Supplementary information

SurE is a *trans*-acting thioesterase cyclizing two distinct non-ribosomal peptides

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General material and methods

^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA 500 (500 MHz for ^{1}H NMR) spectrometer. Chemical shifts are denoted in d (ppm) relative to residual solvent peaks as internal standard (DMSO-d_6, δ_H 2.50, δ_C 39.5). ESI-MS spectra were recorded on a Thermo Scientific Exactive mass spectrometer. Optical rotations were recorded on a JASCO P-1030 polarimeter. LC-MS experiments were performed with amaZon SL-NPC (Bruker Daltonics) coupled with a SHIMADZU HPLC system equipped with a LC-20AD intelligent pump. All reagents were used as purchased from Wako Pure Chemical Industries (Osaka, Japan), Kanto Chemicals (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan) unless otherwise noted.

Deletion of *surE* gene

E. coli DH5α was used as a host for general cloning. The approx. 2 kb upstream and downstream region *surE* gene were amplified by PCR using genomic DNA of *Streptomyces albidoflavus* NBRC12854 as template and cloned into pUC19 vector. A set of primers used to amplify the upstream region are as follow; forward: 5'-cgcgaattcaagcttCCTACTCGATCTCGACAG-3' (underlined characters indicate *Eco*RI site), reverse: 5'- tgctctagaCACTGCGTCCCCTGCGCC-3' (underlined characters indicate *Xba*I site). A set of primers used to amplify the downstream region are as follow; forward: 5'-tgctctagaCGTGCGCCATCAGTTTCG-3' (underlined characters indicate *Xba*I site), reverse: 5'- cccaagcttCCAGGAACCAGAGCCGTTC-3'. The cloned fragments were sequenced for validation. The neomycin resistance marker (*aphII*) flanking *loxP* sequences was amplified by PCR with pKU479 (ref.1) as a template using a set of primers, forward: 5'-tgctctagaCGGCCAGTGAATTCGAGCGACTCGAGT-3' (underlined characters indicate *Xba*I site), reverse: 5'- cccaagctttctagaCCGGGTACCGAGCGAACGCGTT-3' (underlined characters indicate *Xba*I site). These three fragments were integrated to generate a disruption cassette; upstream-*aphII*-downstream. The cassette was inserted into *Hin*dIII site of pGM160Δ*aac1::oriT::pheS(A339G/T278S)::sacB::aph* to yield disruption plasmid, pGM160-Δ*surE*. pGM160Δ*aac1::oriT::pheS(A339G/T278S)::sacB::aph* is a derivative of pGM160*Δaac1::oriT::sacB::aph* (ref.1) equipped with *pheS* (A339G/T278S) gene for counter-selection by 4-chloro-DL-phenylalanine (ref.2). The resultant disruption plasmid pGM160-Δ*surE* was introduced into *E. coli* GM2929 *hsdS::*Tn*10*/pUB307::Tn*7* (ref.1) by electroporation. A 20-fold dilution of overnight culture of *E. coli* strain containing disruption cassette was mixed with spore suspension of *S. albidoflavus* NBRC12854 and spread onto M4-Mg media (10 g of soluble starch, 1.0 g of K_2HPO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NaCl, 2.0 g of $(NH_4)_2SO_4$, 2.0 g of $CaCO_3$, 1 ml of trace metal sol. (1.0 g of $FeSO_4 \cdot 7H_2O$, 1,0 g of $MnCl_2 \cdot 4H_2O$, 1.0 g of $ZnSO_4 \cdot 7H_2O$ in 1 liter of deionized water), 15 g of agar in 1 liter of deionized water, pH was adjusted to 7.2, then autoclaved at 110 °C for 10 min) supplemented with 20 mM $MgCl_2$. After incubation at 30 °C for 18 hours, 1 mL of sterile

water containing 25 μg/ml of neomycin and 2000 μg/ml of aztreonam (TCI chemicals, Tokyo, Japan) were overlaid and further incubated for additional 5 days. Exoconjugants were transferred onto TSB plate containing 25 μg/ml of kanamycin, 12.5 μg/ml of nalidixic acid and 10 mM 4-chloro-DL-phenylalanine (Ark Pharm Inc.). The disruption of *surE* gene was confirmed based on PCR amplification using following primers; forward: 5'-GTGGTGCTCGCCCTCGGC-3'; reverse: 5'-GACCACCTCCGCCGAGCG-3'. The neomycin-resistance marker *aphII* flanking *loxP* sequences on the deletion region in the genome was removed by the expression of Cre recombinase encoded in pKU471 (ref.3). 20-fold dilution of overnight culture of *E. coli* GM2929 *hsdS::*Tn*10*/pUB307::Tn*7* harboring pKU471 was mixed with spore suspension of the disruption mutant and spread onto M4-Mg media supplemented with 20 mM $MgCl_2$. After incubation at 30 °C for 18 hours, 1 mL of sterile water containing 25 μg/ml of thiostrepton and 2000 μg/ml of aztreonam were overlaid and further incubated for additional 5 days. Exoconjugants were transferred to TSB plate containing 12.5 μg/ml of nalidixic acid and 2 w/v% of D-xylose (for induction of *cre* gene). The desired Δ*surE* deletion mutants were selected by the neomycin-sensitive phenotypes and removal of resistance marker was confirmed by PCR amplification using the same primers that were used to check the insertion of neomycin resistance marker.

Procedure for solid-phase peptide synthesis (SPPS)

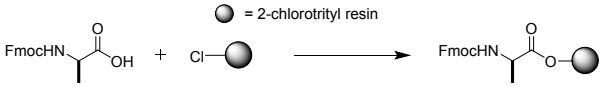
Step 1: Fmoc group of the solid supported peptide was removed by using 20% piperidine/DMF solution (10 min, room temperature).

Step 2: The resin in the reaction vessel was washed with DMF (x 3) and CH_2Cl_2 (x 3).

Step 3: To the solution of carboxylic acid (4 eq) were added DIC (4 eq, 0.50 M in NMP) and Oxyma (4 eq, 0.50 M in DMF). After 2-3 min of pre-activation, the mixture was injected to the reaction vessel. The resulting mixture was stirred for 30 min at 37 °C.

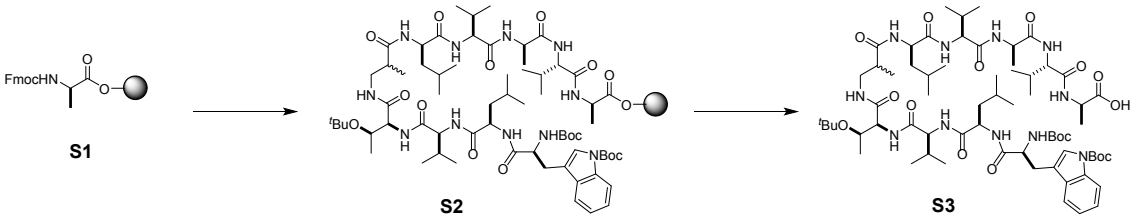
Step 4: The resin in the reaction vessel was washed with DMF (x 3) and CH_2Cl_2 (x 3). Amino acids were condensed onto the solid support by repeating Steps 1–4.

Synthesis of surugamide F-SNAC (7a) and *epi*-surugamide F-SNAC (7b)

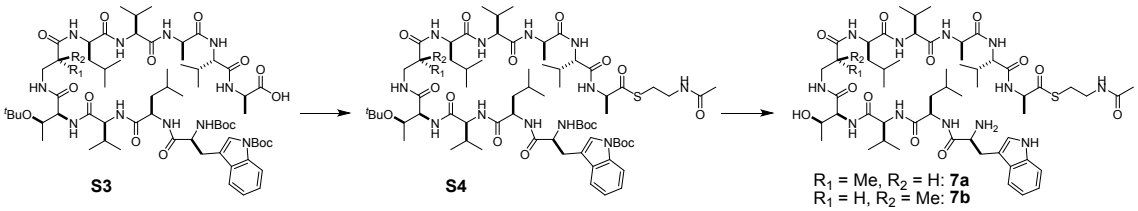


S1

Fmoc-D-Ala-2-chlrotrityl resin (**S1**): 2-Chlorotrityl resin (37.5 mg, 0.05 mmol), in Libra tube was swollen with CH_2Cl_2, and excess solvent was removed by filtration. To the resin were added a solution of Fmoc-D-Ala-OH (15.6 mg, 0.05 mmol) and *i*-Pr_2NEt (26.1 μL, 0.15 mmol) in CH_2Cl_2 (0.5 mL), and stirred for 1 hour. The reaction mixture was filtered, washed with DMF (x 3), CH_2Cl_2 (x 3).



Peptide **S3**: Fmoc-D-Ala-2-chlorotrityl (**S1**) resin in Libra tube was swelled in CH_2Cl_2 for 1 hour, which was subjected to 9 cycles [Fmoc-L-Val-OH, Fmoc-D-Ala-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, Fmoc-DL-AMPA-OH, Fmoc-L-Thr(*t*Bu)-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, N^a-Boc-N^{in}-Boc-L-Trp-OH] of the SPPS protocol (step 1-4) to afford mixture of resin-bound peptides **S2**. To peptides **S2** was added $CH_2Cl_2/(CF_3)_2CHOH$ (= 70 : 30) (0.5 mL), being stirred for 20 min, and then reaction mixture was filtered. This procedure was repeated twice. The filtrate was azeotropically dried with toluene (x 3) to afford crude mixture of peptides **S3**, which was used in the next reaction without further purification.



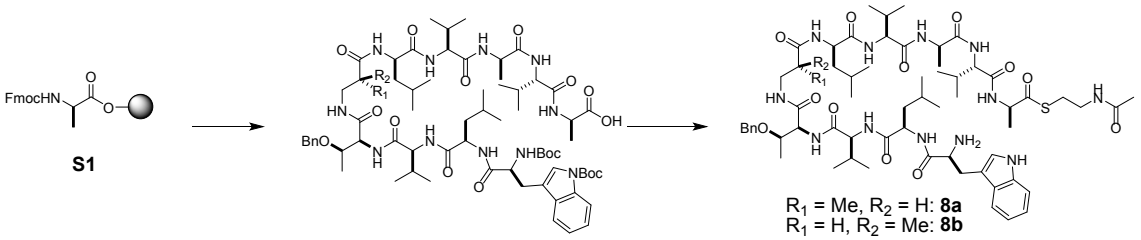
surugamide F-SNAC (**7a**) and its epimer **7b**: To a solution of mixture of peptide **S3** in CH_2Cl_2/DMF (= 9 : 1) (12.5 mL) were added *N*-acetylcysteamine (12 mg, 0.1 mmol), 2,4,6-collidine (6.6 μL, 0.05 mmol), HCTU (22.8 mg, 0.055 mmol). After being stirred overnight, the reaction mixture was concentrated, and the residue was diluted with EtOAc and saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc (x 3), washed with brine, dried over $MgSO_4$, and concentrated. To the residue was added a mixture of TFA/H_2O/iPr$_3$SiH (= 95 : 2.5 : 2.5) (1.0 mL), and the mixture was stirred for 30 min. The reaction mixture was diluted with Et_2O (24 mL), centrifuged at 3,500 x *g* for 15 min at 4 °C, and Et_2O layer was removed by decantation. This procedure was repeated twice. The crude peptides were separated by reverse-phase HPLC to afford **7a** (1.7 mg, 2.9% for 22 steps) and its epimer **7b** (1.5 mg, 2.6% for 22 steps) as a white solid. The separation was accomplished by COSMOSIL 5C_{18}-MS-II 10ID x 250mm (nacalai tesque) eluted with 42% acetonitrile + 0.05% TFA (flow rate: 3.2 mL/min).

7a: $[\alpha]_D^{25}$ - 1.28 (*c* 0.25 MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): see Figure S4, δ 10.99 (s, 1H), δ 8.73 (d, 1H, *J* = 8.5 Hz), δ 8.46 (d, 1H, *J* = 7.5 Hz), δ 8.27 (d, 1H, *J* = 8.5 Hz), δ 8.02-7.95 (m, 7H), δ

7.84 (d, 1H, *J* = 5.0 Hz), δ 7.76 (d, 1H, *J* = 8.5 Hz), δ 7.71-7.69 (m, 2H), δ 7.33 (d, 1H, *J* = 8.0 Hz), δ 7.18 (s, 1H), δ 7.06 (t, 1H, *J* = 7.5 Hz), δ 6.98 (t, 1H, *J* = 7.5 Hz), δ 4.82 (d, 1H, *J* = 4.0 Hz), δ 4.55 (d, 1H, *J* = 7.0 Hz), δ 4.38-4.04 (m, 7H), δ 3.88 (d, 1H, *J* = 5.0 Hz), δ 3.32 (s, 6H), δ 3.19 (dd, 1H, *J* = 14.0, 5.5 Hz), δ 3.11-3.08 (m, 4H), δ 3.00 (dd, 1H, *J* = 14.5, 8.5 Hz), δ 1.99 (m, 3H), δ 1.74 (s, 3H), δ 1.53 (m, 1H), δ 1.43-1.41 (m, 2H), δ 1.30 (m, 2H), δ 1.23 (d, 3H, *J* = 7.5 Hz), δ 1.20 (d, 3H, *J* = 6.5 Hz), δ 0.95 (dd, 6H, *J* = 18, 6.5 Hz), δ 0.85-0.71 (m, 34H); ^{13}C NMR (125 MHz, DMSO-d_6): see Figure S5, δ 201.7, 174.9, 173.0, 172.7, 172.1, 171.6, 171.4, 171.3, 170.6, 169.8, 168.8, 136.8, 127.5, 125.5, 121.7, 119.1, 118.9, 112.0, 107.3, 67.0, 59.0, 58.1, 58.0, 57.8, 55.3, 53.2, 51.5, 49.1, 42.3, 42.0, 41.2, 41.0, 38.7, 31.1, 30.8, 28.2, 24.8, 24.6, 23.5, 23.4, 23.0, 22.2, 22.0, 20.5, 19.8, 19.7, 19.0, 18.5, 18.3, 17.9, 16.0 ; HRMS (ESI) calcd for $C_{56}H_{93}O_{12}N_{12}S$ $[M+H]^+$ 1157.6751, found 1157.6771.

7b: $[\alpha]_D^{22.5}$ + 0.94 (*c* 0.35 MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): see Figure S6, δ 10.97 (s, 1H), δ 8.70 (m, 1H), δ 8.43 (m, 1H), δ 8.26 (m, 1H), δ 8.04-7.98 (m, 6H), δ 7.81-7.67 (m, 5H), δ 7.30-7.18 (m, 14H), δ 7.06 (m, 1H), δ 6.98 (m, 1H), δ 4.98 (s, 1H), δ 4.81 (s, 1H), δ 4.53 (m, 1H), δ 4.33-4.04 (m, 10H), δ 3.84 (m, 1H), δ 3.48 (d, 2H, *J* = 15.0 Hz), δ 3.08 (m, 1H), δ 2.99 (m, 2H), δ 2.81 (m, 2H), δ 1.98 (m, 3H), δ 1.74 (s, 3H), δ 1.52 (m, 1H), δ 1.30-1.15 (m, 13H), δ 0.94 (m, 6H), δ 0.84-0.72 (m, 34H); ^{13}C NMR (125 MHz, DMSO-d_6): see Figure S7, δ 201.7, 174.7, 172.8, 172.8, 172.1, 171.6, 171.4, 171.3, 171.1, 170.5, 169.8, 168.8, 144.6, 137.5, 136.8, 129.6, 128.9, 128.7, 128.3, 128.2, 127.5, 127.4, 125.5, 121.7, 119.1, 118.9, 112.0, 107.3, 70.3, 67.0, 66.8, 65.9, 59.0, 58.1, 57.9, 57.8, 55.4, 53.2, 52.6, 52.1, 52.0, 51.6, 50.2, 49.0, 42.3, 41.2, 40.9, 38.7, 33.1, 31.1, 30.8, 29.5, 28.2, 28.1, 24.8, 24.6, 23.5, 23.3, 23.0, 22.4, 22.0, 20.4, 19.8, 19.7, 19.7, 19.0, 18.6, 18.5, 18.3, 17.9, 15.9; HRMS (ESI) calcd for $C_{56}H_{93}O_{12}N_{12}S$ $[M+H]^+$ 1157.6751, found 1157.6776.

Synthesis of *O*-benzyl surugamide F-SNAC (8a) and its epimer (8b)



<u>*O*-benzyl surugamide F-SNAC (**8a**) and its epimer **8b**</u>: **8a** and **8b** were synthesized following the same procedure as for **7a** and **7b** with the substitution of Fmoc-L-Thr(*t*Bu)-OH for Fmoc-L-Thr(Bn)-OH. The separation was accomplished by COSMOSIL 5C_{18}-MS-II 10ID x 250 mm eluted with 46% acetonitrile + 0.05% TFA (flow rate: 3.2 mL/min). The pure **8a** (2.5 mg, 4.0 % for 22 steps) and **8b**

(4.8 mg, 7.7 % for 22 steps) were obtained as white solids.

8a: $[\alpha]_D^{23}$ + 0.39 (*c* 0.16, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): see Figure S8, δ 10.97 (s, 1H), δ 8.70 (br, 1H), δ 8.45 (d, 1H, *J* = 7.0 Hz), δ 8.22 (d, 1H, *J* = 8.5 Hz), δ 8.02-7.98 (m, 2H), δ 7.92 (m, 1H), δ 7.82 (d, 1H, *J* = 9.0 Hz), δ 7.75 (m, 1H), δ 7.70 (d, 1H, *J* = 7.0 Hz), δ 7.34-7.22 (m, 6H), δ 7.17 (s, 1H), δ 7.06 (t, 1H, *J* = 7.5 Hz), δ 6.98 (t, 1H, *J* = 8.0 Hz), δ 4.54 (m, 1H), δ 4.48 (d, 1H, *J* = 12.0 Hz), δ 4.41 (d, 1H, *J* = 11.5 Hz), δ 4.35-4.29 (m, 5H), δ 4.21-4.13 (m, 2H), δ 4.06-4.01 (m, 2H), δ 3.82 (m, 1H), δ 3.21-2.96 (m, 6H), δ 2.81 (t, 1H, *J* = 7.0 Hz), δ 1.99 (m, 3H), δ 1.74 (s, 3H), δ 8.27 (d, 1H, *J* = 8.5 Hz), δ 1.53 (m, 1H), δ 1.42 (m, 1H), δ 1.30 (m, 2H), δ 1.23 (d, 3H, *J* = 7.0 Hz), δ 1.19 (d, 3H, *J* = 5.5 Hz), δ 1.03 (d, 3H, *J* = 5.5 Hz), δ 0.92 (d, 3H, *J* = 7.0 Hz), δ 0.84 (d, 3H, *J* = 6.5 Hz), δ 0.80-0.78 (m, 33H),; ^{13}C NMR (125 MHz, DMSO-d_6): see Figure S9, δ 201.7, 174.8, 173.0, 172.8, 172.0, 171.7, 171.6, 171.4, 170.2, 169.8, 139.1, 136.9, 128.7, 128.1, 127.9, 127.5, 125.5, 121.7, 119.1, 118.9, 112.0, 75.2, 70.8, 58.0, 57.8, 57.4, 55.4, 53.2, 52.0, 51.6, 49.1, 49.0, 42.4, 42.1, 41.3, 41.0, 38.7, 31.1, 30.8, 28.1, 24.8, 24.6, 23.5, 23.3, 23.0, 22.2, 22.0, 19.8, 19.7, 19.0, 18.5, 18.3, 17.9, 16.8, 16.1; HRMS (ESI) calcd for $C_{63}H_{99}O_{12}N_{12}S$ $[M+H]^+$ 1247.7221, found 1247.7252.

8b: $[\alpha]_D^{23}$ + 7.75 (*c* 0.23, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): see Figure S10, δ 10.98 (s, 1H), δ 8.69 (br, 1H), δ 8.44 (d, 1H, *J* = 7.0 Hz), δ 8.22 (d, 1H, *J* = 8.5 Hz), δ 8.05-7.77 (m, 9H), δ 7.70 (d, 1H, *J* = 8.0 Hz), δ 7.06 (t, 1H, *J* = 8.0 Hz), δ 6.98 (t, 1H, *J* = 6.5 Hz), δ 8.44 (d, 1H, *J* = 7.0 Hz), δ 4.53-4.41 (m, 3H), δ 4.36-4.26 (m, 5H), δ 4.21-4.12 (m, 2H), δ 4.06 (dd, 1H, *J* = 10.5, 5.5 Hz), δ 4.02 (m, 1H), δ 3.78 (m, 1H), δ 3.19 (m, 1H), δ 3.13 (d, 4H, *J* = 5.5 Hz), δ 3.09 (dd, 1H, *J* = 12.5, 5.5 Hz), δ 2.99 (m, 2H), δ 2.81 (t, 2H, *J* = 6.5 Hz), δ 1.98 (m, 3H), δ 2.85 (s, 3H), δ 1.52 (m, 1H), δ 1.40 (m, 2H), δ 1.30 (m, 2H), δ 1.23 (d, 3H, *J* = 7.0 Hz), δ 1.20 (d, 3H, *J* = 7.0 Hz), δ 0.92 (d, 3H, *J* = 6.0 Hz), δ 0.84 (d, 3H, *J* = 6.5 Hz), δ 0.82-0.71 (m, 35H),; ^{13}C NMR (125 MHz, DMSO-d_6): see Figure S11, δ 201.7, 174.7, 172.8, 172.8, 172.1, 171.6, 171.3, 170.1, 169.8, 139.1, 136.9, 128.7, 128.0, 127.9, 12 7.5, 125.5, 121.7, 119.1, 118.9, 112.0, 75.2, 70.8, 58.1, 57.9, 57.3, 55.4, 53.2, 52.1, 51.6, 49.1, 49.0, 42.4, 41.2, 41.0, 38.7, 31.1, 30.9, 28.1, 24.8, 24.6, 23.5, 23.3, 23.0, 22.4, 22.0, 19.8, 19.7, 19.0, 18.5, 18.3, 18.2, 17.9, 16.8, 16.0; HRMS (ESI) calcd for $C_{63}H_{99}O_{12}N_{12}S$ $[M+H]^+$ 1247.7221, found 1247.7234.

Reaction conditions for SurE

The recombinant SurE was expressed in *E. coli* host and purified following the same procedure described in previous report (ref.4). For the cyclization of **7a** and **7b**, the reaction mixtures (100 μL) containing 20 mM Tris-HCl (pH 8.0), 10 μg SurE and substrates (0.3 mM of **7a** or 0.3 mM of **7b**) were prepared. For the cyclization of benzyl substrates **8a** and **8b**, the reaction mixtures (100 μL) containing 20 mM Tris-HCl (pH 8.0), 20 μg SurE and substrates (0.1 mM of **8a** or 0.1 mM of **8b)** were prepared. These samples were incubated at 30 °C for 2 hours and frozen by liquid N_2. The reaction mixtures were dried *in vacuo* and the residues were dissolved into DMSO, then subsequently

analyzed by LC-MS.

Conditions for LC-MS analysis

LC-MS analyses were performed by amaZon SL-NPC operated in positive mode, coupled with a SHIMADZU HPLC system. Separation was accomplished by COSMOSIL Cholester 3.0ID x 150 mm (nacalai tesque). H_2O + 0.05% TFA and acetonitrile + 0.05% TFA were used as mobile phase A and B, respectively. Samples were eluted with the gradient mode: 30 to 70% for mobile phase B in 15 min. Flow rate was 0.5 mL/min.

Chemoenzymatic Synthesis of cyclosurugamide F (10a)

20 μg of SurE was added to a 100 μL solution containing 20 mM Tris-HCl (pH 8.0) and 0.1 mM **8a**. The reaction mixture was incubated at 30 °C for 2 hours and frozen by liquid N_2. This was dried *in vacuo* and the residue was dissolved into 70% AcOH. Removal of benzyl group was accomplished by catalytic hydrogenation using 10% Pd/C under H_2 atmosphere. The resultant mixture was analyzed by LC-MS.

Detection of cyclosurugamide F (10a) in culture broth of *Streptomyces albidoflavus* NBRC12854

Streptomyces albidoflavus NBRC12854 was grown on TSB plate at 30 °C for 2-4 days. A single colony was picked and transferred to 10 mL of TSB liquid media and cultivated at 30 °C for 2 days as pre-culture. The pre-culture was then inoculated to a flask containing 100 mL K media (2.5% soluble starch, 1.5% soybean meal, 0.2% dry yeast, 0.4% $CaCO_3$ and the pH was adjusted to 6.2) and incubated at 30 °C with shaking at 140 rpm. 5 mL volume of broth were withdrawn and cells were harvested by centrifugation at 4,000 rpm and extracted with H_2O/acetone (= 1 : 2). The extract was filtered to remove debris and evaporated to remove solvent. The residue was dissolved into DMSO and subjected to LC-MS analysis.

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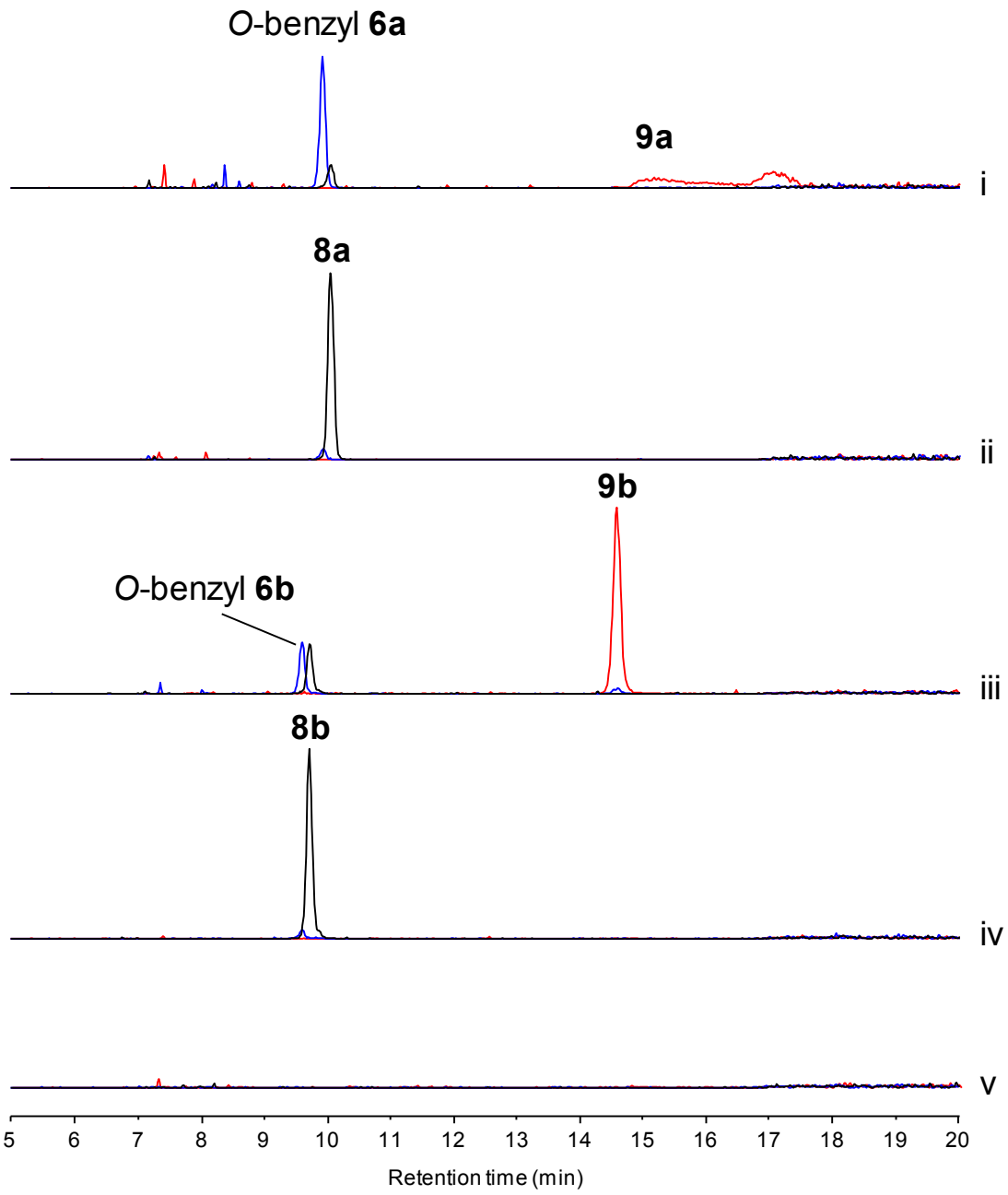

Figure S1: LC-MS analysis of *in vitro* reaction mixture of SurE using **8a** and **8b** as substrates. EICs for **8a** and its epimer **8b** (*m/z* 1247.7, black), EICs for *O*-benzyl **6a** and its epimer **6b** (*m/z* 1146.7, blue) and EICs for **9a** and its epimer **9b** (*m/z* 1128.7, red) are depicted. i: reaction mixture containing SurE and **8a**, ii: reaction mixture with **8a** only; iii: reaction mixture containing SurE and **8b**; iv: reaction mixture with **8b** only; v: reaction mixture with SurE only.

HRMS (ESI) for **9a**: calcd for $C_{59}H_{89}O_{11}N_{11}Na$ $[M+Na]^+$ 1150.6635, found 1150.6651

HRMS (ESI) for **9b**: calcd for $C_{59}H_{89}O_{11}N_{11}Na$ $[M+Na]^+$ 1150.6635, found 1150.6638

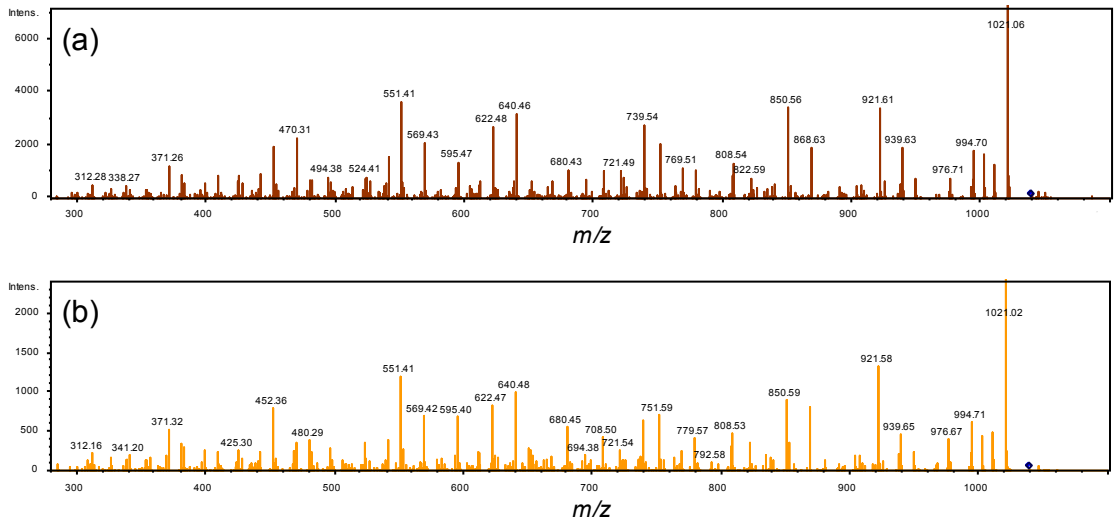

Figure S2: Comparison of MS/MS fragmentation pattern of cyclosurugamide F (**10a**) synthesized *via* two different routes depicted in Scheme 1. (a) MS/MS fragmentation of **10a** synthesized *via* route I, (b) MS/MS fragmentation of **10a** synthesized *via* route II.

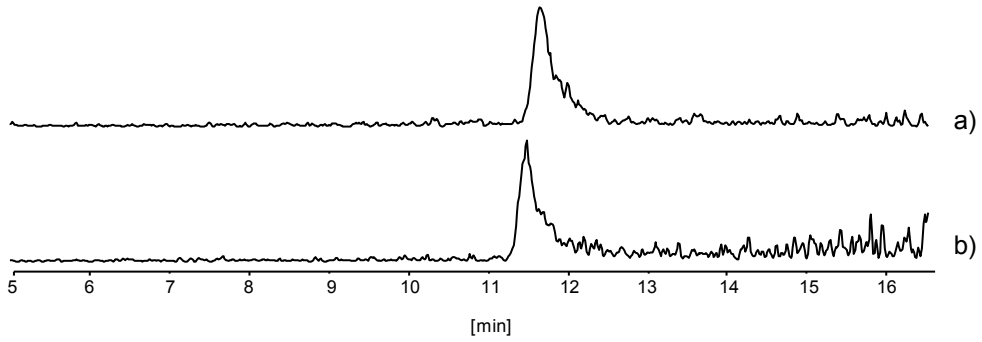

Figure S3: Detection of cyclosurugamide F (**10a**) in culture broth of *S. albidoflavus* NBRC 12854 a) EIC of *m/z* 1038.8 from extracts of *S. albidoflavus* NBRC 12854 grown for two days in K media. b) EIC of *m/z* 1038.8 from *in vitro* reaction mixture of SurE using **7a** as a substrate.

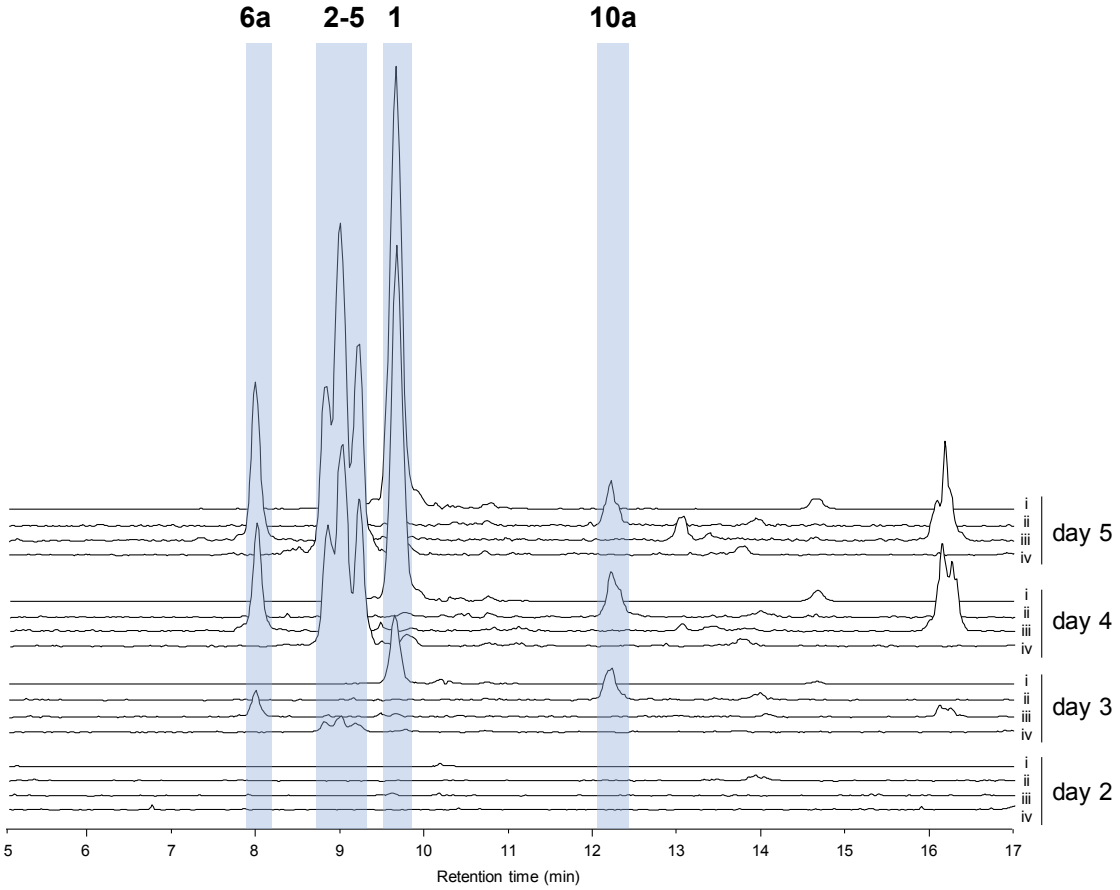

Figure S4: Production of **1**, **2-5**, **6a** and **10a** by *S. albidoflavus* NBRC 12854 from 2 to 5 days of growth. Chromatogram i: EIC of *m/z* 912.8 corresponding to **1**; Chromatogram ii: EIC of *m/z* 1038.8 corresponding to **10a**; Chromatogram iii: EIC of *m/z* 1056.8 corresponding to **6a**, Chromatogram iv: EIC of *m/z* 898.8 corresponding to **2-5**. Days of growth are also described for each chromatogram.

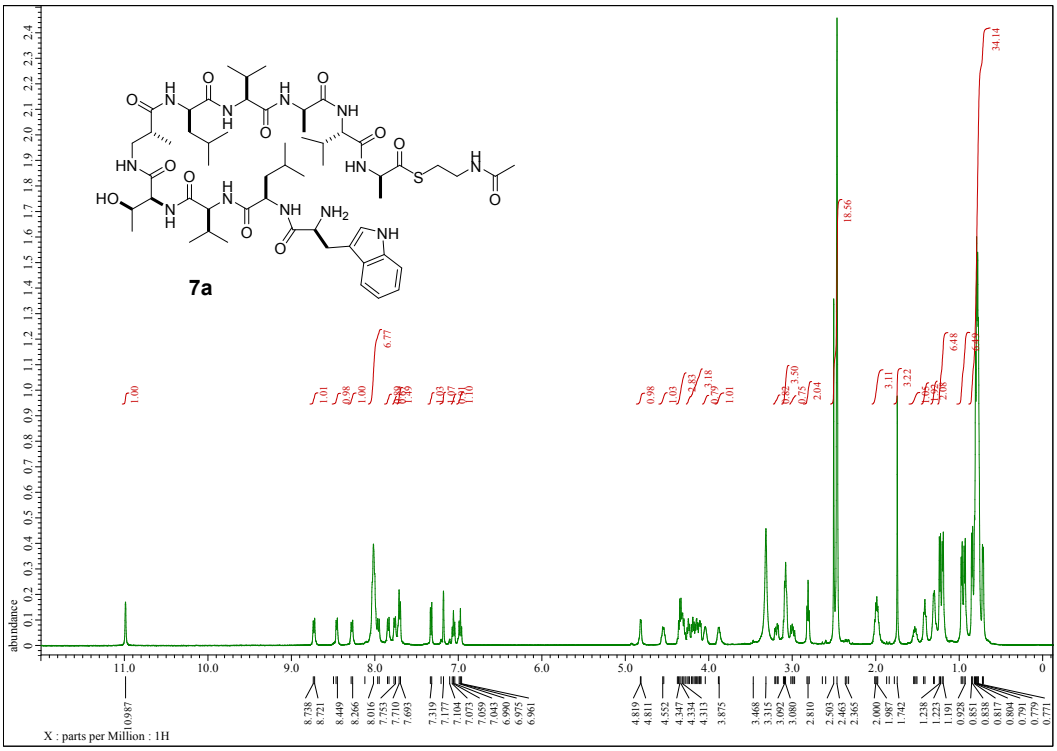

Figure S5: ^{1}H NMR of surugamide F-SNAC (**7a**)

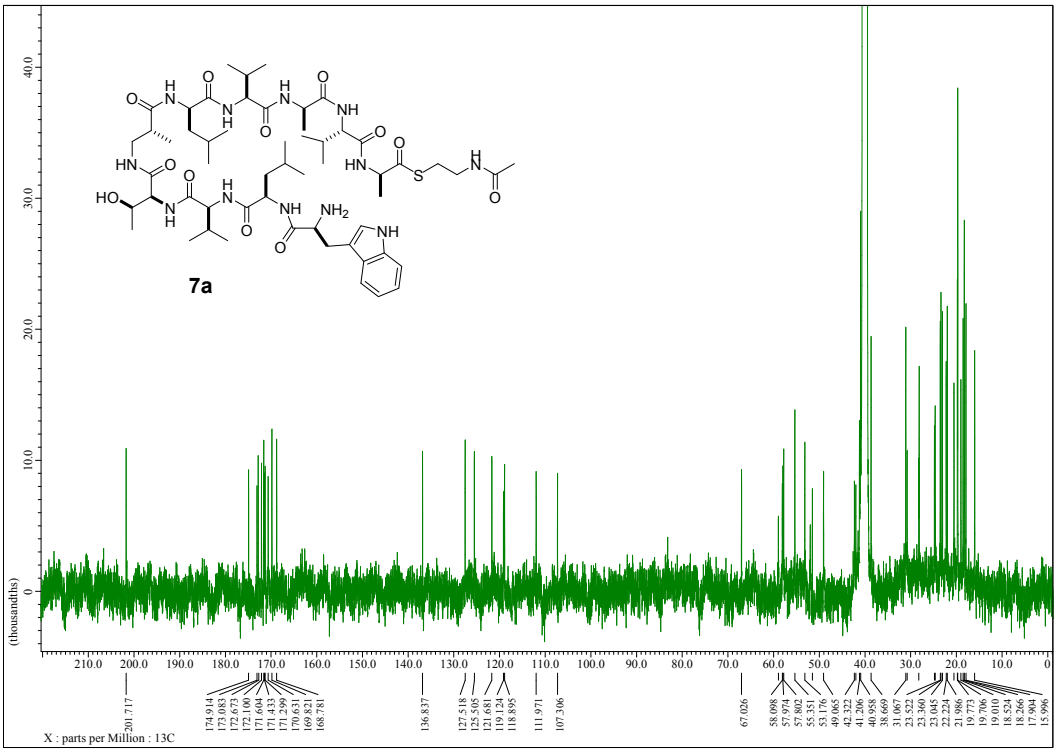

Figure S6: ^{13}C NMR of surugamide F-SNAC (**7a**)

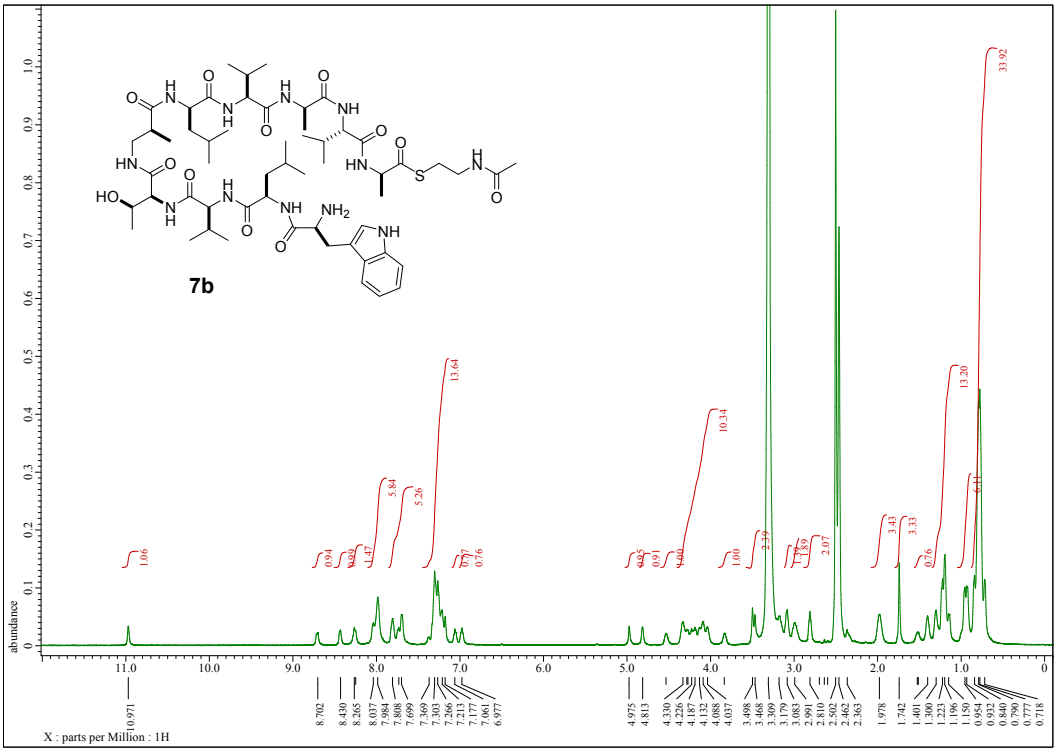

Figure S7: ^{1}H NMR of *epi*-surugamide F-SNAC (**7b**)

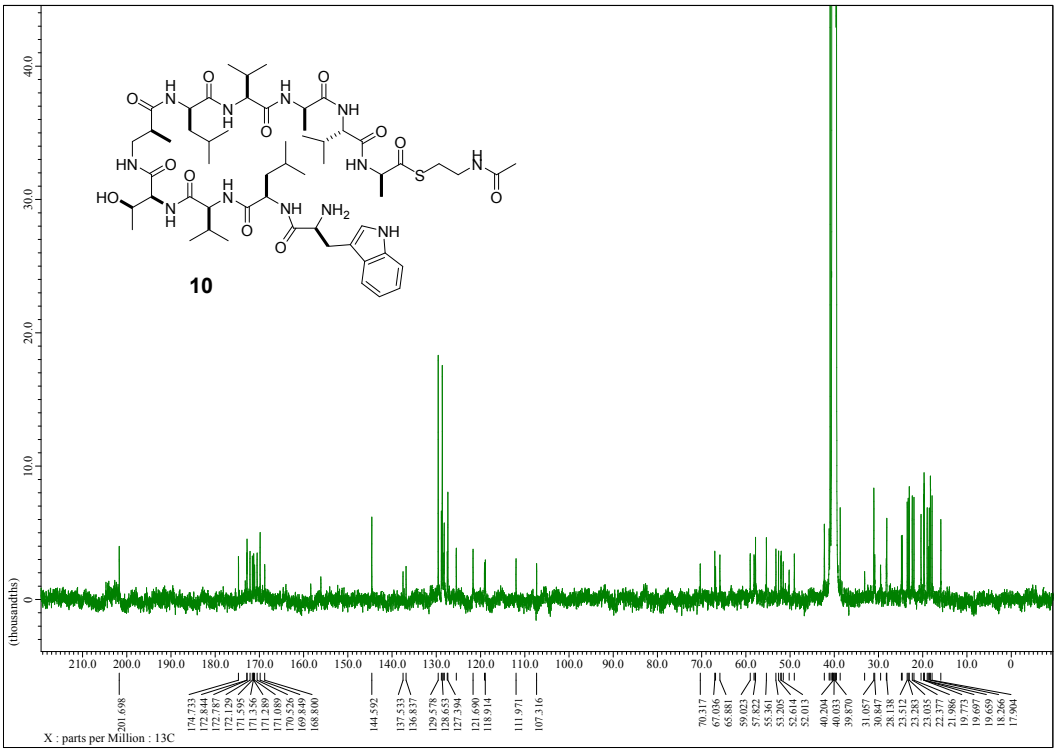

Figure S8: ^{13}C NMR of *epi*-surugamide F-SNAC (**7b**)

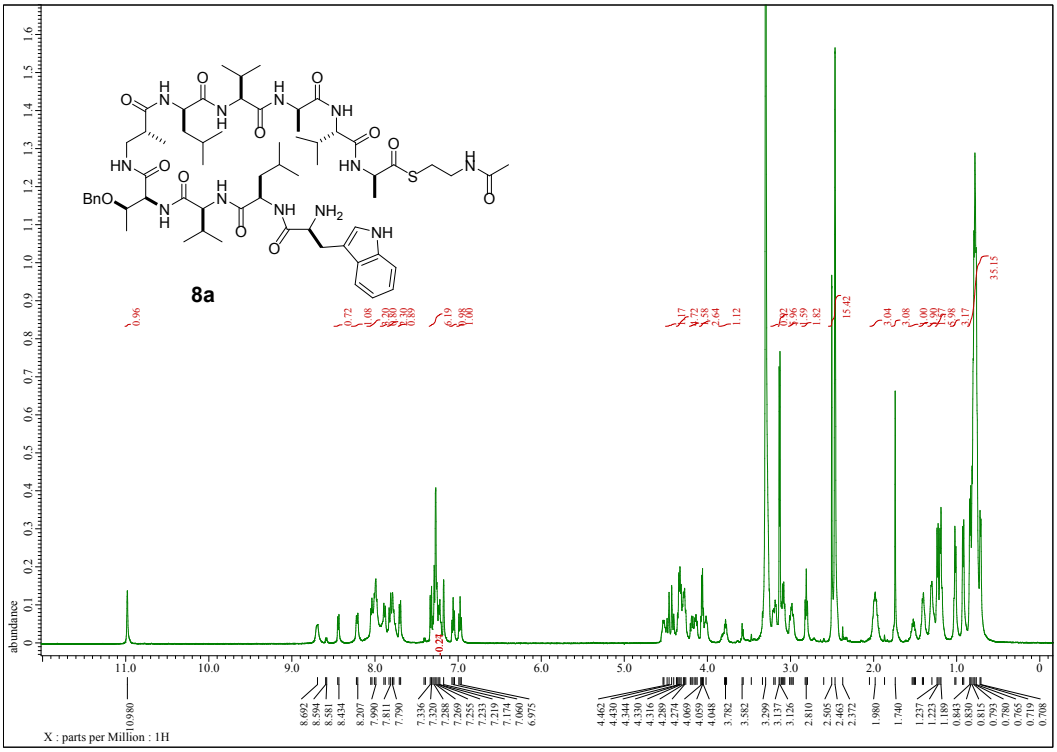

Figure S9: ^{1}H NMR of *O*-benzyl surugamide F-SNAC (**8a**)

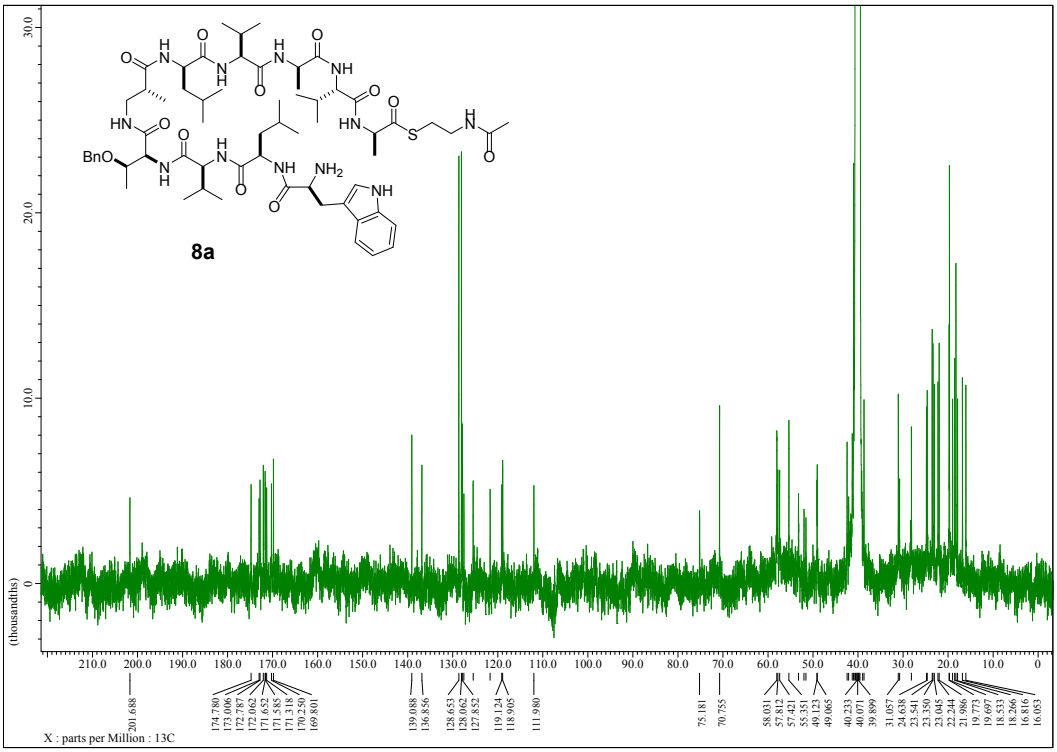

Figure S10: ^{13}C NMR of *O*-benzyl surugamide F-SNAC (**8a**)

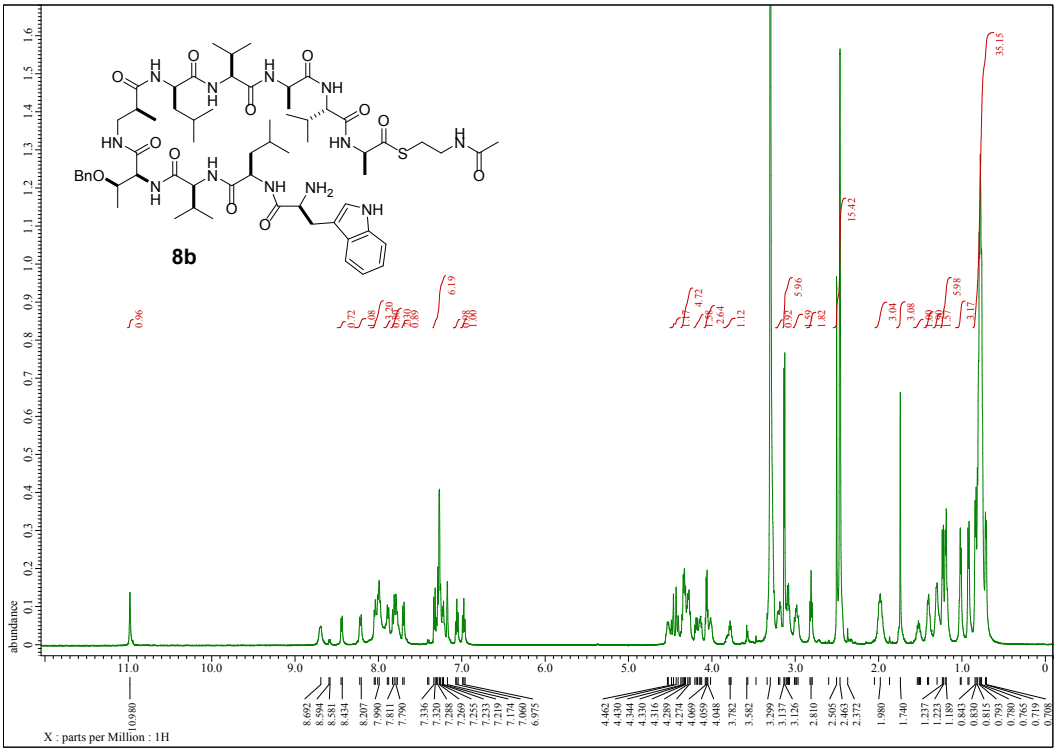

Figure S11: ^{1}H NMR of *O*-benzyl *epi*-surugamide F-SNAC (**8b**)

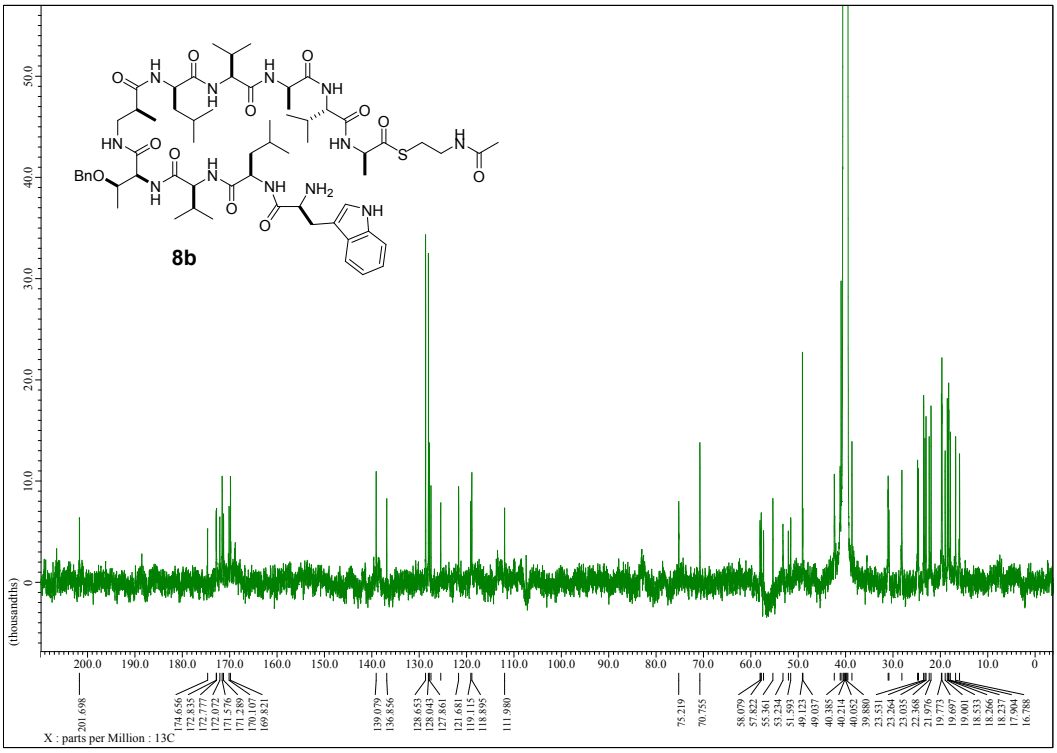

Figure S12: ^{13}C NMR of *O*-benzyl *epi*-surugamide F-SNAC (**8b**)