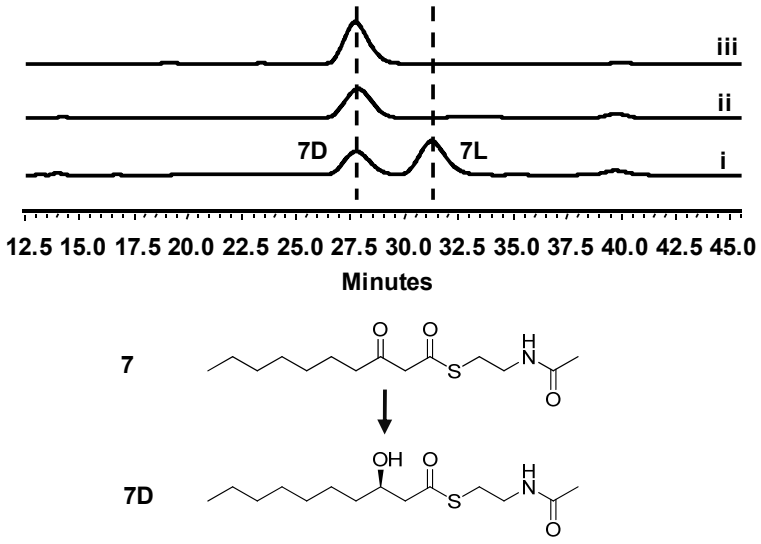


8, exact mass: 315.19 **8D/L**, exact mass: 317.20

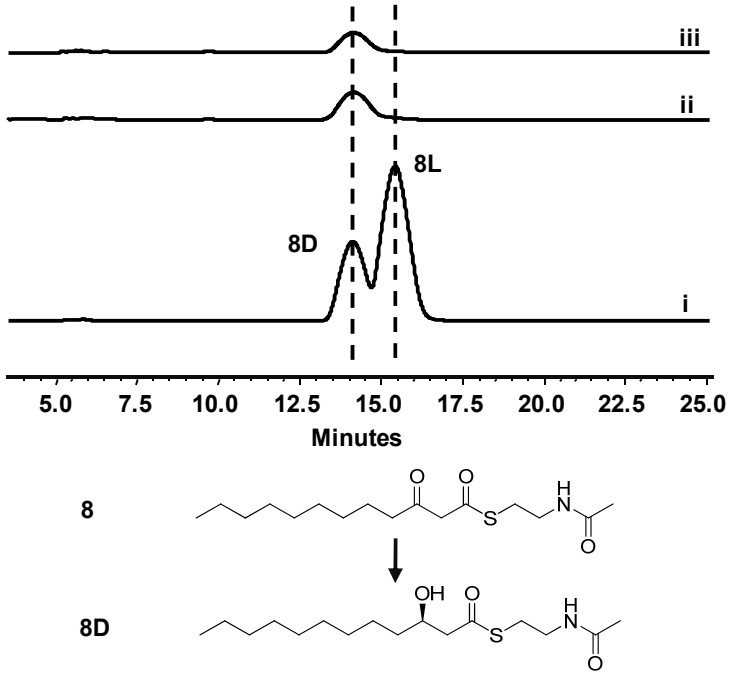
Supplementary Figure 6. The LCMS analysis of the in vitro Hpm8 KR assay on substrate **8**. The traces shown are the selected ion monitoring of desired ions in the positive ionization mode. Trace **a** is $[M+H]^+$ at 318 for **8D/L** and trace **b** is $[M+H]^+$ at 316 for **8**.



Supplementary Figure 7. (i) The chiral HPLC traces of the mixture of the chemical standards **3D** and **3L**, and the chiral HPLC traces of the reductive products by (ii) Hpm8 or (iii) Hpm8B4 assayed with the substrate **3**. The chromatograms above were obtained by monitoring at 240 nm.

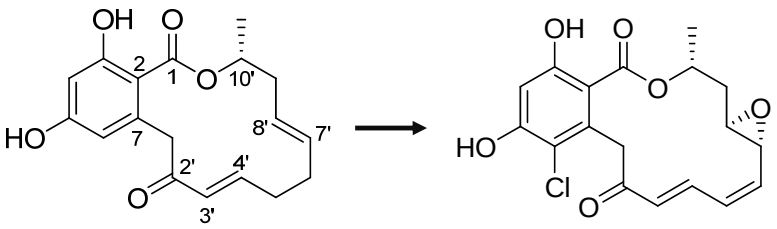


Supplementary Figure 8. (i) The chiral HPLC traces of the mixture of the chemical standards **7D** and **7L**, and the chiral HPLC traces of the reductive products by (ii) Hpm8 or (iii) Hpm8B4 working on the substrate **7**. The chromatograms above were obtained by monitoring at 240 nm.



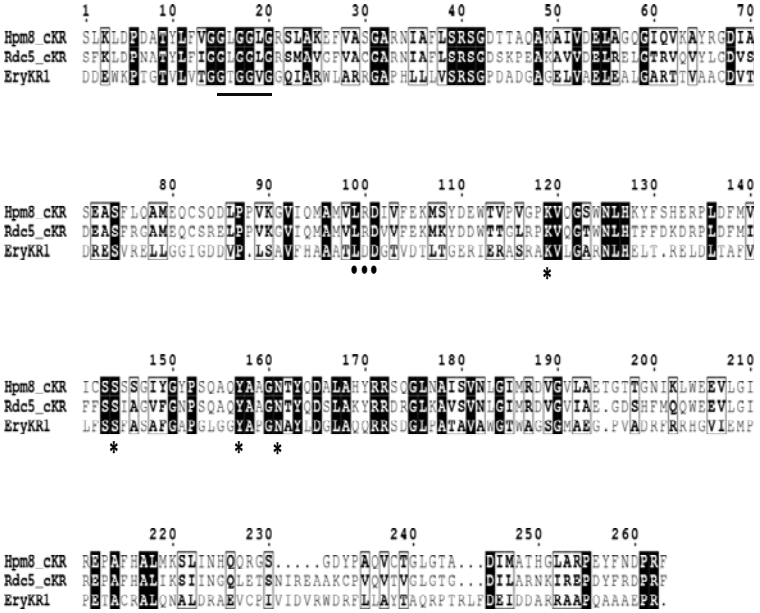
Supplementary Figure 9. (i) The chiral HPLC traces of the mixture of the chemical standards **8D** and **8L**, and the chiral HPLC traces of the reductive products by (ii) Hpm8 or (iii) Hpm8B4 working on the substrate **8**. The chromatograms above were obtained by monitoring at 240 nm.

a

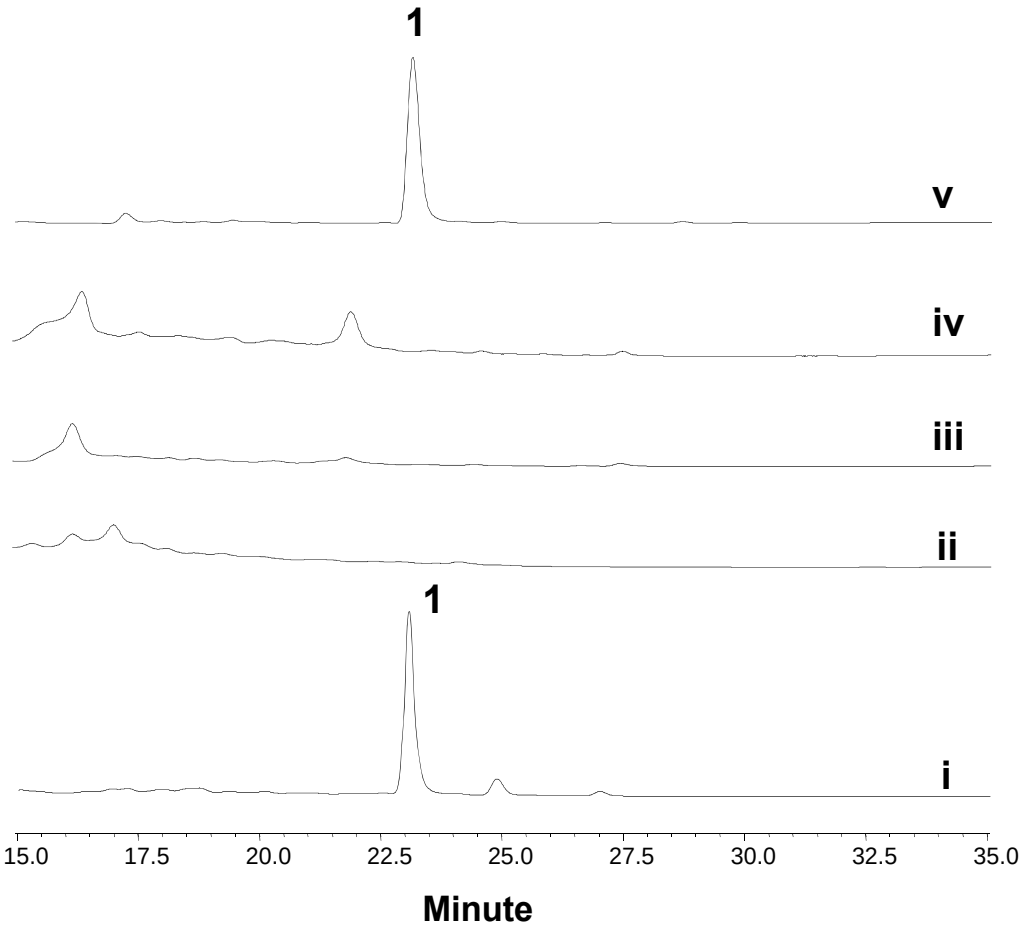


(*R*)-Monocillin II Radicicol

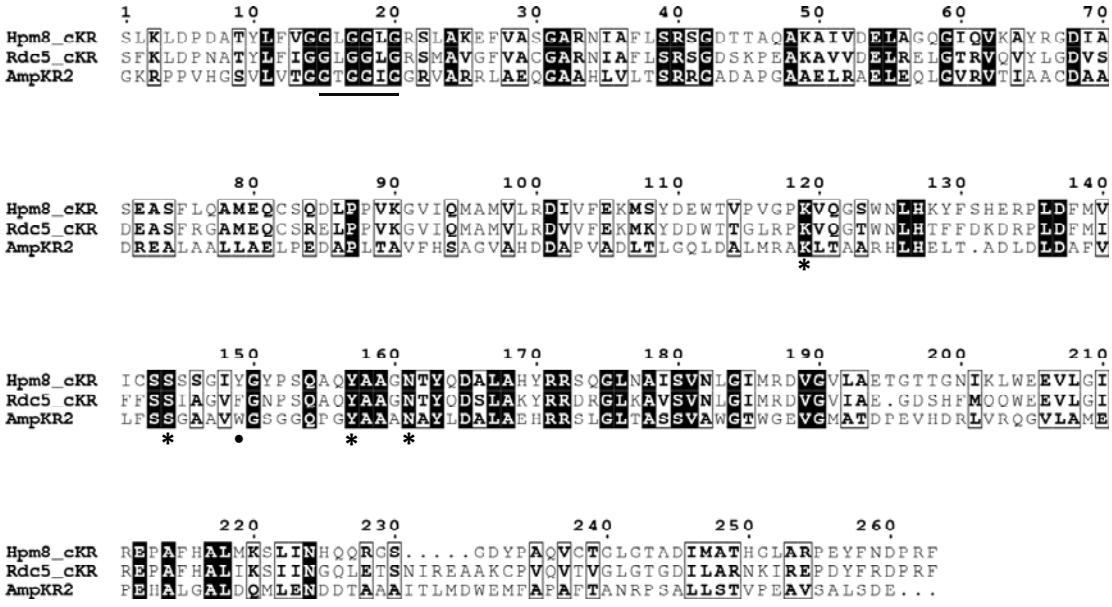
b



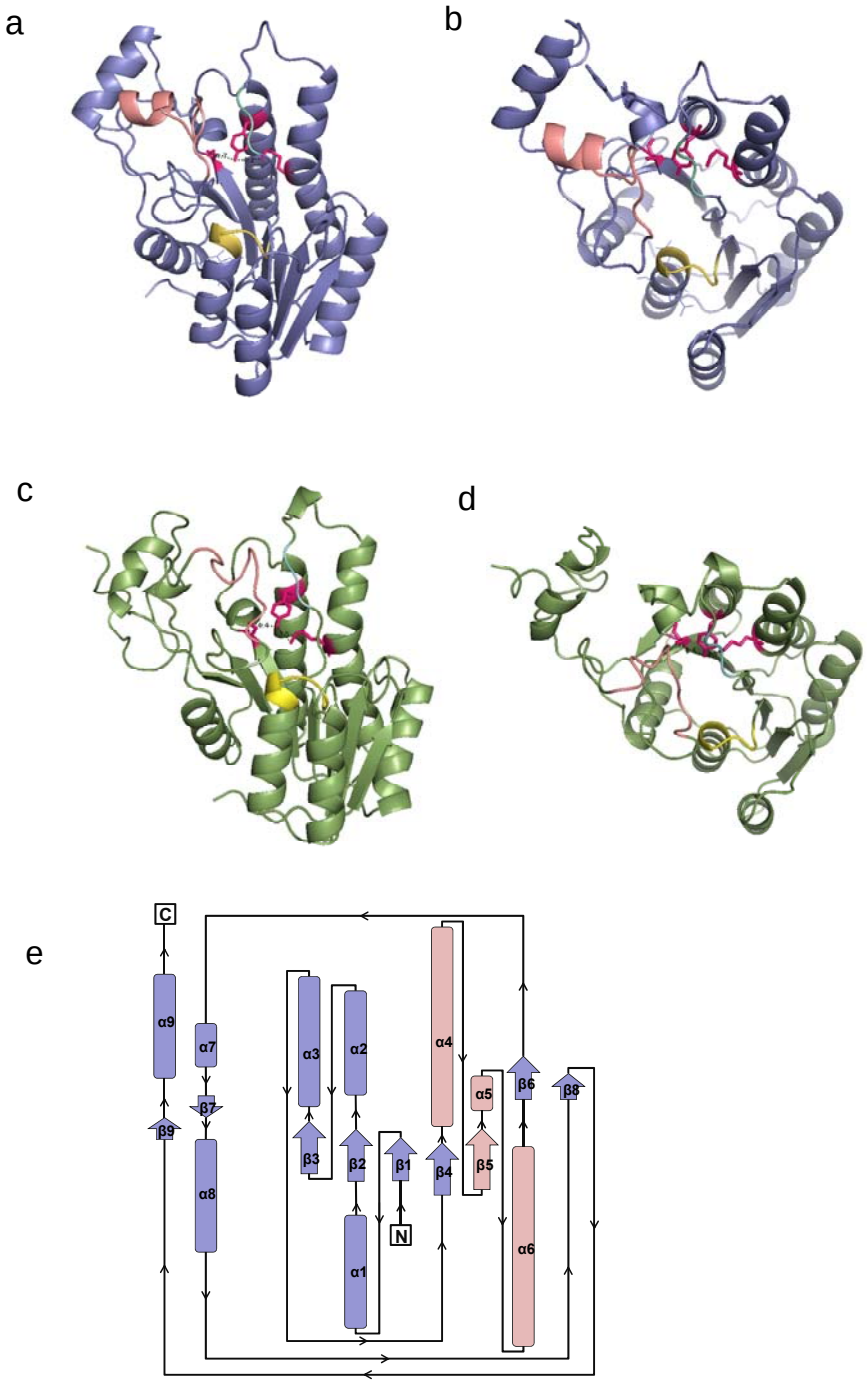
Supplementary Figure 10. **a.** Chemical structures of (R)-monocillin II and radicicol[1]; **b.** The sequence alignment among the catalytic KR domain of DEBS module 1 KR (EryKR1, accession NO.: Q03131), Hpm8_cKR and Rdc5_cKR. The catalytic residues K, S, Y and N are labeled with asterisks. The conserved sequence patch for NADPH binding is underlined. The LDD motif symbolizing B-type KR is highlighted with dots. To match the numbering in entire HRPKS, a plus of 1969 is required for the residue numbers in the above sequence of Hpm8_cKR (a plus of 1994 is required for those in Rdc5_cKR). The catalytic EryKR1 shares 30% sequence similarity with either cKR.



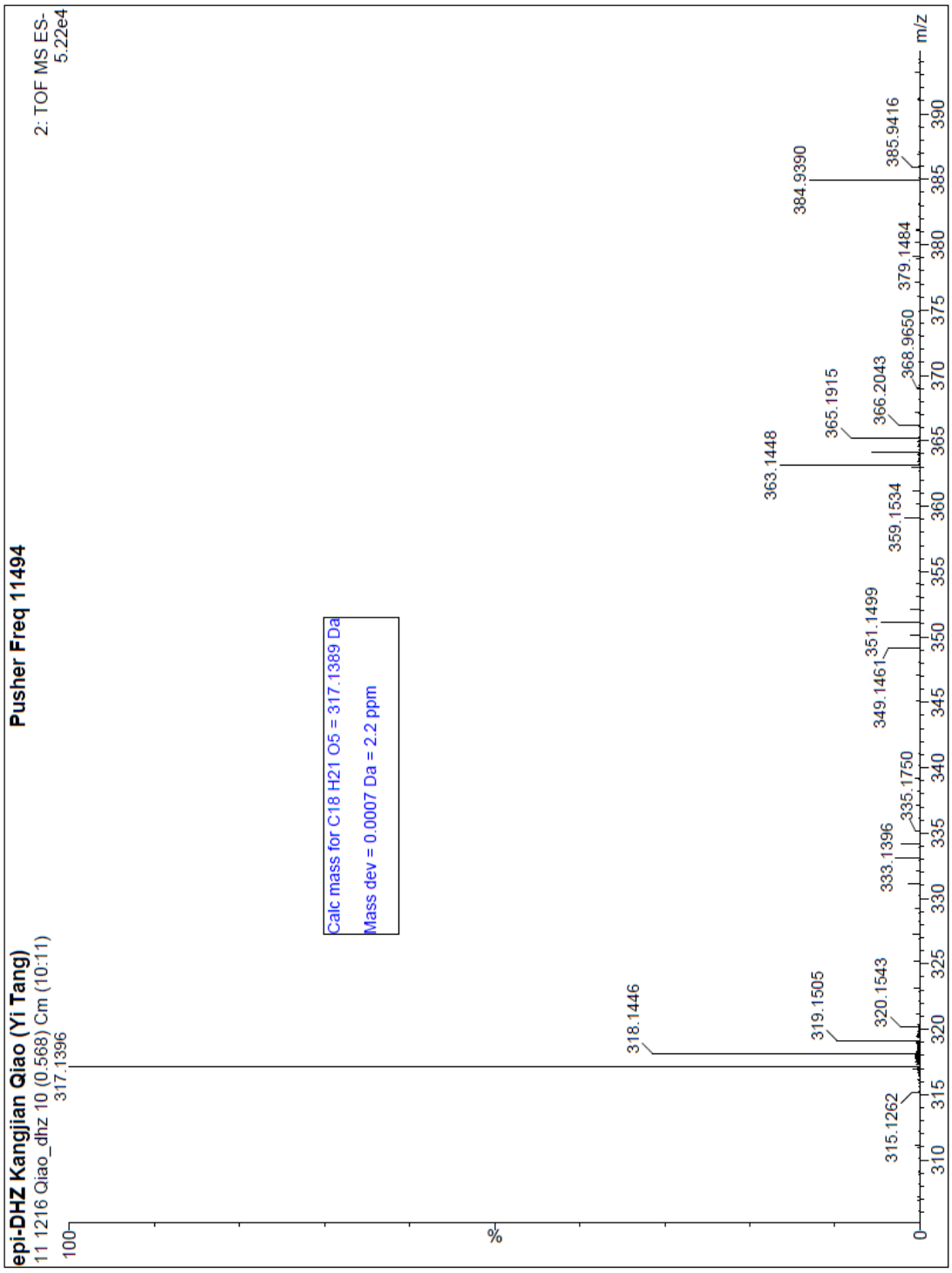
Supplementary Figure 11. The HPLC traces of the in vivo metabolites profiles from the *S. cerevisiae* co-transformants expressing Hpm3 and (i) Hpm8, active site mutants (ii) Hpm8_K^{2088}D, (iii) Hpm8_S^{2113}A, (iv) Hpm8_Y^{2126}A or (v) Hpm8_Y^{2118}F. The chromatograms above were obtained by monitoring at 320 nm. Mutation of K2088, S2113 and Y2126 abolished the activities of Hpm8.



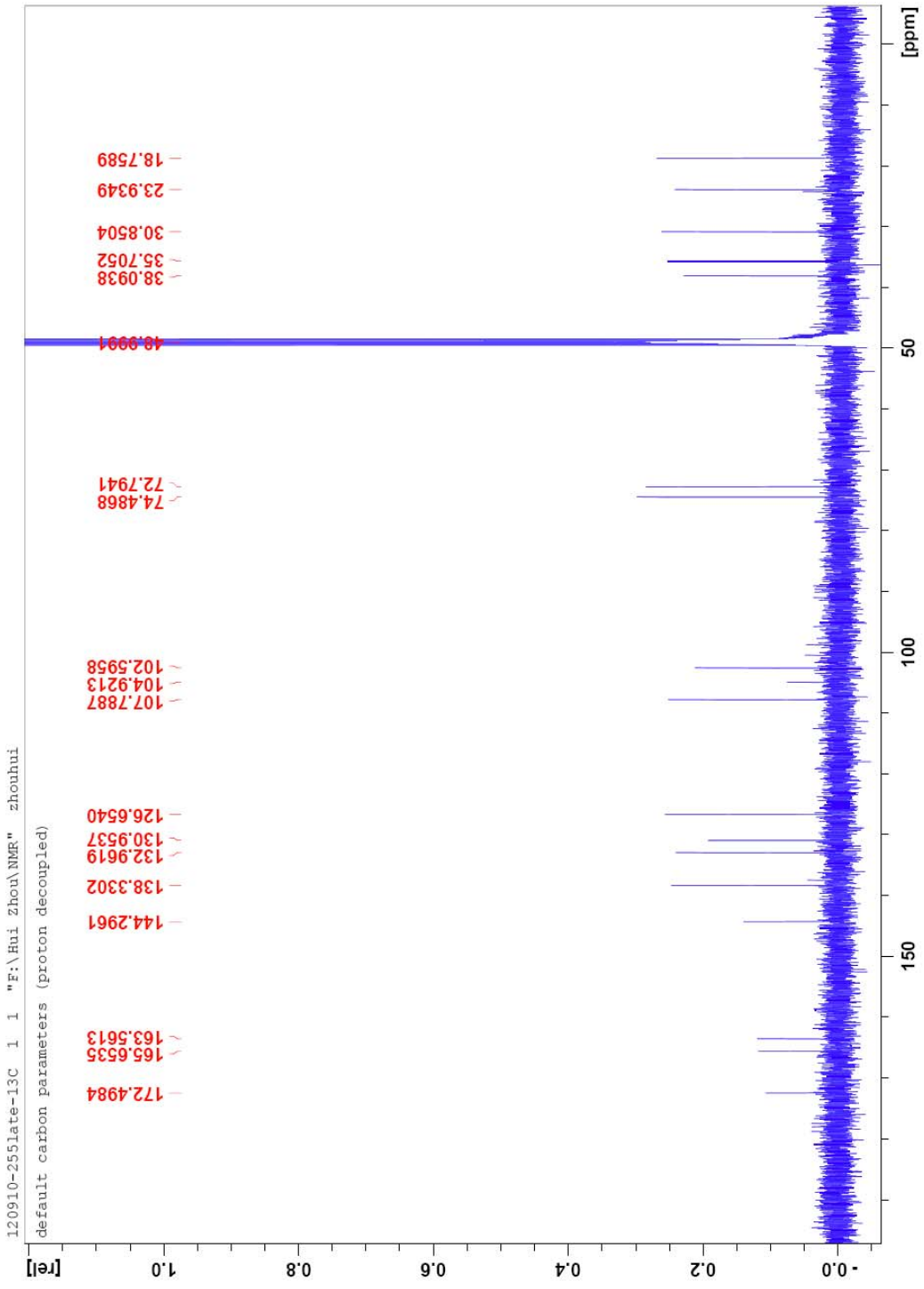
Supplementary Figure 12. The sequence alignment among the catalytic KR domain of AmpKR2 (PDB ID: 3MJE), Hpm8_cKR and Rdc5_cKR. The catalytic residues K, S, Y and N are labeled with asterisks. The conserved sequence patch for NADPH binding is underlined. The conserved W for A-type KR is highlighted with dot. To match the numbering in entire HRPKS, a plus of 1969 is required for the residue numbers in the above sequence of Hpm8_cKR (a plus of 1994 is required for those in Rdc5_cKR).



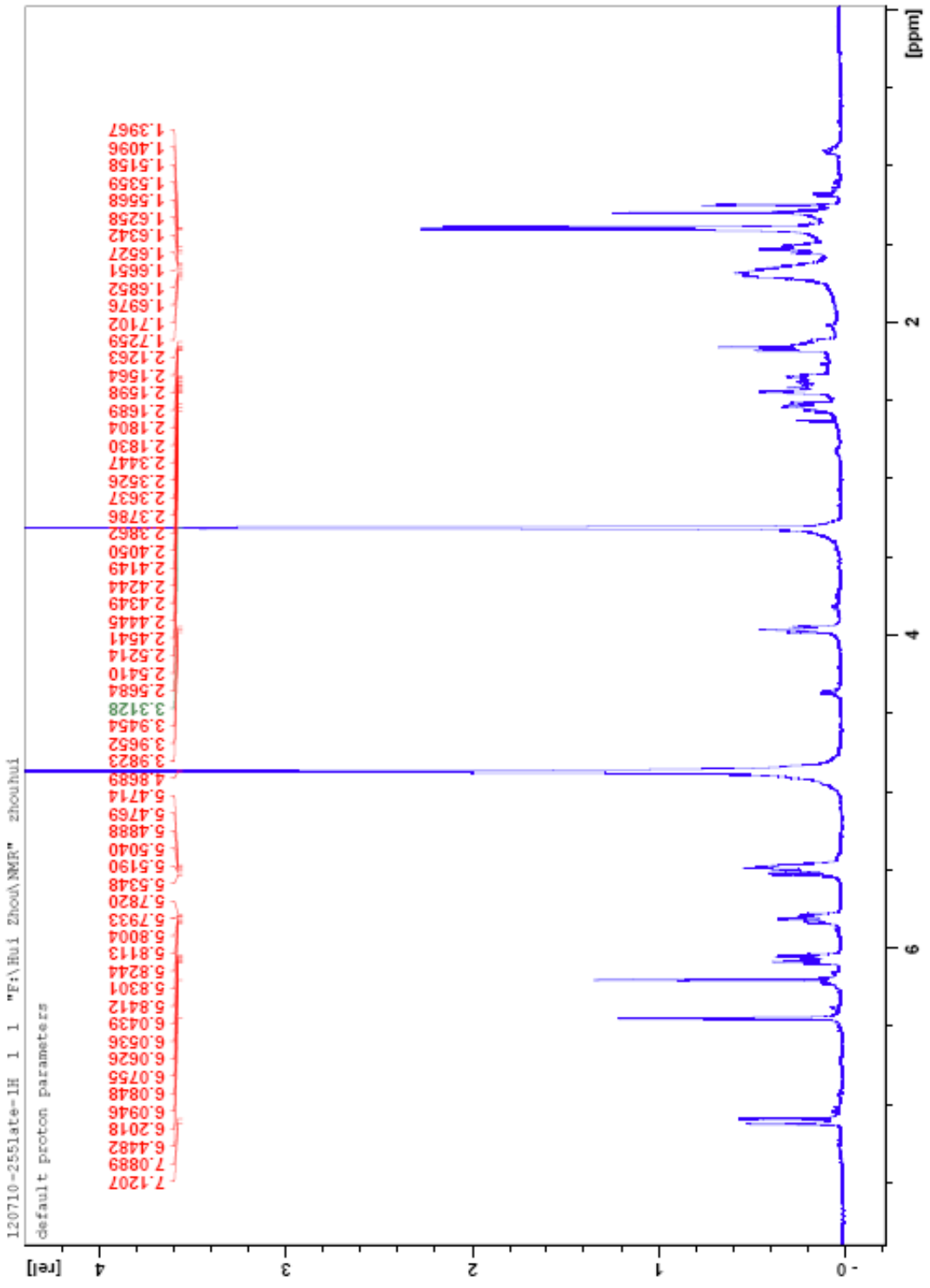
Supplementary Figure 13. The cartoon view of modeled structure of Hpm8_cKR in blue a: from side, b: from top. The cartoon view of modeled structure of Rdc5_cKR in green c: from side, d: from top. The catalytic residues are highlighted in red in all the structures. The conserved motifs (GXGXXG) for NADPH binding are in yellow. The LRD loops are shown in cyan color and the helix lid elements (corresponding to the αFG region in EryKR1) are in salmon pink. e: The topology diagram of the modeled structure of Hpm8_cKR. The secondary structures α4, β5, α5 and α6 are highlighted in salmon.



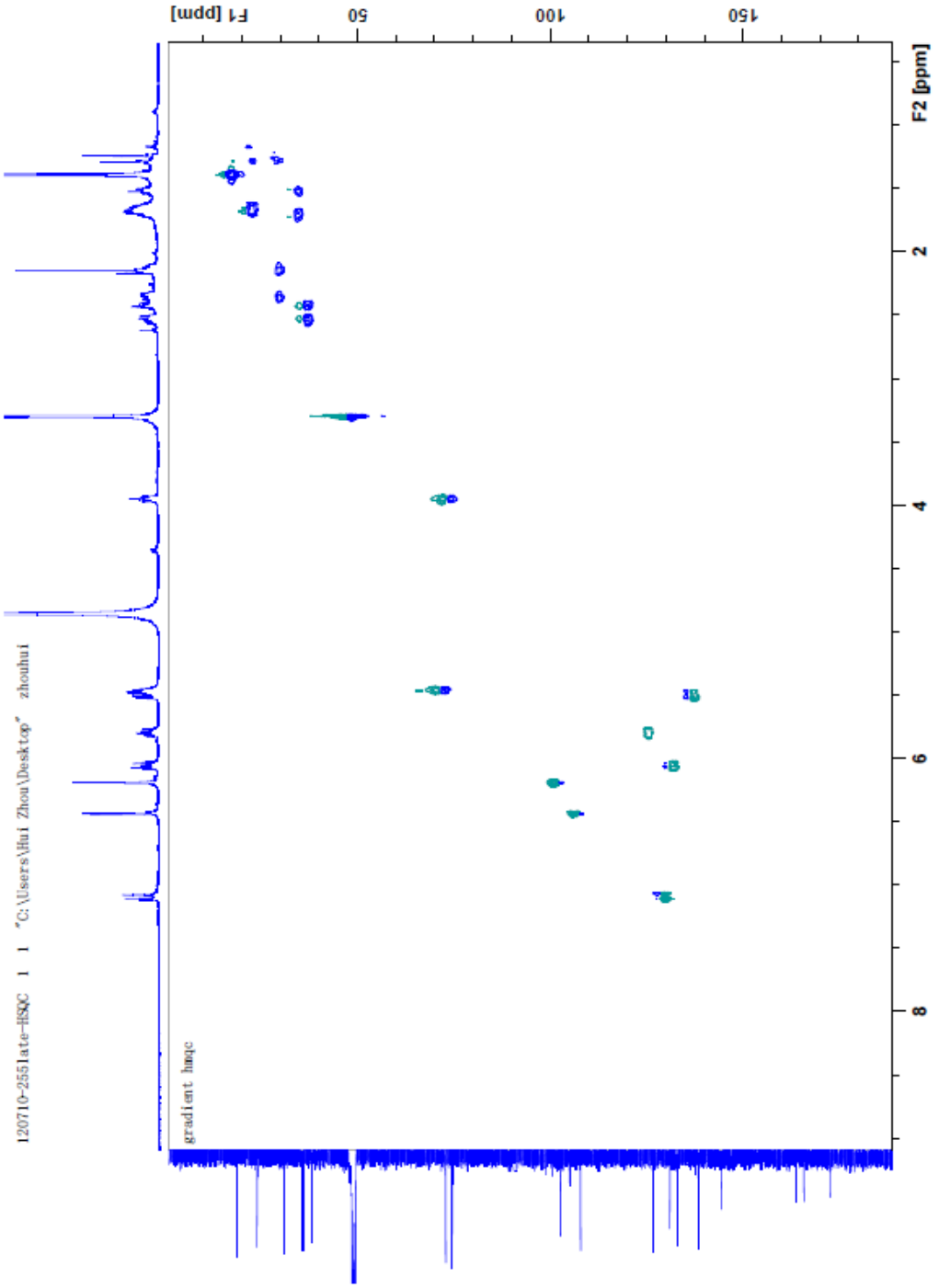
Supplementary Figure 14. High-resolution mass spectrum of compound **9**. The calculated mass is 317.1389 Da.



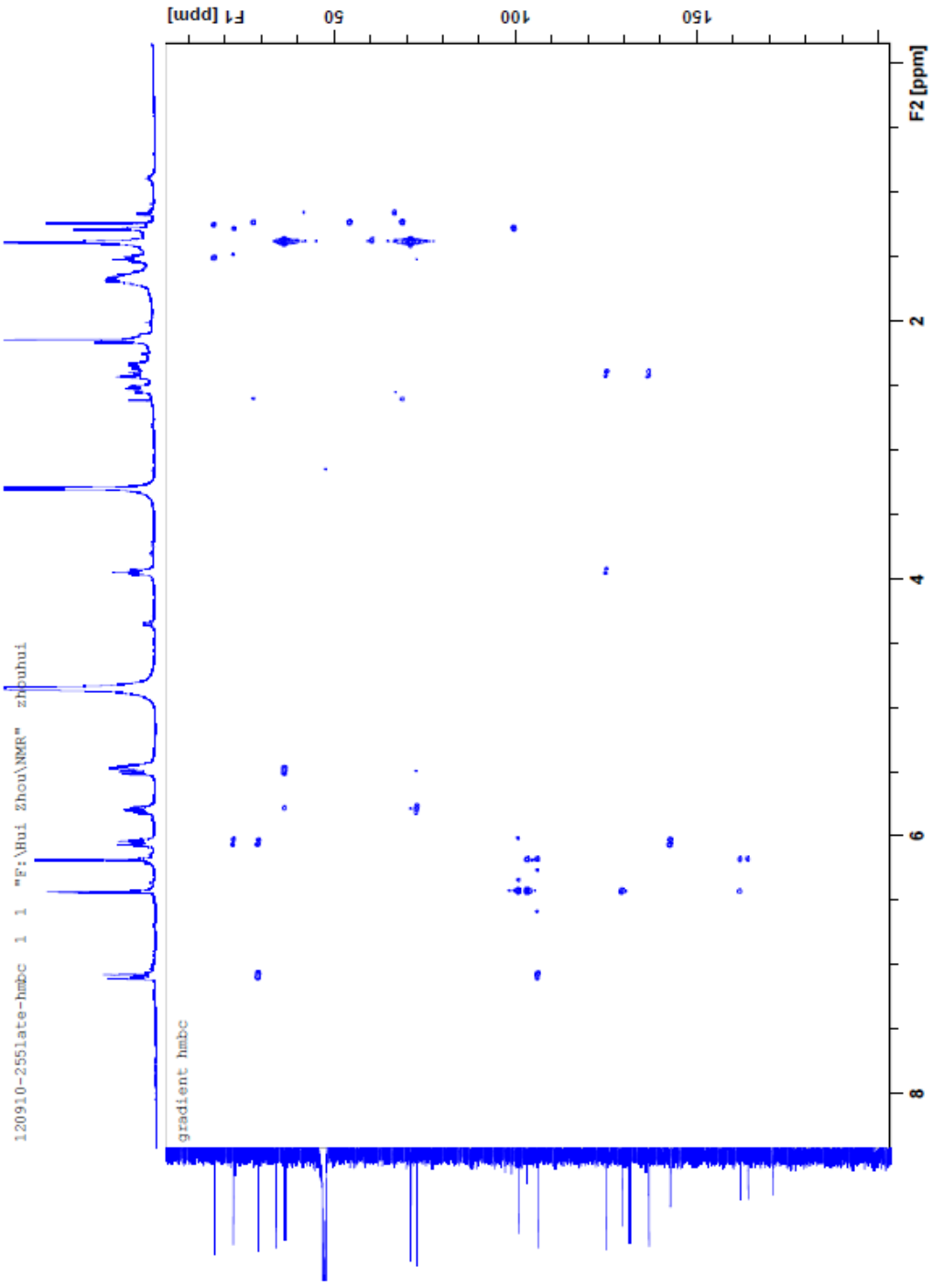
Supplementary Figure 15. ^{13}C NMR spectrum (125 MHz) of **9** in methanol-d_4.



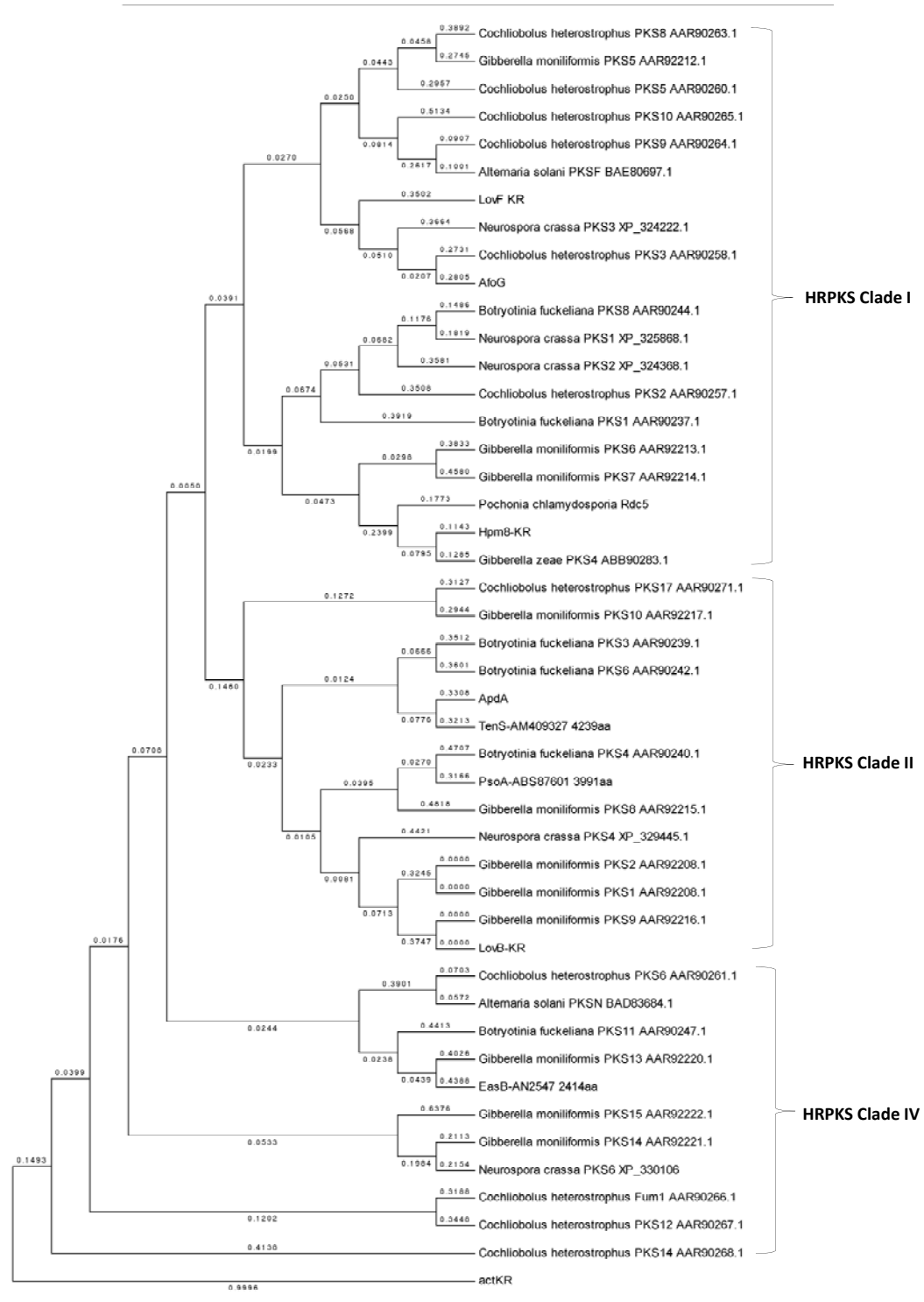
Supplementary Figure 16. ^{1}H NMR spectrum of **9** (500 MHZ) in methanol-d_4.



Supplementary Figure 17. HSQC spectrum of **9** in methanol-d_4 (^{1}H: 500 MHz).

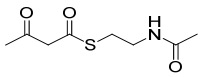
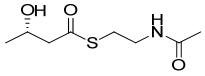
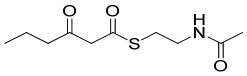
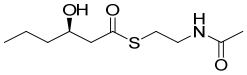
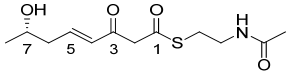
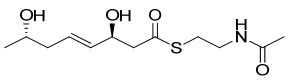
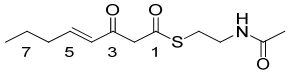
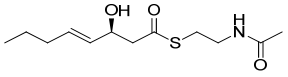
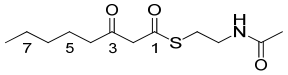
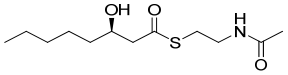
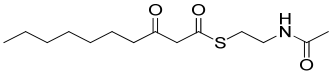
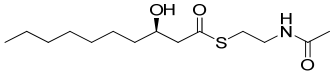
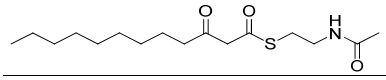
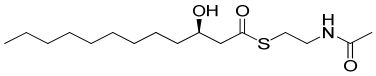


Supplementary Figure 18. HMBC spectrum of **9** in methanol-d_4 (^{1}H: 500 MHz).

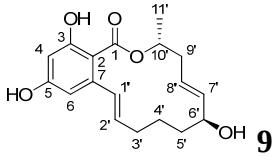
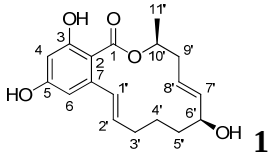


Supplementary Figure 19. Phylogenetic tree of fungal HRPKS catalytic ketoreductase domain with a bacterial actKR as an outgroup. The evolutionary distances were computed using the Poisson correction method[2] and are in the units of the number of amino acid substitutions per site. The branch lengths are also shown along the branches.

Supplementary Table 1. The list of products generated in the KR assay of Hpm8 on different β-keto acyl SNAC substrates.

Substrate structure	D configuration product	L configuration product
Diketide, **2** 	**2D, 8.8%**	**2L, 91.2%** 
Triketide, **3** 	**3D** 	Not Detected
Tetraketide, **4** 	**4D** 	Not Detected
Tetraketide, **5** 	**5D** 	Not Detected
Tetraketide, **6** 	**6D** 	Not Detected
Pentaketide, **7** 	**7D** 	Not Detected
Hexaketide, **8** 	**8D** 	Not Detected

Supplementary Table 2. NMR data comparison between **9** and **1**.

	 **9**			 **1**	
No.	^{13}C δ (ppm)	^{1}H δ (ppm) (m, area, J_{HH} (Hz))		^{13}C δ (ppm)	^{1}H δ (ppm) (m, area, J_{HH} (Hz))
1	171.0	-		172.4	-
2	103.4	-		106.5	-
3	162.0	-		163.0	-
4	101.5	6.44 (d, 1H, 2.4)		102.5	6.40 (d, 1H, 3)
5	164.1	-		164.3	-
6	106.2	6.19 (d, 1H, 2.4)		107.6	6.20 (d, 1H, 3)
7	142.7	-		143.6	-
1’	129.4	7.09 (d, 1H, 15.8)		131.5	6.94 (d, 1H, 15.8)
2’	131.4	6.06 (dt, 1H, 15.8, 5.4)		133.3	5.94 (dt, 1H, 15.8, 6.28)
3’	29.3	2.33-2.39 (m, 2H) 2.15 (1H, m)		31.6	2.14-2.28 (m, 2H)
4’	22.4	1.61-1.66 (m, 2H)		22.6	1.74-1.84 (m, 2H)
5’	34.2	1.49-1.54 (m, 1H) 1.67-1.73 (m, 1H)		34.7	1.52-1.71 (m, 2H)
6’	72.9	3.95 (m, 1H)		72.8	4.23 (m, 1H)
7’	136.8	5.52-5.49 (m, 1H)		137.5	5.59-5.72 (m, 2H)
8’	125.1	5.80 (m, 2H)		126.6	5.59-5.72 (m, 2H)
9’	36.5	2.42-2.44 (m, 1H) 2.50-2.56 (m, 1H)		38.8	2.53 (dt, 1H, 15.8, 4.2)
10’	71.2	5.44-5.48 (m, 1H)		73.2	2.33 (m, 1H)
11’	17.2	1.39 (d, 3H, 6.5)		19.8	5.30 (m, 1H)

[a] Spectra were obtained at 500 MHz for proton and 120 MHz for carbon and were recorded in methanol-d_4.

2. Supplementary Methods

2.1. Molecular cloning

E. coli XL1-Blue and *E. coli* TOP10 (Invitrogen) were used for cloning following standard recombinant DNA techniques. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs). PCR was performed using Phusion® DNA Polymerase (New England Biolabs). The constructs of pCR-Blunt vector (Invitrogen) containing desired PCR products were confirmed by DNA sequencing (Laragen, CA). *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used as the yeast expression hosts[3,4]. The genome integrated *npg*A gene encodes a phosphopantetheinyl transferase required for post-translational activation of the PKS proteins by the addition of phosphopantetheine[5].

The expression plasmid of N-terminus hexahistidine-tagged wild type Hpm8 was constructed based on pKJ31, a 2μ-based yeast-*E.coli* shuttle plasmid with *URA3* auxotrophic marker[6]. The *hpm8* was flanked by 5'-*Nde*I and 3'-*Pml*I sites in this plasmid (pZH126). The cloning vector for constructing all the Hpm8 mutants was prepared by digesting pZH126 with AgeI (The *Age*I site is located in the ER domain of Hpm8, around 2.1kb upstream of the stop codon) and PmlI (The *Pml*I site follows the stop codon of *hpm8*.). The inserts for site-directed mutation were amplified by two-piece slice-overlap extension PCR (SOE). The inserts for the chimeric Hpm8 enzymes (Hpm8B1 to Hpm8B9) were similarly prepared by three-piece SOE PCR. Taking the construction of the expression plasmid pZH327 for the mutant Hpm8_Y^{2126}F as an example, the primer pair of P1-for/P1-Y2126F-rev and the pair of P2-Y2126F-for/P3-rev were used to

amplify fragment I and fragment II. SOE PCR was performed to link these two fragments together, which was ligated into pCR Blunt vector for sequencing. The corresponding insert carrying the $Y^{2126}F$ mutation was cut out by AgeI and PmeI and ligated back to pZH126 derived vector for the construction of pZH327. The Hpm8_$S^{2113}A$, Hpm8_$K^{2088}D$ and Hpm8_$Y^{2118}F$ mutants were constructed similarly to Hpm8_ $Y^{2126}F$. The primer pairs for fragment I replication are P1-for/P1- S2113A-rev, P1-for/P1-K2088D-rev and P1-for/ P1-Y2118F-rev, respectively. The primer pairs for the amplification of fragment II are P2- S2113A-for/P3-rev, P2-K2088D-for/P3-rev and P2-Y2118F-for/P3-rev, respectively.

Similar strategy was applied to construct Hpm8B1 to Hpm8B9. Three pieces SOE PCR was utilized to obtain the insert carrying different regions of Rdc5_cKR. Taking the construction of the expression plasmid pZH213 for Hpm8B1 as an example, fragment I and fragment III were amplified by two pairs of primers including P1-for/ P1-B1-rev and P3-B1-for/P3-rev, respectively. The template gene is wild type *hpm8*. The middle fragment II is replicated by primer pair P2-B1-for/ P2-B1-rev based on the *rdc5* gene as template. After SOE PCR, the insert was ligated into pCR Blunt vector for sequencing. Recovered by cleavage with *Age*I and *Pme*I, the insert was ligated into pZH126-derived vector for the completion of pZH213. The other chimeric Hpm8 enzymes in KR domain were constructed accordingly. The corresponding primers are named with the number of the hybrid enzymes. For instance, the primer pair P2-B2-for/P2-B2-rev are employed for the amplification of fragment II for the insert of Hpm8B2.

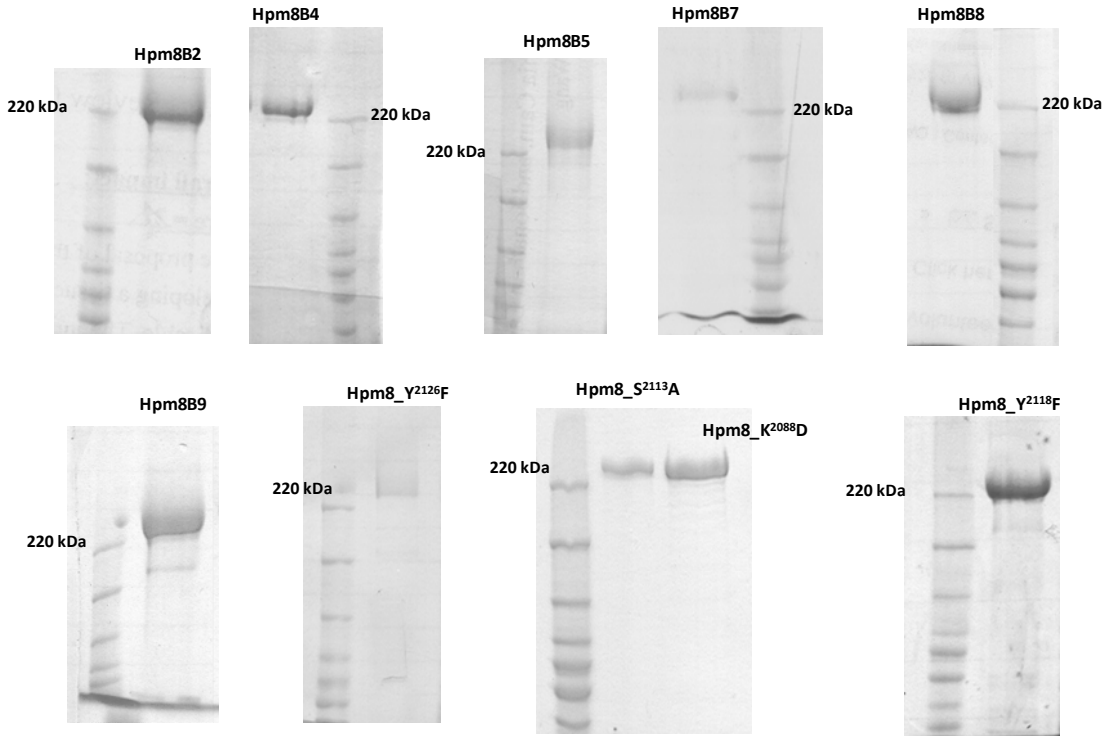
Supplementary Table 3. List of primers.

Primer Name	5’-Primer sequence-3’
P1-for	agaaccggtgcgaaggctacca
P1-Y2126F-rev	atcctggtaagtgttgccagcTgcgAattgagcctgactgggataac
P2-Y2126F-for	gttatcccagtcaggctcaatTcgcAgctggcaacacttaccaggat
P1- S2113A-rev	ataaccgtagataccggagctAGCggagcagatgaccatgaagtc
P2- S2113A-for	gacttcatggtcatctgctccGCTagctccggtatctacggttat
P1-Y2126A-rev	tcctggtaagtgttgccagcCgcgGCttgagcctgactgggataacc
P2-Y2126A-for	gttatcccagtcaggctcaaGCcgcGgctggcaacacttaccaggat
P1-K2088D-rev	caagttccatgaaccttggacGTCggggccaacggggacggtcca
P2-K2088D-for	tggaccgtccccgttggccccGACgtccaaggttcatggaacttg
P1-Y2118F-rev	tgagcctgactgggataaccgAagataccggagcttgaggagc
P2-Y2118F-for	gctcctcaagctccggtatctTcggttatcccagtcaggctca
P2-B1-for	ccttctccgatgacgcaaagGCCCCCGTTCTTTGCAGAGC
P2-B1-rev	GCAGAGCCCTGGCCGTCAGCtgcggcggtggatgtgctgc
P3-B1-for	gcagcacatccaccgccgcaGCTGACGGCCAGGGCTCTGC
P1-B2-rev	ttgctgtcgcctgatcgcgaTAAGAAGGCAATGTTGCGGG
P2-B2-for	CCCGCAACATTGCCTTCTTAtcgcgatcaggcgacagcaa
P2-B2-rev	ACACCGACATCTCGCATGATgcctaggttgacagaaacgg
P3-B2-for	ccgtttctgtcaacctaggcATCATGCGAGATGTCGGTGT
P1-B3-rev	cctacgtctcgcatgatgcccAAGTTGACGGAGATGGCGT
P2-B3-for	ACGCCATCTCCGTCAACTTgggcatcatgcgagacgtagg
P2-B3-rev	GTGGTGACGGCAAGGGGTCCgaaacgagggtcgcggaagt
P3-B3-for	acttccgcgaccctcgtttcGGACCCCTTGCCGTCACCAC
P1-B4-rev	ttgctgtcgcctgatcgcgaTAAGAAGGCAATGTTGCGGG
P2-B4-for	CCCGCAACATTGCCTTCTTAtcgcgatcaggcgacagcaa
P2-B4-rev	TAGTGAGCCAAGGCATCCTGatacgtgttgcctgcggcat
P3-B4-for	atgccgcaggcaacacgtatCAGGATGCCTTGGCTCACTA
P1-B5-rev	ttgctgtcgcctgatcgcgaTAAGAAGGCAATGTTGCGGG
P2-B5-for	CCCGCAACATTGCCTTCTTAtcgcgatcaggcgacagcaa
P2-B5-rev	GAGCTTGAGGAGCAGATGACcatgaagtccagtgggcgat
P3-B5-for	atcgcccactggacttcatgGTCATCTGCTCCTCAAGCTC
P1-B6-rev	aagtccagtgggcgatctttACTGAAGTACTTGTGCAAGT
P2-B6-for	ACTTGCACAAGTACTTCAGTaaagatcgcccactggactt
P2-B6-rev	TAGTGAGCCAAGGCATCCTGatacgtgttgcctgcggcat
P3-B6-for	atgccgcaggcaacacgtatCAGGATGCCTTGGCTCACTA
P1-B7-rev	atcttttcaaataccacatcGCGGAGAACCATGGCCATCT
P2-B7-for	AGATGGCCATGGTTCTCCGCgatgtggtatttgaaaagat
P2-B7-rev	TAGTGAGCCAAGGCATCCTGatacgtgttgcctgcggcat
P3-B7-for	atgccgcaggcaacacgtatCAGGATGCCTTGGCTCACTA
P1-B8-rev	cttttcaaataccacatcGCGGAGAACCATGGCCATCTGG
P2-B8-for	CCAGATGGCCATGGTTCTCCGCgatgtggtatttgaaaag
P2-B8-rev	CCGGAGCTTGAGGAGCAGATgaccatgaagtccagtgggc
P3-B8-for	gcccactggacttcatggtcATCTGCTCCTCAAGCTCCGG
P1-B9-rev	ttgctgtcgcctgatcgcgaTAAGAAGGCAATGTTGCGGG

P2-B9-for	CCCGCAACATTGCCTTCTTAtcgcgatcaggcgacagcaa
P2-B9-rev	ATCTTCTCAAAGACGATATCgcgaagcaccatggccattt
P3-B9-for	aaatggccatggtgcttcgcGATATCGTCTTTGAGAAGAT
P3-rev	GTTTAAACttaaacagtaaccaacttgc

2.2. Protein expression and purification

The expression plasmids harboring the Hpm8 mutants and chimeric HRPKSs were all transformed into *S. cerevisiae* strain BJ5464-NpgA for expression, respectively. For 1 L of yeast culture, the cells were grown at 28°C in YPD media with 1% dextrose for 72 hours. The cells were harvested by centrifugation (4000 rpm, 10 minutes, 4°C), resuspended in 20 mL lysis buffer (50mM NaH_2PO_4 pH = 8.0, 0.15 M NaCl, 10 mM imidazole) and lysed with sonication on ice. Cellular debris was removed by centrifugation (17000 g, 1 hour, 4°C). Ni-NTA agarose resin was added to the supernatant (2 mL/L of culture) and the solution was rotated at 4°C for at least 2 hours. The protein/resin mixture was loaded into a gravity flow column. Buffer A (50 mM Tris-HCl, pH=7.9, 2 mM EDTA, 2 mM DTT) with increasing concentrations of imidazole (10 mM, 20 mM and 30 mM) was used as washing buffers. The desired proteins were eluted with Buffer A containing 250 mM imidazole. Purified proteins were concentrated and buffered exchanged into Buffer A+10% glycerol, concentrated, aliquoted and flash frozen. Protein concentrations were determined using the Bradford dye-binding assay (Biorad).



Supplementary Figure 20. SDS-PAGE of the purified Hpm8B2, B4, B5, B7, B8, B9, Hpm8_Y^{2126}F, Hpm8_S^{2113}A, Hpm8_K^{2088}D and Hpm8_Y^{2118}F.

Supplementary Table 4. List of enzymes constructed in this work.

Enzyme name	Plasmid No.	Expression level (mg/L culture)
Hpm8B1	pZH213	Not solubly expressed
Hpm8B2	pZH305	1.8
Hpm8B3	pZH303	Not solubly expressed
Hpm8B4	pZH255	1.9
Hpm8B5	pZH315	1.7
Hpm8B6	pZH288	Not solubly expressed
Hpm8B7	pZH276	0.9
Hpm8B8	pZH316	1.5
Hpm8B9	pZH254	1.6
Hpm8_Y^{2126}F	pZH327	0.8
Hpm8_S^{2113}A	pZH330	0.38
Hpm8_K^{2088}D	pZH329	1.3
Hpm8_Y^{2118}F	pZH172	1.0

2.3. Homology modeling

Homology modeling of the catalytic KRs of both Hpm8 and Rdc5 are performed by using the online server HHpred[7]. The best template identified by HHpred for both Hpm8_cKR

and Rdc5_cKR is EryKR1 (PDB ID 2FR1). Single-template homology models are constructed for both Hpm8_cKR and Rdc5_cKR based on the same template. In the modeled structure, the distance between catalytic Ser and Tyr (4.7 Å in Hpm8 and 4.4 Å in Rdc5) or Tyr and Lys (4.2 Å in Hpm8 and 3.5 Å in Rdc5) are comparable to the distance of Ser-Tyr (4.5 Å) or Tyr-Lys (4.3 Å) in the crystal structure of EryKR1[8] (SI Fig. 14).

2.4. In vitro assays

For a typical in vitro KR assay, a 100 μL reaction was set up containing 2 μM HRPKS, 2mM NADPH and 100 mM NaH_2PO_4, pH=7.4. After 6 hour incubation, the reactions were quenched and extracted twice with 99% ethyl acetate (EA)/1% acetic acid (AcOH). The resultant organic extracts were evaporated to dryness, redissolved in methanol, and then analyzed by LC-MS. For the chiral HPLC analysis, the dried organic extracts were dissolved in 2-propanol (IPA). In the in vitro assay for Hpm3, 2 mM chemically synthesized hexaketide SNAC thioester **12** and 2 mM malonyl-CoA were co-incubated with 100 μM Hpm3 for an overnight reaction. The same extraction procedure was performed for this assay as the one for KR assays.

2.5. Heterologous reconstitution

The expression plasmids for HRPKSs were co-transformed with Hpm3 (NRPKS) in *S. cerevisiae* strain BJ5464-NpgA. 200 μL of the third day culture was extracted with 99% ethyl acetate (EA)/1% acetic acid (AcOH). The resultant organic extracts were evaporated to dryness, redissolved in methanol, and then analyzed by LC-MS.

2.6. HPLC analysis

LC-MS was conducted with a Shimadzu 2010 EV Liquid Chromatography Mass Spectrometer by using both positive and negative electrospray ionization, and a Phenomenex Luna 5μ 2.0 x 100 mm C18 reverse-phase column. Samples were separated on a linear gradient of 5 to 95% or 5 to 40% CH_3CN (vol/vol) in H_2O supplemented with 0.05% (vol/vol) formic acid at a flow rate of 0.1 ml/min. Chiral compound was analyzed by normal phase HPLC (Lux 3μ Cellulose-1, 150×4.60 mm) under different isocratic condition of IPA in n-Hexane (v/v). All the standard chemicals and reaction extracts were all dissolved in IPA for chiral HPLC analysis. The mixture of each pair of chiral standards contains 10 μl of 5 mM each standard.

For the separation by chiral HPLC, the solvent ratios and flow rates for different pairs of chemical standards are listed in Table 5. The difference in the retention time (ΔRT) for each pair of standards is also calculated.

Supplementary Table 5. Solvent ratios for chiral HPLC and retention time difference.

Pair of standards	IPA/hexane%	flow rate v, ml/mim	ΔRT
2L, 2D	15	1.0	1.2 min
3L, 3D	10	0.8	2.3 min
4L, 4D	15	1.5	1.9 min
5L, 5D	15	1.0	1.0 min
6L, 6D	10	0.6	2.8 min
7L, 7D	10	0.6	2.5 min
8L, 8D	10	0.5	1.5 min

2.7. Phylogenetic analysis of HRPKS catalytic KR domains

Besides Hpm8, Rdc5 and PKS4, the sequences of 42 other HRPKSs (Table 6) were retrieved from National Center for Biotechnology Information (NCBI). The catalytic KR domain of each HRPKS was accordingly identified based on the boundary of Hpm8_cKR. The sequence alignment was then conducted with ClustalW[9], where the sequence of bacterial ketoreductase actKR[10,11] from type II PKs pathway was also included as an out-group. The phylogeny reconstruction was performed on MEGA version 5.0[12] using both the bootstrap minimum evolution method and maximum likelihood method. The evolutionary history was estimated by Minimum Evolution method[13] and by using the Maximum Likelihood method based on the JTT matrix-based model[14]. As shown in SI Fig. 19, the phylogenetic analysis of fungal HRPKS KRs established their phylogenetic relationship that they may co-evolve with their cognate KS domain. While the correlation between sequence and stereochemistry of fungal IPKS KRs is still implicit due to lack of complete stereochemical data for most of the KRs.

Supplementary Table 6. List of HRPKSs used in the phylogenetic analysis.

Protein name	Strain Name	Accession No.
Alternaria solani PKSF	*Alternaria solani*	BAE80697
Alternaria solani PKSN	*Alternaria solani*	BAD83684
LovB	*Aspergillus Terreus*	Q9Y8A5
LovF	*Aspergillus Terreus*	AAD34559
PsoA	*Aspergillus fumigatus Af293*	ABS87601
ApdA	*Aspergillus nidulans FGSC A4*	XP_681681
EasB_AN2547	*Aspergillus nidulans FGSC A4*	CBF87072
AfoG	*Aspergillus nidulans FGSC A4*	XP_658640
TenS	*Beauveria bassiana*	AM409327
Botryotinia fuckeliana PKS1	*Botryotinia fuckeliana*	AAR90237
Botryotinia fuckeliana PKS3	*Botryotinia fuckeliana*	AAR90239
Botryotinia fuckeliana PKS4	*Botryotinia fuckeliana*	AAR90240
Botryotinia fuckeliana PKS6	*Botryotinia fuckeliana*	AAR90242
Botryotinia fuckeliana PKS8	*Botryotinia fuckeliana*	AAR90244
Botryotinia fuckeliana PKS11	*Botryotinia fuckeliana*	AAR90247
Cochliobolus heterostrophus Fum1	*Cochliobolus heterostrophus*	AAR90266
Cochliobolus heterostrophus PKS2	*Cochliobolus heterostrophus*	AAR90257
Cochliobolus heterostrophus PKS3	*Cochliobolus heterostrophus*	AAR90258
Cochliobolus heterostrophus PKS5	*Cochliobolus heterostrophus*	AAR90260
Cochliobolus heterostrophus PKS6	*Cochliobolus heterostrophus*	AAR90261
Cochliobolus heterostrophus PKS8	*Cochliobolus heterostrophus*	AAR90263
Cochliobolus heterostrophus PKS9	*Cochliobolus heterostrophus*	AAR90264
Cochliobolus heterostrophus PKS10	*Cochliobolus heterostrophus*	AAR90265
Cochliobolus heterostrophus PKS12	*Cochliobolus heterostrophus*	AAR90267
Cochliobolus heterostrophus PKS14	*Cochliobolus heterostrophus*	AAR90268
Cochliobolus heterostrophus PKS17	*Cochliobolus heterostrophus*	AAR90271
Gibberella moniliformis PKS1	*Gibberella moniliformis*	AAR92208
Gibberella moniliformis PKS2	*Gibberella moniliformis*	AAR92208
Gibberella moniliformis PKS5	*Gibberella moniliformis*	AAR92212
Gibberella moniliformis PKS6	*Gibberella moniliformis*	AAR92213
Gibberella moniliformis PKS7	*Gibberella moniliformis*	AAR92214
Gibberella moniliformis PKS8	*Gibberella moniliformis*	AAR92215
Gibberella moniliformis PKS9	*Gibberella moniliformis*	AAR92216
Gibberella moniliformis PKS10	*Gibberella moniliformis*	AAR92217
Gibberella moniliformis PKS13	*Gibberella moniliformis*	AAR92220
Gibberella moniliformis PKS14	*Gibberella moniliformis*	AAR92221
Gibberella moniliformis PKS15	*Gibberella moniliformis*	AAR92222
PKS4	*Gibberella zeae*	ABB90283
Hpm8	*Hypomyces subiculosus*	ACD39758
Rdc5	*Pochonia chlamydosporia*	ACD39774
Neurospora crassa PKS1	*Neurospora crassa*	XP_325868
Neurospora crassa PKS2	*Neurospora crassa*	XP_324368
Neurospora crassa PKS3	*Neurospora crassa*	XP_324222
Neurospora crassa PKS4	*Neurospora crassa*	XP_329445

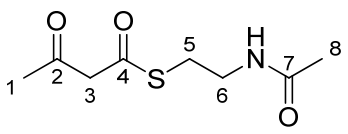
Neurospora crassa PKS6	*Neurospora crassa*	XP_330106
actKR	*Streptomyces coelicolor*	PDB: 2RHC_A

2.8. Compounds syntheses and characterization

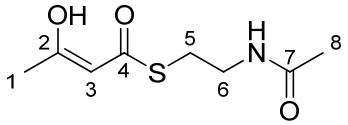
General Synthetic Procedures. All reactions involving air or moisture sensitive reactants were conducted under a positive pressure of dry argon. All solvents and chemicals were reagent grade and used as supplied unless otherwise stated. For anhydrous reactions, solvents were dried according to the procedures detailed in Perrin and Armarego[15]. Removal of solvent was performed under reduced pressure, below 40 °C, using a Büchi rotary evaporator. Chemical reagents were purchased from *Sigma-Aldrich* Chemical Company. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 1.5 cm) precoated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Compounds were visualized by exposure to UV light and by dipping the plates in 1% $Ce(SO_4)_2$•$4H_2O$ 2.5% $(NH_4)Mo_7O_{24}$•$4H_2O$ in 10% H_2SO_4 followed by heating on a hot plate. Flash chromatography was performed on silica gel (EM Science, 60Å, 230-400 mesh).

Spectroscopic Analyses. Nuclear magnetic resonance (NMR) spectra for **2**, **2D**, **2L** and **9** were obtained on a Bruker 500 MHz spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of solvents as reference: CD_3OD δ 3.30 and $CDCl_3$ δ 7.26. ^{13}C NMR chemical shifts are reported relative to CD_3OD δ 49.0 and $CDCl_3$ δ 77.0. NMR spectra of the rest compounds were obtained on a Varian Inova 500 MHz and 600 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of solvents as reference: $CDCl_3$ δ 7.26, and CD_3OD δ 3.30. ^{13}C NMR chemical shifts are

reported relative to $CDCl_3$ δ 77.0, and CD_3OD δ 49.0. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Film Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra were recorded on a Waters LCT-Premier (high resolution, electron impact ionization (EI)), a Kratos IMS-50 (high resolution, electron impact ionization (EI)), and a ZabSpec IsoMass VG (high resolution. Electrospray (ES)).

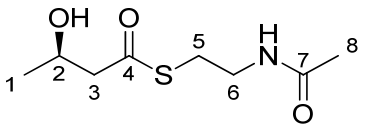


2-keto

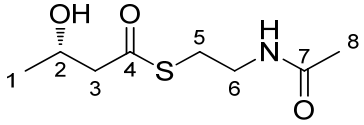


2-enol

The known compound **2** was synthesized by the literature procedures[16]. The main substrate 2, 2, 6-trimethyl-l, 3-dioxin-4-one **13** (95%) was obtained from Sigma-Aldrich. All spectroscopic data and physical properties matched those previously reported.



2D



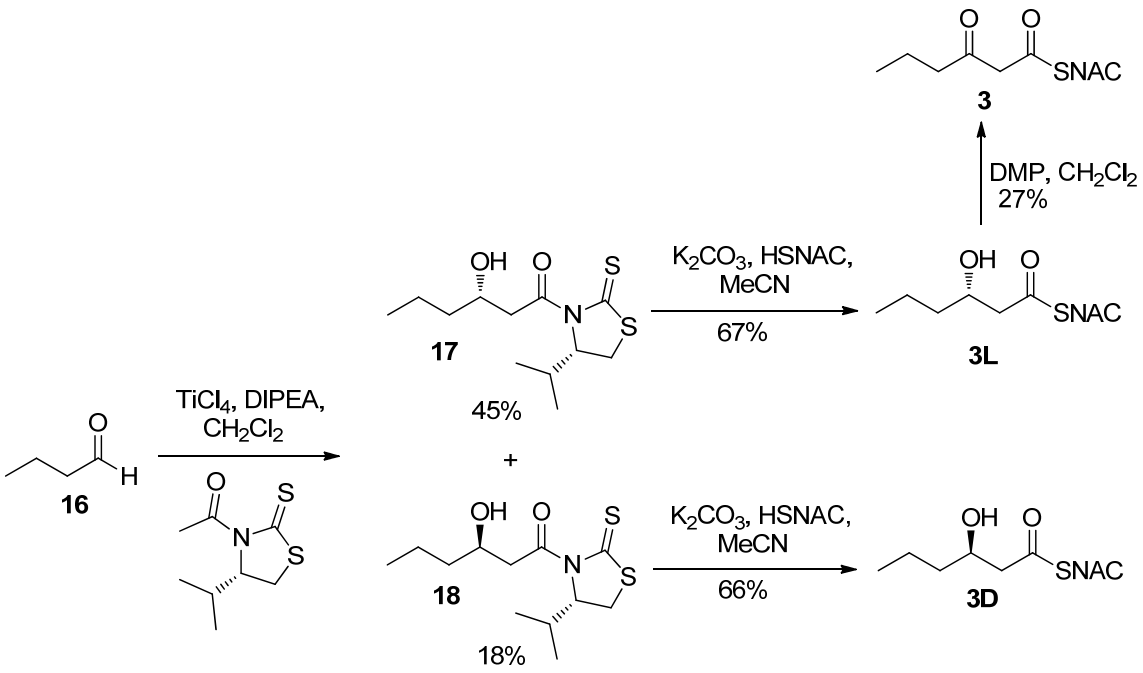
2L

3-(*R*)-hydroxybutyric acid **14** (≥ 98%) and 3-(*S*)-hydroxybutyric acid **15** (≥ 97%) were also purchased from Sigma-Aldrich. **2D** and **2L** were prepared by combining the free acid, diphenylphosphoryl azide, and the free thiol in DMF/triethylamine. Taking the synthesis of **2D** as an example, **14** (104 mg, 1.00 mmol) was dissolved in 10 mL DMF at 0 ^{o}C and then treated with diphenylphosphoryl azide (325 μl, 1.50 mmol) and triethylamine (278 μl, 2.00 mmol) for 2 hours with stirring. *N*-acetylcysteamine (HSNAC, 128.4 μl, 1.20 mmol) was added to the solution. The mixture was stirred at

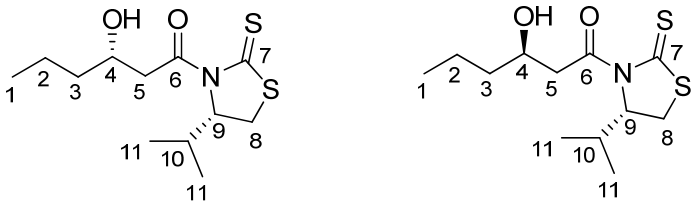
room temperature for additional 3 hours. The reaction was quenched with the addition of 50 ml H_2O and extracted twice with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified with silica gel chromatograph to give 98.4 mg of a light yellow oil.

2D: 98.4 mg, light yellow oil, 48% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.75 (s, 1H, NH), 4.23 (m, 1H, H-2), 3.44 (m, 2H, H-6), 3.03 (m, 2H, H-5), 2.69-2.72 (m, 2H, H-3), 1.95 (s, 3H, H-8), 1.22 (d, 3H, J = 6.30 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.7, 170.9, 65.3, 52.6, 39.5, 29.1, 23.5, 22.9. IR ($CHCl_3$, cast film) 3295, 3087, 2970, 2929, 1686, 1657, 15552 cm^{-1}; α_D^{25} = -33.8 (c = 0.13, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_8H_{15}NSO_3Na$ 228.0670, found 228.0668 $[M+Na]^+$.

2L: 100 mg, light yellow oil, 49% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (s, 1H, NH), 4.23 (m, 1H, H-2), 3.41 (m, 2H, H-6), 3.01 (m, 2H, H-5), 2.67-2.70 (m, 2H, H-3), 1.93 (s, 3H, H-8), 1.20 (d, 3H, J = 6.30 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.5, 170.7, 65.2, 52.8, 39.4, 29.0, 23.4, 22.9. IR ($CHCl_3$, cast film) 3295, 3087, 2970, 2929, 1686, 1657, 15552 cm^{-1}; α_D^{25} = 28.0 (c = 0.49, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_8H_{15}NSO_3Na$ 228.0670, found 228.0700 $[M+Na]^+$.



Supplementary Scheme 1: Synthesis of triketides **3, 3D and 3L**



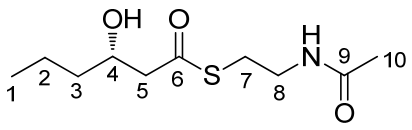
17 **18**

To a stirred solution of (*S*)-4-isopropyl-*N*-acetyl-1, 3-thiazolidine-2-thione (380 mg, 1.87 mmol) in dry dichloromethane (10 mL) was added $TiCl_4$ (1.0 M solution in CH_2Cl_2, 2.05 mL, 2.05 mmol) at 0 °C under Ar. The reaction mixture was stirred for 5 min and then cooled to -78 °C. A solution of DIPEA (291 mg, 2.24 mmol) in dichloromethane (2 mL) was added. The reaction mixture was stirred at -78 °C for 2 h. A solution of aldehyde **16** (333 mg, 1.65 mmol)[6] was added to the reaction mixture, which was then stirred for 15 min at -78 °C. The reaction was quenched with 10 mL saturated ammonium chloride. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4.

The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (1:6 EtOAc/hexanes) to give two diastereomers **17** (98.0 mg, 45% yield) and **18** (40 mg, 18% yield) as yellow oils.

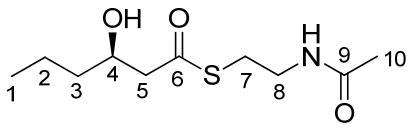
17: 98.0 mg, yellow oil, 45% yield. IR ($CHCl_3$, cast film) 3447, 2961, 2932, 2873, 1694, 1467 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.17 (ddd, 1H, *J* = 7.71, 6.33, 0.92 Hz, H-9), 4.12 (m, 1H, H-4), 3.63 (dd, 1H, *J* = 17.7, 2.38 Hz, H-5), 3.53 (dd, 1H, *J* = 11.5, 7.98 Hz, H-8), 3.12 (dd, 1H, *J* = 17.7, 9.44 Hz, H-5), 3.03 (dd, 1H, *J* = 11.5, 1.01 Hz, H-8), 2.35 (ABX_6, 1H, *J* = 6.78 Hz, H-10), 1.58 - 1.35 (m, 4H, H-2, H-3), 1.05 (d, 3H, *J* = 6.78 Hz, H-11), 0.98 (d, 3H, *J* = 6.97 Hz, H-11), 0.93 (t, 3H, *J* = 7.15Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.1, 173.3, 71.4, 67.7, 45.5, 38.5, 30.8, 30.6, 19.1, 18.7, 17.8, 14.0 ; α_D^{25} = 269 (c = 0.480, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NS_2O_2Na$ 298.0906, found 298.0907 $[M+Na]^+$.

18: 40.0 mg, yellow oil, 18% yield. IR ($CHCl_3$, cast film) 3452, 2961, 2931, 2873, 1697, 1467 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.17 (ddd, 1H, *J* = 7.61, 6.42, 1.01 Hz, H-9), 4.03 (m, 1H, H-4), 3.51 (dd, 1H, *J* = 11.6, 7.98 Hz, H-8), 3.43 (dd, 1H, *J* = 17.4, 9.35 Hz, H-5), 3.32 (dd, 1H, *J* = 17.4, 2.65 Hz, H-5), 3.03 (dd, 1H, *J* = 11.6, 1.10 Hz, H-8), 2.35 (ABX_6, 1H, *J* = 6.78 Hz, H-10), 1.58 - 1.33 (m, 4H, H-2, H-3), 1.05 (d, 3H, *J* = 6.88 Hz, H-11), 0.98 (d, 3H, *J* = 6.77 Hz, H-11), 0.92 (t, 3H, *J* = 6.97 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.1, 173.8, 71.4, 68.2, 45.2, 38.8, 30.8, 30.6, 19.1, 18.7, 17.8, 14.0; α_D^{25} = 233 (c = 0.360, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NS_2O_2Na$ 298.0906, found 298.0907 $[M+Na]^+$.



3L

3L: To a stirred solution of **17** (56.2 mg, 0.204 mmol) in 5 mL MeCN was added K_2CO_3 (109 mg, 0.715 mmol) and *N*-acetylcysteamine (37.8 mg, 0.196 mmol). The reaction mixture was stirred until the yellow color disappeared. The reaction was quenched with 5 mL saturated ammonium chloride. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (EtOAc) to give **3L** (32.0 mg, 67% yield) as a white solid. IR ($CHCl_3$, cast film) 3295, 3085, 2959, 2932, 2873, 1687, 1658, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.01 (s, 1H, NH), 4.05 (m, 1H, H-4), 3.45 (m, 1H, H-8), 3.03 (m, 2H, H-3), 2.80 (d, 1H, J = 4.40 Hz, OH), 2.73 (dd, 1H, J = 15.4, 3.49 Hz, H-5), 2.67 (dd, 1H, J = 15.3, 8.62 Hz, H-5), 1.96 (s, 3H, H-10), 1.53 - 1.33 (m, 4H, H-2, H-3), 0.92 (t, 3H, J = 7.09 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.5, 170.5, 68.5, 51.1, 39.3, 38.9, 28.8, 23.2, 18.6, 13.9; α_D^{25} = 19.1 (c = 0.640, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{10}H_{19}NSO_3Na$ 256.0978, found 256.0979 $[M+Na]^+$.

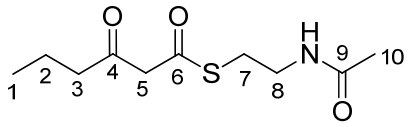


3D

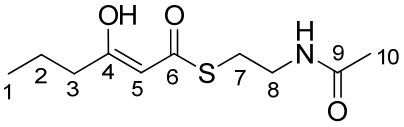
Compound **3D** was synthesized from **18** by the same method for synthesizing **3L**.

3D: 54.6 mg, white solid, yield 66%. IR ($CHCl_3$, cast film) 3290, 3082, 2959, 2933, 2873, 1657, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.20 (s, 1H, NH), 4.04 (m, 1H, H-4),

3.43 (m, 1H, H-8), 3.03 (m, 3H, H-3, OH), 2.71 (dd, 1H, J = 15.2, 3.57 Hz, H-5), 2.63 (dd, 1H, J = 15.0, 7.20 Hz, H-5), 1.93 (s, 3H, H-10), 1.51 - 1.30 (m, 4H, H-2, H-3), 0.90 (t, 3H, J = 6.96 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.3, 170.6, 68.5, 51.2, 39.2, 38.9, 28.8, 23.2, 18.6, 13.9; α_D^{25} = -14.8 (c = 1.10, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{10}H_{19}NSO_3Na$ 256.0978, found 256.0978 $[M+Na]^+$.

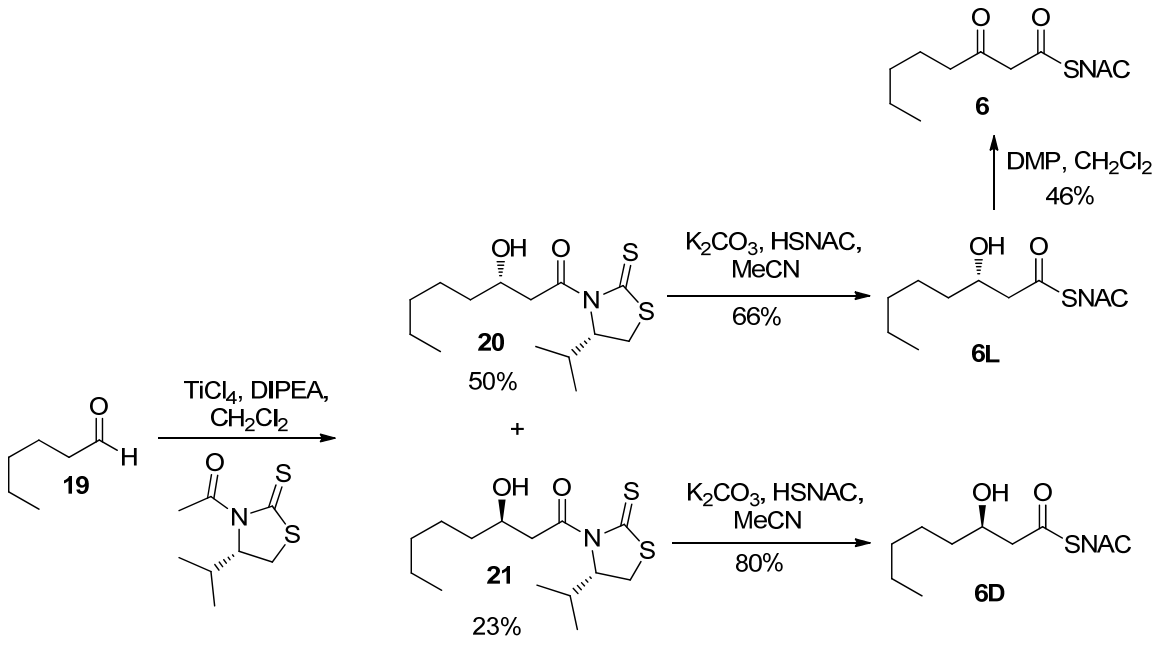


3-keto

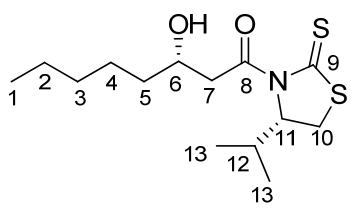


3-enol

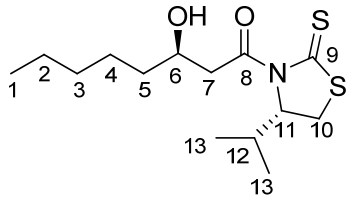
3: To a stirred solution of **3L** (30.0 mg, 0.129 mmol) in 5 mL CH_2Cl_2 was added Dess-Martin periodinane (79 mg, 0.186 mmol). The resulting solution was stirred at 25 °C for 2 h. The reaction was quenched by addition of 5 mL of 1:1 10% $Na_2S_2O_3$: saturated aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (EtOAc) to give **3** (8.00 mg, 27% yield, keto:enol = 3:1) as a white solid. IR ($CHCl_3$, cast film) 3283, 3103, 2958, 2933, 2876, 1716, 1684, 1637, 1562 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (s, 1H, NH), 5.49 (s, 0.25H, enol-H-5), 3.71 (s, 1.5H, keto-H-5), 3.48 (m, 2H, H-8), 3.11 (m, 2H, H-7), 2.53 (t, 1.5H, J = 7.24 Hz, keto-H-3), 2.18 (m, 0.5H, enol-H-3), 2.00 (m, 3H, H-10), 1.65 (m, 2H, H-2), 0.94 (m, 3H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 202.1, 194.3, 192.4, 177.4, 170.6, 170.4, 99.3, 57.2, 45.3, 39.9, 39.3, 36.8, 29.2, 27.8, 23.3, 23.2, 19.6, 16.9, 13.6, 13.5; HRMS (ES) *m/z* calculated for $C_{10}H_{17}NSO_3Na$ 254.0821, found 254.0821 $[M+Na]^+$.



Supplementary Scheme 2: Synthesis of tetraketides **6, 6L, 6D**.



20



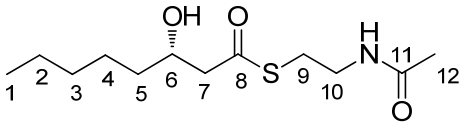
21

Compounds **20** and **21** were synthesized from 1-hexanal by the method for synthesizing **17** and **18**.

20: 240 mg, yellow oil, 50% yield. IR ($CHCl_3$, cast film) 3441, 2959, 2930, 2858, 1690, 1466 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.18 (ddd, 1H, *J* = 7.43, 6.32, 0.91 Hz, H-11), 4.15 (m, 1H, H-6), 3.66 (dd, 1H, *J* = 17.7, 2.39 Hz, H-7), 3.55 (dd, 1H, *J* = 11.5, 7.89 Hz, H-10), 3.15 (dd, 1H, *J* = 17.7, 9.36 Hz, H-7), 3.05 (dd, 1H, *J* = 11.6, 1.10 Hz, H-10), 2.79 (s, 1H, O<u>H</u>), 2.38 (ABX_6, 1H, *J* = 6.78 Hz, H-12), 1.62 - 1.30 (m, 8H, H-2, H-3, H-4, H-5), 1.09 (d, 3H, *J* = 6.79 Hz, H-13), 1.02 (d, 3H, *J* = 6.97 Hz, H-13), 0.86 (t, 3H, *J* = 6.79

Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.1, 173.4, 71.4, 68.1, 45.6, 36.4, 31.8, 30.9, 30.6, 25.2, 22.6, 19.1, 17.9, 14.1; α_D^{25} = 233.76 (c = 1.45, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{25}NS_2O_2Na$ 326.1219, found 326.1225 $[M+Na]^+$.

21: 110 mg, yellow oil, 23% yield. IR ($CHCl_3$, cast film) 3450, 2959, 2930, 2858, 1687, 1466 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.18 (ddd, 1H, *J* = 7.61, 6.33, 1.10 Hz, H-11), 4.03 (m, 1H, H-6), 3.52 (dd, 1H, *J* = 11.6, 7.98 Hz, H-10), 3.45 (dd, 1H, *J* = 17.4, 9.35 Hz, H-7), 3.32 (dd, 1H, *J* = 17.4, 2.66 Hz, H-7), 3.18 (s, 1H, O<u>H</u>), 3.04 (dd, 1H, *J* = 11.6, 1.19Hz, H-10), 2.36 (ABX_6, 1H, *J* = 6.79 Hz, H-12), 1.58 - 1.25 (m, 8H, H-2, H-3, H-4, H-5), 1.07 (d, 3H, *J* = 6.78 Hz, H-13), 0.98 (d, 3H, *J* = 6.97 Hz, H-13), 0.86 (t, 3H, *J* = 6.93 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.2, 173.9, 71.4, 68.5, 45.2, 36.6, 31.8, 30.8, 30.6, 25.2, 22.6, 19.1, 17.9, 14.1; α_D^{25} = 239.47 (c = 0.700, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{25}NS_2O_2Na$ 326.1219, found 326.1225 $[M+Na]^+$.

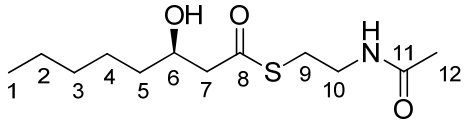


6L

Compound **6L** was synthesized from **20** by the method for synthesizing **3L**.

6L: 80.0 mg, white solid, 66% yield. IR ($CHCl_3$, cast film) 3297, 3086, 2955, 2931, 2859, 1688, 1658, 1553 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H, N<u>H</u>), 4.02 (m, 1H, H-6), 3.39 (m, 1H, H-10), 3.10 (s, 1H, O<u>H</u>), 3.00 (m, 2H, H-9), 2.68 (dd, 1H, *J* = 15.2, 3.67 Hz, H-7), 2.64 (dd, 1H, *J* = 15.2, 8.12 Hz, H-7), 1.92 (s, 3H, H-12), 1.53 - 1.23 (m, 8H, H-2, H-3, H-4, H-5), 0.84 (t, 3H, *J* = 6.87 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.3, 170.7, 68.7, 51.1, 39.2, 36.7, 31.6, 28.7, 25.1, 23.1, 22.5, 13.9; α_D^{25} = 12.31 (c = 3.01,

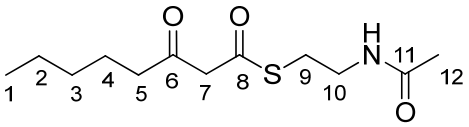
$CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{23}NSO_3Na$ 284.1291, found 284.1295 $[M+Na]^+$.



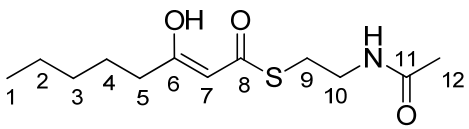
6D

Compound **6D** was synthesized from **21** by the method for synthesizing **3L**.

6D: 56.7 mg, white solid, 80% yield. IR ($CHCl_3$, cast film) 3295, 3084, 2955, 2930, 2859, 1687, 1658, 1552 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H, NH), 4.03 (m, 1H, H-6), 3.42 (m, 1H, H-10), 3.02 (m, 2H, H-9), 2.95 (s, 1H, OH), 2.72 (dd, 1H, *J* = 15.3, 3.57 Hz, H-7), 2.66 (dd, 1H, *J* = 15.2, 8.53 Hz, H-7), 1.95 (s, 3H, H-12), 1.53 - 1.23 (m, 8H, H-2, H-3, H-4, H-5), 0.86 (t, 3H, *J* = 6.88 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.4, 170.6, 68.8, 51.1, 39.2, 36.8, 31.6, 28.8, 25.1, 23.2, 22.6, 14.0; α_D^{25} = -20.09 (c = 0.43, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{23}NSO_3Na$ 284.1291, found 284.1293 $[M+Na]^+$.



6-keto

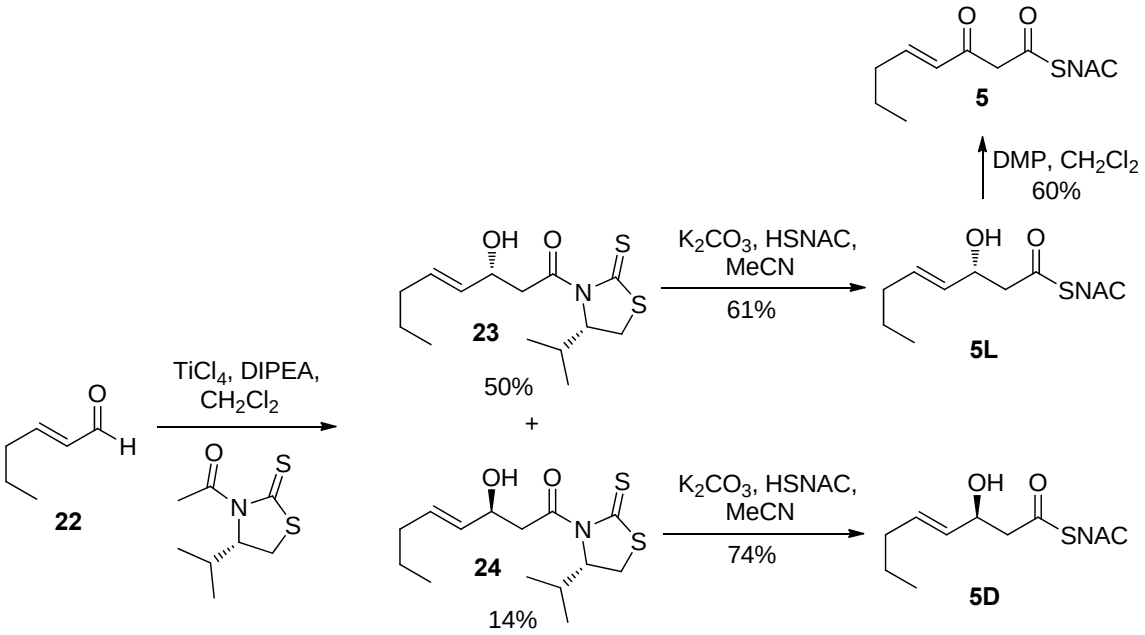


6-enol

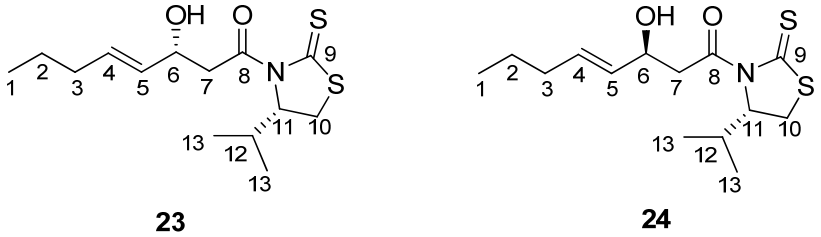
Compound **6** was synthesized from **6L** by the method for synthesizing **3**.

6: 8.2 mg, white solid, 46% yield, keto:enol = 1.85:1. IR ($CHCl_3$, cast film) 3283, 3103, 2958, 2952, 2931, 2867, 1717, 1685, 1637, 1563 cm^{-1};^1H NMR (500 MHz, $CDCl_3$) δ

5.92 (s, 1H, NH), 5.46 (s, 0.35H, enol-H-7), 3.69 (s, 1.3H, keto-H-7), 3.46 (m, 2H, H-10), 3.09 (m, 2H, H-9), 2.52 (t, 1.3H, *J* = 7.34 Hz, keto-H-5), 2.17 (t, 0.7H, *J* = 7.61 Hz, enol-H-5), 1.96 (m, 3H, H-12), 1.59 (m, 2H, H-4), 1.30 (m, 4H, H-2, H-3), 0.89 (m, 3H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 202.3, 194.3, 192.4, 177.7, 170.5, 170.4, 99.1, 57.2, 43.4, 39.9, 39.2, 34.9, 31.3, 31.1, 29.2, 27.8, 25.9, 23.2, 23.1, 23.1, 22.4, 22.3, 13.9, 13.8; HRMS (ES) *m/z* calculated for $C_{12}H_{21}NSO_3Na$ 282.1134, found 282.1137 $[M+Na]^+$.



Supplementary Scheme 3: Synthesis of tetraketides **5, 5L** and **5D**.

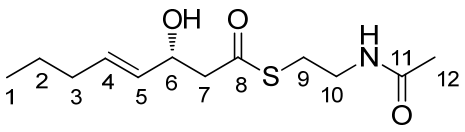


23 **24**

Compounds **23** and **24** were synthesized from hex-2-enal by the method for synthesizing **17** and **18**.

23: 112 mg, yellow oil, 50% yield. IR ($CHCl_3$, cast film) 3426, 2961, 2929, 2872, 1695, 1465cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.75 (m, 1H, H-5), 5.56 (ddt, 1H, *J* = 15.4, 6.42, 1.47 Hz, H-5), 5.17 (ddd, 1H, *J* = 7.43, 6.60, 0.83 Hz, H-11), 4.64 (m, 1H, H-6), 3.63 (dd, 1H, *J* = 17.5, 2.94 Hz, H-7), 3.51 (dd, 1H, *J* = 11.5, 7.88 Hz, H-10), 3.33 (dd, 1H, *J* = 17.6, 8.90 Hz, H-7), 3.05 (dd, 1H, *J* = 11.5, 0.92Hz, H-10), 2.38 (ABX_6, 1H, *J* = 6.79 Hz, H-12), 2.15 (m, 2H, H-3), 1.43 (AB_2X_3, 1H, *J* = 7.43 Hz, H-2), 1.09 (d, 3H, *J* = 6.88 Hz, H-13), 1.01 (d, 3H, *J* = 6.97 Hz, H-13), 0.92 (t, 3H, *J* = 7.42 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 202.9, 172.6, 132.5, 130.6, 71.4, 68.8, 45.5, 34.3, 30.8, 30.6, 22.2, 19.1, 17.8, 13.7; α_D^{25} = 293 (c = 0.470, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{23}NS_2O_3SiNa$ 324.1062, found 324.1063 $[M+Na]^+$.

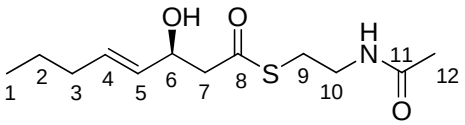
24: 33.0 mg, yellow oil, 14% yield. IR ($CHCl_3$, cast film) 3427, 2961, 2929, 2872, 1694, 1465cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76 (m, 1H, H-5), 5.55 (ddt, 1H, *J* = 15.4, 6.42, 1.37 Hz, H-5), 5.20 (ddd, 1H, *J* = 7.43, 6.33, 1.1 Hz, H-11), 4.56 (m, 1H, H-6), 3.63 (dd, 1H, *J* = 17.3, 8.99 Hz, H-7), 3.53 (dd, 1H, *J* = 11.5, 7.98 Hz, H-10), 3.38 (dd, 1H, *J* = 17.3, 3.21 Hz, H-7), 3.06 (dd, 1H, *J* = 11.5, 1.19 Hz, H-10), 2.38 (ABX_6, 1H, *J* = 6.88 Hz, H-12), 2.15 (m, 2H, H-3), 1.43 (AB_2X_3, 1H, *J* = 7.25 Hz, H-2), 1.09 (d, 3H, *J* = 6.78 Hz, H-13), 1.01 (d, 3H, *J* = 6.87 Hz, H-13), 0.93 (t, 3H, *J* = 7.33 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.1, 173.1, 132.5, 130.7, 71.4, 69.3, 45.3, 34.3, 30.8, 30.6, 22.2, 19.1, 17.8, 13.7; α_D^{25} = 257 (c = 0.500, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{23}NS_2O_3SiNa$ 324.1062, found 324.1063 $[M+Na]^+$.



5L

Compound **5L** was synthesized from **23** by the method for synthesizing **3L**.

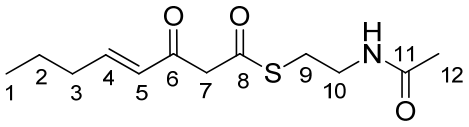
5L: 80.0 mg, yellow oil, 61% yield. IR ($CHCl_3$, cast film) 3295, 3088, 2958, 2930, 2873, 1687, 1657, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) d 6.06 (s, 1H, N<u>H</u>), 5.67 (m, 1H, H-4), 5.45 (m, 1H, H-5), 4.52 (m, 1H, H-6), 3.40 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.82 (s, 1H, O<u>H</u>), 2.75 (m, 2H, H-7), 1.97 (m, 2H, H-3), 1.95 (s, 3H, H-12), 1.37 (AB_2X_3, 1H, J = 7.33 Hz, H-2), 0.87 (t, 3H, J = 7.43 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 198.6, 170.6, 132.8, 130.6, 69.6, 51.2, 39.3, 34.2, 28.7, 23.1, 22.1, 13.6; α_D^{25} = 14.3 (c = 0.430, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NSO_3Na$ 282.1134 , found 282.1136 $[M+Na]^+$.



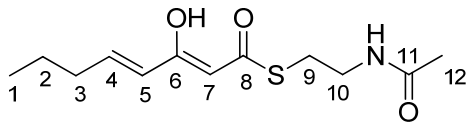
5D

Compound **5D** was synthesized from **24** by the method for synthesizing **3L**.

5D: 52.0 mg, yellow oil, 74% yield. IR ($CHCl_3$, cast film) 3296, 3087, 2958, 2929, 2872, 1687, 1658, 1552 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) d 6.06 (s, 1H, N<u>H</u>), 5.67 (m, 1H, H-4), 5.45 (m, 1H, H-5), 4.52 (m, 1H, H-6), 3.40 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.87 (s, 1H, O<u>H</u>), 2.75 (m, 2H, H-7), 1.97 (m, 2H, H-3), 1.95 (s, 3H, H-12), 1.36 (AB_2X_3, 1H, J = 7.52 Hz, H-2), 0.87 (t, 3H, J = 7.45 Hz, H-1) ;^{13}C NMR (125 MHz, $CDCl_3$) 198.6, 170.6, 132.8, 130.6, 69.6, 51.2, 39.3, 34.2, 28.7, 23.1, 22.1, 13.6; α_D^{25} = -11.3 (c = 0.390, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NSO_3Na$ 282.1134, found 282.1136 $[M+Na]^+$.



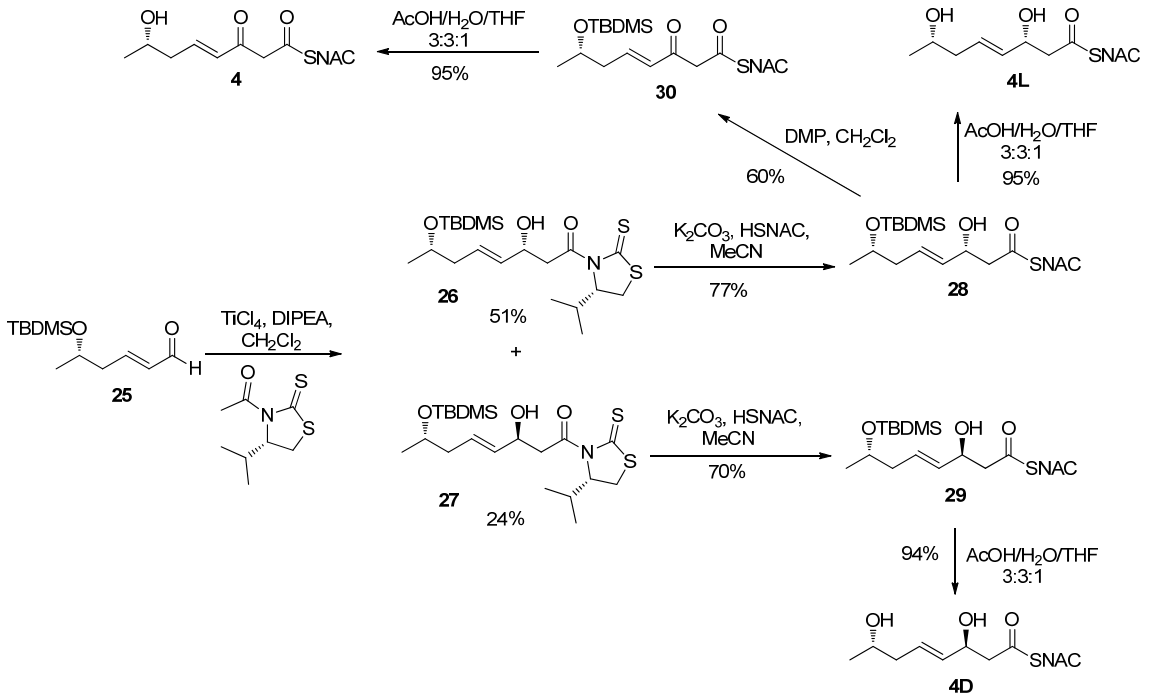
5-keto



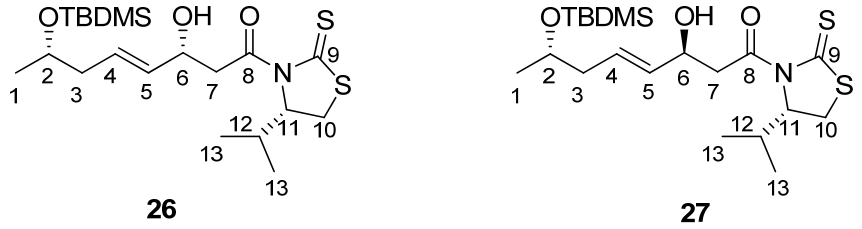
5-enol

Compound **5** was synthesized from **5L** by the method for synthesizing **3L**.

5: 18.0 mg**,** white solid, 60% yield, keto:enol = 1:5.6. IR ($CHCl_3$, cast film) 3298, 3078, 2958, 2956, 2918, 2870, 1650, 1597, 1556 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (dt, 0.14H, *J* = 15.8, 6.87 Hz, keto-H-4), 6.75 (dt, 0.86H, *J* = 15.4, 7.25 Hz, enol-H-4), 6.15 (dt, 0.14H, *J* = 15.9, 1.56 Hz, keto-H-5), 6.08 (s, 1H, N<u>H</u>), 5.72 (dd, 0.86H, *J* = 15.4, 1.46 Hz, enol-H-5), 5.40 (s, 0.85H, enol-H-7), 3.82 (s, 0.3H, keto-H-7), 3.48 (m, 2H, H-10), 3.09 (m, 2H, H-9), 2.18 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.46 (m, 2H, H-2), 0.98 (t, 3H, *J* = 6.24 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 194.5, 192.6, 191.6, 170.5, 170.4, 167.5, 150.8, 143.9, 129.6, 123.9, 99.7, 54.8, 39.9, 39.2, 34.8, 34.6, 29.2, 27.8, 23.2, 23.1, 21.6, 21.2, 13.7; HRMS (ES) *m/z* calculated for $C_{12}H_{19}NSO_3Na$ 280.0978, found 280.0980 $[M+Na]^+$.



Supplementary Scheme 4: Synthesis of tetraketides **4**, **4D**, **4L**.



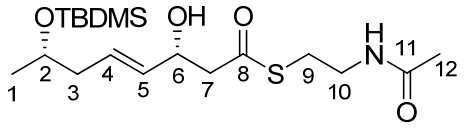
26 **27**

Compounds **26** and **27** were synthesized from **25**[6] by the method for synthesizing **17** and **18**.

26: 190 mg, yellow oil, 51% yield. IR ($CHCl_3$, cast film) 3452, 2959, 2928, 2894, 2856, 1695, 1471 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.74 (m, 1H, H-5), 5.55 (ddt, 1H, *J* = 15.5, 6.22, 1.15 Hz, H-5), 5.15 (ddd, 1H, *J* = 7.51, 6.59, 0.92 Hz, H-11), 4.64 (m, 1H, H-6), 3.83 (AB_2X_3, 1H, *J* = 6.04 Hz, H-2), 3.55 (dd, 1H, *J* = 17.6, 2.93 Hz, H-7), 3.51 (dd, 1H, *J* = 11.5, 7.97 Hz, H-10), 3.30 (dd, 1H, *J* = 17.6, 9.07 Hz, H-7), 3.05 (dd, 1H, *J* = 11.5, 1.01Hz, H-10), 2.38 (m, 1H, H-3), 2.17 (m, 2H, H-3, H-12), 1.11 (d, 3H, *J* = 6.04 Hz, H-1), 1.06 (d, 3H, *J* = 6.87 Hz, H-13), 0.98 (d, 3H, *J* = 6.96 Hz, H-13), 0.88 (s, 9H,

Si-C(C<u>H</u>$_3$)$_3$), 0.05 (s, 6H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 202.8, 172.5, 132.6, 129.1, 71.4, 68.7, 68.3, 45.4, 42.6, 30.8, 30.6, 25.8, 23.5, 19.1, 18.1, 17.8, - 4.49, -4.64; α_D^{25} = 253 (c = 0.690, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{20}H_{37}NS_2O_3SiNa$ 454.1876, found 454.1878 $[M+Na]^+$.

27: 90.0 mg, yellow oil, 24% yield. IR ($CHCl_3$, cast film) 3449, 2928, 2894, 2856, 1695, 1471 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76 (m, 1H, H-5), 5.58 (ddt, 1H, *J* = 15.5, 6.14, 1.28 Hz, H-5), 5.20 (ddd, 1H, *J* = 7.61, 6.24, 1.10 Hz, H-11), 4.56 (m, 1H, H-6), 3.86 (AB_2X_3, 1H, *J* = 6.05 Hz, H-2), 3.65 (dd, 1H, *J* = 17.3, 9.05 Hz, H-7), 3.54 (dd, 1H, *J* = 11.5, 7.97 Hz, H-10), 3.38 (dd, 1H, *J* = 17.3, 3.21 Hz, H-7), 3.05 (dd, 1H, *J* = 11.5, 1.19Hz, H-10), 2.38 (m, 1H, H-3), 2.17 (m, 2H, H-3, H-12), 1.18 (d, 3H, *J* = 6.06 Hz, H-1), 1.05 (d, 3H, *J* = 6.79 Hz, H-13), 1.00 (d, 3H, *J* = 6.96 Hz, H-13), 0.88 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.087 (s, 6H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 203.0, 173.0, 132.7, 129.1, 71.4, 69.1, 68.4, 45.2, 42.6, 30.8, 30.6, 25.9, 23.5, 19.1, 18.2, 17.8, -4.4, -4.6; α_D^{25} = 197 (c = 0.290, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{20}H_{37}NS_2O_3SiNa$ 454.1876, found 454.1882 $[M+Na]^+$.

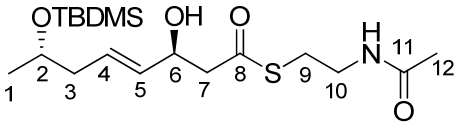


28

Compound **28** was synthesized from **26** by the method for synthesizing **3L**.

28: 196 mg, white solid, 77% yield. IR ($CHCl_3$, cast film) 3290, 2956, 2929, 2897, 2857, 1689, 1657 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (s, 1H, N<u>H</u>), 5.73 (m, 1H, H-4), 5.50 (ddt, 1H, *J* = 15.5, 6.50, 1.28 Hz, H-5), 4.56 (m, 1H, H-6), 3.82 (AB_2X_3, 1H, *J* = 6.04 Hz, H-2), 3.45 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.78 (m, 2H, H-7), 2.48 (s, 1H,

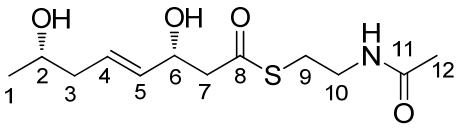
O<u>H</u>), 2.15 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.10 (d, 3H, *J* = 6.05 Hz, H-1), 0.88 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.036 (s, 3H, SiC<u>H</u>$_3$), 0.032 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 198.2, 170.7, 132.7, 129.1, 69.4, 68.2, 51.1, 42.4, 39.1, 28.7, 25.8, 23.3, 23.0, 18.0, -4.6, -4.8; α_D^{25} = 10.3 (c = 0.130, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{18}H_{35}NSO_4SiNa$ 412.1948, found 412.1944 $[M+Na]^+$.



29

Compound **29** was synthesized from **27** by the method for synthesizing **3L**.

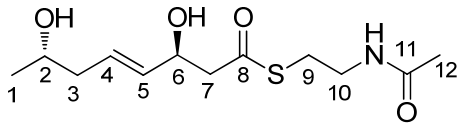
29: 37.0 mg, white solid, 70% yield. IR ($CHCl_3$, cast film) 3298, 2956, 2929, 2895, 2856, 1686, 1657 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (s, 1H, N<u>H</u>), 5.73 (m, 1H, H-4), 5.50 (ddt, 1H, *J* = 15.4, 6.41, 1.19 Hz, H-5), 4.54 (m, 1H, H-6), 3.82 (AB_2X_3, 1H, *J* = 6.04 Hz, H-2), 3.45 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.76 (m, 2H, H-7), 2.71 (s, 1H, O<u>H</u>), 2.15 (m, 2H, H-3), 1.95 (s, 3H, H-12), 1.09 (d, 3H, *J* = 6.24 Hz, H-1), 0.88 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.026 (s, 3H, SiC<u>H</u>$_3$), 0.021 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 198.7, 170.5, 132.5, 129.5, 69.5, 68.2, 51.0, 42.5, 39.3, 28.8, 25.9, 23.5, 23.2, 18.2, -4.5, -4.7; α_D^{25} = -3.67 (c = 0.180, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{18}H_{35}NSO_4SiNa$ 412.1948, found 412.1949 $[M+Na]^+$.



4L

4L: To a flask containing **28** (0.133 mmol) was added 5 mL of a solution of 3:3:1 AcOH/H_2O/THF. The resulting solution was stirred at 25 °C for 12 h. The solvent was

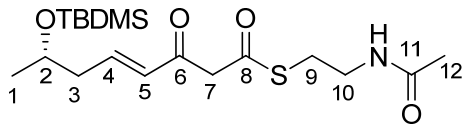
removed *in vacuo* and the residue was purified using flash column chromatography (EtOAc) to give **4L** (6.0 mg, yield 95%) as a white solid. IR ($CHCl_3$, cast film) 3300, 3094, 2965, 2919, 1687, 1658, 1555 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.03 (s, 1H, NH), 5.76 (m, 1H, H-4), 5.60 (ddt, 1H, *J* = 15.5, 6.05, 1.19 Hz, H-5), 4.57 (m, 1H, H-6), 3.82 (m, 1H, H-2), 3.44 (q, 2H, *J* = 6.23 Hz, H-10), 3.04 (td, 2H, *J* = 6.06, 1.93, H-9), 2.87 (s, 1H, OH), 2.81 (m, 2H, H-7), 2.30 – 2.10 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.20 (d, 3H, *J* = 6.14 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 198.6, 170.6, 134.0, 128.5, 69.4, 67.0, 50.9, 42.0, 39.2, 29.1, 23.2, 23.0; α_D^{25} = 12.1 (c = 0.140, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NSO_4Na$ 298.1082, found 298.1083 $[M+Na]^+$.



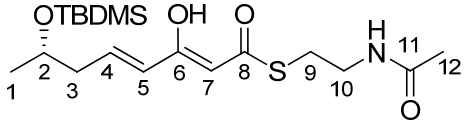
4D

Compound **4D** was synthesized from **29** by the method for synthesizing **4L**.

4D: 8.00 mg, white solid, 94% yield. IR ($CHCl_3$, cast film) 3296, 3094, 2967, 2925, 1687, 1658, 1555 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.00 (s, 1H, NH), 5.76 (m, 1H, H-4), 5.60 (ddt, 1H, *J* = 15.5, 6.33, 1.12 Hz, H-5), 4.58 (m, 1H, H-6), 3.82 (m, 1H, H-2), 3.44 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.87 (s, 1H, OH), 2.82 (m, 2H, H-7), 2.30 – 2.10 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.20 (d, 3H, *J* = 6.24 Hz, H-1),^{13}C NMR (125 MHz, $CDCl_3$) 198.5, 170.6, 134.1, 128.7, 69.6, 67.0, 51.0, 42.0, 39.2, 29.1, 23.2, 23.0; α_D^{25} = 31.3 (c = 0.310, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{12}H_{21}NSO_4Na$ 298.1082, found 298.1083 $[M+Na]^+$.



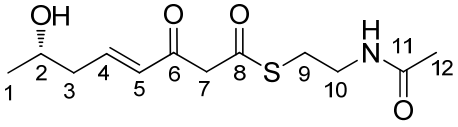
30-keto



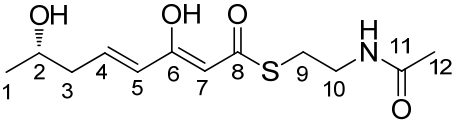
30-enol

Compound **30** was synthesized from **28** by the method for synthesizing **3**.

30: 21.0 mg, white solid, 60% yield, keto:enol = 3:2. IR ($CHCl_3$, cast film) 3287, 3079, 2956, 2930, 2857, 1724, 1656, 1623, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (s, 1H, N<u>H</u>), 5.49 (s, 0.4H, enol-H-5), 4.30 (m, 0.6H, keto-H-2), 4.18 (m, 0.4H, enol-H-2), 3.78 (d, 0.6H, *J* = 15.5 Hz, keto-H-5), 3.72 (d, 0.6H, *J* = 15.5 Hz, keto-H-5), 3.48 (m, 2H, H-8), 3.09 (m, 2H, H-7), 2.70 (dd, 0.6H, *J* = 15.1, 7.25 Hz, keto-H-3), 2.53 (dd, 0.6H, *J* = 15.1, 4.67Hz, keto-H-3), 2.24 (d, 0.8H, *J* = 6.24 Hz, enol-H-3), 1.98 (s, 1.8H, keto-H-10), 1.97 (s, 1.2H, enol-H-10), 1.19 (m, 3H, H-1), 0.88 – 0.89 (m, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.11 – 0.00 (m, 6H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 201.4, 194.3, 192.2, 174.4, 170.5, 170.3, 101.3, 66.1, 65.4, 58.6, 52.6, 45.4, 39.9, 39.2, 29.1, 27.7, 25.8, 25.7, 24.1, 23.9, 23.2, 23.1, 18.0, 17.9, -4.5, -4.6, -4.9, -5.1; HRMS (ES) *m/z* calculated for $C_{16}H_{32}NSO_4SiNa$ 384.1635, found 385.1635 $[M+Na]^+$.



4-keto

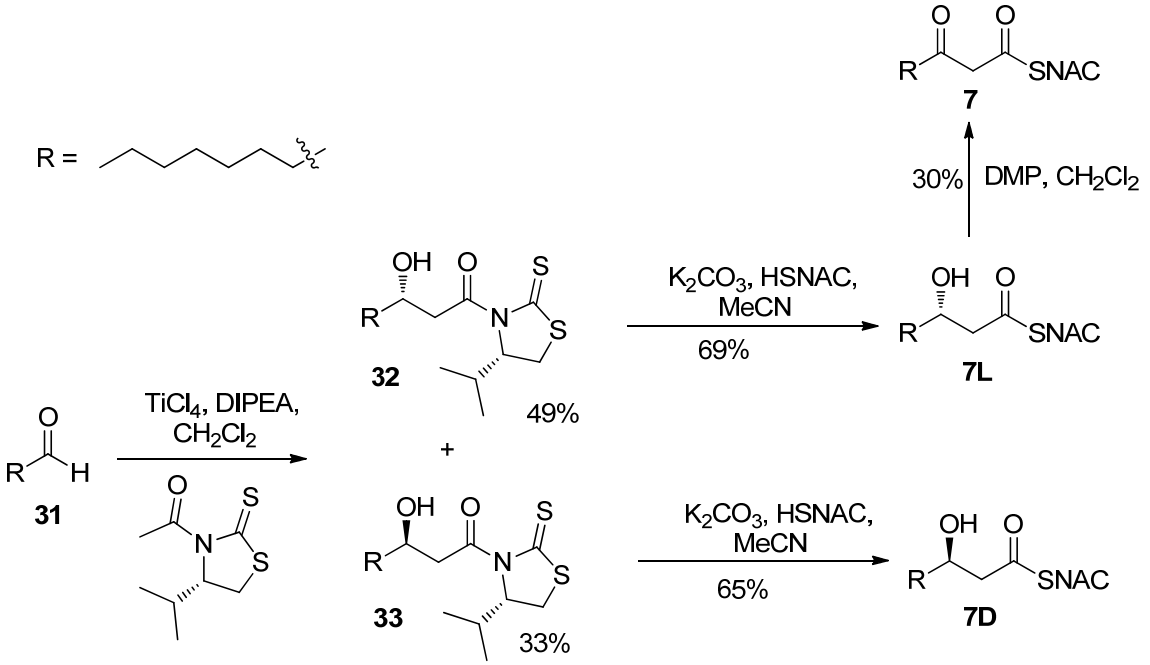


4-enol

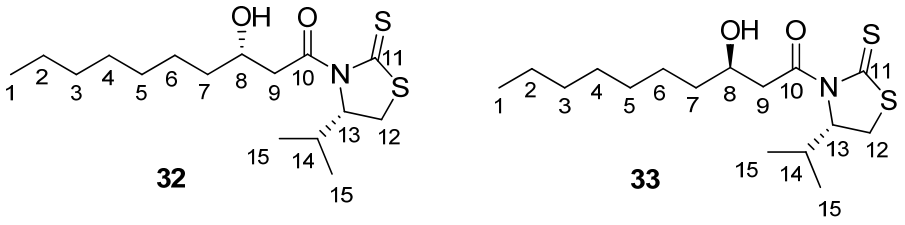
Compound **4** was synthesized from **30** by the method for synthesizing **4L**.

4: 18.0 mg, white solid, 95% yield, keto:enol = 2:3. IR ($CHCl_3$, cast film) 3296, 3086, 2969, 2930, 1657, 1583 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.95 (dt, 0.4H, *J* = 15.9, 7.34 Hz, keto-H-4), 6.78 (dt, 0.6H, *J* = 15.2, 7.52 Hz, enol-H-4), 6.22 (dt, 0.4H, *J* = 15.9, 1.37 Hz, keto-H-5), 6.04 (s, 0.4H, N<u>H</u>), 5.99 (s, 0.6H, N<u>H</u>), 5.82 (d, 0.6H, *J* = 15.5 Hz, enol-H-5), 5.46 (s, 0.6H, enol-H-7), 3.95 (m, 1H, H-2), 3.86 (s, 0.8H, keto-H-7), 3.48 (m,

2H, H-10), 3.09 (m, 2H, H-9), 2.40 (m, 2H, H-3), 1.97 (s, 1.8H, enol-H-12), 1.97 (s, 1.2H, keto-H-12), 1.25 (d, 1.2H, J = 6.24 Hz, keto-H-1), 1.23 (d, 1.8H, J = 6.23 Hz, enol-H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 194.8, 192.6, 191.5, 170.6, 170.5, 167.3, 147.1, 139.6, 131.8, 126.6, 100.3, 67.1, 66.8, 55.1, 42.6, 42.3, 40.0, 39.2, 29.5, 28.1, 23.6, 23.4, 23.4, 23.3; HRMS (ES) *m/z* calculated for $C_{12}H_{19}NSO_4Na$ 269.0927, found 269.0929 $[M+Na]^+$.



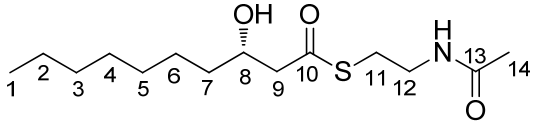
Supplementary Scheme 5: Synthesis of pentaketides **7, 7L** and **7D**.



Compounds **32** and **33** were synthesized from **31** by the method for synthesizing **17** and **18**.

32: 136 mg, yellow oil, 49% yield. IR ($CHCl_3$, cast film) 3437, 2958, 2927, 2857, 1696, 1467 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.15 (ddd, 1H, *J* = 7.68, 6.23, 1.01 Hz, H-13), 4.11 (m, 1H, H-8), 3.61 (dd, 1H, *J* = 17.7, 2.48 Hz, H-9), 3.51 (dd, 1H, *J* = 11.5, 7.89 Hz, H-12), 3.11 (dd, 1H, *J* = 17.7, 9.45 Hz, H-9), 3.02 (dd, 1H, *J* = 11.5, 1.01 Hz, H-12), 2.34 (m, 1H, H-14), 1.58 - 1.1.25 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 1.05 (d, 3H, *J* = 6.79 Hz, H-15), 0.98 (d, 3H, *J* = 6.97 Hz, H-15), 0.85 (d, 3H, *J* = 6.88 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.0, 173.3, 71.4, 68.0, 45.5, 36.4, 31.8, 30.9, 30.6, 29.5, 29.2, 25.5, 22.6, 19.1, 17.8, 14.1; α_D^{25} = 279 (c = 0.470, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{29}NS_2O_2Na$ 354.1532, found 354.1532 $[M+Na]^+$.

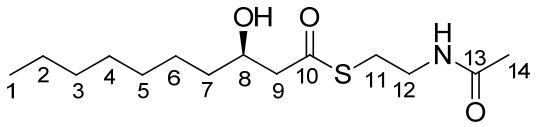
33: 91.3 mg, yellow oil, 33% yield. IR ($CHCl_3$, cast film) 3448, 2958, 2927, 2855, 1697, 1467 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.17 (ddd, 1H, *J* = 7.69, 6.24, 1.10 Hz, H-13), 4.03 (m, 1H, H-8), 3.45 (dd, 1H, *J* = 11.5, 7.97 Hz, H-12), 3.45 (dd, 1H, *J* = 17.4, 9.36 Hz, H-9), 3.32 (dd, 1H, *J* = 17.3, 2.66 Hz, H-9), 3.02 (dd, 1H, *J* = 11.5, 1.10 Hz, H-12), 2.34 (m, 1H, H-14), 1.58 - 1.25 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 1.05 (d, 3H, *J* = 6.87 Hz, H-15), 0.98 (d, 3H, *J* = 6.97 Hz, H-15), 0.86 (t, 3H, *J* = 6.88 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 203.0, 173.8, 71.3, 68.5, 45.1, 36.6, 31.8, 30.7, 30.6, 29.5, 29.2, 25.4, 22.6, 19.0, 17.8, 14.1; α_D^{25} = 212.47 (c = 0.470, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{29}NS_2O_2Na$ 354.1532, found 354.1531 $[M+Na]^+$.



7L

Compound **7L** was synthesized from **32** by the method for synthesizing **3L**.

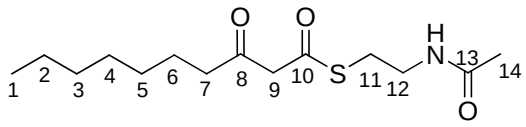
7L: 70.0 mg, white solid, 69% yield. IR ($CHCl_3$, cast film) 3405, 3313, 2955, 2918, 2851, 1685, 1643, 1546 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.03 (s, 1H, NH), 4.10 (m, 1H, H-8), 3.42 (m, 1H, H-12), 3.03 (m, 2H, H-11), 2.87 (d, 1H, *J* = 4.21 Hz OH), 2.72 (dd, 1H, *J* = 15.2, 3.39 Hz, H-9), 2.66 (dd, 1H, *J* = 15.3, 8.62 Hz, H-9), 1.97 (s, 3H, H-14), 1.53 - 1.23 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.86 (t, 3H, *J* = 6.78 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.4, 170.6, 68.8, 51.1, 39.3, 36.8, 31.8, 29.4, 29.2, 28.8, 25.4, 23.2, 22.6, 14.1; α_D^{25} = 14.1 (c = 1.210, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{27}NSO_3Na$ 312.1604, found 312.1604 $[M+Na]^+$.



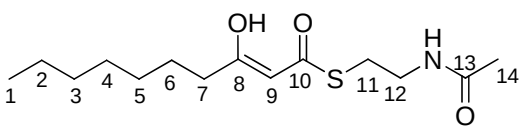
7D

Compound **7D** was synthesized from **33** by the method for synthesizing **3L**.

7D: 45.0 mg, white solid, 65% yield. IR ($CHCl_3$, cast film) 3405, 3313, 2955, 2918, 2851, 1685, 1643, 1546 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.0o (s, 1H, NH), 4.04 (m, 1H, H-8), 3.43 (m, 1H, H-12), 3.03 (m, 2H, H-11), 2.80 (d, 1H, *J* = 4.13 Hz OH), 2.73 (dd, 1H, *J* = 15.3, 3.40 Hz, H-9), 2.66 (dd, 1H, *J* = 15.3, 8.62 Hz, H-9), 1.97 (s, 3H, H-14), 1.53 - 1.23 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.86 (t, 3H, *J* = 6.88 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.5, 170.5, 68.8, 51.1, 39.3, 36.8, 31.8, 29.4, 29.2, 28.8, 25.4, 23.2, 22.6, 14.1; α_D^{25} = -14.3 (c = 1.000, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{14}H_{27}NSO_3Na$ 312.1604, found 312.1603 $[M+Na]^+$.



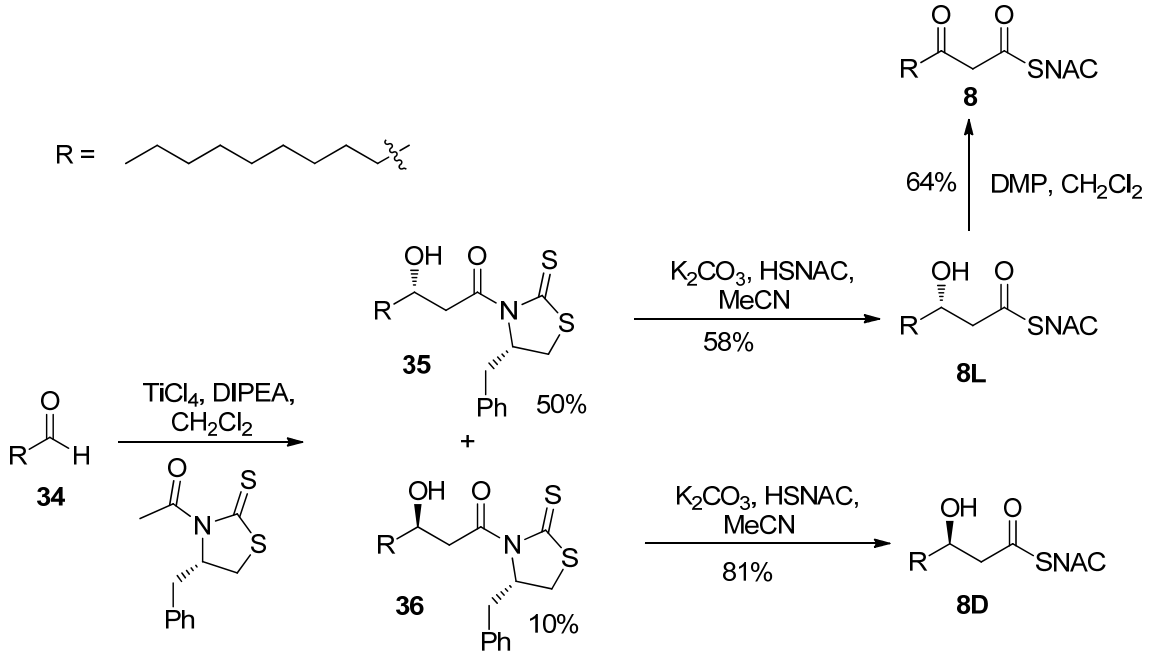
7-keto



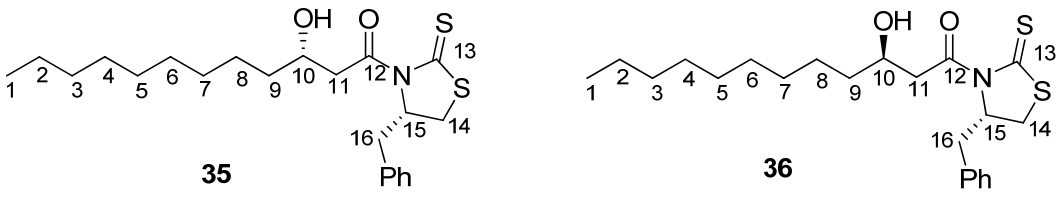
7-enol

Compound **7** was synthesized from **7L** by the method for synthesizing **3**.

7: 15.0 mg, white solid, 30% yield, keto:enol = 1.85:1. IR ($CHCl_3$, cast film) 3281, 3105, 2949, 2923, 2856, 1717, 1687, 1637, 1563 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.95 (s, 1H, NH), 5.45 (s, 0.35H, enol-H-9), 3.69 (s, 1.3H, keto-H-9), 3.46 (m, 2H, H-12), 3.09 (m, 2H, H-11), 2.52 (t, 1.3H, *J* = 7.33 Hz, keto-H-7), 2.17 (t, 0.7H, *J* = 7.62 Hz, enol-H-7), 1.98 (m, 3H, H-14), 1.58 (m, 2H, H-6), 1.33 -1.20 (m, 8H, H-2, H-3, H-4, H-5), 0.89 (m, 3H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 202.3, 194.3, 192.4, 177.7, 170.4, 170.2, 99.1, 57.2, 43.5, 39.9, 39.2, 34.9, 31.7, 31.6, 29.3, 29.1, 29.0, 28.9, 27.9, 26.3, 23.5, 23.3, 23.2, 22.67, 14.1; HRMS (ES) *m/z* calculated for $C_{14}H_{25}NSO_3Na$ 310.1447, found 310.1447 $[M+Na]^+$.



Supplementary Scheme 6: Synthesis of hexaketides **8**, **8L** and **8D**.

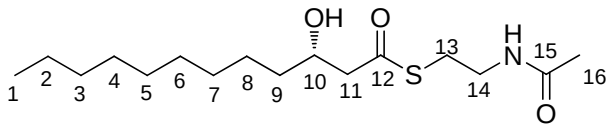


Compounds **35** and **36** were synthesized from **34** by the similar method for synthesizing **17** and **18** where the auxiliary was changed to (*S*)-4-benzyl-*N*-acetyl-1, 3-thiazolidine-2-thione.

35: 203 mg, yellow oil, 50% yield. IR ($CHCl_3$, cast film) 3451, 2925, 2854, 1695, 1496 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.40 (ddd, 1H, *J* = 10.6, 6.97, 4.04 Hz, H-15), 4.05 (m, 1H, H-10), 3.64 (dd, 1H, *J* = 17.7, 2.38 Hz, H-11), 3.40 (dd, 1H, *J* = 11.5, 7.3 Hz, H-14), 3.23 (dd, 1H, *J* = 13.2, 3.76 Hz, H-16), 3.13 (dd, 1H, *J* = 17.7, 9.36 Hz, H-11), 3.05 (dd, 1H, *J* = 13.1, 10.5 Hz, H-16), 2.89 (d, 1H, *J* = 11.6 Hz, H-14), 1.60 - 1.22 (m, 16H, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 0.86 (t, 3H, *J* = 6.61 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 201.4, 173.4, 136.4, 129.5, 128.9, 127.3, 68.4, 67.9, 45.9, 36.9, 36.4, 32.1, 31.9, 29.6, 29.5, 29.5, 29.3, 25.6, 22.7, 14.1; α_D^{25} = 123 (c = 0.280, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{22}H_{33}NS_2O_2Na$ 430.1845, found 430.1847 $[M+Na]^+$.

36: 38.0 mg, yellow oil, 10% yield. IR ($CHCl_3$, cast film) 3449, 2925, 2854, 1697, 1496 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H, Ph), 5.40 (ddd, 1H, *J* = 10.6, 6.97, 4.04 Hz, H-15), 4.05 (m, 1H, H-10), 3.46 (dd, 1H, *J* = 17.4, 9.26 Hz, H-11), 3.40 (dd, 1H, *J* = 11.5, 7.2 Hz, H-14), 3.34 (dd, 1H, *J* = 17.4, 2.56 Hz, H-11),3.23 (dd, 1H, *J* = 13.3, 3.30 Hz, H-16), 3.09 (s, 1H, O<u>H</u>), 3.05 (dd, 1H, *J* = 13.2, 10.5 Hz, H-16), 2.91(d, 1H, *J* = 11.6 Hz, H-14), 1.60 - 1.20 (m, 16H, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 0.86 (t, 3H, *J* = 6.90 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 201.5, 173.9, 136.4, 129.5, 128.9, 127.3, 68.5, 68.3, 45.5, 36.8, 36.7, 32.1, 31.9, 29.6, 29.5, 29.5, 29.3, 25.5, 22.7,

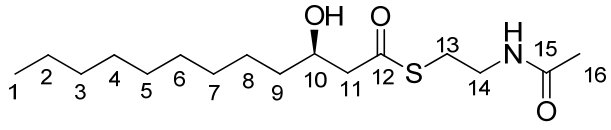
14.1; α_D^{25} = 61.6 (c = 0.260, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{22}H_{33}NS_2O_2Na$ 430.1845, found 430.1847 $[M+Na]^+$.



8L

Compound **8L** were synthesized from **35** by the method for synthesizing **3L**.

8L: 90.0 mg, white solid, 58% yield. IR ($CHCl_3$, cast film) 3408, 3281, 3230, 2954, 2917, 2849, 1683, 1658, 1631, 1557 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.02 (s, 1H, NH), 4.04 (m, 1H, H-10), 3.45 (m, 1H, H-14), 3.04 (m, 2H, H-13), 2.80 (d, 1H, *J* = 4.40 Hz, OH), 2.87 (d, 1H, *J* = 4.21 Hz OH), 2.73 (dd, 1H, *J* = 15.3, 3.40 Hz, H-11), 2.67 (dd, 1H, *J* = 15.3, 8.71 Hz, H-11), 1.97 (s, 3H, H-16), 1.53 - 1.23 (m, 16H, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 0.86 (t, 3H, *J* = 6.87 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.5, 170.5, 68.8, 51.1, 39.3, 36.8, 31.9, 29.6, 29.5, 29.5, 29.3, 28.9, 25.4, 23.2, 22.7, 14.1; α_D^{25} = 29.2 (c = 0.150, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{31}NSO_3Na$ 340.1917, found 340.1919 $[M+Na]^+$.

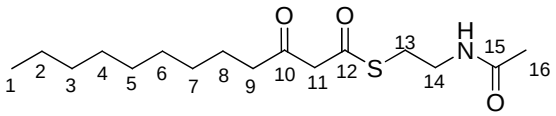


8D

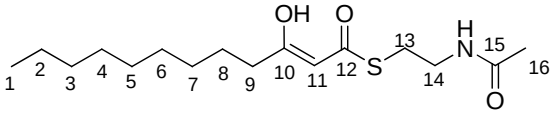
Compound **8D** was synthesized from **36** by the method for synthesizing **3L**.

8D: 22.0 mg, white solid, yield 81%. IR ($CHCl_3$, cast film) 3406, 3313, 2954, 2917, 2845, 1683, 1659, 1638, 1557 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.86 (s, 1H, NH), 4.06 (m, 1H, H-10), 3.45 (m, 1H, H-14), 3.04 (m, 2H, H-13), 2.87 (d, 1H, *J* = 4.21 Hz OH), 2.75

(dd, 1H, *J* = 15.4, 3.30 Hz, H-11), 2.67(dd, 1H, *J* = 15.5, 8.56 Hz, H-11), 2.66 (d, 1H, *J* = 4.22 Hz, OH), 1.97 (s, 3H, H-16), 1.53 - 1.23 (m, 16H, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 0.86 (t, 3H, *J* = 6.88 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 199.6, 170.5, 68.9, 51.1, 39.3, 36.8, 31.9, 29.6, 29.5, 29.5, 29.3, 28.9, 25.4, 23.2, 22.7, 14.1; α_D^{25} = -14.3 (c = 0.260, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{31}NSO_3Na$ 340.1917, found 340.1918 $[M+Na]^+$.



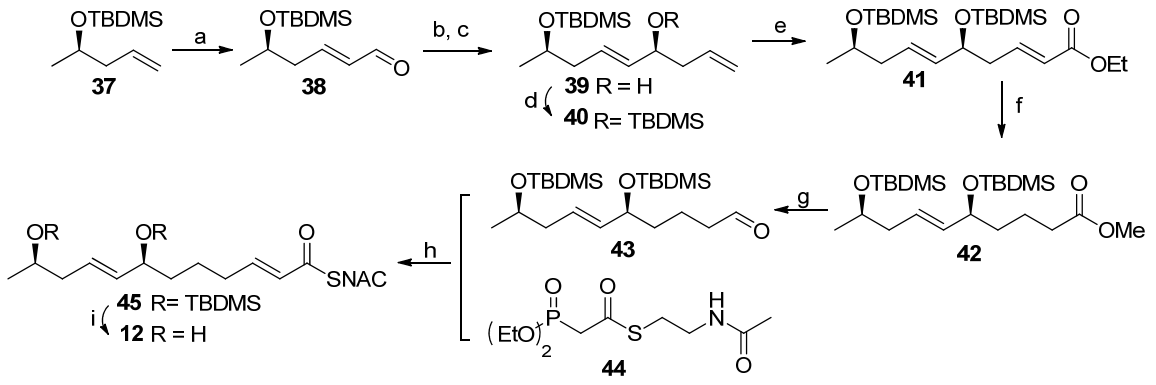
8-keto



8-enol

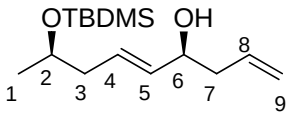
Compound **8** were synthesized from **8L** by the method for synthesizing **3**.

8: 35.0 mg, white solid, 64% yield. IR ($CHCl_3$, cast film) 3279, 3104, 2948, 2920, 2849, 1717, 1687, 1636, 1565 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.86 (s, 1H, NH), 5.45 (s, 0.3H, enol-H-11), 3.69 (s, 1.4H, keto-H-11), 3.46 (m, 2H, H-14), 3.09 (m, 2H, H-13), 2.52 (t, 1.3H, *J* = 7.43 Hz, keto-H-9), 2.17 (t, 0.7H, *J* = 7.65 Hz, enol-H-9), 1.96 (m, 3H, H-16), 1.58 (m, 2H, H-8), 1.33 -1.20 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.89 (m, 3H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) 202.3, 194.3, 192.4, 177.7, 170.4, 170.2, 99.1, 57.2, 43.5, 39.9, 39.2, 34.9, 31.9, 31.3, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.1, 29.0, 27.9, 26.3, 23.5, 23.3, 23.2, 22.7, 14.1; HRMS (ES) *m/z* calculated for $C_{16}H_{29}NSO_3Na$ 338.1760, found 338.1763 $[M+Na]^+$.



Supplementary Scheme 7: Synthesis of hexaketide **12**. Conditions: (a) TBDMSCl, imidazole, DMF, Quant.; (b) Grubbs II, crotonaldehyde, CH_2Cl_2, reflux, 75%; (c) (-)-Ipc_2B(allyl)borane, -100 ^oC, then NaOH, H_2O_2, 25 ^oC, 73%; (d) TBDMSCl, imidazole, DMF, 25 ^oC, Quant.; (e) Grubbs II, ethyl acrylate, CH_2Cl_2, 25 ^oC, 76%; (f) Mg, MeOH, reflux, 90%; (g) DIBAL, CH_2Cl_2, -78 ^oC, 93%; (h) LiBr, Et_3N, **44**, CH_2Cl_2, 78%; (i) $AcOH/H_2O/THF$ = 3:3:1, 25 ^oC, 98%.

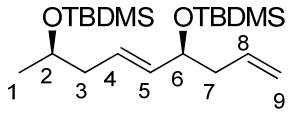
The synthetic scheme for **12** is the same as for its 2-(*S*) diastereomer as reported by Zhou *et al*[6]. The known compound **38**[17] was prepared by a different procedure[6]. All spectroscopic data and physical properties matched those previously reported[17].



39

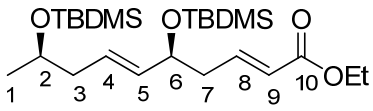
39: 3.30 g, colorless liquid, 73% yield. IR ($CHCl_3$, cast film) 3354, 3077, 2957, 2929, 2897, 2858, 1472, 1466 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.80 (m, 1H, H-8), 5.66 (m, 1H, H-4), 5.52 (ddt, 1H, *J* = 15.3, 6.62, 1.21 Hz, H-5), 5.12 (m, 2H, H-9), 4.12 (m, 1H, H-6), 3.85 (AB_2X_3, 1H, *J* = 6.06 Hz, H-2), 2.30 – 2.15 (m, 4H, H-3, H-7), 1.12 (d, 3H, *J* = 6.06 Hz, H-1), 0.88 (s, 9H, Si-C($C\underline{H}_3)_3$), 0.05 (s, 3H, Si$C\underline{H}_3$), 0.04 (s, 3H, Si$C\underline{H}_3$) ; ^{13}C

NMR (125 MHz, $CDCl_3$) d 134.4 , 134.3, 128.8, 118.0, 71.9, 68.5, 42.6, 41.9, 25.9, 23.5, 18.2, -4.5, -4.8; α_D^{25} = -12.7 (c = 1.13, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{15}H_{30}SiO_2Na$ 293.1907, found 293.1906 $[M+Na]^+$.



40

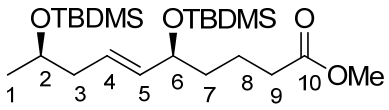
40: 2.10 g, colorless liquid, Quant. IR ($CHCl_3$, cast film) 3078, 2957, 2930, 2897, 2858, 1472, 1463, 1257 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.78 (m, 1H, H-8), 5.55 (m, 1H, H-4), 5.45 (ddt, 1H, *J* = 15.4, 6.51, 1.21 Hz, H-5), 5.03 (m, 2H, H-9), 4.09 (m, 1H), 3.80 (AB_2X_3, 1H, *J* = 6.07 Hz, H-2), 2.27–2.11 (m, 4H, H-3, H-7), 1.10 (d, 3H, *J* = 6.06 Hz, H-1), 0.88 (s, 18H, Si-C($C\underline{H}_3$)$_3$), 0.06 (s, 6H, Si$C\underline{H}_3$), 0.05 (s, 3H, Si$C\underline{H}_3$), 0.03 (s, 3H, Si$C\underline{H}_3$) ; ^{13}C NMR (125 MHz, $CDCl_3$) d 135.4, 135.3, 126.9, 116.6, 73.5, 68.6, 43.1, 42.6, 26.0, 25.9, 23.2, 18.2, 18.1,-4.2, -4.5, -4.6, -4.7; α_D^{25} = -2.16 (c =1.64, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{21}H_{44}Si_2O_2Na$ 407.2772, found 407.2769 $[M+Na]^+$.



41

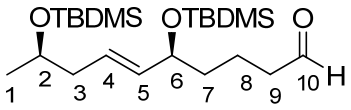
41: 2.10 g, colorless liquid, 75% yield. IR ($CHCl_3$, cast film) 2957, 2930, 2897, 2858, 1725, 1657, 1472, 1463 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.94 (m, 1H, H-8), 5.83 (dt, 1H, *J* = 15.6, 1.32 Hz, H-9), 5.60 (m, 1H, H-4), 5.45 (ddt, 1H, *J* = 15.5, 6.61, 1.10 Hz, H-5), 4.18 (m, 3H, H-6, O$C\underline{H}_2CH_3$), 3.82 (AB_2X_3, 1H, *J* = 6.0 Hz, H-2), 2.38-2.12 (m, 4H, H-3, H-7), 1.28 (t, 3H, *J* = 7.10 Hz, $OCH_2C\underline{H}_3$) 1.10 (d, 3H, *J* = 6.06 Hz, H-1), 0.89 (s, 9H, Si-C($C\underline{H}_3$)$_3$), 0.88 (s, 9H, Si-C($C\underline{H}_3$)$_3$), 0.05 (s, 6H, Si$C\underline{H}_3$), 0.04 (s, 3H, Si$C\underline{H}_3$), 0.02

(s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) d 166.5, 145.9 134.9 127.8 123.6 72.7 68.7 60.1 42.8 41.7 26.1 26.0 23.6 18.2 18.1 14.4 -4.3 -4.5 -4.7 -4.8; α_D^{25} = -3.34 (c =1.39, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{24}H_{48}Si_2O_4Na$ 479.2983, found 479.2987 $[M+Na]^+$.



42

42: 100 mg, colorless liquid, 90% yield. IR ($CHCl_3$, cast film) 2956, 2930, 2897, 2858, 1744, 1472, 1463 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.55 (m, 1H, H-4), 5.42 (m, 1H, H-5), 4.08 (m, 1H, H-6), 3.82 (AB_2X_3, 1H, *J* = 6.06 Hz, H-2), 3.68 (s, 3H, OC<u>H</u>$_3$), 2.32 (t, 2H, *J* = 7.14 Hz, H-9), 2.15 (m, 2H, H-3), 1.70-1.45 (m, 4H, H-7, H-8), 1.11 (d, 3H, *J* = 6.06 Hz, H-1), 0.89 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.88 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.05 (s, 6H, SiC<u>H</u>$_3$), 0.04 (s, 3H, SiC<u>H</u>$_3$), 0.02 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 135.4, 126.8, 73.3, 68.5, 51.4, 42.5, 37.7, 34.0, 26.0, 25.8, 23.2, 20.9, 18.2, 18.1, -4.2, -4.6, -4.7, -4.8; α_D^{25} = -0.77 (c = 0.71, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{23}H_{48}Si_2O_4Na$ 467.2983, found 467.2977 $[M+Na]^+$.

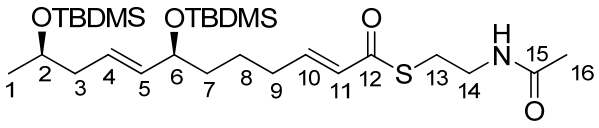


43

43: 70.0 mg, colorless liquid, 93% yield. IR ($CHCl_3$, cast film) 2956, 2930, 2897, 2858, 2710, 1730, 1473, 1255 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, 1H, *J* = 1.84 Hz, H-10), 5.55 (m, 1H, H-4), 5.40 (m, 1H, H-5), 4.08 (m, 1H, H-6), 3.82 (AB_2X_3, 1H, *J* = 6.07 Hz, H-2), 2.42 (td, 2H, *J* = 7.34, 1.84 Hz, H-9), 2.16 (m, 2H, H-3), 1.70-1.45 (m, 4H, H-7,

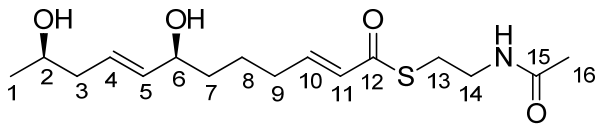
H-8), 1.11 (d, 3H, *J* = 6.05 Hz, H-1), 0.89 (s, 18H, Si-C(C<u>H</u>$_3$)$_3$), 0.06 (s, 3H, SiC<u>H</u>$_3$), 0.05 (s, 3H, SiC<u>H</u>$_3$) 0.04 (s, 3H, SiC<u>H</u>$_3$), 0.02 (s, 3H, SiC<u>H</u>$_3$) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.6, 135.4, 127.0, 73.2, 68.5, 43.8, 42.6, 37.7, 25.9, 25.8, 23.1, 18.1, 18.0, -4.2, -4.5, -4.7, -4.8; α_D^{25} = -5.64 (c = 0.33, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{22}H_{46}Si_2O_3Na$ 437.2878, found 437.2872 $[M+Na]^+$.

44: This known compound was synthesized by literature procedures[6]. All spectroscopic data and physical properties matched those previously reported[6,18].



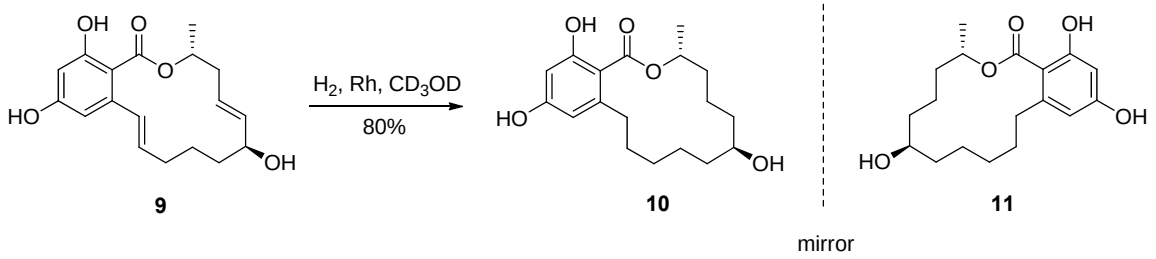
45

45: 150 mg, colorless liquid, 78% yield. IR ($CHCl_3$, cast film) 3289, 2955, 2929, 2896, 2857, 1664, 1635, 1558, 1472, 1289 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.92 dt, 1H, *J* = 15.4, 6.84 Hz, H-10), 6.13 (dt, 1H, *J* = 15.5, 1.54 Hz, H-11 5.86 (s, 1H, N<u>H</u>), 5.54 (m, 1H, H-4), 5.40 (ddt, 1H, *J* = 15.3, 6.73, 1.21 Hz, H-5), 4.05 (dt, 1H, *J* = 6.40, 6.40 Hz, H-6), 3.82 (AB_2X_3, 1H, *J* = 6.06 Hz, H-2), 3.47 (dt, 2H, *J* = 5.96, 5.96 Hz, H-14), 3.10 (t, 2H, *J* = 6.50 Hz, H-13), 2.25 - 2.10 (m, 4H, H-3, H-9), 1.97 (s, 3H, H-16), 1.45 - 1.56 (m, 4H, H-7, H-8), 1.10 (d, 3H, *J* = 6.18 Hz, H-1), 0.88 (s, 18H, Si-C(C<u>H</u>$_3$)$_3$), 0.05 (s, 3H, SiC<u>H</u>$_3$), 0.05 (s, 3H, SiC<u>H</u>$_3$), 0.04 (s, 3H, SiC<u>H</u>$_3$), 0.02 (s, 3H, SiC<u>H</u>$_3$) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.4, 170.2, 146.5, 135.5, 128.4, 126.9, 73.3, 68.5, 42.6, 39.8, 37.8, 32.2, 28.3, 25.9, 25.8, 23.6, 23.4, 23.2, 18.2, 18.1, -4.2, -4.5, -4.7, -4.8; α_D^{25} = -3.21 (c = 1.56, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{28}H_{55}Si_2O_4SNNa$ 580.3283, found 580.3280 $[M+Na]^+$.



12

12: 70.0 mg, colorless liquid, 98% yield. IR ($CHCl_3$, cast film) 3300, 3089, 2965, 2927, 2854, 1660, 1633, 1556, 1436, 1292 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.92 dt, 1H, *J* = 15.4, 6.84 Hz, H-10 6.13 (dt, 1H, *J* = 15.4, 1.44 Hz, H-11 5.96 (s, 1H, N<u>H</u>), 5.67 (m, 1H, H-4), 5.58 (m, 1H, H-5), 4.10 (dt, 1H, *J* = 6.40, 6.40 Hz, H-6), 3.85 (AB_2X_3, 1H, *J* = 6.17 Hz, H-2), 3.45 (dt, 2H, *J* = 5.96, 5.96 Hz, H-14), 3.09 (t, 2H, *J* = 6.23 Hz, H-13), 2.27-2.15 (m, 4H, H-3, H-9), 1.95 (s, 3H, H-16), 1.87 (s, 2H, O<u>H</u>), 1.62-1.48 (m, 4H, H-7, H-8), 1.10 (d, 3H, *J* = 6.28 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.4, 170.4, 146.2, 136.3, 128.6, 128.0, 72.5, 67.3, 42.1, 39.8, 36.5, 32.1, 28.3, 23.9, 23.3, 23.1; α_D^{25} = -5.58 (c = 0.24, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{27}SO_4Na$ 352.1553, found 352.1551 $[M+Na]^+$.



9 **10** **11**

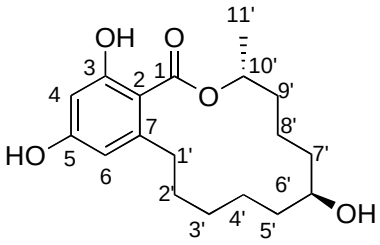
Supplementary Scheme 8: The strategy for confirmation of the stereochemistry of **9**. Hydrogenation of **9** lead to the production of **10**, which is the enantiomer of a commercially available compound **11**.

10: To a stirred solution of epi-DHZ (1.00 mg, 3.10 μmol) in 1 mL CD_3OD was added Rh on alumina (700 μg, 5 wt%). The resulting solution was stirred under 1 atm H_2. The reaction was monitored by NMR until all starting material consumed. The solvent was removed *in vacuo* and the residue was purified using preparative TLC (2:1 Hexane/

EtOAc) to give **10** (0.8 mg, 80% yield). IR (MeOH, cast film) 3362, 2924, 2854, 1646, 1610, 1582, 1436, 1259 cm^{-1}; HRMS (ES) *m/z* calculated for $C_{18}H_{25}O_5Na$ 321.1707, found 321.1706 $[M-H]^-$.

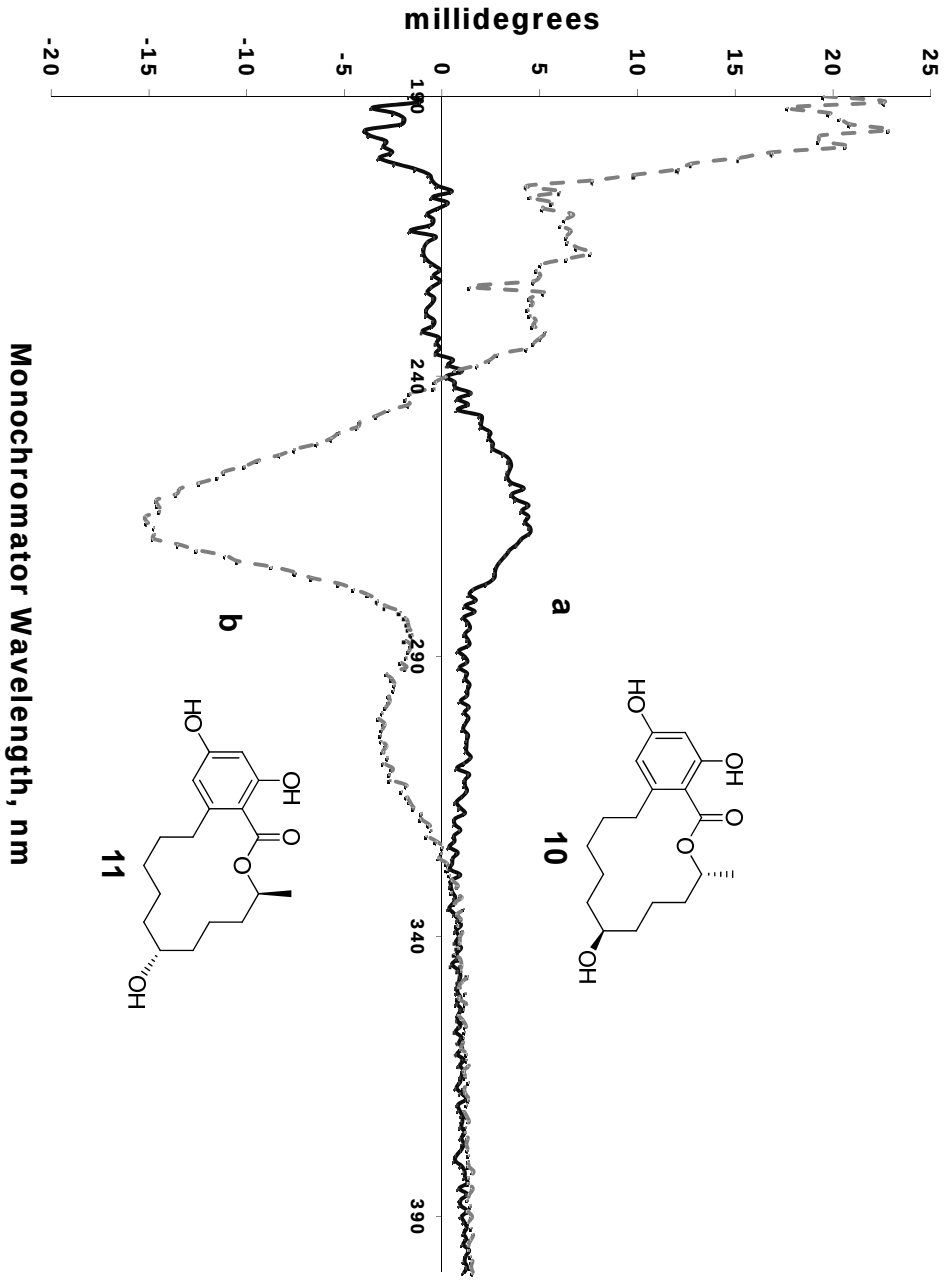
It is noticeable that the extra carbon signals in the ^{13}C spectrum of **10** actually come from the contamination in blank solvent CD_3OD. Both the ^1H and ^{13}C spectra of **10** match with the ones for commercially available **11** (SynInnova, 98%). The circular dichroism spectra of **10** and **11** are mirror imaged, confirming their enantiomeric property.

Supplementary Table 7. Proton and carbon NMR for **10**.

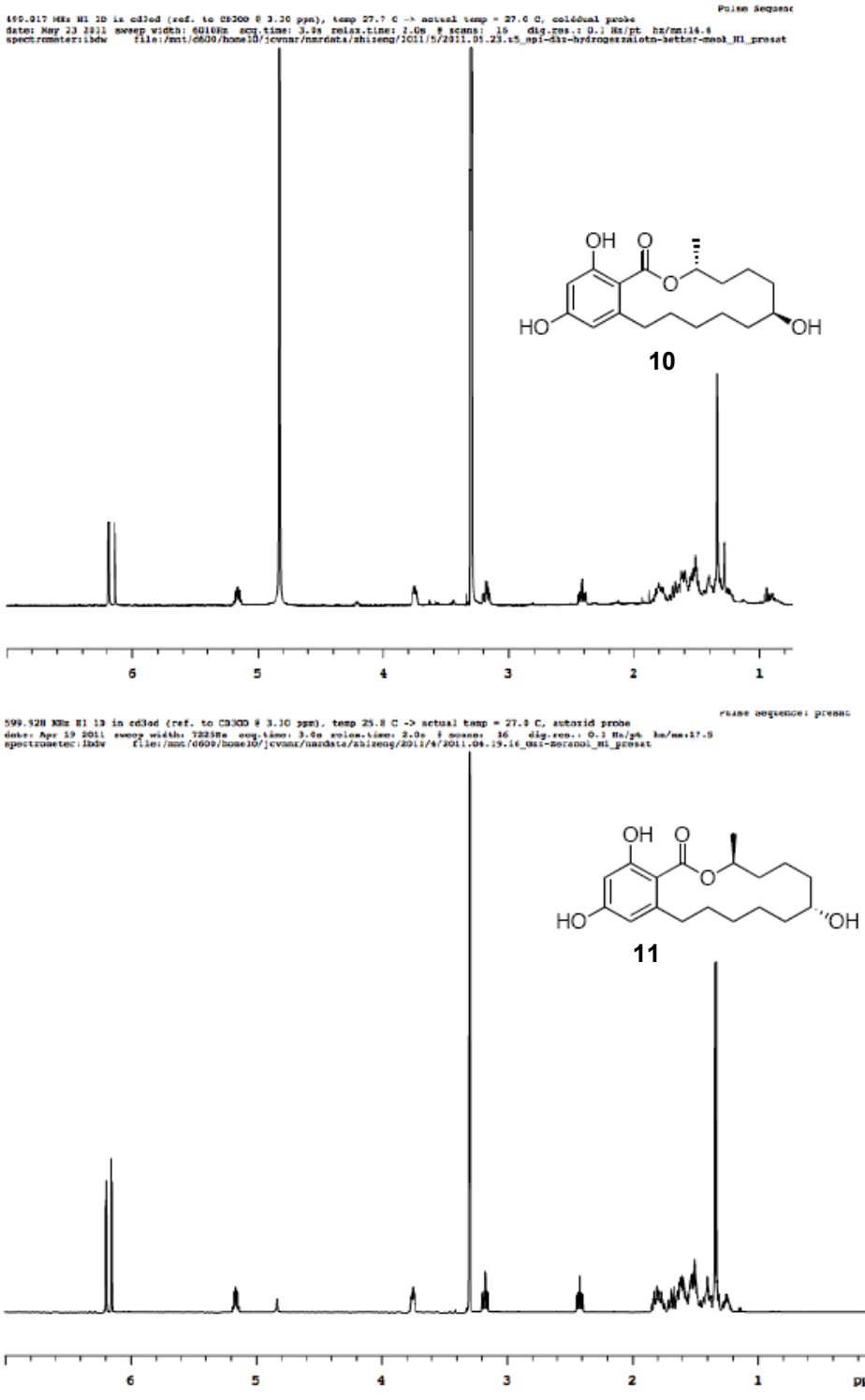


No.	^{13}C δ (ppm)	^{1}H δ (ppm)
1	173.0	-
2	106.0	-
3	166.0	-
4	111.7	6.19 (d, 1H, 2.40)
5	163.7	-
6	102.0	6.15 (d, 1H, 2.40)
7	149.1	-
1’	37.6	3.17 (td, 1H, 12.1, 4.19) 2.42 (td, 1H, 12.5, 5.18)
2’	32.3	1.20-1.30 (m, 1H) 1.75-1.86 (m, 2H)
3’	28.2	1.36-1.46 (m, 2H) 1.46-1.56 (m, 4H)
4’	24.5	1.36-1.46 (m, 2H) 1.56-1.66 (m, 3H)
5’	32.6	1.30-1.35 (m, 4H) 1.66-1.72 (m, 1H)
6’	69.2	3.75 (m, 2H)
7’	36.6	1.46-1.56 (m, 4H) 1.56-1.66 (m, 3H)
8’	22.7	1.46-1.56 (m, 4H)
9’	36.2	1.56-1.66 (m, 3H) 1.74-1.86 (m, 2H)
10’	74.3	5.16 (m, 2H)
11’	21.5	1.33 (d, 3H, 6.17)

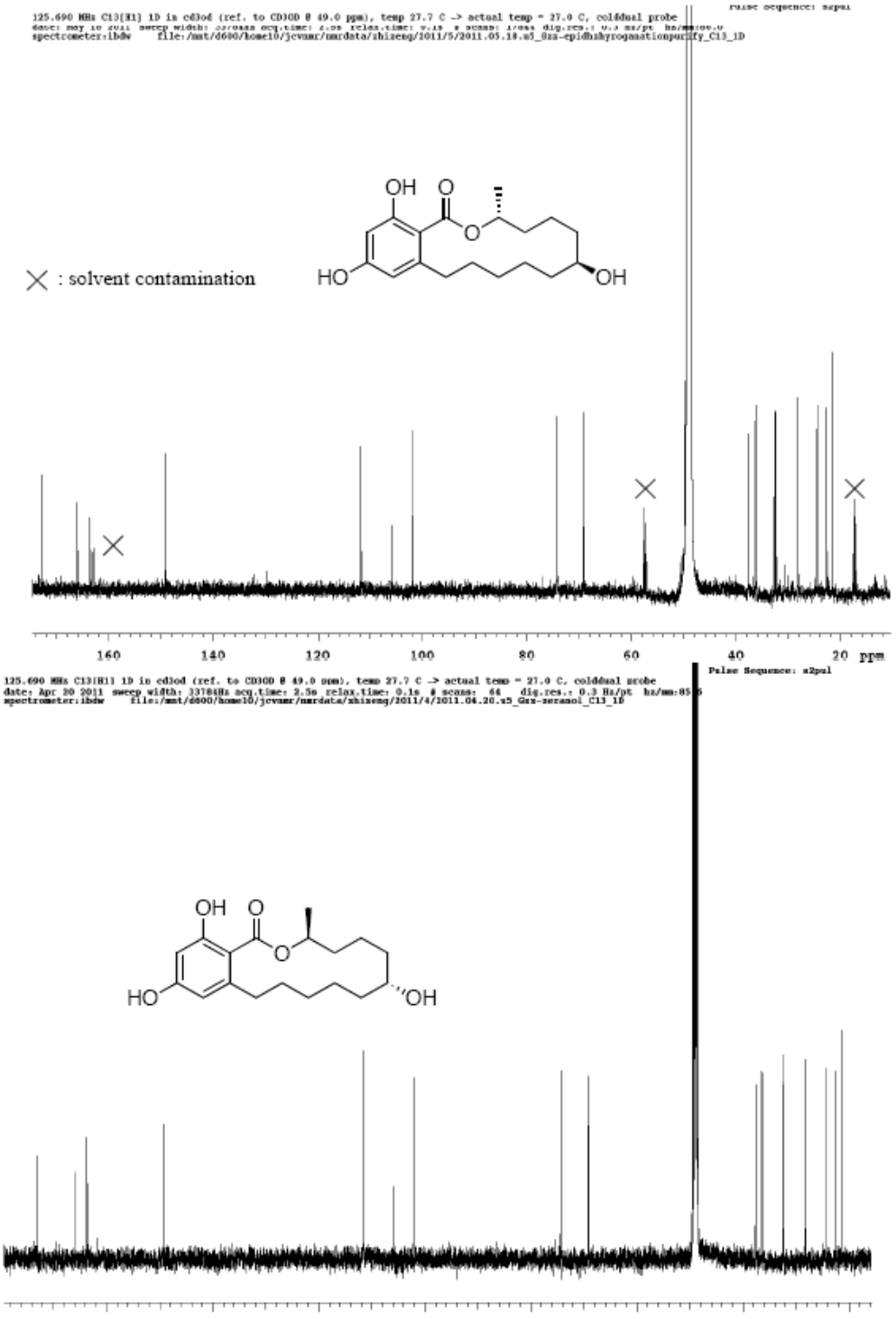
Spectra were obtained at 500 MHz for proton and 125 MHz for carbon and were recorded in CD_3OD.



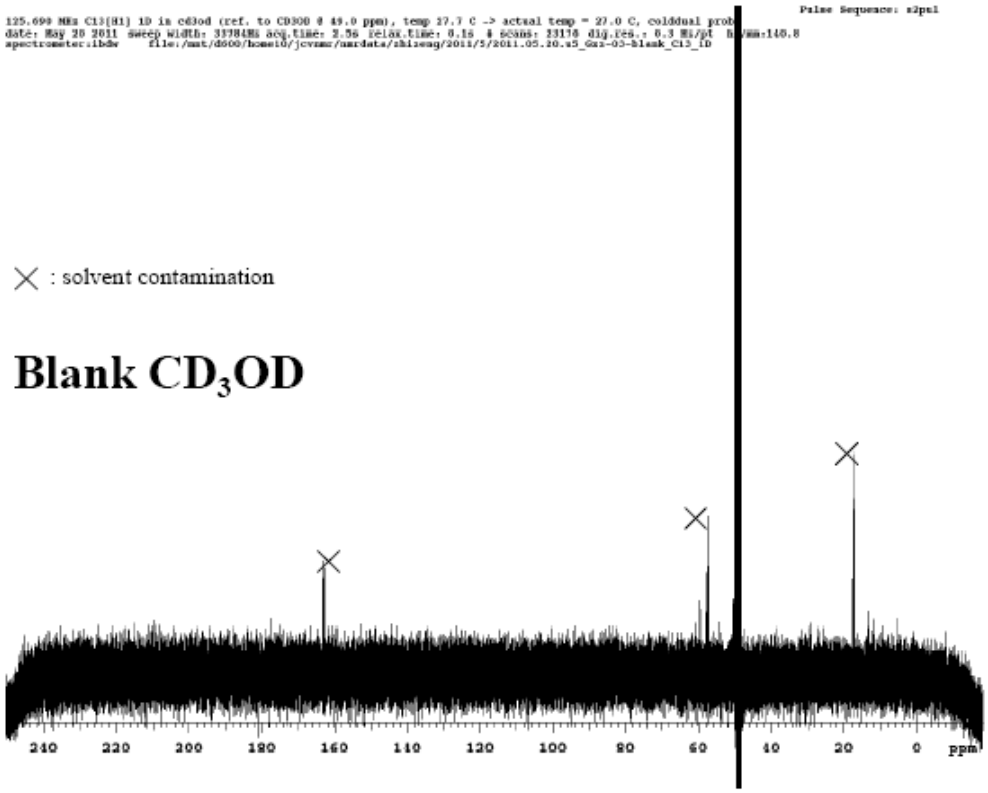
Supplementary Figure 21. The CD spectra of compound **10** (line a, solid) and **11** (line b, dashed). The amount of each compound is 0.67 mg in 1 ml methanol (0.2 mm cell and 5 scans).



Supplementary Figure 22. Proton NMR spectra for **10** and **11**.



Supplementary Figure 23. Carbon NMR spectra for **10** (uppper) and **11** (bottom). The extra carbon signals in the upper spectrum for **10** have been noted compared to Figure 24.



Supplementary Figure 24. The carbon NMR spectrum for solvent CD_3OD.

References

1. Zhou, H., Qiao, K.J., Gao, Z.Z., Vederas, J.C. & Tang, Y. Insights into radicicol biosynthesis via heterologous synthesis of intermediates and analogs. *J. Biol. Chem.* **285**, 41412-41421 (2010).
2. Zuckerkandl, E. & Pauling, L. *Evolutionary divergence and convergence in proteins*, (Academic Press, New York, 1965).
3. Ma, S.M. et al. Complete reconstitution of a highly reducing iterative polyketide synthase. *Science* **326**, 589-592 (2009).
4. Lee, K.K., Da Silva, N.A. & Kealey, J.T. Determination of the extent of phosphopantetheinylation of polyketide synthases expressed in Escherichia coli and Saccharomyces cerevisiae. *Anal. Biochem.* **394**, 75-80 (2009).
5. Mootz, H.D., Schorgendorfer, K. & Marahiel, M.A. Functional characterization of 4 '-phosphopantetheinyl transferase genes of bacterial and fungal origin by complementation of Saccharomyces cerevisiae lys5. *Fems Microbiol. Lett.* **213**, 51-57 (2002).
6. Zhou, H. et al. Enzymatic synthesis of resorcylic acid Lactones by cooperation of fungal iterative polyketide synthases involved in hypothemycin biosynthesis. *J. Am. Chem. Soc.* **132**, 4530-4531 (2010).
7. Soding, J., Biegert, A. & Lupas, A.N. The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res.* **33**, W244-W248 (2005).
8. Keatinge-Clay, A.T. & Stroud, R.M. The structure of a ketoreductase determines the organization of the beta-carbon processing enzymes of modular polyketide synthases. *Structure* **14**, 737-748 (2006).
9. Larkin, M.A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948 (2007).
10. Javidpour, P., Korman, T.P., Shakya, G. & Tsai, S.C. Structural and biochemical analyses of regio- and stereospecificities observed in a type II polyketide ketoreductase. *Biochemistry* **50**, 4638-4649 (2011).
11. McDaniel, R., Ebert-Khosla, S., Fu, H., Hopwood, D.A. & Khosla, C. Engineered biosynthesis of novel polyketides: influence of a downstream enzyme on the catalytic specificity of a minimal aromatic polyketide synthase. *Proc. Natl. Acad. Sci. U S A* **91**, 11542-11546 (1994).
12. Tamura, K. et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **doi: 10.1093/molbev/msr121**(2011).
13. Rzhetsky, A. & Nei, M. A simple method for estimating and testing minimum-evolution trees. *Mol. Biol. Evol.* **9**, 945-967 (1992).
14. Jones, D.T., Taylor, W.R. & Thornton, J.M. The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* **8**, 275-282 (1992).
15. Perrin, D.D. & Armarego, W.L.F. *Purification of laboratory chemicals, 3rd Edition Ed.*, (Pergamon Press, 1988).
16. Gilbert, I.H. et al. Synthesis of β-keto and α,β-unsaturated N-acetylcysteamine thioesters. *Bioorg. Med. Chem. Lett.* **5**, 1587-1590 (1995).

17. Islam, M.S., Ishigami, K. & Watanabe, H. Synthesis of (-)-mellein, (+)-ramulosin, and related natural products. *Tetrahedron* **63**, 1074-1079 (2007).
18. Less, S.L. et al. Biosynthesis of tetronasin .6. Preparation of structural analogues of the diketide and triketide biosynthetic precursors to tetronasin. *Tetrahedron Lett.* **37**, 3515-3518 (1996).