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This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Regular Article

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

Yuichi Masuda,[a] Kazumasa Aoyama,[a] Masahito Yoshida,[a] Keisuke Kobayashi,[b] Taichi Ohshiro,[b] Hiroshi Tomoda,[b] and Takayuki Doi*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Tohoku University; 6–3 Aza-Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan: and* [b] *Graduate School of Pharmaceutical Sciences, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan.*

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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 D-*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 methyldiazirines. In contrast, trifluoromethyldiazirine derivatives were prepared from trifluoromethyl ketones *via* the oxime intermediates, which were transformed into diaziridines. Subsequent oxidation afforded trifluoromethyldiazirines. The synthesized photoreactive amino acids 3a–d were coupled with 3-hydroxy-4-methyloctanoic acid 4 and dipeptide 5, followed by macrolactamization 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).[1] Since CE accumulation in plasma is closely related to artery plaque formation, SOAT inhibitors are expected to be therapeutic agents for atherosclerosis.[2,3] Recent molecular biological studies have revealed the existence of two different SOAT isozymes—SOAT1 and SOAT2—in mammals.[4–7] 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,[8,9] 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.[2,3,10–12]

Beauveriolides I and III (Fig. 1A), isolated from a culture broth of *Beauveria* sp. FO-6979, inhibit lipid droplet formation in mouse peritoneal macrophages.[13,14] These cyclodepsipeptides were found to inhibit CE synthesis by blocking SOAT activity.[15] Ohshiro *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).[10] 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.[10] SOAT1 and SOAT2 share extensive homology, but the topology of the protein domains is thought to be different.[1,5,8,9] 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 (3*S*,4*S*)-3-hydroxy-4-methyloctanoic acid (HMA) moiety.[16] Our intensive structure–activity relationship (SAR) study showed that structural modification of beauveriolide could change the selectivity against SOAT1 and SOAT2.[16–21] 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.[22] 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-

* To whom correspondence should be addressed. e-mail: doi_taka@mail.pharm.tohoku.ac.jp

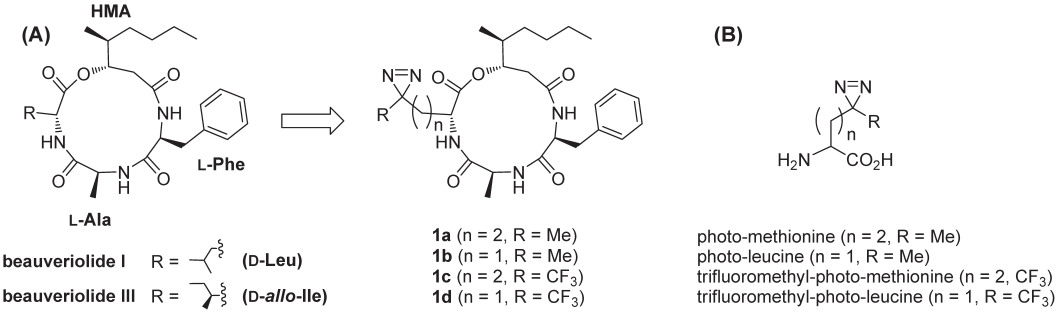

Fig. 1. (A) Beauveriolides and Their Analogues with Methyl- or Trifluoromethyl-Diazirines in the Side Chains of D-Leu or D-*allo*-Ile; (B) Photoreactive Amino Acids with Alkyldiazirines

active groups such as phenylazide,[23] benzophenone,[24] and diazirine[25–30] 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[22,31]: 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 (Fig. 1B), which possess alkyl methyldiazirines in their side chains and exhibit chemical and biological features similar to those of methionine and leucine.[27] Several researchers have reported the successful application of photo-methionine and -leucine in capturing the target molecules of bioactive peptides,[32,33] and in the structural analysis of a β-hairpin-forming peptide.[34]

It has been reported that photo-excited diazirines are first transformed into carbenes or diazoisomers.[25,35–37] As carbenes are highly reactive species with extremely short lifetimes,[38,39] 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.[40,41] In comparison to methyldiazirines, trifluoromethyldiazirines are less prone to the rearrangements,[41,42] the absence of which could increase the efficiency of photoaffinity labeling experiments. The electron-withdrawing effect of the trifluoromethyl group can stabilize the carbenes, preventing them from undergoing rearrangements. Moreover, fluorine does not migrate as readily as hydrogen owing to the greater strength of the C–F bond compared with C–H. Despite many reports on aryl(trifluoromethyl)-diazirines,[31] there is little precedent for alkyl(trifluoromethyl)-diazirines, which possesses a compact structure that is less likely to undergo rearrangement. Recently, Franks and colleagues reported the synthesis of the photoaffinity probe of propofol bearing dimethylene(trifluoromethyl)diazirine.[43]

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 trifluoromethyl photo-methionine and trifluoromethyl photo-leucine (Fig. 1B) are described, along with those of photo-methionine and -leucine. The photochemical properties of the photoreactive amino acids and the SOAT inhibitory activities of the synthesized analogues were also investigated.

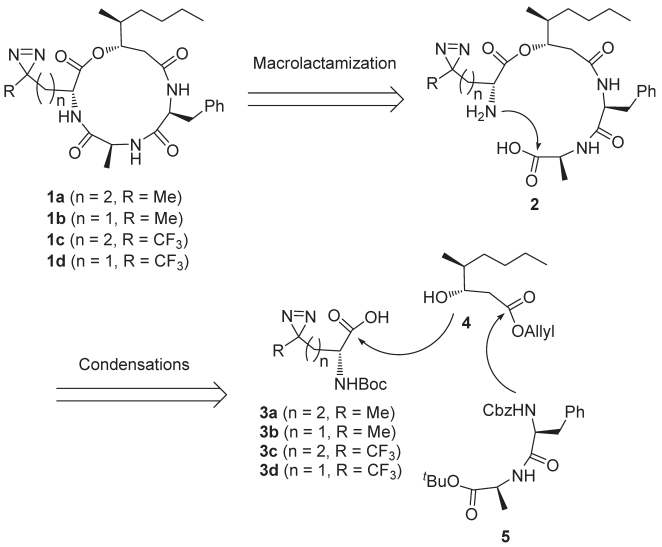

Chart 1. Retrosynthetic Analysis of **1a**–**d**

Results and Discussion

Design of Beauveriolide Analogues Bearing Photoreactive Amino Acids Our SAR studies of beauveriolides[16–21] 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.[21] 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).

Retrosynthesis 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[16] (Chart 1). In principle, **1a**–**d** can be synthesized by macrolactamization 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-photo-methionine from a L-glutamic

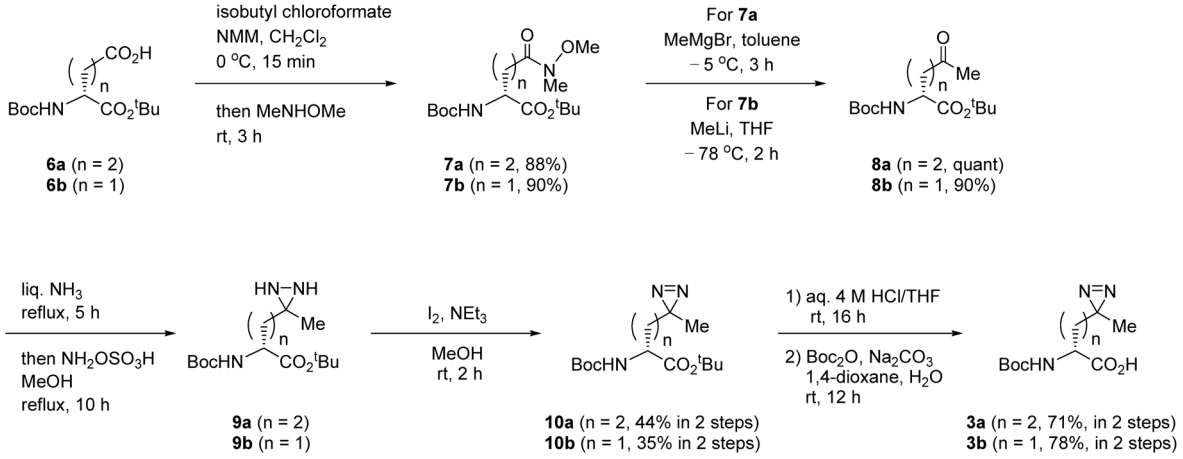

Chart 2. Synthesis of **3a** and **b**

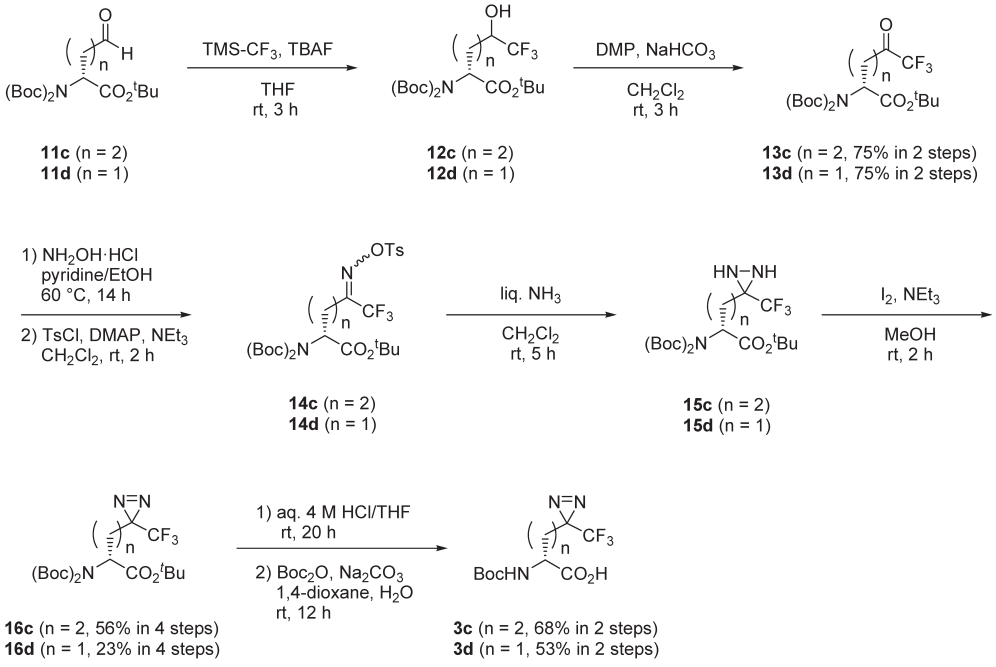

Chart 3. Synthesis of **3c** and **d**

acid derivative.[32] We followed their synthetic route for the preparation of *tert*-butoxycarbonyl (Boc)-D-photo-methionine (**3a**) (Chart 2). Briefly, Boc-D-glutamic acid *α-tert*-butyl ester (**6a**) (the preparation method for this compound is summarized in Chart S1) was transformed into the corresponding *ε*-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 *t*-butyl groups, followed by Boc protection of the resulting amine gave Boc-D-photo-methionine (**3a**). Since the synthesis of L-photo-leucine from an L-aspartic acid derivative has not been reported, we attempted to synthesize Boc-D-photo-leucine (**3b**) in the same manner as that used for **3a** (Chart 2). However, the *δ*-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-photo-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_3),[44] followed by oxidation of the resulting alcohol **12c** with Dess–Martin periodinate (DMP),[45] in 75% yield in three steps. In this synthesis, protection of the *α*-amino group with bis-Boc groups was adopted because mono-Boc protected aldehydes readily cy-

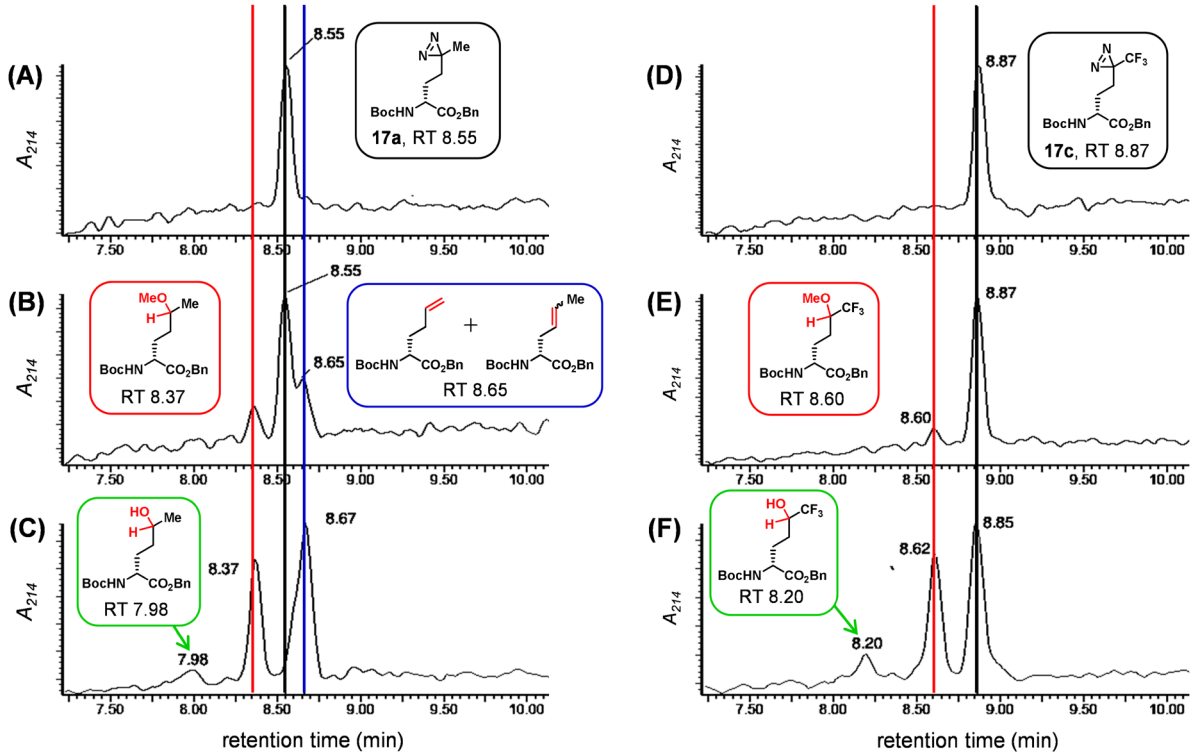

Fig. 2. LC-MS Analysis of the Photolysis of **17a** (A–C) and **17c** (D–F) in MeOH Irradiated with 352 nm Blacklight (Output, 6 W; UV Output, 0.6 W; Distance, 5 cm)

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™ $C_{1,8}$ (3.5 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/H_2O, 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^+).

clize to the aminal, which is then often reluctant to undergo further reactions.[46] Preparation of the trifluoromethyldiazirine moiety in **16c** was unsuccessful using the previous reaction conditions, including the treatment of the ketone **13c** with liquid ammonia and hydroxylamine-*O*-sulfonic acid followed by oxidation. Therefore, we converted **13c** to tosyloxime **14c**; treatment with liquid ammonia then furnished diaziridine **15c**. Due to the instability of **15c** under the purification conditions, the compound was subsequently oxidized with iodine in the presence of triethylamine[47] to afford the desired trifluoromethyldiazirine **16c**. Finally, removal of the Boc and *t*-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 absorption, we attached a benzyl ester to **3a** and **c** 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 black light at 6 W (UV output: 0.6 W) from a distance of 5 cm, and the resultant solution was analyzed by LC-MS (Fig. 2). Although methyldiazirine **17a** rapidly decomposed, a large quantity of alkenes was produced. In contrast, photolysis of trifluoromethyldiazirine **17c** generated much less alkenes, and approximately half of the consumed trifluoromethyldiazirine was cross-linked with MeOH. However, the photolysis of trifluoromethyldiazirine **17c** was sluggish compared with methyldiazirine **17a** (Fig. 2). The UV absorbance spectra showed that the absorbance maximum between 300 and 400 nm is 347 nm for **17a**, and 317 nm for **17c** (Fig. S1). This could be a reason for **17c** being hard to decompose under 352 nm black light. These data indicate that trifluoromethyldiazirine is more efficient for cross linking, but needs 300–330 nm wavelength UV light.

Total Synthesis of 1a–d With the desired photoreactive amino acids **3a–d** in hand, we incorporated them into beauveriolide analogues (Chart 4). After allyl ester protection of the carboxyl group in **18**, which was prepared as previously reported,[16] the resultant alcohol **4** was coupled with **3a–d** using *N*,*N*′-diisopropylcarbodiimide (DIC) and 4-(dimethylamino)-pyridine (DMAP) to afford esters **19a–d** in 81–90% yields. After removal of the allyl groups in **19a–d** and the benzyloxycarbonyl (Cbz) group in **5** (the preparation method for this compound is described in Supplementary materials), the resultant carboxylic acids and amine were coupled by treatment with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIEA) to afford the linear compounds **20a–d**. Finally, macrolactamization was successfully conducted with EDCI for **20a** and **b** (R=CH_3) or 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) (HATU)[48] for **20c** and **d** (R=CF_3) under high-dilution conditions to afford the desired macrocycles **1a–d** in moderate yields. Considerable amount of a dimeric product (*ca.* 25%) was observed only in the formation of **1c**. No serious epimerization at the α-position of the alanine residue in the C-terminus was observed by LC-MS analysis.[16–18,21] The structures of **1a–d** were unambiguously determined by instru-

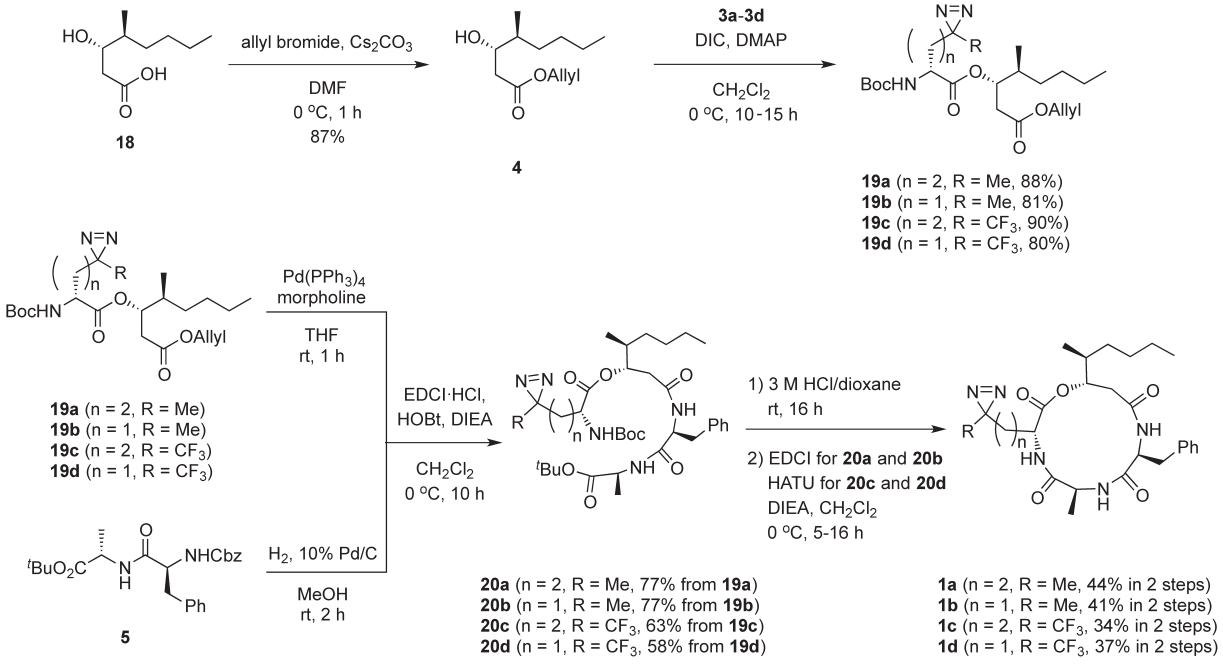

Chart 4. Total Syntheses of **1a–d**

Table 1. SOAT Inhibitory Activities of **1a–d**[a)]

Compound	IC_{50} (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[9)]	0.60	20	2.2	1.9
Beauveriolide III[9)]	0.90	>20	3.0	3.0

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

mental analyses, including 2D NMR.

SOAT Inhibitory Activity The SOAT inhibitory activities of **1a–d** were estimated by a cell-based assay using SOAT1-CHO and SOAT2-CHO. Compounds **1a–d** and [^{14}C]-oleic acid were added to each cell culture, and the resultant radioactivities of [^{14}C]CE, [^{14}C]triacylglycerol (TG), and [^{14}C]-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 IC_{50} 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.

Inhibition of CE syntheses by **1a–d** was also investigated by an enzyme assay. After addition of the compounds and [^{14}C]oleoyl-CoA to a microsomal fraction prepared from SOAT1-CHO or SOAT2-CHO, the generated [^{14}C]CE was measured, and the IC_{50} 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.

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 alkene products 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).

Experimental

General Techniques All commercially available reagents were used as received. Dry THF and CH_2Cl_2 (Kanto Chemical Co., Tokyo, Japan) were obtained by passing through activated alumina column with commercially available predried, 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 H_2SO_4–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). [1-^{14}C]Oleic acid was purchased from PerkinElmer, Inc. Life and Analytical Sciences (U.S.A.). [^{14}C]Oleoyl-CoA was purchased from GE Healthcare Bio-Science (U.S.A.). Fetal bovine serum (FBS) was bought from HyClone (U.S.A.). Ham's F12 was obtained from Sigma-Aldrich (U.S.A.). Geneticin (G-418 sulfate) and MEM vitamin solution were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Penicillin (10000 units/mL)/streptomycin (10000 units/mL) solution was acquired from Invitrogen (U.S.A.). Plastic microplates (48-well) were purchased from Asahi Techno Glass (Japan).

^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded on a JEOL JNM-AL400 (400 MHz for ^{1}H) or a JEOL ECA-600 (600 MHz for ^{1}H) spectrometer in the indicated solvent. Chemical shifts (δ) for ^{1}H-NMR spectra are referenced to signals for internal tetramethylsilane (0 ppm) and residual non-deuterated solvents (chloroform 7.26 ppm; methanol-d_4 3.30 ppm). Chemical shifts (δ) for ^{13}C-NMR spectra are referenced to signals for residual deuterated solvents (chloroform-*d* 77.0 ppm, methanol-d_4 49.0 ppm). Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintet), m (multiplet), dd (double doublet), dt (double triplet), br s (broad singlet). Coupling constants (J) are represented in hertz (Hz).

MS and HR-MS were measured on JEOL JMS-DX303 (for electron ionization (EI)), MS-AX500 (for FAB), SYNAPT G2 HDMS instruments (for electrospray ionization (ESI)). IR spectra were recorded on a JASCO FTIR-4100. Only the strongest and/or structurally important absorptions are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a JASCO P-1010 polarimeter at 589 nm. Melting points were measured on a RFS-10 melting point apparatus (Round Science Inc.) and are uncorrected.

Analytical reversed-phase HPLC (RP-HPLC) was performed on a Waters LC/MS system consisting of a Waters 600 HPLC System equipped with a Waters 2996 Photodiode Array Detector and a Waters Micromass ZQ 2000 unit. Preparative RP-HPLC was carried out on a Waters 1525EF HPLC System equipped with a Waters 2489 UV/Visible detector (monitoring at 214, 254 nm).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-4-[methoxy(methyl)carbamoyl]butanoate (7a)** To a solution of carboxylic acid **6a** (5.30 g, 17.5 mmol, 1.00 equiv.) in dry CH_2Cl_2 (84 mL) at 0°C was added *N*-methylmorpholine (NMM) (4.80 mL, 43.8 mmol, 2.50 equiv.) followed by isobutyl chloroformate (2.90 mL, 21.9 mmol, 1.25 equiv.). The mixture was stirred for 15 min, and then *N,O*-dimethylhydroxylamine hydrochloride (2.05 g, 21.0 mmol, 1.20 equiv.) was added portionwise. The reaction was then allowed to warm up to 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 CH_2Cl_2. The organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=7 : 3) to afford Weinreb amide **7a** (5.33 g, 15.4 mmol, 88%) as a colorless oil. ^{1}H-NMR (600 MHz, $CDCl_3$) 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). ^{13}C-NMR (150 MHz, $CDCl_3$) 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, 1251, 1157, 1051, 1026, 848. HR-ESI-MS *m/z*: 369.1989 (Calcd for $C_{16}H_{30}N_2O_6Na$ [M+Na]$^+$: 369.1996). $[\alpha]_D^{24}$ −5.40 (c=0.950, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-[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). ^{1}H-NMR (400 MHz, $CDCl_3$) 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). ^{13}C-NMR (100 MHz, $CDCl_3$) 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, 1718, 1662, 1496, 1391, 1368, 1252, 1157, 1023, 850. HR-ESI-MS *m/z*: 355.1832 (Calcd for $C_{15}H_{28}N_2O_6Na$ [M+Na]$^+$: 355.1840). $[\alpha]_D^{24}$ −19.8 (c=1.50, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-5-oxohexanoate (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 Na_2SO_4, 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. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.05 (1H, br s), 4.15 (1H, br s), 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). ^{13}C-NMR (100 MHz, $CDCl_3$) 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 $C_{15}H_{27}NO_5Na$ [M+Na]$^+$: 324.1781). $[\alpha]_D^{20}$ −4.00 (c=1.00, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-4-oxopentanoate (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 Na_2SO_4, 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. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.44 (1H, d, J=6.8 Hz), 4.36 (1H, br s), 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). ^{13}C-NMR (100 MHz, $CDCl_3$) 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 (Calcd for $C_{14}H_{25}NO_5Na$ $[M+Na]^+$: 310.1625). $[\alpha]_D^{23}$ −13.8 (*c*=0.95, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-4-(3-methyl-3*H*-diazirin-3-yl)butanoate (10a)** A solution of ketone **8a** (3.30 g, 10.9 mmol, 1.00 equiv.) in liquid ammonia (24 mL) was stirred under reflux (dry ice condenser) for 5 h. Then, the reaction flask was cooled to −50°C and hydroxylamine-*O*-sulfonic acid (1.43 g, 12.7 mmol, 1.15 equiv.) dissolved in dry methanol (6.3 mL) was added over 30 min. The reaction was then allowed to stir under reflux for 10 h until ammonia was evaporated. The resulting slurry was filtered and the filter cake was washed with several portions of methanol. The filtrate was concentrated until ammonia was completely evaporated.

The residue was then diluted with methanol (38 mL) and cooled to 0°C. NEt_3 (1.52 mL, 10.9 mmol, 1.00 equiv.) was added followed by portionwise addition of iodine until a brown color persisted. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=10 : 1) to afford methyldiazirine **10a** (1.50 g, 4.80 mmol, 44% in 2 steps) as white solids. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) *δ*: 5.04 (1H, m), 4.06 (1H, brs), 1.62 (1H, m), 1.39–1.37 (19H, s) 1.29–1.19 (2H, m), 0.943-0.936 (3H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) *δ*: 171.2, 155.1, 81.9, 79.5, 53.2, 30.2, 28.1, 27.8, 27.2, 25.1, 19.5. IR (neat) cm^{-1}: 3362, 2979, 2932, 2870, 1716, 1581, 1501, 1455, 1368, 1253, 1160, 1049, 847. HR-ESI-MS *m/z*: 336.1882 (Calcd for $C_{15}H_{27}N_3O_4Na$ $[M+Na]^+$: 336.1894). $[\alpha]_D^{28}$ −15.7 (*c*=1.00, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-(3-methyl-3*H*-diazirin-3-yl)propanoate (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). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) *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, 6.8 Hz), 1.55 (0.1×9H, s), 1.53 (0.1×9H, s), 1.49 (0.9×9H, s), 1.47 (0.9×9H, s), 1.09 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) *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, 23.8, 19.7, 14.1. IR (neat) cm^{-1}: 3364, 2980, 2933, 1716, 1505, 1456, 1368, 1251, 1156, 1066, 847. HR-ESI-MS *m/z*: 322.1736 (Calcd for $C_{14}H_{25}N_3O_4Na$ $[M+Na]^+$: 322.1737). $[\alpha]_D^{24}$ −13.1 (*c*=1.00, $CHCl_3$).

(*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-4-(3-methyl-3*H*-diazirin-3-yl)butanoic Acid (3a) A solution of Boc-D-photo-Met-O^{t}Bu (**10a**) (1.19 g, 3.79 mmol, 1.00 equiv.) in THF (118 mL) and 8 M 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 a mixture of 1,4-dioxane (3.8 mL) and H_2O (3.8 mL) was added di-*tert*-butyl dicarbonate (910 mg, 4.17 mmol, 1.10 equiv.) and Na_2CO_3 (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 $NaHCO_3$ and brine, dried over $MgSO_4$, 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. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) *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). ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) *δ*: 176.5, 175.6, 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, 1454, 1394, 1369, 1252, 1164, 1051, 1027, 855, 779. HR-FAB-MS 258.1461 (Calcd for $C_{11}H_{20}N_3O_4$ $[MH]^+$: 258.1454). $[\alpha]_D^{27}$ −16.8 (*c*=1.00, $CHCl_3$).

(*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-(3-methyl-3*H*-diazirin-3-yl)propanoic Acid (3b) 3b was prepared from Boc-D-photo-Leu-O^{t}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). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) *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, 4.6 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). ^{13}C-NMR (100 MHz, $CDCl_3$) *δ*: 176.0, 155.4, 80.7, 50.2, 37.5, 28.3, 23.7, 19.7. IR (neat) cm^{-1}: 3323, 2966, 2928, 2857, 1717, 1508, 1455, 1394, 1368, 1254, 1163, 1051, 1025, 878. HR-FAB-MS *m/z*: 244.1302 (Calcd for $C_{14}H_{25}N_3O_4Na$ $[M+Na]^+$: 244.1297). $[\alpha]_D^{24}$ −15 (*c*=0.47, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[Bis(*tert*-Butoxy)carbonyl]amino}-6,6,6-trifluoro-5-oxohexanoate (13c)** To a solution of aldehyde **11c** (350 mg, 0.903 mmol, 1.00 equiv.) in dry THF (4.5 mL) was added tetramethylsilane, or trimethylsilyl (TMS)-CF_3 (266 μL, 1.80 mmol, 2.00 equiv.) and tetrabutylammonium fluoride (TBAF) (90 μL, 1.0 M in THF, 90.3 μmol, 0.10 equiv.) at 0°C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was poured into 1 M aqueous HCl at 0°C and the mixture was stirred for 2 h. The aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with 1 M aqueous HCl and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the above residue in CH_2Cl_2 (9 mL) was added $NaHCO_3$ (567 mg, 6.75 mmol, 7.50 equiv.) and Dess-Martin periodinane (572 mg, 1.35 mmol, 1.50 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into saturated aqueous $Na_2S_2O_3$ and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=8 : 1) to afford trifluoromethyl ketone **13c** (307 mg, 0.677 mmol, 75% in 2 steps) as white solids. ^{1}H-NMR (400 MHz, $CDCl_3$) *δ*: 4.74 (1H, dd, *J*=9.2, 5.6 Hz), 2.89 (1H, m), 2.77 (1H, m), 2.48 (1H, m), 2.19 (1H, m), 1.50 (18H, s), 1.45 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) *δ*: 190.6 (q, *J*=36 Hz), 168.9, 152.4, 115.5 (q, *J*=293 Hz), 83.3, 81.7, 57.6, 33.2, 28.0, 27.9, 22.1. IR (neat) cm^{-1}: 3431, 2981, 2936, 1739, 1699, 1480, 1458, 1369, 1256, 1149, 1007, 849. HR-ESI-MS *m/z*: 478.2001 (Calcd for $C_{20}H_{32}F_3NO_7Na$ $[M+Na]^+$: 478.2023).

$[\alpha]_D^{24}$ +0.92 (c=1.00, $CHCl_3$).

***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). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.44 (1H, dd, J=7.7, 5.2 Hz), 3.76 (1H, dd, J=18.6, 7.8 Hz), 2.95 (1H, dd, J=18.6, 5.2 Hz), 1.51 (18H, s), 1.44 (9H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 188.5 (q, J=36 Hz), 167.9, 152.0, 115.4 (q, J=291 Hz), 83.7, 82.7, 54.0, 37.7, 28.0, 27.8. IR (neat) cm^{-1}: 2982, 2937, 1766, 1739, 1701, 1480, 1458, 1395, 1369, 1258, 1172, 1149, 846. HR-ESI-MS m/z: 464.1847 (Calcd for $C_{19}H_{30}F_3NO_7Na$ $[M+Na]^+$: 464.1867). $[\alpha]_D^{26}$ +23.4 (c=1.00, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[Bis(*tert*-butoxy)carbonyl]amino}-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]butanoate (16c)** To a solution of trifluoromethyl ketone **13c** (240 mg, 0.527 mmol, 1.00 equiv.) in dry pyridine (1 mL) and dry ethanol (0.5 mL) was added $HONH_2 \cdot HCl$ (40.3 mg, 0.58 mmol, 1.10 equiv.) under an argon atmosphere and the mixture was stirred at 60°C for 14 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 dry CH_2Cl_2 (1 mL) was added NEt_3 (190 μL, 1.37 mmol, 2.60 equiv.), tosyl chloride (TsCl) (120 mg, 0.632 mmol, 1.20 equiv.) and DMAP (3.20 mg, 26.3 μmol, 0.05 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into H_2O and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

A solution of the above residue in liquid ammonia (10 mL) and dry CH_2Cl_2 (1 mL) was stirred under reflux equipped with a dry ice condenser for 5 h until the ammonia was evaporated. Resulting slurry was filtered and the filter cake was washed with several portions of methanol. The combined filtrate was concentrated *in vacuo* and 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 NEt_3 (110 μL, 0.760 mmol, 1.50 equiv.) and I_2 (147 mg, 0.580 mmol, 1.10 equiv.) at 0°C under an argon atmosphere and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into saturated aqueous $Na_2S_2O_3$ and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=30 : 1) to afford trifluoromethyldiazirine **16c** (128.1 mg, 0.274 mmol, 56% in 4 steps) as white solids. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.53 (1H, dd, J=8.3, 5.2 Hz), 1.89 (1H, m), 1.72–1.59 (3H, m), 1.43 (18H, s), 1.37 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.8, 152.3, 122.4 (q, J=272 Hz), 83.2, 81.7, 57.8, 27.9, 27.8, 27.2 (q, J=40 Hz), 23.3, 23.1. IR (neat) cm^{-1}: 2982, 2935, 1741, 1701, 1458, 1369, 1269, 1155, 849. HR-ESI-MS m/z: 490.2125 (Calcd for $C_{20}H_{32}F_3N_3O_6Na$ $[M+Na]^+$: 490.2135). $[\alpha]_D^{24}$ +0.58 (c=1.50, $CHCl_3$).

***tert*-Butyl (*R*)-2-{[Bis(*tert*-butoxy)carbonyl]amino}-3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]propanoate (16d) 16d** was prepared from trifluoromethyl ketone **13d** (300 mg, 0.680 mmol) in the same manner to the synthesis of **16c**. Yield 23% in 4 steps (white solids). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 4.74 (1H, t, J=7.3 Hz), 2.43 (2H, d, J=7.6 Hz), 1.52 (18H, s), 1.43 (9H, s). ^{13}C-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=40 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 $C_{19}H_{30}F_3N_3O_6Na$ $[M+Na]^+$: 476.1979). $[\alpha]_D^{28}$ +27.8 (c=1.00, $CHCl_3$).

(*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]butanoic Acid (3c) A solution of **16c** (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 a mixture of 1,4-dioxane (0.28 mL) and H_2O (0.28 mL) was added di-*tert*-butyl dicarbonate (30.0 mg, 0.133 mmol, 1.10 equiv.) and Na_2CO_3 (19.0 mg, 0.182 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 and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=3 : 1) to afford **3c** (25.7 mg, 0.0820 mmol, 68% in 2 steps) as white solids. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 10.55 (1H, br s), 7.03 (0.5×1H, br s), 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); ^{13}C-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 $C_{11}H_{17}F_3N_3O_7$ $[M+H]^+$: 312.1171). $[\alpha]_D^{23}$ −5.01 (c=1.00, $CHCl_3$).

(*R*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]propanoic Acid (3d) 3d was prepared from **16d** (100 mg, 0.221 mmol) in the same manner to the synthesis of **3c**. Yield 53% in 2 steps (white solids). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 9.66 (1H, br s), 6.56 (0.3×1H, br s), 5.08 (0.7×1H, s), 4.21–4.10 (1H, m), 2.47–2.37 (1H, m), 2.21–2.11 (1H, m), 1.47 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ: 174.7 (major) and 174.0 (minor), 156.5 (minor) and 155.2 (major), 129.9 (minor, q, J=211 Hz) and 122.0 (major, q, J=274 Hz), 83.0 (minor) and 81.1 (major), 49.9 (minor) and 49.0 (major), 30.1 (minor) and 29.7 (major), 28.7 (minor) and 28.2 (major), 25.6 (q, J=41 Hz). IR (neat) cm^{-1}: 3334, 2982, 2927, 2853, 1718, 1701, 1523, 1395, 1370, 1319, 1195, 1156, 1078. HR-FAB-MS 320.0828 (Calcd for $C_{10}H_{14}F_3N_3O_4Na$ $[M+Na]^+$: 320.0834). $[\alpha]_D^{23}$ −6.73 (c=0.415, $CHCl_3$).

Allyl (3*S*,4*S*)-3-Hydroxy-4-methyloctanoic 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 $NaHCO_3$ 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 H_2O, brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo*, and then the resulting residue was purified by column chromatography on silica gel (hexane–ethyl acetate=15 : 1) to afford the allyl (3*S*,4*S*)-3-hydroxy-4-methyloctanoic acid **(4)** (2.12 g, 9.89 mmol, 87%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.92 (1H, ddt, J=17.2, 10.4, 5.6 Hz), 5.33 (1H, d, J=17.2 Hz), 5.25 (1H, d, J=10.4 Hz), 4.62 (2H, d, J=5.6 Hz), 3.95 (1H, brs), 2.79 (1H, s), 2.49 (2H, m), 1.50 (2H, m), 1.30 (4H, m), 1.14 (1H, m), 0.90 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.0, 131.9, 118.5, 71.2, 65.3, 38.8, 38.0, 32.3, 29.40, 22.9, 14.2, 14.0. IR (neat) cm^{-1}: 3459, 2958, 2930, 2873, 2860, 1737, 1380, 1276, 1173, 987 cm^{-1}. HR-ESI-MS m/z: 237.1458 (Calcd for $C_{12}H_{22}O_3Na$ $[M+Na]^+$: 237.1461). $[\alpha]_D^{24}$ −55.2 (c=1.50, $CHCl_3$).

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 CH_2Cl_2 (4 mL) was added DMAP (5.7 mg, 47 μmol, 0.10 equiv.) and DIC (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 CH_2Cl_2, washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate=5 : 1) to afford ester unit **19a** (188 mg, 0.414 mmol, 88%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.92 (1H, ddt, J=16.8, 10.0, 5.8 Hz), 5.34 (1H, dd, J=17.2, 1.6 Hz), 5.26 (2H, m), 4.98 (1H, brs), 4.59 (2H, d, J=5.8 Hz), 4.22 (1H, brs), 2.65–2.52 (2H, m), 1.73–1.65 (2H, m), 1.44 (9H, s), 1.42–1.30 (9H, m), 1.01 (3H, s), 0.92–0.87 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.3, 169.9, 155.0, 131.7, 118.3, 79.5, 74.4, 65.2, 52.6, 36.1, 36.0, 31.7, 29.9, 29.0, 28.7, 26.8, 25.0, 22.8, 19.4, 14.3, 13.8. IR (neat) cm^{-1}: 3373, 2962, 2932, 2861, 1741, 1718, 1507, 1454, 1367, 1276, 1250, 1164, 1022. HR-ESI-MS m/z: 476.2713 (Calcd for $C_{23}H_{39}N_3O_6Na$ $[M+Na]^+$: 476.2731). $[\alpha]_D^{21}$ −47.7 (c=1.50, $CHCl_3$).

Ester Unit 19b (n=1, R=Me) **19b** was prepared from **3b** (44 mg, 0.182 mmol, 1.30 equiv.) and **4** (30.0 mg, 0.140 mmol, 1.00 equiv.) in the same manner to the synthesis of **19a**. Yield 81% (a colorless oil). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.90 (1H, ddt, J=16.8, 10.4, 5.8 Hz), 5.32 (1H, d, J=16.8 Hz), 5.26 (2H, m), 5.05 (1H, d, J=7.2 Hz), 4.56 (2H, d, J=5.8 Hz), 4.34 (1H, m), 2.73–2.55 (2H, m), 1.89 (1H, dd, J=5.6 Hz), 1.75 (1H, m), 1.47 (9H, s), 1.31–1.26 (7H, m), 1.08 (3H, s), 0.91 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.1, 170.2, 155.0, 131.8, 118.7, 80.1, 75.0, 65.5, 50.3, 38.0, 36.3, 36.2, 32.1, 29.2, 28.3, 23.8, 22.7, 19.6, 14.4, 14.0. IR (neat) cm^{-1}: 3380, 2961, 2931, 2874, 1741, 1718, 1507, 1457, 1367, 1279, 1248, 1165, 1023. HR-ESI-MS m/z: 462.2562 (Calcd for $C_{22}H_{37}N_3O_6Na$ $[M+Na]^+$: 462.2575). $[\alpha]_D^{24}$ −17.3 (c=1.00, $CHCl_3$).

Ester Unit 19c (n=2, R=CF_3) **19c** was prepared from **3c** (342 mg, 1.10 mmol, 1.10 equiv.) and **4** (214 mg, 1.00 mmol, 1.00 equiv.) in the same manner to the synthesis of **19a**. Yield 90% (a colorless oil). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 5.86–5.74 (1H, m), 5.25–5.07 (4H, m), 4.47 (2H, dd, J=8.5, 7.1 Hz), 4.13 (1H, s), 2.57–2.43 (2H, m), 1.77–1.56 (4H, m), 1.34 (9H, s), 1.20 (7H, m), 0.80 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ: 170.9 (major) and 170.7 (minor), 170.0 (minor) and 169.9 (major), 155.1, 131.7, 122.2 (q, J=274 Hz), 118.5 (minor) and 118.4 (major), 79.9, 74.7, 65.31 (minor) and 65.28 (major), 52.5 (minor) and 52.3 (major), 36.3 (major) and 36.2 (minor), 35.9, 32.1 (major) and 31.8 (minor), 29.1, 28.0, 27.1 (q, J=40 Hz, major) and 27.0 (q, J=40 Hz, minor), 26.2 (minor) and 26.1 (major), 22.5, 22.1 (minor) and 21.9 (major), 14.3 (major) and 14.1 (minor), 13.73 (major) and 13.72 (minor). IR (neat) cm^{-1}: 3370, 2966, 2934, 2874, 2862, 1744, 1735, 1719, 1508, 1500, 1456, 1392, 1367, 1346, 1301, 1276, 1252, 1159, 1104, 1052, 1026, 990, 934. HR-ESI-MS m/z: 530.2432 (Calcd for $C_{23}H_{36}F_3N_3O_6Na$ $[M+Na]^+$: 530.2448). $[\alpha]_D^{25}$ −62 (c=0.90, $CHCl_3$).

Ester Unit 19d (n=1, R=CF_3) **19d** was prepared from **3d** (29.0 mg, 97.6 μmol, 1.10 equiv.) and **4** (19.0 mg, 88.7 μmol, 1.00 equiv.) in the same manner to the synthesis of **19a**. Yield 80% (a colorless oil). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 5.91 (1H, dt, J=16.8, 10.4, 5.6 Hz), 5.35–5.25 (3H, m), 5.05 (1H, d, J=6.8 Hz), 4.57 (2H, d, J=6.0 Hz), 4.14 (1H, m), 2.66 (1H, dd, J=16.0, 8.1 Hz), 2.57 (1H, dd, J=16.0, 4.4 Hz), 2.35 (1H, dd, J=16.2, 4.1 Hz), 2.09 (1H, dd, J=16.2, 8.8 Hz), 1.46 (9H, s), 1.30–1.11 (7H, m), 0.90 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ: 170.2 (major) and 170.1 (minor), 170.0, 154.8, 131.8, 122.0 (q, J=275 Hz), 118.8, 80.5, 75.4 (minor) and 75.4 (major), 65.56 (minor) and 65.54 (major), 48.8, 36.3, 36.2, 32.1, 29.2, 29.0, 28.0, 25.7 (q, J=40 Hz), 22.7, 14.4, 14.0. IR (neat) cm^{-1}: 3373, 2963, 2932, 2876, 2862, 1742, 1728, 1722, 1717, 1507, 1368, 1286, 1204, 1158, 1051. HR-ESI-MS m/z: 516.2285 (Calcd for $C_{22}H_{34}F_3N_3O_6Na$ $[M+Na]^+$: 516.2292). $[\alpha]_D^{22}$ −13.2 (c=0.475, $CHCl_3$).

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 $Pd(PPh_3)_4$ (57.0 mg, 50 μmol, 0.10 equiv.) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was diluted with ethyl acetate, washed with 1 M aqueous HCl and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of dipeptide **5** (260 mg, 0.610 mmol, 1.20 equiv.) in MeOH (11 mL) was added 10% Pd/C (52.0 mg, 20 wt%) under an argon atmosphere and the reaction mixture was purged with hydrogen three times. The reaction 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 CH_2Cl_2 (6 mL) was added HOBt (101 mg, 0.75 mmol, 1.50 equiv.), DIEA (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 $NaHCO_3$, brine, dried over $MgSO_4$, 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. ^{1}H-NMR (400 MHz, $CDCl_3$)

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=7.2 Hz), 2.44 (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). ^{13}C-NMR (100 MHz, $CDCl_3$) 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, 22.6, 19.5, 18.1, 14.2, 13.9. HR-ESI-MS m/z: 710.4091 (Calcd for $C_{36}H_{57}N_5O_8Na$ $[M+Na]^+$: 710.4099). IR (neat) cm^{-1}: 3289, 2977, 2931, 2859, 1735, 1719, 1654, 1647, 1551, 1454, 1367, 1158, 1048, 1024, 755. $[\alpha]_D^{22}$ −17 (c=0.50, $CHCl_3$).

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). ^{1}H-NMR (600 MHz, $CDCl_3$) 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, quint., J=7.8 Hz), 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), 1.44 (9H, s), 1.32–1.18 (9H, m), 1.08 (3H, s), 0.87 (6H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 171.7, 171.5, 170.1, 169.7, 155.4, 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, 1368, 1248, 1222, 1163. HR-ESI-MS m/z: 696.3921 (Calcd for $C_{35}H_{55}N_3O_8Na$ $[M+Na]^+$: 696.3943). $[\alpha]_D^{26}$ −14.2 (c=1.05, $CHCl_3$).

Depsipeptide 20c (n=2, R=CF_3) **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). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) 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, dt, J=8.0, 4.4 Hz), 4.65–4.58 (1H, m), 4.36–4.29 (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). ^{13}C-NMR (150 MHz, $CDCl_3$, mixture of rotamers) 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, 136.47 (minor) and 136.42 (major), 129.2, 128.5, 126.9, 122.2 (q, J=275 Hz), 81.97 (major) and 81.96 (minor), 80.1, 75.9, 75.41 and 75.35 (1 : 1), 54.5, 52.7 and 52.6 (1 : 1), 48.79 and 48.75 (1 : 1), 38.3, 36.4 and 35.9 (1 : 1), 32.2 (major) and 32.1 (minor), 29.2 (major) and 29.1 (minor), 28.2, 27.8, 27.2 (q, J=40.6 Hz), 25.9 (major) and 25.8 (minor), 22.7, 22.3 (minor) and 22.2 (major), 18.4 (minor) and 18.2 (major), 14.40 (minor) and 14.3 (major), 13.9. IR (neat) cm^{-1}: 3291, 2978, 2932, 2875, 1740, 1719, 1645, 1551, 1456, 1368, 1155. HR-ESI-MS m/z: 764.3802 (Calcd for $C_{36}H_{54}F_3N_5O_8Na$ $[M+Na]^+$: 764.3817). $[\alpha]_D^{23}$ −23.0 (c=0.50, $CHCl_3$).

Depsipeptide 20d (n=1, R=CF_3) **20d** was prepared from ester unit **19d** (26.1 mg, 52.7 μmol) and dipeptide **5** (27 mg, 63.2 μmol) in the same manner to the synthesis of **20a**. Yield 58% from **19d** (a colorless oil). ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 7.31–7.19 (5H, m), 6.36 (1H, d, J=7.8 Hz), 6.28 (1H, d, J=7.3 Hz), 5.22 (1H, d, J=7.8 Hz), 5.15 (1H, dt, J=8.3, 4.9 Hz), 4.62 (1H, m), 4.33 (1H, quint, J=7.3 Hz), 4.17 (1H, m), 3.11 (1H, dd, J=13.9, 6.8 Hz), 3.04 (1H, dd, J=13.9, 7.3 Hz), 2.42 (2H, m), 1.83–1.67 (2H, m), 1.44 (18H, s), 1.32 (3H, d, J=7.3 Hz), 1.30–1.18 (7H, m), 0.88 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ: 171.8 (major) and 171.5 (minor), 170.5 (major) and 170.4 (minor), 170.2, 169.5, 155.0, 136.4, 129.2 (major) and 129.1 (minor), 128.6, 127.0, 122.1 (J=273 Hz), 82.1, 80.3, 75.9, 54.6 (minor) and 54.4 (major), 48.8, 38.4, 38.1, 36.4, 32.1 (major) and 31.9 (minor), 29.7, 29.1, 28.2, 27.9, 25.8 (J=38 Hz), 22.7, 18.3, 18.1, 14.4 (major) and 14.3 (minor), 14.1 (minor) and 14.0 (major). IR (neat) cm^{-1}: 3285, 3067, 2963, 2931, 2858, 1738, 1733, 1645, 1557, 1456, 1368, 1284, 1154, 1051, 750. HR-ESI-MS m/z: 750.3651 (Calcd for $C_{35}H_{52}F_3N_5O_8Na$ $[M+Na]^+$: 750.3660). $[\alpha]_D^{22}$ −10.3 (c=1.00, $CHCl_3$).

Beauveriolide Analogue 1a (n=2, R=Me) To a solution of depsipeptide **20a** (140 mg, 0.204 mmol, 1.00 equiv.) in 4 M HCl/dioxane (13 mL) was stirred at room temperature for 16 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 CH_2Cl_2 (210 mL) was added DIEA (142 μL, 0.816 mmol, 4.00 equiv.) and the mixture was stirred at 0°C at 20 min under an argon atmosphere. To the reaction mixture was added EDCI·HCl (117.3 mg, 0.612 mmol, 3.00 equiv.) at 0°C and the mixture was stirred at the same temperature for 8 h and concentrated *in vacuo*. The residue was diluted with organic solvent (ethyl acetate–acetonitrile=4 : 1), washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH=9 : 1) and further purified by reversed-phase HPLC (column, YMC-Pack R&D ODS-A 20 mm×150 mm; solvent, H_2O–MeOH=25 : 75 to 10 : 90 linear gradient (0.0−15.0 min), H_2O–MeOH=10 : 90 isocratic (15.0−20.0 min); flow rate, 12.0 mL/min) to afford beauveriolide analog **1a** (46.1 mg, 89.7 μmol, 44% in 2 steps) as white solids. ^{1}H-NMR (600 MHz, $CDCl_3$–CD_3OD=1 : 1) δ: 7.30–7.18 (5H, m), 4.97 (1H, ddd, J=19.8, 10.8, 4.8 Hz), 4.47 (1H, t, J=7.8 Hz), 4.27 (1H, m), 3.88 (1H, q, J=7.2 Hz), 3.10 (1H, m), 2.99 (1H, m), 2.50 (2H, m), 2.11 (1H, m), 1.63–1.27 (13H, m), 1.01 (3H, s), 0.93–0.89 (6H, m). ^{13}C-NMR (150 MHz, $CDCl_3$–CD_3OD=1 : 1) δ: 171.6, 171.4, 171.3, 168.7, 136.1, 128.7, 128.2, 126.6, 76.3, 56.4, 53.2, 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, 1450, 1371, 1254, 1149, 1006. HR-ESI-MS m/z: Found 536.2837 (Calcd for $C_{27}H_{39}N_5O_5Na$ $[M+Na]^+$: 536.2843). $[\alpha]_D^{24}$ −41 (c=0.25, $CHCl_3$–MeOH=1 : 1).

Beauveriolide Analogue 1b (n=1, 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). ^{1}H-NMR (600 MHz, $CDCl_3$–CD_3OD=1 : 1) δ: 7.24 (5H, 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, m), 1.08 (3H, s), 0.89 (6H, m). ^{13}C-NMR (150 MHz, $CDCl_3$–CD_3OD=1 : 1) δ: 171.8, 171.3, 171.1, 168.3, 136.1, 128.8, 128.3, 126.7, 56.4, 50.1, 49.5, 37.2, 35.9, 35.5, 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,

1682, 1640, 1536, 1375, 1330, 1246, 1002. HR-ESI-MS *m/z*: 522.2679 (Calcd for $C_{26}H_{37}N_5O_5Na$ $[M+Na]^+$: 522.2687). $[\alpha]_D^{24}$ −25.0 (c=0.150, $CHCl_3$–MeOH=1 : 1).

Beauveriolide Analogue 1c (n=2, R=CF_3) To a solution of depsipeptide **20c** (20 mg, 27.0 μmol, 1.00 equiv.) in 4 M HCl/dioxane (2 mL) was stirred at room temperature for 16 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 CH_2Cl_2 (27 mL) was added DIEA (28.2 μL, 162.0 μmol, 6.00 equiv.) and the mixture was stirred at 0°C at 20 min under an argon atmosphere. To the reaction mixture was added HATU (30.8 mg, 81.0 μmol, 3.00 equiv.) at 0°C and the mixture was stirred at the same temperature for 5 h and concentrated *in vacuo*. The residue was diluted with organic solvent (ethyl acetate–acetonitrile=4 : 1), washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH=9 : 1) and further purified by reversed-phase HPLC (column, YMC-Pack R&D ODS-A 20 mm×150 mm; solvent, H_2O–MeOH=25 : 75 to 10 : 90 linear gradient (0.0−15.0 min), H_2O–MeOH=10 : 90 isocratic (15.0−20.0 min); flow rate, 12.0 mL/min) to afford beauveriolide analog **1c** (5.19 mg, 9.10 μmol, 34% in 2 steps) as white solids. ^{1}H-NMR (600 MHz, $CDCl_3$–CD_3OD=1 : 1, mixture of rotamers) δ: 7.24 (5H, m), 4.94 (1H, m), 4.48 (1H, m), 4.30 (1H, t, J=7.2 Hz), 3.80 (1H, m), 3.05 (1H, m), 2.95 (1H, m), 2.59 (1H, m), 2.45 (1H, m), 2.06 (1H, m), 1.81–1.75 (2H, m), 1.61 (1H, m), 1.53–1.28 (7H, m), 1.22 (3H, d, J=6.2 Hz), 0.94–0.90 (6H, m). ^{13}C-NMR (600 MHz, $CDCl_3$–CD_3OD=1 : 1, mixture of rotamers) δ: 173.3, 173.3, 172.7, 169.4, 137.5, 130.1 and 129.9 (1 : 1), 129.4 (minor) and 129.2 (major), 127.8 and 127.6 (1 : 1), 123.4 (q, J=273 Hz), 78.1 (minor) and 77.9 (major), 57.5 and 57.5 (1 : 1), 54.1 (major) and 54.0 (minor), 50.6 (minor) and 50.5 (major), 37.1, 36.8, 36.6, 31.8, 30.5 (minor) and 30.3 (major), 28.0 (q, J=40 Hz), 26.6, 23.7, 23.3, 15.9, 15.3 (minor) and 15.2 (major), 14.3. IR (neat) cm^{-1}: 3296, 2919, 2850, 1727, 1683, 1640, 1537, 1454, 1420, 1375, 1322, 1273, 1236, 1157, 1093. HR-ESI-MS *m/z*: 590.2555 (Calcd for $C_{27}H_{36}F_3N_5O_5Na$ $[M+Na]^+$: 590.2561). $[\alpha]_D^{24}$ −48.4 (c=0.100, $CHCl_3$–MeOH=1 : 1).

Beauveriolide Analogue 1d (n=1, R=CF_3) **1d** was prepared from depsipeptide **20d** (20 mg, 27.5 μmol in the same manner to the synthesis of **1c**. Yield 37% in 2 steps (white solids). ^{1}H-NMR (600 MHz, $CDCl_3$–CD_3OD=1 : 1, mixture of rotamers) δ: 7.20–7.10 (5H, m), 4.77 (1H, m), 4.39–4.34 (1H, m), 4.22–4.16 (1H, m), 3.97 (1H, m), 2.94 (1H, m), 2.86–2.80 (1H, m), 2.48 (1H, m), 2.33 (1H, m), 1.37 (2H, m), 1.23–1.17 (7H, m), 1.15 (3H, d, J=6.8 Hz), 0.85–0.75 (6H, m). ^{13}C-NMR (150 MHz, $CDCl_3$–CD_3OD=1 : 1, mixture of rotamers) δ: 174.4, 173.3 (major) and 173.0 (minor), 172.6 (major) and 172.5 (minor), 167.9 (minor) and 167.8 (major), 137.3 (major) and 137.1 (minor), 129.8 (minor) and 129.7 (major), 129.2, 127.6, 122.9 (J=271 Hz), 57.3 (minor) and 56.1 (major), 52.1(minor) and 50.6 (major), 37.7 37.3, 37.0, 31.9 (major) and 31.8 (minor), 30.3, 30.0 (J=40 Hz), 29.7, 28.7 (minor) and 28.6 (major), 23.5, 16.2, 15.6, 15.1, 14.2. IR (neat) cm^{-1}: 3308, 2958, 2925, 2854, 1730, 1690, 1675, 1641, 1534, 1454, 1378, 1319, 1288, 1259, 1153, 1092, 1029, 803. HR-ESI-MS *m/z*: 576.2390 (Calcd for $C_{26}H_{34}F_3N_5O_5Na$ $[M+Na]^+$: 576.2404). $[\alpha]_D^{23}$ −11 (c=0.080, $CHCl_3$–MeOH=1 : 1).

Photolysis **17a** and **c** in MeOH (1 mg/mL) cooled in an ice bath were irradiated with black light using Toshiba FL-6BL-B black light fluorescent lamp (peak wavelength 352 nm; output 6W; UV output 0.6 W) from a distance of 5 cm. Ten microliter of the resultant solution was injected to a LC-MS system. The analytical conditions are as follows: column, X Bridge™ C18 (3.5 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–H_2O, B: 0.1% HCOOH–MeOH); flow rate, 1.1 mL/min; UV 214 nm.

Culture of SOAT1- and SOAT2-CHO Cells SOAT1- and SOAT2-CHO cells expressing African Green monkey SOAT1 and SOAT2,[10] respectively, which were kind gifts from Dr. Rudel L. L. (Wake Forest University, U.S.A.), were maintained at 37°C in 5% CO_2 in Ham's F-12 medium supplemented with MEM vitamins, geneticin (300 μg/mL) and 10% heat inactivated FBS (hereafter referred to as medium A).

Cell-Based Assay for SOAT Inhibitory Activity SOAT1- or SOAT2-CHO cells (1.25×10^5 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% CO_2. 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-^{14}C]oleic acid (1 nmol, 1.85 KBq, 10% ethanol/phosphate buffered saline (PBS) solution) were added to each culture at 37°C in 5% CO_2. 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.[49] 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 [^{14}C]CE, [^{14}C]TG, and [^{14}C]PL were analyzed by a bioimaging analyzer (FLA-7000, FUJIFILM, Japan). In this cell-based assay, [^{14}C]CE was produced by the reaction of SOAT1 or SOAT2. SOAT inhibitory activity (%) is defined as (1-[^{14}C]CE-drug/[^{14}C]CE-control)×100. The IC_{50} 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 (2×10^8 cells) were homogenized in 10 mL of cold buffered sucrose solution (pH 7.2) containing 100 mM sucrose, 50 mM KCl, 40 mM KH_2PO_4, 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 mg/mL bovine serum albumin (BSA) in buffer A and [1-^{14}C]oleoyl-CoA (20 μM, 3.7 kBq) together with a test sample (added as a 10 μL methanol solution), and the SOAT1 or SOAT2 microsomal fraction (150 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-^{14}C]oleoyl-CoA, and stopped by adding 1.2 mL of $CHCl_3$–MeOH=2 : 1. The produced [^{14}C]CE was extracted by the method of Bligh and Dyer.[49] After the organic solvent was removed by evaporation, lipids was separated on a TLC plate and the radioactivity of [^{14}C]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 ^{1}H- and ^{13}C-NMR spectra.

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