Design, Synthesis, and Biological Evaluation of Beauveriolide Analogues Bearing Photoreactive Amino Acids

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Beauveriolides I and III, which are naturally occurring cyclodepsipeptides, have been reported to bind to sterol O-acyltransferase (SOAT), inhibiting its ability to synthesize cholesteryl esters. To facilitate an analysis of the binding site(s) of these compounds, we designed beauveriolide analogues 1a–d wherein the Leu or n-allo-Ile residue was replaced by photoreactive amino acids possessing methyldiazirine or trifluoromethyldiazirine in the side chains. The methyldiazirine moiety was installed by reaction of methyl ketones with liquid ammonia to provide imine intermediates, followed by treatment with hydroxylamine-O-sulfonic acid to provide the diaziridines. Subsequent oxidation gave methyldiaziridines. In contrast, trifluoromethyldiaziridine derivatives were prepared from trifluoromethyl ketones via the oxime intermediates, which were transformed into diaziridines. Subsequent oxidation afforded trifluoromethyldiaziridines. The synthesized photoreactive amino acids 3a–d were coupled with 3-hydroxy-4-methyloctanoic acid 4 and dipeptide 5, followed by macro lactamization to provide beauveriolide analogues 1a–d. The SOAT inhibitory activities of 1a–d were found to be as potent as those of beauveriolides I and III. Moreover, 1a–d inhibited SOAT1 selectively rather than SOAT2, which was also consistent with the behavior of beauveriolides I and III.

Key words beauveriolide; sterol O-acyltransferase; photoaffinity labeling; diazirine

Sterol O-acyltransferase (SOAT, also known as acyl-CoA:cholesterol acyltransferase (ACAT)) catalyzes conversion of cholesterol and long-chain fatty-acyl-CoA to cholesteryl esters (CE). Since CE accumulation in plasma is closely related to artery plaque formation, SOAT inhibitors are expected to be therapeutic agents for atherosclerosis. Recent molecular biological studies have revealed the existence of two different SOAT isozymes—SOAT1 and SOAT2—in mammals. SOAT1 is ubiquitously expressed in most tissues and cells, including macrophages, and is related to foam cell formation. On the other hand, SOAT2, which is expressed predominantly in the liver and intestine, is related to food cholesterol absorption and secretion of low-density lipoprotein (LDL). Since SOAT1 and SOAT2 function differently, isozyme selectivity is important for developing SOAT inhibitors. So far, a number of SOAT inhibitors have been reported and their isozyme selectivities have been discussed.

Beauveriolides I and III (Fig. 1A), isolated from a culture broth of Beauveria sp. FO-6979, inhibit lipid droplet formation in mouse peritoneal macrophages. These cyclodepsipeptides were found to inhibit CE synthesis by blocking SOAT activity. Oshiro et al. reported that beauveriolides I and III selectively inhibited SOAT1 in a cell-based assay using two Chinese hamster ovary (CHO) cell lines expressing African green monkey SOAT1 (SOAT1-CHO) and SOAT2 (SOAT2-CHO). However, beauveriolides I and III inhibit SOAT2 as well as SOAT1 in an enzyme assay using the microsomal fraction prepared from SOAT1-CHO or SOAT2-CHO. SOAT1 and SOAT2 share extensive homology, but the topology of the protein domains is thought to be different. These data implied that beauveriolides would bind to a homology domain in SOAT1 and SOAT2, but also that the environment wherein the binding site is located might be different for SOAT1 and SOAT2, e.g., inside or outside of the cell membrane. We have accomplished the total synthesis of beauveriolide III and determined the absolute configuration of the (3S,4S)-3-hydroxy-4-methyloctanoic acid (HMA) moiety. Our intensive structure–activity relationship (SAR) study showed that structural modification of beauveriolide could change the selectivity against SOAT1 and SOAT2. Structural modification of beauveriolides might change not only their affinity for each SOAT isozyme but also their distributions in the cell. The mechanism of isozyme selectivity remains unclear because the binding site of beauveriolide to SOAT1 and SOAT2 has not been elucidated by high-resolution (HR) structural analysis due to the structural complexity of SOAT, which is known to be a membrane protein.

Photoaffinity labeling enables direct analysis of a target protein by photo-induced cross-linking between a ligand and its binding protein. Photoaffinity labeling has been utilized to identify the target protein and binding site of low-molecular-weight ligands. Since the analysis of interactions between peptide and protein is expected, many researchers have developed photoreactive amino acids bearing photore-
active groups such as phenylazide, benzophenone, and diazirine in their side chains to probe target molecules and the binding-site structure of bioactive peptides and proteins. Diazirine is frequently used as a useful photoreactive group owing to its practical advantages: relatively high stability under ambient light and various synthetic reaction conditions, quick photolysis under long-wavelength ultraviolet light (350–355 nm), and compact structure with few effects on the chemical properties of the ligand molecules. Suchanek et al. have developed photo-methionine and -leucine, which possess alkyl methyl diazirines in their side chains and exhibit chemical and biological features similar to those of methionine and leucine. Several researchers have reported the successful application of photo-methionine and -leucine in capturing the target molecules of bioactive peptides, and in the structural analysis of a β-hairpin-forming peptide.

It has been reported that photo-excited diazirines are first transformed into carbenes or diazoisomers. As carbenes are highly reactive species with extremely short lifetimes, they can form intermolecular cross-links with the first biomolecule that they encounter. In contrast, diazoisomers of diazirines, which have reduced reactivity and much longer lifetimes, can diffuse from an initial site of generation to other sites in biomolecules. It is a critical problem that photo-excited diazirines, carbenes, and diazoisomers can induce intramolecular 1,2-rearrangements of a hydrogen atom, resulting in the production of alkenes.

Franks and colleagues reported the synthesis of the photoaffinity probe of propofol bearing dimethylene(trifluoromethyl) diazirine. Herein, we have demonstrated the synthesis of beauveriolide analogues bearing a methyldiazirine or trifluoromethyldiazirine moiety in their side chains. Synthetic methods for the preparation of trfifluoromethyl photo-methionine and trifluoromethyl photo-leucine (Fig. 1B) are described, along with those of photo-methionine and -leucine. The photophysical properties of the photoreactive amino acids and the SOAT inhibitory activities of the synthesized analogues were also investigated.

**Results and Discussion**

**Design of Beauveriolide Analogues Bearing Photoreactive Amino Acids**

Our SAR studies of beauveriolides indicated that the HMA moiety is indispensable for SOAT inhibitory activity. The phenylalanine residue was also found to be important for binding activity and SOAT selectivity. Since the L-Ala residue is not critical for this activity, it would not face the target protein and would be left for the incorporation of detecting groups, such as a biotin tag. In contrast, the D-Leu and D-allo-Ile residues in beauveriolides I and III, respectively, are related to the activity, but small modifications of the side chains are allowed while maintaining the activity. Overall, we have designed four kinds of beauveriolide analogues with various photoreactive amino acids in place of the D-Leu and D-allo-Ile residues (1a–d) (Fig. 1A).

**Retrosynthetic Analysis of 1a–d**

The synthesis of 1a–d can be performed in a manner similar to that used in our total synthesis of beauveriolide III (Chart 1). In principle, 1a–d can be synthesized by macro lactamization of the linear compound 2, which can be obtained by sequential couplings of 3–5.

**Synthesis of 3a–d**

First, we conducted the synthesis of the photoreactive amino acids 3a–d. Vila-Perelló et al. reported an efficient synthesis of L-pho-to-methionine from a L-glutamic acid derivative.
acid derivative.\textsuperscript{32} We followed their synthetic route for the preparation of tert-butoxycarbonyl (Boc)-\textgamma-photo-methionine (3a) (Chart 2). Briefly, Boc-\textgamma-glutamic acid \textalpha-tert-butyl ester (6a) (the preparation method for this compound is summarized in Chart S1) was transformed into the corresponding \textepsilon-Weinreb amide 7a, which was then converted into methyl ketone 8a by treatment with a methyl Grignard reagent. The methyldiazirine moiety was then installed by reaction of the ketone with liquid ammonia, followed by treatment with hydroxylamine-O-sulfonic acid to give diaziridine 9a. Subsequent oxidation afforded the desired diazirine 10a in 44% overall yield. Removal of the Boc and \textdelta-butyl groups, followed by Boc protection of the resulting amine gave Boc-\textgamma-photo-methionine (3a). Since the synthesis of \textgamma-photo-leucine from an \textepsilon-aspartic acid derivative has not been reported, we attempted to synthesize Boc-\textgamma-photo-leucine (3b) in the same manner as that used for 3a (Chart 2). However, the \textdelta-Weinreb amide 7b was not completely converted into the corresponding ketone 8b by the methyl Grignard reagent. Our investigation of the methylation conditions found that treatment of the Weinreb amide 7b with methyllithium in tetrahydrofuran at \(-78\)°C gave the desired compound 8b in 90% yield. The resultant ketone 8b was then transformed into 3b in the same manner as that used for the synthesis of 3a.

Next, we attempted the synthesis of trifluoromethyl-\textgamma-methionine 3c (Chart 3). The light-sensitive diazirine moiety should be formed at a late stage in the synthetic protocol, thus we utilized the trifluoromethyl ketone 13c as a key intermediate. Ketone 13c was prepared from aldehyde 11c (the preparation method for this compound is summarized in Chart S2) by nucleophilic addition of the trifluoromethyl group utilizing Ruppert–Prakash reagent (TMS-CF\textsubscript{3}),\textsuperscript{44} followed by oxidation of the resulting alcohol 12c with Dess–Martin periodinate (DMP),\textsuperscript{45} in 75% yield in three steps. In this synthesis, protection of the \textalpha-amino group with bis-Boc groups was adopted because mono-Boc protected aldehydes readily cy-
While the photolysis of trifluoromethyldiazirine was cross-linked with MeOH. However, the photolysis of tri-approximately half of the consumed trifluoromethyldiazirine quantity of alkenes was produced. In contrast, photolysis of Although methyldiazirine and the resultant solution was analyzed by LC-MS (Fig. 2).

Due to the instability of 15c under the purification conditions, the compound was subsequently oxidized with iodine in the presence of triethylamine47) to afford the desired trifluoromethylidyldiazirine 16c. Finally, removal of the Boc and i-butyl groups and subsequent Boc protection furnished 3c in 68% yield over two steps. In a fashion similar to the synthesis of 3c, the synthesis of trifluoromethyl-photo-leucine 3d was also accomplished from the aldehyde 11d (Chart 3).

**Photochemical Properties** The photolysis of the methyl- and trifluoromethyldiazirines was investigated. To quantitate the amounts of substrates and products based on UV absorptions, we attached a benzyl ester to 3a and to afford 17a and c, respectively (Fig. 2, Chart S3). The amino acid derivatives in MeOH, cooled in an ice bath, were irradiated with 352 nm UV light.

Irradiation times were 0 min (A, D), 15 min (B, E), and 90 min (C, F). The analytical conditions are as follows: column, X Bridge (5 µm, 4.6×75 mm); gradient method, 10–95% of B (0.00–4.00 min, 95% of B (4.00–11.0 min, 95–10% of B (11.0–11.1 min, 10% of B (11.1–15.0 min) (A: 0.1% HCOOH/H2O, B: 0.1% HCOOH/MeOH); flow rate, 1.1 mL/min; UV 214 nm. Chemical structures of the observed peaks were estimated by m/z of the molecular ion (MH+).
The SOAT inhibitory activities of 1a–d were estimated by a cell-based assay using SOAT1-CHO and SOAT2-CHO. Compounds 1a–d and [14C]-oleic acid were added to each cell culture, and the resultant radioactivities of [14C] CE, [14C] triacylglycerol (TG), and [14C] phospholipid (PL) were measured. Compounds 1a–d inhibited production of CE in a dose dependent manner, but did not inhibit the formation of TG and PL, even at a concentration of 20 µM. This suggests that 1a–d selectively inhibit CE synthesis in the CHO cells. The IC₅₀ values for CE synthesis are summarized in Table 1. SOAT1 inhibitory activities of 1a–d are as potent as those of beauveriolides I and III, whereas CE synthesis by SOAT2-CHO was not inhibited by 1a–d at 20 µM. The SOAT inhibitory activity and isozyme selectivity of 1a–d were almost identical to those of beauveriolides I and III. These results strongly suggest that the alterations made to the side chain of the Leu or Ile residue in beauveriolides, which resulted in incorporation of alkyl methyldiazirine or trifluoromethyldiazirine, do not affect their SOAT inhibitory activity and isozyme selectivity.

Conclusion
To facilitate an analysis of the binding site in SOAT proteins, we have designed beauveriolide analogues wherein the D-Leu or D-allo-Ile residue was replaced by photoreactive amino acids possessing methyldiazirine (1a, b) or trifluoromethyldiazirine (1c, d) in the side chain (Fig. 1). The methyldiazirine moiety was installed by the reaction of methyl ketones 8a and b with liquid ammonia to give an imine intermediate, followed by treatment with hydroxylamine-O-sulfonic acid to provide the diaziridines 9a and b. Subsequent oxidation gave the desired methyldiazirines 10a and b. In contrast, the trifluoromethyldiazirines were prepared from trifluoromethyl ketones 13c and d via oxime intermediates 14c and d, which were transformed into diaziridines 15c and d. Subsequent oxidation afforded the desired trifluoromethyldiazirines 16c and d. Despite slow photolysis, rearrangement to yield alkenes was not prominent in the alkyl(trifluoromethyl)diazirines, suggesting that such compounds could be effective for photo-affinity labeling. Sequential couplings of 3–5, followed by macrolactamization gave the desired macrocycles 1a–d. The SOAT inhibitory activities of 1a–d were found to be as potent as those of beauveriolides I and III. In addition, 1a–d inhibited SOAT1 selectively rather than SOAT2, which was also consistent with the behavior of beauveriolides I and III (Table 1).

### Table 1. SOAT Inhibitory Activities of 1a–d<sup>a</sup>

| Compound | IC₅₀ (µM) for cholesteryl ester synthesis |
|----------|------------------------------------------|
|          | Cell-based assay | Enzyme assay |
|          | SOAT1-CHO | SOAT2-CHO | SOAT1 | SOAT2 |
| 1a       | 0.98 | >20 | 0.53 | 3.4 |
| 1b       | 2.7 | >20 | 1.4 | 9.5 |
| 1c       | 1.9 | >20 | 3.0 | 2.6 |
| 1d       | 2.1 | >20 | 1.1 | 7.3 |
| Beauveriolide I<sup>9)</sup> | 0.60 | 20 | 2.2 | 1.9 |
| Beauveriolide III<sup>9)</sup> | 0.90 | >20 | 3.0 | 3.0 |

<sup>a</sup> Inhibitions of triacylglycerol and phospholipid syntheses were not observed even at a concentration of 20 µM of 1a–d in cell-based assay.

Inhibition of CE syntheses by 1a–d was also investigated by an enzyme assay. After addition of the compounds and [14C]oleoyl-CoA to a microsomal fraction prepared from SOAT1-CHO or SOAT2-CHO, the generated [14C] CE was measured, and the IC₅₀ values were calculated (Table 1). Compounds 1a–d were found to inhibit CE synthesis catalyzed by both SOAT1 and SOAT2. This indicates that photoaffinity labeling experiments are applicable not only to SOAT1 but also SOAT2 using microsomal fractions.

### Chart 4. Total Syntheses of 1a–d

![Synthesis of 1a–d](image-url)
Experimental

General Techniques  All commercially available reagents were used as received. Dry THF and CH₂Cl₂ (Kanto Chemical Co., Tokyo, Japan) were obtained by passing through activated alumina column with commercially available pre-dried, oxygen-free formulations. All solution-phase reactions were monitored by thin-layer chromatography (TLC) carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by p-anisaldehyde H₂SO₄–ethanol solution, phosphomolybdic acid–ethanol solution, or ninhydrin–acetic acid–1-butanol solution. Column chromatography was carried out with silica gel 60N (Kanto Chemical Co., 100–210 μm).

CH₂Cl₂ (84 mL) at 0°C was added to a solution of carboxylic acid (2.34 g, 18.1 mmol, 2.00 equiv.) in THF (90 mL) at room temperature and stirred under an argon atmosphere for 3 h. The reaction mixture was poured into 1 M aqueous HCl and the aqueous phase was extracted three times with CH₂Cl₂. The organic layers were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate=3:1) to afford Weinreb amide 7a (5.33 g, 15.4 mmol, 88%) as a colorless oil. [1H-NMR (600 MHz, CDCl₃) δ: 5.18 (1H, d, J=7.6 Hz), 4.19 (1H, m), 3.68 (3H, s), 3.18 (3H, s), 2.54 (1H, m), 2.50 (1H, m), 2.15 (1H, m), 1.93 (1H, m), 1.47 (9H, s), 1.44 (9H, s). [13C-NMR (150 MHz, CDCl₃) δ: 173.6, 171.6, 155.5, 81.9, 79.5, 61.2, 53.7, 32.2, 28.3, 28.03, 27.96, 27.6. IR (neat) cm⁻¹: 3340, 2978, 2936, 1794, 1716, 1667, 1512, 1456, 1392, 1367, 1313, 1215, 1157, 1051, 1026, 848. HR-ESI-MS m/z: 369.1989 (Calcd for C₁₅H₂₆NO₅Na [M⁺Na⁺]: 369.1996). [α]D²⁹ = −5.40 (c=0.950, CHCl₃).

 tert-Butyl (R)-2-[[[tert-Butoxy]carbonyl]amino]-4-[methoxy(methyl)carbamoyl]propanoate (7b) 7b was prepared from 6b (8.42 g, 29.1 mmol) in the same manner to the synthesis of 7a. Yield 90% (a colorless oil). [1H-NMR (400 MHz, CDCl₃) δ: 5.66 (1H, d, J=8.4 Hz), 4.47–4.43 (1H, m), 3.68 (3H, s), 3.16 (3H, s), 3.15–3.11 (1H, m), 2.92–2.83 (1H, m), 1.46 (9H, s), 1.44 (9H, s). [13C-NMR (100 MHz, CDCl₃) δ: 171.7, 170.4, 155.6, 81.6, 79.4, 61.1, 50.3, 34.6, 31.9, 28.1, 27.8. IR (neat) cm⁻¹: 3431, 3361, 2978, 2936, 1716, 1662, 1496, 1391, 1368, 1252, 1157, 1023, 850. HR-ESI-MS m/z: 355.1832 (Calcd for C₁₅H₂₈N₂O₆Na [M⁺Na⁺]: 355.1840). [α]D²⁹ = −19.8 (c=1.50, CHCl₃).

tert-Butyl (R)-2-[[[tert-Butoxy]carbonyl]amino]-5-oxo-hexanoate (8a) 8a To a solution of Weinreb amide 7a (6.00 g, 17.3 mmol, 1.00 equiv.) in toluene (57 mL) at ~78°C under an argon atmosphere was added methyl magnesium bromide (43.3 mL, 1 M solution in hexanes, 43.3 mmol, 2.50 equiv.) over 30 min. The reaction was allowed to warm up to ~5°C over 3 h, quenched with 1 M aqueous HCl, and the aqueous layer was extracted three times with ethyl acetate. The organic layers were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate=8:1) to afford ketone 8a (5.21 g, 17.3 mmol, quant.) as a colorless oil. [1H-NMR (400 MHz, CDCl₃) δ: 5.05 (1H, brs), 4.15 (1H, brs), 2.62–2.44 (2H, m), 2.15 (3H, s), 2.12–2.00 (1H, m), 1.89–1.79 (1H, m), 1.46 (9H, s), 1.44 (9H, s). [13C-NMR (100 MHz, CDCl₃) δ: 207.6, 171.5, 155.4, 82.1, 79.7, 53.4, 39.4, 29.9, 28.3, 28.0, 26.8. IR (neat) cm⁻¹: 3361, 2979, 2933, 1716, 1514, 1367, 1252, 1156, 1056, 848. HR-ESI-MS m/z: 324.1777 (Calcd for C₁₅H₂₀N₂O₄Na [M⁺Na⁺]: 324.1781). [α]D²⁹ = −0.40 (c=1.00, CHCl₃).

tert-Butyl (R)-2-[[[tert-Butoxy]carbonyl]amino]-4-oxopentanoate (8b) 8b To a solution of Weinreb amide 7b (3.00 g, 9.03 mmol, 1.00 equiv.) in tetrahydrofuran (THF) (90 mL) at ~78°C under an argon atmosphere was added methyl lithium (18.1 mL, 1 M solution in hexanes, 18.1 mmol, 2.00 equiv.) over 30 min and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with 1 M aqueous HCl, and the aqueous layer was extracted with ethyl acetate. The organic layers were pooled, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate=8:1) to afford ketone 8b (2.34 g, 8.13 mmol, 90%) as a colorless oil. [1H-NMR (400 MHz, CDCl₃) δ: 5.44 (1H, d, J=6.8 Hz), 4.36 (1H, brs), 3.10 (1H, dd, J=17.8, 4.0 Hz), 2.90 (1H, dd, J=17.8, 4.4 Hz), 2.16 (3H, s), 1.44 (18H, s). [13C-NMR (100 MHz, CDCl₃) δ: 206.5, 170.3, 155.6, 82.1, 79.7, 50.1, 45.6, 29.9, 28.3, 27.8. IR
(neat) cm⁻¹: 3364, 2979, 2933, 1719, 1499, 1368, 1252, 1156, 1052, 847. HR-ESI-MS m/z: 310.1620 (Caled for C₁₁H₂₀NO₅Na [M+Na⁺]: 310.1625). [α]D−13.8 (c=0.95, CHCl₃).

tert-Butyl (R)-2-[[( tert-Butyloxy)carbonyl]amino]-4-(3-methyl-3H-diazirin-3-yl)butanoate (10a) A solution of ketone 8a (0.30 g, 2.59 mmol, 1.00 equiv.) in dry benzene (2.6 mL) was added to a solution of tert-butyl (R)-2-[[( tert-Butyloxy)carbonyl]amino]-6,6,6-trifluoro-5-oxohexanoate (13c) (350 mg, 0.903 mmol, 1.00 equiv.) in dry THF (1.5 mL) at 0°C. The mixture was stirred at room temperature for 2 h. The mixture was poured into water (20 mL) and the mixture was washed with ethyl acetate (3×30 mL). The organic layer was washed with water, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was then diluted with methanol (38 mL) and cooled to 0°C. NaOH (1.52 mL, 10.9 mmol, 1.00 equiv.) was added to the mixture, and the mixture was stirred at room temperature for 16 h. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in CH₂Cl₂ (9 mL) was added NaHCO₃ (567 mg, 7.55 mmol) and Dess-Martin periodinane (2.30 g, 5.5 mmol, 2.00 equiv.) in dry THF (118 mL) and stirred at room temperature for 16 h. The mixture was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in a mixture of 1,4-dioxane (3.8 mL) and H₂O (3.8 mL) was added di-tert-butyl dicarbonate (910 mg, 4.17 mmol, 1.10 equiv.) and Na₂CO₃ (603 mg, 5.69 mmol, 1.50 equiv.) at 0°C and the mixture was stirred at room temperature for 12 h. The reaction mixture was acidified with 1 M aqueous HCl to pH 4 and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate=1:1) to afford carboxylic acid 3a (692 mg, 2.69 mmol, 71% in 2 steps) as a slightly yellow oil. ¹H-NMR (400 MHz, CDCl₃, mixture of rotemers) δ: 10.4 (1H, brs), 6.80 (0.4×1H, brs), 5.07 (0.6×1H, brs), 4.29 (0.6×1H, brs), 4.12 (0.4×1H, brs), 1.77 (1H, m), 1.49 (1H, m), 1.45 (10H, m), 1.25 (1H, m), 1.03 (3H, m). ¹³C-NMR (100 MHz, CDCl₃, mixture of rotemers) δ: 176.5, 156.9, 155.5, 82.0, 80.4, 53.7, 52.7, 30.4, 30.0, 28.2, 27.1, 25.2, 19.6, 14.1. IR (neat) cm⁻¹: 3324, 3107, 2979, 2932, 1719, 1514, 1544, 1394, 1369, 1252, 1164, 1051, 1027, 855, 779. HR-FAB-MS 258.1461 (Caled for C₁₁H₁₀N₂O₄ [M⁺]: 258.1454). [α]D−16.8 (c=1.0, CHCl₃).

(R)-2-[[( tert-Butyloxy)carbonyl]amino]-3-(3-methyl-3H-diazirin-3-yl)propanoic Acid (3b) 3b was prepared from Boc-d-photo-Leu-O’Bu (10b) (230 mg, 0.769 mmol) in the same manner to the synthesis of 3a. Yield 78% in 2 steps (a slightly yellow oil). ¹H-NMR (400 MHz, CDCl₃, mixture of rotemers) δ: 9.12 (1H, brs), 6.59 (0.3×1H, brs), 5.10 (0.7×1H, brs), 4.38 (0.7×1H, brs), 4.12 (0.3×1H, brs), 2.06 (0.7×1H, dd, J=15.0, 6.5 Hz), 1.89–1.87 (0.3×1H, m), 1.73–1.68 (0.3×1H, m), 1.61 (0.7×1H, dd, J=15.0, 8.8 Hz), 1.48 (9H, s), 1.10 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ: 176.0, 155.4, 80.7, 50.2, 37.5, 28.3, 23.7, 19.7. IR (neat) cm⁻¹: 3323, 2963, 2928, 2857, 1717, 1508, 1455, 1394, 1268, 1254, 1163, 1051, 1025, 878. HR-FAB-MS m/z: 244.1302 (Caled for C₁₁H₁₉N₂O₄Na [M⁺Na⁺]: 244.1297). [α]D−15 (c=0.47, CHCl₃).

 tert-Butyl (R)-2-[[[( tert-Butyloxy)carbonyl]amino]-3-(3-methyl-3H-diazirin-3-yl)butanoate (10b) 10b was prepared from 8b (2.40 g, 8.35 mmol) in the same manner to the synthesis of 10a. Yield 35% in 2 steps (white solids). ¹H-NMR (400 MHz, CDCl₃, mixture of rotemers) δ: 5.09 (1H, d, J=6.8 Hz), 4.26 (1H, m), 1.82 (1H, dd, J=14.6, 5.6 Hz), 1.61 (1H, dd, J=14.6, 4.4 Hz), 1.55 (0.5×1H, s), 1.53 (0.1×1H, s), 1.49 (0.9×1H, s), 1.47 (0.9×1H, s), 1.09 (3H, s). ¹³C-NMR (100 MHz, CDCl₃, mixture of rotemers) δ: 170.7, 155.0, 146.7, 85.1, 82.5, 79.9, 67.0, 50.9, 38.2, 29.6, 28.3, 27.8, 27.3, 19.7, 14.1. IR (neat) cm⁻¹: 3364, 2980, 2932, 2870, 1716, 1518, 1501, 1455, 1368, 1253, 1160, 1049, 847. HR-ESI-MS m/z: 336.1882 (Caled for C₁₃H₂₁N₂O₅Na [M⁺Na⁺]: 336.1894). [α]D−51.7 (c=1.00, CHCl₃).

(R)-2-[[[( tert-Butyloxy)carbonyl]amino]-4-(3-methyl-3H-diazirin-3-yl)butanoate (3a) A solution of Boc-d-photo-Met-O’Bu (10a) (1.19 g, 3.79 mmol, 1.00 equiv.) in THF (118 mL) and 8a aqueous HCl (118 mL) was stirred at room temperature for 16 h. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in CH₂Cl₂ (9 mL) was added NaHCO₃ (567 mg, 6.75 mmol, 7.50 equiv.) and Dess-Martin periodinane (2.30 g, 5.5 mmol, 2.00 equiv.) in dry THF (118 mL) and stirred at room temperature for 16 h. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in CH₂Cl₂ (9 mL) was added NaHCO₃ (567 mg, 6.75 mmol, 7.50 equiv.) and Dess-Martin periodinane (2.30 g, 5.5 mmol, 2.00 equiv.) in dry THF (118 mL) and stirred at room temperature for 16 h. The mixture was poured into water (20 mL) and the mixture was washed with ethyl acetate (3×30 mL). The organic layer was washed with water, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was then diluted with methanol (38 mL) and cooled to 0°C. NaOH (1.52 mL, 10.9 mmol, 1.00 equiv.) was added to the mixture, and the mixture was stirred at room temperature for 16 h. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.
tert-Butyl (R)-2-[[Bis(tert-butoxy)carbonyl]amino]-5,5,5-
trifluoro-4-oxopentanoate (13d) 13d was prepared from
aldehyde 11d (200 mg, 0.536 mmol) in the same manner to
the synthesis of 13c. Yield 75% in 2 steps (white solids). 1H-NMR (600 MHz, CDCl 3) δ: 7.47 (1H, t, J = 7.3 Hz), 2.43 (2H, d, J = 7.6 Hz), 1.52 (18H, s), 1.43 (9H, s). 13C-NMR (150 MHz, CDCl 3) δ: 168.1, 151.9, 122.3 (q, J = 273 Hz), 83.5, 82.4, 54.0, 28.0, 27.8, 27.1, 25.8 (q, J = 401 Hz). IR (neat) cm⁻¹: 2982, 2933, 1739, 1703, 1394, 1383, 1369, 1281, 1236, 1154, 849. HR-ESI-MS m/z: 476.1967 (Calcd for C₁₅H₂₃F₃NO₇Na [M + Na⁺]: 476.1979). [α]D²⁰ +27.8 (c = 1.00, CHCl₃).

(R)-2-[[Bis(tert-butoxy)carbonyl]amino]-4-[3-
(trifluoromethyl)-3H-diazirin-3-yl]butanoic Acid (3c) 3c was prepared from 16d (56.0 mg, 0.121 mmol, 1.00 equiv) in 4 M HCl/ dioxane (6.6 mL) was stirred at room temperature for 20 h. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in liquid ammonia (10 mL) was added tert-butyl (3-diazirin-3-yl)propanoate (16d) 16d and dry CH₂Cl₂ (1 mL) was added. The reaction mixture was poured into saturated aqueous Na₂S₂O₃ solution and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification.

A solution of the above residue in dry CH₂Cl₂ (1 mL) was added and the mixture was stirred at the same temperature for 1 h under an argon atmosphere. The reaction mixture was poured into H₂O and the aqueous layer was extracted with ethyl acetate. The residue was used for the next reaction without further purification.

A solution of the above residue in dry CHCl₃ (1 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification.

A solution of the above residue in dry MeOH (1.8 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into saturated aqueous Na₂SO₃ and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate = 3: 1) to afford 3f (25.7 mg, 0.0820 mmol, 68% in 2 steps) as white solids.

1H-NMR (400 MHz, CDCl 3, mixture of rotamers) J = 10.55 (1H, br s), 7.03 (0.5×1H, brs), 5.08 (0.5×1H, d, J = 7.3 Hz), 4.29 (0.5×1H, m), 4.14 (0.5×1H, m), 1.87–1.77 (4H, m), 1.47 (0.5×9H, s), 1.45 (0.5×9H, s); 13C-NMR (100 MHz, CDCl 3, mixture of rotamers) δ: 176.0, 174.8, 157.0, 155.6, 122.3 (q, J = 275 Hz), 82.6, 80.8, 53.3, 52.4, 28.2, 27.2 (q, J = 40 Hz), 26.3, 26.2, 22.5, 22.0. IR (neat) cm⁻¹: 3323, 2982, 2936, 1719, 1513, 1456, 1370, 1157, 1056, 849. HR-FAB-MS m/z: 312.1163 (Calcd for C₁₅H₁₇F₃N₂O₇ [M + Na⁺]: 312.1171). [α]D²⁰ −4.43 (c = 0.10, CHCl₃).

Allyl (35, 4S)-3-Hydroxy-4-methyloctanoid Acid (4) To a solution of carboxylic acid 18 (2.00 g, 11.5 mmol, 1.00 equiv) in MeOH (4.1 mL) was added a solution of cesium carbonate (1.87 g, 5.74 mmol, 0.50 equiv) in distilled water (4.1 mL) at 0°C and the mixture was stirred at room temperature for 40 min. The reaction mixture was concentrated in vacuo and diluted with N,N-dimethylformamide (DMF) (57 mL). To the solution was added allyl bromide (1.03 mL, 12.0 mmol, 1.05 equiv) at 0°C and the mixture was stirred at the same temperature for 1 h under an argon atmosphere. The reaction
mixture was quenched with saturated aqueous NaHCO₃ and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with H₂O, brine, dried over MgSO₄ and filtered. The filtrate was concentrated in vacuo, and then the resulting residue was purified by column chromatography on silica gel (hexane–ethyl acetate 1:3) to afford the allyl (3S,4S)-3-hydroxy-4-methyloctanoic acid (4) (2.12 g, 9.89 mmol, 87%) as a colorless oil.

1H-NMR (400 MHz, CDCl₃, δ), H: 5.92 (1H, ddt, J = 16.9, 6.8, 6.0 Hz), 5.35 (2H, m), 5.26 (2H, m), 5.26 (2H, m), 5.26 (2H, m), 2.35 (1H, dd, J = 16.2, 4.1 Hz), 2.09 (1H, dd, J = 16.2, 8.1 Hz), 1.46 (9H, s), 1.30–1.11 (7H, m), 0.90 (6H, m). 1C-NMR (100 MHz, CDCl₃): 190.9 (29.0 mg, 97.6 mmol, 1.10 equiv.) and EDCI·HCl (125 mg, 0.625 mmol, 1.30 equiv.) at room temperature under an argon atmosphere. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH₂Cl₂, washed with 1 M aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄ filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate 1:3) to afford ester unit 19a (188 mg, 0.414 mmol, 88%) as a colorless oil.

IR (neat) cm⁻¹: 3373, 2962, 2932, 2876, 2862, 1744, 1735, 1719, 1508, 1500, 1456, 1392, 1367, 1346, 1301, 1275, 1252, 1159, 1104, 1052, 1026, 990, 934. HR-ESI-MS m/z: 530.2432 (Calcd for C₁₂H₂₁O₆Na [M+Na⁺]: 530.2448). [α]D²⁻ = −55.2 (c=1.50, CHCl₃).

Ester Unit 19a (n=2, R=Me) To a solution of 3a (154 mg, 0.600 mmol, 1.30 equiv.) and 4 (100 mg, 0.470 mmol, 1.00 equiv.) in dry CH₂Cl₂ (4 mL) was added DMAP (57 mg, 0.47 mmol, 1.00 equiv.) and DCC (218 µL, 1.40 mmol, 3.00 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at the same temperature for 10 h. The reaction mixture was diluted with CH₂Cl₂, washed with 1 M aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄ filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate 1:3) to afford ester unit 19a (188 mg, 0.414 mmol, 88%) as a colorless oil.

IR (neat) cm⁻¹: 3373, 2962, 2932, 2876, 2862, 1744, 1735, 1719, 1508, 1500, 1456, 1392, 1367, 1346, 1301, 1275, 1252, 1159, 1104, 1052, 1026, 990, 934. HR-ESI-MS m/z: 530.2432 (Calcd for C₁₂H₂₁O₆Na [M+Na⁺]: 530.2448). [α]D²⁻ = −55.2 (c=1.50, CHCl₃).

Depsipeptide 20a (n=2, R=Me) To a solution of ester unit 19a (227 mg, 0.500 mmol, 1.00 equiv.) and morpholine (109 µL, 1.25 mmol, 2.50 equiv.) in dry THF (15 mL) was added a catalytic amount of Pd(PPh₃)₄ (0.27 mg, 0.23 µmol, 0.01 equiv.) at 0°C under an argon atmosphere and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, washed with 1 M aqueous HCl and brine, dried over MgSO₄ filtered and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of dipeptide 5 (260 mg, 0.616 mmol, 1.20 equiv.) in MeOH (11 mL) was added 10% Pd/C (52.0 mg, 0.20 equiv.) under an argon atmosphere and the reaction mixture was purged with hydrogen three times. The reaction mixture was stirred at room temperature for 2h under a hydrogen atmosphere. After being stirred at the same temperature for 1h, the reaction mixture was diluted with ethyl acetate, washed with 1 M aqueous HCl and brine, dried over MgSO₄ filtered and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the carboxylic acid and amine in CH₂Cl₂ (6 mL) was added HOBt (101 mg, 0.75 mmol, 1.50 equiv.) and DIPEA (235 µL, 1.35 mmol, 2.70 equiv) and EDCI·HCl (125 mg, 0.65 mmol, 1.30 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at the same temperature for 10 h. The reaction mixture was poured into 1 M aqueous HCl and the mixture was stirred at room temperature for 2 h under a hydrogen atmosphere. The suspension was filtered through a pad of Celite® and the filtrate was concentrated in vacuo.

The residue was used for the next reaction without further purification.

To a solution of the carboxylic acid and amine in CH₂Cl₂ (6 mL) was added HOBt (101 mg, 0.75 mmol, 1.50 equiv.) and DIPEA (235 µL, 1.35 mmol, 2.70 equiv) and EDCI·HCl (125 mg, 0.65 mmol, 1.30 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at the same temperature for 10 h. The reaction mixture was poured into 1 M aqueous HCl and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous NaHCO₃, brine, dried over MgSO₄ filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ethyl acetate =3:1) to afford depsipeptide 20a (265 mg, 0.385 mmol, 77% from 19a) as a colorless oil.

1H-NMR (400 MHz, CDCl₃)
δ: 7.32–7.20 (5H, m), 6.83 (1H, d, J=6.8 Hz), 6.67 (1H, d, J=7.2 Hz), 5.26 (1H, d, J=7.2 Hz), 5.15 (1H, m), 4.70 (1H, q, J=7.2 Hz), 4.37–4.30 (1H, dq, J=7.2, 6.8 Hz), 4.17 (1H, m), 3.09 (2H, d), J=2.4 Hz). 2.24 (2H, m), 1.71–1.64 (2H, m), 1.44 (9H, s), 1.32 (9H, s), 1.43–1.20 (12H, m), 0.99 (3H, s), 0.89–0.86 (6H, m). 13C-NMR (100 MHz, CDCl3) δ: 171.6, 171.4, 170.3, 169.4, 155.3, 136.5, 129.1, 128.4, 126.7, 81.7, 79.7, 75.1, 54.4, 52.9, 48.7, 38.3, 38.1, 36.2, 32.0, 30.3, 29.1, 28.2, 27.8, 26.6, 25.1, 19.5, 18.1, 14.2, 13.9. HR-ESI-MS m/z: 710.4091 (Calcd for C36H62N2O2Na [M+Na]+: 710.4099). IR (neat) cm⁻¹: 3289, 2977, 2931, 2859, 1735, 1719, 1645, 1647, 1551, 1454, 1357, 1158, 1048, 724, 705. [α]D28 = −17 (c=0.50, CHCl3).

Depsipeptide 20b (n=1, R=Me) 20b was prepared from ester unit 19b (37.1 mg, 81.6 µmol) and dipeptide 5 (41.8 mg, 97.9 µmol) in the same manner to the synthesis of 20a. Yield 77% from 19b (a colorless oil). 1H-NMR (600 MHz, CDCl3) δ: 7.30–7.20 (5H, m), 6.45 (1H, d, J=7.2 Hz), 6.37 (1H, d, J=6.6 Hz), 5.25 (1H, d, J=8.4 Hz), 5.14–5.11 (1H, m), 4.61 (1H, dt, J=7.8, 7.2 Hz), 4.35–4.30 (1H, q, J=4.8 Hz), 7.8 (4.25 (1H, m), 3.07 (2H, m), 2.50 (2H, dd, J=15.0, 8.4 Hz), 1.88–1.81 (1H, m), 1.68 (1H, m), 1.58 (1H, m), 1.46 (9H, s), 1.32–1.18 (9H, s), 1.08 (3H, s). 1C-NMR (150 MHz, CDCl3) δ: 171.7, 171.5, 170.1, 169.7, 154.5, 136.6, 129.3, 128.7, 127.0, 82.0, 80.2, 75.7, 54.6, 50.5, 48.8, 38.9, 38.1, 37.5, 36.5, 32.3, 29.2, 28.4, 28.0, 23.9, 22.8, 19.7, 18.3, 14.3, 14.0. IR (neat) cm⁻¹: 3287, 2977, 2931, 2859, 1740, 1719, 1644, 1551, 1455, 1392, 1268, 1222, 1163. HR-ESI-MS m/z: 696.3921 (Calcd for C32H53N5O5Na [M+Na]+: 696.3943). [α]D28 = −14.2 (c=1.05, CHCl3).

Depsipeptide 20c (n=2, R=CF3) 20c was prepared from ester unit 19c (300 mg, 0.591 mmol) and dipeptide 5 (302 mg, 0.71 mmol) in the same manner to the synthesis of 20a. Yield 63% from 19c (a colorless oil). 1H-NMR (400 MHz, CDCl3) δ: 7.31–7.19 (5H, m), 6.36 (1H, d, J=7.2 Hz), 6.28 (1H, d, J=7.2 Hz), 5.22 (1H, d, J=8.4 Hz), 5.17–5.13 (1H, m), 4.18–4.12 (1H, quint, J=7.2 Hz), 4.16 (1H, m), 3.10 (1H, dd, J=13.8, 6.8 Hz), 3.04 (1H, dd, J=13.8, 7.2 Hz), 2.50–2.37 (2H, m), 1.86–1.67 (4H, m), 1.44 (18H, s), 1.33–1.25 (10H, m), 0.88 (6H, m). 1C-NMR (150 MHz, CDCl3) δ: 171.54 (major) and 171.45 (minor), 171.31 (major) and 171.26 (minor), 170.2, 169.31 (major) and 169.27 (minor), 155.4, 136.37 (minor) and 136.42 (major), 129.2, 128.5, 126.9, 171.1, 168.3, 136.1, 128.9, 128.0, 126.6, 76.3, 54.5, 49.2, 36.1, 35.4, 35.3, 30.6, 30.3, 29.3, 28.9, 26.4, 22.5, 19.0, 15.0, 14.4, 13.4. IR (neat) cm⁻¹: 3380, 3294, 2958, 2929, 2857, 1724, 1683, 1640, 1537, 1254, 1149, 1006. HR-ESI-MS m/z: 536.2837 (Calcd for C32H36F6N4O4Na [M+Na]+: 536.2834). [α]D28 = −41 (c=0.25, CHCl3–MeOH=1:1).

**Beauveriolide Analogue 1b (n=2, R=Me)** 1b was prepared from depsipeptide 20b (26 mg, 38.6 µmol) in the same manner to the synthesis of 1a. Yield 41% in 2 steps (white solids). 1H-NMR (600 MHz, CDCl3–CD3OD=1:1) δ: 7.25 (4H, m), 4.98–4.95 (1H, m), 4.70 (1H, dd, J=8.4, 7.2 Hz), 4.25 (1H, t, J=8.4 Hz), 3.89 (1H, m), 3.08 (1H, dd, J=13.2, 8.4 Hz), 2.97 (1H, dd, J=13.2, 8.4 Hz), 2.51 (1H, m), 2.44 (1H, dd, J=13.9, 9.5 Hz), 2.11 (1H, m), 1.73 (1H, dd, J=14.8, 7.2 Hz) 1.63 (1H, dd, J=14.8, 8.4 Hz), 1.21 (6H, s), 0.89 (3H, s). 13C-NMR (150 MHz, CDCl3–CD3OD=1:1) δ: 171.8, 171.3, 171.1, 168.3, 136.1, 128.8, 126.8, 56.4, 50.1, 49.5, 37.2, 35.9, 35.5, 30.6, 29.4, 28.9, 23.2, 22.6, 18.8, 15.1, 14.5, 13.6. IR (neat) cm⁻¹: 3380, 3298, 3063, 2959, 2930, 2858, 1726.
Beauveriolide Analogue 1c (n = 2, R = CF₃) To a solution of desipeptide 20c (20 mg, 27.0 µmol, 1.00 equiv.) in 4 mM HCl/dioxane (2 mL) was stirred at room temperature for 1 h under an argon atmosphere. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in dry CH₂Cl₂ (27 mL) was added DIEA (28.2 µL, 162.0 µmol, 6.00 equiv.) and the mixture was stirred at 0°C for 20 min under an argon atmosphere. The suspension was concentrated in vacuo.

The residue was used for the next reaction without further purification. Under an argon atmosphere, the suspension was concentrated in vacuo. The residue was diluted with organic solvent (ethyl acetate–MeOH = 4 : 1), washed with 1 M aqueous NaHCO₃, filtered and dried to remove the salt. The residue was treated with 1 M aqueous HCl, saturated aqueous NaHCO₃, brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (CHC₁₇-MeOH = 9 : 1) and purified by reversed-phase HPLC (column, YMC-Pack R&D ODS-A 75 × 25 mm, 40 µm; gradient method, 10% to 90% linear gradient (0.0–15.0 min), H₂O–MeOH = 90 : 10 isocratic (15.0–20.0 min); flow rate, 12.0 mL/min) to afford beauveriolide analog 1e (5.19 µg, 9.10 µmol, 34% in 2 steps) as white solids.

Beauveriolide Analogue 1d (n = 1, R = CF₃) 1d was prepared from desipeptide 20d (20 mg, 27.5 µmol) in the same manner to the synthesis of 1c. Yield 37% in 2 steps (white solids)

Beauveriolide Analogue 1a (n = 1) To a solution of Beauveriolide Analogue 1c (10 mg, 27.5 µmol, 1.00 equiv.) in dry CH₂Cl₂ (5 mL) was added DIEA (24.0 µL, 162.0 µmol, 6.00 equiv.) and the mixture was stirred at 0°C for 20 min under an argon atmosphere. The suspension was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the above residue in dry CH₂Cl₂ (5 mL) was added DIEA (24.0 µL, 162.0 µmol, 6.00 equiv.) and the mixture was stirred at 0°C for 20 min under an argon atmosphere. The suspension was concentrated in vacuo. The residue was diluted with organic solvent (ethyl acetate–MeOH = 4 : 1), washed with 1 M aqueous NaHCO₃, filtered and dried to remove the salt. The residue was treated with 1 M aqueous HCl, saturated aqueous NaHCO₃, brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (CHC₁₇-MeOH = 9 : 1) and purified by reversed-phase HPLC (column, YMC-Pack R&D ODS-A 75 × 25 mm, 40 µm; gradient method, 10% to 90% linear gradient (0.0–15.0 min), H₂O–MeOH = 90 : 10 isocratic (15.0–20.0 min); flow rate, 12.0 mL/min) to afford beauveriolide analog 1a (15.5 µg, 8.10 µmol, 34% in 2 steps) as white solids.

**Chemical Structure**

Beauveriolide Analogue 1c (n = 2, R = CF₃)

Beauveriolide Analogue 1d (n = 1, R = CF₃)

Beauveriolide Analogue 1a (n = 1)

**Experimental Section**

Preparation of Microsomes from SOAT1- or SOAT2-CHO Cells

SOAT1- or SOAT2-CHO cells (1.25 × 10⁷ cells in 250 µL of medium A) were cultured in a 48-well plastic microplate and allowed to recover overnight at 37°C in 5% CO₂. The assays were performed using cells at least 80% confluent. Following overnight recovery, 2.5 µL of a sample (methanol solution) and 5 µL of [1-¹⁴C]oleic acid (1 nmol, 1.85 KBq, 10% ethanol/phosphate buffered saline (PBS) solution) were added to each culture at 37°C in 5% CO₂. After a 6 h incubation, the medium was removed, and the cells in each well were washed twice with PBS. The cells were lysed by adding 0.25 mL of 10 mM Tris–HCl (pH 7.5) containing 0.1% (w/v) SDS, cellular lipids were extracted by the method of Bligh and Dyer. After the organic phase had been concentrated, the total lipids were separated on a thin layer chromatography (TLC) plate (silica gel F254, 0.5 mm thick, Merck, Germany) and the radioactivities of [¹⁴C]CE, [¹⁴C]TG, and [¹⁴C]PL were analyzed by a bioimaging analyzer (FLA-7000, FUJIFILM, Japan). In this cell-based assay, [¹⁴C]CE was produced by the reaction of SOAT1 or SOAT2, SOAT inhibitory activity (%) is defined as (1–[¹⁴C]CE-drug/[¹⁴C]CE-control) × 100. The IC₅₀ value is defined as the drug concentration causing 50% inhibition of a biological activity.

Preparation of Microsomes from SOAT1- or SOAT2-CHO Cells

SOAT1- or SOAT2-CHO cells (2 × 10⁸ cells) were homogenized in 10 mL of cold buffered sucrose solution (pH 7.2) containing 100 mM sucrose, 50 mM KCl, 40 mM KH₂PO₄, 30 mM ethylenediaminetetraacetic acid (EDTA) and complete protease inhibitor cocktail (Roche, U.S.A.) (hereafter referred to as buffer A) in a Teflon homogenizer. The microsomal fraction was pelleted by centrifugation at 100000 × g for 1 h at 4°C, resuspended in the same buffer at a concentration of 5 mg protein/mL and stored at –80°C until use.

Enzyme Assay for SOAT Inhibitory Activity

SOAT1 and SOAT2 activities were determined by using microsomes prepared as described above as the enzyme source. Briefly, an assay mixture containing 2.5 µg/mL bovine serum albumin (BSA) in buffer A and [1-¹⁴C]oleoyl-CoA (20 µmol, 3.7 kBq) together with a test sample (added as a 10 µL methanol solution), and the SOAT1 or SOAT2 microsomal fraction (15 or 10 µg of protein, respectively) in a total volume of 200 µL were incubated at 37°C for 5 min. The reaction was started by...
adding [1-14C]oleoyl-CoA, and stopped by adding 1.2 mL of CHCl3-MeOH=2:1. The produced [14C]CE was extracted by the method of Bligh and Dyer. After the organic solvent was removed by evaporation, lipids was separated on a TLC plate and the radioactivity of [14C]CE was measured as described above.

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Conflict of Interest  The authors declare no conflict of interest.

Supplementary Materials  The online version of this article contains supplementary materials. They include Fig. S1, Charts S1–S3, supplementary experimental section, and copies of 1-D H- and 13C-NMR spectra.

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